Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 2597–2610

BIOORGANIC & MEDICINAL CHEMISTRY

Design, Synthesis and Biological Activity of YM-60828 Derivatives: Potent and Orally-Bioavailable Factor Xa Inhibitors Based on Naphthoanilide and Naphthalensulfonanilide Templates

Fukushi Hirayama,[a,*] Hiroyuki Koshio,[a] Tsukasa Ishihara,[a] Susumu Watanuki,[a] Shunichiro Hachiya,[a] Hiroyuki Kaizawa,[a] Takahiro Kuramochi,[a] Naoko Katayama,[a] Hiroyuki Kurihara,[a] Yuta Taniuchi,[b] Kazuo Sato,[c] Yumiko Sakai-Moritani,[a] Seiji Kaku,[a] Tomihisa Kawasaki,[a] Yuzo Matsumoto,[a] Shuichi Sakamoto[a] and Shin-ichi Tsukamoto[a]

[a]*Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan*
[b]*Clinical Development Department, Yamanouchi Pharmaceutical Co., Ltd., 3-17-1 Hasune, Itabashi, Tokyo 174-8612, Japan*
[c]*Project Coordination Department, Yamanouchi Pharmaceutical Co., Ltd., 3-17-1 Hasune, Itabashi, Tokyo 174-8612, Japan*

Received 31 January 2002; accepted 16 March 2002

Abstract—Factor Xa (FXa) is a serine protease which plays a pivotal role in the coagulation cascade. The inhibition of FXa has received great interest as a potential target for the development of new antithrombotic drug. Herein we describe a series of novel 7-amidino-2-naphthoanilide and 7-amidino-2-naphthalensulfonanilide derivatives which are potent FXa inhibitors. These scaffolds are rigid and are allowed to adopt an L-shape conformation which was estimated as the active conformation based on a docking study of YM-60828 with FXa. Optimization of the side chain at the central aniline nitrogen of 7-amidino-2-naphthoanilide has led to several potent and orally active FXa inhibitors. **5h** (YM-169964), the best compound of these series, showed potent FXa inhibitory activity ($IC_{50} = 3.9$ nM) and effectively prolonged prothrombin time by 9.6-fold ex vivo at an oral dose of 3 mg/kg in squirrel monkeys.

Introduction

Intravascular clot formation is an essential factor in a number of cardiovascular diseases such as myocardial infarction, unstable angina, deep vein thrombosis, pulmonary embolism, and ischemic stroke. The interruption of the coagulation cascade is one of the most important strategies for inhibition of clot formation which is, in turn, for prevention and treatment of these thrombotic disorders. Warfarin, an inhibitor of the biosynthesis of the vitamin K-dependent coagulation factor, is currently the only marketed oral anticoagulant agent. However due to its mode of action, warfarin has many clinical problems such as slow onset of action, adverse effect on bleeding and interaction with many drugs and foods; hence, it requires individual dose titration and periodic monitoring.[1] Therefore, there is a serious need to develop orally active anticoagulants that are clinically safe and which require less monitoring.

Factor Xa (FXa) is a trypsin-like serine protease that links the intrinsic and extrinsic coagulation cascade. The primary role of FXa is the proteolytic activation of prothrombin to generate thrombin after forming a prothrombinase complex composed of FXa, factor Va, and calcium on the phospholipid membrane. Blood clotting occurs through conversion of fibrinogen to insoluble fibrin and activating platelets by the generated thrombin. Although thrombin inhibitors have been extensively studied as an anticoagulant,[2] only argatroban[3] has been marketed as a parenteral drug and none of the oral thrombin inhibitors has been launched yet. More recently numerous efforts have been made to discover FXa inhibitors.[4] A FXa inhibitor efficiently interrupts both the intrinsic and extrinsic activation cascade. Moreover, since it affects coagulation specifically but not platelet function in contrast to a thrombin inhibitor, these mechanisms should have a reduced effect on abnormal bleeding. Actually, FXa inhibitors have shown good antithrombotic efficacy in animal models of thrombosis.[5–8] Further, in direct comparisons between Factor Xa inhibitors and thrombin inhibitors, the former were shown to be superior antithrombotic agents.[6,7]

*Corresponding author. Tel.: +81-298-54-1563; fax: +81-298-52-2971; e-mail: hirayaf@yamanouchi.co.jp

0968-0896/02/$ - see front matter
PII: S0968-0896(02)00106-2

At present, FXa inhibitors are regarded as potentially ideal anticoagulants.

In our laboratory, we have also made a continuous study of FXa inhibitors and have reported a potent and orally bioavailable FXa inhibitor YM-60828.[9,10] Furthermore, the utility of a FXa inhibitor was confirmed in a number of thrombosis animal models using YM-60828 and its mesylate salt YM-75466.[7,8] In this paper, we describe the results of our work on the synthesis and SAR of YM-60828 derivatives containing carboxamide and sulfonamide linkers.

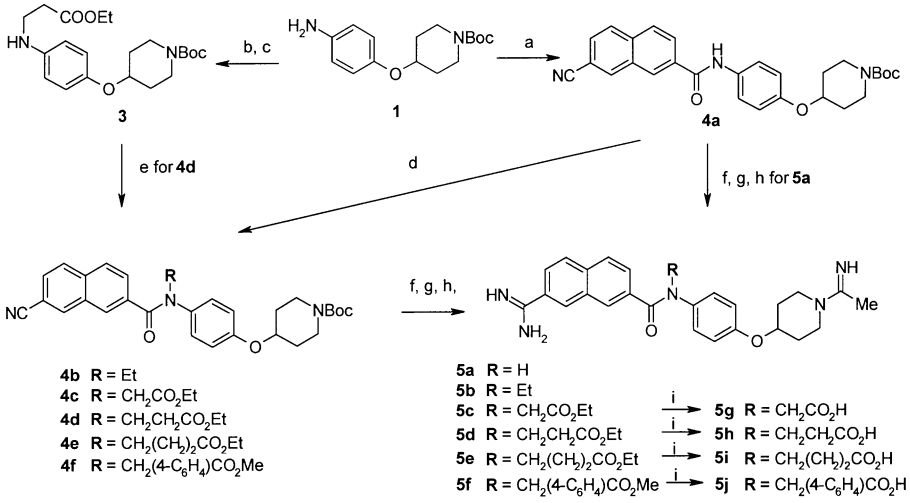
Chemistry

The synthesis of naphthoanilide derivatives **5a**–**5j** is shown in Scheme 1. Key naphthoanilide intermediate **4a** was obtained from condensation of aniline **1**[10] and 7-cyanonaphthalene-2-carboxylic acid (**2**), which was prepared by oxidation of 7-formylnaphthalene-2-carbonitrile.[10] Alkylation of **4a** with various alkyl halides gave *N*-substituted naphthoanilides **4b**, **4c**, **4e** and **4f**. Because the condensation of **4a** and ethyl 3-chloropropionate or ethyl acrylate was not successful, another preparative route to **4d** was employed. Aniline **1** was first coupled with ethyl acrylate to afford *N*-substituted aniline **3** in rather low yield and the nitrogen of piperidine was reprotected by di-*tert*-butyl dicarbonate. This was then condensed with 7-cyanonaphthalene-2-carbonyl chloride to give **4d**. Treatment of the intermediates **4a**–**4f** under Pinner conditions (HCl/MeOH or EtOH) afforded the imidates which were immediately reacted with excess ammonium acetate to give the corresponding mono-amidine derivatives. The *tert*-butoxycarbonyl (BOC) protecting group was simultaneously deprotected to yield a secondary amine under these conditions and then these mono-amidine derivatives were converted to bis-amidines **5a**–**5f** by the reaction with ethyl acetimidate and triethylamine. Hydrolysis of **5c**–**5f** under acidic conditions (HCl/H_2O) gave carboxyl derivatives **5g**–**5j** in good yield.

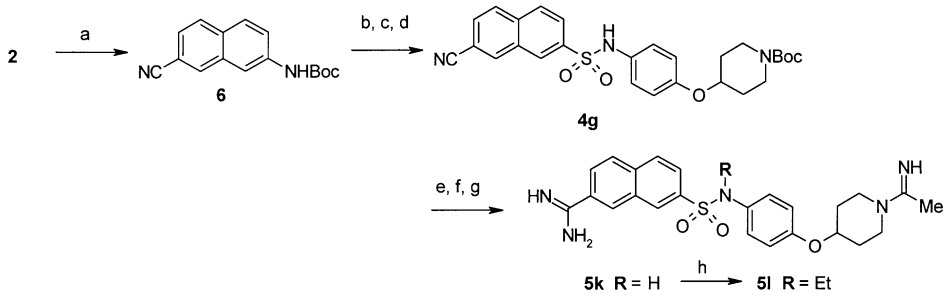
The preparative route to naphthalensulfonanilide derivatives **5k** and **5l** is shown in Scheme 2. Curtius rearrangement of 7-cyanonaphthalene-2-carboxylic acid (**2**) in *tert*-butyl alcohol gave *N*-(*tert*-butoxycarbonyl)amino naphthalene derivative **6**. Deprotection of the BOC group in **6** under acidic conditions, subsequent diazotization with sodium nitrite in concentrated hydrochloric acid generated the diazonium salt, which was then treated with sulfur dioxide and cuprous chloride to give 7-cyanonaphthalen-2-sulfonyl chloride. Condensation of aniline **1** with the sulfonyl chloride afforded naphthalensulfonanilide intermediate **4g**. Subsequent conversion of **4g** to bis-amidine **5k** was accomplished by the same conditions described above. *N*-Ethyl analogue **5l** was prepared from **5k** with ethanol under Mitsunobu condensation conditions (PPh_3, DEAD, THF).

Phenethylsulfonanilide derivative **5m** was also synthesized as shown in Scheme 3 via similar route as naphthalensulfonanilide derivative **5k**. Known phenyl acetaldehyde **7**[11] was reduced by using $NaBH_4$ to phenethyl alchol, which was treated with thionyl chloride to afford phenethyl chloride **8**. It was then reacted with sodium sulfite in water at reflux, followed by treatment with thionyl chloride to provide phenethylsulfonyl chloride. Phenethylsulfonanilide intermediate **9** was obtained by condensation of the sulfonyl chloride and aniline **1** followed by deprotection of the BOC group. Intermediate **9** was carried on following the same methods described above to give bis-amidine **5m**.

