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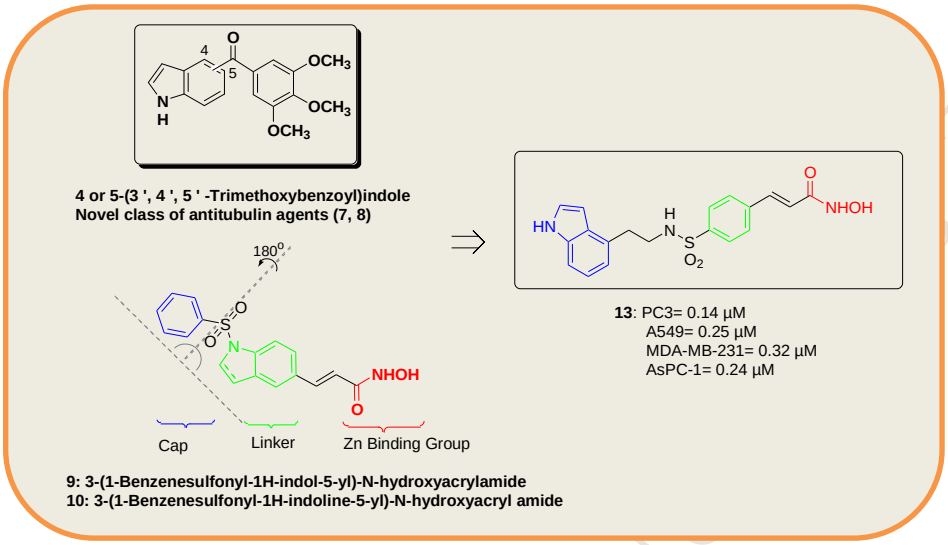
Graphical Abstract



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4-Indolyl-N-hydroxyphenylacrylamides as potent HDAC Class I and IIB inhibitors in Vitro and in Vivo.

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Abstract. A series of 4,5-indolyl-N-hydroxyphenylacrylamides, as HDAC inhibitors, has been synthesized and evaluated *in vitro* and *in vivo*. 4-Indolyl compounds **13** and **17** functions as potent inhibitors of HDAC1 (IC_{50} 1.28 nM and 1.34 nM) and HDAC 2 (IC_{50} 0.90 and 0.53 nM). *N-Hydroxy-3-{4-[2-(1H-indol-4-yl)-ethylsulfamoyl]-phenyl}-acrylamide* (**13**) inhibited the human cancer cell growth of PC3, A549, MDA-MB-231 and AsPC-1 with a GI_{50} of 0.14, 0.25, 0.32, and 0.24 µM, respectively. In *in vivo* evaluations bearing prostate PC3 xenografts nude mice model, compound **13** suppressed tumor growth with a tumor growth inhibition (TGI) of 62.2%. Immunohistochemistry of protein expressions, in PC-3 xenograft model indicated elevated acetyl-histone 3 and prominently inhibited HDAC2 protein expressions. Therefore, compound **13** could be a suitable lead for further investigation and the development of selective HDAC 2 inhibitors as potent anti-cancer compounds.

KEYWORDS. Indole, HDAC, Prostate cancer, Hydroxamic acid, Cancer, Acrylamide

1. Introduction

Prostate cancer, a solid tumor, is the most death causing disease in men second only to lung cancer. Histone deacetylases (HDACs) are overexpressed in many cancer types including prostate cancer and are associated with increased cancer cell proliferation and survival by repressing growth suppressive genes.[1] A study by Weichart et al comprising 192 samples of prostate cancer reported overexpressed class 1 HDAC isoforms in most of them (HDAC1: 69.8%, HDAC2: 74%, HDAC3: 94.8%); concluding the important role of HDAC1 and 2 in controlling prostate tumor dedifferentiation and indicating the highly significant prognostic value of HDAC2.[1f] Recent studies have indicated the role of over expressed HDAC 2 not only in development of prostate cancer but also in neurodegeneration leading to cognitive impairment. Studies explore the potential and thereof the need of HDAC2 inhibitors for the treatment of neurodegenerative diseases such as Alzheimer's disease.[1-3] Upregulated HDAC1 levels [4-6] has been observed in pre malignant and malignant lesions of prostate cancers.

Many studies have concluded that inhibition of class I and II HDACs by histone deacetylase inhibitors (HDIs), such as suberoylanilide hydroxamic acid (SAHA, **1**) and valproic acid (**5**), led to growth arrest, differentiation and/or apoptosis of prostate tumor cells (Figure 1).[5,7-9] PXD101 (**3**), a sulfonamide based HDI has shown promising results for the treatment of prostatic adenocarcinoma and has completed a phase 1 clinical trial for advanced solid tumors including prostate cancer.[10-12] including various other HDIs such as LBH589 (**4**), valproic acid (**5**), MS275 (**6**). Except FK-228 (Romidepsin, **2**), all other FDA approved HDIs possess a similar Zn^{2+} binding Group (ZBG) e.g. hydroxamic acid; however, they differ significantly in their surface recognition cap and linker. Indole moiety has the privilege to be the most explored heterocyclic compound for the development of various anticancer agents e.g. LBH589 (**4**). However, a lot of focus has been laid on exploring the indole C2 and C3 position unlike indole -4, -5, -6, and -7 which remain comparatively unexplored. Some published reports have supported the idea that the position -4, -5, -6 and -7 of indole could be

potential sites for attaching a connector unit with surface recognition cap for developing potent HDAC inhibitors.[13-17]

We previously have explored the regioisomeric effects of various positions of indole on tubulin polymerization inhibition and have developed a novel class of anti-tubulin agents (**7**, **8**).[18] In addition, we have also reported the syntheses of potent HDIs with indole/indoline (**9**, **10**) as a linker and a phenyl group as a surface recognition cap.[19-20] All of these studies have revealed that the compounds with substitution at -4 and -5 position of indole were more potent than other analogues of respective series. Close observation of these HDIs indicated that type of connector unit e.g. rigid (**9** or **10**) or flexible (**13**) could affect the potency of compounds (Figure 2). Prompted from the results we decided to explore the regioisomeric effect of indole -4 and -5 positions on HDAC inhibitory activity by interchanging the cap and linker of our previously reported HDAC inhibitors. This has led to generation of a potent series of 4,5-indolyl-N-hydroxyphenylacrylamides, with indole as surface recognition cap and phenyl as linker, possessing potent activity against prostate cancer (Figure 3). Apart from regioisomeric effect of meta and para substitutions of acrylamide side chain with respect to connector unit (CU); length of CU and substitution at indole N1 has also been studied to complete the SAR of these hydoxamides.

2. Results and discussion

2.1. *Chemistry*

To synthesize amine **20**, 4-nitroindole was reduced using iron (Fe) and ammonium chloride (NH_4Cl) and corresponding amine was extracted using ethyl acetate (EtOAc) and water. Organic layer was then collected, dried and directly used for next step. To synthesize amine **21**, 4-cynoindole was reduced using 10% Pd/C in MeOH over a hydrogenator at 40-42 psi and stirred overnight, removed carefully from hydrogenator, filtered through celite and purified by column chromatography. Reduction using borane-tetrahydrofuran complex or Pd/C at room temperature did not yield satisfactory results (Scheme 1).

To synthesize the amines from **22** to **25**; corresponding aldehydes underwent henry reaction to give corresponding nitro alkenes Attempts to reduce these nitro alkenes using lithium aluminum hydride (LAH) or Pd/C in methanol (MeOH) produced only poor yields of desired products. Thus, nitroalkenes were first reduced to nitroalkanes using $NaBH_4$ in methanol followed by nitro group reduction using Fe-NH_4Cl in a mixture of water and isopropyl alcohol (IPA) (1:4) to give **22** to **25** in quantitative yield (Scheme 1).

The synthesis of compounds **11** to**18** is shown in Scheme 2. Amines **20** to **25** were allowed to react with 3-or 4-bromobenzenesulfonyl chloride or 4- in the presence of pyridine and acetonitrile (ACN) to respectively yield compounds **35** to **42**. Heck coupling with *t*-butyl acrylate in the presence of tris(dibenzylideneacetone)dipalladium [$Pd_2(dba)_3$] and tri-t-butylphosphonium tetrafluoroborate {[(*t*-Bu)$_3$PH]BF_4]} afforded cinnamates **43** to **50**. Hydrolysis of the methyl ester group in **43** to **50** was achieved by treatment with 1N LiOH solution yielding the corresponding acids which were subject to amidation with NH_2OTHP in the presence of EDC•HCl and HOBt affording the corresponding protected N-hydroxyamides. De-protection of the OTHP group was achieved with TFA in dichloromethane (DCM) to yield compounds **11** to **18** in 38 to 56% yield.

2.2. Biological evaluation

2.2.1. HeLa nuclear HDAC enzyme inhibition.

Using HeLa nuclear extract as HDAC source, the ability of 4,5-indolyl-N-hydroxyphenylacrylamides were evaluated to inhibit HDAC activity (Table 1). Compound **11**, where sulfonamide is directly linked to indole *C*-4, failed to show any potent activity against HeLa nuclear extract (HDAC IC_{50} >1000 nM). Increasing the connecter unit (CU) length from n=0 to n=1, by addition of one methylene group between sulfonamide and indole *C*-4 (**12**, HDAC IC_{50}= 7.13 nM), showed a marked increase in HDAC inhibitory activity compared to its precursor **11** with ~18 and ~11 fold more potency compared to SAHA (**1**, HDAC IC_{50}= 131.26 nM) and PXD101 (**3**, HDAC IC_{50}= 80 nM) respectively. **12**, was found to be more potent than reference

compound **9 (**HDAC IC_{50}= 29.5 nM) and MS275 (**6,** HDAC IC_{50} >1000 nM**)** and comparable with LBH589.HCl **(4**, HDAC IC_{50} =4.90 nM**)**. These results indicated that a flexible CU was well tolerated in this series of molecules and addition of one carbon distance was a favorable modification. Compound **13** (n=2, HDAC IC_{50}= 8.56 nM) showed comparable HDAC inhibitory activity with its precursor **12** and **4**.HCl but was found to be more potent against various cancer cell lines compared to **12**. To compare the regioisomeric effect of *N*-hydroxyacrylamide side chain, compound **14** was synthesized. Replacement of *N*-hydroxyacrylamide side chain from *para* (**13)** to *meta* position (**14**) led to decrease in HDAC inhibitory activity indicating that a para substitution is favored unlike PXD101. However **14** (HDAC IC_{50}= 30.45 nM) was still more potent than **1** (HDAC IC_{50}= 131.26 nM), **3** (HDAC IC_{50}= 80 nM), **6 (**HDAC IC_{50} >1000 nM**)**, and comparable with **9 (**HDAC IC_{50}= 29.5 nM). Regioisomers at indole *C*-5 (**15** and **16**) were less potent compared to **13**, however comparable with reference compounds. Substituting the Indole-*N*1 with a methyl group (**17)** maintained HDAC inhibitory activity as **13**. Substituting *N*-1 with 4-methoxybenzenesulfonyl moiety (**18**) led to decrease in HDAC inhibitory activity indicating bulky group substitution at *N*-1 is not a suitable modification. In nutshell, all of these compounds, except **11,** showed overall better HDAC HeLa nuclear extract inhibitory activity compared to standard compounds like SAHA, PXD101 and MS275.

