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Novel N-hydroxyfurylacrylamide-based histone deacetylase (HDAC) inhibitors with branched CAP group (Part 2)

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ABSTRACT

Histone deacetylases (HDACs) are significant enzymes involved in tumor genesis and development. Herein, we report a series of novel N-hydroxyfurylacryl-amide-based HDAC inhibitors, which are marked by introducing branched hydrophobic groups as the capping group. The inhibitory activity of the synthesized compounds against HDACs and several tumor cell lines are firstly determined. Fifteen compounds with promising activities are selected for further evaluation of target selectivity profile against recombinant human HDAC1, HDAC4 and HDAC6. Compounds **10a**, **10b**, **10d** and **16a** exhibit outstanding selectivity against HDAC6. Analysis of HDAC4 X-ray structure and HDAC1, HDAC6 homology model indicates that these enzyme differ significantly in the rim near the surface of the active site. Although TSA has been known as a pan-HDAC inhibitor, it exhibits outstanding selectivity for HDAC6 over HDAC4. For further physicochemical properties study, six compounds are chosen for determination of their physicochemical properties including log$D_{7.4}$ and aqueous solubility. The results suggest that compounds with a smaller framework and with hydrophilicgroups are likely to have better aqueous solubility.

1. Introduction

Epigenetics refers to the heritable changes in gene expression or phenotype that are stable between cell division, and sometimes between generations, but do not involve changes in the underlying DNA sequence of the organism.[1,2] Epigenetic alterations, including DNA methylation, histone modifications, nucleosome positioning, and small noncoding RNAs (miRNA, siRNA), play an important role in cancer progression.[3–5] The histone proteins are widely described as essential players in the control of gene expression via modification through chemical reactions such as acetylation, phosphorylation, and methylation. The amino termini of histones extend from the nucleosomal core and are modified by histone acetyltransferases (HATs) and histone deacetylases (HDACs) during the cell cycle.[6] HATs transfer acetyl groups to the ε-amino group of lysine side chains of the histone protein, leading to local expansion of chromatin and increased accessibility of regulatory proteins to DNA, whereas HDACs oppose the effects of HATs and reverse the acetylation of lysine residues, resulting in condensation of chromosomal DNA and gene transcriptional repression.[7] In addition, many non-histone proteins have been identified to substrate of HDACs. HDAC6 participates in the deacetylation of nonhistone proteins, such as α-tubulin and HSP90, as well as regulating important biological processes including microtubule stability and function, and molecular chaperon activity.[8,9] Abnormality of histone deacetylation has been observed in human tumors, and inhibition of HDAC has become a novel and validated therapeutic strategy against cancers.[10] Therefore, HDACs are widely recognized as promising targets for therapeutic intervention in cancer.

Till now, eighteen HDACs have been reported and they can be divided into four distant classes. Class I (HDAC1–3 and 8), class II (HDAC4–7, 9 and 10) and class IV (HDAC11) HDACs are all Zn^{2+}-dependent metalloproteases, while class III (SIRT1–7) HDACs are NAD^{+}-dependent.[11,12]

Currently, a great number of HDAC inhibitors, including SAHA, ITF2357, MS-275, LAQ824, TSA, Tubacin, MGCD0103, FK228, are reported as antitumor agents (Fig. 1). Among them, Vorinostat (SAHA)[13] and romidepsin (FK228)[14] have been approved by US FDA for the treatment of relapsed cutaneous T-cell lymphoma (CTCL).[15,16] Most HDAC inhibitors, as exemplified by Vorinostat, can be described by the cap, linker, and zinc-binding group (ZBG).[17]

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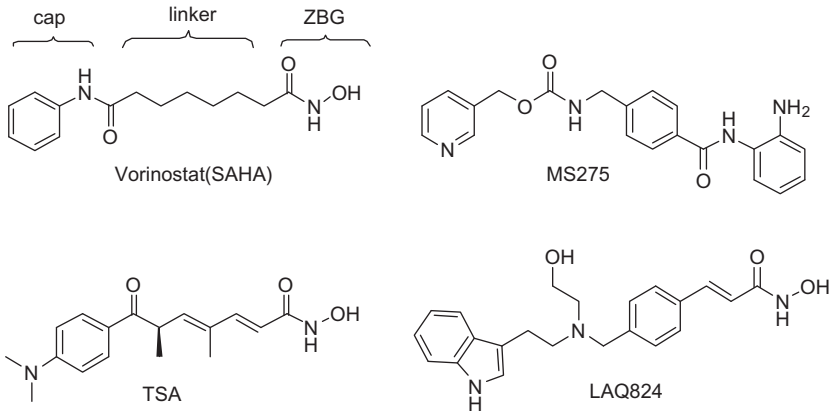


Figure 1. The structures of known HDAC inhibitors.

Half of these inhibitors have the hydroxamic moiety, a typical zinc-binding group. However, hydroxamic acid usually lacks selectivity due to its strong zinc-binding ability, therefore, how to improve the target selectivity of the HDACs inhibitors has been a main problem that attracts the attentions of medicinal chemists all over the world. Sequence aligenment of HDACs indicates that the principal difference among the individual subtype exists in the loops forming shallow grooves around the rim of enzyme pocket.[18,19] Close examination of corresponding crystal structures further indicates that these enzyme differ significantly in the rim near the surface of the active site,[20,21] which suggests that capping group of HDAC inhibitors is quite important to the target selectivity. As a result, rational modification of capping group is a promising strategy for the improvement of selectivity of the HDAC inhibitors.[22] Several groups has reported HDAC selective inhibitors with modified capping group (e.g., compounds **1, 2, 3** and **4**, Fig. 2).[23–29] According to the structure–activity relationship (SAR) data of these reported inhibitors, although the elements account for selectivity are less understood,[30,31] it seemed that the size of capping group is the key point for governing enzyme selectivity.

Our laboratory has focused on the discovery of selective HDAC inhibitors for a long time. In the previous work, we have designed and synthesized a class of novel N-hydroxybenzamide-based HDAC inhibitors with branched capping group, which exhibit outstanding in vitro antitumor activities, and some of them showed selectivity against HDAC1.[30] With the aim to gain further knowledge about the structural requirements for the selectivity of the inhibitors, herein we report the design and synthesis of novel N-hydroxyfurylacrylamide compounds with branched capping group. Compared to the previouly reported compounds,[30] the N-hydroxyfurylacryl-amide was utilized as the zinc binding group. The steric benzyl, 4-phenylbenzyl and homopiperonyl were taken as the hydrophobic group so as to accommodate the binding pocket of HDACs. Another side chain at the amine N (represented by R group in Schemes 1–3) in order to explore the interaction with the shallow grooves near the rim of HDAC catalytic pockets.

The HDACs enzyme inhibitory activity as well as the anti-proliferative effects of the synthesized compounds were initially determined. These compounds displayed good HDAC inhibitory activity and many of them had promising anti-proliferative activity in several tumor cell lines. For further evaluation of our compounds, fifteen compounds were retained for a target selectivity profile study. Human HDAC1, HDAC4 and HDAC6 were used as representative of class I, class IIa and class IIb HDACs, respectively. To elucidate the potential binding mode of the inhibitors, docking studies were performed using HDAC1, HDAC6 homology models and HDAC4 X-ray structure. Through SAR study we found that compounds with branched capping groups tend to selectively inhibit HDAC6. Additionally, compounds with good selective profiles on HDAC6 generally showed decreased antiproliferative

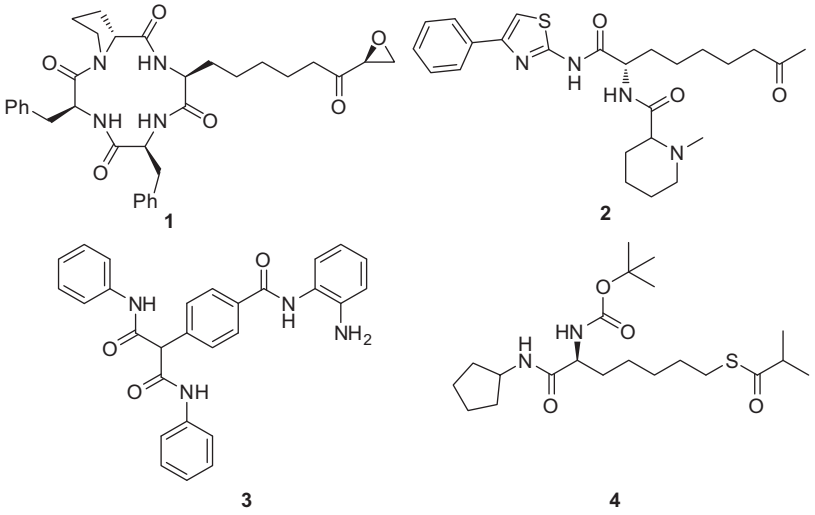


Figure 2. The structures of selective HDAC inhibitors with cyclic peptide or branched capping group.

activities against cancer cells, which was in accordance with previously reported results that HDAC6-selective compounds show reduced antiproliferative properties.[32,33] Finally, Compound **10a**, **10b**, **10d**, **10g**, **10h** and **13f** that show excellent selectivity and acceptable anti-proliferative activities were further evaluated for their physicochemical properties including log$D_{7.4}$ and intrinsic solubility. Compounds **10b** and **10d** that show promising HDAC6 selective profile, drug-like properties which makes them ideal lead compounds for the development of agents treating HDAC6-relevant disease.

2. Result and discussion

2.1. Chemistry

The synthetic route of compounds **10a–10n** was shown in Scheme 1. In detail, furfural **5** was treated with triethyl phosphonacetate in absolute ethanol containing anhydrous potassium carbonate to give (*E*)-ethyl-3-(furan-2-yl)acrylate **6.** (*E*)-Ethyl-3-(5-formylfuran-2-yl)acrylate **7** was prepared through Vilsmeier–Hacck reaction. Benzylamine was reacted with **7** to give Schiff's base, which was further reduced with $NaBH_4$ to provide intermediate **8**. Compound **8** was alkylated with various alkyl bromides to generate **9a–9n**, which was subsequently reacted with hydroxylamine to give target analogs **10a–10n**.

For the synthesis of compounds **13a–13l**, 4-phenylbenzylamine was treated with compound **7** to give Schiff's base, which was reduced with $NaBH_4$ to provide intermediate **11**. Compound **11** was alkylated with various alkyl bromides to generate **12a–12l**, which was subsequently reacted with hydroxylamine to give desired analogs **13a–13l**.

The compounds **16a–16k** were synthesized according to the methods described in Scheme 1. Homopiperonylamine was treated with compound **7** to give Schiff's base, which was reduced with $NaBH_4$ to provide intermediate **14**. Compound **14** was alkylated with various alkyl bromides to generate **15a–15k**, which was subsequently reacted with hydroxylamine to give desired analogs **16a–16k**.

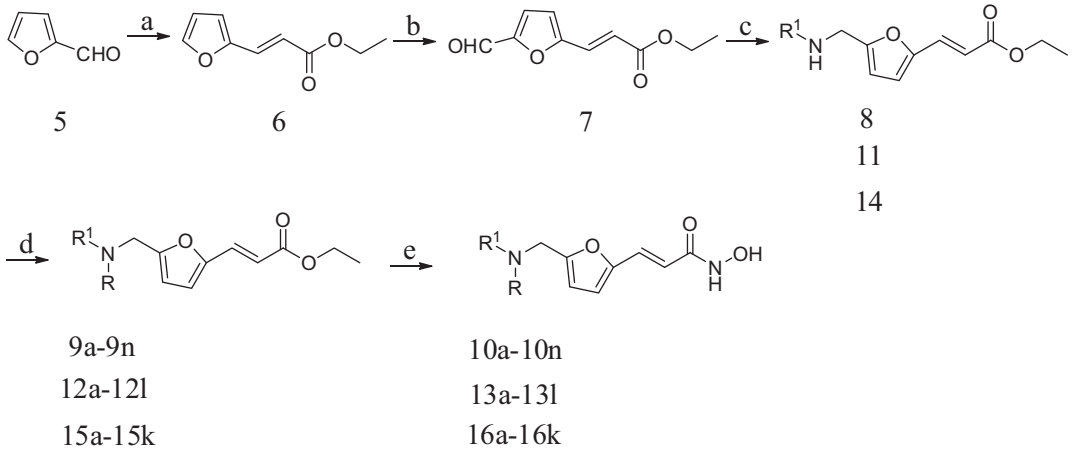
2.2. Biological evaluation

The HDACs enzymes inhibitory activities of these compounds were determined in vitro using the commercially available Drug Discovery Kit AK500 and the results are summarized in Table 1. For compounds **10a–10n**, analogs bearing short alkyl substitutes (**10a–10d**) displayed good potency against the enzymes. Replacing alkyl substitutes with longer alkyl substitutes (**10e–10g**) resulted in decreased inhibitory activity, which is consistent with our previous result that the longer alkyl substitutes might have weaker affinity.[30] We further examined analogs with phenoxyalkyl substitutes to investigate the effects of different substitutions on the inhibition of HDACs. Modification of the R alkyl group of **10a**, **10b**, **10c** seemed detrimental to the activity. In detail, incorporating phenoxy to the ethyl (**10a** and **10i**) and replacing the butyl with aromatic groups (**10d** and **10l**) can cause the decrease in the inhibitory effects. Compound **10h** bearing 2-hydroxy-ethyl showed noticeable HDACs inhibitory activity, indicating that the hydroxyethyl unit may interact with the hydration shell of aspartic acidglutamic acid in the rim of the HDAC binding channel.[34]

Inorder to investgate the effect of capping group upon HDAC activity, we synthesized compounds **13a–13l** and **16a–16k**. When introduced a hydrophobic benzene ring into capping group, **13a–13l** displayed moderate HDAC inhibitory activity. This might be attributed to unfavorable steric effect of 4-phenylbenzyl substitution. In this series, compound **13f** inhibited the HDAC activity with an IC_{50} = 0.089 μM, which is consistant with the above mentioned hypothesis that the 2-hydroxy-ethyl substitution may interact with the hydration shell of aspartic acid and glutamic acid in the rim of the HDAC binding channel.[34]

Compound **16a–16k**, having two methylene groups between the basic N and the homopiperonyl, resulted in the decreased inhibitory activity. In comparison with the activity variation of **13a–13l**, the decreased inhibitory activity was owing to the hydrophobic homopiperonyl and the longer carbon chain. Interestingly, compound **16g** bearing 2-hydroxy-ethyl substitution also displayed maximum HDAC inhibitory activity with an IC_{50} = 0.40 μM in this series.

