European Journal of Medicinal Chemistry 44 (2009) 4470–4476

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis and preliminary biological evaluation of *N*-hydroxy -4-(3-phenylpropanamido)benzamide (HPPB) derivatives as novel histone deacetylase inhibitors

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ARTICLE INFO

Article history:
Received 26 February 2009
Received in revised form
29 May 2009
Accepted 8 June 2009
Available online 17 June 2009

Keywords:
HPPB
N-Hydroxybenzamide
Histone deacetylase inhibitors
Anticancer agents
Cell-cycle arrest

ABSTRACT

A novel series of *N*-hydroxy-4-(3-phenylpropanamido)benzamide (HPPB) derivatives comprising *N*-hydroxybenzamide group as zinc-chelating moiety were designed, synthesized and evaluated for their ability to inhibit histone deacetylases. These compounds possessed inhibitory activity against the enzymes with IC_{50} values as low as 4.0 μM. Among them, the thiophene substituted derivative **5j** ($IC_{50} = 0.3$ μM) and benzo[*d*][1,3]dioxole derivative **5t** ($IC_{50} = 0.4$ μM) exhibited good antiproliferative activity against the growth of human colon carcinoma cell line HCT116 and non-small cell lung cancer cell (NSCLC) line A549. In addition, they were found to potently induce cell-cycle arrest at G2 phase.

1. Introduction

Histone deacetylases (HDACs) take charge of modification and remodeling of chromosomal histones by removing acetyl groups from ε-NH_2 of lysine residues in histones through zinc-dependent hydrolysis [1]. Acetylation results in the positive charge density on the N-termini of nucleosomal histone increases, which strengthens the interaction with the negative-charged DNA chain and blocks the access of RNA polymerase and transcription factor (TF) to DNA [2].

In many cancer cell lines, HDACs are over-expressed resulting in excessive deacetylated histones being tightly packed with DNA to form an abnormal "compact structure" of chromatin. In this process, expression of the cell-cycle inhibitor $p21^{WAF1}$ is inhibited and activity of the onco-suppressor p53 is down-regulated, whereas tumor activators HIF-1 and VEGF are up-regulated. Therefore, the inhibition of HDAC activity is considered as a potential strategy for cancer therapy [3]. HDAC inhibitors (HDACi) become epigenetic anticancer agents due to their capacity to achieve significant biological effects in preclinical models of cancer. They can induce cell growth arrest, differentiation and apoptotic cell death of transformed cells in vitro and vivo [4,5]. Some HDACi have been developed into clinical trials for therapy of various solid tumors and hematological malignancy [6–8]. One inhibitor, Vorinostat (Zolinza®, SAHA), has been approved by FDA for treatment of cutaneous T-cell lymphoma (CTCL) in 2006 and became the first HDACi therapeutic agent [9]. Vorinostat is a representative linear fatty chain HDACi, and the common features of this class of HDACi have been summarized by Manfred and Soon (Fig. 1) [10,11]. There are two rules to the structure–activity relationship (SAR) of Vorinostat-like derivatives: 1) introduction of hydrophobic groups to the *para* position of the benzene ring results in a higher level of activity, and 2) the optimal chain length between the benzene ring and zinc-binding group (ZBG) is 7–8 atoms. As reported in the literature [8,12], three structural units are essential for an HDACi, an aromatic group (Ar), a zinc-binding group (ZBG) and a spacer linking Ar and ZBG. *N*-Hydroxy-4-(3-phenylpropanamido)benzamide (HPPB) [13], another HDACi which contains *N*-hydroxybenzamide as zinc-chelating moiety could provide a molecular framework for the design of novel HPPB-like HDACi. However, so far the structure–activity relationship (SAR) information for HPPB-like derivatives was not reported. In this paper, the design, synthesis and biological evaluation of a novel series of HPPB derivatives as potent HDACi has been described.

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doi:10.1016/j.ejmech.2009.06.010

