Journal Pre-proof

Design, synthesis, and evaluation of N-phenyl-4-(2-phenylsulfonamido)-benzamides as microtubule-targeting agents in drug-resistant cancer cells, displaying HDAC inhibitory response

Wei-Cheng Wu, Yi-Min Liu, Mei-Hsiang Lin, Yu-Hsuan Liao, Mei-Jung Lai, Hsun-Yueh Chuang, To-Yu Hung, Chun-Han Chen, Jing-Ping Liou

PII: S0223-5234(20)30125-2

DOI: https://doi.org/10.1016/j.ejmech.2020.112158

Reference: EJMECH 112158

To appear in: *European Journal of Medicinal Chemistry*

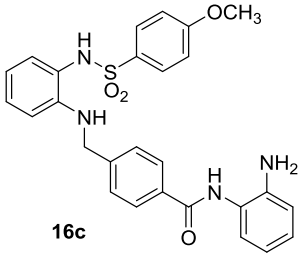
Received Date: 28 November 2019

Revised Date: 17 February 2020

Accepted Date: 17 February 2020

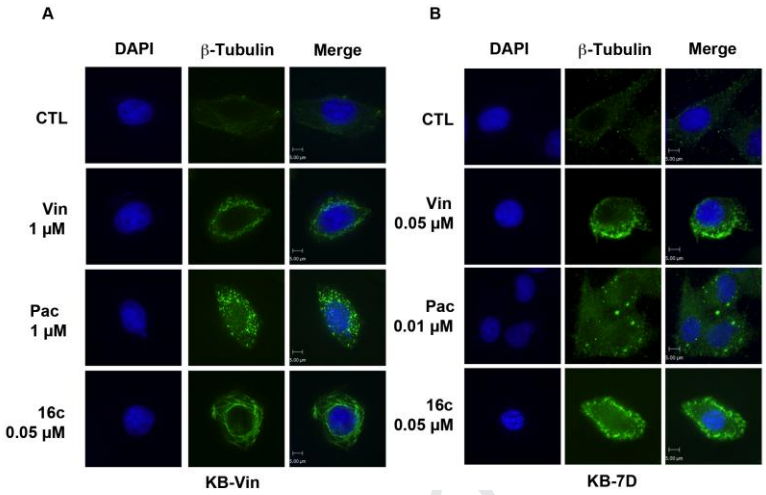
Please cite this article as: W.-C. Wu, Y.-M. Liu, M.-H. Lin, Y.-H. Liao, M.-J. Lai, H.-Y. Chuang, T.-Y. Hung, C.-H. Chen, J.-P. Liou, Design, synthesis, and evaluation of N-phenyl-4-(2-phenylsulfonamido)-benzamides as microtubule-targeting agents in drug-resistant cancer cells, displaying HDAC inhibitory response, *European Journal of Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.ejmech.2020.112158.

Graphical Abstract



16c

GI_{50} = 22 nM (KB-Vin)
GI_{50} = 12 nM (KB-7D)
IC_{50} = 1.07 uM (HDAC1), 1.47 uM (HDAC2), 2.27 uM (HDAC3)



Design, Synthesis, and Evaluation of N-phenyl-4-(2-phenylsulfonamido)-benzamides as Microtubule-targeting agents in Drug-resistant cancer cells, displaying HDAC inhibitory response

Wei-Cheng Wu,[a,1] Yi-Min Liu,[b,f,1] Mei-Hsiang Lin,[a] Yu-Hsuan Liao,[a] Mei-Jung Lai,[b] Hsun-Yueh Chuang,[a] To-Yu Hung,[a] Chun-Han Chen,[*c,e] Jing-Ping Liou[*,a,b,d]

[1] Contributed equally to this work.

* To whom correspondence should be addressed. For J. P. Liou: (Phone) 886-2-2736-1661 ext 6130; (e-mail) jpl@tmu.edu.tw. For C. H. Chen: (Phone) 886-2-27361661 ext 3195, (e-mail) brianchc@tmu.edu.tw

[a] *School of Pharmacy, College of Pharmacy, Taipei Medical University, Taiwan.*
[b] *TMU Biomedical Commercialization Center, Taipei Medical University, Taiwan.*
[c] *Department of Pharmacology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[d] *School of Pharmacy, National Defense Medical Center, Taipei, Taiwan.*
[e] *Cell Physiology and Molecular Image Research Center, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan.*
[f] *Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.*

Abstract

Microtubule-targeting agents (MTA) have enjoyed significant clinical success for decades. However, several mechanisms may cause inactivation of such drugs, leading to acquired resistance in patients treated with them. Therefore, drugs containing a stilbene-like skeleton and possessing dual inhibitory activity may provide a new and differentiated treatment for patients to overcome challenging acquired resistance. A new compound (**16c**) displays promising anticancer activity with GI_{50} of 22 ± 2 and 12 ± 0.1 nM in vincristine-resistant nasopharyngeal (KB-Vin) cancer cells and etoposide-resistant nasopharyngeal (KB-7D) cancer cells and is better than vincristine, etoposide, ABT-751, and MS-275. A mechanistic study revealed that **16c** interferes with the cell cycle distribution and induces cell cycle arrest at the G2/M phase and severe mitotic spindle defects followed by apoptosis. In addition, it produces much more significant cytotoxicity than vincristine and etoposide in the corresponding resistant cells, indicating that it may be a promising candidate to overcome drug resistance in cancer cells. Compound **16c** also displays inhibitory activity against HDAC 1 and HDAC 2 with IC_{50} values of 1.07 μM, and 1.47 μM, respectively. These findings may lead to a new type of structural motif for future development of drugs that could overcome acquired resistance to MTAs.

1. Introduction

Numerous templates and structures have been identified through drug discovery as potent inhibitors of tumor cells.[1] Combination of two different mechanisms by the use of suitable scaffolds or linkers in multi-target drug designs may be more efficacious than drugs that depend on a single mechanism.[2] Stilbene-based compounds, including (*E*)-stilbene (**1**), (*Z*)-stilbene (**2**) and dihydrostilbene (**3**) which occur widely in nature products, have attracted the interest of biologists and chemists.[3] Resveratrol (**4**), a natural trihydroxy stilbene which was isolated from *Veratrum grandiflorum*, has shown anti-inflammatory and anti-oxidative effects, and other promising pharmacological properties relevant to various diseases.[4] However, the major obstacle of resveratrol in translating its effects in patients was poor bioavailability due to its rapid metabolism.[5-6]

Histone deacetylases (HDAC) are drug targets and have been highly explored for decades[7] for their significant roles in different human diseases. SAHA (**5**, vorinostat), PXD101 (**6**, belinostat) and LBH-589 (**7**, panobinostat) all bearing a hydroxamic acid functional group, have been approved for use in the treatment of cutaneous T cell lymphoma, peripheral T cell lymphoma and multiple myeloma, respectively.[8] In addition, benzamide containing HDAC inhibitors, such as MS-275 (**8**,

Entinostat) and MGCD0103 (**9**, Mocetinostat), have also been entered into clinical trials for treatment of various cancers (Figure 1).[8]

Microtubules are protein polymers which play important roles in several cellular processes, such as cell division, cell migration and cell shape maintenance. Anti-mitotic agents, including taxans, vinca alkaloids, and colchicine (**10**), achieve potent activity against cancer cells by interrupting cell cycle progression.[9-10] However, drug resistance caused by different mechanisms has created a great amount of variation between individual patients. The best-known mechanism is P-glycoprotein (P-gp)-mediated multidrug resistance which provides an opportunity for tumor cells to escape cell death induced by chemotherapy.[11-12] In an effort to develop more structurally diverse inhibitors towards microtubules, many effective compounds from natural sources have been examined. Combretastatin A-4 (**11**, CA-4), isolated from the South African tree *Combretum caffrum*, contains a stilbene skeleton and three methoxy groups, binds to the colchicine-binding site and shows strong cytotoxicity against a variety of types of cancer (Figure 1). Similar to combretastatin, ABT-751 (**12**, E7010), an orally bioavailable sulfonamide, also binds to the same colchicine-binding site and shows a marked anticancer effect.[9,13] Notably, neither of these compounds is a transport-related substrate of membrane-bound P-glycoprotein, and this suggests that stilbene could be used as the linker to combine a benzamide with a sulfonamide to generate a series of compounds capable of inhibiting HDAC and microtubules as a therapeutic strategy in drug-resistant nasopharyngeal cancer cells (Figure 2). Our aim therefore, is to optimize an appropriate stilbene as a linker to combine multiple active binding

groups and generate a series of N-phenyl-4-(2-(phenylsulfonamido)phenethyl)benzamides and related substituted compounds as shown in Figure 3.

2. Results and Discussion

2.1 Chemistry

Scheme 1 shows the synthetic routes to compounds **13a-13d**. Commercially available nitrobenzaldehydes (**19a-19c**) underwent a Wittig reaction with (4-(methoxycarbonyl)benzyl)-triphenylphosphonium bromide to produce compounds **20a-20c**, which were then hydrogenated with a catalytic amount of 10% Pd/C to afford compounds **21a-21c**. These compounds were reacted with 4-methoxybenzensulfonyl chloride to produce **22a-22c** which were hydrolyzed by LiOH to afford **18a-18c**. The acid products were reacted with *o*-phenylenediamine to get target compounds **13a-13c**. Compound **23c** also reacted with NH_2OTHP to protect the amide group, and this product was hydrolyzed by 10% TFA, yielding the designed compound **13d**. The synthesis of compounds **14a-14g** is shown in Scheme 2. Various substituted benzenesulfonamide groups were introduced into compound **21c** to give compounds **24a-24g**. Compounds **24a-24g** were hydrolyzed by LiOH to afford acid intermediates **(25a-25g)** which were then reacted with *o*-phenylenediamine to get the target compounds **14a-14g**. In addition, compound **23c** was reacted

with various substituted anilines to produce the designed compounds **15a-15e**, which are shown in Scheme 3. Scheme 4 shows the synthesis of compound **16a** from compound **20c**. Compound **20c** was reduced by iron powder to get an amine product (**26**). Compound **16a** was produced from **26** using the similar reaction in Scheme 1.

Compounds **29a** and **29b** were synthesized from compounds **30a** and **30b** respectively, by a SN_2 reaction, reduction and imine reduction as shown in Scheme 6. The acid compounds **31a** and **31b** were produced by LiOH hydrolysis and then reacted with coupling reagents to give the designed compounds **16b** and **16c**, respectively. A biphenyl compound **16d** was synthesized from compound **32** using tetrakis(triphenylphosphine)palladium and phenylboronic acid under Suzuki coupling reaction conditions, as described in Scheme 6. The biphenyl carboxylate was treated using a method similar to that in Scheme 1 to obtain the desired target compound **16d**. The target compound **17** without a benzamide group was synthesized from compound **19c** as shown in Scheme 7. The target compound **18**, lacking a sulfonamide group, was produced from compound **39** as displayed in Scheme 8. Both of these two designed compounds were afforded by synthetic routes similar to those in Scheme 1.

2.2.1 *In vitro cell growth inhibitory activity*

The synthetic compounds (**13-18**) were evaluated for their antiproliferative activity against human nasopharyngeal cancer KB cells. Compound **17** and **18** were used to examine the importance of the effect of sulfonamide and benzamide functional groups on the cytotoxicity. We found compound **18** lacking the sulfonamide group had diminished antiproliferative activity in an SRB assay (Table 1), indicating that the sulfonamide group is required for anticancer activity against KB cells.

In order to investigate which of the HDAC inhibitors with various functional groups tends to increase the antiproliferative activity toward cancer cells, we introduced a hydroxamic acid and a benzamide group, giving compounds **13a-13d** and examined the anticancer activity in KB cells. As shown in Table 1, **13d** failed to show obvious growth inhibition activity in KB cells, but **13c** showed significant growth inhibitory activity in KB cells, suggesting that the benzamide group may offer an opportunity to improve the anticancer activity. In addition, the GI_{50} values of compounds **13a-13c** indicate the sulfonamide group at the *ortho* position of stilbene together with a benzamide may provide the most potent activity against KB cells. The substituted functional group of the sulfonamide, as in **13c** was also considered. As shown in Table 1, removal or translocation of the methoxy group

(**14a**, **14b**) or its replacement with electron-donating groups (**14c-14f**) resulted in decreased activity. Further, the substituted groups of the benzamide show that one fluorine atom at the 5'-position has an antiproliferative activity similar to that of **13c**.

Variations in the stilbene linker, including a methylene linkage, direct bonding or substitution (**16a** and **16d**) have been explored and heteroatoms have also been introduced (**16b** and **16c**). It was found **16a** and **16d** displayed decreased activity while **16c** exhibited the best antiproliferative activity of these compounds, indicating that nitrogen substitution of the linker has the most potent antiproliferative activity against the KB cells.

2.2.2 In vitro cell growth inhibitory activity in drug-resistant KB cells

We selected several compounds (**13c**, **15c**, **16b and 16c**) with significant antiproliferative activities against KB cells and tested their growth inhibitory activity in vincristine-resistant (KB-vin) and etoposide-resistant (KB-7D) KB cancer cell lines. As shown in Table 2, the GI_{50} values of **12** (ABT751) dramatically increased in KB-vin cells while **8** shows a similar cell growth inhibitory activity compared to the parent KB cells shown in Table 1. The selected compounds (**13c**, **15c**, **16b** and **16c**) also exhibit similar or better potency in inhibition of cell growth in two drug-resistant cancer cells. In a clear result for instance, **15c** shows improved anticancer activity in both KB-Vin and KB-7D cells compared to KB cells. Among the four compounds tested, **16c** exhibits the most potent cell growth inhibitory activity in KB-Vin and KB-7D cells.

2.2.3 Inhibition of HDAC isoforms

In an attempt to evaluate the influence of compounds **13c**, **15c**, **16b** and **16c** on HDAC isoforms, these compounds and the reference compound **8** (MS-275) were assayed for inhibitory activity against HDAC class I isoforms (Table 3). We found all the synthesized compounds showed no inhibitory activity against HDAC 8 while compounds **13c**, **15c**, and **16b** selectively inhibit the activity of HDAC 3. Compound **16c** displays strong inhibitory activity with IC_{50} values of 1.07 μM, and 1.47 μM against HDAC 1 and 2 respectively.

2.2.4 Cell death evaluation in drug-resistant cells

Based on the results in Table 1-3, we selected three compounds (**13c**, **15c**, and **16c**) to examine multiple apoptosis markers in KB-vin and KB-7D cells. As shown in Figure 4, low concentrations of **16c** (0.01 μM) induce significant activation of caspase-3, -8, -9, and PARP in KB-vin cells. In addition, **16c** shows better potency than **15c** and **13c** in activation of apoptotic death markers, indicating **16c** may be a candidate to overcome resistance to vincristine.

2.2.5 Evaluation of cell cycle progression and cell death response in drug-resistant KB cells

We performed flow cytometry and western blot analysis to investigate the effect of **16c** on cell cycle progression and cell death in comparison with **12** (ABT-751), **8** (MS-275), and vincristine or etoposide in KB-vin and KB-7D cells. Compound **16c** failed to generate much more significant cellular subG1 phase accumulation and severe apoptosis than the other drugs in both cell lines after 24 h treatment

(Figure 5A-5D). Although treatment with 0.5 μM **16c** induces more significant subG1 elevation than vincristine (Figure 5A), the activated expression levels of caspase-3, -8 and -9, PARP, and γH2AX were almost identical in vincristine-treated cells (Figure 5C). In addition, the expression of γH2AX was increased significantly in response to **16c** and reference compounds (**8**, **12**, and etoposide) in KB-7D cells (Figure 5D). These results show that there was not much difference between **16c** and those reference compounds. However, **16c** leads to more dramatic apoptotic cell death than the other compounds after 48 h treatment (Figure 6A-6D). It is notable that treatment with 0.05 μM **16c** induces a much more significant subG1 population of cells (Figure 6A) and cell apoptotic biomarkers in KB-vin cells (Figure 6C) than 0.1 μM vincristine. A similar effect can also be detected in KB-7D cells (Figures 6B, 6D). These results confirm that **16c** can effectively overcome resistance in both of vincristine- and etoposide-resistant KB cells.

