Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 8966

Effect of C7-substitution of 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines on the selectivity towards a subclass of histone deacetylases†

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Received 11th March 2014,
Accepted 11th September 2014

DOI: 10.1039/c4ob00542b

www.rsc.org/obc

This study focused on the substitution effect at position C7 of 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)-indolines. Compound **9**, (*E*)-3-(7-amino-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-*N*-hydroxyacrylamide, displayed 4- to 14-fold more potent antiproliferative activity than vorinostat (SAHA, **1**). Notably, **9** possessed specific histone deacetylase (HDAC) inhibitory activity toward HDAC1 and HDAC2, but had no effect on HDAC6, indicating that **9** has the potential to be developed as a class I HDAC inhibitor. In a xenograft tumor model, **9** suppressed the growth of HCT116 cells at 100 mg kg^{-1}, which led to a TGI (tumor growth inhibition) of 40.3%. Taken together, the C7 substitutions have a crucial effect on class I HDACs, which is beneficial for synthesizing efficient anticancer agents.

Introduction

Histone deacetylase (HDAC) is a crucial protein in the epigenetic process. Twelve HDAC isoenzymes (HDAC1-11 and sirtuins) were found having distinct biological functions and were recruited to specific regions of the genome.[1,2] They are grouped into four classes based on their sequence homology to their yeast orthologues, Rpd3, HdaI, and Sir2.[3,4] Class I HDACs includes HDAC1, 2, 3, and 8. Class II HDACs is divided into two subclasses: Class IIa (HDAC4, 5, 7, and 9) and Class IIb (HDAC6 and 10). HDAC11 belongs to Class IV HDACs. Class I, II, and IV are also named "classical" HDACs, whereas class III HDACs is named "non-classical" HDACs or sirtuins.[5] Classical and non-classical HDACs are defined by their mode of action. Classical HDACs are Zn^{2+}-dependent enzymes harboring a catalytic pocket with a Zn^{2+} ion at its base that can be inhibited by Zn^{2+} chelating compounds such as hydroxamic acids. In contrast, class III enzymes have a different mechanism of action requiring NAD^+ as an essential cofactor.

The inhibition of histone deacetylase (HDAC) is highly correlated with the treatment of cancer in recent years, and therefore, it is considered as an attractive target for the development of anticancer agents.[6] Specifically, the approval of vorinostat (SAHA, **1**) and FK-228 (romidepsin, **2**) by the US FDA to treat cutaneous T-cell lymphoma (CTCL) triggered numerous scientific efforts toward this target. Recently, there are many potent HDAC inhibitors entering clinical trials, for example, LBH-589 (**3**), PXD-101 (**4**), and MS-275 (**5**). According to their structural features, HDAC inhibitors are categorized into four families: hydroxamic acids, short-chain fatty acids, benzamides, and cyclic tetrapeptides.[7] Among them, hydroxamic acids attracted scientific attention due to the strong interaction between hydroxamic acid and the zinc ion.[8] Recent research has been performed to further develop isotype-selective HDAC inhibitors due to their efficacy and safety. For instance, compounds **2** and **5** were found to belong to class I HDAC inhibitors, and the other three compounds in Fig. 1 are pan-HDAC inhibitors. Hence, the development of isotype-selective HDAC inhibitors has become significant in the search for effective anticancer agents.

In our previous efforts to develop antitubulin and anti-HDAC agents, the 1-methoxybenzenesulfonylindoline (olive green part in Fig. 2) moiety was considered to be crucial for biological potency.[9] Furthermore, the groups at C5 and C7 guided the molecule towards specific pharmacological mechanisms. For instance, compound **7** with a 4-pyridylcarbonylamino group at the C7 position exhibited potent antiproliferative activity through suppressing the function of tubulin.[10] In addition, the attachment of *N*-hydroxyacrylamide to 1-meth-

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†Electronic supplementary information (ESI) available: Spectral data of compounds **9–19** and HPLC purity data for synthetic compounds. See DOI: 10.1039/c4ob00542b
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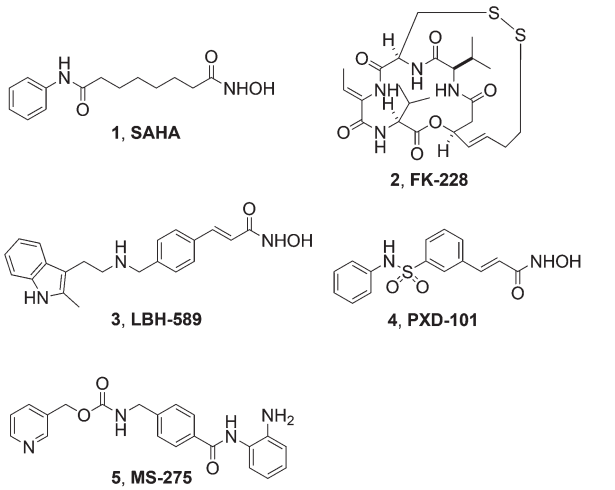


Fig. 1 Examples of histone deacetylase inhibitors.

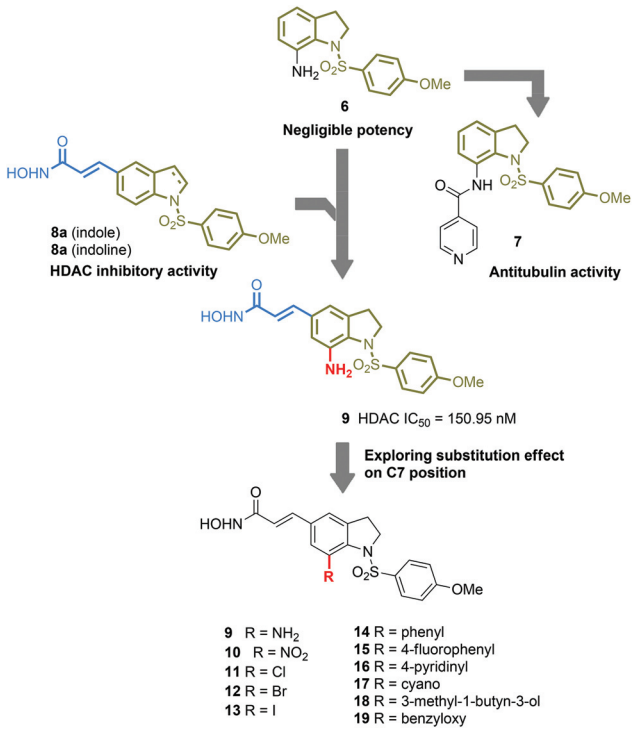


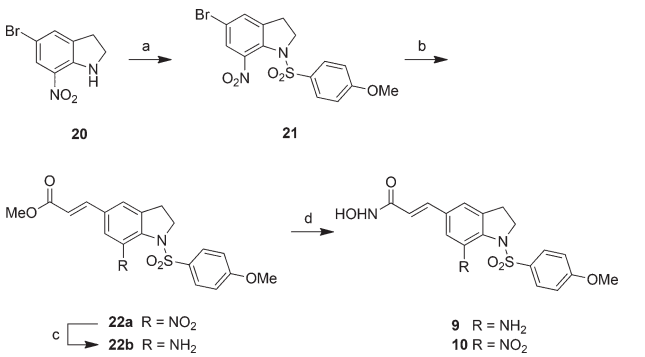
Fig. 2 Diagram for the development of C7-substituted 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines.

oxybenzenesulfonylindole (**8a**) and 1-methoxybenzenesulfonylindoline (**8b**) led to a series of potent HDAC inhibitors,[9,11] and the indoline derivatives exhibited slightly better potency than the indole derivatives. In the early stage of this study, we connected the symbolic *N*-hydroxyacrylamide with inactive compound **6** to yield compound **9**. Surprisingly, **9** was found to exhibit marked HDAC inhibitory activity comparable to **1**. This result indicated that structural hybridization would reactivate this series of products. Therefore, the goal of this study is to develop a series of 7-substituted 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines by utilizing the appropriate indoline chemistry to achieve our goal.

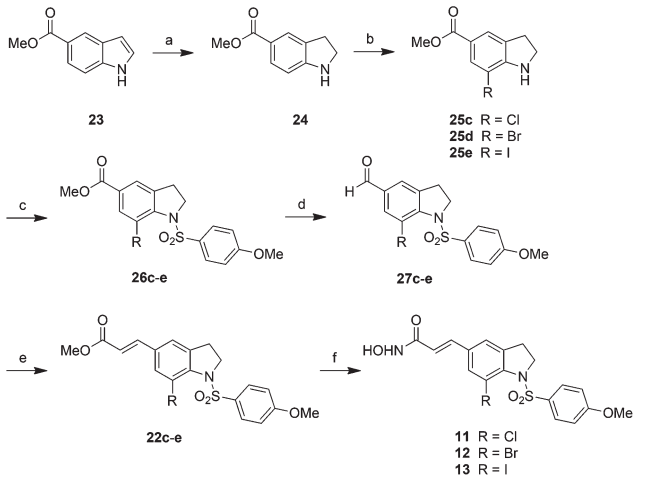
Chemistry

Scheme 1 illustrates the synthesis of designed 7-substituted 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines (**9** and **10**). The commercial 5-bromo-7-nitroindoline (**20**) was reacted with 4-methoxybenzenesulfonyl chloride in the presence of heated pyridine to afford compound **21** (Scheme 1). The following Heck olefination with methyl acrylate yielded compound **22a**. The nitro group was reduced by ferric powder to provide the corresponding amine **22b**. The resulting acrylates (**22a–b**) were hydrolyzed under basic conditions to obtain the corresponding acrylic acids, which were subject to amidation reaction with NH_2OTHP to give *O*-protected *N*-hydroxyacrylamide, which immediately underwent the following reaction. The removal of a protecting group was fulfilled by the treatment of 10% TFA to furnish *N*-hydroxyacrylamides **9** and **10**. This three-step route to convert acrylate into *N*-hydroxyacrylamide (hydrolysis of the ester bond by LiOH, formation of *O*-protected *N*-hydroxyacrylamide, and removal of the THP group) was applied to the following schemes in this study.

