Full Paper

Design, Synthesis, and Preliminary Activity Evaluation of Novel Peptidomimetics as Aminopeptidase N/CD13 Inhibitors

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The synthesis of a series of novel *N*-α-galloylated isoglutamic acid γ-amide peptidomimetics is described. Their enzymatic inhibition against aminopeptidase N (APN/CD13) and matrix metalloproteinase-2 (MMP-2) was tested. The preliminary activity assay revealed that most of the compounds displayed selective inhibition against APN as compared with MMP-2, with IC_{50} values in a micromolar range. Within this series, compound **4** ($IC_{50} = 10.2 \pm 0.9$ μM) demonstrated comparable APN inhibition as compared with the positive control bestatin ($IC_{50} = 13.1 \pm 0.7$ μM), which might be a promising lead for further molecular optimizations.

Keywords: Aminopeptidase N inhibitor / Antiproliferative activity / APN/CD13 / Peptidomimetics

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Introduction

Aminopeptidase N (APN, EC 3.4.11.2), also known as human lymphocyte surface cluster differentiation antigen, CD13, is a 150-kDa monomeric or homodimeric type II membrane-bound glycoprotein which can release neutral or basic amino acids from the N-terminal end of peptides [1]. It is a zinc-dependent exopeptidase that is expressed by myeloid, monocytes, epithelial cells of the intestine and kidney, fibroblasts, and endothelial cells [2]. It is particularly noticeable that APN/CD13 is overexpressed on the epithelium of the tumor and plays critical roles in tumor progression, such as invasion, metastasis, and angiogenesis [3]. Accordingly, APN/CD13 inhibition represents a promising approach to cancer treatment [4, 5].

To date, several natural or synthetic APN inhibitors (APNIs) have been exploited and some of them are currently investigated for clinical usage [6]. Among these inhibitors, bestatin, an antibiotic of microbial origin and best known APNI [7], is a well-established anticancer agent employed clinically in the ancillary treatment of adult acute nonlymphocytic leukemia, which has been a useful tool as positive control in elucidating many physiological conditions [8].

Given most of the reported APNIs are pseudodipeptides or peptide mimics bearing a chelating moiety (such as hydroxamate or carboxylate), inhibitors containing zinc-binding groups (ZBGs), should be optimal selection to inhibit the enzymatic activity of APN/CD13. Moreover, according to the reported binding pattern of bestatin with APN from *Escherichia coli* (ePepN), there are two hydrophobic subsites beside the catalytic zinc(II) ion, called S_1 and S_1' pocket, respectively, which are long and narrow binding domains. Hence, it is appropriate to introduce one or more hydrophobic portion protruding into these pockets so as to generate effective interactions [9, 10].

In our previous work, we have reported a spectrum of peptidomimetic L-Isoglutamine derivatives derived from antineoplaston AS2–5, one of the active degradation products of antineoplaston A-10, which has efficacious antiproliferative activity. The enzymatic evaluation revealed that most compounds displayed good inhibitory activities against APN/CD13 [11, 12]. Considering the side chains of these compounds were all aromatic substituents and not long enough, we herein designed a new series of bi-peptide analogues, which contains amino acid or organic acid residues as elongated hydrophobic substituents inserting into S_1 or S_1' pocket. We hereby hope the elongated side chains could produce improved inhibitory activity towards APN/CD13. Meanwhile, the newly introduced amino acid methyl esters

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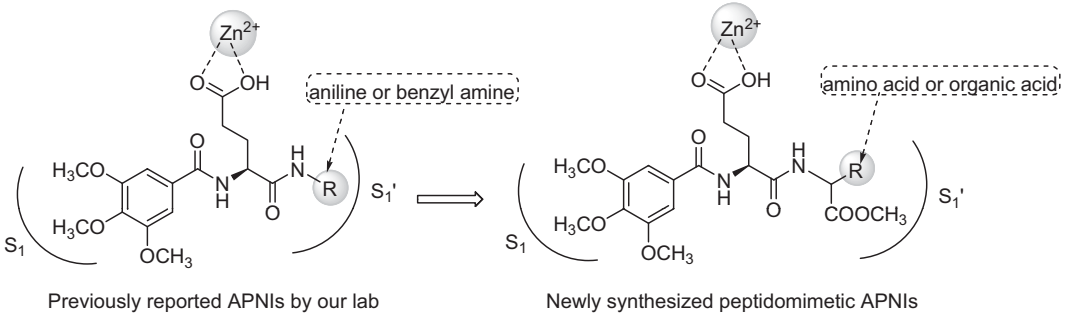


Figure 1. Newly designed peptidomimetic derivatives.

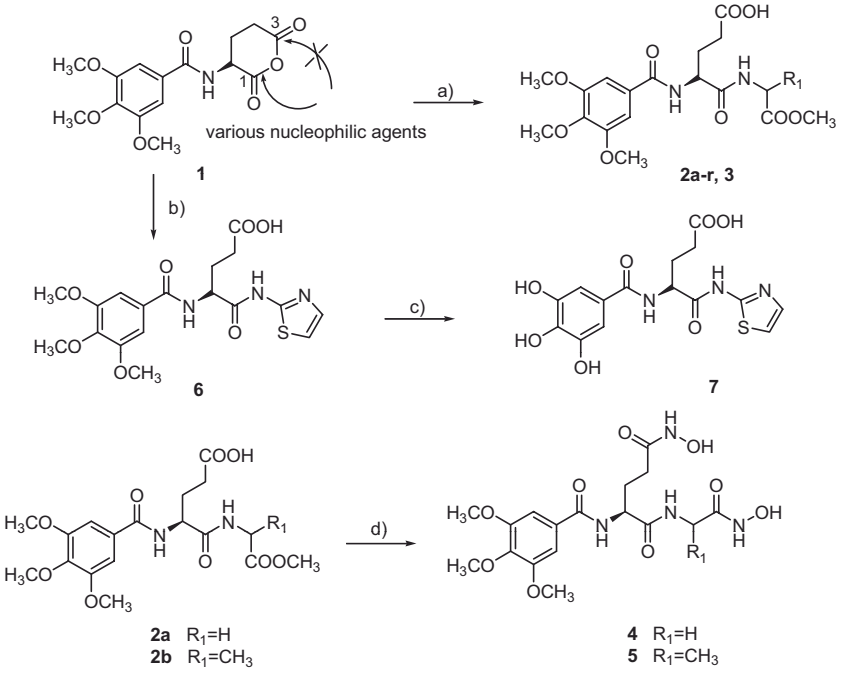
can also serve as possible ZBGs to bind with the catalytic zinc ion (see Fig. 1).

Results and discussion

Chemistry

The target compounds **2a–r** and **3–7**, were synthesized efficiently, following the procedures outlined in Scheme 1. In our synthesis, the starting cyclic anhydride **1** was synthesized in our previous condition [11]. This was followed by coupling with various L-amino acid methyl ester hydrochlorides or primary amines in the presence of Et_3N in anhydrous CH_2Cl_2 to provide the final compounds. In this reaction, other aprotic polar solvent, *e.g.* benzene, is also preferable. In particular, as verified by our previous work [13], when the starting asymmetrically substituted cyclic anhydride was treated with different nucleophilic agents, only L-Isoglutamine derivatives **2a–r** and **3** were finally obtained.

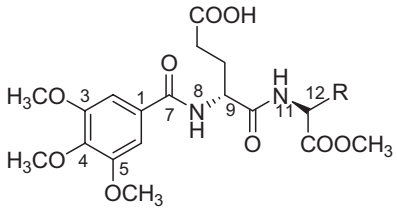
Furthermore, in order to compare the binding affinities of free phenolic hydroxyl group with the galloylated counterparts, compound **6** was chemically converted to its deprotected homologue **7** by using BBr_3 in the mixed solvents of CH_2Cl_2 and DMF. Finally, ammonolysis of **2a** and **2b** with NH_2OK gave the corresponding hydroxamate compounds



Reagents and conditions: a) Various L-amino acid methyl ester hydrochloride, anhydrous CH_2Cl_2, Et_3N, rt. b) thiazol-2-amine, acetic acid, rt. c) BBr_3, CH_2Cl_2, DMF, 0°C. d) NH_2OK in anhydrous CH_3OH, then acetic acid (two steps).

Scheme 1. Synthesis of target compounds from asymmetrically substituted cyclic anhydride **1**.