2.2.6 Compound 16c influences microtubule dynamics in cells

Paclitaxel and vincristine have been recognized as mitotic arrest inducers which induce stabilization or destabilization of microtubules, respectively. We found that treatment with **16c** triggers obvious G2/M (mitotic) arrest between 18-24 h time points in KB-Vin and KB-7D cells (Figures 7A, 7B). In addition, we observed using deconvolution fluorescence microscopy that paclitaxel-induced aggregation of microtubule (round dots) and vincristine-induced abnormal spindles (reticular structure) spread in cells after 24 h treatment. A similar effect of **16c** and vincristine on tubulin polarization change was detected (Figures 8A and 8B), suggesting **16c** may have the same effect as vincristine on disruption of

microtubule dynamics. Further, we carried out intracellular tubulin polymerization assay to directly determine the potency of inhibitory activity of three compounds (paclitaxel, vincristine, and **16c**) on blocking dynamics of tubulins in KB-Vin and KB-7D cells. As shown in Figure 9A and 9B, paclitaxel showed significant induction of polymerized tubulin in cells. Compound **16c** shows significant inhibition of tubulin polymerization in KB-Vin and KB-7D cells. Notably, significant decrease of the polymerized tubulin (P) was detected in cells treated with 0.1 μM of **16c**, indicating compound **16c** is much more potent than vincristine in both cell lines. Taken together, our results demonstrated a consistent outcome by using two different experiments to confirm **16c** shows promising activity in inhibiting tubulin polymerization in cells.

3. Conclusion

In this paper we have described the synthesis of a series of N-phenyl-4-(2-(phenylsulfonamido)phen-ethyl)benzamides (**13-18**). Of all the synthesized compounds, compound **16c** was identified as a potent tubulin inhibitor demonstrating much better cellular cytotoxicity than reference compounds **8** or **12** with a mean GI_{50} value against the KB cell line of 10 nM. In addition, compound **16c** also demonstrated over 100- and 40-fold antitumor efficacy against KB-Vin and KB-7D cancer cells with mean GI_{50} values of 22 nM and 12 nM respectively. It also displayed selective suppression of the function of class I HDAC isoforms. Although **16c** did not exhibit remarkable inhibitory activity against HDACs, its overcoming drug resistance in KB-Vin and KB-7D cells may be caused by the presence of the benzamide functional group. This study provides a

lead compound for development of novel tubulin inhibitors with HDAC inhibition and may provide a new strategy for the treatment of drug-resistance cancer cells.

4.1 Chemistry

Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were obtained with a Bruker DRX-500 spectrometer operating at 500 and 125 MHz and Bruker Fourier 300 and 75MHz. Chemical shifts are reported in parts per million (ppm, δ) downfield from TMS as an internal standard. High-resolution mass spectra (HRMS) were measured with an AB SCIE X (QSTAR® XL) High Resolution Electrospray (ESI) Mass Spectrometry spectrometer. Melting points were measured with Buchi B-545 (Büchi, Switzerland). Purity of the final compounds was achieved with a Hitachi 2000 series HPLC system using C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 µm. 4.6 mm × 150 mm). Flash column chromatography used silica gel: SILICYCLE (SilicaFlash Irregular Silica Gel P60, 40 - 63 µm, 60 Å (R12030B)

N-(2-Aminophenyl)-4-(4-((4-methoxyphenyl)sulfonamido)phenethyl)benzamide (13a)

A mixture of **23a** (0.97 mmole), N,N,N',N'-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, 1.26 mmole), N,N-diisopropylethylamine (DIPEA, 1.26 mmole), *o*-phenylenediamine (1.16 mmole) and DMF (2 mL) was stirred at rt overnight. The reaction was quenched with water and extracted with EtOAc. The residue was purified by flash chromatography over silica gel to afford compound **13a** in 56% yield. ^{1}H-NMR (500 MHz, DMSOd_6) δ (ppm): 2.78-2.80 (m, 2H), 2.85-2.87 (m, 2H), 3.77 (s, 3H), 4.85 (s, 2H), 6.58 (t, *J* = 7.5Hz, 1H), 6.76 (d, *J* = 7.5Hz, 1H), 6.93-6.97 (m 3H), 7.02-7.07 (m, 4H), 7.14 (d, *J* = 7.5Hz, 1H), 7.26 (d, *J* = 8Hz, 2H), 7.64 (d, *J* = 8.5Hz, 2H), 7.85 (d, *J* = 8Hz, 2H), 9.56 (s, 1H) 9.98 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ

(ppm): 36.43, 37.09, 56.08, 114.75, 116.61, 116.75, 120.80, 123.91, 126.87, 127.11, 128.19, 128.75, 129.31, 129.49, 131.77, 132.67, 136.25, 137.35, 143.57, 145.57, 162.78, 165.64. mp = 191.7–192.4°C. HRMS (ESI) for $C_{28}H_{28}N_3O_4S$ $[M+H]^+$: Calcd., 502.1795; Found, 502.1801.

N-(2-Aminophenyl)-4-(3-((4-methoxyphenyl)sulfonamido)phenethyl)benzamide (13b)

The title compound was obtained as a white solid in 54% yield from compound **23b** in a manner similar to that described for the preparation of **13a**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.822.88 (m, 4H), 3.77 (s, 3H), 4.86 (s, 2H), 6.59 (t, *J* = 7.5Hz, 1H), 6.78 (d, *J* = 8Hz, 1H) 6.83-6.88 (m, 2H), 6.95-6.99 (m, 2H), 7.04-7.10 (m, 3H), 7.16 (d, *J* = 8Hz, 1H), 7.26 (d, *J* = 8Hz, 2H), 7.67 (d, *J* = 8.5Hz, 2H), 7.86 (d, *J* = 8Hz, 2H), 9.58 (s, 1H), 10.01 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 36.85, 56.08, 114.76, 116.63, 116.76, 118.08, 120.38, 123.92, 124.56, 126.88, 127.12, 128.20, 128.73, 129.38, 131.73, 132.68, 138.47, 142.57, 143.58, 145.34, 162.83, 165.61. mp = 175.4–176.1°C. HRMS (ESI) for $C_{28}H_{28}N_3O_4S$ $[M+H]^+$: Calcd., 502.1795; Found, 502.1802.

N-(2-Aminophenyl)-4-(2-((4-methoxyphenyl)sulfonamido)phenethyl)benzamide (13c)

The title compound was obtained as a white solid in 55% yield from compound **23c** in a manner similar to that described for the preparation of **13a** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.72, 2.76 m, 2H), 2.79-2.81 (m, 2H), 3.79 (s, 3H), 4.85 (s, 2H), 6.60 (t, *J* = 7.5Hz, 1H), 6.78 (dd, *J* = 1, 8Hz, H), 6.89 (d, *J* = 7.5Hz, 1H), 6.96 (t, *J* = 7.5Hz, 1H), 7.05-7.11 (m, 3H), 7.13 (d, *J* = 7.5Hz, 1H), .22 (d, *J* = 6.5Hz, 1H), 7.27 (d, *J* = 8Hz, 1H), 7.61 (d, *J* = 9Hz, 2H), 7.89 (d, *J* = 7.5Hz, 2H), 9.49 s, 1H), 9.59 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 32.32, 35.72, 56.12, 114.78, 116.65, 116.82, 123.91, 126.92, 126.98, 127.08, 127.13, 128.20, 128.63, 129.26, 130.24, 132.65, 132.83, 135.10,

138.36, 134.56, 145.87, 162.77, 165.71. mp = 195.2-196.0°C HRMS (ESI) for $C_{28}H_{28}N_3O_4S$ $[M+H]^+$: Calcd., 502.1795; Found, 502.1803.

N-Hydroxy-4-(2-((4-methoxyphenyl)sulfonamido)phenethyl)benzamide (13d)

A mixture of **18c** (1.13 mmole), HBTU (1.47 mmole), DIPEA (1.47 mmole) and DMF(3 mL) was stirred briefly then *o*-(tetrahydro-2H-pyran-2-yl)hydroxylamine (1.36 mmole) was added at rt and the mixture was stirred overnight. The residue was purified by flash column over silica gel to afford an oily product which was dissolved in MeOH (4 mL) and 10% $TFA_{(aq)}$ (4 mL) was added at rt. After stirring for 4 h, the mixture was purified by flash chromatography over silica gel to afford compound **13d** in 35% yield. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): .70-2.73 (m, 2H), 2.76-2.80 (m, 2H), 3.79 (s, 3H), 6.89 (dd, *J* = 1, 7.5Hz, 1H), 7.05-7.11 (m, 4H), .20-7.23 (m, 3H), 7.61 (d, *J* = 9Hz, 2H), 7.67 (d, *J* = 8Hz, 2H), 8.96 (s, 1H), 9.48 (s, 1H), 11.13 (s, 1H). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 32.27, 35.70, 56.11, 114.77, 126.95, 127.28, 128.67, 129.24, 130.18, 130.84, 132.90, 135.22, 138.26, 145.54, 162.74. mp = 164.4-164.9°C. HRMS (ESI) for $C_{22}H_{23}N_2O_5S$ $[M+H]^+$: Calcd., 427.1322; Found, 427.1328.

N-(2-Aminophenyl)-4-(2-(phenylsulfonamido)phenethyl)benzamide (14a)

The title compound was obtained as a solid in 60% yield from compound **25a** in a manner similar to that described for the preparation of **13a**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.72-2.79 (m, 4H), 4.88 (s, 2H), 6.61 (t, *J* = 7Hz, 1H), 6.78 (dd, *J* = 1, 8Hz, 1H), 6.88 (dd, *J* = 1, 8Hz, 1H), 6.95 (t, *J* = 7Hz, 1H), 7.08-7.10 (m, 1H), 7.12-7.16 (m, 2H), 7.23 (dd, *J* = 1, 7.5Hz, 1H), 7.27 (d, *J* = 8.5Hz, 2H), 7.54-7.70 (m, 5H), 7.89 (d, *J* = 8Hz, 2H), 9.60 (s, 1H), 9.70 (s, 1H). ^{13}C-NMR (125MHz, DMSO-d_6) δ

(ppm): 32.37, 35.76, 116.60, 116.74, 123.88, 126.90, 127.05, 127.15, 127.17, 128.24, 128.63, 128.96, 129.72, 130.32, 132.68, 133.21, 134.81, 138.48, 141.08, 143.61, 145.80, 165.65. mp = 158.7-159.4°C. HRMS (ESI) for $C_{27}H_{26}N_3O_3S$ $[M+H]^+$: Calcd., 472.1689; Found, 472.1696.

N-(2-Aminophenyl)-4-(2-((3-methoxyphenyl)sulfonamido)phenethyl)benzamide (14b)

The title compound was obtained as solid in 49% yield from compound **25b** in a manner similar to that described for the preparation of **13a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.72-2.79 (m, 4H), 3.73 (s, 3H), 4.87 (s, 2H), 6.61 (t, *J* = 7Hz, 1H), 6.78 (dd, *J* = 1, 8Hz, 1H), 6.93 (dd, *J* = 1.5, 7.5Hz, H), 6.95 (t, *J* = 7Hz, 1H), 7.11-7.19 (m, 5H), 7.21-7.28 (m, 4H), 7.46 (t, *J* = 8.5Hz, 1H), 7.90 (d, *J* = Hz, 2H), 9.58 (s, 1H), 9.67 (s, 1H). ^{13}C-NMR (125MHz, DMSO-d_6) δ (ppm): 32.34, 35.77, 55.99, 11.94, 116.63, 116.76, 119.09, 119.14, 123.93, 126.89, 127.05, 127.13, 127.18, 128.22, 128.59, 130.27, 130.90, 132.69, 134.85, 138.43, 142.32, 143.59, 145.81, 159.84, 165.64. mp = 169.1169.9°C. HRMS (ESI) for $C_{28}H_{28}N_3O_4S$ $[M+H]^+$: Calcd., 502.1795; Found, 502.1800.

N-(2-Aminophenyl)-4-(2-((4-fluorophenyl)sulfonamido)phenethyl)benzamide (14c)

The title compound was obtained as solid in 52% yield from compound **25c** in a manner similar to that described for the preparation of **13a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.77-2.81 (m, 4H), 4.86 (s, 2H), 6.60 (t, *J* = 7.5Hz, 1H), 6.78 (d, *J* = 8Hz, 1H), 6.85 (d, *J* = 7.5Hz, 1H), 6.96 (t, *J* = 7.5Hz, 1H), 7.09 (t, *J* = 7Hz, 1H), 7.14-7.17 (m, 2H), 7.25 (d, *J* = 7.5Hz, 1H), 7.28 (d, *J* = 7.5Hz, 2H), 7.40 (t, *J* = 8.5Hz, 2H), 7.73-7.76 (m, 2H), 7.90 (d, *J* = 8Hz, 2H), 9.59 (s, 1H), 9.72 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 32.39, 35.78, 116.65, 116.78, 116.81, 116.96, 123.91, 126.92, 127.12, 127.31, 127.27, 128.24, 128.61, 130.09, 130.17, 130.38, 132.70, 134.66, 137.44, 138.70, 143.57,

145.79, 163.71, 165.70. mp = 178.9-179.6°C. HRMS (ESI) for $C_{27}H_{25}FN_3O_3S$ $[M+H]^+$: Calcd., 490.1595; Found, 490.1603.

N-(2-Aminophenyl)-4-(2-((4-chlorophenyl)sulfonamido)phenethyl)benzamide (14d)

The title compound was obtained as solid in 47% yield from compound **25d** in a manner similar to that described for the preparation of **13a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.77-2.79 (m, 4H), 4.86 (s, 2H), 6.60 (t, *J* = 7.5Hz, 1H), 6.78 (d, *J* = 8Hz, 1H), 6.86 (d, *J* = 8Hz, 1H), 6.96 (t, *J* = 7.5Hz, H), 7.10 (t, *J* = 7.5Hz, 1H), 7.14-7.16 (m, 2H), 7.25-7.28 (m, 3H), 7.64 (d, *J* = 8.5Hz, 2H), 7.68 (d, *J* = 8.5Hz, 2H), 7.89 (d, *J* = 8Hz, 2H), 9.59 (s, 1H), 9.79 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm): 32.38, 35.74, 116.65, 116.81, 123.91, 126.92, 127.13, 127.15, 127.33, 127.40, 128.25, 128.60, 129.02, 129.88, 130.36, 132.70, 134.59, 137.52, 138.06, 138.69, 139.98, 143.57, 145.77, 165.69. mp = 198.3-199.0°C. HRMS (ESI) for $C_{27}H_{25}ClN_3O_3S$ $[M+H]^+$: Calcd., 506.1300; Found, 506.1305.

N-(2-Aminophenyl)-4-(2-((4-bromophenyl)sulfonamido)phenethyl)benzamide (14e)

The title compound was obtained as a solid in 49% yield from compound **25e** in a manner similar to that described for the preparation of **13a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.69-2.81 (m, 4H), 4.87 (s, 2H), 6.59 (td, *J* = 1, 7.5Hz, 1H), 6.78 (dd, *J* = 1, 7.5Hz, 1H), 6.87 (dd, *J* = 1, 8Hz, 1H), 6.96 (td, *J* = 1.5, 8Hz, 1H), 7.11 (td, *J* = 2, 7.5Hz, 1H), 7.16 (t, *J* = 7.5Hz, 2H), 7.27 (t, *J* = 8Hz, 3H), 7.63 (dd, *J* = 2.5, 9.5Hz, 2H), 7.78 (dd, *J* = 1.5, 6.5Hz, 2H), 7.90 (d, *J* = 8Hz, 2H), 9.58 (s, 1H), 9.79 s, H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 31.25, 32.36, 36.25, 116.62, 116.76, 123.92, 126.88, 27.02, 127.11, 127.15, 127.33, 127.39, 128.26, 128.59, 129.10, 130.35, 132.73, 132.83, 134.58, 138.65,

140.42, 143.58, 145.75, 165.64. mp = 189.5-190.2°C. HRMS (ESI) for $C_{27}H_{25}BrN_3O_3S$ $[M+H]^+$: Calcd., 550.0795; Found, 550.0801.

