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 GI50 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 IC50 values of 12.3, 4.0, and 1.0 nM, respectively, which are comparable to 1. 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 apoptosis 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 lymphoma.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), 4 (PXD101), and 5 (SB939), are undergoing human clinical trials (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-aryloylindoles, 15a 5-aryloylindoles, 15b 1-aryloylindoles, 15c 3-aryltioindoles,15d and 1-aryl(1-benzyl)indoles,15e–f showed substantial anticancer activity. The literature16 reported that the 2-aryl-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...
with the O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (NH2OTHP) in the presence of benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PYBOP) and Et3N 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. The 1-benzenesulfonylation and benzylolation of indole-S-carboxyaldehyde (25) was achieved by using KO-t-Bu as base in the presence of KI and DMF at room temperature. The 1-arlylation of the indole ring was achieved in 28% yield by the treatment of 25 and 4-iodobenzenesulfonyl chloride with CuO/K2CO3 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-S-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, NH2OTHP/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)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-sulfonyl compounds were subject to the Wittig reaction with methyl (triphenylphosphoranylidene)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 aroyl sulfonamide 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, NH2OTHP/PYBOP coupling reaction, and TFA deprotection (Scheme 3). Similar to the preparation of compound 8, compounds 16, 17, and 18, with a benzyl, benzyl, and phenyl group at the N1-position, were obtained in 5–63% yield through a five-step reaction as shown in the Scheme 4. The 1-benzenesulfonylation and benzylolation of indole-S-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/K2CO3 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-S-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, NH2OTHP/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 IC50 values of 29.5 nM on HeLa HDAC enzyme inhibition, which was more potent than 1 (IC50 = 96.4 nM). Shifting of the N-hydroxyacrylamide...
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 IC50 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 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-3-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...
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 GI50 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 GI50 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 IC50 values against HDACs 2 and 6 with IC50 values of 1 and 4 nM, respectively. Compound 11 with 4′-methoxyphenylsulfonyl group displayed high activity on HDAC 6 (IC50 = 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 IC50 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-
Table 1. Inhibition of HeLa Nuclear Extract HDAC Activity and Antiproliferative Activity against Human Cancer Cell Lines by Compounds 6–21 and SAHA (1)

<table>
<thead>
<tr>
<th>compd</th>
<th>ICso ± SD** (nM)</th>
<th>GIso ± SD** (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HeLa nuclear HDAC</td>
<td>Hep3B</td>
</tr>
<tr>
<td>6</td>
<td>&gt;1000</td>
<td>8.87 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>170.9 ± 42.7</td>
<td>4.32 ± 0.1</td>
</tr>
<tr>
<td>8</td>
<td>29.5 ± 4.5</td>
<td>0.41 ± 0.1</td>
</tr>
<tr>
<td>9</td>
<td>254.0 ± 84.6</td>
<td>2.12 ± 0.1</td>
</tr>
<tr>
<td>10</td>
<td>&gt;1000</td>
<td>3.82 ± 0.4</td>
</tr>
<tr>
<td>11</td>
<td>6.8 ± 1.7</td>
<td>0.55 ± 0.1</td>
</tr>
<tr>
<td>12</td>
<td>9.7 ± 2.0</td>
<td>0.36 ± 0.1</td>
</tr>
<tr>
<td>13</td>
<td>16.8 ± 3.8</td>
<td>0.64 ± 0.1</td>
</tr>
<tr>
<td>14</td>
<td>12.6 ± 2.6</td>
<td>0.56 ± 0.1</td>
</tr>
<tr>
<td>15</td>
<td>&gt;1000</td>
<td>&gt;10</td>
</tr>
<tr>
<td>16</td>
<td>757.6 ± 92.8</td>
<td>2.40 ± 0.3</td>
</tr>
<tr>
<td>17</td>
<td>32.1 ± 9.3</td>
<td>0.86 ± 0.1</td>
</tr>
<tr>
<td>18</td>
<td>186.5 ± 20.9</td>
<td>1.32 ± 0.2</td>
</tr>
<tr>
<td>19</td>
<td>&gt;1000</td>
<td>3.65 ± 0.4</td>
</tr>
<tr>
<td>20</td>
<td>407.5 ± 170.8</td>
<td>2.56 ± 0.3</td>
</tr>
<tr>
<td>21</td>
<td>275.2 ± 31.7</td>
<td>1.27 ± 0.1</td>
</tr>
<tr>
<td>1</td>
<td>96.4 ± 10.3</td>
<td>0.69 ± 0.2</td>
</tr>
</tbody>
</table>

