Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Supplementary Information

Design, synthesis, and preliminary bioactivity studies of substituted purine hydroxamic acid derivatives as novel histone deacetylase (HDAC) inhibitors

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Experimental

Chemistry

All microwave reactions took place in a CEM Discover microwave synthesis system. All materials and reagents used in this work are analytical reagents without further purification. Solvents were distilled prior to use and flash chromatography was performed using silica gel (60 Å, 200－300 mesh). All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (GF-254) and visualized with UV light. Melting points were determined on an electrothermal melting point apparatus and thermometer is uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Brucker Avance-300 or Brucker Avance-400 spectrometers using trimethylsilane as an internal standard. High-resolution mass spectral (HRMS) data were reported as *m/z* (relative intensity).

The general synthesis of compounds 2a–2g

The compounds **1a**–**1g** were synthesized according to the literatures [1, 2] and were used in the next step with no further purification.

The mixture of **1** (30 mmol), K_2CO_3 (12.4 g, 90 mmol) and ethyl chloroacetate (4 mL, 36 mmol) in 50 mL DMSO was stirred at room temperature for 4 h. Then five times H_2O was added and extracted with EtOAc. After concentration, the residue was purified by recrystallization or chromatography.

ethyl 2-(6-(benzylamino)-9*H*-purin-9-yl)acetate (2a)

Yield 47%. ^{1}H NMR (400 MHz, DMSO–d_6) δ: 1.22 (t, J=7.2 Hz, 3H), 4.18 (q, J=7.2 Hz, 2H), 4.77 (br s, 2H), 5.08 (s, 2H), 7.20–7.38 (m, 5H), 8.14 (s, 1H), 8.20 (s, 1H), 8.25 (s, 1H).

ethyl 2-(2-amino-6-morpholino-9*H*-purin-9-yl)acetate (2c)

Yield 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.31 (t, J=7.2 Hz, 3H), 3.81–3.83 (m, 4H), 4.26 (q, J=7.2 Hz, 2H), 4.29 (br s, 4H), 4.92 (s, 2H), 7.48 (s, 1H).

ethyl 2-(6-morpholino-2-(phenylamino)-9*H*-purin-9-yl)acetate (2e)

Yield 62%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.31 (t, J=7.2 Hz, 3H), 3.82–3.85 (m, 4H), 4.23–4.31 (m, 6H), 4.85 (s, 2H), 6.83 (br s, 1H), 6.98 (t, J=7.2 Hz, 1H), 7.27–7.33 (m, 2H), 7.59–7.62 (m, 3H).

ethyl 2-(6-(phenylamino)-9*H*-purin-9-yl)acetate (2f)

Yield 47%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30 (t, J=7.2 Hz, 3H), 4.27 (q, J=7.2 Hz, 2H), 4.99 (s, 2H), 7.09–7.15 (m, 1H), 7.36–7.42 (m, 2H), 7.78–7.81 (m, 3H), 7.90 (s, 1H), 8.52 (s, 1H).

ethyl 2-(2,6-bis(phenylamino)-9*H*-purin-9-yl)acetate (2g)

Yield 45%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32 (t, J=7.2 Hz, 3H), 4.29 (q, J=7.2 Hz, 2H), 4.89 (s, 2H), 6.99–7.04 (m, 1H), 7.09–7.14 (m, 2H), 7.29–7.39 (m, 4H), 7.64–7.66 (m, 2H), 7.70 (s, 1H), 7.76–7.79 (m, 2H), 7.94 (br s, 1H).

Compounds **2b** and **2d** were used in the next step without further purification.

The general synthesis of compounds 3a–3g

The compound **2** (15 mmol) was dissolved in 30 mL THF/ MeOH (3:1). Then 2 mol/L NaOH (23 mL, 45 mmol) was added. One hour later, 3 mol/L HCl was used to adjust pH to 2–3 after removing the organic solvent. Precipitation was collected and washed with H_2O repeatedly. And purified by recrystallization with EtOH if necessary.

Compounds **3a**, **3e–3g** were used in the next step with no future purification.

2-(6-morpholino-9*H*-purin-9-yl)acetic acid (3b)

Yield 43%. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 3.72 (t, J=4.8 Hz, 4H), 4.21 (br s, 4H), 4.99 (s, 2H), 8.18 (s, 1H), 8.25 (s, 1H), 13.25 (s, 1H).

2-(2-amino-6-morpholino-9*H*-purin-9-yl)acetic acid (3c)

Yield 89%. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 3.67 (t, J=4.5 Hz, 4H), 4.11 (br s, 4H), 4.30 (s, 2H), 5.81 (s, 2H), 7.63 (s, 1H).

2-(6-(methylamino)-9*H*-purin-9-yl)acetic acid (3d)

Yield 33%. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.96 (s, 3H), 4.81 (s, 2H), 7.65 (br s, 1H), 8.06 (s, 1H), 8.19 (s, 1H), 11.99 (br s, 1H).

The general synthesis of compounds 4a–4x

Compound **3** (3 mmol), amino acid methyl ester hydrochloride (3.6 mmol), *N*-Hydroxy benzotriazole (HOBt, 0.61 g, 4.5 mmol) and triethylamine (TEA, 0.8 mL, 6 mmol) were added in 25 mL anhydrous DCM. Then, the suspension of EDCI (0.86 g, 4.5 mmol) in 10 mL anhydrous DCM was added dropwise to the mixture at 0 ℃. And the reaction was stirred at room temperature over night. Then the reaction was washed with 10% citric acid (30 mL×3), saturated $NaHCO_3$ (30 mL×3) and saturated brine. The solvent was evaporated under reduced pressure after being dried with $MgSO_4$ and purified through chromatography.

methyl 2-(2-(6-(benzylamino)-9*H*-purin-9-yl)acetamido)acetate (4a)

Purified by column chromatography (SiO_2, 20:80 Petroleum ether/EtOAC) to give a white solid. Yield 43%; m.p. 202–204 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.74 (s, 3H), 4.04 (d, J=5.4 Hz, 2H), 4.90 (s, 4H), 6.13 (br s, 1H), 7.09 (br s, 1H), 7.29–7.41 (m, 5H), 7.83 (s, 1H), 8.43 (s, 1H).

