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Design, synthesis and anti-tumor activity study of novel histone deacetylase inhibitors containing isatin-based caps and *o*-phenylenediamine-based zinc binding groups

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Abstract

As a hot topic of epigenetic studies, histone deacetylases (HDACs) are related to lots of diseases, especially cancer. Further researches indicated that different HDAC isoforms played various roles in a wide range of tumor types. Herein a novel series of HDAC inhibitors with isatin-based caps and *o*-phenylenediamine-based zinc binding groups have been designed and synthesized through scaffold hopping strategy. Among these compounds, the most potent compound 9n exhibited similar if not better HDAC inhibition and antiproliferative activities against multiple tumor cell lines compared with the positive control entinostat (MS-275). Additionally, compared with MS-275 (IC_{50} values for HDAC1, 2 and 3 were 0.163, 0.396 and 0.605 μM, respectively), compound 9n with IC_{50} values of 0.032, 0.256 and 0.311 μM for HDAC1, 2 and 3 respectively, showed a moderate HDAC1 selectivity.

Keywords

Anticancer; Epigenetics; HDAC inhibitors; Isatin

1. Introduction

The onset and progression of cancer, traditionally seen as a genetic disease, are revealed to be associated with epigenetic changes along with genetic abnormalities in recent years.[1] Histone deacetylases (HDACs) as novel targets for the discovery of anticancer agents are a series of enzymes that regulate histone deacetylation, which may be the best understood type of epigenetic modifications.[2] HDACs can enrich the histone tails with positive charges via

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Conflict of interest

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

removing an acetyl group from the ε-amino group of lysine residue, forcing the compaction of the chromatin containing electronegative DNA. The packaged chromatin prevents transcription factors from DNA promoters, thereby repressing the expression of genes, including various tumor-suppressor genes.[3] In addition, a mass of non-histone proteins with diverse biological functions are also substrates of HDACs, such as transcription factors (p53), nuclear factors (NF-κB), α-tubulin, heat-shock protein 90 (Hsp90), estrogen receptor (ERα), DNA repair protein Ku70 and so on.[4] 18 mammalian HDACs have been identified and grouped into classes I–IV based on their homology to the yeast (*Saccharomyces cerevisiae*) HDACs.[5] Class I HDACs comprising HDAC1, 2, 3 and 8 are homologous to the yeast transcriptional regulator reduced potassium dependency-3 (RPD3). Class II HDACs which are structurally close to yeast histone deacetylase1 (Had1) are divided into two subfamilies. HDAC4, 5, 7 and 9 compose the class IIa HDACs, while HDAC6 and 10 constitute the class IIb HDACs. Class III HDACs consisting of seven isoforms (SIRT1-7) are most related to the yeast silent information regulator (sir 2) family. HDAC11, the lastly discovered isoform, is the only member of class IV HDAC which shares the homology with class I and II HDACs. The classical HDACs (class I, II, and IV) are Zn^{2+} dependent enzymes, whereas class III HDACs needs the co-factor NAD^{+} for their activity.[3]

Higher expression levels of class I HDACs were observed in tumor cells than in normal cells. HDAC1 has been implicated in the initiation and progression of various types of tumor. For instance, high level of HDAC1 is associated with rapid tumor cell proliferation and genomic instability in prostate cancer.[6] Moreover, the knockdown of HDAC1 in leukemia cells K562, HL-60, and U937 significantly inhibited cell cycle progression and cell proliferation.[7] HDAC2 overexpression is an independent marker of poor prognosis in patients with oral, prostate, ovarian, endometrial or gastric cancer.[8] HDAC3 always forms a stable complex with the nuclear receptor co-repressor (N-CoR) and the silencing mediator of retinoic and thyroid receptors (SMRT), which are relevant to a wide range of biological processes, such as cell differentiation, proliferation and apoptosis.[9–11] Therefore, the discovery of novel class I-selective HDAC inhibitors (HDACIs) as antineoplastic agents are of interest for clinical use.

In recent decades, more than 20 chemical entities have been studied as HDACIs in different clinical trials. Among them, five compounds were successfully accepted by U.S.FDA or Chinese FDA (CFDA) as novel anti-cancer agents (Fig. 1). Vorinostat (SAHA) and romidepsin (FK228) were approved by U.S.FDA for the chemotherapeutics against refractory cutaneous T-cell lymphoma (CTCL). Belinostat (PXD-101) and panobinostat (LBH-589) were permitted by U.S.FDA to remedy for relapsed or refractory peripheral T-cell lymphoma (PTCL) and multiple myeloma, respectively. Unlike the above four pan-HDACIs, chidamide (CS055/HBI-8000) which obtained the approval from CFDA as a monotherapy for relapsed and refractory peripheral T-cell lymphoma (PTCL) is an isoform-selective HDACI.[12] Chidamide, as well as its benzamide derivatives entinostat (MS-275) and CI-994 (Fig. 2), shows more potent inhibition against class I HDACs (HDAC1, 2, and 3) than other classes. The pharmacophore of most HDACIs comprises three units: zinc binding group (ZBG) binding the Zn^{2+} at the bottom of the active site; cap group (CAP) specifically interacting with the amino acid residues around the entrance of the active site; a linker that connects the ZBG and CAP occupying the 11 Å channel of the active site (Fig. 1).[13,14]

As shown in Fig. 2, indoline-2, 3-dione also called isatin is an endogenous molecule with a wide range of biological activities, including antiviral activity, antitumor and antiangiogenic activities, antibacterial, antitubercular, antifungal, anticonvulsant and anxyolytic activities.[15] For example, indirubin as a derivative of isatin is the active ingredient of Danggui Longhui Wan used to treat chronic myelocytic leukemia (CML).[16] In our pioneering work, a series of hydroxamic acid derivatives containing isatin-based caps have been synthesized and evaluated as potential HDACIs. Meanwhile, a benzamide-based molecule **9a** has been obtained by splicing isatin to the structure of the clinical HDACI, CI-994.[17]Although compound **9a** and the positive control MS-275 exhibited similar HDACs inhibition, **9a** exhibited inferior in vitro antiproliferative activity. Herein, structural modification of the lead compound **9a** was carried out through scaffold hopping strategy (Fig. 2) to find more potent HDACIs.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **9a**–**9o** was shown in Scheme 1. Reaction of starting materials **1a**–**1c**, hydroxylamide hydrochloride and chloral hydrate led to compounds **2a**–**2c**, respectively. Sandmeyer reaction was used for the synthesis of compounds **3a**–**3c** under the condition of concentrated sulfuric acid at 80 °C. Compounds **4a**–**4f** obtained from **3a**–**3c** by ketalization reaction in the presence of ethane-1, 2-diol or propane-1, 3-diol were then reacted with methyl 2-bromoacetate or methyl 3-bromo-propanoate to get compounds **5a**–**5h**. The hydrolysis of **5a**–**5h** was implemented to produce intermediates **6a**–**6h**, which further underwent condensation reaction with methyl-4-aminobenzoate or methyl 4-(aminomethyl) benzoate to afford **7a**–**7o**. Compounds **8a**–**8o** were hydrolytic products of **7a**–**7o**, respectively. Finally, target compounds **9a**–**9o** were obtained by condensation of *o*-phenylenediamine and compounds **8a**–**8o** in the presence of 2-(1H-benzotriazole-1-yl)-1, 1, 3, 3-tetramethyluronium tetrafluo-roborate (TBTU).

Condensation of **6a**–**6f** with methyl 6-aminohexanoate hydrochloride, followed by hydrolysis and condensation with *o*-phenylenediamine led to target compounds **12a**–**12f** (Scheme 2).

Unlike Schemes 1 and 2, the synthetic route affording desired compounds with an aromatic $\mathbf{R_1}$ was illustrated in Scheme 3. Firstly, the amino group of 4-bromo-2-nitroaniline (compound **13**) was protected by di-*tert*-butyl dicarbonate to get compound **14**, and then thienyl or phenyl group was introduced through Suzuki coupling to get **15a** or **15b**. Compounds **16a** and **16b** derived from the reduction of **15a** and **15b** were reacted with **19** to get **20a** and **20b**, respectively. At last, the deprotected compounds **21a** and **21b** were coupled with intermediates **6b** and **6e** to give **22a**–**22d**, which were treated with hydrogen chloride in ethyl acetate to remove the Boc group to yield the desired compounds **23a**–**23d**.

The preparation of the compound **30** with fluoro-substituent was illustrated in Scheme 4. Boc protection of the starting material **24** followed by reduction led to **26**. Reaction of **26** with **19** led to **27**, which was transformed to the target compound **30** using similar methods described in Scheme 3.

2.2. HeLa nuclear extract inhibitory assay

HeLa nuclear extract was used as the enzyme source to evaluate the HDACs inhibitory activities of our target compounds with MS-275 as the positive control. Firstly, the **X** group of the isatin-based cap in **9a** was replaced by different halogens to get analogs **9b** and **9c**. Then compounds **9e**–**9f** were synthesized with different sizes of ketal spiro ring at the 3-postion of the isatin-based caps. In the following work, the length of the linker was extended by replacing the methyl-4-aminobenzoate with methyl 4-(aminomethyl) ben-zoate according to the structure of MS-275 so as to gain compounds **9g**–**9l**. Meanwhile, compounds **9m**, **9n** and **9o** with different linkers relative to compounds **9d**, **9e** and **9h** respectively were synthesized to evaluate the effect of linker length on HDAC inhibition.

Results in Table 1 showed that compounds **9a**–**9c** with six-membered spirocycle had better HDAC inhibitory activities than their five-membered spirocycle analogs **9d**–**9f**, respectively. The same structure-activity relationship could also be observed between **9g**–**9i** (n = 1) and **9j**–**9l** (n = 0), though not that robust. Among compounds **9a**–**9c** (m = 0), bromo- or chloro-substituted compounds (**9a**, **9b**) exhibited better HDAC inhibition than fluoro-substituted compound **9c**. The same structure-activity relationship was also observed among **9d**–**9f** (m = 0), but not among **9g**–**9i** (m = 1) or **9j**–**9l** (m = 1). Compounds **9m** and **9n** with the ethidine linker in the **y** position displayed more potent activities than their counterparts **9d** and **9e** with the methylene linker, respectively. However, compound **9o** with the ethidine linker in the **y** position exhibited similar potency to its analogue **9h** with the methylene linker. Above structure-activity relationships indicated that cap part and the linker part could simultaneously affect the HDAC inhibitory potency of these benzamide-based HDACIs.

Then, compounds **12a**–**12f** with the similar aliphatic linker as RG2833 were synthesized.[18] Results in Table 2 showed that the aliphatic linker, however, may decrease the HDAC inhibitory activities of the target compounds. This should be associated with the loss of the π-π stacking interaction between aliphatic linker of **12a**–**12f** and the two parallel phenylalanine residues composing the hydrophobic channel of HDACs active site.[19,20]

Besides a hydrophobic 11 Å channel, class I HDACs active site also contains a 14 Å internal cavity at the bottom of the active site.[21,22] The 14 Å cavity as an escaping route for acetate following deacetylation could be used for the design of HDAC1/2 selective inhibitors.[23,24] Thereby, we designed and synthesized several 5-substituted-*o*-phenylenediamines **23a**–**23d**, which should be HDAC1/2 selective over HDAC3. Moreover, one 4-fluorine-*o*-phenylenediamine analogue **30** was also synthesized. As shown in Table 3, compounds **23a**–**23d** and **30** exhibited significantly decreased inhibitory activities toward HeLa nuclear extract.

2.3. In vitro antiproliferative assay

Based on the results of HDACs inhibitory assay, several compounds were selected to test their antitumor activities against five cancer cell lines, including K562 (chronic myelogenous leukemia cell), Molt-4 (human T lymphoblast; acute lymphoblastic leukemia cell), HEL (human erythrocyte leukemia cell), PC-3 (human prostate cancer cell) and SK-N-BE(2) (human neuroblastoma) (Table 4). Generally, the antiproliferative activities of these

compounds were consistent with their HDACs inhibitory activities. Compounds **9m** and **9n** with the most potent HDACs inhibitory potency also exhibited the most potent antiproliferative activities, which were comparable to that of MS-275.

2.4. HDAC class I and class IIa Whole-cell assay

Whole-cell HDAC inhibition assay in HEL cells was used to evaluate the HDACs class selectivity of five representative compounds. Results in Table 5 showed that except **9a** and **23a**, which was not active for both class I and class IIa HDACs, all tested *o*-phenylene-diamine-based HDACIs exhibited selectivity for class I HDACs over class IIa.

