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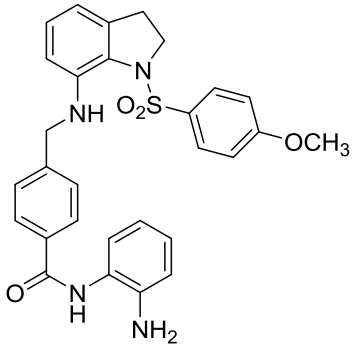
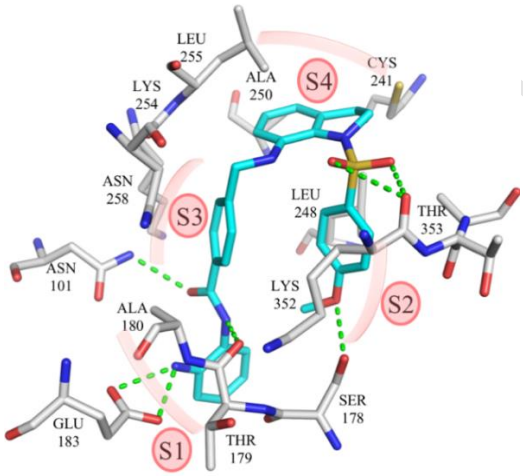
Graphical Abstract



Compound **9**:
Cell growth inhibition: IC_{50} = 49 nM (KB cells)
Tubulin polymerization inhibition: IC_{50} = 1.1 μM
HDAC 1 inhibition: IC_{50} = 0.221 μM



Docking pose of compound 9 in tubulin

European Journal of Medicinal Chemistry

1-Arylsulfonyl Indoline-Benzamides as a New Antitubulin Agents, with Inhibition of Histone Deacetylase

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ABSTRACT. We report structure-activity relationships of 1-arylsulfonyl indoline based benzamides. The benzamide (**9**) exhibits striking tubulin inhibition with an IC_{50} value of 1.1 μM, better than that of combretastain A-4 (**3**), and substantial antiproliferative activity against a variety of cancer cells, including MDR-positive cell lines with an IC_{50} value of 49 nM (KB), 79 nM (A549), 63 nM (MKN45), 64 nM (KB-VIN10), 43 nM (KB-S15), and 46 nM (KB-7D). Dual inhibitory potential of compound **9** was found as it demonstrated significant inhibitory potential against HDAC1, 2 and 6 in comparison to MS-275 (**6**). Some key interactions of **9** with the amino acid residues of the active site of tubulin and with amino acid residues of HDAC 1 isoform have been figured out by molecular modeling. Compound **9** also demonstrated significant *in vivo* efficacy in the human non-small cell lung cancer A549 xenograft model as well as B-cell lymphoma BJAB xenograft tumor model.

Keywords: Tubulin, Indoline, benzamide, HDAC, cancer

1. Introduction

Chemotherapy is one of the major approaches to cancer treatment. The high risk of toxicity, drug resistance, and lack of specificity limit the use of traditional cytotoxic agents in the clinic and this, in turn has triggered the continuing search for new anticancer agents [1]. ABT751 (**1**), colchicine (**2**), combretastatin A-4 (**3**), combretastatin A-4P (**4**), and AVE-8062 (**5**) are some examples of antimitotic agents (Figure 1) which inhibit not only the dynamics of tubulin polymerization but also serve as structural scaffolds for novel potent lead anti- cancer molecules [2,3].

Indolines are structurally essential elements of biologically active natural compounds and as they are widely used as pharmacophores in drug discovery [4-6] they are extremely important in medicinal chemistry. As a part of our drug discovery program, we are actively involved in the design of indoline-based antiproliferative agents. In our previous work, 7-aroyl-aminoindoline-sulfonamides were designed in an effort to rigidify compound **1**, an oral antimitotic agent and vascular disrupting agent [6]. Compound **1** binds competitively to the colchicine site of tubulin and inhibits tubulin polymerization with resultant G2/M arrest and apoptosis. The promising findings of the study motivated us to investigate indoline based constructs exerting anticancer effects via diverse mechanisms.

Recent reports on the antiproliferative potential of benzamides operating with diverse mechanisms [7-15] also prompted us to design *N*-aryl-4-(((1-(arylsulfonyl)-indolin-7-yl)amino)methyl)benzamides as a new class of antiproliferative agents and this is reported in this study. Literature survey reveals that tubulin inhibitors have demonstrated significant flexibility towards the inclusion of diverse antitumor pharmacophores that enables the modulation of multiple targets [16, 17]. Indoline and indole based scaffolds have been widely employed as structural motifs for the design of tubulin inhibitors as well as flexible components of HDAC inhibitory model [6, 18-19]. The benzamide group represents a well-accepted pharmacophore for HDAC inhibition as a zinc binding group that chelates the zinc atom in its active site [10]. MS-275 (**6**) [12 - 13] and Chidamide (**7**) approved by China's FDA represents HDAC inhibitor with a benzamide group [20]. Keeping this in view, fusion of 7-aroyl-aminoindoline-

sulfonamide scaffold with a benzamide functionality appeared to be a rational approach for designing tubulin inhibitors capable of modulating the activity of HDAC enzymes. To add on, the strategy of activating tubulin inhibitory framework towards the HDAC inhibition has been attempted previously by several research groups and optimistic results were attained [17].

Furthermore, number of attempts were made to gain better insight into the SAR of these rigid benzamide analogs and included: a) the influence of electronic effects was observed by placing a differently substituted aryl sulfonyl group at the N1 position of the indoline moiety, b) the 7-anilino/7-aroyl group in previously synthesized analogs was replaced by *N*- and *O*-benzyl functionalities, and an ethyl or vinyl group was introduced as a linker for benzamide functionality to study the influence of induced flexibility in the designed compounds, c) appropriate comparisons were made by synthesizing various indole-based target compounds to evaluate the effect of planarity on the antiproliferative activity, d) regio effects of the benzamide group and comparisons of the designed compounds with the biarylamine type compound were analyzed.

Thus the present study describes the first exploration of the antiproliferative effects of 1-(arylsulfonyl)indolines possessing a benzamide group. The synthesized compounds were evaluated for the *in vitro* cytotoxic activity against a panel of human cancer cell lines including multidrug resistant cancer cell lines and also for their effect on tubulin polymerization. The *in-vivo* efficacy along with HDAC inhibitory potential of the most potent antiproliferative agent and tubulin polymerization inhibitor has also been explored.

2. Results and Discussion

2.1 Chemistry

In Schemes 1-3 the synthetic routes to the designed compounds (**8-28**) are shown. Reaction of compounds **29a-e** with various substituted benzenesulfonyl chlorides yielded 5-bromo-1-(benzenesulfonyl)-7-nitroindolines which were subsequently reduced to provide the corresponding amines. The treatment of the amines (in the case of compounds synthesized from **29a**) with tributyltin

hydride (Bu_3SnH) and 2,2'-azobis-(2-methylpropionitrile) (AIBN) initiated a free radical cascade leading to the debrominated compounds (**30a-30g**). Compounds **30a-30k** were then subjected to reductive amination with 3- and 4-carboxybenzaldehyde and $NaBH_3CN$ to afford the corresponding acids (**31a-g** and **31i-n**). For the synthesis of compound **31h**, **30b** was treated with 4-(methoxycarbonyl)-phenyl)boronic acid in the presence of $Cu(OAc)_2$, myristic acid, and 2,6-lutidine followed by LiOH-mediated ester hydrolysis. The resulting carboxylic acids was further reacted with *o*, *m*, *p*-phenylenediamine and 2-aminophenol using PyBOP as the coupling reagent and triethylamine as the base, to obtain the desired benzamides (**8-25**) (Scheme 1). The synthetic route employed in scheme 1 afforded the target benzamides in moderate to good yields (43 - 76 %). Significant variations were observed in the yields of the benzamides and effect of differently substituted benzene sulfonyl functionality (at N^1 position) along with the different placement of benzamide functionality could not be ascertained on the reaction yields.

The synthetic route to *N*-(2-aminophenyl)-4-(2-(1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)ethyl)benzamide (**26**) is shown in Scheme 2. 1*H*-Indole-7-carboxaldehyde was subjected to a Wittig reaction with 4-methoxycarbonylbenzyltriphenylphosphonium chloride to yield methyl (*E*)-4-(2-(1H-indol-7-yl)vinyl)benzoate. Hydrogenation with Pd/C and subsequent reduction of the indole to an indoline with sodium cyanoborohydride gave methyl 4-(2-(indolin-7-yl)ethyl)-benzoate (**34**). The benzamide (**26**) was produced from compound **35** by a synthetic route similar to that shown in Scheme 1 and involving sulfonylation, ester hydrolysis, and amidation with *o*-phenylenediamine. The benzamide (**27**) with a vinyl linker, was synthesized in a manner similar to that employed for compound **26** keeping the double bond at C7 intact. The synthetic route employed afforded the target benzamides in moderately good yields (65 %). The synthetic route to compound **28** is shown in Scheme 3. *tert*-Butyldimethylsilyl-protected 7-hydroxyindoline was treated with 4-methoxybenzenesulfonyl chloride in pyridine followed by TBAF mediated deprotection of the *tert*-butyldimethylsilyl group in THF to afford the 7-hydroxy-1-(4-methoxybenzene-sulfonyl)indoline (**40**). Compound **40**, on reaction with methyl 4-

(chloromethyl)-benzoate in the presence of K_2CO_3 followed by the synthetic strategy employed in previous schemes yielded the benzamide **28** (Scheme 3). Benzamide 28 was obtained in 52 % yield via synthetic strategy employed in scheme 3.

2.2 Biological Evaluation.

The synthesized compounds were evaluated for growth inhibitory effects *in vitro* against three human cancer cell lines, oral epidermoid carcinoma (KB cells), stomach carcinoma (MKN45 cells) and lung adenocarcinoma (A549 cells). Compounds **1**, **2**, and **6** were used as standards and the results, presented in Table 1 revealed that the oral epidermoid carcinoma KB cells were the most sensitive to these synthetic compounds. Compound **9** exhibited significant inhibitory effects against all the cell lines (IC_{50} = 50 to 80 nM) with the most substantial activity against KB cells (IC_{50} = 50 nM). The inhibitory effects of **9** were more potent than those of **1** and **6**. A decrease in the inhibitory potential was observed with compound **8** which bears an unsubstituted phenyl ring at the N1 position as compared to compound **9** possessing a 4-methoxyphenyl ring at the same position. Use of a dimethoxybenzenesulfonyl ring (**10**) does not favor the activity and a remarkable decrease in the cytotoxicity was observed with **10** as compared to compounds **8** and **9**. Comparison of the cytotoxic effects of **11** with **9** further confirmed that the 4-methoxybenzenesulfonyl group, a structural feature in compound **1**, is required for significant activity. Compound **11** with a 3-methoxyphenyl ring at N1 was found to be 12-16 fold less active than compound **9**. A clear dependence of the cell growth inhibitory activity on the electronic factors could not be established however as compound **12** with a 4-hydroxy substituted benzene sulfonyl group failed to show any potential comparable to that of compound **9** and was even less active than **13** which bears chlorine at the para position of the phenyl ring, the N1 position. Beneficial effects of the benzyl amino functionality at C7 position were clearly demonstrated by the relatively inferior potential of compound **16**, which possesses the biaryl amino moiety, when compared to compound **9**. However, **16** was still more active than the control compounds **1** and **6** against all the cell lines examined. The significant potential of compound **9** further supported our attempt to design relatively flexible chemical

architectures by using *N*-benzyl functionality in place of the biaryl amine and amide groups at C7 of the indoline. A drastic decline in the activity was observed when the benzyl amino group was moved from C7 to the C5 or C6 position of indoline (compare compound **9** with **21** and **22**). The influence of planarity could be easily observed by comparison of the decreased inhibitory effects of **23** and **24**, possessing the indole moiety, with **9** and **22** bearing a non-planar indoline ring. Replacement of –NH-CH_2 functionality (7^{th} position) with –NH-CO- functionality (compound **25**) resulted in drastic decline in the cytotoxic potential against A549 cell lines. Compounds with ethylene and vinyl linkers (**26** and **27**) for the *N*-phenylbenzamide at C7 position were also evaluated. Compound **26** has inhibitory effects comparable to those of the biaryl amino compound (**16**). Better activity induced via an ethylene linker in compound **26** as compared to compound **27** bearing a vinyl linker, further confirmed the favorable trend observed with increased flexibility at the C7 position. Further insights into the structure activity relationships were established by the observation of inhibitory potential of **17**, **18**, and **19**. An interesting revelation was that the shift of the 2-NH_2 group to the 3- or 4-position showed that the amino group at the 4-position was most favorable for the activity as **17** and **18** were found to have reduced activity compared to **9**. The replacement of the amino group with a hydroxy group on *N*-phenylbenzamide ring (**19**) was not useful as the resulting compound displayed complete loss of activity. Compound **20** with a *m*-substituted benzamide group showed weak inhibitory activity. Conversion of the connecting linker at C7 of the indoline from NH-CH_2 to O-CH_2 resulted in inhibitory activities comparable to those of the most potent benzamide (compare **9** and **28**). The structure activity relationships are presented in Figure 3. The efficacy of compounds **9**, **16**, and **28** against P-gp170/MDR (KB-VIN10 and KB-S15) and MRP-overexpressing (KB-7D) drug-resistant cell lines is shown in Table 2. Benzamides **9**, **16**, and **28** were equally effective towards the KB-derived MDR-positive cell lines, even in the presence of high level expression of drug-resistant efflux protein (MDR-P-gp or MRP) in KB-VIN10, KB-S15, and KB-7D.

Further mechanistic studies were performed as per the previous studies [18, 21, 22, 44] to evaluate whether the benzamides inhibits the tubulin assembly. The results are presented in Table 3 and Figure 4.

Among the compounds, compound **9**, **16**, **17**, **26**, **27** and **28** induced significant inhibition of tubulin polymerization with IC_{50} < 3 μM. Compound **9** exhibited the best tubulin inhibitory activity with an IC_{50} value of 1.1 μM (Table 3). The inhibitory effects of compound **9** on the tubulin polymerization were found to be comparable to CA-4 (**3**). Overall, the results indicates that the compounds substoichometrically inhibit tubulin assembly except those inactive. We further compared the binding affinity potency of compound **9**, **16**, **28** at concentration of 1 and 5 μM with reference compounds (**1**, **2** and **3**) using the H3-colchicine competition scintillation proximity assay due to their substantial cytotoxic effects against the cancer cell lines and MDR cell lines along with remarkable inhibition of the tubulin polymerization.The results indicated all the three compounds **9**, **16** and **28** exhibited better affinity for the colchicine binding site than colchicine itself.

To confirm the inhibition by the HDAC isoforms, the effect of compound **9** and the reference compound (**6**) was observed against HDAC isoforms. (Table 4) The benzamide (**9**) possesses remarkable inhibitory effects against all the cell lines and significant tubulin polymerization inhibition. It was also found to possess HDAC inhibitory potential with IC_{50} values of 0.221 μM (HDAC1) and 0.662 μM (HDAC2). Compound **9** was 2.5-fold more potent than **6** (IC_{50} = 0.544 μM) against the HDAC1 isoform and displayed comparable efficacy against the HDAC2 isoform. The benzamide (**9**) also inhibited HDAC6 isoform (IC_{50} = 0.314 μM), however no inhibition against HDAC6 was demonstrated by **6**. This effect was further shown in western blot analysis where treatment with compound **9** resulted in upregulation of acetyl α-tubulin levels. (Figure 5) The results confirmed the dual inhibitory effects of compound **9**.

