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Synthesis and anticancer activities of thieno[3,2-*d*]pyrimidines as novel HDAC inhibitors

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ABSTRACT

A series of thieno[3,2-*d*]pyrimidines bearing a hydroxamic acid moiety as novel HDAC inhibitors were designed and synthesized. The structures of the new synthesized compounds were confirmed using IR, ^{1}H, ^{13}C NMR spectrum. Compounds **11**–**13** showed potent inhibitory activities against HDACs with IC_{50} values at 0.38, 0.49 and 0.61 μM. Most of target compounds displayed strong anti-proliferative activity by a MTT assay on three human cancer cell lines including HCT-116, MCF-7 and HeLa. Compound **11**, having potent inhibitory activities against HDACs, induced apoptosis and G2/M cell cycle arrest in HCT-116 cell line.

1. Introduction

Protein function is regulated by the enzymatic addition and removal of acetyl groups at specific lysine residues. Lysine acetylation is mediated by two classes of enzymes with opposing functions: histone acetyltransferase (HAT) and histone deacetylase (HDAC), which catalyze the addition and removal of acetyl groups, respectively.[1–4] HDACs are associated with many oncogenes and tumor suppressors, leading to altered expression patterns.[5,6] HDAC inhibitors have been shown to cause growth arrest, differentiation and apoptosis in tumor cells and in animal models by inducing histone hyperacetylation and $p21^{waf1}$ expression.[7–10] Several HDAC inhibitors are currently under clinical trials on either monotherapy or combination therapy for cancer treatment.[11,12] The HDAC inhibitor vorinostat (SAHA) is a FDA-approved drug for the treatment of cutaneous T-cell lymphoma (CTCL).[13] Several small molecule HDAC inhibitors were used for clinical trials in a variety of haematological and solid tumors.[14–16] New scaffolds of small molecular inhibitors against HDAC have been constantly reported.[17–23]

Another promising strategy of HDAC inhibition to overcome limitations of targeting the PI3K pathway is to combine HDAC and PI3K inhibitors to achieve synergistic antitumor activity.[24,25] By regulating both histone and nonhistone substrates, HDAC inhibitors can affect a variety of cell functions and synergize with PI3K inhibitors.[26–28]

On the basis of a widely accepted HDAC pharmacophore model, we designed and synthesized a novel series of HDAC inhibitors by incorporating **5b** into a hydroxamic acid moiety (Fig. 1). Compound **11**, one of the synthesized compounds, showing potent HDAC inhibitory activity and antiproliferative activity, is a potential anticancer drug candidate that should be further developed.

2. Results and discussion

2.1. Synthesis

The general synthetic routes used to prepare thieno[3,2-*d*]pyrimidine/HDAC inhibitors are outlined in the Scheme 1.

Compounds **5a** and **5b** were prepared according to the procedure reported previously.[29–32] Alkylation of compound **5a**–**5b** with different chain lengths of ethyl bromoalkanoate yielded ester products **6a**–**6h**. Hydrolysis of the ethyl group of compounds **6a**–**6h** using lithium hydroxide gave corresponding acids **7a**–**7h**. Conversion of carboxylic acid **7a**–**7h** to hydroxamic acids by hydroxylamine hydrochloride yielded targeted compounds **8**–**15** (Scheme 1).

2.2. HDAC inhibition

We tested the HDAC inhibition activity of compounds **8**–**15** using HDAC Fluorimetric Assay/Drug Discovery Kit.[33] These compounds showed inhibitory rate against HDACs from 18.7% to 76.8% at a concentration of 1 μM (Table 1). Among these compounds, compounds **8** and **9** showed the weakest HDAC inhibitory activity, closely followed by compounds **14** and **15**. Compounds **10**–**13** had potent HDAC inhibitory activity, especially compound **11**. The HDAC inhibitory activities of compounds **11**, **12** and **13** with IC_{50} were at 0.38, 0.49 and 0.61 μM, respectively (Table 1).

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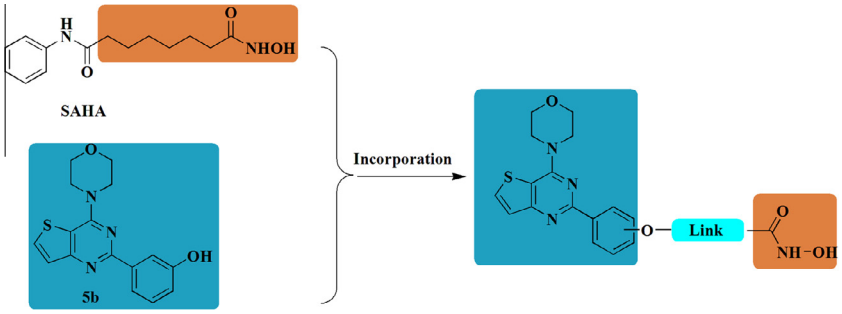
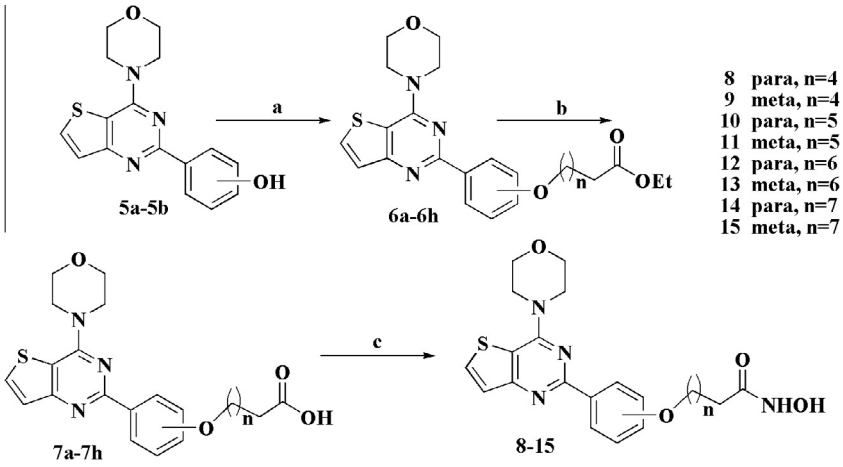


Figure 1. Design of thieno[3,2-*d*]pyrimidine HDAC inhibitors.



Scheme 1. Reagents and conditions: (a) $Br(CH_2)nCO_2Et$, K_2CO_3, DMF, 44–65%; (b) LiOH, THF, 76–100%; (c) isobutyl chloroformate, Et_3N, $NH_2OH{\cdot}HCl$, THF, 40–50% .

