Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Azaindolylsulfonamides, with a More Selective Inhibitory Effect on Histone Deacetylase 6 Activity, Exhibit Antitumor Activity in Colorectal Cancer HCT116 Cells

Hsueh-Yun Lee,[†] An-Chi Tsai,[†] Mei-Chuan Chen,[#] Po-Jung Shen,[†] Yun-Ching Cheng,[‡] Ching-Chuan Kuo,[⊥] Shiow-Lin Pan,[∥] Yi-Min Liu,[†] Jin-Fen Liu,[‡] Teng-Kuang Yeh,[⊥] Jing-Chi Wang,[∥] Chi-Yen Chang,[‡] Jang-Yang Chang,*[,‡,§] and Jing-Ping Liou*[,†]

[†]School of Pharmacy, College of Pharmacy, Taipei Medical University, 250 Wuxing Street, Taipei 11031, Taiwan

[‡]National Institute of Cancer Research, National Health Research Institutes, Tainan 704, Taiwan

[§]Division of Hematology and Oncology, Department of Internal Medicine, National Cheng Kung University Hospital, and College of Medicine, National Cheng Kung University, Tainan, Taiwan

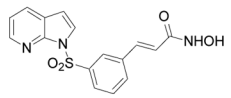
[∥]The Ph.D Program for Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, 250 Wuxing Street, Taipei 11031, Taiwan

[⊥]Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhunan Town, Miaoli County, Taiwan

[#]Ph.D Program for the Clinical Drug Discovery from Botanical Herbs, College of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan

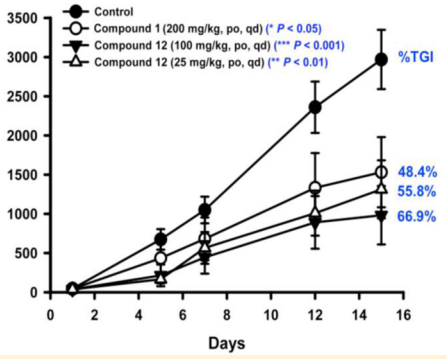
Supporting Information

Graphic for manuscript



12

Compd	HDAC1/HDAC6	HDAC2/HDAC6
12	60	223
1	12	43
5	12	10



ABSTRACT: A series of indolylsulfonylcinnamic hydroxamates has been synthesized. Compound **12**, (*E*)-3-(3-((1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)sulfonyl)phenyl)-*N*-hydroxyacrylamide, which has a 7-azaindole core cap, was shown to have antiproliferative activity against KB, H460, PC3, HSC-3, HONE-1, A549, MCF-7, TSGH, MKN45, HT29, and HCT116 human cancer cell lines. Pharmacological studies indicated that **12** functions as a potent HDAC inhibitor with an IC_{50} value of 0.1 μM. It is highly selective for histone deacetylase 6 (HDAC6) and is 60-fold more active than against HDAC1 and 223-fold more active than against HDAC2. It has a good pharmacokinetic profile with oral bioavailability of 33%. In in vivo efficacy evaluations in colorectal HCT116 xenografts, compound **12** suppresses tumor growth more effectively than SAHA (**1**, *N*-hydroxy-*N*′-phenyloctanediamide) and is therefore seen as a suitable candidate for further investigation.

INTRODUCTION

Epigenetic or chromatin modification is recognized by non-histone proteins and is a code of gene expression. Histone acetyltransferase (HAT) and histone deacetylase (HDAC) regulate epigenetic modifications by respectively executing the acetylation and deacetylation of the lysine residues at the amino terminal of histones.[1] Consequently, histone deacetylases are considered to be important targets in the development of anticancer agents.

Histone deacetylases are grouped into class I, II, III, or IV.[2] HDACs 1, 2, 3, and 8 comprise the class I HDACs and are located mainly in the nucleus. Class II HDACs, located in the cytoplasm, are in two subclasses: class IIa (HDACs 4, 5, 7, and 9) and class IIb (HDACs 6 and 10). Class I and II HDACs, with the assistance of a zinc ion, execute the deacetylation at the N-terminal lysine residue. Class III HDACs, also called nonclassical

Received: December 11, 2013
Published: April 25, 2014

dx.doi.org/10.1021/jm401899x | *J. Med. Chem.* 2014, 57, 4009−4022

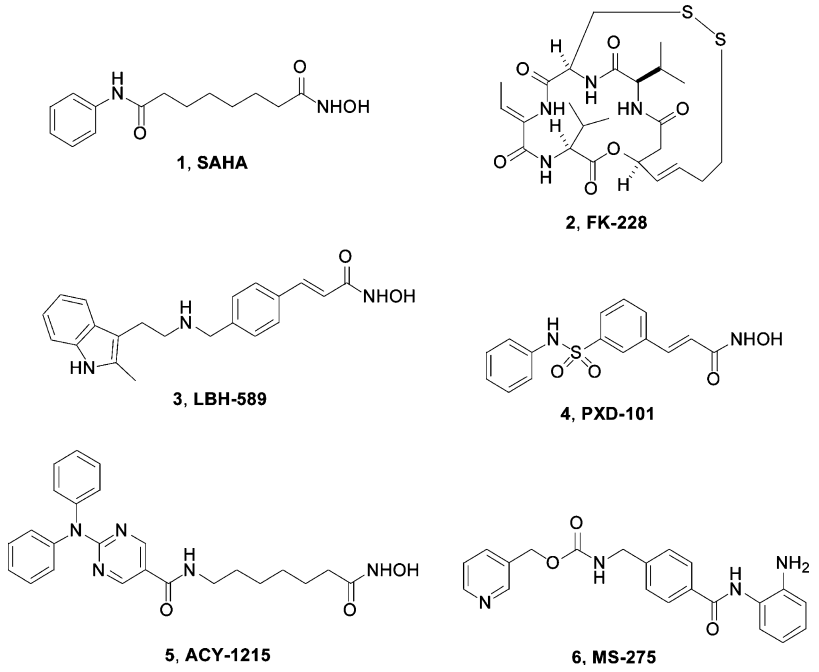


Figure 1. A variety of HDAC inhibitors.

HDACs, are considered to be sirtuins and are involved in the chromatin modification through NAD^+-independent mechanism, and class IV HDACs such as HDAC 11 reside in both nucleus and cytoplasm. Currently, epigenetic aberrance is recognized as a cause of cancer and has attracted much scientific effort to develop anticancer agents targeting HDACs. The discovery of trichostatin A (TSA), an antifungal agent with potent antiproliferative activity as a result of its inhibiting the function of HDAC, has prompted numerous investigations toward the development of HDAC inhibitors.[3]

HDAC inhibitors are grouped chemically into four classes: hydroxamic acids, benzamides, short-chain fatty acids, and cyclic tetrapeptides. To date, many studies on HDAC inhibitor development have focused on the hydroxamic acids. The hydroxamic acid moiety has been identified as a crucial motif interacting with HDAC by chelation of a zinc ion at the catalytic site in the case of trichostatin A and suberoylanilide hydroxamic acid (**1**, *N*-hydroxy-*N*′-phenyloctanediamide or SAHA; Figure 1)[4] and has attracted numerous investigations into the development of hydroxamic acid derivatives. Vorinostat (**1**, Merck & Co.), for example, is a successful HADC inhibitor first approved by the U.S. FDA to treat cutaneous T-cell lymphoma (CTCL).[5] In addition, compounds such as LBH589 (**3**)[6] and belinostat (PXD101, **4**)[7] with an *N*-hydroxyacrylamide moiety exhibit excellent HDAC inhibitory activity and are currently undergoing clinical trials.[8] Development of selective isotype HDAC inhibitors that can avoid the side effects of HDACi treatment is a significant task and an increasing number of investigations are focusing on the development of selective isotype HDAC inhibitors. An example is ACY-1215 (**5**), an HDAC6-selective inhibitor that is currently undergoing human clinical trials for the treatment of multiple myeloma.[9] In addition, some second-generation selective HDAC6 inhibitors, with better HDAC6 inhibition activity compared with tubastatin A, showed immunosuppression activity in murine T cells, suggesting that inhibition of HDAC6 may provide therapeutic benefits in autoimmune disorders.[10]

In our previous study, 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indoles and 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines were identified as potent HDAC inhibitors with potent antitumor and anti-inflammatory activity in vitro and in vivo.[11] Such promising results drew our attention to the possibilities of further modification. The synthetic compounds comprise the following three structural features of HDAC inhibitors: a Zn^{2+}-binding group (ZBG), a hydrophobic linker, and a surface recognition cap group (Figure 2). Literature surveys indicated that indole serves as a cap recognition moiety in many HDAC inhibitors.[6,12] In an attempt to produce HDAC inhibitors with indole as a cap group, we planned to interchange

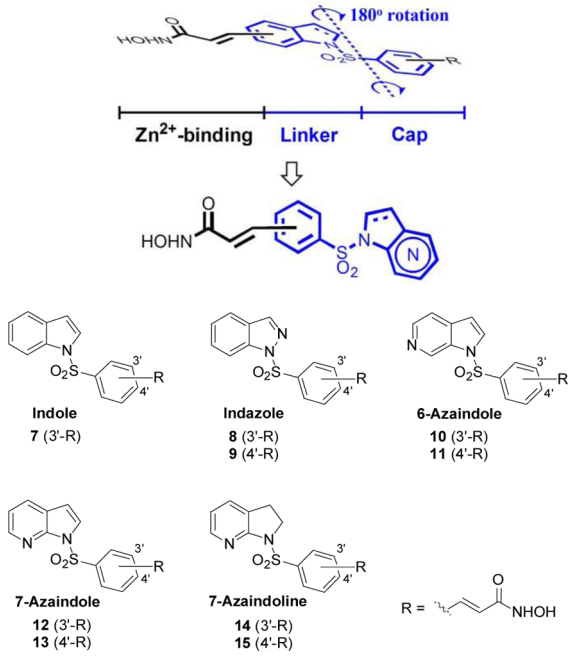


Figure 2. Design of indolyl/azaindolylsulfonylcinnamic hydroxamates.

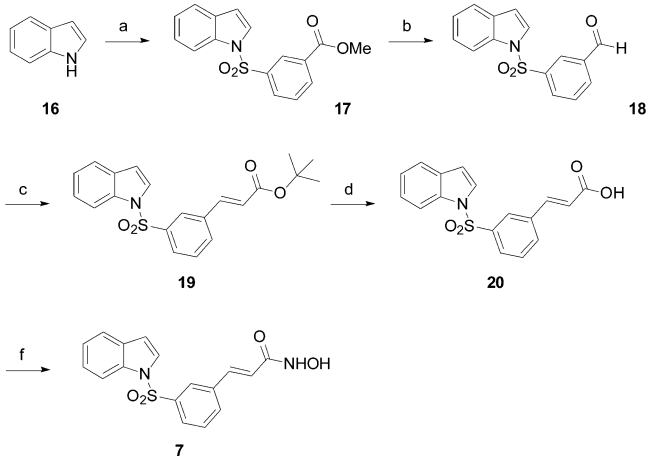
dx.doi.org/10.1021/jm401899x | J. Med. Chem. 2014, 57, 4009−4022

the cap and linker parts (blue in Figure 2) and the effect of various azaindoles on the antiproliferative activity of HDAC inhibitors was considered. As a result, a series of indolyl/azaindolylsulfonylcinnamic hydroxamates (**7**−**15**) were synthesized and the relevant biological assays are discussed below.

RESULTS AND DISCUSSION

Chemistry. The synthetic route to compound **7** containing the indole ring is shown in Scheme 1. Reaction of indole (**16**) with methyl 3-(chlorosulfonyl)benzoate in the presence of KOH and tetrabutylammonium hydrogen sulfate (TBAHS) gave compound **17** whose ester group was subsequently reacted with LAH and PDC, yielding the aldehyde product **18** which underwent a Wittig reaction with (*tert*-butyloxycarbonylmethyl)-triphenylphosphonium chloride to afford the *tert*-butyl acrylate **19**. The conversion of this *tert*-butyl ester into the corresponding carboxylic acid (**20**) was achieved by trifluoroacetic acid (TFA). The resulting product reacted with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (NH_2OTHP), and subsequent hydrolysis yielded compound **7**.

Scheme 1. Synthetic Approaches to Compound 7[a]

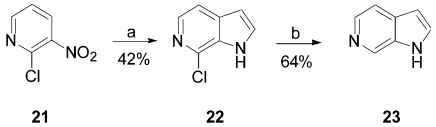


[a]Reagents and conditions: (a) KOH, TBAHS, methyl 3-(chlorosulfonyl)benzoate, CH_2Cl_2, rt; (b) (i) LAH, THF, rt; (ii) PDC, MS, CH_2Cl_2, rt; (c) (*tert*-butyloxycarbonylmethyl)triphenylphosphonium chloride, NaOH, Et_3N, H_2O, CH_2Cl_2, rt; (d) TFA, rt; (e) (i) NH_2OTHP, PyBOP, Et_3N, DMF, rt; (ii) 5% TFA, MeOH, rt.