A molecular docking analysis to elucidate interactions between the synthesized compounds and tubulin was performed. First, *to test the accuracy of the docking program*, the co-crystallized inhibitor, colchicine, was docked into the binding site of the tubulin structure (PDB code: 1SA0) using *LeadIT* [23]. The docking results produced similar docking conformations to the co-crystallized colchicine (Fig. S1) indicating that the docking program is reliable. Next, the synthesized compounds were docked into

the colchicine binding site (Fig. S2). The results of the docking study revealed that the binding site can be separated into four sites (S1-S4) according to the interacting residues and structure of the most potent compound **9** (Fig. 6). It was found that the N-(2-aminophenyl)-4-methylbenzamide moiety occupies sites S1 and S3 where site S1 contains residues Ser178, Thr179 and Ala180 that produces a hydrophobic pocket with the aromatic ring of compound **9** (Fig. 6B) and site S3 consists of residues Leu255 and Asn258 that create hydrophobic contacts with the middle aromatic ring. Hydrogen bonding interaction were observed with S1 residues Asn101, Thr179, and Glu183. Previous research has identified potential inhibitors involved in similar hydrogen-bonding interactions with these residues [24-25]. The second portion of compound **9** (1-(4-methoxybenzenesulfonyl)-2,3-dihydro-1H-indol-7-amine) is located at sites S2 and S4 (Fig. 6B). A hydrogen bond between residue Ser178 of site S2 and the methoxy group is observed. Site S2 contains residues Leu248, Lys352, Thr353, and Ala354 that create a hydrophobic pocket occupied by the anisole moiety. The carbon atoms in the side chain of Lys352, the backbone atoms of Thr353 and hydrophobic residues Leu248 and Ala354 create hydrophobic interactions with anisole ring (Fig.6B). Site S4 creates another hydrophobic pocket with residues Cys241, Ala250, Lys254 and Leu255. Thus, the docking analysis of compound **9** reveals hydrogen bonds and hydrophobic interactions with residues that stabilize the compound within the tubulin binding site.

We further examined the structure activity relationship (SAR) between the colchicine binding site and the designed compounds using the computational software Forge [26]. Using Forge, we identified the “average field of actives”, which highlight common features between the active compounds (Fig. 7A). Forge also produced an activity cliff for each compound using compound **9** as a reference. The results indicates the impact of structural changes in the structure of the compounds on the activity within the tubulin binding site. An “activity cliff summary” summarizes the activity cliff data across the compounds in this study (Fig. 7B).

The average field of actives was produced using the active compound in this study. This model indicates the electrostatic and hydrophobic interactions of compounds in the binding site. The most

potent compound in this study, compound **9**, was used to describe the model. The model contains a negative electrostatic field at site S3 (Cyan, Fig. 7A). Residue Asn101 occupies this region and creates a hydrogen bond with the carbonyl oxygen of compound **9**, which acts as a hydrogen bond acceptor. In contrast, the positive electrostatic potential occupies the S1 site, which consists of residues Ser178, Thr179 and Ala180 (Red, Fig. 7A). Favorable moieties to occupy this site include an amino group to serve as a donor for hydrogen bonds. The aromatic rings of compound **9** occupies the hydrophobic areas within the tubulin binding site (Yellow, Fig. 7A). As a result, compound **9** occupies favorable electrostatic fields within the tubulin binding site.

The "activity cliff summary" of the tubulin binding site combined the activity cliff obtained from all pairs of compounds employing compound **9** as the reference (Fig. 7B). Compounds **19**, **20**, **21** and **22** were selected to further describe the model. At the S1 site, compound **19** (yellow) contains a hydroxyl group on the ortho position in place of the amino group present in compound **9** (grey). In addition, compound **19**'s terminal phenol ring in the S1 region is rotated roughly 90° when compared to compound **9** and is located within an unfavorable hydrophobic region. This may be due to the different characteristics of the aniline and the phenol moiety of compound **9** and **19**, respectively (Fig. 7C). In addition, the hydroxyl group on the terminal ring of compound **19** form a hydrogen bond with residue Glu183.

Compounds **20**, **21** and **22** differ from compound **9** either on the location of the amine linker (at position 7 in compound **9**) or the linkage of the benzene-1,2-diamine (at para position in compound **9**). Compound **20** differs from compound **9** on the linkage of the benzene-1,2-diamine and was found to be rotated away from favorable hydrophobic regions found in sites S2 and S4. The terminal anisole of compound **20** is also positioned outside of the favorable hydrophobic region of site S2. Due to this, the hydrophobic region in S2 repels the secondary amine linker (7-position) and the indole of compound **20** is positioned in an unfavorable hydrophobic region in site S4 (Fig. 7D). Compound **21** contains the amine linker at the 5 position. Unlike compound **9**, the terminal anisole of compound **21** is sandwiched

by unfavorable hydrophobic fields at site S1. The substitution at the indole (5 position) prevents the structure from reaching the favorable hydrophobic field. Therefore, the terminal anisole moiety does not interact with site S2 (Fig. 7E). Finally, compound **22** contains an indole substituted at position 6 by the amine linker. The amine linker of compound **22** is positioned within a field that is favorable for hydrophobic interactions in site S4. This slight difference occupies the hydrophobic region and positions the indole in a more unfavorable region. As a result, the anisole of compound **22** is positioned adjacent to the favorable hydrophobic region in sites S2 and S4 (Fig. 7F). The "activity cliff summary" details the structural differences that have resulted in a variable activity profile of the compounds. Our analysis showed that compound **9** occupies many favorable fields when compared to compounds **19**, **20**, **21** and **22**.

To elucidate binding interactions with HDAC, compound **9** was docked into HDAC 1 (PDB ID: 5ICN) (Fig. 8A). Compound **9** contains typical features of a HDAC inhibitor – a zinc binding group (ZBG), a linker, and a cap to block the entrance to the binding site [27-28]. The ZBG of compound **9** consists of the N-(2-aminophenyl)formamide moiety. This moiety contains a nitrogen and a carbonyl oxygen that coordinates the zinc ion in the binding site (Fig. 8A). The nitrogen that links the two aromatic structures in this moiety forms a hydrogen bond with residue G149. The hydrophobic tunnel is occupied by the benzyl ring. The benzyl ring creates a pi-pi interaction with the hydrophobic F150 residue. Moreover, compound **9** consists of a 1-(4-methoxybenzenesulfonyl)-2,3-dihydroindole moiety that functions as a cap. The cap creates pi-pi stacking interaction with F205 and pi interactions with Y204 and L271. The interactions formed between the cap and the enzyme surface may increase HDAC specificity [27-29]. In contrast, HDAC8 has a M274 residue in place of L271 in HDAC1. The HDAC8 isoform is known to form a specific hydrophobic subpocket [30] and compound **9** could not properly exploit this subpocket. Therefore, the cap construct not only forms less interactions, but is also rotated away from the surface residues when compared to its docking pose in HDAC1. (Fig. 8B). Moreover, docking poses of compound **9** in HDAC1 (PDB ID: 5ICN), 2 (PDB ID: 5IX0), and 6 (PDB ID: 5EF8)

were observed to be similar on superimposition (Fig. 8C). Briefly, the ZBG coordinate the zinc ion and the linker occupies the hydrophobic tunnel, as seen in HDAC1. The cap also creates similar interactions with the surface residue leucine (L271, L276, and L712 for HDAC1, 2, and 6, respectively) when compared to HDAC1 (Fig. 8C). Thus, the interactions with the surface residue leucine observed in HDAC1, 2 and 6 are important for compound **9** to effectively inhibit HDAC function.

The anticancer evaluation of compound **9** was further extended to *in vivo* studies in human non-small cell lung cancer A549 xenograft model as well as BJAB B-cell lymphoma xenograft tumor model. Both tubulin inhibitors as well as HDAC inhibitors have demonstrated clinical benefits in advanced NSCLC [31-32] and lymphomas [33-35]. Of particular mention are the implications of US FDA approved HDAC inhibitors such as SAHA, FK-228, PXD101 and CFDA approved Chidamide (**7**) in the treatment of lymphoma [36-40]. To add on, an indoline sulfonamide based HDAC inhibitor previously synthesised by our research group displayed significant antiproliferative effects against B-cell lymphoma [41]. These factors collectively led us investigate compound **9** in NSCLC and BJAB xenograft tumor models. As shown in Fig. 9a, compound **9** caused significant reduction of the tumor volume in the A549 tumor bearing nude mice (TGI = 62.9 %, 50 mg/kg, ip, qd, $^{**}p < 0.01$). In addition, there was no significant change in body weight of tested animals after treatment with compound **9**. Furthermore**,** *in vitro* studies on B-cell lymphoma cells (BJAB) revealed that compound **9** was endowed with potent cellular activity with an IC_{50} value of 40 nM (results of MTT assay placed in supporting information). The *in vivo* evaluation results of compound **9** (intravenous injection) in subcutaneous xenograft tumor model of BJAB also revealed remarkable inhibition of the tumor growth by benzamide **9.** No significant change in body weight of tested animals was observed in this study also (Fig 10 and 11). Thus the *in vivo* animal model experiments demonstrated that compound **9** possess significant *in vivo* potential against NSCLC and B-Cell lymphoma. The *in vivo* evaluation of compound **9** in oral epidermoid carcinoma (KB cells) is under progress.

3. Conclusion

A series of 1-arylsulfonyl indoline-based benzamides has been synthesized and evaluated against a panel of human cancer cell lines. The results of in vitro cytotoxicity studies indicated that compound **9, 16 and 28** were found to have promising antiproliferative activity with compound **9** displaying striking cell killing effects against the KB cells with an IC_{50} value of 48 nM. Among them, compounds **9** and **28** were found to be more potent tubulin polymerization inhibitors with an IC_{50} of 1.1 and 1.9 μM, respectively, than the reference compounds **2** and **3**. The highlight of the present study demonstrated by these compounds was the unaltered ability to inhibit the KB-derived MDR-positive cell lines, even in the presence of high level expression of drug-resistant efflux protein (MDR-P-gp or MRP) in KB-VIN10, KB-S15, and KB-7D cells. The most potent antiproliferative compound (**9**) possessing the best tubulin polymerization inhibitory activity also inhibited HDAC 1, 2 and 6 confirming its dual inhibitory potential. Some of the important interactions of the active compounds with the amino acid residues of tubulin and HDAC 1 isoform have been figured out by molecular modeling which supports the results of the *in vitro* assays. Compound **9** also demonstrated promising significant *in vivo* efficacy in the human non-small cell lung cancer A549 xenograft model as well as xenograft tumor model of BJAB. These promising findings clearly indicate the need for detailed preclinical and clinical investigation as these scaffold could emerge as templates for further development of potent anticancer agents.

4. Experimental Section

(A) **Chemistry.** Nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were obtained with Bruker Fourier 300 and DRX-500 NMR spectrometers, and are reported as chemical shifts in parts per million (ppm, δ) downfield from TMS as an internal standard. High-resolution mass spectra (HRMS) were recorded with a Finnigan MAT 95S mass spectrometer. The purities of the final compounds were determined using a Hitachi 2000 series HPLC system using an Agilent Zorbax Eclipse XDB-C_{18} column (5 μm, 4.6 mm × 150 mm) with the solvent system consisting of acetonitrile (mobile phase A) and water containing 0.1% formic acid and 10 mmol NH_4OAc (mobile phase B), and were found to be ≥95%.

Flash column chromatography was performed using silica gel (Merck Kieselgel 60, no. 9385, 230−400 mesh ASTM). All reactions were carried out under an atmosphere of dry nitrogen.

***N*-(2-Aminophenyl)-4-(((1-(phenylsulfonyl)indolin-7-yl)amino)methyl)benzamide (8)**

To a solution of **30a** (0.21 g, 0.51 mmol), PyBOP (0.26 g, 0.51 mmol), triethylamine (0.16 ml, 1.15 mmol) in DMF 1.5 mL, benzene-1,2-diamine (0.05 g, 0.48 mmol) was added and stirred at room temperature. After being stirred for 2 h, the reaction was quenched with water, followed by extraction with EtOAc (15 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc) to give 8 as a brown solid in 71% yield; t_R = 43.65. mp: 192-193 °C. ^{1}H NMR (300 MHz, DMSO-d_6) : δ 2.09 (t, *J* = 7.5 Hz, 2H), 4.01 (t, *J* = 7.2 Hz, 2H), 4.54 (d, *J = 4.8 Hz*, 2H), 6.31 (t, *J* = 5.7 Hz, 1H), 6.36 (d, *J* = 6.9 Hz, 1H), 6.47 (d, *J = 8.1 Hz*, 1H), 6.64 (m, 1H), 6.82 (dd, *J = 1.5 and 8.1 Hz*, 1H), 6.91 (t, *J = 7.8 Hz*, 1H), 7.00 (m, 2H), 7.20 (dd, *J = 1.2 and 7.8 Hz*, 1H), 7.50 - 7.74 (m, 7H), 8.01 (d, *J = 8.1 Hz*, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.01, 46.71, 53.72, 111.01, 113.14, 116.79, 117.03, 124.05, 126.93, 127.17, 127.37, 127.45, 127.86, 128.51, 128.55, 129.75, 133.72, 134.41, 136.47, 138.86, 141.49, 143.19, 144.04, 165.67. HRMS (ESI) for $C_{28}H_{26}N_4O_3S$ (M^+): calcd, 498.1726; found, 498.1723.

***N*-(2-Aminophenyl)-4-(((1-((4-methoxyphenyl) sulfonyl)indolin-7-yl)amino)methyl)benzamide (9)**

To a solution of **30b** (0.22 g, 0.51 mmol), PyBOP (0.27 g, 0.51 mmol), Et_3N (0.17 mL, 1.15 mmol) in DMF (1.5 mL), benzene-1,2-diamine (0.05 g, 48 mmol) was added and stirred at room temperature. After being stirred for 2h, the reaction was quenched with H2O, 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 (EtOAc) to give **9** as a white solid (0.18 g, 67% yield); tR = 43.65 min. mp: 171-172 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.14 (t, *J* = 7.2 Hz, 2H), 3.82 (s, 3H), 3.98 (t, *J* = 7.5 Hz, 2H), 4.53 (d, *J = 6.0 Hz*, 2H), 4.91 (s, 2H), 6.28 (t, *J = 6.0 Hz*, 1H), 6.39 (d, *J = 6.6 Hz*, 2H), 6.44 (d, *J = 8.1 Hz*, 1H), 6.61 (m, 1H), 6.79 (dd, *J = 1.5 and 8.1 Hz*, 1H), 6.91 (t, *J = 7.8 Hz*, 1H), 6.98 (m, 1H), 7.05 (d, *J* = 6.9 Hz, 2H), 7.18 (dd, *J = 1.2 and 7.8 Hz*,

1H), 7.55 (d, *J = 9.0 Hz*, 2H), 7.59 (d, *J = 8.4 Hz*, 2H), 7.99 (d, *J* = 8.4 Hz, 2H), 9.61 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.55, 44.14, 51.04, 53.56, 108.34, 110.58, 112.27, 114.05, 121.29, 124.51, 125.85, 127.45, 131.14, 136.30, 138.94, 140.93, 141.41, 161.17, 163.08. HRMS (ESI) for $C_{29}H_{29}N_4O_4S$ ($M + H^+$): calcd, 529.1910; found, 529.1905.

***N*-(2-Aminophenyl)-4-(((1-((3,4-dimethoxyphenyl)sulfonyl)indolin-7-yl)amino)methyl)benzamide (10)**

The title compound **10** was obtained as a white solid in 50% yield in a similar manner as described for the preparation of **9**; t_R = 33.78 min. mp: 199-200 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.09 (t, *J = 7.2 Hz*, 2H), 3.43 (s, 3H), 3.83 (s, 3H), 3.96 (t, *J = 7.5 Hz*, 2H), 4.54 (d, *J = 6.3 Hz*, 2H), 4.90 (s, 2H), 6.31 (t, *J = 6.3 Hz*, 1H), 6.39 (d, *J* = 6.6 Hz, 1H), 6.46 (d, *J* = 8.1 Hz, 1H), 6.61 (m, 1H), 6.76 – 6.81 (m, 2H), 6.90 (d, *J* = 7.5 Hz, 1H), 6.95 - 7.01 (m, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 7.18 (dd, *J* = 1.2 and 7.5 Hz, 1H), 7.34 (dd, *J = 2.1 and 8.4 Hz*, 1H), 7.61 (d, *J = 8.4 Hz*, 2H), 7.99 (d, *J = 8.1 Hz*, 2H), 9.61 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.53, 44.68, 50.87, 53.24, 53.50, 107.29, 107.85, 108.14, 110.61, 115.71, 117.11, 118.81, 121.96, 122.66, 124.57, 124.83, 125.06, 125.18, 125.49, 125.59, 130.31, 136.10, 138.09, 138.74, 141.37, 145.87, 150.57, 163.14. HRMS (ESI) for $C_{30}H_{30}N_4O_5S$ (M^+): calcd, 558.1937; found, 558.1934.