methyl 3-(2-(6-(benzylamino)-9*H*-purin-9-yl)acetamido)propanoate (4b)

Purified by column chromatography (SiO_2, 20:80 Petroleum ether/EtOAC) to give a white solid. Yield 33%; m.p. 167–168 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.47–2.51 (m, 2H), 3.29–3.35 (m, 2H), 3.60 (s, 3H), 4.72 (br s, 2H), 4.82 (s, 2H), 7.17–7.35 (m, 5H), 8.08 (s, 1H), 8.16 (s, 1H), 8.29 (br s, 1H), 8.40 (t, J=5.4 Hz, 1H).

methyl 4-(2-(6-(benzylamino)-9*H*-purin-9-yl)acetamido)butanoate (4c)

Purified by column chromatography (SiO_2, 20:80 Petroleum ether/EtOAC) to give a white solid. Yield 54%; m.p. 186–188 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.67 (quint, J=7.2 Hz, 2H), 2.34 (t, J=7.5 Hz, 2H), 3.10 (q, J=5.7 Hz, 2H), 3.58 (s, 3H), 4.71 (br s, 2H), 4.82 (s, 2H), 7.18–7.35 (m, 5H), 8.08 (s, 1H), 8.16 (s, 1H), 8.40 (t, J=5.4 Hz, 2H).

methyl 6-(2-(6-(benzylamino)-9*H*-purin-9-yl)acetamido)hexanoate (4d)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 60%; m.p. 168–169 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.22–1.31 (m, 2H), 1.36–1.44 (m, 2H), 1.47–1.57 (m, 2H), 2.30 (t, J=7.2 Hz, 2H), 3.06 (q, J=5.7 Hz, 2H), 3.58 (s, 3H), 4.71 (br s, 2H), 4.82 (s, 2H), 7.18–7.35 (m, 5H), 8.08 (s, 1H), 8.16 (s, 1H), 8.27 (t, J=5.4 Hz, 1H), 8.32 (br s, 1H).

methyl 3-(2-(6-morpholino-9*H*-purin-9-yl)acetamido)propanoate (4e)

Purified by column chromatography (SiO_2, 20:80 Petroleum ether/EtOAC) to give a white solid. Yield 57%; m.p. 204–205 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.46–2.48 (m, 2H), 3.28–3.35 (m, 2H), 3.61 (s, 3H), 3.70–3.73 (m, 4H), 4.21 (br s, 4H), 4.84 (s, 2H), 8.12 (s, 1H), 8.23 (s, 1H), 8.40 (t, J=5.4 Hz, 1H).

methyl 4-(2-(6-morpholino-9*H*-purin-9-yl)acetamido)butanoate (4f)

Purified by column chromatography (SiO_2, 20:80 Petroleum ether/EtOAC) to give a white

solid. Yield 40%; m.p. 171–172 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.80 (quint, J=6.9 Hz, 2H), 2.32 (t, J=6.9 Hz, 2H), 3.28 (q, J=5.7 Hz, 2H), 3.65 (s, 3H), 3.82–3.86 (m, 4H), 4.32 (br s, 4H), 4.80 (s, 2H), 6.89 (br s, 1H), 7.83 (s, 1H), 8.34 (s, 1H).

methyl 6-(2-(6-morpholino-9*H*-purin-9-yl)acetamido)hexanoate (4g)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 39%; m.p. 165–166 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23–1.33 (m, 2H), 1.48 (quint, J=7.2 Hz, 2H), 1.60 (quint, J=7.5 Hz, 2H), 2.27 (t, J=7.2 Hz, 2H), 3.23 (q, J=6.0 Hz, 2H), 3.66 (s, 3H), 3.83–3.86 (m, 4H), 4.32 (br s, 4H), 4.80 (s, 2H), 6.72 (br s, 1H), 7.83 (s, 1H), 8.35 (s, 1H).

methyl 2-(4-(2-(6-morpholino-9*H*-purin-9-yl)acetamido)phenyl)acetate (4h)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 46%; m.p. 229–231 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 3.60 (s, 3H), 3.62 (s, 2H), 3.71–3.75 (m, 4H), 4.22 (br s, 4H), 5.09 (s, 2H), 7.21 (d, J=8.4 Hz, 2H), 7.51 (d, J=8.4 Hz, 2H), 8.20 (s, 1H), 8.24 (s, 1H), 10.49 (br s, 1H).

methyl 3-(2-(2-amino-6-morpholino-9*H*-purin-9-yl)acetamido)propanoate (4i)

Purified by column chromatography (SiO_2, 20:80 Petroleum ether/EtOAC) to give a white solid. Yield 19%; m.p. 214–216 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.53 (t, J=6.0 Hz, 2H), 3.50 (t, J=6.0 Hz, 2H), 3.68 (s, 3H), 3.79–3.82 (m, 4H), 4.24 (br s, 4H), 4.65 (s, 2H), 4.89 (br s, 2H), 7.51 (s, 1H), 7.83 (br s, 1H).

methyl 4-(2-(2-amino-6-morpholino-9*H*-purin-9-yl)acetamido)butanoate (4j)

Purified by column chromatography (SiO_2, 20:80 Petroleum ether/EtOAC) to give a white solid. Yield 36%; m.p. 207–208 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.81 (quint, J=6.9 Hz, 2H), 2.34 (t, J=7.2 Hz, 2H), 3.27 (q, J=6.3 Hz, 2H), 3.66 (s, 3H), 3.79–3.82 (m, 4H), 4.24 (br s, 4H), 4.65 (s, 2H), 4.77 (br s, 2H), 7.06 (br s, 1H), 7.50 (s, 1H).

methyl 6-(2-(2-amino-6-morpholino-9*H*-purin-9-yl)acetamido)hexanoate (4k)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 56%; m.p. 193–194 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.24–1.34 (m, 2H), 1.47 (quint, J=7.2 Hz, 2H), 1.60 (quint, J=7.5 Hz, 2H), 2.29 (t, J=7.2 Hz, 2H), 3.21 (q, J=6.6 Hz, 2H), 3.67 (s, 3H), 3.79–3.82 (m, 4H), 4.23 (br s, 4H), 4.65 (s, 2H), 4.75 (br s, 2H), 6.85 (br s, 1H), 7.52 (s, 1H).