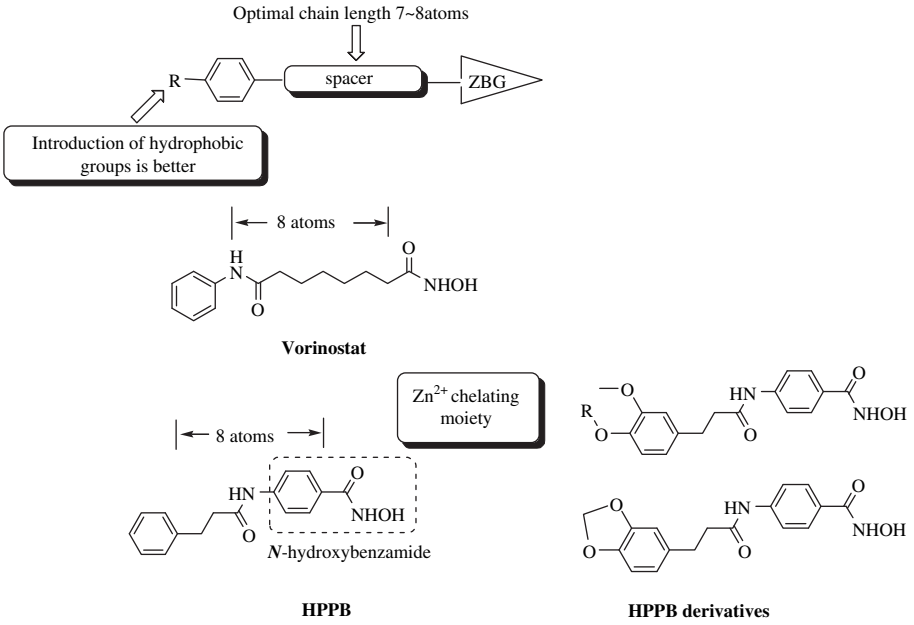


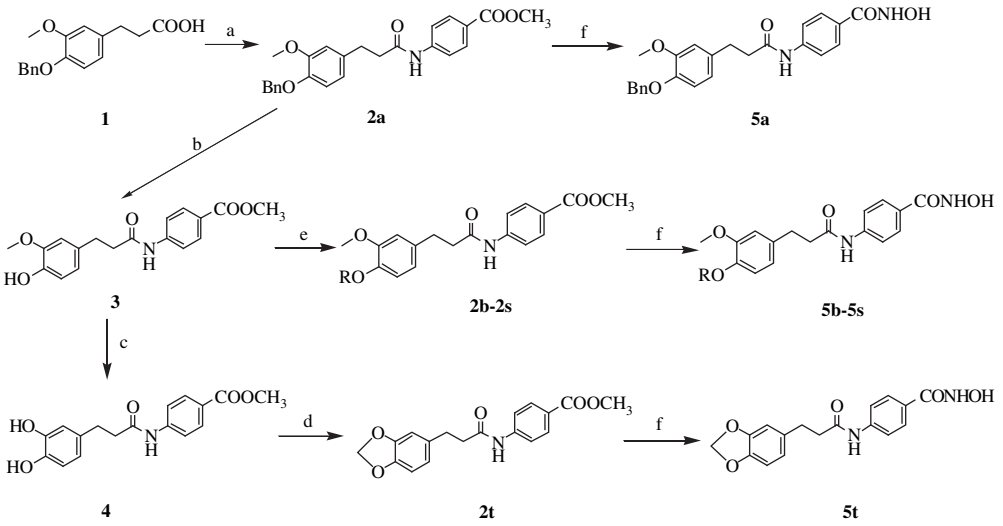
Fig. 1. Structural features of HPPB derivatives.

2. Chemistry

Twenty compounds selected for biological evaluation were prepared as described in Scheme 1. The synthesis of compound **5a** has been described in our previous paper [14], and the synthetic route has been modified based on chemical experimental details of this paper. 3-(4-(Benzyloxy)-3-methoxyphenyl)propanoic acid (**1**) was coupled with methyl 4-aminobenzoate in the presence of dimethylpropyl ethyl carbodiimide hydrochloride (EDCI) to obtain the coupled product (**2a**). Deprotection of **2a** using 10% Pd/C in MeOH yielded the corresponding phenol product (**3**). Etherification of compound **3** with various alkyl bromides and benzyl chloride generated phenolic ether derivatives **2b–s**. Deprotection of methyl group in compound **3** with boron tribromide produced diphenol derivative (**4**), which was closed to form a benzo[*d*][1,3]dioxole ring containing derivative (**2t**) with diiodomethane. Compounds **2a–t** were hydrolyzed with sodium hydroxide to give carboxylic acids, followed by condensation with isobutylchloroformate (ClCOOBu-*i*) and then reaction with hydroxylamine in THF yields the final hydroximic acid compounds **5a–t**.

