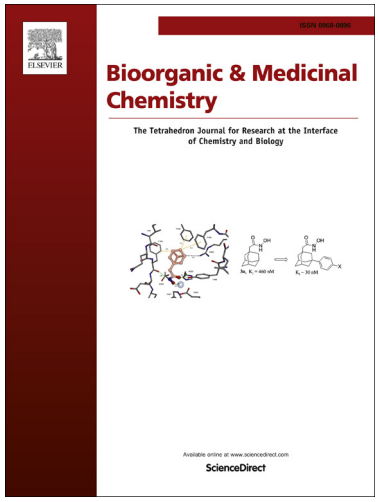


Improved antiproliferative activity of 1,3,4-thiadiazole-containing histone deacetylase (HDAC) inhibitors by introduction of the heteroaromatic surface recognition motif

Peng Guan, Lei Wang, Xuben Hou, Yichao Wan, Wenfang Xu, Weiping Tang, Hao Fang

PII: S0968-0896(14)00685-3
DOI: http://dx.doi.org/10.1016/j.bmc.2014.09.039
Reference: BMC 11826

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 26 July 2014
Revised Date: 17 September 2014
Accepted Date: 18 September 2014

Please cite this article as: Guan, P., Wang, L., Hou, X., Wan, Y., Xu, W., Tang, W., Fang, H., Improved antiproliferative activity of 1,3,4-thiadiazole-containing histone deacetylase (HDAC) inhibitors by introduction of the heteroaromatic surface recognition motif, *Bioorganic & Medicinal Chemistry* (2014), doi: http://dx.doi.org/10.1016/j.bmc.2014.09.039

Improved antiproliferative activity of 1,3,4-thiadiazole-containing histone deacetylase (HDAC) inhibitors by introduction of the heteroaromatic surface recognition motif

Peng Guan[a], Lei Wang[a], Xuben Hou[a], Yichao Wan[a], Wenfang Xu[a], Weiping Tang[b], Hao Fang[a*]

[a]*Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmacy, Shandong University, Jinan, Shandong, 250012, P.R. China*
[b]*Division of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin, Madison 53705, USA.*

Abstract

A series of 1,3,4-thiadiazole-containing hydroxamic acids, in accord with the common pharmacophore of histone deacetylase (HDAC) inhibitors (a Zn^{2+} binding moiety-a linker-a surface recognition motif), was identified as submicromolar HDAC inhibitors by our group. In this study, we continued our efforts to develop 1,3,4-thiadiazole bearing hydroxamate analogues by modifying the surface recognition motif. We found that 1,3,4-thiadiazoles having a heteroaromatic substituent showed better HDAC inhibitory activity in enzymatic assay and higher antiproliferative potency in cellular assay compared to SAHA.

Keywords: 1,3,4-thiadiazole; hydroxamic acid; histone deacetylase inhibitor; surface recognition motif; antiproliferation

1. Introduction

Histone deactylases are a family of epigenetic enzymes which catalyze the removal of acetyl moieties from N-acetyllysine residues of histones and non-histone proteins, many of which are responsible for regulation of gene expression.[1] Histone deacetylases have been found mechanistically linked to tumor onset and progression，the inhibition of which induces cell cycle or growth arrest, differentiation and apoptosis of malignant cells.[2] Histone deactylase (HDAC) inhibitors demonstrated prominent antitumor efficacy on broad spectrum neoplasms in preclinical and clinical studies.[2,3] Strikingly, vorinostat (suberoylanilide hydroxamic acid, SAHA)[4] and romidepsin (depsipeptide)[5] were approved for the treatment of cutaneous T-cell lymphoma by FDA in 2006 and 2009, respectively. (**Figure 1**)

* Corresponding author. Phone: +86-531-88382731. Fax: +86-531-88382548. E-mail: haofangcn@sdu.edu.cn.

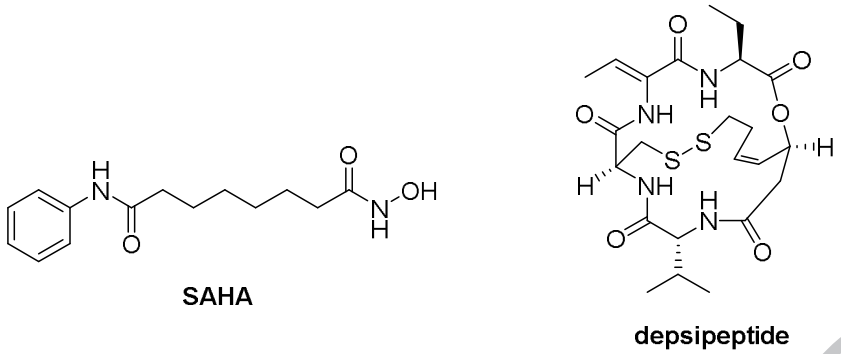


Figure 1. Chemical structures of SAHA and depsipeptide.

In general, the pharmacophore for HDAC inhibitors is composed of a Zn^{2+} binding moiety, a linker, and a surface recognition motif. (**Figure 2**) In the prior work, we reported the discovery of 1,3,4-thiadiazole based hydroxamates as submicromolar HDAC inhibitors, which are comparable to SAHA.[6] (**Figure 2**) The linker length (n) and the surface recognition motif (R) were systematically varied to find the optimum geometric parameters. The structure-activity relationship (SAR) indicated that the presence of 5 or 6 carbon units between the hydroxamate group and the 1,3,4-thiadiazole ring (n = 5 or 6) is optimal for enzymatic efficacy. Compound **1**, containing a phenyl in the surface recognition domain (R = phenyl), showed approximately 2-fold greater potency against HDAC enzymes than SAHA. However, none of these active HDAC inhibitors outperformed SAHA as an antiproliferative agent of cancer cells. In an effort to increase the proliferative inhibition of 1,3,4-thiadiazole bearing hydroxamates, while maintaining n as 5 or 6, we investigated the embellishment and replacement of the surface recognition motif phenyl in **1**.

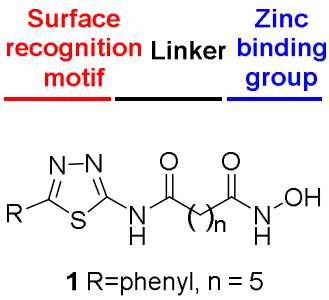
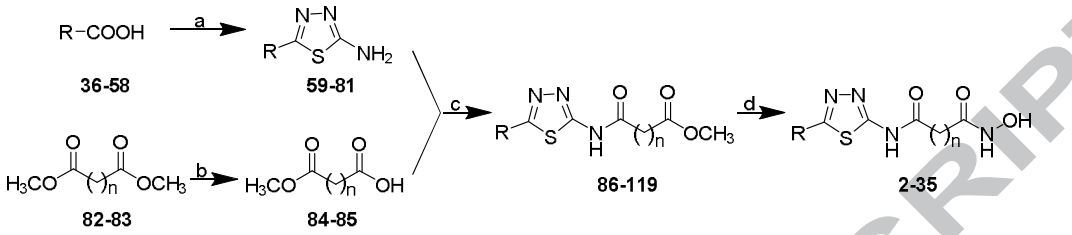


Figure 2. Pharmacophoric characteristics of HDAC inhibitors instantiated by the chemical structure of 1,3,4-thiadiazole-containing hydroxamates.

2. Chemistry

The synthesis of the 1,3,4-thiadiazole bearing hydroxamic acids is shown in **Scheme 1**. The 5-substituted-1,3,4-thiadiazol-2-amines **59**-**81** were prepared by a facile one-pot reaction of the corresponding carboxylic acids, *N*-aminothiourea, and phosphoryl chloride. Saponification of dimethylesters **82**-**83** furnished the

monomethyl esters **84**-**85** in good yields. Sequential treatment of **84**-**85** with refluxing thionyl chloride and then **59**-**81** gave **86**-**119**, which were converted to hydroxamic acids **2**-**35** according to reported procedures by treating with freshly prepared potassium hydroxylamine.[7]



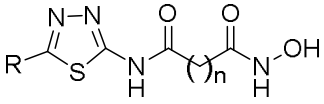
Scheme 1. Reagents and conditions: (a) $NH_2NHCSNH_2$, $POCl_3$, 75 °C, 0.5 h then H_2O, reflux, 4 h; (b) KOH, CH_3OH, 0 °C to rt, 4 h; (c) i) $SOCl_2$, reflux, 2 h; ii) Et_3N, THF, 0 °C to rt, overnight; (d) $NH_2OH{\cdot}HCl$, KOH, CH_3OH, rt,1 h.

3. Results and Discussion

First, we probed the effect of positional and structural modifications of the phenyl on the potency in enzymatic assays. The inhibitory data of total HDAC activity in HeLa nuclear extract in comparison with SAHA as a positive control were compiled in **Tables 1**. Unfortunately, this class of derivatives was all less active towards HDAC enzymes than SAHA, suggesting substitution of the phenyl gave no advantage in terms of enzymatic inhibition. However, some interesting trends can be observed: 1) addition of a bulky group on the phenyl led to a significant loss in HDAC inhibitory activity; 2) compounds with an electron-withdrawing group exhibited generally poorer HDAC inhibition than those with an electron-donating group. Additionally, a tendency in enzymatic potency of para-substitution < meta-substitution < ortho-substitution was noted.

Given that the phenyl-substituted derivatives did not display higher enzymatic potency than SAHA, we turned our attention to a set of alternative aromatic rings. Replacement of the phenyl with a naphthalenyl group resulted in a considerable loss in HDAC inhibition, while heteroaromatic analogues possessed better or similar activity compared to SAHA. Compounds **33** and **35** showed the best activity, with IC_{50} values of approximately 300 nM, suggesting a preference for a heteroaromatic ring adjacent to the 1,3,4-thiadiazole ring as a surface recognition motif. Pyridine- and thiophene- containing compounds had improved HDAC inhibitory efficacy in comparison to SAHA.