methyl 2-(4-(2-(2-amino-6-morpholino-9*H*-purin-9-yl)acetamido)phenyl)acetate (4l)

Purified by column chromatography (SiO_2, 25:75 Petroleum ether/EtOAC) to give a white solid. Yield 55%; m.p. 246–248 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.57 (s, 2H), 3.67 (s, 3H), 3.80–3.82 (m, 4H), 4.28 (br s, 4H), 4.88 (s, 2H), 5.09 (br s, 2H), 7.21 (d, J=8.1 Hz, 2H), 7.52 (d, J=8.1 Hz, 2H), 7.62 (s, 1H), 9.88 (br s, 1H).

methyl 2-(2-(6-(methylamino)-9*H*-purin-9-yl)acetamido)acetate (4m)

Purified by column chromatography (SiO_2, 25:75 Petroleum ether/EtOAC) to give a white solid. Yield 26%; m.p. 196–198 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.97 (br s, 3H), 3.63 (s, 3H), 3.92 (d, J=5.7 Hz, 2H), 4.92 (s, 2H), 7.66 (br s, 1H), 8.06 (s, 1H), 8.20 (s, 1H), 8.75 (t, J=5.7 Hz, 1H).

methyl 3-(2-(6-(methylamino)-9*H*-purin-9-yl)acetamido)propanoate (4n)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 23%; m.p. 214–216 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.47–2.51 (m, 2H), 2.97 (br s, 3H), 3.32 (q, J=5.7 Hz, 2H), 3.61 (s, 3H), 4.81 (s, 2H), 7.65 (br s, 1H), 8.04 (s, 1H), 8.19 (s,

1H), 8.39 (t, *J*=5.4 Hz, 1H).

methyl 4-(2-(6-(methylamino)-9*H*-purin-9-yl)acetamido)butanoate (4o)

Purified by column chromatography (SiO_2, 20:80 Petroleum ether/EtOAC) to give a white solid. Yield 30%; m.p. 214–216 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.79–1.88 (m, 2H), 2.32–2.39 (m, 2H), 3.24–3.34 (m, 2H), 3.65 (s, 3H), 3.68 (s, 3H), 4.85 (s, 2H), 6.85 (br s, 1H), 7.20–7.24 (m, 1H), 7.89 (s, 1H), 8.40 (s, 1H).

methyl 3-(2-(6-morpholino-2-(phenylamino)-9*H*-purin-9-yl)acetamido)propanoate (4p)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 81%; m.p. 204–206 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.49 (t, *J*=6.0 Hz, 2H), 3.48 (q, *J*=6.0 Hz, 2H), 3.61 (s, 3H), 3.82–3.85 (m, 4H), 4.28 (br s, 4H), 4.71 (s, 2H), 6.98–7.03 (m, 1H), 7.23 (br s, 1H), 7.29–7.35 (m, 2H), 7.56 (s, 1H), 7.59–7.62 (m, 2H), 7.71 (br s, 1H).

methyl 4-(2-(6-morpholino-2-(phenylamino)-9*H*-purin-9-yl)acetamido)butanoate (4q)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 46%; m.p. 172–173 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.71 (quint, *J*=6.9 Hz, 2H), 2.26 (t, *J*=7.2 Hz, 2H), 3.23 (q, *J*=6.6 Hz, 2H), 3.61 (s, 3H), 3.82–3.85 (m, 4H), 4.29 (br s, 4H), 4.72 (s, 2H), 7.01–7.06 (m, 1H), 7.13 (br s, 1H), 7.30–7.35 (m, 2H), 7.57–7.60 (m, 3H).

methyl 6-(2-(6-morpholino-2-(phenylamino)-9*H*-purin-9-yl)acetamido)hexanoate (4r)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 67%; m.p. 166–167 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.11–1.22 (m, 2H), 1.28–1.38 (m, 2H), 1.49 (quint, *J*=7.5 Hz, 2H), 2.19 (t, *J*=7.2 Hz, 2H), 3.15 (q, *J*=6.6 Hz, 2H), 3.64 (s, 3H), 3.82–3.85 (m, 4H), 4.28 (br s, 4H), 4.72 (s, 2H), 6.90 (br s, 1H), 6.97 (br s, 1H), 6.99–7.05 (m, 1H), 7.29–7.35 (m, 2H), 7.58–7.61 (m, 3H).

methyl 5-(2-(6-(phenylamino)-9*H*-purin-9-yl)acetamido)pentanoate (4s)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 53%; m.p. 210–212 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47–1.66 (m, 4H), 2.30 (t, *J*=6.9 Hz, 2H), 3.27 (q, *J*=6.3 Hz, 2H), 3.64 (s, 3H), 4.86 (s, 2H), 6.65 (br s, 1H), 7.12–7.17 (m, 1H), 7.38–7.43 (m, 2H), 7.76 (br s, 1H), 7.79–7.82 (m, 2H), 7.96 (s, 1H), 8.54 (s, 1H).

methyl 6-(2-(6-(phenylamino)-9*H*-purin-9-yl)acetamido)hexanoate (4t)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 48%; m.p. 210–212 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.24–1.35 (m, 2H), 1.45–1.52 (m, 2H), 1.55–1.65 (m, 2H), 2.28 (t, *J*=7.2 Hz, 2H), 3.26 (q, *J*=6.6 Hz, 2H), 3.65 (s, 3H), 4.85 (s, 2H), 6.61 (br s,1H), 7.11–7.17 (m, 1H), 7.37–7.43 (m, 2H), 7.78–7.82 (m, 3H), 7.96 (s, 1H), 8.54 (s, 1H).

methyl 7-(2-(6-(phenylamino)-9*H*-purin-9-yl)acetamido)heptanoate (4u)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 76%; m.p. 206–207 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.27–1.35 (m, 4H), 1.43–1.52 (m, 2H), 1.53–1.63 (m, 2H), 2.27 (t, *J*=7.2 Hz, 2H), 3.24 (q, *J*=6.6 Hz, 2H), 3.65 (s, 3H), 4.85 (s, 2H), 6.60 (br s,1H), 7.15 (t, *J*=7.5 Hz, 1H), 7.37–7.42 (m, 2H), 7.79–7.81 (m, 2H), 7.85 (s, 1H), 7.96 (s, 1H), 8.52 (s, 1H).

