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Search for novel histone deacetylase inhibitors. Part II: Design and synthesis of novel isoferulic acid derivatives

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ABSTRACT

Previously, we described the discovery of potent ferulic acid-based histone deacetylase inhibitors (HDACIs) with halogeno-acetanilide as novel surface recognition moiety (SRM). In order to improve the affinity and activity of these HDACIs, twenty seven isoferulic acid derivatives were described herein. The majority of title compounds displayed potent HDAC inhibitory activity. In particular, **IF5** and **IF6** exhibited significant enzymatic inhibitory activities, with IC_{50} values of 0.73 ± 0.08 and 0.57 ± 0.16 μM, respectively. Furthermore, these compounds showed moderate antiproliferative activity against human cancer cells. Especially, **IF6** displayed promising profile as an antitumor candidate with IC_{50} value of 3.91 ± 0.97 μM against HeLa cells. The results indicated that these isoferulic acid derivatives could serve as promising lead compounds for further optimization.

1. Introduction

Histone deacetylase (HDAC) inhibition is a clinically validated therapeutic strategy for cancer treatment. HDAC are involved in remodelling of chromatin and play an essential role in cell proliferation, cell-cycle regulation and apoptosis.[1] An aberrant activity of HDACs has been documented in several human cancers leading to development of histone deacetylase inhibitors (HDACIs) as anticancer agents.[2] Therefore, HDAC is becoming a prominent therapeutic target for the treatment of cancer. Research of HDACIs is becoming an interesting field in anticancer agent design.

Classical HDACs are zinc-dependent enzymes bearing a highly conserved catalytic domain with a zinc ion.[3] So far, a wide range of natural and synthetic derivatives have been identified as potent HDACIs. They are structurally diverse group compounds with attractive antitumor properties. Majority of them consists of a zinc binding group (ZBG) interacting with the zinc ion. Moreover, HDACIs share other common features such as a linker domain, which occupies the narrow channel; a connect unit (CU), which connects SRM and linker; and a surface recognition moiety (SRM), which interacts with residues on the rim of active site (Fig. 1).

Vorinostat (SAHA) is the first HDACI approved by the FDA for the treatment of CTCL in 2006. Belinostat has been granted orphan drug and fast track designation by the FDA. Panobinostat developed by Novartis for the treatment of various cancers is a non-selective HDACI. Entinostat is an oral benzamide HDACI undergoing clinical trials for the treatment of various cancer.[4]

In the course of search for novel HDACIs with novel structure and higher potency, we have developed several ferulic acid derivatives with potent HDAC inhibitory activity and antiproliferative property.[5] It was validated that phenyl-substituted ethylene could serve as rigid linker of HDACIs. Moreover, halogeno-acetanilides were also confirmed to be suitable as SRM of HDACIs. However, in the molecular docking study of ferulic acid derivatives with HDAC, we found that acetanilide did not form any hydrogen binding with SRM as we initially supposed.

On the basis of these observations, we aimed to enhance the structural diversity and affinity with the SRM of HDAC. Rearrangement of acetanilide to *meta*-position was performed to afford corresponding isoferulic acid derivatives. In this study, ferulic acid was replaced by isoferulic acid as rigid linker of HDACIs. The halogeno-acetanilide was incorporated at *meta*-position instead of *para*-position. We supposed that *para*-position might be more suitable for interaction with SRM. These novel HDACIs comprised common hydroxamic acid or 2-aminobenzamide group as ZBG as previously reported (Fig. 2). The conformations of these compounds were more similar to that of SAHA.

As part of our ongoing effort to develop HDACIs with higher affinity and activity, we developed two series of isoferulic acid derivatives bearing halogeno-acetanilide at *meta*-position. The structures of these compounds are quite consistent with common pharmacophore of HDACIs. The binding mode of the most potent

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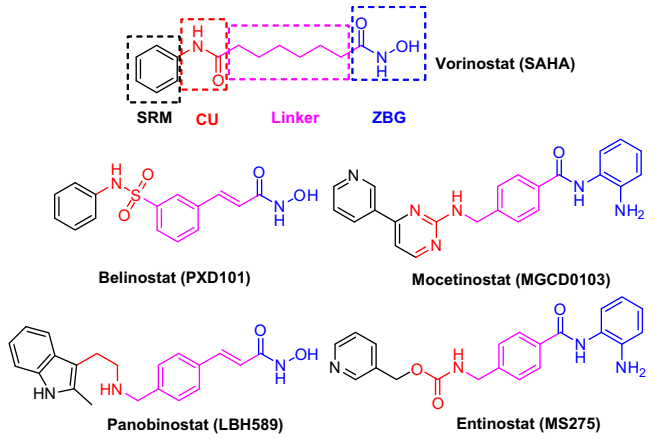


Figure 1. Structures and pharmacophore features of HDACIs.

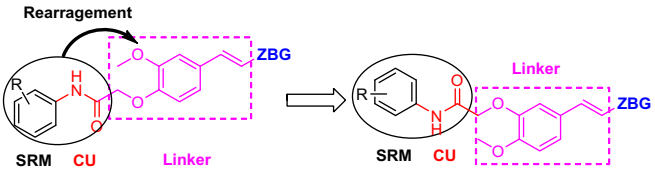


Figure 2. Design strategy and structures of isoferulic acid derivatives.

compound with HDAC was also established in order to analysis the interaction of acetanilide with SRM. Herein, we describe the discovery and evaluation of isoferulic acid-based HDACIs.

