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# Design, synthesis and preliminary bioactivity studies of 1,3,4-thiadiazole hydroxamic acid derivatives as novel histone deacetylase inhibitors

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ABSTRACT

Histone deacetylase (HDAC) inhibitors have emerged as a new class of anticancer agents, targeting the biological processes including cell cycle, apoptosis and differentiation. In the present study, a series of 1,3,4-thiadiazole based hydroxamic acids were developed as potent HDAC inhibitors. Some of them showed good inhibitory activity in HDAC enzyme assay and potent growth inhibition in some tumor cell lines. Among them, compound **6i** ($IC_{50}$ = 0.089 μM), exhibited better inhibitory effect compared with SAHA ($IC_{50}$ = 0.15 μM).



## 1. Introduction

Histone deacetylases are highlighted as one of the pivotal members in tumor epigenome in response to their corroborated competence to deacetylate histone as well as non-histone proteins. In this way histone deacetylases involve in chromatin remodeling and assembly, DNA repair and recombination and the consequent regulation of gene expression.[1] Indeed, extensive studies have recently revealed that histone deacetylases can be tethered mechanistically to the oncogenesis, maintenance and progression of cancer.[2]

Eighteen histone deacetylases have been identified in the mammalian genome and grouped to four classes based on their homology to the respective yeast transcriptional control factor sequence. Class III HDAC (Surtuin1 to -7) share domains with yeast SIR2 protein and their dependence on $NAD^+$ for deacetylase activity attenuates concerns here. Classical HDAC comprising Classes I, II, and IV HDAC family members are $Zn^{2+}$-dependent: Class I HDAC (HDAC1, -2, -3, and -8) are closely related to yeast RPD3; Class II HDAC, including Class IIa (HDAC4, -5, -7, and -9) and Class IIb (HDAC6 and -10), possess sequence similarity to yeast Hda1; HDAC11 is homologs of both RPD3 and Hda1, consequently defining Class IV HDAC. Classes I and IV HDAC are pervasively expressed in diverse tissues and generally localized to the nucleus. Nevertheless, Class II HDAC, which are restricted to certain cell types, display an uncertain cellular localization owing to their ability to shuttle between nucleus and cytoplasm.

Histone deacetylase (HDAC) inhibitors induce, to a variable extent, cell cycle and growth arrest, differentiation or apoptosis of malignant cells of in vitro models and in vivo xenografts. Strikingly, their anticancer efficacy has been clinically substantiated in broad spectrum of neoplasm types from both haematological and solid origins.[2,3] Suberoylanilide hydroxamic acid (SAHA, Vorinostat)[4] (Fig. 1) and depsipeptide (FK228, Romidepsin)[5] have been approved by FDA for the treatment of cutaneous T-cell lymphoma in 2006 and 2009, respectively.

Most of the HDAC inhibitors include three parts: a $Zn^{2+}$ binding group, a linker domain and a surface recognition motif (Fig. 1). Previously, we developed *N*-phenylpropionamide[6] and tetrahydroisoquinoline-based hydroxamic acids[7–9] as novel HDAC inhibitor scaffolds and the latter exhibited higher in vivo antitumor activity than SAHA. In our ongoing study, a 1,3,4-thiadiazole ring has been

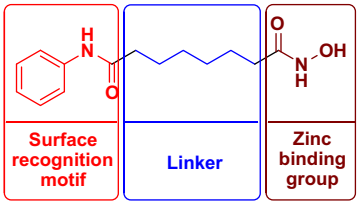


**Figure 1.** The chemical structure of SAHA and pharmacophoric characteristics of HDAC inhibitors exemplified by SAHA.

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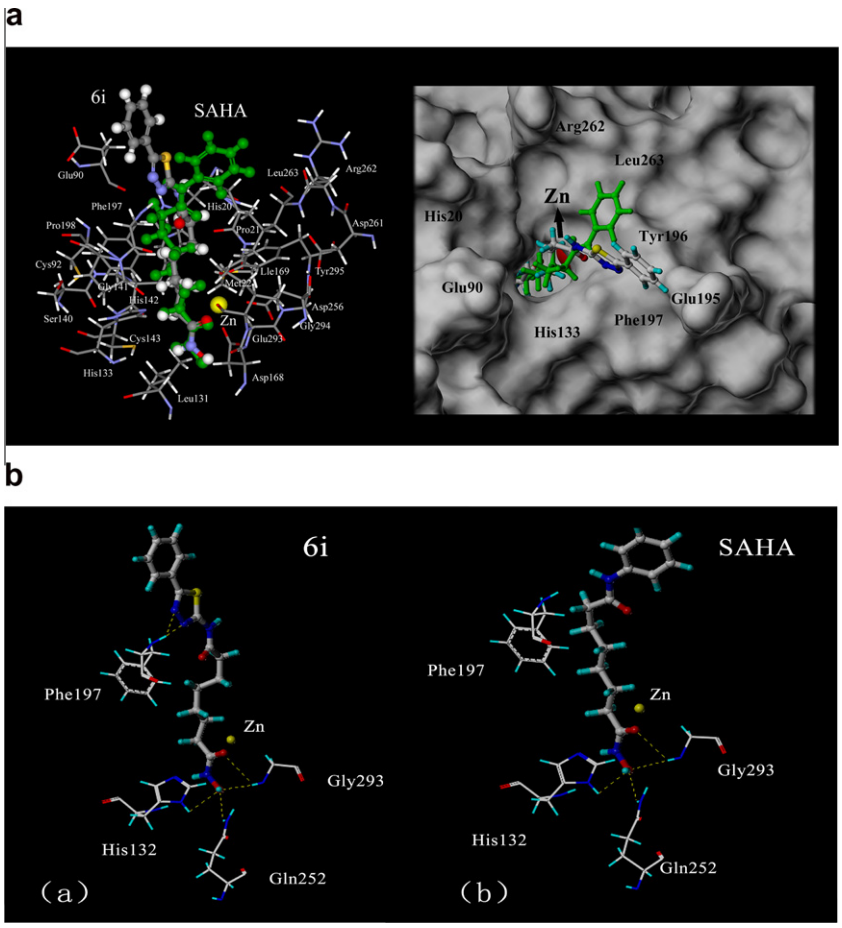



**Figure 2.** (a) The docking modes of compound **6i** (depicted in atom type) and SAHA (depicted in green) in the active site of HDAC1 (left) and their top views with surface representations (right). (b) Hydrogen bond interactions between **6i** (a) and SAHA (b) and HDAC1.

introduced as a substitution of the rigid tetrahydroisoquinoline fragment. This paper will describe the synthesis, enzyme inhibition and antiproliferative activities against some tumor cell lines of the 1,3,4-thiadiazole hydroxamic acid derivatives.

