BIOORGANIC CHEMISTRY **20**, 124–134 (1992)

2-(Peptidamido)-cyclobutanones: A Novel Strategy for the Inhibition of Serine Elastases

HENRY W. PAULS,[1] BILL CHENG, AND LORNE S. REID

Allelix Biopharmaceuticals, 6850 Goreway Dr., Mississauga, Ontario, Canada L4V 1P1

Received January 23, 1992

The synthesis of 2-peptidamidocyclobutanones is described as a novel class of competitive elastase inhibitors. Their inhibitory potency, although modest (i.e., micromolar range), is up to two orders of magnitude better than their acyclic analogues. The enhanced potency is explained, in part, by the tendency of an sp^2 hybridized cyclobutyl carbonyl to release internal ring strain by conversion to an sp^3 hybridized center. © 1992 Academic Press, Inc.

INTRODUCTION

Elastolytic enzymes of the serine proteinase family have received considerable attention recently (*1, 2*), especially as targets for inhibitor design (*3*). This effort has been based upon a general acceptance of the proteinase–antiproteinase hypothesis (*4*) and a good understanding of the mechanism of serine proteinase catalysis (*5–7*).

The so called "transition state analogues" of human neutrophil elastase (HNE) rank as the most potent competitive inhibitors of this enzyme yet discovered (*8*). Peptidyl trifluoromethyl ketones (*9*) and aldehydes (*10*) inhibit HNE in the nanomolar range. For many transition state analogues a carbonyl functionality replaces the scissile amide linkage of a good substrate (*9*). Initial binding is followed by a covalent interaction between the carbonyl of the inhibitor and the hydroxyl of the active site serine. Peptidyl aldehydes (*11*) and peptidyl trifluoromethyl ketones (*12*) form hemiacetal and hemiketal-like structures, respectively, upon interaction with serine proteinases. These tetrahedral species are tightly bound because they mimic a high energy intermediate of the hydrolytic pathway.

Peptidamidoalkyl ketones, in contrast to aldehydes, are sterically encumbered at the carbonyl and are generally poor inhibitors of elastase (*13*). Also, they lack the powerful electron withdrawing groups of the trifluoromethyl ketones and related inhibitors such as esters (*14*) and diketones (*15*). In this work we introduce an alternate strategy, the incorporation of internal ring strain (*16*), for the inhibition of serine proteinases. This is the first instance in which the inherent strain of a cyclobutanone has been exploited for the design of a proteinase inhibitor. A

[1] Current address: Rhone-Poulenc Rorer Central Research, 640 Allendale Rd., King of Prussia, PA 19406.

0045-2068/92 $5.00

related effort on bicyclo[3.2.0]heptan-6-one synthesis led to the discovery of time-dependent β-lactamase inhibitors (*17*).

EXPERIMENTAL

Computer Modeling

All calculations were done on a Silicon Graphics 4D25GT personal IRIS workstation running the Polygen Quanta Rev 2.1A graphics package with CHARMm Rev. 21 molecular minimization/dynamics code and AMPAC Rev 1.00.

Coordinates for the model compounds were calculated from a 2D model with the Chemnote molecule sketching/building package. Partial atomic charges were from an INDOCL calculation. The global energy minimum was found with a grid search in torsional angle space. A grid of 5° was applied around the appropriate torsional angles and an adopted basis Newton Raphson minimization based on the CHARMm parameter set. The coordinates of the lowest energy structure were then used as input into an AMPAC calculation. This calculation employed full geometry optimization and a 100× precision factor on the convergence criterion. Parallel calculations were done for each of the AM1, MNDO, and MINDO3 Hamiltonians.

Synthesis

2-(N-Benzyloxycarbonyl-L-alanyl-L-alanyl-L-prolinamido)-cyclobutanol (**3**). *N*-Benzyloxycarbonyl-L-proline (0.33 g, 1.32 mmol) was dissolved in dry tetrahydrofuran (25 ml), blanketed with argon, and cooled in a dry ice/acetone bath. The solution was treated dropwise with isobutyl chloroformate (154 μl, 1.19 mmol) and *N*-methylmorpholine (128 μl, 1.16 mmol) and stirred for 10 min. The resulting mixture was treated with a solution of *trans*-2-aminocyclobutanol in dry tetrahydrofuran (10 ml) and stirred at room temperature for 4 h. Ethyl acetate (200 ml) was added and the organic layer was washed with 5% HCl (1 × 50 ml), saturated sodium bicarbonate solution (1 × 50 ml), and saturated sodium chloride solution (1 × 50 ml) followed by drying over sodium sulfate. The solvent was evaporated and the residue was applied to a silical gel column. The 2-(*N*-benzyloxycarbonyl-L-prolinamido)cyclobutanol was eluted with MeOH/CH_2Cl_2 (1 : 9) under pressure. Evaporation gave **1** as a syrup (0.165 g, 44.7%) (R_f 0.35; ir 3305, 2972, 1687, 1656, 1421, 1357, 1120, 735 cm^{-1}; ^1H NMR d 1.60–2.35 (m, 8H, H-2, H-2′, H-3, H-3′, $NCH_2(CH_2)_2CH$), 3.4–4.0 (m, 5H, H-1, H-2, $NCH_2(CH_2)_2CH$, OH), 4.31 (bs, 1H, $NCH_2(CH_2)_2CH$), 5.16 (m, 2H, CH_2Ph), 6.24, 7.16 (two d, 1H, NH), 7.36 (s, 5H, CH_2Ph).