2. Chemistry

The synthetic routes of the title compounds were outlined in Schemes 1 and 2. An efficient synthesis of hydroxamic acids was developed in 5-step reaction sequence (Scheme 1). Isoferulic acid **2** was prepared from isovanillin **1** utilizing classical Knoevenagel condensation reaction conditions.[6] Isoferulic acid was esterified in the presence of concentrated H_2SO_4 to afford isoferulic acid methylester **3**. The hydroxyl group at *meta*-position of (**2**) was etherified with various substituted acetanilides in anhydrous acetone in the presence of K_2CO_3 to afford corresponding intermediates **4a–4i**.[7] These resulting esters **4a–4i** were treated with methanolic NH_2OK at room temperature to yield corresponding hydroxamic acid derivatives **IF1–IF9**.[8]

Here again, we have developed a new two-step procedure for preparation of isoferulic acid derivatives bearing 2-aminobenzamide as ZBG (Scheme 2). Isoferulic acid was converted into corresponding imidazolide derivative by reaction with *N*,*N*′-carbonyldiimidazole (CDI) in THF at room temperature. This was further reacted with benzene-1,2-diamine or 4-methylbenzene-1,2-diamine in the presence of trifluoroacetic acid to afford key intermediates **5a–5b**.[9] The hydroxyl group in **5a–5b** was etherified with haloacylanilines in anhydrous acetone in the presence of K_2CO_3 to afford corresponding 2-aminobenzamide-containing derivatives **IF10–IF27**.[10]

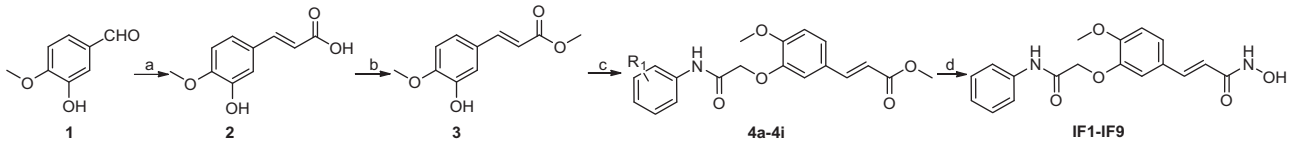
3. Results and discussion

All the synthesized compounds were tested for their HDAC inhibitory activity with SAHA as positive control. It was indicated from Table 1 that majority of them displayed moderate to high inhibitory activity against HDAC. Some of the title compounds were shown to inhibit HDAC with IC_{50} values below the micromolar range. In general, hydroxamic acids were found to be more potent than benzamides. **IF6** was the most potent HDAC inhibitor with an IC_{50} value of 0.57 ± 0.16 μM, while **IF4** and **IF5** also displayed potent HDAC inhibitory activities with IC_{50} values of 0.94 ± 0.26 μM and 0.73 ± 0.08 μM, respectively. The variety and position of halogen-substitution on aniline played important role in biological activity. Benzamides **IF15** and **IF11** were active at higher doses with IC_{50} values of 15.60 ± 3.28 μM and 22.16 ± 2.37 μM, respectively. In contrast, six benzamide derivatives were inactive at 200 μM. According to the results, both substitution on aniline and structure of ZBG played important role in potency. It was indicated that compounds bearing substituents like fluorine, bromine and trifluoromethyl on aniline possessed potent anticancer activity. For ZBG, hydroxamic acid was more suitable for these HDACIs than benzamide.

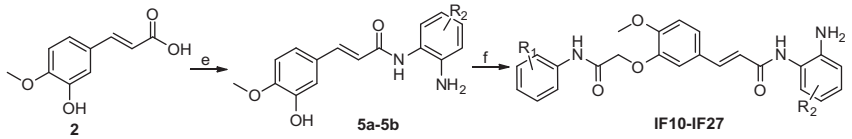
To further investigate their antiproliferative activity, six compounds (five of hydroxamic acids and one of benzamide) were selected to test for their antiproliferative potential by MTT method. The test compounds were evaluated for their anticancer potency against HeLa and MDA-MB-231 cell lines. The results were described in Table 2. It was found that they displayed potent antiproliferative activities with IC_{50} values ranging from 1.90 ± 0.04 μM to 20.51 ± 1.36 μM. Some of them exhibited promising antiproliferative activity against MDA-MB-231. **IF3** displayed the most potent antiproliferative potency against MDA-MB-231 comparable with SAHA. Moreover, **IF6** exhibited the most potent growth inhibition against HeLa cell line. The antiproliferative results were consistent with the HDAC inhibitory assays. It was indicated that inhibition of HDAC might be one of the basis for anticancer activity.

The most potent inhibitor **IF6** was selected for computational studies. Docking studies were carried out to understand interaction between inhibitors and HDAC. **IF6** was docked into active site of HDAC (PDB ID: 3F07) by SYBYL-X 2.0. Molecular insights based on molecular docking indicated favorable binding mode of **IF6** with HDAC (Fig. 3). The results suggested that hydroxamic group was bonded to zinc ion as ZBG. Hydroxamate OH made two hydrogen bond interactions with His142 and His143 with distance of 1.96 Å and 2.41 Å, respectively. N–H could also form a hydrogen bond to His143 with distance of 1.79 Å. Carbonyl group accepted a hydrogen bond from Tyr306 with distance of 1.75 Å. The distance between zinc ion and two oxygen atoms were 1.89 Å and 2.17 Å, respectively.[11] Based on these results, the binding mode of isoferulic acid-based HDACI was the same as ferulic acid-based HDACI.

However, the direction of binding with surface of active site was totally difference (Fig. 4). Moreover, there was an additional hydrogen bond between oxygen atom of acetyl and Gly151 on the surface. This interaction might contribute to affinity and activity of isoferulic

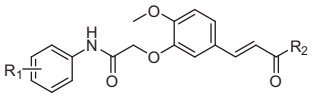


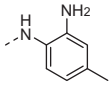
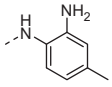
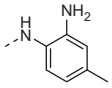
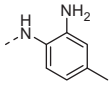
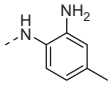
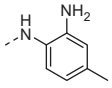
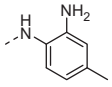
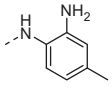
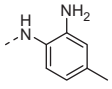
Scheme 1. Preparation of compounds **IF1–IF9**. Reagents and conditions: (a) malonic acid, DBU, pyridine; (b) CH_3OH, H_2SO_4; (c) K_2CO_3, acetone; (d) NH_2OK, NH_2OH, DMF.



Scheme 2. Preparation of compounds **IF10–IF27**. Reagents and conditions: (e) CDI, THF, *O*-phenylenediamine or 3,4-diaminotoluene, TFA; (f) K_2CO_3, acetone.

