



## Design and synthesis of novel chloramphenicol amine derivatives as potent aminopeptidase N (APN/CD13) inhibitors

Kanghui Yang<sup>a</sup>, Qiang Wang<sup>a</sup>, Li Su<sup>a</sup>, Hao Fang<sup>a,\*</sup>, Xuejian Wang<sup>a</sup>, Jianzhi Gong<sup>a</sup>, Binghe Wang<sup>b</sup>, Wenfang Xu<sup>a,\*</sup>

<sup>a</sup> Department of Medicinal Chemistry, School of Pharmacy, Shandong University, 44 West Culture Road, Ji'nan, Shandong 250012, China

<sup>b</sup> Department of Chemistry, Georgia State University, Atlanta, 30303, USA

### ARTICLE INFO

#### Article history:

Received 16 March 2009

Revised 17 April 2009

Accepted 18 April 2009

Available online 24 April 2009

#### Keywords:

Synthesis

Aminopeptidase N

Chloramphenicol amine

Inhibitor

QSAR

### ABSTRACT

Herein we report a series of novel chloramphenicol amine derivatives as aminopeptidase N (APN)/CD13 inhibitors. All compounds were synthesized starting from commercially available (1S,2S)-2-amino-1-(4-nitrophenyl) propane-1,3-diol. The preliminary biological screening showed that some compounds exhibited potent inhibitory activity against APN. It should be noted that one compound, **13b** ( $IC_{50} = 7.1 \mu M$ ), possess similar APN inhibitory activity compared with Bestatin ( $IC_{50} = 3.0 \mu M$ ).

© 2009 Published by Elsevier Ltd.

## 1. Introduction

Aminopeptidase N (APN/CD13) is a  $Zn^{2+}$ -dependent metalloprotease existing in a wide variety of human tissues and cell lines (endothelial, epithelial, fibroblast, leukocyte, etc.).<sup>1</sup> APN expression is dysregulated in inflammatory diseases and in cancers (solid and hematologic tumors). APN is also a receptor for coronaviruses, the pathogenesis of upper respiratory infection. In recent years, the identification of activities of anti-APN/CD13 monoclonal antibody and natural/synthetic APN inhibitors suggests that APN plays a crucial role in modulating bioactive peptide responses (pain management, vasopressin release) and in influencing immune functions and major biological events (cell proliferation, secretion, invasion, angiogenesis).<sup>2</sup> Therefore, the development of APN inhibitors may be of clinical significance for the discovery of anti-cancer and anti-inflammatory agents.

For most APN inhibitors reported in literatures, so far there are four possible binding sites, including a zinc binding group (ZBG), two hydrophobic groups interacting with the  $S_1$  and  $S_1'$  subsites in APN, and one appropriate group interacting with the  $S_2'$  subsite surrounded by several Arg residues.<sup>3–5</sup> It should be emphasized that among these four sites, at least three ones are necessary for a potent inhibitor. For example, AHPA ((2S,3R)-3-amino-2-hydro-

xyl-4-phenylbutanoic acid) is a common scaffold in many natural APN inhibitors. The co-crystal complex of APN and Bestatin (AHPA-Leu) from *Escherichia coli* indicated that the hydroxyl and carbonyl belong to zinc binding group (ZBG). The phenyl ring in AHPA fragment can insert into  $S_1$  pocket and amino group in AHPA can interact with Glu 350 (Fig. 1).

Our group has previously reported several kinds of new APN inhibitors, such as L-lysine derivatives<sup>6</sup>, 3-galloylamido-N'-substituted 2,6-piperidinedione-N-acetamide peptidomimetics<sup>7</sup>, 1,3,4-thiadiazole derivatives<sup>8</sup>, and AHPA ( $\beta$ -amino- $\alpha$ -hydroxy-phenylbutanoic acid) derivatives.<sup>9</sup> In our recent screening, compound (2R,3S)-2-amino-3-hydroxy-3-(4-nitrophenyl)propanoic acid (**1**,

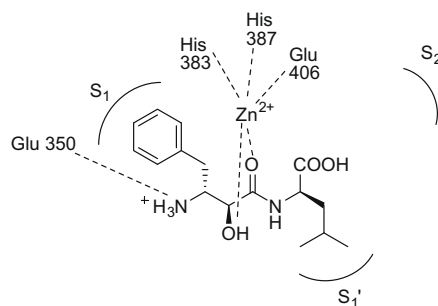


Figure 1. The binding mode of bestatin to the active sites of APN.

\* Corresponding authors. Tel./fax: +86 531 88382264 (W.X.).

E-mail addresses: [haofangcn@sdu.edu.cn](mailto:haofangcn@sdu.edu.cn) (H. Fang), [xuwenf@yahoo.cn](mailto:xuwenf@yahoo.cn), [wfxu@yahoo.cn](mailto:wfxu@yahoo.cn) (W. Xu).

AHNPA) developed from commercially available chloramphenicol amine **2**, exhibited a moderate APN inhibition ( $IC_{50} = 140.2 \mu M$ ). Considering the structural similarity between compound **1** and AHPA, this molecule could act as a new lead compound for further chemical modification and optimization. Therefore, different amino acids and other functional groups can be coupled with compound **1** to generate diverse dipeptide and tripeptide derivatives. In this article, we would like to describe the synthesis, enzymatic evaluation and possible binding mode of the chloramphenicol amine derivatives.

## 2. Chemistry

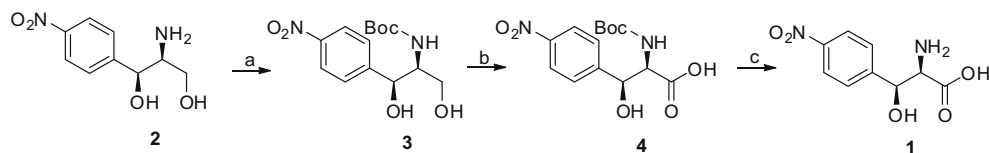
The synthetic route of target compounds is shown in Schemes 1 and 2. Chloramphenicol amine (1S,2R)-2-amino-1-(4-nitrophenyl)propane-1,3-diol, (**2**) was firstly protected by  $(Boc)_2O$ , then was selectively oxidized at the terminal hydroxyl group to give the N-Boc-protected acid **4**.<sup>10</sup> The compound **4** was easily deprotected with 3 N HCl in EtOAc to provide product **1**. The key intermediate **4** can be coupled with different substituted amine by classical EDCI/HOBt method, and then converted into compound **6a–6m** after deprotection. Using the same procedure, different moieties, such as  $\omega$ -amino acids, natural  $\alpha$ -amino acids and dipeptides, were coupled with intermediate **4** to obtain AHNPA derivatives **7a–7b**, **9a–9e**, **12a–12b**. Finally, the esters were hydrolyzed to carboxylic acid

by NaOH/H<sub>2</sub>O in methanol, and then converted into target compounds **8a–8b**, **10a–10e**, and **13a–13b** by deprotecting Boc group. Compounds **11a–11b** containing hydroxamic acid groups were obtained from **9a–9b** by reacting with NH<sub>2</sub>OK and then cleaving Boc.