Table 1
In vitro HDAC inhibition and anti-proliferative activity

No.	Inhibition of HDAC[a] (%)	IC50[a] (μM) HDAC	IC_{50}[c] (μM)		
			HCT-116	HeLa	MCF-7
8	18.8	—[b]	14	12	>20
9	18.7	—	12.2	2.4	9.9
10	61.7	—	5.5	3	5.2
11	76.8	0.38	4	4	4.8
12	73.8	0.49	2.9	7.4	10
13	72.0	0.61	6.1	11.9	11
14	25.4	—	10.1	19	18
15	40.6	—	>20	20	>20
SAHA	87.8	0.065[d]	2.8	2.7	1.6

[a] HDAC inhibitory activity was measured by use of the HDAC Fluorimetric Assay/Drug Discovery Kit.
[b] Not tested.
[c] IC_{50} (μM) data for anti-proliferative activity in HCT-116, HeLa, and MCF-7 cell lines.
[d] The data was obtained from the literature.[29]

2.3. Anti-proliferation

We evaluated the activity of compounds **8**–**15** in human colonic carcinoma cell line (HCT-116), human breast adenocarcinoma cell line (MCF-7) and human cervical carcinoma cell line (HeLa) by a MTT assay. Table 1 showed the IC_{50} values of each compound for above three cell lines. Compounds **9**–**13** displayed strong anti-proliferative activity. SAHA inhibited the proliferation of these tumor cell lines with IC_{50} similar to the values.[29] All compounds displayed their anti-proliferative activities closely matched their HDAC inhibitory activities except compound **15**.

2.4. Structure–activity relationship

From the IC_{50} values, it is clear that most of these derivatives displayed potent anti-proliferative activities against three cancer cell lines. However, these derivatives showed comparatively lesser activity against MCF-7 cell line than HCT-116 and HeLa cell lines. Compound **11** possessed the highest growth inhibitory effect on HCT-116, HeLa and MCF-7 cancer cell lines with IC_{50} values of 4, 4 and 4.8 μM. Anti-proliferative activity of compounds bearing four (**8**, **9**) and seven (**14**, **15**) methylene-linker decreased. Compounds **9** and **11** subsitituted on *meta*-position were found

to be more potent than compounds **8** and **10** subsitituted on *para*-position. Apparently, the *meta*-position substituted on benzene moiety was critical for their anti-proliferative activties. Furthermore, compounds bearing five (**10**, **11**) and six (**12**, **13**) methylene-linker exhibited better HDAC inhibitory properties than four (**8**, **9**) and seven (**14**, **15**) methylene-linked compounds. Compound **11**, bearing five methylene-linker, was found more potent than **10**, which indicated *meta*-position substitute was favorable to HDAC inhibition. Taken together, the results suggested that the inhibition of HDAC played a major role in the anti-proliferative activity of these compounds.

2.5. Cell cycle arrest and apoptosis

As compound **11** displayed the best anti-proliferative activity and HDAC inhibitory activity, we examined the effects of compound **11** on cell cycle by flow cytometry in HCT-116 cells (Fig. 2). Our results demonstrated that the treatment of HCT-116 cells with compound **11** at 0, 5 and 10 μM for 24 h induced G2/M cell cycle arrest. A concentration-dependent increase of cell percentage in subG1 phase was observed from 0% to 44.5%, indicating apoptosis induction. The flow cytometric data clearly indicated that compound **11** induced cell cycle arrest at G2/M phase and apoptosis in HCT-116 cell line.

3. Conclusion

In summary, a series of thieno[3,2-*d*]pyrimidines bearing a hydroxamic acid moiety were synthesized effectively. These compounds exhibited potent HDACs inhibitory activity and anti-proliferative activity in vitro. Compound **11** induced HCT-116 cell cycle arrest at G2/M phase and apoptosis, suggesting it should be further developed as anticancer drug candidate.

4. Experimental part

4.1. Chemical procedures

Solvents were purified in the usual way. TLC was performed on precoated Merck silica Gel 60 F_{254} plates. Flash chromatography was performed on silica gel (100–200 mesh, Qingdao, China). ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker 400 MHz spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts were recorded in ppm values. The high resolution spectra were obtained on a Q-TOF Global Mass (ESIMS).

4.1.1. Thieno[3,2-*d*]pyrimidines-2,4(1*H*,3*H*)-dione (2)

A mixture of 3-amino-2-thiophene carboxylic acid methyl ester (20.8 g, 132.3 mmol) and urea (45.9 g, 661.7 mmol) was heated at 190 °C. After 2 h, 7.5% NaOH solution (100 mL) was poured into the reaction mixture and a white solid precipitated. The solid filtered and the filtrate washed with 2 M HCl. A large amount of white solid was precipitated, filtered and the filter cake was dried to give the white solid product **2** (23.8 g). Yield 98%.

4.1.2. 2,4-Dichlorothieno[3,2-*d*]pyrimidines (3)

Compound **2** (23.8 g, 140.7 mmol) was dissolved in $POC1_3$ (200 mL), the mixture was heated under reflux for 6 h. After

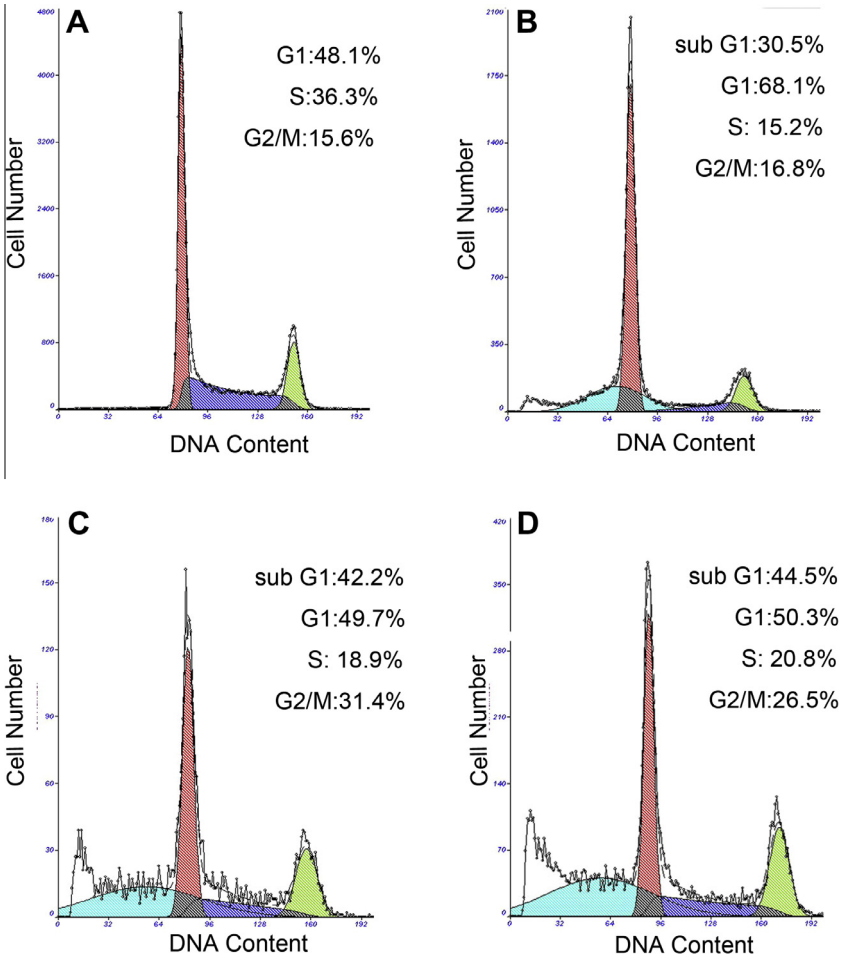


Figure 2. HCT116 cells treated with various concentrations of compound 11 for 24 h. (A) Control, (B) 2.5 μM, (C) 5 μM, (D) 10 μM. Cells were stained by propidium iodide, and cell cycle phases were analyzed by flow cytometry.

removal of most $POC1_3$ under pressure, the remaining reaction solution was slowly poured into ice/water while vigorously stirred yielding a gray precipitate. This was collected by filtration and air-dried to yield a gray solid **3** (16.9 g). Yield 58.3%.

