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Synthesis and Biological Evaluation of 1-Arylsulfonyl-5-(*N*-hydroxyacrylamide)indoles as Potent Histone Deacetylase Inhibitors with Antitumor Activity in Vivo

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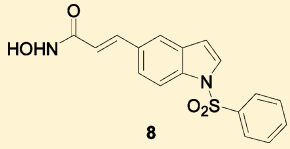
Supporting Information

ABSTRACT: A series of 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indoles has been identified as a new class of histone deacetylase inhibitors. Compounds **8**, **11**, **12**, **13**, and **14** demonstrated stronger antiproliferative activities than **1** (SAHA) with GI_{50} values ranging from 0.36 to 1.21 μM against Hep3B, MDA-MB-231, PC-3, and A549 human cancer cell lines. Lead compound **8** showed remarkable HDAC 1, 2, and 6 isoenzymes inhibitory activities with IC_{50} values of 12.3, 4.0, 1.0 nM, respectively, which are comparable to **1**. In in vivo efficacy evaluation against lung A549 xenograft model, **8** displayed better antitumor activity than compound **1**.



INTRODUCTION

Histone acetylation and deacetylation play a crucial role for regulating protein function of eukaryotic cells, which is correlated with two classes of enzymes: histone deacetylases (HDACs) and histone acetyltransferases (HATs). They affected the removal (HDAC) and addition (HAT) of the acetyl group at specific lysine residues. The acetylation status of histone is important in modulating gene expression and cell life, and its dysregulation is involved in the development of several cancers. Histone hyperacetylation leads to transcriptional activation of suppressed genes, some of them being associated with cell cycle arrest, differentiation, or apotosis in tumor cells. Recent studies have shown that acetylation of non-histone proteins is also relevant for tumorigenesis, cancer cell proliferation, and immune functions. Hence, inhibition of HDAC has arisen as an efficient therapeutic strategy to adjust aberrant epigenetic changes associated with cancer.[1−5]

In October 2006, small molecule **1** (SAHA, vorinostat) was approved by FDA for the treatment of refractory cutaneous T-cell lymphorma.[6] In November 2009, prodrug **2** (FK-228, romidepsin) also gained approval from FDA for the treatment of cutaneous T-cell lymphoma.[7] Many small molecular agents, for example, **3** (LBH-589),[8] **4** (PXD101),[9] and **5** (SB939),[10] are undergoing human clinical trials[11,12] (Figure 1). A close view of these small molecule histone deacetylase inhibitors indicated the hydroxamic acid or the *N*-hydroxyacrylamide group plays an important role for activity.[13,14] Our previous studies of antitubulin agents, for example, 3-aroylindoles,[15a] 5-aroylindoles,[15b] 1-aroylindoles,[15c] 3-arylthioindoles,[15d] and 1-aryl(1-benzyl)indoles,[15e−g] showed substantial anticancer activity. The literature[16] reported that the 2-aroyl-5-(*N*-hydroxyacrylamide)indoles exhibited potent histone deacetylation inhibition. On the basis of these observations, we attempted to explore the structure−activity relationships between the indole moiety and the *N*-hydroxyacrylamide group. Using the 1-arylsulfonylindole as a base coupled with the *N*-hydroxyacrylamide group at the C-3, -4, -5, -6, or -7 position of indole ring provided the regioisomers of indolyl-*N*-hydroxyacrylamides and allowed evaluation of bioactivity (Figure 2). Herein we describe the discovery of 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indoles as a new class of histone deacetylase inhibitors showing antitumor activity in vivo.

RESULTS AND DISCUSSION

Chemistry. The synthesis of 3-(*N*-hydroxyacrylamide)-indole (**6**) is depicted in Scheme 1. Compound **22** was reacted

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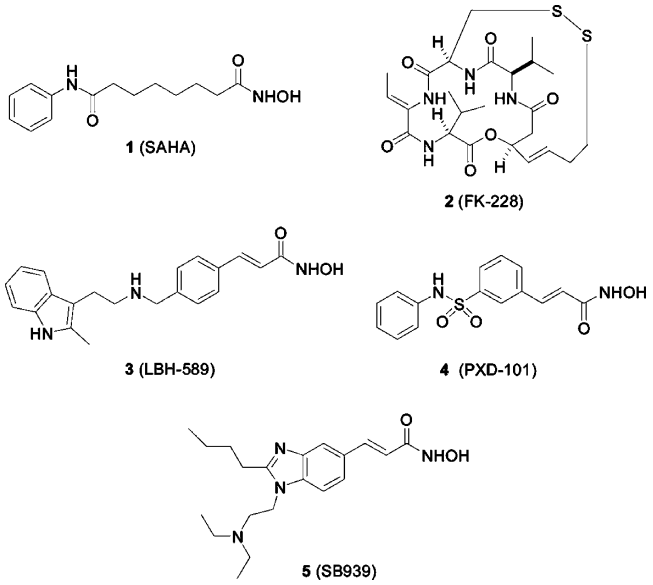

Figure 1. Examples of histone deacetylase inhibitor.

with the *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (NH_2OTHP) in the presence of benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PYBOP) and Et_3N to prepare the tetrahydropyran-protected compound **23**, which was further subjected to the N1-benzenesulfonylation of indole in the presence of KOH and tetrabutylammonium hydrogen sulfate (TBAHS) at room temperature and then trifluoroacetic acid (TFA) mediated deprotection to afford the desired **6**. The general method for the syntheses of 4-, 5-, 6-, and 7-(*N*-hydroxyacrylamide)indoles **7**−**10** and 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indoles **11**−**14** are shown in the Scheme 2. The preparation of compounds **7**−**10** involved a five-step reaction sequence, with an overall yield of 34−52%. The various commercially available indolecarboxyaldehydes (**24**−**27**) with benzenesulfonyl chloride yielded the related 1-benzenesulfonylindoles (**28**−**31**). These indoles-1-sulfonamides were subject to the Wittig reaction with methyl (triphenylphosphorylidene)acetate followed by LiOH hydrolysis and PYBOP-mediated amide formation, and the reaction sequence was completed by TFA-mediated deprotection to afford the desired 1-benzenesulfonyl(*N*-hydroxyacrylamide)-indoles **7**−**10**. The 1-substituted phenylsulfonyl compounds **11**−**14** were synthesized in 41−61% yields from compound **25** by reacting with the corresponding arylsulfonyl chloride in the presence of KOH and tetrabutylammonium hydrogen sulfate (TBAHS) at room temperature. Compound **15**, with a hydroxyamide group at the C-5 position of the indole ring, was synthesized in 40% yield by treatment of indole 5-methylcarboxylate (**44**) with 1-phenylsulfonylation, LiOH-mediated hydroxylation, NH_2OTHP/PYBOP coupling reaction, and TFA deprotection (Scheme 3). Similar to the preparation of compound **8**, compounds **16**, **17**, and **18**, with a benzoyl, benzyl, and phenyl group at the N1-position, were obtained in 5−63% yield through a five-step synthesis as shown in the Scheme 4. The 1-benzoylation and benzylation of indole-5-carboxyaldehyde (**25**) was achieved by using KO-*t*-Bu as base in the presence of KI and DMF at room temperature. The 1-arylation of the indole ring was achieved in 28% yield by the treatment of **25** and 4-iodobenzene with CuO/K_2CO_3 in DMF. As shown in the Scheme 5, the treatment of compound **8** with KOH and Pd/C afforded the desired compounds **19** and **20**, respectively. The synthesis of 3-(benzenesulfonyl)-5-(*N*-hydroxyacrylamide)indole (**21**) is described in Scheme 6. Starting from the indole-5-carboxyaldehyde (**25**), the 3-arylsulfonyl-5-(methylacrylate)indole **54** was obtained by methyl(triphenylphosphoranylidene)acetate-mediated Wittig reaction, diphenyl disulfide mediated electrophilic substitution, and mCPBA oxidation. The conversion of methyl acrylate (**54**) to *N*-hydroxyacrylamide (**21**) was accomplished by a reaction sequence, including the LiOH hydrolysis, NH_2OTHP/PYBOP coupling reaction, and TFA deprotection.

Biological Evaluation. *(A) HeLa Nuclear HDAC Enzyme Inhibition.* First, we evaluated the position effect of the *N*-hydroxyacrylamide group in the 1-benzenesulfonylindole system as shown in Table 1. The regioisomers 3-, 4-, 5-, 6-, and 7-(*N*-hydroxyacrylamide)-1-benzenesulfonylindoles (**6**, **7**, **8**, **9**, and **10**, respectively) were evaluated for the inhibition of HeLa nuclear HDAC activity (which consists of pan-HDAC isoenzymes). The *N*-hydroxyacrylamide moiety located at the C-5 position on the indole ring resulted in the best activity among the five regioisomers with 1-benzenesulfonyl-5-(*N*-hydroxyacrylamide)indole (**8**) showing IC_{50} values of 29.5 nM on HeLa HDAC enzyme inhibition, which was more potent than **1** (IC_{50} = 96.4 nM). Shifting of the *N*-hydroxyacrylamide

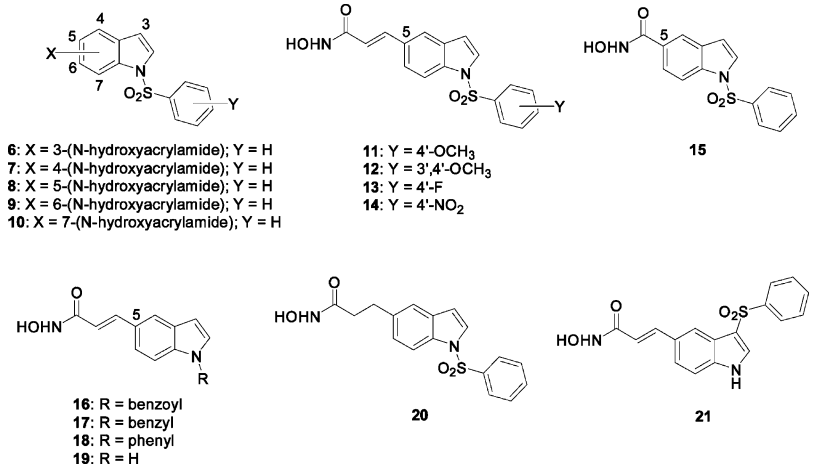

Figure 2. Synthetic indolyl-*N*-hydroxyacrylamides and indolylhydroxyamide.

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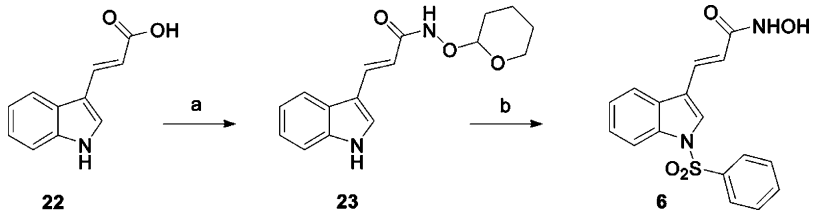
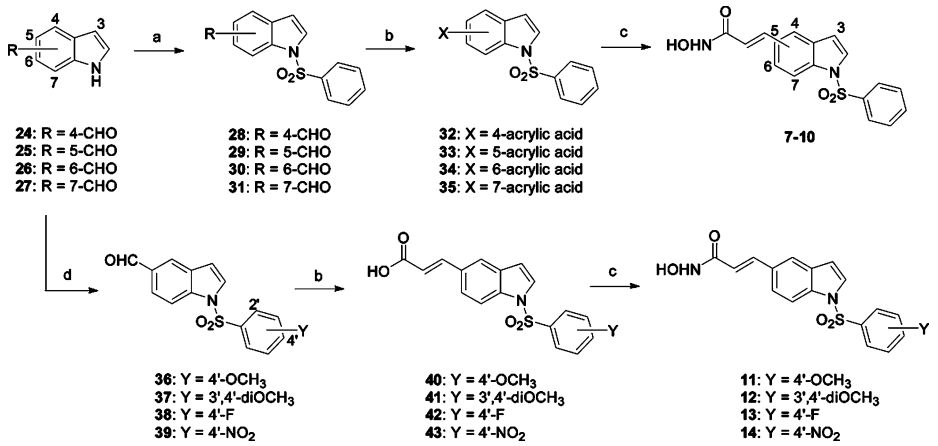
Scheme 1[a]



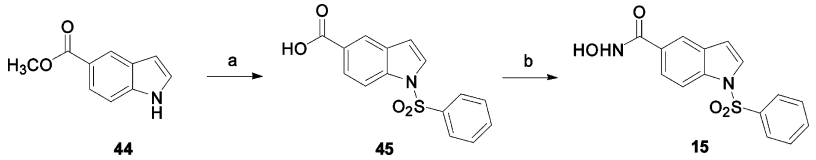
[a]Reagents and conditions: (a) NH_2OTHP, PYBOP, Et_3N, DMF, room temp, 68%; (b) (i) benzenesulfonyl chloride, KOH, TBAHS, CH_2Cl_2, room temp; (ii) TFA, CH_3OH, room temp, two steps, 32%.

