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RESEARCH ARTICLE

Synthesis of a novel series of benzylether-containing cinnamoyl derivatives as histone deacetylase inhibitors

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Abstract

Histone deacetylases (HDACs) play an important role in gene transcription, cellular proliferation, apoptosis, and tumorigenesis. A novel series of benzylether-containing cinnamoyl derivatives were designed and synthesized as inhibitors of histone deacetylases (HDACs). Most of these compounds possessed inhibitory activity against the enzymes with IC_{50} values as low as 30 μM. In addition, compounds **4d** (IC_{50} = 11.1 μM) and **4n** (IC_{50} = 7.7 μM) exhibited high antiproliferative activity against tumor cell growth and effectively induced cell cycle arrest.

Keywords: *Histone deacetylases; benzylether; cinnamoyl derivatives; inhibitors*

Introduction

The human genome is packed in the chromatin, which is made up of repetitive subunits of nucleosomes. A single nucleosomal core is composed of a fragment of DNA chain (146 bp) wrapped around a histone octamer formed by an H3–H4 tetramer and two H2A–H2B dimers[1]. Histones can be in one of two antagonist forms, acetylated or deacetylated, regulated by histone acetylases (HATs) and histone deacetylases (HDACs), respectively. The latter belong to a class of Zn^{2+} dependent metalloproteases that can remove acetyl groups from ε-NH_2 of lysine residues in histones through Zn^{2+}-dependent hydrolysis (Figure 1). Deacetylation results in the positive charge density on the *N*-termini of nucleosomal histones increasing, which strengthens the interaction with the negatively charged DNA chain and blocks the access of transcription factors and RNA polymerases to DNA. Thus the transcription is inhibited[2,3]. Besides histones, other important intracellular proteins including onco-suppressor p53, α-tubulin, and hsp90 are also regulated by HDACs; hyperacetylation of these proteins can result in cell cycle arrest and apoptosis[4].

Inhibition of HDACs represents an effective strategy for cancer therapy, since these enzymes play a fundamental role in regulating gene expression, chromatin remodeling, the cell cycle, and differentiation. Small molecular inhibitors

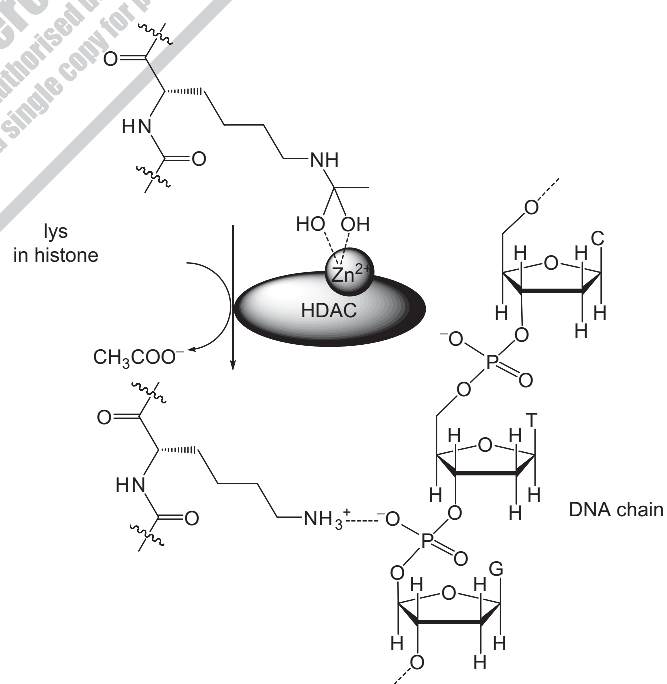

Figure 1. Histone deacetylase (HDAC) function of modification of histones.

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of HDACs have been considered as a new generation of anticancer agents; they are potent inducers of growth arrest, differentiation, and apoptosis of tumor cells[5–7]. Among them, vorinostat (trade name: Zolinza) was approved by the US Food and Drug Administration (FDA) for the treatment of cancer in 2006[8].

An important class of HDAC inhibitors is characterized by the cinnamoyl scaffold[9] as Zn^{2+} chelating moiety, which is essential for all of the small molecular HDAC inhibitors, and this moiety is linked to an aromatic group (Ar) by a connection unit (CU) (Figure 2). The hydroxamic acid derivatives of cinnamic acid have achieved significant biological effects in preclinical models of cancer, and four derivatives (PXD101, CRA 024781, LBH-589, and LAQ-824) have been in clinical trials[10]. The CUs in this class of HDAC inhibitors include benzylamide, methylene, sulfonamide, and cyclic moieties, etc. In this article, we describe the design, synthesis, preliminary biological evaluation, and structure–activity relationship (SAR) study of a novel series of benzylether-containing cinnamoyl derivatives as potent HDAC inhibitors.

The two natural cinnamic acids (*E*)-3-(4-hydroxyphenyl) acrylic acid (**1a**) and (*E*)-3-(4-hydroxy-3-methoxyphenyl) acrylic acid (**1b**, ferulic acid) were used as the starting materials for synthesis of the benzylether derivatives. The COOH group of **1a** and **1b** were protected first, and the OH group retained for modification by various aromatic groups. After etherification of the OH group, the protective COOH group was exposed for conversion to the CONHOH group.

