Synthesis, Structure−Activity Relationships, and Pharmacokinetic Profiles of Nonpeptidic α-Keto Heterocycles as Novel Inhibitors of Human Chymase

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We designed nonpeptidic chymase inhibitors based on the structure of a peptidic compound (**1**) and demonstrated that the combination of a pyrimidinone skeleton as a P_3-P_2 scaffold and heterocycles as P_1 carbonyl-activating groups can function as a nonpeptidic chymase inhibitor. In particular, introduction of heterobicycles such as benzoxazole resulted in more potent chymase-inhibitory activity. Detailed structure−activity relationship studies on the benzoxazole moiety and substituents at the 2-position of the pyrimidinone ring revealed that **2r** (Y-40079) had the most potent chymase-inhibitory activity (K_i = 4.85 nM). This compound was also effective toward chymases of nonhuman origin and showed good selectivity for chymases over other proteases. Pharmacokinetic studies in rats indicated that **2r** was absorbed slowly after oral administration and showed satisfactory bioavailability (BA) (T_{max} = 6.0 ± 2.3 h, BA = 19.3 ± 6.6%, $t_{1/2}$ = 35.7 ± 13.3 h). In conclusion, **2r** is a novel, potent, and orally active chymase inhibitor which would be a useful tool in elucidating the pathophysiological roles of chymase.

Introduction

Chymase (EC 3.4.21.39), which is classified as a chymotrypsin-like serine protease, is secreted from mast cells in response to immunological stimuli.[1−3] While the physiological roles of chymase have not yet been clarified due to uncertainty as to its intrinsic substrates, reports of its possible involvement in the localized production of angiotensin II (Ang II) in several species including humans have provided new insight into the role of proteases in the cardiovascular system.[4−6] In addition, several recent reports have suggested that chymase is involved not only in Ang II formation but also in tissue remodeling in the cardiovascular system[7,8] and that it is probably an endothelin-processing enzyme.[9,10]

Meanwhile, other studies have suggested that, since chymase is localized in mast cells, it may be involved in arthritis and other chronic inflammatory conditions, including various skin disorders and allergies.[11,12] With particular reference to skin inflammation, involvement by chymase in the production of soluble-type stem-cell factor and of interleukin 1β has been suggested,[13−16] while more recently the relation between genetic polymorphism of mast-cell chymase and atopic eczema has been discussed.[17−19] Chymase inhibitors have therefore come to be seen as a potential new type of antiinflammatory and antiallergic agent.

Thus far several peptidic chymase inhibitors have been synthesized, including chloromethyl ketone,[20,21] boronic acid,[11] phosphonate ester,[22] α-ketoester,[20,23] α-ketoamide,[24] and difluoromethylene ketone derivatives.[25,26] Despite their high potency in chymase inhibition, however, their therapeutic use is still limited because of their peptidic nature. Reports on nonpeptidic inhibitors,[20,27,28] on the other hand, have been very few, and none contain descriptions of potent, orally active, nonpeptidic inhibitors.

Our goal has been to develop a nonpeptidic chymase inhibitor characterized by greater potency, higher enzymatic selectivity, and metabolic stability. In seeking such nonpeptidic derivatives, our aim is to elucidate the pathophysiological roles of chymase and to develop therapeutic agents for chymase-induced diseases.

In the study of human leukocyte elastase inhibitors, X-ray crystallographic analysis has demonstrated that nonpeptidic compounds with a pyrimidinone moiety bind to the enzyme in a manner similar to that of peptidic compounds.[29] In chymase, X-ray structure confirms that the P_3-P_1 residue of the irreversible inhibitor succinyl-Ala-Ala-Pro-Phe-CH_2Cl forms the same antiparallel β-sheet binding arrangement as in peptidic elastase inhibitors.[30] We therefore hypothesized that a pyrimidinone structure, which was successfully used in the design of elastase inhibitors,[29,31] would be an effective P_3-P_2 mimetic in peptidic chymase inhibitors of the kind exemplified by compound **1**.

In elastase inhibitors, several functional groups have been used to activate the carbonyl group of peptidyl ketones toward neucleophilic addition by the active site Ser-195 hydroxy group of elastase. In addition to these trifluoromethyl, difluoromethylene, halomethyl, ester, and keto groups,[32] the first examples of heterocycle activation of the carbonyl group in the design of novel elastase inhibitors were described in a seminal paper by Edwards et al.[33−35] The X-ray crystal structure of

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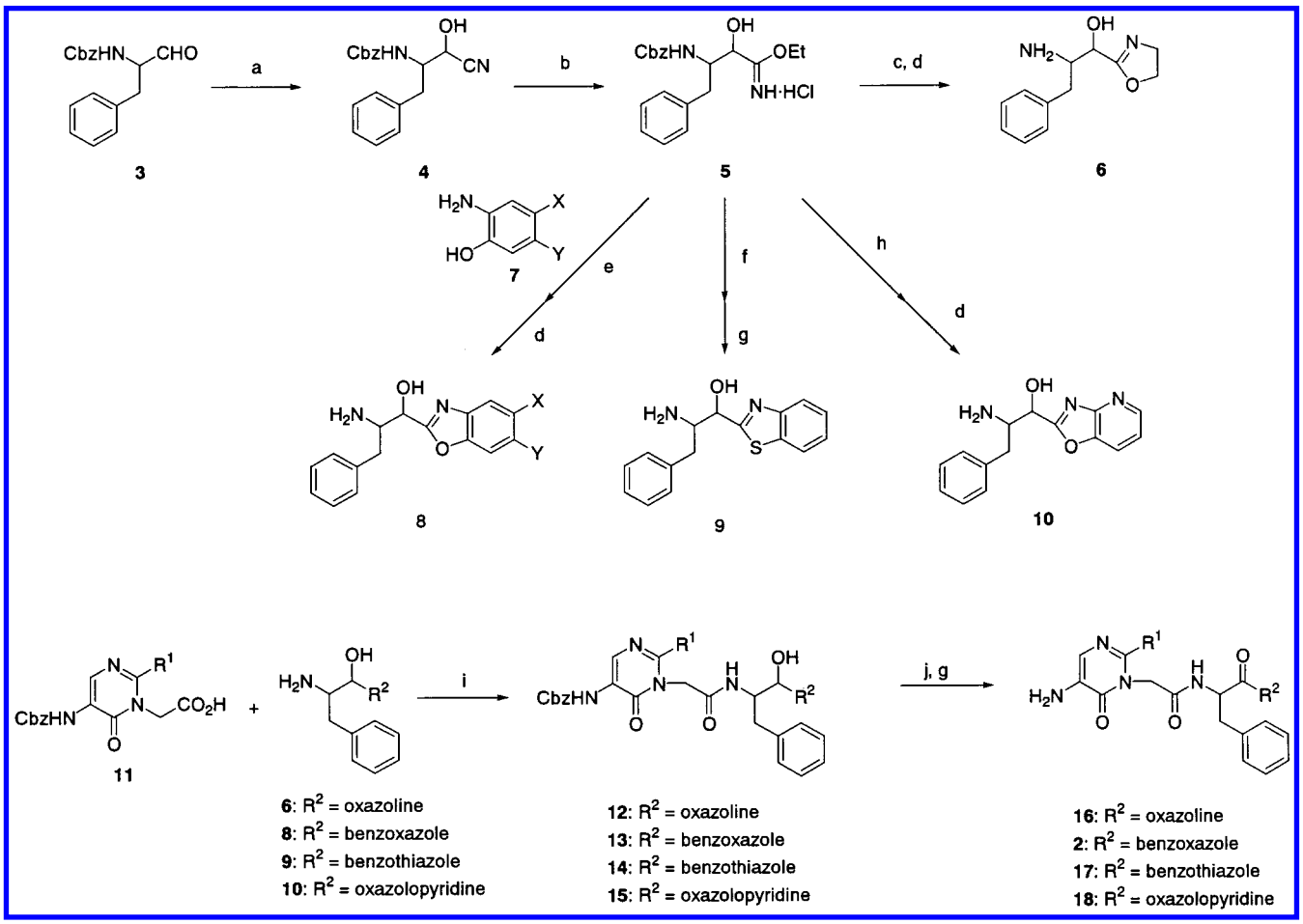
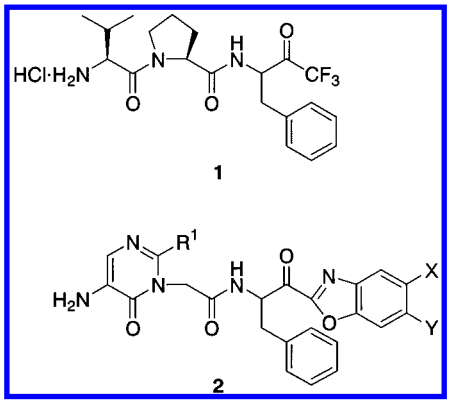
Scheme 1[a]



[a] Generic groups X, Y, and R^1 are defined in Tables 2 and 3. Reagents: (a) acetone cyanohydrin, Et_3N, CH_2Cl_2; (b) HCl, EtOH, $CHCl_3$; (c) monoethanolamine, Et_3N, CH_2Cl_2; (d) H_2, Pd/C, CH_3OH; (e) EtOH; (f) *o*-aminothiophenol, EtOH; (g) CF_3SO_3H, anisole, CH_2Cl_2; (h) 2-amino-3-hydroxypyridine, EtOH; (i) EDC, HOBT, DMF; (j) EDC, Cl_2CHCO_2H, DMSO, toluene.



acetyl-Ala-Pro-Val-(2-benzoxazole) complexed with porcine pancreatic elastase has thus revealed the covalent bonding between the ketone carbonyl carbon atom and the hydroxy group of Ser-195 and, moreover, the formation of a new hydrogen bond between the benzoxazole nitrogen and His-57 of the catalytic triad, which contributes to the stabilization of the enzyme−inhibitor complex. Recently, several reports have described the utilization of such electron-withdrawing heterocycles to activate the carbonyl group in the development of protease inhibitors: for example, the α-keto heterocyclic inhibitors of prolyl endopeptidase used by Tsutsumi et al.[36] and the peptidic thrombin inhibitors used by Castanzo et al.[37] The α-keto heterocycle moiety, in our consideration, shows an analogous function as part of a chymase inhibitor.