2.2.2. In Vitro Cell Growth Inhibitory Activity.

The synthesized 4,5-indolyl-N-hydroxyphenylacrylamides, **11** to **18,** were evaluated for anti-proliferative activities against prostate carcinoma PC-3 cells, lung carcinoma A549 cells, breast carcinoma MDA-MB-231 cells and pancreatic carcinoma AsPC-1 cells (Table 1) The cancer cell growth inhibitory results were consistent with the HDAC extract enzyme inhibitory activity. Compounds such as **13**, **14**, **16** and **17** showed better to comparable activities with standard compounds like SAHA, PXD101 and MS275. Compound **12** (n=1) and **13** (n=2) showed comparable HDAC inhibitory activities, however the anti-proliferative activities of compound **13** (PC-3; GI_{50} =

0.14 µM) was better than **12** (PC-3; GI_{50} = 0.86 µM). Compound **13** was overall more potent than its parent compound **9**. Being consistent with HDAC inhibitory activity, **14** showed diminished anti-cancer activity compared to **13**; indicating that a para substitution is preferred for optimal activity. Substitution of *N*-1 with methyl group (**17**) showed comparable anti-proliferative activity to that of **13,** except against breast carcinoma MDA-MB-231 cells where **17** (IC_{50} = 0.08 µM) showed slight better activity as that of **13** (IC_{50} = 0.32 µM). Compound **18** was overall less potent than its precursor compound **17**.

2.2.3. HDAC Isoform Inhibition.

Compounds **13** and **17** were tested for HDAC isoform enzyme-inhibitory activity including HDAC isoform 1 and 2, which are generally overexpressed in prostate cancer cells, using compound **1, 3** and **6** as reference compounds (Table 2). **13** and **17** showed potent HDAC inhibitory activity against HDAC 1 and 2. These results are consistent with their high efficacy against PC-3 cell lines. **13** (HDAC 1 = 1.28 nM; HDAC 2 = 0.90 nM) and **17** (HDAC 1 = 1.34 nM; HDAC 2 = 0.53 nM) were 15 to 85 fold more potent than standard compounds **1** (HDAC 1 = 110 nM; HDAC 2 = 120 nM) and **3** (HDAC 1 = 20 nM; HDAC 2 = 50 nM) against HDAC 1 and 55 to 226 fold more potent for HDAC 2. Furthermore, results have also indicated that a methyl substitution (**17**) is favored for the HDAC inhibitory activity.

2.2.4. Western blot analysis.

In an effort to further validate the target in the 4,5-indolyl-N-hydroxyphenylacrylamide series, compound **13** was evaluated for the expression of histone 3 and α-tubulin acetylation PC-3 cell line; which were important biomarkers associated with intracellular HDAC inhibition. Western blot analysis indicated that compound **13** increased acetylation levels of histone 3 and *α*-tubulin and was found equipotent as standard compound **1** in PC-3 cells (Fig. 4A-C).

2.2.5. In vivo Efficacy in Human Prostate PC3 Xenograft.

We evaluated the *in vivo* efficacy on compounds **13**.Using human prostate PC3 xenograft nude mouse model (Fig. 5).Once a tumor was approximately 40 mm^3 in size and palpable, the mice were randomized into vehicle control and treatment groups (7-8 mice per group) and control mice received the vehicle (1.0% carboxymethyl cellulose/0.5% Tween80). Results have indicated (Fig. 5A) that administration of **13** (20 mg/kg and 40 mg/kg, ip, q2d) significantly reduced tumor volume dose dependently in nude mice model. The percent of tumor growth inhibition (TGI) of **13** (20 mg/kg and 40 mg/kg) was 51.6% (*$p < 0.05$) and 62.2% (**$p < 0.01$) respectively. In addition, no significant differences in weight loss were observed during **13** treatment periods (Figure 5B).

2.2.6. Effect on Protein Expression in Xenograft Model

After in vivo prostate PC-3 xenograft study, we further conducted the immunohistochemistry of protein expressions as shown Figure 6. Results indicated the effect of **13** on HDAC inhibition marker- acetyl-histone 3(Ac-H3) and expression of HDAC isoforms 1 and 2 in xenograft tumors. Data revealed the information that compound **13** increased the acetyl-histone 3 (Ac-H3) expression dose-dependently and decreased the HDAC 2 expression in 40 mg/kg dose group. No significant change in the expression of HDAC 1 was observed.

3. Conclusion

We have designed and evaluated a novel series of 4,5-indolyl-N-hydroxyphenylacrylamide as potent histone deacetylase inhibitors. Lead compound **13** showed anti-proliferative activity, with GI_{50} values ranging from 0.14 to 0.32 μM in a variety of human cancer cell lines from different organs. **13,** has demonstrated better activity than reference compounds **1, 3** and **6**. Compound **13** exhibited low nanomolar IC_{50} values against HDAC 1 and 2 isoforms with 1.28 and 0.90 respectively. Higher potency against HDAC 1 and 2 corresponds to better activity of **13** against prostate cancer PC-3 cell line. Structure-activity relationship information revealed that the *N*-hydroxyacrylamide group placed at the *C*-4 position of indole displayed the best activity compared

to *C*-5 position and a *para* substitution is a prerequisite for the potent activity. Flexible connector unit (CU) with n=2 was found to be more potent than its parent compound **9** (rigid CU) and other molecules of the series including reference compounds. In *in vivo* efficacy evaluation against human xenograft model in nude mice bearing PC-3 cancer cell line, compound **13** (40 mg/kg, ip, q2d) not only demonstrated substantial antitumor activity with a TGI of 62.2% but also found to upregulate acetyl-histone 3 and α-tubulin expression. Compound **13**, is found to be a potent inhibitor of HDAC Class I and IIB and can decrease level of HDAC2 more selectively than HDAC1 as indicated by immunohistochemistry analysis. In summary, *N-Hydroxy-3-{4-[2-(1H-indol-4-yl)-ethylsulfamoyl]-phenyl}-acrylamide* (**13**) could be a potential lead for further investigation to develop selective HDAC2 inhibitors as potent anticancer agents.

4. Experimental section

4.1. Chemistry

Nuclear magnetic resonance spectra were obtained with Bruker DRX-500 spectrometer (operating at 300 MHz and 500 MHz), with chemical shift in parts per million (ppm, δ) downfield from TMS as an internal standard. High-resolution mass spectra (HRMS) were measured with a JEOL (JMS-700) electron impact (EI) mass spectrometer. Purity of the final compounds were determined using an Hitachi 2000 series HPLC system using C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm. 4.6 mm × 150 mm) and were found to be ≥ 95%. Flash column chromatography was done using silica gel (Merck Kieselgel 60, No. 9385, 230-400 mesh ASTM). All reactions were carried out under an atmosphere of dry nitrogen.

General procedures for reduction of nitro group - Method A. Nitro compound (1 mmol) was dissolve in a 0.1 M mixture of IPA: H_2O (4:1) and iron powder (Fe, 3 mmol), ammonium chloride (NH_4Cl, 2 mmol) were added and refluxed for two hours. After cooling down to room temperature reaction mixture was filtered through celite, extracted with EtOAc (25 mL × 3). The combined

organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure and purified by column chromatography. *Method B.* 4-cynoindole (1.0 mmol) was dissolved in MeOH in a hydrogenator reaction flask, 10% Pd/C (10 mg) was added and hydrogenator was shook at 40-42 psi overnight. Reaction mixture was carefully taken out from hydrogenator, filtered through celite and purified by column chromatography (EtOAc: MeOH; 9.5 : 0.5) with few drops of aqueous ammonia .

*General procedure for the synthesis of amines **22** to **25**.* Henry reaction was performed as reported in the literature.[21] Thus obtained nitroalkene (1 mmol) was dissolved in methanol (15 mL) and this solution was added sodium borohydride ($NaBH_4$, 4.4 mmol) portion wise. Reaction was stirred for 45 mins and was quenched using 1mL acetic acid. Compound was purified from a short column to give an off-white residue. Thus obtained nitro products were reduced using method – A.