Methods

Chemistry

All materials and reagents used in this work were analytical reagents. All reactions were monitored by thin layer chromatography (TLC) on 0.25 mm silica gel plates (60 GF_{254}) and visualized with ultraviolet (UV) light. ^1H nuclear magnetic resonance (NMR) spectra were determined on a Bruker Avance 300 spectrometer using tetramethylsilane (TMS) as an internal standard in dimethylsulfoxide (DMSO)-d_6 solution. Chemical shifts are reported in ppm (δ) downfield from TMS. All the coupling constants (*J*) are in hertz. Infrared (IR) spectra were recorded on a PerkinElmer FTIR 1600 spectrometer. Electrospray ionization-mass spectra (ESI-MS) were determined on an API 4000 spectrometer. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. All reported yields correspond to yields of purified products.

Synthesis of (E)-methyl 3-(4-hydroxyphenyl)acrylate (2a)

(*E*)-3-(4-hydroxyphenyl)acrylic acid (8.2 g, 0.05 mol) was dissolved in dry methanol (200 mL), then paratoluenesulfonic acid (1.7 g, 10 mmol) was added to the stirred solution. The mixture was heated to 80°C under reflux for 10 h. The solvent was removed under reduced pressure and the residue was washed with water several times to obtain the product as a yellow solid (7.5 g, 84.3%), m.p. 136–138°C; ESI-MS *m/z* (%): (MH^+, 179.3).

Synthesis of (E)-methyl 3-(4-hydroxy-3-methoxyphenyl) acrylate (2b)

(*E*)-3-(4-hydroxy-3-methoxyphenyl)acrylic acid (10.0 g, 0.052 mol) was dissolved in dry methanol (200 mL), then paratoluenesulfonic acid (1.7 g, 10 mmol) was added to the stirred solution. The mixture was heated to 80°C under reflux for 10 h. The solvent was removed under reduced pressure and the residue was taken up in ethyl acetate (100 mL), washed with water (3 × 50 mL), and dried over Na_2SO_4. After filtration and evaporation of the solvent *in vacuo*, the (*E*)-methyl 3-(4-hydroxy-3-methoxyphenyl) acrylate (**2b**) was obtained as a yellow solid (10.0 g, 93.3%), m.p. 53–55°C; ESI-MS *m/z* (%): (MH^+, 209.3).

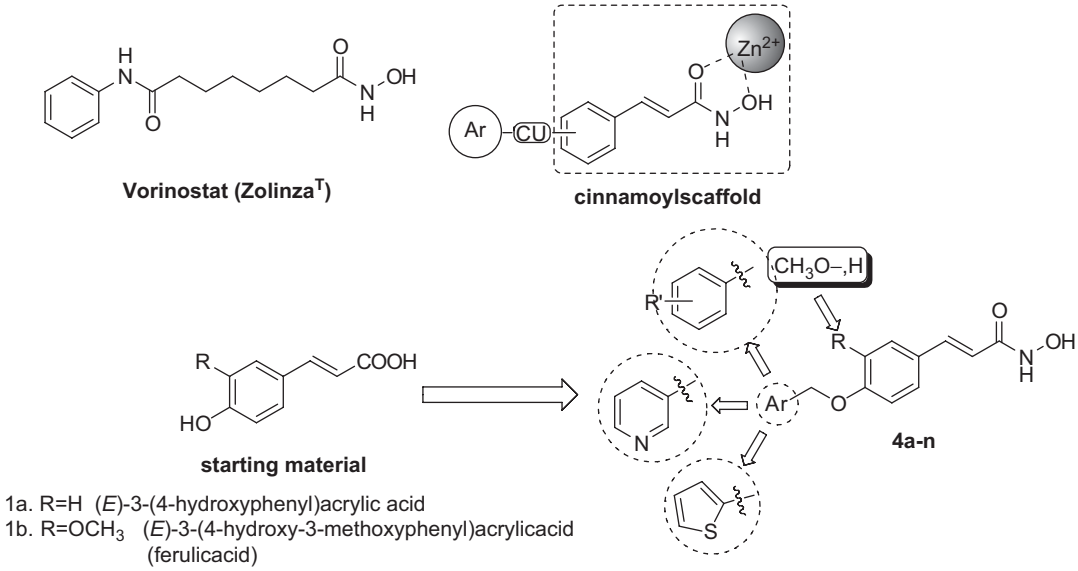


Figure 2. Design of novel cinnamoyl derivatives as HDAC inhibitors.

Synthesis of (E)-methyl 3-(4-(benzyloxy)phenyl) acrylate (3a)

K_2CO_3 (2.1 g, 15.0 mmol) and a little benzyltriethylammonium chloride (TEBA) as phase transfer catalyst were added to a solution of *(E)*-methyl 3-(4-hydroxyphenyl)acrylate (**2a**; 1.8 g, 10 mmol) in dry acetone (50 mL) and the mixture was heated to 70°C, then benzyl chloride ($PhCH_2Cl$; 1.9 g, 15.0 mmol) was added. After reflux for 8 h, the solution was cooled to room temperature. The solvent was evaporated *in vacuo* and the residue was washed with water several times. The crude product was obtained by filtration and then recrystallized with ethyl acetate (EtOAc) to give a yellow solid (1.7 g, 63.0%). m.p. 145–146°C; ^{1}H-NMR: δ 7.42 (d, 1H, *J* = 15.6 Hz, Ar-CH=C), 7.42–7.30 (m, 7H, Ar-H), 7.06 (s, 2H, Ar-H), 6.32 (d, 1H, *J* = 15.6 Hz, =CH-CO-), 5.22 (s, 2H, OCH_3), 3.78 (s, 3H, $COOCH_3$) ppm; ESI-MS *m/z* (%): (MH^+, 269.4); IR (KBr): *ν* 3030.2, 1645.8, 1598.1, 1513.6, 1251.7, 1175.6.