***N*-(2-Aminophenyl)-4-(((1-((3-methoxyphenyl)sulfonyl)indolin-7-yl)amino)methyl)benzamide (11)**

The title compound **11** was obtained as a light brown crystalline solid in 52% yield in a similar manner as described for the preparation of **9**; t_R = 36.12 min. mp: 102-103 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.13 (t, *J* = 7.5 Hz, 2H), 3.56 (s, 3H), 4.00 (t, *J* = 7.5 Hz, 2H), 4.54 (d, *J* = 5.5 Hz, 2H), 6.27 (t, *J* = 6.0 Hz, 1H), 6.39 (d, *J* = 7.5 Hz, 1H), 6.44 (d, *J* = 8.0 Hz, 1H), 6.83 - 6.86 (m, 2H), 6.91 - 6.96 (m, 2H), 7.07 -7.11 (m, 2H), 7.26 - 7.35 (m, 4H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.81 (s, 1H), 7.89 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.46, 44.65, 50.99, 52.85, 108.24, 109.03, 110.61, 115.33, 116.62, 117.12, 117.91, 121.71, 123.20, 124.59, 124.65, 125.22, 125.60, 127.41, 130.25, 134.58, 136.00,

138.62, 138.69, 141.09, 156.79, 163.46, 168.67. HRMS (ESI) for $C_{29}H_{29}N_4O_4S$ ($M + H^+$): calcd, 529.1910; found, 529.1912.

***N*-(2-Aminophenyl)-4-(((1-((4-hydroxyphenyl)sulfonyl)indolin-7-yl)amino)methyl)benzamide (12)**

To a solution of **9** (0.1 g, 0.19 mmol) in dichloromethane (10 mL), bromotribromide (0.024 mL, 0.57 mmol) was added dropwise and the reaction mixture was stirred at room temperature for overnight. The mixture was quenched with water and extracted with dichloromethane. The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford a residue which was purified by silica gel chromatography (EtOAc) to give **12** as a white solid in 76% yield; t_R = 30.95 min. mp: 197-198 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.14 (t, *J = 7.2 Hz*, 2H), 3.94 (t, *J = 7.5 Hz*, 2H), 4.52 (d, *J = 6.0 Hz*, 2H), 4.92 (s, 2H), 6.27 (t, *J = 6.3 Hz*, 1H), 6.39 (d, *J* = 7.2 Hz, 1H), 6.44 (d, *J* = 8.1 Hz, 1H), 6.62 (m, 1H), 6.79 - 6.84 (m, 3H), 6.90 (t, *J = 7.8 Hz*, 1H), 6.96 – 7.01 (m, 1H), 7.18 (m, 1H), 7.43 (d, *J = 9.0 Hz*, 2H), 7.59 (d, *J = 8.1 Hz*, 2H), 8.00 (d, *J = 8.4 Hz*, 2H), 9.64 (s, 1H), 10.87 (bs, 1H); ^{13}C NMR (300 MHz, CD_3OD + DMSO-d_6): δ 30.15, 48.58, 54.85, 112.32, 114.52, 117.37, 117.67, 118.57, 119.30, 125.30, 127.98, 128.59, 128.69, 128.91, 129.35, 129.62, 131.90, 132.03, 134.17, 134.58, 140.04, 142.81, 144.16, 145.60, 168.47, 168.93. HRMS (ESI) for $C_{28}H_{26}N_4O_4S$ (M^+): calcd, 514.1675; found, 514.1678.

***N*-(2-Aminophenyl)-4-(((1-((4-chlorophenyl)sulfonyl)indolin-7-yl)amino)methyl)benzamide (13)**

The title compound **13** was obtained as a white solid in 56% yield in a similar manner as described for the preparation of **9**; t_R = 38.21 min. mp: 101-102 °C. ^{1}H NMR (500 MHz, CD_3OD) δ 2.17 (t, *J = 7.5 Hz*, 2H), 4.01 (t, *J = 7.2 Hz*, 2H), 4.53 (d, *J = 6.0 Hz*, 2H), 4.91 (s, 2H), 6.24 (t, *J = 6.3 Hz*, 1H) 6.40 - 6.48 (m, 2H), 6.61 (m, 1H), 6.79 (dd, *J = 1.5 Hz and 8.1 Hz*, 1H), 6.93 - 6.98 (m, 2H), 7.18 (dd, *J = 1.2 and 7.8 Hz*, 1H), 7.56 - 7.69 (m, 5H), 7.98 (d, *J* = 8.0 Hz, 2H), 9.63 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.49, 44.70, 50.95, 108.32, 110.71, 115.51, 116.85, 121.80, 122.97, 124.59, 124.74, 125.20, 125.38, 125.83, 126.35, 126.60, 130.31, 132.39, 135.60, 137.48, 137.52, 138.39, 138.69, 141.07, 141.31, 163.78. HRMS (ESI) for $C_{28}H_{25}ClN_4O_3S$ (M^+): calcd, 532.1336; found, 532.1339.

***N*-(2-Aminophenyl)-4-(((1-((4-fluorophenyl)sulfonyl)indolin-7-yl)amino)methyl)benzamide (14)**

The title compound **14** was obtained as a white solid in 49% yield in a similar manner as described for the preparation of **9**; t_R = 32.32 min. mp: 199-200 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.15 (t, *J* = 7.2 Hz, 2H), 4.02 (t, *J* = 7.2 Hz, 2H), 4.54 (d, *J* = 6.00 Hz, 2H), 4.92 (s, 2H), 6.25 (t, *J* = 6.0 Hz, 1H), 6.40 (d, *J* = 7.2 Hz, 1H), 6.47 (d, *J* = 7.8 Hz, 1H), 6.61 (m, 1H), 6.80 (dd, *J* = 1.2, 7.8 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 1 H), 6.96 (m, 1H), 7.19 (dd, *J* = 1.2, 7.8 Hz, 1H), 7.40 - 7.43 (m, 2H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.67 - 7.70 (m, 2H), 8.00 (d, *J* = 8.1 Hz, 2H), 9.61 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.47, 44.71, 50.89, 108.28, 110.65, 113.47, 113.77, 115.58, 116.96, 121.86, 122.85, 124.59, 124.79, 124.89, 125.16, 129.86, 129.90, 135.64, 138.28, 138.74, 141.16, 163.26. HRMS (ESI) for $C_{28}H_{26}FN_4O_3S$ (M^+ + H): calcd, 517.1710, found, 517.1708.

***N*-(2-Aminophenyl)-4-(((1-((4-cyanophenyl)sulfonyl)indolin-7-yl)amino)methyl)benzamide (15)**

The title compound **15** was obtained as a pale yellow solid in 59% yield in a similar manner as described for the preparation of **9**; t_R = 43.32 min. mp: 113-114 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.16 (t, *J = 7.2 Hz*, 2H), 4.06 (t, *J = 7.2 Hz*, 2H), 4.54 (d, *J = 6.0 Hz*, 2H), 4.92 (s, 2H), 6.25 (d, *J = 6.0 Hz*, 1H), 6.39 (d, *J = 6.9 Hz*, 1 H), 6.48 (d, *J = 8.1 Hz*, 1H), 6.61 (m, 1H), 6.79 (dd, *J = 1.2 and 7.8 Hz*, 1H), 6.91 - 6.98 (m, 2H), 7.18 (dd, *J = 1.2 and 7.8 Hz*, 1H), 7.59 (d, *J = 8.1 Hz*, 2H), 7.81 (d, *J* = 8.4 Hz, 2 H), 7.98 - 8.05 (m, 4H), 9.63 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.57, 44.71, 51.04, 108.46, 110.77, 114.44, 115.65, 117.02, 121.87, 122.79, 124.17, 124.61, 124.81, 125.19, 125.56, 126.15, 129.96, 130.43, 135.35, 138.05, 138.61, 140.99, 163.17. HRMS (ESI) for $C_{29}H_{26}N_5O_3S$ (M + H^+): calcd, 524.1756; found, 524.1758.

***N*-(2-Aminophenyl)-4-((1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)amino)benzamide (16)**

The title compound **16** was obtained as a brown solid in 51% yield in a similar manner as described for the preparation of **9**; t_R = 34.89 min. mp: 100-101 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.23 (t, *J* = 7.5 Hz, 2H), 3.81 (s, 3H), 4.02 (t, *J* = 7.5 Hz, 2H), 6.70 (d, *J* = 7.0 Hz, 1H), 6.84 - 6.86 (m, 4 H), 7.08 - 7.14 (m, 4H), 7.31 (d, *J* = 7.5 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 9.0 Hz, 1H), 7.73 (s, 1H), 7.81

(d, *J* = 8.5 Hz, 2 H), 8.33 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD + DMSO-d_6): δ 48.91, 55.72, 95.46, 109.59, 112.50, 114.74, 117.02, 117.51, 121.41, 121.92, 123.23, 123.89, 126.92, 127.21, 127.28, 128.30, 129.12, 133.23, 136.55, 143.24, 144.87, 146.73, 164.02. HRMS (ESI) for $C_{28}H_{26}N_4O_4S$ (M^+): calcd, 514.1675; found, 514.1677.

***N*-(3-Aminophenyl)-4-(((1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)amino)methyl)benzamide (17)**

The title compound **17** was obtained as a brown solid in 51% yield in a similar manner as described for the preparation of **9**; t_R = 34.86 min. mp: 171-172 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.14 (t, *J* = 7.2 Hz, 2H), 3.82 (s, 3H), 3.97 (t, *J* = 7.2 Hz, 2H), 4.52 (d, *J* = 6.00 Hz, 2H), 5.08 (s, 2H), 6.30 - 6.34 (m, 2H), 6.38 - 6.46 (m, 2H), 6.85 - 6.99 (m, 3H), 7.05 (d, *J* = 9.0 Hz, 2H), 7.12 (t, *J* = 1.8 Hz, 1H), 7.55 (d, *J* = 8.7 Hz, 2H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 2H), 9.91 (s, 1H). ^{13}C NMR (75 MHz, $CDCl5_3$) δ 26.57, 44.67, 50.85, 52.98, 104.28, 107.53, 108.15, 108.63, 110.56, 111.48, 125.34, 125.41, 125.50, 131.26, 135.86, 136.46, 138.80, 141.11, 144.65, 160.93, 163.15. HRMS (ESI) for $C_{29}H_{29}N_4O_4S$ ($M + H^+$): calcd, 529.1910; found, 529.1905.

***N*-(4-Aminophenyl)-4-(((1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)amino)methyl)benzamide (18)**

The title compound **18** was obtained as a brown solid in 47% yield in a similar manner as described for the preparation of **9**; t_R = 32.62 min. mp: 101-102 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.14 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 4.12 (t, *J* = 7.5 Hz, 2H), 4.51 (d, *J* = 6.0 Hz, 2H), 6.26 (t, *J* = 6.0 Hz, 1H), 6.37 (d, *J* = 7.5 Hz, 1H), 6.42 (d, *J* = 8.0 Hz, 1H), 6.69 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 6.91 (t, *J* = 7.5 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 2 H), 7.50 (d, J = 8.0 Hz, 2H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.65 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.57, 44.69, 50.85, 52.98, 108.16, 110.53, 111.48, 112.80, 119.77, 124.73, 124.79, 125.32, 125.50, 126.76, 127.04, 131.30, 135.84, 138.82, 140.80, 140.84, 160.93, 163.05. HRMS (ESI) for $C_{29}H_{28}N_4O_4S$ ($M + H^+$): calcd, 529.1910; found, 529.1907.

***N*-(2-Hydroxyphenyl)-4-(((1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)methyl)amino)benzamide (19)**

The title compound **19** was obtained with 2-aminophenol as a yellow solid in 49% yield in a similar manner as described for the preparation of **9**; t_R = 43.42 min. mp: 75-76 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.15 (t, *J* = 7.5 Hz, 2H), 3.83 (s, 3H), 4.00 (t, *J* = 7.5 Hz, 2H), 4.57 (d, *J* = 6.0 Hz, 2H), 6.33 (t, *J* = 6.0 Hz, 1H), 6.38-6.41 (m, 2H), 6.84 (d, *J* = 9.0 Hz, 2H), 6.92 (t, *J* = 7.5 Hz, 1H), 7.38 (dd, *J* = 1.5, 8.0 Hz, 1H), 7.43 (m, 1H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.70 (m, 1H), 8.13 (dd, *J* = 1.5, 8.0 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.59, 44.77, 50.87, 52.99, 108.16, 110.65, 111.48, 122.81, 123.19, 123.96, 124.51, 124.71, 125.40, 125.48, 125.52, 127.07, 128.30, 132.05, 135.89, 138.67, 139.40, 141.76, 144.19, 160.93, 161.64. HRMS (ESI) for $C_{29}H_{28}N_4O_4S$ (M^+): calcd, 529.1671; found, 529.1675.

***N*-(2-Aminophenyl)-3-(((1-((4-methoxyphenyl)sulfonyl)indolin-7 yl)amino)methyl)benzamide (20)**

The title compound **20** was obtained as a white solid in 43% yield in a similar manner as described for the preparation of **9**; t_R = 35.73 min. mp: 99-100 °C. ^{1}H NMR (500 MHz, $CDCl_3$) : δ 2.12 (t, *J* = 7.0 Hz, 2H), 3.77 (s, 3H), 3.96 (t, *J* = 7.0 Hz, 2H), 4.55 (d, *J* = 6.5 Hz, 2H), 6.18 (t, *J* = 6.0 Hz, 1H), 6.37 (d, *J* = 7.5 Hz, 1H), 6.45 (d, *J* = 8.5 Hz, 1H), 6.67 - 6.75 (m, 4H), 6.92 - 6.99 (m, 2H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 7.5 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 8.00 (s, 1H), 8.12 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.23, 47.33, 53.51, 55.64, 107.07, 110.34, 110.81, 111.39, 113.22, 114.15, 127.44, 127.99, 129.75, 133.90, 138.52, 139.13, 141.46, 143.76, 163.59, 165.81. HRMS (ESI) for $C_{29}H_{29}N_4O_4S$ ($M + H^+$): calcd, 529.1910; found, 529.1912.

***N*-(2-Aminophenyl)-4-(((1-(4-methoxyphenylsulfonyl)indolin-5-yl)amino)methyl)benzamide (21)**

The title compound **21** was obtained as a brown solid in 68% yield in a similar manner as described for the preparation of **9**; t_R = 32.33 min. mp: 173-174 °C. ^{1}H NMR (300 MHz, DMSO-d_6) : δ 2.59 (t, *J* = 8.1 Hz, 2H), 3.77 (t, *J* = 8.7 Hz, 2H), 3.80 (s, 3H), 4.31 (d, J = 5.7 Hz, 2H), 4.89 (s, 2H), 6.27 (t, J = 6.0 Hz, 1H), 6.37 (d, *J* = 3.1 Hz, 1H), 6.46 (dd, J = 2.1 and 8.7 Hz, 1H), 6.61 (m, 1H), 6.79 (dd, J = 1.2 and 8.1 Hz, 1H), 6.95 - 7.05 (m, 3H), 7.16 - 7.23 (m, 2H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.61 (d, *J* = 9.0 Hz, 2H), 7.93 (d, *J* = 8.1 Hz, 2H), 9.63 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD + DMSO-d_6): δ 28.84, 53.39, 55.16,

110.83, 113.13, 114.12, 117.18, 117.97, 123.90, 126.58, 127.19, 127.31, 127.95, 128.01, 129.72, 133.12, 138.77, 141.43, 142.73, 144.22, 163.95, 167.04. HRMS (EI) for $C_{29}H_{28}N_4O_4S$ (M^+): calcd, 528.1831; found, 528.1829.

***N*-(2-Aminophenyl)-4-(((1-(4-methoxyphenylsulfonyl)indolin-6-yl)amino)methyl)benzamide (22)**

The title compound **22** was obtained as a brown solid in 65% yield in a similar manner as described for the preparation of **9**; t_R = 38.08 min. mp: 173-174 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.72 (t, *J* = 8.4 Hz, 2H), 3.75 (t, *J* = 8.4 Hz, 2H), 3.79 (s, 3H), 4.38 (d, *J* = 6.3 Hz, 2H), 4.88 (s, 2H), 6.25 (dd, *J* = 2.4 and 8.4 Hz, 1H), 6.56 - 6.64 (m, 2H), 6.77 - 6.81 (m, 3H), 6.96-6.99 (m, 3H), 7.16 (dd, *J* = 1.2 and 7.8 Hz, 1H), 7.47 (d, *J* = 9.0 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 8.02 (d, *J* = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD + DMSO-d_6): δ 29.94, 47.49, 57.02, 111.77, 114.08, 115.71, 116.14, 116.33, 117.27, 117.52, 117.68, 117.79, 127.29, 128.51, 128.76, 129.86, 130.76, 130.88, 132.02, 135.72, 136.68, 139.76, 142.28, 145.46, 150.69, 150.91, 164.59, 166.59. HRMS (EI) for $C_{29}H_{28}N_4O_4S$ (M^+): calcd, 528.1831; found, 528.1832.

