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The discovery and optimization of novel dual inhibitors of topoisomerase ii and histone deacetylase

Xuan Zhang [a], Bin Bao [a], Xiuhua Yu [a], Linjiang Tong [b], Yu Luo [d], Qingqing Huang [a], Mingbo Su [c], Li Sheng [c], Jia Li [c], Hong Zhu [e], Bo Yang [e], Xiongwen Zhang [a], Yi Chen [b,*], Wei Lu [a,*]

[a] Institute of Drug Discovery and Development, Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, PR China
[b] Division of Antitumor Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai 201203, PR China
[c] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, SIBS, Chinese Academy of Sciences, Shanghai 201203, PR China
[d] Department of Chemistry, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, PR China
[e] Institute of Pharmacology and Toxicology, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, PR China

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ABSTRACT

A novel class of podophyllotoxin derivatives have been designed and synthesized based on the synergistic antitumor effects of topoisomerase II and histone deacetylase inhibitors. Their inhibitory activities towards histone deacetylases and Topo II and their cytotoxicities in cancer cell lines were evaluated. The aromatic capping group connection, linker length and zinc-binding group were systematically varied and preliminary conclusions regarding structure–activity relationships are discussed. Among all of the synthesized hybrid compounds, compound **24d** showed the most potent HDAC inhibitory activity at a low nanomolar level and exhibited powerful antiproliferative activity towards HCT116 colon carcinoma cells at a low micromolar level. Further exploration of this series led to the discovery of potent dual inhibitor **32**, which exhibited the strongest in vitro cytotoxic activity.

1. Introduction

For decades, cytotoxic drugs have been used in a variety of cancer chemotherapy treatments. However, almost all traditional cytotoxic agents are severely toxic and have other undesirable side effects. In the last decade, the development of targeted medicines as well as monoclonal antibodies has brightened the lives of cancer patients. What was unexpected is that this targeted approach has only proven to be partially successful. The effectiveness of these agents is always hindered by poor response rates and acquired drug resistance. Two decades ago, using a genetic model for colorectal tumorigenesis, Fearon and Vogelstein reported that there is at least one additional mutation in each stage of colorectal cancer.[1] Single target agents can perform excellent during the treatment of diseases with linear pathways. However, systems biology and network analysis have shown that multi-factorial diseases, such as cancer, with complex signaling networks are robust and require the simultaneous perturbation of multiple targets.[2].

Combination therapies can create synergistic antitumor effects by directly blocking the key signaling pathway as well as attenuating multiple compensatory pathways.[3–11] As a result, the efficacy of the individual agents will be improved in combination therapy. However, different drugs always have different pharmacokinetics, including half-lives and distributions. These complex factors have hindered clinical research on combination therapies. Furthermore, these agents may introduce adverse effects associated with drug interactions.

A new strategy to overcome these limitations is the design of multi-target drugs. There are three types of multi-target agents: chimeras, fused molecules and hybrids. Though all of these agents can simultaneously act on several cancer targets and exhibit simpler pharmacokinetics, the hybrid strategy is more likely to result in suitable molecules with lower formula weights and proper liposome–water partition co-efficients.

The histone deacetylase (HDAC) class of epigenetic enzymes is a popular drug target. Together with histone acetyl transferase (HAT), HDAC determines the acetylation status of histones. Inhibition of HDAC can cause growth arrest, differentiation, and apoptosis in tumor cells by inducing histone hyperacetylation and p21 expression.[12–15] Two HDAC inhibitors (HDACi), vorinostat (SAHA) and romidepsin (FK228) were approved by the FDA for the

* Corresponding authors. Fax: +86 2162602475.
E-mail addresses: ychen@jding.dhs.org (Y. Chen), wlu@chem.ecnu.edu.cn (W. Lu).

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treatment of cutaneous T-cell lymphoma (CTCL).[16–18] A typical HDAC inhibitor consists of a capping group, a zinc-binding group (ZBG) and an appropriate linker (Fig. 1). The simple SAR and effectiveness of these molecules in oncotherapy has led many researchers to explore HDAC-involved dual inhibitors.[19–27]

Topoisomerase II (Topo II) is a classical target for cancer chemotherapy treatments.[28–31] Structural modifications of podophyllotoxin (PPT) have significantly contributed to the development of clinically valuable anticancer drugs. Etoposide (VP-16), teniposide (VM-26) and the water-soluble prodrug, etoposide phosphate (Fig. 2) were approved by the FDA.[32,33] These small molecules act on the DNA–enzyme complex and cause errors in DNA synthesis, which promotes apoptosis in cancer cells. However, chromatin DNA is tightly coiled, which may hamper the ability of drug molecules to interact with their DNA targets (Fig. 3). Kim et al., reported the synergistic antitumor effect of HDAC and Topo II inhibitors.[3] HDAC inhibitors enable histones to maintain a high degree of acetylation. The resulting looser state of chromatin DNA may increase the accessibility of DNA drug targets and consequently improve the efficiency of anticancer drugs targeting DNA, such as Topo II inhibitors.[3,34] Recently, Guerrant et al., reported their efforts on Topo-HDAC dual inhibitors and yielded satisfactory results.[35,36]

Herein, we report novel podophyllotoxin derivatives as dual inhibitors of Topo II and HDAC (Fig. 4). The aromatic capping group connection, linker length and zinc-binding group were systematically varied. Their biological evaluation included determination of their inhibitory activities towards HDAC and Topo II and their cytotoxicities in cancer cell lines.

2. Results and discussion

2.1. Chemistry

The structure of podophyllotoxin was first elucidated in the 1930s and its structure–activity relationship (SAR) has been thoroughly studied.[37–44] The biological activities of PPT derivatives are affected by their configurations at the C-4 and C-4′ positions. In general, C-4-β-substituted 4′-*O*-desmethyl-podophyllotoxins show more potent biological activities. As a typical example, etoposide was approved by the FDA in 1983 and used in the chemotherapy of various cancers. By introducing a hydroxamic acid segment at the C-4 position, we speculated that the hydrophilicity of the parent agents could be maintained. Additionally, the podophyllotoxin moiety was expected to enhance the affinity between the cap and the HDAC enzymes. Based on the above theory, we designed three series of PPT derivatives.

The general route used for the synthesis of the PPT–SAHA hybrids is depicted in Scheme 1. Acids **2a–c** were easily synthesized from anhydrides **1a–c**. PPT derivatives **3** and **4** were prepared according to Hu's method.[45] Subsequent amidation of amine **3** or **4** with the corresponding carboxylic acid afforded compounds **5a–c** and **6a–c**. Hydrogenation of these compounds using Pd/C under an atmosphere of hydrogen yielded target molecules **7a–c** and **8a–c**.

Considering the stability of the complex structure found in PPT derivatives, a convergent synthesis strategy was introduced for the synthesis of SAHA-like dual inhibitors (Scheme 2). Preparation of key intermediates **17–19** began with the esterification of bromocarboxylic acids **9a–e**. Subsequently, esters **10a–e** were treated with different nitrophenols in the presence of K_2CO_3 followed by hydrolysis with LiOH to afford acids **11–13**. Treatment of these compounds with thionyl chloride followed by amidation with *N*-Boc-*O*-TBS hydroxylamine gave compounds **14–16**.[46] Reduction of the nitro groups of these compounds using Pd/C under an atmosphere of hydrogen yielded amines **17–19**. Treatment of the synthesized amines with freshly prepared 4-β-iodo-4'-desoxypodophyllotoxin afforded **20–22**. Finally, target molecules **23–25** were obtained after removal of the protecting groups.

To investigate the effect of the zinc-binding group on biological activity, we designed and synthesized a new class of dual inhibitors using aromatic anilides as ZBGs. As shown in Scheme 3, acids **11d** and **12d** were treated with BnBr in the presence of Cs_2CO_3 to afford benzyl esters **26** and **27**. Reduction of these compounds using Fe/NH_4Cl gave corresponding amines **28** and **29**, which were treated with 4-β-iodo-4′-desoxypodophyllotoxin to afford compounds **30** and **31**. Hydrogenation of these compounds gave the corresponding acids, which were subjected to amidation followed by acidification to afford target compounds **32** and **33**.

2.2. Biological assays

2.2.1. In vitro HDAC inhibition and cell growth inhibition activities of PPT–SAHA hybrids

We first tested the HDAC inhibition activity of the PPT–SAHA hybrids towards recombinant human HDAC-1, HDAC-3 and HDAC-6 enzymes using SAHA as the positive control compound (Table 1). Overall, the first generation of designed dual inhibitors exhibited good inhibitory activity against a wide range of HDAC subtypes. A similar trend has been observed, in which the HDAC inhibition activity increased with increasing carbon chain length (**7c** > **7b** > **7a** and **8c** > **8b** > **8a**).[47] The *para*- and *meta*-substituted compounds showed similar anti-HDAC activities (**7a** vs **8a**, **7b** vs **8b** and **7c** vs **8c**). Subsequently, we evaluated their antiproliferative activities towards the HCT-116 human colon cancer cell line using the MTT assay. As shown in Table 1, most of the hybrids exhibited powerful antiproliferative activity against the HCT116 cell line. Interestingly, all *para*-substituted compounds showed stronger antiproliferative activities than the *meta*-substituted analogs despite similar anti-HDAC activities. Among the hybrids tested, compound **7c** showed powerful HDAC inhibitory activity against HDAC-1 (IC_{50} = 0.21 μM), HDAC-3 (IC_{50} = 0.23 μM) and HDAC-6 (IC_{50} = 0.09 μM) and the strongest antiproliferative activity (IC_{50} = 1.27 μM).

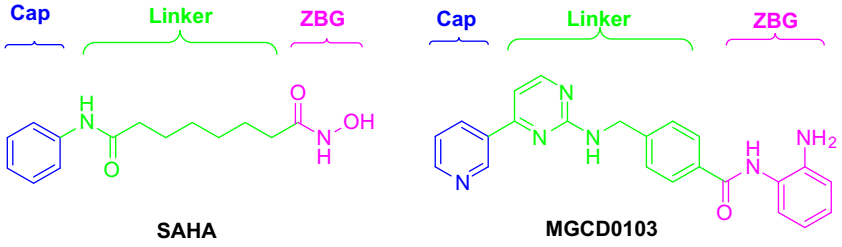


Figure 1. Representative structures of HDAC inhibitors.

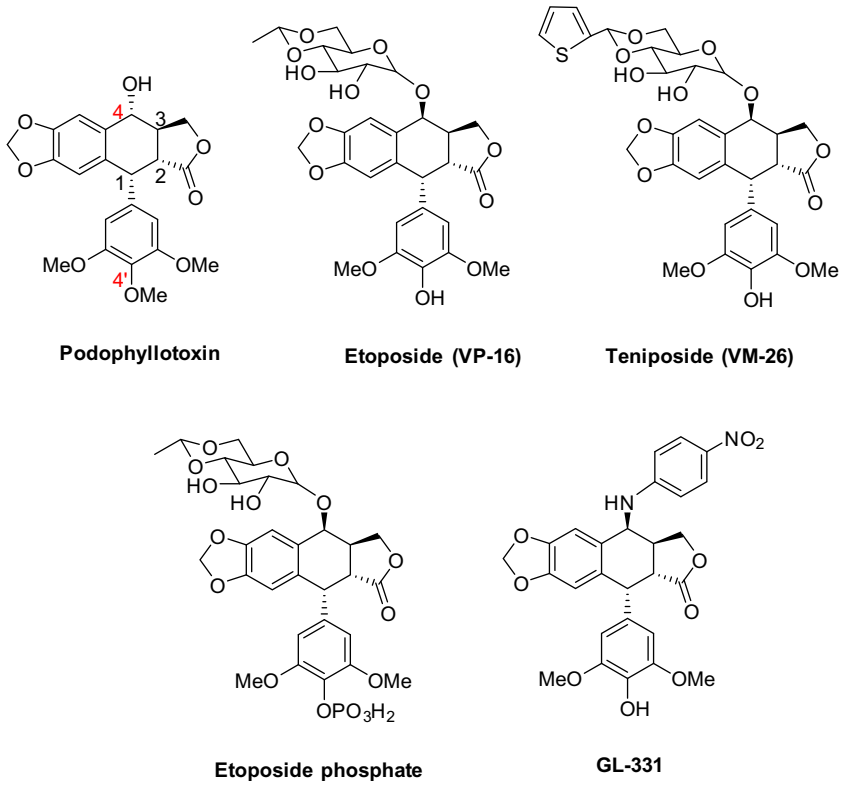


Figure 2. Representative structures of Topo II inhibitors.

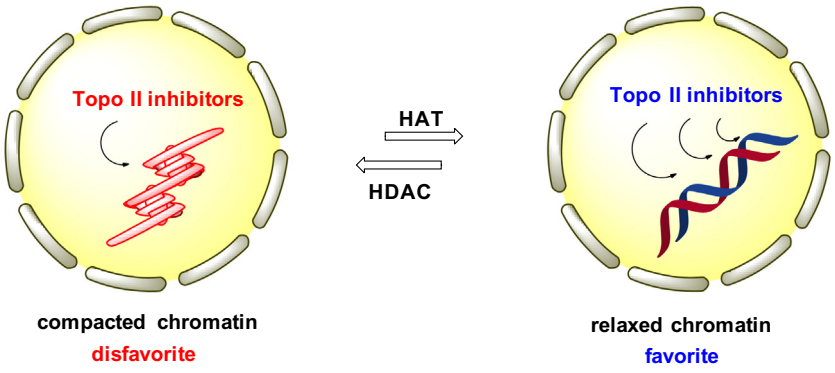


Figure 3. Synergistic antitumor effect of HDAC and Topo II inhibitors.

2.2.2. In vitro HDAC inhibition and cell growth inhibition activities of the SAHA-like series

While it is true that the amide linkage is suitable for SAHA, we hypothesized that a more flexible connection type might increase the affinities of the hybrids for the surfaces of HDAC enzymes. Thus, we designed a new series of hybrids containing an ether linkage.[48,49] The anti-HDAC activities of these compounds were evaluated (Table 2). Because of the different test batches, the HDAC inhibition activity of SAHA slightly fluctuated (Table 2 vs Table 1). However, most of the dual inhibitors exhibited good to excellent inhibitory activity toward HDAC enzymes, which suggests that a larger capping group such as PPT with a flexible connection may promote the affinity between these compounds and the HDAC enzymes. Additionally, the aromatic capping group connection appeared to contribute to the activity. The *meta*-substituted compounds showed better activities when compared to the *para*- and *ortho*-substituted analogs with similar linker lengths (**24e** vs **23e** and **25e**). The weaker observed anti-HDAC activities of the *ortho*-substituted hybrids most likely caused by different spatial orientation. In addition, a parallel trend was observed, in which the HDAC inhibition activity increased with increasing carbon chain length. The structure-activity relationship of the linker length was consistent with what has been previously reported.[48] Among this series of hybrids, compound **24d** showed the most potent anti-HDAC activity against HDAC-1 (IC_{50} = 11 nM), HDAC-3 (IC_{50} = 9.6 nM) and HDAC-6 (IC_{50} = 5.6 nM) and was 10- to 20 fold more potent than the reference compound SAHA.