The synthesis of C7-halogen-containing 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines (**11–13**) is summarized in Scheme 2. The C2–C3 double bond of methyl indole-5-carboxylate (**23**) was reduced by $NaBH_3CN$ in the presence of acetic acid to afford indoline **24**. The halogenation of **24** was carried out by the treatment of NCS, NBS, and NIS to provide the corresponding halogenated products **25c–e**. The treatment of **25c–e** with 4-methoxybenzenesulfonyl chloride yielded compounds **26c–e**. The ester groups of **26c–e** were hydrolyzed, and the resulting carboxylic acids were reduced by BH_3 to afford the alcohol products, which were subsequently oxidized by pyridinium dichromate (PDC) to yield the corresponding aldehydes **27c–e**. The resulting aldehydes were reacted with methyl (triphenylphosphoranylidene)acetate under Witting conditions



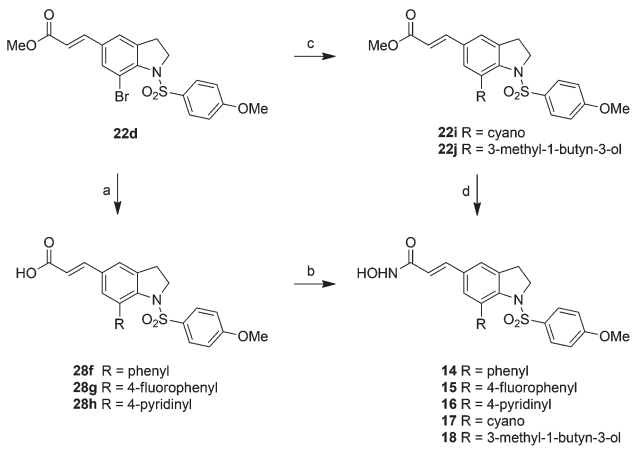
Scheme 1 Synthetic route to 7-substituted 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines (**9** and **10**): a. 4-methoxybenzenesulfonyl chloride, pyridine, 95 °C; b. methyl acrylate, $Pd(OAc)_2$, TPP, TEA, DMF, 90 °C; c. Fe, NH_4Cl, IPA–H_2O, reflux; d. (i) 1 M $LiOH_{(aq)}$, dioxane, 40 °C; (ii) NH_2OTHP, EDC·HCl, NMM, DMF, rt; (iii) 10% $TFA_{(aq)}$, MeOH, rt.



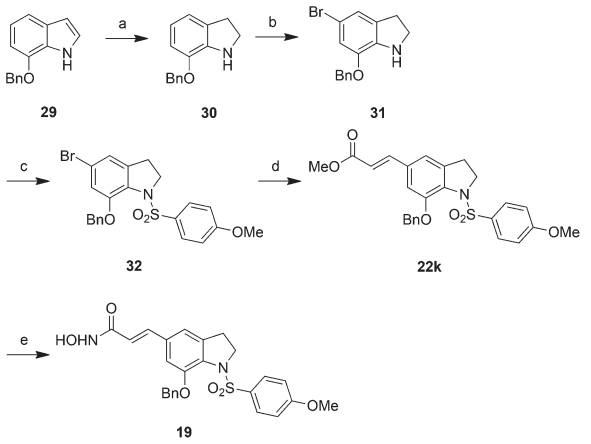
Scheme 2 Synthetic route to 7-substituted 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines (**11–13**): a. $NaBH_3CN$, AcOH, 0 °C to rt; b. *N*-Halosuccinimides, AcOH, dioxane, 0 °C; c. 4-Methoxybenzenesulfonyl chloride, pyridine, 95 °C; d. (i) 1 M $LiOH_{(aq)}$, dioxane, 40 °C; (ii) 1 M BH_3–THF, THF, 0 °C to rt; (iii) PDC, MS, CH_2Cl_2, rt; e. methyl (triphenylphosphoranylidene)acetate, CH_2Cl_2, rt; f. (i) 1 M $LiOH_{(aq)}$, dioxane, 40 °C; (ii) NH_2OTHP, HOBt, NMM, EDC·HCl, DMF, rt; (iii) 10% $TFA_{(aq)}$, MeOH, rt.

to furnish methyl acrylates **22c–e**, which were subject to the general route to generate *N*-hydroxyacrylamides **11–13**.

Scheme 3 outlines the synthesis of compounds **14–18**. Compound **22d** underwent Suzuki arylation with various arylboronic acids with the assistance of microwaves. Interestingly, the acrylates were hydrolyzed probably due to the presence of K_2CO_3 to afford the acrylic acids **28f–h**. The synthetic acids reacted with NH_2OTHP to form *O*-protected *N*-hydroxyacrylamides, which were subject to deprotection by 10% TFA to furnish compounds **14–16**. Compound **22d** underwent Rosenmund–von Braun cyanation and Sonogashira alkynation with CuCN and 3-methyl-1-butyn-3-ol, yielding **22i** and **22j**, respectively. Both **22i** and **22j** followed the general approach cited above to obtain hydroxamic acids **17** and **18**. Scheme 4 outlines the synthetic route to compound **19** with a benzyloxy group at the C7 position. 7-Benzyloxyindole (**29**) was reacted with $NaBH_3CN$ in the presence of acetic acid to afford indoline **30**. The subsequent bromination was achieved by treatment with tetra-*n*-butylammonium tribromide ($TBABr_3$) to provide **31**, which was subject to the methodology cited in Scheme 1 to yield compound **19**.



Scheme 3 Synthetic route to 7-benzyloxy-1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines (**14–18**): a. 2 M $K_2CO_{3(aq)}$, $Pd(PPh_3)_4$, substituted boronic acid, DMF, MW 140 °C; b. (i) NH_2OTHP, EDC·HCl, NMM, DMF, rt; (ii) 10% $TFA_{(aq)}$, MeOH, rt; c. for **22i**: CuCN, DMF, 130 °C; for **22j**: 3-methyl-1-butyn-3-ol, $Pd(PPh_3)_4$, CuI, TEA, DMF, MW 140 °C; d. (i) 1 M $LiOH_{(aq)}$, dioxane, 40 °C; (ii) NH_2OTHP, EDC·HCl, NMM, DMF, rt; (iii) 10% $TFA_{(aq)}$, MeOH, rt.



Scheme 4 Synthetic route to 7-benzyloxy-1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines (**19**): a. $NaBH_3CN$, AcOH, 0 °C to rt; b. $TBAB_3$, CH_2Cl_2, rt; c. 4-Methoxybenzenesulfonyl chloride, pyridine, 95 °C; d. methyl acrylate, $Pd_2(dba)_3$, P(*o*-tol)$_3$, TEA, ACN, 90 °C; e. (i) 1 M $LiOH_{(aq)}$, dioxane, 40 °C; (ii) NH_2OTHP, EDC·HCl, NMM, DMF, rt; (iii) 10% $TFA_{(aq)}$, MeOH, rt.

Biological evaluation

A. HeLa nuclear HDAC enzyme inhibition

The influence of C7 substituents on HDAC enzyme inhibition is summarized in Table 1. The synthesized compounds, with the exception of **12**, displayed comparable HDAC enzyme inhibitory activity to that of SAHA (**1**, IC_{50} = 178 nM). Compound **10** with a nitro group exhibited the most potent anti-HDAC activity with an IC_{50} value of 63 nM, which is 3-fold more potent than **1**. Although C7 replacement caused a slight loss of enzymatic activity, this series of compounds still inhibits HDAC in the same manner as compound **8**. This result indicated that the introduction of *N*-hydroxyacrylamide reactivated compound **6**, which had negligible biological activity, and played a crucial role in determining the potential HDAC inhibitors in this series of compounds (Fig. 2).

B. *In vitro* cell growth inhibitory activity

The antiproliferative activities against four human cancer cell lines, colorectal carcinoma HCT116, hepatocellular carcinoma Hep3B, prostate carcinoma PC3, and non-small cell lung carcinoma A549 cells of the synthetic 7-substituted 1-arylsulfonyl-5-

Table 1 Inhibition of HeLa nuclear extract, HDAC activity, and antiproliferative activity against human cancer cell lines by tested compounds and SAHA

	HeLa HDACs	GI_{50} (μM ± SE[a])			
Compd	IC_{50} (nM ± SE[a])	HCT116	Hep3B	PC3	A549
9	150.95 ± 8.31	0.11 ± 0.02	0.14 ± 0.01	0.17 ± 0.01	0.18 ± 0.01
10	63.40 ± 2.68	0.20 ± 0.01	0.26 ± 0.02	0.31 ± 0.01	0.54 ± 0.08
11	123.35 ± 19.70	0.28 ± 0.03	0.56 ± 0.07	0.50 ± 0.03	0.92 ± 0.04
12	nd	0.67 ± 0.05	1.16 ± 0.07	0.82 ± 0.03	1.01 ± 0.02
13	140.13 ± 14.05	0.43 ± 0.02	1.09 ± 0.06	0.83 ± 0.09	1.46 ± 0.21
14	165.76 ± 39.07	0.63 ± 0.07	1.46 ± 0.35	0.89 ± 0.07	1.18 ± 0.02
15	145.49 ± 14.18	0.96 ± 0.10	1.92 ± 0.30	1.14 ± 0.06	1.73 ± 0.07
16	500.15 ± 66.73	2.83 ± 0.16	5.45 ± 1.02	3.27 ± 0.26	>10
17	228.32 ± 36.15	0.44 ± 0.04	0.64 ± 0.03	0.57 ± 0.01	1.20 ± 0.15
18	160.69 ± 35.75	0.53 ± 0.05	1.10 ± 0.15	0.73 ± 0.03	1.44 ± 0.14
19	271.24 ± 16.86	2.55 ± 0.06	>3.6	>3.6	>3.6
1, SAHA	178.02 ± 16.40	0.44 ± 0.03	0.95 ± 0.04	0.23 ± 0.02	2.62 ± 1.37

[a] SE: standard error. All experiments were independently performed at least three times.

