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The Discovery of YM-60828: A Potent, Selective and Orally-Bioavailable Factor Xa Inhibitor

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Abstract—Since Factor Xa (FXa) is well known to play a central role in thrombosis and hemostasis, inhibition of FXa is an attractive target for antithrombotic strategies. As a part of our investigation of a non-peptide, orally available FXa inhibitor, we found that a series of *N*-[(7-amidino-2-naphthyl)methyl]aniline derivatives possessed potent and selective inhibitory activities. Structure–activity relationship (SAR) of the substituent (R^1) on the central aniline moiety suggested that increasing lipophilicity caused a detrimental effect on anticoagulant activity (prothrombin time assay) in plasma. Several compounds bearing a hydrophilic substituent in R^1 showed not only potent FXa inhibitory activities but also high anticoagulant activities. The best compound in this series was sulfamoylacetic acid derivative **8o** (YM-60828) which was a potent, selective and orally bioavailable FXa inhibitor and was chosen for clinical development.

Introduction

Warfarin, the sole orally-effective anticoagulant therapeutic agent currently available, has been widely used for treatment and/or prevention of thromboembolic disorders. Warfarin exerts its potent anticoagulant effect by inhibiting biosynthesis of a vitamin K-dependent coagulation factor. Due to its indirect mechanism, Warfarin shows slow onset of action, narrow therapeutic range, and interaction with many drugs and foods. Furthermore, the most serious problem is an adverse effect on bleeding and must, therefore, be monitored carefully.[1–3] Consequently, development of a direct acting, orally-available and with no associated hemorrhaging inhibitor of the coagulation factor is very important.

Efforts to discover a specific inhibitor of a protease along the coagulation cascade have been concentrated at inhibition of thrombin, a serine protease which plays a crucial role in thrombosis by not only producing fibrin from fibrinogen for clot formation but also by inducing platelet aggregation.[4–8] Despite numerous efforts, no orally effective thrombin inhibitor has been launched as yet. Recently, inhibition of a thrombin generation "Factor Xa inhibitor" has received much attention as an alternative to direct inhibition of thrombin. The serine protease Factor Xa (FXa) occupies the convergence point of the intrinsic and extrinsic coagulation cascade and combines with factor Va, Ca^{2+} and a phospholipid to form a prothrombinase complex, which generates thrombin by the proteolysis of prothrombin. Because the coagulation cascade is a highly amplified process, FXa is present in blood at a much lower concentration compared with thrombin, leading to the hypothesis that a FXa inhibitor is expected to show therapeutic effect with much smaller doses than a thrombin inhibitor. Moreover, a specific FXa inhibitor does not affect platelet activation and aggregation caused by thrombin; therefore, it could have little hemorrhagic risk. Actually the protein specific FXa inhibitor from the leech (Antistasin)[9,10] and the tick (TAP)[11,12] have been shown to be effective antithrombotics in animal models without prolongation bleeding time when administrated parenterally.

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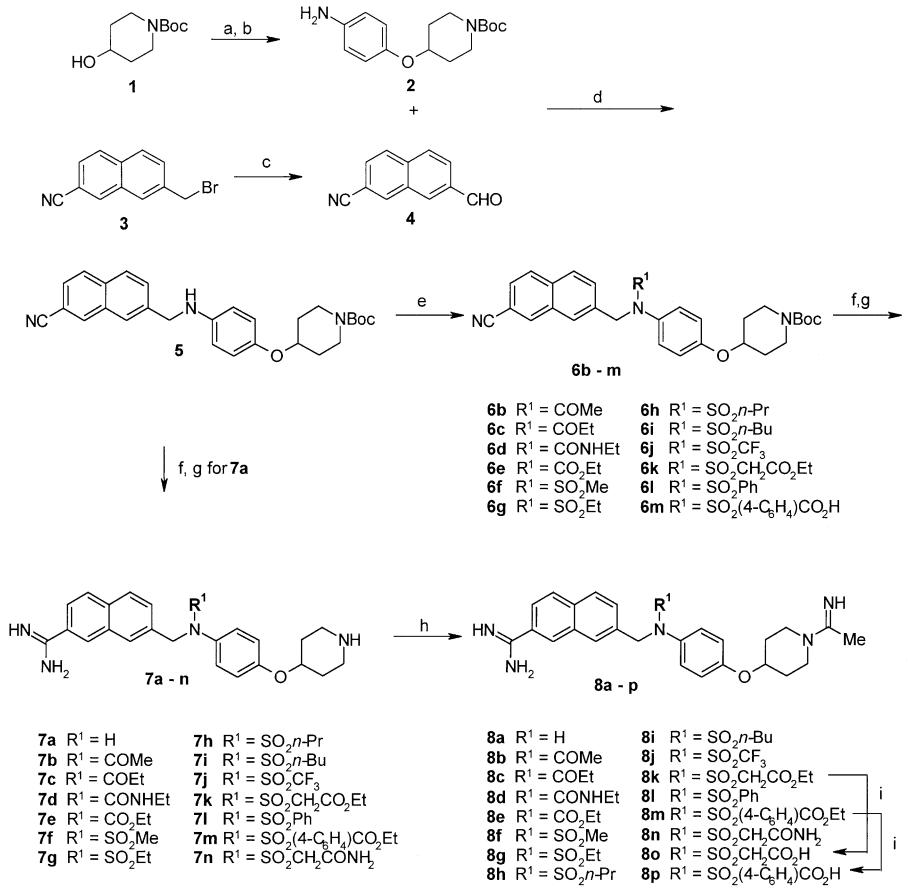


Scheme 1. (a) 4-Nitrophenol, PPh_3, diethyl azodicarboxylate, THF; (b) H_2, 10% Pd/C, EtOH; (c) NMe_3O, $CHCl_3$; (d)$NaB(OAc)_3H$, CH_2Cl_2, AcOH; (e) R^1_2O, base for **6b** and **6j**; EtNCO for **6d**; R^1Cl, base for the other compounds; (f) HCl, EtOH or MeOH; (g) NH_3, EtOH for **7n** from **6k**; NH_4OAc, EtOH or MeOH for the other compounds; (h) ethyl acetimidate hydrochloride, Et_3N, EtOH or MeOH; (i) HCl, H_2O.

Recently, a variety of non-peptide FXa inhibitors have been reported in the literature.[13–15] Among them DX-9065a (Fig. 1) was reported as the first FXa inhibitor which possessed anticoagulant activity after oral administration in rats.[16,17] We planned to create more potent and orally effective FXa inhibitors based on the transformation of three parts of DX-9065a, such as the (7-amidino-2-naphthyl)methyl moiety, 4-[(1-acetimidoyl-4-pyrrolidyl)oxy]phenyl moiety, and carboxylic acid moiety. In order to investigate the variability of SAR, a nitrogen atom was introduced as a juncture of the three parts, which resulted in facilitation of the synthesis, achirality as well as a novel lead compound. In these strategies, we started to explore a series of *N*-{(7-amidino-2-naphthyl)methyl}aniline derivatives. In this paper, we describe the results of our work on the synthesis and SAR of potent and selective FXa inhibitors.

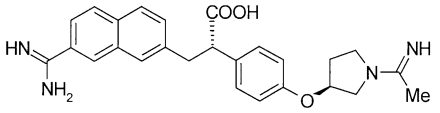
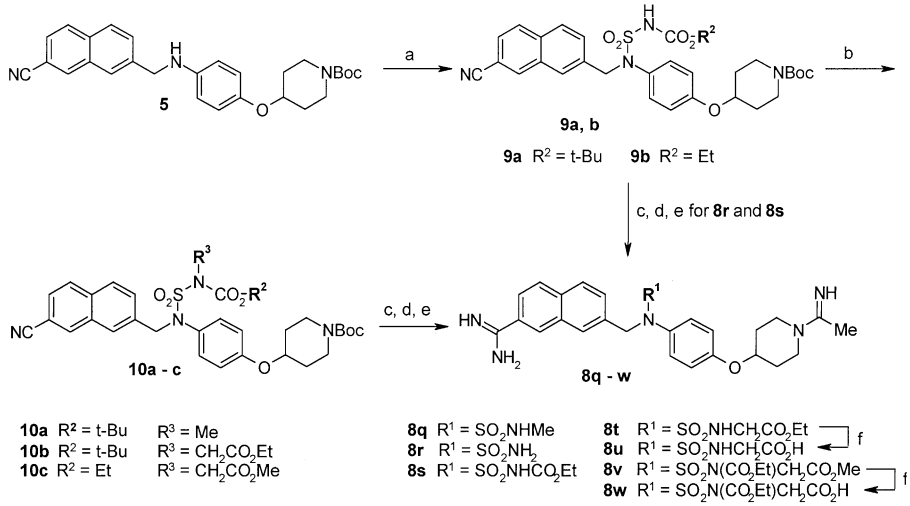


Figure 1. Structure of **DX-9065a**.

Chemistry

Key intermediate **5** was synthesized as depicted in Scheme 1. Mitsunobu condensation of the hydroxypiperidine derivative **1** and 4-nitrophenol followed by reduction of the nitro group afforded aniline **2**. Reductive alkylation of **2** with aldehyde **4**, which was prepared by oxidation of a known naphthylbromide **3**[18] with anhydrous trimethylamine *N*-oxide in $CHCl_3$, gave the versatile intermediate **5**. After reaction of **5** with various acyl chlorides, sulfonyl chlorides or isocyanate, the resulting cyano derivatives were converted to the corresponding amidines **7a**–**7m** under standard Pinner reaction conditions by bubbling HCl gas into a solution of the cyano compounds in ethanol or methanol and subsequent amination of the formed imidate by treatment with ammonium acetate. Carboxamide **7n** was prepared from **6k** using saturated ammonia solution instead of ammonium acetate. The piperidine moieties were treated with ethyl acetimidate hydrochloride and triethylamine in methanol or ethanol to give the corresponding bis-amidines **8a**–**8n**.

Sulfamide derivatives **8q**–**8w** were also synthesized from the intermediate **5** as illustrated in Scheme 2. Following an existing synthetic method[19] of methyl chlorosulfonylcarbamate, chlorosulfonylisocyanate was allowed to react with *tert*-butylalcohol and ethanol to give *tert*-butyl and ethyl chlorosulfonylcarbamate **11a** and **11b**, which was treated with **5** to afford sulfamides **9a** and **9b**. Sulfamides **9a** and **9b** were alkylated with methanol under Mitsunobu condition or with an alkylbromide to



Scheme 2. (a) *t*-BuOCONHSO$_2$Cl (**11a**) for **9a**, EtOCONHSO$_2$Cl (**11b**) for **9b**, pyridine; (b) MeOH, PPh$_3$, diethyl azodicarboxylate, THF for **10a**; R^{3}Br, K$_2$CO$_3$, DMF for **10b** and **10c**; (c) HCl, EtOH or MeOH; (d) NH$_4$OAc, EtOH or MeOH; (e) ethyl acetimidate hydrochloride, Et$_3$N, EtOH or MeOH; (f) HCl, H$_2$O.

afford *N*,*N*,*N*′,*N*′-tetra-substituted sulfamides **10a**–**10c**. The sulfamides **9a, 9b** and **10a**–**10c** were converted to corresponding bis-amidines **8q**–**8t** and **8v** by the same method described above. Acid derivatives **8o**, **8p**, **8u** and **8w** were synthesized from the corresponding esters by acid hydrolysis.

Schemes 3 and 4 depict the synthesis of benzamidine derivatives **16**, **17** and **22**. Intermediate **14** was obtained according to the same procedure as that for **5** using cinnamaldehyde **13** instead of naphthyl aldehyde **4.** Aldehyde **13** was prepared from benzaldehyde **12** and triphenylphosphoranylidene acetaldehyde by Wittig condensation. Compound **14** was reduced by hydrogenation in the presence of 10% palladium on carbon to afford saturated derivative **15.** In the same manner described above, **14** and **15** were converted to methanesulfonamide derivatives and subsequent transformation to bis-amidino compounds to give desired benzamidines **16** and **17**. Another intermediate **21** was prepared by alkylation of sulfonamide **18** with bromoacetanilide **20**. Compound **18** and **20** were easily prepared from compound **2** and aniline **19**, respectively. Compound **21** was also converted to bis-amidine derivative **22** under the same conditions described above.

Results and Discussion

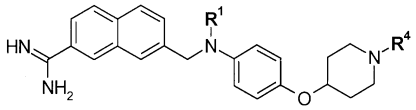
All compounds prepared were evaluated by IC$_{50}$ values for the inhibition of FXa, thrombin, and trypsin enzymatic activities. CT$_2$ values for the prolongation of prothrombin time (PT) are also tabulated in Table 3 as an indicator of anticoagulant activity in vitro. CT$_2$ values were defined as the concentration required to double clotting time.