Syrupy **1** was dissolved in methanol (25 ml) blanketed with argon and treated with 10% palladium on carbon. The argon gas was exchanged for hydrogen (3 atm) and the reaction mixture was stirred at room temperature for 4 h. The contents of the reaction flask were filtered, the cake was washed with MeOH (2 × 30 ml), and the filtrate was concentrated to a syrupy residue. A portion of the residue (0.045 g, 0.19 mmol) was dissolved in dry tetrahydrofuran (7 ml) to make a solution of **2**.

N-Benzyloxy-carbonyl-L-alanyl-L-alanine (0.048 g, 0.20 mmol) was dissolved in dry tetrahydrofuran, blanketed with argon, and cooled in a dry ice/acetone bath. The resulting solution was treated dropwise with isobutylchloroformate (26 μl, 0.20 mmol) and *N*-methylmorpholine (22.6 μl, 0.21 mmol) and stirred for 10 min. The reaction mixture was treated with **2**, in solution, and warmed to room temperature for 4 h.

Ethyl acetate (100 ml) was added and the organic layer washed with 5% HCl (1 × 25 ml) and saturated sodium bicarbonate solution (1 × 25 ml) and dried over sodium sulfate. The organic layer was filtered, evaporated to dryness, and chromatographed under pressure on silica gel using $MeOH/CH_2Cl_2$ (1:9). 2-(*N*-Benzyloxycarbonyl-L-alanyl-L-alanyl-L-prolinamido)cyclobutanol, **3**, was isolated as a syrup (0.036 g, 28%): R_f 0.23; ir 3297, 1703, 1656, 1639, 1544, 1460, 1250, 735 cm^{-1}; ^1H NMR d 1.25–1.4 (m, 6H, $CHCH_3$, $CHCH_3$), 1.5–2.6 (m, 6H, H-3, H-3′, H-4, H-4′, $NCH_2(CH_2)_2CH$), 3.45–3.95, 4.0–4.2 (two m, 5H, H-1, H-2, $NCH_2(CH_2)O_2CH$, OH), 4.25–4.6, 4.83 (m, bs, 3H, CH_3CH, CH_3CH, $NCH_2(CH_2)_2CH$), 5.10 (s, 2H, CH_2Ph), 5.43, 5.69 (two bs, 1H, NH), 7.2–7.5 (bs, 6H, CH_2Ph), 7.89, 7.99 (two bs, 1H, NH); HRMS *m* + 1/*e* calcd. for $C_{23}H_{32}N_4O_6$: 461.2400. Found: 461.2406.

2-(N-Benzyloxycarbonyl-L-alanyl-L-alanyl-L-prolinamido)-cyclobutanone (***4a, 4b***). Dimethyl sulfoxide (208 μl, 2.93 mmol) was added dropwise to a solution of oxalyl chloride (128 μl, 1.46 mmol) at about −78°C in dry dichloromethane (7 ml). The resulting solution was stirred for 30 min then treated dropwise with a solution of 2-(*N*-benzyloxycarbonyl-L-alanyl-L-alanyl-L-prolinamido)cyclobutanol, **3**, (0.021 g, 0.045 mmol) in dry CH_2Cl_2 (7 ml). The reaction mixture was stirred an additional 30 min at −78°C, then treated with triethylamine (407 μl, 2.9 mmol) and warmed to room temperature for 1 h. The reaction mixture was diluted with ethyl acetate (40 ml) then washed with 1 N HCl (10 ml), saturated bicarbonate solution (2 × 10 ml) and brine (3 × 20 ml). The organic layer was dried (Na_2SO_4) and concentrated, the residue was subjected to flash chromatography ($MeOH/CH_2Cl_2$, 1:9) to yield the title compound **4** as a colorless syrup (0.019 g, 92%, R_f 0.35). Separation by HPLC (EtOAc/hexane, 3:1, 4 ml/min, Waters 7.6 mm × 30 cm μPorasil, 10 μm) afforded two fractions.

Short retention isomer **4a**: R_t = 23.5 min; ir 3299, 1793, 1638, 1532, 1460, 1244, 736, 699 cm^{-1}; ^1H NMR d 1.29, 1.35 (two d, 6 *J* = 6.5, 6.8, 2 × CH_3CH), 1.85–2.30 (m, 6, $NCH(CH_2)CH$, H-3, H-3′), 2.83 (m, 2, H-4, H-4′), 3.6–3.8 (m, 2, $NCH_2(CH_2)_2CH$, 5.10 (s, 2, $PhCH_2$), 5.64 (d, 1, *J* = 7, NH), 7.35 (s, 5, $PhCH_2$), 7.60, 7.86 (two d, 2, *J* = 7.2, 7.1, 2 × NH); HRMS *m* + 1/*e* calcd. for $C_{23}H_{30}N_4O_6$: 459.2244. Found: 459.2245.

