Nonpeptidic Inhibitors of Human Leukocyte Elastase. 5. Design, Synthesis, and X-ray Crystallography of a Series of Orally Active 5-Aminopyrimidin-6-one-Containing Trifluoromethyl Ketones

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The effects of changes in substitution in a series of 5-amino-2-pyrimidin-6-ones on both *in vitro* activity and oral activity in an acute hemorrhagic assay have been explored. These compounds contained either a trifluoromethyl ketone or a boronic acid moiety to bind covalently to the Ser-195 hydroxyl of human leukocyte elastase (HLE). Boronic acid-containing inhibitors were found to be more potent than the corresponding trifluoromethyl ketones *in vitro* but were less active upon oral administration. Compound **13b** was found to offer the best combination of oral potency, duration of action, and enzyme selectivity and, as such, was selected for further biological testing. X-ray crystallography of a cocrystallized complex of compound **19m** and porcine pancreatic elastase demonstrated that the inhibitor is bound to the enzyme in a manner similar to that found previously for a closely related series of pyridone-containing inhibitors of HLE.

Introduction

Human leukocyte elastase (HLE) is a serine protease contained within the azurophilic granules of polymorphonuclear leukocytes. The intracellular action of HLE is thought to be an important element of phagocytosis and host defense.[1] In an extracellular role, the ability of elastase to degrade most of the structural proteins found in connective tissues (e.g., elastin and type IV collagen) has led to speculation that elastase aids in the migration of neutrophils into the extravascular spaces, e.g., the airways, in response to chemotatic factors.[2–4] Elastase has been shown to be an extremely potent mucus secretagogue.[5] The effect of elastase on mucus secretion is thought to be due to the proteolytic activity of the enzyme, as it is blocked by active-site-directed inhibitors such as ICI-200,355.[6]

Normally, elastase activity is tightly controlled by a number of proteinaceous inhibitors, such as α_1-protease inhibitor and secretory leukocyte protease inhibitor. However, in a number of pathophysiological states these proteins ineffectively regulate elastase activity. The resulting unrestrained elastolytic activity is associated with the abnormal tissue turnover found in pulmonary emphysema[7] and in diseases such as cystic fibrosis and chronic bronchitis in which mucus hypersecretion and impaired host defense are major components.[8–10]

Our current goal is to develop orally active inhibitors of HLE for use as therapy in diseases in which elastase activity is ineffectively controlled by endogenous inhibitors. We have recently disclosed several series of reversible, nonpeptidic inhibitors of HLE (Figure 1).[11] In this paper, we report the extension of this work to a series of pyrimidinones, which are found to be potent inhibitors of HLE *in vitro* and show promising levels of oral activity in animal models.

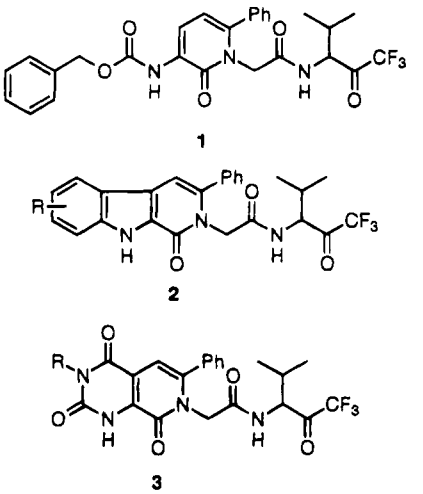

Figure 1. Heterocycle-based elastase inhibitors.

Design Concepts

The compounds shown in Figure 1 were designed, in part, using information gleaned from a number of X-ray crystal structures of elastase containing peptidic inhibitors bound in the enzyme's active site.[12–14] A schematic diagram of the binding interactions found in one of these crystal structures, which is representative of the interactions found in complexes formed with several different peptidic elastase inhibitors, is shown in Figure 2a. This structure is of the complex between acetyl-Ala-Pro-Val-trifluoromethyl ketone and the closely related enzyme porcine pancreatic elastase (PPE).[12,15] As shown in this diagram, the inhibitor makes a number of important interactions with the enzyme. These include (1) the formation of a hemiketal linkage between Ser-195 and the trifluoromethyl ketone carbonyl; (2) hydrogen bonds

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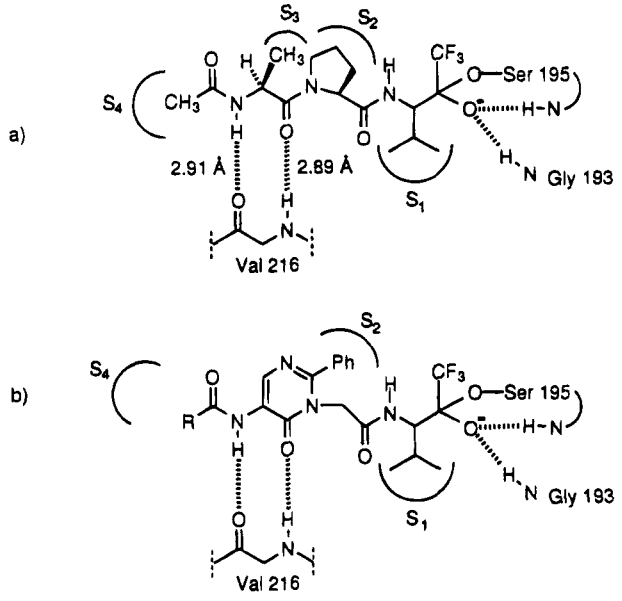


Figure 2. (a) Schematic diagram of the binding interactions found in the crystal structure described in ref 12. (b) Proposed binding mode of pyrimidinone trifluoromethyl ketones.

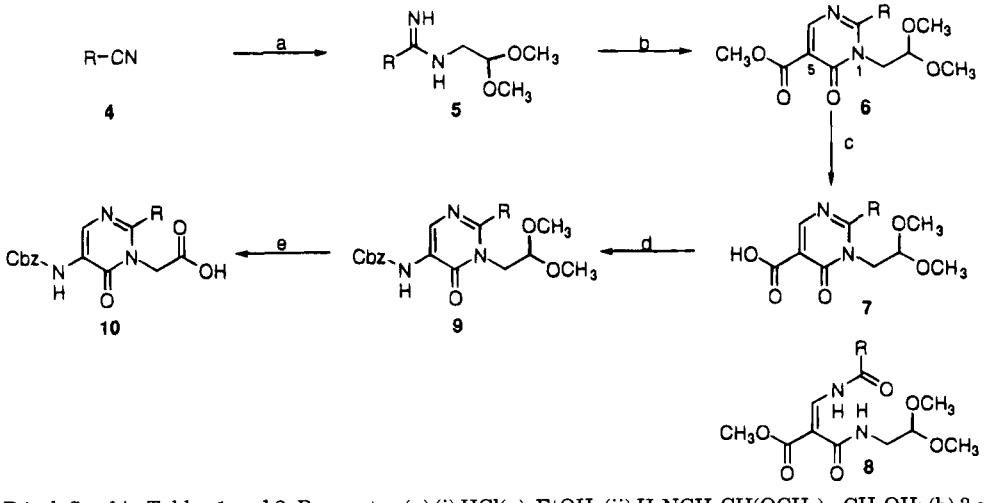
between the oxyanion hole residues Ser-195 and Gly-193 and the hemiketal oxyanion;[16] (3) hydrophobic contacts between the P_1–P_3 side chains and the S_1–S_3 pockets of the enzyme;[17] and (4) a pair of reciprocal hydrogen bonds which exist between the P_3-Ala residue of the inhibitor and Val-216 of the enzyme. An analogous hydrogen-bonding pattern has been reported in the X-ray crystal structure of HLE complexed to the third domain of the turkey ovomucoid inhibitor (TOMI).[18] Examination of this crystal structure (Figure 2a), and several others, revealed two important pieces of structural information which we have used in the design of nonpeptidic inhibitors. The first observation was that the carbonyl (C=O) and amido (N—H) groups in Val-216 have very similar orientations. Secondly, the S_3 subsite in elastase consists of a very shallow dimple on the solvent-exposed surface of the enzyme and is unlikely to provide a critical hydrophobic interaction with the P_3 residue of the inhibitor. This information suggested that it might be possible to replace the P_3 residue of the inhibitor by planar fragments (e.g. the heterocycles present in **1**–**3**) which contained the hydrogen bond acceptor–donor pair but which would not interact with the enzyme's S_3 pocket. The pyrimidinones described in this paper were expected to bind in the active site of elastase in a similar manner as that proposed for other heterocyclic inhibitors (Figure 2b).[11] Support for this binding orientation was obtained from X-ray crystallography of compound **19m** (Table 2) cocrystallized with PPE.[19]