***N*-(2-Aminophenyl)-4-(((1-((4-methoxyphenyl)sulfonyl)-1H-indol-7-yl)amino)methyl)benzamide (23)**

The title compound **23** was obtained as brown solid in 62% yield in a similar manner as described for the preparation of **9**; t_R = 36.87 min. mp: 169-170 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.81 (s, 3H), 4.57 (d, *J* = 5.4 Hz, 2H), 4.92 (s, 2H), 6.49 (d, *J* = 7.5 Hz, 1H), 6.62 (m, 1H), 6.80 - 6.81 (m, 3H), 6.93-7.10 (m, 4H), 7.20 (d, J = 1.2 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.69 – 7.72 (m, 3H), 7.99 (d, *J* = 8.1 Hz, 2H), 9.66 (s, 1H). HRMS (EI) for $C_{29}H_{26}N_4O_4S$ (M^+): calcd, 526.1675; found, 526.1677.

***N*-(2-Aminophenyl)-4-(((1-((4-methoxyphenyl)sulfonyl)-1H-indol-6-yl)amino)methyl)benzamide (24)**

The title compound **24** was obtained as a brown solid in 58% yield in a similar manner as described for the preparation of **9**; t_R = 43.62 min. mp: 179-180 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.75 (s, 3H), 4.45 (d, J = 5.7 Hz, 2H), 4.90 (s, 2H), 6.55 – 6.82 (m, 5H), 6.92 - 7.01 (m, 4H), 7.17 (dd, J = 1.2 and 6.6

Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 6.6 Hz, 2H), 7.48 (d, *J* = 9.0 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 8.05 (d, *J* = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 46.97, 56.24, 95.42, 110.02, 112.55, 115.14, 116.68, 116.80, 121.07, 122.11, 123.46, 123.84, 127.00, 127.17, 127.28, 128.51, 128.92, 129.25, 133.49, 136.51, 143.64, 144.56, 146.93, 163.91, 165.69. HRMS (EI) for $C_{29}H_{26}N_4O_4S$ (M^+): calcd, 526.1675; found, 526.1677.

N^1-(2-aminophenyl)-N^4-(1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)terephthalamide (25)

The title compound **25** was obtained as a yellow solid in 58% yield in a similar manner as described for the preparation of **9.** ^{1}H NMR (300 MHz, DMSO-d_6) : δ 2.31 (t, *J = 6.6 Hz*, 2H), 3.83 (s, 3H), 4.08 (t, *J = 7.5 Hz*, 2H), 4.98 (s, 2H), 6.62 (t, *J = 7.2 Hz*, 1H), 6.80 (dd, *J = 1.2 and 6.9 Hz*, 1H), 6.98 – 7.06 (m, 4H), 7.20 - 7.27 (m, 2H), 7.57 (d, *J = 8.7 Hz*, 2H), 7.99 (d, *J = 8.1 Hz*, 1H), 8.10 (d, *J = 8.4 Hz*, 2H), 8.17 (d, *J = 8.4 Hz*, 2H), 9.82 (s, 1H), 10.24 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) : δ 29.05, 53.69, 56.28, 115.09, 116.58, 116.70, 121.84, 122.80, 123.45, 127.29, 127.61, 127.87, 128.76, 130.16, 131.16, 133.28, 137.24, 138.17, 139.30, 143.73, 164.06, 164.53, 165.18.

***N*-(2-Aminophenyl)-4-(2-(1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)ethyl)benzamide (26)**

Compound **37** (1 g, 2.2 mmol) was dissolved in dioxane (15 mL) and 1M $LiOH_{(aq)}$ (12 mL) was added to the solution. The reaction mixture was stirred at 40 ℃ for 3h and then was evaporated under reduced pressure. The reaction mixture was quenched with water and the pH was adjusted with 3N $HCl_{(aq)}$. The reaction mixture was extracted with ethyl acetate (50 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure to give acid. The obtained acid was subjected to the amide formation in a similar manner to give **25** as described for the preparation of **8** as a brown solid in 65% yield; t_R = 36.87 min. mp: 157-158 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.11 (t, *J* = 7.5 Hz, 2H), 3.09 (t, *J* = 7.5 Hz, 2H), 3.37 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 3.88 (t, *J* = 8.0 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.88 (d, J = 7.5 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 2H), 7.15 (d, *J* = 7.5 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.26, 31.33, 33.34, 50.08, 52.96, 111.35, 115.57, 116.94, 119.89, 122.02, 122.79, 124.41, 124.47, 124.73,

126.31, 126.35, 127.10, 128.97, 133.06, 135.31, 138.25, 138.74, 143.98, 160.74, 163.44. HRMS (ESI) $C_{30}H_{29}N_3O_4S$ (M + H^+): calcd, 528.1957; found, 528.1956.

(E)-*N*-(2-Aminophenyl)-4-(2-(1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)vinyl)benzamide (27)

The title compound **27** was obtained as a brown solid in 65% yield in a similar manner as described for the preparation of **26**; t_R = 43.62 min. mp: 121-122 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.22 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 4.04 (t, *J* = 7.5 Hz, 2H), 6.81 (d, *J* = 9.0 Hz, 2H), 6.85 - 6.87 (m, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 7.08 - 7.11 (m, 1H), 7.16 - 7.19 (m, 2H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 9.0 Hz, 2H), 7.67 - 7.71 (m, 3H), 7.85 - 7.90 (m, 3H). HRMS (EI) for $C_{30}H_{28}N_3O_4S$ (M - H^+): calcd, 524.1644; found,. 524.1648.

***N*-(2-Aminophenyl)-4-(((1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)oxy)methyl)benzamide (28)**

The title compound **28** was synthesized as a light brown solid in 52% yield using compound **41** in a similar manner as that described for the synthesis of **9**; t_R = 33.81 min. mp: 179-180 °C. ^{1}H NMR (300 MHz,DMSO-d_6) δ 2.37 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 4.01 (t, *J* = 7.5 Hz, 2H), 4.93 (s, 2H), 5.27 (s, 2H), 6.62 (t, *J* = 6.6 Hz, 1H), 6.80 (d, *J* = 8.1 Hz, 2H), 6.96 - 7.02 (m, 4H), 7.10 (t, *J* = 7.8 Hz, 2H), 7.20 (d, *J* = 6.9 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.66 (d, *J* = 8.1 Hz, 2H), 8.01 (d, *J* = 8.4 Hz, 2H), 9.67 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.70, 50.29, 52.91, 67.54, 111.25, 111.28, 124.73, 124.99, 125.05, 126.81, 127.21, 128.59, 130.71, 136.42, 138.45, 148.30, 160.55. HRMS (ESI) for $C_{29}H_{28}N_3O_5S$ (M + H^+): calcd, 530.1750; found, 530.1752.

1-(Phenylsulfonyl)indolin-7-amine (30a)

The title compound **30a** was obtained in 80% yield from compound **29a** in a manner similar to that described for the synthesis of compound **30b**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.11 (t, *J* = 7.5 Hz, 2H), 3.98 (t, *J* = 7.5 Hz, 2H), 4.71 (s, 2H), 6.39 - 6.41 (m, 1H), 6.59 (d, *J* = 7.5 Hz, 1H), 6.89 - 6.92 (m, 1H), 7.36 - 7.39 (m, 2H), 7.54-7.57 (m, 1H), 7.61-7.64 (m, 2H).

1-((4-Methoxyphenyl)sulfonyl)indolin-7-amine (30b).

A mixture of 5-bromo-7-nitro-indoline (**29a**) (2.18 g, 9.0 mmol) and 4-methoxybenzene-1-sulfonyl chloride (1.85 g, 9.0 mmol) in pyridine (6 mL) was refluxed overnight. After cooling, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous $MgSO_4$, then concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 4) to give a yellow solid (3.47 g, 93% yield). The solid residue was dissolved in *i*-PrOH (45 mL) and H_2O (5 mL). To this solution, Fe powder (2.31 g, 41.5 mmol) and ammonium chloride (887 mg, 16.6 mmol) was added and the reaction mixture was refluxed for 6 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the reaction was filtered over celite. The filtrate was concentrated and H_2O was added. Extraction was with EtOAc (50 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield the amine (2.73 g, 85% yield). The mixture of the obtained residue, azobisisobutyronitrile (1.19 g, 7.3 mmol) and tributyltin hydride (6.43 g, 22.1 mmol) were refluxed in toluene (30 mL) overnight. After cooling, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure and dried under vacuum. The residue was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 1) to give **30b** (1.82 g, 84% yield). ^{1}HNMR (500 MHz, $CDCl_3$) δ 2.15 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 3.95 (t, *J* = 8.0 Hz, 2H), 4.70 (s, 2H, D_2O exchangeable protons), 6.41 (d, *J* = 7.5 Hz, 1H), 6.58 (d, *J* = 8.0 Hz, 1H), 6.81-6.84 (m, 2H), 6.89 - 6.92 (m, 1H), 7.52 -7.55 (m, 2H).

1-((3,4-Dimethoxyphenyl)sulfonyl)indolin-7-amine (30c)

The title compound **30c** was obtained in 78% yield from compound **29a** in a manner similar to that described for the synthesis of compound **30b**. ^{1}H NMR (500MHz, $CDCl_3$) δ 2.16 (t, *J* = 7.5 Hz, 2H), 3.60 (s, 3H), 3.91 (s, 3H), 3.95 (t, *J* = 7.5 Hz, 2H), 4.39 (s, 2H, D_2O exchangeable protons), 6.54 (d, *J* = 7.5 Hz, 1H), 6.82 - 6.87 (m, 3H), 6.95 - 6.96 (m, 1H), 7.37 (dd, *J* = 2.0, 8.0 Hz, 1H).

1-((3-Methoxyphenyl)sulfonyl)indolin-7-amine (30d)

The title compound **30d** was obtained in 73% yield from compound **29a** in a manner similar to that described for the synthesis of compound **30b**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.13 (t, *J* = 7.5 Hz, 2H), 3.60 (s, 3H), 3.96 (t, *J* = 7.5 Hz, 2H), 4.70 (s, 2H, D_2O exchangeable protons), 6.42 (d, *J* = 7.0 Hz, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 6.91 - 6.97 (m, 3H), 7.07 (m, 1H), 7.42 (m, 1H).

1-((4-Chlorophenyl)sulfonyl)indolin-7-amine (30e)

The title compound **30e** was obtained in 71% yield from compound **29a** in a manner similar to that described for the synthesis of compound **30b**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.23 (t, *J* = 7.5 Hz, 2H), 3.99 (t, *J* = 7.5 Hz, 2H), 4.49 (s, 2H, D_2O exchangeable protons), 6.54 (d, *J* = 7.0 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 6.96 - 6.99 (m, 1H), 7.35 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H).

1-((4-Fluorophenyl)sulfonyl)indolin-7-amine (30f)

The title compound **30f** was obtained in 72% yield from compound **29a** in a manner similar to that described for the synthesis of compound **30b**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.16 (t, *J* = 7.5 Hz, 2H), 3.99 (t, *J* = 7.5 Hz, 2H), 4.69 (s, 2H, D_2O exchangeable protons), 6.42 (d, *J* = 7.5 Hz, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 6.90 (t, *J* = 7.5 Hz, 1H), 7.05 (m, 2H) 7.63 (m, 2H).

4-((7-Aminoindolin-1-yl)sulfonyl)benzonitrile (30g)

The title compound **30g** was obtained in 75% yield from compound **29a** in a manner similar to that described for the synthesis of compound **30b**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.22 (t, *J* = 7.5 Hz, 2H), 4.09 (t, J = 7.5 Hz, 2H), 4.23 (s, 2H, D_2O exchangeable protons), 6.43 (d, *J* = 7.5 Hz, 1H), 6.54 (d, *J* = 8.0 Hz, 1H), 6.97 (t, *J* = 7.5 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 2H), 8.03 (d, *J* = 8.0 Hz, 2H).

1-((4-Methoxyphenyl)sulfonyl)indolin-5-amine (30h)

The mixture of 5-nitro-indoline (**29b**) (1 g, 6.09 mmol) and benzenesulfonyl chloride (1.07 g, 6.09 mmol) in pyridine (3 mL) was refluxed overnight. After cooling, the reaction mixture was quenched with water and extracted with dichloromethane. The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue obtained was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 4). A mixture of the obtained solid (1.83 g, 5.47 mmol), iron

powder (611 mg, 10.9 mmol), ammonium chloride (1.46 g, 27.3 mmol) in isopropanol (45 mL) and water (5 mL) was refluxed for 6 h. The progress of the reaction was monitored on TLC. On completion of the reaction, the reaction was filtered over celite. The filtrate was concentrated and water was added to it. Extraction was done with ethyl acetate (50 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 1) to give compound **30h** in 75% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.66 (t, *J* = 8.5 Hz, 2H), 3.81 (s, 3H), 3.85 (t, *J* = 8.5 Hz, 2H), 6.49 (s, 1H), 6.59 (d, *J* = 8.5 Hz, 1H), 6.85 (d, *J* = 9.0 Hz, 2H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.62-7.64 (m, 2H).

1-((4-Methoxyphenyl)sulfonyl)indolin-6-amine (30i)

The title compound **30i** was obtained in 71% yield from compound **29c** in a manner similar to that described for the preparation of **30h**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.61 (t, *J* = 8.0 Hz, 2H), 3.73 (t, *J* = 8.0 Hz, 2H), 3.81 (s, 3H), 6.74 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 8.00 Hz, 2H), 7.12 (s, 1H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 2H).

1-((4-Methoxyphenyl)sulfonyl)-1H-indol-7-amine (30j)

The title compound **30j** was obtained in 71% yield from compound **29d** in a manner similar to that described for the preparation of **30h**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.79 (s, 3H), 6.61 (d, *J* = 4.5 Hz, 1H), 6.85 - 6.89 (m, 3H), 7.01 (d, *J* = 7.5 Hz, 1H), 7.08 - 7.11 (m, 1H), 7.55 (d, *J* = 4.5 Hz, 1H), 7.79 - 7.81 (d, *J* = 7.5 Hz, 2H).

1-((4-Methoxyphenyl)sulfonyl)-1H-indol-6-amine (30k)

The title compound **30k** was obtained in 69% yield from compound **29e** in a manner similar to that described for the preparation of **29i**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.80 (s, 3H), 6.51 (d, *J* = 3.0 Hz, 1H), 6.74 (dd, *J* = 1.5, 8.5 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 1H), 7.36 (d, *J* = 3.0 Hz, 1H), 7.48 (s, 1H), 7.79 (d, *J* = 8.5 Hz, 2H).

4-(((1-(Phenylsulfonyl)indolin-7-yl)amino)methyl)benzoic acid (31a)

The title compound **31a** was obtained in 66% yield from compound **30a** in a manner similar to that described for the preparation of **31b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.15 (t, *J* = 7.5 Hz, 2H), 3.99 (t, *J* = 7.5 Hz, 2H), 4.61 (d, *J* = 6.0 Hz, 2H), 6.25 - 6.29 (m, 1H), 6.39 (d, *J* = 7.5 Hz, 1H), 6.45 (d, *J* = 8.5 Hz, 1H), 6.89 - 6.93 (m, 1H), 7.23-739 (m, 2H), 7.54 - 7.59 (m, 2H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.98 (d, *J* = 8.5 Hz, 2H).

4-(((1-((4-Methoxyphenyl)sulfonyl)indolin-7-yl)amino)-methyl)benzoic acid (31b)

To a stirred solution of **30b** (1.80 g, 5.94 mmol) and 4-carboxybenzaldehyde (0.885 g, 5.94 mmol) in MeOH (10 mL), few drops of glacial AcOH were added. Sodium cyanoborohydride (560 mg, 8.92 mmol) was added and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with H_2O then extracted with EtOAc. The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 0.95 with 0.5% AcOH) to give compound **31b** (1.76 g, yield 68%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.10 (t, *J* = 7.5 Hz, 2H), 3.79 (s, 3H), 3.93 (t, *J* = 7.5 Hz, 2H), 4.48 (d, *J* = 6.0 Hz, 2H), 6.35 (d, *J* = 7.5 Hz, 1H), 6.38 (d, *J* = 8.5 Hz, 1H), 6.85 - 6.88 (m, 1H), 7.00 (d, *J* = 9 Hz, 2H), 7.49 - 7.51 (m, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.91 (d, *J* = 8.5 Hz, 2H).

