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RESEARCH ARTICLE

Design, synthesis, and biological characterization of tamibarotene analogs as anticancer agents

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In our efforts of developing novel compounds as potential anticancer agents, a series of tamibarotene analogs containing Zn^{2+}-binding moieties were designed and developed. Biological characterization identified compound **7b** as the most potent one with improved antiproliferative activities against multiple cancer cell lines, compared to parent compound tamibarotene. Further characterization also demonstrated that compound **7b** exhibited moderate activities as a histone deacetylase inhibitor with IC_{50} of 1.8 ± 0.1 μm, thus suggesting that this could contribute to the improved antiproliferative activities of **7b**. Pharmacokinetic studies revealed that compound **7b** could release tamibarotene after administration and prolong the circulation time of tamibarotene, and this may also potentially contribute to the improved antiproliferative activities. Collectively, the results demonstrated that compound **7b** could serve as a new lead for further development of more potent analogs as potential anticancer agents.

KEYWORDS
anticancer, histone deacetylases, multitarget drugs, retinoids, tamibarotene

Retinoids have been successfully developed for the treatment of leukemia, such as all-trans retinoic acid (ATRA, **1**) and tamibarotene (**2**, Figure 1A).[1] However, the clinical applications of these agents are limited by associated toxicities such as mucocutaneous dryness, liver toxicity, central nervous system, and hypertriglyceridemia.[2–4] To address this issue, combination therapy is practiced to minimize the toxicity and drug resistance problems.[5,6] For example, the combination of retinoic acid receptor (RAR) agonists with HDAC inhibitors has been reported to show synergistic effects. [7,8] A phase I trial of the 13-*cis*-retinoic acid and HDAC inhibitor MS-275 (Entinostat) combination showed promising results in treating unresectable chemotherapy-resistant pancreatic cancer.[9] Furthermore, prodrugs of ATRA such as compounds **3–5** (Figure 1A) have been developed and demonstrated improved pharmacological activities as anticancer agents compared to ATRA itself.[10,11] Compared to ATRA, tamibarotene exhibited better differentiation induction and lower drug resistance in acute promyelocytic leukemia, probably due to its low affinity to cellular retinoic acid-binding protein.[1,12] However, tamibarotene also suffers undesirable side-effects such as hypertriglyceridemia, hypercholesterolemia, and retinoic acid syndrome, among others.[13] Therefore, it would be of significance to develop novel tamibarotene analogs for further development.

In our previous studies, tamibarotene derivatives containing butyric acid moiety, such as **6** (Figure 1A), were developed and most of them exhibited improved antiproliferative activity against human leukemia cells.[14,15] In continuation of our efforts to develop tamibarotene analogs with novel pharmacology, herein we report the synthesis and biological characterization of a new series of analogs

Abbreviations: AM80, tamibarotene; ATRA, all-trans retinoic acid; HDACs, histone deacetylases; RAR, retinoic acid receptor; ZBG, Zinc-binding group.

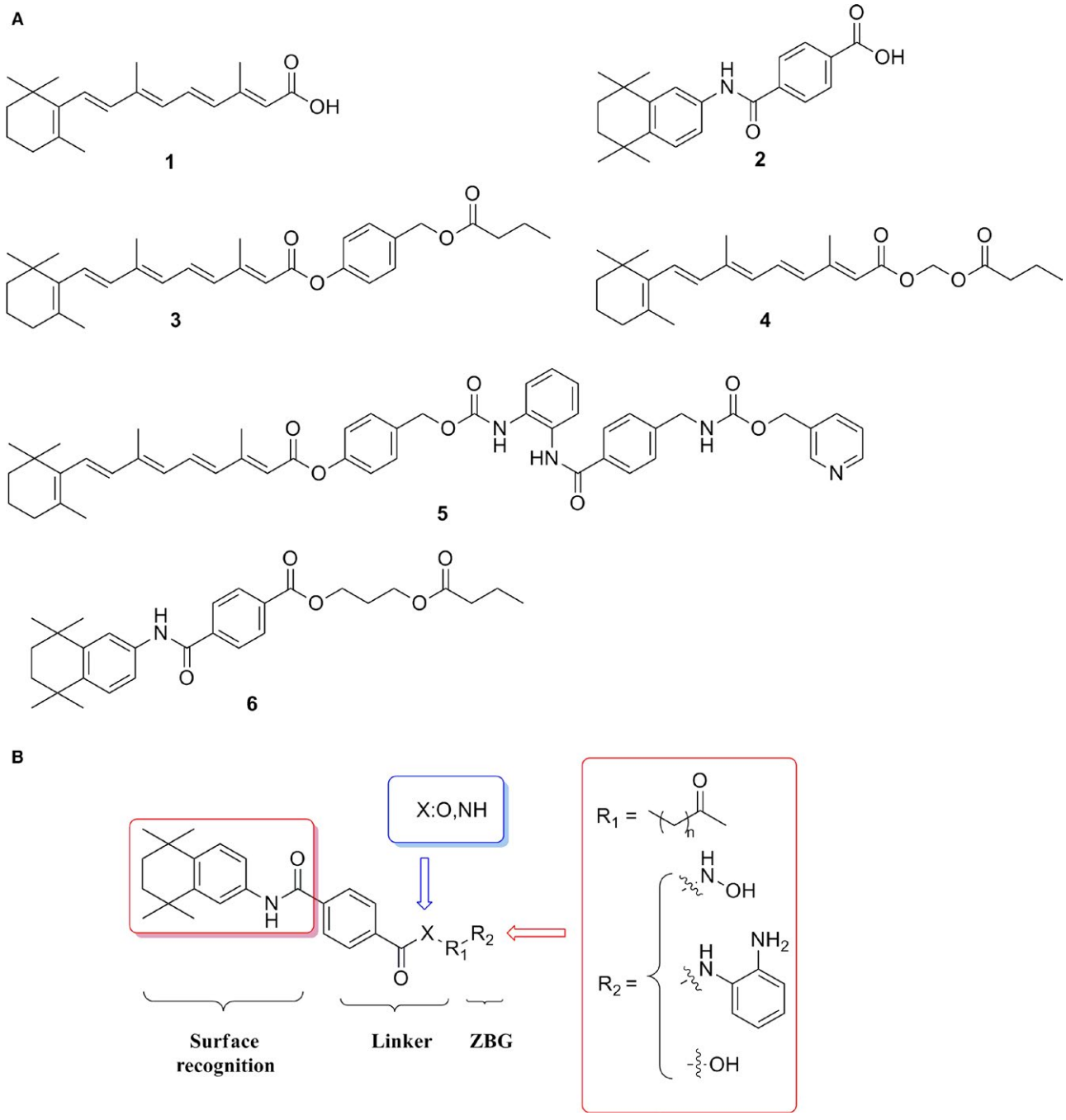


FIGURE 1 (A) Chemical structure of **1**–**6**; (B) schematic representation of the design and chemical structure of target compounds

to incorporate a Zn^{2+}-binding group, given the fact that HDACIs have been added to tamibarotene as combination therapy and Zn^{2+} chelating is an essential part of HDACI design. In the newly designed compounds, the carboxylic acid moiety of tamibarotene was employed to incorporate the Zn^{2+}-chelating moiety such as hydroxamic acid and phenylenediamine via ester or amide linkage (Figure 1B). It was perceived that these novel compounds will function as prodrug to improve pharmacokinetic properties of tamibarotene. Furthermore, the incorporation of Zn^{2+}-chelating moieties may potentially introduce inhibitory activities on HDAC, thus adding another layer of benefits to them as anticancer agents.

1 | METHODS AND MATERIALS

1.1 | Chemistry

All commercially available starting reagents and solvents were used without further purification unless otherwise stated. All reactions were monitored by thin-layer chromatography with 0.25-mm silica gel plates (60GF-254).

UV light and iodine stain were used to visualize the spots. Silica gel or C18 silica gel was used for column chromatography purification. ^{1}H NMR spectra were recorded on a Bruker DRX spectrometer at 600 MHz, while the ^{13}C NMR spectra were recorded at 400 MHz, in parts per million and *J* in hertz, using TMS as an internal standard. High-resolution mass spectra were conducted by Shandong Analysis and Test Center in Ji'nan, China. ESI-MS spectra were recorded on an API 4000 spectrometer. Melting points were determined uncorrected on an electrothermal melting point apparatus. The tested compounds are >95% pure by HPLC analysis, performed on Agilent 1100 HPLC instrument using an ODS HYPERSIL column (5 μm, 4.6 mm × 250 mm), according to the following method (**Figure S1–8**). Compounds **7b**, **12b**, **16a–d**, and **18a–c** were eluted with 72% methanol/20% water (containing 0.5% Phosphoric acid)/8% acetonitrile over 30 min, while the compounds **7a**, **12a**, and **17a–d** were eluted with 74% methanol/18% water/8% acetonitrile. The absorbance was measured at 283 nm, the flow rate was 1 mL/min, and the quantity of injection was 20 μL.