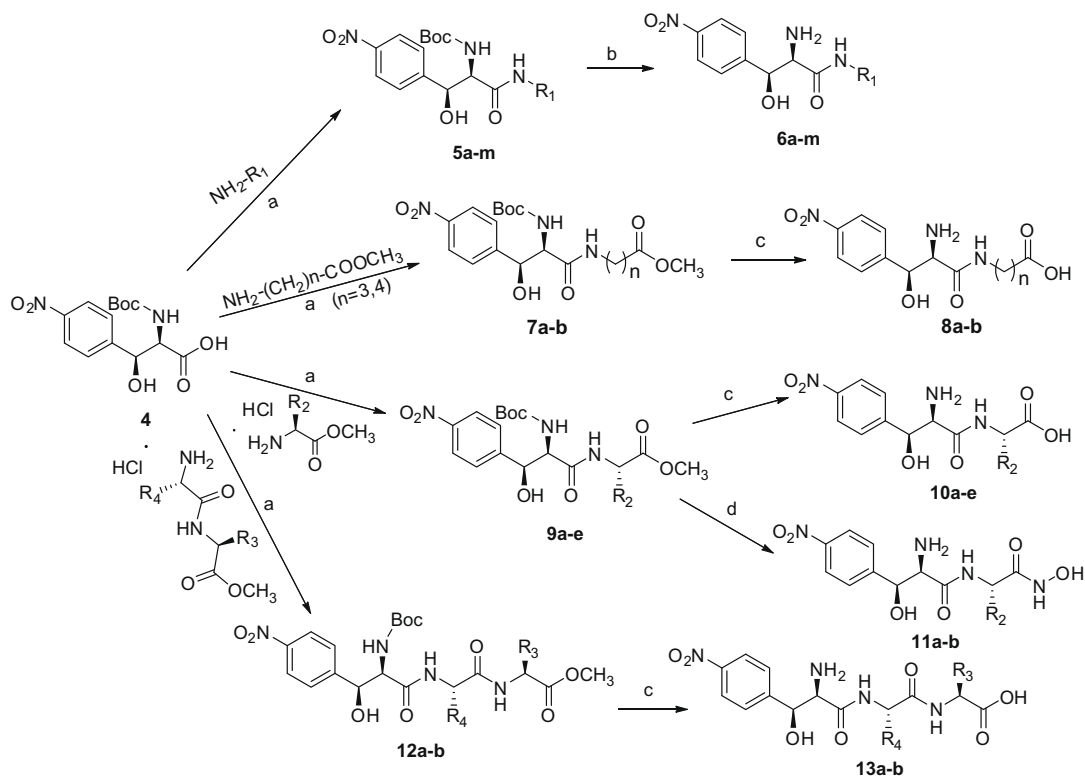
## 3. Results and discussion

All the target compounds were tested for their inhibition against APN as listed in Table 1. As a result, the structural addition of different alkylamine, simple benzylamine and phenethylamine can not significantly enhance the inhibitory activity compared with parent compound AHNPA ( $IC_{50} = 140.2 \mu M$ ). It seems that the substitution of phenyl ring in phenethyl group of R position can slightly contribute to the affinity. For example, *para*-hydroxy substitution shows up to 50  $\mu M$   $IC_{50}$  value.

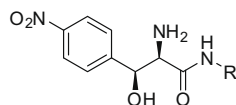
On the other hand, the incorporation of natural amino acids would generate compounds with similar or better inhibitory activities compared with parent compound AHNPA, while  $\omega$  amino acid derivative showed very poor inhibition (Table 2). These results suggest that the introduction of natural amino acids to AHNPA scaffold would be better than of simple nonpeptide fragment. As a zinc binding group (ZBG), hydroxamate group has been used in many enzyme inhibitors especially Zn<sup>2+</sup>-dependent enzyme to improve affinity.<sup>11</sup> In our case, the AHNPA-amino acid derivatives can



**Scheme 1.** Reagents and conditions: (a)  $(Boc)_2O$ /THF; (b) 13% w/w NaOCl, 5%  $NaHCO_3$ , TEMPO/ $CH_3COCH_3$ , H<sub>2</sub>O, 0 °C; (c) HCl/anhydrous EtOAc,  $Na_2CO_3$ .



**Scheme 2.** Reagents and conditions: (a) EDCI, HOBt/anhydrous THF, 0 °C to room temperature; (b) HCl/anhydrous EtOAc,  $Na_2CO_3$ ; (c) (i) 4% NaOH/ $CH_3OH$ ; (ii) HCl/anhydrous EtOAc,  $Na_2CO_3$ ; (d) (i)  $NH_2OK$ /anhydrous  $CH_3OH$ ; (ii) HCl/anhydrous EtOAc,  $Na_2CO_3$ .

**Table 1**The structure and inhibitory activities of compounds **6a–6m**, **8a–8b** against APN

| Compound  | R  | APN/IC <sub>50</sub> (μM) |
|-----------|--|---------------------------|
| <b>6a</b> | –CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> | 183.2                     |
| <b>6b</b> | –CH <sub>2</sub> (CH <sub>3</sub> ) <sub>2</sub> | 250.7                     |
| <b>6c</b> | –CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> | 232.6                     |
| <b>6d</b> | –CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> | 521.7                     |
| <b>6e</b> |  | 251.3                     |
| <b>6f</b> |  | 169.9                     |
| <b>6g</b> |  | 302.5                     |
| <b>6h</b> |  | 144.7                     |
| <b>6i</b> |  | 106.3                     |
| <b>6j</b> |  | 136.8                     |
| <b>6k</b> |  | 51.4                      |
| <b>6l</b> |  | 116.3                     |
| <b>6m</b> |  | 90.3                      |
| <b>8a</b> |  | 2891.2                    |
| <b>8b</b> |  | 1388.6                    |

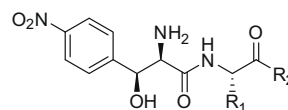
also enhance their activity after converting terminal carboxylate to hydroxamate group. For instance, compounds **11a** and **11b** shows more potent inhibition than compounds **10a** and **10b**.

The enzyme inhibitory results unveiled that AHNPA-dipeptide derivative **13b** shows the best inhibition among all target compounds. This phenomenon suggests that the dipeptide side chain would be helpful to increase the interaction with APN binding sites. Furthermore, compounds **13a** and **13b** were further assessed on their inhibition against HL-60 cell proliferation by using MTT method. The IC<sub>50</sub> values of these two compounds (**13a** 2.02 ± 0.13 mM and **13b** 2.21 ± 0.11 mM) confirmed again that their inhibitory activities are similar with Bestatin (1.65 ± 0.09 mM) (Fig. 2).

In order to determine the interaction between AHNPA derivatives and APN, compound **13b** was docked into the active sites of APN (PDB entry: 2DQM) using SYBYL 7.0.<sup>12</sup> The docking results showed that the carbonyl group and amine group of compound **13b** could chelate with the zinc ion in APN. The nitrophenyl group of the **13b** could insert to the pocket S1 by forming hydrophobic interactions with this subsite. The phenyl ring in the terminal amino acid residue could also insert to the pocket S<sub>2</sub>, in the meantime

**Table 2**

The structure and inhibitory activities of AHNPA-amino acid and AHPNPA-dipeptide derivatives against APN

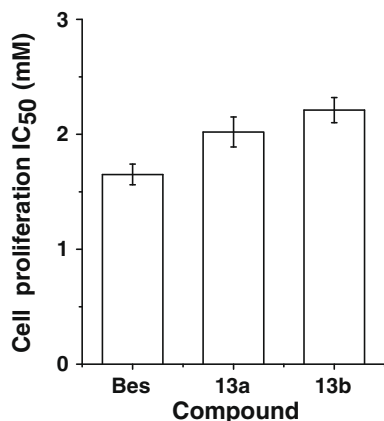


| Compound   | R <sub>1</sub> | R <sub>2</sub> | APN/IC <sub>50</sub> (μM) |
|------------|----------------|----------------|---------------------------|
| <b>10a</b> |                | –OH            | 85.6                      |
| <b>10b</b> |                | –OH            | 60.4                      |
| <b>10c</b> |                | –OH            | 159.9                     |
| <b>10d</b> |                | –OH            | 102.8                     |
| <b>10e</b> |                | –OH            | 162.2                     |
| <b>11a</b> |                | –NHOH          | 54.0                      |
| <b>11b</b> |                | –NHOH          | 31.3                      |
| <b>13a</b> |                |                | 34.6                      |
| <b>13b</b> |                |                | 7.10                      |
| Bestatin   |                |                | 3.00                      |

