Bioorganic & Medicinal Chemistry 20 (2012) 4405–4412

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of *N*-aryl salicylamides with a hydroxamic acid moiety at 5-position as novel HDAC–EGFR dual inhibitors

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ARTICLE INFO

Article history:
Received 22 March 2012
Revised 14 May 2012
Accepted 15 May 2012
Available online 29 May 2012

Keywords:
N-Aryl salicylamides
HDAC inhibitor
EGFR inhibitor
Antiproliferative activity
Synthesis

ABSTRACT

A novel series of *N*-aryl salicylamides with a hydroxamic acid moiety at 5-position were synthesized efficiently. Their activities against EGFR kinase and HDACs were evaluated. All compounds displayed inhibitory activity against EGFR and HDACs. The antiproliferative activities of synthesized compounds were evaluated by MTT method against human cancer cell lines A431, A549 and HL-60. Compound **1o** showed the most potent inhibitory activity against A431 and A549. Compounds **1k** and **1n** exhibited higher potency against HL-60 than gefitinib and SAHA. *N*-Aryl salicylamides with a hydroxamic acid moiety at 5-position is another new HDAC–EGFR dual inhibitors.

1. Introduction

Cancer is a disease characterized by uncontrolled cell growth and proliferation, which usually results from activation of some enzymes. Receptor tyrosine kinases (RTK) have been observed having over-expression and/or constitutive activation in numerous types of tumor, including colon, breast, ovarian, head and neck, and non-small cell lung cancers. RTKs play key roles in the processes of governing cellular proliferation, differentiation and evasion from apoptosis as well. Among the known RTKs, the ErbB family, in particular epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2), have been extensively studied and clinically validated as targets for cancer therapies.[1–4] The ErbB family consists of four receptors, including epidermal growth factor receptor (EGFR/ErbB-1/HER1), HER2 (ErbB-2/neu), HER-3 and HER-4. Current therapies using either monoclonal antibodies (cetuximab and trastuzumab) or small organic molecules (gefitinib, erlotinib) to selectively target EGFR or HER2, or recently, small molecules that target both receptors (lapatinib), have been found successful clinically.[5,6] New compounds for inhibiting both EGFR and HER2 have been constantly discovered.[7]

The reversible acetylation of lysine residues in histone tails plays a critical role in transcriptional activation and repression.[8,9] Histone acetyltransferases (HATs) catalyze the acetylation of lysine residues on histone and non-histone proteins. The reverse reaction is catalyzed by histone deacetylases (HDACs), thus promoting a more closed chromatin structure where transcription is repressed.[10] HDAC inhibitors have been applied to treatment of human cancers.[11] Zolinza (vorinostat, SAHA) was approved by the FDA for the treatment of advanced cutaneous T-cell lymphoma (CTCL).[12] Several small molecule HDAC inhibitors have entered clinical trials for the treatment of a variety haematological and solid tumors.[13,14] New scaffolds of small molecular inhibitors against HDAC have been constantly reported.[15–17]

However, the efficacy of small organic molecules inhibitors such as gefitinib, etc. is restricted to a small subset of patients due to molecular heterogeneity among and within tumors.[18,19] Their effectiveness is also limited by the drug resistance that frequently emerges following treatment.[20] HDAC inhibitors have been shown to synergize with other agents, including RTK inhibitors, to suppress proliferation and induce apoptosis in tumor cells.[21–24] To overcome the low response rate and acquired resistance to RTK inhibitors, a number of strategies have been tested, including combination therapies and multitargeted inhibitors to inhibit multiple pathogenic pathways.[25] One particularly promising approach is incorporating the pharmacophore of HDAC inhibitor (hydroxamic acid) and the pharmacophore of EGFR inhibitor (4-arylaminoquinazoline) into one molecule to obtain new chemical entity. A novel series of compounds with potent, multiacting HDAC, EGFR, and HER2 inhibition were synthesized and evaluated.[26] Meanwhile, CUDC-101 was identified as a drug candidate, which is now in clinical development. Almost simultaneously compound A was synthesized and evaluated by combining the pharmacological activity

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http://dx.doi.org/10.1016/j.bmc.2012.05.034

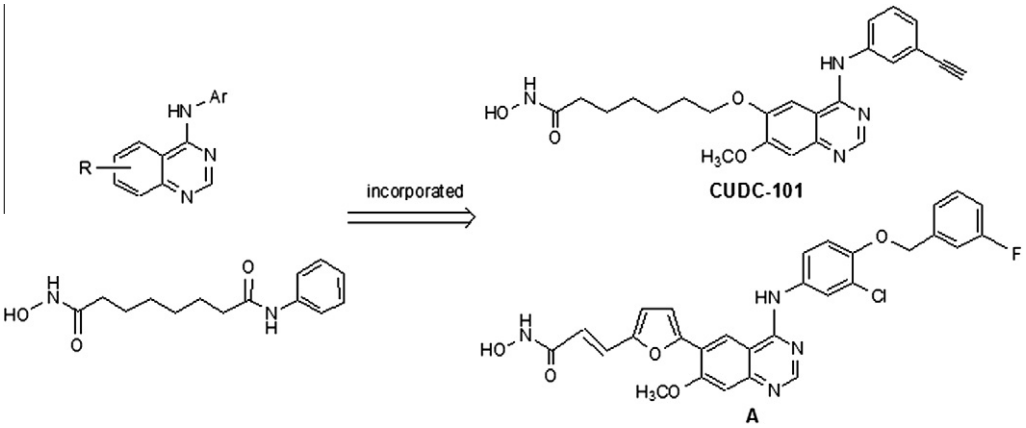


Figure 1. Incorporation of 4-arylaminoquinazoline and SAHA.

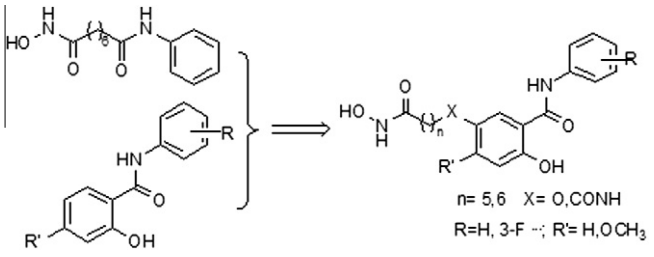


Figure 2. Incorporate strategy of *N*-aryl salicylamide and SAHA.

of EGFR/HER2 kinase inhibition with inhibition of HDAC enzymes (Fig. 1).[27] Thus, chimeric HDAC-kinase inhibitors was able to constitute a new class of experimental cancer drugs worth to be studied in more detail.

The salicylanilide molecule may construct an intramolecular hydrogen bond and form a pseudo six-membered ring. Thus, *N*-aryl salicylamide is supposed to have the same pharmacophore as 4-arylaminoquinazoline, and has been studied as EGFR inhibitors.[28,29] In this study, we selected the scaffold of *N*-aryl salicylamide to incorporate the pharmacophore of HDAC inhibitor (hydroxamic acid) to present a novel class of HDAC–EGFR dual inhibitors. Our strategy was shown in Figure 2. Here *N*-aryl salicylamides with a hydroxamic acid moiety at 5-position were synthesized and their biological activities against EGFR and HDACs and their antiproliferative activities against human carcinoma cell lines A431, A549 and HL-60 were evaluated.

2. Results and discussion

2.1. Chemistry

The target compounds **1a**–**1j** were synthesized from 5-nitrosalicylic acid. The synthetic route was outlined in Scheme 1. The mixture of 5-nitrosalicylic acid, aryl amine, triphenyl phosphite and toluene was refluxed for hours to yield salicylamides 3 with high yield. Hydroxy group was not necessary to protect in this reaction.[30] Reduction of nitro group in compound **3** with iron powder in the acidic condition produced corresponding amines with quantitive yield. The reaction of the amine with acyl chloride, such as 7-ethyoxyl-7-oxoheptanoyl chloride or 8-ethyoxyl-8-oxooctanoyl chloride, afforded compound **4** in the presence of TEA. The yield range from 40% to 60% in the step because the hydroxy group at 2-position may react with acyl chloride in the presence of TEA. Finally, the ethyl ester group in compounds **4** was treated with freshly prepared hydroxylamine in methanol to produce target compounds **1a**–**1j**.