Evaluation of the antiproliferative activities of these compounds was performed using the MTT assay against the HCT116 cancer cell line. As shown in Table 2, the majority of the designed molecules exhibited powerful antiproliferative activity against HCT116 cells at a micromolar level. Interestingly, all *para*-substituted compounds displayed potent antiproliferative activity at the same level despite their different anti-HDAC activities (**23a–e**). In contrast,

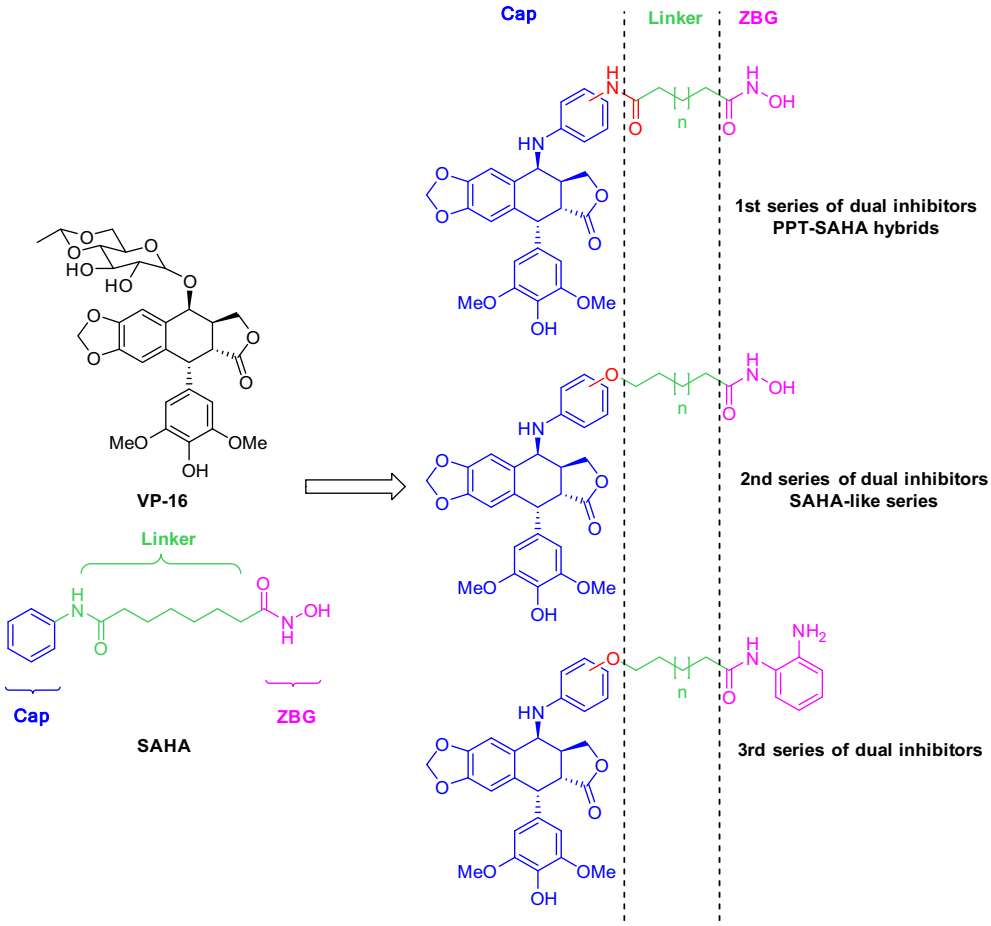
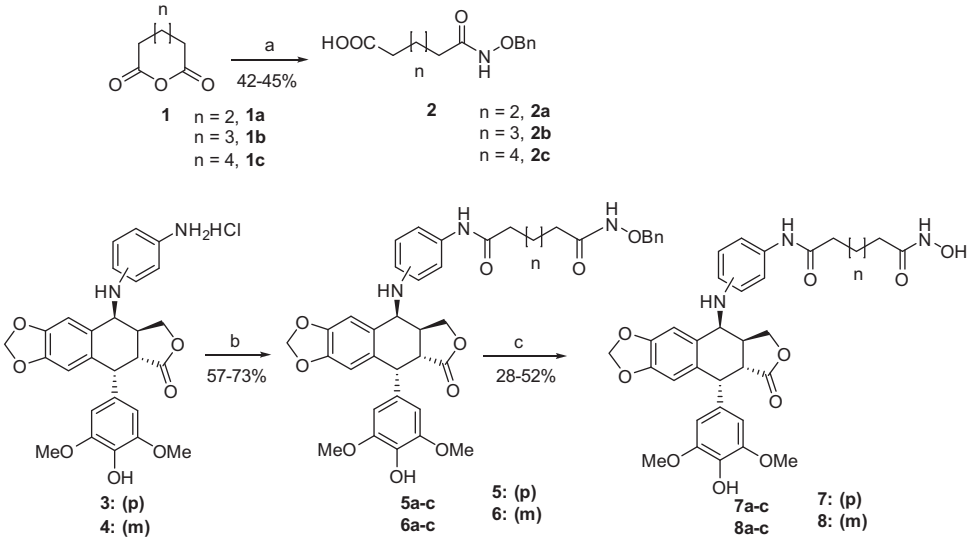


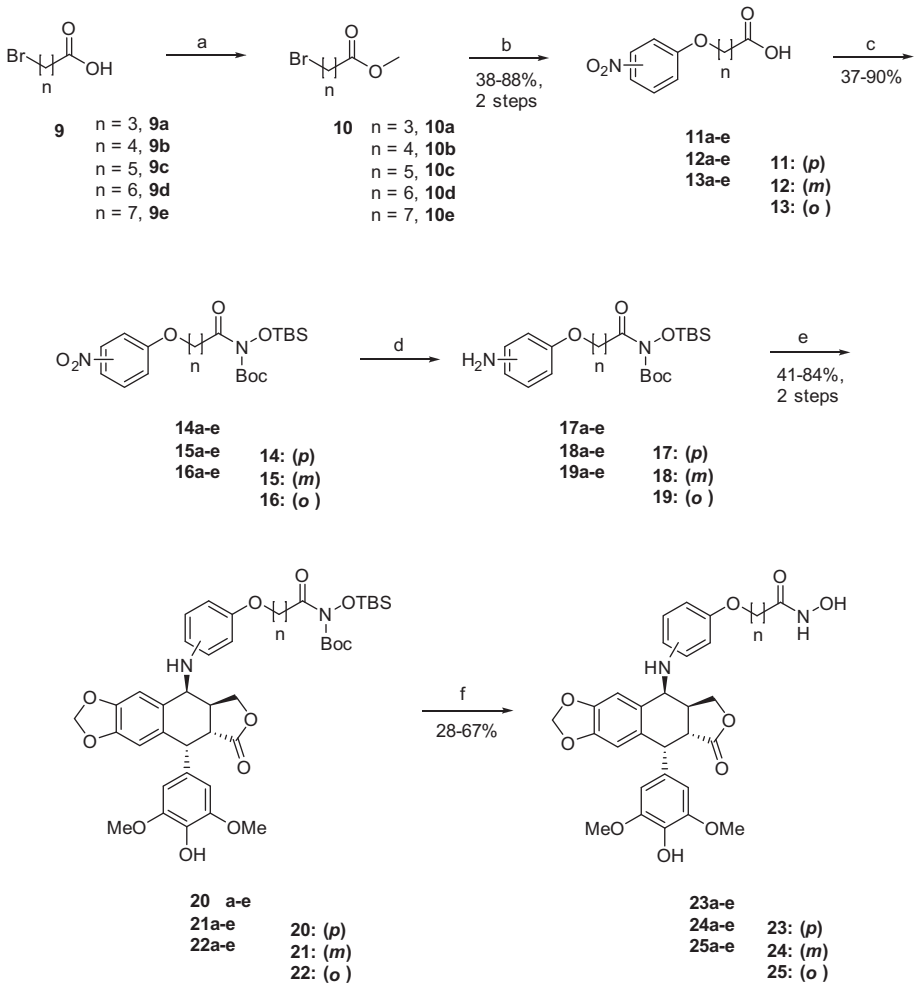
Figure 4. Design of dual inhibitors of Topo II and HDAC.



Reagents and conditions: a) $BnONH_2$, CH_3CN; b) HATU, DIPEA, DCM, c) Pd/C, H_2, MeOH.

Scheme 1. Synthesis of PPT–SAHA hybrids.

ortho-substituted conjugates exhibited lower cytotoxicity due to steric effects (**25b** vs **23b** and **24b**). The most powerful HDAC inhibitor in this series, **24d**, also showed the strongest antiproliferative activity (IC_{50} = 3.33 μM).



Reagents and conditions: a) $SOCl_2$, MeOH; b) nitrophenol, K_2CO_3, DMF, 80 °C; LiOH, MeOH, H_2O; c) $SOCl_2$, toluene, 60 °C; *N*-Boc-*O*-TBS hydroxylamine, TEA, CH_3CN, 0 °C; d) Pd/C, H_2, MeOH; e) 4-β-iodo-4'-desoxypodophyllotoxin, triethylamine, CH_3CN; f) TFA, TBAF, DCM.

Scheme 2. Synthesis of the SAHA-like Topo II-HDAC dual inhibitor series.

2.2.3. In vitro HDAC inhibition and cell growth inhibition activities of the aromatic anilide series

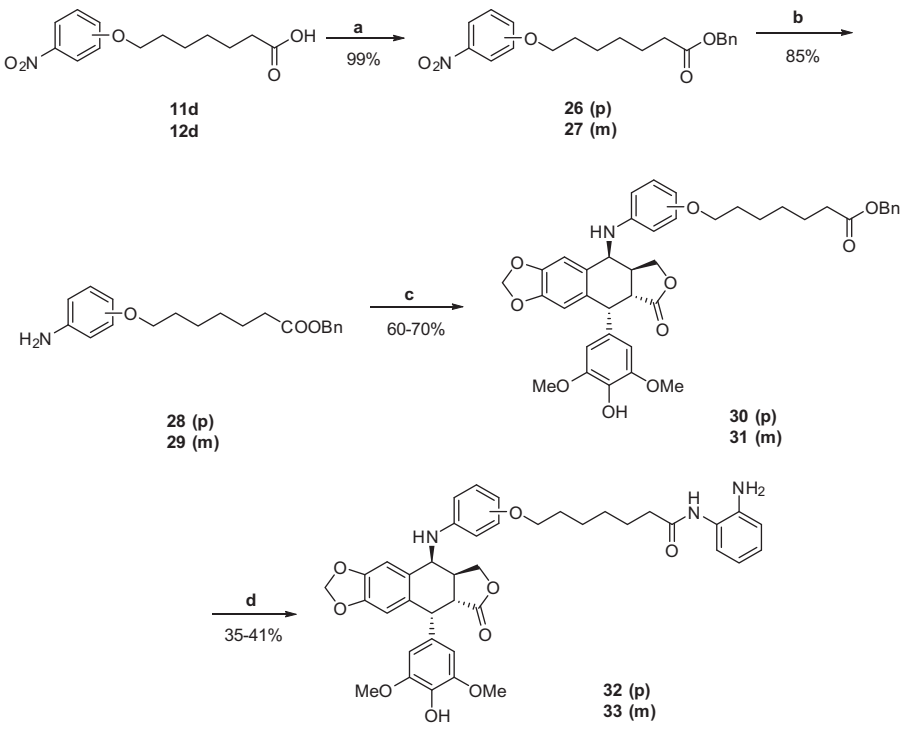
We speculated that the introduction of a PPT moiety as a capping group with an ether connection might facilitate the interactions between the cap and the amino acid side chains at the entrance of the HDAC active site. Thus, we investigated a new series of dual inhibitors characterized by aromatic anilides as ZBGs. When compared with compounds containing hydroxamic acid moieties, compounds containing anilides as ZBGs always displayed weaker in vitro HDAC inhibitory activity. However, we hypothesized that introduction of the arylamine moiety might affect the hydrophile–lipophile balance (HLB) of the dual inhibitors. We also hypothesized that salification of the amine might increase the solubility of the hybrids. Compound **7c** showed powerful antiproliferative activity towards HCT116 cells, but exhibited weaker cytotoxicity in the A549 cell line (Table 3). In contrast, the newly designed hybrids showed more potent antiproliferative activities towards these cell lines. The stronger HDAC inhibitor, **32**, appeared to have better cytotoxicity than its *meta*-substituted analog. Compound **32** showed more potent antiproliferative activity against HCT116 and A549 cells than the reference compounds MGCD0103 and etoposide.

2.2.4. In vitro topoisomerase II relaxation assay

Using pBR322 DNA, we performed a cell-free DNA relaxation assay to determine the Topo II inhibition activities of the synthesized compounds (see Fig. S1 of the Supporting information). Treatment of pBR322 DNA with Topo II resulted in extensive DNA decatenation. A reaction containing only pBR322 DNA was used as the catenated control and reaction containing pBR322 DNA and Topo II was used as the decatenated control. As expected, addition of 100 μM etoposide to the decatenation experiment resulted in a severe impairment of DNA decatenation. In contrast, the addition of 100 μM SAHA or MGCD0103 had no effect on Topo II inhibitory activity. However, all selected hybrids showed obvious anti-Topo II activity. The selected compounds are genuine HDAC-Topo II dual inhibitors.

3. Conclusion

A novel class of PPT derivatives was designed based on the reported synergistic effects of Topo II and HDAC inhibitors. The aromatic capping group connection, linker length and zinc-binding group type were systematically varied. PPT–SAHA hybrids exhibited potent HDAC inhibitory and antiproliferative activities.



Reagents and conditions: a) BnBr, Cs_2CO_3, DMF; b) Fe, NH_4Cl, EtOH, H_2O, room temperature; c) 4-β-iodo-4'-desoxypodophyllotoxin, triethylamine, CH_3CN; d) $Pd(OH)_2$, H_2, MeOH, EtOAc; benzene-1,2-diamine, HATU, DIPEA, DCM.

Scheme 3. Synthesis of the third generation of Topo II–HDAC dual inhibitors.

Table 1
In vitro HDAC inhibition and cell viability assay of PPT–SAHA hybrids

Compound	IC_{50} (μM) ± SD			
	HDAC1	HDAC3	HDAC6	HCT116[a]
SAHA	0.09 ± 0.01	0.11 ± 0.01	0.07 ± 0.00	0.80
7a	2.83 ± 0.57	1.69 ± 0.53	1.06 ± 0.10	3.76
7b	0.59 ± 0.00	0.52 ± 0.04	0.13 ± 0.01	2.94
7c	0.21 ± 0.02	0.23 ± 0.02	0.09 ± 0.02	1.27
8a	1.43 ± 0.46	2.11 ± 0.01	0.52 ± 0.08	14.14
8b	0.38 ± 0.04	0.59 ± 0.06	0.16 ± 0.03	19.62
8c	0.10 ± 0.00	0.13 ± 0.00	0.04 ± 0.00	8.57

[a] Each value is the result of three separate experiments.

