Rational Design, Synthesis, and Structure−Activity Relationships of Novel Factor Xa Inhibitors: (2-Substituted-4-amidinophenyl)pyruvic and -propionic Acids[1]

Kazuyuki Sagi, Tadakiyo Nakagawa, Masahiro Yamanashi, Shingo Makino, Mitsuo Takahashi, Masaru Takayanagi, Kaoru Takenaka, Nobuyasu Suzuki, Seiji Oono, Noriyasu Kataoka, Kohki Ishikawa, Sayaka Shima, Yumiko Fukuda, Takashi Kayahara, Shunji Takehana, Yoichiro Shima, Kazumi Tashiro, Hiroshi Yamamoto, Ryota Yoshimoto, Seinosuke Iwata, Takashi Tsuji, Kuniya Sakurai, and Masataka Shoji*

Pharmaceutical Research Laboratories, Ajinomoto Company Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki-shi 210-8681, Japan

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An inhibitor of factor Xa (fXa), the m-substituted benzamidine AXC1578 (**1a)**, was structurally modified with the aim of increasing its potency. In particular, pyruvic acid and propionic acid substituents were incorporated into the P1 benzamidine moiety to introduce a favorable interaction with the oxy-anion hole in the catalytic triad region of fXa. This strategy was based on computational docking studies using the extracted active site of fXa. The validity of the computational model was supported by the acquisition of X-ray crystal structures of the **1a**−trypsin and **3b**−trypsin complexes (the homology around the active sites of fXa and trypsin is high). The above modifications significantly increased the inhibitory activity toward fXa, whereas the high selectivity for fXa versus thrombin was maintained or enhanced. Compounds **3b**, **3c**, **3e**, and **4b** are considered to be potential lead compounds for the development of orally active anticoagulant drugs because they demonstrated potent activity when administered orally to cynomolgus monkeys.

Introduction

Factor Xa (fXa) is an attractive target for the development of orally active anticoagulants.[2] Although vitamin K antagonists, for example, warfarin, have been used in chronic oral anticoagulant therapy for thromboembolic diseases, there have been numerous reports of serious risks, especially bleeding, associated with the use of these antagonists.[3] Because of the central role of thrombin in thrombosis, thrombin inhibitors have been investigated as alternatives to warfarin. However, direct thrombin inhibitors increase the likelihood of bleeding complications.[4] Factor Xa is a trypsin-like serine protease that converts the prothrombin zymogen to its active form, thrombin. On the basis of studies in preclinical efficacy models, we considered that selective fXa inhibitors might have lower bleeding risks and a more favorable safety/efficacy ratio than thrombin inhibitors.[5]

Initially, we focused on preparing and evaluating new bisamidine compounds because some bisamidine compounds are already known to be selective inhibitors of fXa.[6] This program resulted in the discovery of the m-substituted benzamidine AXC1578 (**1a**) as a selective fXa inhibitor (K_i for fXa = 0.22 μM, K_i for thrombin = 710 μM). The simple structure of **1a** encouraged further study aimed at increasing the potency and selectivity.

Inhibitor Design Based on Computational Models. To facilitate inhibitor design, the structure of the **1a**−fXa complex was modeled computationally by using the X-ray structure of fXa (1HCG),[7] as described in the Experimental Section. The results are illustrated in Figure 1a. To support the proposed model, the **1a**−trypsin complex structure was elucidated by X-ray crystallography. Trypsin was considered to be a reasonable surrogate protein, because the homology around the active sites of fXa and trypsin is high.[8] The similarities in the two structures support the validity of the computational model (Figure 1b).

According to this model, the S4 pocket of fXa is occupied by the 4-amidinophenyl moiety of **1a**, and the S1 pocket is occupied by the other amidinophenyl moiety (see Chart 1). Therefore, we examined the effect of variation of the P4 moiety on the affinity of **1a** for fXa (**1b**,[9] **1c**,[10] **1d**, and **1e** in Table 1). Next, we attempted carboxymethyl substitution on the linker part (**1f**, **1g** in Table 1), on the basis of a visual inspection of the **1a**−fXa complex structure. According to the model of the *R*-isomer **1f**−fXa complex (Figure 2), the carboxylic acid group of **1f** might interact favorably with Gln192[11] and Arg143 of fXa through electrostatic attraction, in contrast to the electrostatic repulsion with Glu192 of thrombin (1DWC).[12] The *S*-isomer **1g** would not interact with Gln192 and Arg143 of fXa, according to the computer modeling (data not shown).

Further study of the **1a**−fXa complex structure indicated the presence of an empty region around the catalytic triad (CT) of fXa (Figure 1a). Appropriate substitutions at the para position of the P1 benzamidine might introduce favorable interactions with the CT region, especially the oxy-anion hole. In connection with this, 4-amidinophenylpyruvic acid (APPA) (**2**) has been reported as a trypsin inhibitor with oral bioavailability.[13] The X-ray crystal structure of the APPA−trypsin complex indicates that the pyruvic acid moiety of APPA interacts with the oxy-anion hole.[14] The benzamidine

* To whom correspondence should be addressed. Tel: +81-44-210-5826. Fax: +81-44-210-5876. E-mail: masataka_shouji@ajinomoto.com

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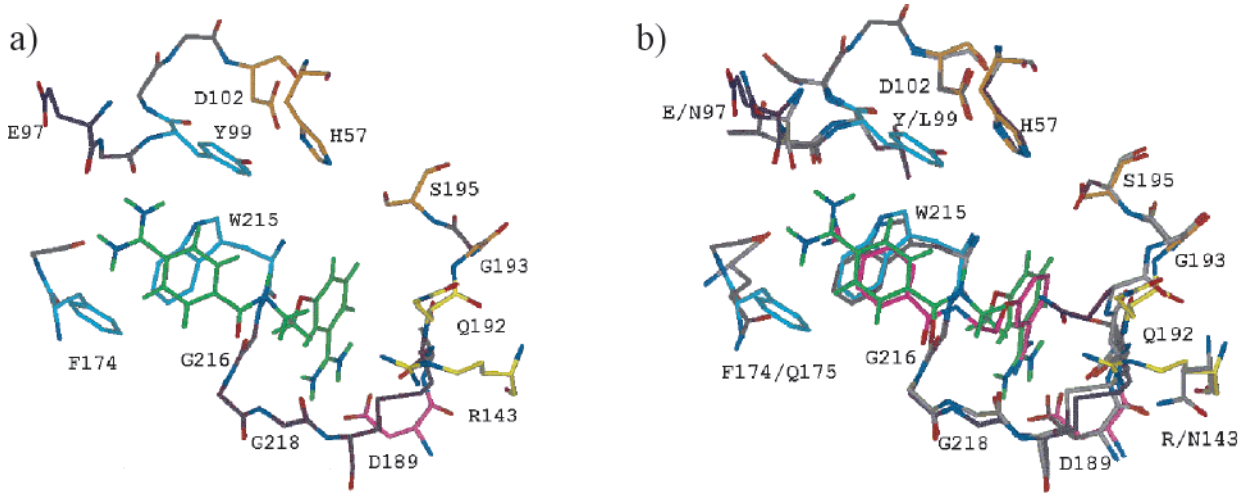
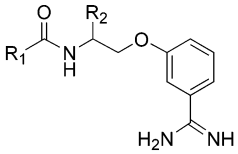
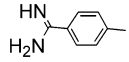
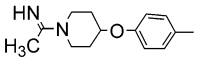
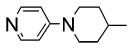
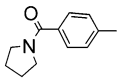
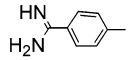
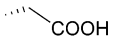
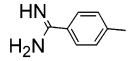
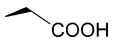


Figure 1. (a) Calculated docking mode of AXC1578 (**1a**) in factor Xa (1HCG). Both the hydrogen and carbon atoms of the inhibitor are colored green. Specific residues relevant to the binding of **1a** are colored. Asp189 at the bottom of the S1 pocket is shown in magenta. Gln192 and Arg143 at the top of the S1 pocket are in yellow. Ser195, His57, and Asp102 forming the catalytic triad and Gly 193 forming the oxy-anion hole are in orange. Residues forming the S4 pocket, Tyr99, Phe174, and Trp215 are in cyan. In this docking mode, the amidine of **1a** hydrogen bonds with both the carboxyl group of Asp189 and the carbonyl group of Gly218, and the carbonyl group of **1a** hydrogen bonds with the amide NH of Gly218. (b) The crystal structure of the complex of **1a** with bovine trypsin is superposed onto Figure 1a. Compound **1a** and the residues of the crystal structure are colored magenta and gray, respectively. The residues that differ between factor Xa and trypsin are depicted as factor Xa/trypsin. The hydrogen bonds of Figure 1a are apparent in this crystal structure.

Table 1. Enzyme-Inhibitory Activity of AXC1578 Analogues without Substitution at the Para-Position of Benzamidine



compound	R_1	R_2	K_i (μM) fXa	K_i (μM) thrombin	selectivity (thrombin/fXa)
1a (AXC1578)		H	0.22	710	3200
1b		H	0.17	440	2600
1c		H	0.29	1000	3500
1d		H	0.020	110	5500
1e[a]		H	2.8	800	290
1f			0.028	>1500	>54000
1g			0.17	>1500	8800

[a] See reference 29.

moieties of **1a** and APPA appear to occupy similar sites of fXa and trypsin, respectively. Therefore, the introduction of a pyruvic acid moiety into **1a** (**3a** in Table 2) should increase the affinity of the compound for the oxyanion hole of fXa. The computational model of **3a**−fXa indicated that the pyruvic acid moiety of **3a** can interact

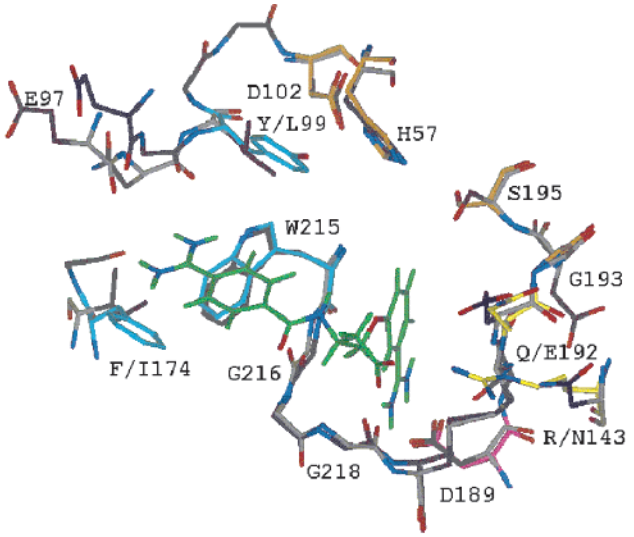


Figure 2. Calculated docking mode of **1f** with factor Xa. The same color scheme as in Figure 1a is adopted. For comparison, the residues of thrombin (1DWC) are superposed and colored gray. The residues that differ between factor Xa and thrombin are depicted as factor Xa or thrombin. The carboxyl group of **1f** is placed in the vicinity of Gln 192 and Arg143 of fXa, which is consistent with hydrogen-bond formation, taking into account the flexibility of the side chains of these amino acids.

with the oxy-anion hole of fXa without interfering with the linker moiety (Figure 3a). According to the X-ray structure of the APPA−trypsin complex, the effect may even be enhanced by nucleophilic interaction of the oxygen atom of the Ser195 side chain with the pyruvic acid moiety. Compound **3a** was therefore synthesized, together with the R_1-modified analogue **3b** (Table 2). The X-ray crystal structure determination of the **3b**−trypsin complex provided support for the proposed binding mode (Figure 3b) based on the high homology between the binding sites of fXa and trypsin.