General procedure for sulfonamide synthesis. To a mixture of amine (1 mmol), pyridine (1 mL) and acetonitrile (0.25 mL) was added 4-bromobenzenesulfonyl chloride (1.1 mmol) portion wise, and then was allowed to stir at room temperature for 2 h. The reaction was quenched with water and was acidified with dropwise addition of 3N HCl. The reaction mixture was then extracted with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (EtOAc: n-hexane = 3: 7)

General procedure for Heck reaction. A mixture of bromo compound (1 mmol), $Pd_2(dba)_3$ (0.1 mmol, $[(t\text{-}Bu)_3PH]BF_4$ (0. 2 mmol), K_2CO_3 (1mmol), TEA (3 mmol), methyl acrylate (1 mmol), and DMF (5 mL) was stirred at 100 ^oC for 12 h. The reaction was quenched with water, extracted with CH_2Cl_2 (20 mL × 3) and the combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified by silica gel

General Procedure for hydroxamic acid synthesis. A mixture of 1N LiOH (3 mL) and Heck ester (1 mmol) was stirred at 40 ^{o}C for 2 hours. The reaction was concentrated under reduced pressure and then was added water. The mixture was acidified with 3N HCl to give off white precipitates. The off-white solid (1mmol) was dissolved in DMF (10 mL) and was added EDC•HCl (1.5 mmol), HOBt hydrate (1.5 mmol) and TEA (3 mmol). After being stirred at room temperature for 30 min, NH_2OTHP (1.2 mmol) was added and allowed to stir for additional 5 h. The reaction mixture was quenched with water and was extracted with EtOAc (25 mL × 3). The combined organic layer was collected, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a light yellow residue, which was purified by silica gel chromatography (EtOAc: n-hexane = 1: 1) to give colorless liquid. To the resulting product dissolved in CH_3OH (5mL) was added of 10% TFA (5 mL) and was allowed to stir at room temperature for 5 h. The reaction mixture was concentrated under reduced pressure to give a white residue, which was recrystallized from CH_3OH to afford the desired compounds.

4.1.1. *Syntheses of amines **20-25**.*

*4.1.1.1. 4-Aminoindole **(20)***. Compound **20** was synthesized using 4-nitroindole (**19**, 0.5 g, 3.08 mmol), iron powder (Fe, 0.5 g, 9.24 mmol) and ammonium chloride (NH_4Cl, 0.3 g, 6.16 mmol) followed by method-A in 89 % yield; ^{1}H NMR (300 MHz, CD_3OD): δ 6.38 (dd, *J* = 6.9 Hz, *J* = 0.9 Hz, 1H), 6.50 (dd, *J* = 6.0 Hz, *J* = 3.3 Hz, 1H), 6.81-6.92 (m, 1H), 7.10 (d, *J* = 3.3 Hz, 1H).

4.1.1.2. 1H-Indol-4-yl-methylamine **(21).** Compound **20** was synthesized using 4-cynoindole (**26,** 0.2 g, 1.40 mmol) and 10 % pd/c followed by method-B in 82% yield; ^{1}H NMR (300 MHz, CD_3OD): δ 4.07 (s, 1H), 6.56 (dd, *J* = 3.0 Hz, *J* = 3.3 Hz, 1H), 7.99 (d, *J* = 7.2 Hz, 1H), 7.09 (d, *J* = 7.2 Hz, 1H), 7.26 (d, *J* = 3.3 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H).

4.1.1.3. 4-(2-amino-ethyl)-1H-indole **(22).** Compound **22** was synthesized using nitro alkene (**29**, 1.32 g, 6.53 mmol) obtained by reported procedure[3] and further followed by general procedure for

the synthesis of amines **22** to **25** in 65% over all yield (from **27**); ^{1}H NMR (500 MHz, MeOD): δ 2.99 - 3.03 (m, 2H), 3.10 (t, *J* = 8.0 Hz, 2H), 6.51 (d, *J* = 3.0 Hz, 1H), 6.83 (d, *J* = 7.0 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 3.5 Hz, 1H),), 7.25 (d, *J* = 8.0 Hz, 1H).

4.1.1.4. 5-(2-amino-ethyl)-1H-indole **(23)** Compound **23** was synthesized using nitro alkene (**30**, 1.32 g, 6.53 mmol) obtained by reported procedure and further followed by general procedure for the synthesis of amines 22 to 25 in 61% over all yield (from **28**); ^{1}H NMR (500 MHz, MeOD): δ 2.84 (t, *J* = 7.0 Hz, 2H), 2.94 (t, *J* = 7.0 Hz, 2H), 6.36 (d, *J* = 3.0 Hz, 1H), 6.96 (dd, *J* = 8.0 Hz, *J* = 8.5 Hz, 1H), 7.18 (d, *J* = 3.0 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H),), 7.38 (s, 1H).

4.1.1.5. N-Methyl-4-formylindole **(31)** To the solution of **28** in dimethylformamide (DMF) maintained at 0 ^{o}C was added sodium hydride (NaH, 0.36 g, 10.32 mmol) and was stirred for 5 mins before adding methylidodide (MeI, 0.44 mL, 8.25 mmol) and the reaction was stirred for 30 mins. Reaction was quenched by slow addition of water at 0 ^{o}C and extracted using EtOAc (25 mL × 3). The combined organic layer was collected, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a pale yellow residue, which was purified by silica gel chromatography (EtOAc: Hexane, 4:1) to give **31** as off white residue in 89% yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 3H), 7.26-7.28 (m, 2H), 7.38 (t, *J* = 8.1 Hz, 1H), 7.46 (t, *J* = 8.1 Hz, 2H), 10.27 (s, 1H).

*4.1.1.6. 1-(4-Methoxy-benzenesulfonyl)-1H-indole-4-*carbaldehyde **(32)** To the solution of **28** (1 g, 6.89 mmol) in dichloromethane (DCM) was added tetrabutylammonium hydrogen sulfate (TBAHS, 0.35 g, 1.03 mmol), potassium hydroxide (KOH, 0.77 g, 13.78 mmol) and stirred for 20 mins before adding 4-Methoxybenzenesulfonyl chloride (2.14 g, 10.33 mmol) and the reaction was stirred for 12 hours at room temperature. Reaction was quenched by addition of water and neutralized with 3N HCl and extracted using EtOAc (25 mL × 3). The combined organic layer was collected, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a pale yellow residue, which was purified by silica gel chromatography (EtOAc: Hexane, 4:1) to give **32** as off-white residue in 45%

yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.81 (s, 3H), 6.90 (d, *J* = 9.0 Hz, 2H), 7.46 - 7.51 (m, 2H), 7.72 - 7.77 (m, 2H), 7.82 – 7.85 (m, 2H), 8.28 (t, *J* = 8.1 Hz, 1H), 10.91 (s, 1H).

4.1.1.7. 2-(1-Methyl-1H-indol-4-yl)-ethylamine **(24)** Compound **24** was synthesized using nitro alkene (**33**, 1.32 g, 6.53 mmol) obtained by reported procedure and further followed by general procedure for the synthesis of amines **22** to **25** in 62% over all yield (from **31**); ^{1}H NMR (300 MHz, MeOD): δ 2.72. (t, *J* = 7.5 Hz, 2H), 2.92 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 6.51 (dd, *J* = 3.3 Hz, *J* = 3.3 Hz, 1H), 6.96 (d, *J* = 7.2 Hz, 1H), 7.12 (d, *J* = 3.0 Hz, 1H), 7.16-722 (m, 1H), 7.27-7.30 (m, 1H).

4.1.1.8. 2-[1-(4-Methoxy-benzenesulfonyl)-1H-indol-4-yl]-ethylamine **(25)** Compound **25** was synthesized using nitro alkene (**34**, 0.51 g, 1.42 mmol) obtained by reported procedure and further followed by general procedure for the synthesis of amines **22** to **25** in 58% over all yield (from **32**); ^{1}H NMR (300 MHz, MeOD): δ 2.74. (t, *J* = 7.0 Hz, 2H), 2.85 (t, *J* = 7.5 Hz, 2H), 3.81 (s, 3H), 6.61 (dd, *J* = 3.3 Hz, *J* = 3.0 Hz, 1H), 6.73 (d, *J* = 6.9 Hz, 2H), 6.89-7.13 (m, 2H), 7.38 (d, *J* = 7.5 Hz, 2H), 7.79 (m, 2H).

*4.1.2. Syntheses of sulfonamides **35-42**.*

4.1.2.1. 4-Bromo-N-(1H-indol-4-yl)-benzenesulfonamide **(35)** Compound **35** was synthesized using a mixture of 4-aminoindole (**20**, 1.0 g, 7.56 mmol), pyridine (4 mL), acetonitrile (1 mL) and 4-bromobenzenesulfonyl chloride (2.12 g, 8.32 mmol) followed by general procedure for sulfonamide synthesis in 61 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.34 (t, *J* = 2.1 Hz, 1H), 6.70 (s, 1H), 7.05 (d, *J* = 6.9 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 1H), 7.15-7.17 (m, 1H), 7.25 (d, *J* = 6.0 Hz, 1H), 7. 51 (d, *J* = 8.7 Hz, 1H), 7.63 (d, *J* = 8.7 Hz, 1H), 8.25 (bs, 1H).

4.1.2.2. 4-Bromo-N-(1H-indol-4-ylmethyl)-benzenesulfonamide **(36)** Compound **36** was synthesized using a mixture of 1H-Indol-4-yl-methylamine (**21**, 1.0 g, 6.84 mmol), pyridine (4 mL), acetonitrile (1 mL) and 4-bromobenzenesulfonyl chloride (2.24 g, 7.52 mmol) followed by general procedure for sulfonamide synthesis in 71 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.45 (d, *J* = 5.7 Hz, 2H), 4.74

(bs, 1H), 6.47 (t, *J* = 1.2 Hz, 1H), 6.90 (d, *J* = 6.9 Hz, 1H), 7.09 (t, *J* = 7.8 Hz, 1H), 7.23 (t, *J* = 2.7 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.59-7.61 (m, 2H), 7.68-7.72 (m, 2H), 8.28 (bs, 1H).

4.1.2.3. 4-Bromo-N-[2-(1H-indol-4-yl)-ethyl]-benzenesulfonamide **(37)** Compound **37** was synthesized using a mixture of 2-(1H-Indol-4-yl)-ethylamine (**22**, 1.0 g, 6.24 mmol), pyridine (4 mL), acetonitrile (1 mL) and 4-bromobenzenesulfonyl chloride (1.78 g, 6.86 mmol) followed by general procedure for sulfonamide synthesis in 78 % yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.04 (t, *J* = 7.0 Hz, 2H), 3.33-3.37 (m, 2H), 6.40 (t, *J* = 1.0 Hz, 1H), 6.79 (d, *J* = 7.5 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 7.17 (t, *J* = 3.0 Hz, 1H), 7.25-7.29 (m, 1H), 7.46-7.47 (m, 4H), 8.27 (bs, 1H).

