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Discovery of 1*H*-benzo[*d*][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate as a potential antiproliferative agent by inhibiting histone deacetylase

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ABSTRACT

Twenty-one benzotriazoles (**3**–**16** and **18**–**24**) were synthesized and half of them (**5**, **8**–**16**, **20**, and **21**) were reported for the first time. Their antiproliferative activities against three human cancer cells were assayed. It revealed that 1*H*-benzo[*d*][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate (**9**) showed considerable activity against three human cancer cell lines with the half maximal inhibitory concentration (IC_{50}) values of 1.2–2.4 nM, which were close to the value of the positive control, doxorubicin. Further investigation indicated compound **9** was a potential histone deacetylase inhibitor (IC_{50} = 9.4 μM) and its binding mode was simulated using docking method.

1. Introduction

vBenzotriazole derivatives have been demonstrated to have an effect on cancer development. Steroidal C-17 benzoazoles inhibit the growth of prostate cancer cells.[1] 4,5,6,7-Tetrabromo-1*H*-benzotriazole (TBB) is a potent inhibitor of protein kinase CK2.[2,3] The complex [2-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-benzotriazole]-dichlorocopper(II) shows very potent superoxide dismutase (SOD) activity and inhibits the growth of seven human tumor cell lines.[4] A series of [4-(2*H*-1,2,3-benzotriazol-2-yl)phenoxy]alkanoic acids have been synthesized and tested as agonists of peroxisome proliferator-activated receptor (PPAR) γ, which has been demonstrated to inhibit growth and/or induce apoptosis in multiple cancer cell lines and in in vivo tumor models.[5,6] 3-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-(4-methoxyphenyl)-1-oxopropan-2-yl benzoate (BmOB) can inhibit proliferation of human hepatocarcinoma cells by increasing oxidative stress concomitant mitochondrial damage.[7]

On the other hand, histone deacetylase (HDAC) catalyzes the deacetylation of lysine (Lys) residues, predominantly in histones H3 and H4.[8] Such chemical modification is one of the key steps in the regulation of expression of target genes affecting proper cell function, differentiation, and proliferation.[9] Abnormal recruitment of HDACs has been clearly linked to carcinogenesis.[10] Many compounds able to inhibit HDAC activity have been shown to have potent antitumor effect in vivo in tumor-bearing animals, and some of them are currently in phase I or phase I/II clinical trials.[11]

In this paper, we designed and synthesized 21 benzotriazole compounds bearing substituted benzoic acids and evaluated their antiproliferative activities. 1*H*-Benzo[*d*][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate (**9**) showed the most potent activity and was further investigated its HDAC inhibitory activity. Docking simulation was also performed using X-ray crystallographic structure of the catalytic core of an archaebacterial HDAC homolog (histone deacetylase-like protein, HDLP), reported in 1999 by Finnin et al.[12] to explore the binding modes of compound **9** at the active site. The results of this study may be useful to researchers attempting to find new potential antiproliferative agents.

Abbreviations: AcOH, acetic acid; Anal., analysis; aq, aqueous; Ar, aryl; BmOB, 3-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(4-methoxyphenyl)-1-oxopropan-2-yl benzoate; Bt, benzotriazolyl; Calcd, calculation; $CDCl_3$, deuterated chloroform (CH_2Cl_2) dichloromethane; CH_3OH, methanol; 3D, three-dimensional; ddH_2O, double distilled water; E_b, binding energy; EDC·HCl, 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride; e.g., exemplī grātia; EI-MS, electron ionization-mass spectrometry; Em., emission wavelength; ESI-MS, electrospray ionization mass spectrometry; EtOAc, ethyl acetate; Ex., excitation wavelength; Fig., figure; HCl, hydrogen chloride; HDAC, histone deacetylase; HDLP, histone deacetylase-like protein; His, histidine; K_i, inhibition constant; MS-275, *N*-[4-[*N*-(2-aminophenyl)carbamoyl]benzyl]carbamic acid 3-pyridylmethyl ester; NMR, nuclear magnetic resonance; IC_{50}, half maximal inhibitory concentration; lit., literature; Lys, lysine; M, molarity; mp, melting point; NaCl, sodium chloride; $NaNO_2$, sodium nitrite; NaOH, sodium hydroxide; PDB, Protein Data Bank; PE, petroleum ether; Phe, phenylalanie; PPAR, peroxisome proliferator-activated receptor; rt, room temperature; SAHA, suberoylanilide hydroxamic acid; SAR, structure–activity relationship; SOD, superoxide dismutase; soln, solution; TBB, 4,5,6,7-tetrabromo-1*H*-benzotriazole; TLC, thin layer chromatography; TMS, tetramethylsilane; TSA, trichostatin A; Tyr, tyrosine; UV, ultraviolet.