Long retention isomer **4b**: R_t = 26 min; ir 3303, 1791, 1639, 1532, 1460, 1244, 736, 700 cm^{-1}; ^1H NMR d 1.28, 1.35 (two d, 6, *J* = 6.6, 6.6, 2 × CH_3CH), 1.7–2.2 (m, 6, $NCH(CH_2)CH$, H-3, H-3′), 2.84 (m, 2, H-4, H-4′), 3.6–3.8 (m, 2, $NCH_2(CH_2)_2CH$), 4.5 (m, 2, 2 × CH_3CH), 4.8–4.9 (m, 2, $NCH_2(CH_2)_2CH$, H-2), 5.09 (s, 2, $PhCH_2$), 5.65 (d, 1, *J* = 6.9, NH), 7.35 (s, 5, $PhCH_2$), 7.67 (d, 1, *J* = 7, NH), 7.92 (bs, 1, NH); HRMS *m* + 1/*e* calcd. for $C_{23}H_{30}N_4O_6$: 459.2244. Found: 459.2240.

3-(N-Benzyloxycarbonyl-L-alanyl-L-alanyl-L-prolinamido)-2-butanone (***8a, 8b***). *N*-Benzyloxycarbonyl-L-proline (0.249 g, 1.0 mmol) was treated, as described for

1, with isobutylchloroformate (137 μl, 1.06 mmol), *N*-methylmorpholine (99 μl, 1.06 mmol) and threo-3-amino-2-butanol (0.090 g, 1.0 mmol). Standard workup and flash chromatography on silica gel ($MeOH/CH_2Cl_2$, 1 : 9) yielded the amidoalcohol **5** (0.265 g, 82.8%, $R_f = 0.34$); ir 3331, 2975, 1701, 1686, 1420, 1357, 1121, 736 cm^{-1}; ^1H NMR d 0.95–1.25 (m, 6, $CH_3CHCHCH_3$), 1.85–2.60 (m, 5, CH_2CH_2, OH), 3.55 (bs, 2, NCH_2), 3.65, 3.85 (2bs, 2, $CH_3CHCHCH_3$), 4.33 (m, 1, NCHCO), 5.16 (s, 2, CH_2Ph), 6.10, 6.60 (2 bs, 1, NH), 7.35 (s, 5, CH_2Ph).

Amidoalcohol **5** (0.260 g, 0.81 mmol) was dissolved in ethanol (125 ml) treated with 10% Pd/C (25 mg) and worked up as described for **2** to give syrupy **6** (0.150 g, 97.5%), which was used without further purification.

N-Benzyloxycarbonyl-L-alanyl-L-alanine (0.119 g, 0.404 mmol) was dissolved in tetrahydrofuran (10 ml) treated with isobutylchloroformate (53 μl, 0.409 mmol), *N*-methylmorpholine (46 μl, 0.418 mmol) and **6** (0.075 g, 0.403 mmol) as previously described. Workup and flash chromatography ($MeOH/CH_2Cl_2$ 1 : 19 to 1 : 9) gave 2-(*N*-benzyloxycarbonyl-L-alanyl-L-alanyl-L-prolinamido)cyclobutanol **7** (0.10 g, 53.7%, $R_f = 0.25$); ir 3305, 2975, 1657, 1638, 1536, 1455, 1265, 735 cm^{-1}; ^1H NMR d 1.04–1.12 (m, 6, $CH_3CHCHCHCH_3$), 1.25, 1.33 (2 d, 6, CH_3CH, CH_3CH), 1.9–2.40 (m, 4, $NCH_2(CH_2)_2CH$), 3.81 (d, 1, OH), 3.5–3.9 (m, 4, $NCH_2(CH_2)_2CH$, $CH_3CHCHCH_3$), 4.3–4.65, 4.84 (m, bs, 3, CH_3CH, CH_3CH, $NCH_2(CH_2)_2CH$), 5.94 (ABq, 2, CH_2Ph), 5.80 (bs, 1, NH), 7.00, 7.09 (2 d, 1H, NH), 7.33 (s, 5, CH_2Ph), 8.05 (d, 1, NH).

Amido butanol **7** (0.09 g, 0.195 mmol) was treated as described for **3** with dimethyl sulfoxide (800 μl, 11.3 mmol), oxalyl chloride (493 μl, 5.65 mmol), and triethylamine (1.60 ml, 11.5 mmol) to give, after chromatography ($MeOH/CH_2Cl_2$, 1 : 19 to 1 : 9), the title compound **8** (0.060 g, 66.4%, $R_f = 0.33$) as a mixture of epimers at C-3. This material was separated into two fractions by HPLC as described above.