4-(((1-((3,4-Dimethoxyphenyl)sulfonyl)indolin-7-yl)amino)methyl)benzoic acid (31c)

The title compound **31c** was obtained in 71% yield from compound **30c** in a manner similar to that described for the preparation of **31b**. ^{1}H NMR (500 MHz, CD_3OD) δ 2.10 (t, *J* = 7.5 Hz, 2H), 3.36 (s, 3H), 3.85 (s, 3H), 3.96 (t, *J* = 7.5 Hz, 2H), 4.53 (s, 2H), 6.36 (d, *J* = 7.5 Hz, 1H), 6.48 (d, *J* = 7.5 Hz, 1H), 6.70 (d, *J* = 2.0 Hz, 1H), 6.88-6.91 (m, 1H), 7.02 (d, *J* = 7.5 Hz, 1H), 7.38 (dd, *J* = 2.0, 7.5 Hz, 1H), 7.60 (d, *J* = 8.5 Hz, 2H), 7.97 (d, *J* = 8.5 Hz, 2H).

4-(((1-((3-Methoxyphenyl)sulfonyl)indolin-7-yl)amino)methyl)benzoic acid (31d)

The title compound **31d** was obtained in 73% yield from compound **30d** in a manner similar to that described for the preparation of **31b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.08 (t, *J* = 7.5 Hz, 2H), 3.53 (s,

3H), 3.96 (t, *J* = 7.5 Hz, 2H), 4.50 (d, *J* = 6.0 Hz, 2H), 6.34 (d, *J* = 7.5 Hz, 1H), 6.40 (d, *J* = 8.5 Hz, 1H), 6.86 - 6.89 (m, 2H), 7.21 - 7.25 (m, 2H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.5 Hz, 2H), 7.96 (d, *J* = 8.5 Hz, 2H).

4-(((1-((4-Chlorophenyl)sulfonyl)indolin-7-yl)amino)methyl)benzoic acid (31e)

The title compound **31e** was obtained in 62% yield from compound **30e** in a manner similar to that described for the preparation of **31b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.13 (t, *J* = 7.5 Hz, 2H), 3.98 (t, *J* = 7.5 Hz, 2H), 4.48 (d, *J* = 6.0 Hz, 2H), 6.36 (d, *J* = 7.0 Hz, 1H), 6.40 (d, *J* = 8.5 Hz, 1H), 6.87 - 6.90 (m, 1H), 7.53 (d, *J* = 8.0 Hz, 2H), 7.55 - 7.57 (m, 4H), 7.91 (d, *J* = 8.0 Hz, 2H).

4-(((1-((4-Fluorophenyl)sulfonyl)indolin-7-yl)amino)methyl)benzoic acid (31f)

The title compound **31f** was obtained in 64% from compound **30f** in a manner similar to that described for the preparation of **31b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.12 (t, *J* = 7.5 Hz, 2H), 3.98 (t, *J* = 7.5 Hz, 2H), 4.49 (d, *J* = 6.0 Hz, 2H), 6.36 (d, *J* = 7.0 Hz, 1H), 6.40 (d, *J* = 8.5 Hz, 1H), 6.89 (m, 1H), 7.36 (t, *J* = 9.0 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.64 - 7.67 (m, 2H), 7.93 (d, *J* = 8.0 Hz, 2H).

4-(((1-((4-Cyanophenyl)sulfonyl)indolin-7-yl)amino)methyl)benzoic acid (31g)

The title compound **31g** was obtained in 65% from compound **30g** in a manner similar to that described for the preparation of **31b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.22 (t, *J* = 7.5 Hz, 2H), 4.09 (t, *J* = 7.5 Hz, 2H), 4.23 (d, *J* = 6.0 Hz, 2H), 6.43 (d, *J* = 7.5 Hz, 1H), 6.53 (d, *J* = 8.0 Hz, 1H), 6.97 (t, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 8.03 (d, *J* = 8.0 Hz, 2H).

4-((1-((4-Methoxyphenyl)sulfonyl)indolin-7-yl)amino)benzoic acid (31h)

To a flame-dried 100 mL pear shaped flask was added (4-(methoxycarbonyl)phenyl)boronic acid (0.18 g, 1.0 mmol), 20 mol % of solid $Cu(OAc)_2$ (0.03 g, 0.16 mmol), crystalline myristic acid (0.75 g, 0.33 mmol) and dry toluene (5 mL).The resulting suspension was stirred for 10 min and 2,6-lutidine (0.20 mL, 1.73 mmol) was added by syringe. After 30 min, **30b** (0.20 g, 0.66 mmol) was added and stirred vigorously at ambient temperature for 24 h. The reaction mixture was filtered and washed by

CH_2Cl_2 and EtOAc. The organic layers were dried over $MgSO_4$, concentrated and purified by flash column chromatography (EtOAc: *n*-hexane = 1: 3) to give the residue. 1M $LiOH_{(aq)}$ (0.37 mL) was added to the solution of the residue (0.08 g, 0.18 mmol) in dioxane (5 mL) and the reaction mixture was heated to 40 ^oC for 18 h. After cooling to room temperature, the reaction mixture was neutralized to pH = 7, extracted with water (10 mL) and chloroform/IPA (chloroform/ IPA = 3/1; 10mL x 3). The organic layers were dried over $MgSO_4$ and the residue was recrystallized from methanol to afford **31h** (0.07 g, 96%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 2.26 (t, *J* = 7.0 Hz, 2H), 3.83 (s, 3H), 4.02 (t, *J* = 7.5 Hz, 2H), 6.75 (d, *J* = 7.0 Hz, 1H), 6.93 (d, *J* = 9.0 Hz, 2H), 7.01 (d, *J* = 8.5 Hz, 2H), 7.11 (m, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 7.50 (d, *J* = 9.0 Hz, 2H), 7.79 (br, 1H), 7.88 (d, *J* = 9.0 Hz, 2H).

3-(((1-((4-Methoxyphenyl)sulfonyl)indolin-7-yl)amino)methyl)benzoic acid (31i)

The title compound **31i** was obtained in 69% yield from compound **30b** using 3-formylbenzoic acid in a manner similar to that described for the preparation of **31b**. ^1H NMR (500 MHz, CD_3OD) δ 2.18 (t, *J* = 7.0 Hz, 2H), 3.88 (s, 3H), 3.99 (t, *J* = 7.0 Hz, 2H), 4.58 (d, *J* = 6.5 Hz, 2H), 6.19 (t, *J* = 6.0 Hz, 1H), 6.39 (d, *J* = 7.5 Hz, 1H), 6.48 (d, *J* = 8.5 Hz, 1H), 6.69 - 6.79 (m, 2H), 7.21 (d, *J* = 7.5 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.59 (t, *J* = 8.0 Hz, 1 H), 7.69 (d, *J* = 7.5 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 8.17 (s, 1H).

4-((1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)carbamoyl)benzoic acid (31j)

To a solution of **30b** (1 g, 3.28 mmol) in dry pyridine (5 mL), methyl 4-(chlorocarbonyl)benzoate (0.652 g, 3.28 mmol) was added. The reaction mixture was stirred at room temperature for 2h. The reaction was then quenched with H_2O and extracted with EtOAc (3 x 50 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The mixture was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 1) to give residue (1.52 g, 65 % yield). The resulting residue was dissolved in dioxane and 10 mL LiOH (aq, 1M) was added to it. The reaction mixture was stirred for 5 h at 40 □ C . The reaction was concentrated under reduced pressure and then was added water. The mixture was acidified with 3 N HCl and extracted with ethyl acetate. The

combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield **31j** in 96% yield. ^{1}H NMR (300 MHz, DMSO-d_6) : δ 2.31 (t, *J* = 7.2 Hz, 2H), 4.08 (t, *J* = 7.5 Hz, 2H), 7.00 - 7.02 (m, 3H), 7.23 (t, J = 7.5 Hz, 1H), 7.55 (d, J = 6.9 Hz, 2H), 7.93 (d, *J* = 7.2 Hz, 1H), 8.09 – 8.18 (m, 4H), 10.25 (s, 1H).

4-(((1-(4-Methoxyphenylsulfonyl)indolin-5-yl)amino)methyl)benzoic acid (31k)

The title compound **31k** was obtained from compound **30h** using 4-formylbenzoic acid in 61% yield in a manner similar to that described for the preparation of **31b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.54 (t, *J* = 8.5 Hz, 2H), 3.73 (t, *J* = 8.5 Hz, 2H), 3.78 (s, 3H), 4.25 (d, *J* = 4.5 Hz, 2H), 6.32 (s, 1H), 6.40 (dd, *J* = 2.0, 8.5 Hz, 1H), 6.99 (d, *J* = 9.0 Hz, 2H), 7.17 (d, *J* = 9.0 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.86 (d, *J* = 8.0 Hz, 2H).

4-((1-(4-Methoxyphenylsulfonyl)indolin-6-yl)amino)benzoic acid (31l)

The title compound **31l** was obtained from compound **30i** in 68% yield in a manner similar to that described for the preparation of **31b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.54 (t, *J* = 8.5 Hz, 2H), 3.79 (t, *J* = 8.5 Hz, 2H), 3.81 (s, 3H), 4.65 (d, *J* = 6.0 Hz, 2H), 6.51 (d, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 2H), 7.12 (s, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 8.5 Hz, 2H), 7.98 (d, *J* = 8.5 Hz, 2H).

4-(((1-((4-Methoxyphenyl)sulfonyl)-1H-indol-7-yl)amino)methyl)benzoic acid (31m)

The title compound **3lm** was obtained in 71% yield from compound **30j** in a similar manner as described for the preparation of **31b**. ^{1}H NMR (500 MHz, CD_3OD) δ 3.80 (s, 3H), 4.53 (s, 2H), 6.44 (d, *J* = 7.5 Hz, 1H), 6.68 (d, *J* = 3.0 Hz, 1H), 6.80 (d, *J* = 7.5 Hz, 1H), 6.93 - 7.00 (m, 3H), 7.40 (d, *J* = 8.5 Hz, 2H), 7.61 - 7.62 (m, 3H), 7.96 (d, *J* = 8.5 Hz, 2H).

4-(((1-((4-Methoxyphenyl)sulfonyl)-1H-indol-6-yl)amino)methyl)benzoic acid (31n)

The title compound **31n** was obtained in 67% yield from compound **30k** in a similar manner as described for the preparation of **31b**. ^{1}H NMR (500 MHz, CD_3OD) δ 3.85 (s, 3H), 4.54 (s, 2H), 7.15 (d, *J* = 3.0 Hz, 1H), 7.35 (dd, *J* = 1.5, 8.5 Hz, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.66 (s, 1H), 7.88 (d, *J* = 8.5

Hz, 1H), 7.93 (d, *J* = 3.0 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 2H), 8.21 (d, *J* = 8.5 Hz, 2H), 8.71 (d, *J* = 8.5 Hz, 2H).

Methyl 4-(2-(1H-indol-7-yl)ethyl)benzoate (33)

To a solution of indole-7-carboxaldehyde (**32**) (1.0 g, 6.8 mmol) and 4-methoxycarbonylbenzyltriphenylphosphonium chloride (3.07 g, 6.8 mmol) stirring in dichloromethane (4 mL), sodium methoxide (0.37 g, 6.8 mmol) was added to the reaction slowly at room temperature with continuous stirring 4h. The reaction mixture was quenched with water and extracted with dichloromethane. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure and dried in vacuum. The residue was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 4) to give a solid residue (1.39 g) in 74% yield. To the mixture of the residue in ethanol was added a catalytic amount of 10% palladium on carbon (139 mg) and the reaction was stirred at room temperature for 3h under hydrogen. The reaction mixture wa3 filtered over celite and the filtrate was concentrated. The residue was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 4) to give compound **33** in 87% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 3.09 - 3.17 (m, 4H), 3.91 (s, 3H), 6.55 (m, 1H), 7.00 (d, *J* = 7.5 Hz, 1H), 7.06 - 7.14 (m, 2H), 7.24 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 2H).

(E)-Methyl 4-(2-(1H-indol-7-yl)vinyl)benzoate (34)

To a solution of indole-7-carboxaldehyde (**32**) (1.0 g, 6.8 mmol) and 4-methoxycarbonyl-benzyltriphenylphosphonium chloride (3.07 g, 6.8 mmol) stirring in dichloromethane (4 mL), sodium methoxide (0.37 g, 6.8 mmol) was added to the reaction slowly at room temperature with continuous stirring 4h. The reaction mixture was quenched with water and extracted with dichloromethane. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure and dried with vacuum. The residue was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 4) to give a solid residue of compound **34** (1.39 g) in 74% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.08 (t, *J* = 8.5 Hz, 2H), 3.63 (t, *J* = 8.5 Hz, 2H), 3.93 (s, 3H), 6.74 (d, *J* = 7.5 Hz, 1H), 7.01 (d, *J* = 16.0 Hz, 1H),

7.07 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.18 (d, *J* = 16.0 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 2H), 8.00 (d, *J* = 8.5 Hz, 2H).

Methyl 4-(2-(indolin-7-yl)ethyl)benzoate (35)

To a solution of compound **34** (1.0 g, 3.5 mmol) in acetic acid (5 ml), sodium cyanoborohydride (0.06 g, 0.93 mmol) was slowly added and the reaction mixture was stirred overnight. The pH of the reaction mixture was adjusted with 1N $NaOH_{(aq)}$ at 0 ^{o}C. The reaction mixture was then quenched with water and extracted with dichloromethane (50 x 3 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 4) to give **35** (805 mg) in 80% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 2.92 - 3.00 (m, 4H), 3.28 (m, 2H), 3.42 (t, *J* = 8.5 Hz, 2H), 3.90 (s, 3H), 6.67 (t, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 7.5 Hz, 1H), 6.98 (t, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.0 Hz, 2H), 7.98 (d, *J* = 7.5 Hz, 2H).

(E)-Methyl 4-(2-(indolin-7-yl)vinyl)benzoate (36)

The title compound **36** was obtained 91% yield in a similar manner as described for the preparation of **34**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.08 (t, *J* = 8.5 Hz, 2H), 3.63 (t, *J* = 8.5 Hz, 2H), 3.93 (s, 3H), 6.74 (d, *J* = 7.5 Hz, 1H), 7.01 (d, *J* = 16.0 Hz, 1H), 7.07 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.18 (d, *J* = 16.0 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 2H), 8.00 (d, *J* = 8.5 Hz, 2H).

Methyl 4-(2-(1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)ethyl)benzoate (37)

Compound **36** (800 mg, 2.84 mmol) was dissolved in pyridine (5 mL) and 4-methoxybenzenesulfonyl chloride (0.58 g, 2.84 mmol) was added to it. The reaction mixture was refluxed overnight and the solvent was evaporated under reduced pressure then the reaction mixture was quenched with water and extracted with dichloromethane. The combined organic layer was dried over an anhydrous $MgSO_4$, concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 4) to give **37** (1.15 g) in 92% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 2.12 (t, *J* = 7.5 Hz, 2H), 3.09 (t, *J* = 8.5 Hz, 2H), 3.37 (t, *J* = 8.5 Hz, 2H), 3.82 (s, 3H), 3.86 (s, 3H), 3.87 (t, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 7.5 Hz, 1H), 7.07 (t, *J* = 7.5 Hz,

1H), 7.16 (d, *J* = 7.5 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.92 (d, *J* = 8.5 Hz, 2H).

Methyl (E)-4-(2-(1-((4-methoxyphenyl)sulfonyl)indolin-7-yl)vinyl)benzoate (38)

The title compound **38** was obtained 91% yield in a similar manner as described for the preparation of **36**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.21 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 3.92 (s, 3H), 4.04 (t, *J* = 7.5 Hz, 2H), 6.80 (d, *J* = 7.5 Hz, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 7.14 - 7.18 (m, 4H), 7.40 (d, *J* = 7.5 Hz, 2H), 7.64-7.67 (m, 1H), 7.85 (d, *J* = 16.0 Hz, 1H), 8.01 (d, *J* = 8.5 Hz, 2H).