General procedure for the synthesis of 3c, 3e, 3g, 3i, 3k, and 3m

K_2CO_3 (2.1 g, 15.0 mmol) and a little benzyltriethylammionium chloride (TEBA) as phase transfer catalyst were added to a solution of *(E)*-methyl 3-(4-hydroxyphenyl)acrylate (**2a**; 1.8 g, 10 mmol) in dry acetone (50 mL) and the mixture was heated to 70°C, then the corresponding benzyl chloride ($ArCH_2Cl$; 15.0 mmol) was added. After reflux for 8 h, the solution was cooled to room temperature. The solvent was evaporated *in vacuo* and the residue was washed with water several times. The crude product was obtained by filtration and purified by recrystallization to be the starting material for the next step of the reaction.

General procedure for the synthesis of 3b, 3d, 3f, 3h, 3j, 3l, and 3n

K_2CO_3 (2.1 g, 15.0 mmol) and a little benzyltriethylammionium chloride (TEBA) as phase transfer catalyst were added to a solution of *(E)*-methyl 3-(4-hydroxy-3-methoxyphenyl) acrylate (**2b**; 2.1 g, 10 mmol) in dry acetone (50 mL) and the mixture was heated to 70°C, then the corresponding benzyl chloride ($ArCH_2Cl$; 15.0 mmol) was added. After reflux for 8 h, the solution was cooled to room temperature. The solvent was evaporated *in vacuo* and the residue was washed with water several times. The crude product was obtained by filtration and purified by recrystallization to be the starting material for the next step of the reaction.

Synthesis of (E)-3-(4-(benzyloxy)phenyl)-N-hydroxyacrylamide (4a)

Compound **3a** (1.3 g, 5.0 mmol) was dissolved in EtOH (50 mL), then 2 mol/L NaOH (10 mL) was added. The solution was stirred at 75°C for 3 h, and then concentrated under reduced pressure. The residue was added to 1 mol/L HCl (50 mL), then the solution was filtered to obtain the sediment, which was washed with water several times. The sediment was dried and dissolved in dry tetrahydrofuran (THF; 50 mL), then Et_3N (1.0 g, 10 mmol) was added. The solution was stirred at room temperature and ClCOOBu-i (0.7 g, 5.3 mmol) was dropped into the solution. After 2 min, NH_2OH/MeOH (5 mL, 2 mmol/mL) was dropped into the reaction mixture. After being stirred for 5 h, the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was added to 1 mol/L HCl (30 mL) and extracted by EtOAc (3 × 20 mL). The organic layer was merged and washed with water (3 × 20 mL) and then dried with $MgSO_4$. The solution was evaporated to give a crude product which was purified by recrystallization from methanol (0.8 g, Yield 56.6%); white crystal solid; m.p. 170–171°C; ^{1}H-NMR: δ 10.67 (s, 1H, OH), 8.98 (s, 1H, NH), 7.42 (d, 1H, *J* = 15.6 Hz, Ar-CH=C), 7.42–7.31 (m, 7H), 7.06 (s, 2H), 6.31 (d, 1H, *J* = 15.6 Hz, =CH-CO-), 5.24 (s, 2H, -OCH_2Ar) ppm; ESI-MS *m/z* (%): (MH^+, 270.4); IR (KBr): *ν* 3188.7, 3031.5, 1650.3, 1596.9, 1511.4, 1256.1, 1172.2. (Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.27; H, 5.57; N, 5.20. Found: C, 71.27; H, 5.69; N, 5.19%.)

General procedure for the synthesis of 4b–n

The corresponding benzylethers **3b–n** (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 3 h, and then concentrated under reduced pressure. The residue was added to 1 mol/L HCl (50 mL), then the solution was filtered to obtain the sediment which was washed with water 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 at 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 being stirred for 5 h, the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was added to 1 mol/L HCl (30 mL) and extracted by EtOAc (3 × 20 mL). The organic layer was merged and washed with water (3 × 20 mL) and then dried with $MgSO_4$. The solution was evaporated to give a crude product which was purified by recrystallization from methanol or acetone.

*(E)-3-(4-(benzyloxy)-3-methoxyphenyl)-N-hydroxyacrylamide (**4b**)*[11] 0.72 g, Yield 47.8%; white solid; recrystallized from CH_3OH; m.p. 164–165°C; ^{1}H-NMR: δ 10.66 (s, 1H, OH), 9.00 (s, 1H, NH), 7.46–7.33 (m, 6H, Ar-H), 7.18–7.04 (m, 3H, Ar-H), 6.35 (d, 1H, *J* = 15.6 Hz, =CH-CO-), 5.12 (s, 2H, -OCH_2Ar), 3.81 (s, 3H, -OCH_3) ppm; ESI-MS *m/z* (%): (MH^+, 300.5); IR (KBr): *ν* 3224.1, 2958.0, 1605.3, 1503.8, 1463.0, 1262.4, 1239.7, 1161.7. (Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.11; H, 5.68; N, 4.67. Found: C, 68.19; H, 5.66; N, 4.44%.)

