Supporting information

Development of novel β-carboline-based hydroxamate derivatives as HDAC inhibitors with DNA damage and apoptosis inducing ability

Ji Liu[a,†], Tingting Wang[a,†], Xinyang Wang[a,b,†], Lin Luo[a], Jing Guo[a], Yanfu Peng[a], Qibing Xu[a], Jiefei Miao[a,c], Yanan Zhang[a,*], Yong Ling[a,b,*]

[a]School of Pharmacy and Jiangsu Province Key Laboratory for Inflammation and Molecular Drug Target, Nantong University, Nantong 226001, P.R. China;

[b]State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, P.R. China;

[c]Tumor-chemotherapy Department, Affiliated Hospital, Nantong University, Nantong 226001, P.R. China

1. Experimental procedures for chemical synthesis

^{1}H NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. MS spectra were recorded on a Mariner Mass Spectrum (ESI). High resolution mass spectra were recorded using an Agilent Technologies LC/MSD TOF. All compounds were routinely checked by TLC and ^{1}H NMR. TLCs and preparative thin-layer chromatography were performed on silica gel GF/UV 254, and the chromatograms were conducted on silica gel (200–300 mesh, Merck) and visualized under UV light at 254 and 365 nm. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. 4-Formylbenzoate **1**, L-tryptophan **4**, differently substituted primary amines **2a-d** and aldehydes were commercially available. Compounds **5a-d** and **4a-d** were synthesized according to literature procedures.[1,2] Solutions after reactions and extractions were concentrated using a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. All compounds were of >95% purity determined by HPLC.

Methyl 4-((methylamino)methyl)benzoate (**3a**)

To a solution of methyl 4-formylbenzoate **1** (6.8 mmol, 1.12 g) in 15ml anhydrous tetrahydrofuran (THF) was added ethanol amine **2a** (8.2 mmol, 0.50 g) and catalytic amount of HOAc.The reaction was stirred at room temperature for 5h. To the mixture was added sodium boron hydride (17.1 mmol, 0.65 g). After the reaction was completed, the solvent was removed under reduced pressure. The crude residue was dissolved in water and was adjusted by 2M HCl to pH = 3. The solution was washed with ethyl acetate (3 × 20 mL). The water layer was adjusted with 2M NaOH to pH = 11. The solution was extracted with ethyl acetate (3 × 20 mL). The organic phase was washed with brine and concentrated to give a yellow oil product **3a**, yield 76%. MS (ESI) *m/z* = 180 $[M+H]^+$.

Methyl 4-((ethylamino)methyl)benzoate (**3b**)

Compound **3b** was synthesized from 4-formylbenzoate **1**, ethanamine **2b**, and sodium boron hydride, according to the synthetic procedure of **3a** in a yield of 62%, yellow oil. MS (ESI) *m/z* = 194 $[M+H]^+$.

Methyl 4-((propylamino)methyl)benzoate (**3c**)

Compound **3c** was synthesized from 4-formylbenzoate **1**, propan-1-amine **2c**, and sodium boron hydride, according to the synthetic procedure of **3a** in a yield of 62%, yellow oil. MS (ESI) *m/z* = 208 $[M+H]^+$.

Methyl 4-((butylamino)methyl)benzoate (**3d**)

Compound **3f** was synthesized from 4-formylbenzoate **1**, butan-1-amine **2d**, and sodium boron hydride, according to the synthetic procedure of **3a** in a yield of 62%, yellow oil. MS (ESI) *m/z* = 222 $[M+H]^+$.

N-(4-(Hydroxycarbamoyl)benzyl)-*N*-methyl-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8a**)

