European Journal of Medicinal Chemistry 127 (2017) 115–127

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Ring-opened tetrahydro-γ-carbolines display cytotoxicity and selectivity with histone deacetylase isoforms

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ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form
17 December 2016
Accepted 19 December 2016
Available online 21 December 2016

Keywords:
Anticancer agents
Histone deacetylase inhibitors

ABSTRACT

This study is focused on modification of the indole moiety and the N1-zinc binding domain of tubastatin A, and the effects of such changes on biological activity. Fourteen *N*-substituted indoles (**5**–**18**) were synthesized and structure-activity relationship studies indicated that the change of the tetrahydro-γ-carboline in tubastatin A led to substituted indoles (compounds **7**, **11**, and **15**) which showed significant improvements of selective inhibition for HDAC6 over HDAC1 and HDAC2 in comparison to ACY1215, a compound undergoing clinical trials. In addition, attachment of different hydroxamic acid groups, the zinc binding motif at the N1 position, contributes to the antiproliferative activity in cancer cells. Several synthetic compounds exhibited potent growth inhibition in a broad spectrum of tumor cell lines, induced irreversible growth arrest capacities by suppressing colony formation ability and activated the apoptosis pathway. The data provide compelling evidence that our newly synthesized compounds with type B to D hydroxamic acid groups as the zinc binding motif at the N1 position are potent selective inhibitors of HDAC6 and could be investigated preclinically as potential anticancer drugs.

1. Introduction

Histone deacetylase (HDAC) is considered as a potential target for regulation of epigenetic aberrance [1]. HDAC inhibitors have been used mainly in treatment of cancer and since 2007, SAHA, PXD101, FK228, and LBH589 have been approved by US FDA for cancer treatment [2–5]. Eighteen iso-types of HDAC and their functions have been identified which are grouped into four classes (I, II, III, and IV) based on distribution and homology with respect to the original yeast enzymes [6,7]. Histone deacetylase 6 (HDAC6) has been recognized as a promising target for several disorders such as tumorigenesis, inflammation, and neurodegeneration. Currently, two HDAC6 inhibitors, ACY1215 (**1**) and ACY241 (**2**), are in clinical trials [8]. ACY1215 (**1**) was developed as an HDAC6 inhibitor and, in combination with bortezomib, has shown potent inhibitory activity against multiple myeloma [9]. ACY241 (**2**) has been used alone and in combination with pomalidomide and dexamethasone to treat multiple myeloma in a clinical study. Significant attention is now being paid to the development of selective HDAC6 inhibitors (Fig. 1).

Tubastatin A (**3**) was identified as a potent HDAC6 inhibitor with neuroprotective functions (Fig. 1) [10]. HDAC6 belongs to class II HDAC which requires the assistance of zinc ion in its catalytic process. The structure of zinc-dependent HDAC inhibitors comprises three domains: cap, linker group, and zinc binding domain [11]. The 3-methyl-tetrahydro-γ-carboline and hydroxamic acid of tubastatin A function as cap group and zinc binding domain, respectively. Second-generation analogs of tubastatin A with diverse substituents at the indole nitrogen (N3) of the tetrahydro-γ-carboline display improved selective HDAC6 activity and show an immunosuppressive effect with Foxp3+ regulatory T cells [12]. Shen et al. replaced the 3-methyl-tetrahydro-γ-carboline of compound **3** with bicyclic heterocyclic units without changing the

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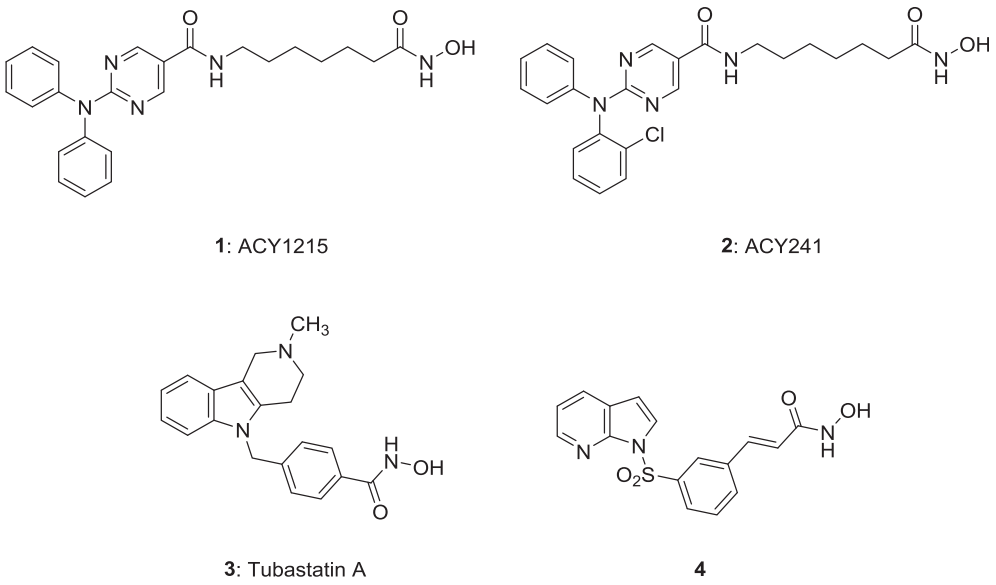


Fig. 1. Structures of selective HDAC6 inhibitors.

linker and the zinc binding domain which resulted in compounds with improved HDAC6 selectivity [13]. Our previous study on azaindolylsulfonamides identified compound **4** as a potent anti-cancer agent with selective HDAC6 activity (Fig. 1) [14]. Literature surveys [12–14] led us to modify the cap of compound **3** using a ring-opening strategy to provide compounds with *N*,*N*-dimethyl-1-(2-methyl-1*H*-indol-3-yl)methanamine, 1-(1*H*-indol-3-yl)-*N*,*N*-dimethylmethanamine, or *N*,*N*-dimethyl-1*H*-indole-3-carboxamide as cap regions in this study. In addition, four types of zinc binding motifs (A-D in Fig. 2) were attached to previously studied caps. As a result, compounds **5**–**18** were developed and their structure-activity relationships has been established in this paper.

2. Results and discussion

2.1. Chemistry

Scheme 1 describes the synthetic approach to compounds **5** and **6**. Substitution of 3-methyl-tetrahydro-γ-carboline (**19**) obtained based on Butler's methodology [10] was carried out using NaH and 3-bromobenzenesulfonyl chloride or 1-bromo-4-(bromomethyl) benzene to give **20a** or **20b**. The Heck reaction between **20a** or **20b** and methyl acrylate gave the corresponding cinnamates (**21a**, **21b**). The resulting products were hydrolyzed in the presence of LiOH to yield the carboxylic acid (**22a**, **22b**) which was subjected to amidation with NH_2OTHP and subsequent deprotection with TFA to

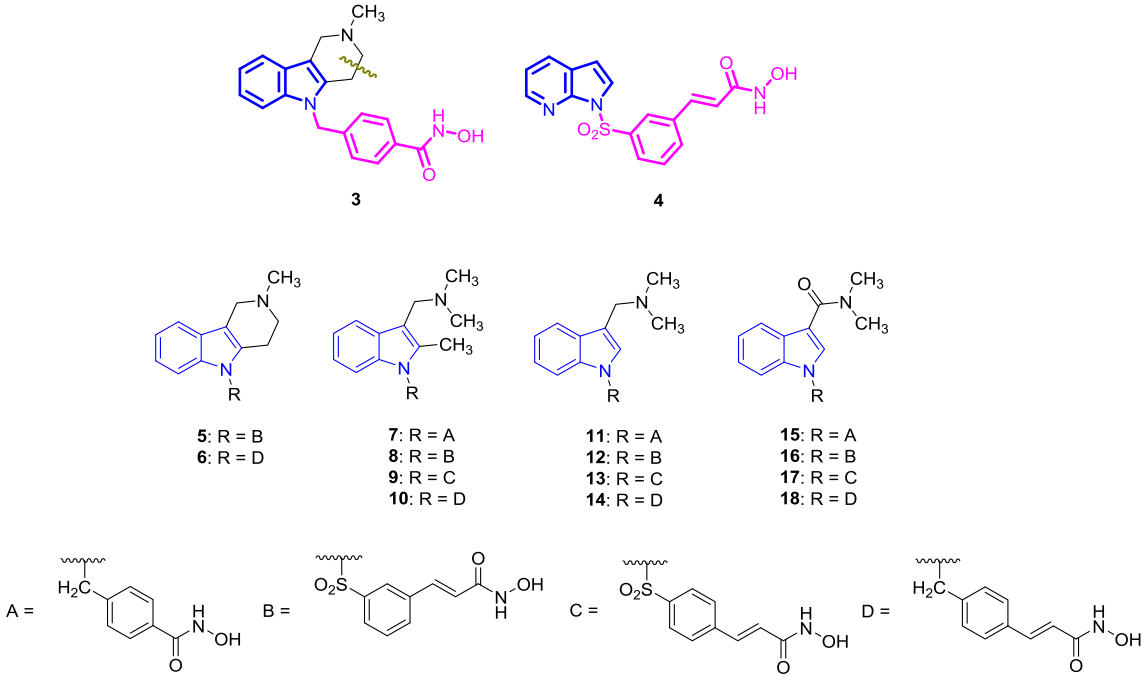
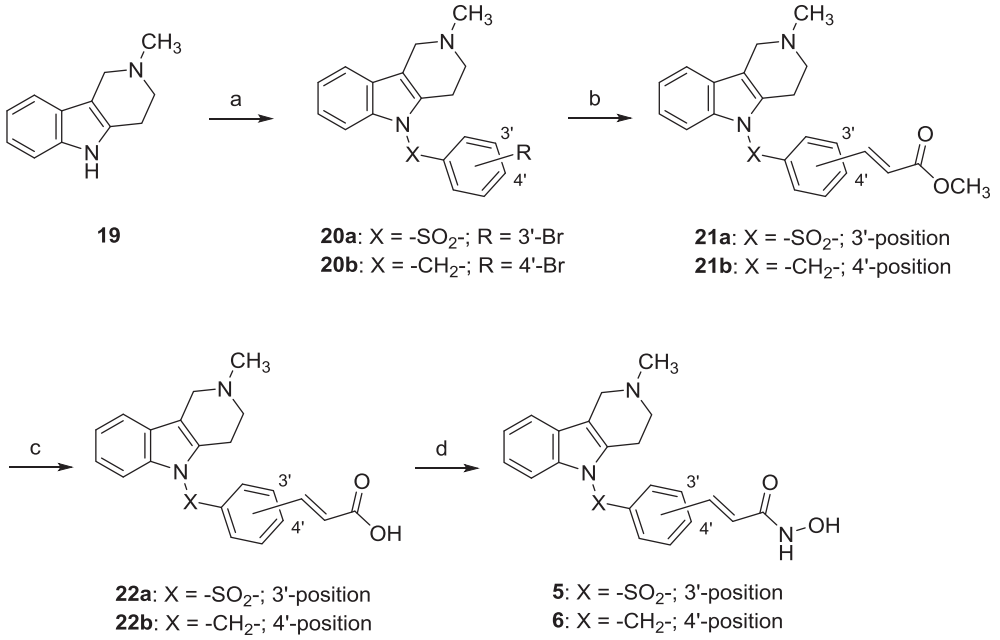


Fig. 2. Designed compounds **5**–**18**.