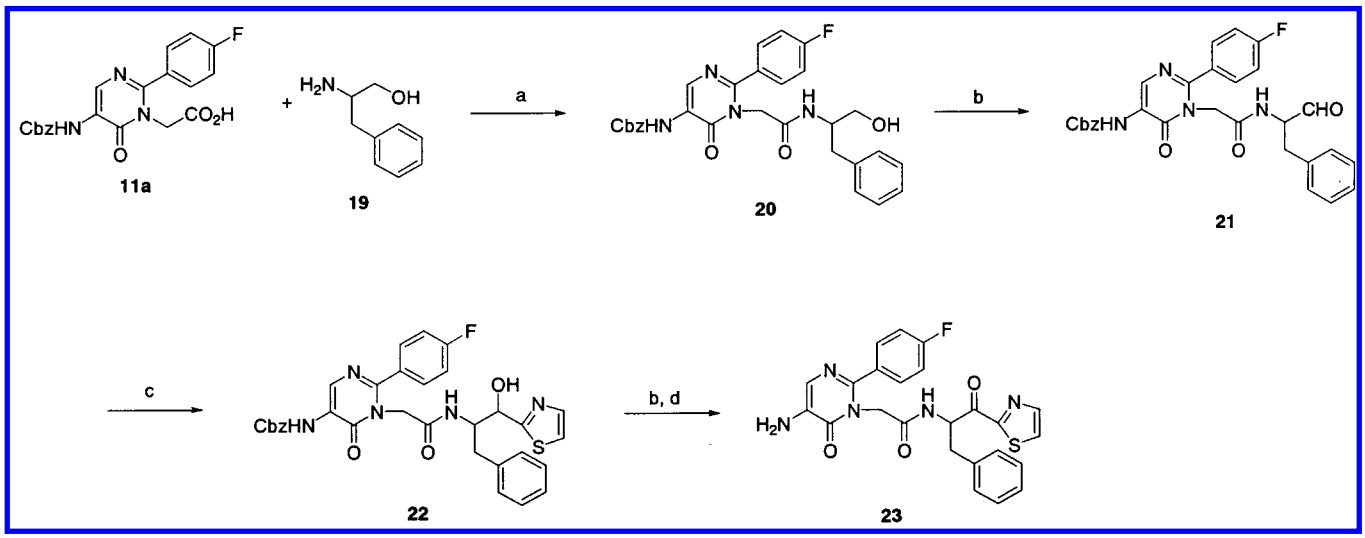
On the basis of the postulation of Kinoshita et al. that the high substrate specificity of human chymase for Ang I is due to the unique conformation of the S′ subsite of chymase,[38] we hypothesized that the introduction to the heterocyclic ring of functional groups able to interact with the S′ subsite would increase affinity and specificity for chymase over other chymotrypsin-type serine proteases. We anticipated that a highly effective nonpeptidic chymase inhibitor would be produced by the combination of a pyrimidinone skeleton acting as a P_3-P_2 scaffold with substituted heterocycles capable of activating the P_1 carbonyl group and interacting with the S′ subsite (general structure **2**).

In the present report, we describe the synthesis and structure−activity relationships (SARs) of a series of 5-amino-6-oxo-1,6-dihydropyrimidine derivatives bearing heterocycles at the position corresponding to the P′ site. We also report on the inhibitory selectivity toward several representative proteases and on the pharmacokinetic profile of the most potent compound, **2r**, which has a 5-methoxycarbonylbenzoxazole moiety.

Chemistry

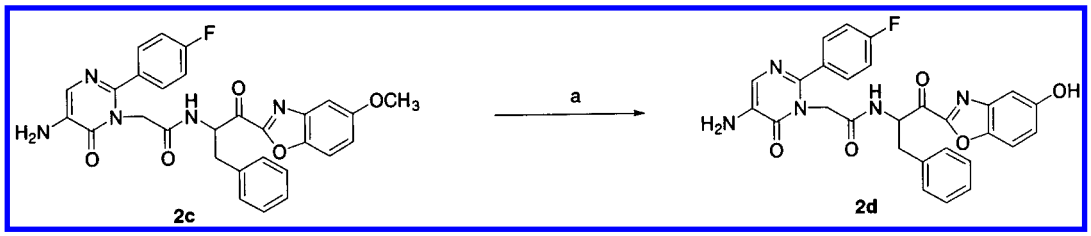
The general method of synthesis for α-keto heterocycle-substituted pyrimidinone derivatives is summarized in

Scheme 2[a]



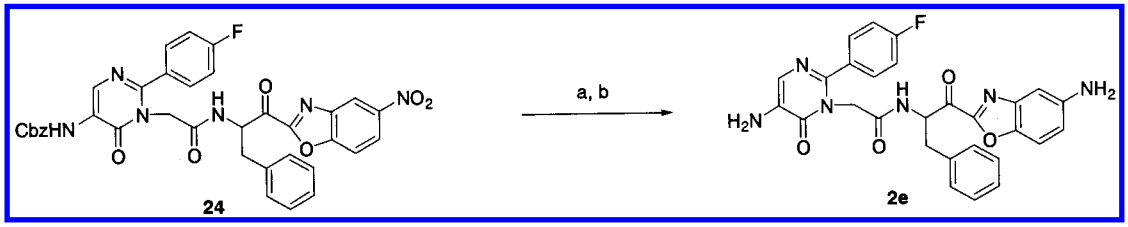
[a] Reagents: (a) EDC, HOBT, DMF; (b) EDC, Cl_2CHCO_2H, DMSO, toluene; (c) 2-trimethylsilylthiazole, CH_2Cl_2 then Bu_4NF; (d) CF_3SO_3H, anisole, CH_2Cl_2.

Scheme 3[a]



[a] Reagents: (a) BBr_3, CH_2Cl_2.

Scheme 4[a]



[a] Reagents: (a) Fe, 1 N HCl, THF, CH_3OH, H_2O; (b) CF_3SO_3H, anisole, CH_2Cl_2.

Scheme 1. The aldehyde **3**[39] was converted into the cyanohydrin **4** with acetone cyanohydrin/Et_3N.[34] Treatment of **4** with anhydrous HCl in ethanol afforded the imidate **5**, which was cyclized with monoethanolamine in the presence of Et_3N to the oxazoline ring. Removal of the carbonylbenzyloxy (Cbz) group by hydrogenolysis furnished the amine **6**. Reaction of the imidate **5** with substituted *o*-aminophenol **7**, *o*-aminothiophenol, or 2-amino-3-hydroxypyridine in refluxing ethanol afforded the corresponding heterobicycles **8**−**10**. EDC-mediated coupling of the amines **6** and **8**−**10** to the pyrimidinylacetic acids **11**[29] followed by modified Pfitzner−Moffatt oxidation[40] and removal of the Cbz group with trifluoromethanesulfonic acid in the presence of anisole afforded the desired α-keto heterocycles **2** and **16**−**18**.

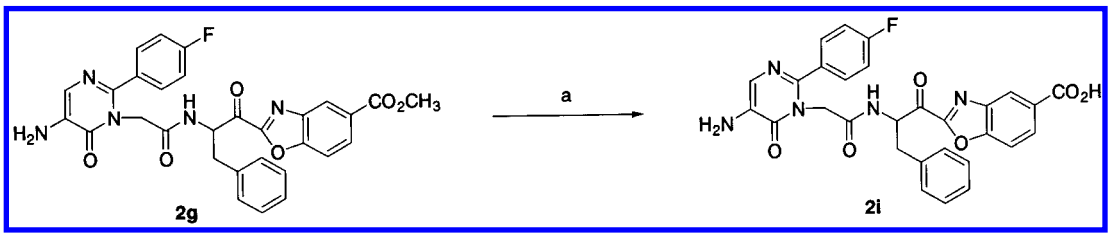
A different approach was necessary for the preparation of α-ketothiazole derivative **23** because of the difficulty of thiazole cyclization (Scheme 2). Condensation of the acetic acid **11a** and the phenylalaninol **19** followed by DMSO oxidation afforded the aldehyde **21**. Reaction of this aldehyde with 2-trimethylsilylthiazole using the method described by Dondoni et al.[41] gave a trimethylsilyl ether which on desilylation by fluoride anion in situ afforded the α-hydroxymethylthiazole **22**. Similarly, oxidation and deprotection provided the desired α-ketothiazole **23**.

Further transformations of the substituents at the 5-position of the benzoxazole ring were achieved by the routes illustrated in Schemes 3−6. The methoxy **2c** was transformed using boron tribromide to the hydroxy **2d**. The amine **2e** was prepared by reduction of the nitro **24** using an Fe−HCl system, as palladium-catalyzed hydrogenolysis reduced the carbonyl group at the α-position of the benzoxazole ring. Demethylation of the methoxycarbonyl **2g** using an aluminum tribromide−dimethyl sulfide system gave the carboxylic acid **2i**.[42] Hydrolysis of the methoxycarbonyl **13g** to the carboxylic acid **13i** followed by condensation with various ammonium salts, oxidation, and deprotection gave the desired carbamoyl analogues **2j**−**l**.