6-Azaindole (**23**) was prepared by the Bartoli indole formation reaction. The synthetic route is shown in Scheme 2. The starting material, 2-chloro-3-nitropyridine (**21**), was reacted with vinylmagnesium bromide, and this was followed by reductive dechlorination to yield 6-azaindole (**23**).[13]

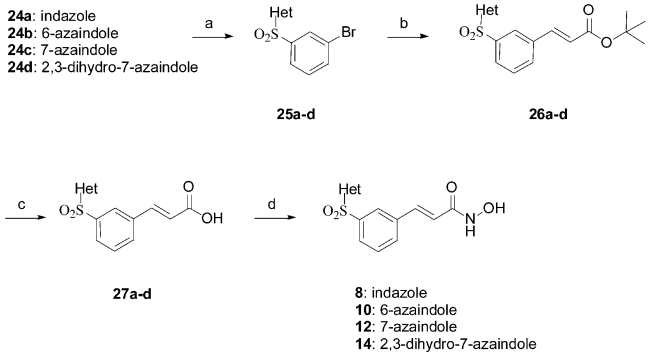
The synthetic route to the cinnamic hydroxamates (**8**, **10**, **12**, and **14**) is summarized in Scheme 3. A variety of [6,5]-bicyclic heteroycles **24a**−**d** were reacted with 3-bromobenzenesulfonyl chloride to yield compounds **25a**−**d**. The resulting products underwent a Heck reaction to afford various *tert*-butyl cinnamates (**26a**−**d**) which were hydrolyzed in the presence of trifluoroacetic acid to furnish the corresponding cinnamic acids (**27a**−**d**). These cinnamic acids underwent amidation via reaction with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine followed by hydrolysis to afford the cinnamic hydroxamates **8**, **10**, **12**, and **14**. In addition, compounds **24a**−**d** were allowed to react with 4-bromobenzenesulfonyl chloride to yield compounds **28a**−**d**. These products underwent reactions similar to those described above (the Heck reaction, hydrolysis, amidation, and deprotection) to afford compounds **9**, **11**, **13**, and **15** bearing hydroxamic acids at the position para to the N-1 sulfonamide groups (Scheme 4).

Scheme 2. Synthetic Approaches to Compound 23[a]



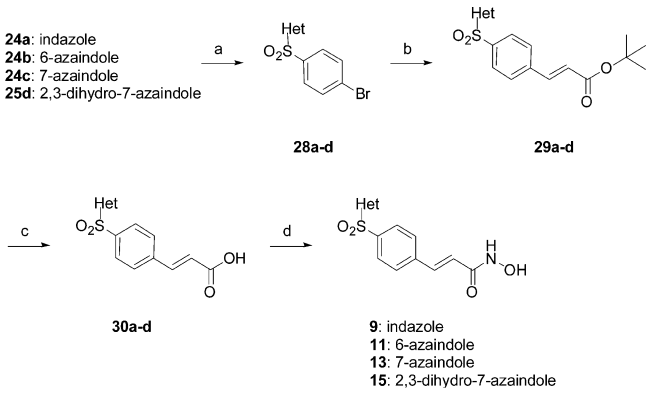
[a]Reagents and conditions: (a) vinylmagnesium bromide, THF, −40 to −50 °C; (b) H_2, Pd/C, EtOH, 4 bar.

Scheme 3. Synthetic Approaches to Compounds 8, 10, 12, and 14[a]



[a]Reagents and conditions: (a) 3-bromobenzenesulfonyl chloride, KOH, TBAHS, CH_2Cl_2, rt, 56%−89%. For **25d**: 3-bromobenzenesulfonyl chloride, pyridine, reflux, 93%. (b) $NaHCO_3$, PPh_3, $Pd(OAc)_2$, *tert*-butyl acrylate, NEt_3, DMF, 80 °C, 37−89%; (c) TFA, rt, 97%−54%; (d) (i) PyBOP, Et_3N, NH_2OTHP, DMF, rt; (ii) 5% TFA, MeOH, rt, 10−33%.

Scheme 4. Synthetic Approaches to Compounds 9, 11, 13, and 15[a]



[a]Reagents and conditions: (a) 4-bromobenzenesulfonyl chloride, KOH, TBAHS, CH_2Cl_2, rt, 88%−56%. For **28d**: 4-bromobenzenesulfonyl chloride, pyridine, reflux, 93%. (b) $NaHCO_3$, PPh_3, $Pd(OAc)_2$, *tert*-butyl acrylate, NEt_3, DMF, 80 °C, 35%−83%; (c) TFA, rt, 97%−53%; (d) (i) PyBOP, Et_3N, NH_2OTHP, DMF, rt; (ii) 5% TFA, MeOH, rt, 9−44%.

dx.doi.org/10.1021/jm401899x | J. Med. Chem. 2014, 57, 4009−4022

Table 1. HDAC Inhibitory Activity (IC_{50}) and Antiproliferative Activity of Compounds 1 and 7−15

compd	HeLa nuclear HDACs IC_{50} (μM)	$IC_{50} \pm SD$ [a] (μM)			
		KB cells	H460 cells	PC3 cells	HCT116 cells
1	0.48 ± 0.02	0.75 ± 0.10	0.82 ± 0.11	1.74 ± 0.31	0.61 ± 0.03
7	0.51 ± 0.03	2.92 ± 0.15	3.01 ± 0.25	4.01 ± 0.22	2.47 ± 0.03
8	0.23 ± 0.11	1.26 ± 0.28	1.21 ± 0.24	3.21 ± 0.54	1.12 ± 0.03
9	0.50 ± 0.09	1.83 ± 0.41	2.79 ± 0.54	3.36 ± 0.58	3.34 ± 0.44
10	0.48 ± 0.04	0.61 ± 0.21	0.56 ± 0.11	1.11 ± 0.06	1.21 ± 0.17
11	0.56 ± 0.04	1.24 ± 0.34	1.67 ± 0.34	1.97 ± 0.29	1.92 ± 0.44
12	0.10 ± 0.05	0.76 ± 0.23	0.57 ± 0.01	1.63 ± 0.41	0.39 ± 0.02
13	0.24 ± 0.11	0.87 ± 0.07	0.91 ± 0.06	1.24 ± 0.18	1.01 ± 0.04
14	0.60 ± 0.09	1.29 ± 0.18	1.82 ± 0.01	4.11 ± 0.43	2.04 ± 0.28
15	0.85 ± 0.11	1.52 ± 0.12	1.98 ± 0.16	3.64 ± 0.67	1.73 ± 0.29

[a]SD: standard deviation. All experiments were independently performed at least three times.

BIOLOGICAL EVALUATION

A. HeLa Nuclear HDAC Enzyme Inhibition. The influence of various indole caps on HDAC enzyme inhibition is summarized in Table 1. The inhibition of HeLa nuclear HDAC activity (which consists of pan-HDAC isoenzymes) by the synthetic azaindolylsulfonylcinnamic hydroxamates (**7**−**15**) and by the reference compound **1** was evaluated. In general, these azaindolylsulfonylcinnamic hydroxamates exhibited HDAC enzyme inhibitory activity comparable to or superior to that of **1** (IC_{50} = 0.48 μM), demonstrating that the strategy of ring closure in the current study is not invalid. Compound **12** is the most active with an IC_{50} value of 0.1 μM on HeLa HDAC enzyme inhibition, a 4-fold improvement over **1** (IC_{50} = 0.48 μM). Compounds **8** and **13** displayed a 2-fold increase in HDAC inhibitory activity compared to **1**. The comparisons of **8** vs **9**, **10** vs **11**, **12** vs **13**, and **14** vs **15** revealed that the position of the *N*-hydroxyacrylamide group on HDAC enzyme inhibition is important, with meta > para. A comparison of the molecules containing a *m*-*N*-hydroxyacrylamide group (**8**, **10**, **12**, and **14**) demonstrated that the order of influence of sequence of central cores on HDAC inhibition is 7-azaindole > 6-azaindole > indazole > 7-azaindoline. This observation also appeared in the class with a *p*-*N*-hydroxyacrylamide group (**13** > **9** ≈ **11** > **15**).

B. In Vitro Cell Growth Inhibition. The antiproliferative activities against four human cancer cell lines, cervical carcinoma KB cells, non-small cell lung carcinoma H460 cells, human prostate cancer PC3 cells, and human colorectal HCT116 cells (Table 1) of the synthetic azaindolylsulfonylcinnamic hydroxamates (**7**−**15**), and the reference compound (**1**) were evaluated. Compound **7** shows a 4-fold decrease of antiproliferative activity compared with **1**, although it exhibits HDAC enzyme inhibitory activity comparable to that of **1**. The scaffold hopping from indole (**7**) to the azaindoles (**8**−**15**) was paralleled by improvement of antiproliferative activity, especially for 6-azaindole and 7-azaindole. With the exceptions of **10**, **11**, **12**, and **13**, compounds with indazole (**8** and **9**) and 7-azaindoline (**14** and **15**) cores have 2- to 3-fold poorer antiproliferative activity compared with **1**. Comparison of **8**, **10**, **12**, and **14** suggested the influence of the central core on cytotoxic activity, demonstrating a trend of influential order that is similar to that of HeLa nuclear HDAC inhibitory activity: 7-azaindole ≈ 6-azaindole > indazole > 7-azaindoline. Compound **12**, which exhibited the best HDAC enzyme inhibitory activity with an IC_{50} value of 0.1 μM, showed antiproliferative activity comparable to that of compound **1**. Compound **10**, whose 7-azaindole was replaced by 6-azaindole, exhibited the best antiproliferative activity against KB cells, H460 cells, PC3 cells, and HCT116 cells with IC_{50} values of 0.61, 0.56, 1.11, and 0.39 μM, respectively. Analysis of two sets of compounds (**8**, **10**, **12**, and **14**; **9**, **11**, **13**, and **15**) revealed that *m*-*N*-hydroxyacrylamide contributes more to enhancement of potency than *p*-*N*-hydroxyacrylamide, consistent with the results of HDAC enzymatic inhibition assays. In this group of *m*-*N*-hydroxyacrylamides (**8**, **10**, **12**, and **14**), compounds with indazole (**8**) and 7-azaindoline (**14**) cores show a 2- to 3-fold loss of antiproliferative activity compared with compound **1**. In addition, compound **12** and the reference compound **1** were selected for further antiproliferation assays against an additional seven cancer cell lines (Table 2).

Table 2. Antiproliferative Activities of 1 and 12 against a Panel of Tumor Cell Lines

cell line	tissue origin	$IC_{50} \pm SD$[a] (μM)	
		1	**12**
KB	oral carcinoma	0.75 ± 0.10	0.76 ± 0.23
H460	non-small-cell lung carcinoma	0.98 ± 0.28	0.57 ± 0.01
PC3	prostate carcinoma	1.74 ± 0.31	1.63 ± 0.41
HCT116	colorectal carcinoma	0.61 ± 0.03	0.39 ± 0.02
HSC-3	oral carcinoma	0.71 ± 0.34	0.66 ± 0.12
HONE-1	nasopharyngeal carcinoma	1.18 ± 0.04	1.20 ± 0.31
A549	non-small-cell lung carcinoma	1.54 ± 0.08	1.33 ± 0.19
MCF-7	breast carcinoma	1.10 ± 0.09	1.33 ± 0.36
TSGH	gastric carcinoma	2.09 ± 0.09	2.10 ± 0.11
MKN-45	gastric carcinoma	1.25 ± 0.04	2.51 ± 0.81
HT-29	colorectal carcinoma	0.72 ± 0.19	1.24 ± 0.40

[a]SD: standard deviation. All experiments were independently performed at least three times.

Interestingly, compound **12**, instead of **1**, induced p53 accumulation in HCT116 cells (p53 wild-type).[14] We further knock down p53 expression level in HCT116 cells and revealed a significant protection against the suppression of cell proliferation and induction of apoptosis by compound **12**, suggesting that compound **12** induced p53-dependent cytotoxicity in HCT116 cells (Supporting Information Figure 1A−C). Thus, it is likely that p53 plays a crucial role to contribute to **12**-induced cell death and to make differential effects of antiproliferative activities between HCT116 and HT29 (p53 mutant) cells.[14] Together, among these cell lines, with the exceptions of MKN-45 and HT-29, compound **12** exhibited antiproliferative activity comparable to that of compound **1**.