19

20a: X = -SO_2-; R = 3'-Br
20b: X = -CH_2-; R = 4'-Br

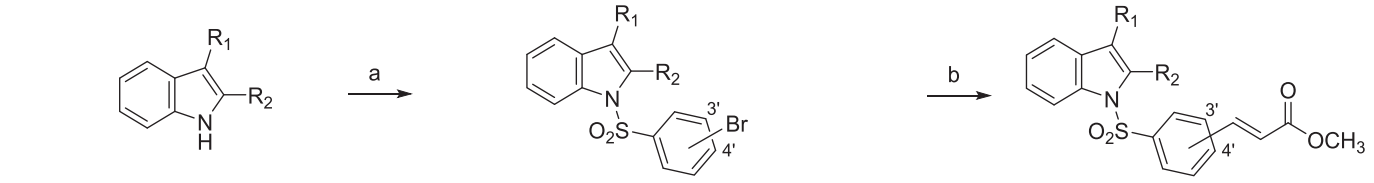
21a: X = -SO_2-; 3'-position
21b: X = -CH_2-; 4'-position

22a: X = -SO_2-; 3'-position
22b: X = -CH_2-; 4'-position

5: X = -SO_2-; 3'-position
6: X = -CH_2-; 4'-position

Scheme 1. Reagents and conditions: (a) 3-bromobenzenesulfonyl chloride or 1-bromo-4-(bromomethyl)benzene, NaH, DMF, rt; (b) $Pd_2(dba)_3$, [(*t*-Bu)$_3$P]BF_4, K_2CO_3, TEA, DMF, 100 °C; (c) 1M LiOH(aq), *p*-dioxane, 40 °C; (d) i. NH_2OTHP, EDC.HCl, HOBt, DIPEA, DMF, rt; ii. 10% TFA(aq), MeOH, rt.

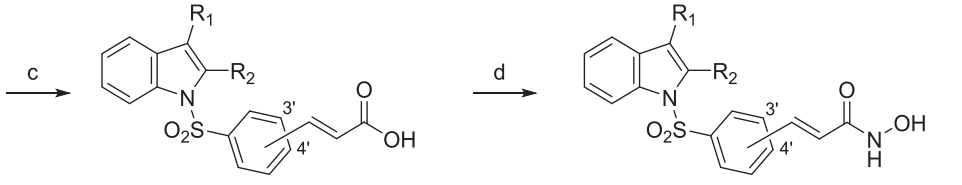
give the anticipated hydroxamic acids (**5**, **6**). Scheme 2 shows the preparation of compounds **8**, **9**, **12**, **13**, **16**, and **17**. Substitution of commercially available 3-formylindoles (**23**, **24**) followed by reductive amination with dimethylamine in the presence of $NaBH(OAc)_3$ gave compounds **26a-26d**. Reaction of **25** which is obtained based on Acheson's methodology [15] with the appropriate



23: R_1 = formyl; R_2 = CH_3
24: R_1 = formyl; R_2 = H
25: R_1 = *N*,*N*-dimethylaminocarbonyl; R_2 = H

26a: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = CH_3; 3'-position
26b: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = CH_3; 4'-position
26c: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = H; 3'-position
26d: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = H; 4'-position
26e: R_1 = *N*,*N*-dimethylaminocarbonyl; R_2 = H; 3'-position
26f: R_1 = *N*,*N*-dimethylaminocarbonyl; R_2 = H; 4'-position

27a-f



28a-f

8: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = CH_3; 3'-position
9: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = CH_3; 4'-position
12: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = H; 3'-position
13: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = H; 4'-position
16: R_1 = *N*,*N*-dimethylaminocarbonyl; R_2 = H; 3'-position
17: R_1 = *N*,*N*-dimethylaminocarbonyl; R_2 = H; 4'-position

Scheme 2. Reagents and conditions: (a) For **26a-d**: i. bromobenzenesulfonyl chlorides, NaH, DMF, rt; ii. dimethylamine, $NaBH(OAc)_3$, CH_2Cl_2, rt. For **26e-f**: bromobenzenesulfonyl chlorides, NaH, DMF, rt; (b) $Pd_2(dba)_3$, [(*t*-Bu)$_3$P]BF_4, K_2CO_3, TEA, DMF, 100 °C; (c) 1M LiOH(aq), *p*-dioxane, 40 °C; (d) i. NH_2OTHP, EDC.HCl, HOBt, DIPEA, DMF, rt; ii. 10% TFA(aq), MeOH, rt.

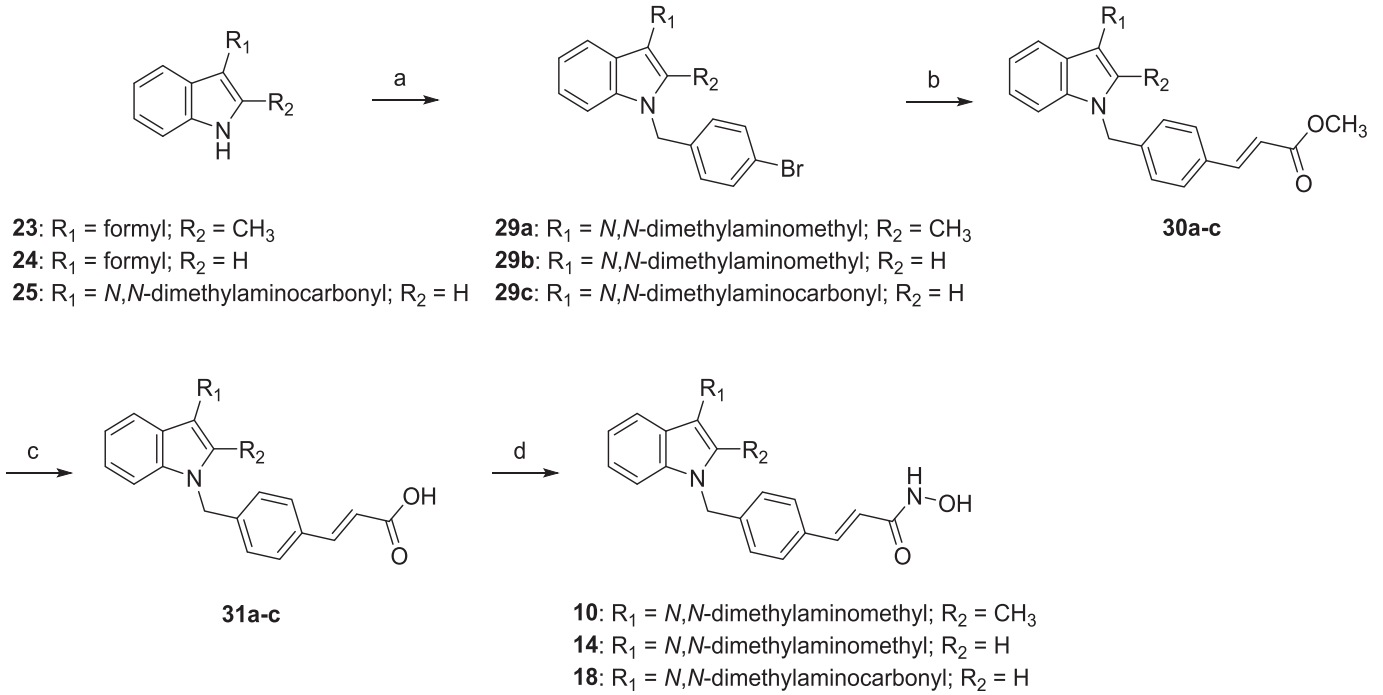
bromobenzenesulfonyl chlorides yielded compounds **26e** and **26f**. The subsequent Heck reaction of **26a-f** with methyl acrylate in the presence of $Pd_2(dba)_3$ and $[(t\text{-}Bu)_3P]BF_4$ yielded the cinnamates **27a-f** which were hydrolyzed to obtain the corresponding carboxylic acids **28a-f**. The resulting products subsequently underwent amidation with NH_2OTHP and TFA-mediated deprotection to afford the anticipated hydroxamic acids (**8**, **9**, **12**, **13**, **16**, and **17**). The synthesis of **10**, **14**, and **18** is shown in Scheme 3. Compounds **23** and **24** underwent substitution with 4-bromobenzyl bromide followed by reductive amination with dimethylamine to afford **29a-b**. Reaction of **25** with 4-bromobenzyl bromide yielded compound **29c**. The resulting products were developed via a synthetic route similar to that shown in Scheme 2, i.e. Heck reaction, ester hydrolysis, amidation with NH_2OTHP, and deprotection with TFA, to yield the designed hydroxamic acids **10**, **14**, and **18**. Compounds **7**, **11**, and **15** lacking a C=C bond between the hydroxamic acid group and the phenyl ring was synthesized based on the approach shown in Scheme 4. Substitution of **23**–**25** with methyl 4-(bromomethyl) benzoate was carried out in the presence of NaH to generate the benzoates **32a-c**, which were subjected to hydrolysis by LiOH to afford the corresponding carboxylic acids **33a-c**. Compounds **33a** and **33b** underwent amidation with NH_2OBn, reductive amination with dimethylamine, and debenzylation, which yielded the designed compounds **7** and **11**. Amidation of compound **33c** with NH_2OBn followed by debenzylation furnished the hydroxamic acid **15**.

2.2. Biological evaluation

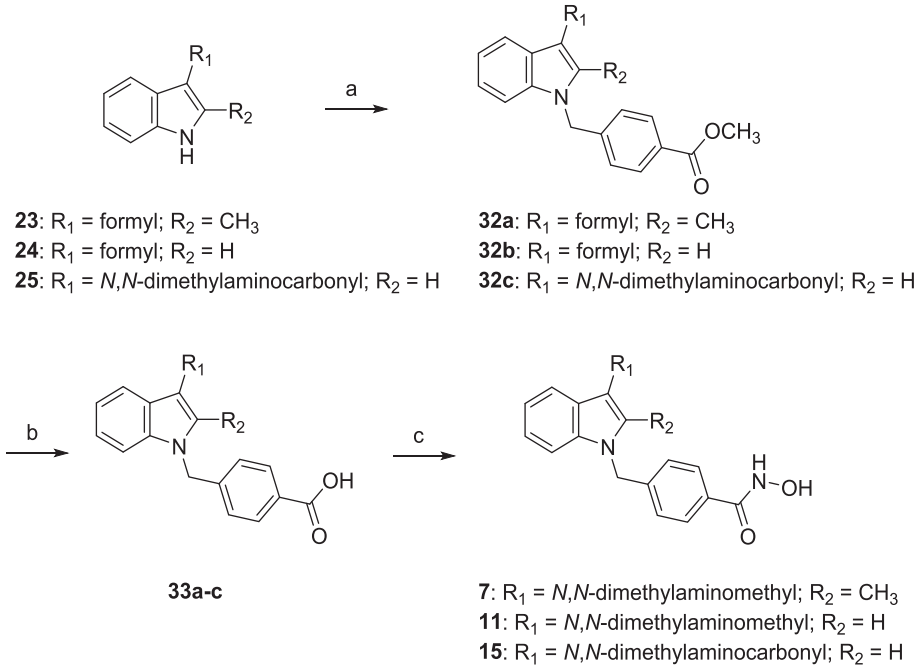
2.2.1. In vitro cell growth inhibitory activity