2.5. In vitro HDACs isoform selectivity fluorescence assay

The compounds were tested for their inhibition against HDAC1, 2 and 3 to further explore their HDAC isoform selective profiles (Table 6). MS-275 was used as the positive control. As expected, 5-substituted-*o*-phenylenediamine **23a** exhibited dramatic HDAC1/2 selectivity over HDAC3. It is worth noting that compared with MS-275, compound **9n** exhibited moderate HDAC1 selectivity over HDAC2 and HDAC3.

2.6. Docking Study

To explore the binding mode of these compounds in HDACs, compounds **9h** and **9n** were docked into the active site of HDAC1 (PDB code 5ICN). Results in Fig. 3 showed that **9h** and **9n** shared a similar binding mode. For both compounds, the isatin-based caps were solvent-exposed (Fig. 3a) and the *o*-phenylenediamine-based zinc binding groups chelated the Zn^{2+} (Fig. 3b). However, compared with **9h**, the phenyl group in the linker part of **9n** could form more favorable p-p stacking interaction with the two parallel phe-nyl groups of Phe 150 and Phe 205 in HDAC1 (Fig. 3b). This could rationalize the better HDAC1 inhibitory activity of **9n.**

3. Conclusions

A novel series of compounds with isatin-based caps and *o*-phenylenediamine-based zinc binding groups as HDACIs were designed and synthesized based on the previous work in our laboratory.[17]All synthesized target compounds were evaluated for their HDAC inhibition using HeLa nuclear extract. Given their better HDACs inhibitory potency, compounds **9d**, **9h**, **9m**, **9n** and **23a** were chosen for the antiproliferative assays, and compounds **9m** and **9n** showed promising antiproliferative effects which were consistent with their potent HDAC inhibitory potency. More importantly, compared with the MS-275, compound **9n** presented moderate HDAC1 selectivity over HDAC2 and HDAC3, which could be used as a lead compound to find more selective HDAC1 inhibitors.

4. Experimental section

4.1. Chemistry

4.1.1. Materials and methods—All materials, reagents and solvents used without further purification unless otherwise stated were purchased from commercial sources. All reactions were monitored by TLC with 0.25 mm silica gel plates (60 GF-254). UV light,

iodine stain and ninhydrin were used to visualize the spots. ^{1}H and ^{13}C were recorded on a Bruker DRX spectrometer at 300/400 MHz with tetramethylsilane (TMS) as an internal reference, δ in parts per million (ppm) and J in Hertz. High-resolution mass spectra were obtained from Shandong Analysis and Test Centre in Ji'nan, China. ESI-MS spectra were recorded on an API 4000 spectrometer. Melting points were determined on an electrothermal melting point apparatus without correcting. Silica gel was used for column chromatography purification. The purity of the tested compounds was assessed by an Agilent 1200 HPLC instrument. An ODS HYPERSIL column (4.6 mm 250 mm, 5 mm) was used with 30% water/70% methanol (method A for compounds **9a**–**9d**, **9n**–**9o**, **12a**–**12f**, **23a**–**23d** and **30**) or 35% water/65% methanol (method B for compounds **9e**–**9m**) over 18 min at a flow rate of 1.0 mL/min with detection at 254 nm. All final compounds were determined to be at least 95% pure by HPLC analysis.

(*E*)-*N*-(4-Bromophenyl)-2-(hydroxyimino) acetamide (**2a**), 5-bromoindoline-2, 3-dione (**3a**), 5-bromospiro [indoline-3, 2′-[1,3] dioxan]-2-one (**4a**), methyl 2-(5-bromo-2-oxospiro [indoline-3, 2′-[1,3]dioxan]-1-yl) acetate (**5a**), 2-(5-bromo-2-oxospiro[indo-line-3,2′-[1,3]dioxan]-1-yl)acetic acid (**6a**), methyl 4-(2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)ben-zoate (**7a**), 4-(2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)benzoic acid (**8a**), *N*-(2-aminophenyl)-4-(2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)ben-zamide (**9a**), methyl 6-(2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]-dioxan]-1-yl)acetamido)hexanoate (**10a**) and their analogues **2b**–**2c**, **3b**–**3c**,**4b**–**4f**, **5b**–**5h**, **6b**–**6h**, **7b**–**7o** and **10b**–**10f** in relevant steps were synthesized as described previously.[17]

4.1.2. General procedure for the preparation of compounds 8b and its analogue 8c–8o

4.1.2.1. 4-(2-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)ac-etamido)benzoic acid (8b): To a solution of compound **7b** (0.29 g, 0.67 mmol) in 6 mL THF and 3 mL CH_3OH was added 3 M aqueous NaOH (0.7 mL). Keep the reaction for 2 h at room temperature (r.t.). Then 1 M aqueous HCl was added to the mixture gradually until the solution became acidic. After filtration, compound **8b** was obtained as white solid (0.20 g, 72% yield). Mp: 226–228 °C, ESI-MS *m/z:* 417.8 $[M+H]^+$.

4.1.2.2. 4-(2-(5-Fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)ac-etamido)benzoic acid (8c): White solid, 91% yield. Mp: >250 °C, ESI-MS *m/z:* 401.1 $[M+H]^+$.

4.1.2.3. 4-(2-(5-Bromo-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl) acetamido)benzoic acid (8d): White solid, 92% yield. Mp: >250 °C, ESI-MS *m/z*: 447.0 $[M+H]^+$.

4.1.2.4. 4-(2-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl) acetamido)benzoic acid (8e): White solid, 78% yield. Mp: >250 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 10.70 (s, 1H), 7.90 (d, J = 8.8 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.52 (d, J = 2.1 Hz, 1H), 7.48 (dd, J = 8.4, 2.2 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 4.56 (s, 2H), 4.37–4.32 (m, 4H).

4.1.2.5. 4-(2-(5-Fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl) acetamido)benzoic acid (8f): White solid, 86% yield. Mp: >250 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 10.71 (s, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.69 (d, J = 8.7 Hz, 2H), 7.37 (dd, J = 7.6, 2.6 Hz, 1H), 7.28 (td, J = 9.2, 2.7 Hz, 1H), 7.08 (dd, J = 8.6, 4.0 Hz, 1H), 4.56 (s, 2H), 4.38–4.31 (m, 4H).

4.1.2.6. 4-((2-(5-Bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl) acetamido)methyl)benzoic acid (8g): White solid, 88% yield. Mp: >250 °C, ESI-MS *m/z*: 475.1 $[M+H]^+$.

4.1.2.7. 4-((2-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl) acetamido)methyl)benzoic acid (8h): White solid, 91% yield. Mp: 228–230 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.88 (s, 1H), 8.85 (t, J = 5.9 Hz, 1H), 7.91 (d, J = 8.3 Hz, 2H), 7.45 (dd, J = 8.4, 2.2 Hz, 1H), 7.42–7.39 (m, 2H), 7.38 (s, 1H), 6.96 (d, J = 8.4 Hz, 1H), 4.72 (td, J = 11.5, 2.4 Hz, 2H), 4.39 (s, 2H), 4.37 (s, 2H), 3.98–3.91 (m, 2H), 2.26–2.12 (m, 1H), 1.74–1.66 (m, 1H).

4.1.2.8. 4-((2-(5-Fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl) acetamido)methyl)benzoic acid (8i): White solid, 90% yield. Mp: >250 °C, ESI-MS *m/z*: 415.1 $[M+H]^+$.

4.1.2.9. 4-((2-(5-Bromo-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl) acetamido)methyl)benzoic acid (8j): White solid, 71% yield. Mp: >250 °C, ESI-MS *m/z*: 461.0 $[M+H]^+$.

4.1.2.10. 4-((2-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)methyl)benzoic acid (8k): White solid, 93% yield. Mp: >250 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.88 (s, 1H), 8.85 (t, J = 5.9 Hz, 1H), 7.91 (d, J = 8.3 Hz, 2H), 7.52–7.45 (m, 2H), 7.38 (d, J = 8.3 Hz, 2H), 6.99 (dd, J = 7.6, 1.3 Hz, 1H), 4.38 (s, 2H), 4.37 (s, 2H), 4.36–4.28 (m, 4H).

4.1.2.11. 4-((2-(5-Fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl) acetamido)methyl)benzoic acid (8l): White solid, 59% yield. Mp: 230–232 °C, ESI-MS *m/z*: 401.1 $[M+H]^+$.

4.1.2.12. 4-(3-(5-Bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl) propanamido)benzoic acid (8m): White solid, 86% yield. Mp: >250 °C, ^{1}H NMHR (400 MHz, DMSO-d_6) δ 12.52 (s, 1H), 10.32 (s, 1H), 7.92–7.85 (m, 2H), 7.65 (d, J = 8.7 Hz, 2H), 7.61 (dd, J = 8.4, 2.1 Hz, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.14 (t, J = 5.7 Hz, 1H), 4.70 (td, J = 11.4, 2.2 Hz, 2H), 3.97–3.88 (m, 4H), 2.69 (t, J = 7.0 Hz, 2H), 2.23–2.10 (m, 1H), 1.72–1.63 (m, 1H).

4.1.2.13. 4-(3-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl) propanamido)benzoic acid (8n): White solid, 95% yield. Mp: >250 °C, ESI-MS *m/z*: 431.1 $[M+H]^+$.

4.1.2.14. 4-((3-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl) propanamido)methyl)benzoic acid (8o): White solid, 98% yield. Mp: >250 °C, ESI-MS *m/z*: 445.1 $[M+H]^+$.

4.1.3. General procedure for the preparation of compounds 9b and its analogue 9c–9o

4.1.3.1. N-(2-Aminophenyl)-4-(2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)benzamide (9b): To a solution of **8b** (0.15 g, 0.36 mmol) in dry THF and CH_2Cl_2 was added TBTU (0.14 g, 0.43 mmol), followed by Et_3N (0.2 mL, 1.44 mmol). After 40 min in room temperature, *o*-phenylenediamine (0.05 g, 0.43 mmol) was added. The mixture was stirred at the same temperature overnight. Then the solvent was evaporated under vacuum. The residue was dissolved in EtOAc and washed with brine (3×20 mL). Organic phase was combined and dried by $MgSO_4$ overnight. The EtOAc was removed under low pressure to give a crude material that was purified via silica-gel column chromatography (CH_2Cl_2/CH_3OH = 100:1), yielding the desired compound **9b** as a reddish white solid (0.25 g, 66% yield). Mp: >250 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 9.60 (s, 1H), 7.98 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.52–7.39 (m, 2H), 7.16 (d, J = 7.5 Hz, 1H), 7.09 (d, J = 8.2 Hz, 1H), 7.02–6.92 (m, 1H), 6.78 (dd, J = 7.9, 0.9 Hz, 1H), 6.65–6.55 (m, 1H), 4.89 (s, 2H), 4.74 (t, J = 10.5 Hz, 2H), 4.59 (s, 2H), 3.99–3.95 (m, 2H), 2.27–2.13 (m, 1H), 1.73–1.69 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.34, 165.66, 165.10, 143.61, 141.80, 141.77, 131.15, 129.82, 129.30, 128.72, 127.41, 127.15, 126.87, 124.45, 123.91, 118.70, 116.74, 116.62, 111.78, 93.44, 61.17, 42.79, 25.21. HRMS (AP-ESI) *m/z* calcd for $C_{26}H_{23}ClN_4O_5$ $[M+H]^+$ 507.1430, found 507.1563. HPLC t_R = 8.37 min, 97.5%.

4.1.3.2. N-(2-Aminophenyl)-4-(2-(5-fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)benzamide (9c): White solid, 51% yield. Mp: >250 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 9.59 (s, 1H), 7.97 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.30 (dd, J = 7.7, 2.6 Hz, 1H), 7.25 (td, J = 9.2, 2.7 Hz, 1H), 7.16 (d, J = 7.5 Hz, 1H), 7.07 (dd, J = 8.6, 4.1 Hz, 1H), 6.96 (t, J = 7.6 Hz, 1H), 6.78 (d, J = 8.7 Hz, 1H), 6.59 (t, J = 7.9 Hz, 1H), 4.88 (s, 2H), 4.75 (dd, J = 11.5, 9.3 Hz, 2H), 4.58 (s, 2H), 3.97 (dd, J = 11.4, 2.9 Hz, 2H), 2.27–2.13 (m, 1H), 1.72–1.69 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.57, 165.77, 165.02, 160.19, 157.81, 143.62, 141.82, 139.05, 129.80, 129.30, 128.47, 128.39, 127.15, 126.87, 123.90, 118.69, 117.73, 117.50, 116.73, 116.61, 112.35, 112.10, 111.27, 111.20, 93.54, 61.09, 42.80, 25.21. HRMS (AP-ESI) *m/z* calcd for $C_{26}H_{23}FN_4O_5$ $[M+H]^+$ 491.1725, found 491.1851. HPLC t_R = 5.64 min, 95.4%.