Table 1. The structures and inhibitory activities of target peptidomimetic compounds **2a–2r** and **3–7**.



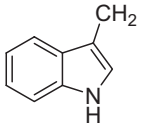
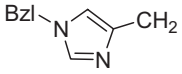
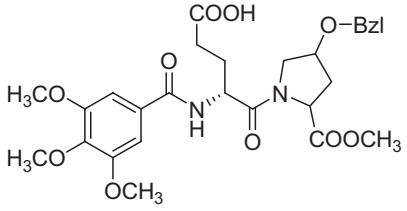
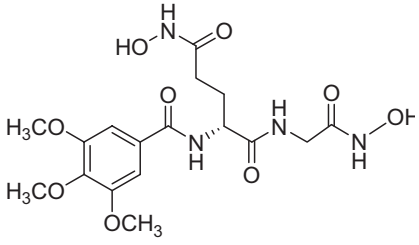
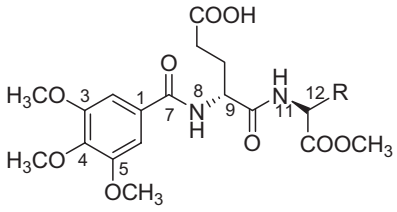
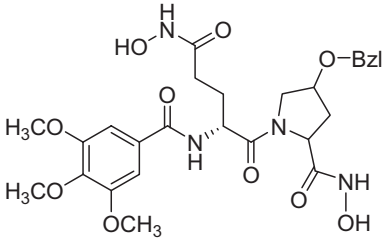
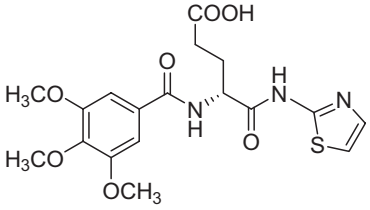
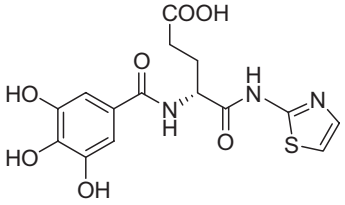
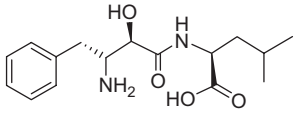
Compd.	R	IC_{50} [μM] [a]	
		APN/CD13	MMP-2 (gelatinase A)
2a	H	33.2 ± 2.2	171.4 ± 8.4
2b	CH_3	59.3 ± 0.7	209.5 ± 10.7
2c	CH_3CH_2	108.2 ± 1.5	380.1 ± 3.5
2d	$CH_3CH_2CH_2$	470.2 ± 5.4	656.7 ± 2.8
2e	$CH_3CH_2CH_2CH_2$	NA [b]	NA [b]
2f	$C_6H_5CH_2$	110.2 ± 12.1	880.5 ± 33.6
2g	*p*-F-$C_6H_4CH_2$	65.8 ± 2.3	309.7 ± 7.9
2h	*p*-Cl-$C_6H_4CH_2$	79.2 ± 1.5	206.5 ± 12.4
2i	*p*-Br-$C_6H_4CH_2$	88.7 ± 0.9	163.3 ± 4.9
2j	*p*-HO-$C_6H_4CH_2$	45.5 ± 3.6	125.0 ± 7.8
2k	$HOCH_2$	36.8 ± 5.1	203.9 ± 3.7
2l	$CH_3SCH_2CH_2$	50.7 ± 1.9	118.4 ± 6.5
2m	$C_6H_5SCH_2CH_2$	37.8 ± 2.2	176.8 ± 15.8
2n	Cbz-$NHCH_2CH_2CH_2$	74.7 ± 8.2	152.4 ± 20.1
2o	Cbz-$NHCH_2CH_2CH_2CH_2$	312.9 ± 11.8	NA [b]
2p	$NO_2NH(NH)CNHCH_2CH_2CH_2$	NA [b]	22.6 ± 10.1
2q		56.6 ± 1.7	110 ± 16.1
2r		29.7 ± 0.8	179.3 ± 5.7
3		30.2 ± 1.4	95.8 ± 8.3
4		10.2 ± 0.9	193.9 ± 5.2

Table 1. (*continued*)



Compd.	R	IC_{50} [μM] [a]	
		APN/CD13	MMP-2 (gelatinase A)
5		18.5 ± 2.4	125.7 ± 9.7
6 [c]		107.6 ± 12.8	369.4 ± 21.4
7		22.8 ± 5.5	206.6 ± 7.9
Bestatin		13.1 ± 0.7	40.5 ± 1.5

[a] Values are means of three experiments, standard deviation is given. [b] NA: no activity. [c] The compound has been reported in our previous work (see [11]).

(**4** and **5**), with the purpose of comparing the chelating effectiveness of different ZBGs. The chemical structures of the target compounds were analytical confirmed by IR, ^{1}H-NMR, ESI-MS, and elemental analysis.

Preliminary *in-vitro* evaluation and SAR discussion

The target compounds were evaluated for their enzymatic inhibition towards APN/CD13 and MMP-2. Similar to APN, MMP-2 (gelatinase A) is also a zinc-dependent proteinase involved in the process of tumor invasion and metastasis [14, 15]. Hence the assay was performed on both of APN/CD13 and MMP-2 so as to identify the potential leads with selectivity. All the inhibition results are summarized in Table 1 and, bestatin was used as the positive control.

The results showed that most compounds, except **2p**, display a better enzymatic inhibition against APN than that of MMP-2, with IC_{50} values lying in micromolar level. This selectivity against APN as compared with MMP-2, to a certain extent, validated our strategy for designing potential APNIs. This might be attributed to their different structures, leading to different requirements for their respective inhibitors. Specifically, APN is a membrane-bound zinc exopeptidase that catalyzed the removal of NH_2-terminal amino acid from the peptide, while MMP-2 is a zinc-dependent endopeptidase that could cut the peptide to parts from the specific amino acid residue of the peptide [14]. As the described selectivity, the following SARs were mainly focused on the APN/CD13 inhibition.

Of these galloylated inhibitors (**2a**∼**r** and **3**∼**6**), compound **4** with dihydroxamate substituents, gave the best inhibitory activity ($IC_{50} = 10.2 \pm 0.9$ μM) and displayed comparable potency with bestatin ($IC_{50} = 13.1 \pm 0.7$ μM). Compounds **5**, **3**, **2a**, **2k**, **2m**, and **2r** exhibited the subsequent activities against APN/CD13, with IC_{50} values ranging from 18.5 ± 2.4 to 37.8 ± 2.2 μM. However, the above six compounds demonstrated less potent in comparison with bestatin.

As to **2a**∼**r**, generally speaking, compounds containing the zinc-binding functional groups, say, **2j** and **2k** (OH), **2l** and **2m** (S), **2n**, **2o**, **2q**, and **2r** (N), offered relatively better potencies. This is because, apart from the contributions of ZBGs, another possible reason might attribute to the electrostatic interaction with the carbonyl group of a specific amino acid residue in the active site of APN/CD13. An interesting case can be used to support this conclusion, that is, sulphur-containing **2l** presented higher affinities than its isosteric aliphatic-containing counterpart **2e**, which even has no APN/CD13 inhibition. Nevertheless, although both containing sulphur atom, **2m** with aromatic side chain gave better activity than that of aliphatic counterpart (**2l**). The most likely reason may due to the π system of the aromatic ring enhancing the interaction with the hydrophobic region of the enzyme.

Comparing **2a–e**, R_1 groups were fixed as H atom, while R_2 was altered as various aliphatic side chains. We can see that the length of R_2 groups was negatively relative with the APN inhibition, suggesting it is unfavorable to increase the length of substituents along the orientation of the R_2 groups. The possible reason is that R_2 groups did not accommodate the active binding domains (S_1 or S_1' pocket) of APN/CD13. Thus, the longer the side chain of R_2 was, to some extent, the less strongly the compounds inhibited the enzyme. The same rule can also be applied to compounds **2n–p**.

Substitution on aromatic ring also has impact on bioactivity. For instance, compounds **2g–j** with halogen substituents at the *para*-position in the aromatic ring, to some extent, exhibited obviously enhanced potencies compared with their predecessor **2f**. This result might be owing to the p-π conjugative effect between halogen atoms and aromatic ring which could enhance the interaction with the enzyme. Moreover, as compared with the fluoro, chloro and bromo substitutions, it seems that the increased bulk might lead to impair activity, implying there is a space requirement in the binding pockets to accommodate the suitable substituents.