The compounds **2** were synthesized following reported procedures.[16]

1.1.1 | 4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl) benzoic acid (2, Am80)

mp: 187–188 °C, ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.63–1.66 (m, 4H), 7.30 (d, J = 8.4 Hz, 1H), 7.59 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 7.68 (d, J = 2.4 Hz, 1H), 8.03–8.08 (m, 4H), 10.26 (s, 1H), 13.26 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 167.24, 164.98, 145.04, 140.56, 139.29, 136.87, 133.65, 129.72, 128.29, 126.94, 118.82, 118.62, 35.10, 34.47, 34.03, 32.13. ESI-MS m/z: 350.5 $[M - H]^-$.

1.2 | General procedure for the preparation of 7a and 7b

1.2.1 | N^1-(2-aminophenyl)-N^4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) terephthalamide (7a)

A solution of **2** (0.35 g, 1.0 mmol), *o*-phenylenediamine (0.11 g, 1.0 mmol), DCC (0.22 g, 1.05 mmol), and DMAP (0.03 g, 0.2 mmol) in dichloromethane (DCM) and acetonitrile (ratio 1:1, 20 mL) was stirred at room temperature (rt) for 5 h, diluted with DCM (20 mL) and filtered. The filtrate was evaporated with the residue being taken up in ethyl acetate (EtOAc; 40 mL). The organic phase was washed with saturated $NaHCO_3$ and brine for three times and dried over anhydrous sodium sulfate over night. After evaporating the solvent, the crude product was purified by recrystallization with EtOAc and petroleum ether (ratio 1:4) to achieve a white pure solid **7a** (0.13 g, yield: 30%). mp: 240–241 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.25–1.26 (m, 12H), 1.64–1.67 (m, 4H), 7.26 (s, 2H), 7.31 (d, J = 8.4 Hz, 1H), 7.44 (S, 1H), 7.62 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 7.71 (d, J = 1.8 Hz, 1H), 8.13 (d, J = 8.4 Hz, 2H), 8.19 (d, J = 7.8 Hz, 2H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 165.51, 164.88, 145.04, 140.55, 138.18, 136.91, 136.77, 128.49, 128.09, 127.66, 127.28, 126.93, 118.89, 118.70, 35.06, 34.49, 34.05, 32.15. HRMS (AP-ESI) m/z calculated for $C_{28}H_{31}N_3O_2$, $[M + H]^+$ 442.2489. Found, 442.2489.

1.2.2 | N^1-hydroxy-N^4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) terephthalamide (7b)

2 (0.7 g, 2.0 mmol) and 4-methylmorpholine (0.24 mL, 2.2 mmol) were dissolved in dry THF (100 mL) under ice bath followed by the addition of isobutyl chloroformate (0.3 mL, 2.1 mmol). The mixture was stirred for 30 min at −10 °C. Hydroxylamine hydrochloride (0.16 g, 2.2 mmol) and 4-methylmorpholine (0.24 mL) dissolved in dry THF (30 mL) were added into the solution directly. The mixture was stirred for 12 h at room temperature. THF was evaporated with the residue being taken up in EtOAc (40 mL). The EtOAc solution was washed with 1 N aqueous citric acid and brine for three times, dried over anhydrous sodium sulfate over night, and evaporated. The residue was purified by column chromatography, using DCM–MeOH (20:1) as the mobile phase, to obtain **7b** as pale yellow solid (0.10 g, yield: 14%). mp: 123–124 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.63–1.66 (m, 4H), 7.30 (d, J = 8.4 Hz, 1H), 7.59 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 7.68 (d, J = 1.8 Hz, 1H), 7.89 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 8.4 Hz, 2H), 9.16 (s, 1H), 10.19 (s, 1H), 11.39 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 164.99, 163.91, 145.02, 140.48, 137.74, 136.93, 135.72, 128.12, 127.36, 126.93, 118.79, 118.56, 35.11, 34.47, 34.03, 32.14. HRMS (AP-ESI) m/z calculated for $C_{22}H_{26}N_2O_3$, $[M + H]^+$ 367.2017. Found, 367.2016.

1.3 | General procedure for the preparation of 9[17]

1.3.1 | Benzyl-2-hydroxyacetate (9)

8 (4.0 g, 52.0 mmol) and Et_3N (8.0 mL, 57.2 mmol) were dissolved in dry acetone (150 mL). After 5 min of stirring, benzyl bromide (6.0 mL, 46.8 mmol) was added to the solution. The mixture was refluxed at 60 °C for 12 h. The solution was filtered and concentrated on vacuo. The crude material was purified via flash chromatography (PE/EtOAc = 1:1) to give **9** (5.44 g, yield: 63%). ESI-MS m/z: 167.4 $[M + H]^+$.

1.4 | General procedure for the preparation of 10

1.4.1 | 2-(benzyloxy)-2-oxoethyl-4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl) benzoate (10)

2 (0.88 g, 2.5 mmol) was dissolved in the mixture of DCM and acetonitrile (ratio 2:1, 50 mL) under ice bath followed by the addition of TBTU (0.9 g, 2.75 mmol), Et_3N (0.4 mL) and stirred for 30 min at room temperature. **9** (0.45 g, 2.75 mmol) and Et_3N (0.4 mL) were added into the solution directly. The mixture was stirred for 4 h at room temperature. The solvent was evaporated under vacuum; the crude product was dissolved with EtOAc, washed by 1 N aqueous citric acid, saturated $NaHCO_3$, and brine for three times, and dried over anhydrous sodium sulfate over night, and the solvent was evaporated under vacuum. The crude product was purified by column chromatography to afford the compound **10** (0.97 g, yield: 78%). mp: 158–160 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.26 (m, 12H), 1.64–1.66 (m, 4H), 5.06 (s, 2H), 5.23 (s, 2H), 7.31 (d, J = 8.4 Hz, 1H), 7.34–7.38 (m, 1H), 7.39–7.40 (m, 3H), 7.60 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 7.68 (d, J = 2.4 Hz, 1H), 8.09–8.14 (m, 4H), 10.32 (s, 1H). ESI-MS m/z: 499.4 $[M + H]^+$.

1.5 | General procedure for the preparation of 11

1.5.1 | 2-((4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl) benzoyl) oxy) acetic acid (11)

10 (3.5 g, 15 mmol) was dissolved in mixture of methanol and THF (ratio 4:1, 200 mL), dry palladium carbon (0.35 g) was added, and the mixture was stirred under a hydrogen atmosphere overnight. The palladium carbon was filtered off over Celite and washed with methanol. After evaporating the solvent, the residue was dried under vacuum to get white solid **11** (2.4 g, yield: 79%). ESI-MS m/z: 408.6 $[M + H]^+$.

1.6 | General procedure for the preparation of 12a and 12b

1.6.1 | 2-((2-aminophenyl) amino)-2-oxoethyl-4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl) benzoate (12a)

A solution of **11** (0.82 g, 2.0 mmol), *o*-phenylenediamine (0.22 g, 2.0 mmol), DCC (0.44 g, 2.1 mmol), and DMAP (0.06 g) in the mixture of DCM and acetonitrile (ratio 4:1, 40 mL) was stirred at room temperature for 5 h, diluted with DCM (40 mL), and filtered, and the filtrate was evaporated with the residue being taken up in EtOAc (80 mL). The organic phase was washed with saturated $NaHCO_3$ and brine for three times and dried over anhydrous sodium sulfate over night. After evaporating the solvent, the crude product was purified by column chromatography to afford the compound **12a** (0.26 g, yield: 26%). mp: 169–170 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.64–1.67 (m, 4H), 5.02 (s, 2H), 6.76 (s, 1H), 6.89–7.04 (m, 3H), 7.24 (d, J = 7.2 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 8.4 Hz, 2H), 7.69 (s, 1H), 8.13–8.19 (m, 4H), 8.98 (s, 1H), 9.69 (s, 1H), 10.34 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 165.18, 164.72, 149.04, 145.05, 140.66, 140.14, 136.81, 133.33, 131.33, 130.28, 128.53, 126.95, 125.56, 118.92, 118.74, 115.80, 58.75, 35.09, 34.48, 34.05, 32.14. HRMS (AP-ESI) m/z calculated for $C_{30}H_{33}N_3O_4$, $[M + H]^+$ 500.2546. Found, 500.2544.

