http://dx.doi.org/10.5012/bkcs.2013.34.5.1487

# Synthesis of Novel *N*-(2-Hydroxyphenyl)arylsulfonamides as Selective HDAC Inhibitory and Cytotoxic Agents

**Jungsu Kim, Pusoon Chun,[†,*] and Hyung Ryong Moon[*]**

*Laboratory of Medicinal Chemistry, College of Pharmacy, Pusan National University, Busan 609-735, Korea*
*[*]E-mail: mhr108@pusan.ac.kr*
*[†]College of Pharmacy, Inje University, Gimhae, Gyeongnam 621-749, Korea. [*]E-mail: pusoon@inje.ac.kr*
*Received February 12, 2013, Accepted February 26, 2013*

Based on the finding that the 2-aminobenzamido group of MS-275 plays a crucial role in inhibiting HDACs through chelation of zinc existing at the active site of HDAC enzymes, novel *N*-(2-hydroxyphenyl)arylsulfonamide derivatives were synthesized for their potential ability to inhibit HDACs and evaluated for anticancer activity against human breast cancer cell line (MCF-7). Although the synthesized arylsulfonamides have failed to significantly inhibit total HDACs activity, phenyl carbamate-linked arylsulfonamide **10** and benzyl thiocarbamate-linked arylsulfonamide **15** exhibited good anticancer activities, which were only 4.3- and 3.6-fold lower anticancer activities, respectively, than MS-275 that is undergoing phase II clinical trials. These results suggest that these compounds may act as a selective HDAC inhibitor and probably *N*-(2-hydroxyphenyl)sulfamoyl group may play an important role in interacting with HDAC enzymes through chelation of zinc ion.

**Key Words :** HDAC inhibition, Anticancer activity, MS-275, *N*-(2-Hydroxyphenyl)sulfamoyl, Arylsulfonamide

## Introduction

All of the human genome is packaged into chromatin that consists of DNA, histones and non-histone proteins.[1] Nucleosomes form the repeating unit of chromatin and consist of DNA wrapped around a histone octamer composed of four histone; H3-H4 tetramer and two H2A-H2B dimers.[2] It has been clear that remodeling of chromatin caused by the opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs) has a key role in epigenetic regulation of gene expression.[3-7] HATs transfer acetyl groups to the ε–amino group of the lysine residues in histones, which results in relaxation of chromatin and increased accessibility of regulatory proteins to DNA, whereas HDACs catalyze the removal of acetyl groups and induce a closed-chromatin configuration and transcriptional repression. The HDACs can be divided into two families: (1) the $Zn^{2+}$ dependent HDAC family composed of class I (HDACs 1, 2, 3 and 8), class II a/b (HDACs 4, 5, 6, 7, 9 and 10), and class IV (HDAC 11) and (2) $Zn^{2+}$ independent $NAD^+$-dependent class III SIRT enzymes.[8] HDAC inhibitors (HDACIs) induce cell-growth arrest in G2 or G2/M, differentiation and/or apoptotic cell death of various tumor cell types with little or no toxicity against normal cells.[9,10] A variety of HDACIs have been purified as natural products or synthetically produced and these inhibitors can be subdivided into five groups based on their chemical structure: (1) hydroxamic acid-derived compounds: trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA) and NVP-LAQ824, (2) cyclic peptides: depsipeptide (FK228), apicidin and trapoxin, (3) short-chain fatty acids: valproic acid (VPA), phenylbutyrate and phenylacetate, (4) benzamides: MS-275 and CI-994, and (5) ketones: trifluoromethyl ketone and α-ketoamides.[10] The fourth class of HDAC inhibitors that contain a benzamide is postulated to enter the active-site of HDACs and chelate the zinc ion by means of its *N*-(2-aminophenyl)-amide. In tumor cell lines, MS-275 (Figure 1), one of the most active derivatives, increases acetylation of histones H3 and H4 and induces overexpression of $p21^{WAF1, CIP1}$ and gelsolin at micromolar concentrations.[11,12,13,14,15] In addition, MS-275 has been shown to have selective inhibitory activity against HDAC enzymes. The studies by Saito *et al.* and Wang *et al.* demonstrated that MS-275 is less active against HDAC3 than HDACs 1, 2 and 4 and has no inhibitory activity toward HDAC8.[12,15,16] To date, vorinostat (SAHA) and romidepsin (FK228) have been licensed by US FDA for

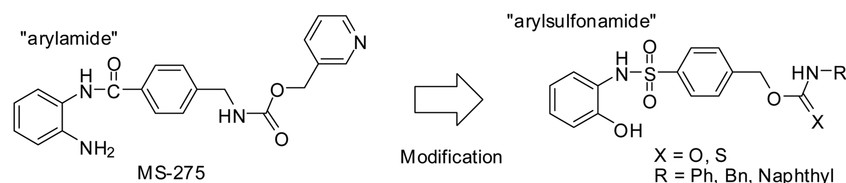
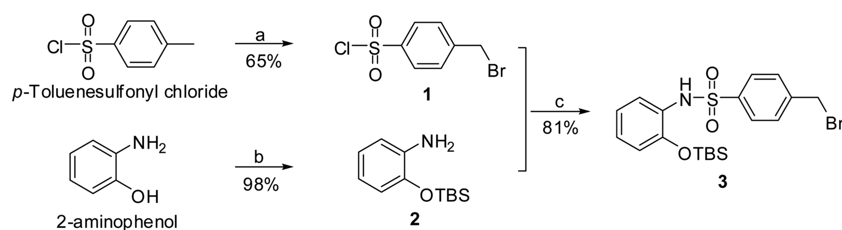