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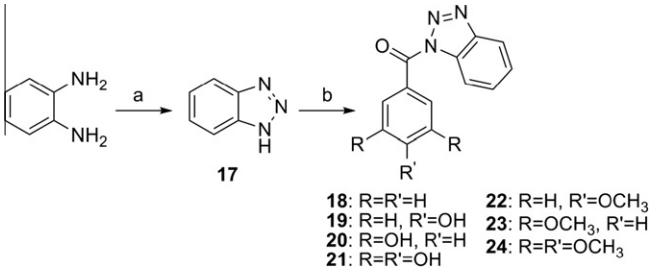
2. Results and discussion

2.1. Chemistry

Benzotriazole ring is the fundamental structure for the anticancer activities of benzotriazole derivatives. On the other hand, benzoic acid and its derivatives have wide biological activities, such as antimicrobials, antioxidant, and antitumor. Many of them have been used as active groups to designed anticancer drugs.[13] In our previous work, we have also synthesized some potential lead compounds as antitumor agents deriving from substituted benzoic acids.[14] Therefore, herein we tried to connect benzotriazole rings with substituted benzoic acids to find some potential antiproliferative agents.

1-Hydroxy benzotriazoles **1** and **2** were prepared as shown in Scheme 1 according to the literature.[15] Benzotriazoles **3**–**16** were prepared in 68–85% yields by treatment of **1** or **2** with commercially available hydroxyl or methoxyl substituted benzoic acids in dichloromethane (CH_2Cl_2) using 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC·HCl) as a condensation agent. To synthesize benzotriazoles **18**–**24**, benzotriazole **17** were prepared as shown in Scheme 2.[16] Compound **17** with hydroxyl or methoxyl substituted benzoic acids in CH_2Cl_2 using EDC·HCl as a condensation agent gave desired **18**–**24** in 75–84% yields. Among these compounds **5**, **8**–**16**, **20**, and **21** were reported for the first time.



Scheme 2. Synthesis of benzotriazoles **18**–**24**. Reagents and conditions: (a) AcOH, $NaNO_2$ aq soln, 70–80 °C; (b) benzoic acids, EDC·HCl, CH_2Cl_2, rt.

2.2. Antiproliferative activity

The synthesized benzotriazoles **3**–**16** and **18**–**24** were evaluated for antiproliferative activities against three types of human cancer cell lines, oral epidermoid carcinoma KB cells, non-small-cell lung carcinoma H460 cells, and stomach carcinoma MKN45 cells, taking doxorubicin as the positive control. The results were summarized in Table 1. All the compounds showed antiproliferative activities against the three lines with the half maximal inhibitory concentration (IC_{50}) values of 1.2–750 nM and compound **9** showed the most potent activity with a mean IC_{50} of 1.7 nM, which was close to the value of the positive control, doxorubicin. Structure–activity relationship (SAR) analysis indicated that ester benzotriazoles **3**–**16** showed stronger activities than the amide benzotriazoles **18**–**24**, with all the IC_{50} values below 300 nM. In the SAR further study of **3**–**16**, the methoxyl substituted benzoate groups played an important role for the activity. Compounds **9** and **16** with 3,4,5-trimethoxyl substitution showed the most potent activities against three cell lines, with the mean IC_{50} of 1.7 and 14 nM, respectively. In addition, methoxyl substitution of benzotriazole group (**10**–**16**) reduced the activities, comparing with compounds **3**–**9**.

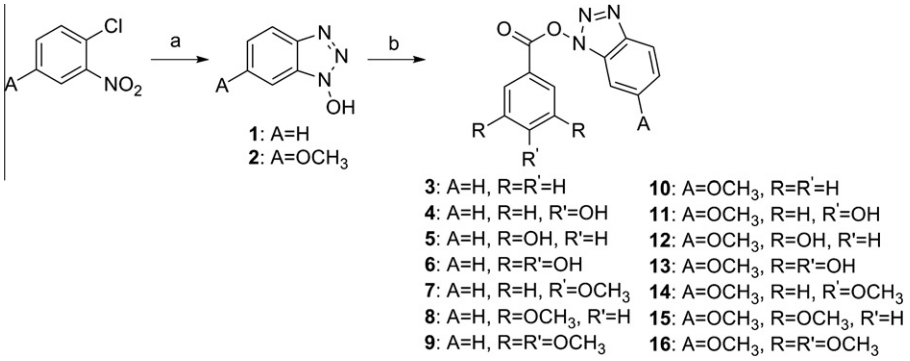
Table 1
The IC_{50} of benzotriazoles **3**–**16** and **18**–**24** for antiproliferative activities

