Bioorganic & Medicinal Chemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of 4-anilinothieno[2,3-*d*]pyrimidine-based hydroxamic acid derivatives as novel histone deacetylase inhibitors

Wei Yang [a,†], Lixuan Li [b,c,†], Xun Ji [a], Xiaowei Wu [a], Mingbo Su [c], Li Sheng [c], Yi Zang [c], Jia Li [b,c,*], Hong Liu [a,*]

[a] CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, PR China
[b] East China Normal University, Institutes for Advanced Interdisciplinary Research, North Zhongshan Road Campus: 3663 N. Zhongshan Rd., Shanghai 200062, PR China
[c] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, SIBS, Chinese Academy of Sciences, Shanghai 201203, PR China

ARTICLE INFO

Article history:
Received 11 June 2014
Revised 22 August 2014
Accepted 26 August 2014
Available online xxxx

Keywords:
HDACs
HDACs inhibitor
Hydroxamic acid
Thieno[2,3-*d*]pyrimidines

ABSTRACT

A series of 4-anilinothieno[2,3-*d*]pyrimidine-based hydroxamic acid derivatives as novel HDACs inhibitors were designed, synthesized and evaluated. Most of these compounds displayed good to excellent inhibitory activities against HDAC1, 3, 6. The IC_{50} values of compound **10r** against HDAC1, HDAC3, HDAC6 was 1.14 ± 0.03 nM, 3.56 ± 0.08 nM, 11.43 ± 0.12 nM. Compound **10r** noticeably up-regulated the level of histone H3 acetylation compared to the SAHA. Most of the compounds showed the strong anti-proliferative activity against human cancer cell lines including RMPI8226 and HCT-116. The IC_{50} values of Compounds **10r** and **10t** against RPMI8226 was 2.39 ± 0.20 μM, 1.41 ± 0.44 μM, respectively, and the HCT-116 was sensitive to the compounds **10h**, **10m**, **10r**, **10w** with the IC_{50} values <1.9 μM.

1. Introduction

The deacetylation and acetylation of histones are important epigenetic modifications, which play a crucial role in controlling chromatin conformation.[1] The acetylation status of histones is determined by two sets of enzymes: histone deacetylases (HDACs) and histone acetyltransferases (HATs). HDACs catalyze the removal of acetyl groups from lysine residues in the *N*-terminal tails of core histones in the nucleosomes and increase the number of protonated lysines that bind closely to the negatively charged DNA phosphate groups leading to chromatin compaction, inaccessibility of transcription factors to DNA and alteration in gene expression.[2] There is growing evidence showing the gene expression controlled by epigenetic modifications is pivotal to the onset and progression of cancer; furthermore, the aberrant overexpression of HDACs have been found in many solid tumors and hematological malignancies.[3]

To date, 18 HDACs isoforms have been found in humans. Based on their homology to yeast HDACs, they can be divided into four classes.[4] Class I (HDAC1-3 and 8), II (HDAC 4–7, 9 and 10), and IV (HDAC11) are Zn^{2+}-dependent enzymes, whereas class III (sirtuins 1–7) are NAD^{+}-dependent enzymes. Given the critical role that HDACs play in tumor cell biology,[5] they have become a major field of research interest as an attractive potential anticancer target.[6] Many HDACs inhibitors have been developed and exhibited excellent therapeutic efficacy in both preclinical and clinical trials (Fig. 1). For example, SAHA[7] and FK228[8] have been approved by FDA for the treatment of cutaneous T-cell lymphoma. Additionally, compounds such as PXD101,[9] LBH589,[10] MS275,[11] and CUDC-101[12] are currently undergoing clinical trials. HDACs inhibitors induce histone hyperacetylation and (or) increase the acetylation

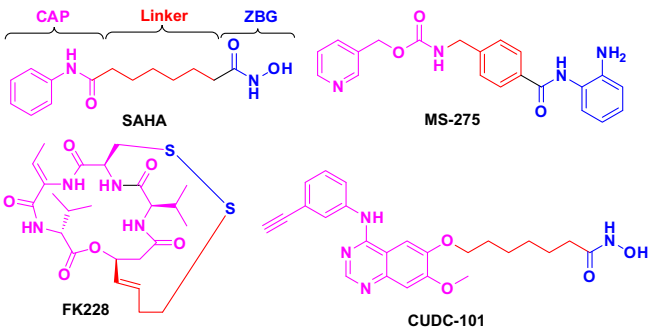


Figure 1. Approved and clinical HDACs inhibitors.

* Corresponding authors. Tel.: +86 21 50801552; fax: +86 21 50800721 (J.L.); tel./fax: +86 21 50807042 (H.L.).
E-mail addresses: jli@mail.shcnc.ac.cn (J. Li), hliu@mail.shcnc.ac.cn (H. Liu).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmc.2014.08.030
0968-0896/

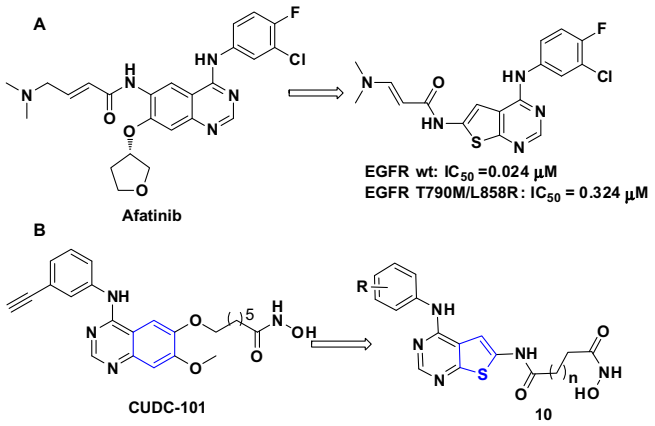
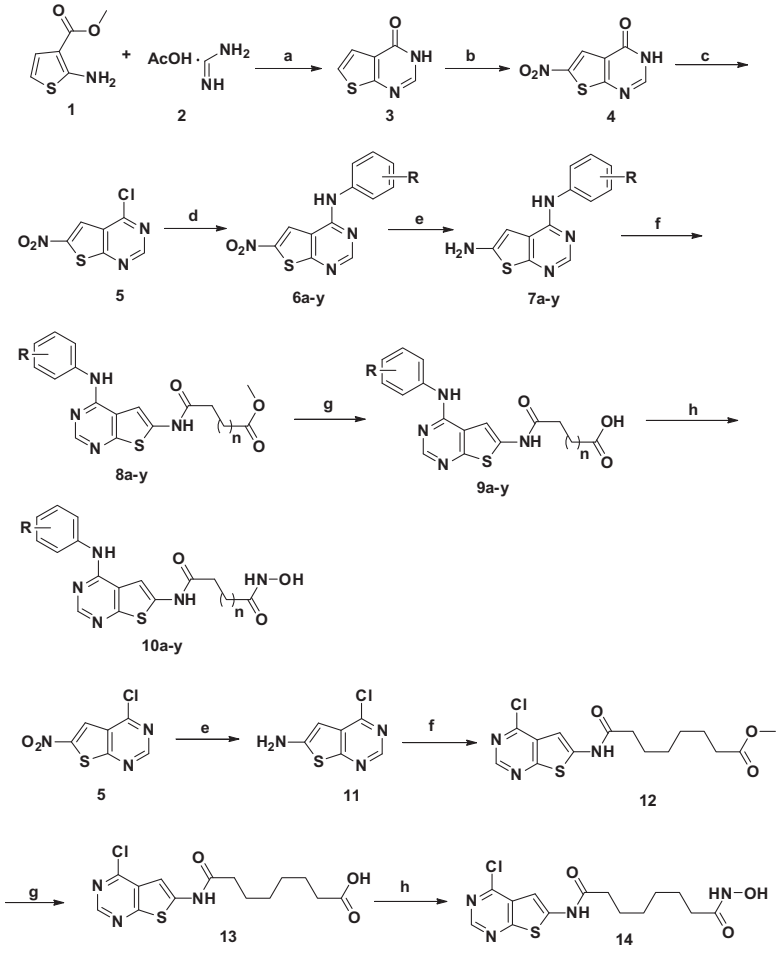


Figure 2. Design strategy and modification of novel HDACs inhibitors.

of nonhistone proteins, leading to cell growth arrest, differentiation and apoptosis.[13] An extensive number of studies have shown that HDACs inhibitors have additional promising potential therapeutic applications in the treatment of neurodegenerative diseases,[14] inflammation[15] and malaria.[16] Although diversity in the structures of HDACs inhibitors, they broadly conform to an accepted pharmacophore depicted by SAHA, that includes a cap group (CAP) for protein surface interactions, a zinc binding group (ZBG) that chelates with the zinc ion to repress the hydrolysis of acetyl group in the lysine residue, and a linker region that connects the CAP and ZBG[17] (Fig. 1).

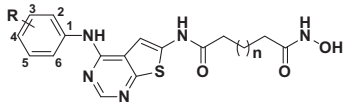
The thieno[2,3-*d*]pyrimidine fragment is widely present in antibacterial agents,[18] antioxidant agents[19] and antitumor agents.[20] In our previous studies, we have reported the replacement of the 4-anilinoquinazolin scaffold in afatinib structure with the 4-anilinothieno[2,3-*d*]pyrimidine fragment to design and synthesis of 6-alkenylamides substituted of 4-anilinothieno[2,3-*d*]pyrimidine derivatives as irreversible EGFR inhibitors that displayed excellent inhibitory activities against wild type and mutant EGFR[21] (Fig. 2A). Furthermore, many literatures have reported modifications of the quinazoline fragment with a thienopyrimidine structure.[22] CUDC-101 has been reported as a dual EGFR and HDACs inhibitor which contains a quinazoline moiety within its structure. On the basis of these observations, we attempted to substitute the 4-anilinoquinazoline fragment with a 4-anilinothieno[2,3-*d*]pyrimidine scaffold to design compounds **10** as novel HDACs inhibitors (Fig. 2B). In this paper, we presented the design, synthesis of thieno[2,3-*d*]pyrimidine-based novel HDACs inhibitors and their preliminary biological evaluation against HDACs and cancer cells. Most of these compounds showed excellent potencies against HDACs. Furthermore, some compounds displayed powerful antiproliferative activities against cancer cell lines in vitro.