To expand the structural diversity of the salicylic acid moiety, we synthesized 4-methoxy substituted *N*-aryl salicylamides **1k** and **1l** from 4-methoxysalicylic acid (Scheme 2) .

The commercial available 4-methoxysalicylic acid was nitrated with concentrated nitric acid in acetic acid to give **5**. Compounds **1k** and **1l** were prepared according to the same procedure described in Scheme 1.

To compare the different substituted group at 2-position of benzoic acid under the influence of activity of final compound, compound **1m** was synthesized from 3-nitrobenzoic acid, compound **1n** was synthesized from **3f**, respectively, (Scheme 3).

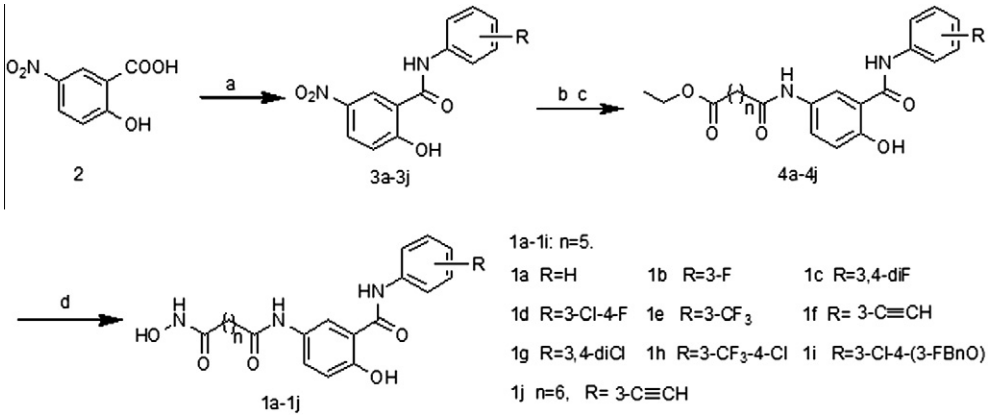
In the structure of compounds **1a**–**1n**, hydroxamic acid moiety is linked with *N*-aryl salicylamides through a amide structure. Preparation of ether linker compound **1o** was shown in Scheme 4. Commercially available 2,5-dihydroxybenzoic acid was converted into amide **6** according to the similar method described in Scheme 1. The refluxing of **6** with ethyl 7-bromoheptanoate in the presence of anhydrous K_2CO_3 and tetrabutylammonium iodide in acetone afforded intermediate **7**. There are two hydroxy groups in compound **6**. Tetrabutylammonium iodide was added to reaction mixture to improve the selectivity and the yield of **7**.[31] Final compound **1o** was obtained by the treatment of **7** with freshly prepared hydroxylamine.

All the synthesized compounds **1a**–**1o** are reported for the first time. Their structures were characterized by ^{1}H NMR and HRMS.

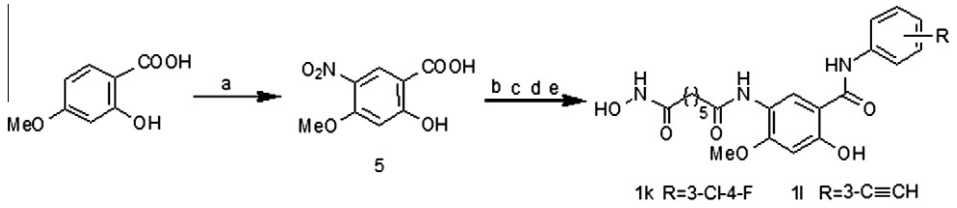
2.2. EGFR and HDAC inhibitory activity

To study biological activity, compounds **1a**–**1o** were evaluated against EGFR kinase activity assays with gefitinib as positive control and HDACs enzyme assay with SAHA as positive control. EGFR was prepared from human A431 carcinoma cell. HDACs were prepared from HeLa cell extracts. Compounds **1a**–**1o** were initially screened at final concentrations of 10.0, 1.0 and 0.1 μM by using ELISA-based EGFR-TK assay and an enzymatic assay measuring total HDACs activity. IC_{50}s of compounds **1a**–**1o** were calculated according to inhibitory rates, the in vitro enzymatic inhibition assay results are summarized in Table 1.

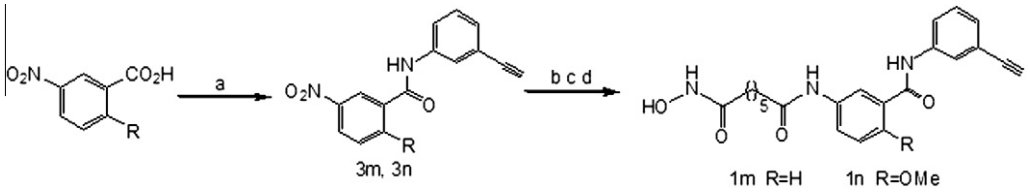
The length of the hydroxamic acid side chain is important for EGFR and HDAC inhibition. The optimal carbon chain length is six ($n = 5$ or 6) in the structure of 4-arylaminoquinazolines.[26] For this reason, short chain analogues were not synthesized in our work. The data in Table 1 demonstrated that compounds **1a–1o** exhibited the inhibitory activity against EGFR and HDACs. The activities of compounds **1b**, **1h**, **1i**, **1k**, **1l** and **1o** against EGFR were



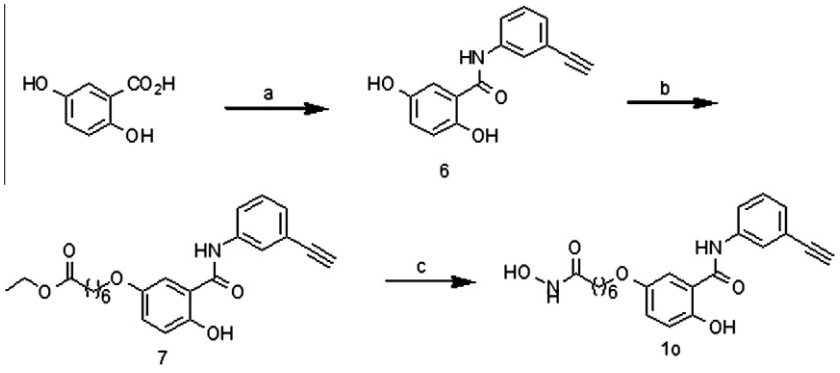
Scheme 1. Reagents and conditions: (a) $ArNH_2$, $P(OPh)_3$, reflux; (b) Fe, HOAc; (c) RCOCl, TEA; (d) NH_2OH, MeOH.



Scheme 2. Reagents and conditions: (a) HNO_3, HOAc; (b) $ArNH_2$, $P(OPh)_3$, reflux; (c) Fe, HOAc; (d) $EtO_2C(CH_2)_5COCl$, TEA; (e) NH_2OH, MeOH.



Scheme 3. Reagents and conditions: (a) 3-EthynylPhNH_2, $P(OPh)_3$, reflux; (b) Fe, HOAc; (c) $EtO_2C(CH_2)_5COCl$, TEA; (d) NH_2OH, MeOH.



Scheme 4. Reagents and conditions: (a) 3-EthynylPhNH_2, $P(OPh)_3$, reflux; (b) ethyl 7-bromoheptylate, K_2CO_3; (c) NH_2OH, MeOH.

close to that of gefitinib. The activities of compounds **1m** and **1n** against EGFR were found to be less potent than gefitinib and other synthesized compounds. Since there is no substituted group at 2-position of benzoic acid ring of **1m** and there is a methoxy group at 2-position of **1n**, a pseudo six-membered ring can not construct in **1m** and **1n**. Consequently the activity of **1m** and **1n** declined in EGFR inhibition. Compounds **1k** and **1l** relatively exhibited higher inhibitory activities than that of **1d** and **1f** against EGFR. These results suggest that a methoxy group at 4-position of salicylic acid is favorable for EGFR inhibition. When *n* is 5 (compound **1f**) or 6 (compound **1j**), EGFR inhibition was close to each other. For EGFR inhibition, an ether linker (compound **1o** IC_{50} = 0.90 μM against EGFR) was more potent than an amide linker (compound **1f** IC_{50} = 3.31 μM against EGFR). The same result was observed in HDAC inhibition.

