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Non-Peptidic Inhibitors of Human Chymase. Synthesis, Structure–Activity Relationships, and Pharmacokinetic Profiles of a Series of 5-Amino-6-oxo-1,6-dihydropyrimidine-containing Trifluoromethyl Ketones

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Abstract—Chymase possesses a wide variety of actions, including promotion of angiotensin II production and histamine release from mast cells. However, due to a lack of effective inhibitors featuring both high inhibitory activity and high metabolic stability, the pathophysiological role of chymase has not been fully elucidated. We designed non-peptidic inhibitors based on the predicted binding mode of the peptidic chymase inhibitor Val-Pro-Phe-CF_3 and demonstrated that the Val-Pro unit is replaceable with a (5-amino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)acetyl moiety. Structure–activity relationship studies revealed that phenyl substitution at the 2-position of the pyrimidinone ring is indispensable for high activity. The most potent compound **1h** ($K_i = 0.0506\ \mu M$) is superior in potency to the parent peptidic inhibitor Val-Pro-Phe-CF_3 and has good selectivity for chymase over other proteases. The related analogue **1e** was orally absorbed and maintained high plasma levels for at least 2 h. These results suggest that the derivatives reported here could be developed as agents for treatment of chymase-induced disease.

Introduction

Chymase (EC 3.4.21.39) is a chymotrypsin-like serine protease which is synthesized and stored in mast cells localized mainly in the heart, blood vessels and skin. Mast cells secrete chymase with other biologically active substances in response to immunological stimuli. Reports on the in vitro activities of chymase have indicated that it degrades IgG and extracellular matrices such as type IV collagen, fibronectin and vitronectin, produces active-type collagenase from procollagenase, and promotes the conversion of macrophages into foam cells by degrading apolipoprotein B-100.[1–3] However, few reports have discussed the physiological and pharmacological importance of chymase. Recently, attention has been focused on the localized production of angiotensin II (Ang II) by human chymase in the cardiovascular system:[4,5] chymase levels have been found to increase in balloon-injured blood vessels of dogs and in the heart of a hamster model of cardiomyopathy,[6,7] while increased chymase-dependent Ang II formation has been observed in human atherosclerotic aorta.[8] In addition, several reports have suggested that chymase is involved not only in Ang II formation but also in tissue remodeling in the cardiovascular system,[9,10] and that chymase is one of the endothelin-processing enzymes.[11,12]

Independently of this, the localization of chymase in mast cells has provoked study of its roles in allergic and inflammatory diseases. Chymase has been reported to be involved in the process of histamine release,[13] and to provoke infiltration of inflammatory cells when injected.[14] With particular reference to skin inflammation, involvement of chymase in the production of soluble

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type stem cell factor and of interleukin 1β has been suggested,[15–18] while more recently the relation between genetic polymorphism of mast-cell chymase and atopic eczema has been discussed.[19–21]

As the various physiological and pharmacological roles of chymase have been discovered, a structurally varied range of inhibitors has emerged: peptide types include chloromethyl ketone,[22,23] boronic acid,[13] phosphonate ester,[24] α-ketoester,[22,25] α-ketoamide[26] and difluoromethylene ketone;[27,28] and non-peptide types sulfonylfluoride[29] and imidazolidine.[22] However, a non-peptidic chymase inhibitor which is highly specific and has high metabolic stability has yet to be identified. In seeking

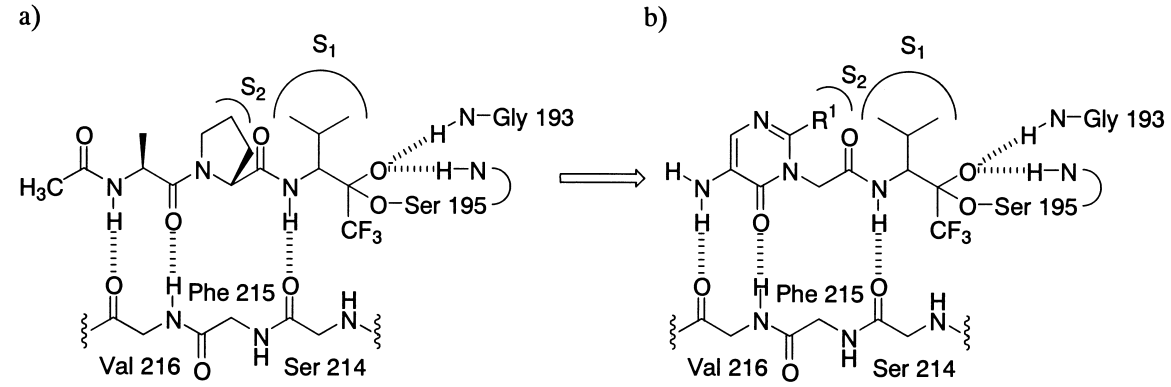


Figure 1. Schematic diagrams of the key interactions within the active site model of human leukocyte elastase with a peptide inhibitor (a) and a pyrimidinone inhibitor (b) found in the crystal structure (refs 31 and 32).

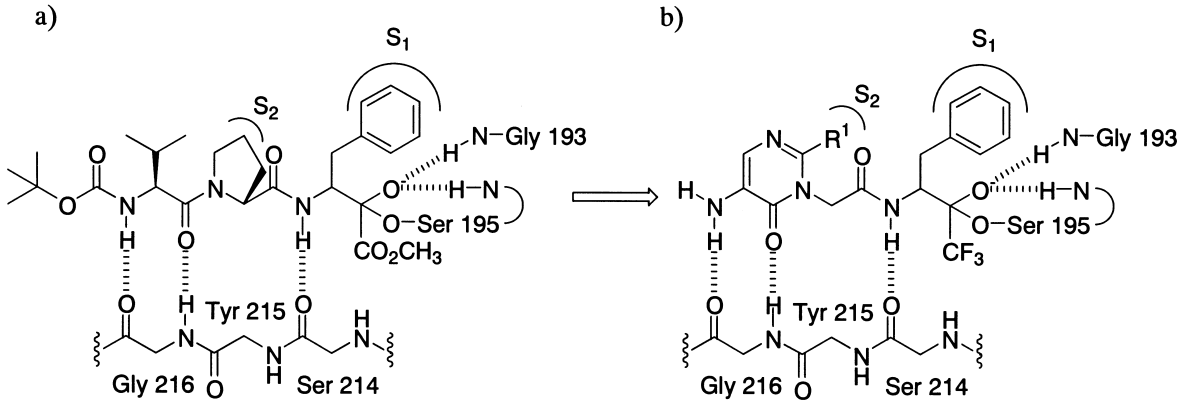
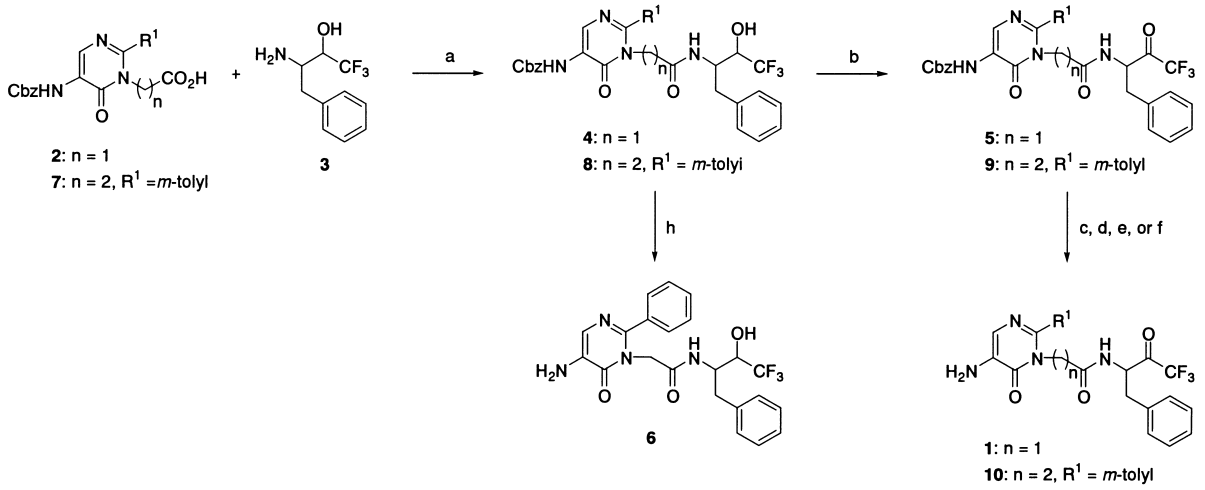


Figure 2. (a) Schematic diagram of the key interactions of a peptide inhibitor complexed to human chymase based on the docking study described in ref 28; (b) hypothetical binding mode of pyrimidinyl trifluoromethyl ketones in human chymase.

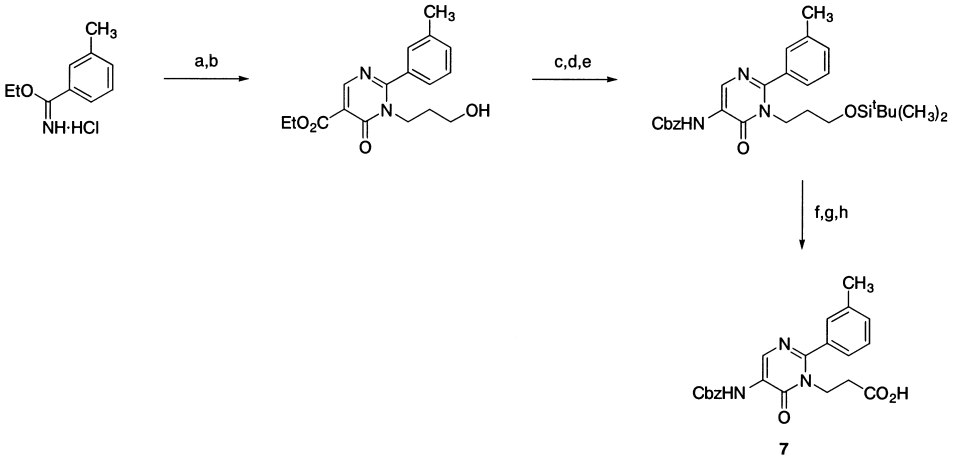


Scheme 1. Generic group R^1 is defined in Table 2. Reagents: (a) EDC, HOBt, DMF; (b) EDC, Cl_2CHCO_2H, DMSO, toluene; (c) CF_3SO_3H, anisole, CH_2Cl_2; (d) HCO_2H, Pd/C, MeOH; (e) H_2, Pd/C, MeOH, THF, 1N HCl; (f) H_2, Pd/C, $HClO_4$, AcOH; (h) H_2, Pd/C, MeOH.

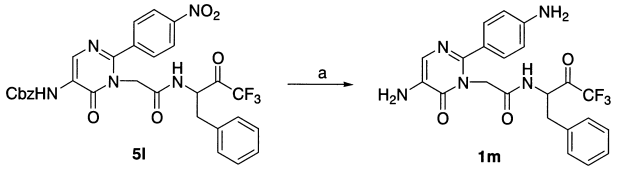
such non-peptidic derivatives, our aim was to elucidate the pathophysiological role of chymase and to develop therapeutic agents for chymase-induced disease.

Initially, we hypothesized that a pyrimidinone structure would function effectively as a scaffold for a non-peptidic chymase inhibitor in the same way as for an elastase inhibitor. Human leukocyte elastase, which is also a serine protease, recognizes α-lower alkyl amino acids such as valine and isoleucine in the S_1 site of the substrate-specific pocket. Recently, Veale et al. reported the development of non-peptidic trifluoromethyl ketone (TFMK) derivatives as elastase inhibitors based on the binding mode of a peptidic inhibitor.[30,31] As shown in Figure 1, the P_2 proline and P_3 alanine residues could be replaced with the proposed dipeptide isostere of pyrimidinone. Indeed, X-ray crystallographic analysis confirms that, like a peptidic inhibitor, this pyrimidinone derivative forms an anti-parallel β-sheet binding arrangement with Ser-214 to Val-216 of elastase.[31,32] Meanwhile, chymase recognizes aromatic amino acids at the S_1 site such as phenylalanine and tyrosine. Burzycki et al. have reported on peptidic chymase inhibitors with the typical structure shown in Figure 2(a).[25] We constructed a three-dimensional model of human chymase utilizing the coordinate data of rat mast cell protease-II,[33] and predicted that the P_3–P_1 residue of the peptidic inhibitor would form an anti-parallel β-sheet binding arrangement with Ser-214 to Gly-216 of chymase, which corresponds to the residues of the elastase inhibitor.[28] This prediction was confirmed by a recent report on the crystal structure of human chymase in complex with peptidic compounds having a chloromethyl ketone.[34] We therefore decided to use a pyrimidinone skeleton as the basic framework of the chymase inhibitor.

