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Selective HDAC inhibitors with potent oral activity against leukemia and colorectal cancer: Design, structure-activity relationship and anti-tumor activity study

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Abstract

Previously, we reported the discovery of a series of *N*-hydroxycinnamamide-based HDAC inhibitors, among which compound **11y** exhibited high HDAC1/3 selectivity. In this current study, structural derivatization of **11y** led to a new series of benzamide based HDAC inhibitors. Most of the compounds exhibited high HDACs inhibitory potency. Compound **11a** (with 4-methoxybenzoyl as *N*-substituent in the cap and 4-(aminomethyl) benzoyl as the linker group) exhibited selectivity against HDAC1 to some extent, and showed potent antiproliferative activity against several tumor cell lines. *In vivo* studies revealed that compound **11a** displayed potent oral antitumor activity in both hematological tumor cell U937 xenograft model and solid tumor cell HCT116 xenograft model with no obvious toxicity. Further modification of benzamide **3**, **11a** and **19** afforded new thienyl and phenyl compounds (**50a**, **50b**, **63a**, **63b** and **63c**) with dramatic HDAC1 and HDAC2 dual selectivity, and the fluorine containing compound **56**, with moderate HDAC3 selectivity.

Keywords

HDACs; HDACIs; Benzamides; Anti-tumor drugs; *In vivo* oral activity

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1. Introduction

It is widely considered that the initiation and progression of cancer are associated with genetic abnormalities, as well as epigenetic alterations that do not change the nucleotide sequence of DNA [1]. Histone deacetylases (HDACs) are one of the most studied epigenetic modulators, modifying the acetylation status of chromatin histones and non-histone proteins [2]. In detail, HDACs remove acetyl groups from lysine residues, resulting in a “closed” chromatin configuration, which blocks the access of the transcription machinery to DNA, and suppresses gene expression including tumor suppressor genes [3].

Currently a total of 18 HDACs have been identified in humans, which can be divided into 4 classes according to their homology. Class I includes HDACs 1, 2, 3, and 8, and they are homologous to yeast *Rpd3*; class IIa (HDACs 4, 5, 7 and 9) and class IIb (HDAC6 and 10) are structurally related to yeast *Hda1*; Class IV has only one member, HDAC11. These HDAC isoforms are all Zn^{2+} -dependent enzymes, which bear a highly conserved catalytic site. The class III HDACs (comprising SIRT 1-7) are related to the yeast protein Sir2 and their activities are depended on NAD^+ concentration [4,5]. The exact physiological role of these HDAC subtypes in cells is far from being completely elucidated, however, the division of labor among them has been studied using knockout and transgenic mice and RNAi techniques, in which, class I and class II are the most well studied [6,7]. The knockout of HDAC1 in mice caused cell proliferation defects and developmental retardation, and this unique function cannot be compensated by HDAC2 and HDAC3 [8,9]. HDAC2 is involved in cardiac morphogenesis until the perinatal period of mice [10]. HDAC3 has been reported to function in proliferative mitosis and the regulation of INF expression [11]. In normal human tissues, HDAC8 is associated with the regulation of the contractile capacity [12]. Unlike class I HDACs, Class IIa HDACs (HDAC4, 5, 7 and 9) display tissue-specific expression and are involved in a variety of signal transduction pathways [13]. The well-known class IIb HDAC6 controls the status of tubulin and hsp90 acetylation [14]. All these indicates each HDAC enzyme has its unique function in normal cells, therefore, most pan-HDAC inhibitors (HDACIs), which inhibit a broad spectrum of HDACs, exhibited significant toxicity. For example, panobinostat (LBH589) caused serious thrombocytopenia, atrial fibrillation, eye swelling, neutropenia, and anemia [15]. Thus, increasing efforts are being directed toward developing selective HDACIs for a single class or a single isoform, which are likely to have fewer adverse reactions and a better tolerance. A few Class I selective HDACIs such as MS-275 and MGCD0103 (HDAC1, 2, and 3-selective) are currently in various stages of clinical trial for cancer therapeutics [16], and Chidamide recently has been approved by China food and drug administration (CFDA).

In our previous study, we designed and synthesized *N*-hydroxycinnamamide-based histone deacetylase 1/3 dual inhibitors with potent oral anti-leukemia activity [17]. Efforts to improve the selectivity of the dually reported the discovery of a new series of benzamides based HDACIs. The structure-activity relationship (SAR) studies, isotype-selectivity, and anticancer activities of benzamide HDACIs were particularly discussed. One representative compound **11a** exhibited selectivity against HDAC1, and showed potent antiproliferative activity against solid and hematological tumor cell lines both *in vitro* and *in vivo*. Further modified thienyl and phenyl compounds all exhibited dramatic HDAC1/2 dual selectivity.

1.1. Chemistry

The synthesis of compound **3** was described in Scheme 1. The synthesis of the starting material **1** was described in our previous study [18]. Deprotection of methyl group of **1** in the presence of KOH aqueous solution yielded **2**, which was then coupled with *o*-phenylenediamine in anhydrous THF to yield the final product **3**.

The synthesis of compounds **11a-11g** was described in Scheme 2. Methyl ester protection of **5** gave rise to **6**, which was then reacted with Boc-protected *L*-Trp by TBTU-mediated amide formation to afford **7**. *N*-deprotection of **7** followed by condensation with different carboxylic acids gave **9a-9g**, which were deprotected in the presence of KOH aqueous solution to obtain **10a-10h**. **10a-10h** were then subjected to hydrolysis reaction and amide formation reaction as **2** in Scheme 1 to yield the final products **11a-11g**, respectively.

Scheme 3 was similar to that of Scheme 2. **12** was reacted with 4-methoxybenzoic acid via condensation to get **13**. **15** was subjected to methyl ester protection to afford **16**. **14**, the hydrolysis products of **13**, was then connected with **16** to get **17** which was subjected to methyl deprotection and amide formation reaction as **3** in Scheme 1 to yield **19**.

Compound **20** of Scheme 4 was reported as an intermediate in our previous study [17], and it was transformed into compound **22** by the same reaction in Scheme 1.

Methyl ester protection of **24a** and **24b** gave **25a** and **25b**, which were connected with **23** through Mitsunobu reaction to give **26a** and **26b** (Scheme 5). Intermediate **27a** and **27b**, the *N*-deprotected product of **26a** and **26b**, were reacted with 4-methoxybenzoic acid to afford **28a** and **28b**, which were further subjected to methyl de-protection and condensation to yield the final products **30a** and **30b**, respectively.

Compound **37** was synthesized following the procedures described in Scheme 6. Condensation between intermediate **14** and *N,O*-dimethylhydroxylamine hydrochloride yielded **31**, which was subsequently converted to aldehyde **32** by LiAlH₄ at -40 °C. **32** was reacted with **6** in the presence of NaBH₃CN to afford **33**. Boc-protection, methyl deprotection, amide formation reaction and *N*-deprotection of **33** yielded the target product **37**.

The reaction for **43a** and **43b** was described in Scheme 7. Boc-protection of **38** gave **39**, which was coupled with thiophene-2-boronic acid or phenylboronic acid using Suzuki reaction in the presence of Pd(OAc)₂ to afford **40a** or **40b**. Catalytic hydrogenation of **40a** and **40b** gave **41a** and **41b**, which were subjected to amide formation reaction and *N*-deprotection to obtain **43a** and **43b**, respectively.

Emmanuel H. Demont et al. described the synthesis of **46** [19], briefly, double Boc-protection of **44** gave **45**, then one Boc-protecting group was removed in the presence of trifluoroacetic acid to afford mono-Boc protected product **46**. Reduction of **46** gave **47**, which was subjected to a similar reaction of **41** in Scheme 7 to yield **49** (Scheme 8).

6 and **50** were treated with trifluoroacetic anhydride to yield trifluoroacetyl protected **51a** and **51b**, respectively. **51a** and **51b** were converted to acyl chloride **52a** and **52b**. **52a** was subsequently reacted with **41a** to afford product **53a**, while **52b** was reacted with **41a** and **41b** to achieve **53b** and **53c**, respectively (Scheme 9). Trifluoroacetyl deprotection of **53a-53c** gave **54a-54c**, which were subjected to a similar reaction of **41** in Scheme 7 to yield **56a-56c**. The synthesis of **60a** and **60b** in Scheme 10 was similar to the reaction of **52a** converting to **56a**, and this route would not be described in this section.

2. Results and discussion

Our previous study described the discovery of several *N*-hydroxycinnamamide-based HDACIs with HDAC 1/3 inhibitory activity [17]. However, due to its strong chelating ability with zinc ions, hydroxamates unavoidably exhibited inhibitory activity against other HDAC subtypes including HDAC 2, 8, 6 and so on. Therefore, we attempted to replace hydroxamic acid with other zinc-binding groups to obtain HDACIs with better isoform selectivity. To the best of our knowledge, most of the sub-class I selective inhibitors currently in research are benzamide HDACIs, such as MS-275, MGCD0103, CI994 and RG2833. Therefore, we kept the cap group (or the cap and the linker groups) of the representative HDAC1/3 selective inhibitor **11y** unchanged, and introduced the linker and zinc binding group (ZBG) of aforementioned sub-class I selective inhibitors to obtain a novel series of hybrid compounds, **3**, **11a**, **19**, **22**, **30a**, **30b**, **37** (Fig. 1). In detail, **3** was designed by combining cap group of **11y** with linker and ZBG group of CI994. Using the same strategy, **11a** and **19** were derived by combining the cap group of **11y** with the linker and the ZBG group of MS275 and RG2833, respectively. Keeping both the cap group and the linker of **11y** unchanged while replacing hydroxamate with the benzamide as ZBG led to **22**. Moreover, **30a**, **30b** and **37** were achieved by further modification of the linker group of **22** and **11a** (Fig. 1).

HDAC class I whole cell assay and antiproliferative assay based on the human leukemia cell line HL60 were conducted to efficiently screen the newly designed compounds (Table 1). Benzamides **3**, **11a**, **19**, **22**, **30a**, **30b** and **37** with different linker groups exhibited diverse class I HDAC inhibitory activities. To be specific, **3**, **11a** and **30a**, bearing linker structure of *N*-(*p*-tolyl)acetamide, *N*-(4-methylbenzyl)acetamide and 1-ethoxy-4-methylbenzene, respectively, displayed submicromolar IC₅₀ values (0.67–0.85 μM), comparable with that of MS275 (0.62 μM). **19** with *N*-hexylacetamide, **22** with (*E*)-1-ethoxy-4-(prop-1-en-1-yl)benzene and **37** with *N*-(4-methylbenzyl)ethanamine as linker group showed low micromolar IC₅₀ values (1.23–2.95 μM), while **30b** having one carbon longer than **30a** in the linker chain length displayed no inhibitory activity up to 10 μM. These results indicated that changes in the linkers had obvious effects on the inhibitory activities of benzamide based HDACs inhibitors. The antiproliferative activity of all the compounds was consistent with their cellular enzymatic activity, **30b** showed no anti-proliferative activity at the concentration up to 20 μM. The other benzamides displayed low micromolar IC₅₀ values, among which, the most potent compounds **3**, **11a** and **30a** also exhibited comparable antiproliferative activities to MS275.

Due to its promising enzymatic and antiproliferative activity, further structural modification was focused on the cap of **11a** to find more potent derivatives. Analogs **11b-11g** were

designed and synthesized by replacing the 4-methoxybenzoyl of **11a** with various *para* substituted benzoic acids or heterocyclic rings. Among these analogs, **11f** (with 2-thiophenecarboxyl), **11g** (with 2-furancarboxyl), and their parent compound **11a** displayed higher enzymatic inhibitory and antiproliferative activity than the other compounds (Table 2).

In order to explore the HDAC isoform selective profile, compounds **3**, **11a**, **19**, **22**, **30a**, **30b**, and **37**, with different linkers, were evaluated in enzyme inhibitory assays against HDAC1, HDAC2 and HDAC3. Results presented in Table 3 revealed that compounds **3**, **11a** and **30a** exhibited moderate HDAC1 selectivity; **19** displayed moderate HDAC1/3 dual selectivity; **37** showed some preference to HDAC1/2; **30b** was not active in cellular assays and also showed no inhibition against HDAC1/2/3 up to 20 μ M; **22** showed almost no selectivity among HDAC1/2/3. Considering its moderate HDAC1 selectivity (Table 3) and promising cellular enzyme activity and antiproliferative activity (Table 1), **11a** was chosen for further evaluation.

The MTT assay results in Table 4 indicated that **11a** exhibited similar *in vitro* antiproliferative activities against several hematological and solid tumor cell lines to MS275. **11a** and MS275 displayed low micromolar or submicromolar IC₅₀ values against HEL, K562, U937, U266 and HCT116 cell lines, while showed poor antiproliferative activity against ES-2.