(*N*-hydroxyacrylamide)indolines were evaluated (**9–19**), and reference compound **1** was evaluated as well (Table 1). The PC3 column of Table 1 shows that only **9** displayed slightly improved activity as compared with **1**, and other adducts showed moderate to less antiproliferative activity. Therefore, the following discussion will focus on the efficacy of the tested products on the growth of the HCT116, Hep3B, and A549 cell lines. In taking into account the substitution effect, the C7 position disfavoured larger groups such as aryl and heteroaryl groups. Of all the synthetic compounds, **9**, **10**, and **11** displayed increased cellular activity as compared to **1**. The order of influence of the substitutions of the preceding compounds is as follows: NH_2 > NO_2 > Cl. Compound **9** with a C7–NH_2 exhibited 4- to 14-fold more potent activity as compared with **1**. It inhibited the growth of HCT116, Hep3B, and A549 cells with GI_{50} values of 0.11, 0.14, and 0.18 μM, respectively. Compound **10** with a C7–NO_2 group showed a 2-fold decrease of potency when compared with **9**, although it exhibited the best HDAC inhibitory activity.

C. Upregulation effect of histone and α-tubulin

Fig. 3 indicates the effect of compounds **9** and **1** on the acetylation level of histone H3 and α-tubulin, which are the substrate of class I HDACs and HDAC6, respectively. Western blot analysis was carried out with HCT116 colorectal cancer cells at different concentrations. Compounds **9** and **1** are able to increase the acetylation levels of histone H3 in a dose-dependent manner (Fig. 3A and B). The treatment with **1** caused the hyperacetylation of α-tubulin, whereas compound **9** had no effect on α-tubulin (Fig. 3A and C). In accordance with these results, compound **9** should probably be classified as a class I HDAC inhibitor.

D. HDAC isoform inhibition

In order to confirm the influence of C7 groups on the selectivity toward HDAC isoforms, the inhibitory activities of compounds **9**, **10**, **11** and SAHA against HDAC 1, 2, 6, and 8 were examined; the

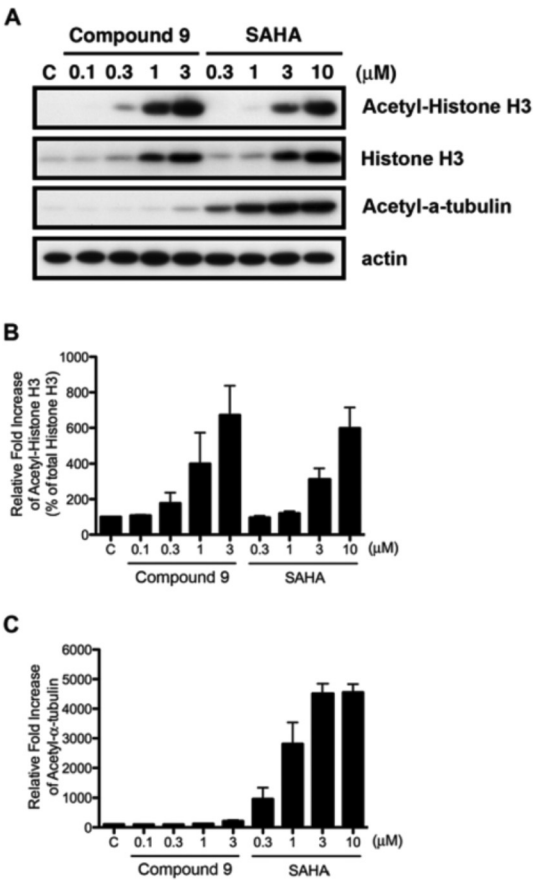


Fig. 3 Effect of compound **9** and SAHA on α-tubulin and histone H3 acetylation. (A) HCT116 cells were treated with compound **9** and SAHA for 24 h at the indicated concentrations. Cell lysates were prepared and subjected to SDS-PAGE and immunoblotting using acetyl-histone H3, histone H3, acetyl-α-tubulin, and α-tubulin antibodies. Quantitative analysis of western blot with ImageQuant (Molecular Dynamics, USA); acetyl-histone H3 (B), and acetyl-α-tubulin (C) were analyzed in HCT116 cells.

Table 2 Activities of compounds **9**, **10**, **11** and SAHA against HDAC isoforms 1, 2, 4, 6, and 8

	HDAC1	HDAC2	HDAC4	HDAC6	HDAC8	Selectivity	
Compd	IC_{50} (nM ± SE[a])					HDAC1/HDAC6	HDAC2/HDAC6
9	75.07 ± 4.65	241.75 ± 47.65	>10 000	4368.22 ± 42.07	3344.43 ± 129.99	58	18
10	29.75 ± 4.49	49.87 ± 3.39	>10 000	869.51 ± 94.24	4284.31 ± 356.74	29	17
11	111.44 ± 8.70	98.49 ± 4.28	>10 000	730.28 ± 41.50	2847.30 ± 627.90	6	7
1, SAHA	115.95 ± 5.51	162.87 ± 2.05	>10 000	78.31 ± 8.96	5741.27 ± 162.56	0.67	0.48

[a] SE: standard error. All experiments were independently performed at least three times.

selectivity ratio is summarized in Table 2. Compound **10** exhibited stronger HDAC1 and HDAC2 inhibitory activity than **1**, with IC_{50} values of 29.75 and 49.87 nM, respectively. Compound **9** showed a 2-fold increase of HDAC1 inhibitory activity as compared with **1**, and a 1.5-fold decrease of HDAC2 inhibitory activity. Notably, **9** and **10** displayed negligible activity against HDAC6, which belongs to class II HDAC. As compared with HDAC6, **9** and **10** were 58- and 29-fold more selective toward HDAC1, and 18- and 17-fold more selective toward HDAC2. These results indicated that this series of compounds are probably classified as class I HDAC inhibitors instead of pan-HDAC inhibitors. As compared to our previous work on 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines (**8**),[9,10] the presence of C7 replacements seems to contribute to isotype-HDAC selectivity.

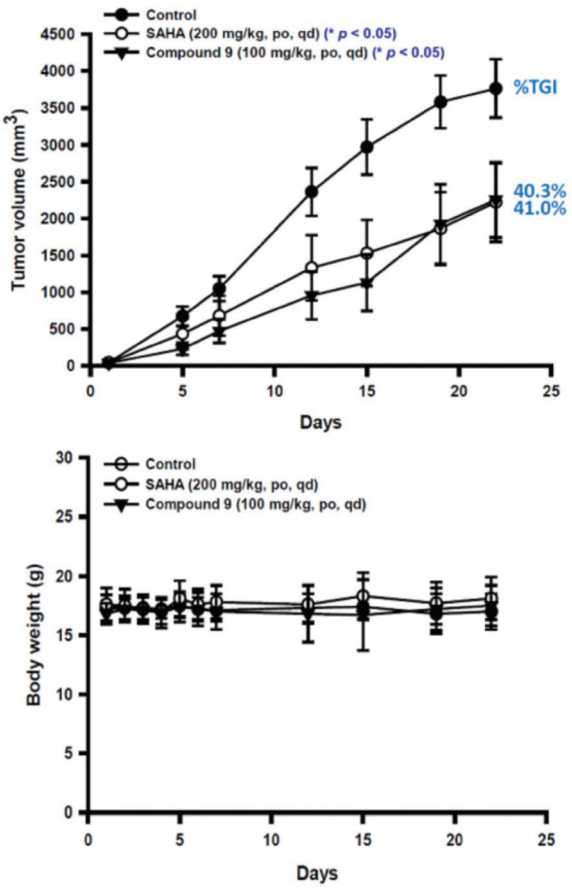


Fig. 4 The effect of compound **9** on the growth of HCT116 cells *in vivo*. (A) Inhibition of tumor growth curves represent a mean ± SEM and percentage change in mean tumor volume. (B) Body weights were measured daily during the first week and then 2 times per week. The body weight ratio was calculated relative to the baseline measurement.

E. *In vivo* efficacy in human xenograft

Compound **9** was further evaluated for the ability to suppress the growth of human HCT116 colorectal adenocarcinoma cells in nude mice. Once a tumor was palpable with a size of approximately 45 mm^3, mice were randomized into vehicle control and treatment groups of seven animals each. Control mice received the vehicle (1.0% carboxymethyl cellulose and 0.5% Tween 80). Compound **9** displayed comparable antitumor activity at lower dose when compared with **1**. It suppressed the growth of HCT116 cancer cell xenografts by a factor of 40.3% (percent tumor growth inhibition [%TGI] values) after oral administration, whereas **1** at 200 mg kg^{-1} orally suppressed tumor growth by 41.0% (%TGI). In addition, treatment with **9** and **1** showed no significant effect on the change in body weight (Fig. 4).

Conclusions

The structural hybridization of the *N*-hydroxyacrylamido motif with 1-arylsulfonylindoline re-activated compound **6** as a potent HDAC inhibitor that is comparable in activity to FDA-approved SAHA. As a result, a series of 7-substituted 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indolines (**9–19**) were generated and were found to possess comparable HDAC inhibition activity to **1**. Among all the synthetic products, compounds **9**, **10**, and **11** exhibited better antiproliferative activity than **1**. Compound **9** suppressed the growth of HCT116 cells in the xenograft tumor model at a dose of 100 mg kg^{-1}. Notably, **9** and **10** displayed remarkable potency to inhibit HDAC1 and HDAC2 but not HDAC6, which revealed that the C7 group probably contributed the selectivity toward class I HDACs. Therefore, this study opens an avenue to develop selective HDAC inhibitors with potent anticancer activity.