Compounds **4** and **5** were found to be more potent than their precursors (**2a** and **3**). This difference was possibly caused by the ZBG, which was the only structural difference between them. It revealed that introduction of hydroxamate (CONHOH) produced increased potency in comparison with its precursor, carboxylate ($COOCH_3$) or carboxyl (COOH), respectively, which is in accordance with our previously SAR results [11, 12]. In addition, the antiproliferative effect of these two compounds (**4** and **5**) on tumor cell HL-60 (myelomonocytic human acute granulocytic leukemia cells expressing high APN level) [16] were assessed by using MTT method. The IC_{50} values of these two compounds (**4**, 2.17 ± 0.39 mM and **5**, 2.61 ± 0.31 mM) confirmed again that their inhibitory activities are similar with bestatin (1.50 ± 0.22 mM, see Fig. 2).

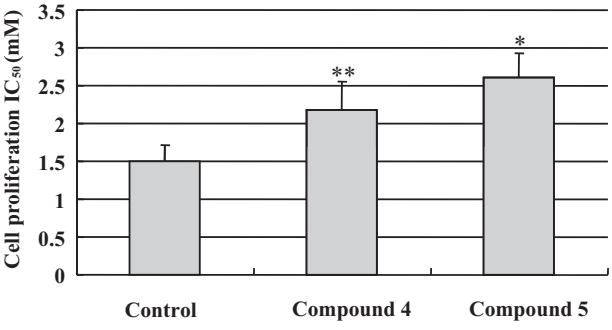


Figure 2. Effects of bestatin (control) and compounds **4** and **5** on proliferation of the HL-60 cell line. Data are expressed as mean values of five independent experiments (±SE). * $p < 0.01$, ** $p < 0.05$ compared to the control.

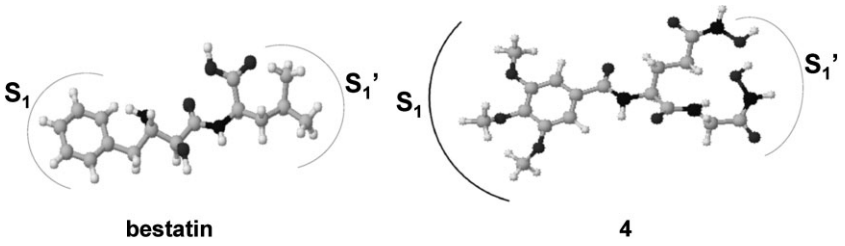


Figure 3. Comparison of preponderant conformations between bestatin and compound **4**.

Consistent with this result, free phenol hydroxyl-substituted compound **7** was prepared derived from its galloylated counterpart (**6**), with the purpose of comparing the chelating effect of hydroxyl group as another ZBG. Compound **6** has been reported in our previous work, (see [15], compound **2C**), for clarity, different denomination was deployed in present report. It revealed that the de-galloylated compound (**7**) provided higher potency than that of the galloylated one (**6**), with nearly 5-fold enhancement towards APN/CD13. Besides the contribution of the phenol hydroxyl group, the hydrogen bond between hydroxyl group and the enzyme, to a certain extent, might also play an important role in the enzymatic inhibition.

Binding studies

The representative compound **4**, which showed the best affinity in our series, was selected to study its binding mode with APN. Firstly, the predicted conformation of **4** was optimized with the Powell Energetic Gradiet method built in the Sketch/Build Edit model (Sybyl 6.91, Linux 7.3) [17]. As compared with bestatin, the comparable results showed the affinity to the same spatial region, suggesting the similar APN/CD13 inhibitory potencies. In precise, both the isopropyl portion of bestatin and the hydroxamate fragment of **4** can adjust their flexibility to occupy the S_1' pocket with their preponderant conformation, as diagrammed in Fig. 3. In contrast, the similar situation can also be observed between the phenyl group of bestatin and the galloylated phenyl portion of **4**.

To further understand the interaction of **4** with APN, the preferred docking study was ongoing performed using a Sybyl/FlexX module. From Figure 4A, we can see both the galloylated phenyl portion and the introduced hydroxamate fragment of **4** can well-orienting interact with the active domain (S_1 and S_1' pocket) of APN/CD13. Additionally, both the nitrogen and oxygen atoms in hydroxymate can coordinate with the zinc ion, with a distance of 2.26 and 1.86 Å, respectively. According to Fig. 4B, the hydroxamate group can form hydrogen bond with His301, one of the essential amino acids of the conserved sequence ($HEXXHX_{18}E$) in the catalytic domain that is well conserved in peptidase M1 family [18], at the distance of 2.65 and 2.73 Å, respectively. Moreover, the amino group of **8** formed a hydrogen bond with the phenolic hydroxyl group of Tyr381 residue, thus stabilizing the reaction intermediate with the zinc ion. In

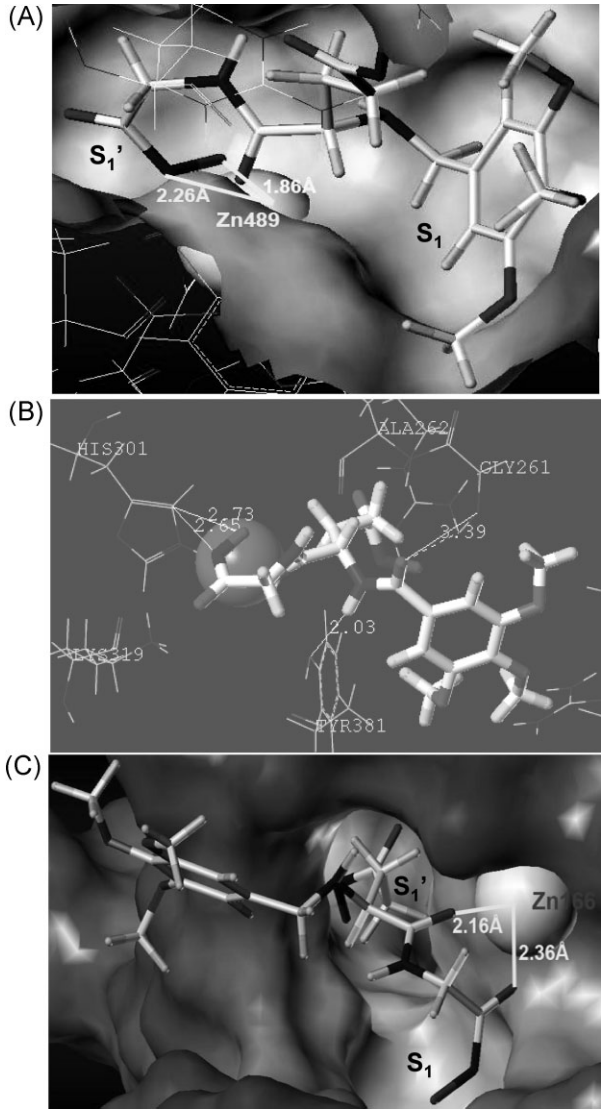


Figure 4. FlexX docking results of compound **4** with the active site of *E. coli* APN (A & B) and MMP-2 (C).

addition, the binding interaction was further enhanced by hydrogen bond with Gly261 (~3.39 Å).

Finally, the selectivity of **4** might be partially explained by the molecular docking with the active site of MMP-2 (PDB code: 1HOV) [19]. As shown in Fig. 4C, although two oxygen atoms of carbonyl groups could coordinate with the zinc ion of MMP-2, with a distance of 2.36 and 2.16 Å, respectively, only the hydroxymate group can partially insert into the S_1' pocket, causing seriously impaired MMP-2 inhibitory activity, in accordance with the enzymatic assay results.

Conclusion

In summary, novel peptidomimetic derivatives were developed and evaluated as potential inhibitors of APN/CD13. Most of the target compounds showed potent and selective activities against APN/CD13 as compared with MMP-2. And else, compound **4** was comparable to bestatin and could be used as a potential lead for further development of APNIs. It should be noted that although our preliminary enzymatic results are not very encouraging (IC_{50} values in the micromole range), their selective inhibition towards APN/CD13 and SAR information provides useful clues for further APNIs exploitation.

Experimental

Biological evaluation

All synthesized compounds were evaluated for their inhibitory properties against APN/CD13 versus MMP-2 (gelatinase A) so as to compare the selectivity. Compounds **4** and **5** were further tested the antiproliferative activity on the HL-60 cell.