1.6.2 | 2-(hydroxyamino)-2-oxoethyl-4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl) benzoate (12b)

11 (0.82 g, 2.0 mmol) and 4-methyl-morpholine (0.24 mL, 2.2 mmol) were dissolved in dry THF (100 mL) under ice bath followed by the addition of isobutyl chloroformate (0.3 mL, 2.1 mmol) and stirred for 30 min at −10 °C. Hydroxylamine hydrochloride (0.16 g, 2.2 mmol) and 4-methylmorpholine (0.24 mL) dissolved in dry THF (30 mL) were added into the solution directly. The mixture was stirred for 12 h at room temperature. THF was evaporated with the residue being taken up in EtOAc (40 mL). The EtOAc solution was washed with 1 N aqueous citric acid and brine for three times, dried over anhydrous sodium sulfate over night, and evaporated, and the residue was chromatographed, using DCM–MeOH (20:1) as the mobile phase, to obtain 0.10 g of pale yellow solid **12b** (0.16 g, yield: 19%). mp: 175–176 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.64–1.67 (m, 4H), 4.72 (s, 2H), 7.31 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 9.0 Hz, 1H), 7.68 (d, J = 1.2 Hz, 1H), 8.08–8.15 (m, 4H), 9.02 (s, 1H), 10.31 (s, 1H), 10.87 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 165.28, 164.80, 163.99, 145.05, 140.63, 139.83, 136.81, 131.96, 129.96, 128.55, 126.96, 118.88, 118.68, 115.80, 62.34, 35.10, 34.48, 34.05, 32.14. HRMS (AP-ESI) m/z calculated for $C_{24}H_{28}N_2O_5$, $[M + H]^+$ 425.2071. Found, 425.2068.

1.7 | General procedure for the preparation of 14a–d

1.7.1 | Methyl-2-aminoacetate hydrochloride (14a)

Glycine (9.0 g, 120 mmol) was dissolved in the dry methanol (MeOH; 200 mL); then, acetyl chloride (25.7 mL, 360 mmol) was added dropwise at 0 °C; the reaction was refluxed at 75 °C

for 3 h. The solvent was evaporated under vacuum; the crude product was purified by recrystallization with ether to achieve a white pure solid **14a**, a white solid (12.41 g, yield: 92%), mp: 176–177 °C (literature reported data[18]: mp: 175–176 °C).

1.7.2 | Methyl-3-aminopropanoate hydrochloride (14b)

White solid, 91% yield, mp: 103–105 °C (literature reported data[19]: mp: 103–104 °C).

1.7.3 | Methyl-4-aminobutanoate hydrochloride (14c)

White solid, 85% yield, mp: 120–122 °C (literature reported data[19]: mp: 122–124 °C).

1.7.4 | Methyl-6-aminohexanoate hydrochloride (14d)

White solid, 88% yield, mp: 117–119°C (literature reported data[20]: mp: 118–122 °C).

1.8 | General procedure for the preparation of 15a–d

1.8.1 | Methyl-2-(4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl) benzamido) acetate (15a)

2 (0.88 g, 2.5 mmol) was dissolved in the mixture of DCM and acetonitrile (ratio 4:1, 50 mL) under ice bath followed by the addition of TBTU (0.9 g, 2.75 mmol), Et_3N (0.4 mL) and stirred for 30 min at room temperature. **14a** (0.34 g, 2.75 mmol) and Et_3N (0.4 mL) were added into the solution directly. The mixture was stirred for 4 h at room temperature. The solvent was evaporated under vacuum; the crude product was dissolved with EtOAc, washed by 1 N aqueous citric acid, saturated $NaHCO_3$, and brine for three times, and dried over anhydrous sodium sulfate over night, and the solvent was evaporated under vacuum. The crude product was purified by column chromatography to give the compound **15a** (0.82 g, yield: 78%). mp: 201–203 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.64–1.67 (m, 4H), 3.67 (s, 3H), 4.02–4.06 (m, 2H), 7.30 (d, J = 9.0 Hz, 1H), 7.60 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz, 1H), 7.69 (d, J = 1.8 Hz, 1H), 8.01 (d, J = 9.0 Hz, 2H), 8.05 (d, J = 9.0 Hz, 2H), 9.13–9.15 (m, 1H), 10.21 (s, 1H). ESI-MS m/z: 423.4 $[M + H]^+$.

1.8.2 | Methyl-3-(4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl) benzamido) propanoate (15b)

White solid, 63% yield. mp: 182–184°C. ^{1}H-NMR (400 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.65 (s, 4H), 2.64 (t, J = 7.0 Hz, 2H), 3.49–4.53 (m, 2H), 3.62 (s, 3H), 7.30 (d, J = 8.6 Hz, 1H), 7.60 (dd, J_1 = 2.1 Hz, J_2 = 8.6 Hz, 1H), 7.68 (d, J = 2.1 Hz, 1H), 7.96 (d, J = 8.4 Hz, 2H), 8.04 (d, J = 8.4 Hz, 2H), 8.75 (t, J = 5.4 Hz, 1H), 10.20 (s, 1H). ESI-MS m/z: 437.4 $[M + H]^+$.

1.8.3 | Methyl-4-(4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl) benzamido) butanoate (15c)

White solid, 71% yield. mp: 149–150 °C. ^{1}H-NMR (400 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.65 (s, 4H), 1.77–1.85 (m, 2H), 2.41 (t, J = 7.4 Hz, 2H), 3.28–3.32 (m, 2H), 3.59 (s, 3H), 7.30 (d, J = 8.6 Hz, 1H), 7.60 (dd, J_1 = 2.1 Hz, J_2 = 8.6 Hz, 1H), 7.69 (d, J = 2.1 Hz, 1H), 7.97 (d, J = 8.4 Hz, 2H), 8.04 (d, J = 8.4 Hz, 2H), 8.66 (t, J = 5.6 Hz, 1H), 10.19 (s, 1H). ESI-MS m/z: 451.6 $[M + H]^+$.

1.8.4 | Methyl-6-(4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carba moyl) benzamido) hexanoate (15d)

White solid, 58% yield. mp: 174–176 °C. ^{1}H-NMR (400 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.32–1.34 (m, 2H), 1.52–1.59 (m, 4H), 1.65 (s, 4H), 2.34 (t, J = 7.4 Hz, 2H), 3.29 (q, J = 6.8 Hz, 2H), 3.58 (s, 3H), 7.30 (d, J = 8.6 Hz, 1H), 7.60 (dd, J_1 = 2.1 Hz, J_2 = 8.6 Hz, 1H), 7.69 (d, J = 2.1 Hz, 1H), 7.97 (d, J = 8.4 Hz, 2H), 8.04 (d, J = 8.4 Hz, 2H), 8.63 (t, J = 5.6 Hz, 1H), 10.20 (s, 1H). ESI-MS m/z: 479.4 $[M + H]^+$.

1.9 | General procedure for the preparation of 16a–d

1.9.1 | 2-(4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl)-benzamido) acetic acid (16a)

15a (0.42 g, 1.0 mmol) was dissolved in the mixture of methanol (15 mL) and 1.5 mol/L NaOH solution (6 mL). The mixture was refluxed at 90 °C for 3 h. After removing THF under vacuum, the solution was acidified to pH 4 with 1 N HCl solution. The white solid **16a** was collected by filtration, washed with water, and dried (0.33 g, yield: 80%). mp: 160–162 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.23–1.25 (m, 12H), 1.65–1.66 (m, 4H), 3.96–3.97 (m, 2H), 7.30 (d, J = 8.4 Hz, 1H), 7.60 (dd, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 7.69 (d, J = 1.8 Hz, 1H), 8.01 (d, J = 8.4 Hz, 2H), 8.06 (d, J = 8.4 Hz, 2H), 9.01–9.03 (m, 1H), 10.22 (s, 1H), 12.80 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 171.66, 166.28, 164.97, 145.02, 140.50, 137.92, 136.91, 136.69, 128.14, 127.73, 126.92, 118.86, 118.66, 41.75, 35.05, 34.47, 34.03,

32.14. HRMS (AP-ESI) m/z calculated for $C_{24}H_{28}N_2O_4$, $[M + H]^+$ 409.2122. Found, 409.2121.

1.9.2 | 3-(4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl)-benzamid-o) propanoic acid (16b)

White solid, 86% yield. mp: 84–85 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.25–1.29 (m, 12H), 1.63–1.66 (m, 4H), 2.50–2.56 (m, 2H), 3.47–3.51 (m, 2H), 7.30 (d, J = 8.4 Hz, 1H), 7.60 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 7.68 (d, J = 2.4 Hz, 1H), 7.97 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 8.73 (t, J = 5.4 Hz, 1H), 10.20 (s, 1H), 12.27 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 173.33, 166.01, 164.99, 145.01, 140.48, 137.68, 137.25, 136.92, 128.03, 127.63, 126.92, 118.85, 118.64, 36.16, 35.11, 34.47, 34.21, 34.03, 32.14. HRMS (AP-ESI) m/z calculated for $C_{25}H_{30}N_2O_4$, $[M + H]^+$ 423.2278. Found, 423.2278.

