Available online at www.sciencedirect.com

ScienceDirect

Chinese Chemical Letters 21 (2010) 159–162

CHINESE CHEMICAL LETTERS

www.elsevier.com/locate/cclet

Optical 2-benzyl-5-hydroxy-4-oxopentanoic acids against carboxypeptidase A: Synthesis, kinetic evaluation and X-ray crystallographic study

Shou Feng Wang [a], Jing-Yi Jin [a], Zong Hao Zeng [b], Guan Rong Tian [a,*]

[a] *Key Laboratory of Organism Functional Factors of Changbai Mountains of Ministry of Education, Yanbian University, Yanji 133002, China*

[b] *Institute of Biophysics, Chinese Academy of Science, Beijing 100871, China*

Received 17 June 2009

Abstract

2-Benzyl-5-hydroxy-4-oxopentanoic acid **1** and its enantiomers were designed, synthesized and assayed for inhibitory activity against carboxypeptidase A (CPA, EC 3.4.17.1). To verify the role of the terminal hydroxyl group in **1** binding to CPA, 2-benzyl-5-benzyloxy-4-oxopentanoic acid **2** was also synthesized and evaluated. The inhibition constants show that both L-**1** and D-**1** were shown to have strong binding affinity with L-**1** being more potent than its enantiomer by 165-fold. On the other hand, the inhibition constant of **2** increases 4-fold comparing with that of **1**. In order to explore the exact binding mode of the hydroxyacteyl group of **1** to the active site zinc ion of CPA, we have solved the crystal structure of CPA complexed with L-**1** up to 1.85 Å resolution. In CPA·L-**1** complex, the phenyl ring is fitted in the substrate recognition pocket at the S_1' subsite, and the carboxylate forms bifurcated hydrogen bonds with the guanidinium moiety of Arg-145 and Arg-127 and a hydrogen bond with the phenolic hydroxyl of the down-positioned Tyr-248. The carbonyl oxygen of L-**1** does coordinate to the active site zinc ion of CPA as expectedly. Unexpectedly, the terminal hydroxyl group of L-**1** is engaged in hydrogen bonding with carbonyl oxygen of Ser-197 instead of coordinating to the active site zinc ion.
© 2009 Guan Rong Tian. Published by Elsevier B.V. on behalf of Chinese Chemical Society. All rights reserved.

Keywords: 2-Benzyl-5-hydroxy-4-oxopentanoic acid; Carboxypeptidase A; X-ray crystallographic study

Zinc proteases, which are widely found in a variety of tissues, play important roles in numerous physiological and pathological processes. The most studied drug design targets of zinc proteases are angiotensin-converting enzyme [1], enkephalinase [2] and matrix metalloproteinases [3]. Inhibitors designed for most zinc proteases have focused on small molecules capable of interacting with the primary recognition pocket, a peptidomimetic backbone and incorporating a zinc-binding group (ZBG). The general strategy of incorporating a ZBG, such as carboxylic acid, thiol, hydroxamic acid and phosphorus, for the designed inhibitors has been proven to be successful [4]. However, the exploitation and discovery of more potent and selective ZBGs is still required [5].

* Corresponding author.
E-mail address: grtn@ybu.edu.cn (G.R. Tian).

1001-8417/$ – see front matter © 2009 Guan Rong Tian. Published by Elsevier B.V. on behalf of Chinese Chemical Society. All rights reserved.
doi:10.1016/j.cclet.2009.09.005

Carboxypeptidase A (CPA, EC 3.4.17.1), the first zinc protease whose crystal structure was solved by X-ray crystallography, is one of the most extensively studied zinc-containing proteolytic enzymes and serves as a prototypical enzyme to exploit ZBGs in the last 30 years [6]. Lee and Kim [7] designed and synthesized hydroxyacetyl-based inactivators of CPA based on the crystal structure of CPA complexed with its substrate Gly-*L*-Tyr (glycyl-*L*-tyrosine) in 1998 and the hydroxyacetyl group was thought to chelate the active site zinc ion. Recently Ślepokura and Lis [8] reported the crystal structures of dihydroxyacetone (DHA) monomer complexes with $CaBr_2$ and $CdCl_2$. The crystal structures show that DHA molecules chelate the cations *via* both the hydroxyl and carbonyl groups. However, the binding mode of hydroxyacetyl group to the active site zinc ion of zinc proteases is still not solved. It is therefore thought to be necessary and interesting to explore the binding mode of the hydroxyacetyl group to the active site zinc ion for further rational designing inhibitors of zinc proteases.