As shown in Table 1, compounds **1a–1o** displayed inhibitory activity against HDACs. Compared with reference compounds, all the synthesized compounds inhibit HDAC activity with reduced potency except compound **1n**. Compound **1n** displayed the most potent activity (IC_{50} = 0.56 μM against HDAC). Contrary to EGFR inhibition, a methoxy group at 2-position of benzoic acid ring improved HDACs inhibition. It seems that the effect of substituted

Table 1
The structures and inhibitory activities of compounds **1a**–**1o**, SAHA and Gifitinib against EGFR and HDACs ($n = 3$, $\bar{x} \pm s$)

Compounds	IC_{50} (μM)	
	EGFR	HDACs
1a	2.34 ± 0.21	1.89 ± 0.30
1b	1.41 ± 0.11	3.59 ± 0.31
1c	2.22 ± 0.18	6.02 ± 0.56
1d	3.53 ± 0.32	3.02 ± 0.28
1e	4.20 ± 0.29	3.19 ± 0.32
1f	3.31 ± 0.60	4.09 ± 0.40
1g	1.80 ± 0.20	3.65 ± 0.51
1h	1.41 ± 0.16	3.48 ± 0.43
1i	1.10 ± 0.13	3.01 ± 0.19
1j	2.25 ± 0.22	2.03 ± 0.27
1k	1.56 ± 0.20	1.09 ± 0.09
1l	1.12 ± 0.15	1.86 ± 0.20
1m	6.17 ± 0.42	3.27 ± 0.55
1n	5.16 ± 0.39	0.56 ± 0.08
1o	0.90 ± 0.11	1.21 ± 0.19
SAHA	Nt	0.98 ± 0.11
Gifitinib	1.08 ± 0.12	Nt

Nt: not tested.

groups on *N*-phenyl ring (*R* in Scheme 1) do not obviously affect EGFR and HDACs inhibitory activity.

2.3. Antiproliferative activity

To test the anticancer activities of the synthesized compounds we evaluated antiproliferative activities of compounds**1a–1o** against human epithelial carcinoma cell line (A431), lung adenocarcinoma epithelial cell line (A549) and promyelocytic leukemia cell line (HL-60) by applying the MTT colorimetric assay. The results are summarized in Table 2. Compounds were tested over a range of concentrations from 10^{-4} to 10^{-8} M, and the calculated IC_{50} values, that is, the concentration (μM) of a compound that was able to cause 50% cell death with respect to the control culture, were reported differently according to different cancer cells.

As expected, compounds **1a**–**1o** exhibited remarked effects on antiproliferative activities in vitro, especially against A431. Compound **1o** showed the most potent inhibitory activity (IC_{50} = 1.88 μM for A431 and IC_{50} = 15.5 μM for A549), and comparable to the positive control erlotinib (IC_{50} = 1.41 μM against A431 and IC_{50} = 11.8 μM against A549). In the case of against A549, all tested compounds, including positive drugs, did not show a strong inhibitory activities (IC_{50} > 10 μM). When tested against HL-60, most compounds were less potent. However, compounds **1k** (IC_{50} = 9.83 μM against HL-60) and **1n** (IC_{50} = 7.10 μM for HL-60) displayed a higher potency than SAHA. This may be related to their high inhibitory activities against HDACs.

Table 2
The antiproliferative activities of compounds **1a**–**1o** and reference compounds ($n = 6$, $\bar{x} \pm s$)

Compounds	IC_{50} (μM)		
	A431	A549	HL-60
1a	5.85 ± 0.66	14.6 ± 2.2	20.3 ± 3.6
1b	4.28 ± 0.58	37.2 ± 3.5	—
1c	4.01 ± 0.51	70.1 ± 8.3	—
1d	3.48 ± 0.36	21.1 ± 6.5	—
1e	2.75 ± 0.44	25.7 ± 3.1	—
1f	5.75 ± 0.27	76.2 ± 9.6	—
1g	3.80 ± 0.61	17.8 ± 1.9	—
1h	4.14 ± 0.32	24.1 ± 4.3	—
1i	2.60 ± 0.18	12.4 ± 2.3	—
1j	6.11 ± 0.98	43.6 ± 5.0	—
1k	6.46 ± 0.12	24.1 ± 3.9	9.83 ± 2.6
1l	4.68 ± 0.81	18.4 ± 1.7	28.6 ± 5.5
1m	7.08 ± 0.57	37.6 ± 2.9	—
1n	4.20 ± 0.39	40.7 ± 8.0	7.10 ± 1.8
1o	1.88 ± 0.09	15.5 ± 1.9	30.9 ± 6.7
SAHA	5.13 ± 0.40	27.8 ± 2.6	76.5 ± 5.6
Gifitinib	1.41 ± 0.12	11.8 ± 1.1	54.5 ± 8.6

'—' Means IC_{50} > 100 μM.

3. Conclusions

In summary, a novel series of *N*-aryl salicylamides with a hydroxamic acid moiety at 5-position were synthesized efficiently as novel HDAC–EGFR dual inhibitors. As expected, these compounds exhibited distinct inhibitory activity against EGFR and HDACs and potent antiproliferative activities in vitro, especially against A431. Compound **1o** showed the most potent inhibitory activity against A431 and A549. Compounds **1k** and **1n** exhibited higher potency against HL-60 than gefitinib and SAHA. By combining two distinct pharmacophores into one molecule, HDAC–EGFR dual inhibitors were postulated to represent a novel approach to cancer therapy.

4. Experimental section

4.1. Chemistry

Unless specified otherwise, all starting materials, reagents and solvents were commercially available. All reactions were monitored by thin-layer chromatography on silica gel plates (GF-254) and visualized with UV light. All the melting points were determined on a Beijing micromelting-point apparatus and thermometer was uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 on a 300 MHz Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal reference. All chemical shifts are reported in parts per million (ppm). High-resolution exact mass measurements were performed using electrospray ionization (positive mode) on a quadrupole time-of-flight (Q-TOF) mass spectrometer (Maxis Q-TOF, Bruker Inc.).

4.1.1. *N*-Aryl-2-hydroxy-5-nitrobenzamide (3)

4.1.1.1. *N*-Phenyl-2-hydroxy-5-nitrobenzamide (3a). A mixture containing 2-hydroxy-5-nitrobenzoic acid (1.83 g, 10 mmol), aniline (1.21 g, 13 mmol), triphenyl phosphite (3.6 mL) and toluene (30 mL) was strirred under refluxing for 8 h, cooled to room temperature. The resulted solid was collected by filtration under reduced pressure, washed with cooled dichlormethane (5 mL × 2), crystallized from ethanol (20 mL) to produce 2.17 g of **3a** as a yellow solid. Yield 84%. Mp 219–221 °C. MS *m/z* 258.9 $[M+H]^+$.

Compounds **3b**–**3l** and **6** were synthesized according to the procedure described above.

4.1.1.2. *N*-(3-Fluorophenyl)-2-hydroxy-5-nitrobenzamide (3b). Yield 94%. Mp 208–210 °C. ^{1}H NMR: δ 10.68 (s, 1H, NH), 8.70 (s, 1H, Ar-H), 8.30 (q, 1H, Ar-H), 7.74 (d, 1H, J = 11.37 Hz, Ar-H), 7.41–7.50 (m, 2H, Ar-H), 7.19 (d, 1H, J = 9.09 Hz, Ar-H), 6.99 (t, 1H, J = 8.86 Hz, Ar-H). HRMS Calcd for $C_{13}H_9FN_2NaO_4$ $[M+Na]^+$: 299.0444. Found 299.0441.

4.1.1.3. *N*-(3, 4-Difluorophenyl)-2-hydroxy-5-nitrobenzamide (3c). Yield 93%. Mp 257–258 °C. ^{1}H NMR: δ 10.68 (s, 1H, NH), 8.69 (s, 1H, Ar-H), 8.30 (q, 1H, Ar-H), 7.44–7.94 (m, 3H, Ar-H), 7.19 (d, 1H, J = 9.06 Hz, Ar-H). HRMS Calcd for $C_{13}H_9F_2N_2O_4$ $[M+H]^+$: 295.0530. Found 295.0522.