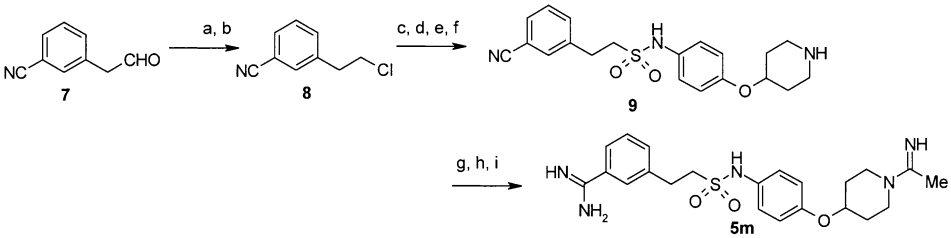
Styrensulfonanilide derivatives **5n** and **5o** were prepared via another methodology outlined in Scheme 4. Aniline **1** was treated with 2-chloro-1-ethanesulfonyl chloride to give unstable vinylsulfonanilide **10**, which was immediately reacted with 3-bromobenzonitrile under standard Heck reaction by heating (140 °C) with palladium(II)



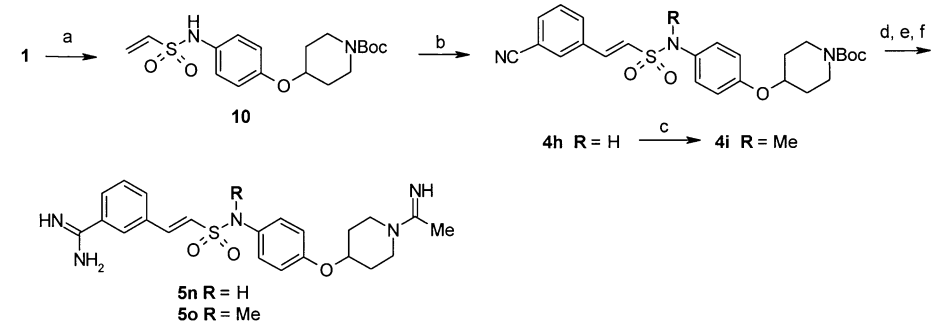
Scheme 1. Synthesis of naphthoanilide derivatives: (a) 7-cyanonaphthalene-2-carboxylic acid (**2**), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, Et_3N, DMF; (b) ethyl acrylate, AcOH; (c) di-*tert*-butyl dicarbonate, $CHCl_3$; (d) EtI for **4b**, RBr for **4c**, **4e**, **4f**, NaH, DMF; (e) **2**, $SOCl_2$, then **3**, pyridine, 4-dimethylaminopyridine, CH_2Cl_2; (f) HCl, EtOH or MeOH; (g) NH_4OAc, EtOH or MeOH; (h) ethyl acetimidate hydrochloride, Et_3N, EtOH or MeOH; (i) HCl, H_2O.



Scheme 2. Synthesis of naphthalensulfonanilide derivatives: (a) diphenylphosphoryl azide, *tert*-butanol, Et_3N; (b) HCl, 1,4-dioxane, EtOAc; (c) $NaNO_2$, HCl, AcOH, H_2O, then SO_2, CuCl; (d) **1**, pyridine, 1,2-dichloroethane; (e) HCl, EtOH; (f) NH_4OAc, EtOH; (g) ethyl acetimidate hydrochloride, Et_3N, EtOH; (h) EtOH, PPh_3, DEAD, THF.



Scheme 3. Synthesis of phenethylsulfonanilide derivative: (a) $NaBH_4$, EtOH (b) $SOCl_2$, CH_2Cl_2; (c) Na_2SO_3, H_2O; (d) $SOCl_2$, benzene; (e) **1**, pyridine, 1,2-dichloroethane; (f) 4 N HCl/EtOAc; (g) HCl, EtOH; (h) NH_4OAc, EtOH; (i) ethyl acetimidate hydrochloride, Et_3N, EtOH.



Scheme 4. Synthesis of styrylsulfonanilide derivatives: (a) 2-chloro-1-ethanesulfonyl chloride, pyridine, DMAP, CH_2Cl_2; (b) PPh_3, $Pd(OAc)_2$, DMF then 3-bromobenzonitrile, **10**, Et_3N; (c) MeI, DMF, K_2CO_3; (d) HCl, EtOH; (e) NH_4OAc, EtOH; (f) ethyl acetimidate hydrochloride, Et_3N, EtOH.

acetate, triphenylphosphine and triethylamine in DMF to yield styrensulfonanilide intermediate **4h**. This was readily *N*-alkylated by methyl iodide and potassium carbonate to give *N*-methylstyrensulfonanilide **4i**. Bisamidine derivatives **5n**, **5o** were obtained from **4h** and **4i**, respectively, by the same manner described above.

Results and Discussion

At the initiation of this program, the docking study of YM-60828 and FXa was performed based on the X-ray crystal structure of it complexed to a related enzyme, trypsin (Fig. 1). This study revealed that YM-60828 takes an L-shape conformation by binding to the FXa active site with three key interactions. First, the naphthamidine moiety is in the S_1 specificity pocket and forms a hydrogen bond network to Asp-189 and Gly-218. There is also a water-mediated hydrogen bond to the carbonyl oxygen of Ile-227. Second, the piperidine group sits in the aryl-binding pocket defined by the three aromatic amino acids Phe-174, Tyr-99 and Trp-215. Third, the acetimidoyl group forms a water-mediated hydrogen bond to the carbonyl oxygen atoms of Thr-98 and Ile-175. On the other hand, little binding contribution was observed for the side chain sulfonylacetic acid moiety which is near the solvent accessible surface at the outer ridge of the active site and is weakly hydrogen bonded to Gln-192. We designed to shift the side chain carboxamide or sulfonamide bond in the series of YM-60828 derivatives[10] to the central anilinomethylnaphthalene linkage (Fig. 2). These transformations afforded naphthoanilide and naphthalensulfonanilide templates as the rigid scaffold that would be able to adopt the L-shape conformation predominantly. Then the structural modification of these two templates was performed to discover some novel, potent and orally bioavailable FXa inhibitors.

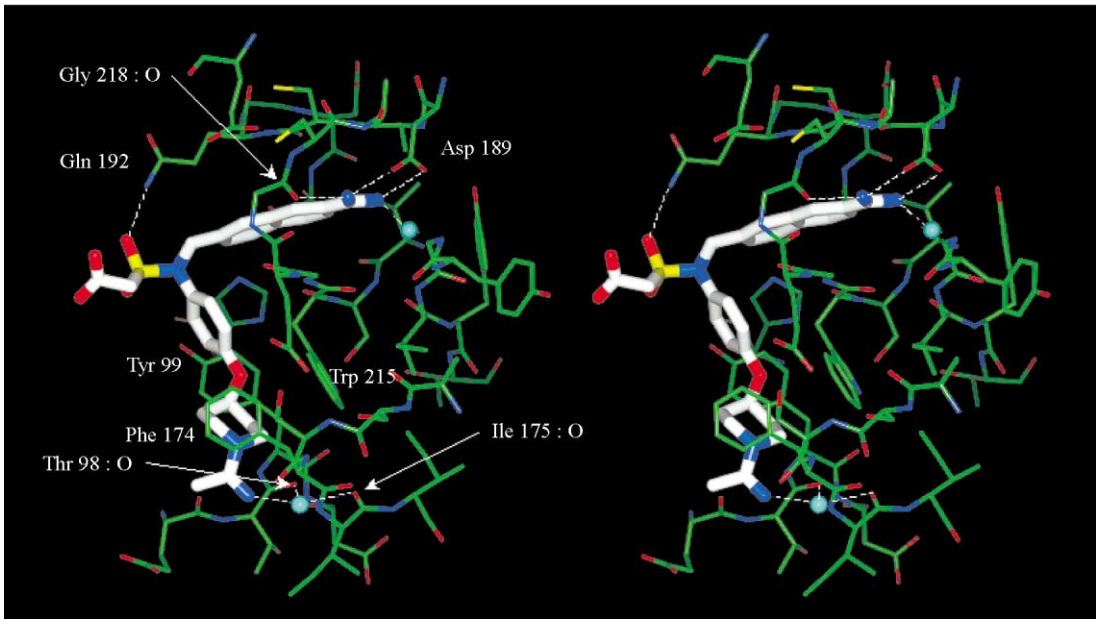


Figure 1. Binding model of YM-60828 to factor Xa.

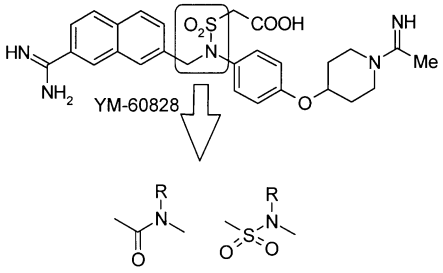
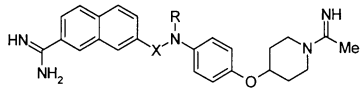


Figure 2.

Table 1 shows evaluations of the templates which contain three types of linkers such as carboxamide (**5a** and **5b**), sulfonamide (**5k** and **5l**) and aminomethylene (**5r**[10] and **5s**). It was noteworthy that the effects of the *N*-ethyl substituent (R) on central aniline nitrogen were quite different depending on the structure of its linkers. In the naphthylmethylaniline derivatives **5r** and **5s**, FXa inhibitory activities were not greatly affected by existence of the ethyl substituent. This result was considered to be reasonable because the modeling experiments of YM-60828 bound with FXa (Fig. 1) suggested that the *N*-alkyl moiety was directed to the solution and made little contact with the enzyme. On the other hand, the ethyl substituent strongly influenced the FXa inhibitory activities of the naphthoanilide and naphthalensulfonanilide derivatives. Unsubstituted naphthoanilide derivative **5a** showed a marked decrease in FXa inhibitory activity compared to that of the ethyl substituted analogue **5b**. In contrast, unsubstituted naphthalensulfonanilide derivative **5k** posessed more potent FXa inhibitory activity than that of the ethyl substituted analogue **5l**. Based on the modeling study of YM-60828 bound with FXa (Fig. 1), naphthalene and benzene ring oriented with *syn* geometry in the active site (L-form). On the other hand, it has been known that *N*-methylbenzanilide exists in the *cis*-amide conformation while unsubstituted benzanilide exists in the *trans*-amide conformation in both crystal and solvent.[12] From these findings, it was considered that *N*-ethylnaphthoanilide **5b** existed predominantly in the *cis*-amide conformation that resulted in its taking the active conformation. But the unsubstituted analogue **5a** was almost locked into the inactive *trans*-amide conformation. The quite different potencies observed between **5a** and **5b** were probably due to switching between these two different conformations. This discussion was supported by the NMR study of **5a** and **5b** in D_2O, in which NOE was observed between protons on naphthalene and benzene in **5b**, but was not observed in **5a** (Fig. 3). Similarly, unsubstituted naphthalensulfonanilide **5k** could exist predominantly in the active *cis* conformation and the ethyl-substituted analogue **5l** took another inactive *trans* conformation in contrast to carboxamide derivatives **5a** and **5b**. Actually, X-ray crystallographic analysis of some benzensulfonanilide derivatives has reported them to exsist as not in a planar conformation.[13]

Table 1. Effect of the ethyl substituent in carboxamide, sulfonamide and aminomethylene derivatives



Compd	R	X	IC_{50} (nM)[a]		
			Factor Xa	Thrombin	Trypsin
5a	H	CO	2242.5	>100,000	26,390
5b	Et	CO	9.1	>100,000	210
5k	H	SO_2	11.7	>100,000	230
5l	Et	SO_2	181.1	>100,000	5248
5r	H	CH_2	19.5[b]	>100,000	278[b]
5s	Et	CH_2	25.1	>100,000	235

[a]Human purified enzymes were used. IC_{50} values represent the average of three determinations with the average standard error of the mean <20%.
[b]Determined by duplicated ($n=1$).