C. Effect on Cell Cycle Progression. To investigate the effect of compound **12** on cell cycle progression, cellular DNA

dx.doi.org/10.1021/jm401899x | J. Med. Chem. 2014, 57, 4009−4022

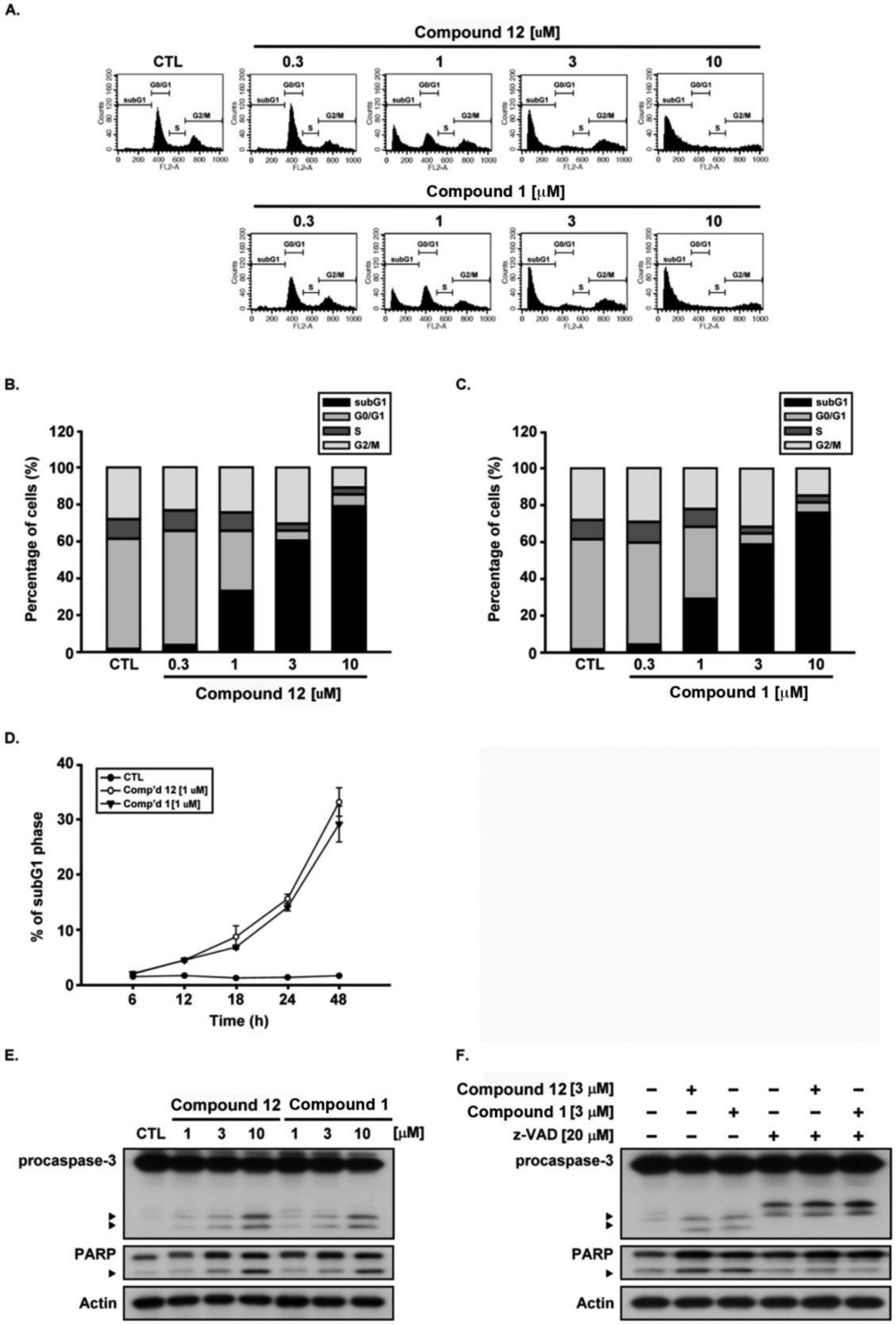


Figure 3. Effects of **12** on cell cycle progression in human HCT116 cells. (A) Concentration-dependent effects of **12** and **1** on cell cycle progression. HCT116 cells were treated with or without the indicated concentrations of **12** or **1** for 24 h and were then analyzed by flow cytometry for cell cycle distribution. (B, C) Data shown are the means values of at least three independent experiments. (D) Time-dependent effects of **12** and **1** on subG1 population. HCT116 cells were treated with or without 1 μM **12** or **1** for the indicated time interval and were analyzed by flow cytometry for subG1 population. (E) **12** induced caspase 3 and PARP activation. HCT116 cells were treated without or with the indicated concentration of **12** or **1** for 24 h then subjected to Western blot for caspase 3 and PARP analysis. (F) **12** induced caspase-dependent cell apoptosis. HCT116 cells were treated without or with 3 μM **12**, **1** or 20 μM z-VAD-fmk for 24 h and subjected to Western blot analysis for caspase 3 and PARP.

content was measured by flow cytometry and compared with compound **1**. Compound **12** increased of the number of cells in the sub-G1 phase of the cell cycle, and its influence was comparable to that of compound **1** (Figure 3A). Compounds **12** and **1** induced the sub-G1 population in a concentration- and time-dependent manner, indicating that compound **12** probably

dx.doi.org/10.1021/jm401899x | J. Med. Chem. 2014, 57, 4009−4022

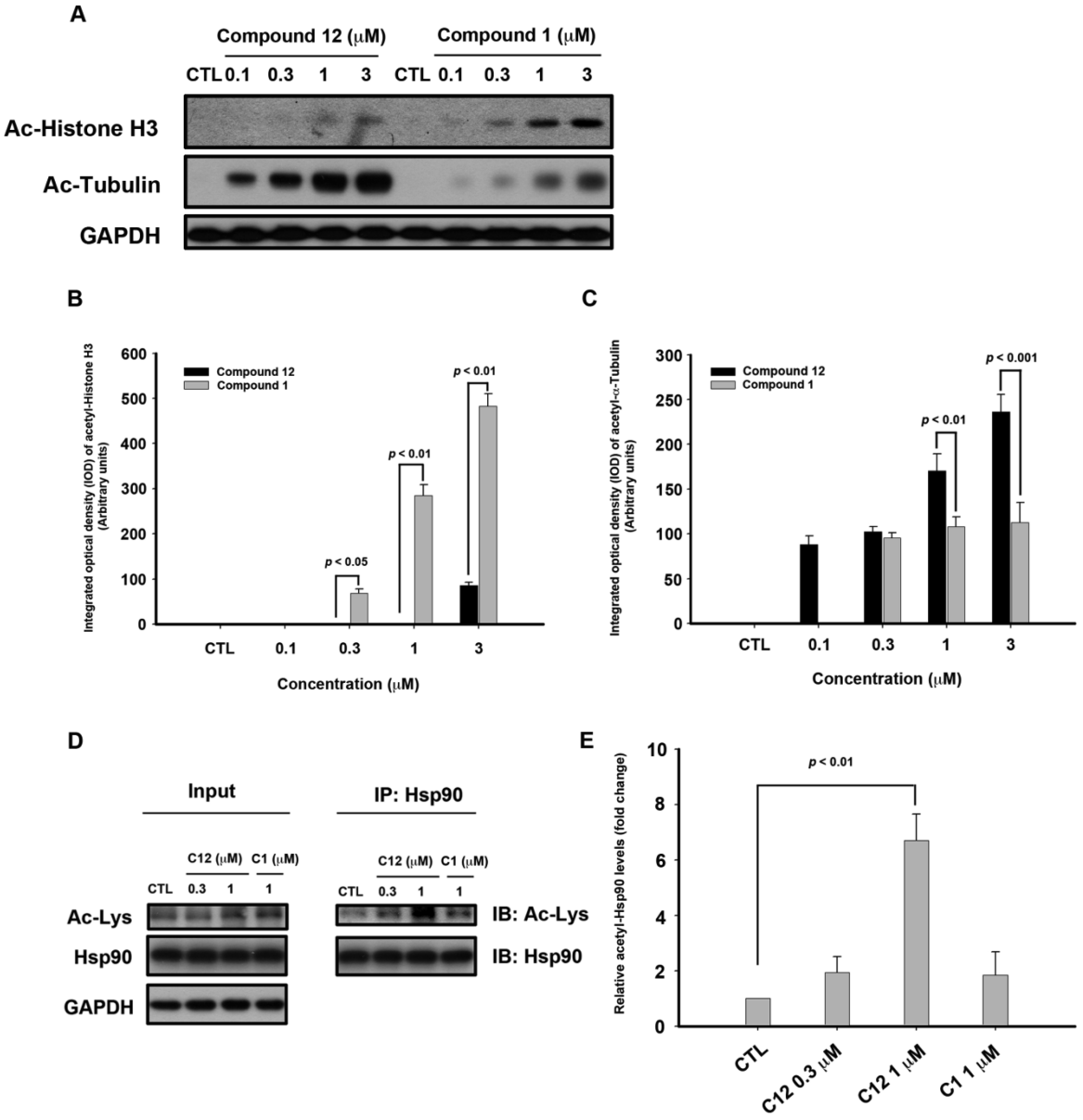


Figure 4. Western blot analyses of histone H3 and α-tubulin acetylation after the treatment with compound **12** or **1**. HCT116 cells were treated with compound **12** or **1** at the indicated concentrations for 3 h. (A) The protein levels of acetylhistone 3, GAPDH, and acetyl tubulin were determined by Western blot analysis. Densitometric analysis of protein bands was performed by using Gel-Pro Analyzer (version 3.1, Media Cybernetics, Bethesda, MD, USA). Acetylhistone H3 (B) and acetyl α-tubulin (C) were analyzed in HCT116 cells. (D) Coimmunoprecipitation analysis of the concentration-dependent effect of compound **12** on Hsp90 acetylation (Ac-Lys) and the protein expression levels of acetylated-Hsp90 were quantified by Gel-Pro Analyzer (E).

induced apoptosis in HCT116 cells (Figure 3B and Figure 3C). Compound **12** also induced the activation of caspase 3 and PARP in a concentration-dependent manner (Figure 3E). Combination of compound **12** with the caspase-inhibitor z-VAD-fmk abolished the incidence of apoptosis (Figure 3F), suggesting that compound **12** induced apoptosis through a caspase-dependent pathway. These results suggested that compound **12** induces cytotoxicity in HCT116 cells through the apoptosis pathway.

D. Upregulation Effect of Histone and α-Tubulin. The effect of the compounds **12** and **1** on the acetylation level of histone H3 and α-tubulin, the biomarkers of HDAC inhibition, is shown in Figure 4. Exposure to compounds **12** and **1** for 3 h induced the acetylation of histone H3 and α-tubulin in a dose-dependent manner (Figure 4A). It is noteworthy that the **1**-induced acetylated histone H3 was more evident compared with compound **12**, while compound **12** was more potent than **1** for induction of tubulin acetylation (Figure 4A–C). Moreover, we further evaluated the drug effect on acetylation of Hsp90 (known substrate of HDAC6) in HCT116 cells by immunoprecipitating with antibody against Hsp90. As shown in Figure 4D and Figure 4E, compound **12** elevated stronger acetylation level of Hsp90 than compound **1** did. However, it has been reported that selective inhibition of HDAC6 did not have effect on acetylated histone H3,[15] suggesting compound **12** may have the ability to inhibit class I HDACs but, unlike compound **1**, showed more selectivity in its inhibition of HDAC6 activity.

E. HDAC Isoform Inhibition. To confirm the selectivity toward HDAC isoforms, the inhibitory activities of compounds **10** and **12** and reference compounds, **1** and **5**, against HDACs 1,

dx.doi.org/10.1021/jm401899x | J. Med. Chem. 2014, 57, 4009–4022

Table 3. HDAC Inhibition Activity and Isoform Selectivity of 10 and 12

	IC_{50} (μM)[a]			selectivity ratio	
compd	HDAC1	HDAC2	HDAC6	HDAC1/HDAC6	HDAC2/HDAC6
10	4.85		0.28	17	
12	0.31	1.16	0.0052	60	223
1	0.11	0.41	0.0094	12	43
5[b]	0.058	0.048	0.0047	12	10

[a]These assays were conducted by the Reaction Biology Corporation, Malvern, PA. All compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 10 μM. [b]Data from ref 9.

2, and 6 were first examined (Table 3). The results were compared with those from **5**, the only HDAC6 inhibitor in clinical trials. In a comparison of HDAC6 activity, compound **12** exhibited potency comparable to that of **1** and **5** with IC_{50} values in the single-digit nanomolar range, while compound **10** caused marked loss of enzymatic activity. Compound **12** inhibited HDAC6 with an IC_{50} value of 5.2 nM. Table 3 shows that HDAC1 and HDAC2 are less sensitive than the reference compounds to compounds **10** and **12**. In a comparison of the selectivity ratio of HDAC1/HDAC6, compound **12** displayed better selectivity of HDAC6 over HDAC1 (60-fold) than **1** (12-fold) and **5** (12-fold). Notably, the selectivity ratio of HDAC2/HDAC6 for compound **12** is 233, which is 5 and 22 times more selective than **1** and **5**, respectively. The effect of compound **12** on other HDAC isoforms was further examined and summarized in Table 4. The result indicated that compound **12** apparently

Table 4. HDAC Inhibition Activity of Compound 12[a]

isoform	IC_{50} (μM),[b] **12**
HDAC1	0.31
HDAC2	1.16
HDAC3	–
HDAC4	–
HDAC5	8.73
HDAC6	0.0052
HDAC7	4.11
HDAC8	1.39
HDAC9	5.34
HDAC10	4.13
HDAC11	–

[a]Dash indicates no inhibition or compound activity and that it could not be fit to an IC_{50} curve. [b]These assays were conducted by the Reaction Biology Corporation, Malvern, PA. All compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 10 μM.

exhibited remarkable inhibitory activity against HDAC6 with IC_{50} value in the single-digit nanomolar range. In addition to compounds **1** and **5**, we also included a FDA-approved thiol containing compound **2** and trichostatin A to compare with compound **12** in inhibition of HDAC isoenzymes (HDAC1−HDAC11) (Supporting Information Table 3). These results confirmed that compound **12** showed more selective inhibitory effect on HDAC6. A previous study has shown that suppression of HDAC1 or HDAC6 leads to downregulaton of Akt phosporylation.[16] In addition, it has been reported that azaindole derivatives inhibit PI3K by downregulating phospho-Akt (Ser473) protein level.[17] As shown, siRNA-mediated knockdown of HDAC1 or HDAC6 resulted in parallel decreases in the protein level of phospho-Akt (Ser473) (Supporting Information Figure 2A). Conversely, ectopic expression of HDAC1 or HDAC6 protected **12**-induced suppression of phospho-Akt (Ser473) in HCT116 cells (Supporting Information Figure 2B), suggesting that the activity of Akt was associated with HDAC inhibition. These results indicate that compound **12** is a potential candidate for further study as a selective HDAC6 inhibitor.

