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Design, Synthesis and Pharmacological Evaluation of 3-Benzylazetidine-2-one-based Human Chymase Inhibitors

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Abstract—3-Benzylazetidine-2-one derivatives were designed and evaluated as a novel series of chymase inhibitors. Structure–activity relationship studies of 3-benzylazetidine-2-ones led to compounds **23**, which exhibited 3.1 nM inhibition of human chymase and enhancement of stability in human plasma ($t_{1/2}$ 6 h).

Introduction

Human chymase is a chymotrypsin-like serine protease that is stored in the secretory granules of mast cells.[1] Although the physiological and pathological roles of chymase have not been fully elucidated, recent studies have demonstrated that this enzyme generates angiotensin II from angiotensin I with greater efficiency than angiotensin I converting enzyme.[2] Chymase has also been shown to participate in histamine release from mast cells,[3] activation of precursor interleukin-1β,[4] and cleavage of type I procollagen[5] and progelatinase B.[6] Thus, chymase is speculated to play an important role in cardiovascular diseases and chronic inflammation following fibrosis, such as cardiac, renal, and pulmonary fibrosis.[7] Chymase inhibitors[8,9] are thought to be potentially useful as tools for elucidating the physiological functions of chymase and therapeutic agents.

In previous papers, we reported on a novel series of 1-oxacephem-based[9a] and 1,3-diazetidine-2,4-dione-based[9b] human chymase inhibitors. 1-Oxacephem **1** and 1,3-diazetidine 2,4-dione **2** exhibited high activities against human chymase (IC_{50}: 27 and 4 nM), but had insufficient stability in human plasma ($t_{1/2}$ 1.5 and <0.5 h). Then, we designed a new class of azetidine-2-one-based human chymase inhibitor **10a** (Fig. 1) on the basis of the insights we gained with regard to the inhibition of human chyamse by 1-oxacephems[9a] and 1,3-diazetidine 2,4-diones,[9b] and the inhibition of human leukocyte elastase by cephalosporin sulfones[10] and 3,3-dialkylazetidine-2-ones[11] developed at Merck. Compound **10a** showed high activity against human chymase, but poor stability in human plasma. Structure–activity relationship studies of 3-benzylazetidine-2-ones such as **10a** led us to compound **23**, which exhibited high potency against human chymase and enhancement of stability in human plasma. We describe herein the design, synthesis and pharmacological evaluation of 3-benzylazetidine-2-one-based human chymase inhibitors.

Chemistry

The molecular structures and synthetic schemes of 3-benzylazetidine-2-one-based human chymase inhibitors are summarized in Scheme 1. Compound **4** was prepared from D-aspartic acid by a known procedure.[12] Deprotonation of **4** with 2 equiv of lithium diisopropylamide (LDA) followed by benzylation with benzyl bromide selectively introduced a benzyl group trans to the carboxyl to afford **5** in 98% yield. Lead tetraacetate (LTA) oxidation of the carboxylic acid **5** followed by removal of the *tert*-butyldimethylsilyl (TBDMS) *N*-protecting group with *n*-tetrabutylammonium fluoride (TBAF) provided the 4α-acetoxy derivative **6** in 53% yield. Displacement of the acetoxy **6** with the preformed sodium salt of diphenylmethyl 4-hydroxybenzoate,

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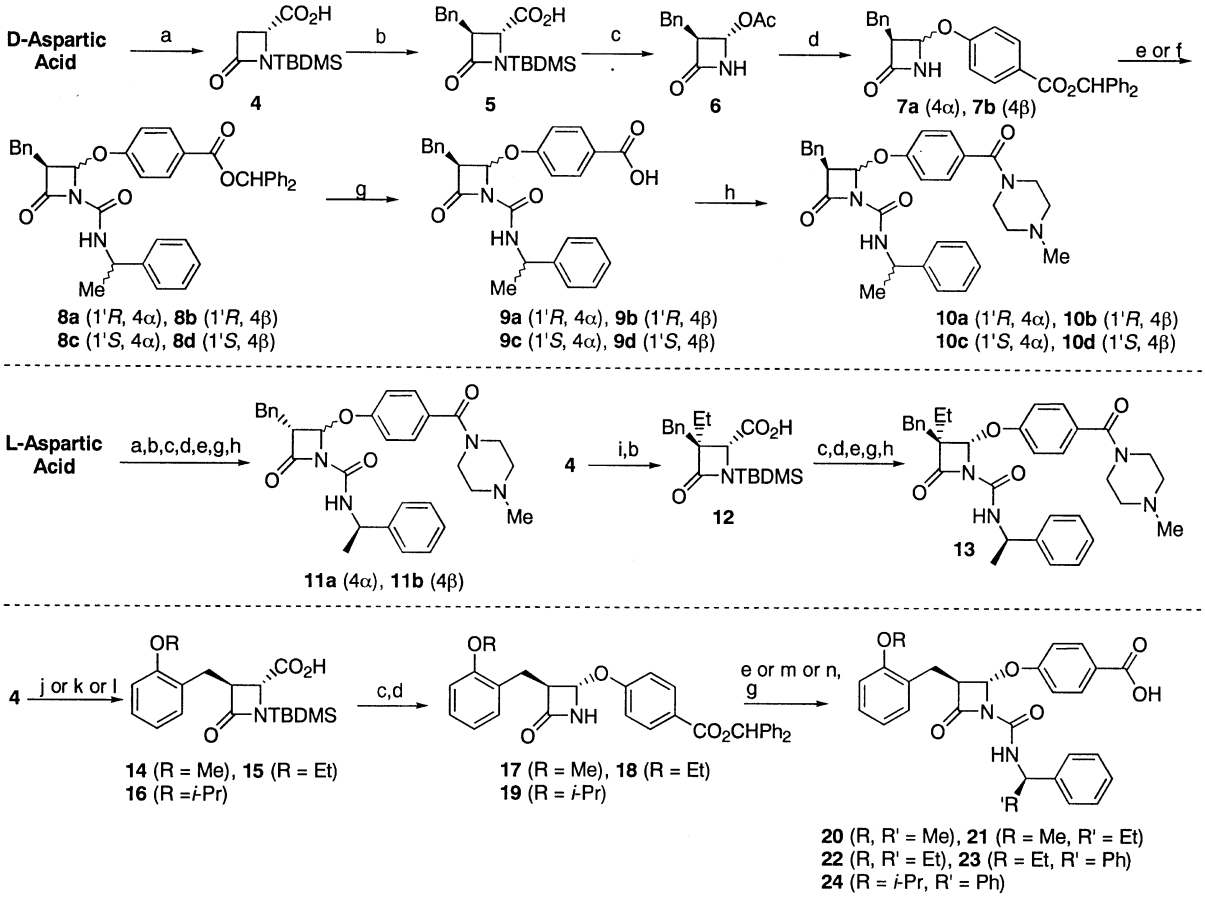


Scheme 1. Reagents and conditions: (a) see ref 12; (b) LDA (2 equiv), THF, BnBr; (c) (i) LTA, DMF, AcOH; (ii) TBAF, THF; (d) 4-$HOC_6H_4CO_2CHPh_2$, NaOH in H_2O–acetone; (e) (*R*)-1-phenylethyl isocyanate, TEA, DMAP, CH_2Cl_2; (f) (*S*)-1-phenylethyl isocyanate, TEA, DMAP, CH_2Cl_2; (g) TFA, anisole; (h) 1-methylpiperadine, WSCD, CH_2Cl_2; (i) LDA (2 equiv), THF, EtI; (j) LDA (2 equiv), THF, 2-$(MeO)C_6H_4CH_2Br$; (k) LDA (2 equiv), THF, 2-$(EtO)C_6H_4CH_2Br$; (l) LDA (2 equiv), THF, 2-(*i*-PrO)$C_6H_4CH_2Br$; (m) (*R*)-phenylpropyl isocyanate, TEA, DMAP, CH_2Cl_2; (n) diphenylmethyl isocyanate, TEA, DMAP, CH_2Cl_2.

prepared from 4-hydroxybenzoic acid and diphenyldiazomethane, afforded 3,4-*trans* isomer **7a** as the major product (74% yield) and 3,4-*cis* isomer **7b** as a minor product (17% yield). Each isomer (**7a** and **7b**) was individually acylated with (*R*)- or (*S*)-1-phenylethyl isocyanate in the presence of triethylamine (TEA) and 4-dimthylaminopyridine (DMAP) to afford the ureas **8a**–**d**. Conversion to 4-benzoic acids **9a**–**d** was achieved by trifluoroacetic acid (TFA) removal of the diphenylmethyl ester in anisole. 4-Amides **10a**–**d** were