**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 Isosforms 1, 2, and 6

<table>
<thead>
<tr>
<th>compd</th>
<th>ICso ± SD** (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HDAC1</td>
</tr>
<tr>
<td>8</td>
<td>12.3 ± 4.2</td>
</tr>
<tr>
<td>11</td>
<td>124.8 ± 9.8</td>
</tr>
<tr>
<td>12</td>
<td>18.1 ± 2.5</td>
</tr>
<tr>
<td>13</td>
<td>33.2 ± 6.6</td>
</tr>
<tr>
<td>14</td>
<td>69.9 ± 12.0</td>
</tr>
<tr>
<td>17</td>
<td>71.2 ± 14.2</td>
</tr>
<tr>
<td>1</td>
<td>8.9 ± 3.3</td>
</tr>
</tbody>
</table>

**SD: standard deviation. All experiments were independently performed at least three times.

Table 4. Pharmacokinetics Parameters of 8 in Rat

<table>
<thead>
<tr>
<th>parameter</th>
<th>iv</th>
<th>po</th>
</tr>
</thead>
<tbody>
<tr>
<td>dose (mg/kg)**</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>CL (mL min⁻¹ kg⁻¹)</td>
<td>35.5 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>Vₐ (L/kg)</td>
<td>21.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>T½/h (h)</td>
<td>2.6 ± 0.2</td>
<td>5.5 ± 1.9</td>
</tr>
<tr>
<td>AUC(0–∞) (ng mL⁻¹ h)</td>
<td>927 ± 80</td>
<td>916 ± 183</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>1.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>C_max (ng/mL)</td>
<td>217 ± 116</td>
<td></td>
</tr>
<tr>
<td>F (%)</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

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

Table 3. CYP Inhibition and Solubility of Compound 8

<table>
<thead>
<tr>
<th>compd</th>
<th>% CYP inhibition** at 10 μM</th>
<th>sol (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H₂O</td>
</tr>
<tr>
<td>8</td>
<td>51</td>
<td>78</td>
</tr>
</tbody>
</table>

**The studies were performed by Ricerca Biosciences.
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 mm³ 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.
I 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 xenograft 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 anti-proliferative activity, with GI_{50} values ranging from 0.41 to 1 μM in a variety of human cancer cell lines from different organ. 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 (1H 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 JEOI JMS-700 mass spectrometer (HRMS-ESI and MS-ESI), Finnigan TSQ 700 mass spectrometer (MS-ESI), and micrOTOF orthogonal ESI-TOF mass spectrometer (HRMS-ESI). Purity of the final compounds was determined using a Hitachi 2000 series HPLC system using C18-column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm) with the solvent system (elution conditions: mobile phase A consisting of acetoni triate; mobile phase B consisting of water containing 0.1% formic acid +10 mmol NH₄OAc) 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.** Synthesis of 3-(1H-indol-3-yl)-(N-tetrahydropyran-2-yloxy)acrylic acid (23). To a solution of trans-3-indolecarboxylic acid (22, 0.50 g, 2.67 mmol), PPh₃ (1.47 g, 2.83 mmol), and triethylamine (0.74 mL, 6.41 mmol) in THF (25 mL) was added NH₂OTHF (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 MgSO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography 23. To give compound 23. Yield 68%. 1H NMR (500 MHz, CDCl₃): δ 7.58–6.75 (m, 2H), 7.15–6.68 (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-Benzensulfonyl-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 CH₂Cl₂ (15 mL) was stirred for 20 min, benzensulfonyl 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 CH₂Cl₂ (20 mL × 3). The combined organic layer was dried over anhydrous MgSO₄, 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 concentrated under reduced pressure for 12 h. The reaction mixture was concentrated under reduced pressure to give a yellow residue, which was recrystallized by CH₂OH to afford the desired compound 6. Yield 32%; mp 195–197°C. 1H 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 (d, J = 8.0 Hz, 1H), 6.62 (d, J = 16.0 Hz, 1H). MS (EI) m/z: 434 (M⁺, 22%), 77 (100%). HRMS (EI) for C₁₇H₁₉N₂O₅S (M⁺): calcd, 342.0674; found, 342.0673.