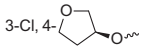
Scheme 1. Reagents and conditions: (a) microwave, 4–5 mins; (b) H_2SO_4/HNO_3, RT-90 °C, 1 h; (c) $POCl_3$, 110 °C or $POCl_3$, TEA, CH_3CN, 70 °C; (d) various substituted anilines, *i*-PrOH, 80 °C; (e) Fe, $NH_4Cl_{(aq)}$, EtOH, 45 °C; (f) $MeOCO(CH_2)nCOCl$, TEA, THF, n = 4, 5, 6; (g) LiOH, MeOH-THF, RT; (h) $NH_2OH.HCl$, DMAP, BOP, DMF, RT.

Please cite this article in press as: Yang, W.; et al. *Bioorg. Med. Chem.* (2014), http://dx.doi.org/10.1016/j.bmc.2014.08.030

Table 1
Structures and in vitro inhibitory activity against HDACs of compound **10** derivatives and compound **14**[a]



10a-y

Compd	R	*n*	IC_{50} (nM) ± SD		
			HDAC1	HDAC3	HDAC6
SAHA	—	—	93.34 ± 2.78	158.17 ± 6.66	78.98 ± 13.19
10a	3-Cl, 4-F	2	131.30 ± 25.21	126.56 ± 9.04	568.76 ± 45.38
10b	3-Cl, 4-F	3	35.89 ± 16.34	37.67 ± 1.61	23.99 ± 0.72
10c	3-Cl, 4-F	4	13.36 ± 1.48	17.91 ± 0.17	21.96 ± 1.42
10d	3-CF_3, 4-Cl	2	218.10 ± 55.35	607.03 ± 52.61	ND[b]
10e	3-CF_3, 4-Cl	3	40.84 ± 8.23	48.26 ± 1.78	30.00 ± 1.14
10f	3-CF_3, 4-Cl	4	21.67 ± 5.65	23.53 ± 2.00	11.49 ± 0.01
10g	2-F, 4-F	4	10.07 ± 0.53	16.85 ± 0.30	7.88 ± 0.22
10h	2-F, 4-Cl	4	12.50 ± 1.11	14.47 ± 0.08	5.58 ± 0.06
10i	2-F, 4-Br	4	13.54 ± 3.08	11.88 ± 0.86	8.87 ± 0.99
10j	2-F, 4-I	4	6.24 ± 1.59	13.52 ± 0.74	6.55 ± 0.13
10k	2-F, 3-Cl	4	7.78 ± 2.14	14.63 ± 1.20	8.46 ± 0.23
10l	3-Cl	4	3.24 ± 0.77	6.46 ± 0.01	8.58 ± 0.04
10n	H	3	11.77 ± 0.50	20.77 ± 0.64	26.99 ± 4.95
10m	H	4	1.90 ± 0.39	3.47 ± 0.26	12.04 ± 0.04
10o	4-CH_3	3	14.01 ± 1.32	9.33 ± 0.10	19.68 ± 1.96
10p	4-CH_3	4	2.01 ± 0.29	2.92 ± 0.06	5.37 ± 0.72
10q	3-CH_3, 4-CH_3	3	29.82 ± 11.51	14.74 ± 0.03	16.87 ± 3.02
10r	3-CH_3, 4-CH_3	4	1.14 ± 0.03	3.56 ± 0.08	11.43 ± 0.12
10s	4- $N(Et)_2$	4	32.87 ± 7.35	39.72 ± 1.62	43.16 ± 0.73
10t	3-F, 4-$N(Et)_2$	4	4.57 ± 0.04	3.52 ± 0.02	3.44 ± 0.06
10u	3-F, 4-$N(CH_3)_2$	4	4.51 ± 0.03	3.22 ± 0.10	2.69 ± 0.01
10v	3-Cl, 4- 	4	2.97 ± 0.07	3.58 ± 0.00	1.90 ± 0.03
10w	3-F,4- 	4	5.39 ± 0.07	8.19 ± 0.24	3.42 ± 0.07
10x	3-F, 4- 	4	14.49 ± 0.85	14.05 ± 0.74	9.28 ± 0.03
10y	4- 	4	13.85 ± 0.55	14.10 ± 0.34	7.19 ± 0.08
14	—	4	25.53 ± 0.15	26.53 ± 0.80	15.10 ± 0.28

[a] IC_{50} values were obtained based on three separate experiments and expressed as means ± SD.
[b] ND indicated no data was obtained.

Table 2
Antiproliferative activities of representative compounds against RPMI 8826 and HCT116 cell lines[a]

Compd	IC_{50} (μM) ± SD	
	RPMI8226	HCT-116
10g	7.49 ± 1.54	ND[b]
10h	3.50 ± 0.29	1.70 ± 0.56
10i	8.12 ± 0.36	ND[b]
10k	8.17 ± 0.45	ND[b]
10l	4.49 ± 0.41	ND[b]
10m	3.79 ± 0.22	1.82 ± 0.76
10p	3.84 ± 0.03	2.34 ± 0.29
10r	2.39 ± 0.20	1.61 ± 0.48
10s	5.95 ± 0.62	4.44 ± 0.96
10t	1.41 ± 0.44	2.55 ± 0.22
10u	3.49 ± 0.28	4.03 ± 0.62
10v	4.20 ± 0.50	2.24 ± 0.70
10w	3.28 ± 0.53	1.44 ± 0.39
10x	4.63 ± 0.47	4.09 ± 0.80
10y	5.42 ± 0.62	3.74 ± 0.41
14	2.18 ± 0.98	2.92 ± 0.15
SAHA	2.39 ± 0.08	3.45 ± 0.20

[a] IC_{50} values were obtained based on three separate experiments and expressed as means ± SD.
[b] ND indicated no data was obtained.

2. Results and discussion

2.1. Chemistry

The route for synthesizing compounds **10** and compound **14** is depicted in Scheme 1 The thieno[2,3-*d*]pyrimidine scaffold **3** was obtained via the cyclization of methyl 2-aminothiophene-3-carboxylate with formamidine acetate assisted by microwave. Then, the nitrification of intermediate **3**, followed by chlorination, treatment with various substituted anilines and reduction of the nitro group afforded the key intermediate **7**, which reacted with acyl chloride to form intermediate **8**. Hydrolysis with lithium hydroxide in a methanol solution gave the acid intermediate **9**, which subsequently was coupled with hydroxylamine hydrochloride in the presence of BOP and DMAP to afford the targeted compounds **10**. Reduction of intermediate **5**, followed by treatment with acyl chloride, formed the intermediate **12** which was subsequently hydrolyzed by lithium hydroxide to give acid intermediate **13**. Intermediate **13** was coupled with hydroxylamine hydrochloride to give the targeted compound **14**.

2.2. In vitro HDAC inhibitory activity

We initially evaluated the inhibitory activities of compounds **10a–10y** and **14** against recombinant human HDAC1, HDAC3,

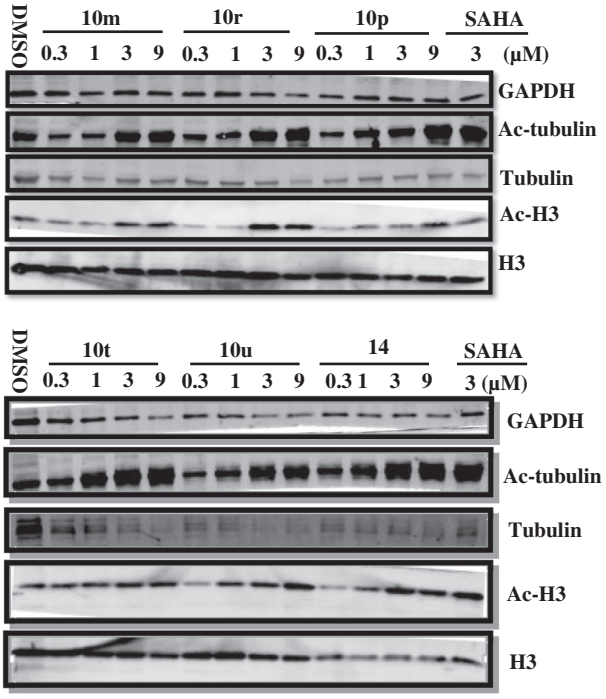


Figure 3. Western blot analysis of Ac-tubulin and Ac-histone H3 from RPMI 8226 cell cultured for 24 h with DMSO control and compounds **10m**, **10r**, **10p**, **10t**, **10u**, **14**and SAHA.

and HDAC6 isoforms using SAHA as positive control. As shown in Table 1, most of the compounds exhibited excellent inhibitory activities against HDAC1, HDAC3 and HDAC6 which indicated the 4-anilinothieno[2,3-*d*]pyrimidine scaffold was an outstanding capping group possessing strong binding affinity with the HDACs surface. Furthermore, these findings confirmed that the design strategy was rational and successful. Increasing the length of the carbon side chain between the two amides increased the inhibitory activities against HDACs. The good to excellent inhibitory activities were obtained when the carbon chain length reached five or six carbons (n = 3 or 4, **10b–c**, **10e–f** vs **10a**, **10d**); moreover, the optimal carbon chain length was six (n = 4) which gave the best inhibition with the minimum IC_{50} values of 1.14 ± 0.03 nM in HDAC1 (compound **10r**), 2.92 ± 0.06 nM in HDAC3 (compound **10p**), and 1.90 ± 0.03 nM in HDAC6 (compound **10v**), respectively. Based on the previous report that halogen-containing substituents on the phenyl ring in the CUDC-101 displayed excellent inhibitory activities,[12] we introduced halogen or halogen-containing groups into the phenyl ring to test their effects on inhibitory activities against HDACs. We found that most of these compounds (**10b–c**, **10e–l**) showed strong potential activities against HDACs with IC_{50} values <50 nM. While, compared to compound **10m** which had an IC_{50} value in the single-digit nanomolar range, these compounds had weaker inhibition on HDACs indicating that the halogen substituents were detrimental to potency. Introduction of a methyl group into the phenyl ring (compounds **10p** and **10r**) displayed similar activities with compound **10m**. In order to improve the aqueous solubility, some polar functional groups were introduced into the phenyl ring (compounds **10s–y**). Most of these compounds had excellent HDACs inhibitory activities for the IC_{50} values, of which compounds **10t, 10u, 10v and 10w** against HDAC1, HDAC3, and HDAC6 were all below 9 nM. When removal of the 4-aniline fragment from the **10** derivatives, compound **14** showed poor inhibitory activity than compounds **10m**, **10p**, **10r**, **10v**. This observed result proved that the presence of the 4-aniline fragment could dramatically increase the lipophilic interaction with HDACs to afford excellent inhibitory activities against HDACs.

