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Article

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Discovery of Novel Class I Histone Deacetylase Inhibitors with Promising *in vitro* and *in vivo* Antitumor Activities

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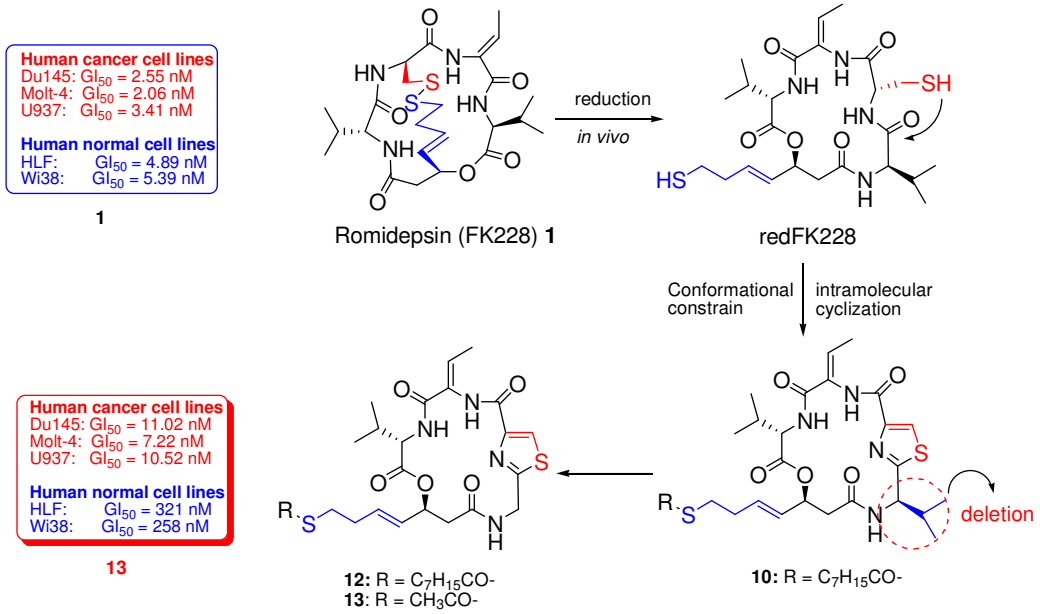
Abstract

A successful structure-based design of novel cyclic depsipeptides that selectively target class I HDAC isoforms is described. Compound **11** has an IC_{50} of 2.78 nM for binding to the HDAC1 protein and the pro-drugs **12** and **13** also exhibit promising antiproliferative activities in the nanomolar range against various cancer cell lines. Compounds **12** and **13** show more than 20-fold selectivity toward human cancer cells over human normal cells in comparison with Romidepsin (FK228) demonstrating low probability of toxic side effects. In addition, compound **13** exhibits excellent *in vivo* anticancer activities in a human prostate carcinoma (Du145) xenograft model with no observed toxicity. Thus, prodrug **13** has therapeutic potential as a new class of anticancer agent for further clinical translation.

Keywords: class I histone deacetylases, inhibitor, cyclic depsipeptides, macrocycles

Graphical abstract



Introduction

Traditional anticancer chemotherapeutics targeting DNA replication and cell division are effective but have systemic side effects due to the lack of selectivity toward cancer cells over normal cells. Development of a new generation of compounds that selectively kill cancer cells without affecting normal tissues and have increased potency and minimal toxic side effects is extremely urgent and necessary.[1] Epigenetics is classically defined as reversible changes in gene expression that are not a result of changes in DNA sequences such as genetic mutations or deletions. The epigenetic regulators of gene expression are cellular support systems and include transcription factors, RNA silencing, DNA methylation, histone modification, chromatin remodeling and nuclear architecture. Cancer cells exploit epigenetic processes to their advantage to support uncontrolled growth and abnormal epigenetic silencing of tumor suppressor genes is a hallmark of many cancers.[2] Currently, targeting epigenetic misregulations, which are as important as genetic defects in the origin of cancers have initiated a new era in cancer treatment. In particular, targeting histone modifiers such as histone deacetylases (HDAC) to restore the expression of tumor suppressor genes has shown clinical benefits. Histone deacetylases are a family of enzymes that catalyze the deacetylation of lysine side chains in chromatin, and these enzymes are involved in a wide range of biological processes, including cell differentiation, proliferation, angiogenesis, and apoptosis.[3-5]

So far, 18 members of the human HDAC family have been identified, and are divided into four distinct classes on the basis of their size, number of catalytically

active sites, subcellular locations, and sequence homology with respect to yeast counterparts.[6-9] Classes I HDACs (1, 2, 3, and 8), class IIa HDACs (4, 5, 7, and 9), class IIb HDACs (6 and 10), and the class IV HDAC (11) are Zn^{2+}-dependent proteases, while class III HDACs (sirtuins 1-7) are NAD^{+} -dependent Sir2-like deacetylases.[6] Class I HDAC isoforms have been intensively studied due to their importance in tumorigenesis and development. They are highly expressed in various cancers, including gastric, pancreatic, colorectal, and prostate cancers and hepatocellular carcinoma,[10-13] but not in resting endothelial cells and normal organs. Therefore, selectively targeting class I HDACs by directly inhibiting their function has recently become a major area of research in cancer therapy.[14-18]

Numerous structurally diverse HDACs inhibitors (HDACi) against Zn^{2+}-dependent HDACs have been exploited for a broad range of tumor indications.[19,20] Currently, over 12 HDACi are in clinical trials against different cancers,[8] and four of which, Romidepsin (FK228)[21,22] (**1**, Figure 1), SAHA (**2**, Figure 1),[23] Belinostat (**3**, Figure 1),[24] and Panobinostat (**4**, Figure 1) [25] have been approved by the US Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma (CTCL), peripheral T-cell lymphoma (PTCL) and multiple myeloma, respectively. Chidamide, an HDACi developed in China, has been recently approved by the China Food and Drug Administration for the treatment of PTCL.[25]

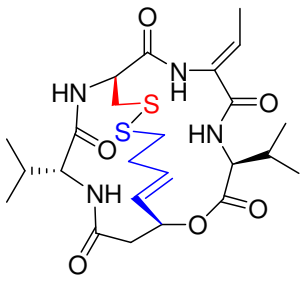
Due to the complexity and diversity of the chemical structures, macrocyclic HDAC inhibitors such as romidepsin, spiruchostatins, thailandepsin A and largazole, were capable of making numerous interactions with the outer rim of the enzyme and

demonstrated excellent HDAC affinity and isoform selectivity. A few reviews summarized natural HDAC inhibitors from the discovery to the synthesis of various analogues.[26-28] The most potent macrocyclic HDAC inhibitor is FK228 (romidepsin),[29] which was isolated from the fermentation broth of Chromobacterium Violaceum (No. 968) by Fujisawa Pharmaceutical Co., Ltd. in 1991. The total syntheses and SAR studies of FK228 were conducted by several research groups, providing additional insight into the biochemistry, and the toxicity of FK228.[30-33] Recently, largazole, isolated from the marine cyanobacterium *Symploca sp*. in 2008, showed promising HDAC1 inhibitory activity and selectivity.[34] The unique structural features and excellent biological properties of largazole have attracted significant attention from the synthetic chemists and medicinal chemists to obtain new molecules of higher potency or selectivity through the investigation of its structure activity relationship (SAR).[35] Mechanistic studies showed that FK228 and largazole are prodrugs due to the fact that the 4-heptenoic acid side chain may be hydrolyzed by cellular esterases or lipases *in vitro* and *in vivo*.[33,35a] The free thiol functional group could chelate with the Zn^{2+} cation of the HDACs, resulting in potent inhibitory effect.