3. Results and discussion

In vitro bioactivity evaluation of compounds **5a–t** was performed by HDAC activity assays using an HDAC colorimetric activity assay kit (AK501, Biomol Research Laboratories). The source of HDACs was HeLa nuclear extracts comprising HDAC1 and HDAC2 (the major contributors to HDAC activity in HeLa nuclear extracts), and the substrate was a type of [^{3}H]acetylated histone peptide. HDAC1 and HDAC2 are known to be the key nucleus enzymes in charge of deacetylation of histones [15]. Assays were performed according to kit instructions. The test results of compounds **5a–t** for inhibitory activity against HDACs *in vitro* have been presented in Table 1. Vorinostat was used as positive control and IC_{50} values for both HPPB and its derivatives have been determined. These compounds showed potent inhibitory activities against HDACs with IC_{50} values as low as 4.0 μM. The alkyl substituted derivatives (**5b–h**) with IC_{50} values ranging from 1.6 μM to 4.0 μM displayed the order of increasing activity of R as: *n*-butyl > *n*-propyl > *s*-butyl > isopentyl ≈ ethyl > methyl > cyclopentyl. The most potent one is *n*-butyl substituted derivative **5e** which is still less potent than the benzylether derivative **5a**. Compound **5j**, the thiophene analogue of **5a**, was 1-fold more potent than **5a**, whereas compound **5i**, the pyridine analogue of **5a**, showed very poor inhibitory potency. Compounds **5k–s** are various substituted derivatives of the lead compound **5a**. Among them, *ortho* bromine substituted benzylether derivative **5n** was the most potent one and nearly as potent as Vorinostat. Shifting the Br from *ortho* position into the *para* (**5p**) or *meta* (**5o**) position resulted in a little loss of inhibitory potency, while the chlorine substituted benzylether derivatives (**5q–s**) and methyl substituted benzylether derivatives (**5k–m**) were all less potent than **5n**. In addition, benzo[*d*][1,3]-dioxole derivative **5t** exhibited higher inhibitory potency than Vorinostat. In general, this series of compounds showed potent inhibitory activities against HDACs. Among them, compounds **5a**, **5j**, **5n**, **5o**, **5p** and **5t** were found to be more potent than the paradigmatic hydroxamate HDACi-HPPB, furthermore **5j** and **5t** exhibited higher inhibitory activity than Vorinostat. Therefore, compounds **5a**, **5j**, **5n** and **5t** were chosen as the representative active ones for evaluation of their antiproliferative activities against the growth of cancer cells. Human colon carcinoma cell line HCT116 was the primary antiproliferative model *in vitro* because HDACs express very highly in this cell line. Besides it, non-small cell lung cancer cell line A549 is a minor model *in vitro* due to its low expressed level of HDACs. The difference of the expressed level of HDACs between the two cancer cell lines was utilized for better elucidation of anticancer activities of the compounds. The antiproliferative activities were determined by MTT assays and the results have been summarized in Table 2. These compounds were found to have potent antiproliferative activities compared with Vorinostat. Compound **5j** greatly inhibits the carcinoma cells' growth with IC_{50} values of 0.03 mM (HCT116) and 0.08 mM (A549), **5t** was a little less potent than **5j** with IC_{50} values of 0.06 mM (HCT116) and 0.5 mM (A549). They are both more potent than Vorinostat with IC_{50} values of 0.08 mM (HCT116) and 0.6 mM (A549). Compound **5a** and its bromine analogue **5n** were nearly as potent as Vorinostat. Further study was made on the effects of **5j** and **5t** upon the cell-cycle by flow cytometric analysis. It has been known that many HDACi including Vorinostat can potently induce cell-cycle arrest at G1 phase or G2 phase which is associated with



Scheme 1. Reagents and conditions: a) EDCI, Et_3N, THF, methyl 4-aminobenzoate, r.t.; b) H_2, 10% Pd–C, CH_3OH, r.t. c) BBr_3, CH_2Cl_2, −40 °C; d) K_2CO_3, CH_2I_2, acetone, reflux; e) KOH, RBr, DMF, 50 °C; f) 2 mol/L NaOH, EtOH, 75 °C (for two steps); ClCOOBu-*s*, Et_3N, THF, $NH_2OH\cdot HCl$, r.t.

activation of cyclin-dependent kinase (CDK) inhibitor p21 expression or apoptotic cell death pathway [14]. The effects of **5j** and **5t** on the cell-cycle were measured against human HCT116 cells at the concentration of 1 μmol/L. The analytic results have been shown in Table 3. The population proportion of HCT116 cells in each phase of cell-cycle has been calculated. The two compounds were found to strongly induce cell-cycle arrest at G_2 phase which is associated with p21-independent apoptotic cell death pathway and the anticancer mechanism of these two compounds is worthy of further scrutiny in the future.

4. Conclusion

In conclusion, we have synthesized a novel series of HPPB derivatives with *N*-hydroxybenzamide as zinc-chelating moiety to inhibit HDACs. Several HPPB derivatives exhibited subnanomolar inhibitory activities against HDACs with IC_{50} values below 1 μM. In this series, the thiophene substituted derivative **5j** ($IC_{50} = 0.3$ μM) and benzo[*d*][1,3]dioxole derivative **5t** ($IC_{50} = 0.4$ μM) were the most active and notably inhibited the growth of human carcinoma cells HCT116 and A549. In addition, they can potently induce cell-cycle arrest in G2 phase which exhibits their good anticancer bioactivities. They can be good lead compounds for the development of novel anticancer agents in future.

5. Experimental procedure

5.1. Chemistry

5.1.1. General

All materials and reagents used in this work are analytical reagents. All reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates ($60GF_{254}$) and visualized with UV light. ^{1}H NMR spectra were obtained on a Brucker Avance 300 spectrometer using TMS as an internal standard in DMSO-d_6 solutions. Chemical shifts were reported in ppm (δ) downfield from TMS. All the coupling constants (J) are in hertz. IR spectra were recorded on a Perkin–Elmer FTIR 1600 spectrometer. ESI-MS were determined on an API 4000 spectrometer. Melting points were determined on an electrothermal melting point apparatus and without correction. All reported yields are for purified products.