F. Antitumor Effect of Compound 12 on 3D Cellular Spheres of HCT116 Colorectal Cancer Cells. An emerging view finds that traditional two-dimensional (2D) cell culture may not accurately mimic the 3D environment in which cancer cells reside, and culturing cells in 3D makes them resistant to chemotherapeutics.[18] Thus, HCT116 cells grown as 3D culture (Figure 5A) were also used to investigate the anticancer efficacy

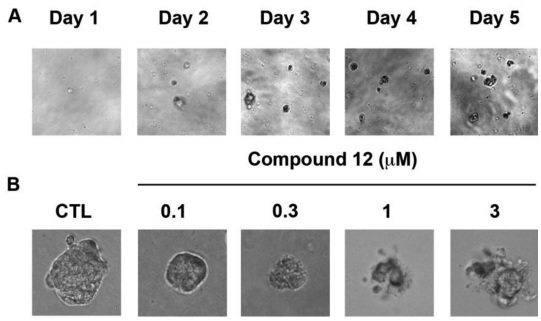


Figure 5. Antitumor effect of compound **12** on 3D cellular spheres of HCT116 colorectal cancer cells. Cellular spheres formed in semisolid and drug effect on cellular spheres were examined using phase-contrast light microscopy. 3D structures of cells were analyzed using 4× magnification from day 1 to day 5 (A) and 20× magnification (B).

of compound **12** in 3D tumors. As shown in Figure 5B, compound **12** disrupted the spheroid structure of HCT116 tumors in a concentration-dependent manner. This result revealed that the cytotoxic effect of compound **12** was equally potent in a 3D spheroid culture and in a 2D culture.

G. Growth Inhibition of Human Colon Cancer Xenografts in Vivo. We evaluated the in vivo efficacy of compounds **12** and **1** using an HCT116 xenograft nude mouse model (Figure 6). Once a tumor was approximately 40 mm^3 in size and palpable, the mice were randomized into vehicle control and treatment groups (7−8 mice per group) and control mice received the vehicle (1.0% carboxymethyl cellulose + 0.5% Tween 80). This study demonstrated that the growth of HCT116 cancer cell xenografts is suppressed by factors of 55.8% and 66.9% (percent tumor growth inhibition [%TGI] values) after oral administration of compound **12** at concentrations of 25 and 100 mg/kg, respectively, whereas compound **1** at 200 mg/kg orally suppressed tumor growth by 48.4% (%TGI) (Figure 6). The tumor growth inhibition by compound **12** is dose-dependent and is greater than that provided by compound **1**. No significant changes in body weight and no other adverse effects were observed upon treatment with compound **12**

dx.doi.org/10.1021/jm401899x | J. Med. Chem. 2014, 57, 4009−4022

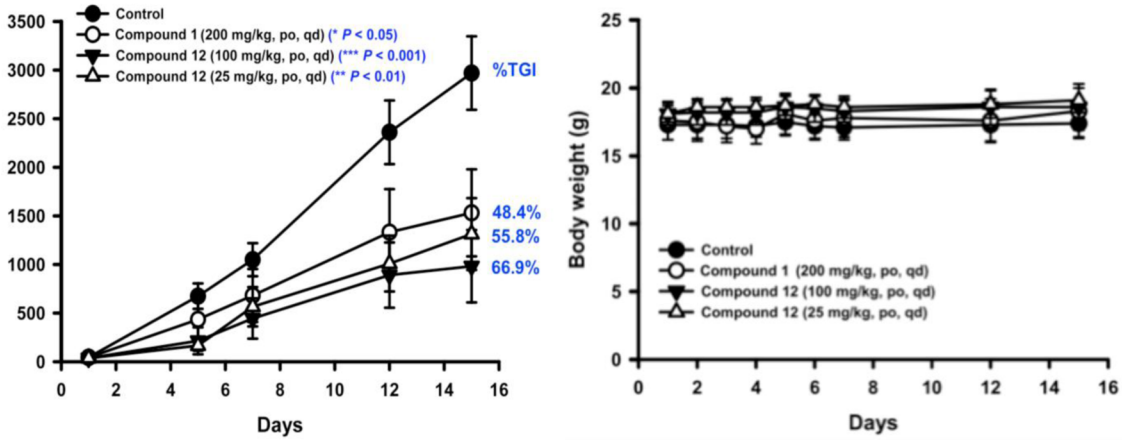


Figure 6. Anticancer activity of compounds **12** and **1** in a xenograft model of human colorectal HCT116. Compounds **12** and **1** were suspended in 1% carboxymethylcellulose and 0.5% Tween 80. Left panel shows the tumor growth of HCT116 xenograft nude mice treated with or without compound **12** (25 and 100 mg/kg) or **1** (200 mg/kg). Tumor growth is tracked by the mean tumor volume (mm^3) ± SE and calculated as % tumor growth inhibition (%TGI). Tumor volume was determined using caliper measurements and was calculated as the product of $^1/_2 \times$ length × width^2. Right panel shows body weight (g) of the mice. (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ as compared with the control group.

(Figure 6). Investigation of the maximal tolerated dose of compound **12** is illustrated in Figure 7. The tested compound

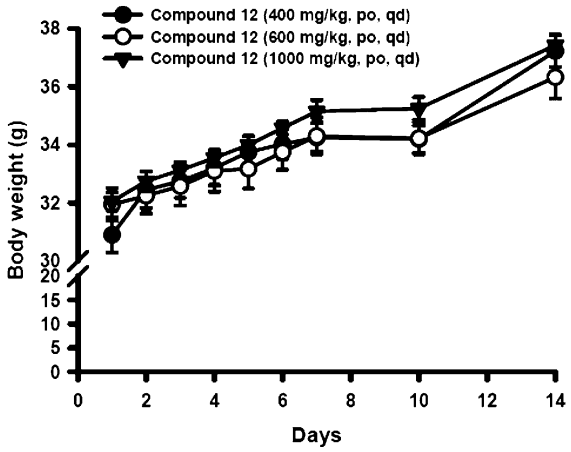


Figure 7. Maximum tolerated dose (MTD) of **12**. Twenty-four animals were randomized into three groups. The treated animals were administered compound **12** at doses of 400, 600, and 1000 mg/kg. The animals were dosed po daily for 7 days and then monitored for 1 week. The animals were weighed daily for 1 week and then twice weekly.

was administered orally at concentrations of 400, 600, and 1000 mg/kg to healthy mice, and body weights were recorded over 14 days. The result revealed that there is no significant toxicity in the treatment with compound **12** at these doses.

H. Pharmacokinetic Studies. Compound **12** was selected for in vivo pharmacokinetic evaluation in mice (Table 5). Compound **12** (dissolved in DMSO/Cremophor/5% dextrose water (5%/5%/90%, v/v/v) for intravenous administration and DMA/PEG400 (20%/80%, v/v) for oral dosing) was administered to imprinting control region (ICR) mice intravenously (iv) at 2 mg/kg body weight and orally at 10 mg/kg body weight. Blood samples were taken, and the plasma was analyzed for concentration of **12** using an LC−MS/MS system. Interestingly, **12** showed better oral bioavailability ($F = 33\%$) compared to **1** ($F = 8.33\%$).[19] Overall, these results suggest that the 7-azaindole (**12**) has potential for development as an orally bioavailable anticancer agent.

Table 5. Pharmacokinetic Characteristics of Compound 12[a]

	mouse	
PK profile[b]	iv at 2 mg/kg	po at 10 mg/kg
AUC_{0-inf} (ng mL^{-1} h)	289	466
$T_{1/2}$ (h)	2.5	2.4
C_{max} (ng/mL)		409
T_{max} (h)		0.3
CL (mL min^{-1} kg^{-1})	127	
V_{ss} (L/kg)	9.8	
F (%)		33

[a]Values are expressed as the mean of at least three independent experiments. [b]AUC, area under curve. CL, clearance. F, oral bioavailability. V_{ss}, volume at steady state. $T_{1/2}$, plasma half-life.

CONCLUSION

To develop HDAC inhibitors with an indole cap group, a series of indolyl/azaindolylsulfonylcinnamic hydroxamate (**7**−**15**) were prepared and evaluated for bioactivity in vitro and in vivo. A SAR analysis revealed that compound **12**, a 7-azaindole, exhibited activity superior to that of indole, indazole, 6-azaindole, and 7-azaindoline. Compound **12** possessed antiproliferative activity with IC_{50} values of 0.39−2.51 μM against 11 diverse human cancer cell lines, probably through induction of the apoptosis pathway. Compound **12** displayed 60-fold selectivity for HDAC6 over HDAC1 and 223-fold over HDAC2 and thus is 5 and 22 times more selective than compound **5**, a HDAC6 selective inhibitor currently in clinical trials. Significantly, compound **12**, with 33% of oral bioavailability in mice, showed substantial antitumor activity with TGI of 55.8% and 66.9% in human colorectal HCT116 xenograft models after oral treatment with 25 and 100 mg/kg, respectively, which was greater than was shown by compound **1** (48.4% TGI, 200 mg/kg, po). 7-Azaindolylsulfonylcinnamic hydroxamate (**12**), with the drug-gable profiles described above, has potential as a promising anticancer agent with selective HDAC6 inhibitory properties.

EXPERIMENTAL SECTION

Chemistry. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Bruker DRX-500 spectrometer (operating at 500 MHz), with chemical shifts in parts per million (δ) downfield from TMS, the

internal standard. High-resolution mass spectra were recorded with a JEOL (JMS-700) electron impact (EI) mass spectrometer. Elemental analyses were performed on a Heraeus varioIII-NCH instrument. The purities of the final compounds were determined using an Agilent 1100 series HPLC system using a C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm) and were found to be ≥95%. Flash column chromatography was conducted using silica gel (Merck Kieselgel 60, no. 9385, 230−400 mesh ASTM). All reactions were conducted under an atmosphere of dry N_2.

(*E*)-3-(3-(1*H*-Indol-1-ylsulfonyl)phenyl)-*N*-hydroxyacrylamide (7). PyBOP (650 mg, 1.25 mmol), DMF (1 mL), *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (160 mg, 1.46 mmol), and triethylamine (1 mL, 2.93 mmol) were added to a mixture of compound **20** (400 mg, 1.22 mmol) and stirred for 5 h under N_2. The mixture was filtered, and the filtrate was extracted with water (30 mL) and CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo to yield an oily product that was purified by flash chromatography over silica gel (1:2 EtOAc/*n*-hexane; R_f = 0.21) to give white solid. This solid was mixed with 5% trifluoroacetic acid (25 mL) and MeOH (1 mL), then stirred for 30 min. The reaction mixture was concentrated in vacuo and filtered to yield a pink product. The resulting solid was purified by flash chromatography over silica gel (1:2 EtOAc/*n*-hexane; R_f = 0.33) and recrystallization with EtOH gave 20 mg (5%) of **7** as a yellow solid: mp = 188−191 °C; ^{1}H NMR (500 MHz, CD_3OD and DMSO) δ 6.66 (d, J = 15.0 Hz, 1H), 6.85 (s, 1H), 7.29 (t, J = 7.0 Hz, 1H), 7.39 (t, J = 8.0 Hz, 2H), 7.57−7.63 (m, 2H), 7.78−7.81 (m, 2H), 7.91 (t, J = 7.5 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 8.10 (s, 1H); MS (EI) m/z 341 (M^+, 1.2%), 344 (100%); HRMS (EI) for $C_{17}H_{14}O_4N_2S$ (M^+) calcd 342.0674, found 342.0676. HPLC purity of 99.65% (retention time = 19.8).