Chemistry

The pyrimidinones described in Tables 1 and 2 were prepared by the general methods shown in Schemes 1–4. The synthesis of the pyrimidinone nucleus is detailed in Scheme 1. In this synthetic sequence, the pyrimidinone ring is formed via the reaction of 3-methoxymethylene malonate and an appropriately substituted amidine (**5**).[20] This route allows the ring to be constructed with complete regiochemical control.[21] The amidines (**5**) are made via the intermediate imidates, which are in turn obtained from commercially available nitriles (**4**) by treatment with ethanolic hydrogen chloride. The heating of amidine **5** with 3-methoxymethylene malonate furnished pyrimidinone **6** in high yield, with none of the alternate N-3 alkyl regioisomer detectable. Base-promoted hydrolysis of the 5-position ester group in **6** led to unacceptable mixtures of the desired acid **7** and a ring-opened compound, **8**. The formation of **8** could be avoided by changing from a hydrolysis reaction to a dealkylation reaction, using lithium iodide in pyridine, which gave **7** exclusively. Transformation of the acid to the corresponding benzyloxycarbonyl-protected amine **9** was accomplished by a modified Curtius reaction.[22] This step was followed by hydrolysis of the acetal group in **9** to the aldehyde and subsequent oxidation with sodium chlorite to afford the desired acid **10**.[23]

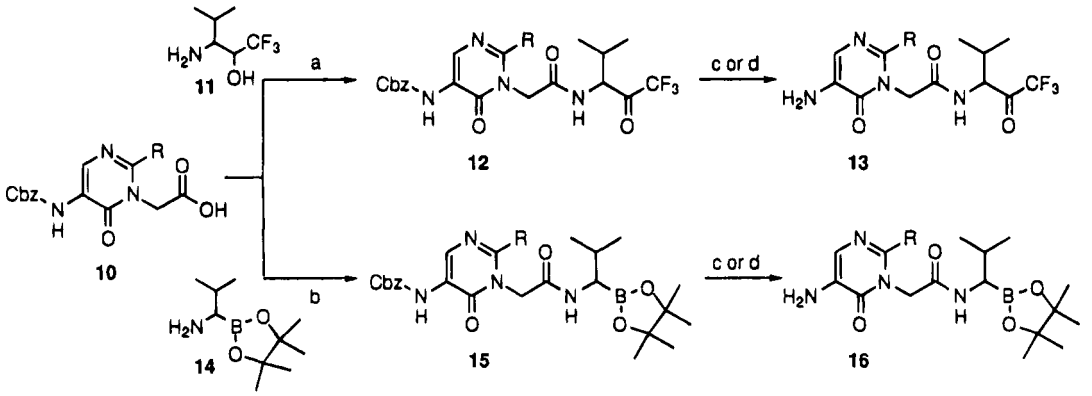
The conversion of the pyrimidinone acids **10** to the corresponding pyrimidinone trifluoromethyl ketones and pyrimidinone boronates is shown in Scheme 2. The trifluoromethyl ketones (TFMK) were obtained by an EDC-mediated[24] coupling of acids **10** to the (trifluoromethyl)valinol **11**[25] followed by a modified Pfitzner–

Scheme 1[a]



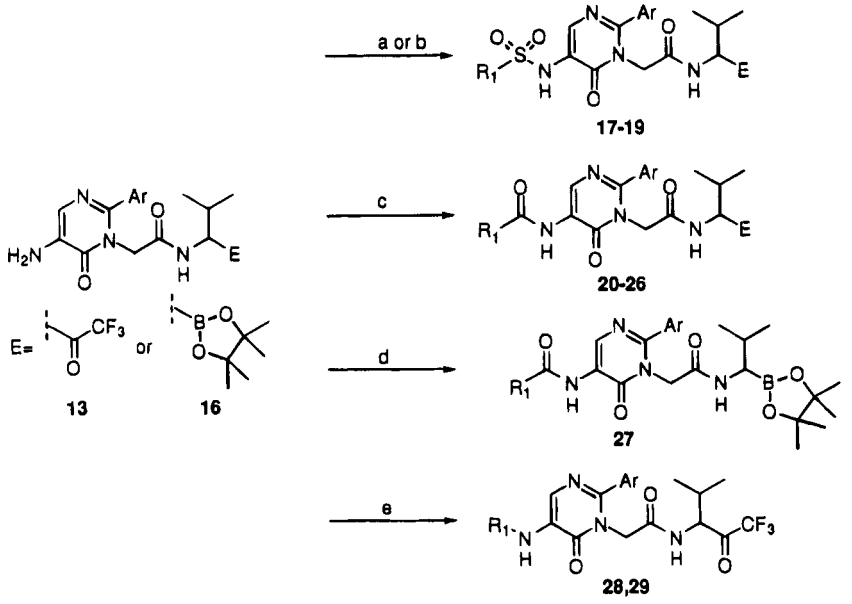
[a] Generic group R is defined in Tables 1 and 2. Reagents: (a) (i) HCl(g), EtOH; (ii) $H_2NCH_2CH(OCH_3)_2$, CH_3OH; (b) 3-methoxymethylene malonate, Δ; (c) LiI, pyridine; (d) diphenyl phosphorazidate, Et_3N, dioxane, benzyl alcohol; (e) (i) H_3O^+; (ii) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-butyl alcohol, THF, H_2O.

Scheme 2[a]



[a] Generic group R is defined in Tables 1 and 2. Reagents: (a) (i) EDC, HOBT, Et_3N, DMF; (ii) EDC, Cl_2CHCO_2H, DMSO, toluene; (b) isobutyl chloroformate, *N*-methylmorpholine, THF; (c) H_2, Pd/C, EtOH; (d) CF_3SO_3H, anisole, CH_2Cl_2.

Scheme 3[a]



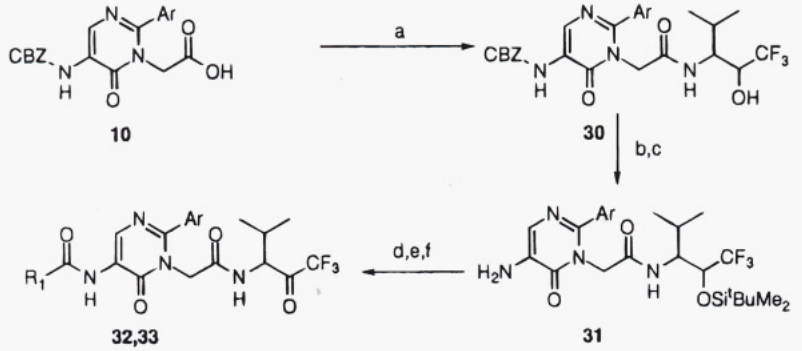
[a] Generic groups R_1 and Ar are defined in Tables 1 and 2. Reagents: (a) R_1SO_2Cl, pyridine; (b) (**19**) (i) CH_3SO_2Cl, pyridine; (ii) H_2, Pd/C, EtOH (reduction of 4-nitrophenyl group); (c) $R_1OC(O)Cl$ or $R_1C(O)Cl$, Et_3N, CH_2Cl_2; (d) triphosgene, Et_3N, ROH or RNH_2, CH_2Cl_2; (e) (i) trifluoroacetic anhydride, Et_3N, CH_2Cl_2; (ii) CH_3I or CH_3CH_2I, Na_2CO_3, DMF; (iii) K_2CO_3, THF, H_2O.

Moffatt oxidation[26] of the trifluoromethyl alcohol to the ketone. Boronates, such as **15**, were similarly obtained from acids **10** by a mixed anhydride coupling with aminoboronate ester **14**.[27] The benzyloxycarbonyl protecting group in **12** or **15** was removed by hydrogenolysis or via treatment with trifluoromethanesulfonic acid to give compounds **13** and **16**. The boronate esters (e.g., **15** or **16**) were hydrolyzed *in situ* to the active boronic acid species by allowing the compounds to stand in aqueous solution for 12 h prior to biological evaluation.