Table 2
In vitro HDAC inhibition cell viability assay of the SAHA-like series

Compound	IC_{50} (nM) ± SD			IC_{50} (μM)
	HDAC-1	HDAC-3	HDAC-6	HCT116 [a]
SAHA	221 ± 23	131 ± 15	129 ± 26	0.81
23a	243 ± 44	193 ± 35	214 ± 34	5.37
24a	385 ± ± 135	767 ± 194	405 ± 68	29.45
25a	578 ± 208	1444 ± 424	357 ± 49	28.88
23b	125 ± 31	242 ± 35	88 ± 8	5.26
24b	93 ± 31	111 ± 21	42 ± 3	6.29
25b	369 ± 119	899 ± 208	84 ± 10	16.46
23c	207 ± 73	69 ± 16	26 ± 2	4.92
24c	45 ± 8	81 ± 6	15 ± 2	5.72
25c	145 ± 35	188 ± 41	20 ± 2	8.10
23d	29 ± 3	49 ± 5	16.0 ± 2	5.03
24d	11 ± 3	9.6 ± 3.2	5.6 ± 0.0	3.33
25d	72 ± 15	155 ± 23	27 ± 2	11.34
23e	87 ± 20	53 ± 8	53 ± 8	3.72
24e	39 ± 8	69 ± 9	69 ± 9	5.19
25e	175 ± 32	217 ± 40	217 ± 40	11.35

[b] IC_{50} > 20 μg/mL.
[a] Each value is the result of three separate experiments.

Further optimization of this series resulted in the PPT–SAHA-like hybrid series, which had remarkably improved anti-HDAC activity. Introduction of the PPT moiety as a capping group might facilitate interactions between the cap and the surfaces of HDAC enzymes. The optimum linker length might promote binding of the hydroxamic acid moiety with the zinc ion at the bottom of the active site. In this series of hybrids, compound **24d** displayed the best HDAC inhibitory activity against HDAC-1 (IC_{50} = 11 nM), HDAC-3 (IC_{50} = 9.6 nM) and HDAC-6 (IC_{50} = 5.6 nM) and was 10- to 20 fold more potent than the reference compound SAHA. Compound **24d** also displayed powerful antiproliferative activity towards the HCT116 cell line at micromolar concentrations (IC_{50} = 3.33 μM). In addition, the zinc-binding group was found to play a significant role in the cytotoxicity. The introduction of anilides as ZBGs resulted in increased antiproliferative activity against HCT116 and A549 cells. Compound **32** showed the most powerful cytotoxicity against these two cancer cell lines. The encouraging results presented here suggest the potential for further optimization of this series of compounds.

4. Experimentals

4.1. Chemistry

Melting points were taken on a Fisher–Johns melting point apparatus, uncorrected and reported in degrees Centigrade. ^1H

Table 3
In vitro HDAC inhibition and cell viability assay

Compound	IC_{50} (μM) ± SD				
	HDAC1	HDAC2	HDAC3	HCT116[a]	A549[a]
MGCD0103	0.95 ± 0.16	0.28 ± 0.02	1.67 ± 0.01	1.57	1.65
Etoposide	—[b]	—	—	0.87	1.71
7c	0.21 ± 0.02	0.14 ± 0.01	0.23 ± 0.02	1.27	18.64
32	14.08 ± 5.41	10.39 ± 1.51	11.62 ± 0.67	0.52	1.16
33	—	53.52 ± 6.56	—	0.92	1.11

[a] Each value is the result of three separate experiments.
[b] IC_{50} > 20 μg/mL.

NMR spectra and ^{13}C NMR were recorded in $CDCl_3$, CD_3OD and acetone-d_6 on a Bruker DRX-500 (500 MHz) or a Bruker DRX-400 (400 MHz) using TMS as internal standard. Chemical shifts were reported as a δ (ppm) and spin–spin coupling constants as J (Hz) values. The mass spectra (MS) were recorded on a Finnigan MAT-95 mass spectrometer. The purity of all tested compounds was established by HPLC to be >95.0%. HPLC analyses were performed on Agilent 1200 series instrument using an Agilent Eclipse XDB-C18 (250 × 4.6 mm).

4.1.1. Representative procedure for 2a–c

Anhydride **2a–c** (1.0 equiv) and $BnONH_2$ (1.0 equiv) were stirred in CH_3CN for 12 h. The mixture was extracted with EtOAc, washed with brine and concentrated to dryness. The crude product was crystallized in a mixture of EtOAc and petroleum ether.

4.1.1.1. 6-((Benzyloxy)amino)-6-oxohexanoic acid (2a). Starting from **1a**, 52% of **2a** was obtained as a white solid according to above-mentioned general procedure. mp 76–78 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 8.20 (br s, 1H), 7.46–7.31 (m, 5H), 5.00–4.73 (m, 2H), 2.43–1.97 (m, 4H), 1.75–1.56 (m, 4H); HRMS (ESI): m/z calcd for $C_{13}H_{17}NO_4Na^+$ ($M+Na^+$): 274.1055, found: 274.1042.

4.1.1.2. 7-((Benzyloxy)amino)-7-oxoheptanoic acid (2b). Starting from **1b**, 45% of **2b** was obtained as a white solid according to above-mentioned general procedure. mp 61–63 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (br s, 1H), 7.49–7.30 (m, 5H), 5.08–4.69 (m, 2H), 2.44–1.94 (m, 4H), 1.75–1.52 (m, 4H), 1.43–1.24 (m, 2H); HRMS (ESI): m/z calcd for $C_{14}H_{19}NO_4Na^+$ ($M+Na^+$): 288.1212, found: 288.1204.

4.1.1.3. 8-((Benzyloxy)amino)-8-oxooctanoic acid (2c). Starting from **1c**, 42% of **2c** was obtained as a white solid according to above-mentioned general procedure. mp 73–76 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (br s, 1H), 7.47–7.31 (m, 5H), 5.27–4.64 (m, 2H), 2.47–1.95 (m, 4H), 1.70–1.54 (m, 4H), 1.42–1.21 (m, 4H); HRMS (ESI): m/z calcd for $C_{15}H_{21}NO_4Na^+$ ($M+Na^+$): 302.1368, found: 302.1354.

4.1.2. Representative procedure for 5a–c and 6a–c

A mixture of amine (1.0 equiv), acid (1.0 equiv), HATU (1.0 equiv) and DIPEA (4.0 equiv) were stirred in DCM for 12 h and then extracted with DCM. Concentration in vacuo gave crude product, which was further purified by column chromatography.

4.1.2.1. Compound 5a. Starting from **2a** and **3**, 65% of **5a** was obtained as a yellow oil according to above-mentioned general procedure. ^1H NMR (500 MHz, $CDCl_3$) δ 8.73 (br s, 1H), 7.66 (br s, 1H), 7.41–7.37 (m, 7H), 6.75 (s, 1H), 6.53–6.49 (m, 3H), 6.34 (s, 2H), 5.97 (d, J = 9.2 Hz, 2H), 5.49 (s, 1H), 4.92–4.89 (m, 2H), 4.65–4.58 (m, 2H), 4.35 (t, J = 8.3 Hz, 1H), 3.97(t, J = 9.5 Hz, 1H), 3.85–3.83 (m, 1H), 3.80 (s, 6H), 3.15 (dd, J = 19.0, 5.0 Hz, 1H), 3.02–2.98 (m, 1H), 2.36–2.32 (m, 2H), 2.13–2.09 (m, 2H), 1.72–1.68 (m, 4H); HRMS (ESI): m/z calcd for $C_{40}H_{41}N_3O_{10}Na^+$ ($M+Na^+$): 746.2684, found: 746.2696.

4.1.2.2. Compound 5b. Starting from **2b** and **3**, 58% of **5b** was obtained as a yellow oil according to above-mentioned general procedure. ^1H NMR (500 MHz, $CDCl_3$) δ 8.66 (br s, 1H), 7.57 (br s, 1H), 7.38–7.36 (m, 7H), 6.74 (s, 1H), 6.52 (s, 1H), 6.48–6.46 (m, 2H), 6.34 (s, 2H), 5.96 (d, J = 9.8 Hz, 2H), 5.50 (s, 1H), 4.94–4.87 (m, 2H), 4.61–4.57 (m, 2H), 4.34–4.31 (m, 1H), 3.96–3.84 (m, 2H), 3.80 (s, 6H), 3.17–3.13 (m, 1H), 2.99–2.95 (m, 1H), 2.35–2.30 (m, 2H), 2.10–2.04 (m, 2H), 1.70–1.65(m, 4H), 1.39–1.34 (m, 2H); HRMS (ESI): m/z calcd for $C_{41}H_{43}N_3O_{10}Na^+$ ($M+Na^+$): 760.2841, found: 760.2835.

4.1.2.3. Compound 5c. Starting from **2c** and **3**, 61% of **2c** was obtained as a yellow oil according to above-mentioned general procedure. ^1H NMR (300 MHz, $CDCl_3$) δ 8.65 (br s, 1H), 7.58 (br s, 1H), 7.40–7.32 (m, 7H), 6.74 (s, 1H), 6.52–6.45 (m, 3H), 6.33 (s, 2H), 5.96 (d, J = 9.6 Hz, 2H), 5.45 (s, 1H), 4.92–4.87 (m, 2H), 4.62–4.57 (m, 2H), 4.33 (t, J = 8.1 Hz, 1H), 3.95 (t, J = 9.3 Hz, 1H), 3.78 (s, 6H), 3.15 (dd, J = 14.1, 4.8 Hz, 1H), 3.02–2.96 (m, 1H), 2.32–2.27 (m, 2H), 2.08–2.00 (m, 2H), 1.58–1.59 (m, 4H), 1.34–1.24 (m, 4H); HRMS (ESI): m/z calcd for $C_{42}H_{45}N_3O_{10}Na^+$ ($M+Na^+$): 774.2997, found: 774.3005.

4.1.2.4. Compound 6a. Starting from **2a** and **4**, 70% of **6a** was obtained as a brown oil according to above-mentioned general procedure. ^1H NMR (500 MHz, $CDCl_3$) δ 8.64 (br s, 1H), 7.92(br s, 1H), 7.35–7.29 (m, 6H), 7.09 (t, J = 8.1 Hz, 1H), 6.74 (s, 1H), 6.65–6.63 (m, 1H), 6.50 (s, 1H), 6.32 (s, 2H), 6.29–6.27 (m, 1H), 5.95 (d, J = 10.5 Hz, 2H), 5.49 (s, 1H), 4.87–4.80 (m, 2H), 4.69–4.68 (m, 1H), 4.57 (d, J = 4.9 Hz, 1H), 4.44 (t, J = 8.3 Hz, 1H), 3.98–3.94 (m, 2H), 3.78(s, 6H), 3.14–3.10(m, 1H), 3.02–2.95(m, 1H), 2.35–2.30 (m, 2H), 2.10–2.04 (m, 2H), 1.70–1.65(m, 4H); HRMS (ESI): m/z calcd for $C_{40}H_{41}N_3O_{10}Na^+$ ($M+Na^+$): 746.2684, found: 746.2681.

4.1.2.5. Compound 6b. Starting from **2b** and **4**, 57% of **6b** was obtained as a yellow oil according to above-mentioned general procedure. ^1H NMR (500 MHz, $CDCl_3$) δ 8.67 (br s, 1H), 7.86(br s, 1H), 7.39–7.32 (m, 6H), 7.12 (t, J = 8.0 Hz, 1H), 6.78 (s, 1H), 6.66–6.65 (m, 1H), 6.55 (s, 1H), 6.38 (s, 2H), 6.33 (d, J = 7.6 Hz, 1H), 5.99 (d, J = 11.1 Hz, 2H), 5.56 (m, 1H), 4.93–4.87 (m, 2H), 4.75–4.70 (m, 1H), 4.62 (d, J = 4.9 Hz, 1H), 4.50–4.46 (m, 1H), 4.02–3.97 (m, 2H), 3.83 (s, 6H), 3.18–3.03 (m, 2H), 2.40–2.32 (m, 2H), 2.13–2.07 (m, 2H), 1.71–1.65 (m, 4H), 1.41–1.37 (m, 2H); HRMS (ESI): m/z calcd for $C_{41}H_{43}N_3O_{10}Na^+$ ($M+Na^+$): 760.2841, found: 760.2820.

4.1.2.6. Compound 6c. Starting from **2c** and **4**, 73% of **6c** was obtained as a yellow oil according to above-mentioned general procedure. ^1H NMR (500 MHz, $CDCl_3$) δ 8.56 (br s, 1H), 7.78 (br s, 1H), 7.38–7.32 (m, 6H), 7.08 (t, J = 7.0 Hz, 1H), 6.74 (s, 1H), 6.58–6.56 (m, 1H), 6.50 (s, 1H), 6.32 (s, 2H), 6.28–6.27 (d, J = 6.6 Hz, 1H), 5.96 (d, J = 10.6 Hz, 2H), 5.50 (s, 1H), 4.92–4.85 (m, 2H), 4.71–4.67 (m, 1H), 4.57 (d, J = 4.9 Hz, 1H), 4.43 (t, J = 7.9 Hz, 1H), 3.97–3.93 (m, 2H), 3.78 (s, 6H), 3.13–3.09 (m, 1H), 3.05–3.00 (m, 1H), 2.31–2.27 (m, 2H), 2.04–2.00 (m, 2H), 1.68–1.64 (m, 2H), 1.62–1.58 (m, 2H), 1.33–1.29 (m, 4H); HRMS (ESI): m/z calcd for $C_{42}H_{45}N_3O_{10}Na^+$ ($M+Na^+$): 774.2997, found: 774.3009.

4.1.3. Representative procedure for 7a–c and 8a–c

A mixture of compound **5a–c**/**6a–c** and 10% Pd/C (10% w/w) in methanol was hydrogenated at room temperature for 10 h. After filtration, the organic layer was evaporated to dryness to give the crude product, which was further purified by column chromatography.

4.1.3.1. Compound 7a. Starting from **5a**, 43% of **7a** was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, CD_3OD) δ 7.28 (d, J = 8.6 Hz, 2H), 6.74 (s, 1H), 6.62 (d, J = 8.7 Hz, 2H), 6.47 (s, 1H), 6.36 (s, 2H), 5.91 (s, 2H), 5.48 (s, 1H), 4.79–4.76 (m, 1H), 4.57–4.53 (m, 1H), 4.38 (t, J = 7.8 Hz, 1H), 3.93–3.89 (m, 1H), 3.73 (s, 6H), 3.30–3.26 (m, 1H), 3.08–3.04 (m, 1H), 2.36–2.32 (m, 2H), 2.16–2.12 (m, 2H), 1.71–1.67 (m, 4H); HPLC: room temperature; eluent, CH_3OH/H_2O (80:20); flow rate, 1.0 mL/min; t_R = 3.60 min, UV_{254} = 98.3%; HRMS (ESI): m/z calcd for $C_{33}H_{35}N_3O_{10}Na^+$ (M+Na^+): 656.2215, found: 656.2227.

4.1.3.2. Compound 7b. Starting from **5b**, 37% of **7b** was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, CD_3OD) δ 7.29 (d, J = 8.8 Hz, 2H), 6.74 (s, 1H), 6.63 (d, J = 8.8 Hz, 2H), 6.47 (s, 1H), 6.36 (s, 2H), 5.91 (s, 2H), 5.48 (s, 1H), 4.78–4.76 (m, 1H), 4.56–4.53 (m, 1H), 4.42–4.37 (m, 1H), 3.93–3.88 (m, 1H), 3.73 (s, 6H), 3.30–3.26 (m, 1H), 3.05–3.00 (m, 1H), 2.33 (t, J = 7.4 Hz, 2H), 2.11 (t, J = 7.4 Hz, 2H), 1.73–1.64 (m, 4H), 1.42–1.35 (m, 2H); HPLC: room temperature; eluent, CH_3OH/H_2O (80:20); flow rate, 1.0 mL/min; t_R = 3.85 min, UV_{254} = 99.6%; HRMS (ESI): m/z calcd for $C_{34}H_{37}N_3O_{10}Na^+$ (M+Na^+): 670.2371, found: 670.2382.