Encouraged by its promising *in vitro* activity, compound **11a** was further progressed to *in vivo* experiments. Firstly, we established a hematological tumor xenograft model, using MS275 as the positive control, to investigate if **11a** was active *in vivo*. 5×10^6 U937 cells were subcutaneously implanted in the right flanks of male nude mice (BALB/c-nu). Once tumor size reached about 100 mm³, mice were randomized into seven per group and were treated with compound **11a** (100 mg/kg/day) or MS275 (50 mg/kg/day) by oral gavage for 13 days. Tumor growth inhibition (TGI) and relative increment ratio (T/C) were calculated at the end of experiment to reveal the antitumor effects via tumor weight and tumor volume, respectively. Meanwhile, tumor volume and mice body weight were also measured during the treatments (Fig. 2, Table 5). Although compound **11a** displayed potent *in vivo* oral antitumor activity with TGI value of 51% and T/C value of 49%, it was a little less potent than the positive control MS275 (TGI = 60%, T/C = 33%). However, we could see from Fig. 2d and Table 5 that during treatment, the mice group administrated with MS275 demonstrated obvious body weight loss compared with the control group, which indicated that MS275 had obvious toxicity in the dose of 50 mg/kg/day. This toxicity didn't appear in the mice treated with **11a** in the dose of 100 mg/kg/day. In fact, at the beginning of the *in vivo* study, mice were treated with MS275 at the same dosage as **11a** (100 mg/kg/day). Three days later, serious body weight loss was observed unexpectedly, and after six days, two of the six mice died. Therefore, we had to setup a new *in vivo* experiment and decreased the dose of MS275 to 50 mg/kg/day. In conclusion, compound **11a** exhibited potent *in vivo* oral antitumor activity in the U937 xenograft model without obvious side effects compared with MS275.

Another subcutaneous colon cancer cell HCT116 xenograft model was also established to test if **11a** was effective against solid tumor *in vivo*. Mice were treated with **11a** (100 mg/kg), SAHA (100 mg/kg) or MS275 (50 mg/kg) by oral gavage for 16 days. Results in Fig. 3 and Table 6 showed that significant tumor growth delays were observed relative to the untreated group for all tested compounds. Compound **11a** (TGI = 49%, T/C = 59%) was much more effective than SAHA (TGI = 32%, T/C = 74%), while was less potent than positive control MS275 (TGI = 62%, T/C = 47%). Again, compared with the control group, serious body weight loss was observed in MS275 group but not in **11a** group and SAHA group (Table 6). Taken together, our benzamide **11a** was orally active not only in the hematological tumor cell U937 xenograft model but also in the solid tumor HCT116 xenograft model. Though less potent, **11a** possessed much better safety profiles than the clinical compound MS275.

Previous reports demonstrated that a 14 Å internal cavity immediately below the zinc binding group could be used to design HDAC1/2 selective HDACs inhibitors effectively [20]. Another report showed the introduction of fluorine in the para position of acid amide could decrease the activity against HDAC1 to afford a HDAC3 selective inhibitor [21]. Therefore, compounds **43a**, **43b**, **49**, **56a-56c**, **60a** and **60b** were designed based on **3**, **11a** or **19**, and these new compounds were expected to display different selective profiles (Fig. 4).

All of the newly designed compounds were evaluated in enzyme inhibitory assays against HDAC1, HDAC2 and HDAC3 along with **19**, **3** and **11a** (Table 7). Results revealed that regardless of the linker, *para* aromatic substituent of compounds **19**, **3** and **11a** could obviously decrease their inhibitory activity against HDAC3, which was consistent with previous reports [20]. Among compounds **49**, **60a** and **60b** with fluorine in the *para* position of acid amide, only **49** displayed moderate HDAC3 selectivity, which indicated the *para* fluorine plus the appropriate linker, such as the linear aliphatic linker in **49**, co-determined the selective profile of HDAC inhibitor. To further ascertain the selectivity of our compounds across the broader family of HDAC isoforms, we next profiled the representative **43a** with *para* aromatic substituent, **49** with fluorine against HDAC8 (class I), HDAC4 (class IIa), and HDAC6 (class IIb). **43a** and **49** displayed almost no activity (>100 µM) against HDAC8, HDAC4 and HDAC6 (see Table 8).

Several HDAC1/2 selective compounds **43a**, **43b**, **56b** and **56c** were tested in antiproliferative assays (Table 9). Results showed all these HDAC1/2 selective compounds displayed much weaker antiproliferative activities than their parent compounds **19** and **11a**. In order to explain this result, we conducted western blot assay to determine the levels of acetylated histone H3, acetylated histone H4 and several apoptosis-related proteins including procaspase-3, cleaved caspase-3 and cleaved PARP (Poly ADP ribose polymerase) in tumor cells treated with **11a**, **56c** and positive control MS275, respectively. Result in Fig. 5 displayed that compound **11a** could markedly increase the level of acetylated histone H3 and acetylated histone H4, which was much superior to **56c** at the same concentration. In addition, compound **11a** and MS275 dramatically reduced the level of procaspase-3 and increased the level of cleaved caspase-3 and cleaved PARP, which indicated the induction of apoptosis, however, the levels of procaspase-3, cleaved caspase-3 and cleaved PARP

could be hardly affected by **56c**. The above western blot results of **11a**, **56c** and MS275 were consistent with their antiproliferative activities.

3. Conclusion

A new series of benzamide-based HDACIs were designed and synthesized, and their structure-activity relationship (SAR), isotype-selectivity, and anticancer activities were described. Most of the compounds exhibited high HDAC inhibitory potency. Any changes in the linker fragment and the *N*-substituent in the cap all had influence in HDAC inhibitory activity and antiproliferative potency of benzamide HDACIs. **11a**, with 4-methoxybenzoyl as *N*-substituent in the cap and 4-(aminomethyl) benzoyl as linker group, exhibited the best activity and exhibited some HDAC1 selective profile. In addition, **11a** also showed potent antiproliferative activity against multiple solid or hematological tumor cell lines. *In vivo* studies revealed that compound **11a** displayed potent oral antitumor activity in the U937 and HCT116 xenograft models. Although it was a little less potent than the positive control MS275, **11a** did have a much better tolerance with almost no toxicity in mice.

The newly designed thienyl and phenyl compounds (**43a**, **43b**, **56a**, **56b** and **56c**) based on **19**, **3** and **11a** exhibited dramatic HDAC1/2 dual selectivity. One representative compound **56c** showed low nanomolar IC₅₀ values against HDAC1 (58.4 nM), submicromolar IC₅₀ values against HDAC2 (311 nM) and micromolar IC₅₀ values against HDAC3 (30850 nM). Compounds **49**, **60a** and **60b** with fluorine in the *para* position of acid amide were also synthesized, among which, **49** displayed moderate HDAC3 selectivity. Disappointingly, our HDAC1/2 selective compounds exhibited poor *in vitro* antiproliferative activity, which was consistent with the western blot results that **56c** displayed much less effects on acetylated histone H3, acetylated histone H4, procaspase-3, cleaved caspase-3 and cleaved PRAP than its parent compound **11a**. Considering that it was reported that thienyl based HDAC1/2 inhibitors exhibited better antiproliferative activity than their parent HDAC1/2/3 inhibitors [20], we supposed that the poor cellular potency of **56c** should be due to its poor transmembrane permeability rather than its lack of HDAC3 inhibition. Actually, our preliminary results showed that the intracellular fluid concentration of **56c** was more than 10 times lower than **11a** (data not shown). Besides, we also detected the solubility of **56c** by testing lip-water partition coefficient (LogP), however, we could hardly detect **56c** in water phase (compound concentration in *n*-octyl alcohol was 5 mM) due to its poor water solubility, which could be partially responsible for its poor transmembrane permeability. Further study focused on the improvement of compounds' water solubility and transmembrane permeability is underway in our lab.

3.1. Experiment section

Materials and Methods—High-resolution mass spectrometry was performed by Shandong Analysis and Test Center in Ji'nan, China. ESI-MS spectra were recorded on an API 4000 spectrometer. The [M+H]⁺ data of HRMS or ESI-MS consisted with the calculated molecular mass of compounds. ¹H NMR and ¹³C NMR spectra were obtained on a Bruker DRX spectrometer at 400 MHz with TMS as an internal standard, δ in parts per million and *J* in hertz. ¹H NMR and ¹³C NMR peaks consisted with the structures of

compounds. All reactions were monitored by TLC using 0.25 mm silica gel plates (60 GF-254). UV light and ferric chloride were used to visualize the spots. Silica gel was used for column chromatography purification. Flash chromatography was accomplished using the automated CombiFlash Rf system from Teledyne ISCO and was done using silica gel of 200–300 mesh. Melting points were determined on an electrothermal melting point apparatus. All tested compounds are >95% pure by HPLC analysis, performed on an Agilent 1100 HPLC instrument using an ODS HYPERSIL column (5 μ m, 4.6 mm $\times$ 250 mm).

3.1.1. General procedure for the preparation of 6 and 16

3.1.1.1. Methyl 4-(aminomethyl)benzoate hydrochloride (6): Compound **5** (1.5 g, 10 mmol) was dissolved in 200 mL MeOH, then acetyl chloride (2.4 g, 30 mmol) was added dropwise at 0 °C; the mixed solution was refluxed at 75 °C for 5 h. The solvent was evaporated under vacuum, the product was washed by diethyl ether to give compound **6**, a white solid powder (1.7 g, 93%).

3.1.1.2. Methyl 6-aminohexanoate hydrochloride (16): Using the synthetic method for **5**, compound **15** gave **16** as a white solid, 94% yield.

3.1.2. General procedure for the preparation of 25a and 25b

3.1.2.1. Methyl 4-hydroxybenzoate (25a): **24a** (2.76 g, 20 mmol) was dissolved in 100 mL MeOH, then acetyl chloride (4.7 g, 60 mmol) was added dropwise at 0 °C; the mixed solution was refluxed at 75 °C for 5 h. The solvent was evaporated under vacuum, the product dissolved with EtOAc, washed by saturated NaHCO₃ solution (2 $\times$ 100 mL), 1 M aqueous citric acid (2 $\times$ 100 mL) and brine (2 $\times$ 100 mL), dried over MgSO₄ overnight and evaporated under vacuum to obtained product compound **25a**, a white solid (7.98 g, 87.5%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.33 (s, 1H), 7.84–7.79 (m, 2H), 6.87–6.82 (m, 2H), 3.78 (s, 3H). ESI-MS *m/z*: 153.2 [M+H]⁺.

3.1.2.2. Methyl 2-(4-hydroxyphenyl)acetate (25b): Using the synthetic method for **25a**, compound **24b** gave **25b** as a white solid, 81% yield.

3.1.3. General procedure for the preparation of 26a and 26b

3.1.3.1. Methyl(S)-4-(2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propoxy)benzoate (26a): Compound **23** (2.90 g, 10 mmol), **25a** (1.82 g, 12 mmol), and PPh₃ (3.95 g, 15 mmol) were dissolved in anhydrous THF, then DEAD (2.61 g, 15 mmol) was added dropwise at 0 °C, the mixture was stirred for 4 h, then THF was evaporated. The crude material was purified via flash chromatography to afford the compound **26a**, a white solid (2.52 g, 60%). ESI-MS *m/z*: 425.4 [M+H]⁺.

3.1.3.2. Methyl(S)-2-(4-(2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propoxy)phenyl) acetate (26b): Using the synthetic method for **26a**, compound **25b** gave **26b** as a white solid, 55% yield. ESI-MS *m/z*: 439.6 [M+H]⁺.

3.1.4. General procedure for the preparation of 7, 9a-9g, 13, 17, 28a, 28b, 31, 39a, 39b

3.1.4.1. Methyl(S)-4-((2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propanamido)methyl) benzoate (7): To a solution of **4** (2.4 g, 8 mmol) in anhydrous CH₂Cl₂, was added 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 2.8 g, 8.8 mmol), followed by Et₃N (0.9 g, 8.8 mmol). After 30 min, compound **6** (1.6 g, 8.8 mmol) was added followed by Et₃N (0.9 g, 8.8 mmol). 4 h later, the solution of CH₂Cl₂ was washed with 1 M HCl (2 × 30 mL), saturated Na₂CO₃ (2 × 30 mL) and brine (2 × 30 mL), dried over MgSO₄ overnight, and the solvent was evaporated under vacuum to afford colorless oil. The crude product was purified by recrystallization with EtOAc and petroleum ether. The desired compound **7** was white solid, 55% yield. ESI-MS *m/z*: 452.2 [M+H]⁺.

3.1.4.2. Methyl (S)-4-((3-(1H-indol-3-yl)-2-(4 methoxybenzamido)propanamido)methyl) benzoate (9a): Using the synthetic method for **7**, compound 4-methoxybenzoic acid and **8** gave **9a** as a white solid, 53% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.80 (s, 1H), 8.68 (t, *J* = 6.0 Hz, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 7.91–7.80 (m, 4H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.32 (dd, *J* = 8.2, 2.2 Hz, 3H), 7.21 (d, *J* = 2.2 Hz, 1H), 7.08–7.04 (m, 1H), 7.00–6.96 (m, 3H), 4.79–4.74 (m, 1H), 4.44–4.32 (m, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.30–3.23 (m, 1H), 3.21–3.13 (m, 1H). ESI-MS *m/z*: 486.5 [M+H]⁺.

3.1.4.3. Methyl (S)-4-((2-(4-(dimethylamino)benzamido)-3-(1H-indol-3yl)propanamido)methyl)benzoate (9b): Using the synthetic method for **7**, compound 4-(dimethylamino)benzoic acid and **8** gave **9b** as a white solid, 55% yield.

3.1.4.4. Methyl (S)-4-((3-(1H-indol-3-yl)-2-(4-(trifluoromethyl)benzamido)propanamido)methyl)benzoate (9c): Using the synthetic method for **7**, compound 4-(trifluoromethyl)benzoic acid and **8** gave **9c** as a white solid, 52% yield. ESI-MS *m/z*: 524.5 [M+H]⁺.