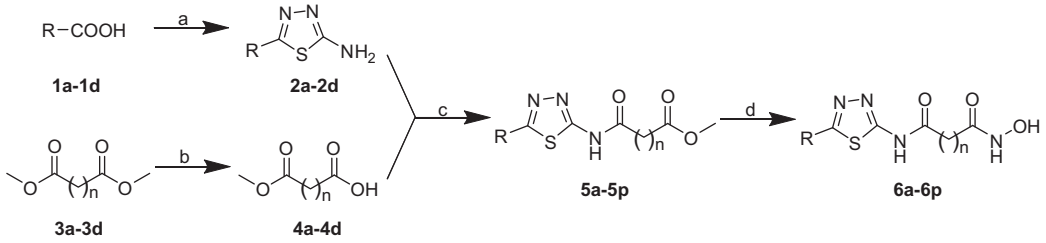
## 2. Chemistry

Synthesis of the 2-amino-1,3,4-thiadiazole-based hydroxamates is described in Schemes 1–3. The 5-substituted-1,3,4-thiadiazol-2-amines **2a–2d** were prepared via a facile one-pot reaction of *N*-aminothiourea with phosphoryl chloride. Saponification of the dimethyl esters **3a–3d** furnished the monomethyl esters **4a–4d** in good yields. Sequential treatment of **4a–4d** with refluxing thionyl chloride and then **2a–2d** gave **5a–5p**, which were converted to hydroxamic acids **6a–6p** by a freshly prepared potassium hydroxylamine according to reported procedures.[10]

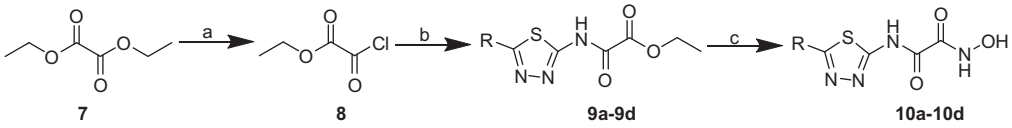
Compounds **10a–10d** were prepared (Scheme 2) from commercially available diethyl oxalate **7**, following the synthetic approach described in Scheme 1. However, compounds **13a** and **13b** could not be obtained using the same method. Thus key intermediates **12a**–**12b** were prepared (Scheme 3) from succinic anhydride **11**. Final hydroxamic acids were generated by the mixed anhydride approach according to literature procedures.[6]

## 3. Results and discussion

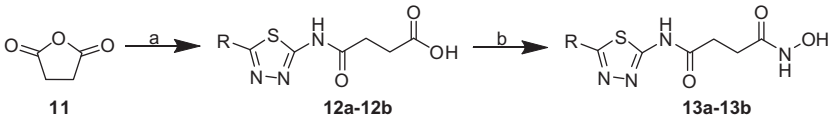
HDAC inhibitory activity of the 1,3,4-thiadiazole based hydroxamates were assessed by the Color de Lys assay and the results are tabulated as $IC_{50}$ values in Table 1. According to the data in Table 1, both the length of the linker chain (*n*) and the substitution in 1,3,4-thiadiazole (*R*) play a role in potency. For example, compounds with the linker comprised of five or six methylene




**Scheme 1.** Reagents: (a) $NH_2NHCSNH_2$, $POCl_3$, then $H_2O$; (b) KOH, $CH_3OH$; (c) (i) $SOCl_2$; (ii) $Et_3N$, THF; (d) $NH_2OK$, $CH_3OH$.

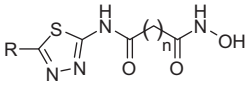


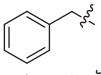
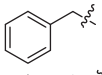
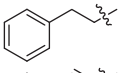
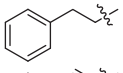
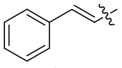
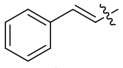
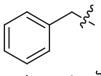
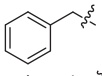
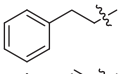
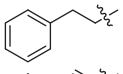
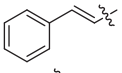
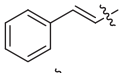
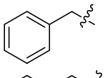
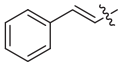
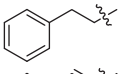
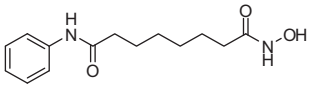
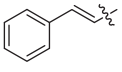

**Scheme 2.** Reagents: (a) (i) $CH_3CO_2K$, $H_2O$; (ii) $SOCl_2$, $Et_2O$; (b) **2a–2d**, $Et_3N$, THF; (c) $NH_2OK$, $CH_3OH$.




**Scheme 3.** Reagents: (a) **2a**, or **2d**, $CH_3CN$; (b) i-BuCOOCl, *N*-methylmorpholine, DMF then $NH_2OH$, $CH_3OH$.

**Table 1**
HDAC inhibitory activity of 1,3,4-thiadiazole hydroxamate derivatives



| Compd | R | *n* | $IC_{50}$ of HDAC[a] (μM) | Compd | R | *n* | $IC_{50}$ of HDAC[a] (μM) |
|---|---|---|---|---|---|---|---|
| **6a** |  | 3 | >5 | **6m** |  | 6 | 0.27 ± 0.004 |
| **6b** |  | 3 | 1.87 ± 0.41 | **6n** |  | 6 | 0.26 ± 0.05 |
| **6c** |  | 3 | 2.71 ± 0.25 | **6o** |  | 6 | 0.32 ± 0.05 |
| **6d** |  | 3 | 0.16 ± 0.03 | **6p** |  | 6 | 3.21 ± 0.10 |
| **6e** |  | 4 | 1.03 ± 0.04 | **10a** |  | 0 | >5 |
| **6f** |  | 4 | 1.12 ± 0.01 | **10b** |  | 0 | >5 |
| **6g** |  | 4 | 3.49 ± 0.04 | **10c** |  | 0 | >5 |
| **6h** |  | 4 | 1.70 ± 0.40 | **10d** |  | 0 | >5 |
| **6i** |  | 5 | 0.089 ± 0.005 | **13a** |  | 2 | >5 |
| **6j** |  | 5 | 0.22 ± 0.04 | **13b** |  | 2 | >5 |
| **6k** |  | 5 | 0.33 ± 0.05 | **SAHA** |  | | 0.15 ± 0.02 |
| **6l** |  | 5 | >5 | | | | |

[a] All of the compounds were assayed three times, and their inhibition results are means of the three independent assays and expressed with standard deviations.

units, such as **6i–6k** and **6m–6o**, inhibited HDAC in nanomolar range, while the rest showed only micromolar activity. In addition, the phenyl and benzyl substitution in 1,3,4-thiadiazole were more potent than the substitution of phenethyl and (*E*)-styryl except for compound **6d**, which exhibited as good enzyme inhibitory activity as SAHA. In general, the substitutions in 1,3,4-thiadiazole ring have a small effect on the inhibitory activities against HDAC compared with the linker between zinc binding group and 1,3,4-thiadiazole ring.