4.1.2.4. 3-Bromo-N-[2-(1H-indol-4-yl)-ethyl]-benzenesulfonamide **(38)** Compound **38** was synthesized using a mixture of 2-(1H-Indol-4-yl)-ethylamine (**22**, 1.0 g, 6.24 mmol), pyridine (4 mL), acetonitrile (1 mL) and 3-bromobenzenesulfonyl chloride (1.78 g, 6.86 mmol) followed by general procedure for sulfonamide synthesis in 75 % yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.06 (t, *J* = 7.0 Hz, 2H), 3.36-3.40 (m, 2H), 6.40 (s, 1H), 6.80 (d, *J* = 7.0 Hz, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.19 (t, *J* = 2.5 Hz, 1H), 7.25-7.29 (m, 3H), 7.62-6.64 (m,; 2H), 7.85 (s, 1H), 8.25 (bs, 1H).

4.1.2.5. 4-Bromo-N-[2-(1H-indol-5-yl)-ethyl]-benzenesulfonamide **(39).** Compound **37** was synthesized using a mixture of 2-(1H-Indol-5-yl)-ethylamine (**23**, 1.0 g, 6.24 mmol), pyridine (4 mL), acetonitrile (1 mL) and 4-bromobenzenesulfonyl chloride (1.78 g, 6.86 mmol) followed by general procedure for sulfonamide synthesis in 66 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.86 (t, *J* = 6.3 Hz, 2H), 3.25-3.31 (m, 2H), 4.40 (t, *J* = 5.7 Hz, 1H), 6.48-6.90 (m, 1H), 6.87-6.89 (m, 1H), 7.24 (d, *J* = 2.7 Hz, 1H), 7.28-7.31 (m, 2H), 7.52-7.60 (m, 4H), 8.27 (bs, 1H).

4.1.2.6. 3-Bromo-N-[2-(1H-indol-5-yl)-ethyl]-benzenesulfonamide **(40).** Compound **38** was synthesized using a mixture of 2-(1H-Indol-5-yl)-ethylamine (**23**, 1.0 g, 6.24 mmol), pyridine (4 mL), acetonitrile (1 mL) and 3-bromobenzenesulfonyl chloride (1.78 g, 6.86 mmol) followed by general

procedure for sulfonamide synthesis in 61 % yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.83 (t, *J* = 7.0 Hz, 2H), 3.25-3.29 (m, 2H), 4.59 (t, *J* = 6.0 Hz, 1H), 6.45 (d, *J* = 2.5 Hz, 1H), 6.86-6.88 (m; 1H), 7.19 (t, *J* = 2.5 Hz, 1H), 7.26-7.29 (m, 3H), 7.62-7.66 (m, 2H), 7.90 (s, 1H), 8.29 (bs, 1H).

4.1.2.7. 4-Bromo-N-[2-(1-methyl-1H-indol-4-yl)-ethyl]-benzenesulfonamide **(41).** Compound **41** was synthesized using a mixture of 2-(1-Methyl-1H-indol-4-yl)-ethylamine (**24**, 1.0 g, 5.74 mmol) in pyridine (4 mL), acetonitrile (1 mL) and 4-bromobenzenesulfonyl chloride (1.88 g, 6.32 mmol) followed by general procedure for sulfonamide synthesis in 68 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.06 (t, *J* = 6.3 Hz, 2H), 3.33-3.40 (m, 2H), 3.80 (s, 1H), 4.35 (t, *J* = 6.0 Hz, 1H), 6.34 (dd, *J* = 3.0 Hz, *J* = 3.3 Hz, 1H), 6.81 (d, *J* = 7.2 Hz, 1H), 7.04 (d, *J* = 3.3 Hz, 1H), 7.11-7.16 (m, 1H), 7.22- 7.27 (m, 1H), 7.49 (bs, 4H).

4.1.2.8. 4-Bromo-N-[2-(1-methyl-1H-indol-4-yl)-ethyl]-benzenesulfonamide **(42).** Compound **42** was synthesized using a mixture of 2-[1-(4-Methoxy-benzenesulfonyl)-1H-indol-4-yl]-ethylamine (**25**, 0.36 g, 1.08 mmol), pyridine (1 mL), acetonitrile (0.5 mL) and 4-bromobenzenesulfonyl chloride (0.31 g, 1.18 mmol) followed by general procedure for sulfonamide synthesis in 49 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01 (t, *J* = 7.2 Hz, 2H), 3.32-3.41 (m, 2H), 3.78 (s, 3H), 6.64-6.67 (m, 3H), 6.92 (dd, *J* = 6.9 Hz, *J* = 3.0 Hz, 1H), 7.12 (dd, *J* = 3.0 Hz, *J* = 3.3 Hz 1H), 7.22 (m, 1H), 7.61-7.64 (m, 2H), 7.68-7.72 (m, 2H), 7.78 (d, *J* = 7.5 Hz, 1H), 7.84-7.89 (m, 2H).

4.1.3. Syntheses of Heck products (methyl esters) ***43-50****.*

4.1.3.1 3-[4-(1H-Indol-4-ylsulfamoyl)-phenyl]-acrylic acid methyl ester **(43).** Compound **43** was synthesized using a mixture of **35** (1.0 g, 2.85 mmol), $Pd_2(dba)_3$, (0.26 g, 0.28 mmol), $[(t\text{-}Bu)_3PH]BF_4$ (0.16 g, 0.57 mmol), K_2CO_3 (0.39 g, 2.85 mmol), TEA (1.17 mL, 8.55 mmol), methyl acrylate (0.30 mL, 3.42 mmol), and DMF (10 mL) followed by general procedure for Heck reaction in 89 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 3H), 6.33-6.35 (m, 1H), 6.45(d, *J* = 16.2 Hz, 1H), 6.75 (s, 1H), 7.04-7.14 (m, 3H), 7.22 -7.25 (m, 1H), 7.49 (d, *J* = 8.7 Hz, 1H), 7. 61 (d,

J = 16.2 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 8.24 (bs, 1H).

4.1.3.2. 3-{4-[(1H-Indol-4-ylmethyl)-sulfamoyl]-phenyl}-acrylic acid methyl ester **(44).** Compound **44** was synthesized using a mixture of **36** (1.2 g, 3.28 mmol), $Pd_2(dba)_3$ (0.30 g, 0.33 mmol**)**, $[(t\text{-}Bu)_3PH]BF_4$ (0.19 g, 0.66 mmol), K_2CO_3 (0.36 g, 2.63 mmol), TEA (1.56 mL, 9.84 mmol), methyl acrylate (0.31 mL, 3.93 mmol) and DMF (10 mL) followed by general procedure for Heck reaction in 61 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (s, 3H), 4.67 (d, *J* = 6.0 Hz, 2H), 4.72 (t, *J* = 6.0 Hz, 1H), 6.47-6.59 (m, 1H), 6.56 (t, *J* = 16.2 Hz, 1H), 6.90 (d, *J* = 7.2 Hz, 1H), 7.08 (t, *J* = 8.1 Hz, 1H), 7.22 (t, *J* = 3.0 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 16.2 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H).

4.1.3.3. 3*-{4-[2-(1H-Indol-4-yl)-ethylsulfamoyl]-phenyl}-acrylic acid methyl ester* **(45).** Compound **45** was synthesized using a mixture of **37** (1.0 g, 2.63 mmol), $Pd_2(dba)_3$ (0.24 g, 0.26 mmol, $[(t\text{-}Bu)_3PH]BF_4$ (0.15 g, 0.52 mmol), K_2CO_3 (0.36 g, 2.63 mmol), TEA (0.74 mL, 5.26 mmol), methyl acrylate (0.28 mL, 2.63 mmol), and DMF (10 mL) followed by general procedure for Heck reaction in 92 % yield; ^{1}H NMR (300 MHz, MeOD): δ 2.99 (t, *J* = 7.2 Hz, 2H), 3.20-3.25 (m, 2H), 3.79 (s, 3H), 6.36-6.37 (m, 1H), 6.61 (d, *J* = 15.6 Hz, 1H), 6.74 (d, *J* = 6.9 Hz, 1H), 6.75-7.00 (m, 1H), 7.17 (d, *J* = 3.3 Hz, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.61-7.66 (m, 3H), 7.75 (d, *J* = 8.7 Hz, 2H).

4.1.3.4. 3-{3-[2-(1H-Indol-4-yl)-ethylsulfamoyl]-phenyl}-acrylic acid methyl ester **(46).** Compound **46** was synthesized using a mixture of **38** (1.0 g, 2.63 mmol), $Pd_2(dba)_3$ (0.24 g, 0.26 mmol, $[(t\text{-}Bu)_3PH]BF_4$ (0.15 g, 0.52 mmol), K_2CO_3 (0.36 g, 2.63 mmol), TEA (0.74 mL, 5.26 mmol), methyl acrylate (0.28 mL, 2.63 mmol), and DMF (10 mL) followed by general procedure for Heck reaction in 88 % yield; ^{1}H NMR (500 MHz, DMSO): δ 2.89 (t, *J* = 7.5 Hz, 2H), 3.03 (t, *J* = 7.5 Hz, 2H),3.72 (s, 3H), 6.30 (s, 1H), 6.68-6.73 (m, 2H), 6.93 (t, *J* = 7.5 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 7.24 (t, *J* = 2.0 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.70 (d, *J* = 16.0 Hz, 1H) 7.78-7.79 (m, 2H), 7.92-7.96 (m,3H), 8.04 (s, 1H).

4.1.3.5. 3-{4-[2-(1H-Indol-5-yl)-ethylsulfamoyl]-phenyl}-acrylic acid methyl ester **(47).** Compound **47** was synthesized using a mixture of **39** (1.0 g, 2.63 mmol), $Pd_2(dba)_3$ (0.24 g, 0.26 mmol, $[(t\text{-}Bu)_3PH]BF_4$ (0.15 g, 0.52 mmol), K_2CO_3 (0.36 g, 2.63 mmol), TEA (0.74 mL, 5.26 mmol), methyl acrylate (0.28 mL, 2.63 mmol), and DMF (10 mL) followed by general procedure for Heck reaction in 89 % yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.84 (t, *J* = 6.5 Hz, 2H), 3.25-3.29 (m, 2H), 3.82 (s, 3H), 6.44 (t, *J* = 2.0 Hz, 1H), 6.48 (d, *J* = 16.0 Hz, 1H), 6.85-6.87 (m, 1H), 7.19 (d, *J* = 2.5 Hz, 1H), 7.25-7.28 (m, 2H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.65 (d, *J* = 16.0 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 8.27 (bs, 1H).