2.3. In vitro inhibition against cancer cells

To further characterize these compounds, the antiproliferative activities of the thieno[2,3-*d*]pyrimidine-based HDAC inhibitors were assessed in two cancer cell lines: the human multiple myeloma cell line, RPMI8226 and the colon cancer cell line, HCT116. The results are summarized in Table 2. Compounds **10g**, **10i and 10k** showed poor antiproliferative activities probably due to potentially limited membrane permeability. On the contrary, compound **10h** showed good activities against both RPMI 8226 and HCT-116 cell lines. Compounds **10r** (IC_{50} = 2.39 ± 0.20 μM) and **10t** (IC_{50} = 1.41 ± 0.44 μM) showed similar antiproliferative activities against RPMI 8226 cells with SAHA, while the other compounds displayed less antiproliferative activities than SAHA. Compared to the RPMI 8226 cancer cells, the HCT116 cells appeared to be more sensitive to our synthesized compounds. Most compounds such as **10h**, **10m**, **10r**, **10p**, **10t**, **10v** and **10w** exhibited higher antiproliferative potency against HCT116 cell than SAHA. Compound **10w** inhibited the HCT-116 cell proliferation most effectively with the IC_{50} value of 1.44 ± 0.39 μM. Compound **14** possessed a slightly higher activities against both RPMI8226 and HCT116 cells than SAHA.

2.4. Analysis of the levels of Ac-tubulin and Ac-histone H3 in RPMI8226 cells by western blotting

Next, some representative compounds were selected to validate the observed biochemical potencies by assessing their effects on

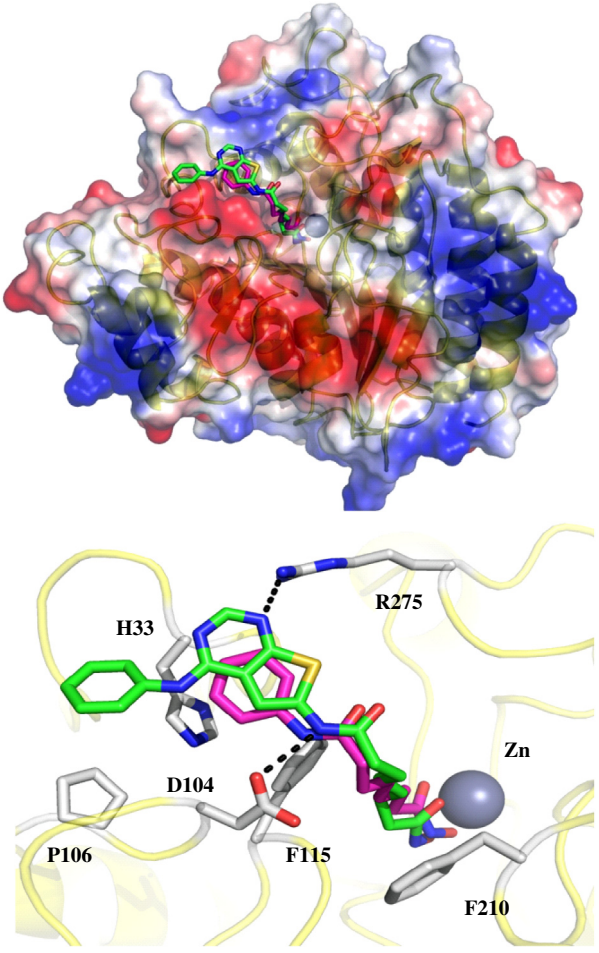


Figure 4. Molecular docking of compound **10m** (green) and SAHA (purple) in the active site of HDAC2.

the levels of α-tubulin and histone H3 acetylation in the multiple myeloma cancer cell line RPMI8226 (Fig. 3). These proteins are important biomarkers that are associated with intracellular HDACs inhibition. Western blot analysis revealed that compounds **10m**, **10r, 10p, 10t, 10u** and **14** dramatically increased the acetylation of α-tubulin and histone H3 in a dose-dependent manner, which was consistent with their inhibitory activities against HDACs and cancer cells. Compound **10r** noticeably up-regulated the level of histone H3 acetylation compared to SAHA at the same concentration. Based on their ability to up-regulate histone H3 and α-tubulin acetylation, our designed compounds, like the reference compound SAHA, were pan-HDACs inhibitors.

2.5. Molecular docking

In order to understand the interaction of these inhibitors and HDACs, we docked the compound **10m** and SAHA in the active site of HDAC2 (PDB code: 4LXZ) using AutoDock4.2[23,24] (Fig. 4). The results showed that compound **10m** displayed a similar binding mode to SAHA in the active site of HDAC2. The hydroxamic acid of the compound **10m** could chelate Zn^{2+} and the nitrogen atom in the pyrimidine ring could form hydrogen bond with R275. In addition, the phenyl moiety could hydrophobically interact with P106 and H33 that could enhance the binding affinity.

3. Conclusion

In summary, we used 4-anilinothieno[2,3-*d*]pyrimidine scaffold to modify the dual inhibitor CUDC-101 to design, synthesize and evaluate compound **10** derivatives and compound **14** as novel HDAC inhibitors. The biological evaluations indicated that when the length of the carbon side chain reached six carbon units, most compounds exhibited excellent inhibitory potencies against HDACs. Some representative compounds could markedly inhibit the proliferation of RPMI 8226 and HCT116 cancer cell lines with low IC_{50} values. These results proved the 4-anilinothieno[2,3-*d*]pyrimidine segment to be a good capping group. Compounds **10t** and **10w** showed the strongest antiproliferative activity against RPMI8226 and HCT116 cancer cells lines respectively. These present results suggested that 4-anilinothieno[2,3-*d*]pyrimidine-based hydroxamic acid derivatives could be lead compounds for further optimization to develop novel anticancer agents.

4. Experimental section

4.1. Chemistry

The reagents (chemicals) were commercially available and used without further purification. Analytical thin-layer chromatography (TLC) was performed on HSGF 254 (0.15–0.2 mm thickness). Column chromatography was performed on silica gel 300–400 mesh to purify the compounds. Nuclear magnetic resonance (NMR) spectra were performed on a Brucker AMX-400 (TMS as IS). Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LRMS and HRMS) were given with electric, electrospray and matrix-assisted laser desorption ionization (EI, ESI and MALDI) produced by Finnigan MAT-95, LCQ-DECA spectrometer and IonSpec4.7 T.

4.2. General procedures for synthesis of HDACs inhibitors

4.2.1. Thieno[2,3-*d*]pyrimidin-4-ol (3)

A mixture of compound **1** (20 g, 0.127 mol) and formimidamide acetate **2** (16 g, 0.153 mol) was reacted in the condition of microwave for 4–5 min. After cooled to RT, water was added and the solid was precipitate, then filtered, and the cake was washed with water, dried under infrared light to obtain the desired compound **3**, 17.2 g as gray solid in 89% yield. ESI-MS *m/z* 153 $[M+H]^+$.

4.2.2. 6-Nitrothieno[2,3-*d*]pyrimidin-4-ol (4)

To a mixture of H_2SO_4 (20 mL) and HNO_3 (20 mL) was added compound **3** (20 g, 0.131 mol) slowly under ice-bath condition within 50 min. Then, the reaction mixture was heated to 90 °C and stirred for 2 h. When the staring material was consumed, the mixture was poured into the ice-water carefully, and the yellow solid was formed, followed by filtration. The cake was washed with water for three times, dried to afford the desired compound **4**, 20.5 g as yellow solid in 80 yield. ESI-MS *m/z* 198 $[M+H]^+$.

4.2.3. 4-Chloro-6-nitrothieno[2,3-*d*]pyrimidine (5)

To a solution of compound **4** (10 g, 0.051 mol) in CH_3CN (100 mL) was added $POCl_3$ (14 mL, 0.15 mol) and TEA (21 mL, 0.15 mol). Then the mixture was heated to 70 °C for 3 h. After the start material was completed, the mixture was poured into ice-water, and extracted with EA, washed by $NaHCO_3$ solution, brine, dried by Na_2SO_4, concentrated and purified by flash silica gel column (0–100% EA in PE gradient) to obtain desired compound **5**, 8.22 g in 75% yield. ESI-MS *m/z* 216 $[M+H]^+$.

4.3. General process for synthesis of intermediate 6 derivatives

4.3.1. *N*-(3-Chloro-4-fluorophenyl)-6-nitrothieno[2,3-*d*]pyrimidin-4-amine 6a

To a solution of compound **5** (10 g, 0.046 mol) in *i*-PrOH (50 mL) was added 3-chloro-4-fluoroaniline (7.43 g, 0.051 mol) at room temperature. Subsequently, the mixture was heated to 80 °C and stirred for 2 h. When the start material was consumed monitored by TLC, the reaction was cooled to room temperature. After removal of the solvent, the residue was suspended in ethyl acetate, filtered and washed with ethyl acetate to afford the intermediate **6**, 14.3 g as yellow solid in a 95% yield. ESI-MS *m/z* 325 $[M+H]^+$.