To further validate the utility of this set of structures at the cellular level, the effect of the exposure of selected compounds was tested on the viability of MDA-MB-231 breast cancer cell and K562 chronic myelogenous leukemia cell. We evaluated compounds **6i**, **6d**, **6j** and **6n** using MTT assay. The $IC_{50}$ values were

**Table 2**
Antiproliferative activities of representative compounds against MDA-MB-231 and K562 cell lines

| Compd | $IC_{50}$ of HDAC (μM) | $IC_{50}$[a] (μM) | |
|---|---|---|---|
| | | MDA-MB-231 | K562 |
| **6i** | 0.089 ± 0.005 | 5.52 ± 2.62 | 12.9 ± 3.18 |
| **6d** | 0.16 ± 0.03 | 5.90 ± 2.75 | 6.75 ± 2.37 |
| **6j** | 0.22 ± 0.04 | 6.14 ± 1.94 | 12.2 ± 5.04 |
| **6n** | 0.26 ± 0.05 | 2.98 ± 0.47 | 9.12 ± 1.10 |
| **SAHA** | 0.15 ± 0.02 | 1.32 ± 0.46 | 1.69 ± 0.04 |

[a] All of the compounds were assayed three times, and their inhibition results are means of the three independent assays and expressed with standard deviations.

summarized in Table 2. Among these compounds, all of them could inhibit the cell proliferation effectively and compound **6n** showed similar antiproliferative activity against the MDA-MB-231 cell compared with SAHA. It seems that these compounds possessed better growth inhibition towards the MDA-MB-231 cell than the K562 cell.

In order to understand the interaction between these inhibitors and HDAC, we modeled the three dimensional structure of HDAC1 (HDAC1 and HDAC2 exhibit a sequence identity of approx. 91% in N-terminal region including the deacetylase catalytic domain) in the MODELLER program[11] following the method in published paper[12] and then docked compound **6i** and SAHA in the active site of homology HDAC1 model using AutoDock4.2[13] (Fig. 2). The result suggested **6i** had a similar binding mode to SAHA in the active site of HDAC1. Both of them did not only chelate $Zn^{2+}$ ion with the hydroxamic acid moiety but also had three hydrogen bonds with His132, Gln252 and Gly293, respectively. It was worth noting that the nitrogen atom of 1,3,4-thiadiazole in **6i** formed two hydrogen bonds with Phe197.

## 4. Conclusions

In summary, we have designed and synthesized a novel series of 1,3,4-thiadiazole based hydroxamic acid HDAC inhibitors which have different linkers and substitutions in 1,3,4-thiadiazole ring as the surface recognition motif. The strong enzymatic inhibition of compound **6d**, **6i**–**6k** and **6m**–**6o** suggested that the presence of 5–6 carbon units between the $Zn^{2+}$ binding group and the 1,3,4-thiadiazole ring is optimal for potency. These results suggest that 1,3,4-thiadiazole hydroxic acid derivatives could be used as lead compounds to develop new potent HDAC inhibitors.

## 5. Experimental section

### 5.1. Chemistry: general procedures

Unless otherwise noted, all starting materials, reagents and solvents were obtained from commercial suppliers and used without further purification. All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light, chloride ferric or iodine vapor. Melting points were determined on an electrothermal melting point apparatus without correction. ESI-MS was determined on an Aglient-1100 series LC/MSD trap spectrometer. $^1$H NMR spectrums were obtained on a Brucker DRX spectrometer (600 MHz). The chemical shifts are expressed in $\delta$ values (parts per million) relative to tetramethylsilane (TMS) as internal standard. Significant $^1$H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) number of protons. HRMS spectrums were conducted on an Agilent 6510 Quadrupole Time-of-Flight LC/MS deliver.

#### 5.1.1. 5-Phenyl-1,3,4-thiadiazol-2-amino (2a)

A stirring mixture of benzoic acid (6.10 g, 50 mmol), *N*-aminothiourea (4.55 g, 50 mmol) and $POCl_3$ (13 ml) was heated at 75 °C for 0.5 h. After cooling down to room temperature, water (55 ml) was added. The reaction mixture was refluxed for 4 h. After cooling, the mixture was basified to pH 8 by the dropwise addition of 50% NaOH solution under stirring. The precipitate was filtered and recrystallized from ethanol to yield 8.14 g of the target compound **2a** as a colorless crystal. Yield: 92%, mp: 224–226 °C. ESI-MS *m*/*z*: 178.2 $[M+H]^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.42–7.45 (m, 3H), 7.47 (m, 2H), 7.75 (m, 2H).

Compounds **2b–2d** were synthesized following the procedure described above.

**5.1.1.1. 5-Benzyl-1,3,4-thiadiazol-2-amine (2b).** Yield: 90%, mp: 193–195 °C. ESI-MS *m*/*z*: 192.4 $[M+H]^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 4.15 (s, 2H), 7.06 (s, 2H), 7.24–7.28 (m, 3H), 7.34 (m, 2H).

**5.1.1.2. 5-Phenethyl-1,3,4-thiadiazol-2-amine (2c).** Yield: 89%, mp: 78–80 °C. ESI-MS *m*/*z*: 206.1 $[M+H]^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.95 (t, *J* = 7.8 Hz, 2H), 3.12 (t, *J* = 7.8 Hz, 2H), 7.02 (s, 2H), 7.20 (m, 1H), 7.24 (m, 2H), 7.29 (m, 2H).

**5.1.1.3. (*E*)-5-Styryl-1,3,4-thiadiazol-2-amine (2d).** Yield: 81%, mp: 236–238 °C. ESI-MS *m*/*z*: 204.2 $[M+H]^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.06 (d, *J* = 16.2 Hz, 1H), 7.30–7.36 (m, 2H), 7.39 (m, 2H), 7.45 (s, 2H), 7.64 (m, 2H).

#### 5.1.2. 5-Methoxy-5-oxopentanoic acid (4a)

A solution of KOH (5.87 g, 104.65 mmol) in MeOH (150 ml) was added to dimethyl glutarate (13.15 g, 90 mmol), and the mixture was stirred for 4 h at rt. The solvent was then removed, and $Et_2O$ (100 ml) and $H_2O$ (200 ml) were added. The organic layer was separated, washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to afford **3a** as a yellow oil (4.61 g, 32%). The aqueous layer was acidified with concentrated HCl to pH 3, and extracted with $Et_2O$ (3 × 100 ml). The combined organic phase was washed with brine (3 × 100 ml) and dried ($MgSO_4$). The solvent was removed to give a mixture of a white solid and an oil. Filtration and concentration in vacuum and purification with silica gel column chromatography gave 5.79 g (44%) of **4a** as a colorless oil.

**5.1.2.1. 6-Methoxy-6-oxohexanoic acid (4b).** Compound **4b** was synthesized following the procedure described above. Yield: 47%.

**5.1.2.2. 7-Methoxy-7-oxoheptanoic acid (4c).** Compound **4c** was synthesized following the procedure described in Section 5.1.2. Yield: 40%.

**5.1.2.3. 8-Methoxy-8-oxooctanoic acid (4d).** Compound **4d** was synthesized following the procedure described in Section 5.1.2. Yield: 35%.