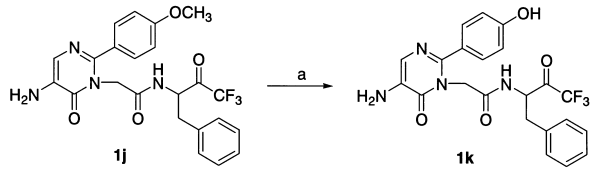
In the present report, we describe the structure–activity relationships of a series of 5-amino-6-oxo-1,6-dihydropyrimidine-containing TFMKs, and enzyme selectivities and pharmacokinetic profiles of a range of compounds representative of the series. As a result of our studies, we discovered a compound with more potent chymase-inhibitory activity than the peptidic



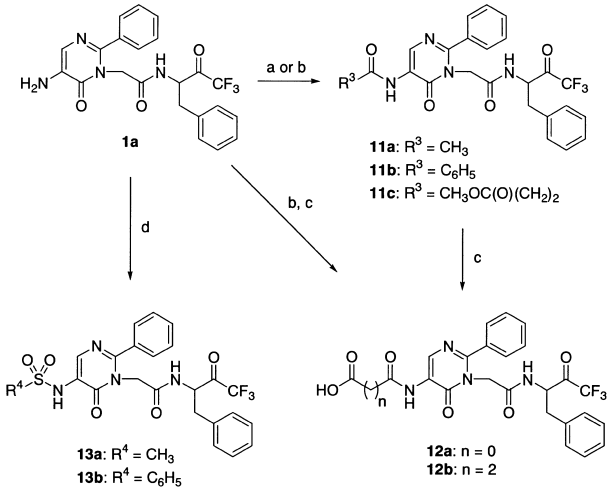
Scheme 2. Reagents: (a) $H_2N(CH_2)_3OH$, EtOH; (b) diethyl ethoxymethylenemalonate, EtOH, Δ; (c) *tert*-butyldimethylsilyl triflate, 2,6-lutidine, CH_2Cl_2; (d) LiI, pyridine, Δ; (e) diphenylphosphoryl azide, Et_3N, 1,4-dioxane, Δ then benzyl alcohol; (f) Bu_4NF, THF; (g) SO_3·pyridine, Et_3N, DMSO, CH_2Cl_2; (h) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^tBuOH, H_2O.



Scheme 3. Reagents: (a) H_2, Pd/C, $HClO_4$, AcOH.



Scheme 4. Reagents: (a) BBr_3, CH_2Cl_2.



Scheme 5. Reagents: (a) $R^3C(O)Cl$, Na_2CO_3, THF; (b) CH_3O-$C(O)(CH_2)_nC(O)Cl$, Na_2CO_3, THF; (c) 0.1N NaOH, THF; (d) R^4SO_2Cl, pyridine, THF.

inhibitor and another compound which was orally absorbed and maintained high plasma levels for several hours.

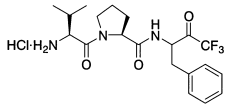
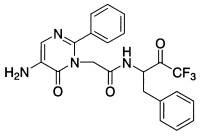
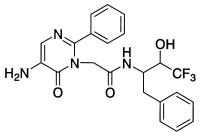
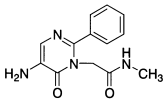
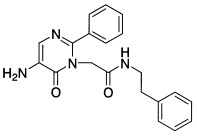
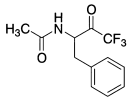
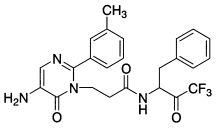
Chemistry

The synthetic method of TFMK-substituted 5-amino-pyrimidine-6-one derivatives used as the basic framework is shown in Scheme 1. The oxopyrimidinylacetic and propionic acids **2** and **7** were prepared using the method reported by Veale et al.,[31] as shown in Scheme 2. The acids **2** and **7** and the amine **3**, prepared by the Peet method,[35] were condensed using EDC and HOBT to give the amides **4** and **8**. Modified Pfitzner–Moffatt oxidation[36] followed by removal of the carbonylbenzyloxy (Cbz) group with trifluoromethanesulfonic acid in the presence of anisole or by hydrogenolysis furnished the desired compounds **1** and **10**. Compound **1m** was directly formed by hydrogenolysis of the 4-nitrophenyl derivative **5l** using perchloric acid–acetic acid as a solvent (Scheme 3). Removal of the Cbz group from compound **4a** afforded the trifluoromethyl alcohol **6**.

The 2-(4-hydroxyphenyl)-substituted pyrimidinone **1k** was obtained by demethylation of **1j** with boron tribromide (Scheme 4).

Modifications of the 5-amino substituent of the pyrimidinone ring are shown in Scheme 5. Acylations and sulfonylations of the amine **1a** were achieved by treatment with the corresponding acylating reagents in the presence of sodium carbonate, or sulfonylating reagents in the presence of pyridine. After introduction of a

Table 1. Enzyme inhibitory activities of peptide compounds and pyrimidinone analogues

Compd	Structure	Inhibitory activity K_i (μM)[a]	
		Chymase	Chymotrypsin
14		0.0821±0.0024	4.40±0.77
1a		0.389±0.032	0.296±0.074
6		38.5±15.1	> 100
15a		84.8±40.3	> 100
15b		111±68	> 100
16		21.6±6.3	24.1±12.2
10		39.9±9.1	> 100
Chymostatin		0.0131±0.0014	0.00936±0.00219

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

methyl oxalyl or a methyl succinyl moiety, basic hydrolysis afforded the terminal carboxy derivatives **12a** and **b**.

^{1}H NMR measurements of TFMK-substituted compounds in DMSO-d_6 indicated that the ketone activated by the trifluoromethyl group was immediately converted to hydrate form by a slight amount of moisture in the solvent (^{1}H NMR data for the hydrate form are reported in the Experimental section).

Results and Discussion

First of all, to confirm that the 5-aminopyrimidinone unit functions as a substitute for the Val-Pro unit and that the interaction with the enzyme shown in Figure 2(b) is essential to potent inhibitors, we investigated the activity of the compounds listed in Table 1. Human heart chymase- and bovine pancreatic α-chymotrypsin-inhibitory activities (K_is) were determined using previously described methods[27] with the synthetic substrate Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide. The structurally optimized peptidic inhibitor **14** had K_i values of 0.0821 and 4.40 μM toward chymase and chymotrypsin, respectively. The compound **1a**, bearing a 2-phenylpyrimidinone unit instead of the Val-Pro unit, was slightly less potent toward chymase and 10-fold more potent toward chymotrypsin than the peptidic compound **14**. This confirmed that the Val-Pro unit is replaceable with 2-phenylpyrimidinone. Compound **6**, which no longer has the carbonyl thought to bind to the Ser-195 of chymase, was almost 100-fold less potent than **1a**. Removal of either the P_1 group and the TFMK unit or the TFMK unit alone resulted in further loss of inhibitory activity (**15a**,**b**). Similarly, removal of the pyrimidinone unit required for interaction with the S_2 pocket and of the hydrogen bond with Gly-216 afforded a 100-fold less potent compound, **16**. Insertion of one methylene between the amide carbonyl and the pyrimidinone unit also had a detrimental effect on chymase-inhibitory activity (**10**). (Substitution of the *m*-tolyl group at the 2-position of the pyrimidinone ring had a better effect on inhibitory activity, as described below.) Accordingly, we decided that **1a** is the minimum essential to inhibit chymase.

We next studied the structure–activity relationship of compounds with substituents at the 2-position of the pyrimidinone ring (Table 2). Compared to the phenyl-substituted compound **1a**, the non-substituted compound **1b** is 100-fold less potent in both chymase- and

Table 2. Enzyme inhibitory activities of 2-substituted pyrimidinone analogues

Compd	R^1	Inhibitory activity K_i (μM)[a]	
		Chymase	Chymotrypsin
1a	C_6H_5	0.389±0.032	0.296±0.074
1b	H	61.8±22.3	49.3±12.5
1c	CH_3	>1000	>100
1d	3-FC_6H_4	0.141±0.009	4.45±0.54
1e	4-FC_6H_4	0.305±0.066	0.463±0.074
1f	3-ClC_6H_4	0.124±0.008	7.07±1.19
1g	4-ClC_6H_4	0.471±0.058	1.30±0.27
1h	3-$(CH_3)C_6H_4$	0.0506±0.0142	0.455±0.117
1i	4-(CH_3) C_6H_4	0.186±0.034	0.446±0.054
1j	4-(CH_3O) C_6H_4	0.487±0.094	0.605±0.136
1k	4-(HO) C_6H_4	0.479±0.069	1.02±0.13
1l	4-(NO_2) C_6H_4	0.547±0.141	2.32±0.48
1m	4-(NH_2) C_6H_4	0.120±0.011	0.254±0.022
1n	3-Pyridyl	0.658±0.105	0.877±0.124
1o	4-Pyridyl	1.22±0.18	1.57±0.11
1p	2-Thienyl	0.377±0.025	0.224±0.045

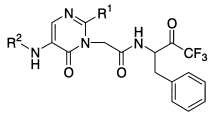
[a]Values are means±SEM of three independent experiments.

Table 4. Enzyme selectivity data for compound **1h**

Enzyme	K_i (μM)[a]
Human chymase	0.0506±0.0142
Bovine pancreatic chymotrypsin	0.455±0.117
Human cathepsin G	6.04±0.66
Human leukocyte elastase	NI (10 μM)[b]
Human plasma thrombin	NI (10 μM)[b]
Human angiotensin-converting enzyme	NI (10 μM)[b]

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

Table 3. Enzyme inhibitory activities of 5-(substituted amino)pyrimidinone analogues



Compd	R^1	R^2	Inhibitory activity K_i (μM)[a]	
			Chymase	Chymotrypsin
1a	C_6H_5	H	0.389±0.032	0.296±0.074
5a	C_6H_5	$C_6H_5CH_2OC(O)$	0.146±0.046	0.101±0.029
11a	C_6H_5	$CH_3C(O)$	0.105±0.011	0.141±0.046
11b	C_6H_5	$C_6H_5C(O)$	0.306±0.029	0.364±0.079
11c	C_6H_5	$CH_3OC(O)$ $(CH_2)_2C(O)$	0.229 ±0.041	3.60±0.50
12a	C_6H_5	$HOC(O)C(O)$	4.47±0.25	16.2±2.9
12b	C_6H_5	$HOC(O)$ $(CH_2)_2C(O)$	0.0656±0.0073	0.0899±0.0141
12c	4-FC_6H_4	$HOC(O)$ $(CH_2)_2C(O)$	0.237±0.030	4.29±0.57
13a	C_6H_5	CH_3SO_2	1.03±0.03	0.334±0.032
13b	C_6H_5	$C_6H_5SO_2$	0.369±0.127	0.188±0.037

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

chymotrypsin-inhibitory activity, while the methyl-substituted compound **1c** completely lacked chymase-inhibitory activity. These results suggest that a phenyl substitution at the 2-position of the pyrimidinone ring generates a lipophilic interaction with the S_2 pocket of the enzyme, as observed with elastase inhibitor.[31]

Next, the effect of substituents on this phenyl ring was investigated. Introduction of fluoro, chloro and methyl at the 4-position of the phenyl ring had no effect on inhibitory activity (**1e**,**g**,**i**), whereas introduction of these groups at the 3-position increased activity (**1d**,**f**,**h**). In particular, the *m*-tolyl analogue **1h** was 10-fold more potent than **1a**, and superior in potency to the parent peptidic inhibitor **14**. Regarding chymotrypsin, on the other hand, introduction of these substituents at the 3-position only maintained or reduced inhibitory activity. Substitution with an electron-releasing methoxy group (**1j**) and an electron-withdrawing nitro group (**1l**) at the 4-position of the phenyl ring resulted in similar levels of chymase-inhibitory activity. This suggests that electronic factors are not involved in the interaction of the phenyl group with the enzyme. The 3-substitutent on the phenyl ring therefore probably acts sterically by improving chymase-binding through additive interaction. Other substitutions (**1k**–**m**) and replacement of phenyl with heteroaryl (**1n**–**p**) did not increase potency.