The ability of synthetic compounds (**5**–**18**) to inhibit the growth of human colorectal HCT116 cells (Fig. 3), human prostate cancer PC3 cells (Fig. 4), and lung adenocarcinoma A549 cells (Fig. 5), was evaluated using compound **3** as a reference compound. The results indicated that compounds with hydroxyaminocarbonyl-*p*-benzyl group (type A hydroxamic acid group; **3**, **7**, **11**, and **15**) exhibited negligible antiproliferative activity against tested cancer cell lines. HCT116 cells were more sensitive than A549 and PC3 cells to compounds **5**–**18** (Figs. 3–5). The data also demonstrated that compounds with a B-, C-and D-type hydroxamic acid group have antiproliferative activity more potent than the compounds with an A-type hydroxamic acid group, indicating the replacement of the type A moiety of compound **3** with B- and D-type hydroxamic acid (**5** and **6**) unit led to improvement of the *in vitro* antitumor activity. Compounds **5** and **6** inhibited the growth of HCT116 cells with GI_{50} values of 0.64 and 0.73 μM, respectively. The attachment of type B, C or D hydroxamic acid groups to *N*,*N*-dimethyl-1-(2-methyl-1*H*-indol-3-yl)methanamine (**8**–**10**), 1-(1*H*-indol-3-yl)-*N*,*N*-dimethylmethanamine (**12**–**14**), or *N*,*N*-dimethyl-1*H*-indole-3-carboxamide (**16**–**18**) also led to enhancement of the antiproliferative activity as compared to compounds bearing A-type hydroxamic acid group (**7**, **11**, and **15**). This result indicated that an N1 hydroxamic acid moiety significantly affects the cellular activity of this series of compounds. The change from rigid tetrahydro-γ-carboline to flexible substituted indoles on the other hand marginally influenced the cellular activity. The antiproliferative activities in three different cell lines (Figs. 3–5) of compounds **5**, **8**, **12**, and **16** revealed that the *N*,*N*-dimethyl-1H-indole-3-carboxamide structure probably results in decrease of cellular activity. It could also be seen that the *N*,*N*-dimethyl-1-(2-methyl-1*H*-indol-3-yl)methanamine and 1-(1*H*-indol-3-yl)-*N*,*N*-dimethylmethan-amine moieties favor the B- and C-type hydroxamic acids, while *N*,*N*-dimethyl-1H-indole-3-carboxamide favors the D-type hydroxamic acid. Collectively, the data suggest that the hydroxamic acid groups at N1 position of substituted indoles play crucial roles in determination of the expression of cytotoxicity.

Based on these results, we chose several compounds from B-type hydroxamic acid moiety (**8** and **12**), C-type hydroxamic acid moiety (**9** and **13**), and D-type hydroxamic acid moiety (**18**) to investigate their effect on the colony forming ability of HCT116 cells. As shown in Fig. 6, the selected compounds show a concentration-dependent decrease in colony forming numbers,



Scheme 3. Reagents and conditions: (a) For **29a-b**: i. 4-bromobenzyl bromide, NaH, DMF, rt; ii. dimethylamine, $NaBH(OAc)_3$, CH_2Cl_2, rt. For **29c**: methyl 4-(bromomethyl)benzoate, NaH, DMF, rt; (b) $Pd_2(dba)_3$, $[(t\text{-}Bu)_3P]BF_4$, K_2CO_3, TEA, DMF, 100 °C; (c) 1M LiOH(aq), *p*-dioxane, 40 °C; (d) i. NH_2OTHP, EDC.HCl, HOBt, DIPEA, DMF, rt; ii. 10% TFA(aq), MeOH, rt.



23: R_1 = formyl; R_2 = CH_3
24: R_1 = formyl; R_2 = H
25: R_1 = *N*,*N*-dimethylaminocarbonyl; R_2 = H

32a: R_1 = formyl; R_2 = CH_3
32b: R_1 = formyl; R_2 = H
32c: R_1 = *N*,*N*-dimethylaminocarbonyl; R_2 = H

33a-c

7: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = CH_3
11: R_1 = *N*,*N*-dimethylaminomethyl; R_2 = H
15: R_1 = *N*,*N*-dimethylaminocarbonyl; R_2 = H

Scheme 4. Reagents and conditions: (a) methyl 4-(bromomethyl)benzoate, NaH, DMF, rt; (b) 1 M LiOH(aq), *p*-dioxane, 40 °C; (c) For **7**and **11**: i. $NH_2OBn.HCl$, EDC.HCl, HOBt, DIPEA, DMF, rt; ii. dimethylamine, $NaBH(OAc)_3$, CH_2Cl_2, rt; 10% Pd/C, H_2, MeOH, rt. For **15**: i. $NH_2OBn.HCl$, EDC.HCl, HOBt, DIPEA, DMF, rt; ii. 10% Pd/C, H_2, MeOH, rt.

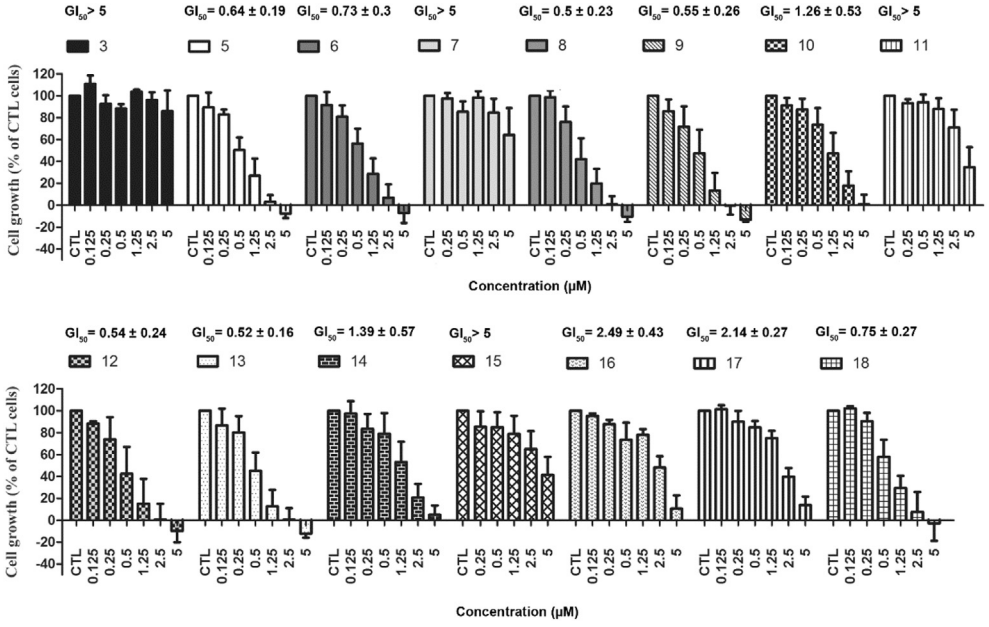


Fig. 3. Antiproliferative activity (GI_{50}) of tested compounds against human colorectalHCT116 cells.

indicating the potential of these compounds to induce irreversible growth arrest capacity in HCT116 cells.

2.2.2. HDAC isoform inhibition

In order to examine the HDAC6 selective inhibitory activity of our compounds, all the synthetic compounds (**5**–**18**), compound **4**, and tubastatin A (**3**) were assayed for their activities on HDAC1, HDAC2, and HDAC6 (Table 1). Compounds **7** and **15** showed slightly more selective HDAC6 activity than compound **3**, and compound **11** displayed selective HDAC6 activity comparable to that of compound **3**. Compounds **7**, **11**, and **15** showed remarkably more HDAC6 selectivity than our previously synthesized compound **4**. This result indicates that the A-type hydroxamic acid plays a vital role, contributing to the HDAC6 selectivity. In addition, the strategy of ring-opening of the tetrahydro-γ-carboline of compound **3** provides potent selective HDAC6 inhibitors. Although replacement of A-type hydroxamic acid moieties with B-, C-, and D-types resulted in a decrease in HDAC6 selectivity, most of this series of compounds showed better HDAC6 selectivity than ACY1215. Taken together the results of cellular and enzymatic activity, it is assumed that A-type hydroxamic acid is significant for HDAC6 selectivity while it causes loss of antiproliferative activity. In addition, B-, C-, and D-types of

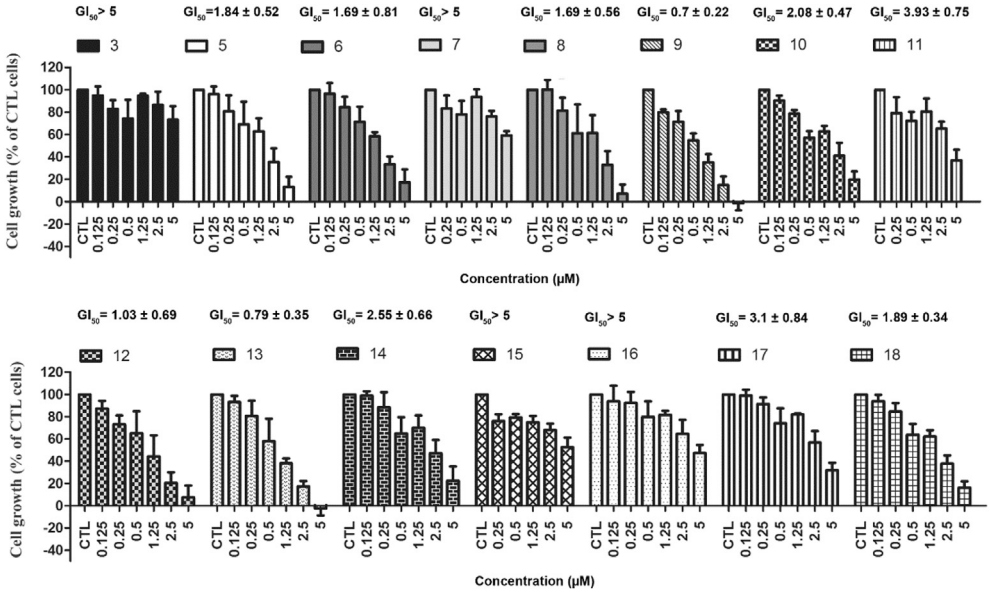


Fig. 4. Antiproliferative activity (GI50) of tested compounds against human prostate cancer PC3 cells.

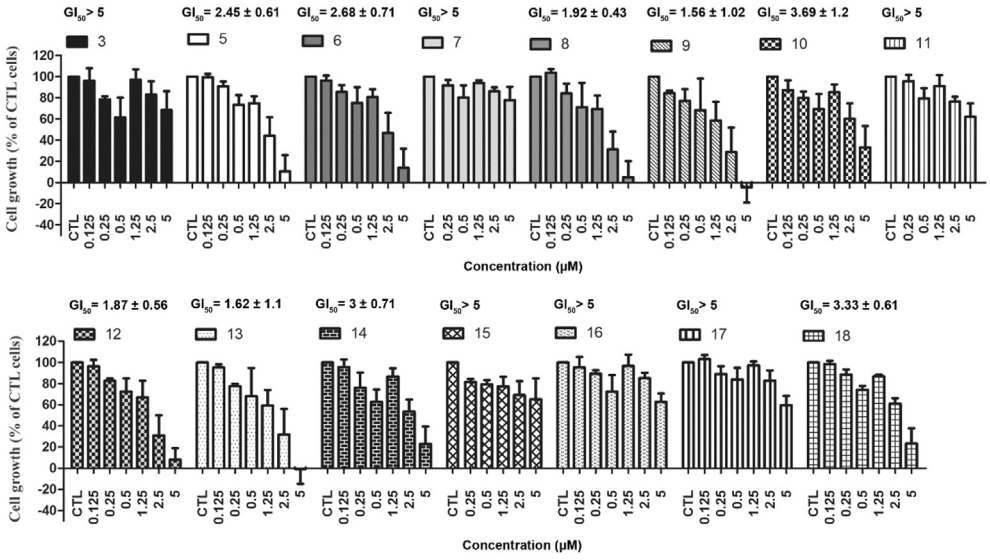


Fig. 5. Antiproliferative activity (GI_{50}) of tested compounds against lung carcinoma A549 cells.

hydroxamic acid are beneficial for cellular activity and have slight effect on HDAC6 selectivity.