N-(2-Aminophenyl)-4-(2-((4-cyanophenyl)sulfonamido)phenethyl)benzamide (14f)

The title compound was obtained as a solid in 57% yield from compound **25f** in a manner similar to that described for the preparation of **13a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.75-2.82 (m, 4H), 4.86 (s, 2H), 6.60 (t, *J* = 7.5Hz, 1H), 6.78 (d, *J* = 8Hz, 1H), 6.81 (d, *J* = 7.5Hz, 1H), 6.96 (t, *J* = 7.5Hz, 1H), 7.10 (t, *J* = 7Hz, 1H), 7.15-7.19 (m, 3H), 7.27 (d, *J* = 8.1Hz, 3H), 7.85 (d, *J* = 8.5Hz, 2H), 7.90 (d, *J* = 7.5Hz, 2H), 8.05 (d, *J* = 8.5Hz, 2H), 9.59 (s, 1H), 10.00 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 32.40, 36.26, 115.59, 116.63, 116.80, 118.12, 123.90, 126.93, 127.14, 127.25, 127.45, 127.67, 127.85, 128.27, 128.58, 130.47, 132.73, 133.95, 134.25, 138.91, 143.58, 145.18, 145.71, 165.68. mp = 132.6-133.4°C. HRMS (ESI) for $C_{28}H_{25}N_4O_3S$ $[M+H]^+$: Calcd., 497.1642; Found, 497.1648.

N-(2-Aminophenyl)-4-(2-((3,4-dimethoxyphenyl)sulfonamido)phenethyl)benzamide (14g)

The title compound was obtained as a white solid in 49% yield from compound **25g** in a manner similar to that described for the preparation of **13a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.722.80 (m, 4H), 3.68 (s, 3H), 3.79 (s, 3H), 4.87 (s, 2H), 6.60 (t, *J* = 7Hz, 1H), 6.78 (d, *J* = 7Hz, 1H), .92-6.98 (m, 2H), 7.02 (d, *J* = 8.4Hz, 1H), 7.09-7.17 (m, 4H), 7.24 (dd, *J* = 2.4, 8.7Hz, 2H), 7.27 (d, *J* = 8.4Hz, 2H), 7.89(d, *J* = 8.1Hz, 2H), 9.49 (s, 1H), 9.59 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 31.15, 32.34, 35.74, 58.07, 56.29, 109.83, 111.52, 116.63, 116.76, 120.80, 123.92, 126.74, 126.88,

126.97, 127.12, 128.20, 128.57, 130.15, 132.67, 138.31, 143.59, 145.87, 149.02, 152.51, 165.64. mp = 171.9-172.4°C. HRMS (ESI) for $C_{29}H_{30}N_3O_5S$ $[M+H]^+$: Calcd., 532.1901; Found, 532.1906.

4-(2-((4-Methoxyphenyl)sulfonamido)phenethyl)-N-phenylbenzamide (15a)

The title compound was obtained as a white solid in 41% yield from compound **23c** in a manner similar to that described for the preparation of **13a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.762.78 (m, 2H), 2.81-2.83 (m, 2H), 3.79 (s, 3H), 6.89 (dd, *J* = 1, 8Hz, 1H), 7.05-7.12 (m, 5H), 7.22 (dd, = 1, 7Hz, 1H), 7.30 (d, *J* = 8.5Hz, 2H), 7.34 (t, *J* = 8Hz, 2H), 7.62 (d, *J* = 9Hz, 2H), 7.77 (d, *J* = 8Hz, 2H), 7.87 (d, *J* = 8.5Hz, 2H), 9.49 (s, 1H), 10.14 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 32.29, 35.74, 56.12, 114.79, 120.82, 124.03, 126.99, 127.03, 127.09, 128.11, 128.71, 129.05, 129.28, 130.25, 132.80, 133.02, 135.04, 138.35, 139.71, 146.06, 162.79, 165.87. mp = 166.4167.3°C. HRMS (ESI) for $C_{28}H_{27}N_2O_4S$ $[M+H]^+$: Calcd., 487.1686 ; Found, 487.1693.

N-(2-Hydroxyphenyl)-4-(2-((4-methoxyphenyl)sulfonamido)phenethyl)benzamide (15b)

A mixture of **23c** (2.21 mmole), HBTU (2.87 mmole), DIPEA (2.87 mmole), 2-benzyloxyaniline (2.65 mmole) and DMF (4 mL) was stirred at rt overnight. The reaction was quenched with water and extracted by EtOAc. The residue was purified by flash chromatography over silica gel to afford an intermediate compound. Then a mixture of the intermediate compound, 10% palladium on carbon (0.4 g) in MeOH (40 mL) was stirred at rt under hydrogen overnight. The organic layer was filtrated and the residue was purified by flash chromatography over silica gel to afford compound **15b** in 32% yield**.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.76-2.81 m, 4H), 3.79 (s, 3H), 6.83 (t, *J* = 7.5Hz, 1H), 6.91 (t, *J* = 7.5Hz, 2H), 7.01-7.11 (m, 5H), 7.21 (d, *J* = .5Hz, 1H), 7.29 (d, *J* = 8Hz, 2H), 7.61 (d,

J = 9Hz, 2H), 7.68 (d, *J* = 8Hz, 1H), 7.88 (d, *J* = 8Hz, H), 9.45 (s, 1H), 9.74 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 32.27, 35.74, 56.11, 69.62, 79.15, 114.77, 116.51, 119.54, 124.42, 126.05, 126.46, 126.97, 127.96, 128.86, 129.24, 130.22, 132.38, 146.25, 149.68, 162.72, 165.63. mp = 175.2-175.9°C. HRMS (ESI) for $C_{28}H_{27}N_2O_5S$ $[M+H]^+$: Calcd., 503.1635; Found, 503.1642.

N-(2-Amino-5-fluorophenyl)-4-(2-((4-methoxyphenyl)sulfonamido)phenethyl)benzamide (15c)

The title compound was obtained as a white solid in 56% yield from compound **23c** in a manner similar to that described for the preparation of **13a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.742.77 (m, 2H), 2.79-2.81(m, 2H), 3.80 (s, 3H), 5.19 (s, 2H), 6.35 (td, *J* = 2.5, 8.5Hz, 1H), 6.54 (dd, *J* 3, 11Hz, 1H), 6.89 (dd, *J* = 1, 7.5Hz, 1H), 7.06 (d, *J* = 9Hz, 2H), 7.05-7.14 (m, 3H), 7.22 (d, *J* = .5Hz, 1H), 7.27 (d, *J* = 8Hz, 2H), 7.61 (d, *J* = 9Hz, 2H), 7.89 (d, *J* = 8Hz, 2H), 9.49 (s, 1H), 9.51 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 32.34, 35.73, 56.12, 101.85, 102.05, 102.43, 102.61, 14.79, 119.86, 126.97, 127.08, 128.23, 128.59, 128.93, 129.01, 129.26, 130.24, 132.56, 132.83, 138.36, 145.87, 145.96, 160.51, 162.41, 162.77, 165.93. mp = 216.5-217.3°C. HRMS (ESI) for $C_{28}H_{27}FN_3O_4S$ $[M+H]^+$: Calcd., 520.1701; Found, 520.1707.

N-(3-Aminophenyl)-4-(2-((4-methoxyphenyl)su lfonamido)phenethyl)benzamide (15d)

A mixture of **23c** (1.67 mmole), HBTU (2.17 mmole), DIPEA (2.17 mmole), *m*-phenylenediamine (2.00 mmole) and DMF (3 mL) was stirred at rt overnight. The reaction was quenched with water and extracted with EtOAc. The residue was purified by flash chromatography over silica gel to afford an intermediate compound. Then a mixture of the intermediate compound, 10% palladium on carbon (0.5 g) in MeOH (50 mL) was stirred at rt under hydrogen overnight. The organic layer was filtrated and

the residue was purified by flash chromatography over silica gel to afford compound **15d** in 45% yield. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.74-2.76 (m, 2H), 2.79-2.81 (m, 2H), 3.78 (s, 3H), 5.03 (s, 2H), 6.30 (dt, *J* = 2, 8Hz, 1H), 6.84, (d, *J* = 8Hz, 1H), 6.88 (dd, *J* = 1.5, 8Hz, 1H), 6.94 (t, *J* = 8Hz, 1H), 7.04-7.11 (m, 5H), 7.21 (dd, *J* = 1.5, 7.5Hz, H), 7.27 (d, *J* = 8Hz, 2H), 7.61 (dd, *J* = 2, 7Hz, 2H), 7.83 (d, *J* = 8Hz, 2H), 9.83 (s, 1H). ^{13}C-NMR 125 MHz, DMSO-d_6) δ (ppm): 32.32, 35.71, 56.11, 106.66, 108.94, 110.21, 114.77, 126.97, 128.04, 128.62, 129.25, 130.21, 133.30, 138.30, 140.22, 145.81, 149.33, 162.74, 165.63. mp 210.3-210.9°C. HRMS (ESI) for $C_{28}H_{28}N_3O_4S$ $[M+H]^+$: Calcd., 502.1795; Found, 502.1801.

N-(4-Aminophenyl)-4-(2-((4-methoxyphenyl)sulfonamido)phenethyl)benzamide (15e)

The title compound was obtained as a white solid in 42% yield from compound **23c** in a manner similar to that described for the preparation of **13a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.732.75 (m, 2H), 2.78-2.81 (m, 2H), 3.79 (s, 3H), 4.88 (s, 2H), 6.53 (d, *J* = 9Hz, 2H), 6.89 (dd, *J* = 1, 7.5Hz, 1H), 7.06 (dd, *J* = 2, 7Hz, 2H), 7.07-7.11 (m, 2H), 7.21 (dd, *J* = 1.5, 7Hz, 1H), 7.25 (d, *J* = 8Hz, 2H), 7.35 (d, *J* = 8.5Hz, 2H), 7.61 (dd, *J* = 1.5, 7Hz, 2H), 7.83 (d, *J* = 8Hz, 2H), 9.49 (s, 1H), 9.77 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 32.30, 35.71, 56.11, 79.17, 79.43, 79.70, 114.15, 114.78, 122.70, 126.90, 127.86, 128.59, 128.65, 129.25, 130.23, 132.82, 133.36, 135.07, 138.33, 145.51, 145.59, 162.77, 165.00. mp = 184.9-185.5°C. HRMS (ESI) for $C_{28}H_{28}N_3O_4S$ $[M+H]^+$: Calcd., 502.1795; Found, 502.1805.

(E)-N-(2-Aminophenyl)-4-(2-((4-methoxyphenyl)sulfonamido)styryl)benzamide (16a)

The title compound was obtained as a white solid in 48% yield from compound **28** in a manner similar to that described for the preparation of **13a**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 3.63 (s, 3H), 4.89 (s, 2H), 6.61 (t, *J* = 7.5Hz, 1H), 6.79 (d, *J* = 8Hz, 1H), 6.91 (d, *J* = 8.5Hz, 2H), 6.96 (t, *J* = 7Hz, 1H), 7.00 (d, *J* = 8.5Hz, 2H), 7.15 (dd, *J* = 2.5, 6.5Hz, 1H), 7.18 (d, *J* = 7.5Hz, 1H), 7.24 (t, *J* = 4Hz, 2H), 7.27 (d, *J* = 16.5Hz, 1H), 7.52 (d, *J* = 1.5Hz, 2H), 7.54 (d, *J* = 1.5Hz, 2H), 7.70 (t, *J* = 9.5Hz, 1H), 7.99 (d, *J* = 8Hz, 2H), 9.66 (s, 1H), 9.76 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 55.90, 114.69, 116.64, 116.78, 123.83, 125.96, 126.07, 126.72, 126.98, 127.19, 128.56, 128.79, 128.88, 129.13, 133.44, 133.75, 140.54, 143.66, 162.70, 165.37. mp = 210.7-211.4°C. HRMS (ESI) for $C_{28}H_{26}N_3O_4S$ $[M+H]^+$: Calcd., 500.1639; Found, 500.1645.

N-(2-Aminophenyl)-4-((2-((4-methoxyphenyl)sulfonamido)phenoxy)methyl)benzamide (16b)

The title compound was obtained as a white solid in 54% yield from compound **31a** in a manner similar to that described for the preparation of **13a**. ^{1}H-NMR (500MHz, DMSO-d_6): 3.74 (s, 3H), 4.87 s, 2H), 5.00 (s, 2H), 6.60 (t, *J* = 7.5Hz, 1H), 6.78 (d, *J* = 8Hz, 1H), 6.86 (t, *J* = 7.5Hz, 1H), 6.90-6.92 m, 3H), 6.97 (t, *J* = 9Hz, 1H), 7.05 (t, *J* = 7.5Hz, 1H), 7.15 (d, *J* = 7.5Hz, 1H), 7.25 (d, *J* = 8Hz, H), 7.41 (d, *J* = 8Hz, 2H), 7.59 (d, *J* = 6Hz, 2H), 7.92 (d, *J* = 8Hz, 2H), 9.38 (s, 1H), 9.65 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 56.00, 69.21, 113.42, 114.42, 116.62, 116.78, 121.15, 123.75, 125.85, 126.80, 127.02, 127.23, 128.12, 129.25, 132.76, 134.23, 139.60, 140.79, 143.64, 151.41, 162.64, 165.57. mp = 144.6-145.4°C. HRMS (ESI) for $C_{27}H_{26}N_3O_5S$ $[M+H]^+$: Calcd., 504.1588; Found, 504.1593.

N-(2-Aminophenyl)-4-(((2-((4-methoxyphenyl)sulfonamido)phenyl)amino)methyl)benzamide (16c)

The title compound was obtained as a solid in 57% yield from compound **31b** in a manner similar to that described for the preparation of **13a**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 3.81 (s, 3H), 4.32 (s, 2H), 4.86 (s, 2H), 5.76 (s, 1H), 6.36-6.41 (m, 2H), 6.59 (t, *J* = 7.5Hz, 1H), 6.65 (d, *J* = 7Hz, 1H), .77 (d, *J* = 7Hz, 1H), 6.89 (t, *J* = 7.5Hz, 1H), 6.96 (td, *J* = 1, 7.5Hz, 1H), 7.06 (d, *J* = 9Hz, 2H), 7.15 d, *J* = 7.5Hz, 1H), 7.32 (d, *J* = 8Hz, 2H), 7.36 (d, *J* = 9Hz, 2H), 7.88 (d, *J* = 8Hz, 2H), 9.20 (s, 1H), 9.58 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 46.42, 56.10, 111.57, 114.62, 116.09, 116.59, 116.75, 121.76, 123.84, 126.91, 127.10, 127.20, 127.83, 128.20, 128.25, 129.59, 132.27, 133.59, 143.56, 143.88, 144.57, 162.83, 165.67. mp = 206.8-207.4°C. HRMS (ESI) for $C_{27}H_{27}N_4O_4S$ $[M+H]^+$: Calcd., 503.1748; Found, 503.1752.

N-(2-Aminophenyl)-2'-((4-methoxyphenyl)sulfonamido)-[1,1'-biphenyl]-4-carboxamide (16d)

The title compound was obtained as a solid in 49% yield from compound **36** in a manner similar to that described for the preparation of **13a**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 3.81 (s, 3H), 4.90 (s, 2H), 6.62 (t, J = 7.5Hz, 1H), 6.80 (d, *J* = 8Hz, 1H), 6.96-7.00 (m, 3H), 7.06 (t, *J* = 5Hz, 1H), 7.19 (d, *J* = 7.5Hz, 1H), 7.29-7.30 (m, 3H), 7.35 (d, *J* = 8.5Hz, 2H), 7.47 (d, *J* = 9Hz, 2H), 7.95 (d, *J* = 8Hz, 2H), 9.42 (s, 1H), 9.71 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 56.07, 114.64, 116.67, 116.81, 123.85, 127.01, 127.24, 127.53, 127.92, 128.92, 129.09, 129.59, 132.77, 133.45, 133.99, 138.51, 142.29, 143.67, 162.65, 165.55. mp = 71.9-72.4°C. HRMS (ESI) for $C_{26}H_{24}N_3O_4S$ $[M+H]^+$: Calcd., 474.1482; Found, 474.1490.

4-Methoxy-N-(2-phenethylphenyl)benzenesulfonamide (17)

A mixture of **38** (2.00 mmole), 4-methoxybenzenesulfonyl chloride (2.20 mmole) and pyridine (4 mL) was stirred at rt overnight. The reaction was quenched with water and extracted with CH_2Cl_2. The residue was purified by flash chromatography over silica gel to afford compound **17** in 61% yield. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.64-2.68 (m, 2H), 2.76-2.79 (m, 2H), 3.79 (s, 3H), 6.88 (d, *J* = 1, 8Hz, 1H), 7.04-7.21 (m, 8H), 7.25-7.28 (m, 2H), 7.61 (dd, *J* = .5, 7Hz, 2H), 9.46 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 32.63, 35.95, 56.10, 114.77, 126.25, 126.87, 126.95, 127.00, 128.64, 128.71, 129.25, 130.17, 132.88, 135.05, 138.60, 142.15, 162.76. mp = 131.9-132.3°C. HRMS (ESI) for $C_{21}H_{22}NO_3S$ $[M+H]^+$: Calcd., 368.1315; Found, 368.1321.