Biological Assays

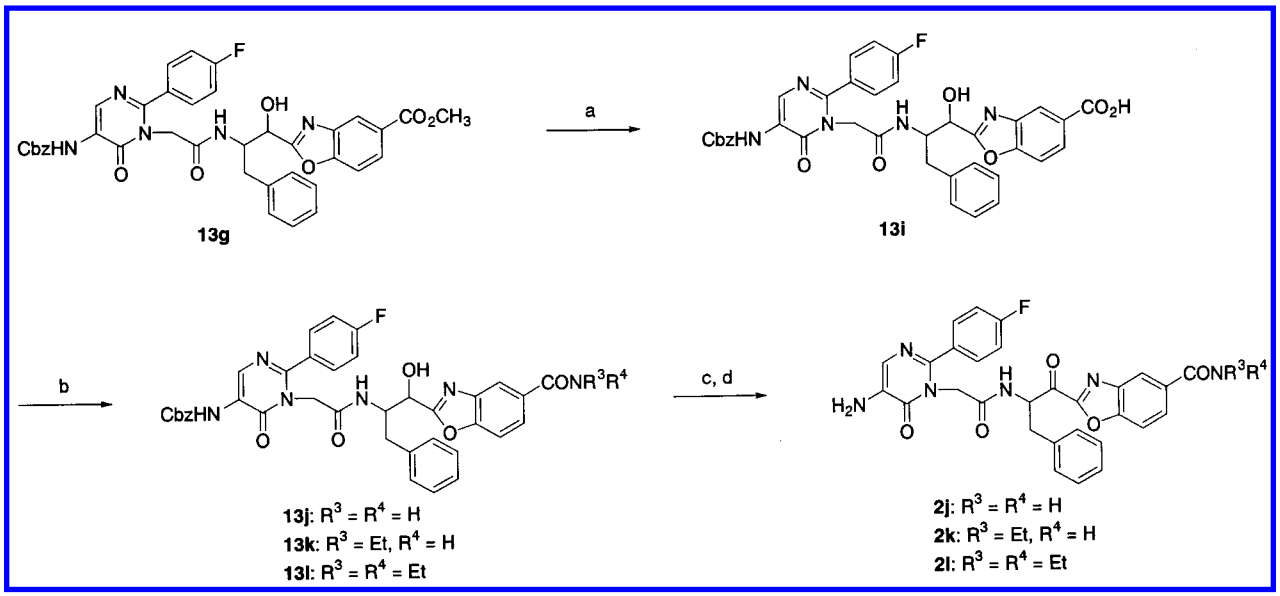
All compounds were evaluated for in vitro inhibitory activity using purified chymase from human heart and

Scheme 5[a]



[a] Reagents: (a) $AlBr_3$, $(CH_3)_2S$, CH_2Cl_2.

Scheme 6[a]



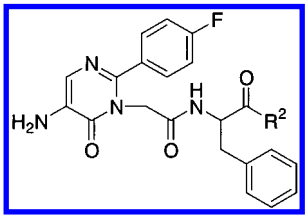
[a] Reagents: (a) 1 N NaOH, DMSO; (b) $R^3R^4NH{\cdot}HCl$, *N*-ethylmorpholine, EDC, HOBT, DMF; (c) Dess-Martin periodinate, DMSO or DMF; (d) CF_3SO_3H, anisole, CH_2Cl_2.

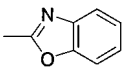
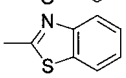
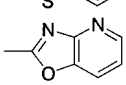
bovine pancreas α-chymotrypsin purchased from Sigma Chemical Co. The assay was based on a published method.[25] Hydrolysis of the substrate succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide was monitored spectrophotometrically by measuring the release of *p*-nitroaniline at 405-nm absorbance.

Results and Discussion

As it had been successfully used in the design of a human leukocyte elastase inhibitor,[29] we hypothesized that a [5-amino-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]acetyl moiety would be an effective $P_3{-}P_2$ (Val-Pro) mimetic in peptidic chymase inhibitors of the kind exemplified by the parent compound **1**. First of all, we investigated the effect of a variety of heterocycles acting as P_1 carbonyl-activating groups on chymase- and chymotrypsin-inhibitory activity (Table 1). The introduction of a monocycle such as oxazoline (**16**) or thiazole (**23**) resulted in reduction of chymase-inhibitory activity, whereas the introduction of a bicycle such as benzoxazole (**2a**), benzothiazole (**17**), or oxazolopyridine (**18**) led to increased activity toward both chymase and chymotrypsin. In particular, compound **18** showed potent activity, with K_i of ca. 60 nM toward both chymase and chymotrypsin. Edwards et al. reported two important SARs between elastase and heterocycles of the inhibitors: (1) the value of the inhibitory constant K_i tends to be positively correlated to the electron-withdrawing properties (σ_I value) of the heterocycle; (2) in the covalent enzyme−inhibitor adduct, the azole nitrogen atom of the inhibitor heterocycle participates in a hydrogen-bonding interaction with the active-site His-57.[33,34] They found that the oxazoline derivative is

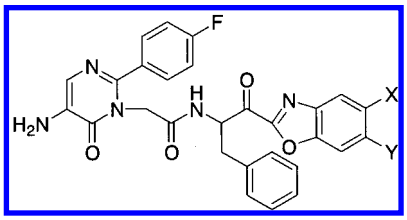
Table 1. Enzyme Inhibitory Activities of α-Keto Heterocycles



		inhibitory activity: K_i (nM)[a]	
compd	R^2	chymase	chymotrypsin
16		589 ± 10	9320 ± 460
23		3850 ± 1000	3500 ± 750
2a		125 ± 58	229 ± 63
17		182 ± 63	166 ± 25
18		60.6 ± 3.4	60.5 ± 8.1
1		82.1 ± 2.4	4400 ± 770
chymostatin		13.1 ± 1.4	9.36 ± 2.19

[a] Values are means ± SEM of three independent experiments.

Table 2. Enzyme Inhibitory Activities of 5- or 6-Substituted Benzoxazole Analogues



			inhibitory activity: K_i (nM)[a]	
compd	X	Y	chymase	chymotrypsin
2a	H	H	125 ± 58	229 ± 63
2b	F	H	93.1 ± 0.2	65.1 ± 1.5
2c	OCH_3	H	73.3 ± 5.2	48.0 ± 17.4
2d	OH	H	2120 ± 1020	2230 ± 90
2e	NH_2	H	183 ± 23	60.0 ± 13.0
2f	C_6H_5	H	174 ± 9	1630 ± 450
2g	CO_2CH_3	H	22.6 ± 8.8	52.9 ± 23.5
2h	CO_2Et	H	30.0 ± 7.5	477 ± 44
2i	CO_2H	H	76.0 ± 8.4	133 ± 3
2j	$CONH_2$	H	94.0 ± 18.0	53.4 ± 20.3
2k	CONHEt	H	68.6 ± 0.2	32.6 ± 1.8
2l	$CONEt_2$	H	59.5 ± 6.8	23.3 ± 5.2
2m	H	CO_2CH_3	56.3 ± 45.0	71.3 ± 21.7

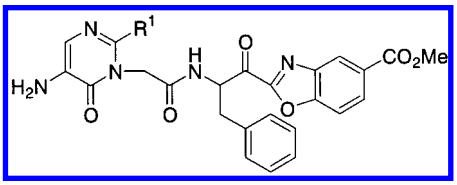
[a] Values are means ± SEM of three independent experiments.

the most effective elastase inhibitor, since the nitrogen atom of the oxazoline is the best hydrogen bond acceptor in a variety of heterocycles.[34] In chymase inhibitors with heteromonocycles, the increase in inhibitory activity for oxazoline compared to thiazole (7-fold) may have some hydrogen-bonding interaction with His-57 of chymase. In contrast to elastase inhibitor, heterobicycles showed more chyamse-inhibitory activity than heteromonocycles. This finding suggests that the aromatic rings of the heterobicycles interact with the S′ subsite of chymase. In Costanzo's study, the best thrombin inhibitor possesses a 2-benzothiazole group.[37] This was explained clearly with reference to novel interactions at the S_1' subsite, where the benzothiazole stacks the Trp-60D of the insertion loop of thrombin in a face-to-edge manner. In chymase inhibitors, it seems possible that aromatic rings of the heterobicycles have such stacking interactions with an aromatic amino acid residue of the enzyme. For further study, we selected the benzoxazole ring as the heterocyclic part due to its ease of synthesis.

In elastase inhibitors of the benzoxazole type, SAR studies have revealed that the electronic effects of various substituents on the benzoxazole ring do not greatly influence inhibitory activity.[35] Increasing the electron-withdrawing ability of the benzoxazole ring substituent does increase the electrophilicity of the ketone carbonyl carbon atom and increase the strength of the covalent bond formed with Oγ of Ser-195, but any increased ketone carbonyl activation by the ring substituent is counterbalanced by a corresponding decrease in the hydrogen-bonding ability between the benzoxazole nitrogen atom and His-57.