To a solution of **6a** (0.25 g, 1.2 mmol), EDCI (0.27 g, 1.44 mmol) and catalytic amount of DMAP in 10 mL anhydrous CH_2Cl_2, was added methyl 4-((methylamino)methyl)benzoate **3a** (0.21 g, 1.2 mmol). The mixture was stirred at room temperature overnight. Then 20 mL of CH_2Cl_2 was added and the mixture was washed with water (30 mL × 3) and brine. The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*, and the crude product was purified by column chromatography to give **7a**, which was then dissolved in 3 mL anhydrous methanol, and poured into a solution of NH_2OK (0.09 g, 4 mmol) in 3 mL of anhydrous methanol. The mixture was stirred for 10-15 h and the solvent was evaporated *in vacuo*. The residue was diluted with saturated NH_4Cl aqueous solution, and then extracted with ethyl acetate (6 mL × 5). The organic layers were combined, dried over anhydrous Na_2SO_4 and evaporated. The resulting residue was purified by column chromatography (eluting with EA followed by 20:1 $CHCl_3$/MeOH followed by 10:1 $CHCl_3$/MeOH) on silica gel to afford **8a** as a pale yellow solid in a yield of 65%. Analytical data for **8a**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ10.62 (s, 1H, NH), 9.05 (s, 1H, NH), 8.87 (s, 1H, Ar-H), 8.72 (s, 1H, Ar-H), 8.21 (m, 1H, Ar-H), 8.07 (d, 2H, J = 7.5 Hz, Ar-H), 7.72 (m, 1H, Ar-H), 7.53-7.58 (m, 3H, Ar-H), 7.31 (m, 1H, Ar-H), 5.05 (s, 1H, NCH_2), 4.90 (s, 1H, NCH_2), 3.73 (s, 3H, NCH_3); MS (ESI) *m/z* = 375 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{21}H_{19}N_4O_3$: 375.1379; found: 375.1393 $[M+H]^+$.

N-Ethyl-*N*-(4-(hydroxycarbamoyl)benzyl)-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8b**)

Compound **8b** was synthesized from **6a**, methyl 4-((ethylamino)methyl)benzoate **3b**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 63%, a pale yellow solid. Analytical data for **8b**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ11.02 (s, 1H, NH), 9.11 (s, 1H, NH), 8.87 (s, 1H, Ar-H), 8.75 (s, 1H, Ar-H), 8.19 (m, 1H, Ar-H), 8.05 (d, 2H, J = 7.5 Hz, Ar-H), 7.72 (m, 1H, Ar-H), 7.51-7.57 (m, 3H, Ar-H), 7.26 (m, 1H, Ar-H), 5.03 (s, 1H, NCH_2), 4.87 (s, 1H, NCH_2), 3.57-3.66 (m, 2H, $NC\underline{H_2}CH_3$), 1.25-1.30 (m, 2H, $NCH_2C\underline{H_3}$); MS (ESI) *m/z* = 389 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{22}H_{21}N_4O_3$: 389.1535; found: 389.1551 $[M+H]^+$.

N-(4-(Hydroxycarbamoyl)benzyl)-*N*-propyl-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8c**)

Compound **8c** was synthesized from **6a**, methyl 4-((propylamino)methyl)benzoate **3c**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 62%, a pale yellow solid. Analytical data for **8c**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ11.21 (s, 1H, NH), 8.89 (s, 1H, Ar-H), 8.71 (s, 1H, Ar-H), 8.27 (m, 1H, Ar-H), 8.07 (d, 2H, J = 7.5 Hz, Ar-H), 7.53-7.61 (m, 3H, Ar-H), 7.32-7.39 (m, 2H, Ar-H), 5.06 (s, 1H, NCH_2), 4.91 (s, 1H, NCH_2), 3.43-3.55 (m, 2H, $NC\underline{H_2}CH_2CH_3$), 1.67-1.71 (m, 2H, $NCH_2C\underline{H_2}$), 0.87-0.95 (m, 3H, $NCH_2CH_2C\underline{H_3}$); MS (ESI) *m/z* = 403 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{23}H_{23}N_4O_3$: 403.1692; found: 403.1705 $[M+H]^+$.

N-Butyl-*N*-(4-(hydroxycarbamoyl)benzyl)-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8d**)

Compound **8d** was synthesized from **6a**, methyl 4-((butylamino)methyl)benzoate **3f**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 66%, a pale yellow solid. Analytical data for **8d**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ11.08 (s, 1H, NH), 8.89 (s, 1H, Ar-H), 8.71 (s, 1H, Ar-H), 8.28 (m, 1H, Ar-H), 8.07 (d, 2H, J = 7.5 Hz, Ar-H), 7.55-7.63 (m, 3H, Ar-H), 7.33 (m, 1H, Ar-H), 5.06 (s, 1H, NCH_2), 4.92 (s, 1H, NCH_2), 3.48-3.56 (m, 2H, $NC\underline{H_2}CH_2CH_2CH_3$), 1.67-1.70 (m, 2H, $NCH_2C\underline{H_2}CH_2CH_3$), 1.38-1.43 (m, 2H, $NCH_2CH_2C\underline{H_2}CH_3$), 0.69-0.75 (m, 3H, $NCH_2CH_2CH_2C\underline{H_3}$); MS (ESI) *m/z* = 417 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{24}H_{25}N_4O_3$: 417.1848; found: 417.1862 $[M+H]^+$.

