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Design, synthesis and preliminary activity assay of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives as novel Histone deacetylases (HDACs) inhibitors

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ABSTRACT

Histone deacetylases (HDACs) are enzymes involved in tumor genesis and development. Herein, we report a novel series of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives as HDACs inhibitors. The preliminary biological screening showed that most of our compounds exhibited potent inhibitory activity against HDACs. Within this series, five compounds, **13a** (IC_{50} = 0.58 ± 0.10 μM), **7d** (IC_{50} = 1.00 ± 0.16 μM), **8l** (IC_{50} = 1.06 ± 0.14 μM), **7i** (IC_{50} = 1.17 ± 0.19 μM) and **7a** (IC_{50} = 1.29 ± 0.15 μM) possessed better HDACs inhibitory activity than Vorinostat (IC_{50} = 1.48 ± 0.20 μM). So these five compounds could be used as novel lead compounds for further design of HDACs inhibitors. The anti-proliferative activities of a few compounds and the structure–activity relationships are also briefly discussed.

1. Introduction

HDACs belong to a family of enzymes that remove the acetyl group from the ε-amide groups of lysine residues located on the nucleosomal histones. The hydrolysis of the acetyl group results in the consequence that the positive charge density on the N-termini of nucleosomal histones increases, which strengthens the interaction with the negatively charged DNA and blocks the access of transcription factors.[1–3] In this way, HDACs involve in the remodeling of chromatin and down-regulate many genes expression. In addition, deacetylation of nucleosomal histones plays roles in the other genome function, including chromatin assembly, DNA repair and recombination.[4,5] Recently, more and more non-histone proteins, such as transcription factors, cytoskeletal proteins, molecular chaperones and nuclear import factors, have been revealed as the HDACs substrates.[6] HDACs are divided into four distinct classes. Class I, II and IV HDACs are zinc-dependent metalloproteinase, whereas class III HDACs (sirtuins) have a unique mechanism of deacetylation dependent on the NAD^+ cofactor.[7]

Because of the multiple functions of HDACs in gene expression and other important cell processes, dysregulation of HDACs will cause many diseases, of which cancer is the most dreadful. It has been shown that global hypoacetylation of histone H4 is a common hallmark of cancer, and changes in H4 acetylation happen early in the tumorigenesis.[8] Many experiments revealed that overexpression of class I and II HDACs, especially class I isozymes, is associated with many kinds of tumors.[9–16]

Up to now, many kinds of HDAC inhibitors (HDACi) have been studied for their therapeutic effects on cancers,[17] including hydroxamates, cyclic peptides, aliphatic acids and benzamides.[18] Suberoylanilide hydroxamic acid (SAHA, Vorinostat) (Fig. 1), the first HDACi approved by the FDA in 2006 for treatment of cutaneous T-cell lymphoma, validated HDACi as a strategy for cancer therapy.[19,20]

Figure 1 also showed the common pharmacophore of HDACi, which includes three parts, the Zn^{2+} binding group, linker and surface recognition domain. In the design of HDACi, much attention has been paid to the 'linker' optimization. One effective strategy is replacing the flexile carbochain linker with conformationally restricted structures. Four clinical trial HDAC inhibitors containing rigid aromatic rings or heterocyclic rings in the 'linker' are represented in Figure 1. Encouraged by these successful compounds, we focused our attention on searching for other rigid active structures as the 'linker' of HDACi. Based on our literatures investigation, we found that Tic, short for 1,2,3,4-tetrahydroiso-

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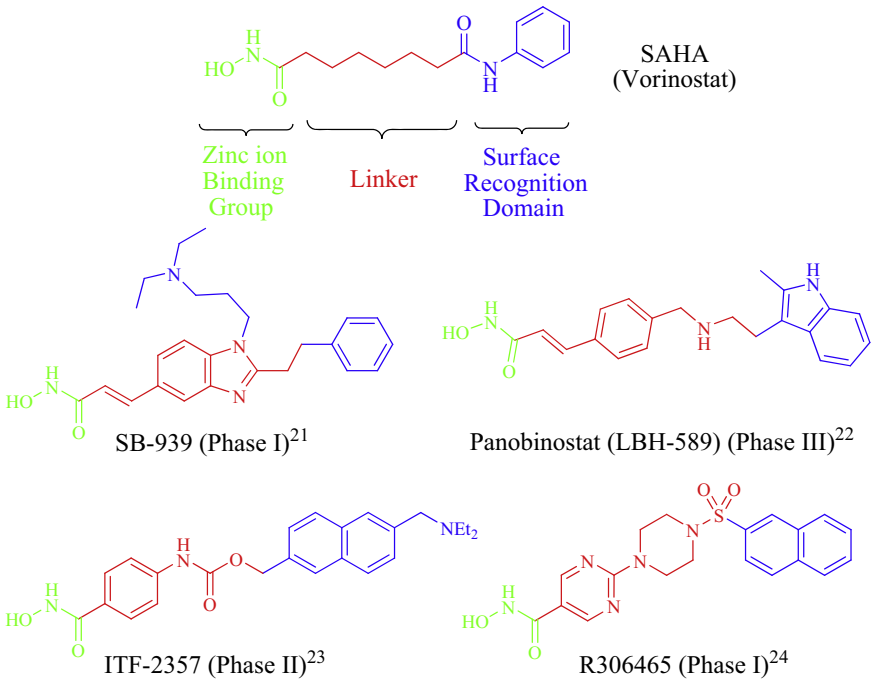


Figure 1. The structures of SAHA and four clinical trial HDACi containing rigid aromatic ring or heterocyclic ring in the 'linker'. The three parts of HDACi pharmacophore are indicated in three different colors, respectively.[21–24]

quinoline-3-carboxylic acid, is a kind of non-protein α-amino acids with distinct geometrical conformation and biological activity. The structure of Tic has been used in many compounds designs (Fig. 2). The most successful example was substituting the Tic residue for the proline residue of enalapril. This modification led to a new approved angiotensin converting enzyme inhibitor (ACEi), quinapril (Fig. 3).[32] However, there is little research on the structure of Tic in HDACi design. In this paper, we introduce the rigid Tic fragment to the 'linker' of HDACi and design a novel HDACi scaffold, which is shown in Figure 4a. The chirality of our target structure is determined by the chirality of HDACs peptide substrate (Fig. 4b). It should be noted that the structure of our designed Tic derivative is quite consistent with the common pharmacophore of HDACi.

2. Chemistry

The target compounds were synthesized following the procedures described in Schemes 1 and 2. The starting material 3, 5-diiodo-L-tyrosine, compound **1** was converted to compound **2** by Pictet–Spengler cyclization according to the literature.[33] The compound **2** was protected by Boc, followed by catalytic hydrogenolysis to give the key intermediate **4**. The compound **4** was coupled

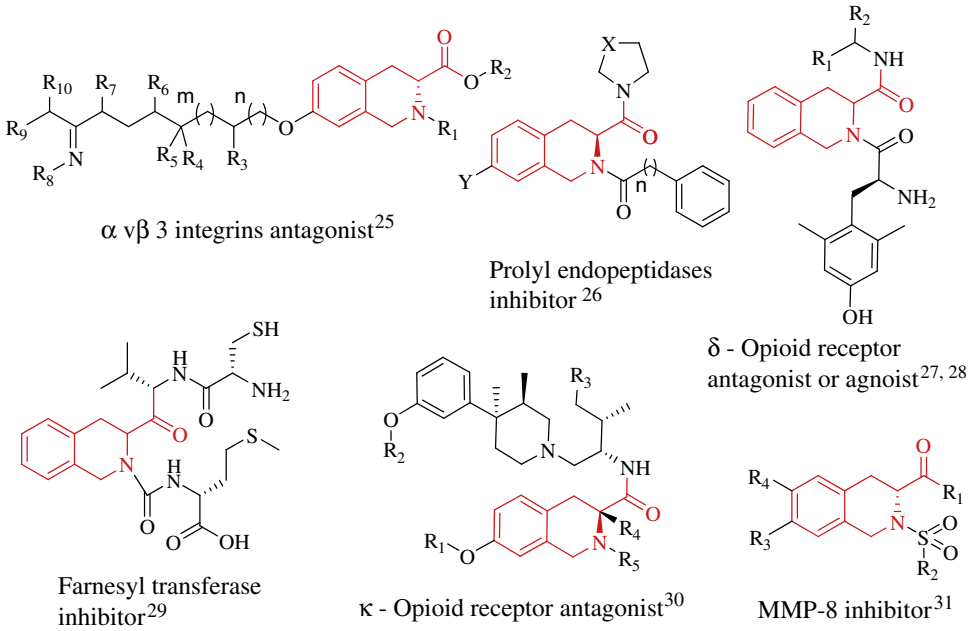


Figure 2. Compounds containing the fragment of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic). The structure of the Tic fragment is indicated in red.[25–31]

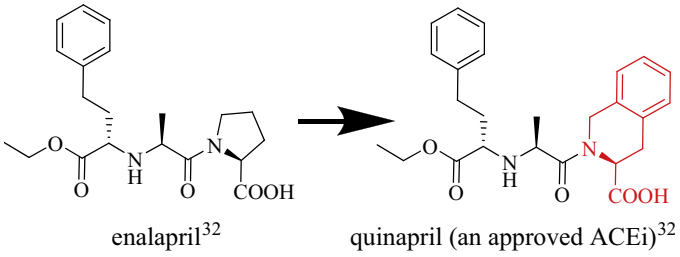


Figure 3. Replacing the proline residue of enalapril led to a new approved drug quinapril.

with a few kinds of aromatic or aliphatic amines by classical DCC/HOBT method to yield **5a–5p**, and then converted to the corresponding **6a–6p**. The ester groups of **6a–6p** were treated with NH_2OK in methanol to get the compounds **7a–7p**. Finally the compounds **8a–8p** were obtained by deprotecting the Boc groups of **7a–7p**.