Because compounds into which a pyruvic acid moiety had been introduced showed good results in the biological activity evaluation (see below), we extended the modification to propionic acid substitution in place of pyruvic acid substitution. Computer modeling indicated that the propionic acid moiety might also interact favorably with Gly193 of the oxy-anion hole and Gln192 of fXa (Figure 4). Because **3b** showed good anticoagulant activity after oral administration, a compound having the same substituent R_1 as **3b** (**4a** in Table 2) was utilized in this modeling.

Chemistry. AXC1578 analogues without substitution at the para position of benzamidines were prepared as follows (Scheme 1). The hydroxyl group of 3-hydroxybenzonitrile (**5**) was alkylated with either alkyl halides or alcohols. Next, the Boc group was removed under acidic conditions, followed by acylation with various carboxylic acids to give **7**. Finally, the nitriles **7** were converted into the target amidines **1** by means of the Pinner reaction.[15]

The benzamidine compounds with pyruvic acid substitution were synthesized as shown in Scheme 2. 3-Hydroxybenzoic acid (**8**) was iodinated to give the 4-iodo compound (**9**).[16] Then, the carboxyl group of **9** was converted into a nitrile group (**10**). Direct iodination of **5** to afford **10** did not proceed well. Compound **10** was subsequently alkylated to afford **11** by using a procedure similar to that in Scheme 1. Compound **11** was coupled with methyl 2-acetamidoacrylate by means of the Heck reaction[17] to give 3-phenyl-2-acetamidoacrylate **12**. Compound **12** was converted into **13** by using a procedure similar to that shown in Scheme 1. The nitrile group of **13** was converted into an amidino group, and the target molecules **3** were obtained by simultaneous hydrolysis[18] of both the enamido and the ester moieties of the 2-acetamido acrylate moiety.

The benzamidine compounds with propionic acid substitution were synthesized according to Scheme 3. The key intermediate **11a** was coupled with ethyl acrylate to afford **14**. Subsequently, amidines **16** were prepared by using a procedure similar to that shown in Scheme 1. Finally, the target compounds **4** were obtained by hydrogenation of the double bond, followed by hydrolysis of the ester.

Biological Results and Discussion

Table 1 summarizes the results of replacement of the 4-amidinobenzoyl group in **1a** (**1b**−**e**). Compounds **1b**

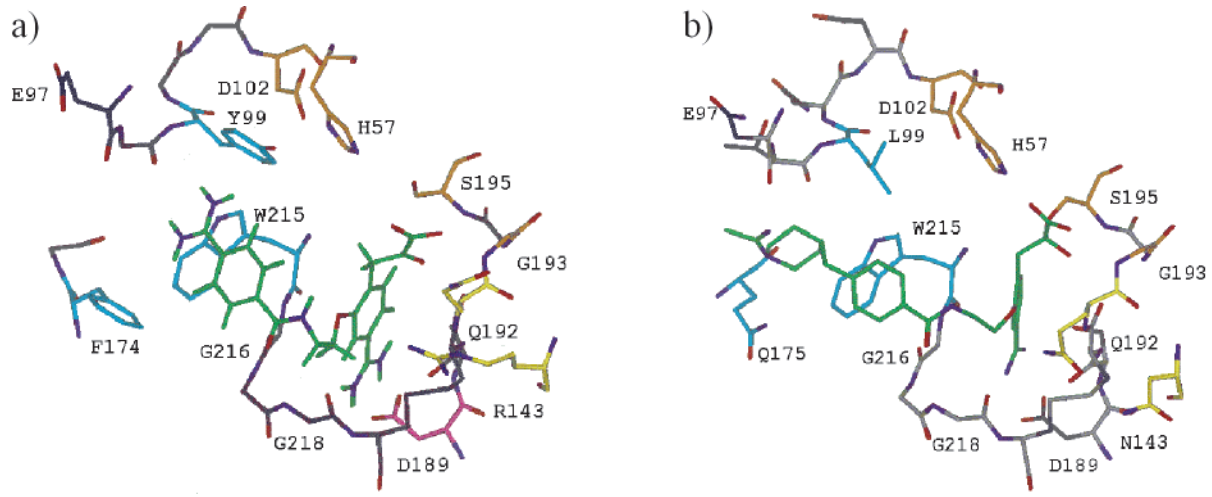
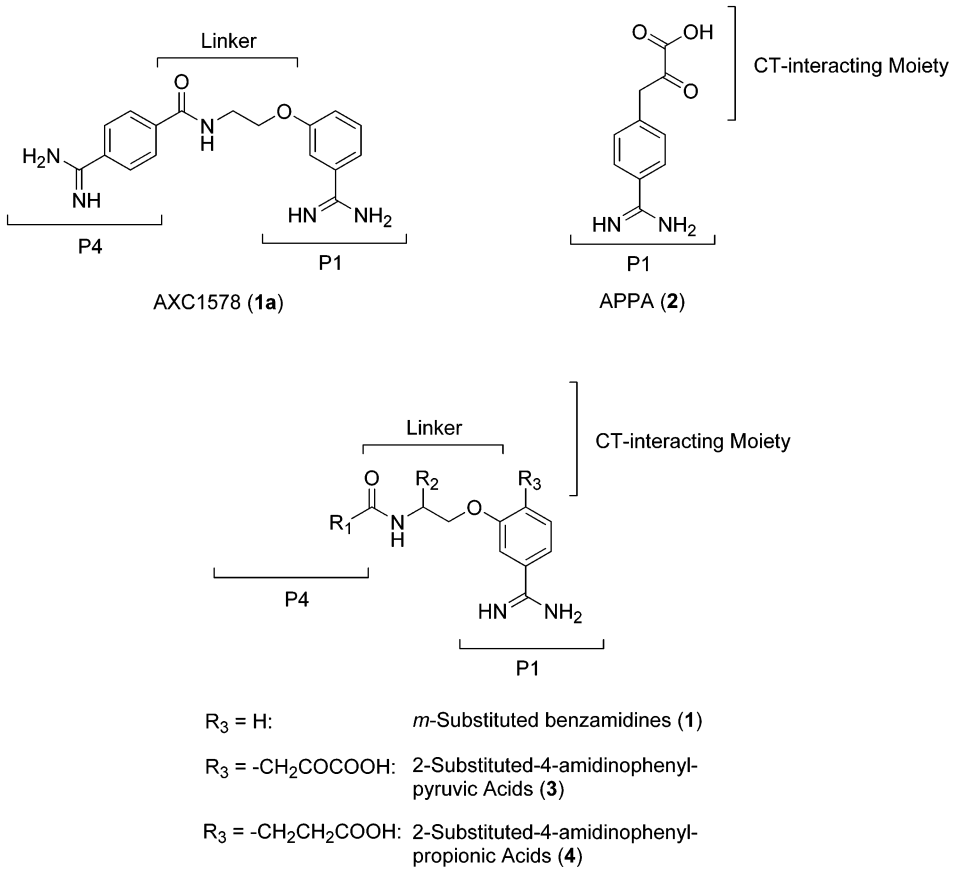


Figure 3. (a) Calculated docking mode of **3a** in factor Xa. The same color scheme as in Figure 1a is adopted. The docking calculation was done assuming formation of a noncovalent Michaelis−Menten complex before the formation of a plausible covalent complex, as observed in the crystal structure of APPA−trypsin complex. (b) Crystal structure of the complex of **3b** with bovine trypsin. The same color scheme as above is adopted. A covalent bond is formed between the carbonyl carbon of **3b** and Oγ of Ser195 of trypsin.

Chart 1

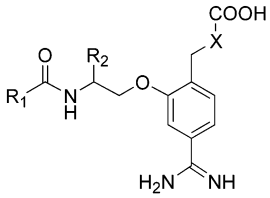


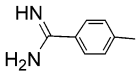
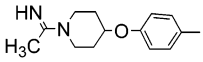
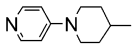
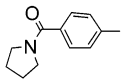
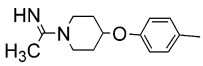
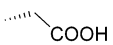
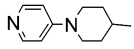
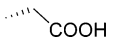
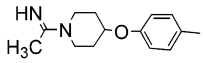
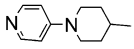
(fXa K_i = 0.17 μM) and **1c** (0.29 μM) are equipotent to the parent compound **1a** (0.22 μM). When R_1 is basic (**1a**−**1c**), the compounds showed high affinity, probably owing to cation−π stabilization between the cationic portions of the protonated R_1 and the aromatic side chains of Tyr99, Phe174, and Trp215 in the S4 pocket (Figure 1a).[19] Especially in the case of **1b**, the affinity may also be owed to hydrogen bonding of the long R_1 moiety with the flexible acidic side chain of Glu97 hanging over the end of the S4 pocket, by induced-fitting as suggested in the DX-9065a−fXa complex.[20] The compound having a neutral R_1, **1d**, is an order of magnitude more potent than **1a** (fXa K_i = 20 nM compared to 0.22 μM). The high potency of **1d** may be attributed to favorable hydrophobic and van der Waals interactions of the pyrrolidine-carbonyl group with the three aromatic side chains mentioned above. Taking into account the low affinity of **1e** (R_1 = Ph, 2.8 μM), it is clear that the R_1 group in **1a**−**1d** greatly enhances the affinity of these compounds for fXa. Table 1 also shows the result of incorporation of a carboxymethyl group in the linker part of **1a** (**1f**, **1g**). As we expected, the affinity for fXa of the *R*-isomer **1f** was 10-fold greater than thatof **1a**, and the selectivity over thrombin was considerably increased (more than 50000-fold selectivity). On the other hand, the affinity of **1a** was not improved by *S*-carboxymethyl substitution (**1g**).

The data in Table 2 demonstrate that the affinity for fXa of **1a** (0.22 μM) was greatly enhanced by the incorporation of the pyruvic acid moiety on P1 benzamidine (**3a**, 26 nM). Next, the R_1 group of **3a** was replaced with the above P4 moieties to estimate the effect of pyruvic acid introduction (comparison between **1a**−**d** and **3a**−**d**). In all cases, the introduction of the pyruvic acid moiety resulted in an approximately 10-fold enhancement of the affinity, indicating that the P4 and pyruvic acid moieties had predominantly independent effects in terms of structure−activity relationships. However, the affinity for thrombin, as well as fXa, was increased by the introduction of the pyruvic acid substituent, presumably because the CT region is highly conserved among these serine proteases. Thus, the selectivity of **3a**−**d** for fXa over thrombin was little changed from that of the corresponding compounds **1a**−**d**. To increase the selectivity, the *R*-carboxymethyl group was further attached to the linker of **3b** and **3c** (**3e** and **3f** in Table 2). To our surprise, this modification did not significantly enhance the affinity for fXa in contrast to the conversion from **1a** to **1f**, but nonetheless, the selectivity for fXa was markedly improved. Thus, the *R*-carboxymethyl and pyruvic acid moieties did not have independent effects in this case, possibly because the binding mode of the compound could not be optimum for both moieties.

