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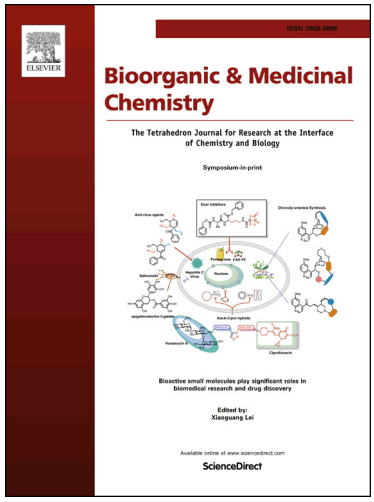

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Design, synthesis and tumor cell growth inhibitory activity of 3-nitro-2*H*-cheromene derivatives as histone deacetylaes inhibitors

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Abstract

As a continuous research for the discovery of coumarin-based targeted anticancer agents, we designed and synthesized a series of novel histone deacetylases (HDAC) inhibitors using the 8-ethoxy-3-nitro-2*H*-chromene as the surface binding or cap group, linear dicarboxylic acid or ω-amino acid moiety with different length as the linking motif, *ortho*-aminoanilides, amides or α-aminoamides as the zinc binding group and the internal cavity motifs. Most of these 3-nitro-2*H*-chromene derivatives exhibited good growth inhibitory activity against K562, A549, MCF-7, PC3 and Hela cells and were more potent than the reference drug SAHA and MS-275. At the concentration of 10 μM, the *ortho*-aminoanilide series and the D-Phe derived α-aminoamide derivatives **16a** and **16b** displayed more potent activity toward HADC1 over HADC2, and only moderate to weak activity over HADC6. In contrast, the amide ZBG analogues, **12a** and **12b**, **14** and **15**, were only moderate HDAC6 inhibitors, but more selective over HDAC1 and HDAC2. The *ortho*-aminoanilides **9b**, **9c**, **10b**, **10c**, **11b**, and the α-aminoamides **16a** and **16b** were potent HADC1 inhibitors with the IC_{50} values in the nanomolar ranges. The *ortho*-aminoanilides **10b** and**10c** with a phenyl internal cavity motif were more potent than MS-275 as HADC1 inhibitors and more selective over HADC2.

Keywords: HDACs, HDAC inhibitors, 3-nitro-2*H*-chromenes, coumarins, anticancer agents, Zinc-dependent hydrolases, isoform selective

1. Introduction

As a family of hydrolases that remove acetyl groups from lysine residues, histone deacetylases (HDACs) play very important roles in the regulation of multiple processes, from gene expression to protein activity [1, 2]. The dysregulation of HDACs is implicated in many diseases, such as cancer, cellular metabolism disorders, neurological disorders, and inflammation. To date, 18 HDACs have been identified in mammals. According to the differences in their sequence identity and catalytic activity, HDACs can be divided into four classes. Class I includes HDAC1, 2, 3 and 8, which are mainly located in the nucleus. Class II includes HDAC4, 5, 6, 7, 9, and 10, which are tissue specific and shuttle between the cytoplasm and the nucleus [3, 4]. Class III, also called sirtuins, are NAD^+-dependent HDACs. Like classes I and II, class IV is metal (Zn^{2+}) dependent and has only one member, HDAC11, which is mainly located in the nucleus. The active site of zinc-dependent HDACs include four prominent binding domains: the surface binding domain, the hydrophobic channel, the catalytic zinc binding domain, and the adjacent internal cavity. The pharmacophore model of HDACs inhibitors (HDACi) can also be divided into four groups: a surface binding or cap group (Cap), a hydrocarbon linking motif (Linker), a zinc binding group (ZBG) and an internal cavity motif correspondingly [5, 6]. A variety of aromatic rings, heterocyclic rings and macrocycles can serve as the cap group. The linker motif is usually an alkyl chain or a substituted aromatic ring. The most common ZBG are hydroxamic acid and *ortho*-aminoanilide. The internal cavity motif can be a variety of aromatic or heterocyclic rings [7, 8]. Some licensed HDACi, including SAHA, Romidepsin, Belinostat, Panobinostat and Chidamide, not only confirmed the safety and efficacy of HDACi as anticancer drugs, but also attracted more attentions for the discovery novel selective HDACi for the treatment of a variety of diseases.

Coumarins are fused benzene and pyrone ring systems that exist abundantly in nature. Both natural and synthetic coumarin derivatives possess a broad spectrum of biological activities, including antioxidant, anti-inflammatory, anticancer, antiviral, antihyperlipidemic, MAO-B inhibitory and antimicrobial activities, among many others [9, 10]. In recent years, the synthesis of coumarins and their derivatives has attracted considerable attention in the development of novel anticancer agents [11, 12, 13]. A coumarin derivative, 8-ethoxy-2-(4-fluorophenyl)-3-nitro-2*H*-chromene (S14161), was discovered as a PI3K/Akt signal pathway inhibitor through high-throughput screening that displayed potent antileukemia and antimyeloma activity with minimal toxicity [14]. Structural simplification and

modification of S14161 by us led to the identification of 6-bromo-8-ethoxy-3-nitro-2*H*-chromene (BENC-511) that exhibited more potent anti-proliferative activities against a panel of cell lines [15]. Compared with S14161, BENC-511 was more potent in blocking the AKT phosphorylation and inducing cancer cell apoptosis, and demonstrated potent oral activity against myeloma *in vivo* and great therapeutic efficacy in a PC3-derived prostate cancer model in nude mice [16, 17]. However, BENC-511 showed little inhibitory effects on the PI3Ks and its molecular targets have not been identified. To develop targeted anticancer drugs, we herein report the design, synthesis and biological evaluation of a series of novel 3-nitro-2-chromene derivatives as potent histone deacetylaes inhibitors. In these HDACi, the 8-ethoxy-3-nitro-2*H*-chromene served as the Cap group, linear dicarboxylic acid or ω-amino acid moiety acted as the Linker, *ortho*-aminoanilides, amides or α-aminoamides behaved as the ZBGs and the internal cavity motifs (Figure 1).

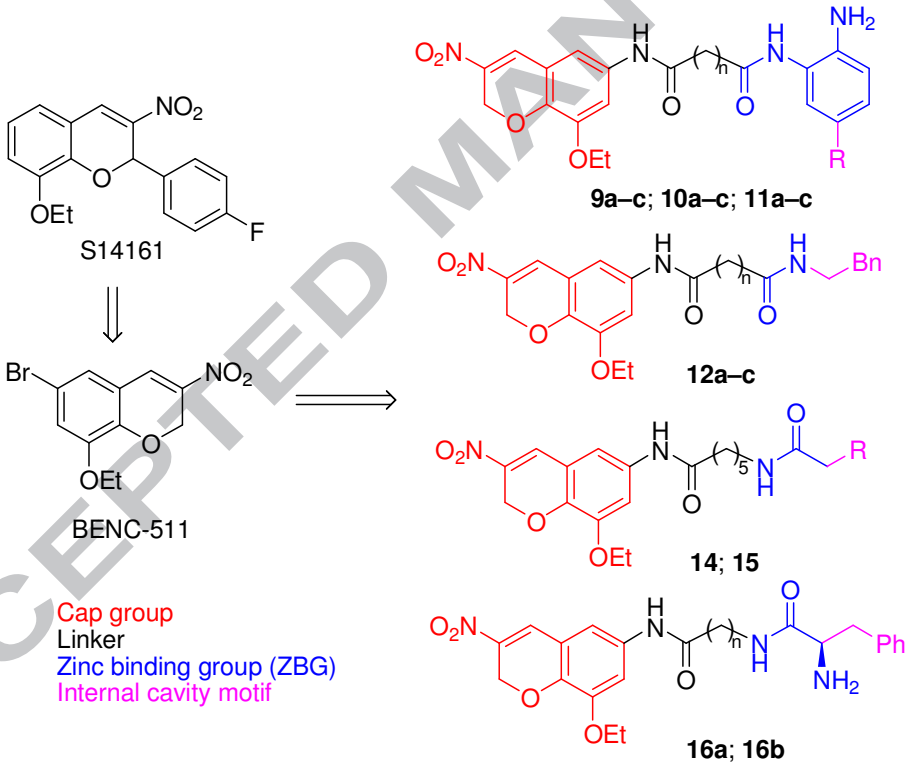


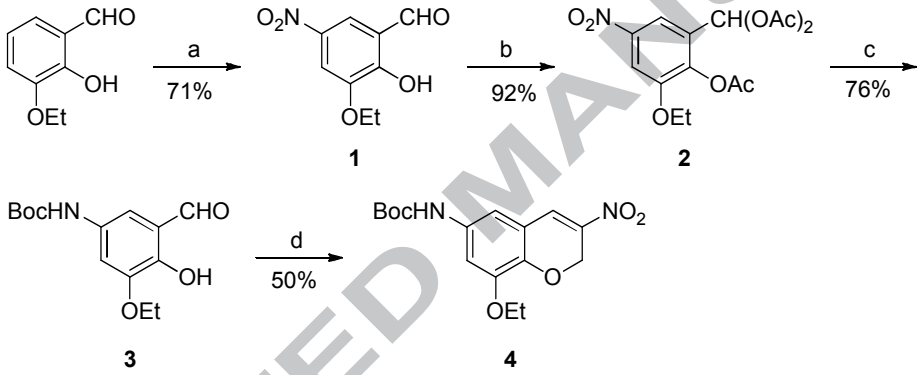
Figure 1. The design of 3-nitro-2*H*-chromene derivatives as HDACi.