**1-Benzensulfonyl-1H-indole-4-carbaldehyde (28).** After a suspension of 1H-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 CH₂Cl₂ (15 mL) was stirred for 20 min, benzensulfonyl 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 CH₂Cl₂ (20 mL × 3). The combined organic layer was dried over anhydrous MgSO₄ 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%. 1H NMR (500 MHz, CDCl₃): δ 10.17 (s, 1H), 8.28 (s, J = 8.1 Hz, 2H), 7.87–8.09 (m, 2H), 7.76 (d, J = 3.6 Hz, 1H), 7.72 (d, J = 7.5 Hz, 1H), 7.44–7.57 (m, 3H). 3-(1-Benzensulfonyl-1H-indol-4-yl)acrylic Acid (32). To a solution of 28 (0.2 g, 0.7 mmol) in CH₂Cl₂ (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 CH₂Cl₂ (25 mL × 3). The combined organic layer was dried over anhydrous MgSO₄ 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 89%. ³¹H NMR (500 MHz, CDCl₃): δ 8.03 (d, J = 8.5 Hz, 1H), 7.95 (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 aqueous solution (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, and concentrated HCl was added to obtain acidic pH to produce a white solid, which was treated with TFA (6.9 mL, 92.90 mmol) in the presence of CH₃OH (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. ¹³C NMR (500 MHz, CDCl₃): δ 179.8, 174.9, 174.7–7.52 (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-Benzensulfonyl-1H-indol-5-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 NH₂OTHP (0.43 g, 3.67 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 MgSO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH₂Cl₂/CH₃OH = 30:1/1% NH₃(aq)) to give a white solid, which was treated with TFA (6.9 mL, 92.90 mmol) to obtain acidic pH to produce the precipitate, which was recrystallized by EtOAc/EtOH to afford the desired 7. Yield 90%. ¹³C NMR (500 MHz, CDCl₃): δ 135.7, 126.7, 126.8, 129.7, 107.7, 107.9, 108.7 (J = 15.9 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-Benzensulfonyl-1H-indol-4-yl)-N-hydroxyacrylamide (8). To a solution of 32 (1.0 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 NH₂OTHP (1.2 mL, 10.77 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 MgSO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH₂Cl₂/CH₃OH = 30:1/1% NH₃(aq)) to give a white solid, which was treated with TFA (6.9 mL, 92.90 mmol) to obtain acidic pH to produce the precipitate, which was recrystallized by EtOAc/EtOH to afford the desired 8. Yield 80% (two steps). ¹H NMR (500 MHz, CDCl₃): δ 8.03 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 2H), 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).
3-[(4-Methoxybenzenesulfonyl)-1H-indol-5-yl]acrylic Acid (40).

To a solution of 36 (1.91 g, 6.06 mmol) in CH2Cl2 (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 CH2Cl2 (25 mL × 3). The combined organic layer was dried over anhydrous MgSO4 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 a yellow residue.

3-[(3,4-Dimethoxybenzenesulfonyl)-1H-indol-5-yl]-N-hydroxyacrylamide (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 NH2OTHP (0.08 g, 0.67 mmol), and 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 with CH2Cl2/MeOH to obtain the desired compound.

Syntheses of 1-Arylsulfonyl-5-(N-hydroxyacrylamide)-indoles 11-14. (4-Methoxybenzenesulfonyl-1H-indol-5-carbaldehyde (36). After a suspension of 1H-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 CH2Cl2 (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 CH2Cl2 (20 mL × 3). The combined organic layer was dried over anhydrous MgSO4 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%. 1H NMR (500 MHz, CDCl3): 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), 3.79 (s, 3H).