The compounds **13a–13c** were synthesized according to the methods described in Scheme 2. The hydantoin structures of compounds **13b** and **13c** could be supported by ^1H NMR, HRMS and many literatures.[34–36] We suppose that the cyclization of **13b** and **13c** might result from the nucleophilic attack of the amide N atom to the carbonyl C atom of Cbz group. However, the exact mechanism of hydantoin formation and the reason why **13a** do not form a hydantoin structure need deep investigation.

3. Result and discussion

Considering the available X-ray crystal structure of human HDAC8 and the high homology of all HDACs isoforms, all the target compounds were tested for their inhibitory ability against HDAC8, with Vorinostat (SAHA) being used as positive control.

From the results listed in Table 1, it could be found that most of our target compounds showed comparable inhibitory activity with Vorinostat, and compounds **13a** (IC_{50} = 0.58 ± 0.10 μM), **7d** (IC_{50} = 1.00 ± 0.16 μM), **8l** (IC_{50} = 1.06 ± 0.14 μM), **7i** (IC_{50} = 1.17 ± 0.19 μM) and **7a** (IC_{50} = 1.29 ± 0.15 μM) were even more potent than Vorinostat (IC_{50} = 1.48 ± 0.20 μM). In the 7 series compounds, although two more potent compounds, **7d** and **7i** were obtained by modification of the R1 phenyl group of **7a**, most modification of the R1 phenyl group of **7a** seemed detrimental to the activity, such as, inserting methylene between the phenyl group and amide group (**7b** and **7c**), introducing substituent groups to the phenyl group (**7e–7h**, **7j–7m**) and replacing the phenyl group with aliphatic groups (**7n–7p**). However, this trend was converse in the 8 series compounds. Compared with compound **8a**, other compounds were more potent except **8p**. Generally, compounds with aromatic R_1 groups (**7a–7m**, **8a–8m**) were more potent than compounds with aliphatic R_1 groups (**7n–7p**, **8n–8p**).

Significantly, almost all 7 series compounds except **7j** and **7l** possessed lower IC_{50} value than that of their corresponding 8 series compounds. For example, the IC_{50} of **7a** was 1.29 ± 0.15 μM, much lower than that of **8a** (8.21 ± 1.68 μM). The most notable exception was compound **8l**, which was much more potent than **7l**. We pre-

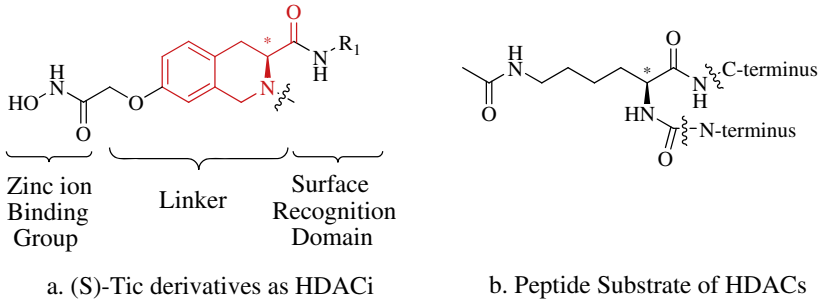
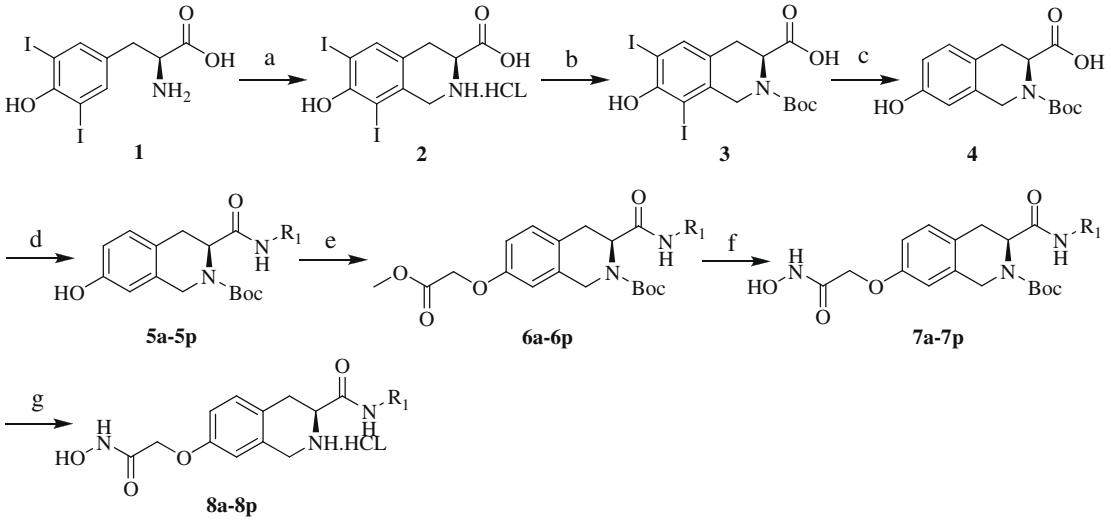
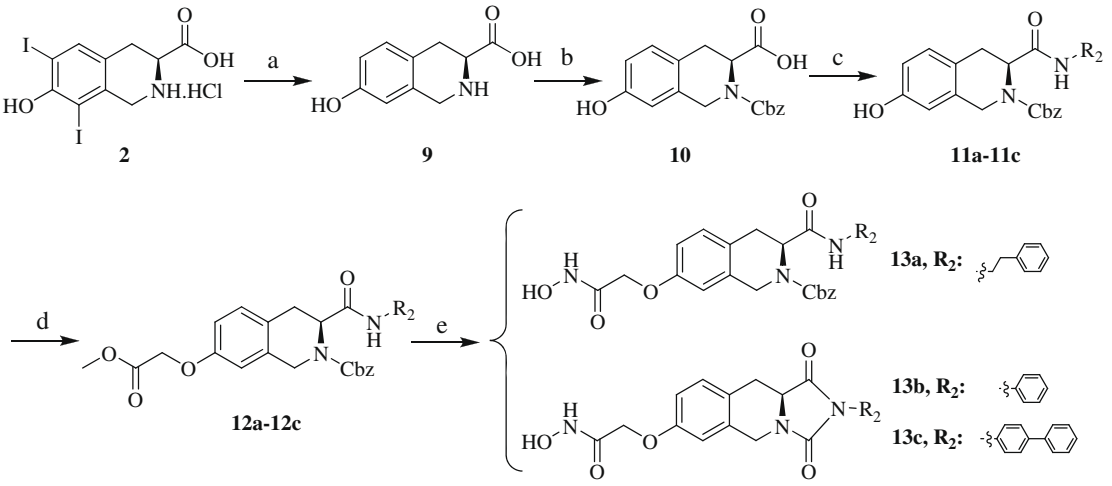


Figure 4. (a) The novel HDACi scaffold containing Tic fragment in the 'linker'. (b) The structure of the HDACs peptide substrate.



Scheme 1. Reagents and conditions: (a) $(CH_2O)_n$, 37% HCl, $CH_3OCH_2CH_2OCH_3$, 72–75 °C, 18 h; (b) $(Boc)_2O$, 1 N NaOH, THF; (c) 10% Pd–C, H_2, Et_3N, MeOH; (d) R_1NH_2, DCC, HOBT, anhydrous THF; (e) $BrCH_2COOHCH_3$, K_2CO_3, anhydrous DMF; (f) NH_2OK, CH_3OH; (g) HCl, anhydrous EtOAc.



Scheme 2. Reagents and conditions: (a) 10% Pd–C, H_2, Et_3N, MeOH, H_2O; (b) Cbz–Cl, 1 M NaOH; (c) R_2NH_2, DCC, HOBT, THF; (d) $BrCH_2COOCH$, K_2CO_3, DMF; (e) NH_2OK, CH_3OH.

sume that the potent activity of **8l** might result from higher affinity between the large fused aromatic naphthalene group of **8l** and the residues of HDAC8 protein. While in compound **7l**, the Boc group with large volume probably restricts the large naphthalene group's free rotation and sufficient interaction with HDAC8 protein, which leads to the decreased activity. In spite of this, the tertiary butyloxycarbonyl group (Boc group) located on the secondary amine atom seemed beneficial to the activity of our compounds. In order to investigate the relationship between the inhibitory activity and the groups located on the secondary amine atom, the benzyloxycarbonyl group (Cbz group) was chose to protect the secondary amine atom as the surrogate of Boc group. As a result, other three compounds were achieved (Scheme 2). Interestingly, under basic condition, Cbz groups of compounds **13b** and **13c** were eliminated to form the hydantoin structures, which could be supported by ^1H NMR, HRMS and many literatures.[34–36] The inhibitory activities of these three compounds are listed in Table 2. The rigid compounds **13b** and **13c** were almost inactive, while **13a** (IC_{50} = 0.58 ± 0.10 μM) exhibited much lower IC_{50} value compared with its corresponding Boc-protecting compound **7c** (IC_{50} = 2.67 ± 0.15 μM). It was remarkable that compound **13a** was even more potent than **7d** (IC_{50} = 1.00 ± 0.16 μM), the most potent compound of all our 7 and 8 series compounds. The result that **13a** possessed the most potent inhibitory activity among all our target compounds further confirmed our hypothesis that the substituent on the secondary amine atom was very crucial to the activity and perhaps more important than the R_1 group of these derivatives.