The results of our investigations of the effect of substituents on the benzoxazole ring are shown in Table 2. Substitution of an electron-withdrawing fluorine atom (**2b**) or an electron-releasing methoxy group (**2c**) at the 5-position of the benzoxazole ring resulted in both cases in ca. 4-fold more potent activity toward chymotrypsin and slightly more potent activity toward chymase than in the nonsubstituted analogue **2a**. The introduction of

Table 3. Enzyme Inhibitory Activities of 2-Substituted Pyrimidinone Analogues



		inhibitory activity: K_i (nM)[a]	
compd	R^1	chymase	chymotrypsin
2g (Y-40613)	4-FC_6H_4	22.6 ± 8.8	52.9 ± 23.5
2n	C_6H_5	5.57 ± 0.57	46.8 ± 17.0
2o	3-FC_6H_4	14.3 ± 2.4	1770 ± 80
2p	3-ClC_6H_4	26.1 ± 3.6	3820 ± 690
2q	3-$(CH_3)C_6H_4$	44.2 ± 24.6	47.7 ± 3.3
2r (Y-40079)	3-$(CH_3O)C_6H_4$	4.85 ± 0.82	943 ± 122
2s	3-$(NO_2)C_6H_4$	41.8 ± 1.0	11500 ± 2900
2t	3-$(NH_2)C_6H_4$	5.60 ± 0.15	855 ± 106
2u	3-pyridyl	77.4 ± 15.2	45.1 ± 9.8
2v	4-pyridyl	43.5 ± 11.7	467 ± 8

[a] Values are means ± SEM of three independent experiments.

a hydroxy group led to a 10-fold decrease in both chymase- and chymotrypsin-inhibitory activity (**2d**), but neither an amino nor a phenyl group had any effect on chymase-inhibitory activity (**2e**,**f**). The methoxycarbonyl analogue **2g** showed 5-fold greater inhibitory activity toward both chymase and chymotrypsin. The replacement of the methoxycarbonyl group of **2g** with an ethoxycarbonyl group did not substantially influence potency (**2h**), while replacement with a carboxyl or carbamoyl group resulted in loss of inhibitory activity (**2i**−**l**). Shifting the methoxycarbonyl group to the 6-position of the benzoxazole ring did not improve inhibitory activity (**2m**). In our chymase inhibitors, as in elastase inhibitors, no correlation was observed between the electronic effect of substituents on the benzoxazole ring and inhibitory activity. Substitution on the benzoxazole ring also had no great effect on selectivity for chymase over chymotrypsin. This suggests that the substituents screened do not interact specifically with a part of the S′ subsite but have some hydrophobic interaction with the S′ subsite of both enzymes.

We subsequently turned our attention to substitution at the 2-position of the pyrimidinone ring of compounds bearing 5-methoxycarbonylbenzoxazole, which had shown the most favorable effect on chymase-inhibitory activity. The results are summarized in Table 3. Substituents at this position are thought to interact with the S_2 pocket of the enzyme. SAR study of 5-amino-6-oxo-1,6-dihydropyrimidine derivatives with a trifluoromethyl ketone group has indicated that an aromatic ring at this position is essential to maintain potent chymase-inhibitory activity and that the introduction of a substituent at the 3-position of the phenyl ring increases activity.[43] We therefore focused on 3-substituted phenyl groups. The nonsubstituted phenyl analogue **2n** was found to be more potent (K_i = 5.57 nM) than compound **2g**. The introduction of various substituents at the 3-position (**2o**−**t**) resulted in maintenance or slight loss of chymase-inhibitory activity; chymotrypsin-inhibitory activity, on the other hand, was dramatically reduced. The 3-methoxyphenyl analogue **2r** showed the most potent chymase-inhibitory activity (K_i = 4.85 nM) and high

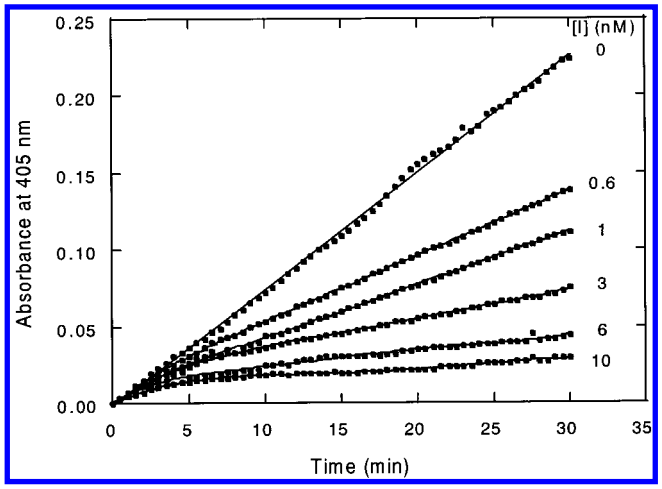


Figure 1. Progress curve of succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide hydrolysis by human chymase in the presence of various concentrations of **2r**. Measurement was done in 20 mM Tris-HCl (pH 7.5) containing 0.1 mM aprotinin and 2 M KCl in the presence of 2.5 mM substrate. The temperature was maintained at 37 °C and reactions were initiated by the addition of enzyme. Values are shown corrected for background absorbance. Numbers on the right correspond to the molar concentration of **2r**.

Table 4. Enzyme Selectivity Data for Compounds **2g**,**r**

enzyme	K_i (nM)[a]	
	2g	**2r**
human heart chymase	22.6 ± 8.8	4.85 ± 0.82
canine skin chymase	3.68 ± 0.48	1.12 ± 0.15
rat peritoneal chymase	103 ± 10	86.6 ± 8.4
mouse peritoneal chymase	40.4 ± 6.6	63.9 ± 26.7
bovine pancreatic chymotrypsin	52.9 ± 23.5	943 ± 122
human cathepsin G	720 ± 58	379 ± 126
human leukocyte elastase	NI (10 μM)[b]	NI (10 μM)[b]
human plasma thrombin	NI (10 μM)[b]	NI (10 μM)[b]
human angiotensin-converting enzyme	NI (10 μM)[b]	NI (10 μM)[b]

[a] Values are means ± SEM of three independent experiments. [b] NI = no inhibition at highest concentration (in parentheses) tested.

chymotrypsin/chymase selectivity [K_i(chymotrypsin)/K_i(chymase) = 194]. These findings clearly indicate that the introduction of substituents at the 3-position of the phenyl ring is highly effective in enhancing enzymatic selectivity for chymase. Accordingly, we assumed that this series of 5-amino-6-oxo-1,6-dihydropyrimidine derivatives utilizes S_2-P_2 interaction to discriminate between related enzymes. Finally, pyridyl substitutions led to a decrease in chymase-inhibitory activity (**2u**,**v**).

In the next step, we investigated the kinetics of compound **2r**. A progress curve for hydrolysis of succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide by chymase displayed a biphasic curve composed of a rapid initial phase and a slower steady-state phase (Figure 1). Although slow-binding inhibition is not a general property of α-keto heterocyclic inhibitors of serine proteases,[32] this

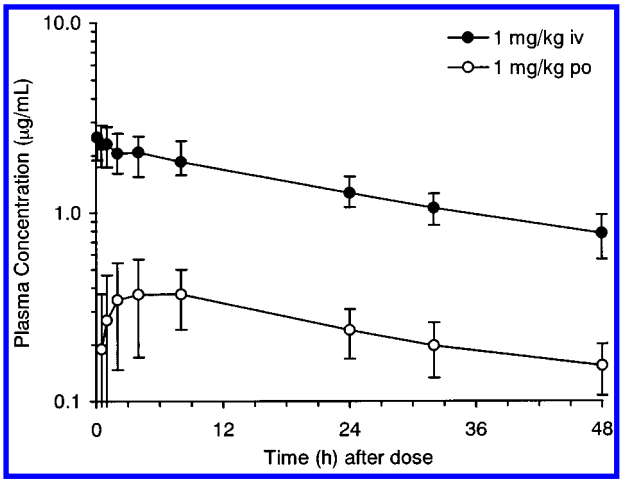


Figure 2. Plasma concentration profile of **2r** in rats.

phenomenon indicated that **2r** is a slow-binding inhibitor[44] like that of an α-keto heterocycle-bearing peptidic elastase inhibitor.[45] The inhibitory constant K_i, the association rate constant k_{on}, and the dissociation constant k_{off} calculated from the progress curve were 1.21 nM, 9.56×10^7 M^{-1} s^{-1}, and 0.030 s^{-1}, respectively. This K_i value differed little from the one determined from the conventional 'stopped' method (incubation of enzyme, inhibitor, and substrate for a given time followed by discontinuance of the reaction)[25] as shown in Tables 1−3. Due to the exceeding shortness of the pre-steady-state of the α-keto heterocycle-bearing inhibitor in comparison with the total reaction time (2 h), we regarded the first phase as having virtually no influence on the determination of K_i.

Compound **2g** and the most potent human chymase inhibitor **2r** were measured for their inhibitory activity toward nonhuman chymases and other representative proteases (Table 4). Both compounds showed strong inhibitory activity toward canine chymase and slightly less potency toward rat and mouse chymases. As expected, inhibitory activity was shown only toward chymotrypsin-type serine proteases. Notably, compound **2r** had 80-fold stronger activity toward human chymase than human catepsin G, which has similar substrate specificity.

Next, we examined plasma levels of compound **2r** after intravenous (iv) and oral (po) administration to rats. The results are shown in Figure 2 and pharmacokinetic parameters summarized in Table 5. The investigations indicated that **2r** was absorbed slowly after oral administration and had satisfactory bioavailability (BA) (T_{max} = 6.0 ± 2.3 h, BA = 19.3 ± 6.6%). Pharmacokinetic half-life and plasma clearance values were 35.7 ± 13.3 h and 10.1 ± 3.2 mL/h/kg, respectively. An in vitro degradation study using an S9 function of rat liver homogenate indicated that the major metabolite was a corresponding alcohol form by reduction of the active carbonyl group.

Table 5. Pharmacokinetic Parameters of **2r** in Rats

route, dose	n[a]	$t_{1/2}$[b] (h)	CL[c] (mL/h/kg)	$V_{d(ss)}$[d] (mL/kg)	AUC[e] (μg·h/mL)	C_{max}[f] (μg/mL)	T_{max}[g] (h)	BA[h] (%)
iv, 1 mg/kg	4	35.7 ± 13.3	10.1 ± 3.2	469 ± 60	107.6 ± 34.3			
po, 1 mg/kg	4				20.8 ± 7.1	0.396 ± 0.164	6.0 ± 2.3	19.3 ± 6.6

[a] n = number of animals. [b] $t_{1/2}$ = pharmacokinetic half-life. [c] CL = plasma clearance. [d] $V_{d(ss)}$ = steady-state volume of distribution. [e] AUC = integrated area under plasma concentration vs time curve from time 0 to time infinity. [f] C_{max} = maximum plasma concentration taken directly from measured values. [g] T_{max} = time to reach C_{max}. [h] BA = oral bioavailability: $(AUC_{po}/AUC_{iv}) \times 100$.