Short retention isomer **8a**: R_t = 23 min; ir 3303, 2926, 1721, 1639, 1530, 1459, 1250, 737 cm^{-1}; ^1H NMR d 1.26–1.38 (m, 9, $CH_3CHCHOCH_3$, $CHCH_3$, $CHCH_3$), 1.9–2.3 (m, 4, $NHCH_2(CH_2)_2CH$), 2.13 (s, 3, $CH_3CHCOCH_3$), 3.55–3.80 (m, 2, $NCH_2(CH_2)_2CH$), 4.35–4.65 (m, 3, CH_3CH, CH_3CH, $CH_3CHCOCH_3$), 4.83 (m, 1, $NCH_2(CH_2)_2CH$), 5.11 (m, 2, CH_2Ph), 5.62 (bs, 1H, NH), 7.34 (s, 5, CH_2Ph), 7.44 (m, 1, NH), 7.63 (bs, 1, NH); HRMS $m + 1/e$ calcd. for $C_{23}H_{33}N_4O_6$: 461.2400. Found: 461.2402.

Long retention isomer **8b**: R_t = 30 min; ir 3283, 2927, 1720, 1640, 1459, 1248, 1028, 700 cm^{-1}, ^1H NMR d 1.24–1.38 (m, 9, $CH_3CHCOCH_3$, CH_3CH, CH_3CH), 2.0–2.25 (m, 4, $NHCH_2(CH_2)_2CH$), 2.18 (s, 3, $CH_3CHCOCH_3$), 3.6–3.8 (m, 2, $NCH_2(CH_2)_2CH$), 4.4–4.55 (m, 3, CH_3CH, CH_3CH, $CH_3CHCOCH_3$), 4.82 (m, 1, $NCH_2(CH_2)_2CH$), 5.12 (m, 2, CH_2Ph), 5.58 (d, 1, NH), 7.35 (bs, 6, CH_2Ph, NH), 7.65 (d, 1, NH); HRMS $m + 1/e$ calcd. for $C_{23}H_{32}N_4O_6$: 461.2400. Found: 461.2402.

3-(N-Benzyloxycarbonyl-L-alanyl-L-prolinamido)-1,1,1-trifluoro-2-butanone (***12***). A solution of *N*-benzyloxycarbonyl-L-proline (0.031 g, 0.13 mmol) in anhydrous tetrahydrofuran was treated with isobutyl chloroformate (16.3 μl, 0.3 mmol), *N*-methylmorpholine (13.8 μl, 0.13 mmol) and a solution of 3-amino-1,1,1-trifluorobutanol (0.020 g, 0.14 mmol) in tetrahydrofuran (5 ml) as described above.

Standard workup yielded a residue: ir 3295, 1681, 1666, 1174, 1138, 734, 696 cm^{-1}. The residue was purified by flash chromatography ($MeOH/CH_2Cl_2$, 1 : 19) to give **9** (0.023 g, 48%) in two fractions.

High R_f fraction: 0.65 ($HOAc/MeOH/CH_2Cl_2$, 0.1 : 1 : 9); ^1H NMR d 1.25 (s, 3, CH_3), 1.9–2.3 (m, 4, $NHCH_2(CH_2)_2CH$), 3.53 (bs, 2, $NCH_2(CH_2)_2CH$), 4.1 (s, 1, OH), 4.22 (s, 2, H-2, H-3), 4.67 (s, 1, $NCH_2(CH_2)_2CH$), 5.14 (s, 2, $PhCH_2$), 6.67 (bs, 1, NH), 7.35 (s, 5, $PhCH_2$).

Low R_f fraction: 0.61 ($HOAc/MeOH/CH_2Cl_2$, 0.1 : 1 : 9); ^1H NMR d 1.26 (s, 3, CH3), 1.9–2.3 (m, 4, $NHCH_2(CH_2)_2CH$), 3.52 (s, 2, $NCH_2(CH_2)_2CH$), 3.85 (s, 1, OH), 4.0–4.4 (m, 2, H-2, H-3), 4.8 (s, 1, $NCH_2(CH_2)_2CH$), 5.14 (s, 2, $PhCH_2$), 6.15–7.1 (bm, 1, NH), 7.35 (s, 5, $PhCH_2$).

A solution of **9** (combined fractions, 0.057 g, 0.15 mmol) in absolute MeOH (10 ml) was treated with 10% Pd/C (20 mg) and hydrogen gas as previously described to give **10** as a residue ir 3325, 1625, 1525, 1175, 1142 cm^{-1}.