As shown in Table 1, compound **8a**, a derivative bearing no substituent R^1 on the juncture–NH– position turned out to have strong FXa inhibitory activity and high degree of selectivity for thrombin. The FXa inhibitory activity was comparable to that of reference compound, DX-9065a. This achiral compound **8a** is positioned to be a valuable and novel lead to explore superior FXa inhibitors.

Some small alkyl groups, methyl or ethyl, were introduced on the juncture –NH– with various linkages such as amide, urea, sulfonamide or sulfamide, to evaluate their inhibitory activities (**8b**–**8g**, and **8q**). Among these compounds, methanesulfonamide **8f** showed 5- and 10-fold enhancements in potency relative to the lead compounds **8a** and DX-9065a, respectively. Sulfonamide and sulfamide derivatives **8f**, **8g** and **8q** (IC$_{50}$ = 3.8–9.7 nM) showed relatively higher potencies than amide, urea and urethane derivatives **8b**–**8e** (IC$_{50}$ = 12.0–31.9 nM).

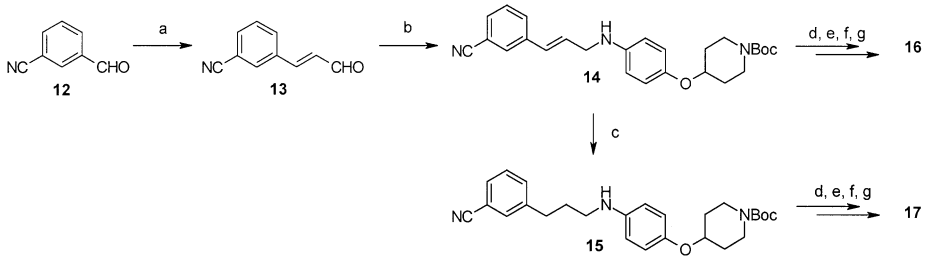
Removal of the acetimidoyl moiety from the piperidine ring (**7f**) resulted in decrease in the activity. In addition,

Table 1. In vitro inhibitory activities (IC$_{50}$) against factor Xa, thrombin, and trypsin

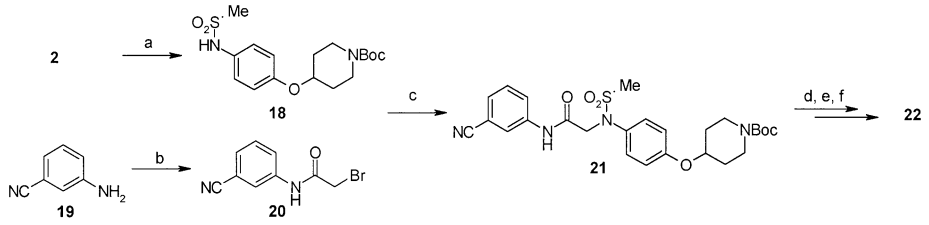


Compd	R^1	R^4	IC$_{50}$ (nM) [a]		
			Factor Xa	Thrombin	Trypsin
8a	H	C(=NH)CH$_3$	19.5	>100,000	278
8b	COMe	C(=NH)CH$_3$	12.4	>100,000	748
8c	COEt	C(=NH)CH$_3$	31.9	>100,000	245
8d	CONHEt	C(=NH)CH$_3$	16.3	>100,000	822
8e	COOEt	C(=NH)CH$_3$	12.0	>100,000	265
8f	SO$_2$Me	C(=NH)CH$_3$	3.8	>100,000	108
8g	SO$_2$Et	C(=NH)CH$_3$	9.7	>100,000	181
8q	SO$_2$NHMe	C(=NH)CH$_3$	6.6	>100,000	171
7f	SO$_2$Me	H	127	>100,000	1410
DX-9065a			39.5±3.7[b]	>100,000	3170

[a]Human purified enzymes were used. IC$_{50}$ values were determined duplicated. The variation from the mean value is ±15% or less.
[b]n = 3.



Scheme 3. (a) Triphenylphosphoranylidene acetaldehyde, benzene; (b) n, $NaB(OAc)_3H$, CH_2Cl_2, AcOH; (c) H_2, Pd/C, MeOH; (d) $MeSO_2Cl$, pyridine; (e) HCl, EtOH; (f)NH_4OAc, EtOH; (g) ethyl acetimidate hydrochloride, Et_3N, EtOH.

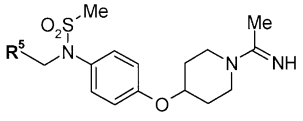


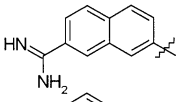
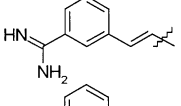
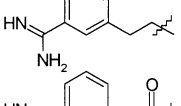
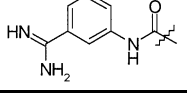
Scheme 4. (a) Methanesulfonyl chloride, pyridine; (b) bromoacetyl bromide, NEt_3, 1,2-dichloroethane; (c) K_2CO_3, CH_3CN; (d) HCl, EtOH; (e) NH_4OAc, EtOH; (f) ethyl acetimidate hydrochloride, Et_3N, EtOH.

introduction of 3-amidinophenyl structures instead of the 7-amidino-2-naphthyl moiety afforded styrene derivative **16** with moderate FXa inhibition, but all these derivatives **16**, **17** and **22** reduced the potencies compared with parent compound **8f** (Table 2).

In an effort to provide understanding of the SARs for these compounds, the binding conformation of **8g** to FXa was studied. Because of the general difficulty in obtaining an X-ray crystal structure of FXa bound with inhibitor, it was modeled based on the crystal structure of **8g** in the complex with the related enzyme, trypsin (Fig. 2). The generated binding mode was similar to that

Table 2. In vitro inhibitory activities (IC_{50}) against factor Xa, thrombin, and trypsin



Compd	R^5	IC_{50} (nM)[a]		
		Factor Xa	Thrombin	Trypsin
8f		3.8	> 100,000	108
16		17.3	> 100,000	972
17		258.7	> 100,000	5806
22		301.0	> 100,000	6540

[a]Refer to Table 1.

of DX-9065a in Trypsin[20] and FXa,[21] which have been solved by Bode et al. The analysis indicates that the naphthamidine moiety occupies deeply the S1 pocket, and the amidine group makes a salt bridge to Asp-189 at the bottom of it. Moreover, the acetimidoylpiperidine moiety is in close contact to an aryl-binding pocket defined by the three aromatic amino acids Phe-174, Tyr-99 and Trp-215, and nitrogen of the acetimidoyl group forms a water-mediated hydrogen bond to the oxygen atoms of Thr-98 and Ile-175. These two parts of compound **8g** mainly participate in the binding to FXa and consequently compound **8g** takes a L-shape conformation. In contrast, the ethanesulfonyl moiety points to the solution away from the enzyme and makes little contact with FXa. Removing the acetimidoyl group results in loss of the hydrogen-bonding interaction; therefore, compound **7f** reduces its inhibitory potencies relative to acetimidoylpiperidine analogue **8f**. Similarly, replacement of the amidinonaphthyl moiety with benzamidine (**16**, **17** and **22**) reduces the activity, possibly due to lowering lipophilic interaction. On the other hand, the role of the substituent R^1 is not in forming additional interaction with FXa, but the sulfonamide and sulfamide linkages are most likely to contribute to stabilization of the L-shape conformation.

From the above modeling study, we considered that there is a possibility to modify the ethyl moiety of sulfonamide in **8g** without markedly affecting FXa inhibitory potency (IC_{50}), and change the physiological profiles of the compounds to improve anticoagulant activities (PT assay) in vitro as well as oral efficacy ex vivo. Since we thought the nM level of FXa inhibitory potency (IC_{50}) was enough to develop an anticoagulant, our synthetic efforts were focused on the modification of sulfonamide and sulfamide alkyl moieties.

With sequential elongation of the alkyl sulfonamide from one to four carbons (**8f**–**8i**), the anticoagulant

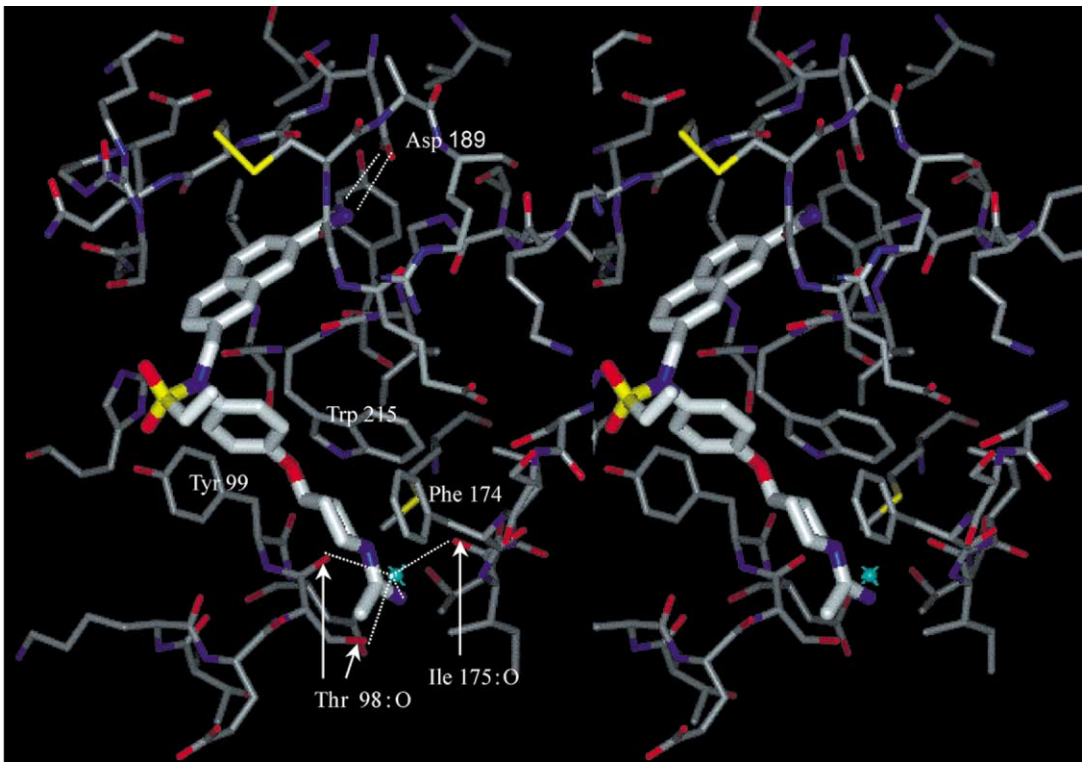
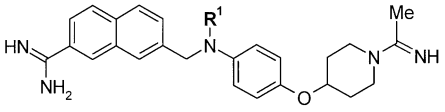


Figure 2. Binding model of **8g** in factor Xa.

Table 3. In vitro inhibitory activities (IC_{50}) against factor Xa, thrombin, trypsin and anticoagulant potencies (PT, CT_2)



Compd	R^1	IC_{50} (nM)[a]			CT_2 (μM)[b] PT[c]
		Factor Xa	Thrombin	Trypsin	
8f	SO_2Me	3.8	> 100,000	108	0.94±0.07
8g	SO_2Et	9.7	> 100,000	181	1.25±0.11
8 h	SO_2n-Pr	7.5	> 100,000	173	1.46±0.10
8i	SO_2n-Bu	4.4	> 100,000	128	2.85±0.26
8j	SO_2CF_3	6.5	> 100,000	215	10.53±0.71
8k	$SO_2CH_2CO_2Et$	8.8	> 100,000	147	0.83±0.06
8l	SO_2Ph	9.7	> 100,000	126	3.30±0.16
8m	$SO_2(4\text{-}C_6H_4)CO_2Et$	0.94	> 100,000	12	1.75±0.29
8n	$SO_2CH_2CONH_2$	4.3	> 100,000	154	0.84±0.04
8o	$SO_2CH_2CO_2H$	2.3±0.5[d]	> 100,000	216±28[d]	0.70±0.06
8p	$SO_2(4\text{-}C_6H_4)CO_2H$	0.074	77,100	133	1.18±0.09
8q	SO_2NHMe	6.6	> 100,000	171	0.81±0.15
8r	SO_2NH_2	3.4	> 100,000	134	0.73±0.04
8s	SO_2NHCO_2Et	2.4	> 100,000	116	1.41±0.25
8t	$SO_2NHCH_2CO_2Et$	2.8	> 100,000	116	0.96±0.04
8u	$SO_2NHCH_2CO_2H$	3.4	> 100,000	148	0.81±0.05
8v	$SO_2N(CO_2Et)CH_2CO_2Me$	2.0	> 100,000	90	1.72±0.17
8w	$SO_2N(CO_2Et)CH_2CO_2H$	5.1	> 100,000	116	0.96[e]
DX-9065a		39.5±3.7[f]	> 100,000	3170	4.87±0.19

[a]Refer to Table 1.
[b]The concentration required to double clotting time ($n = 3$).
[c]Prothrombin time using mouse plasma.
[d]$n = 7$.
[e]$n = 1$.

activities in the PT assay decrease gradually ($CT_2 = 0.94$–2.85 μM; Table 3). Furthermore, the derivatives **8j** and **8l** bearing trifluoromethanesulfonamide and benzenesulfonamide showed poor anticoagulant activities ($CT_2 = 10.53$ and 3.30 μM, respectively). As expected from the X-ray crystallographic analysis, these changes at the sulfonamide moiety didn't greatly affect the FXa inhibitory potencies ($IC_{50} = 3.8$–9.7 nM). Therefore, we speculated that lipophilicities of the alkyl sulfonamide moieties influenced the anticoagulant activities in the PT assay by changing their plasma protein binding. Based on this consideration, polar functional groups were introduced on the alkyl sulfonamide moiety. Consequently compounds, which had ethoxycarbonyl, carbamoyl and calboxyl groups on the methansulfonamide moiety of **8f**, were synthesized (**8k**, **8n**, and **8o**). All these compounds possessed potent anticoagulant activities ($CT_2 = 0.70$–0.84 μM). In this way these sulfonamide series of compounds (**8f**–**8l**, **8n**, and **8o**) showed a relationship between CT_2 values in PT and calculated lipophilicity values (clog*P*),[22] as shown in Figure 3.