Potent FXa inhibitors **5b** and **5k** showed high selectivity (>5000-fold) over thrombin while the selectivity over trypsin was moderate (ca. 20-fold). These two compounds were identified as novel leads for optimization. The data in Table 2 summarize the derivatives of naphthalensulfonanilide **5k**. In addition to the IC_{50} values, CT_2 values of the human plasma prothrombin time (PT) were also tabulated. The CT_2 value is defined as the concentration required to double clotting time. Moreover oral efficacy was also evaluated by prolongation of PT ex vivo after oral administration in mice. Although the potency observed with **5k** in vitro IC_{50} of FXa was well translated into potent activity in vitro PT assays, ex vivo activity following oral dosing was poor. Replacement of the naphthalene in **5k** with a styrene (**5n**) retained the in vitro FXa inhibitory activity and increased the selectivity against trypsin (72-fold), but phenethyl derivative **5m** decreased the FXa inhibitory activity. Following the same trend as for the naphthalensulfonanilide derivatives, the *N*-alkylation of styrensulfonanilide (**5o**) caused a decrease in the activity. Regioisomers of **5n** were also evaluated and the *para*-substituted amidinostyryl derivative **5p** showed poor FXa inhibitory activity, in contrast, the *meta*-substituted central benzene derivative **5q** had comparable FXa inhibitory activity and selectivity over trypsin compared with that of **5n**. Although these two styrensulfonanilide derivatives **5n** and **5q** showed potent and selective FXa inhibitory activities and also have potent PT CT_2 values, the efficacies after oral adminis-

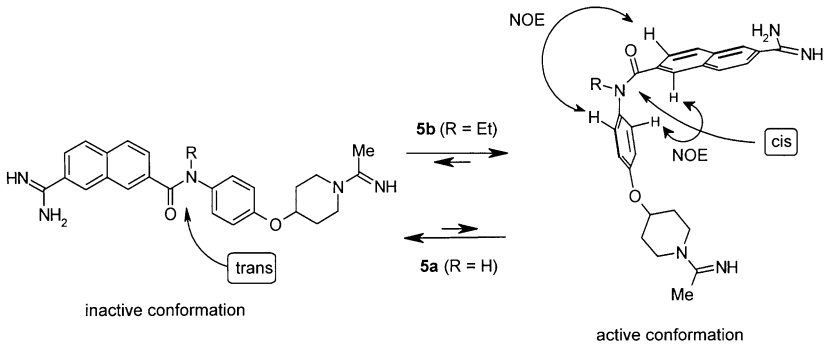
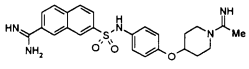
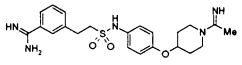
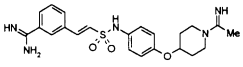
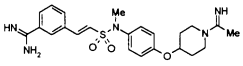
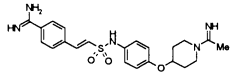
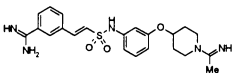


Figure 3.

Table 2. SAR of sulfonamide derivatives

Compd	R	IC_{50} (nM)[a]			CT_2 (μM)[b] PT[c]	PT/control PT[d] mice
		Factor Xa	Thrombin	Trypsin		
5k		11.7	>100,000	230	0.23	1.2
5m		48.7	>100,000	1496	0.49	1.2
5n		12.0	>100,000	865	0.23	1.7
5o		137.3	>100,000	5421	NT[e]	NT[e]
5p		1764.1	>100,000	17,508	NT[e]	NT[e]
5q		19.6	>100,000	1861	0.20	1.3
	YM-60828	2.3	>100,000	216	0.21[f]	2.6

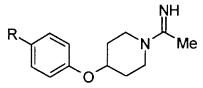
[a]Refer to Table 1.
[b]Values represent the concentration required to double clotting time and represent the average of four determinations with the average standard error of the mean <20%.
[c]Prothrombin time using human plasma.
[d]The relative prothrombin time compared with that measured using normal mice plasma at 0.5 h after oral administration (100 mg/kg, $n=3$).
[e]Not tested.
[f]$n=3$.

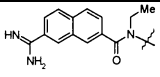
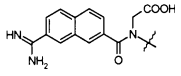
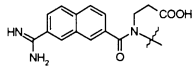
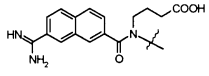
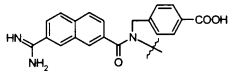
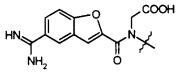
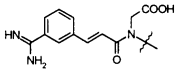
tration were not good. Among them **5n** demonstrated modest oral efficacy ex vivo (prolong PT by 1.7-fold).

The results of SAR studies for the derivatives of **5b** are shown in Table 3. Unfortunately, ex vivo evaluation of **5b** after oral administration in mice failed because six of the 12 mice were dead within 10 min after dosing at 100 mg/kg. This acute lethal toxicity, probably caused by hypotension, has been known as a side effect for strong basic amidine and guanidine compounds such as a thrombin inhibitor[14] and thrombin receptor antagonist.[15] To reduce the basicity of the compound, the ethyl moiety (R) in **5b** was replaced with the acetic acid moiety. The resulting compound **5g** slightly increase the inhibitory activity for FXa and reduce the toxicity, with none of the lethal adverse reaction after oral administration (100 mg/kg) in mice. Furthermore, the previous study on YM-60828 demonstrated that the compounds which contain a carboxyl group had a tendency to have potent ex vivo activity after oral administration in mice.[10] Because of these two desirable effects of a carboxyl group, derivatives bearing the carboxyl moiety were synthesized and evaluated.

Replacement of naphthalene in **5g** with benzofuran (**5t**) caused a large decrease in potency and replacement with styrene (**5u**) was also not well tolerated in contrast to the sulfonamide derivative (**5k** vs **5n**). Extension of the acetic acid moiety in **5g** with methylene (**5h** and **5i**) and phenylene (**5j**) maintained the inhibitory activity against FXa. These SAR may indicate that there is a wide space in this area and naphthoanilide derivatives have the same binding conformation as that of YM-60828. All strong FXa inhibitory potencies of this series of compounds were well translated into submicromolar anticoagulant activities in vitro PT assay (CT_2). The potencies were the same or slightly less than that of YM-60828. The efficacy after oral administration of these compounds was evaluated by prolongation of PT ex vivo in mice and squirrel monkeys. In mice, all compounds (**5g–5j**) showed quite potent activity, which prolonged PT more than 2-fold both at 0.5 h and 2.0 h. In contrast, there was wide variation in efficacy of the test in squirrel monkeys (Fig. 4). Compounds **5g** and **5i** had same or slightly higher efficacy than that of YM-75466 (methanesulfonate salt of YM-60828), but the phenylene insertion compound **5j** showed poor efficacy.

Table 3. SAR of carboxamide derivatives



Compd	R	IC_{50} (nM)[a]			CT_2 (μM)[b] PT[c]	PT/control PT[d]	
		Factor Xa	Thrombin	Trypsin		0.5 h	2.0 h
5b		9.1	> 100,000	210	0.35	ND[g]	ND[g]
5g		4.2	> 100,000	391	0.22	3.7	2.7
5h		3.9	> 100,000	291	0.29	3.1	2.5
5i		3.5	> 100,000	314	0.27	2.9	2.6
5j		2.7	> 100,000	194	0.26	3.2	2.6
5t		1626.8	> 100,000	48,144	NT[e]	NT[e]	NT[e]
5u		101.6	> 100,000	9099	NT[e]	NT[e]	NT[e]
	YM-60828	2.3	> 100,000	216	0.21[f]	2.6	1.7

[a]Refer to Table 1.
[b]Values represent the concentration required to double clotting time and represent the average of four determinations with the average standard error of the mean < 20%.
[c]Prothrombin time using human plasma.
[d]The relative prothrombin time compared with that measured using normal mice plasma at 0.5 h and 2 h after oral administration in mice (100 mg/kg, $n = 3$).
[e]Not tested.
[f]$n = 3$.
[g]Not determined.

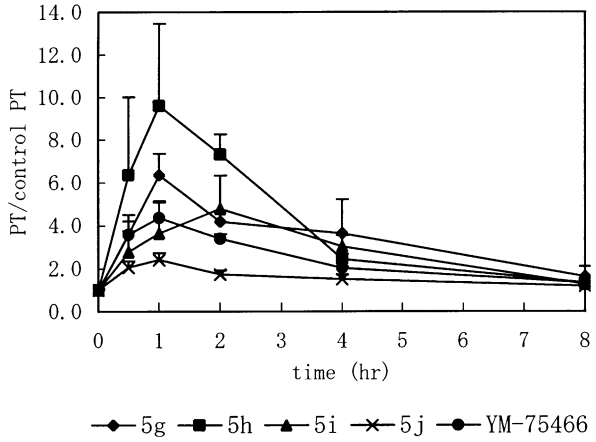


Figure 4. Anticoagulant activity of selected carboxamide derivatives and YM-75466 (methanesulfonate salt of YM-60828) after oral administration at 3 mg/kg in squirrel monkeys. The relative prothrombin time compared with that measured using normal plasma were determined in blood samples taken at the indicated time points after administration (±SEM, $n=3$).