The other substituted anilines was treated with intermediate **5** for preparation of intermediates **6b–y** according to the same procedure described for **6a**.

4.4. General process for synthesis of intermediates 7a–y

4.4.1. N^4-(3-Chloro-4-fluorophenyl)thieno[2,3-*d*]pyrimidine-4,6-diamine 7a

To a solution of intermediate **6a** (10 g, 0.03 mol) in ethanol (50 mL) was added iron powder (8.4 g, 0.15 mol) and saturated NH_4Cl solution (50 mL). The mixture was stirred at 50 °C for 1 h. Then, the mixture was filtered through celite, washed with ethanol followed by the water was added to the mixture to give the solid. Then the solid was filtered, washed and dried to afford the intermediate **7a**, 6.35 g as off-white solid in 70% yield. ESI-MS *m/z* 295 $[M+H]^+$.

The other nitro intermediates **6b–y** was reduced to intermediates **7b–y** according to the same procedure described for **7a**.

4.5. General process for synthesis of intermediates 8a–y

4.5.1. Methyl-6-((4-((4-chloro-3-fluorophenyl)amino)thieno[2,3-d]pyrimidin-6-yl)-amino)-6-oxohexanoate 8a

To a solution of **7a** (147 mg, 0.5 mmol) in dry THF was added methyl-6-chloro-6-oxohexanoate (133 mg, 0.75 mmol) and DIPEA (129 mg, 1 mmol) at 0 °C. Then the mixture was stirred at room temperature overnight. After the start material was consumed, saturated NH_4Cl solution was added to quench the reaction and extracted by EA three times. The organic phase was washed by water and NaCl(aq) respectively, dried by Na_2SO_4, concentrated and

purified by flash silica gel column (0–45% EA in PE gradient) to obtain desired production **8a** as an oil in 75% yield. ESI-MS *m/z* 437 $[M+H]^+$.

The other intermediates **8b–y** was prepared according to the same procedure described for **8a**.

4.6. General process for synthesis of intermediates 9a–y

4.6.1. 6-((4-((4-Chloro-3-fluorophenyl)amino)thieno[2,3-d]pyrimidin-6-yl)amino)-6-oxohexanoic acid 9a

To a solution of **8a** (218 mg, 0.5 mmol) in MeOH was added LiOH H_2O (105 mg, 2.5 mmol), the mixture was stirred at room temperature overnight, followed by concentrated. The residue dissolved in water, extracted by EA. Acetic acid was added to the water phase to form precipitate. Filter and wash the solid to give desired product **9a** in 50% yield. The crude product was used directly to next step without purification. ESI-MS *m/z* 423 $[M+H]^+$.

The other intermediates **9b–y** was prepared according to the same procedure described for **9a**.

4.7. General process for synthesis of the targeted compounds 10a–y

4.7.1. N^1-(4-((4-chloro-3-fluorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^6-hydroxyadipamide 10a

To a solution of 9a (211 mg, 0.5 mmol) in DMF solution was added Hydroxylamine hydrochloride (52 mg, 0.75 mmol), DMAP (122 mg, 1 mmol), BOP (442 mg, 1 mmol). Then, the mixture was stirred at room temperature until the start material was consumed completely. Water was added into the mixture, filtered the formed solid and washed with water. The crude product was purified by flash silica gel column (0–10% MeOH in DCM) to give the targeted compound **10a** as solid in 86% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.62 (s, 1H), 10.40 (s, 1H), 9.66 (s, 1H), 8.70 (s, 1H), 8.45 (s, 1H), 8.20 (s, 1H), 7.80 (s, 1H), 7.49–7.36 (m, 1H), 7.32 (s, 1H), 2.45 (t, J = 6.6 Hz, 2H), 2.00 (t, J = 6.6 Hz, 2H), 1.55–1.60 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 170.95, 169.35, 161.87, 154.11, 153.16, 152.50, 151.89, 137.46, 137.37, 122.71, 121.69, 121.65, 119.33, 119.21, 117.14, 116.99, 115.41, 100.59, 35.40, 32.58, 25.28, 24.97. ESI-MS *m/z* 438 $[M+H]^+$. HRMS calcd for $C_{18}H_{18}ClFN_5O_3S$ $[M+H]^+$ 438.0803, found 438.0808.

4.7.2. N^1-(4-((4-Chloro-3-fluorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^7-hydroxyheptanediamide 10b

Compound **10b** was prepared from **9b** according the same process described for **10a** in 87% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.62 (s, 1H), 10.36 (s, 1H), 9.67 (s, 1H), 8.68 (s, 1H), 8.45 (s, 1H), 8.20 (s, 1H), 7.81 (s, 1H), 7.42 (t, J = 8.8 Hz, 1H), 7.32 (s, 1H), 2.44 (t, J = 6.6 Hz, 2H), 1.97 (t, J = 6.6 Hz, 2H), 1.63–1.62 (m, 2H), 1.54–1.52 (m, 2H), 1.32–1.28 (m, 2H). ^{13}C NMR (151 MHz, DMSO) δ 171.05, 169.48, 161.87, 154.10, 153.16, 152.47, 151.88, 137.47, 137.40, 122.70, 121.68, 121.64, 119.32, 119.20, 117.13, 116.99, 115.42, 100.57, 35.48, 32.59, 28.66, 25.36, 25.01. ESI-MS *m/z* 452 $[M+H]^+$. HRMS calcd for $C_{19}H_{20}ClFN_5O_3S$ $[M+H]^+$ 452.0959, found 452.0962.

4.7.3. N^1-(4-((4-chloro-3-fluorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10c

Compound **10c** was prepared from **9c** according the same process described for **10a** in 84% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.66 (s, 1H), 10.36 (s, 1H), 9.69 (s, 1H), 8.69 (s, 1H), 8.45 (s, 1H), 8.21–8.16 (m, 1H), 7.86–7.81 (m, 1H), 7.41 (t, J = 9.1 Hz, 1H), 7.34 (s, 1H), 2.44 (t, J = 7.3 Hz, 2H), 1.95 (t, J = 7.3 Hz, 2H), 1.67–1.57 (m, 2H), 1.55–1.46 (m, 2H), 1.35–1.27 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 171.11, 169.54, 161.87, 154.09, 153.16, 152.49, 151.86, 137.47, 137.40, 122.69, 121.68, 121.63, 119.31, 119.19, 117.12, 116.98, 115.42, 100.60, 35.57, 32.69, 28.88, 28.82, 25.48, 25.20. ESI-MS *m/z* 466 $[M+H]^+$. HRMS calcd for $C_{20}H_{22}ClFN_5O_3S$ $[M+H]^+$ 466.1116, found 466.1120.

4.7.4. N^1-(4-((4-Chloro-3-(trifluoromethyl)phenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^6-hydroxyadipamide 10d

Compound **10d** was prepared from **9d** according the same process described for **10a** in 78% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.66 (s, 1H), 10.40 (s, 1H), 9.87 (s, 1H), 8.64 (s, 1H), 8.48 (s, 1H), 8.44 (s, 1H), 8.27 (dd, J = 8.8,1.6 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.34 (s, 1H), 2.45 (t, J = 6.8 Hz, 2H), 1.99 (t, J = 6.9 Hz, 2H), 1.67–1.49 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 171.00, 169.36, 162.15, 152.89, 151.68, 139.83, 137.68, 132.18, 127.03, 126.78, 125.64, 124.41, 123.31, 122.27, 119.60, 115.76, 100.49, 35.40, 32.57, 25.27, 24.95. ESI-MS *m/z* 488 $[M+H]^+$. HRMS calcd for $C_{19}H_{18}ClF_3N_5O_3S$ $[M+H]^+$ 488.0771, found 488.0775.

4.7.5. N^1-(4-((4-Chloro-3-(trifluoromethyl)phenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^7-hydroxyheptanediamide 10e

Compound **10e** was prepared from **9e** according the same process described for **10a** in 89% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.64 (s, 1H), 10.39 (s, 1H), 9.85 (s, 1H), 8.62 (s, 1H), 8.46 (s, 1H), 8.44 (s, 1H), 8.23 (dd, J = 8.8,1.6 Hz, 1H), 7.66 (d, J = 8.8 Hz, 1H), 7.34 (s, 1H), 2.43 (t, J = 6.8 Hz, 2H), 2.00 (t, J = 6.9 Hz, 2H), 1.64–1.62 (m, 2H), 1.58–1.49 (m, 2H), 1.33–1.31 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 171.00, 169.36, 162.15, 152.89, 151.68, 139.83, 137.68, 132.18, 127.03, 126.78, 125.64, 124.41, 123.31, 122.27, 119.60, 115.76, 100.49, 35.48, 32.59, 28.66, 25.36, 25.01. ESI-MS *m/z* 502 $[M+H]^+$. HRMS calcd for $C_{20}H_{20}ClF_3N_5O_3S$ $[M+H]^+$ 502.0927, found 502.0928.

4.7.6. N^1-(4-((4-Chloro-3-(trifluoromethyl)phenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10f

Compound **10f** was prepared from **9f** according the same process described for **10a** in 86% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.62 (s, 1H), 10.35 (s, 1H), 9.86 (s, 1H), 8.67 (s, 1H), 8.48 (s, 1H), 8.44 (s, 1H), 8.28 (d, J = 8.7 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.33 (s, 1H), 2.43 (t, J = 7.3 Hz, 2H), 1.94 (t, J = 7.2 Hz, 2H), 1.65–1.56 (m, 2H), 1.50–1.48 (m, 2H), 1.30–1.10 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 171.11, 169.54, 162.15, 152.89, 151.69, 139.87, 137.79, 132.10, 127.04, 126.76, 125.65, 124.45, 123.32, 122.28, 119.55, 115.77, 100.43, 35.61, 32.70,28.85, 28.83, 25.47, 25.10. ESI-MS *m/z* 516 $[M+H]^+$. HRMS calcd for $C_{21}H_{22}ClF_3N_5O_3S$ $[M+H]^+$ 516.1084, found 516.1088.