5.1.2. Methyl 4-(3-(4-(benzyloxy)-3-methoxyphenyl) propanamido)benzoate (**2a**)

To a solution of 3-(4-(benzyloxy)-3-methoxyphenyl)propanoic acid **1** (9.0 g, 32 mmol) in dry THF (200 mL) were added EDCI (7.4 g, 38.4 mmol) and Et_3N (9.0 mL, 64 mmol) at room temperature. The mixture was stirred for 2 h and then a solution of methyl 4-aminobenzoate (4.8 g, 32 mmol) in dry THF (100 mL) was added into this mixture. After stirring for 6 h, the reacted mixture was concentrated under reduced pressure. To the residue was added 1 mol/L HCl (100 mL), and then extracted by ethyl acetate (3 × 100 mL), washed with water (3 × 100 mL) and dried over Na_2SO_4. After filtration and evaporation of the solvent *in vacuo*, methyl 4-(3-(4-(benzyloxy)-3-methoxyphenyl)propanamido)benzoate **2a** was obtained as white crystal. (9.6 g, 72.5%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.25 (s, 1H), 7.92–7.71 (dd, 4H), 7.43–7.31 (m, 5H), 6.94–6.71 (m, 3H), 5.02 (s, 2H), 3.82 (s, 3H), 3.73 (s, 3H), 2.85 (t, 2H, $J = 7.5$ Hz), 2.64 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 420.4 (MH^+).

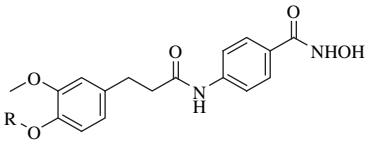
5.1.3. Methyl 4-(3-(4-hydroxy-3-methoxyphenyl) propanamido)benzoate (**3**)

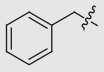
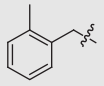
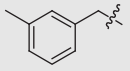
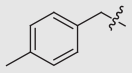
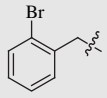
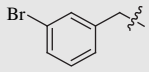
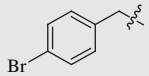
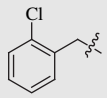
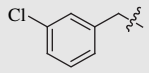
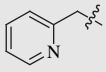
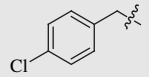
To a solution of methyl 4-(3-(4-(benzyloxy)-3-methoxyphenyl) propanamido)benzoate **2a** (5.0 g, 12 mmol) in MeOH (100 mL) was added 10% wt. Pd–C (0.5 g). The reaction mixture was stirred for 10 h under H_2 pressure at room temperature, filtered with Celite, the solvent was removed *in vacuo* to give methyl 4-(3-(4-hydroxy-3-methoxyphenyl)propanamido)benzoate **3** as a white solid (3.5 g, 88.7%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.24 (s, 1H), 8.70 (s, 1H), 7.92–7.70 (dd, 4H), 6.80–6.60 (m, 3H), 3.82 (s, 3H), 3.72 (s, 3H), 2.81 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 330.3 (MH^+).

5.1.4. Methyl 4-(3-(3,4-dihydroxyphenyl)propanamido) benzoate (**4**)

To a solution of methyl 4-(3-(4-hydroxy-3-methoxyphenyl) propanamido)benzoate **3** (3.0 g, 9 mmol) in dry CH_2Cl_2 (150 mL) was added added KOH powder (0.34 g, 6.0 mmol). at −40 °C. The mixture was stirred for 1 h at −40 °C and then the solvent was removed *in vacuo*. To the residue was added 1 mol/L Na_2CO_3 (100 mL), then extracted by ethyl acetate (3 × 100 mL), washed with water (3 × 50 mL) and dried over $MgSO_4$. After filtration and evaporation of the solvent *in vacuo*, methyl 4-(3-(3,4-dihydroxyphenyl)propana mido)benzoate **4** was obtained as a yellow solid (2.4 g, 83.6%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.23 (s, 2H), 8.71 (s, 1H), 7.90–7.72

Table 1
The inhibitory activity results of HPPB derivatives against HDACs in vitro.



Compd	R	HDACs IC_{50} (μM)	Compd	R	HDACs IC_{50} (μM)
5a		0.7	**5k**		1.8
5b	CH_3–	3.3	**5l**		2.6
5c	C_2H_5–	2.9	**5m**		1.5
5d	$CH_3(CH_2)_2$–	1.7	**5n**		0.6
5e	$CH_3(CH_2)_3$–	1.6	**5o**		0.9
5f	$CH_3CH_2(CH_3)CH$–	2.2	**5p**		0.8
5g	$(CH_3)_2CH(CH_2)_2$–	2.9	**5q**		1.2
5h		4.0	**5r**		1.6
5i		3.9	**5s**		1.4
5j		0.3	**5t**		0.4
HPPB		1.1			
Vorinostat		0.5			

(dd, 4H), 6.79–6.58 (m, 3H), 3.83 (s, 3H), 2.80 (t, 2H, $J = 7.5$ Hz), 2.61 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 316.3 (MH^+).