Anti-proliferative activities of all compounds were evaluated on four tumor cell lines including PC3 (prostatecancer), HCT116 (colon cancer), A549 (lung cancer) and HepG2 (liver cancer). Anti-proliferative cytotoxic activity was measured by MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide] assay. The obtained EC_{50} values of the types of tumer cell lines compared with SAHA are summarized in Table 1. In series **10a–10n**, although analogs **10a–10d** displayed good HDAC inhibitory activity, they just showed moderate growth inhibitory activity to four human tumor cell lines. For examples, analogs **10b** and **10d** exhibited moderate inhibitory against four cell lines with EC_{50} values ranging from 16.72 to 25.03 μM and 14.45 to 24.30 μM, respectively. This might be ascribed to enzyme isoform selectivity



Scheme 1. Synthesis of N-hydroxyfurylacrylamides **10a-10n, 13a-13l, 16a-16k**. Reagents and conditions (a) $(C_2H_5)_2P(O)CH_2CO_2C_2H_5$, K_2CO_3, EtOH, 82%; (b) $POCl_3$, DMF, 82.5%; (c) (1) R^1NH_2, CH_3OH, reflux, 3 h; (2) CH_3OH, $NaBH_4$, 43.7%-76.8%; (d) K_2CO_3, KI, CH_3CN, RX, 35 °C, 36.1%-96.1%; (e) NH_2OH, KOH, CH_3OH, rt, 36.2–46.0%.

Table 1
HDACs inhibitory activities and Anti-proliferative activities of indicated compounds

Compd	R	R^1	HDACs IC_{50} (μM)	PC3 EC_{50} (μM)	HCT116 EC_{50} (μM)	A549 EC_{50} (μM)	HepG2 EC_{50} (μM)
10a	Ethyl	Benzyl	0.13	104.33	6.37	38.37	>166.67
10b	Propyl	Benzyl	0.17	25.03	24.90	23.25	16.72
10c	Iso-propyl	Benzyl	0.18	26.91	22.68	34.52	67.52
10d	Butyl	Benzyl	0.54	20.73	18.32	14.45	24.30
10e	Iso-butyl	Benzyl	3.17	18.84	26.31	20.46	27.68
10f	Hexyl	Benzyl	2.65	23.79	8.96	11.04	21.80
10g	Octyl	Benzyl	2.55	42.32	0.47	6.59	3.96
10h	2-Hydroxy-ethyl	Benzyl	0.17	>138.12	12.07	97.18	128.12
10i	2-Phenoxyethyl	Benzyl	>2.55	>127.55	20.08	52.32	44.72
10j	2-(*p*-Tolyloxy)ethyl	Benzyl	1.87	51.72	14.83	52.59	12.96
10k	3-Phenoxypropyl	Benzyl	1.48	44.85	7.24	25.76	14.95
10l	3-(*p*-Tolyloxy)propyl	Benzyl	2.54	42.17	6.02	7.95	5.95
10m	4-Phenoxybutyl	Benzyl	2.12	35.86	6.43	10.62	14.74
10n	4-(*p*-Tolyloxy)butyl	Benzyl	1.31	15.69	5.62	9.82	16.29
13a	Ethyl	4-Phenylbenzyl	1.25	67.87	1.84	8.40	12.85
13b	Iso-propyl	4-Phenylbenzyl	1.61	24.15	2.74	15.59	15.51
13c	Amyl	4-Phenylbenzyl	>2.39	13.42	8.23	14.71	19.71
13d	Octyl	4-phenylbenzyl	>2.17	36.83	10.43	28.80	36.93
13e	Benzyl	4-Phenylbenzyl	>2.55	14.31	9.01	69.82	66.61
13f	2-Hydroxy-ethyl	4-Phenylbenzyl	0.089	15.91	4.02	8.36	22.56
13g	2-Phenoxyethyl	4-Phenylbenzyl	1.21	15.90	9.53	26.45	18.10
13h	2-(*p*-Tolyloxy)ethyl	4-Phenylbenzyl	1.71	18.82	6.93	23.03	19.40
13i	3-Phenoxypropyl	4-Phenylbenzyl	>2.07	25.22	2.05	16.60	12.74
13j	3-(*p*-Tolyloxy)propyl	4-phenylbenzyl	1.66	18.06	2.22	11.49	11.65
13k	4-Phenoxybutyl	4-Phenylbenzyl	>2.02	23.61	2.76	22.36	32.90
13l	4-(*p*-Tolyloxy)butyl	4-Phenylbenzyl	>1.96	37.65	2.76	15.57	15.92
16a	Ethyl	Homopiperonyl	2.31	66.48	65.96	84.68	81.66
16b	Propyl	Homopiperonyl	1.59	7.57	15.87	50.61	28.72
16c	Butyl	homopiperonyl	>2.69	33.20	16.99	78.23	54.33
16d	Iso-butyl	Homopiperonyl	>2.69	16.14	17.06	39.34	38.01
16e	Amyl	Homopiperonyl	3.49	37.15	74.79	72.51	65.34
16f	Benzyl	Homopiperonyl	2.28	34.51	91.40	35.39	73.20
16g	2-Hydroxy-ethyl	Homopiperonyl	0.40	92.50	69.50	87.92	104.94
16h	2-Phenoxyethyl	Homopiperonyl	2.36	23.72	30.55	37.39	68.26
16i	2-(*p*-Tolyloxy)ethyl	Homopiperonyl	0.84	18.67	28.47	27.04	66.71
16j	3-Phenoxypropyl	Homopiperonyl	>2.22	18.80	37.24	16.29	109.53
16k	4-Phenoxybutyl	Homopiperonyl	1.57	15.54	35.88	46.83	97.69
SAHA	—	—	0.053	>166.67	0.57	8.30	12.20

and cellular membrane permeability. Compared to other cell lines, most of the compounds showed potent inhibitory effect on HCT116, the most potent analogs **10g** exhibited very strong growth inhibition with EC_{50} of 0.47 μM. The data indicate that these new analogs are more sensitive on the HCT116 cells.

Compound series **13a–13l** showed preferable growth inhibitory activity to series **10a–10n**. All compound in this series exhibited more potent growth inhibitory activity relative to SAHA on PC3 cell line. Compound **13a** were comparable to SAHA on A549 and HepG2 cell line. The compound series **16a–16k** which have moderate HDAC inhibitory activity showed poor growth inhibitory activity to four human tumor cell lines, while some compounds were active on the PC-3 cell lines.

We further selected 15 compounds with acceptable activities on both enzyme inhibition and anti-proliferation to evaluate the possible class specificity (Table 2). Human HDAC1, HDAC4 and HDAC6 were taken as markers for class I, class IIa and class IIb HDACs, respectively.[35] These compounds exhibited unexpected potent HDAC6 inhibition with IC_{50} of ranging from 0.18 to 4.65 μM, but displayed poor inhibitory activity against HDAC1 and HDAC4 with IC_{50} of 2.37–31.38 μM and 3.96–68.11 μM, respectively. Therefore, these compounds are highly selective and potent inhibitors of HDAC6, especially compounds **10a**, **10b**, **10d** and **16a**. Analogs bearing different capping group (benzyl, 4-phenylbenzyl and homopiperonyl) showed comparable HDAC6 inhibitory activity (compound **10a**, **13a** and **16a**). For compounds bearing alkyl substitutes (compound **10a**, **10d, 10e** and **10h**), the order of their IC_{50} values against HDAC6 was *n*-butyl < ethyl < hydroxyethyl < isobutyl. Incorporating benzene ring to the end of alkyl resulted in decreased inhibitory activity against HDAC6 (**10a** vs **10j**, **13a** vs **13h**, **16a** vs **16i**). The isoform selectivity analysis shows that capping group of HDAC inhibitors is quite important to selectivity. Tubacin, the HDAC6 selective inhibitor with bulk capping group displays seven fold selectivity for HDAC6 over HDAC1[36] and has been used widely in cell biology studies.[37,38] As a comparision, compound **10b**, the most selective inhibitor, displayed 60-fold selectivity for HDAC6 over HDAC4 and 20-fold selectivity for HDAC6 over HDAC1. Therefore, the data indicate that isoform selective inhibitors can be generated with bulk branched capping group. This is different from the N-hydroxybenzamide-based HDAC inhibitors, which displayed HDAC1 selectivity. Compounds in this study are much more conjugated, compared to N-hydroxybenzamide group, by inserting ethylene link between the C2-furan position and the carbonyl group of the hydroxamic acid. We speculated that the conjugated structure may contribute to the selectivity for directing the cap group of the inhibitor to interact with the rim of the catalytic pocket of the HDAC enzymes. On the other hand, Mai et al. have disclosed a series of aroyl-pyrrolyl-hydroxy-amides (APHAs).[39,40] Some APHAs showed distinct selectivity against HD1-B and HD1-A, two maize deacetylases that are homologous of mammalian class I and class IIa HDACs, respectively. The most selective APHAs showed no inhibitory activity against HDAC1 but was able to inhibit HDAC4. Though APHAs and our compounds have the similarly conjugated structure, the branched cap group of our compounds are more flexible than APHPs. This branched cap group may contribute to the selectivity for comfortably occupying the shallow groove at the rim of the catalytic pocket of the HDAC enzymes. To elucidate

Table 2
HDAC1, HDAC4 and HDAC6 inhibitory activities, log D7.4 and intrinsic solubility of indicated compounds

Compd	HDAC1 IC_{50} (μM)	HDAC4 IC_{50} (μM)	HDAC6 IC_{50} (μM)	HDAC1/HDAC6	HDAC4/HDAC6	log*D*, pH 7.4	Intrinsic solubility (mM)
10a	5.44	10.73	0.25	21.44	42.26	1.95	3.00
10b	4.95	10.63	0.18	28.15	60.50	2.54	2.80
10c	2.37	3.96	0.34	6.94	11.63	ND	ND
10d	4.69	7.26	0.23	20.01	30.94	2.97	2.30
10e	20.62	12.32	1.10	18.68	11.16	ND	ND
10g	ND	ND	ND			5.28	1.90
10h	12.71	11.25	0.43	29.68	26.27	1.62	4.00
10i	20.23	50.56	1.93	10.48	26.18	ND	ND
10j	31.38	68.11	3.13	10.03	21.77	ND	ND
10k	6.48	12.29	0.79	8.21	15.59	ND	ND
10l	9.66	13.22	0.93	10.41	14.24	ND	ND
10n	4.73	12.64	0.55	8.63	23.06	ND	ND
13a	3.42	5.721	0.83	4.11	6.87	ND	ND
13f	ND	ND	ND			1.80	3.11
13h	4.09	9.07	1.14	3.59	7.95	ND	ND
16a	7.88	19.95	0.48	16.57	41.93	ND	ND
16i	17.80	52.45	4.65	3.83	11.29	ND	ND
MS-275	0.31	>100	>100			ND	ND
TSA	0.0049	0.085	0.0016	3.06	53.12	ND	ND

ND = not determined.

the selectivity of compound **10b** for HDAC6 over HDAC1 and HDAC4, we constructed the HDAC1 and HDAC6 homology model. The homology model was constructed by Discovery Studio 3.0 using the Homology Modeling protocol as the method described in the literature.[41] Analysis of HDAC4 X-ray structure and HDAC1, HDAC6 homology modeled catalytic pockets reveals that, while the active site is highly conserved, the shallow grooves S of the catalytic channel rim differ greatly among the three isozymes. Two regions, named A and B, represent boundaries of shallow grooves S rim (Fig. 3). Region A corresponds to Leu262 in HDAC1, Leu299 in HDAC4 and Leu274 in HDAC6; Region B corresponds to Phe196 in HDAC1, Phe227 in HDAC4 and, Phe205 in HDAC6. Compared to Phe196 in HDAC1 and Phe227 in HDAC4, Phe205 in HDAC6 orients in an opposite direction. Variation at the B region produces a significantly wider groove rim in the homology model of HDAC6. The structural manifestation of this variation is that the measured A–B distance is 8.75 Å in HDAC1 and 8.66 Å in HDAC4, compared to10.98 Å in HDAC6. In order to understand the binding mode of the HDAC6 selective inhibitor, compound **10b** was docked into the active site of HDAC1, HDAC6 homology model and HDAC4 X-ray structure using CDOCKER module inbuilt in Discovery Studio 3.0. Data show that compound **10b** with bulk branched capping group is large enough to comfortably occupy the groove S in HDAC6, but not HDAC1 and HDAC4. In the mode of the **10b**–HDAC6 complex, the carbonyl and hydroxyl oxygens of hydroxamate could chelate with the zinc ion, zinc ion was also coordinated to two aspartic acids (Asp267, Asp174) and two histidine (His136, His135). The furan ring was bounded in the tubular hydrophobic pocket (Fig. 4).The 'cap' of **10b** was found to interact with the rim of the HDAC binding channel and the hydrophobic

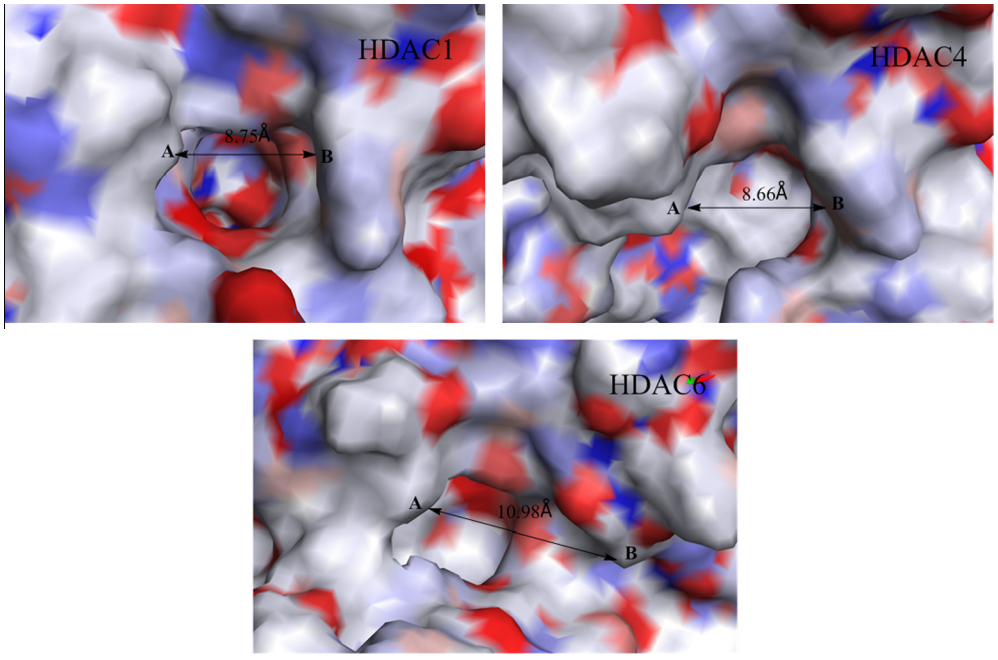


Figure 3. Top-down view of active site for HDAC1, HDAC4 and HDAC6. Distances between boundaries of the grooves S rim are shown with arrows. Letters A–B denote two boundary areas surrounding the grooves rim.