4.1.3.3. Compound 7c. Starting from **5c**, 52% of **7c** was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (300 MHz, CD_3OD) δ 7.29 (d, J = 8.4 Hz, 2H), 6.74 (s, 1H), 6.62 (d, J = 8.4 Hz, 2H), 6.47(s, 1H), 6.36 (s, 2H), 5.91(s, 2H), 4.78–4.76 (m, 1H), 4.57–4.53 (m, 1H), 4.40–4.35 (m, 1H), 3.94–3.88 (m, 1H), 3.73 (s, 6H), 3.30–3.27 (m, 1H), 3.08–3.04 (m, 1H), 2.35–2.30 (m, 2H), 2.12–2.07 (m, 2H), 1.75–1.55(m, 4H), 1.48–1.38 (m, 4H); HPLC: room temperature; eluent, CH_3OH/H_2O (80:20); flow rate, 1.0 mL/min; t_R = 3.91 min, UV_{254} = 99.8%; HRMS (ESI): m/z calcd for $C_{35}H_{39}N_3O_{10}Na^+$ (M+Na^+): 684.2528, found: 684.2515.

4.1.3.4. Compound 8a. Starting from **6a**, 35% of **8a** was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, CD_3OD) δ 7.12–7.07 (m, 2H), 6.79 (s, 1H), 6.73–6.67 (m, 1H), 6.51–6.45 (m, 2H), 6.39 (s, 2H), 5.95 (m, 2H), 4.82–4.80 (m, 1H), 4.61–4.57 (m, 1H), 4.47–4.44 (m, 1H), 3.97–3.92 (m, 1H), 3.77 (s, 6H), 3.30–3.27 (m, 1H), 3.08–3.05 (m, 1H), 2.42–2.38 (m, 2H), 2.19–2.15 (m, 2H), 1.77–1.70 (m, 4H); HPLC: room temperature; eluent, CH_3OH/H_2O (80:20); flow rate, 1.0 mL/min; t_R = 4.43 min, UV_{254} = 98.9%; HRMS (ESI): m/z calcd for $C_{33}H_{35}N_3O_{10}Na^+$ (M+Na^+): 656.2215, found: 656.2223.

4.1.3.5. Compound 8b. Starting from **6b**, 43% of **8b** was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, CD_3OD) δ 7.08–7.04 (m, 2H), 6.75 (s, 1H), 6.68 (d, J = 7.5 Hz, 1H), 6.47 (s, 1H), 6.43 (d, J = 7.9 Hz, 1H), 6.36 (s, 2H), 5.91(s, 2H), 4.78–4.76 (m, 1H), 4.57–4.55 (m, 1H), 4.43–4.41 (m, 1H), 3.91–3.87 (m, 1H), 3.73 (s, 6H), 3.30–3.27 (m, 1H), 3.08–3.00 (m, 1H), 2.36–2.32 (m, 2H), 2.12–2.08 (m, 2H), 1.71–1.62 (m, 4H), 1.41–1.36 (m, 2H); HPLC: room temperature; eluent, CH_3OH/H_2O (80:20); flow rate, 1.0 mL/min; t_R = 4.72 min, UV_{254} = 99.5%; HRMS (ESI): m/z calcd for $C_{34}H_{37}N_3O_{10}Na^+$ (M+Na^+): 670.2371, found: 670.2386.

4.1.3.6. Compound 8c. Starting from **6c**, 28% of **8c** was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, CD_3OD) δ 7.13–7.07 (m, 2H), 6.78–6.71 (m, 2H), 6.50–6.44 (m, 2H), 6.39 (s, 2H), 5.94 (s, 2H), 4.81–4.79 (m, 1H), 4.59–4.56 (m, 1H), 4.46–4.42 (m, 1H), 3.96–3.92 (m, 1H), 3.76 (s, 6H), 3.30–3.27 (m, 1H), 3.10–3.07 (m, 1H), 2.39–2.35 (m, 2H), 2.14–2.10 (m, 2H), 1.75–1.62 (m, 4H), 1.43–1.38 (m, 4H); HPLC: room temperature; eluent, CH_3OH/H_2O (80:20); flow rate, 1.0 mL/min; t_R = 5.43 min, UV_{254} = 99.7%; HRMS (ESI): m/z calcd for $C_{35}H_{39}N_3O_{10}Na^+$ (M+Na^+): 684.2528, found: 684.2543.

4.1.4. Representative procedure for 10a–e

$SOCl_2$ (15.0 mmol) was added dropwise into a solution of bromo carboxylic acid (10.0 mmol) in methanol (30 mL). The mixture was stirred at room temperature for 2 h. The solution was concentrated to give the crude product, which was used directly for the next step.

4.1.5. Representative procedure for 11–13

A mixture of bromo ester (10.0 mmol), nitrophenol (9.1 mmol) and K_2CO_3 (11.0 mmol) in DMF (30 mL) was stirred at 90 °C for 16 h. The reaction mixture was extracted with EtOAc, and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Concentration in vacuo gave crude product, which was crystallized from DCM and diethyl ether. After filtration, the filter cake was dissolved in a mixed solvent of THF and water followed by addition of NaOH (600 mg, 15.0 mmol). The mixture was refluxed for 2 h and acidified with 1 N aqueous HCl till pH 2.0. Then it was extracted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4 and evaporated to dryness.

4.1.5.1. 4-(4-Nitrophenoxy)butanoic acid (11a). Starting from **10a** and 4–nitrophenol, 60% of **11a** was obtained as a white solid according to above-mentioned general procedure. mp 122–124 °C.

4.1.5.2. 5–(4-Nitrophenoxy)pentanoic acid (11b). Starting from **10b** and 4-nitrophenol, 88% of **11b** was obtained as a white solid according to above-mentioned general procedure. mp 100–101 °C.

4.1.5.3. 6-(4-Nitrophenoxy)hexanoic acid (11c). Starting from **10c** and 4-nitrophenol, 47% of **11c** was obtained as a white solid according to above-mentioned general procedure. mp 103–104 °C.

4.1.5.4. 7-(4-Nitrophenoxy)heptanoic acid (11d). Starting from **10d** and 4-nitrophenol, 50% of **11d** was obtained as a yellow solid according to above-mentioned general procedure. mp 84–86 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.1 Hz, 2H), 6.94 (d, J = 8.2 Hz, 2H), 4.05 (t, J = 6.2 Hz, 2H), 2.38 (t, J = 7.2 Hz, 2H), 1.88–1.77 (m, 2H), 1.74–1.64 (m, 2H), 1.56–1.36 (m, 4H); MS (ESI) m/z = 266.0 $(M-H)^-$.

4.1.5.5. 8-(4-Nitrophenoxy)octanoic acid (11e). Starting from **10e** and 4-nitrophenol, 50% of **11e** was obtained as a white solid according to above-mentioned general procedure. mp 94–95 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.3 Hz, 2H), 6.94 (d, J = 8.3 Hz, 2H), 4.04 (t, J = 6.3 Hz, 2H), 2.37 (t, J = 7.3 Hz, 2H), 1.91–1.76 (m, 2H), 1.73–1.60 (m, 2H), 1.56–1.30 (m, 6H); MS (ESI) m/z = 280.1 $(M-H)^-$.

4.1.5.6. 4-(3-Nitrophenoxy)butanoic acid (12a). Starting from **10a** and 3-nitrophenol, 65% of **12a** was obtained as a yellow solid according to above-mentioned general procedure. mp 114–115 °C.

4.1.5.7. 5-(3-Nitrophenoxy)pentanoic acid (12b). Starting from **10b** and 3-nitrophenol, 67% of **12b** was obtained as a yellow solid according to above-mentioned general procedure. mp 77–78 °C.

4.1.5.8. 6-(3-Nitrophenoxy)hexanoic acid (12c). Starting from **10c** and 3-nitrophenol, 79% of **12c** was obtained as a yellow solid according to above-mentioned general procedure. mp 75–78 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.0 Hz, 1H), 7.71 (s, 1H), 7.41 (t, J = 8.1 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 4.04 (t, J = 6.1 Hz, 2H), 2.42 (t, J = 7.2 Hz, 2H), 1.95–1.80 (m, 2H), 1.81–1.67 (m, 2H), 1.64–1.47 (m, 2H); MS (ESI) m/z = 252.0 $(M-H)^-$.

4.1.5.9. 7-(3-Nitrophenoxy)heptanoic acid (12d). Starting from **10d** and 3-nitrophenol, 60% of **12d** was obtained as a yellow solid according to above-mentioned general procedure. mp 65–68 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.1 Hz, 1H), 7.71 (s, 1H), 7.41 (t, J = 8.2 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 4.03 (t, J = 6.2 Hz, 2H), 2.39 (t, J = 7.3 Hz, 2H), 1.91–1.78 (m, 2H), 1.77–1.62 (m, 2H), 1.59–1.35 (m, 4H); MS (ESI) m/z = 266.0 $(M-H)^-$.

4.1.5.10. 8-(3-Nitrophenoxy)octanoic acid (12e). Starting from **10e** and 3-nitrophenol, 64% of **12e** was obtained as a white solid according to above-mentioned general procedure. mp 86–88 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.1 Hz, 1H), 7.71 (s, 1H), 7.41 (t, J = 8.1 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 4.03 (t, J = 6.2 Hz, 2H), 2.37 (t, J = 7.3 Hz, 2H), 1.88–1.77 (m, 2H), 1.73–1.61 (m, 2H), 1.56–1.35 (m, 6H); MS (ESI) m/z = 280.0 $(M-H)^-$.

4.1.5.11. 4-(2-Nitrophenoxy)butanoic acid (13a). Starting from **10a** and 2-nitrophenol, 58% of **13a** was obtained as a white solid according to above-mentioned general procedure. mp 103–104 °C.

4.1.5.12. 5-(2-Nitrophenoxy)pentanoic acid (13b). Starting from **10b** and 2-nitrophenol, 69% of **13b** was obtained as a white solid according to above-mentioned general procedure. mp 80–81 °C.

4.1.5.13. 6-(2-Nitrophenoxy)hexanoic acid (13c). Starting from **10c** and 2-nitrophenol, 77% of **13c** was obtained as a white solid according to above-mentioned general procedure. mp 75–76 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.0 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.11–6.96 (m, 2H), 4.11 (t, J = 6.1 Hz, 2H), 2.41 (t, J = 7.3 Hz, 2H), 1.94–1.81 (m, 2H), 1.81–1.66 (m, 2H), 1.64–1.50 (m, 2H); MS (ESI) m/z = 252.0 $(M-H)^-$.

4.1.5.14. 7-(2-Nitrophenoxy)heptanoic acid (13d). Starting from **10d** and 2-nitrophenol, 38% of **13d** was obtained as a white solid according to above-mentioned general procedure. mp 46–48 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.0 Hz, 1H), 7.50 (t, J = 7.8 Hz, 1H), 7.12–6.96 (m, 2H), 4.10 (t, J = 6.0 Hz, 2H), 2.38 (t, J = 7.2 Hz, 2H), 1.90–1.77 (m, 2H), 1.74–1.61 (m, 2H), 1.61–1.38 (m, 4H); MS (ESI) m/z = 266.0 $(M-H)^-$.

4.1.5.15. 8-(2-Nitrophenoxy)octanoic acid (13e). Starting from **10e** and 2-nitrophenol, 71% of **13e** was obtained as a white solid according to above-mentioned general procedure. mp 87–88 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.1 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.00 (t, J = 7.6 Hz, 1H), 4.09 (t, J = 6.1 Hz, 2H), 2.36 (t, J = 7.3 Hz, 2H), 1.91–1.76 (m, 2H), 1.73–1.59 (m, 2H), 1.56–1.30 (m, 6H); MS (ESI) m/z = 279.8 $(M-H)^-$.

4.1.6. Representative procedure for 14–16

Nitro acid (1.0 equiv) in toluene was treated with $SOCl_2$ (3.0 equiv), the mixture was stirred at 60 °C for 2 h. After removal of solvent by evaporation, the residue was dissolved in CH_3CN and added dropwise into a solution of triethylamine (6.0 equiv), DMAP (0.1 equiv) and *N*-BOC-*O*-TBS hydroxylamine (2.0 equiv) at 0 °C. The mixture was stirred for 0.5 h. Concentration in vacuo gave crude product, which was further purified by column chromatography (EtOAc/petroleum ether = 1:10).

4.1.6.1. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(4-(4-nitrophenoxy)butanoyl)carbamate (14a). Starting from **11a**, 45% of **14a** was obtained as a yellow solid according to above-mentioned general procedure. mp 45–48 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 8.6 Hz, 2H), 4.13 (t, J = 6.1 Hz, 2H), 3.01 (t, J = 6.9 Hz, 2H), 2.19 (p, J = 6.3 Hz, 2H), 1.54 (s, 9H), 1.00 (s, 9H), 0.14 (s, 6H); MS (ESI) m/z = 477.1 $(M+Na^+)$.

4.1.6.2. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(5-(4-nitrophenoxy)pentanoyl)carbamate (14b). Starting from **11b**, 47% of **14b** was obtained as a yellow solid according to above-mentioned general procedure. mp 87–90 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 4.14–4.03 (m, 2H), 2.89 (t, J = 6.2 Hz, 2H), 2.04–1.73 (m, 4H), 1.55 (s, 9H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 491.1 $(M+Na^+)$.

4.1.6.3. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(6-(4-nitrophenoxy)hexanoyl)carbamate (14c). Starting from **11c**, 69% of **14c** was obtained as a yellow solid according to above-mentioned general procedure. mp 36–38 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 7.9 Hz, 2H), 6.93 (d, J = 8.0 Hz, 2H), 4.05 (t, J = 6.2 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 1.95–1.66 (m, 4H), 1.56–1.53 (m, 11H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 505.1 $(M+Na^+)$.

4.1.6.4. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(7-(4-nitrophenoxy)heptanoyl)carbamate (14d). Starting from **11d**, 47% of **14d** was obtained as a yellow solid according to above-mentioned general procedure. mp 55–57 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.2 Hz, 2H), 6.93 (d, J = 8.3 Hz, 2H), 4.04 (t, J = 6.4 Hz, 2H), 2.81 (t, J = 7.2 Hz, 2H), 1.91–1.65 (m, 4H), 1.55 (s, 9H), 1.52–1.38 (m, 4H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 519.1 $(M+Na^+)$.

4.1.6.5. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(8-(4-nitrophenoxy)octanoyl)carbamate (14e). Starting from **11e**, 90% of **14e** was obtained as a yellow solid according to above-mentioned general procedure. mp 54–55 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 8.6 Hz, 2H), 4.03 (t, J = 6.4 Hz, 2H), 2.79 (t, J = 7.3 Hz, 2H), 1.86–1.76 (m, 2H), 1.71–1.63 (m, 2H), 1.54 (s, 9H), 1.50–1.36 (m, 6H), 0.99 (s, 9H), 0.14 (s, 6H); MS (ESI) m/z = 533.2 $(M+Na^+)$.