Compound	IC_{50} (nM)			Compound	IC_{50} (nM)		
	KB	H460	MKN45		KB	H460	MKN45
3	104	195	97	**14**	97	95	93
4	88	98	49	**15**	35	80	36
5	67	52	28	**16**	11	23	8
6	18	41	17	**18**	666	750	668
7	43	52	39	**19**	577	662	385
8	37	42	30	**20**	575	559	521
9	1.2	2.4	1.5	**21**	392	332	334
10	209	295	206	**22**	501	662	500
11	149	216	184	**23**	434	491	419
12	153	195	93	**24**	291	480	275
13	81	117	36	Doxorubicin	0.23	0.38	0.19

2.3. HDAC inhibitory activity and docking simulation

To date, many good HDAC inhibitors were found and they can be classified into five groups according to their chemical structures, including (i) hydroxamic acids (e.g., TSA and SAHA), (ii) cyclic tetrapeptides (e.g., apicidin), (iii) short chain carboxylic acids (e.g., valproic acid), (iv) benzamides (e.g., MS-275) and keto derivatives (e.g., alpha-cétoamide).[17] However, benzotriazole derivatives as HDAC inhibitors were rarely reported.

Compounds **6**–**9**, **15**, and **16**, which showed strong antiproliferative activities, were evaluated for their abilities to inhibit HDAC activity using HeLa Nuclear Extract as the enzyme source. Trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) were tested as reference drugs. The results are expressed as IC_{50} and reported in Table 2. As shown in Table 2, these compounds showed HDAC inhibitory activities of varying degrees, and the activity of compound **9** was much higher than that of the others. This was in accordance with their antiproliferative activities. It indicated



Scheme 1. Synthesis of benzotriazoles **3**–**16**. Reagents and conditions: (a) hydrazine hydrate, 1-heptanol, 110–120 °C; (b) benzoic acids, EDC·HCl, CH_2Cl_2, rt.

Table 2
HDAC inhibitory activities of compounds **6**–**9**, **15**, and **16**

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
6	>100	**15**	87.5
7	73.1	**16**	58.1
8	61.1	TSA	0.015
9	9.4	SAHA	0.12

that the antiproliferative function of compound **9** was possibly associated with its significant HDAC inhibitory activity.

Molecular docking was performed on the binding model based on the crystal structure of HDLP extracted from the HDLP/TSA complex using the AUTODOCK 4.0 software.[18] The structure of the catalytic core of HDLP revealed the mode by which the HDAC inhibitors TSA and SAHA bind to the pocket of the catalytic site of the enzyme.[12] In order to study the binding mode, compound **9**, with the most potent inhibitory activity, was hit by the catalytic core and the results was depicted in Figure 1. In the binding model, amino hydrogen of His170 and Phe198 form hydrogen bond with the two oxygen of ester, respectively. Positive π-stacking interactions can be observed between two benzene rings of compound **9** and Tyr264 (10.659 Å). In addition, the benzotriazole ring and benzene ring of compound **9** may form a hydrophobic interaction with Phe141, Tyr196, Leu265, Lys267, and Tyr297 of the enzyme.

Comparing with compounds **6**–**8**, **15**, and **16**, the binding energy (E_b = −13.13 kcal/mol) and estimated inhibition constant (K_i = 0.238 nM) for compound **9** is the lowest. This is accordant with the result of HDAC assay. Looking into the binding mode of these compounds with HDLP, H-bonds and π–π interaction play an crucial role in stabilizing the three-dimensional (3D) structure of the inhibitor–enzyme complex. The different polarities and sizes of the substituents in the benzene ring and benzotriazole ring can be a key factor to influence the H-bonds and π–π interaction, resulting to the differences of these compounds in HDAC inhibitory activity.

3. Conclusions

Benzotriazoles **3**–**16** and **18**–**24** were synthesized and evaluated their antiproliferative activities against three types of human cancer cell lines. Compound **9** showed the most potent activity with a mean IC_{50} of 1.7 nM. Further investigation of HDAC inhibitory activity showed that compound **9** was a potential HDAC inhibitor. Docking simulation of its binding mode was also carried out.

4. Materials and methods

4.1. Chemistry

4.1.1. Chemistry general

All chemicals (reagent grade) used were purchased from Aldrich (USA). Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM, E. Merck, Germany). The quantity of silica gel used was 50–100 times the weight charged on the column. Thin layer chromatography (TLC) was run on the silica gel coated aluminum sheets (silica gel 60 GF254, E. Merck, Germany) and visualized in ultraviolet (UV) light (254 nm). Melting points (uncorrected) were determined with an XT4 MP apparatus (Taike Corp., Beijing, China). ^{1}H NMR and ^{13}C NMR spectra (300 MHz) were recorded on a ^{1}H-Varian-Mercury-300 spectrometer at 25 °C, using tetramethylsilane (TMS) as the internal standard. ESI-MS were recorded with a Mariner System 5304 mass spectrometer. EI-MS spectra were recorded with a Finnigan Trace MS spectrometer. Elementary analyzes were performed on a CHN-O-Rapid instrument within ±0.4% of the theoretical values.