We reported herein the synthesis of 2-benzyl-5-hydroxy-4-oxopentanoic acid **1** and its enantiomers as inhibitors of CPA. To verify the role of the terminal hydroxyl group in **1** binding to CPA, 2-benzyl-5-benzyloxy-4-oxopentanoic acid **2**, the analogue of **1**, was also synthesized and evaluated. To further explore the exact binding mode of the hydroxyacetyl group in **1** to the active site zinc ion of CPA, the complex of CPA·L-**1** was performed and its crystal structure was investigated using X-ray diffraction method.

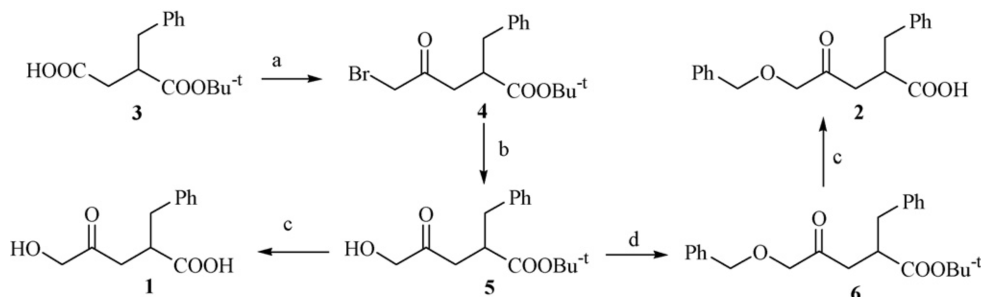
Compound **1** was prepared utilizing 2-benzylsuccinic acid mono *tert*-butyl ester as starting material and its enantiomers were prepared following the same procedure as for **1** starting with optical 2-benzylsuccinic acid mono *tert*-butyl ester. Replacing the terminal bromo group of **4** with sodium formate dihydrate [9] followed by hydrolysis [10] resulted in each of the forms of **1** required (Scheme 1). Compound **2** was synthesized by allowing benzyl bromide to react with **5** in presence of freshly prepared Ag_2O [11] followed by hydrolysis in acidic condition [10] (Scheme 1).

The prepared compounds were evaluated as CPA inhibitors by a standard procedure [15] to find that they inhibit the enzyme in a competitive reversible fashion as can be seen from Fig. 1.

Their inhibitory constants (K_is) obtained from Dixon's plots (Fig. 1) are listed in Table 1. All of these compounds should be active-site-directed inhibitors because they satisfy the essential requirements for CPA recognition, i.e., a terminal carboxylate, a hydrophobic side chain and a zinc-binding group [12]. The finding that the L-**1** ($K_i = 0.86$ μmol L^{-1}) shows 165-fold more potent inhibition against CPA than the corresponding D-**1** ($K_i = 143$ μmol L^{-1}) is consistent with the *L*-specificity of CPA [13]. The inhibition constant of *rac*-**2** ($K_i = 42$ μmol L^{-1}) increases 4-fold comparing with that of *rac*-**1** ($K_i = 8.0$ μmol L^{-1}), which suggests strongly that the hydroxyl group of *rac*-**1** must involve in additional binding interaction in the inhibitor binding to CPA.

The X-ray crystal structure of the CPA·L-**1** complex has been refined at 1.85 Å resolution to a final *R*-factor of 0.238. The atomic coordinates and structure factors of the CPA·L-**1** complex have been deposited with the Brookhaven Protein Data Bank (PDB ID 3FVL).

The final model of the complex exhibits the details of L-**1** binding to CPA (Fig. 2). The carboxylate of L-**1** is engaged in bifurcated hydrogen bonding with one of nitrogen atoms in the guanidinium moiety of Arg-145 (3.15 Å) and Arg-127 (2.76 Å). The aromatic side chain of Tyr-248 is found in the ''so-called'' down position and its phenolic hydroxyl is engaged in hydrogen bonding with the terminal carboxylate of L-**1** (2.54 Å) and the zinc-bound water molecule that is found in the native CPA is absent in the complex (Fig. 2, right). The phenyl ring of L-**1** is accommodated in the S_1' hydrophobic pocket, the primary recognition site of CPA (Fig. 2, left). These binding



Scheme 1. Prepared **1** and **2**: (a) (i) $ClCOOBu^{-t}$, *N*-Me morphorine, Et_2O, −10 °C, 15 min; (ii) CH_2N_2, Et_2O, −10 °C, 15 min and then r.t., overnight; (iii) HBr (48%), CH_2Cl_2, r.t., 15 min; (b) $HCO_2Na·2H_2O$, EtOH, reflux, 12 h; (c) TFA, CH_2Cl_2, r.t., 24 h; (d) BnBr, Ag_2O, CH_2Cl_2, r.t., 12 h.

