Synthesis and Inhibitory Study of *N*-Oxide Containing Substrate Analog Inhibitors of Carboxypeptidase A

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Compounds containing a nitrone moiety were designed, synthesized and evaluated as a new type of active site zinc ligating substrate analog inhibitors for carboxypeptidase A. The kinetic results indicated that they are competitive inhibitors for the enzyme, supporting the design rationale that the oxygen of the nitrone forms a coordinative bond to the active site zinc ion. The present study demonstrates that nitrone is useful as a zinc coordinating ligand in the design of inhibitors for zinc containing proteolytic enzymes.

Introduction

Enzyme inhibitors have received increasing attention as valuable tools for the study of enzymic reaction mechanism and more importantly as therapeutic agents. Over the past few years we have been involved in the development of novel design principles of enzyme inhibitors using well characterized proteases such as carboxypeptidase A and α-chymotrypsin as model target enzymes.[1] The design principles developed with these enzymes bear a special importance because they can be translated to the zinc proteases of medicinal importance such as angiotensin converting enzyme and matrix metalloproteases, leading to the discovery of potential lead compounds for drug development.[2] Carboxypeptidase A (CPA, E.C. 3.4.17.1),[3] a zinc containing metalloprotease, serves as a model for many zinc proteases. The terminal amino acid residue having a hydrophobic side chain, such as Phe is preferably cleaved from peptide substrate by the enzyme. Important residues involved in binding of substrate and catalysis are Arg-71, Arg-127, Asn-144, Arg-145, Tyr-248, Glu-270 and a zinc bound water molecule. The active site zinc ion forms a coordinative bond with the carbonyl oxygen of the scissile peptide bond of substrate. Thus, it is involved in the binding of substrate and activation of the scssile peptide bond for the hydrolytic cleavage. The bound water molecule is known to function as a nucleophile which attacks the scissile peptide bond.[3]

Nitrones (azomethine *N*-oxides) are highly valuable synthetic intermediates.[4] They are excellent 1,3-dipoles for cycloaddition reactions and have been utilized for the synthesis of various nitrogen containing heterocycles. In these 1,3-dipolar cycloaddition reactions of nitrones, significant rate acceleration and high regio- and stereo-specificity were obtained by addition of Lewis acid,[5] suggesting the nitrone oxygen to form a coordinative bond with the Lewis acid.[6] In our continued efforts to develop novel types of inhibitors for zinc containing protease, we have turned our attention to the use of the zinc coordinating property of the *N*-oxide for the inhibitor design. This report describes design, synthesis and evaluation of *N*-oxide containing inhibitors for carboxypeptidase A.

Experimental

General Remarks and materials. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AM 300 (300 MHz) instrument using tetramethylsilane as the internal standard. IR spectra were recorded on BOMEM FT IR M100-C15 spectrometer. High resolution mass spectra were obtained with JEOL JMX-HX-110/110 by the Korea Basic Science Center, Taejon, Korea. Flash chromatography was performed on silica gel 60 (230-400 mesh) and thin layer chromatography (TLC) was carried out on silica coated glass sheets (Merck silica gel 60 F-254). Optical rotations were measured on RUDOLPH RESEARCH AUTOPLOL III digital polarimeter. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Elemental analyses were performed by the Basic Science Center, Kyungbook National University, Taegu, Korea. Kinetic experiments were carried out using a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer.

Kinetic studies. All solutions were prepared by using doubly distilled, deionized water. Stock assay solutions were filtered before use. Carboxypeptidase A (CPA, E.C. 3. 4.17.1) was purchased from Sigma Chemical Co. (Allan form, twice crystallized, from bovine pancreas, aqueous suspension). The hydrolysis of *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyllactate by carboxypeptidase A was monitored spectrophotometrically at 320 nm as described by Suh and Kaiser[7] in a buffer solution of pH 7.5 (50 mM Tris-0.5 M NaCl) at 25 °C. Enzyme concentrations were estimated from the absorbance at 278 nm (ε_{278}=64,200). Initial velocities were calculated from the linear initial slopes of the change in absorbance where the amount of substrate consumed was always less than 10%. The K_i values were determined according to the method of Dixon.[8]