We next examined the influence of substituents on the 5-amino group of the pyrimidinone ring (Table 3). Substitution with Cbz, acetyl, benzoyl and methoxysuccinyl groups did not much affect inhibitory activity (**5a**, **11a**–**c**). Carboxyl groups introduced at an altered distance from the pyrimidinone ring showed some effect on activity. Compound **12a** with an oxalo group was over 10-fold less potent than **1a**, but introduction of a succinyl group resulted in an increase in inhibitory activities toward both chymase and chymotrypsin (**12b**). Methanesulfonyl and benzenesulfonyl substitution did not substantially influence potency (**13a**,**b**). Bernstein et al. reported that, in their elastase inhibitor containing a pyridinone skeleton as a backbone mimetic, substituents of the corresponding amino group did not settle in a specific pocket of the enzyme but protruded above the enzyme surface.[37] They also noted that the increased inhibitory activity of their 5-acyl and sulfonyl amino derivatives was attributable to increased donor activity of the hydrogen on the amino group. Such an acyl effect was not observed in our chymase inhibitors, and the fact that this series of compounds except **12b** did not much increase chymase-inhibitory activity suggests that, in chymase too, substituents on this site may be placed on the solvent-exposed surface. This finding was significant, as it allowed the physicochemical properties of the inhibitors to be improved without loss of activity.

Compound **1h**, the most potent inhibitor of chymase, was measured for its inhibitory activity toward other representative proteases (Table 4). It inhibited only chymotrypsin type serine proteases, with activity toward cathepsin G 100-fold less potent than that toward chymase. Data on enzyme specificity (Tables 2 and 4) suggested that this series of inhibitors utilizes S_2–P_2 interaction to discriminate between related enzymes.

We examined plasma concentration levels after intravenous (iv) and oral administration to rats of the representative compounds **1e** and **12c**, which has a succinyl group and is more water-soluble. The results are shown in Figure 3 and the pharmacokinetic parameters summarized in Tables 5 and 6. Compounds **1e** and **12c** were dosed in aqueous solutions containing DMSO and propylene glycol, respectively. Blood samples were obtained at 5, 10, 30 and 60 min after iv administration and at 10,

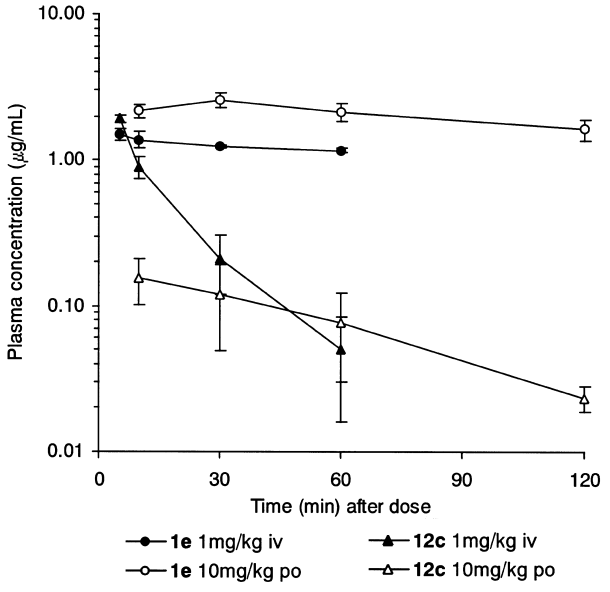


Figure 3. Plasma concentration profiles of **1e** and **12c** in rats.

Table 5. Pharmacokinetic parameters of 1 mg/kg intravenous dose in rats

Compd	n[a]	AUC_{iv}[b] (μg·h/mL)	$t_{1/2}$[c] (h)	CL[d] (L/h/kg)	Vd_{ss}[e] (L/kg)
1e	3	6.74±2.13	3.2±1.2	0.16±0.06	0.68±0.05
12c	3	0.54±0.11	0.2±0.0	1.88±0.34	0.40±0.03

[a]n = number of animals.
[b]Integrated area under plasma concentration versus time curve from time 0 to time infinity.
[c]Pharmacokinetic half-life.
[d]Plasma clearance.
[e]Steady-state volume of distribution.

Table 6. Pharmacokinetic parameters of 10 mg/kg oral dose in rats

Compd	n[a]	C_{max}[b] (μg/mL)	T_{max}[c] (h)	AUC_{po}[d] (μg·h/mL)	BA[e] (%)
1e	5	2.59±0.31	0.5±0.0	9.79±2.39	14.5±3.5
12c	4	0.18±0.05	0.3±0.2	0.18±0.06	3.4±1.1

[a]n = number of animals.
[b]Maximum plasma concentration of unchanged drug in plasma recorded in the period 0–2 h post-dose.
[c]Time of maximum concentration.
[d]Integrated area under plasma concentration versus time curve from time 0 to time infinity.
[e]Oral bioavailability: $(AUC_{po} \times dose_{iv})/(AUC_{iv} \times dose_{po}) \times 100$.

30, 60 and 120 min after oral administration. Levels of **1e** and **12c** were determined by HPLC. It was found that **1e** is absorbed rapidly following oral administration and has moderate plasma clearance, while **12c** is quickly eliminated from plasma. Since another experiment revealed that 59.9% of total iv dosing of **12c** is excreted in bile, an organic anion transporter in hepatocytes may be involved in this elimination.[38,39]

Our findings suggest that the non-peptidic chymase inhibitors reported here may prove useful as tools to elucidate the physiological and pathological roles of chymase and as therapeutic agents for chymase-induced disease.

Conclusion

We designed non-peptidic chymase inhibitors based on the predicted binding mode of the peptidic inhibitor Val-Pro-Phe-CF_3 and demonstrated that the Val-Pro unit is replaceable with a (5-amino-6-oxo-2-phenyl-1-pyrimidinyl)acetyl moiety. The aromatic ring at the 2-position of the pyrimidinone ring is essential to maintain potent chymase-inhibitory activity. The compound **1h**, in particular, has an inhibitory constant of 0.0506 μM toward chymase, an activity superior in potency to that of the parent peptidic inhibitor Val-Pro-Phe-CF_3, and has good selectivity for chymase over other proteases. Pharmacokinetic studies in rats demonstrated that compound **1e** is orally absorbed and maintains high plasma levels for at least 2 h. The derivatives reported here may thus prove useful as tools to elucidate the pathophysiological roles of chymase and as therapeutic agents for chymase-induced disease. Further study of these derivatives with a view to development of clinical candidates is under way and results will be presented shortly.

Experimental

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; br, broad. Mass spectra (MS) were recorded on a Hitachi M-2000 instrument operating in the chemical ionization (CI) mode. Elemental analyses for carbon, hydrogen and nitrogen were determined 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 are 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.

2-(5-Benzyloxycarbonylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)acetamide (4a, $R^1 = C_6H_5$). A solution of (5-benzyloxycarbonylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)acetic acid[31] (**2**, $R^1 = C_6H_5$) (8.57 g, 22.6 mmol) and 3-amino-1,1,1-trifluoro-4-phenyl-2-butanol[35] (**3**) (5.91 g, 27.2 mmol), HOBT (6.10 g, 45.1 mmol), and EDC (5.20 g, 27.2 mmol) in DMF (75 mL) was stirred at room temperature for 16 h. The reaction mixture was poured into 0.5N HCl (500 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 purified by silica gel column chromatography (83:17 chloroform: ethyl acetate) to give **4a** (11.4 g, 87% yield) as colorless crystals: mp 198–202 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.85 (s, 1H, NH), 8.43 (s, 1H, CH=N), 8.32 (d, J = 8.9 Hz, 1H, NH), 7.54–7.08 (m, 15H, ArH), 6.70 (d, J = 7.1 Hz, 1H, OH), 5.19 (s, 2H, CH_2–O), 4.41 (d, J = 16.3 Hz, 1H, CH*H*–N), 4.25 (d, J = 16.3 Hz, 1H, CH*H*–N), 4.07 (m, 1H, CH–O), 3.90 (m, 1H, CH–N), 2.92 (dd, J = 14.1, 2.6 Hz, 1H, CH*H*–Ph), 2.75 (dd, J = 14.1, 10.4 Hz, 1H, CH*H*–Ph).

2-(5-Benzyloxycarbonylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (5a). To a solution of **4** (2.00 g, 3.44 mmol) in DMSO (15 mL) and toluene (15 mL) were added EDC (6.60 g, 34.4 mmol) and then dichloroacetic acid (1.1 mL, 13 mmol). After stirring at room temperature for 2.5 h, the reaction mixture was poured into 1N HCl (150 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 purified by silica gel column chromatography (83:17 chloroform:ethyl acetate) to give **5a** (1.29 g, 65% yield) as colorless crystals. Recrystallization from 1:1 chloroform:hexane afforded 858 mg of colorless crystals: mp 186–188 °C; ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 8.40 (s, 1H, CH=N), 7.50 (t, J = 7.3 Hz, 1H, ArH), 7.44 (d, J = 7.1 Hz, 2H, ArH), 7.42–7.30 (m, 7 H, ArH), 7.22–7.10 (m, 5H, ArH), 5.18 (s, 2H, CH_2–O), 4.43–4.21 (m, 3H, CH_2–N, CH–N), 3.12 (dd, J = 14.1, 2.1 Hz, 1H, CH*H*–Ph), 2.60 (dd, J = 14.1, 11.4 Hz, 1H, CH*H*–Ph); MS (CI) m/z 579 (MH^+). Anal. calcd for $C_{30}H_{25}F_3N_4O_5 \cdot 1.5H_2O$: C, 59.50; H, 4.66; N, 9.25; found: C, 59.27; H, 4.68; N, 9.27.

2-(5-Amino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1a). To a solution of **5a** (7.26 g, 12.5 mmol) and anisole (4.3 mL, 40 mmol) in dichloromethane (75 mL) was added trifluoromethanesulfonic acid (6.0 mL, 68 mmol) with ice-water cooling. The reaction mixture was stirred at 0 °C to room temperature for 1 h and then cooled with ice-water, at which time saturated $NaHCO_3$

(200 mL) was added. The resulting mixture was extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and concentrated. The residue was crystallized with diethyl ether to give **1a** (5.16 g, 92% yield) as pale yellow crystals: mp 208–211 °C; ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 7.45 (t, J = 7.3 Hz, 1H, ArH), 7.35–7.09 (m, 10H, ArH), 4.36–4.18 (m, 3H, CH_2–N, CH–N), 3.12 (dd, J = 14.1, 2.2 Hz, 1H, CH*H*–Ph), 2.61 (dd, J = 14.1, 11.5 Hz, 1H, CH*H*–Ph); MS (CI) m/z 445 (MH^+). Anal. calcd for $C_{22}H_{19}F_3N_4O_3 \cdot 0.5\ H_2O$: C, 58.28; H, 4.45; N, 12.36; found: C, 57.94; H, 4.44; N, 12.22.

