Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2935–2938
Bioorganic & Medicinal Chemistry Letters

Novel 3-galloylamido-*N*′-substituted-2,6-piperidinedione-*N*-acetamide peptidomimetics as metalloproteinase inhibitors

Qianbin Li, Hao Fang and Wenfang Xu*

Institute of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, 44 West Wenhua Road, 250012 Ji'nan, Shandong, PR China

Received 23 September 2006; revised 12 December 2006; accepted 26 December 2006
Available online 5 January 2007

Abstract—Both of aminopeptidase N (APN) and matrix metalloproteinase (MMP) are essential metallopeptidases in the development of tumor invasion and angiogenesis. Novel potent peptidomimetic inhibitors, containing 3-galloylamido-*N*′-substituted-2,6-piperidinedione-*N*-acetamide, have been designed and synthesized according to the conformational constraint strategy. The preliminary biological test showed that most of the compounds displayed high inhibitory activity against MMP-2 and low activity against APN except compounds **6** (IC_{50} = 3.1 μM) and **4l** (IC_{50} = 5.2 μM) which exhibit similar potency to Bestatin (IC_{50} = 2.4 μM).

During the process of angiogenesis and metastatic cascade, tumor cells and endothelial cells pass through several connective tissue barriers. Proteolytic degradation of the extracellular matrix (ECM) is an important step of the process, which involves two classes of zinc-dependent metalloproteinases, amino-peptidase N (APN) and matrix metalloproteinases (MMP).

APN, also known as CD13, is a homodimeric type II membrane-bound glycoprotein and belongs to a member of M1 family ectopeptidase.[1–3] It is widely expressed on the surface of renal and intestinal brush border cells and other cells.[4,5] APN was also shown to be the major receptor for the TGEV and HCV229E, and *Bacillus thuringensis* Cry1A toxin.[6–8] Recently, many experimental results suggest that APN is involved in the down-regulation of several biological active molecules, such as enkephaline, fMLP, IL-8, angiotensin III, and major histocompatibility complex (MHC) class II molecules.[9–14] APN plays an essential role in the entry of HIV into host cells.[15] Furthermore, APN is overexpressed on tumor cells and plays a crucial role in ECM degradation and invasion of tumor cells.[16,17] Therefore, anti-APN/CD13 monoclonal antibody and APN inhibitors have been used to suppress the process of tumor cell invasion.[18,19]

APN inhibitors exhibited prevention of the spread of malignant cells[20] and prove to be clinically efficacious for invasion-protecting therapy. To date, several inhibitors of APN, including Bestatin, Amastatin, and Actinonin, have been developed and some of them are currently investigated for clinical uses.[21] All these natural compounds are pseudodipeptides bearing zinc-chelating functionality in the molecule.

MMP gene family consists of at least 28 structurally related members, among which MMP-2 and -9 are proved to be highly correlated with cancer. MMPs belong to endopeptidase and also play a critical role in the degradation of ECM and tissue remodeling and wound healing.[22,23] Currently, numerous MMP inhibitors are in various developmental stages for different symptoms, mostly in cancer and arthritis. Compounds currently under clinical trials as matrix metalloproteinase inhibitors (MMPIs) include Marimastat, Bay-129566, AG3340, and CGS27023A. All these compounds are applied to treat different types of cancer.[24] However, clinical trials exhibit disappointing results for most of MMPIs and possible reason is due to the inappropriate drug design or poor selectivity.

In general, peptides have drawbacks for clinical application, i.e., proteolytical lability, low bioavailability, rapid excretion, short duration of action, etc. Therefore, it is

Keywords: Aminopeptidase N; Matrix metalloproteinase; Peptidomimetics; Conformational constraint.
* Corresponding author. Tel./fax: +86 531 883 82264; e-mail: drqbli@yahoo.com.cn

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.12.095

important to design and synthesize peptidomimetic derivatives. The concept of conformationally constrained derivatives plays an important role in the design of peptidomimetics in the drug development process due to its powerful ability for probing the bioactive conformations of peptide. In addition, steric effect resulting from conformationally constrained structures could prevent the degradation of hydrolases and minimize possibility of binding to non-target receptors. For example, cyclic peptides can reduce the rate of degradation by peptidases and other enzymes and meanwhile improve the selectivity and affinity to the targets.[25–27]