***N*-Benzylidene-DL-phenylalanine methyl ester *N*-oxide (11).** To a suspension of *N*-benzylphenylalanine methyl ester (3.3 g, 12.3 mmol) and sodium tungstate monohydrate (0.2 g, 0.62 mmol) in MeOH (20 mL) was added 30% hydrogen peroxide (4.2 g, 36.9 mmol) and the mixture was stirred at room temperature for 8 h. Methanol was evaporated and the residue was diluted with 50 mL of dichloromethane and washed with brine (30 mL × 3), dried

($MgSO_4$) and evaporated *in vacuo*. The crude product was recrystallized from ethyl acetate (30% *n*-Hexane) to give the product as a white needle (1.03 g, 30%). mp 174-175 °C; IR (thin film) 1735, 1570 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.55 (dd, *J*=15, 4.5 Hz, 1H), 3.66 (dd, *J*=9.0, 3.0 Hz, 1H), 3.78 (s, 3H), 4.64 (dd, *J*=6.0, 4.0 Hz), 7.06 (s, 1H, C<u>H</u>=N), 7.17-7.38 (m, 8H), 8.11 (m, 2H, ArH ortho to C<u>H</u>=N^+-O^-); ^{13}C NMR ($CDCl_3$) δ 35.5, 53.6, 79.8, 127-137, 167; Anal. Calcd for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94. Found: C, 71.72; H, 6.25; N, 5.14.

***N*-Benzylidene-DL-phenylalanine *N*-oxide (1).** A solution of **11** (0.55 g, 1.94 mmol) in 0.1 N LiOH/THF/MeOH (38 mL/110 mL/30 mL) was stirred at room temperature for 1 h, then acidified with conc. HCl to pH 1. The reaction mixture was diluted with ethyl acetate (150 mL) and washed with brine (30 mL×3), dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was recrystallized from ethyl acetate (30% methanol) to give the product as a white solid (110 mg, 21%). mp 178-180 (dec.); IR (thin film) 3280-3570, 1721, 1660, 1440, 1402 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.11-3.41 (m, 2H), 5.07 (dd, *J*=10.5, 4.2 Hz, 1H), 7.06-7.35 (m, 8H), 7.61 (s, 1H, C<u>H</u>=N^+-O^-), 8.07-8.10 (m, 2H, ArH ortho to C<u>H</u>=N^+-O^-); ^{13}C NMR (DMSO-d_6) δ 34.9, 78.4, 127-137.8, 169.5; Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.54; H, 5.81; N, 5.02. In a similar fashion, optically active **1** was prepared starting with optically active *N*-benzylphenylalanine methyl ester.

***N*-Benzylidene-L-phenylalanine *N*-oxide (L-1):** $[\alpha]_D$= −1.8° (c 1, DMSO). ***N*-Benzylidene-D-phenylalanine *N*-oxide (D-1):** $[\alpha]_D$=+2.1° (c 1, DMSO).

***N*-Benzyl-DL-phenylalanine lithium salt (2).** A mixture of *N*-benzylphenylalanine methyl ester (0.27 g, 1 mmol), 1 N NaOH (0.9 mL) and methanol (5 mL) was heated at 50 °C for 2 h. Methanol was removed and the residue was diluted with water (20 mL), washed with ether (10 mL ×2) and ethyl acetate (10 mL), then water was evaporated *in vacuo* to give the white solid (quantitative). mp 170-175 °C; ^1H NMR (D_2O) δ 2.73-2.87 (m, 2H), 3.26 (t, 1H), 3.48 (d, *J*=12.6 Hz, 1H), 3.67 (d, *J*=12.6 Hz, 1H), 7.10-7.30 (m, 10H).