Although the pyruvic acid moiety is an excellent substituent in terms of affinity, its electrophilic site may not be favorable from the viewpoint of pharmacology.[21] Therefore, we also designed and synthesized corresponding compounds with propionic acid substitution (**4a**, **4b** in Table 2). Their affinity was slightly decreased compared with that of the compounds with pyruvic acid substitution (**3b**, **3c**); however, the affinities of these compounds (**4a**: 33 nM, **4b**: 43 nM) were much higher than those of the compounds without substitution (**1b**: 0.17 μM, **1c**: 0.29 μM), presumably because of the interactions discussed in the design section. On the

Table 2. Enzyme-Inhibitory Activity of Benzamidine Compounds with Pyruvic Acid or Propionic Acid Substitution



compound	R_1	R_2	X	K_i (μM) fXa	K_i (μM) thrombin	selectivity (thrombin/fXa)
3a		H	CO	0.026	38	1500
3b		H	CO	0.020	53	2700
3c		H	CO	0.021	140	6700
3d		H	CO	0.0030	24	8000
3e			CO	0.011	540	49000
3f			CO	0.018	680	38000
4a		H	CH_2	0.033	>1500	>45000
4b		H	CH_2	0.043	>1500	>35000

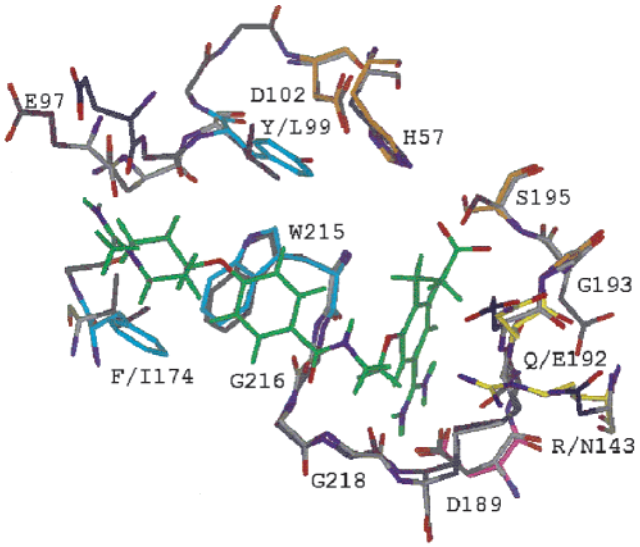
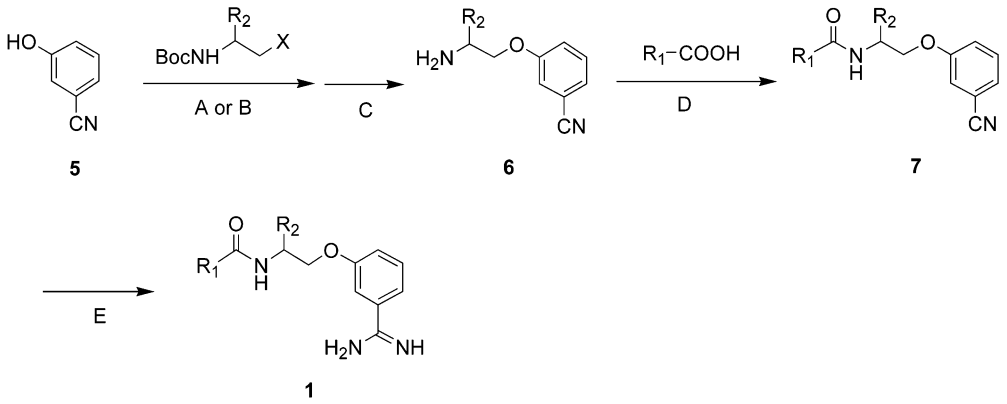


Figure 4. Calculated docking mode of **4a** in fXa. The residues of thrombin are superposed, and the color scheme is the same as in Figure 2. The propionic acid moiety of **4a** is placed in the vicinity of Gly193 and Gln192 of fXa and appears to interact with them.

other hand, the compounds with propionic acid substitution did not show enhanced potency against thrombin, and therefore, the selectivity for fXa over thrombin was significantly improved to more than 35000-fold. This may be due to the repulsive interaction with the corresponding Glu192 of thrombin (Figure 4).

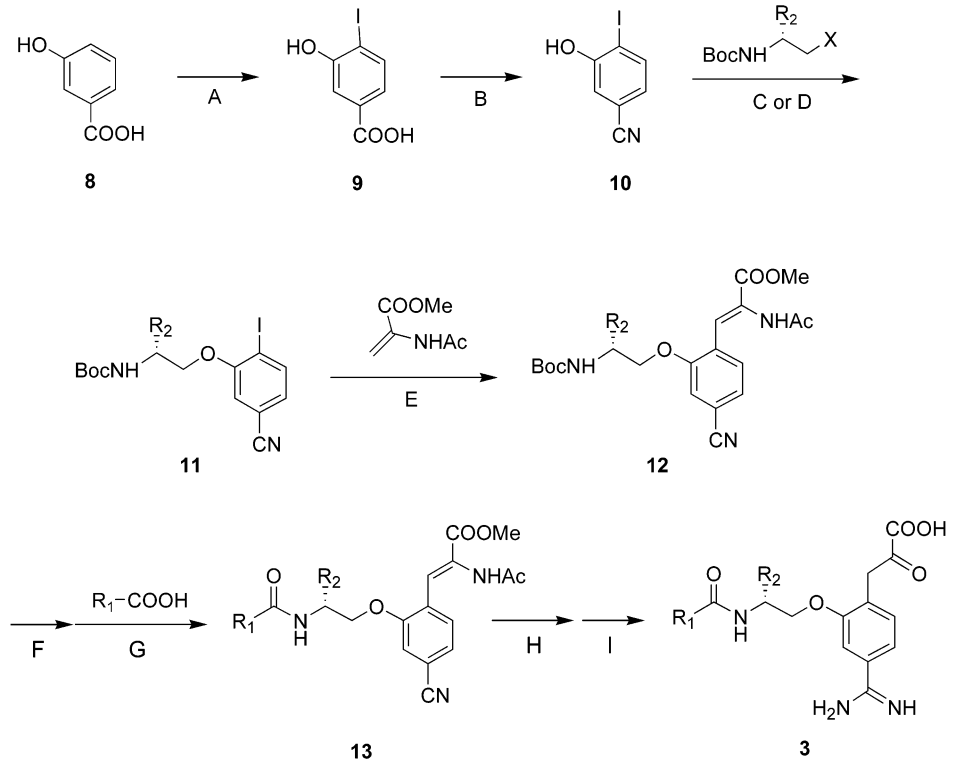
Table 3 shows the concentrations of inhibitors required to double the prothrombin time (PT) in plasma clotting. The selected compounds (**3b**, **3c**, **3e**, and **4b**) showed potent anticoagulant activities. Therefore, the ex vivo anticoagulant activities were further examined in cynomolgus monkeys. In terms of PT prolongation, these compounds exhibited potent anticoagulant activity at the oral dose of 10 mg/kg (Figure 5). The anticoagulant effects of the compounds with pyruvic acid substitution (**3b**, **3c**, **3e**) peaked at 1−2 h and disappeared within 8 h, whereas the compounds with propionic acid substitution (**4b**) had a longer duration of action. The oral bioavailability of **4b**, evaluated by measuring the drug concentration in plasma, was 6%. Compounds **3b**, **3c**, and **3e** had similar bioavailabilities of about 12% and might therefore be potential lead compounds for the

Scheme 1[a]



[a] Reagents and conditions: (A) X = Cl, K_2CO_3, DMF 50 °C; (B) X = OH, DEAD, PPh_3, THF; (C) HCl, dioxane; (D) condensation reaction; (E) i. HCl, EtOH, dioxane; ii. $(NH_4)_2CO_3$, EtOH; iii. ethyl acetimidate hydrochloride, NEt_3 for **1b**; iv. cHCl, 40 °C for **1f** and **1g**.

Scheme 2[a]



[a] Reagents and conditions: (A) ICl, AcOH; (B) i. ClCOOEt, TEA, THF; ii. NH_3, THF; iii. $(CF_3CO)_2O$, pyridine, dioxane; (C) X = Cl, K_2CO_3, DMF, 50 °C; (D) X = OH, *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide, PPh_3, THF; (E) $Pd(OAc)_2$, PPh_3, DMF, 100 °C; (F) HCl, dioxane; (G) EDC, TEA, HOBt, DMF; (H) i. HCl, EtOH, dioxane; ii. $(NH_4)_2CO_3$, EtOH; iii. ethyl acetimidate hydrochloride, TEA for **13b** and **13e**; (I) HCl aq, 80 °C.

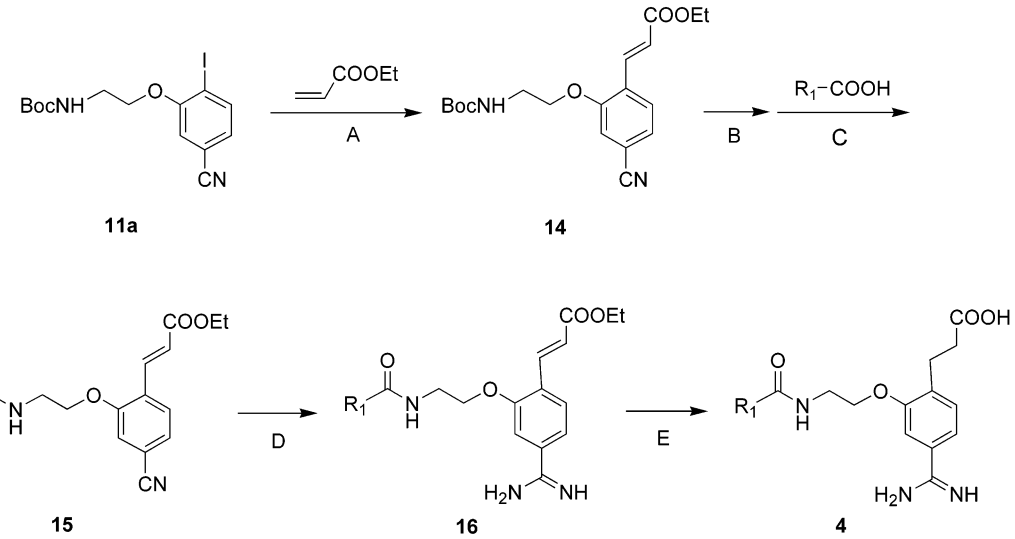
development of anticoagulant drugs for oral administration.

Conclusion

We have computationally designed selective fXa inhibitors based on the three-dimensional structures of fXa and thrombin. This structure-based drug design strategy to improve the affinity and selectivity of **1a** may be summarized as follows: (1) The 4-amidinophenyl group was successfully replaced with other groups designed to interact better with the S4 pocket; (2) *R*-carboxymethyl substitution was introduced into the linker moiety because the computational models predicted electrostatic attraction with fXa and repulsion with thrombin, which was expected to improve the selectivity; and (3) pyruvic and propionic acid moieties were incorporated onto the P1 benzamidine to interact with the CT region, with the aim of enhancing the affinity for fXa.

As a result of this approach, selective fXa inhibitors with high affinity were successfully developed. Some of the compounds (**3b**, **3c**, **3e**, and **4b**) were further examined for anticoagulant activity after oral administration to cynomolgus monkeys, and the results indicated that several of the inhibitors could be potential lead compounds for the development of orally active anticoagulant drugs. Further investigations to improve the pharmacokinetic profile of these compounds are in progress.

Scheme 3[a]



[a] Reagents and conditions: (A) $Pd(OAc)_2$, PPh_3, DMF, 100 °C; (B) HCl, dioxane; (C) EDC, TEA, HOBt, DMF; (D) i. HCl, EtOH, dioxane; ii. $(NH_4)_2CO_3$, EtOH; iii. ethyl acetimidate hydrochloride, TEA for **15a**; (E) i. Pd/C, H_2; ii. cHCl, 50 °C.