On the other hand, unexpected results were obtained with the benzenesulfonamide derivatives. The compounds substituted on the 4-position of benzenesulfonamide with ethoxycarbonyl (**8m**) or carboxyl (**8p**) yielded about 10- and 130-fold enhancement in the FXa inhibitory potency, compared to the mother analogue (**8l**). Particularly quite strong inhibitory potency ($IC_{50} = 0.074$ nM) and excellent selectivity over trypsin (ca. 1800-fold) were observed for compound **8p**. Since non-substituted benzenesulfonamide derivative **8l** showed nearly equal FXa inhibitory potency as that of the other alkylsulfonamide derivatives, the carboxyl group of **8p** seemed to enhance strongly the FXa inhibitory activity and the selectivity. These results are difficult to explain by the expected binding conformation obtained from **8g** in FXa, because this sulfonamide moiety is suggested to be located out of the binding pocket and have little contact with the enzyme. Therefore, these results raise the possibility of a conformational difference between alkyl sulfonamide derivatives and benzene sulfonamide derivatives.

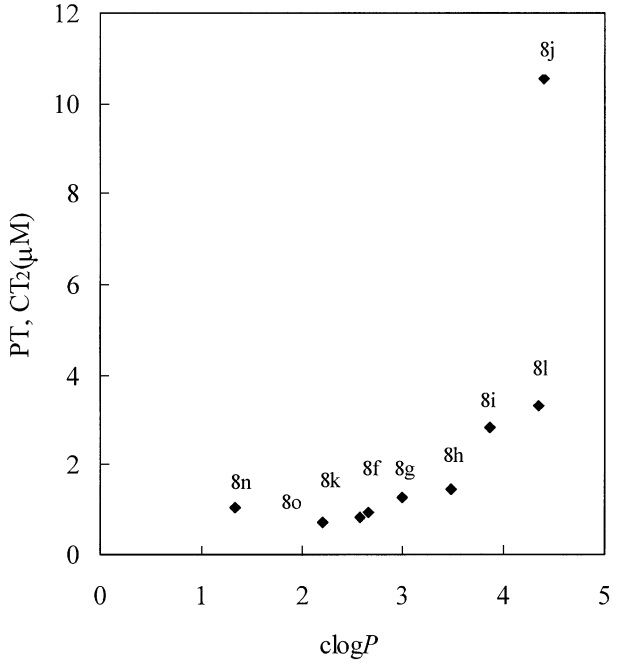


Figure 3. The correlation between clog*P* and CT_2 (PT) of sulfonamide derivatives. clog*P* was calculated using TSAR ver. 3.0 (Oxford Molecular Group). PT, CT_2; see corresponding footnotes b and c in Table 3.

In spite of their excellent FXa inhibitory activities, their anticoagulant activities in the PT assay were not observed to be so potent ($CT_2 = 1.75$ and 1.18 μM, respectively), presumably due to the large lipophilicity of benzensulfonamide derivatives **8m** and **8p** (clog$P = 4.41$ and 4.04, respectively). These results clearly indicate that high FXa inhibitory activity as well as moderate hydrophilicity of the compound is quite important for exhibiting anticoagulant activity in plasma effectively.

From this consideration, limited sulfamide derivatives with low clog *P* values (under 3.0) were synthesized and evaluated. Almost all compounds prepared in this strategy showed not only potent FXa inhibitory activities but also potent anticoagulant activities in the PT assay (**8q**–**8w**).

PT-prolonging effects of these advanced inhibitors following oral administration were evaluated in mice. Male ICR mice were dosed via gastric tube with saline solution of inhibitors; 0.5 and 2.0 h after oral administration, blood was collected and platelet poor plasma was prepared to measure PT. As outlined in Table 4, several compounds (**8f**, **8o**, **8t**, **8u**, and **8w**) prolonged PT more than 2-fold at 0.5 h. In contrast, highly lipophilic compounds **8m** and **8p** showed no activities at the same conditions. For the simple alkyl sulfonamide derivatives, it was observed that the longer the alkyl groups, the weaker the oral activities (**8f**–**8h**). Moreover, compounds having a carboxyl group in its structure

Table 4. Anticoagulant activity after oral administration in mice

Compd	PT/control PT[a]	
	0.5 h	2.0 h
8f	2.1	1.5
8g	1.5	NT[b]
8h	1.3	1.2
8k	1.7	1.4
8m	1.0	0.7
8n	1.6	1.5
8o	2.6	1.7
8p	1.0	0.8
8q	1.5	1.4
8r	1.8	1.7
8s	1.9	1.8
8t	2.2	1.4
8u	2.1	1.6
8w	2.0	1.6
DX-9065a	1.5	1.3

[a]The relative prothrombin time compared with that measured using normal mice plasma at 0.5 and 2.0 h after oral administration (100 mg/kg, $n = 3$).
[b]Not tested.

showed potent activities (**8o**, **8u**, and **8w**). Particularly, compound **8o** exhibited strongest oral activity, which prolonged PT 2.6-fold at 0.5 h, and 1.7-fold at 2.0 h. Compound **8o** was further evaluated by pharmacokinetic studies in guinea pigs and squirrel monkeys, showing the absolute oral bioavailability at 33.4 and 20.4%,[23] respectively. Moreover, it exerted antithrombotic effect in several thrombosis models in rats,[24–29] mice,[30] and guinea pigs[31] with no or little prolongation of bleeding time. These results suggest compound **8o** (YM-60828) may prove to be a valuable, orally-active and potent anti-coagulation drug.

Conclusion

We have synthesized and evaluated a novel series of *N*-[(7-amidino-2-naphthyl)methyl]aniline derivatives as FXa inhibitors. Among this series, from FXa enzymatic assay, quite potent and selective inhibitor **8p** was discovered. However, despite its excellent FXa inhibitory activity in vitro, **8p** showed mediocre anticoagulant activity in vitro and poor activity ex vivo after oral administration in mice. SAR studies indicated that moderate hydrophilicity was an important factor for exhibiting anticoagulant activity in plasma effectively.

From the evaluation of oral administration screening in mice, compound **8o** has been chosen for the best compound as an orally-bioavailable and potent FXa inhibitor. After extensive preclinical evaluation, YM-75466, the methanesulfonate salt of **8o**, is currently undergoing clinical development.

Experimental

Chemistry

^{1}H NMR spectra were measured with a JEOL EX90, EX400 or GX500 spectrometer; chemical shifts are expressed in δ units using tetramethylsilane as the standard (in NMR description, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). Mass spectra were recorded with a Hitachi M-80 or JEOL JMS-DX300 spectrometer. ODS column chromatography was performed on YMC gel (ODS-A 120–230/70).

***tert*-Butyl 4-(4-Aminophenoxy)piperidine-1-carboxylate (2).** To a stirred solution of *tert*-butyl 4-hydroxypiperidine-1-carboxylate **1** (162 g, 805 mmol) and 4-nitrophenol (93.6 g, 673 mmol) in THF (1.3 L) at ambient temperature was added triphenylphosphine (211 g, 805 mmol), and diethyl azodicarboxylate (127 mL, 805 mmol). After stirring at ambient temperature for 21 h, the reaction mixture was concentrated in vacuo. The residue was dissolved in methylene chloride and washed with 1 N aqueous NaOH solution, water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was suspended with diethylether and insoluble material was removed by filtration. The filtrate was concentrated in vacuo and crystallized from ethanol to give *tert*-Butyl 4-(4-Nitrophenoxy)piperidine-1-carboxylate (166.74 g, 77%) as a white solid: mp 93–94 °C; ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s), 1.72–1.86 (2H, m), 1.90–2.03 (2H, m), 3.31–3.44 (2H, m), 3.63–3.77 (2H, m), 4.56–4.66 (1H, m), 6.96 (2H, d, J = 9.0 Hz), 8.20 (2H, d, J = 9.0 Hz); EI Ms m/e $(M)^+$ 322.

To the solution of the nitro compound (10.0 g, 31 mmol) in EtOH (100 mL) was added 10% Pd/C powder (1.0 g). The reaction mixture was stirred in a hydrogen atmosphere at ambient temperature for 1.5 h. The reaction mixture was filtered through a pad of Celite and concentrated in vacuo to give **2** (8.86 g, 98%) as a pale brown solid: mp 89–91 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.60–1.76 (2H, m), 1.80–1.93 (2H, m), 3.10–3.65 (4H, m), 3.65–3.77 (2H, m), 4.21–4.31 (1H, m), 6.63 (2H, d, J = 9.0 Hz), 6.76 (2H, d, J = 9.0 Hz); FAB Ms m/e $(M)^+$ 292.

7-Formylnaphthalene-2-carbonitrile (4). To a stirred solution of 7-bromomethylnaphthalene-2-carbonitrile **3** (2460 mg, 10 mmol) in acetonitrile (40 mL) at ambient temperature was added 4-methylmorpholine *N*-oxide (2340 mg, 20 mmol). After 5 h, water (60 mL) was added and the resulting precipitate was filtered, washed with water and dried in vacuo to give **4** (949 mg, 52%) as a white solid: mp 139–141 °C; ^{1}H NMR ($CDCl_3$) δ 7.78 (1H, dd, J = 1.5, 8.4 Hz), 8.02 (2H, d, J = 8.4 Hz), 8.13 (1H, dd, J = 1.5, 8.4 Hz), 8.41 (2H, s), 10.21 (1H, s); FAB Ms m/e $(M+H)^+$ 182.

7-({4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]anilino}-methyl)naphthalene-2-carbonitrile (5). To a stirred solution of **2** (1370 mg, 4.7 mmol) and **4** (849 mg, 4.7 mmol) in methylene chloride (10 mL) and AcOH (2.7 mL) at ambient temperature was added sodium triacetoxyborohydride (1290 mg, 6.1 mmol). After 45 min the reaction mixture was washed with 2 M potassium carbonate solution, water, and 10% citric acid solution. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was crystallized from methanol to give **5** (1698 mg, 79%) as a white solid: mp 154–155 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.63–1.74 (2H, m), 1.80–1.92 (2H, m), 3.21–3.30 (2H, m), 3.65–3.77 (2H, m), 4.00 (1H, br), 4.21–4.28 (1H, m), 4.49 (2H, s), 6.59 (2H, d, J = 8.8 Hz), 6.79 (2H, d, J = 8.8 Hz), 7.59 (1H, d, J = 8.3 Hz), 7.66 (1H, d, J = 8.8 Hz), 7.84–7.92 (3H, m), 8.19 (1H, s); FAB Ms m/e $(M)^+$ 457.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]acetamide (6b).** To a stirred solution of **5** (150 mg, 0.33 mmol) in pyridine (1 mL) at ambient temperature was added acetic anhydride (268 mg, 2.6 mmol) and 4-dimethylaminopyridine (10 mg, 0.08 mmol). After 15 h, the reaction mixture was diluted with ethyl acetate and washed with 10% citric acid solution and saturated sodium bicarbonate. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was crystallized from ethanol to give **6b** (139 mg, 84%) as a white solid: mp 162–163 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.67–1.77 (2H, m), 1.85–1.97 (5H, m), 3.27–3.36 (2H, m), 3.63–3.76 (2H,

m), 4.37–4.45 (1H, m), 5.02 (2H, s), 6.81 (2H, d, $J=8.8$ Hz), 6.88 (2H, d, $J=8.8$ Hz), 7.56–7.65 (3H, m), 7.83 (1H, d, $J=8.3$ Hz), 7.89 (1H, d, $J=8.3$ Hz), 8.13 (1H, s); FAB Ms m/e $(M+H)^+$ 500.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]propionamide (6c).** To a stirred solution of **5** (150 mg, 0.33 mmol) in 1,2-dichloroethane (5 mL) at ambient temperature was added triethylamine (50 mg, 0.5 mmol) and propionyl chloride (46 mg, 0.5 mmol). After 8 h, the reaction mixture was poured into ice water and extracted with methylene chloride. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (1:12) to give **6c** (167 mg, 98%) as a white solid: mp 194–195 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (3H, t $J=7.0$ Hz), 1.46 (9H, s), 1.54–2.02 (6H, m), 3.01–3.38 (2H, m), 3.50–3.74 (2H, m), 4.34–4.51 (1H, m), 5.01 (2H, s), 6.91–7.12 (4H, m), 7.45–7.88 (5H, m), 8.14 (1H, s); FAB Ms m/e $(M+H)^+$ 514.