***N*-Benzylidene-β-phenylalanine methyl ester *N*-oxide (13) and *N*-β-phenylalanidene methyl ester-benzyl amine *N*-oxide (14).** To a suspension of *N*-benzyl-β-phenylalanine methyl ester (**12**) (3.0 g, 10.6 mmol) and sodium tungstate monohydrate (0.18 g, 0.53 mmol) in MeOH (20 mL) was added 30% hydrogen peroxide (3.6 g, 31.8 mmol) and the mixture was stirred at room temperature for 8 h. Methanol was evaporated and the residue was diluted with 50 mL of dichloromethane and washed with brine (30 mL×3), dried ($MgSO_4$) and evaporated *in vacuo*. The residue was separated by column chromatography (ethyl acetate/*n*-hexane=1/1→ethyl acetate) to give **13** (0.9 g, 29%) as an oil and **14** (1.1 g, 35%) as an oil. For **13**: IR (neat) 1720, 1570, 1555, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.94 (m, 1H), 3.10 (m, 1H), 3.67 (s, 3H), 3.72 (m, 1H), 4.0 (dd, *J*=12.0, 4.8 Hz, 1H), 4.25 (dd, *J*=12.0, 8.7 Hz, 1H), 7.19-7.49 (m, 9H), 8.20-8.23 (m, 2H, ArH ortho to CH=N^+-O^-); ^{13}C NMR ($CDCl_3$) δ 36.2, 44.8, 52.5, 66.9, 127-137, 174. For **14**: IR (neat) 1740, 1590, 1570, 1490 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.11 (m, 1H), 3.21 (m, 1H), 3.68 (s, 3H), 4.21 (q, *J*=6.9 Hz, 1H), 4.88 (s, 2H), 6.79 (d, *J*=6.6 Hz, 1H, -C<u>H</u>=N), 7.04-7.07 (m, 2H), 7.19-7.41 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 35.1, 44.9, 52.6, 69.9, 126.3-137.8, 171.7.

***N*-Benzylidene-β-phenylalanine *N*-oxide (3).** Following the same procedure as that used for the preparation of **1**, **3** was obtained from **13** in 30% yield after recrystallization from ethyl acetate (10% *n*-hexane). mp 181-182 °C; IR (thin film) 3300-3500, 1664, 1443, 1411 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.79-2.96 (m, 2H), 3.7 (m, 1H), 3.96-4.02 (m, 1H), 4.15-4.22 (m, 1H), 7.19-7.43 (m, 8H), 7.83 (s, 1H, -C<u>H</u>=N), 8.21-8.24 (m, 2H, ArH ortho to CH=N^+-O^-); ^{13}C NMR (DMSO-d_6) δ 35.8, 45.4, 67.1, 127, 129-135, 139.2, 174.7; Anal. Calcd for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94. Found: C, 71.75; H, 6.09; N, 4.91.

***N*-β-phenylalanidene-benzylamine *N*-oxide (4).** Following the same procedure as that used for the preparatiion of **1**, **4** was obtained from **14** in 30% yield after recrystallization from ethyl acetate (20% *n*-hexane). mp 177-178 °C; IR (thin film) 3200-3500, 1656, 1023 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.21 (d, 2H), 3.61 (t, 1H), 3.34 (ddd, *J*= 42, 15, 6 Hz, 2H, PhC<u>H</u>$_2$$N^+$-$O^-$), 7.08 (m, 2H), 7.12-7.27 (m, 8H), 7.97 (m, 1H, -C<u>H</u>=N); Anal. Calcd for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94. Found: C, 72.03; H, 6.25; N, 4.95.

***N*-Benzyl-β-phenylalanine lithium salt (5)** was prepared as described for the preparation of **2**. mp 100-105 °C; ^1H NMR (D_2O) δ 2.47-2.72 (m, 5H), 3.66 (s, 2H), 7.05-7.26 (m, 10H).

***N*-(Picolinic acid-*N*-oxidyl)-DL-phenylalanine methyl ester (15).** To a stirred suspension of picolinic acid *N*-oxide (1 g, 7.2 mmol), dicyclohexylcarbodiimide (1.48 g, 7.2 mmol), and hydroxybenzotriazole (0.97 g, 7.2 mmol) in THF/DMF (20 mL/5 mL) were added DL-phenylalanine methylester hydrochloride (1.55 g, 7.2 mmol) and *N*-ethylmorpholine (0.92 mL, 7.2 mmol). The mixture was stirred at room temperature for 28 h, diluted with ethyl acetate (40 mL) and the precipitated dicyclohexylcarbodiimide was removed by filtration. The filtrate was washed with 5% sodium thiosulfate solution (20 mL×3), 10% citric acid (20 mL×2), 10% sidium bicarbonate (20 mL×2) and finally with brine (20 mL×3). The organic extract was dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by column chromatography (ethyl acetate/MeOH= 30/1) to give the product as a yellow oil (1.1 g, 52%). IR (neat) 1730, 1655, 1420 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.15-3.30 (m, 2H), 3.75 (s, 3H), 5.0 (m, 1H), 7.20-7.44 (m, 7H), 8.26 (d, *J*=6.0 Hz, 1H), 8.37 (d, *J*=6.0 Hz, 1H), 11.6 (d, *J*= 6.6 Hz, 1H, CONH).