methyl 5-(2-(2,6-bis(phenylamino)-9*H*-purin-9-yl)acetamido)pentanoate (4v)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 50%; m.p. 132–133 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.38–1.45 (m, 2H), 1.49–1.57 (m, 2H), 2.22 (t, *J*=7.2 Hz, 2H), 3.21 (q, *J*=6.6 Hz, 2H), 3.61 (s, 3H), 4.77 (s, 2H), 6.87 (br s, 1H), 7.03–7.08 (m, 1H), 7.11–7.16 (m, 1H), 7.21 (br s, 1H), 7.30–7.40 (m, 4H), 7.62–7.67 (m, 4H),

7.74–7.76 (m, 2H).

methyl 6-(2-(2,6-bis(phenylamino)-9*H*-purin-9-yl)acetamido)hexanoate (4w)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 75%; m.p. 166–168 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.15–1.24 (m, 2H), 1.32–1.41 (m, 2H), 1.51 (quint, *J*=7.5 Hz, 2H), 2.20 (t, *J*=7.2 Hz, 2H), 3.19 (q, *J*=6.6 Hz, 2H), 3.63 (s, 3H), 4.75 (s, 2H), 6.79 (br s,1H), 7.05 (t, *J*=7.2 Hz, 1H), 7.10–7.17 (m, 2H), 7.30–7.40 (m, 4H), 7.62–7.76 (m, 6H).

methyl 7-(2-(2,6-bis(phenylamino)-9*H*-purin-9-yl)acetamido)heptanoate (4x)

Purified by column chromatography (SiO_2, 35:65 Petroleum ether/EtOAC) to give a white solid. Yield 74%; m.p. 156–158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.17–1.25 (m, 4H), 1.31–1.40 (m, 2H), 1.51 (quint, *J*=7.2 Hz, 2H), 2.22 (t, *J*=7.2 Hz, 2H), 3.18 (q, *J*=6.6 Hz, 2H), 3.64 (s, 3H), 4.77 (s, 2H), 6.77 (br s,1H), 7.05 (t, *J*=7.2 Hz, 1H), 7.12–7.17 (m, 2H), 7.31–7.41 (m, 4H), 7.62–7.64 (m, 3H), 7.69 (s, 1H), 7.74–7.76 (m, 2H).

The general synthesis of compounds 5a–5x

Preparation of potassium hydroxylamine in methanol solution[3]: hydroxylamine hydrochloride (4.67 g, 67 mmol) was dissolved in methanol (24 mL) to form solution A. Potassium hydroxide (5.61 g, 100 mmol) was dissolved in methanol (14 mL) to form solution B. To the solution A at 0 ºC was added solution B dropwise. The mixture was stirred for 30 min at 0 °C. Then the solid was filtered out to afford a solution of hydroxylamine in methanol.

To a flask containing **4** (0.5 mmol) was added the solution of hydroxylamine in methanol (4 mL). The mixture was stirred at room temperature for half an hour. Then it was adjusted to pH 5 with 6 mol/L HCl. The mixture was concentrated to give a residue and washed with water and EtOH to afford the target compounds. And C18 reverse phase column chromatography were used while the compounds were water-soluble.

2-(6-(benzylamino)-9*H*-purin-9-yl)-*N*-(2-(hydroxyamino)-2-oxoethyl)acetamide (5a)

Washed with water and EtOH to afford a white solid. Yield 81%; m.p. 178 °C (dec.). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 3.67 (d, *J*=5.7 Hz, 2H), 4.72 (br s, 2H), 4.90 (s, 2H), 7.18–7.22 (m, 1H), 7.26–7.35 (m, 4H), 8.09 (s, 1H), 8.17 (s, 1H), 8.28 (br s, 1H), 8.61 (t, *J*=5.4 Hz, 1H), 8.84 (s, 1H), 10.57 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 40.2, 42.8, 45.0, 118.5, 126.5, 127.1, 128.1, 140.2, 141.6, 149.2, 152.3, 154.3, 165.3, 166.8; HRMS calcd for $C_{16}H_{17}N_7O_3$ 356.1466, found 356.1458.

3-(2-(6-(benzylamino)-9*H*-purin-9-yl)acetamido)-*N*-hydroxypropanamide (5b)

Washed with water and EtOH to afford a white solid. Yield 78%; m.p. 201–202 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.15 (t, *J*=7.2 Hz, 2H), 3.25–3.29 (m, 2H), 4.72 (br s, 2H), 4.81 (s, 2H), 7.18–7.22 (m, 1H), 7.26–7.35 (m, 4H), 8.07 (s, 1H), 8.16 (s, 1H), 8.23 (br s, 1H), 8.37 (t, *J*=5.4 Hz, 1H), 8.71 (s, 1H), 10.41 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 32.2, 35.4, 43.0, 44.9, 118.5, 126.5, 127.1, 128.1, 140.2, 141.7, 149.3, 152.3, 154.3, 165.3, 167.1; HRMS calcd for $C_{17}H_{19}N_7O_3$ 370.1622, found 370.1615.

4-(2-(6-(benzylamino)-9*H*-purin-9-yl)acetamido)-*N*-hydroxybutanamide (5c)

Washed with water and EtOH to afford a white solid. Yield 84%; m.p. 198–199 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.64 (quint, *J*=7.2 Hz, 2H), 1.98 (t, *J*=7.2 Hz, 2H), 3.08 (q, *J*=6.0 Hz, 2H), 4.72 (br s, 2H), 4.82 (s, 2H), 7.18–7.23 (m, 1H), 7.26–7.35 (m, 4H), 8.08 (s, 1H), 8.17 (s, 1H), 8.27–8.30 (m, 2H), 8.68 (s, 1H), 10.33 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 25.1, 29.8, 38.4, 43.0, 45.0, 118.6, 126.5, 127.1, 128.1, 140.2, 141.7, 149.2, 152.3, 154.4, 166.2, 168.7;

HRMS calcd for $C_{18}H_{21}N_7O_3$ 384.1779, found 384.1785.