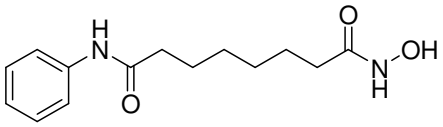
Romidepsin acts as a natural prodrug whose intramolecular disulfide bond is reduced *in vivo* by glutathione reductase to produce a reduced form whose butenyl thiol interacts with zinc ions in the binding pocket of class I histone deacetylases, thus obstructing access of the substrate.[36,37] Although HDACi, like romidepsin, showed promising results in the treatment of cancers of the blood such as CTCL and multiple myeloma, their application in solid tumors has been limited as a result of cardiac

toxicity and ineffective low concentrations in solid tumors.[14] For example, a phase II study of FK228 in gastrointestinal neuroendocrine tumors was recently terminated due to a sudden cardiac death.[38]

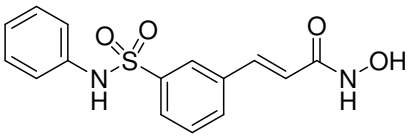
Development of HDACi with increased selectivity toward cancer cells over normal cells and consequent reduction of off-target toxicity is highly desirable. In this paper, we report the design of novel cyclic depsipeptides selectively targeting class I HDAC isoforms based on analysis of structure activity relationships (SAR) and a small library of analogs has been synthesized. The lead compound from this library exhibits potent class I HDAC selectivity, improved selectivity towards tumors over normal tissue and promising therapeutic effects in a human prostate carcinoma (Du145) xenograft model.



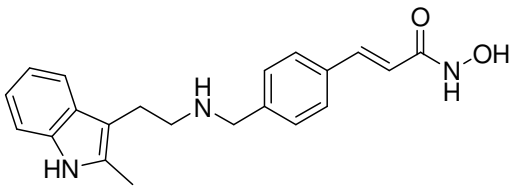
1: Romidepsin (FK228)
(approved in 2009)



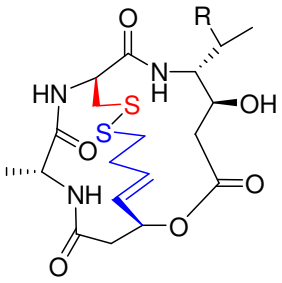
2: SAHA (approved in 2006)



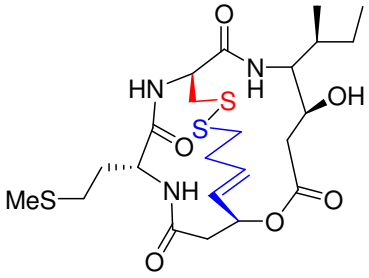
3: Belinostat (approved in 2014)



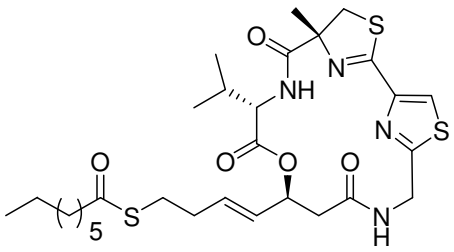
4: Panobinostat (approved in 2015)



5a: Spiruchostatin A (R = Me)
5b: Spiruchostatin B (R = Et)



6: Thailandepsin A



7: Largazole

Figure 1. Selected HDAC inhibitors.

Results and discussion

The basic design strategy for the development of selective class I HDAC isoform inhibitors based on FK228 is shown in Figure **2**. FK228 is converted to its active form

(redFK228) by reduction *in vivo* of the disulfide bond by glutathione reductase (Fig. 2). It has been shown that butenyl thiol group of redFK228 interacts reversibly with zinc ions localized in the active site of HDACs, and its cysteine residue seems to have a weak interaction with enzymes.[36] Our hypothesis is that the toxicity of FK228 may be caused by the relatively high nucleophilic reactivity of its cysteine residue, which may underlie the lack of selectivity of FK228 for cancer cells over normal cells. Accordingly, we designed a class of novel cyclic depsipeptides using a thiol cyclization strategy which removes the cysteine residue of redFK228 and potentially increases the selectivity toward cancer cells over normal cells thus reducing the toxicity associated with FK228.

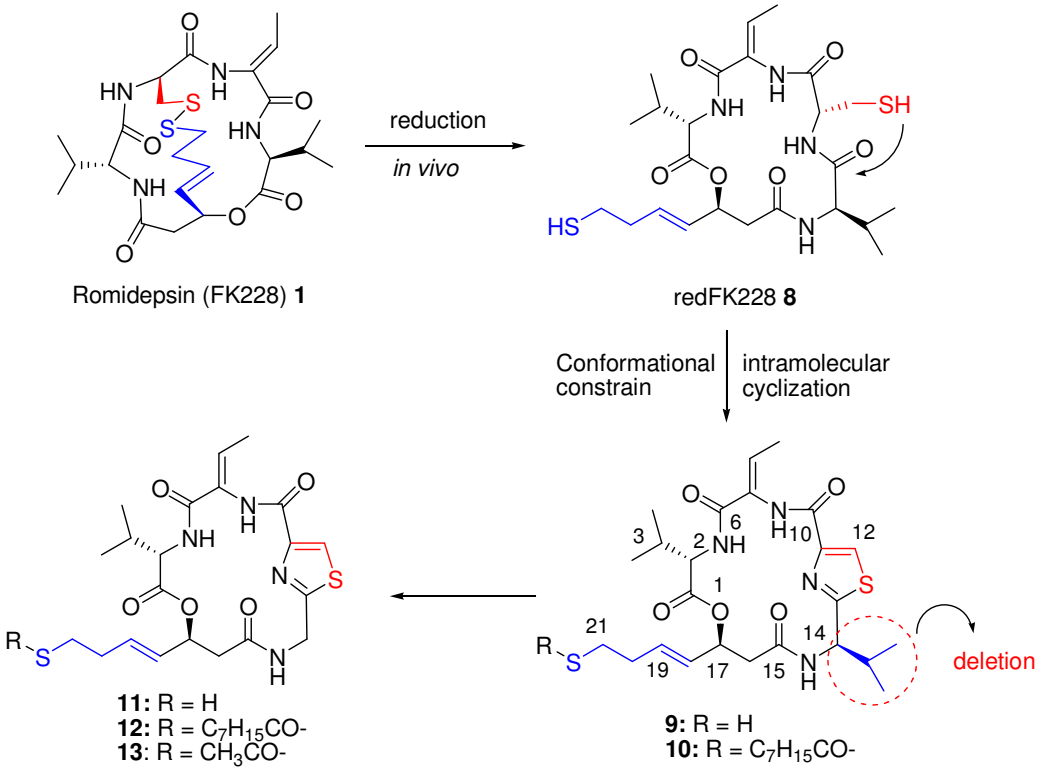


Figure 2. Design of a new class of conformationally constrained FK228 mimetics as selective class I HDAC isoform inhibitors.

Table 1. Inhibition Activities (IC_{50}) of Designed Compounds and SAHA against HDAC Isoforms 1-11.

Compd	IC_{50} (μ M)										
	class I				class IIa				class IIb		class IV
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC5	HDAC7	HDAC9	HDAC6	HDAC10	HDAC11
1 (FK228)	1.05	6.07	0.062	>10	>10	>10	>10	>10	1.22	0.011	>10
8 (redFK228)	0.00012	0.0014	0.0048	0.025	9.57	>10	1.76	>10	0.149	0.0039	2.36
9	0.00218	0.0366	0.00977	2.55	>10	>10	>10	>10	0.459	0.138	>10
10	0.165	2.74	3.15	>10	>10	>10	>10	>10	>10	0.395	>10
11	0.00278	0.045	0.00767	3.55	>10	>10	>10	>10	0.331	0.0374	>10
12	0.158	2.71	1.68	>10	>10	>10	>10	>10	6.95	1.93	>10
13	0.0216	0.328	0.073	>10	>10	>10	>10	>10	4.63	0.751	>10
Largazole	0.0698	1.01	0.859	>10	>10	>10	>10	>10	1.85	2.21	>10
SAHA	0.0885	0.76	0.323	4.89	>10	2.58	6.11	3.57	0.0256	0.686	>10

SAHA was used as the positive control. Values are means of three experiments, the standard error of the IC_{50} was generally less than 10%.