Table 1
The structures and inhibitory activity of isoferulic acid derivatives (IC_{50}, μM)

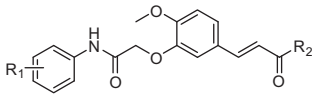


	R_1	R_2	IC_{50} (μM)		R_1	R_2	IC_{50} (μM)
IF1	3-Cl-4-F		5.82 ± 0.61	**IF15**	2-F		15.60 ± 3.28
IF2	3-CF_3		2.96 ± 0.52	**IF16**	3-F		24.01 ± 0.35
IF3	3,4-Cl		1.37 ± 0.07	**IF17**	3,5-CF_3		>200
IF4	4-OCH_3		0.94 ± 0.26	**IF18**	3-CF_3-5-Br		>200
IF5	4-Br		0.73 ± 0.08	**IF19**	3-Cl-4-F		31.32 ± 6.51
IF6	2-F		0.57 ± 0.16	**IF20**	3-CF_3		>200
IF7	3-F		2.81 ± 0.03	**IF21**	3,4-Cl		>200
IF8	3,5-CF_3		4.91 ± 0.13	**IF22**	4-OCH_3		164.61 ± 6.52
IF9	3-CF_3-5-Br		9.04 ± 0.29	**IF23**	4-Br		58.24 ± 1.78
IF10	3-Cl-4-F		66.52 ± 3.86	**IF24**	2-F		29.50 ± 5.44
IF11	3-CF_3		22.16 ± 2.37	**IF25**	3-F		71.02 ± 21.09
IF12	3,4-Cl		>200	**IF26**	3,5-CF_3		56.71 ± 7.65
IF13	4-OCH_3		49.30 ± 11.50	**IF27**	3-CF_3-5-Br		124.67 ± 42.81
IF14	4-Br		>200				
SAHA				**0.26 ± 0.08**			

The bold values mean the lowest IC_{50} value in each series.

acid-based HDACIs. The results indicated that these two kinds of HDACIs had similar binding mode and orientation as APHA in the pocket of HDAC. **IF6** did not only chelate zinc ion but also form four hydrogen bonds with His142, His143, Tyr306, and Gly151.

Table 2
Growth inhibition of HDACIs against a panel of cancer cells (IC_{50}, μM)



	R_1	R_2	HeLa	MDA-MB-231
IF3	3,4-Cl		13.35 ± 2.83	1.90 ± 0.04
IF4	4-OCH_3		4.87 ± 2.52	3.81 ± 0.86
IF5	4-Br		5.39 ± 2.03	5.87 ± 0.81
IF6	2-F		**3.91 ± 0.97**	15.63 ± 3.52
IF7	3-F		6.48 ± 0.04	3.41 ± 1.11
IF15	2-F		20.51 ± 1.36	15.51 ± 9.56
SAHA			**0.72 ± 0.55**	**1.29 ± 0.21**

The bold values mean the lowest IC_{50} value in each series.

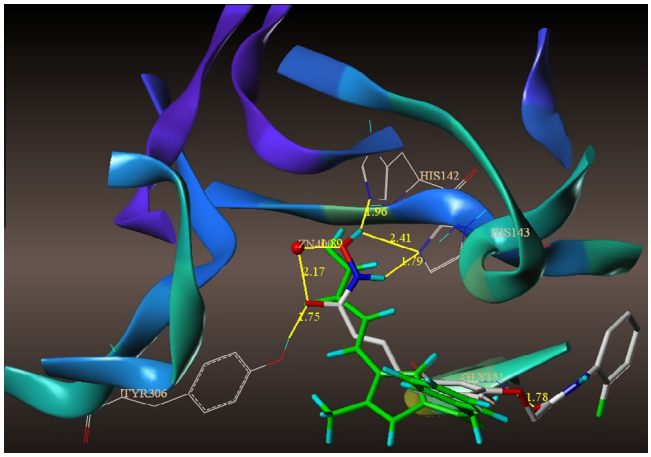


Figure 3. Molecular modeling of **IF6** binding to the active site of HDAC. The original ligands are shown in green. Metal coordination and hydrogen bond interactions are shown as dotted yellow lines.

4. Conclusion

In summary, we proved that ferulic acid and isoferulic acid derivatives with novel SRM as potent HDACIs. All the title compounds displayed varied degree of anticancer potency and some of them exhibited excellent HDAC inhibitory activity comparable to positive control. The results indicated that hydroxamic acid was more suitable for these HDACIs than benzamide. Hydroxamic acid could interact tightly with zinc ion of HDAC. Therefore, hydroxamates were somewhat more potent than benzamides. Moreover, it was confirmed that *meta*-position of styrene was more favorable for incorporation of SRM. Both ferulic acid and isoferulic acid could serve as molecular scaffold and rigid linker. Three compounds (**IF4**, **IF5**, **IF6**) exhibited significant HDAC inhibitory activities, with IC_{50} values below 1 μM. Among them, **IF6** exhibited potency comparable with SAHA. Moreover, some of them exhibited potent antiproliferative activities against MDA-MB-231 and HeLa cell lines. Molecular docking results indicated that isoferulic acid derivatives could interact more tightly with the active site of HDAC than ferulic acid derivatives. In summary, these isoferulic acid derivatives had strong potential to be further developed as novel HDACIs. Further structural optimization of these promising anticancer agents will be reported in due course.

5. Experimental

5.1. Chemistry: general procedure

All solvents and reagents were purified according to standard procedure. All reactions except those in aqueous media were carried out by standard techniques for exclusion of moisture. Petroleum ether used refers to the fraction boiling in the range 60–90 °C. Anhydrous reactions were performed over dried glassware under nitrogen atmosphere. Reactions were monitored by TLC on 0.25-mm silica gel plates (60GF-254) and visualized with UV light. Melting points were determined on electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on Bruker Advance (400 MHz) in $CDCl_3$ or DMSO-d_6 and reference to TMS. Mass spectra were obtained on Shimadzu HPLC–MS-QP2010 instrument.