2. Results and discussion

2.1. Chemistry

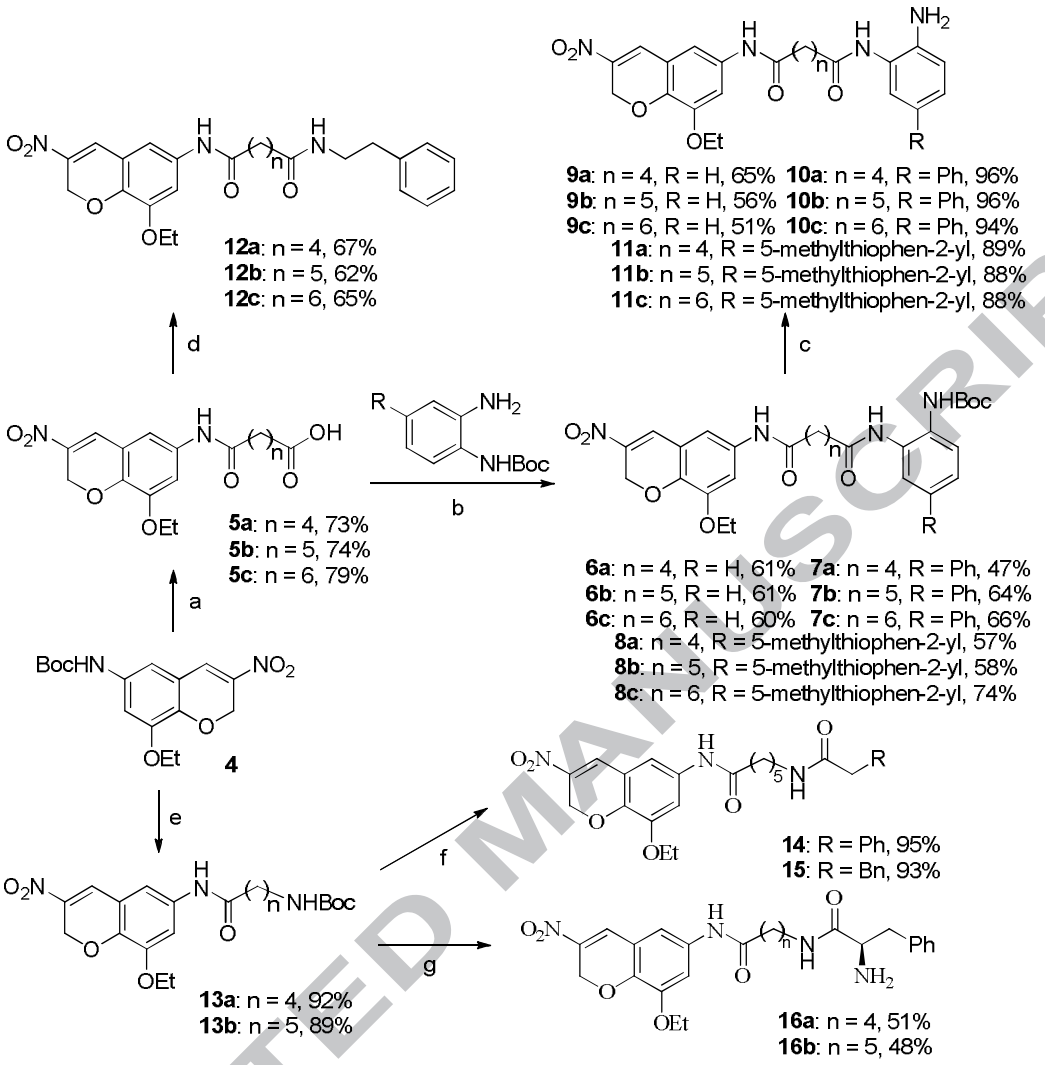
In the design of 3-nitro-2-chromene based histone deacetylaes inhibitors, the 8-ethoxy-3-nitro-2*H*-chromene was designed as the Cap moiety, an amino group was introduced at the

6-position to replace the 6-bromo in BENC-511 through which the dicarboxylic acid or ω-amino acid linker was connected by an amide bond. As shown in Scheme 1, the nitration of 3-ethoxy-2-hydroxybenzaldehyde in glacial acetic acid generated the 6-nitro derivative **1** in 71% yield. The protection of both the hydroxyl and aldehyde group was achieved in high yield (92%) by the treatment of **1** with Ac_2O in the presence of a catalytic amount of concentrated sulfuric acid. The Pd/C catalytic hydrogenation of **2** provided the 6-amino derivative, which was unstable and reacted immediately with $(Boc)_2O$ to protect the resulting amino group, followed by the removal of the acetyl protective groups in refluxing aqueous NaOH solution to give the salicylaldehyde derivative **3** in 76% yield (3 steps). The reaction of salicylaldehyde **3** with 2-nitroethanol in the present of 200% mol of phthalic anhydride and using dibutylamine as a base in toluene under reflux conditions gave the corresponding 6-BocNH-8-ethoxy-3-nitro-2*H*-chromene **4** in 50% yield.



Scheme 1. Synthesis of the 6-BocNH-8-ethoxy-3-nitro-2*H*-chromene **4**. Reagents and conditions: (a) HNO_3, AcOH, 0 °C to rt, 4 h; (b) Ac_2O, H_2SO_4, 2 h; (c) i) 10%Pd/C, H_2, THF, 25 h; ii) $(Boc)_2O$, H_2O, 30 h; iii) NaOH, reflux 8 h; (d) phthalic anhydride, *n*-Bu_2NH, 2-nitroethanol, toluene, reflux, 18 h.

To examine the influences of linker length on the HDAC inhibitory activity, linear dicarboxylic acids of 6-, 7- and 8-carbon lengths were applied. In these dicarboxylic acids derived series, the *ortho*-aminoanilide (**9–11**) and the amide (**12**) were used as the ZBGs. In the *ortho*-aminoanilide series, the phenyl (**10a–c**) and 5-methylthiophen-2-yl (**11a–c**) acted as the internal cavity motifs that were expected to improve both the HDAC inhibitory activity and the isoform selectivity [18, 19]. Besides the linear dicarboxylic acid, the 5-aminopentanoic acid or 6-aminohexanoic acid was used as the linker motif, and the derived ω-amides (**14**, **15**) or the α-amino amides (**16a**, **16b**) behaved as the ZBGs. The synthesis of these 8-ethoxy-3-nitro-2*H*-chromene based HDACi was shown in Scheme 2.



Scheme 2. Synthesis of the 8-ethoxy-3-nitro-2*H*-chromene based HDACi. Reagents and conditions: (a) i) TFA, CH_2Cl_2, 2 h; ii) $(CH_2)_n(CO_2H)_2$, HATU, Et_3N, DMF, 12 h; (b) HATU, Et_3N, DMF, 4 h; (c)TFA, CH_2Cl_2, 2 h; (d) $PhCH_2CH_2NH_2$, HATU, Et_3N, DMF, 4.5 h; (e) i) TFA, CH_2Cl_2, 2 h; ii) $BocNH(CH_2)_nCO_2H$, HATU, Et_3N, DMF, 12 h; (f) i) TFA, CH_2Cl_2, 2 h; ii) RCH_2CO_2H, HATU, Et_3N, DMF, 8 h; (g) i) TFA, CH_2Cl_2, 2 h; ii) Boc-D-Phe, HATU, Et_3N, DMF, 8 h; iii) TFA, CH_2Cl_2, 2 h.

Treatment of **4** with 20% trifluoroacetic acid (TFA) solution in dichloromethane removed the Boc protective group to give the free amine, which was coupled with adipic acid, heptanedioic acid and octanedioic acid, in the presence of HATU and triethylamine in DMF, to afford the acid derivatives **5a**, **5b** and **5c** in 73%, 74% and 79% yield, respectively. Coupling the resulting free amine with the 5-BocNH-pentanoic acid or 6-BocNH-hexanoic acid under the same conditions resulted in **13a** and **13b** in 92% and 89% yield. The reaction of the acids, **5a**, **5b**, and **5c**, with *tert*-butyl 2-aminophenylcarbamate, in the presence of HATU and triethylamine in DMF, provided the amides **6a** (61%), **6b** (61%) and **6c** (60%). By the same method, the reaction of **5a**, **5b**, or **5c** with *tert*-butyl

3-amino-4-(1,1'-biphenyl)-4-yl)carbamate, *tert*-butyl 3-amino-4-(5-methylthiophen-2-yl)phenyl)carbamate or phenylethylamine, respectively, generated the amides **7a** (47%), **7b** (64%), **7c** (66%), **8a** (57%), **8b** (58%), **8c** (74%), or **12a** (67%), **12b** (62%), **12c** (65%). Treatment of **6a–c**, **7a–c**, and **8a–c** with TFA, respectively, produced the corresponding diamides **9a** (65%), **9b** (56%), **9c** (51%), **10a** (96%), **10b** (96%), **10c** (94%), or **11a** (89%), **12b** (88%), **12c** (88%). After the removal of the Boc protective group of **13a** or **13b** with TFA, the resulting amine from **13b** was reacted with phenylacetic acid or 3-phenylpropanoic acid, in the presence of HATU and triethylamine in DMF, to give the ω-amides **14** (95%) and **15** (93%), while the reaction of the amines derived from both **13a** and **13b** with the D-Boc-Phe, followed by the deprotection of the Boc group with TFA in CH_2Cl_2, provided the corresponding ω-(α)-aminoamide **16a** (51%) and **16b** (48%).

2.2. In vitro cell growth inhibitory activity

The newly synthesized 8-ethoxy-3-nitro-2*H*-chromene based HDACi were evaluated for their antiproliferative activity against five human cancer cell lines, K562 (human leukemia), A549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), PC3 (human prostate cancinoma), and HeLa (human cervical carcinoma), by the conventional MTT assay, using the clinical drug SAHA and the typical benzamide HDAC inhibitor MS-275 as the reference drugs [20]. The results are shown in Table 1.

Like SAHA and MS-275, these 8-ethoxy-3-nitro-2*H*-chromene derivatives showed different sensitivity towards K562, A549, MCF-7, PC3 and HeLa cells, and most of them were more sensitive to K562 cells than the others, and they exhibited high potency against K562 with GI_{50} values ranging from 1.53 to 6.04 μM. All of them were more potent than MS-275 and most of them were highly active or comparable to SAHA against K562 cells. Except for a few compounds (**9b**, **11b**), all the others were more active or at least comparable to the reference drugs SAHA and MS-275, toward the A549, MCF-7 and PC3 cell lines. Though they were relatively less sensitive towards the HeLa cells, compounds **12a**, **12b**, **14** and **15** were more potent than SAHA and MS-275, while compounds **9a**, **9b**, **10b**, **10c** and **11a** displayed comparable potency.

In general, the linker, the kind of ZBGs and the internal cavity motifs affected the antiproliferative activity, but in different degrees, depending on the cell types. Among the *ortho*-aminoanilide series (**9~11a–c**), the compounds with a phenyl (**10a–c**) or 5-methylthiophen-2-yl (**11a–c**) internal cavity motif, in most cases, showed increased potency against the K562, A549, MCF-7 and PC3 cell lines in

comparison with their counterparts **9a–c**. Compounds **12a** and **12b** with the amide ZBG groups exhibited relatively higher activity than the *ortho*-aminoanilide series having the same linear dicarboxylic acid derived linkers, especially, they were potent toward all the five tumor cell lines tested. In contrast, their counterpart **12c** with one- or two-carbon elongation in the linker displayed much reduced activity than **12a** and **12b**. The 2-phenylacetamide **14** and the 3-phenylpropanamide **15** with the 6-aminohexanoic acid derived linkers displayed similar potency as that of **12a** and **12b** toward the five tumor cell lines, however, the D-Phe derivatives **16a** and **16b** ere inactive for the HeLa cell line and exhibited a slightly reduced potency than **14** and **15** against the K562, A549, MCF-7 and PC3 cell lines.