1-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-1-[(7-cyano-2-naphthyl)methyl]-3-ethylurea (6d). To a stirred solution of **5** (150 mg, 0.33 mmol) in methylene chloride (2 mL) was added ethyl isocyanate (35 mg, 0.49 mmol). The reaction mixture was stirred at ambient temperature for 15 h. Then, ethyl isocyanate (117 mg, 1.64 mmol) was added again, stirred for 6 h and the reaction mixture was concentrated in vacuo. The crude residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (35:65) to give **6d** (154 mg, 88%) as a white amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, t, $J=7.3$ Hz), 1.46 (9H, s), 1.65–1.78 (2H, m), 1.82–1.95 (2H, m), 3.20–3.37 (4H, m), 3.62–3.75 (2H, m), 4.24 (1H, t, $J=5.5$ Hz), 4.36–4.44 (1H, m), 4.99 (2H, s), 6.83 (2H, d, $J=7.0$ Hz), 6.96 (2H, d, $J=7.3$ Hz), 7.57 (1H, d, $J=8.5$ Hz), 7.62–7.68 (2H, m), 7.83 (1H, d $J=8.5$ Hz), 7.87 (1H, d, $J=8.5$ Hz), 8.13 (1H, s); FAB Ms m/e $(M)^+$ 528.

Ethyl *N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]carbamate (6e). To a stirred solution of **5** (150 mg, 0.33 mmol) in DMF (2 mL) at ambient temperature was added ethyl chloroformate (157 mL, 1.65 mmol) and K_2CO_3 (271 mg, 1.98 mmol). After 3.5 h, water was added and the mixture was extracted with methylene chloride. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (20:80) to give **6e** (169 mg, 97%) as a white amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.15–1.30 (3H, br), 1.46 (9H, s), 1.65–1.80 (2H, m), 1.80–1.95 (2H, m), 3.25–3.37 (2H, m), 3.60–3.75 (2H, m), 4.20 (2H, q, $J=6.8$ Hz), 4.35–4.45 (1H, m), 4.98 (2H, s), 6.79 (2H, d, $J=8.8$ Hz), 6.90–6.71 (2H, br), 7.55–7.64 (2H, m), 7.68 (1H, s), 7.84 (1H, d, $J=8.3$ Hz), 7.89 (1H, d, $J=8.9$ Hz), 8.15 (1H, s); FAB Ms m/e $(M)^+$ 529.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]methanesulfonamide (6f).** To a stirred solution of **5** (250 mg, 0.55 mmol) in pyridine (2 mL) at 3 °C was added methanesulfonyl chloride (315 mg, 2.8 mmol). After 15 h, the reaction mixture was diluted with ethyl acetate and washed with 10% citric acid solution and saturated aqueous sodium bicarbonate. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (20:80–30:70) to give **6f** (264 mg, 90%) as a white amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.63–1.73 (2H, m), 1.80–1.90 (2H, m), 2.99 (3H, s), 3.25–3.35 (2H, m), 3.60–3.70 (2H, m), 4.34–4.40 (1H, m), 4.97 (2H, s), 6.80 (2H, d, $J=8.8$ Hz), 7.17 (2H, d, $J=8.8$ Hz), 7.59 (1H, dd, $J=1.5$, 8.8 Hz), 7.63–7.72 (2H, m), 7.85 (1H, d, $J=8.3$ Hz), 7.88 (1H, d, $J=8.8$ Hz), 8.13 (1H, s); FAB Ms m/e $(M+H)^+$ 536.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]ethanesulfonamide (6g).** Compound **6g** was synthesized from **5** and ethanesulfonyl chloride according to the same procedure as that for **6f**. Compound **6g** was obtained as a white solid (97% yield): mp 144–145 °C; ^{1}H NMR ($CDCl_3$) δ 1.43–1.50 (12H, m), 1.63–1.73 (2H, m), 1.80–1.91 (2H, m), 3.12 (2H, q, $J=7.3$ Hz), 3.25–3.36 (2H, m), 3.60–3.70 (2H, m), 4.33–4.41 (1H, m), 5.00 (2H, s), 6.78 (2H, d, $J=6.8$ Hz), 7.15 (2H, d, $J=6.8$ Hz), 7.58 (1H, d, $J=8.5$ Hz), 7.64 (1H, s), 7.69 (1H, d, $J=8.5$ Hz), 7.84 (1H, d $J=8.3$ Hz), 7.88 (1H, d, $J=8.3$ Hz), 8.13 (1H, s); FAB Ms m/e $(M+H)^+$ 550.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]propanesulfonamide (6h).** Compound **6h** was synthesized from **5** and propanesulfonyl chloride according to the same procedure as that for **6f**. Compound **6h** was obtained as a white solid (71% yield): mp 177–178 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (3H, t, $J=7.3$ Hz), 1.45 (9H, s) 1.63–1.73 (2H, m), 1.80–2.00 (4H, m), 3.02–3.09 (2H, m), 3.24–3.34 (2H, m), 3.59–3.69 (2H, m), 4.33–4.41 (1H, m), 4.98 (2H, s), 6.79 (2H, d, $J=9.3$ Hz), 7.15 (2H, d, $J=9.3$ Hz), 7.58 (1H, dd, $J=8.3$, 1.5 Hz), 7.64 (1H, s), 7.69 (1H, dd, $J=8.8$, 1.5 Hz), 7.84 (1H, d, $J=8.3$ Hz), 7.87 (1H, d, $J=8.3$ Hz), 8.12 (1H, s); FAB Ms m/e $(M)^+$ 563.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]butanesulfonamide (6i).** Compound **6i** was synthesized from **5** and butanesulfonyl chloride according to the same procedure as that for **6f**. Compound **6i** was obtained as a white solid (62% yield): mp 166–167 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t, $J=7.1$ Hz), 1.24–1.36 (2H, m) 1.45 (9H, s), 1.63–1.94 (6H, m), 2.94–3.85 (6H, m), 4.27–4.53 (1H, m), 5.02 (2H, s), 6.71–7.37 (4H, m), 7.42–7.96 (5H, m), 8.07 (1H, s); FAB Ms m/e $(M)^+$ 577.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]trifluoromethanesulfonamide (6j).** Compound **6j** was synthesized from **5** and trifluoromethanesulfonic anhydride according to the same procedure as that for **6f**. Compound **6j** was obtained as a white amorphous powder (54% yield): ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, m), 1.56–1.92 (4H, m), 3.11–3.82 (4H, m), 4.22–4.51 (1H, m), 5.02 (2H, s), 6.65–7.18 (4H,

m), 7.46–7.97 (5H, m), 8.11 (1H, s); FAB Ms *m/e* $(M+H)^+$ 590.

Ethyl (*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]sulfamoyl)acetate (6k). Compound **6k** was synthesized from **5** and ethyl (chlorosulfonyl)acetate[32] according to the same procedure as that for **6f**. Compound **6k** was obtained as a white solid (62% yield): mp 156–157 °C; ^{1}H NMR ($CDCl_3$) δ 1.39 (3H, t, $J=7.2$ Hz), 1.45 (9H, s), 1.57–1.75 (2H, m), 1.78–1.92 (2H, m), 3.24–3.36 (2H, m), 3.59–3.71 (2H, m), 4.05 (2H, s), 4.30–4.42 (3H, m), 5.04 (2H, s), 6.80 (2H, d, $J=9.0$ Hz), 7.29 (2H, d, $J=9.0$ Hz), 7.58 (1H, dd, $J=1.8$, 8.7 Hz), 7.66 (1H, dd, $J=1.8$, 8.7 Hz), 7.80–7.90 (2H, m), 8.12 (1H, s); FAB Ms *m/e* $(M+H)^+$ 608.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]-benzenesulfonamide (6l).** Compound **6l** was synthesized from **5** and benzenesulfonyl chloride according to the same procedure as that for **6f**. Compound **6l** was obtained as a white amorphous powder (91% yield): ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.60–1.70 (2H, m), 3.23–3.09 (2H, m), 3.23–3.33 (2H, m), 3.60–3.70 (2H, m), 4.30–4.38 (1H, m), 4.86 (2H, s), 6.69 (2H, d, $J=8.8$ Hz), 6.86 (2H, d, $J=8.8$ Hz), 7.48–7.73 (8H, m), 7.82 (1H, d, $J=7.8$ Hz), 7.86 (1H, d, $J=8.3$ Hz), 8.09 (1H, s); FAB Ms *m/e* $(M)^+$ 597.

4-(*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]sulfamoyl)benzoic acid (6m). Compound **6m** was synthesized from **5** and 4-(chlorosulfonyl)benzoic acid according to the same procedure as that for **6f**. Compound **6m** was obtained as a white solid (58% yield): mp 226–227 °C; ^{1}H NMR (DMSO-d_6) δ 1.32–1.47 (11H, m), 1.75–1.86 (2H, m), 3.00–3.15 (2H, m), 3.55–3.66 (2H, m), 4.37–4.47(1H, m), 4.97 (2H, s), 6.80 (2H, d, $J=8.8$ Hz), 6.96 (2H, d, $J=9.3$ Hz), 7.67 (1H, d, $J=8.8$ Hz), 7.74 (1H, dd, $J=8.3$, 1.5 Hz), 7.81, (2H, d, $J=8.3$ Hz), 7.89 (1H, s), 8.00 (1H, d, $J=8.3$ Hz), 8.07 (1H, d, $J=8.8$ Hz), 8.15 (2H, d, $J=8.3$ Hz), 8.52 (1H s), 13.52, (1H, s); FAB Ms *m/e* $(M)^+$ 597.

General procedure for synthesis of bis-amidine derivatives 8. *N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]methanesulfonamide (8f). HCl gas was bubbled through a solution of **6f** (711 mg, 1.33 mmol) in EtOH (15 mL) and $CHCl_3$ (10 mL) under −20 °C for 20 min. The mixture was allowed to stir for 30 h at 5 °C, and then concentrated in vacuo. To the crude imidate dissolved in EtOH (15 mL) and $CHCl_3$ (20 mL) was added ammonium acetate (1.0 g, 13.3 mmol) at ambient temperature. The reaction mixture was stirred at ambient temperature for 16 h and concentrated in vacuo. The resulting residue was chromatographed on ODS-gel eluting with MeOH/H_2O (0:100–5:95). MeOH was removed in vacuo, and the aqueous solution was lyophilized after acidification with 1 N HCl. **7f** (312 mg 40%) was obtained as an amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.68–1.81 (2H, m), 1.97–2.07 (2H, m), 2.48–2.52 (2H, m), 3.10–3.18 (5H, m), 4.51–4.61(1H, m), 5.02 (2H, s), 6.92 (2H, d, $J=9.2$ Hz), 7.34 (2H, d, $J=8.8$ Hz), 7.66, (1H, dd, $J=8.8$, 1.4 Hz), 7.80, (1H, dd, $J=8.3$, 1.5 Hz), 7.91 (1H, s), 8.01 (1H, d, $J=8.8$ Hz), 8.09 (1H, d, $J=8.3$ Hz), 8.46 (1H s), 9.23 (5H, br); FAB Ms *m/e* $(M+H)^+$ 453. Anal. calcd for $C_{24}H_{28}N_4O_3S{\cdot}2.0HCl{\cdot}3.2H_2O$: C, 49.43; H, 6.29; N, 9.61; S, 5.50; Cl, 12.16. Found: C, 49.55; H, 6.32; N, 9.60; S, 5.65; Cl, 12.08.