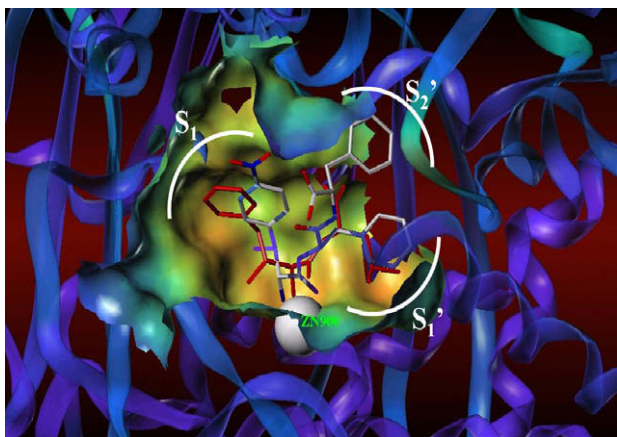
another phenyl group closed to the AHNPA scaffold could have interaction with the pocket S<sub>1</sub>' (Fig. 3). His<sup>297</sup>, Glu<sup>298</sup> and His<sup>301</sup> are the essential amino acids of the conserved sequence (HEX-XHX<sub>18</sub>E) in the catalytic domain of peptidase M1 family.<sup>13</sup> Compound **13b** could form hydrogen bonds with these three residues at the distance of 2.40 Å, 3.06 Å, 2.79 Å, respectively. In addition, the carboxylate group of **13b** could interact with Arg<sup>825</sup> and Tyr<sup>381</sup> residue in the S<sub>2</sub>' pocket by hydrogen bond for stabilizing the reaction intermediate with the zinc ion<sup>14</sup> (Fig. 4).

#### 4. Conclusions

In summary, we reported the synthesis and biological evaluation of a series of novel chloramphenicol amine derivatives as potent APN inhibitors. Compound **13b** is the most active. Among these compounds, compound **13b** not only exhibited similar enzymatic inhibition compared with natural APN inhibitor Bestatin, but



**Figure 2.** Effects of bestatin and compound **13a–13b** on the HL-60 cell line proliferation. Each column represents the mean values with S.E values for five independent experiments.



**Figure 3.** The docking result of **13b** is showed by SYBYL7.0 (Bestatin in the X-ray crystal is showed in red).

also showed good cell-based inhibitory activity. Therefore, **13b** could be a lead compound to search new chloramphenicol amine derivatives as APN inhibitors.

## 5. Experimental

### 5.1. APN inhibition assay

IC<sub>50</sub> values against APN were determined by using L-Leu-p-nitroanilide as substrate and Microsomal aminopeptidase from Porcine Kidney Microsomes (Sigma) as the enzyme in 50 mM PBS, pH 7.2, at 37 °C. The hydrolysis of the substrate was monitored by following the change in the absorbance measured at 405 nm with the UV–vis spectrophotometer Pharmacia LKB, Biochrom 4060. All solutions of inhibitors were prepared in the assay buffer, and pH was neutralized to 7.5 by the addition of 0.1 M HCl or 0.1 M NaOH. All inhibitors were pre-incubated with APN for 30 min at room temperature. The assay mixture, which contained the inhibitor solution (concentration dependent on the inhibitor), the enzyme solution (4 µg/mL final concentration), and the assay buffer, was adjusted to 200 µL.

### 5.2. MTT assay

HL-60 Cell was grown in RPMI1640 medium containing 10% FBS at 37 °C in 5% CO<sub>2</sub> humidified incubator. Cell proliferation was

determined by the MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) assay. Briefly, cells were plated in a 96-well plate at 10,000 cells per well, cultured for 4 h in complete growth medium, then treated with 2000, 1000, 500, 250, 125 µg/mL of compounds for 48 h. 0.5% MTT solution was added to each well. After further incubation for 4 hr, formazan formed from MTT was extracted by adding DMSO and mixing for 15 min. Optical density was read with an ELISA reader at 570 nm.

### 5.3. Chemistry: general procedures

The starting material **2** is a white powder (mp = 159–162 °C,  $[\alpha]_D^{25} = +28.3$  (c 1, MeOH)), purchased from Wuhan Kaitong Fine Chemical Co., Ltd, China. Unless otherwise specified, other materials 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). Melting points are uncorrected. Proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectra were recorded at either 300 MHz. Chemical shifts are reported in delta (δ) units, parts per million (ppm) downfield from trimethylsilane. High-resolution mass spectral (HRMS) data are reported as m/e (relative intensity). Elemental analyses for compounds were performed using an elemental vario EL III CN analyzer (Germany).

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

To a solution of compound **2** (2.12 g, 10 mmol) in THF (10 mL) was added dropwise a solution of di-*tert*-butyl dicarbonate (2.93 g, 11 mmol) in THF (5 mL). After 24 h stirring at room temperature the solvent was concentrated under vacuum to leave a residue. The crude product was recrystallized with EtOAc to give white solid (2.80 g), yield: 90%, mp = 115–116 °C,  $[\alpha]_D^{25} = +22.5$  (c 1, MeOH). ESI-MS *m/z*: 313.3 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.214 (s, 9H), 3.478–3.704 (m, 2H), 4.771–4.790 (m, 1H), 5.607 (d, *J* = 5.40, 1H), 6.194 (d, *J* = 9.00, 1H), 7.564 (d, *J* = 8.40, 2H), 8.181 (d, *J* = 8.40, 2H). Anal. Calcd for C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>6</sub>: C, 53.84; H, 6.45; N, 8.97. Found: C, 53.79; H, 6.31; N, 8.77.

#### 5.3.2. (2R,3S)-2-(*tert*-Butoxycarbonylamino)-3-hydroxy-3-(4-nitrophenyl) propanoic acid (**4**)

To a solution of compound **3** (3.12 g, 10 mmol) in acetone (50 mL) containing TEMPO (0.16 g, 1 mmol) was added a 5% aqueous NaHCO<sub>3</sub> solution (50 mL). The mixture was cooled to 0 °C and NaOCl (20.5 mL, ca. 10% w/w) was added dropwise over 15 min while following the reaction by TLC. The solution was concentrated under vacuum to remove acetone. The remaining mixture was washed with EtOAc (2 × 10 mL), acidified to pH 1–2 by a 1 N citric acid solution, extracted with EtOAc (3 × 20 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude product was purified by flash column chromatography (DCM/MeOH, 20:1 (V/V)) to give the desired compound **4** as a white solid (0.97 g), yield: 30%, mp = 80–82 °C,  $[\alpha]_D^{25} = +29.1$  (c 1, MeOH). ESI-MS *m/z*: 327.5 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.224 (s, 9H), 4.344 (dd, *J* = 9.60, *J* = 3.00, 1H), 5.265 (d, *J* = 3.00, 1H), 6.483 (d, *J* = 9.60, 1H), 7.658 (d, *J* = 8.40, 2H), 8.190 (d, *J* = 8.40, 2H). Anal. Calcd for C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>7</sub>: C, 51.53; H, 5.56; N, 8.59. Found: C, 51.49; H, 5.62; N, 8.37.