4.1.1.4. *N*-(3-Chloro-4-fluorolphenyl)-2-hydroxy-5-nitrobenzamide (3d). Yield: 95%. Mp 259–260 °C. HRMS Calcd for $C_{13}H_8ClFN_2NaO_4$ $[M+Na]^+$: 333.0054. Found 333.0048.

4.1.1.5. *N* -(3-Trifluoromethylphenyl)-2-hydroxy-5-nitrobenzamide (3e). Yield: 97%. Mp 181–182 °C. HRMS Calcd for $C_{14}H_9F_3N_2NaO_4$ $[M+Na]^+$: 349.0407. Found 349.0405.

4.1.1.6. *N*-(3-Ethynylphenyl)-2-hydroxy-5-nitrobenzamide (3f). Yield: 92%. Mp 231–233 °C. ^{1}H NMR: δ 10.64 (s, 1H, NH), 8.74 (s, 1H, Ar-H), 8.30 (m, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 7.15–7.42 (m, 4H, Ar-H), 4.20 (s, 1H, ≡CH). HRMS Calcd for $C_{15}H_{10}N_2NaO_4$ $[M+Na]^+$: 305.0533. Found 305.0535.

4.1.1.7. *N*-(3, 4-Dichlorophenyl)-2-hydroxy-5-nitrobenzamide (3g). Yield: 94%. Mp 270–271 °C. ^{1}H NMR: δ 10.73 (s, 1H, NH), 8.69 (s, 1H, Ar-H), 8.30 (d, 1H, J = 8.94 Hz, Ar-H), 8.11 (s, 1H, Ar-H), 7.64 (m, 2H, Ar-H), 7.18 (d, 1H, J = 9.03 Hz, Ar-H). HRMS Calcd for $C_{13}H_8Cl_2N_2NaO_4$ $[M+Na]^+$: 348.9753. Found 348.9763.

4.1.1.8. *N* -(4-Chloro-3-trifluoromethylphenyl)-2-hydroxy-5-nitrobenzamide (3h). Yield: 85%. Mp 241–242 °C. ^{1}H NMR: δ 10.88 (s, 1H, NH), 8.69 (s, 1H, Ar-H), 8.31 (s, 1H, Ar-H), 8.28 (d, 1H, J = 9.30 Hz, Ar-H), 8.01 (d, 1H, J = 8.40 Hz, Ar-H), 7.25 (d, 1H, J = 8.40 Hz, Ar-H), 7.19 (d, 1H, J = 9.30 Hz, Ar-H). HRMS Calcd for $C_{14}H_8ClF_3N_2NaO_4$ $[M+Na]^+$: 383.0017. Found 383.0027.

4.1.1.9. *N*-[3-Chloro-4-(3-fluorobenzyloxy)phenyl]-2-hydroxy-5-nitrobenzamide (3i). Yield: 92%. Mp 242–243 °C. ^{1}H NMR: δ 10.63 (s, 1H, NH), 8.76 (s, 1H, Ar-H), 8.30 (d, 1H, J = 8.40 Hz, Ar-H), 7.91 (s, 1H, Ar-H), 7.58 (d, 1H, J = 8.40 Hz, Ar-H), 7.14–7.47 (m, 6H, Ar-H), 5.24 (s, 2H, CH_2). HRMS Calcd for $C_{20}H_{15}ClF_3N_2O_6$ $[M+H]^+$: 417.0654. Found 417.0660.

4.1.1.10. *N*-(3-Ethynylphenyl)-2-hydroxy-5-nitrobenzamide (3j= 3f) and *N*-(3-chloro-4-fluorophenyl)-2-hydroxy-4-methoxy-5-nitrobenzamide (3k). 2-Hydroxy-4-methoxybenzoic acid (8.4 g, 50 mmol) was added portionwise into a mixture of glacial acetic acid (31 mL) and nitric acid (31 mL) cooled by ice-water bath. The obtained reaction mixture was stirred overnight, poured into ice-water (about 200 mL), filtered. The solid was washed with water, dried, crystallized from ethanol and water (1:1, v/v) to give 6.40 g of 2-hydroxy-4-methoxy-5-nitrobenzoic acid (**5**) as a yellow solid. Yield 60%. Mp 233–235 °C.

Compounds **3k** was synthesized according to the procedure described in **3a**.

Yield 88%. Mp 250–252 °C. ^{1}H NMR: δ 10.59 (s, 1H, NH), 8.71 (s, 1H, Ar-H), 7.41–8.00 (m, 3H, Ar-H), 6.80 (s, 1H, Ar-H), 3.96 (s, 3H, OCH_3). HRMS Calcd for $C_{14}H_{10}ClFN_2NaO_5$ $[M+Na]^+$: 363.0154. Found 363.0162.

4.1.1.11. *N* -(3-Ethynylphenyl)-2-hydroxy-4-methoxy-5-nitrobenzamide (3l). Yield 78%. Mp 207–209 °C. ^{1}H NMR: δ 10.53 (s, 1H, NH), 8.74 (s, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 7.25–7.72 (m, 3H, Ar-H), 6.81 (s, 1H, Ar-H), 4.20 (s, 1H, ≡CH), 3.96 (s, 3H, O-CH_3). HRMS Calcd for $C_{16}H_{13}N_2O_5$ $[M+H]^+$: 313.0824. Found 313.0826.

4.1.1.12. *N*-(3-Ethynylphenyl)-5-nitrobenzamide (3m). The reaction of 3-nitrobenzoyl chloride (12 mmol) with 3-ethynylbenzenamine (1.4 g, 12 mmol) in dry THF (30 ml) and TEA (0.5 mL, 14 mmol) gave a light-yellow product (3.1 g). Yield 97%. Mp 177–178 °C. HRMS Calcd for $C_{15}H_{11}N_2O_3$ $[M+H]^+$: 267.0770. Found 267.0773.

4.1.1.13. *N*-(3-Ethynylphenyl)-2-methoxy-5-nitrobenzamide (3n). The mixture of **3f** (1.4 g, 5 mmol), Me_2SO_4 (0.96 g, 10 mmol), K_2CO_3 (2.1 g, 15 mmol) and acetone (50 mL) was heated to reflux for 3 h with strirring, cooled to room temperature, added water (30 mL). Then acetone was evaporated. The formed precipitate was collected by filtration, washed with water and dried to give 1.46 g of **3n** as a yellow solid. Yield 98%. Mp 194–196 °C. ^{1}H NMR δ 10.38 (s, 1H, NH), 8.39–8.43 (m, 2H, Ar-H), 7.90 (s, 1H, Ar-H), 7.23–7.74 (m, 4H, Ar-H), 4.19 (s, 1H, ≡CH), 4.03 (s, 3H, O-CH_3). HRMS Calcd for $C_{16}H_{13}N_2O_4$ $[M+H]^+$: 297.0875. Found 297.0877.

4.1.1.14. *N*-(3-Ethynylphenyl)-2, 5-dihydroxybenzamide (6). Yield 73%. Mp 199–201 °C. ^{1}H NMR δ 10.44 (s, 1H, NH), 9.05 (s, 1H, OH), 7.90 (s, 1H, Ar-H), 7.67 (d, 1H, J = 8.17 Hz, Ar-H), 6.81–7.39 (m, 5H, Ar-H), 4.15 (s, 1H, ≡CH). HRMS Calcd for $C_{15}H_{12}NO_3$ $[M+H]^+$: 254.0817. Found 254.0813.

4.1.1.15. Ethyl 7-(4-hydroxy-3-(*N*-phenylcarbamoyl)phenylamino)-7-oxoheptanoate (4a). Reduction of *N*-aryl-2-hydroxy-5-nitrobenzamide: A mixture of *N*-aryl-2-hydroxy-5-nitrobenzamide (10 mmol), iron powder (100 mmol), acetic acid (10 mL), ethanol (40 mL) and THF (40 mL) was stirred under refluxing for 7 h. The solvent was evaporated off under reduced pressure and the residue was adjusted to pH 10 by adding saturated Na_2CO_3 solution. The mixture was extracted with ethyl acetate (50 mL × 3). The combined organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and evaporated to give a grey solid with a quantitive yield. The products were not characterized and directly used for the next step.

7-ethyoxyl-7-oxoheptanoyl chloride (12 mmol) was prepared from 7-ethyoxyl-7-oxoheptanoic acid (12 mmol) and thionyl chlororide (6 mL) according to general procedure.