1.9.3 | 4-(4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl)-benzamido) butanoic acid (16c)

White solid, 81% yield. mp: 175–176 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.65 (s, 4H), 1.77–1.78 (m, 2H), 2.31 (t, J = 6.6 Hz, 2H), 3.32 (q, J = 6.6 Hz, 2H), 7.30 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 9.0 Hz, 1H), 7.69 (s, 1H), 7.97 (d, J = 7.8 Hz, 2H), 8.03 (d, J = 7.8 Hz, 2H), 8.67 (t, J = 5.4 Hz, 1H), 10.20 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 174.70, 166.00, 165.01, 145.01, 140.47, 137.60, 137.47, 136.93, 128.01, 127.63, 126.92, 118.84, 118.63, 39.23, 35.11, 34.47, 34.03, 32.14, 31.64, 24.98. HRMS (AP-ESI) m/z calculated for $C_{26}H_{32}N_2O_4$, $[M + H]^+$ 437.2435. Found, 437.2437.

1.9.4 | 6-(4-((5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) carbamoyl)-benzamido) hexanoic acid (16d)

White solid, 88% yield, mp: 210–211 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.26 (m, 12H), 1.33–1.34 (m, 2H), 1.53–1.55 (m, 4H), 1.63–1.65 (m, 4H), 2.23 (t, J = 7.2 Hz, 2H), 3.27–3.28 (m, 2H), 7.30 (d, J = 8.4 Hz, 1H), 7.59 (dd, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 7.68 (d, J = 1.8 Hz, 1H), 7.96 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 8.60 (s, 1H), 10.18 (s, 1H), 11.99 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 174.89, 165.84, 165.01, 145.00, 140.45, 137.58, 137.53, 136.94, 128.01, 127.60, 126.90, 118.85, 118.64, 35.06, 34.47, 34.10, 34.03, 32.14, 29.28, 26.53, 24.74. HRMS (AP-ESI) m/z calculated for $C_{28}H_{36}N_2O_4$, $[M + H]^+$ 465.2748. Found, 465.2748.

1.10 | General procedure for the preparation of 17a–d

1.10.1 | N^1-(2-((2-aminophenyl) amino)-2-oxoethyl)-N^4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) terephthalamide (17a)

A solution of **16a** (0.82 g, 2.0 mmol), o-phenylenediamine (0.22 g, 2.0 mmol), DCC (0.44 g, 2.1 mmol), and DMAP (0.06 g) in the mixture of DCM and acetonitrile (ratio 4:1, 40 mL) was stirred at room temperature for 5 h, diluted with DCM (40 mL), and filtered, and the filtrate was evaporated with the residue being taken up in EtOAc (80 mL). The organic phase was washed with saturated $NaHCO_3$ and brine for three times and dried over anhydrous sodium sulfate over night. After evaporating the solvent, the crude product was purified via flash chromatography to afford the compound **17a** (0.20 g, yield: 21%). mp: 185–186 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.23–1.25 (m, 12H), 1.63–1.65 (m, 4H), 4.10–4.11 (m, 2H), 4.92 (s, 2H), 6.55 (t, J = 7.2 Hz, 1H), 6.72 (d, J = 7.2 Hz, 1H), 6.93 (t, J = 7.2 Hz, 1H), 7.14 (d, J = 7.2 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.60 (dd, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 7.69 (d, J = 2.4 Hz, 1H), 8.03–8.07 (m, 4H), 9.04 (t, J = 6.0 Hz, 1H), 9.29 (s, 1H), 10.22 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 168.23, 166.53, 164.99, 145.03, 142.94, 140.51, 137.97, 137.85, 136.92, 128.05, 127.85, 126.92, 126.67, 126.32, 123.29, 118.87, 118.66, 116.52, 116.09, 43.67, 35.07, 34.47, 34.03, 32.14. HRMS (AP-ESI) m/z calculated for $C_{30}H_{34}N_4O_3$, $[M + H]^+$ 499.2704. Found, 499.2703.

1.10.2 | N^1-(3-((2-aminophenyl) amino)-3-oxopropyl)-N^4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) terephthalamide (17b)

White solid, 26% yield. mp: 112–114 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.23–1.25 (m, 12H), 1.65–1.66 (m, 4H), 2.66 (t, J = 6.6 Hz, 2H), 3.61 (t, J = 6.6 Hz, 2H), 4.87 (s, 2H), 6.54 (td, J_1 = 1.2 Hz, J_2 = 7.8 Hz, 1H), 6.71 (dd, J_1 = 1.2 Hz, J_2 = 7.8 Hz, 1H), 6.91 (td, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 7.16 (dd, J_1 = 1.2 Hz, J_2 = 7.8 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.60 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 7.67–7.68 (m, 1H), 7.96–7.98 (m, 2H), 8.01–8.03 (m, 2H), 8.79 (t, J = 6.0 Hz, 1H), 9.20 (s, 1H), 10.20 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 169.91, 166.15, 165.02, 145.03, 142.58, 140.49, 137.66, 137.44, 136.92, 128.02, 127.65, 126.92, 126.38, 126.08, 123.75, 118.85, 118.64, 116.56, 116.27, 36.74, 36.25, 35.12, 34.47, 34.03, 32.14. HRMS (AP-ESI) m/z calculated for $C_{31}H_{36}N_4O_3$, $[M + H]^+$ 513.2860. Found, 513.2857.

1.10.3 | N^1-(4-((2-aminophenyl) amino)-4-oxobutyl)-N^4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) terephthalamide (17c)

White solid, 23% yield. mp: 124–126 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.65 (s, 4H), 1.84–1.88 (m, 3H), 1.99 (s, 1H), 2.42 (t, J = 7.2 Hz, 2H), 4.88 (s, 2H), 6.54 (t, J = 7.2 Hz, 1H), 6.72 (d, J = 7.8 Hz, 1H), 6.90 (t, J = 7.8 Hz, 1H), 7.16 (d, J = 7.2 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.70 (s, 1H), 7.98–8.04 (m, 4H), 8.71 (s, 1H), 9.15 (s, 1H), 10.21 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 171.32, 166.03, 165.00, 145.02, 142.50, 140.48, 137.59, 137.53, 136.93, 128.00, 127.65, 126.91, 126.22, 125.92, 123.93, 118.86, 118.65, 116.55, 116.27, 47.99, 35.07, 34.47, 34.03, 32.14, 25.81, 24.93. HRMS (AP-ESI) m/z calculated for $C_{32}H_{38}N_4O_3$, $[M + H]^+$ 527.3017. Found, 527.3016.

1.10.4 | N^1-(6-((2-aminophenyl) amino)-6-oxohexyl)-N^4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) terephthalamide (17d)

White solid, 19% yield. mp: 104–105°C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.37–1.39 (m, 2H), 1.57–1.62 (m, 2H), 1.65–1.66 (m, 6H), 2.34 (t, J = 6.6 Hz, 2H), 3.31 (q, J = 6.6 Hz, 2H), 4.82 (s, 2H), 6.54 (td, J_1 = 1.2 Hz, J_2 = 8.4 Hz, 1H), 6.72 (dd, J_1 = 1.2 Hz, J_2 = 8.4 Hz, 1H), 6.90 (td, J_1 = 1.2 Hz, J_2 = 8.4 Hz, 1H), 7.15 (dd, J_1 = 1.2 Hz, J_2 = 8.4 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.59 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz, 1H), 7.68 (d, J = 2.4 Hz, 1H), 7.97 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 8.65 (t, J = 6.0 Hz, 1H), 9.10 (s, 1H), 10.19 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 171.58, 165.87, 165.03, 145.02, 142.33, 140.48, 137.61, 137.56, 136.93, 128.00, 127.59, 126.91, 126.14, 125.75, 124.09, 118.85, 118.64, 116.65, 116.37, 36.22, 35.08, 34.48, 34.04, 32.14, 29.39, 26.70, 25.57. HRMS (AP-ESI) m/z calculated for $C_{34}H_{42}N_4O_3$, $[M + H]^+$ 555.3333. Found, 555.3335.