6-(2-(6-(benzylamino)-9*H*-purin-9-yl)acetamido)-*N*-hydroxyhexanamide (5d)

Washed with water and EtOH to afford a white solid. Yield 91%; m.p. 192–194 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.19–1.29 (m, 2H), 1.36–1.53 (m, 4H), 1.93 (t, J=7.2 Hz, 2H), 3.06 (q, J=6.0 Hz, 2H), 4.71 (br s, 2H), 4.82 (s, 2H), 7.18–7.22 (m, 1H), 7.26–7.35 (m, 4H), 8.09 (s, 1H), 8.17 (s, 1H), 8.25–8.29 (m, 2H), 8.67 (s, 1H), 10.34 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 24.8, 25.9, 28.6, 32.2, 42.9, 45.0, 118.5, 126.5, 127.1, 128.1, 140.2, 141.7, 149.2, 152.3, 154.4, 166.1, 169.0; HRMS calcd for $C_{20}H_{25}N_7O_3$ 412.2092, found 412.2097.

***N*-hydroxy-3-(2-(6-morpholino-9*H*-purin-9-yl)acetamido)propanamide (5e)**

Washed with water and EtOH to afford a white solid. Yield 43%; m.p. 232–234 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.15 (t, J=7.2 Hz, 2H), 3.25–3.27 (m, 2H), 3.72 (t, J=4.8 Hz, 4H), 4.21 (br s, 4H), 4.83 (s, 2H), 8.11 (s, 1H), 8.23 (s, 1H), 8.37 (t, J=5.4 Hz, 1H), 8.73 (s, 1H), 10.42 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 32.2, 35.4, 44.9, 45.2, 66.2, 118.6, 141.1, 150.8, 151.7, 153.1, 166.2, 167.0; HRMS calcd for $C_{14}H_{19}N_7O_4$ 350.1571, found 350.1573.

***N*-hydroxy-4-(2-(6-morpholino-9*H*-purin-9-yl)acetamido)butanamide (5f)**

Washed with water and EtOH to afford a white solid. Yield 54%; m.p. 206–208 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.64 (quint, J=7.2 Hz, 2H), 1.97 (t, J=7.8 Hz, 2H), 3.07 (q, J=5.7 Hz, 2H), 3.72 (t, J=4.8 Hz, 4H), 4.21 (br s, 4H), 4.84 (s, 2H), 8.12 (s, 1H), 8.23 (s, 1H), 8.29 (t, J=5.4 Hz, 1H), 8.67 (s, 1H), 10.33 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 25.1, 29.8, 38.4, 45.0, 45.2, 66.2, 118.7, 141.1, 150.8, 151.7, 153.1, 166.1, 168.7; HRMS calcd for $C_{15}H_{21}N_7O_4$ 364.1728, found 364.1719.

***N*-hydroxy-6-(2-(6-morpholino-9*H*-purin-9-yl)acetamido)hexanamide (5g)**

Washed with water and EtOH to afford a white solid. Yield 67%; m.p. 192–194 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.24–1.32 (m, 2H), 1.36–1.43 (m, 2H), 1.46–1.53 (m, 2H), 1.93 (t, J=7.2 Hz, 2H), 3.06 (q, J=5.7 Hz, 2H), 3.72 (t, J=4.8 Hz, 4H), 4.21 (br s, 4H), 4.83 (s, 2H), 8.12 (s, 1H), 8.23–8.27 (m, 2H), 8.63 (s, 1H), 10.32 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 24.8, 25.9, 28.6, 32.2, 45.0, 45.2, 66.2, 118.7, 141.1, 150.9, 151.7, 153.2, 166.0, 167.0; HRMS calcd for $C_{17}H_{25}N_7O_4$ 392.2041, found 392.2048.

***N*-hydroxy-2-(4-(2-(6-morpholino-9*H*-purin-9-yl)acetamido)phenyl)acetamide (5h)**

Washed with water and EtOH to afford a white solid. Yield 71%; m.p. 202–204 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 3.23 (s, 2H), 3.73 (t, J=4.8 Hz, 4H), 4.22 (br s, 4H), 5.09 (s, 2H), 7.19 (d, J=8.4 Hz, 2H), 7.49 (d, J=8.4 Hz, 2H), 8.20 (s, 1H), 8.25 (s, 1H), 10.44 (s, 1H), 10.60 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 38.7, 45.4, 45.7, 66.1, 118.6, 119.0, 129.3, 131.2, 136.9, 141.3, 150.6, 151.2, 152.8, 164.8, 167.0; HRMS calcd for $C_{19}H_{21}N_7O_4$ 412.1728, found 412.1732.

3-(2-(2-amino-6-morpholino-9*H*-purin-9-yl)acetamido)-*N*-hydroxypropanamide (5i)

Washed with water and EtOH to afford a white solid. Yield 42%; m.p. 214–215 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.15 (t, J=7.2 Hz, 2H), 3.24–3.28 (m, 2H), 3.68 (t, J=4.8 Hz, 4H), 4.11 (br s, 4H), 4.62 (s, 2H), 5.88 (s, 2H), 7.67 (s, 1H), 8.23 (t, J=5.4 Hz, 1H), 8.73 (s, 1H), 10.42 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 32.2, 35.4, 44.5, 45.0, 66.2, 112.9, 137.8, 153.3, 153.6, 159.5, 166.6, 167.2; HRMS calcd for $C_{14}H_{20}N_8O_4$ 365.1680, found 365.1672.

4-(2-(2-amino-6-morpholino-9*H*-purin-9-yl)acetamido)-*N*-hydroxybutanamide (5j)

Washed with water and EtOH to afford a white solid. Yield 57%; m.p. 183–185 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.64 (quint, J=7.2 Hz, 2H), 1.97 (t, J=7.2 Hz, 2H), 3.06 (q, J=6.3 Hz, 2H), 3.68 (t, J=4.8 Hz, 4H), 4.11 (br s, 4H), 4.63 (s, 2H), 5.87 (s, 2H), 7.68 (s, 1H), 8.15 (t, J=5.4

Hz, 1H), 8.68 (s, 1H), 10.34 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 25.2, 29.8, 38.4, 44.6, 45.0, 66.2, 113.0, 137.8, 153.3, 153.6, 159.5, 166.5, 168.8; HRMS calcd for $C_{15}H_{22}N_8O_4$ 379.1837, found 379.1840.