Further elaboration of the 5-position amino group of generic compounds **13** and **16** is shown in Schemes 3 and 4. Conversion to the sulfonamides **17–19** was found to be best achieved using the sulfonyl chloride in the presence of a weak base such as pyridine. Employing stronger bases such as triethylamine in this reaction led to the formation of extensive amounts of *N,N*-bis-sulfonylated products. This result was presumably due to the acidity of the sulfonamido group NH ($pK_a \approx 8-9$), which can be deprotonated by bases such as Et_3N. The resulting anion can then successfully compete with the starting amines **13** or **16** for unreacted sulfonyl chloride.[28,29] Compounds **20–26** were prepared by acylation of the amines **13** or **16** with the appropriate acylating agent in the presence of Et_3N. Transformation of boronates **16** into urethanes or ureas (**27**) could also be achieved by treatment of **16** with triphosgene and triethylamine followed by addition of the appropriate alcohol or amine. Alkylation of the 5-position nitrogen was best effected by first converting the amine to the trifluoroacetamide followed by alkylation and then subsequent alkaline hydrolysis to provide compounds **28** and **29**.

In the case of trifluoromethyl ketones (**13**), conversion to urethanes or ureas by the triphosgene procedure (step d, Scheme 3) was found to be low yielding. The route shown in Scheme 4, in which the trifluoromethyl alcohol has been protected as the silyl ether, was found to be a superior method for the preparation of these compounds. The silyl ether protecting group could be easily removed by treatment with tetrabutylammonium fluoride buffered with acetic acid. The resulting trifluoromethyl alcohols were then oxidized to the ketones as described in Scheme 2.

Scheme 4[a]



[a] Generic groups R_1 and Ar are defined in Tables 1 and 2. Reagents: (a) **11**, EDC, HOBT, Et_3N, DMF; (b) $tBuMe_2SiOTf$, 2,6-lutidine, CH_2Cl_2; (c) H_2, Pd/C, EtOH; (d) triphosgene, Et_3N, ROH or RNH_2, CH_2Cl_2; (e) Bu_4NF, AcOH, THF; (f) EDC, Cl_2CHCO_2H, toluene, DMSO.

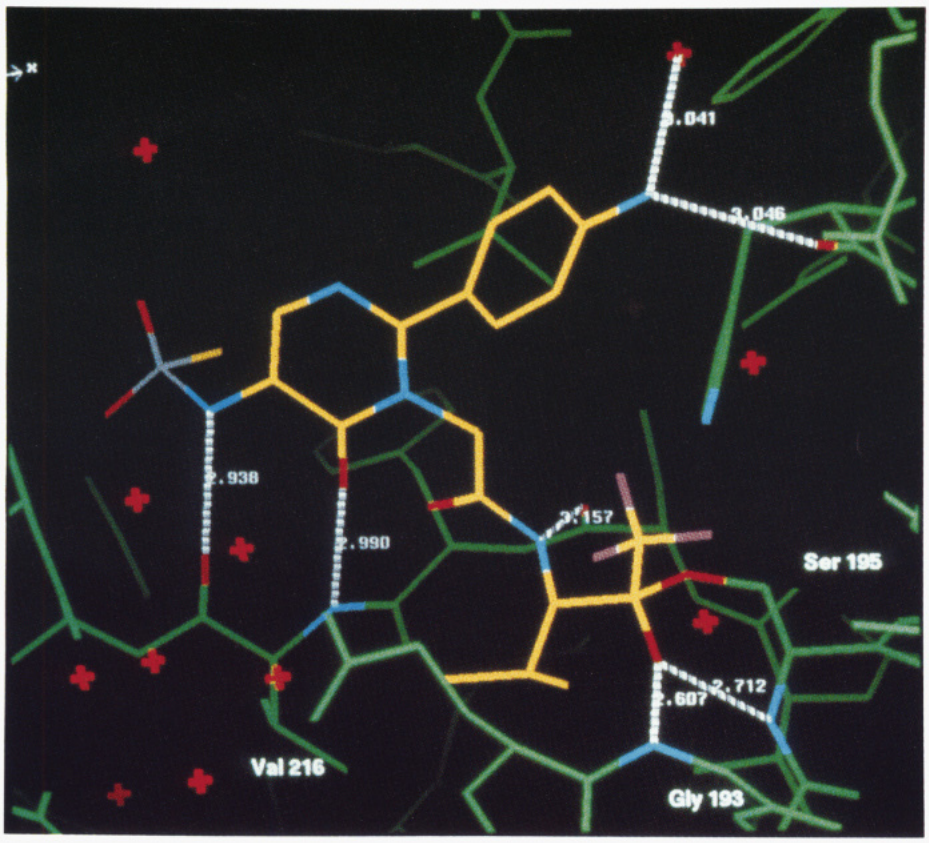
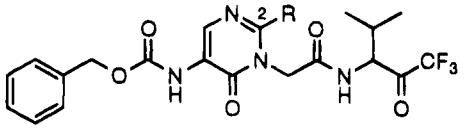


Figure 3. X-ray crystal structure of the complex between compound **19m** and porcine pancreatic elastase. The backbone of the inhibitor is shown in yellow and the enzyme in green. The indicated hydrogen bond distances refer to heteroatom to heteroatom values. The red crosses represent positions for ordered water molecules.

Results and Discussion

X-ray Crystallographic Analysis of the Enzyme–Inhibitor Complex of Compound 19m Bound to Porcine Pancreatic Elastase (PPE). A crystal structure of inhibitor **19m** bound to PPE, determined at 2.0 Å with a final *R*-factor of 16.7%, is shown in Figure 3.[30] The structure obtained agreed closely with that predicted by molecular modeling studies.[11] In this complex, the inhibitor is found to be covalently bound to the enzyme via the trifluoromethyl ketone carbonyl. The bond length of the hemiketal linkage between the trifluoromethyl ketone carbonyl carbon and Oγ of Ser-195 is 1.45 Å, and the geometry of the carbon is tetrahedral. The hemiketal oxygen atom is pointing toward the oxyanion hole of the enzyme and is within hydrogen-bonding distance of the amido nitrogen moieties of Gly-193 (2.61 Å) and Ser-195 (2.71 Å). A pair of reciprocal hydrogen bonds is observed between the pyrimidinone carbonyl oxygen and the amido hydrogen of Val-216 (2.99 Å) and between the 5-position sulfonamido hydrogen and the carbonyl oxygen of Val-216 (2.94 Å). The P_1 isopropyl group is nestled in the S_1 pocket of the enzyme, while the 2-aryl substituent of the pyrimidinone is found in the S_2 pocket. Additional close contacts involving inhibitor, enzyme, and solvent are also observed. The P_1 amino nitrogen of **19m** is

Table 1. Modifications of the 2-Position Substituent



compd	R	K_i (nM)[a]	lung hemorrhage, % inhibition[b]	K_i ratio,[c] chymotrypsin/HLE	solubility[d] (μg/mL)	log P [e]
12a	Ph	6.6 ± 1.4	54	145	13	2.1
12b	4-F-Ph	8.2 ± 1.9	54	378	6.9	2.2
12c	2-thienyl	11 ± 3	57	162	48	2.0
12d	4-CF_3-Ph	42 ± 6	NA[f]	164	25	2.3
12e	4-CH_3O-Ph	8.1 ± 1.9	NA	272		
12f	4-Cl-Ph	18 ± 1	NA	322		
12g	3-pyridyl	33 ± 5	59	245	580	1.3
12h	4-NO_2-Ph	17 ± 5	NA	406		
12i	3,5-F-Ph	20 ± 2	43	230		
12j	3,5-$(CF_3)_2$-Ph	72 ± 5	NA			
12k	cyclohexyl	57 ± 11	NA		42	2.9
12l	isopropyl	170 ± 20	NA	100	40	2.6
12m	4-NH_2-Ph	3.6 ± 0.7	NA			
12n	4-$CF_3C(O)NH$-Ph	43 ± 8	NA			
12o	4-HC(O)NH-Ph	9 ± 3.2	NA			
1	6-Ph-pyridone	5.6 ± 1.6	NA	11	1.8	>4

[a] Method reported in ref 30. [b] Percent inhibition of elastase-induced lung hemorrhage when the compound was dosed orally at 20 mg/kg, 30 min prior to instillation of elastase. [c] Selectivity ratio between bovine pancreatic chymotrypsin and HLE. [d] Solubility of compound in 0.01 M sodium phosphate buffer at pH = 7.4. [e] Measured log P in octanol/water. [f] Not active.

hydrogen-bound to the carbonyl oxygen of Ser-214 (3.16 Å). The *p*-amino group on the 2-position aryl substituent is involved in a pair of close contacts with the carbonyl oxygen of His-57 (3.05 Å, although the orientation is not ideal for a hydrogen bond) and an active-site water molecule (3.04 Å). This same water is, in turn, hydrogen-bonded to Oδ1 of Asp-60 (2.55 Å).