N-(4-(Hydroxycarbamoyl)benzyl)-*N*,9-dimethyl-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8e**)

Compound **8e** was synthesized from **6b**, methyl 4-((methylamino)methyl)benzoate **3a**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 61%, a pale yellow solid. Analytical data for **8e**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ9.13 (s, 1H, NH), 8.72 (s, 1H, Ar-H), 8.29 (m, 1H, Ar-H), 8.03-8.07 (m, 3H, Ar-H), 7.52-7.59 (m, H, Ar-H), 7.28 (m, 1H, Ar-H), 5.08 (s, 1H, NCH_2), 4.92 (s, 1H, NCH_2), 3.76(s, 1H, NCH_3), 2.89 (s, 3H, CH_3); MS (ESI) *m/z* = 389 $[M+H]^+$; HRMS

(ESI): m/z calcd for $C_{22}H_{21}N_4O_3$: 389.1535; found: 389.1549 $[M+H]^+$.

N-Ethyl-*N*-(4-(hydroxycarbamoyl)benzyl)-9-methyl-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8f**)

Compound **8f** was synthesized from **6b**, methyl 4-((ethylamino)methyl)benzoate **3b**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 65%, a pale yellow solid. Analytical data for **8f**: ^1H NMR (DMSO-d_6, 300 MHz): δ9.16 (s, 1H, NH), 8.76 (m, 1H, Ar-H), 8.28 (m, 1H, Ar-H), 8.04-8.10 (m, 3H, Ar-H), 7.52-7.56 (m, 1H, Ar-H), 7.30 (m, 1H, Ar-H), 5.05 (s, 1H, NCH_2), 4.91 (s, 1H, NCH_2), 3.56-3.67 (m, 2H, $N\underline{CH_2}CH_3$), 2.91 (s, 3H, CH_3), 1.25-1.33 (m, 3H, $NCH_2C\underline{H_3}$); MS (ESI) *m/z* = 403 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{23}H_{23}N_4O_3$: 403.1692; found: 403.1677 $[M+H]^+$.

N-(4-(Hydroxycarbamoyl)benzyl)-9-methyl-*N*-propyl-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8g**)

Compound **8g** was synthesized from **6b**, methyl 4-((propylamino)methyl)benzoate **3c**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 70%, a pale yellow solid. Analytical data for **8g**: ^1H NMR (DMSO-d_6, 300 MHz): δ11.26 (s, 1H, NH), 8.70 (s, 1H, Ar-H), 8.30 (m, 1H, Ar-H), 8.03-8.09 (m, 3H, Ar-H), 7.63 (m, H, Ar-H), 7.51 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.28 (m, 1H, Ar-H), 5.03 (s, 1H, NCH_2), 4.87 (s, 1H, NCH_2), 2.89 (s, 3H, CH_3), 3.38-3.53 (m, 2H, $N\underline{CH_2}CH_2CH_3$), 1.63-1.70 (m, 2H, $NCH_2C\underline{H_2}CH_3$), 0.85-0.91 (m, 3H, $NCH_2CH_2C\underline{H_3}$); MS (ESI) *m/z* = 417$[M+H]^+$; HRMS (ESI): m/z calcd for $C_{24}H_{25}N_4O_3$: 417.1848; found: 417.1861 $[M+H]^+$.