4.1.3.3. N-(2-Aminophenyl)-4-(2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)benzamide (9d): White solid, 60% yield. Mp: >250 °C,^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 9.59 (s, 1H), 7.95 (t, J = 12.3 Hz, 2H), 7.69 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 6.8 Hz, 2H), 7.15 (d, J = 7.6 Hz, 1H), 7.07 (d, J = 9.0 Hz, 1H), 6.96 (t, J = 7.4 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 6.59 (t, J = 7.4 Hz, 1H), 4.89 (s, 2H), 4.57 (s, 2H), 4.39–4.30 (m, 4H). 13C NMR (100 MHz, DMSO-d_6) δ 172.84, 165.60, 165.11, 143.70, 143.60, 141.77, 134.68, 129.86, 129.28, 127.88, 127.13, 126.85, 126.81, 123.93, 118.73, 116.74,

116.62, 115.27, 112.57, 101.52, 66.39, 46.18, 43.09. HRMS (AP-ESI) *m/z* calcd for $C_{25}H_{21}BrN_4O_5$ $[M+H]^+$ 537.0768, found 537.0769. HPLC t_R = 6.50 min, 95.3%.

4.1.3.4. N-(2-Aminophenyl)-4-(2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)benzamide (9e): White solid, 55% yield. Mp: >250 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 9.58 (s, 1H), 7.97 (d, J = 8.7 Hz, 2H), 7.69 (d, J = 8.7 Hz, 2H), 7.53 (d, J = 2.1 Hz, 1H), 7.50 (dd, J = 8.4, 2.2 Hz, 1H), 7.16 (d, J = 7.0 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 7.00–6.93 (m, 1H), 6.78 (dd, J = 8.0, 1.2 Hz, 1H), 6.59 (td, J = 7.8, 1.2 Hz, 1H), 4.90 (s, 2H), 4.58 (s, 2H), 4.39–4.30 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.96, 165.63, 165.10, 143.61, 143.26, 141.77, 131.87, 129.85, 129.29, 127.70, 127.14, 126.86, 126.47, 125.18, 123.91, 118.72, 116.74, 116.61, 112.10, 101.56, 66.38, 43.11. HRMS (AP-ESI) *m/z* calcd for $C_{25}H_{21}ClN_4O_5$ $[M+H]^+$ 493.1273, found 493.1410. HPLC t_R = 8.36 - min, 97.7%.

4.1.3.5. N-(2-Aminophenyl)-4-(2-(5-fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)benzamide (9f): White solid, 66% yield. Mp: >250 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.25 (t, J = 5.4 Hz, 1H), 7.26 (dd, J = 7.7, 2.7 Hz, 1H), 7.23–7.14 (m, 2H), 6.93–6.83 (m, 2H), 6.71 (dd, J = 8.0, 1.2 Hz, 1H), 6.53 (td, J = 7.7, 1.4 Hz, 1H), 4.81 (s, 2H), 4.73 (td, J = 11.5, 2.4 Hz, 2H), 4.26 (s, 2H), 3.99–3.88 (m, 2H), 3.09 (q, 6.6 Hz, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.25–2.10 (m, 1H), 1.72–1.65 (m, 1H), 1.65–1.55 (m, 2H), 1.52–1.41 (m, 2H), 1.39–1.29 (m, 2H). 13C NMR (100 MHz, DMSO-d_6) δ 172.95, 171.55, 166.13, 142.28, 140.55, 126.19, 125.76, 124.09, 118.35, 118.11, 116.69, 116.40, 112.88, 112.64, 111.41, 111.33, 101.67, 66.23, 42.39, 39.12, 36.16, 29.26, 26.57, 25.47. HRMS (AP-ESI) *m/z* calcd for $C_{25}H_{21}FN_4O_5$ $[M+H]^+$ 477.1569, found 477.1717. HPLC t_R = 11.55 min, 95.2%.

4.1.3.6. N-(2-Aminophenyl)-4-((2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)methyl)benzamide (9g): White solid, 34% yield. Mp: 219–221 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (s, 1H), 8.88 (t, J = 5.7 Hz, 1H), 7.95 (d, J = 8.0 Hz, 2H), 7.59 (dd, J = 8.4, 1.9 Hz, 1H), 7.52 (d, J = 1.9 Hz, 1H), 7.40 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 7.7 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 6.60 (t, J = 7.4 Hz, 1H), 4.86 (s, 2H), 4.72 (t, J = 10.6 Hz, 2H), 4.39 (d, J = 7.0 Hz, 4H), 3.94 (dd, J = 11.1, 2.9 Hz, 2H), 2.27–2.09 (m, 1H), 1.69 (d, J = 13.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.09, 166.58, 165.54, 143.61, 143.00, 142.23, 133.82, 129.08, 128.33, 127.45, 127.17, 127.11, 126.66, 123.80, 116.66, 114.88, 112.13, 93.36, 61.13, 42.52, 42.13, 25.21. HRMS (AP-ESI) *m/z* calcd for $C_{27}H_{25}BrN_4O_5$ $[M+H]^+$ 565.1081, found 565.1086. HPLC t_R = 11.94 min, 98.3%.

4.1.3.7. N-(2-Aminophenyl)-4-((2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)methyl)benzamide (9h): White solid, 31% yield. Mp: 203–205 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (s, 1H), 8.87 (t, J = 5.8 Hz, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.46 (dd, J = 8.4, 2.1 Hz, 1H), 7.44–7.41 (m, 2H), 7.40 (s, 1H), 7.18 (d, J = 7.6 Hz, 1H), 6.98 (t, J = 7.2 Hz, 2H), 6.79 (d, J = 7.3 Hz, 1H), 6.61 (t, J = 7.4 Hz, 1H), 4.90 (s, 2H), 4.73 (t, J = 10.5 Hz, 2H), 4.41 (s, 2H), 4.39 (s, 2H), 3.97–3.94 (m, 2H), 2.27–2.12 (m, 1H), 1.72–1.69 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.19, 166.61, 165.55, 143.62, 143.00,

141.79, 133.72, 131.05, 128.75, 128.33, 127.45, 127.33, 127.18, 126.96, 124.41, 123.78, 116.72, 116.59, 111.64, 93.40, 61.11, 42.52, 42.15, 25.20. HRMS (AP-ESI) *m/z* calcd for $C_{27}H_{25}ClN_4O_5$ $[M+H]^+$ 521.1586, found 521.1702. HPLC t_R = 10.54 min, 98.1%.

4.1.3.8. N-(2-Aminophenyl)-4-((2-(5-fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)methyl)benzamide (9i): White solid, 39% yield. Mp: 198–200 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (s, 1H), 8.87 (t, J = 5.7 Hz, 1H), 7.95 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 7.28 (dd, J = 7.6, 2.3 Hz, 1H), 7.25–7.20 (m, 1H), 7.17 (d, J = 7.6 Hz, 1H), 7.01–6.91 (m, 2H), 6.79 (d, J = 7.7 Hz, 1H), 6.60 (t, J = 7.4 Hz, 1H), 4.90 (s, 2H), 4.74 (dd, J = 11.2, 9.7 Hz, 2H), 4.43–4.36 (m, 4H), 3.95 (dd, J = 10.8, 3.0 Hz, 2H), 2.25–2.11 (m, 1H), 1.71–1.68 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.41, 166.71, 165.54, 160.15, 157.77, 143.63, 143.03, 139.05, 133.72, 128.32, 127.45, 127.18, 126.95, 123.78, 117.63, 117.40, 116.72, 116.59, 112.32, 112.07, 111.12, 111.04, 93.52, 61.03, 42.51, 42.18, 25.21. HRMS (AP-ESI) *m/z* calcd for $C_{27}H_{25}FN_4O_5$ $[M+H]^+$ 505.1882, found 505.1986. HPLC t_R = 6.89 min, 97.2%.

4.1.3.9. N-(2-Aminophenyl)-4-((2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)methyl)benzamide (9j): White solid, 18% yield. Mp: 231–233 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 8.86 (t, J = 5.8 Hz, 1H), 7.95 (d, J = 8.1 Hz, 2H), 7.65–7.58 (m, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.17 (d, J = 7.5 Hz, 1H), 7.01–6.92 (m, 2H), 6.78 (d, J = 9.1 Hz, 1H), 6.60 (t, J = 7.5 Hz, 1H), 4.91 (s, 2H), 4.39 (s, 2H), 4.37 (s, 2H), 4.39–4.31 (m, 4H).^{13}C NMR (100 MHz, DMSO-d_6) δ 172.68, 166.53, 165.57, 143.72, 143.57, 142.98, 134.56, 133.77, 128.32, 127.82, 127.46, 127.13, 126.90, 123.85, 116.75, 116.62, 115.18, 112.41, 101.51, 66.35, 42.55, 42.47. HRMS (AP-ESI) *m/z* calcd for $C_{26}H_{23}BrN_4O_5$ $[M+H]^+$ 551.0928, found 551.0926. HPLC t_R = 7.93 min, 95.8%.

4.1.3.10. N-(2-Aminophenyl)-4-((2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)methyl)benzamide (9k): White solid, 41% yield. Mp: >250 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 8.85 (t, J = 5.9 Hz, 1H), 7.95 (d, J = 8.1 Hz, 2H), 7.53–7.46 (m, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.17 (d, J = 7.3 Hz, 1H), 7.03–6.93 (m, 2H), 6.79 (dd, J = 8.0, 1.3 Hz, 1H), 6.60 (td, J = 7.7, 1.2 Hz, 1H), 4.89 (s, 2H), 4.39 (s, 2H), 4.37 (s, 2H), 4.36–4.28 (m, 4H). 13C NMR (100 MHz, DMSO-d_6) δ 172.80, 166.56, 165.56, 143.60, 143.27, 142.99, 133.75, 131.70, 128.32, 127.62, 127.46, 127.16, 126.94, 126.56, 125.12, 123.81, 116.73, 116.61, 111.94, 101.55, 66.34, 42.53, 42.48. HRMS (AP-ESI) *m/z* calcd for $C_{26}H_{23}$-ClN_4O_5 $[M+H]^+$ 507.1430, found 507.1538. HPLC t_R = 7.17 min, 98.3%.

4.1.3.11. N-(2-Aminophenyl)-4-((2-(5-fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)methyl)benzamide (9l): White solid, 50% yield. Mp: 230–232 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.62 (s, 1H), 8.84 (t, J = 5.9 Hz, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 7.34 (dd, J = 7.7, 2.7 Hz, 1H), 7.31–7.23 (m, 1H), 7.20–7.14 (m, 1H), 6.97 (ddd, J = 9.5, 6.1, 1.7 Hz, 2H), 6.78 (dd, J = 8.0, 1.3 Hz, 1H), 6.60 (td, J = 7.7, 1.3 Hz, 1H), 4.88 (s, 2H), 4.42–4.36(m, 4H), 4.35–4.30 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.04, 166.66, 165.55, 160.34, 157.96, 143.61, 143.02, 140.55, 133.74, 128.32, 128.06, 127.76, 127.46, 127.17, 126.94, 123.80, 118.36, 118.12, 116.72, 116.60, 112.94, 112.69,

111.46, 111.39, 66.26, 42.49. HRMS (AP-ESI) *m/z* calcd for $C_{26}H_{23}FN_4O_5$ $[M+H]^+$ 491.1725, found 491.1836. HPLC t_R = 9.09 min, 95.2%.

4.1.3.12. N-(2-Aminophenyl)-4-(3-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)propanamido)benzamide (9m): White solid, 44% yield. Mp: 218–220 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 9.57 (s, 1H), 7.95 (d, J = 8.6 Hz, 2H), 7.67 (d, J = 8.7 Hz, 2H), 7.62 (dd, J = 8.4, 2.0 Hz, 1H), 7.52 (d, J = 2.0 Hz, 1H), 7.19–7.13 (m, 2H), 7.00–6.94 (m, 1H), 6.79 (dd, J = 7.9, 0.9 Hz, 1H), 6.63–6.57 (m, 1H), 4.89 (s, 2H), 4.72 (dd, J = 11.5, 9.4 Hz, 2H), 3.98–3.89 (m, 4H), 2.70 (t, J = 7.0 Hz, 2H), 2.25–2.11 (m, 1H), 1.69 (d, J = 13.4 Hz, 1H). 13C NMR (100 MHz, DMSO-d_6) δ 170.98, 169.46, 165.17, 143.58, 142.14, 141.58, 134.07, 129.54, 129.32, 129.14, 127.25, 127.12, 126.85, 123.96, 118.78, 116.76, 116.62, 114.98, 112.18, 93.20, 61.07, 36.16, 34.72, 25.19. HRMS (AP-ESI) *m/z* calcd for $C_{27}H_{25}BrN_4O_5$ $[M+H]^+$ 565.1081, found 565.1078. HPLC t_R = 18.11 min, 97.5%.