1-((4-Methoxyphenyl)sulfonyl)indolin-7-ol (40)

To a solution of **39** (0.42 g, 3.11 mmol) and *tert*-butyldimethylsilyl chloride (0.56 g, 3.73 mmol) in dichloromethane (30 mL), DIPEA (1.10 mL, 6.21 mmol) was added to the reaction at room temperature. The reaction mixture was stirred for overnight, quenched with water and extracted with dichloromethane (20 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure to give a residue (387 mg, 85% yield). The mixture of crude residue and 4-methoxybenzenesulfonyl chloride (320 mg, 1.5 mmol) in pyridine (2 mL) was heated at 100 ^{o}C overnight. The reaction solvent was evaporated under reduced pressure to give a black residue, which was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 1) to afford a yellow liquid, yield 91%. To a solution of the obtained residue in THF (10 mL), 1M tetrabutylammonium fluoride in THF (3 mL) was added to the reaction dropwise at 0 ^{o}C. The reaction mixture was stirred at room temperature for 1 h and then quenched with water and extracted with dichloromethane (15 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure. The residue obtained was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 1) to afford **40** in 93% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.38 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 3.99 (t, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 7.5 Hz, 1H), 6.84 - 6.88 (m, 2H), 6.98 - 7.01 (m, 1H), 7.56 - 7.57 (m, 2H), 8.20 (s, 1H).

4-(((1-((4-Methoxyphenyl)sulfonyl)indolin-7-yl)oxy)methyl)benzoic acid (41)

The mixture of **40** (0.40 g, 1.3 mmol), 4-chloromethylbenzoic acid methyl ester (239 mg, 1.3 mmol) and potassium carbonate (269 mg, 1.9 mmol) in acetone (20 mL) was refluxed for 6h. The reaction mixture was quenched with water and extracted with dichloromethane (20 mL x 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a residue, which was purified by silica gel chromatography (EtOAc: *n*-hexane = 1: 4) in 76% yield. To the solution of obtained residue (451 mg, 0.9 mmol) in dioxane (10 mL), 1M $LiOH_{(aq)}$ (5 mL) was added and stirred at 40 ^{o}C. After being stirred overnight, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in water and concentrated HCl was added up to acidic pH to give **41** as white precipitates in 91% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 2.41 (t, *J* = 7.5 Hz, 2H), 3.80 (s, 3H), 4.03 (t, *J* = 7.5 Hz, 2H), 5.23 (s, 2H), 6.74 (d, *J* = 7.5 Hz, 1H), 6.87 - 6.92 (m, 3H), 7.04-7.07 (m, 1H), 7.52 - 7.53 (m, 2H), 7.59 - 7.61 (d, *J* = 8.0 Hz, 2H), 7.98 (d, *J* = 8.0 Hz, 2H).

(B) Biology

Reagents for cell culture were obtained from Gibco-BRL Life Technologies (Gaitherburg, MD). Microtubule-associated protein (MAP)-rich tubulin was purchased from Cytoskeleton, Inc. (Denver, CO). [^{3}H]Colchicine (specific activity, 60-87 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA).

Cell culture

Human cancer cell lines, oral epidermoid carcinoma (KB cells), stomach carcinoma (MKN45 cells) and lung adenocarcinoma (A549 cells) were maintained in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/mL penicillin, 100 mg/mL streptomycin and 2.5 mg/mL amphotericin B. Cells were cultured in tissue culture flasks in a humidified incubator at 37 °C, in an atmosphere of 5% CO2 and 95% air.

The sulforhodamine B assays

Counted cells were seeded in 96-well plates in medium with 5% FBS. After 24 h, cells were fixed with 10% trichloroacetic acid (TCA) to represent cell population at the time of compound addition (T0).

After additional incubation of DMSO or test compound for 48 h, cells were fixed with 10% TCA and then stained with SRB at 0.4% (w/ v) in 1% acetic acid. Unbound SRB was washed out by 1% acetic acid and SRB-containing cells were solubilized with 10mMTrizma base. The absorbance was read at a wavelength of 515 nm. Using the following absorbance measurements, such as time zero (T0), control growth (C), and cell growth in the presence of the compound (Tx), the percentage of growth was calculated at each of the compound concentration levels. Growth inhibition of 50% (GI_{50}) was calculated from the equation [(Ti - Tz)/(C-Tz)] x 100 = 50, which provides the compound concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the incubation with the compound.

Tubulin polymerization in vitro assay [42, 43]

Turbidimetric assays of microtubules were performed as described by Bollag *et al* [44]. In brief, microtubule-associated protein (MAP)-rich tubulin (from bovine brain, Cytoskeleton, Denver, C.O.) had been dissolved in reaction buffer (100 mM PIPES (pH 6.9), 2 mM $MgCl_2$, 1 mM GTP) in preparing of 4 mg/mL tubulin solution. Tubulin solution (240 g MAP-rich tubulin per well) was placed in 96-well microtiter plate in the presence of test compounds or 2% (v/v) DMSO as vehicle control. The increase in absorbance was measured at 350 nm in a PowerWave X Microplate Reader (BIO-TEK Instruments, Winooski, VT) at 37 °C and recorded every 30 s for 30 min. The area under the curve (AUC) used to determine the concentration that inhibited tubulin polymerization to 50% (IC_{50}). The AUC of the untreated control and 10 μM of colchicine was set to 100% and 0% polymerization, respectively, and the IC_{50} was calculated by nonlinear regression in at least three experiments.

Tubulin competition binding scintillation proximity assay ([^{3}H] Colchicine Binding Assay)

The assay was performed according to the method reported in previous studies [45-47]. In the study, 1 μM tubulin was incubated with 5.0 μM [^{3}H]-colchicine at either 1.0 or 5.0 μM concentrations of test compounds in a buffer containing 0.05 M PIPES (pH 6.8), 1mM EGTA, 10 % glycerol, 1mM $MgCl_2$, and 1 mM GTP. Streptavidin-labeled poly (vinyl toluene) SPA beads were then added to the reaction

mixture. The radioactive counts were then directly measured by a scintillation counter, and the inhibition constant (K_i) was calculated using the Cheng-Prusoff equation.

HDAC enzymes inhibition assays

Enzyme inhibition assays were performed by the Reaction Biology Corporation, Malvern, PA. (http://www.reactionbiology.com). The substrate for HDAC-1, -2, -3, -4, -6, -7, -8, -9, and -10 is a fluorogenic peptide derived from p53 residues 379–382 [RHKK(Ac)]. Compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 10 μM. Trichostatin A (TSA) and MS-275 were used as the reference compounds.

Western blot analysis

Cell lysates were prepared, and proteins were separated by 7.5–15% SDS-PAGE, transferred onto PVDF membrane, and then immunoblotted with specific antibodies. Proteins were visualized with an ECL detection system. Compound tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 10 μM.

Molecular modeling studies

The crystal structure (PDB ID: 1SA0) of tubulin was downloaded from Protein Data Bank [48]. The structure was prepared using the drug design platform *LeadIT* [23]. The binding site was defined as a radius of 10Å from the co-crystallized ligand. The compounds were protonated in aqueous solution and were docked into the binding site using FlexX docking module in LeadIT. The docking strategy was performed by the hybrid (enthalpy and entropy) approach. The scoring parameters were used with the default settings.

The LeadIT docking poses were then added to the computational program Forge [26] to generate molecular force fields. The activity miner [49] methodology of Forge identifies the “average field” and “activity cliff”. The average field compares active molecules to identify common electrostatic and hydrophobic fields. The activity cliff represents compound pairs where small structural differences that causes large changes in activity. The activity cliff for each compound pair was summarized into a global activity atlas model. The default settings were used to generate the molecular force fields.

Antitumor activity *in vivo*

The human non-small cell lung cancer A549 cells were implanted subcutaneously (s.c.) with 10^8 cells into the flank of the 4-week-old male balb/c nude mice. After tumor volume average around 200 mm^3, mice were separated into two groups (n = 6), control (vehicle) and compound **9** (intraperitoneal injection daily, dissolved in 5% DMSO + 5% Cremophor + 90% dextrose). Tumor volumes were monitored daily at first week and then twice weekly until tumor volumes of control group approached 1000 mm^3. Tumor volume was calculated from (W^2 x L)/2. W, width and L, length.

Male nude mice (NOD-SCID) were inoculated subcutaneously with the same volume of BD Matrigel Matrix HC (catalog 354248, BD bioscience), and BJAB cells (1 x 10^7 cell/mouse) into the flank of each animal. When the tumors had grown to around 200 mm^3, animals were divided into two groups (n = 5) and received the following treatments (a) control Ctrl (10% DMSO, 20% Cremophor EL, and 70% Normal Saline) (b) intravenous injection of compound **9** at 50 mg/kg daily for 5 days in 1 week. Compound **9** was dissolved in vehicle (10% DMSO, 20% Cremophor EL and 70% Normal Saline). 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-h light cycle at 21 - 23^0 C and 60-85% humidity. Nude mice were maintained in accordance with the Institutional Animal Care and Use Committee procedures and guidelines.

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.

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ABBREVIATIONS USED

HDAC, histone deacetylases; CA-4, combretastatin A-4; CA-4P, combretastatin A-4P; AIBN, 2,2'-azobis-(2-methylpropionitrile); LiOH, lithium hydroxide; IPA, isopropanol; TBDMSCl, *tert*-Butyldimethylsilyl chloride; DIPEA, *N*,*N*-Diisopropylethylamine; TBAF, Tetra-*n*-butylammoniumfluoride; PyBOP, (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.

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Figure Captions

Figure 1. Structures of antimitotic agents and benzamide-based HDAC inhibitors

Figure 2. Structures of designed compounds (**8-27**)

Figure 3. Structure activity relationships for 1-arylsulfonyl indoline-benzamides

Figure 4. The effect of compounds **9**, **16**, and **27** on in vitro tubulin polymerization. MAP-rich tubulins were incubated at 37 °C in the absence [DMSO as a control] or presence of colchicine (**2**) or serial concentrations of synthetic compounds). Absorbance at 350 nm was measured every 30s for 30 min and is presented as the increased polymerized microtubule.

Figure 5. Expression pattern of acetylated α-tubulin in A549 cells. Cells were treated with various concentrations of indicated compounds for 6 h, then assessed by western blot analysis.

Figure 6. Docking pose of compound 9 in Tubulin. (A) The stick model of compound **9** (blue) with interacting tubulin residues (grey). Residues are grouped into four sections as shown. Dotted green lines denotes hydrogen bond. (B) 2D representation of compound **9** interaction in tubulin. Dotted red lines indicate hydrogen bonds. Residues in green denote hydrophobic interactions. Residues are grouped and listed as shown. Residues are grouped and listed as shown.

Figure 7. Average molecular fields and activity cliff summary of compounds in tubulin. (A) Field regions obtained from active tubulin inhibitors. Red color shows positive field region, blue color shows negative field region, and yellow color shows hydrophobic interactions sites required for activity. (B) Analysis of the electrostatic and hydrophobic areas for tubulin binding site with compounds 19 (C), 20 (D), 21 (E), and 22 (F) were superimposed with compound 9 (grey). Red circle denotes regions where compounds are misaligned in electrostatic or hydrophobic areas. Red and cyan color indicates positive or negative electrostatics, respectively. Green color shows favorable hydrophobic regions, while pink indicates unfavorable hydrophobic energies.

Figure 8. Docking poses of compound 9 in HDAC isozymes. (A) The docking pose of compound **9** in HDAC 1 (PDB ID: 5ICN). (B) Superimposed docking results of compound 9 in HDAC1 (yellow), 2 (PDB ID: 5IX0, green), and 6 (PDB ID: 5EF8, pink). (C) Docking results of compound 9 in HDAC8 (PDB ID: 3SFH). Dotted green line represents chelation to zinc. Zinc ion represented as grey sphere. Red circle highlights contrasting surface residue in HDAC isozymes.

Figure 9 Anti-cancer activity of compound **9** in human non-small cell lung cancer A549 xenograft model.

Figure 10 The anticancer effect of Intravenous injection of compound **9** to subcutaneous xenograft tumor model of BJAB.

Figure 11 The mouse (a) and the tumor (b) images of the control and 50 mg/kg compound **9** groups in the day 25 after the first dose. The results of tumor weight in the day 25 after the first dose (c).

Table 1. IC_{50} values (nM ± SD[a]) of compounds and reference compounds

	Cell Type (IC_{50} ± SD,[a] nM)		
compd	KB	A549	MKN45
8	260±50	870±30	780±140
9	49±46	79±8	63±32
10	1400±630	2700±790	2300±1300
11	780±250	1100±86	800±230
12	440±90	860±23	780±65
13	210±11	560±170	460±37
14	540±24	930±79	870±14
15	560±130	920±88	880±29
16	120±4	190±32	140±49
17	200 ± 51	450±20	420±17
18	260±61	570±160	480±100
19	8700±1900	10500±820	10000±230
20	2200±860	5700±2100	3900±360
21	2100±380	8900±1400	5000±550
22	870±310	2400±450	2100±130
23	130±23	640±170	440±46
24	1440±300	3900±570	2500±280
25	-	> 10000	-
26	110±30	200±13	130±45
27	230±0	270±101	200±10
28	54±7	100±5	78±23
1	250±65	-	170±9
2[b]	13±4	-	15±3
6	520±130	1200±88	1200±11

[a]SD: standard deviation, all experiments were independently performed at least three times. [b]Data from reference 17

- not determined

Table 2. Growth inhibition by **9**, **16**, and **28** against KB-derived drug-resistant cell lines

	IC_{50} ± SD[a]						
Compd	Vincristine nM[b]	Paclitaxel nM[b]	VP-16 μM[b]	**2** nM[b]	**9** nM	**16** nM	**28** nM
KB (Parental)	0.6 ± 0.2	4.1 ± 1.6	1.1 ± 0.5	10.4± 2.5	48.6 ± 46.4	116.2 ± 3.9	54.2 ± 6.8
KB-VIN10 (MDR ↑)	90.1 ± 7.4	16500 ± 707	23 ± 3	122 ± 9.4	64.7 ± 24.5	114.3 ± 4.8	64.9 ± 3.8
KB-S15 (MDR ↑)	17.6 ± 0.5	273 ± 15	3.5 ± 0.3	35.4 ± 3.8	43.9 ± 2.8	106.0 ± 11.5	55.7 ± 4.6
KB-7D (MRP ↑)	1.2 ± 0.4	7.9 ± 0.5	54 ± 3.5	4.2 ± 1.9	46.6 ± 1.0	78.3 ± 9.2	54.2 ± 6.8

[a]SD: standard deviation. All experiments were independently performed at least three times. [b]Data from reference 18.

Table 3. Inhibition of tubulin polymerization and colchicine binding inhibition by compounds **9**, **16**, **27**, and references **1**, **2**, and **3**.

Compound	Tubulin[a] IC_{50} (μM)	% Colchicine competition binding[b] at concentration of:	
		1 μM	5 μM
8	3.2	-	-
9	1.1	69.9 ± 3.1	85.2 ± 5.5
10	>10	-	-
11	3.5	-	-
12	3.3	-	-
13	4	-	-
14	3.7	-	-
15	6.4	-	-
16	2.3	64.3 ± 2.9	98.8 ± 7.9
17	2.3	-	-
18	3.7	-	-
19	>10	-	-
20	8.5	-	-
21	>10	-	-
22	>10	-	-
23	3.7	-	-
24	>10	-	-
25	-	-	-
26	2.5	-	-
27	2.7	-	-
28	1.9	32.5 ± 6.2	77.7 ± 10.2
1	3.2	40.2 ± 1.3	71.6 ± 2.3
2	4.9	21.8 ± 7.8	53.9 ± 2.5
3	2.1	80.3 ± 1.3	84.1 ± 15.4

[a]Inhibition of tubulin polymerization.[b]Inhibition of [^{3}H] colchicine binding. Tubulin was at 1 μM; [^{3}H] colchicine was at 5 μM; test compounds were at 1 or 5 μM;

- Not determined

Table 4. HDAC inhibition activity and isoform selectivity of benzamide **9** and reference **6**

	IC_{50} (μM)[a]								
Compd	HDAC1	HDAC2	HDAC3	HDAC4	HDAC6	HDAC7	HDAC8	HDAC9	HDAC10
9	0.221	0.662			0.314				10
6	0.544	0.613			>30		9.884		
Trichostatin A	0.0249	0.0391	0.0262	ND	0.00355	ND	0.427	ND	0.071

[a]These assays were conducted by the Reaction Biology Corporation, Malvern, PA. All compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 10 μM.