2.2.3. *Western blotting and flow cytometry*

The biomarkers of HDAC inhibition are acetylation of histone and non-histone proteins. Class I HDAC enzymatic inhibition results in hyperacetylation of histone H3 and HDAC6 inhibition could lead to an increase in acetylated tubulin. To confirm the HDAC isozyme selectivity, we performed western blot analysis to evaluate the protein levels of HDAC inhibition biomarkers, acetyl-histone H3 and acetyl-tubulin, in HCT116 cells treated with different compounds. The synthetic compounds (**8**, **9**, **12**, **13**, and **18**), which have been evaluated in previous colony formation assay, exhibited stronger inhibition of the HDAC6 enzyme, resulting in upregulation of acetyl-tubulin without significant induction of acetyl-histone H3 (Fig. 7A).

To investigate the underlying mechanism of cell growth repression by these compounds, the effects of compounds on cell cycle progression were assessed by flow cytometry. As shown in Fig. 7B and C, treatment with 1.25 μM or 2.5 μM of the selected compounds for 48 h caused accumulation of HCT116 cells subG1 phase in a dose-dependent manner and exposure to higher concentrations (5 μM) of compound **3** for 48 h had a similar effect (Fig. 7B), indicating that synthetic compounds (**8**, **9**, **12**, **13**, and **18**) exerted a more potent cell cycle deregulation effect than tubastatin A (**3**). In addition, the selected compounds (**8**, **9**, **12**, **13**, and **18**) also activate apoptotic markers PARP and caspase 3 whereas tubastatin A failed to cause apparent cytotoxicity to HCT116 cells (Fig. 7D). These results indicated that the novel synthetic compounds (**8**, **9**, **12**, **13**, and **18**) exhibit a broad spectrum of antitumor activities with selectivity for HDAC6 over HDAC1 and HDAC2.

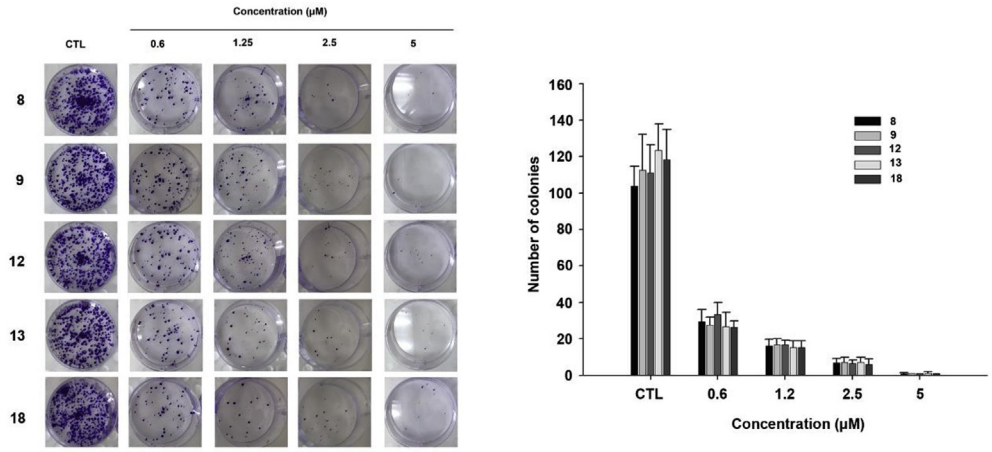


Fig. 6. Colony formation ability of compounds in HCT116 cells. (A) The compounds selected suppressed colony formation and the numbers of colonies were determined and are shown in (B). Clonogenic survival was assessed as described in the Experimental section.

Table 1
HDAC inhibition Activity and isoform selectivity of compounds **4**–**18**.

compd	IC50(M ± SD)[a]			Selectivity ratio	
	HDAC1	HDAC2	HDAC6	HDAC1/HDAC6	HDAC2/HDAC6
4[b]	3.10×10^{-7}	1.16×10^{-6}	5.20×10^{-9}	60	223
5	2.70×10^{-6}	3.13×10^{-6}	1.89×10^{-7}	14	16
6	1.60×10^{-6}	1.65×10^{-6}	4.18×10^{-8}	38	39
7	9.73×10^{-6}	$>1.0 \times 10^{-5}$	6.17×10^{-9}	1576	>1620
8	1.85×10^{-6}	1.34×10^{-6}	8.49×10^{-8}	21	15
9	4.01×10^{-6}	1.27×10^{-6}	6.30×10^{-8}	63	20
10	2.13×10^{-6}	2.38×10^{-6}	9.64×10^{-8}	22	24
11	7.13×10^{-6}	1.22×10^{-5}	2.17×10^{-8}	328	562
12	1.51×10^{-6}	1.64×10^{-6}	8.93×10^{-8}	16	18
13	1.93×10^{-6}	9.70×10^{-7}	1.67×10^{-8}	115	58
14	2.25×10^{-6}	1.12×10^{-6}	4.31×10^{-8}	52	25
15	5.72×10^{-6}	$>1.0 \times 10^{-5}$	8.01×10^{-9}	714	>1248
16	6.91×10^{-6}	$>1.0 \times 10^{-5}$	2.01×10^{-7}	34	>49
17	2.01×10^{-6}	1.46×10^{-6}	1.08×10^{-8}	186	135
18	6.27×10^{-7}	1.04×10^{-6}	8.50 × 10-9	73	122
Tubastatin A	$>1.0 \times 10^{-5}$	$>1.0 \times 10^{-5}$	1.37×10^{-8}	>729	>729
ACY1215[c]	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 IC50mode with 3-fold serial dilution starting at 10 μM.
[b] Data from ref [14].
[c] Datafrom ref [9].

3. Conclusion

This study modified the cap of tubastatin A using a ring-opening strategy, which gave three substituted indoles (*N*,*N*-dimethyl-1-(2-methyl-1*H*-indol-3-yl)methanamine, 1-(1*H*-indol-3-yl)-*N*,*N*-dimethylmethanamine, and *N*,*N*-dimethyl-1*H*-indole-3-carboxamide). Meanwhile, four types of zinc binding moieties (A-D) were bound to the preceding compounds, and their biological effects were examined. Structure-activity relationship studies revealed that compounds **7**, **11**, and **15** with substituted indoles as the cap motif also showed selective HDAC6 activity, comparable to that of tubastatin A (**3**). The replacement of A-type hydroxamic acid group with B-, C-, or D-type groups led to enhancement of anti-proliferative activity; for example, in compounds **8**, **9**, **12**, **13**, and **18**, and activation of apoptosis. All compounds in this study displayed better HDAC6 selectivity than the compound in clinical trials (ACY1215). This study led to novel HDAC inhibitors exhibiting not only potent antiproliferative activity but also HDAC6 selectivity.

4. Experimental section

4.1. Chemistry

Nuclear magnetic resonance (^{1}H and ^{13}CNMR) spectra were obtained with Bruker Fourier 300 NMR spectrometers, and are reported as chemical shifts in parts per million (ppm, δ) downfield from TMS as an internal standard. High-resolution mass spectra (HRMS) were recorded with a Finnigan MAT 95S mass spectrometer. The purities of the final compounds were determined using a Hitachi 2000 series HPLC system using an Agilent Zorbax Eclipse XDB-C_{18} column (5 μm, 4.6 mm × 150 mm) with the solvent system consisting of acetonitrile (mobile phase A) and water containing 0.1% formic acid and 10 mmol NH_4OAc (mobile phase B), and were 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.

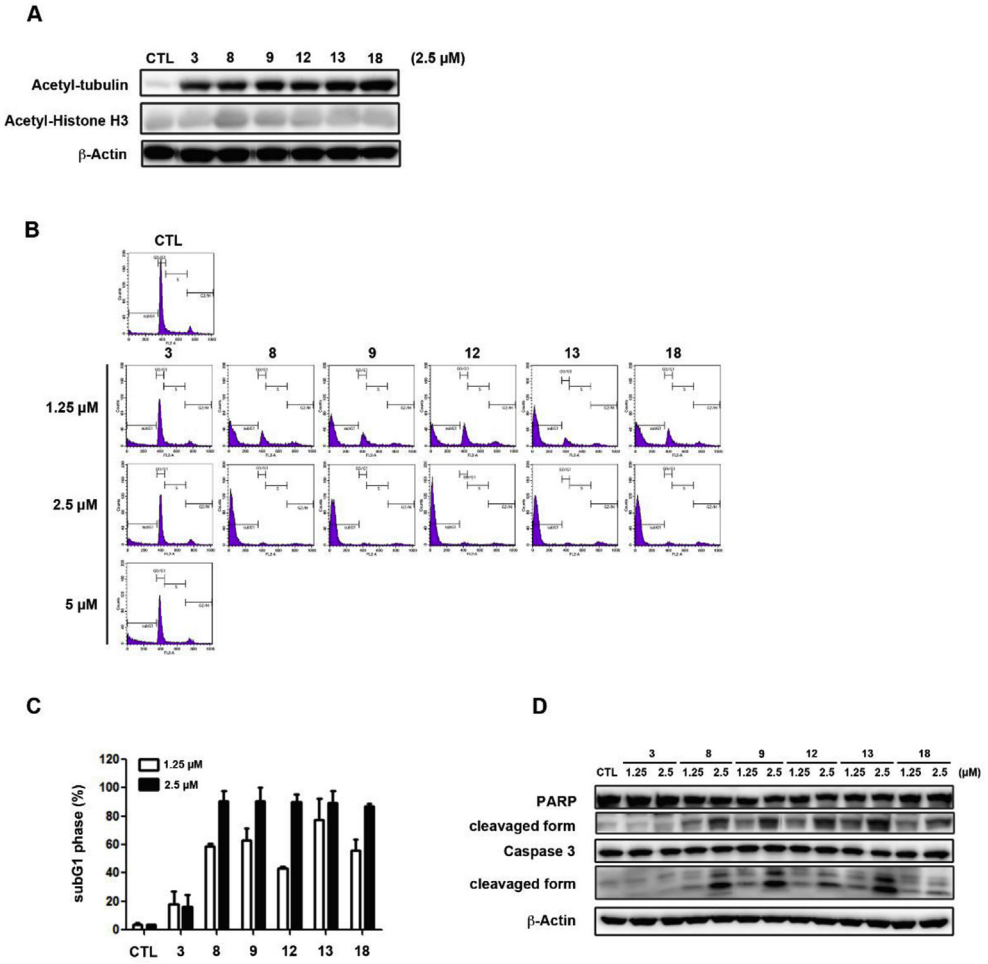


Fig. 7. Effect of selected compounds on HDAC inhibition biomarkers, cell cycle distribution, and activation of apoptosis. (A)Accumulation of acetyltubulin after 6 h treatment with selected compounds. (B) Sub-G1 phase induction and (C) the mean percentage of subG1 fraction populations with 1.25 μM and 2.5 μM of compounds for 48 h in HCT116 cells. (D) Apoptosis induction with different concentrations of compounds for 48 h in HCT116 cells. Cells were treated with indicated concentrations for indicated times, then assessed by flow cytometry or western blot analysis as described in Materials and Methods.