1.11 | General procedure for the preparation of 18a–c

1.11.1 | N^1-(2-(hydroxyamino)-2-oxoethyl)-N^4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) terephthalamide (18a)

KOH (28 g, 509 mmol) and $NH_2OH{\cdot}HCl$ (23.35 g, 343 mmol) were dissolved, respectively, in 70 and 120 mL of MeOH to get solution A and solution B. Next, solution A was added dropwise to solution B. After filtering the precipitate (KCl), a NH_2OK solution was obtained. **15a** (0.42 g, 1 mmol) was dissolved in the NH_2OK solution and stirred at room temperature for 6 h. After removing MeOH under vacuum, the residue was acidified with 1 N HCl to a pH 3 and then extracted with EtOAc. The organic phase was washed with brine for three times and dried over anhydrous sodium sulfate over night. After evaporating the solvent, the crude product was purified via flash chromatography to afford the compound **18a** (0.16 g, yield: 38%). mp: 198–200 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.63–1.67 (m, 4H), 3.81–3.82 (m, 2H), 7.30 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 1.8 Hz, 1H), 8.01 (d, J = 8.4 Hz, 2H), 8.05 (d, J = 8.4 Hz, 2H), 8.84 (s, 1H), 8.93 (d, J = 5.4 Hz, 1H), 10.21 (s, 1H), 10.65 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 171.75, 166.27, 165.00, 145.02, 140.50, 137.92, 136.92, 136.73, 128.13, 128.00, 127.84, 127.72, 126.92, 118.86, 118.67, 41.81, 35.12, 34.47, 34.03, 32.14. HRMS (AP-ESI) m/z calculated for $C_{24}H_{29}N_3O_4$, $[M + H]^+$ 424.2231. Found, 424.2232.

1.11.2 | N^1-(3-(hydroxyamino)-3-oxopropyl)-N^4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) terephthalamide (18b)

White solid, 26% yield. mp: 123–125 °C. ^{1}H-NMR (600 MHz DMSO-d_6): δ 1.24–1.25 (m, 12H), 1.65–1.66 (m, 4H), 2.30 (t, J = 7.2 Hz, 2H), 3.46–3.49 (m, 2H), 7.30 (d, J = 8.4 Hz, 1H), 7.59 (dd, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 7.68 (d, J = 1.8 Hz, 1H), 7.96 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 8.73 (t, J = 6.0 Hz, 1H), 8.77 (d, J = 1.2 Hz, 1H), 10.20 (s, 1H), 10.48 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 167.76, 165.99, 165.00, 145.03, 140.50, 137.67, 137.32, 136.91, 128.02, 127.61, 126.91, 118.85, 118.65, 36.61, 35.12, 34.47, 34.03, 32.84, 32.14. HRMS (AP-ESI) m/z calculated for $C_{25}H_{31}N_3O_4$, $[M + H]^+$ 438.2387. Found, 438.2386.

1.11.3 | N^1-(4-(hydroxyamino)-4-oxobutyl)-N^4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl) terephthalamide (18c)

White solid, 31% yield. mp: 205–207 °C. 1H-NMR (600 MHz DMSO): δ 1.24–1.25 (m, 12H), 1.63–1.66 (m, 4H), 1.76–1.78 (m, 2H), 2.05 (t, J = 7.8 Hz, 2H), 3.29 (q, J = 6.6 Hz, 2H), 7.30 (d, J = 8.4 Hz, 1H), 7.60 (dd, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 7.69 (d, J = 1.8 Hz, 1H), 7.98 (d, J = 8.4 Hz, 2H), 8.04 (d, J = 8.4 Hz, 2H), 8.70 (t, J = 6.0 Hz, 1H), 8.73 (d, J = 1.8 Hz 1H), 10.22 (s, 1H), 10.43 (s, 1H). ^{13}C-NMR (100 MHz DMSO-d_6): δ 169.38, 165.96, 165.01, 145.00, 140.45, 137.55, 137.46, 136.95, 128.02, 127.65, 126.88, 118.88, 118.70, 39.48, 35.07, 34.47, 34.02, 32.14, 30.50, 25.72. HRMS (AP-ESI) m/z calculated for $C_{26}H_{33}N_3O_4$, $[M + H]^+$ 452.2544. Found, 452.2543.

1.12 | *In vitro* antiproliferative assay

Cells were maintained in RPMI1640 medium containing 10% FBS at 37 °C in a 5% CO_2 humidified incubator. Cell proliferation was determined by the MTT method. Briefly, cells were seeded into a 96-well cell plates, allowed to grow for 12 h and then treated with different concentrations of compounds for 48 h. A 0.5% MTT solution was added to each well. After incubation for another 4 h, formazan formed was extracted by adding 150 μL of DMSO. Absorbance was then recorded at 570 nm.

1.13 | *In vitro* HDACs inhibition fluorescence assay

In vitro HDACs inhibition assays were conducted as previously described.[21] In brief, 10 μL of enzyme solution (HeLa nuclear extract contain HDAC1, 2) was mixed with compound at various concentrations (50 μL). The mixture was incubated at 37 °C for 5 min, then 40 μL of fluorogenic substrate Boc-Lys(acetyl)-AMC (300 μM) was added. After incubation at 37 °C for 1 h, the reaction was stopped by the addition of 100 μL of developer containing trypsin (10 μg/mL) and TSA (2 μM). Fluorescence intensity was measured by a microplate reader at excitation and emission wavelengths of 390 and 460 nm, respectively. The IC_{50} values were calculated using a regression analysis of the concentration/inhibition data.

1.14 | *In vitro* HDAC8 assay

The HDAC8 was expressed and purified as previously described.[22] Briefly, HDAC8 gene was amplified by using a polymerase chain reaction, the products were subcloned into pGEX-6p-1 plasmid. Subsequently, the constructs were used to transform Escherichia coli cells. The human HDAC8 proteins were purified using a GST column. The purity of hHDAC8 was above 95%, and the specific activity of hHDAC8 was 0.73 ± 0.23 U/mg. *In vitro* HDAC8 inhibition assays were conducted as previously described.[23] IC_{50} values against HDAC8 were determined using HDAC8 expressed in Escherichia coli as the enzyme. Briefly, the HDAC8 enzyme was diluted 20 times. Then, 10 μL of HDAC8 solution was mixed with compounds at various concentrations (50 μL). Then, 40 μL of the substrate [Boc-Lys-(acetyl)-AMC, 300 μM] was added, and the mixture was incubated at 37 °C for 30 min. Subsequently, the reaction was stopped by the addition of 100 μL of developer containing trypsin (10 μg/mL) and TSA (2 μM). Fluorescence intensity was measured by a microplate reader at excitation and emission wavelengths of 390 and 460 nm, respectively. The IC_{50} values were calculated using a regression analysis of the concentration/inhibition data.

1.15 | APN inhibition assay

The inhibitory activity of target compounds toward APN was determined using microsomal aminopeptidase from porcine kidney microsomes (Sigma) as the enzyme.[24] Briefly, the assay was performed in 96-well plates in 50 mM PBS, pH 7.2 as the assay buffer, at 37 °C. The APN solution was mixed with compounds at various concentrations and incubated at 37 °C for 5 min. Subsequently, the substrate (L-leucine-*p*-nitroanilide) was added and the mixture was incubated for 30 min at 37°C. Finally, the hydrolysis of the substrate was measured by a microplate reader (Thermo Fisher, Shanghai, China) at 405 nm.