4.1.3. 2-Chloro-4-(morpholin-4-yl)-thieno[3,2-*d*]pyrimidines (4)

To a stirred solution of compound **3** (16.9 g, 83.2 mmol) in dry methanol (300 mL) and morphine (15.9 g, 183.1 mmol) was added. After the addition, the reaction mixture was stirred at room temperature for 1 h. Separated gray solid was filtered and washed with water and methanol. The gray solid was dried and filtered. The crude product was found to be pure and taken to the next step without purification, yield a solid **4** (15.6 g). Yield 73.5%.

4.1.4. 2-Phenol-4-(morpholin-4-yl)-thieno[3,2-*d*]pyrimidines (5a)

A mixture of **4** (1.5 g, 5.9 mmol), 4-hydroxyphenyl boronic acid (1.4 g, 9.9 mmol), K_2CO_3 (1.2 g, 8.8 mmol), Pd $(PPh_3)_4$ (0.2 g, 0.2 mmol) and DMF (20 mL) was heated at 85 °C under nitrogen. After 6 h, the reaction was was poured to H_2O (30 mL) and extracted with ethyl acetate. The combined organic was washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated under vacuum. The residue was recrystallized from ethanol to give a yellowed solid **5a**. Yield 80%.

4.1.5. Ethyl-6-[4-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (6a)

A mixture of **5a** (1.5 g, 4.8 mmol), K_2CO_3 (1.6 g, 11.5 mmol) and 6-bromohexanoate (1.6 g, 7.2 mmol) and DMF (10 mL) was heated at 60 °C. After 12 h, the reaction was was poured to H_2O (30 mL) and extracted with ethyl acetate. The combined organic was washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated under vacuum. The residues were purified by flash column chromatography (petroleum/EtOAc = 3:1) to give compound **6a** (1.4 g) as yellow Oil. Yield 65%. Mp: 86–88 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.35 (d, J = 8.8 Hz, 2H, ArH), 8.23 (d, J = 5.2 Hz, 1H, 6-H), 7.49 (d, J = 5.6 Hz, 1H, 7-H), 7.02 (d, J = 8.8 Hz, 2H, ArH), 4.08 (m, 2H, $-CH_2-$), 4.04 (d, J = 14.1 Hz, 2H, $-CH_2-$), 4.00 (t, J = 4.8 Hz, 4H, 2′-CH_2, 6′-CH_2), 3.81 (t, J = 5.2 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.33 (t, J = 7.2 Hz, 2H, $-CH_2-$), 1.78 (m, 2H, $-CH_2-$), 1.63 (m, 2H, $-CH_2-$), 1.47 (m, 2H, $-CH_2-$), 1.19 (t, J = 7.2 Hz, 3H, $-CH_3$). ^{13}C NMR (DMSO): δ 173.30, 162.99, 160.84, 159.47, 158.25, 148.44, 134.14, 130.79, 129.75, 125.23, 114.55, 114.32, 112.01, 67.84, 67.09, 66.47, 60.14, 40.40, 40.20, 33.92, 28.83, 25.53, 24.71, 14.60. HRMS (ESI) calcd for $C_{24}H_{29}N_3O_4S$ $(M+H)^+$ 456.1952, found 456.1931. IR (KBr) ν (cm^{-1}) = 3447, 2935, 2862, 1731, 1605, 1585, 1306, 1246, 1114, 758.

4.1.6. 6-[4-(4-Morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoa-te (7a)

To a solution of **6a** (0.5 g, 1.1 mmol) in THF (20 mL), $LiOH{\cdot}H_2O$ (0.3 g, 6.6 mmol) was added with stirring at 55 °C. After 3 h, the mixture was cooled to 0 °C and acidified with 2 M HCl. The solid was filtered, washed with H_2O, and dried to give the product, which was taken to next step without purification (0.4 g). Yield 81.7%. Mp: 148–151 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.48 (d, J = 5.6 Hz, 1H, 6-H), 8.43 (d, J = 9.2 Hz, 2H, ArH), 7.77(d, J = 5.2 Hz, 1H, 7-H), 7.13 (d, J = 8.8 Hz, 2H, ArH), 4.15 (d, J = 4.4 Hz, 4H, 2′-CH_2, 6′-CH_2), 4.10 (t, J = 6.4 Hz, 2H, $-CH_2-$), 3.86 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.27 (t, J = 7.2 Hz, 2H, $-CH_2-$), 1.80 (m, 2H, $-CH_2-$), 1.62 (m, 2H, $-CH_2-$), 1.49 (m, 2H, $-CH_2-$). ^{13}C NMR (DMSO): δ 174.92, 161.00, 158.83, 158.18, 148.82, 132.44, 129.84, 128.05, 123.90, 114.62, 112.41, 112.05, 109.80, 67.93, 67.17, 66.45, 40.60, 40.40, 34.10, 28.90, 25.63, 24.76. HRMS (ESI) calcd for $C_{22}H_{25}N_3O_4S$ $(M+H)^+$ 428.1639, found 428.1628. IR (KBr) ν (cm^{-1}) = 3446, 2939, 2360, 2341, 1607, 1585, 1306, 1247, 1172, 935.

4.1.7. 1-Hydroxyamino-6-[4-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (12)

A solution of **7a** (0.4 g, 0.9 mmol), isobutyl chloroformate (0.1 g, 1 mmol), Et_3N (0.2 g, 1.8 mmol) and DMF (10 mL) was added at 0 °C. After 10 min, the reaction was added hydroxylamine hydrochloride (0.1 g, 1.8 mmol) solution in methanol (4 mL), allowed to warm naturally to room temperature and stirred. After 2 h, the reaction was diluted with ethyl acetate, washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The product was purified by column chromatography to give a white solid. Yield 50.5%. R_f = 0.3 (acetone/petroleumther = 1:1). Mp: 176–178 °C. ^{1}H NMR (400 Hz, DMSO): δ 10.37 (s, 1H, –OH), 8.69 (s, 1H, –NH), 8.35 (d, J = 8.8 Hz, 2H, ArH), 8.23 (d, J = 5.6 Hz, 1H, 6-H), 7.49 (d, J = 5.6 Hz, 1H, 7-H), 7.02 (d, J = 8.8 Hz, 2H, ArH), 4.03 (m, 6H, $-CH_2-$, 2′-CH_2, 6′-CH_2), 3.81 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.00 (t, J = 7.2 Hz, 2H, $-CH_2-$), 1.77 (m, 2H, $-CH_2-$), 1.60 (m, 2H, $-CH_2-$), 1.44 (m, 2H, $-CH_2-$). ^{13}C NMR ($CDCl_3$): δ 169.50, 162.98, 160.86, 159.47, 158.26, 134.18, 130.77, 129.76, 125.22, 114.58, 112.01, 67.91, 66.47. HRMS (ESI) calcd for $C_{22}H_{26}N_4O_4S$ $(M+H)^+$ 443.1675, found 443.1755. IR (KBr) ν (cm^{-1}) = 3436, 2241, 2937, 2861, 1657, 1584, 1533, 1400, 1245, 650.