Encouraged by the potent anti-HDACs activities of compounds **7d**, **7i**, **8l** and **13a**, we then evaluated their anti-proliferative activities against HCT116, SKOV3 and HL60 cell lines using MTT assay. As can be seen in Figure 5, for each compound, the order of its IC_{50} values against these three tumor cell lines was HCT116 < SKOV3 < HL60, which was due to the HDACs expression levels in these three cell lines (HCT116 > SKOV3 > HL60). These results indirectly provided proof that the observed anti-proliferative effect on the cells was through inhibition of HDACs. The activities against all the three cell lines of compounds **7d**, **7i** and **8l** were better than that of SAHA. Unexpectedly, the anti-proliferative activity of **13a** was not as excellent as its anti-HDACs enzyme activity. We suppose that the easiness of benzyl ester hydrolysis in **13a** might result in its decreased cell activity.

In order to determine the interaction between our compounds and HDACs, compounds **13a** and **7d** were docked into the active site of HDAC8 (PDB entry: 1T64) using SYBYL 7.3 (Fig. 6). Molecular docking study was carried out via Sybyl/FlexX module. The molecular structures of **13a** and **7d** were generated with Sybyl/Sketch module and optimized using Powell's method with the Tripos force field with convergence criterion set at 0.05 kcal/(Å mol), and assigned with Gasteiger–HÜckel method. The residues in a radius of 8.0 Å around TSA in the co-crystal structure of HDAC8 and TSA (PDB entry: 1T64) were selected as the active site. Other docking parameters implied in the program were kept default. The docking results showed that the carbonyl and hydroxyl oxygens of hydroxamate could chelate with the zinc ion in HDACs. Both the methoxyphenyl group of **7d** and the phenethyl group of **13a** could form π–π interaction with Tyrosine 100 (Y100). This could explain why compounds with aromatic R_1 groups (**7a–7m**, **8a–8m**) were more potent than compounds with aliphatic R_1 groups (**7n–7p**, **8n–8p**). Because the Cbz group of **13a** was longer than the Boc group of **7d**, the Cbz group could insert into pocket X to form stronger hydrophobic interaction. Although the docking results were consistent with our inference and revealed the reason why compound **13a** was more active than **7d**, the exact binding mode of our compounds with HDAC8 should be verified from further X-ray co-crystal studies. In order to find more potent compounds having stronger affinity with pocket X and to validate the docking results, derivatives with diverse N-substituted groups are under research in our lab.

4. Conclusions

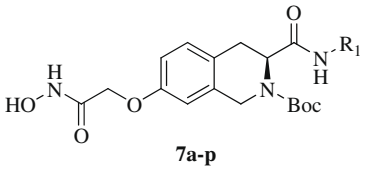
In summary, we developed a novel series of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives as potent HDACs inhibitors. Among them, compounds **13a**, **7d**, **8l**, **7i** and **7a** exhibited better anti-HDACs and anti-proliferative activity than Vorinostat. Therefore, using these compounds as leads, efforts are currently underway in our laboratoty to search new 1,2,3,4-trtrahydroisoquinoline-3-carboxylic acid derivatives as more potent HDACs inhibitors.

5. Experimental section

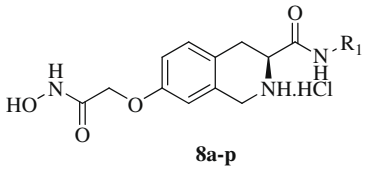
5.1. Chemistry: general procedures

Unless specified otherwise, all starting materials, reagents and solvents were commercially available. All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. All reactions were monitored by thin-

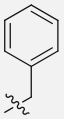
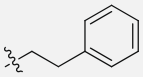
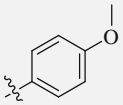
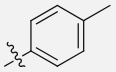
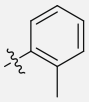
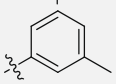
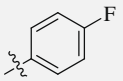
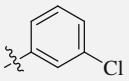
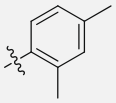
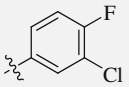
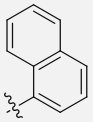
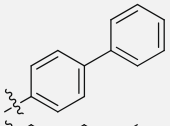
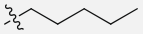
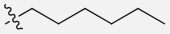
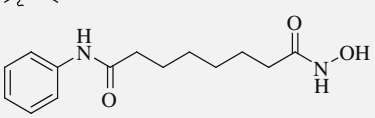
Table 1
The structures and inhibitory activities of compounds **7a–7p**, **8a–8p** against HDAC8



7a-p

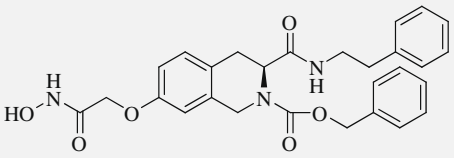
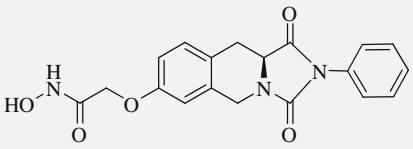
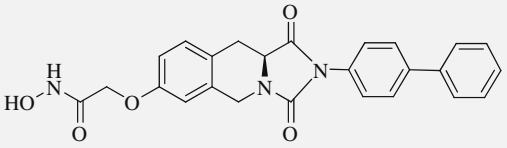
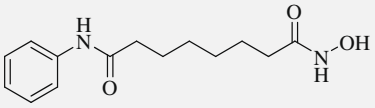


8a-p

Compounds	IC_{50} [a] of HDAC8 (μM)	R_1
7a	1.29 ± 0.15	
8a	8.21 ± 1.68	
7b	3.41 ± 0.21	
8b	5.10 ± 0.49	
7c	2.67 ± 0.15	
8c	4.07 ± 0.61	
7d	1.00 ± 0.16	
8d	5.57 ± 0.44	
7e	1.65 ± 0.18	
8e	3.82 ± 0.29	
7f	4.00 ± 0.38	
8f	4.13 ± 0.51	
7g	1.77 ± 0.25	
8g	3.62 ± 0.37	
7h	2.56 ± 0.33	
8h	3.23 ± 0.25	
7i	1.17 ± 0.19	
8i	3.20 ± 0.41	
7j	3.78 ± 0.66	
8j	3.39 ± 0.0.39	
7k	1.55 ± 0.24	
8k	3.34 ± 0.23	
7l	4.25 ± 0.27	
8l	1.06 ± 0.14	
7m	1.98 ± 0.23	
8m	2.21 ± 0.19	
7n	3.02 ± 0.25	
8n	3.54 ± 0.67	
7o	4.42 ± 0.42	
8o	5.77 ± 1.09	
7p	4.58 ± 0.57	
8p	12.17 ± 1.87	
Vorinostat	1.48 ± 0.20	

[a] Data represent mean values of at least three independent experiments and standard deviations are calculated based on the results of these experiments.

Table 2
The structures and inhibitory activities of compounds **13a**–**13c** against HDAC8

Compounds	Structures	IC_{50} [a] of HDAC8 (μM)
13a		0.58 ± 0.10
13b		>50
13c		>50
Vorinostat		1.48 ± 0.20

[a] Data represent mean values of at least three independent experiments and standard deviations are calculated based on the results of these experiments.

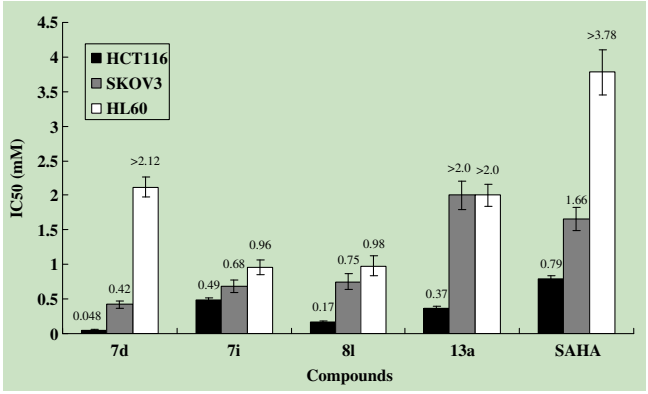


Figure 5. Anti-proliferative activities of the indicated compounds against three tumor cell lines. The columns represent the mean values of five independent experiments.

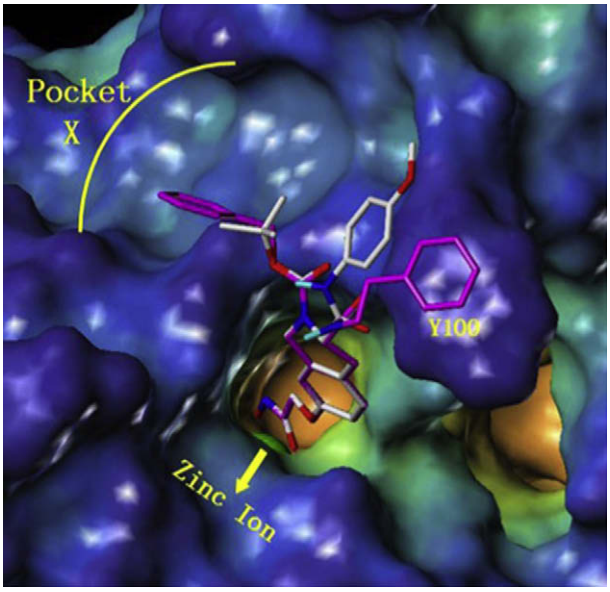


Figure 6. The docking modes of compounds **13a**, **7d** with HDAC8. The protein is represented by molecular surface. Zinc ion is indicated. Y100 stands for the tyrosine 100 residue. The yellow arc indicates the pocket X of the protein. **13a** is depicted in purple and **7d** in white. (atom types: polar H, sky-blue; N, dark blue; O, red).

layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light, chloride ferric or iodine vapor. Proton NMR spectrums were determined on a Brucker DRX spectrometer (600 MHz), δ in parts per million and J in Hertz, using TMS as an internal standard. Measurements were made in DMSO-d_6 solutions. ESI-MS were determined on an API 4000 spectrometer. HRMS spectrums were conducted by Shandong Analysis and Test Center. Melting points were determined on an electrothermal melting point apparatus and were uncorrected.