***N*-(Nicotinic acid-*N*-oxidyl)-DL-phenylalanine methyl ester (16).** Following the same procedure as that used for the preparation of **15**, **16** was obtained from nicotinic acid *N*-oxide in 55% yield as a colorless oil. IR (neat) 1730, 1655, 1420 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.20-3.37 (m, 2H), 3.80 (s, 3H), 5.01 (m, 1H), 7.21-7.37 (m, 6H), 7.64 (d, *J*=8.0 Hz, 1H), 7.80 (d, *J*=7.4 Hz, CON<u>H</u>, 1H), 8.31 (d, *J*=6.4 Hz, 1H), 8.74 (s, 1H).

***N*-(Picolinic acid-*N*-oxidyl)-DL-phenylalanine (6).** A mixture of **15** (0.55 g, 1.83 mmol) and 1 N NaOH (5.5 mL) in methanol (20 mL) was heated at 50 °C for 1 h, then acidified with 3 N HCl to pH of 1. The reaction mixture was extracted with ethyl acetate (20 mL×3), washed with brine (20 mL×2), dried ($MgSO_4$) and evaporated *in vacuo*

to give the product as hygroscopic pale yellow fluffy solid (0.5 g, 95%). IR (film) 3000-3300, 1725, 1650 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.18-3.38 (m, 2H), 5.01 (m, 1H), 7.19-7.49 (m, 7H), 8.28-8.41 (m, 2H), 9.1 (s, br, 1H), 11.55 (d, *J*=7.2 Hz, 1H, CONH).

***N*-(Nicotinic acid-*N*-oxidyl)-DL-phenylalanine (7).** Following the same procedure as that used for the preparation of **6**, **7** was obtained from **16** in quantitative yield. It was recrystallized from ethyl acetate (30% methanol). mp 232-235 °C; IR (thin film) 3100-3300, 1725, 1645; ^1H NMR (DMSO-d_6) δ 2.98-3.24 (m, 2H), 4.61 (m, 1H), 7.16-7.36 (m, 5H), 7.53 (m, 1H), 7.65 (d, *J*=8.0 Hz, 1H), 8.35 (m, 1H), 8.43 (s, 1H), 9.05 (d, *J*=8.1 Hz, 1H, CONH), 13.0 (s, br, COOH); Anal. Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.78. Found: C, 62.52; H, 5.01; N, 9.56. In a similar fashion, optically active **7** was prepared starting with optically active phenylalanine methyl ester. ***N*-(Nicotinic acid-*N*-oxidyl)-L-phenylalanine (L-7):** $[\alpha]_D$= − 61.5° (c 0.4, DMSO). ***N*-(Nicotinic acid-*N*-oxidyl)-D-phenylalanine (D-7):** $[\alpha]_D$=+69.8° (c 1, DMSO).

2-(2-Pyridine-*N*-oxidyl)hydrocinnamic acid methyl ester (17). A mixture of 2-(2-pyridyl)hydrocinnamic acid methyl ester (0.8 g, 3.3 mmol) and 30% hydrogen peroxide (0.7 g, 5.6 mmol) in acetic acid (10 mL) was heated at 60 °C for 24 h. Acetic acid was removed *in vacuo* and the residue was diluted with ethyl acetate (20 mL), washed with 3 N HCl (10 mL × 2) and brine (10 mL × 2) then dried ($MgSO_4$) and evaporated *in vacuo* to give the product as a yellow oil (230 mg, 30%). IR (neat) 1730, 1480, 1420, 1200-1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.34-3.50 (m, 2H), 3.70 (s, 3H), 4.43 (t, *J*=7.6 Hz, 1H), 7.11-7.47 (m, 8H), 8.34 (d, *J*=5.9 Hz, 1H).