Table 1. HDAC inhibitory activity of 1,3,4-thiadiazole hydroxamic acid derivatives.



compd	R	n	IC_{50} of HDACs (nM)[a]
2	4-morpholino-Ph	5	>5000
3	4-$N(CH_3)_2$-Ph	5	>5000
4	4-OCH_3-Ph	5	437±55
5	3,4-OCH_3-Ph	5	487±56
6	4-CH_3-Ph	5	422±53
7	(1,1'-biphenyl)-4-yl	5	>5000
8	4-F-Ph	5	741±52
9	4-Cl-Ph	5	988±81
10	3-Cl-Ph	5	847±43
11	2-Cl-Ph	5	507±19
12	4-Br-Ph	5	1285±127
13	4-I-Ph	5	875±45
14	4-SO_2CH_3-Ph	5	721±55
15	4-NO_2-Ph	5	709±40
16	4-CF_3-Ph	5	1154±92
17	4-morpholino-Ph	6	>5000
18	(1,1'-biphenyl)-4-yl	6	>5000
19	3-Br-Ph	6	1215±297
20	2-Br-Ph	6	526±42
21	4-I-Ph	6	>5000
22	4-SO_2CH_3-Ph	6	598±51
23	4-CF_3-Ph	6	>5000
24	naphthalen-1-yl	5	3186±475
25	naphthalen-2-yl	5	>5000
26	pyridin-3-yl	5	325±33
27	pyridin-4-yl	5	411±39
28	furan-2-yl	5	434±33
29	thiophen-2-yl	5	411±43
30	naphthalen-1-yl	6	>5000
31	naphthalen-2-yl	6	>5000
32	pyridin-3-yl	6	386±53
33	pyridin-4-yl	6	286±8
34	furan-2-yl	6	436±53
35	thiophen-2-yl	6	310±42
SAHA			416±20

[a] Inhibitory data are means of three independent determinations and expressed with standard deviations.

Recently, genetic studies suggested that HDAC1 and HDAC2 should be most closely related to tumor cell survival.[8] Therefore inhibition of HDAC1 and HDAC2 might be helpful to block cancer cell proliferation. In our studies, the enzyme for HDAC inhibitory assay came from HeLa nuclear extracts which mainly include HDAC1 and HDAC2. Considering that the crystal structure of HDAC2 and SAHA complex has been reported (PDB code 4LXZ), further molecular docking was performed using most active compound **33** and the program of Surflex-Dock (**Figure 3**). The result showed that the hydroxamic acid of compound **33** could chelate Zn^{2+} ion in the active site of HDAC2, which indicated that compound **33** could have a similar binding mode to SAHA. On the other hand, 1,3,4-thiadiazole of **33** could form additional π-π interaction with Tyr209 and hydrogen bond with Phe210, which suggested that interactions between the surface recognition motif and HDAC may lead to the better potency of compound **33** compared with SAHA.

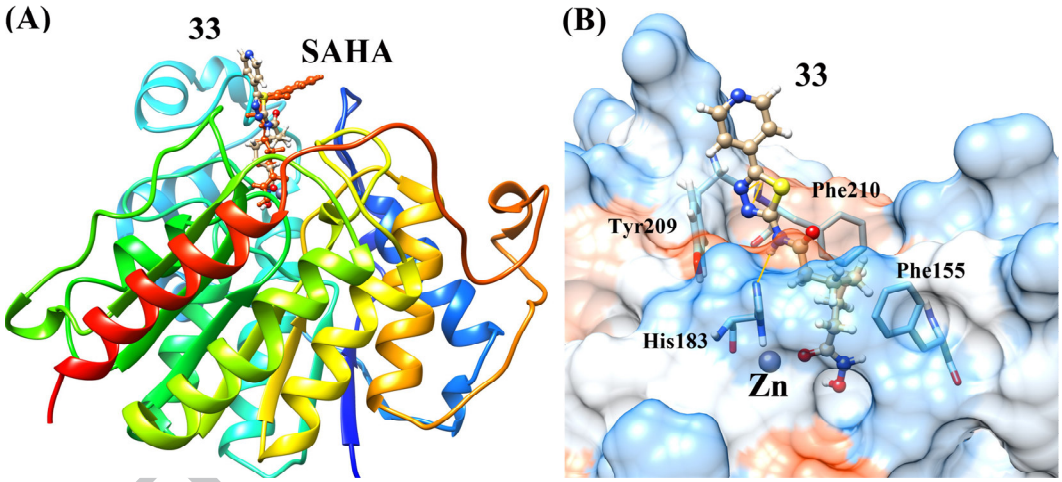
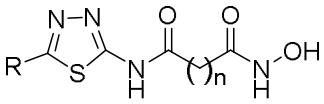


Figure 3. (A) Docked compound **33** (depicted in atom type) into the crystal structure of HDAC2 complexed with SAHA (depicted in red). (B) Binding mode of compound **33** in the active site of HDAC2.

To further ascertain the utility of heteroaromatic HDAC inhibitors at the cellular level, the efficacy of pyridine and thiophene analogues on the viability of cancer cells was measured by MTT assay. **Table 2** showed their IC_{50} values and SAHA was used as a reference. The 6 carbon unit linker (compound **32**, **33** and **35**) afforded appreciably potent cell growth inhibition in the same order of magnitude with SAHA. It was noteworthy that the thiophene variant **35** exerted higher antiproliferative activity toward all three cell lines than SAHA. In addition, the pyridine derivative **32** was more active towards the MDA-MB-231 cell than SAHA.

Table 2. Antiproliferative activities of heteroaromatic compound towards MDA-MB-231, K562 and PC3 cell lines.



compd	R	n	IC_{50} of HDACs (nM)	IC_{50} (μM)[a]		
				MDA-MB-231	K562	PC3
33	pyridin-4-yl	6	286±8	4.69±0.61	4.15±1.19	7.75±1.04
35	thiophen-2-yl	6	310±42	1.21±0.18	1.56±0.06	3.60±0.83
26	pyridin-3-yl	5	325±33	11.7±2.80	>60	54.4±7.08
32	pyridin-3-yl	6	386±53	1.87±0.07	4.36±0.85	7.98±1.19
27	pyridin-4-yl	5	411±39	40.1±10.6	>60	>60
29	thiophen-2-yl	5	411±43	15.2±0.82	>60	>60
SAHA			416±20	2.29±0.19	1.61±0.08	5.79±1.23

[a] Inhibitory data are means of three independent determinations and expressed with standard deviations.

4. Conclusions

Through an optimization process of the surface recognition motif of 1,3,4-thiadiazole-containing hydroxamate HDAC inhibitors, we discovered that heteroaromatic derivatives had higher or similar inhibitory activity in both enzymatic and cellular assays compared to SAHA. Thiophene-containing compound **35** was identified as a more potent inhibitor of HDAC enzymes and tumor cell growth than SAHA, representing a highly promising antitumor agent with the potential for the treatment of human cancers.

5. Experimental section

5.1. Chemistry

All starting materials, reagents, and solvents from commercial suppliers were used without further purification unless otherwise noted. All reactions were monitored by thin layer chromatography (TLC) on 0.25 mm silica gel (60GF-254) plates and visualized with iodine vapor, UV light, or ferric chloride. Melting points were determined by the RY-1 electrothermal melting point apparatus without correction. ESI-MS spectras were recorded on an Aglient-1100 series LC/MSD trap spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker DRX spectrometer (300 MHz). The chemical shifts were expressed by δ values (parts per million) with tetramethylsilane (TMS) as internal standard and significant data were reported in an order of multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. HRMS spectra were performed on an Agilent 6510 Quadrupole Time-of-Flight LC/MS deliver.

5.1.1. 5-(4-Morpholinophenyl)-1,3,4-thiadiazol-2-amine (59)

A mixture of 4-morpholinobenzoic acid (5.18 g, 25 mmol) and N-aminothiourea (2.28 g, 25 mmol) in $POCl_3$ (7 ml) was stirred vigrously at 75 °C for 0.5 h. After addition of H_2O (30 ml), the reaction mixture was heated under reflux for 4 h and basified to pH 8 by 50% NaOH solution. The mixture was filtered and the filter cake was recystallized from ethanol to yield 3.90 g of compound **59** as a yellow crystal. Yield: 59%, mp: 293-295 °C (EtOH). ESI-MS m/z: 263.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 3.19 (t, J = 5.4 Hz, 4H), 3.74 (t, J = 5.4 Hz, 4H), 7.00 (m, 2H), 7.23 (s, 2H) , 7.59 (m, 2H).

The synthetic procedures of compounds **60**-**81** were the same as that described above.

5.1.1.1. 5-(4-(Dimethylamino)phenyl)-1,3,4-thiadiazol-2-amine (60)

Yield: 97%, mp: 249-250 °C (EtOH). ESI-MS m/z: 221.3 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 2.96 (s, 6H), 6.74-6.76 (m, 2H), 7.15 (s, 2H), 7.54-7.55 (m, 2H).

5.1.1.2. 5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-amine (61)

Yield: 82%, mp: 219-220 °C (EtOH). ESI-MS m/z: 208.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 3.80 (s, 3H), 7.01-7.03 (m, 2H), 7.23 (s, 2H), 7.67-7.69 (m, 2H).

5.1.1.3. 5-(3,4-Dimethoxyphenyl)-1,3,4-thiadiazol-2-amine (62)

Yield: 77%, mp: 195-197 °C (EtOH). ESI-MS m/z: 238.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 3.80 (s, 3H), 3.82 (s, 3H), 7.02 (d, J = 8.4 Hz, 1H), 7.19-7.21 (dd, $J1$ = 8.4 Hz, $J2$ = 1.8 Hz, 1H), 7.31 (s, 2H), 7.36 (d, J = 1.8 Hz, 1H).

5.1.1.4. 5-(p-Tolyl)-1,3,4-thiadiazol-2-amine (63)

Yield: 98%, mp: 194-195 °C (EtOH). ESI-MS m/z: 192.3 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H), 7.27 (d, J = 8.4 Hz, 2H), 7.35 (s, 2H), 7.64 (d, J = 8.4 Hz, 2H).

5.1.1.5. 5-([1,1'-Biphenyl]-4-yl)-1,3,4-thiadiazol-2-amine (64)

Yield: 43%, mp: 245-247 °C (EtOH). ESI-MS m/z: 254.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.40 (t, J = 7.8 Hz, 1H), 7.45 (s, 2H), 7.48-7.51 (m, 2H), 7.72-7.73 (m, 2H), 7.77-7.78 (m, 2H), 7.83-7.85 (m, 2H).

5.1.1.6. 5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-amine (65)

Yield: 84%, mp: 238-240 °C (EtOH). ESI-MS m/z: 196.3 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.30-7.33 (m, 2H), 7.43 (s, 2H), 7.80-7.82 (m, 2H).

5.1.1.7. 5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-amine (66)

Yield: 98%, mp: 226-228 °C (EtOH). ESI-MS m/z: 212.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.48 (s, 2H), 7.52-7.54 (m, 2H), 7.77-7.78 (m, 2H).

5.1.1.8. 5-(3-Chlorophenyl)-1,3,4-thiadiazol-2-amine (67)
Yield: 75%, mp: 212-214 °C (EtOH). ESI-MS m/z: 212.1 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 7.49-7.50 (m, 2H), 7.54 (s, 2H), 7.70-7.71 (m, 1H), 7.80 (s, 1H).

5.1.1.9. 5-(2-Chlorophenyl)-1,3,4-thiadiazol-2-amine (68)
Yield: 88%, mp: 190-192 °C (EtOH). ESI-MS m/z: 212.1 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 7.45-7.50 (m, 4H), 7.60-7.62 (m, 1H), 7.99-8.01 (m, 1H).