To a stirred solution of **7f** (165 mg, 0.28 mmol) in EtOH (4 mL) at ambient temperature was added ethyl acetimidate hydrochloride (90 mg, 0.73 mmol) and triethylamine (157 μL, 1.13 mmol). The mixture was allowed to stir for 4 days at ambient temperature, and then concentrated in vacuo. The resulting residue was chromatographed on ODS-gel eluting with MeOH/H_2O (0:100–5:95). MeOH was removed in vacuo, and the aqueous solution was lyophilized after acidification with 1 N HCl. **8f** (135 mg 78%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.60–1.74 (2H, m) 1.94–2.03 (2H, m) 2.27 (3H, s), 3.13 (3H, s), 3.43–3.55 (2H, m), 3.63–3.73 (1H, m), 3.73–3.80 (1H, m), 4.60–4.65 (1H, m), 5.03 (2H, s), 6.93 (2H, d, $J=9.2$ Hz), 7.34 (2H, d, $J=8.5$ Hz), 7.66, (1H, dd, $J=8.6$, 1.2 Hz), 7.81, (1H, dd, $J=8.6$, 1.8 Hz), 7.91 (1H, s), 8.01 (1H, d, $J=8.6$ Hz), 8.09 (1H, d, $J=8.5$ Hz), 8.47 (1H, s), 8.74 (1H, s), 9.20–9.31 (3H, m), 9.48 (2H, s); FAB Ms *m/e* $(M+H)^+$ 494. Anal. calcd for $C_{26}H_{31}N_5O_3S{\cdot}2.0HCl{\cdot}3.0H_2O$: C, 50.32; H, 6.33; N, 11.28; S, 5.17; Cl, 11.43. Found: C, 50.58; H, 6.22; N, 11.39; S, 5.07; Cl, 11.87.

7-({4-[(1-Acetimidoyl-4-piperidyl)oxy]anilino}methyl)-naphthalene-2-carboxamidine (8a). Compound **8a** was synthesized from **5** according to the same procedure as that for **8f**. Compound **8a** was obtained as a pale brown amorphous powder (25% yield): ^{1}H NMR (DMSO-d_6) δ 1.60–1.73 (2H, m), 1.88–1.99 (2H, m), 2.27 (3H, s), 3.42–3.55 (2H, m), 3.63–3.77 (2H, m), 4.35–4.42 (1H, m), 4.45 (2H, d, $J=6.1$ Hz), 6.16 (1H, t, $J=6.1$ Hz), 6.55 (2H, d, $J=8.5$ Hz), 6.75 (2H, d, $J=8.5$ Hz), 7.74, (1H, d, $J=8.5$ Hz), 7.79, (1H, d, $J=8.5$ Hz), 7.99 (1H, s), 8.03 (1H, d, $J=8.5$ Hz), 8.11 (1H, d, $J=8.5$ Hz), 8.43 (1H s), 9.0-9.6 (7H, br); FAB Ms *m/e* $(M+H)^+$ 416. Anal. calcd for $C_{25}H_{29}N_5O{\cdot}3.2HCl{\cdot}4.0H_2O$: C, 49.69; H, 6.71; N, 11.59; Cl, 18.77. Found: C, 49.69; H, 6.54; N, 11.84; Cl, 18.98.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]acetamide (8b).** Compound **8b** was synthesized from **6b** according to the same procedure as that for **8f**. Compound **8b** was obtained as a white amorphous powder (69% yield): ^{1}H NMR (DMSO-d_6) δ 1.61–1.76 (2H, m), 1.87 (3H, s), 1.95–2.06 (2H, m), 2.28 (3H, s), 3.43–3.55 (2H, m), 3.63–3.73 (1H, m), 3.75–3.84 (1H, m), 4.61–4.69 (1H, m), 5.03 (2H, s), 6.96 (2H, d, $J=8.5$ Hz), 7.15 (2H, d, $J=8.5$ Hz), 7.59 (1H, d, $J=8.5$ Hz), 7.81 (1H, d, $J=8.5$ Hz), 7.85 (1H, s), 8.01 (1H, d, $J=8.5$ Hz), 8.11 (1H, d, $J=8.5$ Hz), 8.48 (1H s), 8.80 (1H, s), 9.30 (2H, s), 9.33 (1H, s), 9.51 (2H, s); FAB Ms *m/e* $(M+H)^+$ 458. Anal. calcd for $C_{27}H_{31}N_5O2{\cdot}2.3HCl{\cdot}4.1H_2O$: C, 52.71; H, 6.80; N, 11.38; Cl, 13.23. Found: C, 52.47; H, 6.54; N, 11.60; Cl, 12.88.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]propionamide (8c).** Compound **8c** was synthesized from **6c** according to the same procedure as that for **8f**. Compound **8c** was obtained as a white amorphous powder (27% yield): ^{1}H NMR (DMSO-d_6) δ 0.99 (3H, t, J = 9.3 Hz), 1.64–1.80 (2H, m) 1.94–2.14 (4H, m), 2.28 (3H, s), 3.02–3.09 (2H, m), 3.62–3.80 (2H, m), 4.60–4.64 (1H, m), 5.03 (2H, s), 6.95 (2H, d, J = 9.2 Hz), 7.12 (2H, d, J = 8.5 Hz), 7.48 (1H, d, J = 8.5 Hz), 7.78–7.86 (2H, m), 8.00 (1H, d, J = 8.4 Hz), 8.11 (1H, d, J = 8.6 Hz), 8.48 (1H s), 8.82 (1H, s), 9.30 (3H, s), 9.51 (2H, s); FAB Ms m/e $(M+H)^+$ 472. Anal. calcd for $C_{28}H_{33}N_5O_2 \cdot 2.4HCl \cdot 3.5H_2O$: C, 54.05; H, 6.87; N, 11.26; Cl, 13.68. Found: C, 54.03; H, 6.78; N, 11.06; Cl, 13.50.

1-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-1-[(7-amidino-2-naphthyl)methyl]-3-ethylurea (8d). Compound **8d** was synthesized from **6d** according to the same procedure as that for **8f**. Compound **8d** was obtained as a white amorphous powder (62% yield): ^{1}H NMR (DMSO-d_6) δ 0.99 (3H, t, J = 7.3 Hz), 1.61–1.73 (2H, m), 1.95–2.05 (2H, m), 2.28 (3H, s), 3.01–3.13 (2H, m), 3.45–3.59 (2H, m), 3.64–3.84 (2H, m), 4.58–4.65 (1H, m), 4.97 (2H, s), 5.73 (1H, t, J = 5.5 Hz), 6.93 (2H, d, J = 8.6 Hz), 7.08 (2H, d, J = 9.2 Hz), 7.60 (1H, d, J = 8.5 Hz), 7.75–7.86 (2H, m), 8.00 (1H, d, J = 8.5 Hz), 8.10 (1H, d, J = 9.2 Hz), 8.46 (1H, s), 8.85 (1H, s), 9.23–9.40 (3H, m), 9.51 (2H, s); FAB Ms m/e $(M+H)^+$ 487. Anal. calcd for $C_{28}H_{34}N_6O_2 \cdot 2.1HCl \cdot 2.5H_2O$: C, 55.29; H, 6.81; N, 13.82; Cl, 12.24. Found: C, 55.10; H, 6.77; N, 13.81; Cl, 12.36.

Ethyl *N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]carbamate (8e). Compound **8e** was synthesized from **6e** according to the same procedure as that for **8f**. Compound **8e** was obtained as a white amorphous powder (51% yield): ^{1}H NMR (DMSO-d_6) δ 1.16 (3H, t, J = 7.0 Hz), 1.61–1.77 (2H, m), 1.95–2.06 (2H, m), 2.29 (3H, s), 3.43–3.57 (2H, m), 3.65–3.75 (1H, m), 3.75–3.83 (1H, m), 4.12 (2H, q, J = 7.0 Hz), 4.58–4.65 (1H, m), 5.03 (2H, s), 6.91 (2H, d, J = 8.5 Hz), 7.15 (2H, d, J = 8.5 Hz), 7.60 (1H, d, J = 8.5 Hz), 7.82 (1H, d, J = 8.5 Hz), 7.86 (1H, s), 8.03 (1H, d, J = 8.5 Hz), 8.11 (1H, d, J = 8.5 Hz), 8.50 (1H, s), 8.81 (1H, s), 9.33 (2H, s), 9.35 (1H, s), 9.53 (2H, s); FAB Ms m/e $(M+H)^+$ 488. Anal. calcd for $C_{28}H_{33}N_5O_3 \cdot 2.0HCl \cdot 3.0H_2O$: C, 54.72; H, 6.72; N, 11.40; Cl, 11.54. Found: C, 54.91; H, 6.60; N, 11.43; Cl, 11.66.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]ethanesulfonamide (8g):.** Compound **8g** was synthesized from **6g** according to the same procedure as that for **8f** Compound **8g** was obtained as a white amorphous powder (42% yield): ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, J = 7.3 Hz), 1.59–1.73 (2H, m) 1.93–2.03 (2H, m), 2.27 (3H, s), 3.26 (2H, q, J = 7.3 Hz), 3.43–3.54 (2H, m), 3.63–3.71 (1H, m), 3.71–3.80 (1H, m), 4.58–4.64 (1H, m), 5.07 (2H, s), 6.91 (2H, d, J = 9.2 Hz), 7.33 (2H, d, J = 9.2 Hz), 7.65 (1H, d, J = 8.5 Hz), 7.81 (1H, d, J = 8.5 Hz), 7.90 (1H, s), 8.01 (1H, d, J = 8.5 Hz), 8.09 (1H, d, J = 9.2 Hz), 8.47 (1H, s), 8.74 (1H, s), 9.26 (2H, s), 9.28 (1H, s), 9.50 (2H, s); FAB Ms m/e $(M+H)^+$ 508. Anal. calcd for $C_{27}H_{33}N_5O_3S \cdot 2.0HCl \cdot 3.0H_2O$: C, 51.10; H, 6.51; N, 11.04; S, 5.50; Cl, 11.17. Found: C, 50.91; H, 6.32; N, 11.17; S, 5.08; Cl, 11.41.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]-propanesulfonamide (8h).** Compound **8h** was synthesized from **6h** according to the same procedure as that for **8f**. Compound **8h** was obtained as a white amorphous powder (43% yield): ^{1}H NMR (DMSO-d_6) δ 1.03 (3H, t, J = 7.6 Hz), 1.60–1.73 (2H, m), 1.74–1.85 (2H, m), 1.93–2.03 (2H, m), 2.27 (3H, s), 3.21–3.25 (2H, m), 3.42–3.56 (2H, m), 3.63–3.71 (1H, m), 3.75–3.82 (1H, m), 4.59–4.63 (1H, m), 5.05 (2H, s), 6.92 (2H, d, J = 8.5 Hz), 7.33 (2H, d, J = 8.5 Hz), 7.66 (1H, dd, J = 8.6, 1.2 Hz), 7.82 (1H, dd, J = 8.5, 1.8 Hz), 7.89 (1H, s), 8.01 (1H, d, J = 8.6 Hz), 8.09 (1H, d, J = 8.5 Hz), 8.48 (1H, s), 8.80 (1H, s), 9.28–9.38 (3H, m), 9.52 (2H, s); FAB Ms m/e $(M+H)^+$ 522. Anal. calcd for $C_{28}H_{35}N_5O_3S \cdot 2.0HCl \cdot 3.0H_2O$: C, 51.85; H, 6.68; N, 10.80; S, 4.94; Cl, 10.93. Found: C, 51.44; H, 6.58; N, 10.79; S, 4.90; Cl, 11.31.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]butanesulfonamide (8i).** Compound **8i** was synthesized from **6i** according to the same procedure as that for **8f**. Compound **8i** was obtained as a white amorphous powder (35% yield): ^{1}H NMR (DMSO-d_6) δ 0.92 (3H, t, J = 7.3 Hz), 1.41–1.46 (2H, m), 1.62–1.78 (4H, m), 1.91–2.01 (2H, m), 2.27 (3H, s), 3.24 (2H, t, J = 7.9 Hz), 3.41–3.55 (2H, m), 3.63–3.80(2H, m), 4.58–4.62 (1H, m), 5.06 (2H, s), 6.91 (2H, d, J = 9.2 Hz), 7.33 (2H, d, J = 9.2 Hz), 7.65 (1H, d, J = 8.5 Hz), 7.80 (1H, d, J = 6.7 Hz), 7.90 (1H, s), 8.01 (1H, d, J = 8.6 Hz), 8.09 (1H, d, J = 8.5 Hz), 8.46 (1H, s), 8.72 (1H, s), 9.18–9.30 (3H, m), 9.47 (2H, s); FAB Ms m/e $(M+H)^+$ 536. Anal. calcd for $C_{29}H_{37}N_5O_3S \cdot 2.1HCl \cdot 2.0H_2O$: C, 53.73; H, 6.70; N, 10.80; S, 4.95; Cl, 11.48. Found: C, 53.96; H, 6.47; N, 10.90; S, 4.65; Cl, 11.56.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]trifluoromethanesulfonamide (8j).** Compound **8j** was synthesized from **6j** according to the same procedure as that for **8f**. Compound **8j** was obtained as a white amorphous powder (14% yield): ^{1}H NMR (DMSO-d_6) δ 1.60–1.74 (2H, m), 1.92–2.05 (2H, m), 2.26 (3H, s), 3.44–3.50 (2H, m), 3.67–3.76 (2H, m), 4.63–4.65 (1H, m), 5.25 (2H, s), 6.95 (2H, d, J = 9.2 Hz), 7.30 (2H, d, J = 9.2 Hz), 7.60 (1H, d, J = 6.7 Hz), 7.83 (1H, d, J = 7.3 Hz), 7.92 (1H, s), 8.04 (1H, d, J = 8.6 Hz), 8.12 (1H, d, J = 8.6 Hz), 8.48 (1H, s), 8.68 (1H, s), 9.20 (3H, s), 9.47 (2H, s); FAB Ms m/e $(M+H)^+$ 548. Anal. calcd for $C_{26}H_{28}N_5O_3SF_3 \cdot 2.3HCl \cdot 3.6H_2O$: C, 44.85; H, 5.43; N, 10.06; S, 4.61; Cl, 11.71; F, 8.19. Found: C, 45.21; H, 5.29; N, 10.43; S, 4.49; Cl, 11.51; F, 7.78.