N-(2-Aminophenyl)-4-phenethylbenzamide (18)

The title compound was obtained as a white solid in 54% yield from compound **39** in a manner similar to that described for the preparation of **13a**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 2.912.96 (m, 4H), 4.84 (s, 2H), 6.59 (t, *J* = 7.5Hz, 1H), 6.77 (d, *J* = 7.5Hz, 1H), 6.95 (td, *J* = 0.5, 8Hz, H), 7.13-7.18 (m, 2H), 7.22-7.28 (m, 4H), 7.33 (d, *J* = 8Hz, 2H), 7.87 (d, *J* = 8Hz, 2H), 9.58 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 37.12, 37.20, 116.64, 116.80, 123.91, 126.36, 126.90, 127.12, 128.22, 128.72, 128.79, 128.88, 132.65, 141.68, 143.56, 145,68, 165.69. mp = 185.1-185.7°C. HRMS (ESI) for $C_{21}H_{21}N_2O$ $[M+H]^+$: Calcd., 317.1648; Found, 317.1655.

Methyl-4-(4-nitrostyryl)benzoate (20a)

A mixture of **19a** (16.5 mmole), (4-(methoxycarbonyl)benzyl)triphenylphosphonium bromide (16.5 mmole), NaOH (21.5 mmole) and THF (40 mL) was stirred at rt overnight. The reaction was

quenched with water and extracted with CH_2Cl_2. The residue was purified by flash chromatography over silica gel to afford compound **20a** in 67% yield. ^{1}H-NMR (300 MHz, DMSOd_6) δ (ppm): 3.86 (s, 3H), 7.52 (s, 1H), 7.60 (s, 1H), 7.81 (d, *J* = 8.4Hz, 2H), 7.91 (d, *J* = 9Hz, 2H), 7.99 (d, *J* = 8.4Hz, 2H), 8.25 (d, *J* = 9Hz, 2H).

Methyl-4-(3-nitrostyryl)benzoate (20b)

The title compound was obtained as a white solid in 54% yield from compound **19b** in a manner similar to that described for the preparation of **20a**. ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 3.89 (s, 3H), 6.82 (d, *J* = 12.3Hz, 1H), 6.88 (d, *J* = 12.3Hz, 1H), 7.31 (d, *J* = 8.4Hz, 2H), 7.48 (d, *J* = 7.8Hz, 1H), 7.57 (d, *J* = 7.8Hz, 1H), 7.90 (d, *J* = 4.8Hz, 2H), 8.04-8.06 (m, 2H).

Methyl-4-(2-nitrostyryl)benzoate (20c)

The title compound was obtained as a white solid in 56% yield from compound **19c** in a manner similar to that described for the preparation of **20a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.86 (s, 3H), 6.78 (d, *J* = 12Hz, 1H), 7.00 (d, *J* = 12Hz, 2H), 7.10 (d, *J* = 8.1Hz, 2H), 7.17-7.20 (m, 1H), 7.35-7.44 (m, 2H), 7.82 (dd, *J* = 1.5, 6.6Hz, 2H), 8.09-8.12 (m, 1H).

Methyl 4-(4-aminophenethyl)benzoate (21a)

A mixture of methyl-4-(4-nitrostyryl)benzoate (12.4 mmole), 10% palladium on carbon (0.5 g) in MeOH (50 mL) was stirred at rt under hydrogen overnight. The organic layer was filtered and the residue was purified by flash chromatography over silica gel to afford compound **0a** in 88% yield. ^{1}H-NMR (300 MHz, DMSO-d_6)) δ (ppm): 2.68-2.71 (m, 2H), 2.81-2.84 (m, 2H), 3.81 (s, 3H), 4.79 (s, 2H), 6.43 (d, *J* = 8.4Hz, 2H), 6.82 (d, *J* = 8.4Hz, 2H), 7.31 (d, *J* = 8.4Hz, 2H), 7.83 (d, *J* = 8.4Hz, 2H).

The title compound was obtained as solid in 81% yield from compound **20b** in a manner similar to that described for the preparation of **21a**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.69-2.74 (m, 2H), .86-2.92 (m, 2H), 3.82 (s, 3H), 4.90 (s, 2H), 6.34-6.41 (m, 3H), 6.88 (t, *J* = 7.5Hz, 1H), 7.35 (d, *J* = 8.4Hz, 2H), 7.85 (dd, *J* = 1.8, 6.6Hz, 2H).

Methyl 4-(2-aminophenethyl)benzoate (21c)

The title compound was obtained as a white solid in 77% yield from compound **20c** in a manner similar to that described for the preparation of **21a**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.682.74 (m, 2H), 2.84-2.89 (m, 2H), 3.82 (s, 3H), 4.87 (s, 2H), 6.44 (td, *J* = 1.2, 7.2Hz, 1H), 6.60 (dd, *J* = 0.9, 8.1Hz, 1H), 6.84-6.89 (m, 2H), 7.41 (d, *J* = 8.1Hz, 2H), 7.85 (d, *J* = 8.1Hz, 2H).

Methyl 4-(4-((4-methoxyphenyl)sulfonamido)phenethyl)benzoate (22a)

The title compound was obtained as a solid in 82% yield from compound **21a** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.75-2.78 (m, 2H), 2.83-2.85 (m, 2H), 3.77 (s, 3H), 3.81 (s, 3H), 6.92 (d, *J* = 8.4Hz, 2H), 7.01 (d, *J* = 9.3Hz, 4H), 7.25 (d, *J* = 8.4Hz, 2H), 7.62 (d, *J* = 9Hz, 2H), 7.80 (d, *J* = 8.4Hz, 2H).

Methyl 4-(3-((4-methoxyphenyl)sulfonamido)phenethyl)benzoate (22b)

The title compound was obtained as a solid in 88% yield from compound **21b** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.79-2.84 (m, 4H), .77 (s, 3H), 3.82 (s, 3H), 6.80 (d, *J* = 7.5Hz, 1H), 6.85 (dd, *J* = 2.1, 7.2Hz, 1H), 6.94 (t, *J* = 1.8Hz,

1H), 7.01-7.09 (m, 3H), 7.24 (d, *J* = 8.4Hz, 2H), 7.64 (d, *J* = 9Hz, 2H), 7.81 (d, *J* = 9.3Hz, 2H), 10.04 (s, 1H).

Methyl 4-(2-((4-methoxyphenyl)sulfonamido)phenethyl)benzoate (22c)

The title compound was obtained as a solid in 91% yield from compound **21c** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 2.74-2.80(m, 4H), 3.83(s, 3H), 3.91(s, 3H), 5.99(s, 1H), 6.88(d, *J* = 8.7Hz, 2H), 7.08-7.13(m, 6H), 7.63(d, *J* = 9Hz, 2H), 7.93(d, *J* = 8.4Hz, 2H).

4-(4-((4-Methoxyphenyl)sulfonamido)phenethyl)benzoic acid (23a)

The mixture of **22a** (1.88mmole), 1N lithium hydroxide solution (4ml) and 1,4-dioxane (8 ml) was stirred at 40°C overnight. The reaction was concentrated and quenched with 3N hydrogen chloride solution and water. The water layer was filtered to obtain compound **23a** in 84% yield without further purification. ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.75-2.77 (m, 2H), 2.81-2.84 (m, 2H), 3.77 (s, 3H), 6.93 (d, *J* = 8.4Hz, 2H), 7.00-7.04 (m, 4H), 7.21 (d, *J* = 8.4Hz, 2H), 7.62 (d, *J* = 9.0Hz, 2H), 7.77 (d, *J* = 8.4Hz, 2H).

4-(3-((4-Methoxyphenyl)sulfonamido)phenethyl)benzoic acid (23b)

The title compound was obtained as a solid in 76% yield from compound **22b** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.80 (s, 4H), 3.77 (s, 3H), 6.79-6.87 (m, 2H), 6.95 (s, 1H), 7.01-7.09 (m, 3H), 7.17 (d, *J* = 8.1Hz, 2H), 7.64 (d, *J* = 8.7Hz, 2H), 7.78 (d, *J* = 8.1Hz, 2H).

4-(2-((4-Methoxyphenyl)sulfonamido)phenethyl)benzoic acid (23c)

The title compound was obtained as a solid in 89% yield from compound **23c** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 2.81-2.85 (m, 4H), .83 (s, 3H), 6.94 (d, *J* = 7.5Hz, 1H), 6.99 (dd, *J* = 2.1, 7.2Hz, 2H), 7.01-7.15 (m, 3H), 7.21 (d, *J* = 8.1Hz, 2H), 7.63 (d, *J* = 9Hz, 2H), 7.90 (d, *J* = 8.1Hz, 2H).

Methyl 4-(2-(phenylsulfonamido)phenethyl)benzoate (24a)

The title compound was obtained as a solid in 82% yield from compound **21c** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.73-2.74 (m, 4H), 3.82 (s, 3H), 6.89 (dd, *J* = 1.5, 7.2Hz, 1H), 7.05-7.12 (m, 5H), 7.22 (d, *J* = 6.9Hz, 1H), 7.29-7.37 (m, 4H), 7.61 (dd, *J* = 1.8, 6.6Hz, 2H), 7.77 (dd, *J* = 1.2, 8.7Hz, 2H), 7.87 (d, *J* = 8.4Hz, 2H), 9.50 (s, 1H).

Methyl 4-(2-((3-methoxyphenyl)sulfonamido)phenethyl)benzoate (24b)

The title compound was obtained as a solid in 86% yield from compound **21c** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, CDCl3) δ (ppm): 2.70-2.77 (m, 4H), 3.71 (s, 3H), 3.91 (s, 3H), 6.02 (s, 1H), 7.07-7.16 (m, 8H), 7.17-7.34 (m, 2H), 7.93 (d, *J* = 8.4Hz, 2H).

Methyl 4-(2-((4-fluorophenyl)sulfonamido)phenethyl)benzoate (24c)

The title compound was obtained as solid in 85% yield from compound **21c** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.77 (s, 4H), 3.83 (s, 3H), 6.84-6.87 (m, 1H), 7.07-7.10 (m, 2H), 7.18-7.22 (m, 1H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.37 (t, *J* = 8.7Hz, 2H), 7.70-7.75 (m, 2H), 7.87 (d, *J* = 8.4Hz, 2H).

Methyl 4-(2-((4-chlorophenyl)sulfonamido)phenethyl)benzoate (24d)

The title compound was obtained as solid in 89% yield from compound **21c** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 2.74-2.82 (m, 4H), 3.91 (s, 3H), 6.04 (s, 1H), 7.08-7.15 (m, 6H), 7.40 (d, *J* = 8.7Hz, 2H), 7.62 (d, *J* = 8.7Hz, 2H), 7.94 (d, *J* = 8.4Hz, 2H).

Methyl 4-(2-((4-bromophenyl)sulfonamido)phenethyl)benzoate (24e)

The title compound was obtained as a solid in 88% yield from compound **21c** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz $CDCl_3$) δ (ppm): 2.74-2.82 (m, 4H), 3.92 (s, 3H), 5.95 (s,1H), 7.08-7.16 (m, 6H), 7.56-7.60 (m, 4H), 7.95 (d, *J* = 8.4Hz, 2H).

Methyl 4-(2-((4-cyanophenyl)sulfonamido)phenethyl)benzoate (24f)

The title compound was obtained as a solid in 77% yield from compound **21c** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 2.75-2.84 (m, 4H), 3.91 (s, 3H), 6.99 (d, *J* = 7.8Hz, 1H), 7.08-7.20 (m, 5H), 7.76 (dd, *J* = 1.2, 8.1Hz, 4H), 7.94 (d, *J* = 8.1Hz, 2H).

Methyl 4-(2-((3,4-dimethoxyphenyl)sulfonamido)phenethyl)benzoate (24g)

The title compound was obtained as solid in 84% yield from compound **21c** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.74 (s, 4H), 3.65 (s, 3H), 3.78 (s, 3H), 3.84 (s, 3H), 6.93-6.96 (m, 1H), 7.03-7.10 (m, 3H), 7.15-7.23 (m, 3H), 7.28 (d, *J* = 8.4Hz, 2H), 7.86 (d, *J* = 8.1Hz, 2H).

4-(2-(Phenylsulfonamido)phenethyl)benzoic acid (25a)

The title compound was obtained as a solid in 73% yield from compound **24a** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.68-2.78 (m, 4H), 6.86-6.89 (m, 1H), 7.05-7.15 (m, 2H), 7.19-7.26 (m, 3H), 7.54-7.60 (m, 2H), 7.63-7.72 (m, 2H), 7.84 (d, *J* = 8.1Hz, 2H), 9.67 (s, 1H), 12.77 (s, 1H).

4-(2-((3-Methoxyphenyl)sulfonamido)phenethyl)benzoic acid (25b)

The title compound was obtained as a solid in 89% yield from compound **24b** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.65-2.77 (m, 4H), 3.71 (s, 3H), 6.92 (d, *J* = 2.4Hz, 1H), 6.93-7.26 (m, 8H), 7.45 (td, *J* = 2.4, 8.1Hz, 1H), 7.84 (dd, *J* = 2.4, 8.1Hz, 2H).

4-(2-((4-Fluorophenyl)sulfonamido)phenethyl)benzoic acid (25c)

The title compound was obtained as solid in 84% yield from compound **24c** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.76 (s, 4H), 6.85 (dd, *J* = 1.5, 7.5Hz, 1H), 7.06-7.15 (m, 2H), 7.22 (dd, *J* = 2.4, 7.8Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.39 (t, *J* = 8.7Hz, 2H), 7.73 (dd, *J* = 5.1, 8.7Hz, 2H), 7.85 (d, *J* = 8.4Hz, 2H).

4-(2-((4-Chlorophenyl)sulfonamido)phenethyl)benzoic acid (25d)

The title compound was obtained as a solid in 82% yield from compound **24d** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.75 (s, 4H), 6.87 (dd, *J* = 1.5, 7.2Hz, 1H), 7.08-7.11 (m, 2H), 7.22-7.26 (m, 3H), 7.61-7.69 (m, 4H), 7.85 (d, *J* = 8.1Hz, 2H).

4-(2-((4-Bromophenyl)sulfonamido)phenethyl)benzoic acid (25e)

The title compound was obtained as a solid in 80% yield from compound **24e** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.70-2.80 (m, 4H), 6.87 (dd, *J* = 1.8, 7.5Hz, 1H), 7.07-7.17 (m, 2H), 7.22-7.25 (m, 3H), 7.59 (d, *J* = 8.4Hz, 2H), 7.77 (d, *J* = 8.7Hz, 2H), 7.85 (d, *J* = 8.4Hz, 2H), 9.78 (s, 1H).

4-(2-((4-Cyanophenyl)sulfonamido)phenethyl)benzoic acid (25f)

The title compound was obtained as a solid in 81% yield from compound **24f** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, MeOD) δ (ppm): 2.84-2.88 (m, 4H), 6.87 (dd, *J* = 7.8, 14.4Hz, 1H), 7.05-7.12 (m, 1H), 7.16-7.27 (m, 4H), 7.80 (d, *J* = 8.7Hz, 1H), 7.89-7.94 (m, 4H), 7.99 (d, *J* = 8.7Hz, 1H).

4-(2-((3,4-Dimethoxyphenyl)sulfonamido)phenethyl)benzoic acid (25g)

The title compound was obtained as a solid in 90% yield from compound **24g** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.72 (s, 4H), 3.65 (s, 3H), 3.77 (s, 3H), 6.92-6.95 (m, 1H), 7.03-7.15 (m, 4H), 7.19-7.25 (m, 4H), 7.83 (d, *J* = 8.4Hz, 2H).

***Methyl (E)-4-(2-aminostyryl)benzoate* (26)**

A mixture of **15c** (1.67mmole), iron powder (5.01mmole), ammonium chloride (3.34mmole), water (4ml) and isopropyl alcohol (16ml) was stirred reflux for 3hrs. The reaction was quenched with water and extracted by ethyl acetate. The residue was purified by flash chromatography over silica gel to afford **26** in 45% yield. ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 3.86 (s, 3H), 6.54 (td, *J* = 1.5, 7.8Hz, 1H), 6.67 (s, 2H), 6.77 (dd, *J* = 0.9, 8.1Hz, 1H), 6.90 (dd, *J* = 1.5, 7.8Hz, 1H), 7.03 (td, *J*

= 1.5, 8.7Hz, 1H), 7.31 (d, *J* = 8.7Hz, 2H), 7.80 (d, *J* = 8.7Hz, 2H).