6-(2-(2-amino-6-morpholino-9*H*-purin-9-yl)acetamido)-*N*-hydroxyhexanamide (5k)

Washed with water and EtOH to afford a light yellow solid. Yield 74%; m.p. 167–169 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.21–1.29 (m, 2H), 1.37–1.54 (m, 4H), 1.94 (t, J=7.2 Hz, 2H), 3.06 (q, J=5.7 Hz, 2H), 3.72 (t, J=4.5 Hz, 4H), 4.19 (br s, 4H), 4.73 (s, 2H), 7.83 (s, 1H), 8.21 (t, J=5.4 Hz, 1H), 10.34 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 24.7, 25.9, 28.6, 32.1, 45.2, 45.8, 66.0, 112.5, 138.8, 150.4, 151.1, 155.8, 165.8, 169.1; HRMS calcd for $C_{17}H_{26}N_8O_4$ 407.2150, found 407.2159.

2-(2-amino-6-morpholino-9*H*-purin-9-yl)-*N*-(4-(2-(hydroxyamino)-2-oxoethyl)phenyl) acetamide (5l)

Washed with water and EtOH to afford a white solid. Yield 79%; m.p. 271 °C (dec.). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 3.23 (s, 2H), 3.69 (t, J=4.5 Hz, 4H), 4.12 (br s, 4H), 4.88 (s, 2H), 5.89 (s, 2H), 7.19 (d, J=8.4 Hz, 2H), 7.48(d, J=8.4 Hz, 2H), 7.75 (s, 1H), 8.79 (s, 1H), 10.32 (s, 1H), 10.60 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 38.7, 45.0, 45.1, 66.2, 113.0, 118.9, 129.3, 131.1, 137.0, 137.9, 153.4, 153.6, 159.5, 165.3, 167.1; HRMS calcd for $C_{19}H_{22}N_8O_4$ 427.1837, found 427.1830.

***N*-hydroxy-2-(2-(6-(methylamino)-9*H*-purin-9-yl)acetamido)acetamide (5m)**

Purified by reverse phase column chromatography (C18, MeOH/H_2O, Gradient elution) to give a white solid. Yield 53%; m.p. 189–190 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.97 (br s, 3H), 3.68 (d, J=5.7 Hz, 2H), 4.89 (s, 2H), 7.65 (br s, 1H), 8.05 (s, 1H), 8.19 (s, 1H), 8.61 (t, J=5.7 Hz, 1H), 8.85 (s, 1H), 10.58 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 27.0, 40.2, 45.0, 118.7, 141.4, 148.7, 152.3, 154.9, 165.3, 166.8; HRMS calcd for $C_{10}H_{13}N_7O_3$ 280.1153, found 280.1154.

***N*-hydroxy-3-(2-(6-(methylamino)-9*H*-purin-9-yl)acetamido)propanamide (5n)**

Purified by reverse phase column chromatography (C18, MeOH/H_2O, Gradient elution) to give a white solid. Yield 51%; m.p. 210–211 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.15 (t, J=7.2 Hz, 2H), 2.98 (br s, 3H), 3.25–3.28 (m, 2H), 4.80 (s, 2H), 7.63 (br s, 1H), 8.03 (s, 1H), 8.18 (s, 1H), 8.36 (t, J=5.4 Hz, 1H), 8.73 (s, 1H), 10.42 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 27.0, 32.2, 35.4, 44.8, 118.7, 141.4, 148.8, 152.4, 154.9, 166.3, 167.1; HRMS calcd for $C_{11}H_{15}N_7O_3$ 294.1309, found 294.1313.

***N*-hydroxy-4-(2-(6-(methylamino)-9*H*-purin-9-yl)acetamido)butanamide (5o)**

Washed with water to afford a white solid. Yield 57%; m.p. 184–186 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.64 (quint, J=7.2 Hz, 2H), 1.97 (t, J=7.2 Hz, 2H), 2.97 (br s, 3H), 3.07 (q, J=6.0 Hz, 2H), 4.82 (s, 2H), 7.66 (br s, 1H), 8.05 (s, 1H), 8.19 (s, 1H), 8.28 (t, J=5.4 Hz, 1H), 8.68 (br s, 1H), 10.34 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 25.1, 27.1, 29.8, 38.4, 44.9, 118.7, 141.5, 148.8, 152.2, 154.8, 166.2, 168.7; HRMS calcd for $C_{12}H_{17}N_7O_3$ 308.1466, found 308.1473.

***N*-hydroxy-3-(2-(6-morpholino-2-(phenylamino)-9*H*-purin-9-yl)acetamido)propanamide (5p)**

Washed with water and EtOH to afford a white solid. Yield 78%; m.p. 210–211 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 2.17 (t, J=7.2 Hz, 2H), 3.31 (q, J=6.0 Hz, 2H), 3.73 (t, J=4.5 Hz, 4H), 4.19 (br s, 4H), 4.74 (s, 2H), 6.86 (t, J=7.2 Hz, 1H), 7.23 (t, J=7.5 Hz, 2H), 7.74 (d, J=7.2 Hz, 2H), 7.84 (s, 1H), 8.39 (t, J=5.4 Hz, 1H), 8.99 (s, 1H), 10.45 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 32.3, 35.5, 45.0, 45.3, 66.2, 113.9, 118.2, 120.3, 128.3, 138.8, 141.4, 152.4, 153.2, 155.3, 166.4, 167.1; HRMS calcd for $C_{20}H_{24}N_8O_4$ 441.1993, found 441.1986.