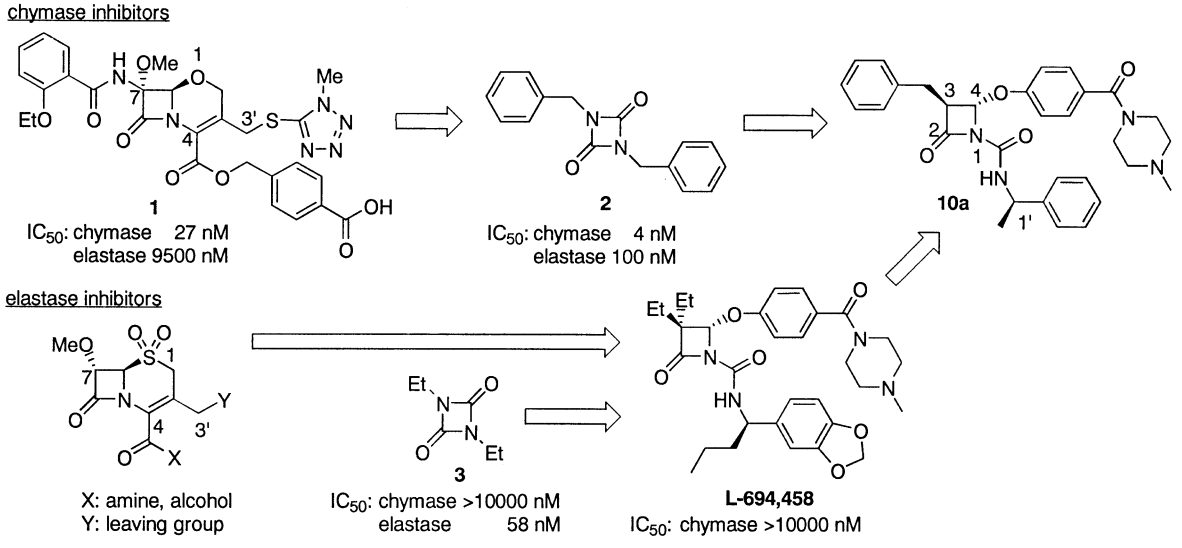


Figure 1. Design of 3-benzylazetidine-2-one-based human chymase inhibitor.

obtained by treatment with **9a**–**d** and 1-methylpiperadine in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSCD). Compounds **11a** and **11b** were prepared from L-aspartic acid by the above procedures. Alkylation of the dianion of compound **4** with ethyl iodide provided 3β-ethyl derivative and second alkylation with benzyl bromide selectively introduced a benzyl group *trans* to the carboxyl to afford compound **12**. 3,3-Disubstituted 2-azetidinone **13** was prepared from compound **12** by the above procedures. Alkylation of dianion of compound **4** with *ortho*-alkoxybenzyl bromide, prepared from the corresponding benzyl alcohol and hydrogen bromide, provided carboxylic acids **14**, **15** and **16**. Compounds **17**, **18** and **19** were prepared from compounds **14**, **15** and **16** respectively. Each isomer (**17**, **18** and **19**) was individually acylated with (*R*)-1-phenylethyl isocyanate or (*R*)-1-phenylpropyl isocyanate or diphenylmethyl isocyanate, prepared from the corresponding carboxylic acids by the Shioiri method,[13] in the presence of triethylamine and 4-dimthylaminopyridine to afford the ureas **20**–**24**.

Results and Discussion

We designed compound **10a** as an azetidin-2-one-based human chymase inhibitor based on the following considerations (Fig. 1).

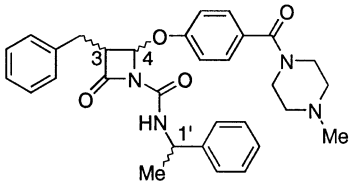
i. Human chymase and human elastase belong to the same family of serine proteases. With regard to β-lactam-based chymase or elastase inhibitors, substituents at the α-position of β-lactam carbonyl are situated in the S1 pocket of the enzymes.[9–11] 1,3-Diazetidine 2,4-dione **3**[9b] and 3,3-diethylazetidine-2-one **L-694,458**[11c] are elastase inhibitors, which do not possess inhibitory activities against human chymase.[9b,14] The *N*-ethyl of **3** and the 3-ethyl of **L-694,458**, substituents at the α-position of the β-lactam carbonyl group, determine the selectivity against human elastase. The 7β-phenyl ring of 1-oxaxephems such as compound **1** and *N*-benzyl substituent of 1,3-diazetidine 2,4-diones such as compound **2** are essential for inhibition against human chymase.[9] These results agree with the primary substrate specificities of human chymase (Phe, Thy)[9b,15] and human elastase (Val, Leu).[9b,16] Accordingly, the 3β-benzyl substituent is thought to be required for azetidine-2-one-based human chymase inhibitors.

ii. The structure–activity relationships at the 3′- and 4-positions of 1-oxacephem chymase inhibitors displayed a similar tendency compared to those of the cephalosporin elastase inhibitors.[9a,10] 4-Substituents of 1-oxacephems and cephalosporins occupy the prime site region (S′ region) of each enzyme and 3-substituents function as the leaving group and do not strictly interact with enzymes. This may suggest that the structure of the S′ region of human chymase resembles that of human leukocyte elastase. The 1-substituent of azetidine-2-one-based elastase inhibitors such as **L-694,458** occupies the S′ region of elastase and the 4-substituent acts as the leaving group and does not strictly interact with the enzyme.[11] Based on the above results, the substituent effects at the 1- and 4-positions of azetidine-2-one-based chymase inhibitors are thought to be similar to those of azetidine-2-one-based elastase inhibitors. We selected (*R*)-1-phenylethyl urea easily prepared from commercially available (*R*)-1-phenylethyl isocyanate and the same substituent as that of **L-694,458** as the 1- and 4-substituents of azetidine-2-one-based chymase inhibitors, respectively.

First, we examined the inhibitory activity of the designed compound **10a** (Table 1). As expected, compound **10a** possessed high potency against human chymase (IC_{50} 0.46 nM). Next, diastereomers of **10a** were evaluated. (3β, 4β)-Isomer **10b** displayed 3-fold increase compared to (3β, 4α)-isomer **10a**. (3α, 4α)-Isomer **11a** and (3α, 4β)-isomer **11b** were 80- and 2-fold less potent than (3β, 4α)-isomer **10a**, respectively. This suggests that 3β-isomers are more potent than 3α-isomers. (1′*S*)-Isomers **10c** and **10d** led to a greater loss of potency than the corresponding (1′*R*)-isomers **10a** and **10b**. Compound **10b** displayed the best IC_{50} value of 0.17 nM, but stereoselective synthesis of the 3,4-*cis* isomer such as **10b** is more difficult than that of the 3,4-*trans* isomer such as **10a** as shown in Scheme 1. The above results suggest the optimal compound to be (1′*R*, 3β, 4α)-isomer **10a**. However, compound **10a** was unstable in human plasma. We next investigated enhancement of the stability in human plasma by modifying the 1′-, 3- and 4-positions of compound **10a** (Table 2).

First, 3,3-disubstituent compound **13** was prepared and evaluated, because the Merck group reported that 3,3-disubstituents improved the hydrolytic stability of azetidin-2-one.[11b] As expected, the 3α-ethyl group of compound **13** dramatically enhanced the stability in human plasma, however, it markedly decreased the potency against human chymase. Next, 4-substituent was modified. 4-Carboxylic acid **9a** mildly improved the stability.

Table 1. Modifications at 1′-, 3-, 4-positions



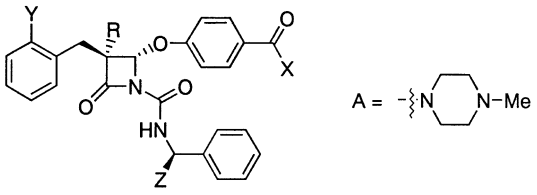
Compd	1′-position	3-position	4-position	IC_{50} (nM)	Stability $t_{1/2}$ (h)
10a	*R*	β	α	0.46	1.0
10b	*R*	β	β	0.17	0.9
10c	*S*	β	α	0.66	0.5
10d	*S*	β	β	13	2.1
11a	*R*	α	α	36	2.1
11b	*R*	α	β	0.80	<0.5

The 3-substituent was then modified with the 4-carboxylic acid. Introduction of an alkoxy group at the *ortho*-position of the 3-benzyl substituent was tried, because an *ortho*-alkoxy group on 7β-benzamide of 1-oxacephem-based human chymase inhibitors such as compound **1** (Scheme 1) increased the potency against human chymase and the stability in human plasma.[9a] As expected, 3-*ortho*-(methoxy)benzyl **20** showed enhancement of the stability. We next examined the substituent effect at the 1′-position. Replacement of the methyl group by an ethyl group at the 1′-position of **21** resulted in dramatic improvement of the stability. Introduction of an ethoxy group at the 3-position of **22** led to further enhancement of the stability. Finally, 1-diphenylmethyl urea **23**, which did not possess an asymmetric carbon atom at the 1-position, offered sufficient potency against human chymase (IC_{50} 3.1 nM) and stability in human plasma ($t_{1/2}$ 6 h). 3-*ortho*-(*iso*-Propoxy)benzyl **24** displayed remarkable improvement of the stability, but considerable reduction of the activity. Additional enzymatic work showed that compound **23** was a selective inhibitor, causing weak or no inhibition of several other serine proteases (Table 3).