3.1.4.5. Methyl (S)-4-((3-(1H-indol-3-yl)-2-(picolinamido)propanamido)methyl)benzoate (9d): Using the synthetic method for **7**, compound picolinic acid and **8** gave **9d** as a white solid, 58% yield.

3.1.4.6. Methyl (S)-4-((3-(1H-indol-3-yl)-2-(pyrazine-2-carboxamido)propanamido)methyl) benzoate (9e): Using the synthetic method for **7**, compound pyrazine-2-carboxylic acid and **8** gave **9e** as a white solid, 54% yield.

3.1.4.7. Methyl(S)-4-((3-(1H-indol-3-yl)-2-(thiophene-2-carboxamido)propanamido)methyl)benzoate (9f): Using the synthetic method for **7**, compound thiophene-2-carboxylic acid and **8** gave **9f** as a white solid, 54% yield.

3.1.4.8. Methyl (S)-4-((2-(furan-2-carboxamido)-3-(1H-indol-3-yl)propanamido)methyl) benzoate (9g): Using the synthetic method for **7**, compound furan-2-carboxylic acid and **8** gave **9g** as a white solid, 56% yield.

3.1.4.9. Methyl (4-methoxybenzoyl)-L-tryptophanate (13): Using the synthetic method for **7**, compound 4-methoxybenzoic acid and **12** gave **13** as a white solid, 52% yield.

3.1.4.10. Methyl (S)-6-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)hexanoate (17): Using the synthetic method for **7**, compound **14** and **16** gave **17** as a white solid, 52% yield.

3.1.4.11. Methyl (S)-4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propoxy)benzoate (28a): Using the synthetic method for **7**, compound 4-methoxybenzoic acid and **27a** gave **28a** as a white solid, 52% yield.

3.1.4.12. Methyl(S)-2-(4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propoxy)phenyl)acetate (28b): Using the synthetic method for **7**, compound 4-methoxybenzoic acid and **27b** gave **28b** as a white solid, 52% yield.

3.1.4.13. (S)-N-(3-(1H-indol-3-yl)-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)-4-methoxybenzamide (31): Using the synthetic method for **7**, compound **14** and *N,O*-dimethylhydroxylamine gave **31** as a white solid, 78% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.79 (s, 1H), 8.52 (d, *J* = 7.6 Hz, 1H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 7.24 (d, *J* = 2.1 Hz, 1H), 7.06 (t, *J* = 7.1 Hz, 1H), 7.02–6.96 (m, 3H), 5.11 (s, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 3.16 (s, 3H), 3.14–3.10 (m, 2H).

3.1.4.14. Tert-butyl (S)-(2-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)ethyl) carbamate (39a): Using the synthetic method for **7**, compound **14** and **38a** gave **39a** as a white solid, 70% yield.

3.1.4.15. Tert-butyl (S)-(3-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)propyl) carbamate (39b): Using the synthetic method for **7**, compound **14** and **38b** gave **39b** as a white solid, 65% yield.

3.1.5. General procedure for the preparation of **8**, **27a**, **27b** and **37**

3.1.5.1. Methyl(S)-4-((2-amino-3-(1H-indol-3-yl)propanamido)methyl)benzoate hydrochloride (8): Compound **7** (2.0 g, 4.4 mmol) was dissolved in a solution of EtOAc (20 mL) saturated by dry HCl gas. The solution was stirred at room temperature overnight. The filtered precipitate, was washed by diethyl ether to give the compound **8**, a white solid powder (1.4 g, 80%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.09 (d, *J* = 1.6 Hz, 1H), 9.14 (t, *J* = 5.8 Hz, 1H), 8.35 (s, 2H), 7.84 (d, *J* = 8.3 Hz, 2H), 7.67, (d, *J* = 7.9 Hz, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.22–7.19 (m, 3H), 7.13–7.06 (m, 1H), 7.04–6.97 (m, 1H), 4.40 (dd, *J* = 15.9, 6.0 Hz, 1H), 4.31 (dd, *J* = 15.9, 5.6 Hz, 1H), 4.06 (s, 1H), 3.85 (s, 3H), 3.31–3.15 (m, 2H). ESI-MS *m/z*: 352.5 [M+H]⁺.

3.1.5.2. Methyl (S)-4-(2-amino-3-(1H-indol-3-yl)propoxy)benzoate hydrochloride (27a): Using the synthetic method for **7**, compound **26a** gave **27a** as a white solid, 78% yield.

3.1.5.3. Methyl (S)-2-(4-(2-amino-3-(1H-indol-3-yl)propoxy)phenyl) acetate

(27b): Using the synthetic method for **7**, compound **26b** gave **27b** as a white solid, 82% yield.

3.1.5.4. (S)-4-(((3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propyl) amino)methyl)-N-(2-aminophenyl)benzamide dihydrochloride (37):

Using the synthetic method for **7**, compound **36** gave **37** as a white solid, 78% yield. Mp: 180–182 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.92 (s, 1H), 10.59 (s, 1H), 9.43 (s, 2H), 8.55 (d, *J* = 8.1 Hz, 1H), 8.13 (d, *J* = 8.1 Hz, 2H), 7.94 (d, *J* = 8.7 Hz, 2H), 7.69–7.65 (m, 3H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 7.4 Hz, 1H), 7.41–7.29 (m, 3H), 7.19 (d, *J* = 1.6 Hz, 1H), 7.07 (t, *J* = 7.4 Hz, 1H), 7.01–6.98 (m, 3H), 4.66 (s, 1H), 4.31–4.28 (m, 1H), 4.21–4.16 (m, 1H), 3.81 (s, 5H), 3.13–3.05 (m, 1H), 3.02–2.97 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.69, 165.60, 162.18, 136.67, 135.94, 134.50, 130.55, 129.96, 128.70, 127.79, 127.75, 127.08, 126.92, 124.07, 123.92, 121.47, 118.98, 118.82, 113.80, 111.90, 110.49, 55.86, 50.22, 49.95, 47.82, 28.84, 21.54. HRMS (AP-ESI) *m/z* calcd for C₃₃H₃₃N₅O₃ [M+H]⁺ 548.2656, found 548.2659.

3.1.6. General procedure for the preparation of 32

3.1.6.1. (S)-N-(1-(1H-indol-3-yl)-3-oxopropan-2-yl)-4-methoxybenzamide (32): At –40 °C, to a solution of **31** (3.81 g, 10 mmol) in anhydrous THF (150 mL) was added LiAlH₄ (0.45 g, 12 mmol) in batch. After 4 h, the reaction was quenched by adding 1 M aqueous citric acid slowly. Then THF was evaporated with the residue being extracted by EtOAc (3 × 50 mL). The EtOAc solution was washed with saturated Na₂CO₃ (3 × 30 mL) and brine (3 × 30 mL), dried over MgSO₄ and evaporated under vacuum to afford **32** (2.58 g, 8 mmol), the crude product was used for the following reaction without any purification.

3.1.7. General procedure for the preparation of 33

3.1.7.1. Methyl(S)-4-(((3-(1H-indol-3-yl)-2-(4-methoxybenzamido) propyl)amino)methyl)benzoate (33): Solution of compound **6** (1.6 g, 8 mmol) in 50 mL CH₃OH, was added with Et₃N (0.81 g, 8 mmol) followed by **32** (2.58 g, 8 mmol). After the addition of AcOH (0.48 g, 8 mmol), NaBH₃CN (0.75 g, 12 mmol) was added in batch. The reaction was stirred at room temperature overnight. After the reaction finished, the solvent was evaporated under vacuum and the crude material was purified via flash chromatography to afford the compound to yield **33** (1.51 g, 40%). ESI-MS *m/z*: 472.5 [M+H]⁺.

3.1.8. General procedure for the preparation of 29

3.1.8.1. Methyl(S)-4-(((3-(1H-indol-3-yl)-2-(4-methoxybenzamido) propyl)(tert-butoxy carbonyl)amino)methyl)benzoate (34): At room temperature, to a solution of **33** (1.41 g, 3 mmol) in anhydrous CH₂Cl₂ (20 mL), was added Et₃N (0.91 g, 9 mmol) followed by (Boc)₂O (0.78 g, 3.6 mmol). After stirring the mixture at room temperature for 8 h, the solution was washed with 1 M aqueous citric acid (3 × 10 mL) and brine (3 × 10 mL), dried over MgSO₄, and evaporated under vacuum to yield the crude product compound **34** (1.54 g, 90% yield) as a white solid. This product was used for the following reaction without further purification. ESI-MS *m/z*: 572.4 [M+H]⁺.

3.1.9. General procedure for the preparation of **2**, **10a**–**10 g**, **14**, **18**, **21**, **29a**, **29b**, **35**

3.1.9.1. (S)-4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)benzoic acid (2**):** Compound **1** (0.56 g, 1.2 mmol) was dissolved in 5 mL CH₃OH, then 2 mL 3 M KOH aqueous solution was added. The mixture was refluxed at 85 °C for 2 h. After the reaction completed, it was evaporated under vacuum. The residue was acidified with 1 N HCl to pH 3–4, then filtered. The precipitate was **2**, a white solid, 85% yield.

3.1.9.2. (S)-4-((3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)methyl)benzoic acid (10a**):** Using the synthetic method for **2**, compound **9a** gave **10a** as a white solid, 87% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.83 (s, 1H), 10.81 (s, 1H), 8.68 (t, *J* = 5.9 Hz, 1H), 8.42 (d, *J* = 7.9 Hz, 1H), 7.90–7.80 (m, 4H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.33–7.28 (m, 3H), 7.21 (d, *J* = 2.1 Hz, 1H), 7.05 (t, *J* = 7.2 Hz, 1H), 6.99–6.96 (m, 3H), 4.79–4.73 (m, 1H), 4.38–4.37 (m, 2H), 3.80 (s, 3H), 3.28–3.12 (m, 2H). ESI-MS *m/z*: 472.3 [M+H]⁺.

3.1.9.3. (S)-4-((2-(4-(dimethylamino)benzamido)-3-(1H-indol-3-yl)propanamido)methyl) benzoic acid (10b**):** Using the synthetic method for **2**, compound **9b** gave **10b** as a white solid, 87% yield.

3.1.9.4. (S)-4-((3-(1H-indol-3-yl)-2-(4-(trifluoromethyl)benzamido)propanamido)methyl) benzoic acid (10c**):** Using the synthetic method for **2**, compound **9c** gave **10c** as a white solid, 80% yield.

3.1.9.5. (S)-4-((3-(1H-indol-3-yl)-2-(picolinamido)propanamido)methyl)benzoic acid (10d**):** Using the synthetic method for **2**, compound **9d** gave **10d** as a white solid, 85% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.86 (s, 1H), 8.76 (t, *J* = 5.9 Hz, 1H), 8.67 (d, *J* = 8.2 Hz, 1H), 8.63 (d, *J* = 4.6 Hz, 1H), 8.06–7.95 (m, 2H), 7.84 (d, *J* = 8.2 Hz, 2H), 7.65–7.57 (m, 2H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 8.2 Hz, 2H), 7.13 (d, *J* = 2.2 Hz, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 6.94 (t, *J* = 7.4 Hz, 1H), 4.88–4.83 (m, 1H), 4.43–4.27 (m, 2H), 3.27–3.14 (m, 2H). ESI-MS *m/z*: 443.5 [M+H]⁺.

3.1.9.6. (S)-4-((3-(1H-indol-3-yl)-2-(pyrazine-2-carboxamido)propanamido)methyl) benzoic acid (10e**):** Using the synthetic method for **2**, compound **9e** gave **10e** as a white solid, 83% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.66 (s, 1H), 10.85 (s, 1H), 9.16 (d, *J* = 1.3 Hz, 1H), 8.87 (d, *J* = 2.5 Hz, 1H), 8.76 (t, *J* = 5.9 Hz, 1H), 8.72–8.71 (m, 1H), 8.69 (d, *J* = 8.2 Hz, 1H), 7.85 (d, *J* = 8.2 Hz, 2H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.27 (d, *J* = 8.2 Hz, 2H), 7.14 (d, *J* = 2.1 Hz, 1H), 7.05 (t, *J* = 7.2 Hz, 1H), 6.93 (t, *J* = 7.3 Hz, 1H), 4.89–4.83 (m, 1H), 4.36 (d, *J* = 5.8 Hz, 2H), 3.31–3.26 (m, 2H). ESI-MS *m/z*: 444.5 [M+H]⁺.

3.1.9.7. (S)-4-((3-(1H-indol-3-yl)-2-(thiophene-2-carboxamido)propanamido)methyl)benzoic acid (10f**):** Using the synthetic method for **2**, compound **9f** gave **10f** as a white solid, 86% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.82 (s, 1H), 8.73 (t, *J* = 5.9 Hz, 1H), 8.66 (d, *J* = 8.1 Hz, 1H), 7.89 (d, *J* = 2.9 Hz, 1H), 7.85 (d, *J* = 8.2 Hz, 2H), 7.73 (dd, *J* = 5.0, 0.7 Hz, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 8.0 Hz,

1H), 7.26 (d, J = 8.2 Hz, 2H), 7.20 (d, J = 2.1 Hz, 1H), 7.13 (dd, J = 4.9, 3.8 Hz, 1H), 7.06 (t, J = 7.1 Hz, 1H), 6.98 (t, J = 7.1 Hz, 1H), 4.77–4.71 (m, 1H), 4.37 (t, J = 5.8 Hz, 2H), 3.20–3.10 (m, 2H). ESI-MS m/z : 448.4 $[M+H]^+$.