Table 3. Anticoagulant Activities of fXa Inhibitors

	2× PT (μM)[a]	
compound	human	monkey
3b	0.20	0.12
3c	0.22	0.22
3e	0.38	0.38
4b	0.42	0.70

[a] Concentration required to double the prothrombin time (PT).

Experimental Section

Molecular Modeling. The coordinate sets for the protein structures used in this investigation were 1HCG[7] for fXa and 1DWC[12] for thrombin. Compounds **1a**, **1f**, **3a**, and **4a** were docked into the fXa active site using an in-house docking program. This method is composed of three steps. Initially, alignment of an anchor fragment in a target protein is searched, namely P1 benzamidine in the case of fXa. Second, the remaining part (excluding the anchor) is put into incremental conformational searches in the protein environment. Finally, the alignment of the resulting whole ligand is optimized. The docking program was created using the Sybyl Programming Language and utilizes the Sybyl commands, systematic search, and Leapfrog search.[24] Full details of these docking studies have been reported.[1c,25]

Determination of Trypsin−Inhibitor Crystal Structure. Crystallization and Inhibitor Soaking. Bovine pancreatic trypsin (Sigma T8003) was solubilized to final concentration of 30 mg/mL in a buffer containing 0.5 mM $CaCl_2$, 20 mM benzamidine, and 50 mM cacodylate at pH 6.0. The sitting-drop crystallization method was used to form crystals. Single crystals were obtained with ammonium sulfate (2.0−2.2 M) at pH 6.0 in the open form (*P*212121, $a = 63.7$ Å, $b = 63.5$ Å, $c = 68.9$ Å), wherein the binding site is free of any contact with crystallographic symmetry-related molecules.[26] The crystals were soaked in 500 μL of a buffer containing 3.0 M ammonium sulfate, 1 mM $CaCl_2$, and 50 mM cacodylate at pH 6.0 for 1 h five times to exclude benzamidine, and then they were soaked in 500 μL of the same solution with 2 mM **1a** or **3b** for 2 d.

Data Collection and Processing. X-ray intensity data were collected using an R-AXIS II at room temperature (Rigaku, Japan). Data processing was done with PROCESS (Rigaku), and structure refinement was performed using X-PLOR.3.851.[27] The starting point was the uncomplexed trypsin structure at 1.50 Å resolution (1TLD). The final R-factors of **1a** and **3b** were 0.188 and 0.193, respectively, in the resolution range of 10−1.88 Å.

Chemistry. Workup included drying over magnesium sulfate, filtering, and concentrating in vacuo. Column chromatography of intermediates was performed using silica gel (Merck, particle size 0.063−0.200 mm). Final products were purified by preparative reversed-phase HPLC, which was performed on a Waters 600 system with a C-18 reversed-phase column (Inertsil ODS-3, GL Sciences Inc.), using a mixture of acetonitrile and water, both of which contained 0.1% TFA. Fractions containing the desired material were concentrated and lyophilized to obtain the final products as a white solid. NMR spectra were recorded on a Varian EM-390 at 300 MHz. Keto−enol tautomerism was observed in the NMR spectra of all the benzamidine compounds with pyruvic acid substitution (**3**). Mass spectra (ESI and FAB) were measured on JEOL JMS-DX300 instruments. Where analyses are indicated only by the symbols of the elements, the results obtained were within 0.4% of the theoretical values. Reagent abbreviations: HOBT, 1-hydroxybenzotriazole; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl; TEA, triethylamine; TFA, trifluoroacetic acid.

***tert*-Butyl (2-Chloroethyl)carbamate (17a).** A mixture of TEA (30 mL, 0.2 mol), di-*tert*-butyl carbonate (40 g, 0.18 mol), and 2-chloroethylamine HCl (25 g, 0.2 mol) in dichloromethane (300 mL) was stirred for 3 d. Then 0.5 M HCl was added to the mixture. The organic layer was separated, washed with saturated NaCl, and worked up to yield **17a** (35.6 g, 99%): ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 3.45 (2H, dt), 3.60 (2H, t), 5.00 (1H, br).

Benzyl (3*R*)-3-*tert*-Butoxycarbonylamino-4-hydroxybutyrate (17b). Ethyl chloroformate (3.08 mL, 32.2 mmol) was added to a mixture of Boc-*D*-aspartic acid β-benzyl ester (10.4 g, 32.3 mmol) and TEA (4.49 mL, 32.2 mmol) in tetrahydrofuran (100 mL) at 0 °C. The reaction mixture was stirred for 10 min and then filtered to remove precipitates. A small amount of crushed ice and $NaBH_4$ (1.23 g, 32.5 mmol) in water was added to the filtrate at 0 °C. The mixture was stirred for 3 min and then diluted with 1 N HCl. EtOAc was added to the solution, and then the organic layer was washed with saturated NaCl and worked up. The crude product was purified by chromatography (EtOAc/hexane) to give **17b** (5.28 g, 51%): ^{1}H NMR ($CDCl_3$) δ 1.42 (9H, s), 2.66 (2H, d), 3.65 (2H, dd), 4.00 (1H, ddt), 5.14 (2H, s), 7.35−7.40 (5H, m).

Benzyl (3*S*)-3-*tert*-Butoxycarbonylamino-4-hydroxybutyrate (17c). The title compound was prepared as described above, with Boc-*L*-aspartic acid β-benzyl ester as starting material.

3-(2-Aminoethoxy)benzonitrile HCl (6a). Part A. A mixture of 3-cyanophenol (6.64 g, 55.7 mmol), K_2CO_3 (7.70 g,

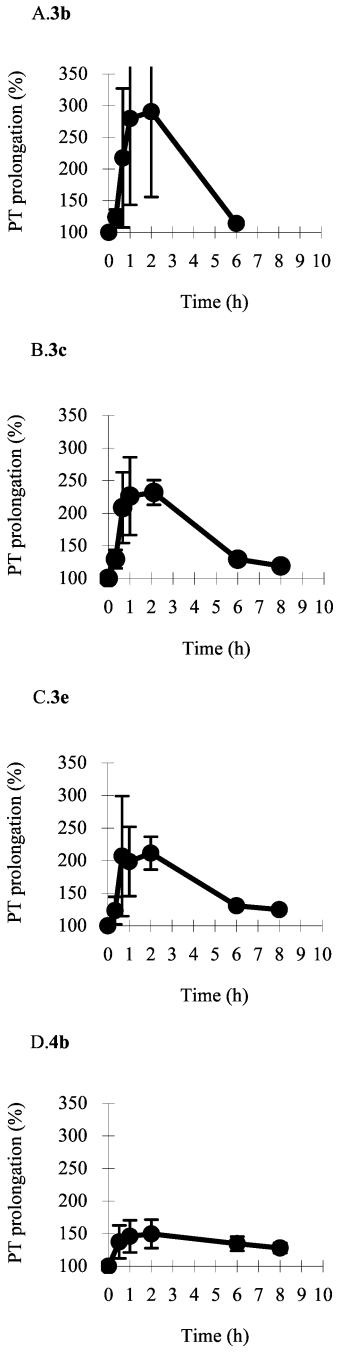


Figure 5. Anticoagulant activities of fXa inhibitors after oral administration at 10 mg/kg in cynomolgus monkeys. The results are expressed as mean ± SEM of three determinations for (A) **3b**, (B) **3c**, (C) **3e**, and (D) **4b**.

55.7 mmol), and **17a** (10 g, 55.7 mmol) in DMF (100 mL) was heated to 60 °C for 4 d. The solution was concentrated in vacuo and diluted with EtOAc. The solution was washed with 1 N HCl, 1 N NaOH, and saturated NaCl and then worked up. The crude product was purified by chromatography (EtOAc/hexane) to give 3-[2-(*tert*-butoxycarbonylamino)ethoxy]benzonitrile (10.9 g, 75%): ^{1}H NMR ($CDCl_3$) δ 1.44 (1H, s), 3.55 (2H, dt), 4.05 (2H, t), 4.95 (1H, br), 7.12 (1H, d), 7.14 (1H, s), 7.26 (1H, d), 7.38 (1H, t).

Part B. A solution of 4.0 M HCl in 1,4-dioxane (100 mL) was added to the product of Part A (23.9 g, 91.2 mmol). The reaction mixture was stirred for 2 h and concentrated in vacuo. The residue was washed with EtOAc to give **6a** (15.4 g, 85%): ^{1}H NMR (DMSO-d_6) δ 3.20 (2H, t), 4.30 (2H, t), 7.35 (1H, d), 7.45 (1H, d), 7.50 (1H, s), 7.55 (1H, t), 8.35 (3H, br).

Benzyl (3*R*)-3-Amino-4-(3-cyanophenoxy)butyrate HCl (6b). Part A. Diethyl azodicarboxylate 40% solution in toluene (7.84 g, 18 mmol) was added to a mixture of **17b** (5.28 g, 18 mmol), 3-cyanophenol (2.38 g, 20 mmol), and triphenylphosphine (4.72 g, 18 mmol) in tetrahydrofuran (60 mL) at 0 °C. The mixture was stirred for 2 d and concentrated in vacuo. The residue was diluted with EtOAc, washed with 1 N NaOH and saturated NaCl, and then worked up. The crude product was purified by chromatography (EtOAc/hexane) to give benzyl (3*R*)-3-*tert*-butoxycarbonylamino-4-(3-cyanophenoxy)butyrate (4.32 g, 58%): ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 2.79 (2H, d), 4.00 (1H, dd), 4.06 (1H, dd), 4.41 (1H, br), 5.13 (2H, s), 5.56 (1H, br), 7.05−7.18 (4H, m), 7.21−7.38 (5H, m).

Part B. The product of Part A was treated as described for **6a**, Part B, to give **6b**: ^{1}H NMR (DMSO-d_6) δ 2.90 (2H, d), 3.90 (1H, m), 4.15−4.30 (2H, m), 5.20 (2H, s), 7.30−7.40 (7H, m), 7.40−7.60 (3H, m), 8.35 (3H, br).

Benzyl (3*S*)-3-Amino-4-(3-cyanophenoxy)butyrate HCl (6c). The title compound was prepared as described above, with benzyl (3*S*)-3-*tert*-butoxycarbonylamino-4-hydroxybutyrate (**17c**) as the starting material.

4-(1-*tert*-Butoxycarbonyl-4-piperidyloxy)benzoic Acid (18a). Part A. Diethyl azodicarboxylate (1.62 g, 9.3 mmol), ethyl 4-hydroxybenzoate (1.70 g, 10.2 mmol), 1-*tert*-butoxycarbonyl-4-hydroxypiperidine (1.76 g, 9.3 mmol), and triphenylphosphine (2.44 g, 9.3 mmol) were treated as described for **6b**, Part A, to give ethyl 4-(1-*tert*-butoxycarbonyl-4-piperidyloxy)benzoate (1.57 g, 44%): ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t), 1.50 (9H, s)1.70−1.80 (2H, m), 1.90−2.00 (2H, m), 3.30−3.41 (2H, m), 3.63−3.75 (2H, m), 4.35 (2H, q), 4.55 (1H, m), 6.90 (2H, d), 8.00 (2H, d).

Part B. A 1.0 N solution of NaOH (5 mL) was added to a solution of the product of Part A (847 mg, 2.43 mmol) in ethanol (50 mL). The reaction mixture was stirred for 3 d and concentrated in vacuo. The residue was diluted with EtOAc, then washed with 1 N HCl and saturated NaCl, and worked up to yield **18a** (697 mg, 92%): ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 1.70−2.00 (4H, m), 3.30−3.40 (2H, m), 3.65−3.75 (2H, m), 4.60 (1H, s), 6.95 (2H, d), 8.05 (2H, d).