Experiments

General information

(A) Chemistry. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were obtained with a Bruker Fourier 300 and

DRX-500 spectrometer (operating at 300 and 500 MHz), with the chemical shift in parts per million (ppm, δ) downfield from tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HRMS) were measured with a JEOL (JMS-700) electron impact (EI) mass spectrometer. The purity of the final compounds was determined using an Agilent 1100 series HPLC system with a C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm) and was found to be ≥95%. Flash column chromatography was performed using silica gel (Merck Kieselgel 60, no. 9385, 230–400 mesh ASTM). All reactions were carried out under an atmosphere of dry nitrogen.

*(E)-3-(7-Amino-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-N-hydroxyacrylamide (**9**).* To a solution of compound **21b** (0.3 g, 0.77 mmol) in dioxane (10 mL), 1 M $LiOH_{(aq)}$ (1.5 mL) was added and stirred at 40 °C overnight. The mixture was concentrated under reduced pressure. The residue was acidified by 3 N HCl to give the precipitation, which was collected by filtration and formed a yellow solid (0.25 g, 86%). A mixture of the resulting solid (0.25 g, 0.67 mmol), EDC·HCl (0.19 g, 1.0 mmol), HOBt (0.11 g, 0.8 mmol), NMM (0.18 mL, 1.6 mmol), and DMF (1 mL) was stirred at room temperature for 10 min. NH_2OTHP (0.09 g, 0.8 mmol) was added to the mixture, which was stirred at room temperature overnight. The mixture was quenched with water and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ followed by filtration, and then the filtrate was dried under reduced pressure. A mixture of the residue, 10% $TFA_{(aq)}$ (3 mL), and MeOH (5 mL) was stirred at room temperature for 5 h. The mixture was purified by column chromatography (EtOAc–*n*-hexane = 4 : 1) to afford **9** (0.19 g, 76%) as a pale orange solid. mp 130 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 2.19 (t, J = 7.5 Hz, 2H), 3.82 (s, 3H), 3.98 (t, J = 7.5 Hz, 2H), 6.30 (d, J = 16.0 Hz, 1H), 6.64 (s, 1H), 6.88 (s, 1H), 6.95 (d, J = 9.0 Hz, 2H), 7.39 (d, J = 16.0 Hz, 1H), 7.57 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO): 28.50, 53.09, 55.76, 112.03, 114.25, 114.53, 118.34, 127.80, 129.50, 134.04, 138.46, 139.14, 141.21, 162.90, 163.28. MS (EI) *m*/*z*: 389 (M^+, 0.31%), 203 (100%). HRMS (EI) for $C_{18}H_{19}O_5N_3S$ (M^+): calcd, 389.1045; found, 389.1048.

*(E)-N-Hydroxy-3-(1-(4-methoxyphenylsulfonyl)-7-nitroindolin-5-yl)acrylamide (**10**).* The title compound was obtained in 37% overall yield from **22a** in a similar manner as that described for the preparation of **9**; mp 165 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.41 (t, J = 7.5 Hz, 2H), 3.81 (s, 3H), 4.04 (t, J = 7.5 Hz, 2H), 6.52 (d, J = 15.5 Hz, 1H), 7.06 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 17.0 Hz, 1H), 7.46 (d, J = 8.5 Hz, 2H), 7.72 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (75 MHz, DMSO): 28.19, 51.81, 55.85, 114.87, 121.54, 122.54, 127.55, 127.86, 129.51, 134.32, 134.81, 135.77, 141.01, 142.24, 162.15, 163.61. HRMS (EI) for $C_{18}H_{17}O_7N_3S$ (M^+): calcd, 419.0787; found, 419.0788.

*(E)-3-(7-Chloro-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-N-hydroxyacrylamide (**11**).* The title compound was obtained in 36% overall yield from **22c** in a similar manner as that described for the preparation of **9**; mp 132 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.31 (t, J = 7.5 Hz, 2H), 3.82 (s, 3H), 3.98 (t, J = 7.5 Hz, 2H), 6.44 (d, J = 16.0 Hz, 1H), 7.06 (d, J = 9.0 Hz, 2H), 7.35 (s, 1H), 7.38 (d, J = 16.0 Hz, 1H), 7.54 (d, J = 9.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO): 29.03, 52.91, 55.81, 114.62, 120.57, 122.20, 126.35, 128.53, 128.59, 129.65, 135.13, 136.34, 140.37, 141.12, 162.31, 163.35. HRMS (EI) for $C_{18}H_{17}O_5N_2ClS$ (M^+): calcd, 408.0547; found, 408.0548.

*(E)-3-(7-Bromo-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-N-hydroxyacrylamide (**12**).* The title compound was obtained in 57% overall yield from **22d** in a similar manner as that described for the preparation of **9**; mp 138 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.30 (t, J = 7.0 Hz, 2H), 3.82 (s, 3H), 3.96 (t, J = 7.0 Hz, 2H), 6.44 (d, J = 16.0 Hz, 1H), 7.01 (d, J = 9.0 Hz, 2H), 7.36–7.39 (m, 2H), 7.53 (d, J = 9.0 Hz, 2H), 7.70 (s, 1H). ^{13}C NMR (75 MHz, DMSO): 29.24, 52.73, 55.82, 114.62, 115.23, 120.58, 122.73, 128.47, 129.74, 131.56, 135.40, 136.25, 141.13, 142.23, 162.32, 163.38. MS (EI) *m*/*z*: 452 (M^+, 5.02%), 214 (100%); HRMS (EI) for $C_{18}H_{17}O_5N_2BrS$ (M^+): calcd, 452.0042; found, 452.0042.

*(E)-N-Hydroxy-3-(7-iodo-1-(4-methoxyphenylsulfonyl)indolin-5-yl)acrylamide (**13**).* The title compound was obtained in 33% overall yield from **22e** in a similar manner as that described for the preparation of **9**; mp 141 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.25 (t, J = 7.0 Hz, 2H), 3.82 (s, 3H), 3.92 (t, J = 7.0 Hz, 2H), 6.42 (d, J = 16.0 Hz, 1H), 7.06 (d, J = 8.5 Hz, 2H), 7.34 (d, J = 16.0 Hz, 1H), 7.38 (s, 1H), 7.50 (d, J = 8.5 Hz, 2H), 7.90 (s, 1H). ^{13}C NMR (75 MHz, DMSO): 29.44, 52.27, 55.83, 89.25, 114.61, 120.32, 123.39, 128.37, 129.80, 135.40, 136.22, 137.84, 139.99, 146.09, 162.33, 163.39. HRMS (EI) for $C_{18}H_{17}O_5N_2IS$ (M^+): calcd, 499.9903; found, 499.9907.

*(E)-N-Hydroxy-3-(1-(4-methoxyphenylsulfonyl)-7-phenylindolin-5-yl)acrylamide (**14**).* A mixture of **28f** (0.31 g, 0.71 mmol), EDC·HCl (0.21 g, 1.07 mmol), HOBt (0.12 g, 0.85 mmol), NMM (0.19 mL, 1.71 mmol), and DMF (1 mL) was stirred at room temperature for 10 min. NH_2OTHP (0.10 g, 0.85 mmol) was added to the mixture, which was stirred at room temperature overnight. The mixture was quenched with water and extracted with ethyl acetate (EA). The organic layer was dried over anhydrous MgSO4 and then filtered. The filtrate was dried under reduced pressure. To the resulting residue was added MeOH (20 mL) and 10% TFA (5 mL), and the mixture was stirred at room temperature for 5 h. The mixture was purified by column chromatography (EtOAc–*n*-hexane = 3 : 1) to afford **14** (0.15 g, 47%) as an orange solid. mp 165 °C. ^{1}H NMR (500 MHz, DMSO-*d*6): δ 2.28 (t, J = 7.5 Hz, 2H), 3.79 (s, 3H), 4.01 (t, J = 7.5 Hz, 2H), 6.46 (d, J = 15.5 Hz, 1H), 6.98 (d, J = 9.0 Hz, 2H), 7.31 (d, J = 9.0 Hz, 2H), 7.29–7.34 (m, 2H), 7.39–7.46 (m, 4H), 7.64 (d, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO): 28.39, 51.88, 55.76, 114.39, 119.39, 122.50, 126.98, 128.07, 128.20, 128.56, 128.99, 129.43, 134.05, 135.12, 137.63, 139.62, 140.01, 140.71, 162.73, 163.13. MS (EI) *m*/*z*: 450 (M^+, 0.34%), 139 (100%). HRMS (EI) for $C_{24}H_{22}O_5N_2S$ (M^+): calcd, 450.1249; found, 450.1248.

*(E)-3-(7-(4-Fluorophenyl)-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-N-hydroxyacrylamide (**15**).* The title compound was obtained in 19% overall yield from **28g** in a similar manner as that described for the preparation of **14**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.35 (t, J = 7.5 Hz, 2H), 3.82 (s, 3H), 4.06 (t, J = 7.5 Hz, 2H), 6.44 (d, J = 16.0 Hz, 1H), 6.92 (d, J = 9.0 Hz, 2H),

7.12 (t, J = 9.0 Hz, 2H), 7.31 (s, 1H), 7.35 (d, J = 9.0 Hz, 2H), 7.45 (s, 1H), 7.55 (d, J = 16.0 Hz, 1H), 7.63–7.65 (m, 2H). ^{13}C NMR (75 MHz, DMSO): 28.37, 51.91, 55.76, 114.40, 114.87, 115.16, 119.47, 122.56, 128.45, 128.89, 129.43, 130.03, 130.13, 131.45, 131.58, 134.01, 134.08, 136.35, 137.53, 139.65, 140.67, 159.81, 162.68, 163.16. MS (EI) m/z: 468 (M^+, 1.24%), 263 (100%). HRMS (EI) for $C_{24}H_{21}O_5N_2FS$ (M^+): calcd, 468.1155; found, 468.1158.