4.1.3.6. 3-{3-[2-(1H-Indol-5-yl)-ethylsulfamoyl]-phenyl}-acrylic acid methyl ester **(48).** Compound **48** was synthesized using a mixture of **40** (1.0 g, 2.63 mmol), $Pd_2(dba)_3$ (0.24 g, 0.26 mmol, $[(t\text{-}Bu)_3PH]BF_4$ (0.15 g, 0.52 mmol), K_2CO_3 (0.36 g, 2.63 mmol), TEA (0.74 mL, 5.26 mmol), methyl acrylate (0.28 mL, 2.63 mmol), and DMF (10 mL) followed by general procedure for Heck reaction in 81 % yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.84 (t, *J* = 6.5 Hz, 2H), 3.25-3.29 (m, 2H), 3.83 (s, 3H), 6.42-6.45 (m, 2H), 6.83-6.85 (m, 1H), 7.20 (t, *J* = 2.5 Hz, 1H), 7.26-7.29 (m, 2H), 7.45 (t, *J* = 7.5 Hz, 1H), 7.57 (d, *J* = 16.5 Hz, 1H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.74-7.75 (m, 2H).

4.1.3.7. 3-{4-[2-(1-Methyl-1H-indol-4-yl)-ethylsulfamoyl]-phenyl}-acrylic acid methyl ester **(49).** Compound **49** was synthesized using a mixture of **41** (1.0 g, 2.85 mmol), $Pd_2(dba)_3$ (0.26 g, 0.28 mmol), $[(t\text{-}Bu)_3PH]BF_4$] (0.16 g, 0.56 mmol), K_2CO_3 (0.39 g, 2.85 mmol), TEA (0.93 mL, 8.55 mmol), methyl acrylate (0.42 mL, 3.42 mmol), and DMF (10 mL) followed by general procedure for Heck reaction in 56 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.67 (t, *J* = 6.9 Hz, 2H), 3.17 (t, *J* = 7.2 Hz, 2H), 3.69 (s, 3H), 3.84 (s, 3H), 6.50 (d, *J* = 15.9 Hz, 1H), 6.54-6.56 (m, 1H), 6.89 (d, *J* = 6.3 Hz, 1H), 6.75 (t, *J* = 3.0 Hz, 1H),7.14-7.23 (m, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 15.9 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 2H).

4.1.3.8. 3-(4-{2-[1-(4-Methoxy-benzenesulfonyl)-1H-indol-4-yl]-ethylsulfamoyl}-phenyl)-acrylic acid

methyl ester **(50).** Compound **50** was synthesized using a mixture of **42** (0.3 g, 0.62 mmol), $Pd_2(dba)_3$ (0.06 g, 0.06 mmol), $[(t\text{-}Bu)_3PH]BF_4$] (0.03 g, 0.12 mmol), K_2CO_3 (0.08 g, 0.62 mmol), TEA (0.23 mL, 1.67 mmol), methyl acrylate (0.11 mL, 0.93 mmol), and DMF (5 mL) followed by general procedure for Heck reaction in 61 % yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.95 - 2.97 (m, 2H), 3.13 - 3.16 (m, 2H), 3.80 (s, 3H), 3.82 (s, 3H), 6.63 - 6.72 (m, 2H), 6.97-6.99 (m, 3H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.62-7.72 (m, 6H), 7.81-7.99 (m, 4H).

*4.1.4. Syntheses of hydroxamic acids **11**-**18**.*

4.1.4.1. N-Hydroxy-3-[4-(1H-indol-4-ylsulfamoyl)-phenyl]-acrylamide **(11).** Compound **11** was synthesized using free acid (0.82 g, 2.39 mmol), obtained after hydrolysis of compound **43** (1 g, 1.81 mmol), and a mixture of EDC•HCl (0.55 g, 3.58 mmol), HOBt (0.48 g, 3.58 mmol), NMM (0.92 mL, 8.36 mmol) and NH_2OTHP (0.31 g, 2.63 mmol) followed by general procedure for hydroxamic acid synthesis in 49% yield (from **43**); mp: 158-159 ^{o}C; purity: 95.13 %. ^{1}H NMR (300 MHz, CD_3OD): δ 6.40 (t, *J* = 3.0 Hz, 1H), 6.53 (d, *J* = 15.9 Hz, 1H), 6.84 (d, *J* = 6.9 Hz, 1H), 6.93 (t, *J* = 7.8 Hz, 1H), 7.05 (d, *J* = 3.3 Hz, 1H), 7.11 (d, *J* = 7.1 Hz, 1H), 7.22 (d, *J* = 15.9 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H). MS (ESI) m/z: 356.1 ($M\text{-}H^+$). HRMS (ESI) for $C_{17}H_{15}N_3O_4S$ ($M\text{-}H^+$): calcd, 356.0895; found, 356.1869.

4.1.4.2. N-Hydroxy-3-{4-[(1H-indol-4-ylmethyl)-sulfamoyl]-phenyl}-acrylamide **(12).** Compound **12** was synthesized using free acid (1.1 g, 2.08 mmol), obtained after hydrolysis of compound **44** (1.2 g, 3.02 mmol), and a mixture of EDC•HCl (0.72 g, 7.28 mmol), HOBt hydrate (0.62 g, 3.12 mmol), NMM (1.2 mL, 7.28 mmol) and NH_2OTHP (0.40 g, 3.08 mmol) followed by general procedure for hydroxamic acid synthesis 53% yield (overall from **44**); mp: 161-163 ^{o}C; purity: 98.45 %. ^{1}H NMR (300 MHz, CD_3OD): δ 4.35 (s, 2H), 6.46 (d, *J* = 3.0 Hz, 1H), 6.55 (d, *J* = 15.6 Hz, 1H), 6.84 (d, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 8.1 Hz, 1H), 7.19 (d, *J* = 3.0 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 1H), 7.55-7.61 (m, 3H), 7.76 (d, *J* = 8.1 Hz, 2H). MS (ESI) m/z: 370.1 ($M\text{-}H^+$). HRMS (ESI) for $C_{18}H_{17}N_3O_4S$ ($M\text{-}H^+$):

calcd, 370.0940; found, 370.0862.

4.1.4.3. N-Hydroxy-3-{4-[2-(1H-indol-4-yl)-ethylsulfamoyl]-phenyl}-acrylamide **(13).** Compound **13** was synthesized using free acid (1.0 g, 2.70 mmol), obtained after hydrolysis of compound **45** (1 g, 2.60 mmol), and a mixture of EDC•HCl (0.77 g, 4.05 mmol), HOBt hydrate (0.62 g, 4.05 mmol), NMM (1.13 mL, 8.1 mmol) and NH_2OTHP (0.38 g, 3.24 mmol) followed by general procedure for hydroxamic acid synthesis 56% yield (overall from **45**); mp: 164-165 ^oC; purity: 97.52 %. ^1H NMR (300 MHz, CD_3OD): δ 2.96-3.01 (m, 2H), 3.23 (t, *J* = 8.1 Hz, 2H), 6.37 (d, *J* = 3.0 Hz, 1H), 6.57 (d, *J* = 15.6 Hz, 1H), 6.74 (d, *J* = 6.9 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 7.17 (d, *J* = 3.0 Hz, 1H), 7.21 (d, *J* = 7.2 Hz, 1H), 7.56-7.64 (m, 3H), 6.74 (d, *J* = 8.1 Hz, 2H). MS (ESI) m/z: 386.1 ($M+H^+$). HRMS (ESI) for $C_{19}H_{19}N_3O_4S$ ($M+H^+$): calcd, 386.1095; found, 386.1169.

4.1.4.4. N-Hydroxy-3-{3-[2-(1H-indol-4-yl)-ethylsulfamoyl]-phenyl}-acrylamide **(14).** Compound **14** was synthesized using free acid (1.0 g, 2.70 mmol), obtained after hydrolysis of compound **46** (1 g, 2.60 mmol), and a mixture of EDC•HCl (0.77 g, 4.05 mmol), HOBt hydrate (0.62 g, 4.05 mmol), NMM (1.13 mL, 8.1 mmol) and NH_2OTHP (0.38 g, 3.24 mmol) followed by general procedure for hydroxamic acid synthesis 51% yield (overall from **46**); mp: 161-163 ^oC; purity: 100.00 %. ^1H NMR (500 MHz, DMSO): δ 2.70 (t, *J* = 7.0 Hz, 2H), 2.93-2.98 (m, 2H), 6.29 (s, 1H), 6.62 (d, *J* = 16.0 Hz, 1H), 6.83 (t, *J* = 8.5 Hz, 1H), 7.23-7.25 (m, 3H), 7.59 (d, *J* = 16.0 Hz, 1H), 7.75 (d, *J* = 8.5 Hz, 3H), 7.84 (d, *J* = 8.0 Hz, 2H). MS (ESI) m/z: 386.1 ($M+H^+$). HRMS (ESI) for $C_{19}H_{19}N_3O_4S$ ($M+H^+$): calcd, 386.1095; found, 386.1169.

4.1.4.5. N-Hydroxy-3-{4-[2-(1H-indol-5-yl)-ethylsulfamoyl]-phenyl}-acrylamide **(15).** Compound **15** was synthesized using free acid (1.0 g, 2.70 mmol), obtained after hydrolysis of compound **47** (1 g, 2.60 mmol), and a mixture of EDC•HCl (0.77 g, 4.05 mmol), HOBt hydrate (0.62 g, 4.05 mmol), NMM (1.13 mL, 8.1 mmol) and NH_2OTHP (0.38 g, 3.24 mmol) followed by general procedure for hydroxamic acid synthesis 48 % yield (overall from **47**); mp: 159-160 ^oC; purity: 96.10 %. ^1H NMR

(300 MHz, MeOD): δ 2.78 (t, *J* = 7.2 Hz, 2H), 3.15 (t, *J* = 7.5 Hz, 2H), 7.73 (d, *J* = 3.0 Hz, 1H), 6.57 (d, *J* = 15.6 Hz, 1H), 6.81-6.85 (m, 1H), 7.18-7.24 (m, 1H), 7.44 (d, *J* = 15.6 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.71 (d, *J* = 8.1 Hz, 2H). MS (ESI) m/z: 386.1 ($M+H^+$). HRMS (ESI) for $C_{19}H_{19}N_3O_4S$ ($M+H^+$): calcd, 386.1095; found, 386.1169.