Ethyl (*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl)acetate (8k). Compound **8k** was synthesized from **6k** according to the same procedure as that for **8f**. Compound **8k** was obtained as a white amorphous powder (52% yield): ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.3 Hz), 1.61–1.74

(2H, m), 1.94–2.04 (2H, m), 2.26 (3H, s), 3.43–3.51 (2H, m), 3.64–3.78 (2H, m), 4.23 (2H, q, $J=7.3$ Hz), 4.43 (2H, s), 4.59–4.65 (1H, m), 5.05 (2H, s), 6.94 (2H, d, $J=9.2$ Hz), 7.32 (2H, d, $J=9.2$ Hz), 7.64 (1H, d, $J=8.5$ Hz), 7.79 (1H, d, $J=8.6$ Hz), 7.92 (1H, s), 8.01 (1H, d, $J=8.0$ Hz), 8.10 (1H, d, $J=8.5$ Hz), 8.45(1H, s), 8.64 (1H, s), 9.12 (3H, s), 9.43 (2H, s); FAB Ms *m*/*e* $(M+H)^+$ 566. Anal. calcd for $C_{29}H_{35}N_5O_5S\cdot2.2HCl\cdot1.8H_2O$: C, 51.35; H, 6.06; N, 10.32; S, 4.73; Cl, 11.50. Found: C, 51.38; H, 6.14; N, 10.31; S, 4.78; Cl, 11.60.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]-benzenesulfonamide (8l).** Compound **8l** was synthesized from **6l** according to the same procedure as that for **8f**. Compound **8l** was obtained as a white amorphous powder (45% yield): ^{1}H NMR (DMSO-d_6) δ 1.58–1.70 (2H, m), 1.91–2.03 (2H, m), 2.27 (3H, s), 3.42–3.49 (2H, m), 3.67–3.77 (2H, m), 4.58–4.61 (1H, m), 4.99 (2H, s), 6.84 (2H, d, $J=9.2$ Hz), 6.98 (2H, d, $J=8.5$ Hz), 7.64–7.76 (6H, m), 7.82 (1H, d, $J=10.4$ Hz), 7.92 (1H, s), 8.01 (1H, d, $J=8.6$ Hz), 8.09 (1H, d, $J=8.5$ Hz), 8.46(1H, s), 8.81 (1H, s), 9.34 (3H, s), 9.53 (2H, s); FAB Ms *m*/*e* $(M+H)^+$ 570. Anal. calcd for $C_{31}H_{33}N_5O_3S\cdot2.1HCl\cdot2.2H_2O$: C, 55.42; H, 5.93; N, 10.42; S, 4.77; Cl, 11.08. Found: C, 55.16; H, 5.77; N, 10.43; S, 4.84; Cl, 11.12.

Ethyl 4-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl)benzoate (8m). Compound **8m** was synthesized from **6m** according to the same procedure as that for **8f**. Compound **8m** was obtained as a white amorphous powder (44% yield): ^{1}H NMR (DMSO-d_6) δ 1.37 (3H, t, $J=7.0$ Hz), 1.57–1.73 (2H, m), 1.92–2.02 (2H, m), 2.26 (3H, s), 3.40–3.50 (2H, m), 3.62–3.80 (2H, m), 4.39 (2H, q, $J=7.0$ Hz), 4.55–4.62 (1H, m), 5.00 (2H, s), 6.85 (2H, d, $J=9.2$ Hz), 6.99 (2H, d, $J=8.6$ Hz), 7.66 (1H, d, $J=8.6$ Hz), 7.81(1H, dd, $J=1.8$, 9.2 Hz), 7.85 (2H, d, $J=8.5$ Hz), 7.92 (1H, s), 8.02 (1H, d, $J=8.6$ Hz), 8.09 (1H, d, $J=8.5$ Hz), 8.18 (2H, d, $J=8.6$ Hz), 8.44 (1H, s), 8.71 (1H, s), 9.13–9.27 (3H, m), 9.47 (2H, s); FAB Ms *m*/*e* $(M+H)^+$ 628. Anal. calcd for $C_{34}H_{37}N_5O_5S\cdot2.0HCl\cdot1.3H_2O$: C, 56.40; H, 5.79; N, 9.67; S, 4.43; Cl, 9.79. Found: C, 56.66; H, 6.09; N, 9.67; S, 4.52; Cl, 10.09.

(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl)acetamide (8n). Compound **8n** was synthesized from **6k** according to the same procedure as that for **8f**, but using saturated ammonia solution in ethanol instead of ammonium acetate for conversion of the imidate to the amidine and ester to amide simultaneously. Compound **8n** was obtained as a white amorphous powder (68% yield): ^{1}H NMR (DMSO-d_6) δ 1.60–1.72 (2H, m), 1.94–2.02 (2H, m), 2.26 (3H, s), 3.42–3.51 (2H, m), 3.64–3.78 (2H, m), 4.07 (2H, s), 4.58–4.64 (1H, m), 5.01 (2H, s), 6.93 (2H, d, $J=8.6$ Hz), 7.38 (2H, d, $J=9.2$ Hz), 7.52 (1H,s), 7.66 (1H, d, $J=8.6$ Hz), 7.80 (1H, d, $J=8.6$ Hz), 7.84 (1H, s), 7.90 (1H, s), 8.00 (1H, d, $J=8.60$ Hz), 8.08 (1H, d, $J=9.2$ Hz), 8.44 (1H, s), 8.84–9.14 (5H, br); FAB Ms *m*/*e* $(M+H)^+$ 537. Anal. calcd for $C_{27}H_{32}N_6O_4S\cdot2.0HCl\cdot2.0H_2O$: C, 50.23; H, 5.93; N, 13.02; S, 4.97; Cl, 10.98. Found: C, 50.10; H, 6.01; N, 12.97; S, 5.05; Cl, 10.58.

(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl)acetic acid (8o). A solution of **8k** (4.00 g, 5.9 mmol) in 1 N HCl (100 mL) was stirred under reflux for 1.5 h. The reaction mixture was concentrated in vacuo and the residue was chromatographed on ODS-gel eluting with CH_3CN/H_2O (0:100–1:99). The product containing fractions were acidified with 1 N HCl (pH = 1), combined and concentrated in vacuo. The resulting residue was crystallized from EtOH to give **8o** (1.11 g, 28%) as a white solid: mp 203–206 °C, ^{1}H NMR (DMSO-d_6) δ 1.59–1.76 (2H, m), 1.92–2.05 (2H, m), 2.28 (3H, s), 3.40–3.57 (2H, m), 3.64–3.85 (2H, m), 4.30 (2H, s), 4.59–4.68 (1H, m), 5.05 (2H, s), 6.94 (2H, d, $J=8.8$ Hz), 7.33 (2H, d, $J=8.8$ Hz), 7.66 (1H, d, $J=8.0$ Hz), 7.83(1H, d, $J=8.4$ Hz), 7.90 (1H, s), 8.02 (1H, d, $J=8.0$ Hz), 8.10 (1H, d, $J=8.4$ Hz), 8.49(1H, s), 8.85 (1H, s), 9.40 (3H, s), 9.55 (2H, s); FAB Ms *m*/*e* $(M+H)^+$ 538. Anal. calcd for $C_{27}H_{31}N_5O_5S\cdot2.0HCl\cdot3.0H_2O$: C, 48.80; H, 5.91; N, 10.54; S, 4.82; Cl, 10.67. Found: C, 48.83; H, 5.53; N, 10.55; S, 4.95; Cl, 10.92.

4-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]-sulfamoyl)benzoic acid (8p). A solution of **8m** (1.85 g, 2.55 mmol) in 1 N HCl (200 mL) was refluxed for 7 h. The reaction mixture was concentrated and dried in vacuo. Compound **8p** (1.74 g, 97%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.57–1.72 (2H, m), 1.91–2.02 (2H, m), 2.26 (3H, s), 3.40–3.51 (2H, m), 3.62–3.80 (2H, m), 4.55–4.62 (1H, m), 5.01 (2H, s), 6.85 (2H, d, $J=9.2$ Hz), 7.00 (2H, d, $J=9.2$ Hz), 7.67 (1H, d, $J=9.5$ Hz), 7.77–7.85 (3H, m), 7.92 (1H, s), 8.02 (1H, d, $J=8.6$ Hz), 8.09 (1H, d, $J=8.5$ Hz), 8.16 (2H, d, $J=8.6$ Hz), 8.45 (1H, s), 8.74 (1H, s), 9.18–9.34 (3H, m), 9.49 (2H, s), 13.53 (1H, s); FAB Ms *m*/*e* $(M+H)^+$ 600. Anal. calcd for $C_{32}H_{33}N_5O_5S\cdot2.1HCl\cdot1.4H_2O$: C, 54.79; H, 5.45; N, 9.98; S, 4.57; Cl, 10.61. Found: C, 54.63; H, 5.34; N, 10.07; S, 4.55; Cl, 10.57.

***tert*-Butyl *N*-(*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-N-[(7-cyano-2-naphthyl)methyl]-sulfamoyl)carbamate (9a).** To a stirred solution of chlorosulfonylisocyanate (5.0 g, 35.3 mmol) in benzene (15 mL) at ambient temperature was added *tert*-butyl alcohol (2.6 g, 35.3 mmol) dropwise. After 1 h, *n*-hexane (30 mL) was added and the resulting precipitate was filtered, washed with *n*-hexane and dried in vacuo. *tert*-Butyl chlorosulfonylcarbamate (**11a**) (4.1 g, 54%) was obtained as a hygroscopic white solid. ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s). Compound **9a** was synthesized from **5** and **11a** according to the same procedure as that for **6f**. Compound **9a** was obtained as a white amorphous powder (86% yield): ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.57 (9H, s), 1.63–1.73 (2H, m), 1.80–1.91 (2H, m), 3.25–3.35 (2H, m), 3.60–3.70 (2H, m), 4.33–4.43 (1H, m), 5.21 (2H, s), 6.80 (2H, d, $J=9.3$ Hz), 7.00 (1H, s), 7.18 (2H, d, $J=8.8$ Hz), 7.58 (1H, dd, $J=1.7$, 8.5 Hz), 7.62–7.70 (2H, m), 7.84 (1H, d, $J=6.6$ Hz), 7.87 (1H, d, $J=8.3$ Hz), 8.13 (1H, s); FAB Ms *m*/*e* $(M)^+$ 636.

Ethyl *N*-(*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]-phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]sulfamoyl)carbamate (9b). Ethyl chlorosulfonylcarbamate (**11b**) was synthesized from chlorosulfonylisocyanate and ethanol

according to the same procedure as that for **11a**. Compound **11b** was obtained as a hygroscopic white solid (88% yield). ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t, J=9.5 Hz), 4.39 (2H, q, J=9.5 Hz). Compound **9b** was synthesized from **5** and **11b** according to the same procedure as that for **6f**. Compound **9b** was obtained as a white amorphous powder (72% yield): ^{1}H NMR ($CDCl_3$) δ 1.37 (3H, t, J=7.1 Hz), 1.45 (9H, s), 1.62–1.74 (2H, m), 1.80–1.91 (2H, m), 3.25–3.34 (2H, m), 3.60–3.71 (2H, m), 4.29–4.42 (3H, m), 5.21 (2H, s), 6.80 (2H, d, J=8.8 Hz), 7.08–7.20 (3H, m), 7.58 (1H, dd, J=1.5, 8.3 Hz), 7.62–7.69 (2H, m), 7.84 (1H, d, J=8.8 Hz), 7.87 (1H, d, J=8.3 Hz), 8.13 (1H, s); FAB Ms m/e $(M)^+$ 608.