(*E*)-3-(3-(1*H*-Indazol-1-ylsulfonyl)phenyl)-*N*-hydroxyacrylamide (8). PyBOP (770 mg, 1.52 mmol), DMF (1 mL), *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (150 mg, 1.29 mmol), and triethylamine (1 mL, 3.52 mmol) were added to a mixture of compound **27a** (420 mg, 1.28 mmol), and the mixture was stirred for 5 h under N_2. The mixture was filtered, and the filtrate was extracted with water (30 mL) and CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo to yield an oily product. The residue was purified by flash column over silica gel (1:2 EtOAc/*n*-hexane; R_f = 0.39) to give a white solid to which 5% trifluoroacetic acid (60 mL) and MeOH (3 mL) were added and then stirred for 30 min. This reaction mixture was concentrated in vacuo and filtered to yield a white product. The mixture was washed with H_2O/CH_2Cl_2 to give 80 mg (18%) of **8** as a purple solid: mp = 166−168 °C; ^{1}H NMR (500 MHz, CD_3OD and DMSO) δ 6.52 (d, J = 16.0 Hz, 1H), 7.39 (t, J = 7.5 Hz, 1H), 7.51−7.54 (m, 2H), 7.64 (t, J = 8.0 Hz, 1H), 7.79 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 8.0 Hz, 1H), 8.11 (s, 1H), 8.21 (d, J = 8.5 Hz, 1H), 8.35 (s, 1H); HRMS (ESI) for $C_{16}H_{12}O_4N_3S$ (M − H) calcd 342.0549, found 342.0541. HPLC purity of 99.76% (retention time = 23.29).

(*E*)-3-(4-(1*H*-Indazol-1-ylsulfonyl)phenyl)-*N*-hydroxyacrylamide (9). A mixture of compound **30a** (640 mg, 1.96 mmol), PyBOP (1170 mg, 2.31 mmol), DMF (1 mL), *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (230 mg, 1.96 mmol), and triethylamine (1 mL, 5.35 mmol) was stirred under nitrogen for 5 h. The mixture was then filtered, and the filtrate was extracted with H_2O (30 mL) and CH_2Cl_2 (30 mL × 3). The organic layer was dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ through filtration, the filtrate was concentrated in vacuo to yield an oily product. The residue was purified by flash chromatography over silica gel (EtOAc/*n*-hexane = 1: 2; R_f = 0.40) to give a white solid. Then 5% trifluoroacetic acid (60 mL) and methanol (3 mL) were added to the solid, and the mixture was stirred for 30 min. This reaction mixture was concentrated in vacuo and filtered to yield a white product. The mixture was washed with H_2O/CH_2Cl_2 to give 110 mg (16%) of **9** as a purple solid: mp = 140−142 °C; ^{1}H NMR (500 MHz, CD_3OD and DMSO) δ 6.53 (d, J = 16.0 Hz, 1H), 7.39 (t, J = 7.5 Hz, 1H), 7.52 (d, J = 16.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.67 (d, J = 8.0 Hz, 2H), 7.79 (d. J = 8.0 Hz, 1H), 7.94 (d, J = 8.5 Hz, 2H), 8.20 (d, J = 9.0 Hz, 1H), 8.34 (s, 1H); HRMS (EI) for $C_{16}H_{13}O_4N_3S$ (M^+) calcd 343.0627, found 343.0627. HPLC purity of 97.87% (retention time = 22.47).

(*E*)-3-(3-(1*H*-Pyrrolo[2,3-*c*]pyridin-1-ylsulfonyl)phenyl)-*N*-hydroxyacrylamide (10). The title compound was obtained in 33% overall yield from compound **27b** in a manner similar to that described for the preparation of **8**: mp = 128−129 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 6.58 (d, J = 17.0 Hz, 1H), 7.19 (d, J = 3.5 Hz, 1H), 7.59 (d, J = 15.5 Hz, 1H), 7.65 (t, J = 8.0 Hz, 1H), 7.95 (d, J = 7.5 Hz, 1H), 8.13 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 6.0 Hz, 1H), 8.30 (s, 1H), 8.49 (d, J = 6.5 Hz, 1H), 8.54 (d, J = 3.5 Hz, 1H), 9.56 (s, 1H); MS (EI) m/z 342.0 (M^+, 3.57%), 118 (100%); HRMS (EI) for $C_{16}H_{13}O_4N_3S$ (M^+) calcd 343.0627, found 343.0629. HPLC purity of 99.50% (retention time = 19.65).

(*E*)-3-(4-(1*H*-Pyrrolo[2,3-*c*]pyridin-1-ylsulfonyl)phenyl)-*N*-hydroxyacrylamide (11). The title compound was obtained in 22% overall yield from compound **30b** in a manner similar to that described for the preparation of **9**: mp = 167−169 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 6.53 (d, J = 16.0 Hz, 1H), 7.15 (d, J = 3.5 Hz, 1H), 7.54 (d, J = 15.5 Hz, 1H), 7.76 (d, J = 8.5 Hz, 2H), 8.12 (d, J = 6.5 Hz, 1H), 8.14 (d, J = 8.5 Hz, 2H), 8.46 (t, J = 3.5 Hz, 1H), 8.48 (s, 1H), 9.51 (s, 1H); HRMS (ESI) for $C_{16}H_{12}O_4N_3S$ (M − H) calcd 342.0549, found 342.0542. HPLC purity of 95.46% (retention time = 19.78).

(*E*)-3-(3-(1*H*-Pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)phenyl)-*N*-hydroxyacrylamide (12). This compound was obtained in 20% overall yield from compound **27c** in a manner similar to that described for the preparation of **8**: mp = 188−191 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 6.55 (d, J = 15.5 Hz, 1H), 6.74 (d, J = 4.0 Hz, 1H), 7.25 (dd, J = 5.0, 7.5 Hz, 1H), 7.54 (m, 1H), 7.57 (d, J = 4.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 4.0 Hz, 1H), 7.97 (dd, J = 1.5, 7.5 Hz, 1H), 8.06 (d, J = 7.5 Hz, 1H), 8.33 (dd, J = 1.5, 4.5 Hz, 1H), 8.36 (s, 1H); MS (EI) m/z 342.0 (M^+, 2.20%), 118 (100%); ^{13}C NMR (125 MHz, DMSO-d_6) δ 106.22, 119.47, 121.87, 122.47, 125.53, 126.76, 127.68, 130.29, 133.69, 136.01, 136.18, 138.15, 144.66, 146.56, 161.85; HRMS (EI) for $C_{16}H_{13}O_4N_3S$ (M^+) calcd 343.0627, found 343.0626. HPLC purity of 99.46% (retention time = 19.91).

(*E*)-3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)phenyl)-*N*-hydroxyacrylamide (13). This compound was obtained in 9% overall yield from compound **30c** in a manner similar to that described for the preparation of **9**: mp = 169−170 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 6.54 (d, J = 16.0 Hz, 1H), 6.74 (d, J = 3.5 Hz, 1H), 7.25 (dd, J = 5.0, 9.0 Hz, 1H), 7.53 (d, J = 16.0 Hz, 1H), 7.68 (d, J = 8.5 Hz, 2H), 7.81 (d, J = 4.0 Hz, 1H), 8.97 (dd, J = 1.5, 8.0 Hz, 1H), 8.11 (d, J = 8.5 Hz, 2H), 8.31 (dd, J = 1.0, 4.5 Hz, 1H); HRMS (EI) for $C_{16}H_{13}O_4N_3S$ (M^+) calcd 343.0627, found 343.0627. HPLC purity of 97.27% (retention time = 19.85).

(*E*)-3-(3-(2,3-Dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)-phenyl)-*N*-hydroxyacrylamide (14). This compound was obtained in 10% overall yield from compound **27d** in a manner similar to that described for the preparation of **8**: mp = 228−230 °C; ^{1}H NMR (500 MHz, CD_3OD and $CDCl_3$) δ 3.04 (t, J = 8.0 Hz, 2H), 4.04 (t, J = 8.0 Hz, 2H), 6.50 (dd, J = 5.0, 8.0 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.55 (d, J = 16.0 Hz, 1H), 7.72 (d, J = 7.0 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 4.5 Hz, 1H), 8.21 (s, 1H); MS (EI) m/z 347.0 (M^+, 0.90), 116 (100%); HRMS (EI) for $C_{16}H_{15}O_4N_3S$ (M^+) calcd 345.0783; found 345.0783. HPLC purity of 99.21% (retention time = 16.87).

(*E*)-3-(4-(2,3-Dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)-phenyl)-*N*-hydroxyacrylamide (15). This compound was obtained in 28% overall yield from compound **30d** in a manner similar to that described for the preparation of **9**: mp = 220−223 °C; ^{1}H NMR (500 MHz, CD_3OD and DMSO) δ 3.04 (t, J = 8.0 Hz, 2H), 4.04 (t, J = 8.0 Hz, 2H), 6.54 (d, J = 16.0 Hz, 1H), 6.51 (dd, J = 5.0, 7.5 Hz, 1H), 7.49 (d, J = 9.0 Hz, 1H), 7.53−7.58 (m, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 7.5 Hz, 1H), 8.04 (d, J = 5.0 Hz, 1H), 8.23 (s, 1H); HRMS (EI) for $C_{16}H_{13}O_4N_3S$ (M^+) calcd 345.0783, found 345.0783. HPLC purity of 99.21% (retention time = 16.87).

Methyl 3-(1*H*-indol-1-ylsulfonyl)benzoate (17). A mixture of indole (500 mg, 4.26 mmol), potassium hydroxide (480 mg, 8.52 mmol), tetrabutylammonium hydrogen sulfate (220 mg, 6.39 mmol), and dry CH_2Cl_2 (25 mL) was stirred under N_2 for 30 min. Methyl 3-

(chlorosulfonyl)benzoate (1500 mg, 6.39 mmol) was added to the mixture, which was stirred overnight. The mixture was filtered, and the filtrate was extracted with H_2O (30 mL) and CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo to yield an oily product. The residue was purified with flash column over silica gel (1:2 EtOAc/*n*-hexane; R_f = 0.31) to give 930 mg (69%) of **17** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.93 (s, 3H), 6.69 (d, J = 2.7 Hz, 1H), 7.24 (t, J = 7.5 Hz, 1H), 7.33 (t, J = 7.9 Hz, 1H), 8.01 (d, J = 8.3 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 8.17 (d, J = 7.8 Hz, 1H), 8.55 (s, 1H).

3-(1*H*-Indol-1-ylsulfonyl)benzaldehyde (18). LAH (100 mg, 2.86 mmol) was added to a mixture of compound **17** (900 mg, 2.86 mmol) and THF (30 mL) in an ice bath and under N_2, and the mixture was stirred for 2 h at room temperature. The mixture was filtered and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$ followed by concentration in vacuo to yield an oily product. PDC (2360 mg, 5.72 mmol), MS (2360 mg), and CH_2Cl_2 (30 mL) were added to the reaction mixture which was then stirred under N_2 for 5 h. The PDC was filtered off through Celite−silica gel−Celite packing, and the filtrate was extracted with H_2O (30 mL) and CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo to yield an oily product. The residue was purified by flash column over silica gel (1:2 EtOAc/*n*-hexane; R_f = 0.31) to give 350 mg (43%) of **18** as a solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.70 (d, J = 3.6 Hz, 1H), 7.25 (d, J = 7.5 Hz, 1H), 7.34 (t, J = 7.7 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.57 (d, J = 3.7 Hz, 1H), 7.62 (t, J = 7.8 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 7.6 Hz, 1H), 8.10 (d, J = 7.9 Hz, 1H), 8.36 (s, 1H), 9.99 (s, 1H).

(*E*)-*tert*-Butyl 3-(3-(1*H*-Indol-1-ylsulfonyl)phenyl)acrylate (19). A mixture of compound **18** (570 mg, 1.99 mmol), NaOH (320 mg, 7.99 mmol), (*tert*-butyloxycarbonylmethyl)triphenylphosphonium (1640 mg, 3.98 mmol), triethylamine (0.3 mL, 1.99 mmol), CH_2Cl_2 (15 mL), and H_2O (5 mL) was stirred for 12 h. The mixture was filtered, and the filtrate was extracted with H_2O (30 mL) and CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo to yield an oily product. The residue was purified by flash column over silica gel (1:4 EtOAc/*n*-hexane; R_f = 0.21) to give 420 mg (59%) of **19** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.54 (s, 9H), 6.37 (d, J = 16.1 Hz, 1H), 6.66 (d, J = 3.6 Hz, 1H), 7.22 (d, J = 7.7 Hz, 1H), 7.38 (t, J = 4.3 Hz, 1H), 7.47−7.52 (m, 2H), 7.55−7.58 (m, 2H), 7.81 (d, J = 8.3 Hz, 1H), 7.99 (d, J = 9.4 Hz, 2H).

(*E*)-3-(3-(1*H*-Indol-1-ylsulfonyl)phenyl)acrylic Acid (20). A mixture of compound **19** (420 mg, 1.10 mmol) and trifluoroacetic acid (4 mL, 0.06 mmol) was stirred for 30 min. The mixture was filtered off and water was added, followed by extraction with CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo to yield an oily product. The residue was purified by flash column over silica gel (1:4 EtOAc/*n*-hexane; R_f = 0.27) to give 300 mg (84%) of **20** as a solid: ^{1}H NMR (500 MHz, $CDCl_3$ and CD_3OD) δ 6.44 (d, J = 16.0 Hz, 1H), 6.69 (d, J = 3.6 Hz, 1H), 7.24 (t, J = 7.41 Hz, 1H), 7.34 (t, J = 7.7 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.56 (d, J = 3.7 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.69 (d, J = 16.1 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 8.01 (s, 1H).