N-Bytyl-*N*-(4-(hydroxycarbamoyl)benzyl)-9-methyl-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8h**)

Compound **8h** was synthesized from **6b**, methyl 4-((butylamino)methyl)benzoate **3f**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 68%, a pale yellow solid. Analytical data for **8h**: ^1H NMR (DMSO-d_6, 300 MHz): δ9.12 (s, 1H, NH), 8.75 (s, 1H, Ar-H), 8.28 (m, 1H, Ar-H), 8.03-8.10 (m, 3H, Ar-H), 5.51-7.56 (m, 3H, Ar-H), 7.30 (m, 1H, Ar-H), 5.09 (s, 1H, NCH_2), 4.95 (s, 1H, NCH_2), 3.52-3.61 (m, 2H, $NC\underline{H_2}CH_2$), 2.91 (s, 3H, CH_3), 1.65-1.71 (m, 2H, $NCH_2C\underline{H_2}CH_2$), 1.40-1.44 (m, 2H, $NCH_2CH_2C\underline{H_2}$), 0.67-0.72 (m, 3H, $NCH_2CH_2CH_2C\underline{H_3}$); MS (ESI) *m/z* =431 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{25}H_{27}N_4O_3$: 431.2005; found: 431.1989 $[M+H]^+$.

N-(4-(Hydroxycarbamoyl)benzyl)-9-(4-methoxyphenyl)-*N*-methyl--9*H*-pyrido[3,4-b]indole-3-carboxamide (**8i**)

Compound **8i** was synthesized from **6c**, methyl 4-((methylamino)methyl)benzoate **3a**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 62%, a pale yellow solid. Analytical data for **8i**: ^1H NMR (DMSO-d_6, 300 MHz): δ11.06 (s, 1H, NH), 8.79 (s,1H, Ar-H), 8.44 (m, 1H, Ar-H), 8.16 (m, 1H, Ar-H), 7.96-8.05 (m, 3H, Ar-H), 7.53-7.59 (m, 3H, Ar-H), 7.46 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.35 (m, 1H, Ar-H), 7.12 (d, 2H, *J* = 7.5 Hz, Ar-H), 5.07 (s, 1H, NCH_2), 4.91 (s, 1H, NCH_2), 3.82 (s, 1H, NCH_3); MS (ESI) *m/z* = 481 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{28}H_{25}N_4O_4$: 481.1798; found: 481.1814 $[M+H]^+$.

N-Ethyl-*N*-(4-(hydroxycarbamoyl)benzyl)-9-(4-methoxyphenyl)-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8j**)

Compound **8j** was synthesized from **6c**, methyl 4-((ethylamino)methyl)benzoate **3b**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 62%, a pale yellow solid. Analytical data for **8j**: ^1H NMR (DMSO-d_6, 300 MHz): δ9.15 (s, 1H, NH), 8.76 (s, 1H, Ar-H), 8.48 (s, 1H, Ar-H), 8.16 (m, 1H, Ar-H), 8.05 (d, 1H, *J* = 6.3 Hz, Ar-H), 8.01 (d, 1H, *J* = 6.3 Hz, Ar-H), 7.94 (m, 1H, Ar-H), 7.50-7.60 (m, 4H, Ar-H), 7.34 (m, 1H, Ar-H), 7.11 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.91 (m, 1H, NH), 5.04 (s, 1H, NCH_2), 4.93 (s, 1H, NCH_2), 3.86 (s, 3H, $OC\underline{H_3}$), 3.59-3.68 (m, 2H, NC$\underline{H}_2$CH$_3$), 1.26-1.31 (m, 3H, NCH$_2$C$\underline{H}_3$); MS (ESI) *m/z* = 495 [M+H]$^+$; HRMS (ESI): m/z calcd for $C_{29}H_{27}N_4O_4$: 495.1954; found: 495.1971 [M+H]$^+$.

N-(4-(Hydroxycarbamoyl)benzyl)-9-(4-methoxyphenyl)-*N*-propyl-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8k**)

Compound **8k** was synthesized from **6c**, methyl 4-((propylamino)methyl)benzoate **3c**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 62%, a pale yellow solid. Analytical data for **8k**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ8.63 (s, 1H, Ar-H), 8.40 (m, 1H, Ar-H), 7.91-8.09 (m, 3H, Ar-H), 7.84 (m, 1H, Ar-H), 7.39-7.50 (m, 3H, Ar-H), 7.32 (m, 1H, Ar-H), 7.06 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.85 (m, 1H, NH), 5.08 (s, 1H, NCH$_2$), 4.94 (s, 1H, NCH$_2$), 3.86 (s, 3H, OCH$_3$), 3.40-3.54 (m, 2H, NC$\underline{H}_2$CH$_2$CH$_3$), 1.65-1.70 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_3$), 0.88-0.93 (m, 3H, NCH$_2$CH$_2$C$\underline{H}_3$); MS (ESI) *m/z* = 509 [M+H]$^+$; HRMS (ESI): m/z calcd for $C_{30}H_{29}N_4O_4$: 509.2111; found: 509.2130 [M+H]$^+$.