*(E)-N-hydroxy-3-(4-(thiophen-2-ylmethoxy)phenyl)acrylamide (**4c**)*[11] 0.52 g, Yield 37.7%; white solid; recrystallized from CH_3OH; m.p. 168–169°C; ^{1}H-NMR: δ 10.69 (s, 1H, OH), 9.00 (s, 1H, NH), 7.66–7.50 (m, 3H, Ar-H), 7.42 (d, 1H, *J* = 15.6 Hz, Ar-CH=C), 7.23 (s, 1H, Ar-H), 7.08–7.04 (m, 3H, Ar-H), 6.34 (d, 1H, *J* = 15.9 Hz, =CH-CO-), 5.33 (s, 2H, -OCH_2Ar) ppm; ESI-MS *m/z* (%): (MH^+, 276.3); IR (KBr): *ν* 3108.1, 3016.9, 1650.5, 1598.9, 1511.5, 1250.0, 1178.1. (Anal. Calcd for $C_{14}H_{13}NO_3S$: C, 61.02; H, 4.72; N, 5.09. Found: C, 61.12; H, 5.02; N, 4.94%.)

*(E)-N-hydroxy-3-(3-methoxy-4-(thiophen-2-ylmethoxy) phenyl)acrylamide (**4d**)* 0.69 g, Yield 45.2%; yellow solid;

recrystallized from acetone; m.p. 151–153°C; ^{1}H-NMR: δ 10.66 (s, 1H, OH), 9.00 (s, 1H, NH), 7.56 (s, 1H, Ar-H), 7.40 (d, 1H, *J* = 15.6 Hz, Ar-CH=C), 7.27 (s, 1H, Ar-H), 7.22 (s, 1H, Ar-H), 7.11–7.02 (m, 3H, Ar-H), 6.38 (d, 1H, *J* = 15.6 Hz, =CH-CO-), 5.30 (s, 2H, -OCH$_2$Ar), 3.80 (s, 3H, -OCH$_3$) ppm; ESI-MS *m/z* (%): (MH$^+$, 306.4); IR (KBr): *ν* 3167.0, 3024.3, 1645.8, 1601.1, 1510.8, 1424.3, 1268.5, 1140.4. (Anal. Calcd for $C_{15}H_{15}NO_4S$: C, 58.94; H, 4.91; N, 4.58. Found: C, 58.97; H, 5.16; N, 4.56%.)

*(E)-N-hydroxy-3-(4-(pyridin-3-ylmethoxy)phenyl)acrylamide (**4e**)* 0.52 g, Yield 38.3%; white solid; recrystallized from CH$_3$OH; m.p. 187–188°C; ^{1}H-NMR: δ 10.65 (s, 1H, OH), 9.07 (s, 1H, NH), 8.68 (s, 1H, Ar-H), 8.56 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.88 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.54–7.38 (m, 4H, Ar-H), 7.07 (s, 2H, Ar-H), 6.33 (d, 1H, *J* = 15.6 Hz, =CH-CO-), 5.19 (s, 2H, -OCH$_2$Ar) ppm; ESI-MS *m/z* (%): (MH$^+$, 271. 4); IR (KBr): *ν* 3193.0, 3044.8, 1646.1, 1600.9, 1510.3, 1250.2, 1178.4. (Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.57; H, 5.18; N, 10.36. Found: C, 66.12; H, 5.58; N, 10.08%.)

*(E)-N-hydroxy-3-(3-methoxy-4-(pyridin-3-ylmethoxy) phenyl)acrylamide (**4f**)* 0.71 g, Yield 47.6%; white solid; recrystallized from CH$_3$OH; m.p. 173–174°C; ^{1}H-NMR: δ 10.65 (s, 1H, OH), 8.98 (s, 1H, NH), 8.58 (s, 1H, Ar-H), 7.84 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.53–7.33 (m, 3H, Ar-H), 7.20–7.03 (m, 3H, Ar-H), 6.35 (d, 1H, *J* = 15.9 Hz, =CH-CO-), 5.19 (s, 2H, -OCH$_2$Ar), 3.83 (s, 3H, -OCH$_3$) ppm; ESI-MS *m/z* (%): (MH$^+$, 301.4); IR (KBr): *ν* 3191.4, 1651.4, 1621.1, 1600.6, 1517.1, 1269.3, 1140.3. (Anal. Calcd for $C_{16}H_{16}N_2O_4$: C, 63.91; H, 5.33; N, 9.32. Found: C, 64.14; H, 5.24; N, 9.27%.)

*(E)-3-(4-(4-methylbenzyloxy)phenyl)-N-hydroxyacrylamide (**4g**)* 0.53 g, Yield 37.7%; yellow solid; recrystallized from CH$_3$OH; m.p. 171–172°C; ^{1}H-NMR: δ 10.66 (s, 1H, OH), 8.97 (s, 1H, NH), 7.51–7.32 (m, 5H, Ar-H), 7.21–7.01 (dd, 4H, Ar-H), 6.30 (d, 1H, *J* = 15.9 Hz, =CH-CO-), 5.09 (s, 2H, -OCH$_2$Ar), 2.30 (s, 3H, p-CH$_3$-Ph) ppm; ESI-MS *m/z* (%): (MH$^+$, 284.3); IR (KBr): *ν* 3190.5, 1651.1, 1618.0, 1597.6, 1511.4, 1254.9, 1172.5. (Anal. Calcd for $C_{17}H_{17}NO_3$: C, 72.01; H, 6.00; N, 4.94. Found: C, 71.58; H, 6.11; N, 4.90%.)