3.1.9.8. (S)-4-((2-(furan-2-carboxamido)-3-(1H-indol-3-yl)propanamido)methyl)benzoic acid (10 g): Using the synthetic method for **2**, compound **9g** gave **10g** as a white solid, 82% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 12.84 (s, 1H), 10.81 (s, 1H), 8.71 (t, J = 5.9 Hz, 1H), 8.31 (d, J = 8.1 Hz, 1H), 7.86–7.81 (m, 3H), 7.65 (d, J = 7.9 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 7.28 (d, J = 2H), 8.2 Hz, 7.18–7.15 (m, 2H), 7.05 (t, J = 11.2, 7.2 Hz, 1H), 6.97 (t, J = 7.2 Hz, 1H), 6.60 (dd, J = 3.4, 1.7 Hz, 1H), 4.78–4.72 (m, 1H), 4.36 (d, J = 5.9 Hz, 2H), 3.29–3.24 (m, 1H), 3.19–3.13 (m, 1H). ESI-MS m/z : 432.4 $[M+H]^+$.

3.1.9.9. (4-methoxybenzoyl)-L-tryptophan (14): Using the synthetic method for **2**, compound **13** gave **14** as a white solid, 78% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 10.79 (s, 1H), 8.34 (d, J = 7.7 Hz, 1H), 7.82–7.76 (m, 2H), 7.59 (d, J = 7.8 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.18 (d, J = 2.2 Hz, 1H), 7.07–7.01 (m, 1H), 6.97–6.93 (m, 3H), 4.63–4.57 (m, 1H), 3.79 (s, 3H), 3.35–3.30 (m, 1H), 3.26–3.16 (m, 1H).

3.1.9.10. (S)-6-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)hexanoic acid (18): Using the synthetic method for **2**, compound **17** gave **18** as a white solid, 83% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 12.03 (s, 1H), 10.76 (s, 1H), 8.28 (d, J = 8.1 Hz, 1H), 8.02 (t, J = 5.5 Hz, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 7.8 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.19 (d, J = 2.1 Hz, 1H), 7.04 (dd, J = 11.0, 3.9 Hz, 1H), 6.99–6.95 (m, 3H), 4.69–4.63 (m, 1H), 3.80 (s, 3H), 3.23–2.97 (m, 4H), 2.17 (t, J = 7.4 Hz, 2H), 1.55–1.43 (m, 2H), 1.41–1.34 (m, 2H), 1.26–1.20 (m, 2H). ESI-MS m/z : 474.4 $[M+Na]^+$.

3.1.9.11. (S,E)-3-(4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propoxy)phenyl)acrylic acid (21): Using the synthetic method for **2**, compound **20** gave **21** as a white solid, 80% yield. ESI-MS m/z : 471.5 $[M+H]^+$.

3.1.9.12. (S)-4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propoxy)benzoic acid (29a): Using the synthetic method for **2**, compound **28a** gave **29a** as a white solid, 83% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 10.83 (s, 1H), 8.37 (d, J = 7.9 Hz, 1H), 7.84 (dd, J = 8.9, 2.5 Hz, 4H), 7.64 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.18 (d, 1H), (t, J = 2.2 Hz, 7.06 J = 7.5 Hz, 1H), 7.00–6.94 (m, 5H), 4.59 (dd, J = 12.9, 6.5 Hz, 1H), 4.21 (dd, J = 9.8, 6.5 Hz, 1H), 4.10 (dd, J = 9.9, 5.1 Hz, 1H), 3.80 (s, 3H), 3.10 (d, J = 6.9 Hz, 2H). ESI-MS m/z : 445.6 $[M+H]^+$.

3.1.9.13. (S)-2-(4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propoxy)phenyl)acetic acid (29b): Using the synthetic method for **2**, compound **28b** gave **29b** as a white solid, 80% yield.

3.1.9.14. (S)-4-(((3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propyl)(tert-butoxycarbonyl) amino)methyl)benzoic acid (35): Using the synthetic method for **2**, compound **34** gave **35** as a white solid, 80% yield.

3.1.10. General procedure for the preparation of 39 and 45

3.1.10.1. Tert-butyl (4-bromo-2-nitrophenyl)carbamate (39): At room temperature, to a solution of **38** (10.85 g, 50 mmol) in anhydrous CH_2Cl_2 (300 mL), Et_3N (15.17 g, 150 mmol) was added followed by $(\text{Boc})_2\text{O}$ (2.18 g, 100 mmol) and DMAP (4-dimethylaminopyridine) (0.06 g, 0.5 mmol). After stirring the mixture at room temperature for 2 h, the solution was washed with 1 M aqueous citric acid (3×100 mL) and brine (3×100 mL), dried over MgSO_4 , and evaporated under vacuum to get the crude product. The crude product was recrystallized by EtOAc and petroleum ether to achieve a white pure solid (8.71 g, 55%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.34 (d, $J = 2.3$ Hz, 1H), 8.02 (dd, $J = 8.5$, 2.3 Hz, 1H), 7.60 (d, $J = 8.5$ Hz, 1H), 1.33 (s, 9H).

3.1.10.2. Di-tert-butyl (5-fluoro-2-nitrophenyl)carbamate (45): Using the synthetic method for **39**, compound **44** gave **45** as a white solid, 87% yield. ESI-MS m/z : 357.3 [$\text{M} + \text{H}$] $^+$.

3.1.11. General procedure for the preparation of 46

3.1.11.1. Tert-butyl (5-fluoro-2-nitrophenyl)carbamate (46): A solution of **45** (3.57 g, 10 mmol) in CH_2Cl_2 (100 mL) at room temperature was treated with TFA (1.14 g, 10 mmol) drop wise, and the resulting mixture was stirred at this temperature for 80 min before being treated with a saturated NaHCO_3 aqueous solution. The layers were separated, and the organic phase was washed with a saturated NaHCO_3 aqueous solution, dried over Na_2SO_4 concentrated in vacuo to give **46** (0.23 g, 90%) as a yellow solid, which was used in the next step without further purification. ESI-MS m/z : 255.4 [$\text{M} - \text{H}$] $^-$.

3.1.12. General procedure for the preparation of 40a and 40b

3.1.12.1. Tert-butyl (2-nitro-4-(thiophen-2-yl)phenyl)carbamate (40a): To a solution of compound **39** (6.34 g, 20 mmol), thiophene-2-boronic acid (3.07 g, 24 mmol), PPh_3 (0.11 g, 0.4 mmol), $\text{Pd}(\text{OAc})_2$ (0.04 g, 0.2 mmol) in 20 mL DMSO Na_2CO_3 (4.24 g, 40 mmol) was added dissolving in 15 mL of water under N_2 atmosphere. The N_2 protected reaction mixture was heated at 100 $^\circ\text{C}$ for 5 h, after the reaction finished, the mixture was poured on 200 mL water and extracted by EtOAc. The organic phase was washed with saturated 1 M HCl (2×30 mL), NaHCO_3 (2×30 mL) and brine (2×30 mL), dried over Na_2SO_4 overnight, and the solvent was evaporated under vacuum. The crude material was purified via flash chromatography to afford the compound **40a** (3.71 g, 58%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.65 (s, 1H), 8.15 (d, $J = 2.2$ Hz, 1H), 7.95 (dd, $J = 8.6$, 2.2 Hz, 1H), 7.70 (d, $J = 8.6$ Hz, 1H), 7.66–7.59 (m, 2H), 7.17 (dd, $J = 5.0$, 3.7 Hz, 1H), 1.46 (s, 9H). ESI-MS m/z : 343.3 [$\text{M} + \text{Na}$] $^+$.

3.1.12.2. Tert-butyl (3-nitro-[1,1'-biphenyl]-4-yl)carbamate (40b): Using the synthetic method for **40a**, compound **39** and phenylboronic acid gave **40b** as a white solid, 48% yield. ESI-MS m/z : 337.6 [$\text{M} + \text{Na}$] $^+$.

3.1.13. General procedure for the preparation of 41a, 41b and 47

3.1.13.1. Tert-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate (41a): To a solution of **40a** (3.43 g, 10 mmol) in CH₃OH (200 mL), was added dry palladium-carbon (0.34 g), the mixture was stirred under a hydrogen atmosphere overnight. The palladium-carbon was filtered off over celite, after evaporating the solvent, the residue was dried under vacuum to give **41a** (2.46 g, 85%), a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.35 (s, 1H), 7.45–7.43 (m, 1H), 7.30 (dd, *J* = 3.6, 1.1 Hz, 1H), 7.27 (d, *J* = 8.2 Hz, 1H), 7.08 (dd, *J* = 5.1, 3.6 Hz, 1H), 6.98 (d, *J* = 2.1 Hz, 1H), 6.85 (dd, *J* = 8.2, 2.1 Hz, 1H), 5.01 (s, 2H), 1.47 (s, 9H). ESI-MS *m/z*: 291.3 [M+H]⁺.

3.1.13.2. Tert-butyl (3-amino-[1,1'-biphenyl]-4-yl)carbamate (41b): Using the synthetic method for **41a**, compound **40b** gave **41b** as a white solid, 86% yield. ESI-MS *m/z*: 307.4 [M + Na]⁺.

3.1.13.3. Tert-butyl (2-amino-5-fluorophenyl)carbamate (47): Using the synthetic method for **41a**, compound **46** gave **47** as a white solid, 80% yield. ESI-MS *m/z*: 227.2 [M+H]⁺.

3.1.14. General procedure for the preparation of 51a and 51b

3.1.14.1. 4-(2,2,2-trifluoroacetamido)benzoic acid (51a): **50** (2.74 g, 20 mmol) was dissolved in 10 mL TFA slowly at 0 °C, the solution was stirred at room temperature for 2 h. After the reaction finished, the mixture was quenched by adding 100 mL ice water and filtered, the residue was dried under infrared drying oven to afford **51a** (4.43 g, 95%).

3.1.14.2. 4-((2,2,2-trifluoroacetamido)methyl)benzoic acid (51b): Using the synthetic method for **51a**, compound **6** gave **51b** as a white solid, 98% yield. mp: 214–215 °C, ¹H NMR (400 MHz DMSO-*d*₆): δ 4.47 (d, *J* = 6.0 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.94 (d, *J* = 8.2 Hz, 2H), 10.07 (s, 1H), 12.94 (s, 1H). ESI-MS *m/z*: 248.5 [M+H]⁺.

3.1.15. General procedure for the preparation of 53a–53c, 57a and 57b

3.1.15.1. Tert-butyl (4-(thiophen-2-yl)-2-(4-(2,2,2-trifluoroacetamido)benzamido)phenyl)carbamate (53a): Compound **51a** (1.16 g, 5 mmol) was dissolved in 15 mL SOCl₂, the mixed solution was refluxed at 80 °C for 5 h. The solvent was evaporated under vacuum, the product (**52a**) was dissolved with anhydrous CH₂Cl₂ and was dropped in the CH₂Cl₂ solution of **41a** (1.45 g, 5 mmol) at 0 °C followed by Et₃N (0.51 g, 5 mmol). The mixture reacted at room temperature overnight, the solution was washed with 1 N HCl (2 × 30 mL), saturated NaHCO₃ (2 × 30 mL) and brine (2 × 30 mL), dried over Na₂SO₄ overnight, and the solvent was evaporated under vacuum. The crude product was recrystallized by EtOAc and petroleum ether to achieve a white pure solid (1.57 g, 62%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.54 (s, 1H), 9.93 (s, 1H), 8.75 (s, 1H), 8.04 (d, *J* = 8.7 Hz, 2H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.82 (d, *J* = 2.2 Hz, 7.63 (d, *J* = 8.5 Hz, 1H), 7.55–7.49 (m, 2H), 7.46 (dd, *J* = 3.6, 1.0 Hz, 1H), 7.14 (dd, *J* = 5.1, 3.6 Hz, 1H), 1.46 (s, 9H). ESI-MS *m/z*: 506.5 [M+H]⁺.

3.1.15.2. Tert-butyl(4-(thiophen-2-yl)-2-(4-((2,2,2-trifluoroacetamido)methyl)benzamido)phenyl)carbamate (53b): Using the synthetic

method for **53a**, compound **51b** and **41a** gave **53b** as a white solid, 58% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 10.11 (t, J = 6.0 Hz, 1H), 9.91 (s, 1H), 8.75 (s, 1H), 7.97 (d, J = 8.3 Hz, 2H), 7.83 (d, J = 2.1 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.55–7.50 (m, 2H), 7.46–7.44 (m, 3H), 7.14 (dd, J = 5.0, 3.6 Hz, 1H), 4.49 (d, J = 6.0 Hz, 2H), 1.46 (s, 9H).

3.1.15.3. Tert-butyl (3-(4-((2,2,2-trifluoroacetamido)methyl)benzamido)-[1,1'-biphenyl]-4-yl)carbamate (53c): Using the synthetic method for **53a**, compound **51b** and **41b** gave **53c** as a white solid, 58% yield. ESI-MS m/z : 514.5 $[\text{M}+\text{H}]^+$.

3.1.15.4. Tert-butyl (5-fluoro-2-(4-(2,2,2-trifluoroacetamido)benzamido)phenyl)carbamate (57a): Using the synthetic method for **53a**, compound **52a** and **47** gave **57a** as a white solid, 56% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 11.52 (s, 1H), 9.79 (s, 1H), 8.79 (s, 1H), 8.01 (t, J = 6.9 Hz, 2H), 7.82 (t, J = 8.2 Hz, 2H), 7.54 (dd, J = 11.2, 2.8 Hz, 1H), 7.45 (dd, J = 8.8, 6.3 Hz, 1H), 6.97 (td, J = 8.4, 2.9 Hz, 1H), 1.45 (s, 9H). ESI-MS m/z : 442.4 $[\text{M}+\text{H}]^+$.