**Figure 1.** The rationale for the design of the desired arylsulfonamide derivatives.

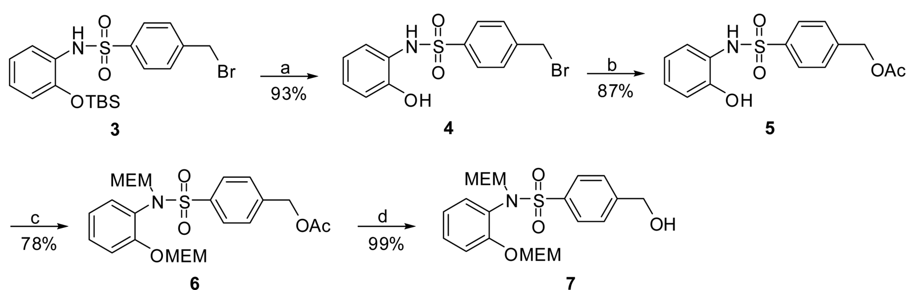
the treatment of cutaneous T-cell lymphoma, whereas entinostat (MS-275) is in phase II clinical trials for various cancers. Although hydroxamates exhibit potent enzymatic activity, their biologically labile nature and short half-lives *in vivo* have prompted us to look for alternative zinc-binding groups.[14,17,18] On the basis of the structure-activity relationship that reported by Suzuki *et al.*[11] and *Saito et al.*,[12] all the compounds were designed and synthesized to have an *N*-(2-hydroxyphenyl)sulfamoyl group, in which the 2-hydroxyl and sulfonamide groups have replaced the 2-amino and amido groups of MS-275, respectively, as shown in Figure 1. Thus, the purpose of this study was to synthesize potential HDAC inhibitors having an *N*-(2-hydroxyphenyl)sulfamoyl moiety that might chelate the zinc ion in the active site of HDACs. Additionally, the evaluation on the anticancer potentiality was performed against MCF-7 breast cancer cell line.

## Results and Discussion

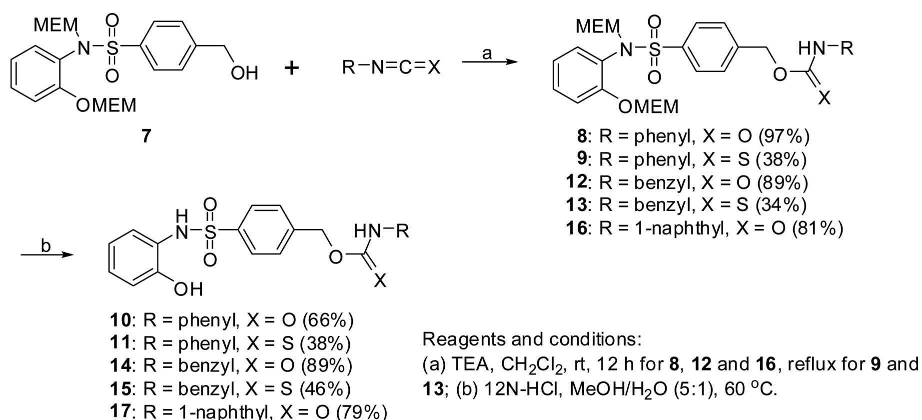
**Chemistry.** A series of novel arylsulfonamide derivatives **10**, **11**, **14**, **15** and **17** with an *N*-(2-hydroxyphenyl)sulfamoyl group were successfully synthesized for their potential inhibitory activity against HDACs, as shown in Schemes 1, 2 and 3. It was envisioned that compound **7** would be an appropriate key intermediate for the preparation of the target compounds. It was thought that the key intermediate **7** would be derived from compound **3**. *p*-Toluenesulfonyl chloride and 2-aminophenol, commercially available, were used as starting materials. Bromination of *p*-toluenesulfonyl chloride using *N*-bromosuccinimide in the presence of a catalytic amount of benzoylperoxide gave α-bromotosyl chloride **1** in 65% yield (Scheme 1). Reaction of 2-aminophenol with TBSCl in the presence of imidazole exclusively gave *O*-silylated product **2**, which was condensed with α-bromotosyl chloride **1** in the presence of pyridine to give arylsulfonamide **3** in a good yield. Scheme 2 shows the synthetic route of key intermediate **7**. TBS deprotection with 6 *N* HCl in THF afforded phenolic compound **4**, which was treated with sodium acetate to form the corresponding acetate **5** in 87% yield. Before reacting with isocyanate or isothiocyanate, the sulfonamido and hydroxyl groups of **5** should be protected. Reaction of **5** with MEMCl produced *N*,*O*-diMEM protected compound **6** in 78% yield. The acetyl protecting group was easily removed by treatment with methylamine in THF to give compound **7**, which was ready to couple with various isocyanate and isothiocyanate. In Scheme 3, the last steps for the synthesis of novel *N*-(2-hydroxyphenyl)arylsulfonamides with phenylcarbamate and phenylthiocarbamate, respectively, are outlined. In the presence of TEA, the coupling reactions of compound **7** with phenyl isocyanate and phenyl isothiocyanate gave the corresponding compounds **8** (97%) and **9** (38%), respectively. Under acidic conditions, the MEM groups were removed from those compounds to give phenyl carbamate-linked arylsulfonamide **10** (66%) and phenyl thiocarbamate-linked arylsulfonamide **11** (38%), respectively. Similarly, the coupling reactions between compound **7** and benzyl isocyanate and benzyl isothiocyanate gave compound **12** and **13**, respectively, which then lost their MEM groups under acidic conditions, affording the desired benzyl carbamate-linked arylsulfonamide **14** and benzyl thiocarbamate-linked arylsulfonamide **15** in 89% and 46% yield, respectively. Likewise, a reaction with 1-naphthyl isocyanate followed by