5.1.5. *Methyl 4-(3-(3,4-dimethoxyphenyl)propanamido) benzoate (**2b**)*

To a solution of methyl 4-(3-(4-hydroxy-3-methoxyphenyl) propanamido)benzoate **3** (1.0 g, 3 mmol) in DMF (10 mL) was added KOH powder (0.34 g, 6.0 mmol). The reaction mixture was stirred for 1 h and then CH_3I (1.3 g, 9.0 mmol) was added at room temperature. The mixture was heated to 50 °C and stirred for 5 h, then was added H_2O (50 mL) and then extracted by ether (3 × 50 mL). The organic layer was merged and washed with water (3 × 50 mL) and dried over $MgSO_4$. After filtration and evaporation of the solvent *in vacuo*, methyl 4-(3-(3,4-dimethoxyphenyl) propanamido)benzoate **2b** was obtained as a yellow solid (0.9 g, 87.4%). ^1H NMR (DMSO-d_6, 300 MHz) δ 8.70 (s, 1H), 7.90–7.68

Table 2
Growth inhibitory results of HDACi against HCT116 and A549 cell lines (IC_{50}, mM).

Compd	HCT116	A549
5a	0.12	0.6
5j	0.03	0.08
5n	0.10	0.6
5t	0.06	0.5
Vorinostat	0.08	0.6

Table 3
The effects of **5j** and **5t** on the cell-cycle were measured against human HCT116 cells at the concentration of 1 μmol/L, and the population proportion of HCT116 cells in each phase of cell-cycle has been calculated.

Compd concentration (1 μmol/L)	Proportion of HCT116 cells (%)		
	G_1 phase	S phase	G_2 phase
5j	28.51	24.33	47.16
5t	17.09	34.11	48.80
Control	34.60	46.55	18.85

(dd, 4H), 6.79–6.63 (m, 3H), 3.83 (s, 3H), 3.73 (s, 6H), 2.80 (t, 2H, $J = 7.5$ Hz), 2.65 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 344.4 (MH^+).

*5.1.6. General procedure for the synthesis of ethers **2c**–**t***

To a solution of methyl 4-(3-(4-hydroxy-3-methoxyphenyl) propanamido)benzoate **3** (1.0 g, 3 mmol) in DMF (10 mL) was added KOH powder (0.34 g, 6.0 mmol). The reaction mixture was stirred for 1 h and then corresponding alkyl halide (9.0 mmol) was added at room temperature. The mixture was heated to 50 °C and stirred for 5 h, then H_2O was added (50 mL) and extracted by ether (3×50 mL). The organic layer was merged and washed with water (3×50 mL) and dried over $MgSO_4$. After filtration and evaporation of the solvent *in vacuo*, the ethers **2c**–**s** were obtained.

*5.1.7. General procedure for the synthesis of hydroxamic acids **5a**–**t** from methyl esters **2a**–**t***

The corresponding methyl esters **2a**–**t** (5.0 mmol) were dissolved in EtOH (50 mL), then 2 mol/L NaOH (10 mL) was added. The solution was stirred at 75 °C for 5–6 h, and then concentrated under reduced pressure. To the residue was added 1 mol/L HCl (50 mL), then the solution was filtered to get the sediment which was washed with water for several times. The sediment was dried and dissolved in dry THF (50 mL), then Et_3N (1.0 g, 10 mmol) was added. The solution was stirred under room temperature and ClCOOBu-*i* (0.7 g, 5.3 mmol) was dropped into the solution. After 1–3 min, NH_2OH/MeOH (5 mL, 2 mmol/mL) was dropped into the reaction mixture. After stirring for 5 h, the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. To the residue was added 1 mol/L HCl (30 mL) and extracted by EtOAc (3×20 mL) for 3 times. The organic layer was merged and washed with water (3×20 mL) and then dried with $MgSO_4$. The solution was filtered and evaporated to give a crude product which was purified by column chromatography.

*5.1.7.1. 4-(3-(4-(Benzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5a**).* Yield: 28.6%. M.p. 193–195 °C. IR (KBr):ν (cm^{-1}) 3266, 1674, 1513, 1256. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.09 (s, 1H), 10.12 (s, 1H), 8.94 (s, 1H), 7.71–7.62 (m, 4H), 7.44–7.29 (m, 5H), 6.92 (d, 1H, $J = 8.1$ Hz), 6.88 (s, 1H, $J = 8.1$ Hz), 6.73 (d, 1H, $J = 8.1$ Hz), 5.02 (s, 2H), 3.73 (s, 3H), 2.85 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 421.4 (MH^+).