4.7.7. N^1-(4-((2,4-Difluorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10g

Compound **10g** was prepared from **9g** according the same process described for **10a** in 86% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.62 (s, 1H), 10.36 (s, 1H), 9.44 (s, 1H), 8.67 (s, 1H), 8.26 (s, 1H), 7.57 (m, 1H), 7.35 (m, 1H), 7.17 (s, 1H), 7.13 (m, 1H), 2.42 (t, J = 7.0 Hz, 2H), 1.95 (t, J = 7.1 Hz, 2H), 1.60–1.58 (m, 2H), 1.55–1.43 (m, 2H), 1.33–1.23 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 171.01, 169.55, 161.79, 159.22, 159.13, 158.39, 158.29, 156.41, 156.31, 154.43, 152.28, 137.19, 129.77, 129.69, 123.46, 123.37, 114.80, 111.81, 111.61, 105.12, 104.91, 104.71, 100.39, 35.57, 32.70, 28.83, 28.82, 25.48, 25.20. ESI-MS *m/z* 450 $[M+H]^+$. HRMS calcd for $C_{20}H_{22}F_2N_5O_3S$ $[M+H]^+$ 450.1411, found 450.1409.

4.7.8. N^1-(4-((4-Chloro-2-fluorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10h

Compound **10h** was prepared from **9h** according the same process described for **10a** in 84% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.55 (s, 1H), 10.35 (s, 1H), 9.47 (s, 1H), 8.68 (s, 1H), 8.29 (s, 1H),

7.63 (t, J = 8.5 Hz, 1H), 7.53 (dd, J = 10.2, 1.9 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.18 (s, 1H), 2.41 (t, J = 7.3 Hz, 2H), 1.95 (t, J = 7.3 Hz, 2H), 1.61 (m, 2H), 1.55–1.45 (m, 2H), 1.33–1.24 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 171.02, 169.56, 161.98, 157.74, 155.75, 153.99, 152.18, 137.34, 130.12, 130.05, 129.21, 126.24, 126.15, 125.00, 117.09, 116.90, 115.05, 100.34, 35.59, 32.70, 28.84, 28.82, 25.48, 25.18. ESI-MS m/z 466 [M+H]$^+$. HRMS calcd for $C_{20}H_{22}ClFN_5O_3S$ [M+H]$^+$ 466.1116, found 466.1112.

4.7.9. N^1-(4-((4-Bromo-2-fluorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10i

Compound **10h** was prepared from **9h** according the same process described for **10a** in 90% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.60 (s, 1H), 10.36 (s, 1H), 9.50 (s, 1H), 8.68 (s, 1H), 8.29 (s, 1H), 7.64 (d, J = 9.5 Hz, 1H), 7.57 (t, J = 8.1 Hz, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.19 (s, 1H), 2.42 (t, J = 6.5 Hz, 2H), 1.96 (t, J = 6.5 Hz, 2H), 1.62 (m, 2H), 1.56–1.48 (m, 2H), 1.33–1.25 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 170.98, 169.49, 161.99, 157.78, 155.79, 153.91, 152.16, 137.33, 137.32, 129.51, 127.92, 126.69, 126.59, 119.82, 119.63, 115.08, 100.37, 35.47, 32.58, 28.66, 25.35, 25.00. ESI-MS m/z 510[M+H]$^+$. HRMS calcd for $C_{20}H_{22}BrFN_5O_3S$ [M+H]$^+$ 510.0611, found 510.0612.

4.7.10. N^1-(4-((2-Fluoro-4-iodophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10j

Compound **10j** was prepared from **9j** according the same process described for **10a** in 86% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.54 (s, 1H), 10.34 (s, 1H), 9.44 (s, 1H), 8.67 (s, 1H), 8.28 (s, 1H), 7.72 (dd, J = 9.8, 1.7 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.41 (t, J = 8.3 Hz, 1H), 7.18 (s, 1H), 2.41 (t, J = 7.4 Hz, 2H), 1.94 (t, J = 7.3 Hz, 2H), 1.68–1.56 (m, 2H), 1.55–1.44 (m, 2H), 1.34–1.24 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 171.01, 169.54, 162.00, 157.62, 155.62, 153.88, 152.16, 137.33, 133.81, 129.71, 127.13, 127.04, 125.18, 125.00, 115.10, 100.36, 35.59, 32.70, 28.84, 28.82, 25.48, 25.18. ESI-MS m/z 558 [M+H]$^+$. HRMS calcd for $C_{20}H_{22}FIN_5O_3S$ [M+H]$^+$ 558.0472, found 558.0473.

4.7.11. N^1-(4-((3-Chloro-2-fluorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10k

Compound **10k** was prepared from **9k** according the same process described for **10a** in 70% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.65 (s, 1H), 10.37 (s, 1H), 9.63 (s, 1H), 8.68 (s, 1H), 8.31 (s, 1H), 7.56 (t, J = 6.3 Hz, 1H), 7.44 (t, J = 6.2 Hz, 1H), 7.25 (t, J = 7.7 Hz, 1H), 7.21 (s, 1H), 2.43 (t, J = 6.3 Hz, 2H), 1.95 (t, J = 6.3 Hz, 2H), 1.66–1.58 (m, 2H), 1.56–1.48 (m, 2H), 1.32–1.22 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 171.01, 169.50, 162.05, 153.91, 153.60, 152.19, 151.62, 137.41, 128.67, 128.58, 127.29, 126.94, 125.34, 125.30, 120.64, 120.51, 115.11, 100.38, 35.47, 32.58, 28.65, 28.63, 25.35, 25.01. ESI-MS m/z 466 [M+H]$^+$. HRMS calcd for $C_{20}H_{22}ClFN_5O_3S$ [M+H]$^+$ 466.1116, found 466.1118.

4.7.12. N^1-(4-((3-Chlorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10l

Compound **10l** was prepared from **9l** according the same process described for **10a** in 88% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.59 (s, 1H), 10.35 (s, 1H), 9.63 (s, 1H), 8.68 (s, 1H), 8.47 (s, 1H), 8.10 (s, 1H), 7.81 (d, J = 8.1 Hz, 1H), 7.38 (t, J = 8.1 Hz, 1H), 7.35 (s, 1H), 7.11 (d, J = 7.9 Hz, 1H), 2.43 (t, J = 7.3 Hz, 2H), 1.95 (t, J = 7.3 Hz, 2H), 1.64–1.60 (m, 2H), 1.53–1.48 (m, 2H), 1.35–1.25 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 171.10, 169.55, 161.98, 153.12, 151.87, 141.76, 137.41, 133.27, 130.58, 122.68, 120.54, 119.58, 115.60, 100.61, 35.60, 32.70, 28.84, 28.83, 25.48, 25.19. ESI-MS m/z 448[M+H]$^+$. HRMS calcd for $C_{20}H_{23}ClN_5O_3S$ [M+H]$^+$ 448.1210, found 448.1212.

4.7.13. N^1-Hydroxy-N^7-(4-(phenylamino)thieno[2,3-*d*]pyrimidin-6-yl)heptanediamide 10n

Compound **10n** was prepared from **9n** according the same process described for **10a** in 80% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.61 (s, 1H), 10.38 (s, 1H), 9.51 (s, 1H), 8.70 (s, 1H), 8.39 (s, 1H), 7.83 (d, J = 7.3 Hz, 2H), 7.39–7.34 (m, 3H), 7.07 (t, J = 6.3 Hz, 1H), 2.43 (t, J = 6.3 Hz, 2H), 1.97 (t, J = 6.3 Hz, 2H), 1.67–1.57 (m, 2H), 1.58–1.48 (m, 2H), 1.36–1.25 (m, 2H). ^{13}C NMR (151 MHz, DMSO) δ 171.00, 169.51, 161.72, 153.60, 152.07, 140.05, 137.00, 128.93, 123.30, 121.76, 115.32, 100.85, 35.47, 32.59, 28.66, 25.36, 25.04. ESI-MS m/z 400[M+H]$^+$. HRMS calcd for $C_{19}H_{22}N_5O_3S$ [M+H]$^+$ 400.1443, found 400.1440.

4.7.14. 21N^1-Hydroxy-N^8-(4-(phenylamino)thieno[2,3-*d*]pyrimidin-6-yl)octanediamide 10m

Compound **10m** was prepared from **9m** according the same process described for **10a** in 82% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.55 (s, 1H), 10.35 (s, 1H), 9.49 (s, 1H), 8.68 (s, 1H), 8.39 (s, 1H), 7.83 (d, J = 7.8 Hz, 2H), 7.36 (t, J = 7.5 Hz, 2H), 7.32 (s, 1H), 7.08 (t, J = 7.0 Hz, 1H), 2.43 (t, J = 7.0 Hz, 2H), 1.95 (t, J = 6.9 Hz, 2H), 1.68–1.56 (m, 2H), 1.54–1.46 (m, 2H), 1.35–1.27 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 171.07, 169.55, 161.72, 153.51, 152.08, 140.05, 137.00, 128.94, 123.37, 121.75, 115.32, 100.74, 35.59, 32.71, 28.85, 28.83, 25.49, 25.21. ESI-MS m/z 414 [M+H]$^+$. HRMS calcd for $C_{20}H_{24}N_5O_3S$ [M+H]$^+$ 414.1600, found 414.1604.