2-(5-Amino-6-oxo-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1b). To a solution of **5b** (R^1 = H, 385 mg, 0.766 mmol) in methanol (6 mL) and formic acid (0.3 mL) was added 10% palladium/carbon (165 mg) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 22 h. Palladium/carbon was removed by filtration and washed with ethyl acetate. The filtrate was poured into saturated $NaHCO_3$ (50 mL) and 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 **1b** (100 mg, 35% yield) as colorless crystals. Recrystallization from chloroform afforded 65 mg of colorless crystals: mp 208–211 °C; ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 7.28 (s, 1H, CH=N), 7.27–7.21 (m, 4H, ArH), 7.18–7.14 (m, 2H, ArH), 4.53 (d, J = 15.9 Hz, 1H, CH*H*–N), 4.37 (d, J = 15.9 Hz, 1H, CH*H*–N), 4.23 (dd, J = 11.5, 2.4 Hz, 1H, CH–N), 3.12 (dd, J = 13.7, 2.4 Hz, 1H, CH*H*–Ph), 2.65 (dd, J = 13.7, 11.5 Hz, 1H, CH*H*–Ph); MS (CI) m/z 369 (MH^+). Anal. calcd for $C_{16}H_{15}F_3N_4O_3 \cdot 1.0H_2O$: C, 49.74; H, 4.44; N, 14.50; found: C, 49.87; H, 4.54; N, 14.35.

2-(5-Amino-2-methyl-6-oxo-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1c). Compound **5c** (R^1 = CH_3, 1.42 g, 2.75 mmol) was deprotected as described for **1b** to give **1c** (435 mg, 41% yield) as colorless crystals: mp 117–120 °C; ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 7.27–7.21 (m, 4H, ArH), 7.17 (m, 1H, ArH), 7.10 (s, 1H, CH=N), 4.77 (d, J = 16.8 Hz, 1H, CH*H*–N), 4.36–4.28 (m, 2H, CH*H*–N, CH–N), 3.15 (dd, J = 13.6, 2.7 Hz, 1H, CH*H*–Ph), 2.64 (dd, J = 13.6, 12.1 Hz, 1H, CH*H*–Ph), 1.78 (s, 3H, CH_3); MS (CI) m/z 383 (MH^+). Anal. calcd for $C_{17}H_{17}F_3N_4O_3 \cdot 1.0H_2O$: C, 51.00; H, 4.78; N, 13.99; found: C, 51.03; H, 4.77; N, 13.87.

2-[5-Amino-2-(3-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1d). Compound **5d** (R^1 = 3-F–C_6H_4, 4.02 g, 6.74 mmol) was deprotected as described for **1a** to give **1d** (1.72 g, 55% yield) as colorless crystals: mp 198–201 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (d, J = 9.7 Hz, 1H, NH), 7.40–7.00 (m, 12H, ArH×10, OH×2), 5.18 (br s, 2H, NH_2), 4.45–4.10 (m, 3H, CH_2–N, CH–N), 3.18–3.00 (m, 1H, CH*H*–Ph), 2.70–2.48 (m, 1H, CH*H*–Ph). Anal. calcd for $C_{22}H_{18}F_4N_4O_3 \cdot 1.0H_2O$: C, 55.00; H, 4.20; N, 11.66; found: C, 55.13; H, 4.27; N, 11.65.

2-[5-Amino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1e). Compound **5e** (R^1 = 4-F–C_6H_4, 2.02 g, 3.39 mmol) was deprotected as described for **1a** to give **1d** (1.18 g, 75% yield) as colorless crystals: mp 133–135 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.10 (d, J = 9.7 Hz, 1H, NH), 7.30 (dd, J = 8.7, 5.5 Hz, 2H), 7.26 (s, 1H, CH=N), 7.20–7.13 (m, 5H, ArH), 7.12–7.05 (m, 4H, ArH×2, OH×2), 5.12 (s, 2H, NH_2), 4.35 (d, J = 16.6 Hz, 1H, CH*H*–N), 4.27–4.18 (m, 2H, CH*H*–N, CH–N), 3.11 (dd, J = 14.0, 2.2 Hz, 1H, CH*H*–Ph), 2.60 (dd, J = 14.0, 11.4 Hz, 1H, CH*H*–Ph); MS (CI) m/z 481 (MH^+). Anal. calcd for $C_{22}H_{18}F_4N_4O_3 \cdot 1.2H_2O$: C, 54.59; H, 4.25; N, 11.58; found: C, 54.22; H, 4.06; N, 11.36.

2-[5-Amino-2-(3-chlorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1f). Compound **5f** (R^1 = 3-Cl–C_6H_4, 2.22 g, 3.62 mmol) was deprotected as described for **1a** to give **1f** (1.18 g, 68% yield) as colorless crystals: mp 127–130 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (d, J = 9.7 Hz, 1H, NH), 7.70–6.90 (m, 12H, ArH×10, OH×2), 5.19 (br s, 2H, NH_2), 4.50–4.10 (m, 3H, CH_2–N, CH–N), 3.20–2.45 (m, 2H, CH_2–Ph). Anal. calcd for $C_{22}H_{18}ClF_3N_4O_3 \cdot 0.8H_2O$: C, 53.57; H, 4.01; N, 11.36; found: C, 53.92; H, 4.11; N, 11.21.

2-[5-Amino-2-(4-chlorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1g). Compound **5g** (R^1 = 4-Cl–C_6H_4, 561 mg, 0.915 mmol) was deprotected as described for **1a** to give **1g** (372 mg, 85% yield) as colorless crystals: mp 197–200 °C; ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 7.33 (d, J = 8.5 Hz, 2H, ArH), 7.29 (s, 1H. CH=N), 7.26 (d, J = 8.5 Hz, 2H, ArH), 7.20–7.13 (m, 5H, ArH), 4.38 (d, J = 16.4 Hz, 1H, CH*H*–N), 4.27–4.17 (m, 2H, CH*H*–N, CH–N), 3.11 (dd, J = 14.0, 2.4 Hz, 1H, CH*H*–Ph), 2.60 (dd, J = 14.0, 11.6 Hz, 1H, CH*H*–Ph); MS (CI) m/z 479, 481 (MH^+). Anal. calcd for $C_{22}H_{18}ClF_3N_4O_3 \cdot 0.7H_2O$: C, 53.77; H, 3.98; N, 11.40; found: C, 53.67; H, 4.09; N, 11.13.

2-[5-Amino-6-oxo-2-(*m*-tolyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1h). To a solution of **5h** (R^1 = 3-CH_3–C_6H_4, 1.28 g, 2.16 mmol) in methanol (20 mL) and THF (20 mL) were added 1N HCl (0.4 mL), followed by 10% palladium/carbon (460 mg) under a nitrogen atmosphere. The resulting mixture was stirred at room temperature for 6 h under a hydrogen atmosphere. Palladium/carbon was removed by filtration and washed with methanol. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (95:5 chloroform:methanol) to give **1h** (330 mg, 33% yield) as pale yellow crystals. Recrystallization from 4:1 chloroform: hexane afforded 149 mg of colorless crystals: mp 177–181 °C; ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 7.34 (s, 1H, CH=N), 7.30 (d, J = 7.7 Hz, 1H, ArH), 7.22 (t, J = 7.7 Hz, 1H, ArH), 7.10–7.21 (m, 6H, ArH), 7.02 (d, J = 7.7 Hz, 1H, ArH), 4.39 (d, J = 16.2 Hz, 1H, CH*H*–N), 4.31 (d, J = 16.2 Hz, 1H, CH*H*–N), 4.21 (dd, J = 11.3, 2.3 Hz, 1H, CH–N), 3.12 (dd, J = 14.1, 2.3 Hz,

1H, CH*H*–Ph), 2.61 (dd, $J=14.1$, 11.3 Hz, 1H, CH*H*–Ph), 2.29 (s, 3H, CH_3); MS (CI) m/z 459 (MH^+). Anal. calcd for $C_{23}H_{21}F_3N_4O_3\cdot0.5H_2O$: C, 59.10; H, 4.74; N, 11.99; found: C, 58.99; H, 4.72; N, 11.89.

2-[5-Amino-6-oxo-2-(*p*-tolyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1i). Compound **5i** ($R^1=4$-CH_3–C_6H_4, 500 mg, 0.844 mmol) was deprotected as described for **1b** to give **1i** (235 mg, 61% yield) as colorless crystals: mp 200–203 °C; ^{1}H NMR (500 MHz, DMSO-d_6+D_2O) δ 7.31 (s, 1H, CH=N), 7.22–7.09 (m, 9H, ArH), 4.36 (d, $J=16.3$ Hz, 1H, CH*H*–N), 4.28–4.20 (m, 2H, CH*H*–N, CH–N), 3.13 (dd, $J=14.2$, 2.4 Hz, 1H, CH*H*–Ph), 2.62 (dd, $J=14.2$, 11.4 Hz, 1H, CH*H*–Ph), 2.36 (s, 3H, CH_3); MS (CI) m/z 459 (MH^+). Anal. calcd for $C_{23}H_{21}F_3N_4O_3\cdot0.2H_2O$: C, 59.79; H, 4.67; N, 12.13; found: C, 59.63; H, 4.54; N, 12.08.

2-[5-Amino-2-(4-methoxyphenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)-acetamide (1j). Compound **5j** ($R^1=4$-CH_3O–C_6H_4, 497 mg, 0.817 mmol) was deprotected as described for **1a** to give **1j** (377 mg, 97% yield) as colorless crystals: mp 137–140 °C; ^{1}H NMR (500 MHz, DMSO-d_6+D_2O) δ 7.30 (s, 1H, CH=N), 7.24–7.15 (m, 7H, ArH), 6.82 (d, $J=8.8$ Hz, 2H, ArH), 4.37 (d, $J=16.3$ Hz, 1H, CH*H*–N), 4.31–4.23 (m, 2H, CH*H*–N, CH–N), 3.81 (s, 3H, CH_3–O), 3.13 (dd, $J=14.2$, 2.2 Hz, 1H, CH*H*–Ph), 2.63 (dd, $J=14.2$, 11.5 Hz, 1H, CH*H*–Ph); MS (CI) m/z 475 (MH^+). Anal. calcd for $C_{23}H_{21}F_3N_4O_4\cdot1.0H_2O$: C, 56.10; H, 4.71; N, 11.38; found: C, 56.25; H, 4.58; N, 11.51.

2-[5-Amino-2-(4-hydroxyphenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)-acetamide (1k). To a solution of **1j** (375 mg, 0.790 mmol) in dichloromethane (10 mL) was added a solution of boron tribromide in dichloromethane (1.0M, 16 mL, 16 mmol). The resulting mixture was stirred at room temperature for 24 h, at which time methanol (3 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) and further purified by reverse phase column chromatography (67:33 water:acetonitrile) to give **1k** (168 mg, 46% yield) as a pale brown solid: ^{1}H NMR (500 MHz, DMSO-d_6+D_2O) δ 7.31 (s, 1H, CH=N), 7.25–7.12 (m, 5H, ArH), 7.08 (d, $J=8.6$ Hz, 2H, ArH), 6.72 (d, $J=8.6$ Hz, 2H, ArH), 4.38 (d, $J=16.2$ Hz, 1H, CH*H*–N), 4.31 (d, $J=16.2$ Hz, 1H, CH*H*–N), 4.25 (dd, $J=11.3$, 2.1 Hz, 1H, CH–N), 3.13 (dd, $J=13.8$, 2.1 Hz, 1H, CH*H*–Ph), 2.62 (dd, $J=13.8$, 11.3 Hz, 1H, CH*H*–Ph); MS (CI) m/z 461 (MH^+). Anal. calcd for $C_{22}H_{19}F_3N_4O_4\cdot1.0H_2O$: C, 55.23; H, 4.42; N, 11.71; found: C, 55.34; H, 4.51; N, 11.51.

2-[5-Amino-2-(4-nitrophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1l). Compound **5l** ($R^1=4$-NO_2–C_6H_4, 587 mg, 0.941 mmol) was deprotected as described for **1a** to give **1l** (287 mg, 62% yield) as pale brown crystals: mp 130–134 °C; ^{1}H NMR (500 MHz, DMSO-d_6+D_2O) δ 8.12 (d, $J=8.8$ Hz, 2H, ArH), 7.52 (d, $J=8.8$ Hz, 2H, ArH), 7.33 (s, 1H, CH=N), 7.18–7.08 (m, 5H, ArH), 4.45 (d, $J=16.4$ Hz, 1H, CH*H*–N), 4.27–4.18 (m, 2H, CH*H*–N, CH–N), 3.10 (dd, $J=14.1$, 2.5 Hz, 1H, CH*H*–Ph), 2.57 (dd, $J=14.1$, 11.7 Hz, 1H, CH*H*–Ph); MS (CI) m/z 490 (MH^+). Anal. calcd for $C_{22}H_{18}F_3N_5O_5\cdot0.5H_2O$: C, 53.02; H, 3.84; N, 14.05; found: C, 52.94; H, 3.68; N, 14.02.