Biological Results. The compounds listed in Tables 1 and 2 were tested both *in vitro* and *in vivo* for their ability to inhibit elastase activity. *In vitro* testing consisted of determination of the inhibition constant (K_i) corresponding to the compounds' ability to inhibit hydrolysis of the synthetic substrate MeO-Suc-Ala-Ala-Pro-Val-pNA.[31] *In vivo* testing was done using an acute hemorrhagic assay (A.H.A.) conducted in hamsters.[32] This assay measures the ability of an orally administered inhibitor to protect the lung from hemorrhage induced by a subsequent intratracheal challenge of a 50 μg/animal dose of HLE. Increasing the time interval between oral administration of inhibitor and instillation of HLE in this assay was used as a measure of the compound's duration of action.

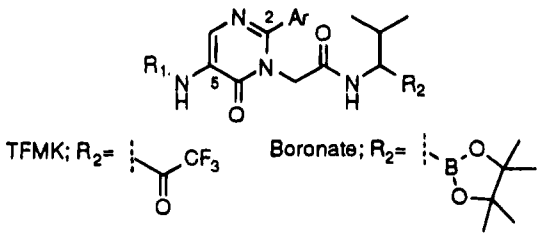
We began by exploring the impact of modifications at the 2-position of the pyrimidinone ring on biological activity and physical properties. We previously reported a study on the effect of changes at the corresponding position in a series of pyridone-based inhibitors (e.g., **1,** Figure 1), but the extent of modifications had been narrow in scope due to synthetic limitations.[11c] That study had found that incorporation of a phenyl substituent into the analogous position in a pyridone molecule conferred a large increase in *in vitro* activity. However, while compound **1** was found to have a high level of selectivity for HLE over a number of other proteases from the serine, cysteine, aspartic acid, and metalloprotease classes, unacceptable affinity for bovine pancreatic chymotrypsin (BPC) was demonstrated (Table 1). Since BPC might be representative of related human "chymotrypsin-like" proteases, we considered improvement in this selectivity ratio to be an important parameter. Additionally, measurable activity was not obtained upon oral administration of a 20 mg/kg dose of **1** in the acute hemorrhagic assay. Subsequent studies implied that the lack of oral activity was related to poor oral absorption.[11d] Measurement of the log P and solubility of **1** suggested that poor aqueous solubility and high lipophilicity were likely limiting factors.

We were pleased to find that, upon changing from pyridone to pyrimidinone-based inhibitors (e.g., **1** to **12a**, Table 1), several desirable attributes were obtained. For example, while the lipophilicity of **12a** compared to **1** decreased by >100-fold, essentially no diminution of *in vitro* potency against HLE resulted, and significant oral activity was obtained. Additionally, the selectivity ratio between BPC and HLE significantly improved from 11 to 145.

A number of other 2-substituted pyrimidinones were synthesized and several showed *in vitro* affinities similar to that obtained with **12a**, while enzyme selectivity ratios further improved. Several general trends emerged. (1) Large substituents on the aromatic ring such as trifluoromethyl in **12d,j** were found to be deleterious to binding. (Presumably, these substituents are too large for optimal binding of the aromatic ring in the enzyme's S_2 pocket). (2) Compounds in which the phenyl ring had been replaced by cyclohexyl (**12k**) or isopropyl (**12l**) were also found to be inimical to *in vitro* potency. (3) Several compounds (e.g., **12a–c,g,i**) were found to show significant oral activity.

Having identified several 2-position substituents which had promising profiles, we next explored modifications of the substituent on the 5-position amino group (R_1). Molecular dynamics simulations conducted in the pyridone series[11] had suggested that the Cbz group in **1** was not involved in tight, specific interactions with the enzyme but was rather mobile in nature. This observation suggested that the 5-position would be an appropriate place for incorporation of functionality to vary physicochemical parameters and improve oral activity. We expected that a similar situation would exist for the Cbz group in **12a**, so modifications were made in this region of the molecule to vary factors such as solubility,

Table 2. *In Vitro* and *in Vivo* Activities[a]



compd	R_1	Ar	R_2	K_i (nM)	lung hemorrhage, % inhibition: 20 mg/kg (predose time interval)	2.5 mg/kg 30 min predose	2.5 mg/kg 90 min predose
13b	H	4-F-Ph	TFMK	101 ± 10	66 (30)	43	49
13c	H	2-thienyl	TFMK	52 ± 14	75 (30)	47	37
13i	H	3,5-F-Ph	TFMK	270 ± 90	72 (30)	NT[b]	45
16b	H	4-F-Ph	boronate	6.2 ± 0.5	39 (30)	NA	NT
16c	H	2-thienyl	boronate	4.9 ± 1.1	50 (30)	NA	NT
17b	cyclohexyl-$NHSO_2$	4-F-Ph	TFMK	1.6 ± 0.9	NT	NA	NT
18b	cyclohexyl-$NHSO_2$	4-F-Ph	boronate	0.21 ± 0.09	NT	31	NA
19m	CH_3SO_2	4-NH_2-Ph	TFMK	15 ± 1.6	NA (30)	NT	NT
20b	$CH_3OC(O)$	4-F-Ph	TFMK	40 ± 7	73 (30)	39	NT
20c	$CH_3OC(O)$	2-thienyl	TFMK	42 ± 10	80 (30)	49	NA
21a	$CH_3SC(O)$	Ph	TFMK	16 ± 8	57 (90)	NT	32
21b	$CH_3SC(O)$	4-F-Ph	TFMK	23 ± 8	NT	NT	50
22c	iPROC(O)	2-thienyl	TFMK	27 ± 4	92 (30)	50	33
23a	$CF_3CH_2OC(O)$	Ph	TFMK	40 ± 8	47 (90)	NT	39
23c	$CF_3CH_2OC(O)$	2-thienyl	TFMK	48 ± 4	60 (90)	NT	41
24c	HC(O)	2-thienyl	TFMK	100 ± 20	58 (90)	NT	37
25a	$CF_3C(O)$	Ph	TFMK	94 ± 7	73 (30)	37	NA
26b	$CH_3OC(O)$	4-F-Ph	boronate	7.7 ± 3.6	NT	NA	NT
27b	3,5-dimethyl-4-pyridinyl-CH_2OCO	4-F-Ph	boronate	0.96 ± 0.17	NT	NA	NT
28b	CH_3	4-F-Ph	TFMK	46 ± 2	89 (30)	43	NA
29c	Et	2-thienyl	TFMK	26 ± 5	52 (90)	NT	32
32b	2-pyridinyl-CH_2OCO	4-F-Ph	TFMK	22 ± 4	NA (30)	NT	NT
33b	4-pyridinyl-CH_2OCO	4-F-Ph	TFMK	23 ± 7	NA (30)	NT	NT

[a] See footnotes for Table 1. [b] Not tested.

lipophilicity, and molecular weight. This effort resulted in a number of compounds which were very potent inhibitors of HLE *in vitro* (Table 2). Of a large number of 5-position substituents tried, the cyclohexylsulfamoyl substituent (**17b**) was found to confer the best *in vitro* potency among R_1 substituents.

Peptidyl boronic acids have been reported to be excellent inhibitors of HLE *in vitro* and have typically been found to be more potent than the corresponding trifluoromethyl ketones.[33] Boronic ester analogs were made of several promising compounds in the pyrimidinone series including **17b** and were found, following *in situ* hydrolysis to the boronic acids, to further increase *in vitro* activity. For example, compound **18b** (0.21 nM) is approximately 8-fold more potent than the corresponding trifluoromethyl ketone and is one of the most potent, reversible, nonpeptidic inhibitors of HLE to be reported. However, the boronic acids did not convey commensurate levels of oral activity.