7-Chloro-6-azaindole (22). A mixture of 2-chloro-3-nitropyridine (500 mg, 3.15 mmol) and THF (20 mL) was mixed with vinylmagnesium bromide (15 mL, 25.24 mmol) at −40 to −50 °C under N_2 and stirred for 2 h. The reaction was quenched with H_2O and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo to give an oily product which was purified with flash chromatography over silica gel (1:2 EtOAc/*n*-hexane; R_f = 0.26) to give 200 mg (42%) of **22** as a yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.66 (t, J = 2.3 Hz, 1H), 7.48 (t, J = 2.6 Hz, 1H), 7.52 (d, J = 5.5 Hz, 1H), 8.04 (d, J = 5.5 Hz, 1H).

6-Azaindole (23). A mixture of **22** (100 mg, 0.66 mmol), Pd/C (20 mg, 0.05 mmol), and EtOH (2 mL) was stirred under hydrogen with a pressure of 4 bar for 12 h. The catalyst was then filtered off through Celite packing, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography over silica gel (6:1 CH_2Cl_2/MeOH; R_f = 0.35) to give 50 mg (64%) **23** of an orange solid: ^{1}H NMR (500 MHz, CD_3OD) δ 6.70 (d, J = 3.0 Hz, 1H), 7.69 (d, J = 3.0 Hz, 1H), 7.73 (d, J = 5.0 Hz, 1H), 8.16 (d, J = 5.5 Hz, 1H), 8.81 (s, 1H).

1-(3-Bromophenylsulfonyl)-1*H*-indazole (25a). A mixture of indazole (500 mg, 4.23 mmol), potassium hydroxide (470 mg, 8.46 mmol), and tetrabutylammonium hydrogen sulfate (220 mg, 0.63 mmol) in dry CH_2Cl_2 (30 mL) was stirred for 30 min under N_2 gas. The mixture was filtered, and the filtrate was extracted with water (30 mL) and CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo to yield an oily product. The residue was purified by flash chromatography over silica gel (3:1 EtOAc/*n*-hexane; R_f = 0.28) to give 1270 mg (89%) of **25a** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (t, J = 8.0 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 7.59 (td, J = 1.5, 8.5 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 8.13 (t, J = 2.0 Hz, 1H), 8.19 (d, J = 8.5 Hz, 1H), 8.22 (s, 1H).

1-(3-Bromophenylsulfonyl)-1*H*-pyrrolo[2,3-*c*]pyridine (25b). The title compound was obtained in 62% overall yield from compound **24b** in a manner similar to that described for the preparation of **25a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.70 (d, J = 3.5 Hz, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.47 (d, J = 5.5 Hz, 1H), 7.65 (d, J = 3.5 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 8.03 (s, 1H), 8.42 (d, J = 5.5 Hz, 1H), 9.29 (s, 1H).

1-(3-Bromophenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridine (25c). This compound was obtained in 85% overall yield from compound **24c** in a manner similar to that described for the preparation of **25a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.62 (d, J = 4.0 Hz, 1H), 7.20 (dd, J = 4.5, 7.5 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.68 (d, J = 9.0 Hz, 1H), 7.69 (d, J = 12.0 Hz, 1H), 7.85 (dd, J = 2, 8 Hz, 1H), 8.15 (dd, J = 1.5, 8.0 Hz, 1H), 8.36 (t, J = 2.0 Hz, 1H), 8.44 (dd, J = 1.5, 5.0 Hz, 1H).

1-(3-Bromophenylsulfonyl)-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]-pyridine (25d). This compound was obtained in 93% overall yield from compound **24d** in a manner similar to that described for the preparation of **25a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.06 (t, J = 8.5 Hz, 2H), 4.06 (t, J = 8.5 Hz, 2H), 6.84 (dd, J = 5.0, 7.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 7.0 Hz, 1H), 7.67 (d, J = 7.0 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 5.0 Hz, 1H), 8.28 (t, J = 2.0 Hz, 1H).

(*E*)-*tert*-Butyl 3-(3-(1*H*-Indazol-1-ylsulfonyl)phenyl)acrylate (26a). A mixture of compound **25a** (1250 mg, 3.71 mmol), *tert*-butyl acrylate (0.60 mL, 4.45 mmol), triethylamine (0.75 mL, 6.32 mmol), triphenylphosphine (480 mg, 1.86 mmol), palladium acetate (410 mg, 1.86 mmol), and sodium bicarbonate (310 mg, 3.70 mmol) was heated to 80 °C in DMF (2 mL) for 5 h. Then the reaction was quenched with H_2O and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo. The residue was purified by flash chromatography over silica gel (3:1 EtOAc/*n*-hexane; R_f = 0.28) to give 1180 mg (81%) of **26a** as a solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.51 (s, 9H), 6.38 (dd, J = 6.0, 16.5 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 7.48 (d, J = 16.0 Hz, 1H), 7.55 (d, J = 8.0 Hz, 2H), 7.58 (td, J = 1.0, 7.0 Hz, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 2.0 Hz, 2H), 8.20 (s, 1H), 8.22 (d, J = 8.5 Hz, 1H).

(*E*)-*tert*-Butyl 3-(3-(1*H*-Pyrrolo[2,3-*c*]pyridin-1-ylsulfonyl)-phenyl)acrylate (26b). The title compound was obtained in 68% overall yield from compound **25b** in a manner similar to that described for the preparation of **26a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53 (s, 9H), 6.38 (d, J = 16.5 Hz, 1H), 6.70 (d, J = 3.5 Hz, 1H), 7.46−7.51 (m, 3H), 7.66 (d, J = 7.5 Hz, 1H), 7.69 (d, J = 3.5 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 8.01 (t, J = 2.0 Hz, 1H), 8.42 (d, J = 5.0 Hz, 1H), 9.32 (s, 1H).

(*E*)-*tert*-Butyl-3-(3-(1*H*-pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)-phenyl)acrylate (26c). This compound was obtained in 37% overall yield from compound **25c** in a manner similar to that described for the preparation of **26a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50 (s, 9H), 6.41 (d, J = 15.9 Hz, 1H), 6.56 (d, J = 3.6 Hz, 1H), 7.12 (m, 1H), 7.43 (t, J = 7.43 Hz, 1H), 7.51 (d, J = 16.0 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.67 (d, J =

3.6 Hz, 1H), 7.79 (d, J = 7.5 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.33 (s, 1H), 8.39 (d, J = 4.3 Hz, 1H).

(*E*)-*tert*-Butyl 3-(3-(2,3-Dihydro-1*H*-pyrrolo[2,3-*b*]pyridine-1-ylsulfonyl)phenyl)acrylate (26d). Compound **26d** was prepared in 72% overall yield from compound **25d** in a manner similar to that described for the preparation of **26a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53 (s, 9H), 3.05 (t, J = 8.5 Hz, 2H), 4.06 (t, J = 8.5 Hz, 2H), 6.44 (d, J = 16.0 Hz, 1H), 6.82 (dd, J = 5.0, 7.0 Hz, 1H), 7.35 (dd, J = 1.0, 7.5 Hz, 1H), 7.48 (t, J = 8.0 Hz, 1H), 7.56 (d, J = 16.0 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 4.5 Hz, 1H), 8.27 (s, 1H).

(*E*)-3-(3-(1*H*-Indazol-1-ylsulfonyl)phenyl)acrylic Acid (27a). Trifluoroacetic acid (8.60 mL, 0.13 mmol) was added to compound **26a** (1030 mg, 2.60 mmol) and stirred for 30 min. Then H_2O (20 mL) was added to afford a white solid. The resulting solid was collected by filtration and purified by recrystallization with EtOH to give 450 mg (53%) of **27a** as a pink solid: ^{1}H NMR (500 MHz, CD_3OD and $CDCl_3$) δ 6.07 (d, J = 16.0 Hz, 1H), 6.97 (t, J = 7.5 Hz, 1H), 7.11 (t, J = 8.0 Hz, 1H), 7.19 (d, J = 7.0 Hz, 1H), 7.22 (d, J = 7.5 Hz, 1H), 7.36 (dd, J = 8.0, 13.5 Hz, 2H), 7.52 (d, J = 8.0 Hz, 1H), 7.69 (s, 1H), 7.78 (d, J = 8.5 Hz, 1H), 7.89 (s, 1H).

(*E*)-3-(3-(1*H*-Pyrrolo[2,3-*c*]pyridin-1-ylsulfonyl)phenyl)acrylic Acid (27b). The title compound was obtained in 94% overall yield from compound **26b** in a manner similar to that described for the preparation of **27a**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.64 (d, J = 16.0 Hz, 1H), 7.16 (d, J = 3.5 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.69 (d, J = 16.0 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 8.14 (d, J = 6.0 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 8.37 (d, J = 1.5 Hz, 1H), 8.48 (d, J = 6.5 Hz, 1H), 8.54 (d, J = 3.5 Hz, 1H), 9.57 (s, 1H).

(*E*)-3-(3-(1*H*-Pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)phenyl)-acrylic Acid (27c). This compound was obtained in 97% overall yield from compound **26c** in a manner similar to that described for the preparation of **27a**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.05 (d, J = 12.9 Hz, 1H), 6.21 (d, J = 2.4 Hz, 1H), 6.75 (m, 1H), 7.06–7.15 (m, 2H), 7.27 (d, J = 8.0 Hz, 1H), 7.28 (d, J = 3.0 Hz, 1H), 7.43 (s, 1H), 7.66 (s, 1H), 7.89 (s, 1H), 7.94 (s, 1H).

(*E*)-3-(3-(2,3-Dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)-phenyl)acrylic Acid (27d). The title compound was obtained in 92% overall yield from compound **26d** in a manner similar to that described for the preparation of **27a**: ^{1}H NMR (500 MHz, CD_3OD and $CDCl_3$) δ 3.08 (t, J = 8.0 Hz, 2H), 4.07 (t, J = 8.0 Hz, 2H), 6.51 (d, J = 16.0 Hz, 1H), 6.90 (dd, J = 5.0, 8.0 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.63–7.67 (m, 3H), 8.06–8.10 (m, 3H).

1-(4-Bromophenylsulfonyl)-1*H*-indazole (28a). 4-Bromobenzenesulfonyl chloride (1300 mg, 5.10 mmol) was added to a mixture of indazole (500 mg, 4.23 mmol), KOH (470 mg, 8.46 mmol), and tetrabutylammonium hydrogen sulfate (220 mg, 0.63 mmol) in dry CH_2Cl_2 (30 mL), and the mixture was stirred overnight. The mixture was then filtered, and water was added followed by extraction with CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$ and concentrated in vacuo to yield an oily product. The residue was purified by flash column chromatography over silica gel (EtOAc/*n*-hexane = 3: 1; R_f = 0.30) to give 1250 mg (88%) of **28a** as a solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (td, J = 2.5, 8.5 Hz, 1H), 7.31 (dd, J = 2.0, 7.5 Hz, 1H), 7.64 (dd, J = 9.0, 16.0 Hz, 2H), 7.68 (d, J = 9.0 Hz, 2H), 7.96 (dd, J = 1.5, 7.0 Hz, 2H), 8.62 (s, 1H).

1-(4-Bromophenylsulfonyl)-1*H*-pyrrolo[2,3-*c*]pyridine (28b). The title compound was obtained in 67% overall yield from compound **24b** in a manner similar to that described for the preparation of **28a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.69 (d, J = 3.5 Hz, 1H), 7.46 (dd, J = 1.0, 5.5 Hz, 1H), 7.59 (m, 2H), 7.64 (dd, J = 1.0, 3.5 Hz, 1H), 7.76 (dd, J = 2.0, 4.0 Hz, 2H), 8.42 (dd, J = 2.0, 5.0 Hz, 1H), 9.29 (s, 1H).

1-(4-Bromophenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridine (28c). The title compound was obtained in 79% overall yield from compound **24c** in a manner similar to that described for the preparation of **28a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.61 (d, J = 4.5 Hz, 1H), 7.19 (dd, J = 5.0, 8.0 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 4.0 Hz, 1H), 7.85 (dd, J = 1.5, 8.0 Hz, 1H), 8.07 (d, J = 8.5 Hz, 2H), 8.42 (dd, J = 1.5, 4.5 Hz, 1H).

1-(4-Bromophenylsulfonyl)-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]-pyridine (28d). This compound was obtained in 93% overall yield from compound **24d** in a manner similar to that described for the preparation of **28a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.03 (t, J = 8.5 Hz, 2H), 4.01 (t, J = 8.0 Hz, 2H), 6.81(dd, J = 5.5, 7.5 Hz, 1H), 7.35 (dd, J = 1.5, 8.0 Hz, 1H), 7.57–7.60 (m, 2H), 7.95–7.98 (m, 2H), 8.12 (d, J = 5.0 Hz, 1H).