2-[5-Amino-2-(4-aminophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1m). To a solution of **5l** (196 mg, 0.314 mmol) in acetic acid (3 mL) and perchloric acid (70%, 3 drops) was added 10% palladium/carbon (100 mg) under a nitrogen atmosphere. The resulting mixture was stirred at room temperature for 65 h under a hydrogen atmosphere. Palladium/carbon was removed by filtration and washed with ethyl acetate. The filtrate was poured into saturated $NaHCO_3$ and extracted with ethyl acetate. The extract was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (97.5:2.5 ethyl acetate:methanol) and recrystallized from chloroform:ethyl acetate:hexane to give **1m** (68 mg, 44% yield) as pale yellow fine crystals: mp >210 °C dec.; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.17 (d, $J=9.6$ Hz, 1H, NH), 7.35–7.15 (m, 6H, ArH×5, CH=N), 7.15 (s, 1H, OH), 7.11 (s, 1H, OH), 6.94 (d, $J=8.5$ Hz, 2H, ArH), 6.45 (d, $J=8.5$ Hz, 2H, ArH), 5.46 (s, 2H, NH_2), 4.92 (s, 2H, NH_2), 4.38 (d, $J=16.1$ Hz, 1H, CH*H*–N), 4.31 (d, $J=16.1$ Hz, 1H, CH*H*–N), 4.23 (br t, $J=9.7$ Hz, 1H, CH–N), 3.14 (dd, $J=14.1$, 2.0 Hz, 1H, CH*H*–Ph), 2.65 (dd, $J=14.1$, 11.2 Hz, 1H, CH*H*–Ph); MS (CI) m/z 460 (MH^+). Anal. calcd for $C_{22}H_{20}F_3N_5O_3\cdot1.2H_2O$: C, 54.93; H, 4.69; N, 14.56; found: C, 54.71; H, 4.64; N, 14.57.

2-[5-Amino-6-oxo-2-(3-pyridyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1n). Compound **5n** ($R^1=3$-pyridyl, 200 mg, 0.345 mmol) was deprotected as described for **1h** to give **1n** (111 mg, 72% yield) as colorless crystals: mp 88–91 °C; ^{1}H NMR (500 MHz, DMSO-d_6+D_2O) δ 8.63 (dd, $J=4.8$, 1.5 Hz, 1H, ArH), 8.50 (d, $J=1.8$ Hz, 1H, ArH), 7.61 (m, 1H, ArH), 7.33 (s, 1H, CH=N), 7.32 (dd, $J=7.9$, 4.8 Hz, 1H, ArH), 7.20–7.11 (m, 5H, ArH), 4.38 (d, $J=16.9$ Hz, 1H, CH*H*–N), 4.32 (d, $J=16.9$ Hz, 1H, CH*H*–N), 4.20 (dd, $J=11.3$, 2.3 Hz, 1H, CH–N), 3.10 (dd, $J=14.0$, 2.3 Hz, 1H, CH*H*–Ph), 2.58 (dd, $J=14.0$, 11.3 Hz, 1H, CH*H*–Ph); MS (CI) m/z 446 (MH^+). Anal. calcd for $C_{21}H_{18}F_3N_5O_3\cdot1.0H_2O$: C, 54.43; H, 4.35; N, 15.11; found: C, 54.13; H, 4.19; N, 15.06.

2-[5-Amino-6-oxo-2-(4-pyridyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1o). Compound **5o** ($R^1=4$-pyridyl, 250 mg, 0.431 mmol) was deprotected as described for **1h** to give **1o** (161 mg, 84% yield) as a pale yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6+D_2O) δ 8.52 (d, $J=6.0$ Hz, 2H, ArH), 7.32 (s, 1H, CH=N), 7.24 (d, $J=6.0$ Hz, 2H, ArH), 7.19–7.13 (m, 5H, ArH), 4.38 (d, $J=16.9$ Hz, 1H, CH*H*–N), 4.29 (m, 1H, CH*H*–N), 4.25 (dd, $J=11.5$, 2.5 Hz, 1H,

CH–N), 3.11 (dd, $J=14.1$, 2.5 Hz, 1H, CH*H*–Ph), 2.59 (dd, $J=14.1$, 11.5 Hz, 1H, CH*H*–Ph); MS (CI) m/z 446 (MH^+). Anal. calcd for $C_{21}H_{18}F_3N_5O_3\cdot 1.0H_2O$: C, 54.43; H, 4.35; N, 15.11; found: C, 54.34; H, 4.33; N, 15.04.

2-[5-Amino-6-oxo-2-(2-thienyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (1p). Compound **5p** (R^1 = 2-thienyl, 760 mg, 1.30 mmol) was deprotected as described for **1a** to give **1p** (415 mg, 71% yield) as pale yellow crystals: mp 198–201 °C; ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 7.60 (d, $J=5.1$ Hz, 1H, ArH), 7.28–7.22 (m, 6H, ArH×5, CH=N), 6.91 (dd, $J=5.1$, 3.7 Hz, 1H, ArH), 6.64 (br s, 1H, ArH), 4.68 (d, $J=16.5$ Hz, 1H, CH*H*–N), 4.43 (d, $J=16.5$ Hz, 1H, CH*H*–N), 4.37 (dd, $J=11.6$, 2.4 Hz, 1H, CH–N), 3.18 (dd, $J=13.9$, 2.4 Hz, 1H, CH*H*–Ph), 2.65 (dd, $J=13.9$, 11.6 Hz, 1H, CH*H*–Ph); MS (CI) m/z 481 (MH^+). Anal. calcd for $C_{20}H_{17}F_3N_4O_3S\cdot 0.8H_2O$: C, 51.68; H, 4.03; N, 12.05; found: C, 51.75; H, 4.09; N, 11.95.

2-(5-Amino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)acetamide (6). To a solution of **4a** (290 mg, 0.500 mmol) in methanol (15 mL) was added 10% palladium/carbon (106 mg) under a nitrogen atmosphere. The resulting mixture was stirred at room temperature for 2.5 h under a hydrogen atmosphere. Palladium/carbon was removed by filtration and washed with methanol. The filtrate was concentrated, and then the residue was purified by silica gel column chromatography (91:9 chloroform:methanol) to give **6** (202 mg, 90% yield) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (d, $J=8.5$ Hz, 0.6H, NH), 8.19 (d, $J=9.2$ Hz, 0.4H, NH), 7.48–7.09 (m, 11H, ArH), 6.73 (br s, 1H, OH), 5.14 (s, 2H, NH_2), 4.47–3.82 (m, 4H, CH–O, CH_2–N, CH–N), 2.98–2.61 (m, 2H, CH_2–Ph). Anal. calcd for $C_{22}H_{21}F_3N_4O_3$: C, 59.19; H, 4.74; N, 12.55; found: C, 59.30; H, 4.78; N, 12.29.

3-[5-Benzyloxycarbonylamino-6-oxo-2-(*m*-tolyl)-1,6-dihydro-1-pyrimidinyl]propionic acid (7). To a solution of ethyl 3-methylbenzimidate hydrochloride (15.0 g, 75.1 mmol) in ethanol (75 mL) was added 3-aminopropanol (6.9 mL, 90 mmol) with ice-water cooling. After stirring at room temperature for 16 h, the reaction mixture was concentrated under reduced pressure. The residue was poured into 1N NaOH (200 mL) and extracted with chloroform. The extract was dried ($MgSO_4$) and concentrated to give *N*-(3-hydroxypropyl)-3-methylbenzamidine (17.1 g) as a colorless oil. To a solution of this amidine in ethanol (300 mL) was added dropwise diethyl ethoxymethylenemalonate (18.0 mL, 89.1 mmol). The reaction mixture was heated at 90 °C for 3 h, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (95:5 chloroform:methanol) to give ethyl 1-(3-hydroxypropyl)-2-(*m*-tolyl)pyrimidin-6(1*H*)-one-5-carboxylate (23.5 g, 99% yield) as a pale yellow oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.50 (s, 1H, CH=N), 7.45–7.35 (m, 4H, ArH), 4.39 (t, $J=5.3$ Hz, 1H, OH), 4.26 (q, $J=7.1$ Hz, 2H, CH_2–O), 3.91 (m, 2H, CH_2–N), 3.25 (t, $J=5.3$ Hz, 2H, CH_2–O), 2.38 (s, 3H, CH_3), 1.68 (m, 2H, CH_2), 1.29 (t, $J=7.1$ Hz, 3H, CH_3).

To a solution of the above intermediate (23.1 g, 73.0 mmol) and 2,6-lutidine (13 mL, 110 mmol) in dichloromethane (250 mL) was added *tert*-butyldimethylsilyl triflate (20 mL, 87 mmol) with ice-water cooling. After stirring at 0 °C to room temperature for 5 h, the reaction mixture was poured into 1N HCl (400 mL), and then extracted with chloroform. The extract was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel column chromatography (99:1 chloroform: methanol) to give ethyl 1-{3-[(*tert*-butyldimethylsilyl) oxy]propyl}-2-(*m*-tolyl)pyrimidin-6(1*H*)-one-5-carboxylate (29.6 g, 94% yield) as a pale yellow oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.49 (s, 1H, CH=N), 7.43–7.35 (m, 4H, ArH), 4.26 (q, $J=7.1$ Hz, 2H, CH_2–O), 3.94 (m, 2H, CH_2–N), 3.46 (t, $J=5.6$ Hz, 2H, CH_2–O), 2.36 (s, 3H, CH_3), 1.70 (m, 2H, CH_2), 1.28 (t, $J=7.1$ Hz, 3H, CH_3), 0.72 (s, 9H, CH_3×3), −0.11 (s, 6H, CH_3–Si×2).

A solution of the above intermediate (29.3 g, 68.0 mmol) and lithium iodide (22.0 g, 164 mmol) in pyridine (120 mL) was heated at reflux temperature for 10 h, at which time organic solvents were evaporated. The residue was poured into 2N HCl (450 mL) and then extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and concentrated. The residue was crystallized from 1:3 diethyl ether:hexane to give 1-{3-[(*tert*-butyldimethylsilyl)oxy]propyl}-2-(*m*-tolyl)pyrimidin-6(1*H*)-one-5-carboxylic acid (14.6 g, 53% yield) as colorless crystals: mp 65–66 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.15 (br s, 1H, OH), 8.66 (s, 1H, CH=N), 7.46–7.37 (m, 4H, ArH), 4.01 (m, 2H, CH_2–N), 3.48 (t, $J=5.7$ Hz, 2H, CH_2–O), 2.37 (s, 3H, CH_3), 1.74 (m, 2H, CH_2), 0.73 (s, 9H, CH_3×3), −0.10 (s, 6H, CH_3–Si×2).

To a mixture of the above intermediate (14.3 g, 35.5 mmol), 1,4-dioxane (150 mL), and triethylamine (10 mL, 72 mmol) was added dropwise diphenylphosphoryl azide (9.6 mL, 43 mmol). The resulting mixture was heated at reflux temperature for 2 h, at which time benzyl alcohol (4.8 mL, 46 mmol) was added. The mixture was further heated at reflux temperature for 12 h, cooled to room temperature, and then concentrated under reduced pressure. The residue was poured into saturated NH_4Cl (300 mL) and extracted with ethyl acetate. The extract was washed with 1N NaOH (300 mL) and brine, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel column chromatography (83:17 hexane:ethyl acetate) to give 1-[5-benzyloxycarbonylamino-6-oxo-2-(*m*-tolyl)-1,6-dihydro-1-pyrimidinyl]-3-[(*tert*-butyldimethylsilyl)oxy]propane (12.4 g, 61% yield) as a colorless oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (s, 1H, NH), 8.39 (s, 1H, CH=N), 7.44 (d, $J=7.2$ Hz, 2H, ArH), 7.42–7.30 (m, 7H, ArH), 5.18 (s, 2H, CH_2–O), 3.95 (m, 2H, CH_2–N), 3.45 (t, $J=5.8$ Hz, 2H, CH_2–O), 2.36 (s, 3H, CH_3), 1.69 (m, 2H, CH_2), 0.73 (s, 9H, CH_3×3), −0.10 (s, 6H, CH_3–Si×2).