In vivo testing of the compounds in Table 2 was initially done at a 20 mg/kg oral dose, using a 30 min time interval between administration of inhibitor and subsequent enzyme challenge. As better inhibitors were obtained, the screening criteria for new compounds was tightened by first lowering the dose of inhibitor to 2.5 mg/kg for initial testing and then extending the time interval to 90 min. After preparing a large number of compounds which spanned a wide range of physical properties and *in vitro* potencies,[34] it was found that the inhibitors with the best *in vivo* activity were generally those which contained either a small R_1 substituent or none at all (e.g., **13b,i**, **21c**). This was especially true in relation to the compound's duration of action in the acute hemorrhagic assay. It was also apparent that the compounds which exhibited the best *in vitro* potency were not also the ones which had the best oral activity (e.g., compare **13b–i** to **17b** or **18b**). This finding is not surprising considering the impact that absorption, distribution, and elimination factors have on *in vivo* activity. The precise factors which limit the expression of oral activity with the more potent (K_i) compounds in Table 2 are presently not clear.

Table 3. *In Vitro* Enzyme Selectivity of Compound **13b**

enzyme	selectivity ratio[a]
porcine pancreatic elastase	231
bovine pancreatic chymotrypsin	720
human pancreatic chymotrypsin	>4000
human pancreatic trypsin	>4000
human plasma thrombin	4600
human leukocyte cathepsin G	>4000
human mast cell tryptase	>4000
human plasma kallikrein	>4000
human urine kallikrein	>4000
human plasma plasmin	>4000
human spleen cathepsin B	>4000
human lung angiotensin converting enzyme	>4000

[a] Selectivity ratio refers to the ratio K_i(enzyme)/K_iHLE.

Of the compounds listed in Table 2, **13b** was found to offer the best combination of oral potency, duration of activity, and enzyme selectivity (see Table 3) and, as such, was selected for further study.[35] Evaluation of **13b** at 100, 30, 10, 3, and 1 mg/kg using a 90 min predose interval in the acute hemorrhagic assay gave

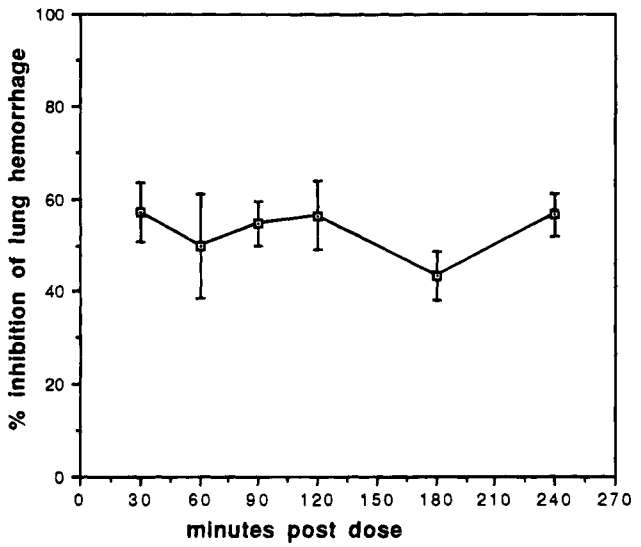


Figure 4. Duration of action study of compound **13b**. Minutes postdose refers to the time interval between oral administration of a 30 mg/kg dose of compound **13b** and the it administration of a 50 μg challenge of HLE.

Table 4. Pharmacokinetic Parameters for **13b**[a]

species (n)[b]	$t_{1/2}$ (min)[c]	C_{max}[d] (μg/mL)	T_{max}[e] (min)	AUC[f] (min μg/mL)	oral bioavailability (%)
hamster (3)	81	0.75	15	71.5	77
rat (3)	96	1.90	60	310	62
dog (1)	58	3.23	60	357	88

[a] All data have been normalized to a 10 mg/kg oral dose. [b] n = number of animals. [c] Pharmacokinetic half-life. [d] Maximum concentration of unchanged drug in plasma recorded in the period 0–6 h postdose. [e] Time of maximum concentration. [f] Integrated area under the concentration vs time curve.

an ED_{50} of approximately 7.5 mg/kg. A duration of action study is shown in Figure 4. In this study, the interval between oral administration of **13b** and enzyme challenge was extended out to 4 h, with the activity remaining essentially unchanged.

Bioavailability and Metabolism Studies. Studies were undertaken to measure the oral bioavailability of compound **13b** in three species: hamster, rat, and dog. Compound **13b** was dosed in an aqueous solution containing 10% PEG by both oral and intravenous routes. Blood samples were obtained over a 6 h time period and the levels of intact **13b** determined by HPLC. These studies found **13b** to have excellent oral bioavailability in all three species tested (Table 4). In these studies, two major metabolites were observed and identified as the diastereomeric trifluoromethyl alcohols corresponding to reduction of the ketone in **13b**. Approximately equal amounts of the erythro and threo alcohols were found in the blood samples, suggesting that a nonselective reduction of the ketone was occurring. In these studies, reduction of the ketone to the corresponding alcohol appeared to be the major factor limiting the pharmacokinetic half-life of **13b** in plasma.

Summary

The analysis of several protein crystal structures of elastase complexed to peptidic inhibitors has provided insight regarding important binding interactions occurring between enzyme and inhibitor. This led to the development of several series of nonpeptidic inhibitors of HLE, including the pyrimidinone-containing molecules described in this paper. X-ray crystallography of a complex obtained by cocrystallization of one of these compounds, **19m**, with porcine pancreatic elastase revealed that the inhibitor binds in the active site of the enzyme in the manner predicted by molecular modeling studies. The work presented here also demonstrates that this series of pyrimidinone-containing trifluoromethyl ketones (e.g., **13b**) has achieved promising levels of oral activity.

Experimental Section

General Methods. Analytical samples were homogeneous by TLC and afforded spectroscopic results consistent with the assigned structures. Proton NMR spectra were obtained using either a Bruker WM-250 or AM-300 spectrometer. Chemical shifts are reported in parts per million relative to Me_4Si as internal standard. Mass spectra (MS) were recorded on a Kratos MS-80 instrument operating in the chemical ionization (CI) mode. Elemental analyses for carbon, hydrogen, and nitrogen were determined by the ZENECA Pharmaceuticals Analytical Department on a Perkin-Elmer 241 elemental analyzer and are within ±0.4% of theory for the formulas given. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates (60F-254, 0.2 mm thick; E. Merck). Visualization of the plates was accomplished by using UV light or phosphomolybdic acid/ethanol-charring procedures. Chromatography refers to flash chromatography conducted on Kieselgel 60 230–400 mesh (E. Merck, Darmstadt), using the indicated solvents. Solvents used for reactions or chromatography were either reagent grade or HPLC grade. Reactions were run under an argon atmosphere at ambient temperature unless otherwise noted. Solutions were evaporated under reduced pressure on a rotary evaporator. The following abbreviations are used, THF, tetrahydrofuran; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; TFA, trifluoroacetic acid.

***N*-(2,2-Dimethoxyethyl)-4-fluorobenzamidine (5, Scheme 1, R = 4-fluorophenyl).** A solution of 4-fluorobenzonitrile (76.0 g, 628 mmol) in ethanol (900 mL) was cooled to 0 °C and saturated with dry HCl gas. The solution was allowed to warm to room temperature and stand for 16 h. The solvent was removed, and the resulting solid was triturated with ether. The material was collected and dried to yield the imidate hydrochloride salt (103.1 g) as a white solid. To a solution of this imidate in methanol (300 mL) at 0 °C was added dropwise a solution of aminoacetaldehyde dimethyl acetal (71.6 g, 681 mmol) in methanol (75 mL). The resulting solution was allowed to stand for 3 days at 5 °C and then was evaporated to provide the amidine hydrochloride salt (93 g) as a white solid. This solid was dissolved in 1 N NaOH and extracted into dichloromethane. The organic solution was dried and the solvent removed to give 76 g (53%) of **5** as a colorless, viscous oil: ^{1}H NMR (250 MHz, DMSO) δ 7.26 (4H, m), 6.25 (1H, bs), 4.59 (1H, bs), 4.34 (1H, bs), 3.33 (2H, d, J = 7.1 Hz), 3.24 (6H, s); MS (CI) 227 (M + H).