In our previous work, Wang et al. have reported that the novel L-iso-glutamine derivatives can serve as a potential antitumor agent and possess potent inhibitory activity toward APN, but the selectivity between APN and MMP is low.[28] In our ongoing work, the strategy of conformational constraint was used to cyclize the carboxyl group with the nitrogen of the amide hoping for exploring new metalloproteinase inhibitors with antitumor activity (Fig. 1). To improve the bioactivity, the gallic acid moiety was introduced its derivatives possess anti-tumor and anti-oxidative activities.[29,30] In this study, we report the preparation and in vitro inhibitory activity assay of 3-galloylamido-*N*′-substituted-2,6-piperidinedione-*N*-acetamide peptidomimetic derivatives.

In order to study the SAR of these novel peptidomimetic compounds, different amide compounds **4a**–**r** and hydroxamate derivatives **6** were designed and synthesized via the route outlined in Scheme 1. The synthesis of *N*′-substituted-2,6-piperidinedione-*N*-acetamide peptidomimetic derivatives was started from readily available dicarboxylic acid **1**.[28] Compound **2** was obtained by dehydration of **1** and then reacted with glycine under microwave irradiation to generate piperidinedione **3**. This was followed by coupling with various amino acid methyl esters using EDCI to give target compounds **4a**–**r**.[31] In addition, compound **3** reacted with thionyl chloride in methanol to yield methyl ester **5**. Finally hydroxymate **6** was prepared by the reaction of **5** with NH_2OH.

The target compounds were evaluated for inhibitory activity toward APN and MMP-2. APN inhibitory activity has been evaluated by measuring *p*-nitroanilide liberated from L-Leu-*p*-nitroanilide[32] and MMP assay was performed according to the literature.[33] All the inhibition results are summarized in Table 1.

The inhibition results showed that all the compounds display excellent potency toward MMP-2 with IC_{50} values lying in micromolar level. Comparing **4g**, **4i**, **4j**, **4r**, we could confirm that the length of side chains of R was positively related with the inhibitory activities. This could be due to its bulky side chain exhibiting good interaction with the enzymes, hydrophobic domains.

In addition, as for the inhibitory activity toward APN, most of compounds showed low affinity except

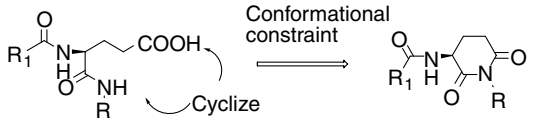
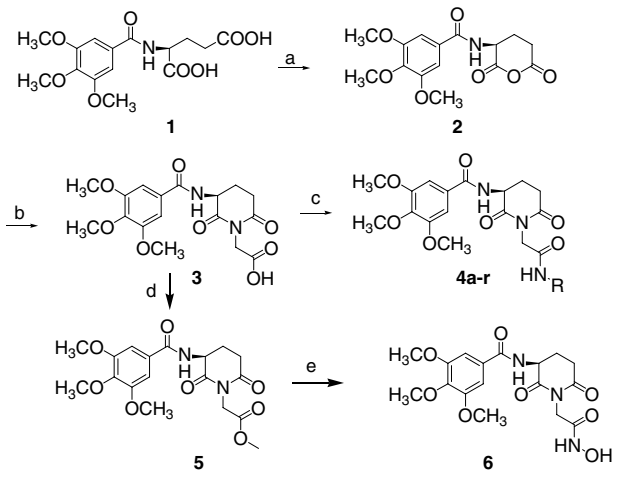
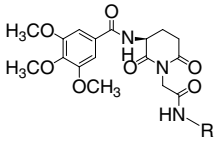


Figure 1. Cyclization of L-iso-glutamine derivatives of metalloproteinase inhibitors.



Scheme 1. Reagents and conditions: (a) Ac_2O, 55–60 °C, 60%; (b) glycine, DMF, Microwave, 110 °C, 1 h, 57%; (c) methyl ester of amino acids, EDCI, HOBt, DCM/DMSO, 56–85%; (d) $SOCl_2$, CH_3OH, 98%; (e) NH_2OH, KOH, CH_3OH; 60%.