Compound **2r**, which displayed the most potent human chymase-inhibitory activity, is also a good inhibitor of nonhuman chymases. Its high selectivity for chymases over other proteases as well as their distinctive pharmacokinetic profiles suggest that the nonpeptidic chymase inhibitors reported here would be useful tools in elucidating the pathophysiological roles of chymase.

Conclusion

Starting from the peptidic chymase inhibitor **1**, we synthesized a series of 5-amino-6-oxo-1,6-dihydropyrimidine derivatives bearing heterocycles and evaluated their human chymase-inhibitory activity. We found that compounds with heterobicycles such as benzoxazole were more potent than those with heteromonocycles such as oxazoline or thiazole and that the introduction of a methoxycarbonyl group at the 5-position on the benzoxazole ring led to a 5-fold increase in chymase-inhibitory activity. These findings suggest that the S′ subsite of chymase not only has such a stacking interaction with the aromatic ring of the heterobicycles but also participates in a hydrophobic interaction with the substituents on the heterobicycles. Further SAR studies at the 2-position of the pyrimidinone ring revealed that compound **2r** (Y-40079) had the most potent chymase-inhibitory activity (K_i = 4.85 nM). This compound was also effective toward chymases of nonhuman orgins and showed good selectivity for chymases over other proteases. Pharmacokinetic studies in rat indicated that **2r** was absorbed slowly after oral administration and showed satisfactory bioavailability (BA) (T_{max} = 6.0 ± 2.3 h, BA = 19.3 ± 6.6%, $t_{1/2}$ = 35.7 ± 13.3 h). This biological profile suggests that compound **2r** will prove useful in elucidating the pathophysiological roles of chymase.

Experimental Section

Chemistry. Melting points were determined with a Yanaco melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on either a Bruker DPX-300 or AMX-500 instrument with tetramethylsilane as the internal standard; chemical shifts are reported in parts per million (ppm, δ units). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; or br, broad. Mass spectra (MS) were recorded on a Hitachi M-2000 or PE Sciex API-165 instrument operating in the secondary ionization mass spectrometry (SIMS) and electrospray ionization (ESI) modes, respectively. Elemental analyses for carbon, hydrogen, and nitrogen were conducted with a Yanaco MT-6 and are within ±0.4% of theory for formulas given. Chromatography refers to flash chromatography conducted on Kieselgel 60 230−400 mesh (E. Merck, Darmstadt) using the indicated solvents. All chemicals and solvents were reagent grade unless otherwise specified. Reactions were run under a nitrogen atmosphere at ambient temperature unless otherwise noted. The following abbreviations are used: THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBT, 3-hydroxybenztriazole hydrate.

3-Benzyloxycarbonylamino-2-hydroxy-4-phenylbutylnitrile (4). To a solution of *N*-(benzyloxycarbonyl)phenylalaninal (**3**) (5.00 g, 17.6 mmol) and acetone cyanohydrin (4.8 mL, 53 mmol) in dichloromethane (50 mL) was added triethylamine (1.5 mL, 11 mmol). The reaction mixture was stirred at room temperature for 4 h, at which time the solvent was evaporated. The residue was poured into water (100 mL) and then extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (66:34 hexanes−ethyl acetate) to give **4** (5.15 g, 94% yield) as a pale yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.68 (d, J = 8.8 Hz, 0.5H, NH), 7.54 (d, J = 9.4 Hz, 0.5H, NH), 7.35−7.17 (m, 10H, ArH), 6.85 (m, 0.5H,OH), 6.81 (m, 0.5H, OH), 5.00−4.90 (m, 2H, CH_2−O), 4.61 (m, 0.5H, CH−O), 4.41 (m, 0.5H, CH−O), 3.91 (m, 1H, CH−N), 2.99 (m, 1H, CH*H*-Ph), 2.71 (dd, J = 13.5, 11.4 Hz, 0.5H, CH*H*-Ph), 2.61 (dd, J = 13.7, 11.2 Hz, 0.5H, CH*H*-Ph).

2-(2-Amino-1-hydroxy-3-phenylpropyl)benzoxazole (8a). A solution of chloroform (30 mL) and absolute ethanol (28 mL, 0.48 mol) at 0 °C under nitrogen was treated with acetyl chloride (31 mL, 0.44 mol) dropwise over 20 min. The cyanohydrin **4** (4.50 g, 14.5 mmol) in chloroform (30 mL) was added, the mixture stirred at 0 °C for 3 h, and the solvent evaporated while maintaining the temperature at 25 °C or below. The crude imidate in anhydrous ethanol (100 mL) was treated with *o*-aminophenol (1.90 g, 17.4 mmol) and heated under reflux for 6 h and the solvent evaporated. The residue was taken up in ethyl acetate, washed successively with 0.5 N NaOH, 0.5 N HCl, saturated $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (98:2 chloroform−methanol) to give 2-(2-benzyloxycarbonylamino-1-hydroxy-3-phenylpropyl)benzoxazole (5.12 g, 88% yield) as a pale brown solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.76−7.66 (m, 2H, ArH), 7.42−7.00 (m, 13H, ArH), 6.43 (d, J = 6.2 Hz, 0.4H, OH), 6.25 (d, J = 5.4 Hz, 0.6H, OH), 4.93−4.71 (m, 3H, CH_2−O, CH−O), 4.16 (m, 1H, CH−N), 3.15 (dd, J = 13.8, 2.5 Hz, 0.4H, CH*H*-Ph), 3.01 (dd, J = 13.8, 4.7 Hz, 0.6H, CH*H*-Ph), 2.80−2.72 (m, 1H, CH*H*-Ph).

To a solution of the above intermediate (3.63 g, 9.02 mmol) in methanol (50 mL) was added 10% palladium−carbon (480 mg) under a nitrogen atmosphere. The reaction mixture was stirred under a hydrogen atmosphere at room temperature for 18 h. Palladium−carbon was removed by filtration and washed with methanol. The filtrate was concentrated to give **8a** (2.43 g, 100% yield) as a pale brown solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.74−7.68 (m, 2H, ArH), 7.41−7.15 (m, 7H, ArH), 6.17 (m, 0.4H, OH), 6.08 (m, 0.6H, OH), 4.61 (m, 0.6H, CH−O), 4.54 (m, 0.4H, CH−O), 3.34 (m, 1H, CH−N), 3.05 (dd, J = 13.4, 3.8 Hz, 0.4H, CH*H*-Ph), 2.78 (dd, J = 13.4, 5.9 Hz, 0.6H, CH*H*-Ph), 2.60 (dd, J = 13.4, 7.9 Hz, 0.6H, CH*H*-Ph), 2.53 (dd, J = 13.4, 8.9 Hz, 0.4H, CH*H*-Ph), 1.47 (br s, 2H, NH_2).

2-[5-Benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(2-benzoxazolyl)hydroxymethyl]-2-phenylethyl]acetamide (13a). To a solution of [5-benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]acetic acid (**11a**) (1.78 g, 4.48 mmol) and 2-(2-amino-1-hydroxy-3-phenylpropyl)benzoxazole (**8a**) (1.37 g, 5.11 mmol) in DMF (15 mL) were added HOBT (1.21 g, 8.95 mmol) and EDC (1.03 g, 5.37 mmol). After stirring at room temperature for 4 h, the reaction mixture was poured into 0.5 N HCl (100 mL), and then extracted with ethyl acetate. The extract was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated. The residue was washed with diethyl ether and dried in vacuo to give **13a** (2.43 g, 84% yield) as colorless crystals: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.79 (br s, 0.7H, NH), 8.68 (br s, 0.3H, NH), 8.39 (s, 1H, CH=N), 8.34 (d, J = 8.7 Hz, 0.3H, NH), 8.30 (d, J = 8.7 Hz, 0.7H, NH), 7.73−7.64 (m, 2H, ArH), 7.47−7.31 (m, 9H, ArH), 7.28−7.07 (m, 7H, ArH), 6.44 (d, J = 6.0 Hz, 0.3H, OH), 6.38 (d, J = 5.1 Hz, 0.7H, OH), 5.19 (s, 2H, CH_2−O), 4.81 (m, 1H, CH−O), 4.48−4.18 (m, 3H, CH_2−N, CH−N), 3.00 (dd, J = 13.8, 5.9 Hz, 0.7H, CH*H*-Ph), 2.93 (dd, J = 14.1, 3.5 Hz, 0.3H, CH*H*-Ph), 2.83 (dd, J = 14.1, 9.6 Hz, 0.3H, CH*H*-Ph), 2.69 (dd, J = 13.8, 8.4 Hz, 0.7H, CH*H*-Ph).