Enzyme inhibition assay

APN/CD13 inhibition

The IC_{50} values against APN/CD13 were determined 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 [20]. 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 adjusted to 7.5 by the addition of 0.1 M HCl or 0.1 M NaOH. All inhibitors were preincubated 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.

MMP-2 inhibition

MMP-2 (gelatinase A) assay was performed as described by Baragi *et al*. [21]. The gelatinase, substrate, and inhibitor were dissolved in sodium borate (pH 8.5, 50 mmol/L) and incubated for 30 min at 37°C, and then 0.03% trinitrobenzenesulfonic acid (TNBS, Sigma) was added and incubated for another 20 min, the resulting solution was detected under 450 nm wavelength to gain absorption.

MTT assay

The cell lines were grown in RPMI1640 medium containing 10% FBS at 37°C in a humidified incubator with 5% CO_2. Cell proliferation was determined by the MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazolium bromide) assay. Briefly, cells were plated in a 96-well plate at 10 000 cells per well, cultured for 4 h in complete growth medium, then treated with 1600, 800, 400, 200, or 100 μg/mL 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.

Computational-docking

The docking study was performed as follows: The selected compound was constructed with a Sybyl/Sketch module and optimized using Powell Energetic Gradient method with a Tripos force field with the convergence criterion set at 0.05 kcal/(mol Å), and assigned with Gasteiger-Hückel method [22, 23]. When it comes to the docking assay of APN, the residues in a radius of 7.0 Å around bestatin in the co-crystal structure (PDB code: 2DQM) [24] were selected as the active site, and other docking parameters implied in the program were kept as the defaults. As to MMP-2, residues in a radius of 4.0 Å around SC-74020 (the provided ligand of MMP-2 in the crystal structure, PDB code: 1HOV) [19] were considered as the active site, including the catalytic zinc(II) ion.

General procedures for chemistry

Silica gel for column chromatography (CC) and TLC plates precoated with silica gel GF_{254} were commercial available from Qingdao Haiyang Chemical Company, Qingdao, China. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on 0.25 mm silicagel 60 F_{254} plates and visualized under ultraviolet (UV) light (254 nm), or iodine (I_2) vapor. Flash-chromatography (FC) was performed using 200–300 mesh silica gel and the solvent system was indicated in the procedure. All solvents were of reagent grade and, when necessary, were purified and dried by standard methods. Melting points (mp) were determined on an X-6 micro melting point apparatus with no correction. Infrared spectra (IR) were recorded in the range of 4000–600 cm^{-1} using a Nicolet Nexus 470FT-IR spectrometer, and KBr disks were used as indicated. ^{1}H-NMR spectra were determined on a Bruker Avance DRX-600 spectrometer, with chemical shift (δ) given in ppm upfield from Me_4Si (TMS) as an internal standard, and coupling constants *J* were recorded in hertz (Hz). Electrospray ionization mass spectrometry (ESI-MS) was performed on an API-4000 triple-stage quadrupole instrument. Measurements were made in D_2O or CD_3OD solutions. Elemental analyses were carried out on a Perkin-Elmer C, H, N elemental analyzer. Anhydrous reactions were carried out in oven-dried glassware under a nitrogen atmosphere, and all anhydrous solvents were distilled over CaH_2 or Na/benzophenone prior to use. Yields refer to purified products and are not optimized.

General procedure for the preparation of L-amino acid methyl ester hydrochloride

The preparation of methyl 2-aminoacetate hydrochloride is reported here as a representative example: To a suspension of glycine (15.0 g, 200 mmol) in anhydrous methanol (150 mL) at 0°C was pumped slowly in dried HCl gas until solution was completely clear. Cool in a refrigerator, preferably overnight. Remove the solvent and small amount methanol was added again to remove the excess HCl. The collected white precipitate was recrystallized from methanol/diethyl ether (1:4) to give methyl 2-aminoacetate hydrochloride (20 g, 80%). M.p. 175–176°C; IR (KBr, cm^{-1}): 3215.0–3523.0 (NH), 2935.2 (CH), 1676.3 (C=O), 1129.3 (C–O).

Similarly, reaction of other L-amino acids and anhydrous methanol under dry HCl atmosphere gave methyl ester hydrochloride as follows.

Methyl 2-amino-4-methylpentanoate hydrochloride: Yield: 69%, m.p. 115–117°C

Methyl 2-aminopropanoate hydrochloride: Yield: 84%, m.p. 97–100°C

Methyl 2-amino-3-phenylpropanoate hydrochloride: Yield: 70%, m.p. 101–105°C

Methyl 2-amino-3-(4-chlorophenyl)propanoate hydrochloride: Yield: 66%, m.p. 180–182°C

Methyl 2-amino-3-(4-fluorophenyl)propanoate hydrochloride: Yield: 79%, m.p. 174–176°C

Methyl 2-amino-4-methylpentanoate hydrochloride: Yield: 80%, m.p. 129–132°C

Methyl 2-amino-3-(4-hydroxyphenyl)propanoate hydrochloride: Yield: 76%, m.p. 118–121°C

Methyl 2-(methylamino)acetate hydrochloride: Yield: 52%, m.p. 111–115°C

Methyl 2-amino-3-hydroxypropanoate hydrochloride: Yield: 62%, m.p. 89–92°C

Methyl 2-amino-4-(methylthio)butanoate hydrochloride: Yield: 79%, m.p. 187–193°C

Methyl 3-(2-amino-1H-indol-3-yl)propanoate hydrochloride: Yield: 63%, m.p. 220–223°C

Methyl 2-amino-3-methylbutanoate hydrochloride: Yield: 83%, m.p. 90–95°C

(R)-5-(2-Methoxy-2-oxoethylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***2a***

To a suspension of compound **1** (3.23 g, 10 mmol) and methyl 2-aminoacetate hydrochloride (2.5 g, 20 mmol) in CH_2Cl_2 (30 mL) at room temperature was added 5 mL of Et_3N. The mixture was stirred at this temperature until the disappearance of the starting material, checking via TLC. The solvent was evaporated *in vacuo* and poured into ice-cold water (50 mL). The mixture was acidified with 2 N HCl to pH 2. Cool in a refrigerator, collect the white flaky crystals on a Buchner funnel, wash with ice cold water, and dry to give **2a** (0.94 g, 74%). M.p. 169–172°C; IR (KBr, cm^{-1}): 3325.1 (NH), 2939.6 (CH), 1745.7 & 1714.5 (O=C–NH), 1637.8 (O=C–O), 1584.3 (C=C), 1131.3 (C–O). $[\alpha]_D^{25} = +39.5$ (c 1, MeOH); ^{1}H–NMR (DMSO-d_6, ppm): δ 8.45 (d, 1H, $J = 7.5$ Hz, NH), 8.38 (t, 1H, $J = 5.4$ Hz, NH), 7.23 (s, 2H, Ar–H), 4.47 (m, 1H, CH), 3.88 (d, 2H, $J = 5.4$ Hz, CH_2), 3.83 (s, 6H, 2-OCH_3), 3.69 (s, 3H, OCH_3), 3.62 (s, 3H, $COOCH_3$), 2.34 (t, 2H, $J = 8.0$ Hz, CH_2), 2.06, 1.92 (m, 2H, CH_2). ESI–MS *m/z*: 412.8 $[M + H]^+$. Anal. calcd. for $C_{18}H_{24}N_2O_9$: C, 52.43; H, 5.83; N, 6.80. Found: C, 52.88; H, 5.91; N, 6.72.

(R)-5-((S)-1-Methoxy-1-oxopropan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido) pentanoic acid ***2b***

Yield: 35.0%, m.p. 153–155°C, IR (KBr, cm^{-1}): 3304.8 (NH), 2933.5 (CH), 1751.1 & 1719.9 (O=C–NH), 1647.7 (O=C–O), 1584.2 (C=C), 1129.9 (C–O). $[\alpha]_D^{25} = +28.9$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.74 (d, 1H, $J = 7.8$ Hz, NH), 8.33 (d, 1H, $J = 6.0$ Hz, NH), 7.24 (s, 2H, Ar-H), 4.36 (m, 1H, CH), 4.23 (m, 1H, CH), 3.85 (s, 6H, 2-OCH_3), 3.73 (s, 3H, OCH_3), 3.60 (s, 3H, $COOCH_3$), 2.23 (t, 2H, $J = 7.8$ Hz, CH_2), 2.06, 1.83 (m, 2H, CH_2), 1.23 (d, 3H, $J = 7.2$ Hz, CH_3). ESI-MS *m/z*: 425.1 $[M + H]^+$. Anal. calcd. for $C_{19}H_{26}N_2O_9$: C, 53.52; H, 6.10; N, 6.57. Found: C, 54.07; H, 6.18; N, 6.46.

