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Design, synthesis and biological evaluation of novel 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives as aminopeptidase N/CD13 inhibitors

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ABSTRACT

A series of novel 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives were designed, synthesized and assayed for their activities against aminopeptidase N (APN/CD13) and MMP-2. The results showed that most compounds exhibited higher inhibitory activities against APN than that of MMP-2. Within this series, compound **12h** (IC_{50} = 6.28 ± 0.11 μM) showed similar inhibitory activities compared with Bestatin (IC_{50} = 5.55 ± 0.01 μM), and it could be used as novel lead compound for the future APN inhibitors development as anticancer agents.

1. Introduction

Aminopeptidase N (APN; EC 3.4.11.2) is a 150 kDa membrane-bond zinc-dependent type II ectopeptidase, and belongs to the M1 family of MA clan of peptidase.[1,2] APN can preferentially release neutral or basic amino acids from the N-terminal of unsubstituted oligopeptides, amides and arylamide, with the exception of peptides with Pro in the penultimate position.[3] APN is widely distributed in most of mammalian tissues such as kidney,[4] intestine,[5] liver,[6] lung,[7] brain,[8] fibroblasts,[9] vessels of smooth muscle,[10] haematopoietic system,[11] and so on. The widely distribution of APN associated with its broad substrate specificity suggests that APN has probably several physiological functions, such as protein modification, activation, and degradation as well as in the metabolism of biologically active peptides in tumor metastasis and leukemia.[12,13] So the design and synthesis of APN inhibitors may result in potential therapeutic agents.

To date, many natural or synthetic small molecule APN inhibitors have been reported, such as amastatin, bestatin, probestin, phebestin, AHPA-Val and so on.[14–16] Among these inhibitors, bestatin is the only one being used in clinical as anti-cancer agent. Bestatin is a dipeptide immunomodulator which was first isolated from the culture filtrate of *Streptomyces olivoreticuli* in 1976,[17] and now is widely used as the positive control in the search of small molecule APN inhibitors.

Our group has reported some novel APN inhibitors such as 3-amino-2-hydroxy-4-phenyl butanoic acid (AHPA) derivatives,[18–20] chloramphenicol amine derivatives,[21] 3-phenylpropane-1,2-diamine derivatives,[22,23] L-lysine derivatives,[24] L-arginine derivatives,[25,26] 1,3,4-thiadiazole derivatives[27] and so on. In our previous work, we have reported a series of novel APN inhibitors with cyclic-imide scaffold, and found that compound **13f** showed the best APN inhibitory activity with the IC_{50} value to 1.8 μM.[28] In order to find better APN inhibitors, we modified **13f** by replacing the hydroxamate group with different amino acid benzyl ester, amino acid or organic amine, but the result was not satisfied.[29] The 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) is a constrained analogue of phenylalanine with distinct geometrical conformation and biological activity, has been used for many compounds design. And our group has successfully used it in the design of Histone deacetylases (HDACs) inhibitors.[30,31] There is a phenylalanine residue in the structure of **13f**, considering the conformation constrained of Tic may be beneficial for APN inhibitors, we introduced Tic for the modification of **13f**. Furthermore, we also used Tic as the key scaffold, by coupling it with natural or unnatural amino acids or bi-peptides, we got a series of novel APN inhibitors with 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid scaffold (Fig. 1).

2. Chemistry

The target compound **9** was synthesized following the procedures as shown in Scheme 1. The key intermediate compound **3**

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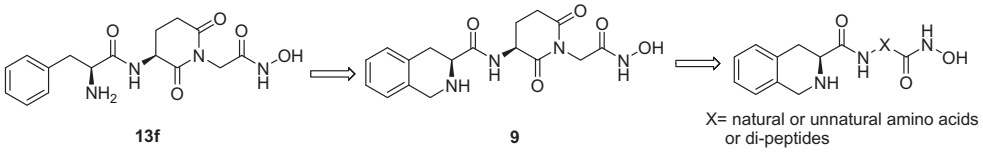
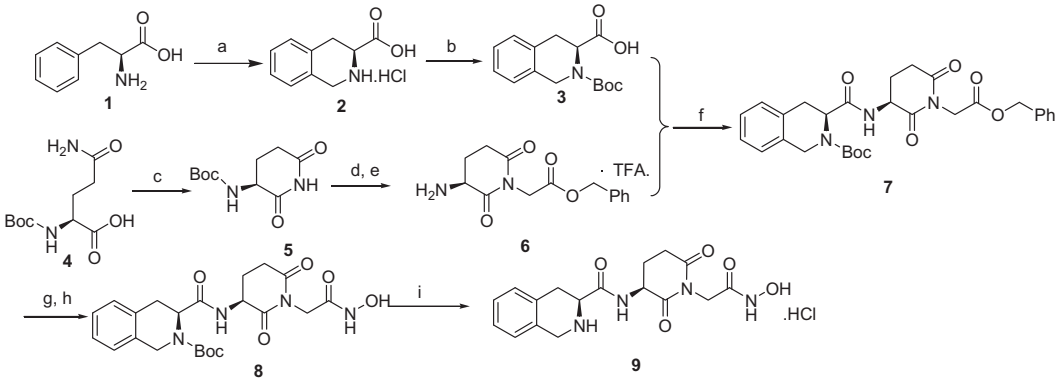
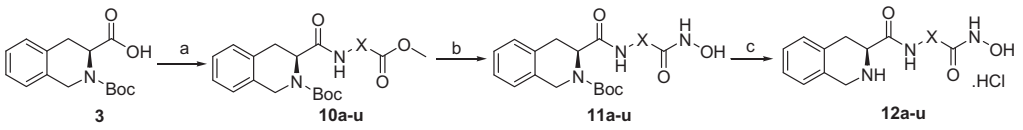


Figure 1. The strategy for the design of APN inhibitors with 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid scaffold.



Scheme 1. Reagents and conditions: (a) HCHO, HCl (concd), 90 °C; (b) NaOH, $(Boc)_2O$; (c) DCC, HOSu, THF, 65 °C; (d) K_2CO_3, KI, acetone, $BrCH_2COOCH_2Ph$, 56 °C; (e) TFA, DCM; (f) isobutyl chloroformate, NMM, THF, −20 °C; (g) 10%Pb/C, H_2, EtOH; (h) isobutyl chloroformate, NMM, $H_2NOH{\cdot}HCl$, THF, −20 °C; (i) 3 N HCl–EtOAc.



Scheme 2. Reagents and conditions: (a) EDCI, HOBT, DCM, NH_2-X-$COOCH_3{\cdot}HCl$; (b) NH_2OK, CH_3OH; (c) 3 N HCl–EtOAc.

was easily prepared from L-Phe **1** via Pictet–Spengler cyclization and amidation according to the literature.[32] Compound **6** was prepared as our early report.[28] Then coupled compound **3** with compound **6** by mixed anhydride method to give compound **7**, which was then catalytically hydrogenated using 10% Pd/C to afford compound **8**. Coupled compound **8** with hydroxylamine hydrochloride and then deprotected the Boc group to give the target compound **9**.

Compounds **12a**–**12u** were synthesized according to the methods described in Scheme 2. Compound **3** coupled with different methyl ester of amino acids or bi-peptides by classical EDCI/HOBt method to give compounds **10a**–**10u**. The ester groups of **10a**–**10u** can be easily converted to hydroximic acid groups by treated **10a**–**10u** with NH_2OK in methanol to get the compounds **11a**–**11u**. Finally the compounds **12a**–**12u** were obtained by deprotecting the Boc groups of **11a**–**11u** in 3 N HCl–EtOAc.