Methyl 1-(2,2-Dimethoxyethyl)-2-(4-fluorophenyl)pyrimidin-6(1*H*)-one-5-carboxylate (6, Scheme 1, R = 4-fluorophenyl). A mixture 3-methoxymethylene malonate (66.5 g, 382 mmol) and **5** (95 g, 420 mmol) in methanol (100 mL) was heated at 100 °C for 1.5 h (distilling off the solvent as the reaction progressed). The solution was cooled, diluted with ethyl acetate, and washed with saturated aqueous ammonium chloride (2×), H_2O, and brine. The organic solution was dried and the solvent removed to give an oil which crystallized upon standing. The solid was collected, washed with ether/hexane (1:1), and dried to give 93.6 g (73%) of **6** as a light tan solid: ^{1}H NMR (250 MHz, DMSO) δ 8.58 (1H, s), 7.72 (2H, m), 7.40 (2H, m), 4.56 (1H, t, J = 3.6 Hz), 4.06 (2H, d, J = 5.2 Hz), 3.81 (3H, s), 3.17 (6H, s); MS (CI) 337 (M + H).

1-(2,2-Dimethoxyethyl)-2-(4-fluorophenyl)pyrimidin-6(1*H*)-one-5-carboxylic Acid (7, Scheme 1, R = 4-fluorophenyl). To a solution of **6** (93.6 g, 278 mmol) in pyridine

(400 mL) was added lithium iodide (93 g, 695 mmol), and the resulting solution was heated at reflux for 2 h. The reaction mixture was cooled to room temperature and the pyridine removed under vacuum. To the resulting gum was added 1 N HCl (300 mL) followed by extraction with dichloromethane/methanol (4:1, three extractions). The organic extracts were combined, washed with 6 M HCl, and dried, and the solvent was removed. The resulting material was triturated with ether to give a solid which was collected and dried to give 79.5 g (89%) of **7** as a tan solid: ^{1}H NMR (300 MHz, DMSO) δ 8.66 (1H, s), 7.72 (2H, m), 7.41 (2H, t, J = 8.9 Hz), 4.58 (1H, t, J = 5.4 Hz), 4.09 (2H, d, J = 5.3 Hz), 3.17 (6H, s); MS (CI) 323 (M + H).

[5-[(Benzyloxycarbonyl)amino]-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]acetaldehyde Dimethyl Acetal (9, Scheme 1, R = 4-fluorophenyl). A solution of **7** (79.5 g, 247 mmol), triethylamine (69 mL, 494 mmol), and diphenyl phosphorazidate (59 mL, 272 mmol) was heated at 100 °C for 2 h. Benzyl alcohol (51 mL, 494 mmol) was added, and the temperature was maintained at 100 °C for 12 h. The solution was then cooled and the solvent evaporated. The resulting residue was dissolved in ethyl acetate and washed with saturated aqueous ammonium chloride (2×) and 1 N NaOH. The solution was dried and the solvent removed to yield an oil which crystallized upon the addition of ether/hexane. The resulting solid was collected and washed with ether/hexane to give 78.4 g (74%) of **9** as a tan solid: ^{1}H NMR (250 MHz, DMSO) δ 8.94 (1H, s), 8.43 (1H, s), 7.63 (2H, m), 7.38 (7H, m), 5.18 (2H, s), 4.52 (1H, t, J = 5.5 Hz), 4.04 (1H, d, J = 5.4 Hz), 3.13 (6H, s); MS (CI) 428 (M + H).

[5-[(Benzyloxycarbonyl)amino]-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]acetic Acid (10, Scheme 1, R = 4-fluorophenyl). A solution of **9** (78.4 g, 184 mmol) in THF (450 mL) and 1 N HCl (150 mL) was heated to reflux for 8 h. The solution was cooled to room temperature, the THF was removed under vacuum, and H_2O (200 mL) was added. The pH was adjusted to 7 by addition of solid $NaHCO_3$. The resulting emulsion was extracted with dichloromethane (3×). The organic extracts were combined, washed with H_2O, and dried ($MgSO_4$), and the solvent was removed resulting in a solid. The solid was triturated with ether and dried to give the corresponding aldehyde (61.0 g). The crude aldehyde was dissolved in a mixture of THF (265 mL), *tert*-butyl alcohol (265 mL), and 2-methyl-2-butene (340 mL) and cooled to 5 °C. To this was added a solution of sodium chlorite (130.2 g, 1440 mmol) and sodium dihydrogen phosphate monohydrate (154.6 g, 1120 mmol) in H_2O (450 mL). The solution was warmed to room temperature and stirred for 1 h. The solvents were removed under vacuum, the residue was diluted with H_2O (300 mL), and the pH was adjusted to 3 by addition of a saturated solution of sodium bicarbonate. The aqueous solution was extracted with THF/dichloromethane (1:2, three extractions), the organic extracts were combined and dried ($MgSO_4$), and the solvent was removed to provide a solid. The solid was triturated with ether, collected, and dried to give 52.4 g (72%) of **10** as an off-white solid: ^{1}H NMR (300 MHz, DMSO) δ 13.34 (1H, bs) 9.06 (1H, s), 8.46 (1H, s), 7.56 (2H, m), 7.39 (7H, m), 5.19 (2H, s), 4.53 (2H, s); MS (CI) 398 (M + H).

2-[5-[(Benzyloxycarbonyl)amino]-6-oxo-2-(2-thienyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (12c). To a solution of the acid **10** (R = 2-thienyl; 9.5 g, 25 mmol), the amino alcohol **11** (7.78 g, 37.5 mmol), 1-hydroxybenzotriazole hydrate (6.76 g, 50 mmol), and triethylamine (10.5 mL, 75 mmol) in DMF (50 mL) was added EDC (9.58 g, 50 mmol), and the solution was stirred for 16 h. The solution was diluted with ethyl acetate and washed with a saturated aqueous solution of ammonium chloride (2×) and H_2O. The solution was dried and the solvent removed. The resulting oil crystallized upon the addition of dichloromethane/ether (1:1) and was collected and dried to give the corresponding alcohol (10.0 g). To a solution of this alcohol (3 g, 5.58 mmol) in a mixture of toluene (28 mL) and DMSO (28 mL) was added EDC (10.7 g, 55.8 mmol) followed by the dropwise addition of dichloroacetic acid (1.84 mL, 22.3 mmol). The solution was stirred (2 h) and then diluted with ethyl acetate. The solution was washed with a saturated aqueous solution of ammonium chloride (2×) and H_2O. The solution was dried, the solvent was removed, and the resulting oil was chromatographed (gradient elution: 15% ethyl acetate in dichloromethane to 30% ethyl acetate in dichloromethane) to provide a colorless oil which crystallized upon the addition of dichloromethane/hexane to give 2.3 g (57%, two steps) of **12c** as a white solid: mp 174–176 °C; ^{1}H NMR (300 MHz, DMSO) δ 9.03 (2H, m), 8.44 (1H, s), 7.85 (1H, m), 7.37 (6H, m), 7.16 (1H, m), 5.18 (2H, s), 4.95 (2H, dd, J = 16.9 Hz), 4.74 (1H, t, J = 6.3 Hz), 2.25 (1H, m), 0.93 (6H, m). Anal. ($C_{24}H_{23}F_3N_4O_5S\cdot 0.75H_2O$) C,H,N.

2-[5-Amino-6-oxo-2-(2-thienyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (13c). To a solution of **12c** (1.85 g, 3.45 mmol) and anisole (1.2 mL, 11.07 mmol) in dichloromethane (35 mL) at 5 °C was added trifluoromethanesulfonic acid (1.64 mL, 18.56 mmol) dropwise. The solution was warmed to room temperature, and after 15 min, the reaction was returned to 5 °C and quenched by addition of a saturated solution of sodium bicarbonate. The product was extracted into ethyl acetate and the organic layer washed with brine. The solution was dried, the solvent was removed, and the resulting material was chromatographed (gradient elution: 5% methanol in dichloromethane to 7% methanol in dichloromethane) to give 1.04 g (75%) of **13c** as a yellow solid: mp 192–194 °C; ^{1}H NMR (300 MHz, DMSO/D_2O) δ 7.67 (1H, d, J = 10.4 Hz), 7.31 (1H, s), 7.23 (1H, d, J = 3.7 Hz), 7.02 (1H, m), 4.81 (2H, m), 4.09 (1H, d, J = 2.8 Hz), 2.27 (1H, m), 0.92 (3H, d, J = 6.8 Hz), 0.78 (3H, d, J = 6.8 Hz). Anal. ($C_{16}H_{17}F_3N_4O_3S\cdot 0.5H_2O$) C,H,N.