Table 1. In vitro enzyme assay (APN and MMP-2) results for compounds **3**–**6** and Bestatin



Compound	R	IC_{50}[a] (μM)		IC_{50} (APN)/ IC_{50} (MMP-2)
		APN	MMP-2	
3		49.8 ± 5.5	4.0 ± 0.5	12.45
4a	Gly-OMe	55.4 ± 3.5	4.4 ± 1.1	12.59
4b	Val-OMe	21.4 ± 2.6	1.0 ± 0.2	21.40
4c	Leu-OMe	46.2 ± 3.2	3.8 ± 0.5	12.16
4d	Ile-OMe	49.3 ± 2.9	4.3 ± 0.4	11.47
4e	β-Ala-OMe	53.7 ± 8.4	2.2 ± 0.6	24.41
4f	Ala-OMe	18.9 ± 4.4	1.2 ± 0.3	15.75
4g	Arg (NO_2)-OMe	42.0 ± 7.6	0.3 ± 0.1	140.00
4h	Met-OMe	20.7 ± 2.7	1.2 ± 0.3	17.25
4i	Z-Lys-OMe	38.1 ± 3.8	0.6 ± 0.2	63.50
4j	Tyr-OMe	44.8 ± 5.2	0.3 ± 0.1	149.33
4k	Trp-OMe	43.1 ± 3.2	1.2 ± 0.4	35.92
4l	His-OMe	5.2 ± 2.1	13.0 ± 2.9	0.40
4m	D-Phe-OMe	46.2 ± 6.4	1.4 ± 0.5	33.00
4n	Thr-OMe	51.9 ± 3.9	1.8 ± 0.6	28.83
4o	L-Phe-OMe	11.0 ± 3.0	1.3 ± 0.3	8.46
4p	Cys-OMe	50.3 ± 4.3	1.7 ± 0.5	29.59
4q	2-Cl-Ala-OMe	50.0 ± 4.2	15.6 ± 2.3	3.21
4r	Asp-$(OMe)_2$	21.7 ± 2.1	0.5 ± 0.1	43.40
5		63.4 ± 3.2	10.6 ± 2.1	5.98
6	–OH	3.1 ± 0.7	2.2 ± 0.6	1.41
	Bestatin	2.4 ± 0.5	3.4 ± 0.6	0.71

[a] Values are means of three experiments, standard deviation is given.

compound **4l** and compound**6.** Comparing **5** and **6**, it was shown that introducing strong zinc binding group, hydroxymate, could significantly enhance the inhibition against APN and compound **6** (IC_{50} = 3.1 μM) exhibited almost equivalent potency to Bestatin (IC_{50} = 2.4 μM). The docking results of **6** with the active site of *Escherichia coli* APN are shown in Figure 2a. The two oxygen atoms in hydroxymate (OH) and 6-carbonyl group of piperidinedione can interact with the metal ion with the distance of 1.03 Å and 1.88 Å, respectively.

Compound **4l** also displays potent activity against APN with the IC_{50} of 5.2 μM. The docking results of **4l** with *E. coli* APN show that sp^2 nitrogen of the imidazole ring can be coordinated with the zinc ion of APN (Fig. 2b, the distance from zinc ion is 1.69 Å).

In conclusion, we developed a series of novel metalloproteinase inhibitors. Most of compounds seem to be selective to MMP and could be used as lead compounds for the development of low molecular-weight peptidomimetic MMP inhibitors. And else, two target compounds (**4l** and **6**) showed similar inhibitory activity compared with natural APN inhibitor Bestatin. With the combination of the requirement of the active site of APN, novel conformationally constrained peptidomimetic inhibitors against APN will also be investigated.