5.1.1.10. 5-(4-Bromophenyl)-1,3,4-thiadiazol-2-amine (69)
Yield: 98%, mp: 216-218 °C (EtOH). ESI-MS m/z: 256.1 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.49 (s, 2H), 7.66-7.67 (m, 2H), 7.70-7.71 (m, 2H).

5.1.1.11. 5-(4-Iodophenyl)-1,3,4-thiadiazol-2-amine (70)
Yield: 77%, mp: 240-241 °C (EtOH). ESI-MS m/z: 304.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.48 (s, 2H), 7.54-7.55 (m, 2H), 7.83-7.84 (m, 2H).

5.1.1.12. 5-(4-(Methylsulfonyl)phenyl)-1,3,4-thiadiazol-2-amine (71)
Yield: 41%, mp: 241-242 °C (EtOH). ESI-MS m/z: 256.1 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 3.26 (s, 3H), 7.65 (s, 2H), 7.99-8.02 (m, 4H).

5.1.1.13. 5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-amine (72)
Yield: 53%, mp: 256-258 °C (EtOH). ESI-MS m/z: 223.4 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.74 (s, 2H), 8.02 (d, J = 9.0 Hz, 2H), 8.30 (d, J = 9.0 Hz, 2H).

5.1.1.14. 5-(4-(Trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-amine (73)
Yield: 75%, mp: 242-243 °C (EtOH). ESI-MS m/z: 246.3 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.61 (s, 2H), 7.82-7.83 (m, 2H), 7.97-7.98 (m, 2H).

5.1.1.15. 5-(3-Bromophenyl)-1,3,4-thiadiazol-2-amine (74)
Yield: 89%, mp: 222-224 °C (EtOH). ESI-MS m/z: 256.1 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.43 (t, J = 7.8 Hz, 1H), 7.54 (s, 2H), 7.62-7.64 (m, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.93 (t, J = 1.8 Hz, 1H).

5.1.1.16. 5-(2-Bromophenyl)-1,3,4-thiadiazol-2-amine (75)
Yield: 85%, mp: 202-203 °C (EtOH). ESI-MS m/z: 256.1 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 7.39-7.42 (td, $J1$ = 7.8 Hz, $J2$ = 1.8 Hz, 1H), 7.45 (s, 2H), 7.49-7.51 (td, $J1$ = 7.8 Hz, $J2$ = 1.2 Hz, 1H), 7.77-7.79 (dd, $J1$ = 7.8 Hz, $J2$ = 1.2 Hz, 1H), 7.86-7.88 (dd, $J1$ = 7.8 Hz, $J2$ = 1.8 Hz, 1H).

5.1.1.17. 5-(Naphthalen-1-yl)-1,3,4-thiadiazol-2-amine (76)
Yield: 62%, mp: 208-210 °C (EtOH). ESI-MS m/z: 228.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.49 (s, 2H), 7.58-7.66 (m, 3H), 7.74-7.76 (m, 1H), 8.04 (t, J = 8.4 Hz, 2H), 8.80 (d, J = 9.0 Hz, 1H).

5.1.1.18. 5-(Naphthalen-2-yl)-1,3,4-thiadiazol-2-amine (77)

Yield: 95%, mp: 230-231 °C (EtOH). ESI-MS m/z: 228.3 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.49 (s, 2H), 7.55-7.59 (m, 2H), 7.95-7.96 (m, 1H), 7.99-8.00 (m, 2H), 8.03-8.05 (m, 1H), 8.24 (s, 1H).

5.1.1.19. 5-(Pyridin-3-yl)-1,3,4-thiadiazol-2-amine (78)

Yield: 70%, mp: 234-236 °C (EtOH). ESI-MS m/z: 179.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.50-7.52 (m, 1H), 7.56 (s, 2H), 8.13-8.15 (m, 1H), 8.61-8.62 (dd, *J1* = 4.8 Hz, *J2* = 1.8Hz, 1H), 8.95 (d, *J* = 2.4 Hz, 1H).

5.1.1.20. 5-(Pyridin-4-yl)-1,3,4-thiadiazol-2-amine (79)

Yield: 62%, mp: 238-241 °C (EtOH). ESI-MS m/z: 179.1 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.70 (s, 2H), 7.71-7.72 (m, 2H), 8.64-8.65 (m, 2H).

5.1.1.21. 5-(Furan-2-yl)-1,3,4-thiadiazol-2-amine (80)

Yield: 97%, mp: 247-249 °C (EtOH). ESI-MS m/z: 168.3 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 6.65-6.66 (m, 1H), 6.95 (d, *J* = 3.6 Hz, 1H), 7.45 (s, 2H), 7.83-7.84 (m, 1H).

5.1.1.22. 5-(Thiophen-2-yl)-1,3,4-thiadiazol-2-amine (81)

Yield: 61%, mp: 204-206 °C (EtOH). ESI-MS m/z: 184.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 7.12-7.14 (m, 1H), 7.41-7.42 (dd, *J1* = 3.6 Hz, *J2* = 0.6 Hz, 1H), 7.43 (s, 2H), 7.63-7.64 (dd, *J1* = 4.8 Hz, *J2* = 0.6 Hz, 1H).

5.1.2. 7-Methoxy-7-oxoheptanoic acid (84)

A solution of KOH (5.87 g, 104.65 mmol) in CH_3OH (150 ml) was added dropwise to dimethyl heptanedioate **82** (16.94 g, 90 mmol) at 0 °C. The reaction mixture was allowed to be stirred for 4 h at room temperature. After removal of the solvent under reduced pressure, Et_2O (100 ml) and H_2O (200 ml) were added and the organic phase was concentrated to give **82** as yellow oil (5.08 g, 30%). The aqueous phase was acidified to pH 3 by concentrated HCl and extracted with Et_2O (100 ml×3). The combined organic layer was washed with brine (100 ml×3) and dried over $MgSO_4$. The solvent was concentrated in vacuum. Filtration and purification with silica gel column chromatography gave 5.96 g (38%) of compound **84** as colorless oil. ESI-MS m/z: 173.3 $[M-H]^-$; ^1H NMR (DMSO-d_6) δ 1.23-1.31 (m, 4H), 1.44-1.57 (m, 4H), 2.19 (t, *J* = 7.2 Hz, 2H), 2.29 (t, *J* = 7.2 Hz, 2H), 3.58 (s, 3H), 11.97 (s, 1H).

The synthetic procedures of compounds **85** were the same as that described above.

5.1.2.1. 8-Methoxy-8-oxooctanoic acid (85)

Yield: 44%. ESI-MS m/z: 187.4 $[M-H]^-$; ^1H NMR (DMSO-d_6) δ 1.21-1.32 (m, 4H), 1.43-1.53 (m, 4H), 2.18 (t, *J* = 7.2 Hz, 2H), 2.29 (t, *J* = 7.2 Hz, 2H), 3.58 (s, 3H), 11.97 (s, 1H).

5.1.3. Methyl

7-((5-(4-morpholinophenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (86)

A soln. of **84** (1.74 g, 10 mmol) in $SOCl_2$ (4 ml) was refluxed for 2 h. The removal of $SOCl_2$ under reduced pressure yielded orange oil, which was dissolved in dichloromethane. The resulting solution was added dropwise to a solution of **59** (2.10 g, 8 mmol) and Et_3N (3.5 mL, 25 mmol) in dichloromethane (65 mL) at 0 °C. The resulting reaction mixture was stirred overnight at room temperature, washed by 1M H_3PO_4 (3×80 mL) and brine (3×80 mL), and dried ($MgSO_4$). Concentration and recrystallization from EtOAc gave 1.22 g of compound **86** as a colorless crystal. Yield: 36%, mp: 214-216 °C (EtOAc). ESI-MS m/z: 419.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.47-1.57 (m, 2H), 1.68-1.78 (m, 2H), 1.82-1.92 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.83 (t, *J* = 7.5 Hz, 2H), 3.28 (t, *J* = 4.8 Hz, 4H), 3.63 (s, 3H), 3.89 (t, *J* = 4.8 Hz, 4H), 6.97 (d, *J* = 8.7 Hz, 2H), 7.83 (d, *J* = 8.7 Hz, 2H), 13.27 (s, 1H).

The synthetic procedures of compounds **87-119** were the same as that described above.

5.1.3.1. Methyl 7-((5-(4-(dimethylamino)phenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (87)

Yield: 62%, mp: 192-194 °C (EtOAc). ESI-MS m/z: 377.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.48-1.57 (m, 2H), 1.68-1.78 (m, 2H), 1.81-1.92 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 3.05 (s, 6H), 3.63 (s, 3H), 6.75 (d, *J* = 9.0 Hz, 2H), 7.79 (d, *J* = 9.0 Hz, 2H), 13.32 (s, 1H).

5.1.3.2. Methyl 7-((5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (88)

Yield: 59%, mp: 177-179 °C (EtOAc). ESI-MS m/z: 364.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.47-1.57 (m, 2H), 1.68-1.78 (m, 2H), 1.82-1.92 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 3.63 (s, 3H), 3.88 (s, 3H), 6.98-7.03 (m, 2H), 7.84-7.89 (m, 2H), 13.31 (s, 1H).

5.1.3.3. Methyl 7-((5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (89)

Yield: 55%, mp: 179-181 °C (EtOAc). ESI-MS m/z: 394.3 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.47-1.57 (m, 2H), 1.67-1.77 (m, 2H), 1.82-1.92 (m, 2H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 3.63 (s, 3H), 3.96 (s, 3H), 3.97 (s, 3H), 6.96 (d, *J* = 8.4 Hz, 1H), 7.45-7.49 (dd, *J1* = 8.4 Hz, *J2* = 1.8 Hz, 1H), 7.51 (d, *J* = 1.8 Hz, 1H), 13.15 (s, 1H).

5.1.3.4. Methyl 7-oxo-7-((5-(p-tolyl)-1,3,4-thiadiazol-2-yl)amino)heptanoate (90)

Yield: 53%, mp: 160-162 °C (EtOAc). ESI-MS m/z: 348.3 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.47-1.57 (m, 2H), 1.68-1.78 (m, 2H), 1.82-1.92 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.42 (s, 3H), 2.85 (t, *J* = 7.5 Hz, 2H), 3.63 (s, 3H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.1 Hz, 2H), 13.28 (s, 1H).

5.1.3.5. Methyl 7-((5-([1,1'-biphenyl]-4-yl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (91)

Yield: 60%, mp: 181-183 °C (EtOAc). ESI-MS m/z: 410.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.50-1.60 (m, 2H), 1.70-1.80 (m, 2H), 1.85-1.95 (m, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 3.63 (s, 3H), 7.37-7.43 (m, 1H), 7.46-7.51 (m, 2H), 7.64-7.66 (m, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 8.01 (d, *J* = 8.4 Hz, 2H), 13.37 (s, 1H).