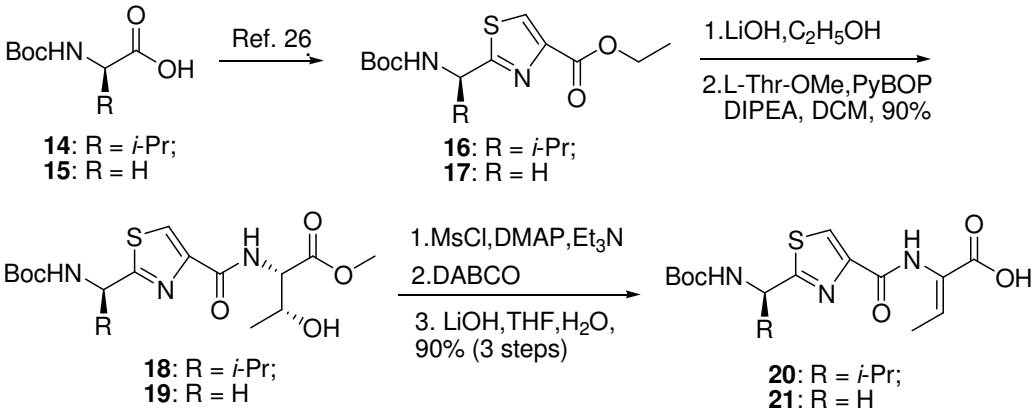
First, FK228, redFK228 and compounds **9**-**13** were tested against HDAC isoforms 1-11 using SAHA and largazole as the positive control. The results are outlined in table 1 and exhibited the interesting HDACs isoform selectivity. In our fluorescence fret-based binding assay, compound **9** strongly inhibited HDAC1, 2 or 3 with IC_{50} values of 2.18 nM, 36.6 nM, and 9.77 nM, respectively, more effectively than HDAC4, 5, 7, 9 or 11 (IC_{50} > 10 μM), confirming that this cyclization strategy is feasible. However, compound **9** is substantially less potent than redFK228, due to slight conformational differences when bound to HDAC1. We masked the thiol group

of **9** with octanoyl chloride, and the resulting prodrug **10** is less potent than **9** against HDAC1, HDAC2 or HDAC3. In view of the disadvantage of a hydrophobic group, we investigated the possibility of removing the isopropyl group from the 14 position of **9**, leading to the design of **11** (Figure **2**). Indeed, it was determined that compound **11** has IC_{50} values of 2.78, 45.0 and 7.67 nM, respectively, against HDAC1, 2 and 3 (Table **1**). The binding data thus confirm our assumption that the isopropyl group of **9** does not play key role in the binding with HDAC1 and consequently compound **11** represents a potent, novel lead compound for HDAC1 inhibition. In addition, compound **11** was synthesized in much higher yield than that of compound **9**. Subsequently, acetylation of the thiol group[37] in **11** led to the prodrug **13**, which is 48 and 18 times more potent than FK228 against HDAC1 and 2, respectively. When the thiol group of **11** is masked with octanoyl chloride, the resulting prodrug **12** is 6 and 2 times more potent than FK228 against HDAC1 and HDAC2. The acetyl and octanoyl groups in prodrug **12** and **13** could make the compounds more membrane permeable and more stable under physiological conditions than the free thiol, which would be released by the action of a thioesterase within the cells.

The chemical synthesis of compounds **9**-**13** is shown in Schemes **1** and **2**. The intermediates **16** and **17** were synthesized from *t*-Boc protected glycine **14** and D-valine **15**, respectively, using the modified Hantzsch procedure.[38] Hydrolysis of the ethyl ester group in **16** or **17** with 1M aqueous LiOH gave the corresponding acid. The resulting acids were then coupled with L-threonine methyl ester under PyBOP conditions in the presence of diisopropylethylamine to furnish dipeptides **18** and **19**,

which were mesylated, and subsequently by eliminated and saponified to provide the acids **20** and **21**, respectively.

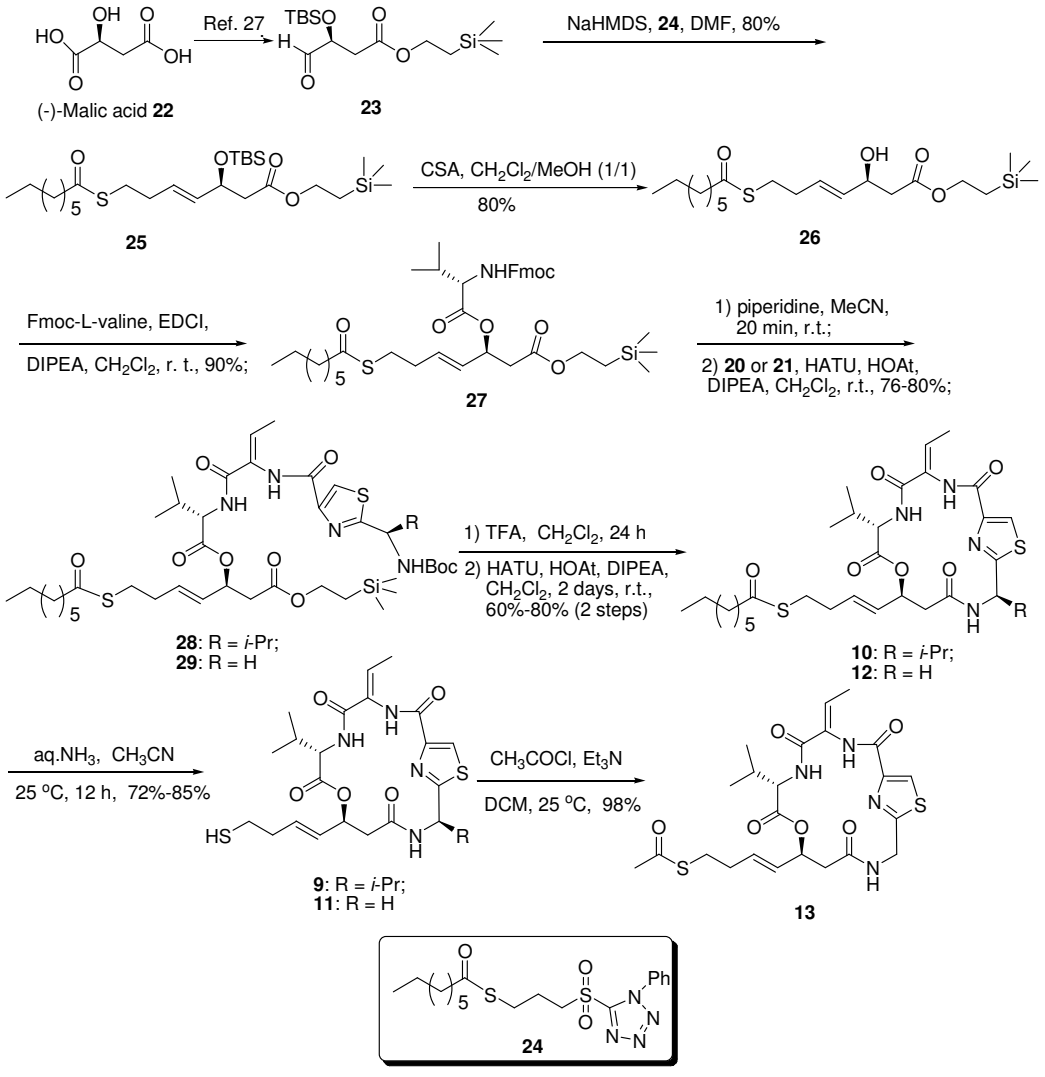
Scheme 1. Synthesis of the Key Fragments 20 and 21