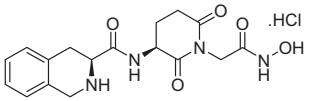
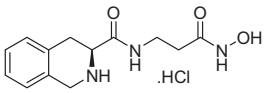
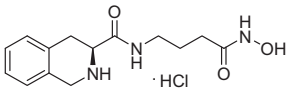
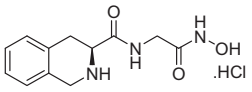
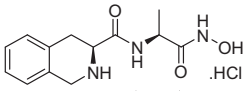
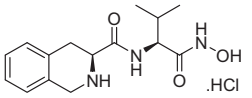
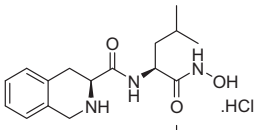
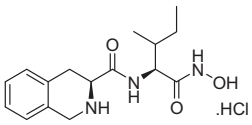
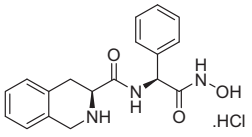
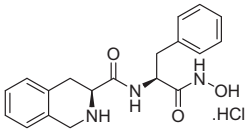
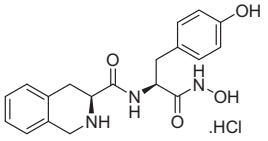
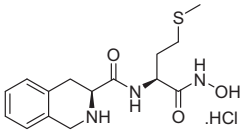
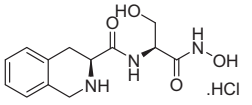
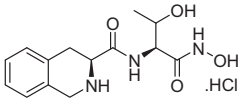
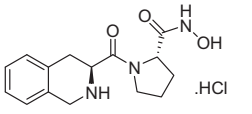
3. Results and discussion

The newly synthesized 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives were assayed for the enzymatic inhibitions both on APN from porcine kidney (Microsomal, Sigma) and MMP-2. The results are listed in Table 1. MMP-2 is also a zinc-dependent metalloproteinase and associated with the process of tumor invasion and metastasis. The difference between them is that APN is an exopeptidase while MMP-2 is an endopeptidase. In order to observe the selectivity, all the target compounds were assayed for the inhibitory activities both on APN and MMP-2 with bestatin as the positive control.

As shown in Table 1, most compounds exhibited higher inhibitory activities against APN than that of MMP-2, which to some extent validating our strategy for designing APN special inhibitors. This may due to the structure difference between APN and MMP-2 may lead to different requirements for their respective inhibitors. APN is a membrane-bound zinc exopeptidase, which catalyzed the removal of NH_2-terminal amino acid from the peptide, while MMP-2 is an endopeptidase which could cut the peptide to parts from the specific amino acid residue of the peptide. As these 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives exhibited highly selective inhibition against APN, the following structure–activity relationships (SARs) were mainly focused on the APN/CD13 inhibition.

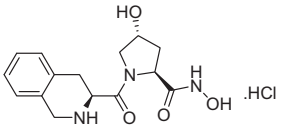
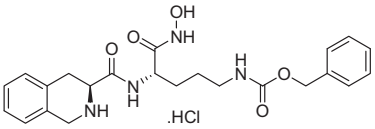
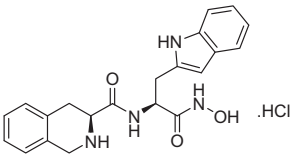
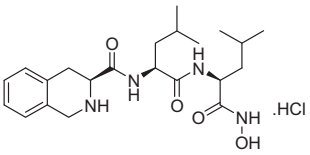
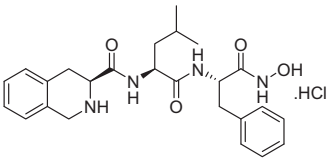
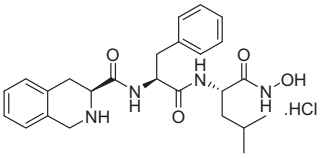
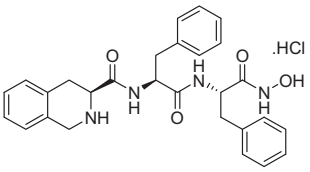
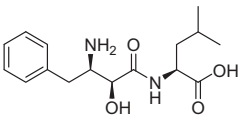
Compound **9** did not show expected activity towards APN, which may indicated that the replacement of the Phe residue of **13f** with Tic could not improve the affinity. This may due to the cyclization of the amino group with the phenyl group could constraint the freedom of the amino group (which may be important for APN interaction). In order to study whether the other substitution could improve the activity, we replaced the cyclic-imide scaffold of compound **9** with other natural or unnatural amino acids or dipeptides to get compounds **12a**–**12u**. Of these compounds, compound **12h** with phenylglyine residue, gave the best inhibitory activity with the IC_{50} value to 6.28 ± 0.11 μM. Comparing compounds **12a**, **12b** and **12c**, we can see that the length of X group was negatively relative with the APN inhibition. And from compounds **12d**, **12e**, **12f** and **12h**, we can see that the APN inhibitory activity is associated with the volume of hydrophobic side chain of the amino acid, compounds with bigger volume hydrophobic side chain of amino acid had better APN inhibitory activities. This may imply that there is a space requirement in the binding pocket to accommodate the suitable substituent. Comparing compounds

Table 1
The structures and in vitro APN inhibitory activities of the target compounds and positive control bestatin

Compd	Structure	IC_{50}[a](μM)	
		APN	MMP-2
9		20.38 ± 0.79	NA[b]
12a		37.08 ± 0.52	516.83 ± 3.49
12b		44.50 ± 0.27	251.91 ± 0.01
12c		21.68 ± 3.84	249.24 ± 0.12
12d		83.85 ± 3.12	463.38 ± 0.92
12e		36.58 ± 0.28	307.27 ± 0.96
12f		27.52 ± 0.04	836.49 ± 3.84
12g		NA[b]	671.56 ± 2.69
12h		6.28 ± 0.11	393.14 ± 0.32
12i		12.82 ± 0.16	939.57 ± 3.27
12j		9.39 ± 0.19	540.03 ± 3.51
12k		8.77 ± 0.17	166.11 ± 0.04
12l		40.19 ± 0.08	267.73 ± 0.31
12m		46.55 ± 1.09	200.63 ± 0.73
12n		104.41 ± 2.72	157.42 ± 0.79

(*continued on next page*)

Table 1 (*continued*)

Compd	Structure	IC_{50}[a](μM)	
		APN	MMP-2
12o		NA[b]	276.44 ± 1.93
12p		15.87 ± 0.11	NA[b]
12q		43.44 ± 0.04	NA[b]
12r		480.72 ± 7.86	NA[b]
12s		465.28 ± 1.05	656.77 ± 3.37
12t		555.15 ± 3.01	NA[b]
12u		435.28 ± 0.25	NA[b]
Bestatin		5.55 ± 0.01	163.48 ± 1.13

[a] Values are means of three experiments, standard derivation is given.
[b] NA, No activity.

12h and **12i**, there is only one carbon difference, compound **12h** showed better APN activity, this may indicate that the phenylglycine residue would be more suitable for APN interaction. Compound **12j** with a hydroxyl group in the *para*-position of the phenyl group showed better activity than that of compound **12i**, this may due to the hydrogen bond formation between the hydroxyl group and APN could enhance the interaction. Compound **12k** with the methionine residue also showed better APN inhibitory activity among these compounds. However, the pseudotripeptides **12r**–**12u** with di-peptide substituents showed very poor APN activity, this may due to that the large side chain interfered the interaction of the hydroxamic acid group with the zinc ion in the active site of APN.

In order to investigate the interaction of the target compounds with APN, the most active compound **12h** was constructed with a 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 docking study performed using Sybyl7.3/FlexX module, the residues in a radius of 7.0 Å around Bestatin in the co-crystal structure (PDB code: 2DQM) were selected as the active site. Other docking parameters implied in the program were kept default. As diagrammed in Figure 2, the backbone of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid can insert into the S_2' pocket, the hydroxamic acid group of **12h** can interact with the zinc ion in the active site of APN, and the phenyl group of L-phenylglycine moiety can insert into the S_1 pocket. For a further and detail understanding of the binding mode of **12h** with APN, a 2D picture was also created with the program LIGPLOT (Fig. 3), we can see that the phenyl group of L-phenylglycine moiety could form hydrophobic interaction with Tyr^{376},

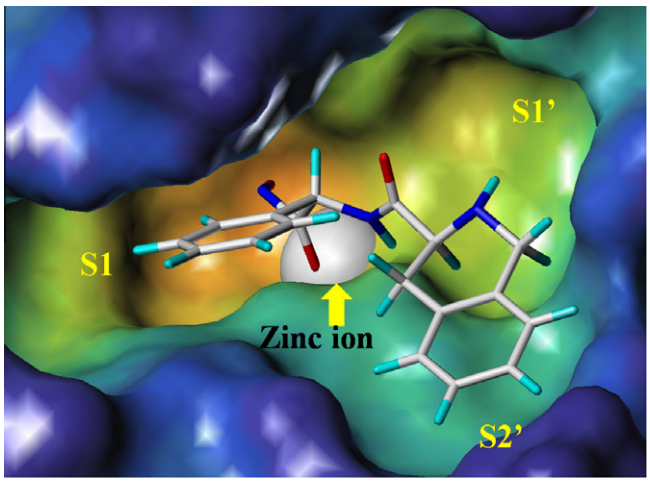


Figure 2. The FlexX docking result of **12h** with the active site of APN (PDB: 2DQM). Zinc ion is shown as pale sphere.

Met[260] and Tyr[381] of the S_1 pocket the two oxygen atoms of hydroxamic acid could chelate the zinc ion of APN. The carbonyl group could form hydrogen bond with His[297] at a distance of 3.06 Å, and the hydroxyl group could form hydrogen bond with Glu[264], Glu[298] and His[301] at the distance of 2.20, 2.25 and 2.94 Å, respectively. The phenyl group of the Tic moiety could form hydrophobic interaction with the S'_2 pocket that formed by Leu[378], Thr[377], and Glu[382]. The other compounds had less activities than compound **12h**, which may due to the different binding energy or interaction modes with APN. In order to verify the assumption, the docking study of the representative compound **12j** was also investigated by using the same method as compound **12h**. As is shown in Figure 4, we can see that in contrast to compound **12h**, the backbone 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid of **12j** is insert into the S_1 pocket, though the hydroxamic acid group can interact well with the zinc ion in the active site of APN, the Tyr residue cannot occupy the S1' pocket, the inhibitory activity of **12j** is less than that of **12h**.

Although the computed information partially supported our assumption, the exact binding mode of the 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives with APN should be obtained from further X-ray crystal studies.

In order to study the inhibitory activity of the target compounds towards human APN, we also tested the enzymatic inhibitory activity of compound **12h** towards APN expressed on the surface of human ovary clear cell carcinoma cell ES-2. The results showed that, just like bestatin, compound **12h** is also a dose-dependent APN inhibitor in the concentration range of 0.39–400 μM with the IC_{50} value to 32.69 ± 1.17 μM which is similar with that bestatin (IC_{50} = 29.67 ± 0.11 μM) (Fig. 5).

In addition, the effects of compound **12h** on the proliferation of two human tumor cell lines (ES-2 cells and MDA-MB-231cells) compared with bestatin were further assessed by using MTT assay (Fig. 6). The results showed that just like bestatin, the anti-proliferative effect of **12h** against ES-2 cells is better than that of MDA-MB-231cells, which may due to the higher APN expression on ES-2 cells than that of MDA-MB-231 cells. Additionally, consistent with the results of enzyme inhibition, compound **12h** also showed slightly lower anti-proliferative effect on ES-2 cells than that of

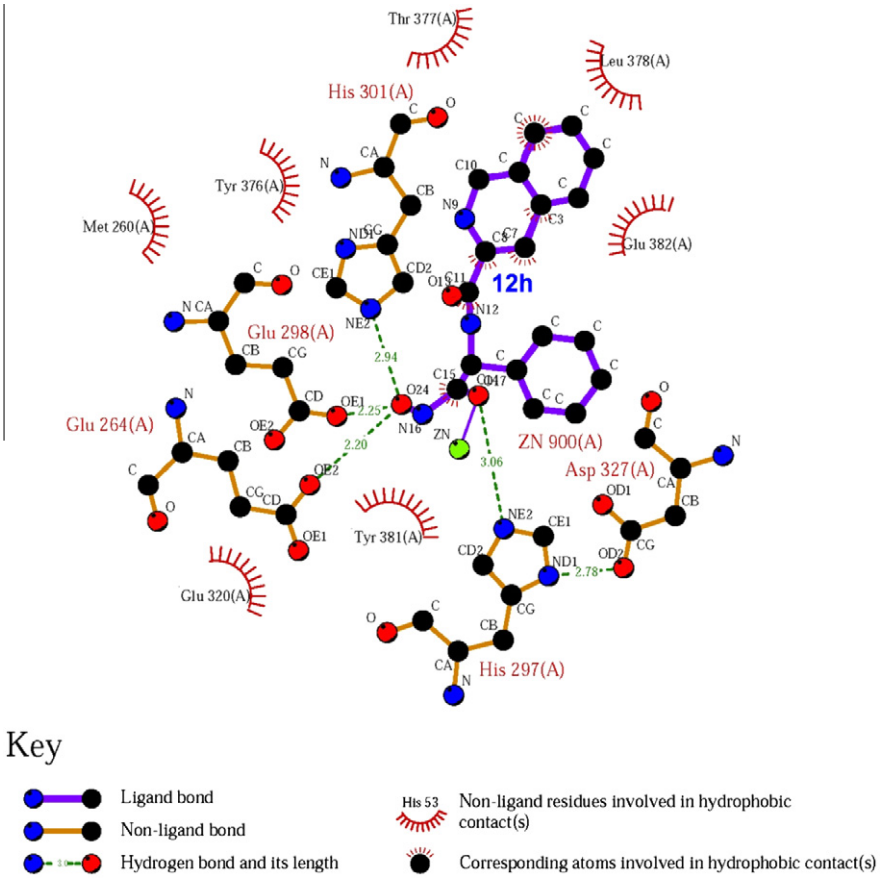


Figure 3. The docking result of **12h** with APN showed by LIGPLOT. Compound **12h** is shown in violet.

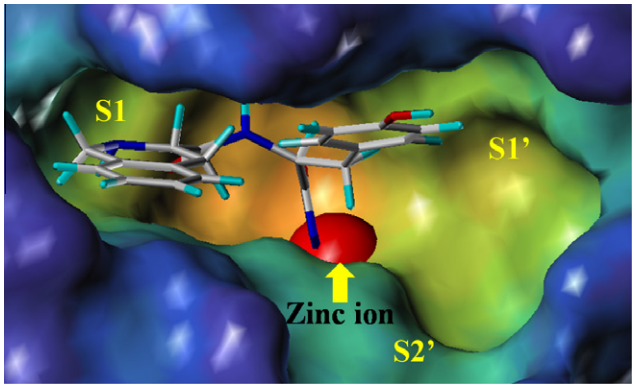


Figure 4. The FlexX docking result of **12j** with the active site of APN (PDB: 2DQM).

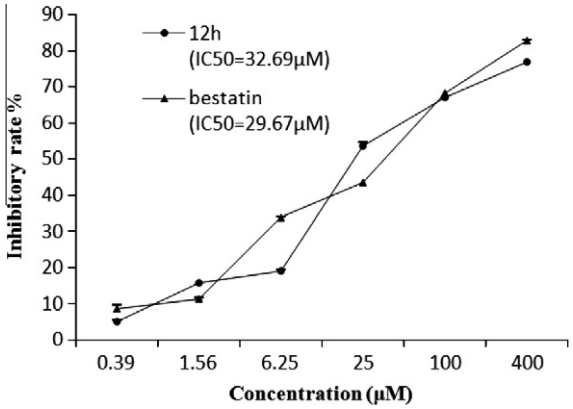


Figure 5. Inhibition of APN/CD13 activity on ES-2 cells induced by **12h** or bestatin. Data are expressed as mean values of three independent experiments (±SE).

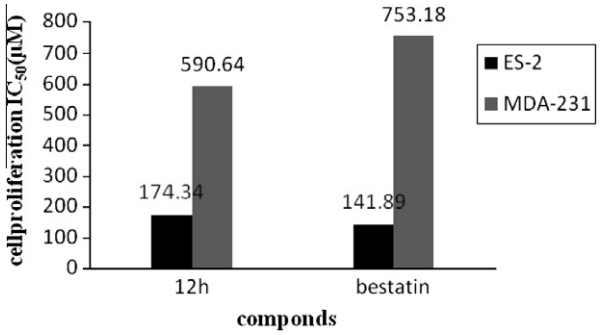


Figure 6. Anti-proliferative activities of compound **12h** and bestatin against two tumor cell lines (ES-2 cells and MDA-MB-231 cells). The columns represent the mean values of three independent experiments.

bestatin with the IC_{50} value of 174.34 ± 0.09 μM versus 141.89 ± 8.12 μM.

4. Conclusion

In summary, we have described the synthesis and properties of a series of novel 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives as APN inhibitors. Most of the target compounds showed potent inhibitory activities towards APN, and compound **12h** showed the best inhibition both in the enzymatic assay and cell-based assay, which makes it a good novel lead for future APN inhibitors development as anticancer agents.

5. Experiment

5.1. Chemistry: general procedures

All the material we used were purchased from commercial suppliers and used without further purification. Solvents were distilled prior to use and flash chromatography was performed using silica gel (60 Å, 200 ± 300 mesh). All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light, or chloride ferric. Melting points were determined on an electrothermal melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Brucker Avance 600 spectrometer using TMS as an internal standard in DMSO-d_6 solutions. Chemical shifts are reported in delta (δ) units, parts per million (ppm) downfield from trimethylsilane. ESI-MS were determined on an API 4000 spectrometer. High-resolution mass spectral (HRMS) data were conducted by Shandong Analysis and Test Center, and are reported as *m/z* (relative intensity).

5.1.1. (*S*)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid (2)

The title compound was prepared as described by Cheng et al.[32] ESI-MS *m/z*: 178.3 $[M+H]^+$.

5.1.2. (*S*)-2-(*tert*-Butoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (3)

The title compound was prepared as described by Cheng et al.[32] ESI-MS *m/z*: 278.4 $[M+H]^+$.

5.1.3. (*S*)-Benzyl 2-(3-amino-2,6-dioxopiperidin-1-yl)acetate trifluoroacetate (6)

The title compound was prepared as described by Li et al.[28]

5.1.4. (*S*)-*tert*-Butyl-3-(((*S*)-1-(2-(benzyloxy)-2-oxoethyl)-2,6-dioxopiperidin-3-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7)

To a solution of compound **3** (0.54 g, 2 mmol) and *N*-methylmorpholine (0.26 ml, 2.2 mmol) in 30 ml anhydrous THF was added isobutyl chloroformate (0.30 ml, 2.2 mmol) at −20 °C. The mixture was stirred for 30 min at the same temperature. A solution of compound **6** (2 mmol) in DCM (5 ml) (which was previous neutralized with *N*-methylmorpholine) was added dropwise to the reaction mixture. Keep the reaction for 1 h at −20 °C and then remove the cooling bath. The reaction was continued for another 4 h at room temperature. Filtrated and concentrated with a rotary evaporator. The residue was dissolved in 50 ml EtOAc and washed with saturated $NaHCO_3$ (10 ml × 3), saturated citric acid (10 ml × 3) and brine (10 ml × 2) in turn. The organic phase was dried over anhydrous Na_2SO_4 and concentrated with a rotary evaporator to afford crude product. The crude product was recrystallized by EtOAc/diethyl ether to give compound **7** as white solid (0.81 g, yield 75.6%), mp = 118–120 °C. $[\alpha]_D^{20} = -41.2$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.38–1.45 (m, 9H), 1.77–1.81 (m, 1H), 1.86–1.93 (m, 1H), 2.67–2.70 (m, 1H), 2.89–2.93 (m, 1H), 2.96–3.00 (m, 1H), 3.12–3.16 (m, 1H), 4.36–4.50 (m, 4H), 4.58–4.63 (m, 1H), 4.68–4.73 (m, 1H), 5.14 (s, 2H), 7.16–7.20 (m, 5H), 7.34–7.40 (m, 4H), 8.33–8.38 (m, 1H). ESI-MS *m/z*: 536.6 $[M+H]^+$.

5.1.5. 2-((*S*)-3-((*S*)-2-(*tert*-Butoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido)-2,6-dioxopiperidin-1-yl)acetic acid (8)

Compound **7** (1.07 g, 2 mmol) and catalytic amount of 10% Pd/C in methanol (30 ml) was hydrogenated in the presence of H_2 at room temperature. After 16 h, the catalyst was filtered and the solvent was removed under vacuum. The residue was then dissolved

in 30 ml anhydrous THF at −20 °C, *N*-methylmorpholine (0.26 ml, 2.2 mmol) was added, 2 min later, isobutyl chloroformate (0.30 ml, 2.2 mmol) was added to the mixture. The reaction was then stirred for 30 min at −20 °C. A solution of hydroxylamine hydrochloride (0.14 g, 2 mmol) in MeOH (2 ml) (which was previous neutralized with *N*-methylmorpholine) was added dropwise to the reaction mixture. Keep the reaction for 1 h at −20 °C and then remove the cooling bath. The reaction was continued for another 4 h at room temperature. Filtrated and concentrated with a rotary evaporator. The residue was dissolved in 50 ml EtOAc and washed with saturated $NaHCO_3$ (10 ml × 3), saturated citric acid (10 ml × 3) and brine (10 ml × 2) in turn. The organic phase was dried over anhydrous Na_2SO_4 and concentrated with a rotary evaporator. The residue was purified by column chromatography to give compound **8** in white solid (0.46 g, yield 50.0%), mp = 164–166 °C. $[\alpha]_D^{20} = -84.3$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.38–1.48 (m, 9H), 1.75–1.76 (m, 1H), 1.86–1.91 (m, 1H), 2.62–2.68 (m, 1H), 2.81–2.86 (m, 1H), 2.96–3.00 (m, 1H), 3.12–3.17 (m, 1H), 4.09–4.17 (m, 2H), 4.37–4.51 (m, 2H), 4.57–4.63 (m, 1H), 4.65–4.74 (m, 1H), 7.16–7.20 (m, 4H), 8.27–8.32 (m, 1H), 8.83 (s, 1H), 10.54 (s, 1H). ESI-MS *m/z*: 461.6 $[M+H]^+$.

5.1.6. (*S*)-*N*-((*S*)-1-(2-(Hydroxyamino)-2-oxoethyl)-2,6-dioxopiperidin-3-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (9)

Compound **8** (0.2 g, 0.43 mmol) was dissolved in 10 ml HCl–EtOAc (3 mol/L) at 0 °C. The reaction solution was stirred at 0 °C for 2 h, and then the temperature was raised to room temperature. After 5 h, the solvent was filtrated and the precipitate was washed with EtOAc to get compound **9** as white solid (0.15 g, yield 88.2%), mp = 198–200 °C. $[\alpha]_D^{20} = -37.3$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.02–2.09 (m, 2H), 2.75–2.78 (m, 1H), 2.94–3.00 (m, 1H), 3.06–3.11 (m, 1H), 3.36–3.40 (m, 1H), 4.13–4.22 (m, 2H), 4.24–4.25 (m, 1H), 4.30–4.32 (m, 1H), 4.36–4.40 (m, 1H), 4.88–4.93 (m, 1H), 7.27–7.32 (m, 4H), 9.13 (d, *J* = 9.0 Hz, 1H), 9.56 (d, *J* = 6.6 Hz, 1H), 9.78 (s, 1H), 10.64 (s, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{17}H_{21}N_4O_5$ $[M+H]^+$ 361.1507, found 361.1504.

5.1.7. (*S*)-*tert*-Butyl-3-(3-methoxy-3-oxopropylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10a)

To a solution of Compound **3** (1.62 g, 6 mmol), β-Alanine methyl ester hydrochloride (0.74 g, 7.2 mmol), HOBt (0.97 g, 7.2 mmol) and DMAP (0.07 g, 0.6 mmol) in dry DCM, was added TEA (1.82 g, 18 mmol). The reaction mixture was gently cooled to 0 °C in ice bath. To the reaction mixture was added dropwise a solution of EDCI (2.3 g, 12 mmol) in DCM for 30 min. After removal of the ice bath, the reaction mixture was stirred at room temperature for 12 h and filtered to remove the precipitate. The filtrate was washed with 1 N citric acid solution, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated in vacuo to give the crude product compound **10a** (1.4 g, yield 64.5%), mp = 80–82 °C. $[\alpha]_D^{20} = -70.6$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.36–1.45 (m, 9H), 2.25 (s, 1H), 2.36–2.38 (m, 1H), 2.90–2.94 (m, 1H), 3.03–3.07 (m, 1H), 3.17–3.33 (m, 2H), 3.54–3.56 (m, 3H), 4.33–4.36 (m, 1H), 4.46–4.64 (m, 2H), 7.12–7.19 (m, 4H), 7.85–7.96 (m, 1H). ESI-MS: *m/z*: 363.5 $[M+H]^+$.

The other compounds (**10b–10u**) were prepared in the same procedure as described above.

5.1.7.1. (*S*)-*tert*-Butyl-3-((4-methoxy-4-oxobutyl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10b).

Yield 74.2%, mp = 79–80 °C. ESI-MS *m/z*: 377.5 $[M+H]^+$.

5.1.7.2. (*S*)-*tert*-Butyl-3-((2-methoxy-2-oxoethyl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10c).

Yield 78.5%, mp = 125–126 °C. ESI-MS *m/z*: 349.5 $[M+H]^+$.

5.1.7.3. (*S*)-*tert*-Butyl-3-(((*S*)-1-methoxy-1-oxopropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10d).

Yield 68.5%, mp = 84–85 °C. ESI-MS *m/z*: 363.5 $[M+H]^+$.

5.1.7.4. (*S*)-*tert*-Butyl-3-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10e).

Yield 64.9%, oil. ESI-MS *m/z*: 391.5 $[M+H]^+$.

5.1.7.5. (*S*)-*tert*-Butyl-3-(((S)-1-methoxy-4-methyl-1-oxopentan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10f).

Yield 70.3%, mp = 81–83 °C. ESI-MS *m/z*: 405.5 $[M+H]^+$.

5.1.7.6. (*S*)-*tert*-Butyl-3-(((2*S*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10g).

Yield 73.5%, mp = 73–74 °C. ESI-MS *m/z*: 405.5 $[M+H]^+$.

5.1.7.7. (*S*)-*tert*-Butyl-3-(((*S*)-2-methoxy-2-oxo-1-phenylethyl) carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10h).

Yield 75.3%, mp = 134–135 °C. ESI-MS *m/z*: 425.5 $[M+H]^+$.

5.1.7.8. (*S*)-*tert*-Butyl-3-(((*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10i).

Yield 68.3%, mp = 91–93 °C. ESI-MS *m/z*: 439.5 $[M+H]^+$.

5.1.7.9. (*S*)-*tert*-Butyl-3-(((*S*)-3-(4-hydroxyphenyl)-1-methoxy-1-oxopropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10j).

Yield 62.4%, mp = 62–65 °C. ESI-MS *m/z*: 455.5 $[M+H]^+$.

5.1.7.10. (*S*)-*tert*-Butyl-3-(((*S*)-1-methoxy-4-(methylthio)-1-oxobutan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10k).

Yield 60.9%, mp = 148–149 °C. ESI-MS *m/z*: 423.5 $[M+H]^+$.

5.1.7.11. (*S*)-*tert*-Butyl-3-(((*S*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10l).

Yield 67.6%, mp = 69–71 °C. ESI-MS *m/z*: 379.5 $[M+H]^+$.

5.1.7.12. (*S*)-*tert*-Butyl-3-(((2*S*)-3-hydroxy-1-methoxy-1-oxobutan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10m).

Yield 72.5%, mp = 80–81 °C. ESI-MS *m/z*: 393.5 $[M+H]^+$.

5.1.7.13. (*S*)-*tert*-Butyl-3-((*S*)-2-(carbonyl)pyrrolidine-1-carbonyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10n).

Yield 70.5%, mp = 103–104 °C. ESI-MS *m/z*: 389.5 $[M+H]^+$.

5.1.7.14. (*S*)-*tert*-Butyl-3-((2*S*,4*R*)-2-(carbonyl)-4-hydroxypyrrolidine-1-carbonyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10o).

Yield 72.4%, mp = 169–170 °C. ESI-MS *m/z*: 405.5 $[M+H]^+$.

5.1.7.15. (*S*)-*tert*-Butyl-3-(((*S*)-6-(benzyloxycarbonyl)-1-methoxy-1-oxohexan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10p).

Yield 73.2%, oil. ESI-MS *m/z*: 554.7 $[M+H]^+$.

5.1.7.16. (*S*)-*tert*-Butyl-3-(((*S*)-3-(1*H*-indol-2-yl)-1-methoxy-1-oxopropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10q).

Yield 72.6%, mp = 71–72 °C. ESI-MS *m/z*: 478.6 $[M+H]^+$.

5.1.7.17. (*S*)-*tert*-Butyl-3-(((*S*)-1-((*S*)-1-methoxy-4-methyl-1-oxopentan-2-ylamino)-4-methyl-1-oxopentan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10r).

Yield 67.4%, mp = 79–80 °C. ESI-MS *m/z*: 518.7 $[M+H]^+$.

5.1.7.18. (*S*)-*tert*-Butyl-3-(((*S*)-1-((*S*)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-4-methyl-1-oxopentan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10s).

Yield 73.6%, mp = 69–71 °C. ESI-MS *m/z*: 552.7 $[M+H]^+$.

5.1.7.19. (*S*)-*tert*-Butyl-3-(((*S*)-1-((*S*)-1-methoxy-4-methyl-1-oxopentan-2-ylamino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10t).

Yield 75.3%, mp = 164–165 °C. ESI-MS *m/z*: 552.7 $[M+H]^+$.

5.1.7.20. (*S*)-*tert*-Butyl-3-(((*S*)-1-((*S*)-1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10u).

Yield 72.8%, mp = 73–74 °C. ESI-MS *m/z*: 587.7 $[M+H]^+$.

5.1.8. (*S*)-*tert*-Butyl-3-((3-(hydroxyamino)-3-oxopropyl) carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11a)

To a solution of compound **10a** (1.08 g, 3 mmol) in methanol (7 ml) at room temperature was added dropwise a solution of NH_2OK (6 mmol) in methanol (3.4 ml). The mixture was stirred for 12 h and the solvent was evaporated in vacuum. The residue was purified by column chromatography to get compound **11a** (0.3 g, yield 27.5%), mp = 120–122 °C. $[\alpha]_D^{20} = -74.5$ (*c* 0.5, DMSO). 1H NMR (600 MHz, DMSO-d_6) δ 1.33–1.47 (m, 9H), 1.97–2.05 (m, 2H), 2.91–2.94 (m, 1H), 3.04 (s, 1H), 3.13–3.28 (m, 2H), 4.31–4.34 (m, 1H), 4.48–4.64 (m, 2H), 7.19–7.31 (m, 4H), 7.88–7.95 (m, 1H), 10.37–10.42 (m, 1H). ESI-MS *m/z*: 364.5 $[M+H]^+$.

The other compounds (**11b–11u**) were prepared in the same procedure as described above.

5.1.8.1. (*S*)-*tert*-Butyl-3-((3-(hydroxyamino)-4-oxopropyl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11b).

Yield 25.6%, mp = 79–80 °C. ESI-MS *m/z*: 378.5 $[M+H]^+$.

5.1.8.2. (*S*)-*tert*-Butyl-3-((3-(hydroxyamino)-2-oxopropyl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11c).

Yield 27.3%, mp = 121–122 °C. ESI-MS *m/z*: 350.4 $[M+H]^+$.

5.1.8.3. (*S*)-*tert*-Butyl-3-(((*S*)-1-(hydroxyamino)-1-oxopropan-2-yl) carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11d).

Yield 26.5%, mp = 116–117 °C. ESI-MS *m/z*: 364.5 $[M+H]^+$.

5.1.8.4. (*S*)-*tert*-Butyl-3-(((*S*)-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11e).

Yield 28.3%, mp = 88–89 °C. ESI-MS *m/z*: 392.5 $[M+H]^+$.

5.1.8.5. (*S*)-*tert*-Butyl-3-(((*S*)-1-(hydroxyamino)-4-methyl-1-oxopentan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11f).

Yield 30.1%, mp = 92–93 °C. ESI-MS *m/z*: 406.5 $[M+H]^+$.

5.1.8.6. (*S*)-*tert*-Butyl-3-(((2*S*)-1-(hydroxyamino)-3-methyl-1-oxopentan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11g).

Yield 28.4%, mp = 97–98 °C. ESI-MS *m/z*: 406.5 $[M+H]^+$.

5.1.8.7. (*S*)-*tert*-Butyl-3-(((*S*)-2-(hydroxyamino)-2-oxo-1-phenylethyl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11h).

Yield 28.6%, mp = 94–95 °C. ESI-MS *m/z*: 426.5 $[M+H]^+$.

5.1.8.8. (*S*)-*tert*-Butyl-3-(((*S*)-1-(hydroxyamino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11i).

Yield 26.5%, mp = 86–87 °C. ESI-MS *m/z*: 440.5 $[M+H]^+$.

5.1.8.9. (*S*)-*tert*-Butyl-3-(((*S*)-1-(hydroxyamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11j).

Yield 25.6%, mp = 203–204 °C. ESI-MS *m/z*: 456.5 $[M+H]^+$.

5.1.8.10. (*S*)-*tert*-Butyl-3-(((S)-1-(hydroxyamino)-4-(methylthio)-1-oxobutan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11k).

Yield 28.3%, mp = 73–74 °C. ESI-MS *m/z*: 424.5 $[M+H]^{+\cdot}$

5.1.8.11. (*S*)-*tert*-Butyl-3-(((*S*)-3-hydroxy-1-(hydroxyamino)-1-oxopropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11l).

Yield 26.4%, mp = 82–83 °C. ESI-MS *m/z*: 380.5 $[M+H]^+$.

5.1.8.12. (*S*)-*tert*-Butyl-3-(((2*S*)-3-hydroxy-1-(hydroxyamino)-1-oxobutan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11m).

Yield 27.3%, mp = 196–197 °C. ESI-MS *m/z*: 394.5 $[M+H]^+$.

5.1.8.13. (*S*)-*tert*-Butyl-3-((*S*)-2-(hydroxycarbamoyl)pyrrolidine-1-carbonyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11n).

Yield 26.8%, mp = 95–96 °C. ESI-MS *m/z*: 390.5 $[M+H]^+$.

5.1.8.14. (*S*)-*tert*-Butyl-3-((2*S*,4*R*)-4-hydroxy-2-(hydroxycarbamoyl)pyrrolidine-1-carbonyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11o).

Yield 27.3%, mp = 122–124 °C. ESI-MS *m/z*: 406.5 $[M+H]^+$.

5.1.8.15. (*S*)-*tert*-Butyl-3-(((*S*)-6-(benzyloxycarbonyl)-1-(hydroxyamino)-1-oxohexan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11p).

Yield 29.7%, mp = 77–78 °C. ESI-MS *m/z*:555.6 $[M+H]^+$.

5.1.8.16. (*S*)-*tert*-Butyl-3-(((*S*)-1-(hydroxyamino)-3-(1*H*-indol-2-yl)-1-oxopropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11q).

Yield 30.6%, mp = 114–115 °C. ESI-MS *m/z*: 479.6 $[M+H]^+$.

5.1.8.17. (*S*)-*tert*-Butyl-3-(((*S*)-1-((*S*)-1-(hydroxyamino)-4-methyl-1-oxopentan-2-ylamino)-4-methyl-1-oxopentan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11r).

Yield 30.4%, mp = 172–173 °C. ESI-MS *m/z*: 519.7 $[M+H]^+$.

5.1.8.18. (*S*)-*tert*-Butyl-3-(((*S*)-1-((*S*)-1-(hydroxyamino)-1-oxo-3-phenylpropan-2-ylamino)-4-methyl-1-oxopentan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11s).

Yield 32.1%, mp = 207–208 °C. ESI-MS *m/z*: 553.7 $[M+H]^+$.

5.1.8.19. (*S*)-*tert*-Butyl-3-(((*S*)-1-((*S*)-1-(hydroxyamino)-4-methyl-1-oxopentan-2-ylamino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11t).

Yield 35.8%, mp = 198–201 °C. ESI-MS *m/z*: 553.7 $[M+H]^+$.

5.1.8.20. (*S*)-*tert*-Butyl-3-(((*S*)-1-((*S*)-1-(hydroxyamino)-1-oxo-3-phenylpropan-2-ylamino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (11u).

Yield 40.2%, mp = 209–211 °C. ESI-MS *m/z*: 586.7 $[M+H]^+$.

5.1.9. (*S*)-*N*-(3-(Hydroxyamino)-3-oxopropyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12a)

Compound **11a** (0.20 g, 0.55 mmol) was dissolved in 10 ml HCl–EtOAc (3 mol/L) at 0 °C. The reaction solution was stirred at 0 °C for 2 h, and then the temperature was raised to room temperature. After 5 h, the solvent was filtrated and the prepicitate was washed with EtOAc to get compound **12a** as white solid (0.15 g, yield 91.0%), mp = 134–135 °C. $[\alpha]_D^{20} = -117.6$ (*c* 0.5, DMSO). 1H NMR (600 MHz, DMSO-d_6) δ 2.21 (t, *J* = 7.2 Hz, 2H), 2.96–3.01 (m, 1H), 3.26–3.30 (m, 1H), 3.36–3.39 (t, *J* = 7.2 Hz, 2H), 4.11 (dd, *J* = 4.8 Hz, 12 Hz, 1H), 4.28–4.36 (m, 2H), 7.23–7.28 (m, 4H). HRMS (AP-ESI) *m/z*: calcd for $C_{13}H_{18}N_3O_3$ $[M+H]^+$ 264.1343, found 264.1375.

The other compounds (**12b**–**12u**) were synthesized in the same procedure as described above.

5.1.10. (*S*)-*N*-(4-(Hydroxyamino)-4-oxobutyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12b)

Yellow solid, yield 94.8%, mp = 112–115 °C. $[\alpha]_D^{20} = -117.6$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.67–1.72 (m, 2H), 2.02–2.05 (m, 2H), 2.98–3.03 (m, 1H), 3.14–3.17 (m, 2H), 3.31–3.35 (m, 1H), 4.11–4.15 (m, 1H), 4.27–4.34 (m, 2H), 7.24–7.32 (m, 4H), 8.77–8.79 (m, 1H), 9.46–9.47 (m, 1H), 9.80–9.82 (m, 1H), 10.48 (s, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{14}H_{20}N_3O_3$ $[M+H]^+$ 278.1499, found 278.1532.

5.1.11. (*S*)-*N*-(2-(Hydroxyamino)-2-oxoethyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12c)

White solid, yield 90.1%, mp = 175–177 °C. $[\alpha]_D^{20} = -117.6$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.68–3.03 (m, 1H), 3.12–3.35 (m, 1H), 3.71–3.81 (m, 2H), 4.19–4.22 (m, 1H), 4.29–4.32 (m, 1H), 4.36–4.39 (m, 1H), 7.25–7.30 (m, 4H), 8.93–8.95 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{12}H_{16}N_3O_3$ $[M+H]^+$ 250.1186, found 250.1214.

5.1.12. (*S*)-*N*-((*S*)-1-(Hydroxyamino)-1-oxopropan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12d)

White solid, yield 88.8%, mp = 130–133 °C. $[\alpha]_D^{20} = -68.6$ (c 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.27 (d, *J* = 7.2 Hz, 3H), 2.94–2.99 (m, 1H), 3.30–3.33 (m, 1H), 4.13–4.16 (m, 1H), 4.18–4.36 (m, 3H), 7.25–7.29 (m, 4H). HRMS (AP-ESI) *m/z*: calcd for $C_{13}H_{18}N_3O_3$ $[M+H]^+$ 264.1343, found 264.1375.

5.1.13. (*S*)-*N*-((*S*)-1-(Hydroxyamino)-3-methyl-1-oxobutan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12e)

White solid, yield 93.2%, mp = 229–230 °C. $[\alpha]_D^{20} = -86.3$ (c 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.87–0.94 (m, 6H), 1.90–1.99 (m, 1H), 2.90–2.95 (m, 1H), 3.27–3.30 (m, 1H), 4.04–4.07 (t, *J* = 8.4 Hz, 1H), 4.17–4.21 (m, 1H), 4.28–4.30 (m, 1H), 4.34–4.38 (m, 1H), 7.24–7.28 (m, 4H), 8.77–8.79 (m, 1H), 9.00 (s, 1H), 9.42–9.44 (m, 1H), 9.73 (s, 1H), 10.80 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{15}H_{22}N_3O_3$ $[M+H]^+$ 292.1656, found 296.1687.

5.1.14. (*S*)-*N*-((*S*)-1-(Hydroxyamino)-4-methyl-1-oxopentan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12f)

White solid, yield 92.1%, mp = 207–209 °C. $[\alpha]_D^{20} = -94.1$ (c 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.88 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 1.42–1.47 (m, 1H), 1.51–1.56 (m, 1H), 1.59–1.64 (m, 1H), 2.53–3.00 (m, 1H), 3.29–3.37 (m, 1H), 4.13–4.16 (m, 1H), 4.27–4.36 (m, 3H), 7.24–7.29 (m, 4H), 8.84–8.85 (m, 1H), 9.48–9.50 (m, 1H), 9.84 (s, 1H), 10.86 (s, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{16}H_{24}N_3O_3$ $[M+H]^+$ 306.1812, found 306.1844.

5.1.15. (*S*)-*N*-((2*S*)-1-(Hydroxyamino)-3-methyl-1-oxopentan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12g)

White solid, yield 95.6%, mp = 219–220 °C. $[\alpha]_D^{20} = -98.0$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.84–0.87 (m, 6H), 1.08–1.13 (m, 1H), 1.51–1.55 (m, 1H), 1.70–1.74 (m, 1H), 2.90–2.95 (m, 1H), 3.27–3.30 (m, 1H), 4.08–4.10 (m, 1H), 4.18–4.20 (m, 1H), 4.28–4.37 (m, 2H), 7.25–7.28 (m, 4H), 8.81–8.83 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{16}H_{24}N_3O_3$ $[M+H]^+$ 306.1812, found 306.1855.

5.1.16. (*S*)-*N*-((*S*)-2-(Hydroxyamino)-2-oxo-1-phenylethyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12h)

White solid, yield 90.8%, mp = 178–179 °C. $[\alpha]_D^{20} = -64.7$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.88–2.93 (m, 1H), 2.99–3.04 (m, 1H), 4.25–4.36 (m, 3H), 5.45–5.50 (m, 1H), 7.24–7.26 (m, 3H), 7.27–7.29 (m, 1H) 7.32–7.34 (m, 1H), 7.37–7.40 (m, 2H), 7.41–7.47 (m, 2H), 9.12 (s, 1H), 9.32–9.53 (m, 1H), 9.84 (s, 1H), 11.23 (s, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{18}H_{20}N_3O_3$ $[M+H]^+$ 324.1354, found 324.1349.

5.1.17. (*S*)-*N*-((*S*)-1-(Hydroxyamino)-1-oxo-3-phenylpropan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12i)

White solid, yield 89.5%, mp = 186–188 °C. $[\alpha]_D^{20} = -76.5$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.86–3.00 (m, 3H), 3.34–3.35 (m, 1H), 4.09–4.12 (m, 1H), 4.24–4.27 (m, 1H), 4.33–4.36 (m, 1H), 4.46–4.50 (m, 1H), 7.21–7.31 (m, 9H), 8.99–9.00 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{19}H_{22}N_3O_3$ $[M+H]^+$ 340.1656, found 340.1659.

5.1.18. (*S*)-*N*-((*S*)-1-(Hydroxyamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12j)

White solid, yield 86.8%, mp = 170–172 °C. $[\alpha]_D^{20} = -74.5$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.74–2.77 (m, 1H), 2.85–2.88 (m, 1H), 2.90–2.95 (m, 1H), 3.34–3.39 (m, 1H), 4.09–4.12 (m, 1H), 4.24–4.27 (m, 1H), 4.33–4.36 (m, 1H), 4.37–4.41 (m, 1H), 6.68 (d, *J* = 8.4 Hz, 2H), 7.06 (d, *J* = 8.4 Hz, 2H), 7.23–7.28 (m, 4H), 8.94–8.95 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{19}H_{22}N_3O_4$ $[M+H]^+$ 356.1605, found 356.1637.

5.1.19. (*S*)-N-((S)-1-(hydroxyamino)-4-(methylthio)-1-oxobutan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12k)

White solid, yield 93.5%, mp = 140–143 °C. $[\alpha]_D^{20} = -58.8$ (c 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.85–1.91 (m, 2H), 2.06 (s, 3H), 2.42–2.54 (m, 2H), 2.94–2.99 (m, 1H), 3.30–3.34 (m, 1H), 4.17–4.20 (m, 1H), 4.29–4.38 (m, 3H), 7.26–7.29 (m, 4H), 8.93–8.95 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{15}H_{22}N_3O_3S$ $[M+H]^+$ 324.1376, found 324.1403.

5.1.20. (S)-N-((S)-3-hydroxy-1-(hydroxyamino)-1-oxopropan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12l)

White solid, yield 92.8%, mp = 153–156 °C. $[\alpha]_D^{20} = -68.6$ (c 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.95–2.30 (m, 1H), 3.32–3.40 (m, 1H), 3.52–3.60 (m, 3H), 4.15–4.19 (m, 1H), 4.28–4.36 (m, 3H), 7.24–7.28 (m, 4H), 8.87–8.89 (m, 1H), 9.48–9.55 (m, 1H), 9.82–9.89 (m, 1H), 10.78 (s, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{13}H_{18}N_3O_4$ $[M+H]^+$ 280.1292, found 280.1328.

5.1.21. (S)-N-((2S)-3-hydroxy-1-(hydroxyamino)-1-oxobutan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12m)

White solid, yield 94.8%, mp = 148–150 °C. $[\alpha]_D^{20} = -82.4$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.10 (d, *J* = 6.6 Hz, 3H), 2.95–3.00 (m, 1H), 3.37–3.40 (m, 1H), 3.91–3.93 (m, 1H), 4.13–4.15 (m, 1H), 4.23–4.26 (m, 1H), 4.28–4.36 (m, 2H), 7.24–7.29 (m, 4H), 8.80–8.82 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{14}H_{20}N_3O_4$ $[M+H]^+$ 294.1448, found 294.1479.

5.1.22. (S)-N-hydroxy-1-((S)-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)pyrrolidine-2-carboxamide hydrochloride (12n)

White solid, yield 94.5%, mp = 155–157 °C. $[\alpha]_D^{20} = -49.0$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.80–1.84 (m, 1H), 1.86–1.91 (m, 1H), 2.01–2.10 (m, 1H), 2.11–2.15 (m, 1H), 2.88–2.93 (m, 1H), 3.34–3.40 (m, 1H), 3.60–3.64 (m, 1H), 3.69–3.73 (m, 1H), 4.25–4.05 (m, 1H), 4.32 (s, 2H), 4.60–4.62 (m, 1H), 7.25–7.30 (m, 4H). HRMS (AP-ESI) *m/z*: calcd for $C_{15}H_{20}N_3O_3$ $[M+H]^+$ 290.1499, found 290.1537.

5.1.23. (2S,4R)-N,4-dihydroxy-1-((S)-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)pyrrolidine-2-carboxamide hydrochloride (12o)

White solid, yield 87.8%, mp = 169–171 °C. $[\alpha]_D^{20} = -94.1$ (c 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.87–1.91 (m, 1H), 2.06–2.10 (m, 1H), 2.85–2.90 (m, 1H), 3.33–3.39 (m, 1H), 3.61–3.63 (m, 1H), 3.68–3.70 (m, 1H), 4.32–4.34 (m, 3H), 4.41 (s, 1H), 4.63–4.66 (m, 1H), 7.27–7.29 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{15}H_{20}N_3O_4$ $[M+H]^+$ 306.1448, found 306.1487.

5.1.24. Benzyl-(S)-6-(hydroxyamino)-6-oxo-5-((S)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido)hexylcarbamate hydrochloride (12p)

White solid, yield 86.9%, mp = 174–175 °C. $[\alpha]_D^{20} = -94.1$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.25–1.28 (m, 1H), 1.31–1.36 (m, 1H), 1.40–1.42 (m, 1H), 1.57–1.62 (m, 1H), 2.92–2.99 (m, 3H), 3.29–3.32 (m, 1H), 4.16–4.36 (m, 4H), 7.25–7.38 (m, 9H), 8.06–8.10 (m, 1H), 8.85–8.93 (m, 1H), 9.47–9.54 (m, 1H), 9.77–9.84 (m, 1H), 10.81–10.85 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{24}H_{31}N_4O_5$ $[M+H]^+$ 455.2289, found 455.2296.

5.1.25. (S)-N-((S)-1-(hydroxyamino)-3-(1H-indol-2-yl)-1-oxopropan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12q)

Pink solid, yield 90.3%, mp = 180–182 °C. $[\alpha]_D^{20} = -82.4$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.92–2.97 (m, 1H), 2.99–3.03 (m, 1H), 3.10–3.14 (m, 1H), 3.34–3.40 (m, 1H), 4.10–4.12 (m, 1H), 4.25–4.27 (m, 1H), 4.32–4.35 (m, 1H), 4.53–4.56 (m, 1H), 7.00 (t, *J* = 7.8 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 7.19 (s, 1H), 7.22–7.29 (m, 4H), 7.34 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 8.98–8.99 (m, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{21}H_{23}N_4O_3$ $[M+H]^+$ 379.1765, found 379.1801.

5.1.26. (S)-N-((S)-1-((S)-1-(hydroxyamino)-4-methyl-1-oxopentan-2-ylamino)-4-methyl-1-oxopentan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12r)

White solid, yield 90.8%, mp = 154–156 °C. $[\alpha]_D^{20} = -74.5$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.78–0.93 (m, 12H), 1.38–1.45 (m, 1H), 1.46–1.58 (m, 4H), 1.64–1.66 (m, 1H), 2.91–2.96 (m, 1H), 3.33–3.40 (m, 1H), 4.16–4.23 (m, 2H), 4.26–4.29 (m, 1H), 4.33–4.35 (m, 1H), 4.37–4.44 (m, 1H), 7.19–7.28 (m, 4H), 8.15 (d, *J* = 8.4 Hz, 1H), 8.77 (d, *J* = 8.4 Hz, 1H), 9.47–9.54 (m, 1H), 9.81–9.83 (m, 1H), 10.72 (s, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{22}H_{35}N_4O_4$ $[M+H]^+$ 419.2653, found 419.2689.

5.1.27. (S)-N-((S)-1-((S)-1-(hydroxyamino)-1-oxo-3-phenylpropan-2-ylamino)-4-methyl-1-oxopentan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12s)

White solid, yield 90.1%, mp = 158–160 °C. $[\alpha]_D^{20} = -74.5$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.83–0.93 (m, 6H), 1.38–1.48 (m, 2H), 1.58–1.62 (m, 1H), 2.82–2.88 (m, 1H), 2.90–2.94 (m, 2H), 3.27–3.30 (m, 1H), 4.13–4.17 (m, 1H), 4.26–4.42 (m, 4H), 7.18–7.31 (m, 9H), 8.33 (d, *J* = 8.4 Hz, 1H), 8.74 (d, *J* = 8.4 Hz, 1H), 9.45–9.53 (m, 1H), 9.78–9.79 (m, 1H), 10.74 (s, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{25}H_{33}N_4O_4$ $[M+H]^+$ 453.2496, found 453.2533.

5.1.28. (S)-N-((S)-1-(1-(hydroxyamino)-4-methyl-1-oxopentan-2-ylamino)-1-oxo-3-phenylpropan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12t)

White solid, yield 90.6%, mp = 150–152 °C. $[\alpha]_D^{20} = -70.6$ (c 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.85 (d, *J* = 6.0 Hz, 3H), 0.90 (d, *J* = 6.0 Hz, 3H), 1.41–1.46 (m, 1H), 1.48–1.52 (m, 1H), 1.54–1.61 (m, 1H), 2.74–2.85 (m, 1H), 2.88–2.97 (m, 1H), 3.05–3.13 (m, 1H), 3.36–3.42 (m, 1H), 4.06–4.10 (m, 1H), 4.20–4.27 (m, 1H), 4.31–4.35 (m, 1H), 4.63–4.66 (m, 1H), 7.20–7.22 (m, 2H), 7.24–7.25 (m, 2H), 7.27–7.30 (m, 1H), 7.32–7.36 (m, 2H), 8.33–8.35 (m, 1H), 8.83–8.84 (m, 1H), 9.41–9.42 (m, 1H), 9.59–9.61 (m, 1H), 10.75 (s, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{25}H_{33}N_4O_4$ $[M+H]^+$ 453.2496, found 453.2530.

5.1.29. (S)-N-((S)-1-((S)-1-(hydroxyamino)-1-oxo-3-phenylpropan-2-ylamino)-1-oxo-3-phenylpropan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (12u)

White solid, yield 90.8%, mp = 171–173 °C. $[\alpha]_D^{20} = -56.9$ (*c* 0.5, DMSO). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.74–2.83 (m, 1H), 2.84–2.90 (m, 2H), 2.94–3.00 (m, 1H), 3.01–3.04 (m, 1H), 3.33–3.36 (m, 1H), 4.06–4.19 (m, 1H), 4.20–4.23 (m, 1H), 4.31–4.54 (m, 1H), 4.61–4.64 (m, 1H), 7.14–7.32 (m, 14H), 8.51 (d, *J* = 8.4 Hz, 1H), 8.82–8.83 (m, 1H), 9.37–9.38 (m, 1H), 9.59 (s, 1H), 10.74 (s, 1H). HRMS (AP-ESI) *m/z*: calcd for $C_{28}H_{31}N_4O_4$ $[M+H]^+$ 487.2340, found 487.2370.

5.2. Biological evaluation

5.2.1. In vitro APN inhibition assay

IC_{50} values against APN were determined as previously described[29] by using L-Leu-*p*-nitroanilide as substrate and Microsomal aminopeptidase from Porcine Kidney Microsomes (Sigma) as enzyme in 50 mM PBS (pH 7.4) or suspension of ES-2 cells in PBS (1×10^5/well). After incubation with various concentrations of detected compounds at 37 °C, hydrolysis of the substrate was measured using a plate reader (Varioskan, Thermo, USA) by observing the change of OD values at 405 nm.

5.2.2. In vitro MMP inhibition assay

Gelatinase A (MMP-2) and TNBS were purchased from Sigma, and the substance was synthesized as described by Vijaykumar et al.[33] The gelatinase, substance, and inhibitor were dissolved in sodium borate (pH8.5, 50 mmol/L) and incubated for 30 min at 37 °C, and then 0.03% TNBS was added and incubated for another 20 min, the resulting solution was detected under 450 nm wavelength to gain absorption.

5.2.3. MTT assay

ES-2 cells and MDA-MB-231 cells were grown in RPMI1640 medium with 10% fetal bovine serum at 37 °C in 5% CO2 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 5000 cells per well, cultured for 4 h in complete growth medium, then treated with 4000, 800, 160, 32, or 6.4 μmol/L of the compounds for 48 h. Following this, 0.5% MTT solution was added to each well. After further incubation for 4 h, the formazan formed from MTT was extracted by adding DMSO and mixing for 15 min. The optical density was read with ELISA reader at 570 nm.

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