4.1.4.6. N-Hydroxy-3-{3-[2-(1H-indol-5-yl)-ethylsulfamoyl]-phenyl}-acrylamide **(16).** Compound **16** was synthesized using free acid (1.0 g, 2.70 mmol), obtained after hydrolysis of compound **48** (1 g, 2.60 mmol), and a mixture of EDC•HCl (0.77 g, 4.05 mmol), HOBt hydrate (0.62 g, 4.05 mmol), NMM (1.13 mL, 8.1 mmol) and NH_2OTHP (0.38 g, 3.24 mmol) followed by general procedure for hydroxamic acid synthesis 38 % yield (overall from **48**); mp: 162-163 ^oC; purity: 97.86 %. ^1H NMR (500 MHz, MeOD): δ 2.73 (t, *J* = 7.5 Hz, 2H), 3.15 (t, *J* = 7.5 Hz, 2H), 6.30 (d, *J* = 3.0 Hz, 1H), 6.50 (d, *J* = 16.0 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 7.15 (d, *J* = 3.0 Hz, 1H), 7.20-7.21 (m, 2H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 15.5 Hz, 1H), 7.67-7.71 (m, 2H), 7.88 (s, 1H). MS (ESI) m/z: 386.1 ($M+H^+$). HRMS (ESI) for $C_{19}H_{19}N_3O_4S$ ($M+H^+$): calcd, 386.1095; found, 386.1169.

4.1.4.7. N-Hydroxy-3-{4-[2-(1-methyl-1H-indol-4-yl)-ethylsulfamoyl]-phenyl}-acrylamide **(17).** Compound **17** was synthesized using free acid (0.15 g, 0.39 mmol), obtained after hydrolysis of compound **49** (1 g, 2.50 mmol), and a mixture of EDC•HCl (0.09 g, 0.58 mmol), HOBt hydrate (0.08 g, 0.58 mmol), NMM (0.11 mL, 1.05 mmol) and NH_2OTHP (0.05 g, 0.43 mmol) followed by general procedure for hydroxamic acid synthesis 55 % yield (overall from **49**); mp: 147-148 ^oC; purity: 98.80 %. ^1H NMR (300 MHz, MeOD): δ 2.98 (t, *J* = 6.9 Hz, 2H), 3.22 (t, *J* = 7.1 Hz, 2H), 3.75 (s, 3H), 6.34 (d, *J* = 3.3 Hz, 1H), 6.57(d, *J* = 15.6 Hz, 1H), 6.78 (d, *J* = 7.2 Hz, 1H), 7.03 (d, *J* = 7.8 Hz, 1H), 7.07-7.09 (m, 2H), 7.19 (d, *J* = 8.1 Hz, 1H), 7.56-7.62 (m, 3H), 7.72 (d, *J* = 8.1Hz, 2H). MS (ESI) m/z: 400.1 ($M+H^+$). HRMS (ESI) for $C_{20}H_{22}N_3O_4S$ ($M+H^+$): calcd, 400.1331; found, 400.1321.

4.1.4.8.

N-Hydroxy-3-(4-{2-[1-(4-methoxy-benzenesulfonyl)-1H-indol-4-yl]-ethylsulfamoyl}-phenyl)-acrylamide **(18).** Compound **18** was synthesized using free acid (0.2 g, 0.38 mmol), obtained after hydrolysis of compound **50** (0.21 g, 0.34 mmol), and a mixture of EDC•HCl (0.09 g, 0.58 mmol), HOBt hydrate (0.08 g, 0.58 mmol), NMM (0.11 mL, 1.05 mmol) and NH_2OTHP (0.05 g, 0.43 mmol) followed by general procedure for hydroxamic acid synthesis 49 % yield (overall from **50**); mp: 142-144 ^oC; purity: 97.13 %. ^1H NMR (300 MHz, MeOD): δ 2.95 - 2.97 (m, 2H), 3.12- 3.15 (m, 2H), 3.80 (s, 3H), 6.57 (d, *J* = 15.3 Hz, 1H), 6.72 (s, 1H), 6.97- 7.00 (m, 3H), 7.18(t, *J* = 7.2 Hz, 1H), 7.56-7.63 (m, 4H), 7.70 - 7.73 (m, 2H), 7.79 -7.86 (m, 3H). MS (ESI) m/z: 554.2 ($M-H^+$). HRMS (ESI) for $C_{26}H_{25}N_3O_7S_2$ ($M-H^+$): calcd, 554.1134; found, 555.1053.

4.2. Biology

4.2.1. Tumor Cell Culture.

All human cancer cells were maintained in RPMI 1640 medium supplemented with 10% FBS and penicillin (100 units/ml)/streptomycin (100 μg/ml)/amphotericin B (0.25 μg/ml). All cells were maintained in humidified air containing 5% CO_2 at 37°C and cultured every 2-3 days. All cells were cultured in tissue culture flasks in a humidified air containing 5% CO_2 and 95% air at 37 ° C and cultured every 2-3 days.

4.2.2. The Sulforhodamine B Assays

Cells were seeded at the density of 5000 cells/well into 96-plate overnight. Basal 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 (C) or different doses of test compounds (Tx) for 48 h, cells were fixed with 10 % TCA and stained with SRB at 0.4 % (w/v) in 1 % acetic acid. Unbound SRB was washed out using 1 % acetic acid and SRB bound cells were solubilized with 10

mM Trizma base. The absorbance was read at a wavelength of 515 nm. The 50% growth inhibition (GI_{50}) was calculated by $100 - [(T_x - T_0) / (C - T_0)] \times 100$.

4.2.3. HeLa Nuclear Extract HDAC Activity Assay

HDAC Fluorescent Activity Assay Kit (BioVision, CA) was used to detect HeLa nuclear extract HDAC activity according to 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. A fluorescence plate reader with excitation at 355 nm and emission at 460 nm was used to quantify HDAC activity.

4.2.4. HDAC Biochemical Assays.

The HDACs *in vitro* activities of human recombinant HDAC 1, 2, 4, 6, and 8 were conducted by Eurofin Panlabs (Taipei, Taiwan). In brief, indicated compounds were incubated with specific HDAC enzyme and Fluor-de-Lys deacetylase substrate. Fluor-de-Lys deacetylsubstrate were spectrofluorimetric quantitated compared to control.

4.2.5. Western Blot Analysis

Cells were incubated with indicated compounds for 24 h and lysed with ice-cold lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% Sodium Deoxycholate, 0.1% SDS, 2.5 mM β-glycerolphosphate, 1 mM $Na_4P_2O_7$, 5 mM NaF, 1 mM Na_3VO_4 and protease inhibitor cocktail from Millipore) on ice for 30 min followed by centrifugation at 13000 rpm for 30 min. Protein concentrations were determined and equal amounts of protein were separated by 8-15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to poly(vinylidene difluoride) (PVDF) membranes. Membranes were immunoblotted with specific antibodies overnight at 4°C and then applied to appropriate horseradish

peroxidase-conjugated anti-mouse or anti-rabbit IgG secondary antibodies for 1 h at room temperature. Signals were detected using an enhanced chemiluminescence (Amersham, Buckinghamshire, UK).

4.2.6. Immunohistochemistry (IHC).

Formalin-fixed paraffin-embeded tumor sections were submitted to Rapid Science Co., Ltd. to conduct the immunohistochemistry staining. In brief, tumor sections were deparaffinized, rehydration and immersed in 3% H_2O_2 to inactivate endogenous peroxidase. For antigen retrival, slides were incubated in buffer with pH 9 at 80°C overnight. After PBS washing, the slides were blocked with fatty-free milk and incubated with primary antibody at 4°C overnight. The secondary antibody was added and then Mayer's Hematoxylin solution was used for counterstaining. For hematoxylin and eosin stain (H&E stain), the tumor sections were incubated in hematoxylin solution and then counterstained with eosin.

4.2.7. Antitumor Activity in vivo.

Male nude mice were injected subcutaneously with the same volume of BD Matrigel Matrix HC (catalog 354248, BD bioscience), and PC3 cells (1×10^7cell/mouse) into the flank of each animal. When the tumors had grown to around 100 mm^3, animals were divided into three groups (n=6) and receive the following treatment by intraperitoneal injection for 18 days during the study: (a) vehicle alone, Compound 13 at (b) 20 mg/kg or (c) 40 mg/kg every other day (q2d). Compound 13 was dissolved in vehicle (5% Cremophor EL + 5% DMSO + 90% dextrose). Tumor size was measured twice weekly and calculated from $V = l*w^2/2$, where w = width (w) and l = length (l). The mice were housed on Taipei Medical University Laboratory Animal Center, TMU, on a 12-hour light cycle at 21–23 °C and 60–85% humidity. Nude mice were maintained in accordance with the Institutional Animal Care and Use Committee procedures and guidelines (IACUC no. LAC-2013-0139).

4.2.8. Statistical and Graphical Analyses.

Each experiment was performed independently at least three times and the data are presented as mean ± SEM for the indicated number of separate experiments. Student's t-test was used to compare the mean of each group with that of the control group in experiments and one-way ANOVA was used in animal study. P-values less than 0.05 were considered significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Acknowledgments

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Abbreviations

HDAC, histone deacetylase; NH_2OTHP, O-(tetrahydro-2H-pyran-2-yl)hydroxylamine; EDC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; NMM, N-methylmorpholine; DMF, N,N-dimethylformamide; TGI, tumor growth inhibition; IP, intraperitoneal.