7-ethyoxyl-7-oxoheptanoyl chloride (about 12 mmol, freshly prepared) diluted with dry THF (15 mL) was added dropwise to the solution of *N*-phenyl-2-hydroxy-5-aminobenzamide (10 mmol) and TEA (1.7 mL, 12 mmol) in dry THF (40 mL) cooled by an ice-water bath under stirring. After addition, the mixture was stirred at the same temperature for another 3 h. Then the solvent was evaporated. The residue was dissolved in ethyl acetate (150 mL). The organic phase was washed with saturated $NaHCO_3$ solution, H_2O and brine, dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatographic using $CHCl_3$:MeOH (40:1, v/v) as eluent to give 1.95 g of **4a** as a pale yellow solid. Yield 49%. Mp 199–200 °C. ^{1}H NMR δ 10.44 (s, 1H, NH), 9.75 (s, 1H, NH), 8.02 (s, 1H, Ar-H), 6.90–7.70 (m, 7H, Ar-H), 4.03 (q, 2H, O-CH_2), 2.27 (m, 4H, 2×CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.31 (m, 2H, CH_2), 1.17 (t, 3H, J = 7.68 Hz,CH_3). HRMS Calcd for $C_{22}H_{26}N_2NaO_5$ $[M+Na]^+$: 421.1739. Found 421.1736.

Compounds **4b–4m** were synthesized according to the procedure described above.

4.1.1.16. Ethyl 7-[4-hydroxy-3-(*N* -3-fluorophenylcarbamoyl) phenylamino]-7-oxoheptanoate (4b). Yield 45%. Mp 203–204 °C. ^{1}H NMR: δ 11.16 (s, 1H, OH), 10.55 (s, 1H, NH), 9.84 (s, 1H, NH), 8.00 (s, 1H, Ar-H), 7.76 (d, 1H, J = 11.49 Hz, Ar-H), 7.65 (d, 1H, J = 8.43 Hz, Ar-H), 6.93–7.46 (m, 4H, Ar-H), 4.03 (q, 2H, O-CH_2), 2.28 (m, 4H, 2×CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.31 (m, 2H, CH_2), 1.17 (t, 3H, J = 8.08 Hz, CH_3). HRMS Calcd for $C_{22}H_{25}FN_2NaO_5$ $[M+Na]^+$: 439.1645. Found 439.1648.

4.1.1.17. Ethyl 7-[4-hydroxy-3-(*N*-3,4-difluorophenylcarbamoyl) phenylamino]-7-oxoheptanoate (4c). Yield 43%. Mp 208–209 °C. ^{1}H NMR: δ 10.54 (s, 1H, NH), 9.84 (s, 1H, NH), 8.00 (s, 1H, Ar-H), 7.9 (m, 1H, Ar-H), 7.64 (1H, J = 8.49 Hz, Ar-H), 7.41–7.47 (m, 2H, Ar-H), 6.96 (d, 1H, J = 8.85 Hz, Ar-H), 4.05 (q, 2H, O-CH_2), 2.28 (m, 4H, 2×CO-CH_2), 1.57 (m, 4H, 2×CH_2), 1.31 (m, 2H, CH_2), 1.17 (t, 3H, J = 7.66 Hz, CH_3). HRMS Calcd for $C_{22}H_{24}F_2N_2NaO_5$ $[M+Na]^+$: 457.1551. Found 457.1552.

4.1.1.18. Ethyl 7-[4-hydroxy-3-(*N* -3-chloro-4-fluorophenylcarbamoyl)phenylamino]-7-oxoheptanoate (4d). Yield 59%. Mp

183–184 °C. ^{1}H NMR: δ 11.06 (s, 1H, OH), 10.50 (s, 1H, NH), 9.83 (s, 1H, NH), 6.95–8.06 (m, 6H, Ar-H), 4.03 (q, 2H, O-CH_2), 2.27 (m, 4H, 2×CO-CH_2), 1.57 (m, 4H, 2×CH_2), 1.31 (m, 2H, CH_2), 1.17 (t, 3H, J = 7.76 Hz,CH_3). HRMS Calcd for $C_{22}H_{24}ClFN_2NaO_5$ $[M+Na]^+$: 473.1255. Found 473.1262.

4.1.1.19. Ethyl 7-[4-hydroxy-3-(*N* -3-trifluoromethylphenylcarbamoyl)phenylamino]-7-oxoheptanoate (4e). Yield 40%. Mp 186–187 °C. ^{1}H NMR: δ 11.07 (s, 1H, OH), 10.65 (s, 1H, NH), 9.85 (s, 1H, NH), 8.25 (s, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 7.46–7.94 (m, 4H, Ar-H), 6.96 (d, 1H, J = 8.73 Hz, Ar-H), 4.04 (q, 2H, O-CH_2), 2.28 (m, 4H, 2×CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.31 (m, 2H, CH_2), 1.18 (t, 3H, J = 8.02 Hz,CH_3). HRMS Calcd for $C_{23}H_{25}F_3N_2NaO_5$ $[M+Na]^+$: 489.1613. Found 489.1617.

4.1.1.20. Ethyl 7-[4-hydroxy-3-(*N* -3-ethynylphenylcarbamoyl) phenylamino]-7-oxoheptanoate (4f). Yield 56%. Mp 187–188 °C. ^{1}H NMR: δ 11.12 (s, 1H, OH), 10.44 (s, 1H, NH), 9.81 (s, 1H, NH), 8.00 (s, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 6.93–7.70 (m, 5H, Ar-H), 4.20 (s, 1H, ≡CH), 4.05 (q, 2H, O-CH_2), 2.28 (m, 4H, 2×CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.30 (m, 2H, CH_2), 1.18 (t, 3H, J = 7.77 Hz,CH_3). HRMS Calcd for $C_{24}H_{26}N_2NaO_5$ $[M+Na]^+$: 445.1739. Found 445.1738.

4.1.1.21. Ethyl 7-[4-hydroxy-3-(*N*-3,4-dichlorophenylcarbamoyl) phenylamino]-7-oxoheptanoate (4g). Yield: 49%. Mp 193–194 °C. ^{1}H NMR: δ 10.60 (s, 1H, NH), 9.83 (s, 1H, NH), 8.13 (s, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 7.59–7.68 (m, 3H, Ar-H), 6.95 (d, 1H, J = 8.71 Hz, Ar-H), 4.04 (q, 2H, O-CH_2), 2.28 (m, 4H, 2×CO-CH_2), 1.57 (m, 4H, 2×CH_2), 1.31 (m, 2H, CH_2), 1.17 (t, 3H, J = 8.00 Hz,CH_3). HRMS Calcd for $C_{22}H_{24}Cl_2N_2NaO_5$ $[M+Na]^+$: 489.0960. Found 489.0968.

4.1.1.22. Ethyl 7-[4-hydroxy-3-(*N*-4-chloro-3-trifluoromethylphenylcarbamoyl) phenylamino]-7-oxoheptanoate (4h). Yield: 40%. Mp 151–152 °C. ^{1}H NMR: δ 10.69 (s, 1H, NH), 9.80 (s, 1H, NH), 8.69 (s, 1H, Ar-H), 8.33 (s, 1H, Ar-H), 8.00 (m, 2H, Ar-H), 6.94–7.72 (m, 3H, Ar-H), 4.03 (q, 2H, O-CH_2), 2.28 (m, 4H, 2×CO-CH_2), 1.57 (m, 4H, 2×CH_2), 1.31 (m, 2H, CH_2), 1.17 (t, 3H, J = 7.84 Hz,CH_3). HRMS Calcd for $C_{23}H_{24}ClF_3N_2NaO_5$ $[M+Na]^+$: 523.1224. Found 523.1220.

4.1.1.23. Ethyl 7-[4-hydroxy-3-(*N*-3-chloro-4-(3-fluorobenzyloxy) phenyl)carbamoyl] phenylamino)-7-oxoheptanoate (4i). Yield 54%. Mp 173–174 °C. ^{1}H NMR: δ 11.16 (s, 1H, OH), 10.36 (s, 1H, NH), 9.81 (s, 1H, NH), 8.01 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 7.17–7.63 (m, 7H, Ar-H), 6.95 (d, 1H, J = 8.37 Hz, Ar-H), 5.24 (s, 2H, CH_2), 4.05 (q, 2H, O-CH_2), 2.28 (m, 4H, 2×CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.31 (m, 2H, CH_2), 1.17 (t, 3H, J = 8.02 Hz,CH_3). HRMS Calcd for $C_{29}H_{30}ClFN_2NaO_6$ $[M+Na]^+$: 579.1674. Found 579.1676.