(*E*)-*tert*-Butyl 3-(4-(1*H*-Pyrazolo[3,4-*b*]pyridin-1-ylsulfonyl)-phenyl)acrylate (29a). A mixture of **28a** (1270 mg, 3.78 mmol), *tert*-butyl acrylate (0.60 mL, 4.54 mmol), triethylamine (0.75 mL, 6.45 mmol), triphenylphosphine (490 mg, 1.90 mmol), palladium acetate (420 mg, 1.90 mmol), and sodium bicarbonate (320 mg, 3.78 mmol) was heated to 80 °C in DMF (2 mL) for 5 h. The mixture was then filtered, and the filtrate was extracted with H_2O (30 mL) and CH_2Cl_2 (3 × 30 mL). The organic layer was collected and dried over anhydrous $MgSO_4$. After the removal of $MgSO_4$ by filtration, the filtrate was concentrated in vacuo to yield an oily product which was purified by flash chromatography over silica gel (1:10 EtOAc/*n*-hexane) to give 680 mg (47%) of **29a** as a yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.57 (s, 9H), 6.38 (d, J = 16.0 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.50–7.59 (m, 3H), 7.70 (dd, J = 3.5, 8.5 Hz, 1H), 7.98 (dd, J = 9.0, 17.0 Hz, 2H), 8.19–8.23 (m, 2H).

(*E*)-*tert*-Butyl 3-(4-(1*H*-Pyrrolo[2,3-*c*]pyridin-1-ylsulfonyl)-phenyl)acrylate (29b). The title compound was obtained in 83% overall yield from compound **28b** in a manner similar to that described for the preparation of **29a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.51 (s, 9H), 6.37 (d, J = 16.0 Hz, 1H), 6.69 (d, J = 3.5 Hz, 1H), 7.46–7.49 (m, 2H), 7.55 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 4.0 Hz, 1H), 7.91 (d, J = 8.5 Hz, 1H), 8.42 (d, J = 5.0 Hz, 1H), 9.32 (s, 1H).

(*E*)-*tert*-Butyl 3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)-phenyl)acrylate (29c). The title compound was obtained in 35% overall yield from compound **28c** in a manner similar to that described for the preparation of **29a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (s, 9H), 6.30 (d, J = 16.0 Hz, 1H), 6.51 (d, J = 5.5 Hz, 1H), 7.08 (dd, J = 4.5, 7.5 Hz, 1H), 7.38 (d, J = 16.0 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 4.0 Hz, 1H), 7.75 (dd, J = 1.5, 8.0 Hz, 1H), 8.03 (d, J = 8.5 Hz, 2H), 8.26 (dd, J = 1.5, 5.0 Hz, 1H).

(*E*)-*tert*-Butyl 3-(4-(2,3-Dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)phenyl)acrylate (29d). This compound was obtained in 72% overall yield from compound **28d** in a manner similar to that described for the preparation of **29a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.91 (s, 9H), 3.04 (t, J = 8.0 Hz, 2H), 4.05 (t, J = 8.0 Hz, 2H), 6.54 (d, J = 16.0 Hz, 1H), 6.91 (dd, J = 5.0, 8.0 Hz, 1H), 7.50 (dd, J = 1.5, 8.0 Hz, 1H), 7.57 (d, J = 16.0 Hz, 1H), 7.72 (d, J = 8.0 Hz, 2H), 8.02 (d, J = 8.5 Hz, 3H).

(*E*)-3-(4-(1*H*-Indazol-1-ylsulfonyl)phenyl)acrylic Acid (30a). Trifluoroacetic acid (4.80 mL, 0.07 mmol) was added to compound **29a** (560 mg, 1.46 mmol), and the mixture was stirred for 30 min. Then H_2O (20 mL) was added, affording a white solid. This solid was collected by filtration and purified by recrystallization from MeOH to give 410 mg (86%) of **30a** as a white solid: ^{1}H NMR (500 MHz, CD_3OD) δ 6.44 (d, J = 16.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.53–7.62 (m, 4H), 7.71 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 2H), 8.16 (d, J = 8.5 Hz, 1H), 8.23 (s, 1H).

(*E*)-3-(4-(1*H*-Pyrrolo[2,3-*c*]pyridin-1-ylsulfonyl)phenyl)acrylic Acid (30b). The title compound was obtained in 94% overall yield from compound **29b** in a manner similar to that described for the preparation of **30a**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.61 (d, J = 16.0 Hz, 1H), 7.15 (d, J = 3.5 Hz, 1H), 7.64 (d, J = 16.0 Hz, 1H), 7.82 (d, J = 8.5 Hz, 2H), 8.12 (d, J = 6.5 Hz, 1H), 8.15 (d, J = 8.5 Hz, 2H), 8.46–8.48 (m, 2H), 9.52 (s, 1H).

(*E*)-3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)phenyl)-acrylic Acid (30c). This compound was obtained in 97% overall yield from compound **29c** in a manner similar to that described for the preparation of **30a**: ^{1}H NMR (500 MHz, $CDCl_3$ and CD_3OD) δ 6.24 (d, J = 16.0 Hz, 1H), 7.15 (t, J = 7.5 Hz, 1H), 7.29 (t, J = 7.9 Hz, 1H), 7.37 (d, J = 5.7 Hz, 1H), 7.40 (d, J = 6.6 Hz, 1H), 7.52 (d, J = 7.9 Hz, 2H), 7.72 (d, J = 8.0 Hz, 1H), 7.88 (s, 1H), 7.97 (d, J = 8.5 Hz, 1H), 8.04 (s, 1H).

(*E*)-3-(4-(2,3-Dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-1-ylsulfonyl)-phenyl)acrylic Acid (30d). The title compound was obtained in 88% overall yield from compound **29d** in a manner similar to that described for the preparation of **30a**: ^{1}H NMR (500 MHz, CD_3OD and DMSO) δ

dx.doi.org/10.1021/jm401899x | *J. Med. Chem.* 2014, 57, 4009–4022

2.96 (t, J = 8.5 Hz, 2H), 3.96 (t, J = 8.5 Hz, 2H), 6.50 (d, J = 16.0 Hz, 1H), 6.81 (dd, J = 5.0, 7.0 Hz, 1H), 7.41 (dd, J = 1.5, 7.0 Hz, 1H), 7.54 (d, J = 16.0 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.92−7.95 (m, 3H).

(B) Biology. (a) HeLa Nuclear HDAC Enzyme Inhibition. *HeLa Nuclear HDAC Inhibitory Assay.* The IC_{50} values of HDAC inhibitors were determined by a fluorimetric histone deacetylase assay following the manufacturer's instructions. For the pan-HDAC assay, HeLa nuclear extracts were used as a source of histone deacetylase (BioVision Inc.). Briefly, 0.25 mg of protein per mL of HeLa nuclear extract was incubated for 30 min with the test compound and 500 μM HDAC kit substrate (Boc-Lys(Ac)-AMC). The reaction was stopped by adding lysine developer, and then the mixture was incubated for 30 min. The fluorescence generated by the deacetylated substrate was measured with a SpectraMax M5 multidetection microplate reader (Molecular Devices) at a wavelength of 360 nm and an emission wavelength of 460 nm. The IC_{50} is determined as the concentration of compound that results in 50% reduction of HDAC activity increase in control wells during the compound incubation. The reaction was done in triplicate for each sample. Each point represents the mean ± SD of replicates.

(b) In Vitro Cell Growth Inhibitory Activity. *Culture Medium and Culture Condition of Cell Lines.* Human lung adenocarcinoma A549 and H460 cells, head and neck carcinoma KB and HSC-3 cells, colorectal carcinoma HT29 and HCT116 cells, and gastric MKN-45 and TSGH carcinoma cells were maintained in RPMI 1640 medium supplied with 5% fetal bovine serum. Human prostatic adenocarcinoma PC-3 cells were maintained in F-12K medium supplied with 10% fetal bovine serum. Human nasopharyngeal carcinoma HONE-1 cells were maintained in DMEM medium supplied with 10% fetal bovine serum. Human breast carcinoma MCF-7 cells were maintained in MEM medium supplemented with 10% FBS. Cells were cultured at 37 °C in a humidified atmosphere and 5% CO_2.

Cell Growth Inhibitory Assay. Cells in their logarithmic phase were cultured in completed medium in 96-well plates at 37 °C with 5% CO_2 for overnight incubation and treated with appropriate concentrations of test compounds for three generative times. Cells were then incubated with a serum free medium containing MTT at a final concentration of 0.5 mg/mM for 4 h. The conversion of MTT to formazan by metabolically viable cells was solublized using 50% DMF solution containing 20% SDS at 37 °C overnight. Absorbance was measured at 570 nm using a SpectraMax M5 microplate reader (Molecular Devices, U.K.).

(c) Effect on Cell Cycle Progression. *Flow Cytometric Analysis.* Cell cycle progression was determined by flow cytometric analysis. Cells were plated onto six-well plates at a final density of 250 000 cells/well and treated with test compounds, then trypsinized, collected, and fixed in ice-cold 75% ethanol overnight at −20 °C. After centrifugation, the fixed cells were rinsed with ice-cold phosphate buffered saline (PBS) and incubated with 0.1 M phosphate/citric acid buffer (0.2 M Na_2HPO_4, 0.1 M citric acid, pH 7.8) at room temperature for 30 min. The cells were then centrifugated and resuspended with propidium iodide (PI) staining buffer containing RNase (100 μg/mL), PI (80 μg/mL), and 0.1% Triton X-100 (v/v) in the dark. The DNA content was analyzed by FACScan flow cytometer with Cell-Quest software (Becton Dickinson, San Jose, CA).

SDS−PAGE and Western Blot Analysis. Cells were harvested by lysis buffer (2.5 mM sodium pyrophosphate, 1 mM EGTA, 1 mM EDTA, 150 mM NaCl, 1 mM PMSF, 1 mM Na_3VO_4, 1 mM NaF, 0.1% Triton X-100 in 20 mM Tris-HCl buffer, pH 7.5) and centrifuged at 13 000 rpm for 30 min. Cell lysates were then collected and analyzed by immunoblotting for specific antibodies. The signals were visualized by enhanced chemiluminescence (ECL) detection system (Amersham, Buckinghamshire, U.K.).

(d) HDAC Enzymes Inhibition Assays. Enzyme inhibition assays were conducted by the Reaction Biology Corporation, Malvern, PA (www.reactionbiology.com). The substrate for HDACs 1, 2, and 6 is a fluorogenic peptide derived from p53 residues 379−382 (RHKK(Ac)). Compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 10 μM. HDAC control compound trichostatin A was tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 10 μM as shown in Supporting Information.

(e) Cell Viability on Three-Dimensional (3D) Cellular Spheres. HCT116 cancer cells (4×10^3 /well) were cultured in a semisolid culture medium comprising GFR-Matrigel (BD Biosciences, catalog no. 356231) and 10% FBS-DMEM medium (v/v = 1:1) for 5 days to allow the formation of 3D cellular spheres. The cellular spheres were treated with compound **12** for 72 h. The semisolid cellular spheres and the effect of the drug on them were examined by phase-contrast light microscopy.

(f) Antitumor Activity in Vivo. Eight-week-old female nude athymic mice were housed in groups under conditions of constant photoperiod (12 h light/12 h dark at 21−23 °C and 60−85% humidity) with ad libitum access to sterilized food and water. All animal experiments followed ethical standards, and protocols have been reviewed and approved by Animal Use and Management Committee of National Taiwan University (IACUC Approval No. 20100225). Each mouse was inoculated sc with 1×10^7 HCT116 cells in a total volume of 0.1 mL of serum-free medium. As tumors became established at ∼40 mm^3 in volume, mice were randomized into four groups (n = 7−8) that received the following treatments: (a) control, 1% carboxymethylcellulose/0.5% Tween 80 vehicle, (b) compound **1** at 200 mg kg^{-1} day^{-1}, (c) compound **12** at 25 mg kg^{-1} day^{-1}, and (d) compound **12** at 100 mg kg^{-1} day^{-1}. Mice received treatments by gavage for the duration of the study. Tumors were measured weekly using calipers. Tumor size, in mm^3, was calculated as volume = $(w^2l)/2$, where w is the width and l is the length in mm of the tumor.

(g) Cell Transfection. The small interfering RNA (siRNA) for p53 and negative control (scrambled siRNA) were purchased from Invitrogen (Carlsbad, CA, USA). Plasmids encoding Flag-tagged HDAC1 and HDAC6 were purchased from Addgene (Cambridge, MA, USA). Transfection was done using lipofectamine reagent according to the manufacturer's instructions. Following transfection, cells were allowed to recover for 24 h and then started the treatment.