#### 5.1.3. Methyl 5-oxo-5-((5-phenyl-1,3,4-thiadiazol-2-yl)amino)pentanoate (5a)

A soln of **4a** (1.46 g, 10 mmol) in $SOCl_2$ (4 ml) was refluxed for 1 h. The $SOCl_2$ was removed under reduced pressure to afford an orange oil, which was added dropwise to a stirred solution of **2a** (1.42 g, 8 mmol) and $Et_3N$ (3.5 ml, 25 mmol) in anhydrous tetrahydrofuran (65 ml) at 0 °C. The reaction mixture was allowed to be stirred overnight at room temperature. The solvent was removed in vacuum and the residue was diluted with dichloromethane (150 ml), washed with 1 M $H_3PO_4$ (3 × 80 ml) and brine (3 × 80 ml) and dried over with $MgSO_4$. Filtration and concentration in vacuum and recrystallization from AcOEt gave 1.29 g of

**5a** as a white crystal. Yield: 53%, mp: 182–185 °C. ESI-MS *m*/*z*: 306.4 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.18–2.23 (m, 2H), 2.56 (t, $J$ = 7.2 Hz, 2H), 2.93 (t, $J$ = 7.2 Hz, 2H), 3.65 (s, 3H), 7.49–7.50 (m, 3H), 7.94–7.95 (m, 2H), 13.63 (s, 1H).

Compounds **5b–5p** were synthesized following the procedure described above.

**5.1.3.1. Methyl 5-((5-benzyl-1,3,4-thiadiazol-2-yl)amino)-5-oxopentanoate (5b).** Yield: 68%, mp: 159–161 °C. ESI-MS *m*/*z*: 320.3 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.08–2.13 (m, 2H), 2.47 (t, $J$ = 7.5 Hz, 2H), 2.81 (t, $J$ = 7.5 Hz, 2H), 3.65 (s, 3H), 4.33 (s, 2H), 7.28–7.35 (m, 5H), 13.41 (s, 1H).

**5.1.3.2. Methyl 5-oxo-5-((5-phenethyl-1,3,4-thiadiazol-2-yl)amino)pentanoate (5c).** Yield: 71%, mp: 131–133 °C. ESI-MS *m*/*z*: 334.4 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.09–2.14 (m, 2H), 2.48 (t, $J$ = 7.2 Hz, 2H), 2.80 (t, $J$ = 7.2 Hz, 2H), 3.12 (t, $J$ = 8.0 Hz, 2H), 3.34 (t, $J$ = 8.0 Hz, 2H), 3.65 (s, 3H), 7.22–7.24 (m, 3H), 7.30–7.32 (m, 2H), 13.39 (s, 1H).

**5.1.3.3. (*E*)-Methyl 5-oxo-5-((5-styryl-1,3,4-thiadiazol-2-yl)amino)pentanoate (5d).** Yield: 55%, mp: 217–219 °C. ESI-MS *m*/*z*: 332.3 $[M+H]^+$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.87–1.89 (m, 2H), 2.39 (t, $J$ = 7.5 Hz, 2H), 2.55 (t, $J$ = 7.5 Hz, 2H), 3.60 (s, 3H), 7.37 (m, 1H), 7.41–7.47 (m, 3H), 7.52 (d, $J$ = 16.2 Hz, 1H), 7.72 (m, 2H), 12.59 (s, 1H).

**5.1.3.4. Methyl 6-oxo-6-((5-phenyl-1,3,4-thiadiazol-2-yl)amino)hexanoate (5e).** Yield: 65%, mp: 203–205 °C. ESI-MS *m*/*z*: 320.4 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.82–1.94 (m, 4H), 2.44 (t, $J$ = 7.2 Hz, 2H), 2.89 (t, $J$ = 7.2 Hz, 2H), 3.65 (s, 3H), 7.48–7.50 (m, 3H), 7.93–7.95 (m, 2H), 13.62 (s, 1H).

**5.1.3.5. Methyl 6-((5-benzyl-1,3,4-thiadiazol-2-yl)amino)-6-oxohexanoate (5f).** Yield: 60%, mp: 138–139 °C. ESI-MS *m*/*z*: 334.4 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.73–1.84 (m, 4H), 2.39 (t, $J$ = 7.4 Hz, 2H), 2.77 (t, $J$ = 7.4 Hz, 2H), 3.64 (s, 3H), 4.34 (s, 2H), 7.27–7.35 (m, 5H), 13.40 (s, 1H).

**5.1.3.6. Methyl 6-oxo-6-((5-phenethyl-1,3,4-thiadiazol-2-yl)amino)hexanoate (5g).** Yield: 67%, mp: 160–162 °C. ESI-MS *m*/*z*: 348.4 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.74–1.86 (m, 4H), 2.40 (t, $J$ = 7.2 Hz, 2H), 2.76 (t, $J$ = 7.2 Hz, 2H), 3.13 (t, $J$ = 8.0 Hz, 2H), 3.35 (t, $J$ = 8.0 Hz, 2H), 3.63 (s, 3H), 7.21–7.24 (m, 3H), 7.29–7.32 (m, 2H), 13.39 (s, 1H).

**5.1.3.7. (*E*)-Methyl 6-oxo-6-((5-styryl-1,3,4-thiadiazol-2-yl)amino)hexanoate (5h).** Yield: 70%, mp: 200–203 °C. ESI-MS *m*/*z*: 346.2 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.80–1.91 (m, 4H), 2.45 (t, $J$ = 7.4 Hz, 2H), 2.84 (t, $J$ = 7.4 Hz, 2H), 3.66 (s, 3H), 7.25 (d, $J$ = 16.8 Hz, 1H), 7.34–7.41 (m, 4H), 7.55 (m, 2H), 13.51 (s, 1H).

**5.1.3.8. Methyl 7-oxo-7-((5-phenyl-1,3,4-thiadiazol-2-yl)amino)heptanoate (5i).** Yield: 69%, mp: 160–162 °C. ESI-MS *m*/*z*: 334.4 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.51–1.56 (m, 2H), 1.71–1.76 (m, 2H), 1.86–1.91 (m, 2H), 2.34 (t, $J$ = 7.5 Hz, 2H), 2.87 (t, $J$ = 7.5 Hz, 2H), 3.63 (s, 3H), 7.49–7.51 (m, 3H), 7.93–7.94 (m, 2H), 13.58 (s, 1H).

**5.1.3.9. Methyl 7-((5-benzyl-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (5j).** Yield: 52%, mp: 122–123 °C. ESI-MS *m*/*z*: 348.4 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.42–1.47 (m, 2H), 1.66–1.71 (m, 2H), 1.76–1.81 (m, 2H), 2.31 (t, $J$ = 7.5 Hz, 2H), 2.74 (t, $J$ = 7.5 Hz, 2H), 3.64 (s, 3H), 4.34 (s, 2H), 7.28–7.35 (m, 5H), 13.27 (s, 1H).