To a solution of the above intermediate (12.0 g, 21.0 mmol) in THF (100 mL) was added tetrabutylammonium fluoride (1.0M solution in THF,

24 mL, 24 mmol). After stirring for 1.5 h, the reaction mixture was poured into water (150 mL) and extracted with ethyl acetate. The extract was washed with water and brine, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel column chromatography (50:50 hexane:ethyl acetate) to give 3-[5-benzyloxycarbonylamino-6-oxo-2-(*m*-tolyl)-1,6-dihydro-1-pyrimidinyl]propanol (8.19 g, 99% yield) as a colorless oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.79 (s, 1H, NH), 8.40 (s, 1H, CH=N), 7.44 (d, J = 7.0 Hz, 2H, ArH), 7.42–7.32 (m, 7H, ArH), 5.19 (s, 2H, CH_2–O), 4.40 (t, J = 5.3 Hz, OH), 3.91 (m, 2H, CH_2–N), 3.24 (t, J = 5.3 Hz, 2H, CH_2–O), 2.37 (s, 3H, CH_3), 1.67 (m, 2H, CH_2).

To a solution of the above intermediate (7.99 g, 20.3 mmol) in dichloromethane (60 mL) were added successively triethylamine (8.5 mL, 61 mmol) and a solution of sulfur trioxide–pyridine complex (9.70 g, 60.9 mmol) in DMSO (60 mL) with ice-water cooling. After stirring at 0 °C for 3 h, the reaction mixture was poured into a mixture of ice and brine, and then extracted with ethyl acetate. The extract was washed successively with 0.5N HCl and brine, dried ($MgSO_4$), and concentrated to give a pale brown oil (7.95 g). To a mixture of this oil, 2-methyl-2-propanol (130 mL) and 2-methyl-2-butene (22 mL, 0.21 mol) was added a solution of sodium dihydrogen phosphate dihydrate (22.2 g, 0.142 mol) and sodium chlorite (85%, 15.8 g, 0.148 mol) in water (60 mL). The resulting mixture was stirred at room temperature for 3 h, at which time organic solvents were evaporated. The residue was acidified with 2N HCl to pH 3 and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel column chromatography (95:5 chloroform:methanol) to give **7** (8.16 g, 99% yield) as a colorless solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.3 (br s, 1H, OH), 8.85 (s, 1H, NH), 8.41 (s, 1H, CH=N), 7.44 (d, J = 7.2 Hz, 2H, ArH), 7.32–7.42 (m, 7H, ArH), 5.19 (s, 2H, CH_2–O), 4.03 (t, J = 7.8 Hz, 2H, CH_2–N), 2.59 (t, J = 7.8 Hz, 2H, CH_2–C=O), 2.37 (s, 3H, CH_3).

3-[5-Benzyloxycarbonylamino-6-oxo-2-(*m*-tolyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)propanamide (8). Compound **7** (2.37 g, 5.82 mmol) was coupled with **3** using a method similar to that described for **4a** to give **8** (3.05 g, 86% yield) as colorless crystals: mp 165–168 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (s, 1H, NH), 8.39 (s, 1H, CH=N), 7.98 (d, J = 8.8 Hz, 1H, NH), 7.44 (d, J = 7.1 Hz, 2H, ArH), 7.40 (t, J = 7.1 Hz, 2H, ArH), 7.37–7.06 (m, 10H, ArH), 6.58 (d, J = 7.3 Hz, 1H, OH), 5.19 (s, 2H, CH_2–O), 4.01 (m, 1H, CH–N), 3.96–3.78 (m, 3H, CH_2–N, CH–O), 2.93 (dd, J = 13.9, 2.7 Hz, 1H, CH*H*–Ph), 2.62 (dd, J = 13.9, 11.0 Hz, 1H, CH*H*–Ph), 2.43–2.28 (m, 5H, CH_3, CH_2–C=O).

3-[5-Benzyloxycarbonylamino-6-oxo-2-(*m*-tolyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)propanamide (9). Compound **8** (2.00 g, 3.29 mmol) was oxidized using conditions described for the preparation of **5a** to give **9** (1.57 g, 79% yield) as colorless crystals: mp 119–123 °C; ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 8.78 (s, 1H, NH), 8.38 (s, 1H, CH=N), 7.80 (d, J = 9.8 Hz, 1H, NH), 7.44 (d, J = 7.1 Hz, 2H, ArH), 7.40 (t, J = 7.1 Hz, 2H, ArH), 7.37–7.32 (m, 3H, ArH), 7.29–7.25 (m, 2H, ArH), 7.11–7.05 (m, 7H, ArH×5, OH×2), 5.19 (s, 2H, CH_2–O), 4.12 (m, 1H, CH–N), 3.86 (m, 1H, CH*H*–N), 3.71 (m, 1H, CH*H*–N), 3.04 (dd, J = 13.5, 2.5 Hz, 1H, CH*H*–Ph), 2.56 (dd, J = 13.5, 11.8 Hz, 1H, CH*H*–Ph), 2.47–2.30 (m, 5H, CH_3, CH_2–C=O); MS (CI) m/z 607 (MH^+).

3-[5-Amino-6-oxo-2-(*m*-tolyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)propanamide (10). Compound **9** (500 mg, 0.824 mmol) was deprotected using conditions described for the preparation of **1a** to give **10** (1.57 g, 79% yield) as a colorless solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.78 (d, J = 9.8 Hz, 1H, NH), 7.36–7.04 (m, 12H, ArH×9, CH=N, OH×2), 5.11 (s, 2H, NH_2), 4.11 (m, 1H, CH–N), 3.84 (m, 1H, CH*H*–N), 3.68 (m, 1H, CH*H*–N), 3.04 (dd, J = 13.5, 2.6 Hz, 1H, CH*H*–Ph), 2.56 (dd, J = 13.5, 11.9 Hz, 1H, CH*H*–Ph), 2.45–2.27 (m, 5H, CH_3, CH_2–C=O); MS (CI) m/z 473 (MH^+). Anal. calcd for $C_{24}H_{23}F_3N_4O_3 \cdot 0.6H_2O$: C, 59.65; H, 5.05; N, 11.59; found: C, 59.60; H, 5.10; N, 11.54.

2-(5-Acetylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (11a). To a solution of **1a** (559 mg, 1.26 mmol) and sodium carbonate (372 mg, 3.51 mmol) in THF (10 mL) was added acetyl chloride (0.10 mL, 1.4 mmol) with ice-water cooling. After stirring at 0 °C for 2 h, the resulting mixture was poured into saturated $NaHCO_3$, and then extracted with ethyl acetate. The organic layer was washed with brine and concentrated. The residue was purified by silica gel column chromatography (95:5 chloroform:methanol) to give **11a** (509 mg, 83% yield) as colorless crystals. Recrystallization from 5:2 chloroform:hexane afforded 414 mg of colorless crystals: mp 110–112 °C; ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O) δ 8.73 (s, 1H, CH=N), 7.53 (t, J = 7.5 Hz, 1H, ArH), 7.38 (t, J = 7.5 Hz, 2H, ArH), 7.29 (d, J = 7.5 Hz, 2H, ArH), 7.23–7.13 (m, 5H, ArH), 4.42 (d, J = 15.9 Hz, 1H, CH*H*–N), 4.33 (d, J = 15.9 Hz, 1H, CH*H*–N), 4.26 (dd, J = 11.5, 2.2 Hz, 1H, CH–N), 3.13 (dd, J = 13.9, 2.2 Hz, 1H, CH*H*–Ph), 2.59 (dd, J = 13.9, 11.5 Hz, 1H, CH*H*–Ph), 2.15 (s, 3H, CH_3); MS (CI) m/z 487 (MH^+). Anal. calcd for $C_{24}H_{21}F_3N_4O_4 \cdot 1.3H_2O$: C, 56.54; H, 4.67; N, 10.99; found: C, 56.43; H, 4.51; N, 10.88.

2-(5-Benzoylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (11b). Compound **1a** (300 mg, 0.678 mmol) was reacted with benzoyl choride (126 mg, 0.908 mmol) using conditions described for the preparation of **11a** to give **11b** (271 mg, 73% yield) as colorless crystals: mp 225–227 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.42 (s, 1H, NH), 8.75 (s, 1H, CH=N), 8.21 (d, J = 9.7 Hz, 1H, NH), 7.96 (m, 2H, ArH), 7.67–7.49 (m, 4H, ArH), 7.38–7.36 (m, 4H, ArH), 7.23–7.18 (m, 5H, ArH), 7.16 (s, 1H, OH), 7.14 (s, 1H, OH), 4.44 (d, J = 16.6 Hz, 1H, CH*H*–N), 4.35 (d, J = 16.6 Hz, 1H, CH*H*–N), 4.27 (m, 1H, CH–N), 3.14 (dd, J = 14.1, 2.1 Hz, 1H, CH*H*–Ph), 2.61

(dd, $J=14.1$, 11.4 Hz, 1H, CH*H*–Ph); MS (CI) m/z 549 (MH^+). Anal. calcd for $C_{29}H_{23}F_3N_4O_4\cdot1.2H_2O$: C, 61.09; H, 4.49; N, 9.83; found: C, 60.92; H, 4.47; N, 9.64.

2-(5-Methoxysuccinylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)-acetamide (11c). Compound **1a** (600 mg, 1.36 mmol) was reacted with methyl succinyl chloride (0.22 mL, 1.8 mmol) using conditions described for the preparation of **11a** to give **11c** (576 mg, 76% yield) as colorless crystals: mp 78.5–79.5 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.52 (s, 1H, NH), 8.75 (s, 1H, CH=N), 8.15 (d, $J=9.7$ Hz, 1H, NH), 7.49 (m, 1H, ArH), 7.34 (t, $J=7.8$ Hz, 2H, ArH), 7.29 (d, $J=7.3$ Hz, 2H, ArH), 7.21–7.14 (m, 5H, ArH), 7.12 (s, 1H, OH), 7.11 (s, 1H, OH), 4.42 (d, $J=16.1$ Hz, 1H, CH*H*–N), 4.31–4.24 (m, 2H, CH*H*–N, CH–N), 3.59 (s, 3H, CH_3–O), 3.14–3.11 (m, 1H, CH*H*–Ph), 2.77 (t, $J=5.6$ Hz, 2H, CH_2–C=O), 2.62–2.56 (m, 3H, CH_2–C=O, CH*H*–Ph); MS (CI) m/z 559 (MH^+). Anal. calcd for $C_{27}H_{25}F_3N_4O_6\cdot0.5H_2O$: C, 57.14; H, 4.62; N, 9.87; found: C, 57.34; H, 5.01; N, 9.65.

2-(5-Oxaloamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (12a). Compound **1a** (600 mg, 1.36 mmol) was reacted with methyl oxalyl chloride (0.17 mL, 1.8 mmol) using conditions described for the preparation of **11a** to give 2-(5-methoxalylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)-acetamide (467 mg, 65% yield) as pale yellow crystals: mp 210–211 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.66 (s, 1H, NH), 8.73 (s, 1H, CH=N), 8.19 (d, $J=9.8$ Hz, 1H, NH), 7.52 (m, 1H, ArH), 7.39–7.32 (m, 4H, ArH), 7.21–7.14 (m, 5H, ArH), 7.13 (s, 1H, OH), 7.11 (s, 1H, H), 4.41 (d, $J=16.3$ Hz, 1H, CH*H*–N), 4.33 (d, $J=16.3$ Hz, 1H, CH*H*–N), 4.26 (m, 1H, CH–N), 3.86 (s, 3H, CH_3–O), 3.13 (dd, $J=14.2$, 2.4 Hz, 1H, CH*H*–Ph), 2.60 (dd, $J=14.2$, 11.4 Hz, 1H, CH*H*–Ph); MS (CI) m/z 531 (MH^+).