Table 2 suggests that the α-branched substituent at the 1-benzylurea and the *ortho*-alkoky substituent on the 3-benzene ring affect the activity against human chymase and the stability in human plasma. Steric hindrance at the 1- and 3-positions may prevent a variety of nucleophiles in human plasma from attacking the β-lactam carbonyl group followed by cleavage of the β-lactam ring and result in enhancement of the stability in human plasma (Fig. 2). However, the steric hindrance also hinders nucleophilic substitution of the active serine hydroxyl group in human chymase followed by generation of an acyl-enzyme, which is necessary for inhibition of human chymase activity (Fig. 3). Accordingly, enhancement of the stability in human plasma is inversely proportional to the increase of the potency against human chymase (Table 2). This tendency was also observed for 1-oxacephem-based human chymase inhibitors.[9a]

A model for the inhibition by compound **23** of human chymase, using atomic coordinates from the published X-ray structure of the enzyme,[17] is shown in Figure 4.[18] In this model, the inhibitor **23** is bound to the active site of the enzyme with the hydroxy of Ser195 and the resulting hemiketal oxygen is in the oxyanion hole formed by the backbone amide NH of Ser195 and Gly193. The benzyl substituent at the 3-position is fully enclosed by the residues of the S1 pocket and capped with the side chain of Phe191. The phenoxy substituent at the 4-position occupies the S2 site with the carboxyl group extending into the solution. The urea substituent

Table 2. Modifications at 1′-, 3-, 4-positions



Compd	R	X	Y	Z	IC_{50} (nM)	Stability $t_{1/2}$ (h)
10a	H	A	H	Me	0.46	1.0
13	Et	A	H	Me	190	>24
9a	H	OH	H	Me	11	1.3
20	H	OH	OMe	Me	1.0	1.7
21	H	OH	OMe	Et	0.5	3.8
22	H	OH	OEt	Et	2.1	5
23	H	OH	OEt	Ph	3.1	6
24	H	OH	OPr-*i*	Ph	40	12

Table 3. Selectivity of **23** as an inhibitor of human chymase compared to other serine proteases

Enzyme	IC_{50} (nM)	Enzyme	IC_{50} (nM)
Chymase	3.1	Thrombin	>10,000
α-Chymotrypsin	15.3	Elastase	>10,000
CathepsinG	35.4	Plasmin	>10,000
Trypsin	26,600		

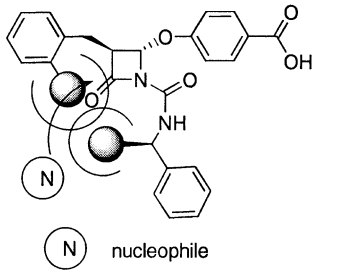


Figure 2. Schematic drawing of steric effects at 1- and 3-positions.

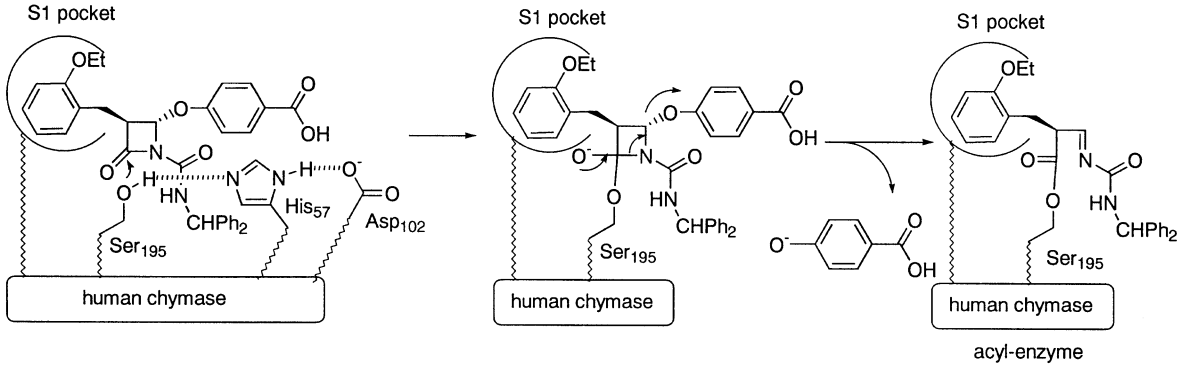


Figure 3. Proposed mechanism for inhibition of human chymase by compound **23**.

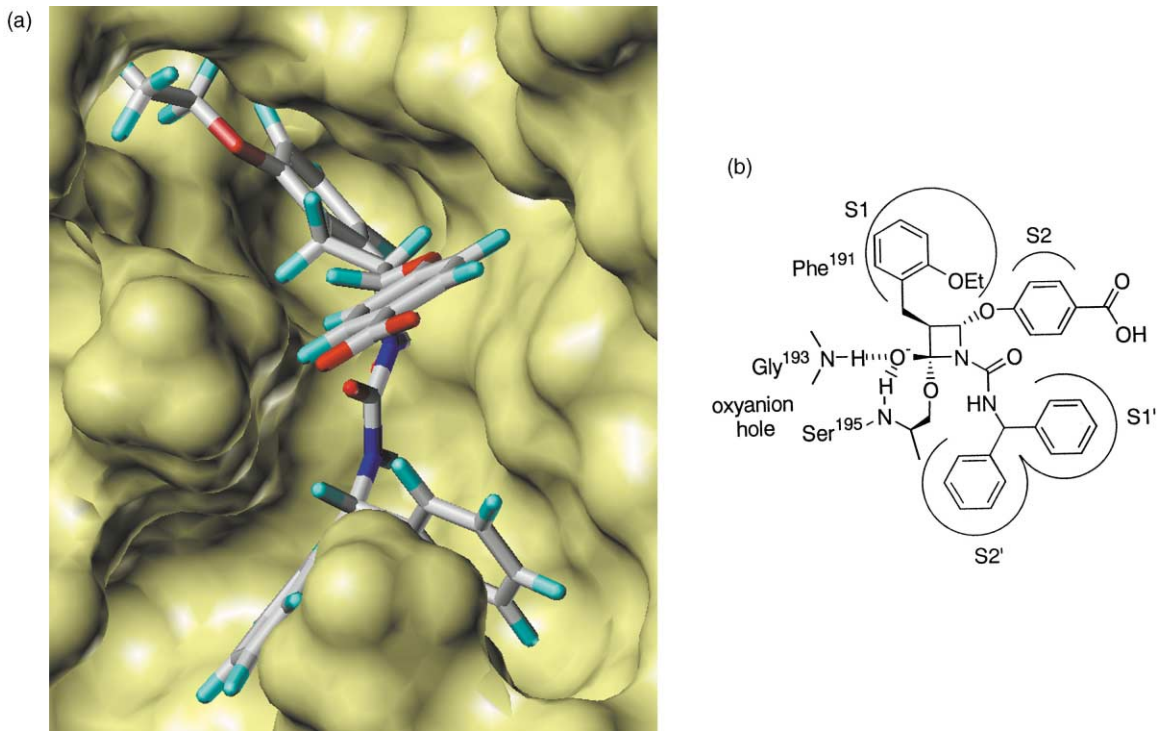


Figure 4. Docking model of inhibitor **23** into human chymase: (a) surface representation; (b) schematic drawing.

at the 1-position occupies the hydrophobic prime site region (S′) of the enzyme. The S′ region is made up of two regions (S1′ and S2′). The two pairs of benzene rings are projected into different hydrophobic regions (S1′ and S2′).

Conclusion

We have described here the design, synthesis and pharmacological evaluation of human chymase inhibition based on 3-benzylazetidine-2-one. The designed compound **10a** possessed high potency against human chymase. Modifications of compound **10a** led to compound **23** which displayed high activity against human chymase (IC_{50} 3.1 nM) and sufficient stability in human plasma ($t_{1/2}$ 6 h), and accordingly, is a promising candidate for in vivo testing.

Experimental

General

IR spectra were recorded in $CHCl_3$ or 99.5% EtOH on a JASCO FT/IR-700. ^1H NMR spectra were recorded on a Varian GEMINI 2000 at 300 MHz with tetramethylsilane as internal standard (δ scale) and in $CDCl_3$. *J* values are given in Hertz. Optical rotations were measured on a Perkin-Elmer polarimeter. HR-FAB/MS were recorded on a JEOL LMS-SX/SX 102A. Analytical thin layer chromatography (TLC) was carried out on Merck precoated TLC plates of silica gel 60 F_{254} and visualized with UV light or 10% H_2SO_4 containing 5% ammonium molybdate and 0.2% ceric sulfate. Flash chromatography was performed with Merck silica gel 60 (230–400 mesh). Reactions were carried out under a nitrogen atmosphere in anhydrous solvents (dried over molecular sievses type 4A). Extractions were routinely carried out twice with the given solvent and each extract was washed with a portion of water and/or sodium bicarbonate ($NaHCO_3$) solution followed by a portion of brine. The organic layers were then combined, dried over sodium sulfate (Na_2SO_4) and concentrated in vacuo on a rotary evaporator.