***Methyl (E)-4-(2-((4-methoxyphenyl)sulfonamido)styryl)benzoate* (27)**

The title compound was obtained as solid in 85% yield from compound **26** in a manner similar to that describe for the preparation of **17.** ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.73 (s, 3H), 3.96 (s, 3H), 6.57 (d, *J* = 9Hz, 2H), 7.16-7.33 (m, 4H), 7.45-7.49 (m, 4H), 7.66-7.70 (m, 1H), 7.93 (d, *J* = 8.4Hz, 2H), 8.10 (s, 1H), 8.62 (d, *J* = 4.8Hz, 1H).

***(E)-4-(2-((4-methoxyphenyl)sulfonamido)styryl)benzoic acid* (28)**

The title compound was obtained as solid in 66% yield from compound **27** in a manner similar to that describe for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.66 (s, 3H), 6.58-6.70 (m, 2H), 6.92-7.02 (m, 4H), 7.15-7.19 (m, 2H), 7.61-7.71 (m, 4H), 8.01-8.12 (m, 2H), 9.61(s, 1H).

Methyl 4-((2-((4-methoxyphenyl)sulfonamido)phenoxy)methyl)benzoate (30a)

A mixture of **29a** (2 mmole), methyl 4-(bromomethyl)benzoate (2.06 mmole), K_2CO_3 (2.2 mmole) and DMF (4 mL) was stirred at rt overnight. The reaction was quenched with water and filtered with gravity to get a white solid. Then a mixture of the white solid, 10% palladium on carbon (0.5 g) in MeOH (50 mL) was stirred at rt under hydrogen overnight. Remove the palladium was removed and the residue and 4-methoxybenzenesulfonyl chloride (2.20 mmole) and pyridine (4 mL) was stirred at rt overnight. The reaction was quenched with water and extracted with CH_2Cl_2. The residue was purified by flash chromatography over silica gel to afford compound **30a** in 54% yield. ^{1}H-NMR (300 MHz, DMSOd_6) δ (ppm): 3.73 (s, 3H), 3.85 (s, 3H), 4.98 (s, 2H), 6.85-6.90 (m, 4H), 7.06 (t, *J* = 7.2Hz,

1H), 7.27 (d, *J* = 8.1Hz, 1H), 7.38 (d, *J* = 8.1Hz, 1H), 7.55 (d, *J* = 8.7Hz, 2H), 7.87 (d, *J* = 8.1Hz, 2H), 9.40 (s, 1H).

Methyl 4-(((2-((4-methoxyphenyl)sulfonamido)phenyl)amino)methyl)benzoate (30b)

A mixture of **29b** (2 mmole), 4-methoxybenzenesulfonyl chloride (2.20 mmole) and pyridine (4 mL) was stirred at rt overnight. The reaction was quenched with water and extracted with CH_2Cl_2. The organic layer was collected and dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield an oily intermediate. Then a mixture of the oily intermediate, 10% palladium on carbon (0.5 g) in MeOH (50 mL) was stirred at rt under hydrogen overnight. The palladium was removed and the organic layer was concentrated *in vacuo* to yield an orange solid. A mixture of orange solid, methyl 4-formylbenzoate (2.02 mmole), sodium cyanoborohydride (2.4 mmole) and MeOH (40 mL) was stirred at rt overnight. The reaction was filtered with gravity to afford compound **30b** in 74% yield. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 3.81 (s, 3H), 3.83 (s, 3H), 4.31 (s, 2H), 5.75 (s, 1H), 6.31 (d, *J* = 8Hz, 1H), 6.40 (d, *J* = 7.5Hz, 1H), 6.71 (d, *J* = 7.5Hz, 1H), 6.87 (t, *J* = 8Hz, 1H), 7.05 (d, *J* = 9Hz, 2H), 7.27 (d, *J* = 8Hz, 2H), 7.61 (d, *J* = 9Hz, 2H), 7.85 (d, *J* = 8Hz, 2H), 9.18 (s, 1H).

4-((2-((4-Methoxyphenyl)sulfonamido)phenoxy)methyl)benzoic acid (31a)

The title compound was obtained as a solid in 82% yield from compound **30a** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 3.73 (s, 3H), 4.98 (s, 2H), 6.86-6.91 (m, 4H), 7.06 (t, *J* = 7.5Hz, 1H), 7.27 (d, *J* = 8Hz, 1H), 7.36 (d, *J* = 8Hz, 2H), 7.56 (d, *J* = 9Hz, 2H), 7.88 (d, *J* = 8.5Hz, 2H), 9.38 (s, 1H).

4-(((2-((4-Methoxyphenyl)sulfonamido)phenyl)amino)methyl)benzoic acid (31b)

The title compound was obtained as a solid in 83% yield from compound **30b** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.80 (s, 3H), 4.25 (s, 2H), 6.32-6.41 (m, 2H), 6.73 (dd, *J* = 1.5, 7,8Hz, 1H), 6.83 (t, *J* = 7.5Hz, 1H), 7.02 (d, *J* = 8.7Hz, 2H), 7.18 (d, *J* = 8.1Hz, 2H), 7.61 (d, *J* = 9Hz, 2H), 7.80 (d, *J* = 8.4Hz, 2H).

Methyl 2'-nitro-[1,1'-biphenyl]-4-carboxylate (33)

A mixture of 1-bromo-2-nitrobenzene (2 mmole), tetrakis (0.2 mmole), 2N $K_2CO_{3(aq)}$ (6 mL) and (4-(methoxycarbonyl)phenyl)boronic acid (3 mmole) in EtOH (10 mL) and toluene (10 mL) was stirred at 105 °C overnight. The residue was purified by flash chromatography over silica gel to afford compound **33** in 65% yield. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 4.83 (s, 3H), 7.43 (d, *J* =8Hz, 2H), 7.52 (dd, *J* = 1.5, 8Hz, 1H), 7.62 (td, *J* = 1, 8Hz 1H), 7.74 (td, *J* = 1, 7.5Hz 1H), 7.96 (dd, *J* = 1, 8Hz, 1H), 8.08 (d, *J* = 8Hz, 2H).

Methyl 2'-amino-[1,1'-biphenyl]-4-carboxylate (34)

The title compound was obtained as a solid in 86% yield from compound **33** in a manner similar to that described for the preparation of **21a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.86 (s, 3H), 4.88 (s, 2H), 6.64 (td, *J* = 1.2, 7.2Hz, 1H), 6.77 (dd, *J* = 1.2, 8.1Hz, 1H), 7.01 (dd, *J* = 1.5, 7.5Hz, 1H), 7.06 (td, *J* = 1.8, 9Hz, 1H), 7.57 (d, *J* = 8.4Hz, 2H), 8.00 (d, *J* = 8.4Hz, 2H).

Methyl 2'-((4-methoxyphenyl)sulfonamido)-[1,1'-biphenyl]-4-carboxylate (35)

The title compound was obtained as a solid in 85% yield from compound **34** in a manner similar to that described for the preparation of **17.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.80 (s, 3H), 3.88 (s,

3H), 6.93 (d, *J* = 8.7Hz, 2H), 7.09-7.16 (m, 1H), 7.27-7.31 (m, 5H), 7.34 (d, *J* = 8.4Hz, 2H), 7.42 (d, *J* = 9Hz, 2H), 7.88 (d, *J* = 8.4Hz, 2H), 9.46 (s, 1H).

2'-((4-Methoxyphenyl)sulfonamido)-[1,1'-biphenyl]-4-carboxylic acid (36)

The title compound was obtained as a solid in 82% yield from compound **35** in a manner similar to that described for the preparation of **23a.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.80 (s, 3H), 6.94 (d, *J* = 9Hz, 2H), 7.07-7.10 (m, 1H), 7.25-7.32 (m, 5H), 7.43 (d, *J* = 9Hz, 2H), 7.87 (d, *J* = 8.4Hz, 2H), 9.46 (s, 1H).

1-Nitro-2-styrylbenzene (37)

The title compound was obtained as a solid in 76% yield from compound **19c** in a manner similar to that described for the preparation of **20a.** ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 6.77 (d, *J* = 12Hz, 1H), 6.90 (d, *J* = 12Hz, 1H), 7.03-7.07 (m, 2H), 7.15-7.17 (m, 3H), 7.27-7.28 (m, 1H), 7.37-7.40 (m, 2H), 8.07-8.10 (m, 2H).

2-Phenethylaniline (38)

The title compound was obtained as a solid in 66% yield from compound **37** in a manner similar to that described for the preparation of **21a.** ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.87-2.90 (m, 2H), 2.94-2.97 (m, 2H), 6.87-6.92 (m, 2H), 7.07-7.10 (m, 2H), 7.17-7.25 (m, 5H).

4.2.1 Cell lines and reagents

We thank Dr. Jang-Yang Chang (National Cheng Kung University, Taiwan) for generous gifts of KB, KB-vin, and KB-7D cells. Cells were maintained in 5% fetal bovine serum (FBS)-supplemented RPMI 1640 medium (GIBCO, Grand Island, NY, USA) and 1% penicillin–streptomycin (GIBCO) at 37 °C in a humidified incubator containing 5% CO_2. Antibodies against various proteins were obtained from the following sources: PARP (Poly-ADP-ribose polymerase) was obtained from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). Caspase 8, caspase 9, and γH2AX were obtained from Cell signaling (Danvers, MA, USA). GAPDH and β-Actin were from Millipore (Billerica, MA, USA). Caspase 3 and was obtained from Novous (Littleton, CO, USA). Anti-mouse and anti-rabbit IgGs were from Jackson ImmunoResearch Laboratories (West Grove, PA, USA).

4.2.2 HDAC Isoform Inhibition

HDAC Enzyme Inhibition Assays. Enzyme inhibition assays were conducted by the Reaction Biology Corporation (Malvern, PA, USA, www.reactionbiology.com). Compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 10 μM.

4.2.3 SRB (Sulforhodamine B) assay

Cells were seeded in 96-well plates and cultured overnight followed by the exposure to gradient concentrations of different compounds for 48 h. Briefly, cells in Tz group were fixed *in situ* with 10% trichloroacetic acid (TCA) to represent a measurement of the cell population at the time of drug addition (T_0). After an additional 48 h incubation with or without compounds in medium with 5%

FBS, the assay was terminated by 10% TCA in CTL and treatment groups. SRB dye purchased from Sigma (St. Louis, MO, USA) at 0.4% (w/v) in 1% acetic acid was added to stain the cells. Unbound dye was removed with 1% acetic acid and the plates were air dried. Bound dye was subsequently solubilized with 10 mM trizma base, and the absorbance was read at a wavelength of 515 nm.

4.2.4 FACScan Flow Cytometric analysis

Cells were seeded in 6-well plates ($2.5x10^5$/well) and treated with DMSO or indicated compounds with various concentrations for indicated times. Cells were washed with phosphate-buffered saline, fixed in ice cold 70% EtOH at -20 °C overnight, and stained with propidium iodide (80 μg/mL) containing Triton X-100 (0.1%, v/v) and RNase A (100 μg/mL) in phosphate-buffered saline. DNA content was analyzed with the FACScan and CellQuest software (Becton Dickinson, Mountain View, CA, USA).

4.2.5 Immunoblotting

Cells were seeded in dishes and allowed to attach overnight. The cells were treated with drugs at indicated concentrations for indicated times. After the indicated exposure time, cells were lysed and the immunoblotting was performed as described previously.[14]

4.2.6 Deconvolution microscopy

Cells were grown on glass coverslips. After treatment with indicated drugs for 24 h, the cells were fixed with 4% paraformaldehyde for 15 min then permeabilized with 0.5% Triton X-100. The coverslips were washed with phosphate-buffered saline (PBS) and blocked with PBS containing 2% bovine serum albumin, incubated with an antibody specific to beta-tubulin (1:250 dilution, T4026)

(Sigma, St. Louis, MO, USA) followed by Alexa 488–conjugated anti-mouse (1:250) (Biotium, Fremont, CA, USA) and Antifade Mounting Medium with DAPI (Vector Laboratories, Inc., Burlingame, CA, USA). For images captured, cells were assessed by Wide-field Delta Vision deconvolution microscope (Applied Precision Inc., Eagle, ID, USA), equipped with an inverted microscope (IX-71; Olympus, Tokyo, Japan), 100x/1.42 NA oil immersion objective lens, and camera (CoolSnap ES2; Photometrics, Tucson, AZ, USA). Images were reconstructed by using SoftWorx v6.1.1 software (Applied Precision lnc.), and analyzed with Volocity software (Perkin-Elmer, Waltham, MA, USA), as described previously.[15]

4.2.7 Intracellular tubulin polymerization assay

The intracellular tubulin polymerization assay was carried out as previously described[16]. Briefly, following treatment with paclitaxel, vincristine or **16c** in KB-Vin and KB-7D cells for 16 h, the cells were harvested collected by low-speed centrifugation and immediately lysed at 37 °C for 5 min in the dark in a hypotonic buffer containing 1% Nonidet P-40, 1 mmol/L $MgCl_2$, 2 mmol/L EGTA, 50 mmol/L Tris–HCL (pH 6.8), and 10 μL/mL protease inhibitor cocktail (Sigma, St. Louis, MO, USA). The resulting cell lysate was centrifuged at 13000 rpm for 10 min at room temperature to separate soluble tubulin from polymerized tubulin, and the pellet containing the polymerized tubulin was resuspended in an equal volume of hypotonic lysate buffer. The supernatant and pellet fractions were then electrophoresed on SDS–polyacrylamide gels.

Acknowledgments

This research was supported by the Ministry of Science and Technology, Taiwan (grant no. MOST 106-2113-M-038 -002; 107-2113-M-038 -001; 108-2320-B-038-056). We highly appreciate Dr. Jang-Yang Chang (Division of Hematology/Oncology, Department of Internal Medicine, National Cheng Kung University Hospital) for providing KB, KB-Vin, and KB-7D cells

References

1. Juanita S Lopez and Udai Banerji, Combine and conquer: challenges for targeted therapy combinations in early phase trials, Nat Rev Clin Oncol. 2017 Jan; 14(1): 57–66.
2. Rona R. Ramsay,corresponding, Marija R. Popovic-Nikolic,2 Katarina Nikolic, Elisa Uliassi, and Maria Laura Bolognesi, A perspective on multi-target drug discovery and design for complex diseases, Clin Transl Med. 2018; 7: 3.
3. Giacomini E, Rupiani S, Guidotti L, Recanatini M, Roberti M, The Use of Stilbene Scaffold in Medicinal Chemistry and Multi- Target Drug Design, Curr Med Chem. 2016;23(23):2439-89.
4. Baur JA and Sinclair DA., Therapeutic potential of resveratrol: the in vivo evidence, Nat Rev Drug Discov. 2006 Jun;5(6):493-506.
5. Chandra K. Singh, Mary A. Ndiaye, and Nihal Ahmad, Resveratrol and cancer: Challenges for clinical translation, Biochim Biophys Acta. 2015 Jun; 1852(6): 1178–1185.
6. Adi Y. Berman, Rachel A. Motechin, Maia Y. Wiesenfeld, and Marina K. Holz, The therapeutic potential of resveratrol: a review of clinical trials, NPJ Precis Oncol. 2017; 1: 35.
7. Marks P1, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK, Histone deacetylases and cancer: causes and therapies, Nat Rev Cancer. 2001 Dec;1(3):194-202.
8. Tomas Eckschlager, Johana Plch, Marie Stiborova, and Jan Hrabeta, Histone Deacetylase Inhibitors as Anticancer Drugs, Int J Mol Sci. 2017 Jul; 18(7): 1414.
9. Liu YM, Chen HL, Lee HY, Liou JP, Tubulin inhibitors: a patent review, Expert Opin Ther Pat. 2014 Jan;24(1):69-88.