Among them, the best compound was **5h** (YM-169964), which remarkably prolonged PT by 9.6-fold at 1.0 h. **5h** was further evaluated in pharmacokinetic study following oral administration in dogs (10 mg/kg, $n=3$). It demonstrated good profiles with C_{max} (2429 ng/mL), AUC (10246 ng h/mL) and T_{max} (0.8 h).

Conclusion

We have designed and synthesized a series of naphthoanilide and naphthalensulfonanilide series of compounds as FXa inhibitors. Among these compounds, naphthoanilide derivatives (**5g–j**) showed low nanomolar potency in FXa inhibitory activities, and potent ex vivo activities after oral administration in mice. The best compound was **5h** (YM-169964) which had excellent ex vivo activity following oral dosing in mice and squirrel monkeys and good pharmacokinetic profiles in dogs. Subsequent communications from our laboratories will describe further results of the investigation of related scaffolds.

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. Melting points were measured with a Yanaco MP-500D melting point apparatus without correction. ODS column chromatography was performed on YMC gel (ODS-A 120–230/70).

7-Cyanonaphthalene-2-carboxylic acid (2). To a stirred suspension of 7-formylnaphthalene-2-carbonitrile[10] (10.00 g, 55.2 mmol) in acetonitrile (250 mL) and H_2O (250 mL) at ambient temperature was added sodium chlorite (purity 80%, 31.20 g, 276 mmol). After stirring under reflux for 20 h, the reaction mixture was allowed to reach ambient temperature and 0.7 N aqueous NaOH (700 mL) was added. The mixture was washed with ethyl acetate and the aqueous solution was acidified with 12 N HCl. The resulting precipitate was filtered, washed with H_2O and dried in vacuo to give **2** (10.08 g, 92%) as a white solid: mp 276–278 °C; ^{1}H NMR (DMSO-d_6) δ 7.91 (1H, dd, $J=1.5$, 8.3 Hz), 8.10–8.22 (3H, m), 8.72 (1H, s), 8.79 (1H, s); FAB MS m/e $(M-1)^-$ 196.

Ethyl 3-{4-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]anilino}propionate (3). A solution of *tert*-butyl 4-(4-aminophenoxy)piperidine-1-carboxylate (**1**) (4.07 g, 13.9 mmol)[10] and ethyl acrylate (1.39 g, 13.9 mmol) in acetic acid (28 mL) was stirred at 100 °C for 12 h. After the reaction mixture was concentrated in vacuo, to the resulting residue was added $CHCl_3$, which was washed with 10% aqueous K_2CO_3, dried over Na_2SO_4 and concentrated in vacuo. To a stirred solution of the residue in $CHCl_3$ (28 mL) at ambient temperature was added di-*tert*-butyl dicarbonate (1.53 g, 7.0 mmol). After the reaction mixture was concentrated in vacuo, the resulting residue was chromatographed on silica gel eluting with ethyl acetate (EtOAc)/*n*-hexane(Hex) (17:83) to give **3** (700 mg, 13%) as a pale brown viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, $J=6.9$ Hz), 1.43 (9H, s), 1.61–1.71 (2H, m), 1.79–1.89 (2H, m), 2.56 (2H, t, $J=6.3$ Hz), 3.19–3.28 (2H, m), 3.36 (2H, t, $J=6.3$ Hz), 3.63–3.72 (2H, m), 4.12 (2H, q, $J=6.9$ Hz), 4.18–4.26 (1H, m), 6.54 (2H, d, $J=4.0$ Hz), 6.76 (2H, d, $J=9.0$ Hz); FAB MS m/e $(M+1)^+$ 393.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-7-cyano-2-naphthamide (4a).** To a stirred solution of 7-cyanonaphthalene-2-carboxylic acid (**2**) (1.5 g, 7.6 mmol) and *tert*-butyl 4-(4-aminophenoxy)piperidine-1-carboxylate (**1**) (2.2 g, 7.6 mmol) in *N*,*N*-dimethylformamide (DMF) (30 mL) at ambient temperature was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.75 g, 9.1 mmol), 1-hydroxybenzotriazole (1.03 g, 9.1 mmol) and triethylamine (Et_3N) (1.26 mL, 9.1 mmol). After stirring at ambient temperature for 24 h, the reaction mixture was filtered and concentrated in vacuo. The resulting residue was crystallized from ethanol (EtOH) to give **4a** (2.78 g, 78%) as a white solid: mp 199–201 °C; ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s), 1.70–1.80 (2H, m), 1.85–1.98 (2H, m), 3.28–3.40 (2H, m), 3.65–3.70 (2H, m), 4.41–4.50 (1H, m), 6.94 (2H, d, $J=8.8$ Hz), 7.59 (2H, d, $J=8.8$ Hz), 7.71 (1H, d, $J=9.0$ Hz), 7.94–8.03 (2H, m), 8.06–8.15 (2H, m), 8.29 (1H, s), 8.41 (1H, s); FAB MS m/e $(M)^+$ 471.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-7-cyano-*N*-ethyl-2-naphthamide (4b).** To a stirred solution of **4a** (707 mg, 1.5 mmol) in DMF (10 mL) at ambient temperature was added 60% NaH in paraffin liquid (120 mg, 3.0 mmol). After 20 min ethyl iodide (0.36 mL, 4.5 mmol) was added to the reaction mixture and stirred for 1 h at ambient temperature. The reaction was

quenched by the addition of aqueous NH_4Cl and concentrated in vacuo. To the resulting residue was added H_2O followed by extracting with $CHCl_3$. The organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/Hex (30:70) to give **4b** (663 mg, 89%) as a white amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, J = 6.8 Hz), 1.45 (9H, s), 1.59–1.73 (2H, m), 1.78–1.89 (2H, m), 3.21–3.31 (2H, m), 3.60–3.70 (2H, m), 3.99 (2H, q, J = 6.8 Hz), 4.29–4.37 (1H, m), 6.73 (2H, d, J = 8.8 Hz), 6.97 (2H, d, J = 8.8 Hz), 7.49 (1H, d, J = 8.3 Hz), 7.59 (1H, dd, J = 1.4, 8.3 Hz), 7.65 (1H, d, J = 8.3 Hz), 7.81 (1H, d, J = 8.3 Hz), 7.89 (1H, s), 8.09 (1H, s); FAB MS m/e $(M+1)^+$ 500.

Ethyl *N*-{4-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-(7-cyano-2-naphthoyl)glycinate (4c). Compound **4c** was synthesized from **4a** and ethyl bromoacetate according to the same procedure as that for **4b**. Compound **4c** was obtained as a white amorphous powder (98% yield): ^{1}H NMR ($CDCl_3$) δ 1.32 (3H, t, J = 6.9 Hz), 1.45 (9H, s), 1.57–1.72 (2H, m), 1.76–1.89 (2H, m), 3.20–3.32 (2H, m), 3.57–3.71 (2H, m), 4.26 (2H, q, J = 6.9 Hz), 4.28–4.38 (1H, m), 4.60 (2H, s), 6.72 (2H, d, J = 9.0 Hz), 7.10 (2H, d, J = 9.0 Hz), 7.53 (1H, d, J = 8.4 Hz), 7.60 (1H, dd, J = 1.5, 8.4 Hz), 7.67 (1H, d, J = 8.7 Hz), 7.82 (1H, d, J = 8.4 Hz), 7.95 (1H, s), 8.10 (1H, s); FAB MS m/e $(M+1)^+$ 558.

Ethyl 3-(*N*-{4-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-(7-cyano-2-naphthoyl)amino)propionate (4d). A suspended solution of 7-cyano-2-naphthalenecarboxylic acid (**1**) (246 mg, 1.2 mmol) in thionyl chloride (2.5 mL) was stirred at 90 °C for 4 h. The reaction mixture was concentrated in vacuo, and to a suspended solution of the resulting residue in CH_2Cl_2 (1.3 mL) was added a solution of **3** (540 mg, 1.4 mmol) in pyridine (14 mL) and 4-dimethylaminopyridine (17 mg). The reaction mixture was stirred at 60 °C for 12 h and concentrated in vacuo. The resulting residue was dissolved in $CHCl_3$ and washed with 10% aqueous K_2CO_3. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel eluting with EtOAc/Hex (40:60) to give **4d** (450 mg, 66%) as a white solid: mp 115–116 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.2 Hz), 1.45 (9H, s), 1.59–1.74 (2H, m), 1.78–1.90 (2H, m), 2.72 (2H, t, J = 7.2 Hz), 3.22–3.31 (2H, m), 3.58–3.72 (2H, m), 4.10 (2H, q, J = 7.2 Hz), 4.23 (2H, t, J = 7.2 Hz), 4.29–4.39 (1H, m), 6.73 (2H, d, J = 8.7 Hz), 7.00 (2H, d, J = 8.7 Hz), 7.47 (1H, dd, J = 1.5, 8.4 Hz), 7.59 (1H, dd, J = 1.5, 8.4 Hz), 7.65 (1H, d, J = 8.4 Hz), 7.81 (1H, d, J = 8.4 Hz), 7.89 (1H, s), 8.09 (1H, s); FAB MS m/e $(M+1)^+$ 572.

Ethyl 4-(*N*-{4-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-(7-cyano-2-naphthoyl)amino)buthylate (4e). Compound **4e** was synthesized from **4a** and ethyl 4-bromobutylate according to the same procedure as that for **4b**. Compound **4e** was obtained as a pale yellow viscous oil (17% yield): ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.2 Hz), 1.45 (9H, s), 1.59–1.73 (2H, m), 1.77–1.88 (2H, m), 2.03 (2H, quintet, J = 7.2 Hz), 2.43 (2H, t, J = 7.5 Hz), 3.21–3.33 (2H, m), 3.58–3.71 (2H, m), 3.97 (2H, t, J = 7.2 Hz), 4.13 (2H, q, J = 7.2 Hz), 4.29–4.37 (1H, m), 6.72 (2H, d, J = 8.6 Hz), 6.98 (2H, d, J = 8.6 Hz), 7.47 (1H, d, J = 8.4 Hz), 7.59 (1H, dd, J = 1.8, 8.4 Hz), 7.65 (1H, d, J = 9.0 Hz), 7.81 (1H, d, J = 8.4 Hz), 7.88 (1H, s), 8.01 (1H, s); FAB MS m/e $(M)^+$ 585.