**5.1.3.10. Methyl 7-oxo-7-((5-phenethyl-1,3,4-thiadiazol-2-yl)amino)heptanoate (5k).** Yield: 58%, mp: 112–115 °C. ESI-MS *m*/*z*: 362.5 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.43–1.48 (m, 2H), 1.67–1.72 (m, 2H), 1.78–1.83 (m, 2H), 2.32 (t, $J$ = 7.5 Hz, 2H), 2.74 (t, $J$ = 7.5 Hz, 2H), 3.13 (t, $J$ = 7.7 Hz, 2H), 3.35 (t, $J$ = 7.7 Hz, 2H), 3.63 (s, 3H), 7.21–7.24 (m, 3H), 7.29–7.32 (m, 2H), 13.32 (s, 1H).

**5.1.3.11. (*E*)-Methyl 7-oxo-7-((5-styryl-1,3,4-thiadiazol-2-yl)amino)heptanoate (5l).** Yield: 51%, mp: 176–178 °C. ESI-MS *m*/*z*: 360.3 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.50–1.54 (m, 2H), 1.71–1.76 (m, 2H), 1.83–1.88 (m, 2H), 2.36 (t, $J$ = 7.5 Hz, 2H), 2.81 (t, $J$ = 7.5 Hz, 2H), 3.65 (s, 3H), 7.28 (d, $J$ = 16.2 Hz, 1H), 7.35–7.37 (m, 1H), 7.40–7.43 (m, 3H), 7.55 (m, 2H), 13.27 (s, 1H).

**5.1.3.12. Methyl 8-oxo-8-((5-phenyl-1,3,4-thiadiazol-2-yl)amino)octanoate (5m).** Yield: 49%, mp: 157–159 °C. ESI-MS *m*/*z*: 348.3 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.40–1.45 (m, 2H), 1.49–1.54 (m, 2H), 1.63–1.68 (m, 2H), 1.84–1.89 (m, 2H), 2.29 (t, $J$ = 7.5 Hz, 2H), 2.86 (t, $J$ = 7.5 Hz, 2H), 3.64 (s, 3H), 7.49–7.52 (m, 3H), 7.93–7.94 (m, 2H), 13.56 (s, 1H).

**5.1.3.13. Methyl 8-((5-benzyl-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (5n).** Yield: 70%, mp: 112–114 °C. ESI-MS *m*/*z*: 362.4 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.37–1.45 (m, 4H), 1.60–1.65 (m, 2H), 1.74–1.79 (m, 2H), 2.29 (t, $J$ = 7.2 Hz, 2H), 2.74 (t, $J$ = 7.2 Hz, 2H), 3.64 (s, 3H), 4.34 (s, 2H), 7.28–7.35 (m, 5H), 13.32 (s, 1H).

**5.1.3.14. Methyl 8-oxo-8-((5-phenethyl-1,3,4-thiadiazol-2-yl)amino)octanoate (5o).** Yield: 56%, mp: 129–130 °C. ESI-MS *m*/*z*: 376.5 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.37–1.46 (m, 4H), 1.61–1.66 (m, 2H), 1.76–1.81 (m, 2H), 2.29 (t, $J$ = 7.5 Hz, 2H), 2.74 (t, $J$ = 7.5 Hz, 2H), 3.13 (t, $J$ = 7.8 Hz, 2H), 3.35 (t, $J$ = 7.8 Hz, 2H), 3.64 (s, 3H), 7.21–7.24 (m, 3H), 7.29–7.32 (m, 2H), 13.34 (s, 1H).

**5.1.3.15. (*E*)-Methyl 8-oxo-8-((5-styryl-1,3,4-thiadiazol-2-yl)amino)octanoate (5p).** Yield: 69%, mp: 168–170 °C. ESI-MS *m*/*z*: 374.4 $[M+H]^+$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.39–1.44 (m, 2H), 1.47–1.51 (m, 2H), 1.64–1.69 (m, 2H), 1.81–1.86 (m, 2H), 2.32 (t, $J$ = 7.2 Hz, 2H), 2.81 (t, $J$ = 7.2 Hz, 2H), 3.64 (s, 3H), 7.27 (t, 1H, $J$ = 16.8 Hz), 7.41–7.47 (m, 4H), 7.55 (m, 2H), 13.40 (s, 1H).

### 5.1.4. $N^1$-Hydroxy-$N^5$-(5-phenyl-1,3,4-thiadiazol-2-yl)glutaramide (6a)

Preparation of hydroxylamine in methanol solution: hydroxylamine hydrochloride (4.67 g, 67 mmol) was dissolved in methanol (24 ml) to form solution A. Potassium hydroxide (5.61 g, 100 mmol) was dissolved in methanol (14 ml) to form solution B. To the solution A at 0 °C was added solution B dropwise. The mixture was stirred for 30 min at 0 °C, and the solid was filtered out to afford a solution of hydroxylamine in methanol. To a flask containing **5a** (0.61 g, 2 mmol) was added the solution of hydroxylamine in methanol (10 ml). The mixture was stirred at room temperature for 1 h. Then it was adjusted to pH 7 with concentrated HCl. The mixture was concentrated to give a residue washed with water and dichloromethane to afford 0.54 g of **6a** as a white solid. Yield: 88%, mp: 189–191 °C. $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.81–1.88 (m, 2H), 2.02 (t, $J$ = 7.3 Hz, 2H), 2.54 (t, $J$ = 7.3 Hz, 2H), 7.52–7.54 (m, 3H), 7.93–7.95 (m, 2H), 8.72 (s, 1H), 10.39 (s, 1H), 13.64 (s, 1H); HRMS (AP-ESI) *m*/*z* Calcd for $C_{13}H_{14}N_4O_3S$ $[M+H]^+$ 307.0859. Found: 307.0854.

Compounds **6a–6p** were synthesized following the procedure described above.

**5.1.4.1. $N^1$-(5-Benzyl-1,3,4-thiadiazol-2-yl)-$N^5$-hydroxyglutaramide (6b).** Yield: 91%, mp: 168–170 °C. $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.76–1.81 (m, 2H), 1.96 (t, $J$ = 7.3 Hz, 2H), 2.44 (t, $J$ = 7.3 Hz,

2H), 4.33 (s, 2H), 7.26–7.37 (m, 5H), 8.69 (s, 1H), 10.35 (s, 1H), 12.40 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{14}H_{16}N_4O_3S$ $[M+H]^+$ 321.1016. Found: 321.1025.

**5.1.4.2. $N^1$-Hydroxy-$N^5$-(5-phenethyl-1,3,4-thiadiazol-2-yl)glutaramide (6c).** Yield: 76%, mp: 166–167 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.78–1.82 (m, 2H), 1.99 (t, $J$ = 7.3 Hz, 2H), 2.45 (t, $J$ = 7.3 Hz, 2H), 3.03 (t, $J$ = 7.7 Hz, 2H), 3.29 (t, $J$ = 7.7 Hz, 2H), 7.18–7.31 (m, 5H), 8.68 (s, 1H), 10.36 (s, 1H), 12.33 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{15}H_{18}N_4O_3S$ $[M+H]^+$ 335.1172. Found: 335.1172.