2-[5-[(Benzyloxycarbonyl)amino]-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-*N*-[2-methyl-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]acetamide (15, Scheme 2, R = 4-fluorophenyl). To a solution of the acid **10** (R = *p*-fluorophenyl; 1.0 g, 2.52 mmol) and *N*-methylmorpholine (0.29 mL, 2.62 mmol) in dry THF (13 mL) at −25 °C was added isobutyl chloroformate (0.34 mL, 2.59 mmol), and the resulting solution was stirred for 30 min. A second portion of *N*-methylmorpholine (0.29 mL 2.62 mmol) was added followed by the aminoboronate **14** (0.77 g, 2.47 mmol). The solution was maintained at −25 °C for 15 min and then allowed to warm to room temperature and stirred for 16 h. The solution was filtered free of solids, and the solvent was removed. The resulting oil was chromatographed (gradient elution: 4% THF in dichloromethane to 10% THF in dichloromethane), and the product crystallized upon the addition of dichloromethane/hexane to give 1.05 g (74%) of **15** as a white powder: mp 131–135 °C; ^{1}H NMR (250 MHz, DMSO) δ 8.98 (1H, s), 8.44 (1H, s), 8.27 (1H, d, J = 5.4 Hz), 7.59 (2H, m), 7.36 (7H, m), 5.18 (2H, s), 4.49 (2H, s), 2.68 (1H, t, J = 5.6 Hz), 1.75 (1H, m), 1.16 (12H, s), 0.84 (6H, d, J = 6.7 Hz). Anal. ($C_{30}H_{36}BFN_4O_6$) C,H,N.

2-[5-Amino-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-*N*-[2-methyl-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]acetamide (16b). To a solution of **15** (Scheme 2, R = 4-fluorophenyl; 700 mg, 1.21 mmol) in THF (15 mL) was added 10% Pd/C (165 mg), and the mixture was stirred for 1.5 h under a hydrogen atmosphere at atmospheric pressure. The catalyst was filtered and the solvent removed. The resulting residue was chromatographed (gradient elution: 4% methanol in dichloromethane to 6% methanol in dichloromethane) to give a foam which solidified upon the addition of ether/hexane (1:1). The solid was dried to give 475 mg (89%) of **16b** as an off-white solid: mp 100–104 °C; ^{1}H NMR (300 MHz, DMSO) δ 8.30 (1H, d, J = 5.2 Hz), 7.52 (2H, m), 7.31 (1H, s), 7.23 (2H, m), 5.19 (2H, s), 4.45 (2H, s), 2.62 (1H, t, J = 5.5 Hz), 1.75 (2H, m), 1.16 (12H, s), 0.84 (6H, m). Anal. ($C_{22}H_{30}BFN_4O_4$) C,H,N.

2-[2-(4-Fluorophenyl)-6-oxo-5-[(cyclohexylsulfamoyl)amino]-1,6-dihydro-1-pyrimidinyl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (17b). To a solution of **13b** (251 mg, 0.61 mmol) and pyridine (0.2 mL, 2.46 mmol) in THF (3 mL) was added cyclohexylaminesulfonyl chloride (162 mg, 0.82 mmol) followed by stirring for 16 h. The solution was poured into 0.5 N HCl and extracted into ethyl acetate. The organic layer was washed sequentially with 0.5 N HCl, H_2O, and brine. The solution was dried and the solvent

removed. The resulting solid was triturated from ether, collected, and dried to give 225 mg (64%) of **17b** as a white solid: mp 179–179.5 °C; ^{1}H NMR (300 MHz, DMSO) δ 8.92 (1H, s), 8.84 (1H, d, J = 7.1 Hz), 7.96 (1H, s), 7.66 (1H, d, J = 7.6 Hz), 7.53 (2H, m), 7.31 (2H, m), 4.61 (3H, m), 2.16 (1H, m), 1.79 (2H, bs), 1.62 (2H, bs), 1.51 (1H, m), 1.18 (6H, m), 0.89 (3H, d, J = 6.8 Hz), 0.83 (3H, d, J = 6.8 Hz); MS (CI) 576 (M + H). Anal. ($C_{24}H_{29}F_4N_5O_5S$) C,H,N.

2-[2-(2-Thienyl)-6-oxo-5-[(2-propoxycarbonyl)amino]-1,6-dihydro-1-pyrimidinyl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (22c). To a solution of **13c** (600 mg, 1.5 mmol) in THF (10 mL) containing ground sodium carbonate (420 mg, 4.0 mmol) was added a solution of isopropyl chloroformate (2 mL, 2.0 mmol) in toluene (1 M solution) followed by stirring for 16 h. The solution was diluted with ethyl acetate and washed with H_2O (2×), a saturated solution of ammonium chloride, and brine. The solution was dried, the solvent removed, and the resulting oil chromatographed (15% ethyl acetate/dichloromethane) to provide 480 mg (66%) of **22c** as a yellow solid: ^{1}H NMR (250 MHz, DMSO/D_2O) δ 8.41 (1H, s), 7.79 (1H, d, J = 5.0 Hz), 7.39 (1H, d, J = 3.6 Hz), 7.14 (1H, t, J = 4.5 Hz), 4.87 (3H, m), 4.09 (1H, m), 2.27 (1H, m), 1.28 (6H, d, J = 6.2 Hz), 0.95 (3H, d, J = 6.7 Hz), 0.81 (3H, d, J = 6.8 Hz); MS (CI) 489 (M + H). Anal. ($C_{20}H_{23}F_3N_4O_5S$) C,H,N.

2-[2-(4-Fluorophenyl)-5-[[[(2,6-dimethylpyrid-4-yl)methoxy]carbonyl]amino]-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[2-methyl-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]acetamide (27b). To a solution of **16b** (1.0 g, 2.25 mmol) and triphosgene (1.0 g, 3.37 mmol) in dry dichloromethane (23 mL) at 0 °C was added triethylamine (1.58 mL, 11.25 mmol) in dichloromethane (1.5 mL) followed by stirring for 25 min. A solution of (3,5-dimethyl-4-pyridyl)carbinol (0.99 g, 7.22 mmol) in dichloromethane (4 mL) was added and the solution stirred for 16 h. The reaction mixture was diluted with ethyl acetate and washed with H_2O and the aqueous wash extracted with dichloromethane. The organic extracts were combined and dried, and the solvent was removed. The resulting oil was chromatographed (gradient elution: 15% THF in dichloromethane to 50% THF in dichloromethane) to yield an oil which solidified upon the addition of hexane to give 492 mg (36%) of **27b** as an off-white solid: mp 97–100 °C; ^{1}H NMR (250 MHz, DMSO) δ 9.17 (1H, s), 8.47 (1H, s), 8.26 (1H, s), 7.81 (2H, m), 7.30 (2H, t, J = 8.0 Hz), 7.10 (2H, s), 5.15 (2H, s), 4.51 (2H, s), 2.70 (1H, t, J = 5.4 Hz), 2.43 (6H, s), 1.75 (1H, m), 1.17 (12H, s), 0.85 (6H, d, J = 6.7 Hz); MS (CI) 608 (M + H). Anal. ($C_{31}H_{39}BFN_5O_6$) C,H,N.