*(E)-N-Hydroxy-3-(1-(4-methoxyphenylsulfonyl)-7-(pyridin-4-yl)-indolin-5-yl)acrylamide (**16**).* The title compound was obtained in 31% overall yield from **28h** in a similar manner as that described for the preparation of **14**; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.27 (t, J = 7.0 Hz, 2H), 3.79 (s, 3H), 4.04 (t, J = 7.0 Hz, 2H), 6.49 (d, J = 16.0 Hz, 1H), 6.98 (d, J = 9.0 Hz, 2H), 7.32 (d, J = 9.0 Hz, 2H), 7.40–7.42 (m, 2H), 7.54 (s, 1H), 7.66 (d, J = 5.5 Hz, 2H), 8.60 (d, J = 5.5 Hz, 2H). ^{13}C (75 MHz, DMSO): 28.22, 51.89, 55.81, 114.50, 119.82, 123.02, 123.71, 128.08, 128.85, 129.48, 132.19, 134.34, 137.34, 139.87, 140.75, 147.66, 149.44, 162.68, 163.28. MS (EI) m/z: 451 (M^+, 0.09%), 236 (100%). HRMS (EI) for $C_{24}H_{22}O_5N_2S$ (M^+): calcd, 451.1202; found, 451.1201.

*(E)-3-(7-Cyano-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-N-hydroxyacrylamide (**17**).* The title compound was obtained in 27% overall yield from **22i** in a similar manner as that described for the preparation of **9**; ^{1}H NMR (500 MHz, CD_3OD): δ 2.40 (t, J = 7.5 Hz, 2H), 3.79 (s, 3H), 4.03 (t, J = 7.5 Hz, 2H), 6.44 (d, J = 16.0 Hz, 1H), 6.96 (d, J = 9.0 Hz, 2H), 7.46 (d, J = 16.0 Hz, 1H), 7.51 (d, J = 9.0 Hz, 2H), 7.58 (s, 1H), 7.76 (s, 1H). ^{13}C (75 MHz, DMSO): 28.04, 52.19, 55.85, 104.85, 114.83, 116.63, 121.08, 127.77, 127.83, 129.61, 132.22, 134.02, 135.75, 139.68, 143.92, 162.16, 163.56. MS (EI) m/z: 389 (M^+, 0.27%), 171 (100%). HRMS (EI) for $C_{19}H_{17}O_5N_3S$ (M^+): calcd, 399.0889; found, 399.0887.

*(E)-N-Hydroxy-3-(7-(3-hydroxy-3-methylbut-1-ynyl)-1-(4-methoxyphenylsulfonyl)indolin-5-yl)acrylamide (**18**).* The title compound was obtained in 7% overall yield from **22j** in a similar manner as that described for the preparation of **9**; ^{1}H NMR (500 MHz, CD_3OD): δ 1.59 (s, 6H), 2.30 (t, J = 7.5 Hz, 2H), 3.83 (s, 3H), 3.98 (t, J = 7.5 Hz, 2H), 6.40 (d, J = 15.5 Hz, 1H), 6.97 (d, J = 9.0 Hz, 2H), 7.29 (s, 1H), 7.44–7.48 (m, 2H), 7.54 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO): 28.27, 31.50, 52.39, 55.79, 63.89, 78.34, 99.36, 114.54, 116.67, 123.52, 128.72, 128.88, 129.55, 131.24, 131.46, 131.59, 132.11, 133.35, 138.97, 163.19. MS (EI) m/z: 456 (M^+, 0.71%), 57 (100%). HRMS (EI) for $C_{23}H_{24}O_6N_2S$ (M^+): calcd, 456.1355; found, 456.1354.

*(E)-3-(7-(Benzyloxy)-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-N-hydroxyacrylamide (**19**).* The title compound was obtained in 31% overall yield from **22k** in a similar manner as that described for the preparation of **9**; mp 151 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.51 (t, J = 7.5 Hz, 2H), 3.80 (s, 3H), 4.00 (t, J = 7.5 Hz, 2H), 5.18 (s, 2H), 6.40 (d, J = 16.0 Hz, 1H), 6.94 (s, 1H), 6.97 (d, J = 9.0 Hz, 2H), 7.12 (s, 1H), 7.13–7.34 (m, 1H), 7.39 (t, J = 7.5 Hz, 2H), 7.51 (d, J = 7.5 Hz, 2H), 7.55 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO): 28.74, 52.78, 55.71, 69.81, 113.01, 114.27, 116.23, 118.96, 127.61, 127.75, 128.35, 129.32, 129.93, 131.79, 134.21, 136.94, 137.96, 139.31, 150.41, 162.75, 162.87. HRMS (EI) for $C_{25}H_{24}O_6N_2S$ (M^+): calcd, 480.1355; found, 480.1353.

*5-Bromo-1-(4-methoxyphenylsulfonyl)-7-nitroindoline (**21**).* To a solution of **20** (2.0 g, 8.23 mmol) in pyridine (10 mL), 4-methoxybenzenesulfonyl chloride (3.4 g, 16.46 mmol) was added, and the mixture was stirred at 95 °C overnight. The mixture was quenched with $NaHCO_{3(aq)}$ and extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by column chromatography (EtOAc–n-hexane = 1 : 2) to afford **21** (2.96 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.70 (t, J = 7.5 Hz, 2H), 3.87 (s, 3H), 4.05 (t, J = 7.5 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 7.50 (s, 1H), 7.66 (d, J = 9.0 Hz, 2H), 7.90 (s, 1H).

*(E)-Methyl 3-(1-(4-methoxyphenylsulfonyl)-7-nitroindolin-5-yl)-acrylate (**22a**).* To a suspension of TEA (1.03 mL, 7.40 mmol), PPh_3 (0.26 g, 0.99 mmol), and $Pd(OAc)_2$ (0.11 g, 0.49 mmol), compound **21** (2.04 g, 4.94 mmol) in DMF (5 mL), methyl acrylate (0.53 mL, 5.92 mmol) was added, and the mixture was stirred at 90 °C overnight. The mixture was cooled to room temperature and filtered through a pad of Celite. The filtrate was extracted with CH_2Cl_2 and $NaHCO_{3(aq)}$. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by column chromatography (EtOAc–n-hexane = 1 : 4) to afford **22a** (1.81 g, 88%) as a yellow solid. mp 155.2 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.70 (t, J = 7.5 Hz, 2H), 3.80 (s, 3H), 3.85 (s, 3H), 4.07 (t, J = 7.5 Hz, 2H), 6.44 (d, J = 16.0 Hz, 1H), 6.92 (d, J = 9.0 Hz, 2H), 7.50 (s, 1H), 7.60 (d, J = 16.0 Hz, 1H), 7.62 (d, J = 9.0 Hz, 2H), 7.86 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 29.12, 52.08, 55.84, 114.56, 120.21, 123.47, 127.75, 128.83, 129.91, 133.28, 136.74, 140.70, 141.69, 142.45, 163.98, 166.75. HRMS (ESI) for $C_{19}H_{18}N_2NaO_7S$ ($M + Na^+$): calcd, 441.0732; found, 441.0711.

*(E)-Methyl 3-(7-amino-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-acrylate (**22b**).* A suspension of **21a** (0.4 g, 0.96 mmol), iron powder (0.16 g, 2.87 mmol), and NH_4Cl (0.1 g, 1.91 mmol) in IPA (9.5 mL) and water (1.9 mL) was heated to reflux for 4 h. The mixture was cooled to room temperature and filtered through a pad of Celite. The filtrate was evaporated under reduced pressure and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by column chromatography (EtOAc–n-hexane = 1 : 3) to afford **22b** (0.3 g, 80%) as a yellow solid. mp 70.4 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.20 (t, J = 7.5 Hz, 2H), 3.79 (s, 3H), 3.83 (s, 3H), 3.99 (t, J = 7.5 Hz, 2H), 6.30 (d, J = 16.0 Hz, 1H), 6.62 (s, 1H), 6.74 (d, J = 1.0 Hz, 1H), 6.85 (d, J = 8.5 Hz, 2H), 7.52 (d, J = 16.0 Hz, 1H), 7.57 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 28.98, 51.75, 53.35, 55.68, 113.77, 114.25, 115.95, 117.45, 128.28, 129.65, 129.88, 133.98, 139.38, 140.54, 144.56, 163.64, 167.53. HRMS (ESI) for $C_{19}H_{21}N2O_5S$ ($M + H^+$): calcd, 389.1171; found, 389.1155.

*(E)-Methyl 3-(7-chloro-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-acrylate (**22c**).* The title compound was obtained in 91% overall yield from **27c** in a similar manner as that described for the preparation of **22d**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.49 (t, J = 7.5 Hz, 2H), 3.80 (s, 3H), 3.85 (s, 3H), 4.03 (t, J = 7.5 Hz, 2H), 6.37 (d, J = 16.0 Hz, 1H), 6.90 (d, J = 9.0 Hz, 2H), 7.17 (s, 1H),

7.43 (s, 1H), 7.56 (d, J = 16.0 Hz, 1H), 7.65 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 29.76, 51.89, 53.24, 55.72, 114.25, 119.25, 122.33, 127.67, 129.57, 129.62, 129.79, 134.17, 140.61, 141.90, 142.66, 163.61, 167.00. HRMS (ESI) for $C_{19}H_{19}ClNO_5S$ ($M + H^+$): calcd, 408.0672; found, 408.0654.