A partial suspension of benzyloxycarbonyl-L-alanyl-L-alanine (0.040 g, 0.136 mmol) in dry tetrahydrofuran was treated with isobutylchloroformate (17.6 μl, 0.136 mmol) and *N*-methylmorpholine (14.9 μl, 0.136 mmol) and 3-(L-prolinamido)-1,1,1-trifluorobutan-2-ol, **10** (0.36 g, 0.15 mmol). After stirring at room temperature 3 h, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide · HCl (0.013 g, 0.068 mmol) was added and the suspension was stirred overnight at room temperature. Standard workup gave a residue which was purified by flash chromatography ($MeOH/CH_2Cl_2$, 1 : 19–1 : 9) to yield **11** as a solid (0.052 g, 74%): R_f 0.48, 0.44 ($MeOH/CH_2Cl_2$, 1 : 9): ir 3305, 1704, 1635, 1270, 1176, 1143, 737, 699 cm^{-1}; ^1H NMR d 1.1–1.4 (m, 9, CH_3), 2.0–2.1 (m, 4, $NCH_2(CH_2)_2CH$), 3.61, 3.74 (m, 1, $NCH_2(CH_2)_2CH$), 3.9–4.5 (m, 3, 2 × $CHCH_3$, H-2), 4.82 (d, 1, J = 7.0, $NCH_2(CH_2)_2CH$), 5.10 (s, 2, $PhCH_2$), 5.45 (bs 1, H-2), 5.74 (d, 1, J = 7.7, NH), 7.34 (s, 5, $PhCH_2$), 7.56 (s, 1, NH), 8.09 (d, 1, J = 7.6, NH).

The amido trifluoromethylbutanol **11** was treated as described above with oxalyl chloride (140 μl, 1.60 mmol), dimethyl sulfoxide (230 μl, 3.24 mmol), and triethylamine (450 μl, 3.23 mmol). Workup followed by flash chromatography ($MeOH/CH_3Cl_2$, 1 : 9) gave the title **12** as a solid (0.024 g, 72%): R_f 0.60–0.67; ir 3302, 1697, 1634, 1266, 1182, 738, 702 cm^{-1}; ^1H NMR d 1.31 (m, 9, CH3), 1.9–2.2 (m, 3, 2 × CH_3CH, H-2), 3.6–3.8 (m, 2, $NCH_2(CH)_2CH$), 4.3–4.6 (m, 3, 2 × CH_3CH H-2), 4.82 (m, 1, $NCH_2(CH_2)_2CH$), 5.10 (Abq, J = 12, 1, $PhCH_2$) 5.55 (d, 1, J = 7.9, NH), 7.35 (s, 5, $PhCH_2$), 7.6 (m, 2, NH); HRMS m + 1/e calcd: for $C_{23}H_{29}N_4O_6F_3$: 515.2118. Found: 515.2115. A sample of this material was further purified by HPLC; one diffuse peak with R_t = 14.8 minutes was recovered.

Elastase Inhibition Assay

The compounds were assayed for inhibitory activity against human neutrophil elastase obtained from Elastin Products (lot SE 563) and porcine pancreatic elastase from Boehringer-Manheim, West Germany (lot 11111223-02). The elastase inhibition assay employed the following: buffer, 1.0 M NaCl, 0.2 M Hepes, pH 7.5; substrate, methoxysuccinyl-L-alanyl-L-alanyl-L-prolyl-L-valyl-4-nitroanalide dissolved in DMSO; test compound, dissolved in DMSO and elastase dissolved in

TABLE 1

Inhibition Constants of Elastase Inhibitors

Inhibitor	Isomer	K_i (mM) HNE	PPE
3 Z—A—A—P—NH⟨□⟩OH	—	2.0	0.85
4a Z—A—A—P—NH⟨□⟩=O	Short retention	0.55	0.025
4b Z—A—A—P—NH⟨□⟩=O	Long retention	1.2	ND
8a Z—A—A—P—NH⟨CH(C=O)⟩	Short retention	>2.5	>2.0
8b Z—A—A—P—NH⟨CH(C=O)⟩	Long retention	>5.0	1.6
12 Z—A—A—P—NH⟨CH(C=O)CF$_3$⟩	—	0.005	0.0001

buffer. Assays were monitored at 410 nm in a 25°C, temperature-stabilized six-position Beckman DU-40 spectrophotometer.

To conduct the assay, the following were mixed in a 500-μl quartz cuvette: 20 μl buffer; 150 μl distilled, deionized water; 20 μl substrate; 20 μl inhibitor; and 10 μl elastase. Absorbance was monitored for 30 min and the slope of the linear range was calculated. For the purpose of calculating an inhibition constant, these steps were repeated for the following solution conditions: elastase, 5–20 nM; substrate, 0-500 μM; and inhibitor, 0–2000 μM. The inhibition constant, K_i, was then calculated from the Dixon plot (1/velocity vs [I]) for each concentration of substrate. The K_i s obtained are presented in Table 1.

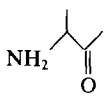
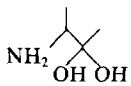
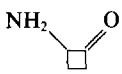
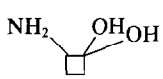
RESULTS AND DISCUSSION

Nucleophilic addition to aldehydes and ketones has been chosen as a model system for understanding the inhibitory potential of peptidamido ketones and aldehydes (*18*). For example, trifluoromethyl ketones are extensively hydrated in aqueous systems (i.e., low K_{H_2O}), alkyl ketones are not (*19*). This correlates with the inhibitory potency of their peptidyl analogues, even though a linear relationship between K_i and K_{H_2O} cannot be demonstrated (*18*).