2-[5-Amino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(2-benzoxazolyl)carbonyl]-2-phenylethyl]-acetamide (2a). To a solution of **13a** (2.12 g, 3.27 mmol) in DMSO (20 mL) and toluene (20 mL) were added EDC (3.13 g, 16.3 mmol) and then dichloroacetic acid (0.54 mL, 6.5 mmol). After stirring at room temperature for 3.5 h, the reaction mixture was poured into 1 N HCl (100 mL), and then extracted with ethyl acetate. The extract was washed with saturated

$NaHCO_3$ and brine and dried ($MgSO_4$). The solvent was evaporated and the residue purified by silica gel column chromatography (83:17 dichloromethane−ethyl acetate) to give 2-[5-benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(2-benzoxazolyl)carbonyl]-2-phenylethyl]acetamide (1.69 g, 80% yield) as colorless crystals. Recrystallization from ethyl acetate afforded colorless crystals (1.43 g): mp 222−225 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.98 (d, J = 6.9 Hz, 1H, NH), 8.86 (s, 1H, NH), 8.40 (s, 1H, CH═N), 8.00 (d, J = 8.0 Hz, 1H, ArH), 7.91 (d, J = 8.0 Hz, 1H, ArH), 7.66 (t, J = 8.0 Hz, 1H, ArH), 7.55 (t, J = 8.0 Hz, 1H, ArH), 7.46−7.40 (m, 4H, ArH), 7.38 (t, J = 7.1 Hz, 2H, ArH), 7.33 (t, J = 7.1 Hz, 1H, ArH), 7.25 (t, J = 6.7 Hz, 2H, ArH), 7.22−7.13 (m, 5H, ArH), 5.56 (m, 1H, CH−CO), 5.17 (s, 2H, CH_2−O), 4.53 (d, J = 16.6 Hz, 1H, CH*H*-N), 4.45 (d, J = 16.6 Hz, 1H, CH*H*-N), 3.31 (dd, J = 14.2, 4.8 Hz, 1H, CH*H*-Ph), 2.96 (dd, J = 14.2, 9.0 Hz, 1H, CH*H*-Ph); MS (SIMS, positive) m/z 646 (MH^+).

To a solution of the above intermediate (581 mg, 0.900 mmol) and anisole (0.31 mL, 2.9 mmol) in dichloromethane (9 mL) was added trifluoromethanesulfonic acid (0.48 mL, 5.4 mmol) with ice-water cooling. The reaction mixture was stirred at 0 °C to room temperature for 1 h, after which saturated $NaHCO_3$ (10 mL) was added. After stirring at room temperature for 1.5 h, the reaction mixture was poured into saturated $NaHCO_3$ (50 mL) and then extracted with ethyl acetate. The extract was washed with brine and dried ($MgSO_4$). The solvent was evaporated and the residue purified by silica gel column chromatography (97:3 chloroform−methanol) to give **2a** (437 mg, 95% yield) as colorless crystals: mp 233−236 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.92 (d, J = 6.8 Hz, 1H, NH), 8.01 (d, J = 8.0 Hz, 1H, ArH), 7.91 (d, J = 8.0 Hz, 1H, ArH), 7.66 (t, J = 8.0 Hz, 1H, ArH), 7.56 (t, J = 8.0 Hz, 1H, ArH), 7.35 (dd, J = 8.6, 5.5 Hz, 2H, ArH), 7.28 (s, 1H, CH═N), 7.26 (t, J = 8.0 Hz, 2H, ArH), 7.23−7.17 (m, 3H, ArH), 7.09 (t, J = 8.0 Hz, 2H, ArH), 5.55 (m, 1H, CH−C═O), 5.13 (s, 2H, NH_2), 4.48 (d, J = 16.7 Hz, 1H, CH*H*-N), 4.41 (d, J = 16.7 Hz, 1H, CH*H*-N), 3.30 (dd, J = 14.1, 4.9 Hz, 1H, CH*H*-Ph), 2.97 (dd, J = 14.1, 8.9 Hz, 1H, CH*H*-Ph); MS (SIMS, positive) m/z 512 (MH^+). Anal. ($C_{28}H_{22}FN_5O_4$) C, H, N.

The compounds **2b**,**c**,**f**−**h**,**m**−**v** were prepared using the same procedures as described above.

2-[5-Amino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(5-hydroxybenzoxazol-2-yl)carbonyl]-2-phenylethyl]acetamide (2d). To a solution of **2c** (452 mg, 0.835 mmol) in dichloromethane (10 mL) was added a solution of boron tribromide in dichloromethane (1.0 M, 8.4 mL, 8.4 mmol) with ice−water cooling. The resulting mixture was stirred at 0 °C−room temperature for 4 h, at which time methanol (1.5 mL) was added. After stirring for 10 min, the reaction mixture was poured into saturated $NaHCO_3$ (50 mL), and then extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (91:9 chloroform−methanol) to give **2d** (340 mg, 77% yield) as a yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.95 (brs, 1H, OH), 8.89 (d, J = 6.9 Hz, 1H, NH), 7.69 (d, J = 8.9 Hz, 1H, ArH), 7.35 (dd, J = 8.5, 5.6 Hz, 2H, ArH), 7.29−7.07 (m, 10H, ArH), 5.54 (m, 1H, CH−C═O), 5.15 (s, 2H, NH_2), 4.48 (d, J = 16.5 Hz, 1H, CH*H*-N), 4.41 (d, J = 16.5 Hz, 1H, CH*H*-N), 3.27 (dd, J = 14.1, 4.7 Hz, 1H, CH*H*-Ph), 2.94 (dd, J = 14.1, 9.0 Hz, 1H, CH*H*Ph); MS (SIMS, positive) m/z 528 (MH^+). Anal. ($C_{28}H_{22}FN_5O_5$·0.2H_2O) C, H, N.

2-[5-Amino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(5-aminobenzoxazol-2-yl)carbonyl]-2-phenylethyl]acetamide (2e). To a solution of 2-[5-benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(5-nitrobenzoxazol-2-yl)carbonyl]-2-phenylethyl]acetamide (**24**) (1.34 g, 1.94 mmol) in THF (40 mL), water (7 mL) and methanol (7 mL) were added successively iron powder (2.73 g, 49.0 mmol) and 1 N HCl (1.64 mL). The resulting mixture was stirred at room temperature for 18 h. The iron powder was removed by filtration and washed with chloroform. The filtrate was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (97:3 chloroform−methanol) to give 2-[5-benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(5-aminobenzoxazol-2-yl)carbonyl]-2-phenylethyl]acetamide (1.00 g, 78% yield) as red brown crystals: mp 163−169 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.93 (s, 1H, NH), 8.92 (d, J = 6.6 Hz, 1H, NH), 8.41 (s, 1H, CH═N), 7.54 (d, J = 8.9 Hz, 1H, ArH), 7.48−7.13 (m, 14H, ArH), 6.95 (d, J = 2.0 Hz, 1H, ArH), 6.92 (dd, J = 8.9, 2.0 Hz, 1H, ArH), 5.56 (m, 1H, CH−C═O), 5.37 (s, 2H, NH_2), 5.17 (s, 2H, NH_2), 4.53 (d, J = 16.7 Hz, 1H, CH*H*-N), 4.44 (d, J = 16.7 Hz, 1H, CH*H*-N), 3.25 (dd, J = 14.0, 4.4 Hz, 1H, CH*H*-Ph), 2.92 (dd, J = 14.0, 8.9 Hz, 1H, CH*H*Ph).

The above intermediate was deprotected using a method similar to that described for **2a** to give **2e** (83% yield) as yellow crystals: mp 163−169 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.83 (d, J = 7.0 Hz, 1H, NH), 7.53 (d, J = 8.8 Hz, 1H, ArH), 7.36 (m, 2H, ArH), 7.28 (s, 1H, ArH), 7.26 (t, J = 7.3 Hz, 2H, ArH), 7.23−7.17 (m, 3H, ArH), 7.13 (t, J = 8.8 Hz, 2H, ArH), 6.96 (d, J = 2.2 Hz, 1H, ArH), 6.92 (dd, J = 8.8, 2.2 Hz, 1HV), 5.56 (m, 1H, CH−C═O), 5.35 (s, 2H, NH_2), 5.14 (s, 2H, NH_2), 4.48 (d, J = 16.6 Hz, 1H, CH*H*-N), 4.41 (d, J = 16.6 Hz, 1H, CH*H*-N), 3.25 (dd, J = 14.1, 4.8 Hz, 1H, CH*H*-Ph), 2.93 (dd, J = 14.1, 8.8 Hz, 1H, CH*H*-Ph); MS (SIMS, positive) m/z 527 (MH^+). Anal. ($C_{28}H_{22}FN_5O_5$·0.2H_2O) C, H, N.

2-[5-Amino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(5-carboxybenzoxazol-2-yl)carbonyl]-2-phenylethyl]acetamide (2i). To a solution of **2g** (180 mg, 0.316 mmol) in dimethyl sulfide (5 mL) and dichloromethane (5 mL) was added aluminum bromide (680 mg, 2.55 mmol) with ice−water cooling. The reaction mixture was stirred at 0 °C-room temperature for 5 h, after which water (10 mL) and 1 N HCl (10 mL) were added. After stirring at room temperature for 1 h, the resulting suspension was filtrated. The solid was washed with water and chloroform and purified by silica gel column chromatography (67:33 chloroform−methanol) to give **2i** (146 mg, 83% yield) as yellow crystals: mp 207−214 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.94 (d, J = 6.8 Hz, 1H, NH), 8.46 (s, 1H, ArH), 8.24 (d, J = 8.6 Hz, 1H, ArH), 7.87 (d, J = 8.6 Hz, 1H, ArH), 7.35 (dd, J = 8.7, 5.5 Hz, 2H, ArH), 7.29−7.18 (m, 6H, ArH), 7.11 (t, J = 8.7 Hz, 2H, ArH), 5.55 (m, 1H, CH−C═O), 5.13 (s, 2H, NH_2), 4.48 (d, J = 16.6 Hz, 1H, CH*H*-N), 4.42 (d, J = 16.6 Hz, 1H, CH*H*-N), 3.30 (m, 1H, CH*H*-Ph), 2.97 (dd, J = 14.1, 8.8 Hz, 1H, CH*H*-Ph); MS (SIMS, positive) m/z 556 (MH^+). Anal. ($C_{29}H_{22}FN_5O_6$·2.3H_2O) C, H, N.