Methyl 4-[(*N*-{4-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]phenyl}-*N*-(7-cyano-2-naphthoyl)amino)methyl]benzoate (4f). Compound **4f** was synthesized from **4a** and methyl 4-(bromomethyl)benzoate according to the same procedure as that for **4b**. Compound **4e** was obtained as a white amorphous powder (95% yield): ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.55–1.68 (2H, m), 1.77–1.86 (2H, m), 3.19–3.31 (2H, m), 3.58–3.71 (2H, m), 3.89 (3H, s), 4.25–4.35 (1H, m), 5.19 (2H, s), 6.66 (2H, d, J = 8.7 Hz), 6.85 (2H, d, J = 8.7 Hz), 7.43 (2H, d, J = 8.7 Hz) 7.53 (1H, d, J = 8.4 Hz), 7.57 (1H, dd, J = 1.5, 8.4 Hz), 7.66 (1H, d, J = 8.4 Hz), 7.80 (1H, d, J = 8.4 Hz), 7.94 (1H, s), 8.00 (2H, d, J = 8.7 Hz), 8.08 (1H, s); FAB MS m/e $(M)^+$ 620.

General procedure for conversion to bis-amidine derivatives. *N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-7-amidino-*N*-ethyl-2-naphthamide (5b). HCl gas was bubbled through a solution of **4b** (606 mg, 1.2 mmol) in EtOH (10 mL) under −20 °C for 20 min. The mixture was allowed to stir for 24 h at 5 °C, and then concentrated in vacuo. To the crude imidate dissolved in EtOH (10 mL) at ambient temperature was added ammonium acetate (931 mg, 12.1 mmol). The reaction mixture was stirred at ambient temperature for 24 h and concentrated in vacuo. The resulting residue was chromatographed on ODS-gel eluting with methanol (MeOH)/H_2O (0:100–10:90). MeOH was removed in vacuo, and the aqueous solution was lyophilized after being acidified with 1 N HCl to give **7-amidino-*N*-ethyl-*N*-[4-(4-piperidyloxy)phenyl]-2-naphthamide** as a white amorphous powder (527 mg): ^{1}H NMR (DMSO-d_6) δ 1.14 (3H, t, J = 7.4 Hz), 1.63–1.80 (2H, m), 1.93–2.08 (2H, m), 2.88–3.05 (2H, m), 3.05–3.20 (2H, m), 3.73–3.98 (2H, m), 4.45–4.60 (1H, m), 6.86 (2H, d, J = 8.3 Hz), 7.13 (2H, d, J = 8.3 Hz), 7.48 (1H, d, J = 7.3 Hz), 7.79–7.90 (2H, m), 8.00–8.10 (2H, m), 8.43 (1H, s), 9.09 (2H, br-s), 9.30 (2H, s), 9.54 (2H, s); FAB MS m/e $(M+1)^+$ 417.

To a stirred solution of the mono-amidine intermediate (481 mg) in EtOH (10 mL) and MeOH (10 mL) at ambient temperature was added ethyl acetimidate hydrochloride (847 mg, 6.9 mmol) and Et_3N (893 mg, 8.8 mmol). The mixture was allowed to stir for 24 h 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 being acidified with 1N HCl. **5b** (430 mg, 67%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.2 Hz), 1.55–1.72 (2H, m), 1.89–2.03 (2H, m), 2.27 (3H, s), 3.38–3.55 (2H, m), 3.61–3.81 (2H, m), 3.81–3.95 (2H, m), 4.53–4.63 (1H, m), 6.86 (2H, d, J = 7.8 Hz), 7.14 (2H, d,

$J = 7.8$ Hz), 7.42–7.54 (1H, m), 7.80–7.90 (2H, m), 8.03–8.10 (2H, m), 8.43 (1H, s), 8.75 (1H, s), 9.27 (3H, s), 9.52 (2H, s); FAB MS m/e $(M+1)^+$ 458. Anal. calcd for $C_{27}H_{31}N_5O_2 \cdot 2.1HCl \cdot 2.5H_2O$: C, 55.99; H, 6.63; N, 12.09; Cl, 12.85. Found: C, 56.07; H, 6.64; N, 12.22; Cl, 13.02.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-7-amidino-2-naphthamide (5a).** Compound **5a** was synthesized from **4a** according to the same procedure as that for **5b**. Compound **5a** was obtained as a white amorphous powder (41% yield): ^{1}H NMR (DMSO-d_6) δ 1.70–1.85 (2H, m), 2.00–2.14 (2H, m), 2.30 (3H, s), 3.48–3.62 (2H, m), 3.70–3.85 (2H, m), 4.65–4.73 (1H, m), 7.05 (2H, d, $J = 9.2$ Hz), 7.76 (2H, d, $J = 9.2$ Hz), 7.94 (1H, dd, $J = 8.4$, 1.6 Hz), 8.18–8.28 (3H, m), 8.60–8.70 (2H, m), 8.72 (1H, s), 9.20 (3H, s), 9.55 (2H, s), 10.48 (1H, s); FAB MS m/e $(M+1)^+$ 430. Anal. calcd for $C_{25}H_{27}N_5O_2 \cdot 2.1HCl \cdot 3.3H_2O$: C, 53.10; H, 6.36; N, 12.38; Cl, 13.16. Found: C, 53.07; H, 6.12; N, 12.36; Cl, 13.45.

Ethyl *N*-{4-[(1-acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-(7-amidino-2-naphthoyl)glycinate (5c). Compound **5c** was synthesized from **4c** according to the same procedure as that for **5b**. Compound **5c** was obtained as a white amorphous powder (40% yield): ^{1}H NMR (DMSO-d_6) δ 1.24 (3H, t, $J = 7.2$ Hz), 1.53–1.71 (2H, m), 1.88–2.02 (2H, m), 2.27 (3H, s), 3.38–3.55 (2H, m), 3.62–3.82 (2H, m), 4.17 (2H, q, $J = 7.2$ Hz), 4.53–4.63 (3H, m), 6.86 (2H, d, $J = 8.4$ Hz), 7.17 (2H, d, $J = 8.4$ Hz), 7.45 (1H, d, $J = 8.4$ Hz), 7.82–7.92 (2H, m), 8.05–8.12 (2H, m), 8.48 (1H, s), 8.78 (1H, s), 9.31 (3H, s), 9.54 (2H, s); FAB MS m/e $(M+1)^+$ 516. Anal. calcd for $C_{29}H_{33}N_5O_4 \cdot 1.6HCl \cdot 3.0H_2O$: C, 55.47; H, 6.52; N, 11.15; Cl, 9.03. Found: C, 55.29; H, 6.43; N, 11.13; Cl, 9.05.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-(7-amidino-2-naphthoyl)glycine (5g).** A solution of **5c** (170 mg, 0.27 mmol) in 3 N HCl (50 mL) was stirred at 80 °C for 2 h. The reaction mixture was concentrated in vacuo and the residue was chromatographed on ODS-gel eluting with MeOH/H_2O (0:100–10:90). MeOH was removed in vacuo, and the aqueous solution was lyophilized after being acidified with 1 N HCl. **5g** (135 mg, 81%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.55–1.73 (2H, m), 1.88–2.05 (2H, m), 2.26 (3H, s), 3.40–3.55 (2H, m), 3.61–3.78 (2H, m), 4.49–4.62 (3H, m), 6.85 (2H, d, $J = 7.8$ Hz), 7.16 (2H, d, $J = 7.8$ Hz), 7.40–7.49 (1H, m), 7.81–7.90 (2H, m), 8.05–8.12 (2H, m), 8.46 (1H, s), 8.69 (1H, s), 9.17–9.25 (3H, m), 9.49 (2H, s), 12.75–13.00 (1H, br); FAB MS m/e $(M+1)^+$ 488. Anal. calcd for $C_{27}H_{29}N_5O_4 \cdot 2.4HCl \cdot 2.2H_2O$: C, 52.76; H, 5.87; N, 11.36; Cl, 13.84. Found: C, 53.09; H, 6.13; N, 11.60; Cl, 13.88.

Ethyl 3-(*N*-{4-[(1-acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-(7-amidino-2-naphthoyl)amino)propionate (5d). Compound **5d** was synthesized from **4d** according to the same procedure as that for **5b**. Compound **5d** was obtained as a white amorphous powder (59% yield): ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, $J = 7.3$ Hz), 1.55–1.70 (2H, m), 1.89–2.01 (2H, m), 2.25 (3H, s), 2.63 (2H, t, $J = 6.8$ Hz), 3.47–3.55 (2H, m), 3.63–3.70 (1H, m), 3.76–3.84 (1H, m), 4.01 (2H, q, $J = 7.3$ Hz), 4.11 (2H, t, $J = 6.8$ Hz), 4.56–4.63 (1H, m), 6.87 (2H, d, $J = 8.3$ Hz), 7.16 (2H, d, $J = 8.6$ Hz), 7.46 (1H, d, $J = 7.3$ Hz), 7.85–7.89 (2H, m), 8.02–8.08 (2H, m), 8.49 (1H, s), 8.91 (1H, s), 9.45 (3H, s), 9.63 (2H, s); FAB MS m/e $(M+1)^+$ 530. Anal. calcd for $C_{30}H_{35}N_5O_4 \cdot 2.1HCl \cdot 2.5H_2O$: C, 55.33; H, 6.52; N, 10.75; Cl, 11.43. Found: C, 55.65; H, 6.30; N, 10.46; Cl, 11.26.

3-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-(7-amidino-2-naphthoyl)amino)propionic acid (5h). A solution of **5d** (705 mg, 1.1 mmol) in 12 N HCl (30 mL) was stirred at ambient temperature for 5 h. The reaction mixture was concentrated in vacuo and the residue was chromatographed on ODS-gel eluting with CH_3CN/H_2O (0:100–10:90). CH_3CN was removed in vacuo, and the aqueous solution was lyophilized after being acidified with 1 N HCl. **5h** (592 mg, 87%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.55–1.70 (2H, m), 1.89–2.00 (2H, m), 2.28 (3H, s), 2.54–2.61 (2H, m), 3.39–3.55 (2H, m), 3.63–3.70 (1H, m), 3.76–3.83 (1H, m), 4.03–4.08 (2H, m), 4.55–4.62 (1H, m), 6.86 (2H, d, $J = 7.8$ Hz), 7.17 (2H, d, $J = 7.8$ Hz), 7.44–7.50 (1H, m), 7.84–7.88 (2H, m), 8.02–8.08 (2H, m), 8.47 (1H, s), 8.87 (1H, s), 9.41 (3H, s), 9.60 (2H, s); FAB MS m/e $(M+1)^+$ 502. Anal. calcd for $C_{28}H_{31}N_5O_4 \cdot 2.4HCl \cdot 1.5H_2O$: C, 54.59; H, 5.95; N, 11.37; Cl, 13.81. Found: C, 54.68; H, 6.03; N, 11.15; Cl, 13.86.