*4.1.1. N-Hydroxy-3-[3-(2-methyl-1,2,3,4-tetrahydro-pyrido[4,3-b] indole-5-sulfonyl)-phenyl]-acrylamide (**5**)*

A mixture of compound **22a** (0.55 g, 1.4 mmol), EDC.HCl (0.32 g, 2.1 mmol), HOBt (0.22 g, 1.7 mmol) and DIPEA (0.48 mL, 2.8 mmol) in DMF (5.0 mL) was stirred at room temperature for 30 min before adding NH_2OTHP (0.19 g, 1.7 mmol). After being stirred for a further 5 h, the reaction mixture was quenched with water and extracted with EtOAc (50 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$ and was purified by silica gel chromatography (EtOAc) to give a semisolid residue. The resulting product was dissolved in CH_3OH (10 mL) and to this was added 10% $TFA_{(aq)}$ (5 mL). The reaction was stirred at room temperature for 5 h and the reaction mixture was concentrated under reduced pressure to give a white precipitate which was recrystallized from CH_3OH to afford compound **5** in 52% yield; HPLC Purity: 97.98% (t_R = 19.26 min). mp: 224–225 °C. ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.52 (s, 3H), 2.73 (bs, 2H), 3.18 (bs, 2H), 4.02 (s, 2H), 6.63 (d, J = 15.9 Hz, 1H), 7.29 (t, J = 7.5 Hz 1H), 7.38 (t, J = 7.5 Hz, 1H), 7.45–7.54 (m, 2H), 7.60 (t, J = 8.1 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 8.04 (d, J = 8.4 Hz, 2H), 9.18 (s, 1H, NH, D_2O exchangeable proton), 10.92 (s, 1H, NH, D_2O exchangeable proton). ^{13}CNMR (75 MHz, DMSO-d_6) δ 20.93, 43.15, 49.26, 50.81, 113.72, 118.44, 122.17, 123.74, 124.67, 125.17, 126.63, 127.29, 130.47, 132.12, 134.67, 134.98, 135.59, 136.46, 137.78, 161.73. HRMS (ESI) for $C_{21}H_{20}N_3O_4S$ ($M\text{-}H^+$): calcd, 410.1175; found, 410.1171.

*4.1.2. (E)-3-(4-((1,2,3,4-Tetrahydro-2-methylpyrido[4,3-b]indol-5-yl)methyl)phenyl)-N-hydroxyacrylamide (**6**)*

The title compound was obtained in 51% yield from compound **22b** in a manner similar to that described for the synthesis of compound **5**; HPLC purity: 97.56% (t_R = 16.25 min). mp: 213–214 °C. ^{1}HNMR (300 MHz, CD_3OD) δ 2.79 (s, 3H), 2.98 (t, J = 6.0 Hz, 2H), 3.23 (t, J = 6.0 Hz, 2H), 4.09 (s, 2H), 5.37 (s, 2H), 6. 42 (d, J = 15.9 Hz, 1H), 7.04 (d, J = 8.1 Hz, 2H), 7.08–7.17 (m, 2H), 7.32 (d, J = 7.5 Hz, 1H), 7.44–7.54 (m, 4H). ^{13}CNMR (75 MHz, DMSO-d_6) 21.27, 40.91, 46.41, 47.17, 48.72, 111.73, 116.48, 120.09, 121.79, 122.76, 124.67, 125.18, 128.48, 129.94, 130.67, 132.98, 133.73, 134.48, 135.76, 159.75. HRMS (ESI) for $C_{22}H_{22}N_3O_2$ ($M\text{-}H^+$): calcd, 360.1712; found, 360.1707.

*4.1.3. 4-((3-((Dimethylamino)methyl)-2-methyl-1H-indol-1-yl) methyl)-N-hydroxybenzamide (**7**)*

To the stirred solution of compound **33a** (0.5 g, 1.2 mmol) in CH_3OH (10 mL) was added a catalytic amount of 10% palladium on carbon and the reaction mixture was stirred at room temperature for 6 h under hydrogen. The reaction mixture was filtered over celite and the filtrate was dried in vacuum to afford compound **7** in 75% yield; HPLC purity: 95.05% (t_R = 19.04 min). mp: 130–131 °C.

^{1}HNMR (300 MHz, DMSO-d_6) δ 2.18 (s, 6H), 2.33 (s, 3H), 3.57 (s, 2H), 5.48 (s, 2H), 6.99–7.01 (m, 4H), 7.34 (m, 1H), 7.57 (dd, J = 3.0, 6.9 Hz, 1H), 7.66 (d, J = 8.1 Hz, 2H). ^{13}CNMR (75 MHz, DMSO-d_6) δ 7.98, 42.15, 43.31, 50.60, 105.70, 107.14, 116.21, 116.81, 118.41, 123.44, 123.78, 126.03, 127.33, 129.52, 133.18, 133.79, 139.49, 161.67. HRMS (ESI) for $C_{20}H_{22}N_3O_2$ (M-H$^+$): calcd, 336.1712; found, 336.1709.

4.1.4. 3-[3-(3-Dimethylaminomethyl-2-methyl-indole-1-sulfonyl)-phenyl]-N-hydroxy-acrylamide (**8**)

The title compound was obtained in 58% yield from compound **28a** in a manner similar to that described for the synthesis of compound **5**. HPLC purity: 97.18% (t_R = 14.51 min). mp: 180–181 °C. ^{1}HNMR (300 MHz, CD_3OD) δ 2.76 (s, 3H), 2.81 (s, 6H), 4.41 (s, 2H), 6.53 (d, J = 15.6 Hz, 1H), 7.36–7.47 (m, 2H), 7.51–7.68 (m, 2H), 7.69 (d, J = 6.9 Hz, 1H), 7.84–7.85 (m, 2H), 8.02 (bs, 1H), 8.27 (d, J = 8.1 Hz, 1H). ^{13}CNMR (75 MHz, CD_3OD) δ 9.65, 39.34, 47.59, 108.30, 111.74, 115.74, 117.72, 121.45, 122.24, 122.42, 124.11, 126.46, 127.50, 129.88, 133.39, 133.97, 134.73, 136.45, 136.55, 161.09. HRMS (ESI) for $C_{21}H_{22}N_3O_4S$ (M-H$^+$): calcd, 412.1331; found, 412.1329.

4.1.5. 3-[4-(3-Dimethylaminomethyl-2-methyl-indole-1-sulfonyl)-phenyl]-N-hydroxy-acrylamide (**9**)

The title compound was obtained in 58% yield from compound **28b** in a manner similar to that described for the synthesis of compound **5**. HPLC purity: 98.07% (t_R = 17.04 min). mp: 191–192 °C. ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.71 (s, 9H), 4.39 (s, 2H), 6.59 (d, J = 15.9 Hz, 1H), 7.36–7.41 (m, 2H), 7.46 (d, J = 15.9 Hz, 1H), 7.75 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 7.2 Hz, 1H) 7.93 (d, J = 8.4 Hz, 2H), 8.11 (d, J = 7.5 Hz, 1H), 9.17 (s, 1H, D_2O exchangeable proton), 10.95 (s, 1H, D_2O exchangeable proton). ^{13}CNMR (75 MHz, CD_3OD) δ 9.84, 39.25, 47.43, 107.57, 111.69, 112.27, 115.84, 116.16, 118.96, 121.51, 122.26, 124.13, 125.74 126.41, 133.20, 134.79, 135.60, 136.95, 138.05, 158.88. HRMS (ESI) for $C_{21}H_{22}N_3O_4S$ (M-H$^+$): calcd, 412.1331; found, 412.1330.

4.1.6. (E)-3-(4-((3-((Dimethylamino)methyl)-2-methyl-1H-indol-1-yl)methyl)phenyl)-N-hydroxyacrylamide (**10**)

The title compound was obtained in 52% yield from compound **31a** in a manner similar to that described for the synthesis of compound **5**. HPLC purity: 97.05% (t_R = 16.99 min). mp: 174–175 °C. ^{1}HNMR (300 MHz, CD_3OD) δ 2.44 (s, 3H) 2.90 (s, 6H), 4.53 (s, 2H), 5.41 (s, 2H), 6.46 (d, J = 15.6 Hz, 1H), 6.97–7.05 (m, 2H) 7.16–7.18 (m, 2H), 7.32 (d, J = 6.0 Hz, 1H), 7.39–7.49 (m, 3H), 7.71 (d, J = 6.3 Hz, 1H). ^{13}CNMR (75 MHz, CD_3OD) δ 6.86, 38.59, 43.30, 48.77, 97.88, 106.97, 114.91, 117.85, 119.26, 123.78, 124.98, 125.26, 125.48, 131.36, 131.70, 134.09, 136.53, 136.69, 162.21. HRMS (ESI) for $C_{22}H_{24}N_3O_2$ (M-H$^+$): calcd, 362.1869; found, 362.1866.

4.1.7. 4-((3-((Dimethylamino)methyl)-1H-indol-1-yl)methyl)-N-hydroxybenzamide (**11**)

The title compound was obtained in 72% yield from compound **33b** in a manner similar to that described for the synthesis of compound **7**. HPLC purity: 98.70% (t_R = 18.69 min). mp: 171–172 °C. ^{1}HNMR (300 MHz, CD_3OD) δ 2.37 (s, 6H), 3.81 (s, 2H), 5.48 (s, 2H), 7.09–7.18 (m, 2H), 7.22 (d, J = 8.1 Hz, 2H), 7.30–7.34 (m, 2H), 7.67–7.70 (m, 3H). ^{13}CNMR (75 MHz, CD_3OD) δ 40.57, 46.29, 50.14, 106.73, 106.96, 112.55, 115.99, 116.71, 118.68, 123.96, 124.41, 125.24, 125.91, 126.43, 133.85, 139.28, 163.62. HRMS (ESI) for $C_{19}H_{20}N_3O_2$ (M-H$^+$): calcd, 322.1556; found, 322.1553.

4.1.8. 3-[3-(3-Dimethylaminomethyl-indole-1-sulfonyl)-phenyl]-N-hydroxy-acrylamide (**12**)

The title compound was obtained in 53% yield from compound **28c** in a manner similar to that described for the synthesis of compound **5**. HPLC purity: 97.54% (t_R = 17.01 min). mp: 160–161 °C. ^{1}HNMR (300 MHz, CD_3OD) δ 2.52 (s, 6H), 4.00 (s, 2H), 6.55 (d, J = 15.9 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 7.42 (t, J = 8.1 Hz, 1H), 7.52–7.58 (m, 2H), 7.71 (d, J = 7.8 Hz, 1H), 7.80–7.85 (m, 2H), 7.94 (d, J = 7.2 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 8.11 (s, 1H). ^{13}CNMR (75 MHz, CD_3OD) δ 40.27, 49.19, 110.79, 113.57, 116.96, 117.66, 121.01, 122.44, 122.83, 124.55, 124.63, 127.25, 127.69, 129.82, 132.44, 133.87, 134.79, 135.74, 161.19. HRMS (ESI) for $C_{20}H_{20}N_3O_4S$ (M-H$^+$): calcd, 398.1175; found, 398.1167.