2-[2-(4-Fluorophenyl)-6-oxo-5-(methylamino)-1,6-dihydro-1-pyrimidinyl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (28b). To a solution of **13b** (512 mg, 1.24 mmol) in dichloromethane (20 mL) at −5 °C was added trifluoroacetic anhydride (0.38 mL, 2.65 mmol), and the solution was allowed to warm to room temperature. After 4 h, the solvent was removed to provide a gummy oil (631 mg). A portion of the oil (567 mg, 1.11 mmol) was dissolved in DMF (10 mL), and to this was added sodium carbonate (335 mg, 3.16 mmol) and methyl iodide (0.52 mL, 8.27 mmol). The reaction mixture was then stirred for 16 h at room temperature. The reaction mixture was diluted with dichloromethane and filtered free of solids. The filtrate was washed with H_2O and a saturated solution of ammonium chloride. The solution was dried, the solvent was removed, and the resulting oil was chromatographed (gradient elution: 5% acetone in dichloromethane to 10% acetone in dichloromethane) to give a viscous oil. The oil was dissolved in a mixture of THF (5 mL) and H_2O (11 mL), and to this was added potassium carbonate (764 mg, 5.53 mmol) followed by stirring for 16 h. The reaction mixture was diluted with dichloromethane, washed with H_2O and brine, and dried, and the solvent was removed. The resulting oil was chromatographed (1.5% methanol in dichloromethane) to give 264 mg (53%) of **28b** as a white solid: mp 234–235 °C; ^{1}H (250 MHz, DMSO/D_2O) δ 7.48 (2H, m), 7.23 (2H, t, J = 9.0 Hz), 7.08 (1H, s), 4.66 (1H, d, J = 16.7 Hz), 4.52 (1H, d, J = 16.5 Hz), 4.04 (1H, d, J = 2.9 Hz), 2.75 (3H, s), 2.22 (1H, m), 0.86 (3H, d, J = 6.8 Hz), 0.77 (3H, d, J = 6.8 Hz); MS (CI) 429 (M + H). Anal. ($C_{19}H_{20}F_4N_4O_3 \cdot 0.4H_2O$) C,H,N.

2-[5-[(Benzyloxycarbonyl)amino]-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-*N*-(3,3,3-trifluoro-2-hydroxy-1-isopropylpropyl)acetamide (30). To a solution of **10** (Scheme 4, Ar = 4-fluorophenyl) (8.0 g, 20.2 mmol), amino alcohol **11** (6.29 g, 30.3 mmol), 1-hydroxybenzotriazole hydrate (5.46 g, 40.4 mmol), and triethylamine (8.5 mL, 60.6 mmol) in DMF (40 mL) was added EDC (7.74 g, 40.4 mmol), and the solution was stirred for 16 h. The reaction mixture was diluted with ethyl acetate and washed with a saturated solution of ammonium chloride and H_2O. The solution was dried and the solvent removed. The resulting solid was triturated from ether, collected, and dried to give 9.6 g (86%) of **30** as an off-white solid: ^{1}H NMR (250 MHz, DMSO) δ 8.94 (1H, s), 8.44 (1H, s), 8.02 (1H, d, J = 9.7 Hz), 7.54 (2H, m), 7.32 (7H, m), 6.54 (1H, d, J = 6.8 Hz), 5.18 (2H, s), 4.65 (1H, d, J = 16.7 Hz), 4.45 (1H, d, J = 17.2 Hz), 4.09 (1H, t, J = 7.1 Hz), 3.80 (1H, t, J = 9.0 Hz), 1.72 (1H, m), 0.88 (3H, d, J = 6.7 Hz), 0.79 (3H, d, J = 6.7 Hz); MS (CI) 551 (M + H).

2-[5-Amino-2-(4-fluorophenyl)-6-oxo-1,6-dihydro-1-pyrimidinyl]-*N*-[2-[(*tert*-butyldimethylsilyl)oxy]-3,3,3-trifluoro-1-isopropylpropyl]acetamide (31). To a solution of **30** (Scheme 4, Ar = 4-fluorophenyl) (5.0 g, 9.09 mmol) and 2,6-lutadine (1.59 mL, 13.64 mmol) in dry dichloromethane (90 mL) at 5 °C was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (4.39 mL, 19.09 mmol). The solution was allowed to warm to room temperature and stirred for 45 min. The reaction mixture was diluted with ethyl acetate and washed with 1 N HCl and brine. The solution was dried and the solvent removed. The resulting solid was triturated from ether, collected, and dried to give the desired silyl ether (5.06 g). A portion of this material (500 mg, 0.75 mmol) was dissolved in a mixture of ethanol (7.5 mL) and THF (2.5 mL), and to this was added 10% Pd/C (50 mg). The suspension was shaken under a hydrogen atmosphere (45 psi) for 1 h. The mixture was filtered free of catalyst and the solvent removed, yielding an oil which crystallized upon the addition of ether/hexane to give 370 mg (90%, two steps) of **31** as an off-white solid: ^{1}H NMR (250 MHz, DMSO) δ 7.71 (1H, d, J = 9.7 Hz), 7.46 (2H, m), 7.30 (1H, s), 7.19 (2H, t, J = 8.8 Hz), 5.18 (2H, s), 4.68 (1H, d, J = 16.6 Hz), 4.27 (2H, m), 3.78 (1H, t, J = 10.2 Hz), 1.73 (1H, m), 0.92 (3H, d, J = 6.6 Hz), 0.86 (9H, s), 0.79 (3H, d, J = 6.5 Hz), 0.09 (6H, s); MS (CI) 531 (M + H).

2-[2-(4-Fluorophenyl)-6-oxo-5-[[(4-pyridylmethoxy)carbonyl]amino]-1,6-dihydro-1-pyrimidinyl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (33b). To a solution of **31** (Scheme 4, Ar = 4-fluorophenyl; 1.06 g, 2.0 mmol) and triphosgene (0.89 g, 3.0 mmol) in dry dichloromethane (20 mL) at 5 °C was added a solution of triethylamine (1.96 mL, 14.0 mmol) in dichloromethane (2 mL). The solution was stirred for 25 min followed by addition of a solution of 4-pyridinemethanol (0.70 g, 6.4 mmol) in dichloromethane (3 mL). The solution was stirred for an additional 16 h, diluted with ethyl acetate, and washed with saturated sodium bicarbonate and H_2O. The solution was dried and the solvent removed. The resulting oil was chromatographed (5% methanol in dichloromethane) to provide the desired urethane as a solid. This solid (0.94 g, 1.41 mmol) was dissolved in THF (14 mL), and to this was added tetrabutylammonium fluoride (1.69 mL, 1 M solution) followed by stirring for 1 h. The reaction mixture was diluted with ethyl acetate and washed with H_2O. The aqueous wash was extracted with dichloromethane, the organic portions were combined and dried, and the solvent was removed. The resulting residue was chromatographed (gradient elution: 5% methanol in dichloromethane to 7% methanol in dichloromethane) to give an oil. This oil was dissolved in a mixture of toluene (4.5 mL) and DMSO (4.5 mL), and to this was added EDC (1.74 g, 9.1 mmol) followed by the dropwise addition of dichloroacetic acid (0.30 mL, 3.64 mmol). The reaction mixture was stirred for 1 h; the solution was diluted with ethyl acetate and washed three times with saturated sodium bicarbonate. The solution was dried, the solvent was removed, and the resulting oil was chromatographed (gradient elution: dichloromethane to 7% methanol in dichloromethane) to give an oil which crystallized upon the addition of ether/hexane (1:1) to provide 383 mg (35%) of **33b** as a white solid: mp 187–191 °C; ^{1}H NMR (250 MHz,

DMSO/D_2O) δ 8.57 (2H, d, J = 6.0 Hz), 8.44 (1H, s), 7.54 (2H, m), 7.45 (2H, d, J = 5.6 Hz), 7.26 (2H, d, J = 8.8 Hz), 5.23 (2H, s), 4.70 (1H, d, J = 16.6 Hz), 4.50 (1H, d, J = 16.4 Hz), 4.03 (1H, d, J = 2.8 Hz), 2.21 (1H, m), 0.85 (3H, d, J = 6.7 Hz), 0.75 (3H, d, J = 6.8 Hz); MS (CI) 550 (M + H). Anal. ($C_{25}H_{23}F_4N_5O_5 \cdot 0.5H_2O$) C,H,N.

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Supplementary Material Available: Experimental procedures for the bioavailability determinations on compound **13b** and a ΔF map for the X-ray crystallographic structure determination of the complex between PPE and compound **19m** (8 pages). Ordering information is given on any current masthead page.

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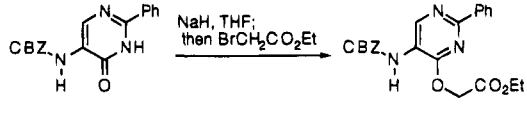

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