2-[5-Benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(5-carboxylbenzoxazol-2-yl)hydroxymethyl]-2-phenylethyl]acetamide (13i). To a solution of 2-[5-benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[[5-(methoxycarbonyl)-benzoxazol-2-yl]hydroxymethyl]-2-phenylethyl]acetamide (**13g**) (2.00 g, 2.83 mmol) in DMSO (250 mL) was added 1 N NaOH (30 mL). After stirring at room temperature for 1 h, the reaction mixture was poured into 0.1 N HCl (1000 mL) and then extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated. The residue was washed with diethyl ether and dried in vacuo to give **13i** (1.58 g, 81% yield) as a colorless solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.0 (br s, 1H, OH), 8.79 (s, 0.6H, NH), 8.65 (s, 0.4H, NH), 8.39−8.32 (m, 2H, CH═N, NH), 8.24 (d, J = 1.2 Hz, 0.4H, NH), 8.21 (d, J = 1.2 Hz, 0.6H, NH), 8.04−7.97 (m, 1H, ArH), 7.82−7.77 (m, 1H, ArH), 7.46−7.07 (m, 14H, ArH), 6.53 (d, J = 6.0 Hz, 0.4H, OH), 6.46 (d, J = 4.9 Hz, 0.6H, OH), 5.19 (s, 2H, CH_2−O), 4.87−4.80 (m, 1H, CH−O), 4.52−4.18 (m, 3H, CH_2−N, CH−N), 3.02 (dd, J = 13.8, 6.0 Hz, 0.6H, CH*H*-Ph), 2.96 (dd, J = 14.0, 3.7 Hz, 0.4H, CH*H*-Ph), 2.82 (dd, J = 14.0, 9.5 Hz, 0.4H, CH*H*-Ph), 2.71 (dd, J = 13.8, 8.6 Hz, 0.6H, CH*H*-Ph).

2-[5-Benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(5-carbamoylbenzoxazol-2-yl)hydroxymethyl]-2-phenylethyl]acetamide (13j). To a solution of **13i** (1.23 g, 1.77 mmol), NH_4Cl (190 mg, 3.55 mmol) and *N*-ethylmorpholine (0.45 mL, 3.5 mmol) in DMF

(30 mL) were added HOBT (479 mg, 3.55 mmol) and EDC (408 mg, 2.13 mmol). After stirring at room temperature for 15 h, the reaction mixture was poured into 1 N HCl (100 mL), and then extracted with ethyl acetate. The extract was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated. The residue was washed with diethyl ether and dried in vacuo to give **13j** (952 mg, 78% yield) as a pale brown solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.84 (br s, 0.6H, NH), 8.75 (br s, 0.4H, NH), 8.42−8.31 (m, 2H, CH═N, NH), 8.26 (s, 0.4H, NH), 8.22 (s, 0.6H, NH), 8.08 (br s, 1H, NH*H*), 7.94−7.92 (m, 1H, ArH), 7.77 (d, J = 8.6 Hz, 0.4H, ArH), 7.73 (d, J = 8.6 Hz, 0.6H, ArH), 7.47−7.07 (m, 15H, ArH, NH*H*), 6.52 (br s, 0.4H, OH), 6.47 (br s, 0.6H, OH), 5.19 (s, 2H, CH_2−O), 4.82 (br s, 1H, CH−O), 4.52−4.18 (m, 3H, CH_2−N, CH−N), 3.03−2.63 (m, 2H, CH_2−Ph).

2-[5-Amino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(5-carbamoylbenzoxazol-2-yl)carbonyl]-2-phenylethyl]acetamide (2j). To a solution of **13j** (687 mg, 0.995 mmol) in DMF (10 mL) was added Dess−Martin periodinate (1.06 g, 2.49 mmol). The reaction mixture was stirred at room temperature for 4 h, after which saturated $NaHCO_3$ (12 mL) containing sodium thiosulfate (2.64 g) was added with ice−water cooling. After stirring at 0 °C for 30 min, the reaction mixture was poured into saturated $NaHCO_3$ (100 mL) and extracted with ethyl acetate. The extract was washed with 10% citric acid aqueous solution, saturated $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (95:5 chloroform−ethanol) and recrystallization from chloroform−diethyl ether to give 2-[5-benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-[(5-carbamoylbenzoxazol-2-yl)carbonyl]-2-phenylethyl]acetamide (295 mg, 43% yield) as pale yellow crystals: mp 264−266 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.01 (d, J = 7.1 Hz, 1H, NH), 8.89 (s, 1H, NH), 8.50 (s, 1H, ArH), 8.40 (s, 1H, CH═N), 8.22−8.10 (m, 2H, ArH), 7.98 (d, J = 8.7 Hz, 1H, ArH), 7.62−7.10 (m, 16H, ArH, NH_2), 5.60−5.42 (m, 1H, CH−C═O), 5.17 (s, 2H, CH_2−O), 4.60−4.38 (m, 2H, CH_2−N), 3.45−2.85 (m, 2H, CH_2−Ph).

The above intermediate was deprotected using a method similar to that described for **2a** to give **2j** (58% yield) as colorless crystals: mp 270 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.96 (d, J = 6.8 Hz, 1H, NH), 8.51 (s, 1H, ArH), 8.24−8.11 (m, 2H, ArH), 7.98 (d, J = 8.7 Hz, 1H, ArH), 7.56 (br s, 1H NH*H*), 7.35 (dd, J = 8.4, 5.6 Hz, 2H, ArH), 7.30−7.15 (m, 5H, ArH, NH*H*), 7.10 (t, J = 8.7 Hz, 2H, ArH), 5.60−5.40 (m, 1H, CH−C═O), 5.14 (s, 2H, NH_2), 4.58−4.30 (m, 2H, CH_2−N), 3.40−3.20 (m, 1H, CH*H*-Ph), 3.00−2.80 (m, 1H, CH*H*-Ph). Anal. ($C_{29}H_{23}FN_6O_5$·0.8H_2O) C, H, N.

The compounds **2k**,**l** were prepared using the same procedures as described above.

2-(2-Amino-1-hydroxy-3-phenylpropyl)oxazoline (6). To the crude imidate **5** [prepared from the cyanohidrin **4** (1.33 g, 4.27 mmol)] in dichloromethane (18 mL) were added monoethanolamine (0.51 mL, 8.5 mmol) and triethylamine (1.2 mL, 8.6 mmol). After stirring at room temperature for 18 h, the reaction mixture was poured into 1 N NaOH (50 mL), and then extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (97:3 chloroform−methanol) to give 2-(2-benzyloxycarbonylamino-1-hydroxy-3-phenylpropyl)oxazoline (509 mg, 33% yield) as a colorless solid. A solution of this intermediate (505 mg, 1.42 mmol) was deprotected using a method similar to that described for **8a** to give **6** (284 mg, 91% yield) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33−7.12 (m, 5H, ArH), 4.20 (m, 2H, CH_2−O), 3.90 (dd, J = 8.4, 4.6 Hz, 1H, CH−O), 3.72 (m, 2H, CH_2−N), 3.04 (m, 1H, CH−N), 2.90 (dd, J = 13.4, 4.1 Hz, 0.4H, CH*H*-Ph), 2.80−2.65 (m, 1.2H, CH_2−Ph), 2.42 (dd, J = 13.4, 8.8 Hz, 0.4H, CH*H*-Ph).

Compounds **16**−**18** were prepared using a similar procedure as described for **2a**.

2-[5-Benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-hydroxymethyl-2-phenylethyl]acetamide (20). Compound **11a** (6.00 g, 15.1 mmol) and phenylalaninol (**19**) (2.51 g, 16.6 mmol) were coupled using a similar procedure as described for **13a** to give **20** (6.51 g, 81% yield) as colorless crystals: mp 232−235 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (s, 1H, NH), 8.43 (s, 1H, CH═N), 8.05 (d, J = 8.2 Hz, 1H, NH), 7.51 (dd, J = 8.6, 5.5 Hz, 1H, ArH), 7.44 (d, J = 7.1 Hz, 2H, ArH), 7.39 (t, J = 7.1 Hz, 2H, ArH), 7.34 (t, J = 7.1 Hz, 1H, ArH), 7.28−7.21 (m, 4H, ArH), 7.18 (t, J = 7.1 Hz, 1H, ArH), 5.19 (s, 2H, CH_2−O), 4.78 (br s, 1H, OH), 4.47 (d, J = 16.4 Hz, 1H, CH*H*-N), 4.35 (d, J = 16.6 Hz, 1H, CH*H*-N), 3.87 (m, 1H, CH−N), 3.28 (m, 2H, CH_2−O), 2.79 (dd, J = 13.7, 5.8 Hz, 1H, CH*H*-Ph), 2.58 (dd, J = 13.7, 7.9 Hz, 1H, CH*H*-Ph).

2-[5-Benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[1-formyl-2-phenylethyl]-acetamide (21). Compound **20** (5.89 g, 11.1 mmol) was oxidized using a similar procedure as described for **2a** to give **21** (5.45 g, 93% yield) as colorless crystals: mp 138−141 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.62 (s, 1H, CH═O), 8.77 (br s, 1H, CH═N), 7.53 (dd, J = 8.7, 5.2 Hz, 2H, ArH), 7.48 (s, 1H, ArH), 7.43−7.33 (m, 5H, ArH), 7.28−7.08 (m, 7H, ArH), 6.52 (d, J = 6.8 Hz, 1H, ArH), 5.24 (s, 2H, CH_2−O), 4.77 (q, J = 6.6 Hz, 1H, CH−N), 4.52 (AB-q, J = 15.4 Hz, 2H, CH*H*-N), 3.17 (m, 2H, CH_2−Ph); MS (SIMS, positive) m/z 529 (MH^+).