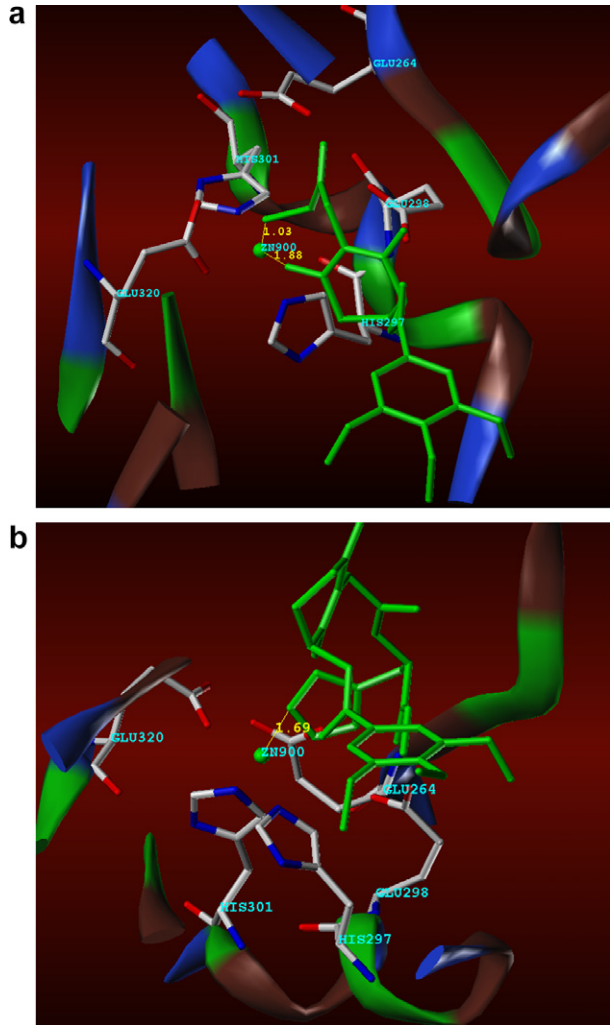


Figure 2. (a and b) The docking result of **6** and **4l** with the active site of *E. coli* APN (PDB:2DQM).

Acknowledgments

This work was supported by National Nature Science Foundation of China (Grant No. 36072541) and the Ph.D. Programs Foundation of Ministry of Education of P.R. China (No. 20060422029) and Shandong Nature Science Foundation of China (Grant No. Y2004C02).