3.1.15.5. Tert-butyl(5-fluoro-2-(4-(2,2,2-trifluoroacetamido)methyl)benzamido)phenyl)carbamate (57b): Using the synthetic method for **53a**, compound **52b** and **47** gave **57b** as a white solid, 48% yield. ESI-MS m/z : 456.4 $[\text{M}+\text{H}]^+$.

3.1.16. General procedure of compound **54a-54c**, **58a** and **58b**

3.1.16.1. Tert-butyl (2-(4-aminobenzamido)-4-(thiophen-2-yl)phenyl)carbamate (54a): To a solution of **53a** (1.51 g, 3 mmol) in the mixture of 10 mL CH_3OH and 10 mL H_2O , K_2CO_3 (1.24 g, 9 mmol) was added. The mixture was then stirred at room temperature overnight, and the CH_3OH was evaporated under vacuum. The residue was extracted by EtOAc. The organic phase was washed with brine (2×30 mL), dried over Na_2SO_4 overnight, and the solvent was evaporated under vacuum to afford **54a** (0.98 g, 80%). ^1H NMR (400 MHz, DMSO- d_6) δ 9.57 (s, 1H), 8.71 (s, 1H), 7.82 (d, J = 2.1 Hz, 1H), 7.71 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 8.5 Hz, 1H), 7.52 (dd, J = 5.1, 0.9 Hz, 1H), 7.47 (dd, J = 8.5, 2.1 Hz, 1H), 7.44 (dd, J = 3.5, 0.9 Hz, 1H), 7.13 (dd, J = 5.0, 3.6 Hz, 1H), 6.62 (d, J = 8.6 Hz, 2H), 5.85 (s, 2H), 1.47 (s, 9H).

3.1.16.2. Tert-butyl (2-(4-(aminomethyl)benzamido)-4-(thiophen-2-yl)phenyl)carbamate (54b): Using the synthetic method for **54a**, compound **53b** gave **54b** as a white solid, 79% yield.

3.1.16.3. Tert-butyl (3-(4-(aminomethyl)benzamido)-[1,1'-biphenyl]-4-yl)carbamate (54c): Using the synthetic method for **54a**, compound **53c** gave **54c** as a white solid, 83% yield.

3.1.16.4. Tert-butyl (2-(4-aminobenzamido)-5-fluorophenyl)carbamate (58a): Using the synthetic method for **54a**, compound **57a** gave **58a** as a white solid, 78% yield. ESI-MS m/z : 346.3 $[\text{M}+\text{H}]^+$.

3.1.16.5. Tert-butyl (2-(4-(aminomethyl)benzamido)-5-fluorophenyl)carbamate

(58b): Using the synthetic method for **54a**, compound **57b** gave **58a** as a white solid, 84% yield.

3.1.17. General procedure for the preparation of 3, 11a-11 g, 19, 22, 30a, 30b, 36, 49a, 42b, 48, 55a-55c, 59a and 59b

3.1.17.1. (S)-4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)-N-(2-aminophenyl) benzamide (3): To a solution of **2** (0.46 g, 1 mmol) in anhydrous THF, 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate (TBTU, 0.35 g, 1.1 mmol) was added, followed by Et₃N (0.15 g, 1.5 mmol). 30 min later, compound benzene-1,2-diamine (0.12 g, 1.1 mmol) was added. After the reaction finished, the solution was evaporated under vacuum, the residue was dissolved with CH₂Cl₂, and washed with saturated NaHCO₃ (2 × 30 mL) and brine (2 × 30 mL), dried over Na₂SO₄ overnight, and the solvent was evaporated under vacuum. The crude material was purified via flash chromatography to afford the compound **3** (0.16 g, 30%). Mp: 212–214 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.81 (s, 1H), 10.51 (s, 1H), 9.61 (s, 1H), 8.55 (d, *J* = 7.7 Hz, 1H), 7.98 (d, *J* = 8.6 Hz, 2H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.7 Hz, 3H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 1.9 Hz, 1H), 7.18 (d, *J* = 7.4 Hz, 1H), 7.07 (t, *J* = 7.2 Hz, 1H), 7.03–6.94 (m, 4H), 6.84–6.77 (m, 1H), 6.62 (t, *J* = 7.1 Hz, 1H), 5.03 (s, 2H), 4.94–4.88 (m, 1H), 3.80 (s, 3H), 3.29–3.20 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.97, 166.40, 165.23, 162.19, 143.26, 142.30, 136.54, 129.86, 129.57, 129.12, 127.74, 127.13, 126.86, 126.58, 124.30, 124.17, 121.42, 119.13, 119.06, 118.71, 117.06, 116.82, 113.89, 111.80, 110.67, 55.83, 55.66, 27.97. HRMS (AP-ESI) *m/z* calcd for C₃₂H₂₉N₅O₄ [M+H]⁺ 548.2292, found 548.2293.

3.1.17.2. (S)-4-((3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)methyl)-N-(2-aminophenyl)benzamide (11a): Using the synthetic method for **3**, compound **10a** gave **11a** as a white solid, 32% yield. Mp: 187–188 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.80 (s, 1H), 9.64 (s, 1H), 8.70 (t, *J* = 5.9 Hz, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 7.92 (d, *J* = 8.1 Hz, 2H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.39–7.30 (m, 3H), 7.22 (d, *J* = 2.0 Hz, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 7.06 (t, *J* = 7.4 Hz, 1H), 7.01–6.96 (m, 4H), 6.83–6.77 (m, 1H), 6.63 (t, *J* = 7.4 Hz, 1H), 5.09 (s, 2H), 4.80–4.74 (m, 1H), 4.39 (d, *J* = 5.8 Hz, 2H), 3.28–3.12 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.57, 166.24, 165.65, 162.11, 143.57, 143.10, 136.58, 133.47, 129.81, 128.22, 127.75, 127.23, 127.13, 126.92, 126.81, 124.18, 124.11, 121.36, 118.98, 118.71, 117.11, 116.86, 113.82, 111.80, 111.04, 55.82, 54.93, 42.39, 28.05. HRMS (AP-ESI) *m/z* calcd for C₃₃H₃₁N₅O₄ [M+H]⁺ 562.2449, found 562.2449.

3.1.17.3. (S)-N-(2-aminophenyl)-4-((2-(4-(dimethylamino)benzamido)-3-(1H-indol-3-yl)propanamido)methyl)benzamide (11b): Using the synthetic method for **3**, compound **10b** gave **11b** as a white solid, 28% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.80 (s, 1H), 9.70 (s, 1H), 8.65 (t, *J* = 5.9 Hz, 1H), 8.15 (d, *J* = 7.9 Hz, 1H), 7.92 (d, *J* = 8.1 Hz, 2H), 7.73 (d, *J* = 8.9 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.35–7.31 (m, 3H), 7.21–7.19 (m, 2H), 7.08–7.97 (m, 3H), 6.85 (d, *J* = 7.3 Hz, 1H), 6.69–6.67 (m, 3H), 5.53 (s, 2H), 4.78–4.73 (m, 1H), 4.39 (d, *J* = 5.8 Hz, 2H), 3.22–3.12 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.82,

166.64, 165.70, 152.65, 143.66, 136.57, 133.37, 129.32, 128.23, 127.78, 127.24, 127.19, 126.94, 124.63, 124.15, 121.34, 121.20, 118.97, 118.69, 117.86, 117.36, 111.79, 111.12, 54.80, 42.37, 28.08. HRMS (AP-ESI) m/z calcd for $C_{34}H_{34}N_6O_3$ $[M+H]^+$ 575.2765, found 575.2767.

3.1.17.4. (S)-4-((3-(1H-indol-3-yl)-2-(4-(trifluoromethyl)benzamido)propanamido)methyl)-N-(2-aminophenyl)benzamide

(11c): Using the synthetic method for **3**, compound **10c** gave **11c** as a white solid, 34% yield. 1H NMR (400 MHz, DMSO- d_6) δ 10.81 (s, 1H), 9.68 (s, 1H), 8.89 (d, J = 7.9 Hz, 1H), 8.78 (t, J = 5.8 Hz, 1H), 8.06 (d, J = 8.1 Hz, 2H), 7.93 (d, J = 8.0 Hz, 2H), 7.83 (d, J = 8.2 Hz, 2H), 7.71 (d, J = 7.8 Hz, 1H), 7.35–7.32 (m, 3H), 7.25–7.15 (m, 2H), 7.11–7.04 (m, 1H), 7.01–6.98 (m, 2H), 6.83 (d, J = 7.8 Hz, 1H), 6.66 (t, J = 7.4 Hz, 1H), 5.37 (s, 2H), 4.84–4.79 (m, 1H), 4.41 (d, J = 5.6 Hz, 2H), 3.23–3.11 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 172.15, 165.63, 143.50, 143.08, 138.29, 136.57, 133.46, 128.90, 128.26, 127.67, 127.20, 126.94, 125.66, 124.22, 124.08, 121.40, 118.98, 118.73, 117.11, 116.86, 111.83, 110.86, 55.16, 42.39, 27.99. HRMS (AP-ESI) m/z calcd for $C_{33}H_{28}F_3N_5O_3$ $[M+H]^+$ 600.2217, found 600.2220.

3.1.17.5. (S)-N-(1-((4-((2-aminophenyl)carbamoyl)benzyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)picolinamide (11d):

Using the synthetic method for **3**, compound **10d** gave **11d** as a white solid, 31% yield. Mp: 187–189 °C. 1H NMR (400 MHz, DMSO- d_6) δ 10.99 (s, 1H), 9.75 (s, 1H), 8.94–8.81 (m, 1H), 8.68 (d, J = 8.2 Hz, 1H), 8.63 (d, J = 4.6 Hz, 1H), 8.04–7.94 (m, 4H), 7.63–7.59 (m, 2H), 7.34 (d, J = 8.0 Hz, 1H), 7.30 (t, J = 7.1 Hz, 2H), 7.20 (d, J = 7.5 Hz, 1H), 7.12–7.10 (m, 1H), 7.05 (t, J = 7.5 Hz, 1H), 6.98–6.91 (m, 2H), 6.80 (d, J = 7.6 Hz, 1H), 6.61 (t, J = 7.3 Hz, 1H), 5.05 (s, 2H), 4.90–4.85 (m, 1H), 4.45–4.25 (m, 2H), 3.33–3.28 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 171.58, 165.62, 163.69, 149.83, 148.94, 143.20, 138.36, 136.62, 133.54, 128.30, 128.12, 127.84, 127.19, 126.81, 124.18, 122.27, 121.36, 119.00, 118.71, 116.89, 116.80, 111.84, 109.91, 54.06, 42.46, 28.74. HRMS (AP-ESI) m/z calcd for $C_{31}H_{28}N_6O_3$ $[M+H]^+$ 533.2296, found 533.2299.

3.1.17.6. (S)-N-(1-((4-((2-aminophenyl)carbamoyl)benzyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)pyrazine-2-carboxamide (11e):

Using the synthetic method for **3**, compound **10e** gave **11e** as a white solid, 36% yield. Mp: 206–208 °C. 1H NMR (400 MHz, DMSO- d_6) δ 10.84 (s, 1H), 9.64 (s, 1H), 9.17 (s, 1H), 8.87 (d, J = 2.1 Hz, 1H), 8.80 (t, J = 5.7 Hz, 1H), 8.72–8.70 (m, 2H), 7.92 (d, J = 7.9 Hz, 2H), 7.62 (d, J = 7.9 Hz, 1H), 7.37–7.28 (m, 3H), 7.18 (d, J = 7.7 Hz, 1H), 7.15 (s, 1H), 7.05 (t, J = 7.5 Hz, 1H), 7.01–6.90 (m, 2H), 6.80 (d, J = 7.9 Hz, 1H), 6.62 (t, J = 7.4 Hz, 1H), 5.07 (s, 2H), 4.87 (dd, J = 13.8, 7.3 Hz, 1H), 4.38 (d, J = 5.6 Hz, 2H), 3.32–3.26 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 171.38, 165.63, 162.88, 148.19, 144.70, 143.89, 143.81, 143.29, 143.20, 136.62, 133.59, 128.25, 127.81, 127.36, 127.13, 126.93, 124.23, 124.00, 121.43, 118.97, 118.76, 116.97, 116.77, 111.83, 110.03, 54.18, 42.51, 28.44. HRMS (AP-ESI) m/z calcd for $C_{30}H_{27}N_7O_3$ $[M+H]^+$ 534.2248, found 534.2248.