4.7.15. N^1-Hydroxy-N^7-(4-(p-tolylamino)thieno[2,3-*d*]pyrimidin-6-yl)heptanediamide 10o

Compound **10m** was prepared from **9m** according the same process described for **10a** in 87% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.55 (s, 1H), 10.36 (s, 1H), 9.42 (s, 1H), 8.69 (s, 1H), 8.36 (s, 1H), 7.69 (d, J = 8.0 Hz, 2H), 7.30 (s, 1H), 7.17 (d, J = 8.0 Hz, 2H), 2.43 (t, J = 7.2 Hz, 2H), 2.29 (s, 3H), 1.97 (t, J = 7.2 Hz, 2H), 1.67–1.58 (m, 2H), 1.58–1.48 (m, 2H), 1.35–1.26 (m, 2H). ^{13}C NMR (151 MHz, DMSO) δ 170.94, 169.48, 161.56, 153.70, 152.16, 137.42, 136.84, 132.40, 129.36, 121.95, 115.14, 100.84, 35.47, 32.59, 28.66, 25.36, 25.03, 20.97. ESI-MS m/z 414[M+H]$^+$. HRMS calcd for $C_{20}H_{24}N_5O_3S$ [M+H]$^+$ 414.1600, found 414.1598.

4.7.16. N^1-Hydroxy-N^8-(4-(p-tolylamino)thieno[2,3-*d*]pyrimidin-6-yl)octanediamide 10p

Compound **10p** was prepared from **9p** according the same process described for **10a** in 86% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.52 (s, 1H), 10.35 (s, 1H), 9.41 (s, 1H), 8.67 (s, 1H), 8.36 (s, 1H), 7.68 (d, J = 8.3 Hz, 2H), 7.29 (s, 1H), 7.17 (d, J = 8.2 Hz, 2H), 2.42 (t, J = 7.4 Hz, 2H), 2.30 (s, 3H), 1.95 (t, J = 7.3 Hz, 2H), 1.64–1.59 (m, 2H), 1.56–1.47 (m, 2H), 1.33–1.25 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 170.98, 169.54, 161.56, 153.70, 152.16, 137.42, 136.85, 132.41, 129.37, 122.00, 115.14, 100.82, 35.59, 32.70, 28.85, 28.83, 25.48, 25.21, 20.97. ESI-MS m/z 428 [M+H]$^+$. HRMS calcd for $C_{21}H_{26}N_5O_3S$ [M+H]$^+$ 428.1756, found 428.1760.

4.7.17. N^1-(4-((3,4-Dimethylphenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^7-hydroxyheptanediamide 10q

Compound **10q** was prepared from **9q** according the same process described for **10a** in 79% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.56 (s, 1H), 10.36 (s, 1H), 9.36 (s, 1H), 8.71 (s, 1H), 8.36 (s, 1H), 7.55 (m, 2H), 7.29 (s, 1H), 7.11 (d, J = 7.9 Hz, 1H), 2.43 (t, J = 7.2 Hz, 2H), 2.24 (s, 3H), 2.21 (s, 3H), 1.97 (t, J = 7.2 Hz, 2H), 1.61 (m, 2H), 1.54 (m, 2H), 1.30 (m, 2H). ^{13}C NMR (151 MHz, DMSO) δ 170.94, 169.49, 161.52, 153.77, 152.20, 137.64, 136.78, 136.46, 131.28, 129.84, 123.17, 119.57, 115.10, 100.86, 35.46, 32.59, 28.66, 25.36, 25.04, 20.12, 19.29. ESI-MS m/z 428 [M+H]$^+$. HRMS calcd for $C_{21}H_{26}N_5O_3S$ [M+H]$^+$ 428.1756, found 428.1760.

4.7.18. N^1-(4-((3,4-Dimethylphenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10r

Compound **10r** was prepared from **9r** according the same process described for **10a** in 79% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.52 (s, 1H), 10.35 (s, 1H), 9.34 (s, 1H), 8.68 (s, 1H), 8.36 (s, 1H), 7.55 (m, 2H), 7.28 (s, 1H), 7.11 (d, J = 8.0 Hz, 1H), 2.42 (t, J = 7.4 Hz, 2H), 2.24 (s, 3H), 2.21 (s, 3H), 1.95 (t, J = 7.3 Hz, 2H), 1.61 (m, 2H), 1.55–1.44 (m, 2H), 1.35–1.26 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 170.97, 169.55, 161.52, 153.76, 152.20, 137.64, 136.79, 136.50, 131.28, 129.84, 123.17, 119.56, 115.10, 100.84, 35.58, 32.70, 28.85, 28.83, 25.48, 25.21, 20.12, 19.29. ESI-MS m/z 442 [M+H]$^+$. HRMS calcd for $C_{22}H_{28}N_5O_3S$ [M+H]$^+$ 442.1913, found 442.1910.

4.7.19. N^1-(4-((4-(Diethylamino)phenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10s

Compound **10s** was prepared from **9s** according the same process described for **10a** in 81% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.50 (s, 1H), 10.37 (s, 1H), 9.22 (s, 1H), 8.70 (s, 1H), 8.26 (s, 1H), 7.46 (d, J = 8.6 Hz, 2H), 7.18 (s, 1H), 6.54 (d, J = 8.8 Hz, 2H), 3.11 (q, J = 7.0 Hz, 4H), 2.40 (t, J = 7.4 Hz, 2H), 1.97 (t, J = 7.3 Hz, 2H), 1.65–1.58 (m, 2H), 1.54–1.45 (m, 2H), 1.34–1.20 (m, 4H), 1.09 (t, J = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO) δ 170.88, 169.57, 161.08, 154.23, 152.32, 145.20, 136.30, 128.05, 124.53, 114.63, 111.82, 100.93, 46.80, 35.60, 32.70, 28.85, 28.83, 25.48, 25.20, 12.70. ESI-MS m/z 485 [M+H]$^+$. HRMS calcd for $C_{24}H_{33}N_6O_3S$ [M+H]$^+$ 485.2335, found 485.2330.

4.7.20. N^1-(4-((4-(Diethylamino)-3-fluorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10t

Compound **10t** was prepared from **9t** according the same process described for **10a** in 86% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.53 (s, 1H), 10.34 (s, 1H), 9.47 (s, 1H), 8.67 (s, 1H), 8.40 (s, 1H), 7.77 (m, 1H), 7.46 (d, J = 6.9 Hz, 1H), 7.28 (s, 1H), 7.05 (s, 1H), 3.13 (q, J = 7.0 Hz, 4H), 2.42 (t, J = 7.4 Hz, 2H), 1.94 (t, J = 7.3 Hz, 2H), 1.65–1.58 (m, 2H), 1.54–1.45 (m, 2H), 1.35–1.22 (m, 4H), 0.99 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 171.01, 169.55, 161.63, 156.48, 154.59, 153.34, 152.03, 137.09, 122.00, 117.43, 117.41, 115.26, 110.00, 109.76, 100.70, 46.20, 35.60, 32.70, 28.85, 28.83, 25.48, 25.20, 12.70. ESI-MS m/z 503 [M+H]$^+$. HRMS calcd for $C_{24}H_{32}FN_6O_3S$ [M+H]$^+$ 503.2241, found 503.2243.

4.7.21. N^1-(4-((4-(Dimethylamino)-3-fluorophenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10u

Compound **10u** was prepared from **9u** according the same process described for **10a** in 86% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.51 (s, 1H), 10.35 (s, 1H), 9.44 (s, 1H), 8.67 (s, 1H), 8.38 (s, 1H), 7.76 (m, 1H), 7.43 (d, J = 8.3 Hz, 1H), 7.27 (s, 1H), 6.97 (t, J = 9.5 Hz, 1H), 2.74 (s, 6H), 2.41 (t, J = 7.2 Hz, 2H), 1.95 (t, J = 7.2 Hz, 2H), 1.68–1.55 (m, 2H), 1.54–1.45 (m, 2H), 1.35–1.23 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 171.00, 169.50, 161.46, 155.18, 153.42, 153.25, 152.08, 137.01, 136.39, 136.32, 133.95, 133.86, 118.64, 117.65, 115.17, 110.13, 109.98, 100.71, 43.12, 35.60, 32.70, 28.85, 28.83, 25.48, 25.19. ESI-MS m/z 475[M+H]$^+$. HRMS calcd for $C_{22}H_{28}FN_6O_3S$ [M+H]$^+$ 475.1928, found 475.1930.

4.7.22. (S)-N^1-(4-((3-Chloro-4-((tetrahydrofuran-3-yl)oxy)phenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10v

Compound **10v** was prepared from **9v** according the same process described for **10a** in 70% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.52 (s, 1H), 10.35 (s, 1H), 9.34 (s, 1H), 8.68 (s, 1H), 8.36 (s, 1H), 7.55 (m, 2H), 7.28 (s, 1H), 7.11 (d, J = 8.0 Hz, 1H), 5.32 (m, 1H), 4.18–4.03 (m, 2H), 4.07–3.92 (m, 2H), 2.47–2.27 (m, 4H), 1.95 (t, J = 7.3 Hz, 2H), 1.61–1.58 (m, 2H), 1.55–1.44 (m, 2H), 1.35–1.26 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 170.97, 169.55, 161.52, 153.76, 152.20, 137.64, 136.79, 136.50, 131.28, 129.84, 123.17, 119.56, 115.10, 100.84, 80.3, 79.6, 67.5, 35.61, 32.69, 32.2, 28.85, 28.80, 25.45, 25.09. ESI-MS m/z 534 [M+H]$^+$. HRMS calcd for $C_{24}H_{29}ClN_5O_5S$ [M+H]$^+$ 534.1578, found 534.1572.