5.1.3.6. Methyl 7-((5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (92)

Yield: 80%, mp: 200-201 °C (EtOAc). ESI-MS m/z: 352.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.46-1.56 (m, 2H), 1.68-1.78 (m, 2H), 1.82-1.92 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.82 (t, *J* = 7.5 Hz, 2H), 3.64 (s, 3H), 7.17-7.23 (m, 2H), 7.91-7.96 (m, 2H), 12.98 (s, 1H).

5.1.3.7. Methyl 7-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (93)

Yield: 75%, mp: 201-204 °C (EtOAc). ESI-MS m/z: 368.2 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.47-1.57 (m, 2H), 1.68-1.78 (m, 2H), 1.83-1.93 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 3.64 (s, 3H), 7.45-7.50 (m, 2H), 7.85-7.89 (m, 2H), 13.32 (s, 1H).

5.1.3.8. Methyl 7-((5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (94)

Yield: 72%, mp: 172-173 °C (EtOAc). ESI-MS m/z: 368.3 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.47-1.58 (m, 2H), 1.68-1.79 (m, 2H), 1.83-1.93 (m, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 2.83 (t, *J* = 7.5 Hz, 2H), 3.64 (s, 3H), 7.41-7.48 (m, 2H), 7.77-7.80 (dt, *J1* = 6.9 Hz, *J2* = 1.8 Hz, 1H), 7.97 (s, 1H), 13.02 (s, 1H).

5.1.3.9. Methyl 7-((5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (95)

Yield: 67%, mp: 155-157 °C (EtOAc). ESI-MS m/z: 368.2 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.45-1.55 (m, 2H), 1.66-1.76 (m, 2H), 1.82-1.92 (m, 2H), 2.32 (t, *J* = 7.2 Hz, 2H), 2.84 (t, *J* = 7.2 Hz, 2H), 3.63 (s, 3H), 7.40-7.46 (m, 2H), 7.51-7.57 (m, 1H), 8.14-8.19 (m, 1H), 13.17 (s, 1H).

5.1.3.10. Methyl 7-((5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (96)

Yield: 57%, mp: 201-202 °C (EtOAc). ESI-MS m/z: 412.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.47-1.57 (m, 2H), 1.68-1.78 (m, 2H), 1.82-1.93 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 3.64 (s, 3H), 7.61-7.66 (m, 2H), 7.78-7.83 (m, 2H), 13.24 (s, 1H).

5.1.3.11. Methyl 7-((5-(4-iodophenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (97)

Yield: 67%, mp: 207-209 °C (EtOAc). ESI-MS m/z: 460.3 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.46-1.56 (m, 2H), 1.67-1.77 (m, 2H), 1.82-1.92 (m, 2H), 2.33 (t, J = 7.5 Hz, 2H), 2.83 (t, J = 7.5 Hz, 2H), 3.64 (s, 3H), 7.64-7.68 (m, 2H), 7.82-7.86 (m, 2H), 13.17 (s, 1H).

5.1.3.12. Methyl 7-((5-(4-(methylsulfonyl)phenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (98)

Yield: 39%, mp: 219-221 °C (EtOAc). ESI-MS m/z: 412.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.48-1.58 (m, 2H), 1.69-1.79 (m, 2H), 1.84-1.94 (m, 2H), 2.35 (t, J = 7.2 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 3.12 (s, 3H), 3.66 (s, 3H), 8.08-8.11 (m, 2H), 8.15-8.17 (m, 2H), 12.94 (s, 1H).

5.1.3.13. Methyl 7-((5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (99)

Yield: 46%, mp: 229-239 °C (EtOAc). ESI-MS m/z: 379.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.48-1.58 (m, 2H), 1.69-1.79 (m, 2H), 1.85-1.95 (m, 2H), 2.36 (t, J = 7.5 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 3.66 (s, 3H), 8.11-8.16 (m, 2H), 8.36-8.40 (m, 2H), 12.86 (s, 1H).

5.1.3.14. Methyl 7-oxo-7-((5-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)amino)heptanoate (100)

Yield: 61%, mp: 195-196 °C (EtOAc). ESI-MS m/z: 402.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.47-1.57 (m, 2H), 1.68-1.79 (m, 2H), 1.84-1.94 (m, 2H), 2.35 (t, J = 7.2 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 3.64 (s, 3H), 7.77 (d, J = 8.1 Hz, 2H), 8.07 (d, J = 8.1 Hz, 2H), 12.98 (s, 1H).

5.1.3.15. Methyl 8-((5-(4-morpholinophenyl)-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (101)

Yield: 33%, mp: 235-237 °C (EtOAc). ESI-MS m/z: 433.5 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.31-1.48 (m, 4H), 1.54-1.64 (m, 2H), 1.73-1.83 (m, 2H), 2.22 (t, J = 7.5 Hz, 2H), 2.75 (t, J = 7.5 Hz, 2H), 3.21 (t, J = 4.8 Hz, 4H), 3.57 (s, 3H), 3.82 (t, J = 4.8 Hz, 4H), 6.91 (d, J = 9.0 Hz, 2H), 7.76 (d, J = 9.0 Hz, 2H), 13.18 (s, 1H).

5.1.3.16. Methyl 8-((5-([1,1'-biphenyl]-4-yl)-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (102)

Yield: 51%, mp: 201-204 °C (EtOAc). ESI-MS m/z: 424.5 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.43-1.58 (m, 4H), 1.62-1.72 (m, 2H), 1.83-1.93 (m, 2H), 2.30 (t, J = 7.5 Hz, 2H), 2.85 (t, J = 7.5 Hz, 2H), 3.62 (s, 3H), 7.38-7.43 (m, 1H), 7.46-7.51 (m, 2H), 7.65-7.67 (m, 2H), 7.74 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 8.4 Hz, 2H), 13.08 (s, 1H).

5.1.3.17. Methyl 8-((5-(3-bromophenyl)-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (103)

Yield: 38%, mp: 200-202 °C (EtOAc). ESI-MS m/z: 426.2 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.39-1.56 (m, 4H), 1.62-1.71 (m, 2H), 1.81-1.91 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.81 (t, *J* = 7.5 Hz, 2H), 3.64 (s, 3H), 7.37 (t, *J* = 7.8 Hz, 1H), 7.60-7.64 (m, 1H), 7.81-7.85 (m, 1H), 8.13 (t, *J* = 1.8 Hz, 1H), 12.94 (s, 1H).

5.1.3.18. Methyl 8-((5-(2-bromophenyl)-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (104)

Yield: 68%, mp: 133-135 °C (EtOAc). ESI-MS m/z: 426.2 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.35-1.52 (m, 4H), 1.58-1.67 (m, 2H), 1.79-1.89 (m, 2H), 2.27 (t, *J* = 7.5 Hz, 2H), 2.81 (t, *J* = 7.5 Hz, 2H), 3.65 (s, 3H), 7.32-7.38 (td, *J1* = 7.8 Hz, *J2* = 1.8 Hz, 1H), 7.44-7.49 (td, *J1* = 7.8 Hz, *J2* = 1.2 Hz, 1H), 7.73-7.76 (dd, *J1* = 7.8 Hz, *J2* = 1.2 Hz, 1H), 7.97-8.01 (dd, *J1* = 7.8 Hz, *J2* = 1.8 Hz, 1H), 12.93 (s, 1H).

5.1.3.19. Methyl 8-((5-(4-iodophenyl)-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (105)

Yield: 77%, mp: 198-200 °C (EtOAc). ESI-MS m/z: 474.2 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.40-1.54 (m, 4H), 1.60-1.70 (m, 2H), 1.80-1.90 (m, 2H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.79 (t, *J* = 7.5 Hz, 2H), 3.66 (s, 3H), 7.65-7.68 (m, 2H), 7.83-7.86 (m, 2H), 12.81 (s, 1H).

5.1.3.20. Methyl 8-((5-(4-(methylsulfonyl)phenyl)-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (106)

Yield: 40%, mp: 234-236 °C (EtOAc). ESI-MS m/z: 426.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.41-1.52 (m, 4H), 1.64-1.71 (m, 2H), 1.80-1.90 (m, 2H), 2.31 (t, *J* = 7.2 Hz, 2H), 2.75 (t, *J* = 7.2 Hz, 2H), 3.12 (s, 3H), 3.66 (s, 3H), 8.07-8.10 (m, 2H), 8.14-8.17 (m, 2H), 11.96 (s, 1H).

5.1.3.21. Methyl 8-oxo-8-((5-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)amino)octanoate (107)

Yield: 49%, mp: 202-203 °C (EtOAc). ESI-MS m/z: 416.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.38-1.56 (m, 4H), 1.61-1.71 (m, 2H), 1.82-1.92 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.82 (t, *J* = 7.5 Hz, 2H), 3.65 (s, 3H), 7.76-7.79 (m, 2H), 8.05-8.08 (m, 2H), 12.91 (s, 1H).

5.1.3.22. Methyl 7-((5-(naphthalen-1-yl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (108)

Yield: 75%, mp: 169-170 °C (EtOAc). ESI-MS m/z: 384.3 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.43-1.54 (m, 2H), 1.63-1.73 (m, 2H), 1.84-1.94 (m, 2H), 2.25 (t, *J* = 7.5 Hz, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 3.61 (s, 3H), 7.55-7.61 (m, 3H), 7.83-7.86 (dd, *J1* = 7.2 Hz, *J2* = 0.9 Hz, 1H), 7.92-7.97 (m, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 8.75-8.78 (m, 1H), 13.21 (s, 1H).

5.1.3.23. Methyl 7-((5-(naphthalen-2-yl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (109)

Yield: 33%, mp: 200-201 °C (EtOAc). ESI-MS m/z: 384.4 $[M+H]^+$; ^{1}H NMR ($CDCl_3$) δ 1.51-1.61 (m, 2H), 1.71-1.81 (m, 2H), 1.86-1.96 (m, 2H), 2.36 (t, *J* = 7.5 Hz, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 3.62 (s, 3H), 7.55-7.59 (m, 2H), 7.87-7.97 (m, 3H), 8.09-8.13 (m, 1H), 8.35 (s, 1H), 12.93 (s, 1H).

5.1.3.24. Methyl 7-oxo-7-((5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)amino)heptanoate (110)

Yield: 60%, mp: 173-174 °C (EtOAc). ESI-MS m/z: 335.5 $[M+H]^+$; ^{1}H NMR ($CDCl_3$) δ 1.47-1.57 (m, 2H), 1.68-1.78 (m, 2H), 1.83-1.93 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.83 (t, *J* = 7.5 Hz, 2H), 3.64 (s, 3H), 7.47-7.51 (m, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 8.74 (d, *J* = 4.2 Hz, 1H), 9.22 (s, 1H), 12.89 (s, 1H).