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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 x 3). The combined organic layer was dried over anhydrous MgSO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH₂Cl₂/CH₃OH = 30:1) to give a white solid, which was treated with TFA (1.2 mL, 16.1 mmol) in the presence of CH₃OH (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 CH₃OH to afford the desired compound 12. Yield 53%; mp 146–146 °C. ¹H NMR (500 MHz, CD₃OD): 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), 7.35 (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, 99%), 372 (100%). HRMS (EI) for C₁₉H₁₈N₂O₆S (M⁺): calcd, 402.0884; found, 402.0884.

1-(4-Fluorobenzensulfonyl)-1H-indole-5-carboxaldehyde (38). After a suspension of 1H-indole-5-carboxaldehyde (25.1 g, 1.0 g, 6.89 mmol), TBAB (0.35 g, 1.03 mmol), and KOH (0.77 g, 13.78 mmol) in CH₂Cl₂ (20 mL) was stirred for 20 min, 4-fluorobenzensulfonyl chloride (2.01 g, 10.33 mmol) was added. The mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with water, and extraction was with CH₂Cl₂ (20 mL x 3). The combined organic layer was dried over anhydrous MgSO₄ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (CH₂Cl₂/CH₃OH = 30:1) to afford compound 38. Yield 76%; mp 138–140 °C. ¹H NMR (500 MHz, CD₃OD): 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), 7.5 (d, J = 8.5 Hz, 1H), 7.24 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 3.5 Hz, 1H), 6.67 (d, J = 15.5 Hz, 1H), 3.81 (s, 3H) (EI) m/z: 387 (M⁺ – 15, 99%), 372 (100%). HRMS (EI) for C₁₉H₁₈N₂O₆S (M⁺): calcd, 402.0880; found, 402.0886.

1-(4-Fluorobenzensulfonyl)-1H-indole-5-carboxaldehyde (38). After a suspension of 1H-indole-5-carboxaldehyde (25.1 g, 1.0 g, 6.89 mmol), TBAB (0.35 g, 1.03 mmol), and KOH (0.77 g, 13.78 mmol) in CH₂Cl₂ (20 mL) was stirred for 20 min, 4-fluorobenzensulfonyl chloride (2.01 g, 10.33 mmol) was added. The mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with water, and extraction was with CH₂Cl₂ (20 mL x 3). The combined organic layer was dried over anhydrous MgSO₄ and concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (CH₂Cl₂/CH₃OH = 30:1) to afford compound 38. Yield 76%; mp 138–140 °C. ¹H NMR (500 MHz, CD₃OD): 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), 7.5 (d, J = 8.5 Hz, 1H), 7.24 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 3.5 Hz, 1H), 6.67 (d, J = 15.5 Hz, 1H), 3.81 (s, 3H) (EI) m/z: 387 (M⁺ – 15, 99%), 372 (100%). HRMS (EI) for C₁₉H₁₈N₂O₆S (M⁺): calcd, 402.0880; found, 402.0886.

To a solution of crude methyl ester in dioxane (30 mL) was added 1 M LiOH (0.50 g, 1.51 mmol) 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). ¹H NMR (500 MHz, DMSO-d₆): δ 8.34–8.35 (2H), 8.24–8.27 (m, 2H), 7.94 (d, J = 8.5 Hz, 1H), 7.82 (s, 1H). MS (EI) m/z: 87 (100%). HRMS (EI) for C₁₇H₁₃N₃O₆S (M⁺): calcd, 387 (M⁺, 0.21%), 372 (100%).
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%. 1H NMR (500 MHz, CD3OD): 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), 7.68 (d, J = 3.7 Hz, 1H), 7.51–7.54 (m, 2H), 6.83 (d, J = 3.7 Hz, 1H).

1-Benzylsulfonyl-1H-indole-5-carboxylic Acid Hydroxymide (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 NH2OTHP (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 MgSO4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH2Cl2/CH3OH = 30:1)/1% NH3(aq)) to give a white solid, which was treated with TFA (0.70 mL, 10.5 mmol) in the presence of CH2OH (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 CH2OH to afford the desired 16. Yield 49%; mp 146–148 °C. 1H 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), 7.09 (s, J = 7.5 Hz, 1H), 7.54–7.61 (m, 4H), 7.32 (d, J = 3.5 Hz, 1H), 6.68 (d, J = 15.5 Hz, 1H). MS (EI) m/z: 306 (M+, 17%), 105 (100%). HRMS (EI) for C18H14N2O3 (M+): calc, 306.1004; found, 306.1006.