N-Butyl-*N*-(4-(hydroxycarbamoyl)benzyl)-9-(4-methoxyphenyl)-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8l**)

Compound **8l** was synthesized from **6c**, methyl 4-((butylamino)methyl)benzoate **3d**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 62%, a pale yellow solid. Analytical data for **8l**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ8.79 (s,1H, Ar-H), 8.46 (m, 1H, Ar-H), 8.15 (m, 1H, Ar-H), 7.95-8.05 (m, 3H, Ar-H), 7.53-7.59 (m, 3H, Ar-H), 7.48 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.35 (m, 1H, Ar-H), 7.12 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.91 (m, 1H, NH), 5.11 (s, 1H, NCH$_2$), 4.97 (s, 1H, NCH$_2$), 3.92 (s, 3H, OCH$_3$), 3.51-3.58 (m, 2H, NC$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 1.67(m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$CH$_3$), 1.41 (m, 2H, NCH$_2$CH$_2$C$\underline{H}_2$CH$_3$), 0.7-0.74 (m, 3H, NCH$_2$CH$_2$CH$_2$C$\underline{H}_3$); MS (ESI) *m/z* = 523 [M+H]$^+$; HRMS (ESI): m/z calcd for $C_{31}H_{31}N_4O_4$: 523.2267; found: 523.2288 [M+H]$^+$.

N-(4-(Hydroxycarbamoyl)benzyl)-9-(3-methoxyphenyl)-*N*-propyl-9*H*-pyrido[3,4-b]indole-3-carboxamide(**8m**)

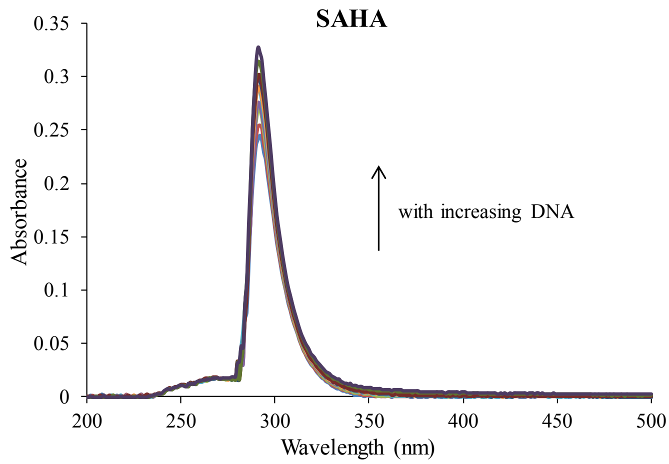
Compound **8m** was synthesized from **6d**, methyl 4-((propylamino)methyl)benzoate **3c**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 62%, a pale yellow solid. Analytical data for **8m**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ8.86 (s, 1H, Ar-H), 8.67 (m, 1H, Ar-H), 8.49 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.16 (m, 1H, Ar-H), 8.03 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.96 (m, 1H, Ar-H), 7.46-7.58 (m, 4H, Ar-H), 7.23-7.37 (m, 3H, Ar-H), 6.96 (m, 1H, NH), 5.06 (s, 1H, NCH$_2$), 4.92 (s, 1H, NCH$_2$), 3.91 (s, 3H, OCH$_3$), 3.46-3.49 (m, 2H, NC$\underline{H}_2$CH$_2$CH$_3$), 1.73 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_3$), 0.87 (m, 3H, NCH$_2$CH$_2$C$\underline{H}_3$); MS (ESI) *m/z* =509 [M+H]$^+$; HRMS (ESI): m/z calcd for $C_{30}H_{29}N_4O_4$: 509.2111; found: 509.2098 [M+H]$^+$.