5.1.3.25. Methyl 7-oxo-7-((5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)amino)heptanoate (111)

Yield: 51%, mp: 170-172 °C (EtOAc). ESI-MS m/z: 335.5 $[M+H]^+$; ^{1}H NMR ($CDCl_3$) δ 1.48-1.58 (m, 2H), 1.69-1.79 (m, 2H), 1.84-1.94 (m, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 3.65 (s, 3H), 7.83-7.85 (m, 2H), 8.79-8.81 (m, 2H), 13.01 (s, 1H).

5.1.3.26. Methyl 7-((5-(furan-2-yl)-1,3,4-thiadiazol-2-yl)amino)-7-oxoheptanoate (112)

Yield: 60%, mp: 179-181 °C (EtOAc). ESI-MS m/z: 324.4 $[M+H]^+$; ^{1}H NMR ($CDCl_3$) δ 1.45-1.55 (m, 2H), 1.67-1.77 (m, 2H), 1.80-1.90 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 3.65 (s, 3H), 6.57-6.59 (m, 1H), 7.07-7.08 (dd, *J1* = 3.6 Hz, *J2* = 0.6 Hz, 1H), 7.59-7.60 (m, 1H), 12.77 (s, 1H).

5.1.3.27. Methyl 7-oxo-7-((5-(thiophen-2-yl)-1,3,4-thiadiazol-2-yl)amino)heptanoate (113)

Yield: 49%, mp: 175-177 °C (EtOAc). ESI-MS m/z: 340.4 $[M+H]^+$; ^{1}H NMR ($CDCl_3$) δ 1.48-1.58 (m, 2H), 1.69-1.79 (m, 2H), 1.81-1.91 (m, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 2.80 (t, *J* = 7.5 Hz, 2H), 3.64 (s, 3H), 7.12-7.15 (m, 1H), 7.46-7.48 (dd, *J1* = 5.1 Hz, *J2* = 1.2 Hz, 1H), 7.50-7.51 (dd, *J1* = 3.6 Hz, *J2* = 0.9 Hz, 1H), 12.93 (s, 1H).

5.1.3.28. Methyl 8-((5-(naphthalen-1-yl)-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (114)

Yield: 77%, mp: 191-193 °C (EtOAc). ESI-MS m/z: 398.4 $[M+H]^+$; ^{1}H NMR ($CDCl_3$) δ 1.36-1.50 (m, 4H), 1.52-1.62 (m, 2H), 1.82-1.92 (m, 2H), 2.19 (t, *J* = 7.5 Hz, 2H), 2.87 (t, *J* = 7.5 Hz, 2H), 3.62 (s, 3H), 7.56-7.62 (m, 3H), 7.83-7.86 (dd, *J1* = 7.2 Hz, *J2* = 1.2 Hz, 1H), 7.92-7.97 (m, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 8.76-8.08 (m, 1H), 13.19 (s, 1H).

5.1.3.29. Methyl

8-((5-(naphthalen-2-yl)-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (115)
Yield: 60%, mp: 199-200 °C (EtOAc). ESI-MS m/z: 398.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.41-1.57 (m, 4H), 1.63-1.73 (m, 2H), 1.84-1.94 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.85 (t, *J* = 7.5 Hz, 2H), 3.63 (s, 3H), 7.54-7.61 (m, 2H), 7.88-7.98 (m, 3H), 8.10-8.14 (m, 1H), 8.35 (s, 1H), 12.81 (s, 1H).

5.1.3.30. Methyl 8-oxo-8-((5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)amino)octanoate (116)
Yield: 42%, mp: 165-166 °C (EtOAc). ESI-MS m/z: 349.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.38-1.56 (m, 4H), 1.60-1.70 (m, 2H), 1.82-1.92 (m, 2H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.85 (t, *J* = 7.5 Hz, 2H), 3.64 (s, 3H), 7.45-7.50 (m, 1H), 8.23-8.27 (m, 1H), 8.73-8.75 (m, 1H), 9.19 (d, *J* = 1.8 Hz, 1H), 13.32 (s, 1H).

5.1.3.31. Methyl 8-oxo-8-((5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)amino)octanoate (117)
Yield: 50%, mp: 186-187 °C (EtOAc). ESI-MS m/z: 349.4 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.39-1.56 (m, 4H), 1.61-1.71 (m, 2H), 1.82-1.92 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.82 (t, *J* = 7.5 Hz, 2H), 3.65 (s, 3H), 7.81-7.83 (m, 2H), 8.78-8.80 (m, 2H), 12.89 (s, 1H).

5.1.3.32. Methyl 8-((5-(furan-2-yl)-1,3,4-thiadiazol-2-yl)amino)-8-oxooctanoate (118)
Yield: 67%, mp: 160-171 °C (EtOAc). ESI-MS m/z: 338.5 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.38-1.53 (m, 4H), 1.60-1.70 (m, 2H), 1.78-1.88 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 3.65 (s, 3H), 6.58-6.59 (m, 1H), 7.06-7.07 (dd, *J1* = 3.6 Hz, *J2* = 0.6 Hz, 1H), 7.60-7.61 (m, 1H), 12.82 (s, 1H).

5.1.3.33. Methyl 8-oxo-8-((5-(thiophen-2-yl)-1,3,4-thiadiazol-2-yl)amino)octanoate (119)
Yield: 62%, mp: 163-164 °C (EtOAc). ESI-MS m/z: 354.3 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.40-1.56 (m, 4H), 1.61-1.71 (m, 2H), 1.79-1.89 (m, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 3.65 (s, 3H), 7.13-7.15 (m, 1H), 7.48 (d, *J* = 5.1 Hz, 1H), 7.51 (d, *J* = 3.6 Hz, 1H), 12.90 (s, 1H).

5.1.4. N^1-hydroxy-N^7-(5-(4-morpholinophenyl)-1,3,4-thiadiazol-2-yl)heptanediamide (2)

Solution A was prepared by dissolution of hydroxylamine hydrochloride (4.67 g, 67 mmol) in methanol (24 mL). Potassium hydroxide (6.60 g, 100 mmol) was diluted in methanol (14 mL) to develop solution B, which was added dropwise to the solution A at 0 °C . The reaction mixture was stirred for 0.5 h at 0 °C and filtered to give a solution of hydroxylamine in methanol. **86** (0.42 g, 1 mmol) was dissolved in the solution of hydroxylamine in methanol (10 mL). The resulting mixture was stirred for 1 h at room temperature and then adjusted to pH 7 by concentrated HCl. After

concentration under reducd pressure, the residue was washed with water to afford 0.34 g of compound **2** as a white solid. Yield: 81%, mp: 239-240 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.22-1.32 (m, 2H), 1.46-1.66 (m, 4H), 1.95 (t, J = 7.2 Hz, 2H), 2.48 (t, J = 7.2 Hz, 2H), 3.23 (t, J = 4.5Hz, 4H), 3.75 (t, J = 4.5Hz, 4H), 7.03-7.06 (m, 2H), 7.76-7.79 (m, 2H), 8.65 (s, 1H), 10.32 (s, 1H), 12.46 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.32, 24.80, 28.05, 32.07, 34.70, 47.35, 65.87, 114.58, 120.28, 127.84, 152.32, 157.12, 161.83, 168.99, 171.26; HRMS (AP-ESI) m/z calcd for $C_{19}H_{25}N_5O_4S$ $[M+H]^+$ 420.1700, found: 420.1707.

The synthetic procedures of compounds **3-35** were the same as that described above.

5.1.4.1.
N^1-(5-(4-(dimethylamino)phenyl)-1,3,4-thiadiazol-2-yl)-N^7-hydroxyheptanediamide (3)

Yield: 98%, mp: 214-215 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.22-1.32 (m, 2H), 1.46-1.66 (m, 4H), 1.95 (t, J = 7.2 Hz, 2H), 2.44-2.52 (m, 2H), 2.99 (s, 6H), 6.79 (d, J = 9.0 Hz, 2H), 7.72 (d, J = 9.0 Hz, 2H), 8.64 (s, 1H), 10.32 (s, 1H), 12.40 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.33, 24.79, 28.05, 32.07, 34.70, 39.70, 111.98, 117.40, 127.89, 151.58, 156.64, 162.26, 168.97, 171.17; HRMS (AP-ESI) m/z calcd for $C_{17}H_{23}N_5O_3S$ $[M+H]^+$ 378.1594, found: 378.1594.

5.1.4.2.
N^1-hydroxy-N^7-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)heptanediamide (4)

Yield: 85%, mp: 212-213 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.22-1.32 (m, 2H), 1.46-1.67 (m, 4H), 1.95 (t, J = 7.2 Hz, 2H), 2.46-2.49 (m, 2H), 3.83 (s, 3H), 7.05-7.10 (m, 2H), 7.85-7.89 (m, 2H), 8.64 (s, 1H), 10.32 (s, 1H), 12.51 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.30, 24.79, 28.04, 32.07, 34.69, 55.35, 114.68, 122.75, 128.38, 157.63, 160.98, 161.43, 168.99, 171.35; HRMS (AP-ESI) m/z calcd for $C_{16}H_{20}N_4O_4S$ $[M+H]^+$ 365.1278, found: 365.1282.

5.1.4.3.
N^1-(5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazol-2-yl)-N^7-hydroxyheptanediamide (5)

Yield: 89%, mp: 213-215 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.22-1.32 (m, 2H), 1.46-1.66 (m, 4H), 1.95 (t, J = 7.5 Hz, 2H), 2.46-2.49 (m, 2H), 3.83 (s, 3H), 3.86 (s, 3H), 7.08 (d, J = 8.4 Hz, 1H), 7.43-7.46 (dd, $J1$ = 8.4 Hz, $J2$ = 2.1 Hz, 1H), 7.50 (d, J = 2.1 Hz, 1H), 8.65 (s, 1H), 10.32 (s, 1H), 12.54 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.30, 24.79, 28.04, 32.07, 34.69, 55.58, 55.64, 109.31, 112.02, 120.29, 122.81, 149.10, 150.79, 157.70, 161.62, 168.97, 171.37; HRMS (AP-ESI) m/z calcd for $C_{17}H_{22}N_4O_5S$ $[M+H]^+$ 395.1384, found: 395.1388.

5.1.4.4. N^1-hydroxy-N^7-(5-(p-tolyl)-1,3,4-thiadiazol-2-yl)heptanediamide (6)

Yield: 72%, mp: 201-202 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.22-1.32 (m, 2H), 1.46-1.67 (m, 4H), 1.95 (t, J = 7.2 Hz, 2H), 2.37 (s, 3H), 2.49 (t, J = 7.2 Hz, 2H),

7.34 (d, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.1 Hz, 2H), 8.64 (s, 1H), 10.32 (s, 1H), 12.55 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 20.90, 24.29, 24.79, 28.04, 32.07, 34.70, 126.75, 127.51, 129.82, 140.36, 157.97, 161.67, 168.99, 171.41; HRMS (AP-ESI) m/z calcd for $C_{16}H_{20}N_4O_3S$ $[M+H]^+$ 349.1329, found: 349.1334.