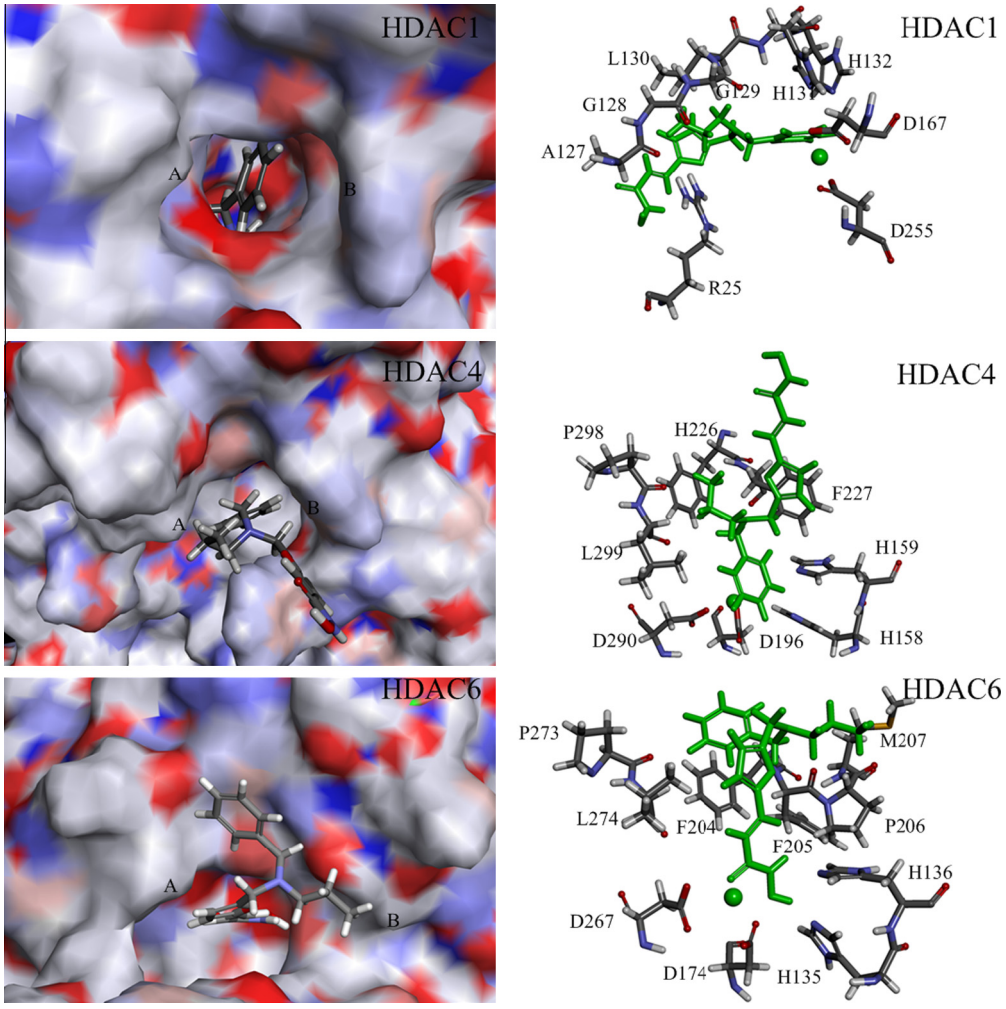


Figure 4. The docking modes of compounds **10b** with HDAC4 X-ray structure and HDAC1, HDAC6 homology models. The protein is represented by molecular surface. Letters A–B denote two boundary areas surrounding the grooves S. Compound **10b** and zinc ion is indicated green.

surface. The aromatic benzene ring of **10b** showed favorable π–π stacking interaction with Phe204. Favorable van der Waals interactions of the aromatic benzene ring with Pro273 and Leu274 were observed. Favorable van der Waals interactions of the hydrophobic propyl group of **10b** with Pro206 and Met207 were also observed at the rim of groove S. The furan ring could form hydrophobic interaction with Leu274. Therefore, the bulk branched capping group gives the extra stabilization energy by occupying the shallow groove S to form stronger hydrophobic interaction. These observations can explain the high binding energy of **10b** with HDAC6 (–CDOCKER energy 42.88 kcal/mol). In the mode of the **10b**–HDAC1 complex, **10b** completely inserted into the long internal cavity, forming an unsuitable molecular conformation and unpleasant binding energy (–CDOCKER energy −35.33 kcal/mol). Compound **10b** was surrounded by the hydrophobic residue Ala127, Gly128, Gly129 and Leu130. Arg25 could form hydrogen bonds with the hydroxamic acid of the inhibitors. The zinc ion was stably coordinated to two aspartic acids (Asp255, Asp167) and two histidine (His132, His131). However, the carbonyl and hydroxyl oxygens of hydroxamate did not chelate with the zinc ion. Additionally, it did not interact with the rim of the HDAC binding channel and the hydrophobic surface. As for HDAC4, **10b** also binded with an unrational conformation, which showed an reverse orientation compared to that in the **10b**–HDAC6 complex. The zinc ion was stably coordinated to two aspartic acids (Asp196, Asp290) and two histidine (His159, His158). However, the carbonyl and hydroxyl oxygens of hydroxamate did not chelate with the zinc ion. The N-hydroxyfurylacrylamide group had little direct contact with the enzyme. Weak van der Waals interactions of the hydrophobic propyl group with Leu299 could be observed. The furan group of **10b** could form π–π interaction with Phe227. In addition, the shallow groove S of HDAC4 was also not occupied by **10b**, which was ascribed to the decreased binding energy (19.29 kcal/mol). Taken together, the comparison of the binding conformations and the interaction energies in the three complexes has confirmed the target selective profile of our compound.

Interestingly, The HDAC4 IC_{50}/HDAC6 IC_{50} value of **TSA** is 53, although **TSA** has been known as a pan-HDAC inhibitor. To better understand the selectivity of TSA for HDAC6 over HDAC4, **TSA** were docked into HDAC4 X-ray structure and HDAC6 homology model. Analysis of HDAC4 binding mode showed that the hydroxamic acid interacted with the zinc ion and polar residue His198 (Fig. 5). Phe168 and Phe227 sandwiched the hydrophobic alkyl chain of the inhibitors. The methyl group near the benzene ring could form hydrophobic interaction with Pro298 and Leu299. The cap group of **TSA** was outside the peptide binding groove and did not make any interactions. Additional, the shallow groove S of HDAC4 was not occupied by **TSA**. The analysis of the binding mode obtained for the HDAC6 homology model showed that His136 could form hydrogen bonds with the hydroxamic acid of

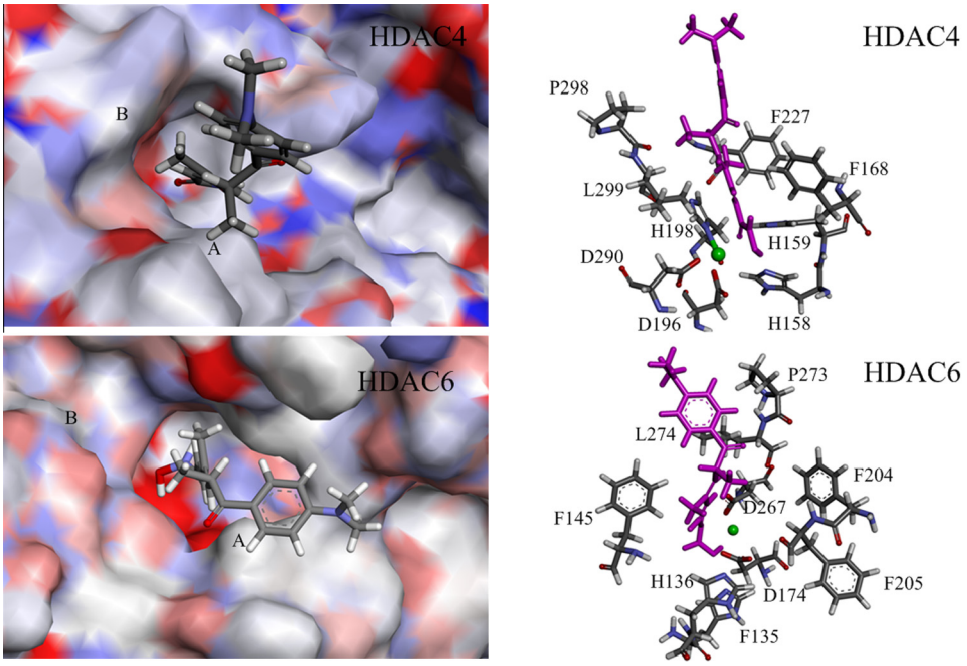


Figure 5. The docking modes of compounds **TSA** with HDAC4 X-ray structure and HDAC6 homology models. The protein is represented by molecular surface. Letters A-B denote two boundary areas surrounding the grooves S. Compound **TSA** is indicated magenta. Zinc ion is indicated green.

the inhibitors. Phe145 and Phe204 in HDAC6 sandwiched the alkyl chain linker between their hydrophobic portions. The aromatic cap group of **TSA** could form hydrophobic interaction with Pro273 and Leu274. In addition, the shallow groove S of HDAC6 was also not occupied by **TSA**. Docking analysis showed that there was no significant difference in **TSA**–HDAC binding mode between HDAC4 and HDAC6 at the entrance of the binding pocket. Also, the binding mode of **TSA** was diferent from compound **10b**. Then, we analysis the chelation geometry of the central zinc cation to explore this selectivity of **TSA** (Fig. 6). The measured distance between carbonyl and hydroxyl oxygens of hydroxamate and zinc ion is 2.18 Å, 4.19 Å in HDAC4 and 2.70 Å, 2.55 Å in HDAC6. HDAC6 ligand–protein complexes shows the expected bidentate chelation geometry of the central zinc cation. However, the observed geometry in the HDAC4 structures is more in line with the weaker monodentate binding mode of the central zinc cation. Therefore, the comparison of the chelation mode of hydroxamate with the zinc ion in the two complexes has confirmed the target selective profile of **TSA**.

On the other hand, Bradner et al. described that hydroxamates such as vorinostat, belinostat, and panobinostat showed much reduced potency against class IIa enzymes including HDAC4. They interpreted this observation based on the available crystal structures of HDAC4 (PDB ID: 2VQM) and HDAC7 (PDB ID: 3C0Z and 3C10) bound to hydroxamate inhibitors. None of the ligand–protein complexes showed the expected bidentate chelation geometry of the central zinc cation, as observed in the structures of ligand-bound human HDAC8 (PDB ID: 1T64 and 1T69) and bacterial homologs (PDB ID: 1ZZ1). The observed geometry in the published HDAC4 and HDAC7 structures, however, was more in line with the weaker monodentate binding mode of the neutral form of the hydroxamic acid.[42] This could also help us to understand the selectivity of TSA in our study.

In an effort to identify potential drug like compounds, six compounds were chosen for determination of their physicohemical properties including $\log D_{7.4}$ and aqueous solubility. The partition coefficient ($\log D$) at pH 7.4 and aqueous solubility were determined according to the method of Avdeef and Tsinman[43] on a Gemini Profiler instrument (pION) by the 'goldstandard' Avdeef–Bucher potentiometric titration method.[44]

As shown in Table 2, all six compounds displayed good aqueous solubility. Generally, the introduction of hydrophilic groups resulted in a significant increase in solubility. For instance, comparing the solubility of **10h** and **10a**, showed that the former compound were more soluble than the latter compound. Introduction of hydrophobic aliphatic chain with bulk volume led to poor

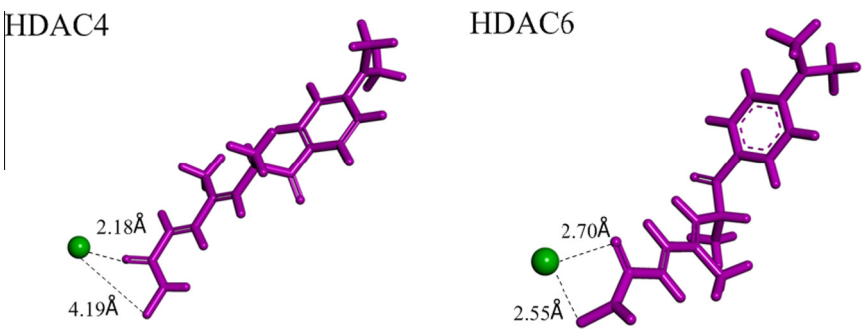


Figure 6. Examination of Zinc chelation by **TSA**. Compound **TSA** is indicated magenta. Zinc ion is indicated green.

solubility. For examples, compound **10g** with octyl group displayed reduced aqueous solubility compared to the compound **10a** with ethyl substituent. Furthermore, it was shown that compound **10h** with a benzyl as capping group were more soluble than compound **13f** with a 4-phenylbenzyl substituent. There was significant negative correlation between the $\log D_{7.4}$ and aqueous solubility of the six tested compounds. These results suggest that compounds with a smaller framework and with hydrophilic groups are likely to have better aqueous solubility.