Table 1: Antiproliferative activity and HDACs inhibitory potency *in vitro*

Compd.	GI_{50} (μM)[a]					% inhibition of HDACs[b]		
	K562	A549	MCF-7	PC-3	HeLa	HDAC1	HDAC2	HDAC6
9a	3.68	9.63	17.32	18.91	13.07	89	91	68
9b	3.96	12.18	19.45	> 20	14.93	95	96	57
9c	3.36	11.67	13.47	8.63	> 20	100	93	52
10a	5.27	7.46	19.93	14.59	> 20	95	59	25
10b	3.21	4.86	7.03	7.08	19.79	100	81	28
10c	2.00	5.40	9.37	10.74	16.24	94	72	29
11a	2.94	13.75	7.91	13.48	18.35	66	32	8
11b	2.92	5.07	> 20	11.38	> 20	90	62	36
11c	3.11	5.83	5.33	6.36	> 20	83	55	19
12a	2.90	6.42	2.82	6.46	9.33	5	3	54
12b	3.47	5.32	6.12	7.09	6.08	11	0	40
12c	4.12	18.21	19.11	10.94	> 20	23	8	14
14	1.53	4.80	4.17	13.15	5.80	5	0	51
15	3.32	5.23	5.96	7.17	6.74	0	8	43
16a	3.56	5.95	8.72	11.17	> 20	99	90	60
SAHA	3.66	19.45	17.90	18.83	19.21	100	100	100
MS-275	9.32	18.37	19.26	19.09	19.24	95	98	3

[a]three replicates with SD values <20% of the mean; [b]at 10 μM.

2.3. In vitro HDACs inhibitory activity

Selective HDAC inhibitors would provide powerful chemical tools to dissect the individual functions of the HDAC isoforms, in addition to providing lead antitumor drug candidates. To assess the isoform selectivity of the 8-ethoxy-3-nitro-2*H*-chromene analogues, HDAC1 and HDAC2 representing class I and HDAC6 representing class II were used in the *in vitro* inhibitory assay. At the concentration

of 10 μM, these 8-ethoxy-3-nitro-2*H*-chromene derivatives exhibited quite different inhibitory rates (Table 1), depending on mainly on the ZBG type and internal cavity group. The *ortho*-aminoanilides **9a–c** with no internal cavity group showed good inhibiting rates for HDAC1 and HDAC2, and moderate activity for HDAC6, while the *ortho*-aminoanilides with a phenyl (**10a–c**) or 5-methylthiophen-2-yl (**11a–c**) internal cavity group exhibited comparable potency for HDAC1, but reduced activity for HDAC2 and HDAC6. The linker length had slight influences on the activity of these *ortho*-aminoanilides, and in general, the heptanedioic acid derived linker was preferred. In comparison with the *ortho*-aminoanilide series, the weak amide ZGB derivative **12a** and **12b**, **14** and **15**, showed some selectivity toward HDAC6, since they had moderate inhibitory activity for HDAC6, but little inhibition against HDAC1 and HDAC2. It is of notice that the D-Phe derived analogues **16a** and **16b** showed similar potency as that as the *ortho*-aminoanilides against HDAC1, 2 and HADC6. It is apparent that the amino group at the α-position of the amide can contribute to the binding with the Zn^{2+}.

The IC_{50} values for those compounds that showed relatively good inhibitory activity toward HADC1, 2 at 10 μM had been determined (Table 2). The *ortho*-aminoanilides **9b**, **9c**, **10b**, **10c**, **11b**, and the α-aminoamides **16a** and **16b** were potent HADC1 inhibitors with the IC_{50} values in the nanomolar ranges. The *ortho*-aminoanilides **10b** and**10c** with a phenyl internal cavity motif were more potent than MS-275 and more selective of HADC1 to HADC2.

Table 2: The IC_{50} values of the selected compounds for HDAC1 and HDAC2

HDACs	IC_{50}(nM)								
	9b	**9c**	**10b**	**10c**	**11b**	**16a**	**16b**	MS275	SAHA
HDAC1	288	232	128	179	366	742	448	271	9
HDAC2	759	439	659	827	1788	2915	1294	505	17

3. Conclusion

In summary, a series of 8-ethoxy-3-nitro-2*H*-chromene based HDACIs were designed, synthesized and evaluated for their inhibitory activity against K562, A549, MCF-7, PC3 and HeLa cell lines and HDAC1, 2 and HDAC6. Most of these coumarin derivatives displayed good antiproliferative activity and were more potent toward the five tumor cell lines rested than the reference drug SAHA and

MS-275. The *ortho*-aminoanilide and the D-Phe derived α-amnoamide derivatives (**16a** and **16b**) displayed more potent activity toward HADC1 over HADC2, and only moderate to weak activity over HADC6. In contrast, the amide ZBG analogues (**12a** and **12b**, **14** and **15**) were only moderate HDAC6 inhibitors, but more selective over HDAC1 and HDAC2. The *ortho*-aminoanilides **9b**, **9c**, **10b**, **10c**, **11b**, and the α-aminoamides **16a** and **16b** were potent HADC1 inhibitors with the IC_{50} values in the nanomolar ranges. The *ortho*-aminoanilides **10b** and**10c** with a phenyl internal cavity motif were more potent than MS-275 as HADC1 inhibitors and more selective over HADC2. In comparison with the amide ZBG derivatives, the D-Phe derived analogues were more potent HDAC inhibitors. The application of amino acids as both ZBG and internal cavity motifs will be hopeful in the discovery potent and selective HDAC inhibitors.

4. Experiment sections

4.1. Chemmistry

Melting points were determined on an X-6 micro-melting point apparatus (Beijing Tech. Co., Ltd, China, Beijing) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a AVANCE 600 (Bruker, Switzerland) instrument with a 600/150 MHz operating frequency at room temperature for $CDCl_3$ solutions, unless otherwise stated. All chemical shifts were reported as δ values (ppm) relative to Me_4Si (0.00 ppm) as internal standards. Electrospray-ionization mass spectrometry (ESI-MS) was performed on an API 4000 (AB SCIEX, USA) instrument. Column chromatography was performed on silica gel (200−300 mesh). Independent analysis of purity for synthetic products was achieved using analytical HPLC system (Agilent 1100 series, Agilent Technologies, Germany). All the compounds for the biological assay are of high purities (>98.5%). All the reactions involving oxygen- or moisture-sensitive compounds were carried out under a dry N_2 atmosphere. Unless otherwise noted, reagents were added by syringe. THF was distilled from sodium/benzophenone immediately prior to use.

4.1.1. Synthesis of 3-ethoxy-2-hydroxy-5-nitrobenzaldehyde (1)

To a solution of 3-ethoxy-2-hydroxybenzaldehyde (6.7 g, 40.3 mmol) in glacial acetic acid (20 mL) was added the mixture solution of nitric acid (4.85 mL) and glacial acetic acid (20 mL) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was poured into ice water (150 mL). The resulting precipitate was filtered, washed with a small amount of ice water. The crude product was

recrystallized from EtOAc and petroleum (1:2) to give 6.0 g of **1** as a yellow solid, yield 71%, mp. 159–161 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.54 (s, 1H), 10.33 (s, 1H), 8.11 (d, 1H, J = 2.4Hz), 7.91 (d, 1H, J = 2.4 Hz), 4.26 (q, 2H, J = 7.2 Hz),1.41 (t, 3H, J = 6.6 Hz); HRESI-MS calcd. for $C_9H_{10}NO_5$ $[M+H]^+$ 212.0153, found 212.0149.

4.1.2 Synthesis of (2-acetoxy-3-ethoxy-5-nitrophenyl)methylene diacetate (2)

To a solution of **1** (4.1 g, 19.4 mmol) in Ac_2O (100 mL) was added 6 drops of concentrated sulfuric acid. After stirring at room temperature for 2 h, the reaction mixture was poured into ice and water (500 mL). After stirring for 40 min, the resulting precipitate was filtered, washed with a small amount of icy water, dried, to give 6.20 g of **2** as a white solid, yield 92%, mp. 113–114 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.00 (dd, 2H, J = 1.8, 6.0 Hz), 7.76 (s, 1H), 7.91 (d, 1H, J = 2.4 Hz), 4.23 (q, 2H, J = 7.2 Hz), 2.32 (s, 1H), 2.11 (s, 6H), 1.31 (t, 3H, J = 6.6 Hz); HRESI-MS calcd. for $C_{15}H_{18}NO_9$ $[M+H]^+$ 356.0976, found 356.0978.

4.1.3. Synthesis of tert-butyl (3-ethoxy-5-formyl-4-hydroxyphenyl)carbamate (3)

A mixture of **2** (3.0 g, 8.45 mmol) and 10% Pd/C (0.3 g) in THF (10 mL) was stirred under H_2 atmosphere at room temperature for 25 h. The reaction mixture was filtered, and the filtrate was evaporated. To the resulting residue, water (20 mL) and Boc_2O (4.4 g，40.3 mmol) were added. After stirring at room temperature for 12 h, additional Boc_2O (2.2 g，80.6 mmol) was added, and the stirring was continued for 18 h. EtOAc (30 mL) was added. The water phase was extracted with EtOAc (30 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was dissolved in THF (130 mL), and a 10 M aqueous NaOH solution (15 mL) was added. After refluxing for 8 h, 10% aqueous citric acid was added to adjust the pH to 5. After the extraction with EtOAc, the organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by column chromatography on silica gel (petroleum/EtOAc: 5/1) to afford 1.81 g of **3** as a light yellow solid, yield 76%, mp. 125–126 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.23 (s, 1H), 9.75 (s, 1H), 9.26 (s, 1H), 7.35 (s, 2H), 4.03 (q, 2H, J = 7.2 Hz), 1.47 (s, 9H), 1.37 (t, 3H, J = 6.6 Hz); HRESI-MS calcd. for $C_{14}H_{20}NO_5$ $[M+H]^+$ 282.1336, found 282.1342.

4.1.4. Synthesis of tert-butyl (8-ethoxy-3-nitro-2H-chromen-6-yl)carbamate (4)

To a solution of **3** (4.68 g, 17.79 mmol) in anhydrous toluene (150 mL) was added phthalic anhydride (4.93 g, 36.05 mmol) and di-*n*-butylamine (1.60 mL, 9.5 mmol). The mixture was refluxed

with a Dean and Stark apparatus to remove the water. A total of 2.10 mL of 2-nitroethanol (29.3 mmol) was added portionwise within 12 h. After refluxing for another 36 h, EtOAc (150 mL) was added. The organic layer was washed with water, brine, and dried over anhydrous $NaSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc/hexane: 1/20) to give 2.8 g of **4** as a pale red solid, yield 50%, mp. 203–204 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.33 (s, 1H), 8.00 (s, 1H), 7.22 (s, 1H), 5.17 (s, 2H), 3.98 (q, 2H, J = 6.6 Hz), 1.47 (s, 9H), 1.33 (t, 3H, J = 7.2 Hz); HRESI-MS calcd. for $C_{16}H_{21}N_2O_6$ $[M+H]^+$ 337.1394, found 337.1390.