4.1.2. Synthesis of 1*H*-benzo[*d*][1,2,3]triazol-1-ol (1)

To a solution of *o*-nitrochlorobenzene (788 mg, 5 mmol) in 1-heptanol (10 mL), hydrazine hydrate (1.2 mL, 25 mmol) was added

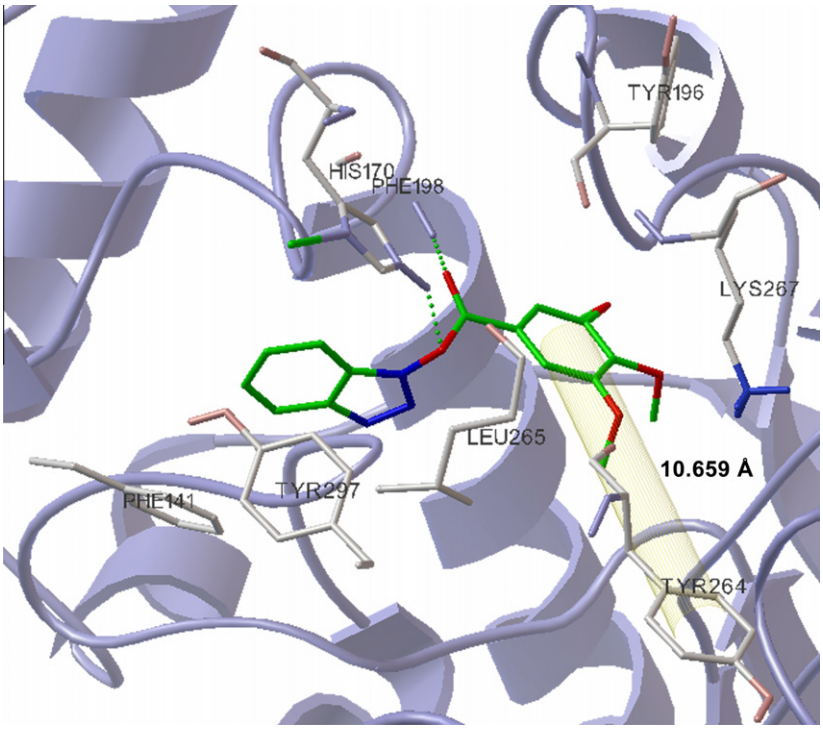


Figure 1. Binding model of compound **9** and HDLP.

and the solution was stirred at 110–120 °C for 5 h. The reaction mixture was then neutralized with 40% NaOH aq sol. Excess hydrazine and 1-heptanol were removed under reduced pressure, and then the pH value was adjusted to 3.2–3.5 with 1 M HCl. The precipitated product was collected by filtration, washed by 5% ice NaCl aq soln and recrystallized (CH_2Cl_2/CH_3OH) to afford compound **1** as white powder, yield 95%, mp 155–157 °C (lit.[15] mp 154–155 °C).

4.1.3. Synthesis of 6-methoxy-1*H*-benzo[*d*][1,2,3]triazol-1-ol (2)

Compound **2** was obtained as white powder in 90% yield from 1-chloro-4-methoxy-2-nitrobenzene (938 mg, 5 mmol), 1-heptanol (10 mL), and hydrazine hydrate (1.2 mL, 25 mmol) in a similar manner as described for the preparation of compound **1**, mp 169–171 °C (lit.[19] mp 168–170 °C).

4.1.4. Synthesis of 1*H*-benzo[*d*][1,2,3]triazole (17)

To a solution of benzene-1,2-diamine (541 mg, 5 mmol) in AcOH (10 mL), $NaNO_2$ aq soln (25 mL, 1 M) was added and the solution was stirred at 70–80 °C for 1 h. And then the pH value was adjusted to 4.4–4.6 by 40% NaOH aq soln and 1 M HCl. The precipitated product was collected by filtration, washed by 5% ice NaCl aq soln and recrystallized (CH_2Cl_2/CH_3OH) to afford compound **17** as white powder, yield 90%, mp 98–100 °C (lit.[20] mp 99–101 °C).

4.1.5. General synthesis method of benzotriazoles 3–16 and 18–24

To a solution of triazole (**1**, **2**, or **17**, 2.5 mmol) and substituted benzoic acid (hydroxyl or methoxyl, 2.5 mmol) in CH_2Cl_2 (30 mL), EDC·HCl (575 mg, 3 mmol) was added at rt. After being stirred for 24 h, the mixture was evaporated under reduced pressure to give a residue. The residue was purified by silica gel chromatography (PE/EtOAc = 1:1) and recrystallized from PE/EtOAc to afford benzotriazole (**3–16** or **18–24**).