3. Conclusions

We have synthesized and evaluated a series of hydroxamic acids with branched capping group. Although the HDACs inhibitory activity of most compounds is weaker than SAHA, compounds **13f** exhibited comparable HDACs inhibitory activity with SAHA. Interestingly, compounds (**10h, 13f, 16g**) with hydroxyethyl unit all displayed maximum HDACs inhibitory activity. Docking study on LAQ824 carried out on homology modeling of HDAC1 revealed that the hydroxyethyl unit was found to interact with the hydration shell of aspartic acid and glutamic acid in the rim of the HDAC binding channel.[34] Thus, the hydroxyethyl group is crucial in enzyme inhibitory activity. We also evaluated anti-proliferative activities of all compounds on four tumor cell lines using PC3, HCT116, A549 and HepG2. These compounds displayed good HDAC inhibitory activity, and many of them had promising anti-proliferative activity in several tumor cell lines. We next tested the isoform selectivity of fifteen selected compounds against human HDAC1, HDAC4 and HDAC6. Compounds **10a, 10b, 10d** and **16a** demonstrated outstanding selectivity against HDAC6. Furthermore, previously reported works indicated that single-treatment of HDAC6 selective inhibitors did not show anti-proliferative activity in general.[32,33] On the other hand, although compounds **10a, 10b** and **10d** demonstrated outstanding selectivity against HDAC6, their HDAC1 inhibitory activity was not vanished. The two reasons could explain why compounds with good selective profiles on HDAC6 showed decreased, but not lost, antiproliferative activities on tumor cells. Homology model and docking analysis showed that compound **10b** with bulk branched capping group was large enough to comfortably occupy the groove S in HDAC6, but not HDAC1 and HDAC4. These results emphasized the importance of cap group for the target selectivity and provide guidance for the design of HDAC6 selectivity inhibitors. In an effort to identify potential drug like compounds, six compounds that show excellent selectivity and acceptable anti-proliferative activities were chosen for determination of their physicochemical properties including $\log D_{7.4}$ and aqueous solubility. Compound **10b** and **10d** displayed good aqueous solubility with an intrinsic solubility of 2.80 mM and 2.30 mM. The results suggest that compounds with a smaller framework and with hydrophilic groups are likely to have better aqueous solubility. Creating isoform selectivity HDAC inhibitors has been challenging.[45] However, selective inhibitors would provide powerful chemical tools to elucidate the individual functions of the HDAC isoforms.[37] In addition, isoform selective inhibitors have the potential to improve therapeutic outcomes in the clinic compared to broad spectrum inhibitors. Unlike other histone deacetylase, inhibition of HDAC6 does not appear to be associated with any serious toxicity, making it an excellent drug target.[44] Despite the involvement of HDAC6 in disease states, there are relativity few examples of HDAC6 selective inhibitors.[46–50] Therefore, compounds **10b** and **10d** that show promising HDAC6 selective profile, drug-like properties which makes them ideal lead compounds for the development of agents treating other HDAC6-relevant disease such as autoimmune disorders, neurodegenerative disorders.

4. Experimental

4.1. General

Melting points were determined on a Mel-TEMP II melting point apparatus and are uncorrected. Infrared (IR) spectra (KBr) were recorded on a Nicolet Impact 410 instrument (KBr pellet). ^{1}H NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. MS spectra were recorded on a Shimadzu GC–MS 2010 (EI) or a Mariner Mass Spectrum (ESI), or a LC/MSD TOF HR-MS Spectrum. All compounds were routinely checked by TLC and ^{1}H NMR. TLCs and preparative thin-layer chromatography were performed on silica gel GF/UV 254, and the chromatograms were performed on silica gel (200–300 mesh) visualized under UV light at 254 and 365 nm. All solvents were reagent grade and, when necessary, were purified and dried by standards methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results are within (0.40% of the theoretical values).

4.1.1. (*E*)-Ethyl 3-(furan-2-yl)acrylate (6)

Furfural (**5**, 0.1 mol, 8.28 mL) in absolute ethanol (10 mL) was added in one portion to a mixture of triethyl 2-phosphonobutyrate (0.12 mol, 23.8 mL) and anhydrous potassium carbonate (0.3 mol, 41.4 g) in absolute ethanol (100 mL). After being stirred at 70 °C for 2 h, the reaction mixture was cooled to room temperature, filtrated and washed with ethanol (2 × 20 mL). The filtrate was concentrated and distilled under reduced pressure to give pure yellowish solid (13.6 g) in 82% yield; mp 24–26 °C [lit.[51] 24 °C].

4.1.2. (*E*)-Ethyl 3-(5-formylfuran-2-yl)acrylate (7)

$POCl_3$ (0.13 mol, 11.9 mL) was added to a cooled (0 °C) mixture of (*E*)-ethyl 3-(furan-2-yl)acrylate (0.12 mol, 19.6 g) and DMF (0.13 mol, 10.0 mL) over a period of 20 min. After stirring at 60 °C for 2 h, the mixture was cooled to room temperature and poured onto crushed ice (200 g). The pH of the mixture was adjusted to **7** with 10% NaOH. The resulted precipitate was filtered and purified to give brown solid (19.2 g) in 82.5% yield by crystallization with a mixture of ethanol and water; mp 73.4–74 °C [lit.[52] 74 °C].

4.1.3. General procedure for the synthesis of compounds 8, 11, 14

4.1.3.1. (*E*)-Ethyl 3-{5-[(benzylamino)methyl]furan-2-yl}acrylate (8). A solution of **7** (9.7 g, 0.05 mol) and benzylamine (5.5 mL, 0.05 mol) in methanol (50 mL) was refluxed for 3 h. After cooling to room temperature, $NaBH_4$ (1.9 g, 0.05 mol) was added slowly in three batches to the mixture and stirred for 30 min. The solution was evaporated to dryness and treated with water (50 mL). The mixture was extracted with ethyl acetate (2 × 30 mL). The combined organic solution was washed with brine (10 mL) and the solution was treated with hydrochloric acid to give hydrochloride of the product. The precipitated was filtered and washed with ethyl acetate. The filter cake was moved into a beaker, 2 N NaOH was added into the beaker. Stir until the solid dissolve. The resulted mixture was extracted with ethyl acetate (50 mL × 2). The combined organic phase was washed with saturated brine (10 mL), dried and evaporated to dryness to furnish the product (9.1 g). Yield: 63.8%; oil. MS (EI) *m*/*z* 285 (M).

4.1.3.2. (*E*)-Ethyl 3-{5-[(biphenyl-4-ylmethylamino)methyl]-furan-2-yl}acrylate (11). Compound **11** (15.25 g, 76.8% yield) was synthesized from **7** (10.7 g, 0.055 mol) and 4-biphenylethylamine (10.1 g, 0.055 mol) according to the procedure used to synthesize **8**; mp 63–65 °C; MS (EI) *m*/*z* 361 (M).

4.1.3.3. (*E*)-Ethyl 3-{5-{[2-(benzo[*d*][1,3]dioxol-5-yl)ethylamino]methyl}furan-2-yl}acrylate (14). Compound **14** (6.0 g, 43.7% yield) was synthesized from **7** (8.54 g, 0.044 mol) and homopiperonylamine (6.61 g, 0.04 mol) according to the procedure used to synthesize **8**; oil; MS (EI) *m*/*z* 343 (M+).

4.1.4. General procedure for the synthesis of compounds 9a–9p, 12a–12p, 15a–15p

4.1.4.1. (*E*)-Ethyl 3-{5-{[benzyl(ethyl)amino]methyl}furan-2-yl}acrylate (9a). Bromoethane (0.45 mL, 6 mmol) was dropped to the mixture of **8** (0.86 g, 3 mmol), K_2CO_3 (0.83 g, 6 mmol) and KI (catalytic amount) in CH_3CN. The mixture was stirred at 35 °C for 8 h. The reaction mixture was filtered. The filtrate was concentrated and purified by chromatography on a silica gel column, eluting with a mixture of ethyl acetate and petroleum ether to give the desired product (0.60 g). Yield: 64.1%, oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 0.99 (t, 3H, *J* = 6.6, CH_2CH_3), 1.06 (t, 3H, *J* = 6.0, $COOCH_2CH_3$), 2.46 (q, 2H, *J* = 6.6, CH_2CH_3), 3.57 (s, 2H, CH_2), 3.61 (s, 2H, CH_2), 3.98 (q, *J* = 6.0, 2H, $COOCH_2CH_3$), 6.24 (d, 1H, *J* = 15.6, vinyl-H), 6.39 (s, 1H, furan-H), 6.69 (s, 1H, furan-H), 7.24 (d, 1H, *J* = 15.0, vinyl-H), 7.27–7.33 (m, 5H, Ar-H); MS (EI) *m*/*z* 313 (M+).

4.1.4.2. (*E*)-Ethyl 3-{5-{[benzyl(propyl)amino]methyl}furan-2-yl}acrylate (9b). Compound **9b** (1.10 g, 96.1% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and 1-bromopropane (0.96 mL, 10.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 327 (M+).

4.1.4.3. (*E*)-Ethyl 3-{5-{[benzyl(isopropyl)amino]methyl}furan-2-yl}acrylate (9c). Compound **9c** (0.41 g, 36.1% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and 2-bromopropane (0.96 mL, 10.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 327 (M+).

4.1.4.4. (*E*)-Ethyl 3-{5-{[benzyl(butyl)amino]methyl}furan-2-yl}acrylate (9d). Compound **9d** (0.93 g, 77.9% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and 1-bromobutane (1.13 mL, 10.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 341 (M+).

4.1.4.5. (*E*)-Ethyl 3-{5-{[benzyl(isobutyl)amino]methyl}furan-2-yl}acrylate (9e). Compound **9e** (0.83 g, 69.2% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and 1-bromo-2-methylpropane (1.13 mL, 10.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 341 (M+).

4.1.4.6. (*E*)-Ethyl 3-{5-{[benzyl(hexyl)amino]methyl}furan-2-yl}acrylate (9f). Compound **9f** (1.10 g, 85.2% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and bromohexane (0.99 mL, 7 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 369 (M+).

4.1.4.7. (*E*)-Ethyl-3-{5-{[benzyl(octyl)amino]methyl}furan-2-yl}acrylate (9g). Compound **9g** (0.91 g, 65.5% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and bromooctane (1.22 mL, 7 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 397 (M+).

4.1.4.8. (*E*)-Ethyl 3-{5-{[benzyl(2-hydroxyethyl)amino]methyl}-furan-2-yl}acrylate (9h). Compound **9h** (1.08 g, 93.8% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and 2-bromoethanol (0.77 mL, 10.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 329 (M+).

4.1.4.9. (*E*)-Ethyl 3-{5-{[benzyl(2-phenoxyethyl)amino]methyl}-furan-2-yl}acrylate (9i). Compound **9i** (0.91 g, 74.8% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and (2-bromoethoxy)benzene (0.72 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 405 (M+).

4.1.4.10. (*E*)-Ethyl 3-{5-{benzyl[2-(*p*-tolyloxy)ethyl]amino}-methyl}furan-2-ylacrylate (9j). Compound **9j** (0.79 g, 62.9% yield) was synthesized from **8** (0.85 g, 3 mmol) and 1-(2-bromoethoxy)-4-methylbenzene (0.77 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 419 (M+).

4.1.4.11. (*E*)-Ethyl 3-{5-[benzyl(3-phenoxypropyl)amino]-methyl}furan-2-ylacrylate (9k). Compound **9k** (1.09 g, 86.6% yield) was synthesized from **8** (0.85 g, 3 mmol) and (3-bromopropoxy)benzene (0.77 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 419 (M+).

4.1.4.12. (*E*)-Ethyl 3-{5-{{benzyl[3-(*p*-tolyloxy)propyl]amino}-methyl}furan-2-yl}acrylate (9l). Compound **9l** (1.01 g, 77.9% yield) was synthesized from **8** (0.85 g, 3 mmol) and 1-(3-bromopropoxy)-4-methylbenzene (0.82 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 433 (M+).

4.1.4.13. (*E*)-Ethyl 3-{5-{[benzyl(4-phenoxybutyl)amino]methyl}-furan-2-yl}acrylate (9m). Compound **9m** (0.90 g, 59.4% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and (4-bromobutoxy)benzene (0.86 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 433 (M+).

4.1.4.14. (*E*)-Ethyl 3-{5-{{benzyl[4-(*p*-tolyloxy)butyl]amino}-methyl}furan-2-yl}acrylate (9n). Compound **9n** (1.26 g, 80.5% yield) was synthesized from **8** (1.0 g, 3.5 mmol) and 1-(4-bromobutoxy)-4-methylbenzene(0.87 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 447 (M+).

4.1.4.15. (*E*)-Ethyl 3-{5-{[(biphenyl-4-ylmethyl)(ethyl)amino]-methyl}furan-2-yl}acrylate (12a). Compound **12a** (1.26 g, 92.2% yield) was synthesized from **11** (1.26 g, 3.5 mmol) and 1-bromoethane (0.78 mL, 10.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 389 (M+).

4.1.4.16. (*E*)-Ethyl 3-{5-{[(biphenyl-4-ylmethyl)(isopropyl)amino]methyl}furan-2-yl}acrylate (12b). Compound **12b** (0.51 g, 36.1% yield) was synthesized from **11** (1.26 g, 3.5 mmol) and 2-bromopropane (0.96 mL, 10.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 403 (M+).

4.1.4.17. (*E*)-Ethyl 3-{5-{[(biphenyl-4-ylmethyl)(pentyl)amino]-methyl}furan-2-yl}acrylatee (12c). Compound **12c** (1.36 g, 90.1% yield) was synthesized from **11** (1.26 g, 3.5 mmol) and bromopentane (0.87 mL, 7 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 431 (M+).