4.1.1.24. Ethyl 8-[4-hydroxy-3-(*N* -3-ethynylphenyllcarbamoyl) phenylamino]-8-oxooctanoate (4j). The reaction of *N*-3-ethynylphenyl-2-hydroxy-5-aminobenzamide with 8-ethyoxyl-8-oxooctanoyl chloride gave **4j**. Yield 38%. Mp 179–180 °C. ^{1}H NMR: δ 11.09 (s, 1H, OH), 10.46 (s, 1H, NH), 9.78 (s, 1H, NH), 8.01 (s, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 6.92–7.70 (m, 5H, Ar-H), 4.18 (s, 1H, ≡CH), 4.05 (q, 2H, O-CH_2), 2.27 (m, 4H, 2×CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.30 (m, 4H, 2×CH_2), 1.19 (t, 3H, J = 7.94 Hz, CH_3). HRMS Calcd for $C_{25}H_{28}N_2NaO_5$ $[M+Na]^+$: 459.1896. Found 459.1894.

4.1.1.25. Ethyl 7-[5-(*N* -3-chloro-4-fluorophenylcarbamoyl)-4-hydroxy-2-methoxy-phenylamino]-7-oxoheptanoate (4k). Yield 43%. Mp 82–83 °C. ^{1}H NMR: δ 12.09 (s, 1H, OH), 10.38 (s, 1H, NH), 9.10 (s, 1H, NH), 8.20 (s, 1H, Ar-H), 7.39–8.00 (m, 3H, Ar-H), 6.61 (s, 1H, Ar-H), 4.06 (q, 2H, O-CH_2), 3.84 (s, 3H, O-CH_3), 2.32 (m, 4H, 2×CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.35 (m, 2H, CH_2), 1.17 (t, 3H, J = 8.08 Hz, CH_3). HRMS Calcd for $C_{23}H_{26}ClFN_2NaO_6$ $[M+Na]^+$: 503.11361. Found 503.1333.

4.1.1.26. Ethyl 7-[5-(*N* -3-ethynylphenylcarbamoyl)-4-hydroxy-2-methoxy-phenylamino]-7-oxoheptanoate (4l). Yield 60%. Mp 96–98 °C. ^{1}H NMR: δ 10.35 (s, 1H, NH), 9.10 (s, 1H, NH), 8.22 (s, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 7.71 (d, 1H, J = 7.88 Hz, Ar-H), 7.38 (t, 1H, Ar-H), 7.24 (d, 1H, J = 7.30 Hz, Ar-H), 6.61 (s, 1H, Ar-H), 4.22 (s, 1H, ≡CH), 4.08 (q, 2H, O-CH_2), 3.84 (s, 3H, O-CH_3), 2.32 (m, 4H, 2×CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.34 (m, 2H, CH_2), 1.17 (t, 3H, J = 7.87 Hz,CH_3). HRMS Calcd for $C_{25}H_{28}N_2NaO_6$ $[M+Na]^+$: 475.1845. Found 475.1815.

4.1.1.27. Ethyl 7-[3-(*N*-3-ethynylphenylcarbamoyl)phenylamino]-7-oxoheptanoate (4m). Yield 83%. Mp 148–149 °C. ^{1}H NMR: δ 10.36 (s, 1H, NH), 10.10 (s, 1H, NH), 8.10 (s, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 7.84 (d, 1H, J = 8.10 Hz, Ar-H), 7.81 (d, 1H, J = 7.50 Hz, Ar-H), 7.62 (d, 1H, J = 7.20 Hz, Ar-H), 7.46 (t, 1H, Ar-H), 7.37 (t, 1H, Ar-H), 7.22 (d, 1H, J = 7.50 Hz, Ar-H), 4.20 (s,1H, ≡CH), 4.05 (q, 2H, O-CH_2), 2.29 (m, 4H, 2×CO-CH_2), 1.58 (m, 4H, 2×CH_2), 1.32 (m, 2H, CH_2), 1.17 (t, 3H, J = 7.88 Hz, CH_3). HRMS Calcd for $C_{24}H_{26}N_2NaO_4$ $[M+Na]^+$: 429.1790. Found 429.1753.

4.1.1.28. 7-[3-(*N* -Ethynylphenylcarbamoyl)-4-methoxyphenylamino]-7-oxoheptanoic acid (4n). The mixture of 5-amino-*N*-(3-ethynylphenyl)-2-methoxybenzamide (4.3 mmol), pimelic acid anhydride (0.74 g, 5.1 mmol), anhydrous THF (20 mL) and TEA (0.9 mL) was stirred overnight. Then the solvent was evaporated and the product was purified by silica gel column chromatographic using $CHCl_3$:MeOH (50:1, v/v) as eluent to give 1.20 g of **4n** as a white solid. Yield 68%. Mp > 260 °C. ^{1}H NMR δ 11.95 (s, 1H, OH), 10.21 (s, 1H, NH), 9.89 (s, 1H, NH), 7.90 (s, 1H, Ar-H), 7.84 (s, 1H, Ar-H), 7.11–7.77 (m, 5H, Ar-H), 4.20 (s, 1H, ≡CH), 3.87 (s, 3H, O-CH_3), 2.18–2.30 (m, 4H, CO-CH_2), 1.52–1.58 (m, 4H, 2×CH_2), 1.30 (m, 2H, CH_2). HRMS Calcd for $C_{23}H_{24}N_2NaO_5$ $[M+Na]^+$: 431.1583. Found 431.1559.

4.1.1.29. Ethyl 7-[3-(*N*-ethynylphenyl)carbamoyl-4-hydroxyphenoxy]heptanoate (7). The mixture of **6** (0.51 g, 2 mmol), Ethyl 7-bromoheptanoate (0.47 g, 2 mol), K_2CO_3 (0.83 g, 6 mmol), tetrabutylammonium iodide (1.48 g, 4 mmol) and acetone (15 mL) was strirred under refluxing for 4 h. The solvent was evaporated. The residue was dissolved in water (30 mL) and ethyl acetate (100 mL). The organic phase was washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated. The residue was purified by silica gel column chromatographic using $CHCl_3$:MeOH (50:1, v/v) as eluent to produce 0.53 g of **7** as a pale yellow solid. Yield 65%. Mp 117–118 °C. ^{1}H NMR δ 10.17 (s, 1H, NH), 9.26 (s, 1H, OH), 7.87 (s, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 6.86–7.68 (m, 5H, Ar-H), 4.15 (s, 1H, ≡CH), 4.02 (m, 4H, 2×O-CH_2), 2.20 (m, 2H, CH_2), 1.73 (m, 2H, CH_2), 1.42 (m, 4H, 2×CH_2), 1.30 (m, 2H, CH_2), 1.16 (t, 3H, J = 7.79 Hz, CH_3). HRMS Calcd for $C_{24}H_{27}NNaO_5$ $[M+Na]^+$: 432.1787. Found 432.1759.

4.1.1.30. *N* -Phenyl-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido)benzamide (1a). KOH (2.64 g, 40 mmol) was added to a solution of hydroxyamine hydrochloride (1.88 g, 27 mmol) in methanol (15 mL) cooled by an ice bath. The mixture was stirred for another 1 h. The resulting precipitate was filtered off, and the solution of free hydroxylamine was prepared.

The above freshly prepared hydroxyamine solution was placed in a round bottom flask cooled by an ice bath. **4a** (1.08 g, 2.7 mmol) was added to the solution. Then the mixture was stirred over night, water (50 mL) and AcOH were added to adjust pH to 4–5, kept for 3 h in a refrigerator. The precipitate was collected by filtration, washed with water, then crystallized from acetone/THF (1:1, v/v)

to afford 0.65 g of **1a** as a carneous solid. Yield 63%. Mp 249–251 °C. ^{1}H NMR δ 10.68 (s, 1H, NH), 10.35 (s, 1H, NH), 9.81 (s, 1H, NH), 8.04 (s, 1H, Ar-H), 6.92–7.70 (m, 7H, Ar-H), 2.27 (m, 2H, CO-CH_2), 1.95 (m, 2H, CO-CH_2), 1.55 (m, 4H, 2×CH_2), 1.29 (m, 2H, CH_2). HRMS Calcd for $C_{20}H_{23}N_3NaO_5$ $[M+Na]^+$: 408.1535. Found 408.1540.