4.1.3.13. N-(2-Aminophenyl)-4-(3-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)propanamido)benzamide (9n): White solid, 25% yield. Mp: 220–222 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 9.55 (s, 1H), 7.94 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 7.49 (dd, J = 8.4, 2.2 Hz, 1H), 7.41 (d, J = 2.2 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.16 (d, J = 6.9 Hz, 1H), 7.02–6.92 (m, 1H), 6.78 (dd, J = 8.0, 1.2 Hz, 1H), 6.60 (td, J = 7.7, 1.3 Hz, 1H), 4.89 (s, 2H), 4.72 (td, J = 11.5, 2.4 Hz, 2H), 4.03–3.87 (m, 4H), 3.32 (s, 2H), 2.70 (t, J = 7.0 Hz, 2H), 2.24–2.10 (m, 1H), 1.74–1.63 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.08, 169.46, 165.18, 143.52, 142.14, 141.16, 131.20, 129.55, 129.13, 129.02, 127.39, 127.10, 126.84, 124.55, 124.00, 118.79, 116.80, 116.65, 111.68, 93.26, 61.06, 36.18, 34.73, 25.19. HRMS (AP-ESI) *m/z* calcd for $C_{27}H_{25}ClN_4O_5$ $[M+H]^+$ 521.1586, found 521.1579. HPLC t_R = 10.44 min, 95.8%.

4.1.3.14. N-(2-Aminophenyl)-4-((3-(5′-chloro-2′-oxospiro[[1,3]diox-ane-2,3′-indolin]-1′-yl)propanamido)methyl)benzamide (9o): White solid, 37% yield. Mp: 171–173 °C,^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H), 8.56 (t, J = 5.9 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.44 (dd, J = 8.3, 2.2 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.29 (d, J = 8.2 Hz, 2H), 7.16 (d, J = 7.3 Hz, 1H), 7.11 (d, J = 8.3 Hz, 1H), 7.01–6.93 (m, 1H), 6.78 (dd, J = 8.0, 1.3 Hz, 1H), 6.65–6.56 (m, 1H), 4.89 (s, 2H), 4.74 (td, J = 11.5, 2.5 Hz, 2H), 4.32 (d, J = 5.8 Hz, 2H), 3.98–3.91 (m, 2H), 3.88 (t, J = 7.0 Hz, 2H), 2.52 (t, J = 8.7 Hz, 2H), 2.25–2.11 (m, 1H), 1.75–1.66 (m, 1H). 13C NMR (100 MHz, DMSO-d_6) δ 171.04, 170.16, 165.54, 143.55, 143.21, 141.17, 133.58, 131.15, 128.98, 128.23, 127.37, 127.12, 126.91, 124.50, 123.87, 116.76, 116.63, 111.72, 93.27, 61.08, 42.35, 36.49, 33.66, 25.22. HRMS (AP-ESI) *m/z* calcd for $C_{28}H_{27}ClN_4O_5$ $[M+H]^+$ 535.1743, found 535.1742. HPLC t_R = 8.14 min, 97.3%.

4.1.4. General procedure for the preparation of compounds 11a and its analogue 11a–11f

4.1.4.1. 6-(2-(5-Bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)ac-etamido)hexanoic acid (11a): To a solution of compound 10a (0.20 g, 0.43 mmol) in 6 mL THF and 3 mL CH3OH was added 3 M aqueous NaOH (0.42 mL). Keep the reaction for 2 h at room temperature. Then 1 M aqueous HCl was added to the mixture gradually until the solution

became acidic. After filtration, the compound 11a was obtained as white solid (0.18 g, 93% yield). ESI-MS *m/z*: 455.1 $[M+H]^+$.

4.1.4.2. 6-(2-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)ac-etamido)hexanoic acid (11b): White solid, 89% yield. Mp: 143–145 °C, ESI-MS *m/z*: 411.1 $[M+H]^+$.

4.1.4.3. 6-(2-(5-Fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)ac-etamido)hexanoic acid (11c): White solid, 93% yield. Mp: 144–146 °C, ESI-MS *m/z*: 395.2 $[M+H]^+$.

4.1.4.4. 6-(2-(5-Bromo-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl) acetamido)hexanoic acid (11d): White solid, 74% yield. Mp: 165–167 °C, ESI-MS *m/z*: 441.1 $[M+H]^+$.

4.1.4.5. 6-(2-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl) acetamido)hexanoic acid (11e): White solid, 98% yield. Mp: 170–172 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 8.22 (t, J = 5.5 Hz, 1H), 7.50–7.44 (m, 2H), 6.92 (dd, J = 8.0, 0.6 Hz, 1H), 4.38–4.28 (m, 4H), 4.25 (s, 2H), 3.05 (q, J = 6.7 Hz, 2H), 2.19 (t, J = 7.4 Hz, 2H), 1.54–1.45 (m, 2H), 1.45–1.37 (m, 2H), 1.32–1.22 (m, 2H).

4.1.4.6. 6-(2-(5-Fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl) acetamido)hexanoic acid (11f): White solid, 79% yield. Mp: 133–135 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.98 (s, 1H), 8.24 (t, J = 5.4 Hz, 1H), 7.32 (dd, J = 7.7, 2.7 Hz, 1H), 7.30–7.20 (m, 1H), 6.90 (dd, J = 8.6, 4.1 Hz, 1H), 4.41–4.26 (m, 4H), 4.24 (s, 2H), 3.06 (q, 6.6 Hz, 2H), 2.20 (t, J = 7.3 Hz, 2H), 1.59–1.46 (m, 2H), 1.45–1.36 (m, 2H), 1.33–1.19 (m, 2H).

4.1.5. General procedure for the preparation of compounds 12a and its analogue 12b–12f

4.1.5.1. N-(2-Aminophenyl)-6-(2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)hexanamide (12a): To a solution of **11a** (0.20 g, 0.44 mmol) in dry THF and CH_2Cl_2 was added TBTU (0.17 g, 0.53 mmol), followed by Et_3N (0.25 mL, 1.76 mmol). After 40 min in room temperature, *o*-phenylenediamine (0.06 g, 0.53 mmol) was added. The mixture was stirred at the same temperature overnight. Then the solvent was evaporated under vacuum. The residue was dissolved in EtOAc and washed with brine (3×20 mL). Organic phase was combined and dried by $MgSO_4$ overnight. The EtOAc was removed under vacuum to give a crude material that was purified via silica-gel column chromatography (CH_2Cl_2/CH_3OH = 100:1), yielding the desired compound **12a** as white solid (0.25 g, 54% yield). Mp: 139–140 °C, 1H NMR (400 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.24 (t, J = 5.0 Hz, 1H), 7.58–7.48 (m, 2H), 7.16 (d, J = 7.7 Hz, 1H), 6.93–6.80 (m, 2H), 6.71 (d, J = 7.9 Hz, 1H), 6.54 (t, J = 7.5 Hz, 1H), 4.80 (s, 2H), 4.71 (t, J = 10.6 Hz, 2H), 4.26 (s, 2H), 3.92 (d, J = 2.8 Hz, 2H), 3.09 (dd, J = 12.2, 6.3 Hz, 2H), 2.31 (t, J = 7.3 Hz, 2H), 2.25–2.10 (m, 1H),1.68 (d, J = 13.4 Hz, 1H), 1.60 (dt, J = 14.7, 7.4 Hz, 2H), 1.51–1.41 (m, 2H), 1.38–1.28 (m, 2H). 13C NMR (100 MHz, DMSO-d_6) δ 171.54, 171.02, 166.04, 142.34, 142.25, 133.90, 129.06, 127.13, 126.15, 125.75, 124.08, 116.66, 116.38, 114.80, 112.06, 93.35,61.11, 60.21, 42.02, 39.15, 36.17, 29.25, 26.58, 25.47, 25.21. HRMS (AP-

ESI) *m/z* calcd for $C_{25}H_{29}BrN_4O_5$ $[M+H]^+$ 545.1394, found 545.1395. HPLC t_R = 8.54 min, 96.6%.

4.1.5.2. N-(2-Aminophenyl)-6-(2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)hexanamide (12b): White solid, 53% yield. Mp: 123–125 °C, ^{1}H ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.26 (t, J = 5.4 Hz, 1H), 7.45–7.39 (m, 2H), 7.16 (d, J = 7.2 Hz, 1H), 6.89 (dd, J = 7.8, 3.7 Hz, 2H), 6.71 (d, J = 7.2 Hz, 1H), 6.54 (t, J = 7.5 Hz, 1H), 4.72 (t, J = 10.5 Hz, 2H), 4.27 (s, 2H), 3.95–3.92 (m, 2H), 3.09 (q, J = 6.4 Hz, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.25–2.11 (m, 1H), 1.70–1.67 (m, 1H), 1.65–1.55 (m, 2H), 1.50–1.43 (m, 2H), 1.37–1.29 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.54, 171.11, 166.07, 142.35, 141.81, 131.04, 128.70, 127.25, 126.16, 125.77, 124.41, 124.04, 116.65, 116.37, 111.58, 93.39, 61.08, 42.02, 39.13, 36.16, 29.26, 26.57, 25.48, 25.20. HRMS (AP-ESI) *m/z* calcd for $C_{25}H_{29}ClN_4O_5$ $[M+H]^+$ 501.1899, found 501.2034. HPLC t_R = 7.71 min, 98.5%.

4.1.5.3. N-(2-Aminophenyl)-6-(2-(5-fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)hexanamide (12c): White solid, 42% yield. Mp: 129–131 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.25 (t, J = 5.4 Hz, 1H), 7.26 (dd, J = 7.7, 2.7 Hz, 1H), 7.23–7.14 (m, 2H), 6.93–6.83 (m, 2H), 6.71 (dd, J = 8.0, 1.2 Hz, 1H), 6.53 (td, J = 7.7, 1.4 Hz, 1H), 4.81 (s, 2H), 4.73 (td, J = 11.5, 2.4 Hz, 2H), 4.26 (s, 2H), 3.99–3.88 (m, 2H), 3.09 (q, 6.6 Hz, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.25–2.10 (m, 1H), 1.72–1.65 (m, 1H), 1.65–1.55 (m, 2H), 1.52–1.41 (m, 2H), 1.39–1.29 (m, 2H). 13C NMR (100 MHz, DMSO-d_6) δ 171.54, 171.33, 166.17, 142.36, 139.08, 128.46, 128.39, 126.16, 125.77, 124.05, 117.61, 117.37, 116.65, 116.37, 112.25, 112.00, 111.05, 110.97, 61.01, 42.06, 39.12, 36.16, 29.27, 26.58, 25.48, 25.21. HRMS (AP-ESI) *m/z* calcd for $C_{25}H_{29}FN_4$-O $[M+H]^+$ 485.2195, found 485.2249. HPLC t_R = 5.46 min, 96.6%.

4.1.5.4. N-(2-Aminophenyl)-6-(2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)hexanamide (12d): White solid, 31% yield. Mp: 158–160 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.25 (s, 1H), 7.58 (d, J = 7.8 Hz, 2H), 7.16 (d, J = 7.6 Hz, 1H), 6.88 (t, J = 9.2 Hz, 2H), 6.71 (d, J = 7.7 Hz, 1H), 6.53 (t, J = 7.3 Hz, 1H), 4.81 (s, 2H), 4.37–4.28 (m, 4H), 4.25 (s, 2H), 3.13–3.02 (m, 2H), 2.31 (t, J = 7.2 Hz, 2H), 1.63–1.56 (m, 2H), 1.47–1.42 (m, 2H), 1.37–1.28 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 172.59, 171.55, 166.00, 143.73, 142.34, 134.56, 127.76, 126.83, 126.15, 125.76, 124.08, 116.67, 116.38, 115.10, 112.36, 101.50, 66.32, 42.36, 39.15, 36.17, 29.24, 26.57, 25.47. HRMS (AP-ESI) *m/z* calcd for $C_{24}H_{27}BrN_4O_5$ $[M+H]^+$ 531.1238, found 531.1656. HPLC t_R = 6.00 min, 95.9%.