10. Jordan MA, Wilson L., Microtubules as a target for anticancer drugs, Nat Rev Cancer. 2004 Apr;4(4):253-65.
11. Kavallaris M, Tait AS, Walsh BJ, He L, Horwitz SB, Norris MD, Haber M., Multiple microtubule alterations are associated with Vinca alkaloid resistance in human leukemia cells, Cancer Res. 2001 Aug 1;61(15):5803-9.
12. Kavallaris M, Microtubules and resistance to tubulin-binding agents, Nat Rev Cancer. 2010 Mar;10(3):194-204.
13. Nam NH, Combretastatin A-4 analogues as antimitotic antitumor agents, Curr Med Chem. 2003 Sep;10(17):1697-722.
14. Nnnn Chen CH, Changou CA, Hsieh TH, Lee YC, Chu CY, Hsu KC, Wang HC, Lin YC, Lo YN, Liu YR, Liou JP, Yen Y. Dual Inhibition of PIK3C3 and FGFR as a New Therapeutic Approach to Treat Bladder Cancer. Clin Cancer Res. 2018 Mar 1;24(5):1176-1189.
15. Wu TY, Cho TY, Lu CK, Liou JP, Chen MC. Identification of 7-(4'-Cyanophenyl)indoline-1-benzenesulfonamide as a mitotic inhibitor to induce apoptotic cell death and inhibit autophagy in human colorectal cancer cells. Sci Rep. 2017 Sep 29;7(1):12406.
16. Giannakakou P, Sackett DL, Kang YK, Zhan Z, Buters JT, Fojo T, Poruchynsky MS. Paclitaxel-resistant human ovarian cancer cells have mutant beta-tubulins that exhibit impaired paclitaxel-driven polymerization. J Biol Chem. 1997 Jul 4;272(27):17118-25.

Figure Captions

Figure 1. Reported stilbene-based compounds, and HDAC and tubulin inhibitors.

Figure 2. Rational design of target compounds.

Figure 3. Structures of synthetic compounds (**13-18**).

Figure 4. Compound **16c** induces significant apoptosis in KB-vin and KB-7D cells.

Figure 5. Effect of Compound **16c** and the comparators on cell cycle progression and cell death for 24 h.

Figure 6. Effect of Compound **16c** and the comparators on cell cycle progression and cell death for 48 h.

Figure 7. Compound **16c** induces G2/M arrest followed by subG1 accumulation.

Figure 8. Compound **16c** induces changes in microtubule assembly.

Figure 9. Effect of **16c** on tubulin polymerization in KB-Vin and KB-7D cells.

Table 1. Antiproliferative activity (GI_{50}) of compounds (**13-18**)

Compounds	KB cancer cell line GI_{50} (μM ± SD[a])	Compounds	KB cancer cell line GI_{50} (μM ± SD[a])
13a	>10	**15a**	0.137 ± 0.0069
13b	0.678 ± 0.2174	**15b**	0.472 ± 0.0408
13c	0.023 ± 0.0008	**15c**	0.066 ± 0.0007
13d	>10	**15d**	0.697 ± 0.1000
14a	0.456 ± 0.0425	**15e**	0.286 ± 0.0133
14b	0.462 ± 0.0525	**16a**	0.660 ± 0.0262
14c	0.884 ± 0.1364	**16b**	0.060 ± 0.0043
14d	0.448 ± 0.0151	**16c**	0.012 ± 0.0006
14e	0.124 ± 0.0096	**16d**	0.442 ± 0.0180
14f	0.790 ± 0.2840	**17**	4.409 ± 0.2760
14g	0.888 ± 0.1349	**18**	>10
12 (ABT-751)	0.801 ± 0.0800	**Etoposide**	8.392 ± 1.3804
Vincristine	0.00186 ± 0.0030	**8 (MS-275)**	6.632 ± 0.2197

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

Table 2. Antiproliferative activity (GI_{50}) of compounds (**13c**, **15c**, **16b and 16c**) against drug-resistant KB cancer cell lines

Compounds	KB-Vin cancer cell GI_{50} (μM ± SD[a])	KB-7D cancer cell GI_{50} (μM ± SD[a])
13c	0.031 ± 0.0011	0.022 ± 0.0001
15c	0.029 ± 0.0018	0.012 ± 0.0006
16b	0.042 ± 0.0021	0.062 ± 0.0022
16c	0.022 ± 0.0023	0.012 ± 0.0001
12 (ABT-751)	2.073 ± 0.0257	0.444 ± 0.0545
8 (MS-275)	7.713 ± 1.4524	5.685 ± 0.2587
Vincristine	0.219 ± 0.0344	-
Etoposide	-	98.627 ± 1.2395

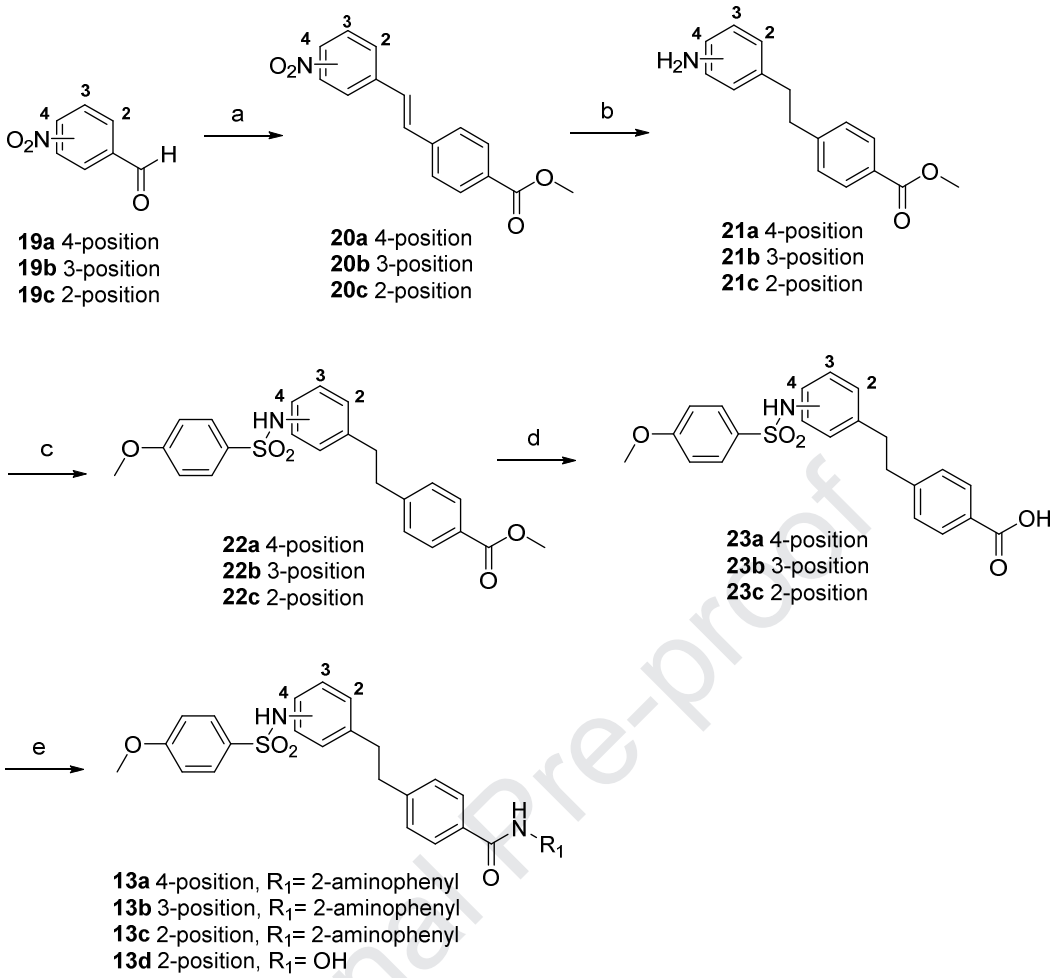
a SD: standard deviation. All experiments were independently performed at least three times.

Table 3. HDACs 1, 2, 3, 8 isoform inhibition[a]

Compound	HDAC IC_{50} (μM)			
	HDAC 1	HDAC 2	HDAC 3	HDAC 8
13c	1.19	>10	2.55	>10
15c	>10	9.08	1.75	>10
16b	5.45	4.95	7.92	>10
16c	1.07	1.47	2.27	>10
8	0.54	0.61	0.62	9.88

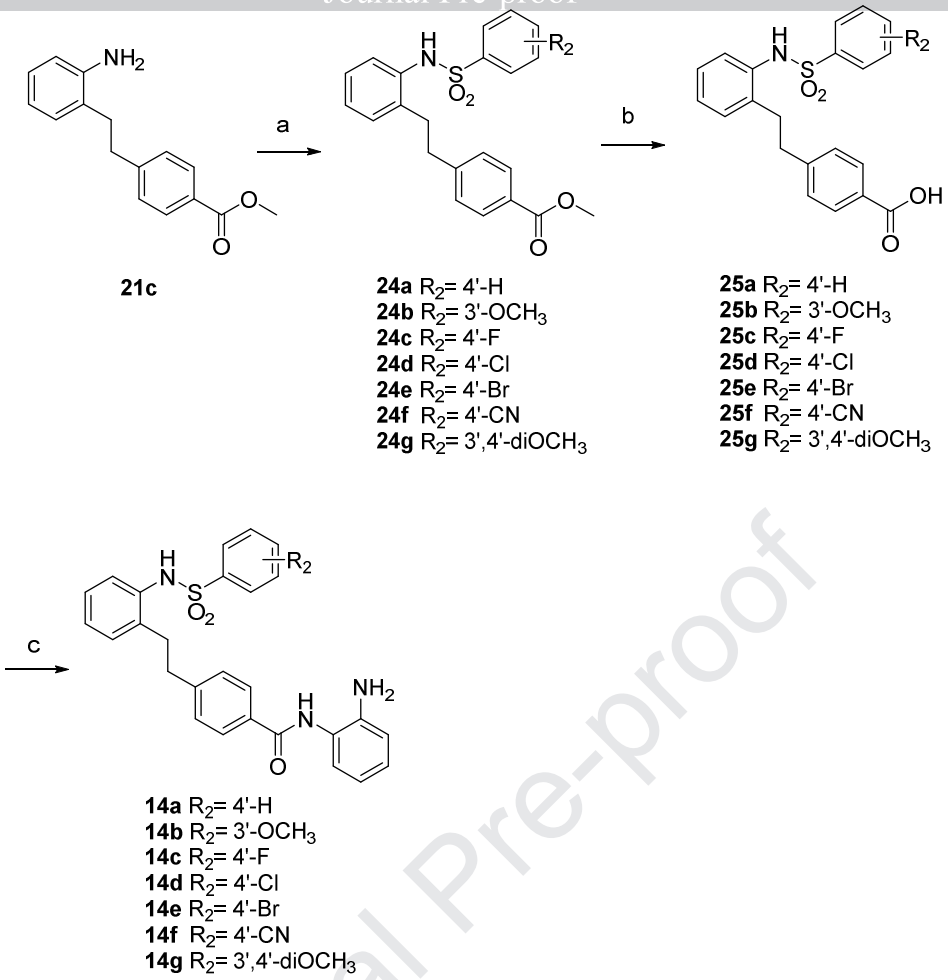
a These assays were conducted by the Reaction Biology Corporation, Malvern, PA. All compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 10 μM.

Scheme 1. Synthetic route to compounds **13a-13d**[a]



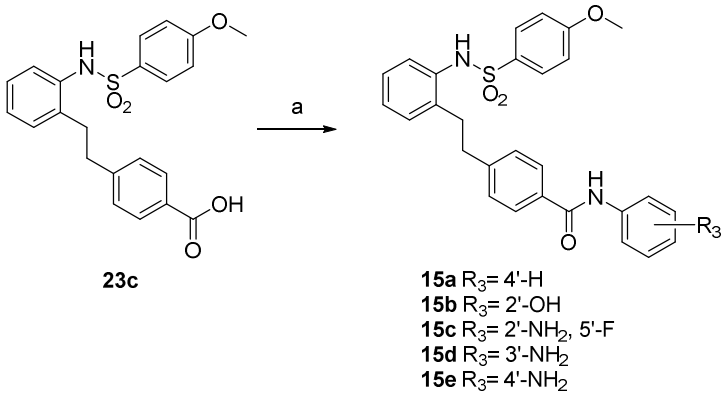
[a]Reagents and conditions: a) (4-(methoxycarbonyl)benzyl)triphenylphosphonium bromide, NaOH, THF, rt; b) 10% Pd/C, H_2, MeOH, rt; c) 4-methoxybenzenesulfonyl chloride, pyridine, rt; d) 1M $LiOH_{(aq.)}$, *p*-dioxane, 40°C; e) for **13a**-**13c**, HBTU, DIPEA, *o*-phenylenediamine, DMF, rt; for **13d**, i. NH_2OTHP, HBTU, DIPEA, DMF, rt, ii. 10%$TFA_{(aq.)}$, MeOH, rt

Scheme 2. Synthetic route to compounds **14a-14g**[a]



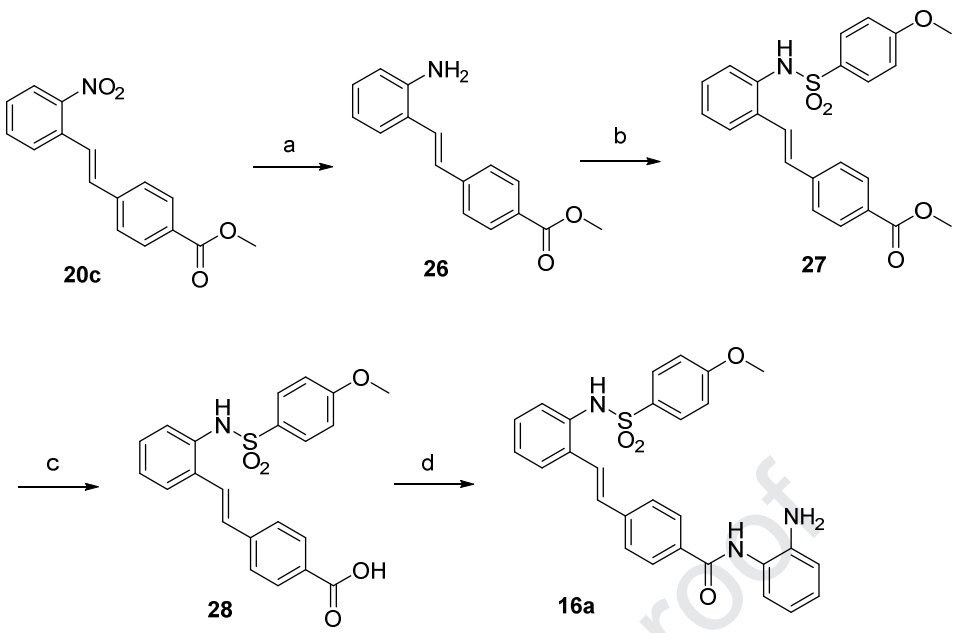
[a]Reagents and conditions: a) substituted benzenesulfonyl chloride, pyridine, rt; b) 1M $LiOH_{(aq.)}$, *p*-dioxane, 40°C; c) HBTU, DIPEA, *o*-phenylenediamine, DMF, rt

Scheme 3. Synthetic route to compounds **15a-15e**[a]



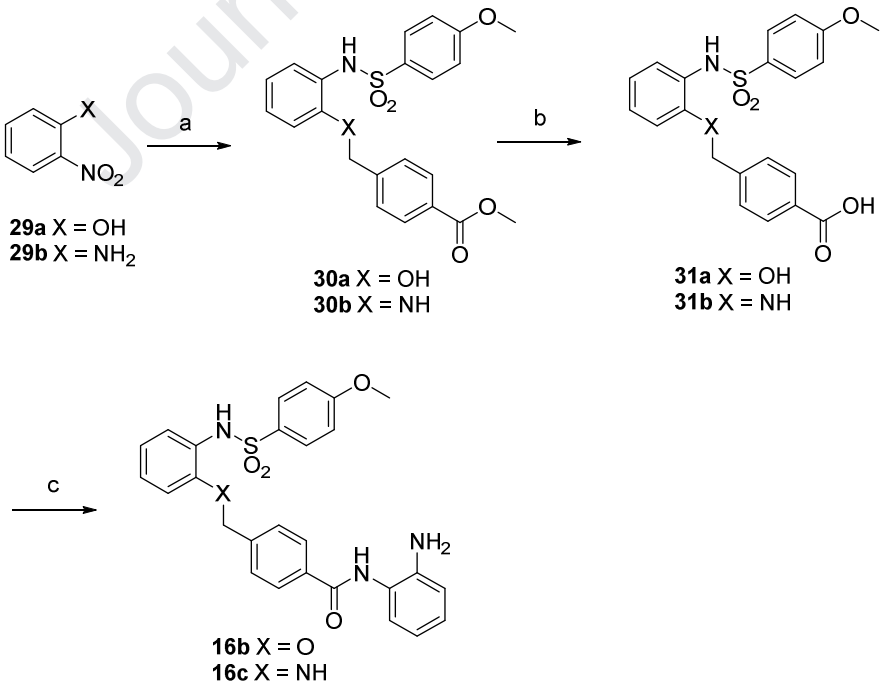
[a]Reagents and conditions: a) for **15a**, **15c** and **15e**, substituted aniline, DMF, rt; for **15b** and **15d**, i. HBTU, DIPEA, substituted aniline, DMF, rt, ii. 10%Pd/C, H_2, MeOH, rt