4-(1-Pyrrolidinecarbonyl)benzoic acid (18b). A mixture of methyl 4-chlorocarbonylbenzoate (29.0 g, 0.146 mmol), pyrrolidine (14.2 g, 200 mmol), and TEA (21.0 g, 208 mmol) in dichloromethane (300 mL) was stirred. The solution was washed with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl, and then it was worked up to give crude methyl 4-(1-pyrrolidinecarbonyl)benzoate. NaOH (12.0 g, 0.3 mmol) was added to a solution of the crude ester in a mixture of water, methanol, and tetrahydrofuran (70 mL:70 mL:70 mL). The reaction mixture was stirred and concentrated in vacuo. The residue was diluted with CH_2Cl_2, washed with saturated NaCl, and then worked up to yield **18b** (23.7 g, 74%): ^{1}H NMR (DMSO-d_6) δ 1.75−1.90 (4H, m), 3.30−3.50 (4H, m), 7.62 (2H, d), 7.99 (2H, d), 13.14 (1H, br).

***N*-[2-(3-Cyanophenoxy)ethyl]-4-cyanobenzamide (7a).** Ethyl chloroformate (0.67 mL, 7.1 mmol) was added to a solution of 4-cyanobenzoic acid (**18c**) (1.13 g, 7.68 mmol) and *N*-methylmorpholine (1.6 mL, 14.1 mmol) in DMF at 0 °C. After 10 min, **6a** (1.27 g, 6.41 mmol) was added, and the mixture was stirred for 5 h. The reaction mixture was diluted with EtOAc, washed with 1 N HCl, 1 N NaOH, and saturated NaCl, and then worked up. The residue was washed with Et_2O and EtOAc to give **7a** (1.29 g, 69%): ^{1}H NMR ($CDCl_3$) δ 3.91 (2H, dt), 4.19 (2H, t), 6.78 (1H, br), 7.14 (1H, d), 7.17 (1H, s), 7.28 (1H, d), 7.39 (1H, t), 7.75 (2H, d), 7.90 (2H, d).

***N*-[2-(3-Cyanophenoxy)ethyl]-4-(1-*tert*-butoxycarbonyl-4-piperidyloxy)benzamide (7b).** The title compound was prepared from **18a** (211 mg, 0.65 mmol) and **6a** (129 mg, 0.65 mmol) as described for **7a**. The crude product was purified by chromatography (EtOAc/hexane) to give **7b** (167 mg, 55%): ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 1.65−1.80 (2H, m), 1.85−2.00 (2H, m), 3.30−3.40 (2H, m), 3.60−3.75 (2H, m), 3.90 (2H, dt), 4.20 (2H, t), 4.55 (1H, m), 6.45 (1H, t), 6.94 (2H, d), 7.15 (1H, d), 7.17 (1H, s), 7.26 (1H, d), 7.38 (1H, t), 7.74 (2H, d).

***N*-[2-(3-Cyanophenoxy)ethyl]-1-(4-pyridyl)piperidine-4-carboxamide (7c).** A mixture of 1-(4-pyridyl)piperidine-4-carboxylic acid HCl (**18d**)[10] (412 mg, 1.48 mmol), **6a** (350 mg, 1.77 mmol), TEA (0.25 mL, 1.77 mmol), HOBT (240 mg, 1.77 mmol), and EDC (340 mg, 1.77 mmol) in DMF (3 mL) was stirred overnight. The mixture was diluted with EtOAc, washed with 1 N NaOH and saturated NaCl, and then worked up to give **7c** (470 mg, 91%): ^{1}H NMR (DMSO-d_6) δ 1.52 (2H, m), 1.75 (2H, d), 2.40 (1H, m), 2.80 (2H, m), 3.40 (2H, dt), 3.90 (2H, m), 4.08 (2H, t), 6.80 (2H, d), 7.31 (1H, d), 7.40 (1H, d), 7.42 (1H, s), 7.51 (1H, t), 8.09 (1H, t), 8.13 (2H, d).

***N*-[2-(3-Cyanophenoxy)ethyl]-4-(pyrrolidine-1-carbonyl)benzamide (7d).** The title compound was prepared from **6a** and **18b** as described for **7c**: ^{1}H NMR ($CDCl_3$) δ 1.80−2.00 (4H, m), 3.30−3.70 (4H, m), 3.85 (2H, dt), 4.20 (2H, t), 7.14−7.28 (4H, m), 7.38 (1H, t), 7.48 (2H, d), 7.79 (2H, d).

***N*-[2-(3-Cyanophenoxy)ethyl]benzamide (7e).** Benzoyl chloride (421 mg, 3.00 mmol) was added to a solution of **6a** and TEA (2.09 mL, 15.0 mmol) in DMF (15 mL) at 0 °C. The reaction mixture was stirred overnight and diluted with EtOAc. The solution was washed with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl, and then worked up. The residue was purified by chromatography to give **7e** (710 mg, 89%): ^{1}H NMR ($CDCl_3$) δ 3.91 (2H, q), 4.19 (2H, t), 6.54 (1H, m), 7.18 (2H, d), 7.28 (1H, d), 7.38 (1H, d), 7.46 (2H, d), 7.52 (1H, d), 7.78 (2H, d).

Benzyl (3*R*)-3-(4-Cyanobenzoylamino)-4-(3-cyanophenoxy)butyric Acid (7f). Compound **6b** (1.52 g, 4.38 mmol), 4-cyanobenzoyl chloride (1.09 g, 6.58 mmol), TEA (1.22 mL, 8.76 mmol), and CH_2Cl_2 (5 mL) were treated as described for **7e** to give **7f** (1.21 g, 63%): ^{1}H NMR ($CDCl_3$) δ 2.86 (1H, dd), 2.95 (1H, dd), 4.12 (1H, dd), 4.20 (1H, dd), 4.85 (1H, br), 5.16 (2H, s), 7.09 (1H, d), 7.11 (1H, dd), 7.24−7.40 (7H, m), 7.72 (2H, d), 7.83 (2H, d).

Benzyl (3*S*)-3-(4-Cyanobenzoylamino)-4-(3-cyanophenoxy)butyric Acid (7g). The title compound was prepared as described above, with benzyl (3*S*)-3-amino-4-(3-cyanophenoxy)-butyrate HCl **(6c)** as the starting material.

***N*-[2-(3-Amidinophenoxy)ethyl]-4-amidinobenzamide Bistrifluoroacetate (1a).** A mixture of 30% HCl solution in ethanol (24 mL), **7a** (2.43 g, 8.35 mmol), and a 4.0 M HCl solution in 1,4-dioxane (56 mL) was stirred for 4 d and concentrated in vacuo. The crude imidate was diluted with 10% ammonia solution in ethanol. The reaction mixture was stirred for 24 h and concentrated in vacuo to yield the crude product. Purification by reversed-phase HPLC yielded **1a** (1.19 g, 26%): ^{1}H NMR (DMSO-d_6) δ 3.69 (2H, dt), 4.24 (2H, t), 7.32 (1H, d), 7.39 (1H, d), 7.40 (1H, s), 7.53 (1H, t), 7.90 (2H, d), 8.05 (2H, d), 9.02 (1H, t), 9.18 (2H, br), 9.30 (4H, br), 9.43 (2H, br). MS (FAB): 326 (MH^+). Anal. ($C_{17}H_{19}N_5O_2$·2.0TFA·2.0H_2O) C, H, N.

***N*-[2-(3-Amidinophenoxy)ethyl]-4-(1-acetimidoyl-4-piperidyloxy)benzamide Bistrifluoroacetate (1b). Part A.** Compound **7b** (165 mg, 0.35 mmol) was treated as described for **1a** to yield *N*-[2-(3-amidinophenoxy)ethyl]-4-(4-piperidyloxy)benzamide bistrifluoroacetate (124 mg, 0.20 mmol): ^{1}H NMR (DMSO-d_6) δ 1.80−1.90 (2H, m), 2.08−2.18 (2H, m), 3.02−3.30 (4H, m), 3.62 (2H, q), 4.21 (2H, t), 4.75 (1H, m), 7.06 (2H, d), 7.30−7.42 (3H, m), 7.53 (1H, t), 7.85 (2H, d), 8.58 (2H, br), 8.61 (1H, br), 9.12 (2H, br), 9.28 (2H, br). MS (ESI): 383 (MH^+).

Part B. Ethyl acetimidate HCl (147 mg, 1.2 mmol) was added to a solution of the Part A product (124 mg, 0.20 mmol) and TEA (183 mg, 1.8 mmol) in ethanol (5 mL). The reaction mixture was stirred for 6 d and concentrated in vacuo. The residue was purified by reversed-phase HPLC to give **1b** (120 mg, 92%): ^{1}H NMR (DMSO-d_6) δ 1.70−1.82 (2H, m), 2.02−2.14 (2H, m), 2.30 (3H, s), 3.50−3.60 (2H, m), 3.65 (2H, q), 3.70−3.80 (2H, m), 4.20 (2H, t), 4.80 (1H, m), 7.07 (2H, d), 7.30−7.40 (3H, m), 7.53 (1H, t), 7.85 (2H, d), 8.57−8.63 (2H, m), 9.11−9.18 (3H, m), 9.28 (2H, br). MS (ESI): 424 (MH^+). Anal. ($C_{23}H_{29}N_5O_3$·2.0TFA·1.2H_2O) C, H, N.

***N*-[2-(3-Amidinophenoxy)ethyl]-1-(4-pyridyl)piperidine-4-carboxamide Bistrifluoroacetate (1c).** Compound **7c** (460 mg, 1.31 mmol) was treated as described for **1a** to yield **1c** (402 mg, 52%): ^{1}H NMR (DMSO-d_6) δ 1.57 (2H, m), 1.82 (2H, m), 2.51−2.60 (1H, m), 3.20 (2H, m), 3.40 (2H, dt), 4.09 (2H, t), 4.23 (2H, m), 7.18 (2H, d), 7.30 (1H, d), 7.40 (1H, d), 7.40 (1H, s), 7.55 (1H, t), 8.20 (3H, m), 9.25 (2H, br), 9.29 (2H, br). MS (ESI): 368 (MH^+). Anal. ($C_{20}H_{25}N_5O_2$·2.0TFA·1.5H_2O) C, H, N.

***N*-[2-(3-Amidinophenoxy)ethyl]-4-(1-pyrrolidinecarbonyl)benzamide Trifluoroacetate (1d).** To 4.0 M HCl solution in 1,4-dioxane (5 mL) and ethanol (0.5 mL) was added **7d** (270 mg, 0.74 mmol). The reaction mixture was stirred for 5 d and concentrated in vacuo. The crude imidate was diluted with ethanol (10 mL), and ammonium carbonate (300 mg) was added to the solution. The reaction mixture was stirred for 2 d and concentrated in vacuo. Purification by reversed-phase HPLC yielded **1d** (238 mg, 65%): ^{1}H NMR (DMSO-d_6) δ 1.75−1.90 (4H, m), 3.30−3.50 (4H, m), 3.70 (2H, dt), 4.20 (2H, t), 7.34 (1H, d), 7.39 (1H, d), 7.40 (1H, s), 7.54 (1H, t), 7.59 (2H, d), 7.91 (2H, d), 8.80 (1H, t), 9.10 (2H, br), 9.30 (2H, br). MS (ESI): 381 (MH^+). Anal. ($C_{21}H_{24}N_4O_3$·TFA·0.35H_2O) C, H, N.