5.1.1. Preparation of isoferulic acid (2) from isovanillin (1) through Knoevenagel condensation reaction

To a solution of isovanillin (**1**) (22.81 g, 150 mmol) and malonic acid (62.44 g, 600 mmol) in pyridine (150 mL) was added 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU, 34.26 g). The stirred

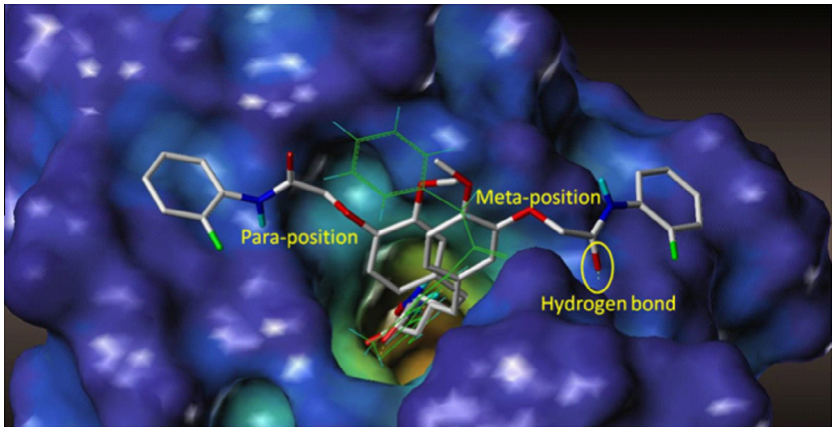


Figure 4. Molecular modeling of isoferulic acid-based HDACIs showing additional hydrogen bond with surface of active site.

reaction solution was heated at reflux for 4 h, then cooled to room temperature. The cooled reaction mixture was poured into a solution (500 mL) of concentrated HCl and ice (v/v = 1:1) and the resulting precipitate was filtered and washed with acidified water to give a crude product. The crude product was dissolved in aqueous NaOH (1 M, 200 mL) and extracted with CH_2Cl_2) (3 × 50 mL). The aqueous solution was acidified to pH = 1 and the resultant precipitate washed with acidified water to afford 22.56 g (77.2%) of isoferulic acid as white solid. Mp: 228–230 °C.

5.1.2. Methyl (2*E*)-3-(3-hydroxy-4-methoxyphenyl)acrylate (3)[12]

Isoferulic acid (6.80 g, 35 mmol) was dissolved in methanol (80 mL) containing catalytic of 2 mL concentrated H_2SO_4, and then solution was heated at reflux for about 4 h. After cooling at room temperature, solvent was evaporated under vacuum. The residue was taken up in EtOAc (150 mL) and washed with saturated aqueous solution of $NaHCO_3$ until neutral pH. The organic layer was then washed with distilled water and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to afforded (**3**) (6.65 g, 91.2%) as white solid. Mp: 74–76 °C.

5.1.3. Methyl (2*E*)-3-(3-{2-[3,5-bis(trifluoromethyl)phenyl] amino]-2-oxoethoxy}-4-methoxyphenyl)acrylate (4h)[13]

To a suspension of (**3**) (1.67 g, 8 mmol) in dehydrated acetone (100 mL) was added anhydrous potassium carbonate (3.31 g, 24 mmol). The mixture was stirred at room temperature for 30 min and then 2-chloro-*N*-[3,5-bis(trifluoromethyl)]acetamide (2.69 g, 8.8 mmol) was added. The reaction mixture was refluxed for another 10 h. Filtration and evaporation of acetone was done in vacuum. The residue was extracted with EtOAc (120 mL). The combined layers were washed with water, 2 M NaOH, 2 M HCl and brine, dried over Na_2SO_4 and solvent was removed. The crude product was purified by silica gel column chromatography (petroleum/AcOEt = 5:1 to 1:1) to yield (**4h**) as white solid (1.98 g, 71.7%). Mp: 180–182 °C. EI-MS (*m*/*z*): 476.9 (M^+). ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 2H), 7.67 (s, 1H), 7.63 (d, *J* = 8 Hz, 1H), 7.29 (d, *J* = 6 Hz, 1H), 7.20 (s, 1H), 7.00 (d, *J* = 4 Hz, 1H), 6.35 (d, *J* = 8 Hz, 1H), 4.73 (s, 2H), 4.02 (s, 3H), 3.83 (s, 3H).

Compounds (**4a**–**4l**) were prepared by using the same procedure described above.

5.1.4. (2*E*)-3-[3-(2-{[3,5-Bis(trifluoromethyl)phenyl]amino}-2-oxoethoxy)-4-methoxyphenyl]-*N*-hydroxyacrylamide (IF8)[14]

NH_2OK/NH_2OH solution was prepared as previously described.

To a solution of (**4h**) (1.70 g, 3.50 mmol) in 10 mL of anhydrous DMF was added NH_2OK/NH_2OH solution (7 mL). The mixture was stirred at room temperature for one day. The reaction mixture was taken up in dilute aqueous HCl (pH = 2), extracted with EtOAc (120 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (AcOEt/MeOH = 20:1) to give hydroxamic acid **IF8** as white solid (0.85 g, 49.7%). Mp: 158–160 °C. EI-MS (*m*/*z*): 478.0 (M^+). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 8.37 (s, 2H), 7.82 (s, 1H), 7.38 (d, *J* = 8 Hz, 1H), 7.23 (s, 1H), 7.22 (d, *J* = 4 Hz, 1H), 7.07 (d, *J* = 4 Hz, 1H), 6.32 (d, *J* = 8 Hz, 1H), 4.81 (s, 2H), 3.84 (s, 3H).

Compounds (**IF1**–**IF9**) were synthesized following the same procedure described above.

5.1.4.1. (2*E*)-3-(3-{2-[(3-Chloro-4-fluorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)-*N*-hydroxyacrylamide (IF1).

Mp: 139–141 °C. EI-MS (*m*/*z*): 395.0 (M^+). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 7.97 (d, *J* = 2 Hz, 1H), 7.55 (d, *J* = 2 Hz, 1H), 7.42 (d, *J* = 6 Hz, 1H), 7.37 (d, *J* = 4 Hz, 1H), 7.21 (s, 2H), 7.06 (d, *J* = 4 Hz, 1H), 6.30 (d, *J* = 8 Hz, 1H), 4.74 (s, 2H), 3.84 (s, 3H).

5.1.4.2. (2*E*)-*N*-Hydroxy-3-[4-methoxy-3-(2-oxo-2-{[3-(trifluoromethyl)phenyl]amino}ethoxy)phenyl]acrylamide (IF2).