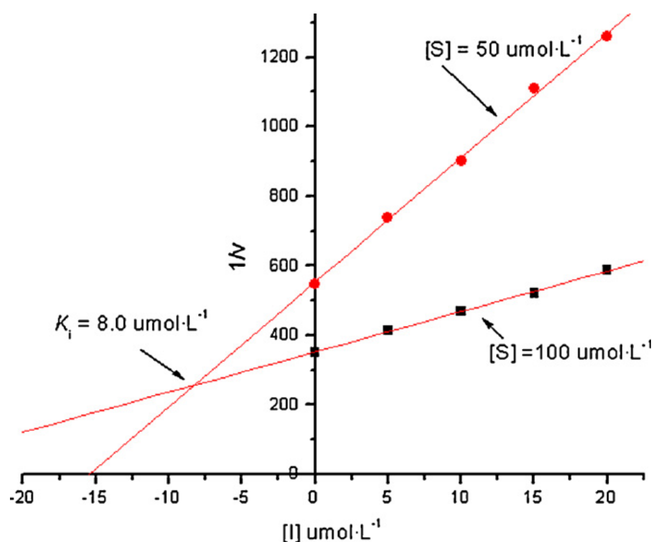


Fig. 1. The Dixon plot.

Table 1
Inhibitory constants for CPA inhibition.

Compounds	K_i (μmol L^{-1})
rac-**1**	8.0
L-**1**	0.86
D-**1**	143
rac-**2**	42

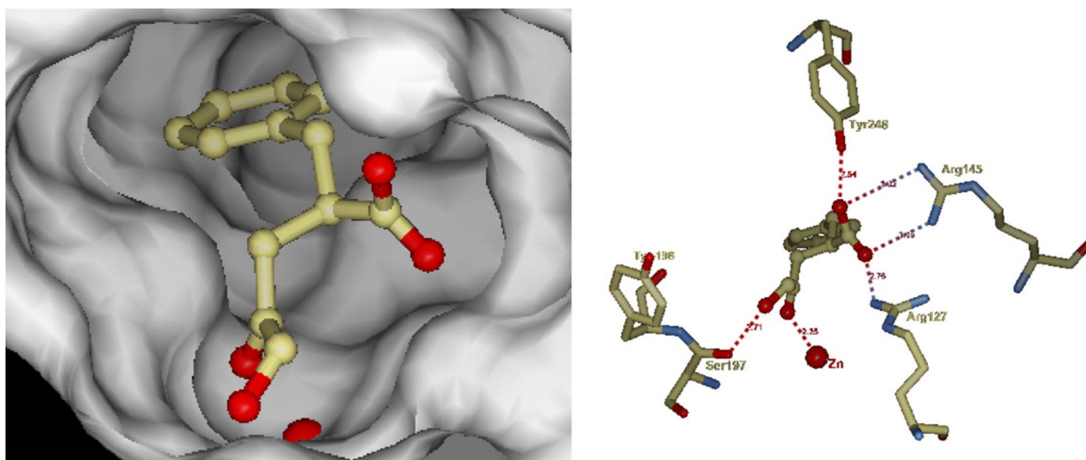


Fig. 2. The stereo-view of the CPA·L-**1** complex (left) and the interactions of L-**1** with the residue of CPA [distance (Å)] (right).

interactions are reminiscent of those reported the complexes of CPA with its inhibitors [14]. It is interestingly to notice that the carbonyl oxygen of L-**1** is separated from the active site zinc ion by 2.25 Å, which suggests that it is ligated to the zinc ion. However, the terminal hydroxyl group of L-**1** is separated from the active site zinc ion by 4.04 Å, suggesting that it is not ligated to the zinc ion. In addition, its carbonyl oxygen is involved in hydrogen bonding with one of carboxylate oxygen atoms of Glu-270 with bonding distance of 2.63 Å. Most interestingly, a hydrogen bond is formed between the terminal hydroxyl group and the carbonyl oxygen of Ser-197 with bonding distance of 2.71 Å

(Fig. 2, right). It should attribute to the additional hydroxyl-carbonyl-type hydrogen bond that makes the inhibitory activity of *rac*-**1** against CPA more potent than that of *rac*-**2**. The binding mode of hydroxyacetyl group of L-**1** with CPA presented is not consistent with the binding mode proposed by Lee and Kim [7] or the exact binding mode of DHA to metal ion such as Cd (II) and Ca (II) reported by Ślepokura and Lis [8].