The intermediate **23** was synthesized from commercially available (-)-malic acid **22** according to our previously reported method (Scheme **2**).[35k] Swern oxidation led to the aldehyde **23** which was subjected to Julia-Kocienski olefination coupling with the sulfone **24** to obtain the olefin **25**. For the synthesis of olefin **25**, several bases (*e.g.,* LiHMDS, NaHMDS, KHMDS, or LDA) and solvents (THF, DCM, DMF and toluene) were tested at temperatures ranging from -78 °C to room temperature. We found that utilization of NaHMDS in DMF gave the most favorable E/Z (20/1) ratio, which is better than our previous reported method.[35k] After selective removal of the TBS protecting group, condensation of **26** with Fmoc-L-valine in the presence of EDCI and HOAt at room temperature gave intermediate **27**. Removal of the Fmoc group followed by EDC-mediated coupling to the acids **20** and **21** in different combinations Subsequently, TFA-mediated removal of the *t*-Boc and 2-(trimethylsilyl)ethoxy groups were achieved. After examining several macrocyclization conditions, **10** and

12 were obtained in 60-80% yield, respectively, using HATU as the condensation reagent in anhydrous DCM solution (0.7 mM). In addition, aminolysis of **10** or **12**, using aqueous NH_3 or CH_3CN, proceeded smoothly to afford the thiol analogue **9** and **11** in 72% and 85% yield, respectively. Acetylation of the thiol group of **11** with acetyl chloride under standard conditions led to the prodrug **13** in 98% yield.

Scheme 2. Synthesis of compounds 9-13



To further investigate the antiproliferative activities of these derivatives, correlate them with their HDAC inhibitory activity and test their selectivity toward cancer cells over normal cells, compounds **10, 12** and **13** were evaluated with CCK8 assays for their activity in inhibition of cell growth against the human prostate carcinoma cells Du145, the human acute lymphoblastic leukemia cells Molt-4, and the human leukemic monocyte lymphoma cells U937, and human normal cell lines lung fibroblast cells HLF and fibroblast-like fetal lung cells Wi38, with SAHA, largazole and FK-228 as the positive controls. The results are summarized in Table **2.** Compounds **12** and **13** showed promising anti-proliferative activities and good cancer cell selectivity.

Table 2. Antiproliferative activity of SAHA, largazole, FK-228 and the designed compounds 10, 12 and 13

Sample	GI_{50} (nM)					
	Cancer cell lines			Normal cell lines		
	Du145	Molt-4	U937	HLF	Wi38	Selectivity (Du145/HLF)
FK-228	2.55	2.06	3.41	4.89	5.39	0.52
10	20.5	22	31.7	710	561	0.029
12	6.99	5.87	6.59	312	157	0.022
13	11.02	7.22	10.52	321	258	0.034

Largazole	11	16.2	16.8	39.7	82.5	0.28
SAHA	1590	542	1460	1650	6080	0.96

[a]SAHA largazole and FK-228 were used as a positive control. [b]Inhibition of cell growth by the listed compounds was determined by using CCK8 assay. [c]Values are means of three experiments, and standard error of the GI_{50} was generally less than 20%.

In our cellular assay (Table 2), FK-228 exhibits very potent activities not only for the cancer cell lines (Du145, GI_{50} = 2.55 nM; Molt-4, GI_{50} = 2.06 nM; U937, GI_{50} = 3.41 nM) but also for the normal cell lines (HLF, GI_{50} = 4.89 nM; Wi38, GI_{50} = 5.39 nM). Our assay showed that compound **10** is 8-11 times less potent against cancer cell lines in comparison to FK-228 but has improved selectivity for cancer cells over normal cells. Dramatically increased selectivity toward human cancer cells over normal cells was observed with compounds **12** and **13**. Intramolecular cyclization and removal of the isopropyl group at the 14 position led to compound **12** (selectivity 0.022) with 22-fold and 20 fold more selective to cancer cells when compared to FK228 (selectivity 0.52) and largazole (selectivity 0.28), respectively, with similar inhibitory effect on cancer cell lines, on the order of nM, compared to FK228, further confirming our design strategy. Compound **13**, with an acetyl side chain, also retains similar selectivity toward human cancer cells over normal cells with potency in the nanomolar range against cancer cells.

To further investigate selectivity of these subtypes at the cellular level, compounds **12** and **13** were evaluated for the expression of the acetylation status of histone H3 and H4 in comparison to that of the HDAC6-specific substrate α-tubulin in U937

cancer cell lines, which are important biomarkers associated with intracellular HDAC inhibition. FK228 and SAHA were used as positive control. We found that both compounds **12** and **13** have significant, dose-dependent effect on histone H3 and H4 acetylation, whereas much less effect to induce significant acetylation of α-tubulin comparing to SAHA and FK228 confirmed that they are not very effective inhibitors of HDAC6 (Figure **3**). This result further suggests that both compounds **12** and **13** are class I selective inhibitors.

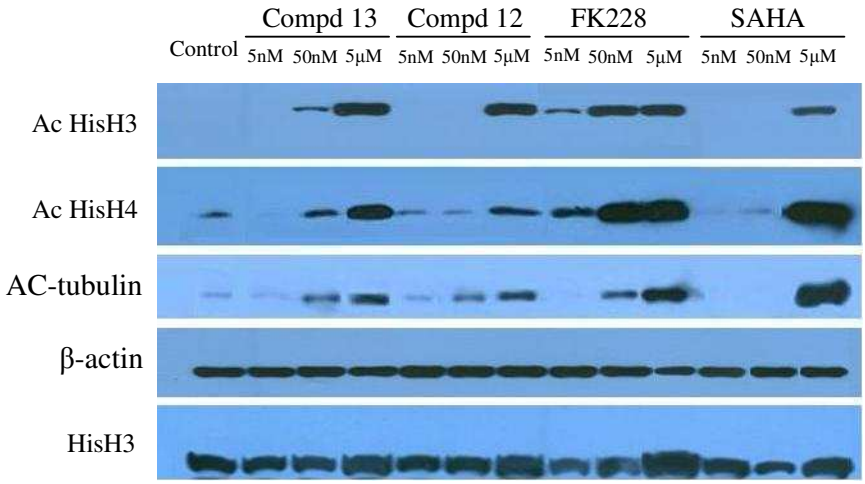


Figure 3. Effect of histone H3 and H4 acetylation and α-tubulin acetylation in cultured human lymphoma cells (U937) after 24 h treatment with compounds **12**, **13**, FK228 and SAHA using Western blot analysis. β-actin and histone H3 (bottom) serves as a control for protein loading.

Compound **13** was chosen for a further *in vivo* efficacy study of its high binding affinities to class I HDACs, potent cell growth inhibitory activity and good aqueous solubility. The human prostate cancer cell line (Du145) obtained from ATCC was tested negative for rodent pathogens. The BALB/c nude mice (6 weeks old) were inoculated with suspensions of 5×10^6 Du145 cells at the right flank. When the mean

tumor size reached approximately 110 mm^3 14 days after inoculation, compound **13** was administered *via* i.v. injection 5 days a week for 4 weeks at dosages of 20, 40, and 80 mg/kg with SAHA (50 mg/kg) as a positive control. The result of tumor growth inhibition in different groups at different time points after treatment is shown in Figure **4**. Compound **13** causes tumor growth delay in a dose-dependent manner, such that doses of 20, 40, and 80 mg/kg/day cause reductions of 51.7%, 75.2%, and 81.2%, respectively, compared with untreated control group. The activity of **13** at the low dosage (20 mg/kg, tumor growth inhibition (TGI) = 51.7%) was greater than that of the control compound SAHA (50 mg/kg, TGI = 41.7%). The toxicity of **13** was assessed by monitoring the body weight and survival of the mice. Before sacrifice, the mouse body weights showed no significant change in different groups. All doses of **13** are well-tolerated, with no mortality or significant loss of body weight observed during treatment (Figure **5**).