1-Benzyl-1H-indole-5-carbaldehyde (47). After a suspension of 1H-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 CH2Cl2 (20 mL × 3). The combined organic layer was dried over anhydrous MgSO4, concentrated under reduced pressure, and vacuum to give a yellow residue, which was purified by silica gel chromatography (EtOAc/n-hexane = 1:3) to afford compound 47. Yield 85%. 1H NMR (500 MHz, CDCl3): 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).

Syntheses of 1-Benzyl, Benzyl, and Phenyl-(N-hydroxyacrylamid)eindoles 16–18. 1-Benzyl-1H-indole-5-carbaldehyde (46). After a suspension of 1H-indole-5-carbaldehyde (25, 0.5 g, 2.85 mmol), KI (0.64 g, 4.13 mmol), and potassium tert-butoxide (0.64 g, 5.71 mmol) in DMF (2 mL) was stirred for 15 min, benzyl bromide (0.50 mL, 4.28 mmol) was added, and the mixture was stirred at room temperature for 24 h. The reaction was quenched with water, and extraction was with CH2Cl2 (20 mL × 3). The combined organic layer was dried over anhydrous MgSO4, 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%. 1H NMR (500 MHz, CDCl3): δ 10.10 (s, 1H), 8.50 (d, J = 8.5 Hz, 1H), 8.15 (dd, 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 (50). To a solution of 47 (0.5 g, 2.85 mmol) in CH2Cl2 (10 mL) was added benzaldehyde (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 CH2Cl2 (25 mL × 3). The combined organic layer was dried over anhydrous MgSO4, concentrated under reduced pressure to give a yellow residue, which was purified by silica gel chromatography (EtOAc/n-hexane = 1:2) to afford compound 46. Yield 87%. 1H NMR (500 MHz, CDCl3): 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%. 1H NMR (500 MHz, CDCl3): 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-Benzoyl-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 NH2OTHP (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 MgSO4, concentrated under reduced pressure. The residue was purified by silica gel chromatography ((CH2Cl2/CH3OH = 30:1)/1% NH3(aq)) to give a white solid, which was treated with TFA (0.92 mL, 12.4 mmol) in the presence of CH2OH (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 CH2OH to afford the desired 17. Yield 81%; mp 133–135 °C. 1H NMR (500 MHz, CDCl3): 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), 6.57 (d, J = 15.0 Hz, 1H), 5.38 (s, 2H). MS (EI) m/z: 292 (M+, 19%), 91 (100%). HRMS (EI) for C16H13N2O2 (M+): calc, 292.1212; found, 292.1213.

1-Phenyl-1H-indole-5-carbaldehyde (48). A suspension of 1H-indole-5-carbaldehyde (25, 0.70 g, 4.82 mmol), 4-iodobenzene (0.65 mL, 5.79 mmol), and K2CO3 (0.93 g, 6.67 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 MgSO₄ 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%. "H NMR (500 MHz, CDCl₃): 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), 6.41 (d, J = 15.9 Hz, 1H), 4.62 (d, J = 15.9 Hz, 1H). 3-(1-Phenyl-1H-indol-5-yl)acrylic Acid (51). To a solution of 48 (0.29 g, 1.31 mmol) in CH₂Cl₂ (10 mL) was added methyl compound as a yellow liquid. Yield 92%. "H NMR (500 MHz, CDCl₃): δ 8.21 (d, J = 1.5 Hz, 1H), 7.78 (d, 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).