A solution of the above intermediate (350 mg, 0.660 mmol) and 0.1N NaOH (6.6 mL) in THF (20 mL) was stirred at room temperature for 2 h, at which time 1N HCl (0.7 mL) was added. The organic solvents were evaporated, after which the suspension was extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated. The residue was recrystallized from 1:5 ethyl acetate:hexane to give **12a** (313 mg, 92% yield) as pale yellow crystals: mp 193–195 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.54 (s, 1H, NH), 8.76 (s, 1H, CH=N), 8.19 (d, $J=9.8$ Hz, 1H, NH), 7.52 (m, 1H, ArH), 7.39–7.32 (m, 4H, ArH), 7.20–7.14 (m, 5H, ArH), 7.13 (s, 1H, OH), 7.11 (s, 1H, OH), 4.40 (d, $J=16.4$ Hz, 1H, CH*H*–N), 4.34 (d, $J=16.4$ Hz, 1H, CH*H*–N), 4.25 (m, 1H, CH–N), 3.13 (dd, $J=14.2$, 2.4 Hz, 1H, CH*H*–Ph), 2.60 (dd, $J=14.2$, 11.4 Hz, 1H, CH*H*–Ph); MS (CI) m/z 517 (MH^+). Anal. calcd for $C_{24}H_{19}F_3N_4O_6\cdot1.0H_2O$: C, 53.94; H, 3.96; N, 10.48; found: C, 53.95; H, 3.95; N, 10.26.

2-(5-Hydroxysuccinylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)-acetamide (12b). Compound **11c** (360 mg, 0.645 mmol) was hydrolyzed using conditions described for the preparation of **12a** to give **12b** (311 mg, 88% yield) as colorless crystals: mp 100–101 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.0–11.5 (br, 1H, OH), 9.46 (s, 1H, NH), 8.76 (s, 1H, CH=N), 8.14 (d, $J=9.7$ Hz, 1H, NH), 7.49 (m, 1H, ArH), 7.35 (t, $J=8.0$ Hz, 2H, ArH), 7.29 (d, $J=7.1$ Hz, 2H, ArH), 7.21–7.14 (m, 5H, ArH), 7.13 (s, 1H, OH), 7.11 (s, 1H, OH), 4.42 (d, $J=16.0$ Hz, 1H, CH*H*–N), 4.30 (d, $J=16.0$ Hz, 1H, CH*H*–N), 4.26 (m, 1H, CH–N), 3.13 (d, $J=14.1$, 2.2 Hz, 1H, CH*H*–Ph), 2.71 (t, $J=7.2$ Hz, 2H, CH_2–C=O), 2.60 (d, $J=14.1$, 11.4 Hz, 1H, CH*H*–Ph), 2.51–2.48 (m, 2H, CH_2–C=O); MS (CI) m/z 545 (MH^+). Anal. calcd for $C_{26}H_{23}F_3N_4O_6$: C, 57.35; H, 4.26; N, 10.29; found: C, 57.09; H, 4.52; N, 10.00.

2-[2-(4-Fluorophenyl)-5-hydroxysuccinylamino-6- oxo-1,6-dihydro-1-pyrimidinyl]-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (12c). Compound **1e** (600 mg, 1.30 mmol) was reacted with methyl succinyl chloride (0.18 mL, 1.5 mmol) using conditions described for the preparation of **11a** to give 2-[2-(4-fluorophenyl)-5-methoxysuccinylamino-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N* - (1 - benzyl - 3,3,3 - trifluoro - 2 - oxopropyl)acetamide (736 mg, 98% yield) as colorless solid. This intermediate (347 mg, 0.602 mmol) was hydrolyzed using conditions described for the preparation of **12a** to give **12c** (295 mg, 87% yield) as colorless crystals: mp 140–147 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.11 (br s, 1H, OH), 9.50 (s, 1H, NH), 8.76 (s, 1H, CH=N), 8.16 (d, $J=9.8$ Hz, 1H, NH), 7.35 (dd, $J=8.5$, 5.5 Hz, 2H, ArH), 7.21–7.11 (m, 9H, ArH, OH×2), 4.44 (d, $J=15.8$ Hz, 1H, CH*H*–N), 4.26 (m, 2H, CH*H*–N, CH–N), 3.12 (d, $J=14.1$, 2.2 Hz, 1H, CH*H*–Ph), 2.71 (t, $J=6.5$ Hz, 2H, CH_2–C=O), 2.59 (d, $J=14.1$, 11.6 Hz, 1H, CH*H*–Ph), 2.48 (t, $J=6.5$ Hz, 2H, CH_2–C=O); MS (CI) m/z 561 (MH^+). Anal. calcd for $C_{26}H_{22}F_4N_4O_6\cdot0.9H_2O$: C, 53.96; H, 4.15; N, 9.68; found: C, 53.63; H, 4.14; N, 9.48.

2-(5-Methanesulfonylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)-acetamide (13a). To a solution of **1a** (400 mg, 0.900 mmol) and pyridine (0.36 mL, 4.5 mmol) in THF (30 mL) was added methanesulfonyl chloride (0.14 mL, 1.8 mmol). After stirring at room temperature for 17 h, the reaction mixture was extracted with ethyl acetate. The organic solvents were washed with saturated potassium dihydrogen phosphate and brine, and dried ($MgSO_4$). Concentration followed by silica gel column chromatography (4:3 dichloromethane:ethyl acetate) gave a colorless oil, which was crystallized from 1:20 ethyl acetate:hexane to afford **13a** (307 mg, 65% yield) as colorless crystals: mp 93–96 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.27 (s, 1H, NH), 8.19 (d, $J=9.8$ Hz, 1H, NH), 7.95 (s, 1H, CH=N), 7.54 (m, 1H, ArH), 7.40 (m, 2H, ArH), 7.33 (m, 2H, ArH), 7.24–7.15 (m, 5H, ArH), 7.13 (s, 1H, OH), 7.10 (s, 1H, OH), 4.37 (s, 2H, CH_2–N), 4.28 (m, 1H, CH–N), 3.14 (dd, $J=14.0$, 2.0 Hz, 1H, CH*H*–Ph), 3.06 (s, 3H, CH_3–S), 2.61 (dd, $J=14.2$, 11.6 Hz, 1H, CH*H*–Ph); MS (CI) m/z 523 (MH^+). Anal. calcd for $C_{23}H_{21}F_3N_4O_5S\cdot1.0H_2O$: C, 51.11; H, 4.29; N, 10.37; found: C, 50.85; H, 4.31; N, 10.27.

2-(5-Benzenesulfonylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)-acetamide (13b). Compound **1a** (400 mg, 0.900 mmol) was reacted with benzenesulfonyl chloride (0.28 mL, 4.3 mmol) using conditions described for the preparation of **13a** to give **13b** (396 mg, 75% yield) as colorless crystals: mp 92–94 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.05 (s, 1H, NH), 8.13 (d, J = 9.7 Hz, 1H, NH), 7.94–7.90 (m, 2H, ArH), 7.87 (s, 1H, CH=N), 7.68–7.54 (m, 3H, ArH), 7.51–7.46 (m, 1H, ArH), 7.42–7.23 (m, 4H, ArH), 7.20–7.12 (m, 5H, ArH), 7.11 (s, 1H, OH), 7.10 (s, 1H, OH), 4.35–4.19 (m, 3H, CH_2–N, CH–N), 3.12 (dd, J = 14.0, 2.2 Hz, 1H, CH*H*–Ph), 2.57 (dd, J = 14.0, 11.4 Hz, 1H, CH*H*–Ph); MS (CI) m/z 585 (MH^+). Anal. calcd for $C_{28}H_{23}F_3N_4O_5S{\cdot}0.8H_2O$: C, 56.15; H, 4.14; N, 9.35; found: C, 56.11; H, 3.88; N, 9.14.

L-Valyl-*N*-(1-benzyl-3,3,3-trifluoro-2-oxopropyl)-L-prolinamide hydrochloride (14). To a solution of *tert*-butoxycarbonyl - L - valyl - *N* - (1 - benzyl - 3,3,3 - trifluoro - 2 - oxopropyl)-L-prolinamide (143 mg, 0.278 mmol) in 1,4-dioxane (2 mL) was added hydrogen chloride (4N solution in 1,4-dioxane, 5 mL, 20 mmol) with ice-water cooling. The resulting mixture was stirred at 0 °C–room temperature for 2 h, and then concentrated under reduced pressure. To the residue was added 1:1 ethyl acetate-hexane and then the mixture was concentrated under reduced pressure to give a colorless oil, which was crystallized from 1:1 ethyl acetate:hexane to afford **14** (117 mg, 94% yield) as colorless crystals: mp 125–140 °C; ^{1}H NMR (300 MHz, DMSO-d_6 + D_2O) δ 7.35–7.08 (m, 5H, ArH), 4.50–4.15 (m, 2H, CH–N×2), 3.90 (m, 1H, CH–N), 3.70–3.07 (m, 3H, CH_2–N, CH*H*–Ph), 2.75–2.60 (m, 1H, CH*H*–Ph), 2.15–1.50 (m, 4H, CH_2–C*H*H, C*H*–CH_3), 1.15–0.80 (m, 7H, CH_3×2, C*H*H); MS (CI) m/z 414 (MH^+). Anal. calcd for $C_{20}H_{26}F_3N_3O_3{\cdot}HCl{\cdot}1.0H_2O$: C, 51.34; H, 6.25; N, 8.98; found: C, 51.03; H, 6.17; N, 8.78.

2-(5-Amino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-methylacetamide (15a). Compound **2a** (759 mg, 2.00 mmol) was coupled with methylamine using a method similar to that described for **4a** to give 2-(5-benzyloxycarbonylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-methylacetamide (704 mg, 90% yield) as colorless crystals. This intermediate (589 mg, 1.50 mmol) was deprotected using conditions described for the preparation of **1b** to give **15a** (352 mg, 91% yield) as colorless crystals: mp 252–254 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.99 (q, J = 4.5 Hz, 1H, NH), 7.45 (s, 5H, ArH), 7.33 (s, 1H, CH=N), 5.14 (s, 2H, NH_2), 4.36 (s, 2H, CH_2–N), 2.57 (d, J = 4.5 Hz, 3H, CH_3–N). Anal. calcd for $C_{13}H_{14}N_4O_2{\cdot}0.2H_2O$: C, 59.62; H, 5.54; N, 21.39; found: C, 59.39; H, 5.22; N, 21.37.

2-(5-Amino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(2-phenethyl)acetamide (15b). Compound **2a** (759 mg, 2.00 mmol) was coupled with phenethylamine (0.30 mL, 2.4 mmol) using a method similar to that described for **4a** to give 2-(5-benzyloxycarbonylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl)-*N*-(2-phenethyl)acetamide (859 mg, 89% yield) as colorless crystals. This intermediate (724 mg, 1.50 mmol) was deprotected using conditions described for the preparation of **1b** to give **15b** (449 mg, 86% yield) as colorless crystals: mp 198–200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16 (t, J = 5.5 Hz, 1H, NH), 7.50–7.40 (m, 5H, ArH), 7.33 (s, 1H, CH=N), 7.30–7.12 (m, 5H, ArH), 5.16 (s, 2H, NH_2), 4.37 (s, 2H, CH_2–N), 3.25 (m, 2H, CH_2–N), 2.66 (t, J = 7.3 Hz, 2H, CH_2–Ph). Anal. calcd for $C_{20}H_{20}N_4O_2$: C, 8.95; H, 5.79; N, 16.08; found: C, 69.23; H, 5.77; N, 16.03.

***N*-(1-Benzyl-3,3,3-trifluoro-2-oxopropyl)acetamide (16).** Compound **3** (438 mg, 2.00 mmol) was coupled with acetic acid (0.12 mL, 2.1 mmol) using a method similar to that described for **4a** to give *N*-(1-benzyl-3,3,3-trifluoro-2-hydroxypropyl)acetamide (503 mg, 96% yield) as colorless crystals. This intermediate (459 mg, 1.77 mmol) was oxidized using conditions described for the preparation of **5a** to give **16** (292 mg, 64% yield) as colorless crystals: mp 121–122 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.73 (d, J = 5.9 Hz, 1H, NH), 7.33–7.10 (m, 5H, ArH), 4.77 (m, 1H, CH–N), 3.06 (dd, J = 13.8, 5.9 Hz, 1H, CH*H*–Ph), 2.94 (dd, J = 13.8, 8.8 Hz, 1H, CH*H*–Ph), 1.81 (s, 3H, CH_3). Anal. calcd for $C_{12}H_{12}F_3NO_2{\cdot}0.2H_2O$: C, 54.84; H, 4.76; N, 5.33; found: C, 54.73; H, 4.77; N, 5.24.