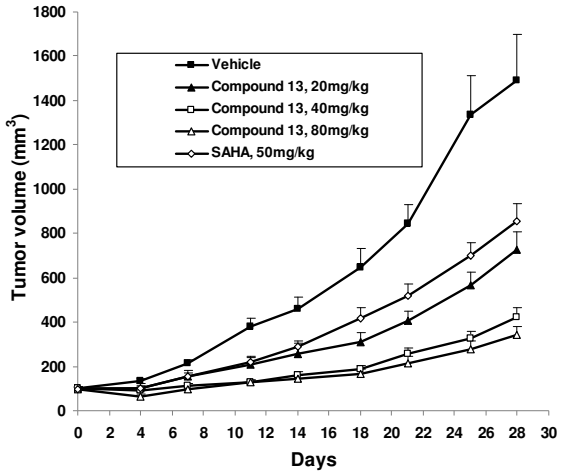


Figure 4. Growth curve of the Du145 prostate cancer xenograft in nude mice treated with i.v. injections of vehicle alone or compound **13** (20, 40, or 80 mg/kg) with

SAHA (50 mg/kg) as positive control ($p < 0.01$). Data are expressed as the mean tumor volume ± SE of the animals in each treatment group.

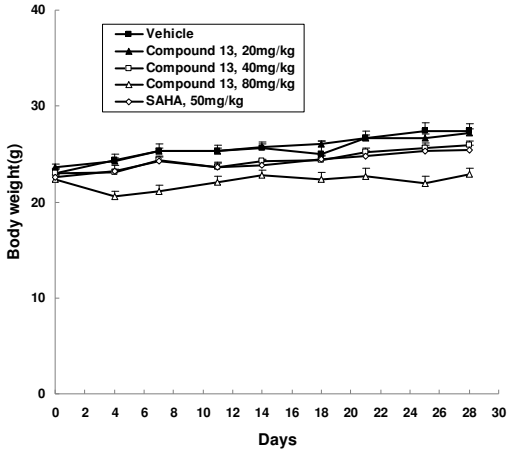


Figure 5. Body weight of mice during treatment with compound **13** and SAHA ($p < 0.01$).

Conclusion

A new class of histone deacetylase inhibitors (**9**-**13**) has been designed based upon the thiol cyclization strategy. The lead drug candidates, compounds **12** and **13** have good HDAC class I selectivity in the inhibition assay against HDAC isoforms and sub-nanomolar inhibitory efficacy on cancer cell lines. These two compounds showed improved selectivity toward human cancer cells over normal cells in comparison with FK228, which makes them attractive lead compounds for cancer therapy. More importantly, compound **13** exhibited potent *in vivo* antitumor activities with no observed toxicity in prostate cancer Du145 xenograft model. In addition, the synthesis

of compound **13** is well-suited to scale-up and much easier than that of FK-228, which needs over 12 synthetic steps with low overall yield. Taken together, our results indicate that the compound **13** is a promising new class of HDAC targeted anticancer agents and has potential for clinical translation. Further evaluation of safety and toxicology of compound **13** is ongoing. The strategy for the design of novel HDAC drug candidate **13** presented in this report may accelerate further discovery of more potent and selective anti-tumor agents.

Experimental procedure

1. Chemistry. General. Reagents and solvents were used as purchased without further purification. The progress of all reactions was monitored by TLC using ethyl acetate/n-hexane as solvent system, and spots were visualized by irradiation with ultraviolet light (254 nm). Flash chromatography was performed using silica gel (300−400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance ARX-400 (or Bruker Avance ARX-500). The low or high resolution of ESIMS was recorded on an Agilent 1200 HPLC-MSD mass spectrometer or Applied Biosystems Q-STAR Elite ESI-LC-MS/MS mass spectrometer, respectively. Anhydrous solvents were obtained as follows: THF by distillation from sodium and benzophenone; dichloromethane, toluene and N, N- dimethylformamide from CaH_2. All other solvents were reagent grade. All moisture sensitive reactions were carried out in flame dried flask under argon atmosphere. The purity of the final compounds was determined by Agilent 1260 series HPLC system using the following conditions: C-18

column (DicKma, 4.6 mm × 250 mm) with the solvent system (elution conditions: mobile phase A consisting of MeOH; mobile phase B consisting of water containing 0.1% ammonia), with monitoring between 190 and 800 nm. A flow rate of 1.0 mL/min was used. The retention time was reported as t_R (min). The purity of final compounds is >95%.

(*R*,*Z*)-2-(2-(1-(tert-butoxycarbonyl)-2-methylpropyl)thiazole-4-carboxamido)but-2-enoic acid (20)

To a solution of **18** (785 mg, 1.9 mmol) in anhydrous CH_2Cl_2 (10 mL) was added DMAP (46 mg, 0.4 mmol), Et_3N (0.56 mL, 4 mmol), and MsCl (0.24 ml, 2.9 mmol) at 0 ^oC. After stirring for 1 h, sat aq $NaHCO_3$ (10 mL) was added followed by dichloromenthane (10 mL). The phase was separated and the aqueous phase extracted with dichloromenthane (3 × 10 mL). The combined organic phases were dried over Na_2SO_4, filtered, evaporated in vacuo. The result oil was dissolved in anhydrous CH_2Cl_2 (10 ml) and cooled to 0 ^oC, DABCO (448 mg, 4 mmol) was added. After stirring for 30 min, the solution was warmed to rt, and stirred for 2 h. The solution was washed with sat aq $NaHCO_3$, saturated NaCl solution and dried over Na_2SO_4, filtered, evaporated in vacuo and purified by chromatography to give a white solid. To a solution of above solid in MeOH (10 mL) was added an aqueous solution of LiOH (161 mg, 3.8 mmol) in H_2O (5 ml) at 0 ^oC. After stirring for 2 h, the solution was neutralized by adding 1M aq HCl and evaporated in vacuo. The residue was diluted with ethyl acetate and saturated NaCl solution, separated aqueous layer was extracted

with ethyl acetate (3 × 10 mL). The combined organic layers dried over Na_2SO_4, and evaporated to afford **20** as white solid (655 mg, 90%). $[\alpha]^{23}_D$: 4.68 (c 0.83 , $CHCl_3$). ^{1}H NMR (400MHz, CDCl3): δ 8.68 (s, 1H), 8.01 (s, 1H), 7.00 (q, *J* = 6.8 Hz, 1H), 5.16 (d, *J* = 8.8 Hz, 1H), 4.89 (d, *J* = 7.2 Hz, 1H), 2.37 (brs, 1H), 1.90 (d, *J* = 7.2 Hz, 3H), 1.47 (s, 9H), 1.24 (m, 1H), 1.00 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 6.4 Hz, 3H) ppm. ^{13}C NMR (125 MHz, CDCl3): δ 173.0, 167.9, 159.0, 155.5, 149.3, 135.9, 125.7, 124.0, 80.4, 58.0, 33.2, 28.3, 19.3, 17.4, 15.1 ppm. MS (EI, m/z): 383.1 $[M+H]^+$. HRMS (ESI) Calcd m/z for C17H25N3O5S $[M+H]^+$ 384.1588, found 384.1586.

(*Z*)-2-(2-((tert-butoxycarbonyl)methyl) thiazole-4-carboxamido)but-2-enoic acid (21)

The procedure was the same as described above. ^{1}H NMR (400 MHz, MeOD-D4): δ 8.19 (s, 1H), 6.96 (q, *J* = 7.2Hz, 1H), 5.56(s, 2H), 1.82 (d, *J* = 7.2Hz, 3H), 1.47 (s, 9H) ppm. ^{13}C NMR (125 MHz, MeOD-D4): δ 173.0, 167.4, 161.9, 158.4, 150.0, 137.1, 128.1, 125.9, 81.2, 43.1, 28.7, 14.5 ppm. MS (EI, m/z): 341.0 $[M]^+$. HRMS (ESI) Calcd m/z for C14H19N3NaO5S $[(M + Na)^+]$ 364.1251, found 364.1251.