2-(2-Pyridine-*N*-oxidyl)hydrocinnamic acid (9). A mixture of **17** (0.22 g, 0.85 mmol) and 1 N NaOH (2 mL) in methanol (10 mL) was heated at 60 °C for 1 h, then acidified with 3 N HCl to pH of 1. The resulting mixture was extracted with ethyl acetate (20 mL × 3), washed with brine (20 mL × 2), dried ($MgSO_4$) and evaporated *in vacuo* and the crude product was recrystallized from a mixture of ethyl acetate and methanol (9 : 1 by vol) to give the product as a white solid (0.14 g, 70%). mp 142-144 °C; ^1H NMR (DMSO-d_6) δ 3.23-3.31 (m, 2H), 4.30 (t, *J*=6.8 Hz, 1H), 6.83-7.29 (m, 8H), 8.23 (d, *J*=6.3 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 34, 49.2, 111.5, 126, 127, 128, 129, 129.5, 140, 149.5, 173; Anal. Calcd for $C_{14}H_{13}NO_3$: C, 69.10; H, 5.39; N, 5.76. Found: C, 68.80; H, 5.69; N, 5.48.

2-(2-Pyridyl)hydrocinnamic acid hydrochloride (10) was prepared from 2-(2-pyridyl)hydrocinnamic acid methyl ester in a fashion that used for the preparation of **2**. ^1H NMR (D_2O) δ 3.46-3.60 (m, 2H), 4.38 (m, 1H), 7.0-7.2 (m, 5H), 7.76-7.85 (m, 2H), 8.32 (t, *J*=8.0 Hz, 1H), 8.48 (d, *J*=6.0 Hz, 1H).

Results and Discussion

Compounds **1**, **3**, **4**, **6**, **7**, and **9** were designed as potential competitive inhibitors for carboxypeptidase A. The rationale for designing them as CPA inhibitors is illustrated in Figure 1. As substrate analogs,[9] the carboxylate in these inhibitors would form hydrogen bonds with the guanidinium moiety of Arg-145 and the benzyl group anchors in the primary substrate recognition pocket. Then the oxygen of the nitrone would be rested at a position proximal to the active site zinc ion, thereby to form a coordinative bond with the metal ion.

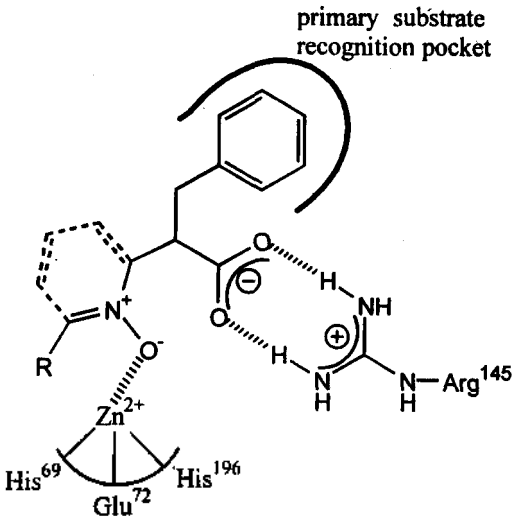
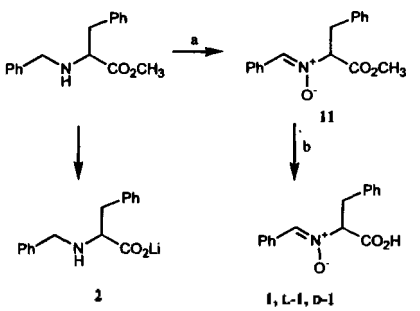
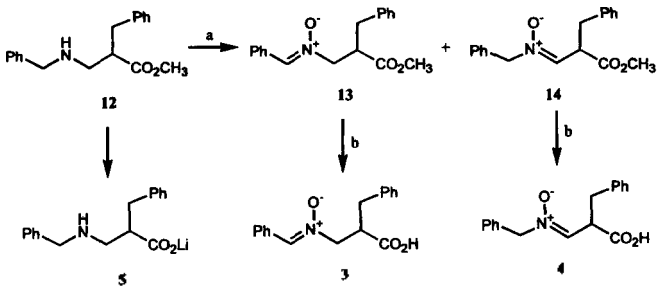


Figure 1. Schematic representation of binding of *N*-oxide containing inhibitors to the active site of CPA.