#### 5.3.3. (2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)propanoic acid (**1**)

To a solution of compound **4** (3.26 g, 10 mmol) in dry EtOAc at 0 °C was added dropwise a solution of EtOAc (10 mL) saturated with dry HCl gas. The reaction solution was stirred at 0 °C for 2 h, and then the temperature is raised to room temperature and the reaction proceeds for 5 h before being concentrated in vacuo.

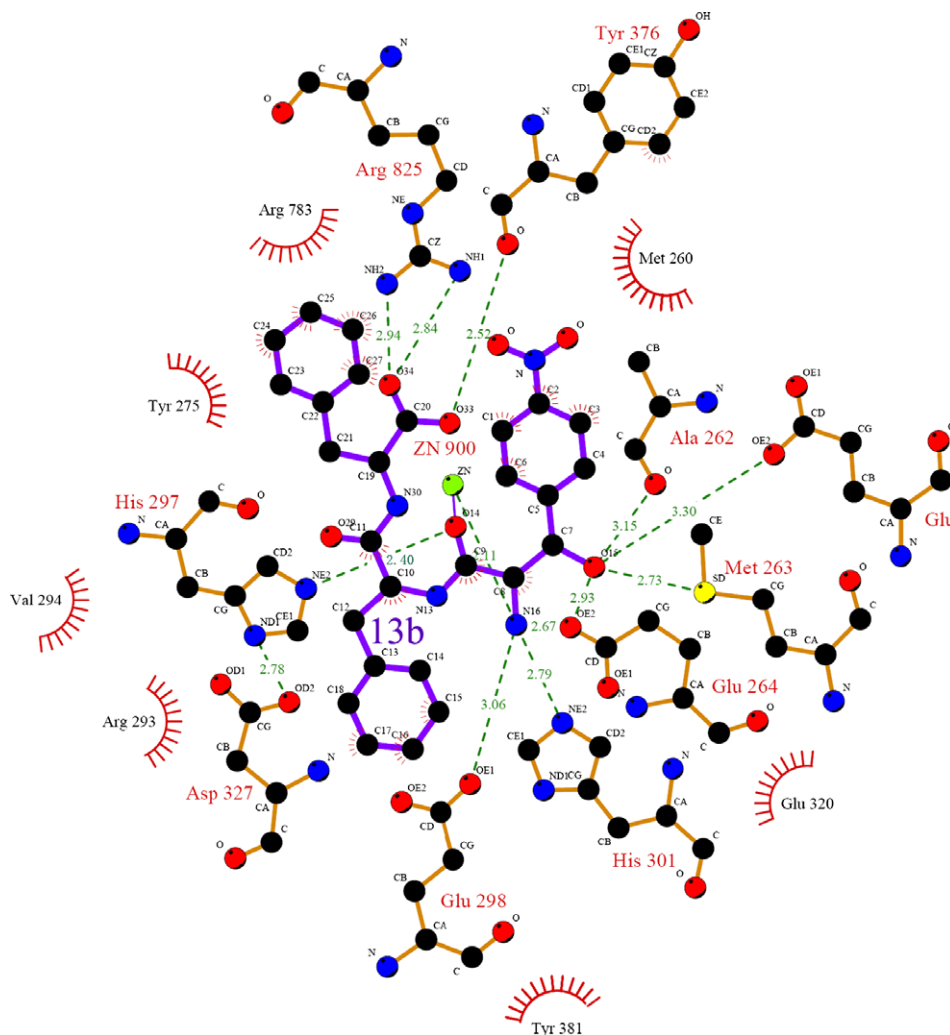


Figure 4. The docking result of **13b** is showed by Ligplot.

The residue was dissolved in water (5 mL), and then washed with EtOAc (2 × 5 mL). 1 N NaOH was added to the mixture until pH 6–7, and the solid was crystallized out. The solution was removed by filtration, and the remaining solid was sequentially washed with water and dried overnight, then recrystallized with MeOH to give a yellow crystals, yield: 74.2%, mp = 187–189 °C,  $[\alpha]_D^{25} = +20.8$  (c 1, MeOH). ESI-MS  $m/z$ : 227.3 (M+H);  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  3.425 (d,  $J = 3.90$ , 1H), 5.193 (d,  $J = 3.90$ , 1H), 7.659 (d,  $J = 8.70$ , 2H), 8.194 (d,  $J = 8.70$ , 2H). Anal. Calcd for  $\text{C}_9\text{H}_{10}\text{N}_2\text{O}_5$ : C, 47.79; H, 4.46; N, 12.39. Found: C, 47.58; H, 4.31; N, 12.27.

**5.3.3.1. (2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)-N-propylpropanamide (6a).** To a 150 mL solution of Compound **4** (3.26 g, 10 mmol), 1-propylamine (0.71 g, 12 mmol), and HOBt (1.62 g, 12 mmol) in dry THF, was added TEA (1.11 g, 11 mmol). The reaction mixture was gently cooled to 0 °C in ice bath. To the reaction mixture was added dropwise a solution of EDCI (3.82 g, 20 mmol) in THF for 1 h. 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<sub>3</sub> and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated in vacuo to give the crude product compound **5a** (3.15 g, yield 86%). This product was used for the following reaction without further purification, yield 84%, mp = 104–106 °C,  $[\alpha]_D^{25} = +27.9$  (c 1, MeOH). ESI-MS  $m/z$ : 368.2 (M+H);  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  0.828–0.835 (m, 3H), 1.251 (s, 9H), 1.301–1.405 (m, 2H), 2.843–2.904 (m, 2H), 3.203 (d,  $J = 3.90$ , 1H),

5.009 (d,  $J = 3.90$ , 1H), 7.895 (m, 2H), 7.621 (d,  $J = 8.70$ , 2H), 8.187 (d,  $J = 8.70$ , 2H). Anal. Calcd for  $\text{C}_{17}\text{H}_{25}\text{N}_3\text{O}_6$ : C, 55.58; H, 6.86; N, 11.44. Found: C, 55.67; H, 6.59; N, 11.83.

To a solution of compound **5a** (1.83 g, 5 mmol) in dry EtOAc at 0 °C was added dropwise a solution of EtOAc (10 mL) saturated by dry HCl gas. The reaction solution was stirred at 0 °C for 2 h, and then the temperature is raised to room temperature and the reaction proceeds for 5 h before being concentrated in vacuo. The residue was dissolved by saturated Na<sub>2</sub>CO<sub>3</sub> (10 mL), the aqueous solution was extracted with ethyl ether (3 × 30 mL). The extractions were combined, washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated in vacuo. The residue was purified by flash column chromatography (DCM/MeOH, 6/1) to give the desired compound **6a** as a white solid (1.12 g), yield 84%, mp = 118–120 °C,  $[\alpha]_D^{25} = +24.3$  (c 1, MeOH). ESI-MS  $m/z$ : 268.3 (M+H);  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  0.738–0.787 (m, 3H), 1.290–1.407 (m, 2H), 2.898–3.104 (m, 2H), 3.234 (d,  $J = 3.90$ , 1H), 5.018 (d,  $J = 3.90$ , 1H), 7.894 (t,  $J = 6.00$ , 1H), 7.613 (d,  $J = 8.70$ , 2H), 8.187 (d,  $J = 8.70$ , 2H). Anal. Calcd for  $\text{C}_{12}\text{H}_{17}\text{N}_3\text{O}_4$ : C, 53.92; H, 6.41; N, 15.72. Found: C, 53.67; H, 6.41; N, 15.72.