5.1.1. (*S*)-7-Hydroxy-6,8-diiodo-Tic hydrochloride (2)[33]

A suspension of 3,5-diiodo-L-tyrosine (**1**, 30.0 g, 69.3 mmol) in concentrated HCl (250 mL), 1,2-dimethoxyethane (20 mL) and paraformaldehyde (7.8 g, 260.0 mmol) was stirred vigorously and slowly heated to 72 °C. After 0.5 h, concentrated HCl (50 mL), 1,2-dimethoxyethane (10 mL) and paraformaldehyde (5.2 g, 173.3 mmol) were added and stirring was continued for 18 h at 72–75 °C. The suspension was then cooled in an ice bath and filtered. The filter cake was washed thoroughly with 1,2-dimethoxyethane and dried under vacuum to get 19.41 g of compound **2** as a white powder. Yield: 58%, ESI-MS *m/z*: 446.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 3.07 (dd, J = 16.8 Hz, 10.8 Hz, 1H), 3.22 (dd, J = 16.8 Hz, 4.8 Hz, 1H), 4.02 (d, J = 16.2 Hz, 1H), 4.15 (d, J = 16.2 Hz, 1H), 4.32 (dd, J = 4.8 Hz, 10.8 Hz, 1H), 7.73 (s, 1H), 9.68 (s, 1H), 10.00 (br s, 2H), 14.17 (br s, 1H).

5.1.2. (*S*)-2-(*tert*-Butoxycarbonyl)-7-hydroxy-6,8-diiodo-Tic (3)[33]

To a solution of compound **2** (4.81 g, 10.0 mmol) in 22 mL of 1 N NaOH, was added a solution of $(Boc)_2O$ (2.40 g, 11.0 mmol) in 5 mL THF. The solution was kept between pH 9 and 11 by addition of 1 N NaOH. After stirring the mixture at room temperature for 6 h, the THF was evaporated in vacuum with the residues being adjusted

to pH 4–5 with 1 N aqueous citric acid. Then the mixture was extracted with EtOAc (3 × 15 mL). The extractions were combined, washed with brine (3 × 10 mL), dried over Mg_2SO_4 and evaporated to give 4.99 g of crude product compound **3** as a light yellow powder. This product was used for the following reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.34 + 1.40 (s, 9H, cis/trans), 2.87–3.00 (m, 2H), 4.13–4.41 (m, 2H), 4.61–4.75 (m, 1H), 7.57 (s, 1H), 9.41 (br s, 1H), 12.71 (br s, 1H).

5.1.3. (*S*)-2-(*tert*-Butoxycarbonyl)-7-hydroxy-Tic (4)[33]

To a solution of compound **3** (2.73 g, 5.0 mmol) in 30 mL of anhydrous methanol, were added triethylamine (1.11 g, 11.0 mmol) and 10% Pd/C (0.23 g). After stirring the mixture under hydrogen atmosphere for 5 h, the solution was filtered on Celite, the filtrate concentrated and acidified with 1 N aqueous citric acid until pH 4–5 then extracted with EtOAc (3 × 20 mL). The organic layers were combined, washed with brine (3 × 20 mL), dried over Mg_2SO_4 and evaporated to give 1.10 g of crude product compound **4** as a light yellow powder. This product was used for the following reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.39 + 1.45 (s, 9H, cis/trans), 2.92–3.04 (m, 2H), 4.26–4.51 (m, 2H), 4.57–4.82 (m, 1H), 6.52 (s, 1H), 6.57 (d, J = 8.4 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 9.28 (s, 1H), 12.60 (s, 1H).

5.1.4. (*S*)-*tert*-Butyl 7-hydroxy-3-(phenylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5a)

At 0 °C, to a solution of compound **4** (2.93 g, 10.0 mmol) and HOBT (1.49 g, 11.0 mmol) in 40 mL of anhydrous THF, was added a solution of DCC (2.27 g, 11.0 mmol) in 10 mL of anhydrous THF, and after 30 min, the aniline (1.02 g, 11.0 mmol). After stirring the mixture at room temperature over night, THF was evaporated with the residues being taken up in EtOAc (40 mL) and freezed in refrigerator. Then DCU was filtered off, the filtrate washed with saturated Na_2CO_3 (3 × 10 mL), 1 N HCl (3 × 10 mL), brine (3 × 10 mL) and dried over Mg_2SO_4. The solvent was evaporated to give 2.55 g of crude product compound **5a** as a yellow powder. This product was used for the following reaction without further purification. ESI-MS m/z: 369.5 $[M+H]^+$; ^{1}H NMR (DMSO-d_6) δ 1.30 + 1.45 (s, 9H, cis/trans), 2.85–3.11 (m, 2H), 4.27–4.52 (m, 2H), 4.55–4.74 (m, 1H), 6.57–6.64 (m, 2H), 6.96–7.04 (m, 2H), 7.25–7.30 (m, 2H), 7.49–7.56 (m, 2H), 9.29 (s, 1H), 9.96 (s, 1H).

5.1.4.1. (*S*)-*tert*-Butyl 3-(benzylcarbamoyl)-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5b). Compound **5b** was synthesized following the procedure described above.

5.1.4.2. (*S*)-*tert*-Butyl 7-hydroxy-3-(phenethylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5c). Compound **5c** was synthesized following the procedure described in Section 5.1.4.

5.1.4.3. (*S*)-*tert*-Butyl 7-hydroxy-3-(4-methoxyphenylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5d). Compound **5d** was synthesized following the procedure described in Section 5.1.4.

5.1.4.4. (*S*)-*tert*-Butyl 7-hydroxy-3-(*p*-tolylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5e). Compound **5e** was synthesized following the procedure described in Section 5.1.4.

5.1.4.5. (*S*)-*tert*-Butyl 7-hydroxy-3-(*o*-tolylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5f). Compound **5f** was synthesized following the procedure described in Section 5.1.4.

5.1.4.6. (*S*)-*tert*-Butyl 7-hydroxy-3-(*m*-tolylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5g). Compound **5g** was synthesized following the procedure described in Section 5.1.4.

5.1.4.7. (*S*)-*tert*-Butyl 3-(4-fluorophenylcarbamoyl)-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5h). Compound **5h** was synthesized following the procedure described in Section 5.1.4.

5.1.4.8. (*S*)-*tert*-Butyl 3-(3-chlorophenylcarbamoyl)-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5i). Compound **5i** was synthesized following the procedure described in Section 5.1.4.

5.1.4.9. (*S*)-*tert*-Butyl 3-(2,4-dimethylphenylcarbamoyl)-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5j). Compound **5j** was synthesized following the procedure described in Section 5.1.4.

5.1.4.10. (*S*)-*tert*-Butyl 3-(3-chloro-4-fluorophenylcarbamoyl)-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5k). Compound **5k** was synthesized following the procedure described in Section 5.1.4.

5.1.4.11. (*S*)-*tert*-Butyl 7-hydroxy-3-(naphthalen-1-ylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5l). Compound **5l** was synthesized following the procedure described in Section 5.1.4.

5.1.4.12. (S)-*tert*-Butyl 3-(biphenyl-4-ylcarbamoyl)-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5m). Compound **5m** was synthesized following the procedure described in Section 5.1.4.

5.1.4.13. (S)-*tert*-Butyl 7-hydroxy-3-(pentylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5n). Compound **5n** was synthesized following the procedure described in Section 5.1.4.

5.1.4.14. (*S*)-*tert*-Butyl 3-(hexylcarbamoyl)-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5o). Compound **5o** was synthesized following the procedure described in Section 5.1.4.

5.1.4.15. (*S*)-*tert*-Butyl 3-(*tert*-butylcarbamoyl)-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (5p). Compound **5p** was synthesized following the procedure described in Section 5.1.4.

5.1.5. (S)-*tert*-Butyl 7-(2-methoxy-2-oxoethoxy)-3-(phenylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6a)

A mixture of **5a** (2.55 g, 6.93 mmol), K_2CO_3 (1.91 g, 13.86 mmol) and methyl bromoacetate (2.12 g, 13.86 mmol) in 40 mL of anhydrous DMF was stirred at room temperature for 3 h. The mixture was poured to 300 mL of H_2O and extracted with EtOAc (3 × 40 mL). The organic layers were combined, washed with brine (3 × 20 mL), dried over Mg_2SO_4 and evaporated under vacuum. The residues were purified by flash column chromatography (petroleum/EtOAc 3:1) to give 1.16 g of desired compound **6a** as colorless oil. Yield: 38%, ESI-MS m/z: 441.6 $[M+H]^+$; ^{1}H NMR (DMSO-d_6) δ 1.33 + 1.47 (s, 9H, cis/trans), 2.93–3.16 (m, 2H), 3.70 (s, 3H), 4.34–4.58 (m, 2H), 4.62–4.88 (m, 1H), 4.78 (s, 2H), 6.72–6.90 (m, 2H), 7.02–7.16 (m, 2H), 7.26–7.31 (m, 2H), 7.49–7.56 (m, 2H), 10.00 (s, 1H).

