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Zn(II)-Chelating Inhibitors of Carboxypeptidase A

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Abstract: New structural elements for Zn(II)-chelating inhibitors of carboxypeptidase A are deduced. Based on K_i values for 21 new inhibitors prepared in this study, 2-mercaptoethylthio group and mercaptophenyl group are proposed to be effective Zn(II) chelators.

Carboxypeptidase A (CPA),[1-5] a Zn(II)-containing exopeptidase, has been exploited as a model of other Zn(II)-peptidases such as angiotensin converting enzyme (ACE), enkephalinase, aminopeptidases, and collagenase. For example, ACE inhibitors such as captopril were designed by mimicking benzylsuccinate, a potent CPA inhibitor.[5] A large number of inhibitors are known for CPA, and most of them may be classified as substrate analogues, products/products analogues, or transition state analogues. Few systematic investigations, however, have been performed with regard to inhibitors containing various functional groups capable of coordination to the Zn(II) ion. Knowledge of the structural features of Zn(II)-binding inhibitors common to various Zn(II) peptidases is valuable for the design of new pharmaceuticals based on inhibition of the physiological activities of Zn(II) peptidases.

In the present study, compounds **1-21** were prepared and tested for their inhibitory activity in CPA action in order to gain insights into the structural elements for the Zn(II)-binding inhibitors. Except **17-19**, the inhibitors contain terminal carboxyl groups and benzyl side chains and, therefore, can be considered as analogues of substrates or products of CPA. Dissociation constants for the complexes formed between CPA and most of its substrates or products are about 10^{-4} M. If the inhibition constant (K_i) for an inhibitor is considerably smaller than 10^{-4} M, it may be assumed that the inhibitor possesses extra structural elements which facilitate complexation with CPA.

In the kinetic studies for inhibition by **1-21**, the CPA-catalyzed hydrolysis of *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyllactate (ClCPL)[6] was studied spectrophotometrically at pH 7.5 and 25 ℃ in the presence of 0.5 M NaCl and 0.05 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonate. Stock solutions of the inhibitors were made in either dimethyl sulfoxide or acetonitrile and the content of the organic solvents in the reaction mixtures of the kinetic measurements was 2 % (v/v). Initial rates (v_o) of the CPA-catalyzed hydrolysis of ClCPL were calculated by the method reported in the literature.[7] From the dependence of v_o on S_o (the initially added substrate concentration), values of k_{cat}^{app} and K_m^{app} were obtained for each kinetic run carried out in the presence of an inhibitor added at various concentrations (I_o). From the dependence of k_{cat}^{app} and K_m^{app} on I_o, the mode of inhibition and the values of K_i were determined for each inhibitor.[8] Inhibition constants and modes of inhibition determined for **1-21** are summarized in Table 1. Hydrolysis of *N*-acyl L-Phe derivatives **1-3** and **5** was not