3.1.17.7. (S)-N-(1-((4-((2-aminophenyl)carbamoyl)benzyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)thiophene-2-carboxamide (11f):

Using the synthetic method for **3**,

compound **10f** gave **11f** as a white solid, 34% yield. Mp: 134–136 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.81 (s, 1H), 9.67 (s, 1H), 8.76 (t, *J* = 5.9 Hz, 1H), 8.65 (d, *J* = 8.1 Hz, 1H), 8.00–7.86 (m, 3H), 7.78–7.67 (m, 2H), 7.33 (d, *J* = 8.1 Hz, 3H), 7.21–7.18 (m, 2H), 7.15–7.13 (m, 1H), 7.06 (t, *J* = 7.1 Hz, 1H), 6.99 (t, *J* = 7.4 Hz, 2H), 6.82 (d, *J* = 7.9 Hz, 1H), 6.65 (t, *J* = 7.1 Hz, 1H), 5.31 (s, 2H), 4.78–4.73 (m, 1H), 4.39 (d, *J* = 5.8 Hz, 2H), 3.19–3.13 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.24, 165.67, 161.59, 143.50, 142.70, 140.11, 136.57, 133.47, 131.39, 129.09, 128.31, 128.24, 127.70, 127.25, 127.14, 126.94, 124.31, 124.21, 121.39, 118.98, 118.74, 117.42, 117.06, 111.82, 110.84, 54.83, 42.41, 28.09. HRMS (AP-ESI) *m/z* calcd for C₃₀H₂₇N₅O₃S [M+H]⁺ 538.1907, found 538.1903.

3.1.17.8. (S)-N-(1-((4-((2-aminophenyl)carbamoyl)benzyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)furan-2-carboxamide (11g): Using the synthetic method for **3**, compound **10g** gave **11g** as a white solid, 36% yield. Mp: 202–204 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.81 (s, 1H), 9.64 (s, 1H), 8.74 (t, *J* = 5.9 Hz, 1H), 8.32 (d, *J* = 8.1 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.85–7.78 (m, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 3H), 7.22–7.13 (m, 3H), 7.06 (t, *J* = 7.4 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 2H), 6.80 (d, *J* = 7.8 Hz, 1H), 6.66–6.57 (m, 2H), 5.01 (s, 2H), 4.79–4.73 (m, 1H), 4.39 (d, *J* = 5.7 Hz, 2H), 3.24–3.15 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.04, 165.65, 158.10, 148.00, 145.54, 143.37, 136.59, 133.54, 128.23, 127.73, 127.29, 127.13, 126.93, 124.19, 123.99, 121.40, 118.96, 118.74, 116.97, 116.75, 114.22, 112.29, 111.82, 110.64, 54.12, 42.44, 28.13. HRMS (AP-ESI) *m/z* calcd for C₃₀H₂₇N₅O₄ [M+H]⁺ 522.2136, found 522.2137.

3.1.17.9. (S)-N-(1-((6-((2-aminophenyl)amino)-6-oxohexyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-methoxybenzamide (19): Using the synthetic method for **3**, compound **18** gave **19** as a white solid, 31% yield. Mp: 133–135 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.76 (s, 1H), 9.13 (s, 1H), 8.29 (d, *J* = 8.1 Hz, 1H), 8.05 (t, *J* = 5.3 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.22–7.13 (m, 2H), 7.04 (t, *J* = 7.4 Hz, 1H), 6.99–6.94 (m, 3H), 6.89 (t, *J* = 7.1 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.55 (t, *J* = 7.1 Hz, 1H), 5.21 (s, 2H), 4.74–4.58 (m, 1H), 3.79 (s, 3H), 3.23–2.96 (m, 4H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.62–1.55 (m, 2H), 1.45–1.39 (m, 2H), 1.34–1.21 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.11, 171.63, 166.04, 162.05, 142.01, 136.53, 129.75, 127.80, 126.87, 126.15, 125.78, 124.25, 124.06, 121.30, 119.01, 118.63, 116.91, 116.53, 113.80, 111.74, 111.10, 55.80, 54.75, 39.07, 36.23, 29.35, 28.23, 26.59, 25.54. HRMS (AP-ESI) *m/z* calcd for C₃₁H₃₅N₅O₄ [M+H]⁺ 542.2762, found 542.2764.

3.1.17.10. (S)-4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propoxy)-N-(2-aminophenyl) benzamide (30a): Using the synthetic method for **3**, compound **29a** gave **30a** as a white solid, 33% yield. Mp: 106–108 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.81 (s, 1H), 9.54 (s, 1H), 8.37 (d, *J* = 7.9 Hz, 1H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.84 (d, *J* = 8.9 Hz, 2H), 7.64 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 2.1 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.08–7.04 (m, 3H), 7.01–6.93 (m, 4H), 6.81–6.76 (m, 1H), 6.60 (t, *J* = 7.5 Hz, 1H), 4.96 (s, 2H), 4.63–4.58 (m, 1H), 4.25–4.10 (m, 2H), 3.80 (s, 3H), 3.17–3.07 (m, 2H). HRMS (AP-ESI) *m/z* calcd for C₃₂H₃₀N₄O₄ [M+H]⁺ 535.2340, found 535.2343.

3.1.17.11. (S)-N-(1-(4-(2-((2-aminophenyl)amino)-2-oxoethyl)phenoxy)-3-(1H-indol-3-yl)propan-2-yl)-4-methoxybenzamide (30b): Using the synthetic method for **3**, compound **29b** gave **30b** as a white solid, 35% yield. Mp: 194–196 °C. ¹H NMR (400 MHz, DMSO-d₆) δ 10.79 (s, 1H), 9.31 (s, 1H), 8.33 (d, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.17–7.13 (m, 2H), 7.05 (t, *J* = 7.5 Hz, 1H), 7.00–6.86 (m, 6H), 6.72 (d, *J* = 7.9 Hz, 1H), 6.54 (t, *J* = 7.5 Hz, 1H), 4.94 (s, 2H), 4.60–4.55 (m, 1H), 4.15–4.00 (m, 2H), 3.80 (s, 3H), 3.56 (s, 2H), 3.15–3.03 (m, 2H). ¹³C NMR (100 MHz, DMSO-d₆) δ 169.91, 166.24, 162.00, 157.77, 142.29, 136.65, 130.55, 129.63, 128.92, 127.92, 127.26, 126.33, 125.75, 123.92, 123.82, 121.38, 118.83, 118.77, 116.75, 116.40, 114.92, 113.85, 111.84, 111.41, 69.48, 55.81, 50.27, 42.30, 27.26. HRMS (AP-ESI) *m/z* calcd for C₃₃H₃₂N₄O₄ [M+H]⁺ 549.2496, found 549.2500.

3.1.17.12. (S,E)-N-(1-(4-(3-((2-aminophenyl)amino)-3-oxoprop-1-en-1-yl)phenoxy)-3-(1H-indol-3-yl)propan-2-yl)-4-methoxybenzamide (22): Using the synthetic method for **3**, compound **21** gave **22** as a white solid, 30% yield. Mp: 98–100 °C. ¹H NMR (400 MHz, DMSO-d₆) δ 10.81 (s, 1H), 9.33 (s, 1H), 8.36 (d, *J* = 7.9 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 2H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.56–7.48 (m, 3H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.18 (d, *J* = 2.0 Hz, 1H), 7.09–6.89 (m, 7H), 6.77–6.74 (m, 2H), 6.59 (t, *J* = 8.0 Hz, 1H), 5.07 (s, 2H), 4.62–4.57 (m, 1H), 4.22–4.07 (m, 2H), 3.80 (s, 3H), 3.14–3.05 (m, 2H). ¹³C NMR (100 MHz, DMSO-d₆) δ 166.28, 164.29, 162.03, 160.27, 141.88, 139.81, 136.67, 129.72, 129.64, 128.04, 127.90, 127.21, 126.15, 125.16, 124.24, 123.86, 121.41, 120.32, 118.78, 116.91, 116.58, 115.55, 113.87, 111.87, 111.32, 69.56, 55.82, 50.22, 27.23. HRMS (AP-ESI) *m/z* calcd for C₃₄H₃₂N₄O₄ [M+H]⁺ 561.2496, found 561.2495.

3.1.17.13. Tert-butyl (S)-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propyl)(4-((2-aminophenyl)carbamoyl)benzyl)carbamate (36): Using the synthetic method for **3**, compound **35** gave **36** as a white solid, 38% yield. ESI-MS *m/z*: 648.6 [M+H]⁺.

3.1.17.14. Tert-butyl (S)-(2-(6-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)hexanamido)-4-(thiophen-2-yl)phenyl)carbamate (42a): Using the synthetic method for **3**, compound **18** and **41a** gave **42a** as a white solid, 65% yield. ¹H NMR (400 MHz, DMSO-d₆) δ 10.75 (s, 1H), 9.49 (s, 1H), 8.44 (s, 1H), 8.28 (d, *J* = 8.1 Hz, 1H), 8.05 (t, *J* = 5.5 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 2.0 Hz, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.51 (dd, *J* = 5.1, 1.0 Hz, 1H), 7.44 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.41 (dd, *J* = 3.5, 0.8 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 2.2 Hz, 1H), 7.11 (dd, *J* = 5.1, 3.6 Hz, 1H), 7.07–7.02 (m, 1H), 7.00–6.93 (m, 3H), 4.70–4.64 (m, 1H), 3.79 (s, 3H), 3.19–3.06 (m, 4H), 2.36 (t, *J* = 7.4 Hz, 2H), 1.69–1.56 (m, 2H), 1.50–1.40 (m, 11H), 1.36–1.25 (m, 2H).

3.1.17.15. Tert-butyl (S)-(3-(6-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)hexanamido)-[1,1'-biphenyl]-4-yl)carbamate (42b): Using the synthetic method for **3**, compound **18** and **41b** gave **42b** as a white solid, 63% yield.

3.1.17.16. Tert-butyl (S)-(2-(6-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)hexanamido)-5-fluorophenyl)carbamate (48): Using

the synthetic method for **3**, compound **18** and **47** gave **48** as a white solid, 58% yield. ¹H NMR (400 MHz, DMSO-d₆) δ 10.75 (s, 1H), 9.37 (s, 1H), 8.49 (s, 1H), 8.28 (d, *J* = 8.1 Hz, 1H), 8.05 (t, *J* = 5.5 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.49 (dd, *J* = 11.1, 2.8 Hz, 1H), 7.36 (dd, *J* = 8.9, 6.2 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 2.1 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.99–6.94 (m, 3H), 6.86 (td, *J* = 8.5, 3.0 Hz, 1H), 4.71–4.61 (m, 1H), 3.79 (s, 3H), 3.19–3.05 (m, 4H), 2.32 (t, *J* = 7.4 Hz, 2H), 1.61–1.55 (m, 2H), 1.51–1.37 (m, 11H), 1.35–1.21 (m, 2H). ESI-MS *m/z*: 660.6 [M+H]⁺.

3.1.17.17. Tert-butyl (S)-(2-(4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)benzamido)-4-(thiophen-2-yl)phenyl)carbamate (55a): Using the synthetic method for **3**, compound **14** and **54a** gave **55a** as a white solid, 58% yield. ESI-MS *m/z*: 730.6 [M+H]⁺.

3.1.17.18. Tert-butyl(S)-(2-(4-((3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)methyl)benzamido)-4-(thiophen-2-yl)phenyl)carbamate (55b): Using the synthetic method for **3**, compound **14** and **54b** gave **55b** as a white solid, 58% yield.

3.1.17.19. Tert-butyl(S)-(3-(4-((3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)methyl)benzamido)-[1,1'-biphenyl]-4-yl)carbamate (55c): Using the synthetic method for **3**, compound **14** and **54c** gave **55c** as a white solid, 58% yield. ¹H NMR (400 MHz, DMSO-d₆) δ 10.79 (s, 1H), 9.89 (s, 1H), 8.80 (s, 1H), 8.72 (t, *J* = 6.0 Hz, 1H), 8.42 (d, *J* = 7.9 Hz, 1H), 7.91 (d, *J* = 8.3 Hz, 2H), 7.88 (d, *J* = 2.0 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 2H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.66–7.63 (m, 3H), 7.52 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.41–7.36 (m, 3H), 7.35–7.30 (m, 1H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.08–7.04 (m, 1H), 7.01–6.97 (m, 3H), 4.80–4.75 (m, 1H), 4.41 (d, *J* = 5.8 Hz, 2H), 3.80 (s, 3H), 3.30–3.14 (m, 2H), 1.46 (s, 9H). ESI-MS *m/z*: 738.7 [M+H]⁺.

3.1.17.20. Tert-butyl(S)-(2-(4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)benzamido)-5-fluorophenyl)carbamate (59a): Using the synthetic method for **3**, compound **14** and **58a** gave **59a** as a white solid, 56% yield.

3.1.17.21. Tert-butyl(S)-(2-(4-((3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)methyl)benzamido)-5-fluorophenyl)carbamate (59b): Using the synthetic method for **3**, compound **14** and **58b** gave **59b** as a white solid, 50% yield. ESI-MS *m/z*: 680.5 [M+H]⁺.

3.1.18. General procedure of compound 43a, 43b, 49, 56a-56c, 60a and 60b

3.1.18.1. (S)-N-(1-((6-((2-amino-5-(thiophen-2-yl)phenyl)amino)-6-oxohexyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-methoxybenzamide (43a): To a solution of **42a** (0.72 g, 1 mmol) in CH₂Cl₂, TFA (0.23 g, 2 mmol) was added, the mixture was then reacted overnight. After the reaction finished, Et₃N (0.21 g, 2 mmol) was added to neutralize the solution. The solvent was washed with brine and dried over Na₂SO₄ overnight, and the solvent was evaporated under vacuum to get crude product. The crude product was recrystallized by EtOAc and petroleum ether to achieve a white pure solid (0.40 g, 65%).