4.1.5.5. N-(2-Aminophenyl)-6-(2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)hexanamide (12e): White solid, 69% yield. Mp: 170–172 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.25 (t, J = 5.2 Hz, 1H), 7.48 (s, 1H), 7.47–7.42 (m, 1H), 7.16 (d, J = 7.8 Hz, 1H), 6.93–6.87 (m, 2H), 6.71 (d, J = 7.9 Hz, 1H), 6.55 (t, 7.4 Hz, 1H), 4.84 (s, 2H), 4.38–4.27 (m, 4H), 4.25 (s, 2H), 3.08 (dd, J = 12.5, 6.4 Hz, 2H), 2.31 (t, J = 7.3 Hz, 2H), 1.65–1.54 (m, 2H), 1.49–1.42 (m, 2H), 1.36–1.28 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 172.71, 171.54, 166.02, 143.28, 142.33, 131.71, 127.54, 126.48, 126.16,

125.77, 125.06, 124.05, 116.67, 116.38, 111.89, 101.54, 66.31, 42.36, 39.19, 36.15, 29.25, 26.57, 25.54. HRMS (AP-ESI) *m/z* calcd for $C_{24}H_{27}ClN_4O_5$ $[M+H]^+$ 487.1743, found 487.1842. HPLC t_R = 5.53 min, 95.3%.

4.1.5.6. N-(2-Aminophenyl)-6-(2-(5-fluoro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)hexanamide (12f): White solid, 46% yield. Mp: 183–185 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.73 (s, 1H), 8.87 (t, J = 5.4 Hz, 1H), 7.98 (d, J = 7.8 Hz, 2H), 7.63–7.50 (m, 3H), 7.45–7.37 (m, 4H), 7.37–7.32 (m, 2H), 7.31–7.22 (m, 2H), 6.98 (dd, J = 8.4, 3.8 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 5.13 (s, 2H), 4.45–4.37 (m, 4H), 4.37–4.25 (m, 4H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 173.05, 166.67, 165.72, 160.34, 157.96, 143.20, 143.09, 140.65, 140.53, 133.70, 129.27, 128.64, 128.38, 127.47, 126.50, 125.98, 125.27, 125.17, 124.09, 118.36, 118.13, 117.02, 112.96, 112.71, 111.47, 111.39, 101.68, 66.31, 42.52. HRMS (AP-ESI) *m/z* calcd for $C_{24}H_{27}FN_4O_5$ $[M+H]^+$ 471.2083, found 471.2183. HPLC t_R = 4.26 min, 97.6%.

4.1.6. tert- Butyl (4-Bromo-2-nitrophenyl) carbamate (14)—4-Bromo-2-nitroaniline (**13**) (1.0 g, 4.61 mmol) was dissolved in dry CH_2Cl_2 (15 mL), and then Et_3N (1.92 mL, 13.82 mmol) was added followed by $(Boc)_2O$ (1.51 g, 6.91 mmol) and DMAP (0.06 g, 0.50 mmol) solubilized in dry CH_2Cl_2. After 1 h in room temperature, the solution was washed with water, dried by Na_2SO_4 and concentrated under low pressure. The residue was washed with petroleum ether (PE) and EtOAc to afford the product **14** as a yellow solid (1.80 g, 55% yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (d, J = 2.2 Hz, 1H), 8.11 (dd, J = 8.8, 2.5 Hz, 1H), 7.64 (d, J = 8.3 Hz, 1H), 1.35 (s, 9H).

4.1.7. tert-Butyl (2-nitro-4-(thiophen-2-yl) phenyl) carbamate (15a) and tert-butyl (3-nitro-[1, 1′-biphenyl]-4-yl) carbamate (15b)

4.1.7.1. tert-Butyl (2-nitro-4-(thiophen-2-yl) phenyl) carbamate (15a): Compound **14** (0.40 g, 1.26 mmol) and 2-thienylboronic acid were dissolved in degassed 1, 4-dioxane, and then Pd[P $(pH_3)_4$] was added quickly. Degassed 3 M aqueous Na_2CO_3 (1.25 mL) was added into the solution, which was degassed for 10 min by sparging with argon gas. The reaction mixture was heated at 90 °C for 5 h under argon atmosphere. The solvent was removed with rotary evaporator. EtOAc was added. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and purified by chromatography on a silica gel column (PE/EtOAc = 90:1) to obtain compound **15a** as a pure yellow powder (0.35 g, 87% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 8.17 (d, J = 2.4 Hz, 1H), 7.94 (dd, J = 8.5, 2.3 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.63–7.58 (m, 2H), 7.16 (dd, J = 5.2, 3.5 Hz, 1H), 1.45 (s, 9H).

4.1.7.2. tert-Butyl (3-Nitro-[1, 1′-biphenyl]-4-yl) carbamate (15b): Yellow pure solid, 25% yield. ESI-MS *m/z*: 337.6 $[M+Na]^+$.

4.1.8. tert-Butyl (2-Amino-4-(thiophen-2-yl)phenyl)carbamate (16a) and tert-butyl (3-amino-[1,1′-biphenyl]-4-yl)carbamate (16b)

4.1.8.1. tert-Butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate (16a): Compound **15a** (0.53 g, 1.66 mmol) was solubilized in methanol and EtOAc (6 + 16 mL) and stirred at room

temperature for 20 h with Pd/C (0.05 g, 10% w/w) under hydrogen atmosphere. The catalyst was filtered off, and the solvent was removed and washed with PE to give compound **16a** as a white solid (0.47 g, 98% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.44–7.42 (m, 1H), 7.29 (dd, J = 3.5, 1.2 Hz, 1H), 7.26 (d, J = 8.5 Hz, 1H), 7.11 (dd, J = 5.3, 3.5 Hz, 1H), 6.97 (d, J = 2.0 Hz, 1H), 6.78 (dd, J = 8.5, 2.2 Hz, 1H), 5.04 (s, 2H), 1.45 (s, 9H).

4.1.8.2. tert-Butyl (3-amino-[1,1′-biphenyl]-4-yl)carbamate (16b): White solid, 90% yield. Mp: 171–173 °C, ESI-MS *m/z*: 307.4 $[M+Na]^+$.

4.1.9. 4-((2,2,2-Trifluoroacetamido)methyl)benzoic acid (18)—Trifluoroacetic anhydride (4 mL) was dropwise added to compound **17** (1.00 g, 6.62 mmol) under the ice baths. The suspension was stirred at room temperature for 2 h, and quenched by adding ice water. Compound **18** was collected via filtration and dried under vacuum to get a white solid (1.50 g, 93% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H), 10.09 (t, J = 5.9 Hz, 1H), 7.93 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 4.47 (d, J = 6.0 Hz, 2H).

4.1.10. tert-Butyl (4-(Thiophen-2-yl)-2-(4-((2,2,2-trifluoroacetamido) methyl) benzamido) phenyl) carbamate (20a) and tert-butyl (3-(4-((2,2,2-trifluoroacetamido)methyl) benzamido)-[1,1′-biphenyl]-4-yl) carbamate (20b)

4.1.10.1. tert-Butyl (4-(Thiophen-2-yl)-2-(4-((2,2,2-trifluoroac-etamido) methyl) benzamido) phenyl) carbamate (20a): To a solution of compound **18** (0.5 g, 2.02 mmol) in anhydrous THF (7 mL) was added $SOCl_2$ (0.74 mL, 10.11 mmol) slowly under ice baths. The mixture was heated at reflux for 3 h, and concentrated under vacuum to get crude material **19** as yellow oil. The residue solubi-lized in pyridine (3 mL) was added to the solution of the compound **16a** (0.43 g, 1.50 mmol) in pyridine (3 mL) slowly under ice baths. The mixture was stirred overnight at room temperature. Pyridine was removed. The residue was extracted with CH_2Cl_2 (3×20 mL) and washed with 1 M HCl (3×20 mL), saturated $NaHCO_3$ (3×20 mL) and brine (3×20 mL). The under solvent was evaporated vacuum to give the crude product which was recrystallized by EtOAc. Finally, the desired compound **20a** was achieved as a white solid (0.55 g, 71% yield).

4.1.10.2. tert-Butyl (3-(4-((2,2,2-Trifluoroacetamido)methyl) benza-mido)-[1,1′-biphenyl]-4-yl) carbamate (20b): White solid, 52% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.12 (t, J = 6.0 Hz, 1H), 9.96 (s, 1H), 8.80 (s, 1H), 8.00 (d, J = 8.3 Hz, 2H), 7.88 (d, J = 2.1 Hz, 1H), 7.71–7.62 (m, 3H), 7.53 (dd, J = 8.5, 2.2 Hz, 1H), 7.50–7.43 (m, 4H), 7.36 (t, J = 7.3 Hz, 1H), 4.50 (d, J = 5.9 Hz, 2H), 1.47 (s, 9H).

4.1.11. tert-Butyl (2-(4-(aminomethyl)benzamido)-4-(thiophen-2-yl) phenyl)carbamate (21a) and tert-butyl (3-(4-(aminomethyl) benzamido)-[1,1′-biphenyl]-4-yl)carbamate (21b)

4.1.11.1. tert-Butyl (2-(4-(aminomethyl)benzamido)-4-(thiophen-2-yl)phenyl)carbamate (21a): To a solution of compound **20a** (0.37 g, 0.72 mmol) in THF (4 mL), methanol (3 mL) and water (2 mL) was added K_2CO_3 (0.40 g, 2.88 mmol). The mixture was kept at reflux for 6 h, and then cooled to room temperature. The solution was concentrated and dissolved in

CH_2Cl_2 (30 mL) again. After washing with water, the product **21a** precipitated from the solvent and filtered to obtain a white solid (0.30 g, 100% yield).

4.1.11.2. tert-Butyl (3-(4-(aminomethyl)benzamido)-[1,1′-biphenyl]-4-yl)carbamate (21b): White solid, 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, J = 8.1 Hz, 1H), 7.93 (d, J = 8.1 Hz, 2H), 7.58–7.48 (m, 3H), 7.45 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.1 Hz, 1H), 6.94–6.86 (m, 1H), 4.24 (d, J = 6.3 Hz, 1H), 3.79 (s, 2H), 1.45 (s, 9H).

4.1.12. General procedure of preparation of 22a and its analogues 22b–22d

4.1.12.1. tert-Butyl (2-(4-((2-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)methyl)benzamido)-4-(thiophen-2-yl) phenyl)carbamate (22a): Compound **6b** (0.19 g, 0.65 mmol) was dissolved in anhydrous THF and CH_2Cl_2, then TBTU (0.25 g, 0.77 mmol) was added followed by Et_3N (0.33 mL, 2.36 mmol). After 40 min, another reactant, **22a** (0.25 g, 0.59 mmol) was added. The mixture solution was stirred at room temperature overnight. Solvent was concentrated and dissolved in EtOAc. The organic layer was washed with saturated $NaHCO_3$ (3×20 mL) and brine (3×20 mL), dried by $MgSO_4$, and the solution was evaporated in vacuum. The crude product was purified by silica chromatography column (CH_2Cl_2/CH_3OH = 100:1) to get compound **22a** as a white solid (0.41 g, 89% yield). Mp: 176–178 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.93 (t, J = 5.9 Hz, 1H), 8.77 (s, 1H), 7.97 (d, J = 8.3 Hz, 2H), 7.84 (d, J = 2.1 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.56–7.51 (m, 2H), 7.48–7.41 (m, 5H), 7.14 (dd, J = 5.1, 3.6 Hz, 1H), 6.98 (d, J = 8.3 Hz, 1H), 4.73 (td, J = 11.3, 2.1 Hz, 2H), 4.42 (s, 2H), 4.41 (s, 2H), 3.99–3.90 (m, 2H), 2.28–2.10 (m, 1H), 1.74–1.65 (m, 1H), 1.46 (s, 9H).

4.1.12.2. tert-Butyl (2-(4-((2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]-dioxolan]-1-yl)acetamido)methyl)benzamido)-4-(thiophen-2-yl) phenyl)carbamate (22b): White solid, 89% yield. Mp: 174–176 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.99 (t, J = 5.9 Hz, 1H), 8.80 (s, 1H), 7.98 (d, J = 8.3 Hz, 2H), 7.83 (d, J = 2.1 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.54 (dd, J = 3.6, 1.5 Hz, 2H), 7.51–7.45 (m, 4H), 7.43 (s, 1H), 7.13 (dd, J = 5.1, 3.6 Hz, 1H), 7.02 (dd, J = 7.7, 1.1 Hz, 1H), 4.41 (s, 2H), 4.39 (s, 2H), 4.37–4.30 (m, 4H), 1.46 (s, 9H).

4.1.12.3. tert-Butyl (3-(4-((2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]-dioxan]-1-yl)acetamido)methyl)benzamido)-[1,1′-biphenyl]-4-yl)car-bamate (22c): White solid, 60% yield. Mp: 141–143 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.91 (s, 1H), 8.88 (t, J = 5.9 Hz, 1H), 8.78 (s, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.87 (d, J = 2.1 Hz, 1H), 7.65 (d, J = 8.3 Hz, 3H), 7.55–7.41 (m, 7H), 7.40–7.33 (m, 1H), 6.98 (d, J = 8.3 Hz, 1H), 4.79–4.67 (m, 2H), 4.41 (s, 4H), 4.00–3.90 (m, 2H), 2.26–2.10 (m, 1H), 1.75–1.65 (m, 1H), 1.47 (s, 9H).