4.1.6.6. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(4-(3-nitrophenoxy)butanoyl)carbamate (15a). Starting from **12a**, 49% of **15a** was obtained as a yellow solid according to above-mentioned general procedure. mp 44–47 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.0 Hz, 1H), 7.72 (s, 1H), 7.41 (t, J = 8.1 Hz, 1H), 7.21 (d, J = 8.3 Hz, 1H), 4.11 (t, J = 6.0 Hz, 2H), 3.02 (t, J = 7.0 Hz, 2H), 2.19 (p, J = 6.3 Hz, 2H), 1.55 (s, 9H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 477.1 $(M+Na^+)$.

4.1.6.7. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(5-(3-nitrophenoxy)pentanoyl)carbamate (15b). Starting from **12b**, 43% of **15b** was obtained as a yellow solid according to above-mentioned general procedure. mp 56–57 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.1 Hz, 1H), 7.71 (s, 1H), 7.41 (t, J = 8.2 Hz, 1H), 7.20 (d, J = 8.3 Hz, 1H), 4.12–4.01 (m, 2H), 2.94–2.80 (m, 2H), 1.96–1.80 (m, 4H), 1.55 (s, 9H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 491.1 $(M+Na^+)$.

4.1.6.8. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(6-(3-nitrophenoxy)hexanoyl)carbamate (15c). Starting from **12c**, 39% of **15c** was obtained as a yellow solid according to above-mentioned general procedure. mp 33–35 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80

(d, J = 8.1 Hz, 1H), 7.71 (s, 1H), 7.41 (t, J = 8.1 Hz, 1H), 7.20 (d, J = 8.2 Hz, 1H), 4.03 (t, J = 6.3 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 1.94–1.68 (m, 4H), 1.60–1.56 (m, 2H), 1.55 (s, 9H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 505.1 ($M+Na^+$).

4.1.6.9. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(7-(3-nitrophenoxy)heptanoyl)carbamate (15d). Starting from **12d**, 42% of **15d** was obtained as a yellow solid according to above-mentioned general procedure. mp 27–29 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.0 Hz, 1H), 7.71 (s, 1H), 7.41 (t, J = 8.2 Hz, 1H), 7.21 (d, J = 8.1 Hz, 1H), 4.02 (t, J = 6.3 Hz, 2H), 2.81 (t, J = 7.3 Hz, 2H), 1.92–1.65 (m, 4H), 1.55 (s, 9H), 1.52–1.37 (m, 4H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 519.1 ($M+Na^+$).

4.1.6.10. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(8-(3-nitrophenoxy)octanoyl)carbamate (15e). Starting from **12e**, 40% of **15e** was obtained as a yellow solid according to above-mentioned general procedure. mp 26–28 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.0 Hz, 1H), 7.71 (s, 1H), 7.41 (t, J = 8.2 Hz, 1H), 7.20 (d, J = 8.3 Hz, 1H), 4.02 (t, J = 6.3 Hz, 2H), 2.79 (t, J = 7.3 Hz, 2H), 1.90–1.62 (m, 4H), 1.55 (s, 9H), 1.43 (d, J = 34.5 Hz, 6H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 533.1 ($M+Na^+$).

4.1.6.11. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(4-(2-nitrophenoxy)butanoyl)carbamate (16a). Starting from **13a**, 50% of **16a** was obtained as a yellow solid according to above-mentioned general procedure. mp 40–43 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 8.0 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.00 (t, J = 7.7 Hz, 1H), 4.19 (t, J = 5.9 Hz, 2H), 3.04 (t, J = 6.8 Hz, 2H), 2.19 (p, J = 6.3 Hz, 2H), 1.55 (s, 9H), 0.99 (s, 9H), 0.13 (s, 6H); MS (ESI) m/z = 477.1 ($M+Na^+$).

4.1.6.12. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(5-(2-nitrophenoxy)pentanoyl)carbamate (16b). Starting from **13b**, 51% of **16b** was obtained as a yellow solid according to above-mentioned general procedure. mp 56–58 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.0 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.00 (t, J = 7.7 Hz, 1H), 4.12 (t, J = 5.2 Hz, 2H), 2.88 (t, J = 6.3 Hz, 2H), 1.97–1.82 (m, 4H), 1.55 (s, 9H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 491.1 ($M+Na^+$).

4.1.6.13. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(6-(2-nitrophenoxy)hexanoyl)carbamate (16c). Starting from **13c**, 46% of **16c** was obtained as a yellow solid according to above-mentioned general procedure. mp 36–38 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.1 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.00 (t, J = 7.7 Hz, 1H), 4.10 (t, J = 6.3 Hz, 2H), 2.82 (t, J = 7.3 Hz, 2H), 1.96–1.65 (m, 4H), 1.60–1.56 (m, 2H), 1.55 (s, 9H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 505.1 ($M+Na^+$).

4.1.6.14. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(7-(2-nitrophenoxy)heptanoyl)carbamate (16d). Starting from **13d**, 37% of **16d** was obtained as a yellow solid according to above-mentioned general procedure. mp 58–60 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.1 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.06 (d, J = 8.5 Hz, 1H), 7.00 (t, J = 7.8 Hz, 1H), 4.09 (t, J = 6.4 Hz, 2H), 2.80 (t, J = 7.3 Hz, 2H), 1.89–1.63 (m, 4H), 1.55 (s, 9H), 1.53–1.38 (m, 4H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 519.0 ($M+Na^+$).

4.1.6.15. *tert*-Butyl (*tert*-butyldimethylsilyl)oxy(8-(2-nitrophenoxy)octanoyl)carbamate (16e). Starting from **13e**, 40% of **16e** was obtained as a colourless oil according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.1 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.05 (d, J = 8.5 Hz, 1H), 6.99 (t, J = 7.7 Hz, 1H), 4.08 (t, J = 6.4 Hz, 2H), 2.78 (t, J = 7.4 Hz, 2H), 1.87–1.78 (m, 2H), 1.72–1.61 (m, 2H), 1.54 (s, 9H), 1.51–1.46 (m, 2H), 1.42–1.33 (m, 4H), 0.99 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 533.2 ($M+Na^+$).

4.1.7. Representative procedure for 17–19

A mixture of nitro compound and 10% Pd/C (10% w/w) in methanol was hydrogenated at room temperature for 10 h. The mixture was filtered and the filtrate was evaporated to dryness to give the corresponding compound, which was used directly in the next step.

4.1.8. Representative procedure for 20–22

TMSCl (143 mg, 1.32 mmol) was slowly dropped into a mixture of VP-16 (194 mg, 0.33 mmol) and NaI (198 mg, 1.32 mmol) in CH_3CN (10 mL) under the protection of nitrogen. After stirring at room temperature for 1 h, triethylamine (117 mg, 1.16 mmol) and Ba_2CO_3 (197 mg, 0.99 mmol) was added into the flask. Then corresponding aromatic amine in CH_3CN (3 mL) was added dropwise into the mixture and stirred for 12 h. Subsequently, the mixture was filtered and extracted with EtOAc. Concentration in vacuo gave crude product, which was further purified by column chromatography (EtOAc/petroleum ether = 1:2).

4.1.8.1. Compound 20a. Starting from **17a**, 40% of **20a** was obtained as a brown foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.83–6.74 (m, 3H), 6.52 (s, 1H), 6.47 (d, J = 7.3 Hz, 2H), 6.33 (s, 2H), 5.96 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.62–4.53 (m, 2H), 4.36 (t, J = 7.7 Hz, 1H), 4.07–3.92 (m, 3H), 3.79 (s, 6H), 3.20–3.13 (m, 1H), 3.03–2.90 (m, 3H), 2.18–2.07 (m, 2H), 1.55 (s, 9H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 829.3 ($M+Na^+$).

4.1.8.2. Compound 20b. Starting from **17b**, 59% of **20b** was obtained as a brown foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.83–6.74 (m, 3H), 6.55–6.45 (m, 3H), 6.33 (s, 2H), 5.96 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.58 (s, 2H), 4.36 (t, J = 7.4 Hz, 1H), 4.03 (t, J = 10.1 Hz, 1H), 3.95–3.89 (m, 2H), 3.79 (s, 6H), 3.21–3.12 (m, 1H), 3.03–2.91 (m, 1H), 2.91–2.83 (m, 2H), 1.87–1.79 (m, 4H), 1.55 (s, 9H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 843.2 ($M+Na^+$).

4.1.8.3. Compound 20c. Starting from **17c**, 68% of **20c** was obtained as a brown foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.82–6.74 (m, 3H), 6.54–6.45 (m, 3H), 6.33 (s, 2H), 5.96 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.58 (s, 2H), 4.36 (t, J = 7.4 Hz, 1H), 4.03 (t, J = 9.6 Hz, 1H), 3.93–3.87 (m, 2H), 3.79 (s, 6H), 3.24–3.10 (m, 1H), 3.03–2.91 (m, 1H), 2.82 (t, J = 7.1 Hz, 2H), 1.84–1.68 (m, 4H), 1.55 (s, 9H), 1.53–1.48 (m, 2H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 857.4 ($M+Na^+$).

4.1.8.4. Compound 20d. Starting from **17d**, 61% of **20d** was obtained as a brown foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.83–6.74 (m, 3H), 6.56–6.45 (m, 3H), 6.34 (s, 2H), 5.96 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.63–4.55 (m, 2H), 4.36 (t, J = 7.5 Hz, 1H), 4.03 (t, J = 9.5 Hz, 1H), 3.89 (t, J = 5.9 Hz, 2H), 3.79 (s, 6H), 3.17 (dd, J = 14.2, 3.5 Hz, 1H), 3.03–2.91 (m, 1H), 2.80 (t, J = 7.1 Hz, 2H), 1.82–1.64 (m, 4H), 1.55 (s, 9H), 1.51–1.37 (m, 4H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 871.3 ($M+Na^+$).

4.1.8.5. Compound 20e. Starting from **17e**, 84% of **20e** was obtained as a brown foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.80 (d, J = 7.6 Hz, 2H), 6.76 (s, 1H), 6.52 (s, 1H), 6.48 (d, J = 7.9 Hz, 2H), 6.33 (s, 2H), 5.96 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.61–4.55 (m, 2H), 4.36 (t, J = 8.0 Hz, 1H), 4.04 (t, J = 9.7 Hz, 1H), 3.89 (t, J = 5.9 Hz, 2H), 3.79 (s, 6H), 3.20–3.13 (m, 1H), 3.02–2.91 (m, 1H), 2.79 (t, J = 7.3 Hz,

2H), 1.79–1.62 (m, 4H), 1.55 (s, 9H), 1.42 (m, 6H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 885.3 ($M+Na^+$).

4.1.8.6. Compound 21a. Starting from **17a**, 59% of **20a** was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, J = 7.3 Hz, 1H), 6.77 (s, 1H), 6.52 (s, 1H), 6.37–6.27 (m, 3H), 6.19–6.06 (m, 2H), 5.97 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.66 (s, 1H), 4.61–4.57 (m, 1H), 4.39 (t, J = 7.8 Hz, 1H), 4.05–3.96 (m, 3H), 3.80 (s, 6H), 3.16–2.93 (m, 4H), 2.25–2.06 (m, 2H), 1.54 (s, 9H), 1.02–0.90 (m, 9H), 0.17–0.08 (m, 6H); MS (ESI) m/z = 829.2 ($M+Na^+$).

4.1.8.7. Compound 21b. Starting from **18b**, 78% of **21b** was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, J = 7.9 Hz, 1H), 6.78 (s, 1H), 6.52 (s, 1H), 6.36–6.29 (m, 3H), 6.15 (d, J = 8.1 Hz, 1H), 6.09 (s, 1H), 5.97 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.67 (s, 1H), 4.60 (d, J = 3.0 Hz, 1H), 4.39 (t, J = 7.6 Hz, 1H), 4.01 (t, J = 9.6 Hz, 1H), 3.97–3.91 (m, 2H), 3.80 (s, 6H), 3.13 (dd, J = 14.0, 3.6 Hz, 1H), 3.04–2.94 (m, 1H), 2.91–2.82 (m, 2H), 1.88–1.80 (m, 4H), 1.54 (s, 9H), 1.00 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 843.2 ($M+Na^+$).

4.1.8.8. Compound 21c. Starting from **18c**, 76% of **21c** was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, J = 7.7 Hz, 1H), 6.78 (s, 1H), 6.52 (s, 1H), 6.37–6.28 (m, 3H), 6.14 (d, J = 7.8 Hz, 1H), 6.09 (s, 1H), 5.97 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.67 (s, 1H), 4.59 (d, J = 4.0 Hz, 1H), 4.39 (t, J = 7.9 Hz, 1H), 4.02 (t, J = 9.8 Hz, 1H), 3.96–3.89 (m, 2H), 3.79 (s, 6H), 3.13 (dd, J = 14.5, 4.3 Hz, 1H), 3.05–2.91 (m, 1H), 2.82 (t, J = 7.0 Hz, 2H), 1.84–1.69 (m, 4H), 1.55 (s, 9H), 1.53–1.47 (m, 2H), 0.99 (s, 9H), 0.15 (s, 6H); MS (ESI) m/z = 857.3 ($M+Na^+$).

4.1.8.9. Compound 21d. Starting from **18d**, 67% of **21d** was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, J = 8.0 Hz, 1H), 6.78 (s, 1H), 6.52 (s, 1H), 6.36–6.28 (m, 3H), 6.14 (d, J = 7.9 Hz, 1H), 6.09 (s, 1H), 5.97 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.67 (s, 1H), 4.59 (d, J = 4.2 Hz, 1H), 4.39 (t, J = 7.8 Hz, 1H), 4.02 (t, J = 9.6 Hz, 1H), 3.91 (t, J = 6.1 Hz, 2H), 3.79 (s, 6H), 3.13 (dd, J = 14.0, 4.5 Hz, 1H), 3.03–2.92 (m, 1H), 2.80 (t, J = 7.1 Hz, 2H), 1.82–1.64 (m, 4H), 1.54 (s, 9H), 1.49–1.37 (m, 4H), 0.99 (s, 9H), 0.14 (s, 6H); MS (ESI) m/z = 871.3 ($M+Na^+$).

4.1.8.10. Compound 21e. Starting from **18e**, 54% of **21e** was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.10 (t, J = 7.9 Hz, 1H), 6.78 (s, 1H), 6.52 (s, 1H), 6.36–6.30 (m, 3H), 6.14 (d, J = 8.1 Hz, 1H), 6.10 (s, 1H), 5.97 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.67 (s, 1H), 4.59 (d, J = 4.1 Hz, 1H), 4.39 (t, J = 7.7 Hz, 1H), 4.02 (t, J = 9.4 Hz, 1H), 3.94–3.88 (m, 2H), 3.79 (s, 6H), 3.13 (dd, J = 13.9, 3.9 Hz, 1H), 3.03–2.92 (m, 1H), 2.78 (t, J = 7.0 Hz, 2H), 1.81–1.72 (m, 2H), 1.71–1.64 (m, 2H), 1.54 (s, 9H), 1.47–1.36 (m, 6H), 0.99 (s, 9H), 0.14 (s, 6H); MS (ESI) m/z = 885.3 ($M+Na^+$).