2-[5-Benzyloxycarbonylamino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[2-phenyl-1-[(2-thiazolyl)-hydroxymethyl]ethyl]acetamide (22). To a solution of **21** (724 mg, 1.37 mmol) in dichloromethane (15 mL) was added 2-trimethylsilylthiazole (0.23 mL, 1.4 mmol), and the mixture stirred at room temperature for 1 day. Tetrabutylammonium fluoride (1.0 M solution in THF, 2.5 mL, 2.5 mmol) was then added. After stirring at room temperature for 30 min, the reaction mixture was poured into saturated $NaHCO_3$ (50 mL), and then extracted with chloroform. The extract was washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (98:2 chloroform−methanol) to give **22** (665 mg, 79% yield) as a colorless solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (s, 1H, NH), 8.42 (s, 0.25H, CH═N), 8.40(s, 0.75H, CH═N), 8.33 (d, J = 10.7 Hz, 0.25H, NH), 8.21 (d, J = 9.0 Hz, 0.75H, NH), 7.78 (d, J = 3.2 Hz, 0.25H, ArH), 7.68 (d, J = 3.2 Hz, 0.75H, ArH), 7.65 (d, J = 3.2 Hz, 0.25H, ArH), 7.56 (d, J = 3.2 Hz, 0.75H, ArH), 7.46−7.12 (m, 13.5H, ArH), 7.04 (d, J = 6.8 Hz, 0.5H, ArH), 6.64 (d, J = 5.1 Hz, 0.75H, OH), 6.61 (d, J = 5.6 Hz, 0.25H, OH), 5.18 (s, 2H, CH_2−O), 4.85 (dd, J = 5.6, 3.8 Hz, 0.25H, CH−O), 4.78 (dd, J = 5.1, 2.6 Hz, 0.75H, CH−O), 4.57−4.22 (m, 3H, CH_2−N, CH−N), 2.89 (dd, J = 13.5, 7.4 Hz, 0.75H, CH*H*-Ph), 2.68 (dd, J = 14.4, 10.4 Hz, 0.25H, CH*H*-Ph), 2.58 (dd, J = 13.5, 7.1 Hz, 0.75H, CH*H*-Ph), 2.5 (m, 0.25H, CH*H*-Ph).

2-[5-Amino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[2-phenyl-1-[(2-thiazolyl)carbonyl]ethyl]acetamide (23). Compound **22** was oxidized using a similar procedure as described for **2a** to give **23** as colorless crystals: mp 199−203 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.79 (d, J = 7.5 Hz, 1H, NH), 8.30 (d, J = 2.9 Hz, 1H, ArH), 8.21 (d, J = 2.9 Hz, 1H, ArH), 7.36 (dd, J = 8.5, 5.6 Hz, 2H, ArH), 7.29−7.14 (m, 8H, ArH), 5.67 (m, 1H, CH−C═O), 5.15 (s, 2H, NH_2), 4.50 (d, J = 16.6 Hz, 1H, CH*H*-N), 4.39 (d, J = 16.6 Hz, 1H, CH*H*-N), 3.19 (dd, J = 14.0, 4.3 Hz, 1H, CH*H*-Ph), 2.89 (dd, J = 14.0, 9.2 Hz, 1H, CH*H*-Ph); MS (SIMS, positive) m/z 478 (MH^+). Anal. ($C_{24}H_{20}FN_5O_3S$) C, H, N.

Protease Inhibition Assay. Human heart chymase was purified according to the method of Urata et al.[5] Bovine pancreatic α-chymotrypsin and human cathepsin G were purchased from Sigma Chemical Co., St. Louis, MO, and Athens Research and Technologies Inc., respectively. Human thrombin, elastase, and angiotensin-converting enzyme (ACE) were from Welfide Corp., Osaka, Japan, Elastins Products Co. Inc., Owensville, MO, and Nippon Zoki Pharmaceutical Co., Osaka, Japan, respectively.

Inhibitory activities for chymase, chymotrypsin, and cathepsin G were measured using previously described methods[25] through measurement at 405-nm absorbance of *p*-nitroaniline release from the synthetic substrate succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (Sigma Chemical Co.). In the case of thrombin

and elastase, buffer conditions previously described[46] were used with the synthetic substrates S-2238 (Chromogenix AB, Mölndal, Sweden) and CH_3O-Suc-Ala-Ala-Pro-Val-*p*-nitroaniline (Sigma Chemical Co.), respectively. Enzymatic activity of ACE was measured using the methods described by Miyazaki et al.[47] Calculations of K_i's were performed using previously described methods.[25]

Kinetic Studies for Representative Compound by Progress Curve Method. The progress of human chymase inhibition by **2r** was measured under a pseudo-first-order inhibition condition, i.e., $[I_0] \geq 10[E_0]$, by reacting chymase with a mixture of inhibitor and substrate and recording the liberation of free *p*-nitroaniline at 405-nm absorbance. Reactions were conducted in 20 mM Tris-HCl (pH 7.5) containing 0.1 mM aprotinin and 2 M KCl at 37 °C. Under these conditions, K_m (Michaelis−Menton constant) for succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide was 741 nM. Reaction progress was monitored in a 96-well fluorimetric plate using a fluorimetric plate reader (Spectrafluor Tecan Japan; Tokyo, Japan). Reaction was preformed in 200 μL of final volume and initiated by the addition of a 20 μL aliquot of enzyme stock. Readings were taken every 30 s for a total of 1800 s, and initial absorbance values were subtracted from the data prior to subsequent calculations. Data were fitted to the integrated rate equation for slow-binding inhibition (eq 1) according to the method described by Williams and Morrison,[48] by nonlinear regression analysis:

$$A = v_s t + (v_0 - v_s)(1 - e^{-k't})/k' + A_0 \quad (1)$$

Values for v_0 (initial rate), v_s (final steady-state rate), k' (apparent rate constant for the transition from v_0 to v_s), and A_0 (the initial absorbance at 405 nm) were obtained for each progress curve. Mean values for k', obtained from three independent assays, were plotted against the inhibitor concentration, $[I_0]$. A linear dependency between $[I_0]$ and k' was observed and fitted to eq 2 to estimate k_{on}' (apparent association rate constant) and k_{off} (dissociation rate constant). Subsequently a value for k_{on} (association rate constant) was determined from the correction of k_{on}' for the competition of the substrate using eq 3:

$$k' = k_{off} + k_{on}'[I_0] \quad (2)$$

$$k_{on} = k_{on}'(1 + [S_0]/K_m) \quad (3)$$

where $[S_0]$ is the concentration of the chromogenic substrate (2.5 mM). K_i' values were determined using a direct, nonlinearizing plot of v_s vs [I], fitted to eq 4. Subsequently K_i was calculated from K_i' according to eq 5:

$$K_i' = [I_0]/(v_0/v_s - 1) \quad (4)$$

$$K_i = K_i'/(1 + [S_0]/K_m) \quad (5)$$

Pharmacokinetic Measurements. Male Sprague−Dawley rats (approximate weight 200 g) were used for the pharmacokinetic study. The test compound **2r** was administered at a dose of 1 mg/kg intravenously (via bolus injection into tail vein) or orally (via gavage to the stomach). For intravenous administration the test compound was formulated as a solution (0.4 mg/mL) in DMSO−PEG-400−0.1 M HCl−saline (1:8:10:81, v/v). For oral administration the test compound was formulated as a suspension (0.25 mg/mL) in 0.5% hydroxypropylmethyl cellulose. Rats were fasted for 18 h before dosing and 4 h after dosing. Blood was collected from the jugular vein into a heparinized syringe at several time points up to 48 h after dosing. Plasma sample was separated by centrifugation and kept frozen (−20 °C) until analysis.

Plasma concentration of the test compound was determined by the internal standard method. Calibration standards were prepared in plasma by adding known amounts of the test compound to control rat plasma. Plasma sample (100 μL) was mixed with 0.2 M glycine−NaOH buffer (pH 9.0) (0.5 mL) and internal standard solution (0.6 μM/mL **2n**, 50 μL). The sample was extracted with ethyl acetate (5 mL). After phase separation through centrifugation, the organic layer was transferred and evaporated to dryness under nitrogen steam. The residue was reconstituted in 100 μL of 10 mM ammonium acetate (pH 6.8)−acetonitrile (50:50, v/v), and 50 μL was injected onto an LC−MS/MS system. Chromatographic separation was performed using a Develosil ODS-MG-3 (50 mm × 2 mm i.d., 3 μm, Nomura Chemical Co., Japan) at a flow rate of 0.2 mL/min. The mobile phase consisted of 10 mM ammonium acetate (pH 6.8) (A) and acetonitrile (B). The following gradient system was used: 20% B to 80% B linearly over the first 2.5 min; isocratic 80% B for 2.5 min. Protonated analyte ions were generated using electron spray ionization. For quantitation, the ion signal was recorded by selective reaction monitoring. Pharmacokinetic parameters were determined by noncompartmental methods.

In Vitro Degradation Study by Rat Liver Homogenate. The S9 protein (2.5 mg) of rat liver was incubated with **2r** (final concentration, 50 μM; added as 5 μL in DMSO) in 100 mM phosphate buffer (pH 7.4, final volume; 0.5 mL) consisting of 4 mM NADPH, 4 mM NADH, 8 mM $MgCl_2$, 5 mM glucose-6-phosphate, and 1 U/mL glucose-6-phosphate dehydrogenase at 37 °C for 15 min. The reaction was terminated by the addition of acetonitrile (2 mL). After centrifugation the supernatant was analyzed under the above HPLC conditions.

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Supporting Information Available: Experimental data and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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