(3*S*,4*R*)-3-Benzyl-1-(*tert*-butyldimethylsilyl)-2-oxoazetidine-4-carboxylic acid (5). To a solution of (4*R*)-1-(*tert*-butyldimethylsilyl)-2-oxoazetidine-4-carboxylic acid (**4**)[12] (12.8 g, 56 mmol) in tetarahydrofuran (THF) (64 mL) at −55 °C was added 2.0 M lithium diisopropylamide in heptane/THF/ethylbenzene (58.8 mL, 0.12 mol) over 15 min. After 20 min, benzylbromide (14.7 mL, 0.12 mol) was added at −55 °C. The reaction mixture was kept between −40 and −15 °C for 1.5 h and then poured into 1 M $NaHSO_4$ aqueous solution. The layers were separated, and the aqueous layer was extracted with ethyl acetate (EtOAc). The organic layers were combined and extracted with six portions of 5% sodium bicarbonate solution. The aqueous layers were combined, adjusted to pH 2.0 and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, evaporated under reduced pressure to dryness and treated with hexane. The precipitate was collected by filtration, washed with hexane and dried at room temperature under atmospheric pressure to give 17.5 g (98%) of **5**. ^1H NMR ($CDCl_3$): δ 0.01 (3H, s), 0.21 (3H, s), 0.78 (9H, s), 3.03 (1H, dd, *J* = 14.5, 5.5 Hz), 3.15 (1H, dd, *J* = 14.5, 6.2 Hz), 3.65 (1H, ddd, *J* = 6.0, 5.7, 2.8 Hz), 3.78 (1H, d, *J* = 2.8 Hz), 6.36 (1H, br s), 7.20–7.40 (5H, m).

(3*S*,4*S*)-4-Acetoxy-3-benzylazetidin-2-one (6). To a solution of **5** (17.3 g, 54 mmol) in dimethylformamide (DMF; 50 mL) and acetic acid (HOAc, 10 mL) under N_2 was added lead tetraacetate (25.2 g, 54 mmol). The reaction mixture was heated at 50–55 °C for 40 min and then cooled to 25 °C. To the mixture was added 1.0 M *n*-tetrabutylammonium fluoride in THF (43 mL, 43 mmol) at 25 °C. The reaction mixture was stirred at room temperature for 1 h before it was poured into dilute HCl and extracted with EtOAc. The EtOAc layer was washed with sodium bicarbonate solution, and brine, dried over Na_2SO_4, and evaporated. The residue was purified by elution (EtOAc/toluene) through a silica gel column and gave 6.24 g (53%) of **6** as an oil, 0.65 g (6%) of (3*S*,4*R*)-4-acetoxy-3-benzylazetidin-2-one and 1.07 g (9%) of a mixture of C-4 isomers. ^{1}H NMR ($CDCl_3$): δ 2.08 (SH, s), 3.02 (1H, dd, J = 14.8, 8.0 Hz), 3.14 (1H, dd, J = 14.7, 6.0 Hz), 3.51 (1H, ddd, J = 7.5, 6.2, 1.3 Hz), 5.51 (1H, d, J = 1.1 Hz), 6.42 (1H, br s), 7.18–7.40 (5H, m).

(3*S*,4*S*)- and (3*S*,4*R*)-3-Benzyl-4-[4-(1,1-diphenylmethoxycarbonyl)phenoxy]azetidin-2-one (7a,b). To a solution of 4-hydroxybenzoic acid (27.6 g, 0.20 mol) in methylene chloride (221 mL) and methanol (69 mL) was added a solution of diphenyldiazomethane (58.3 g, 0.30 mol) in methylene chloride (200 mL) in an ice bath. The reaction mixture was stirred at room temperature for 16 h and concentrated. The residue was crystallized from *n*-hexane/EtOAc to give 50.0 g (82%) of diphenylmethyl 4-hydroxybenzoate. To a solution of diphenylmethyl 4-hydroxybenzoate (2.77 g, 9.1 mmol) in acetone (11 mL) and 1 N sodium hydroxide (8.4 mL, 8.4 mmol) was slowly added a solution of **6** (1.54 g, 7.0 mmol) in acetone (8 mL) at 5–10 °C. The mixture was stirred at 10–15 °C for 3 h and then diluted with water and extracted with two portions of EtOAc. The EtOAc layers were each washed with brine and then combined, dried over Na_2SO_4 and evaporated to give 4.22 g of oil. The residue was purified by elution (EtOAc/toluene) through a silica gel column and gave 2.38 g (74%) of **7a** as a solid and 0.56 g (17%) of **7b** as a solid. **7a**, ^{1}H NMR ($CDCl_3$): δ 3.07 (1H, dd, J = 14.8, 8.4 Hz), 3.23 (1H, dd, J = 14.9, 5.7 Hz), 3.63 (1H, ddd, J = 8.3, 5.2, 1.2 Hz), 5.40 (1H, d, J = 1.2 Hz), 6.55 (1H, br s), 6.87 (2H, d, J = 8.6 Hz), 7.08 (1H, s), 7.13–7.48 (15H, m), 8.02 (2H, d, J = 8.5 Hz). **7b**, ^{1}H NMR ($CDCl_3$): δ 3.20 (2H, d, J = 7.6 Hz), 3.71–3,85 (1H, m), 5.75 (1H, d, J = 4.2 Hz), 6.70 (1H, br s), 6.86 (2H, J = 8.9 Hz), 7.09 (1H, s), 7.10–7.49 (15H, m), 8.04 (2H, d, J = 8.9 Hz).

(3*S*,4*S*)-3-Benzyl-4-[4-(1,1-diphenylmethoxycarbonyl)phenoxy]-1-[(*R*)-1-(phenylethyl)aminocarbonyl]azetidin-2-one (8a). To a solution of **7a** (1.85 g, 4.0 mmol) in methylene chloride (18 mL) were added (*R*)-1-phenylethyl isocyanate (1.13 mL, 8.0 mmol), triethylamine (1.12 mL, 8.0 mmol) and 4-(dimethylamino)pyridine (DMAP) (25 mg, catalyst). The reaction mixture was stirred at room temperature for 16 h and then poured into dilute HCl and extracted twice with EtOAc. The EtOAc layers were washed with brine, combined, dried over Na_2SO_4, and evaporated. The residue was purified by elution (EtOAc/toluene) through a silica gel column and gave 2.02 g (83%) of **8a** as a solid. ^{1}H NMR ($CDCl_3$): δ 1.53 (3H, d, J = 6.9 Hz), 3.02 (1H, dd, J = 14.7, 9.1 Hz), 3.22 (1H, dd, J = 14.9, 6.2 Hz), 3.65 (1H, ddd, J = 8.9, 6.0, 1.2 Hz), 5.01 (1H, quintet, J = 7.4 Hz), 5.72 (1H, d, J = 1.3 Hz), 6.58 (1H, d, J = 8.0 Hz), 6.94 (2H, d, J = 8.8 Hz), 7.07 (1H, s), 7.12–7.48 (20H, m), 7.99 (2H, d, J = 8.9 Hz).

Preparation of 8b–d. Using essentially the same procedures as for the preparation of **8a**, the following compounds were prepared from **7a** or **7b**.

(3*S*,4*R*)-3-Benzyl-4-[4-(1,1-diphenylmethoxycarbonyl)phenoxy]-1-[(*R*)-1-(phenylethyl)aminocarbonyl]azetidin-2-one (8b). This was derived from **7b** and (*R*)-1-phenylethyl isocyanate; ^{1}H NMR ($CDCl_3$): δ 1.54 (3H, d, J = 6.4 Hz), 3.19 (1H, dd, J = 14.8, 8.6 Hz), 3.23 (1H, dd, J = 14.9, 6.1 Hz), 3.87 (1H, ddd, J = 8.4, 7.0, 4.5 Hz), 5.04 (1H, quintet, J = 7.4 Hz), 6.13 (1H, d, J = 4.6 Hz), 6.85 (1H, d, J = 8.3 Hz), 7.08 (1H, s), 7.12–7.48 (22H, m), 8.06 (2H, d, J = 9.0 Hz).

(3*S*,4*S*)-3-Benzyl-4-[4-(1,1-diphenylmethoxycarbonyl)phenoxy]-1-[(*S*)-1-(phenylethyl)aminocarbonyl]azetidin-2-one (8c). This was derived from **7a** and (*S*)-1-phenylethyl isocyanate; ^{1}H NMR ($CDCl_3$): δ 1.53 (3H, d, J = 7.6 Hz), 3.05 (1H, dd, J = 14.6, 9.0 Hz), 3.25 (1H, dd, J = 14.9, 6.2 Hz), 3.63 (1H, ddd, J = 7.5, 6.9, 1.3 Hz), 5.02 (1H, quintet, J = 7.4 Hz), 5.77 (1H, d, J = 1.3 Hz), 6.79 (1H, d, J = 8.0 Hz), 6.92 (2H, d, J = 8.9 Hz), 7.07 (1H, s), 7.12–7.48 (20H, m), 7.98 (2H, d, J = 8.9 Hz).

(3*S*,4*R*)-3-Benzyl-4-[4-(1,1-diphenylmethoxycarbonyl)phenoxy]-1-[(*S*)-1-(phenylethyl)aminocarbonyl]azetidin-2-one (8d). This was derived from **7b** and (*S*)-1-phenylethyl isocyanate; ^{1}H NMR ($CDCl_3$): δ 1.53 (3H, d, J = 7.0 Hz), 3.22 (1H, dd, J = 14.8, 8.4 Hz), 3.25 (1H, dd, J = 14.8, 7.0 Hz), 3.84 (1H, ddd, J = 8.6, 7.1, 4.7 Hz), 5.03 (1H, quintet, J = 7.4 Hz), 6.09 (1H, d, J = 4.7 Hz), 6.88 (1H, d, J = 8.2 Hz), 7.08 (1H, s), 7.12–7.48 (22H, m), 8.07 (2H, d, J = 9.0 Hz).