5.1.4.5.
N^1-(5-([1,1'-biphenyl]-4-yl)-1,3,4-thiadiazol-2-yl)-N^7-hydroxyheptanediamide (7)
Yield: 78%, mp: 254-256 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.24-1.34 (m, 2H), 1.48-1.58 (m, 2H), 1.58-1.68 (m, 2H), 1.96 (t, *J* = 7.5 Hz, 2H), 2.49-2.54 (m, 2H), 7.39-7.44 (m, 1H), 7.51 (t, *J* = 7.2 Hz, 2H), 7.74-7.77 (m, 2H), 7.84 (d, *J* = 8.4 Hz, 2H), 8.03 (d, *J* = 8.4 Hz, 2H), 8.66 (s, 1H), 10.33 (s, 1H), 12.58 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.82, 25.31, 28.57, 32.59, 35.29, 127.18, 127.91, 127.97, 128.54, 129.55, 129.77, 139.51, 142.47, 158.94, 161.73, 169.49, 172.09; HRMS (AP-ESI) m/z calcd for $C_{21}H_{22}N_4O_3S$ $[M+H]^+$ 411.1485, found: 411.1492.

5.1.4.6. N^1-(5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)-N^7-hydroxyheptanediamide (8)
Yield: 77%, mp: 299-302 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.23-1.33 (m, 2H), 1.47-1.67 (m, 4H), 1.95 (t, *J* = 7.5 Hz, 2H), 2.48-2.52 (m, 2H), 7.33-7.41 (m, 2H), 7.96-8.03 (m, 2H), 8.65 (s, 1H), 10.32 (s, 1H), 12.60 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.27, 24.78, 28.03, 32.06, 34.70, 116.20, 116.50, 126.82, 126.86, 129.09, 129.21, 158.38, 160.53, 161.60, 164.89, 168.98, 171.51; HRMS (AP-ESI) m/z calcd for $C_{15}H_{17}FN_4O_3S$ $[M+H]^+$ 353.1078, found: 353.1072.

5.1.4.7. N^1-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-N^7-hydroxyheptanediamide (9)
Yield: 81%, mp: 313-316 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.22-1.33 (m, 2H), 1.47-1.67 (m, 4H), 1.95 (t, *J* = 7.2 Hz, 2H), 2.48-2.53 (m, 2H), 7.57-7.62 (m, 2H), 7.94-7.98 (m, 2H), 8.65 (s, 1H), 10.32 (s, 1H), 12.63 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.26, 24.78, 28.03, 32.06, 34.72, 128.52, 129.10, 129.36, 135.05, 158.61, 160.46, 168.96, 171.57; HRMS (AP-ESI) m/z calcd for $C_{15}H_{17}ClN_4O_3S$ $[M+H]^+$ 369.0783, found: 369.0779.

5.1.4.8. N^1-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)-N^7-hydroxyheptanediamide (10)
Yield: 92%, mp: 285-288 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.23-1.33 (m, 2H), 1.47-1.57 (m, 2H), 1.57-1.67 (m, 2H), 1.95 (t, *J* = 7.2 Hz, 2H), 2.48-2.53 (m, 2H), 7.53-7.61 (m, 2H), 7.87-7.91 (dt, *J1* = 6.9 Hz, *J2* = 1.8 Hz, 1H), 7.99 (d, *J* = 1.8 Hz, 1H), 8.65 (s, 1H), 10.32 (s, 1H), 12.66 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.26, 24.78, 28.03, 32.06, 34.73, 125.71, 126.07, 130.21, 131.24, 132.17, 133.97, 158.84, 160.14, 168.96, 171.62; HRMS (AP-ESI) m/z calcd for $C_{15}H_{17}ClN_4O_3S$ $[M+H]^+$ 369.0783, found: 369.0785.

5.1.4.9. N^1-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)-N^7-hydroxyheptanediamide

(11)

Yield: 98%, mp: 210-212 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.23-1.33 (m, 2H), 1.47-1.57 (m, 2H), 1.57-1.67 (m, 2H), 1.96 (t, J = 7.5 Hz, 2H), 2.49-2.54 (m, 2H), 7.50-7.59 (m, 2H), 7.67-7.70 (m, 1H), 8.09-8.12 (m, 1H), 8.65 (d, J = 1.2 Hz, 1H), 10.32 (s, 1H), 12.67 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.25, 24.79, 28.03, 32.07, 34.64, 127.83, 128.99, 130.55, 130.83, 131.02, 131.74, 157.59, 159.92, 168.97, 171.64; HRMS (AP-ESI) m/z calcd for $C_{15}H_{17}ClN_4O_3S$ $[M+H]^+$ 369.0783, found: 369.0783.

5.1.4.10. N^1-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)-N^7-hydroxyheptanediamide (12)

Yield: 87%, mp: 318-322 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.23-1.32 (m, 2H), 1.47-1.67 (m, 4H), 1.95 (t, J = 7.5 Hz, 2H), 2.48-2.52 (m, 2H), 7.71-7.75 (m, 2H), 7.87-7.91 (m, 2H), 8.66 (s, 1H), 10.32 (s, 1H), 12.68 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.28, 24.79, 28.04, 32.07, 34.76, 123.75, 128.68, 129.46, 132.26, 158.71, 160.50, 168.97, 171.62; HRMS (AP-ESI) m/z calcd for $C_{15}H_{17}BrN_4O_3S$ $[M+H]^+$ 413.0278, found: 413.0270.

5.1.4.11. N^1-hydroxy-N^7-(5-(4-iodophenyl)-1,3,4-thiadiazol-2-yl)heptanediamide (13)

Yield: 80%, mp: 309-312 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.22-1.32 (m, 2H), 1.46-1.67 (m, 4H), 1.95 (t, J = 7.5 Hz, 2H), 2.47-2.52 (m, 2H), 7.70-7.75 (m, 2H), 7.88-7.92 (m, 2H), 8.64 (s, 1H), 10.32 (s, 1H), 12.61 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.27, 24.79, 28.03, 32.07, 34.74, 97.26, 128.57, 129.71, 138.08, 158.58, 160.79, 168.98, 171.58; HRMS (AP-ESI) m/z calcd for $C_{15}H_{17}IN_4O_3S$ $[M+H]^+$ 461.0139, found: 461.0140.

5.1.4.12. N^1-hydroxy-N^7-(5-(4-(methylsulfonyl)phenyl)-1,3,4-thiadiazol-2-yl)heptanediamide (14)

Yield: 69%, mp: 270-272 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.23-1.33 (m, 2H), 1.47-1.57 (m, 2H), 1.58-1.68 (m, 2H), 1.96 (t, J = 7.5 Hz, 2H), 2.50-2.54 (m, 2H), 3.29 (s, 3H), 8.05-8.08 (m, 2H), 8.20-8.23 (m, 2H), 8.65 (s, 1H), 10.32 (s, 1H), 12.73 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.27, 24.78, 28.03, 32.06, 34.77, 43.35, 127.62, 127.99, 134.72, 141.96, 159.37, 160.01, 168.97, 171.77; HRMS (AP-ESI) m/z calcd for $C_{16}H_{20}N_4O_5S_2$ $[M+H]^+$ 413.0948, found: 413.0952.

5.1.4.13. N^1-hydroxy-N^7-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)heptanediamide (15)

Yield: 95%, mp: 296-299 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.23-1.33 (m, 2H), 1.47-1.57 (m, 2H), 1.58-1.68 (m, 2H), 1.96 (t, J = 7.5 Hz, 2H), 2.53 (t, J = 7.5 Hz, 2H), 8.20-8.24 (m, 2H), 8.33-8.37 (m, 2H), 8.65 (s, 1H), 10.32 (s, 1H), 12.77 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.23, 24.77, 28.03, 32.06, 34.73,

124.47, 127.94, 136.02, 148.17, 159.58, 168.97, 171.76; HRMS (AP-ESI) m/z calcd for $C_{15}H_{17}N_5O_5S$ $[M+H]^+$ 380.1023, found: 380.1027.

5.1.4.14.
N^1-hydroxy-N^7-(5-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)heptanediamide (16)
Yield: 82%, mp: 283-286 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.23-1.33 (m, 2H), 1.47-1.57 (m, 2H), 1.58-1.68 (m, 2H), 1.95 (t, J = 7.2 Hz, 2H), 2.49-2.54 (m, 2H), 7.88-7.90 (m, 2H), 8.15-8.18 (m, 2H), 8.64 (s, 1H), 10.32 (s, 1H), 12.70 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.24, 24.78, 28.03, 32.06, 34.72, 122.06, 125.67, 126.15, 126.20, 127.54, 129.56, 129.98, 130.41, 130.84, 133.97, 159.10, 160.12, 168.98, 171.65; HRMS (AP-ESI) m/z calcd for $C_{16}H_{17}F_3N_4O_3S$ $[M+H]^+$ 403.1046, found: 403.1054.

5.1.4.15.
N^1-hydroxy-N^8-(5-(4-morpholinophenyl)-1,3,4-thiadiazol-2-yl)octanediamide (17)
Yield: 76%, mp: 224-226 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.27-1.28 (m, 4H), 1.44-1.54 (m, 2H), 1.56-1.63 (m, 2H), 1.94 (t, J = 7.2 Hz, 2H), 2.47 (t, J = 7.2 Hz, 2H), 3.23 (d, J = 4.8 Hz, 4H), 3.75 (d, J = 4.8 Hz, 4H), 7.04 (d, J = 8.7 Hz, 2H), 7.77 (d, J = 8.7 Hz, 2H), 8.64 (s, 1H), 10.31 (s, 1H), 12.44 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.47, 24.92, 28.20, 28.25, 32.18, 34.81, 47.37, 65.88, 114.59, 120.30, 127.84, 152.33, 157.16, 161.80, 169.04, 171.33; HRMS (AP-ESI) m/z calcd for $C_{20}H_{27}N_5O_4S$ $[M+H]^+$ 434.1857, found: 434.1864.

5.1.4.16.
N^1-(5-([1,1'-biphenyl]-4-yl)-1,3,4-thiadiazol-2-yl)-N^8-hydroxyoctanediamide (18)
Yield: 88%, mp: 265-266 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.28-1.29 (m, 4H), 1.45-1.54 (m, 2H), 1.58-1.65 (m, 2H), 1.95 (t, J = 7.5 Hz, 2H), 2.49-2.53 (m, 2H), 7.39-7.44 (m, 1H), 7.48-7.53 (m, 2H), 7.74-7.77 (m, 2H), 7.84 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 8.64 (s, 1H), 10.32 (s, 1H), 12.62 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.44, 24.92, 28.21, 28.25, 32.18, 34.83, 126.67, 127.41, 127.46, 128.04, 129.04, 129.23, 139.00, 141.98, 158.33, 161.27, 169.04, 171.57; HRMS (AP-ESI) m/z calcd for $C_{22}H_{24}N_4O_3S$ $[M+H]^+$ 425.1642, found: 425.1645.