4.1.9. 3-[4-(3-Dimethylaminomethyl-indole-1-sulfonyl)-phenyl]-N-hydroxy-acrylamide (**13**)

The title compound was obtained in 58% yield from compound **28d** in a manner similar to that described for the synthesis of compound **5**; HPLC purity: 98.66% (t_R = 18.40 min). mp: 179–180 °C. ^{1}HNMR (300 MHz, CD_3OD) δ 2.58 (s, 6H), 4.08 (s, 2H), 6.56 (d, J = 15.6 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 7.39 (t, J = 7.2 Hz, 1H), 7.47 (d, J = 15.6 Hz, 1H), 7.62 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 7.8 Hz, 1H), 7.88 (s, 1H), 7.93 (d, J = 8.1 Hz, 2H), 8.01 (d, J = 8.4 Hz, 1H). ^{13}CNMR (75 MHz, DMSO-d_6) δ 41.20, 49.51, 111.03, 118.43, 121.23, 121.47, 123.07, 125.05, 125.90, 126.36, 127.06, 128.02, 132.13, 133.65, 134.34, 138.72, 164.86. HRMS (ESI) for $C_{20}H_{20}N_3O_4S$ (M-H$^+$): calcd, 398.1175; found, 398.1168.

4.1.10. (E)-3-(4-((3-((Dimethylamino)methyl)-1H-indol-1-yl)methyl)phenyl)-N-hydroxyacrylamide (**14**)

The title compound was obtained in 55% yield from compound **31b** in a manner similar to that described for the synthesis of compound **5**; HPLC purity: 99.14% (t_R = 19.14 min). mp: 168–169 °C. ^{1}HNMR (300 MHz, CD_3OD) δ 2.86 (s, 6H), 4.50 (s, 2H), 5.46 (s, 2H), 6.48 (d, J = 15.9 Hz, 1H), 7.18–7.24 (m, 4H), 7.41 (d, J = 6.9 Hz, 1H), 7.46–7.54 (m, 3H), 7.62 (s, 1H), 7.77 (dd, J = 2.7, 6.3 Hz, 1H). ^{13}CNMR (75 MHz, CD_3OD) δ 38.49, 46.67, 49.30, 100.58, 107.68, 114.68, 115.51, 117.83, 119.83, 124.12, 124.39, 125.14, 125.20, 128.77, 131.73, 134.00, 136.85, 161.62. HRMS (ESI) for $C_{21}H_{22}N_3O_2$ (M-H$^+$): calcd, 348.1712; found, 348.1714.

4.1.11. 1-(4-(Hydroxycarbamoyl)benzyl)-N,N-dimethyl-1H-indole-3-carboxamide (**15**)

The title compounds was obtained in 70% yield from compound **33c** in a manner similar to that described for the synthesis of compound **7**. HPLC purity: 98.12% (t_R = 17.06 min). mp: 197–198 °C. ^{1}HNMR (300 MHz, DMSO-d_6) δ 3.12 (s, 6H), 5.54 (s, 2H), 7.10–7.20 (m, 2H), 7.31 (d, J = 8.1 Hz, 2H), 7.48 (dd, J = 1.2, 6.9 Hz, 1H), 7.70 (d, J = 8.1 Hz, 2H). 7.82 (dd, J = 1.8, 8.1 Hz, 1H), 8.00 (s, 1H). ^{13}CNMR (75 MHz, DMSO-d_6) δ 37.06, 46.80, 107.47, 108.39, 118.33, 118.98, 119.95, 124.85, 125.10, 129.23, 129.90, 133.20, 138.55, 161.75, 163.54. HRMS (ESI) for $C_{19}H_{18}N_3O_3$ (M-H$^+$): calcd, 336.1348; found, 336.1345.

4.1.12. 1-[3-(2-Hydroxycarbamoyl-vinyl)-benzenesulfonyl]-1H-indole-3-carboxylic acid dimethylamide (**16**)

The title compound was obtained in 53% yield from compound **28e** in a manner similar to that described for the synthesis of compound **5**. HPLC purity: 97.41% (t_R = 17.09 min). mp: 129–130 °C. ^{1}HNMR (300 MHz, CD_3OD) δ 3.15 (s, 6H), 6.56 (d, J = 15.9 Hz, 1H), 7.34 (t, J = 7.2 Hz, 1H), 7.43 (t, J = 7.2 Hz, 1H), 7.53 (d, J = 15.9 Hz, 1H), 7.63–7.71 (m, 3H), 8.01–8.04 (m, 4H). ^{13}CNMR (75 MHz, CD_3OD) δ 46.75, 107.00, 107.57, 114.66, 117.93, 118.13, 119.75, 124.33, 124.52, 124.91, 125.12, 128.30, 131.69, 133.37, 136.43, 136.91, 162.14, 165.71. HRMS (ESI) for $C_{20}H_{18}N_3O_5S$: (M-H$^+$): calcd, 412.0967; found, 412.0963.

4.1.13. 1-[4-(2-Hydroxycarbamoyl-vinyl)-benzenesulfonyl]-1H-indole-3-carboxylic acid dimethylamide (**17**)

The title compound was obtained in 59% yield from compound

28f in a manner similar to that described for the synthesis of compound **5**. HPLC purity: 99.12% (t_R = 18.71 min). mp: 141–142 °C. ^{1}HNMR (300 MHz, DMSO-d_6) δ 3.06 (s, 6H), 6.58 (d, J = 15.9 Hz, 1H), 7.33 (t, J = 7.2 Hz, 1H), 7.40–7.47 (m, 2H), 7.68 (d, J = 7.5 Hz, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 8.7 Hz, 2H), 8.13 (s, 1H). ^{13}CNMR (75 MHz, DMSO-d_6) δ 38.16, 110.95, 115.07, 119.38, 121.90, 123.29, 124.48, 125.40, 126.43, 126.60, 131.42, 133.51, 134.20, 134.24, 139.00, 159. 42, 161.45. HRMS (ESI) for $C_{20}H_{18}N_3O_5S$: (M-H$^+$): calcd, 412.0967; found, 412.0966.

4.1.14. 1-(4-((E)-2-(Hydroxycarbamoyl)vinyl)benzyl)-N,N-dimethyl-1H-indole-3-carboxamide (**18**)

The title compound was obtained in 54% yield from compound **31c** in a manner similar to that described for the synthesis of compound **5**; HPLC purity: 95.19% (t_R = 17.42 min). mp: 180–181 °C. ^{1}HNMR (300 MHz, CD_3OD) δ 3.22 (s, 6H), 5.50 (s, 2H), 6.45 (d, J = 15.9 Hz, 1H), 7.19–7.25 (m, 4H), 7.40 (m, 1H), 7.50–7.58 (m, 3H), 7.77–7.81 (m, 2H). ^{13}CNMR (75 MHz, CD_3OD) δ 44.12, 46.75, 107.00, 107.57, 114.66, 117.93, 118.13, 119.75, 124.33, 124.52, 125.12, 128.30, 131.69, 133.37, 136.43, 136.91, 162.14, 165.71. HRMS (ESI) for $C_{21}H_{20}N_3O_3$: (M-H$^+$): calcd, 362.1505; found, 362.1502.

4.1.15. 5-(3-Bromo-benzenesulfonyl)-2-methyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (**20a**)

To a mixture of NaH (0.12 g, 3.0 mmol), **19** (0.5 g, 2.6 mmol), and DMF (3 mL), 3-bromobenzenesulfonyl chloride (0.75 g, 3.0 mmol) was added dropwise and stirred at room temperature for 2.5 h. The reaction was quenched with water and then extracted with EtOAc (50 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (EtOAc: n-hexane = 4: 1) to give compound **20a** in 80% yield; ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.57 (s, 3H), 2.85 (t, J = 5.7 Hz, 2H), 3.19 (t, J = 5.7 Hz, 2H), 3. 59 (s, 2H), 7.32–7.35 (m, 2H), 7.64–7.70 (m, 3H), 8.00 (t, J = 3.0 Hz, 1H), 8.12–8.15 (m, 2H).

4.1.16. 5-(4-Bromobenzyl)-2,3,4,5-tetrahydro-2-methyl-1H-pyrido[4,3-b]indole (**20b**)

The title compound was obtained in 74% yield from compound **19** by using 4-bromobenzyl bromide in a manner similar to that described for the synthesis of compound **20a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.62 (s, 3H), 2.81–2.85 (m, 4H), 3.81 (s, 2H), 5.29 (s, 2H), 7.07 (d, J = 8.1 Hz, 2H), 7.09–7.23 (m, 3H), 7.43 (d, J = 8.1 Hz, 2H), 7.47 (m, 1H).

4.1.17. 3-[3-(2-Methyl-1,2,3,4-tetrahydro-pyrido [4,3-b]indole-5-sulfonyl)-phenyl]-acrylic acid methyl ester (**21a**)

A mixture of **20a** (0.8 g, 2.0 mmol), tris(dibenzylideneacetone) dipalladium (0.36 g, 0.39 mmol), tri-*t*-butylphosphonium tetrafluoroborate (0.23 g, 0.79 mmol), K_2CO_3 (0.55 g, 4.0 mmol), TEA (0.68 mL, 4.9 mmol), methyl acrylate (0.26 mL, 3.0 mmol), and DMF (15 mL) was stirred at 100 °C for 12 h. The reaction was quenched with water and extracted with EtOAc (50 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (EtOAc) to give compound **21a** in 83% yield; ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.47 (s, 3H), 2.75 (t, J = 6.0 Hz, 2H), 3.13 (t, J = 6.0 Hz, 2H), 3.48 (bs, 2H), 3.76 (s, 3H), 6.37 (d, J = 15.9 Hz, 1H), 7.18–7.35 (m, 3H), 7.38–7.59 (m, 3H), 7.89–7.97 (m, 2H), 8.09 (d, J = 8.1 Hz, 1H).

4.1.18. (E)-Methyl 3-(4-((1,2,3,4-tetrahydro-2-methylpyrido[4,3-b]indol-5-yl)methyl)phenyl)acrylate (**21b**)

The title compound was obtained in 74% yield from compound **20b** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.60 (s, 3H), 2.86–2.87 (m, 4H), 3.79 (s, 2H), 3.82 (s, 3H), 5.27 (s, 2H), 6.40 (d, J = 16.2 Hz, 1H), 7.01 (d, J = 8.1 Hz, 2H), 7.11–7.20 (m, 3H), 7.41 (d, J = 8.1 Hz, 2H), 7.49 (m, 1H), 7.65 (d, J = 16.2 Hz, 1H).

4.1.19. 3-[3-(2-Methyl-1,2,3,4-tetrahydro-pyrido[4,3-b]indole-5-sulfonyl)-phenyl]-acrylic acid (**22a**)

A mixture of **21a** (0.6 g, 1.5 mmol), 1 M $LiOH_{(aq)}$ (6.0 mL), and dioxane (15 mL) was stirred at 40 °C for 2 h. The reaction was concentrated under reduced pressure and water was added. The mixture was acidified with 3 N HCl and extracted with EtOAc (50 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield compound **22a** in 94% yield; ^{1}HNMR (300 MHz, CD_3OD) δ 2.51 (s, 3H), 2.83 (t, J = 6.0 Hz, 2H), 3.21 (t, J = 6.0 Hz, 2H), 3.57 (bs, 2H), 6.42 (d, J = 15.9 Hz, 1H), 7.19–7.31 (m, 3H), 7.35–7.57 (m, 3H), 7.87–7.99 (m, 2H), 8.11 (d, J = 8.1 Hz, 1H).