(R)-5-((S)-1-Methoxy-1-oxobutan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido) pentanoic acid ***2c***

Yield: 28.5%, m.p. 105–107°C, IR (KBr, cm^{-1}): 3305.2 (NH), 2942.2 (CH), 1746.1 & 1717.9 (O=C-NH), 1644.7 (O=C–O), 1586.7 (C=C), 1127.6 (C–O). $[\alpha]_D^{25} = +22.6$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.73 (d, 1H, $J = 7.8$ Hz, NH), 8.37 (d, 1H, $J = 6.0$ Hz, NH), 7.00 (s, 2H, Ar-H), 4.36 (m, 1H, CH), 4.27 (m, 1H, CH), 3.73 (s, 6H, 2-OCH_3), 3.67 (s, 3H, OCH_3), 3.82 (s, 3H, $COOCH_3$), 2.23 (t, 2H, $J = 7.0$ Hz, CH_2), 2.06, 1.83 (m, 2H, CH_2), 1.92 (m, 2H, CH_2), 0.98 (d, 3H, $J = 7.2$ Hz, CH_3). ESI-MS *m/z*: 439.4 $[M + H]^+$. Anal. calcd. for $C_{20}H_{28}N_2O_9$: C, 54.55; H, 6.36; N, 6.36. Found: C, 54.64; H, 6.45; N, 6.22.

(R)-5-((S)-1-Methoxy-1-oxopentan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido) pentanoic acid ***2d***

Yield: 23.4%, m.p. 110–112°C, IR (KBr, cm^{-1}): 3307.8 (NH), 2940.6 (CH), 1755.4 & 1718.1 (O=C–NH), 1640.9 (O=C–O), 1589.2 (C=C), 1125.5 (C–O). $[\alpha]_D^{25} = +25.2$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.88 (d, 1H, $J = 7.8$ Hz, NH), 8.24 (d, 1H, $J = 8.1$ Hz, NH), 6.98 (s, 2H, Ar-H), 4.33 (m, 1H, CH), 4.53 (m, 1H, CH), 3.73 (s, 6H, 2-OCH_3), 3.62 (s, 3H, OCH_3), 3.72 (s, 3H, $COOCH_3$), 2.10 (t, 2H, $J = 15.3$ Hz, CH_2), 2.11, 1.79 (m, 2H, CH_2), 1.32 (m, 2H, CH_2), 1.90 (m, 2H, CH_2), 0.97 (d, 3H, $J = 8.4$ Hz, CH_3). ESI-MS *m/z*: 453.3 $[M + H]^+$. Anal. calcd. for $C_{21}H_{30}N_2O_9$: C, 55.51; H, 6.61; N, 6.17. Found: C, 55.63; H, 6.70; N, 6.02.

(R)-5-((S)-1-Methoxy-1-oxohexan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido) pentanoic acid ***2e***

Yield: 61.2%, m.p. 99–101°C, IR (KBr, cm^{-1}): 3321.4 & 3256.9 (NH), 2956.2 (CH), 1724.1 (O=C–NH), 1647.6 (O=C–O), 1584.3 (C=C), 1130.5 (C–O). $[\alpha]_D^{25} = +21.7$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 7.82 (d, 1H, $J = 7.6$ Hz, NH), 7.15 (s, 1H, Ar-H), 7.12 (s, 1H, Ar-H), 6.44 (d, 1H, $J = 7.2$ Hz, NH), 4.93 (m, 1H, CH), 4.60 (m, 1H, CH), 3.92 (s, 6H, 2-OCH_3), 3.80 (s, 3H, OCH_3), 3.68 (s, 3H, $COOCH_3$), 2.28 (t, 2H, $J = 7.2$ Hz, CH_2), 2.06 (m, 2H, CH_2), 1.92 (m, 2H, CH_2), 1.29 (br, 4H, 2-CH_2), 0.87 (t, 3H, $J = 7.8$ Hz, CH_3). ESI-MS *m/z*: 467.1 $[M + H]^+$. Anal. calcd. for $C_{22}H_{32}N_2O_9$: C, 56.41; H, 6.84; N, 5.98. Found: C, 56.51; H, 6.90; N, 5.89.

(R)-5-((S)-1-Methoxy-1-oxo-3-phenylpropan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxy-benzamido) pentanoic acid ***2f***

Yield: 56.3%, m.p. 141–144°C, IR (KBr, cm^{-1}): 3416.0 NH), 2939.9 (CH), 1743.7 & 1718.8 (O=C–NH), 1646.4 (O=C–O), 1584.1 & 1536.7 (C=C), 1128.9 (C–O). $[\alpha]_D^{25} = +33.8$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.69 (d, 1H, $J = 4.3$ Hz, NH), 8.38 (d, 1H, $J = 6.8$ Hz, NH), 7.24 (m, 2H, Ar-H), 7.18 (m, 5H, Ar-H), 4.44

(m, 1H, CH), 4.34 (m, 1H, CH), 3.85 (s, 6H, 2-OCH_3), 3.70 (s, 3H, OCH_3), 3.57 (s, 3H, $COOCH_3$), 2.18 (t, 2H, $J = 7.6$ Hz, CH_2), 2.11 (d, 2H, $J = 7.4$ Hz, CH_2), 1.98, 1.76 (2m, 2H, CH_2). ESI-MS *m/z*: 501.1 $[M + H]^+$. Anal. calcd. for $C_{25}H_{30}N_2O_9$: C, 59.76; H, 5.98; N, 5.58. Found: C, 59.85; H, 6.09; N, 5.47.

(R)-5-((S)-3-(4-Fluorophenyl)-1-methoxy-1-oxopropan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***2g***

Yield: 61.4%, m.p. 107–111°C, IR (KBr, cm^{-1}): 3296.3 (NH), 2942.1 (CH), 1743.0 O=C–NH), 1648.6 (O=C–O), 1584.2 & 1537.3 (C=C), 1127.6 (C–O). $[\alpha]_D^{25} = +45.1$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 7.78 (d, 1H, $J = 6.6$ Hz, NH), 7.26 (s, 2H, Ar-H), 7.12 (m, 2H, Ar-H), 6.95 (m, 2H, Ar-H), 6.47 (d, 1H, $J = 8.0$ Hz, NH), 4.78 (m, 1H, CH), 4.57 (m, 1H, CH), 3.93 (s, 6H, 2-OCH_3), 3.86 (s, 3H, OCH_3), 3.68 (s, 3H, $COOCH_3$), 3.10 (d, 2H, $J = 8.2$ Hz, CH_2), 2.27 (t, 2H, $J = 7.2$ Hz, CH_2), 1.89 (m, 2H, CH_2). ESI-MS *m/z*: 519.2 $[M + H]^+$. Anal. calcd. for $C_{25}H_{29}N_2O_9F$: C, 57.69; H, 5.58; N, 5.38. Found: C, 57.77; H, 5.65; N, 5.30.

(R)-5-((S)-3-(4-Chlorophenyl)-1-methoxy-1-oxopropan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***2h***

Yield: 67.2%, m.p. 143–145°C, IR (KBr, cm^{-1}): 3300.1 (NH), 2942.3 (CH), 1742.9 & 1718.6 (O=C–NH), 1647.0 (O=C–O), 1584.0 C=C), 1129.0 (C–O). $[\alpha]_D^{25} = +19.8$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 7.74 (d, 1H, $J = 6.4$ Hz, NH), 7.29 (s, 2H, Ar-H), 7.22 (m, 2H, Ar-H), 7.10 (m, 2H, Ar-H), 6.54 (d, 1H, $J = 7.8$ Hz, NH), 4.79 (m, 1H, CH), 4.55 (m, 1H, CH), 3.93 (s, 6H, 2-OCH_3), 3.89 (s, 3H, OCH_3), 3.72 (s, 3H, $COOCH_3$), 3.12 (d, 2H, $J = 7.6$ Hz, CH_2), 2.31 (t, 2H, $J = 7.6$ Hz, CH_2), 1.95 (m, 2H, CH_2). ESI-MS *m/z*: 535.1 $[M + H]^+$. Anal. calcd. for $C_{25}H_{29}N_2O_9Cl$: C, 55.92; H, 5.41; N, 5.22. Found: C, 56.04; H, 5.52; N, 5.18.