4.1.5. Synthesis of 6-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-6-oxohexanoic acid (5a)

To a solution of **4** (130 mg, 0.39 mmol) in dichloromethane (1.6 mL) was added TFA (0.4 mL). After stirring at room temperature for 2 h, the mixture was concentrated under reduce pressure. The residue was dissolved in anhydrous DMF, and adipic acid (169 mg, 1.20 mmol), HATU (163 mg, 0.43 mmol) and triethylamine (0.43 mL, 3.09 mmol) were added. After stirring at room temperature under nitrogen atmosphere for 12 h, the reaction mixture was poured into 20 mL brine. The precipitate was filtered, washed with a small amount of water, and then dissolved in 40 mL ethyl acetate and 20 mL methanol. The insoluble residues were filtered and the filtrate was concentrated under reduced pressure. Recrystallization from EtOAc and hexane (1:2) afforded 103 mg of **5a** as an orange-red solid, yield 73%, mp. 181–183 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.02 (s, 1H), 9.90 (s, 1H), 8.02 (s, 1H), 7.39 (d, J = 1.4 Hz, 1H), 7.30 (d, J = 1.6 Hz, 1H), 5.18 (s, 2H), 4.00 (q, J = 6.8 Hz, 3H), 2.28 (t, J = 7.1 Hz, 2H), 2.23 (t, J = 7.1 Hz, 2H), 1.63–1.48 (m, 4H), 1.33 (t, J = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.8, 171.4, 147.0, 140.4, 139.8, 134.5, 129.9, 119.3, 112.7, 110.1, 64.6, 62.8, 36.5, 33.9, 25.1, 24.6, 15.1; HRESI-MS calcd. for $C_{17}H_{21}N_2O_7$ $[M+H]^+$ 365.1343, found 365.1336.

4.1.6. 7-((8-Ethoxy-3-nitro-2H-chromen-6-yl)amino)-7-oxoheptanoic acid (5b)

Following the same procedures described for **5a**, intermediate **5b** was prepared from **4** (130 mg, 0.39 mmol), TFA (0.4 mL); heptanedioic acid (192 mg, 1.20 mmol), HATU (163 mg, 0.43 mmol) and triethylamine (0.43 mL, 3.09 mmol), in 74% yield (110 mg), as an orange-red solid, mp. 186–187 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.99 (s, 1H), 9.89 (s, 1H), 8.02 (s, 1H), 7.39 (d, J = 2.2 Hz, 1H), 7.29 (d, J = 2.2 Hz, 1H), 5.18 (d, J = 0.7 Hz, 2H), 4.00 (q, J = 6.9 Hz, 2H), 2.26 (t, J = 7.4 Hz, 2H), 2.20 (t, J = 7.3 Hz, 2H), 1.60–1.47 (m, 4H), 1.33 (t, J = 7.0 Hz, 3H), 1.30–1.24 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.5, 171.1, 146.6, 139.9, 139.3, 134.1, 129.5, 118.8, 112.3, 109.6, 64.1, 62.3, 36.2, 33.6, 28.2, 24.8, 24.3, 14.7; HRESI-MS calcd. for $C_{18}H_{23}N_2O_7$ $[M+H]^+$ 379.1500, found

379.1498.

4.1.7. 8-((8-Ethoxy-3-nitro-2H-chromen-6-yl)amino)-8-oxooctanoic acid (5c)

Following the same procedures described for **5a**, intermediate **5c** was prepared from **4** (120 mg, 0.34 mmol), TFA (0.4 mL); octanedioic acid (186 mg, 1.06 mmol), HATU (137 mg, 0.36 mmol) and triethylamine (0.4 mL, 2.85 mmol), in 79% yield (106 mg), as an orange-red solid, mp. 196–198 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.97 (s, 1H), 9.89 (s, 1H), 8.03 (s, 1H), 7.40 (d, J = 2.0 Hz, 1H), 7.31 (s, 1H), 5.19 (s, 2H), 4.01 (t, J = 10.4 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 2.20 (t, J = 7.3 Hz, 2H), 1.62–1.47 (m, 4H), 1.34 (t, J = 7.0 Hz, 4H), 1.36–1.28 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.5, 171.2, 146.6, 139.9, 139.3, 134.1, 129.5, 118.8, 112.3, 109.6, 64.1, 62.3, 36.3, 33.6, 28.4, 28.4, 25.0, 24.4, 14.7; HRESI-MS calcd. for $C_{19}H_{25}N_2O_7$ $[M+H]^+$ 393.1656, found 393.1648.

4.1.8. Synthesis of tert-butyl

(2-(6-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-6-oxohexanamido)phenyl)carbamate (6a)

To a stirred solution of **5a** (92 mg, 0.25 mmol) in DMF (1.5 mL) was added *tert*-butyl 2-aminophenylcarbamate (53 mg, 0.25 mmol), HATU (106 mg, 0.28 mmol), and triethylamine (0.07 mL, 0.52 mmol). After stirring at room temperature for 4 h under nitrogen atmosphere, the mixture was poured into 40 mL water. The precipitate was filtered, washed with a small amount of water. Purification by column chromatography on silica gel (dichloromethane/methanol: 100/1) gave 85 mg of **6a** as an orange-solid, yield 61%, mp. 141–142 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.93 (s, 1H), 9.46 (s, 1H), 8.32 (s, 1H), 8.02 (s, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.42–7.39 (m, 2H), 7.30 (d, J = 2.1 Hz, 1H), 7.13 (td, J = 7.8, 1.2 Hz, 1H), 7.06 (td, J = 7.7, 1.4 Hz, 1H), 5.18 (s, 2H), 4.00 (t, J = 10.4 Hz, 2H), 1.66–1.62 (m, 4H), 1.64 (s, 4H), 1.43 (s, 9H), 1.33 (t, J = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.6, 171.0, 153.1, 146.6, 139.9, 139.3, 134.1, 131.1, 129.6, 129.4, 125.1, 124.9, 123.9, 123.7, 118.8, 112.2, 109.6, 79.3, 64.1, 62.3, 36.1, 35.8, 28.0, 24.9, 24.7, 14.6; HRESI-MS calcd. for $C_{28}H_{35}N_4O_8$ $[M+H]^+$ 555.2449, found 555.2446.

4.1.9. tert-Butyl

(2-(7-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-7-oxoheptanamido)phenyl)carbamate (6b)

Following the same procedures described for **6a**, intermediate **6b** was prepared from **5b** (100 mg, 0.26 mmol), *tert*-butyl 2-aminophenylcarbamate (55 mg, 0.26 mmol), HATU (106 mg, 0.28 mmol), and triethylamine (0.07 mL, 0.52 mmol), in 61% yield (91 mg), as an orange-red solid, mp. 138–140 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 9.43 (s, 1H), 8.33 (s, 1H), 8.02 (s, 1H), 7.52 (d, J = 7.8 Hz,

1H), 7.42–7.41 (m, 2H), 7.30 (d, *J* = 2.2 Hz, 1H), 7.12 (td, *J* = 7.9, 1.2 Hz, 1H), 7.05 (td, *J* = 7.7, 1.4 Hz, 1H), 5.18 (s, 2H), 3.99 (q, *J* = 7.0 Hz, 2H), 2.35 (t, *J* = 7.3 Hz, 2H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.65–1.62 (m, 4H), 1.45 (s, 9H), 1.41–1.32 (m, 5H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.7, 171.1, 153.1, 146.6, 139.9, 139.3, 134.1, 131.1, 129.7, 129.5, 125.0, 124.8, 123.9, 123.7, 118.8, 112.2, 109.6, 79.3, 64.1, 62.3, 40.0, 36.1, 35.8, 28.1, 25.0, 24.8, 14.6; HRESI-MS calcd. for $C_{29}H_{37}N_4O_8$ $[M+H]^+$ 569.2606, found 569.2606.

4.1.10. tert-Butyl (2-(8-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-8-oxooctanamido)phenyl)carbamate (6c)

Following the same procedures described for **6a**, intermediate **6c** was prepared from **5c** (100 mg, 0.25 mmol), *tert*-butyl 2-aminophenylcarbamate (53 mg, 0.25 mmol), HATU (106 mg, 0.28 mmol), and triethylamine (0.07 mL, 0.52 mmol), in 60% yield (89 mg), as an orange-red solid, mp. 174–175 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 9.44 (s, 1H), 8.32 (s, 1H), 8.03 (s, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.41 (d, *J* = 2.2 Hz, 2H), 7.31 (d, *J* = 2.1 Hz, 1H), 7.14 (t, *J* = 7.0 Hz, 1H), 7.07 (td, *J* = 7.7, 1.4 Hz, 1H), 5.20 (s, 2H), 4.01 (q, *J* = 6.9 Hz, 2H), 2.35 (t, *J* = 7.2 Hz, 2H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.64–1.59 (m, 4H), 1.46 (s, 9H), 1.36–1.32 (m, 7H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.2, 171.6, 153.5, 147.0, 140.4, 139.8, 134.5, 131.6, 130.2, 129.9, 125.5, 125.3, 124.4, 124.2, 119.3, 112.7, 110.1, 79.8, 64.6, 62.8, 36.8, 36.4, 29.0, 28.8, 28.5, 25.6, 25.5, 15.1; HRESI-MS calcd. for $C_{30}H_{39}N_4O_8$ $[M+H]^+$ 583.2762, found 583.2760.

4.1.11. tert-Butyl (3-(6-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-6-oxohexanamido)-(1,1'-biphenyl)-4-yl)carbamate (7a)

Following the same procedures described for **6a**, intermediate **7a** was prepared from **5a** (81 mg, 0.22 mmol), *tert*-butyl 3-amino-4-(1,1'-biphenyl)-4-yl)carbamate [21] (63 mg, 0.22 mmol), HATU (92 mg, 0.28 mmol), and triethylamine (0.06 mL, 0.44 mmol), in 47% yield (65 mg), as an orange-red solid, mp. 199–201 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.94 (s, 1H), 9.53 (s, 1H), 8.45 (s, 1H), 8.02 (s, 1H), 7.74 (d, *J* = 1.9 Hz, 1H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.60 (d, *J* = 7.3 Hz, 2H), 7.47–7.42 (m, 4H), 7.34 (t, *J* = 7.3 Hz, 1H), 7.30 (d, *J* = 2.1 Hz, 1H), 5.18 (s, 2H), 3.99 (q, *J* = 6.9 Hz, 2H), 2.41 (t, *J* = 7.3 Hz, 2H), 2.34 (t, *J* = 7.4 Hz, 2H), 1.68–1.84 (m, 4H), 1.46 (s, 9H), 1.32 (t, *J* = 6.9 Hz, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.8, 171.0, 153.0, 146.6, 139.9, 139.4, 139.3, 135.6, 134.1, 130.5, 129.8, 129.4, 129.0, 127.3, 126.3, 123.7, 123.2, 122.9, 118.8, 112.2, 109.6, 79.5, 64.1, 62.3, 36.2, 35.9, 28.1, 24.9, 24.7,

14.6; HRESI-MS calcd. for $C_{34}H_{39}N_4O_8$ $[M+H]^+$ 631.2762, found 231.2762.