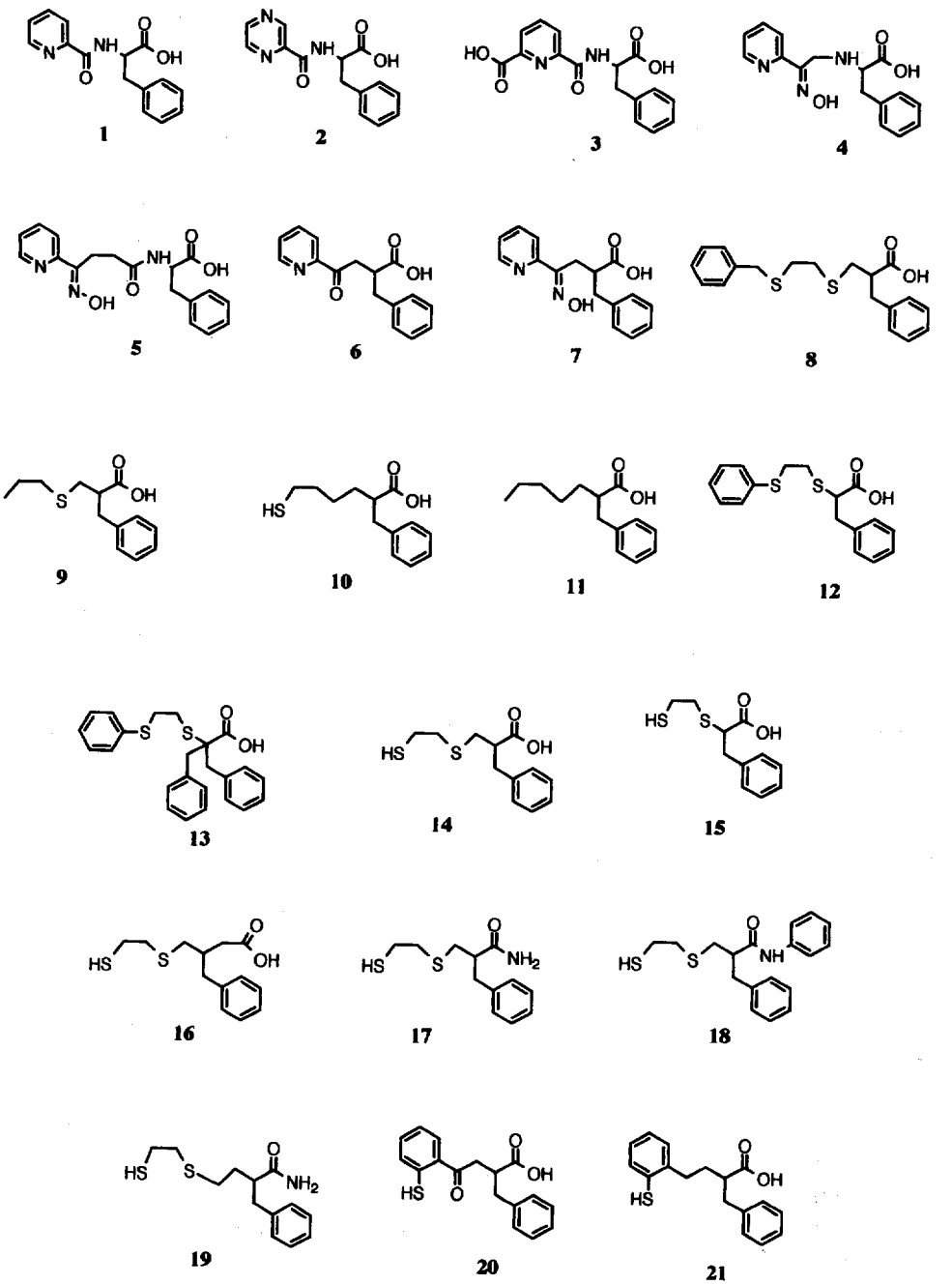

Fig. 1. Structures of Inhibitors of CPA Prepared in this Study

detected under the conditions of kinetic measurements. Standard deviations are usually less than 20 % of the K_i values. For poor inhibitors with K_i values greater than 2 X 10^{-4} M, however, the K_i values are less accurate.

Table 1. Inhibition Constants and Modes of Inhibition for Various Inhibitors of CPA Prepared in this Study

Inhibitor[a]	K_i, 10^{-4} M^{-1}	Mode[b]	Inhibitor[a]	K_i, 10^{-4} M^{-1}	Mode[b]
1	3.7	n	**11**	6.2	u
2	1.8	c	**12**	6.7	n
3	7.9	c	**13**	4.5	c
4	5.8	c	**14**	0.042	n
5	>>3		**15**	0.21	n
6	0.82	n	**16**	0.19	c
7	>>3		**17**	0.29	n
8	6.6	n	**18**	0.34	u
9	3.4	c	**19**	0.92	n
10	0.45	n	**20**	0.044	u
			21	0.058	c

[a]Compounds **1-5** were prepared with L-Phe. Other compounds were racemates. When racemates were used as inhibitors, K_i was estimated by assuming that only the L-isomer is active since it is known that D-compounds are not bound effectively by CPA. Compounds **1-21** were characterized by ^{1}H NMR, ^{13}C NMR, and elemental analysis.
[b]c; competitive, n; noncompetitive, u; uncompetitive.

Pyridyl or pyrazinyl derivatives **1-7** were prepared in order to examine whether the aromatic nitrogen atoms in combination with carbonyl oxygen or oxime nitrogen atoms act as effective bidentate chelators for the Zn(II) ion of CPA. Complexation of the pyridyl or pyrazinyl derivatives with transition metal ions is well documented.[9,10] The K_i values summarized in Table 1, however, reveal that these pyridyl or pyrazinyl derivatives do not manifest extra binding features towards CPA.

Mercaptans and sulfides are good ligands of transition metal ions.[10] Derivatives of mercaptoacetate or 3-mercaptopropionate have been reported as potent inhibitors of CPA.[11,12] In order to examine whether 1,2-ethanedithio derivatives can be exploited as Zn(II)-chelating inhibitors for CPA, compounds **8-19** were prepared. The highest affinity for CPA was observed with **14**. Comparison of K_i values for **8-14** indicates that the 2-mercaptoethylthio group of **14** is responsible for the high affinity of **14**. Thus, when either of the two sulfur atoms of the 2-mercaptoethylthio group is substituted, binding became very weak.

Compounds **14-19** contain 2-mercaptoethylthio moieties. Compounds **14-16** contain carboxyl groups. Compared with **14**, **15** contains one less methylene group and **16** one more methylene group. Insertion or deletion of one methylene unit between the 2-mercaptoethylthio group and the carboxyl group leads to about 5-fold increase in K_i. On the other hand, 100-fold difference in K_i has been reported for 2-benzyl-3-mercaptopropionate and 2-benzyl-2-mercaptoacetate,[11,12] whose structures differ from each other by one methylene unit. It may be proposed, therefore, interaction of both mercapto group and carboxylate group with

CPA is important for 2-benzyl-3-mercaptopropionate and 2-benzyl-2-mercaptoacetate whereas interaction of carboxylate group with CPA is not so important in the interaction of **14-16** with CPA. This is further supported by K_i values for **17-19** in which carboxyl group is substituted with carboxamide group.[13]

When 2-mercaptoethylthio group forms a chelate with the active site Zn(II) ion of CPA, the interaction of carboxylate groups of inhibitors with the enzyme makes only a secondary contribution to the overall binding. This suggests that 2-mercaptoethylthio group may be exploited as effective Zn(II)-chelating sites in the design of inhibitors for other Zn(II)-metalloenzymes.

The K_i for **20** is similar to that of **14**. When the carbonyl group of **20** is converted into a methylene group to obtain **21**, K_i is not affected considerably.[15] Thus, the mercaptophenyl group instead of the mercaptobenzoyl group appears to provide the extra structural element for complexation. Although a systematic study has not been carried out with inhibitors containing mercaptophenyl groups, the present results suggest that the mercaptophenyl group can be also exploited as Zn(II)-chelating sites of inhibitors for Zn(II)-metalloenzymes.

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