4.1.5.1. 1*H*-Benzo[*d*][1,2,3]triazol-1-yl benzoate (3). White solid, yield 80%, mp 82–83 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.29–8.25 (m, 2H, Ar–H), 8.11 (d, J = 8.3 Hz, 1H, Bt Ar–H), 7.80–7.76 (m, 1H, Ar–H), 7.61–7.58 (m, 2H, Ar–H), 7.55–7.51 (m, 1H, Bt Ar–H), 7.32–7.29 (m, 2H, Bt Ar–H). ESI-MS: 240.1 $[M+H]^+$. Anal. Calcd for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.79; N, 17.56. Found: C, 65.36; H, 3.95; N, 17.34. All spectral data agreed with previously reported data.[21]

4.1.5.2. 1*H*-Benzo[*d*][1,2,3]triazol-1-yl 4-hydroxybenzoate (4). White solid, yield 78%, mp 185–186 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.16 (d, J = 8.3 Hz, 1H, Bt Ar–H), 8.08 (d, J = 8.5 Hz, 2H, Ar–H), 7.64–7.59 (m, 1H, Bt Ar–H), 7.50–7.45 (m, 2H, Bt Ar–H), 7.38 (d, J = 8.5 Hz, 2H, Ar–H). ESI-MS: 256.1 $[M+H]^+$. Anal. Calcd for $C_{13}H_9N_3O_3$: C, 61.18; H, 3.55; N, 16.46. Found: C, 61.36; H, 3.68; N, 16.40.

4.1.5.3. 1*H*-Benzo[*d*][1,2,3]triazol-1-yl 3,5-dihydroxybenzoate (5). White solid, yield 75%, mp 193–194 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.11 (d, J = 8.1 Hz, 1H, Bt Ar–H), 7.60–7.57 (m, 1H, Bt Ar–H), 7.46–7.41 (m, 2H, Bt Ar–H), 7.46 (d, J = 2.2 Hz, 2H, Ar–H), 6.90 (d, J = 2.2 Hz, 1H, Ar–H). ESI-MS: 272.1 $[M+H]^+$. Anal. Calcd for $C_{13}H_9N_3O_4$: C, 57.57; H, 3.34; N, 15.49. Found: C, 57.42; H, 3.61; N, 15.32.

4.1.5.4. 1*H*-Benzo[*d*][1,2,3]triazol-1-yl 3,4,5-trihydroxybenzoate (6). White solid, yield 70%, mp 199–200 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.14 (d, J = 8.3 Hz, 1H, Bt Ar–H), 7.70–7.66 (m, 1H, Bt Ar–H), 7.57 (s, 2H, Ar–H), 7.52–7.44 (m, 2H, Bt Ar–H). ESI-MS: 288.0 $[M+H]^+$. Anal. Calcd for $C_{13}H_9N_3O_5$: C, 54.36; H, 3.16; N, 14.63. Found: C, 54.56; H, 3.26; N, 14.36.

4.1.5.5. 1*H*-Benzo[*d*][1,2,3]triazol-1-yl 4-methoxybenzoate (7). White crystal, yield 82%, mp 158–159 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.12 (d, J = 8.3 Hz, 1H, Bt Ar–H), 7.96 (d, J = 8.5 Hz, 2H, Ar–H), 7.57–7.52 (m, 1H, Bt Ar–H), 7.40–7.37 (m, 2H, Bt Ar–H), 7.16–7.12 (d, J = 8.5 Hz, 2H, Ar–H), 4.01 (s, 3H, OCH_3). ESI-MS: 270.1 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.36; H, 4.04; N, 15.71.

4.1.5.6. 1*H*-Benzo[*d*][1,2,3]triazol-1-yl 3,5-dimethoxybenzoate (8). White crystal, yield 84%, mp 162–163 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.10 (d, J = 8.2 Hz, 1H, Bt Ar–H), 7.58–7.54 (m, 1H, Bt Ar–H), 7.45–7.40 (m, 2H, Bt Ar–H), 7.36 (d, J = 2.2 Hz, 2H, Ar–H), 6.86 (d, J = 2.2 Hz, 1H, Ar–H), 3.98 (s, 6H, OCH_3). ESI-MS: 300.1 $[M+H]^+$. Anal. Calcd for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04. Found: C, 60.26; H, 4.16; N, 14.28.