(*S*, *E*)-2-(trimethylsilyl)ethyl 3-((S)-2-(2-((R)-1-(tert-butoxycarbonyl)-2-methylpropyl) thiazole-4-carboxamido)but-2-enamido)-3-methyl butanoyloxy)-7-(octanoylthio)hept-4-enoate (28).

To the solution of **27** (860 mg, 1.19 mmol) in MeCN (10 mL) at 0 ^{o}C was added piperidine (0.5 mL). After stirring for 1 h at rt, the reaction mixture was carefully quenched with sat aq NH_4Cl (10 ml). The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was dried over anhydrous Na_2SO_4,

concentrated in vacuo, and purified by chromatography to give the amine as oil for the next step.

To the solution of acid **20** (455 mg, 1.19 mmol) in CH_2Cl_2 (15 mL) was added HATU (542 mg, 1.43 mmol), HOAt (194 mg, 1.43 mmol) followed by addition of the above amine (475 mg, 0.95 mmol) in CH_2Cl_2 (5 mL), DIEA (0.47 mL, 2.85 mmol) at 0 ^oC. And the reaction mixture was allowed to warm to rt and stirred for 12 h. The reaction mixture was concentrated *in vacuo* and subjected to flash column chromatography to give **28** (783 mg, 76%) as an oil. $[\alpha]^{23}_D$: -2.2 (*c* 0.50, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.61 (s, 1H), 8.07 (s, 1H), 6.58 (m, 1H), 6.56 (m, 1H), 5.77 (m, 1H), 5.62 (q, *J* = 6.8 Hz, 1H), 5.50 (dd, *J* = 15.6, 7.2 Hz, 1H), 5.28 (brs, 1H), 4.86 (brs, 1H), 4.60 (q, *J* = 4.0 Hz, 1H), 4.15 (m, 2H), 2.85 (t, *J* = 7.2 Hz, 2H), 2.72 (dd, *J* = 15.6, 8.0 Hz, 1H), 2.66 (dd, *J* = 15.6, 5.2 Hz, 1H), 2.56 (t, *J* = 7.6 Hz, 2H), 2.48 (brs, 2H), 2.27 (q, *J* = 7.2 Hz, 2H), 2.18 (m, 1H), 1.89 (d, *J* = 7.2 Hz, 3H), 1.63 (m, 2H), 1.44 (s, 9H), 1.27 (m, 8H), 0.95 (t, *J* = 4.0 Hz, 2H), 0.90 (t, 6H), 0.87 (t, 3H), 0.03 (s, 9H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 199.2, 173.0, 170.7, 169.5, 164.4, 159.2, 155.4, 149.2, 132.3, 129.5, 128.8, 128.7, 128.3, 123.9, 80.2, 71.8, 63.1, 60.3, 57.9, 57.1, 44.1, 39.6, 38.5, 33.1, 32.1, 31.6, 31.5, 28.8, 28.3 27.8, 25.6, 22.5, 19.3, 18.9, 17.5, 17.3, 14.1, 14.0, 13.9, 0.95 ppm. MS (EI, *m/z*): 867.3 $[M+H]^+$. HRMS (ESI) Calcd *m/z* for $C_{42}H_{70}N_4O_9S_2Si$ $[M+H]^+$ 867.3519, found 867.3521.

_S_-(_E_)-4-((7_S_, 10_S_, 14_R_, _Z_)-4-ethylidene-7, 14-diisopropyl-2, 5, 8, 12-tetraoxo-9-16 –thia-3, 6, 13, 18-tetraaza-bicyclo [13.2.1]octadec-1(17)-en-10-yl) but-3-enyl octanethioate (10).

To a solution of **28** (693 mg, 0.8 mmol) in CH_2Cl_2 (10 mL) was added TFA (2 mL) at 0 ^oC, and the mixture was stirred at rt for 24 h. The reaction mixture was concentrated *in vacuo* to give the crude amino acid. This amino acid was then dissolved in DMF (800 mL), and HATU (1.52 g, 4 mmol), HOAt (0.55 g, 4 mmol) and DIEA (1.3 mL, 8 mmol) were added to the solution successively. The reaction mixture was stirred for 48 h at rt and then the solvent was evaporated under reduced pressue and dissolved in ethyl acetate (20 ml). This solution was washed with sat aq NaCl and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo to give an oil which was subjected to column chromatography to give **10** (311 mg, 60%) as a white solid. $[\alpha]^{23}_D$: 12.5 (*c* 0.30 , $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.50 (s, 1H), 8.20 (s, 1H), 7.07 (q, *J* = 7.2 Hz, 1H), 6.36 (d, *J* = 10.4 Hz, 1H), 6.03 (d, *J* = 9.2 Hz, 1H), 5.76 (m, 1H), 5.69 (dd, *J* = 15.6, 7.2 Hz, 1H), 5.50 (dd, *J* = 15.6, 7.2 Hz, 1H), 5.35 (dd, *J* = 9.2, 3.6 Hz, 1H), 4.78 (dd, *J* = 10.4, 3.6 Hz, 1H), 2.89 (t, *J* = 7.2 Hz, 2H), 2.73 (d, *J* = 6.4 Hz, 2H), 2.52 (t, *J* = 7.2 Hz, 2H), 2.54 (m, 2H), 2.29 (m, 4H), 1.92 (d, *J* = 7.2 Hz, 3H), 1.63 (m, 2H), 1.28 (m, 8H), 1.10 (d, *J* = 7.2 Hz, 3H), 0.92 (d, *J* = 7.2 Hz, 3H), 0.87 (t, *J* = 7.2 Hz, 3H), 0.78 (d, *J* = 7.2 Hz, 3H), 0.54 (d, *J* = 7.2 Hz, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 199.4, 171.4, 169.8, 168.9, 163.3, 158.6, 147.8, 135.0, 132.6, 128.4, 127.1, 125.1, 71.8, 57.0, 56.4, 44.1, 40.8, 33.6, 32.2, 31.6, 28.9,

27.8, 25.6, 22.6, 19.5, 18.9, 16.5, 16.2, 14.7, 14.0 ppm. MS (EI, *m/z*): 648.7 $[M+H]^+$. HRMS (ESI) Calcd *m/z* for $C_{32}H_{48}N_4NaO_6S_2$ $[M + Na]^+$ 671.2907, found 671.2908. HPLC analysis: 97.2% in purity.

(*S*, *E*)-2-(trimethylsilyl)ethyl 3-((*S*)-2-(2-((*Z*)-2-(2-((tert-butoxycarbonyl)methyl) thiazole-4-carboxamido)but-2-enamido)-3-methylbutanoyloxy)-7-(octanoylthio) hept-4-enoate (29).

To a stirred solution of **29** (3.5 g, 4.7 mmol) in MeCN (30 mL) at 0 ^oC was added piperidine (2 mL). After stirring for 2 h at rt, the reaction mixture was concentrated under reduce pressure to afford the amine for the next step.