Scheme 2[a]



[a]Reagents and conditions: (a) benzenesulfonyl chloride, KOH, TBAHS, CH_2Cl_2, room temp, 66−91%; (b) (i) methyl (triphenylphosphoranylidene)acetate, CH_2Cl_2, room temp; (ii) 1 M LiOH(aq), dioxane, 40 °C, two steps, 79−90%; (c) (i) NH_2OTHP, PYBOP, Et_3N, DMF, room temp; (ii) TFA, CH_3OH, room temp, two steps, 53−78%; (d) substituted arylsulfonyl chloride, KOH, TBAHS, CH_2Cl_2, room temp, 76−82%.

Scheme 3[a]



[a]Reagents and conditions: (a) (i) benzenesulfonyl chloride, KOH, TBAHS, CH_2Cl_2, room temp, two steps, 76%; (ii) 1 M LiOH(aq), dioxane, 40 °C; (b) (i) NH_2OTHP, PYBOP, Et_3N, DMF, room temp; (ii) TFA, CH_3OH, room temp, two steps, 53%.

group to the C-4 (**7**) and C-6 (**9**) positions resulted in a decrease in enzyme activity to the 100 nM level, while moving to the C-3 and C-7 positions, as in compounds **6** and **10**, respectively, resulted in the loss of HDAC enzyme activity. Second, we expanded the panel construction using indole **8** as motif. Further investigation of substitution effects on the N1-benzenesulfonyl group of compound **8** revealed that **11** with an additional methoxy group at the C-4′ position, **12** with the dimethoxy substitution at the C-3′ and C-4′ positions, **13** with a fluoro group at the C-4′ position, and **14** with a nitro group at C-4′ position all exhibited substantial activities with IC_{50} values of 6.8, 9.7, 16.8, and 12.6 nM, respectively. In an effort to further understand the role of *N*-hydroxyacrylamide substituent at the C-5 position of indole ring, compound **15** (with two carbons shorter than **8**) and compound **20** (with a saturated double from **8**) were prepared. They exhibited a decrease in potency, thus emphasizing the importance of *N*-hydroxyacrylamide at the C-5 position of indole in inhibiting HDAC enzyme activity. In order to understand the role of 1-sulfonyl functionality in **8**, 1-benzoyl, 1-benzyl, and 1-phenyl substituted compounds **16**, **17**, and **18**, respectively, were prepared. Compound **17**, with a benzyl group, retained HDAC enzyme activity comparable to that of **8**, but benzoyl (**16**) or phenyl (**18**) substitution resulted in a decrease in activity, thus revealing that the 1-benzenesulfonyl and 1-benzyl group in the indol-5-yl-*N*-hydroxyacrylamide series were preferable. The removal of 1-benzenesulfonyl group in **8**, for example, compound **19**, resulted in a dramatic loss of activity, indicating the 1-arylsulfonyl group played a vital role for activity in this series. Changing the position of arylsulfonyl group from N-1 to C-3 on the indole ring, for example, compound **21**, caused a reduced potency.

(B) In Vitro Cell Growth Inhibitory Activity. The synthesized indolyl-*N*-hydroxyacrylamides **6**−**14**, **16**−**19**, **21** and indolylhydroxamic acids **15**, **20** were evaluated for antiproliferative activities against human liver carcinoma Hep3B cells, breast

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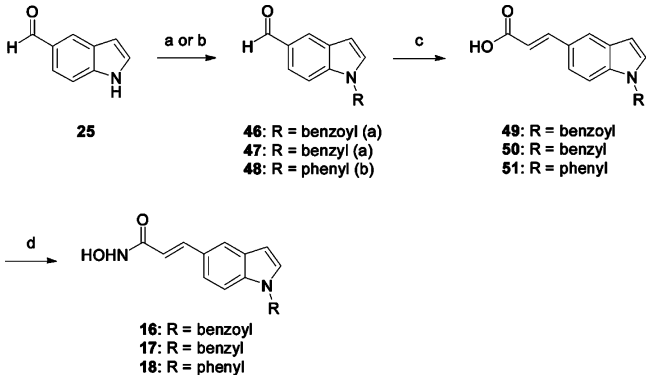
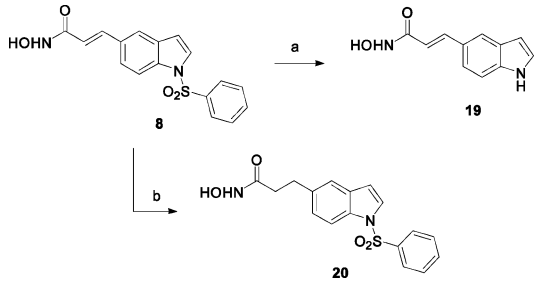
Scheme 4[a]



[a]Reagents and conditions: (a) benzoyl bromide or benzyl chloride, KO-*t*-Bu, KI, DMF, room temp, 85−87%; (b) 4-iodobenzene, K_2CO_3, CuO, DMF, reflux, 28%; (c) (i) methyl (triphenylphosphoranylidene)acetate, CH_2Cl_2, room temp; (ii) 1 M $LiOH_{(aq)}$, dioxane, 40 °C; (d) (i) NH_2OTHP, PYBOP, Et_3N, DMF, room temp; (ii) TFA, CH_3OH, room temp, two steps, 44−81%.

Scheme 5[a]



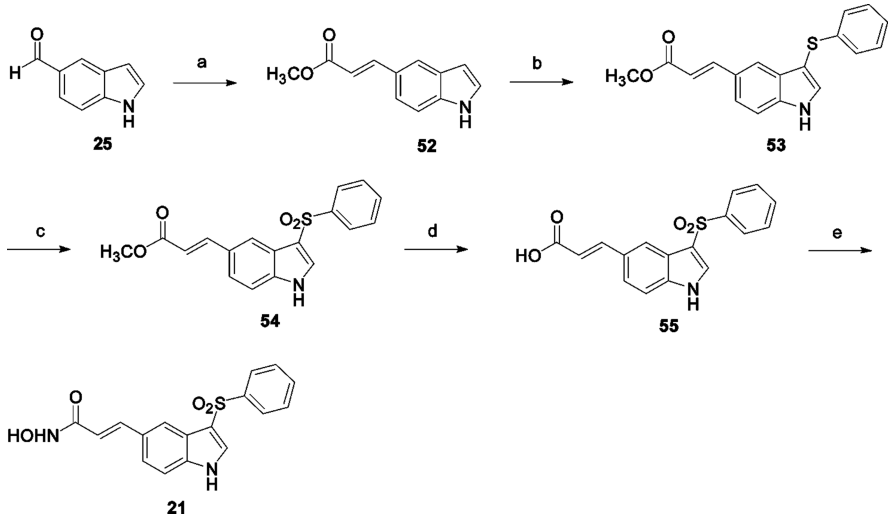
[a]Reagents and conditions: (a) 1 M $KOH_{(aq)}$, CH_3OH, reflux, 51%; (b) H_2, 10% Pd/C, CH_2Cl_2, room temp, 30%.

carcinoma MDA-MB-231 cells, prostate carcinoma PC-3 cells, and lung carcinoma A549 cells (Table 1). The cancer cell growth inhibitory results were proportional to the HDAC extract enzyme activity except for compound **17**. Only the series of 1-(arylsulfonyl)-5-(*N*-hydroxyacrylamide)indoles **8**, **11**, **12**, **13**, and **14** showed more potent antiproliferative activities than SAHA (**1**). For example, compound **8** demonstrated GI_{50} of 0.41, 0.48, 0.62, and 1 μM against the Hep3B, MDA-MB-231, PC-3, and A549 cell lines, respectively. Compound **12** with dimethoxybenzenesulfonyl group displayed antiproliferative activity in four cancer cell lines with GI_{50} values of 0.36, 0.37, 0.93, and 0.56 μM, respectively. The 1-(arylbenzyl)-5-(*N*-hydroxyacrylamide)indole **17** displayed about 2-fold decrease in activities compared to **8** and slight decrease in activities compared to **1**. Other compounds with the *N*-hydroxyacrylamide group at the C-3, C-4, C-6, C-7 position of 1-(arylsulfonyl)indoles (**6**, **7**, **9**, **10**), benzoyl, phenyl, and no substitution at the N1 of 5-(*N*-hydroxyacrylamide)indoles (**16**, **18**, **19**), and modifications to the C-5 position from **8** (**15**, **20**) had significantly less antiproliferative activities.

(C) HDAC Isoform Inhibition. The 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indoles **8**, **11**, **12**, **13**, **14**, and **17** were tested for HDAC isoform enzyme-inhibitory activity (HDACs 1, 2, and 6) using compound **1** as a reference (Table 2). Compound **8** with 1-benzenesulfonyl group had low nanomolar IC_{50} values against HDACs 2 and 6 with IC_{50} values of 1 and 4 nM, respectively. Compound **11** with 4′-methoxyphenylsulfonyl group displayed high activity on HDAC 6 (IC_{50} = 3.3 nM) and 40- to 100-fold selectivity for HDACs 1 and 2. However, compound **12** with a dimethoxy group at both C-3′ and C-4′ positions exhibited selective and potent inhibitory activity for HDAC 2, with IC_{50} of 2.3 nM, among HDACs 1, 2, and 6. The results indicated that the position of introduced methoxy group in the 1-arylsulfonyl group of 5-(*N*-hydroxyacrylamide)indoles seems to affect selectivity between HDAC isoforms. Compounds **13** and **14**, with a fluoro and nitro substitution at the 4′-position of the arylsulfonyl moiety, respectively, exhibited diminished activities against HDACs 1, 2, and 6 compared with parent compound **8**.

(D) Up-Regulation Effect of Histone and α-Tubulin. In an effort to further validate the target in the 1-(arylsulfonyl)-5-(*N*-

Scheme 6[a]



[a]Reagents and conditions: (a) methyl (triphenylphosphoranylidene)acetate, CH_2Cl_2, room temp, 99%; (b) NaH, Ph-S-S-Ph, DMF, 0 °C to room temp, 42%; (c) mCPBA, CH_2Cl_2, 0 °C to room temp, 46%; (d) 1 M $LiOH_{(aq)}$, MeOH, 40 °C, 52%; (e) (i) NH_2OTHP, EDC−HCl, HOBt, *N*-methylmorphine, DMF, room temp; (ii) 10% $TFA_{(aq)}$, MeOH, room temp, two steps, 26%.