*(E)-3-(4-(4-methylbenzyloxy)-3-methoxyphenyl)-N-hydroxyacrylamide (**4h**)* 0.60 g, Yield 38.1%; yellow solid; recrystallized from acetone; m.p. 168–169°C; ^{1}H-NMR: δ 10.64 (s, 1H, OH), 8.97 (s, 1H, NH), 7.4 2(d, 1H, *J* = 15.6 Hz, Ar-CH=C), 7.34–7.18 (dd, 4H, Ar-H), 7.17–7.03 (m, 3H, Ar-H), 6.34 (d, 1H, *J* = 15.6Hz, =CH-CO-), 5.06 (s, 2H, -OCH$_2$Ar), 3.80 (s, 3H, -OCH$_3$), 2.31 (s, 3H, p-CH$_3$-Ph) ppm; ESI-MS *m/z* (%): (MH$^+$, 314.3); IR (KBr): *ν* 3192.1, 3018.0, 1652.0, 1595.3, 1519.4, 1260.1, 1134.7. (Anal. Calcd for $C_{18}H_{19}NO_4$: C, 68.94; H, 6.06; N, 4.47. Found: C, 68.71; H, 6.26; N, 4.30%.)

*(E)-3-(4-(4-methoxybenzyloxy)phenyl)-N-hydroxyacrylamide (**4i**)* 0.58 g, Yield 38.6%; white solid; recrystallized from CH$_3$OH; m.p. 179–180°C; ^{1}H-NMR: δ 10.66 (s, 1H, OH), 8.98 (s, 1H, NH), 7.64–7.37 (m, 5H, Ar-H), 7.04–6.93 (dd, 4H, Ar-H), 6.31 (d, 1H, *J* = 15.9 Hz, =CH-CO-), 5.05 (s, 2H, -OCH$_2$Ar), 3.76 (s, 3H, p-OCH$_3$-Ph) ppm; ESI-MS *m/z* (%): (MH$^+$, 300.4); IR (KBr): *ν* 3130.4, 3014.0, 1651.0, 1597.9, 1516.8, 1248.1, 1172.1. (Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.14; H, 5.68; N, 4.68. Found: C, 68.07; H, 5.85; N, 4.14%.)

*(E)-3-(4-(4-methoxybenzyloxy)-3-methoxyphenyl)-N-hydroxyacrylamide (**4j**)* 0.94 g, Yield 57.1%; yellow solid; recrystallized from CH$_3$OH; m.p. 163–164°C; ^{1}H-NMR: δ 10.63 (s, 1H, OH), 8.96 (s, 1H, NH), 7.42 (d, 1H, *J* = 15.6 Hz, Ar-CH=C), 7.38–7.04 (m, 3H, Ar-H), 7.07–6.93 (dd, 4H, Ar-H), 6.34 (d, 1H, *J* = 15.9 Hz, =CH-CO-), 5.03 (s, 2H, -OCH$_2$Ar), 3.79 (s, 3H, -OCH$_3$), 3.76 (s, 3H, p-OCH$_3$-Ph) ppm; ESI-MS *m/z* (%): (MH$^+$, 330.4); IR (KBr): *ν* 3192.5, 1595.0, 1517.0, 1465.0, 1253.0, 1138.0. (Anal. Calcd for $C_{18}H_{19}NO_5$: C, 65.57; H, 5.77; N, 4.25. Found: C, 65.34; H, 5.69; N, 4.55%.)

*(E)-3-(4-(4-chlorobenzyloxy)phenyl)-N-hydroxyacrylamide (**4k**)* 0.59 g, Yield 39.0%; yellow solid; recrystallized from acetone; m.p. 165–167°C; ^{1}H-NMR: δ 10.66 (s, 1H, OH), 9.04 (s, 1H, NH), 7.52–7.37 (m, 5H, Ar-H), 7.21–7.02 (dd, 4H, Ar-H), 6.32 (d, 1H, *J* = 15.6 Hz, =CH-CO-), 5.15 (s, 2H, -OCH$_2$Ar); ESI-MS *m/z* (%): (MH$^+$, 304.3); IR (KBr): *ν* 3245.8, 1662.7, 1603.4, 1511.8, 1256.3, 1174.9. (Anal. Calcd for $C_{16}H_{14}ClNO_3$: C, 63.30; H, 4.62; N, 4.62. Found: C, 62.66; H, 4.45; N, 4.24%.)

*(E)-3-(4-(4-chlorobenzyloxy)-3-methoxyphenyl)-N-hydroxyacrylamide (**4l**)* 0.57 g, Yield 34.3%; yellow solid; recrystallized from CH$_3$OH; m.p. 160–161°C; ^{1}H-NMR: δ 10.65 (s, 1H, OH), 8.98 (s, 1H, NH), 7.47–7.36 (m, 5H, Ar-H), 7.18–7.03 (m, 3H, Ar-H), 6.35 (d, 1H, *J* = 15.9 Hz, =CH-CO-), 5.12 (s, 2H, -OCH$_2$Ar), 3.81 (s, 3H, -OCH$_3$) ppm; ESI-MS *m/z* (%): (MH$^+$, 334.4); IR (KBr): *ν* 3196.2, 1651.5, 1594.6, 1518.6, 1268.5, 1133.7. (Anal. Calcd for $C_{17}H_{16}ClNO_4$: C, 61.19; H, 4.80; N, 4.20. Found: C, 59.86; H, 4.91; N, 4.16%.)