4.1.4.18. (*E*)-Ethyl 3-{5-{[(biphenyl-4-ylmethyl)(octyl)amino]-methyl}furan-2-yl}acrylate (12d). Compound **12d** (1.08 g, 65.5% yield) was synthesized from **11** (1.26 g, 3.5 mmol) and bromooctane (1.22 mL, 7 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 473 (M+).

4.1.4.19. (*E*)-Ethyl 3-{5-{[benzyl(biphenyl-4-ylmethyl)amino]-methyl}furan-2-yl}acrylate (12e). Compound **12e** (1.14 g,

72.4% yield) was synthesized from **11** (1.26 g, 3.5 mmol) and benzylbromide (0.5 mL, 4.2 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 451 (M+).

4.1.4.20. (*E*)-Ethyl 3-{5-{[(biphenyl-4-ylmethyl)(2-hydroxyethyl)amino]methyl}furan-2-yl}acrylate (12f). Compound **12f** (1.33 g, 93.8% yield) was synthesized from **11** (1.26 g, 3.5 mmol) and 2-bromoethanol (0.77 mL, 10.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 405 (M+).

4.1.4.21. (*E*)-Ethyl 3-{5-{[(biphenyl-4-ylmethyl)(2-phenoxyethyl)amino]methyl}furan-2-yl}acrylate (12g). Compound **12g** (1.08 g, 74.8% yield) was synthesized from **11** (1.26 g, 3 mmol) and (2-bromoethoxy)benzene (0.72 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 481 (M+).

4.1.4.22. (*E*)-Ethyl 3-{5-{(biphenyl-4-ylmethyl)[2-(*p*-tolyloxy)ethyl]amino}methyl}furan-2-ylacrylate (12h). Compound **12h** (0.93 g, 62.9% yield) was synthesized from **11** (1.26 g, 3 mmol) and 1-(2-bromoethoxy)-4-methylbenzene (0.77 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 495 (M+).

4.1.4.23. (*E*)-Ethyl 3-{5-[(biphenyl-4-ylmethyl)(3-phenoxypropyl)amino]methyl}furan-2-ylacrylate (12i). Compound **12i** (1.29 g, 86.6% yield) was synthesized from **11** (1.26 g, 3 mmol) and (3-bromopropoxy)benzene (0.77 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 495 (M+).

4.1.4.24. (*E*)-Ethyl 3-{5-{{(biphenyl-4-ylmethyl)[3-(*p*-tolyloxy)propyl]amino}methyl}furan-2-yl}acrylate (12j). Compound **12j** (1.19 g, 77.9% yield) was synthesized from **11** (1.26 g, 3 mmol) and 1-(3-bromopropoxy)-4-methylbenzene (0.82 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 509 (M+).

4.1.4.25. (*E*)-Ethyl 3-{5-{[(biphenyl-4-ylmethyl)(4-phenoxybutyl)amino]methyl}furan-2-yl}acrylate (12k). Compound **12k** (1.06 g, 59.4% yield) was synthesized from **11** (1.26 g, 3.5 mmol) and (4-bromobutoxy)benzene (0.86 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 509 (M+).

4.1.4.26. (*E*)-Ethyl 3-{5-{{(biphenyl-4-ylmethyl)[4-(*p*-tolyloxy)butyl]amino}methyl}furan-2-yl}acrylate (12l). Compound **12l** (1.47 g, 80.5% yield) was synthesized from **11** (1.26 g, 3.5 mmol) and 1-(4-bromobutoxy)-4-methylbenzene (0.87 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 523 (M+).

4.1.4.27. (*E*)-Ethyl 3-{5-{[(2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](ethyl)amino}methyl}furan-2-ylacrylate (15a). Compound **15a** (0.52 g, 46.4% yield) was synthesized from **14** (1.0 g, 3 mmol) and 1-bromoethane (0.67 mL, 9 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 371 (M+).

4.1.4.28. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](propyl)amino}methyl}furan-2-yl}acrylate (15b). Compound **15b** (0.60 g, 62.3% yield) was synthesized from **14** (0.86 g, 2.5 mmol) and 1-bromopropane (0.91 mL, 10 mmol) according to the procedure used to synthesize **9a**; oil; MS(EI) *m*/*z* 385 (M+).

4.1.4.29. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](butyl)amino}methyl}furan-2-yl}acrylate (15c). Compound **15c** (0.58 g, 58.1% yield) was synthesized from **14** (0.86 g, 2.5 mmol) and 1-bromobutane (0.81 mL, 7.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 399 (M+).

4.1.4.30. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](isobutyl)amino}methyl}furan-2-yl}acrylate (15d). Compound **15d** (0.42 g, 42.1% yield) was synthesized from **14** (0.86 g, 2.5 mmol) and 1-bromo-2-methylpropane (1.1 mL, 10 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 399 (M+).

4.1.4.31. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](pentyl)amino}methyl}furan-2-yl}acrylate (15e). Compound **15e** (0.46 g, 58.6% yield) was synthesized from **14** (0.65 g, 1.9 mmol) and bromopentane (1.0 mL, 8 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 413 (M+).

4.1.4.32. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](benyl)amino}methyl}furan-2-yl}acrylate (15f). Compound **15f** (0.94 g, 72.4% yield) was synthesized from **14** (1.0 g, 3 mmol) and benzylbromide (0.43 mL, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 433 (M+).

4.1.4.33. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](2-hydroxyethyl)amino}methyl}furan-2-yl}acrylate (15g). Compound **15g** (0.56 g, 47.9% yield) was synthesized from **14** (1.0 g, 3 mmol) and 2-bromoethanol (0.64 mL, 9 mmol) according to the procedure used to synthesize **9a**; oil; MS(EI) *m*/*z* 387 (M+).

4.1.4.34. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](2-phenoxyethyl)amino}methyl}furan-2-yl}acrylate (15h). Compound **15h** (0.96 g, 69.1% yield) was synthesized from **14** (1.0 g, 3 mmol) and (2-bromoethoxy)benzene (0.91 g, 4.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 463 (M+).

4.1.4.35. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl][2-(*p*-tolyloxy]ethyl}amino}methyl}furan-2-yl}acrylate (15i). Compound **15i** (0.78 g, 53.6% yield) was synthesized from **14** (1.0 g, 3 mmol) and 1-(2-bromoethoxy)-4-methylbenzene (0.77 g, 3.6 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 477 (M+).

4.1.4.36. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](3-phenoxypropyl)amino}methyl}furan-2-yl}acrylate (15j). Compound **15j** (1.28 g, 89.4% yield) was synthesized from **14** (1.0 g, 3 mmol) and (3-bromopropoxy)benzene (0.97 g, 4.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 477 (M+).

4.1.4.37. (*E*)-Ethyl 3-{5-{{[2-(benzo[*d*][1,3]dioxol-5-yl)ethyl](4-phenoxybutyl)amino}methyl}furan-2-yl}acrylate (15k). Compound **15k** (1.0 g, 67.9% yield) was synthesized from **14** (1.0 g, 3 mmol) and (4-bromobutoxy)benzene (1.0 g, 4.5 mmol) according to the procedure used to synthesize **9a**; oil; MS (EI) *m*/*z* 491 (M+).

4.1.5. General procedure for the synthesis of compounds 10a–10p, 13a–13p, 16a–16p

4.1.5.1. (*E*)-3-{5-{[Benzyl(ethyl)amino]methyl}furan-2-yl}-N-hydroxyacrylamide (10a). Hydroxylamine hydrochloride (1.28 g, 18.4 mmol) in methanol (3.2 mL) was mixed with KOH (1.03 g, 18.4 mmol) at 40 °C in methanol (6.0 mL), after stirring for 5 min the mixture was cooled to 0 °C for 30 min. The precipitate was filtered. Compound **9a** (0.60 g, 2.0 mmol) was then added to the filtrate followed by addition of KOH (0.06 g, 1.0 mmol). The mixture was stirred at room temperature for 1 h. The solution was added to stirring cold water (20 mL), and the pH was adjusted to 7 by adding 2 N HCl. The mixture was extracted with ethyl acetate (15 mL × 2). The combined organic solution was washed with saturated brine (5 mL), dried and evaporated to dryness. The residue

solid was purified by recrystallization. Yield 43.3% (0.26 g); oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 0.99 (t, 3H, J = 6.6, CH_2CH_3), 2.46 (q, 2H, J = 6.6, CH_2CH_3), 3.57 (s, 2H,CH_2), 3.61 (s, 2H, CH_2), 6.24 (d, 1H, J = 15.6, vinyl-H), 6.39 (s, 1H, furan-H), 6.69 (s, 1H, furan-H), 7.24 (d, 1H, J = 15.0, vinyl-H), 7.27–7.33 (m, 5H, Ar-H), 9.0 (s, 1H, CONHOH), 10.0 (s, 1H, CONHOH); MS (ESI) m/z 299 (M−H); HRMS calcd for $C_{17}H_{21}N_2O_3$ $[MH]^+$: 301.1547; found: 301.1544; IR (KBr) ν (cm^{-1}) = 3416, 3224, 2968, 1621. HPLC purity: 99. 0% (t_R = 4.54 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.2. (*E*)-3-{5-{[Benzyl(propyl)amino]methyl}furan-2-yl}-N-hydroxyacrylamide (10b). Compound **10b** (0.25 g, 39.3% yield) was synthesized from **9b** (0.62 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.82 (t, 3H, J = 7.1, CH_3), 1.47 (m, 2H, CH_2CH_3), 2.36 (t, 2H, J = 6.9, $CH_2CH_2CH_3$), 3.58 (s, 2H, CH_2), 3.61 (s, 2H, CH_2), 6.20 (d, 2H, J = 15.3, vinyl-H), 6.39 (s, 1H, furan-H), 6.70 (s, 1H, furan-H), 7.23 (d, 2H, J = 15.6, vinyl-H), 7.25–7.34 (m, 5H, Ar-H), 8.99 (s, 1H, CONHOH), 10.75 (s, 1H, CONHOH); MS (ESI) m/z 313 (M−H); HRMS calcd for $C_{18}H_{23}N_2O_3$ $[MH]^+$: 315.1703; found: 315.1700; IR (KBr) ν (cm^{-1}) = 3430, 3231, 2975, 1665, 1456. HPLC purity: 98. 7% (t_R = 5.44 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.3. (*E*)-3-{5-{[Benzyl(isopropyl)amino]methyl}furan-2-yl}-N-hydroxyacrylamide (10c). Compound **10c** (0.12 g, 37.5% yield) was synthesized from **9c** (0.31 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6; 300 MHz, δ ppm): 1.01 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 2.91 (m, 1H, CH), 3.58 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 6.20 (d, 1H, J = 15.6, vinyl-H), 6.34 (d, 1H, J = 2.7, furan-H), 6.59 (s, 1H, furan-H), 7.11 (d, 1H, J = 15.0, vinyl-H), 7.18–7.36 (m, 5H, Ar-H); MS (ESI) m/z 313 (M−H); HRMS calcd for $C_{18}H_{23}N_2O_3$ $[MH]^+$: 315.1703; found: 315.1712; IR (KBr) ν (cm^{-1}) = 3438, 3267, 2954, 1616, 1495. HPLC purity: 99. 1% (t_R = 4.86 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.4. (*E*)-3-{5-{[Benzyl(butyl)amino]methyl}furan-2-yl}-N-hydroxyacrylamide (10d). Compound **10d** (0.28 g, 43.4% yield) was synthesized from **9d** (0.65 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.81 (t, 3H, J = 7.2, CH_3), 1.25 (m, 2H, CH_2), 1.45 (m, 2H, CH_2), 2.38 (t, 2H, J = 7.1, CH_2), 3.58 (s, 2H, CH_2), 3.61 (s, 2H, CH_2), 6.21 (d, 1H, J = 15.3, vinyl-H), 6.39 (d, 1H, J = 3.3, furan-H), 6.70 (d, 1H, J = 3.0, furan-H), 7.23 (d, 1H, J = 15.6, vinyl-H), 7.23–7.34 (m, 5H, Ar-H), 9.02 (s, 1H, CONHOH), 10.76 (s, 1H, CONHOH); MS (ESI) m/z 327 (M−H); HRMS calcd for $C_{19}H_{25}N_2O_3$ $[MH]^+$: 329.1860; found: 329.1866; IR (KBr) ν (cm^{-1}) = 3416, 3246, 2968, 1659, 1449. HPLC purity: 98. 0% (t_R = 6.78 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.5. (*E*)-3-{5-{[Benzyl(isobutyl)amino]methyl}furan-2-yl}-N-hydroxyacrylamide (10e). Compound **10e** (0.30 g, 45.1% yield) was synthesized from **9e** (0.65 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.81 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 1.81 (m, 1H, CH), 2.15 (d, 2H, J = 7.5, CH_2CH), 3.57 (s, 2H, CH_2), 3.59 (s, 2H, CH_2), 6.20 (d, 1H, J = 15.3, vinyl-H), 6.38 (d, 1H, J = 3.3, furan-H), 6.70 (d, 1H, J = 3.0, furan-H), 7.23 (d, 1H, J = 15.3, vinyl-H), 7.22–7.35 (m, 5H, Ar-H), 8.98 (s, 1H, CONHOH), 10.76 (s, 1H, CONHOH); MS (ESI) m/z 327 (M−H); HRMS calcd for $C_{19}H_{25}N_2O_3$ $[MH]^+$: 329.1860; found: 329.1863; IR (KBr) ν (cm^{-1}) = 3473, 3437, 3238, 2953, 1658. HPLC purity: 98. 7% (t_R = 6.57 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.6. (*E*)-3-{5-{[Benzyl(hexyl)amino]methyl}furan-2-yl}-N-hydroxyacrylamide (10f). Compound **10f** (0.32 g, 45.1% yield) was synthesized from **9f** (0.71 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.82 (t, 3H, J = 6.9, CH_3), 1.13–1.24 (m, 6H, $CH_2 \times 3$), 1.40–1.46 (m, 2H, CH_2), 2.37 (t, 2H, J = 7.1, CH_2), 3.58 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 6.21 (d, 1H, J = 15.6, vinyl-H), 6.39 (d, 1H, J = 3.0, furan-H), 6.71 (d, 1H, J = 3.0, furan-H), 7.23 (d, 1H, J = 15.9, vinyl-H), 7.23–7.35 (m, 5H, Ar-H), 9.03 (s, 1H, CONHOH), 10.77 (s, 1H, CONHOH); MS (ESI) m/z 357 (M+H); HRMS calcd for $C_{21}H_{29}N_2O_3$ $[MH]^+$: 357.2173; found: 357.218; IR (KBr) ν (cm^{-1}) = 3260, 3024, 2861, 1580. HPLC purity: 98. 6% (t_R = 5.65 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.7. (*E*)-3-{5-{[Benzyl(octyl)amino]methyl}furan-2-yl}-N-hydroxyacrylamide (10g). Compound **10g** (0.32 g, 41.2% yield) was synthesized from **9g** (0.76 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.90 (t, 3H, J = 6.6, CH_3), 1.26 (m, 10H, $CH_2 \times 5$), 1.50 (m, 2H,CH_2), 2.43 (t, 2H, J = 6.7,CH_2), 3.64 (s, 2H, CH_2), 3.67 (s, 2H, CH_2), 6.27 (d, 1H, J = 15.6, vinyl-H), 6.45 (d,1H, J = 3.0, furan-H), 6.76 (d, 1H, J = 3.0, furan-H), 7.28 (d, 1H, J = 15.6, vinyl-H), 7.31–7.41 (m, 5H, Ar-H), 9.08 (s, 1H, CONHOH), 10.08 (s, 1H, CONHOH); MS (ESI) m/z 383 (M−H); HRMS calcd for $C_{23}H_{33}N_2O_3$ $[MH]^+$: 385.2486; found: 385.2475; IR (KBr) ν (cm^{-1}) = 3416, 3253, 2940, 1620, 1534. HPLC purity: 98. 1% (t_R = 6.34 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.8. (*E*)-3-{5-{[Benzyl(2-hydroxyethyl)amino]methyl}furan-2-yl}-N-hydroxyacrylamide (10h). Compound **10h** (0.24 g, 37.5% yield) was synthesized from **9h** (0.63 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.53 (t, 2H, J = 6.0, CH_2), 3.51 (t, 2H, J = 6.0, OCH_2), 3.63 (s, 2H, CH_2),3.67 (s, 2H, CH_2), 4.41 (t, 1H, J = 5.1, OH), 6.21 (d, 1H, J = 15.6, vinyl-H), 6.42 (s, 1H, furan-H), 6.72 (s,1H, furan-H), 7.25 (d, 1H, J = 15.6, vinyl-H), 7.2–7.35 (m, 5H, Ar-H), 9.01 (s, 1H, CONHOH), 10.75 (s, 1H, CONHOH); MS (ESI) m/z 315 (M−H); HRMS calcd for $C_{17}H_{21}N_2O_4$ $[MH]^+$: 317.1496; found: 317.1486; IR (KBr) ν (cm^{-1}) = 3217, 2925, 2847, 1740. HPLC purity: 98.3% (t_R = 3.17 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.9. (*E*)-3-{5-{[Benzyl(2-phenoxyethyl)amino]methyl}furan-2-yl}-N-hydroxyacrylamide (10i). Compound **10i** (0.34 g, 43.1% yield) was synthesized from **9i** (0.80 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.84 (t, 2H, J = 5.6, NCH_2), 3.72 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 4.06 (t, 2H, J = 5.4, CH_2O), 6.24 (d, 1H, J = 15.6, vinyl-H), 6.45 (d, 1H, J = 2.1, furan-H), 6.72 (d, 1H, J = 1.8, furan-H), 6.88–6.93 (m, 3H, Ar-H), 7.27 (d, 1H, J = 15.6, vinyl-H), 7.22–7.39 (m, 7H, Ar-H), 9.04 (s, 1H, CONHOH), 10.76 (s, 1H, CONHOH); MS (ESI) m/z 393 (M+H); HRMS calcd for $C_{23}H_{25}N_2O_4$ $[MH]^+$: 393.1809; found: 393.1816; IR (KBr) ν (cm^{-1}) = 3032, 2918, 1722, 1641. HPLC purity: 98. 9% (t_R = 4.48 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.10. (*E*)-3-{5-{{Benzyl[2-(*p*-tolyloxy)ethyl]amino}methyl}-furan-2-yl}-N-hydroxyacrylamide (10j). Compound **10j** (0.35 g, 43.6% yield) was synthesized from **9j** (0.81 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.20 (s, 3H, CH_3), 2.81 (t, 2H, J = 6.8, NCH_2), 3.70 (s, 2H, CH_2), 3.74 (s, 2H, CH_2), 4.00 (t, 2H, J = 6.8, CH_2O), 6.21 (d, 1H, J = 15.6, vinyl-H), 6.43 (d, 1H, J = 3.0, furan-H), 6.69 (d, 1H, J = 2.7, furan-H), 6.76 (d, 2H, J = 8.4, Ar-H), 7.03 (d, 2H, J = 8.1, Ar-H), 7.22 (d, 1H, J = 15.3, vinyl-H), 7.21–7.37 (m, 5H, Ar-H); MS (ESI) m/z 405 (M−H); HRMS calcd for $C_{24}H_{27}N_2O_4$ $[MH]^+$: 407.1965; found: 407.1969; IR (KBr) ν (cm^{-1}) = 3181,