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Table 1. Inhibition of HeLa Nuclear Extract HDAC Activity and Antiproliferative Activity against Human Cancer Cell Lines by Compounds 6−21 and SAHA (1)

compd	IC_{50} ± SD [a] (nM) HeLa nuclear HDAC	GI_{50} ± SD [a] (μM) Hep3B	MDA-MB-231	PC-3	A549
6	>1000	8.87 ± 0.2	8.14 ± 0.7	5.77 ± 0.5	7.06 ± 0.1
7	170.9 ± 42.7	4.32 ± 0.1	4.16 ± 0.3	3.67 ± 0.3	6.70 ± 0.2
8	29.5 ± 4.5	0.41 ± 0.1	0.48 ± 0.1	0.62 ± 0.2	1.02 ± 0.2
9	254.0 ± 84.6	2.12 ± 0.1	2.41 ± 0.3	2.40 ± 0.3	5.30 ± 0.7
10	>1000	3.82 ± 0.4	7.71 ± 0.6	4.75 ± 0.4	5.50 ± 0.4
11	6.8 ± 1.7	0.55 ± 0.1	0.75 ± 0.1	0.80 ± 0.2	1.18 ± 0.2
12	9.7 ± 2.0	0.36 ± 0.1	0.37 ± 0.1	0.93 ± 0.3	0.56 ± 0.1
13	16.8 ± 3.8	0.64 ± 0.1	0.66 ± 0.2	0.39 ± 0.1	0.75 ± 0.7
14	12.6 ± 2.6	0.56 ± 0.1	0.75 ± 0.2	0.85 ± 0.2	1.21 ± 0.2
15	>1000	>10	5.68 ± 0.3	7.26 ± 0.6	>10
16	757.6 ± 92.8	2.40 ± 0.3	3.18 ± 0.4	2.32 ± 0.2	5.80 ± 0.8
17	32.1 ± 9.3	0.86 ± 0.1	1.18 ± 0.2	1.23 ± 0.2	2.37 ± 0.1
18	186.5 ± 20.9	1.32 ± 0.2	1.73 ± 0.1	1.54 ± 0.2	2.85 ± 0.8
19	>1000	3.65 ± 0.4	4.37 ± 0.5	4.80 ± 0.1	9.07 ± 0.9
20	407.5 ± 170.8	2.56 ± 0.3	2.21 ± 0.4	2.07 ± 0.1	4.04 ± 0.2
21	275.2 ± 31.7	1.27 ± 0.1	1.25 ± 0.1	1.26 ± 0.1	3.33 ± 0.2
1	96.4 ± 10.3	0.69 ± 0.2	0.97 ± 0.2	1.21 ± 0.3	1.85 ± 0.3

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

Table 2. Activities of Compounds 8, 11−14, 17 and Reference Compound 1 against HDAC Isoforms 1, 2, and 6

compd	IC_{50} ± SD [a] (nM) HDAC1	HDAC2	HDAC6
8	12.3 ± 4.2	4.0 ± 0.7	1.0 ± 0.2
11	124.8 ± 9.8	361.8 ± 22.2	3.3 ± 0.7
12	18.1 ± 2.5	2.3 ± 0.3	45.4 ± 6.4
13	33.2 ± 6.6	199.4 ± 24.7	59.2 ± 1.5
14	69.9 ± 12.0	189.3 ± 70.6	18.3 ± 3.6
17	71.2 ± 14.2	381.1 ± 36.8	40.4 ± 12.4
1	8.9 ± 3.3	1.7 ± 0.2	5.0 ± 0.4

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

Table 3. CYP Inhibition and Solubility of Compound 8

compd	% CYP inhibition[a] at 10 μM 1A2	2C19	2C8	2C9	2D6	2E1	3A4	sol (μg/mL) H_2O	EtOH
8	51	78	78	22	31	22	65	<1	939

[a]The studies were performed by Ricerca Biosciences.

Table 4. Pharmacokinetics Parameters of 8 in Rat

parameter	iv	po
dose (mg/kg)[a]	2	20
CL (mL min^{-1} kg^{-1})	35.5 ± 3.7	
V_{ss} (l/kg)	2.1 ± 0.1	
$T_{1/2}$ (h)	2.6 ± 0.2	5.5 ± 1.9
$AUC_{(0-inf)}$ (ng mL^{-1} h)	927 ± 80	916 ± 183
T_{max} (h)		1.7 ± 0.6
C_{max} (ng/mL)		217 ± 116
F (%)		10

[a]Compound was dissolved in PEG400/DMSO (80:20,v/v) for intravenous administration and in 1% carboxylmethyl cellulose and 0.5% Tween 80 for oral dosing.

hydroxyacrylamide)indole series, compound **8** was evaluated for the expression of histone and α-tubulin acetylation in PC-3 prostate cancer cell lines and A549 non-small-cell lung cancer cells, which were important biomarkers associated with intracellular HDAC inhibition. Western blot analysis of the cell lysates revealed that compound **8**, like compound **1**, was a pan-HDAC inhibitor on the basis of its ability to up-regulate H3 and tubulin acetylation in dose-dependent manner (Figure 3).

*(E) CYP Inhibition and Pharmacokinetic Study. (E.1) Effect of **8** on Cytochrome P450 Isozymes.* Compound **8** was tested for its inhibition of CYP1A2, 2C19, 2C8, 2C9, 2D6, 2E1, and 3A4 at 10 μM (Table 3). The CYP inhibition of **8** was greatest for CYP2C19 and CYP2C8 with 78% at 10 μM. Both CYP3A4 and CYP1A2 were also affected to a lesser extent, 65% and 51% at 10 μM, respectively. Compound **8** has a solubility of <1 μg/mL in water and 939 μg/mL in EtOH.

*(E.2) In Vivo Rat PK Profiling of **8**.* The PK parameters of **8** in male Sprague−Dawley rats, following single dose intravenous (iv) and oral dosing, are shown in Table 4. Following iv administration, **8** showed a mean $T_{1/2}$ of 2.6 h. After a single oral dose, compound **8** showed rapid absorption with T_{max} = 1.7 h, $T_{1/2}$ = 5.5 h, and bioavailability F = 10%.

(F) In Vivo Efficacy in Human Xenograft. Compound **8** and reference compound **1** were evaluated for in vivo efficacy of tumor xenograft in nude mice bearing human A549 cancer cell line expressed by tumor growth delay (TGD; Figure 4A, Table 5) and tumor growth inhibition (TGI; Figure 4B). Once tumor was palpable with a size of ~65 mm^3, mice were randomized into vehicle control and treatment groups of seven animals each. Control mice received vehicle (0.5% carboxymethyl cellulose). Compounds **8** and **1** were suspended in the 0.5% carboxymethyl cellulose. All tumors in mice grew to the 1000 mm^3 end point volume. The median TTE (time to end point) for control mice was 27.0 days. Compound **1**, at 200 mg/kg po daily to end, produced a median TTE of 30.0 days, corresponding to a 3.0 day $T - C$ and a TGD (tumor growth delay) of 11%. On the basis of the log rank analysis, compound

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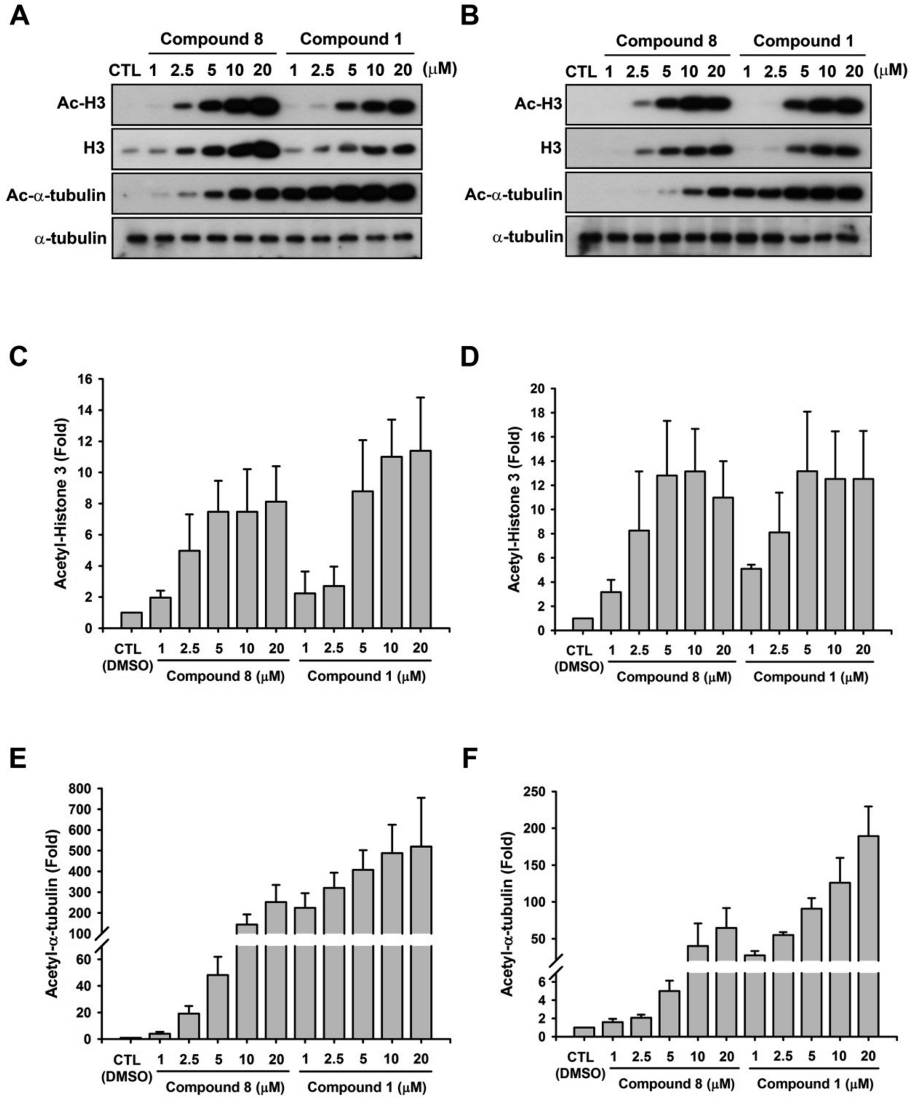

Figure 3. Effect of α-tubulin acetylation and histone H3 acetylation in cultured (A) human prostate cells (PC-3) and (B) non-small-cell lung cancer cells (A549) by compounds **8** and **1** using Western blot analysis. Quantitative analysis of Western blot was done with ImageQuant (Molecular Dynamics, U.S.). Acetyl-histone H3 (C, D) and acetyl α-tubulin (E, F) were analyzed in PC-3 and A549 cells, respectively.

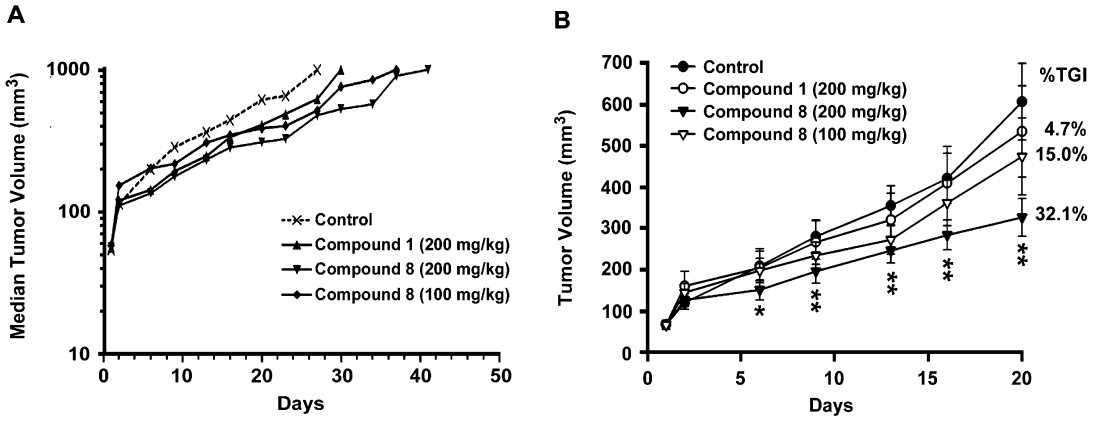

Figure 4. Inhibition of human A549 lung cancer xenograft growth in nude mice (n = 7). Compounds **8** and **1** were suspended in the 0.5% carboxymethyl cellulose. All tumors in mice grew to the 1000 mm^3 end point volume. (A) Tumor growth delay (TGD): 100 mg/kg compound **8** po daily (◆); 200 mg/kg compound **8** po daily (▼); 200 mg/kg compound **1** po daily (▲). (B) % of tumor growth inhibition (%TGI): control (●); 100 mg/kg compound **8** po daily (▽); 200 mg/kg compound **8** po daily (▼); 200 mg/kg compound **1** po daily (○). (*) $P < 0.05$, (**) $P < 0.01$ compared with control group at the indicated times.