***N*-hydroxy-4-(2-(6-morpholino-2-(phenylamino)-9*H*-purin-9-yl)acetamido)butanamide (5q)**

Washed with water and EtOH to afford a white solid. Yield 83%; m.p. 182–184 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.66 (quint, J=7.2 Hz, 2H), 1.98 (t, J=7.5 Hz, 2H), 3.09 (q, J=6.3 Hz, 2H), 3.73 (t, J=4.8 Hz, 4H), 4.19 (br s, 4H), 4.75 (s, 2H), 6.85 (t, J=7.5 Hz, 1H), 7.22 (t, J=7.5 Hz, 2H), 7.74 (d, J=7.8 Hz, 2H), 7.85 (s, 1H), 8.32 (t, J=5.4 Hz, 1H), 8.69 (s, 1H), 10.37 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 25.2, 29.8, 38.4, 45.0, 45.2, 66.2, 114.0, 118.2, 120.2, 128.3, 138.8, 141.5, 152.5, 153.3, 155.4, 166.4, 168.7; HRMS calcd for $C_{21}H_{26}N_8O_4$ 455.2150, found 455.2155.

***N*-hydroxy-6-(2-(6-morpholino-2-(phenylamino)-9*H*-purin-9-yl)acetamido)hexanamide (5r)**

Washed with water and EtOH to afford a white solid. Yield 85%; m.p. 182–183 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.22–1.29 (m, 2H), 1.38–1.52 (m, 4H), 1.93 (t, J=7.2 Hz, 2H), 3.08 (q, J=6.0 Hz, 2H), 3.73 (t, J=4.5 Hz, 4H), 4.20 (br s, 4H), 4.75 (s, 2H), 6.86 (t, J=7.5 Hz, 1H), 7.22 (t, J=7.8 Hz, 2H), 7.75 (d, J=7.8 Hz, 2H), 7.85 (s, 1H), 8.31 (t, J=5.1 Hz, 1H), 8.67 (br s, 1H), 8.98 (s, 1H), 10.36 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 25.0, 26.2, 29.0, 32.4, 38.9, 45.2, 45.4, 66.5, 114.3, 118.4, 120.4, 128.5, 139.1, 141.7, 152.8, 153.6, 155.7, 166.5, 169.3; HRMS calcd for $C_{23}H_{30}N_8O_4$ 483.2463, found 483.2451.

***N*-hydroxy-5-(2-(6-(phenylamino)-9*H*-purin-9-yl)acetamido)pentanamide (5s)**

Washed with water and EtOH to afford a white solid. Yield 53%; m.p. 202–204 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.41–1.57 (m, 4H), 1.96 (t, J=7.2 Hz, 2H), 3.09 (q, J=6.0 Hz, 2H), 4.90 (s, 2H), 7.03 (t, J=7.2 Hz, 1H), 7.33 (t, J=7.8 Hz, 2H), 7.95 (d, J=7.8 Hz, 2H), 8.25 (s, 1H), 8.33 (t, J=5.4 Hz, 1H), 8.37 (s, 1H), 8.67 (br s, 1H), 9.85 (s, 1H), 10.35 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 22.6, 28.5, 31.9, 38.6, 45.1, 119.3, 120.7, 122.5, 128.3, 139.7, 142.7, 149.9, 151.8, 151.9, 166.0, 169.0; HRMS calcd for $C_{18}H_{21}N_7O_3$ 384.1779, found 384.1777.

***N*-hydroxy-6-(2-(6-(phenylamino)-9*H*-purin-9-yl)acetamido)hexanamide (5t)**

Washed with water and EtOH to afford a white solid. Yield 87%; m.p. 197–198 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.21–1.30 (m, 2H), 1.38–1.54 (m, 4H), 1.94 (t, J=7.2 Hz, 2H), 3.08 (q, J=6.0 Hz, 2H), 4.89 (s, 2H), 7.03 (t, J=7.5 Hz, 1H), 7.33 (t, J=7.8 Hz, 2H), 7.95 (d, J=7.5 Hz, 2H), 8.25 (s, 1H), 8.31 (t, J=5.7 Hz, 1H), 8.38 (s, 1H), 8.67 (br s, 1H), 9.85 (s, 1H), 10.34 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 24.8, 26.0, 28.7, 32.2, 38.7, 45.1, 119.3, 120.7, 122.5, 128.3, 139.7, 142.7, 149.9, 151.8, 151.9, 166.0, 169.1; HRMS calcd for $C_{19}H_{23}N_7O_3$ 398.1935, found 398.1950.

***N*-hydroxy-7-(2-(6-(phenylamino)-9*H*-purin-9-yl)acetamido)heptanamide (5u)**

Washed with water and EtOH to afford a white solid. Yield 87%; m.p. 186–188 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.26 (br s, 4H), 1.40–1.51 (m, 4H), 1.94 (t, J=7.2 Hz, 2H), 3.09 (q, J=6.0 Hz, 2H), 4.89 (s, 2H), 7.03 (t, J=7.5 Hz, 1H), 7.33 (t, J=7.8 Hz, 2H), 7.95 (d, J=7.8 Hz, 2H), 8.25 (s, 1H), 8.30 (t, J=5.1 Hz, 1H), 8.37 (s, 1H), 8.66 (br s, 1H), 9.85 (s, 1H), 10.33 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 25.0, 26.0, 28.2, 28.8, 32.2, 38.8, 45.1, 119.3, 120.7, 122.5, 128.3, 139.7, 142.7, 149.9, 151.8, 151.9, 166.0, 169.1; HRMS calcd for $C_{20}H_{25}N_7O_3$ 412.2092, found 412.2092.

5-(2-(2,6-bis(phenylamino)-9*H*-purin-9-yl)acetamido)-*N*-hydroxypentanamide (5v)

Washed with water and EtOH to afford a white solid. Yield 88%; m.p. 199–201 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.42–1.57 (m, 4H), 1.95 (t, J=7.2 Hz, 2H), 3.12 (q, J=6.0 Hz, 2H), 4.80 (s, 2H), 6.90 (t, J=7.2 Hz, 1H), 7.04 (t, J=7.2 Hz, 1H), 7.22 (t, J=7.8 Hz, 2H), 7.32 (t, J=7.8 Hz, 2H), 7.78 (d, J=7.8 Hz, 2H), 7.97–8.00 (m, 3H), 8.32 (t, J=4.8 Hz, 1H), 9.14 (s, 1H), 9.65 (s, 1H), 10.35 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 22.6, 28.6, 31.8, 38.6, 45.0, 113.9, 118.7, 120.6, 120.8, 122.3, 128.2, 128.3, 139.8, 140.0, 141.2, 151.2, 151.8, 155.9, 166.2, 168.9; HRMS calcd

for $C_{24}H_{26}N_8O_3$ 475.2201, found 475.2202.