- Empty cells indicate no inhibition or compound activity that could not be fit to an IC_{50} curve

ND, not determined

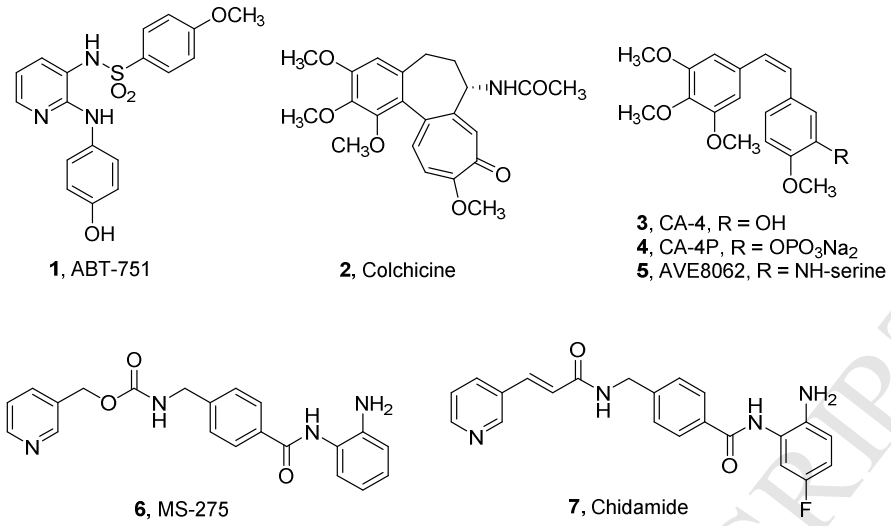
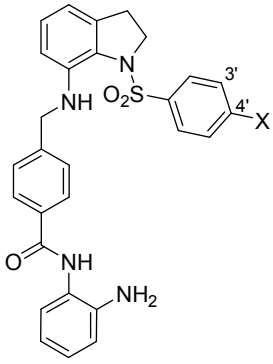
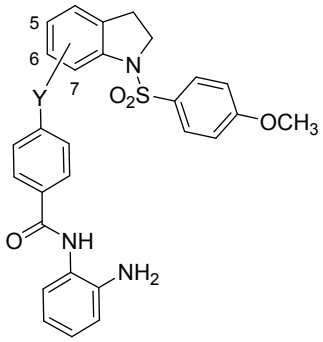


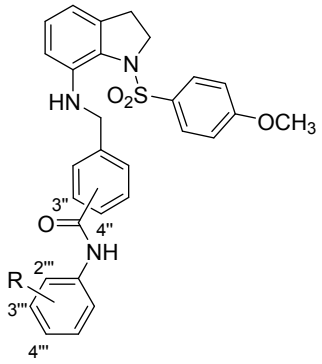
Figure 1. Structures of antimitotic agents and benzamide-based HDAC inhibitors



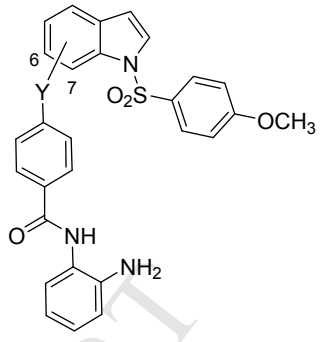
8, X = H
9, X = 4'-OCH_3
10, X = 3',4'-OCH_3
11, X = 3'-OCH_3
12, X = 4'-OH
13, X = 4'-Cl
14 X = 4'-F
15 X = 4'-CN



16, Y = -NH- (7-position)
21, Y = -NH-CH_2- (5-position)
22, Y = -NH-CH_2- (6-position)
25, Y = -NH-CO- (7-position)
26, Y = -CH_2-CH_2- (7-position)
27, Y = -CH=CH- (7-position)
28, Y = -O-CH_2- (7-position)



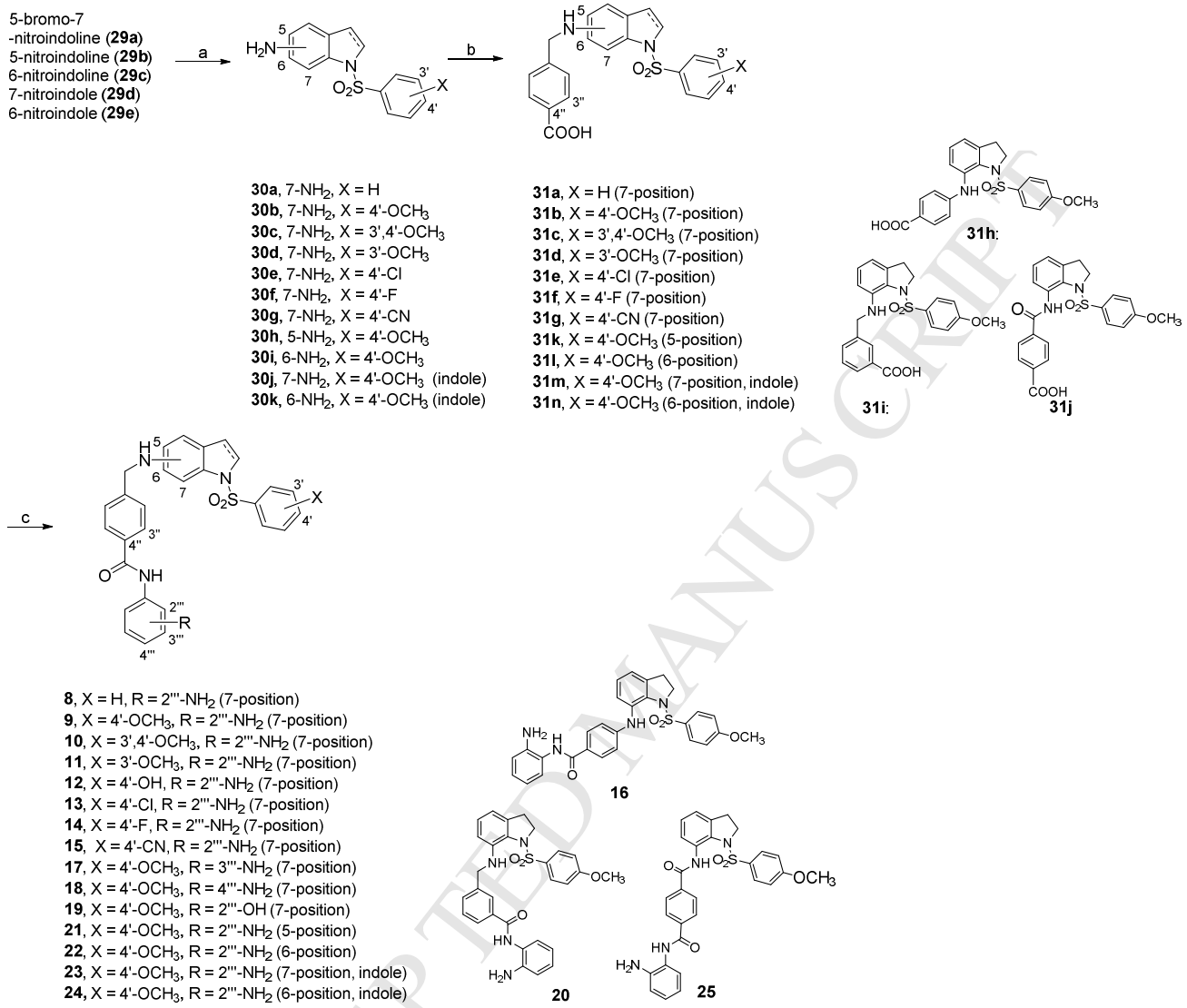
17, R = 3'''-NH_2 (4''-position)
18, R = 4'''-NH_2 (4''-position)
19, R = 2'''-OH (4''-position)
20, R = 2'''-NH_2 (3''-position)



23, Y = -NH-CH_2- (7-position)
24, Y = -NH-CH_2- (6-position)

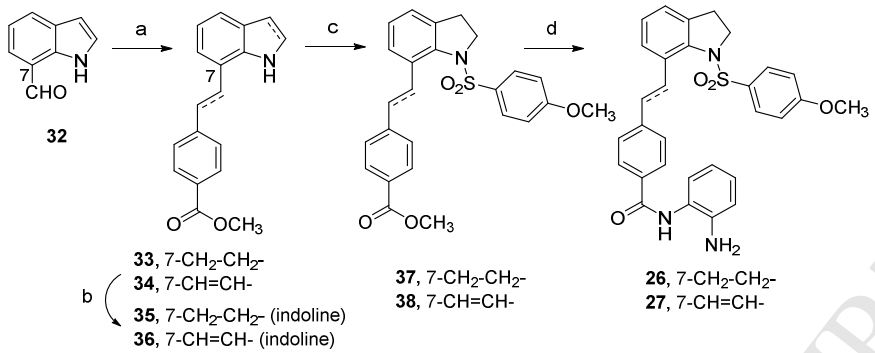
Figure 2. Structures of designed compounds (**8-27**)

Scheme1. Synthetic Approaches to Compounds **8-24**



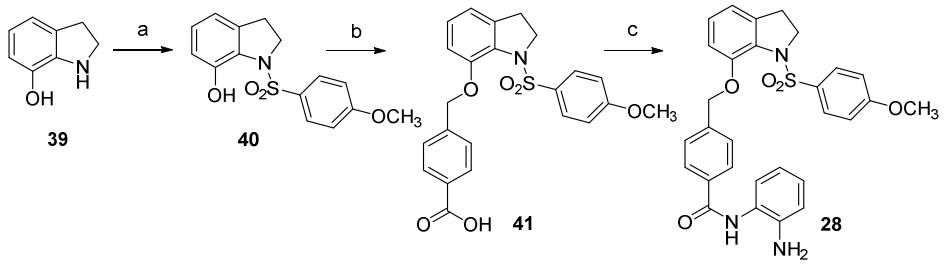
[a]Reagents and conditions: (a) i. substituted benzenesulfonyl chlorides, pyridine, reflux; ii. Fe, NH_4Cl, IPA/H_2O, reflux; iii. AIBN, Bu_3SnH, toluene, reflux; for **30h-k**: (a) i. 4-methoxybenzenesulfonyl chloride, pyridine, reflux; ii. Fe, NH_4Cl, IPA/H_2O, reflux; (b) 4-carboxybenzaldehyde, $NaBH_3CN$, MeOH, AcOH, rt; for **31h**: $Cu(OAc)_2$, myristic acid, 2,6-lutidine, 4-(methoxycarbonyl)phenyl)boronic acid, toluene, rt; for **31i**: i) 3-carboxybenzaldehyde, $NaBH_3CN$, MeOH, AcOH, rt; for **31j:** methyl 4-(chlorocarbonyl)benzoate , pyridine, rt; ii) LiOH (aq), dioxane, 40 □ C (c) *o*-phenylenediamine, PyBOP, Et_3N, DMF, rt; for **12**: BBr_3, CH_2Cl_2, rt; for **17**: *m*-phenylenediamine, PyBOP, Et_3N, DMF, rt; for **18***: p*-phenylenediamine, PyBOP, Et_3N, DMF, rt; for **19**: 2-aminophenol, PyBOP, Et_3N, DMF, rt.

Scheme 2. Synthetic Approaches to Compounds **25-26**[a]



[a]Reagents and conditions: For **26**: (a) i. 4-methoxycarbonylbenzyltriphenylphosphonium chloride, sodium methoxide, CH_2Cl_2, rt; ii. Pd/C, H_2, CH_3OH, rt; for **27**: 4-methoxycarbonylbenzyltriphenylphosphonium chloride, sodium methoxide, CH_2Cl_2, rt; (b) $NaCNBH_3$, AcOH, rt; (c) 4-methoxybenzenesulfonyl chloride, pyridine, reflux; (d) i. 1M $LiOH_{(aq)}$, dioxane, 40 ^oC; ii. *o*-phenylenediamine, PyBOP, Et_3N, DMF, rt.

Scheme 3. Synthetic Approaches to Compound **27**[a]



[a]Reagents and conditions: (a) i. TBDMSCl, DIPEA, CH_2Cl_2, rt; ii. 4-methoxybenzene sulfonyl chloride, pyridine, reflux; iii. TBAF, THF, 0 ^oC to rt; (b) i. methyl 4-(chloromethyl)benzoate, K_2CO_3, KI, acetone, reflux; ii. 1M $LiOH_{(aq)}$, dioxane, 40 ^oC; (c) *o*-phenylenediamine, PyBOP, Et_3N, DMF, rt.

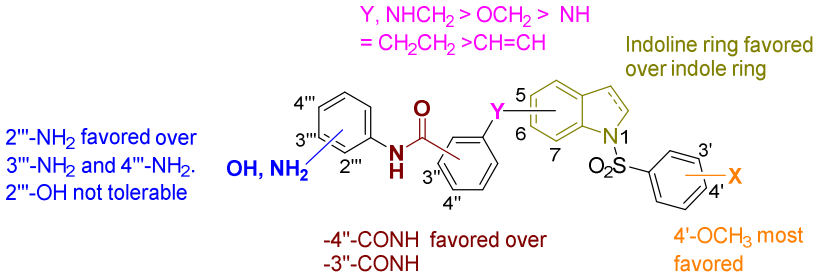


Figure 3. Structure activity relationships for 1-arylsulfonyl indoline-benzamides

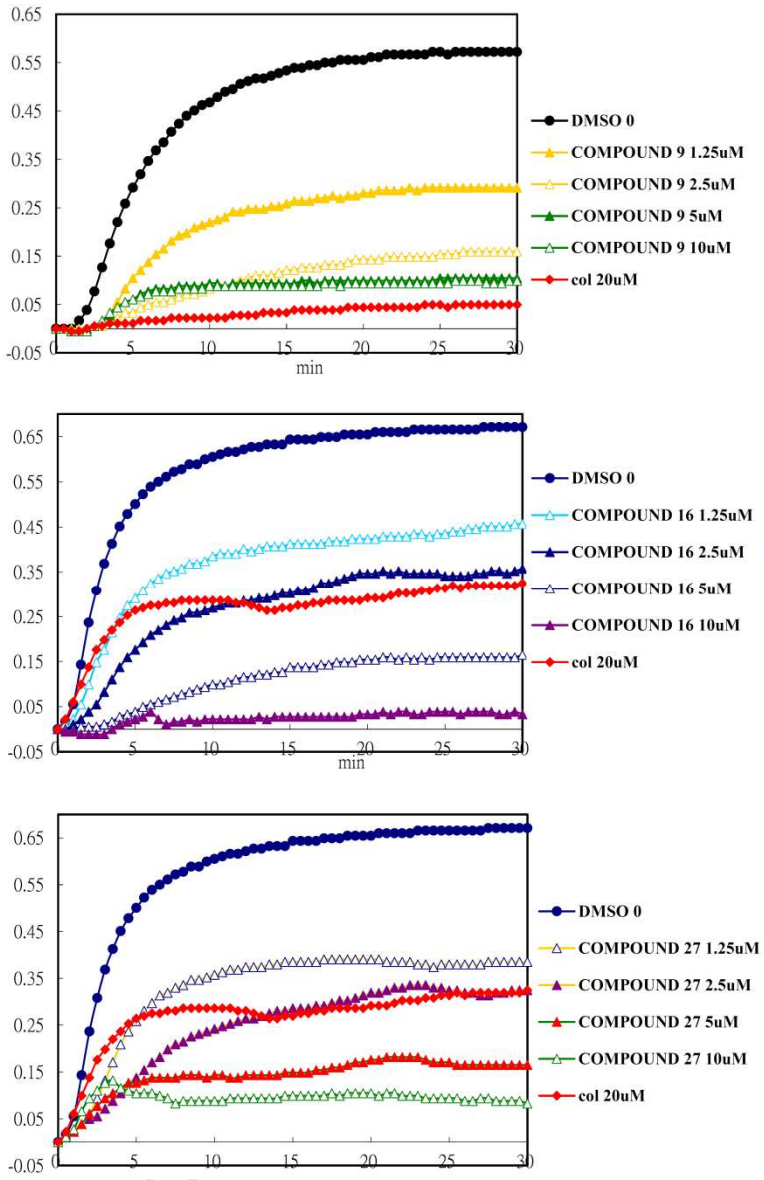


Figure 4. The effect of compounds **9**, **16**, and **28** on in vitro tubulin polymerization. MAP-rich tubulins were incubated at 37 °C in the absence [DMSO as a control] or presence of colchicine (**2**) or serial concentrations of synthetic compounds). Absorbance at 350 nm was measured every 30 s for 30 min and is presented as the increased polymerized microtubule.

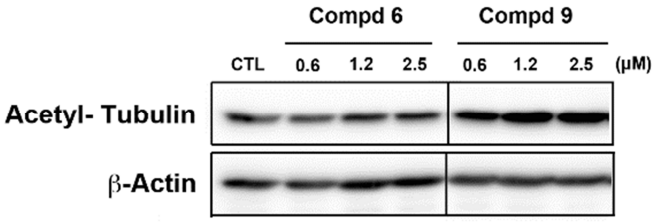


Figure 5. Expression pattern of acetylated α-tubulin in A549 cells. Cells were treated with various concentrations of indicated compounds for 6 h, then assessed by western blot analysis.