4.1.5.7. 1*H*-Benzo[*d*][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate (9). White crystal, yield 85%, mp 175–176 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.12 (d, J = 8.4 Hz, 1H, Bt Ar–H), 7.60–7.54 (m, 1H, Bt Ar–H), 7.52 (s, 2H, Ar–H), 7.50–7.43 (m, 2H, Bt Ar–H), 4.00 (s, 3H, OCH_3), 3.97 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 162.4, 153.4 (2C), 144.2, 143.6, 128.9, 128.7, 124.8, 120.6, 119.1, 108.4, 108.1 (2C), 61.1, 56.5 (2C). EI-MS *m/z* (%): 212 (100), 197 (56), 195 (27), 119 (12), 91 (9). ESI-MS: 330.1 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_5$: C, 58.36; H, 4.59; N, 12.76. Found: C, 58.65; H, 4.62; N, 12.64.

4.1.5.8. 6-Methoxy-1*H*-benzo[*d*][1,2,3]triazol-1-yl benzoate (10). White crystal, yield 81%, mp 96–97 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.26–8.24 (m, 2H, Ar–H), 8.02 (d, J = 8.4 Hz, 1H, Bt Ar–H), 7.78–7.74 (m, 1H, Ar–H), 7.60–7.57 (m, 2H, Ar–H), 7.46–7.42 (m, 1H, Bt Ar–H), 7.10–7.05 (m, 1H, Bt Ar–H), 3.92 (s, 3H, OCH_3). ESI-MS: 270.1 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.35; H, 4.02; N, 15.76.

4.1.5.9. 6-Methoxy-1*H*-benzo[*d*][1,2,3]triazol-1-yl 4-hydroxybenzoate (11). White crystal, yield 78%, mp 185–186 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.05 (d, J = 8.2 Hz, 1H, Bt Ar–H), 7.95 (d, J = 8.4 Hz, 2H, Ar–H), 7.49–7.44 (m, 1H, Bt Ar–H), 7.18–7.13 (m, 1H, Bt Ar–H), 7.21 (d, J = 8.4 Hz, 2H, Ar–H), 3.95 (s, 3H, Bt OCH_3). ESI-MS: 286.1 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}N_3O_4$: C, 58.95; H, 3.89; N, 14.73. Found: C, 58.81; H, 3.78; N, 14.85.

4.1.5.10. 6-Methoxy-1*H*-benzo[*d*][1,2,3]triazol-1-yl 3,5-dihydroxybenzoate (12). White crystal, yield 72%, mp 205–206 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.01 (d, J = 8.4 Hz, 1H, Bt Ar–H), 7.45–7.41 (m, 1H, Bt Ar–H), 7.36 (d, J = 2.3 Hz, 2H, Ar–H), 7.12–7.07 (m, 1H, Bt Ar–H), 6.85 (d, J = 2.3 Hz, 1H, Ar–H), 3.91 (s, 3H, Bt OCH_3). ESI-MS: 302.1 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}N_3O_5$: C, 55.82; H, 3.68; N, 13.95. Found: C, 56.02; H, 3.78; N, 13.78.

4.1.5.11. 6-Methoxy-1*H*-benzo[*d*][1,2,3]triazol-1-yl 3,4,5-trihydroxybenzoate (13). White crystal, yield 68%, mp 208–209 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 7.99 (d, J = 8.5 Hz, 1H, Bt Ar–H), 7.58 (s, 2H, Ar–H), 7.49–7.45 (m, 1H, Bt Ar–H), 7.21–7.15 (m, 1H, Bt Ar–H), 3.95 (s, 3H, Bt OCH_3). ESI-MS: 318.1 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}N_3O_6$: C, 53.00; H, 3.49; N, 13.24. Found: C, 53.07; H, 3.62; N, 13.05.

4.1.5.12. 6-Methoxy-1*H*-benzo[*d*][1,2,3]triazol-1-yl 4-methoxybenzoate (14). White crystal, yield 83%, mp 161–162 °C. ^{1}H NMR ($CDCl_3$, δ ppm): 8.03 (d, J = 8.3 Hz, 1H, Bt Ar–H), 7.92 (d, J = 8.5 Hz, 2H, Ar–H), 7.47–7.43 (m, 1H, Bt Ar–H), 7.15–7.12 (m, 1H, Bt Ar–H), 7.18 (d, J = 8.5 Hz, 2H, Ar–H), 4.02 (s, 3H, Ar OCH_3), 3.93 (s, 3H, Bt

OCH_3). ESI-MS: 300.1 $[M+H]^+$. Anal. Calcd for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04. Found: C, 60.02; H, 4.56; N, 13.92.