4.1.8. 3-Phenol-4-(morpholin-4-yl)-thieno[3,2-*d*]pyrimidines (5b)

Compound **5b** was prepared from **4** according to the same procedure described for **5a** using 3-hydroxyphenyl boronic acid. Yield 73%. ^{1}H NMR (400 Hz, DMSO): δ 9.51 (s, 1H, 6-H), 8.25 (d, J = 5.6 Hz, 1H, ArH), 7.87 (dd, J = 1.6 Hz, 6.8 Hz, 2H, 7-H, ArH), 7.51 (d, J = 5.6 Hz, 1H, ArH), 7.29 (t, J = 7.6 Hz, 1H, ArH), 6.87 (m, 1H, –OH), 4.01 (t, J = 4.8 Hz, 4H, 2′-CH_2, 6′-CH_2), 3.82 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2. ESI-MS: m/z, 314.0 $[M+H]^+$.

4.1.9. Ethyl-6-[3-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (6b)

Compound **6b** was prepared from **5b** according to the same procedure described for **6a** to give a white solid. Yield 64.2%. Mp: 80–83 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.26 (d, J = 5.6 Hz, 1H, ArH), 8.00 (d, J = 8.0 Hz, 1H, 6-H), 7.93 (m, 1H, ArH), 7.54 (d, J = 5.6 Hz, 1H, ArH), 7.41 (t, J = 8.0 Hz, 7-H), 7.05 (dd, J = 1.6 Hz, 7.2 Hz, 1H, ArH), 4.08 (m, 8H, 2′-CH_2, 6′-CH_2), 3.82 (t, J = 5.2 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.34 (t, J = 7.6 Hz, 2H, $-CH_2-$), 1.79 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), 1.49 (m, 2H, $-CH_2-$), 1.19 (t, J = 7.2 Hz,3H, $-CH_3$). ^{13}C NMR (DMSO): δ 173.32, 162.86, 159.33, 159.24, 158.28, 139.95, 134.38, 129.88, 125.31, 120.57, 116.53, 113.95, 112.78, 67.77, 66.46, 60.13, 46.33, 40.61, 40.40, 33.94, 28.89, 25.58, 24.74, 16.60. HRMS (ESI) calcd for $C_{24}H_{29}N_3O_4S$ $(M+H)^+$ 456.1952, found 456.1934. IR (KBr) ν (cm^{-1}) = 3433, 2970, 2870, 1726, 1595, 1471, 1311, 1233, 1116, 794.

4.1.10. 6-[3-(4-Morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (7b)

Compound **7b** was prepared from **6b** according to the same procedure described for **7a** to give a white solid. Yield 76%. Mp: 169–171 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.50 (d, J = 5.6 Hz, 1H, 6-H), 8.02 (d, J = 7.2 Hz, 2H, ArH), 7.83 (d, J = 5.2 Hz, 1H, 7-H), 7.51 (t, J = 8.0 Hz, 1H, ArH), 7.21 (d, J = 9.2 Hz, 1H, ArH), 4.16 (t, J = 4.4 Hz, 4H, 2′-CH_2, 6′-CH_2-), 4.12 (t, J = 6.4 Hz, 2H, $-CH_2-$), 3.87 (t, J = 4.8 Hz, 5H, 3′-CH_2, 5′-CH_2, –OH), 2.27 (t, J = 7.2 Hz, 2H, $-CH_2-$), 1.80 (m, 2H, $-CH_2-$), 1.63 (m, 2H, $-CH_2-$), 1.50 (m, 2H, $-CH_2-$).^{13}C NMR (DMSO): δ 174.89, 159.42, 157.08, 155.32, 152.81, 138.59, 130.56, 121.50, 120.29, 119.44, 114.62, 113.34, 105.2, 68.25, 66.23, 47.22, 40.60, 40.39, 34.13, 28.89, 25.66, 24.77. HRMS (ESI) calcd for $C_{22}H_{25}N_3O_4S$ $(M+H)^+$ 428.1639, found 428.1632. IR (KBr) ν (cm^{-1}) = 3447, 2952, 2873, 1727, 1571, 1542, 1499, 1453, 1267, 1045.

4.1.11. 1-Hydroxyamino-6-[3-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (13)

Compound **13** was prepared from **7b** according to the same procedure described for **12** to give a white solid. Yield 41%. R_f = 0.3 (acetone/petroleumether = 1:1). Mp: 168–170 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.38 (d, J = 8.8 Hz, 2H, ArH), 8.27 (d, J = 5.2 Hz, 1H, 6-H), 8.11(s, 1H, –NH), 8.01 (d, J = 5.6 Hz, 1H, 7-H), 7.02 (d, J = 8.8 Hz, 2H, ArH), 4.03 (m, 6H, –CH_2–, 2′-CH_2, 6′-CH_2), 3.81 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.00 (t, J = 7.2 Hz, 2H, –CH_2–) 1.77 (m, 2H, –CH_2–), 1.60 (m, 2H, –CH_2–), 1.44 (m, 2H, –CH_2–). ^{13}C NMR ($CDCl_3$): δ 162.85, 159.31, 159.24, 158.26, 139.93, 134.43, 129.90, 125.30, 120.56, 116.51, 113.89, 112.76, 67.81, 66.45, 63.51, 40.46, 40.35, 32.66, 28.96, 25.68, 25.43. HRMS (ESI) calcd for $C_{22}H_{26}N_4O_4S$ $(M+H)^+$ 443.1675, found 443.1759. IR (KBr) ν (cm^{-1}) = 3405, 2931, 2860, 1609, 1540, 1489, 1409, 1275, 1232, 656.

4.1.12. Ethyl-7-[4-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (6c)

Compound **6c** was prepared from **5a** according to the same procedure described for **6a** using 7-bromoheptanoate ethyl to give a yellow solid. Yield 44.5%. Mp: 90–91 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.35 (d, J = 9.2 Hz, 2H, ArH), 8.23 (d, J = 5.2 Hz, 1H, 6-H), 7.49 (d, J = 5.6 Hz, 1H, 7-H), 7.02 (d, J = 9.2 Hz, 2H, ArH), 4.07 (dd, J = 7.2, 14.4 Hz, 4H, –CH_2–, –CH_2–), 4.0 (t, J = 4.4 Hz, 4H, 2′-CH_2, 6′-CH_2), 3.81 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.31 (t, J = 7.2 Hz, 2H, –CH_2–), 1.76 (m, 2H, –CH_2–), 1.59 (m, 2H, –CH_2–), 1.46 (m, 2H, –CH_2–), 1.37 (m, 2H, –CH_2–), 1.19 (t, J = 7.2 Hz, 3H, –CH_3). ^{13}C NMR (DMSO): δ 173.34, 162.99, 160.86, 159.27, 158.25, 134.15, 130.17, 129.75, 125.22, 114.56, 112.01, 112.00, 102,21, 67.91, 66.47, 60.12, 46.32, 40.61, 40.40, 33.90, 28.98, 28.66, 25.67, 24.87, 14.60. HRMS (ESI) calcd for $C_{25}H_{31}N_3O_4S$ $(M+H)^+$ 470.2108, found 470.2089. IR (KBr) ν (cm^{-1}) = 2946, 2863, 1731, 1608, 1537, 1488, 1302, 1278, 1115, 656.