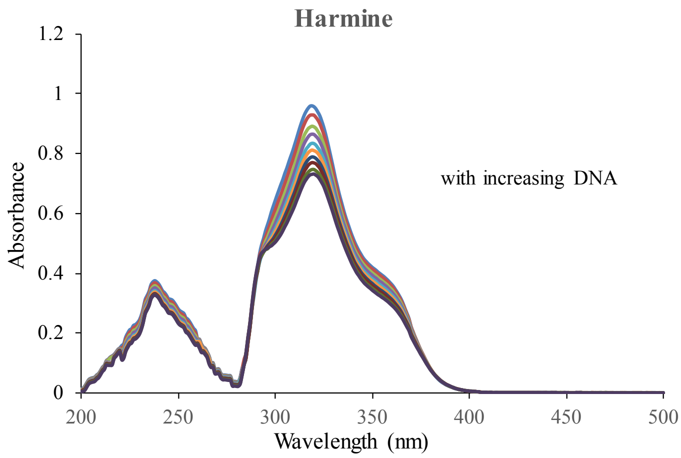
N-Butyl-*N*-(4-(hydroxycarbamoyl)benzyl)-9-(3-methoxyphenyl)-9*H*-pyrido[3,4-b]indole-3-carboxamide (**8n**)

Compound **8n** was synthesized from **6d**, methyl 4-((butylamino)methyl)benzoate **3d**, EDCI, and NH_2OK, according to the synthetic procedure of **8a** in a yield of 62%, a pale yellow solid. Analytical data for **8n**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ8.75 (s, 1H, Ar-H), 8.51 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.15 (m, 1H, Ar-H), 8.02-8.06 (m, 2H, Ar-H), 7.46-7.58 (m, 5H, Ar-H), 7.26-7.37 (m, 3H, Ar-H), 6.96 (m, 1H, NH), 5.06 (s, 1H, NCH$_2$), 4.91 (s, 1H, NCH$_2$), 3.91 (s, 3H, OCH$_3$), 3.48-3.56 (m, 2H, NC$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 1.67 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$CH$_3$), 1.08 (m, 2H, NCH$_2$CH$_2$C$\underline{H}_3$), 0.88 (m, 3H,

$NCH_2CH_2C\underline{H}_3$); MS (ESI) *m/z* =523 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{31}H_{31}N_4O_4$: 523.2267; found: 523.2283 $[M+H]^+$.

2. UV-visible absorption spectra of SAHA and harmine in the presence of increasing amounts of CT DNA. Arrows indicate the changes in absorbance with increasing the concentration of DNA.





3. Experimental procedures for biological evaluation

Cell Culture and Reagents. Human hepatocellular carcinoma cells (HepG2, SMMC-7721), human colon cancer cells (HCT116, LOVO), and Hela cells were maintained in DMEM supplemented with 10% fetal bovine serum (Invitrogen), 100 units/ml of penicillin, and 0.1 μg/ml of streptomycin in a humid atmosphere incubator with 5% CO_2 at 37 °C. All cell lines were originally from the Shanghai Institute of Cell Biology (Shanghai, China). Cells were routinely subcultured twice weekly. PARP antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), monoclonal anti-actin antibody, and goat peroxidase-conjugated anti-rabbit IgG antibody, and goat peroxidase-conjugated anti-mouse IgG antibody were purchased from Sigma-Aldrich (St. Louis, MO). FITC-Annexin V and PI are from (BioVision). The chemiluminescence (ECL) kit was purchased from Thermo Fisher Scientific (Rockford, IL). Antibodies such as acetyl-H3, acetyl-tubulin, Bax, Bcl-2, and cleaved-caspase 3 are from Cell Signaling Technology (Danvers, MA).

Cell viability assay. Anti-proliferative activities of synthesized compounds were evaluated *in vitro* against four human cancer cell lines (HepG2, SMMC-7721, HCT116, and LOVO). Briefly, 100 µl of different colon cancer cells were plated in a 96-well flat bottom tissue culture plate at a density of 10^4 cells/ml, respectively, in DMEM medium and 10% fetal bovine serum and allowed to adhere overnight at 37°C in 5% CO_2. The cells were treated by adding 100 µL of different compounds at various concentrations into the respective well. The reagent DMSO (0.1%) was used as a negative control. The cell viability assay (MTT assay) was carried out at 48 hours after drug treatment. The concentration, which inhibited 50% of cellular growth (IC50 value), was calculated by the following formula: Cell inhibition rate (%) = (1 − OD of treatment group/OD of control group) × 100%. The cytotoxicity potency of tested compounds on colon cancer cells was expressed as IC_{50} values or by a histogram (The bars are the mean ± SD). All the data were derived from three independent measurements.