The other compounds of **6** series were synthesized following the general procedure as described above.

**5.3.3.2. (2R,3S)-2-Amino-3-hydroxy-N-isopropyl-3-(4-nitrophenyl)propanamide (6b).** White solid, yield 79%, mp = 132–134 °C,  $[\alpha]_D^{25} = +16.5$  (c 1, MeOH). ESI-MS  $m/z$ : 268.3 (M+H);  $^1\text{H}$  NMR (DMSO-



$\delta$  0.926 (d,  $J$  = 6.60, 3H), 1.033 (d,  $J$  = 6.60, 3H), 3.193 (d,  $J$  = 4.20, 1H), 3.748–3.862 (m, 1H), 4.963 (d,  $J$  = 4.20, 1H), 7.591 (d,  $J$  = 8.70, 2H), 7.651 (d,  $J$  = 7.80, 1H), 8.182 (d,  $J$  = 8.70, 2H). Anal. Calcd for  $C_{12}H_{17}N_3O_4$ : C, 53.92; H, 6.41; N, 15.72. Found: C, 53.89; H, 6.48; N, 15.91.

**5.3.3.3. (2R,3S)-2-Amino-N-butyl-3-hydroxy-3-(4-nitrophenyl)propanamide (6c).** White solid, yield 83%, mp = 88–89 °C,  $[\alpha]_D^{25}$  = +24.2 (c 1, MeOH). ESI-MS  $m/z$ : 282.4 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  0.786–0.835 (m, 3H), 1.082–1.204 (m, 2H), 1.242–1.333 (m, 2H), 2.908–3.233 (m, 2H), 3.234 (d,  $J$  = 3.90, 1H), 4.982 (d,  $J$  = 3.90, 1H), 7.591 (d,  $J$  = 8.70, 2H), 7.854 (t,  $J$  = 6.00, 1H), 8.188 (d,  $J$  = 8.70, 2H). Anal. Calcd for  $C_{13}H_{19}N_3O_4$ : C, 55.50; H, 6.81; N, 14.94. Found: C, 55.31; H, 6.76; N, 14.83.

**5.3.3.4. (2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)-N-pentylpropanamide (6d).** White solid, yield 83%, mp = 112–144 °C,  $[\alpha]_D^{25}$  = +26.8 (c 1, MeOH). ESI-MS  $m/z$ : 296.4 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  0.795–0.842 (m, 3H), 1.098–1.319 (m, 6H), 2.908–2.970 (m, 1H), 3.044–3.108 (m, 1H), 3.228 (d,  $J$  = 3.60, 1H), 4.968 (d,  $J$  = 3.60, 1H), 7.597 (d,  $J$  = 8.70, 2H), 7.849 (t,  $J$  = 6.00, 1H), 8.176 (d,  $J$  = 8.70, 2H). Anal. Calcd for  $C_{14}H_{21}N_3O_4$ : C, 56.94; H, 7.17; N, 14.23. Found: C, 56.92; H, 7.07; N, 14.29.

**5.3.3.5. (2R,3S)-2-Amino-N-cyclohexyl-3-hydroxy-3-(4-nitrophenyl)propanamide (6e).** White solid, yield 83%, mp = 127–129 °C,  $[\alpha]_D^{25}$  = +13.2 (c 1, MeOH). ESI-MS  $m/z$ : 308.5 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  0.679–1.312 (m, 6H), 1.437–1.990 (m, 4H), 3.417 (d,  $J$  = 4.50, 1H), 3.393–4.064 (m, 1H), 4.988 (d,  $J$  = 4.50, 1H), 7.659 (d,  $J$  = 9.00, 1H), 8.238 (d,  $J$  = 9.00, 2H), 8.283 (d,  $J$  = 7.80, 1H). Anal. Calcd for  $C_{15}H_{21}N_3O_4$ : C, 58.62; H, 6.89; N, 13.67. Found: C, 58.42; H, 6.40; N, 13.99.

**5.3.3.6. (2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)-N-propylpropanamide (6f).** White solid, yield 88%, mp = 125–127 °C,  $[\alpha]_D^{25}$  = +26.4 (c 1, MeOH). ESI-MS  $m/z$ : 316.3 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  3.355 (d,  $J$  = 3.90, 1H), 4.217 (dd,  $J$  = 5.70,  $J$  = 15.70, 1H), 4.326 (dd,  $J$  = 5.70,  $J$  = 15.70, 1H), 5.040 (d,  $J$  = 3.90, 1H), 7.181–7.285 (m, 5H), 7.618 (d,  $J$  = 8.70, 2H), 8.157 (d,  $J$  = 8.70, 2H), 8.442 (t,  $J$  = 5.70, 1H). Anal. Calcd for  $C_{16}H_{17}N_3O_4$ : C, 60.94; H, 5.43; N, 13.33. Found: C, 60.44; H, 5.73; N, 12.97.

**5.3.3.7. (2R,3S)-2-Amino-N-(4-fluorobenzyl)-3-hydroxy-3-(4-nitrophenyl)propanamide (6g).** White solid, yield 81.5%, mp = 115–118 °C,  $[\alpha]_D^{25}$  = +24.5 (c 1, MeOH). ESI-MS  $m/z$ : 334.3 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  3.327 (d,  $J$  = 3.60, 1H), 4.193 (dd,  $J$  = 6.00,  $J$  = 15.00, 1H), 4.294 (dd,  $J$  = 6.00,  $J$  = 15.00, 1H), 5.053 (d,  $J$  = 3.60, 1H), 7.052–7.111 (m, 2H), 7.187–7.235 (m, 2H), 7.593 (d,  $J$  = 8.70, 2H), 8.155 (d,  $J$  = 8.70, 2H), 8.458 (t,  $J$  = 6.30, 1H). Anal. Calcd for  $C_{16}H_{16}FN_3O_4$ : C, 57.56; H, 4.84; N, 12.61. Found: C, 57.09; H, 4.82; N, 12.30.

**5.3.3.8. (2R,3S)-2-Amino-3-hydroxy-N-(4-methoxybenzyl)-3-(4-nitrophenyl)propanamide (6h).** White solid, yield 66.7%, mp = 141–143 °C,  $[\alpha]_D^{25}$  = +21.3 (c 1, MeOH). ESI-MS  $m/z$ : 346.3 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  3.330 (d,  $J$  = 3.30, 1H), 3.717 (s, 3H), 4.114 (dd,  $J$  = 6.30,  $J$  = 15.70, 1H), 4.225 (dd,  $J$  = 6.30,  $J$  = 15.70, 1H), 5.014 (d,  $J$  = 3.30, 1H), 7.061 (d,  $J$  = 8.70, 2H), 7.592 (d,  $J$  = 8.70, 2H), 7.907 (d,  $J$  = 8.70, 2H), 8.147 (d,  $J$  = 8.70, 2H), 8.325 (t,  $J$  = 6.30, 1H). Anal. Calcd for  $C_{17}H_{19}N_3O_5$ : C, 59.12; H, 5.55; N, 12.17. Found: C, 59.15; H, 5.61; N, 12.48.

**5.3.3.9. (2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)-N-phenethylpropanamide (6i).** White solid, yield 76%, mp = 126–128 °C,  $[\alpha]_D^{25}$  = +27.4 (c 1, MeOH). ESI-MS  $m/z$ : 330.3 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  2.664 (t,  $J$  = 7.50, 2H), 3.246 (d,  $J$  = 3.60, 1H), 3.286–3.349 (m, 2H), 5.059 (d,  $J$  = 3.60, 1H), 7.167–7.213 (m, 3H), 7.225–7.306 (m, 2H), 7.593 (d,  $J$  = 8.70, 2H), 8.037 (t,  $J$  = 5.70, 1H),

8.182 (d,  $J$  = 8.70, 2H). Anal. Calcd for  $C_{17}H_{19}N_3O_4$ : C, 62.00; H, 5.81; N, 12.76. Found: C, 62.13; H, 5.80; N, 12.65.