Mp: 158–161 °C. EI-MS (*m*/*z*): 411.0 (M^+). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 8.14 (s, 1H) 7.86 (d, *J* = 4 Hz, 1H), 7.61–7.57 (m, 1H), 7.45 (d, *J* = 4 Hz, 1H), 7.39 (d, *J* = 8 Hz, 1H), 7.22 (s, 1H), 7.21 (d, *J* = 4 Hz, 1H), 7.06 (d, *J* = 4 Hz, 1H), 6.31 (d, *J* = 8 Hz, 1H), 4.78 (s, 2H), 3.84 (s, 3H).

5.1.4.3. (2*E*)-3-(3-{2-[(3,4-Dichlorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)-*N*-hydroxyacrylamide (IF3).

Mp: 153–154 °C. EI-MS (*m*/*z*): 394.0 $[M-OH]^+$. ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 8.05 (d, *J* = 4 Hz, 1H), 7.61 (d, *J* = 6 Hz, 1H), 7.57 (d, *J* = 2 Hz, 1H), 7.38 (d, *J* = 8 Hz, 1H), 7.21 (s, 2H), 7.06 (d, *J* = 4 Hz, 1H), 6.30 (d, *J* = 8 Hz, 1H), 4.75 (s, 2H), 3.84 (s, 3H).

5.1.4.4. (2*E*)-*N*-hydroxy-3-(4-methoxy-3-{2-[(4-methoxyphenyl) amino]-2-oxoethoxy}phenyl)acrylamide (IF4).

Mp: 160–162 °C. EI-MS (*m*/*z*): 372.1 (M^+). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 7.54 (d, *J* = 4 Hz, 2H), 7.37 (d, *J* = 8 Hz, 1H), 7.22 (s, 1H), 7.21 (d, *J* = 6 Hz, 1H), 7.06 (d, *J* = 6 Hz, 1H), 6.91 (d, *J* = 6 Hz, 2H), 6.31 (d, *J* = 8 Hz, 1H), 4.70 (s, 2H), 3.84 (s, 3H), 3.73 (s, 3H).

5.1.4.5. (2*E*)-3-(3-{2-[(4-Bromophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)-*N*-hydroxyacrylamide (IF5).

Mp: 175–177 °C. EI-MS (*m*/*z*): 421.9 (M^+). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 7.62 (d, *J* = 4 Hz, 2H), 7.52 (d, *J* = 4 Hz, 2H), 7.37 (d, *J* = 8 Hz, 1H), 7.21 (s, 1H), 7.20 (d, *J* = 4 Hz, 1H), 7.05 (d, *J* = 4 Hz, 1H), 6.30 (d, *J* = 8 Hz, 1H), 4.74 (s, 2H), 3.84 (s, 3H).

5.1.4.6. (2*E*)-3-(3-{2-[(2-Fluorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)-*N*-hydroxyacrylamide (IF6).

Mp: 149–151 °C. EI-MS (*m*/*z*): 360.0 (M^+). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 7.95 (d, *J* = 2 Hz, 1H), 7.38 (d, *J* = 8 Hz, 1H), 7.33–7.28 (m, 1H), 7.25 (s, 1H), 7.22–7.19 (m, 1H), 7.20 (d, *J* = 2 Hz, 1H), 7.19 (d, *J* = 2 Hz, 1H), 7.07 (d, *J* = 4 Hz, 1H), 6.33 (d, *J* = 8 Hz, 1H), 4.81 (s, 2H), 3.85 (s, 3H).

5.1.4.7. (2*E*)-3-(3-{2-[(3-Fluorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)-*N*-hydroxyacrylamide (IF7).

Mp: 151–152 °C. EI-MS (*m*/*z*): 344.1 $[M-OH]^+$. ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 7.63 (d, *J* = 6 Hz, 1H), 7.39 (d, *J* = 2 Hz, 1H), 7.36 (s, 1H), 7.35 (d, *J* = 4 Hz, 1H), 7.21 (s, 1H), 7.20 (d, *J* = 2 Hz, 1H), 7.06 (d, *J* = 4 Hz, 1H), 6.95–6.90 (m, 1H), 6.31 (d, *J* = 6 Hz, 1H), 4.75 (s, 2H), 3.84 (s, 3H).

5.1.4.8. (2*E*)-3-[3-(2-{[3-Bromo-5-(trifluoromethyl)phenyl]amino}-2-oxoethoxy)-4-methoxyphenyl]-*N*-hydroxyacrylamide (IF9).

Mp: 151–153 °C. EI-MS (*m*/*z*): 474.9 $[M-CH_3]^+$. ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 8.21 (s, 1H), 8.07 (s, 1H), 7.68 (s, 1H), 7.38 (d, *J* = 8 Hz, 1H), 7.22 (s, 2H), 7.07 (d, *J* = 6 Hz, 1H), 6.31 (d, *J* = 8 Hz, 1H), 4.78 (s, 2H), 3.84 (s, 3H).

5.1.5. (2*E*)-*N*-(2-Aminophenyl)-3-(3-hydroxy-4-methoxyphenyl)-acrylamide (5a)

To a solution of isoferulic acid (5.83 g, 30 mmol) in anhydrous THF (120 mL) was added CDI (5.35 g, 33 mmol) portionwise at room temperature. The reaction mixture was stirred for 1 h to form acylimidazole followed by addition of 1,2-phenylenediamine (25.96 g, 240 mmol) and TFA (3.49 g, 30 mmol). The reaction mixture was stirred at room temperature for another 16 h. The mixture was then filtered to give crude product which was purified by silica gel column chromatography (petroleum/AcOEt = 3:1 to 1:3) to yield **5a** as white solid (6.95 g, 81.5%). Mp: 171–173 °C. EI-MS (*m*/*z*): 284.1 (M^+). ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8 Hz, 1H), 7.33 (d, *J* = 4 Hz, 1H), 7.05 (s, 1H), 7.04 (d, *J* = 4 Hz, 1H), 6.97

(d, J = 4 Hz, 1H), 6.92–6.88 (m, 1H), 6.75 (d, J = 4 Hz, 1H), 6.68 (d, J = 6 Hz, 1H), 6.60–6.56 (m, 1H), 3.81 (s, 3H).

Compound **5b** was prepared following the same procedure as **5a**. Then it was purified by silica gel column chromatography (petroleum/AcOEt = 3:1 to 1:3).