Cyclobutanones are generally more susceptible to nucleophilic attack than their

TABLE 2

AMPAC Calculations on Model Compounds

Compound	Hamiltonian	Heat of formation (H_f) (kcal/mol)	ΔH_f[a] (kcal/mol)	$\Delta\Delta H_f$[b] (kcal/mol)
	AM1	−50.9		
	MNDO	−465		
	MINDO3	−53.5		
	AM1	−122.3	71.4	
	MNDO	−104.8	58.3	
	MINDO3	−108.5	55.0	
	AM1	−21.7		
	MNDO	−31.1		
	MINDO3	−39.1		
	AM1	−97.2	75.5	4.1
	MNDO	−97.5	66.4	8.1
	MINDO3	−102.1	63.0	8.0

[a] (H_f of ketone)-(H_f of corresponding hydrate).

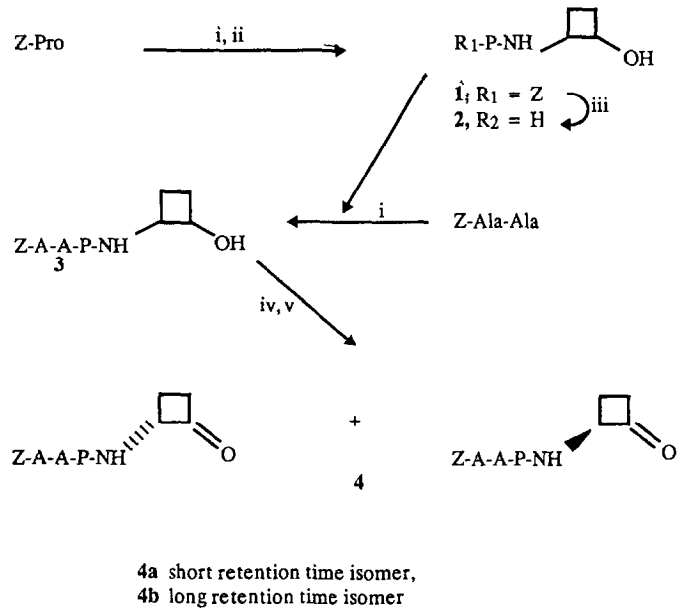
[b] Difference in ΔH_f between acyclic and cyclic forms.

acyclic counterparts. A tentative result (*20*) indicates that the four-membered ring is significantly more hydrated than its acyclic counterpart. Moreover, the equilibrium value for thiol addition to cyclobutanone, K(RSD) is about 20 times the value for acetone. Reaction rates are also affected; the reduction of cyclobutanone by sodium borohydride is about 500 times faster than it is for open chain ketones (*21*).

These phenomena are attributed to the strain introduced by incorporating an sp^2-hybridized carbonyl (120° preferred) into a four-membered ring (90° angles). Some of this "I strain" (*16*) is released by conversion to an sp^3 hybridized center (109.5° preferred), i.e., through reduction, thiohemiketal formation, or, presumably, formation of a tetrahedral hemiketal in the enzyme active site.

The energetics of the cyclobutanone/hydrate pair were modeled and compared to their acyclic counterparts. Results of the AMPAC calculation are given in Table 2. The MNDO and MINDO3 Hamiltonians predict that the "I strain" effect would favor the hydration of the cyclobutanone over its acyclic analogue by some 8 kcal/mol. The calculation based on the AM1 Hamiltonian also favors cyclobutanone hydration but by only 4 kcal/mol. This analysis ignores any entropic effects, which should also favor the cyclobutanone hydration. The conformationally restricted four-membered ring has fewer degrees of rotational freedom than its acyclic analogue.

The above considerations led to the formulation of potential PPE inhibitors **4a**, **4b** in which a cyclobutanone is grafted onto peptidyl chains complementary to the elastase active site. The peptidyl backbone of **4** (A-A-P) is recognized as one of the preferred sequences for binding into the S2–S4 subsites of porcine pancreatic elastase (*22*). The N-blocking group, benzyloxycarbonyl (Z), was chosen for syn-