**5.3.3.10. (2R,3S)-2-Amino-N-(2-chlorophenethyl)-3-hydroxy-3-(4-nitrophenyl)propanamide (6j).** White solid, yield 72.3%, mp = 140–142 °C,  $[\alpha]_D^{25}$  = +25.2 (c 1, MeOH). ESI-MS  $m/z$ : 364.2 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  2.730 (t,  $J$  = 6.30, 1H), 3.220–3.391 (m, 2H), 3.398 (d,  $J$  = 3.9, 1H), 5.041 (d,  $J$  = 3.90, 1H), 7.226–7.260 (m, 3H), 7.400–7.432 (m, 1H), 7.601 (d,  $J$  = 8.70, 2H), 8.184 (t,  $J$  = 5.70, 1H), 8.213 (d,  $J$  = 8.70, 2H). Anal. Calcd for  $C_{17}H_{18}ClN_3O_4$ : C, 56.13; H, 4.99; N, 11.55. Found: C, 56.52; H, 5.01; N, 11.54.

**5.3.3.11. (2R,3S)-2-Amino-3-hydroxy-N-(4-hydroxyphenethyl)-3-(4-nitrophenyl)propanamide (6k).** White solid, yield 83%, mp = 99–100 °C,  $[\alpha]_D^{25}$  = +21.0 (c 1, MeOH). ESI-MS  $m/z$ : 346.3 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  2.538–2.563 (m, 2H), 3.145–3.164 (m, 2H), 3.188 (d,  $J$  = 3.90, 1H), 5.067 (d,  $J$  = 3.90, 1H), 6.657 (d,  $J$  = 8.70, 2H), 6.953 (d,  $J$  = 8.70, 2H), 7.591 (d,  $J$  = 8.70, 2H), 7.988 (t,  $J$  = 6.00, 1H), 8.187 (d,  $J$  = 8.70, 2H). Anal. Calcd for  $C_{17}H_{19}N_3O_5$ : C, 59.12; H, 5.55; N, 12.17. Found: C, 59.08; H, 5.57; N, 12.46.

**5.3.3.12. (2R,3S)-2-Amino-3-hydroxy-N-((S)-1-hydroxy-3-phenylpropan-2-yl)-3-(4-nitrophenyl)propanamide (6l).** White solid, yield 80.3%, mp = 133–134 °C,  $[\alpha]_D^{25}$  = +22.3 (c 1, MeOH). ESI-MS  $m/z$ : 360.3 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  3.259–2.809 (m, 2H), 3.176–3.343 (m, 2H), 3.882 (d,  $J$  = 3.30, 1H), 5.047 (d,  $J$  = 3.30, 1H), 7.151–7.176 (m, 3H), 7.223–7.272 (m, 2H), 7.593 (d,  $J$  = 8.70, 2H), 7.901 (d,  $J$  = 8.70, 1H), 8.164 (d,  $J$  = 8.70, 2H). Anal. Calcd for  $C_{18}H_{21}N_3O_5$ : C, 60.16; H, 5.89; N, 11.69. Found: C, 60.19; H, 5.88; N, 11.48.

**5.3.3.13. (2R,3S)-2-Amino-N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-hydroxy-3-(4-nitrophenyl)propanamide (6m).** White solid, yield 85.9%, mp = 136–137 °C,  $[\alpha]_D^{25}$  = +18.7 (c 1, MeOH). ESI-MS  $m/z$ : 374.4 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  2.581 (t,  $J$  = 7.20, 2H), 3.171–3.214 (m, 2H), 3.236 (d,  $J$  = 3.30, 1H), 5.060 (d,  $J$  = 3.30, 1H), 5.961 (s, 2H), 6.622 (d,  $J$  = 7.80, 1H), 6.756–6.819 (m, 2H), 7.696 (d,  $J$  = 8.70, 2H), 7.998 (t,  $J$  = 5.40, 1H), 8.183 (d,  $J$  = 8.70 Hz, 2H). Anal. Calcd for  $C_{18}H_{19}N_3O_6$ : C, 57.90; H, 5.13; N, 11.25. Found: C, 57.84; H, 5.11; N, 11.20.

Compounds **8a** and **8b** were synthesized following the general procedure as described below (preparation of **12a**).

**5.3.3.14. 4-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)propanamido)butanoic acid (8a).** White solid, yield 43.3%, mp = 141–143 °C,  $[\alpha]_D^{25}$  = +20.5 (c 1, MeOH). ESI-MS  $m/z$ : 312.3 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  1.186–1.210 (m, 2H), 2.137 (t,  $J$  = 7.50, 2H), 2.996–3.020 (m, 2H), 3.581 (d,  $J$  = 5.10, 1H), 5.024 (d,  $J$  = 5.10, 1H), 7.649 (d,  $J$  = 8.70, 2H), 8.204 (d,  $J$  = 8.70, 2H), 8.292 (d,  $J$  = 7.80, 1H). Anal. Calcd for  $C_{13}H_{17}N_3O_6$ : C, 50.16; H, 5.50; N, 13.50. Found: C, 50.12; H, 5.53; N, 13.60.

**5.3.3.15. 6-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)propanamido)hexanoic acid (8b).** White solid, yield 43.3%, mp = 150–153 °C,  $[\alpha]_D^{25}$  = +25.6 (c 1, MeOH). ESI-MS  $m/z$ : 340.3 (M+H);  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  1.066–1.165 (m, 2H), 1.214–1.430 (m, 4H), 2.134 (t,  $J$  = 7.50, 2H), 3.006–3.055 (m, 2H), 3.583 (d,  $J$  = 4.80, 1H), 5.028 (d,  $J$  = 4.80, 1H), 7.633 (d,  $J$  = 8.70, 2H), 8.204 (d,  $J$  = 8.70, 2H), 8.259 (d,  $J$  = 7.00, 1H). Anal. Calcd for  $C_{15}H_{21}N_3O_6$ : C, 53.09; H, 6.24; N, 12.38. Found: C, 53.04; H, 6.28; N, 12.36.

**5.3.3.16. (S)-2-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)propanamido)-4-methylpentanoic acid (10a).** To a 150 mL solution of compound **4** (3.26 g, 10 mmol), L-leucine methyl ester hydrochloride (1.81 g, 10 mmol), and HOBt (1.62 g, 12 mmol) in

dry THF, was added TEA (2.22 g, 22 mmol). The reaction mixture was gently cooled to 0 °C in ice bath, to the reaction mixture was added dropwise a solution of EDCI (3.82 g, 20 mmol) in THF for 1 h. 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 M citric acid solution, saturated NaHCO<sub>3</sub> and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated in vacuo. The residue was purified by flash column chromatography (EtOAc/PE, 1:4(V/V)) to give the desired compound **9a** as a white solid (4.08 g), yield 88.7%, mp = 141–143 °C,  $[\alpha]_D^{25} = +29.1$  (c 1, MeOH). ESI-MS *m/z*: 454.3 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.789 (d, *J* = 20.40, 6H), 1.235 (s, 9H), 1.397–1.423 (m, 1H), 1.442–1.529 (m, 2H), 3.628 (s, 3H), 4.234 (d, *J* = 4.50, 1H), 4.313–4.359 (m, 1H), 5.033 (d, *J* = 4.50, 1H), 6.423 (s, *J* = 9.60, 1H), 7.625 (d, *J* = 8.40, 2H), 8.194 (d, *J* = 8.40, 2H), 8.472 (d, *J* = 7.80 Hz, 1H). Anal. Calcd for C<sub>21</sub>H<sub>31</sub>N<sub>3</sub>O<sub>8</sub>: C, 55.62; H, 6.89; N, 9.27. Found: C, 55.42; H, 6.83; N, 9.23.