5.1.6. (2*E*)-*N*-(2-Aminophenyl)-3-[3-(2-{[3-bromo-5-(trifluoromethyl)phenyl]amino}-2-oxoethoxy)-4-methoxyphenyl]acrylamide (IF18)

To a suspension of **5a** (0.85 g, 3 mmol) in dehydrated acetone (70 mL) was added anhydrous potassium carbonate (1.30 g, 9 mmol). The mixture was stirred at room temperature for 30 min and then 2-chloro-*N*-(3-bromo-5(trifluoromethyl)acetamide (3.33 g, 15 mmol) was added. The reaction mixture was refluxed for another 10 h. Filtration and evaporation of acetone was done in vacuum. The residue was extracted with EtOAc (120 mL). The combined layers were washed with water, 2 M NaOH, 2 M HCl and brine, dried over Na_2SO_4 and solvent was removed. The crude product was purified by silica gel column chromatography (PE/AcOEt = 3:1 to 1:3) to yield (**IF18**) as white solid (0.98 g, 58.0%). Mp: 193–195 °C. EI-MS (m/z): 563.0 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.22 (s, 1H), 8.08 (s, 1H), 7.69 (s, 1H), 7.49 (d, J = 8 Hz, 1H), 7.33–7.28 (m, 2H), 7.29 (d, J = 4 Hz, 1H), 7.11 (d, J = 4 Hz, 1H), 6.91 (d, J = 6 Hz, 1H), 6.76 (s, 1H), 6.74 (d, J = 2 Hz, 1H), 6.58 (d, J = 8 Hz, 1H), 4.80 (s, 2H), 3.86 (s, 3H).

Compounds (**IF10–IF27**) were prepared following the procedure described above.

5.1.6.1. (2*E*)-*N*-(2-Aminophenyl)-3-(3-{2-[(3-chloro-4-fluorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)acrylamide (IF10).

Mp: 191–193 °C. EI-MS (m/z): 469.1. (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.99 (d, J = 6 Hz, 1H), 7.91 (d, J = 4 Hz, 1H), 7.59–7.55 (m, 1H), 7.50 (s, 1H), 7.42 (d, J = 4 Hz, 1H), 7.40 (d, J = 6 Hz, 1H), 7.21–7.19 (m, 1H), 6.91 (d, J = 6 Hz, 1H), 6.76 (s, 1H), 6.73 (d, J = 4 Hz, 1H), 6.58 (d, J = 6 Hz, 1H), 4.94 (s, 2H), 3.86 (s, 3H).

5.1.6.2. (2*E*)-*N*-(2-Aminophenyl)-3-[4-methoxy-3-(2-oxo-2-{[3-(trifluoromethyl)phenyl]amino}ethoxy)phenyl]acrylamide (IF11).

Mp: 188–189 °C. EI-MS (m/z): 485.1 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.15 (s, 1H), 7.87 (d, J = 4 Hz, 1H), 7.61–7.57 (m, 1H), 7.49 (d, J = 8 Hz, 1H), 7.46 (d, J = 6 Hz, 1H), 7.33 (d, J = 2 Hz, 1H), 7.29 (s, 1H), 7.28 (d, J = 2 Hz, 1H), 7.11 (d, J = 4 Hz, 1H), 6.93–6.89 (m, 1H), 6.77–6.73 (m, 2H), 6.57 (d, J = 6 Hz, 1H), 4.80 (s, 2H), 3.86 (s, 3H).

5.1.6.3. (2*E*)-*N*-(2-Aminophenyl)-3-(3-{2-[(3,4-dichlorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)acrylamide (IF12).

Mp: 209–210 °C. EI-MS (m/z): 485.0 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.06 (s, 1H), 7.61 (d, J = 4 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 7.51 (d, J = 4 Hz, 1H), 7.33 (d, J = 6 Hz, 1H), 7.28 (d, J = 4 Hz, 1H), 7.27 (s, 1H), 7.10 (d, J = 4 Hz, 1H), 6.93–6.89 (m, 2H), 6.73 (d, J = 4 Hz, 1H), 6.57 (d, J = 6 Hz, 1H), 4.77 (s, 2H), 3.86 (s, 3H).

5.1.6.4. (2*E*)-*N*-(2-Aminophenyl)-3-(4-methoxy-3-{2-[(4-methoxyphenyl)amino]-2-oxoethoxy}phenyl)acrylamide (IF13).

Mp: 190–191 °C. EI-MS (m/z): 447.1 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.56 (d, J = 4 Hz, 2H), 7.48 (d, J = 8 Hz, 1H), 7.33 (d, J = 4 Hz, 1H), 7.28 (s, 1H), 7.27 (d, J = 4 Hz, 1H), 7.10 (d, J = 4 Hz, 1H), 6.93 (d, J = 2 Hz, 1H), 6.91 (d, J = 4 Hz, 2H), 6.77–6.73 (m, 2H), 6.58 (d, J = 8 Hz, 1H), 4.72 (s, 2H), 3.86 (s, 3H), 3.73 (s, 3H).

5.1.6.5. (2*E*)-*N*-(2-Aminophenyl)-3-(3-{2-[(4-bromophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)acrylamide (IF14).

Mp: 217–219 °C. EI-MS (m/z): 497.1 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.62 (d, J = 6 Hz, 2H), 7.52 (d, J = 4 Hz, 2H), 7.50 (d, J = 4 Hz, 1H), 7.35 (d, J = 4 Hz, 1H), 7.28 (s, 1H), 7.17 (d, J = 4 Hz, 1H), 6.99 (d, J = 4 Hz, 1H), 6.94–6.90 (m, 1H), 6.80 (d, J = 8 Hz, 1H), 6.76 (d, J = 4 Hz, 1H), 6.61–6.57 (m, 1H), 4.76 (s, 2H), 3.87 (s, 3H).

5.1.6.6. (2*E*)-*N*-(2-Aminophenyl)-3-(3-{2-[(2-fluorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)acrylamide (IF15).

Mp: 193–194 °C. EI-MS (m/z): 435.1 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.98 (d, J = 8 Hz, 1H), 7.49 (d, J = 8 Hz, 1H), 7.35–7.28 (m, 4 H), 7.20 (d, J = 2 Hz, 1H), 7.18 (d, J = 2 Hz, 1H), 7.11 (d, J = 4 Hz, 1H), 6.93 (d, J = 2 Hz, 1H), 6.77 (d, J = 4 Hz, 1H), 6.74 (s, 1H), 6.58 (d, J = 8 Hz, 1H), 4.83 (s, 2H), 3.87 (s, 3H).