HDAC activity assay. HDAC activity assays were performed as previously reported.[3] HeLa cell nuclear extract which is a rich source of HDACs was prepared using the EpiQuik nuclear extraction kit (OP-0002, Epigentek Group Inc). The HDAC activity was determined using the HDAC fluorimetric activity assay kit (Enzo Life Sciences Inc.) according to the manufacturer's instructions. Briefly, HDAC enzyme solution (HeLa nuclear extract) was incubated with test compounds at different concentrations in the presence of HDAC substrate (Boc-Lys (Ac)-AMC) at 37 °C for 60 min. Then the lysine developer was added to stop the reaction. After 30 min, the data was recorded in a fluorescence plate reader with excitation at 355 nm and emission at 460 nm. The HDAC activity was calculated as a percentage of activity compared with the control group. The concentration required for 50% inhibition (IC_{50}) was calculated using the software GraphPad Prism (Version 4.03).

UV–visible spectroscopy titrations. UV–visible spectroscopy titrations were performed on an ultraviolet spectrophotometer (UV 2500, Shimadzu, Tokyo, Japan) at 25 °C. Stock solutions of 20 µM of CT DNA (calf thymus DNA, which can form perfect double stranded DNA structure) were prepared in 5mM Tris buffer (60.50 mg Tris, 292.5 mg NaCl, Ph 7.0, with 100 ml Milli Q water). Stock solutions of 20 µM synthesized derivatives were prepared by dissolving them in 1:1000 DMSO/Milli Q water. UV–visible absorption titrations were done by adding 100 µl CT DNA solution each time to the quartz cuvette containing about 4 ml active compound solution. Titrations were carried out until the complex absorption band remains at a fixed wavelength upon five successive additions of CT DNA. Absorption spectra were recorded from 200 nm to 500 nm.

Flow cytometry assay of cell apoptosis. HepG2 cells cells were cultured overnight and incubated in triplicate with different concentrations of **8k** (1.0, 2.0, and 4.0 µM), SAHA (5.0 µM), or vehicle for 48 h. The cells were harvested and stained with FITC-Annexin V and PI at room temperature for 15 min. The percentage of apoptotic cells was determined by flow cytometry (Epics XL-MCL, Beckman Coulter, Indianapolis, USA) analysis. The FITC signal detector (FL1) and PI staining signal detector (FL3) were used to detect the cells with the flow cytometer (Ex = 488 nm; Em = 530 nm). Ten thousand cells were counted for three independent experiments. The data were analyzed using WinList 3D (version7.1) and the histogram was plotted using Excel 2010.

Western Blot Analysis. HepG2 cells with or without **8k**, or SAHA treatment at indicated time and doses were washed with PBS and lysed on ice for 30 minutes in PBS containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mg/ml PMSF, and 20 mM leupeptin. The protein concentrations were determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Up to 50 μg of total protein were separated onto an SDS-PAGE and transferred to polyvinylidenedifluoride membranes. After blocking with 5% fat free milk for 2 h, membranes were incubated overnight at 4 °C with a primary antibody in TBS-T and then reacted with a peroxidase-conjugated secondary antibody for 1 h. Immuno reactive proteins were detected with the ECL Western blotting Detection System.

Metabolic stability

Stock solutions of studied compounds were prepared at concentration of 10 mM in DMSO. Working solutions were prepared by dilution of stock with reaction buffer or acetonitrile, final concentration of organic solvent did not exceed 1%. Incubation mixture contained 1.0 μM of a studied derivative, 1 mM of NADPH (Sigma-Aldrich) and 0.5 mg/mL of rat liver microsomes (BD Gentest) in potassium phosphate buffer (0.1 M, pH 7.4). Incubation was carried out in thermostat at 37 °C and started by addition of studied compound. 50 μL samples were taken after 5, 15, 30, and 45 min. Enzymatic reaction was terminated by the addition of the equal volume of ice-cold acetonitrile containing 1.0 μM of ketanserin (Sigma-Aldrich) serving as internal standard (IS). Control incubations were performed without NADPH to assess chemical instability. After collection, samples were immediately centrifuged (10 min, 10,000 rpm) and resulted supernatant was directly analyzed or kept in −80 °C until LC-MS analysis. Natural logarithm of a compound over IS peak area ratio was plotted versus incubation time. Metabolic half-life (t1/2) was calculated from the slope of the linear regression.

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