(3*S*,4*S*)-3-Benzyl-4-(4-carboxy)phenoxy-1-[(*R*)-1-(phenylethyl)aminocarbonyl]azetidin-2-one (9a). To a solution of **8a** (1.88 g, 3.1 mmol) in anisole (9.4 mL) was added precooled trifluoroacetic acid (TFA; 2.4 mL) at 5 °C. After 1.5 h at 5 °C, the reaction mixture was poured into sodium bicarbonate solution and extracted with two portions of EtOAc. The EtOAc layers were each washed with brine, combined, dried over Na_2SO_4 and evaporated to give 10 g of oil. The residue was crystallized from hexane to give 1.23 g (90%) of **9a**, $[\alpha]_D$ +89.4° (*c* 0.51, $CHCl_3$). IR ν_{max} cm^{-1}: 1779, 1708, 1691; ^{1}H NMR ($CDCl_3$): δ 1.54 (3H, d, J = 6.9 Hz), 1.80 (1H, br s), 3.03 (1H, dd, J = 14.6, 9.5 Hz), 3.24 (1H, dd, J = 14.4, 6.0 Hz), 3.66 (1H, ddd, J = 9.2, 6.0, 1.2 Hz), 5.02 (1H, quintet, J = 7.4 Hz), 5.74 (1H, d, J = 1.2 Hz), 6.83 (1H, d, J = 8.4 Hz), 6.90 (2H, d, J = 9.3 Hz), 7.18–7.40 (10H, m), 7.92 (2H, d, J = 8.7 Hz); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{26}H_{24}O_5N_2+H]^+$ 445.1763, found 445.1768.

Preparation of 9b–d. Using essentially the same procedures as for the preparation of **9a**, the following compounds were prepared from **8b**, **8c** or **8d**.

(3*S*,4*R*)-3-Benzyl-4-(4-carboxy)phenoxy-1-[(*R*)-1-(phenylethyl)aminocarbonyl]azetidin - 2 - one (9b). This was derived from **8b**; ^{1}H NMR ($CDCl_3$): δ 1.55 (3H, d, J = 6.9 Hz), 2.20 (1H, br s), 3.16 (1H, dd, J = 14.9, 8.9 Hz), 3.25 (1H, dd, J = 14.6, 7.1 Hz), 3.89 (1H, ddd, J = 10.2, 7.2, 4.5 Hz), 5.06 (1H, quintet, J = 7.2 Hz), 6.17 (1H, d, J = 4.5 Hz), 6.89 (1H, d, J = 7.8 Hz), 7.11 (2H, d, J = 8.7 Hz), 7.16–7.45 (10H, m), 7.96 (2H, d, J = 8.7 Hz).

(3*S*,4*S*)-3-Benzyl-4-(4-carboxy)phenoxy-1-[(*S*)-1-(phenylethyl)aminocarbonyl]azetidin - 2 - one (9c). This was derived from **8c**; ^{1}H NMR ($CDCl_3$): δ 1.53 (3H, d, J = 6.9 Hz), 1.85 (1H, br s), 3.06 (1H, dd, J = 14.6, 9.5 Hz), 3.27 (1H, dd, J = 14.4, 6.0 Hz), 3.66 (1H, ddd, J = 9.2, 6.0, 1.2 Hz), 5.02 (1H, quintet, J = 7.4 Hz), 5.76 (1H, d, J = 1.2 Hz), 6.83 (1H, d, J = 8.4 Hz), 6.90 (2H, d, J = 9.0 Hz), 7.18–7.40 (10H, m), 7.92 (2H, d, J = 9.0 Hz).

(3*S*,4*R*)-3-Benzyl-4-(4-carboxy)phenoxy]-1-[(*S*)-1-(phenylethyl)aminocarbonyl]azetidin - 2 - one (9d). This was derived from **8d**; ^{1}H NMR ($CDCl_3$): δ 1.55 (3H, d, J = 7.2 Hz), 1.80 (1H, br s), 3.19 (1H, dd, J = 14.9, 8.6 Hz), 3.27 (1H, dd, J = 14.7, 7.2 Hz), 3.86 (1H, ddd, J = 8.6, 7.1, 4.5 Hz), 5.04 (1H, quintet, J = 7.2 Hz), 6.12 (1H, d, J = 4.8 Hz), 6.91 (1H, d, J = 8.4 Hz), 7.16 (2H, d, J = 9.0 Hz), 7.18–7.38 (10H, m), 7.99 (2H, d, J = 9.0 Hz).

(3*S*,4*S*)-3-Benzyl-4-(4-methylpiperazincarbonyl)phenoxy-1-[(*R*)-1-(phenylethyl)aminocarbonyl]azetidin-2-one (10a). To a solution of **9a** (120 mg, 0.27 mmol) in methylene chloride (1.2 mL) were added 1-methylpiperazine (36 μL, 0.32 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (62 mg, 32 mmol) at 5 °C. The reaction mixture was stirred at room temperature for 5 h and then poured into sodium bicarbonate solution and extracted with two portions of EtOAc. The EtOAc layers were each washed with brine, combined, dried over Na_2SO_4 and evaporated to give 143 mg of oil. The residue was crystallized from hexane to give 130 mg (92%) of **10a** as an amorphous solid, $[\alpha]_D$ +43.2° (*c* 0.77, $CHCl_3$); IR ν_{max} cm^{-1}: 1780, 1710, 1620; ^{1}H NMR ($CDCl_3$): δ 1.54 (3H, d, J = 6.9 Hz), 2.32 (3H, s), 2.30–2.54 (4H, br), 3.04 (1H, dd, J = 14.7, 9.3 Hz), 3.25 (1H, dd, J = 14.7, 6.0 Hz), 3.32–3.90 (4H, br), 3.64 (1H, ddd, J = 9.2, 6.0, 1.2 Hz), 5.03 (1H, quintet, J = 7.3 Hz), 5.70 (1H, d, J = 1.2 Hz), 6.83 (1H, d, J = 7.5 Hz), 6.88 (2H, d, J = 8.4 Hz), 7.19–7.40 (12H, m); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{31}H_{34}O_4N_4+H]^+$ 527.2658, found 527.2662.

Preparation of 10b–d. Using essentially the same procedures as for the preparation of **10a**, the following compounds were prepared from **9b**, **9c** or **9d**.

(3*S*,4*R*)-3-Benzyl-4-(4-methylpiperazincarbonyl)phenoxy-1 - [(*R*) - 1 - (phenylethyl)aminocarbonyl]azetidin - 2 - one (10b). This was derived from **9b**; ^{1}H NMR ($CDCl_3$): δ 1.54 (3H, d, J = 6.9 Hz), 2.33–2.56 (4H, br), 2.34 (3H, s), 3.21 (1H, dd, J = 15.3, 8.4 Hz), 3.24 (1H, dd, J = 14.7, 6.9 Hz), 3.32–3.90 (4H, br), 3.86 (1H, ddd, J = 8.6, 6.9, 4.6 Hz), 5.05 (1H, quintet, J = 7.4 Hz), 6.07 (1H, d, J = 4.8 Hz), 6.89 (1H, d, J = 7.5 Hz), 7.14 (2H, d, J = 8.4 Hz), 7.18–7.38 (12H, m); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{31}H_{34}O_4N_4+H]^+$ 527.2658, found 527.2659.

(3*S*,4*S*)-3-Benzyl-4-(4-methylpiperazincarbonyl)phenoxy-1-[(*S*)-1-(phenylethyl)aminocarbonyl]azetidin-2-one (10c). This was derived from **9c**; ^{1}H NMR ($CDCl_3$): δ 1.53 (3H, d, J = 6.6 Hz), 2.25–2.55 (4H, br), 2.32 (3H, s), 3.03 (1H, dd, J = 14.9, 9.5 Hz), 3.10–3.90 (4H, br), 3.24 (1H, dd, J = 14.7, 5.7 Hz), 3.63 (1H, ddd, J = 9.2, 6.2, 1.2 Hz), 5.02 (1H, quintet, J = 7.2 Hz), 5.70 (1H, d, J = 1.2 Hz), 6.76 (1H, d, J = 8.1 Hz), 6.87 (2H, d, J = 8.4 Hz), 7.15–7.38 (12H, m); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{31}H_{34}O_4N_4+H]^+$ 527.2658, found 527.2661.

(3*S*,4*R*)-3-Benzyl-4-(4-methylpiperazincarbonyl)phenoxy] - 1 - [(*S*) - 1 - (phenylethyl)aminocarbonyl]azetidin - 2 - one (10d). This was derived from **9d**; ^{1}H NMR ($CDCl_3$): δ 1.54 (3H, d, J = 6.9 Hz), 2.33 (3H, s), 2.33–2.53 (4H, br), 3.23 (1H, dd, J = 14.7, 8.4 Hz), 3.27 (1H, dd, J = 14.9, 7.1 Hz), 3.40–3.85 (4H, br), 3.84 (1H, ddd, J = 8.6, 7.4, 4.6 Hz), 5.03 (1H, quintet, J = 7.4 Hz), 6.02 (1H, d, J = 4.5 Hz), 6.92 (1H, d, J = 8.4 Hz), 7.16 (2H, d, J = 8.7 Hz), 7.19–7.38 (12H, m); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{31}H_{34}O_4N_4+H]^+$ 527.2658, found 527.2669.