4a short retention time isomer,
4b long retention time isomer

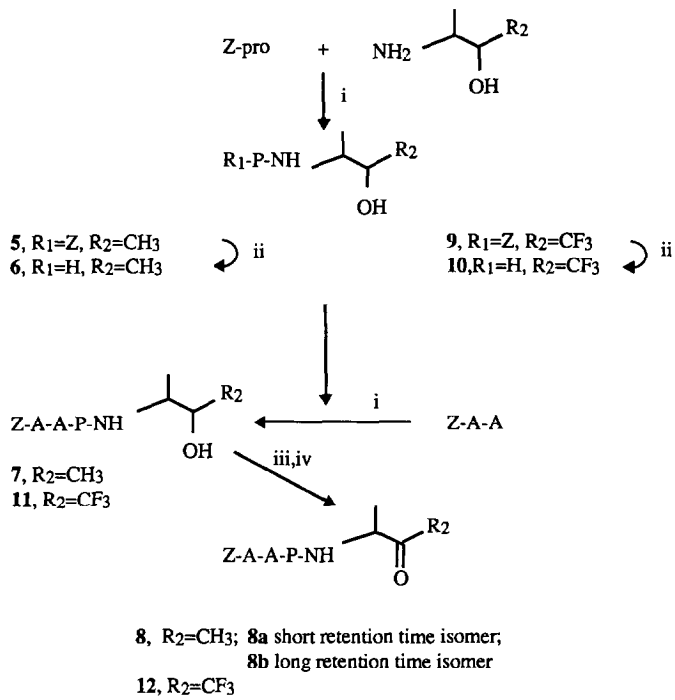
SCHEME 1. Synthesis of 2-peptidamidocyclobutanones: (i) NMM, isobutylchloroformate; (ii) *trans*-2-amino-cyclobutanol; (iii) H_2, Pd/C; (iv) DMSO, oxalylchloride, Et_3N; (v) HPLC.

thetic convenience and because it has been successfully incorporated into human neutrophil elastase inhibitors (*23*). Retrosynthetic analysis yielded three synthons; Z-proline and -Ala–Ala are commercially available and *trans*-2-aminocyclobutanol was prepared by the method of Hartmann *et al.* (*24*) (Scheme 1).

Aminocyclobutanol, **1**, was coupled to Z-proline using EDCl or the mixed anhydride procedure (*25*); the latter gave more consistent results. The diastereomeric mixture obtained was deprotected and used for subsequent coupling to Z-Ala–Ala without further purification. This coupling reaction followed the first in all respects and gave **3** as a mixture of stereoisomers. The final oxidation step was accomplished with PCC or Swern conditions (*26*); the latter was cleaner and the method of choice. This protocol gave **4** as a mixture of stereoisomers at C-2, which were separated by HPLC. The stereochemistry of **4a** and **4b** at C-2 was not assigned.

An entirely analogous procedure, beginning with 3-amino-2-butanol (*27*) yielded the ketones **8a** and **8b**, which were assayed as controls against both PPE and HNE. Similarly, **12** was obtained from the previously described 3-amino-1,1,1-trifluoro-2-butanol (*13*) as a mixture which, in our hands, could not be separated into its constituent isomers (Scheme 2).

The compounds were tested against both PPE and HNE (see Table 1). The inhibition constants are generally much lower against PPE than HNE. PPE prefers to bind small aliphatic side chains such as alanine at the S-1 subsite; HNE



SCHEME 2. Synthesis of 2-peptidamidobutanones: (i) NMM, isobutylchloroformate; (ii) H_2, Pd/C; (iii) DMSO, oxalylchloride, Et_3N; (iv) HPLC.

prefers larger aliphatic side chains such as valine (*3, 28*). The 3-methyl groups of **8a**, **8b**, and **12** mimic an alanine side chain. The PPE preference for cyclobutanone is less easily explained; however, the ring is small and may not be any more sterically demanding than an alanine mimic.

The striking feature, from the viewpoint of inhibitor design, is the real difference in inhibitory potency between the peptidaminocyclobutanones, **4a** and **4b** and the acyclic counterparts **8a** and **8b**. Against PPE the difference in K_i between **4a** and **8b**, the most potent isomers, approximates two orders of magnitude. A similar enhancement is seen against HNE, with a lower limit of an order of magnitude difference. This difference in inhibitor potency may be driven by an I strain effect which enhances hemiketal formation (sp^3 hybridization) in the four-membered ring.

However, an alternative explanation is hinted at by the weak inhibitory potency of the cyclobutanol **3**. The cyclobutyl compounds can be viewed as conformationally restricted analogues of **8a** and **8b**. Thus entropic factors, in addition to the I-strain (enthalpy), may be contributing toward lower K_i s. This study does not allow us to deduce the relative importance of these nonexclusive rationalizations.

Even the best peptidyl cyclobutanone inhibitor is about two orders of magnitude less potent than comparable trifluoromethyl ketones. This result could have

been predicted from the hydration studies of Burkey and Fahey (*19, 20*) and also suggests a strategy for lowering the K_i s of the cyclobutyl series. Electron withdrawing groups at C-4 of the cyclobutyl ring would render the carbonyl more susceptible to nucleophilic attack.

We conclude that the cyclobutanone functionality can be exploited for the inhibition of serine proteinases such as elastase. The application of cyclobutanones for the inhibition of other hydrolytic enzymes is an obvious extension of this work (*29*). For example, tetrahedral structures have been demonstrated in the enzyme inhibitor complexes formed between peptidyl alkyl ketones and the metalloproteinase carboxypeptidase A (*30*). Alternatively, we are exploring the preparation of novel cysteine proteinase inhibitors based upon the ready addition of thiols to cyclobutanones (*20*).