**5.1.4.3. (*E*)-$N^1$-Hydroxy-$N^5$-(5-styryl-1,3,4-thiadiazol-2-yl)glutaramide (6d).** Yield: 89%, mp: 238–242 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.80–1.87 (m, 2H), 2.02 (t, $J$ = 7.3 Hz, 2H), 2.53 (t, $J$ = 7.3 Hz, 2H), 7.36 (m, 1H), 7.40–7.47 (m, 3H), 7.52 (d, $J$ = 16.1 Hz, 1H), 7.71 (m, 2H), 8.72 (s, 1H), 10.39 (s, 1H), 12.56 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{15}H_{16}N_4O_3S$ $[M+H]^+$ 333.1016. Found: 333.1016.

**5.1.4.4. $N^1$-Hydroxy-$N^6$-(5-phenyl-1,3,4-thiadiazol-2-yl)adipamide (6e).** Yield: 90%, mp: 192–196 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.52–1.64 (m, 4H), 1.98 (t, $J$ = 7.0 Hz, 2H), 2.52 (t, $J$ = 7.0 Hz, 2H), 7.51–7.54 (m, 3H), 7.91–7.94 (m, 2H), 8.57 (s, 1H), 10.28 (s, 1H), 12.52 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{14}H_{16}N_4O_3S$ $[M+H]^+$ 321.1016. Found: 321.1024.

**5.1.4.5. $N^1$-(5-Benzyl-1,3,4-thiadiazol-2-yl)-$N^6$-hydroxyadipamide (6f).** Yield: 87%, mp: 184–185 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.44–1.57 (m, 4H), 1.94 (t, $J$ = 7.0 Hz, 2H), 2.42 (t, $J$ = 7.0 Hz, 2H), 7.26–7.36 (m, 5H), 8.65 (s, 1H), 10.33 (s, 1H), 12.36 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{15}H_{18}N_4O_3S$ $[M+H]^+$ 335.1172. Found: 335.1179.

**5.1.4.6. $N^1$-Hydroxy-$N^6$-(5-phenethyl-1,3,4-thiadiazol-2-yl)adipamide (6g).** Yield: 94%, mp: 186–188 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.47–1.59 (m, 4H), 1.95 (t, $J$ = 7.0 Hz, 2H), 2.43 (t, $J$ = 7.0 Hz, 2H), 3.03 (t, $J$ = 7.7 Hz, 2H), 3.29 (t, $J$ = 7.7 Hz, 2H), 7.18–7.31 (m, 5H), 8.65 (s, 1H), 10.33 (s, 1H), 12.31 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{16}H_{20}N_4O_3S$ $[M+H]^+$ 349.1329. Found: 349.1321.

**5.1.4.7. (*E*)-$N^1$-Hydroxy-$N^6$-(5-styryl-1,3,4-thiadiazol-2-yl)adipamide (6h).** Yield: 84%, mp: 210–213 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.50–1.64 (m, 4H), 1.98 (t, $J$ = 7.1 Hz, 2H), 2.47 (t, $J$ = 7.1 Hz, 2H), 7.36 (m, 1H), 7.40–7.46 (m, 3H), 7.51 (d, $J$ = 16.1 Hz, 1H), 7.71 (m, 2H), 8.66 (s, 1H), 10.35 (s, 1H), 12.49 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{16}H_{18}N_4O_3S$ $[M+H]^+$ 347.1172. Found: 347.1179.

**5.1.4.8. $N^1$-Hydroxy-$N^7$-(5-phenyl-1,3,4-thiadiazol-2-yl)heptanediamide (6i).** Yield: 80%, mp: 172–174 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.23–1.31 (m, 2H), 1.47–1.53 (m, 2H), 1.58–1.65 (m, 2H), 1.95 (t, $J$ = 7.3 Hz, 2H), 2.47 (t, $J$ = 7.3 Hz, 2H), 7.52–7.55 (m, 3H), 7.92–7.95 (m, 2H), 8.68 (s, 1H), 10.34 (s, 1H), 12.60 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{15}H_{18}N_4O_3S$ $[M+H]^+$ 335.1172. Found: 335.1178.

**5.1.4.9. $N^1$-(5-Benzyl-1,3,4-thiadiazol-2-yl)-$N^7$-hydroxyheptanediamide (6j).** Yield: 79%, mp: 247–249 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.20–1.25 (m, 2H), 1.45–1.57 (m, 4H), 1.92 (t, $J$ = 7.3 Hz, 2H), 2.41 (t, $J$ = 7.3 Hz, 2H), 4.33 (s, 2H), 7.25–7.39 (m, 5H), 8.65 (s, 1H), 10.31 (s, 1H), 12.37 (br s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{16}H_{20}N_4O_3S$ $[M+H]^+$ 349.1329. Found: 349.1325.

**5.1.4.10. $N^1$-Hydroxy-$N^7$-(5-phenethyl-1,3,4-thiadiazol-2-yl)heptanediamide (6k).** Yield: 84%, mp: 176–177 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.20–1.28 (m, 2H), 1.45–1.61 (m, 4H), 1.93 (t, $J$ = 7.3 Hz, 2H), 2.42 (t, $J$ = 7.3 Hz, 2H), 3.03 (t, $J$ = 7.8 Hz, 2H), 3.28 (t, $J$ = 7.8 Hz, 2H), 7.17–7.31 (m, 5H), 8.65 (br s, 1H), 10.31 (br s, 1H), 12.29 (br s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{17}H_{22}N_4O_3S$ $[M+H]^+$ 363.1485. Found: 363.1489.

**5.1.4.11. (*E*)-$N^1$-Hydroxy-$N^7$-(5-styryl-1,3,4-thiadiazol-2-yl)heptanediamide (6l).** Yield: 90%, mp: 198–200 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.23–1.30 (m, 2H), 1.47–1.55 (m, 2H), 1.57–1.65 (m, 2H), 1.95 (t, $J$ = 7.3 Hz, 2H), 2.49 (t, $J$ = 7.3 Hz, 2H), 7.36 (m, 1H), 7.40–7.47 (m, 3H), 7.71 (d, $J$ = 16.5 Hz, 2H), 7.71 (m, 2H), 8.67 (s, 1H), 10.33 (s, 1H), 12.55 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{17}H_{20}N_4O_3S$ $[M+H]^+$ 361.1329. Found: 361.1338.

**5.1.4.12. $N^1$-Hydroxy-$N^8$-(5-phenyl-1,3,4-thiadiazol-2-yl)octanediamide (6m).** Yield: 91%, mp: 204–205 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.26–1.28 (m, 4H), 1.45–1.52 (m, 2H), 1.58–1.63 (m, 2H), 1.94 (t, $J$ = 7.4 Hz, 2H), 2.48 (t, $J$ = 7.4 Hz, 2H), 7.51–7.56 (m, 3H), 7.92–7.95 (m, 2H), 8.67 (s, 1H), 10.34 (s, 1H), 12.63 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{16}H_{20}N_4O_3S$ $[M+H]^+$ 349.1329. Found: 349.1336.