6-(2-(2,6-bis(phenylamino)-9*H*-purin-9-yl)acetamido)-*N*-hydroxyhexanamide (5w)

Washed with water and EtOH to afford a white solid. Yield 85%; m.p. 182–185 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.26–1.28 (m, 2H), 1.42–1.53 (m, 4H), 1.93 (t, J=7.2 Hz, 2H), 3.10 (q, J=5.7 Hz, 2H), 4.81 (s, 2H), 6.90 (t, J=7.5 Hz, 1H), 7.04 (t, J=7.5 Hz, 1H), 7.22 (t, J=7.5 Hz, 2H), 7.32 (t, J=7.5 Hz, 2H), 7.79 (d, J=8.1 Hz, 2H), 7.98 (d, J=8.1 Hz, 2H), 8.04 (s, 1H), 8.32 (t, J=4.8 Hz, 1H), 9.16 (s, 1H), 9.69 (s, 1H), 10.34 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 25.2, 26.4, 29.2, 32.6, 39.2, 45.6, 114.1, 119.2, 121.1, 121.2, 122.8, 128.7, 128.8, 140.2, 140.4, 141.6, 151.6, 152.1, 156.3, 166.6, 169.5; HRMS calcd for $C_{25}H_{28}N_8O_3$ 489.2357, found 489.2377.

7-(2-(2,6-bis(phenylamino)-9*H*-purin-9-yl)acetamido)-*N*-hydroxyheptanamide (5x)

Washed with water and EtOH to afford a white solid. Yield 82%; m.p. 158–160 °C. ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.24–1.25 (m, 4H), 1.42–1.52 (m, 4H), 1.93 (t, J=7.2 Hz, 2H), 3.10 (q, J=6.0 Hz, 2H), 4.83 (s, 2H), 6.91 (t, J=7.2 Hz, 1H), 7.05 (t, J=7.2 Hz, 1H), 7.23 (t, J=7.5 Hz, 2H), 7.33 (t, J=7.5 Hz, 2H), 7.79 (d, J=7.8 Hz, 2H), 7.99 (d, J=7.8 Hz, 2H), 8.11 (s, 1H), 8.35 (t, J=5.4 Hz, 1H), 9.19 (s, 1H), 9.76 (s, 1H), 10.35 (s, 1H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 25.0, 26.1, 28.2, 28.9, 32.2, 38.8, 45.1, 113.6, 118.7, 120.6, 120.7, 122.3, 128.2, 128.3, 139.8, 140.0, 141.2, 151.1, 151.6, 155.9, 166.1, 169.1; HRMS calcd for $C_{26}H_{30}N_8O_3$ 503.2514, found 503.2539.

Enzymatic inhibition assay *in vitro*

In vitro HDAC assay

The HDAC inhibitors were screened using a HDAC colorimetric assay kit (BML-AK501, Enzo® Life Sciences). Tested compounds were dissolved in dimethylsulphoxide (DMSO) to give a 25 mM working stock concentration and diluted with assay buffer.

Each assay was setup as instructions. The assay was beginning from mixing undiluted HeLa extract (5 μL/well), assay buffer (10 μL/well), 10 μL of various concentrations of samples and 25 μL of substrate (1 mM) on the 96-well plate and incubating at 37 °C for 30 min. Then the fresh prepared Color de Lys™ Developer (50 μL/well) was added to stop the reaction. Incubated plate at 37 °C for 30 min and then read the plate in a microtiter-plate reader at 405 nm. The inhibition rates were calculated from the ultraviolet absorption readings of inhibited wells related to those of control wells. Finally, the IC_{50} values were determined using a regression analysis of the concentration and inhibition data.

HDAC1 (or HDAC6) inhibition assay

The assay was performed by quantitating the fluorescent product in solution followed by a enzyme reaction. The enzymes were purchased from Abcam (HDAC1, #AB101661 and HDAC6, #AB42632). Tested compound (5 μL/well) was added to a total of 50 μL reaction mixture, which contained assay buffer (25 mM Tris, pH 8.0, 1 mM $MgCl_2$, 137 mM NaCl, 2.7 mM KCl), 0.1 mg/mL BSA, HDAC1 (or HDAC6) and the specific fluorescent substrate. The mixture was incubated at 37 °C for 30 min. Then the fluorescence was analyzed at SpectraMax M5 microtiter plate reader (Ex: 350–360 nm, Em: 450–460 nm). The IC_{50} values were calculated using nonlinear regression with normalized dose-response fit.

HDAC8 inhibition assay

The inhibition to HDAC8 was tested with HDAC-Glo® Luminescent kit (G6420, Promega). The enzyme HDAC8 was purchased from SignalChem (H90-30H). HDAC8 was diluted in HDAC-Glo buffer and incubated with the HDAC inhibitors at room temperature for at least 30 min. The fresh prepared HDAC-Glo reagent was then added and incubated for 15–45min. Read

the assay plate on Envision and calculated the IC_{50} values using nonlinear regression with normalized dose-response fit.

MTT Assay

The cancer cells were respectively cultured in RPMI1640 medium containing 10% FBS at 37 °C in 5% CO_2 humidified incubator. In test, cells were plated in a 96-well plate at 5,000–10,000 cells/well and cultured for 5–8 h in complete growth medium, then treated with various concentrations of compounds for 48 h. After that, 0.5% MTT solution was added to each well and incubation for another 4 h. Formazan formed from MTT was extracted by adding 150 μL/well DMSO and mixing for 10 min. Optical density was read in a microtiter-plate reader at 570 nm.

Molecular Docking

AutoDock4.2 [4-8] was used for all docking calculations. One hundred runs were performed using Lamarckian Genetic Algorithm (LGA) with default parameters. The molecular structures were generated with Sybyl/Sketch module and optimized using semiempirical MOPAC/AM1 method and then assigned with AM1-BCC charges. Results differing less than 0.5Å in positional root-mean-square deviation (RMSD) were clustered together. Conformations in first cluster with the most favorable free binding energy were selected as the best docking result.

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