Compounds **6b–p** were synthesized following the procedure described above.

5.1.5.1. (*S*)-*tert*-Butyl 3-(benzylcarbamoyl)-7-(2-methoxy-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6b). Yield: 38%.

5.1.5.2. (*S*)-*tert* -Butyl 7-(2-methoxy-2-oxoethoxy)-3-(phenethylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6c). Yield: 40%.

5.1.5.3. (*S*)-*tert* -Butyl 7-(2-methoxy-2-oxoethoxy)-3-(4-methoxyphenylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6d). Yield: 36%.

5.1.5.4. (*S*)-*tert*-Butyl 7-(2-methoxy-2-oxoethoxy)-3-(*p*-tolylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6e). Yield: 33%.

5.1.5.5. (*S*)-*tert*-Butyl 7-(2-methoxy-2-oxoethoxy)-3-(*o*-tolylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6f). Yield: 40%.

5.1.5.6. (*S*)-*tert* -Butyl 7-(2-methoxy-2-oxoethoxy)-3-(*m*-tolylcarbamoyl)-3, 4-dihydroisoquinoline-2(1*H*)-carboxylate (6g). Yield: 39%.

5.1.5.7. (*S*)-*tert* -Butyl 3-(4-fluorophenylcarbamoyl)-7-(2-methoxy-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6h). Yield: 34%.

5.1.5.8. (*S*)-*tert*-Butyl 3-(3-chlorophenylcarbamoyl)-7-(2-methoxy-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6i). Yield: 31%.

5.1.5.9. (*S*)-*tert*-Butyl 3-(2,4-dimethylphenylcarbamoyl)-7-(2-methoxy-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6j). Yield: 48%.

5.1.5.10. (*S*)-*tert*-butyl 3-(3-chloro-4-fluorophenylcarbamoyl)-7-(2-methoxy-2-oxoethoxy)-3, 4-dihydroisoquinoline-2(1H)-carboxylate (6k). Yield: 37%.

5.1.5.11. (*S*)-*tert*-Butyl 7-(2-methoxy-2-oxoethoxy)-3-(naphthalen-1-ylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6l). Yield: 29%.

5.1.5.12. (*S*)-*tert*-Butyl 3-(biphenyl-4-ylcarbamoyl)-7-(2-methoxy-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6m). Yield: 31%.

5.1.5.13. (*S*)-*tert* -Butyl 7-(2-methoxy-2-oxoethoxy)-3-(pentylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6n). Yield: 39%.

5.1.5.14. (*S*)-*tert*-Butyl 3-(hexylcarbamoyl)-7-(2-methoxy-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6o). Yield: 41%.

5.1.5.15. (*S*)-*tert*-Butyl 3-(*tert* -butylcarbamoyl)-7-(2-methoxy-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6p). Yield: 38%.

5.1.6. (*S*)-*tert*-Butyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(phenylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7a)

To a solution of compound **6a** (0.88 g, 2.0 mmol) in 10 mL of anhydrous methanol, was added a solution of NH_2OK (0.14 g, 6 mmol) in 3.5 mL of anhydrous methanol. The mixture was stirred for 0.5 h and the solvent was evaporated under vacuum. The residues were acidified with 2 N HCl until pH 3–4 then extracted with EtOAc (3×10 mL). The organic layers were combined, washed with brine (3×10 mL), dried over Mg_2SO_4 and evaporated with the residues being recrystallized with EtOAc/EtOH to give 0.23 g of title compound **7a** as a white powder. Yield: 26%, mp: 179–181 °C. IR (KBr) 3329, 3260, 2976, 2924, 2855, 1699, 1663, 1601 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 + 1.46 (s, 9H, cis/trans), 2.89–3.19 (m, 2H), 4.34–4.65 (m, 2H), 4.44 (s, 2H), 4.57–4.80 (m, 1H), 6.70–6.88 (m, 2H), 7.01–7.17 (m, 2H), 7.24–7.32 (m, 2H), 7.49–7.56 (m, 2H), 8.97 (s, 1H), 10.01 (s, 1H), 10.81 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{23}H_{28}N_3O_6$ $[M+H]^+$ 442.1978, found: 442.1980.

Compounds **7b**–**p** were synthesized following the procedure described above.

5.1.6.1. (*S*)-*tert* -Butyl 3-(benzylcarbamoyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7b). Yield: 22%, mp: 153–156 °C. IR (KBr) 3299, 2976, 2928, 2868, 1670 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33 + 1.47 (s, 9H, cis/trans), 2.95–3.11 (m, 2H), 4.03–4.20 (m, 2H), 4.36–4.53 (m, 2H), 4.40 (s, 2H), 4.56–4.76 (m, 1H), 6.80–6.89 (m, 2H), 6.99–7.11 (m, 2H), 7.15–7.20 (m, 2H), 7.23–7.34 (m, 2H), 8.33 (s, 1H), 9.02 (s, 1H), 10.76 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{24}H_{30}N_3O_6$ $[M+H]^+$ 456.2135, found: 456.2137.

5.1.6.2. (*S*)-*tert* -Butyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(phenethylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7c). Yield: 29%, mp: 155–157 °C. IR (KBr) 3315, 3200, 2978, 2931, 2864, 1664 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.35 + 1.45 (s, 9H, cis/trans), 2.54–2.64 (m, 2H), 2.83–2.95 (m, 2H), 3.13–3.27 (m, 2H), 4.28–4.57 (m, 2H), 4.44 (s, 2H), 4.52–4.76 (m, 1H), 6.76–6.81 (m, 2H), 7.02–7.19 (m, 3H), 7.24–7.28 (m, 2H), 7.92 (s, 1H), 8.97 (s, 1H), 10.81 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{25}H_{31}N_3O_6$-Na $[M+Na]^+$ 492.2111, found: 492.2129.

5.1.6.3. (*S*)-*tert* -Butyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(4-methoxyphenylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7d). Yield: 25%, mp: 167–169 °C. IR (KBr) 3268, 3150, 2978, 2933, 2915, 2854, 2836, 1702, 1671, 1613 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 + 1.46 (s, 9H, cis/trans), 2.89–3.17 (m, 2H), 3.72 (s, 3H), 4.32–4.65 (m, 2H), 4.44 (s, 2H), 4.60–4.80 (m, 1H), 6.75–6.90 (m, 4H), 7.06–7.24 (m, 1H), 7.38–7.47 (m, 2H), 8.98 (s, 1H), 9.86 (s, 1H), 10.82 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{24}H_{30}N_3O_7$ $[M+H]^+$ 472.2084, found: 472.2090.

5.1.6.4. (*S*)-*tert*-Butyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(*p*-tolylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7e). Yield: 27%, mp: 164–166 °C. IR (KBr) 3267, 3138, 2980, 2916, 2859, 1700, 1664, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 + 1.45 (s, 9H, cis/trans), 2.23 (s, 3H), 2.87–3.17 (m, 2H), 4.32–4.65 (m, 2H), 4.44 (s, 2H), 4.56–4.79 (m, 1H), 6.74–6.88 (m, 2H), 7.05–7.17 (m, 3H), 7.35–7.46 (m, 2H), 8.97 (s, 1H), 9.91 (s, 1H), 10.81 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{24}H_{30}N_3O_6$ $[M+H]^+$ 456.2135, found: 456.2138.

5.1.6.5. (*S*)-*tert*-Butyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(*o*-tolylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7f). Yield: 26%, mp: 166–168 °C. IR (KBr) 3252, 2975, 2911, 2859, 1694, 1660, 1616 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.39 + 1.47 (s, 9H, cis/trans), 1.96 + 2.05 (s, 3H, cis/trans), 3.01–3.20 (m, 2H), 4.43 (s, 2H), 4.45–4.55 (m, 2H), 4.60–4.86 (m, 1H), 6.78–6.87 (m, 2H), 7.05–7.19 (m, 5H), 8.97 (s, 1H), 9.28 (s, 1H), 10.81 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{24}H_{30}N_3O_6$ $[M+H]^+$ 456.2135, found: 456.2131.

5.1.6.6. (*S*)-*tert*-Butyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(*m*-tolylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7g). Yield: 33%, mp: 183–184 °C. IR (KBr) 3332, 3150, 2976, 2928, 2865, 1696, 1664, 1612 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 + 1.46 (s,

9H, cis/trans), 2.30 (s, 3H), 2.91–3.15 (m, 2H), 4.35–4.63 (m, 2H), 4.41 (s, 2H), 4.56–4.82 (m, 1H), 6.75–6.88 (m, 3H), 7.09–7.18 (m, 2H), 7.25–7.29 (m, 2H), 8.98 (s, 1H), 9.93 (s, 1H), 10.82 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{24}H_{29}N_3O_6Na$ $[M+Na]^+$ 478.1954, found: 478.1971.

5.1.6.7. (*S*)-*tert* -Butyl 3-(4-fluorophenylcarbamoyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7h). Yield: 28%, mp: 167–169 °C. IR (KBr) 3261, 3164, 2979, 2933, 2917, 2856, 1700, 1663, 1619 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 + 1.46 (s, 9H, cis/trans), 2.91–3.16 (m, 2H), 4.35–4.63 (m, 2H), 4.44 (s, 2H), 4.50–4.77 (m, 1H), 6.76–6.88 (m, 2H), 7.10–7.20 (m, 3H), 7.51–7.58 (m, 2H), 9.07 (s, 1H), 10.64 (s, 1H), 10.76 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{23}H_{27}FN_3O_6$ $[M+H]^+$ 482.1703, found: 482.1733.