4.1.12. tert-Butyl

(3-(7-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-7-oxoheptanamido)-(1,1'-biphenyl-4-yl)carbamate (7b)

Following the same procedures described for **6a**, intermediate **7b** was prepared from **5b** (64 mg, 0.17 mmol), *tert*-butyl 3-amino-4-(1,1'-biphenyl)-4-yl)carbamate (48 mg, 0.17 mmol), HATU (61 mg, 0.19 mmol), and triethylamine (0.05 mL, 0.33 mmol), in 64% yield (70 mg), as an orange-red solid, mp. 180–181 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.47 (s, 1H), 8.41 (s, 1H), 7.96 (s, 1H), 7.69 (s, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.55 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.7 Hz, 3H), 7.35 (d, J = 2.2 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 7.25 (d, J = 2.1 Hz, 1H), 5.13 (d, J = 0.4 Hz, 2H), 3.94 (q, J = 7.0 Hz, 2H), 2.35 (t, J = 7.3 Hz, 2H), 2.26 (t, J = 7.4 Hz, 2H), 1.69–1.60 (m, 4H), 1.43 (s, 9H), 1.41–1.37 (m, 3H), 1.28 (t, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 171.2, 146.6, 141.4, 140.4, 139.9, 139.3, 134.1, 129.5, 128.9, 128.1, 126.0, 125.5, 123.9, 123.8, 123.3, 118.8, 116.2, 112.3, 109.6, 64.1, 62.3, 36.3, 35.8, 28.5, 25.2, 25.0, 14.7; HRESI-MS calcd. for $C_{35}H_{41}N_4O_8$ $[M+H]^+$ 645.2919, found 645.2920.

4.1.13. tert-Butyl

(3-(8-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-8-oxooctanamido)-(1,1'-biphenyl)-4-yl)carbamate (7c)

Following the same procedures described for **6a**, intermediate **7c** was prepared from **5c** (90 mg, 0.23 mmol), *tert*-butyl 3-amino-4-(1,1'-biphenyl)-4-yl)carbamate (66 mg, 0.23 mmol), HATU (96 mg, 0.28 mmol), and triethylamine (0.06 mL, 0.46 mmol), in 66% yield (101 mg), as an orange-red solid, yield 66.3%, mp165–166 °C.. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.86 (s, 1H), 9.48 (s, 1H), 8.39 (s, 1H), 7.97 (s, 1H), 7.68 (d, J = 1.8 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.7 Hz, 3H), 7.35 (d, J = 2.1 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 7.26 (d, J = 2.1 Hz, 1H), 5.14 (s, 2H), 3.95 (q, J = 7.0 Hz, 2H), 2.34 (t, J = 7.3 Hz, 2H), 2.25 (t, J = 7.4 Hz, 2H), 1.60–1.55 (m, 4H), 1.42 (s, 9H), 1.32–1.31(m, 4H), 1.29 (t, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.8, 171.0, 153.0, 146.6, 139.9, 139.4, 139.3, 135.6, 134.1, 130.5, 129.8, 129.4, 129.0, 127.3, 126.3, 123.8, 123.2, 122.9, 118.8, 112.2, 109.6, 79.5, 64.1, 62.3, 36.2, 35.9, 28.1, 24.9, 24.7, 14.6; HRESI-MS calcd. for $C_{36}H_{43}N_4O_8$ $[M+H]^+$ 659.7480, found 659.7472.

4.1.14. tert-Butyl

(2-(6-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-6-oxohexanamido)-4-(5-methylthiophen-2-yl)phenyl)carbamate (8a)

Following the same procedures described for **6a**, intermediate **8a** was prepared from **5a** (81 mg, 0.22 mmol), *tert*-butyl 3-amino-4-(5-methylthiophen-2-yl)phenyl)carbamate [22] (68 mg, 0.22 mmol), HATU (93 mg, 0.24 mmol), and triethylamine (0.06 mL, 0.44 mmol), in 57% yield (82 mg), as an orange-red solid, mp. 137–139 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.91 (s, 1H), 9.48 (s, 1H), 8.42 (s, 1H), 8.01 (s, 1H), 7.66 (s, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.40 (d, J = 1.7 Hz, 1H), 7.36 (dd, J = 8.5, 1.4 Hz, 1H), 7.31 (d, J = 1.8 Hz, 1H), 7.18 (d, J = 3.3 Hz, 1H), 6.79 (d, J = 2.8 Hz, 1H), 5.19 (s, 2H), 4.00 (q, J = 6.9 Hz, 2H), 2.45 (s, 3H), 2.39 (t, J = 7.2 Hz, 2H), 2.31 (t, J = 7.3 Hz, 2H), 1.68–1.62 (m, 4H), 1.47 (s, 9H), 1.41–1.36 (m, 2H), 1.33 (t, J = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.8, 171.0, 153.0, 146.6, 140.3, 139.9, 139.3, 138.8, 134.1, 130.0, 129.9, 129.7, 129.4, 126.8, 124.0, 123.1, 121.6, 121.1, 118.8, 112.2, 109.6, 79.5, 64.1, 62.3, 36.1, 35.8, 28.0, 24.8, 24.7, 15.1, 14.6; HRESI-MS calcd. for $C_{33}H_{39}N_4O_8S$ $[M+H]^+$ 651.2483, found 651.2479.

4.1.15. tert-Butyl (2-(7-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-7-oxoheptanamido)-4-(5-methylthiophen-2-yl)phenyl)carbamate (8b)

Following the same procedures described for **6a**, intermediate **8b** was prepared from **5b** (81 mg, 0.21 mmol), *tert*-butyl 3-amino-4-(5-methylthiophen-2-yl)phenyl)carbamate [22] (65 mg, 0.21 mmol), HATU (87 mg, 0.23 mmol), and triethylamine (0.06 mL, 0.44 mmol), in 58% yield (82 mg), as an orange-red solid, mp. 132–133 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 9.48 (s, 1H), 8.42 (s, 1H), 8.01 (s, 1H), 7.65 (d, J = 1.8 Hz, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H), 7.36 (dd, J = 8.5, 2.0 Hz, 1H), 7.30 (d, J = 2.1 Hz, 1H), 7.18 (d, J = 3.5 Hz, 1H), 6.79 (dd, J = 3.5, 1.0 Hz, 1H), 5.18 (s, 2H), 3.99 (q, J = 6.9 Hz, 2H), 2.45 (s, 3H), 2.38 (t, J = 7.2 Hz, 2H), 2.31 (t, J = 7.3 Hz, 2H), 1.69–1.60 (m, 4H), 1.46 (s, 9H), 1.41–1.37 (m, 2H), 1.33 (t, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.8, 171.0, 153.0, 146.6, 140.3, 139.9, 139.3, 138.8, 134.1, 130.0, 129.9, 129.7, 129.4, 126.8, 124.0, 123.1, 121.6, 121.0, 118.8, 112.2, 109.6, 79.5, 64.1, 62.3, 36.1, 35.8, 28.0, 24.8, 24.7, 15.1, 14.6; HRESI-MS calcd. for $C_{34}H_{41}N_4O_8S$ $[M+H]^+$ 665.2640, found 665.2632.

4.1.16. tert-Butyl (2-(8-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-8-oxooctanamido)-4-(5-methylthiophen-2-yl)phenyl)carbamate (8c)

Following the same procedures described for **6a**, intermediate **8c** was prepared from **5c** (80 mg, 0.20 mmol), *tert*-butyl 3-amino-4-(5-methylthiophen-2-yl)phenyl)carbamate (62 mg, 0.20 mmol), HATU (84 mg, 0.20 mmol), and triethylamine (0.06 mL, 0.44 mmol), in 74% yield (102 mg), as an orange-red solid, mp. 153–155 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 9.48 (s, 1H), 8.39 (s, 1H), 8.02 (s, 1H), 7.64 (s, 1H), 7.55 (d, J = 8.1 Hz, 1H), 7.40 (d, J = 1.7 Hz, 1H), 7.36 (dd, J = 8.4, 1.8 Hz, 1H), 7.30 (d, J = 1.7 Hz, 1H), 7.19 (d, J = 3.3 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 5.18 (s, 2H), 3.99 (q, J = 6.9 Hz, 2H), 2.45 (s, 3H), 2.37 (t, J = 7.2 Hz, 2H), 2.29 (t, J = 7.3 Hz, 2H), 1.64–1.59 (m, 4H), 1.45 (s, 9H), 1.36–1.35 (m, 7H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.4, 171.6, 153.5, 147.0, 140.8, 140.4, 139.7, 139.2, 134.5, 130.5, 130.2, 129.9, 127.2, 124.5, 123.6, 122.0, 121.5, 119.3, 112.7, 110.0, 80.0, 64.6, 62.8, 36.8, 36.4, 29.0, 28.9, 28.5, 25.5, 15.5, 15.1; HRESI-MS calcd. for $C_{35}H_{43}N_4O_8S$ $[M+H]^+$ 679.2796, found 679.2790.

4.1.17. Synthesis of N^1-(2-aminophenyl)-N^6-(8-ethoxy-3-nitro-2H-chromen-6-yl)adipamide (9a)

To a solution of **6a** (30 mg, 0.054 mmol) in DCM (1.6 mL) was added TFA (0.4 mL). After stirring at room temperature under nitrogen atmosphere for 4 h, the mixture was concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate, washed with saturation $NaHCO_3$, water and brine, dried over Na_2SO_4, filtered, and concentrated. Recrystallization with DCM and hexane (1/2) afforded 16 mg of **9a** (65%) as an orange-yellow solid, mp. 201–203 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.95 (s, 1H), 9.12 (s, 1H), 8.03 (s, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.31 (d, J = 2.0 Hz, 1H), 7.15 (dd, J = 7.8, 0.8 Hz, 1H), 6.88 (td, J = 7.8, 1.2 Hz, 1H), 6.71 (dd, J = 7.9, 1.0 Hz, 1H), 6.53 (td, J = 7.8, 1.2 Hz, 1H), 5.19 (s, 2H), 4.83 (s, 2H), 4.00 (q, J = 6.9 Hz, 2H), 2.35–2.31 (m, 4H), 1.63–1.62 (m, 4H), 1.33 (t, J = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 171.0, 146.6, 141.9, 139.9, 139.3, 134.1, 129.5, 125.7, 125.3, 123.5, 118.9, 116.1, 115.9, 112.3, 109.6, 64.1, 62.3, 36.2, 35.6, 25.1, 24.8, 14.7; HRESI-MS calcd. for $C_{23}H_{27}N_4O_6$ $[M+H]^+$ 455.1925, found 455.1919.