**Scheme 1.** Synthesis of the intermediate **3**.




**Scheme 2.** Synthesis of the key intermediate **7**.




**Scheme 3.** Synthesis of the target compounds **10**, **11**, **14**, **15** and **17**.

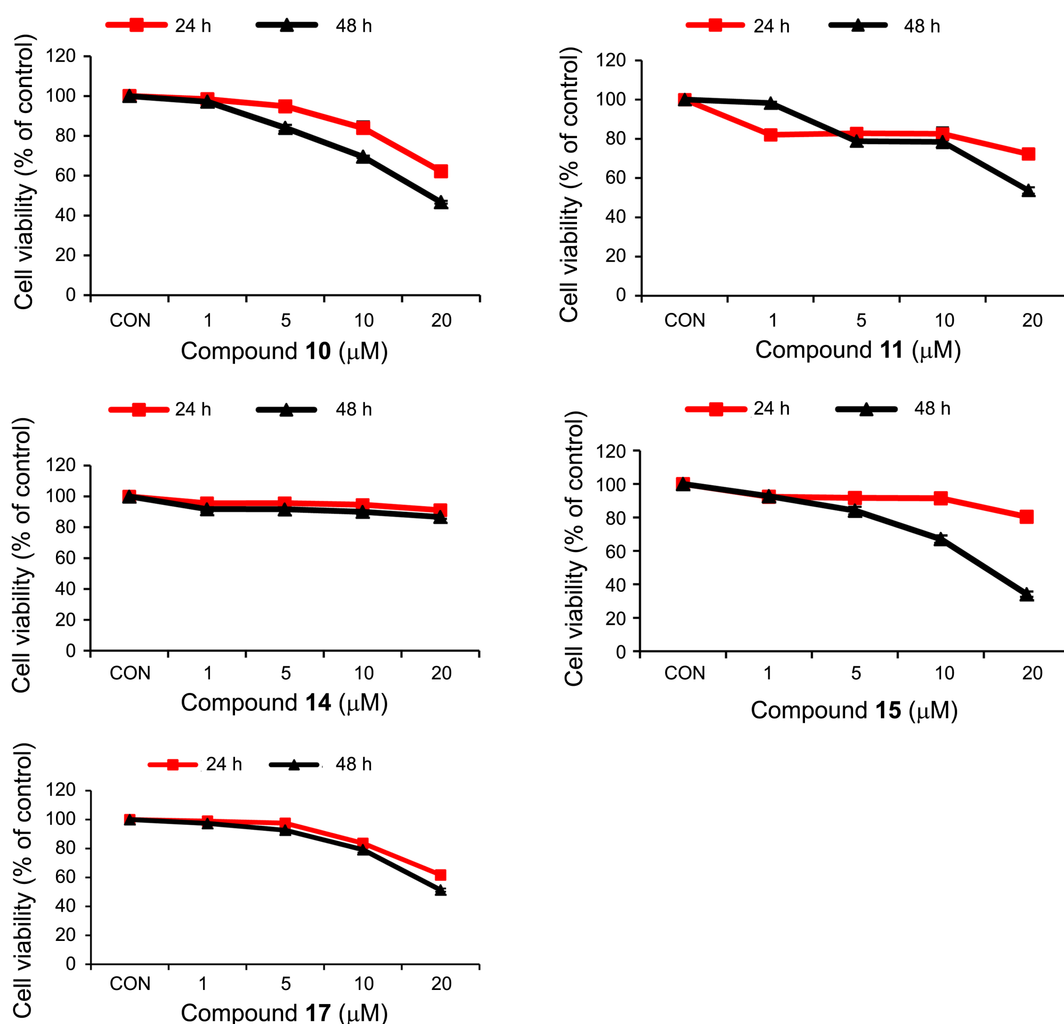



**Figure 2.** Effect of compounds **10**, **11**, **14**, **15** and **17** on cytotoxicity of MCF-7 breast cancer cell line.

deprotection of MEM groups gave naphthyl carbamate-linked arylsulfonamide **17** in a moderate yield. All of the synthesized compounds were characterized by $^{1}H$ and $^{13}C$ NMR and mass spectra analysis.

**HDAC Enzymes Inhibition.** The inhibitory activity of the final compounds **10**, **11**, **14**, **15** and **17** against total HDACs *in vitro* was evaluated using the Fluorometric HDAC activity assay kit. No significant inhibition of total HDACs activity was observed for all synthesized compounds up to a concentration of 100 μM (not shown here). Although they failed to inhibit total HDACs activity significantly, phenyl carbamate-linked arylsulfonamide **10** and benzyl thiocarbamate-linked arylsulfonamide **15** showed approximately 20% inhibition of total HDACs activity at a concentration of 100

μM compared with a control.

**Anticancer Activity.** The cytotoxicity of arylsulfonamide derivatives **10**, **11**, **14**, **15** and **17** against MCF-7 breast cancer cell line was determined by MTT assay (Figure 2). Weak to mild effects were shown for compounds **11**, **14**, and **17** against MCF-7 breast cancer cell line. However, good anticancer activities were observed for phenyl carbamate-linked arylsulfonamide **10** and benzyl thiocarbamate-linked arylsulfonamide **15** with $IC_{50}$ values of 18.6 μM for **10** and 15.4 μM for **15**, respectively, after 48 h treatment. Considering the inhibitory activity of MS-275 ($IC_{50}$ = 4.3 μM),[19] compounds **10** and **15** showed only 4.3- and 3.6-fold lower activities, respectively. Interestingly, although compounds **10** and **15** exhibited very low inhibition of total HDACs activity, the compounds displayed good anti-cancer activities. This result implies that compounds **10** and **15** may inhibit only specific HDACs selectively, like MS-275, which is known as a selective HDAC inhibitor. In addition, this result suggests that the derivatives bearing an *N*-(2-hydroxyphenyl)sulfamoyl group may act as an effective zinc-chelator. To find the clear inhibition mechanism of these compounds, evaluation of inhibitory activity on each subtype HDAC enzyme will be performed in the close future with the anticancer activity evaluation against other cancer cell lines.

In summary, on the basis of the chemical structure of MS-275, an *N*-(2-hydroxyphenyl)sulfamoyl group was designed as a potential novel zinc chelator and various arylsulfonamide derivatives bearing the functional group were synthesized starting from *p*-toluenesulfonyl chloride and 2-aminophenol. Whereas compounds **10** and **15** did not show potent total HDACs inhibitory activity, the compounds exhibited potent anticancer activities in MCF-7 breast cancer cell line. This indicates that these compounds may inhibit just specific HDAC enzymes and the *N*-(2-hydroxyphenyl)sulfamoyl group can functionally replace the *N*-(2-aminophenyl)amido group of MS-275.

## Experimental Section

All chemical reagents were commercially available. Melting points are uncorrected. Nuclear Magnetic Resonance (NMR) data were recorded on Varian Unity INOVA 400 spectrometer and Varian Unity AS 500 spectrometer, using $CDCl_3$, $CD_3OD$, DMSO-$d_6$, or $D_2O$ and chemical shifts were reported in parts per million (ppm) with reference to the respective residual solvent or deuteriated peaks ($\delta_H$ 3.30 and $\delta_C$ 49.0 for $CD_3OD$, $\delta_H$ 7.26 and $\delta_C$ 77.0 for $CDCl_3$). Coupling constants are reported in hertz. The abbreviations used are as follows: s (singlet), d (doublet), q (quartet), td (triplet of doublets), m (multiplet), dd (doublet of doublets), br s (broad singlet), br t (broad triplet) or br d (broad doublet). All the reactions described below were performed under nitrogen or argon atmosphere and monitored by thin-layer chromatography (TLC). TLC was performed on Merck precoated $60F_{254}$ plates. Column chromatography was performed using silica gel 60 (230-400 mesh, Merck). All anhydrous solvents were distilled over $CaH_2$ or Na/benzophenone prior to use.