3039, 2875, 1609. HPLC purity: 98. 4% (t_R = 6.42 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.11. (*E*)-3-{5-{[Benzyl(3-phenoxypropyl)amino]methyl}-furan-2-yl}-N-hydroxyacrylamide (10k). Compound **10k** (0.36 g, 43.8% yield) was synthesized from **9k** (0.81 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.89 (m, 2H, CH_2), 2.58 (t, 2H, J = 6.7, CH_2), 3.63 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 3.96 (t, 2H, J = 6.3,CH_2), 6.14 (d, 1H, J = 15.6, vinyl-H), 6.41 (d, 1H, J = 3.0, furan-H), 6.69 (d, 1H, J = 3.0, furan-H), 6.77–6.93 (m, 3H, Ar-H), 7.28 (d, 1H, J = 15.6, vinyl-H), 7.20–7.35 (m, 7H, Ar-H), 9.03 (s, 1H, CON*H*OH), 10.73 (s, 1H, CONHO*H*); MS (ESI) m/z 407 (M+H); HRMS calcd for $C_{24}H_{27}N_2O_4$ $[MH]^+$: 407.1965; found: 407.1971; IR (KBr) ν (cm^{-1}) = 3452, 2085, 1655, 1634. HPLC purity: 97. 9% (t_R = 5.91 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.12. (*E*)-3-{5-{{Benzyl[3-(*p*-tolyloxy)propyl]amino}methyl}-furan-2-yl}-N-hydroxyacrylamide (10l). Compound **10l** (0.28 g, 43.6% yield) was synthesized from **9l** (0.63 g, 1.5 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.86 (t, 2H, J = 6.5, CH_2), 2.01 (s, 3H, CH_3), 2.61 (m, 2H, CH_2),3.62 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.90 (t, 2H, J = 6.9, CH_2O), 6.22 (d, 1H, J = 15.6, vinyl-H), 6.35 (d, 1H, J = 3.0, furan-H), 6.69 (d, 1H, J = 3.0, furan-H), 6.74 (d, 2H, J = 8.4, Ar-H), 7.05 (d, 2H, J = 8.4, Ar-H), 7.24 (d, 1H, J = 15.0, vinyl-H), 7.12–7.35 (m, 5H, Ar-H), 9.08 (s, 1H, CON*H*OH), 10.72 (s, 1H,CONHO*H*); MS (ESI) m/z 421 (M+H); HRMS calcd for $C_{25}H_{29}N_2O_4$ $[MH]^+$: 421.2122; found: 421.2119; IR (KBr) ν (cm^{-1}) = 3573, 3423, 1730, 1570. HPLC purity: 98. 5% (t_R = 6.84 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.13. (*E*)-3-{5-{[Benzyl(4-phenoxybutyl)amino]methyl}-furan-2-yl}-N-hydroxyacrylamide (10m). Compound **10m** (0.25 g, 40.2% yield) was synthesized from **9m** (0.63 g, 1.5 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.62–1.73 (m, 4H, $CH_2 \times 2$), 2.48 (t, 2H, J = 6.4, CH_2), 3.60 (s, 2H, CH_2), 3.62 (s, 2H, CH_2), 3.88 (t, 2H, J = 6.2, CH_2O), 6.22 (d, 1H, J = 15.6, vinyl-H), 6.40 (d, 1H, J = 3.0, furan-H, 6.70 (d, 1H, J = 3.0, furan-H), 6.88 (d, 1H, J = 15.6, vinyl-H), 6.78–6.90 (m, 3H, Ar-H), 6.99–7.59 (m, 7H, Ar-H), 9.04 (s, 1H, CON*H*OH), 10.77 (s, 1H, CONHO*H*); MS (ESI) m/z 421 (M+H); HRMS calcd for $C_{25}H_{29}N_2O_4$ $[MH]^+$: 421.2122; found: 421.2120; IR (KBr) ν (cm^{-1}) = 3566, 3437, 1698, 1552. HPLC purity: 98. 8% (t_R = 5.09 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.14. (*E*)-3-{5-{{Benzyl[4-(*p*-tolyloxy)butyl]amino}methyl}-furan-2-yl}-N-hydroxyacrylamide (10n). Compound **10n** (0.28 g, 43.3% yield) was synthesized from **9n** (0.65 g, 1.5 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.61–1.68 (m, 4H, $CH_2 \times 2$), 2.21 (s, 3H, CH_3), 2.44 (t, 2H, J = 6.4, CH_2), 3.59 (s, 2H, CH_2), 3.62 (s, 2H, CH_2), 3.84 (t, 2H, J = 5.9, CH_2O), 6.22 (d, 1H, J = 15.6, vinyl-H), 6.39 (d, 1H, J = 3.0, furan-H), 6.68 (d, 1H, J = 2.7, furan-H), 6.75 (d, 2H, J = 8.4, Ar-H), 7.05 (d, 2H, J = 8.4, Ar-H), 7.26 (d, 1H, J = 15.6, vinyl-H), 7.19–7.34 (m, 5H, Ar-H), 9.04 (s, 1H, CON*H*OH), 10.77 (s,1H, CONHO*H*); MS (ESI) m/z 435 (M+H); HRMS calcd for $C_{26}H_{31}N_2O_4$ $[MH]^+$: 435.2278; found: 435.2276; IR (KBr) ν (cm^{-1}) = 3480, 3423, 2925, 1634. HPLC purity: 97. 6% (t_R = 7.74 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.15. (*E*)-3-{5-{[(Biphenyl-4-ylmethyl)(ethyl)amino]methyl}-furan-2-yl}-N-hydroxyacrylamide (13a). Compound **13a** (0.33 g, 44.3% yield) was synthesized from **12a** (0.75 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.12 (t, 3H, J = 7.1, CH_2CH_3), 2.56 (q, 2H, J = 7.1, CH_2CH_3), 3.63 (s, 2H, CH_2), 3.73 (s, 2H, CH_2), 6.29 (d, 2H, J = 15.6, vinyl-H), 6.48 (d, 1H, J = 3.3, furan-H), 6.77 (d, 1H, J = 3.0, furan-H), 7.29 (d, 2H, J = 15.6, vinyl-H), 7.39–7.77 (m, 9H, Ar-H), 9.06 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 375 (M−H); HRMS calcd for $C_{23}H_{25}N_2O_3$ $[MH]^+$: 377.1860; found: 377.1861; IR (KBr) ν(cm^{-1}) = 3440, 2968, 2918, 2839, 1630. HPLC purity: 97. 3% (t_R = 4.76 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.16. (*E*)-3-{5-{[(Biphenyl-4-ylmethyl)(isopropyl)amino]-methyl}furan-2-yl}-N-hydroxyacrylamide (13b). Compound **13b** (0.15 g, 38.3% yield) was synthesized from **12b** (0.39 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.03 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 2.93 (m, 1H, CH), 3.62 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 6.08 (d, 1H, J = 15.6, vinyl-H), 6.41 (d, 1H, J = 2.7, furan-H), 6.71 (d, 1H, J = 2.7, furan-H), 7.24(d, 1H, J = 15.6, vinyl-H), 7.33–7.68(m, 9H, Ar-H), 9.01(s, 1H, CON*H*OH), 10.8(s, 1H, CONHO*H*); MS (ESI) m/z 389 (M−H); HRMS calcd for $C_{24}H_{27}N_2O_3$ $[MH]^+$: 391.2016; found: 391.2013; IR (KBr) ν (cm^{-1}) = 3423, 2968, 1655, 1630. HPLC purity: 99. 1% (t_R = 5.13 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.17. (*E*)-3-{5-{[(Biphenyl-4-ylmethyl)(pentyl)amino]-methyl}furan-2-yl}-N-hydroxyacrylamide (13c). Compound **13c** (0.34 g, 40.4% yield) was synthesized from **12c** (0.84 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.85 (s, 3H, CH_3), 1.23 (m, 4H, $CH_2 \times 2$), 1.48 (m, 2H, CH_2), 2.41 (q, 2H, J = 4.5, CH_2), 3.63 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 6.23 (d, 1H, J = 15.6, vinyl-H), 6.41 (d, 1H, J = 3.0, furan-H), 6.71 (d, 1H, J = 3.0, furan-H), 7.25 (d, 1H, J = 15.6, vinyl-H), 7.33–7.49 (m, 5H, Ar-H), 7.65–7.68 (m, 4H, Ar-H), 9.0 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 419 (M+H); HRMS calcd for $C_{26}H_{31}N_2O_3$ $[MH]^+$: 419.2329; found: 419.2335; IR (KBr) ν (cm^{-1}) = 3200, 3039, 2928, 1641. HPLC purity: 98. 1% (t_R = 4.26 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.18. (*E*)-3-{5-{[(Biphenyl-4-ylmethyl)(octyl)amino]methyl}-furan-2-yl}-N-hydroxyacrylamide (13d). Compound **13d** (0.37 g, 40.1% yield) was synthesized from **12d** (0.92 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.82 (t, 3H, J = 6.4, CH_3), 1.19 (m, 10H, $CH_2 \times 5$), 1.46 (m, 2H,CH_2), 2.41 (t, 2H, J = 6.7, CH_2), 3.63 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 6.25 (d, 1H, J = 15.3, vinyl-H), 6.41 (d, 1H, J = 2.7, furan-H), 6.71 (d, 1H, J = 2.7, furan-H), 7.26 (d, 1H, J = 15.6, vinyl-H), 7.33–7.48 (m, 5H, Ar-H), 7.65–7.67 (m, 4H, Ar-H), 9.0 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 461 (M+H); HRMS calcd for $C_{29}H_{37}N_2O_3$ $[MH]^+$: 461.2799; found: 461.2812; IR (KBr) ν (cm^{-1}) = 3025, 2925, 2854, 1637, 1505. HPLC purity: 98. 2% (t_R = 5.49 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.19. (*E*)-3-{5-{[Benzyl(biphenyl-4-ylmethyl)amino]methyl}-furan-2-yl}-N-hydroxyacrylamide (13e). Compound **13e** (0.33 g, 37.7% yield) was synthesized from **12e** (0.88 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 3.60 (s, 6H, $CH_2 \times 3$), 6.26 (d, 1H, J = 15.6, vinyl-H), 6.38 (d, 1H, J = 3.0, furan-H), 6.58 (d, 1H, J = 3.0, furan-H), 7.21 (d, 1H, J = 15.6, vinyl-H), 7.34–7.45 (m, 10H, Ar-H), 7.64–7.67 (m, 4H, Ar-H); MS (ESI) m/z 437 (M−H); HRMS calcd for $C_{28}H_{27}N_2O_3$ $[MH]^+$: 439.2016; found: 439.2022; IR (KBr) ν (cm^{-1}) = 3027, 2925, 2871, 1598. HPLC purity: 98. 0% (t_R = 4.81 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.20. (*E*)-3-{5-{[(Biphenyl-4-ylmethyl)(2-hydroxyethyl)-amino]methyl}furan-2-yl}-N-hydroxyacrylamide (13f). Compound **13f** (0.28 g, 36.2% yield) was synthesized from **12f** (0.80 g, 2.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.57 (t, 2H, J = 6.3, NCH_2), 3.53 (t, 2H, J = 5.1, CH_2OH), 3.68 (s, 2H, CH_2), 3.71 (s, 2H, CH_2), 4.43 (t, 1H, J = 4.8, CH_2OH), 6.24 (d, 1H, J = 15.6, vinyl-H), 6.44 (d, 1H, J = 2.4, furan-H), 6.72 (s, 1H), 7.24 (d, 1H, J = 15.6, vinyl-H), 7.33–7.38 (m, 1H, Ar-H), 7.44–7.49 (m, 4H, Ar-H), 7.64 (d, 1H, J = 8.4, Ar-H), 7.66 (d, 1H, J = 7.8, Ar-H), 9.03 (s, 1H, CON*H*OH), 10.77 (s, 1H, CONHO*H*); MS (ESI) m/z 393 (M+H); HRMS calcd for $C_{23}H_{25}N_2O_4$ [MH]$^+$: 393.1809; found: 393.1816; IR (KBr) ν (cm^{-1}) = 3409, 2932, 1630, 1559. HPLC purity: 99. 3% (t_R = 3.94 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.21. (*E*)-3-{5-{[(Biphenyl-4-ylmethyl)(2-phenoxyethyl)-amino]methyl}furan-2-yl}-N-hydroxyacrylamide (13g). Compound **13g** (0.31 g, 43.9% yield) was synthesized from **12g** (0.70 g, 1.5 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.87 (t, 2H, J = 5.3, NCH_2), 3.77 (s, 2H, CH_2), 3.80 (s, 2H, CH_2), 4.10 (t, 2H, J = 5.4, CH_2O), 6.25 (d, 1H, J = 15.6, vinyl-H), 6.49 (s, 1H, furan-H), 6.73 (s, 1H, furan-H), 6.89–6.92 (m, 3H, Ar-H), 7.26 (d, 1H, J = 15.0, vinyl-H), 7.24–7.29 (m, 2H, Ar-H), 7.33–7.38 (m, 1H, Ar-H), 7.45 (d, 2H, J = 7.9,Ar-H), 7.47 (d, 2H, J = 8.1, Ar-H), 7.64 (d, 2H, J = 8.1, Ar-H), 7.66 (d, 2H, J = 7.8, Ar-H), 9.04 (s,1H, CON*H*OH), 10.77 (s, 1H, CONHO*H*); MS (ESI) m/z 469 (M+H); HRMS calcd for $C_{29}H_{29}N_2O_4$ [MH]$^+$:469.2122; found: 469.2128; IR (KBr) ν (cm^{-1}) = 3200, 2918, 1602, 1495. HPLC purity: 98. 9% (t_R = 4.73 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.22. (*E*)-3-{5-{{(Biphenyl-4-ylmethyl)[2-(*p*-tolyloxy)ethyl]-amino}methyl}furan-2-yl}-N-hydroxyacrylamide (13h). Compound **13h** (0.33 g, 46.0% yield) was synthesized from **12h** (0.72 g, 1.5 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.12 (s, 3H, CH_3), 2.85 (t, 2H, J = 5.2, NCH_2), 3.76 (s, 2H, CH_2), 3.79 (s, 2H, CH_2), 4.06 (t, 2H, J = 5.1, CH_2O), 6.25 (d, 1H, J = 15.6, vinyl-H), 6.48 (s, 1H, furan-H), 6.73 (s, 1H, furan-H), 6.79 (d, 2H, J = 7.8, Ar-H), 7.05 (d, 2H, J = 8.2, Ar-H), 7.25 (d, 1H, J = 15.6, vinyl-H), 7.33–7.38 (m, 1H, Ar-H), 7.46–7.47 (m, 4H, Ar-H), 7.63 (d, 2H, J = 8.1, Ar-H), 7.65 (d, 2H, J = 7.9, Ar-H), 9.03 (s, 1H, CON*H*OH), 10.77 (s, 1H, CONHO*H*); MS (ESI) m/z 481 (M−H); HRMS calcd for $C_{30}H_{31}N_2O_4$ [MH]$^+$:483.2278; found: 483.2289; IR (KBr) ν (cm^{-1}) = 3210, 3032, 2918, 1612. HPLC purity: 99. 6% (t_R = 6.41 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.23. (*E*)-3-{5-{[(Biphenyl-4-ylmethyl)(3-phenoxypropyl)-amino]methyl}furan-2-yl}-N-hydroxyacrylamide (13i). Compound **13i** (0.30 g, 41.6% yield) was synthesized from **12i** (0.72 g, 1.5 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.93 (t, 2H, J = 5.7, NCH_2), 2.61 (m, 2H, CH_2), 3.67 (s, 2H, CH_2), 3.69 (s, 2H, CH_2), 3.99 (t, 2H, J = 6.0, CH_2O), 6.24 (d, 1H, J = 15.6, vinyl-H), 6.44 (d, 1H, J = 2.1, furan-H), 6.70 (s, 1H, furan-H), 6.88 (d, 1H, J = 15.3, vinyl-H), 6.84–6.90 (m, 2H, Ar-H), 7.21–7.27 (m, 3H, Ar-H), 7.33–7.37 (m, 1H, Ar-H), 7.41–7.48 (m, 4H, Ar-H), 7.59 (d, 2H, J = 8.1, Ar-H), 7.64 (d, 2H, J = 8.1, Ar-H), 9.01 (s, 1H, CON*H*OH), 10.75 (s, 1H, CONHO*H*); MS (ESI) m/z 483 (M+H); HRMS calcd for $C_{30}H_{31}N_2O_4$ [MH]$^+$:483.2278; found: 483.2280; IR (KBr) ν (cm^{-1}) = 3025, 2939, 1602, 1580. HPLC purity: 99. 2% (t_R = 5.14 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.24. (*E*)-3-{5-{{(Biphenyl-4-ylmethyl)[3-(*p*-tolyloxy)propyl]amino}methyl}furan-2-yl}-N-hydroxyacrylamide (13j). Compound **13j** (0.31 g, 42.0% yield) was synthesized from **12j** (0.74 g, 1.5 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.90 (m, 2H, CH_2), 2.21 (s, 3H, CH_3), 2.58 (t, 2H, J = 6.3, NCH_2), 3.66 (s, 2H, CH_2), 3.68 (s, 2H, CH_2), 3.94 (t, 2H, J = 5.8, CH_2O), 6.24 (d, 1H, J = 15.6, vinyl-H), 6.44 (s, 1H, furan-H), 6.72 (s, 1H, furan-H), 6.74 (d, 2H, J = 8.4, Ar-H), 7.03 (d, 2H, J = 8.1, Ar-H), 7.24 (d, 1H, J = 15.6, vinyl-H), 7.33–7.49 (m, 5H, Ar-H), 7.59 (d, 2H, J = 8.1, Ar-H), 7.64 (d, 2H, J = 7.5, Ar-H), 9.03 (s, 1H, CON*H*OH), 10.77 (s, 1H, CONHO*H*); MS (ESI) m/z 497 (M+H); HRMS calcd for $C_{31}H_{33}N_2O_4$ [MH]$^+$:497.2435; found: 497.2444; IR (KBr) ν (cm^{-1}) = 3388, 3196, 2918, 1614, 1580. HPLC purity: 97. 6% (t_R = 4.94 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.25. (*E*)-3-{5-{[(Biphenyl-4-ylmethyl)(4-phenoxybutyl)-amino]methyl}furan-2-yl}-N-hydroxyacrylamide (13k). Compound **13k** (0.32 g, 43.5% yield) was synthesized from **12k** (0.74 g, 1.5 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.66 (m, 2H, CH_2), 1.73 (m, 2H, CH_2), 2.50 (t, 2H, J = 6.1, NCH_2), 3.64 (s, 2H, CH_2), 3.67 (s, 2H, CH_2), 3.89 (t, 2H, J = 6.0, CH_2O), 6.24 (d, 1H, J = 15.6, vinyl-H), 6.43 (d, 1H, J = 3.0, furan-H), 6.71 (d, 1H, J = 3.0, furan-H), 6.85–6.89 (m, 3H, Ar-H), 7.23 (d, 1H, J = 15.6, vinyl-H), 7.24–7.26 (m, 2H, Ar-H), 7.35–7.38 (m, 1H, Ar-H), 7.42–7.49 (m, 3H, Ar-H), 7.62 (d, 2H, J = 7.8, Ar-H), 7.65 (d, 2H, J = 7.5, Ar-H); MS (ESI) m/z 497 (M+H); HRMS calcd for $C_{31}H_{33}N_2O_4$ [MH]$^+$:497.2435; found: 497.2446; IR (KBr) ν (cm^{-1}) = 3024, 2939, 2861, 1599, 1495. HPLC purity: 98.7% (t_R = 4.57 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.26. (*E*)-3-{5-{{[Biphenyl-4-ylmethyl][4-(*p*-tolyloxy)butyl]amino}methyl}furan-2-yl}-N-hydroxyacrylamide (13l). Compound **13l** (0.31 g, 41.2% yield) was synthesized from **12l** (0.76 g, 1.5 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.65–1.70 (m, 4H, $CH_2 \times 2$), 2.20 (s, 3H, CH_3), 3.34 (m, 2H, CH_2), 3.63 (t, 2H, J = 6.6, CH_2), 3.66 (s, 2H, CH_2), 3.84 (t, 2H, J = 6.1, CH_2O), 6.25 (d, 1H, J = 15.6, vinyl-H), 6.42 (s, 1H, furan-H), 6.73 (s, 1H, furan-H), 6.74 (d, 2H, J = 7.8, Ar-H), 7.02 (d, 2H, J = 8.0, Ar-H), 7.25 (d, 1H, J = 15.0, vinyl-H), 7.35–7.45 (m, 5H, Ar-H), 7.61 (d, 1H, J = 7.8, Ar-H), 7.64 (d, 1H, J = 7.8, Ar-H), 9.04 (s, 1H, CON*H*OH), 10.77 (s, 1H, CONHO*H*); MS (ESI) m/z 511 (M+H); HRMS calcd for $C_{32}H_{35}N_2O_4$ [MH]$^+$:511.2591; found: 511.2594; IR (KBr) ν (cm^{-1}) = 3438, 3260, 2954, 2769, 1630. HPLC purity: 99. 0% (t_R = 7.53 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.27. (*E*)-3-{5-{{[2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](ethyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16a). Compound **16a** (0.14 g, 40.6% yield) was synthesized from **15a** (0.36 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.0 (t, 3H, J = 6.4, CH_3), 2.51 (q, 2H, J = 6.4, NCH_2), 2.52–2.64 (m, 4H,$CH_2 \times 2$), 3.68 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.20 (d, 1H, J = 15.4, vinyl-H), 6.37 (d, 1H, J = 3.0, furan-H), 6.62–6.65 (m, 1H, Ar-H), 6.68 (d, 1H, J = 3.0, furan-H), 6.76–6.79 (m, 2H, Ar-H), 7.21 (d, 1H, J = 15.5, vinyl-H), 8.96 (s, 1H, CON*H*OH), 10.67 (s, 1H, CONHO*H*); MS (ESI) m/z 357 (M−H); HRMS calcd for $C_{19}H_{23}N_2O_5$ [MH]$^+$:359.1601; found: 359.1609; IR (KBr) ν(cm^{-1}) = 3200, 2932, 1609, 1488. HPLC purity: 98. 1% (t_R = 4.68 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.28. (*E*)-3-{5-{{[2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](propyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16b). Compound **16b** (0.15 g, 42.0% yield) was synthesized from **15b** (0.37 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.82 (t, 3H, J = 6.1, CH_3), 1.42 (m, 2H, CH_2), 2.42 (t, 2H, J = 6.4, NCH_2), 2.50–