5.1.6.8. (*S*)-*tert*-Butyl 3-(3-chlorophenylcarbamoyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7i). Yield: 23%, mp: 182–184 °C. IR (KBr) 3314, 3188, 3114, 2977, 2932, 2918, 1699, 1664, 1620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 + 1.46 (s, 9H, cis/trans), 2.91–3.17 (m, 2H), 4.37–4.57 (m, 2H), 4.44 (s, 2H), 4.60–4.78 (m, 1H), 6.75–6.89 (m, 2H), 7.08–7.17 (m, 2H), 7.30–7.35 (m, 1H), 7.37–7.45 (m, 1H), 7.71–7.76 (m, 1H), 8.97 (s, 1H), 10.23 (s, 1H), 10.82 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{23}H_{26}ClN_3O_6Na$ $[M+Na]^+$ 498.1408, found: 498.1431.

5.1.6.9. (*S*)-*tert*-Butyl 3-(2, 4-dimethylphenylcarbamoyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7j). Yield: 28%, mp: 154–156 °C. IR (KBr) 3247, 3150, 2977, 2919, 2861, 1696, 1666, 1618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.39 + 1.47 (s, 9H, cis/trans), 1.91 + 2.00 (s, 3H, cis/trans), 2.21 (s, 3H), 3.00–3.18 (m, 2H), 4.41–4.54 (m, 2H), 4.44 (s, 2H), 4.57–4.84 (m, 1H), 6.77–6.86 (m, 2H), 6.89–7.04 (m, 3H), 7.11–7.16 (m, 1H), 8.97 (s, 1H), 9.21 (s, 1H), 10.82 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{25}H_{31}N_3O_6Na$ $[M+Na]^+$ 492.2111, found: 492.2118.

5.1.6.10. (*S*)-*tert* -Butyl 3-(3-chloro-4-fluorophenylcarbamoyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7k). Yield: 40%, mp: 163–165 °C. IR (KBr) 3326, 3145, 2979, 2930, 2852, 1694, 1675, 1628 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 + 1.46 (s, 9H, cis/trans), 2.91–3.17 (m, 2H), 4.37–4.62 (m, 2H), 4.44 (s, 2H), 4.54–4.77 (m, 1H), 6.76–6.89 (m, 2H), 7.10–7.16 (m, 1H), 7.32–7.46 (m, 2H), 7.82–7.87 (m, 1H), 8.97 (s, 1H), 10.25 (s, 1H), 10.82 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{23}H_{25}ClFN_3O_6Na$ $[M+Na]^+$ 516.1314, found: 516.1310.

5.1.6.11. (*S*)-*tert* -Butyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(naphthalen-1-ylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7l). Yield: 35%, mp: 142–144 °C. IR (KBr) 3263, 2976, 2930, 2868, 1746, 1677, 1627 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.41 + 1.49 (s, 9H, cis/trans), 3.11–3.28 (m, 2H), 4.41–4.64 (m, 2H), 4.45 (s, 2H), 4.67–4.97 (m, 1H), 6.66–6.91 (m, 2H), 7.18–7.23 (m, 1H), 7.38–7.53 (m, 3H), 7.59–7.52 (m, 3H), 8.05–8.13 (m, 1H), 8.98 (s, 1H), 9.91 (s, 1H), 10.82 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{27}H_{30}N_3O_6$ $[M+H]^+$ 492.2135, found: 492.2131.

5.1.6.12. (*S*)-*tert*-Butyl 3-(biphenyl-4-ylcarbamoyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7m). Yield: 29%, mp: 144–146 °C. IR (KBr) 3428, 3255, 2976, 2923, 2860, 1701, 1662, 1594 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.32 + 1.47 (s, 9H, cis/trans), 2.93–3.19 (m, 2H), 4.37–4.65 (m, 2H), 4.44 (s, 2H), 4.55–4.83 (m, 1H), 6.76–6.90 (m, 2H), 7.11–7.18 (m, 1H), 7.31–7.33 (m, 1H), 7.42–7.45 (m, 2H), 7.60–7.67 (m, 6H), 8.97 (s, 1H), 10.12 (s, 1H), 10.82 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{29}H_{32}N_3O_6$ $[M+H]^+$ 518.2291, found: 518.2294.

5.1.6.13. (*S*)-*tert* -Butyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(pentylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7n). Yield: 25%, mp: 159–161 °C. IR (KBr) 3363, 3235, 2962, 2931, 2861, 1693, 1669, 1647 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.79–0.86 (m, 3H), 1.08–1.14 (m, 2H), 1.17–1.23 (m, 2H), 1.30–1.36 (m, 2H), 1.39 + 1.46 (s, 9H, cis/trans), 2.95–3.05 (m, 4H), 4.31–4.65 (m, 2H), 4.48 (s, 2H), 4.60–4.87 (m, 1H), 6.74 (s, 1H), 6.81 (d, J = 8.4 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 7.90 (s, 1H), 9.07 (s, 1H), 10.69 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{22}H_{34}N_3O_6$ $[M+H]^+$ 435.2369, found: 435.2364.

5.1.6.14. (*S*)-*tert* -Butyl 3-(hexylcarbamoyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7o). Yield: 26%, mp: 105–107 °C. IR (KBr) 3311, 3180, 2957, 2931, 2858, 1690, 1661, 1639 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.81–0.85 (m, 3H), 1.07–1.23 (m, 8H), 1.36 + 1.45 (s, 9H, cis/trans), 2.86–3.01 (m, 4H), 4.31–4.56 (m, 2H), 4.14 (s, 2H), 4.44–4.75 (m, 1H), 6.74 (s, 1H), 6.81 (d, J = 8.4 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 7.75 (s, 1H), 8.97 (s, 1H), 10.81 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{23}H_{36}N_3O_6$ $[M+H]^+$ 450.2604, found: 450.2610.

5.1.6.15. (*S*)-*tert*-Butyl 3-(*tert*-butylcarbamoyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7p). Yield: 28%, mp: 176–178 °C. IR (KBr) 3307, 3239, 2978, 2920, 2854, 2697, 1675, 1640 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.14 + 1.21 (s, 9H, cis/trans), 1.36 + 1.44 (s, 9H, cis/trans), 2.74–3.03 (m, 2H), 4.25–4.57 (m, 2H), 4.45 (s, 2H), 4.19–4.76 (m, 1H), 6.77 (s, 1H), 6.80 (d, J = 8.4 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 7.31 (s, 1H), 8.96 (s, 1H), 10.80 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{21}H_{31}N_3O_6Na$ $[M+Na]^+$ 444.2111, found: 444.2119.

5.1.7. (*S*)-7-(2-(Hydroxyamino)-2-oxoethoxy)-*N*-phenyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8a)

To a solution of compound **7a** (0.15 g, 0.34 mmol) in 5 mL of dry EtOAc, was added a solution of EtOAc (10 mL) saturated by dry HCl gas. The reaction solution was stirred at room temperature for 8 h when the precipitation appeared. The suspension was filtered with the filter being washed with ether to give 0.12 g of desired compound **8a** as a white powder. Yield: 91%, mp: 187–189 °C. IR (KBr) 3192, 3134, 3056, 2981, 2808, 2606, 2489, 1677, 1606 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.98–3.37 (m, 2H), 4.28–4.30 (m, 2H), 4.32–4.37 (m, 1H), 4.45 (s, 2H), 6.89–6.92 (m, 2H), 7.12–7.23 (m, 2H), 7.36–7.41 (m, 2H), 7.65–7.67 (m, 2H), 9.00 (br s, 1H), 9.60 (br s, 1H), 9.81 (br s, 1H), 10.77 (s, 1H), 10.87 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{18}H_{20}N_3O_4$ $[M+H]^+$ 342.1454, found: 342.1459.

Compounds **8b–p** were synthesized following the procedure described above.

5.1.7.1. (*S*)-*N*-Benzyl-7-(2-(hydroxyamino)-2-oxoethoxy)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8b). Yield: 94%, mp: 175–177 °C. IR (KBr) 3216, 3063, 3033, 2926, 2851, 2812, 2604, 2485, 1672, 1617 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.91–3.28 (m, 2H), 4.15–4.27 (m, 2H), 4.31–4.41 (m, 3H), 4.45 (s, 2H), 6.87–6.90 (m, 2H), 7.17–7.20 (m, 1H), 7.28–7.39 (m, 5H), 9.90 (br s, 1H), 9.22 (s, 1H), 9.53 (br s, 1H), 9.74 (br s, 1H), 10.87 (s, 1H); HRMS (AP-ESI) *m/z* calcd for $C_{19}H_{22}N_3O_4$ $[M+H]^+$ 356.1610, found: 356.1680.

5.1.7.2. (*S*)-7-(2-(Hydroxyamino)-2-oxoethoxy)-*N*-phenethyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8c). Yield: 92%, mp: 167–169 °C. IR (KBr) 3222, 3062, 3028, 2933, 2802, 2604, 2484, 1669, 1615 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.75–2.80 (m, 2H), 2.79–3.16 (m, 2H), 3.38–3.49 (m, 2H), 4.06–

4.30 (m, 3H), 4.44 (s, 2H), 6.84 (s, 1H), 6.88 (d, J = 8.4 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.22–7.26 (m, 3H), 7.31–7.34 (m, 2H), 8.70 (s, 1H), 8.99 (br s, 1H), 9.42 (br s, 1H), 9.58 (br s, 1H), 10.86 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{21}H_{26}N_3O_3$ $[M+H]^+$ 370.1767, found: 370.1778.