Compounds **1b**–**1m** and **1o** were synthesized according to the procedure described above.

4.1.1.31. *N*-(3-Fluorophenyl)-2-hydroxy-5-(7-hydroxyamino-7-oxohep-tanamido) benzamide (1b). Yield 63%. Mp 260–262 °C. ^{1}H NMR δ 10.76 (s, 1H, NH), 10.34 (s, 1H, NH), 9.81 (s, 1H, NH), 8.00 (s, 1H, Ar-H), 7.76 (d, 1H, *J* = 11.37 Hz, Ar-H), 7.64 (d, 1H, *J* = 8.37 Hz, Ar-H), 6.92–7.42 (m, 4H, Ar-H), 2.26 (m, 2H, CO-CH_2), 1.95 (m, 2H, CO-CH_2), 1.55 (m, 4H, 2×CH_2), 1.29 (m, 2H, CH_2). HRMS Calcd for $C_{20}H_{23}FN_3NaO_5$ $[M+Na]^+$: 404.1622. Found 404.1632.

4.1.1.32. *N*-(3, 4-Difluorophenyl)-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido) benzamide (1c). Yield 71%. Mp 254–256 °C. ^{1}H NMR δ 10.74 (s, 1H, NH), 10.34 (s, 1H, NH), 9.81 (s, 1H, NH), 8.00 (s, 1H, Ar-H), 7.43–7.94 (m, 4H, Ar-H), 6.94 (d, 1H, *J* = 8.76 Hz, Ar-H), 2.26 (m, 2H, CO-CH_2), 1.96 (m, 2H, CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.29 (m, 2H, CH_2). HRMS Calcd for $C_{20}H_{21}F_2N_3NaO_5$ $[M+Na]^+$: 444.1347. Found 444.1354.

4.1.1.33. *N*-(3-Chloro-4-fluorophenyl)-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido)benzamide (1d). Yield 63%. Mp 219–222 °C. ^{1}H NMR δ 11.07 (s, 1H, Ar-OH), 10.51 (s, 1H, NH), 10.33 (s, 1H, NH), 9.82 (s, 1H, NH), 8.65 (s, 1H, N-OH), 6.92–8.06 (m, 6H, Ar-H), 2.26 (m, 2H, CO-CH_2), 1.95 (m, 2H, CO-CH_2), 1.55 (m, 4H, 2×CH_2), 1.28 (m, 2H, CH_2). HRMS Calcd for $C_{20}H_{21}ClFN_3NaO_5$ $[M+Na]^+$: 460.1051. Found 460.1062.

4.1.1.34. *N*-(3-Trifluoromethylphenyl-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido)benzamide (1e). Yield 60%. Mp 212–214 °C. ^{1}H NMR δ 10.70 (s, 1H, NH), 10.36 (s, 1H, NH), 9.85 (s, 1H, NH), 8.25 (s, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 7.46–7.94 (m, 4H, Ar-H), 6.96 (d, 1H, *J* = 8.55 Hz, Ar-H), 2.28 (m, 2H, CO-CH_2), 1.97 (m, 2H, CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.29 (m, 2H, CH_2). HRMS Calcd for $C_{21}H_{22}F_3N_3NaO_5$ $[M+Na]^+$: 476.1409. Found 476.1424.

4.1.1.35. *N*-(3-Ethynylphenyl)-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido) benzamide (1f). Yield 73%. Mp > 260 °C. ^{1}H NMR δ 10.60 (s, 1H, NH), 10.34 (s, 1H, NH), 9.80 (s, 1H, NH), 8.02 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 6.93–7.70 (m, 5H, Ar-H), 4.18 (s, 1H, ≡CH), 2.27 (m, 2H, CO-CH_2), 1.96 (m, 2H, CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.28 (m, 2H, CH_2). HRMS Calcd for $C_{22}H_{23}N_3NaO_5$ $[M+Na]^+$: 432.1535. Found 432.1519.

4.1.1.36. *N*-(3,4-Dichlorophenyl)-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido) benzamide (1g). Yield 67%. Mp 225–227 °C. ^{1}H NMR δ 10.63 (s, 1H, NH), 10.35 (s, 1H, NH), 9.84 (s, 1H, NH), 8.71 (s, 1H, OH), 8.14 (s, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 7.59–7.68 (m, 3H, Ar-H), 6.95 (d, 1H, *J* = 8.76 Hz, Ar-H), 2.25 (m, 2H, CO-CH_2), 1.96 (m, 2H, CO-CH_2), 1.55 (m, 4H, 2×CH_2), 1.28 (m, 2H, CH_2). HRMS Calcd for $C_{20}H_{21}Cl_2N_3NaO_5$ $[M+Na]^+$: 476.0756. Found 476.0737.

4.1.1.37. *N*-(4-Chloro-3-trifluoromethylphenyl)-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido)benzamide (1h). Yield 67%. Mp 197–198 °C. ^{1}H NMR δ 10.82 (s, 1H, NH), 10.30 (s, 1H, NH), 9.78 (s, 1H, NH), 8.32 (s, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 6.92–7.97 (m, 4H, Ar-H), 2.28 (m, 2H, CO-CH_2), 1.96 (m, 2H, CO-CH_2), 1.58 (m, 4H, 2×CH_2), 1.28 (m, 2H, CH_2). HRMS Calcd for $C_{21}H_{21}ClF_3N_3NaO_5$ $[M+Na]^+$: 5710.1020. Found 510.1001.

4.1.1.38. *N*-[3-Chloro-4-(3-fluorobenzyloxy)phenyl]-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido)benzamide (1i). Yield 69%. Mp 228–231 °C. ^{1}H NMR δ 10.38 (s, 1H, NH), 9.85 (s, 1H, NH), 8.04 (s, 1H, Ar-H), 7.94 (s, 1H, Ar-H), 7.17–7.64 (m, 7H, Ar-H), 6.97 (d, 1H, *J* = 8.37 Hz, Ar-H), 5.24 (s, 2H, CH_2), 2.28 (m, 2H, CO-CH_2), 1.97 (m, 2H, CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.29 (m, 2H, CH_2). HRMS Calcd for $C_{27}H_{27}ClFN_3NaO_6$ $[M+Na]^+$: 566.1470. Found 566.1445.

4.1.1.39. *N*-(3-Ethynylphenyl)-2-hydroxy-5-(8-hydroxyamino-8-oxooctanamido)benzamide (1j). Yield 72%. mp > 260 °C. ^{1}H NMR δ 10.61 (s, 1H, NH), 10.31 (s, 1H, NH), 9.79 (s, 1H, NH), 8.65 (s, 1H, OH), 8.00 (s, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 6.91–7.69 (m, 5H, Ar-H), 4.19 (s, 1H, ≡CH), 2.26 (m, 2H, CO-CH_2), 1.94 (m, 2H, CO-CH_2), 1.49–1.57 (m, 4H, 2×CH_2), 1.29 (m, 4H, 2×CH_2). HRMS Calcd for $C_{23}H_{25}N_3NaO_5$ $[M+Na]^+$: 466.1692. Found 466.1675.

4.1.1.40. *N*-(3-Chloro-4-fluorophenyl)-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido)-4-methoxybenzamide (1k). Yield 89%. Mp 193–195 °C [crystallized from ethyl acetate/THF (3:1, v/v)]. ^{1}H NMR δ 10.47 (s, 1H, NH), 10.38 (s, 1H, NH), 9.09 (s, 1H, NH), 8.20 (s, 1H, Ar-H), 7.39–8.01 (m, 3H, Ar-H), 6.60 (s, 1H, Ar-H), 3.84 (s, 3H, O-CH_3), 2.32 (m, 2H, CO-CH_2), 1.97 (m, 2H, CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.29 (m, 2H, CH_2). HRMS Calcd for $C_{21}H_{23}ClFN_3NaO_6$ $[M+Na]^+$: 490.1157. Found 490.1135.