2.60 (m, 4H, $CH_2 \times 2$), 3.68 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.18 (d, 1H, J = 15.6, vinyl-H), 6.38 (s, 1H, furan-H), 6.63–6.79 (m, 4H, Ar-H), 7.21 (d, 1H, J = 15.6, vinyl-H), 9.0 (s, 1H, CON*H*OH), 10.7 (s, 1H, CONHO*H*); MS (ESI) m/z 373 (M+H); HRMS calcd for $C_{20}H_{25}N_2O_5$ $[MH]^+$:373.1758; found: 373.1770; IR (KBr) ν (cm^{-1}) = 3209, 2968, 2868, 1439. HPLC purity: 98. 5% (t_R = 5.66 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.29. (*E*)-3-{5-{{[2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](butyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16c). Compound **16c** (0.15 g, 39.4% yield) was synthesized from **15c** (0.38 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.84 (t, 3H, J = 7.2, CH_3), 1.22 (m, 2H, CH_2), 1.27 (m, 2H, CH_2), 2.44 (t, 2H, J = 7.1, NCH_2), 2.55–2.64 (m, 4H, $CH_2 \times 2$), 3.67 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.19 (d,1H, J = 15.6, vinyl-H), 6.37 (d, 1H, J = 3.3, furan-H), 6.62–6.66 (m, 1H, Ar-H), 6.69 (d, 1H, J = 3.0, furan-H), 6.77–6.79 (m, 2H, Ar-H), 7.21 (d, 1H, J = 15.6, vinyl-H), 9.0 (s, 1H, CON*H*OH), 10.7 (s,1H, CONHO*H*); MS (ESI) m/z 387 (M+H); HRMS calcd for $C_{21}H_{27}N_2O_5$ $[MH]^+$:387.1914; found: 387.1926; IR (KBr) ν (cm^{-1}) = 3180, 2954, 2875, 1637, 1577. HPLC purity: 98. 8% (t_R = 6.19 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.30. (*E*)-3-{5-{{[2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](isobutyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16d). Compound **16d** (0.15 g, 39.2% yield) was synthesized from **15d** (0.38 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.86 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 1.77 (m, 1H, CH), 2.26 (d, 2H, J = 7.5, NCH_2), 2.62–2.67 (m, 4H, $CH_2 \times 2$), 3.73 (s, 2H, CH_2), 6.0 (s, 2H, OCH_2O), 6.25 (d, 1H, J = 15.6, vinyl-H), 6.43 (d, 1H, J = 3.0, furan-H), 6.68 (d, 1H, J = 3.0, furan-H), 6.71 (m, 3H, Ar-H), 7.26 (d, 1H, J = 15.6, vinyl-H); MS (ESI) m/z 385 (M−H); HRMS calcd for $C_{21}H_{27}N_2O_5$ $[MH]^+$:387.1914; found: 387.1923; IR (KBr) ν (cm^{-1}) = 3293, 2952, 2927, 1730. HPLC purity: 98. 0% (t_R = 6.71 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.31. (*E*)-3-{5-{{[2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](pentyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16e). Compound **16e** (0.16 g, 41.5% yield) was synthesized from **15e** (0.40 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 0.84 (t, 3H, J = 6.4, CH_3), 1.21–1.63 (m, 6H, $CH_2 \times 3$), 2.43 (t, 2H, J = 6.7, NCH_2), 2.50–2.60 (m, 4H, $CH_2 \times 2$), 3.68 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.09 (d, 1H, J = 15.3, vinyl-H), 6.37 (s, 1H, furan-H), 6.64–6.80 (m, 4H, 3Ar-H, 1 furan-H), 7.23 (d, 1H, J = 15.6, vinyl-H); MS (ESI) m/z 401 (M+H); HRMS calcd for $C_{22}H_{29}N_2O_5$ $[MH]^+$:401.2071; found: 401.2080; IR (KBr) ν (cm^{-1}) = 3200, 2968, 2861, 1744, 1609. HPLC purity: 97. 6% (t_R = 6.26 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.32. (*E*)-3-5-{{[2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](benzyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16f). Compound **16f** (0.18 g, 44.2% yield) was synthesized from **15f** (0.40 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.59–2.67 (m, 4H, $CH_2 \times 2$), 3.66 (s, 2H, CH_2), 3.68 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.23 (d, 1H, J = 15.6, vinyl-H), 6.41 (d, 1H, J = 3.0, furan-H), 5.59–6.61 (m, 1H, Ar-H), 6.70 (d, 1H, J = 3.0, furan-H), 6.76–6.85 (m, 2H, Ar-H), 7.21–7.42 (m, 5H, Ar-H), 9.04 (s, 1H, CON*H*OH), 10.77 (s, 1H, CONHO*H*); MS (ESI) m/z 405 (M−H); HRMS calcd for $C_{24}H_{25}N_2O_5$ $[MH]^+$:421.1758; found: 421.1767; IR (KBr) ν (cm^{-1}) = 3032, 2940, 1630, 1488. HPLC purity: 99. 2% (t_R = 6.52 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.33. (*E*)-3-{5-{{[2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](2-hydroxyethyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16g). Compound **16g** (0.14 g, 38.5% yield) was synthesized from **15g** (0.37 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.51 (t, 2H, J = 6.0, CH_2), 2.57–2.62 (m, 4H, $CH_2 \times 2$), 3.46 (t, 2H, J = 5.1, CH_2), 3.73 (s, 2H, CH_2), 4.35 (t, 1H, J = 4.8, CH_2OH), 5.94 (s, 2H, OCH_2O), 6.19 (d, 1H, J = 15.3, vinyl-H), 6.4 (d, 1H, J = 3.0, furan-H), 6.62–6.65 (m, 1H, Ar-H), 6.69 (d, 1H, J = 3.0, furan-H), 6.77–6.78 (m, 2H, Ar-H), 7.21 (d, 1H, J = 15.6, vinyl-H), 9.0 (s, 1H, CON*H*OH), 10.7 (s, 1H, CONHO*H*); MS (ESI) m/z 375 (M+H); HRMS calcd for $C_{19}H_{23}N_2O_6$ $[MH]^+$:375.1551; found: 375.1563; IR (KBr) ν (cm^{-1}) = 3224, 2889, 1726, 1662. HPLC purity: 98. 8% (t_R = 4.29 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.34. (*E*)-3-{5-{{[2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](2-phenoxyethyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16h). Compound **16h** (0.17 g, 39.1% yield) was synthesized from **15h** (0.45 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.68 (m, 4H, $CH_2 \times 2$), 2.91 (m, 2H, CH_2), 3.82 (s, 2H, CH_2), 4.1 (m, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.23 (d, 1H, J = 15.6, vinyl-H), 6.42 (d, 1H, J = 3.0, furan-H), 6.65 (d, 1H, J = 3.0, furan-H), 6.68–6.92 (m, 6H, Ar-H), 7.30 (d, 1H, J = 15.3, vinyl-H), 7.09–7.53 (m, 2H, Ar-H); MS (ESI) m/z 449 (M−H); HRMS calcd for $C_{25}H_{27}N_2O_6$ $[MH]^+$:451.1864; found: 451.1880; IR (KBr) ν (cm^{-1}) = 3200, 2968, 2861, 1744, 1609. HPLC purity: 97. 5% (t_R = 4.36 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.35. (*E*)-3-{5-{{ [2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](2-(*p*-tolyloxy)ethyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16i). Compound **16i** (0.21 g, 45.3% yield) was synthesized from **15i** (0.46 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 2.28 (s, 3H, CH_3), 2.73–2.74 (m, 4H, $CH_2 \times 2$), 2.95 (t, 2H, J = 5.2, CH_2), 3.87 (s, 2H, CH_2), 4.04 (t, 2H, J = 5.1, CH_2), 5.99 (s, 2H, OCH_2O), 6.27 (d, 1H, J = 15.6, vinyl-H), 6.48 (d, 1H, J = 3.0, furan-H), 6.70–6.85 (m, 5H, Ar-H), 6.76 (d, 1H, J = 3.0, furan-H), 6.86 (d, 2H, J = 8.4, Ar-H), 7.13 (d, 2H, J = 8.4, Ar-H), 7.27 (d, 1H, J = 15.6, vinyl-H); MS (ESI) m/z 465 (M+H); HRMS calcd for $C_{26}H_{29}N_2O_6$ $[MH]^+$:465.2020; found: 465.2034; IR (KBr) ν (cm^{-1}) = 3025, 2918, 1612, 1510. HPLC purity: 98. 3% (t_R = 7.16 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.36. (*E*)-3-{5-{{ [2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](3-phenoxypropyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16j). Compound **16j** (0.17 g, 37.5% yield) was synthesized from **15j** (0.46 g, 1.0 mmol) according to the procedure used to synthesize **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MH, δ ppm): 0.91 (m, 2H, CH_2), 1.87 (t, 2H, J = 5.8, CH_2), 2.56–2.74 (m, 4H, $CH_2 \times 2$), 3.88 (s, 2H, CH_2), 3.96 (t, 2H, J = 6.0, CH_2), 5.95 (s, 2H, OCH_2O), 6.26 (d, 1H, J = 15.3, vinyl-H),6.43 (d, 1H, J = 3.0, furan-H), 6.73 (d, 1H, J = 2.4, furan-H), 6.80–7.25 (m, 6H, Ar-H), 7.30 (d, 1H, J = 15.3, vinyl-H), 7.25–7.4 (m, 2H, Ar-H); MS (ESI) m/z 463 (M−H); HRMS calcd for $C_{26}H_{29}N_2O_6$ $[MH]^+$:465.2020; found: 465.2024; IR (KBr) ν (cm^{-1}) = 2932, 1601, 1491, 1442. HPLC purity: 98. 8% (t_R = 5.73 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.1.5.37. (*E*)-3-{5-{{[2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl](4-phenoxybutyl)amino}methyl}furan-2-yl}-N-hydroxyacrylamide (16k). Compound **16k** (0.18 g, 37.2% yield) was synthesized from **15k** (0.48 g, 1.0 mmol) according to the procedure used to synthe-