**4-(Bromomethyl)benzene-1-sulfonyl Chloride (1).** To a stirred of TsCl (20 g, 104.9 mmol) in $CCl_4$ (500 mL) was added NBS (20.6 g, 115.7 mmol) and benzoyl peroxide (2.54 g, 10.84 mmol) at room temperature, and the reaction mixture was refluxed for 10 h. The reaction mixture was filtered through a pad of Celite and filterate was evaporated under reduced pressure to give yellow oil. The residue was recrystallized with hexane as the eluent to give a-bromotosyl chloride **1** (20.6 g, 65%) as a white solid: mp 75.9-76.5 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, 2 H, *J* = 8.4 Hz, 2-H, 6-H), 7.26 (d, 2 H, *J* = 8.4 Hz, 3-H, 5-H), 4.50 (s, 2 H, $CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 145.7, 144.1, 130.4, 127.8, 30.9.

**2-(*tert*-Butyldimethylsilyloxy)aniline (2).** To a stirred solution of 2-aminophenol (209.4 mg, 1.92 mmol) and imidazole (195.8 mg, 2.87 mmol) in DMF (3 mL) was treated dropwise TBSCl (346.6 mg, 2.29 mmol) at 0 °C. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was extracted with ether and washed with brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using hexane and ethyl acetate (20:1) as the eluent to give **2** (420.9 mg, 98%) as a yellow oil: $^1$H NMR (400 MHz, $CDCl_3$) δ 6.81-6.71 (m, 3 H, 3-H, 5-H, 6-H), 6.63 (m, 1 H, 4-H), 3.68 (br s, 2 H, $NH_2$), 1.03 (s, 9 H, *tert*-butyl), 0.25 (s, 6 H, 2 × $CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 143.2, 138.3, 122.1, 118.7, 118.6, 115.9, 26.1, 18.5, −4.0.

**4-(Bromomethyl)-*N*-(2-(*tert*-butyldimethylsilyloxy)phenyl)benzenesulfonamide (3).** To a stirred solution of **2** (990.5 mg, 4.43 mmol) and pyridine (0.35 mL, 4.43 mmol) in methylene chloride (5 mL) was added portionwise compound **1** (1.193 g, 4.43 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was quenched with 0.5 *N* HCl (3 mL). The aqueous layer was extracted with methylene chloride and washed with saturated $NaHCO_3$ solution and the organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using hexane and ethyl acetate (10:1) as the eluent to give arylsulfonamide **3** (1.630 g, 81%) as a yellow oil: $^1$H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, 2 H, *J* = 8.0 Hz, 2'-H, 6'-H), 7.58 (dd, 1 H, *J* = 2.0, 8.0 Hz, 6-H), 7.41 (d, 2 H, *J* = 8.0 Hz, 3'-H, 5'-H), 6.96 (td, 1 H, *J* = 2.0, 8.0 Hz, 5-H), 6.92 (td, 1 H, *J* = 1.5, 7.5 Hz, 4-H), 6.85 (s, 1 H, NH), 6.72 (dd, 1 H, *J* = 1.5, 7.5 Hz, 3-H), 4.55 (s, 2 H, $CH_2$), 0.94 (s, 9 H, *tert*-butyl), 0.12 (s, 6 H, 2 × $CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 145.8, 142.7, 139.4, 129.7, 129.1, 127.7, 125.4, 121.9, 121.2, 117.9, 45.0, 26.0, 18.3, −4.2.

**4-(Bromomethyl)-*N*-(2-hydroxyphenyl)benzenesulfonamide (4).** To a solution of **3** (1.855 g, 4.06 mmol) in THF (7 mL) was added 6 *N* HCl (4.62 mL, 6.90 mmol) and the reaction mixture was stirred at room temperature for 12 h. And then the reaction mixture was treated with saturated $NaHCO_3$ solution (10 mL) and partitioned between ethyl

acetate and brine and the organic layer was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using hexane and ethyl acetate (2.5:1) as the eluent to give **4** (1.292 g, 93%) as a yellow solid: mp 137.7-139.4 °C; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, 2 H, *J* = 8.5 Hz, 2'-H, 6'-H), 7.45 (d, 2 H, *J* = 8.0 Hz, 3'-H, 5'-H), 7.05 (td, 1 H, *J* = 1.5, 8.0 Hz, 4-H), 6.94 (dd, 1 H, *J* = 1.5, 8.0 Hz, 6-H), 6.87 (dd, 1 H, *J* = 1.5, 8.0 Hz, 3-H), 6.78 (s, 1 H, NH), 6.61 (td, 1 H, *J* = 1.5, 8.0 Hz, 5-H), 6.50 (br s, 1 H, OH), 4.57 (s 2 H, $CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 149.9, 142.9, 138.2, 129.2, 128.1, 128.1 125.2, 123.1, 121.3, 116.9, 45.0.

**4-(*N*-(2-Hydroxyphenyl)sulfamoyl)benzyl Acetate (5).** To a solution of **4** (46.4 mg, 0.14 mmol) and TBAI (25 mg, 0.07 mmol) in DMF (2 mL) was added sodium acetate (16.7 mg, 0.20 mmol) and acetic acid (0.04 mL. 0.66 mmol) and the reaction mixture was stirred at 50 °C for 12 h. After cooling, the reaction mixture was evaporated *in vacuo* and the resulting residue was partitioned between ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using hexane and ethyl acetate (2:1) as the eluent to give acetate **5** (37.7 mg, 87%) as a yellow solid: mp 148.4-150.0 °C; $^1$H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, 2 H, *J* = 8.4 Hz, 3'-H, 5'-H), 7.36 (d, 2 H, *J* = 8.4 Hz, 2'-H, 6'-H), 7.02 (t, 1 H, *J* = 8.0 Hz, 4-H), 6.93 (d, 1 H, *J* = 8.0 Hz, 6-H), 6.83 (d, 1 H, *J* = 8.0 Hz, 3-H), 6.74 (t, 1 H, *J* = 8.4 Hz, 5-H), 6.64 (br s, 2 H, OH, NH), 5.10 (s, 2 H, $CH_2$), 2.10 (s, 3 H, $CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.9, 150.3, 141.8, 137.9, 128.2, 128.1, 127.9, 125.6, 123.1, 121.3, 117.0, 65.2, 21.1.