4.1.18. N^1-(2-Aminophenyl)-N^7-(8-ethoxy-3-nitro-2H-chromen-6-yl)heptanediamide (9b)

Following the same procedures described for **9a**, diamide **9b** was prepared from **6b** (80 mg, 0.14 mmol) and TFA (0.4 mL) in 56% yield (37 mg), as an orange-yellow solid, mp. 195–197 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 9.09 (s, 1H), 8.03 (s, 1H), 7.40 (s, 1H), 7.31 (s, 1H), 7.14 (d, J = 7.4 Hz, 1H), 6.88 (t, J = 7.4 Hz, 1H), 6.71 (d, J = 7.7 Hz, 1H), 6.52 (t, J = 7.3 Hz, 1H), 5.19 (s, 2H), 4.81 (s, 2H), 4.00 (q, J = 6.8 Hz, 2H), 2.31 (dd, J = 15.2, 7.5 Hz, 4H), 1.66–1.61 (m, 4H), 1.39–1.34 (m, 5H), ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.6, 171.5, 147.0, 142.3, 140.4, 134.5, 129.9, 126.1, 125.7,

124.0, 119.30, 116.6, 116.3, 112.7, 110.1, 64.6, 62.8, 36.7, 36.1, 28.8, 25.6, 25.4, 15.1; HRESI-MS calcd. for $C_{24}H_{29}N_4O_6$ $[M+H]^+$ 469.2082, found 469.2082.

4.1.19. N^1-(2-Aminophenyl)-N^8-(8-ethoxy-3-nitro-2H-chromen-6-yl)octanediamide (9c)

Following the same procedures described for **9a**, diamide **9c** was prepared from **6c** (80 mg, 0.137 mmol) and TFA (0.2 mL) in 51% yield (34 mg), as an orange-yellow solid, mp. 192–194 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.07 (s, 1H), 8.02 (s, 1H), 7.40 (s, 1H), 7.31 (s, 1H), 7.14 (d, J = 7.7 Hz, 1H), 6.88 (t, J = 7.1 Hz, 1H), 6.71 (d, J = 7.8 Hz, 1H), 6.53 (t, J = 7.5 Hz, 1H), 5.19 (s, 2H), 4.80 (s, 2H), 4.00 (q, J = 6.9 Hz, 2H), 2.30 (dd, J = 15.2, 7.4 Hz, 4H), 1.62–1.58 (m, 4H), 1.35–1.32 (m, 7H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 171.5, 147.0, 142.3, 140.4, 139.8, 134.5, 129.9, 126.1, 126.7, 124.1, 119.3, 116.6, 116.4, 112.8, 110.1, 64.6, 62.8, 36.8, 36.2, 29.0, 25.7, 25.5, 15.1; HRESI-MS calcd. for $C_{25}H_{31}N_4O_6$ $[M+H]^+$ 483.2238, found 483.2228.

4.1.20. N^1-(4-Amino-(1,1'-biphenyl)-3-yl)-N^6-(8-ethoxy-3-nitro-2H-chromen-6-yl)adipamide (10a)

Following the same procedures described for **9a**, diamide **10a** was prepared from **7a** (45 mg, 0.07 mmol) and TFA (0.4 mL) in 96% yield (40 mg), as an orange-yellow solid, mp. 210–212 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.08 (s, 1H), 9.33 (s, 1H), 8.02 (s, 1H), 7.57 (d, J = 1.7 Hz, 1H), 7.45 (d, J = 1.7 Hz, 1H), 7.38 (t, J = 7.7 Hz, 2H), 7.36 (d, J = 1.6 Hz, 1H), 7.23–7.21 (m, 2H), 5.19 (s, 2H), 5.10 (s, 2H), 4.00 (q, J = 6.9 Hz, 2H), 2.41 (t, J = 6.3 Hz, 2H), 2.36 (t, J = 6.4 Hz, 2H), 1.66 (s, 4H), 1.33 (t, J = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.3, 171.2, 146.5, 141.3, 140.4, 139.9, 139.3, 134.2, 129.5, 128.8, 127.9, 125.9, 125.5, 124.0, 123.7, 123.1, 118.8, 116.2, 112.3, 109.7, 64.1, 62.3, 36.0, 35.5, 24.9, 14.7; HRESI-MS calcd. for $C_{29}H_{31}N_4O_6$ $[M+H]^+$ 531.2238, found 531.2240.

4.1.21. N^1-(4-Amino-(1,1'-biphenyl)-3-yl)-N^7-(8-ethoxy-3-nitro-2H-chromen-6-yl)heptanediamide (10b)

Following the same procedures described for **9a**, diamide **10b** was prepared from **7b** (50 mg, 0.08 mmol) and TFA (0.4 mL) in 96% yield (45 mg), as an orange-yellow solid, mp. 181–183 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.96 (s, 1H), 9.22 (s, 1H), 8.00 (s, 1H), 7.54 (d, J = 2.0 Hz, 1H), 7.50 (d, J = 7.4 Hz, 2H), 7.41 (d, J = 1.9 Hz, 1H), 7.37 (t, J = 7.7 Hz, 2H), 7.32 (d, J = 2.0 Hz, 1H), 7.23–7.21 (m, 2H), 6.79 (d, J = 8.3 Hz, 1H), 5.17 (s, 2H), 5.04 (s, 2H), 3.99 (q, J = 6.9 Hz, 2H), 2.36 (t, J = 7.4 Hz, 2H), 2.31 (t, J = 7.4 Hz, 2H), 1.66–1.61 (m, 4H), 1.39–1.34 (m, 2H), 1.32 (t, J = 6.9 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.4, 171.2, 146.6, 141.4, 140.4, 139.9, 139.3, 134.2, 129.5, 128.8, 128.0,

125.9, 125.5, 123.9, 123.8, 123.2, 118.8, 116.2, 112.3, 109.7, 64.1, 62.3, 36.2, 35.7, 28.3, 25.1, 24.9, 14.7; HRESI-MS calcd. for $C_{30}H_{33}N_4O_6$ $[M+H]^+$ 545.2395, found 545.2395.

4.1.22. N^1-(4-Amino-(1,1'-biphenyl)-3-yl)-N^8-(8-ethoxy-3-nitro-2H-chromen-6-yl)octanediamide (10c)

Following the same procedures described for **9a**, diamide **10c** was prepared from **7c** (80 mg, 0.12 mmol) and TFA (0.4 mL) in 94% yield (71 mg), as an orange-yellow solid, mp. 172–173°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.91 (s, 1H), 9.17 (s, 1H), 8.01 (s, 1H), 7.52 (d, J = 1.8 Hz, 1H), 7.50 (d, J = 7.4 Hz, 2H), 7.40 (d, J = 2.0 Hz, 1H), 7.37 (t, J = 7.7 Hz, 2H), 7.31 (d, J = 1.9 Hz, 1H), 7.24–7.20 (m, 2H), 6.79 (d, J = 8.3 Hz, 1H), 5.17 (s, 2H), 5.02 (s, 2H), 3.99 (q, J = 6.9 Hz, 2H), 2.34 (t, J = 7.4 Hz, 2H), 2.28 (t, J = 7.3 Hz, 2H), 1.63–1.58 (m, 4H), 1.35–1.30 (m, 7H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.8, 171.7, 147.0, 141.9, 140.8, 140.3, 139.7, 134.6, 129.9, 129.2, 128.5, 126.4, 126.0, 124.4, 124.3, 123.7, 119.3, 116.7, 112.7, 110.1, 64.6, 62.8, 36.8, 36.8, 29.0, 25.7, 25.5, 15.1; HRESI-MS calcd. for $C_{31}H_{35}N_4O_6$ $[M+H]^+$ 559.2551, found 559.2543.

4.1.23. N^1-(2-Amino-5-(5-methylthiophen-2-yl)phenyl)-N^6-(8-ethoxy-3-nitro-2H-chromen-6-yl)adipamide (11a)

Following the same procedures described for **9a**, diamide **11a** was prepared from **8a** (40 mg, 0.06 mmol) and TFA (0.2 mL) in 89% yield (30 mg), as a yellow solid, mp. 214–215 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.93 (s, 1H), 9.15 (s, 1H), 8.02 (s, 1H), 7.40 (t, J = 2.0 Hz, 2H), 7.30 (d, J = 2.1 Hz, 1H), 7.12 (dd, J = 8.2, 2.2 Hz, 1H), 6.95 (d, J = 3.5 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.69 (d, J = 1.2 Hz, 1H), 5.17 (s, 2H), 5.03 (s, 2H), 3.99 (q, J = 6.9 Hz, 3H), 2.39 (s, 3H), 2.35–2.32 (m, 4H), 1.65–1.61 (m, 4H), 1.32 (t, J = 6.9 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 171.1, 146.6, 142.0, 141.3, 139.9, 139.3, 136.4, 134.0, 129.5, 126.3, 123.6, 122.7, 122.5, 122.0, 120.6, 118.8, 116.1, 112.3, 109.7, 64.1, 62.3, 36.2, 35.6, 25.0, 24.8, 15.0, 14.6; HRESI-MS calcd. for $C_{28}H_{31}N_4O_6S$ $[M+H]^+$ 551.1959, found 551.1961.

4.1.24. N^1-(2-Amino-5-(5-methylthiophen-2-yl)phenyl)-N^7-(8-ethoxy-3-nitro-2H-chromen-6-yl)heptanediamide (11b)

Following the same procedures described for **9a**, diamide **11b** was prepared from **8b** (42 mg, 0.06 mmol) and TFA (0.4 mL) in 88% yield (30 mg), as a yellow solid, mp. 163–165 °C. ^{1}H NMR (600

MHz, DMSO-d_6) δ 9.91 (s, 1H), 9.13 (s, 1H), 8.01 (s, 1H), 7.40 (dd, J = 6.8, 2.1 Hz, 2H), 7.30 (d, J = 2.1 Hz, 1H), 7.12 (dd, J = 8.3, 2.1 Hz, 1H), 6.96 (d, J = 3.4 Hz, 1H), 6.71 (d, J = 8.4 Hz, 1H), 6.69 (d, J = 1.1 Hz, 1H), 5.18 (s, 2H), 5.02 (s, 2H), 3.99 (dd, J = 13.6, 6.6 Hz, 2H), 2.40 (s, 3H), 2.36–2.26 (m, 4H), 1.66–1.59 (m, 4H), 1.39–1.35 (m, 5H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.3, 171.1, 146.6, 142.0, 141.3, 139.9, 139.3, 136.4, 134.1, 129.5, 126.3, 123.7, 122.7, 122.5, 121.9, 120.6, 118.8, 116.08, 112.3, 109.7, 64.1, 62.3, 36.2, 35.6, 28.3, 25.0, 24.9, 15.0, 14.6; HRESI-MS calcd. for $C_{29}H_{33}N_4O_6S$ $[M+H]^+$ 565.2115, found 565.2110.