4.1.8.11. Compound 22a. Starting from **17b**, 55% of **20b** was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.88 (t, J = 7.9 Hz, 1H), 6.82–6.68 (m, 3H), 6.53 (s, 1H), 6.47 (d, J = 7.6 Hz, 1H), 6.36 (s, 2H), 5.96 (s, 2H), 5.41 (s, 1H), 4.65 (s, 1H), 4.62 (d, J = 3.9 Hz, 1H), 4.36 (t, J = 7.4 Hz, 1H), 4.04 (t, J = 6.3 Hz, 2H), 3.96 (t, J = 9.5 Hz, 1H), 3.80 (s, 6H), 3.19 (dd, J = 14.0, 3.7 Hz, 1H), 3.04–2.87 (m, 3H), 2.17–2.07 (m, 2H), 1.54 (s, 9H), 0.99 (s, 9H), 0.13 (s, 6H); MS (ESI) m/z = 807.2 ($M+H^+$).

4.1.8.12. Compound 22b. Starting from **19b**, 67% of **22b** was obtained as a white foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.87 (t, J = 7.0 Hz, 1H), 6.82–6.68 (m, 3H), 6.52 (s, 1H), 6.47 (d, J = 7.8 Hz, 1H), 6.36 (s, 2H), 5.97 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.63 (s, 2H), 4.37 (t, J = 7.7 Hz, 1H), 4.07–3.93 (m, 3H), 3.80 (s, 6H), 3.20 (dd, J = 14.1, 3.1 Hz, 1H), 3.06–2.93 (m, 1H), 2.81 (t, J = 6.6 Hz, 2H), 1.86–1.69 (m, 4H), 1.54 (s, 9H), 1.00 (s, 9H), 0.13 (s, 6H); MS (ESI) m/z = 843.2 ($M+Na^+$).

4.1.8.13. Compound 22c. Starting from **19c**, 65% of **22c** was obtained as a white foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.87 (t, J = 8.1 Hz, 1H), 6.82–6.69 (m, 3H), 6.53 (s, 1H), 6.47 (d, J = 7.3 Hz, 1H), 6.36 (s, 2H), 5.98 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.62 (s, 2H), 4.40–4.34 (m, 1H), 4.07–3.91 (m, 3H), 3.80 (s, 6H), 3.24–3.14 (m, 1H), 3.10–2.91 (m, 1H), 2.76 (t, J = 7.0 Hz, 2H), 1.85–1.74 (m, 2H), 1.72–1.65 (m, 2H), 1.55 (s, 9H), 1.46–1.39 (m, 2H), 1.00 (s, 9H), 0.14 (s, 6H); MS (ESI) m/z = 857.2 ($M+Na^+$).

4.1.8.14. Compound 22d. Starting from **19d**, 75% of **22d** was obtained as a white foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.87 (t, J = 7.4 Hz, 1H), 6.82–6.68 (m, 3H), 6.53 (s, 1H), 6.46 (d, J = 7.7 Hz, 1H), 6.36 (s, 2H), 5.99 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.63 (s, 2H), 4.37 (t, J = 7.9 Hz, 1H), 4.06–3.90 (m, 3H), 3.80 (s, 6H), 3.23–3.15 (m, 1H), 3.07–2.93 (m, 1H), 2.76 (t, J = 7.2 Hz, 2H), 1.80–1.69 (m, 2H), 1.67–1.59 (m, 2H), 1.54 (s, 9H), 1.41–1.33 (m, 4H), 1.00 (s, 9H), 0.14 (s, 6H); MS (ESI) m/z = 871.3 ($M+Na^+$).

4.1.8.15. Compound 22e. Starting from **19e**, 41% of **22e** was obtained as a white foam according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 6.87 (t, J = 7.3 Hz, 1H), 6.82–6.69 (m, 3H), 6.54 (s, 1H), 6.47 (d, J = 7.6 Hz, 1H), 6.36 (s, 2H), 5.97 (s, 1H), 5.95 (s, 1H), 5.41 (s, 1H), 4.63 (s, 2H), 4.37 (t, J = 7.6 Hz, 1H), 4.05–3.91 (m, 3H), 3.80 (s, 6H), 3.19 (dd, J = 13.9, 3.5 Hz, 1H), 3.05–2.93 (m, 1H), 2.76 (t, J = 7.1 Hz, 2H), 1.78–1.69 (m, 2H), 1.66–1.60 (m, 2H), 1.55 (s, 9H), 1.42–1.27 (m, 6H), 1.00 (s, 9H), 0.14 (s, 6H); MS (ESI) m/z = 885.3 ($M+Na^+$).

4.1.9. Representative procedure for 23–25

Compound **20**–**22** (1.0 equiv) and CsF (2.5 equiv) were added into a mixed solvent of TFA and DCM (1:4 v/v). The mixture was stirred at room temperature for 24 h. Then it was extracted with EtOAc, washed with 5% $NaHCO_3$ (aq) followed by brine and dried over anhydrous Na_2SO_4. Concentration in vacuo gave crude product, which was further purified by column chromatography (DCM/methanol = 1:25) to give pure target compound.

4.1.9.1. Compound 23a. Starting from **20a** (100 mg, 0.12 mmol), **23a** (25 mg, 34%) was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (500 MHz, acetone-d_6) δ 6.65–6.85 (m, 5H), 6.47 (s, 1H), 6.39 (s, 2H), 5.97 (s, 1H), 5.96 (s, 1H), 4.80–4.98 (m, 2H), 4.55 (d, J = 5.0 Hz, 1H), 4.37 (t, J = 7.8 Hz, 1H), 3.85–4.00 (m, 3H), 3.64 (s, 6H), 3.26 (dd, J = 14.2, 5.0 Hz, 1H), 3.05–3.15 (m, 1H), 2.20–2.35 (m, 2H), 1.95–2.05 (m, 2H); HPLC: room temperature; eluent, CH_3OH–H_2O (65:35); flow rate, 1.0 mL/min; t_R = 4.43 min, UV_{254} = 97.7%; HRMS (ESI): m/z calcd for $C_{31}H_{32}N_2O_{10}Na^+$ ($M+Na^+$): 615.1949, found: 615.1934.

4.1.9.2. Compound 23b. Starting from **20b** (150 mg, 0.19 mmol), **23b** (35 mg, 32%) was obtained as a yellow foam according to above-mentioned general procedure.^1H NMR (500 MHz, acetone-d_6) δ 6.70–6.80 (m, 5H), 6.52 (s, 1H), 6.39 (s, 2H), 5.97 (s, 1H), 5.96 (s, 1H), 4.80–5.00 (m, 2H), 4.30–4.55 (m,

2H), 3.80–4.00 (m, 3H), 3.70 (s, 6H), 3.05–3.30 (m, 2H), 2.15–2.25 (m, 2H), 1.75–1.80 (m, 4H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 8.00 min, UV_{254} = 100.0%; HRMS (ESI): *m*/*z* calcd for $C_{32}H_{34}N_2O_{10}Na^+$ ($M+Na^+$): 629.2106, found: 629.2112.

4.1.9.3. Compound 23c. Starting from **20c** (150 mg, 0.18 mmol), **23c** (40 mg, 36%) was obtained as a yellow foam according to above-mentioned general procedure.^{1}H NMR (500 MHz, acetone-d_6) δ 10.01 (br s, 1H), 8.30–8.45 (br s, 1H), 7.14 (br s, 1H), 6.65–6.80 (m, 5H), 6.51 (s, 1H), 6.39 (s, 2H), 5.97 (s, 1H), 5.96 (s, 1H), 4.80–4.90 (m, 2H), 4.54 (d, *J* = 4.6Hz, 1H), 4.36 (t, *J* = 7.9 Hz, 1H), 3.75–3.95 (m, 3H), 3.70 (s, 6H), 3.26 (dd, *J* = 14.2, 4.8, 1H), 3.10–3.15 (m, 1H), 2.05–2.15 (m, 2H), 1.60–1.80 (m, 4H), 1.40–1.50 (m, 2H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 11.37 min, UV_{254} = 97.3%; HRMS (ESI): *m*/*z* calcd for $C_{33}H_{36}N_2O_{10}Na^+$ ($M+Na^+$): 643.2262, found: 643.2274.

4.1.9.4. Compound 23d. Starting from **20d** (120 mg, 0.14 mmol), **23d** (30 mg, 39%) was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (400 MHz, acetone-d_6) δ 6.81–6.76 (m, 3H), 6.73–6.67 (m, 2H), 6.52 (s, 1H), 6.40 (s, 2H), 5.97 (s, 1H), 5.96 (s, 1H), 4.81 (d, *J* = 3.9 Hz, 1H), 4.55 (d, *J* = 4.8 Hz, 1H), 4.38 (t, *J* = 7.7 Hz, 1H), 3.99–3.86 (m, 3H), 3.71 (s, 6H), 3.27 (dd, *J* = 14.2, 4.9 Hz, 1H), 3.17–3.10 (m, 1H), 2.23–2.10 (m, 2H), 1.76–1.68 (m, 2H), 1.67–1.59 (m, 2H), 1.50–1.44 (m, 2H), 1.42–1.35 (m, 2H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 17.88 min, UV_{254} = 100.0%; HRMS (ESI): *m*/*z* calcd for $C_{34}H_{38}N_2O_{10}Na^+$ ($M+Na^+$): 657.2419, found: 657.2423.

4.1.9.5. Compound 23e. Starting from **20e** (100 mg, 0.12 mmol), **23e** (25 mg, 33%) was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.96 (br s, 1H), 8.19 (br s, 1H), 7.11 (br s, 1H), 6.82–6.66 (m, 5H), 6.52 (s, 1H), 6.39 (s, 2H), 5.97 (d, *J* = 3.5 Hz, 2H), 4.92–4.78 (m, 2H), 4.55 (d, *J* = 4.2 Hz, 1H), 4.37 (t, *J* = 7.7 Hz, 1H), 3.98–3.86 (m, 3H), 3.70 (s, 6H), 3.27 (dd, *J* = 14.2, 4.3 Hz, 1H), 3.18–3.07 (m, 1H), 2.15–2.07 (m, 2H), 1.76–1.56 (m, 4H), 1.50–1.31 (m, 6H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 30.15 min, UV_{254} = 98.3%; HRMS (ESI): *m*/*z* calcd for $C_{35}H_{40}N_2O_{10}Na^+$ ($M+Na^+$): 671.2575, found: 671.2574.

4.1.9.6. Compound 24a. Starting from **21a** (100 mg, 0.12 mmol), **24a** (22 mg, 30%) was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, acetone-d_6) δ 7.02 (m, 1H), 6.81 (s, 1H), 6.52 (s, 1H), 6.40 (s, 2H), 6.20–6.35 (m, 3H), 5.97 (s, 1H), 5.96 (s, 1H), 4.85–4.95 (m, 1H), 4.53–4.55 (m, 1H), 4.37–4.41 (m, 1H), 3.85–4.04 (m, 3H), 3.70 (s, 6H), 3.05–3.30 (m, 2H), 2.00–2.50 (m, 4H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 7.86 min, UV_{254} = 98.4%; HRMS (ESI): *m*/*z* calcd for $C_{31}H_{32}N_2O_{10}Na^+$ ($M+Na^+$): 615.1949, found: 615.1934.

4.1.9.7. Compound 24b. Starting from **21b** (90 mg, 0.11 mmol), **24b** (29 mg, 44%) was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, acetone-d_6) δ 10.03 (br s, 1H), 8.25–8.40 (br s, 1H), 7.11 (br s, 1H), 7.02 (t, *J* = 8.0 Hz, 1H), 6.81 (s, 1H), 6.52 (s, 1H), 6.39 (s, 2H), 6.23–6.35 (m, 3H), 5.97 (s, 1H), 5.96 (s, 1H), 5.30 (d, *J* = 8.0 Hz, 1H), 4.90–4.95 (m, 1H), 4.54 (d, *J* = 4.5Hz, 1H), 4.38 (t, *J* = 8.0 Hz, 1H), 3.80–4.00 (m, 3H), 3.70 (s, 6H), 3.25 (dd, *J* = 14.5, 5.0 Hz, 1H), 3.11–3.20 (m, 1H), 2.07–2.15 (m, 2H), 1.70–1.80 (m, 4H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 9.83 min, UV_{254} = 98.0%; HRMS (ESI): *m*/*z* calcd for $C_{32}H_{34}N_2O_{10}Na^+$ ($M+Na^+$): 629.2106, found: 629.2097.

4.1.9.8. Compound 24c. Starting from **21c** (95 mg, 0.11 mmol), **24c** (20 mg, 28%) was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, acetone-d_6) δ 10.00 (br s, 1H), 8.00–8.25 (br s, 1H), 7.10 (br s, 1H), 7.02 (t, *J* = 8.0 Hz, 1H), 6.82 (s, 1H), 6.52 (s, 1H), 6.39 (s, 2H), 6.13–6.30 (m, 3H), 5.98 (s, 1H), 5.97 (s, 1H), 5.29 (d, *J* = 7.7 Hz, 1H), 4.90–4.95 (m, 1H), 4.30–4.45 (m, 2H), 3.90–4.00 (m, 3H), 3.70 (s, 6H), 3.05–3.35 (m, 2H), 2.05–2.13 (m, 2H), 1.60–1.80 (m, 4H), 1.40–1.50 (m, 2H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 14.00 min, UV_{254} = 98.1%; HRMS (ESI): *m*/*z* calcd for $C_{33}H_{36}N_2O_{10}Na^+$ ($M+Na^+$): 643.2262, found: 643.2274.

4.1.9.9. Compound 24d. Starting from **21d** (80 mg, 0.09 mmol), **24d** (26 mg, 44%) was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.98 (br s, 1H), 8.14 (br s, 1H), 7.11 (br s, 1H), 7.02 (t, *J* = 8.4 Hz, 1H), 6.81 (s, 1H), 6.52 (s, 1H), 6.39 (s, 2H), 6.34–6.30 (m, 2H), 6.26–6.21 (m, 1H), 5.97 (s, 1H), 5.96 (s, 1H), 5.29 (d, *J* = 7.7 Hz, 1H), 4.92 (dd, *J* = 7.4, 4.1 Hz, 1H), 4.54 (d, *J* = 4.9 Hz, 1H), 4.37 (t, *J* = 7.7 Hz, 1H), 3.96–3.88 (m, 3H), 3.70 (s, 6H), 3.26 (dd, *J* = 14.2, 5.0 Hz, 1H), 3.20–3.10 (m, 1H), 2.14–2.07 (m, 2H), 1.77–1.69 (m, 2H), 1.66–1.58 (m, 2H), 1.50–1.42 (m, 2H), 1.42–1.33 (m, 2H); ^{13}C NMR (100 MHz, acetone-d_6) δ 175.5, 170.8, 161.5, 150.8, 148.7, 148.1, 148.0, 136.1, 133.1, 132.5, 131.6, 130.8, 110.3, 110.2, 109.8, 105.9, 103.9, 102.3, 99.5, 69.6, 68.2, 56.7, 52.4, 44.4, 42.3, 39.8, 33.2, 30.6, 30.0, 26.6, 26.2; HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 21.58 min, UV_{254} = 97.7%; HRMS (ESI): *m*/*z* calcd for $C_{34}H_{38}N_2O_{10}Na^+$ ($M+Na^+$): 657.2419, found: 657.2415.