***N*-[2-(3-Cyanophenoxy)ethyl]benzamide Trifluoroacetate (1e).** Compound **7e** (460 mg, 1.73 mmol) was treated as described for **1a** to yield **1e** (117 mg, 14%): ^{1}H NMR (DMSO-d_6) δ 3.69 (2H, dt), 4.23 (2H, t), 7.35−7.53 (7H, m), 7.89 (2H, d), 8.78 (1H, br), 9.21 (2H, br), 9.33 (2H, br). MS (ESI): 284 (MH^+). Anal. ($C_{16}H_{17}N_3O_2$·TFA·0.5H_2O) C, H, N.

(3*R*)-3-(4-Amidinobenzoylamino)-4-(3-amidinophenoxy)-butyric Acid Bistrifluoroacetate (1f). Part A. Compound **7f** (1.21 g, 2.75 mmol) was treated as described for **1a** to yield ethyl (3*R*)-3-(4-amidinobenzoylamino)-4-(3-amidinophenoxy)-butyrate bistrifluoroacetate (456 mg, 26%): ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t), 2.82 (2H, d), 4.07 (2H, q), 4.12 (1H, dd), 4.24 (1H, dd), 4.72 (1H, br), 7.33 (1H, d), 7.39 (1H, s), 7.40 (1H, d), 7.54 (1H, dd), 7.91 (2H, d), 8.02 (2H, d), 8.84 (1H, d), 9.16 (2H, s), 9.28 (4H, s), 9.42 (2H, s). MS (ESI): 412 (MH^+).

Part B. A mixture of the Part A product (466 mg, 0.73 mmol) and hydrochloric acid (10 mL) was stirred at 40 °C for 6 h and concentrated in vacuo. The residue was purified by reversed-phase HPLC to give **1f** (151 mg, 46%): ^{1}H NMR (DMSO-d_6) δ 2.74 (2H, d), 4.13 (1H, dd), 4.24 (1H, dd), 4.69 (1H, ddt), 7.35 (1H, d), 7.40 (1H, d), 7.41 (1H, s), 7.55 (1H, dd), 7.91 (2H, d), 8.03 (2H, d), 8.81 (1H, d), 9.20 (2H, s), 9.28 (2H, s), 9.33 (2H, s), 9.43 (2H, s). MS (ESI): 384 (MH^+). Anal. ($C_{19}H_{21}N_5O_4$·2.0TFA·1.0H_2O) C, H, N.

(3*S*)-3-(4-Amidinobenzoylamino)-4-(3-amidinophenoxy)-butyric Acid Bistrifluoroacetate (1 g). The title compound was prepared as described above, with benzyl (3*S*)-3-amino-4-(3-cyanophenoxy)butyrate HCl (**6c**) as the starting material. Anal. ($C_{19}H_{21}N_5O_4$·2.0TFA·1.0H_2O) C, H, N.

3-Hydroxy-4-iodobenzoic Acid (9). Iodine monochloride (53.0 g, 326 mmol) was added to a solution of 3-hydroxybenzoic acid (**8**) (30.0 g, 217 mmol) in acetic acid (200 mL). The reaction mixture was stirred at 45 °C for 15 h, and concentrated in vacuo. The residue was washed with 1% sodium thiosulfate and water to give **9** (17.2 g 30%):^{1}H NMR (DMSO-d_6) δ 7.13 (1H, dd), 7.43 (1H, d), 7.80 (1H, d); the spectrum was identical to the reported spectrum.[16b]

3-Hydroxy-4-iodobenzonitrile (10). Part A. Ethyl chloroformate (19.7 mL, 206 mmol) was added to a solution of **9** (22.3 g, 89.7 mmol) and TEA (28.7 mL, 206 mmol) in tetrahydrofuran (300 mL) at 0 °C. The reaction mixture was stirred for 15 min and filtered to remove precipitates. The filtrate was added to a NH_3 solution in tetrahydrofuran at 0 °C. The mixture was stirred for 10 h and concentrated in vacuo to give the crude benzamide.

Part B. The product of Part A was diluted with dioxane (450 mL), and then trifluoroacetic anhydride (17.4 mL, 117 mmol) and pyridine (21.8 mL 269 mmol) were added to the solution at 0 °C. The reaction mixture was stirred for 18 h at room temperature and concentrated in vacuo. The residue was diluted with $CHCl_3$, washed with saturated NaCl, and worked up to give the crude benzonitrile.

Part C. The residue of Part B was diluted with a mixture (180 mL) of tetrahydrofuran and methanol (1:1). A 1 N NaOH

solution (90 mL) was added, and the reaction mixture was stirred for 4 h to hydrolyze the remaining ethyl phenyl carbonate. The solution was concentrated, and the residue was diluted with EtOAc. This solution was washed with 1 N HCl and saturated NaCl and worked up. The crude product was purified by chromatography (EtOAc/hexane) to give **10** (9.29 g, 42%): ^{1}H NMR ($CDCl_3$) δ 5.63 (1H, br), 6.96 (1H, dd), 7.23 (1H, d), 7.79 (1H, d).

***tert*-Butyl [2-(5-Cyano-2-iodophenoxy)ethyl]carbamic Acid (11a).** Compound **10** (28.7 g, 117 mmol), **17a** (25 g, 140 mmol), K_2CO_3 (48.4 g, 350 mmol), and KI (39 g, 235 mmol) were treated as described for **6a**, Part A, to give **11a** (37 g, 81%). ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 3.62 (2H, dt), 4.10 (2H, t), 5.05 (1H, br), 6.96−7.06 (2H, m), 7.90 (1H, d).

Benzyl (3*R*)-3-*tert*-Butoxycarbonylamino-4-(5-cyano-2-iodophenoxy)butyric Acid (11b). *N*,*N*,*N*′,*N*′-Tetramethylazodicarboxamide[28] (7.4 g, 42.7 mmol) was added to a mixture of **17b** (10.16 g, 32.8 mmol), **10** (10.5 g, 42.7 mmol), and triphenylphosphine (11.2 g, 42.7 mmol) in toluene (100 mL) at 0 °C. The reaction mixture was stirred overnight and concentrated in vacuo. The residue was purified by chromatography (EtOAc/hexane) to give **11b** (11.9 g, 67%): ^{1}H NMR ($CDCl_3$) δ 2.93 (1H, dd), 3.08 (1H, dd), 4.13 (1H, dd), 4.30 (1H, dd), 4.94 (1H, m), 5.14 (1H, d), 5.19 (1H, d), 6.96 (1H, d), 7.03 (1H, dd), 7.28−7.33 (5H, m), 7.72 (2H, d), 7.86 (2H, d), 7.88 (1H, d).

Methyl 2-Acetamido-3-[2-(2-*tert*-butoxycarbonylaminoethoxy)-4-cyanophenyl]acrylate (12a). A mixture of **11a** (15.33 g, 39.5 mmol), TEA (7.99 g, 79.0 mmol), methyl 2-acetamidoacrylate (9.05 g, 63.2 mmol), tri-*o*-tolylphosphine (7.30 g, 24 mmol), and $Pd(OAc)_2$ in CH_3CN (150 mL) was heated to reflux overnight. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was diluted with EtOAc, and the organic layer was washed with 1 N HCl and saturated NaCl and worked up. The crude product was washed with EtOAc/hexane to give **12a** (9.63 g, 60%): ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 1.95 (3H, s), 3,35 (2H, dt), 3.70 (3H, s), 4.10 (2H, t), 7.03 (1H, t), 7.20 (1H, s), 7.43 (1H, d), 7.55 (1H, s), 7.68 (1H, d), 9.65 (1H, s).

Methyl 2-Acetamido-3-[2-(2-aminoethoxy)-4-cyanophenyl]acrylate HCl (19a). Compound **12a** (9.63 g, 23.9 mmol) was treated as described for **6a**, Part B, to give **19a** (6.31 g, 78%): ^{1}H NMR (DMSO-d_6) δ 1.95 (3H, s), 3.25 (2H, dt), 3.70 (3H, s), 4.30 (2H, t), 7.28 (1H, s), 7.48 (1H, d), 7.62 (1H, s), 7.70 (1H, d), 8.20 (3H, br), 9.75 (1H, s).

Methyl 2-Acetamido-3-[(2-butoxycarbonylamino-3-benzyloxycarbonylpropoxy)-4-cyanophenyl]acrylate (12b). Compound **11b** (12.0 g, 22.4 mmol), TEA (6.24 mL), methyl 2-acetamidoacrylate (6.40 g, 44.7 mmol), tri-*o*-tolylphosphine (1.47 g, 4.83 mmol), $Pd(OAc)_2$ (0.49 g, 10 mol %), and DMF (100 mL) were treated as described in **12a** to give **12b** (9.8 g, 79%): ^{1}H NMR (DMSO-d_6) δ 1.35 (9H, s), 1.95 (3H, s), 2.61−2.73 (2H, m), 3.68 (3H, s), 4.05 (2H, m), 4.24 (1H, m), 5.10 (2H, s), 7.11 (1H, d), 7.19 (1H, s), 7.35 (5H, m), 7.44 (1H, d), 7.56 (1H, s), 7.67 (1H, d), 9.69 (br, 1H).

Methyl 2-Acetamido-3-[(2-amino-3-benzyloxycarbonylpropoxy)-4-cyanophenyl]acrylate HCl (19b). 12b (530 mg, 0.96 mmol) was treated as described for **6a**, Part B, to give **19b** (470 mg, quant.): MS (ESI): 452 (MH^+).

Methyl 2-Acetamido-3-[2-[2-(4-cyanobenzoylamino)ethoxy]-4-cyanophenyl]acrylate (13a). 4-Cyanobenzoic acid (**18c**) (44 mg, 0.3 mmol), **19a** (100 mg, 0.3 mmol), TEA (61 mg, 0.6 mmol), HOBT (46 mg, 0.3 mmol), EDC (58 mg, 0.3 mmol), and DMF (5 mL) were treated as described for **7c** to give **13a** (120 mg, 92%): ^{1}H NMR (DMSO-d_6) δ 1.95 (3H, s), 3.65 (3H, s), 3.70 (2H, dt), 4.25 (2H, t), 7.20 (1H, s), 7.45 (1H, d), 7.65 (1H, s), 7.70 (1H, d), 8.00 (4H, s), 8.90 (1H, t), 9.65 (1H, s).

Methyl 2-Acetamido-3-[2-[2-[4-(1-*tert*-butoxycarbonyl-4-piperidyloxy)benzoylamino]ethoxy]-4-cyanophenyl]-acrylate (13b). Compound **18a** (6.8 g, 21 mmol), **19a** (6.75 g, 20 mmol), HOBT (3.42 g, 21 mmol), TEA (9 mL), EDC (58 mg, 0.3 mmol), and DMF (80 mL) were treated as described for **7c** to give **13b** (10.3 g, 85%): ^{1}H NMR ($CDCl_3$) δ 1.45 (9H,s), 1.65−1.80 (2H, m), 1.85−2.00 (2H, m), 2.02 (3H, s), 3.30−3.40 (2H, m), 3.60−3.75 (2H, m), 3.80 (3H, s), 4.35 (2H, t), 4.55 (1H, m), 6.82 (2H, d), 6.99 (1H, t), 7.18−7.22 (2H, m), 7.33 (1H, s), 7.44 (1H, s), 7.69 (2H, d), 7.87 (1H, d).