5.1.4.17. N^1-(5-(3-bromophenyl)-1,3,4-thiadiazol-2-yl)-N^8-hydroxyoctanediamide (19)
Yield: 70%, mp: 245-247 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.28-1.29 (m, 4H), 1.45-1.54 (m, 2H), 1.57-1.64 (m, 2H), 1.94 (t, J = 7.5 Hz, 2H), 2.47-2.52 (m, 2H), 7.49 (t, J = 7.8 Hz, 1H), 7.70-7.73 (m, 1H), 7.92 (d, J = 7.8 Hz, 1H), 8.12 (t, J = 1.8 Hz, 1H), 8.63 (s, 1H), 10.32 (s, 1H), 12.63 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.45, 24.92, 28.20, 28.24, 32.18, 34.91, 122.40, 126.07, 128.86, 131.46, 132.44, 133.06, 159.07, 159.92, 169.04, 171.79; HRMS (AP-ESI) m/z calcd for $C_{16}H_{19}BrN_4O_3S$ $[M+H]^+$ 427.0434, found: 427.0439.

5.1.4.18. N^1-(5-(2-bromophenyl)-1,3,4-thiadiazol-2-yl)-N^8-hydroxyoctanediamide (20)

Yield: 80%, mp: 165-167 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.28-1.29 (m, 4H), 1.45-1.54 (m, 2H), 1.57-1.64 (m, 2H), 1.94 (t, *J* = 7.2 Hz, 2H), 2.49-2.54 (m, 2H), 7.45-7.50 (td, *J1* = 7.8 Hz, *J2* = 1.8 Hz, 1H), 7.53-7.58 (td, *J1* = 7.8 Hz, *J2* = 1.2 Hz, 1H), 7.83-7.86 (dd, *J1* = 7.8 Hz, *J2* = 1.2 Hz, 1H), 7.93-7.96 (dd, *J1* = 7.8 Hz, *J2* = 1.8 Hz, 1H), 8.63 (d, *J* = 1.2 Hz, 1H), 10.31 (s, 1H), 12.65 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.41, 24.92, 28.20, 28.25, 32.18, 34.75, 121.31, 128.19, 131.06, 131.63, 131.86, 133.76, 159.05, 159.72, 169.05, 171.67; HRMS (AP-ESI) m/z calcd for $C_{16}H_{19}BrN_4O_3S$ $[M+H]^+$ 427.0434, found: 427.0430.

5.1.4.19. N^1-hydroxy-N^8-(5-(4-iodophenyl)-1,3,4-thiadiazol-2-yl)octanediamide (21)

Yield: 89%, mp: 284-286 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.27-1.28 (m, 4H), 1.44-1.53 (m, 2H), 1.56-1.66 (m, 2H), 1.93 (t, *J* = 7.2 Hz, 2H), 2.47-2.51 (m, 2H), 7.70-7.75 (m, 2H), 7.88-7.92 (m, 2H), 8.63 (d, *J* = 1.5 Hz, 1H), 10.31 (s, 1H), 12.64 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.93, 25.42, 28.70, 28.75, 32.69, 35.32, 97.80, 129.10, 130.21, 138,61, 159.02, 161.34, 169.55, 172.10; HRMS (AP-ESI) m/z calcd for $C_{16}H_{19}IN_4O_3S$ $[M+H]^+$ 475.0295, found: 475.0284.

5.1.4.20.
N^1-hydroxy-N^8-(5-(4-(methylsulfonyl)phenyl)-1,3,4-thiadiazol-2-yl)octanediamide (22)

Yield: 66%, mp: 253-255 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.28-1.29 (m, 4H), 1.47-1.54 (m, 2H), 1.60-1.65 (m, 2H), 1.94 (t, *J* = 7.5 Hz, 2H), 2.50-2.54 (m, 2H), 3.29 (s, 3H), 8.05-8.08 (m, 2H), 8.20-8.23 (m, 2H), 8.64 (s, 1H), 10.31 (s, 1H), 12.75 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.41, 24.91, 28.18, 28.23, 32.18, 34.83, 43.35, 127.63, 127.98, 134.70, 141.97, 159.30, 160.05, 169.04, 171.76; HRMS (AP-ESI) m/z calcd for $C_{17}H_{22}N_4O_5S_2$ $[M+H]^+$ 427.1104, found: 427.1098.

5.1.4.21.
N^1-hydroxy-N^8-(5-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)octanediamide (23)

Yield: 86%, mp: 281-284 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.28-1.29 (m, 4H), 1.45-1.54 (m, 2H), 1.58-1.65 (m, 2H), 1.95 (t, *J* = 7.2 Hz, 2H), 2.50-2.54 (m, 2H), 7.89 (d, *J* = 8.1 Hz, 2H), 8.17 (d, *J* = 8.1 Hz, 2H), 8.64 (s, 1H), 10.32 (s, 1H), 12.71 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.40, 24.91, 28.19, 28.24, 32.17, 34.84, 122.08, 125.69, 126.13, 126.18, 126.23, 126.28, 127.56, 129.56, 129.99, 130.41, 130.84, 134.00, 159.16, 160.12, 169.03, 171.74; HRMS (AP-ESI) m/z calcd for $C_{17}H_{19}F_3N_4O_3S$ $[M+H]^+$ 417.1203, found: 417.1203.

5.1.4.22.
N^1-hydroxy-N^7-(5-(naphthalen-1-yl)-1,3,4-thiadiazol-2-yl)heptanediamide (24)

Yield: 99%, mp: 200-201 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.25-1.35 (m,

2H), 1.48-1.58 (m, 2H), 1.59-1.69 (m, 2H), 1.97 (t, *J* = 7.5 Hz, 2H), 2.53 (t, *J* = 7.5 Hz, 2H), 7.61-7.69 (m, 3H), 7.85 (d, *J* = 6.6 Hz, 1H), 8.04-8.09 (m, 1H), 8.12 (d, *J* = 8.1 Hz, 1H), 8.65-8.70 (m, 2H), 10.34 (s, 1H), 12.70 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.33, 24.80, 28.05, 32.07, 34.74, 125.30, 125.48, 126.62, 126.78, 127.58, 128.55, 129.22, 129.96, 130.69, 133.54, 158.69, 160.94, 168.96, 171.66; HRMS (AP-ESI) m/z calcd for $C_{19}H_{20}N_4O_3S$ $[M+H]^+$ 385.1329, found: 385.1323.

5.1.4.23.
N^1-hydroxy-N^7-(5-(naphthalen-2-yl)-1,3,4-thiadiazol-2-yl)heptanediamide (25)
Yield: 83%, mp: 209-210 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.25-1.34 (m, 2H), 1.48-1.58 (m, 2H), 1.59-1.69 (m, 2H), 1.97 (t, *J* = 7.2 Hz, 2H), 2.50-2.55 (m, 2H), 7.58-7.64 (m, 2H), 7.96-8.02 (m, 1H), 8.04-8.14 (m, 3H), 8.52 (s, 1H), 8.66 (s, 1H), 10.33 (s, 1H), 12.65 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.32, 24.79, 28.05, 32.08, 34.76, 123.54, 126.96, 127.03, 127.40, 127.69, 127.73, 128.54, 128.89, 132.86, 133.65, 158.38, 161.78, 168.98, 171.54; HRMS (AP-ESI) m/z calcd for $C_{19}H_{20}N_4O_3S$ $[M+H]^+$ 385.1329, found: 385.1334.

5.1.4.24. N^1-hydroxy-N^7-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)heptanediamide (26)
Yield: 86%, mp: 233-234 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.23-1.33 (m, 2H), 1.47-1.57 (m, 2H), 1.58-1.68 (m, 2H), 1.96 (t, *J* = 7.2 Hz, 2H), 2.49-2.54 (m, 2H), 7.55-7.59 (m, 1H), 8.31-8.35 (m, 1H), 8.65 (s, 1H), 8.69-8.71 (dd, *J1* = 4.8 Hz, *J2* = 1.5 Hz, 1H), 9.12 (d, *J* = 2.1 Hz, 1H), 10.32 (s, 1H), 12.69 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.26, 24.78, 28.02, 32.06, 34.71, 124.26, 126.48, 134.30, 147.37, 151.12, 158.89, 168.99, 171.64; HRMS (AP-ESI) m/z calcd for $C_{14}H_{17}N_5O_3S$ $[M+H]^+$ 336.1125, found: 336.1128.

5.1.4.25. N^1-hydroxy-N^7-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)heptanediamide (27)
Yield: 98%, mp: 230-231 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.23-1.33 (m, 2H), 1.47-1.57 (m, 2H), 1.58-1.68 (m, 2H), 1.96 (t, *J* = 7.2 Hz, 2H), 2.50-2.55 (m, 2H), 7.90-7.92 (m, 2H), 8.65 (s, 1H), 8.72-8.74 (m, 2H), 10.33 (s, 1H), 12.79 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.22, 24.77, 28.02, 32.06, 34.72, 120.75, 137.14, 150.69, 159.50, 159.53, 168.97, 171.75; HRMS (AP-ESI) m/z calcd for $C_{14}H_{17}N_5O_3S$ $[M+H]^+$ 336.1125, found: 336.1125.

5.1.4.26. N^1-(5-(furan-2-yl)-1,3,4-thiadiazol-2-yl)-N^7-hydroxyheptanediamide (28)
Yield: 65%, mp: 198-200 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.22-1.32 (m, 2H), 1.46-1.67 (m, 4H), 1.95 (t, *J* = 7.5 Hz, 2H), 2.47-2.52 (m, 2H), 6.72-6.74 (m, 1H), 7.19 (d, *J* = 3.0 Hz, 1H), 7.94 (d, *J* = 1.2 Hz, 1H), 8.64 (s, 1H), 10.32 (s, 1H), 12.67 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.21, 24.77, 28.02, 32.05, 34.66, 110.75, 112.50, 145.12, 145.31, 152.38, 157.56, 168.98, 171.54; HRMS (AP-ESI) m/z calcd for $C_{13}H_{16}N_4O_4S$ $[M+H]^+$ 325.0965, found: 325.0965.