size **10a**; oil. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.64 (m, 2H, CH_2), 1.74 (m, 2H, CH_2), 2.56 (t, 2H, J = 6.2, CH_2), 2.58–2.67 (m, 4H, $CH_2 \times 2$), 3.76 (s, 2H, CH_2), 3.97 (t, 2H, J = 6.3, CH_2), 5.99 (s, 2H, OCH_2O), 6.26 (d, 1H, J = 15.6, vinyl-H), 6.44 (d, 1H, J = 3.3, furan-H), 6.72–6.74 (m, 1H, Ar-H), 6.74 (d, 1H, J = 3.0, furan-H), 6.82–6.85 (m, 2H, Ar-H), 6.94–6.99 (m, 3H, Ar-H), 7.25–7.35 (m, 2H, Ar-H), 7.32 (d, 1H, J = 15.6,vinyl-H); MS (ESI) m/z 479 (M+H); HRMS calcd for $C_{27}H_{31}N_2O_6$ $[MH]^+$:479.2177; found: 479.2171; IR (KBr) ν (cm^{-1}) = 2960, 2875, 1602, 1488. HPLC purity: 99. 2% (t_R = 6.93 min, 80% MeCN/20% H_2O/0.1% TFA), 0.5 mL/min in 20 min.

4.2. Bioactivity test

4.2.1. General

4.2.1.1. Ligands and materials. SAHA (kind gift of Merck) was dissolved in DMSO and used at 5 μM. MS-275 (kind gift from Schering AG) was dissolved in ethanol and used at 5 μM. All other compounds were dissolved in DMSO and used at the indicated concentrations.

4.2.1.2. Fluorimetric HDACs assays. The HDAC Fluorescent Activity Assay is based on the Fluor deLys Substrate and Developer combination (BML-AK500-0001, New York, USA) and has been carried out according to supplier's instructions. First, the Fluor deLys Substrate, which comprises an acetylated lysine side chain, has been incubated with HeLa nuclear extractin presence or absence of the inhibitors. Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the Developer produces a fluorophore. Fluorescence has been quantified with a TECAN inphinite M200 station.

4.2.1.3. Cellular proliferation assay. All compounds were dissolved in DMSO with the stock concentration of 10 mg/mL, and diluted with medium freshly before drug administration. Cell lines were seeded into 96-well flat bottom plates at density of 4000 cells/well. Twenty-four hours after seeding, each compound dilution was added in duplicate and incubation continued at 37 °C in a humidified atmosphere containing 5% CO_2. After 48 h, add 20 μL MTT at 5 mg/mL in PBS (filter sterilized, light protected, and stored at 4 °C) per well, and after 4 h of incubation at 37 °C, the fluorescence was measured at 570 nm using Thermo Multiskan Spectrum.

4.2.1.4. HDAC1, HDAC4 and HDAC6 enzyme inhibition assays. HDAC1, HDAC4 and HDAC6 enzyme inhibition assays were performed by the Reaction Biology Corporation, Malvern, PA, using the Reaction Biology HDAC Spectrum platform. (www.reactionbiology.com) The HDAC1, HDAC 4, HDAC 6 assays used isolated recombinant human protein. Substrate for HDAC1, HDAC4 and HDAC6 assays is a fluorogenic peptide from p53 residues 379–382 (RHKKAc). Compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 100 μM. Control Compound Trichostatin A (TSA) was tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 10 μM. Control Compound MS275 was tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 100 μM. IC_{50} values were extracted by curve-fitting the dose/response slopes.

4.3. Computational docking

All computational work was done using Discovery Studio 3.0 package (Accelrys Inc.) Compound **10b** was built using the 'Sketch and Edit Molecule' module. The HDAC1 and HDAC6 homology model was constructed by Discovery Studio 3.0 using the Homology Modeling protocol as the method described in the literature.[41] The crystal structure deposited in the Protein Data Bank (PDB ID 2VQM) was used for the structural model of HDAC4. Water molecules were removed from three structure. Then, CDOCKER was used to perform the docking of **10b** to the catalytic site of HDAC1, HDAC4 and HDAC6. All default parameters were used in the docking process. CHARMm-based molecular dynamics (1000 steps) were used to generate random ligand **10b** conformations, and the position of any ligand **10b** was optimized in the binding site using rigid body rotation followed by simulated annealing at 700 K. Final energy minimization was set as the full potential mode. The final binding conformation of **10b** was determined on the basis of energy, other parameters were set as default. Docking study of ligand **TSA** was carried out as described above.

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