Methyl 2-Acetamido-3-[2-[2-[1-(pyridin-4-yl)piperidine-4-carbonylamino]ethoxy]-4-cyanophenyl]acrylate Trifluoroacetate (13c). Bromotripyrrolidinophosphonium hexafluorophosphate (1.51 g, 3.24 mmol) was added to a mixture of 1-(pyridin-4-yl)piperidine-4-carboxylic acid HCl (**18d**) (0.79 g, 3.24 mmol), **19a** (1.0 g, 2.94 mmol), and TEA (1.64 mL) in DMF at 0 °C. The reaction mixture was stirred overnight at ambient temperature and concentrated in vacuo. The residue was purified by HPLC to give **13c** (360 mg, 25%): ^{1}H NMR (DMSO-d_6) δ 1.50−1.70 (2H, m), 1.75−1.90 (2H, m), 1.95 (3H, s), 2.50 (1H, m), 3.15−3.30 (2H, m), 3.42 (2H, dt), 3.65 (3H, s), 4.10−4.22 (4H, m), 7.15−7.21 (3H, m), 7.44 (1H, d), 7.58 (1H, s), 7.68 (1H, d), 8.09 (1H, t), 8.21 (2H, d), 9.65 (1H, s).

Methyl 2-Acetamido-3-[2-[2-[4-(pyrrolidine-1-carbonyl)benzoylamino]ethoxy]-4-cyanophenyl]acrylate (13d). Compound **18b** (1.56 g, 8.1 mmol), **19a** (2.5 g, 7.4 mmol), HOBT (1.1 g, 8.1 mmol), TEA (1.53 mL), EDC (1.55 g, 8.1 mmol), and DMF were treated as described for **7c** to give **13d** (1.80 g, 50%): ^{1}H NMR (DMSO-d_6) δ 1.78−1.90 (4H, m), 1.95 (3H, s), 3.30−3.50 (4H, m), 3.60−3.65 (3H, s), 3.70 (2H, dt), 4.30 (2H, t), 7.20 (1H, s), 7.41 (1H, d), 7.55−7.70 (4H, m), 7.85 (2H, d), 8.75 (1H, t), 9.65 (1H, s).

Methyl 2-Acetamido-3-[2-[(2*R*)-2-[4-(1-*tert*-butoxycarbonylpiperidyl-4-oxy)benzoyl]amino-3-benzyloxycarbonylpropoxy]-4-cyanophenyl]acrylate (13e). Compound **19b** was treated as described for **13b** to give **13e**: ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 1.65−2.05 (4H, m), 2.00 (3H, s), 2.80−3.10 (2H, m), 3.30−3.40 (2H,m), 3.60−3.80 (2H, m), 3.80 (3H, s), 4.25 (2H, d), 4.55 (1H, m), 4.85 (1H, m), 5.15 (2H, s), 6.80−7.90 (15H, m).

Methyl 2-Acetamido-3-[2-[(2*R*)-2-[1-(4-pyridyl)piperidine-4-carbonyl]amino-3-benzyloxycarbonylpropoxy]-4-cyanophenyl]acrylate Trifluoroacetate (13f). Compound **19b** (2.84 g, 5.9 mmol), 1-(4-pyridinyl)piperidine-4-carboxylic acid (**18d**) (1.4 g, 5.9 mmol), bromotripyrrolidinophosphonium hexafluorophosphate (2.80 g, 6.0 mmol), TEA (3.3 mL, 23.7 mmol), and DMF (50 mL) were treated as described for **13c** to give **13f** (1.78 g, 40%): ^{1}H NMR (DMSO-d_6) δ 1.45−1.65 (2H, m), 1.70−1.85 (2H, m), 1.95 (3H, s), 2.55−2.80 (2H, m), 3.16 (1H, m), 3.24 (1H, br d), 3.60 (2H, m), 3.67 (3H, s), 4.05 (1H, m), 4.17 (2H, m), 4.50 (1H, m), 5.09 (2H, d), 7.18 (3H, m), 7.32−7.40 (5H, m), 7.45 (1H, m), 7.58 (1H,m), 7.70 (1H, m), 8.10 (1H, d), 8.22 (2H, d), 9.69 (1H, br).

3-[4-Amidino-2-[2-(4-amidinobenzoylamino)ethoxy]-phenyl]-2-oxopropionic Acid Bistrifluoroacetate (3a). Part A. Compound **13a** (120 mg, 0.3 mmol) was treated as described for **1d** to give the crude amidine.

Part B. The crude amidine was diluted with 3 N HCl. The mixture was stirred at 80°C and concentrated in vacuo. Purification by reversed-phase HPLC gave **3a** (65 mg, 34%): ^{1}H NMR (DMSO-d_6) δ 3.72 (2H, dt), 4.15 (2H, s, keto form), 4.31 (2H, t), 6.81 (1H, s, enol form), 7.36−7.48 (2H, m), 7.88 (2H, d), 8.04 (2H, d), 8.33 (1H, d), 9.02 (1H, t), 9.07 (2H, m), 9.25 (4H, br), 9.40 (2H, br). MS (FAB): 412 (MH^+). Anal. ($C_{20}H_{21}N_5O_5$·2.0TFA·1.0H_2O) C, H, N.

3-[4-Amidino-2-[2-[4-(1-acetimidoyl-4-piperidyloxy)-benzoylamino]ethoxy]phenyl]-2-oxopropionic Acid Bistrifluoroacetate (3b). Part A. Compound **13b** (10.3 g, 17.0 mmol) was treated as described in **1d** to give crude ethyl 2-acetamido-3-[2-[2-[4-(4-piperidyloxy)benzoylamino]ethoxy]-4-cyanophenyl]acrylate.

Part B. The crude product of Part A was treated as described for **1b**, Part B to give crude methyl 2-acetamido-3-[2-[2-[4-(1-acetimidoyl-4-piperidyloxy)benzoylamino]ethoxy]-4-cyanophenyl]acrylate.

Part C. The crude product in Part B was treated as described for **3a**, Part B, to give **3b** (4.09 g, 33%): ^{1}H NMR (DMSO-d_6) δ 1.65−1.85 (2H, m), 2.02−2.19 (2H, m), 2.25 (3H, s), 3.58−3.82 (6H, m), 4.23 (2H, s, keto form), 4.30 (2H, t), 4.79 (1H, m), 6.80 (1H, s, enol form), 7.07 (2H, d), 7.38−7.47 (2H,

m), 7.83 (2H, d), 8.33 (1H, d), 8.55−8.67 (2H, m), 9.05−9.34 (5H, br), 9.75 (1H, br, enol form). MS (ESI): 510 (MH^+). Anal. ($C_{26}H_{31}N_5O_6$·2.0TFA·1.0H_2O) C, H, N.

3-[4-Amidino-2-[2-[1-(pyridin-4-yl)piperidine-4-carbonylamino]ethoxy]phenyl]-2-oxopropionic Acid Bistrifluoroacetate (3c). Compound **13c** (360 mg, 0.73 mmol) was treated as described for **3a** to give **3c** (205 mg, 41%): ^{1}H NMR (DMSO-d_6) δ 1.50−1.70 (2H, m), 1.80−1.95 (2H, m), 2.60 (1H, m), 3.18−3.30 (2H, m), 3.43−3.60 (2H, m), 4.10−4.30 (4H, m), 6.80 (1H, s, enol form), 7.18 (2H, d), 7.35−7.48 (2H, m), 8.22 (2H, d), 8.34 (1H, d), 9.15 (2H, br), 9.30 (2H, br), 9.80 (1H, br, enol form). MS (ESI): 454 (MH^+). Anal. ($C_{23}H_{27}N_5O_5$·2.0TFA·2.0H_2O) C, H, N.

3-[4-Amidino-2-[2-[4-(pyrrolidine-1-carbonyl)benzoylamino]ethoxy]phenyl]-2-oxopropionic Acid Trifluoroacetate (3d). Compound **13d** (1.80 g, 3.68 mmol) was treated as described for **3a** to give **3d** (345 mg, 16%): ^{1}H NMR (DMSO-d_6) δ 1.75−1.95 (4H, m), 3.30−3.50 (4H, m), 3.72 (2H, dt), 4.25 (2H, s, keto form), 4.30 (2H, t), 6.81 (1H, s, enol form), 7.35−7.50 (2H, m), 7.60 (2H, d), 7.89 (2H, d), 8.33 (1H, d), 8.85 (1H, br), 9.01 (2H, br), 9.27 (2H, br), 9.80 (1H, br, enol form). MS (ESI): 467 (MH^+). Anal. ($C_{24}H_{26}N_4O_6$·TFA·2.0H_2O) C, H, N.

(3*R*)-4-[5-Amidino-2-(2-carboxy-2-oxoethyl)phenoxy]-3-[4-(1-acetimidoylpiperidyl-4-oxy)benzoylamino]butyric Acid Bistrifluoroacetate (3e). Part A. Compound **13e** (12.0 g, 16.0 mmol) was treated as described for **1d** to give methyl 2-acetamido-3-[4-amidino-2-[(2*R*)-3-ethoxycarbonyl-2-[4-(piperidyl-4-oxy)benzoylamino]propoxy]phenyl]acrylate bistrifluoroacetate (3.35 g, 25%): ^{1}H NMR (DMSO-d_6) δ 1.16 (3H, t), 1.72−1.89 (2H, m), 1.95 (3H, s), 2.04−2.17 (2H, m), 2.79 (2H, d), 3.03−3.18 (2H, m), 3.20−3.32 (2H, m), 3.66 (3H, s), 4.04 (2H, q), 4.23 (2H, d), 4.66−4.79 (1H, m), 7.06 (2H, d), 7.28 (1H, s), 7.44 (1H, dd), 7.53 (1H, d), 7.73 (1H, d), 7.81 (2H, d), 8.46 (1H, d), 9.18 (2H, s), 9.34 (2H, s), 9.67 (1H, br s).

Part B. The product of Part A (3.35 g, 5.5 mmol) was treated as described for **1b**, Part B, and subsequently the crude product was treated as described for **3a**, Part B, to give **3e** (1.73 g, 39%): ^{1}H NMR (DMSO-d_6) δ 1.70−1.87 (2H, m), 2.02−2.15 (2H, m), 2.29 (3H, s), 2.68 (1H, d), 2.74 (1H, d), 3.48−3.58 (2H, m), 4.11−4.28 (2H, m), 4.60−4.72 (1H, m), 4.81 (1H, br), 6.80 (1H, s, enol form), 7.07 (2H, d), 7.45 (1H, d), 7.48 (1H, s), 7.82 (2H, d), 8.33 (1H, d), 8.62 (1H, s), 9.14 (2H, s), 9.17 (1H, s), 9.26 (2H, s). MS (ESI): 568 (MH^+). Anal. ($C_{28}H_{33}N_5O_8$·2.0TFA·1.5H_2O) C, H, N.

(3*R*)-4-[5-Amidino-2-(2-carboxy-2-oxoethyl)phenoxy]-3-[1-(pyridin-4-yl)piperidine-4-carbonylamino]butyric Acid Bistrifluoroacetate (3f). Compound **13f** (1.78 g, 2.8 mmol) was treated as described for **3a** to give **3f** (745 mg, 43%): ^{1}H NMR (DMSO-d_6) δ 1.79 (2H, m), 1.82 (2H, m), 2.55 (2H, m), 3.25−3.70 (5H, m), 4.00−4.25 (4H, m), 4.45 (1H, m), 6.76 (1H, s, enol), 7.20 (2H, m), 7.42 (3H, m), 8.23 (2H, d), 8.35 (1H, d), 9.15 (2H, br), 9.28 (2H, br), 9.80 (1H, br). MS (ESI): 512 (MH^+). Anal. ($C_{25}H_{29}N_5O_7$·2.0TFA·1.5H_2O) C, H, N.