**4-(*N*-(2-((2-Methoxyethoxy)methoxy)phenyl)-*N*-((2-methoxyethoxy) methyl)sulfamoyl)benzyl acetate (6).** To a stirred solution of **5** (685.2 mg, 2.45 mmol) in DMF (5 mL) were added ($i$-Pr)$_2$NEt (0.61 mL, 5.39 mmol) and MEMCl (1.41 mL, 8.09 mmol) and the reaction mixture was stirred at room temperature for 12 h. After the reaction mixture was concentrated *in vacuo*, the resulting residue were partitioned between ethyl acetate and water and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using hexane and ethyl acetate (1:1) as the eluent to give **6** (955 mg, 78%) as a white oil: $^1$H NMR (500 MHz, $CDCl_3$) δ 7.67 (d, 2 H, *J* = 7.5 Hz, 3'-H, 5'-H), 7.39 (d, 2 H, *J* = 8.0 Hz, 2'-H, 6'-H), 7.27 (td, 1 H, *J* = 1.5, 7.5 Hz, 5-H), 7.23 (dd, 1 H, *J* = 2.0, 8.0 Hz, 3-H), 7.13 (dd, 1 H, *J* = 1.5, 8.5 Hz, 6-H), 6.97 (td, 1 H, *J* = 1.5, 7.5 Hz, 4-H), 5.13 (s, 4 H, $CH_2$, $OCH_2O$), 4.78 (s, 2 H, $NCH_2O$), 3.83 (m, 2 H, $OCH_2OCH_2CH_2$), 3.58 (m, 2 H, $OCH_2OCH_2CH_2$), 3.54 (m, 2 H, $NCH_2OCH_2CH_2$), 3.47 (m, 2 H, $NCH_2OCH_2CH_2$), 3.35 (s, 3 H, $OCH_3$), 3.34 (s, 3 H, $OCH_3$), 2.13 (s, 3 H, $COCH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.7, 154.5, 141.0, 140.6, 133.0, 130.5, 128.0, 127.9, 126.6, 122.1, 115.4, 93.6, 81.4, 71.7, 71.6, 67.8, 67.6, 65.3, 59.2, 21.0.

**4-(Hydroxymethyl)-*N*-(2-((2-methoxyethoxy)methoxy)-phenyl)-*N*-((2-methoxyethoxy)methyl)benzenesulfonamide (7).** To a solution of **6** (249.9 mg, 0.50 mmol) in THF was added methylamine (0.6 mL, 40% in $H_2O$) and the reaction mixture was stirred at room temperature for 12 h. And then the reaction mixture was evaporated *in vacuo* and the resulting residue was partitioned between ethyl acetate and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The resulting residue was purified by silica gel column chromatography using hexane and ethyl acetate (2:1) as the eluent to give **7** (228.4 mg, 99%) as a white oil: $^1$H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, 2 H, *J* = 8.5 Hz, 2'-H, 6'-H), 7.40 (d, 2 H, *J* = 8.0 Hz, 3'-H, 5'-H), 7.29 (dd, 1 H, *J* = 2.0, 8.0 Hz, 3-H), 7.25 (td, 1 H, *J* = 1.5, 8.0 Hz, 5-H), 7.08 (dd, 1 H, *J* = 1.5, 8.0 Hz, 6-H), 6.98 (td, 1 H, *J* = 1.5, 8.0 Hz, 4-H), 5.15 (br s, 2 H, $OCH_2O$), 4.81 (s, 2 H, $CH_2$), 4.71 (s, 2 H, $NCH_2O$), 3.85 (m, 2 H, $OCH_2OCH_2CH_2$), 3.54 (m, 2 H, $NCH_2OCH_2CH_2$), 3.44 (m, 4 H, $OCH_2OCH_2CH_2$, $NCH_2OCH_2CH_2$), 3.35 (s, 3 H, $OCH_3$), 3.35 (s, 3 H, $OCH_3$), 3.34 (br s, 1 H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 154.4, 146.3, 139.8, 133.4, 130.4, 127.9, 126.8, 126.4, 122.1, 115.3, 93.7, 81.3, 71.7, 71.5, 67.6, 67.5, 64.4, 59.2, 59.2; LRMS(ESI) *m/z* 456 $(M+H)^+$.

**4-(*N*-(2-((2-Methoxyethoxy)methoxy)phenyl)-*N*-((2-methoxyethoxy) methyl)sulfamoyl)benzyl phenylcarbamate (8).** To a stirred solution of **7** (80 mg, 0.17 mmol) in anhydrous methylene chloride (2 mL) were added TEA (0.12 mL, 0.86 mmol) and phenyl isocyanate (0.05 mL, 0.45 mmol) and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was partitioned between methylene chloride and $H_2O$ and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using hexane and ethyl acetate (1.5:1) as the eluent to give phenyl carbamate **8** (98.2 mg, 97%) as a white oil: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, 2 H, *J* = 8.8 Hz, 3'-H, 5'-H), 7.38 (d, 2 H, *J* = 8.8 Hz, 2'-H, 6'-H), 7.29-7.21 (m, 5 H, 2-H, 3-H, 5-H, 6-H, 5"-H), 7.07 (dd, 1 H, *J* = 1.6, 8.0 Hz, 3"-H), 7.07 (dd, 1 H, *J* = 1.2, 8.4 Hz, 6"-H), 7.02 (t, 1 H, *J* = 7.6 Hz, 4-H), 6.94 (td, 1 H, *J* = 1.2, 7.6 Hz, 4"-H), 5.17 (s, 2 H, $CH_2$), 5.12 (br s, 2 H, $OCH_2OCH_2$), 4.70 (s, 2 H, $NCH_2OCH_2$), 3.81 (m, 2 H, $OCH_2OCH_2CH_2$), 3.50 (m, 2 H, $NCH_2OCH_2CH_2$), 3.43 (m, 4 H, $OCH_2OCH_2CH_2$, $NCH_2OCH_2CH_2$), 3.31 (s, 3 H, $OCH_3$), 3.30 (s, 3 H, $OCH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 154.4, 153.3, 141.3, 140.7, 138.0, 129.3, 128.2, 127.9, 127.0, 124.4, 123.8, 122.1, 120.2, 118.9, 115.4, 93.7, 81.4, 71.7, 71.6, 67.8, 67.6, 65.9, 59.2, 59.1.