5.1.7.3. (*S*)-7-(2-(Hydroxyamino)-2-oxoethoxy)-*N*-(4-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8d). Yield: 94%, mp: 176–178 °C. IR (KBr) 3195, 3055, 3006, 2934, 2836, 2606, 2487, 1675, 1614 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.99–3.42 (m, 2H), 3.74 (s, 3H), 4.28–4.30 (m, 2H), 4.36–3.38 (m, 1H), 4.46 (s, 2H), 6.89–6.91 (m, 2H), 6.94–6.96 (m, 2H), 7.20–7.21 (m, 1H), 7.57–7.59 (m, 2H), 8.99 (br s, 1H), 9.60 (br s, 1H), 9.83 (br s, 1H), 10.79 (s, 1H), 10.89 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{19}H_{22}N_3O_5$ $[M+H]^+$ 372.1559, found: 372.1563.

5.1.7.4. (*S*)-7-(2-(Hydroxyamino)-2-oxoethoxy)-*N*-*p*-tolyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8e). Yield: 90%, mp: 182–184 °C. IR (KBr) 3188, 3123, 3038, 2922, 2806, 2604, 2483, 1677, 1612 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.78 (s, 3H), 2.99–3.44 (m, 2H), 4.28–4.38 (m, 3H), 4.46 (s, 2H), 6.85 (s, 1H), 6.89 (d, J = 8.4 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 7.8 Hz, 2H), 7.56 (d, J = 7.8 Hz, 2H), 9.00 (br s, 1H), 9.62 (br s, 1H), 9.89 (br s, 1H), 10.82 (s, 1H), 10.90 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{19}H_{22}N_3O_4$ $[M+H]^+$ 356.1610, found: 356.1661.

5.1.7.5. (*S*)-7-(2-(Hydroxyamino)-2-oxoethoxy)-*N*-*o*-tolyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8f). Yield: 96%, mp: 177–179 °C. IR (KBr) 3187, 3025, 2918, 2800, 2739, 2606, 2489, 1675, 1615 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.25 (s, 3H), 3.05–3.45 (m, 2H), 4.28–4.40 (m, 3H), 4.46 (s, 2H), 6.83–6.92 (m, 2H), 7.17–7.37 (m, 5H), 9.00 (br s, 1H), 9.59 (br s, 1H), 9.78 (br s, 1H), 10.20 (s, 1H), 10.88 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{19}H_{22}N_3O_4$ $[M+H]^+$ 356.1610, found: 356.1652.

5.1.7.6. (*S*)-7-(2-(Hydroxyamino)-2-oxoethoxy)-*N*-*m*-tolyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8g). Yield: 89%, mp: 173–175 °C. IR (KBr) 3235, 3150, 3028, 2920, 2723, 2598, 2477, 1679, 1617 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.31 (s, 3H), 2.99–3.42 (m, 2H), 4.29–4.32 (m, 2H), 4.37–4.39 (m, 1H), 4.45 (s, 2H), 6.87 (s, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.96–6.97 (m, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.25–7.27 (m, 1H), 7.45–7.46 (m, 2H), 8.99 (s, 1H), 9.60 (br s, 1H), 9.71 (br s, 1H), 10.71 (s, 1H), 10.87 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{19}H_{22}N_3O_4$ $[M+H]^+$ 356.1610, found: 356.1668

5.1.7.7. (*S*)-*N*-(4-Fluorophenyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8h). Yield: 91%, mp: 171–173 °C. IR (KBr) 3209, 3152, 3059, 2809, 2605, 2487, 1679, 1620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.00–3.43 (m, 2H), 4.29–4.32 (m, 2H), 4.36–4.39 (m, 1H), 4.45 (s, 2H), 6.89 (s, 1H), 6.90–6.92 (m, 1H), 7.20–7.29 (m, 3H), 7.68–7.70 (m, 2H), 9.04 (s, 1H), 9.63 (br s, 1H), 9.74 (br s, 1H), 10.83 (s, 1H), 10.97 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{18}H_{19}FN_3O_4$ $[M+H]^+$ 360.1360, found: 360.1377.

5.1.7.8. (*S*)-*N*-(3-chlorophenyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8i). Yield: 92%, mp: 193–195 °C. IR (KBr) 3234, 3182, 3057, 2984, 2930, 2604, 2485, 1677, 1618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.00–3.47 (m, 2H), 4.27–4.40 (m, 3H), 4.46 (s, 2H), 6.89 (s, 1H), 6.90–6.91 (m, 1H), 7.19–7.22 (m, 2H), 7.40–7.43 (m, 1H), 7.59–7.61 (m, 1H), 7.88 (s, 1H), 9.00 (br s, 1H), 9.67 (br s, 1H), 9.90 (br s, 1H), 10.90 (s, 1H), 11.36 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{18}H_{19}ClN_3O_4$ $[M+H]^+$ 376.1064, found: 376.1078.

5.1.7.9. (*S*)-*N*-(2,4-Dimethylphenyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8j). Yield: 88%, mp: 163–165 °C. IR (KBr) 3443, 3190, 3022, 2975, 2921, 2601, 2484, 1673, 1616 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.19 (s, 3H), 2.27 (s, 3H), 2.99–3.42 (m, 2H), 4.22–4.25 (m, 2H), 4.30–4.33 (m, 1H), 4.45 (s, 2H), 6.85 (s, 1H), 6.88–6.89 (m, 1H), 7.02–7.03 (m, 1H), 7.08 (s, 1H), 7.20–7.33 (m, 2H), 8.99 (s, 1H), 9.60 (br s, 1H), 9.71 (br s, 1H), 9.98 (s, 1H), 10.87 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{20}H_{24}N_3O_4$ $[M+H]^+$ 370.1767, found: 370.1770.

5.1.7.10. (*S*)-*N*-(3-Chloro-4-fluorophenyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8k). Yield: 90%, mp: 173–175 °C. IR (KBr) 3196, 3135, 3047, 2980, 2935, 2607, 2486, 1681, 1615 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.00–3.47 (m, 2H), 4.27–4.40 (m, 3H), 4.46 (s, 2H), 6.85 (s, 1H), 6.88–6.89 (m, 1H), 7.17–7.20 (m, 1H), 7.46–7.47 (m, 1H), 7.64–7.66 (m, 1H), 7.99–8.00 (m, 1H), 9.00 (br s, 1H), 9.67 (br s, 1H), 9.91 (br s, 1H), 10.90 (s, 1H), 11.50 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{18}H_{18}ClFN_3O_4$ $[M+H]^+$ 394.0970, found: 394.0987.

5.1.7.11. (*S*)-7-(2-(Hydroxyamino)-2-oxoethoxy)-N-(naphthalen-1-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8l). Yield: 85%, mp: 230–232 °C. IR (KBr) 3422, 3176, 3050, 3013, 2981, 2611, 2489, 1738, 1672 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.15–3.59 (m, 2H), 4.15–4.35 (m, 3H), 4.47 (s, 2H), 6.91 (s, 1H), 6.94 (d, J = 8.4 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.56–7.62 (m, 3H), 7.68–7.71 (m, 1H), 7.87–7.89 (m, 1H), 7.99–8.01 (m, 1H), 8.14–8.16 (m, 1H), 9.01 (br s, 1H), 9.66 (br s, 1H), 9.80 (br s, 1H), 10.78 (s, 1H), 10.89 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{22}H_{22}N_3O_4$ $[M+H]^+$ 392.1610, found: 392.1629.

5.1.7.12. (*S*)-*N*-(Biphenyl-4-yl)-7-(2-(hydroxyamino)-2-oxoethoxy)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8m). Yield: 87%, mp: 178–180 °C. IR (KBr) 3240, 3179, 3104, 3032, 2977, 2601, 2486, 1677, 1607 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.02–3.47 (m, 2H), 4.32–4.42 (m, 3H), 4.46 (s, 2H), 6.90 (s, 1H), 6.91 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 8.4 Hz, 1H), 7.34–7.37 (m, 1H), 7.46 (d, J = 7.8 Hz, 2H), 7.67 (d, J = 7.8 Hz, 2H), 7.71 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.4 Hz, 2H), 9.00 (br s, 1H), 9.64 (br s, 1H), 9.82 (br s, 1H), 10.89 (s, 1H), 11.04 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{24}H_{24}N_3O_4$ $[M+H]^+$ 418.1767, found: 418.1759.

5.1.7.13. (*S*)-7-(2-(Hydroxyamino)-2-oxoethoxy)-*N*-pentyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8n). Yield: 91%, mp: 168–170 °C. IR (KBr) 3234, 3076, 2955, 2930, 2861, 2598, 2482, 1670, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.88 (t, J = 6.6 Hz, 3H), 1.24–1.30 (m, 4H), 1.47 (q, J = 6.6 Hz, 2H), 2.87–3.25 (m, 2H), 3.16 (t, J = 6.0 Hz, 2H), 4.09–4.32 (m, 3H), 4.44 (s, 2H), 6.81 (s, 1H), 6.77 (d, J = 8.4 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 8.69 (s, 1H), 9.00 (br s, 1H), 9.44 (br s, 1H), 9.68 (br s, 1H), 10.88 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{17}H_{26}N_3O_4$ $[M+H]^+$ 336.1923, found: 336.1932.