4.7.23. N^1-(4-((3-Fluoro-4-(piperidin-1-yl)phenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10w

Compound **10w** was prepared from **9w** according the same process described for **10a** in 70% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.56 (s, 1H), 10.35 (s, 1H), 9.47 (s, 1H), 8.67 (s, 1H), 8.39 (s, 1H), 7.78 (m, 1H), 7.46 (m, 1H), 7.29 (m, 1H), 7.02 (t, J = 9.0 Hz, 1H), 2.92 (m, 4H), 2.46 (t, J = 7.2 Hz, 2H), 1.99 (t, J = 7.2 Hz, 2H), 1.71–1.56 (m, 4H), 1.55–1.44 (m, 6H), 1.35–1.20 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 171.02, 169.55, 161.58, 155.83, 153.37, 152.06, 137.06, 136.68, 136.61, 134.87, 134.78, 119.66, 119.64, 117.51, 115.23, 109.88, 109.68, 100.74, 52.28, 35.59, 32.70, 28.85, 28.82, 26.23, 25.47, 25.20, 24.26. ESI-MS m/z 515[M+H]$^+$. HRMS calcd for $C_{25}H_{32}FN_6O_3S$ [M+H]$^+$ 515.2241, found 515.2243.

4.7.24. N^1-(4-((3-Fluoro-4-(pyrrolidin-1-yl)phenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 10x

Compound **10x** was prepared from **9x** according the same process described for **10a** in 75% yield. ^{1}H NMR (500 MHz, DMSO) δ 11.48 (s, 1H), 10.34 (s, 1H), 9.35 (s, 1H), 8.66 (s, 1H), 8.35 (s, 1H), 7.67 (m, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.24 (s, 1H), 6.75 (t, J = 9.3 Hz, 1H), 3.28 (m, 4H), 2.45 (t, J = 7.0 Hz, 2H), 1.98 (t, J = 7.0 Hz, 2H), 1.92–1.86 (m, 4H), 1.63–1.57 (m, 2H), 1.54–1.45 (m, 2H), 1.33–1.25 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 170.95, 169.55, 161.32, 153.51, 152.20, 150.28, 136.81, 133.58, 133.53, 130.60, 130.45, 118.37, 115.73, 115.68, 114.98, 110.78, 110.40, 100.75, 50.03, 35.59, 32.70, 28.85, 28.83, 25.48, 25.20, 25.01. ESI-MS m/z 501 [M+H]$^+$. HRMS calcd for $C_{24}H_{30}FN_6O_3S$ [M+H]$^+$ 501.2084, found 501.2080.

4.7.25. N^1-Hydroxy-N^8-(4-((4-(pyrrolidin-1-yl)phenyl)amino)thieno[2,3-*d*]pyrimidin-6-yl)octanediamide 10y

Compound **10y** was prepared from **9y** according the same process described for **10a** in 79% yield. ^{1}H NMR (600 MHz, DMSO) δ 11.50 (s, 1H), 10.37 (s, 1H), 9.22 (s, 1H), 8.70 (s, 1H), 8.26 (s, 1H), 7.46 (d, J = 8.6 Hz, 2H), 7.18 (s, 1H), 6.54 (d, J = 8.8 Hz, 2H), 3.38 (m, 4H), 2.41 (t, J = 7.3 Hz, 2H), 1.98–1.93 (m, 6H), 1.64–1.57 (m, 2H), 1.53–1.47 (m, 2H), 1.32–1.25 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 170.88, 169.57, 161.08, 154.23, 152.32, 145.20, 136.30, 128.05, 124.53, 114.63, 111.82, 100.93, 47977, 35.56, 32.70, 28.86, 28.83, 25.49, 25.41, 25.23. ESI-MS m/z 483 [M+H]$^+$. HRMS calcd for $C_{24}H_{31}N_6O_3S$ [M+H]$^+$ 483.2178, found 483.2180.

4.7.26. 4-Chlorothieno[2,3-*d*]pyrimidin-6-amine 11

To a solution of **5** (215 mg, 1 mmol) in EtOH was added Iron power (280 mg, 5 mmol) and saturated NH_4Cl solution (4 mL). The mixture was stirred at 50 °C for 1 h. Then, the mixture was filtered through celite, washed with ethanol, concentrated. The residue was purified by flash silica gel column (0–25% EA in PE gradient) to afford the intermediate 11 as an oil in 75% yield. ESI-MS m/z 186 [M+H]$^+$.

4.7.27. Methyl 8-((4-chlorothieno[2,3-*d*]pyrimidin-6-yl)amino)-8-oxooctanoate 12

Compound **12** was prepared according to the same procedure described for **8a–y** to give an oil, yield 78%. ESI-MS m/z 356 [M+H]$^+$.

4.7.28. 8-((4-Chlorothieno[2,3-*d*]pyrimidin-6-yl)amino)-8-oxooctanoic acid 13

Compound **13** was prepared according to the same procedure described for **9a–y** to give an white solid in 59% yield which was used without purification for next step. ESI-MS m/z 341 [M+H]$^+$.

4.7.29. N^1-(4-Chlorothieno[2,3-*d*]pyrimidin-6-yl)-N^8-hydroxyoctanediamide 14

Compound **14** was prepared according to the same process described for **10a** in 86% yield.^{1}H NMR (600 MHz, DMSO) δ 11.58 (s, 1H), 10.34 (s, 1H), 8.67 (s, 1H), 8.56 (s, 1H), 6.76 (s, 1H), 2.41 (t, J = 7.2 Hz, 2H), 1.95 (t, J = 7.2 Hz, 2H), 1.64–1.58 (m, 2H), 1.54–1.46 (m, 2H), 1.34–1.26 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 171.05, 169.55, 163.10, 162.26, 151.97, 138.99, 116.71, 98.75, 35.61, 32.69, 28.85, 28.80, 25.45, 25.09. ESI-MS m/z 357 $[M+H]^+$. HRMS calcd for $C_{14}H_{18}ClN_4O_3S$ $[M+H]^+$ 357.0788, found 357.0784.

4.8. Biological evaluation

4.8.1. Materials

Cell counting kit-8 was purchased from Dojindo, anti-histone H3 primary antibody (#4499) was purchased from Cell Signaling Technology Corporation, anti-tubulin primary antibody (#1878-1) was purchased from epitomics, anti-acetyl-histone H3primary antibody (#06-599) was purchased from Millipore, anti-acetyl-tubulin primary antibody (#T7451) was purchased from Sigma-Aldrich. Both anti-Rabbit and anti-mouse IgG (H+L), DyLight 800 labeled secondary antibodies were purchased from KPL (Kirkegaard & Perry Laboratories. Inc.), PVDF transfer membrane was purchased from Amersham Biosciences Corporation.

4.8.2. In vitro HDAC activity assay

All three full-length recombinant human HDACs (rhHDACs) 1, 3 and 6 were expressed in insect High5 cells using a baculoviral expression system, and all His_6-tagged and GST-fusion proteins was purified using Ni-NTA (QIAGEN). The deacetylase activity of rhHDACs 1 and 3 were assayed with a HDAC substrate (Ac-Lys-Tyr-Lys(-acetyl)-AMC), and HDAC6 was assayed with another HDAC substrate (Boc-Lys(-acetyl)-AMC). The total HDAC assay volume was 25 μl and all the assay components were diluted in Hepes buffer (25 mM Hepes, 137 mM NaCl, 2.7 mM KCl and 4.9 mM $MgCl_2$, pH 8.0). The reaction was carried out in black 384-well plates (OptiPlateTM-384F, PerkinElmer). In brief, the HDAC assay mixture contained the substrate (5–50 μM, 5 μl), rhHDAC isoforms (20–200 nM) and inhibitors (1 μl). Positive controls contained all the above components except the inhibitors. The negative controls contained neither enzymes nor inhibitors. The HDAC6 assay components were incubated at room temperature for 3 h, and HDAC1 or 3 were incubated for 24 h. The reaction was quenched with the addition of 25 μl Trypsin with the final concentration of 0.3125%. After 30 min incubation at room temperature, the 384 micro-well plates were read at wavelengths 355 nm (excitation) and 460 nm (emission) using Envision (PerkinElmer). Each experiment was done in triplicate.

4.8.3. Cell proliferation assay

Cell proliferation assay was determined with the Cell Counting Kit-8 (CCK-8) which utilized Dojindo's highly water-soluble tetrazolium salt. WST-8. WST-8 is reduced by dehydrogenases in cells to give an orange colored product (formazan) which is soluble in the tissue culture medium. The amount of the formazan dye generated by dehydrogenases in cells is directly proportional to the number of living cells. According to the manufacturer's instructions, cells were plated overnight on 96-well plates at 10,000 cells per well in RPMI1640 growth medium. Then, the cells were treated with the compounds at indicated concentrations (0.00064 μM, 0.032 μM, 0.16 μM, 0.8 μM, 4 μM and 20 μM) .After 72 h treatment, 10 μL of CCK-8 solution was added to each well. Plates were returned to the incubator and left in the dark for 3 hour. The absorbance was measured on a SpectraMax 340 microplate reader (Molecular Devices, USA) at 450 nm with a reference at 690 nm. Each experiment was done in triplicate

4.8.4. Western blot analysis

20–100 μg of protein per lane was loaded onto a 10% SDS-polyacrylamide gel and then transferred to a PVDF membrane. The membrane was incubated with the following primary antibodies: anti-tubulin, anti-ac tubulin, anti-actin, anti-H3 and anti-ac H3 at 4 °C overnight. Then, the membrane was washed with TBST three times and incubated with Anti-Rabbit or mouse IgG (H+L), DyLight 800 labeled secondary antibody for 60 min at room temperature. After three times washing with TBST, The immunoblots were visualized by Odyssey® Infrared Imaging System (LI-COR Biosciences).

Acknowledgements

We gratefully acknowledge financial support from the National Natural Science Foundation of China (21021063, 91229204, 81125023, 81173033 and 81025017), National S&T Major Projects (2012ZX09103101-072, 2012ZX09301001-005, and 2013ZX09507-001), Chinese Academy of Science 'strategic leader in science and technology projects' (XDA01040303), Program of Shanghai Subject Chief Scientist (12XD1407100, 13XD1404300), the National Science & Technology Major Project 'Key New Drug Creation and Manufacturing Program' of China (2014ZX09507-002).