***O*-4-(*N*-(2-((2-Methoxyethoxy)methoxy)phenyl)-*N*-((2-methoxyethoxy) methyl)sulfamoyl)benzyl phenylcarbamothioate (9).** To a stirred solution of **7** (100 mg, 0.22 mmol) in anhydrous methylene chloride (2 mL) was added TEA (0.12 mL, 1.07 mmol) and phenyl isothiocyanate (0.07 mL, 0. 58 mmol) and the reaction mixture was refluxed for 12 h. The reaction mixture was partitioned between methylene chloride and $H_2O$ and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using hexane and ethyl acetate (1.5:1) as the eluent to give

phenyl thiocarbamate **9** (40.2 mg, 38%) as a white oil: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, 2 H, *J* = 8.0 Hz, 3'-H, 5'-H), 7.39-7.12 (m, 10 H, 2-H, 3-H, 4-H, 5-H, 6-H, 2'-H, 6'-H, 3''-H, 5''-H, 6''-H), 6.93 (t, 1 H, *J* = 7.6 Hz, 4''-H), 5.63 (br s, 2 H, $CH_2$), 5.10 (br s, 2 H, $OC\mathit{H}_2OCH_2$), 4.67 (br s, 2 H, $NC\mathit{H}_2OCH_2$), 3.80 (m, 2 H, $OCH_2OC\mathit{H}_2CH_2$), 3.50 (m, 4 H, $OCH_2OCH_2C\mathit{H}_2$, $NCH_2OCH_2C\mathit{H}_2$), 3.43 (m, 2 H, $NCH_2OC\mathit{H}_2$), 3.31 (s, 3 H, $OCH_3$), 3.29 (s, 3 H, $OCH_3$).

**4-(*N*-(2-Hydroxyphenyl)sulfamoyl)benzyl phenylcarbamate (10).** To a solution of **8** (65.8 mg, 0.11 mmol) in methanol/$H_2O$ (5:1) was added dropwise 12 *N* HCl until the pH became 1-2. The reaction mixture was stirred at 60 ºC for 24 h. After cooling, the reaction mixture was evaporated *in vacuo*, the resulting residue was partitioned between ethyl acetate and $H_2O$, and the organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give an oil, which was purified by silica gel column chromatography using hexane and ethyl acetate (2:1) as the eluent to give final phenyl carbamate linked arylsulfonamide **10** (30.2 mg, 66%) as a white solid: mp 171.3-172.6 ºC; $^{1}$H NMR (400 MHz, $CD_3OD$) δ 7.72 (d, 2 H, *J* = 8.4 Hz, 3'-H, 5'-H), 7.42 (d, 2 H, *J* = 8.4 Hz, 2'-H, 6'-H), 7.39 (d, 2 H, *J* = 7.6 Hz, 2-H, 6-H), 7.27-7.22 (m, 3 H, 3-H, 5-H, 6''-H), 6.99 (t, 1 H, *J* = 7.6 Hz, 4-H), 6.89 (td, 1 H, *J* = 1.6, 7.2 Hz, 4''-H), 6.69 (td, 1 H, *J* = 1.6, 7.6 Hz, 5''-H), 6.65 (dd, 1 H, *J* = 1.2, 8.0 Hz, 3''-H), 5.17 (s, 2 H, $CH_2$); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 154.3, 150.0, 142.1, 139.5, 138.8, 128.7, 127.4, 127.4, 126.3, 124.3, 124.2, 123.0, 119.3, 118.7, 115.1, 65.2; LRMS (ESI) *m/z* 397 (M-H)$^{-}$.

***O*-4-(*N*-(2-Hydroxyphenyl)sulfamoyl)benzyl phenylcarbamothioate (11).** Phenyl thiocarbamate linked arylsulfonamide **11** (38%, a white solid) was prepared by a similar procedure for the synthesis of **10**: mp 133.8-134.9 ºC; $^{1}$H NMR (400 MHz, $CD_3OD$) δ 7.72-7.11 (m, 10 H, 2-H, 3-H, 4-H, 5-H, 6-H, 2'-H, 3'-H, 5'-H, 6'-H, 5''-H), 6.90 (td, 1 H, *J* = 1.6, 7.6 Hz, 4''-H), 6.70 (td, 1 H, *J* = 1.2, 7.6 Hz, 6''-H), 6.67 (dd, 1 H, *J* = 1.6, 8.4 Hz, 3''-H), 5.54 (s, 2 H, $CH_2$); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 188.1, 149.9, 141.8, 141.0, 139.5, 128.4, 127.7, 127.4, 126.3, 125.2, 124.3, 124.1, 122.9, 119.4, 115.2, 71.4; LRMS (ESI) *m/z* 413 (M-H)$^{-}$.

**4-(*N*-(2-((2-Methoxyethoxy)methoxy)phenyl)-*N*-((2-methoxyethoxy) methyl)sulfamoyl)benzyl benzylcarbamate (12).** Benzyl carbamate **12** (89%, a white oil) was prepared by a similar procedure for the synthesis of **8**: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, 2 H, *J* = 8.0 Hz, 3'-H, 5'-H), 7.37 (d, 2 H, *J* = 8.0 Hz, 2'-H, 6'-H), 7.31-7.22 (m, 7 H, Ph, 3''-H, 5''-H), 7.08 (br d, 1 H, *J* = 8.0 Hz, 6''-H), 6.95 (td, 1 H, *J* = 1.2, 7.6 Hz, 4''-H), 5.13 (s, 4 H, $CH_2OC(=O)$, -$OC\mathit{H}_2OCH_2$), 4.70 (s, 2 H, -$NC\mathit{H}_2OCH_2$), 4.36 (d, 2 H, *J* = 5.6 Hz, $NC\mathit{H}_2Ph$), 3.82 (m, 2 H, $OCH_2OC\mathit{H}_2CH_2$), 3.52 (m, 2 H, $NCH_2OC\mathit{H}_2CH_2$), 3.48 (m, 2 H, $OCH_2OCH_2C\mathit{H}_2$), 3.41 (m, 2 H, $NCH_2OCH_2C\mathit{H}_2$), 3.33 (s, 3 H, $OCH_3$), 3.27 (s, 3 H, $OCH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 156.2, 154.4, 143.0, 141.6, 140.6, 138.4, 133.3, 130.5, 128.9, 128.0, 127.9, 127.8, 126.5, 122.1, 115.4, 94.6, 81.4, 71.7, 71.6, 67.8, 67.6, 65.9, 59.2, 59.1, 45.4.