REFERENCES

1. BIETH, J. G. (1986) *in* Regulation of Matrix Accumulation (Mecham, R. P., Ed.), pp. 218–320, Academic Press, New York.
2. STEIN, R. L., TRAINOR, D. A., AND WILDONGER, R. A. (1985) *in* Annual Reports in Medicinal Chemistry (Bailey, D. M., Ed.), pp. 237–246, Academic Press, New York.
3. POWERS, J. C., AND HARPER, J. W. (1986) *in* Proteinase Inhibitors (Barrett, A. J., and Salvesen, G., Eds.), pp. 55–152, Elsevier Science Publishers, B. V., New York.
4. MITTMAN, C. (Ed.) (1972) Pulmonary Emphysema and Proteloysis, Academic Press, New York.
5. BODE, W., MEYER, E., JR., AND POWERS, J. C. (1989) *Biochemistry*, **28,** 1952–1963.
6. WARSHEL, A., NARAY-SZABO, G., SUSSMAN, F., AND HWANG, J-K. (1989) *Biochemistry* **28,** 3629–3637.
7. STEIN, R. L. (1985) *J. Am. Chem. Soc.* **107,** 5767–5775.
8. TRAINOR, D. A. (1987) *Trends Pharm. Sci.* **8,** 303–307.
9. STEIN, R. L., STRIMPLER, A. M., EDWARDS, P. D., LEWIS, J. J., MAUGER, R. C., SCHWARTZ, J. A., STEIN, M. M., TRAINOR, D. A., WILDONGER, R. A., AND ZOTTALA, M. A. (1987) *Biochemistry* **26,** 2682–2689.
10. HASSAL, C. H., JOHNSON, W. H., KENNEDY, A. H., AND ROBERTS, N. A. (1985) *FEBS Lett.* **183,** 201–205.
11. MEYER, E. F., JR., RADHAKRISHNAN, R., COLE, G. M., AND PRESTA, L. G. (1986) *J. Mol. Biol.* **189,** 533–539.
12. TAKAHASHI, H., RADHAKRISHNAN, R., ROSENFIELD, R. E., MEYER, E. F. JR., TRAINOR, D. A., AND STEIN, M. (1988) *J. Mol. Biol.* **201,** 423–428.
13. IMPERIALI, B. AND ABELES, R. H. (1986) *Biochemistry* **25,** 3760–3767.
14. HORI, H., YASUTAKE, A., MINEMSU, Y., AND POWERS, J. C. (1985) *in* Peptides, Structure and Function (Proceedings of the Ninth American Peptide Symposium) (Deber, C. M., Hruby, V. J., and Kipple, K. D., Eds.), pp. 819–822, Pierce Chemical Co., Rockford, IL.
15. ANGELASTRO, M. R., PEET, N. P., AND BEY, P. (1989) *J. Org. Chem.* **54,** 3913–3916.
16. BROWN, H. C., FLETCHER, R. S., AND JOHANNSEN, R. B. (1951) *J. Am. Chem. Soc.* **73,** 212–221.
17. LOWE, G., AND SWAIN, S. (1983) *J. Chem. Soc. Chem. Commun.* 1279–1281.
18. SMITH, R. A., COPP, L. J., DONNELLY, S. L. SPENCER, R. W., AND KRANTZ, A. (1988) *Biochemistry* **27,** 6568–6573.
19. BURKEY, T. J., AND FAHEY, R. C. (1983) *J. Am. Chem. Soc.* **105,** 868–871.
20. BURKEY, T. J., AND FAHEY, R. C. (1985) *J. Org. Chem.* **50,** 1304–1306.
21. BROWN, H. C., AND ICHIKAWA, K. (1957) *Tetrahedron* **1,** 221–230.
22. POWERS, J. C., AND TUHY, P. M. (1973) *Biochemistry* **12,** 4767–4774.
23. DUNLAP, R. P., STONE, P. J., AND ABELES, R. H. (1987) *Biochem. Biophys. Res. Commun.* **145,** 509–513.

24. HARTMANN, W., SCHOLZ, K. H., AND HEINE, H. G. (1980) *Chem. Ber.* **113,** 1884–1889.
25. BODANSKEY, M., AND BODANSKY, A. (1984) The Practice of Peptide Synthesis, Sringer-Verlag, New York.
26. OMURA, K., AND SWERN, D. (1978) *Tetrahedron* **34,** 1651–1660.
27. DICKEY, F. H., FICKETT, W., AND LUCAS, H. J. (1952) *J. Am. Chem. Soc.* **74,** 944–949.
28. POWERS, J. C., GUPTON, B. F., HARLEY, A. D., NICHINO, N., AND WITLEY, R. J. (1977) *Biochim. Biophys. Acta* **485,** 156–166.
29. PAULS, H. W., AND REID, L. S. (1989) Proteinase Inhibitors U.S. Patent Application 435,560, filed Nov. 13, 1989.
30. CHRISTIANSON, D. A., AND LIPSCOMB, W. N. (1989) *Acc. Chem. Res.* **22,** 62–69.