4-(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-(7-amidino-2-naphthoyl)amino)butyric acid (5i). Compound **5i** was synthesized from **4e** according to the same procedure as that for **5h**. Compound **5i** was obtained as a white amorphous powder (55% yield): ^{1}H NMR (DMSO-d_6) δ 1.53–1.71 (2H, m), 1.72–1.83 (2H, m), 1.88–2.02 (2H, m), 2.27 (3H, s), 2.27–2.37 (2H, m), 3.37–3.54 (2H, m), 3.60–3.94 (4H, m), 4.52–4.63 (1H, m), 6.86 (2H, d, $J = 8.3$ Hz), 7.15 (2H, d, $J = 8.3$ Hz), 7.48 (1H, d, $J = 6.8$ Hz), 7.80–7.89 (2H, m), 8.01–8.10 (2H, m), 8.44 (1H, s), 8.81 (1H, s), 9.34 (3H, s), 9.55 (2H, s); FAB MS m/e $(M+1)^+$ 516. Anal. calcd for $C_{29}H_{33}N_5O_4 \cdot 2.0HCl \cdot 3.0H_2O$: C, 54.21; H, 6.43; N, 10.90; Cl, 11.03. Found: C, 53.92; H, 6.20; N, 10.90; Cl, 11.41.

4-[(*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-(7-amidino-2-naphthoyl)amino)methyl]benzoic acid (5j). Compound **5j** was synthesized from **4f** according to the same procedure as that for **5h**. Compound **5j** was obtained as a white solid (41% yield): ^{1}H NMR (DMSO-d_6) δ 1.46–1.68 (2H, m), 1.84–2.02 (2H, m), 2.14 (3H, s), 3.35–3.55 (2H, m), 3.61–3.84 (2H, m), 4.46–4.59 (1H, m), 5.19 (2H, s), 6.78 (2H, d, $J = 8.8$ Hz), 7.06 (2H, d, $J = 8.8$ Hz), 7.48 (2H, d, $J = 8.0$ Hz), 7.55 (1H, d, $J = 8.0$ Hz), 7.84 (1H, dd, $J = 2.0$, 8.8 Hz), 7.88 (1H, d, $J = 8.0$ Hz), 7.91 (2H, d, $J = 8.0$ Hz), 8.08 (1H, d, $J = 8.8$ Hz), 8.18 (1H, s), 8.44 (1H, s), 8.56–8.76 (1H, br), 9.09–9.65 (5H, m), 12.70–13.15 (1H, br); FAB MS m/e $(M+1)^+$ 564 . Anal. calcd for $C_{33}H_{33}N_5O_4 \cdot 2.0HCl \cdot 3.6H_2O$: C, 56.51; H, 6.06; N, 9.98; Cl, 10.11. Found: C, 56.29; H, 5.96; N, 10.01; Cl, 9.96.

7-[*N*-(*tert*-Butoxycarbonyl)amino]naphthalene-2-carbonitrile (6). To a stirred solution of Et_3N (5.58 mL,

40 mmol) in *tert*-butanol was added 4 Å molecular sieves (ca. 40 mL) and refluxed for 30 min. Then **2** (5.91 g, 30 mmol) and diphenylphosphoryl azide (8.09 mL, 37.5 mmol) was added and refluxed for 3.5 h. The reaction mixture was allowed to cool to ambient temperature, filtered and concentrated in vacuo. The resulting residue was suspended in ether and washed with H_2O, saturated aqueous $NaHCO_3$, 10% aqueous citric acid, brine, dried over Na_2SO_4 and concentrated in vacuo to provide **6** (8.42 g) as a white amorphous powder which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.56 (9H, s), 6.77 (1H, s), 7.42–7.52 (2H, m), 7.74–7.85 (2H, m), 8.02–8.13 (2H, m); FAB MS *m*/*e* $(M+1)^+$ 269.

***N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-7-cyanonaphthalene-2-sulfonamide (4g).** To a stirred solution of **6** (8.10 g) in 1,4-dioxane at ambient temperature was added 4 N HCl–EtOAc (10 mL). The reaction mixture was stirred at ambient temperature for 12 h and concentrated in vacuo. The residue was suspended in Et_2O, filtered and dried in vacuo. The crude 7-aminonaphthalene-2-carbonitrile hydrochloride (4.72 g) was used in the next step without purification: ^{1}H NMR (DMSO-d_6) δ 7.61 (1H, d, J = 8.7 Hz), 7.74 (1H, d, J = 8.7 Hz), 7.83 (1H, s), 8.04–8.15 (2H, m), 8.59 (1H, s), 9.50 (3H, br-s); GC MS *m*/*e* $(M)^+$ 168.

To a stirred solution of the aniline (1.0 g) in acetic acid (3 mL) and concd HCl (10 mL) at −10 °C was added dropwise a solution of $NaNO_2$ in H_2O (373 mg/0.5 mL). The reaction mixture was stirred at −5 °C for 45 min (diazotization reaction mixture). SO_2 gas was bubbled through acetic acid (30 mL) with stirring at ambient temperature for 30 min, then to the solution was added CuCl (1.5 g) and introduction of SO_2 gas was continued for a further 30 min. The reaction mixture was cooled to 10 °C and the diazotization reaction mixture was added to this stirred solution in portions. After stirring at ambient temperature for 1 h, the reaction mixture was poured into ice water and extracted with Et_2O. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$, H_2O and dried over Na_2SO_4, then concentrated in vacuo to afford crude 7-cyanonaphthalen-2-sulfonyl chloride (0.81 g) which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 7.79 (1H, dd, J = 1.5, 8.4 Hz), 7.90 (1H, dd, J = 1.5, 8.4 Hz), 8.01 (1H, d, J = 8.4 Hz), 8.10 (1H, d, J = 8.4 Hz), 8.31 (1H, s), 8.68 (1H, s); EI MS *m*/*e* $(M)^+$ 251, 253.

To a stirred solution of **1** (819 mg, 2.8 mmol) in 1,2-dichloroethane (10 mL) at 3 °C was added pyridine (234 mL) and the sulfonyl chloride (700 mg). After stirring for 3 h, the reaction mixture was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was recrystallized from EtOAc–Hex to afford **4g** (881 mg, 62%) as a brown amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.35–1.47 (2H, m), 1.38 (9H, s), 1.74–1.85 (2H, m), 3.04–3.17 (2H, m), 3.54–3.67 (2H, m), 4.33–4.43 (1H, m), 6.81 (2H, d, J = 8.7 Hz), 6.97 (2H, d, J = 8.7 Hz), 7.91 (1H, d, J = 8.7 Hz), 7.96 (1H, d, J = 8.7 Hz), 8.18–8.25 (2H, m), 8.47 (1H, s), 8.79 (1H, s), 10.15 (1H, s); FAB MS *m*/*e* $(M)^+$ 507.

7-Amidino-*N*-{4-[(1-acetimidoyl-4-piperidyl)oxy]phenyl}-naphthalene-2-sulfonamide (5k). Compound **5k** was synthesized from **4g** according to the same procedure as that for **5b**. Compound **5k** was obtained as a white amorphous powder (68% yield): ^{1}H NMR (DMSO-d_6) δ 1.57–1.72 (2H, m), 1.90–2.20 (2H, m), 2.27 (3H, s), 3.40–3.53 (2H, m), 3.63–3.83 (2H, m), 4.50–4.58 (1H, m), 6.85 (2H, d, J = 9.0 Hz), 7.02 (2H, d, J = 9.0 Hz), 7.95 (1H, dd, J = 1.2, 8.8 Hz), 7.99 (1H, dd, J = 1.6, 8.8 Hz), 8.20–8.28 (2H, m), 8.47 (1H, s), 8.68 (1H, s), 9.20–9.80 (6H, m), 10.30 (1H, br-s); FAB MS *m*/*e* $(M+1)^+$ 466. Anal. calcd for $C_{24}H_{27}N_5O_3 \cdot 2.1HCl \cdot 2.5H_2O$: C, 49.09; H, 5.85; N, 11.93; S, 5.46; Cl, 12.68. Found: C, 48.80; H, 5.69; N, 11.93; S, 5.56; Cl, 12.47.

7-Amidino-*N*-{4-[(1-acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-ethylnaphthalene-2-sulfonamide (5l). To a stirred solution of **5k** (100 mg, 0.17 mmol) in tetrahydrofuran (2 mL) and EtOH (5 mL) at ambient temperature was added triphenylphosphine (PPh_3) (67 mg, 0.25 mmol) and diethyl azodicarboxylate (DEAD) (40 μL, 0.25 mmol). After stirring at ambient temperature for 30 h, PPh_3 (223 mg, 0.85 mmol) and DEAD (133 μL, 0.85 mmol) was added again and stirred at ambient temperature for 20 h. The reaction mixture was diluted with 0.5 N HCl and EtOAc and the aqueous layer was concentrated in vacuo. The resulting residue was chromatographed on ODS-gel eluting with CH_3CN/0.005 N aqueous HCl (2:98–6:94). CH_3CN was removed in vacuo, and the aqueous solution was lyophilized to give **5l** (40 mg, 37%) as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.00 (3H, t, J = 7.3 Hz), 1.64–1.81 (2H, m), 1.99–2.11 (2H, m), 2.29 (3H, s), 3.41–3.58 (2H, m), 3.63 (2H, q, J = 7.3 Hz), 3.68–3.88 (2H, m), 4.64–4.72 (1H, m), 6.97 (4H, s), 7.77 (1H, dd, J = 1.5, 8.3 Hz), 8.01 (1H, d, J = 1.5, 8.3 Hz), 8.26 (1H, d, J = 8.7 Hz), 8.30 (1H, d, J = 8.8 Hz), 8.48 (1H, s), 8.72 (2H, s), 9.20–9.33 (3H, m), 9.57 (2H, s); FAB MS *m*/*e* $(M+1)^+$ 466. Anal. calcd for $C_{26}H_{31}N_5O_3S \cdot 2.6HCl \cdot 2.2H_2O$: C, 49.72; H, 6.10; N, 11.15; S, 5.11; Cl, 14.68. Found: C, 49.55; H, 6.14; N, 11.44; S, 5.15; Cl, 14.83.