***O*-4-(*N*-(2-((2-Methoxyethoxy)methoxy)phenyl)-*N*-((2-methoxyethoxy) methyl)sulfamoyl)benzyl benzylcarbamothioate (13).** Benzyl thiocarbamate **13** (34%, a white oil) was prepared by a similar procedure for the synthesis of **9**: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, 2 H, *J* = 8.0 Hz, 3'-H, 5'-H), 7.38 (d, 2 H, *J* = 8.0 Hz, 2'-H, 6'-H), 7.33-7.20 (m, 7 H, Ph, 3''-H, 5''-H), 7.06 (d, 1 H, *J* = 8.0 Hz, 6''-H), 6.95 (t, 1 H, *J* = 8.4 Hz, 4''-H), 5.52 (s, 2 H, $CH_2OC(=S)$), 5.11 (br s, 2 H, $OC\mathit{H}_2OCH_2$), 4.74 (d, 2 H, *J* = 5.6 Hz, $NC\mathit{H}_2Ph$), 4.69 (s, 2 H, $NC\mathit{H}_2OCH_2$), 3.82 (m, 2 H, $OCH_2OC\mathit{H}_2CH_2$), 3.51 (m, 2 H, $NCH_2OC\mathit{H}_2CH_2$), 3.42 (m, 2 H, $OCH_2OCH_2C\mathit{H}_2$), 3.39 (m, 2 H, $NCH_2OCH_2C\mathit{H}_2$), 3.32 (s, 3 H, $OCH_3$), 3.23 (s, 3 H, $OCH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 190.1, 154.3, 140.9, 140.8, 140.6, 136.8, 133.4, 130.5, 129.0, 128.3, 128.2, 127.9, 126.4, 122.2, 115.4, 93.6, 81.4, 71.7, 71.6, 70.7, 67.8, 67.6, 59.2, 59.1, 49.8.

**4-(*N*-(2-Hydroxyphenyl)sulfamoyl)benzyl benzylcarbamate (14).** Benzyl carbamate linked arylsulfonamide **14** (89%, a white solid) was prepared by a similar procedure for the synthesis of **10**: mp 127.9-129.0 ºC; $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.67 (d, 2 H, *J* = 7.5 Hz, 3'-H, 5'-H), 7.33-7.23 (m, 7 H, Ph, 2'-H, 6'-H), 7.00 (m, 2 H, 4''-H, 6''-H), 6.92 (br s, 1 H, $NHSO_2$), 6.86 (br s, 1 H, OH), 6.80 (d, 1 H, *J* = 8.0 Hz, 3''-H), 6.74 (t, 1 H, *J* = 7.5 Hz, 5''-H), 5.33 (br t, 1 H, *J* = 6.0 Hz, 4-$CH_2N\mathit{H}$), 5.09 (s, 2 H, $CH_2OC(=O)$), 4.35 (d, 2 H, *J* = 6.0 Hz, $NC\mathit{H}_2Ph$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 156.5, 149.9, 142.3, 138.1, 138.0, 129.0, 127.9, 127.8, 127.8, 127.8, 127.7, 125.1, 123.4, 121.1, 116.7, 65.8, 45.4; LRMS (ESI) *m/z* 411 (M-H)$^{-}$.

***O*-4-(*N*-(2-Hydroxyphenyl)sulfamoyl)benzyl benzylcarbamothioate (15).** Benzyl thiocarbamate linked arylsulfonamide **15** (46%, a white solid) was prepared by a similar procedure for the synthesis of **10**: mp 129.6-131.1 ºC; $^{1}$H NMR (400 MHz, $CD_3OD$) δ 7.70 (d, 2 H, *J* = 8.4 Hz, 3'-H, 5'-H), 7.42 (d, 2 H, *J* = 8.4 Hz, 2'-H, 6'-H), 7.28-7.13 (m, 6 H, Ph, 6''-H), 6.90 (td, 1 H, *J* = 1.6, 7.6 Hz, 4''-H), 6.71-6.64 (m, 2 H, 3''-H, 5''-H), 5.12 (s, 2 H, $CH_2OC(=S)$), 4.69 (s, 2 H, $NC\mathit{H}_2Ph$); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 190.8, 150.0, 142.0, 139.4, 137.9, 128.3, 127.4, 127.3, 127.2, 127.1, 126.3, 124.1, 124.1, 119.3, 115.1, 69.8, 46.3; LRMS (ESI) *m/z* 427 (M-H)$^{-}$.

**4-(*N*-(2-((2-Methoxyethoxy)methoxy)phenyl)-*N*-((2-methoxyethoxy) methyl)sulfamoyl)benzyl naphthalen-1-ylcarbamate (16).** Naphthyl carbamate **16** (81%, a white oil) was prepared by a similar procedure for the synthesis of **8**: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.86 (m, 2 H), 7.67 (m, 3 H), 7.53-7.41 (m, 5 H), 7.24 (m, 3 H), 7.08 (dd, 1 H, *J* = 1.2, 8.8 Hz, 6''-H), 6.95 (td, 1 H, *J* = 1.2, 7.6 Hz, 4''-H), 5.25 (s, 2 H, $CH_2OC(=O)$), 5.12 (br s, 2 H, $OC\mathit{H}_2OCH_2$), 4.73 (s, 2 H, $NC\mathit{H}_2OCH_2$), 3.82 (m, 2 H, $OCH_2OC\mathit{H}_2CH_2$), 3.52 (m, 2 H, $NCH_2OC\mathit{H}_2CH_2$), 3.48 (m, 2 H, $OCH_2OCH_2C\mathit{H}_2$), 3.41 (m, 2 H, $NCH_2OCH_2C\mathit{H}_2$), 3.32 (s, 3 H, $OCH_3$), 3.25 (s, 3 H, $OCH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 154.4, 141.2, 140.8, 134.3, 133.2, 132.4, 130.5, 129.0, 128.2, 128.0, 128.0, 126.6, 126.5, 126.3, 125.9, 125.8, 122.2, 120.8, 119.6, 115.4, 93.7, 81.4, 71.7, 71.6, 67.9, 67.6, 66.3, 59.2, 59.1.

**4-(*N*-(2-Hydroxyphenyl)sulfamoyl)benzylnaphthalen-1-ylcarbamate (17).** Naphthyl carbamate linked arylsulfonamide **17** (79%, a white solid) was prepared by a similar

procedure for the synthesis of **10**: mp 87.6-89.9 °C; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.86 (m, 2 H), 7.71 (m, 3 H), 7.52-7.42 (m, 6 H), 7.09 (br s, 1 H, $NHSO_2$), 7.01 (td, 1 H, $J$ = 1.5, 8.0 Hz, 4''-H), 6.94 (d, 1 H, $J$ = 7.5 Hz, 6''-H), 6.79 (dd, 1 H, $J$ = 1.5, 8.0 Hz, 3''-H), 6.74 (td, 1 H, $J$ = 1.5, 7.5 Hz, 5''-H), 6.64 (s, 1 H, OH), 6.43 (s, 1 H, C(=O)NH), 5.26 (s, 2 H, $CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 150.1, 142.0, 138.1, 134.3, 132.1, 129.0, 128.1, 128.0, 127.9, 127.9, 126.7, 126.4, 125.9, 125.4, 123.2, 121.2, 120.7, 120.1, 116.8, 66.2; LRMS (ESI) *m/z* 447 $(M-H)^-$.