4.1.13. 7-[4-(4-Morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (7c)

Compound **7c** was prepared from **6c** according to the same procedure described for **7a** to give a white solid. Yield 98%. Mp: 128–130 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.52 (d, J = 5.6 Hz, 1H, 6-H), 8.47 (d, J = 9.2 Hz, 2H, ArH), 7.84 (d, J = 5.6 Hz, 1H, 7-H), 7.15 (d, J = 8.8 Hz, 2H, ArH), 4.18 (t, J = 4.0 Hz, 4H, 2′-CH_2, 6′-CH_2), 4.11 (t, J = 6.8 Hz, 2H, –CH_2–), 3.87 (t, J = 5.2 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.24 (t, J = 7.2 Hz, 2H, –CH_2–), 1.79 (m, 2H, –CH_2–), 1.57 (m, 2H, –CH_2–), 1.48 (m, 2H, –CH_2–), 1.38 (m, 2H, –CH_2–). ^{13}C NMR (DMSO): δ 174.93, 166.65, 158.10, 157.30, 149.98, 137.34, 135.73, 130.88, 128.18, 115.03, 113.11, 112.48, 102.20, 68.26, 66.29, 46.93, 40.57, 40.36, 34.09, 28.90, 28.75, 25.68, 24.90. HRMS (ESI) calcd for $C_{23}H_{27}N_3O_4S$ $(M+H)^+$ 442.1795, found 442.1783. IR (KBr) ν (cm^{-1}) = 3424, 2926, 2853, 1652, 1608, 1585, 1302, 1245, 1112, 654.

4.1.14. 1-Hydroxyamino-7-[4-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (14)

Compound **14** was prepared from**7c** according to the same procedure described for **12** to give a white solid. Yield 42%. R_f = 0.3 (acetone/petroleumether = 1:1). Mp: 179–183 °C. ^{1}H NMR (400 Hz, DMSO): δ 10.40 (s, 1H, –OH), 8.68 (s, 1H, –NH), 8.35 (d, J = 8.8 Hz, 2H, ArH), 8.23 (d, J = 5.6 Hz, 1H, 6-H), 7.49 (d, J = 5.6 Hz, 1H, 7-H), 7.02 (d, J = 8.8 Hz, 2H, ArH), 4.03 (m, 6H, –CH_2–, 2′-CH_2, 6′-CH_2), 3.81 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 1.98 (t, J = 7.2 Hz, 2H, –CH_2–), 1.76 (m, 2H, –CH_2–), 1.55 (m, 2H, –CH_2–), 1.45 (m, 2H, –CH_2–), 1.35 (m, 2H, –CH_2–). ^{13}C NMR ($CDCl_3$): δ 169.58, 169.47, 162.98, 160.87, 159.48, 158.25, 134.21, 130.45, 129.77, 128.1, 125.22, 114.69, 112.01, 67.96, 66.47, 49.05, 46.31, 45.80, 32.67, 29.03, 28.81, 25.72, 25.54. HRMS (ESI) calcd for $C_{23}H_{28}N_4O_4S$ $(M+H)^+$ 457.1831, found 457.1911. IR (KBr) ν (cm^{-1}) = 3424, 2926, 2853, 1652, 1608, 1585, 1302, 1245, 1112, 654.

4.1.15. Ethyl-7-[3-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (6d)

Compound **6d** was prepared from **5b** according to the same procedure described for **6a** using 7-bromoheptanoate ethyl to give a white solid. Yield 48%. Mp: 88–90 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.26 (d, J = 5.6 Hz, 1H, 6-H), 8.00 (d, J = 7.6 Hz, 1H, ArH), 7.93 (m, 1H, ArH), 7.54 (d, J = 5.6 Hz, 1H, 7-H), 7.41 (t, J = 8.0 Hz, 1H, ArH), 7.05 (dd, J = 2.8, 8.4 Hz, 1H, ArH), 4.07 (dd, J = 7.2, 14.4 Hz, 4H, –CH_2, –CH_2), 4.01 (t, J = 4.0 Hz, 4H, 2′-CH_2, 6′-CH_2), 3.82 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.31 (t, J = 7.2 Hz, 2H, –CH_2–), 1.78 (m, 2H, –CH_2–), 1.59 (m, 2H, –CH_2–), 1.48 (m, 2H, –CH_2–), 1.38 (m, 2H, –CH_2–), 1.18 (t, J = 7.2 Hz, 3H, –CH_3). ^{13}C NMR (DMSO): δ 173.35, 162.86, 159.34, 159.26, 158.28, 139.95, 134.39, 129.87, 125.30, 120.56, 116.55, 113.93, 112.78, 67.84, 66.46, 60.11, 46.33, 40.61, 40.40, 33.91, 29.03, 28.68, 25.71, 24.88, 14.60. HRMS (ESI) calcd for $C_{25}H_{31}N_3O_4S$ $(M+H)^+$ 470.2108, found 470.2091. IR (KBr) ν (cm^{-1}) = 3446, 3090, 2936, 2589, 1731, 1601, 1538, 1352, 1229, 1116, 656.

4.1.16. 7-[3-(4-Morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (7d)

Compound **7d** was prepared from **6d** according to the same procedure described for **7a** to give a white solid. Yield 98%. Mp: 130–132 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.27 (d, J = 5.2 Hz, 1H, 6-H), 8.01 (d, J = 7.6 Hz, 1H, ArH), 7.94 (t, J = 2.0 Hz, 1H, ArH), 7.55 (d, J = 5.6 Hz, 1H, 7-H), 7.41 (t, J = 8.0 Hz, 1H, ArH), 7.06 (dd, J = 2.4, 8 Hz, 1H, ArH), 4.06 (t, J = 6.4 Hz, 2H, –CH_2–), 4.02 (t, J = 4.8 Hz, 4H, 2′-CH_2, 6′-CH_2), 3.83 (t, J = 5.2 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.24 (t, J = 7.2 Hz, 2H, –CH_2–), 1.79 (m, 2H, –CH_2–), 1.58 (m, 2H, –CH_2–), 1.49 (m, 2H, –CH_2–), 1.39 (m, 2H, –CH_2–). ^{13}C NMR (DMSO): δ 174.99, 162.86, 159.34, 159.26, 158.28, 139.94, 134.38, 129.88, 125.31, 120.55, 116.55, 113.93, 112.77, 67.87, 66.45, 46.33, 40.60, 40.39, 34.13, 29.08, 28.82, 25.78, 24.95. HRMS (ESI) calcd for $C_{23}H_{27}N_3O_4S$ $(M+H)^+$ 442.1795, found 442.1779. IR (KBr) ν (cm^{-1}) = 3474, 3093, 2936, 2863, 1671, 1604, 1538, 1352, 1228, 1117, 656.