Mp: 118–120 °C. ¹H NMR (400 MHz, DMSO-d₆) δ 10.81 (s, 1H), 9.75 (s, 1H), 8.42–8.30 (m, 1H), 8.12 (t, *J* = 6.0 Hz, 1H), 7.84–7.79 (m, 2H), 7.71–7.65 (m, 1H), 7.64–7.61 (m, 1H), 7.46 (d, *J* = 4.7 Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 3.0 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.19 (s, 1H), 7.12–7.01 (m, 3H), 7.00–6.91 (m, 3H), 4.72–4.59 (m, 1H), 3.78 (s, 3H), 3.17–3.09 (m, 4H), 2.38 (t, *J* = 7.4 Hz, 2H), 1.71–1.54 (m, 2H), 1.50–1.39 (m, 2H), 1.33–1.31 (m, 2H). HRMS (AP-ESI) *m/z* calcd for C₃₅H₃₇N₅O₄S [M+H]⁺ 624.2639, found 624.2642.

3.1.18.2. (S)-N-(1-((6-((4-amino-[1,1'-biphenyl]-3-yl)amino)-6-oxohexyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-methoxybenzamide (43b): Using the synthetic method for **43a**, compound **42b** gave **43b** as a white solid, 63% yield. Mp: 124–126 °C. ¹H NMR (400 MHz, DMSO-d₆) δ 10.81 (s, 1H), 9.86 (s, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.71–7.65 (m, 2H), 7.57 (d, *J* = 7.5 Hz, 2H), 7.42 (dd, *J* = 13.7, 6.7 Hz, 3H), 7.30 (d, *J* = 7.6 Hz, 2H), 7.22–7.13 (m, 2H), 7.07–7.00 (m, 1H), 6.99–6.94 (m, 3H), 4.68–4.65 (m, 1H), 3.79 (s, 3H), 3.16–3.00 (m, 4H), 2.44–2.34 (m, 2H), 1.63–1.61 (m, 2H), 1.44–1.42 (m, 2H), 1.33–1.31 (m, 2H). HRMS (AP-ESI) *m/z* calcd for C₃₇H₃₉N₅O₄ [M+H]⁺ 618.3075, found 618.3077.

3.1.18.3. (S)-N-(1-((6-((2-amino-4-fluorophenyl)amino)-6-oxohexyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-4-methoxybenzamide (49): Using the synthetic method for **43a**, compound **48** gave **49** as a white solid, 49% yield. Mp: 172–174 °C. ¹H NMR (400 MHz, DMSO-d₆) δ 10.78 (s, 1H), 9.08 (s, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 8.07 (t, *J* = 5.1 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.30 (d, *J* = 7.9 Hz, 1H), 7.19 (s, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 7.04 (t, *J* = 7.4 Hz, 1H), 6.99–6.96 (m, 3H), 6.49 (dd, *J* = 11.1, 2.1 Hz, 1H), 6.28 (td, *J* = 8.4, 2.3 Hz, 1H), 5.19 (s, 2H), 4.70–4.65 (m, 1H), 3.79 (s, 3H), 3.19–3.02 (m, 4H), 2.29 (t, *J* = 7.3 Hz, 2H), 1.64–1.51 (m, 2H), 1.49–1.37 (m, 2H), 1.30–1.28 (m, 2H). ¹³C NMR (100 MHz, DMSO-d₆) δ 172.13, 171.85, 166.03, 162.05, 159.71, 144.65, 144.53, 136.53, 129.75, 127.80, 127.53, 127.43, 126.87, 124.05, 121.28, 120.13, 119.00, 118.62, 113.80, 111.74, 111.09, 102.48, 102.26, 101.98, 101.73, 55.80, 54.79, 39.06, 36.12, 29.34, 28.24, 26.59, 25.48. HRMS (AP-ESI) *m/z* calcd for C₃₁H₃₄FN₅O₄ [M+H]⁺ 560.2668, found 560.2670.

3.1.18.4. (S)-4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)-N-(2-amino-5-(thiophen-2-yl)phenyl)benzamide (56a): Using the synthetic method for **43a**, compound **55a** gave **56a** as a white solid, 40% yield. Mp: 150–152 °C. ¹H NMR (400 MHz, DMSO-d₆) δ 10.82 (s, 1H), 10.54 (s, 1H), 9.81 (s, 1H), 8.56 (d, *J* = 7.6 Hz, 1H), 8.01 (d, *J* = 8.6 Hz, 2H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.80–7.76 (m, 3H), 7.53 (s, 1H), 7.40 (d, *J* = 5.0 Hz, 1H), 7.38–7.25 (m, 4H), 7.08–7.04 (m, 2H), 7.02–6.98 (m, 3H), 6.93 (d, *J* = 8.2 Hz, 1H), 4.93–4.88 (m, 1H), 3.81 (s, 3H), 3.28–3.23 (m, 2H). ¹³C NMR (100 MHz, DMSO-d₆) δ 172.01, 166.39, 165.46, 162.19, 144.40, 142.49, 136.53, 129.86, 129.24, 128.76, 127.73, 126.57, 124.30, 124.20, 121.97, 121.41, 119.14, 119.05, 118.70, 113.89, 111.80, 110.66, 55.83, 27.96. HRMS (AP-ESI) *m/z* calcd for C₃₆H₃₁N₅O₄S [M+H]⁺ 630.2170, found 630.2175.

3.1.18.5. (S)-4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)methyl)-N-(2-amino-5-(thiophen-2-yl)phenyl)benzamide (56b): Using the synthetic method for **43a**,

compound **55b** gave **56b** as a white solid, 39% yield. Mp: 158–160 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.84 (s, 1H), 10.16 (s, 1H), 8.74 (t, *J* = 5.9 Hz, 1H), 8.45 (d, *J* = 7.9 Hz, 1H), 8.01 (d, *J* = 8.2 Hz, 2H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 1.6 Hz, 1H), 7.50–7.45 (m, 2H), 7.40–7.36 (m, 3H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 1.9 Hz, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 7.12–7.10 (m, 1H), 7.06 (t, *J* = 7.1 Hz, 1H), 7.01–6.96 (m, 3H), 4.81–4.75 (m, 1H), 4.41 (d, *J* = 5.7 Hz, 2H), 3.80 (s, 3H), 3.31–3.17 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.60, 166.24, 166.01, 162.10, 144.04, 143.49, 136.58, 132.84, 129.83, 128.91, 128.45, 127.75, 127.27, 126.80, 125.29, 124.27, 124.18, 124.10, 123.22, 121.35, 118.97, 118.70, 113.82, 111.82, 111.01, 55.82, 55.02, 42.39, 28.05. HRMS (AP-ESI) *m/z* calcd for C₃₇H₃₃N₅O₄S [M+H]⁺ 644.2326, found 644.2326.

3.1.18.6. (S)-4-((3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)methyl)-N-(4-amino-[1,1'-biphenyl]-3-yl)benzamide (56c): Using the synthetic method for **43a**, compound **55c** gave **56c** as a white solid, 39% yield. Mp: 168–170 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.86 (s, 1H), 10.35 (s, 1H), 8.76 (t, *J* = 5.7 Hz, 1H), 8.47 (d, *J* = 7.9 Hz, 1H), 8.06 (d, *J* = 7.8 Hz, 2H), 7.87 (d, *J* = 8.2 Hz, 2H), 7.79 (s, 1H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.55 (d, *J* = 7.7 Hz, 1H), 7.47 (t, *J* = 7.4 Hz, 2H), 7.38–7.33 (m, 5H), 7.22 (s, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.01–6.97 (m, 3H), 4.81–4.76 (m, 1H), 4.41 (d, *J* = 5.5 Hz, 2H), 3.80 (s, 3H), 3.31–3.18 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.60, 166.24, 166.04, 162.10, 144.11, 139.72, 136.59, 132.77, 129.84, 129.46, 128.49, 127.75, 127.28, 126.81, 126.75, 125.53, 125.09, 124.18, 121.34, 118.98, 118.70, 113.82, 111.83, 110.99, 55.82, 55.04, 42.39, 28.06. HRMS (AP-ESI) *m/z* calcd for C₃₉H₃₅N₅O₄ [M+H]⁺ 638.2762, found 638.2763.

3.1.18.7. (S)-4-(3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)-N-(2-amino-4-fluorophenyl)benzamide (60a): Using the synthetic method for **43a**, compound **59a** gave **60a** as a white solid, 35% yield. Mp: 170–172 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.83 (s, 1H), 10.56 (s, 1H), 9.54 (s, 1H), 8.59 (d, *J* = 7.7 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.78 (d, *J* = 8.7 Hz, 3H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 1.9 Hz, 1H), 7.14–7.10 (m, 1H), 7.10–7.03 (m, 1H), 7.01–6.97 (m, 3H), 6.55 (dd, *J* = 11.2, 2.8 Hz, 1H), 6.36 (td, *J* = 8.5, 2.8 Hz, 1H), 5.23 (s, 2H), 4.90 (dd, *J* = 14.3, 8.0 Hz, 1H), 3.81 (s, 3H), 3.32–3.23 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.97, 166.37, 165.50, 162.19, 160.26, 145.97, 145.86, 142.34, 136.54, 129.87, 129.46, 129.13, 128.92, 127.75, 126.58, 124.29, 121.40, 119.95, 119.14, 119.05, 118.70, 113.88, 111.80, 110.67, 102.65, 102.42, 102.12, 101.87, 55.83, 55.70, 27.98. HRMS (AP-ESI) *m/z* calcd for C₃₂H₂₈FN₅O₄ [M+H]⁺ 566.2198, found 566.2196.

3.1.18.8. (S)-4-((3-(1H-indol-3-yl)-2-(4-methoxybenzamido)propanamido)methyl)-N-(2-amino-4-fluorophenyl)benzamide (60b): Using the synthetic method for **43a**, compound **59b** gave **60b** as a white solid, 35% yield. Mp: 152–154 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.84 (s, 1H), 9.74 (s, 1H), 8.73 (t, *J* = 5.8 Hz, 1H), 8.45 (d, *J* = 7.9 Hz, 1H), 7.94 (d, *J* = 7.8 Hz, 2H), 7.85 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.35–7.32 (m, 3H), 7.22–7.19 (m, 2H), 7.06 (t, *J* = 7.2 Hz, 1H), 7.00–6.96 (m, 3H), 6.70–6.67 (m, 1H), 6.50 (s, 1H), 4.80–4.75 (m, 1H), 4.39 (d, *J* = 5.7 Hz, 2H), 3.79 (s, 3H), 3.30–3.17 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 172.62, 166.22, 165.92, 162.08, 160.08, 143.67, 136.56, 133.22, 129.85,

129.14, 129.04, 128.30, 127.71, 127.18, 126.75, 124.21, 121.35, 120.71, 118.99, 118.69, 113.80, 111.82, 111.01, 103.78, 102.92, 55.81, 55.01, 42.35, 28.04. HRMS (AP-ESI) m/z calcd for $C_{33}H_{30}FN_5O_4$ $[M+H]^+$ 580.2355, found 580.2352.

4. HDAC class I cellular activity

The cellular HDAC assay was based on such assay described on patent WO20080952A1 with minor modifications. Briefly, human prostate cancer cell line HL60 were seeded into a 96-well tissue culture plates (Corning, Germany) at a density of 1.2×10^4 cells/well and allowed to grow for 24 h. Then cells were treated with increasing concentrations of test compounds for 3 h. The reaction was started by adding substrate Boc-Ac-Lys-AMC. 3 h later under cell culture conditions, stop solution (50 mM Tris-HCl (pH 8.0), 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1% NP-40, 2.0 mg/mL trypsin, 10 μ M TSA) was added and the mixture was incubated for another 3 h. Fluorescence intensity was measured using a microplate reader at excitation and emission wavelengths of 360 and 470 nm, respectively.

5. *In vitro* HDACs inhibition fluorescence assay

In vitro HDACs inhibition assays were conducted as previously described.¹⁷ In brief, 10 μ L of enzyme solution was mixed with different concentrations of tested compound (50 μ L). The mixture was incubated at 37 °C for 10 min, then 40 μ L fluorogenic substrate (Boc-Lys (acetyl)-AMC was used for HDAC1, HDAC2, HDAC3, HDAC8 and HDAC6 while Boc-Lys (trifluoroacetyl)-AMC substrate was used for HDAC4) was added. After incubation at 37 °C for 30 min, the mixture was stopped by addition of 100 μ L of developer containing trypsin and Trichostatin A (TSA). 20 min later, fluorescence intensity was measured using a microplate reader at excitation and emission wavelengths of 390 and 460 nm, respectively. The inhibition ratios were calculated from the fluorescence intensity readings of tested wells relative to those of control wells, and the IC_{50} values were calculated using a regression analysis of the concentration/inhibition data.

6. *In vitro* antiproliferative assay

In vitro antiproliferative assays were determined by the MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) method as previously described.¹⁷ All cell lines were maintained in RPMI1640 medium containing 10% FBS at 37 °C in a 5% CO_2 humidified incubator. Cells were passaged the day before dosing into a 96-well cell plate, allowed to grow for 12 h, and then treated with different concentrations of compound for 48 h. A 0.5% MTT solution was added to each well. After incubation for another 4 h, formazan formed from MTT was extracted by adding 150 μ L of DMSO rocking for 15 min. Absorbance was then determined using an ELISA reader at 570 nm.