***tert*-Butyl *N*-(*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]sulfamoyl)-*N*-methylcarbamate (10a).** To a stirred solution of **9a** (172 mg, 0.27 mmol) in THF (0.7 mL) at 3 °C was added triphenylphosphine (139 mg, 0.53 mmol), methanol (32 μL, 0.79 mmol) and diethyl azodicarboxylate (83 μL, 0.53 mmol). After stirring at ambient temperature for 40 min, the reaction mixture was concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (15:85) to give **10a** (153 mg, 87%) as a white amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.57–1.73 (11H, m), 1.80–1.92 (2H, m), 2.93 (3H, s), 3.24–3.35 (2H, m), 3.60–3.72 (2H, m), 4.33–4.42 (1H, m), 5.19 (2H, s), 6.79 (2H, d, J=9.3 Hz), 7.15 (2H, d, J=8.8 Hz), 7.57 (1H, dd, J=1.5, 8.3 Hz), 7.64–7.70 (2H, m), 7.84 (1H, d, J=8.3 Hz), 7.87 (1H, d, J=8.3 Hz), 8.13 (1H, s); FAB Ms m/e $(M)^+$ 650.

Ethyl *N*-(*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]-phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]sulfamoyl)-*N*-*tert*-butoxycarbonyl glycinate (10b). To a stirred solution of **9a** (1.5 g, 2.36 mmol) of DMF (15 mL) at ambient temperature was added ethyl bromoacetate (0.79 mL, 7.08 mmol) and K_2CO_3 (978 mg, 7.08 mmol). The mixture was allowed to stir for 18 h at ambient temperature, and then concentrated in vacuo. Water was added, and the mixture was extracted with ethyl acetate. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (10:90–17:83) to give **10b** (1.26 g, 74%) as a white amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J=7.1 Hz), 1.45 (9H, s), 1.58 (9H, s), 1.60–1.76 (2H, m), 1.80–1.92 (2H, m), 3.24–3.35 (2H, m), 3.60–3.71 (2H, m), 4.08–4.20 (4H, m), 4.32–4.42 (1H, m), 5.19 (2H, s), 6.77 (2H, d, J=8.8 Hz), 7.15 (2H, d, J=8.8 Hz), 7.57 (1H, d, J=8.3 Hz), 7.62–7.70 (2H, m), 7.83 (1H, d, J=8.3 Hz), 7.87 (1H, d, J=8.3 Hz), 8.12 (1H, s); FAB Ms m/e $(M)^+$ 722.

Methyl *N*-(*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-[(7-cyano-2-naphthyl)methyl]sulfamoyl)-*N*-ethoxycarbonyl glycinate (10c). Compound **10c** was synthesized from **9b** and methyl bromoacetate according to the same procedure as that for **10b**. Compound **10c** was obtained as a white amorphous powder (98% yield): ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t, J=7.1 Hz), 1.45 (9H, s), 1.62–1.74(2H, m), 1.80–1.92 (2H, m), 3.24–3.35 (2H, m), 3.60–3.71 (5H, m), 4.21 (2H, s), 4.34–4.44 (3H, m), 5.19 (2H, s), 6.78 (2H, d, J=8.8 Hz), 7.13 (2H, d, J=8.8 Hz), 7.58 (1H, d, J=8.5 Hz), 7.62–7.69 (2H, m), 7.84 (1H, d, J=8.8 Hz), 7.88 (1H, d, J=8.8 Hz), 8.12 (1H, s); FAB Ms m/e $(M)^+$ 680.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]-*N'*-methylsulfamide (8q).** Compound **8q** was synthesized from **10a** according to the same procedure as that for **8f**. Compound **8q** was obtained as a white amorphous powder (54% yield): ^{1}H NMR (DMSO-d_6) δ 1.59–1.73 (2H, m) 1.92–2.03 (2H, m), 2.27 (3H, s), 2.67 (3H, d, J=4.9 Hz), 3.41–3.55 (2H, m), 3.63–3.72 (1H, m), 3.72–3.81 (1H, m), 4.56–4.63 (1H, m), 4.96 (2H, s), 6.89 (2H, d, J=9.2 Hz), 7.27 (2H, d, J=8.5 Hz), 7.39–7.46 (1H, m), 7.66 (1H, d, J=8.6 Hz), 7.81 (1H, dd, J=1.5, 8.8 Hz), 7.89 (1H, s), 8.00 (1H, d, J=8.5 Hz), 8.09 (1H, d, J=8.6 Hz), 8.45 (1H, s), 8.79 (1H, s), 9.23–9.40 (3H, m), 9.52 (2H, m); FAB Ms m/e $(M+H)^+$ 509. Anal. calcd for $C_{26}H_{32}N_6O_3S{\cdot}2.1HCl{\cdot}2.3H_2O$: C, 49.83; H, 6.22; N, 13.41; S, 5.12; Cl, 11.88. Found: C, 49.81; H, 6.13; N, 13.54; S, 4.87; Cl, 11.81.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamide (8r).** Compound **8r** was synthesized from **9a** according to the same procedure as that for **8f**. Compound **8r** was obtained as a white amorphous powder (47% yield): ^{1}H NMR (DMSO-d_6) δ 1.59–1.74 (2H, m), 1.93–2.03 (2H, m), 2.27 (3H, s), 3.43–3.57 (2H, m), 3.63–3.71 (1H, m), 3.73–3.81 (1H, m), 4.56–4.63 (1H, m), 4.90 (2H, s), 6.89 (2H, d, J=9.2 Hz), 7.20 (2H, s), 7.27 (2H, d, J=9.2 Hz), 7.71 (1H, d, J=7.6 Hz), 7.80 (1H, dd, J=1.8, 8.6 Hz), 7.92 (1H, s), 7.99 (1H, d, J=8.5 Hz), 8.08 (1H, d, J=8.6 Hz), 8.44 (1H, s), 8.80 (1H, s), 9.24–9.37 (3H, m), 9.51 (2H, m); FAB Ms m/e $(M+H)^+$ 495. Anal. calcd for $C_{25}H_{30}N_6O_3S{\cdot}2.0HCl{\cdot}2.7H_2O$: C, 48.73; H, 6.12; N, 13.64; S, 5.20; Cl, 11.51. Found: C, 48.83; H, 6.05; N, 13.67; S, 5.15; Cl, 11.84.

Ethyl *N*-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl)carbamate (8s). Compound **8s** was synthesized from **9b** according to the same procedure as that for **8f**. Compound **8s** was obtained as a white amorphous powder (45% yield): ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, J=7.3 Hz), 1.60–1.75 (2H, m), 1.95–2.03 (2H, m), 2.27 (3H, s), 3.41–3.57 (2H, m), 3.63–3.73 (1H, m), 3.73–3.82 (1H, m), 4.23 (2H, q, J=7.3 Hz), 4.60–4.67 (1H, m), 5.17 (2H, s), 6.96 (2H, d, J=9.2 Hz), 7.23 (2H, d, J=8.5 Hz), 7.65 (1H, d, J=7.3 Hz), 7.82 (1H, d, J=8.5 Hz), 7.91 (1H, s), 8.03 (1H, d, J=8.5 Hz), 8.10 (1H, d, J=8.5 Hz), 8.47 (1H, s), 8.81 (1H, s), 9.23–9.40 (3H, m), 9.51(2H, s), 11,52 (1H, s); FAB Ms m/e $(M+H)^+$ 567. Anal. calcd for $C_{28}H_{34}N_6O_5S{\cdot}2.0HCl{\cdot}2.6H_2O$: C, 48.99; H, 6.05; N, 12.24; S, 4.67; Cl, 10.33. Found: C, 48.71; H, 5.98; N, 12.24; S, 4.68; Cl, 10.73.

Ethyl *N*-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl)glycinate (8t). Compound **8t** was synthesized from **10b** according to the same procedure as that for **8f**. Compound **8t** was

obtained as a white amorphous powder (33% yield): ^{1}H NMR (DMSO-d_6) δ 1.21 (3H, t, J = 7.3 Hz), 1.60–1.73 (2H, m), 1.92–2.02 (2H, m), 2.27 (3H, s), 3.42–3.55 (2H, m), 3.63–3.70 (1H, m), 3.70–3.80 (1H, m), 3.83 (2H, d, J = 6.1 Hz), 4.14 (2H, q, J = 7.3 Hz), 4.56–4.62 (1H, m), 4.94 (2H, s), 6.89 (2H, d, J = 9.2 Hz), 7.28 (2H, d, J = 9.2 Hz), 7.65 (1H, d, J = 8.5 Hz), 7.80 (1H, d, J = 8.6 Hz), 7.89 (1H, s), 7.99 (1H, d, J = 8.5 Hz), 8.04–8.10 (2H, m), 8.43 (1H, s), 8.74 (1H, s), 9.20–9.31 (3H, m), 9.48 (2H, s); FAB Ms m/e $(M+H)^+$ 581. Anal. calcd for $C_{29}H_{36}N_6O_5S\cdot 2.2HCl\cdot 2.5H_2O$: C, 49.34; H, 6.17; N, 11.90; S, 4.54; Cl, 11.05. Found: C, 49.24; H, 6.04; N, 12.06; S, 4.56; Cl, 10.97.

***N*-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl)glycine (8u).** A solution of **8t** (109 mg, 0.15 mmol) in concd HCl (50 mL) was stirred at ambient temperature for 2 h. The reaction mixture was concentrated in vacuo and the residue was chromatographed on ODS-gel eluting with MeOH/H_2O (0:100–12:88). MeOH was removed in vacuo, and the aqueous solution was lyophilized after acidification with 1 N HCl. **8u** (88 mg 85%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.60–1.71 (2H, m), 1.90–2.02 (2H, m), 2.26 (3H, s), 3.40–3.55 (2H, m), 3.61–3.81 (4H, m), 4.56–4.62 (1H, m), 4.94 (2H, s), 6.88 (2H, d, J = 9.2 Hz), 7.28 (2H, d, J = 8.6 Hz), 7.64 (1H, d, J = 8.5 Hz), 7.78 (1H, d, J = 8.5 Hz), 7.81–7.93 (2H, m), 7.97 (1H, d, J = 8.6 Hz), 8.07 (1H, d, J = 8.6 Hz), 8.42 (1H, s), 8.71 (1H, s), 9.26 (1H, s), 9.30–9.50 (4H, m), 12.8 (1H, s); FAB Ms m/e $(M+H)^+$ 553. Anal. calcd for $C_{27}H_{32}N_6O_5S\cdot 1.9HCl\cdot 2.7H_2O$: C, 48.36; H, 5.91; N, 12.53; S, 4.78; Cl, 10.05. Found: C, 48.21; H, 5.68; N, 12.52; S, 4.89; Cl, 9.66.

Methyl *N*-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl)-*N*-ethoxycarbonylglycinate (8v). Compound **8v** was synthesized from **10c** according to the same procedure as that for **8f**, but using methanol as a solvent instead of ethanol. Compound **8v** was obtained as a white amorphous powder (46% yield): ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, J = 7.0 Hz), 1.60–1.75 (2H, m), 1.95–2.05 (2H, m), 2.27 (3H, s), 3.41–3.53 (2H, m), 3.62 (3H, s), 3.65–3.72 (1H, m), 3.75–3.82 (1H, m), 4.17 (2H, s), 4.35 (2H, q, J = 7.0 Hz), 4.60–4.67 (1H, m), 5.19 (2H, s), 6.96 (2H, d, J = 9.2 Hz), 7.23 (2H, d, J = 9.2 Hz), 7.63 (1H, d, J = 8.6 Hz), 7.83 (1H, d, J = 8.6 Hz), 7.91 (1H, s), 8.04 (1H, d, J = 8.6 Hz), 8.11(1H, d, J = 8.6 Hz), 8.47 (1H, s), 8.79 (1H, s), 9.26–9.35 (3H, m), 9.52 (2H, s); FAB Ms m/e $(M+H)^+$ 639. Anal. calcd for $C_{31}H_{38}N_6O_7S\cdot 2.1HCl\cdot 2.3H_2O$: C, 49.20; H, 5.95; N, 11.11; S, 4.24; Cl, 9.84. Found: C, 49.05; H, 5.80; N, 11.23; S, 4.29; Cl, 10.16.

***N*-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl)-*N*-ethoxycarbonylglycine (8w).** Compound **8w** was synthesized from **8v** according to the same procedure as that for **8u**. Compound **8w** was obtained as a white amorphous powder (80% yield): ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, J = 7.0 Hz), 1.61–1.73 (2H, m), 1.95–2.05 (2H, m), 2.27 (3H, s), 3.41–3.53 (2H, m), 3.62–3.72 (1H, m), 3.72–3.82 (1H, m), 4.01 (2H, s), 4.34 (2H, q, J = 7.0 Hz), 4.59–4.68 (1H, m), 5.19 (2H, s), 6.96 (2H, d, J = 9.2 Hz), 7.23 (2H, d, J = 9.2 Hz), 7.64 (1H, d, J = 8.5 Hz), 7.82 (1H, d, J = 8.5 Hz), 7.92 (1H, s), 8.03 (1H, d, J = 8.5 Hz), 8.11 (1H, d, J = 8.6 Hz), 8.46 (1H, s), 8.74 (1H, s), 9.20–9.40 (3H, m), 9.48 (2H, s), 13.01 (1H, br-s); FAB Ms m/e $(M+H)^+$ 625. Anal. calcd for $C_{30}H_{36}N_6O_7S\cdot 2.0HCl\cdot 2.6H_2O$: C, 48.40; H, 5.85; N, 11.29; S, 4.31; Cl, 9.52. Found: C, 48.62; H, 5.75; N, 11.37; S, 4.41; Cl, 9.66.