Preparation of 11a,b. Using essentially the same procedures as for the preparation of **10a,b**, the following compounds were prepared from L-aspartic acid.

(3*R*,4*S*)-3-Benzyl-4-(4-methylpiperazincarbonyl)phenoxy-1 - [(*R*) - 1 - (phenylethyl)aminocarbonyl]azetidin - 2 - one (11a). ^{1}H NMR ($CDCl_3$) δ 1.54 (3H, d, J = 6.9 Hz), 2.33–2.55 (4H, br), 2.34 (3H, s), 3.23 (1H, dd, J = 14.7, 8.7 Hz), 3.26 (1H, dd, J = 15.0, 7.5 Hz), 3.36–3.90 (4H, br), 3.84 (1H, ddd, J = 8.7, 7.2, 4.6 Hz), 5.04 (1H, quintet, J = 7.4 Hz), 6.02 (1H, d, J = 4.5 Hz), 6.92 (1H, d, J = 8.4 Hz), 7.16 (2H, d, J = 9.0 Hz), 7.19–7.38 (12H, m); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{31}H_{34}O_4N_4+H]^+$ 527.2658, found 527.2660.

(3*R*,4*R*)-3-Benzyl-4-(4-methylpiperazincarbonyl)phenoxy - 1 - [(*R*) - 1 - (phenylethyl)aminocarbonyl]azetidin - 2 - one (11b). ^{1}H NMR ($CDCl_3$) δ 1.54 (3H, d, J = 6.9 Hz), 2.30–2.54 (4H, br), 2.33 (3H, s), 3.04 (1H, dd, J = 14.7, 9.3 Hz), 3.25 (1H, dd, J = 14.7, 6.0 Hz), 3.32–3.90 (4H, br), 3.64 (1H, ddd, J = 9.2, 6.0, 1.2 Hz), 5.03 (1H, quintet, J = 7.3 Hz), 5.70 (1H, d, J = 1.2 Hz), 6.83 (1H, d, J = 7.5 Hz), 6.88 (2H, d, J = 8.4 Hz), 7.19–7.40 (12H, m); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{31}H_{34}O_4N_4+H]^+$ 527.2658, found 527.2660.

(3*S*,4*R*)-3-Benzyl-3-ethyl-1-(*tert*-butyldimethylsilyl)-2-oxoazetidine-4-carboxylic acid (12). To a solution of diisopropylamine (9.8 mL, 70 mmol) in THF (53 mL) at −35 °C was added 1.6 M *n*-butyllithium in hexane (42.6 mL, 68 mmol). After 15 min, the solution was cooled to −55 °C and a solution of **4** (7.11 g, 31 mmol) in tetarahydrofuran (THF) (31 mL) was added over 5 min. The solution was warmed to −25 °C for 30 min before ethyl iodide (6.20 mL, 78 mmol) was added. The reaction mixture was kept between −25 and 0 °C for 1.5 h and then diluted with ether and poured into a

mixture of ice and 1 N HCl. The layers were separated, and the aqueous layer was extracted with ether. The ether layers were each washed with brine, combined, dried over Na_2SO_4 and evaporated. The residue was crystallized from hexane to give 7.00 g (84%) of (3*S*,4*R*)-1-(*tert*-butyldimethylsilyl)-3-ethyl-2-oxoazetidine-4-carboxylic acid. ^{1}H NMR ($CDCl_3$): δ 0.14 (3H, s), 0.32 (3H, s), 0.97 (9H, s), 1.06 (3H, t, J = 7.4 Hz), 1.62–1.99 (2H, m), 3.29 (1H, ddd, J = 8.3, 5.7, 2.6 Hz), 3.79 (1H, d, J = 2.7 Hz), 6.90 (1H, br s). To a solution of diisopropylamine (9.8 mL, 70 mmol) in THF (53 mL) at −35 °C was added 1.6 M *n*-butyllithium in hexane (42.6 mL, 68 mmol). After 15 min, the solution was cooled to −55 °C and a solution of (3*S*,4*R*)-1-(*tert*-butyldimethylsilyl)-3-ethyl-2-oxoazetidine-4-carboxylic acid (7.00 g, 26 mmol) in THF (31 mL) was added over 5 min. The solution was warmed to −25 °C for 30 min before benzyl bromide (8.13 mL, 68 mmol) was added. The reaction mixture was kept between −25 and 0 °C for 1.5 h and then poured into a mixture of ice and 1N HCl and extracted with EtOAc. The organic layer was extracted with eleven portions of 5% sodium bicarbonate solution. The aqueous layer was adjusted to pH 1.0 and extracted with two portions of EtOAc. The organic layers were each washed with brine, combined, dried over Na_2SO_4 and evaporated to give 8.74 g (79%) of **12**. ^{1}H NMR ($CDCl_3$): δ −0.10 (3H, s), 0.26 (3H, s), 0.92 (9H, s), 1.29 (3H, t, J = 7.5 Hz), 1.70–2.10 (2H. m), 2.96 (1H, d, J = 14.1 Hz), 3.40 (1H, d, J = 14.0 Hz), 4.11 (1H, s), 6.30 (1H, br s), 7.28–7.50 (5H, m).

(3*S*,4*S*)-3-Benzyl-3-ethyl-4-(4-methylpiperazincarbonyl)-phenoxy-1-[(*R*)-1-(phenylethyl)aminocarbonyl]azetidin-2-one (13). Using essentially the same procedures as for the preparation of **10a**, compound **13** was prepared from **12**. ^{1}H NMR ($CDCl_3$): δ 1.19 (3H, t, J = 7.5 Hz), 1.50 (3H, d, J = 6.6 Hz), 1.84 (1H, quintet, J = 7.2 Hz), 2.05 (1H, quintet, J = 7.3 Hz), 2.28–2.53 (4H, br), 2.33 (3H, s), 2.95 (1H, d, J = 14.1 Hz), 3.15 (1H, d, J = 14.1 Hz), 3.30–3.90 (4H, m), 4.94 (1H, quintet, J = 7.5 Hz), 5.62 (1H, s), 6.82 (1H, d, J = 8.1 Hz), 7.03 (2H, d, 8.4 Hz), 7.11–7.39 (12H, m); HRMS (FAB, $[M+H]^+$) calcd for $[C_{33}H_{38}O_4N_4+H]^+$ 555.2971, found 555.2967.

(3*S*,4*R*)-1-(*tert*-Butyldimethylsilyl)-3-(2-methoxybenzyl)-2-oxoazetidine-4-carboxylic acid (14). The mixture of 2-methoxybenzyl alcohol (25.4 g, 0.18 mol) and 48% hydrobromic acid (64.2 mL, 0.57 mol) was vigorously stirred at room temperature for 2.5 h. The layers were separated and the organic layer was dried over $CaCl_2$ to give 32.6 g (90%) of 2-methoxybenzyl bromide. To a solution of diisopropylamine (15.8 mL, 0.11 mol) in THF (85 mL) at −35 °C was added 1.54 M *n*-butyllithium in hexane (71.4 mL, 0.11 mol). After 15 min, the solution was cooled to −55 °C and a solution of **4** (11.5 g, 0.050 mol) in tetarahydrofuran (THF) (50 mL) was added over 15 min. The solution was warmed to −25 °C for 30 min before 2-methoxybenzyl bromide (25.1 g, 0.12 mol) was added. The reaction mixture was kept between −25 and 0 °C for 1.5 h and then poured into a mixture of ice and 1 N HCl and extracted with EtOAc. The organic layer was extracted with six portions of 7% sodium bicarbonate solution. The aqueous layer was adjusted to pH 2.0 and extracted with two portions of EtOAc. The organic layers were each washed with brine, combined, dried over Na_2SO_4 and evaporated to give 17.5 (95%) g of **14** as an oil. ^{1}H NMR ($CDCl_3$): δ 0.03 (3H, s), 0.24 (3H, s), 0.83 (9H, s), 2.97 (1H, dd, J = 14.2, 7.7 Hz), 3.22 (1H, dd, J = 14.2, 5.3 Hz), 3.62 (1H, ddd, J = 7.8, 5.3, 2.4 Hz), 3.82 (3H, s), 3.83 (1H, d, J = 2.7 Hz), 5.60 (1H, br s), 6.80–6.95 (2H, m), 7.15–7.25 (2H, m).

(3*S*,4*R*)-1-(*tert*-Butyldimethylsilyl)-3-(2-ethoxybenzyl)-2-oxoazetidine-4-carboxylic acid (15). Using essentially the same procedures as for the preparation of **14**, compound **15** was prepared from 2-ethoxybenzyl alcohol, hydrobromic acid and **4**. ^{1}H NMR ($CDCl_3$): δ 0.01 (3H, s), 0.23 (3H, s), 0.81 (9H, s), 1.42 (3H, t, J = 7.1 Hz), 2.96 (1H, dd, J = 14.1, 7.2 Hz), 3.25 (1H, dd, J = 14.3, 5.3 Hz), 3.63 (1H, ddd, J = 7.7, 5.4, 3.0 Hz), 3.88 (1H, d, J = 2.7 Hz), 4.04 (2H, q, J = 7.1 Hz), 6.83 (1H, d, J = 8.4 Hz), 6.87 (1H, td, J = 7.5, 1.2 Hz), 7.18 (1H, td, J = 7.2, 1.5 Hz), 7.19 (1H, d, J = 7.2 Hz).

