Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 367–381

BIOORGANIC & MEDICINAL CHEMISTRY

Design, Synthesis and Biological Activity of YM-60828 Derivatives. Part 2: Potent and Orally-Bioavailable Factor Xa Inhibitors Based on Benzothiadiazine-4-one Template

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Received 16 July 2002; accepted 13 September 2002

Abstract—Compound YM-60828 was previously characterized in our laboratory as a potent, selective and orally-bioavailable Factor Xa (FXa) inhibitor. The L-shape conformation of this compound in the active site of FXa was recognized as an important factor in displaying its FXa inhibitory activity. This led to the exploration of conformationally restricted cyclic scaffolds bearing a similar active conformation. The current study investigated a novel series of benzothiadiazine-4-one based compounds as FXa inhibitors. Structure–activity relationship (SAR) investigations revealed some potent FXa inhibitors that were selected for further in vitro and ex vivo anticoagulant studies. Among them, compound **6j** (YM-169920) was proved to be most effective anticoagulant in this series. The synthesis and SAR in addition to docking studies of this class of inhibitors are described.

Introduction

Factor Xa (FXa) is a serine protease which plays a pivotal role in the sequence of blood coagulation events. Intrinsic and extrinsic coagulation cascades intersect at FXa, and activate prothrombin to generate thrombin through proteolytic cleavage. Thrombin, in turn, promotes blood clot formation by the conversion of fibrinogen to insoluble fibrin and activating platelets. Although small molecule thrombin inhibitors have been intensely investigated as a treatment for thromboembolic disorders,[1] only argatroban[2] has been marketed as a parenteral drug and none of the orally effective thrombin inhibitors have been successfully developed. Recently, a number of reports suggested that direct FXa inhibitors were more suitable antithrombotic agents compared to direct thrombin inhibitors, based on abnormal bleeding side effects in the animal thrombotic models.[3,4] Therefore, exploration of FXa inhibitor is recently quite attractive field for the discovery of antithrombotic agents.

We have previously reported a series of *N*-[(7-amidino-2-naphthyl)methyl]aniline derivatives as potent and orally-bioavailable FXa inhibitors.[5] Furthermore, we revealed compound YM-60828 and its mesylate salt YM-75466 which displayed optimum properties, possessing potent efficacies for various thrombosis models without side effect bleeding.[4,6] Moreover, the docking study of YM-60828 in FXa revealed that the active conformation of YM-60828 was L-shaped and the introduction of a rigid scaffolding which could adopt the active conformation, such as naphthoanilide and naphthalensulfonanilide templates, has led to discovery of a series of YM-60828 derivatives with potent FXa activities (Fig. 1a).[7] Our design of potent and selective FXa inhibitors was focused on developing molecules with a similar conformationally restricted scaffold which adopts an active L-shape conformation. The docking study showed negligible interaction of the carboxyl moiety of YM-60828 to FXa.[7] We subsequently synthesized the derivatives of YM-60828 cyclized at the sulfamoylacetic acid side chain and central benzene ring (Fig. 1b). In this report we describe the SAR of the cyclic derivatives of YM-60828 and discuss its conformations bound to FXa.

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PII: S0968-0896(02)00462-5

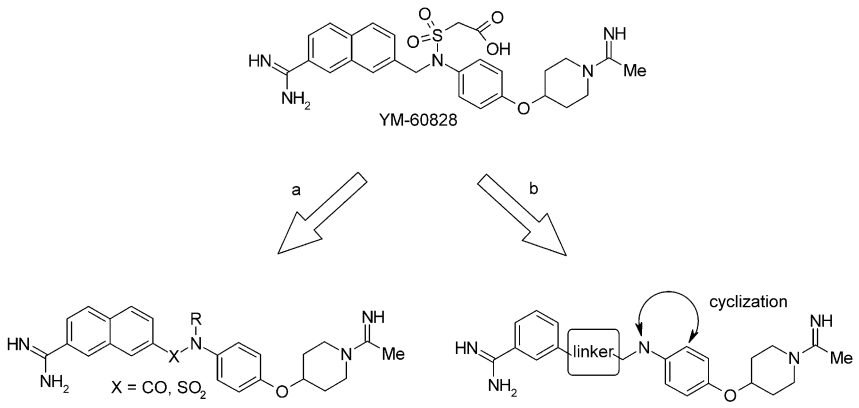


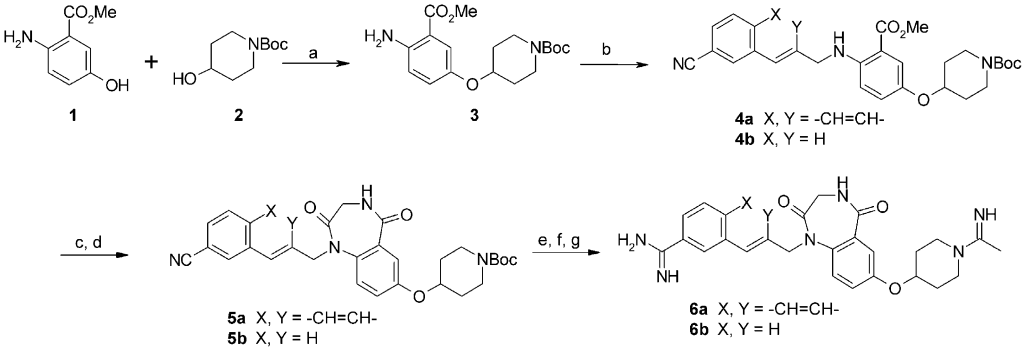
Figure 1.