5.1.7.14. (*S*)-*N*-hexyl-7-(2-(hydroxyamino)-2-oxoethoxy)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8o). Yield: 95%, mp: 173–175 °C. IR (KBr) 3230, 3074, 2955, 2930, 2857, 2601, 2484, 1669, 1618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.88 (t, J = 6.6 Hz, 3H), 1.29–1.43 (m, 6H), 1.46 (q, J = 6.6 Hz, 2H), 2.87–3.25 (m, 2H), 3.13–3.19 (m, 2H), 4.07–4.32 (m, 3H), 4.44 (s, 2H), 6.86 (s, 1H), 6.88 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 8.66 (s, 1H), 9.00 (br s, 1H), 9.45 (br s, 1H), 9.67 (br s, 1H), 10.89 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{18}H_{28}N_3O_4$ $[M+H]^+$ 350.2080, found: 350.2075.

5.1.7.15. (*S*)-*N*-*tert* -Butyl-7-(2-(hydroxyamino)-2-oxoethoxy)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (8p). Yield: 93%, mp: 179–181 °C. IR (KBr) 3466, 3234, 3069,

2973, 2932, 2615, 2491, 1671, 1618 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33 (s, 9H), 2.83–3.25 (m, 2H), 3.97–3.99 (m, 1H), 4.21 (d, J = 15.6 Hz, 1H), 4.30 (d, J = 15.6 Hz, 1H), 4.44 (s, 2H), 6.85 (s, 1H), 6.87 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 8.25 (s, 1H), 8.99 (s, 1H), 9.36 (br s, 1H), 9.62 (br s, 1H), 10.88 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{16}H_{24}N_3O_4$ $[M+H]^+$ 322.1767, found: 322.1756.

5.1.8. (*S*)-7-Hydroxy-Tic (9)[33]

A solution of compound **2** (4.45 g, 9.24 mmol) in EtOH (150 mL) and H_2O (50 mL) containing triethylamine (3.08 g, 30.5 mmol) was hydrogenated in the presence of 10% Pd/C (0.55 g) for 4 h. The catalyst was filtered off with the solution being evaporated until crystals started to appear. The pH was adjusted to 6 with 2 N HCl. After cooling overnight in the refrigerator, the crystals were filtered, washed with cold water and dried to give 1.23 g of desired compound **9**. This product was used for the following reaction without further purification. Mp: 253–255 °C. ^1H NMR (DMSO-d_6) δ 2.79 (dd, J = 16.8 Hz, 10.8 Hz, 1H), 3.03 (dd, J = 16.8 Hz, 4.8 Hz, 1H), 3.32 (br s, 1H), 3.47 (dd, J = 4.8 Hz, 10.8 Hz, 1H), 4.04–4.09 (m, 2H), 6.57 (s, 1H), 6.64 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 8.92 (br s, 1H), 9.47 (br s, 1H).

5.1.9. (*S*)-2-(Benzyloxycarbonyl)-7-hydroxy-Tic (10)[37]

At 0 °C, to a solution of compound **9** (1.23 g, 6.38 mmol) in 7 mL of 1 N NaOH, was added Cbz–Cl (1.20 g, 7.01 mmol) dropwise. The solution was kept between pH 9–11 by addition of 1 N NaOH. After stirring the mixture for 3 h, the solution was adjusted to pH 4–5 with 1 N HCl. Then the mixture was extracted with EtOAc (3 × 10 mL). The extractions were combined, washed with brine (3 × 10 mL), dried over Mg_2SO_4 and evaporated to give 2.00 g of crude product compound **10** as light yellow oil. This product was used for the following reaction without further purification. ^1H NMR (DMSO-d_6) δ 3.00–3.07 (m, 2H), 4.38–4.65 (m, 2H), 4.84–4.85 (m, 1H), 5.10–5.19 (m, 2H), 6.59–6.63 (m, 2H), 6.98–7.00 (m, 1H), 7.30–7.43 (m, 5H), 9.33 (br s, 1H), 12.65 (br s, 1H).

Compounds **11a–c** were synthesized according to the same procedure as compound **5a**.

5.1.10. (*S*)-Benzyl 7-hydroxy-3-(phenethylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11a)

Yield: 67%, ESI-MS m/z: 431.2 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 3.04–3.22 (m, 2H), 4.65–4.90 (m, 3H), 5.04–5.18 (m, 2H), 6.75–6.79 (m, 1H), 6.89–6.94 (m, 1H), 7.02–7.06 (m, 1H), 7.11–7.54 (m, 10H), 9.33 (br s, 1H), 10.06 (s, 1H).

5.1.10.1. (*S*)-Benzyl 7-hydroxy-3-(phenylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11b). Yield: 69%.

5.1.10.2. (*S*)-Benzyl 3-(biphenyl-4-ylcarbamoyl)-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11c). Yield: 71%.

Compounds **12a–c** were synthesized according to the same procedure as compound **6a**.

5.1.11. (*S*)-Benzyl 7-(2-methoxy-2-oxoethoxy)-3-(phenethylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (12a)

Yield: 37%, ESI-MS m/z: 503.3 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ 3.03–3.21 (m, 2H), 3.69 (s, 3H), 4.64–4.89 (m, 3H), 4.76 (s, 2H), 5.04–5.18 (m, 2H), 6.74–6.78 (m, 1H), 6.87–6.92 (m, 1H), 7.01–7.05 (m, 1H), 7.10–7.53 (m, 10H), 10.06 (s, 1H).

5.1.11.1. (*S*)-Benzyl 7-(2-methoxy-2-oxoethoxy)-3-(phenylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (12b). Yield: 39%.

5.1.11.2. (*S*)-Benzyl 3-(biphenyl-4-ylcarbamoyl)-7-(2-methoxy-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (12c). Yield: 40%.

Compounds **13a–c** were synthesized according to the same procedure as compound **7a**.

5.1.12. (*S*)-Benzyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(phenethylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (13a)

Yield: 23%, mp: 151–153 °C. IR (KBr) 3264, 3233, 3027, 2929, 2857, 1671, 1649 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.53–2.57 (m, 2H), 2.93–3.01 (m, 2H), 3.15–3.18 (m, 2H), 4.41 (s, 2H), 4.51–4.72 (m, 3H), 5.06–5.19 (m, 2H), 6.78–6.83 (m, 2H), 7.03–7.08 (m, 2H), 7.16–7.44 (m, 9H), 8.01 (s, 1H), 8.96 (s, 1H), 10.81 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{28}H_{30}N_3O_6$ $[M+H]^+$ 504.2135, found: 504.2125.

5.1.12.1. (*S*)-Benzyl 7-(2-(hydroxyamino)-2-oxoethoxy)-3-(phenylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (13b). Yield: 25%, mp: 145–147 °C. IR (KBr) 3050, 2933, 1768, 1709, 1617 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.98–3.18 (m, 2H), 4.40–4.52 (m, 2H), 4.45 (s, 2H), 4.87–4.90 (m, 1H), 6.86–6.93 (m, 2H), 7.22–7.23 (m, 1H), 7.40–7.51 (m, 5H), 8.97 (s, 1H), 10.83 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{19}H_{18}N_3O_5$ $[M+H]^+$ 368.1246, found: 368.1249.

5.1.12.2. (*S*)-Benzyl 3-(biphenyl-4-ylcarbamoyl)-7-(2-(hydroxyamino)-2-oxoethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (13c). Yield: 20%, mp: 187–189 °C. IR (KBr) 3466, 1772, 1718, 1614 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.01–3.19 (m, 2H), 4.42–4.45 (m, 2H), 4.67 (s, 2H), 4.89–4.92 (m, 1H), 6.82–6.92 (m, 2H), 7.21–7.24 (m, 1H), 7.39–7.41 (m, 1H), 7.49–7.52 (m, 4H), 7.71–7.80 (m, 4H), 8.97 (s, 1H), 10.83 (s, 1H); HRMS (AP-ESI) m/z calcd for $C_{25}H_{22}N_3O_5$ $[M+H]^+$ 444.1553, found: 444.1559.

5.2. Biological materials and methods

5.2.1. In vitro HDAC8 assay

IC_{50} values against HDAC8 were determined in 15 mM Tris–HCl, pH 8.0, at 37 °C, using Boc-Lys (acetyl)-AMC as substrate and HDAC8 expressed in *Escherichia coli* as the enzyme. The HDAC8 was expressed and purified essentially as the protocol described in the supporting materials and methods of Ref. 38. The compound samples and the positive control drug were diluted to various concentrations. Firstly, on the 96-well plate, HDAC8 was incubated at 37 °C with compound sample for 5 min, and then the substrate was added. The HDAC8 reaction was allowed to proceed for 30 min, during which Boc-Lys (acetyl)-AMC could be hydrolyzed to Boc-Lys-AMC. Secondly, trypsin solution was added to transform Boc-Lys-AMC to AMC (4-amino-7-methylcoumarin) for another 20 min. Finally, fluorescence intensity was measured using a microplate reader (ex: 390 nm and em: 460 nm). The inhibition ratios were calculated from the fluorescence intensity readings of inhibited wells relative to those of control wells, and the IC_{50} values were determined using a regression analysis of the concentration/inhibition data.

5.2.2. MTT Assay

HCT116, SKOV3 and HL60 cells were respectively grown in RPMI1640 medium containing 10% FBS at 37 °C in 5% CO_2 humidified incubator. Cell proliferation was determined by the MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazolium bromide) assay. Briefly, cells were plated in a 96-well plate at 10,000 cells per well, cultured for 4 h in complete growth medium, then treated with 2000, 400, 80, 16, 3.2, 0.64 μg/mL of compounds for 48 h. 0.5% MTT solution was added to each well. After further incubation for 4 h, formazan formed from MTT was extracted by adding 200 μL DMSO and mixing for 15 min. Optical density was read with an ELISA reader at 570 nm.

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