Scheme 4. Synthetic route to compound **16a**[a]



[a]Reagents and conditions: a) Fe powder, ammonium chloride, IPA, H_2O, reflux; b) *p*-methoxybenzenesulfonyl chloride, pyridine, rt; c) 1M $LiOH_{(aq.)}$, *p*-dioxane, 40°C; d) HBTU, DIPEA, *o*-phenylenediamine, DMF, rt

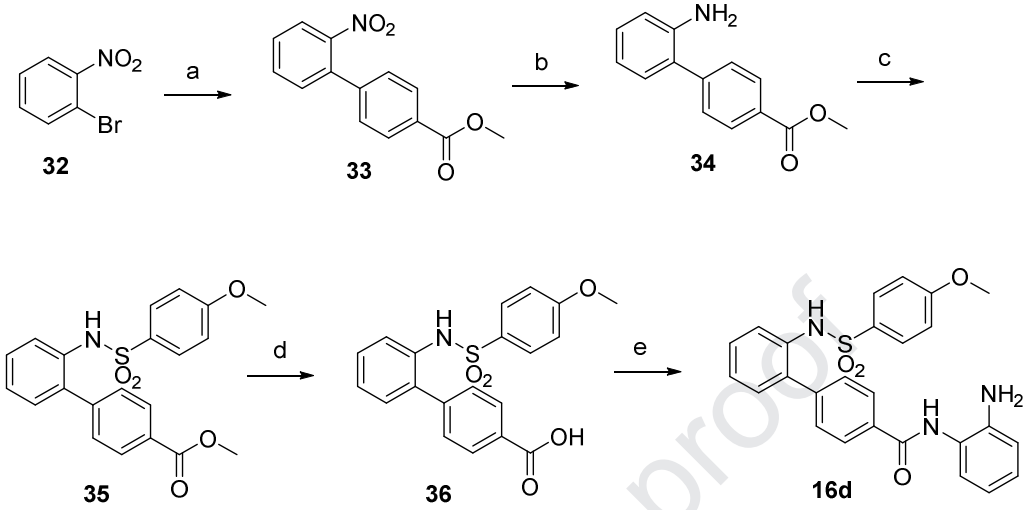
Scheme 5. Synthetic route to compounds **16b** and **16c**[a]



[a]Reagents and conditions: a) for **30a**, i. K_2CO_3, methyl 4-formylbenzoate, DMF, rt; ii. H_2, 10%Pd/C, MeOH, rt; iii. 4-methoxybenzenesulfonyl chloride, pyridine, rt; for **30b**, i. 4-methoxybenzenesulfonyl chloride, pyridine, rt; ii. H_2, 10%Pd/C, MeOH, rt; iii. $NaBH_3CN$, methyl

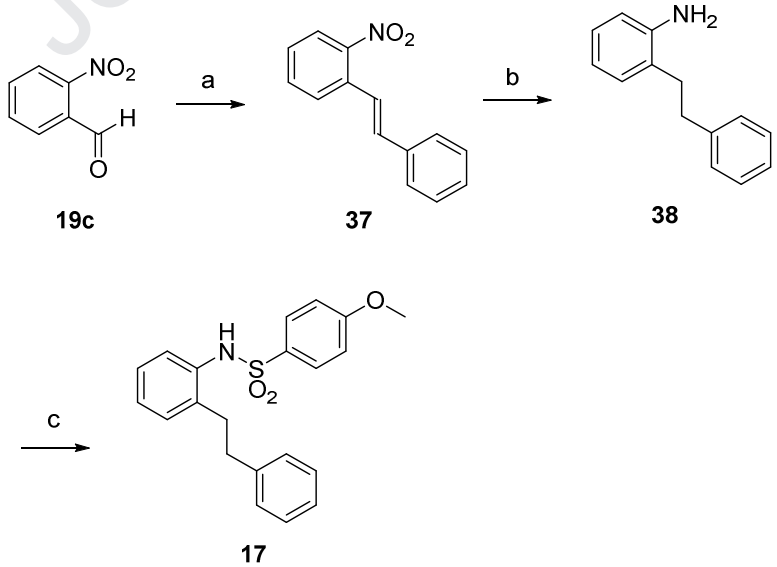
4-formylbenzoate, MeOH, rt; b) 1M $LiOH_{(aq.)}$, *p*-dioxane, 40°C; c) HBTU, DIPEA, *o*-phenylenediamine, DMF, rt

Scheme 6. Synthetic route to compound **16d**[a]



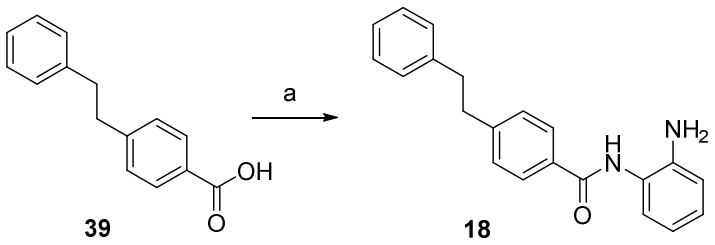
[a]Reagents and conditions: a) $Pd(PPh_3)_4$, 2N $K_2CO_{3(aq.)}$, 4-(methoxycarbonyl)phenylboronic acid, EtOH, toluene, reflux; b) Fe powder, ammonium chloride, IPA, H_2O, reflux; c) 4-methoxybenzenesulfonyl chloride, pyridine, rt; d) 1M $LiOH_{(aq.)}$, *p*-dioxane, 40°C; e) HBTU, DIPEA, *o*-phenylenediamine, DMF, rt

Scheme 7. Synthetic route to compound **17**[a]



[a]Reagents and conditions: a) Benzyltriphenylphosphonium bromide, NaOH, THF, rt; b) 10%Pd/C, H_2, MeOH, rt; c) 4-methoxybenzenesulfonyl chloride, pyridine, rt

Scheme 8. Synthetic route of compounds **18**[a]



[a]Reagents and conditions: a) HBTU, DIPEA, *o*-phenylenediamine, DMF, rt

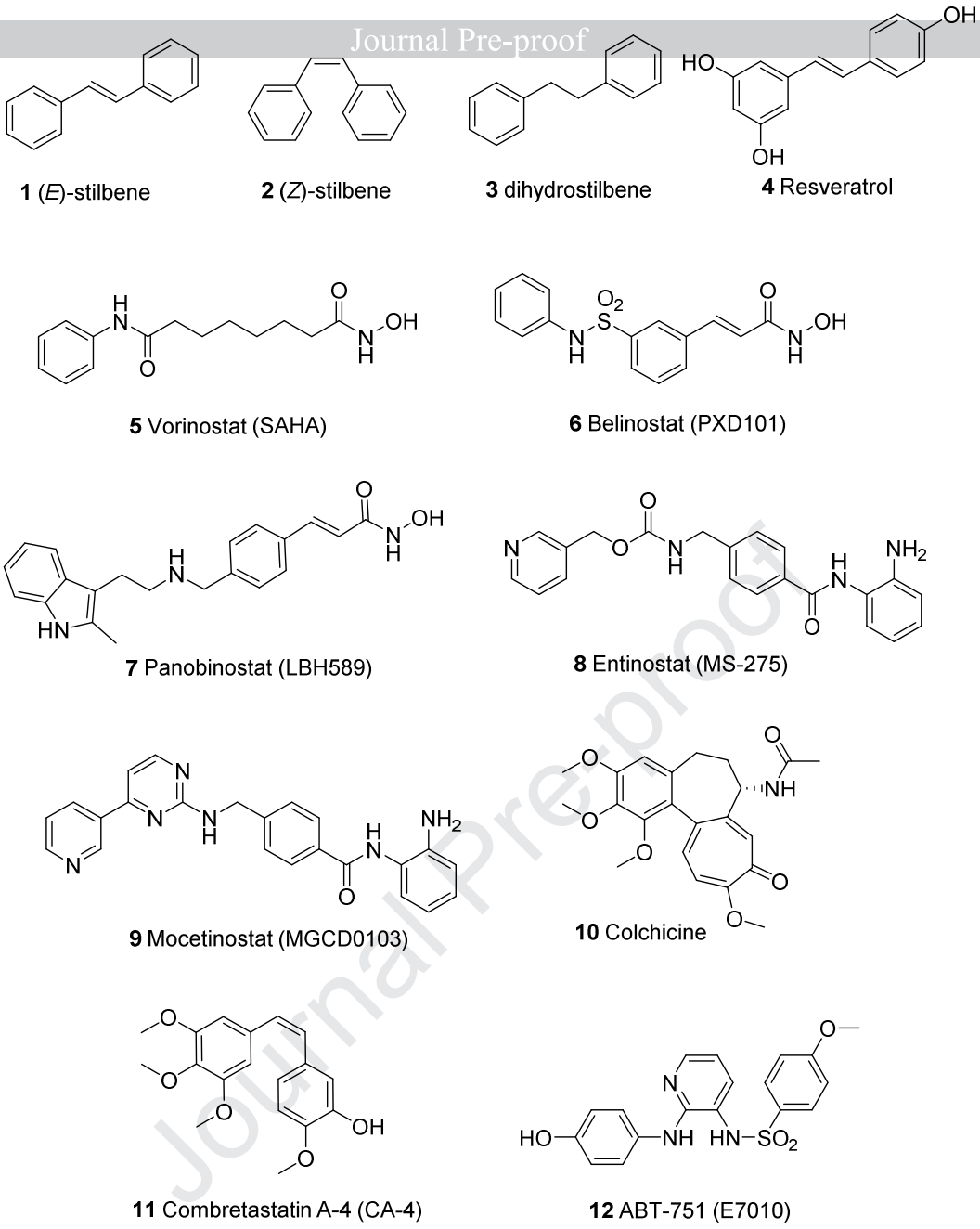


Figure 1. Reported stilbene-based compounds, and HDAC and tubulin inhibitors

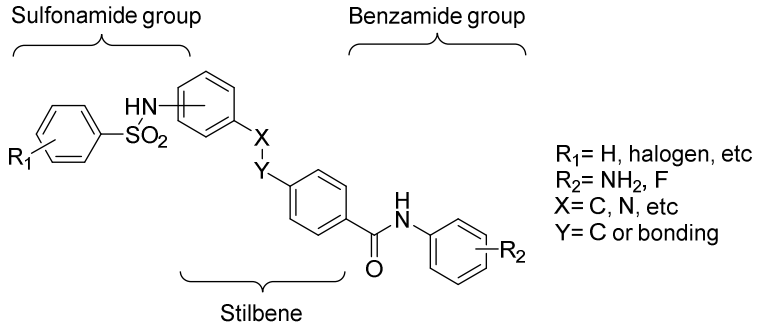


Figure 2. Rational design of target compounds.

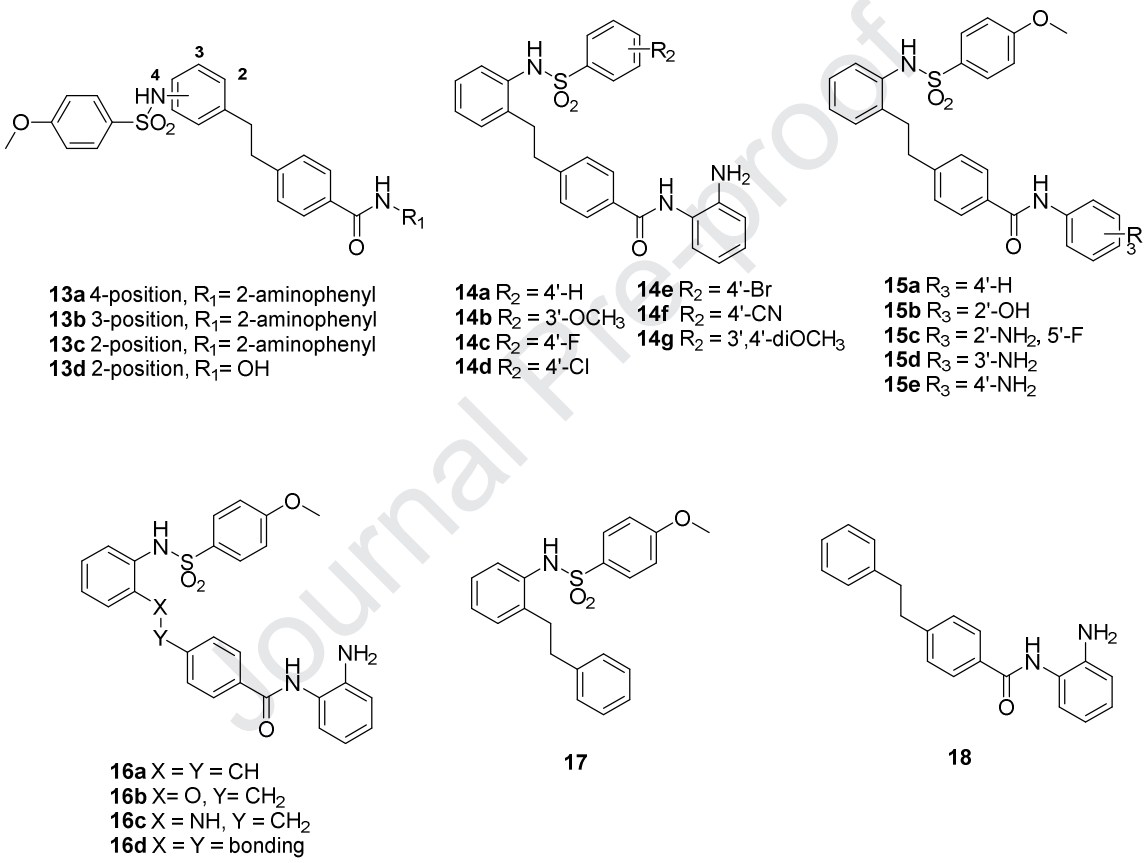


Figure 3. Structures of synthetic compounds (**13-18**).

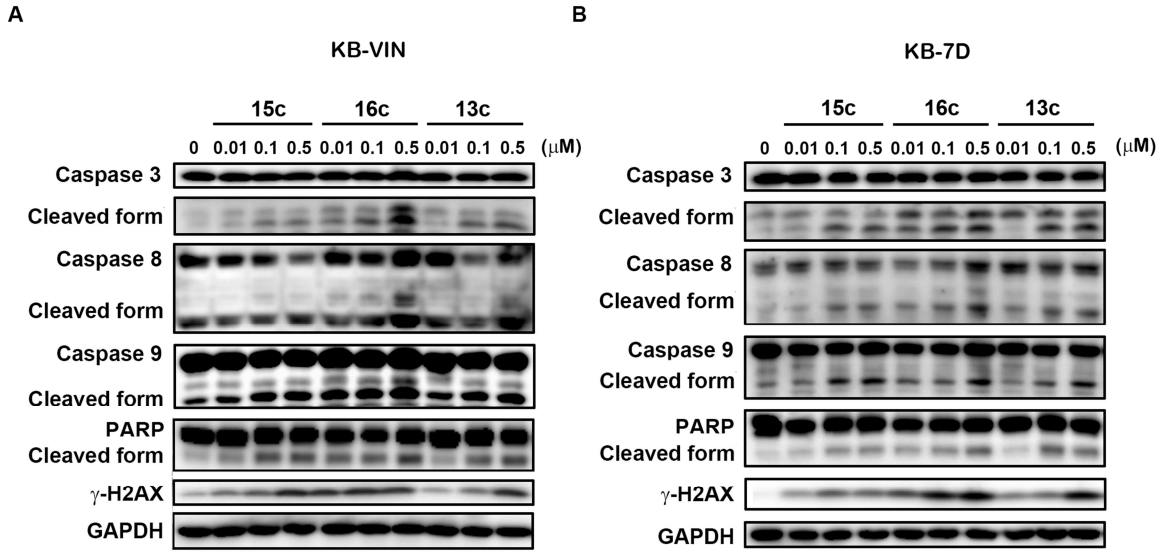


Figure 4. Compound **16c** induces significant apoptosis in KB-vin and KB-7D cells. (A-B) Effects of indicated compounds on apoptosis in KB-vin and KB-7D cells. Compound **16c** increased levels of the cleaved (activated) forms of PARP, γH2AX, caspase-3, -8, and -9 in a concentration-dependent manner.