*(E)-Methyl 3-(7-bromo-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-acrylate (**22d**).* To a solution of **27d** (3.45 g, 8.71 mmol) in CH_2Cl_2 (30 mL) was added methyl (triphenylphosphoranylidene)acetate (4.36 g, 13.06 mmol), and then the mixture was stirred at room temperature overnight. The reaction was quenched with H_2O (30 mL) and extracted with CH_2Cl_2. The organic layer was collected and purified by column chromatography (EtOAc–*n*-hexane = 1 : 3) to afford **22d** (3.07 g, 78%) as a white solid. mp 120.2 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.48 (t, J = 7.0 Hz, 2H), 3.81 (s, 3H), 3.86 (s, 3H), 4.01 (t, J = 7.0 Hz, 2H), 6.37 (d, J = 16.0 Hz, 1H), 6.91 (d, J = 9.0 Hz, 2H), 7.21 (s, 1H), 7.56 (d, J = 16.0 Hz, 1H), 7.63 (s, 1H), 7.65 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO): 29.23, 51.58, 52.74, 55.82, 114.63, 115.26, 119.15, 123.42, 128.51, 129.74, 132.77, 134.49, 141.05, 142.45, 143.12, 163.40, 166.45. HRMS (ESI) for $C_{19}H_{19}BrNO_5S$ ($M + H^+$): calcd, 452.0167; found, 452.0151.

*(E)-Methyl 3-(7-iodo-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-acrylate (**22e**).* The title compound was obtained in 93% overall yield from **27e** in a similar manner as that described for the preparation of **22d**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.43 (t, J = 7.0 Hz, 2H), 3.81 (s, 3H), 3.86 (s, 3H), 3.97 (t, J = 7.0 Hz, 2H), 6.37 (d, J = 16.0 Hz, 1H), 6.90 (d, J = 9.0 Hz, 2H), 7.23 (s, 1H), 7.55 (d, J = 16.0 Hz, 1H), 7.63 (d, J = 9.0 Hz, 2H), 7.89 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 30.22, 51.92, 52.71, 55.77, 88.50, 114.29, 118.99, 123.70, 129.14, 130.06, 134.73, 139.31, 139.82, 142.47, 147.52, 163.72, 167.01. HRMS (ESI) for $C_{19}H_{19}INO_5S$ ($M + H^+$): calcd, 500.0029; found, 500.0011.

*(E)-Methyl 3-(7-cyano-1-(4-methoxyphenylsulfonyl)indolin-5-yl)-acrylate (**22i**).* A mixture of **22d** (0.5 g, 1.11 mmol), CuCN (0.32 g, 3.54 mmol), and DMF (2 mL) was stirred at 130 °C for 18 h. The mixture was cooled to room temperature and filtered through a pad of Celite. The filtrate was added H_2O (30 mL) and extracted with EtOAc. The organic layer was collected and purified by column chromatography (EtOAc–*n*-hexane = 1 : 2) to afford **22i** (0.36 g, 82%) as a yellow solid. mp 169.6 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.68 (t, J = 8.0 Hz, 2H), 3.82 (s, 3H), 3.86 (s, 3H), 4.08 (t, J = 8.0 Hz, 2H), 6.40 (d, J = 16.0 Hz, 1H), 6.94 (d, J = 9.0 Hz, 2H), 7.48 (s, 1H), 7.58 (d, J = 16.0 Hz, 1H), 7.67 (s, 1H), 7.73 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 28.69, 51.97, 52.31, 55.78, 105.44, 114.53, 116.54, 119.64, 127.82, 128.88, 129.75, 132.80, 139.09, 141.63, 145.27, 163.85, 166.73. ^{13}C NMR (75 MHz, $CDCl_3$): 28.69, 51.97, 52.31, 55.78, 105.44, 114.53, 116.54, 119.64, 127.82, 128.88, 129.75, 132.80, 139.09, 141.63, 145.27, 163.85, 166.73. HRMS (ESI) for $C_{19}H_{16}N_2NaO_5S$ ($M + Na^+$): calcd, 421.0834; found, 421.0818.

*(E)-Methyl-3-(7-(3-hydroxy-3-methylbut-1-ynyl)-1-(4-methoxyphenylsulfonyl) indolin-5-yl)acrylate (**22j**).* A mixture of **22d** (0.4 g, 0.88 mmol), $Pd(PPh_3)_4$ (0.51 g, 0.44 mmol), CuI (0.08 g, 0.44 mmol), 3-methyl-1-butyn-3-ol (0.43 mL, 4.42 mmol), TEA (1.09 mL, 8.84 mmol), and DMF (3 mL) was heated with the assistance of microwaves at 140 °C for 30 min. The mixture was cooled to room temperature and filtered through a pad of Celite. The filtrate was extracted with CH_2Cl_2 and $NaHCO_{3(aq)}$. The organic layer was collected and purified by column chromatography (EtOAc–*n*-hexane = 1 : 2) to afford **22j** (0.27 g, 67%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.63 (s, 6H), 2.35 (t, J = 7.5 Hz, 2H), 3.78 (s, 3H), 3.81 (s, 3H), 3.99 (t, J = 7.5 Hz, 2H), 6.34 (d, J = 16.0 Hz, 1H), 6.84 (d, J = 8.5 Hz, 2H), 7.16 (s, 1H), 7.45 (d, J = 1.0 Hz, 1H), 7.54 (d, J = 16.0 Hz, 1H), 7.56 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 28.75, 31.17, 51.88, 52.89, 55.71, 65.65, 79.62, 98.95, 114.25, 116.81, 118.35, 123.72, 129.41, 129.68, 132.59, 132.65, 138.37, 143.23, 144.95, 163.52, 167.25. HRMS (ESI) for $C_{24}H_{25}NNaO_6S$ ($M + Na^+$): calcd, 478.1300; found, 473.1280.

*(E)-Methyl 3-(7-(benzyloxy)-1-(4-methoxyphenylsulfonyl)indolin-5-yl)acrylate (**22k**).* A mixture of **32** (0.5 g, 1.05 mmol), $Pd_2(dba)_3$ (0.39 g, 0.42 mmol), P(*o*-tol)$_3$ (0.51 g, 1.69 mmol), TEA (0.59 mL, 4.22 mmol), methyl acrylate (0.14 mL, 1.58 mmol), and CH_3CN (10 mL) was stirred at 90 °C for 2 days. The mixture was cooled to room temperature and filtered through a pad of Celite. The filtrate was extracted with CH_2Cl_2 and $NaHCO_{3(aq)}$ The organic layer was collected and purified by column chromatography (EtOAc–*n*-hexane = 1 : 3) to afford **22k** (0.35 g, 76%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.62 (t, J = 7.5 Hz, 2H), 3.79 (s, 3H), 3.81 (s, 3H), 4.12 (t, J = 7.5 Hz, 2H), 5.17 (s, 2H), 6.28 (d, J = 16.0 Hz, 1H), 6.78 (d, J = 9.0 Hz, 2H), 6.94 (s, 1H), 6.96 (s, 1H), 7.30–7.33 (m, 1H), 7.35–7.39 (m, 2H), 7.48 (d, J = 7.0 Hz, 2H), 7.55 (d, J = 16.0 Hz, 1H), 7.61 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 29.46, 51.76, 53.04, 55.59, 70.99, 113.83, 113.90, 117.28, 117.46, 127.66, 127.96, 128.59, 129.42, 130.96, 133.47, 133.55, 136.59, 139.02, 144.27, 150.62, 163.06, 167.39. HRMS (ESI) for $C_{26}H_{25}NNaO_6S$ ($M + Na^+$): calcd, 502.1300; found, 502.1280.

*Methyl indoline-5-carboxylate (**24**).* To a suspension of methyl **23** (6.0 g, 34.25 mmol) in AcOH (16 mL), $NaBH_3CN$ (4.30 g, 68.50 mmol) was added to the reaction at 0 °C. The mixture was warmed to room temperature and stirred overnight. The mixture was quenched with ice water and basified with concentrated $NaOH_{(aq)}$. The aqueous layer was extracted with EA. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The yellow residue was purified by column chromatography (EtOAc–*n*-hexane = 1 : 5) to afford **24** (5.37 g, 88%). ^{1}H NMR (500 MHz, CD_3OD): δ 2.99 (t, J = 8.5 Hz, 2H), 3.57 (t, J = 8.5 Hz, 2H), 3.79 (s, 3H), 6.50 (d, J = 8.0 Hz, 1H), 7.64 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H).

*Methyl 7-chloroindoline-5-carboxylate (**25c**).* To a solution of **24** (1.0 g, 5.64 mmol) in AcOH (2 mL) in an ice bath was added dropwise a solution of NCS (1.13 g, 8.47 mmol) in dioxane (23 mL). The reaction was warmed to room temperature and stirred overnight. The solvent was removed in vacuum; the residue was basified by 2N NaOH and then was extracted with EtOAc. The organic layer was collected and purified by column chromatography (EtOAc–*n*-hexane = 1 : 6) to afford **25c** (0.36 g, 29%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.16 (t, J = 8.5 Hz, 2H), 3.73 (t, J = 8.5 Hz, 2H), 3.85 (s, 3H), 4.35 (s, 1H), 7.64 (s, 1H), 7.77 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 29.77, 47.06, 51.88, 113.22, 120.53, 120.55, 124.35, 130.00, 152.74, 166.62.

HRMS (ESI) for $C_{10}H_{11}ClNO_2$ ($M + H^+$): calcd, 212.0478; found, 212.0466.

*Methyl 7-bromoindoline-5-carboxylate (**25d**).* The title compound was obtained in 75% overall yield from the reaction of **24** with NBS in a similar manner as that described for the preparation of **25c**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.19 (t, J = 8.5 Hz, 2H), 3.72 (t, J = 8.5 Hz, 2H), 3.85 (s, 3H), 4.35 (s, 1H), 7.67 (s, 1H), 7.91 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 30.10, 46.77, 51.91, 101.18, 120.95, 124.91, 129.65, 132.97, 154.19, 166.47. HRMS (ESI) for $C_{10}H_{11}BrNO_2$ ($M + H^+$): calcd, 255.9973; found, 255.9960.