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Table 5. Summary Responses of Compounds 1 and 8 in the Human A549 Lung Xenograft Model

agent (n [a])	dose (mg/kg)	route	schedule[b]	median TTE[c] (day)	$T - C$[d] (day)	% TGD[e]	log rank significance[f]	P
control (7)		po	q.d. to end point	27.0				
1 (7)	200	po	q.d. to end point	30.0	3.0	11	ns[g]	0.3925
8 (7)	200	po	q.d. to end point	39.8	12.8	47	*[h]	0.0274
8 (7)	100	po	q.d. to end point	36.2	9.2	34	ns	0.1688

[a] n = number of animals. [b] Study end point = 1000 mm^3. Days in progress = 48. [c] TTE = time to end point. [d] $T - C$ = difference between median TTE (days) of treated versus control group. [e] % TGD (tumor growth delay) = $[(T - C)/C] \times 100$. [f] Statistical significance = log rank test. [g] ns = not significant. [h] * indicates $P < 0.05$ compared with control.

1 did not produce significant antitumor activity ($P = 0.3925$). However, compound **8**, at 200 and 100 mg/kg po daily to end, produced a median TTE of 39.8 and 36.2 days, corresponding to a 12.8 and 9.2 day $T - C$ and a TGD of 47% and 34%, respectively. On the basis of the log rank analysis, compound **8** exhibits significant antitumor activity at 200 mg/kg ($P = 0.0274$) but not at 100 mg/kg ($P = 0.1668$). Results indicated that there was a dose-dependent delay of tumor growth in **8**. Compound **8** with TGI of 32.1% at 200 mg/kg, po ($P < 0.01$) apparently demonstrated more potent antitumor activity than reference compound **1** with TGI of 4.7% at 200 mg/kg po on day 20. In summary, compound **8** displayed better efficacy in in vivo lung A549 xenografts model than **1**. No significant body weight difference as indicated in the Supporting Information and no adverse effects were observed.

CONCLUSION

We have identified a new class of 1-arylsulfonyl-5-(*N*-hydroxyacrylamide)indoles as potent histone deacetylase inhibitors. Lead compound **8** (MPT0E014) showed antiproliferative activity, with GI_{50} values ranging from 0.41 to 1 μM in a variety of human cancer cell lines from different organs. It has better activity than compound **1**. Compound **8** also exhibited low nanomolar IC_{50} values against HDACs 1, 2, and 6 enzymes with 1.0, 4.0, and 12.3 nM, respectively, comparable to compound **1**. Structure−activity relationship information revealed that the *N*-hydroxyacrylamide group located at the C-5 position of indole ring displayed the best activity compared to other positions such as the C-3, -4, -6, and -7 positions. The 1-arylsulfonyl substitution pattern contributed to a significant extent for maximal activity, for example, compounds **8**, **11**, **12**, **13**, and **14** showed better activity compared to benzoyl, benzyl, or phenyl group substituted compounds. In an in vivo efficacy evaluation against human xenograft model in nude mice bearing lung A549 cancer cell line, compound **8** demonstrated greater antitumor activity than compound **1**. There was a 47% tumor growth delay (32% tumor growth inhibition) on treatment with **8** daily at 200 mg/kg po. In summary, compound **8** has potential in further investigations as anticancer agents.

EXPERIMENTAL SECTION

Chemistry. Nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker DRX-500 spectrometer (operating at 500 MHz), with chemical shift in parts per million (ppm, δ) downfield from TMS as an internal standard. Mass spectrometry (MS) data were measured on a JEOL JMS-700 mass spectrometer (HRMS-EI and MS-EI), Finnigan TSQ 700 mass spectrometer (MS-EI), and micrOTOF orthogonal ESI-TOF mass spectrometer (HRMS-ESI). Purity of the final compounds was determined using a Hitachi 2000 series HPLC system using C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm) with the solvent system (elution conditions: mobile phase A consisting of acetonitrile; mobile phase B consisting of water containing 0.1% formic acid +10 mmol NH_4OAc) and was found to be ≥95%. Flash column chromatography was done using silica gel (Merck Kieselgel 60, no. 9385, 230−400 mesh ASTM). All reactions were carried out under an atmosphere of dry nitrogen.

Syntheses of 1-Phenylsulfonyl-(*N*-hydroxyacrylamide)-indoles 6−10. *3-(1H-Indol-3-yl)-N-(tetrahydropyran-2-yloxy)-acrylamide (**23**).* To a solution of *trans*-3-indoleacrylic acid (**22**, 0.50 g, 2.67 mmol), PYBOP (1.47 g, 2.83 mmol), and triethylamine (0.74 mL, 6.41 mmol) in THF (25 mL) was added NH_2OTHP (0.38 g, 3.21 mmol), and the mixture was stirred at room temperature. After being stirred for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc and quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((EtOAc/*n*-hexane = 1.5:1)/1% $NH_{3(aq)}$) to give compound **23**. Yield 68%. ^{1}H NMR (500 MHz, CD_3OD): δ 7.84−7.87 (m, 2H), 7.59 (s, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.15−7.21 (m, 2H), 6.47 (d, J = 14.9 Hz, 1H), 4.97−4.98 (m, 1H), 4.03−4.08 (m, 1H), 3.63−3.65 (m, 1H), 1.79−1.90 (m, 3H), 1.58−1.70 (m, 3H).

*3-(1-Benzenesulfonyl-1H-indol-3-yl)-N-hydroxyacrylamide (**6**).* After a suspension of **23** (0.52 g, 1.82 mmol), TBAHS (0.09 g, 0.27 mmol), and KOH (0.20 g, 3.63 mmol) in CH_2Cl_2 (15 mL) was stirred for 20 min, benzenesulfonyl chloride (0.35 mL, 2.72 mmol) was added. The mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and dried with vacuum to give a yellow residue without purification, which was dissolved in methanol (50 mL) and treated with TFA (2.2 mL, 29.8 mmol). The mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure to give a yellow residue, which was recrystallized by CH_3OH to afford the desired compound **6**. Yield 32%; mp 195−197 °C. ^{1}H NMR (500 MHz, DMSO): δ 10.62 (s, 1H), 9.03 (s, 1H), 8.27 (s, 1H), 7.97−8.01 (m, 3H), 7.85 (d, J = 8.0 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.56−7.61 (m, 3H), 7.42 (t, J = 8.0 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 6.62 (d, J = 16.0 Hz, 1H). MS (EI) m/z: 342 (M^+, 22%), 77 (100%). HRMS (EI) for $C_{17}H_{14}N_2O_4S$ (M^+): calcd, 342.0674; found, 342.0673.

*1-Benzenesulfonyl-1H-indole-4-carbaldehyde (**28**).* After a suspension of 1*H*-indole-4-carbaldehyde (**24**, 0.5 g, 3.44 mmol), TBAHS (0.18 g, 0.52 mmol), and KOH (0.39 g, 6.89 mmol) in CH_2Cl_2 (15 mL) was stirred for 20 min, benzenesulfonyl chloride (0.66 mL, 5.17 mmol) was added, and the mixture was stirred at room temperature overnight. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:3) to afford compound **28**. Yield 85%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.17 (s, 1H), 8.28 (d, J = 8.4 Hz, 2H), 7.87−7.89 (m, 2H), 7.76 (d, J = 3.6 Hz, 1H), 7.72 (d, J = 7.5 Hz, 1H), 7.44−7.57 (m, 5H).

*3-(1-Benzenesulfonyl-1H-indol-4-yl)acrylic Acid (**32**).* To a solution of **28** (0.2 g, 0.7 mmol) in CH_2Cl_2 (10 mL) was added methyl (triphenylphosphoranylidene)acetate (0.28 g, 0.84 mmol), and the mixture was stirred at room temperature. After being stirred for 16

h, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:2) to give the acrylic acid methyl ester compound. Yield 88%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03 (d, J = 8.3 Hz, 1H), 7.96 (d, J = 16.0 Hz, 1H), 7.88−7.89 (m, 2H), 7.68 (d, J = 3.7 Hz, 1H), 7.54−7.57 (m, 1H), 7.44−7.49 (m, 3H), 7.32−7.35 (m, 1H), 6.94 (d, J = 3.7 Hz, 1H), 6.51 (d, J = 16.0 Hz, 1H), 3.82 (s, 3H).

To a solution of ester compound (0.2 g, 0.58 mmol) in dioxane (10 mL), 1 M $LiOH_{(aq)}$ (1.2 mL) was added. The mixture was stirred at 40 °C. After being stirred for 12 h, the mixture was concentrated under reduced pressure. The residue was dissolved in water. Concentrated HCl was added to obtain acidic pH to produce a precipitate, which was recrystallized by CH_3OH and dried by vacuum to afford compound **32**. Yield 90%. ^{1}H NMR (500 MHz, CD_3OD): δ 8.03 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 15.9 Hz, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.87 (d, J = 3.8 Hz, 1H), 7.59−7.62 (m, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.49−7.52 (m, 2H), 7.33−7.34 (m, 1H), 7.03 (d, J = 3.8 Hz, 1H), 6.54 (d, J = 15.9 Hz, 1H).

*3-(1-Benzenesulfonyl-1H-indol-4-yl)-N-hydroxyacrylamide (**7**).* To a solution of **32** (0.2 g, 0.61 mmol), PYBOP (0.34 g, 0.65 mmol), and triethylamine (0.2 mL, 1.47 mmol) in DMF (1.5 mL) was added NH_2OTHP (0.09 g, 0.73 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 2 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (1.9 mL, 25.58 mmol) in the presence of CH_3OH (35 mL). The mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized by CH_3OH to afford the desired **7**. Yield 72%; mp 100−102 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 7.96 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.84 (d, J = 16.0 Hz, 1H), 7.73 (d, J = 3.5 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.44−7.48 (m, 3H), 7.29 (t, J = 7.5 Hz, 1H), 6.98 (d, J = 3.5 Hz, 1H), 6.50 (d, J = 16.0 Hz, 1H). MS (EI) m/z: 342 (M^+, 17%), 77 (100%). HRMS (EI) for $C_{17}H_{14}N_2O_4S$ (M^+): calcd, 342.0674; found, 342.0674.

*1-Benzenesulfonyl-1H-indole-5-carbaldehyde (**29**).* After a suspension of 1*H*-indole-5-carbaldehyde (**25**, 1.00 g, 6.89 mmol), TBAHS (0.35 g, 1.03 mmol), and KOH (0.77 g, 13.78 mmol) in CH_2Cl_2 (30 mL) was stirred for 20 min, benzenesulfonyl chloride (1.32 mL, 10.33 mmol) was added. The mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:2) to afford compound **29**. Yield 91%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.03 (s, 1H), 8.11 (d, J = 8.6 Hz, 1H), 8.06 (s, 1H), 7.89 (d, J = 7.6 Hz, 2H), 7.85−7.87 (m, 1H), 7.67 (d, J = 3.7 Hz, 1H), 7.55−7.58 (m, 1H), 7.45−7.48 (m, 2H), 6.78 (d, J = 3.7 Hz, 1H).

*3-(1-Benzenesulfonyl-1H-indol-5-yl)acrylic Acid (**33**).* To a solution of **29** (1.79 g, 6.27 mmol) in CH_2Cl_2 (25 mL), methyl (triphenylphosphoranylidene)acetate (2.52 g, 7.53 mmol) was added, and the mixture was stirred at room temperature. After being stirred overnight, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the methyl ester compound as a yellow residue for the next reaction.

To a solution of methyl ester residue in dioxane (20 mL), 1 M LiOH aqueous solution (11.7 mL) was added. The mixture was stirred at 40 °C. After being stirred for 16 h, the mixture was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was recrystallized by CH_3OH and dried by vacuum to afford compound **33**. Yield 80% (two steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, J = 8.7 Hz, 1H), 7.89 (d, J = 8.9 Hz, 2H), 7.67−7.72 (m, 2H), 7.61 (d, J = 3.7 Hz, 1H), 7.55−7.58 (m, 1H), 7.52 (dd, J = 8.7, 1.4 Hz, 1H), 7.45−7.48 (m, 2H), 6.71 (d, J = 3.7 Hz, 1H), 6.39 (d, J = 16.1 Hz, 1H).