**General for Cell Culture, Cell Viability Test and Assay of HDAC Enzyme Activity.** All chemicals used for bioassay were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trichostatin A and test compounds **10**, **11**, **14**, **15** and **17** were dissolved in sterile dimethyl sulfoxide (DMSO) to generate a 10 mM stock solution. The solution was stored at 80 °C. Subsequent dilutions were made in RPMI-1640 (Gibco, Rockville, MD, USA).

**Cell Lines and Culture.** The cell line, MCF-7 cells were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). All cells were maintained as monolayers at 37 °C in an atmosphere containing 5% $CO_2$/air in RPMI-1640 (Gibco, Rockville, MD) containing 2 mM L-glutamate, 10% heat-inactivated fetal bovine serum (FBS, Gibco), 1.25 mM HEPES (Gibco) and 100 U/mL penicillin/streptomycin (Gibco).

**Cell Viability Test.** Cytotoxicity was assessed by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. For the compound treatment, the cells were plated for 48 h in RPMI-1640 containing 10% FBS. The medium was then changed to RPMI-1640 containing 5% charcoal-dextran treated FBS (CD-FBS) with various concentrations of test compounds, **10**, **11**, **14**, **15** and **17**. The cultures were initiated in 96-well microtiter plates at a density of $1 \times 10^4$ cells per well. Cells were allowed to attach for 48 h before treatment with **10**, **11**, **14**, **15** and **17**. At the end of the treatment period, 15 μL of the MTT reagent (5 mg/mL) was added to each well. After 4 h incubation at 37 °C, the supernatant was aspirated, and formazan crystals were dissolved in 100 μL DMSO at 37 °C for 10 min with gentle agitation. The absorbance per well was measured at 540 nm with a VERS Amax Microplate Reader (Molecular Devices Corp.). Assay was done in triplicate. The $IC_{50}$ values were then determined for each compounds from a plot of log (compound concentration) versus percentage of cell killed.

**Assay of HDAC Enzyme Activity.** The HDAC enzymes were purchased from BPS Bioscience (San Diego, CA, USA) and the HDAC enzyme assay was performed using a Fluorogenic HDAC Assay Kit (BPS Bioscience) according to the manufacturer's instructions. Briefly, HDAC enzymes were incubated with vehicle or various concentrations of trichostatin A, **10**, **11**, **14**, **15** and **17** for 30 min at 37 °C in the presence of an HDAC fluorimetric substrate. The HDAC assay developer (which produces a fluorophore in the reaction mixture) was added, and the fluorescence was measured using VICTOR 3 (PerkinElmer, Waltham, MA, USA) with excitation at 360 nm and emission at 460 nm. The measured activities were substracted by calculated using GraphPad Prism (Graphpad Software, San Diego, CA, USA).

**Acknowledgments.** This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2010-0012038).

## References


1. Wu, J.; Grunstein, M. *Trends Biochem. Sci.* **2000**, *25*, 619.
2. Luger, K.; Mader, A. W.; Richmond, R. K.; Sargent, D. F.; Richmond, T. J. *Nature* **1997**, *389*, 251.
3. Marmorstein, R.; Roth, S. Y. *Curr. Opin. Genet. Dev.* **2001**, *11*, 155.
4. Hassig, C. A.; Schreiber, S. L. *Curr. Opin. Chem. Biol.* **1997**, *1*, 300.
5. Johnstone, R. W. *Nat. Rev. Drug Discov.* **2002**, *1*, 287.
6. Thiagalingam, S.; Cheng, K.-H.; Lee, H. J.; Mineva, N.; Thiagalingam, A.; Ponte, J. F. *Ann. N. Y. Acad. Sci.* **2003**, *983*, 84.
7. Roth, S. Y.; Denu, J. M.; Allis, C. D. *Ann. Rev. Biochem.* **2001**, *70*, 81.
8. Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat. Rev. Drug Discov.* **2006**, *5*, 769.
9. Marks, P. A.; Rifkind, R. A.; Richon, V. M.; Breslow, R.; Miller, T.; Kelly, W. K. *Nat. Rev. Cancer.* **2001**, *1*, 194.
10. Lindemann, R. K.; Gabrielli, B.; Johnstone, R. W. *Cell Cycle* **2004**, *3*, 777.
11. Suzuki, T.; Ando, T.; Tsuchiya, K.; Fukazawa, N. *J. Med. Chem.* **1999**, *42*, 3001.
12. Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; Ando, T.; Suzuki, T.; Tsuruo, T.; Nakanishi, O. *Proc. Natl. Acad. Sci.* **1999**, *96*, 4592.
13. Meinke, P. T.; Liberator, P. *Curr. Med. Chem*. **2001**, *8*, 211.
14. Bouchain, G.; Delorme, D. *Curr. Med. Chem*. **2003**, *10*, 2359.
15. Hess-Stumpp, H.; Bracker, T. U.; Henderson, D.; Politz, O. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 1388.
16. Wang, D.-F.; Helquist, P.; Wiech, N. L.; Wiest, O. *J. Med. Chem.* **2005**, *48*, 6936.
17. Michaelides, M. R.; Dellaria, J. F.; Gong, J.; Holms, J. H.; Bouska, J. J.; Stacey, J.; Wada, C. K.; Heyman, H. R.; Curtin, M. L.; Guo, Y.; Goodfellow, C. L.; Elmore, I. B.; Albert, D. H.; Magoc, T. J.; Marcotte, P. A.; Morgan, D. W.; Davidsen, S. K. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1553.
18. Curtin, M.; Glaser, K. *Curr. Med. Chem*. **2003**, *10*, 2373.
19. Nagaoka, Y.; Maeda, T.; Kawai, Y.; Nakashima, D.; Oikawa, T.; Shimoke, K.; Ikeuchi, T.; Kuwajima, H.; Uesato, S. *Eur. J. Med. Chem*. **2006**, *41*, 697.