1.16 | Stability of representative compound in human plasma, fresh rat plasma, and rat liver homogenate

Preparation of human plasma, fresh rat plasma, and rat liver homogenate was conducted as previously described.[25] Human plasma, fresh rat plasma, and rat liver homogenate were added to the stock solution of **7b** (4 mg/mL in methanol) and incubated at 37 °C for 48 h. At scheduled times, sample aliquots were collected and the enzymatic reaction was quenched by adding acetonitrile. Human plasma and fresh rat plasma samples underwent extraction using 300 μL of acetonitrile, while rat liver homogenate samples underwent extraction using 400 μL of acetonitrile. The samples were filtered (0.22 μm) after shocking 30 seconds and centrifugation at 12 000 rpm for 10 min. Analytical HPLC was performed on Agilent 1200 HPLC instrument using a Alltima C_{18} column (5 μm, 4.6 mm × 250 mm), and the compound was eluted with 72% methanol/20% water (containing 0.5% Phosphoric acid)/8% acetonitrile over 15 min. The absorbance was measured at 283 nm, the flow rate was 1 mL/min, and the quantity of injection was 20 μL. Retention times (min) for **7b** and tamibarotene were as follows: 8.064 (**7b**) and 11.845 (tamibarotene).

1.17 | *In vivo* pharmacokinetics

For pharmacokinetic studies, six Wistar rats, weight 200 ± 20 g, were randomly divided into two groups (injected through tail vein with 20 mg/kg **7b** and tamibarotene). The rats were fasted for 12 h before the experiment, free drinking water. For test and control groups, 0.3 mL of blood sample from the carotid sinus before the administration of 0 h and after the administration of 0.05, 0.083, 0.167, 0.333, 0.667, l, 2, 4, 6, 10, and 24 h, respectively, was added in the centrifuge tube handled by heparin sodium, centrifuged at 4000 rpm for l0 min, and stored at −80 °C until sample extraction. The test samples were prepared for quantification by protein precipitation and evaporation. **7b** and tamibarotene were quantified by HPLC analysis.

1.18 | Determination of 7b in whole blood, plasma, and blood cells

Three Wistar rats were injected through tail vein with 20 mg/kg **7b**. Before the experiment, the rats were forbidden to eat anything but drink water. We got 0.5 mL of blood sample from the jugular sinus, after the time of administration of 5 min and 10 min, and added the blood sample in the centrifuge tube handled by heparin sodium. Preparation of whole blood samples: 0.2 mL of acetonitrile was added into the 0.1 mL of rat blood. The samples were filtered (0.22 μm) after shocking 30 seconds and centrifugation at 12 000 rpm for 10 min. Preparation of plasma and blood cells samples: 0.35 mL of rat blood was centrifuged at 4000 rpm for 10 min, and then, the plasma and blood cells were prepared. About 0.2 mL of acetonitrile was added into the 0.15 mL of each of plasma and blood cells. The samples were filtered (0.22 μm) after shocking 30 seconds and centrifugation at 12 000 rpm for 10 min, and then, we obtained the cell samples. To determine the concentration of compound **7b** in 20 μL of each of samples of whole plasma and blood cell samples at 5 and 10 min, the amount of drug extracted from whole blood was taken as the full, then the plasma and the distribution of drug in blood cells were calculated.

2 | RESULTS AND DISCUSSION

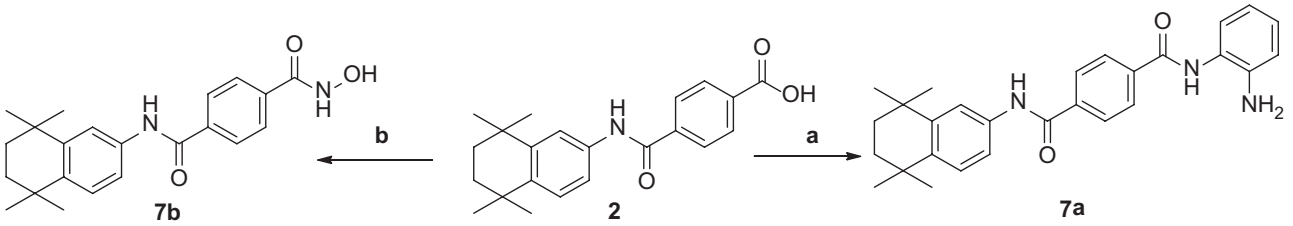
2.1 | Chemistry

To prepare the target compounds, key intermediate **2** (**tamibarotene/Am80**) was synthesized following reported procedures.[16] Coupling reactions of **2** with hydroxylamine or *o*-phenylenediamine accomplished the synthesis of compounds **7a** and **7b**, respectively, following the conditions outlined in Scheme 1.

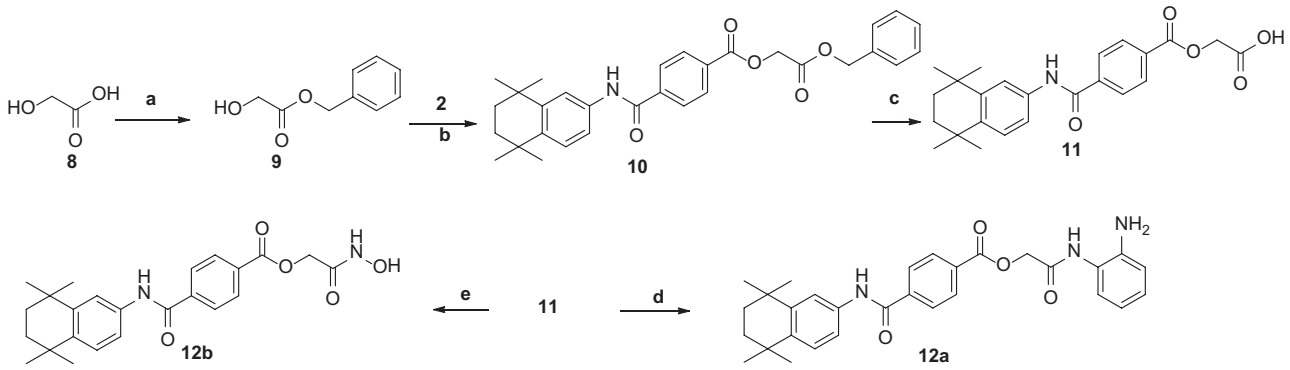
The synthesis of target compounds **12a–b** was shown in Scheme 2. Briefly, reaction of **8** with benzyl bromide in acetone in the presence of triethylamine (Et_3N) yielded **9**, which was followed by reaction with **2** to give intermediate **10** in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU). Deprotection of **10** followed by coupling reactions with hydroxylamine or *o*-phenylenediamine finally gave target compounds **12a–b**. To make analogs **16a–d**, **17a–d**, and **18a–c**, coupling reactions of **2** with various amino esters **14a–d** produced **15a–d**. Reactions of **15a–d** with NH_2OK in dry methanol directly delivered hydroxamic acids **18a–c**. Hydrolysis of **15a–d** followed by coupling reactions with *o*-phenylenediamine gave **17a–d** (Scheme 3).