*Methyl 7-iodoindoline-5-carboxylate (**25e**).* The title compound was obtained in 42% overall yield from the reaction of **24** with NIS in a similar manner as that described for the preparation of **25e**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.19 (t, J = 8.5 Hz, 2H), 3.68 (t, J = 8.5 Hz, 2H), 3.83 (s, 3H), 4.29 (s, 1H), 7.65 (s, 1H), 8.06 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 30.56, 46.14, 51.88, 72.37, 121.33, 125.70, 128.22, 138.86, 157.36, 166.20. HRMS are not available due to the ready decomposition of this compound while being exposed to air.

*Methyl 7-chloro-1-(4-methoxyphenylsulfonyl)indoline-5-carboxylate (**26c**).* The title compound was obtained in 91% overall yield from **25c** in a similar manner as that described for the preparation of **21**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.53 (t, J = 7.5 Hz, 2H), 3.86 (s, 3H), 3.91 (s, 3H), 4.08 (t, J = 7.5 Hz, 2H), 6.90 (d, J = 9.0 Hz, 2H), 7.64 (d, J = 9.0 Hz, 2H), 7.68 (s, 1H), 7.98 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 29.68, 52.52, 53.37, 55.74, 114.31, 124.26, 127.15, 129.47, 129.67, 129.73, 131.39, 140.05, 144.42, 163.65, 165.54. HRMS (ESI) for $C_{17}H_{16}ClNO_5S$ ($M + H^+$): calcd, 382.0516; found, 382.0500.

*Methyl 7-bromo-1-(4-methoxyphenylsulfonyl)indoline-5-carboxylate (**26d**).* The title compound was obtained in 95% overall yield from **25d** in a similar manner as that described for the preparation of **21**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.50 (t, J = 7.5 Hz, 2H), 3.86 (s, 3H), 3.91 (s, 3H), 4.04 (t, J = 7.5 Hz, 2H), 6.90 (d, J = 9.0 Hz, 2H), 7.63 (d, J = 9.0 Hz, 2H), 7.72 (s, 1H), 8.16 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 29.91, 52.55, 53.25, 55.77, 114.32, 115.55, 124.87, 129.47, 129.78, 129.90, 134.58, 140.21, 146.29, 163.72, 165.43. HRMS (ESI) for $C_{17}H_{17}BrNO_5S$ ($M + H^+$): calcd, 426.0011; found, 426.0004.

*Methyl 7-iodo-1-(4-methoxyphenylsulfonyl)indoline-5-carboxylate (**26e**).* The title compound was obtained in 96% overall yield from **25e** in a similar manner as that described for the preparation of **21**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.43 (t, J = 7.5 Hz, 2H), 3.86 (s, 3H), 3.91 (s, 3H), 4.01 (t, J = 7.5 Hz, 2H), 6.90 (d, J = 9.0 Hz, 2H), 7.60 (d, J = 9.0 Hz, 2H), 7.73 (d, J = 1.0 Hz, 1H), 8.43 (d, J = 1.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 30.07, 52.52, 52.80, 55.78, 87.46, 114.35, 125.70, 129.08, 129.91, 130.01, 139.35, 140.99, 149.95, 163.77, 165.27. HRMS (ESI) for $C_{17}H_{16}INO_5S$ ($M + H^+$): calcd, 473.9872; found, 473.9854.

*7-Chloro-1-(4-methoxyphenylsulfonyl)indoline-5-carbaldehyde (**27c**).* The title compound was obtained in 72% overall yield from **26c** in a similar manner as that described for the preparation of **27d**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.63 (t, J = 7.5 Hz, 2H), 3.86 (s, 3H), 4.11 (t, J = 7.5 Hz, 2H), 6.92 (d, J = 9.0 Hz, 2H), 7.56 (s, 1H), 7.68 (d, J = 9.0 Hz, 2H), 7.78 (s, 1H), 9.90 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 29.76, 53.45, 55.80, 114.39, 123.19, 127.87, 129.77, 129.95, 132.57, 135.73, 140.84, 145.78, 163.75, 190.02. HRMS (ESI) for $C_{16}H_{15}ClNO_4S$ ($M + H^+$): calcd, 352.0410; found, 352.0396.

*7-Bromo-1-(4-methoxyphenylsulfonyl)indoline-5-carbaldehyde (**27d**).* A mixture of **26d** (5.06 g, 11.87 mmol), 1 M $LiOH_{(aq)}$ (23.74 mL), and dioxane (100 mL) was stirred at 40 °C overnight. The mixture was concentrated under reduced pressure, and the residue was acidified with 3N $HCl_{(aq)}$. The precipitation was collected by filtration to give a white solid that was subject to the next reaction without purification. The product was used for the next step without further purification. The resulting white solid was dissolved in THF (4.88 g, 11.85 mmol), and then 1 M BH_3–THF (23.7 mL) at 0 °C was added. The mixture was warmed to room temperature and stirred at the same temperature for 4 h. The solvent was evaporated under reduced pressure, and the residue was extracted with EA. The organic layer was collected and purified by column chromatography (EtOAc–*n*-hexane = 2 : 1) to afford a white solid (4.58 g, 97%). To the resulting product (4.58 g, 11.5 mmol) was added CH_2Cl_2 (30 mL), molecular sieves (8.65 g), and PDC (8.65 g, 23.0 mmol); the mixture was then stirred at room temperature for 2 h. The mixture was filtered through a pad of Celite, and the filtrate was purified by column chromatography (EtOAc–*n*-hexane = 1 : 3) to afford **27d** (3.45 g, 75%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.58 (t, J = 7.5 Hz, 2H), 3.88 (s, 1H), 4.07 (t, J = 7.5 Hz, 2H), 6.92 (d, J = 9.0 Hz, 2H), 7.60 (s, 1H), 7.67 (d, J = 9.0 Hz, 2H), 7.97 (s, 1H), 9.90 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 29.85, 53.24, 55.77, 114.37, 116.18, 123.76, 129.47, 129.82, 135.63, 135.97, 141.04, 147.51, 163.76, 189.90. HRMS (ESI) for $C_{16}H_{15}BrNO_4S$ ($M + H^+$): calcd, 395.9905; found, 395.9893.

*7-Iodo-1-(4-methoxyphenylsulfonyl)indoline-5-carbaldehyde (**27e**).* The title compound was obtained in 72% overall yield from **26e** in a similar manner as that described for the preparation of **27d**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.50 (t, J = 7.0 Hz, 2H), 3.87 (s, 3H), 4.02 (t, J = 7.0 Hz, 2H), 6.91 (d, J = 9.0 Hz, 2H), 7.60 (s, 1H), 7.64 (d, J = 9.0 Hz, 2H), 8.22 (s, 1H), 9.88 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 30.03, 52.82, 55.82, 88.14, 114.41, 124.46, 129.12, 130.03, 136.16, 140.23, 142.41, 151.25, 163.86, 189.88. HRMS (ESI) for $C_{16}H_{15}INO_4S$ ($M + H^+$): calcd, 443.9767; found, 443.9751.

*(E)-3-(1-(4-Methoxyphenylsulfonyl)-7-phenylindolin-5-yl)acrylic acid (**28f**).* A mixture of **22d** (0.5 g, 1.11 mmol), $Pd(PPh_3)_4$ (0.63 g, 0.55 mmol), phenylboronic acid (0.27 g, 2.21 mmol), 2 M $K_2CO_{3(aq)}$ (1.66 mL), and DMF (2 mL) was heated with the assistance of microwaves at 140 °C for 30 min. The mixture was cooled to room temperature and filtered through a pad of Celite. The filtrate was extracted with CH_2Cl_2 and $NaHCO_{3(aq)}$. The organic layer was collected and purified by column chromatography (CH_2Cl_2–MeOH = 30 : 1) to afford **28f** (0.31 g, 64%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.42 (t, J = 7.5 Hz, 2H), 3.83 (s, 3H), 4.06 (t, J = 7.5 Hz, 2H), 6.42 (d, J = 16.0 Hz, 1H), 6.80 (d, J = 9.0 Hz, 2H), 7.25 (s, 1H), 7.35–7.37 (m, 3H), 7.45 (t, J = 8.0 Hz, 2H), 7.47 (s, 1H), 7.66 (d, J = 7.0 Hz, 2H), 7.74 (d, J = 16.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): 29.25,

52.43, 55.78, 114.19, 117.55, 123.50, 127.63, 128.34, 128.64, 129.57, 129.75, 130.87, 133.23, 136.29, 139.48, 140.01, 142.71, 146.29, 163.53, 171.98. HRMS (ESI) for $C_{24}H_{20}NO_5S$ ($M - H^+$): calcd, 434.1062; found, 434.1068.

*(E)-3-(7-(4-Fluorophenyl)-1-(4-methoxyphenylsulfonyl)indolin-5-yl)acrylic acid (**28g**).* The title compound was obtained in 66% overall yield from **22d** in a similar manner as that described for the preparation of **28f**; ^{1}H NMR (500 MHz, CD_3OD): δ 2.36 (t, J = 7.5 Hz, 2H), 3.82 (s, 3H), 4.06 (t, J = 7.5 Hz, 2H), 6.46 (d, J = 16.0 Hz, 1H), 6.92 (d, J = 8.5 Hz, 2H), 7.11 (t, J = 8.5 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.37 (s, 1H), 7.46 (s, 1H), 7.63–7.66 (m, 3H). ^{13}C NMR (75 MHz, DMSO): 49.06, 56.04, 60.21, 103.36, 107.16, 110.23, 119.16, 123.11, 124.69, 127.70, 127.86, 128.68, 131.05, 133.73, 134.17, 137.93, 138.03, 139.19, 140.68, 152.54, 162.77, 194.95. HRMS (ESI) for $C_{24}H_{19}FNO_5S$ ($M - H^+$): calcd, 452.0968; found, 452.0975.