*(E)-3-(4-(3,4,5-trimethoxybenzyloxy)phenyl)-N-hydroxyacrylamide (**4m**)* 0.70g, Yield 39.2%; white solid; recrystallized from acetone; m.p. 185–186°C; ^{1}H-NMR: δ 10.68 (s, 1H, OH), 8.99 (s, 1H, NH), 7.54 (d, 1H, *J* = 15.9 Hz, Ar-CH=C), 7.25–7.04 (dd, 4H, Ar-H), 6.78 (s, 2H, Ar-H), 6.39 (d, 1H, *J* = 15.9Hz, =CH-CO-), 5.06 (s, 2H, -OCH$_2$Ar), 3.78 (s, 6H, 4,5-dimethoxyl), 3.65 (s, 3H, 3-methoxyl) ppm; ESI-MS *m/z* (%): (MH$^+$, 360.4); IR (KBr): *ν* 3165.4, 1684.8, 1623.3, 1601.0, 1512.2, 1466.2, 1425.5, 1245.3, 1125.8. (Anal. Calcd for $C_{19}H_{21}NO_6$: C, 63.44; H, 5.84; N, 3.90. Found: C, 63.36; H, 5.94; N, 3.85%.)

*(E)-3-(4-(3,4,5-trimethoxybenzyloxy)-3-methoxyphenyl)-N-hydroxyacrylamide (**4n**)* 0.76 g, Yield 38.8%; white solid; recrystallized from CH$_3$OH; m.p. 178–179°C; ^{1}H-NMR: δ 10.66 (s, 1H, OH), 8.99 (s, 1H, NH), 7.40 (d, 1H, *J* = 15.6 Hz, Ar-CH=C), 7.18–6.06 (m, 3H, Ar-H), 6.78 (s, 2H, Ar-H), 6.35 (d, 1H, *J* = 15.6 Hz, =CH-CO-), 5.03 (s, 2H, -OCH$_2$Ar), 3.81 (s, 3H, -OCH$_3$), 3.77 (s, 6H, 4,5-dimethoxyl), 3.65 (s, 3H, 3-methoxyl) ppm; ESI-MS *m/z* (%): (MH$^+$, 390.4); IR (KBr): *ν* 3166.1, 1649.8, 1592.2, 1509.4, 1461.4, 1422.9, 1259.5, 1129.6. (Anal. Calcd for $C_{20}H_{23}NO_7$: C, 61.63; H, 5.91; N, 3.60. Found: C, 61.49; H, 6.07; N, 3.54%.)

Enzyme 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 [3H]acetylated histone peptide. HDAC1 and HDAC2 are both known to be nucleus proteins in charge of the deacetylation

of histones2. The compound samples and the control drug were diluted to various concentrations: 20 μg/mL, 4 μg/mL, 0.8 μg/mL, and 0.16 μg/mL. On a 96-well plate, HDACs (5 μL/well) were incubated at 37°C with 10 μL of various concentrations of sample 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 percent 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.

Cell viability assays

Cells, maintained in McCoy's 5a medium with 10% fetal bovine serum, were plated in 96-well plates (50 μL/well) at a density of 2.0×10^5/mL. After 4 h, compounds of various concentrations (400 μg/mL, 200 μg/mL, 100 μg/mL, 50 μg/mL, 25 μg/mL) were dosed, and the cells were cultured for 2 days. Then 0.5% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 10 μL/well) was added into each well. After an additional 4 h incubation, OD570 and OD630 (optical densities at 570 nm and 630 nm) as references were measured, and the IC_{50} values were calculated according to a regression analysis of the concentration/inhibition data.

Results and discussion

Chemistry

Compounds selected for biological evaluation were prepared as described in Scheme 1. The compounds with general structure **4a–n** were synthesized starting from the two cinnamic acids **1a** and **1b**, which were esterified with paratoluenesulfonic acid (PTS) in methanol to yield the methyl esters **2a** and **2b**. Compounds **2a** and **2b** were treated with various benzyl chlorides ($ArCH_2Cl$) to yield the benzylethers **3a–n** by the Williamson reaction method. Hydrolysis of these benzylethers was carried out to obtain the corresponding benzylether-containing cinnamic acids, which were reacted with isobutyl chloroformate (ClCOOBu-s) first to form the mixed-anhydride then treated with hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) dissolved in methanol to yield the hydroxamic acid products **4a–n**.

HDAC activity assays

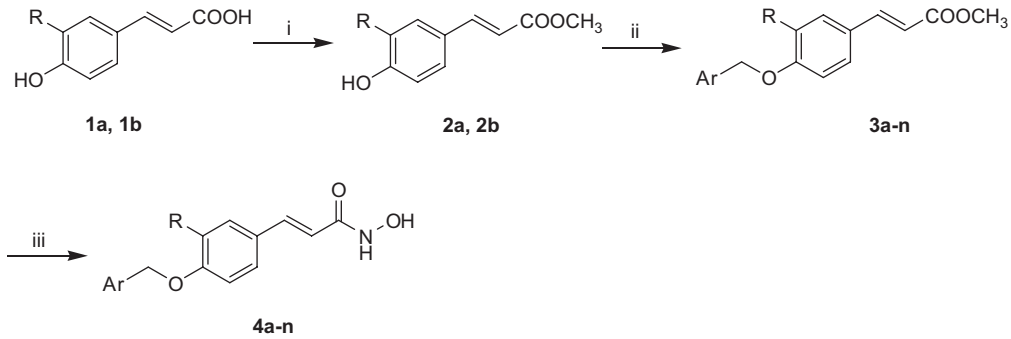
In vitro bioactivity evaluation of compounds **4a–n** was performed by HDAC activity assays using a HDAC colorimetric activity assay kit (AK501; Biomol Research Laboratories). The source of HDACs was HeLa nuclear extracts, including HDAC1 and HDAC2. Assays were performed according to kit instructions. The results of the *in vitro* testing of compounds **4a–n** for inhibitory activity against HDACs are presented in Table 1. The positive control drug was Zolinza, and IC_{50} values were determined for most of the active compounds.