4.1.25. N^1-(2-Amino-5-(5-methylthiophen-2-yl)phenyl)-N^8-(8-ethoxy-3-nitro-2H-chromen-6-yl)octanediamide (11c)

Following the same procedures described for **9a**, diamide **11c** was prepared from **8c** (80 mg, 0.12 mmol) and TFA (0.4 mL) in 88% yield (60 mg), as a yellow solid, mp. 164–166 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 9.14 (s, 1H), 8.02 (s, 1H), 7.40 (dd, J = 7.9, 2.1 Hz, 2H), 7.31 (d, J = 2.1 Hz, 1H), 7.13 (dd, J = 8.3, 2.1 Hz, 1H), 6.96 (d, J = 3.4 Hz, 1H), 6.71 (d, J = 8.4 Hz, 1H), 6.70 (dd, J = 3.4, 1.1 Hz, 1H), 5.18 (s, 2H), 5.02 (s, 2H), 3.99 (q, J = 7.0 Hz, 2H), 2.40 (s, 3H), 2.33 (t, J = 7.4 Hz, 2H), 2.29 (t, J = 7.4 Hz, 2H), 1.63–1.59 (m, 4H), 1.36–1.35 (m, 7H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.3, 171.2, 146.6, 142.0, 141.3, 139.9, 139.3, 136.4, 134.1, 129.5, 126.4, 123.7, 122.7, 122.5, 122.0, 120.6, 118.8, 116.1, 112.3, 109.7, 64.1, 62.3, 36.3, 35.8, 28.5, 25.1, 25.0, 24.9, 15.0, 14.6; HRESI-MS calcd. for $C_{30}H_{35}N_4O_6S$ $[M+H]^+$ 579.2272, found 579.2260.

4.1.26. Synthesis of N^1-(8-ethoxy-3-nitro-2H-chromen-6-yl)-N^6-phenethyladipamide (12a)

To a solution of **5a** (60 mg, 0.16 mmol) and HATU (68 mg, 0.18 mmol) in anhydrous DMF (2 mL) was added triethylamine (0.05 mL, 0.32 mmol). After stirring for 30 min at room temperature under nitrogen atmosphere, phenylethylamine (0.021 mL, 0.16 mmol) was added. The resulting mixture was stirred for another 4 h. The mixture was poured into 20 mL water and filtered. The precipitate was dissolved in ethyl acetate and filtered, and the filtrate was concentrated under reduced pressure. Recrystallization with EtOAc and hexane (1/2) afforded 50 mg of **12a** (67%) as a orange-red solid, mp. 233–234 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.01 (s, 1H), 7.87 (t, J = 5.1 Hz, 1H), 7.39 (d, J = 1.4 Hz, 1H), 7.29 (d, J = 1.5 Hz, 1H), 7.25 (t, J = 7.5 Hz, 2H), 7.18–7.14 (m, 3H), 5.17 (s, 2H), 3.99 (q, J = 6.9 Hz, 2H), 3.24 (dd, J = 13.3, 6.8 Hz, 2H), 2.67 (t, J = 7.3 Hz, 2H), 2.25 (t, J = 6.6 Hz, 2H), 2.05 (t, J = 6.6 Hz, 2H), 1.51–1.48 (m, 4H), 1.32 (t, J = 6.9 Hz, 3H); ^{13}C NMR (150 MHz,

DMSO-d_6) δ 171.9, 171.1, 146.6, 139.9, 139.5, 139.3, 134.1, 129.5, 128.7, 128.3, 126.1, 118.9, 112.2, 109.9, 64.1, 62.3, 40.1, 36.2, 35.3, 35.2, 25.1, 24.9, 14.7; HRESI-MS calcd. for $C_{25}H_{30}N_3O_6$ $[M+H]^+$ 468.2129, found 468.2120.

4.1.27. N^1-(8-Ethoxy-3-nitro-2H-chromen-6-yl)-N^7-phenethylheptanediamide (12b)

Following the same procedures described for **12a**, diamide **12b** was prepared from **5b** (78 mg, 0.21 mmol), phenylethylamine (0.026 mL, 0.21 mmol), HATU (87 mg, 0.21 mmol), and triethylamine (0.06 mL, 0.44 mmol), in 62% yield (60 mg), as an orange-red solid, mp. 180–182 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 7.99 (s, 1H), 7.84 (s, 1H), 7.38 (s, 1H), 7.28 (s, 1H), 7.25 (t, J = 7.4 Hz, 2H), 7.16 (d, J = 7.3 Hz, 3H), 5.16 (s, 2H), 3.97 (dd, J = 13.6, 6.7 Hz, 2H), 3.22 (dd, J = 13.1, 6.6 Hz, 2H), 2.65 (t, J = 7.2 Hz, 2H), 2.24 (t, J = 7.1 Hz, 2H), 2.01 (t, J = 7.1 Hz, 2H), 1.56–1.51 (m, 2H), 1.49 – 1.44 (m, 2H), 1.30 (t, J = 6.8 Hz, 3H), 1.24–1.19 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.2, 171.4, 146.8, 140.1, 139.7, 139.5, 134.3, 129.7, 128.8, 128.5, 126.2, 119.0, 112.4, 109.8, 64.3, 62.5, 40.3, 36.4, 35.5, 35.4, 28.5, 25.3, 25.1, 14.8; HRESI-MS calcd. for $C_{26}H_{32}N_3O_6$ $[M+H]^+$ 482.2286, found 482.2278.

4.1.28. N^1-(8-Ethoxy-3-nitro-2H-chromen-6-yl)-N^8-phenethyloctanediamide (12c)

Following the same procedures described for **12a**, diamide **12c** was prepared from **5c** (57 mg, 0.15 mmol), phenylethylamine (0.018 mL, 0.15 mmol), HATU (65 mg, 0.17 mmol), and triethylamine (0.04 mL, 0.29 mmol), in 65% yield (47 mg), as an orange-red solid, mp. 211–212 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.02 (s, 1H), 7.84 (s, 1H), 7.40 (s, 1H), 7.31 (s, 1H), 7.27 (t, J = 7.5 Hz, 2H), 7.19 (d, J = 7.0 Hz, 3H), 5.18 (s, 2H), 4.00 (q, J = 6.8 Hz, 2H), 3.24 (dd, J = 13.1, 6.7 Hz, 2H), 2.68 (t, J = 7.2 Hz, 2H), 2.50 (s, 3H), 2.26 (s, 2H), 2.02 (t, J = 7.2 Hz, 2H), 1.55 (dd, J = 14.1, 7.0 Hz, 2H), 1.47–1.44 (m, 2H), 1.33 (t, J = 6.9 Hz, 3H), 1.27–1.22 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.0, 171.2, 146.6, 139.9, 139.6, 139.3, 134.1, 129.5, 128.6, 128.3, 126.0, 118.9, 112.3, 109.6, 64.1, 62.3, 40.1, 36.3, 35.4, 35.2, 28.5, 28.4, 25.2, 25.0, 14.7; HRESI-MS calcd. for $C_{27}H_{34}N_3O_6$ $[M+H]^+$ 496.2442, found 496.2442.

4.1.29. Synthesis of tert-butyl (4-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-4-oxobutyl)carbamate (13a)

To a solution of **4** (100 mg, 0.30 mmol) in DCM (1.6 mL) was added TFA (0.4 mL). After stirring at room temperature for 2 h, the mixture was concentrated under reduced pressure. The resulting residue was dissolved in anhydrous DMF (2 mL), 5-(*tert*-butoxycarbonylamino)pentanoic acid (71 mg,

0.33 mmol), HATU (125 mg, 0.33 mmol) and triethylamine (0.17 mL, 1.2 mmol) were added. After stirring for 12 h, the mixture was poured into 40 mL water. The precipitate was filtered and dried to give 120 mg of **13a** (92%) as a yellow solid, mp. 199–200 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.66 (s, 1H), 7.35 (d, J = 1.3 Hz, 1H), 6.99 (s, 1H), 5.22 (s, 2H), 4.65 (s, 1H), 4.06 (q, J = 7.0 Hz, 2H), 3.15 (dd, J = 12.3, 6.0 Hz, 2H), 2.36 (t, J = 7.5 Hz, 2H), 1.77–1.72 (m, 2H), 1.56–1.53 (m, 3H), 1.41 (t, 3H), 1.40 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.6, 156.7, 147.5, 140.5, 139.7, 133.2, 129.2, 118.8, 112.5, 110.2, 79.5, 64.9, 63.1, 39.1, 36.6, 29.5, 28.5, 22.5, 14.7; HRESI-MS calcd. for $C_{21}H_{30}N_3O_7$ $[M+H]^+$ 436.2078, found 436.2076.

4.1.30. tert-Butyl (5-((8-ethoxy-3-nitro-2H-chromen-6-yl)amino)-5-oxopentyl)carbamate (13b)

Following the same procedures described for **13a**, intermediate **13b** was prepared from **4** (131 mg, 0.39 mmol), TFA (0.4 mL); 5-(*tert*-butoxycarbonylamino)hexanoic acid (100 mg, 0.43 mmol), HATU (164 mg, 0.43 mmol) and triethylamine (0.22 mL, 1.56 mmol), in 89% yield (155 mg), as an orange-yellow solid, mp. 160–161 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.48 (s, 1H), 7.39 (d, J = 1.9 Hz, 1H), 7.00 (s, 1H), 5.26 (s, 2H), 4.60 (s, 1H), 4.10 (q, J = 7.0 Hz, 2H), 3.13 (d, J = 6.4 Hz, 2H), 2.35 (t, J = 7.5 Hz, 2H), 1.76–1.73 (m, 2H), 1.55–1.51 (m, 2H), 1.46 (t, J = 7.5 Hz, 3H), 1.44 (s, 9H), 1.42–1.36 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 156.2, 147.5, 140.6, 139.7, 133.1, 129.2, 118.8, 112.5, 110.2, 79.3, 65.0, 63.1, 40.1, 37.3, 29.6, 28.4, 26.1, 24.9, 14.7; HRESI-MS calcd. for $C_{22}H_{32}N_3O_7$ $[M+H]^+$ 450.2235, found 450.2227.