3-(2-Chloroethyl)benzonitrile (8). To a stirred solution of 3-(2-oxoethyl)benzonitrile (**7**) (1.0 g, 6.89 mmol) in EtOH (10 mL) at 3 °C was added sodium borohydride (0.4 g, 10.6 mmol). After the mixture was stirred for 3 h at 3 °C, H_2O was added and extracted with EtOAc. The EtOAc extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo to give 3-(2-hydroxyethyl)benzonitrile (1.02 g, 100%) as a colorless oil which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 2.90 (2H, t, J = 6.5 Hz), 3.90 (2H, q, J = 6.5 Hz), 7.38–7.65 (4H, m).

To a stirred solution of 3-(2-hydroxyethyl)benzonitrile (1.17 g, 7.95 mmol) in CH_2Cl_2 (15 mL) at ambient temperature was added thionyl chloride (0.3 mL, 41.1 mmol) and DMF (20 mg). The mixture was allowed to warm to 50 °C, stirred for 1 h and then concentrated in vacuo. The resulting residue was suspended in H_2O

and extracted with EtOAc. The EtOAc extracts were dried over Na_2SO_4 and concentrated in vacuo to give **8** (1.26 g, 96%) as a pale brown oil: ^{1}H NMR ($CDCl_3$) δ 3.10 (2H, t, J = 6.7 Hz), 3.73 (2H, t, J = 6.7 Hz), 7.35–7.70 (4H, m); GC MS m/e $(M)^+$ 165, 167.

2-(3-Cyanophenyl)-*N*-{4-[(4-piperidyl)oxy]phenyl}ethanesulfonamide (9). To a stirred solution of **8** (1.26 g, 7.61 mmol) in H_2O (15 mL) at ambient temperature was added sodium sulfite (1.20 g, 9.52 mmol). The mixture was allowed to reflux for 25 h and concentrated in vacuo. After the resulting residue was dissolved in benzene, thionyl chloride (0.7 mL, 9.6 mmol) and DMF (20 mg) was added at ambient temperature. The mixture was refluxed for 25 h, poured into ice water and extracted with EtOAc. The EtOAc extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo to give 2-(3-cyanophenyl)ethanesulfonyl chloride (1.09 g, 62%) as a brown oil which was used without further purification. To a stirred solution of **1** (1.50 g, 5.15 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added pyridine (0.5 mL, 6.18 mmol), the sulfonyl chloride (1.09 g, 4.75 mmol) and 4-dimethylaminopyridine (17 mg). After the reaction mixture was stirred for 29 h at ambient temperature, it was quenched with 10% aqueous citric acid and extracted with $CHCl_3$. The $CHCl_3$ extracts were dried over Na_2SO_4 and concentrated in vacuo to give crude *N*-{4-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-phenyl}-2-(3-cyanophenyl)ethanesulfonamide as a solid. To the solution of this compound in EtOAc (15 mL) was added 4N HCl/EtOAc (10 mL) at 0 °C and the reaction mixture was stirred for 13 h at ambient temperture. The reaction mixture was quenched with H_2O, neutralized with saturated aqueous $NaHCO_3$ and extracted with MeOH/$CHCl_3$ (10:90). The extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was dissolved in EtOAc and the solution was washed with H_2O and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was crystallized from EtOAc to give **9** (1.09 g, 60%) as a pale brown solid: mp 138–140 °C; ^{1}H NMR (DMSO-d_6) 1.33–1.48 (2H, m) 1.83–1.93 (2H, m), 2.52–2.60 (2H, m), 2.88–2.98 (2H, m), 3.01–3.09 (2H, m), 3.28–3.37 (2H, m), 4.25–4.36 (1H, m), 6.90 (2H, d, J = 8.7 Hz), 7.12 (2H, d, J = 8.7 Hz), 7.48 (1H, dd, J = 7.8, 8.1 Hz), 7.56 (1H, d, J = 7.8 Hz), 7.67 (1H, d, J = 8.1 Hz), 7.71 (1H, s); FAB MS m/e $(M+1)^+$ 386.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-2-(3-amidinophenyl)ethanesulfonamide (5m).** Compound **5m** was synthesized from **9** according to the same procedure as that for **5b**. Compound **5m** was obtained as a white amorphous powder (80% yield): ^{1}H NMR (DMSO-d_6) δ 1.65–1.80 (2H, m) 1.95–2.10 (2H, m), 2.32 (3H, s), 3.05–3.13 (2H, m), 3.35–3.43 (2H, m), 3.47–3.65 (2H, m), 3.68–3.90 (2H, m), 4.60–4.70 (1H, m), 6.97 (2H, d, J = 8.8 Hz), 7.19 (2H, d, J = 8.7 Hz), 7.51 (1H, dd, J = 7.9, 8.0 Hz), 7.58 (1H, d, J = 7.9 Hz), 7.71 (1H, d, J = 8.0 Hz), 7.73 (1H, s), 8.93 (1H, s), 9.36 (2H, s), 9.48 (3H, s), 9.76 (1H, s); FAB MS m/e $(M+1)^+$ 444. Anal. calcd for $C_{22}H_{29}N_5O_3S \cdot 2.3HCl \cdot 0.8H_2O$: C, 48.77; H, 6.12; N, 12.93; S, 5.92; Cl, 15.05. Found: C, 48.87; H, 6.31; N, 13.04; S, 5.93; Cl, 15.15.

(*E*)-*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-2-(3-cyanophenyl)-ethenesulfonamide (4h). To a stirred solution of **1** (500 mg, 1.72 mmol) in CH_2Cl_2 (5 mL) at ambient temperature was added pyridine (0.40 mL, 4.95 mmol), 2-chloro-1-ethanesulfonylchloride (0.20 mL, 1.90 mmol), 4-dimethylaminopyridine (20 mg). After stirring at ambient temperature for 20 h, the reaction mixture was concentrated in vacuo. To the resulting residue was added citric acid aqueous solution which was then extracted with $CHCl_3$. The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The resulting crude ***N*-{4-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]phenyl}ethenesulfonamide (10)** (0.46 g) was not stable and used in the next step without purification: FAB MS m/e $(M-H)^-$ 381.

To a stirred solution of PPh_3 (50 mg, 0.19 mmol) in DMF (5 mL) at ambient temperature was added palladium(II) acetate (20 mg, 0.089 mmol) under argon atmosphere. After stirring at ambient temperature for 5 min, 3-bromobenzonitrile (360 mg, 1.98 mmol), the DMF solution of crude **10** and Et_3N (0.70 mL, 5.02 mmol) was added and stirred at 140 °C for 17 h. The reaction mixture was concentrated in vacuo. To the resulting residue was added H_2O followed by extracting with EtOAc. The organic solution was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/Hex (33:67–50:50) to give **4h** (0.35 g, 42%) as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.39 (9H, s), 1.40–1.56 (2H, m) 1.75–1.90 (2H, m), 3.06–3.20 (2H, m), 3.57–3.68 (2H, m), 4.38–4.50 (1H, m), 6.90 (2H, d, J = 8.7 Hz), 7.11 (2H, d, J = 8.7 Hz), 7.38 (1H, d, J = 15.3 Hz), 7.43 (1H, d, J = 15.3 Hz), 7.61 (1H, dd, J = 7.5, 7.8 Hz), 7.86 (1H, d, J = 7.8 Hz), 8.02 (1H, d, J = 7.5 Hz), 8.26 (1H, s), 9.79 (1H, s); FAB MS m/e $(M-H)^-$ 482.

(*E*)-*N*-{4-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]phenyl}-2-(3-cyanophenyl)-*N*-methylethenesulfonamide (4i). To a stirred solution of **4h** (0.79 g, 1.63 mmol) in DMF (10 mL) at ambient temperature was added K_2CO_3 (0.45 g, 3.26 mmol) and methyl iodide (0.35 g, 2.47 mmol). After stirring at ambient temperature for 1 h, the reaction mixture was concentrated in vacuo. The resulting residue was suspended in H_2O and extracted with EtOAc. The EtOAc extracts were dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/Hex (25:75–33:67) to give **4i** (0.57 g, 70%) as a pale yellow viscous oil: ^{1}H NMR (DMSO-d_6) δ 1.40 (9H, s), 1.40–1.56 (2H, m) 1.75–1.95 (2H, m), 3.06–3.20 (2H, m), 3.20 (3H, s), 3.57–3.70 (2H, m), 4.48–4.58 (1H, m), 6.96 (2H, d, J = 8.7 Hz), 7.27 (2H, d, J = 8.7 Hz), 7.33 (1H, d, J = 15.3 Hz), 7.54 (1H, d, J = 15.3 Hz), 7.64 (1H, dd, J = 7.8, 8.1 Hz), 7.89 (1H, d, J = 7.8 Hz), 8.07 (1H, d, J = 8.1 Hz), 8.33 (1H, s); FAB MS m/e $(M+1)^+$ 498.

(*E*)-*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-2-(3-amidinophenyl)ethenesulfonamide (5n). Compound **5n** was synthesized from **4h** according to the same procedure as that for **5b**. Compound **5n** was obtained as a pale yellow amorphous powder (34% yield): ^{1}H NMR

(DMSO-d_6) δ 1.60–1.80 (2H, m), 1.92–2.10 (2H, m), 2.28 (3H, s), 3.43–3.61 (2H, m), 3.62–3.75 (1H, m), 3.75–3.85 (1H, m), 4.56–4.68 (1H, m), 6.95 (2H, d, J = 8.8 Hz), 7.15 (2H, d, J = 8.8 Hz), 7.42 (2H, br-s), 7.65 (1H, dd, J = 7.8, 7.9 Hz), 7.88 (1H, d, J = 7.8 Hz), 8.00 (1H, d, J = 7.9 Hz), 8.21 (1H, s), 8.84 (1H, s), 9.28–9.44 (3H, m), 9.52 (2H, s), 9.92 (1H, s); FAB MS m/e $(M+1)^+$ 442. Anal. calcd for $C_{22}H_{27}N_5O_3S \cdot 2.3HCl \cdot 1.5H_2O$: C, 47.83; H, 5.89; N, 12.68; S, 5.80; Cl, 14.76. Found: C, 47.98; H, 5.99; N, 12.79; S, 5.96; Cl, 15.03.