**5.1.4.13. $N^1$-(5-Benzyl-1,3,4-thiadiazol-2-yl)-$N^8$-hydroxyoctanediamide (6n).** Yield: 70%, mp: 161–163 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.22–1.23 (m, 4H), 1.44–1.47 (m, 2H), 1.53–1.56 (m, 2H), 1.92 (t, $J$ = 7.4 Hz, 2H), 2.41 (t, $J$ = 7.4 Hz, 2H), 4.33 (s, 2H), 7.25–7.37 (m, 5H), 8.64 (s, 1H), 10.31 (s, 1H), 12.37 (br s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{17}H_{22}N_4O_3S$ $[M+H]^+$ 363.1485. Found: 363.1495.

**5.1.4.14. $N^1$-Hydroxy-$N^8$-(5-phenethyl-1,3,4-thiadiazol-2-yl)octanediamide (6o).** Yield: 74%, mp: 165–166 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.24–1.25 (m, 4H), 1.44–1.51 (m, 2H), 1.53–1.58 (m, 2H), 1.93 (t, $J$ = 7.4 Hz, 2H), 2.42 (t, $J$ = 7.4 Hz, 2H), 3.03 (t, $J$ = 7.7 Hz, 2H), 3.28 (t, $J$ = 7.7 Hz, 2H), 7.17–7.31 (m, 5H), 8.63 (br s, 1H), 10.31 (br s, 1H), 12.30 (br s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{18}H_{24}N_4O_3S$ $[M+H]^+$ 377.1642. Found: 377.1647.

**5.1.4.15. (*E*)-$N^1$-Hydroxy-$N^8$-(5-styryl-1,3,4-thiadiazol-2-yl)octanediamide (6p).** Yield: 95%, mp: 206–207 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 1.28–1.29 (m, 4H), 1.46–1.53 (m, 2H), 1.58–1.65 (m, 2H), 1.94 (t, $J$ = 7.3 Hz, 2H), 2.47 (t, $J$ = 7.3 Hz, 2H), 7.35 (m, 1H), 7.40–7.45 (m, 3H), 7.49 (d, $J$ = 16.5 Hz, 1H), 7.69 (m, 2H), 8.54 (s, 1H), 10.25 (s, 1H), 12.44 (s, 1H); HRMS (AP-ESI) *m/z* Calcd for $C_{18}H_{22}N_4O_3S$ $[M+H]^+$ 375.1485. Found: 375.1487.

### 5.1.5. Ethyl 2-chloro-2-oxoacetate (8)

A mixture of potassium acetate (20 g), water (30 ml) and diethyl oxalate (29.2 g, 0.2 mol) was stirred at 70–80 °C for 2 h. The reaction mixture was cooled and condensed to 30 ml. After ethanol (50 ml) and diethyl ether (150 ml) were added, the solution was filtrated to give 23.61 g of potassium monoethyl oxalate (76%). $SOCl_2$ (30 g, 0.25 mol) was added slowly to this dry potassium salt (20.6 g, 0.13 mol) previously mixed with diethyl ether (20 ml) in an ice bath. The mixture was then heated under reflux for 15 h. The white solid of produced potassium chloride was filtrated, the filtrate was fractionated and a fraction of 125–130 °C was collected to give 12.22 g of **8** (69%).

### 5.1.6. Ethyl 2-oxo-2-((5-phenyl-1,3,4-thiadiazol-2-yl)amino)acetate (9a)

Eight (1.36 g, 10 mmol) was added dropwise to a stirred solution of **2a** (1.77 g, 10 mmol) and $Et_3N$ (3.5 ml, 25 mmol) in anhydrous tetrahydrofuran (65 ml) at 0 °C. The reaction mixture was allowed to be stirred overnight at room temperature. The solvent was removed in vacuum and the residue was diluted with dichloromethane (150 ml), washed with 1M $H_3PO_4$ (3 × 80 ml) and brine (3 × 80 ml) and dried over with $MgSO_4$. Filtration and concentration in vacuum and recrystallization from methanol gave 1.41 g

of **5a** as a white crystal. Yield: 51%, mp: 194–196 °C. ESI-MS *m*/*z*: 304.3 $[M+H]^+$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.35 (t, $J$ = 7.2 Hz, 3H), 4.34–4.37 (m, 2H), 7.44–7.58 (m, 3H), 7.98–8.00 (m, 2H), 13.68 (br s, 1H).

**5.1.6.1. Ethyl 2-((5-benzyl-1,3,4-thiadiazol-2-yl)amino)-2-oxoacetate (9b).** Yield: 58%, mp: 131–133 °C. ESI-MS *m*/*z*: 292.4 $[M+H]^+$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.30 (t, $J$ = 7.2 Hz, 3H), 4.29–4.32 (m, 2H), 4.42 (s, 2H), 7.27–7.39 (m, 5H), 13.48 (br s, 1H).

**5.1.6.2. Ethyl 2-oxo-2-((5-phenethyl-1,3,4-thiadiazol-2-yl)amino)acetate (9c).** Yield: 64%, mp: 135–138 °C. ESI-MS *m*/*z*: 306.4 $[M+H]^+$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.31 (t, $J$ = 7.2 Hz, 3H), 3.06 (t, $J$ = 7.8 Hz, 2H), 3.35 (t, $J$ = 7.8 Hz, 2H), 4.29–4.33 (m, 2H), 4.42, 7.19–7.31 (m, 5H), 13.40 (br s, 1H).

**5.1.6.3. (*E*)-Ethyl 2-oxo-2-((5-styryl-1,3,4-thiadiazol-2-yl)amino)acetate (9d).** Yield: 48%, mp: 191–193 °C. ESI-MS *m*/*z*: 304.3 $[M+H]^+$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.34 (t, $J$ = 7.2 Hz, 3H), 7.94–7.95 (m, 2H), 7.38–7.39 (m, 1H), 7.42–7.45 (m, 2H), 7.56 (m, 2H), 7.74–7.75 (m, 2H), 13.60 (s, 1H).

**5.1.7. $N^1$-Hydroxy-$N^2$-(5-phenyl-1,3,4-thiadiazol-2-yl)oxalamide (10a)**

To a flask containing **9a** (0.55 g, 2 mmol) was added the solution of hydroxylamine in methanol (10 ml). The mixture was stirred at room temperature for 0.5 h. The precipitate was filtrated out and washed with water and dichloromethane to afford 0.82 g of **10a** as a white solid. Yield: 88%, mp: 192–193 °C. $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.53–7.56 (m, 3H), 7.96–7.99 (m, 2H), 9.57 (s, 1H), 11.93 (s, 1H), 13.23 (br s, 1H); HRMS (AP-ESI) *m*/*z* Calcd for $C_{10}H_8N_4O_3S$ $[M+H]^+$ 265.0390. Found: 265.0395.

**5.1.7.1. $N^1$-(5-Benzyl-1,3,4-thiadiazol-2-yl)-$N^2$-hydroxyoxalamide (10b).** Yield: 91%, mp: 174–175 °C. $^1H$ NMR (DMSO-$d_6$) $\delta$ 4.39 (s, 2H), 7.26–7.37 (m, 5H), 9.52 (s, 1H), 11.84 (s, 1H), 12.95 (br s, 1H); HRMS (AP-ESI) *m*/*z* Calcd for $C_{11}H_{10}N_4O_3S$ $[M+H]^+$ 279.0546. Found: 279.0551.