4.1.12.4. tert-Butyl (3-(4-((2-(5-Chloro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)methyl)benzamido)-[1,1′-biphenyl]-4-yl)carbamate (22d): White solid, 62% yield. Mp: 183–185 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 9.04 (s, 1H), 8.84 (s, 1H), 8.00 (dd, J = 8.2, 3.9 Hz, 2H), 7.88 (d, J = 1.3 Hz, 1H), 7.66 (dd, J = 8.2, 3.2 Hz, 3H), 7.52 (dd, J = 8.5, 2.2 Hz, 1H), 7.51–7.43 (m, 6H), 7.36 (t, J = 7.3 Hz, 1H), 7.02 (d, J = 7.9 Hz, 1H), 4.44–4.38 (m, 4H), 4.38–4.30 (m, 4H), 1.47 (s, 9H).

4.1.13. General procedure for the preparation of 23a and its analogues 23b–23d

4.1.13.1. N-(2-Amino-5-(thiophen-2-yl)phenyl)-4-((2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)methyl)benza-mide (23a): Compound **22a** (0.40 g, 0.57 mmol) was solubilized in the solution of EtOAc (4 mL) saturated by anhydrous HCl gas. After 5 h, the precipitate was collected by filter as a yellow solid. The crude product was washed with saturated $NaHCO_3$, extracted with EtOAc, and purified by chromatography on a silica gel column (CH_2Cl_2/CH_3OH = 80:1) to afford compound **23a** as a white solid (0.11 g, 32% yield). Mp: 208–210 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.73 (s, 1H), 8.88 (t, J = 5.8 Hz, 1H), 7.99 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 1.6 Hz, 1H), 7.46 (dd, J = 8.4, 2.2 Hz, 1H), 7.44–7.43 (m, 2H), 7.41 (s, 1H), 7.36 (dd, J = 5.0, 0.7 Hz, 1H), 7.31 (dd, J = 8.3, 2.1 Hz, 1H), 7.25 (d, J = 2.7 Hz, 1H), 7.06 (dd, J = 5.0, 3.7 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 5.16 (s, 2H), 4.73 (dd, J = 11.5, 9.4 Hz, 2H), 4.41 (s, 2H), 4.40 (s, 2H), 3.96 (dd, J = 11.4, 2.9 Hz, 2H), 2.27–2.13 (m, 1H), 1.72–1.69 (m, 1H). 13C NMR (100 MHz, DMSO-d_6) δ 171.19, 166.62, 165.72, 144.71, 143.51, 143.12, 141.80, 133.59, 131.05, 128.75, 128.69, 128.40, 127.45, 127.33, 124.42, 123.85, 123.70, 122.72, 121.42, 116.79, 111.64, 93.41, 61.11, 42.53, 42.16, 25.21. HRMS (AP-ESI) *m/z* calcd for $C_{31}H_{27}ClN_4O_5S$ $[M+H]^+$ 603.1463, found 603.1598. HPLC t_R = 16.93 min, 95.5%.

4.1.13.2. N-(2-Amino-5-(thiophen-2-yl)phenyl)-4-((2-(5-chloro-2-oxospiro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)methyl)benza-mide (23b): White solid, 47% yield. Mp: 198–200 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.90 (t, J = 5.9 Hz, 1H), 7.99 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 1.7 Hz, 1H), 7.52–7.47 (m, 2H), 7.43 (s, 1H), 7.39 (dd, J = 6.5, 1.1 Hz, 2H), 7.36 (dd, J = 8.3, 2.0 Hz, 1H), 7.29 (d, J = 3.3 Hz, 1H), 7.07 (dd, J = 5.0, 3.7 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.3 Hz, 1H), 4.40 (s, 2H), 4.39(s, 2H), 4.37–4.27 (m, 4H).^{13}C NMR (100 MHz, DMSO-d_6) δ 172.80, 166.59, 165.81, 144.36, 143.27, 143.23, 133.44, 131.71, 128.76, 128.44, 127.62, 127.47, 126.55, 125.13, 124.36, 124.14, 121.96, 117.92, 111.95, 101.55, 66.34, 42.53, 42.48. HRMS (AP-ESI) *m/z* calcd for $C_{30}H_{25}ClN_4O_5S$ $[M+H]^+$ 589.1307, found 589.1436. HPLC t_R = 11.31 min, 95.4%.

4.1.13.3. N-(4-Amino-[1,1′-biphenyl]-3-yl)-4-((2-(5-chloro-2-oxos-piro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)methyl)benzamide (23c): White solid, 65% yield. Mp: 218–220 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.73 (s, 1H), 8.88 (t, J = 5.9 Hz, 1H), 7.99 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 7.5 Hz, 2H), 7.54 (d, J = 1.5 Hz, 1H), 7.46 (dd, J = 8.4, 2.2 Hz, 1H), 7.44–7.36 (m, 5H), 7.34 (dd, J = 8.3, 2.1 Hz, 1H), 7.24 (t, J = 7.3 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 5.11 (s, 2H), 4.73 (t, J = 10.5 Hz, 2H), 4.41 (s, 2H), 4.39(s, 2H), 3.97–3.93 (m, 2H), 2.19 (m, 1H), 1.71–1.68 (m, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ 171.20, 166.62, 165.71, 143.27, 143.07, 141.80, 140.66, 133.70, 131.05, 129.27, 128.75, 128.60, 128.38, 127.47, 127.33, 126.50, 125.97, 125.28, 125.17, 124.42, 124.05, 116.99, 111.64, 93.41, 61.11, 42.53, 42.16, 25.21. HRMS (AP-ESI) *m/z* calcd for $C_{33}H_{29}ClN_4O_5$ $[M+H]^+$ 597.1899, found 597.1966. HPLC t_R = 19.26 min, 95.1%.

4.1.13.4. N-(4-Amino-[1,1′-biphenyl]-3-yl)-4-((2-(5-chloro-2-oxos-piro[indoline-3,2′-[1,3]dioxolan]-1-yl)acetamido)methyl)benzamide (23d): White solid, 98% yield. Mp: 191–192 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74 (s, 1H), 8.89 (t, J = 5.9 Hz, 1H), 7.99

(d, J = 8.2 Hz, 2H), 7.55 (m, 3H), 7.51–7.47 (m, 2H), 7.43–7.37 (m, 4H), 7.34 (dd, J = 8.3, 2.2 Hz, 1H), 7.24 (t, J = 7.3 Hz, 1H), 7.00 (d, J = 8.6 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 5.11 (s, 2H), 4.40 (s, 2H), 4.38 (s, 2H), 4.37–4.28 (m, 4H).^{13}C NMR (100 MHz, DMSO-d_6) δ 172.80, 166.58, 165.73, 143.27, 143.07, 140.67, 133.72, 131.71, 129.27, 128.60, 128.38, 127.62, 127.47, 126.56, 126.49, 125.98, 125.27, 125.13, 124.07, 116.99, 111.94, 101.55, 66.34, 42.54, 42.48. HRMS (AP-ESI) m/z calcd for $C_{32}H_{27}ClN_4O_5$ $[M+H]^+$ 583.1743, found 583.1787. HPLC t_R = 12.89 min, 97.3%.

4.1.14. tert-Butyl (5-fluoro-2-(4-((2,2,2-trifluoroacetamido)methyl) benzamido)phenyl)carbamate (27)—Using the synthetic method for compounds **20a**, compounds **19** and **26** gave the compound **27** as a white solid, 57% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 9.81 (s, 1H), 8.80 (s, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.53 (dd, J = 11.1, 2.9 Hz, 1H), 7.45 (dd, J = 12.4, 7.3 Hz, 3H), 6.97 (td, J = 8.5, 3.0 Hz, 1H), 4.48 (s, 2H), 1.45 (s, 9H).

4.1.15. tert-Butyl (2-(4-(aminomethyl)benzamido)-5-fluorophenyl)carbamate (28)—Using the synthetic method for compounds **21a**, compound **27** gave the compound **28** as a white solid, 98% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, J = 8.1 Hz, 1H), 7.93 (d, J = 8.1 Hz, 2H), 7.58–7.48 (m, 3H), 7.45 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.1 Hz, 1H), 6.94–6.86 (m, 1H), 4.24 (d, J = 6.3 Hz, 1H), 3.79 (s, 2H), 1.45 (s, 9H).

4.1.16. tert-Butyl (2-(4-((2-(5-bromo-2-oxospiro[indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)methyl)benzamido)-5-fluorophenyl)carbamate (29)—Using the synthetic method for compounds **22a**, compounds **6a** and **28** gave the compound **29** as a white solid, 46% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (s, 1H), 8.88 (t, J = 5.9 Hz, 1H), 8.77 (s, 1H), 7.94 (d, J = 8.1 Hz, 2H), 7.58 (dd, J = 8.4, 2.0 Hz, 1H), 7.52 (td, J = 6.0, 3.0 Hz, 2H), 7.49–7.41 (m, 3H), 7.00–6.91 (m, 2H), 4.72 (dd, J = 11.6, 9.3 Hz, 2H), 4.39 (d, J = 4.1 Hz, 4H), 3.95 (dd, J = 8.1, 6.4 Hz, 2H), 2.25–2.15 (m, 1H), 1.71–1.67 (m, 1H), 1.45 (s, 9H).

4.1.17. N-(2-Amino-4-fluorophenyl)-4-((2-(5-bromo-2-oxospiro [indoline-3,2′-[1,3]dioxan]-1-yl)acetamido)methyl)benzamide (30)—Using the synthetic method for compounds **23a**, compound **29** gave the compound **30** as a white solid, 98% yield. Mp: 237–239 °C, 1H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.88 (t, J = 5.7 Hz, 1H), 7.95 (d, J = 8.1 Hz, 2H), 7.59 (dd, J = 8.4, 1.9 Hz, 1H), 7.53 (d, J = 1.9 Hz, 1H), 7.39 (d, J = 8.1 Hz, 2H), 7.11 (dd, J = 8.3, 6.6 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.54 (dd, J = 11.2, 2.8 Hz, 1H), 6.36 (td, J = 8.5, 2.7 Hz, 1H), 5.24 (s, 2H), 4.72 (dd, J = 11.5, 9.6 Hz, 2H), 4.39 (d, J = 8.5 Hz, 4H), 3.94 (dd, J = 11.3, 2.9 Hz, 2H), 2.25–2.11 (m, 1H), 1.69 (d, J = 13.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.08, 166.59, 165.82, 143.03, 142.22, 133.91, 133.60, 129.07, 128.35, 127.41, 127.11, 119.74, 114.88, 112.13, 102.60, 102.38, 102.04, 101.78, 93.34, 61.21, 42.52, 42.11, 25.20. HRMS (AP-ESI) m/z calcd for $C_{27}H_{24}BrFN_4O_5$ $[M+H]^+$ 583.0987, found 583.0983. HPLC t_R = 8.81 min, 97.2%.

4.2. Biological materials and methods

4.2.1. In vitro HDAC inhibition fluorescence assay—In vitro HDAC inhibition assays were conducted as previously described.[25] In brief, 10 lL of enzyme solution (HeLa nuclear extract, HDAC1, 2 or 3) was mixed with various concentrations of 50 μL tested compounds (0.039, 0.39, 1.56, 6.25, 25, 100 μM). The mixture was incubated at 37 °C for 1 h, then fluorogenic substrate Boc-Lys (acetyl)-AMC (40 μL) was added, and the suspension was incubated at 37 °C for another 1 h. The reaction was ended by addition of 100 μL of developer containing trypsin and TSA. After incubation under the same situation for 20 min, fluorescence intensity was monitored through 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, and the IC_{50} values were calculated using a regression analysis of the concentration/inhibition data.

4.2.2. In vitro antiproliferative assay—Cell antiproliferative assay was determined by MTT [(3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide)] method as previously described.[25] All cell lines were cultured in RPMI 1640 medium containing 10% FBS at 37 °C in a 5% CO_2 humidified incubator. Cells were plated into 96-well plates (5000/well), and permitted to grow for at least 4 h. After addition of different concentrations of compounds, cells were maintained for 48 h. A solution of 0.5% MTT was added to each well. After incubation for another 4 h, the formazan formed from MTT was extracted by adding 200 μL of DMSO for 15 min. Then the optical density values were measured using a UV–vis spectrophotometer at 570 nm.