(*E*)-*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-2-(3-amidinophenyl)-*N*-methylethenesulfonamide (5o). Compound **5o** was synthesized from **4i** according to the same procedure as that for **5b**. Compound **5o** was obtained as a white amorphous powder (51% yield): ^{1}H NMR (DMSO-d_6) δ 1.65–1.80 (2H, m), 1.96–2.10 (2H, m), 2.30 (3H, s), 3.23 (3H, s), 3.46–3.60 (2H, m), 3.68–3.77 (1H, m), 3.77–3.86 (1H, m), 4.65–4.74 (1H, m), 7.00 (2H, d, J = 8.8 Hz), 7.35 (2H, d, J = 8.7 Hz), 7.37 (1H, d, J = 15.1 Hz), 7.59 (1H, d, J = 15.1 Hz), 7.68 (1H, dd, J = 7.8, 7.9 Hz), 7.90 (1H, d, J = 7.8 Hz), 8.05 (1H, d, J = 7.9 Hz), 8.34 (1H, s), 8.81 (1H, s), 9.33 (3H, br-s), 9.55 (2H, s); FAB MS m/e $(M+1)^+$ 456. Anal. calcd for $C_{23}H_{29}N_5O_3S \cdot 2.1HCl \cdot 2.5H_2O$: C, 47.86; H, 6.30; N, 12.13; S, 5.56; Cl, 12.90. Found: C, 48.03; H, 6.17; N, 12.16; S, 5.55; Cl, 12.86.

(*E*)-*N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-2-(4-amidinophenyl)ethenesulfonamide (5p). Compound **5p** was synthesized according to the same procedure as that for **5n**. Compound **5p** was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.60–1.80 (2H, m) 1.92–2.10 (2H, m), 2.28 (3H, s), 3.44–3.60 (2H, m), 3.62–3.80 (2H, m), 4.55–4.65 (1H, m), 6.94 (2H, d, J = 9.3 Hz), 7.13 (2H, d, J = 9.3 Hz), 7.43 (1H, d, J = 15.6 Hz), 7.47 (1H, d, J = 15.6 Hz), 7.85 (2H, d, J = 8.3 Hz), 7.94 (2H, d, J = 8.3 Hz), 8.70 (1H, s), 9.15 (2H, s), 9.24 (1H, s), 9.43 (2H, s), 9.85 (1H, s); FAB MS m/e $(M+1)^+$ 442. Anal. calcd for $C_{22}H_{27}N_5O_3S \cdot 2.0HCl \cdot 2.6H_2O$: C, 47.08; H, 6.14; N, 12.48; S, 5.71; Cl, 12.63. Found: C, 46.89; H, 5.86; N, 12.32; S, 5.77; Cl, 12.67.

(*E*)-*N*-{3-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-2-(3-amidinophenyl)ethenesulfonamide (5q). Compound **5q** was synthesized according to the same procedure as that for **5n**. Compound **5q** was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) 1.65–1.78 (2H, m) 1.95–2.07 (2H, m), 2.28 (3H, s), 3.48–3.57 (2H, m), 3.66–3.78 (2H, m), 4.58–4.67 (1H, m), 6.72 (1H, d, J = 7.8 Hz), 6.78–6.83 (2H, m), 7.20 (1H, dd, J = 7.8, 8.6 Hz), 7.47 (1H, d, J = 15.7 Hz), 7.56 (1H, d, J = 15.7 Hz), 7.66 (1H, dd, J = 7.8, 8.3 Hz), 7.86 (1H, d, J = 8.3 Hz), 8.03 (1H, d, J = 7.8 Hz), 8.22 (1H s), 8.74 (1H, s), 9.23 (2H, s), 9.28 (1H, s), 9.44 (2H, s), 10.24 (1H, s); FAB MS m/e $(M+1)^+$ 442. Anal. calcd for $C_{22}H_{27}N_5O_3S \cdot 2.1HCl \cdot 1.7H_2O$: C, 48.15; H, 5.97; N, 12.76; S, 5.84; Cl, 13.57. Found: C, 48.33; H, 6.03; N, 12.78; S, 5.76; Cl, 13.34.

7-({4-[(1-Acetimidoyl-4-piperidyl)oxy]-*N*-ethyl-anilino}-methyl)naphthalene-2-carboxamidine (5s). Compound **5s** was synthesized from 7-({4-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]anilino}methyl)naphthalene-2-carbonitrile[10] according to the same procedure as that for **5b**. Compound **5s** was obtained as a pale brown amorphous powder: ^{1}H NMR (DMSO-d_6) 1.15 (3H, t, J = 7.3 Hz), 1.52–1.85 (2H, m), 1.85–2.15 (2H, m), 2.29 (3H, s), 3.30–4.00 (6H, m), 4.35–5.13 (3H, br), 6.50–7.20 (4H, br), 7.50–8.30 (5H, m), 8.44 (1H s), 8.90 (1H, s), 9.42 (3H, s), 9.64 (2H, s), FAB MS m/e $(M+1)^+$ 444. Anal. calcd for $C_{27}H_{33}N_5O \cdot 3.0HCl \cdot 2.8H_2O$: C, 53.92; H, 6.64; N, 11.65; Cl, 17.69. Found: C, 53.85; H, 7.07; N, 11.68; Cl, 18.07.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-(5-amidinobenzofuran-2-carbonyl) glycine (5t).** Compound **5t** was synthesized from 5-cyanobenzofuran-2-carboxylic acid[16] and **2** according to the same procedure as that for **5g**. Compound **5t** was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) 1.77–1.83 (2H, m), 2.00–2.12 (2H, m), 2.30 (3H, s), 3.35–3.40 (2H, m), 3.70–3.87 (2H, m), 4.47 (2H, s), 4.69–4.77 (1H, m), 6.50 (1H, s), 7.07 (2H, d, J = 9.1 Hz), 7.35 (2H, d, J = 8.8 Hz), 7.76 (1H, d, J = 8.6 Hz), 7.81 (1H, dd, J = 1.6, 8.6 Hz), 8.14 (1H, s), 8.77 (1H, s), 9.11 (2H, s), 9.28 (1H, s), 9.36 (2H, s), 12.95 (1H, s); FAB MS m/e $(M+1)^+$ 478. Anal. calcd for $C_{25}H_{27}N_5O_5 \cdot 2.0HCl \cdot 3.0H_2O$: C, 49.67; H, 5.84; N, 11.59; Cl, 11.73. Found: C, 49.81; H, 5.72; N, 11.81; Cl, 11.47.

***N*-{4-[(1-Acetimidoyl-4-piperidyl)oxy]phenyl}-*N*-[(*E*)-3-(3-amidinophenyl)acryloyl]glycine (5u).** Compound **5u** was synthesized from (*E*)-3-cyanocinnamic acid[17] and **2** according to the same procedure as that for **5g**. Compound **5u** was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) 1.70–1.87 (2H, m), 2.01–2.16 (2H, m), 2.32 (3H, s), 3.45–3.65 (2H, m), 3.70–3.91 (2H, m), 4.38 (2H, s), 4.69–4.80 (1H, m), 6.55 (1H, d, J = 15.7 Hz), 7.10 (2H, d, J = 8.8 Hz), 7.31 (2H, d, J = 8.8 Hz), 7.55–7.63 (2H, m), 7.77 (2H, d, J = 7.8 Hz), 7.85 (1H, s), 8.84 (1H, s), 9.26 (2H, s), 9.34 (1H, s), 9.43 (2H, s), 12.6–12.9 (1H, br-s); FAB MS m/e $(M+1)^+$ 464. Anal. calcd for $C_{25}H_{29}N_5O_4 \cdot 2.3HCl \cdot 3.0H_2O$: C, 49.93; H, 6.25; N, 11.64; Cl, 13.56. Found: C, 49.72; H, 5.95; N, 11.65; Cl, 13.86.

X-ray crystallographic experiment

Crystals of the YM-60828/bovine pancreatic trypsin complex were prepared using the same method as reported previously.[18] The X-ray diffraction data were collected with a Rigaku R-AXIS IIc image-plate system. The data set covers 79% of the theoretically calculated number of reflections up to 2.3 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). Model building, electron density calculation, and model refinement were carried out using program O[19] and CNX2000 (Accelrys Inc.). The model has been refined to a crystallographic R-value of 18.3% (R_{free} = 23.6%) with good stereochemistry (r.m.s.d. of bonds = 0.007 Å and angles = 1.4° from ideality). We will deposit the crystallographic data for this structure in the Protein Data Bank after this manuscript is accepted.

Modeling study

YM-60828 was initially placed in the active site of factor Xa by superimposing the crystal structure of the YM-60828/trypsin complex on to that of ZK-807834/factor Xa[20] complex (1FJS) using corresponding Ca atoms. ZK-807834 and two water molecules around the active site were then removed from the model because they seem to have unsuitable contacts with the YM-60828 initial model. After manual adjustment of the position of the side chains, energy minimization of the complex model was performed with program DISCOVER (Accelrys Inc.). YM-60828, water molecules and side chain atoms within 10 Å from YM-60828 were allowed to move during the minimization.

Biology 1. 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 portion of the 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.

2. Plasma clotting time assay. Prothrombin time (PT) was performed using a KC10A coagulometer (Amelung Co., Lehbringsweg, Germany). 50 μL of citrated plasma from human, mice and squirrel monkey were 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.

3. Ex vivo studies. Male ICR mice weighing 20–30 g and squirrel monkeys of both sexes weighing 660–775 g were fasted overnight. Inhibitors were dissolved in saline and administered orally to the mice at 100 mg/kg, and to squirrel monkeys at 3 mg/kg using a gastric tube. Several times after oral administration of the inhibitor, citrated blood was collected from the abdominal vena cava (mice) or the femoral vein (squirrel monkeys), and platelet poor plasma was prepared by centrifugation to measure PT. All data were expressed as relative fold values, compared with the baseline value (squirrel monkeys) or the vehicle group (mice).

Acknowledgements

The authors deeply acknowledge Dr. Yuuji Mano for pharmacokinetic studies and Dr. Masaya Orita for NMR studies. The authors are also grateful to the staff of the Division of Analytical Science Laboratories for the elemental analysis and spectral measurements.

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