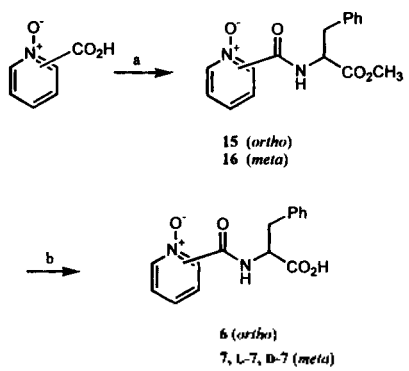
Nitrone derivatives thus designed as inhibitors for CPA were synthesized by oxidation of the corresponding secondary amine with hydrogen peroxide in the presence of a catalytic amount of sodium tungstate[10] as shown in Scheme 1. In the oxidation of **12**, regioisomers **13** and **14** were obtained in a nearly equal ratio, and these isomers were separated easily by column chromatography (Scheme 2). Differentiation of **13** from **14** was possible on the basis of ^1H NMR signals of two *ortho* hydrogens in the phenyl ring next to the carbon-nitrogen bond: The resonance signals in **13** appeared at δ 8.2 ppm, whereas the corresponding protons in **14** gives the signals at δ 7.2-7.4 ppm. Inhibitors **6** and **7** were prepared from picolinic acid *N*-oxide or nicotinic acid *N*-oxide with phenylalanine methyl ester under the standard peptide coupling conditions and subsequent hydrolysis (Scheme 3). Compound **9** was synthesized by oxidation of 2-(2-pyridyl)hydrocinnamic acid methyl ester using hydrogen peroxide and acetic acid followed by base hydrolysis (Scheme 4). Aldonitrones such as those synthesized in this study are known to exist in the thermodynamically more stable *trans*-form.[11]



Scheme 1. Reagents, conditions and yields: (a) Na_2WO_4 (5 mol%), 30% H_2O_2, MeOH, r.t. 8 h, 30%; (b) 0.1 N LiOH, THF/MeOH/H_2O (3/1/1), r.t. 1 h, 21%.



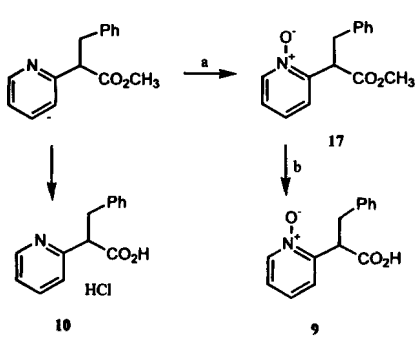
Scheme 2. Reagents, conditions and yields: (a) Na_2WO_4 (5 mol%), 30% H_2O_2, MeOH, r.t. 8 h, (13 : 14=29%, 35%); (b) 0.1 N LiOH, THF/MeOH/H_2O (3/1/1), r.t. 1 h, 30%.



Scheme 3. Reagents, conditions and yields: (a) L-Phe-OCH_3-HCl, DCC, HOBt, NEM, THF, 28 h, 52%. (b) 1 N NaOH, MeOH, 50 °C, 95%.

Indeed, they were shown to be competitive inhibitors for CPA as were demonstrated by their Dixon plots, for which Figure 2 is a typical example. The inhibitory constant (K_i) of each inhibitor was determined from the respective Dixon plot and is listed in Table 1. In the Dixon plot, $[I]_o$ represents the concentration of inhibitor and v_o represents initial velocity, monitored spectrophotometrically at 320 nm, of the CPA catalyzed hydrolysis of *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyllactate (substrate) in the presence of each inhibitor in a solution of pH 7.5 Tris buffer at 25 °C.

Inhibitor **1** is a potent inhibitor of CPA, potency of which is 12 times that of the corresponding secondary amine **2**. Inhibitor **3** also exhibits much more potent binding affinity towards the enzyme than its parent compound **5**, extent of which is almost the same as **1** is over **2**. These observations



Scheme 4. Reagents, conditions and yields: (a) 30% H_2O_2, AcOH, 60 °C, 24 h, 30%; (b) 1 N NaOH, MeOH, 50 °C, 1 h, 70%.