Chemistry

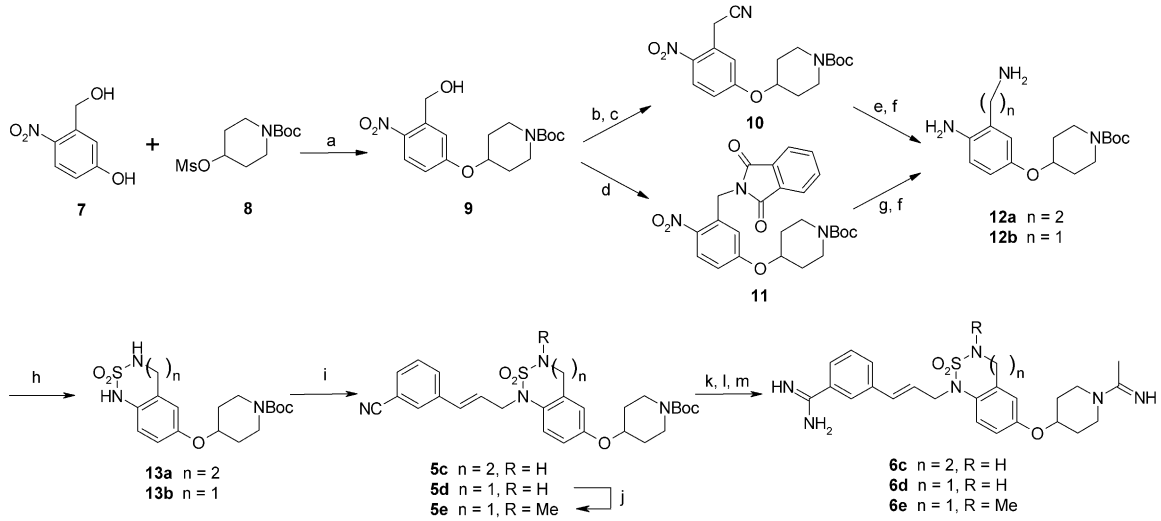
The synthesis of benzodiazepine derivatives **6a** and **6b** are shown in Scheme 1. Construction of the benzodiazepine skeletons **5a** and **5b** were accomplished by treatment of anthranilic acid derivatives **4a** and **4b** with bromoacetylbromide, followed by cyclization with ammonia. Aniline **3** was prepared from phenol **1** and alcohol **2** under Mitsunobu condensation conditions (PPh_3, DEAD, THF). The anthranilic acid derivatives **4a** and **4b** were synthesized by the condensation of aldehydes 7-formylnaphthalene-2-carbonitrile[5] and (*E*)-3-cyanocinnamaldehyde[5] respectively with **3**. Benzodiazepines **5a** and **5b** were subjected to the standard Pinner reactions and aminations, followed by treatment with ethyl acetimidate to give bis-amidines **6a** and **6b**, respectively.

The preparative route to benzothiadiazepine (**6c**) and benzothiadiazine (**6d**, **6e**) derivatives are shown in Scheme 2. Rings **13a** and **13b** were constructed from diamine derivatives **12a** and **12b** by treatment with sulfamide in pyridine under reflux. Compound **9** was prepared by coupling phenol **7** with methanesulfonate **8** which was subsequently used as the common starting material for the synthesis of the diamines **12a** and **12b**. Alcohol **9** was converted to cyano-substituted derivative **10** via nucleophilic addition of sodium cyanide to the methanesulfonate intermediate, followed by stepwise reduction of cyano and nitro groups to afford diamine **12a**. Alternatively, alcohol **9** was subjected to a Mitsunobu reaction with phthalimide followed by hydrazine deprotection and subsequent reduction of the nitro group to yield diamine **12b**. The benzothiadiazepine **13a** and benzothiadiazine **13b** were each coupled with 3-((*E*)-3-hydroxypropenyl)benzonitrile by a Mitsunobu reaction to afford cyanocinnamyl derivatives **5c** and **5d**, respectively. Methyl substituted derivative **5e** was prepared by the reaction of **5d** with methyl iodide under basic conditions (K_2CO_3). Bis-amidines **6c–6e** were obtained from **5c–5e**, respectively, in the same manner as described for **6a** and **6b**.