References and notes

1. Look, A. T.; Ashmun, R. A.; Shapiro, L. H.; Stephen, C.; Peiper, S. C. *J. Clin. Invest.* **1989**, *83*, 1299.
2. Antczak, C.; De Meester, I.; Bauvois, B. *BioEssays* **2001**, *23*, 251.
3. Taylor, A. *FASEB J.* **1993**, *7*, 290.
4. Maroux, S.; Louvard, D.; Barrati, H. *Biochim. Biophys. Acta* **1973**, *321*, 282.
5. Dixon, J.; Kaklamanis, L.; Turley, H.; Hickson, I. D.; Leek, R. D.; Harris, A. L.; Gatter, K. C. *J. Clin. Pathol.* **1994**, *47*, 43.
6. Delmas, B.; Gelfi, J.; L'Haridon, R.; SjöströmNorén, H.; Laude, H. *Nature* **1992**, *357*, 417.
7. Yeager, C. L.; Ashmun, R. A.; Williams, R. K.; Cardellichio, C. B.; Shapiro, L. H.; Thomas Look, A.; Holmes, K. V. *Nature* **1992**, *357*, 420.
8. Nakanishi, K.; Yaoi, K.; Nagino, Y.; Hara, H.; Kitami, M.; Atsumi, S.; Miura, N.; Sato, R. *FEBS Lett.* **2002**, *519*, 215.
9. Irazusta, J.; Valdivia, A.; Fernandez, D.; Agirregoitia, E.; Ochoa, C.; Casis, L. *J. Androl.* **2004**, *25*, 733.
10. Shipp, M. A.; Look, A. T. *Blood* **1993**, *82*, 1052.
11. Kanayama, N.; Kajiwara, Y.; Goto, J.; el Maradny, E.; Maehara, K.; Andou, K.; Terao, T. *J. Leukoc. Biol.* **1995**, *57*, 129.
12. Mitsui, T.; Nomura, S.; Itakura, A.; Mizutani, S. *Biol. Pharm. Bull.* **2004**, *27*, 768.
13. Larsen, Ś. L.; Pedersen, L. O.; Buus, S.; Stryhn, A. *J. Exp. Med.* **1996**, *184*, 183.
14. Hattori, A.; Tsujimoto, M. *Biol. Pharm. Bull.* **2004**, *27*, 777.
15. Pulido-Cejudo, G.; Conway, B.; Proulx, P.; Brown, R.; Izaguirre, C. A. *Antiviral Res.* **1997**, *36*, 167.
16. Saiki, I.; Fujii, H.; Yoneda, J.; Abe, F.; Nakajima, M.; Tsuruo, T.; Azuma, I. *Int. J. Cancer* **1993**, *54*, 137.
17. Menrad, A.; Speicher, D.; Wacker, J.; Herlyn, M. *Cancer Res.* **1993**, *53*, 1450.
18. O'Connell, P. J.; Gerkis, V.; d'Apice, A. J. *J. Biol. Chem.* **1991**, *266*, 4593.
19. Fujii, H.; Nakajima, M.; Aoyagi, T.; Tsuruo, T. *Biol. Pharm. Bull.* **1996**, *19*, 6.
20. Sato, Y. *Biol. Pharm. Bull.* **2004**, *772*, 776.
21. Xu, W.; Li, Q. *Curr. Med. Chem.-Anti Cancer Agents* **2005**, *5*, 281.
22. Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. *Curr. Med. Chem.* **2001**, *8*, 425.
23. Ramnath, N.; Creaven, P. J. *Curr. Oncol. Rep.* **2004**, *6*, 96.
24. Sridhar, S. S.; Shepherd, F. A. *Lung Cancer* **2003**, *42*, 81.
25. Kha, A. R.; Parrish, J. C.; Fraser, M. E.; Smith, W. W.; Bartlett, P. A.; James, M. N. *Biochemistry* **1998**, *37*, 16839.
26. Dinsmore, C. J.; Bell, I. M. *Curr. Top. Med. Chem.* **2003**, *3*, 1075.

27. Yeung, K. S.; Meanwell, N. A.; Qiu, Z.; Hernandez, D.; Zhang, S.; McPhee, F.; Weinheimer, S.; Clark, J. M.; Janc, J. W. *J. Comput. Chem.* **2004**, *25*, 1133.
28. Wang, J. L.; Xu, W. F. *J. Chem. Res. Synop.* **2003**, *12*, 789.
29. Pellegrina, C. D.; Padovani, G.; Mainente, F.; Zoccatelli, G.; Bissoli, G.; Mosconi, S.; Veneri, G.; Peruffo, A.; Andrighetto, G.; Rizzi, C.; Chignola, R. *Cancer Lett.* **2005**, *226*, 17.
30. Ohno, T.; Inoue, M.; Ogihara, Y. *Anticancer Res.* **2001**, *21*, 3875.
31. All new compounds **4a**–**r** provided acceptable MS data and ^{1}H NMR spectra that exhibit no discernible impurities. Compound **4a**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (1H, d), 8.88 (1H, t), 7.17 (2H, s), 4.53 (1H, m), 4.37 (2H, s), 4.16 (2H, s), 3.83 (6H, s), 3.71 (3H, s), 3.67 (3H, s), 2.91 (2H, m), 2.18 (1H, m), 2.11 (1H, m); MS (ESI+) *m*/*z* 452.6 $[M+1]^+$; mp 98.7–100.6 °C.
32. Lejczak, B.; Kafarski, P.; Zygmunt, J. *Biochemistry* **1989**, *28*, 3549, IC_{50} values against APN were determined as previously described 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.
33. Vijaykumar, M. B.; Bonnie, J. S.; Richard, R. R.; Paul, J. K.; Howard, G. W.; Mahesh, M.; Jon, R. C.; Srinivasa, K. R. *Matrix Biol.* **2000**, *19*, 26, Gelatinase (MMP-2) and TNBS were purchased from Sigma, and the substance was synthesized as described by Vijaykumar et al. The gelatinase, substance, 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% TNBS was added and incubated for another 20 min, the resulting solution was detected under 450 nm wavelength to gain absorption.