*(E)-3-(1-(4-Methoxyphenylsulfonyl)-7-(pyridin-4-yl)indolin-5-yl)-acrylic acid (**28h**).* The title compound was obtained in 78% overall yield from **22d** in a similar manner as that described for the preparation of **28f**; ^{1}H NMR (500 MHz, CD_3OD): δ 2.35 (t, J = 7.5 Hz, 2H), 3.82 (s, 3H), 4.09 (t, J = 7.5 Hz, 2H), 6.52 (d, J = 14.0 Hz, 1H), 6.93 (d, J = 9.0 Hz, 2H), 7.35 (d, J = 9.0 Hz, 2H), 7.48 (s, 1H), 7.59 (s, 1H), 7.67 (d, J = 16.0 Hz, 1H), 7.78 (s, 2H), 8.58 (s, 2H). ^{13}C NMR (75 MHz, DMSO): 28.20, 51.91, 55.84, 114.55, 120.10, 123.04, 124.36, 128.12, 129.50, 129.68, 132.24, 133.80, 139.86, 141.42, 142.80, 147.38, 149.56, 163.32, 167.66.

*7-Benzyloxyindoline (**30**).* To a suspension of **29** (2.5 g, 11.2 mmol) in AcOH (16 mL) at 0 °C, $NaBH_3CN$ (1.41 g, 22.39 mmol) was added. The mixture was warmed to room temperature and stirred overnight. The mixture was quenched with ice water and basified with concentrated $NaOH_{(aq)}$. The aqueous layer was extracted with EA. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The yellow residue was purified by column chromatography (EtOAc–*n*-hexane = 1 : 5) to afford **30** (2.17 g, 86%) as a violet oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.07 (t, J = 8.5 Hz, 2H), 3.58 (t, J = 8.5 Hz, 2H), 3.85 (s, 1H), 5.07 (s, 2H), 6.65–6.72 (m, 2H), 6.80–6.82 (m, 1H), 7.30–7.33 (m, 1H), 7.36–7.39 (m, 2H), 7.43–7.44 (m, 2H).

*7-Benzyloxy-5-bromoindoline (**31**).* To a solution of **30** (1.5 g, 6.66 mmol) in CH_2Cl_2–MeOH (9 mL/3 mL) was added $TBABr_3$ (6.42 g, 13.32 mmol), and the mixture was stirred at room temperature overnight. The mixture was quenched with 5% $NaS_2O_{3(aq)}$ and extracted with CH_2Cl_2. The organic layer was collected and purified by column chromatography (EtOAc–*n*-hexane = 1 : 8) to afford **31** (1.22 g, 60%) as a gray oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.04 (t, J = 8.5 Hz, 2H), 3.57 (t, J = 8.5 Hz, 2H), 3.82 (s, 1H), 5.02 (s, 2H), 6.85 (s, 1H), 6.93 (s, 1H), 7.35–7.36 (m, 1H), 7.38–7.43 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): 30.29, 47.74, 70.47, 109.89, 114.08, 120.58, 127.62, 128.05, 128.11, 128.56, 131.96, 136.60, 140.21, 144.79. HRMS (ESI) for $C_{15}H_{15}BrNO$ ($M + H^+$): calcd, 304.0337; found, 304.0324.

*7-Benzyloxy-5-bromo-1-(4-methoxyphenylsulfonyl)indoline (**32**).* The title compound was obtained in 99% overall yield from **31** in a similar manner as that described for the preparation of **21**; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.50 (t, J = 7.5 Hz, 2H), 3.82 (s, 3H), 4.04 (t, J = 7.5 Hz, 2H), 5.15 (s, 2H), 6.79 (d, J = 9.0 Hz, 2H), 6.88 (s, 1H), 7.00 (s, 1H), 7.31–7.34 (m, 1H), 7.36–7.39 (m, 2H), 7.50 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 29.64, 53.04, 55.62, 71.23, 113.97, 117.13, 119.58, 120.76, 127.76, 128.03, 128.60, 129.59, 130.45, 130.97, 136.34, 140.43, 151.59, 163.15. HRMS (ESI) for $C_{22}H_{20}BrNNaO_4S$ ($M + Na^+$): calcd, 496.0194; found, 496.0178.

(B) Biology

HeLa nuclear extract HDAC activity assay.[12] The HeLa nuclear extract HDAC activity was measured using an HDAC Fluorescent Activity Assay Kit (BioVision, CA) according to the manufacturer's instructions. Briefly, the HDAC fluorometric substrate and assay buffer were added to HeLa nuclear extracts in a 96-well format and incubated at 37 °C for 30 min. The reaction was stopped by adding lysine developer, and the mixture was incubated for another 30 min at 37 °C. Additional negative controls included incubation without the nuclear extract, without the substrate, or without both. Trichostatin A (TSA) at 1 mM served as the positive control. A fluorescence plate reader with excitation at 355 nm and emission at 460 nm was used to quantify HDAC activity.

HDAC biochemical assays.[13] The HDAC *in vitro* activities of recombinant human HDAC 1, 2, 6, and 8 (BPS Biosciences) were detected by fluorigenic release of 7-amino-4-methylcoumarin from the substrate upon deacetylase enzymatic activity.

Cell culture. All human cancer cells were maintained in RPMI 1640 medium containing 100 units mL^{-1} penicillin G sodium, 100 mg mL^{-1} streptomycin sulfate, 0.25 mg mL^{-1} amphotericin B, and 25 mg mL^{-1} gentamicin. The medium was supplemented with 10% fetal bovine serum and 2 mM glutamine. RAW 264.7 murine macrophage cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin sulfate. The cells were cultured in tissue culture flasks in a humidified incubator at 37 °C, in an atmosphere of 5% CO_2 and 95% air.

The sulforhodamine B assays.[14] Human cancer A549 (non-small cell lung cancer), Hep3B (hepatoma), HCT116 (colorectal), and PC-3 (prostate cancer) cells were seeded in 96-well plates in medium with 5% FBS. After 24 h, cells were fixed with 10% trichloroacetic acid (TCA) to represent the cell population at the time of compound addition (T0). After additional incubation of DMSO or test compound for 48 h, cells were fixed with 10% TCA, and SRB at 0.4% (w/v) in 1% acetic acid was added to stain the cells. Unbound SRB was removed with 1% acetic acid, and SRB bound cells were solubilized with 10 mM Trizma base. The absorbance was read at a wavelength of 515 nm. Using the following absorbance measurements, such as time zero (T0), control growth (C), and cell growth in the presence of the compound (Tx), the percentage growth was calculated at each of the compound concentrations levels. Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which was the compound concentration resulting in a 50% reduction in the net protein increase (as

measured by SRB staining) in control cells during the compound incubation.

Western blot analysis.[15] HCT116 cells were treated with a test compound at 1, 3, and 10 μM in RPMI 1640 medium supplemented with 10% FBS for 48 hours. The cells were collected and sonicated. Protein concentrations in the resultant lysates were determined with a Bradford protein assay kit (Bio-Rad, Hercules, CA). The protein lysates, containing the same amount of proteins, were subjected to 10% SDS-polyacrylamide gel (10%) electrophoresis. The proteins on the gel were then transferred onto an Immobilon nitrocellulose membrane (Millipore, Bellerica, MA) using a semi-dry transfer cell. The transblotted membrane was washed twice with Tris-buffered saline containing 0.1% Tween 20 (TBST). After being blocked with TBST containing 5% nonfat milk for 40 min, the membrane was incubated with a primary antibody specific to Acetyl-H3, H3 (Upstate Biotechnology Inc., Lake Placid, NY), in TBST/1% nonfat milk at 4 °C overnight. The membrane was washed three times with TBST for a total of 15 min and then incubated with a goat anti-rabbit or anti-mouse IgG antibody conjugated with horseradish (diluted 1 : 3000) for 1 h at room temperature. After being washed at least three times with TBST, the signal intensity for each protein band was determined.

Antitumor activity in vivo.[16] Male nude mice (NTUH Animal Facility) were 8 weeks old. The animals were fed ad libitum water (reverse osmosis, 1 ppm Cl) and PicoLab Rodent Diet 20 Modified and Irradiated Lab Diet® consisting of 20.0% crude protein, 9.9% crude fat, and 4.7% crude fiber. The mice were housed at the National Taiwan University Laboratory Animal Center, NTUMC, on a 12 hour light cycle at 21–23 °C and 60–85% humidity. Nude mice were maintained in accordance with the Institutional Animal Care and Use Committee procedures and guidelines. Mice were sorted into three groups of 7 mice. All doses were administered at a volume of 10 mL kg^{-1} (0.2 mL/20 g mouse), scaled to the body weight of each animal. Control Group 1 mice received vehicle daily p.o. to endpoint. Group 2 received reference **1** daily p.o. at 200 mg kg^{-1} to endpoint. Group 3 received compound **9** daily p.o. at 100 mg kg^{-1} to the end schedule.

The human HCT116 colorectal adenocarcinoma cells used for implantation were harvested during log phase growth and resuspended in phosphate-buffered saline at 5×10^7 cells mL^{-1}. Each mouse was injected s.c. in the right flank with 1×10^7 cells (0.2 mL cell suspension). Tumor size, in mm^3, was calculated from: Tumor Volume = $(w^2 \times l)/2$, where w = width and l = length in mm of the tumor. Tumor weight can be estimated with the assumption that 1 mg is equivalent to 1 mm^3 of the tumor volume. Mice were sorted into five groups of 7 mice. Animals were weighed daily for the first five days, and then twice weekly until the completion of the study. The mice were examined frequently for overt signs of any adverse, drug-related side effects.

Acknowledgements

This research were supported by the National Science Council, Taiwan (grant no. MOST 103-2113-M-038-001-MY3).

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