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Figure Caption

Figure 1. Various examples of HDIs

Figure 2. Design and rationale of the study

Figure 3. Series of 4,5-indolyl-N-hydroxyphenylacrylamides

Figure 4. (A) Effect of α-tubulin acetylation and histone H3 acetylation in cultured human prostate cells (PC-3) by compound **13** and reference **1**. Cells were treated with 1 μM of compound **13** and reference **1** for 24 h and subjected to western blot analysis. Quantification analysis of western blot using ImageJ software; (B) acetyl-α-tubulin and (C) acetyl-histone 3 were analyzed in both cells

Figure 5. Inhibition of prostate cancer (PC-3) cells xenograft growth in nude mice (n = 7-8). Compounds **12** was suspended in 1% carboxymethyl cellulose and 0.5% Tween 80. *Left panel*, tumor growth of PC3 xenograft nude mice treated with or without compound **13** by ip (20 and 40 mg/kg) with a frequency of q2d. Tumor growth is tracked by the mean tumor volume (mm^3) ± S.E and calculated as % tumor growth inhibition (%TGI). Tumor volume was determined using caliper measurements and was calculated as the product of 1/2 x length x $width^2$. *Right panel*, body weight (g) of the mice. *, $p < 0.05$, and ** $p < 0.01$ as compared with the control group.

Figure 6. Immunohistochemistry of protein expressions in PC-3 xenograft model. Formalin-fixed paraffin-embedded sections from PC3 xenograft tumors were stained with H&E stain, acetyl-hisotne 3, HDAC 1 and 2 antibodies.

Table 1 Inhibition of HeLa Nuclear Extract HDAC Activity and Antiproliferative Activity against Human Cancer Cell Lines by **11** to **18** and Reference Compounds

Cpds.	IC_{50} ± SD (nM[a]) HeLa nuclear HDAC	Cell type (GI_{50} ± SD, μM[a]) Prostate PC-3	Lung A549	Breast MDA-MB-231	Pancreatic AsPC-1
11	> 1000	3.49 ± 0.17	>10	>10	>10
12	7.17 ± 3.13	0.86 ± 0.07	1.65 ± 0.27	3.55 ± 0.29	4.56 ± 1.91
13	8.56 ± 3.67	0.14 ± 0.02	0.25 ± 0.01	0.32 ± 0.07	0.24 ± 0.03
14	30.45 ± 7.93	0.31 ± 0.08	0.87 ± 0.03	2.06 ± 0.66	3.85 ± 1.05
15	76.13 ± 33.3	0.31 ±0.05	0.60 ± 0.16	9.91 ± 2.04	3.97 ± 1.22
16	52.17 ± 10.2	0.33 ± 0.01	0.82 ± 0.02	2.47 ± 0.38	2.78 ± 1.36
17	14.3 ± 3.91	0.15 ± 0.03	0.25 ± 0.01	0.08 ± 0.01	0.16 ± 0.02
18	60 ± 12	2.88 ± 0.39	4.35 ± 0.63	5.85 ± 0.09	12.46 ± 2.24
9[19]	29.5 ± 4.5	0.62 ± 0.2	1.02 ± 0.2	0.48 ± 0.1	ND
SAHA (**1**)	131.26 ± 30.0	0.60 ± 0.06	1.73 ± 0.02	2.67 ± 0.50	5.44 ± 0.49
PXD101 (**3**)	81 ± 21	0.31 ± 0.02	0.23 ± 0.01	0.28 ± 0.04	0.65 ± 0.12
LBH589.HCl (4)	4.90±1.74	0.07±0.01	0.15±0.01	0.06±0.03	0.03±0.002
MS-275 (**6**)	> 1000	0.43 ± 0.06	5.76 ± 0.57	1.41 ± 0.13	6.90 ± 1.30

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

Table 2 Activities of **13** and **17** and Reference Compounds **1**, **3** and **6** Against HDAC Isoforms 1, 2, 4, 6, and 8.

Compd	HDAC Isoforms IC_{50} (nM)[a]				
	1	**2**	**4**	**6**	**8**
13	1.28	0.90	> 100	8.32	> 100
17	1.34	0.53	> 100	6.80	> 100
1	110.0	120.0	> 1000	110.0	> 1000
3	20	50	>1000	9.85	70
4	1.06	0.18	> 100	14.4	> 100
6	99	740	>10000	>10000	>10000

[a]These assays were conducted by Eurofins Panlabs Taiwan Ltd. All compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 10 μM All experiments were independently performed at least three times.

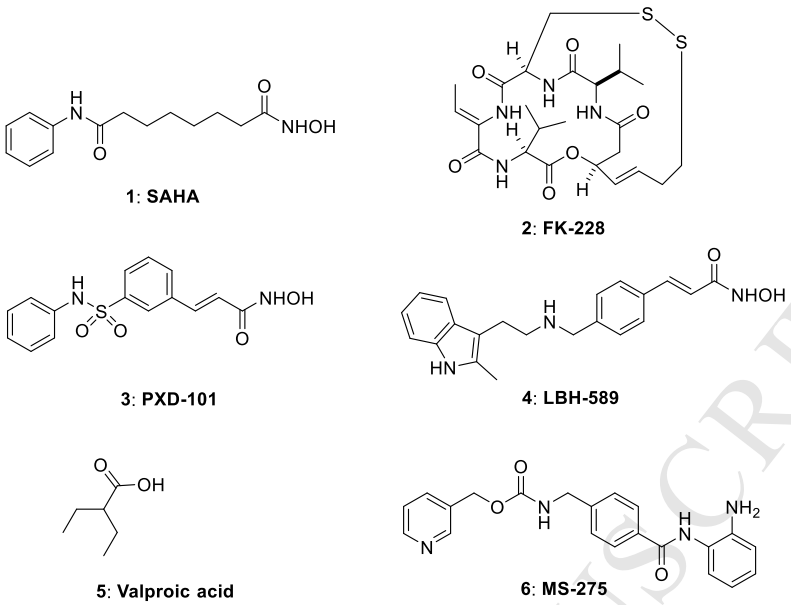


Figure 1. Various examples of HDIs

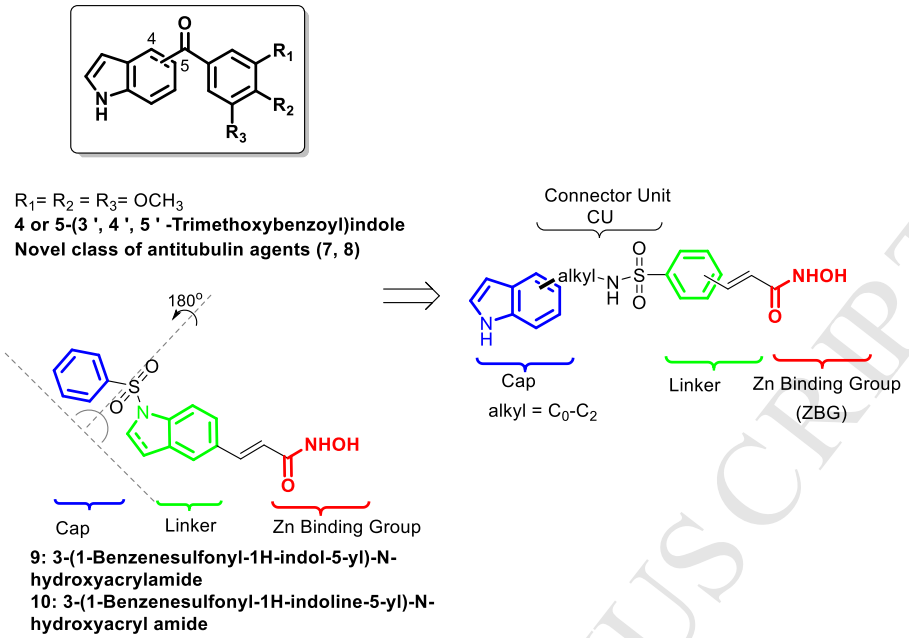
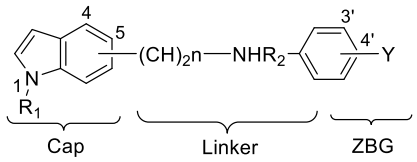


Figure 2. Design and rationale of the study



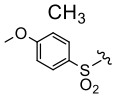
	indole position	R_1	R_2	n	Y
11:	4	H	SO_2	0	4'-(N-hydroxyacrylamide)
12:	4	H	SO_2	1	4'-(N-hydroxyacrylamide)
13:	4	H	SO_2	2	4'-(N-hydroxyacrylamide)
14:	4	H	SO_2	2	3'-(N-hydroxyacrylamide)
15:	5	H	SO_2	2	4'-(N-hydroxyacrylamide)
16:	5	H	SO_2	2	3'-(N-hydroxyacrylamide)
17:	4	CH_3	SO_2	2	4'-(N-hydroxyacrylamide)
18:	4		SO_2	2	4'-(N-hydroxyacrylamide)