2.2 | Antiproliferative activities of the designed analogs

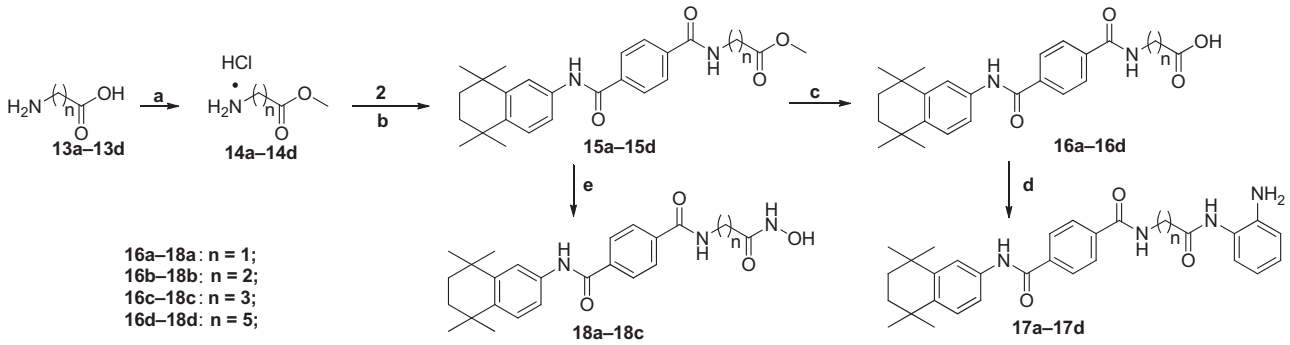
To evaluate whether the incorporation of Zn^{2+}-chelating moiety into tamibarotene will improve anticancer activities, we examined the antiproliferative activities of these compounds in human leukemia K562 cells, which was most frequently used in evaluating tamibarotene derivatives.[14] Initially, a single concentration of 30 μM was chose for the screening to identify compounds with reasonable activity. As shown in Table 1, in general, the analogs with hydroxamic acid moiety exhibited better antiproliferative activities at the tested concentration. Analogs with carboxylic acid moiety (**16a–d**) lost antiproliferative activities compared to tamibarotene or SAHA. This may suggest that the Zn^{2+}-chelating moiety is important for the biological activities within the designed analogs. Based on the initial screening results, compounds **7a**, **7b**, and **18a** were selected for further dose–response studies to obtain potency against six cancer cells lines including solid and hematological tumor cells, which were most frequently used in evaluating tamibarotene derivatives and HDACIs (Table 2). Overall, compounds **7a**, **7b**, and **18a** showed improved antiproliferation potency against six cancer cell lines, compared to tamibarotene. When compared to SAHA, they exhibited comparable potencies against K562, HL60, and ES-2 cell lines. Among these three compounds, **7b** displayed an overall optimal potency than the other two analogs, specifically for HL60, MDA-MB-231, HCT116, and ES-2 cancer cells. This may suggest that the observed activity of **7b** could be due to both of the tamibarotene moiety and the hydroxamic acid through interactions with retinoid receptors and HDAC. Additionally, cytotoxicity of **7b** against human liver cell HL-7702 was tested in our lab, and the IC_{50} value was 108.9 ± 15.4 μM, which revealed the selectivity of **7b** between non-transformed cells and tumor cells. The compound **7b** has been reported as an HDACI, but no further



SCHEME 1 Synthesis of **7a–b**. Reagents and conditions: (a) DCC/DMAP, DCM; (b) isobutyl chloroformate, THF, hydroxylamine hydrochloride



SCHEME 2 Synthesis of **12a–b**. Reagents and conditions: (a) benzyl bromide, acetone, Et_3N, **60** °C; (b) TBTU/Et_3N; (c) Pd/C, H_2; (d) DCC/DMAP, DCM; (e) isobutyl chloroformate, THF, hydroxylamine hydrochloride



SCHEME 3 Synthesis of **16a–d**, **17a–d** and **18a–c**. Reagents and conditions: (a) acetyl chloride, MeOH, **70** °C; (b) TBTU/Et_3N; (c) MeOH/NaOH, **90** °C; (d) DCC/DMAP, DCM; (e) NH_2OK/MeOH, THF

characterization and biological results available from the early report.[26]

2.3 | HeLa cell extract inhibition by the target compounds

Given the fact that the hydroxamic acid moiety is essential for HDACIs as a Zn^{2+}-chelating group, we next examined the inhibitory effects of these compounds on HDAC. To this end, HeLa cell nuclear extract (which contains primarily HDAC1 and HDAC2) was used as the HDACs enzyme source.[25] As shown in Table 3, compounds **12**, **17**, and **18** exhibited moderate inhibitory activities on HDACs and no inhibitory effects were observed for compounds **16**, consistent with the antiproliferative activities (Table 1). This again may suggest the important roles of the Zn^{2+}-chelating moieties on the newly designed analogs. Overall, compounds containing hydroxamic acid (**18a–c**) were more potent than compounds with benzamide moiety (**17a–d**) under the current testing conditions. Notably, compound **7b** exhibited the most potent inhibition on HDACs with single-digit micromolar potency (IC_{50}: 1.8 ± 0.1 μM). Although the inhibitory potency on HDAC is lower than SAHA, the combination of dual functions from **7b**, namely RAR agonist and HDACI, may explain the overall improved cytotoxic effects compared

TABLE 1 Growth inhibition rates toward K562 cells at concentration of **30** μM

Compound	Inhibition (%)[a]	Compound	Inhibition (%)[a]	Compound	Inhibition (%)[a]
7a	77.0	**16c**	8.1	**18a**	66.4
7b	67.2	**16d**	32.3	**18b**	58.4
12a	41.8	**17a**	49.1	**18c**	60.7
12b	58.4	**17b**	56.7	**SAHA**	49.4
16a	35.8	**17c**	45.2	**Tamibarotene**	47.1
16b	38.3	**17d**	60.1		

[a]Assays were performed in replicate ($n \geq 2$); the SD values are <20% of the mean.

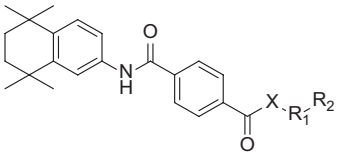
TABLE 2 Antiproliferative activities of **7**a, **7**b, **18a**, **tamibarotene**, and **SAHA** against six tumor cell lines (IC_{50} in μM[a])

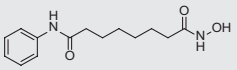
Compound	IC_{50}					
	HCT116	**ES-2**	**K562**	**HL60**	**PC-3**	**MDA-MB-231**
7a	26.9	17.5	4.6	14.7	>100	13.7
7b	7.8	12.9	34.6	4.0	11.0	5.2
18a	12.8	26.8	31.1	10.7	6.5	98.6
Tamibarotene	81.3	ND	42.4	16.1	79.4	>100
SAHA	0.3	13.4	40.3	5.7	21.4	0.7

ND, Not determined.

[a]Assays were performed in replicate ($n \geq 2$); the SD values are <20% of the mean.

TABLE 3 HDAC inhibition activity of compounds **7a–b**, **12a–b**, **16a–d**, **17a–d**, **18a–c**, **tamibarotene**, and **SAHA**



Compound	X	R_1	R_2	IC_{50} against HeLa extract (μM)[a]
7a	–	–	*o*-H_2NPhNH	>100
7b	–	–	NHOH	1.8 ± 0.1
12a	O	CH_2CO	*o*-H_2NPhNH	51.6 ± 2.4
12b	O	CH_2CO	NHOH	15.1 ± 0.5
16a	NH	CH_2CO	OH	>100
16b	NH	$(CH_2)_2CO$	OH	>100
16c	NH	$(CH_2)_3CO$	OH	>100
16d	NH	$(CH_2)_5CO$	OH	>100
17a	NH	CH_2CO	*o*-H_2NPhNH	75.2 ± 4.8
17b	NH	$(CH_2)_2CO$	*o*-H_2NPhNH	46.1 ± 2.1
17c	NH	$(CH_2)_3CO$	*o*-H_2NPhNH	57.8 ± 3.6
17d	NH	$(CH_2)_5CO$	*o*-H_2NPhNH	32.4 ± 1.3
18a	NH	CH_2CO	NHOH	9.3 ± 0.4
18b	NH	$(CH_2)_2CO$	NHOH	21.4 ± 0.5
18c	NH	$(CH_2)_3CO$	NHOH	12.1 ± 0.3
SAHA				0.1 ± 0.0
Tamibarotene	OH	–	–	>100

[a]Assays were performed in replicate ($n \geq 2$); values are shown as mean ± SD.

TABLE 4 *In vitro* inhibition of HDAC-**8** and APN of **7b**[a]

IC_{50} (μM)[a]	**Compound**			
	7b	**SAHA**	**Tamibarotene**	**Bestatin**
HDAC-8	4.3 ± 0.1	1.1 ± 0.0	>100	ND
APN	>100	>100	>100	2.5 ± 0.3

[a]Assays were performed in replicate ($n \geq 2$); values are shown as mean ± SD.

to tamibarotene or SAHA alone. This further supports our design rationale of such tamibarotene analogs.

Compound **7b** was further evaluated for its inhibitory activity against HDAC8 and APN. Similar to HDACs, APN is zinc-dependent metalloproteinase as well and associated closely with of tumorigenesis. Bestatin was used as the positive control to APN. The result in Table 4 showed that compound **7b** exhibited low micromolar IC_{50} value against HDAC8, which was similar to its inhibitory activity toward HeLa cell nuclear extract (which contains primarily HDAC1 and HDAC2) and no inhibitory effects were observed for APN. These results validated our strategy for rational designing of potential HDACIs.