*3-(1-Benzenesulfonyl-1H-indol-5-yl)-N-hydroxyacrylamide (**8**).* To a solution of **33** (1.00 g, 3.05 mmol), PYBOP (1.69 g, 3.24 mmol), and triethylamine (1.02 mL, 7.33 mmol) in DMF (1.5 mL) was added NH_2OTHP (0.43 g, 3.67 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 3 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (6.90 mL, 92.90 mmol) in the presence of CH_3OH (140 mL) and stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized by EtOAc/EtOH to afford the desired **8**. Yield 71%; mp 165−167 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 7.98 (d, J = 8.5 Hz, 1H), 7.93 (d, J = 7.5 Hz, 2H), 7.68−7.72 (m, 2H) 7.59−7.63 (m, 2H), 7.49−7.54 (m, 3H), 6.75 (d, J = 3.5 Hz, 1H), 6.43 (d, J = 16.0 Hz, 1H). MS (EI) m/z: 342 (M^+, 3%), 327 (100%). HRMS (EI) for $C_{17}H_{14}N_2O_4S$ (M^+): calcd, 342.0674; found, 342.0673.

*1-Benzenesulfonyl-1H-indole-6-carbaldehyde (**30**).* After a suspension of 1*H*-indole-6-carbaldehyde (**26**, 0.5 g, 3.44 mmol), TBAHS (0.18 g, 0.52 mmol), and KOH (0.39 g, 6.89 mmol) in CH_2Cl_2 (15 mL) was stirred for 20 min, benzenesulfonyl chloride (0.66 mL, 5.17 mmol) was added, and the mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:3) to afford compound **30**. Yield 87%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.08 (s, 1H), 8.49 (s, 1H), 7.91 (dd, J = 8.5, 1.0 Hz, 2H), 7.77−7.79 (m, 2H), 7.65 (d, J = 8.5 Hz, 1H), 7.56−7.59 (m, 1H), 7.46−7.49 (m, 2H), 6.74 (d, J = 3.5 Hz, 1H).

*3-(1-(Phenylsulfonyl)-1H-indol-6-yl)acrylic Acid (**34**).* To a solution of **30** (1.0 g, 3.5 mmol) in CH_2Cl_2 (20 mL) was added methyl (triphenylphosphoranylidene)acetate (1.4 g, 4.2 mmol), and the mixture was stirred at room temperature. After being stirred for 16 h, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the methyl ester compound as a yellow residue for the next reaction.

To a solution of crude methyl ester in dioxane (20 mL) was added 1 M $LiOH_{(aq)}$ (7 mL), and the mixture was stirred at 40 °C. After being stirred for 12 h, the mixture was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to give acidic pH to produce the precipitate, which was recrystallized by CH_3OH and dried by vacuum to afford compound **34**. Yield 79% (two steps). ^{1}H NMR (500 MHz, CD_3OD, DMSO-d_6): δ 8.14 (s, 1H), 7.96 (d, J = 7.6 Hz, 2H), 7.77 (d, J = 15.9 Hz, 1H), 7.75 (d, J = 3.6 Hz, 1H), 7.51−7.63 (m, 5H), 6.76 (d, J = 3.6 Hz, 1H), 6.49 (d, J = 15.9 Hz, 1H).

*3-(1-Benzenesulfonyl-1H-indol-6-yl)-N-hydroxyacrylamide (**9**).* To a solution of **34** (0.3 g, 0.92 mmol), PYBOP (0.51 g, 0.97 mmol), and triethylamine (0.31 mL, 2.2 mmol) in DMF (2 mL) was added NH_2OTHP (0.13 g, 1.10 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 3 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (2.1 mL, 28.27 mmol) in the presence of CH_3OH (42 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a red residue, which was recrystallized by CH_3OH to afford the desired **9**. Yield 64%; mp 166−168 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 8.13 (s, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.72 (d, J = 3.5 Hz, 1H), 7.67 (d, J = 15.5 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.50−7.56 (m, 3H), 7.44 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 3.5 Hz, 1H),

6.52 (d, J = 16.0 Hz, 1H). MS (EI) m/z: 342 (M^+, 11%), 77 (100%). HRMS (EI) for $C_{17}H_{14}N_2O_4S$ (M^+): calcd, 342.0674; found, 342.0674.

*1-Benzenesulfonyl-1H-indole-7-carbaldehyde (**31**).* After a suspension of 1*H*-indole-7-carbaldehyde (**27**, 0.8 g, 5.51 mmol), TBAHS (0.28 g, 0.83 mmol), and KOH (0.62 g, 11.0 mmol) in CH_2Cl_2 (20 mL) was stirred for 20 min, benzenesulfonyl chloride (1.05 mL, 8.27 mmol) was added, and the mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:4) to afford compound **31**. Yield 66%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.69 (s, 1H), 7.80 (d, J = 7.5 Hz, 1H), 7.69 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 3.7 Hz, 1H), 7.57 (d, J = 7.7 Hz, 2H), 7.49−7.52 (m, 1H), 7.35−7.38 (m, 3H), 6.78 (d, J = 3.7 Hz, 1H).

*3-(1-Benzenesulfonyl-1H-indol-7-yl)acrylic Acid (**35**).* To a solution of **31** (1.04 g, 3.65 mmol) in CH_2Cl_2 (20 mL) was added methyl (triphenylphosphoranylidene)acetate (1.46 g, 4.38 mmol), and the mixture was stirred at room temperature. After being stirred overnight, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the methyl ester as a yellow residue for the next reaction.

To a solution of crude methyl ester in dioxane (25 mL) was added 1 M $LiOH_{(aq)}$ (7.3 mL), and the mixture was stirred at 40 °C. After being stirred for 12 h, the mixture was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was recrystallized and dried by vacuum to afford compound **35**. Yield 85% (two steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.72 (d, J = 15.5 Hz, 1H), 7.85 (d, J = 3.8 Hz, 1H), 7.74 (d, J = 7.5 Hz, 2H), 7.53−7.61 (m, 2H), 7.38−7.43 (m, 3H), 7.25−7.27 (m, 1H), 6.75 (d, J = 3.8 Hz, 1H), 6.14 (d, J = 15.5 Hz, 1H).

*3-(1-Benzenesulfonyl-1H-indol-7-yl)-N-hydroxyacrylamide (**10**).* To a solution of **35** (0.2 g, 0.61 mmol), PYBOP (0.34 g, 0.65 mmol), and triethylamine (0.2 mL, 1.47 mmol) in DMF (1.5 mL) was added NH_2OTHP (0.09 g, 0.73 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 3 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (1.4 mL, 18.85 mmol) in the presence of CH_3OH (28 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized by CH_3OH to afford the desired compound **10**. Yield 62%; mp 177−178 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 8.55 (d, J = 15.5 Hz, 1H), 7.88 (d, J = 3.5 Hz, 1H), 7.73 (m, 2H), 7.55−7.59 (m, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.30 (d, J = 7.5 Hz, 1H), 7.21 (t, J = 7.5 Hz, 1H), 6.81 (d, J = 3.5 Hz, 1H), 6.00 (d, J = 15.5 Hz, 1H). MS (EI) m/z: 342 (M^+, 16%), 77 (100%). HRMS (EI) for $C_{17}H_{14}N_2O_4S$ (M^+): calcd, 342.0674; found, 342.0672.

Syntheses of 1-Arylsulfonyl-5-(*N*-hydroxyacrylamide)-indoles 11−14. *1-(4-Methoxybenzenesulfonyl)-1H-indole-5-carbaldehyde (**36**).* After a suspension of 1*H*-indole-5-carbaldehyde (**25**, 1.0 g, 6.89 mmol), TBAHS (0.35 g, 1.03 mmol), and KOH (0.77 g, 13.78 mmol) in CH_2Cl_2 (20 mL) was stirred for 20 min, 4-methoxybenzenesulfonyl chloride (2.14 g, 10.33 mmol) was added, and the mixture was stirred at room temperature overnight. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:2) to afford compound **36**. Yield 88%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.03 (s, 1H), 8.10 (d, J = 8.6 Hz, 1H), 8.06 (s, 1H), 7.83−7.86 (m, 3H), 7.66 (d, J = 3.6 Hz, 1H), 6.89−6.92 (m, 2H), 6.77 (d, J = 3.6 Hz, 1H), 3.80 (s, 3H).

*3-[1-(4-Methoxybenzenesulfonyl)-1H-indol-5-yl]acrylic Acid (**40**).* To a solution of **36** (1.91 g, 6.06 mmol) in CH_2Cl_2 (20 mL) was added methyl (triphenylphosphoranylidene)acetate (2.43 g, 7.27 mmol), and the mixture was stirred at room temperature. After being stirred overnight, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the methyl ester as a yellow residue for the next reaction.

To a solution of crude methyl ester in dioxane (40 mL) was added 1 M $LiOH_{(aq)}$ (12.2 mL), and the mixture was stirred at 40 °C. After being stirred for 12 h, the mixture was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was recrystallized and dried by vacuum to afford compound **40**. Yield 89% (two steps). ^{1}H NMR (500 MHz, CD_3OD): δ 7.98 (d, J = 8.7 Hz, 1H), 7.85−7.88 (m, 2H), 7.76 (s, 1H), 7.71 (d, J = 16.0 Hz, 1H), 7.66 (d, J = 3.6 Hz, 1H), 7.56 (d, J = 8.5 Hz, 1H), 6.98−7.01 (m, 2H), 6.74 (d, J = 3.6 Hz, 1H), 6.44 (d, J = 16.0 Hz, 1H), 3.79 (s, 3H).

*N-Hydroxy-3-[1-(4-methoxybenzenesulfonyl)-1H-indol-5-yl]-acrylamide (**11**).* To a solution of **40** (0.2 g, 0.56 mmol), PYBOP (0.32 g, 0.62 mmol), and triethylamine (0.19 mL, 1.34 mmol) in DMF (2 mL) was added NH_2OTHP (0.08 g, 0.67 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 3 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (1.3 mL, 17.34 mmol) in the presence of CH_3OH (27 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized by CH_3OH to afford compound **11**. Yield 78%; mp 90−92 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 7.96 (d, J = 8.5 Hz, 1H), 7.84−7.87 (m, 2H), 7.71 (s, 1H), 7.64 (d, J = 3.5 Hz, 1H), 7.61 (d, J = 15.5 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 6.97−6.99 (m, 2H), 6.72 (d, J = 3.5 Hz, 1H), 6.43 (d, J = 15.5 Hz, 1H), 3.78 (s, 3H). MS (EI) m/z: 372 (M^+, 5%), 171 (100%). HRMS (EI) for $C_{18}H_{16}N_2O_5$ (M^+): calcd, 372.0780; found, 372.0779.

*3-[1-(3,4-Dimethoxybenzenesulfonyl)-1H-indol-5-yl]acrylic Acid (**41**).* After a suspension of 1*H*-indole-5-carbaldehyde (**25**, 0.4 g, 2.76 mmol), TBAHS (0.14 g, 0.41 mmol), and KOH (0.31 g, 5.51 mmol) in CH_2Cl_2 (15 mL) was stirred for 20 min, 3,4-dimethoxybenzenesulfonyl chloride (0.98 g, 4.13 mmol) was added, and the mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue **37**, which was dissolved in CH_2Cl_2 (15 mL) and then treated with methyl (triphenylphosphoranylidene)acetate (1.11 g, 3.31 mmol) at room temperature. After being stirred overnight, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the methyl ester compound as a yellow residue for the next reaction.

To a solution of crude methyl ester in dioxane (25 mL) was added 1 M $LiOH_{(aq)}$ (5.5 mL), and the mixture was stirred at 40 °C. After the mixture was stirred for 12 h, the reaction was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was recrystallized and dried by vacuum to afford compound **41**. Yield 78% (three steps). ^{1}H NMR (500 MHz, CD_3OD): δ 8.00 (d, J = 8.7 Hz, 1H), 7.77 (s, 1H), 7.71 (d, J = 16.0 Hz, 1H), 7.68 (d, J = 3.6 Hz, 1H), 7.55−7.59 (m, 2H), 7.34 (d, J = 2.3 Hz, 1H), 6.99−7.02 (m, 1H), 6.74 (d, J = 3.6 Hz, 1H), 6.44 (d, J = 16.0 Hz, 1H), 3.81 (s, 3H), 3.78 (s, 3H).