(R)-5-((S)-3-(4-Bromophenyl)-1-methoxy-1-oxopropan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxy benzamido)pentanoic acid ***2i***

Yield: 50.7%, m.p. 166–168°C, IR (KBr, cm^{-1}): 3303.4 (NH), 2946.1 (CH), 1744.2 & 1719.0 (O=C–NH), 1647.4 (O=C–O), 1583.8 & 1534.6 (C=C), 1128.7 (C–O). $[\alpha]_D^{25} = +22.5$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 7.77 (d, 1H, $J = 6.8$ Hz, NH), 7.26 (s, 2H, Ar-H), 7.18 (m, 2H, Ar-H), 7.05 (m, 2H, Ar-H), 6.62 (d, 1H, $J = 4.5$ Hz, NH), 4.83 (m, 1H, CH), 4.57 (m, 1H, CH), 3.91 (s, 6H, 2-OCH_3), 3.80 (s, 3H, OCH_3), 3.77 (s, 3H, $COOCH_3$), 3.01 (d, 2H, $J = 8.4$ Hz, CH_2), 2.39 (t, 2H, $J = 5.8$ Hz, CH_2), 1.98 (m, 2H, CH_2). ESI-MS *m/z*: 580.3 $[M + H]^+$. Anal. calcd. for $C_{25}H_{29}N_2O_9Br$: C, 51.64; H, 4.99; N, 4.82. Found: C, 51.73; H, 5.06; N, 4.73.

(R)-5-((S)-3-(4-Hydroxyphenyl)-1-methoxy-1-oxopropan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)-pentanoic acid ***2j***

Yield: 49.0%, m.p. 87–89°C, IR (KBr, cm^{-1}): 3355.2 & 3300.7 (NH), 2952.3 (CH), 1744.1 (O=C–NH), 1647.7 (O=C–O), 1585.9 & 1498.7 (C=C), 1257.7 & 1126.5 (C–O). $[\alpha]_D^{25} = +33.4$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.72 (d, 1H, $J = 7.0$ Hz, NH), 8.33 (d, 1H, $J = 6.4$ Hz, NH), 7.23 (d, 2H, $J = 3.4$ Hz, Ar-H), 6.95 (m, 2H, Ar-H), 6.64 (m, 2H, Ar-H), 4.34 (m, 1H, CH), 3.83 (s, 6H, 2-OCH_3), 3.70 (s, 3H, OCH_3), 3.56 (s, 3H, $COOCH_3$), 3.49 (m, 1H, CH), 2.20 (t, 2H, $J = 7.5$ Hz, CH_2), 2.11 (d, 2H, $J = 6.8$ Hz, CH_2), 2.00, 1.78 (2m, 2H, CH_2). ESI-MS *m/z*: 517.1 $[M + H]^+$. Anal. calcd. for $C_{25}H_{30}N_2O_{10}$: C, 57.92; H, 5.79; N, 5.41. Found: C, 58.03; H, 5.86; N, 5.32.

(R)-5-((S)-3-Hydroxy-1-methoxy-1-oxopropan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido) pentanoic acid ***2k***

Yield: 60.0%, m.p. 85–87°C, IR (KBr, cm^{-1}): 3266.1 (NH), 2941.7 (CH), 1744.2 (O=C–NH), 1632.2 (O=C–O), 1584.3 (C=C), 1129.3 (C–O). $[\alpha]_D^{25} = +40.8$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.76 (d, 1H, $J = 6.5$ Hz, NH), 8.26 (d, 1H, $J = 6.8$ Hz, NH), 7.24 (m, 2H, Ar-H), 5.02 (m, 1H, CH), 4.33 (m, 1H, CH), 3.82 (s, 6H, 2-OCH_3), 3.71 (s, 3H, OCH_3), 3.61 (s, 3H, $COOCH_3$), 3.05 (d, 2H, $J = 5.5$ Hz, CH_2), 2.28 (t, 2H, $J = 6.0$ Hz, CH_2), 2.05, 1.83 (2m, 2H, CH_2). ESI-MS *m/z*: 441.1 $[M + H]^+$. Anal. calcd. for $C_{19}H_{26}N_2O_{10}$: C, 51.58; H, 5.88; N, 6.33. Found: C, 51.66; H, 5.95; N, 6.25.

(R)-5-((S)-1-Methoxy-4-(methylthio)-1-oxobutan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***2l***

Yield: 59.1%, m.p. 124–127°C, IR (KBr, cm^{-1}): 3289.3 (NH), 2941.8 (CH), 1750.9 & 1717.8 (O=C-NH), 1647.2 (O=C–O), 1584.5 (C=C), 1129.7 (C–O). $[\alpha]_D^{25} = +37.3$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.72 (d, 1H, $J = 5.7$ Hz, NH), 8.32 (d, 1H, $J = 7.3$ Hz, NH), 7.24 (s, 2H, Ar-H), 4.47 (m, 1H, CH), 4.37 (m, 1H, CH), 3.78 (s, 6H, 2-OCH_3), 3.70 (s, 3H, OCH_3), 3.61 (s, 3H, $COOCH_3$), 2.26 (t, 2H, $J = 7.8$ Hz, CH_2), 2.13 (t, 2H, $J = 7.4$ Hz, CH_2), 2.06 (m, 4H, 2-CH_2), 1.83 (s, 3H, CH_3). ESI-MS *m/z*: 486.1 $[M + H]^+$. Anal. calcd. for $C_{21}H_{30}N_2O_9S$: C, 51.85; H, 6.17; N, 5.76. Found: C, 51.97; H, 6.24; N, 5.68.

(R)-5-((R)-1-Methoxy-1-oxo-3-(phenylthio)propan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***2m***

Yield: 54.0%, m.p. 77–79°C, IR (KBr, cm^{-1}): 3272.9 (NH), 2940.6 (CH), 1746.5 & 1722.0 (O=C–NH), 1648.5 (O=C–O), 1584.0 (C=C), 1128.4 (C–O). $[\alpha]_D^{25} = +20.5$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.77 (d, 1H, $J = 6.8$ Hz, NH), 8.46 (d, 1H, $J = 7.2$ Hz, NH), 7.34 (d, 2H, $J = 4.1$ Hz, Ar-H), 7.30 (m, 5H, Ar-H), 4.47 (m, 1H, CH), 4.20 (m, 1H, CH), 3.83 (s, 6H, 2-OCH_3), 3.75 (s, 3H, OCH_3), 3.60 (s, 3H, $COOCH_3$), 3.32 (s, 2H, CH_2), 3.06 (d, 2H, $J = 6.8$ Hz, CH_2), 2.26 (t, 2H, $J = 7.8$ Hz, CH_2), 2.07 & 1.84 (2m, 2H, CH_2). ESI-MS *m/z*: 547.1 $[M + H]^+$. Anal. calcd. for $C_{26}H_{32}N_2O_9S$: C, 56.93; H, 5.84; N, 5.11. Found: C, 57.04; H, 5.91; N, 5.01.

(R)-5-((S)-5-(Benzyloxycarbonyl)-1-methoxy-1-oxopentan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***2n***

Yield: 43.0%, m.p. 108–112°C, IR (KBr, cm^{-1}): 3333.2 & 3276.9 (NH), 2945.9 (CH), 1745.0 O=C–NH), 1688.4, 1584.1 (C=C), 1128.2 (C–O). $[\alpha]_D^{25} = +15.8$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.74 (d, 1H, $J = 5.6$ Hz, NH), 8.28 (d, 1H, $J = 6.9$ Hz, NH), 7.34 (m, 5H, Ar-H), 7.24 (s, 2H, Ar-H), 4.99 (s, 2H, CH_2), 4.36 (m, 1H, CH), 4.19 (m, 1H, CH), 3.83 (s, 6H, 2-OCH_3), 3.71 (s, 3H, OCH_3), 3.59 (s, 3H, $COOCH_3$), 2.96 (t, 2H, $J = 5.2$ Hz, CH_2), 2.25 (t, 2H, $J = 7.8$ Hz, CH_2), 2.06 & 1.83 (2m, 2H, CH_2), 1.66 & 1.54 (2m, 2H, CH_2), 1.45 (m, 2H, CH_2). ESI-MS *m/z*: 602.1 $[M + H]^+$. Anal. calcd. for $C_{29}H_{37}N_3O_{11}$: C, 57.71; H, 6.14; N, 6.97. Found: C, 57.84; H, 6.21; N, 6.89.