5.1.6.7. (2*E*)-*N*-(2-Aminophenyl)-3-(3-{2-[(3-fluorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)acrylamide (IF16).

Mp: 199–201 °C. EI-MS (m/z): 435.1 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.65 (d, J = 6 Hz, 1H), 7.48 (d, J = 8 Hz, 1H), 7.39 (d, J = 2 Hz, 1H), 7.39–7.37 (m, 1H), 7.33 (d, J = 4 Hz, 1H), 7.28 (d, J = 4 Hz, 1H), 7.27 (s, 1H), 7.10 (d, J = 4 Hz, 1H), 6.94–6.90 (m, 2H), 6.76 (s, 1H), 6.73 (d, J = 4 Hz, 1H), 6.58 (d, J = 8 Hz, 1H), 4.77 (s, 2H), 3.86 (s, 3H).

5.1.6.8. (2*E*)-*N*-(2-Aminophenyl)-3-[3-(2-{[3,5-bis(trifluoromethyl)phenyl]amino}-2-oxoethoxy)-4-methoxyphenyl]acrylamide (IF17).

Mp: 231–233 °C. EI-MS (m/z):553.3 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.38 (s, 2H), 7.83 (s, 1H), 7.49 (d, J = 8 Hz, 1H), 7.33–7.29 (m, 2H), 7.30 (d, J = 4 Hz, 1H), 7.12 (d, J = 4 Hz, 1H), 6.91 (d, J = 8 Hz, 1H), 6.76 (d, J = 4 Hz, 1H), 6.76 (s, 1H), 6.57 (d, J = 8 Hz, 1H), 4.83 (s, 2H), 3.86 (s, 3H).

5.1.6.9. (2*E*)-*N*-(2-Amino-4-methylphenyl)-3-(3-{2-[(3-chloro-4-fluorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl) acrylamide (IF19).

Mp: 170–171 °C. EI-MS (m/z): 483.1 (M^+). ^{1}H NMR (400 MHz, $(CD3)_2SO$) δ 7.98 (d, J = 4 Hz, 1H), 7.44 (d, J = 4 Hz, 1H), 7.40 (d, J = 6 Hz, 1H), 7.26 (s, 2H), 7.17 (d, J = 4 Hz, 1H), 7.10 (d, J = 4 Hz, 1H), 6.75 (d, J = 2 Hz, 1H), 6.65 (d, J = 4 Hz, 1H), 6.56 (s, 1H), 6.39 (d, J = 4 Hz, 1H), 4.76 (s, 2H), 3.86 (s, 3H), 2.16 (s, 3H).

5.1.6.10. (2*E*)-*N*-(2-Amino-4-methylphenyl)-3-[4-methoxy-3-(2-oxo-2-{[3-(trifluoromethyl)phenyl]amino}ethoxy)phenyl] acrylamide (IF20).

Mp: 192–194 °C. EI-MS (m/z): 499.1 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.15 (s, 1H), 7.87 (d, J = 4 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 7.47 (d, J = 4 Hz, 1H), 7.45 (d, J = 4 Hz, 1H), 7.28 (s, 1H), 7.27 (d, J = 4 Hz, 1H), 7.17 (d, J = 4 Hz, 1H), 7.10 (d, J = 4 Hz, 1H), 6.76–6.70 (m, 1H), 6.56 (s, 1H), 6.39 (d, J = 4 Hz, 1H), 4.79 (s, 2H), 3.86 (s, 3H), 2.17 (s, 3H).

5.1.6.11. (2*E*)-*N*-(2-Amino-4-methylphenyl)-3-(3-{2-[(3,4-dichlorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)acrylamide (IF21).

Mp: 178–180 °C. EI-MS (m/z): 500.3 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.05 (s, 1H), 7.61 (d, J = 4 Hz, 1H), 7.59 (d, J = 6 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 7.27 (d, J = 4 Hz, 1H), 7.26 (s, 1H), 7.17 (d, J = 4 Hz, 1H), 7.10 (d, J = 4 Hz, 1H), 6.72 (d, J = 4 Hz, 1H), 6.56 (s, 1H), 6.39 (d, J = 4 Hz, 1H), 4.77 (s, 2H), 3.86 (s, 3H), 2.17 (s, 3H).

5.1.6.12. (2*E*)-*N*-(2-Amino-4-methylphenyl)-3-(4-methoxy-3-{2-[(4-methoxyphenyl)amino]-2-oxoethoxy}phenyl)acrylamide (IF22).

Mp: 184–186 °C. EI-MS (m/z): 461.2 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.56 (d, J = 6 Hz, 2H), 7.49 (d, J = 2 Hz, 1H), 7.27 (s, 1H), 7.26 (d, J = 4 Hz, 1H), 7.18 (d, J = 4 Hz, 1H), 7.10 (d, J = 6 Hz, 1H), 6.74 (d, J = 6 Hz, 1H), 6.56 (s, 1H), 6.39 (d, J = 4 Hz, 1H), 4.71 (s, 2H), 3.87 (s, 3H), 3.73 (s, 3H), 2.17 (s, 3H).

5.1.6.13. (2*E*)-*N*-(2-Amino-4-methylphenyl)-3-(3-{2-[(4-bromophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl)acrylamide (IF23). Mp: 198–200 °C. EI-MS (*m*/*z*): 509.0 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.64 (d, *J* = 6 Hz, 2H), 7.55 (d, *J* = 6 Hz, 2H), 7.46 (d, *J* = 8 Hz, 1H), 7.26 (s, 1H), 7.17 (d, *J* = 4 Hz, 1H), 7.10 (d, *J* = 4 Hz, 1H), 6.73 (d, *J* = 8 Hz, 1H), 6.67 (d, *J* = 8 Hz, 1H), 6.56 (s, 1H), 6.39 (d, *J* = 4 Hz, 1H), 4.75 (s, 2H), 3.86 (s, 3H), 2.17 (s, 3H).