4.1.5.13. 6-Methoxy-1*H*-benzo[*d*][1,2,3]triazol-1-yl 3,5-dimethoxybenzoate (15). White crystal, yield 85%, mp 176–177 °C. ^1H NMR ($CDCl_3$, δ ppm): 7.98 (d, J = 8.4 Hz, 1H, Bt Ar–H), 7.44–7.40 (m, 1H, Bt Ar–H), 7.32 (d, J = 2.2 Hz, 2H, Ar–H), 7.10–7.06 (m, 1H, Bt Ar–H), 6.82 (d, J = 2.2 Hz, 1H, Ar–H), 3.96 (s, 6H, Ar OCH_3), 3.88 ppm (s, 3H, Bt OCH_3). ESI-MS: 330.1 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_5$: C, 58.36; H, 4.59; N, 12.76. Found: C, 58.55; H, 4.74; N, 12.41.

4.1.5.14. 6-Methoxy-1*H*-benzo[*d*][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate (16). White crystal, yield 81%, mp 182–183 °C. ^1H NMR ($CDCl_3$, δ ppm): 7.96 (d, J = 8.5 Hz, 1H, Bt Ar–H), 7.55 (s, 2H, Ar–H), 7.46–7.42 (m, 1H, Bt Ar–H), 7.18–7.14 (m, 1H, Bt Ar–H), 4.02 (s, 3H, Ar OCH_3), 3.99 (s, 6H, Ar OCH_3), 3.91 (s, 3H, Bt OCH_3). ESI-MS: 360.1 $[M+H]^+$. Anal. Calcd for $C_{17}H_{17}N_3O_6$: C, 56.82; H, 4.77; N, 11.69. Found: C, 56.71; H, 4.88; N, 11.42.

4.1.5.15. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(phenyl)methanone (18). White crystal, yield 78%, mp 111–112 °C. ^1H NMR ($CDCl_3$, δ ppm): 8.36 (d, J = 8.3 Hz, 1H, Bt Ar–H), 8.25–8.20 (m, 2H, Ar–H), 8.19–8.14 (m, 1H, Bt Ar–H), 7.74–7.72 (m, 1H, Ar–H), 7.69–7.64 (m, 2H, Ar–H), 7.62–7.55 (m, 2H, Bt Ar–H). ESI-MS: 224.1 $[M+H]^+$. Anal. Calcd for $C_{13}H_9N_3O$: C, 69.95; H, 4.06; N, 18.82. Found: C, 69.78; H, 4.09; N, 18.90. All spectral data agreed with previously reported data.[22]

4.1.5.16. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-hydroxyphenyl)methanone (19). White crystal, yield 80%, mp 115–116 °C. ^1H NMR ($CDCl_3$, δ ppm): 8.42 (d, J = 8.3 Hz, 1H, Bt Ar–H), 8.30 (d, J = 8.5 Hz, 2H, Ar–H), 8.22–8.18 (m, 1H, Bt Ar–H), 7.81–7.76 (m, 1H, Bt Ar–H), 7.60–7.55 (m, 1H, Bt Ar–H), 7.26 (d, J = 8.5 Hz, Ar–H). ESI-MS: 240.1 $[M+H]^+$. Anal. Calcd for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.79; N, 17.56. Found: C, 65.35; H, 3.61; N, 17.72.

4.1.5.17. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(3,5-dihydroxyphenyl)methanone (20). White crystal, yield 78%, mp 144–145 °C. ^1H NMR ($CDCl_3$, δ ppm): 8.36 (d, J = 8.4 Hz, 1H, Bt Ar–H), 8.19–8.16 (m, 1H, Bt Ar–H), 7.73–7.69 (m, 1H, Bt Ar–H), 7.55–7.51 (m, 1H, Bt Ar–H), 7.46 (d, J = 2.3 Hz, 2H, At–H), 6.95 (d, J = 2.3 Hz, 1H, Ar–H). ESI-MS: 256.1 $[M+H]^+$. Anal. Calcd for $C_{13}H_9N_3O_3$: C, 61.18; H, 3.55; N, 16.46. Found: C, 61.26; H, 3.42; N, 16.39.

4.1.5.18. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(3,4,5-trihydroxyphenyl)methanone (21). White crystal, yield 75%, mp 157–158 °C. ^1H NMR ($CDCl_3$, δ ppm): 8.35 (d, J = 8.3 Hz, 1H, Bt Ar–H), 8.16–8.13 (m, 1H, Bt Ar–H), 7.78–7.75 (m, 1H, Bt Ar–H), 7.59–7.55 (m, 1H, Bt Ar–H), 7.27 (s, 2H, At–H). ESI-MS: 272.1 $[M+H]^+$. Anal. Calcd for $C_{13}H_9N_3O_4$: C, 57.57; H, 3.34; N, 15.49. Found: C, 57.51; H, 3.51; N, 15.58.