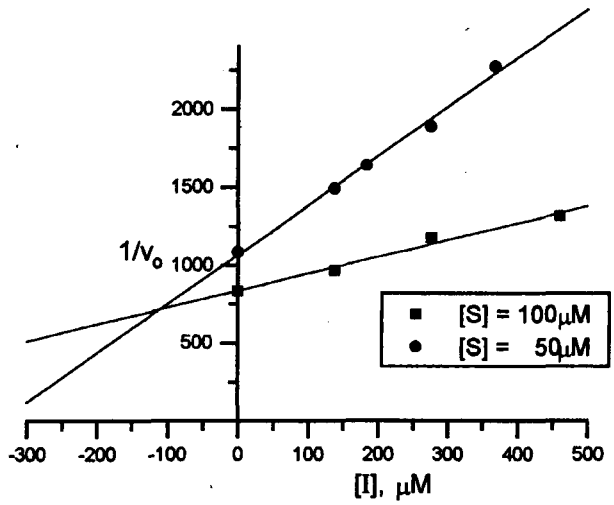


Figure 2. Dixon plot for the inhibition of CPA catalyzed hydrolysis of *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyl-lactate (substrate) by L-1 at $[CPA]_o=6.9\times10^{-9}$ M.

strongly suggest that the oxygen of nitrone in these inhibitors is capable of coordinating to the active site zinc ion. Futhermore, the finding that **3** is more potent than **2** suggests that the nitrone in the enzyme bound **3** is more favorably positioned for coordination to the active site zinc ion than that in **2**. In the case of **4**, however, the enhancement of the binding affinity is much reduced, which may be a consequence of the unfavorable orientation of the nitrone oxygen for its coordination to the zinc ion due to the azomethine double bond. In contrast to **1** and **3**, inhibitors containing the pyridine *N*-oxide (**6**, **7**, and **9**) showed only marginal enhancement in binding to the enzyme compared with the respective pyridine parent compound. It appears that the *N*-oxide oxygen is not properly rested for it to coordinate to the active site zinc ion upon binding to CPA by virtue of bulkiness of the pyridine ring. The S_1 subsite

Table 1. Inhibition constants of Inhibitors

Inhibitor	K_i (10^{-4} M)
1	2.60
L-1	1.11
D-1	NI[a]
2	31
3	1.49
4	9.30
5	16.9
6	4.70
7	5.06
L-7	2.53
D-7	NI[a]
8[b]	7.40
9	2.70
10	5.95

[a] NI=No Inhibition was detected up to the conc. of 5×10^{-4} M. [b] *N*-(2-pyridylcarbonyl)phenylalanine: Suh, J.; Lee, S. H.; Uh, J. Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 585-588.

which should accommodate the pyridine ring when these inhibitors bind the enzyme may accept the aromatic ring but the pyridine *N*-oxide cannot be fitted in properly with its *N*-oxide being directed to the zinc ion for coordination. To see the effect of stereochemistry at the α-carbon of inhibitors, optically active **1** and **7** were prepared and their binding constants were determined (Table 1) to show that the "L" stereomers are more potent by 2 fold than the racemic form. It is well established that CPA shows high "L" stereospecificity for substrate.[9,12] In consistent with the substrate stereospecificity, the inhibitory activity of these compounds rest primarily on the L-isomers.

Conclusion

Numerous analogs of substrate for CPA have been known to exhibit competitive inhibitory activity toward the enzyme: 2-benzylsuccinic acid[9a] and 2-benzyl-3-mercaptopropanoic acid[13] are the representative examples. Recently, we have reported that imidazole can be a viable zinc ligating group, producing potent inhibitors for CPA upon incorporating it into substrate basic skeleton.[1j] In the present study we report another new type of substrate analog inhibitors for CPA containing nitrone group. The design protocol developed in this study may be useful in designing inhibitors of medicinally interesting zinc containing proteases such as angiotensin converting enzyme and matrix metalloproteases, generating inhibitors of therapeutic applications.