To the solution of acid **21** (1.92 g, 5.63 mmol) in CH_2Cl_2 (50 mL) was added HATU (2.68 g, 7.04 mmol), HOAt (0.96 g, 7.04 mmol) at 0 ^oC, followed by addition of the above amine in CH_2Cl_2 (10 mL), DIEA (2.33 mL, 14.07 mmol) and the reaction mixture was allowed to warm to rt and stirred for 12 h. The reaction mixture was concentrated in vacuo and subjected to flash column chromatography to give **29** (3.10 g, 80%) as an oil. $[\alpha]^{23}_D$: -7.57 (*c* 7.5, $CHCl_3$). ^1H NMR (400MHz, $CDCl_3$, ppm): δ 8.60 (s, 1H), 8.10 (s, 1H), 6.60 (m, 2H), 5.77 (m, 1H), 5.62 (dd, *J* = 13.7, 7.2 Hz, 1H), 5.49 (dd, *J* = 13.7, 7.5 Hz, 1H), 5.42 (m, 1H), 4.58 (m, 3H), 4.14 (t, *J* = 8.6Hz, 2H), 2.85 (t, *J* = 7.2Hz, 2H), 2.68 (dd, *J* = 15.7, 7.7 Hz, 2H), 2.50 (t, *J* = 7.5 Hz, 2H), 2.26 (q, *J* = 7.1 Hz, 2H), 2.18 (m, 1H), 1.80 (d, *J* = 7.2Hz, 3H), 1.60 (m, 2H), 1.46 (m, 8H), 1.26 (m, 9H), 0.96 (m, 3H), 0.86 (t, *J* = 6.4 Hz, 6H), 0.02 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 199.2, 170.7, 169.6, 164.3, 159.3, 148.9, 133.3, 129.4, 129.2, 128.3, 124.8, 71.8, 63.1, 57.2, 44.1, 39.6, 38.6, 32.1, 31.6, 31.6, 28.8, 28.3, 27.8, 25.6, 22.5,

18.9, 17.6, 17.3, 14.0, 13.9, 0.96, -1.57. MS (EI, *m/z*): 847 $[M+Na]^+$. HRMS (ESI): calcd *m/z* for $C_{39}H_{64}N_4NaO_9S_2Si$ $[M+Na]^+$ 847.3779, found 847.3777.

S-(*E*)-4-((7*S*,10*S*,*Z*)-4-ethylidene-7-isopropyl-2,5,8,12-tetraoxo-9-oxa-16-thia-3,6, 13,18-tetraaza-bicyclo[13.2.1]octadec-1(17)-en-10-yl)but-3-enyl octanethioate (12).

To a solution of **29** (659 mg, 0.8 mmol) in CH_2Cl_2 (10 mL) was added TFA (2 mL) at 0 ^oC, and the mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated *in vacuo* to give the crude amino acid. This amino acid was then dissolved in DCM (800 mL), and HATU (1.52 g, 4 mmol), HOAt (0.55 g, 4 mmol) and DIEA (1.3 mL, 8 mmol) were added to the solution successively. The reaction mixture was stirred for 48 h at rt and then the solvent was evaporated under reduced pressue and dissolved in ethyl acetate (20 mL). This solution was washed with sat aq NaCl and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo to give an oil which was subjected to column chromatography to give **12** (388 mg, 80%) as white solid. $[\alpha]^{23}_D$: 19.2 (*c* 0.9, $CHCl_3$). ^1H NMR (400MHz, $CDCl_3$): δ 8.54 (s, 1H), 8.08 (s, 1H), 6.97 (q, *J* = 7.2 Hz, 1H), 6.82 (m, 1H), 6.51 (d, *J* = 10.2 Hz, 1H), 5.74-5.6 (m, 2H), 5.47 (dd, *J* = 15.5, 6.8 Hz, 1H), 5.14 (dd, *J* = 16, 8.2 Hz,1H), 4.70 (dd, *J* = 10.1, 3.2 Hz ,1H), 4.32 (dd, *J* = 17.4, 3.5 Hz, 1H), 2.82 (t, *J* = 7.2 Hz, 2H), 2.75-2.59 (m, 2H), 2.47 (t, *J* = 7.5 Hz, 2H), 2.24 (m, 2H), 1.82 (d, *J* = 7.2 Hz, 3H), 1.58 (m, 2H), 1.24 (m, 8H), 0.83 (m, 3H), 0.74 (d, *J* = 6.7 Hz, 3H), 0.55

(d, J = 6.7 Hz, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 199.4, 169.7, 169.0, 167.5, 163.4, 158.8, 147.8, 134.6, 132.5, 128.2, 127.1, 124.4, 71.8, 57.0, 44.0, 40.7, 40.0, 32.1, 31.5, 28.7, 27.7, 25.5, 22.4, 18.9, 16.3, 14.5, 13.9 ppm. MS (EI, *m/z*): 607.1 $[M+H]^+$. HRMS (ESI): calcd *m/z* for $C_{29}H_{42}N_4NaO_6S_2$ $[(M+Na)^+]$ 629.2438, found 629.2440. HPLC analysis: 98.8% in purity.

(7S,10S,14R,Z)-4-ethylidene-7,14-diisopropyl-10-((E)-4-mercaptobut-1-en-1-yl)-9-oxa-16-thia-3,6,13,18-tetraazabicyclo[13.2.1]octadeca-1(17),15(18)-diene-2,5,8,12-tetraone (9).

To a solution of **10** (40 mg, 0.06 mmol) in CH_3CN (5 mL) was added aqueous NH_3 (28.9%, 0.5 mL). The resulting mixture was stirred at room temperature for 18 h and concentrated in vacuo. The residue was purified by column chromatography to afford thiol **9** (23 mg, 72%) as a white solid. $[\alpha]^{23}_D$: 19.8 (*c* 0.17 , $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.49 (s, 1H), 8.20 (s, 1H), 7.09 (q, J = 7.2 Hz, 1H), 6.37 (d, J = 10.0 Hz, 1H), 5.99 (d, J = 9.2 Hz, 1H), 5.83-5.69 (m, 2H), 5.56 (dd, J = 15.6, 6.8 Hz, 1H), 4.80 (dd, J = 10.4, 3.6 Hz, 1H), 4.79 (dd, J = 10.4, 3.6 Hz, 1H), 2.77 (s, 1H), 2.75 (d, J = 2.4 Hz, 1H), 2.57 (q, J = 7.2 Hz, 2H), 2.38-2.28 (m, 5H), 1.93 (d, J = 7.2 Hz, 3H), 1.38 (t, J = 8.0 Hz, 1H), 1.11 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8Hz, 3H), 0.80 (d, J = 6.8Hz, 3H), 0.55 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.4, 169.9, 168.9, 163.3, 158.6, 147.8, 135.1, 132.4, 130.9, 128.8, 127.0, 125.1, 71.9, 57.0, 56.4, 40.9, 38.7, 36.2, 31.6, 23.0, 19.6, 19.0, 16.5, 16.2, 14.7 ppm. MS (EI, *m/z*): 523.2 $[M+H]^+$. HRMS (ESI) Calcd *m/z* for $C_{24}H_{34}N_4O_5S_2$ M^+ 522.1971, found 522.1973. HPLC analysis: 97.7% in purity.

(7S,10S,Z)-4-ethylidene-7-isopropyl-10-((E)-4-mercaptobut-1-en-1-yl)-9-oxa-16-thia-3,6,13,18-tetraazabicyclo[13.2.1]octadeca-1(17),15(18)-diene-2,5,8,12-tetraone (11).

To a solution of **12** (606 mg, 1 mmol) in CH_3CN (5 mL) was added aqueous NH_3 (28.9%, 0.5 mL). The resulting mixture was stirred at room temperature for 18 h and concentrated *in vacuo*. The residue was purified by column chromatography to afford thiol **11** (407 mg, 85%) as white solid. $[\alpha]^{23}_{D}$: 15.9 (*c* 0.26 , $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.53 (s, 1H), 8.13 (s, 1H), 7.03 (q, *J* = 7.2 Hz, 1H), 6.56 (brs, 1H), 6.48 (d, *J* = 10.4 Hz, 1H), 5.78-5.67 (m, 2H), 5.55 (dd, *J* = 15.6, 7.2 Hz, 1H), 5.19 (dd, *J* = 15.6, 7.2 Hz, 1H), 4.77 (dd, *J* = 10.4, 3.2 Hz, 1H), 4.35 (dd, *J* = 17.6, 4.0 Hz, 1H), 2.78-2.72 (m, 2H), 2.55 (q, *J* = 7.2 Hz, 2H), 2.33(q, *J* = 6.4 Hz, 3H), 1.88 (d, *J* = 7.2 Hz, 3H), 1.37 (t, *J* = 8.0 Hz, 1H), 0.82 (d, *J* = 6.8 Hz, 3H), 0.62 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8, 168.9, 167.4, 163.4, 158.5, 148.0, 134.6, 132.4, 128.8, 127.0, 124.5, 71.7, 57.1, 40.8, 40.5, 36.1, 31.6, 23.7, 19.0, 16.5, 14.7 ppm; MS (EI, *m/z*): 480.2 $[M+H]^+$. HRMS (ESI) Calcd *m/z* for $C_{21}H_{28}N_4O_5S_2$ $[(M + H)^+]$ 481.1571, found 481.1574. HPLC analysis: 98.2% in purity.