4.1.5.19. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-methoxyphenyl)methanone (22). White crystal, yield 84%, mp 108–110 °C. ^1H NMR ($CDCl_3$, δ ppm): 8.35 (d, J = 8.2 Hz, 1H, Bt Ar–H), 8.27 (d, J = 8.5 Hz, 2H, Ar–H), 8.18–8.14 (m, 1H, Bt Ar–H), 7.74–7.70 (m, 1H, Bt Ar–H), 7.55–7.53 (m, 1H, Bt Ar–H), 7.05 (d, J = 8.5 Hz, Ar–H), 3.95 (s, 3H, OCH_3). ESI-MS: 254.1 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}N_3O_2$: C, 66.40; H, 4.38; N, 16.59. Found: C, 66.54; H, 4.62; N, 16.49. All spectral data agreed with previously reported data.[22]

4.1.5.20. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(3,5-dimethoxyphenyl)methanone (23). White crystal, yield 82%, mp 116–118 °C. ^1H NMR ($CDCl_3$, δ ppm): 8.34 (d, J = 8.3 Hz, 1H, Bt Ar–H), 8.16–8.14 (m, 1H, Bt Ar–H), 7.70–7.67 (m, 1H, Bt Ar–H), 7.53–7.50 (m, 1H, Bt Ar–H), 7.44 (d, J = 2.3 Hz, 2H, At–H), 6.92 (d, J = 2.3 Hz, 1H, Ar–H), 3.99 (s, 6H, OCH_3). ESI-MS: 284.1 $[M+H]^+$. Anal. Calcd for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.29; H, 4.22; N, 15.06.

4.1.5.21. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(3,4,5-trimethoxyphenyl)methanone (24). White crystal, yield 83%, mp 126–128 °C. ^1H NMR ($CDCl_3$, δ ppm): 8.32 (d, J = 8.3 Hz, 1H, Bt Ar–H), 8.15–8.13 (m, 1H, Bt Ar–H), 7.76–7.74 (m, 1H, Bt Ar–H), 7.56–7.53 (m, 1H, Bt Ar–H), 7.26 (s, 2H, At–H), 3.97 (s, 6H, OCH_3), 3.94 (s, 3H, OCH_3). ESI-MS: 314.1 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41. Found: C, 61.21; H, 4.48; N, 13.55.

4.2. Antiproliferative activity

Regents for cell culture were obtained from Gibco-BRL Life Technologies (Gaithersburg, Maryland). Human oral epidermoid carcinoma KB cells, non-small cell lung carcinoma H460 cells, and stomach carcinoma MKN45 cells were maintained in RPMI-1640 medium supplied with 5% fetal bovine serum. Cell in logarithmic phase were cultured at a density of 5000 cells/mL/well in a 24-well plate. The cells were exposed to various concentrations of the test drugs for 72 h. The methylene blue dye assay was used to evaluate the effect of the test compounds on cell growth as described previously.[23] The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically as a comparison with the control.

4.3. HDAC inhibitory activity

HDAC inhibitor drug screening kit was purchased from BioVision (USA). HDAC assay were carried out according its protocol. Briefly, test samples were diluted into 85 μL with double distilled water (ddH_2O) in each well. For reference drug, 83 μL of ddH_2O and 2 μL of TSA or SAHA were added in the well. Then, 10 μL of the 10 × HDAC assay buffer and 2 μL of HeLa nuclear extract were added to each well, mixing thoroughly. After that, 5 μL of the HDAC fluorometric substrate was added to each well, mixing thoroughly. The plates were incubated at 37 °C for 30 min. And then, the reaction was stopped by adding 10 μL of lysine developer and the matrixes were mixed well. The plates were then incubated at 37 °C for 30 min. Finally, the samples were read in a fluorescence plate reader with Ex. = 350–380 nm and Em. = 440–460 nm.

4.4. Docking simulations

Molecular docking of compound **9** into 3D X-ray structure of HDLP (PDB code: 1c3r) was carried out using the AUTODOCK software package (Version 4.0) as implemented through the graphical user interface AutoDockTools (ADT 1.4.6).[18]

Declaration

The material is original and has not been submitted for publication elsewhere. There is no conflict of interest in the manuscript. The main work in synthesis of benzotriazoles compounds, evaluation their biological activities, data analysis, and paper writing were finished by Jie Fu. Docking simulations was done by Ying Yang and Wen-Jun Mao. Xue-Wei Zhang and Zhi-Ming Zhang contributed to synthesis, biological assay, and data analysis. Hai-Liang Zhu is the corresponding author.

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Supplementary data

Supplementary data (the contents include ^{1}H NMR, ^{13}C NMR, ESI-MS, and EI-MS spectra of compound **9**, 3D X-ray structure of HDLP) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.10.049.

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