Figure 3. Series of 4,5-indolyl-N-hydroxy phenyl- acrylamides

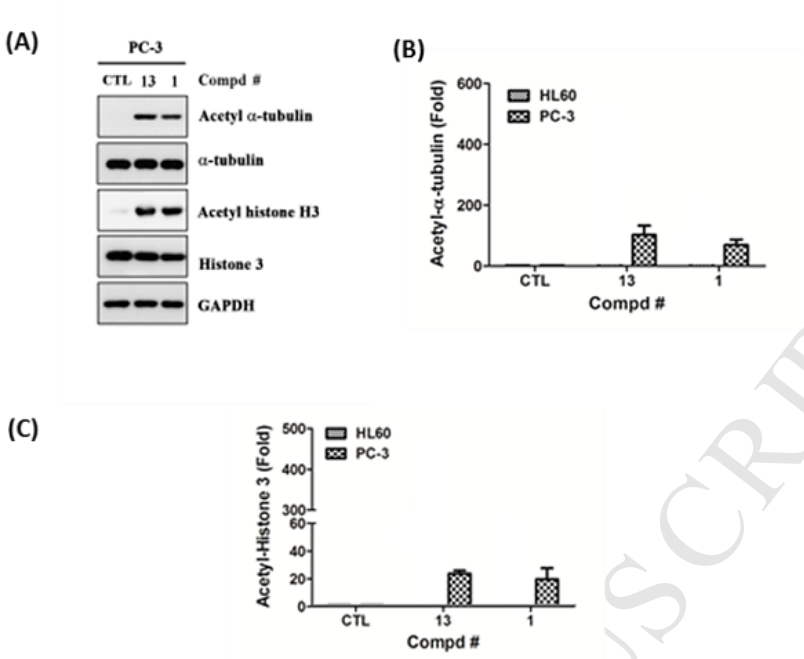
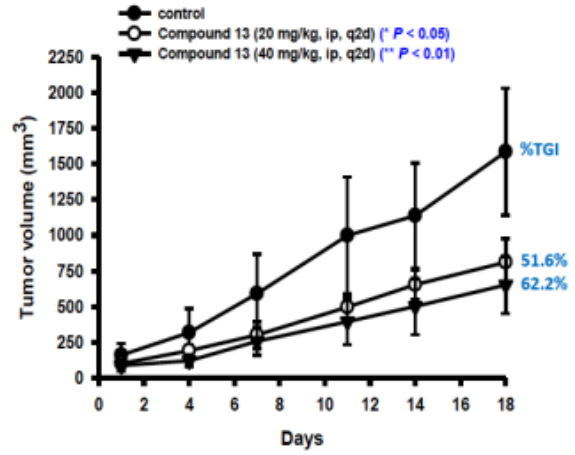
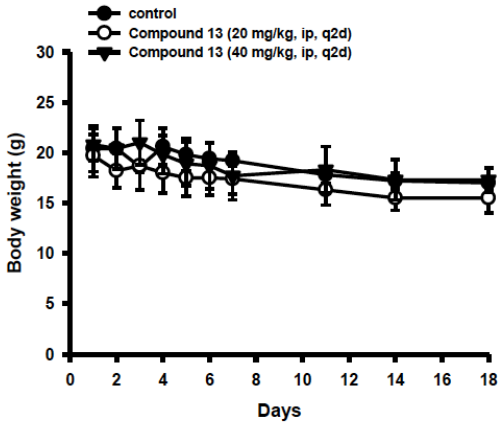


Figure 4. (A) Effect of α-tubulin acetylation and histone H3 acetylation in cultured human prostate cells (PC-3) by compound **13** and reference **1**. Cells were treated with 1 μM of compound **13** and reference **1** for 24 h and subjected to western blot analysis. Quantification analysis of western blot using ImageJ software; (B) acetyl-α-tubulin and (C) acetyl-histone 3 were analyzed in both cells

A



B



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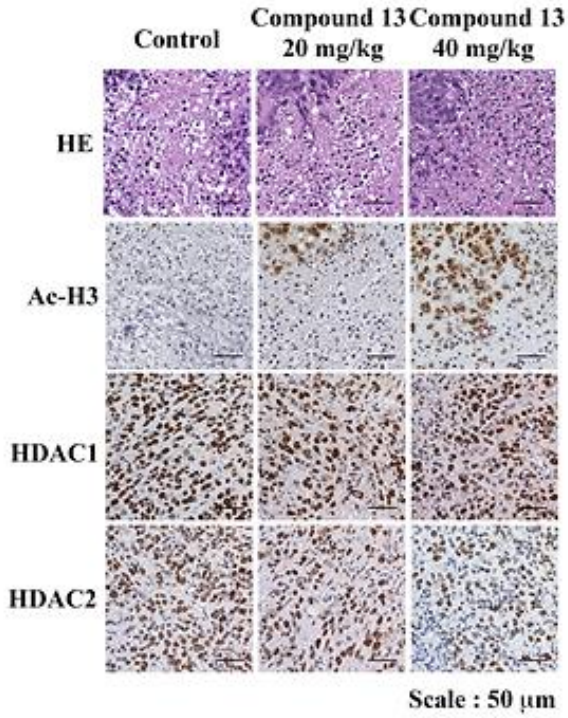
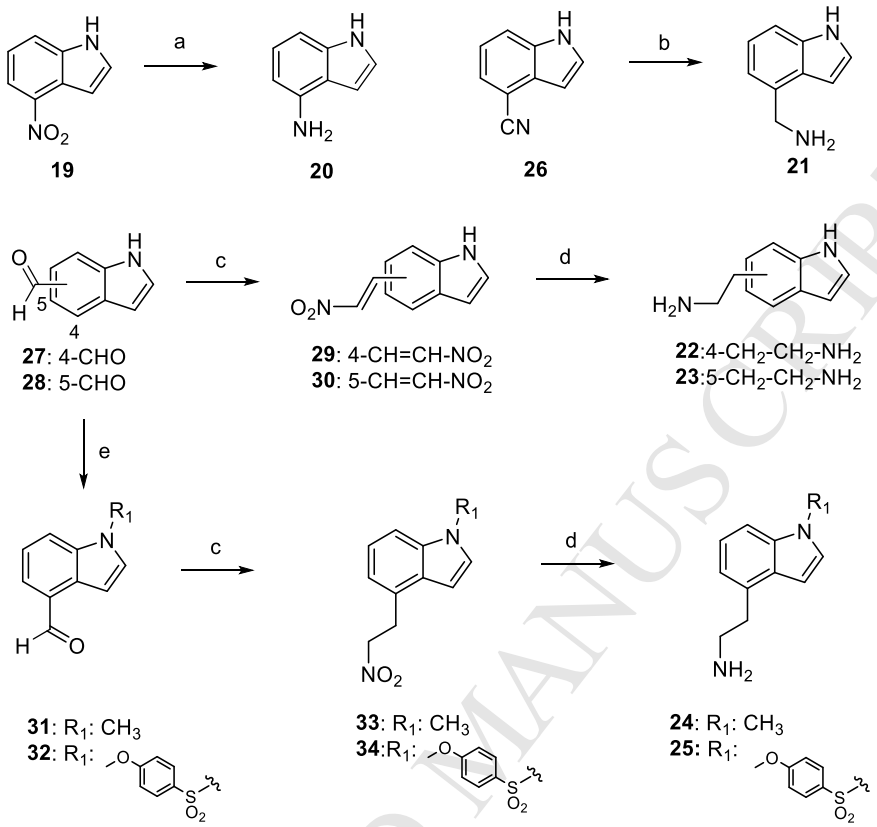


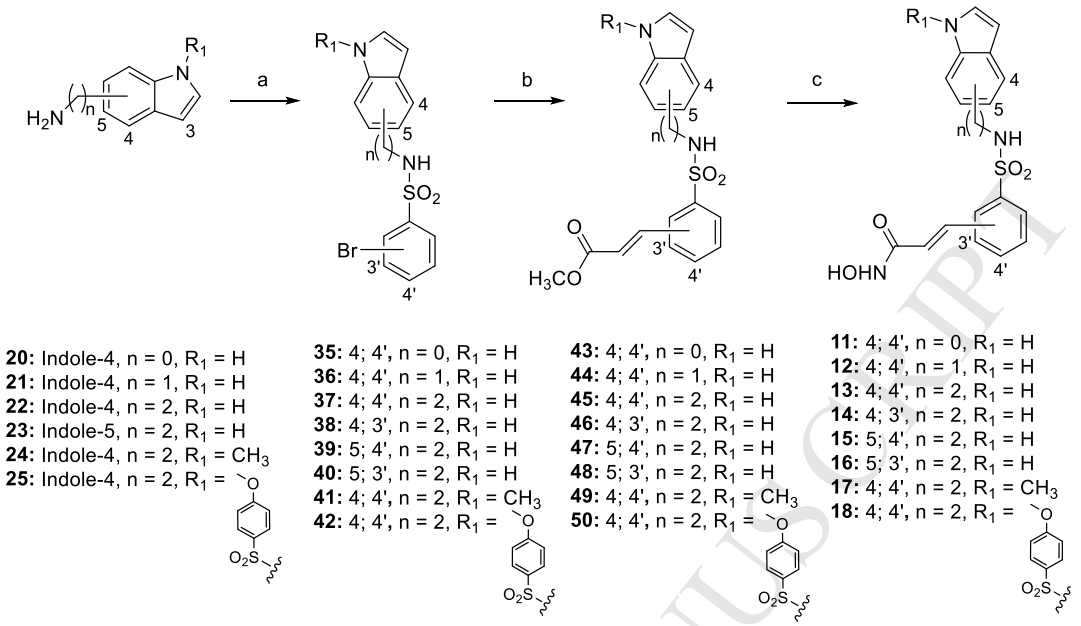
Figure 6. Immunohistochemistry of protein expressions in PC-3 xenograft model. Formalin-fixed paraffin-embedded sections from PC3 xenograft tumors were stained with H&E stain, acetyl-hisotne 3, HDAC 1 and 2 antibodies.

Scheme 1. Synthetic approaches to amines **20 to 25** [a]



[a]Reagents and conditions: (a) Fe, NH_4Cl, Isopropanol: H_2O (4:1), reflux (b) Pd/C, H_2, 40-42 psi, CH_3OH, rt; (c) CH_3NO_2, ammonium acetate, reflux; (d) $NaBH_4$, MeOH, [2] Fe, NH_4Cl, IPA: H_2O (4:1), reflux; (e). NaH, MeI (**31**) or 4-methoxybeznzenesulfonylchloride (**32**), DMF, 0^oC to rt.

Scheme 2. Synthetic approaches to compounds **11 to 18**.[a]



[a]Reagents and conditions: (a) bromobenzenesulfonyl chlorides, pyridine, ACN, rt; (b) methyl acrylate, $Pd_2(dba)_3$, [(*t*-Bu)$_3$P]BF_4, K_2CO_3, TEA, DMF, 100-105 ^oC; (c) (i) 1N LiOH (aq.), 40 ^oC; [ii] EDC•HCl, HOBt, NMM, NH_2OTHP, NMM, rt; [iii] 10% $TFA_{(aq)}$, CH_3OH, rt

Research Highlights

1. Rationally designed 4-Indolyl-N-hydroxyphenylacrylamides compounds evaluated for their anticancer activity.
2. These compounds have shown potent inhibitory activity against HDAC isoforms1, 2 and 6 in HDAC isoform assay.
3. Human prostate cancer xenograft model indicated potent activity of these compounds against prostate cancer with 62.2 % dose dependent TGI.
4. Immunohistochemistry analysis revealed that these compounds selectively inhibit expression of HDAC 2 in PC-3 cancer model.
5. Activities attributed to increased HDAC3 Acetylation and increases α-tubulin expression as indicated by western blot analysis.