To a solution of compound **9a** (4.53 g, 10 mmol) in MeOH at 5 °C was added dropwise a solution of 1 N NaOH (12 mL). The reaction was warmed slowly to room temperature and stirred for 5 h. The solvent was concentrated under vacuum to remove the MeOH. The remaining mixture was added water (10 mL) and acidified to pH 1–2 by a 1 N citric acid solution. The precipitate was collected, washed with water (20 mL), and dried overnight. The crude product was then recrystallized with EtOAc to give the desired compound as white solid (4.39 g), yield 93.1%, mp = 128–130 °C,  $[\alpha]_D^{25} = +27.2$  (c 1, MeOH). ESI-MS *m/z*: 440.7 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.809 (d, *J* = 18.60, 6H), 1.231 (s, 9H), 1.330–1.420 (m, 1H), 1.421–1.439 (m, 2H), 4.165 (d, *J* = 4.50, 1H), 4.318–4.363 (m, 1H), 5.054 (d, *J* = 4.50, 1H), 6.398 (d, *J* = 9.60, 1H), 7.633 (d, *J* = 8.40, 2H), 8.197 (d, *J* = 8.40, 2H), 8.332 (d, *J* = 8.10, 1H). Anal. Calcd for C<sub>20</sub>H<sub>29</sub>N<sub>3</sub>O<sub>8</sub>: C, 54.66; H, 6.65; N, 9.56. Found: C, 54.51; H, 6.62; N, 9.57.

The compound **10a** was synthesized from following the general procedure as described above (preparation of **1**), white solid, yield 60%, mp = 203–205 °C,  $[\alpha]_D^{25} = +36.2$  (c 1, MeOH). ESI-MS *m/z*: 340.4 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.731 (d, *J* = 12.10, 6H), 1.121–1.142 (m, 1H), 1.336–1.354 (m, 2H), 3.417 (d, *J* = 4.50, 1H), 4.094–4.113 (m, 1H), 4.882 (d, *J* = 4.50, 1H), 7.631 (d, *J* = 8.70, 2H), 8.092 (d, *J* = 7.50, 1H), 8.180 (d, *J* = 8.70, 2H). Anal. Calcd for C<sub>15</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>: C, 53.09; H, 6.24; N, 12.38. Found: C, 53.50; H, 6.43; N, 12.30.

The other compounds of **10** series were synthesized following the general procedure as described above (preparation of **10a**).

**5.3.3.17. (S)-2-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)-propanamido)-3-phenylpropanoic acid (10b).** White solid, yield 85.9%, mp = 215–218 °C,  $[\alpha]_D^{25} = +28.4$  (c 1, MeOH). ESI-MS *m/z*: 374.4 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 2.854–3.013 (m, 2H), 3.462 (d, *J* = 3.90, 1H), 4.375–4.442 (m, 1H), 5.061 (d, *J* = 3.90, 1H), 7.079–7.105 (m, 2H), 7.132–7.243 (m, 3H), 7.606 (d, *J* = 8.70, 2H), 8.165 (d, *J* = 8.70, 2H), 8.272 (d, *J* = 7.20, 1H). Anal. Calcd for C<sub>18</sub>H<sub>19</sub>N<sub>3</sub>O<sub>6</sub>: C, 57.90; H, 5.13; N, 11.25. Found: C, 57.85; H, 5.16; N, 11.23.

**5.3.3.18. (S)-2-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)-propanamido)-3-methylbutanoic acid (10c).** White solid, yield 85.9%, mp = 179–180 °C,  $[\alpha]_D^{25} = +21.6$  (c 1, MeOH). ESI-MS *m/z*: 326.3 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.688–0.731 (m, 6H), 1.912–1.919 (m, 1H), 3.493 (d, *J* = 3.00, 1H), 4.055–4.059 (m, 1H), 5.001–5.003 (d, *J* = 3.00, 1H), 7.632 (d, *J* = 8.70, 2H), 8.087 (d, *J* = 6.00, 1H), 8.177 (d, *J* = 8.70, 2H). Anal. Calcd for C<sub>18</sub>H<sub>19</sub>N<sub>3</sub>O<sub>6</sub>: C, 51.69; H, 5.89; N, 12.92. Found: C, 51.67; H, 5.49; N, 12.61.

**5.3.3.19. (S)-2-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)-propanamido)-3-methylbutanoic acid (10d).** White solid, yield 85.9%, mp = 178–179 °C,  $[\alpha]_D^{25} = +24.8$  (c 1, MeOH). ESI-MS *m/z*: 340.3 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.685 (d, *J* = 6.60, 3H), 0.752 (t, *J* = 7.50, 3H), 0.939–0.940 (m, 1H), 1.210–1.213 (m, 1H),

1.591–1.594 (m, 1H), 3.504 (d, *J* = 3.00, 1H), 4.095–4.098 (m, 1H), 4.964 (d, *J* = 3.00, 1H), 7.632 (d, *J* = 8.70, 2H), 8.081 (d, *J* = 6.00, 1H), 8.195 (d, *J* = 8.70, 2H). Anal. Calcd for C<sub>15</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>: C, 53.09; H, 6.24; N, 12.38. Found: C, 53.51; H, 6.19; N, 12.40.

**5.3.3.20. (S)-2-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)-propanamido)-3-(1H-indol-2-yl) propanoic acid (10e).** White solid, yield 85.9%, mp = 179–180 °C,  $[\alpha]_D^{25} = +26.1$  (c 1, MeOH). ESI-MS *m/z*: 413.4 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 3.064–3.113 (m, 2H), 3.163 (d, *J* = 3.00, 1H), 4.487–4.490 (m, 1H), 5.106 (d, *J* = 3.00, 1H), 6.965–7.030 (m, 1H), 7.054–7.078 (m, 2H), 7.327 (d, *J* = 8.10, 1H), 7.504 (d, *J* = 7.50, 1H), 7.599 (d, *J* = 8.70, 2H), 8.132 (d, *J* = 8.70, 2H), 8.275 (d, *J* = 6.50, 1H). Anal. Calcd for C<sub>20</sub>H<sub>20</sub>N<sub>4</sub>O<sub>6</sub>: C, 58.25; H, 4.89; N, 13.59. Found: C, 58.60; H, 4.73; N, 13.54.

**5.3.3.21. (S)-2-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)-propanamido)-N-hydroxy-4-methylpentanamide (11a).** To a solution of compound **10a** (4.53 g, 10 mmol) in MeOH at room temperature was added dropwise a solution of 1.5 N H<sub>2</sub>N–OK (12 mL). The reaction mixture was stirred for 1 h, and then acidified to pH 7 by acetic acid. The solvent was evaporated in vacuo to give the yellow solid. The crude product was purified by flash column chromatography (DCM/MeOH, 40:1 (V/V)) to give the desired compound as a white solid (3.15 g), yield 70.7%, mp = 153–155 °C,  $[\alpha]_D^{25} = +20.1$  (c 1, MeOH). ESI-MS *m/z*: 455.3 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.545–0.610 (m, 6H), 0.703–0.801 (m, 1H), 0.934–1.187 (m, 2H), 3.954 (d, *J* = 3.50, 1H), 4.062–4.082 (m, 1H), 4.903 (d, *J* = 3.50, 1H), 7.652 (d, *J* = 8.70, 2H), 8.246 (d, *J* = 8.70, 2H), 8.746 (m, 2H).