4.1.17. 1-Hydroxyamino-7-[3-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (15)

Compound **15** was prepared from **7d** according to the same procedure described for **12** to give a white solid. Yield 40.3%. R_f = 0.3 (acetone/petroleumether = 1:1). Mp: 180–185 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.38 (d, J = 8.8 Hz, 2H, ArH), 8.27 (d, J = 5.2 Hz, 1H, 6-H), 8.01 (s, 1H, –NH), 8.01 (d, J = 5.6 Hz, 1H, 7-H), 7.02 (d, J = 8.8 Hz, 2H, ArH), 4.03 (m, 6H, –CH_2–, 2′-CH_2, 6′-CH_2), 3.81 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.00 (t, J = 7.2 Hz, 2H, –CH_2–), 1.77 (m, 2H, –CH_2–), 1.60 (m, 2H, –CH_2–), 1.44 (m, 2H, –CH_2–), 1.35 (m, 2H, –CH_2–). ^{13}C NMR ($CDCl_3$): δ 171.57, 162.00, 160.23, 159.29, 158.14, 139.45, 132.79, 130.95, 128.98, 124.01, 116.16, 113.69, 112.79, 77.51, 67.59, 66.43, 47.91, 47.70, 32.38, 29.38, 28.91, 25.51, 25.34. HRMS (ESI) calcd for $C_{23}H_{28}N_4O_4S$ $(M+H)^+$ 457.1831, found 457.1910. IR (KBr) ν (cm^{-1}) = 3424, 2930, 2853, 2360, 1633, 1540, 1351, 1278, 1115, 656.

4.1.18. Ethyl-4-[4-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (6e)

Compound **6e** was prepared from **5a** according to the same procedure described for **6a** using 4-bromobutyrate to give a white solid. Yield 85%. Mp: 87–88 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.36 (d, J = 8.8 Hz, 2H, ArH), 8.23 (d, J = 5.6 Hz, 1H, 6-H), 7.50 (d, J = 5.6 Hz, 1H, 7-H), 7.03 (d, J = 8.8 Hz, 2H, ArH), 4.11 (m, 4H, –CH_2, –CH_2), 4.00 (t, J = 4.8 Hz, 4H, 2′-CH_2, 6′-CH_2), 3.82 (t, J = 5.2 Hz, 4H,

3′-CH_2, 5′-CH_2), 2.50 (t, J = 8.8 Hz, 2H, –CH_2–), 2.04 (m, 2H, –CH_2–), 1.21 (t, J = 7.2 Hz, 3H, ––CH_3). ^{13}C NMR (DMSO): δ 173.01, 162.98, 160.64, 159.44, 158.26, 145.88, 134.16, 130.95, 129.77, 125.23, 114.58, 114.27, 112.04, 67.05, 66.47, 64.28, 60.36, 40.61, 40.41, 30.61, 24.7, 14.58. HRMS (ESI) calcd for $C_{22}H_{25}N_3O_3S$ $(M+H)^+$ 428.1639, found 428.1634. IR (KBr) ν (cm^{-1}) = 3097, 3076, 2957, 2906, 2850, 1735, 1608, 1364, 1248, 1114, 762.

4.1.19. 4-[4-(4-Morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (7e)

Compound **7e** was prepared from **6e** according to the same procedure described for **7a** to give a white solid. Yield 94%. Mp: 183–185 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.53 (d, J = 5.6 Hz, 1H, ArH), 8.45 (d, J = 8.8 Hz, 2H, 6-H), 7.82 (d, J = 5.6 Hz, 1H, 7-H), 7.16 (d, J = 8.8 Hz, 2H, ArH), 4.19 (t, J = 4.4 Hz, 4H, 2′-CH_2, 6′-CH_2), 4.14 (t, J = 6.4 Hz, 2H, –CH_2), 3.87 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.44 (t, J = 7.2 Hz, 2H, –CH_2–), 2.03 (m, 2H, –CH_2–).^{13}C NMR (DMSO): δ 174.50, 162.77, 156.98, 156.69, 138.50, 136.66, 131.23, 128.29, 118.29, 115.23, 113.57, 112.66, 102.39, 67.58, 67.37, 66.24, 40.60, 40.39, 30.50, 24.56. HRMS (ESI) calcd for $C_{20}H_{21}N_3O_4S$ $(M+H)^+$ 400.1339, found 400.1325. IR (KBr) ν (cm^{-1}) = 3460, 3299, 2909, 2867, 1731, 1590, 1306, 1263, 1115, 759.

4.1.20. 1-Hydroxyamino-4-[4-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (8)

Compound **8** was prepared from **7e** according to the same procedure described for **12** to give a white solid. Yield 66%. R_f = 0.3 (acetone/petroleumether = 1:1). Mp: 165–168 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.53 (d, J = 5.6 Hz, 1H, 6-H), 8.45 (d, J = 8.8 Hz, 2H, ArH), 7.83 (d, J = 5.6 Hz, 1H, 7-H), 7.15 (d, J = 8.8 Hz, 2H, ArH), 4.18 (s, 4H, 2′-CH_2, 6′-CH_2), 4.12 (t, J = 6.4 Hz, 2H, –CH_2–), 3.86 (s, 4H, 3′-CH_2, 5′-CH_2), 3.62 (s, 1H, –NH), 2.19 (t, J = 7.2 Hz, 2H, –CH_2–), 2.00 (t, J=6.4 Hz, 2H, –CH_2–). ^{13}C NMR ($CDCl_3$): δ 169.02, 161.81, 157.9, 157.5, 131.11, 130.32, 115.27, 114.76, 112.62, 99.99, 67.10, 66.27, 56.61, 40.49, 40.38, 29.07, 25.12. HRMS (ESI) calcd for $C_{20}H_{22}N_4O_4S$ $(M+H)^+$ 414.1445, found 414.1362. IR (KBr) ν (cm^{-1}) = 3417, 3201, 2924, 2873, 1726, 1676, 1592, 1309, 1263, 1196, 777.

4.1.21. Ethyl-4-[3-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (6f)

Compound **6f** was prepared from **5a** according to the same procedure described for **6a** using 4-bromobutyrate to give a white solid. Yield 80%. Mp: 90–92 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.36 (d, J = 8.8 Hz, 2H, ArH), 8.23 (d, J = 5.2 Hz, 1H, 6-H), 7.50 (d, J = 5.6 Hz, 1H, 7-H), 7.03 (d, J = 9.2 Hz, 2H, ArH), 4.11 (m, 4H, –CH_2, –CH_2), 4.01 (t, J = 4.8 Hz, 4H, 2′-CH_2, 6′-CH_2), 3.82 (t, J = 5.2 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.50 (t, J = 8.4 Hz, 2H, –CH_2–), 2.04 (m, 2H, –CH_2–), 1.21 (t, J = 6.8 Hz, 3H, –CH_3). ^{13}C NMR (DMSO): δ 173.02, 162.98, 160.64, 159.44, 158.26, 147.93, 134.18, 130.94, 129.77, 128.08, 125.23, 114.58, 112.04, 74.96, 67.06, 66.47, 60.36, 40.61, 40.40, 30.61, 24.7, 14.58. HRMS (ESI) calcd for $C_{22}H_{25}N_3O_3S$ $(M+H)^+$ 428.1639, found 428.1630. IR (KBr) ν (cm^{-1}) = 3435, 3097, 2962, 2897, 2858, 1725, 1608, 1302, 1251, 1119, 762.