*5.1.7.2. 4-(3-(3,4-Dimethoxyphenyl)propanamido)-N-hydroxybenzamide (**5b**).* Yield: 19.0%. M.p. 178–179 °C. IR (KBr): ν (cm^{-1}) 3302, 1662, 1608, 1592, 1515, 1255. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.10 (s, 1H), 10.13 (s, 1H), 8.95 (s, 1H), 7.75–7.62 (dd, 4H), 6.86–6.73 (m, 3H), 3.71 (s, 3H), 3.70 (s, 3H), 2.85 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 345.3 (MH^+).

*5.1.7.3. 4-(3-(4-Ethoxy-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5c**).* Yield: 57.6%. M.p. 179–180 °C. IR (KBr): ν (cm^{-1}) 3299, 1660, 1609, 1515, 1258. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.10 (s, 1H), 10.13 (s, 1H), 8.95 (s, 1H), 7.75–7.62 (dd, 4H), 6.85–6.70 (m, 3H), 3.94 (d, 2H, $J = 6.9$ Hz), 3.71 (s, 3H), 2.84 (s, 2H), 2.63 (d, 2H, $J = 7.2$ Hz), 1.29 (t, 3H, $J = 6.9$ Hz). ESI-MS m/z 359.5 (MH^+).

*5.1.7.4. N-Hydroxy-4-(3-(3-methoxy-4-propoxyphenyl)propanamido)-benzamide (**5d**).* Yield: 19.2%. M.p. 182–183 °C. IR (KBr): ν (cm^{-1}) 3306, 1671, 1639, 1610, 1592, 1514, 1258. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.10 (s, 1H), 10.13 (s, 1H), 8.94 (s, 1H), 7.72–7.62 (dd, 4H), 6.85–6.71 (m, 3H), 3.84 (t, 2H, $J = 6.6$ Hz), 3.72 (s, 3H), 2.85 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz), 1.75–1.63 (m, 2H), 0.95 (t, 3H, $J = 7.5$ Hz). ESI-MS m/z 373.2 (MH^+).

*5.1.7.5. 4-(3-(4-Butoxy-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5e**).* Yield: 28.2%. M.p. 163–165 °C. IR (KBr): ν (cm^{-1}) 3302, 1668, 1514, 1255. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.10 (s, 1H), 10.13 (s, 1H), 8.95 (s, 1H), 7.71–7.62 (m, 4H), 6.85–6.82 (m, 2H), 6.72 (d, 1H, $J = 8.1$), 3.88 (t, 2H, $J = 6.6$), 3.71 (s, 3H), 2.84 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz), 1.70–1.61 (m, 2H), 1.47–1.35 (m, 2H), 0.91 (t, 3H, $J = 7.5$ Hz). ESI-MS m/z 387.4 (MH^+).

*5.1.7.6. 4-(3-(4-sec-Butoxy-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5f**).* Yield: 38.4%. M.p. 156–158 °C. IR (KBr): ν (cm^{-1}) 3261, 1664, 1626, 1608, 1512, 1259. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.94 (s, 1H), 7.71–7.62 (dd, 4H), 6.85–6.70 (m, 3H), 4.25–4.19 (m, 1H, $J = 6.0$ Hz), 3.71 (s, 3H), 2.84 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz), 1.67–1.46 (m, 2H), 1.16 (d, 3H, $J = 6.0$ Hz), 0.90 (t, 3H, $J = 7.5$ Hz). ESI-MS m/z 387.4 (MH^+).

*5.1.7.7. N-Hydroxy-4-(3-(4-(isopentyloxy)-3-methoxyphenyl)propanamido)benzamide (**5g**).* Yield: 38.4%. M.p. 171–172 °C. IR (KBr): ν (cm^{-1}) 3301, 1670, 1643, 1609, 1590, 1515, 1255. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.08 (s, 1H), 10.12 (s, 1H), 8.94 (s, 1H), 7.71–7.62 (dd, 4H), 6.87–6.71 (m, 3H), 3.91 (t, 2H, $J = 6.6$ Hz), 3.71 (s, 3H), 2.84 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz), 1.81–1.72 (m, 1H), 1.61–1.54 (q, 2H, $J = 6.6$ Hz), 0.92 (s, 3H), 0.90 (s, 3H). ESI-MS m/z 401.5 (MH^+).

*5.1.7.8. 4-(3-(4-(Cyclopentyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5h**).* Yield: 77.0%. M.p. 188–190 °C. IR (KBr): ν (cm^{-1}) 3309, 1670, 1641, 1609, 1593, 1511, 1256. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.11 (s, 1H), 10.13 (s, 1H), 8.99 (s, 1H), 7.72–7.63 (dd, 4H), 6.84–6.71 (m, 3H), 4.70 (s, 1H), 3.70 (s, 3H), 2.83 (d, 2H, $J = 7.5$ Hz), 2.63 (d, 2H, $J = 7.5$ Hz), 1.81–1.54 (m, 8H). ESI-MS m/z 399.4 (MH^+).