(3*S*,4*R*)-1-(*tert*-Butyldimethylsilyl)-2-oxo-3-(2-*iso*-propoxybenzyl)azetidine-4-carboxylic acid (16). Using essentially the same procedures as for the preparation of **14**, compound **16** was prepared from 2-*iso*-propoxybenzyl alcohol, hydrobromic acid and **4**. ^{1}H NMR ($CDCl_3$): δ 0.01 (3H, s), 0.23 (3H, s), 0.81 (9H, s), 1.35 (6H, d, J = 6.0 Hz), 2.92 (1H, dd, J = 14.0, 7.4 Hz), 3.26 (1H, dd, J = 14.3, 5.4 Hz), 3.63 (1H, ddd, J = 7.7, 5.4, 3.0 Hz), 3.91 (1H, d, J = 2.7 Hz), 4.58 (1H, quintet, J = 6.0 Hz), 6.85 (1H, d, J = 8.4 Hz), 6.86 (1H, td, J = 6.8, 1.5 Hz), 7.18 (1H, td, J = 7.6, 1.5 Hz), 7.20 (1H, d, J = 7.6 Hz).

Preparation of 17, 18 and 19. Using essentially the same procedures as for the preparation of **7a**, the following compounds were prepared from **14**, **15** or **16**.

(3*S*,4*S*)-4-[4-(1,1-Diphenylmethoxycarbonyl)phenoxy]-3-(2-methoxybenzyl)azetidin-2-one (17). This was derived from **14**; ^{1}H NMR ($CDCl_3$): δ 3.00 (1H, dd, J = 14.4, 8.8 Hz), 3.28 (1H, dd, J = 14.4, 5.2 Hz), 3.59 (1H, ddd, J = 8.8, 5.3, 1.0 Hz), 3.76 (3H, s), 5.46 (1H, d, J = 1.0 Hz), 6.52 (1H, s), 6.76 (2H, d, J = 8.9 Hz), 6.85 (1H, dd, J = 8.4, 1.2 Hz), 6.92 (1H, td, J = 7.8, 1.2 Hz), 7.08 (1H, s), 7.12–7.48 (12H, m), 8.01 (2H, d, J = 8.9 Hz).

(3*S*,4*S*)-4-[4-(1,1-Diphenylmethoxycarbonyl)phenoxy]-3-(2-ethoxybenzyl)azetidin-2-one (18). This was derived from **15**; ^{1}H NMR ($CDCl_3$): δ 1.35 (3H, t, J = 6.9 Hz), 3.01 (1H, dd, J = 14.6, 8.9 Hz), 3.30 (1H, dd, J = 14.4, 5.4 Hz), 3.62 (1H, ddd, J = 8.9, 5.3, 1.0 Hz), 4.00 (2H, q, J = 6.9 Hz), 5.50 (1H, d, J = 0.9 Hz), 6.36 (1H, s), 6.74 (2H, d, J = 8.7 Hz), 6.84 (1H, dd, J = 8.4, 1.2 Hz), 6.91 (1H, td, J = 7.8, 1.2 Hz), 7.08 (1H, s), 7.19–7.45 (12H, m), 8.00 (2H, d, J = 9.0 Hz).

(3*S*,4*S*)-4-[4-(1,1-Diphenylmethoxycarbonyl)phenoxy]-2-oxo-3-(2-*iso*-propoxybenzyl)azetidin (19). This was derived from **16**; ^{1}H NMR ($CDCl_3$): δ 1.23 (3H, d,

$J=6.0$ Hz), 3.01 (1H, dd, $J=14.6$, 8.9 Hz), 3.30 (1H, dd, $J=14.4$, 5.4 Hz), 3.62 (1H, ddd, $J=8.9$, 5.3, 1.0 Hz), 4.52 (1H, quintet, $J=6.0$ Hz), 5.50 (1H, d, $J=0.9$ Hz), 6.36 (1H, s), 6.74 (2H, d, $J=8.7$ Hz), 6.84 (1H, dd, $J=8.4$, 1.2 Hz), 6.91 (1H, td, $J=7.8$, 1.2 Hz), 7.08 (1H, s), 7.19–7.45 (12H, m), 8.00 (2H, d, $J=9.0$ Hz).

(3*S*,4*S*)-4-(4-Carboxy)phenoxy-3-(2-methoxybenzyl)-1-[(*R*)-1-(phenylethyl)aminocarbonyl]azetidin-2-one (20). Using essentially the same procedures as for the preparation of **9a**, the following compound was prepared from **17**. White amorphous solid; $[\alpha]_D$ +102.3° (*c* 0.51, $CHCl_3$). IR ν_{max} cm^{-1}: 1778, 1708, 1691; ^1H NMR ($CDCl_3$): δ 1.54 (3H, d, $J=7.2$ Hz), 1.80 (1H, br s), 3.00 (1H, dd, $J=14.3$, 8.7 Hz), 3.24 (1H, dd, $J=14.4$, 5.4 Hz), 3.64 (1H, ddd, $J=8.7$, 5.4, 1.2 Hz), 3.68 (3H, s), 5.03 (1H, quintet, $J=7.4$ Hz), 5.81 (1H, d, $J=1.2$ Hz), 6.83 (1H, d, $J=8.1$ Hz), 6.85 (1H, t, $J=8.3$ Hz), 6.92 (2H, d, $J=8.7$ Hz), 7.17 (1H, dd, $J=7.5$, 1.5 Hz), 7.23–7.40 (7H, m), 7.92 (2H, d, $J=9.0$ Hz); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{27}H_{26}O_6N_2+H]^+$ 475.1869, found 475.1870.

(3*S*,4*S*)-4-(4-Carboxy)phenoxy-3-(2-methoxybenzyl)-1-[(*R*)-1-(phenylpropyl)aminocarbonyl]azetidin-2-one (21). To a solution of (*R*)-phenylbutyric acid (616 mg, 3.8 mmol) in methylene chloride (6 mL) were added triethylamine (523 μL, 3.8 mmol) and diphenylphosphoryl azide (808 μL, 3.7 mmol) and then the reaction mixture was stirred at room temperature for 2.5 h. To the reaction mixture were added a solution of **17** (740 mg, 1.5 mmol) in methylene chloride (4 mL), triethylamine (523 μL, 3.8 mmol) and DMAP (10 mg, catalyst). The reaction mixture was stirred at 45 °C for 5 h, diluted with EtOAc and poured into a mixture of ice and 1 N HCl. The layers were separated and the aqueous layer was reextracted with EtOAc. The organic layers were each washed with brine, combined, dried over Na_2SO_4 and evaporated. The residue was purified by elution (toluene/EtOAc) through a silica gel column and gave 880 mg (90%) of (3*S*,4*S*)-4-(4-diphenylmethoxycarbonyl)phenoxy-3-(2-methoxybenzyl)-1-[(*R*)-1-(phenylpropyl)aminocarbonyl]azetidin-2-one **25**. ^1H NMR ($CDCl_3$): δ 0.91 (3H, t, $J=7.4$ Hz), 1.81 (1H, qdd, $J=7.6$, 13.8, 1.4 Hz), 1.88 (1H, qdd, $J=7.6$, 13.8, 1.4 Hz), 3.00 (1H, dd, $J=14.1$, 9.1 Hz), 3.21 (1H, dd, $J=14.3$, 5.5 Hz), 3.59–3.70 (1H, m), 3.64 (3H, s), 4.76 (1H, q, $J=7.1$ Hz), 5.78 (1H, d, $J=1.3$ Hz), 6.78 (1H, d, $J=8.1$ Hz), 6.87 (1H, t, $J=7.8$ Hz), 6.95 (2H, d, $J=9.0$ Hz), 7.07 (1H, s), 7.10–7.48 (18H, m), 7.98 (2H, d, $J=9.0$ Hz). To a solution of **25** (655 mg, 1.0 mmol) in anisole (3.2 mL) was added precooled TFA (0.8 mL) at 5 °C. After 1.5 h at 5 °C, the reaction mixture was poured into sodium bicarbonate solution and extracted with two portions of EtOAc. The EtOAc layers were each washed with brine, dried over Na_2SO_4 and evaporated. The residue was crystallized from hexane to give 415 g (85%) of **21** as a white amorphous solid, $[\alpha]_D$ +2.0° (*c* 0.50, 99.5% EtOH); IR ν_{max} cm^{-1}: 1778, 1709, 1691; ^1H NMR ($CDCl_3$): δ 0.92 (3H, t, $J=7.5$ Hz), 1.84 (1H, qdd, $J=7.7$, 13.8, 3.0 Hz), 1.87 (1H, qdd, $J=7.7$, 13.8, 3.0 Hz), 2.00 (1H, br s), 3.00 (1H, dd, $J=14.1$, 9.3 Hz), 3.23 (1H, dd, $J=14.1$, 5.7 Hz), 3.59–3.70 (1H, m), 3.66 (3H, s), 4.77 (1H, q, $J=7.5$ Hz), 5.80 (1H, d, $J=1.2$ Hz), 6.81 (1H, d, $J=8.4$ Hz), 6.90 (1H, t, $J=7.5$ Hz), 6.91 (2H, d, $J=9.0$ Hz), 7.13–7.39 (8H, m), 7.91 (2H, d, $J=9.0$ Hz); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{28}H_{28}O_6N_2+H]^+$ 489.2026, found 489.2024.