2.4 | Stability of compound 7b in human plasma, fresh rat plasma, and rat liver homogenate

Given the improved antiproliferative activities of **7b**, we next set out to assess its *in vivo* pharmacokinetic profiles. To this end, *in vitro* stability of **7b** in human plasma, fresh rat plasma,

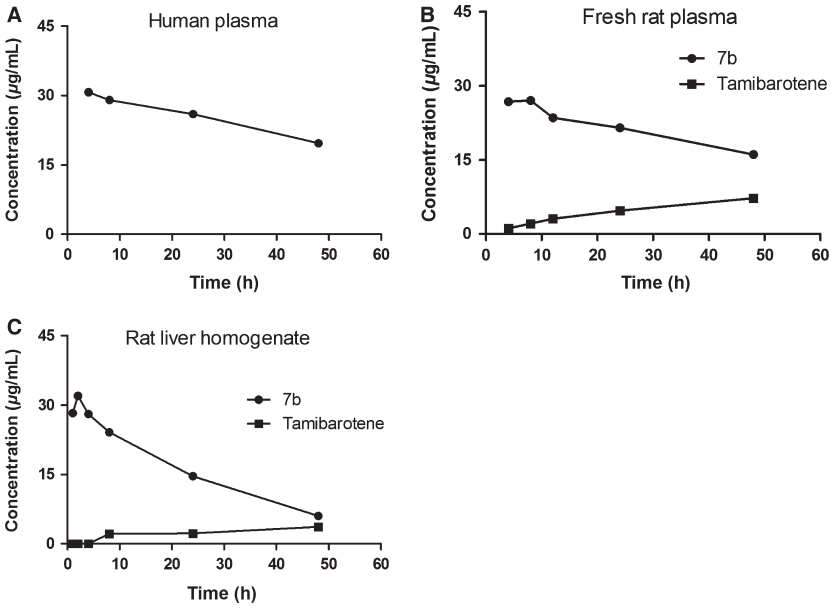


FIGURE 2 (A–B) Stability of compound **7b** in human plasma and fresh rat plasma; Points were achieved after **4**, **8**, **12**, **24**, and **48** h, respectively. (C) Stability of compound **7b** in rat liver homogenate; Points were achieved after **1**, **2**, **4**, **8**, **24**, and **48** h, respectively

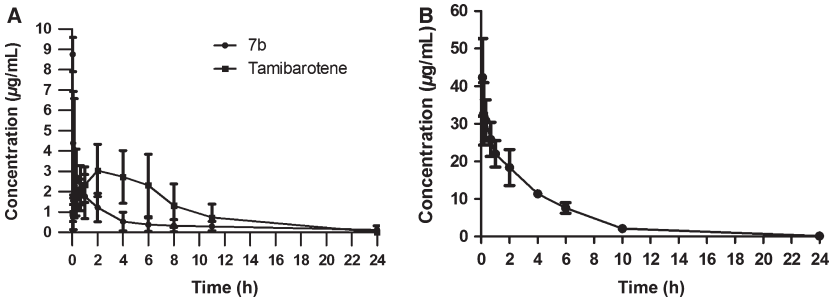


FIGURE 3 (A) Concentration–time curve after intravenous injection of **7b** (**20** mg/kg) in rats. (B) Concentration–time curve after intravenous injection of tamibarotene (**20** mg/kg) in rats

TABLE 5 Main pharmacokinetic parameters of **7b** and **tamibarotene** after i.v. administration (**20** mg/kg) in rat (mean ± SD, $n = 3$)

	7b administration		Tamibarotene administration
Pharmacokinetic parameters	**7b**	**Tamibarotene**	**Tamibarotene**
$t_{1/2\alpha}$ (h)	0.08 ± 0.05	1.38 ± 1.39	0.80 ± 1.23
$t_{1/2\beta}$ (h)	2.28 ± 1.03	3.88 ± 1.76	2.22 ± 38.20
V_1 (L/kg)	2.21 ± 0.77	1.11 ± 1.40	0.409 ± 0.11
MRT (h)	15.70 ± 20.31	18.09 ± 14.05	4.04 ± 1.04
$AUC_{0-\infty}$ (mg/L*h)	16.58 ± 14.87	92.16 ± 79.64	136.67 ± 9.41
T_{max} (h)	0.06 ± 0.02	4.00 ± 2.83	0.08 ± 0.01
C_{max} (mg/L)	6.70 ± 3.61	3.89 ± 0.02	42.40 ± 10.29
CL (L/h/kg)	3.65 ± 3.91	0.35 ± 0.30	0.15 ± 0.01

$t_{1/2\alpha}$, absorption half-life; $t_{1/2\beta}$, elimination half-life; V_1, apparent volume of distribution; MRT, mean residence time; $AUC_{0-\infty}$, area under curve; T_{max}, peak time; C_{max}, maximum plasma drug concentration; CL, clearance.

and rat liver homogenate was studied using a HPLC method. As shown in Figure 2, **7b** was partially cleaved in human plasma, fresh rat plasma, and rat liver homogenate within 48 h. Data showed that compound **7b** released significant levels of tamibarotene *in vitro*. To evaluate whether **7b** will function as a prodrug to release the parent compound tamibarotene upon metabolism, we also determined the level of tamibarotene under these experimental conditions. As shown in Figure 2B and C, there is a steady increase of tamibarotene accompanied

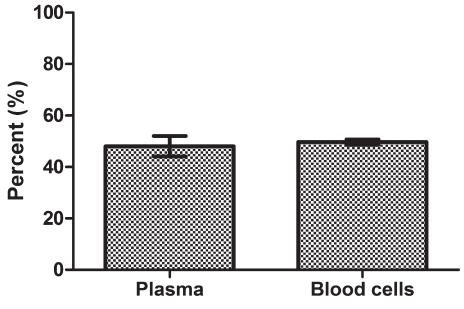


FIGURE 4 The distribution of **7b** in plasma and blood cells

with the degradation of **7b**, thus suggesting that **7b** gradually release the parent compound tamibarotene upon metabolism. This is consistent with our initial design rationale.

2.5 | The pharmacokinetic study of 7b *in vivo*

Given the promising results of **7b** from the *in vitro* studies, we decided to study the *in vivo* pharmacokinetic properties of **7b** that will help prepare further preclinical studies of **7b** as well as guide development of more potent analogs of **7b**.

As shown in Figure 3A and Table 5, after the intravenous administration of **7b**, at 20 mg/kg dose, tamibarotene was detected 5 min later, and it reached the peak concentration at 4 h postadministration of **7b**. This clearly indicated that **7b** was metabolized to release tamibarotene *in vivo*. Compared to the profile of tamibarotene, administration of **7b** significantly improved the half-life of tamibarotene (from 2.22 to 3.88 h), mean residence time (MRT; from 4.44 to 18.09 h), and peak time (T_{max}; from 0.08 to 4 h; Table 5). Collectively, the results suggest that compound **7b** could provide more beneficial properties as a potential anticancer agent compared to tamibarotene by exhibiting the properties such as RAR agonist and HDAC inhibitor and extending the residence time of tamibarotene.

2.6 | Determination of 7b in whole blood, plasma, and blood cells

The pharmacokinetic results of **7b** *in vivo* indicated that the total level of **7b** and its metabolic product tamibarotene was lower than the given dose of **7b** (Table 5). This may suggest that parent **7b** is partially distributed. Therefore, we decided to determine the recovery rate of **7b** in the plasma, whole blood, and blood cells. As shown in Figure 4, the level of **7b** in the plasma accounted only about 48% and the rest of given **7b** was distributed into blood cells.

3 | CONCLUSION

In summary, a series of tamibarotene analogs containing Zn^{2+}-binding moieties were designed and synthesized. The compound **7b** exhibited more improved antiproliferation potency than tamibarotene-tested cancer cells. Further enzymatic studies suggested that additional inhibition on HDAC could potentially contribute to the improved antiproliferative activities of **7b**. Pharmacokinetic studies showed that **7b** could be metabolized to release tamibarotene and prolong the half-time of tamibarotene. Taken together, the results strongly suggest that compound **7b** could serve as a new lead compound for development of more potent analogs as treatment agents for leukemic patients.

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CONFLICT OF INTEREST

The authors confirmed that this article content has no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:
Figure S1. HPLC analysis chromatogram of **7a.**
Figure S2. HPLC analysis chromatogram of **7b.**
Figure S3. HPLC analysis chromatogram of **16a.**
Figure S4. HPLC analysis chromatogram of **16b.**
Figure S5. HPLC analysis chromatogram of **17a.**
Figure S6. HPLC analysis chromatogram of **17b.**
Figure S7. HPLC analysis chromatogram of **18b.**
Figure S8. HPLC analysis chromatogram of **18c.**