References

1. For example, (a) Kim, D. H.; Kim, K. B. *J. Am. Chem. Soc.* **1991**, *113*, 3200-3202. (b) Yun, M.; Park, C.; Kim, S.; Nam, D.; Kim, S. C.; Kim, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 2281-2282. (c) Kim, D. H.; Li, Z.-H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2297-2302. (d) Kim, D. H.; Ryoo, J. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1287-1292. (e) Lee, S. S.; Li, Z.-H.; Lee, D. H.; Kim, D. H. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2877-2882. (f) Kim, D. H.; Chung, S. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1667-1672. (g) Kim, D. H.; Kim, K. R. *Bull. Korean Chem. Soc.* **1996**, *17*, 34-38. (h) Lee, K. J.; Kim, D. H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2431-2436. (i) Ryu, S.-E.; Choi, H.-J.; Kim, D. H. *J. Am. Chem. Soc.* **1997**, *119*, 967-969. (j) Lee, K. J.; Joo, K. C.; Kim, E.-J.; Lee, M.; Kim, D. H. *Bioorg. Med. Chem.* in press.
2. (a) Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441-444. (b) Kim, D. H.; Guinosso, C. I.; Buzby, G. C. Jr.; Herbst, D. R.; Mccaully, R. J.; Wicks, T. C.; Wendt, R. L. *J. Med. Chem.* **1983**, *26*, 394-403.
3. (a) Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62-69. (b) Lipscomb, W. N.; Strüter, N. *Chem. Rev.* **1996**, *96*, 2375-2433.
4. (a) Goti, A.; Cicchi, S.; Fedi, V.; Nannelli, L.; Brandi, A. *J. Org. Chem.* **1997**, *62*, 3119-3125. (b) Murahashi, S.-I. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2443-2465.
5. (a) Gothelf, K. V.; Thomsen, I.; Jörgensen, K. A. *J. Am. Chem. Soc.* **1996**, *118*, 59-64. (b) Hori, K.; Kodama, H.; Ohta, T.; Furukawa, I. *Tetrahedron Lett.* **1996**, *37*, 5947-5950. (c) Tamura, O.; Gotanda, K.; Terashima, R.; Kikuchi, M.; Miyawaki, T.; Sakamoto, M. *J. Chem. Soc., Chem. Commun.* **1996**, 1861-1862. (d) Ukaji, Y.; Shimizu, Y.; Kenmoku, Y.; Ahmed, A.; Inomata, K. *Chem. Lett.* **1997**, 59-60.
6. (a) Carlin, R. L.; Jongh, L. J. D. *Chem. Rev.* **1986**, *86*, 659-680. (b) Chiumia, G. C.; Phillips, D. J.; Rae, A. D. *Inorganica Chimica Acta* **1995**, *238*, 197-201. (c) Antolovich, M.; Phillips, D. J.; Rae, A. D. *Inorganica Chimica Acta* **1989**, *156*, 189-193. (d) Chen, X.; Hu, Y.; Wu, D.; Weng, L.; Kang, B. *Polyhedron* **1991**, *10*, 2651-2657.
7. Suh, J.; Kaiser, E. T. *J. Am. Chem. Soc.* **1976**, *98*, 1940-1947.
8. Dixon, M. *Biochem. J.* **1953**, *55*, 170-171.
9. (a) Byers, L. D.; Wolfenden, R. *Biochemistry* **1973**, *12*, 2070-2078. (b) Grobelny, D.; Goli, U. B.; Galardy, R. E. *Biochem. J.* **1985**, *232*, 15-19. (c) Holmquist, B.; Vallee, B. L. *Proc. Natl. Acad. Sci. U. S. A.* **1979**, *76*, 6216-6220.
10. Murahashi, S.-I.; Mitsui, H.; Shiota, T.; Tsuda, T.; Watanabe, S. *J. Org. Chem.* **1990**, *55*, 1736-1744.
11. Hamer, J.; Macaluso, A. *Chem. Rev.* **1964**, *64*, 473-595.
12. (a) Kim, D. H.; Kim, Y. J. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2681-2684. (b) Mangani, S.; Carloni, P.; Orioli, P. *J. Mol. Biol.* **1992**, *223*, 573-578.
13. Ondetti, M. A.; Condon, M. E.; Reid, J.; Sabo, E. F.; Cheung, H. S.; Cushman, D. W. *Biochemistry* **1979**, *18*, 1427-1430.