*3-[1-(3,4-Dimethoxybenzenesulfonyl)-1H-indol-5-yl]-N-hydroxyacrylamide (**12**).* To a solution of **41** (0.2 g, 0.52 mmol), PYBOP (0.3 g, 0.57 mmol), and triethylamine (0.17 mL, 1.25 mmol) in DMF (2 mL) was added NH_2OTHP (0.07 g, 0.62 mmol), and the mixture

was stirred at room temperature. After the mixture was stirred for 2 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (1.2 mL, 16.1 mmol) in the presence of CH_3OH (25 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a yellow residue, which was recrystallized by CH_3OH to afford the desired compound **12**. Yield 53%; mp 146−148 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 7.99 (d, J = 8.5 Hz, 1H), 7.72 (s, 1H), 7.67 (d, J = 3.5 Hz, 1H) 7.62 (d, J = 16.0 Hz, 1H), 7.55 (dd, J = 2.0, 8.5 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.33 (d, J = 2.0 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 3.5 Hz, 1H), 6.43 (d, J = 16.0 Hz, 1H), 3.81 (s, 3H), 3.78 (s, 3H). MS (EI) m/z: 387 (M^+ − 15, 59%), 137 (100%). HRMS (EI) for $C_{19}H_{18}N_2O_6S$ (M^+): calcd, 402.0886; found, 402.0884.

*1-(4-Fluorobenzenesulfonyl)-1H-indole-5-carbaldehyde (**38**).* After a suspension of 1*H*-indole-5-carbaldehyde (**25**, 1.0 g, 6.89 mmol), TBAHS (0.35 g, 1.03 mmol), and KOH (0.77 g, 13.78 mmol) in CH_2Cl_2 (20 mL) was stirred for 20 min, 4-fluorobenzenesulfonyl chloride (2.01 g, 10.33 mmol) was added. The mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:5) to afford compound **38**. Yield 76%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.04 (s, 1H), 8.08−8.11 (m, 2H), 7.92−7.94 (m, 2H), 7.87 (d, J = 8.7 Hz, 1H), 7.65 (d, J = 3.6 Hz, 1H), 7.08−7.16 (m, 2H), 6.80 (d, J = 3.6 Hz, 1H).

*3-[1-(4-Fluorobenzenesulfonyl)-1H-indol-5-yl]acrylic Acid (**42**).* To a solution of **38** (1.0 g, 3.30 mmol) in CH_2Cl_2 (25 mL) was added methyl (triphenylphosphoranylidene)acetate (1.32 g, 3.96 mmol), and the mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the methyl ester yellow residue for the next reaction.

To a solution of crude methyl ester in dioxane (30 mL) was added 1 M $LiOH_{(aq)}$ (6.6 mL) was added, and the mixture was stirred at 40 °C. After being stirred for 12 h, the mixture was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was recrystallized and dried by vacuum to afford compound **42**. Yield 90% (two steps). ^{1}H NMR (500 MHz, CD_3OD): δ 8.00−8.03 (m, 3H), 7.79 (s, 1H), 7.71 (d, J = 15.9 Hz, 1H), 7.69 (d, J = 3.7 Hz, 1H), 7.59 (d, J = 8.8 Hz, 1H), 7.24−7.28 (m, 2H), 6.78 (d, J = 3.7 Hz, 1H), 6.45 (d, J = 15.9 Hz, 1H).

*3-[1-(4-Fluorobenzenesulfonyl)-1H-indol-5-yl]-N-hydroxyacrylamide (**13**).* To a solution of **42** (0.2 g, 0.58 mmol), PYBOP (0.33 g, 0.64 mmol), and triethylamine (0.19 mL, 1.39 mmol) in DMF (1.5 mL) was added NH_2OTHP (0.08 g, 0.70 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 3 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (1.3 mL, 18 mmol) in the presence of CH_3OH (28 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized by CH_3OH to afford the desired compound **13**. Yield 76%; mp 138−140 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 7.97−8.01 (m, 3H), 7.72 (s, 1H), 7.67 (d, J = 3.5 Hz, 1H), 7.62 (d, J = 15.5 Hz, 1H), 7.54 (t, J = 8.5 Hz, 1H), 7.24 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 3.5 Hz, 1H), 6.44 (d, J = 15.5 Hz, 1H). MS (EI) m/z: 360 (M^+, 0.6%), 345 (100%). HRMS (EI) for $C_{17}H_{13}FN_2O_4S$ (M^+): calcd, 360.0580; found, 360.0580.

*1-(4-Nitrobenzenesulfonyl)-1H-indole-5-carbaldehyde (**39**).* After a suspension of 1*H*-indole-5-carbaldehyde (**25**, 1.5 g, 10.33 mmol), TBAHS (0.53 g, 1.55 mmol), and KOH (1.16 g, 20.67 mmol) in CH_2Cl_2 (25 mL) was stirred for 20 min, 4-nitrobenzenesulfonyl chloride (3.44 g, 15.5 mmol) was added. The mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:3) to afford compound **39**. Yield 82%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.04 (s, 1H), 8.29−8.32 (m, 2H), 8.07−8.13 (m, 4H), 7.89 (dd, J = 9.0, 2.0 Hz, 1H), 7.65 (d, J = 3.5 Hz, 1H), 6.85 (d, J = 3.5 Hz, 1H).

*3-[1-(4-Nitrobenzenesulfonyl)-1H-indol-5-yl]acrylic Acid (**43**).* To a solution of **39** (0.50 g, 1.51 mmol) in CH_2Cl_2 (15 mL) was added methyl (triphenylphosphoranylidene)acetate (0.61 g, 1.82 mmol), and the mixture was stirred at room temperature. After being stirred overnight, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a methyl ester yellow residue for the next reaction.

To a solution of crude methyl ester in dioxane (15 mL) was added 1 M $LiOH_{(aq)}$ (3.1 mL), and the mixture was stirred at 40 °C. After being stirred for 12 h, the mixture was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was recrystallized and dried by vacuum to afford compound **43**. Yield 82% (two steps). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.34−8.35 (m, 2H), 8.24−8.27 (m, 2H), 7.94 (d, J = 8.5 Hz, 1H), 7.92 (s, 1H), 7.89 (d, J = 3.5 Hz, 1H), 7.70 (dd, J = 9.0, 1.5 Hz, 1H), 7.61 (d, J = 16.0 Hz, 1H), 6.91 (d, J = 3.5 Hz, 1H), 6.48 (d, J = 16.0 Hz, 1H).

*N-Hydroxy-3-[1-(4-nitrobenzenesulfonyl)-1H-indol-5-yl]-acrylamide (**14**).* To a solution of **43** (0.2 g, 0.54 mmol), PYBOP (0.31 g, 0.59 mmol), and triethylamine (0.18 mL, 1.3 mmol) in DMF (1.5 mL) was added NH_2OTHP (0.08 g, 0.65 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 3 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (1.2 mL, 16.7 mmol) in the presence of CH_3OH (27 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized by CH_3OH to afford the desired compound **14**. Yield 65%; mp 154−156 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 8.33 (d, J = 8.5 Hz, 2H), 8.19 (d, J = 8.5 Hz, 2H), 8.01 (d, J = 8.5 Hz, 1H), 7.72−7.74 (m, 2H), 7.61 (d, J = 16.0 Hz, 1H), 7.57 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 3.5 Hz, 1H), 6.45 (d, J = 16.0 Hz, 1H). MS (EI) m/z: 387 (M^+, 0.21%), 372 (100%). HRMS (EI) for $C_{17}H_{13}N_3O_6S$ (M^+): calcd, 387.0525; found, 387.0523.

Synthesis of 1-Phenylsulfonyl-5-hydroxyamideindole (15). *1-Benzenesulfonyl-1H-indole-5-carboxylic Acid (**45**).* After a suspension of methyl indole-5-carboxylate (**44**, 0.30 g, 1.71 mmol), TBAHS (0.19 g, 0.26 mmol), and KOH (0.19 g, 3.42 mmol) in CH_2Cl_2 (15 mL) was stirred for 20 min, benzenesulfonyl chloride (0.32 mL, 2.57 mmol) was added, and the mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:4) to afford the 1-phenylsulfonyl indole compound as a white solid. Yield 89%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.26 (s, 1H), 7.99−8.04 (m, 2H), 7.87−7.89 (m, 2H), 7.62 (d, J = 3.5 Hz, 1H), 7.54−7.57 (m, 1H), 7.43−7.47 (m, 2H), 6.73 (d, J = 3.5 Hz, 1H), 3.91 (s, 3H).

To a solution of 1-phenylsulfonylindole (0.52 g, 1.65 mmol) in dioxane (15 mL) was added 1 M LiOH aqueous solution (3.8 mL), and the mixture was stirred at 40 °C. After being stirred overnight, the mixture was concentrated under reduced pressure. The residue was

dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was dried by vacuum to afford compound **45**. Yield 85%. ^{1}H NMR (500 MHz, CD_3OD): δ 8.25 (d, J = 0.8 Hz, 1H), 8.03 (d, J = 8.9 Hz, 1H), 7.97 (dd, J = 8.9, 1.5 Hz, 1H), 7.95 (d, J = 7.6 Hz, 2H), 7.75 (d, J = 3.7 Hz, 1H), 7.60−7.63 (m, 1H), 7.51−7.54 (m, 2H), 6.83 (d, J = 3.7 Hz, 1H).

*1-Benzenesulfonyl-1H-indole-5-carboxylic Acid Hydroxyamide (**15**).* To a solution of **45** (0.18 g, 0.60 mmol), PYBOP (0.33 g, 0.63 mmol), and triethylamine (0.20 mL, 1.43 mmol) in DMF (1.5 mL) was added NH_2OTHP (0.08 g, 0.72 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 2 h, the reaction was quenched with water, followed by extraction with EtOAc (15 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (1.70 mL, 22.89 mmol) in the presence of CH_3OH (31 mL). The mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was purified by silica gel chromatography (CH_2Cl_2/CH_3OH = 30:1) to afford the desired **15** as a colorless oil. Yield 53%. ^{1}H NMR (500 MHz, CD_3OD): δ 8.05 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 7.5 Hz, 3H), 7.74 (d, J = 3.5 Hz, 1H), 7.68 (d, J = 7.5 Hz, 1H), 7.61 (t, J = 7.5 Hz, 1H), 7.51 (t, J = 8.0 Hz, 2H), 6.81 (d, J = 3.5 Hz, 1H). MS (EI) m/z: 316 (M^+, 6%), 315 (24%), 77 (100%). HRMS (EI) for $C_{15}H_{12}N_2O_4S$ (M^+): calcd, 316.0518; found, 316.0518.

Syntheses of 1-Benzoyl, Benzyl, and Phenyl-5-(*N*-hydroxyacrylamide)indoles 16−18. *1-Benzoyl-1H-indole-5-carbaldehyde (**46**).* After a suspension of 1*H*-indole-5-carbaldehyde (**25**, 0.50 g, 2.85 mmol), KI (0.64 g, 1.43 mmol), and potassium *tert*-butoxide (0.64 g, 5.71 mmol) in DMF (2 mL) was stirred for 15 min, benzoyl bromide (0.50 mL, 4.28 mmol) was added, and the mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and dried with vacuum to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:2) to afford compound **46**. Yield 87%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.10 (s, 1H), 8.50 (d, J = 8.5 Hz, 1H), 8.15 (d, J = 2.0 Hz, 1H), 7.92 (dd, J = 9.0, 2.0 Hz, 1H), 7.75−7.77 (m, 2H), 7.63−7.66 (m, 1H), 7.54−7.57 (m, 2H), 7.42 (d, J = 3.5 Hz, 1H), 6.74 (d, J = 3.5 Hz, 1H).