4.1.9.10. Compound 24e. Starting from **21e** (80 mg, 0.09 mmol), **24e** (28 mg, 46%) was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.03 (t, *J* = 7.9 Hz, 1H), 6.82 (s, 1H), 6.53 (s, 1H), 6.40 (s, 2H), 6.33 (s, 2H), 6.24 (d, *J* = 7.8 Hz, 1H), 5.97 (s, 2H), 4.93 (s, 1H), 4.56 (d, *J* = 4.1 Hz, 1H), 4.39 (t, *J* = 7.9 Hz, 1H), 3.97–3.89 (m, 3H), 3.71 (s, 6H), 3.30–3.22 (m, 1H), 3.22–3.10 (m, 1H), 2.14–2.07 (m, 2H), 1.79–1.69 (m, 2H), 1.64–1.56 (m, 2H), 1.52–1.30 (m, 6H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 35.99 min, UV_{254} = 98.3%; HRMS (ESI): *m*/*z* calcd for $C_{35}H_{40}N_2O_{10}Na^+$ ($M+Na^+$): 671.2575, found: 671.2568.

4.1.9.11. Compound 25a. Starting from **22a** (100 mg, 0.12 mmol), **25a** (18 mg, 24%) was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, acetone-d_6) δ 6.60–6.80 (m, 5H), 6.52 (s, 1H), 6.41 (s, 2H), 5.98 (s, 1H), 5.97 (s, 1H), 4.85–4.99 (m, 2H), 4.40–4.55 (m, 2H), 3.70–4.05 (m, 3H), 3.64 (s, 6H), 3.44 (dd, *J* = 13.7, 4.2 Hz, 1H), 3.14–3.20 (m, 1H), 1.95–2.25 (m, 4H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 7.53 - min, UV_{254} = 100.0%; HRMS (ESI): *m*/*z* calcd for $C_{31}H_{32}N_2O_{10}Na^+$ ($M+Na^+$): 615.1949, found: 615.1943.

4.1.9.12. Compound 25b. Starting from **22b** (90 mg, 0.11 mmol), **25b** (38 mg, 57%) was obtained as a yellow foam according to above-mentioned general procedure. ^{1}H NMR (500 MHz, acetone-d_6) δ 10.00 (br s, 1H), 8.25–8.50 (br s, 1H), 7.12 (br s, 1H), 6.60–6.90 (m, 5H), 6.51 (s, 1H), 6.41 (s, 2H), 5.98 (s, 1H), 5.95 (s, 1H), 4.80–4.90 (m, 2H), 4.57 (d, *J* = 5.0 Hz, 1H), 4.38 (t, *J* = 8.0 Hz, 1H), 3.89–4.02 (m, 2H), 3.85 (t, *J* = 9.5Hz, 1H), 3.71 (s, 6H), 3.34 (dd, *J* = 14.5, 5.0 Hz, 1H), 3.13–3.20 (m, 1H),

2.05–2.20 (m, 2H), 1.70–1.80 (m, 4H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 9.64 min, UV_{254} = 100.0%; HRMS (ESI): *m*/*z* calcd for $C_{32}H_{34}N_2O_{10}Na^+$ ($M+Na^+$): 629.2106, found: 629.2094.

4.1.9.13. Compound 25c. Starting from **22c** (80 mg, 0.10 mmol), **25c** (40 mg, 67%) was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (500 MHz, acetone-d_6) δ 9.97 (s, 1H), 8.25–8.50 (br s, 1H), 7.02–7.15 (br s, 1H), 6.60–6.85 (m, 5H), 6.52 (s, 1H), 6.41 (s, 2H), 5.99 (s, 1H), 5.96 (s, 1H), 4.75–4.85 (m, 2H), 4.59 (d, *J* = 4.9 Hz, 1H), 4.40 (t, *J* = 7.7 Hz, 1H), 3.85–4.00 (m, 3H), 3.71 (s, 6H), 3.32 (dd, *J* = 14.2, 4.9 Hz, 1H), 3.15–3.25 (m, 1H), 2.00–2.10 (m, 2H), 1.55–1.75 (m, 4H), 1.35–1.50 (m, 2H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 14.24 min, UV_{254} = 100.0%; HRMS (ESI): *m*/*z* calcd for $C_{33}H_{36}N_2O_{10}Na^+$ ($M+Na^+$): 643.2262, found: 643.2277.

4.1.9.14. Compound 25d. Starting from **22d** (90 mg, 0.11 mmol), **25d** (31 mg, 44%) was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (400 MHz, acetone-d_6) δ 6.91–6.81 (m, 2H), 6.79 (s, 1H), 6.72 (d, *J* = 7.6 Hz, 1H), 6.66 (t, *J* = 7.7 Hz, 1H), 6.55 (s, 1H), 6.41 (s, 2H), 6.02 (s, 1H), 5.97 (s, 1H), 4.88–4.75 (m, 2H), 4.60 (d, *J* = 3.7 Hz, 1H), 4.42 (t, *J* = 7.7 Hz, 1H), 4.09–3.93 (m, 2H), 3.87 (t, *J* = 9.4 Hz, 1H), 3.71 (s, 6H), 3.31 (dd, *J* = 14.1, 4.1 Hz, 1H), 3.19 (s, 1H), 2.08–2.06 (m, 2H), 1.74–1.65 (m, 2H), 1.59–1.50 (m, 2H), 1.42–1.26 (m, 4H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 25.58 min, UV_{254} = 100.0%; HRMS (ESI): *m*/*z* calcd for $C_{34}H_{38}N_2O_{10}Na^+$ ($M+Na^+$): 657.2419, found: 657.2427

4.1.9.15. Compound 25e. Starting from **22e** (104 mg, 0.12 mmol), **25e** (34 mg, 44%) was obtained as a yellow foam according to above-mentioned general procedure. ^1H NMR (400 MHz, acetone-d_6) δ 6.90–6.81 (m, 2H), 6.79 (s, 1H), 6.71 (d, *J* = 7.6 Hz, 1H), 6.65 (t, *J* = 7.5 Hz, 1H), 6.54 (s, 1H), 6.42 (s, 2H), 5.99 (s, 1H), 5.96 (s, 1H), 4.87–4.77 (m, 2H), 4.60 (d, *J* = 3.5 Hz, 1H), 4.41 (t, *J* = 7.5 Hz, 1H), 4.08–3.92 (m, 2H), 3.86 (t, *J* = 9.5 Hz, 1H), 3.71 (s, 6H), 3.29 (dd, *J* = 14.3, 3.8 Hz, 1H), 3.23–3.12 (m, 1H), 2.07–2.02 (m, 2H), 1.73–1.63 (m, 2H), 1.59–1.49 (m, 2H), 1.39–1.21 (m, 6H); HPLC: room temperature; eluent, CH_3OH–H_2O (60:40); flow rate, 1.0 mL/min; t_R = 45.36 min, UV_{254} = 100.0%; HRMS (ESI): *m*/*z* calcd for $C_{35}H_{40}N_2O_{10}Na^+$ ($M+Na^+$): 671.2575, found: 671.2578.

4.1.10. Representative procedure for 26–27

A mixture of acid compound **11d**/**12d** (1.0 equiv), BnBr (1.0 equiv) and Cs_2CO_3 (1.05 equiv) in DMF was stirred at room temperature for 1 h. Then the solution was poured into water and extracted with EtOAc. The organic layer was concentrated to give the crude product, which was further purified by column chromatography.

4.1.10.1. Benzyl 7-(4-nitrophenoxy)heptanoate (26). Starting from **11d**, 93% of compound **26** was obtained as a yellow solid according to above-mentioned general procedure. mp 57–60 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 9.2 Hz, 2H), 7.39–7.33 (m, 5H), 6.92 (d, *J* = 9.2 Hz, 2H), 5.12 (s, 2H), 4.03 (t, *J* = 6.4 Hz, 2H), 2.38 (t, *J* = 7.4 Hz, 2H), 1.87–1.76 (m, 2H), 1.75–1.63 (m, 2H), 1.53–1.36 (m, 4H); HRMS (ESI): *m*/*z* calcd for $C_{20}H_{23}NO_5Na^+$ ($M+Na^+$): 380.1474, found: 380.1464.

4.1.10.2. Benzyl 7-(3-nitrophenoxy)heptanoate (27). Starting from **12d**, 98% of compound **27** was obtained as a yellow oil according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.86–7.77 (m, 1H), 7.70 (t, *J* = 2.3 Hz, 1H), 7.45–7.27 (m, 6H), 7.22–7.17 (m, 1H), 5.12 (s, 2H), 4.01 (t, *J* = 6.4 Hz, 2H), 2.38 (t, *J* = 7.5 Hz, 2H), 1.86–1.65 (m, 4H), 1.55–1.35 (m, 4H); HRMS (ESI): *m*/*z* calcd for $C_{20}H_{23}NO_5Na^+$ ($M+Na^+$): 380.1474, found: 380.1477.

4.1.11. Representative procedure for 28–29

A mixture of nitro compounds **26/27** (1.0 equiv), iron powder (5.0 equiv) and NH_4Cl (5.0 equiv) in EtOH–H_2O was stirred at 60 °C for 1 h. Then the solution was cooled to room temperature and filtered. Subsequently, the filtrate was extracted with EtOAc and the organic layer was evaporated to dryness to give the crude product.

4.1.11.1. Benzyl 7-(4-aminophenoxy)heptanoate hydrochloride (28). Starting from **26**, 82% of compound **28** was obtained as a white solid according to above-mentioned general procedure. The resulting oil was dissolved in EtOH and concentrated hydrochloric acid was dropped into the solution. The crystals are collected by vacuum filtration and washed by EtOH. mp 138–141 °C, ^1H NMR (400 MHz, DMSO) δ 9.87 (br s, 2H), 7.44–7.30 (m, 5H), 7.27 (d, *J* = 8.7 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 5.08 (s, 2H), 3.95 (t, *J* = 6.4 Hz, 2H), 2.36 (t, *J* = 7.3 Hz, 2H), 1.78–1.64 (m, 2H), 1.63–1.53 (m, 2H), 1.45–1.26 (m, 4H); HRMS (ESI): *m*/*z* calcd for $C_{20}H_{26}NO_3$ ($M+H^+$): 328.1913, found: 328.1917.

4.1.11.2. Benzyl 7-(3-aminophenoxy)heptanoate (29). Starting from **27**, 95% of compound **29** was obtained as a brown oil according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.43–7.28 (m, 5H), 7.04 (t, *J* = 8.0 Hz, 1H), 6.29 (t, *J* = 9.5 Hz, 2H), 6.23 (s, 1H), 5.12 (s, 2H), 3.89 (t, *J* = 6.5 Hz, 2H), 3.63 (br s, 2H), 2.37 (t, *J* = 7.4 Hz, 2H), 1.79–1.63 (m, 4H), 1.50–1.33 (m, 4H); HRMS (ESI): *m*/*z* calcd for $C_{20}H_{26}NO_3$ ($M+H^+$): 328.1913, found: 328.1901.

4.1.12. Representative procedure for 30–31

TMSCl (4.0 equiv) was slowly dropped into a mixture of VP-16 (1.0 equiv) and NaI (4.0 equiv) in CH_3CN (10 mL) under nitrogen atmosphere. After stirring at room temperature for 1 h, TEA (3.5 equiv) and Ba_2CO_3 (3.0 equiv) was added into the flask. Then corresponding aromatic amine **28**/**29** was dissolved in CH_3CN and added into the mixture dropwise. The mixture was stirred for 12 h and extracted with EtOAc. Concentration in vacuo gave crude product, which was further purified by column chromatography.

4.1.12.1. Compound 30. Starting from **28**, 70% of compound **30** was obtained as a colourless oil according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.41–7.29 (m, 5H), 6.84–6.74 (m, 3H), 6.53–6.46 (m, 3H), 6.33 (s, 2H), 5.99–5.93 (m, 2H), 5.43 (s, 1H), 5.12 (s, 2H), 4.64–4.53 (m, 2H), 4.36 (t, *J* = 7.9 Hz, 1H), 4.03 (dd, *J* = 10.6, 8.6 Hz, 1H), 3.88 (t, *J* = 6.5 Hz, 2H), 3.79 (s, 6H), 3.63–3.52 (m, 1H), 3.16 (dd, *J* = 14.0, 5.0 Hz, 1H), 3.05–2.88 (m, 1H), 2.37 (t, *J* = 7.5 Hz, 2H), 1.81–1.64 (m, 4H), 1.52–1.34 (m, 4H); HRMS (ESI): *m*/*z* calcd for $C_{40}H_{43}NO_{10}Na^+$ ($M+Na^+$): 732.2785, found: 732.2761.

4.1.12.2. Compound 31. Starting from **29**, 70% of compound **31** was obtained as a colourless oil according to above-mentioned general procedure. ^1H NMR (400 MHz, $CDCl_3$) δ 7.42–7.29 (m, 5H), 7.10 (t, *J* = 8.1 Hz, 1H), 6.78 (s, 1H), 6.52 (s, 1H), 6.38–6.26 (m, 3H), 6.19–6.12 (m, 1H), 6.12–6.07 (m, 1H), 5.96 (d, *J* = 7.0 Hz, 2H), 5.43 (s, 1H), 5.11 (s, 2H), 4.70–4.65 (m, 1H), 4.59 (d, *J* = 4.8 Hz, 1H), 4.38 (t, *J* = 8.0 Hz, 1H), 4.05–3.97 (m, 1H), 3.91 (t, *J* = 6.4 Hz, 2H), 3.80 (s, 6H), 3.13 (dd, *J* = 14.0, 4.9 Hz, 1H), 3.04–2.92 (m, 1H), 2.37 (t, *J* = 7.5 Hz, 2H), 1.80–1.64 (m, 4H), 1.50–1.35 (m, 4H); HRMS

(ESI): m/z calcd for $C_{40}H_{43}NO_{10}Na^+$ ($M+Na^+$): 732.2785, found: 732.2794.

4.1.13. Representative procedure for 32–33

A mixture of benzyl ester (71 mg, 0.1 mmol) and 7 mg $Pd(OH)_2$ (20% w/w) in MeOH–EtOAc was hydrogenated at room temperature for 48 h. After filtration, the organic layer was evaporated to dryness to give the corresponding acids. The intermediate was dissolved in 5 mL DCM and benzene-1,2-diamine (33 mg, 0.3 mmol), HATU (40 mg, 0.105 mmol), DIPEA (39 mg, 0.3 mmol) were added into the solution. The mixture was stirred for 12 h and extracted with DCM. Concentration in vacuo gave crude product, which was further purified by column chromatography (DCM/MeOH = 30:1).