(R)-5-((S)-6-(Benzyloxycarbonyl)-1-methoxy-1-oxohexan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)-pentanoic acid ***2o***

Yield: 48.0%, m.p. 147–150°C, IR (KBr, cm^{-1}): 3325.6 (NH), 2940.1 (CH), 1720.9 (O=C–NH), 1648.8 (O=C–O), 1584.8 & 1498.2 (C=C), 1234.6 & 1126.5 (C–O). $[\alpha]_D^{25} = +19.0$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.72 (t, 1H, $J = 6.0$ Hz, NH), 8.24 (t, 1H, $J = 7.2$ Hz, NH), 7.33 (m, 5H, Ar-H), 7.24 (s, 2H, Ar-H), 7.20 (s, 1H, Ar-H), 4.99 (s, 2H, CH_2), 4.37 (m, 1H, CH), 4.17 (m, 1H, CH), 3.83 (s, 6H, 2-OCH_3), 3.74 (s, 3H, OCH_3), 3.61 (s, 3H, $COOCH_3$), 2.94 (t, 2H, $J = 6.0$ Hz, CH_2), 2.27 (t, 2H, $J = 7.8$ Hz, CH_2), 2.13 (s, 3H, CH_3), 2.05 (m, 1H, CH), 1.85 (m, 1H, CH), 1.56 (m, 2H, CH_2), 1.36 (m, 2H, CH_2), 1.26 (m, 2H, CH_2). ESI-MS *m/z*: 616.1 $[M + H]^+$. Anal. calcd. for $C_{30}H_{39}N_3O_{11}$: C, 58.35; H, 6.32; N, 6.81. Found: C, 58.44; H, 6.41; N, 6.75.

(R)-5-((S)-1-Methoxy-5-(3-nitroguanidino)-1-oxopentan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***2p***

Yield: 52.2%, m.p. 123–126°C, IR (KBr, cm^{-1}): 3307.4 (NH), 2943.8 (CH), 1739.8 (O=C–NH), 1649.1 (O=C–O), 1584.7 & 1499.7 (C=C), 1236.4 & 1127.5 (C–O). $[\alpha]_D^{25} = +34.2$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.66 (d, 1H, $J = 6.6$ Hz, NH), 8.22 (t, 1H, $J = 6.0$ Hz, NH), 7.24 (s, 2H, Ar-H), 4.37 (m, 1H, CH), 4.24 (m, 1H, CH), 3.84 (s, 6H, 2-OCH_3), 3.72 (s, 3H, OCH_3), 3.61 (s, 3H, $COOCH_3$), 3.13 (d, 2H, $J = 5.4$ Hz, CH_2), 2.27 (t, 2H, $J = 7.2$ Hz, CH_2), 2.13 (s, 2H, CH_2), 2.07 (m, 1H, CH), 1.85 (m, 1H, CH), 1.71 (m, 1H, CH), 1.58 (m, 2H, CH_2), 1.51 (m, 1H, CH). ESI-MS *m/z*: 555.1 $[M + H]^+$. Anal. calcd. for $C_{22}H_{32}N_6O_{11}$: C, 47.48; H, 5.76; N, 15.11. Found: C, 47.54; H, 5.85; N, 15.03.

(R)-5-((S)-3-(1H-indol-3-yl)-1-methoxy-1-oxopropan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***2q***

Yield: 44.1%, m.p. 167–169°C, IR (KBr, cm^{-1}): 3362.5 (NH), 2948.5 (CH), 1739.8 (O=C–NH), 1652.4 (O=C–O), 1584.7 & 1497.1 (C=C), 1233.3 & 1126.5 (C–O). $[\alpha]_D^{25} = +22.4$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.14 (s, 1H, NH), 8.09 (s, 1H, OH), 7.91 (d, 1H, $J = 6.1$ Hz, NH), 7.84 (d, 1H, $J = 6.3$ Hz, NH), 7.51 (t, 2H, $J = 8.4$ Hz, Ar-H), 7.16 (s, 2H, Ar-H), 7.14 (s, 2H, Ar-H), 7.08 (m, 1H, =CH), 4.76 (m, 1H, CH), 4.55 (m, 1H, CH), 3.92 (s, 6H, 2-OCH_3), 3.90 (s, 3H, OCH_3), 3.71 (s, 3H, $COOCH_3$), 2.41 (m, 1H, CH), 2.27 (m, 2H, CH_2), 2.20 (m, 2H, CH_2), 1.96 (m, 1H, CH). ESI-MS *m/z*: 540.1 $[M + H]^+$. Anal. calcd. for $C_{27}H_{31}N_3O_9$: C, 59.89; H, 5.73; N, 7.76. Found: C, 59.95; H, 5.81; N, 7.62.

(R)-5-((S)-3-(1-(Benzyloxycarbonyl)-1H-imidazol-4-yl)-1-methoxy-1-oxopropan-2-ylamino)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***2r***

Yield: 56.3%, m.p. 112–116°C, IR (KBr, cm^{-1}): 3241.5 (NH), 2947.2 (CH), 1744.7 (O=C–NH), 1661.9 (O=C–O), 1584.4 & 1498.5 (C=C), 1233.9 & 1125.8 (C–O). $[\alpha]_D^{25} = +26.1$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.40 (d, 1H, $J = 5.7$ Hz, NH), 8.27 (d, 1H, $J = 6.0$ Hz, NH), 7.35 (s, 2H, Ar-H), 7.34 (s, 1H, =CH), 7.52 (d, 1H, $J = 5.3$ Hz, Ar-H), 7.22 (d, 1H, $J = 2.6$ Hz, Ar-H), 7.12 (d, 1H, $J = 6.6$ Hz, Ar-H), 6.67 (s, 1H, =CH), 5.03 (m, 2H, Ar-CH_2), 4.74 (m, 1H, CH), 4.59 (m, 1H, CH), 3.92 (s, 6H, 2-OCH_3), 3.91 (s, 3H, OCH_3), 3.89 (s, 3H, $COOCH_3$), 3.07 (m, 2H, CH_2), 2.53 (m, 2H, CH_2), 2.46 (m, 2H, CH_2). ESI-MS *m/z*: 581.2 $[M + H]^+$. Anal. calcd. for $C_{29}H_{34}N_4O_9$: C, 59.79; H, 5.84; N, 9.62. Found: C, 59.88; H, 5.91; N, 9.53.

(4R)-5-(4-(Benzyloxy)-2-(methoxycarbonyl)pyrrolidin-1-yl)-5-oxo-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***3***

Yield: 65.4%, m.p. 98–101°C; IR (KBr, cm^{-1}): 3237.4 (NH), 3099.1 & 2944.7 (CH), 1745.6 and 1717.7 (O=C–NH), 1657.8 (O=C–O), 1577.8 & 1484.6 (C=C), 1231.7 and 1121.96 (C–O). $[\alpha]_D^{25} = +40.2$ (c 1, MeOH); ^{1}H-NMR (DMSO-d_6, ppm): δ 8.66 (d, 1H, $J = 4.0$ Hz, NH), 7.26–7.32 (m, 5H, Ar-H), 7.23 (s, 1H, Ar-H), 4.50 (s, 2H, CH_2), 4.39 (m, 1H, CH), 4.32 (t, 1H, $J = 7.8$ Hz, CH), 3.83 (s, 6H, 2-OCH_3), 3.72 (s, 3H, OCH_3), 3.61 (s, 3H, $COOCH_3$), 3.23 (s, 1H, CH), 2.39 (m, 2H, CH_2), 2.32 (m, 1H, CH), 2.12 (d, 1H, $J = 7.8$ Hz, CH_2), 2.06 (m, 1H, CH), 1.98 (m, 1H, CH), 1.87 (m, 1H, CH). ESI-MS *m/z*: 557.1 $[M + H]^+$. Anal. calcd. for $C_{28}H_{34}N_2O_{10}$: C, 60.22; H, 6.09; N, 5.02. Found: C, 60.31; H, 6.17; N, 4.98.