7. *In vivo* antitumor assay against U937 and HCT116 xenograft

All experiments involving laboratory animals were conducted with the approved of the local ethics committee. For *in vivo* anti-tumor efficacy research, 1×10^7 U937 or HCT116 Cells were inoculated subcutaneously in the right flank of male nude mice (5–6 weeks old, SLAC LABORATORY ANIMAL, Shanghai). Ten days after injection, tumors were palpable and

mice were randomized into treatment and control groups (6 or 7 mice per group). The treatment groups received compound **9b**, MS275 or SAHA by oral administration, and the blank control group received equal volume of PBS solution containing 5% DMSO. During treatment, subcutaneous tumors were measured with vernier caliper every three days, and body weight was monitored regularly. After treatment, mice were sacrificed and dissected to weigh the tumor tissues and to examine the internal organ injury. Tumor growth inhibition (TGI) and relative increment ratio (T/C) were used as the evaluation indicators to reveal the antitumor effects in tumor weight and tumor volume, respectively. Data were analyzed by Student's two-tailed *t*-test. A *P* level <0.05 was considered statistically significant.

$$\text{TGI} = (\text{the mean tumor weight of control group} - \text{the mean tumor weight of treated group}) / \text{the mean tumor weight of control group}.$$

Tumor volumes (V) were estimated using equation ($V = ab^2/2$, where *a* and *b* stand for the longest and shortest diameter, respectively). T/C was calculated according to the following formula:

$$\text{T/C} = \text{the mean RTV of treated group} / \text{the mean RTV of control group}.$$

RTV, namely relative tumor volume = V_t/V_0 (V_t : the tumor volume measured at the end of treatment; V_0 : the tumor volume measured at the beginning of treatment).

8. Western blot analysis

HL60 cells were plated at 500,000 cells/mL, 3 mL in flat bottom 6-well plates and allowed to grow for 12 h, and then treated with different concentrations of compounds. 48 h later, cells were harvested, washed by PBS, and lysed with RIPA buffer which was comprised of 50 mM Tris Base, 150 mM NaCl, 5 mM EDTA, 0.1% (v/v) SDS, 0.5% (v/v) Sodium Deoxycholate, and 1% (v/v) Triton-x-100. After lysing, the suspension was ultra-sonicated and centrifuged at 14000 RPM for 15 min at 4 °C. The mixture of 75 µL of supernatant and 25 µL of β-mercaptoethanol:LDS solution (15:85) was heated at 90 °C for 15 min and normalization according to BCA test before loading. Lysates were run on Invitrogen NuPAGE 4–12% Bis-Tris 15 well gels at 170 V for approximately 60 min in MES buffer. Gels were transferred to methylcellulose and ran at 30 V for 3 h. Membranes were incubated at 4 °C overnight with 1/1000 primary antibodies, which were diluted in 2.5% (w/v) milk or 5% (w/v) Bovine Serum Albumin. The membrane was washed twice with TBST buffer before incubated with secondary antibodies. Images were acquired using a GE ImageQuant LAS 4000.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2017.03.069>.

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Fig. 1.
Design of benzamides bearing HDACIs.

Fig. 2. Antitumor activity comparison of **11a** and MS275 against U937 human tumor xenografts implanted in mice. (a) Picture of dissected U937 tumor tissues; (b) Tumor weight in different mice group; (c) Mean tumor volume during mice treatment; (d) Mice body weight change after administration.

Fig. 3. Antitumor activity comparison of **11a**, SAHA and MS275 against HCT116 human tumor xenografts implanted in mice. (a) Picture of dissected HCT116 tumor tissues; (b) Mean tumor volume during mice treatment.

Fig. 4.
Design of the new series compounds.

Fig. 5. Western blot analysis of acetylated histone H3 (AcHH3), acetylated histone H4 (AcHH4), procaspase-3, cleaved caspase-3 and cleaved PARP in HL60 cell lines after 48 h treatment with compounds using MS275 as positive control. β-actin was used as a loading control.

Scheme 1.

Synthesis of Compound 3.

Reagents and conditions: (a) CH₃OH/H₂O, KOH, reflux, 85% yield; (b) TBTU, Et₃N, anhydrous THF, 30–33% yield.

Scheme 2. Synthesis of Compounds **11a-11g**.

Reagents and conditions: (a) CH_3COCl , CH_3OH , 95% yield; (b) TBTU, Et_3N , anhydrous CH_2Cl_2 , 60–65% yield; (c) AcOEt/HCl , 80% yield; (d) ROOH , TBTU, Et_3N , anhydrous CH_2Cl_2 , 60–65% yield; (e) $\text{CH}_3\text{OH}/\text{H}_2\text{O}$, KOH , reflux, 80% yield; (f) TBTU, Et_3N , anhydrous THF, 30–33% yield.

Scheme 3. Synthesis of Compound **19**.

Reagents and conditions: (a) TBTU, Et₃N, anhydrous CH₂Cl₂, 60–65% yield; (b) CH₃OH/H₂O, KOH, reflux, 80% yield; (c) CH₃COCl, CH₃OH, 95% yield; (d) TBTU, Et₃N, anhydrous THF, 30–33% yield.

Scheme 4. Synthesis of Compound **22**.

Reagents and conditions: (a) $\text{CH}_3\text{OH}/\text{H}_2\text{O}$, KOH , reflux, 90% yield; (b) TBTU, Et_3N , anhydrous THF, 30–33% yield.

Scheme 5.

Synthesis of Compounds **30a**, **30b**.

Reagents and conditions: (a) CH_3COCl , CH_3OH , 95% yield; (b) PPh_3 , DEAD, anhydrous THF, 57% yield; (c) AcOEt/HCl , 80% yield; (d) TBTU, Et_3N , anhydrous CH_2Cl_2 , 60–65% yield; (e) $\text{CH}_3\text{OH}/\text{H}_2\text{O}$, KOH, reflux, 80% yield; (f) TBTU, Et_3N , anhydrous THF, 30–33% yield.

Scheme 6.**Synthesis of Compound 37.**

Reagents and conditions: (a) TBTU, Et₃N, anhydrous CH₂Cl₂, 60–65% yield; (b) LiAlH₄, 2 h, 70% yield; (c) NaBH₃CN, AcOH, Et₃N, CH₃OH, 53% yield; (d) (Boc)₂O, Et₃N, CH₂Cl₂, 90% yield; (e) CH₃OH/H₂O, KOH, reflux, 80% yield; (f) TBTU, Et₃N, anhydrous THF, 48% yield; (g) AcOEt/HCl, 65% yield.

40a, 41a, 42a, 43a R = 2-thienyl; **40b, 41b, 42b, 43b** R = phenyl

Scheme 7.

Synthesis of Compounds **43a** and **43b**.

Reagents and conditions: (a) (Boc)₂O, Et₃N, CH₂Cl₂, DMAP, 90% yield; (b) Thiophene-2-boronic acid or phenylboronic acid, Pd(OAc)₂, PPh₃, Na₂CO₃, H₂O, DMSO, 48% yield; (c) Pd/C, H₂, CH₃OH, 85% yield; (d) TBTU, Et₃N, anhydrous THF, 60–65% yield; (e) CF₃COOH, 68% yield.

Scheme 8.

Synthesis of Compound 49.

Reagents and conditions: (a) (Boc)₂O, Et₃N, CH₂Cl₂, DMAP, 90% yield; (b) CF₃COOH, CH₂Cl₂, 78% yield; (c) Pd/C, H₂, ethyl alcohol, 85% yield; (d) TBTU, Et₃N, anhydrous CH₂Cl₂, 60–65% yield; (e) CF₃COOH, 65% yield.

50, 51a, 52a $n=0$; **6, 51b, 52b** $n=1$; **41a** $R = 2\text{-thienyl}$; **41b** $R = \text{phenyl}$

53a-56a $n = 0$ $R = 2\text{-thienyl}$; **53b-56b** $n = 1$ $R = 2\text{-thienyl}$; **53c-56c** $n = 1$ $R = \text{phenyl}$

Scheme 9.

Synthesis of Compounds **56a-56c**.

Reagents and conditions: (a) (CF₃CO)₂O, 95% yield; (b) SOCl₂, reflux, 83% yield; (c) pyridine, 78% yield; (d) K₂CO₃, CH₃OH/H₂O, 67% yield; (e) TBTU, Et₃N, anhydrous CH₂Cl₂, 60–65% yield; (f) CF₃COOH, 65% yield.

57a-60a $n = 0$; **57b-60b** $n = 1$

Scheme 10.

Synthesis of Compounds **60a** and **60b**.

Reagents and conditions: (a) pyridine, 78% yield; (b) K_2CO_3 , CH_3OH/H_2O , 67% yield; (c) TBTU, Et_3N , anhydrous CH_2Cl_2 , 60–65% yield; (d) CF_3COOH , 65% yield.

Table 1

HDAC class I cellular activity and *in vitro* antiproliferative activity for synthesized HDAC inhibitors **3**, **11a**, **19**, **22**, **30a**, **30b** and **37**.^a

Cpd No.	Structure	IC ₅₀ of HDAC cellular class I (μM) ^b	IC ₅₀ of HL60 cell (μM)
3		0.85	2.14
11a		0.67	1.84
19		1.23	7.94
30a		0.82	1.93
30b		>10	>20
22		1.75	5.97

Cpd No.	Structure	IC ₅₀ of HDAC cellular class I (μM) ^b	IC ₅₀ of HL60 cell (μM)
37		2.95	8.04
MS275		0.62	1.52

^a Assays were performed in HL60 cells; Values are average of at least two determinations, the SD values are < 20% of the mean.

^b Boc-Lys (acetyl)-AMC substrate.

Table 2HDAC class I cellular activity and *in vitro* antiproliferative activity for synthesized HDAC inhibitors **11a-11g**.^a

Cpd No.	Structure	IC ₅₀ of HDAC cellular class I (μM) ^b	IC ₅₀ of HL60 cell (μM)
11a		0.67	1.84
11b		1.51	2.38
11c		1.98	3.09
11d		1.61	6.09
11e		1.02	5.85
11f		0.78	1.74
11g		0.91	2.40

^a Assays were performed in HL60 cells; Values are average of at least two determinations, the SD values are < 20% of the mean.^b Boc-Lys (acetyl)-AMC substrate.

Table 3*In Vitro* inhibition of HDACs isoforms of representative Compounds.^a

Cpd No.	R ₁	IC ₅₀ (nM)		
		HDAC1	HDAC2	HDAC3
3		49.6	441	227
11a		20.6	157	138
19		58.6	296	42.9
30a		14.7	111	90.0
30b		>20000	>20000	>20000
22		284	217	261
37		29.9	21.2	223

^a Assays were performed in replicate (n = 2); The SD values are < 20% of the mean.

Table 4

In *Vitro* antiproliferative Activity of **11a** and **MS275**.^a

Cpd	IC ₅₀ (μM) ^a				
	HEL	K562	U937	U266	HCT116 ES-2
11a	3.02	0.98	1.10	2.23	4.23
MS275	1.29	1.68	0.77	6.10	2.07
					>20
					>20

^a Assays were performed in replicate (n = 2); The SD values are < 20% of the mean.

Table 5Data of *in vivo* study with U937 xenograft model.

Cpd No.	Dose (mg/kg)	Tumor growth inhibition (TGI %)	Relative increment ratio (T/C %)	Mice body weight (g)
11a	100	51	49	25.04 ± 1.31
MS275	50	60	32	19.77 ± 0.61
Ctrl	/	/	/	25.39 ± 1.18

Table 6Data of *in vivo* study with HCT116 xenograft model.

Cpd No.	Doses (mg/kg)	Tumor growth inhibition (TGI %)	Relative increment ratio (T/C %)	Mice body weight (g)
11a	100	49	59	21.59 ± 1.05
SAHA	100	32	73	21.23 ± 0.63
MS275	50	62	46	18.87 ± 0.80
Ctrl	/	/	/	19.91 ± 1.26

Table 7

In *Vitro* inhibition of HDACs isoforms of representative Compounds.^a

Cpd No.	R ₁	R ₂	R ₃	IC ₅₀ (nM)		
				HDAC1	HDAC2	HDAC3
19		H	H	58.7	296	42.9
43a			H	48.0	338	3569
43b			H	55.5	490	5626
49		H	F	511	909	111
3		H	H	49.7	441	227

Cpd No.	R ₁	R ₂	R ₃	IC ₅₀ (nM)		
				HDAC1	HDAC2	HDAC3
56a			H	36.5	314	62540
60a		H	F	91.7	121	167
11a		H	H	20.6	157	138
56b			H	58.8	320	8007
56c			H	58.4	311	30850
60b		H	F	171	179	243

^a Assays were performed in replicate (n = 2); The SD values are < 20% of the mean.

Table 8

In *Vitro* inhibition of HDACs isoforms of representative compounds **43a** and **49**.^a

Cpd No.	Class I (μM)			Class IIa (μM)			Class IIb (μM)
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC6	HDAC6
43a	0.048	0.337	3.57	>100	>100	>100	>100
49	0.511	0.908	0.110	>100	>100	>100	>100
11r [17]	0.012	0.50	0.004	2.0	5.7	0.31	0.31

^a Assays were performed in replicate (n = 2); The SD values are < 20% of the mean.

Table 9

In *Vitro* Antiproliferative Activity of representative and MS275.^a

Cpd	IC ₅₀ (μM) ^d				
	HEL	K562	U937	U266	HCT116 ES-2
10	15.4	8.51	13.20	12.7	10.6
43a	16.6	16.0	>20	>20	>20
43b	25.2	15.7	>20	>20	>20
11a	3.02	0.98	1.10	2.23	4.23
56b	>20	13.5	>20	>20	>20
56c	>20	14.0	>20	>20	>20
MS275	1.29	1.68	0.77	6.10	2.07

^a Assays were performed in replicate (n = 2); The SD values are < 20% of the mean.