4.1.22. 4-[3-(4-Morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (7f)

Compound **7f** was prepared from **6f** according to the same procedure described for **7a** to give a white solid. Yield 80%. Mp: 179–180 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.51 (d, J = 5.6 Hz, 1H, ArH), 8.43 (d, J = 8.8 Hz, 2H, 6-H), 7.78 (d, J = 5.6 Hz, 1H, 7-H), 7.16 (d, J = 9.2 Hz, 2H, ArH), 4.18 (t, J = 4.4 Hz, 4H, 2′-CH_2, 6′-CH_2), 4.14 (t, J = 6.4 Hz, 2H, –CH_2), 3.87 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.44 (t, J = 7.2 Hz, 2H, –CH_2–), 2.03 (m, 2H, –CH_2–). ^{13}C NMR (DMSO): δ 174.53, 172.38, 157.60, 150.35, 130.54, 136.65, 131.24, 128.28, 118.27, 115.22, 114.95, 112.37, 102.34, 67.38, 67.36, 66.34, 40.59, 40.38, 30.54, 24.62. HRMS (ESI) calcd for $C_{20}H_{21}$ N_3O_4S $(M+H)^+$ 400.1339, found 400.1321. IR (KBr) ν (cm^{-1}) = 3454, 2922, 2867, 1731, 1608, 1590, 1307, 1262, 1182, 778.

4.1.23. 1-Hydroxyamino-4-[3-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (9)

Compound **9** was prepared from **7f** according to the same procedure described for **12** to give a white solid. Yield 89.6%. R_f = 0.3 (acetone/petroleumether = 1:1). Mp: 175–177 °C. ^{1}H NMR (400 Hz, DMSO): δ 10.35 (s, 1H, –OH), 8.55 (d, J = 5.6 Hz, 1H, 6-H), 8.47 (d, J = 8.4 Hz, 2H, ArH), 7.89 (d, J = 5.6 Hz, 1H, 7-H), 7.13 (d, J = 8.8 Hz, 2H, ArH), 4.18 (s, 4H, 2′-CH_2, 6′-CH_2), 4.10 (t, J = 6.0 Hz, 2H, –CH_2–), 3.86 (s, 4H, 3′-CH_2, 5′-CH_2), 3.08 (m,1H, –NH–), 2.19 (t, J = 7.2 Hz, 2H, –CH_2–), 2.00 (t, J = 6.8 Hz, 2H, –CH_2–). ^{13}C NMR ($CDCl_3$): δ 174.74, 169.31, 158.93, 157.34, 147.86, 136.18, 130.99, 129.22, 115.18, 114.02, 106.15, 99.99, 69.37, 67.58, 66.29, 40.56, 40.35, 29.07, 25.72. HRMS (ESI) calcd for $C_{20}H_{22}N_4O_4S$ $(M+H)^+$ 414.1459, found 414.1362. IR (KBr) ν (cm^{-1}) = 3416, 3200, 2924, 2873, 1726, 1677, 1592, 1264, 1196, 777.

4.1.24. Ethyl-5-[4-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (6g)

Compound **6g** was prepared from **5b** according to the same procedure described for **6a** using 5-bromo-pentanoate ethyl to give a white solid. Yield 96%. Mp 96–98 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.36 (d, J = 8.8 Hz, 2H, ArH), 8.22 (d, J = 5.6 Hz, 1H, 6-H), 7.50 (d, J = 5.6 Hz, 1H, 7-H), 7.02 (d, J = 8.8 Hz, 2H, ArH), 4.09 (m, 6H, –CH_2, –CH_2, –CH_2), 4.00 (t, J = 4.8 Hz, 4H, 2′-CH_2, 6′-CH_2), 3.82 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.51(d, J = 1.6 Hz, 2H, –CH_2–), 2.40 (t, J = 7.2 Hz, 2H, –CH_2–), 1.20 (m, 3H, –CH_3).^{13}C NMR (DMSO): δ 173.23, 162.98, 160.78, 159.46, 158.26,134.14, 133.51, 130.83, 130.66, 129.75, 125.22, 114.57, 112.02, 67.62, 66.47, 60.20, 46.31, 40.61, 40.41, 33.62, 28.49, 21.67, 14.60. HRMS (ESI) calcd for $C_{22}H_{25}N_3O_3S$ $(M+H)^+$ 442.1795, found 442.1779. IR (KBr) ν (cm^{-1}) = 3449, 3090, 2984, 2869, 1736, 1606, 1306, 1252, 1118, 740.

4.1.25. 5-[4-(4-Morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (7g)

Compound **7g** was prepared from **6g** according to the same procedure described for **7a** to give a white solid. Yield 94%. Mp: 195–197 °C. ^{1}H NMR (400 Hz, DMSO): δ 8.52 (d, J = 5.6 Hz, 1H, 6-H), 8.43 (d, J = 8.8 Hz, 2H, ArH), 7.79 (d, J = 5.6 Hz, 1H, 7-H), 7.16 (d, J = 8.8 Hz, 2H, ArH), 4.19 (t, J = 5.2 Hz, 4H, 2′-CH_2, 6′-CH_2), 4.13 (t, J = 6.4 Hz, 2H, –CH_2), 3.87 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.33 (t, J = 7.6 Hz, 2H, –CH_2–), 1.82 (m, 2H, –CH_2–), 1.71 (m, 2H, –CH_2–). ^{13}C NMR (DMSO): δ 174.81, 167.03, 162.83, 159.46, 157.05, 140.07, 136.38, 131.19, 124.82, 115.22, 113.20, 112.63, 92.26, 68.13, 67.05, 66.25, 40.59, 40.38, 33.74, 28.44, 21.62. HRMS (ESI) calcd for $C_{21}H_{23}$ N_3O_4S $(M+H)^+$ 414.1482, found 414.1480. IR (KBr) ν (cm^{-1}) = 3466, 3100, 2933, 2874, 1721, 1608, 1591, 1306, 1263, 1115, 776.

4.1.26. 1-Hydroxyamino-5-[4-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (10)

Compound **10** was prepared from **7g** according to the same procedure described for **12** to give a white solid. Yield 50.6%. R_f = 0.3 (acetone/petroleumether = 1:1). Mp: 144–147 °C. ^{1}H NMR (400 Hz, DMSO): δ 10.45 (s, 1H, –OH), 8.54 (d, J = 5.6 Hz, 1H, 6-H), 8.45 (d, J = 8.8 Hz, 2H, ArH), 7.83 8(d, J = 5.6 Hz, 1H, 7-H), 7.16 (d, J = 8.8 Hz, 2H, ArH), 4.18 (s, 4H, 2′-CH_2, 6′-CH_2), 4.12 (t, J = 6.0 Hz, 2H, –CH_2–), 3.87 (t, J = 8.8 Hz, 5H, 3′-CH_2, 5′-CH_2, –NH–), 2.51 (t, J = 1.6 Hz, 4H, –CH_2–, –CH_2–), 3.08 (m, 1H, –NH), 2.08

(m, 2H, $-CH_2-$). ^{13}C NMR ($CDCl_3$): δ 171.94, 169.35, 164.34, 162.86, 157.06, 153.70, 136.13, 131.14, 124.61, 119.35, 115.28, 112.63, 68.09, 67.48, 66.25, 40.59, 40.38, 32.33, 28.50, 22.20. HRMS (ESI) calcd for $C_{21}H_{24}N_4O_4S$ $(M+H)^+$ 428.1601, found 428.1518. IR (KBr) ν (cm^{-1}) = 3423, 3170, 2923, 2869, 1651, 1560, 1373, 1278, 1182, 776.