*3-(1-Benzoyl-1H-indol-5-yl)acrylic Acid (**49**).* To a solution of **46** (0.62 g, 2.03 mmol) in CH_2Cl_2 (15 mL) was added methyl (triphenylphosphoranylidene)acetate (0.81 g, 2.44 mmol), and the mixture was stirred at room temperature. After being stirred for 16 h, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a methyl ester yellow residue for the next reaction.

To a solution of crude methyl ester in dioxane (10 mL) was added 1 M $LiOH_{(aq)}$ (4.1 mL), and the mixture was stirred at 40 °C. After being stirred for 12 h, the mixture was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was recrystallized and dried by vacuum to afford compound **49**. Yield 82% (two steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (d, J = 8.6 Hz, 1H), 7.66−7.76 (m, 4H), 7.47−7.58 (m, 4H), 7.27 (d, J = 3.7 Hz, 1H), 6.58 (d, J = 3.7 Hz, 1H), 6.39 (d, J = 15.9 Hz, 1H).

*3-(1-Benzoyl-1H-indol-5-yl)-N-hydroxyacrylamide (**16**).* To a solution of **49** (0.1 g, 0.34 mmol), PYBOP (0.05 g, 0.41 mmol), and triethylamine (0.12 mL, 0.82 mmol) in DMF (1.5 mL) was added NH_2OTHP (0.05 g, 0.41 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 3 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (0.78 mL, 10.5 mmol) in the presence of CH_3OH (16 mL). The mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized by CH_3OH to afford the desired **16**. Yield 49%; mp 146−148 °C. ^{1}H NMR (500 MHz, DMSO): δ 9.00 (s, 1H), 8.25 (d, J = 8.5 Hz, 1H), 7.85 (s, 1H), 7.76 (d, J = 7.5 Hz, 2H), 7.69 (t, J = 7.5 Hz, 1H), 7.54−7.61 (m, 4H), 7.42 (d, J = 3.5 Hz, 1H), 6.78 (d, J = 3.5 Hz, 1H), 6.48 (d, J = 15.5 Hz, 1H). MS (EI) m/z: 306 (M^+, 17%), 105 (100%). HRMS (EI) for $C_{18}H_{14}N_2O_3$ (M^+): calcd, 306.1004; found, 306.1006.

*1-Benzyl-1H-indole-5-carbaldehyde (**47**).* After a suspension of 1*H*-indole-5-carbaldehyde (**25**, 0.5 g, 3.44 mmol), KI (0.29 g, 1.72 mmol), and potassium *tert*-butoxide (0.77 g, 6.89 mmol) in DMF (3 mL) was stirred for 15 min, benzyl chloride (0.8 mL, 6.89 mmol) was added. The mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and extraction was with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and dried with vacuum to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:3) to afford compound **47**. Yield 85%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.02 (s, 1H), 8.17 (d, J = 1.1 Hz, 1H), 7.73 (dd, J = 8.6, 1.1 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.26−7.33 (m, 3H), 7.22 (d, J = 3.0 Hz, 1H), 7.10−7.12 (m, 2H), 6.71 (d, J = 3.0 Hz, 1H), 5.36 (s, 2H).

*3-(1-Benzyl-1H-indol-5-yl)acrylic Acid (**50**).* To a solution of **47** (0.3 g, 1.28 mmol) in CH_2Cl_2 (10 mL) was added methyl (triphenylphosphoranylidene)acetate (0.51 g, 1.53 mmol), and the mixture was stirred at room temperature. After being stirred for 16 h, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and dried with vacuum to give a brown residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:3) to afford the methyl ester compound as an orange solid. Yield 85%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.82 (d, J = 16.0 Hz, 1H), 7.80 (s, 1H), 7.39 (dd, J = 8.5, 1.5 Hz, 1H), 7.27−7.32 (m, 4H), 7.14 (d, J = 3.0 Hz, 1H), 7.10 (d, J = 7.0 Hz, 2H), 6.58 (d, J = 3.0 Hz, 1H), 6.39 (d, J = 16.0 Hz, 1H), 5.32 (s, 2H), 3.81 (s, 3H).

To a solution of methyl ester (0.32 g, 1.10 mmol) in dioxane (10 mL) was added 1 M $LiOH_{(aq)}$ (2.2 mL), and the mixture was stirred at 40 °C. After being stirred overnight, the mixture was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was dried by vacuum to afford compound **50**. Yield 91%. ^{1}H NMR (500 MHz, CD_3OD): δ 7.76−7.79 (m, 2H), 7.39 (dd, J = 8.5, 1.5 Hz, 1H), 7.22−7.34 (m, 5H), 7.12 (d, J = 7.0 Hz, 2H), 6.55 (d, J = 3.0 Hz, 1H), 6.35 (d, J = 16.0 Hz, 1H), 5.39 (s, 2H).

*3-(1-Benzyl-1H-indol-5-yl)-N-hydroxyacrylamide (**17**).* To a solution of **50** (0.1 g, 0.4 mmol), PYBOP (0.23 g, 0.44 mmol), and triethylamine (0.13 mL, 0.96 mmol) in DMF (1 mL) was added NH_2OTHP (0.06 g, 0.48 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 1 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (0.92 mL, 12.4 mmol) in the presence of CH_3OH (19 mL). The mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized by CH_3OH to afford the desired **17**. Yield 81%; mp 133−135 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.66 (d, J = 15.0 Hz, 1H), 7.20−7.36 (m, 6H), 7.12 (d, J = 7.0 Hz, 2H), 6.53 (d, J = 3.0 Hz, 1H), 6.37 (d, J = 15.0 Hz, 1H), 5.38 (s, 2H). MS (EI) m/z: 292 (M^+, 19%), 91 (100%). HRMS (EI) for $C_{18}H_{16}N_2O_2$ (M^+): calcd, 292.1212; found, 292.1213.

*1-Phenyl-1H-indole-5-carbaldehyde (**48**).* A suspension of 1*H*-indole-5-carbaldehyde (**25**, 0.70 g, 4.82 mmol), 4-iodobenzene (0.65 mL, 5.79 mmol), and K_2CO_3 (0.93 g, 6.75 mmol), CuO (0.04 g, 0.48 mmol) in DMF (2 mL) was refluxed for 2 days. The reaction mixture

was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:4) to afford compound **48**. Yield 28%. ^{1}H NMR (500 MHz, CD_3OD): δ 10.06 (s, 1H), 8.21 (d, J = 1.5 Hz, 1H), 7.78 (dd, J = 9.0, 1.5 Hz, 1H), 7.54−7.60 (m, 3H), 7.49−7.51 (m, 2H) 7.42−7.44 (m, 2H), 6.83 (d, J = 3.2 Hz, 1H).

*3-(1-Phenyl-1H-indol-5-yl)acrylic Acid (**51**).* To a solution of **48** (0.29 g, 1.31 mmol) in CH_2Cl_2 (10 mL) was added methyl (triphenylphosphoranylidene)acetate (0.52 g, 1.57 mmol), and the mixture was stirred at room temperature. After being stirred for 16 h, the reaction mixture was quenched with water and extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and dried with vacuum to give a brown residue, which was purified by silica gel chromatography (EtOAc/*n*-hexane = 1:5) to afford the methyl ester compound as a yellow liquid. Yield 92%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83−7.86 (m, 2H), 7.35−7.55 (m, 8H), 6.71 (d, J = 3.1 Hz, 1H), 6.42 (d, J = 15.9 Hz, 1H), 3.81 (s, 3H).

To a solution of the methyl ester compound (0.31 g, 1.12 mmol) in dioxane (10 mL) was added 1 M $LiOH_{(aq)}$ (2.3 mL), and the mixture was stirred at 40 °C. After being stirred for 12 h, the mixture was concentrated under reduced pressure. The residue was dissolved in water, and concentrated HCl was added to obtain acidic pH to produce the precipitate, which was dried by vacuum to afford compound **51**. Yield 48%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85 (s, 1H), 7.78 (d, J = 15.9 Hz, 1H), 7.47−7.57 (m, 7H), 7.38−7.41 (m, 1H), 6.71 (d, J = 3.0 Hz, 1H), 6.41 (d, J = 15.9 Hz, 1H).

*N-Hydroxy-3-(1-phenyl-1H-indol-5-yl)acrylamide (**18**).* To a solution of **51** (0.2 g, 0.76 mmol), PYBOP (0.43 g, 0.84 mmol), and triethylamine (0.25 mL, 1.82 mmol) in DMF (1.5 mL) was added NH_2OTHP (0.11 g, 0.91 mmol), and the mixture was stirred at room temperature. After the mixture was stirred for 2 h, the reaction was quenched with water, followed by extraction with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH_2Cl_2/CH_3OH = 30:1)/1% $NH_{3(aq)}$) to give a white solid, which was treated with TFA (1.8 mL, 24.2 mmol) in the presence of CH_3OH (36 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized by CH_3OH to afford the desired **18**. Yield 44%; mp 97−100 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 7.82 (s, 1H), 7.70 (d, J = 15.5 Hz, 1H), 7.51−7.58 (m, 5H), 7.48 (d, J = 3.0 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.39−7.42 (m, 1H), 6.71 (d, J = 3.0 Hz, 1H), 6.42 (d, J = 15.5 Hz, 1H). HRMS (EI) for $C_{17}H_{14}N_2O_2$ (M^+): calcd, 278.1055; found, 278.1055.

Synthesis of 5-(*N*-Hydroxyacrylamide)indole 19. *N-Hydroxy-3-(1H-indol-5-yl)acrylamide (**19**).* **8** (0.20 g, 0.58 mmol) was dissolved in MeOH (8 mL), and to the mixture was added 1 M KOH (aq) (1.16 mL, 1.16 mmol). The mixture was stirred and refluxed for 1 h. The reaction was quenched by water, and extraction was with ethyl acetate (30 mL × 3). The organic layer was collected and dried over anhydrous $MgSO_4$ and concentrated in vacuo to yield a colorless product. The residue was purified by flash column chromatography over silica gel (EtOAc/*n*-hexane = 2:1) to afford **19**. Yield 51%; mp 162−164 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 8.17 (d, J = 9.0 Hz, 1H), 7.75 (s, 1H), 7.65−7.68 (m, 2H), 7.52 (d, J = 3.0 Hz, 1H), 6.65 (d, J = 4.0 Hz, 1H), 6.46 (d, J = 15.5 Hz, 1H). HRMS (ESI) for $C_{11}H_9N_2O_2$ ($M - H$)$^-$: calcd, 201.0670; found, 201.0681.

Synthesis of 1-Benzenesulfonyl-5-(*N*-hydroxypropanamide)indole 20. *N-Hydroxy-3-(1-(phenylsulfonyl)-1H-indol-5-yl)propanamide (**20**).* **8** (0.20 g, 0.58 mmol) was dissolved in CH_2Cl_2 (10 mL), and to the mixture was added 10% Pd/C (0.01 g, 0.06 mmol). The mixture was stirred at room temperature for 1 h and filtered via Celite by CH_2Cl_2 wash. The residue was purified by flash column over silica gel (EtOAc/*n*-hexane = 2:1) to afford **20**. Yield 30%; mp 116−118 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 7.86−7.89 (m, 3H), 7.57−7.60 (m, 2H), 7.48 (t, J = 7.0 Hz, 2H), 7.37 (s, 1H), 7.17 (dd, J = 1.5 Hz, 8.5 Hz, 1H), 6.66 (d, J = 3.5 Hz, 1H), 2.96 (t, J = 7.5 Hz, 2H), 2.36 (t, J = 7.5 Hz, 2H). HRMS (ESI) for $C_{17}H_{15}N_2O_4S$ ($M - H$)$^-$: calcd, 343.0758; found, 343.0737.