SAR study

Most of these compounds had potent inhibitory activity against HDACs with IC_{50} values as low as 30 μM. The structure–activity relationship (SAR) analysis of them is summarized in Figure 3. Compound **4a** was less potent than its CH_3O substituted analog **4b**, while the other benzylether derivatives (**4c**, **4e**, **4g**, **4i**, **4k**, and **4m**) also showed less potency than their CH_3O substituted analogs (**4d**, **4f**, **4h**, **4j**, **4l**, and **4n**). This proves that introducing CH_3O to the *meta*-position of the cinnamoyl scaffold results in higher inhibitory activity of the compounds. Compound **4d**, the thiophen analog of **4b**, was found to be more potent than **4b** and **4f**, which is the pyridine analog of **4b**. As for compounds **4g–l** with R′ substituted benzylether, the results proved that the order of increasing activity of R′ is: $OCH_3 > CH_3 \approx H > Cl$. In addition, the 3,4,5-trimethoxybenzyl derivative **4n** was as potent as the 4-methoxybenzyl derivative **4j**, with IC_{50} value below 10 μM. Compounds **4d**, **4j**, and **4n** were chosen as representative compounds for evaluation of their antiproliferative activity against tumor cell growth.

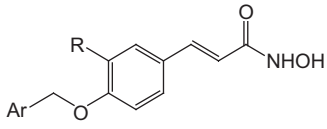
Antiproliferative activity assays and cell cycle analysis

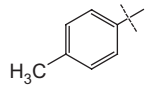
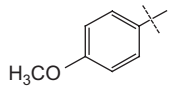
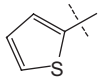
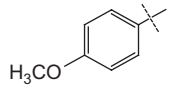
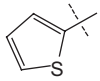
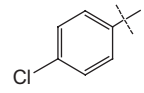
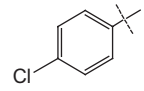
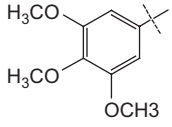
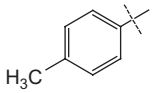
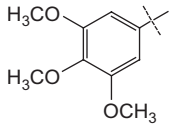
The cell line chosen for antiproliferative activity assays was a human colon cancer cell line (HCT116), in which HDACs express very highly, usually used in *in vitro* models for MTT assay of HDAC inhibitors. Besides this, a non-small cell lung cancer (NSCLC) cell line (A549) was chosen as an additional model for MTT assay of the compounds. The results of *in vitro* testing of compounds **4d**, **4j**, and **4n** for inhibitory activity against HCT116 and A549 cell growth are presented in Table 2.



Scheme 1. Reaction conditions: (i) PTS, CH_3OH, (ii) K_2CO_3, TEBA, $ArCH_2Cl$, CH_3COCH_3, (iii) NaOH, H_2O, EtOH; ClCOOBu-i, Et_3N, THF, $NHOH{\cdot}HCl$, CH_3OH.

Table 1. *In vitro* inhibitory activity against HDACs.



Compd	R	Ar	HDAC IC_{50} (μM)	Compd	R	Ar	HDAC IC_{50} (μM)
4a	H		20.0 ± 0.5	**4h**	OCH_3		13.3 ± 0.5
4b	OCH_3		13.4 ± 0.8	**4i**	H		14.5 ± 0.5
4c	H		12.5 ± 0.4	**4j**	OCH_3		3.4 ± 0.4
4d	OCH_3		11.1 ± 0.3	**4k**	H		>100
4e	H		29.5 ± 0.4	**4l**	OCH_3		27.8 ± 0.6
4f	OCH_3		21.6 ± 0.7	**4m**	H		20.6 ± 0.4
4g	H		23.5 ± 1.0	**4n**	OCH_3		7.7 ± 0.3
Zolinza			1.1 ± 0.3				

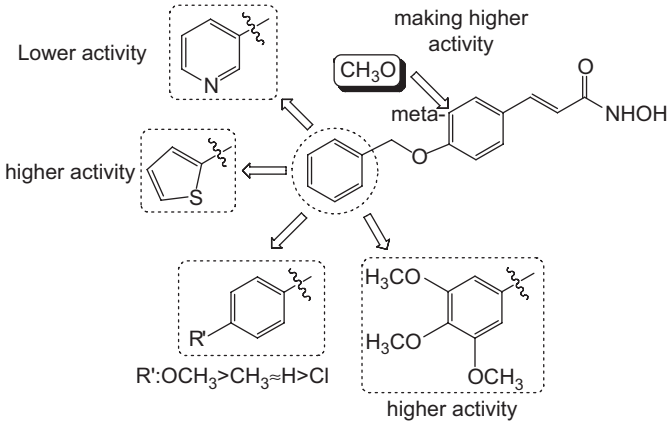


Figure 3. SAR analysis of the benzylether derivatives.