In conclusion, compound **1** and its enantiomers capable of hydroxyacetyl group were synthesized and evaluated. The inhibitory constants show that L-**1** has more potent binding affinity than its corresponding D-**1** by 165-fold. The crystal structure of CPA·L-**1** complex reveals that L-**1** does with its carbonyl oxygen coordinating to the active site zinc ion of CPA. Unexpectedly, the terminal hydroxyl group of L-**1** is involved in hydrogen bonding with the carbonyl oxygen of Ser-197 instead of coordinating to the active site zinc ion, which is not consistent with the binding mode proposed in previous papers. The design strategy demonstrated and the exact binding mode of hydroxyacetyl group of L-**1** to the active site zinc ion of CPA in this work may be of considerable interest to those who are engaged in the rational design of inhibitors for zinc-containing proteases.

References

[1] R. Behnia, A. Molteni, Curr. Pharm. Des. 9 (2000) 763;
H.P. Lefebvre, S.A. Brown, V. Chetboul, Curr. Pharm. Des. 13 (2007) 1347.
[2] V. Thanawala, V.J. Kadam, R. Ghosh, Curr. Drug Targets 9 (2008) 887.
[3] J.W. Skiles, N.C. Gonnella, A.Y. Jeng, Curr. Med. Chem. 8 (2001) 425;
B.G. Rao, Curr. Pharm. Des. 11 (2005) 295.
[4] S.P. Gupta, Chem. Rev. 107 (2007) 3042.
[5] F. Mannello, G. Tonti, S. Papa, Curr. Cancer Drug Targets 5 (2005) 285.
[6] W.N. Lipscomb, N. Sträter, Chem. Rev. 96 (1996) 2375;
D.H. Kim, Curr. Top. Med. Chem. 4 (2004) 1217.
[7] D.H. Kim, K.J. Lee, Bioorg. Med. Chem. Lett. 7 (1997) 2607;
K.J. Lee, D.H. Kim, Bioorg. Med. Chem. 6 (1998) 1613.
[8] K. Ślepokura, T. Lis, Acta Cryst. C 64 (2008) m127.
[9] Z.G. Tang, H.Q. Tian, D.W. Ma, Tetrahedron 60 (2004) 10547.
[10] C.B. Stephen, A.I. Khadiga, Synthesis 2000 (2000) 1369.
[11] B. Abderrahim, S. Gilles, Tetrahedron Lett. 38 (1997) 5945.
[12] D.W. Christianson, W.N. Lipscomb, Acc. Chem. Res. 22 (1989) 62.
[13] D.H. Kim, S. Chung, Tetrahedron: Asymmetry 10 (1999) 3769.
[14] S. Mangani, Coordin. Chem. Rev. 120 (1992) 309.
[15] Determination of K_i value. CPA was purchased from Sigma Chemical Co. (Allan form, twice crystallized from bovine pancrease, aqueous suspension in toluene) and used without further purification. CPA stock solutions were prepared by dissolving the enzyme in 0.05 mol L^{-1} Tris/ 0.5 mol L^{-1} NaCl, pH 7.5 buffer solution. *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyllactate (Cl-CPL), which was synthesized as described in the literature [16], was used as substrate. Typically, the enzyme stock solution was added to a solution containing Cl-CPL (final concentrations: 50 μmol L^{-1} and 100 μmol L^{-1}) and inhibitor (five different final concentrations in the range of $0.5K_i$ to $2K_i$ μmol L^{-1}) in 0.05 mol L^{-1} Tris/ 0.5 mol L^{-1} NaCl, pH 7.5 buffer solution (1 mL cuvette), and the change in absorbance at 320 nm was measured immediately using a Perkin–Elmer HP 8453 UV/Vis spectrometer at 25 °C. Initial velocities were then calculated from the linear initial slopes of the change in absorbance. The K_i values were then estimated from the semireciprocal plot of the initial velocity *versus* the concentration of the inhibitor according to the method of Dixon [17]. The correlation coefficients for the Dixon plots were above 0.990.
[16] J. Suh, E.T. Kaiser, J. Am. Chem. Soc. 98 (1976) 1940.
[17] M. Dixon, Biochem. J. 55 (1953) 170.