4.1.20. (E)-3-(4-((1,2,3,4-Tetrahydro-2-methylpyrido[4,3-b]indol-5-yl)methyl)phenyl)acrylic acid (**22b**)

The title compound was obtained in 96% yield from compound **21b** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 2.75 (s, 3H), 2.92 (t, J = 6.0 Hz, 2H), 3.19 (t, J = 6.0 Hz, 2H), 4.07 (s, 2H), 5.31 (s, 2H), 6.41 (d, J = 15.9 Hz, 1H), 7.01–7.23 (m, 4H), 7.29 (d, J = 7.5 Hz, 2H), 7.37–7.43 (m, 3H).

4.1.21. [1-(3-Bromo-benzenesulfonyl)-2-methyl-1H-indol-3-ylmethyl]-dimethyl-amine (**26a**)

A mixture of **23** (0.5 g, 3.1 mmol), NaH (0.15 g, 3.7 mmol), DMF (3 mL), and 3-bromobenzenesulfonyl chloride (0.8 g, 3.2 mmol) was added dropwise and stirred at room temperature for 2.5 h. The reaction was quenched with water and then extracted with EtOAc (50 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting residue was dissolved in DCM (25 mL), and then sodium triacetoxyborohydride (1.0 g, 4.7 mmol) and dimethyl amine (5 M solution in EtOH, 1.0 mL, 5.0 mmol) were added and stirred at room temperature for 12 h. The reaction mixture was quenched with water and was extracted with DCM (50 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$ and purified by silica gel chromatography to give compound **26a** in 68% yield; ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.18 (s, 6H), 2.54 (s, 3H) 3.39 (s, 2H), 7.19–7.23 (m, 2H), 7.36–7.53 (m, 4H), 7.69 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 7.8 Hz, 1H).

4.1.22. [1-(4-Bromo-benzenesulfonyl)-2-methyl-1H-indol-3-ylmethyl]-dimethyl-amine (**26b**)

The title compounds was obtained in 71% yield from compound **23** in a manner similar to that described for the synthesis of compound **26a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.29 (s, 6H), 2.41 (s, 3H), 3.59 (s, 2H), 7.24–7.41 (m, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.67 (m, 1H), 7.78 (d, J = 8.7 Hz, 2H), 8.01 (m, 1H).

4.1.23. [1-(3-Bromo-benzenesulfonyl)-1H-indol-3-ylmethyl]-dimethyl-amine (**26c**)

The title compound was obtained in 65% yield from compound **24** in a manner similar to that described for the synthesis of compound **26a.** ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.29 (s, 6H), 3.59 (s, 2H), 7.35–7.37 (m, 2H), 7.38–7.47 (m, 2H), 7.65–7.67 (m, 2H), 7.89 (d, J = 1.2 Hz, 1H), 8.00–8.03 (m, 2H).

4.1.24. [1-(4-Bromo-benzenesulfonyl)-1H-indol-3-ylmethyl]-dimethyl-amine (**26d**)

The title compound was obtained in 68% yield from compound

24 in a manner similar to that described for the synthesis of compound **26a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.28 (s, 6H), 3.56 (s, 2H), 7.26–7.39 (m, 2H), 7.47 (s, 1H), 7.58 (d, J = 8.7 Hz, 2H), 7.65 (m, 1H), 7.74 (d, J = 8.7 Hz, 2H), 7.97 (m, 1H).

*4.1.25. 1-(3-Bromo-benzenesulfonyl)-1H-indole-3-carboxylic acid dimethylamide (**26e**)*

The title compound was obtained in 80% yield from compound **24** in a manner similar to that described for the synthesis of compound **20a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 3.16 (s, 6H), 7.17–7.39 (m, 3H), 7.42–7.48 (m, 3H), 7.79 (m, 1H), 7.82–7.89 (m, 2H).

*4.1.26. 1-(4-Bromo-benzenesulfonyl)-1H-indole-3-carboxylic acid dimethylamide (**26f**)*

The title compound was obtained in 82% yield from compound **25** in a manner similar to that described for the synthesis of compound **20a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 3.18 (s, 6H), 7.25–7.32 (m, 2H), 7.49 (d, J = 8.1 Hz, 2H), 7.58 (m, 1H), 7.75 (d, J = 8.1 Hz, 2H), 7.79–7.85 (m, 2H).

*4.1.27. 3-[3-(3-Dimethylaminomethyl-2-methyl-indole-1-sulfonyl)-phenyl]-acrylic acid methyl ester (**27a**)*

The title compound was obtained in 65% yield from compound **26a** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.11 (s, 6H), 2.52 (s, 3H), 3.37 (s, 2H), 3.71 (s, 3H), 6.35 (d, J = 16.2 Hz, 1H), 7.17–7.21 (m, 2H), 7.39–7.52 (m, 5H), 7.66 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 7.8 Hz, 1H).

*4.1.28. 3-[4-(3-Dimethylaminomethyl-2-methyl-indole-1-sulfonyl)-phenyl]-acrylic acid methyl ester (**27b**)*

The title compound was obtained in 67% yield from compound **26b** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.22 (s, 6H), 2.54 (s, 3H), 3.37 (s, 2H), 3.71 (s, 3H), 6.35 (d, J = 16.2 Hz, 1H), 7.17–7.21 (m, 2H), 7.39–7.52 (m, 4H), 7.66 (d, J = 8.4 Hz, 2H), 8.12 (d, J = 7.8 Hz, 1H).

*4.1.29. 3-[3-(3-Dimethylaminomethyl-indole-1-sulfonyl)-phenyl]-acrylic acid methyl ester (**26c**)*

The title compound was obtained in 62% yield from compound **26c** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.29 (s, 6H), 3.59 (s, 2H), 3.84 (s, 3H), 6.44 (d, J = 15.9 Hz, 1H), 7.35–7.37 (m, 2H), 7.38–7.47 (m, 2H), 7.65–7.67 (m, 3H), 7.89 (d, J = 1.2 Hz, 1H), 8.00–8.03 (m, 2H).

*4.1.30. 3-[4-(3-Dimethylaminomethyl-indole-1-sulfonyl)-phenyl]-acrylic acid methyl ester (**27d**)*

The title compound was obtained in 65% yield from compound **26d** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.28 (s, 6H), 3.56 (s, 2H), 3.82 (s, 3H), 6.45 (d, J = 15.9 Hz, 1H), 7.24–7.38 (m, 2H), 7.50–7.58 (m, 5H), 7.64 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 8.1 Hz, 1H).

*4.1.31. 3-[3-(3-Dimethylcarbamoyl-indole-1-sulfonyl)-phenyl]-acrylic acid methyl ester (**27e**)*

The title compound was obtained in 64% yield from compound **26e** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 3.16 (s, 6H), 3.85 (s, 3H), 6.48 (d, J = 16.2 Hz, 1H), 7.29–7.54 (m, 3H), 7.62–7.68 (m, 4H), 7.91 (m, 1H), 7.92–8.00 (m, 2H).

*4.1.32. 3-[4-(3-Dimethylcarbamoyl-indole-1-sulfonyl)-phenyl]-acrylic acid methyl ester (**27f**)*

The title compound was obtained in 67% yield from compound **26f** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 3.16 (s, 6H), 3.82 (s, 3H), 6.47 (d, J = 16.2 Hz, 1H), 7.35–7.42 (m, 2H), 7.60 (d, J = 8.1 Hz, 2H), 7.62–7.77 (m, 2H), 7.95 (d, J = 8.1 Hz, 2H), 7.99–8.01 (m, 2H).

*4.1.33. 3-[3-(3-Dimethylaminomethyl-2-methyl-indole-1-sulfonyl)-phenyl]-acrylic acid (**28a**)*

The title compound was obtained in 95% yield from compound **27a** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 2.79 (s, 3H), 2.89 (s, 6H), 4.51 (s, 2H), 6.53 (d, J = 16.2 Hz, 1H), 7.40–7.46 (m, 2H), 7.60–7.71 (m, 3H), 7.89–7.91 (m, 2H), 8.06 (m, 1H), 8.28 (m, 1H).

*4.1.34. 3-[4-(3-Dimethylaminomethyl-2-methyl-indole-1-sulfonyl)-phenyl]-acrylic acid (**28b**)*

The title compound was obtained in 95% yield from compound **27b** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 2.56 (s, 3H), 2.89 (s, 6H), 4.59 (s, 2H), 6.67 (d, J = 16.2 Hz, 1H), 7.37–7.82 (m, 4H), 8.06 (d, J = 8.4 Hz, 2H), 8.10–8.11 (m, 2H), 8.29 (s, 1H).

*4.1.35. 3-[3-(3-Dimethylaminomethyl-indole-1-sulfonyl)-phenyl]-acrylic acid (**28c**)*

The title compound was obtained in 92% yield from compound **27c** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 2.91 (s, 6H), 4.54 (s, 2H), 6.55 (d, J = 16.2 Hz, 1H), 7.42–7.7.49 (m, 2H), 7.60–7.71 (m, 4H), 7.90–7.93 (m, 2H), 8.09 (m, 1H), 8.31 (m, 1H).

*4.1.36. 3-[4-(3-Dimethylaminomethyl-indole-1-sulfonyl)-phenyl]-acrylic acid (**28d**)*

The title compound was obtained in 90% yield from compound **27d** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 2.88 (s, 6H), 4.54 (s, 2H), 6.65 (d, J = 16.2 Hz, 1H), 7.39–7.47 (m, 2H), 7.56 (d, J = 16.2 Hz, 1H), 7.74–7.80 (m, 2H), 8.02 (d, J = 8.4 Hz, 2H), 8.08–8.10 (m, 2H), 8.27 (s, 1H).

*4.1.37. 3-[3-(3-Dimethylcarbamoyl-indole-1-sulfonyl)-phenyl]-acrylic acid (**28e**)*

The title compound was obtained in 94% yield from compound **27e** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 3.21 (s, 6H), 6.58 (d, J = 16.2 Hz, 1H), 7.39–7.59 (m, 3H), 7.68–7.79 (m, 4H), 7.98 (m, 1H), 8.00–8.11 (m, 2H).

*4.1.38. 3-[4-(3-Dimethylcarbamoyl-indole-1-sulfonyl)-phenyl]-acrylic acid (**28f**)*

The title compound was obtained in 90% yield from compound **27f** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 3.26 (s, 6H), 6.59 (d, J = 16.2 Hz, 1H), 7.39–7.48 (m, 2H), 7.66 (d, J = 8.1 Hz, 2H), 7.69–7.81 (m, 2H), 7.99 (d, J = 8.1 Hz, 2H), 8.01–8.18 (m, 2H).

*4.1.39. (1-(4-Bromobenzyl)-2-methyl-1H-indol-3-yl)-N,N-dimethylmethanamine (**29a**)*

The title compound was obtained in 68% yield from compound **23** in a manner similar to that described for the synthesis of compound **26a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.39 (s, 6H), 2.41 (s, 3H), 3.75 (s, 2H), 5.34 (s, 2H), 6.98 (d, J = 8.1 Hz, 2H), 7.15 (m, 3H), 7.43 (d, J = 8.1 Hz, 2H), 7.70 (m, 1H).

*4.1.40. (1-(4-bromobenzyl)-1H-indol-3-yl)-N,N-dimethylmethanamine (**29b**)*

The title compounds was obtained in 70% yield from compound **24** in a manner similar to that described for the synthesis of

compound **26a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.39 (s, 6H), 3.65 (s, 2H), 5.37 (s, 2H), 7.15–7.26 (m, 6H), 7.48 (d, J = 8.1 Hz, 2H), 7.71 (m, 1H).