4.1.13.1. Compound 32. Starting from **30**, compound **32** (29 mg, 41%) of was obtained as a yellow solid according to above-mentioned general procedure. mp 119–122 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21–7.03 (m, 3H), 6.86–6.73 (m, 5H), 6.56–6.44 (m, 3H), 6.33 (s, 2H), 5.96 (d, J = 6.6 Hz, 2H), 5.43 (s, 1H), 4.66–4.55 (m, 2H), 4.37 (t, J = 7.8 Hz, 1H), 4.03 (t, J = 9.5 Hz, 1H), 3.97–3.78 (m, 10H), 3.60–3.53 (m, 1H), 3.22–3.13 (m, 1H), 3.04–2.92 (m, 1H), 2.43 (t, J = 7.3 Hz, 2H), 1.86–1.74 (m, 4H), 1.55–1.43 (m, 4H); HPLC: room temperature; eluent, CH_3OH–H_2O (70:30); flow rate, 1.0 mL/min; t_R = 10.49 min, UV_{254} = 95.0%; HRMS (ESI): m/z calcd for $C_{40}H_{43}N_3O_9Na^+$ ($M+Na^+$): 732.2897, found: 732.2889.

4.1.13.2. Compound 33. Starting from **31**, compound **33** (25 mg, 35%) was obtained as a yellow solid according to above-mentioned general procedure. mp 126–129 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20–7.02 (m, 4H), 6.84–6.75 (m, 3H), 6.52 (s, 1H), 6.37–6.31 (m, 3H), 6.19–6.07 (m, 2H), 5.96 (d, J = 6.9 Hz, 2H), 5.44 (s, 1H), 4.70–4.64 (m, 1H), 4.58 (d, J = 4.0 Hz, 1H), 4.39 (t, J = 8.0 Hz, 1H), 4.01 (t, J = 9.7 Hz, 1H), 3.97–3.91 (m, 2H), 3.90–3.76 (m, 9H), 3.13 (dd, J = 13.9, 4.0 Hz, 1H), 3.03–2.90 (m, 1H), 2.42 (t, J = 7.2 Hz, 2H), 1.88–1.69 (m, 4H), 1.56–1.41 (m, 4H); HPLC: room temperature; eluent, CH_3OH–H_2O (70:30); flow rate, 1.0 mL/min; t_R = 14.32 min, UV_{254} = 97.0%; HRMS (ESI): m/z calcd for $C_{40}H_{44}N_3O_9$ ($M+H^+$): 710.3078, found: 710.3078.

4.2. Biological assays

4.2.1. HDAC enzymatic assay in vitro

Recombinant human HDAC1, HDAC3 and HDAC6 were cloned and expressed in High5 insect cells using a baculovirus expression system and purified using Ni-NTA (QIAGEN). The histone deacetylase inhibitory activity of HDAC1 and HDAC3 was determined using the HDAC substrate Ac-Lys-Tyr-Lys (ε-acetyl)-AMC while the inhibitory activity of HDAC6 was assayed with the HDAC substrate Boc-Lys (ε-acetyl)-AM). The reaction was carried out in black 384-well plates (OptiPlateTM-384F, PerkinElmer) at room temperature. The typical inhibition assay was carried out in 25 μL of buffer containing 25 mM HEPES, 137 mM NaCl, 2.7 mM KCl and 4.9 mM $MgCl_2$, pH 8.0, HDAC protein (20–200 nM), HDAC substrate (5–50 μM) and 20 μg/mL individual compound. Positive controls contained SAHA and all the above components except the inhibitor. The negative controls contained neither enzyme nor inhibitor. After incubation for 24 and 3 h, respectively, the reaction of HDAC1, HDAC3 and HDAC6 was quenched with the addition of 25 μL Trypsin (diluted to a final concentration of 0.3125%). The plates were incubated for 30 min at room temperature to allow the fluorescence signal to develop. The fluorescence generated was monitored at 355 nm (excitation) and 460 nm (emission) using an EnVision multilabel plate reader (PerkinElmer Life Sciences, Boston, MA, USA).

4.2.2. Cell culture and cytotoxicity/proliferation assay

Cells were kept at logarithmic growth phase in 5% CO_2 at 37 °C with the corresponding medium supplemented with 10% fetal bovine serum and 100 units/mL each of penicillin G and streptomycin. Cells were seeded onto a 96-well plate at a concentration of 2000–3000 cells/well and incubated at 37 °C in 5% CO_2 for 24 h. A range of concentrations of the test compounds were added and the plate was incubated at 37 °C for 72 h before 20 μL MTT (5 mg/mL)/well was added. After 3 h of incubation, the medium was removed and 100 μL DMSO was added to each well. The absorbance was measured using a SpectraMax 340 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 550 nm with a reference at 690 nm. The optical density of the result of the MTT assay was directly proportional to the number of viable cells.[24]

4.2.3. In vitro topoisomerase II relaxation assay

The relaxation of pBR322 DNA was assayed according to TopoGen protocol in order to determine topoisomerase II activity. The substrate pBR322 DNA (200 ng) and 100 μM drug were combined in assay buffer (50 mM Tris−HCl, pH 8.0, 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol, 300 μg/mL bovine serum albumin (BSA)) and incubated for 30 min on ice. Subsequently, 4 units of topoisomerase II (TopoGEN, Port Orange, Florida, USA) were added and the reaction was allowed to proceed for 10 min at 37 °C. The reaction was quenched via the addition of loading buffer (1% sarkosyl, 0.025% bromophenol blue, and 5% glycerol) and was analyzed by electrophoresis on a 1% agarose gel in TBE buffer (89 mM Tris, 89 mM borate, and 2 mM Na-EDTA, pH 8.3) for 3.5 h at 40 V. The gel was stained with ethidium bromide (EB) for 30 min and was visualized under UV illumination.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.09.023.

References and notes

1. Fearon, E. R.; Vogelstein, B. *Cell* **1990**, *61*, 759.
2. Costantino, L.; Barlocco, D. *Future Med. Chem.* **2013**, *5*, 5.
3. Kim, M. S.; Blake, M.; Baek, J. H.; Kohlhagen, G.; Pommier, Y.; Carrier, F. *Cancer Res.* **2003**, *63*, 7291.
4. Bruzzese, F.; Rocco, M.; Castelli, S.; Di Gennaro, E.; Desideri, A.; Budillon, A. *Mol. Cancer Ther.* **2009**, *8*, 3075.
5. Bevins, R. L.; Zimmer, S. G. *Cancer Res.* **2005**, *65*, 6957.
6. Ocker, M.; Alajati, A.; Ganslmayer, M.; Zopf, S.; Luders, M.; Neureiter, D.; Hahn, E. G.; Schuppan, D.; Herold, C. *J. Cancer Res. Clin. Oncol.* **2005**, *131*, 385.
7. Sarcar, B.; Kahali, S.; Chinnaiyan, P. *J. Neurooncol.* **2010**, *99*, 201.
8. Dokmanovic, M.; Clarke, C.; Marks, P. A. *Mol. Cancer Res.* **2007**, *5*, 981.
9. Lin, H. Y.; Chen, C. S.; Lin, S. P.; Weng, J. R.; Chen, C. S. *Med. Res. Rev.* **2006**, *26*, 397.
10. Lee, M. J.; Kim, Y. S.; Kummar, S.; Giaccone, G.; Trepel, J. B. *Curr. Opin. Oncol.* **2008**, *20*, 639.
11. Chen, C. S.; Weng, S. C.; Tseng, P. H.; Lin, H. P.; Chen, C. S. *J. Biol. Chem.* **2005**, *280*, 38879.
12. Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; Ando, T.; Suzuki, T.; Tsuruo, T.; Nakanishi, O. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4592.
13. Glick, R. D.; Swendeman, S. L.; Coffey, D. C.; Rifkind, R. A.; Marks, P. A.; Richon, V. M.; La Quaglia, MP *Cancer Res.* **1999**, *59*, 4392.
14. Butler, L. M.; Agus, D. B.; Scher, H. I.; Higgins, B.; Rose, A.; Cordon-Cardo, C.; Thaler, H. T.; Rifkind, R. A.; Marks, P. A.; Richon, V. M. *Cancer Res.* **2000**, *60*, 5165.

15. Oyelere, A. K.; Chen, P. C.; Guerrant, W.; Mwakwari, S. C.; Hood, R.; Zhang, Y.; Fan, Y. *J. Med. Chem.* **2009**, *52*, 456.
16. Mann, B. S.; Johnson, J. R.; Cohen, M. H.; Justice, R.; Pazdur, R. *Oncologist* **2007**, *12*, 1247.
17. Grant, C.; Rahman, F.; Piekarz, R.; Peer, C.; Frye, R.; Robey, R. W.; Gardner, E. R.; Figg, W. D.; Bates, S. E. *Expert Rev. Anticancer Ther.* **2010**, *10*, 997.
18. Mwakwari, S. C.; Patil, V.; Guerrant, W.; Oyelere, A. K. *Curr. Top. Med. Chem.* **2010**, *10*, 1423.
19. Cai, X.; Zhai, H. X.; Wang, J.; Forrester, J.; Qu, H.; Yin, L.; Lai, C. J.; Bao, R.; Qian, C. *J. Med. Chem.* **2000**, *2010*, 53.
20. Lai, C. J.; Bao, R.; Tao, X.; Wang, J.; Atoyan, R.; Qu, H.; Wang, D. G.; Yin, L.; Samson, M.; Forrester, J.; Zifcak, B.; Xu, G. X.; DellaRocca, S.; Zhai, H. X.; Cai, X.; Munger, W. E.; Keegan, M.; Pepicelli, C. V.; Qian, C. *Cancer Res.* **2010**, *70*, 3647.
21. Mahboobi, S.; Dove, S.; Sellmer, A.; Winkler, M.; Eichhorn, E.; Pongratz, H.; Ciossek, T.; Baer, T.; Maier, T.; Beckers, T. *J. Med. Chem.* **2009**, *52*, 2265.
22. Mahboobi, S.; Sellmer, A.; Winkler, M.; Eichhorn, E.; Pongratz, H.; Ciossek, T.; Baer, T.; Maier, T.; Beckers, T. *J. Med. Chem.* **2010**, *53*, 8546.
23. Thaler, F. *Pharm. Pat. Analyst* **2012**, *1*, 75.
24. Zhang, X.; Zhang, J.; Tong, L.; Luo, Y.; Su, M.; Zang, Y.; Li, J.; Lu, W.; Chen, Y. *Bioorg. Med. Chem.* **2013**, *21*, 3240.
25. Zhang, X.; Su, M.; Chen, Y.; Li, J.; Lu, W. *Molecules* **2013**, *18*, 6491.
26. Gryder, B. E.; Rood, M. K.; Johnson, K. A.; Patil, V.; Raftery, E. D.; Yao, L.-P. D.; Rice, M.; Azizi, B.; Doyle, D. F.; Oyelere, A. K. *J. Med. Chem.* **2013**, *56*, 5782.
27. Ko, K. S.; Steffey, M. E.; Brandvold, K. R.; Soellner, M. B. *ACS Med. Chem. Lett.* **2013**, *4*, 779.
28. Liu, L. F. *Annu. Rev. Biochem.* **1989**, *58*, 351.
29. Xu, D.; Cao, J.; Qian, S.; Li, L.; Hu, C.; Weng, Q.; Lou, J.; Zhu, D.; Zhu, H.; Hu, Y.; He, Q.; Yang, B. *Invest. New Drugs* **2011**, *29*, 786.
30. Hu, C.; Xu, D.; Du, W.; Qian, S.; Wang, L.; Lou, J.; He, Q.; Yang, B.; Hu, Y. *Mol. Biosyst.* **2010**, *6*, 410.
31. Yang, X. C.; Qian, S. J.; Wang, L.; Liao, S. D.; Cao, J.; Hu, Y. Z.; He, Q. J.; Zhu, H.; Yang, B. *Transl. Oncol.* **2013**, *6*, 75.
32. Hande, K. R. *Eur. J. Cancer* **1998**, *34*, 1514.
33. Hande, K. R. *Bba-Gene Struct. Expr.* **1998**, *1400*, 173.
34. Catalano, M. G.; Fortunati, N.; Pugliese, M.; Poli, R.; Bosco, O.; Mastrocola, R.; Aragno, M.; Boccuzzi, G. *J. Endocrinol.* **2006**, *191*, 465.
35. Guerrant, W.; Patil, V.; Canzoneri, J. C.; Oyelere, A. K. *J. Med. Chem.* **2012**, *55*, 1465.
36. Guerrant, W.; Patil, V.; Canzoneri, J. C.; Yao, L. P.; Hood, R.; Oyelere, A. K. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3283.
37. Long, B. H.; Musial, S. T.; Brattain, M. G. *Biochemistry* **1984**, *23*, 1183.
38. Wang, Z. Q.; Kuo, Y. H.; Schnur, D.; Bowen, J. P.; Liu, S. Y.; Han, F. S.; Chang, J. Y.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1990**, *33*, 2660.
39. Zhang, Y. L.; Guo, X.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1994**, *37*, 446.
40. Zhu, X. K.; Guan, J.; Tachibana, Y.; Bastow, K. F.; Cho, S. J.; Cheng, H. H.; Cheng, Y. C.; Gurwith, M.; Lee, K. H. *J. Med. Chem.* **1999**, *42*, 2441.
41. Xiao, Z.; Bastow, K. F.; Vance, J. R.; Sidwell, R. S.; Wang, H. K.; Chen, M. S.; Shi, Q.; Lee, K. H. *J. Med. Chem.* **2004**, *47*, 5140.
42. Barret, J. M.; Kruczynski, A.; Vispe, S.; Annereau, J. P.; Brel, V.; Guminski, Y.; Delcros, J. G.; Lansiaux, A.; Guilbaud, N.; Imbert, T.; Bailly, C. *Cancer Res.* **2008**, *68*, 9845.
43. Kamal, A.; Suresh, P.; Ramaiah, M. J.; Srinivasareddy, T.; Kapavarapu, R. K.; Rao, B. N.; Imthiajali, S.; Lakshminarayanreddy, T.; Pushpavalli, S. N. C. V. L; Shankaraiah, N.; Pal-Bhadra, M. *Bioorg. Med. Chem.* **2013**, *21*, 5198.
44. Chen, Y.-F.; Lin, Y.-C.; Huang, P.-K.; Chan, H.-C.; Kuo, S.-C.; Lee, K.-H.; Huang, L.-J. *Bioorg. Med. Chem.* **2013**, *21*, 5064.
45. Wang, L.; Yang, F.; Yang, X.; Guan, X.; Hu, C.; Liu, T.; He, Q.; Yang, B.; Hu, Y. *Eur. J. Med. Chem.* **2011**, *46*, 285.
46. Altenburger, J. M.; Mioskowski, C.; Dorchymont, H.; Schirlin, D.; Schalk, C.; Tarnus, C. *Tetrahedron Lett.* **1992**, *33*, 5055.
47. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188.
48. Vasudevan, A.; Ji, Z.; Frey, R. R.; Wada, C. K.; Steinman, D.; Heyman, H. R.; Guo, Y.; Curtin, M. L.; Guo, J.; Li, J.; Pease, L.; Glaser, K. B.; Marcotte, P. A.; Bouska, J. J.; Davidsen, S. K.; Michaelides, M. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3909.
49. Glaser, K. B.; Li, J.; Aakre, M. E.; Morgan, D. W.; Sheppard, G.; Stewart, K. D.; Pollock, J.; Lee, P.; O'Connor, C. Z.; Anderson, S. N.; Mussatto, D. J.; Wegner, C. W.; Moses, H. L. *Mol. Cancer. Ther.* **2002**, *1*, 759.