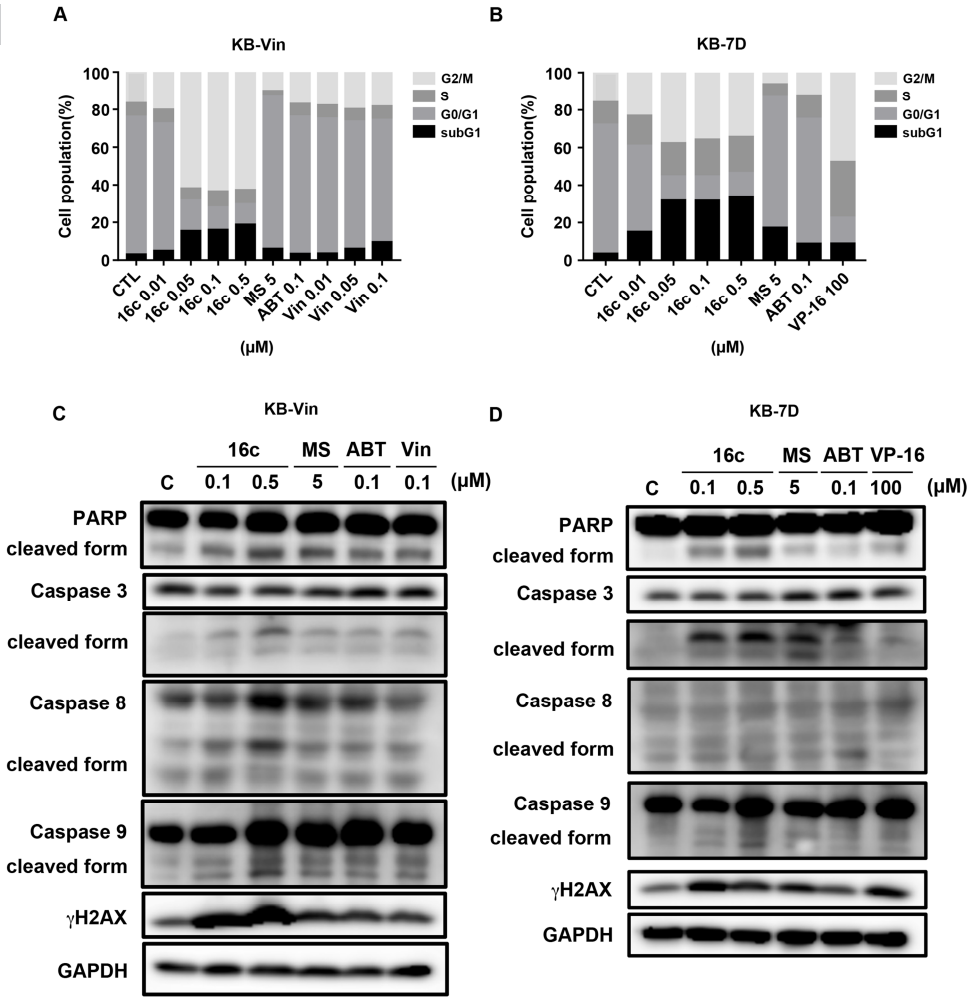


Figure 5. Effect of Compound **16c** and the comparators on cell cycle progression and cell death for 24 h. (A,C) Effects of indicated compounds on cell cycle alteration (A) and apoptosis (C) in KB-Vin cells. (B, D) Effects of indicated compounds on cell cycle alteration (B) and apoptosis (D) in KB-7D cells. Cells were treated with indicated compounds with indicated concentrations for 24 h, and cell lysates were analyzed and immunoblotted using flow cytometry or indicated antibodies to observe cell cycle changes and protein expression levels. MS, MS-275 (**8**). ABT, ABT-751 (**12**). Vin, vincristine.

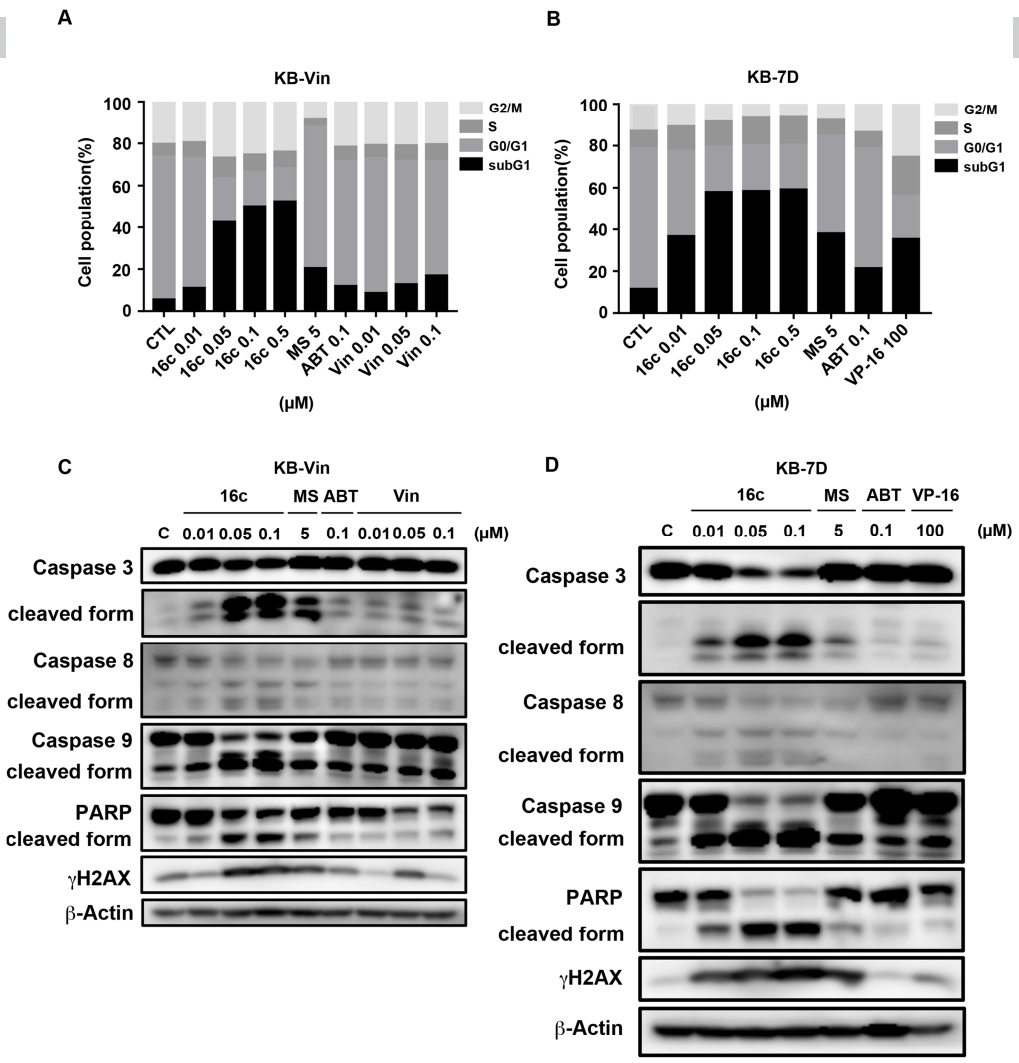


Figure 6. Effect of Compound **16c** and the comparators on cell cycle progression and cell death for 48h. (A,C) Effects of indicated compounds on cell cycle alteration (A) and apoptosis (C) in KB-Vin cells. (B, D) Effects of indicated compounds on cell cycle alteration (B) and apoptosis (D) in KB-7D cells. Cells were treated with indicated compounds with indicated concentrations for 48 h, and cell lysates were analyzed and immunoblotted using flow cytometry or indicated antibodies to observe cell cycle changes and protein expression levels. MS, MS-275 (**8**). ABT, ABT-751 (**12**). Vin, vincristine.

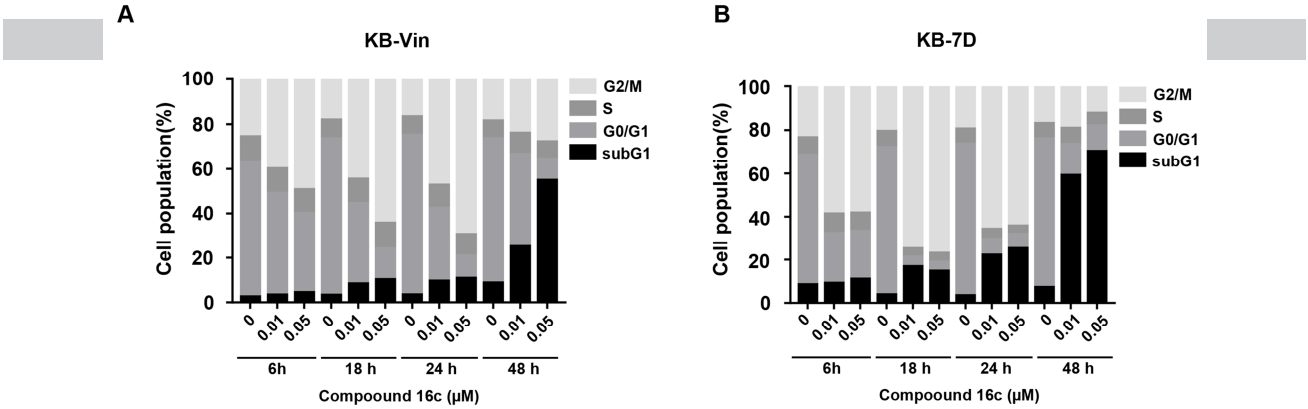


Figure 7. Compound **16c** induces G2/M arrest followed by subG1 accumulation. (A,B) Effects of **16c** on cell cycle alterations in KB-Vin (A) and KB-7D (B) cells. Cells were treated with indicated compounds with indicated concentrations for indicated times, and cell lysates were analyzed using flow cytometry to observe cell cycle changes.

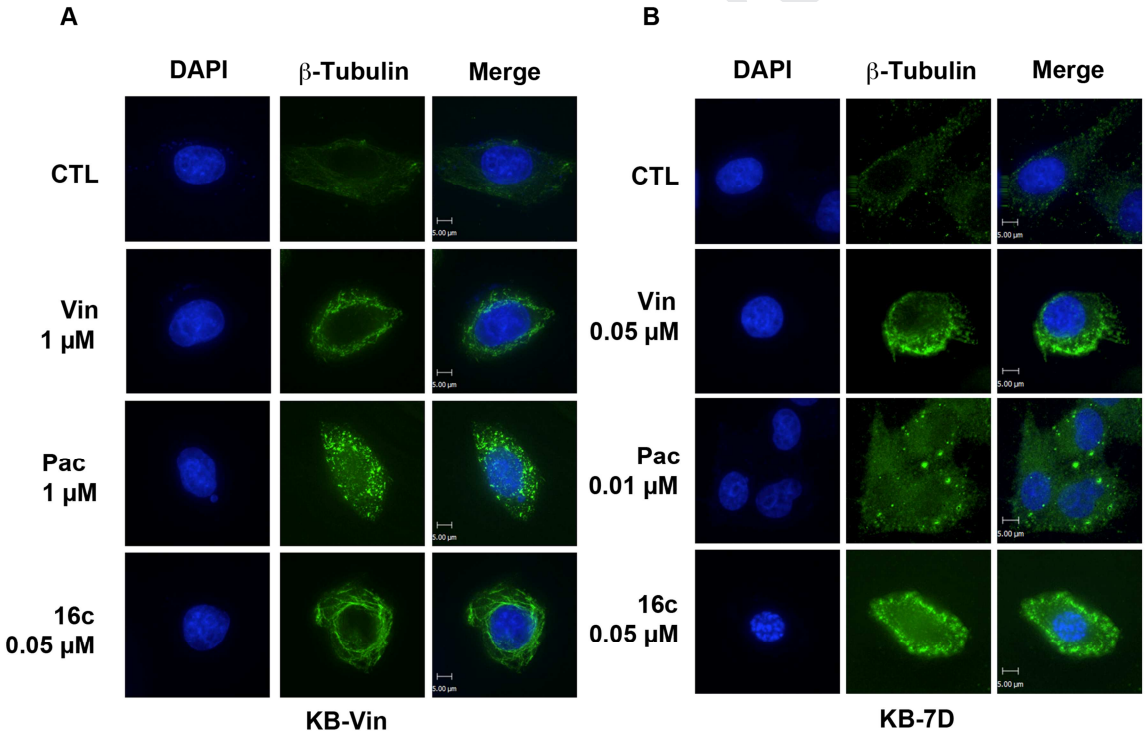


Figure 8. Compound **16c** induces changes in microtubule assembly. Effects of **16c** on β-tubulin cytoskeleton morphology in KB-Vin (A) and KB-7D (B) cells. Cells were treated with indicated compounds with indicated concentrations for 24 h, and cells were fixed and stained with indicated antibody as described in Materials and Methods. Vin, vincristine. Pac, paclitaxel. Left panel, nuclei labeled with DAPI (blue fluorescence). Central panel, microtubule networks (green fluorescence). Right panel, merged images. Scale bar, 5 μm.

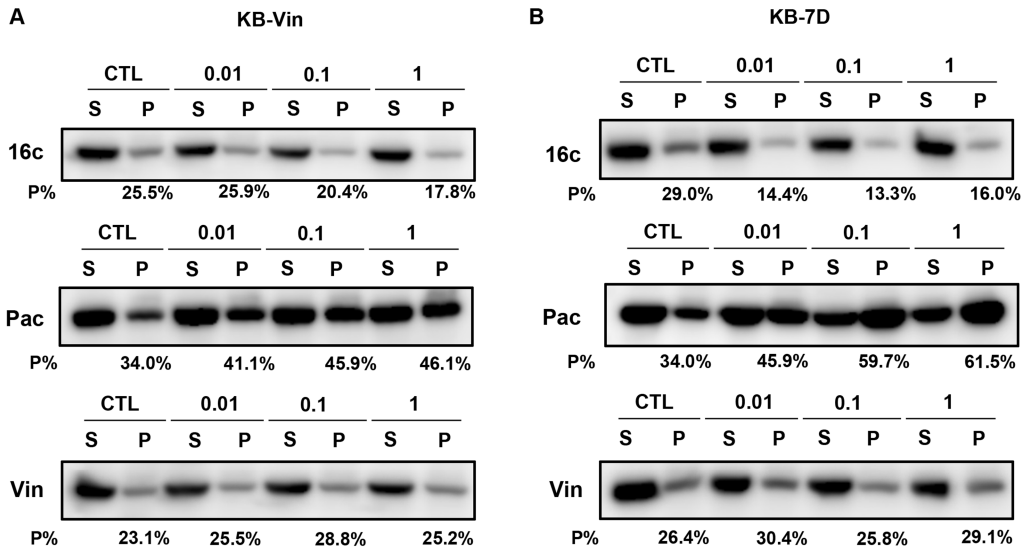


Figure 9. Effect of 16c on tubulin polymerization in KB-Vin and KB-7D cells. Drug resistant cell line KB-Vin (A) and KB-7D (B) were lysed with a hypotonic buffer in response to different drug treatments for 16 h. Following cell lysis the polymerized (P) and the soluble (S) protein fractions were separated by centrifugation, and each fraction was resolved on adjacent lanes by electrophoresis and stained with β-tubulin. The percentage of polymerized tubulin was obtained by dividing the densitometric value of the polymerized tubulin by the total tubulin content: P/(S+P)*100%. Vin, vincristine. Pac, paclitaxel.

Research highlights

1. N-phenyl-4-(2-phenylsulfonamido)-benzamides act as Microtubule-targeting agents.
2. **16c** displays promising anticancer activity against resistant cancer cells.
3. **16c** induces cell cycle arrest at the G2/M phase.
4. **16c** significantly inhibits microtubule polymerization.
5. **16c** exhibited inhibitory potential against HDAC 1, 2 and 3.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: