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Novel aminopeptidase N (APN/CD13) inhibitors derived from chloramphenicol amine

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ABSTRACT

Aminopeptidase N (APN) is involved in different physiological and pathological processes of tumor cells, including proliferation, invasion, apoptosis and metastasis. Herein one series of compounds derived from commercially available (1*S*,2*S*)-2-amino-1-(4-nitrophenyl) propane-1,3-diol have been designed and synthesized. Furthermore, preliminary activity evaluation showed that some compounds elicited moderate inhibitory activity against APN with compounds **10e** (IC_{50} = 6.1 ± 0.5 μM) possessing the best efficacy, which could be used as the lead compound in the future for anticancer agents research.

1. Introduction

Aminopeptidase N (APN/CD13: EC 3.4.11.2) is a Zn^{2+}-containing exopeptidase consisting of 967 amino acids in primary structure with highly conserved sequence $HEXXHX_{18}E$ in its active sites.[1] APN as a ubiquitous enzyme, is widely expressed on different kinds of cells such as epithelial cells of the intestine and kidney, neuronal synaptic membranes, macrophages, granulocytes and so on. It was identified to partially cleave neutral or basic amino acids from the N-terminus of oligopeptidases. In accordance to its overexpression in tumor cells, APN has intimate relationships with tumor invasion, metastasis and angiogenesis.[2–4] Therefore, inhibition of APN may lead to the development of anti-cancer agents.

Various of natural and synthetic inhibitors of APN have been reviewed.[5–7] Of these effective inhibitors, Bestatin (Fig. 1(a)) is the only marketed drug, which was isolated by Umezawa et al.[8] from *Streptomyces oliuoreticuli*. The *Escherichia coli*'s crystal structure of APN was first illustrated in ligand free form and enzyme–Bestatin complex form, respectively, by Kiyoshi et al.[9] Our group have studied mainly functional sites of APN based on this co-crystal complex published for years and several potent APN inhibitors possessing various scaffolds have been synthesized and reported.[10–15] Generally, the binding sites of APN with Bestatin contain three counterparts as shown in Figure 2. Viz. a hydrophobic pocket (S1) interacting with the phenyl group of Bestatin; a zinc binding group (ZBG) coordinated by the 2-hydroxy group and 1-carbonyl oxygen of Bestatin, and final another hydrophobic pocket in opposite which can be divided into two subsites (S1′ and S2′).

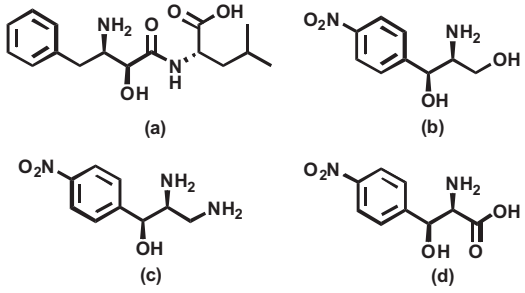

Figure 1. Chemical structures of (a) Bestatin, (b) chloramphenicol amine, (c) AHNPA, and (d) DANP.

Some previous work focusing on the derivation of (1*S*,2*S*)-2-amino-1-(4-nitrophenyl) propane-1,3-diol (chloramphenicol amine) (Fig. 1(b)) has been conducted in our group.[16] Some compounds sharing (2*R*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propanoic acid (AHNPA) (Fig. 1(c)) as the key intermediate have exhibited moderate inhibitory activity against APN in the micromolar range, and the docking study further manifested high affinities of AHNPA with APN.[16] Encouraged by this result, another new modification direction towards chloramphenicol amine is conducted. In this Letter, we described the synthesis and biological activity evaluation of newly designed compounds sharing (1*S*,2*S*)-2,3-diamino-1-(4-nitrophenyl) propan-1-ol (DANP) (Fig. 1(d)) as the common scaffold and docking studies of interactions are also discussed.

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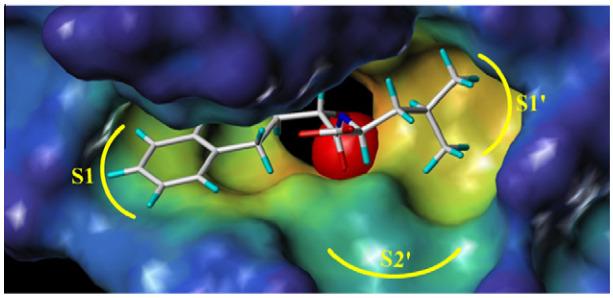
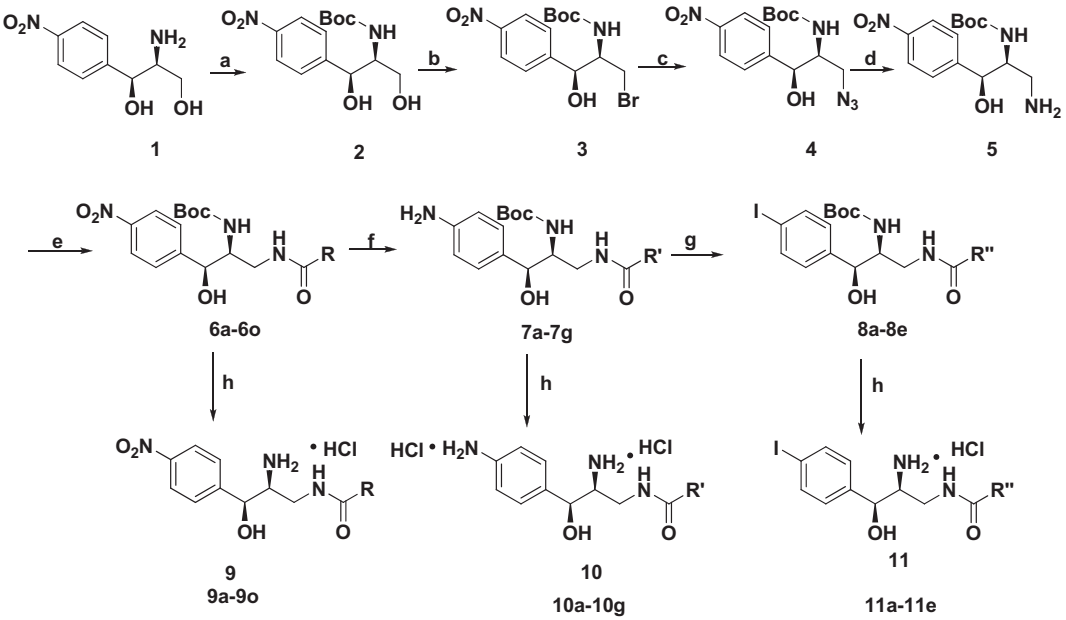


Figure 2. The interaction mode of Bestatin and APN (Bestatin in the X-ray crystal is showed in capped sticks mode).

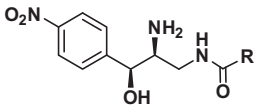
2. Chemistry

All the compounds are synthesized via the route shown in Scheme 1. The starting material, commercially available (1*S*,2*S*)-2-amino-1-(4-nitrophenyl) propane-1,3-diol, was firstly protected by $(Boc)_2O$, then the product was selectively bromized at the primary hydroxyl group to give the mono-bromide **3**. The following trinitride **4** was prepared with sodium azide in situ, by the next hydrogenation step leading to the key intermediate compound **5**. Several Boc-protected amino acids or Boc-protected dipeptides were condensed with compound **5** adopting classic EDCI/HOBt protocol. The target compounds **9a–9o** were readily obtained after the deprotection step in the presence of saturated HCl/anhydrous



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, THF; (b) NBS, Ph_3P, pyridine, THF; (c) NaN_3, DMF, 60 °C; (d) Ph_3P, THF, H_2O; (e) *N*-Boc amino acids or N-Boc dipeptides, Et_3N, EDCI, HOBt, DCM; (f) $N_2H_4{\cdot}H_2O$ (80%), $FeCl_3{\cdot}6H_2O$, active C; (g) *p*-TsOH, KI, $NaNO_2$, H_2O; (h) HCl/anhydrous EtOAc.

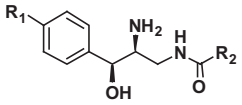
Table 1
The structures and inhibitory activity of target compounds **9a–9o** against APN and MMP-2

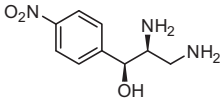
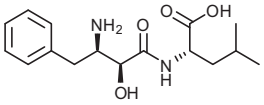


Compd	R	IC_{50}[a] (μM)		IC_{50} (MMP-2)/IC_{50} (APN)
		APN	MMP-2	
9a	-L-Ser-NH_2	422.5 ± 9.9	331.1 ± 5.6	0.8
9b	-L-isoSer-NH_2	163.5 ± 4.9	100.1 ± 3.4	0.06
9c	-L-Cys-NH_2	12.4 ± 1.5	151.6 ± 3.1	12.7
9d	-L-Leu-NH_2	80.6 ± 6.0	142.9 ± 2.9	1.8
9e	-L-Lys-NH_2	50.1 ± 3.5	84.3 ± 1.2	1.7
9f	-D-Phe-NH_2	42.2 ± 1.0	67.4 ± 1.0	1.6
9g	-L-Tyr-NH_2	39.2 ± 2.2	61.3 ± 1.5	1.6
9h	-L-Hpro-NH_2	441.3 ± 1.8	>1000	>2.3
9i	-L-Leu-L-isoSer-NH_2	19.6 ± 2.3	83.9 ± 2.2	4.2
9j	-D-Phe-L-Leu-NH_2	9.4 ± 1.6	173.1 ± 6.9	19.2
9k	-L-Ser-D-Phe-NH_2	21.6 ± 3.2	110.1 ± 3.7	5.0
9l	-L-isoSer-D-Phe-NH_2	17.6 ± 2.1	108.9 ± 2.8	6.1
9m	-D-Phe-D-Phe-NH_2	133.6 ± 10.1	44.1 ± 0.9	0.3
9n	-L-Leu-D-Phe-NH_2	74.7 ± 5.4	105.6 ± 3.2	1.4
9o	-D-Phe-L-isoSer-NH_2	6.5 ± 0.7	40.6 ± 1.7	6.3

[a] Mean values of triplicate experiments and standard deviations are given.

Table 2
The structures and inhibitory activity of target compounds **10a**–**10g**, **11a**–**11e** and control Bestatin against APN and MMP-2



Compd	R_1	R_2	IC_{50}[a] (μM)		IC_{50} (MMP-2)/IC_{50} (APN)
			APN	MMP-2	
10a	NH_2	-L-Leu-NH_2	63.2 ± 5.4	119.3 ± 4.5	1.9
10b	NH_2	-L-Lys-NH_2	135.6 ± 6.7	157.4 ± 3.9	1.2
10c	NH_2	-D-Phe-NH_2	141.3 ± 18.3	149.2 ± 2.1	1.1
10d	NH_2	-L-Tyr-NH_2	113.7 ± 5.6	111.3 ± 1.8	1.0
10e	NH_2	-D-Phe-L-Leu-NH_2	6.1 ± 0.5	133.8 ± 1.6	22.3
10f	NH_2	-D-Phe-D-Phe-NH_2	30.6 ± 3.9	40.4 ± 0.9	1.3
10g	NH_2	-L-Leu-D-Phe-NH_2	31.6 ± 1.9	94.0 ± 1.3	3.0
11a	I	-L-Leu-NH_2	30.6 ± 5.6	119.5 ± 4.6	3.9
11b	I	-L-Tyr-NH_2	21.6 ± 1.8	42.2 ± 2.9	2.0
11c	I	-D-Phe-L-Leu-NH_2	11.3 ± 0.5	86.6 ± 2.1	7.7
11d	I	-D-Phe-D-Phe-NH_2	>1000	107.1 ± 2.8	<0.1
11e	I	-L-Leu-D-Phe-NH_2	105.4 ± 3.9	52.7 ± 1.1	0.5
DANP			893.9 ± 10.8	>1000	>1.1
Bestatin			3.7 ± 0.4	120.4 ± 2.6	32.5

[a] Mean values of triplicate experiments and standard deviations are given.

EtOAc. Meanwhile, the anilides **7** produced by reduction were converted into target compounds **10a**–**10g** after deprotection. Similarly, expected compounds **11a**–**11e** were successfully synthesized.

3. Results and discussion

All the target compounds synthesized have been tested for the potential enzymatic activity and results are exhibited in Tables 1 and 2. Similarly to APN, MMP-2 is also a Zn^{2+}-dependent metalloprotease related to tumor invasion and metastasis.[17,18] Thus the assay was performed both on APN and MMP-2 as to study the compounds' selectivities using Bestatin as the positive control.

From data shown in Tables 1 and 2, we can see that most of target compounds had higher affinities with APN than MMP-2, which to some extent promoted our strategy for further rational APNIs design. Moreover, all the compounds assayed possessed better APN inhibitory activity compared with parent compound DANP (IC_{50} = 893.9 ± 10.8 μM) demonstrating that the structural additions of mono-amino acids or dipeptides lead to better efficacy in inhibiting APN.

Comparing compounds **9a**–**9o**, DANP-amino acid derivatives (compounds **9a**–**9h**) generally displayed worse activity than those DANP-dipeptide derivatives (compounds **9i**–**9o**), and this may be explained by longer hydrophobic side chains binding more tightly with APN's S1′ and S2′ active pockets. Furthermore, compound **9c** with IC_{50} value to 12.4 ± 1.5 μM has the best activity among compounds **9a**–**9h**, which may be contributed to sulfhydryl group's positive hydrophobic effect with APN pockets. In contrary, too large side chain led to comparatively poorer activity of compound **9m** (IC_{50} = 133.6 ± 10.1 μM) since APN's active cavities are relatively deeper requiring ligands having suitable volumes.[9]

The electronic and steric effects of nitro group of DNAP were also studied by converting nitro group into amino group and iodine, respectively. The enzymatic assay results unveiled that nitro group, owing to its strong hydrogen-bonding interactions with APN, was helpful to increase affinities of target compounds in contrary to amino group by comparing the IC_{50} values of compounds **9d**–**9e** to compounds **10a**–**10d** correspondingly. Also, the corresponding iodides **11a**, **11b** have better activity than **10a**, **10d**,

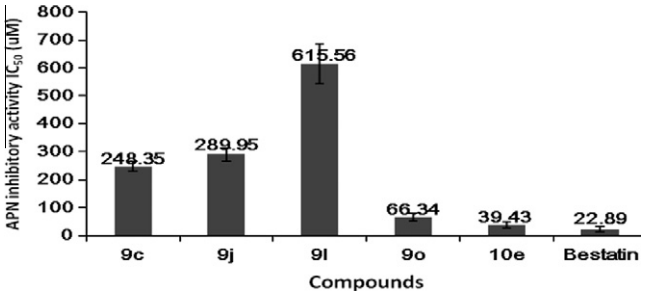


Figure 3. APN inhibitory activity of compounds **9c**, **9j**, **9l**, **9o**, **10e** and Bestatin against HL-60 cells. Data given are mean values of three independent experiments.

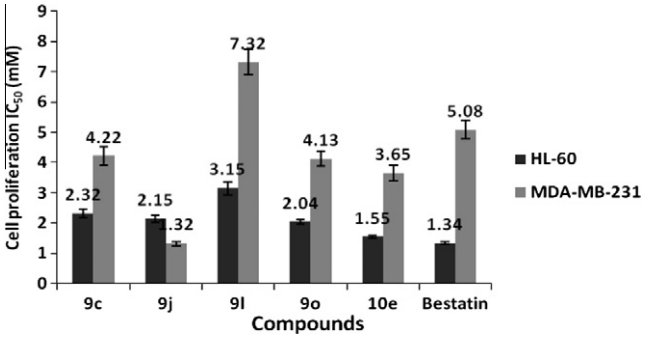


Figure 4. Anti-proliferation activity of compounds **9c**, **9j**, **9l**, **9o**, **10e** and Bestatin against two tumor cell lines (HL-60 cells and MDA-MB-231 cells). Data given are mean values of three independent experiments.

respectively. However, this conclusion is not in accordance with DNAP-dipeptide derivates, anilides **10e**–**10g** showing better activity. It is speculated that here amino group decreased tripeptides' obvious steric hindrances, and that compounds **11c**–**11e** sharing a larger group iodine than nitro group are less efficacious supported previous speculation in return.

Considering all the factors concerned, it is expected that compounds **10e** has the best inhibition (IC_{50} = 6.1 ± 0.5 μM) similar with Bestatin (IC_{50} = 3.7 ± 0.4 μM).

Moreover, the enzymatic inhibitory activity of compounds **9c**, **9j**, **9l**, **9o** and **10e** against APN was also determined with HL-60 cells high-expressing APN. Results are shown in Figure 3, from which it can be learned that none of these five inhibitors exhibited better activity than the control Bestatin which is consistent with the assay in enzymatic level described above. However, compounds **9c** performed stronger effect than **9j** contrary to the previous result in Table 1, which is assumed owing to distinct characteristics of APNs among species.[9]

Additionally, compounds **9c**, **9j**, **9l**, **9o** and **10e** are detected for their potential effects on proliferation of two tumor cell lines (HL-60 cells and MDA-MB-231 cells) with Bestatin as control via MTT assay. The result depicted in Figure 4 indicated that all compounds' anti-proliferative effects against HL-60 cells are better than against MDA-MB-231 cells mainly owing to APN's higher expression level on HL-60 cells than the other. Compound **10e** shows the best effect against HL-60 cells with the IC_{50} of 1.34 ± 0.08 mM complying with the enzymatic assay. However, except compound **9l**, all of the others exhibited better anti-proliferation activity against MDA-MB-231 cells than Bestatin as the positive control. Compared with others,[16] compounds with DANP scaffold may be the main contributions to the more obvious inhibitory activity towards tumor cell proliferation, possibly implying a

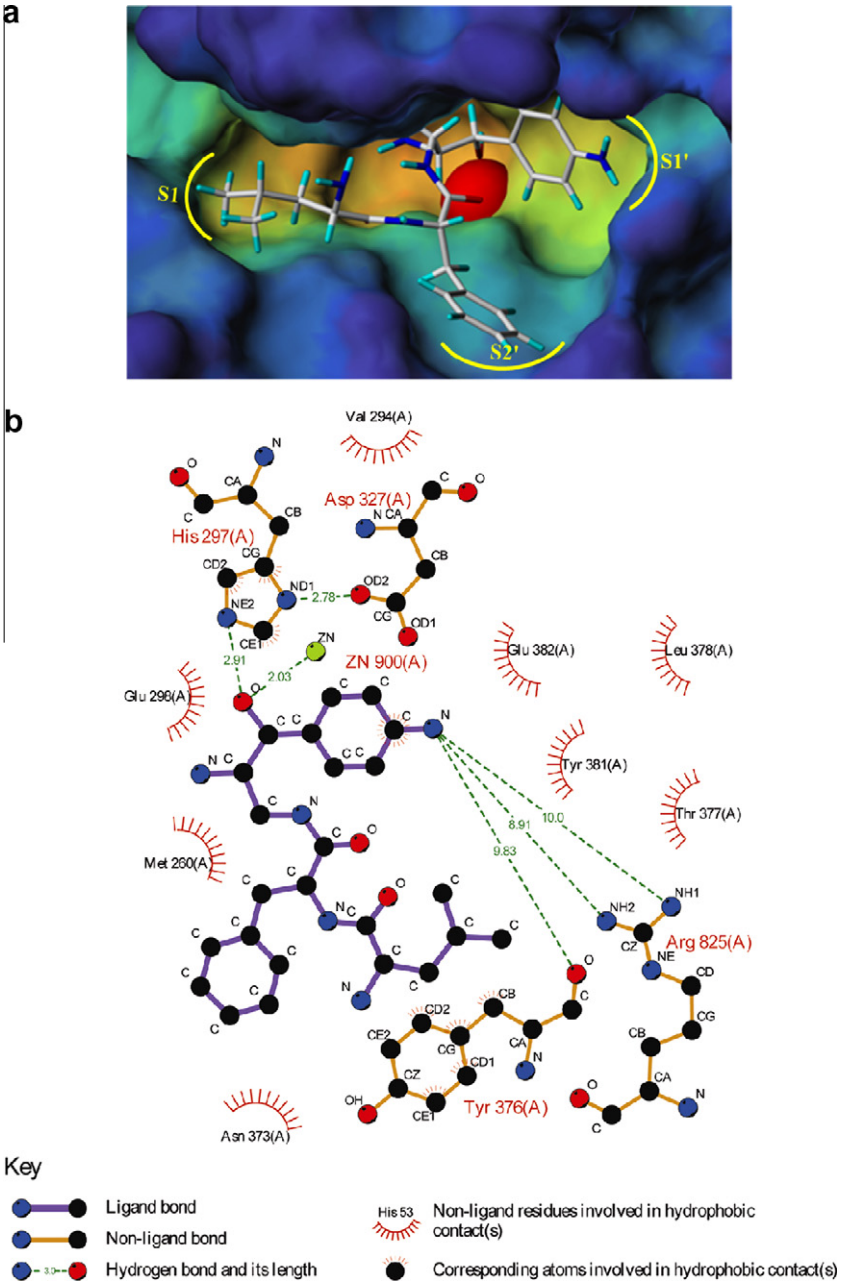


Figure 5. (a) The docking result of **10e** with APN (**10e** is showed in capped sticks mode). (b) The docking result of compound **10e** is shown by LIGPLOT.

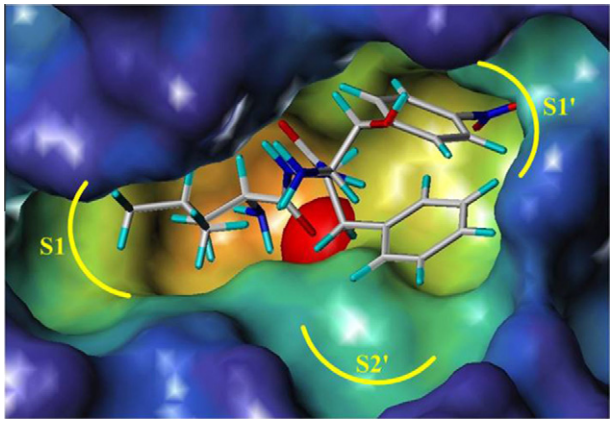


Figure 6. The docking result of **9j** with APN (**9j** is showed in capped sticks mode).

different mechanism from interactions with APN requiring further identification.

Aiming to investigate the interaction modes of DANP-derivatives with APN, the most active compound **10e** was sketched and docked into active sites of APN (PDB code: 2DQM) using Sybyl 7.0 via Sybyl/Sketch module and optimized using Powell's method in presence of the Tripos force field with convergence criterion set at 0.05 kcal/Å mol, and assigned with Gasteiger–Hückel charges. The docking study was performed using Sybyl/FlexX module. Owing to crucial functions of the zinc ion in ligand–receptor interactions, active sites of APN was defined as 13.1 Å radius circles around the zinc ion. Other docking parameters applied in the program were kept default. As illustrated in Figure 5, the crucial catalytic zinc ion was chelated by **10e**'s hydroxyl and carbonyl groups. Besides, aniline group of **10e** deeply inserted into S1′ pocket of APN. Also, D-phenyl moiety of phenylalanine residue and leucine residue could plunge into S1 and S2′ pockets, respectively, via hydrophobic interaction. It is also noted that compound **10e** could form hydrogen bonds with His^{297} at a distance of 2.91 Å. Furthermore, the stability of this protein–ligand complex could be reinforced by further interactions of amino group of compound **10e** with Tyr^{381} and Arg^{825}. Notably, the observed hydrophobic interactions of **10e** with Glu^{298}, Ala^{262}, Glu^{382}, Met^{260} contributed much to improve binding affinities as well. Additionally, the docking study of **9j** was synchronously investigated following the same protocol as **10e** (shown in Fig. 6), through which the hydrophobic and steric contributions of nitro group to affinities of **9j** and APN being further verdicted to approve our previous assumption.

Finally, although the computed information assay basically supported our speculation, the exact binding modes of the DANP-derivatives with APN should be finally confirmed through further X-ray crystal study.

4. Conclusion

In all, one series of compounds containing (1*S*,2*S*)-2,3-diamino-1-(4-nitrophenyl) propan-1-ol block have been synthesized and evaluated. Most of target compounds elicited moderate and selective APN inhibitory activity. Among them, **10e** possessed the best inhibition both in the enzymatic assay and cell-based assay, making it a good lead for searching better chloramphenicol amine derivatives as APN inhibitors.

5. Experimental section

5.1. Chemistry: general procedures

All the materials involved were commercially available. All solvents were distilled before used. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light or ninhydrin. 200–300 Mesh silica gel was used to purify the products. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Brucker DRX spectrometer (600 MHz), and chemical shifts are reported in delta (δ) units, parts per million (ppm) downfield from trimethylsilane and hertz. Preparations were made in DMSO-d_6 solvent. ESI-MS were determined on an API 4000 spectrometer. High-resolution mass spectra (HRMS) spectra were conducted by Shandong Analysis and Test Center. Melting points were tested on an electrothermal melting point apparatus (uncorrected).

5.1.1. *N*-Protected (1*S*,2*S*)(+)-2-amino-1-(4-nitrophenyl)-1,3-propanediol (2)

A solution of di-*tert*-butyl dicarbonate (2.93 g, 11 mmol) in THF (5 mL) was added dropwise to a solution of compound **1** (2.12 g, 10 mmol) in THF (10 mL) and the mixture was stirred for 24 h at ambient temperature. Then the solvent was evaporated in vacuum and the residue was recrystallized with EtOAc to afford white solid (2.75 g), yield: 88%, mp: 115–116 °C, $[\alpha]_D^{20} = +23.1$ (c = 1, MeOH), ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.29 (s, 9H), 3.37–3.49 (m, 2H), 4.55–4.59 (m, 1H), 5.20 (d, J = 5.9 Hz, 1H), 6.09 (d, J = 9.0 Hz, 1H), 7.59 (d, J = 9.0 Hz, 2H), 8.18 (d, J = 9.0 Hz, 2H). ESI-MS *m/z*: 313.3 $[M+H]^+$.

5.1.2. *tert*-Butyl(1*S*,2*R*)-3-bromo-1-hydroxy-1-(4-nitrophenyl) propan-2-ylcarbamate (3)

The mixture of compound **2** (3.12 g, 10 mmol) and Ph_3P (3.93 g, 15 mmol) was dissolved in newly distilled THF (100 mL) in ice-bath condition in a flask. Then pyridine of 0.8 mL (0.79 g, 10 mmol) was added dropwise, subsequently, solid *N*-bromosuccinimide (NBS) (2.67 g, 15 mmol) was also added into the flask in three equal parts by 10 min interval. The solution was stirred at 0 °C for 1 h, and then continuously stirred at room temperature for 8 h. The solid was filtrated and solvent was evaporated in vacuum to leave residue, which was washed by ether (100 mL) and solid produced here was filtrated and rejected. The filtrate was washed with 1 N H_3PO_4, saturated $NaHCO_3$, and brine in turn, then the organic phase was dried with anhydrous $MgSO_4$ and concentrated to give a maroon oil. Finally, the crude product was depurated by fast column chromatograph (EtOAc/PE = 3:1) to afford compound **3** as a white solid (1.87 g), yield: 50%, mp: 116–119 °C, $[\alpha]_D^{20} = -34.35$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.21 (s, 9H), 3.34 (s, 1H), 3.51–3.54 (m, 1H), 3.63–3.68 (m, 1H), 4.72–4.79 (m, 1H), 4.93–4.97 (m, 1H), 5.60 (d, J = 5.4 Hz, 1H), 6.19 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H); ESI-MS *m/z:* 375.3, 377.4 $[M+1]^+$.

5.1.3. *tert*-Butyl(1*S*,2*S*)-3-azido-1-hydroxy-1-(4-nitrophenyl)propan-2-ylcarbamate (4)

Solid sodium azide (0.97 g, 15 mmol) was added slowly into the solution of compound **3** (3.75 g, 10 mmol) in anhydrous DMF (10 mL). Keep the reaction at 65 °C for 12 h with nitrogen protection in situ. Then cool the solution to room temperature, and pour the mixture into ice-water (60 mL), stir quickly with a glass bar to get white solid precipitation. And separate the solid by filtration in the reduced pressure and dry it completely in vacuum to get compound **4** (2.93 g), yield: 87%, mp: 143–145 °C, $[\alpha]_D^{20} = +46.05$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.22 (s, 9H), 3.34 (s, 1H), 3.50–3.55 (m, 1H), 3.64–3.67 (m, 1H), 4.75–4.80 (m, 1H), 4.92–4.96 (m, 1H), 5.60 (d, J = 5.4 Hz, 1H), 6.19 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H); ESI-MS *m/z:* 338.5 $[M+1]^+$.

5.1.4. *tert*-Butyl(1*S*,2*S*)-3-amino-1-hydroxy-1-(4-nitrophenyl) propan-2-ylcarbamate (5)

To a solution of compound **4** (3.37 g, 10 mmol) in THF (80 mL, 0.5% water), PPh_3 (3.14 g, 12 mmol) was added at ambient

temperature and reacted for 24 h, then most of the solvent was evaporated off, and the residue was redissolved in EtOAc (40 mL). Then extract the product with 1 N H_3PO_4 (10 mL × 3), incorporate the inorganic phase and adjust the pH to 10, then reextract the product with EtOAc (20 mL × 3). Following, the organic phase was incorporated and concentrated in vacuum to give light yellow solid, which was recrystallized by EtOAc/petrol ether to give compound **5** in white solid form (2.27 g), yield: 73%, mp: 197–201 °C, $[\alpha]_D^{20} = +35.21$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.23 (s, 9H), 3.34 (s, 1H), 3.52–3.55 (m, 1H), 3.62–3.67 (m, 1H), 4.75–4.81 (m, 1H), 4.94–4.96 (m, 1H), 5.60 (d, J = 5.4 Hz, 1H), 6.19 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H); ESI-MS m/z: 312.2 $[M+1]^+$.

5.1.5. *N*-Protected(*S*)-2-amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propyl)-4-methylpentanamide (6d)

Compound **5** (3.11 g, 10 mmol), Boc-Leu (2.54 g, 11 mmol), and HOBt (1.62 g, 12 mmol) were mixed and dissolved in distilled DCM (80 mL) in ice-bath condition and then TEA (1.11 g, 11 mmol) was added followed the addition of EDCI (3.82 g, 20 mmol) slowly. Then keep the reaction at 0 °C for 1 h, remove the ice bath and stir the solution for 12 h at room temperature. The solvent was evaporated off in vacuo and the residue was redissolved with EtOAc (80 mL), which was washed with 1 N citric acid, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to give the crude product, which was purified by fast column chromatograph (EtOAc/petrol ether = 2:1) to afford compound **6d** as a white solid (3.67 g), yield: 70%, mp: 200–201 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.81 (d, J = 6.6 Hz, 6H), 1.08–1.23 (m, 2H), 1.30 (s, 9H), 1.35 (s, 9H), 1.56–1.62 (m, 1H), 2.84–2.89 (m, 1H), 3.52–3.57 (m, 1H), 3.63–3.68 (m, 1H), 4.73–4.80 (m, 1H), 4.91–4.97 (m, 1H), 5.60 (d, J = 5.4 Hz, 1H), 6.19 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H); ESI-MS m/z: 525.4 $[M+1]^+$.

5.1.6. *N*-Protected(*S*)-2-amino-*N*-((2*S*,3*S*)-2-amino-3-(4-aminophenyl)-3-hydroxypropyl)-4-methylpentanamide (7a)

Compound **6d** (5.25 g, 10 mmol) was mixed with activated carbon (0.12 g, 10 mmol) with quantum sufficit $FeCl_3{\cdot}6H_2O$ as catalyst in situ in MeOH (100 mL). When the solution was heated to boil, hydrazine hydrate (80%) (9.6 g, 300 mmol) was added dropwise slowly to it and keep the reaction refluxing for 3 h until the yellow color disappeared. Then cool down the solution to room temperature, and solid was filtrated. The filtrate was concentrated by a rotary evaporator and residue was washed with water and saturated $NaHCO_3$ in turn and extracted by EtOAc (30 mL × 3). Subsequently, the organic phase was concentrated to give the crude product, which was recrystallized with EtOAc/petrol ether to give pure compound **7a** (4.45 g), yield: 90%, mp: 199–200 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.81 (d, J = 6.6 Hz, 6H), 1.09–1.23 (m, 2H), 1.30 (s, 9H), 1.35 (s, 9H), 1.56–1.62 (m, 1H), 2.87–2.89 (m, 1H), 3.51–3.57 (m, 1H), 3.62–3.68 (m, 1H), 4.75–4.79 (m, 1H), 4.93–4.97 (m, 1H), 5.60 (d, J = 5.4 Hz, 1H), 6.19 (d, J = 9.0 Hz, 1H), 6.47 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 8.4 Hz, 2H); ESI-MS m/z: 495.5 $[M+1]^+$.

5.1.7. *N*-Protected(*S*)-2-amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-iodophenyl)propyl)-4-methylpentanamide (8a)

To a solution of *p*-TsOH (5.16 g, 30 mmol) in chromatographic pure MeCN (30 mL) was added compound **7a** (4.94 g, 10 mmol) and the mixture was cooled to 10–15 °C. To this suspension, a solution of $NaNO_2$ (1.38 g, 20 mmol) and KI (4.15 g, 25 mmol) in H_2O (6 mL) was gradually added. The reaction mixture was stirred for 10 min, then allowed to come to room temperature for continuously reacting for 30 min. H_2O (100 mL) was then added and pH was adjusted to about 10 over $NaHCO_3$. The product was then extracted with EtOAc and purified by fast column chromatograph (EtOAc/petrol ether = 3:1) to obtain compound **8a** as a stramineous solid (5.44 g), yield: 90%, mp: 120–122 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.81 (d, J = 6.6 Hz, 6H), 1.07–1.20 (m, 2H), 1.30 (s, 9H), 1.35 (s, 9H), 1.56–1.62 (m, 1H), 2.84–2.89 (m, 1H), 3.51–3.56 (m, 1H), 3.64–3.67 (m, 1H), 4.74–4.79 (m, 1H), 4.92–4.96 (m, 1H), 5.60 (d, J = 5.4 Hz, 1H), 6.19 (d, J = 9.0 Hz, 1H), 7.77 (d, J = 8.4 Hz, 2H), 8.31 (d, J = 8.4 Hz, 2H); ESI-MS m/z: 606.3 $[M+1]^+$.

5.1.8. (*S*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propyl)-4-methylpentanamide (9d)

A solution of compound **6d** (5.25 g, 10 mmol) in distilled EtOAc was added dropwise to HCl gas saturated EtOAc solution. Keep the reaction at room temperature for 4 h, and evaporate the liquid to get the crude product, which was recrystallized to produce a white solid **9d** (3.08 g), yield: 95%, mp: 194–195 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.81 (d, J = 6.6 Hz, 6H), 1.09–1.21 (m, 2H), 1.54–1.60 (m, 1H), 2.83–2.87 (m, 1H), 3.52–3.56 (m, 1H), 3.68–3.71 (m, 1H), 4.76–4.80 (m, 1H), 5.04–5.08 (m, 1H), 6.29 (d, J = 9.0 Hz, 1H), 7.66 (d, J = 8.8 Hz, 2H), 8.18 (d, J = 8.8 Hz, 2H); HRMS (AP-ESI) m/z Calcd for $C_{15}H_{24}N_4O_4$ $[M+H]^+$ 325.1798. Found: 325.1788.

The other compounds of **9** series were synthesized via the same route as described above.

5.1.9. (*S*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propyl)-3-hydroxypropanamide (9a)

White solid, yield: 87%, mp: 175–176 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.30–3.32 (m, 1H), 3.33–3.35 (m, 1H), 3.41–3.44 (m, 1H), 3.75–3.78 (m, 1H), 3.83–3.84 (m, 1H), 3.85–3.87 (m, 1H), 4.95 (d, J = 6.0 Hz, 1H), 6.70 (d, J = 9.0 Hz, 1H), 7.66 (d, J = 8.8 Hz, 2H), 8.18 (d, J = 8.8 Hz, 2H); HRMS (AP-ESI) m/z Calcd for $C_{12}H_{18}N_4O_5$ $[M+H]^+$ 299.1277. Found: 299.1285.

5.1.10. (*S*)-3-Amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propyl)-2-hydroxypropanamide (9b)

White solid, yield: 88%, mp: 158–160 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.92–2.94 (m, 1H), 3.06–3.09 (m, 1H), 3.22–3.24 (m, 1H), 3.34–3.37 (m, 1H), 3.46–3.49 (m, 1H), 4.17–4.20 (m, 1H), 4.88 (d, J = 6.0 Hz, 1H), 6.45 (d, J = 9.0 Hz, 1H), 7.73 (d, J = 9.0 Hz, 2H), 8.26 (d, J = 9.0 Hz, 2H); HRMS (AP-ESI) m/z Calcd for $C_{12}H_{18}N_4O_5$ $[M+H]^+$ 299.1277. Found: 299.1265.

5.1.11. (*R*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propyl)-3-mercaptopropanamide (9c)

White solid, yield: 54%, mp: 156–159 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.30–3.34 (m, 1H), 3.33–3.36 (m, 1H), 3.42–3.45 (m, 1H), 3.76–3.79 (m, 1H), 3.83–3.84 (m, 1H), 3.85–3.87 (m, 1H), 4.96 (d, J = 6.0 Hz, 1H), 6.90 (d, J = 9.0 Hz, 1H), 7.73 (d, J = 9.0 Hz, 2H), 8.26 (d, J = 9.0 Hz, 2H); HRMS (AP-ESI) m/z Calcd for $C_{12}H_{18}N_4O_4S$ $[M+H]^+$ 315.1049. Found: 315.1056.

5.1.12. (*S*)-2,6-Diamino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propyl)hexanamide (9e)

White solid, yield: 64%, mp: 155–158 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.34–1.38 (m, 2H), 1.56–1.59 (m, 2H), 1.74–1.78 (m, 2H), 2.74–2.76 (m, 2H), 3.17–3.20 (m, 1H), 3.41–3.46 (m, 1H), 3.47–3.50 (m, 1H), 3.79–3.81 (m, 1H), 4.99 (d, J = 7.2 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 8.4 Hz, 2H), 8.27 (d, J = 8.4 Hz, 2H); HRMS (AP-ESI) m/z Calcd for $C_{15}H_{25}N_5O_4$ $[M+H]^+$ 340.1907. Found: 340.1926.

5.1.13. (*R*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propyl)-3-phenylpropanamide (9f)

White solid, yield: 87%, mp: 185–187 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.99–3.03 (m, 1H), 3.06–3.07 (m,1H), 3.08–3.13 (m, 1H), 3.31–3.35 (m, 1H), 3.42–3.46 (m, 1H), 4.03–4.05 (m, 1H), 4.92 (d, J = 6.0 Hz, 1H), 7.23–7.32 (m, 5H), 7.72 (d, J = 8.4 Hz, 2H),

8.22 (d, J = 8.4 Hz, 2H), 9.16 (t, J = 5.4 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{18}H_{22}N_4O_4$ $[M+H]^+$ 359.1641. Found: 359.1655.

5.1.14. (*S*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitro phenyl)propyl)-3-(4-hydroxyphenyl)propanamide (9g)

White solid, yield: 87%, mp: 189–191 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.99–3.04 (m, 1H), 3.08–3.10 (m, 1H), 3.12–3.13 (m, 1H), 3.32–3.36 (m, 1H), 3.42–3.47 (m, 1H), 4.03–4.05 (m, 1H), 4.92 (d, J = 6.0 Hz, 1H), 6.75 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H), 8.25 (d, J = 8.4 Hz, 2H), 9.01 (t, J = 6.0 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{18}H_{22}N_4O_5$ $[M+H]^+$ 375.1590. Found: 375.1575.

5.1.15. (*S*)-*N*-((2*S*,3*S*)-2-Amino-3-hydroxy-3-(4-nitrophenyl) propyl)-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (9h)

White solid, yield: 73%, mp: 159–161 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.87–2.89 (m, 1H), 2.90–3.09 (m, 1H), 3.11–3.13 (m, 1H), 3.32–3.35 (m, 1H), 3.36–3.38 (m, 2H), 3.41–3.42 (m, 1H), 4.29–4.40 (m, 1H), 4.88 (d, J = 6.0 Hz, 1H), 6.56 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 7.8 Hz, 1H), 6.92 (s, 1H), 7.13 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 8.4 Hz, 2H), 8.25 (d, J = 8.4 Hz, 2H); HRMS (AP-ESI) m/z Calcd for $C_{19}H_{22}N_4O_5$ $[M+H]^+$ 387.1590. Found: 387.1580.

5.1.16. (*S*)-2-((*S*)-3-Amino-2-hydroxypropanamido)-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propyl)-4-methylpentanamide (9i)

White solid, yield: 80%, mp: 107–109 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.85 (d, J = 6.0 Hz, 3H), 0.88 (d, J = 6.0 Hz, 3H), 1.49–1.57 (m, 3H), 2.78–2.81 (m, 1H), 3.05–3.10 (m, 2H), 3.13–3.16 (m, 2H), 4.19–4.21 (m, 1H), 4.26–4.29 (m, 1H), 4.76 (d, J = 4.2 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 1H), 8.22 (d, J = 8.4 Hz, 2H), 8.24 (d, J = 8.4 Hz, 2H); HRMS (AP-ESI) m/z Calcd for $C_{18}H_{29}N_5O_6$ $[M+H]^+$ 412.2118. Found: 412.2132.

5.1.17. (*S*)-2-Amino-*N*-((*R*)-1-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propylamino)-1-oxo-3-phenylpropan-2-yl)-4-methylpentanamide (9j)

White solid, yield: 82%, mp: 165–166 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.79 (d, J = 6.0 Hz, 3H), 0.82 (d, J = 6.0 Hz, 3H), 1.22–1.24 (m, 1H), 1.29–1.30 (m, 1H), 1.46–1.48 (m, 1H), 2.76–2.80 (m, 1H), 2.93–2.98 (m, 2H), 3.81–3.82 (m, 1H), 3.89–3.93 (m, 1H), 4.49–4.53 (m, 1H), 4.75 (d, J = 3.4 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 7.15–7.23 (m, 5H), 7.53 (d, J = 8.4 Hz, 2H), 7.83 (d, J = 7.8 Hz, 1H), 8.17 (d, J = 8.4 Hz, 2H); HRMS (AP-ESI) m/z Calcd for $C_{24}H_{33}N_5O_5$ $[M+H]^+$ 472.2482. Found: 472.2486.

5.1.18. (*R*)-2-Amino-*N*-((*S*)-1-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propylamino)-3-hydroxy-1-oxopropan-2-yl)-3-phenylpropanamide (9k)

White solid, yield: 76%, mp: 103–105 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.59–2.61 (m, 1H), 2.81–2.84 (m, 1H), 2.98–3.04 (m, 2H), 3.07–3.12 (m, 1H), 3.44–3.47 (m, 2H), 3.60–3.63 (m, 1H), 4.21–4.24 (m, 1H), 4.64 (d, J = 4.0 Hz, 1H), 7.18–7.28 (m, 5H), 7.59 (d, J = 9 Hz, 2H), 7.85 (t, J = 5.4 Hz, 1H), 8.07 (d, J = 7.8 Hz, 1H), 8.18 (d, J = 9 Hz, 2H); HRMS (AP-ESI) m/z Calcd for $C_{21}H_{27}N_5O_6$ $[M+H]^+$ 446.1961. Found: 446.1962.

5.1.19. (*R*)-2-Amino-*N*-((*S*)-3-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propylamino)-2-hydroxy-3-oxopropyl)-3-phenylpropanamide (9l)

White solid, yield: 80%, mp: 174–175 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.93–2.97 (m, 1H), 3.09–3.13 (m, 2H), 3.24–3.27 (m, 1H), 3.30–3.34 (m, 1H), 3.45–3.46 (m, 1H), 3.48–3.52 (m, 1H), 4.03–4.05 (m, 2H), 4.88 (t, J = 4.8 Hz, 1H), 7.24–7.32 (m, 5H), 7.70 (d, J = 9 Hz, 2H), 8.17 (t, J = 6 Hz, 1H), 8.24 (d, J = 9 Hz, 2H), 8.71 (t, J = 6 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{21}H_{27}N_5O_6$ $[M+H]^+$ 446.1961. Found: 446.1975.

5.1.20. (*R*)-2-Amino-*N*-((*R*)-1-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propylamino)-1-oxo-3-phenylpropan-2-yl)-3-phenylpropanamide (9m)

White solid, yield: 86%, mp: 121–122 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.62–2.65 (m, 1H), 2.72–2.76 (m, 1H), 2.82–2.85 (m, 1H), 3.12–3.14 (m, 1H), 3.29–3.39 (m, 5H), 4.49–4.52 (m, 1H), 6.59 (d, J = 3.6 Hz, 1H), 7.18–7.27 (m, 10H), 7.68 (d, J = 8.4 Hz, 2H), 8.26 (d, J = 8.4 Hz, 1H), 9.12 (d, J = 7.8 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{27}H_{31}N_5O_5$ $[M+H]^+$ 506.2325. Found: 506.2332.

5.1.21. (*S*)-*N*-((2*S*,3*S*)-2-Amino-3-hydroxy-3-(4-nitrophenyl) propyl)-2-((*R*)-2-amino-3-phenylpropanamido)-4-methylpentanamide (9n)

White solid, yield: 80%, mp: 118–119 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.85 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 1.05–1.08 (m, 1H), 1.44–1.49 (m, 2H), 2.94–2.98 (m, 2H), 3.01–3.15 (m, 1H), 3.15–3.18 (m, 1H), 4.02–4.07 (m, 1H), 4.21–4.24 (m, 1H), 4.92 (d, J = 4.2 Hz, 1H), 6.64 (d, J = 4.2 Hz, 1H), 7.29–7.32 (m, 5H), 7.66 (d, J = 7.8 Hz, 2H), 8.25 (d, J = 8.4 Hz, 2H), 8.84 (d, J = 7.8 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{24}H_{33}N_5O_5$ $[M+H]^+$ 472.2482. Found: 472.2488.

5.1.22. (*S*)-3-Amino-*N*-((*R*)-1-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-nitrophenyl)propylamino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxypropanamide (9o)

White solid, yield: 78%, mp: 210–212 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.49–2.51 (m, 1H), 2.83–2.85 (m, 1H), 2.90–2.94 (m, 1H), 3.16–3.19 (m, 2H), 3.41–3.43 (m, 1H), 3.8 (br s, 1H), 4.16 (t, J = 4.8 Hz, 1H), 4.50 (d, J = 4.2 Hz, 1H), 4.94 (t, J = 4.8 Hz, 1H), 7.18–7.26 (m, 5H), 7.69 (d, J = 9 Hz, 2H), 8.14 (d, J = 8.4 Hz, 1H), 8.27 (d, J = 9 Hz, 2H), 8.52 (d, J = 6 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{21}H_{27}N_5O_6$ $[M+H]^+$ 446.1961. Found: 446.1971.

5.1.23. (*S*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-(4-aminophenyl)-3-hydroxypropyl)-4-methylpentanamide (10a)

A solution of compound **7a** (4.94 g, 10 mmol) in distilled EtOAc was added dropwise to HCl gas saturated EtOAc solution. Keep the reaction at room temperature for 8 h, and evaporate the liquid to get the crude product, which was recrystallized to produce a white solid **10a** (2.65 g), yield: 90%, mp: 184–185 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.83 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 1.17–1.22 (m, 1H), 1.36–1.40 (m, 1H), 1.66–1.71 (m, 1H), 2.70–2.76 (m, 1H), 2.98–3.02 (m, 1H), 3.02–3.13 (m, 1H), 4.15 (br s, 1H), 6.50 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 7.84 (s, 1H); HRMS (AP-ESI) m/z Calcd for $C_{15}H_{26}N_4O_2$ $[M+H]^+$ 295.2056. Found: 295.2064.

The other compounds of **10** series were synthesized via the same route as described above.

5.1.24. (*S*)-2,6-Diamino-*N*-((2*S*,3*S*)-2-amino-3-(4-aminophenyl)-3-hydroxypropyl)hexanamide (10b)

Light yellow solid, yield: 70%, mp: 167–168 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.33–1.36 (m, 2H), 1.52–1.53 (m, 2H), 1.73–1.78 (m, 2H), 2.64–2.66 (m, 2H), 3.07–3.12 (m, 1H), 3.40–3.44 (m, 1H), 3.47–3.50 (m, 1H), 3.76–3.81 (m, 1H), 4.78 (d, J = 6.8 Hz, 1H), 6.53 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H), 8.07 (d, J = 7.8 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{15}H_{27}N_5O_2$ $[M+H]^+$ 310.2165. Found: 310.2172.

5.1.25. (*R*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-(4-aminophenyl)-3-hydroxypropyl)-3-phenylpropanamide (10c)

White solid, yield: 80%, mp: 190–191 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.91–2.93 (m, 1H), 2.96–2.98 (m, 1H), 3.00–3.12 (m,

1H), 3.19 (s, 1H), 3.33–3.38 (m, 1H), 3.99 (d, J = 5.4 Hz, 1H), 4.64 (d, J = 7.2 Hz, 1H), 7.01 (d, J = 8.4 Hz, 2H), 7.23–7.32 (m, 5H), 7.45 (d, J = 8.4 Hz, 2H), 9.03 (t, J = 6.0 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{18}H_{24}N_4O_2$ $[M+H]^+$ 329.1899. Found: 329.1889.

5.1.26. (*S*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-(4-aminophenyl)-3-hydroxypropyl)-3-(4-hydroxyphenyl)propanamide (10d)

Yellow solid, yield: 74%, mp: 123–124 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.45–2.47 (m, 1H), 2.63–2.66 (m, 1H), 2.69–2.73 (m, 1H), 2.77–2.80 (m, 1H), 2.98–3.02 (m, 1H), 3.25–2.27 (m, 2H), 4.10 (d, J = 6.0 Hz, 1H), 6.50 (d, J = 9.0 Hz, 2H), 6.65 (d, J = 9.0 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.4 Hz, 2H), 7.74 (t, J = 5.4 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{18}H_{24}N_4O_3$ $[M+H]^+$ 345.1848. Found: 345.1859.

5.1.27. (*S*)-2-Amino-*N*-((*R*)-1-((2*S*,3*S*)-2-amino-3-(4-aminophenyl)-3-hydroxypropylamino)-1-oxo-3-phenylpropan-2-yl)-4-methylpentanamide (10e)

White solid, yield: 82%, mp: 156–158 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.77 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H), 1.04–1.09 (m, 1H), 1.22–1.26 (m, 1H), 1.58–1.60 (m, 1H), 2.64–2.67 (m, 1H), 2.73–2.82 (m, 2H), 2.94–2.99 (m, 2H), 3.11–3.26 (m, 1H), 4.47–4.48 (m, 1H), 6.50 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 7.15–7.25 (m, 5H), 7.77–7.91 (t, J = 5.4 Hz, 1H), 8.05 (d, J = 7.8 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{24}H_{35}N_5O_3$ $[M+H]^+$ 442.2740. Found: 442.2754.

5.1.28. (*R*)-2-Amino-*N*-((*R*)-1-((2*S*,3*S*)-2-amino-3-(4-aminophenyl)-3-hydroxypropylamino)-1-oxo-3-phenylpropan-2-yl)-3-phenylpropanamide (10f)

White solid, yield: 80%, mp: 183–184 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.61–2.65 (m, 1H), 2.73–2.77 (m, 1H), 2.82–2.85 (m, 1H), 3.14–3.17 (m, 1H), 3.20–3.25 (m, 2H), 3.37–3.39 (m, 1H), 4.08–4.12 (m, 1H), 4.42–4.45 (m, 1H), 4.74 (d, J = 6.6 Hz, 1H), 7.18 (m, 1H), 7.21–7.29 (m, 5H), 7.38 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 8.20 (br s, 5H), 9.11 (d, J = 9 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{27}H_{33}N_5O_3$ $[M+H]^+$ 476.2583. Found: 476.2589.

5.1.29. (*S*)-*N*-((2*S*,3*S*)-2-Amino-3-(4-aminophenyl)-3-hydroxypropyl)-2-((*R*)-2-amino-3-phenylpropanamido)-4-methylpentanamide (10g)

White solid, yield: 79%, mp: 176–178 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.83 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H), 1.04–1.09 (m, 1H), 1.40–1.45 (m, 2H), 2.84–2.87 (m, 2H), 3.01–3.10 (m, 1H), 3.15–3.18 (m, 1H), 4.01–4.06 (m, 1H), 4.20–4.22 (m, 1H), 4.82 (d, J = 4.2 Hz, 1H), 6.50 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 7.29–7.32 (m, 5H), 7.91 (d, J = 5.8 Hz, 1H), 8.84 (d, J = 7.8 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{24}H_{35}N_5O_3$ $[M+H]^+$ 442.2740. Found: 442.2766.

5.1.30. (*S*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-iodophenyl)propyl)-4-methylpentanamide (11a)

A solution of compound **8a** (6.05 g, 10 mmol) in distilled EtOAc was added dropwise to HCl gas saturated EtOAc solution. Keep the reaction at room temperature for 8 h, and evaporate the liquid to get the crude product, which was recrystallized to produce a white solid **11a** (3.65 g), yield: 89%, mp: 154–155 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.87 (d, J = 6.0 Hz, 3H), 0.89 (d, J = 6.0 Hz, 3H), 1.49–1.51 (m, 1H), 1.51–1.53 (m, 2H), 2.99–3.03 (m, 1H), 3.44–3.48 (m, 1H), 3.71–3.72 (m, 1H), 4.70 (d, J = 6.0 Hz, 1H), 8.16 (d, J = 10.4 Hz, 2H), 8.36 (d, J = 10.4 Hz, 2H), 9.01 (t, J = 6.0 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{15}H_{24}IN_3O_2$ $[M+H]^+$ 406.0913. Found: 406.0923.

The other compounds of **11** series were synthesized via the same route as described above.

5.1.31. (*S*)-2-Amino-*N*-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-iodophenyl)propyl)-3-(4-hydroxyphenyl)propanamide (11b)

White solid, yield: 92%, mp: 175–177 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.83–2.88 (m, 1H), 2.93–3.00 (m, 2H), 3.05–3.10 (m, 1H), 3.89–3.93 (m, 2H), 4.68 (d, J = 5.4 Hz, 1H), 6.70 (d, J = 8.4 Hz, 2H), 7.04 (d, J = 8.4 Hz, 2H), 8.16 (d, J = 9.0 Hz, 2H), 8.33 (d, J = 9.0 Hz, 2H), 9.01 (t, J = 6.0 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{18}H_{22}IN_3O_3$ $[M+H]^+$ 456.0706. Found: 456.0722.

5.1.32. (*S*)-2-Amino-*N*-((*R*)-1-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-iodophenyl)propylamino)-1-oxo-3-phenylpropan-2-yl)-4-methylpentanamide (11c)

White solid, yield: 85%, mp: 140–141 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.80 (d, J = 6.0 Hz, 3H), 0.83 (d, J = 6.0 Hz, 3H), 1.21–1.22 (m, 1H), 1.25–1.28 (m, 1H), 1.42–1.44 (m, 1H), 2.74–2.78 (m, 1H), 2.93–2.97 (m, 2H), 3.82–3.85 (m, 1H), 3.89–3.95 (m, 1H), 4.59–4.62 (m, 1H), 4.69 (br s, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.21–7.23 (m, 5H), 7.43 (d, J = 9.0 Hz, 2H), 7.97 (d, J = 9.0 Hz, 2H), 8.83 (d, J = 7.8 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{24}H_{33}IN_4O_3$ $[M+H]^+$ 553.1597. Found: 553.1605.

5.1.33. (*R*)-2-Amino-*N*-((*R*)-1-((2*S*,3*S*)-2-amino-3-hydroxy-3-(4-iodophenyl)propylamino)-1-oxo-3-phenylpropan-2-yl)-3-phenylpropanamide (11d)

White solid, yield: 88%, mp: 148–149 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.56–2.61 (m, 1H), 2.70–2.74 (m, 1H), 2.80–2.83 (m, 1H), 3.08–3.11 (m, 1H), 3.17–3.20 (m, 1H), 3.27–3.32 (m, 2H), 4.03–4.05 (m, 1H), 4.51–4.55 (m, 1H), 4.65–4.88 (m, 1H), 7.18 (d, J = 8.4 Hz, 2H), 7.22–7.28 (m, 6H), 7.76 (d, J = 8.4 Hz, 2H), 8.06 (br s, 5H), 8.97 (d, J = 8.4 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{27}H_{31}IN_4O_3$ $[M+H]^+$ 587.1441. Found: 587.1456.

5.1.34. (*S*)-*N*-((2*S*,3*S*)-2-Amino-3-hydroxy-3-(4-iodophenyl)propyl)-2-((*R*)-2-amino-3-phenylpropanamido)-4-methylpentanamide (11e)

White solid, yield: 84%, mp: 148–150 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.83 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 1.06–1.07 (m, 1H), 1.40–1.42 (m, 2H), 2.84–2.87 (m, 2H), 3.11–3.15 (m, 1H), 3.25–3.29 (m, 1H), 4.06–4.09 (m, 1H), 4.31–4.35 (m, 1H), 4.85 (d, J = 4.2 Hz, 1H), 6.95 (d, J = 5.2 Hz, 1H), 7.27–7.30 (m, 5H), 7.46 (d, J = 9.0 Hz, 2H), 7.92 (d, J = 9.0 Hz, 2H), 8.75 (d, J = 8.4 Hz, 1H); HRMS (AP-ESI) m/z Calcd for $C_{24}H_{33}IN_4O_3$ $[M+H]^+$ 553.1597. Found: 553.1610.

5.2. Biological screening

5.2.1. In vitro APN inhibition assay

IC_{50} values against APN were determined as previously described[19,20] by using L-Leu-*p*-nitroanilide as substrate and Microsomal aminopeptidase from Porcine Kidney Microsomes (Sigma) as enzyme in 50 mM PBS (pH 7.2) or suspension of HL-60 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 changes of OD values at 405 nm.

5.2.2. In vitro MMP inhibition assay

Enzyme MMP-2 and developer TNBS were both purchased from Sigma, and the substance was synthesized as described by Baragi et al.[21] The enzyme, substance and inhibitor were dissolved in 50 mM sodium borate (pH 8.5) in a 96-well plate and incubated for 30 min at 37 °C, and then TNBS (0.03%, m/v) was added and incubated for another 20 min at room temperature, the resulting solution was detected under 450 nm wavelength to gain absorption.

5.2.3. MTT assay

HL-60 cells (high APN expression) and MDA-MB-231 cells (low APN expression) were cultured in RPMI1640 medium containing 10% FBS in situ at 37 °C in 5% CO_2 humidified incubator. Cell proliferation was determined by MTT [(3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazolium bromide)] assay. Briefly, cells were seeded in a 96-well plate (1×10^4/well) and cultured for 4 h, followed by addition of different concentrations of inhibitors. After another 48 h treatment, 1% MTT (0.5 mg/mL) solution was added to form the formazan product. Finally, DMSO was used to solve the formazan and concentrations were monitored by detecting OD values at 570 nm through a plate reader (Varioskan, Thermo, USA).

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References and notes

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