(R)-N-(5-(Hydroxyamino)-1-(2-(hydroxyamino)-2-oxoethylamino)-1,5-dioxopentan-2-yl)-3,4,5-trimethoxybenzamide ***4***

A warm solution of KOH (5.6 g, 100 mmol) in anhydrous methanol (15 mL) was added to a hot solution of $NH_2OH \cdot HCl$ (4.67 g, 67.2 mmol) in anhydrous methanol (25 mL), maintaining the temperature at less than 35°C. Filtered and the filtrate (solution of NH_2OK in methanol) was allowed to cool to room temperature. Successively the obtained filtrate was added dropwise to a solution of compound **6a** (0.5 g, 1.2 mmol) in anhydrous methanol (10 mL). After being stirred for 4 h, the mixture was acidified with a small amount of AcOH to pH 5–6, and extracted with CH_2Cl_2. The aqueous layer was evaporated and dried to give compound **4** (0.4 g, 80%) as white solid, which appeared prunosus in $FeCl_3$ developer. M.p. 176–179°C; IR (KBr, cm^{-1}): 3299.7 (NH), 2959.1 (CH), 1749.4 and 1718.9 (O=C–NH), 1645.6 (O=C–O), 1584.1 (C=C), 1236.3 and 1128.9 (C–O). $[\alpha]_D^{25} = +20.7$ (c 1, MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6, ppm): δ 10.59 (s, 1H, OH), 10.46 (s, 1H, OH), 8.75 (s, 1H, NH), 8.43 (d, 1H, $J = 8.4$ Hz, NH), 8.05 (t, 2H, $J = 6.0$ Hz, 2NH), 6.98 (s, 2H, 2Ar-H), 4.57 (m, 1H, CH), 3.84 (s, 6H, 2-OCH_3), 3.70 (s, 3H, OCH_3), 4.09 (m, 2H, CH_2), 3.58 (m, 2H, CH_2), 2.19 (m, 2H, CH_2). ESI-MS *m/z*: 427.1 $[M + H]^+$. Anal. calcd. for $C_{17}H_{24}N_4O_9$: C, 47.66; H, 5.61; N, 13.08. Found: C, 47.75; H, 5.70; N, 12.99.

4-(Benzyloxy)-N-hydroxy-1-(5-(hydroxyamino)-5-oxo-2-(3,4,5-trimethoxybenzamido)pentanoyl)pyrrolidine-2-carboxamide ***5***

Yield: 52.2%, m.p. 173–175°C, IR (KBr, cm^{-1}): 3287.3 (NH), 3004.7 & 2954.4 (CH), 1752.7 and 1716.6 (O=C–NH), 1640.1 (O=C–O), 1646.2 & 1580.2 (C=C), 1237.1 & 1130.0 (C–O). $[\alpha]_D^{25} = +29.8$ (c 1, MeOH), ^{1}H–NMR (DMSO-d_6, ppm): δ 10.52 (s, 1H, OH), 10.48 (s, 1H, OH), 8.70 (t, 2H, 2NH), 8.03 (t, 2H, $J = 6.7$ Hz, 2NH), 7.19 (m, 5H, 5Ar-H), 6.92 (s, 2H, 2Ar-H), 4.68 (s, 2H, CH_2), 4.51 (m, 1H, CH), 4.44 (t, 1H, CH), 3.79 (s, 6H, 2-OCH_3), 3.73 (s, 3H, OCH_3), 3.71 (m, 2H, CH_2N), 3.32 (m, 1H, CH-OCH_2), 2.46 (dd, 2H, $J = 8.6$ Hz, $J = 6.2$ Hz, CH_2), 2.18 (m, 2H, CH_2), 2.01 (m, 2H, CH_2). ESI-MS *m/z*: 573.2 $[M + H]^+$. Anal. calcd. for $C_{27}H_{34}N_4O_{10}$: C, 56.45; H, 5.92; N, 9.76. Found: C, 56.54; H, 6.01; N, 9.62.

(R)-5-Oxo-5-(thiazol-2-ylamino)-4-(3,4,5-trimethoxybenzamido)pentanoic acid ***6***

A suspension of compound **1** (3.23 g, 10 mmol) and 4,5-dihydrothiazol-2-amine (2.8 g, 11 mmol) in acetic acid (30 mL) was allowed to reach ambient temperature. After completion of

the reaction, the white precipitate was collected and dried *in vacuo* to give **6** (3.45 g, 81.4%). M.p. 204–205°C; IR (KBr, cm^{-1}): 3411.7 & 3328.3 (NH), 2940.7 (CH), 1696.1 (O=C–NH), 1583.0 C=C), 1128.3 (C–O). $[\alpha]_D^{25} = +16.5$ (c 1, MeOH), ^{1}H–NMR (DMSO-d_6, ppm): δ 12.27 (s, 1H, COOH), 12.20 (s, 1H, NH), 8.62 (d, 1H, $J = 7.0$ Hz, NH), 7.48 (d, 1H, $J = 3.5$ Hz, =CH), 7.25 (s, 2H, Ar-H), 7.22 (d, 1H, $J = 3.6$ Hz, =CH), 4.63 (m, 1H, CH), 3.83 (s, 6H, 2-OCH_3), 3.71 (s, 3H, -OCH_3), 2.41 (m, 1H, CH), 2.34 (m, 1H, CH), 2.09 (m, 2H, CH_2). ESI-MS *m/z*: 423.8 $[M + H]^+$. Anal. calcd. for $C_{18}H_{21}N_3O_7S$: C, 51.06; H, 4.96; N, 9.93. Found: C, 51.14; H, 5.05; N, 9.81.

(R)-5-Oxo-5-(thiazol-2-ylamino)-4-(3,4,5-trihydroxy-benzamido)pentanoic acid ***7***

Method A: Place 0.43 g (1 mmol) of compound **6** and 15 mL of anhydrous CH_2Cl_2 in a 250-mL conical flask until the solid has almost completely dissolved. The mixture was cooled and stirred in an ice bath to achieve temperature 0°C under N_2 atmosphere, followed by addition of a solution of BBr_3 (1.2 g, 4.5 mmol) in CH_2Cl_2 (25 mL). The reaction mixture was stirred at 0°C for 1 h and after that at room temperature for 10 h. The mixture was cooled to 0°C and quenched with distilled water (50 mL). The resulting reaction mixture was successively diluted with ice-cold water (100 mL) and partitioned. The filtrate was extracted with EtOAc (3 × 50 mL), and the combined organic fraction dried (Na_2SO_4). After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromatography (SiO_2; *n*-hexane/acetone, 1:1) to present **7** (0.25 g, 66%) as off-white crystals: M.p. 186–188°C.

Method B: A solution of compound **6** (0.43 g, 1 mmol) in anhydrous CH_2Cl_2 (10 mL) was added solution of BBr_3 (1.2 g, 4.5 mmol) in CH_2Cl_2 (10 mL) dropwise under N_2 atmosphere, maintaining the temperature between −5 to 0°C. After stirring vigorously at this temperature for 1 h, the mixture was heated at 50°C for 12 h and at room temperature for 2 h. Concentrated HCl (5 mL) was slowly added and the mixture was allowed to stir at room temperature for another 30 min. The solvent was removed under reduced pressure which afforded an oily residue. This oil was dissolved in $CHCl_3$, and the subsequent careful addition of a small amount of anhydrous ethanol caused a precipitate of a white solid which was collected by fraction to afford 0.2 g (53%) of compound **7**. M.p. 187–188°C, IR (KBr, cm^{-1}): 3375.5 & 3289.6 (NH), 3139.7 (CH-OH), 2930.1 (CH), 1599.2 (O=C–NH), 1546.9 & 1488.3 (C=C), 1137.1 (C–O). $[\alpha]_D^{25} = +41.2$ (c 1, MeOH), ^{1}H-NMR (DMSO-d_6, ppm): δ 12.01 (s, 1H, COOH), 10.5 (s, 3H, 3Ar-OH), 8.90 (s, 1H, NH), 8.56 (d, 1H, $J = 8.5$ Hz, NH), 7.54 (d, 1H, $J = 6.8$ Hz, =CH), 6.58 (d, 1H, $J = 7.2$ Hz, =CH), 4.58 (m, 1H, CH), 2.33 (m, 2H, CH_2), 2.34 (m, 1H, CH), 2.11 (m, 2H, CH_2). ESI-MS *m/z*: 381.8 $[M + H]^+$. Anal. calcd. for $C_{15}H_{15}N_3O_7S$: C, 47.24; H, 3.94; N, 11.02. Found: C, 47.31; H, 4.02; N, 10.97.

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