4.2.3. Whole-cell HDAC inhibition assay—The whole-cell HDAC inhibition assay was based on assays published by Tessier P. et al. [26], Hildmann C. et al.[27] and Marek L. et al.[28] with minor modifications. Different subtypes of HDACs have intrinsic differences in substrate recognition. The lysine analogs Boc-Lys (acetyl)-AMC and Boc-Lys (triflouroacetyl)-AMC could permeate into cells and function as cellular class I and class IIa HDAC substrates, respectively. After deacetylation of the lysine residue, the product could be hydrolyzed by trypsin to release 7-amino-4-methylcoumarin (AMC), a well-studied fluorophore detectable by standard fluorescence measurements.

HEL cells were seeded into 96-well black tissue culture plates at a density of 8×10^3 cells/well in a total volume of 81 μL of culture medium and cultivated for 24 h under standard cell culture conditions. The volume of 9 μL of tested compounds with various concentrations was added to each well. After 3 h, 10 μL of 3 mM Boc-Lys (Ac)-AMC or Boc-Lys-(ε-trifluormethylacetyl)-AMC was added to reach a final concentration of 300 μM. Cells were placed with the substrates for 3 h in 37 °C incubator with 5% CO_2. The reaction was stopped by adding 100 μL/well lysis/developer buffer mix (50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1% Nonidet-P40, 2.0 mg/mL trypsin, 10 μM TSA). After final incubation for 3 h, fluorescence was measured at excitation of 360 nm and emission of 470 nm.

4.2.4. Molecular docking studies—Compounds were docked into the active site of HDAC1 (PDB entry: 5ICN) using Tripos SYBYL-X 1.3. Before docking process, the

protein structure retrieved from PDB site was treated by deleting water molecules, FF99 charges. A 100-step minimization process was performed to further optimize the protein structure. The molecular structures were generated with Sybyl/Sketch module and optimized using Powell's method with the Tripos force field with convergence criterion setting at 0.005 kcal/(Åmol) and assigned charges with the Gasteiger-Hückel method. Molecular docking was carried out via the Sybyl/Surflex-Dock module. Other docking parameters were kept to the default values.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2017.03.036.

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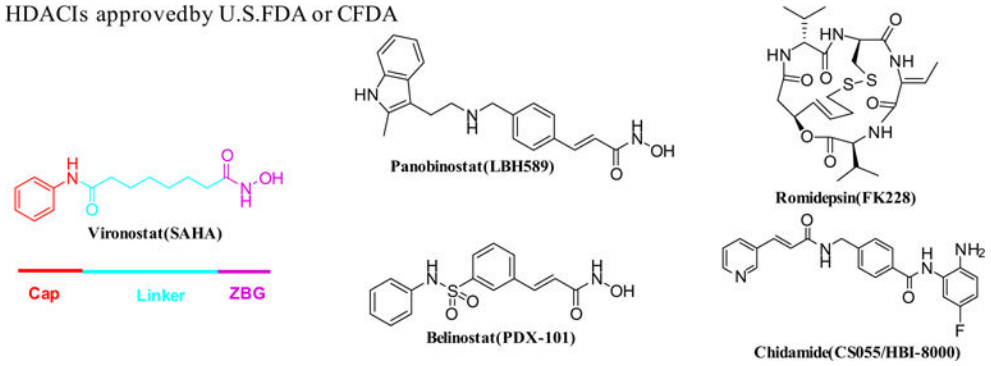


Fig. 1.
Pharmacophore model and chemical structures of representative HDACIs.

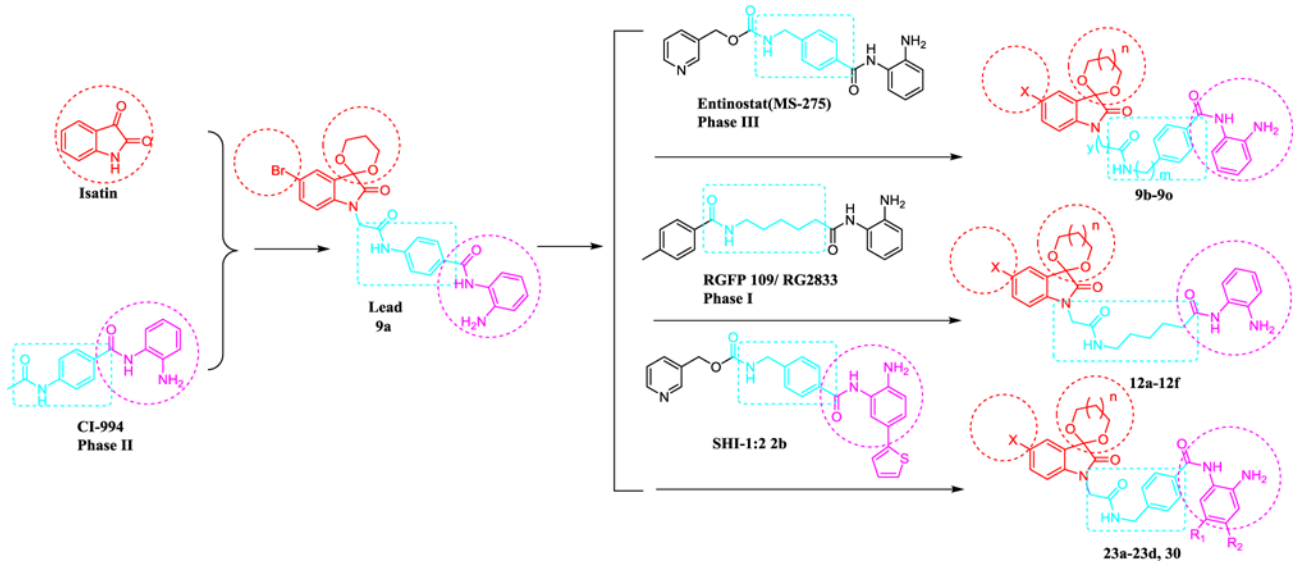


Fig. 2.
The design of HDACIs through scaffold splicing and hopping strategy.

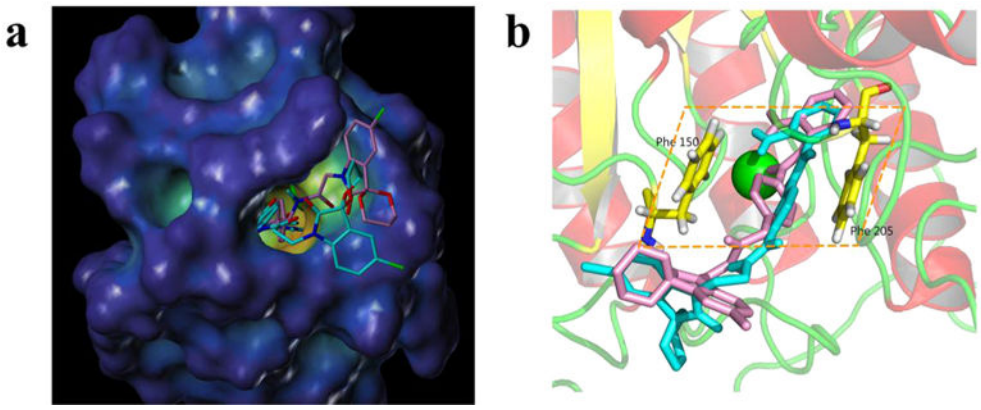
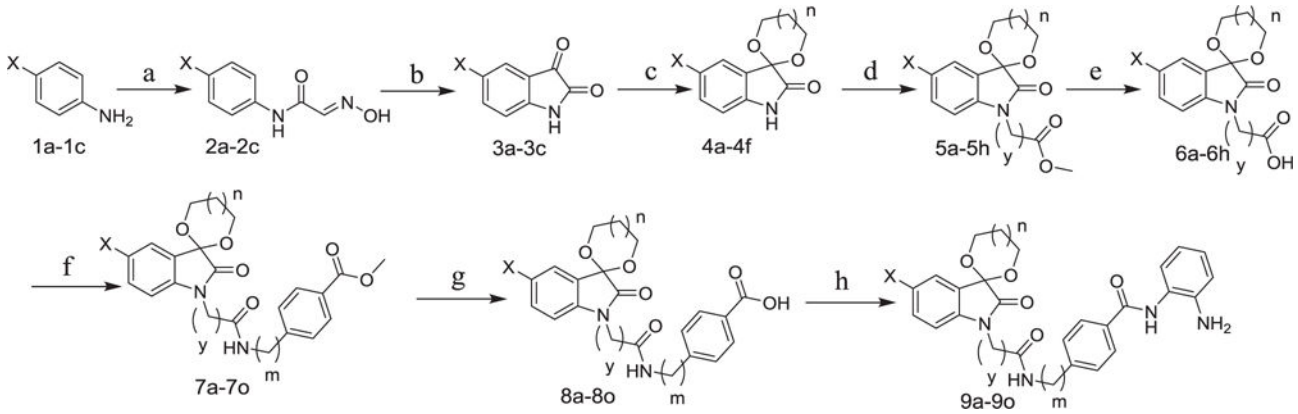


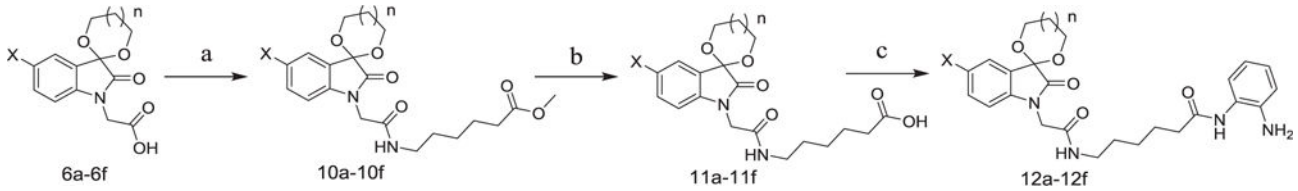
Fig. 3.

Proposed binding modes of compounds **9h** (pink) and **9n** (blue) in the active site of HDAC1 (derived by modification of PDB code 5ICN using Tripos SYBYL-X 1.3). The zinc ion is shown as a green sphere.



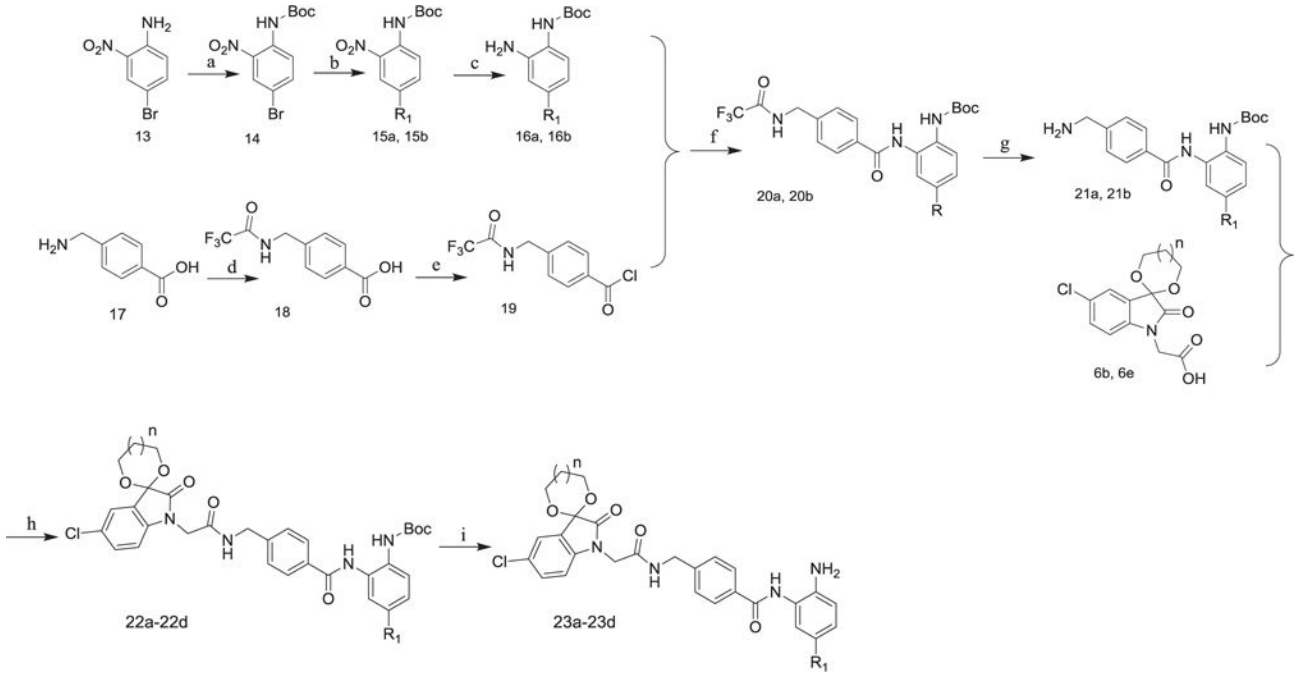
Scheme 1.