The three compounds were found to have potent antiproliferative activity, inhibiting HCT116 cell growth compared with Zolinza (Figure 4), while **4d** and **4n** showed good activity in both cell line assays. Compound **4j** exhibited good activity in inhibiting HDACs; however, it was less potent in inhibition of tumor cell growth than **4d** and **4n**.

Histone acetylation by inhibition of HDACs is known to precede gene transcription. Among genes, the most important is the cell cycle gene CDKN1A, which encodes cyclin-dependent kinase (CDK) inhibitor $p21^{WAF1}$. This cytokine inhibits cell-cycle progression by blocking CDK activity and arresting the cell cycle at G1 phase, and most HDAC inhibitors including Zolinza can potently induce the expression of p21 and cell cycle arrest at G1 phase[7]. The effects of compounds **4d** and **4n** on the cell cycle were measured by flow cytometric analysis against human HCT116 cells after 16 h exposure at the concentration of 1 μmol/L. The analytical results are shown in Table 3. The population proportion of HCT116 cells in each phase of the cell cycle has been calculated. The results of cell-cycle analysis elucidate that the two compounds exert their anti-cancer effects in different manners. Compound **4d** induced accumulation of cells in the G1 phase, which indicates that inhibition of proliferation of cancer cells by this compound is associated

Table 2. *In vitro* antiproliferative inhibiting potency of compounds **4d**, **4j**, and **4n**.

Compd	HCT116 IC_{50} (mM)	A549 IC_{50} (mM)
4d	0.09 ± 0.01	0.35 ± 0.03
4j	0.26 ± 0.02	>1000
4n	0.15 ± 0.03	0.59 ± 0.07
Zolinza	0.17 ± 0.02	0.60 ± 0.05

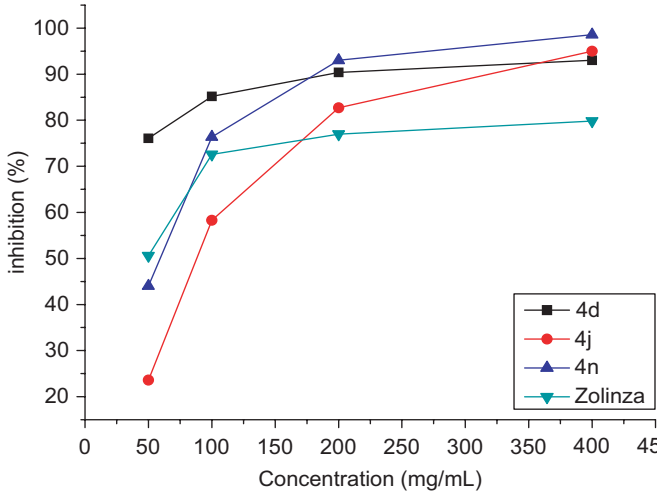


Figure 4. Activity to inhibit HCT116 cell growth.

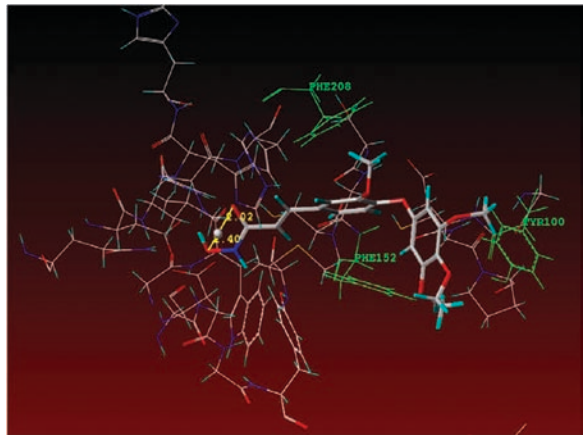


Figure 5. Docking result of **4n** with the active site of HDAC8 (PDB: 1t64).

Table 3. Cell cycle analysis of human HCT116 cancer cells.

	Proportion of HCT116 cells (%)		
Compd	G_1 phase	S phase	G_2 phase
4d	44.20	45.45	10.35
4n	19.96	17.69	62.35
Control	34.60	46.55	18.85

with expression of CDK inhibitor $p21^{WAF1}$. Interestingly, compound **4n** strongly caused G2 phase detention of cancer cells, which manifests that this compound can potently induce apoptosis of cancer cells. The pharmacological action mechanism of these two compounds is worthy of further investigation.

Binding analysis

We examined the interactions between compound **4n** and the catalytic site of HDAC8 (Protein Data Bank (PDB): 1t64). The docking work was done using software Sybyl 7.0, and the results are shown in Figure 5. The –CONHOH group chelates the zinc ion and the distances are 2.02 Å and 2.40 Å. The amino-acid residues of Phe208 and Phe152 have π–π conjugated interaction with the Ph ring in the scaffold of cinnamic acid, and the benzylether-containing side chain makes hydrophobic interaction with the amino-acid residue of Tyr100.

Conclusions

In summary, we have described the synthesis and SAR study of a novel series of benzylether-containing cinnamoyl derivatives as inhibitors of HDACs. Most of these compounds showed inhibitory activity against HDACs with IC_{50} values as low as 30 μM, and among them two compounds (**4d** and **4n**) were found to have potent antiproliferative activity against tumor cell growth and effectively induced cell cycle arrest. They could be used as potential lead compounds for the development of new anti-cancer agents and are worthy of further research in the future.

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***Declaration of interest*:** The authors report no conflicts of interest.

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