Ethyl 3-[2-[2-(1-*tert*-Butoxycarbonylamino)ethoxy]-4-cyanophenyl]acrylate (14). A mixture of **11a** (11.0 g, 28.4 mmol), ethyl acrylate (15.4 mL, 142 mmol), TEA (20 mL, 142 mmol), and $Pd(OAc)_2$ (127 mg, 0.57 mmol) in DMF (200 mL) was stirred overnight at 100 °C. The reaction mixture was conentrated in vacuo and diluted with EtOAc. The solution was washed with 1 N HCl, saturated $NaHCO_3$ and saturated NaCl, and then worked up. The residue was purified by chromatography to give **14** (9.6 g, 94%): ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t), 1.46 (9H, s), 3.62 (2H, dt), 4.16 (2H, t),4.28 (2H, q), 6.56 (1H, d), 7.16 (1H, d), 7.27 (1H, d), 7.60 (1H, d), 7.96 (1H, d).

Ethyl 3-[2-(2-aminoethoxy)-4-cyanophenyl]acrylate HCl (20). Compound **14** was treated as described for **6a**, Part B, to give **20**: ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t), 3.25 (2H, br), 4.20 (2H, q), 4.40 (2H, t), 6.75 (1H, d), 7.50 (1H, d), 7.65 (1H, d), 7.95 (1H, d), 8.00 (1H, d), 8.40 (3H, br).

Ethyl 3-[2-[2-[4-(1-*tert*-Butoxycarbonyl-4-piperidyloxy)-benzoylamino]ethoxy]-4-cyanophenyl]acrylate (15a). Compound **20** (2.24 g, 7.56 mmol), **18a** (2.67 g, 8.32 mmol), EDC (1.59 g, 8.32 mmol), HOBT (1.12 g, 8.32 mmol), TEA (3.16 mL, 22.7 mmol), and CH_2Cl_2 (50 mL) were treated as described for **7c** to give **15a** (3.0 g, 71%): ^{1}H NMR ($CDCl_3$) δ 1.33 (3H, t), 1.47 (9H, s), 1.64−1.79 (2H, m), 1.86−1.98 (2H, m), 3.24−3.42 (2H, m), 3.60−3.73 (2H, m), 3.92 (2H, dt), 4.24 (2H, q), 4.28 (2H, t), 4.45−4.53 (1H, m), 6.57 (1H, d), 6.77 (1H, t), 6.88 (2H, d), 7.18 (1H, d), 7.23 (1H, d), 7.58 (1H, d), 7.77 (2H, d), 7.97 (1H, d).

Ethyl 3-[2-[2-[1-(4-Pyridyl)piperidine-4-carbonylamino]ethoxy]-4-cyanophenyl]acrylate (15b). Compound **20** (2.47 g, 8.33 mmol), 1-(4-pyridyl)piperidine-4-carboxylic acid HCl (**18d**) (2.22 g, 9.17 mmol), bromotripyrrolidinophosphonium hexafluorophosphate (4.27 g, 9.17 mmol), TEA (3.48 mL, 25.0 mmol), and DMF (50 mL) were treated as described for **13c**. The crude product was purified by chromatography to give **15b** (2.3 g, 62%): ^{1}H NMR (DMSO-d_6) δ 1.26 (3H, t), 1.50−1.68 (2H, m), 1.68−1.73 (2H, m), 2.62−2.68 (1H, m), 2.94−3.06 (2H, m), 3.40−3.53 (2H, m), 3.95−4.25 (6H, m), 6.76 (1H, dd), 6.94 (2H, d), 7.44 (1H, dd), 7.62 (1H, br), 7.83 (1H, dd), 7.90 (1H, d), 9.01 (1H, t), 8.15 (2H, d).

3-[2-[2-[(1-Acetimidoyl-4-piperidyloxy)benzoylamino]-ethoxy]-4-amidinophenyl]propionic Acid Bistrifluoroacetate (4a). Part A. Compound **15a** (3.0 g, 5.33 mmol) was treated as described for **1d** to give crude ethyl 3-[2-[2-[4-(4-piperidyloxy)benzoylamino]ethoxy]-4-amidinophenyl]acrylate. The crude product was treated as described for **1b**, Part B, to give ethyl 3-[2-[2-[(1-acetimidoyl-4-piperidyloxy)benzoylamino]ethoxy]-4-amidinophenyl]acrylate bistrifluoroacetate (**16a**) (2.3 g, 37%): ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t), 1.67−1.87 (2H, m), 2.00−2.25 (2H, m), 2.29 (3H, s), 3.45−3.60 (2H, m), 3.66−3.77 (4H, m), 4.17 (2H, q), 4.34 (2H,t), 4.73−4.76 (1H, m), 6.79 (1H, d), 7.05 (2H, t), 7.43 (1H, d), 7.56 (1H, d), 7.84 (2H, d), 7.92 (1H, br), 7.97 (1H, d), 8.64 (2H, br), 9.19 (1H, br), 9.37 (4H, br). MS (ESI, *m*/*z*): 522 (MH^+).

Part B. A mixture of 10% Pd−C (100 mg) and **16a** (1.2 g, 1.60 mmol) in methanol (50 mL) was stirred under a hydrogen atmosphere overnight. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was purified by reversed-phase HPLC to give ethyl 3-[2-[2-[(1-acetimidoyl-4-piperidyloxy)benzoylamino]ethoxy]-4-amidinophenyl]propionate bistrifluoroacetate (1.1 g, 91%): ^{1}H NMR (DMSO-d_6) δ 1.16 (3H, t), 1.67−1.76 (2H, m), 2.00−2.25 (2H, m), 2.29 (3H, s), 2.58 (2H, t), 2.90 (2H, t), 3.46−3.58 (2H, m), 3.63−3.84 (4H, m), 3.98 (2H, q), 4.23 (2H, t), 4.74−4.87 (1H, m), 7.08 (1H, d), 7.36 (1H, br), 7.38 (2H, d), 7.84 (2H, d), 8.61 (2H, br), 9.11 (2H, br), 9.16 (1H, br), 9.24 (2H, br). MS (ESI, *m*/*z*): 524 (MH^+).

Part C. A mixture of the product from Part B (600 mg, 0.8 mmol) and hydrochloric acid (10 mL) was stirred at 50 °C for 4 h and concentrated in vacuo. The residue was purified by reversed-phase HPLC to give **4a** (480 mg, 77%): ^{1}H NMR (DMSO-d_6) δ 1.66−1.87 (2H, m), 2.02−2.17 (2H, m), 2.29 (3H, s), 2.52 (2H, t), 2.78 (2H, t), 3.44−3.62 (2H, m), 3.64−3.75 (4H, m), 4.42 (2H, t), 4.74−4.86 (1H, m), 7.06 (2H, d), 7.37 (1H, br), 7.39 (2H, d), 7.85 (2H, d), 8.64 (2H, br), 9.19 (1H, d), 9.23 (2H, br), 9.25 (2H, br). MS (ESI, *m*/*z*): 496 (MH^+). Anal. ($C_{26}H_{33}N_5O_5$·2.0TFA·2.0H_2O) C, H, N.

3-[2-[2-[1-(4-Pyridyl)piperidine-4-carbonylamino]-ethoxy]-4-amidinophenyl]propionic Acid Bistrifluoroacetate (4b). Part A. Compound **15b** (2.3 g, 5.13 mmol) was treated as described for **1d** to give the crude amidine (**16b**). The amidine was treated as described for **4a**, Part B, to give ethyl 3-[2-[2-[1-(4-pyridyl)piperidine-4-carbonylamino]ethoxy]-4-amidinophenyl]propionate bistrifluoroacetate (1.5 g, 42%): ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t), 1.50−1.67 (2H, m), 1.76−1.81 (2H, m), 2.52−2.60 (1H, m), 2.62 (2H, dd), 2.89 (2H, dd), 3.15−3.28 (2H, m), 3.49 (2H, dt), 4.03 (2H, q), 4.12 (2H, t), 4.20 (2H, d), 7.19 (2H, d), 7.37 (3H, br), 8.18 (1H, d), 8.21 (2H, d), 9.23 (2H, br), 9.25 (2H, br). MS (ESI, *m*/*z*): 468 (MH^+).

Part B. The product of Part A (250 mg, 0.36 mmol) was treated as described for **4a**, Part C, to give **4b** (480 mg, 92%):

^{1}H NMR (DMSO-d_6) δ 1.50−1.67 (2H, m), 1.76−1.92 (2H, m), 2.54 (2H, dd), 2.55−2.67 (1H, m), 2.88 (2H, dd), 3.12−3.29 (2H, m), 3.49 (2H, dt), 4.12 (2H, t), 4.20 (2H, d), 7.18 (2H, d), 7.36 (2H, br), 7.37 (1H, d), 8.18 (1H, d), 8.20 (2H, d), 9.14 (2H, br), 9.24 (2H, br). MS (ESI, *m/z*): 440 (MH^+). Anal. ($C_{23}H_{29}N_5O_4$·2.0TFA) C, H, N.

Enzyme Assays. Human fXa and thrombin were obtained from Enzyme Research Laboratories, Inc. and Sigma Chemical Co., respectively. The chromogenic substrates used were S-2222 (Daiichi Pure Chemical, Japan) for fXa and S-2238 (Daiichi Pure Chemical) for thrombin. FXa and thrombin were assayed in a buffer containing 0.1 M Tris, 0.2 M NaCl, 0.1% PEG-6000, and 0.02% Tween20 at pH 8.4.

The final substrate concentrations in the reaction were 200 and 100 μM for S-2222 and S-2238, respectively. All enzyme assays were carried out at room temperature in 96-well microtiter plates with final concentrations of 4.5 nM for fXa and 1.1 nM for thrombin. Compounds at appropriate dilutions were added to wells containing buffer and enzyme and preincubated for 10 min. The enzyme reactions were initiated by the addition of substrate, and color development, due to the release of *p*-nitroaniline from each chromogenic substrate, was monitored continuously for 16 min at 405 nm on a microplate reader (model 3550-UV, Bio-Rad). The measured initial velocities were used to determine the amount of inhibitor required to reduce the control velocity by 50%; this concentration was defined as the IC_{50} of the inhibitor. The apparent K_i values of compounds were then calculated according to the relationship: $(IC_{50})_1/(IC_{50})_2 = (K_i)_1/(K_i)_2$.[22] In this equation, the K_i value of **3e**, which was determined from a double reciprocal plot,[23] was used as the standard.

Coagulation Assay. PT was measured with coagulometer CA3000 (Sysmex, Japan). Citrated human plasma, which was collected from healthy volunteers, and cynomolgus monkey plasma (A.T. VIRI, Inc., the Philippines) were used in the assays. Plasma (45 μL) was mixed with 5 μL of compound dilutions followed by the automatic addition of 100 μL of prothrombin reagent (Thromboplastin C plus, Dade Behring). The concentrations required to double the clotting time (2 × PT) were estimated from each dose−response curve.

Anticoagulant and Pharmacokinetic Studies in Cynomolgus Monkeys. Male cynomolgus monkeys were fasted overnight. Compounds were administered orally at 10 mg/kg using a gastric tube and intravenously at 0.3 mg (**3b**, **3c**, and **4b**) or 1.0 mg/kg (**3e**). Blood (1.5 mL) was collected from the saphenous vein in the leg prior to treatment and at multiple time points for up to 24 h after treatment. Collected blood samples were immediately transferred into sample tubes containing 167 μL of 3.8% trisodium citrate solution, and platelet-poor plasma was prepared by centrifugation to measure PT. Plasma drug concentrations were also measured by HPLC.

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