S-((E)-4-((7S,10S,Z)-4-ethylidene-7-isopropyl-2,5,8,12-tetraoxo-9-oxa-16-thia-3,6,13,18-tetraazabicyclo[13.2.1]octadeca-1(17),15(18)-dien-10-yl)but-3-en-1-yl) ethanethioate (13).

To a solution of **11** (208 mg, 0.43 mmol) in CH_2Cl_2 (10 ml) cooled to 0 ^oC was added Et_3N (0.1 ml, 1.3 mmol), AcCl (0.2 ml, 1.3 mmol). The reaction mixture was stirred at room temperature for 4 h and concentrate *in vacuo*. The residue was purified

by column chromatography to afford **13** (220 mg, 98%) as white solid. $[\alpha]^{23}_{D}$: 19.6 (*c* 0.16 , $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.50 (s, 1H), 8.15 (s, 1H), 7.06 (q, *J* = 7.2 Hz, 1H), 6.41 (d, *J* = 10.4 Hz, 1H), 6.27 (s, 1H), 5.78-5.67 (m, 2H), 5.55 (dd, *J* = 15.6, 7.2 Hz, 1H), 5.26 (dd, *J* = 15.6, 7.2 Hz, 1H), 4.82 (dd, *J* = 10.4, 3.2 Hz, 1H), 4.38 (dd, *J* = 17.6, 4.0 Hz, 1H), 2.90 (t, *J* = 7.2 Hz, 2H), 2.72 (d, *J* = 6.4 Hz, 2H), 2.30 (m, 6H), 1.91 (d, *J* = 7.2 Hz, 3H), 0.82 (d, *J* = 6.8 Hz, 3H), 0.62 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.6, 169.7, 170.0, 167.4, 163.3, 158.5, 148.0, 134.6, 132.5, 128.3, 127.0, 124.5, 71.6, 57.0, 40.8, 40.3, 32.1, 31.6, 30.6, 28.1, 19.0, 16.4, 14.7 ppm; MS (EI, *m/z*): 523.2 $[M+H]^+$. HRMS (ESI) Calcd *m/z* for $C_{23}H_{30}N_4NaO_6S_2$ $[(M + Na)^+]$ 545.1499, found 545.1499. HPLC analysis: 98.9% in purity.

2. Biological Assays.

In Vitro HDAC Inhibition Assay. The Purified recombinant Human HDACs 1-11 and their corresponding substrates were purchased from BPS Bioscience (BPS Bioscience Inc., USA). The assays were carried out in 384-well format using the BPS fluorescent-based HDAC activity assay according to the manufacturer's protocol (BPS Bioscience Inc., USA). The HDAC reaction mixture was composed of HDAC assay buffer (BPS Bioscience Inc., USA), BSA, serial diluted test compounds, appropriate concentration of HDACs, and 20 μM fluorogenic substrate, the mixture was incubated at 37 ^{o}C for 60 min, and then stopped by addition of developer containing trypsin and TSA. After 20 min incubation, the fluorescence was detected at

the excitation wavelength of 360 nm and the emission wave length of 460 nm using EnVision Multilabel Reader (PerkinElmer Inc., USA). The analytical software, GraphPad Prism 5.0 (GraphPad Software, Inc., USA) was used to generate IC_{50} value via non-linear regression analysis.

Cell-Based anti-proliferation Assay. Cell lines, DU145, Molt-4, U937, Wi38 and HLF were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. Cells were grown and maintained in mediums, RPMI for Molt-4 and U937, HAM'S/F-12 for DU145, DMEM for HLF, and MEM for Wi38, with 10% FBS and with 1% penicillin/streptomycin or without antibiotics (only for Wi38). All cell lines were incubated in a Thermo/Forma Scientific CO_2 Water Jacketed Incubator with 5% CO_2 in air at 37 °C. Cell proliferation assay was determined by the CCK8 (DOjinDo, Japan) assay. Cells were seeded at a density of 800-1000 cells/ well in 384 well plates and treated with various concentration of compounds or solvent control. After 72 h incubation, CCk8 reagent was added, and absorbance was measured at 450 nm using Envision 2104 multi-label Reader (Perkin Elmer, USA). Dose response curves were plotted to determine the IC_{50} values using Prism 5.0 (GraphPad Software Inc., USA).

Western blot Analysis. Human leukemic monocyte lymphoma cell lines (U937) were cultivated in 1640 culture medium with antibiotics and 10% fetal bovine serum. Cells were seeded at a destiny of 6×10^4 /well in six well plates and incubated overnight. The U937 cells were treated with various concentrations of compounds **13**, **12**, FK228 and SAHA for 24h at 37 °C. After treatment, the cells were washed, harvested and lysed. The 10 μg total proteins for each sample were loaded on 15%

SDS-polyacrylamide gel, then transfered onto PVDF membranes. The transferred membranes were blocked for 1h with 5% skimmed milk, and then incubated overnight at 4 ^{o}C with Acetyl-Histone H3 Antibody (Cell Signaling) (1:1000), Acetyl-Histone H4 Antibody (Cell Signaling) (1:1000), Anti-Acetylated Tubulin Antibody (Sigma) (1:1000), or β-actin (Cell Signaling,1:1000), Histone H3 (Cell Signaling) (1:2000). After incubated, the PVDF membranes were washed four times with PBST buffer, then incubated with goat anti-rabbit or anti-mouse IgG-horseradish peroxidase conjugates (1:2000) for 1h at room temperature and washed 4 times. The immunoblots were visualized by enhanced chemiluminescence.

In Vivo Antitumor Efficacy in Prostate Tumor Mode. Male BALB/c nude mice between 6 to 8 weeks of age were housed in individual HEPA-ventilated cages on a 12 hours light–dark cycle at 21 °C to 23 °C and 40% to 60% humidity, and used for human tumor xenografts. Tumors were established by subcutaneous injection of 5 x 10^6 Du145 cells on the right rear flank of a nude mouse in a volume of 0.1 ml of PBS. The treatments were started when the mean tumor size reaches approximately 110 mm^3 at 10 days after inoculation. Tumor-bearing mice (n = 8 per group) were injected with various doses of compound **13** (20, 40, and 80 mg/kg), SAHA (50 mg/kg) or solvent control by intravenously injection once a day for 5 consecutive days per week. Tumor dimensions were measured daily by using calipers, and tumor volumes were calculated by using the formula TV = width^2 × length × 0.5. Beginning on Day 0, tumor dimensions and body weight were measured daily during the experiments. Tumor mass weight was measured at the end of study.

ASSOCIATED CONTENTS

Supporting Information

Experimental details including biological assays, syntheses and characterization data.

This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no completing financial interest.

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ABBREVIATIONS USED

HDAC, histone deacetylases; CTCL, cutaneous T-cell lymphoma; PTCL, peripheral T-cell lymphoma; SAR, structure activity relationships; SAHA, suberoylanilide hydroxamic acid; ZBG, zinc binding group; DCM, dichloromethane; THF, tetrahydrofuran; DMF, N,N-dimethylformamide; DIPEA, *N,N'*-diisopropylethyl amine; NaHMDS, sodium bis(trimethylsilyl)amide; IC_{50}, the half maximal inhibitory concentration; DMSO, dimethylsulfoxide; EDCI, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, Hydroxybenzotriazole; HATU, 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; GI_{50}, concentration at which cell growth is inhibited by 50%;

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