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 Corporation, Osaka, Japan, Elastins Products Co. Inc., Owensville, MO and Nippon Zoki Pharmaceutical Co., Osaka, Japan, respectively.

Chymase-, chymotrypsin- and cathepsin G-inhibitory activities were determined using previously described methods[27] through measurement at 405 nm absorbance of *p*-nitroaniline release from the synthetic substrate MeO-Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide (Sigma Chemical Co.). In the case of thrombin and elastase, previously described buffer conditions[40] were used involving the synthetic substrates S-2238 (Chromogenix AB) and MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (Sigma Chemical Co.), respectively. Enzymatic activity of ACE was measured using previously described methods.[41] Calculations of K_is were performed using previously described methods.[27] Since protease inhibitors containing trifluoromethyl ketone are known to need more than 10 min for interaction with enzymes (slow binding inhibition),[42,43] two-hour incubation was used to measure inhibition in steady state.

Pharmacokinetic determinations

The test compound **1e** was formulated as a solution (1 mg/mL) in DMSO and aqueous solution (84 mM HCl containing 34 mM NaCl) cosolvent (5:95 v/v). The test compound **12c** was formulated as a solution (1 mg/mL) in propylene glycol and 2M Na_2CO_3 aqueous solution

(50:50 v/v) cosolvent. Male Sprague–Dawley rats (approximate weight 250 g) were given either a 1 mg/kg dose of the test compound iv or a 10 mg/kg dose orally. Serial blood samples were collected from the jugular vein at several time points up to 2 h after dosing. Plasma was harvested from the blood samples by centrifugation, and the samples were stored at −20 °C until analysis.

To each plasma sample (100 μL) was added acetonitrile (500 μL) to precipitate protein. After centrifugation, the supernatant was evaporated to dryness under N_2. The residue was reconstituted in 200 μL of HPLC mobile phase (**1e**: 20 mM phosphate buffer pH 6.8:acetonitrile 68:32 v/v; **12c**: water:acetonitrile 65:35 v/v containing 5 mM tetrabutylammonium phosphate). HPLC analysis was carried out on a Waters Symmetry octyl column (150 mm×4.6 mm) at 40 °C with a flow rate of 1.0 mL/min and an injection volume of 150 μL. Detection of test compounds was achieved by monitoring UV absorption at 305 nm. All pharmacokinetic parameters were calculated using non-compartment methods.

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References and Notes

1. Schechter, N. M. In *Mast Cell Proteases in Immunology and Biology*. *Clinical Allergy and Immunology*; Caughey, G. H., Ed.; Marcel Dekker, Inc.: New York, 1995; pp 47–69.
2. Redington, A. E.; Polosa, R.; Walls, A. F.; Howarth, P. H.; Holgate, S. T. In *Human Basophils and Mast Cells: Clinical Aspects*. *Chemical Immunology*; Marone, G., Ed.; Karger AG: Basel, 1995; pp 22–59.
3. Kovanen, P. T. *Curr. Opin. Lipid* **1996**, *7*, 281.
4. Okunishi, H.; Miyazaki, M.; Toda, N. *J. Hypertens.* **1984**, *2*, 277.
5. Urata, H.; Kinoshita, A.; Misono, K. S.; Bumpus, F. M.; Husain, A. *J. Biol. Chem.* **1990**, *265*, 22348.
6. Takai, S.; Shiota, N.; Kobayashi, S.; Matsumura, E.; Miyazaki, M. *FEBS Lett.* **1997**, *412*, 86.
7. Shiota, N.; Fukamizu, A.; Takai, S.; Okunishi, H.; Murakami, K.; Miyazaki, M. *J. Hypertens.* **1997**, *15*, 431.
8. Ihara, M.; Urata, H.; Kinoshita, A.; Suzumiya, J.; Sasaguri, M.; Kikuchi, M.; Ideishi, M.; Arakawa, K. *Hypertension* **1999**, *33*, 1399.
9. Kokkonen, J. O.; Saarinen, J.; Kovanen, P. T. *Ann. Med.* **1998**, *30* (Suppl. 1), 9.
10. Liao, Y.; Husain, A. *Can. J. Cardiol.* **1995**, *11* (Suppl. F), 13F–19F.
11. Wypij, D. M.; Nichols, J. S.; Novak, P. J.; Stacy, D. L.; Berman, J.; Wiseman, J. S. *Biochem. Pharmacol.* **1992**, *43*, 845.
12. Takai, S.; Shiota, N.; Jin, D.; Miyazaki, M. *Eur. J. Pharmacol.* **1998**, *358*, 229.
13. Kato, Y.; Kido, H.; Fukusen, N.; Katunuma, N. *J. Biochem.* **1988**, *103*, 820.
14. Shaoheng, H.; Walls, A. F. *Br. J. Pharmacol.* **1998**, *125*, 1491.
15. Mizutani, H.; Schechter, N.; Lazarus, G.; Black, R. A.; Kupper, T. S. *J. Exp. Med.* **1997**, *174*, 821.
16. Brattsand, M.; Egelrud, T. *Cytokine* **1998**, *10*, 506.
17. Longley, B. J.; Tyrrell, L.; Ma, Y.; Williams, D. A.; Halaban, R.; Langley, K.; Lu, H. S.; Schechter, N. M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 9017.
18. de Paulis, A.; Minopoli, G.; Piaz, F. D.; Pucci, P.; Russo, T.; Marone, G. *Int. Arch. Allergy Immunol.* **1999**, *118*, 422.
19. Mao, X. Q.; Shirakawa, T.; Yoshikawa, T.; Yoshikawa, K.; Kawai, M.; Sasaki, S.; Enomoto, T.; Hashimoto, T.; Furuyama, J.; Hapkin, J. M.; Morimoto, K. *Lancet* **1996**, *348*, 581.
20. Kawashima, T.; Noguchi, E.; Arinami, T.; Kobayashi, K.; Otsuka, F.; Hamaguchi, H. *Hum. Hered.* **1998**, *48*, 271.
21. Tanaka, K.; Sugiura, H.; Uehara, M.; Sato, H.; Hashimoto-Tamaoki, T.; Furuyama, J. *Clin. Exp. Allergy* **1999**, *29*, 800.
22. Powers, J. C.; Tanaka, T.; Harper, J. W.; Minematsu, Y.; Barker, L.; Lincoln, D.; Crumley, K. V.; Fraki, J. E.; Schechter, N. M.; Lazarus, G. G.; Nakajima, K.; Nakashino, K.; Neurath, H.; Woodbury, R. G. *Biochemistry* **1985**, *24*, 2048.
23. Hayashi, Y.; Iijima, K.; Katada, J.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 199.
24. Oleksyszyn, J.; Powers, J. C. *Biochemistry* **1991**, *30*, 485.
25. Burzycki, T. A.; Hoover, K. W.; Thomsen, D. L. III; Sneddon, S. F.; Rauch, A. L.; Hoover, D. J. *IBC Conference on Developmental Therapy for Hypertension: Beyond ACE Inhibitors and Calcium Antagonists*; Philadelphia, PA, 25 January 25, 1993.
26. Bastos, M.; Maeji, N. J.; Abeles, R. H. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 6738.
27. Eda, M.; Ashimori, A.; Akahoshi, F.; Yoshimura, T.; Inoue, Y.; Fukaya, C.; Nakajima, M.; Fukuyama, H.; Imada, T.; Takai, S.; Shiota, N.; Miyazaki, M.; Nakamura, N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 913.
28. Eda, M.; Ashimori, A.; Akahoshi, F.; Yoshimura, T.; Inoue, Y.; Fukaya, C.; Nakajima, M.; Fukuyama, H.; Imada, T.; Takai, S.; Shiota, N.; Miyazaki, M.; Nakamura, N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 919.
29. Niwata, S.; Fukami, H.; Sumida, M.; Ito, A.; Kakutani, S.; Saitoh, M.; Suzuki, K.; Imoto, M.; Shibata, H.; Imajo, S.; Kiso, Y.; Tanaka, T.; Nakazato, H.; Ishihara, T.; Takai, S.; Yamamoto, D.; Shiota, N.; Miyazaki, M.; Okunishi, H.; Kinoshita, A.; Urata, H.; Arakawa, K. *J. Med. Chem.* **1997**, *40*, 2156.
30. Brown, F. J.; Andisik, D. W.; Bernstein, P. R.; Bryant, C. B.; Ceccarelli, C.; Damewood, J. R. Jr.; Edwards, P. D.; Earley, R. A.; Feeney, S.; Green, R. C.; Gomes, B.; Kosmider, B. J.; Krell, R. D.; Shaw, A.; Steelman, G. B.; Thomas, R. M.; Vacek, E. P.; Veale, C. A.; Tuthill, P. A.; Warner, P.; Williams, J. C.; Wolanin, D. J.; Woolson, S. A. *J. Med. Chem.* **1994**, *37*, 1259.
31. Veale, C. A.; Bernstein, P. R.; Bryant, C.; Ceccarelli, C.; Damewood, J. R.; Earley, R. Jr.; Feeney, S. W.; Gomes, B.; Kosmider, B. J.; Steelman, G. B.; Thomas, R. M.; Vacek, E. P.; Williams, J. C.; Wolanin, D. J.; Woolson, S. *J. Med. Chem.* **1995**, *38*, 98.
32. Takahashi, L. H.; Radhakrishnan, R.; Rosenfield, R. E.; Meyer, E. F. Jr.; Trainor, D. A.; Stein, M. *J. Mol. Biol.* **1988**, *201*, 423.
33. Remington, S. J.; Woodbury, R. G.; Reynolds, R. A.; Matthews, B. W.; Neurath, H. *Biochemistry* **1988**, *27*, 8097.
34. Pereira, P. J. B.; Wang, Z.; Rubin, H.; Huber, R.; Bode, W.; Schechter, N. M.; Strobl, S. *J. Mol. Biol.* **1999**, *286*, 163.
35. Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P.; Kolb, M.; Neises, B.; Schirlin, D. *J. Med. Chem.* **1990**, *33*, 394.
36. Fearon, K.; Spaltenstein, A.; Hopkins, P. B.; Gelb, M. H. *J. Med. Chem.* **1987**, *30*, 1617.

37. Bernstein, P. R.; Andisik, D.; Bradley, P. K.; Bryant, C. B.; Ceccarelli, C.; Damewood, J. R.; Earley, R. Jr.; Edwards, P. D.; Feeney, S.; Gomes, B. C.; Kosmider, B. J.; Steelman, G. B.; Thomas, R. M.; Vacek, E. P.; Veale, C. A.; Williams, J. C.; Wolanin, D. J.; Woolson, S. A. *J. Med. Chem.* **1994**, *37*, 3313.
38. Yamazaki, M.; Suzuki, H.; Sugiyama, Y. *Pharm. Res.* **1996**, *13*, 497.
39. Kusuhara, H.; Suzuki, H.; Sugiyama, Y. *J. Pharm. Sci.* **1998**, *87*, 1025.
40. Ono, S.; Kuwahara, S.; Takeuchi, M.; Sakashita, H.; Naito, Y.; Kondo, T. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3285.
41. Miyazaki, M.; Okunishi, H.; Nishimura, K.; Toda, N. *Clin. Sci.* **1984**, *66*, 39.
42. Williams, J. C.; Falcone, R. C.; Knee, C.; Stein, R. L.; Strimpler, A. M.; Reaves, B.; Giles, R. E.; Krell, R. D. *Am. Rev. Respir. Dis.* **1991**, *144*, 875.
43. Shinguh, Y.; Imai, K.; Yamazaki, A.; Inamura, N.; Shima, I.; Wakabayashi, A.; Higashi, Y.; Ono, T. *Eur. J. Pharmacol.* **1997**, *337*, 63.