(*E*)-3-Cyanocinnamaldehyde (13). A solution of 3-cyanobenzaldehyde (**12**, 14.53 g, 110.8 mmol) and triphenylphosphoranylidene acetaldehyde (33.72 g, 110.8 mmol) in benzene was refluxed for 16 h. To the reaction mixture was added triphenylphosphoranylidene acetaldehyde (6.74 g, 37.0 mmol) again and the reaction mixture was further refluxed for 19 h. After the reaction mixture was concentrated in vacuo, the crude residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (10:90–30:70) to give **13** (13.49 g, 78%) as a pale yellow solid: mp 101–102 °C; ^{1}H NMR ($CDCl_3$) δ 6.75 (1H, dd, J = 7.3, 16.1 Hz), 7.45 (1H, d, J = 16.1 Hz), 7.57 (1H, t, J = 7.9 Hz), 7.73 (1H, dt, J = 1.1, 7.9 Hz), 7.78–7.84 (2H, m), 9.75 (1H, d, J = 7.3 Hz); EI Ms m/e $(M)^+$ 157.

3-((*E*)-3-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]anilino}propenyl)benzonitrile (14). Compound **14** was synthesized from **2** and **13** according to the same procedure as that for **5.** Compound **14** was obtained as a pale yellow viscous oil (59% yield): ^{1}H NMR (DMSO-d_6) δ 1.46 (9H, s), 1.60–2.00 (4H, m), 3.22–3.31 (2H, m), 3.66–3.76 (2H, m), 3.93 (2H, d, J = 5.4 Hz), 4.22–4.31 (1H, m), 6.40 (1H, dt, 16.1, 5.4 Hz), 6.57–6.64 (3H, m), 6.81 (2H, d, J = 9.3 Hz), 7.41 (1H, t, J = 7.8 Hz), 7.50 (1H, d, J = 7.8 Hz), 7.57 (1H, d, J = 7.8 Hz), 7.76 (1H, s); FAB Ms m/e $(M)^+$ 433.

3-(3-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]anilino}propyl)benzonitrile (15). To a solution of **14** (540 mg, 1.24 mmol) in EtOH (12 mL) was added 10% Pd/C powder (50 mg), the reaction mixture was stirred in a hydrogen atmosphere at ambient temperature for 1 h. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (75:25) to give **15** (334 mg, 62%) as a colorless viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.61–1.78 (2H, m), 1.80–1.99 (4H, m), 2.77 (2H, t, J = 7.8 Hz), 3.11 (2H, t, J = 6.8 Hz), 3.22–3.31 (2H, m), 3.65–3.76 (2H, m), 4.21–4.29 (1H, m), 6.53 (2H, d, J = 8.8 Hz), 6.79 (2H, d, J = 8.7 Hz), 7.39 (1H, t, J = 7.8 Hz), 7.44 (1H, d, J = 7.8 Hz), 7.47–7.52 (2H, m); FAB Ms m/e $(M)^+$ 435.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(*E*)-3-(3-amidinophenyl)allyl]methanesulfonamide (16).** Compound **16** was synthesized from **14** according to the same procedure as that for **8f.** Compound **16** was obtained as a white amorphous powder (9% yield): ^{1}H NMR (DMSO-d_6) δ 1.65–1.81 (2H, m), 1.98–2.10 (2H, m), 2.28 (3H, s), 3.04 (3H, s), 3.40–3.57 (2H, m), 3.66–3.82 (2H, m), 4.42 (2H, d, J = 5.9 Hz), 4.65–4.73 (1H, m), 6.45 (1H, dt, J = 5.9, 16.1 Hz), 6.58 (1H, d, J = 16.1

Hz), 7.01 (2H, d, $J=9.2$ Hz), 7.38, (2H, d, $J=8.8$ Hz), 7.54 (1H, t, $J=7.8$ Hz), 7.66 (1H, d, $J=7.8$ Hz), 7.72 (1H, d, $J=7.8$ Hz), 7.86 (1H, s), 8.64 (1H s), 9.02 (2H, s), 9.18 (1H, s), 9.33 (2H, s); FAB Ms *m/e* $(M+H)^+$ 470. Anal. calcd for $C_{24}H_{31}N_5O_3S{\cdot}2.1HCl{\cdot}1.6H_2O$: C, 50.13; H, 6.36; N, 12.18; S, 5.58; Cl, 12.95. Found: C, 50.05; H, 6.35; N, 12.29; S, 5.68; Cl, 13.09.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl} - *N* - [3 - (3 - amidinophenyl)propyl]methanesulfonamide (17).** Compound **17** was synthesized from **15** according to the same procedure as that for **8f.** Compound **17** was obtained as a white amorphous powder (3% yield): ^{1}H NMR (DMSO-d_6) δ 1.62–1.83 (4H, m), 2.00–2.13 (2H, m), 2.31 (3H, s), 2.63–2.74 (2H, m), 2.95 (3H, s), 3.48–3.88 (6H, m), 4.68–4.77 (1H, m), 7.05 (2H, d, $J=8.8$ Hz), 7.34, (2H, d, $J=8.8$ Hz), 7.47–7.54 (2H, m), 7.60–7.65 (2H, m), 8.79 (1H, s), 9.16 (2H, s), 9.27–9.37 (3H, br); FAB Ms *m/e* $(M+H)^+$ 472. Anal. calcd for $C_{24}H_{33}N_5O_3S{\cdot}2.7HCl{\cdot}2.0H_2O$: C, 47.56; H, 6.60; N, 11.55; S, 5.29; Cl, 15.79. Found: C, 47.79; H, 6.96; N, 11.74; S, 5.53; Cl, 15.70.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}methanesulfonamide (18).** Compound **18** was synthesized from **2** according to the same procedure as that for **6f.** Compound **18** was obtained as a white solid (79% yield): mp 145–146 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.68–1.82 (2H, m), 1.83–1.98 (2H, m), 2.96 (3H, s), 3.28–3.39 (2H, m), 3.63–3.74 (2H, m), 4.39–4.57 (1H, m), 6.44 (1H, s), 6.89 (2H, d, $J=8.8$ Hz), 7.19 (2H, d, $J=8.8$ Hz); FAB Ms *m/e* $(M)^+$ 370.

2-Bromo-*N*-(3-cyanophenyl)acetamide (20). To a stirred solution of 3-aminobenzonitrile (**19**, 2.00 g, 16.9 mmol) and triethylamine (7.12 mL, 51.1 mmol) in 1,2-dichloroethane (20 mL) at 3 °C was added bromoacetyl bromide (2.54 mL, 25.4 mmol). After stirring at ambient temperature for 3 h, the reaction mixture was poured into water and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (1:3–1:2) to give **20** (1.15 g, 28%) as a pale brown solid: mp 124–125 °C; ^{1}H NMR ($CDCl_3$) δ 4.04 (2H, s), 7.44–7.50 (2H, m), 7.70–7.77 (1H, m), 7.97 (1H, s), 8.23 (1H, br-s); FAB Ms *m/e* $(M+1)^+$ 239, 241.

2-(*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N* - (methylsulfonyl)amino)-*N*-(3-cyanophenyl)acetamide (21). To a stirred solution of **18** (1.80 g, 4.8 mmol) and **20** (1.15 g, 4.8 mmol) in acetonitrile (35 mL) at ambient temperature was added K_2CO_3 (750 mg, 5.4 mmol). After the reaction mixture was refluxed for 6 h, it was filtered and concentrated in vacuo. The residue was chromatographed on silica gel eluting with ethyl acetate/*n*-hexane (1:2–1:1) to give **21** (1.24 g, 49%) as a pale brown amorphous powder: ^{1}H NMR ($CDCl_3$) 1.47 (9H, s), 1.67–1.82 (2H, m), 1.83–1.98 (2H, m), 3.05 (3H, s), 3.28–3.40 (2H, m), 3.62–3.73 (2H, m), 4.01–4.50 (3H, m), 6.93 (2H, d, $J=9.3$ Hz), 7.37 (2H, d, $J=8.8$ Hz), 7.42–7.47 (2H, m), 7.65–7.68 (1H, m), 7.98 (1H, s), 8.21 (1H, s); FAB Ms *m/e* $(M-H)^-$ 527.

2-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-(methylsulfonyl)amino)-*N*-(3-amidinophenyl)acetamide (22). Compound **22** was synthesized from **21** according to the same procedure as that for **8f.** Compound **22** was obtained as a white amorphous powder (8% yield): ^{1}H NMR (DMSO-d_6) δ 1.63–1.80 (2H, m), 1.99–2.10 (2H, m), 2.30 (3H, s), 3.13 (3H, s), 3.45–3.60 (2H, m), 3.70–3.82 (2H, m), 4.52 (2H, s), 4.68–4.76 (1H, m), 7.04 (2H, d, $J=9.2$ Hz), 7.42–7.50, (3H, m), 7.54 (1H, t, $J=7.9$ Hz), 7.84 (1H, d, $J=8.6$ Hz), 8.11 (1H s), 9.29 (6H, br-s); FAB Ms *m/e* $(M+H)^+$ 487. Anal. calcd for $C_{23}H_{30}N_6O_4S{\cdot}1.9HCl{\cdot}2.0H_2O$: C, 46.67; H, 6.11; N, 14.20; S, 5.42; Cl, 11.38. Found: C, 47.07; H, 6.18; N, 14.22; S, 4.44; Cl, 11.10.

X-ray crystallographic experiment

Crystals of **8g**/bovine pancreatic trypsin complex were prepared using the same method as reported previously.[33] The X-ray diffraction data were collected with a Rigaku R-AXIS IIc image-plate system. The data set covers 80% of theoretically calculated number of reflections up to 1.85 Å. The structural analysis of the inhibitor complex was achieved by the Patterson search method based on a molecular model of the bovine pancreatic trypsin/NAPAP complex (1ppc.pdb). Model building, electron density calculation, and model refinement were carried out using program O[34] and CNX2000 (Accelrys Inc.). The model has been refined to a crystallographic R-value of 20.8% ($R_{free}=23.5\%$) with good stereochemistry (r.m.s.d. of bonds = 0.006 Å and angles = 1.3° from ideality). We will deposit the crystallographic data for this structure with the Cambridge Crystallographic Data Center after this manuscript is accepted.

Modeling study

Compound **8g** was initially placed in the active site of factor Xa by superimposing the crystal structure of the **8g**/trypsin complex on to that of factor Xa[35] (1 hcg.pdb) using corresponding Ca atoms. The EGF-like domain of factor Xa and some water molecules around the active site were then removed from the model because they seem to have unsuitable contacts with the inhibitor. After manual adjustment of the position of the side chains, energy minimization of the complex model was performed with program DISCOVER (Accelrys Inc.). During the minimization, only **8g**, water molecule and side chain atoms within 10 Å from the inhibitor were allowed to move.

Biology

Chromogenic assay. The hydrolysis rates of synthetic substrates were assayed by continuously measuring absorbance at 405 nm at 37 °C with a microplate reader (Model 3550, Bio-Rad, Richmond, USA). Reaction mixtures (125 μL) were prepared in 96-well plates containing chromogenic substrates and an inhibitor in either 0.05 M Tris–HCl, pH 8.4, 0.15 M NaCl. Reactions were initiated with a 25 μL of enzyme solution. Enzymes and substrates were used as follows: factor Xa and chromogenic Xa; thrombin and S-2238; trypsin and

S-2222. The concentration of an inhibitor required to inhibit enzyme activity by 50% (IC_{50}) was calculated from dose–response curves in which the logit transformation of residual activity was plotted against the logarithm of inhibitor concentration.

Plasma clotting time assay. Prothrombin time (PT) was performed using a KC10A coagulometer (Amelung Co., Lehbringsweg, Germany). Fifty μL of citrated mice pool plasma was incubated for 1 min at 37 °C with 50 μL of diluted compound, followed by the addition of 50 μL of PT reagent (Ortho Diagnostic Systems Co., Tokyo, Japan) to initiate clot formation. The concentration required to double clotting time (CT_2) was estimated from each individual dose–response curve.

Anticoagulant assays in mice. Male ICR mice weighing 20–30 g were fasted overnight. Inhibitors were dissolved in saline and administered orally to the mice at 100 mg/kg using a gastric tube. Several times after oral administration of the inhibitor, blood (0.9 mL) was collected from the abdominal vena cava into syringes containing 0.1 mL of 3.8% citrate, and platelet poor plasma was prepared by centrifugation to measure PT.

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