**5.1.7.2. $N^1$-Hydroxy-$N^2$-(5-phenethyl-1,3,4-thiadiazol-2-yl)oxalamide (10c).** Yield: 89%, mp: 164–166 °C. $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.05 (t, $J$ = 7.6 Hz, 2H), 3.35 (t, $J$ = 7.6 Hz, 2H), 7.18–7.31 (m, 5H), 9.52 (br s, 1H), 11.85 (br s, 1H), 12.90 (br s, 1H); HRMS (AP-ESI) *m*/*z* Calcd for $C_{12}H_{12}N_4O_3S$ $[M+H]^+$ 293.0703. Found: 293.0707.

**5.1.7.3. (*E*)-$N^1$-Hydroxy-$N^2$-(5-styryl-1,3,4-thiadiazol-2-yl)oxalamide (10d).** Yield: 93%, mp: 195–196 °C. $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.37 (m, 1H), 7.43 (m, 2H), 7.51 (d, $J$ = 16.5 Hz, 1H), 7.57 (d, $J$ = 16.5 Hz, 1H), 7.73 (m, 2H), 9.56 (br s, 1H), 11.91 (s, 1H), 13.14 (br s, 1H); HRMS (AP-ESI) *m*/*z* Calcd for $C_{12}H_{10}N_4O_3S$ $[M+H]^+$ 291.0546. Found: 291.0552.

**5.1.8. 4-Oxo-4-((5-phenyl-1,3,4-thiadiazol-2-yl)amino)butanoic acid (12a)**

Succinic anhydride (2 g, 0.02 mol) was added to a solution of **2a** (3.54 g, 0.02 mol) in hot acetonitrile (150 ml). The solution was heated for 1 h under reflux. After cooling, the precipitated product was collected to give 2.88 g of **12a** as a white solid. Yield: 52%, mp: 267–270 °C. ESI-MS *m*/*z*: 278.3 $[M+H]^+$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.60 (t, $J$ = 6.6 Hz, 2H), 2.74 (t, $J$ = 6.6 Hz, 2H), 7.53–7.55 (m, 3H), 7.93–7.94 (m, 2H), 12.25 (s, 1H), 12.70 (s, 1H).

**5.1.8.1. 4-Oxo-4-((5-phenyl-1,3,4-thiadiazol-2-yl)amino)butanoic acid (12b).** Compound **11b** was synthesized following the procedure described above. Yield: 59%, mp: 228–232 °C. ESI-MS *m*/*z*: 304.3 $[M+H]^+$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.61 (t, $J$ = 6.6 Hz, 2H), 2.75 (t, $J$ = 6.6 Hz, 2H), 7.37 (m, 1H), 7.41–7.46 (m, 3H), 7.52 (d, $J$ = 16.8 Hz, 1H), 7.72 (m, 2H), 12.30 (br s, 1H), 12.63 (s, 1H).

**5.1.9. $N^1$-Hydroxy-$N^4$-(5-phenyl-1,3,4-thiadiazol-2-yl)succinamide (13a)**

To a −20 °C cooled solution of **12a** (2.12 g, 7 mmol) and *N*-methylmorpholine (1.6 ml, 14 mmol) in anhydrous *N*,*N*-dimethylformamide (15 ml) was added ClCOOBu-i (1.1 ml, 8.4 mmol), and the mixture was stirred for 0.5 h. The solid was filtered out and filtrate was added to freshly prepared $NH_2OK$ in methanol (5 ml, 1.54 mol/L). The resulting mixture was stirred at room temperature overnight, then was filtered and the residue was washed with water to give 1.29 g of **13a** as a white solid. Yield: 63%, mp: 197–198 °C. $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.35 (t, $J$ = 6.8 Hz, 2H), 2.75 (t, $J$ = 6.8 Hz, 2H), 7.53–7.55 (m, 3H), 7.93–7.94 (m, 2H), 8.76 (s, 1H), 10.47 (s, 1H), 12.68 (s, 1H); HRMS (AP-ESI) *m*/*z* Calcd for $C_{12}H_{12}N_4O_3S$ $[M+H]^+$ 293.0703. Found: 293.0698.

**5.1.9.1. (*E*)-$N^1$-Hydroxy-$N^4$-(5-styryl-1,3,4-thiadiazol-2-yl)succinamide (13b).** Compound **12b** was synthesized following the procedure described above. Yield: 49%, mp: 196–197 °C. $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.35 (t, $J$ = 7.0 Hz, 2H), 2.74 (t, $J$ = 7.0 Hz, 2H), 7.36 (m, 1H), 7.40–7.45 (m, $J$ = 16.3 Hz, 3H), 7.51 (d, $J$ = 16.3 Hz, 1H), 7.71 (m, 2H), 8.73 (s, 1H), 10.45 (s, 1H), 12.57 (br s, 1H); HRMS (AP-ESI) *m*/*z* Calcd for $C_{14}H_{14}N_4O_3S$ $[M+H]^+$ 319.0859. Found: 319.0853.

### 5.2. In vitro HDAC assay

We performed assays according to the kit instruction. HDAC came from HeLa cell nucleus extracts, mainly including HDAC1 and HDAC2, and the substrate was a type of [$^3H$] acetylated histone peptide. The tested compounds and the control drug SAHA were diluted to various concentrations. On the 96-well plate, HDAC (5 μL/well) were incubated at 37 °C with 10 μL of various concentrations of samples and 25 μL of substrate. After reacting for 30 min, Color de Lys Developer (50 μL/well) was added. Then, after 15 min the ultraviolet absorption of the wells was measured on a microtiter-plate reader at 405 nm. The inhibition rates were calculated from the ultraviolet absorption readings of inhibited wells related to those of control wells. Finally, the $IC_{50}$ values were determined using a regression analysis of the concentration/inhibition data.

### 5.3. MTT Assay

MDA-MB-231 and K562 cells were respectively cultured in RPMI1640 medium containing 10% FBS at 37 °C in 5% $CO_2$ humidified incubator. Cell proliferation was determined by the MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) assay. Briefly, cells were plated in a 96-well plate at 10,000 cells per well, cultured for 4 h in complete growth medium, then treated with 2000, 400, 80, 16, 3.2, 0.64 mg/mL of compounds for 48 h. 0.5% MTT solution was added to each well. After further incubation for 4 h, formazan formed from MTT was extracted by adding 200 μL DMSO and mixing for 15 min. Optical density was read with an microtiter-plate reader at 570 nm.

### 5.4. Docking study

AutoDock 4.2 was used for all docking calculations. One hundred runs were performed using Lamarckian Genetic Algorithm (LGA) with default parameters. The molecular structures were generated with Sybyl/Sketch module and optimized using semi-empirical MOPAC/AM1 method and then assigned with AM1-BCC

charges. Results differing less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together. Conformations in first cluster with the most favorable free binding energy were selected as the best docking result.

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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.2012.04.032. These data include MOL files and InChiKeys of the most important compounds described in this article.

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