(3*S*,4*S*)-3-(2-Ethoxybenzyl)4-(4-carboxy)phenoxy-1-[(*R*)-1-(phenylpropyl)aminocarbonyl]azetidin-2-one (22). Using essentially the same procedures as for the preparation of **21**, the following compound was prepared from **18**. White amorphous solid, $[\alpha]_D$ +2.0° (*c* 0.50, 99.5% EtOH); IR ν_{max} cm^{-1}: 1778, 1709, 1691; ^1H NMR ($CDCl_3$): δ 0.92 (3H, t, $J=7.2$ Hz), 1.28 (3H, t, $J=7.1$ Hz), 1.84 (1H, qdd, $J=7.4$, 13.8, 3.3 Hz), 1.87 (1H, qdd, $J=7.4$, 13.8, 3.3 Hz), 2.00 (1H, br s), 2.98 (1H, dd, $J=13.8$, 9.3 Hz), 3.27 (1H, dd, $J=14.0$, 5.6 Hz), 3.70 (1H, ddd, $J=9.0$, 5.7, 1.4 Hz), 3.93 (2H, q, $J=7.1$ Hz), 4.77 (1H, q, $J=7.8$ Hz), 5.78 (1H, d, $J=1.2$ Hz), 6.80 (1H, d, $J=7.8$ Hz), 6.88 (1H, t, $J=7.5$ Hz), 6.89 (2H, d, $J=9.0$ Hz), 7.16 (1H, dd, $J=7.5$, 1.8 Hz), 7.19–7.39 (7H, m), 7.90 (2H, d, $J=9.0$ Hz); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{29}H_{30}O_6N_2+H]^+$ 503.2182, found 503.2188.

(3*S*,4*S*)-4-(4-Carboxy)phenoxy-1-[diphenylmethyl)aminocarbonyl]-3-(2-ethoxybenzyl)azetidin-2-one (23). To a solution of diphenylacetic acid (182 mg, 0.86 mmol) in methylene chloride (2 mL) were added triethylamine (144 μL, 1.0 mmol) and diphenylphosphoryl azide (222 μL, 1.0 mmol) and then the reaction mixture was stirred at room temperature for 2.5 h. To the reaction mixture were added a solution of **18** (300 mg, 0.59 mmol) in methylene chloride (5 mL), triethylamine (144 μL, 1.0 mmol) and DMAP (10 mg, catalyst). The reaction mixture was stirred at 45 °C for 5 h, diluted with EtOAc and poured into a mixture of ice and 1 N HCl. The layers were separated and the aqueous layer was reextracted with EtOAc. The organic layers were each washed with brine, combined, dried over Na_2SO_4 and evaporated. The residue was purified by elution (toluene/EtOAc) through a silica gel column and gave 398 mg (94%) of (3*S*,4*S*)-4-(4-diphenylmethoxycarbonyl)phenoxy-1-[(diphenylmethyl)aminocarbonyl]-3-(2-ethoxybenzyl)azetidin-2-one **26**. ^1H NMR ($CDCl_3$): δ 1.28 (3H, t, $J=6.9$ Hz), 3.00 (1H, dd, $J=14.3$, 8.6 Hz), 3.29 (1H, dd, $J=14.1$, 6.1 Hz), 3.65–3.75 (1H, m), 3.94 (2H, q, $J=6.8$ Hz), 5.83 (1H, d, $J=1.5$ Hz), 6.15 (1H, d, $J=8.4$ Hz), 6.81 (1H, d, $J=8.0$ Hz), 6.89 (1H, t, $J=7.8$ Hz), 6.94 (2H, d, $J=8.9$ Hz), 7.07 (1H, s), 7.10–7.48 (18H, m), 7.97 (2H, d, $J=8.9$ Hz). To a solution of **26** (392 mg, 0.55 mmol) in anisole (0.42 mL) was added precooled TFA (0.42 mL) at 5 °C. After 1.5 h at 5 °C, the reaction mixture was poured into sodium bicarbonate solution and extracted with two portions of EtOAc. The EtOAc layers were each washed with brine, dried over Na_2SO_4 and evaporated. The residue was crystallized from hexane to give 242 g (80%) of **23** as a white amorphous solid, $[\alpha]_{365}$−2.0° (*c* 0.51, $CHCl_3$). IR ν_{max} cm^{-1}: 1778, 1709, 1691; ^1H NMR ($CDCl_3$): δ 1.29 (3H, t, $J=7.0$ Hz), 2.97 (1H, dd, $J=14.3$, 9.3 Hz), 3.30 (1H, dd, $J=13.7$, 5.9 Hz), 3.66–3.76 (1H, m), 3.95 (2H, q, $J=7.0$ Hz), 5.84 (1H, d, $J=1.3$ Hz), 6.16 (1H, d, $J=8.4$ Hz), 6.79–6.95 (4H, m), 7.15–7.40 (12H, m), 7.91

(2H, d, $J=8.9$ Hz); HRMS (FAB, $[M+H]^+$) calcd for $[C_{33}H_{30}O_6N_2+H]^+$ 551.2182, found 551.2175.

(3*S*,4*S*)-4-(4-Carboxy)phenoxy-1-[(diphenylmethyl)aminocarbonyl]-2-oxo-3-(2-*iso*-propoxybenzyl)azetidine (24). Using essentially the same procedures as for the preparation of **23**, the following compound was prepared from **19**. White amorphous solid, $[\alpha]_D-1.2°$ (*c* 0.51, 99.5% EtOH); IR ν_{max} cm^{-1}: 1780, 1711, 1691; ^1H NMR ($CDCl_3$): δ 1.21 (3H, d, $J=6.1$ Hz), 1.26 (3H, d, $J=6.1$ Hz), 2.97 (1H, dd, $J=14.3$, 9.3 Hz), 3.30 (1H, dd, $J=13.7$, 5.9 Hz), 3.73 (1H, ddd, $J=9.0$, 5.7, 1.4 Hz), 4.52 (1H, quintet, $J=6.3$ Hz), 5.84 (1H, d, $J=1.2$ Hz), 6.15 (1H, d, $J=8.4$ Hz), 6.80–6.95 (4H, m), 7.15–7.40 (12H, m), 7.89 (2H, d, $J=8.9$ Hz); HR-MS (FAB, $[M+H]^+$) calcd for $[C_{34}H_{32}O_6N_2+H]^+$ 565.2339, found 565.2344.

Enzyme assay

The human chymase assay was performed as follows. First, human chymase was purified according to the method of Takai.[19] The purified chymase was preincubated with test compounds dissolved in DMSO at 37 °C for 30 min in 0.1 M Tris–HCl (pH 8.0) containing 1.8 M NaCl, then the chymase reaction was started by adding succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilides (Sigma Chemical Co.). The change of absorbance was measured at 405 nm after 2 h incubation at 37 °C. The IC_{50} value was calculated from the inhibition of *p*-nitroaniline formation at each concentration of the test compound.

The inhibitory effects of compound **23** on the enzymatic activities of seven serine proteases were evaluated using the purified enzymes and chromogenic substrates. The enzymes and substrates used here were as follows: *N*-succinyl-Ala-Ala-Pro-Phe-pNA (Bachem) for bovine pancreatic α-chymotrypsin (Sigma) and human cathepsin G (Wako); Chromozyme TH (Boehringer Mannheim) for human thrombin (Sigma); *N*-succinyl-Ala-Ala-Phe-Arg-pNA (Bachem) for bovine pancreatic trypsin (Sigma); *N*-succinyl-Ala-Ala-Val-pNA (Bachem) for human neutrophil elastase (Athens Research and Technology, Inc.); Chromozym PL (Boehringer Mannheim) for human plasmin (Sigma). The assay buffer used here was as follows: 50 mM Tris–HCl (pH = 8.0) containing 2 mM $CaCl_2$ for α-chymotrypsin, trypsin and elastase; 50 mM Tris–HCl (pH = 7.5) containing 2 mM $CaCl_2$ for cathepsin G; 50 mM Tris–HCl (pH = 7.5) containing 50 mM NaCl for plasmin; 0.1 M Tris–HCl (pH = 8.0) containing 10 mM $CaCl_2$ and 0.1 M NaCl for thrombin.

Stability assay

The stability assay in human plasma was performed as follows. Test compounds dissolved in DMSO were added at 20 μg/mL to human plasma and incubated at 37 °C. Aliquots were removed at intervals of 30 min, quenched with 3-fold volume of AcOH–MeOH (1/1) or AcOH–MeCN (1/1), and analyzed by reverse-phase HPLC after removal of the proteins. The disappearance of test compounds was then monitored for the half-life period and the results were fitted to a first-order decay curve from which the reported $t_{1/2}$ value was derived.

Molecular modeling

Molecular modeling was performed using the SYBYL 6.6 software package. The three-dimensional model of compound **23** was generated by CONCORD and optimized by the Tripos force field. The model of inhibitor **23** was manually docked into the active site of human chymase on the basis of the structure–activity relationships of 3-benzylazetidine-2-one derivatives against human chymase and the docking study for the binding of 3,3-diethyl-2-azetidinone to human leukocyte elastase.[11b] The resulting hemiketal intermediate (human chymase-**23** complex) was energy-minimized using the Tripos force field.

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