(1) X = Br, Cl or F; n = 0 or 1. (2) Reagents and conditions: (a) $Cl_3CH(OH)$ 2, NH_2OH, Na_2SO_4, 60–85 °C; (b) concentrated H_2SO_4, 80 °C; (c) ethane-1, 2-diol or propane-1, 3-diol, TsOH, toluene, 140 °C; (d) $BrCH_2COOCH_3$ or $BrCH_2CH_2COOCH_3$, TBAB, K_2CO_3, KI, acetone, 60 °C; (e) KOH, H_2O, THF/CH_3OH, r.t., then acidified to pH 1 with HCl; (f) methyl-4-aminobenzoate or methyl 4-(aminomethyl) benzoate, TBTU, Et_3N, THF/CH_2Cl_2, r.t.; (g) NaOH, H_2O, THF/CH_3OH, reflux; then acidified to pH 1 with HCl; (h) *o*-phenylenediamine, TBTU, Et_3N, THF/CH_2Cl_2, r.t.



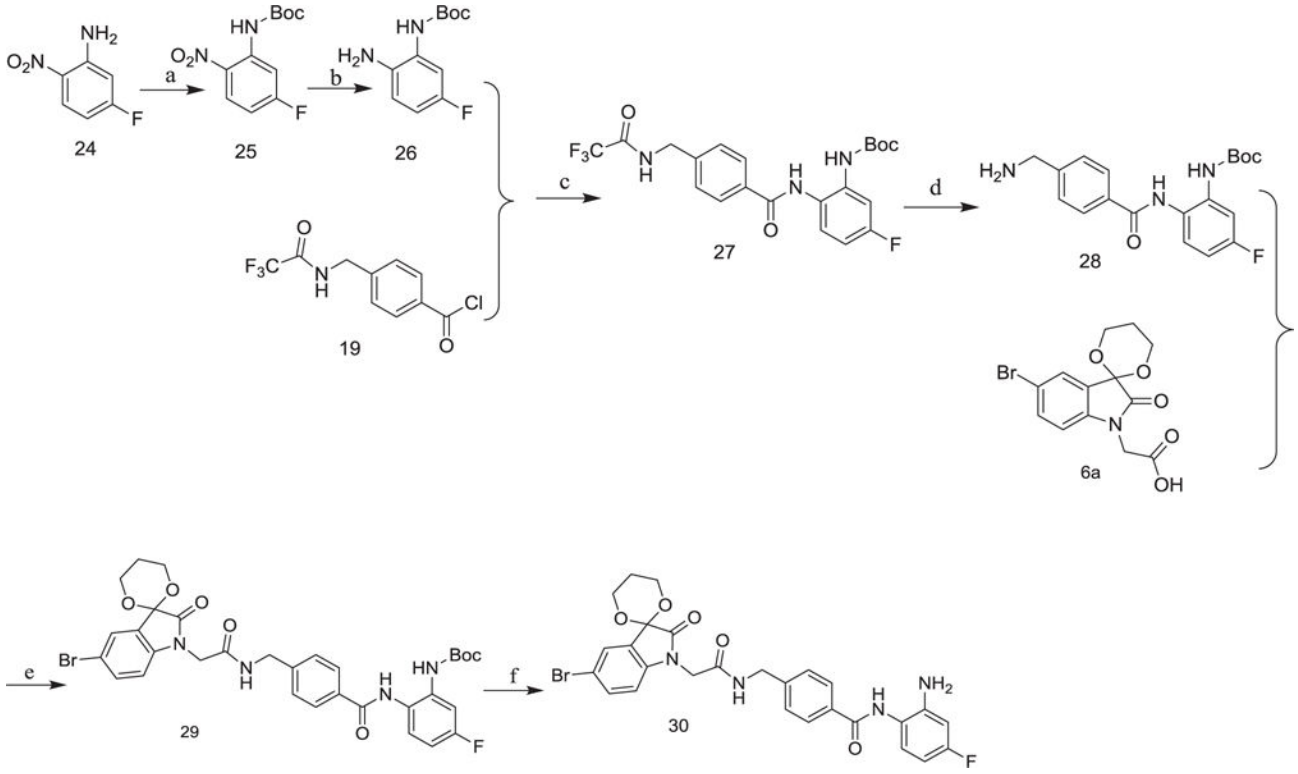
Scheme 2.

(1) X = Br, Cl or F; n = 0 or 1. (2) Reagents and conditions: (a) Methyl 6-aminohexanoate hydrochloride, TBTU, Et_3N, THF/CH_2Cl_2, r.t.; (b) NaOH, H_2O, THF/CH_3OH, reflux; then acidified to pH 1 with HCl; (c) *o*-phenylenediamine, TBTU, Et_3N, THF/CH_2Cl_2, r.t.



Scheme 3.

(1) R_1 = thienyl or phenyl. (2) Reagents and conditions: (a) $(Boc)_2O$, Et_3N, DMAP, CH_2Cl_2, r.t.; (b) thiophene-2-boronic acid or phenylboronic acid, $Pd[P(pH_3)_4]$, Na_2CO_3, 1, 4-dioxane, H_2O, 90 °C; (c) Pd/C, H_2, CH_3OH/EtOAc, r.t.; (d) $(CF_3CO)_2O$, 0 °C; (e) $SOCl_2$, THF; reflux; (f) pyridine, r.t.; (g) K_2CO_3, H_2O, THF/CH_3OH, 70 °C; (h) *o*-phenylenediamine, TBTU, Et_3N, THF/CH_2Cl_2, r.t.; (i) EtOAc/HCl, r.t.



Scheme 4.

Reagents and conditions: (a) $(Boc)_2O$, Et_3N, DMAP, CH_2Cl_2, r.t.; (b) Pd/C, H_2, CH_3OH/EtOAc, r.t.; (c) pyridine, r.t.; (d) K_2CO_3, H_2O, THF/CH_3OH, 70 °C; (e) *o*-phenylenediamine, TBTU, Et_3N, THF/CH_2Cl_2, r.t.; (f) EtOAc/HCl, r.t.

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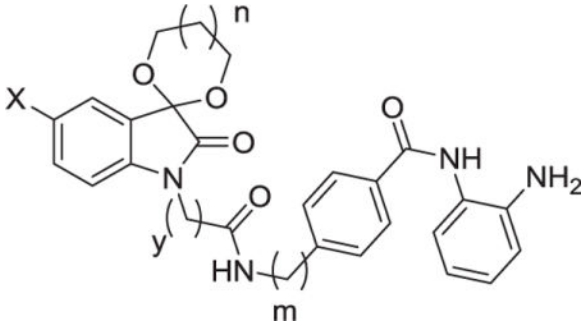
Table 1

HDAC Inhibitory Activity of Compounds **9a**–**9o**, and **MS-275**.

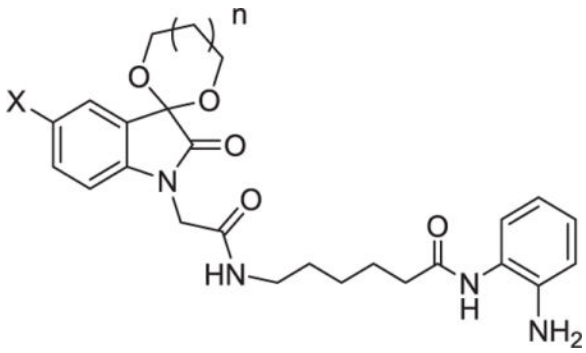


Compd	X	n	m	y	IC_{50} of HeLa nuclear extract (μM)[a]
9a	−Br	1	0	1	1.44 ± 0.42
9b	−Cl	1	0	1	1.08 ± 0.08
9c	−F	1	0	1	3.75 ± 0.65
9d	−Br	0	0	1	2.66 ± 0.45
9e	−Cl	0	0	1	2.17 ± 0.18
9f	−F	0	0	1	>6.00
9g	−Br	1	1	1	2.00 ± 0.13
9h	−Cl	1	1	1	1.25 ± 0.08
9i	−F	1	1	1	1.56 ± 0.72
9j	−Br	0	1	1	2.14 ± 0.73
9k	−Cl	0	1	1	1.93 ± 0.34
9l	−F	0	1	1	1.84 ± 0.62
9m	Br	1	0	2	0.51 ± 0.03
9n	Cl	1	0	2	0.43 ± 0.19
9o	Cl	1	1	2	1.03 ± 0.25
MS-275					1.12 ± 0.21

[a]Results expressed as the mean ± standard deviation of at least three separate determinations.

Table 2

HDAC Inhibitory Activity of Compounds **12a**–**12f**, and **MS-275**.



Compd	X	n	IC_{50} of HeLa nuclear extract (mM)[a]
12a	−Br	1	8.42 ± 1.59
12b	−Cl	1	8.30 ± 0.87
12c	−F	1	6.50 ± 0.09
12d	−Br	0	7.53 ± 1.32
12e	−Cl	0	9.70 ± 2.83
12f	−F	0	27.18 ± 0.28
MS-275			1.12 ± 0.21

[a]Results expressed as the mean ± standard deviation of at least three separate determinations.

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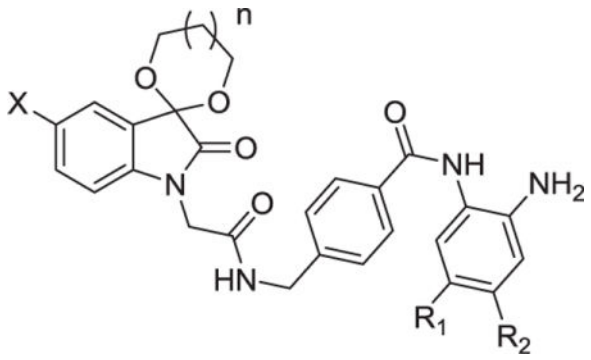
Table 3

HDAC Inhibitory Activity of Compounds **23a**–**23d**, **30**, and **MS-275**.



Compd	X	n	R_1	R_2	IC_{50} of HeLa nuclear extract (μM)[a]
23a	Cl	1		H	>100.0
23b	Cl	0		H	>100.0
23c	Cl	1		H	>100.0
23d	Cl	0		H	>100.0
30	Br	1	H	F	>100.0
MS-275					1.12 ± 0.21

[a]Results expressed as the mean ± standard deviation of at least three separate determinations.

Table 4

In vitro antiproliferative activity for selected HDAC inhibitors.

Compd	IC_{50} (μM)[a]				
	K562	Molt-4	HEL	PC-3	SK-N-BE(2)
9a	ND[b]	ND	2.27	ND	ND
9d	1.75	1.24	0.69	1.27	0.93
9h	2.83	2.46	1.15	11.35	1.92
9m	1.54	0.75	0.53	4.82	0.96
9n	1.48	0.70	0.58	3.23	1.01
23a	5.28	5.13	2.75	10.19	8.98
MS-275	1.32	0.65	0.48	4.89	0.33

[a]Assays were performed in replicate (n ≥ 2), the SD values are <20% of the mean.

[b]Not determined.

Table 5

HDAC class I and IIa cellular activity for selected HDAC inhibitors.[a]

Compd	HDAC class I cellular IC_{50} (μM)[b]	HDAC class IIa cellular IC_{50} (μM)[c]
9a	>2.00	>2.00
9d	0.61	>2.00
9h	0.93	>2.00
9m	0.37	>2.00
9n	0.51	>2.00
23a	>2.00	>2.00
MS-275	0.43	>2.00

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

[b] Boc-Lys (acetyl)-AMC substrate.

[c] Boc-Lys (triflouroacetyl)-AMC substrate.

Table 6

HDACs inhibitory activity and isoform selectivity for selected HDAC inhibitors.

Compd	IC_{50} of HDAC1 (μM)[a]	IC_{50} of HDAC2 (μM)[a]	IC_{50} of HDAC3 (μM)[a]	HDAC2/HDAC1 isoform selectivity	HDAC3/HDAC1 isoform selectivity
9d	0.046	0.128	0.667	2.8	14.5
9h	0.137	0.223	0.096	1.6	0.7
9m	0.012	0.026	0.019	2.2	1.6
9n	0.032	0.256	0.311	8.0	9.7
23a	0.023	0.179	151.8	7.8	6600
MS-275	0.163	0.396	0.605	2.3	3.7

[a]Assays were performed in replicate (n ≥ 2), the SD values are <20% of the mean.