4.1.1.41. *N*-(3-Ethynylphenyl)-2-hydroxy-5-(7-hydroxyamino-7-oxoheptanamido)-4-methoxybenzamide (1l). Yield 85%. Mp 157–159 °C. ^{1}H NMR δ 10.63 (s, 1H, NH), 10.36 (s, 1H, NH), 9.06 (s, 1H, NH), 8.70 (s, 1H, OH), 8.19 (s, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 7.70 (d, 1H, *J* = 7.95 Hz, Ar-H), 7.37 (t, 1H, Ar-H), 7.23 (d, 1H, *J* = 7.35 Hz, Ar-H), 6.58 (s, 1H, Ar-H), 4.22 (s, 1H, ≡CH), 3.83 (s, 3H, O-CH_3), 2.31 (m, 2H, CO-CH_2), 1.96 (m, 2H, CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.28 (m, 2H, CH_2). HRMS Calcd for $C_{23}H_{25}N_3NaO_6$ $[M+Na]^+$: 462.1641. Found 462.1620.

4.1.1.42. *N*-(3-Ethynylphenyl)-3-(7-hydroxyamino-7-oxoheptanamido)benzamide (1m). Yield 81%. Mp > 250 °C (crystallized from ethyl acetate). ^{1}H NMR δ 10.36 (s, 1H, NH), 10.11 (s, 1H, NH), 9.76 (s, 1H, NH), 8.69 (s, 1H, OH), 8.09 (s, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 7.86 (d, 1H, *J* = 8.10 Hz, Ar-H), 7.80 (d, 1H, *J* = 7.80 Hz, Ar-H), 7.61 (d, 1H, *J* = 7.50 Hz, Ar-H), 7.48 (t, 1H, Ar-H), 7.37 (t, 1H, *J* = 6.82 Hz,Ar-H), 7.22 (d, 1H, *J* = 7.20 Hz, Ar-H), 4.21 (s, 1H, ≡CH), 2.32 (m, 2H, CO-CH_2), 1.95 (m, 2H, CO-CH_2), 1.57 (m, 4H, 2×CH_2), 1.28 (m, 2H, CH_2). HRMS Calcd for $C_{22}H_{23}N_3NaO_4$ $[M+Na]^+$: 416.1586. Found 416.1568.

4.1.1.43. *N*-(3-Ethynylphenyl)-5-(7-hydroxyamino-7-oxoheptanamido)-2-methoxy benzamide (1n). A solution of methyl chloroformate (2 drops) in anhydrous THF (1 mL) was added dropwise to the solution of **4n** (0.2 g, 0.49 mmol) and TEA (0.1 mL) in dry THF (15 mL) cooled by an ice-water bath under N_2 atmosphere with stirring. The mixture was stirred at the same temperature for another 1 h, added the freshly prepared hydroxyamine (10 equiv) solution, then stirred for 1 h, adjusted pH to 4–5 by adding AcOH. Then the solvent was evaporated and the residue was purified by silica gel column chromatographic using $CHCl_3$/MeOH (30:1, v/v) as eluent to give 0.11 g of **1n** as a pale yellow solid. Yield 53%. Mp 154–155 °C. ^{1}H NMR δ 10.62 (s, 1H, NH), 10.28 (s, 1H, NH), 9.86 (s, 1H, NH), 7.91 (s, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.1,5–7.76 (m, 5H, Ar-H), 4.19 (s, 1H, ≡CH), 3.85 (s, 3H, O-CH_3), 2.27 (m, 2H, CO-CH_2), 1.96 (m, 2H, CO-CH_2), 1.56 (m, 4H, 2×CH_2), 1.28

(m, 2H, CH_2). HRMS Calcd for $C_{23}H_{26}N_3O_5$ $[M+H]^+$: 424.1872. Found 424.1853.

4.1.1.44. *N*-(3-Ethynylphenyl)-2-hydroxy-5-(7-hydroxyamino-7-oxoheptyloxy) benzamide (1o). Yield 80%. Mp 149–151 °C (crystallized from ethyl acetate). ^{1}H NMR δ 10.33 (s, 1H, NH), 10.21 (s, 1H, NH), 8.71 (s, 1H, OH), 7.89 (s, 1H, Ar-H), 7.67 (d, 1H, J = 8.07 Hz, Ar-H), 7.37 (t, 1H, Ar-H), 7.20 (d, 1H, J = 7.41 Hz, Ar-H), 7.13 (s, 1H, Ar-H), 7.00 (d, 1H, J = 8.73 Hz, Ar-H), 6.90 (d, 1H, J = 8.07 Hz, Ar-H), 4.20 (s, 1H, ≡CH), 4.02 (t, 2H, O-CH_2), 1.90 (t, 2H, CO-CH_2), 1.73 (m, 2H, CH_2), 1.42 (m, 4H, 2×CH_2), 1.26 (m, 2H, CH_2). HRMS Calcd for $C_{22}H_{24}N_2NaO_5$ $[M+Na]^+$: 419.1583. Found 419.1565.

4.2. Biological materials and methods

4.2.1. ELISA-based EGFR-TK assay

In vitro EGFR-TK inhibition assays were carried out as described in reference.[32] EGFR-TK was prepared from shed membrane vesicles of human A431 carcinoma cell. Briefly, 96-well plates were precoated with a synthetic substrate poly-Glu-Tyr (Sigma, 0.25 mg/mL) overnight at 37 °C. Ten microlitres of EGFR-TK extract, reaction medium and tested compound (20 μL) were added. The reaction mixtures were incubated for 30 min at room temperature while being shaken. Kinase reaction was quenched by removal of the reaction mixture, then the wells were washed with washing buffer for three times. Phosphorylated tyrosine substrate was blocked in PBS containing 3% BSA for 30 min, washed with washing buffer (PBS containing 0.1% Tween 20) for three times, detected by adding anti-phosphotyrosine antibody for 1 h. Then antibody was removed, and wells were washed with washing buffer for three times. Anti-mouse lgG (ZSGB-BIO; ZB-2305; 100 μL /well) coupled with horseradish peroxidase (HRP) was added and incubated for 1 h. HRP substrate was added (100 μL per well) and incubated for 10−20 min. The TMB reaction was quenched by addition of 50 μL of 0.1 M H_2SO_4. The optical density was measured at 450 nm by an ELISA reader. Experiment with triplicate data were performed. IC_{50} values were calculated for test compounds by using a regression analysis of the concentration/inhibition data.

4.2.2. In vitro HDACs inhibition fluorescence assay

In vitro HDACs inhibition assays were conducted as described in reference.[15] Briefly, 10 μL of HeLa nuclear extract was mixed with tested compound (50 μL). Five minutes later, fluorogenic substrate Boc-Lys (acetyl)-AMC (40 μL) was added, and the mixture was incubated at 37 °C for 30 min and then stopped by addition of 100 μL of developer containing trypsin and TSA. After incubation at 37 °C for 20 min, fluorescence intensity was measured using a microplate reader at excitation and emission wavelengths of 390 and 460 nm, respectively. The inhibition ratios were calculated from the fluorescence intensity readings of tested wells relative to those of control wells. Experiment with triplicate data were performed. The IC_{50} values were calculated using a regression analysis of the concentration/inhibition data.

4.2.3. In vitro antiproliferative assay

Cellular chemosensitivity was determined by using a modified MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazolium bromide) method assay in vitro. In brief, A431, A549 and HL-60 cells in 100 μL culture medium were seeded into 96-well microplates, respectively, and incubated at 37 °C for 24 h prior to drug exposure. Cell numbers were titrated to keep control cells growing in the exponential phase throughout the 48 h incubation period. Cells were treated with final concentrations of 100.0, 10.0, 1.0, 0.1 and 0.01 μM of tested compounds simultaneously and incubated for 48 h and then 50 μL of MTT solution (5 mg/ml in medium) was added to each well and incubated for 2.5 h. The formed blue formazan crystals were pelleted to the bottom of the well by centrifugation, separated from the supernatant, and dissolved in 150 μL of DMSO. The optical density at 570 nm was determined by an ELISA reader. Two separate experiments with triplicate data were performed to obtain mean cell viability. The IC_{50} values were calculated according to the inhibition ratios.

Acknowledgment

Financial support from National Natural Science Foundation of China (Grant No. 21072156) is gratefully acknowledged.

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