(h) Coimmunoprecipitation Analysis. Cells were treated with compound **12** for 24 h and lysed by lysis buffer (2.5 mM sodium pyrophosphate, 1 mM EGTA, 1 mM EDTA, 150 mM NaCl, 1 mM PMSF, 1 mM Na_3VO_4, 1 mM NaF, 0.1% Triton X-100 in 20 mM Tris-HCl buffer, pH 7.5) on ice for 30 min. After centrifugation at 13000g for 20 min, $^1/_{10}$ volume of supernatant was stored at 4 °C for use as input and the remainder was incubated with protein A/G-Sepharose beads for 1 h to eliminate nonspecific binding. The mixture was centrifuged at 1000g for 5 min, and the supernatants were incubated with anti-Hsp90 antibodies and protein A/G Sepharose overnight. The immunocomplexes were resolved by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), and proteins were detected with indicated antibodies.

(i) Maximum Tolerated Dose (MTD) Analysis. The ICR mice were kept fasting overnight supplied only with water. Then the vehicle was administered orally 0.2 mL/20 g mouse (n = 10), and the test compound was administered orally at doses of 400, 600, and 1000 mg/kg, respectively (n = 10 for each group) and observed for 14 days. The animals were dosed po daily for 7 days and then monitored for 1 week. The animals were weighed daily for 1 week and thereafter twice weekly. If mortality occurred in 2 out of 10 animals, then this dose was taken as the toxic dose.

(j) Statistics and Data Analysis. Results are expressed as the mean ± SE for the indicated number of separate experiments. Mean values were assessed for significant differences using t-test, and P-values of <0.05 were considered significant.

(k) In Vivo Pharmacokinetics Evaluation in Mice. The in vivo pharmacokinetic study was approved by the Institutional Animal Care and Use Committee of National Health Research Institutes. A 0.5 and 1.25 mg/mL dosing solution was preparing by dissolving the appropriate amount of the compound in a mixture of DMSO/Cremophor/5% dextrose water (5%/5%/90%, v/v/v) for iv administration and DMA/PEG400 (20%/80%, v/v) for oral dosing. ICR mice, weighing 20−25 g each, were obtained from BioLASCO, Ilan, Taiwan. Each tested compound was separately administered intravenously to a group of three mice per time point (2 mg/kg dose) by a bolus injection (10 mg/kg dose) to the tail vein or periorally. The volume of dosing solution administered was 100 μL for intravenous administration and 200 μL for the oral route. At time points 0 (prior to dosing), 2, 5 (iv

only), 15, and 30 min and 1, 2, 4, 6, 8, and 24 h after dosing, a blood sample was collected from each animal via cardiac puncture and stored in ice (0−4 °C). Plasma was separated from the blood by centrifugation (14000*g* for 15 min at 4 °C) and stored in a freezer at −80 °C. All samples were analyzed for the test compound by LC−MS/MS (ABI4000). Data were acquired via monitoring of multiple reactions. Plasma concentration data were analyzed by a standard non-compartmental method.

(l) Human Ether-a-Go-Go Related Gene (hERG) Assay. The assay was conducted by Eurofins Panlabs Taiwan, Ltd. [^{3}H]Astemizole binding to HERG transfected human embryonic kidney HEK293 cells membrane was performed in incubation buffer (10 mM HEPES, pH 7.4, 0.1% BSA, 5 mM KCl, 0.8 mM $MgCl_2$, 130 mM NaCl, 1 mM EGTA, 10 mM Glucose) with DMSO or test drugs as described previously.[20] [^{3}H]Astemizole binding to hERG transfected membrane is inhibited by compounds known to prolong QT-interval.

■ ASSOCIATED CONTENT

Supporting Information

HPLC results of target compounds **7**−**15**, ^{1}H NMR spectra for compounds **7**−**15**, HDAC isozymes activity of compound **2** and trichostatin A, and the hERG assay of compound **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*J.-Y.C.: phone, 886-6-700-0123, extension 65100; e-mail, jychang@nhri.org.tw.

*J.-P.L.: phone, 886-2-2736-1661, extension 6130; e-mail, jpl@tmu.edu.tw.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the National Science Council of the Republic of China (Grants NSC 100-2628-M-038-001-MY3 and NSC 102-2325-B-038-002).

■ ABBREVIATIONS USED

HDAC, histone deacetylase; HAT, histone acetyltransferase; NH_2OTHP, *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine; PYBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; TBAHS, tetrabutylammonium hydrogen sulfate; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; TSA, trichostatin A; TGI, tumor growth inhibition

■ REFERENCES

(1) (a) Bolden, J. E.; Peart, M. J.; Johnstone, R. W. Anticancer activities of histone deacetylase inhibitors. *Nat. Rev. Drug Discovery* **2006**, *5*, 769−784. (b) O'Connor, O. A.; Heaney, M. L.; Schwartz, L.; Richardson, S.; Willim, R.; MacGregor-Cortelli, B.; Curly, T.; Moskowitz, C.; Portlock, C.; Horwitz, S.; Zelenetz, A. D.; Frankel, S.; Richon, V.; Marks, P.; Kelly, W. K. Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. *J. Clin. Oncol.* **2006**, *24*, 166−173.

(2) de Ruijter, A. J.; van Gennip, A. H.; Caron, H. N.; Kemp, S.; van Kuilenburg, A. B. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem. J.* **2003**, *370*, 737−749.

(3) (a) 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. (b) Yoshida, M.; Horinouchi, S.; Beppu, T. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *BioEssays* **1995**, *17*, 423−430.

(4) 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.

(5) Duvic, M.; Talpur, R.; Ni, X.; Zhang, C.; Hazarika, P.; Kelly, C.; Chiao, J. H.; Reilly, J. F.; Ricker, J. L.; Richon, V. M.; Frankel, S. R. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). *Blood* **2007**, *109*, 31−39.

(6) (a) Giles, F.; Fischer, T.; Cortes, J.; Garcia-Manero, G.; Beck, J.; Ravandi, F.; Masson, E.; Rae, P.; Laird, G.; Sharma, S.; Kantarjian, H.; Dugan, M.; Albitar, M.; Bhalla, K. A phase I study of intravenous LBH589, a novel cinammic hydroxamid acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. *Clin. Cancer Res.* **2006**, *12*, 4628−4635. (b) Atadja, P. Development of the pan-HDAC inhibitor panobinostat (LBH589): success and challenges. *Cancer Lett.* **2009**, *280*, 233−241.

(7) (a) Steele, N. L.; Plumb, J. A.; Vidal, L.; Tjørnelund, J.; Knoblauch, P.; Rasmussen, A.; Ooi, C. E.; Buhl-Jensen, P.; Brown, R.; Evans, T. R.; Debono, J. S. A phase 1 pharmacokinetic and pharmacodynamic study of the histone deacetylase inhibitor belinostat in patients with advanced solid tumors. *Clin. Cancer Res.* **2008**, *14*, 804−810. (b) Plumb, J. A.; Finn, P. W.; Williams, R. J.; Bandara, M. J.; Romero, M. R.; Watkins, C. J.; Thangue, N. B. L.; Brown, R. Pharmacodynamic response and inhibition of growth of human tumor xenografts by the novel histone deacetylase inhibitor PXD101. *Mol. Cancer Ther.* **2003**, *2*, 721−728.

(8) Wagner, J. M.; Hackanson, B.; Lübbert, M.; Jung, M. Histone deacetylase (HDAC) inhibitors in recent clinical trials for cancer therapy. *Clin. Epigenet.* **2010**, *1*, 117−136.

(9) Santo, L.; Hideshima, T.; Kung, A. L.; Tseng, J. C.; Tamang, D.; Yang, M.; Jarpe, M.; Duzer, J. H. V.; Mazitschek, R.; Ogier, W. C.; Cirstea, D.; Rodig, S.; Eda, H.; Scullen, T.; Canavese, M.; Bradner, J.; Anderson, K. C.; Jones, S. S.; Raje, N. Preclinical activity, pharmacodynamic, and pharmacokinetic properties of a selective HDAC6 inhibitor, ACY-1215, in combination with bortezomib in multiple myeloma. *Blood* **2012**, *119*, 2579−2589.

(10) Kalin, J. H.; Butler, K. V.; Akimova, T.; Hancock, W. W.; Kozikowski, A. P. Second-generation histone deacetylase 6 inhibitors enhance the immunosuppressive effects of Foxp3+ T-regulatory cells. *J. Med. Chem.* **2012**, *55*, 639−651.

(11) (a) Lai, M. J.; Huang, H. L.; Pan, S. L.; Liu, Y. M.; Peng, C. Y.; Lee, H. Y.; Yeh, T. K.; Huang, P. H.; Teng, C. M.; Chen, C. S.; Chuang, H. Y.; Liou, J. P. Synthesis and biological evaluation of 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indoles as potent histone deacetylase inhibitors with antitumor activity in vivo. *J. Med. Chem.* **2012**, *55*, 3777−3791. (b) Lee, H. Y.; Yang, C. R.; Lai, M. J.; Huang, H. L.; Hsieh, Y. L.; Liu, Y. M.; Yeh, T. K.; Li, Y. H.; Mehndiratta, S.; Teng, C. M.; Liou, J. P. 1-Arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines histone deacetylase inhibitors are potent cytokine release suppressors. *ChemBioChem* **2013**, *14*, 1248−1254.

(12) (a) Zhang, Y.; Yang, P.; Xu, W.; Chou, C. J.; Liu, C.; Wang, X. Development of *N*-hydroxycinnamamide-based histone deacetylase inhibitors with an indole-containing cap group. *ACS Med. Chem. Lett.* **2013**, *4*, 235−238. (b) Giannini, G.; Marzi, M.; Di Marzo, M.; Battistuzzi, G.; Pezzi, R.; Brunetti, T.; Cabri, W.; Vesci, L.; Pisano, C. Exploring bis-(indolyl)methane moiety as an alternative and innovative CAP group in the design of histone deacetylase (HDAC) inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2840−2843. (c) Attenni, B.; Ontoria, J. M.; Cruz, J. C.; Rowley, M.; Schultz-Fademrecht, C.; Steinkuhler, C.; Jones, P. Histone deacetylase inhibitors with a primary amide zinc binding group display antitumor activity in xenograft model. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3081−3084.

(13) Zhang, Z.; Yang, Z.; Meanwell, N. A.; Kadow, J. F.; Wang, T. A general method for the preparation of 4- and 6-azaindoles. *J. Org. Chem.* **2002**, *67*, 2345−2347.

(14) Chen, W. S.; Lee, Y. J.; Yu, Y. C.; Hsaio, C. H.; Yen, J. H.; Yu, S. H.; Tsai, Y. J.; Chiu, S. J. Enhancement of p53-mutant human colorectal

cancer cells radiosensitivity by flavonoid fisetin. *Int. J. Radiat. Oncol., Biol., Phys.* **2010**, *77*, 1527−1535.
(15) (a) Namdar, M.; Perez, G.; Ngo, L.; Marks, P. A. Selective inhibition of histone deacetylase 6 (HDAC6) induces DNA damage and sensitizes transformed cells to anticancer agents. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 20003−20008. (b) Kaliszczak, M.; Trousil, S.; Åberg, O.; Perumal, M.; Nguyen, Q. D.; Aboagye, E. O. A novel small molecule hydroxamate preferentially inhibits HDAC6 activity and tumour growth. *Br. J. Cancer.* **2013**, *108*, 342−350.
(16) Chen, C. S.; Weng, S. C.; Tseng, P. H.; Lin, H. P.; Chen, C. S. Histone acetylation-independent effect of histone deacetylase inhibitors on Akt through the reshuffling of protein phosphatase 1 complexes. *J. Biol. Chem.* **2005**, *280*, 38879−38887.
(17) (a) Hong, S.; Lee, S.; Kim, B.; Lee, H.; Hong, S. S.; Hong, S. Discovery of new azaindole-based PI3Kα inhibitors: apoptotic and antiangiogenic effect on cancer cells. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7212−7215. (b) Hong, S.; Kim, J.; Seo, J. H.; Jung, K. H.; Hong, S. S.; Hong, S. Design, synthesis, and evaluation of 3,5-disubstituted 7-azaindoles as Trk inhibitors with anticancer and antiangiogenic activities. *J. Med. Chem.* **2012**, *55*, 5337−5349.
(18) Horning, J. L.; Sahoo, S. K.; Vijayaraghavalu, S.; Dimitrijevic, S.; Vasir, J. K.; Jain, T. K.; Panda, A. K.; Labhasetwar, V. 3-D tumor model for in vitro evaluation of anticancer drugs. *Mol. Pharmaceutics* **2008**, *5*, 849−862.
(19) Yeo, P.; Xin, L.; Goh, E.; New, L. S.; Zeng, P.; Wu, X.; Venkatesh, P.; Kantharaj, E. Development and validation of high-performance liquid chromatography−tandem mass spectrometry assay for 6-(3-benzoyl-ureido)-hexanoic acid hydroxyamide, a novel HDAC inhibitor, in mouse plasma for pharmacokinetic studies. *Biomed. Chromatogr.* **2007**, *21*, 184−189.
(20) Finlayson, K.; Turnbull, L.; January, C. T.; Sharkey, J.; Kelly, J. S. [^{3}H]Dofetilide binding to HERG transfected membranes: a potential high throughput preclinical screen. *Eur. J. Pharmacol.* **2001**, *430*, 147−148.