*5.1.7.9. N-Hydroxy-4-(3-(3-methoxy-4-(pyridin-2-ylmethoxy)phenyl)-propanamido)benzamide (**5i**).* Yield: 57.9%. M.p. 205–206 °C. IR (KBr): ν (cm^{-1}) 3290, 1655, 1641, 1603, 1592, 1518, 1257. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.92 (s, 1H), 8.55 (s, 1H), 7.83 (t, 1H, $J = 7.5$ Hz), 7.71–7.62 (dd, 4H), 7.50 (d, 1H, $J = 7.8$ Hz), 7.33 (s, 1H), 6.93–6.71 (m, 3H), 5.10 (s, 2H), 3.76 (s, 3H), 2.83 (d, 2H, $J = 7.5$ Hz), 2.63 (d, 2H, $J = 7.5$ Hz). ESI-MS m/z 422.4 (MH^+).

*5.1.7.10. N-Hydroxy-4-(3-(3-methoxy-4-(thiophene-2-ylmethoxy)phenyl)-propanamido)benzamide (**5j**).* Yield: 19.4%. M.p. 169–170 °C. IR (KBr): ν (cm^{-1}) 3305, 1674, 1638, 1609, 1589, 1512, 1255. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.94 (s, 1H),

7.71–7.62 (dd, 4H), 7.53 (d, 1H, $J = 4.8$ Hz), 7.15 (s, 1H), 7.01 (t, 1H, $J = 4.2$ Hz), 6.97–6.72 (m, 3H), 5.20 (s, 2H), 3.73 (s, 3H), 2.85 (t, 2H, $J = 7.5$ Hz), 2.63 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 427.5 (MH^+).

*5.1.7.11. 4-(3-(4-(2-Methylbenzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5k**).* Yield: 48.3%. M.p. 173–175 °C. IR (KBr): ν (cm^{-1}) 3215, 1667, 1608, 1515, 1257. ^1H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.96 (s, 1H), 7.71–7.62 (dd, 4H), 7.38–7.22 (m, 4H), 6.98–6.76 (m, 3H), 5.00 (s, 2H), 3.73 (s, 3H), 2.86 (t, 2H, $J = 7.5$ Hz), 2.63 (t, 2H, $J = 7.5$ Hz), 2.31 (s, 3H). ESI-MS m/z 435.5 (MH^+).

*5.1.7.12. 4-(3-(4-(3-Methylbenzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5l**).* Yield: 19.4%. M.p. 178–179 °C. IR (KBr): ν (cm^{-1}) 3308, 1673, 1643, 1610, 1590, 1513, 1257. ^1H NMR (DMSO-d_6, 300 MHz) δ 10.99 (s, 1H), 10.12 (s, 1H), 8.94 (s, 1H), 7.71–7.62 (dd, 4H), 7.29–7.13 (m, 4H), 6.93–6.71 (m, 3H), 4.97 (s, 2H), 3.71 (s, 3H), 2.85 (t, 2H, $J = 7.5$ Hz), 2.63 (t, 2H, $J = 7.5$ Hz), 2.31 (s, 3H). ESI-MS m/z 435.4 (MH^+).

*5.1.7.13. 4-(3-(4-(4-Methylbenzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5m**).* Yield: 58.0%. M.p. 216–218 °C. IR (KBr): ν (cm^{-1}) 3314, 1675, 1592, 1513, 1254. ^1H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.94 (s, 1H), 7.87–7.62 (m, 4H), 7.31–7.16 (dd, 4H), 6.92–6.70 (m, 3H), 4.97 (s, 2H), 3.73 (s, 3H), 2.85 (t, 2H, $J = 7.5$ Hz), 2.64 (t, 2H, $J = 7.5$ Hz), 2.30 (s, 3H). ESI-MS m/z 435.5 (MH^+).

*5.1.7.14. 4-(3-(4-(2-Bromobenzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5n**).* Yield: 16.2%. M.p. 162–164 °C. IR (KBr): ν (cm^{-1}) 3246, 1661, 1595, 1514, 1558. ^1H NMR (DMSO-d_6, 300 MHz) δ 11.07 (s, 1H), 10.11 (s, 1H), 8.93 (s, 1H), 7.72–7.29 (m, 8H), 6.94–6.74 (m, 3H), 5.05 (s, 2H), 3.75 (s, 3H), 2.87 (s, 2H), 2.64 (s, 2H). ESI-MS m/z 499.3/501.3 (MH^+).

*5.1.7.15. 4-(3-(4-(3-Bromobenzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5o**).* Yield: 16.2%. M.p. 200–202 °C. IR (KBr): ν (cm^{-1}) 3285, 1671, 1611, 1512, 1256. ^1H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.93 (s, 1H), 7.71–7.62 (m, 5H), 7.53–7.32 (m, 3H), 6.91 (d, 2H, $J = 8.7$ Hz), 6.73 (d, 1H, $J = 8.1$ Hz), 5.04 (s, 2H), 3.75 (s, 3H), 2.85 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz). ESI-MS 499.3/501.3 (MH^+).