*4.1.41. 1-(4-Bromobenzyl)-N,N-dimethyl-1H-indole-3-carboxamide (**29c**)*

The title compound was obtained in 84% yield from compound **25** in a manner similar to that described for the synthesis of **26e**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 3.21 (s, 6H), 5.43 (s, 2H), 7.28 (d, J = 8.1 Hz, 2H), 7.23–7.41 (m, 3H), 7.69 (d, J = 8.1 Hz, 2H), 7.79 (s, 1H), 8.32 (m, 1H).

*4.1.42. (E)-Methyl 3-(4-((3-((dimethylamino)methyl)-2-methyl-1H-indol-1-yl)methyl)phenyl)acrylate (**30a**)*

The title compounds was obtained in 68% yield from compound **29a** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.37 (s, 6H), 2.39 (s, 3H), 3.75 (s, 2H), 3.82 (s, 3H), 5.36 (s, 2H), 6.43 (d, J = 15.9 Hz, 1H), 6.99 (d, J = 8.1 Hz, 2H), 7.15–7.21 (m, 3H), 7.41 (d, J = 8.1 Hz, 2H), 7.69 (d, J = 15.9 Hz, 1H), 7.70 (m, 1H).

*4.1.43. (E)-Methyl 3-(4-((3-((dimethylamino)methyl)-1H-indol-1-yl)methyl)phenyl)acrylate (**30b**)*

The title compound was obtained in 65% yield from compound **29b** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.34 (s, 6H), 3.69 (s, 2H), 3.83 (s, 3H), 5.33 (s, 2H), 6.44 (d, J = 16.2 Hz, 1H), 7.11–7.26 (m, 6H), 7.46 (d, J = 8.1 Hz, 2H), 7.74 (d, J = 16.2 Hz, 1H), 7.78 (dd, J = 1.2, 7.2 Hz, 1H).

*4.1.44. (E)-Methyl 3-(4-((3-(dimethylcarbamoyl)-1H-indol-1yl) methyl)phenyl)acrylate (**30c**)*

The title compound was obtained in 64% yield from compound **29c** in a manner similar to that described for the synthesis of compound **21a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 3.19 (s, 6H), 3.83 (s, 3H), 5.38 (s, 2H), 6.43 (d, J = 16.2 Hz, 1H), 7.16 (d, J = 8.1 Hz, 2H), 7.24–7.27 (m, 3H), 7.48–7.50 (m, 3H), 7.68 (d, J = 16.4 Hz, 1H), 7.82 (m, 1H).

*4.1.45. (E)-3-(4-((3-((Dimethylamino)methyl)-2-methyl-1H-indol-1-yl)methyl)phenyl)acrylic acid (**31a**)*

The title compound was obtained in 93% yield from compounds **30a** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 2.36 (s, 6H), 2.41 (s, 3H), 3.79 (s, 2H), 5.32 (s, 2H), 6.48 (d, J = 15.9 Hz, 1H), 7.01 (d, J = 8.1 Hz, 2H), 7.19–7.25 (m, 3H), 7.46 (d, J = 8.1 Hz, 2H), 7.78 (d, J = 15.9 Hz, 1H), 7.81 (m, 1H).

*4.1.46. (E)-3-(4-((3-((Dimethylamino)methyl)-1H-indol-1-yl) methyl)phenyl)acrylic acid (**31b**)*

The title compound was obtained in 95% yield from compound **30b** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 2.39 (s, 6H), 3.78 (s, 2H), 5.29 (s, 2H), 6.49 (d, J = 16.2 Hz, 1H), 7.14–7.29 (m, 6H), 7.49 (d, J = 8.1 Hz, 2H), 7.72 (d, J = 16.2 Hz, 1H), 7.76 (dd, J = 1.2, 7.2 Hz, 1H).

*4.1.47. (E)-3-(4-((3-(Dimethylcarbamoyl)-1H-indol-1-yl)methyl) phenyl)acrylic acid (**31c**)*

The title compound was obtained in 94% yield from compound **30c** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 3.31 (s, 6H), 5.47 (s, 2H), 6.51 (d, J = 16.2 Hz, 1H), 7.23 (d, J = 8.1 Hz, 2H), 7.29–7.41 (m, 3H), 7.53–7.59 (m, 3H), 7.78 (d, J = 16.4 Hz, 1H), 7.92 (m, 1H).

*4.1.48. Methyl 4-((3-formyl-2-methyl-1H-indol-1-yl)methyl) benzoate (**32a**)*

The title compound was obtained in 82% yield from compound **23** in a manner similar to that described for the synthesis of compound **20a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.78 (s, 3H), 3.91 (s, 3H), 5.41 (s, 2H), 7.23 (d, J = 8.1 Hz, 2H), 7.28–7.39 (m, 3H), 7.68 (d, J = 8.1 Hz, 2H), 8.28 (m, 1H), 10.22 (s, 1H).

*4.1.49. Methyl 4-((3-formyl-1H-indol-1-yl)methyl)benzoate (**32b**)*

The title compound was obtained in 80% yield from compound **24** in a manner similar to that described for the synthesis of compound **20a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 3.88 (s, 3H), 5.44 (s, 2H), 7.21 (d, J = 8.1 Hz, 2H), 7.32–7.46 (m, 3H), 7.66 (d, J = 8.1 Hz, 2H), 7.75 (s, 1H), 8.35 (m, 1H), 10.05 (s, 1H).

*4.1.50. Methyl 4-((3-(dimethylcarbamoyl)-1H-indol-1-yl)methyl) benzoate (**32c**)*

The title compound was obtained in 68% yield from compound **25** in a manner similar to that described for the synthesis of compound **20a**. ^{1}HNMR (300 MHz, $CDCl_3$) δ 3.20 (s, 6H), 3.93 (s, 3H), 5.42 (s, 2H), 7.20–7.29 (m, 5H), 7.51 (s, 1H), 7.81 (dd, J = 2.4, 9.0 Hz, 1H), 8.01 (d, J = 8.4 Hz, 2H).

*4.1.51. 4-((3-Formyl-2-methyl-1H-indol-1-yl)methyl)benzoic acid (**33a**)*

The title compound was obtained in 88% yield from compounds **32a** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 2.81 (s, 3H), 5.47 (s, 2H), 7.28 (d, J = 8.1 Hz, 2H), 7.31–7.36 (m, 3H), 7.79 (d, J = 8.1 Hz, 2H), 8.32 (m, 1H), 10.27 (s, 1H).

*4.1.52. 4-((3-Formyl-1H-indol-1-yl)methyl)benzoic acid (**33b**)*

The title compound was obtained in 90% yield from compounds **32b** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 5.49 (s, 2H), 7.27 (d, J = 8.1 Hz, 2H), 7.29–7.49 (m, 3H), 7.69 (d, J = 8.1 Hz, 2H), 7.72 (s, 1H), 8.39 (m, 1H), 10.15 (s, 1H).

*4.1.53. 4-((3-(Dimethylcarbamoyl)-1H-indol-1-yl)methyl)benzoic acid (**33c**)*

The title compound was obtained in 90% yield from compound **32c** in a manner similar to that described for the synthesis of compound **22a**. ^{1}HNMR (300 MHz, CD_3OD) δ 3.39 (s, 6H), 5.56 (s, 2H), 7.25–7.39 (m, 5H), 7.59 (s, 1H), 7.87 (dd, J = 2.4, 9.0 Hz, 1H), 8.21 (d, J = 8.4 Hz, 2H).

4.2. Biology

4.2.1. Materials

Sulforhodamine B (SRB), crystal violet, and propidium iodide (PI) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies to acetyl-tubulin was purchased from Cell Signaling (Danvers, MA, USA), PARP was from Santa Cruz (Dallas, Texas, USA), caspase 3 was from Novus (Littleton, CO, USA), acetyl-Histone H3, and β-actin were purchased from Millipore (Billerica, MA, USA).

4.2.2. Cell culture

Human NSCLC cell line A549, colorectal carcinoma HCT116 cells, and human prostate cancer cell line PC-3 cells were obtained from American Type Culture Collection (ATCC) (Manassas, VA). Cells were maintained in RPMI 1640 medium with 10% fetal bovine serum (FBS) and penicillin (100 U/ml)/streptomycin (100 μg/mL)/amphotericin (0.25 μg/mL) at 37 °C in a humidified incubator with 5% CO_2.

4.2.3. Sulforhodamine B (SRB) assay

All cells were seeded in 96-well culture plates at a density of 3–5 x 10^3 cells/well. After attachment, cells were fixed with 10% trichloroacetic acid (TCA) to provide a measurement of the cell population at the time of drug addition (T0). After additional incubation of DMSO or compounds for 48 h, cells were fixed with 10% TCA and stained with 0.4% (w/v) SRB dissolved in 1% AcOH. The protein-bound dye was subsequently extracted with 10 mM trizma base to determine the absorbance at a wavelength of 515 nm. The percentage of cell growth was calculated with O.D. value from different treatments, such as time zero (T0), control growth (C), and cell growth in the presence of the drug (Tx). Percentage growth was calculated as [(Tx-T0)/(C-T0)]*100. Growth inhibition of 50% (GI_{50}) is determined at the drug concentration that results in 50% reduction of total protein increase in control cells during the compound incubation.

4.2.4. HDAC enzymes inhibition assays

Enzyme inhibition assays were performed by the Reaction Biology Corporation, Malvern, PA (http://www.reactionbiology.com). The substrate for HDAC-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. Trichostatin A (TSA) was the reference compound tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 10 μM.

4.2.5. Colony formation assay

Cells were treated with various concentrations of compounds or DMSO vehicle for 24 h. The drugs were washed out and the cells were trypsinized and plated at 500 cells/well in 6 well plates. After 14 days, the colonies were fixed and stained with crystal violet (0.5% in 70% EtOH). Colonies containing more than 50 cells were scored. The survival fraction was calculated based on the number of colonies formed in drug-treated cells relative to that of the DMSO control. Each dose was carried out in triplicate, and the experiments were repeated at least twice.

4.2.6. Western blot analysis

For Western blot analysis, cell lysates were prepared, and proteins were separated by 7.5–15% SDS-PAGE, transferred onto PVDF membrane, and then immunoblotted with specific antibodies. Proteins were visualized with an ECL detection system.

4.2.7. Flow cytometry

Cells were treated with indicated concentration of indicated compounds for 48 h and then fixed in EtOH (75%, v/v) overnight at −20 °C. After centrifugation, fixed cells were washed with ice-cold PBS once and incubated in 0.1 M of phosphate–citric acid buffer (0.2 M $NaHPO_4$, 0.1 M citric acid, pH 7.8) for 30 min at room temperature, and then stained with propidium iodide (PI) staining buffer containing Triton X-100 (0.1%, v/v), RNase A (100 μg/mL) and propidium iodide (80 μg/mL). Cell cycle distribution was performed using a FACScan flow cytometry with CellQuest software (Becton Dickinson, Mountain View, CA, USA).

4.2.8. Statistical analysis

Results are expressed as the mean ± SD for the indicated number of separate experiments. Means were assessed for significant differences using *t*-test and *P*-values < 0.05 were considered significant.

Acknowledgments

This research were supported by the Ministry of Science and Technology of the Republic of China (grant no. MOST 103-2113-M-038-001-MY3 and MOST-103-2320-B-038-030-MY3), and TMU102-AE1-B43 by Taipei Medical University.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.12.039.

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