Synthesis of 3-Benzenesulfonyl-5-(*N*-hydroxypropanamide)indole 21. *Methyl 3-(1H-Indol-5-yl)-acrylate (**52**).* To a mixture of compound **25** (2.00 g, 13.78 mmol) and CH_2Cl_2 (20 mL) was added methyl (triphenylphosphoranylidene)acetate (6.91 g, 20.67 mmol). The mixture was stirred at room temperature for overnight. The reaction was quenched by water, and extraction was with CH_2Cl_2 (30 mL × 3). The organic layer was collected and dried over anhydrous $MgSO_4$ and concentrated in vacuum to yield a yellow product. The residue was purified by flash column over silica gel (EtOAc/*n*-hexane = 2:1) to afford **52**. Yield 99%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.32 (br, 1H), 7.84 (d, J = 15.5 Hz, 1H), 7.81 (s, 1H), 7.43 (dd, J = 2.0 Hz, 10.5 Hz, 1H), 7.38 (d, J = 8.5 Hz, 1H), 7.24 (t, d, J = 3.0 Hz, 1H), 6.59 (s, 1H), 6.42 (d, J = 15.5 Hz, 1H), 3.80 (s, 3H).

*Methyl 3-(3-(Phenylthio)-1H-indol-5-yl)acrylate (**53**).* To a mixture of NaH (0.30 g, 2.24 mmol) and DMF (2 mL) was added **52** (0.30 g, 1.49 mmol) at 0 °C, and the mixture was stirred at room temperature for 90 min. To the mixture was added phenyl disulfide (0.36 g, 1.64 mmol), and the mixture was stirred at room temperature for overnight. The reaction was quenched by water at 0 °C, and extraction was with ethyl acetate (30 mL × 3). The organic layer was collected and dried over anhydrous $MgSO_4$ and concentrated in vacuum to yield an orange product. The residue was purified by flash column over silica gel (EtOAc/*n*-hexane = 1:4) to afford **53**. Yield 42%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.58 (br, 1H), 7.76−7.80 (m, 2H), 7.52 (d, J = 2.5 Hz, 1H), 7.48 (dd, J = 1.0 Hz, 8.5 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.17 (t, J = 8.0 Hz, 2H), 7.11 (d, J = 7.0 Hz, 2H), 7.07 (t, J = 7.0 Hz, 1H), 6.39 (d, J = 16.0 Hz, 1H), 3.78 (s, 3H).

*Methyl 3-(3-(Phenylsulfonyl)-1H-indol-5-yl)acrylate (**54**).* To a mixture of **53** (0.30 g, 097 mmol) and CH_2Cl_2 (10 mL) was added mCPBA (0.50 g, 2.91 mmol) at 0 °C, and the mixture was stirred at room temperature overnight. The reaction was quenched by saturated $NaHCO_3$ (aq), and extraction was with CH_2Cl_2 (30 mL × 3). The organic layer was collected and dried over anhydrous $MgSO_4$ and concentrated in vacuum to yield an orange product. The residue was purified by flash column over silica gel (EtOAc/*n*-hexane = 1:1) to afford **54**. Yield 46%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.22 (s, 1H), 8.03−8.07 (m, 3H), 7.81 (d, J = 16.0 Hz, 1H), 7.67 (dd, J = 1.5 Hz, 8.0 Hz, 1H), 7.54−7.60 (m, 3H), 7.51 (d, J = 8.5 Hz, 1H), 6.57 (d, J = 16.0 Hz, 1H), 3.73 (s, 3H).

*3-(3-(Phenylsulfonyl)-1H-indol-5-yl)acrylic Acid (**55**).* To a mixture of **54** (0.20 g, 0.59 mmol) and dioxane (1 mL) was added 1 M LiOH (aq) (1.18 mL, 1.18 mmol), and the mixture was stirred overnight at 40 °C. Solvent was removed, and the remainder was dissolved in water. To the water layer was added 3 N HCl (aq), and gravity filtration yielded a white product. The product, without more purification, was **55**. Yield 52%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.21 (s, 1H), 8.03−8.05 (m, 2H), 7.97 (s, 1H), 7.80 (d, J = 16.0 Hz, 1H), 7.54−7.62 (m, 4H), 7.51 (d, J = 8.5 Hz, 1H), 6.46 (d, J = 16.0 Hz, 1H).

*3-(3-Benzenesulfonyl-1H-indol-5-yl)-N-hydroxyacrylamide (**21**).* A mixture of **55** (0.60 g, 1.83 mmol), EDC (0.53 g, 2.75 mmol), HOBt (0.30 g, 2.20 mmol), *N*-methylmorphine (0.48 mL, 4.39 mmol), and DMF (1.5 mL) was stirred for a while. Then to the mixture was added *o*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (0.26 g, 2.20 mmol), and the mixture was stirred at room temperature overnight. The residue was purified by flash column over silica gel (EtOAc/*n*-hexane = 4: 1) to yield the colorless oil product. The product was dissolved in MeOH (2 mL), and to the mixture was added 10% TFA (aq) (2 mL). The mixture was stirred at room temperature overnight. To the mixture was added water, and gravity filtration afforded **21**. Yield 26%; mp 180−182 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.21 (s, 1H), 8.01 (d, J = 7.0 Hz, 2H), 7.95 (s, 1H), 7.54−7.60 (m, 4H), 7.51 (d, J = 9.0 Hz, 1H), 7.45 (d, J = 8.5 Hz,

1H), 6.45 (d, J = 15.5 Hz, 1H). MS (EI) m/z: 342 (M^+, 3%), 327 (100%). HRMS (EI) for $C_{17}H_{14}N_2O_4S$ (M^+): calcd, 342.0674; found, 342.0673.

Biology. HeLa Nuclear Extract HDAC Activity Assay.[17] The HeLa nuclear extract HDAC activity was measured by using the 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. TSA at 1 μM 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.[18] The HDAC in vitro activities of recombinant human HDACs 1, 2, 6, and 8 (BPS Biosciences) were detected by fluorigenic release of 7-amino-4-methylcoumarin from substrate upon deacetylase enzymatic activity.

Tumor Cell Culture. All human cancer cells were maintained in RPMI 1640 medium containing 100 units/mL penicillin G sodium, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B, and 25 μg/mL gentamicin. The medium was supplemented with 10% fetal bovine serum and 2 mM glutamine. 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.

Sulforhodamine B Assays.[19] Human cancer A549 (non-small-cell lung cancer), MDA-MB-231 (estrogen-independent breast cacner), Hep 3B (hepatoma), and PC-3 (prostate) 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 cell population at the time of compound addition (T_0). 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 cells. Unbound SRB was washed out by 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 absorbance parameters time zero (T_0), control growth (C), and cell growth in the presence of the compound (T_x), the percentage growth was calculated at each of the compound concentrations levels. Growth inhibition of 50% (GI_{50}) was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, 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.[20] PC-3 and A549 cells were treated with vehicle (0.1% DMSO) and a test compound at 1, 2.5, 5, 10, and 20 μM in RPMI 1640 supplemented with 10% FBS for 48 h. The cells were collected and sonicated. Protein concentrations in the resultant lysates were determined by 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) in a semidry 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 (antibody obtained from Upstate Biotechnology, Inc., Lake Placid, NY), H3 (Upstate Biotechnology), acetyl α-tubulin (Sigma-Aldrich, St. Louis, MO), or α-tubulin (Sigma-Aldrich, St. Louis, MO) (1:3000 dilution) 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 the samples were washed at least three times with TBST, the signals were developed using the enhanced chemiluminescence system (Pierce). Quantitative analysis of Western blot was done with ImageQuant (Molecular Dynamics, U.S.)

Solubility Determination in Water and Ethanol. Solubility was determinations by the shake-flask method. Briefly, 1 mg of a test compound was added into water or ethanol (1 mL). The mixture was sonicated for 10 min and then shaken for 24 h at room temperature. After centrifugation, an aliquot of the clear supernatant was transferred into vials and the target compound present in the sample was determined by reversed phase HPLC and UV detection at 328 nm.

Pharmacokinetic Evaluation. The in vivo pharmacokinetic study was approved by Institutional Animal Care and Use Committee of National Health Research Institutes. A 1 and 5- mg/mL doing solution was preparing by dissolving appropriate amount compound in a mixture of PEG400/DMSO (80:20,v/v) for intravenous administration and 1% carboxylmethyl cellulose, 0.5% Tween 80 for oral dosing, respectively. Male Sprague–Dawley rats, weighing 250–350 g each (8–10 weeks old), were obtained from BioLASCO, Ilan, Taiwan. Tested compound was separately administered to group of three male rats intravenously (2 mg/kg dose) by a bolus injection to the jugular vein or periorally (20 mg/kg dose). The volume of dosing solution administered was adjusted according to the body weight recorded before dose administration. At 0 (prior to dosing), 2, 5 (IV only), 15, and 30 min and at 1, 2, 4, 6, 8, and 24 h after dosing, a blood sample (~150 μL) was collected from each animal via the jugular-vein cannula and stored in ice (0–4 °C). Plasma was separated from the blood by centrifugation (14,000 × g for 15 min at 4 °C) and stored in a freezer (−80 °C). All samples were analyzed for the test compound by LC-MS/MS (ABI4000). Data were acquired via multiple reactions monitoring. Plasma concentration data were analyzed with standard noncompartmental method.

Antitumor Activity in Vivo.[21] Female 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 LabDiet consisting of 20.0% crude protein, 9.9% crude fat, and 4.7% crude fiber. The mice were housed at National Taiwan University Laboratory Animal Center, NTUMC, on a 12 h 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 four groups of seven mice. All doses were administered at a volume of 10 mL/kg (0.2 mL/20 g mouse), scaled to the body weight of each animal. Control group 1 mice received vehicle daily po to the end point. Group 2 received reference **1** daily at 200 mg/kg po to the end point. Groups 3 and 4 received compound **8** daily at 200 and 100 mg/kg po, respectively, to the end schedule.

The human A549 lung adenocarcinoma cells used for implantation were harvested during log phase growth and resuspended in phosphate-buffered saline at 5 × 10^7 cells/mL. Each mouse was injected sc in the right flank with 1 × 10^7 cells (0.2 mL cell suspension). Tumor size, in mm^3, was calculated from the following: tumor volume = $w^2l/2$, where w is the width and l is the length in mm of the tumor. Tumor weight can be estimated with the assumption that 1 mg is equivalent to 1 mm^3 of tumor volume. Mice were sorted into four groups of seven mice. Each animal was euthanized when the tumors reached the predetermined end point size of 1000 mm^3. The time to end point (TTE) for each mouse was calculated by the following equation: TTE = $(\log_{10}(\text{end point volume}) - b)/m$, where TTE is expressed in days, end point volume is in mm^3, b is the intercept, and m is the slope of the line obtained by linear regression of a log-transformed tumor growth data set. The data set is comprised of the first observation that exceeded the study end point volume and the three consecutive observations that immediately preceded the attainment of the end point volume. The calculated TTE is usually less than the day on which an animal is euthanized for tumor size. Treatment efficacy was determined from tumor growth delay (TGD), which is defined as the increase in the median TTE for a treatment group compared to the control group, TGD = $T - C$, expressed in days, or as a percentage of the median TTE of the control group, % TGD = $(T - C)/C \times 100$, where T is the median TTE for a treatment group, C is the median TTE for control group 1. Animals were weighed daily for the first 5 days, then twice weekly until the completion of the study. In addition, we also determined tumor growth inhibition (TGI), whose antitumor effects are expressed as % T/C (treated versus control), dividing the tumor volumes from

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treatment groups by those of the control groups and multiplied by 100. The mice were examined frequently for overt signs of any adverse, drug-related side effects.

Statistical and Graphical Analyses. The log rank test was used to determine the statistical significance of the difference between the TTE values of two groups. Statistical and graphical analyses were performed with Prism 3.03 (GraphPad) for Windows. The two-tailed statistical analyses were conducted at $P = 0.05$. Kaplan–Meier plots show the percentage of animals remaining in the study versus time. The Kaplan–Meier plots use the same data set as the log rank test.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra of compound **8**, HPLC purity data for target compounds, animal body weight change of compounds **8** and **1** treatment in vivo, in vivo efficacy evaluation of compounds **8**, **11**, **13** (oral route), and **1** (by ip and oral routes) against A549 xenografts, activities of compounds **8**, **11**–**14**, **17**, and **1** against HDAC 8, the CYP inhibition and solubility of compounds **11**–**14**, and in vitro metabolic stability in rat microsomes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

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

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