4.1.27. Ethyl-5-[3-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (6h)

Compound **6h** was prepared from **5b** according to the same procedure described for **6a** using 5-bromo-pentanoate ethyl to give a white solid. Yield 80%. Mp: 75–77 °C. ^1H NMR (400 Hz, DMSO): δ 8.25 (d, J = 5.6 Hz, 1H, ArH), 8.01 (d, J = 7.6 Hz, 1H, 6-H), 7.93 (s, 1H, ArH), 7.54 (d, J = 5.2 Hz, 1H, 7-H), 7.41 (t, J = 8 Hz, 1H, ArH), 7.05 (dd, J = 2.4, 8 Hz, 1H, ArH), 4.09 (m, 5H, $-CH_2$, $-CH_2$, $-CH_2$), 4.01 (t, J = 4.4 Hz, 4H, 2′-CH_2, 6′-CH_2), 3.82 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.51 (d, J = 1.6 Hz, 1H, $-CH_2-$), 2.40 (t, J = 7.2 Hz, 2H, $-CH_2-$), 2.34 (t, J = 7.6 Hz, 1H, $-CH_2-$), 1.20 (t, J = 7.2 Hz, 4H, $-CH_2-$, $-CH_3$). ^{13}C NMR ($CDCl_3$): δ 173.26, 162.83, 159.31, 159.18, 158.27, 139.95, 134.36, 129.87, 125.29, 120.61, 116.52, 113.97, 112.78, 67.54, 66.46, 60.19, 46.33, 40.41, 40.20, 33.65, 28.56, 21.72, 14.59. HRMS (ESI) calcd for $C_{22}H_{25}N_3O_3S$ $(M+H)^+$ 442.1793, found 442.1779. IR (KBr) ν (cm^{-1}) = 3101, 2974, 2935, 2868, 1713, 1537, 1304, 1268, 1187, 746.

4.1.28. 5-[3-(4-Morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (7h)

Compound **7h** was prepared from **6h** according to the same procedure described for **7a** to give a white solid. Yield 80%. Mp: 180–182 °C. ^1H NMR (400 Hz, DMSO): δ 8.47 (d, J = 5.2 Hz, 1H, 6-H), 8.00 (m, 2H, ArH), 7.74 (d, J = 5.6 Hz, 1H, 7-H), 7.21 (dd, J = 2.8 Hz, 1H, ArH), 4.15 (t, J = 4.8 Hz, 4H, 2′-CH_2, 6′-CH_2), 4.11 (t, J = 6 Hz, 2H, $-CH_2$), 3.86 (t, J = 4.8 Hz, 4H, 3′-CH_2, 5′-CH_2), 2.34 (t, J = 7.2 Hz, 2H, $-CH_2-$), 1.80 (m, 2H, $-CH_2-$), 1.73 (m, 2H, $-CH_2-$). ^{13}C NMR (DMSO): δ 174.70, 167.03, 162.83, 159.44, 157.05, 140.07, 136.38, 130.01, 121.19, 114.42, 113.16, 112.63, 92.26, 68.12, 67.84, 66.41, 40.90, 40.60, 33.80, 28.62, 21.73 HRMS (ESI) calcd for $C_{21}H_{23}$ N_3O_4S $(M+H)^+$ 414.1486, found 414.1485. IR (KBr) ν (cm^{-1}) = 3424, 3106, 2939, 2864, 1716, 1586, 1570, 1323, 1263, 1113, 776.

4.1.29. 1-Hydroxyamino-5-[3-(4-morpholin-4-yl-thieno[3,2-*d*]pyrimidines-2-yl)phenoxy]hexanoate (11)

Compound **11** was prepared from **7h** according to the same procedure described for **12** to give a white solid. Yield 25.4%, R_f = 0.3 (acetone/petroleumether =1:1). Mp 144–146 °C. ^1H NMR (400 Hz, DMSO): δ 10.45 (s, 1H, –OH), 8.54 (d, J = 5.6 Hz, 1H, 6-H), 8.45 (d, J = 8.8 Hz, 2H, ArH), 7.83 (d, J = 5.6 Hz, 1H, 7-H), 7.16 (d, J = 8.8 Hz, 2H, ArH), 4.18 (s, 4H, 2′-CH_2, 6′-CH_2), 4.12 (t, J = 6.0 Hz, 2H, $-CH_2-$), 3.87 (t, J = 8.8 Hz, 5H, 3′-CH_2, 5′-CH_2, –NH), 2.51 (t, J = 1.6 Hz, 4H, $-CH_2-$, $-CH_2-$), 3.08 (m, 1H, –NH), 2.08 (m, 2H, $-CH_2-$). ^{13}C NMR ($CDCl_3$): δ 169.38, 165.94, 159.38, 157.21, 139.81, 132.92, 130.62, 120.91, 117.61, 114.63, 113.29, 106.80, 68.60, 66.87, 66.26, 40.54, 40.33, 32.33, 28.55, 22.27. HRMS (ESI) calcd for $C_{21}H_{24}N_4O_4S$ $(M+H)^+$ 428.1604, found 428.1518. IR (KBr) ν (cm^{-1}) = 3422, 3205, 2928, 2866, 1715, 1648, 1587, 1375, 1228, 778.

4.2. Biological methods

4.2.1. In vitro HDAC inhibition

In vitro HDAC inhibition was measured by the HDAC Fluorimetric Assay/Drug Discovery Kit as previously described.[24] Briefly, 15 μL of HeLa nuclear extract was mixed with 10 μL of 5× compound. Fluorogenic substrate (25 μL) was added, and reaction was allowed to proceed for 15 min at room temperature and then stopped by addition of a developer containing TSA. Fluorescence was monitored after 15 min at excitation and emission wavelengths of 360 and 460 nm, respectively.

4.2.2. In vitro antiproliferative assays

Antiproliferative cellular assays were conducted as described using HCT-116, MCF-7 and HeLa cells. These cancer cells were a generous gift from Jiangsu University. All cell lines were cultured in a 37 °C incubator with a 5% CO_2 environment. Compounds were dissolved in DMSO with a concentration of 10 mM. Cells were seeded into 96-well plates at a concentration of 2×10^3 per well, cultured for 24 h, exposed to the compounds at various concentrations for cancer cell viability experiments, cells were cultured for 72 h and viability was determined through the use of the MTT assay.

4.2.3. Cell cycle analysis by FACS

HCT-116 cells were seeded into six-well plates at a concentration of 2×10^5 per well, exposed to the compounds at various concentrations, cultured for 24 h, collected, fixed with 70% cold ethanol,stained with PI-solution (50 μg/mL PI from 50× stock solution (2.5 mg/mL), 0.1 mg/mL RNase A, 0.05% Tritin X-100), incubated for 40 min at 37 °C and analyzed by FACS using the Gallios flow cytometry system (Beckman Coulter, Brea, CA, USA).

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