Synthesis of 3-Benzensulfonyl-5-(N-hydroxypropanamine)dione 21. Methyl 3-(1H-indol-5-yl)acrylate (52). To a mixture of compound 25 (2.00 g, 13.78 mmol) and CH₂Cl₂ (20 mL) was added methyl (triphenylphosphoranylidene)acetate (0.52 g, 1.57 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched by water, and extraction was with CH₂Cl₂ (30 mL × 3). The organic layer was collected and dried over anhydrous MgSO₄ 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%. "H NMR (500 MHz, CDCl₃): δ 8.32 (br, 1H), 7.84 (d, J = 15.5 Hz, 1H), 7.81 (s, 1H), 7.43 (d, J = 2.0 Hz, 10.5 Hz, 1H), 7.38 (d, J = 8.5 Hz, 1H), 7.24 (t, J = 3.0 Hz, 1H), 6.59 (s, 1H), 6.42 (d, J = 15.5 Hz, 1H), 3.80 (s, 3H).

Methyl 3-(Phenylthio)-1H-indol-5-ylacrylate (53). To a mixture of NaH (0.30 g, 2.44 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 (0.40 g, 2.14 mmol) at room temperature and stirred 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 MgSO₄ 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%. "H NMR (500 MHz, CDCl₃): δ 8.58 (br, 1H), 7.76–7.80 (m, 2H), 7.52 (d, J = 2.5 Hz, 1H), 7.48 (d, 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 (t, 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-(Phenyldisulfonyl)-1H-indol-5-ylacrylate (54). To a mixture of 53 (0.30 g, 0.97 mmol) and CH₂Cl₂ (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 NaHCO₃ (aq), and extraction was with CH₂Cl₂ (30 mL × 3). The organic layer was collected and dried over anhydrous MgSO₄ 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%. "H NMR (500 MHz, DMSO-d₆): δ 8.22 (s, 1H), 8.03–8.07 (m, 3H), 7.81 (d, J = 16.0 Hz, 1H), 7.67 (d, J = 1.5 Hz, 8.0 Hz, 1H), 7.54–7.60 (m, 1H), 7.51 (d, J = 8.5 Hz, 1H), 6.57 (d, J = 16.0 Hz, 1H), 3.73 (s, 3H).

3-(Phenyldisulfonyl)-1H-indol-5-ylacrylic Acid (55). To a mixture of 54 (0.20 g, 0.59 mmol) and dioxane (1 mL) was added 1 M LiOH (aq) (0.18 mL, 1.18 mmol), and the mixture was stirred overnight at 40 °C. Solvent was removed, and the remainder was dissolved in MeOH (2 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 MgSO₄ and concentrated in vacuo to yield an orange product. The residue was purified by flash column over silica gel (EtOAc/n-hexane = 1:1) to afford 55. Yield 52%. "H NMR (500 MHz, DMSO-d₆): δ 7.81 (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-(Phenyldisulfonyl)-1H-indol-5-ylacrylamide (56). 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-2H-pyran-2-yl)hydroxylamine (0.26 g, 2.20 mmol), and the mixture was stirred at room temperature overnight. 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) (0.2 mL) to 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. "H NMR (500 MHz, DMSO-d₆): 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, 2H).
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 fluorescent 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.

The HDAC in vitro activities of recombinant human HDACs 1, 2, 6, and 8 (BPS Biosciences) were detected by fluorogenic 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% CO2 and 95% air.

Sulforhodamine B Assays.19 Human cancer cell A549 (non-small-cell lung cancer), MDA-MB-231 (estrogen-independent breast cancer), 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 time zero. HRMS (EI) for C17H14N2O4S (M+) calcd, 342.0674; found, 342.0673.

The human A549 lung adenocarcinoma cells used for implantation were harvested during log phase growth and resuspended in phosphate-buffered saline at 5 × 106 cells/mL. Each mouse was injected sc in the right flank with 1 × 107 cells (0.2 mL cell suspension). The tumor size, in mm3, was calculated from the following equation: tumor volume = w2/2, 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 mm3 of tumor volume. 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 compound 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 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 dosing solution was preparing by dissolving appropriate amount compound in a mixture of PEG400/DMSO (80:20, v/v) for intravenous administration and 1% carboxymethyl 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 perorally (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 (AB4000). 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 compound 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.

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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; NH₂OTHP, (N-(tetrahydro-2H-pyran-2-yl)hydroxylamine); PYBOP, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; TBAHS, tetrabutylammonium hydrogen sulfate; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; TTE, time to end point; TGD, tumor growth delay; TGI, tumor growth inhibition

REFERENCES