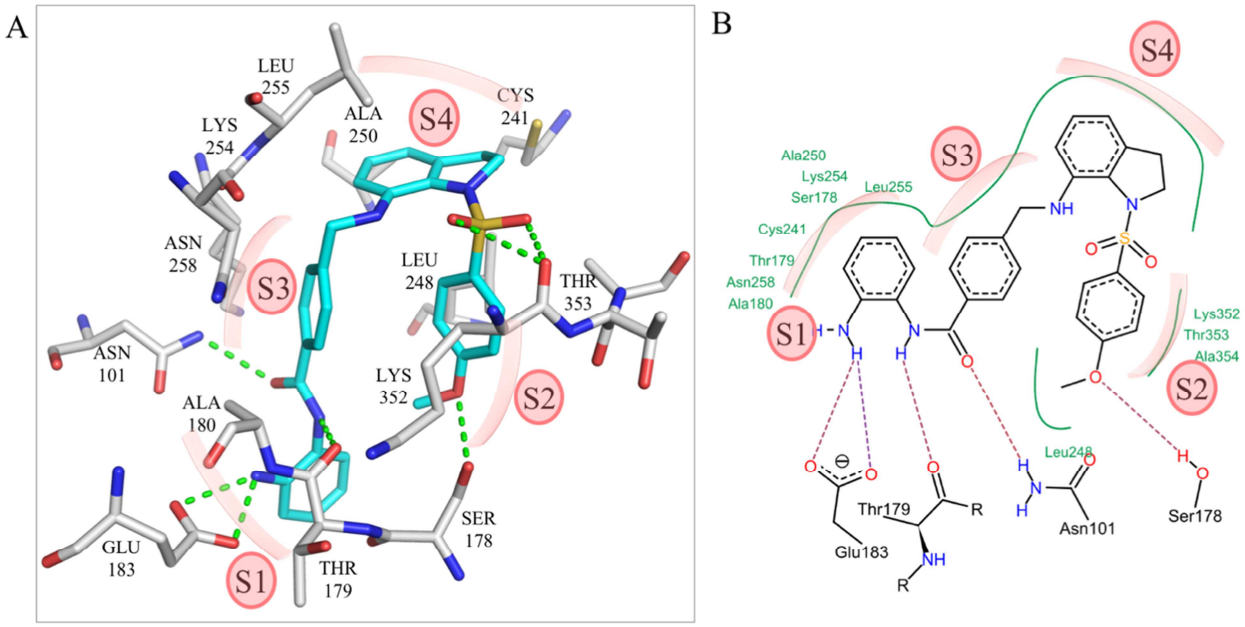


Figure 6. Docking pose of compound 9 in Tubulin. (A) The stick model of compound **9** (blue) with interacting tubulin residues (grey). Residues are grouped into four sections as shown. Dotted green lines denotes hydrogen bond. (B) 2D representation of compound **9** interaction in tubulin. Dotted red lines indicate hydrogen bonds. Residues in green denote hydrophobic interactions. Residues are grouped and listed as shown.

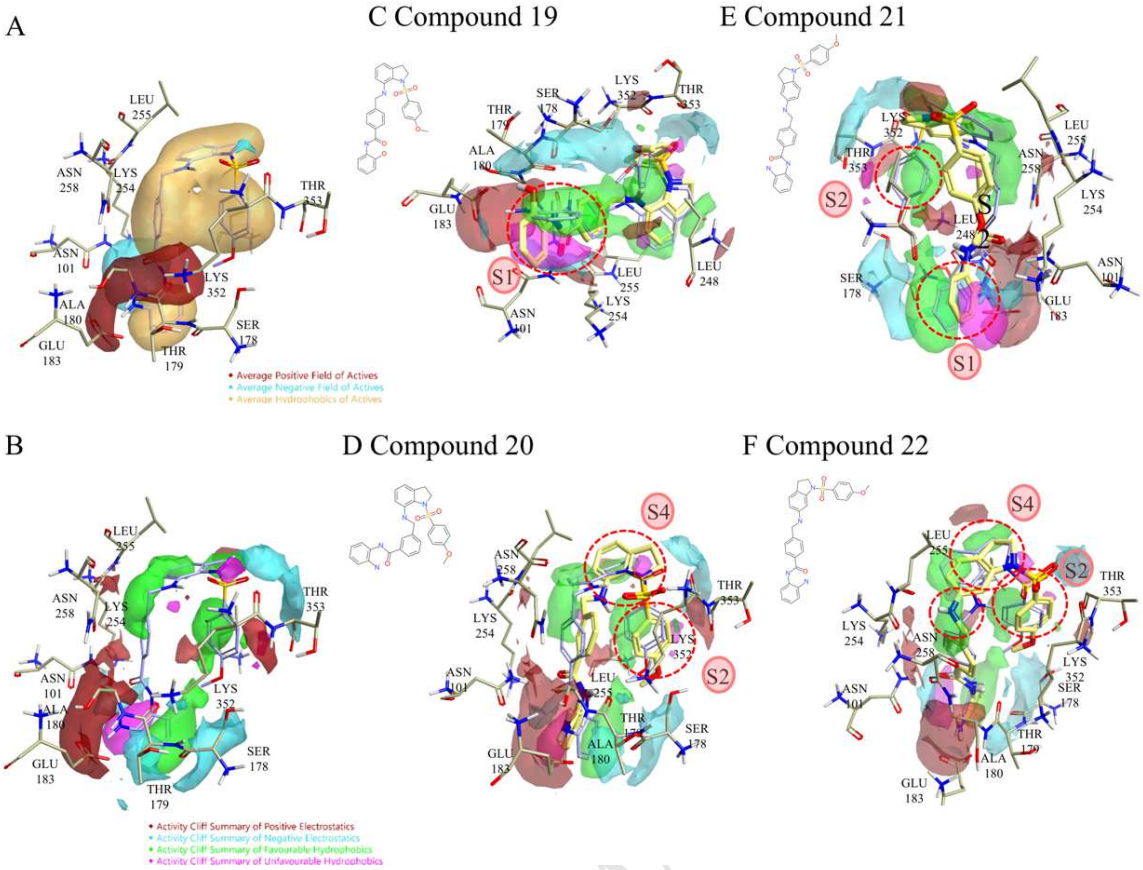


Figure 7. Average molecular fields and activity cliff summary of compounds in tubulin. (A) Field regions obtained from active tubulin inhibitors. Red color shows positive field region, blue color shows negative field region, and yellow color shows hydrophobic interactions sites required for activity. (B) Analysis of the electrostatic and hydrophobic areas for tubulin binding site with compounds 19 (C), 20 (D), 21 (E), and 22 (F) were superimposed with compound 9 (grey). Red circle denotes regions where compounds are misaligned in electrostatic or hydrophobic areas. Red and cyan color indicates positive or negative electrostatics, respectively. Green color shows favorable hydrophobic regions, while pink indicates unfavorable hydrophobic energies.

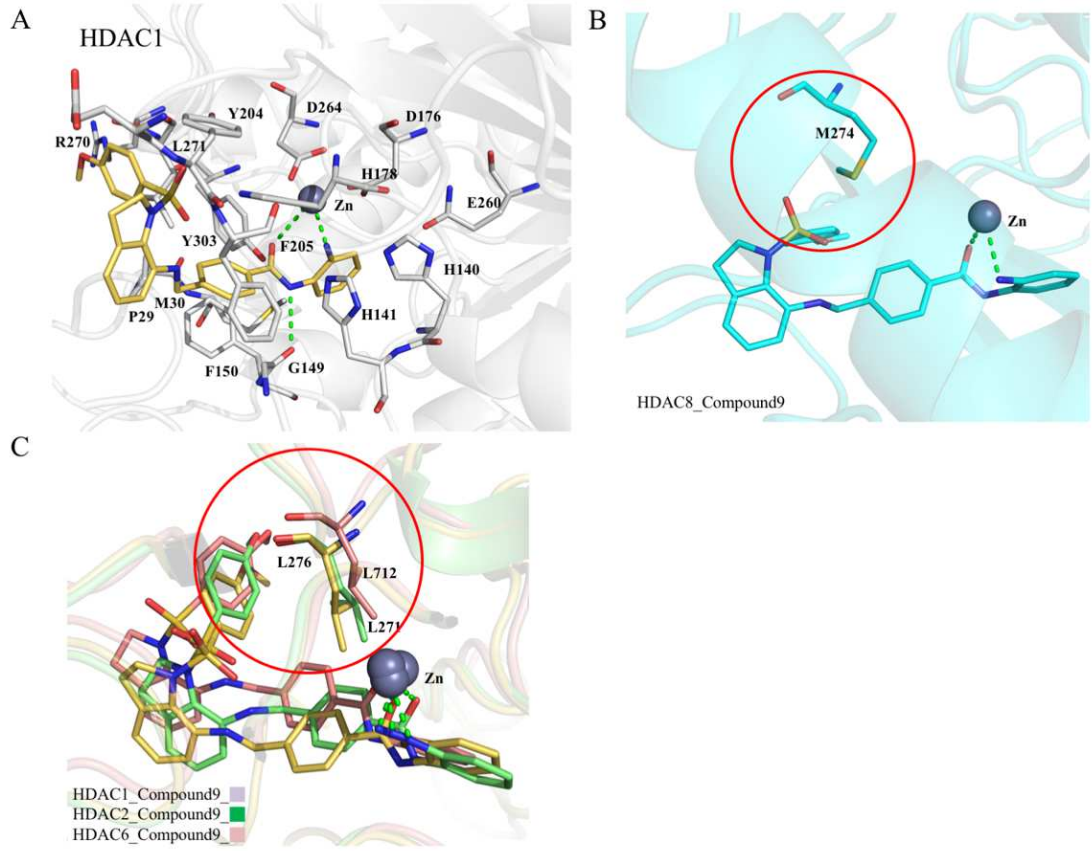


Figure 8. Docking poses of compound 9 in HDAC isozymes. (A) The docking pose of compound **9** in HDAC 1 (PDB ID: 5ICN). (B) Superimposed docking results of compound 9 in HDAC1 (yellow), 2 (PDB ID: 5IX0, green), and 6 (PDB ID: 5EF8, pink). (C) Docking results of compound 9 in HDAC8 (PDB ID: 3SFH). Dotted green line represents chelation to zinc. Zinc ion represented as grey sphere. Red circle highlights contrasting surface residue in HDAC isozymes.

a) b)

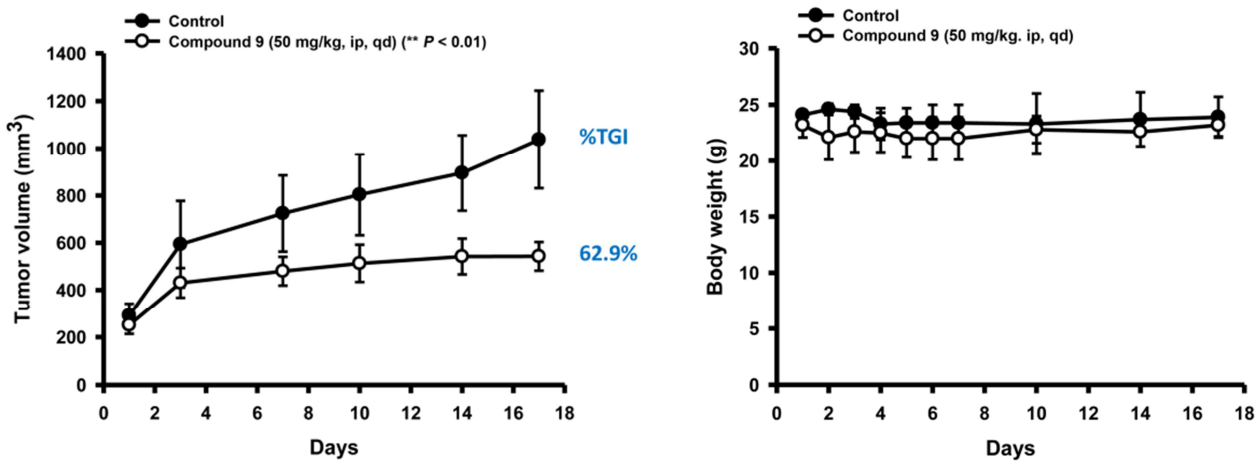


Figure 9 Anti-cancer activity of compound **9** in human non-small cell lung cancer A549 xenograft model.

a) b)

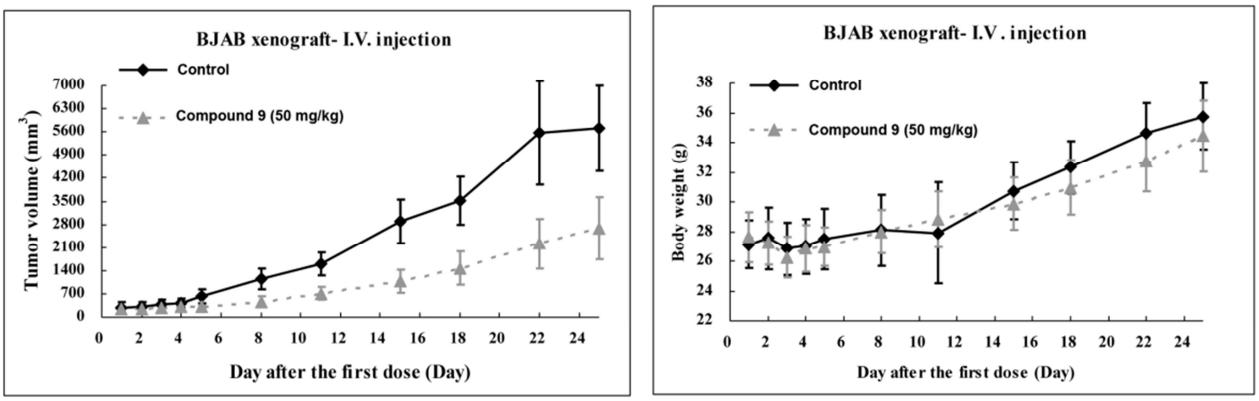


Figure 10 The anticancer effect of Intravenous injection of compound **9** to subcutaneous xenograft tumor model of BJAB.

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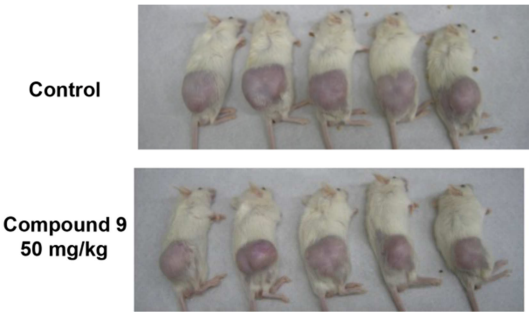
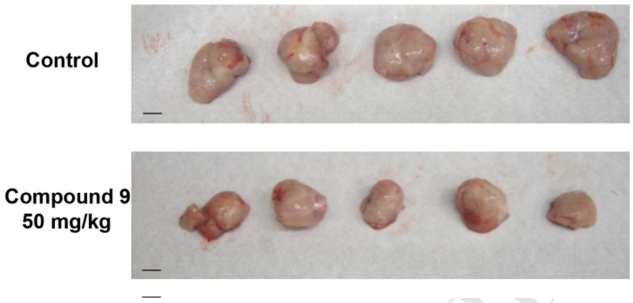
a)



b)



c)

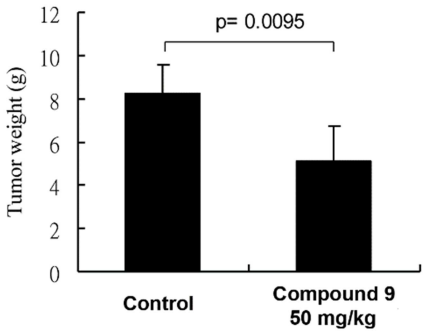


Figure 11 The mouse (a) and the tumor (b) images of the control and 50 mg/kg compound **9** groups in the day 25 after the first dose. The results of tumor weight in the day 25 after the first dose (c).

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Research highlights

1. A series of 1-Arylsulfonyl Indoline-Benzamides has been synthesised.
2. Compound **9** remarkably suppressed the growth of cancer cell lines.
3. The benzamide **9** displayed striking tubulin inhibition.
4. Compound **9** exhibited significant inhibitory potential against HDAC 1, 2 and 6.
5. Compound **9** also demonstrated significant *in vivo* efficacy