References and notes

1. (a) Gallinari, P.; Di Marco, S.; Jones, P.; Pallaoro, M.; Steinkuhler, C. *Cell. Res.* **2007**, *17*, 195; (b) Grunstein, M. *Nature* **1997**, *389*, 349; (c) Kouzarides, T. *Cell* **2007**, *128*, 693; (d) Li, E. *Nat. Rev. Genet.* **2002**, *3*, 662.
2. Peserico, A.; Simone, C. *J. Biomed. Biotechnol.* **2011**, *2011*, 371832.
3. (a) Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat. Rev. Drug. Discov.* **2006**, *5*, 769; (b) Pandolfi, P. P. *Cancer Chemother. Pharmcol.* **2001**, *48*, S17; (c) Weichert, W. *Cancer Lett.* **2009**, *280*, 168; (d) Weichert, W.; Roske, A.; Gekeler, V.; Beckers, T.; Stephan, C.; Jung, K.; Fritzsche, F. R.; Niesporek, S.; Denkert, C.; Dietel, M.; Kristiansen, G. *Br. J. Cancer* **2008**, *98*, 604.
4. (a) De Ruijter, A. J. M.; Van Gennip, A. H.; Caron, H. N.; Kemp, S.; Van Kuilenburg, A. B. P. *Biochem. J.* **2003**, *370*, 737; (b) Gregoretti, I. V.; Lee, Y. M.; Goodson, H. V. *J. Mol. Biol.* **2004**, *338*, 17; (c) Michan, S.; Sinclair, D. *Biochem. J.* **2007**, *404*, 1.
5. Witt, O.; Deubzer, H. E.; Milde, T.; Oehme, I. *Cancer Lett.* **2009**, *277*, 8.
6. (a) Johnstone, R. W. *Nat. Rev. Drug Discov.* **2002**, *1*, 287; (b) Ropero, S.; Esteller, M. *Mol. Oncol.* **2007**, *1*, 19.
7. Butler, L. M.; Agus, D. B.; Scher, H. I.; Higgins, B.; Rose, A.; Cordon-Cardo, C.; Thaler, H. T.; Rifkind, R. A.; Marks, P. A.; Richon, V. M. *Cancer Res.* **2000**, *60*, 5165.
8. (a) Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K. H.; Nishiyama, N.; Nakajima, I.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. *Cancer Res.* **2002**, *62*, 4916; (b) Li, K. W.; Wu, J.; Xing, W. N.; Simon, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 7237.
9. Steele, N. L.; Plumb, J. A.; Vidal, L.; Tjornelund, J.; Knoblauch, P.; Rasmussen, A.; Ooi, C. E.; Buhl-Jensen, P.; Brown, R.; Evans, T. R. J.; DeBono, J. S. *Clin. Cancer Res.* **2008**, *14*, 804.
10. Prince, H. M.; Bishton, M. J.; Johnstone, R. W. *Future Oncol.* **2009**, *5*, 601.
11. (a) Parodi, S.; Santi, I.; Marani, E.; Casella, C.; Puppo, A.; Vercelli, M.; Stagnaro, E. *Arch. Environ. Occup. Health* **2014**, *69*, 139; (b) Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; Ando, T.; Suzuki, T.; Tsuruo, T.; Nakanishi, O. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4592; (c) Suzuki, T.; Ando, T.; Tsuchiya, K.; Fukazawa, N.; Saito, A.; Mariko, Y.; Yamashita, T.; Nakanishi, O. *J. Med. Chem.* **1999**, *42*, 3001.
12. Cai, X.; Zhai, H. X.; Wang, J.; Forrester, J.; Qu, H.; Yin, L.; Lai, C. J.; Bao, R. D.; Qian, C. G. *J. Med. Chem.* **2000**, *2010*, 53.
13. (a) Insinga, A.; Monestiroli, S.; Ronzoni, S.; Gelmetti, V.; Marchesi, F.; Viale, A.; Altucci, L.; Nervi, C.; Minucci, S.; Pelicci, P. G. *Nat. Med.* **2005**, *11*, 233; (b) Petrella, A.; Festa, M.; Ercolino, S. F.; Zerilli, M.; Stassi, G.; Solito, E.; Parente, L. *Cancer Biol. Ther.* **2006**, *5*, 643; (c) Xu, W. S.; Ngo, L.; Perez, G.; Dokmanovic, M.; Marks, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15540; (d) Zhao, Y.; Tan, J.; Zhuang, L.; Jiang, X.; Liu, E. T.; Yu, Q. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 16090.
14. (a) Burli, R. W.; Luckhurst, C. A.; Aziz, O.; Matthews, K. L.; Yates, D.; Lyons, K. A.; Beconi, M.; McAllister, G.; Breccia, P.; Stott, A. J.; Penrose, S. D.; Wall, M.; Lamers, M.; Leonard, P.; Muller, I.; Richardson, C. M.; Jarvis, R.; Stones, L.; Hughes, S.; Wishart, G.; Haughan, A. F.; O'Connell, C.; Mead, T.; McNeil, H.; Vann, J.; Mangette, J.; Maillard, M.; Beaumont, V.; Munoz-Sanjuan, I.; Dominguez, C. *J. Med. Chem.* **2013**, *56*, 9934; (b) Graff, J.; Tsai, L. H. *Annu. Rev. Pharmacol.* **2013**, *53*, 311; (c) Kazantsev, A. G.; Thompson, L. M. *Nat. Rev. Drug Discov.* **2008**, *7*, 854; (d) Sweatt, J. D. *Int. J. Dev. Neurosci.* **2010**, *28*, 639; (e) Yu, C. W.; Chang, P. T.; Hsin, L. W.; Chern, J. W. *J. Med. Chem.* **2013**, *56*, 6775.
15. Dekker, F. J.; van den Bosch, T.; Martin, N. I. *Drug Discov. Today* **2014**, *19*, 654.

16. (a) Andrews, K. T.; Haque, A.; Jones, M. K. Immunol. Cell Bio. **2012**, *90*, 66; (b) Sumanadasa, S. D. M.; Goodman, C. D.; Lucke, A. J.; Skinner-Adams, T.; Sahama, I.; Haque, A.; Do, T. A.; McFadden, G. I.; Fairlie, D. P.; Andrew, K. T. *Antimicrob. Agents Chemother.* **2012**, *56*, 3849; (c) Andrews, K. T.; Tran, T. N.; Wheatley, N. C.; Fairlie, D. P. *Curr. Top. Med. Chem.* **2009**, *9*, 292; (d) Mukherjee, P.; Pradhan, A.; Shah, F.; Tekwani, B. L.; Avery, M. A. *Bioorg. Med. Chem.* **2008**, *16*, 5254.
17. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188.
18. Dewal, M. B.; Wani, A. S.; Vidaillac, C.; Oupický, D.; Rybak, M. J.; Firestine, S. M. *Eur. J. Med. Chem.* **2012**, *51*, 145.
19. Kotaiah, Y.; Harikrishna, N.; Nagaraju, K.; Venkata, R. C. *Eur. J. Med. Chem.* **2012**, *58*, 340.
20. (a) Deng, X.; Okram, B.; Ding, Q.; Zhang, J.; Choi, Y.; Adrián, F. J.; Wojciechowski, A.; Zhang, G.; Che, J.; Bursulaya, B.; Cowan-Jacob, S. W.; Rummel, G.; Sim, T.; Gray, N. S. *J. Med. Chem.* **2010**, *53*, 6934; (b) Zhao, A.; Gao, X.; Wang, Y.; Ai, J.; Wang, Y.; Chen, Y.; Geng, M.; Zhang, A. *Bioorg. Med. Chem.* **2011**, *19*, 3906; (c) Rheault, T. R.; Caferro, T. R.; Dickerson, S. H.; Donaldson, K. H.; Gaul, M. D.; Goetz, A. S.; Mullin, R. J.; McDonald, O. B.; Petrov, K. G.; Rusnak, D. W.; Shewchuk, L. M.; Spehar, G. M.; Truesdale, A. T.; Vanderwall, D. E.; Wood, E. R.; Uehling, D. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 817.
21. Ji, X.; Peng, T.; Zhang, X.; Li, J.; Yang, W.; Tong, L.; Qu, R.; Jiang, H.; Ding, J.; Xie, H.; Liu, H. *Bioorg. Med. Chem.* **2014**, *22*, 2366.
22. (a) Verheijen, J. C.; Yu, K.; Toral-Barza, L.; Hollander, I.; Zask, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 375; (b) Phoujdar, M. S.; Kathiravan, M. K.; Bariwac, J. B.; Shah, A. K.; Jain, K. S. *Tetrahedron Lett.* **2008**, *49*, 1269; (c) Hubbard, R. D.; Dickerson, S. H.; Emerson, H. K.; Griffin, R. J.; Reno, M. J.; Hornberger, K. R.; Rusnak, D. W.; Wood, E. R.; Uehling, D. E.; Waterson, A. G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5738; (d) Boschelli, D. H.; Wu, B. Q.; Sosa, A. C. B.; Durutlic, H.; Ye, F.; Raifeld, Y.; Golas, J. M.; Boschelli, F. *J. Med. Chem.* **2004**, *47*, 6666.
23. Lauffer, B. E. L.; Mintzer, R.; Fong, R. N.; Mukund, S.; Tam, C.; Zilberleyb, I.; Flicke, B.; Ritscher, A.; Fedorowicz, G.; Vallero, R.; Neumann, L.; Koth, C. M.; Lupardus, P. J.; Kaminker, J. S.; Heise, C. E.; Steiner, P. *J. Biol. Chem.* **2013**, *37*, 26926.
24. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. *J. Comput. Chem.* **2009**, *30*, 2785.

Please cite this article in press as: Yang, W.; et al. *Bioorg. Med. Chem.* (2014), http://dx.doi.org/10.1016/j.bmc.2014.08.030