5.1.6.14. (2*E*)-*N*-(2-Amino-4-methylphenyl)-3-(3-{2-[(2-fluorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl) acrylamide (IF24). Mp: 184–186 °C. EI-MS (*m*/*z*): 449.1 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.97 (d, *J* = 8 Hz, 1H), 7.47 (d, *J* = 8 Hz, 1H), 7.31 (d, *J* = 4 Hz, 2H), 7.30 (s, 2H), 7.28 (d, *J* = 2 Hz, 1H), 7.21 (d, *J* = 2 Hz, 1H), 7.20 (d, *J* = 2 Hz, 1H), 7.11 (d, *J* = 4 Hz, 1H), 6.78–6.62 (m, 2H), 6.56 (s, 1H), 6.39 (d, *J* = 4 Hz, 1H), 4.83 (s, 2H), 3.87 (s, 3H), 2.17 (s, 3H).

5.1.6.15. (2*E*)-*N*-(2-Amino-4-methylphenyl)-3-(3-{2-[(3-fluorophenyl)amino]-2-oxoethoxy}-4-methoxyphenyl) acrylamide (IF25). Mp: 162–164 °C. EI-MS (*m*/*z*): 450.1 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.65 (d, *J* = 6 Hz, 1H), 7.58 (d, *J* = 6 Hz, 1H), 7.46 (d, *J* = 8 Hz, 1H), 7.38 (s, 1H), 7.37 (d, *J* = 4 Hz, 1H), 7.27 (s, 1H), 7.17 (d, *J* = 4 Hz, 1H), 7.10 (d, *J* = 4 Hz, 1H), 6.93–6.90 (m, 1H), 6.73 (d, *J* = 2 Hz, 1H), 6.56 (s, 1H), 6.39 (d, *J* = 4 Hz, 1H), 4.76 (s, 2H), 3.87 (s, 3H), 2.17 (s, 3H).

5.1.6.16. (2*E*)-*N*-(2-Amino-4-methylphenyl)-3-[3-(2-{[3,5-bis(trifluoromethyl)phenyl]amino}-2-oxoethoxy)-4-methoxyphenyl] acrylamide (IF26). Mp: 225–227 °C. EI-MS (*m*/*z*): 567.1 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.38 (s, 2H), 7.82 (s, 1H), 7.47 (d, *J* = 8 Hz, 1H), 7.29 (d, *J* = 2 Hz, 1H), 7.28 (s, 1H), 7.17 (d, *J* = 4 Hz, 1H), 7.11 (d, *J* = 4 Hz, 1H), 6.72 (d, *J* = 8 Hz, 1H), 6.55 (s, 1H), 6.39 (d, *J* = 2 Hz, 1H), 4.82 (s, 2H), 3.86 (s, 3H), 2.17 (s, 3H).

5.1.6.17. (2*E*)-*N*-(2-Amino-4-methylphenyl)-3-[3-(2-{[3-bromo-5-(trifluoromethyl)phenyl]amino}-2-oxoethoxy)-4-methoxyphenyl]acrylamide (IF27). Mp: 229–231 °C. EI-MS (*m*/*z*): 579.0 (M^+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.22 (s, 1H), 8.07 (s, 1H), 7.69 (s, 1H), 7.46 (d, *J* = 8 Hz, 1H), 7.29 (d, *J* = 4 Hz, 1H), 7.27 (s, 1H), 7.17 (d, *J* = 4 Hz, 1H), 7.11 (d, *J* = 6 Hz, 1H), 6.72 (d, *J* = 8 Hz, 1H), 6.56 (s, 1H), 6.39 (d, *J* = 4 Hz, 1H), 4.79 (s, 2H), 3.86 (s, 3H), 2.17 (s, 3H).

5.2. HDAC inhibitory activity assays[15]

The HDAC inhibitory activity assay was carried out using Color-de-Lys™ HDAC colorimetric activity assay kit (Enzo Life Sciences, Inc.) according to the manufacturer's instructions. The kit is useful for inhibitors screening using HDAC from HeLa nuclear extract. The *Color de Lys*™ substrate which comprises an acetylated lysine is incubated with sample containing HDAC activity. Deacetylation of substrate sensitizes the substrate. The mixing with the *Color de Lys*™ developer causes an increase in color intensity at 405 nm.

The title compounds and positive control were diluted in buffer to various concentrations (20 μg/mL, 4 μg/mL, 0.8 μg/mL, 0.16 μg/mL, 0.032 μg/mL). HDACs (5 μL) were incubated at 37 °C with 10 μL of compounds and 25 μL of substrate on 96-well plates. After incubation for 30 min, *Color de Lys*™ developer (50 μL/well) was added to stop HDAC reactions. Incubate plate at 37 °C for 15 min and read plate in microtiter-plate reader at 405 nm. The inhibition rates were calculated from ultraviolet absorption readings of inhibited wells related to those of control wells. The IC_{50} values were calculated according to inhibition ratios.

5.3. Cell growth inhibitory activity in cancer cells[16]

The six potent compounds were tested for their antiproliferative potency against breast cancer cell line (MDA-MB-231) and cervical cancer cell (HeLa) using MTT method. Exponentially growing cells were harvested and plated in 96-well plates at a concentration of 1×10^4 cells/well, and then incubated for 24 h at 37 °C. The cells in the wells were treated with the title compounds respectively at various concentrations for 48 h. Then, 20 mL MTT (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. Supernatant was discarded, and 150 mL DMSO was added to each well. Absorbance values were determined by a microplate reader (Bio-Rad Instruments) at 570 nm. The IC_{50} values were calculated according to inhibition ratios.

5.4. Molecular docking modeling[17]

Molecule docking was carried out using Sybyl/Surflex-dock based on crystal structures of HDAC (PDB ID: 3F07). Hydrogen was added and minimized using Tripos force field and Pullman charges. All the waters were removed as well as all ions except for catalytic zinc ion. The residues in a radius 5.0 Å around APHA (ligand of HDAC in crystal complex) were selected as active site. Compound (**IF6**) was depicted with Sybyl/Sketch module (Tripos Inc.) and optimized applying Powell's method with Tripos force field with convergence criterion set at 0.05 kcal/(Å mol), and assigned with Gasteiger–Hückel method. Other docking parameters were kept at default.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.03.019.

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