4.1.31. Synthesis of N-(8-ethoxy-3-nitro-2H-chromen-6-yl)-6-(2-phenylacetamido)hexanamide (14)

To a solution of **13b** (100 mg, 0.22 mmol) in CH_2Cl_2 (1.6 mL) was added TFA (0.4 mL). After stirring at room temperature for 2 h, the mixture was concentrated under reduced pressure. The resulting residue was dissolved in anhydrous DMF (1.5 mL). Phenylacetic acid (36 mg, 0.27 mmol), anhydrous triethylamine (0.16 mL, 1.11 mmol) and HATU (101 mg, 0.27 mmol) were added to the mixture. After stirring for 8 h, the reaction mixture was poured into 20 mL water. The precipitate was filtered and dried. Recrystallization with CH_2Cl_2 and hexane (1/2) provided 99 mg of **14** as an orange-red solid, yield 95%, mp. 220–222 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.01 (s, 2H), 7.39 (d, J = 1.5 Hz, 1H), 7.28 (d, J = 1.6 Hz, 1H), 7.26 (t, J = 7.4 Hz, 2H), 7.22 (d, J = 7.1 Hz, 2H), 7.18 (t, J = 7.1 Hz, 1H), 5.17 (s, 2H), 3.98 (q, J = 6.9 Hz, 2H), 3.35 (s, 2H), 3.02 (dd, J = 12.7, 6.5 Hz, 2H), 2.24 (t, J = 7.3 Hz, 2H), 1.57–1.52 (m, 2H), 1.42–1.37 (m, 2H), 1.31 (t, J = 6.9 Hz, 3H), 1.28–1.23 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 169.9, 146.6, 139.9, 139.3, 136.6, 134.1,

129.5, 128.9, 128.2, 126.3, 118.9, 112.3, 109.6, 64.1, 42.5, 38.5, 36.2, 28.9, 26.1, 24.8, 14.7; HRESI-MS calcd. for $C_{25}H_{30}N_3O_6$ $[M+H]^+$ 468.2129, found 468.2121.

4.1.32. N-(8-Ethoxy-3-nitro-2H-chromen-6-yl)-6-(3-phenylpropanamido)hexanamide (15)

Following the same procedures described for **14**, phenylpropanamide **15** was prepared from **13b** (100 mg, 0.22 mmol), TFA (0.4 mL); 3-phenylpropanoic acid (40 mg, 0.27 mmol), HATU (101 mg, 0.27 mmol) and triethylamine (0.16 mL, 1.11 mmol), in 93% yield (100 mg), as an orange-yellow solid, mp. 189–190 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.00 (s, 1H), 7.77 (t, J = 5.3 Hz, 1H), 7.38 (d, J = 1.9 Hz, 1H), 7.28 (d, J = 1.9 Hz, 1H), 7.24 (t, J = 7.5 Hz, 2H), 7.16–7.13 (m, 3H), 5.16 (s, 2H), 3.98 (q, J = 6.9 Hz, 2H), 3.00 (dd, J = 12.8, 6.6 Hz, 2H), 2.77 (t, J = 7.8 Hz, 2H), 2.32 (t, J = 7.8 Hz, 2H), 2.24 (t, J = 7.3 Hz, 2H), 1.56–1.51 (m, 2H), 1.38–1.34 (m, 2H), 1.31 (t, J = 6.9 Hz, 3H), 1.24–1.19 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.2, 171.1, 146.6, 141.4, 139.9, 139.3, 134.1, 129.5, 128.3, 128.2, 125.9, 118.9, 112.3, 109.6, 64.1, 62.3, 38.3, 37.1, 36.2, 31.2, 29.0, 26.1, 24.8, 14.7; HRESI-MS calcd. for $C_{26}H_{32}N_3O_6$ $[M+H]^+$ 482.2286, found 482.2278.

4.1.33. Synthesis of

(R)-5-(2-amino-3-phenylpropanamido)-N-(8-ethoxy-3-nitro-2H-chromen-6-yl)pentanamide (16a)

To a solution of **13a** (100 mg, 0.23 mmol) in DCM (1.6 mL) was added TFA (0.4 mL). After stirring for 2 h at room temperature, the mixture was concentrated under reduced pressure. The resulting residue was dissolved in anhydrous DMF (1.5 mL). D-Boc-Phe (74 mg, 0.28 mmol), triethylamine (0.15 mL, 1.11 mmoland HATU (106 mg, 0.28 mmol) were added to the mixture. After stirring for 8 h at room temperature, the resulting mixture was poured into 20 mL water. After filtration, the precipitate was dried and then dissolved in a 20% TFA in CH_2Cl_2 (2.0 mL). After stirring for 2 h, the reaction mixture was diluted with CH_2Cl_2. The organic phase was washed with saturation $NaHCO_3$, distilled water, brine, and dried over Na_2SO_4, filtered and concentrated under reduced pressure. Recrystallization with DCM and hexane (1/2) afforded 40 mg of **16a** as an orange-yellow solid, yield 51%, mp 191–192 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 8.29 (t, J = 5.6 Hz, 1H), 8.08 (s, 2H), 8.00 (s, 1H), 7.39 (s, 1H), 7.31–7.28 (m, 3H), 7.22 (t, J = 7.4 Hz, 1H), 7.18 (s, 2H), 5.17 (s, 2H), 3.97 (q, J = 7.0 Hz, 2H), 3.87 (t, J = 7.2 Hz, 1H), 3.12 (td, J = 13.2, 6.6 Hz, 1H), 3.00–2.92 (m, 3H), 2.23 (t, J = 7.3 Hz, 2H), 1.47–1.41 (m, 2H), 1.34–1.26 (m, 5H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.0, 167.7, 146.6, 140.0, 139.3, 135.0, 134.1, 129.4, 128.5, 127.1, 118.9, 116.4, 112.2, 109.5, 64.1, 62.4, 53.7, 38.4, 37.2, 35.8, 28.3, 22.4, 14.7; HRESI-MS calcd. for $C_{25}H_{31}N_4O_6$ $[M+H]^+$ 483.2238,

found 483.2236.

4.1.34. (R)-6-(2-Amino-3-phenylpropanamido)-N-(8-ethoxy-3-nitro-2H-chromen-6-yl)hexanamide (16b)

Following the same procedures described for **16a**, 6-hexanamide **16b** was prepared from **13b** (100 mg, 0.22 mmol), 20% TFA in CH_2Cl_2 (2.0 mL); D-Boc-Phe (72 mg, 0.27 mmol), HATU (101 mg, 0.27 mmol) and triethylamine (0.15 mL, 1.11 mmol); 20% TFA in CH_2Cl_2 (2.0 mL), in 48% yield (37 mg), as an orange-yellow solid, mp. 113–115 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.18 (t, J = 5.4 Hz, 1H), 7.98 (s, 1H), 7.38 (d, J = 2.1 Hz, 1H), 7.32–7.30 (m, 3H), 7.23 (t, J = 7.3 Hz, 1H), 7.18 (d, J = 7.2 Hz, 2H), 5.16 (s, 2H), 3.97 (q, J = 6.9 Hz, 2H), 3.77 (t, J = 7.0 Hz, 1H), 3.15–3.09 (m, 1H), 2.96–2.93 (m, 2H), 2.91–2.87 (m, 1H), 2.23 (t, J = 7.3 Hz, 2H), 1.55–1.50 (m, 2H), 1.31–1.28 (m, 5H), 1.19–1.14 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 168.6, 146.6, 139.9, 139.3, 135.6, 134.1, 129.5, 129.4, 128.5, 127.0, 118.9, 112.2, 109.6, 64.1, 62.3, 54.1, 38.5, 37.8, 36.2, 28.6, 26.0 24.7, 14.7; HRESI-MS calcd. for $C_{26}H_{33}N_4O_6$ $[M+H]^+$ 497.2395, found 497.2387.

4.2. In vitro antiproliferative assay

The 8-ethoxy-3-nitro-*2H*-chromene derivatives were subjected to in *vitro* growth inhibitory assay against A549, MCF-7, K562, HeLa and PC3 cells by conventional MTT method [23]. The values of GI_{50}, the effective concentration at which 50% of the tumor cells were inhibited, were calculated by the GraphPad Prism 5 software. Tumor cells were seeded in 96-well plates at density of 4×10^3 cells per well. Following the overnight adherence, cells were incubated with medium alone or with the 8-ethoxy-3-nitro-*2H*-chromene derivatives or the reference drugs at the indicated concentrations for 48 h at 37 °C. Cells were treated with the MTT solution (final concentration, 0.5 mg/mL) for 4 h at 37 °C. The supernatants were removed carefully, followed by the addition of 100 μL DMSO to each well to dissolve the precipitate. The absorbance was measured at 570 nm on a microplate reader. Triplicate experiments were performed.

4.3. In vitro HDACs inhibition assay

In vitro HDACs inhibition assays were conducted as previously described [24]. In brief, 10 μL of enzyme solution (HDAC1, HDAC2, or HDAC6) was mixed with different concentrations of 8-ethoxy-3-nitro-*2H*-chromene derivatives (50 μL), positive drug control SAHA and MS275, using 100% and none HDACs groups as control group, and the mixture. After incubation at 37 °C for 10 min, fluorogenic substrate Ac-Leu-GlyLys(Ac)-AMC (40 μL) was added and then the mixture was

incubated at 37 °C for 30 min. The reaction was quenched with 100 μL of stop buffer containing trypsin and TSA. After incubation at 37 °C for 20 min, fluorescence intensity was measured using a microplate reader at excitation and emission wavelengths of 390 and 460 nm, respectively. The inhibition ratios were calculated from the fluorescence intensity readings of tested wells relative to those of control wells. In the IC_{50} determination, each compound was tested at 8 concentrations starting from 10 μM with 3-fold-dilution in singlet. The IC_{50} values were calculated using a regression analysis of the concentration/inhibition data.

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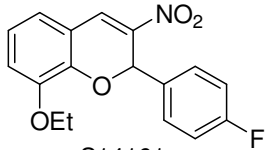
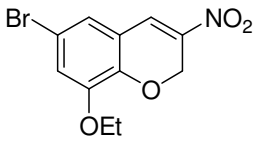
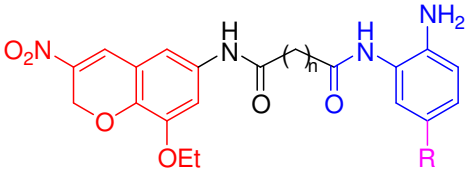


S14161

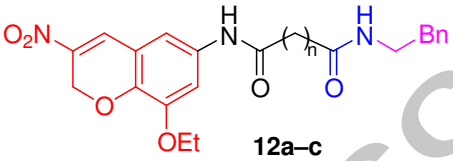


BENC-511

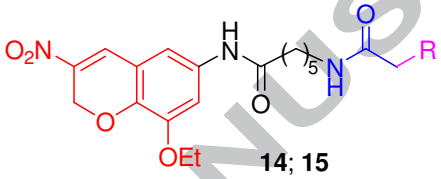
Cap group
Linker
Zinc binding group (ZBG)
Internal cavity motif



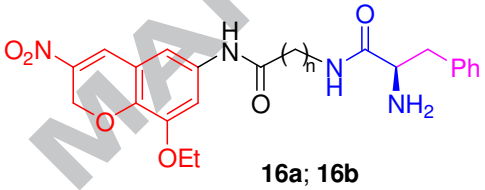
9a–c; **10a–c**; **11a–c**



12a–c



14; **15**



16a; **16b**