*5.1.7.16. 4-(3-(4-(4-Bromobenzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5p**).* Yield: 16.2%. M.p. 208–210 °C. IR (KBr): ν (cm^{-1}) 3313, 1674, 1637, 1607, 1591, 1514, 1256. ^1H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.93 (s, 1H), 7.71–7.56 (m, 6H), 7.38 (d, 2H, $J = 7.8$ Hz), 6.90 (d, 1H, $J = 8.4$ Hz), 6.74 (s, 1H), 5.01 (s, 2H), 3.74 (s, 3H), 2.85 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 499.2/501.2 (MH^+).

*5.1.7.17. 4-(3-(4-(2-Chlorobenzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5q**).* Yield: 35.0%. M.p. 206–208 °C. IR (KBr): ν (cm^{-1}) 3253, 1663, 1609, 1594, 1514, 1259. ^1H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.93 (s, 1H), 7.71–7.56 (m, 4H), 7.52–7.35 (m, 4H), 6.95–6.70 (m, 3H), 5.08 (s, 2H), 3.74 (s, 3H), 2.86 (t, 2H, $J = 7.5$ Hz), 2.61 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 455.3 (MH^+).

*5.1.7.18. 4-(3-(4-(3-Chlorobenzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5r**).* Yield: 35.0%. M.p. 238–240 °C. IR (KBr): ν (cm^{-1}) 3260, 1671, 1644, 1610, 1591, 1513, 1256. ^1H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.94 (s, 1H), 7.71–7.62 (m, 4H), 7.49–7.39 (m, 3H), 6.93–6.72 (m, 3H), 5.05 (s, 2H), 3.74 (s, 3H), 2.86 (t, 2H, $J = 7.5$ Hz), 2.63 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 455.3 (MH^+).

*5.1.7.19. 4-(3-(4-(4-Chlorobenzyloxy)-3-methoxyphenyl)propanamido)-N-hydroxybenzamide (**5s**).* Yield: 17.6%. M.p. 240–242 °C. IR (KBr): ν (cm^{-1}) 3307, 1671, 1641, 1607, 1592, 1514, 1256. ^1H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.12 (s, 1H), 8.94 (s, 1H), 7.71–7.65 (m, 4H), 7.44 (s, 4H), 6.91 (d, 2H, $J = 8.7$ Hz), 6.73 (d, 1H, $J = 7.8$ Hz), 5.03 (s, 2H), 3.74 (s, 3H), 2.86 (t, 2H, $J = 7.5$ Hz), 2.62 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 455.3 (MH^+).

*5.1.7.20. 4-(3-(Benzo[d][1,3]dioxol-5-yl)propanamido)-N-hydroxybenzamide (**5t**).* Yield: 19.0%. M.p. 230–232 °C. IR (KBr): ν (cm^{-1}) 3280, 1658, 1608, 1534, 1501, 1255. ^1H NMR (DMSO-d_6, 300 MHz) δ 11.09 (s, 1H), 10.10 (s, 1H), 8.93 (s, 1H), 7.71–7.61 (dd, 4H), 6.83–6.69 (m, 3H), 5.95 (s, 2H), 2.83 (t, 2H, $J = 7.5$ Hz), 2.60 (t, 2H, $J = 7.5$ Hz). ESI-MS m/z 329.4 (MH^+).

5.2. HDAC inhibitory activity assays

We performed assays according to kit instructions. The source of HDACs was HeLa nuclear extracts including HDAC1 and HDAC2 (the major contributors to HDAC activity in HeLa nuclear extracts), and the substrate was a type of [^{3}H]acetylated histone peptide. Both HDAC1 and HDAC2 are known to be nucleus proteins in charge of the deacetylation of histones. The compound samples and the control drug were diluted to various concentrations. On the 96-well plate, HDACs (5 μL/well) were incubated at 37 °C with 10 μL of various concentrations of samples and 25 μL of substrate. After reacting for 30 min, Color de Lys Developer (50 μL/well) was added. Then, after 15 min the ultraviolet absorption of the wells was measured on a microtiter-plate reader at 405 nm. The % inhibition was calculated from the ultraviolet absorption readings of inhibited wells relative to those of control wells. Finally, the IC_{50} values were determined using a regression analysis of the concentration/inhibition data.

5.3. Antiproliferative activity evaluation of HDAC inhibitors by MTT assays

HCT116 cells were maintained in McCoy's 5a medium with 10% fetal bovine serum (FBS) while A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. Appropriate numbers of cells (2.0×10^5/mL) were inoculated into 96-well plates (50 μL/well). After 4 h, compounds of various concentrations were dosed, and the cells were cultured for 2 days. Then 0.5% MTT (10 μL/well) was added into each well. After additional 4 h incubation, OD570 and OD630 as references were measured, and the IC_{50} values were calculated according to a regression analysis of the concentration/inhibition data.

Acknowledgments

This work was supported by National "863" Foundation (No. 2007AA02Z314) of PR China and the National Natural Foundation Research Grant (Grant Nos. 30772654; 36072541).

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