5.1.4.27. N^1-hydroxy-N^7-(5-(thiophen-2-yl)-1,3,4-thiadiazol-2-yl)heptanediamide (29)

Yield: 88%, mp: 206-208 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.22-1.32 (m, 2H), 1.46-1.66 (m, 4H), 1.95 (t, *J* = 7.5 Hz, 2H), 2.49 (t, *J* = 7.5 Hz, 2H), 7.19-7.22 (m, 1H), 7.67-7.70 (dd, *J1* = 3.6 Hz, *J2* = 0.9 Hz, 1H), 7.75-7.77 (dd, *J1* = 5.1 Hz, *J2* = 0.9 Hz, 1H), 8.64 (s, 1H), 10.32 (s, 1H), 12.63 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.26, 24.78, 28.03, 32.06, 34.68, 128.35, 128.94, 129.07, 132.29, 156.05, 157.75, 168.97, 171.53; HRMS (AP-ESI) m/z calcd for $C_{13}H_{16}N_4O_3S_2$ $[M+H]^+$ 341.0737, found: 341.0748.

5.1.4.28. N^1-hydroxy-N^8-(5-(naphthalen-1-yl)-1,3,4-thiadiazol-2-yl)octanediamide (30)

Yield: 90%, mp: 201-203 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.30-1.31 (m, 4H), 1.46-1.55 (m, 2H), 1.59-1.66 (m, 2H), 1.95 (t, *J* = 7.5 Hz, 2H), 2.53 (t, *J* = 7.5 Hz, 2H), 7.61-7.69 (m, 3H), 7.87-7.90 (dd, *J1* = 6.9 Hz, *J2* = 0.9 Hz, 1H), 8.04-8.08 (m, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 8.65-8.69 (m, 2H), 10.32 (s, 1H), 12.66 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.48, 24.92, 28.21, 28.26, 32.19, 34.82, 125.30, 125.48, 126.62, 126.78, 127.58, 128.55, 129.22, 129.96, 130.70, 133.54, 158.67, 160.96, 169.05, 171.68; HRMS (AP-ESI) m/z calcd for $C_{20}H_{22}N_4O_3S$ $[M+H]^+$ 399.1485, found: 399.1490.

5.1.4.29. N^1-hydroxy-N^8-(5-(naphthalen-2-yl)-1,3,4-thiadiazol-2-yl)octanediamide (31)

Yield: 98%, mp: 237-239 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.29-1.30 (m, 4H), 1.46-1.55 (m, 2H), 1.59-1.66 (m, 2H), 1.95 (t, *J* = 7.2 Hz, 2H), 2.52 (t, *J* = 7.2 Hz, 2H), 7.58-7.64 (m, 2H), 7.97-8.02 (m, 1H), 8.04-8.14 (m, 3H), 8.51 (s, 1H), 8.67 (s, 1H), 10.33 (s, 1H), 12.63 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.99, 25.44, 28.73, 28.77, 32.70, 35.41, 124.04, 127.44, 127.52, 127.89, 128.23, 129.04, 129.39, 133.37, 134.15, 158.99, 162.23, 169.56, 172.14; HRMS (AP-ESI) m/z calcd for $C_{20}H_{22}N_4O_3S$ $[M+H]^+$ 399.1485, found: 399.1490.

5.1.4.30. N^1-hydroxy-N^8-(5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl)octanediamide (32)

Yield: 86%, mp: 197-198 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.28-1.29 (m, 4H), 1.45-1.54 (m, 2H), 1.58-1.65 (m, 2H), 1.95 (t, *J* = 7.5 Hz, 2H), 2.51 (t, *J* = 7.5 Hz, 2H), 7.55-7.59 (m, 1H), 8.31-8.35 (m, 1H), 8.64 (s, 1H), 8.69-8.71 (dd, *J* = 4.8 Hz, *J* = 1.2 Hz, 1H), 9.12 (d, *J* = 1.8 Hz, 1H), 10.32 (s, 1H), 12.69 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.41, 24.91, 28.18, 28.24, 32.18, 34.81, 124.26, 126.49, 134.30, 147.38, 151.13, 158.89, 169.04, 171.68; HRMS (AP-ESI) m/z calcd for $C_{15}H_{19}N_5O_3S$ $[M+H]^+$ 350.1281, found: 350.1275.

5.1.4.31. N^1-hydroxy-N^8-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)octanediamide (33)

Yield: 97%, mp: 248-250 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.28-1.29 (m,

4H), 1.45-1.54 (m, 2H), 1.58-1.65 (m, 2H), 1.95 (t, *J* = 7.5 Hz, 2H), 2.52 (t, *J* = 7.5 Hz, 2H), 7.90-7.92 (m, 2H), 8.64 (s, 1H), 8.72-8.74 (m, 2H), 10.32 (s, 1H), 12.65 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.38, 24.91, 28.18, 28.23, 32.17, 34.82, 120.75, 137.15, 150.69, 159.52, 169.05, 171.79; HRMS (AP-ESI) m/z calcd for $C_{15}H_{19}N_5O_3S$ $[M+H]^+$ 350.1281, found: 350.1289.

5.1.4.32. N^1-(5-(furan-2-yl)-1,3,4-thiadiazol-2-yl)-N^8-hydroxyoctanediamide (34)

Yield: 98%, mp: 203-204 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.27-1.28 (m, 4H), 1.44-1.54 (m, 2H), 1.56-1.63 (m, 2H), 1.94 (t, *J* = 7.2 Hz, 2H), 2.47-2.52 (m, 2H), 6.72-6.73 (m, 1H), 7.18 (d, *J* = 3.3 Hz, 1H), 7.94 (d, *J* = 1.5 Hz, 1H), 8.63 (s, 1H), 10.31 (s, 1H), 12.63 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.41, 24.91, 28.19, 28.23, 32.17, 34.80, 128.32, 128.89, 129.02, 132.32, 156.02, 157.82, 169.06, 171.59; HRMS (AP-ESI) m/z calcd for $C_{14}H_{18}N_4O_4S$ $[M+H]^+$ 339.1122, found: 339.1130.

5.1.4.33. N^1-hydroxy-N^8-(5-(thiophen-2-yl)-1,3,4-thiadiazol-2-yl)octanediamide (35)

Yield: 87%, mp: 205-206 °C. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 1.27-1.29 (m, 4H), 1.44-1.54 (m, 2H), 1.56-1.63 (m, 2H), 1.94 (t, *J* = 7.5 Hz, 2H), 2.47-2.51 (m, 2H), 7.19-7.22 (m, 1H), 7.68-7.70 (dd, *J1* = 3.6 Hz, *J2* = 0.9 Hz, 1H), 7.74-7.76 (dd, *J1* = 5.1 Hz, *J2* = 0.9 Hz, 1H), 8.63 (s, 1H), 10.31 (s, 1H), 12.51 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 24.38, 24.90, 28.19, 28.23, 32.17, 34.80, 110.70, 112.49, 145.16, 145.27, 152.33, 157.69, 169.06, 171.65; HRMS (AP-ESI) m/z calcd for $C_{14}H_{18}N_4O_3S_2$ $[M+H]^+$ 355.0893, found: 355.0897.

5.2. In vitro HDAC assay

According to the instruction of Color de Lys assay kits, HeLa cell nuclear extracts (5 μL/well) and different concentrations of compounds (10 μL/well) were incubated at 37 ℃ for 5 minutes in the 96-well plate. After addition of Color de Lys substrate (25 μL), the resulting mixture reacted at 37 °C for 30 minutes. At the end Color de Lys Developer (50 μL/well) was added. After incubation for 15 minutes, enzymatic activity was measured in a microtiter-plate reader at 405 nm.

5.3. Molecular Docking

Surflex-Dock program in Sybyl 7.3 was used with default values except specially referred to. The structure of compound **33** was optimized using Powell's method and then assigned with AM1-BCC atomic charges. The active site was generated based on the ligand in the co-crystal structure of HDAC2-SAHA (PDB code: 4LXZ). The top-scored conformation was selected as the best docking result.

5.4. MTT Assay

MDA-MB-231 breast cancer cell, K562 chronic myelogenous leukaemia cell and PC3 prostate cancer cell were cultured in RPMI1640 medium (10% FBS) in 5% CO_2 humidified incubator at 37 °C. Cancer cells were plated at 5,000 cells per well in

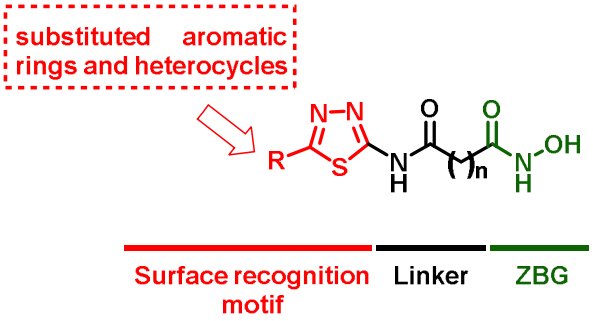
96-well plates and treated with 60, 20, 6.67, 2.22, 0.74, 0.25 μM of compounds for 48 h. After addition of 5 mg/mL MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl -2H-tetrazolium bromide) solution (20 μL) and further incubation for 4 h, DMSO (150 μL) was added for extraction of utiformazan. Antiproliferative activity was determined in a microtiter-plate reader at 570 nm.

Acknowledgments

This work was supported by National Natural Science Foundation China (No. 81373281), Shandong Natural Science Fund for Distinguished Young Scholars (No. JQ201319), the Program for New Century Excellent Talents in University (No. NCET-12-0337), Independent Innovation Foundation of Shandong University, IIFSDU (No. 2012JC003), and Program for Changjiang Scholars and Innovative Research Team in University, PCSIRT (No. IRT13028).

References

1. Minucci, S.; Pelicci, P. G. *Nat Rev Cancer* **2006,** *6*, 38.
2. Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat Rev Drug Discov* **2006,** *5*, 769.
3. West, A. C.; Johnstone, R. W. *J. Clin. Invest.* **2014,** *124*, 30.
4. Iwamoto, M.; Friedman, E. J.; Sandhu, P.; Agrawal, N. G. B.; Rubin, E. H.; Wagner, J. A. *Cancer Chemother. Pharmacol.* **2013,** *72*, 493.
5. Prince, H. M.; Dickinson, M.; Khot, A. *Future Oncol.* **2013,** *9*, 1819.
6. Guan, P.; Sun, F.; Hou, X.; Wang, F.; Yi, F.; Xu, W.; Fang, H. *Bioorg Med Chem* **2012,** *20*, 3865.
7. Qian, C.; Cai, X.; Zhai, H. WO2009036016A1, **2009**.
8. Haberland, M.; Johnson, A.; Mokalled, M. H.; Montgomery, R. L.; Olson, E. N. *Proc Natl Acad Sci U S A* **2009,** *106*, 7751.



	IC_{50} (μM)			
	HDACs	MDA-MB-231	K562	PC3
35	0.310	1.21	1.56	3.60
SAHA	0.416	2.29	1.61	5.79