The compound **11a** was synthesized following the general procedure as described above (preparation of **1**), yield 74.2%, mp = 212–213 °C,  $[\alpha]_D^{25} = +27.7$  (c 1, MeOH). ESI-MS *m/z*: 355.6 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.548–0.609 (m, 6H), 0.701–0.811 (m, 1H), 0.911–1.198 (m, 2H), 3.967 (d, *J* = 3.50, 1H), 4.063–4.081 (m, 1H), 4.968 (d, *J* = 3.50, 1H), 7.651 (d, *J* = 8.70, 2H), 8.244 (d, *J* = 8.70, 2H), 8.746 (d, *J* = 8.10, 2H). Anal. Calcd for C<sub>15</sub>H<sub>22</sub>N<sub>4</sub>O<sub>6</sub>: C, 50.84; H, 6.26; N, 15.81. Found: C, 50.90; H, 6.06; N, 16.17.

**5.3.3.22. (2R,3S)-2-Amino-3-hydroxy-N-((S)-1-(hydroxyamino)-1-oxo-3-phenylpropan-2-yl)-3-(4-nitrophenyl)propanamide (11b).** The compound **11b** was synthesized following the general procedure as described above, yield 74.2%, mp = 215–217 °C,  $[\alpha]_D^{25} = +26.7$  (c 1, MeOH). ESI-MS *m/z*: 389.5 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 2.553–2.763 (m, 2H), 4.071 (d, *J* = 3.30, 1H), 4.317–4.366 (m, 1H), 5.063 (d, *J* = 3.30, 1H), 7.043–7.065 (m, 2H), 7.118–7.268 (m, 3H), 7.636 (d, *J* = 8.70, 2H), 8.199 (d, *J* = 8.70, 2H), 8.958 (d, *J* = 8.10, 2H). Anal. Calcd for C<sub>18</sub>H<sub>20</sub>N<sub>4</sub>O<sub>6</sub>: C, 55.67; H, 5.19; N, 14.43. Found: C, 55.34; H, 5.16; N, 14.30.

**5.3.3.23. (S)-2-((S)-2-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)propanamido)-4-methylpentanamido)-3-phenylpropanoic acid (13a).** Compounds **13a**, **13b** were synthesized following the general procedure as described above (preparation of **10a**). White solid, yield 85.9%, mp = 115–116 °C,  $[\alpha]_D^{25} = +27.2$  (c 1, MeOH). ESI-MS *m/z*: 487.4 (M+H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.692 (d, *J* = 12.10, 6H), 1.023–1.110 (m, 1H), 1.218–1.287 (m, 2H), 2.849–3.053 (m, 2H), 3.742 (d, *J* = 4.80, 1H), 4.114–4.225 (m, 1H), 4.252–4.295 (m, 1H), 4.919 (d, *J* = 4.80, 1H), 7.179–7.279 (d, *J* = 8.70, 5H), 7.604 (d, *J* = 8.70, 2H), 8.021 (t, *J* = 7.50, 1H), 8.150 (t, *J* = 7.30, 1H), 8.185 (d, *J* = 8.70, 2H). Anal. Calcd for C<sub>24</sub>H<sub>30</sub>N<sub>4</sub>O<sub>7</sub>: C, 59.25; H, 6.22; N, 11.52. Found: C, 59.19; H, 6.34; N, 11.28.

**5.3.3.24. (S)-2-((S)-2-((2R,3S)-2-Amino-3-hydroxy-3-(4-nitrophenyl)propanamido)-3-phenylpropanamido)-3-phenylpropanoic acid (13b).** White solid, yield 85.9%, mp = 169–170 °C,  $[\alpha]_D^{25} = +22.1$  (c 1, MeOH). ESI-MS *m/z*: 521.3 (M+H); <sup>1</sup>H NMR

(DMSO- $d_6$ )  $\delta$  2.722–2.933 (m, 2H), 2.941–3.097 (m, 2H), 3.427 (d,  $J$  = 3.60, 1H), 4.322–4.441 (m, 1H), 4.457–4.511 (m, 1H), 5.004 (d,  $J$  = 3.60, 1H), 7.088–7.563 (m, 10H), 7.498 (d,  $J$  = 8.70, 2H), 8.139 (d,  $J$  = 8.70, 2H), 8.281 (t,  $J$  = 7.30, 2H). Anal. Calcd for  $C_{27}H_{28}N_4O_7$ : C, 62.30; H, 5.42; N, 10.76. Found: C, 62.28; H, 5.37; N, 11.12.

## Acknowledgments

This work was supported by National Natural Foundation Research Grant (Grant Nos. 9071304 and 30772654) and National High Technology Research and Development Program of China (863 Project; Grant No. 2007AA02Z314) and Doctoral Foundation of Ministry of Education of the People's Republic of China (Grant No. 20060422029).

## References and notes

- Xu, W. F.; Li, Q. B. *Curr. Med. Chem. Anti-Cancer Agents* **2005**, 5, 281.
- Bauvois, B.; Dauzonne, D. *Med. Res. Rev.* **2006**, 26, 88.
- Ito, K.; Nakajima, Y.; Onohara, Y.; Takeo, M.; Nakashima, K.; Matsubara, F.; Ito, T.; Yoshimoto, T. *J. Biol. Chem.* **2006**, 281, 33664.
- Onohara, Y.; Nakajima, Y.; Ito, K.; Xu, Y.; Nakashima, K.; Ito, T.; Yoshimoto, T. *Acta Crystallgr.*, **2006**, 62, 699.
- Addlagatta, A.; Gay, L.; Matthews, B. W. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, 103, 13339.
- Wang, Q.; Chen, M. Y.; Zhu, H. W.; Zhang, J.; Fang, H.; Wang, B. H.; Xu, W. F. *Bioorg. Med. Chem.* **2008**, 16, 5473.
- Li, Q. B.; Fang, H.; Xu, W. F. *Bioorg. Med. Chem. Lett.* **2007**, 17, 2935.
- Tu, G. G.; Li, S. H.; Huang, H. M.; Li, G.; Xiong, F.; Mai, X.; Zhu, H. W.; Kuang, B. H.; Xu, W. F. *Bioorg. Med. Chem.* **2008**, 16, 6663.
- Ma, T.; Xu, W. F.; Wang, J. L.; Yuan, Y. M. *Chinese J. Med. Chem.* **2003**, 13, 70.
- Testa, M. L.; Ciriminna, R.; Hajji, C.; Garcia, E. Z.; Ciclosi, M.; Arques, J. S.; Pagliaro, M. *Adv. Synth. Catal.* **2004**, 346, 655.
- Flipo, M.; Beghyn, T.; Charton, J.; Leroux, V. A.; Deprez, B. P.; Deprez-Poulain, R. F. *Bioorg. Med. Chem.* **2007**, 15, 63.
- Zhang, J.; Wang, Q.; Fang, H.; Xu, W. F.; Liu, A.; Du, G. H. *Bioorg. Med. Chem.* **2008**, 16, 3839.
- Hooper, N. M. *FEBS Lett.* **1994**, 354, 1.
- Kiyoshi, I.; Yoshitaka, N.; Tadashi, Y., et al. *J. Biol. Chem.* **2006**, 281, 33664.