The benzothiadiazine-4-one derivatives **6f–6k** were prepared as shown in Scheme 3. All compounds were synthesized via key sulfamide intermediates **14a–14f**. A general synthesis of this series of compounds was exemplified by the preparation of compounds **6f** and **6g**. The anthranilic acid derivatives **4a** and **4b** were reacted with *tert*-butyl chlorosulfonylcarbamate[5] in pyridine to give sulfamide derivatives **14a** and **14b**, respectively. The conversion of intermediates **14a** and **14b** to corresponding benzothiadiazine-4-ones **15a** and **15b** was achieved by TFA deprotection from the BOC protecting group



Scheme 1. Synthesis of benzodiazepine derivatives: (a) PPh_3, diethyl azodicarboxylate (DEAD), THF; (b) aldehydes, $NaB(OAc)_3H$, AcOH, CH_2Cl_2; (c) bromoacetylbromide, pyridine, diethylether; (d) NH_3, MeOH; (e) HCl, EtOH; (f) NH_4OAc, EtOH, MeOH; (g) ethyl acetimidate hydrochloride, Et_3N, EtOH.



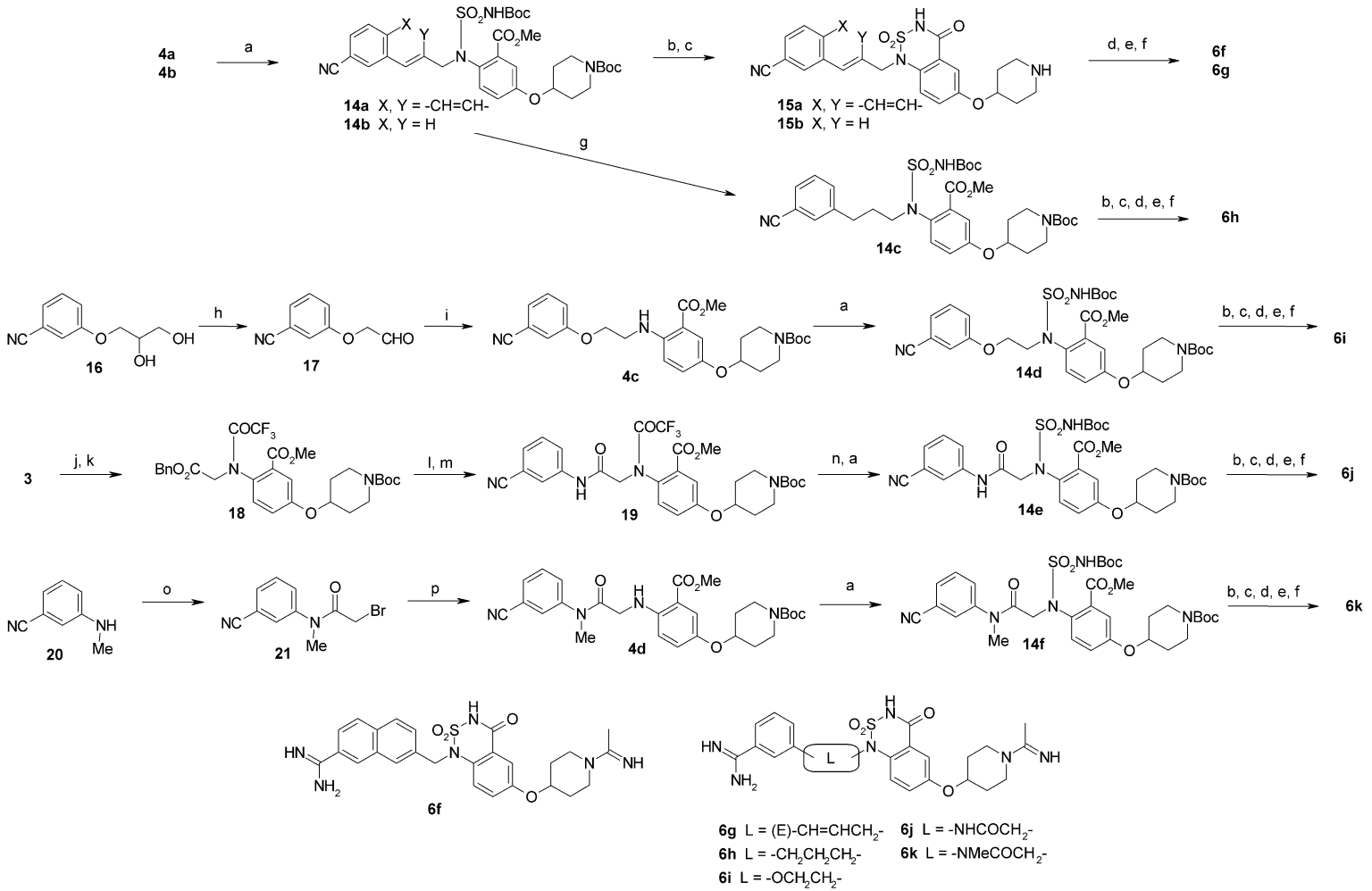
Scheme 2. Synthesis of benzothiadiazepine and benzothiadiazine derivatives: (a) K_2CO_3, DMF; (b) methanesulfonylchloride, Et_3N, 1,2-dichloroethane; (c) NaCN, DMF; (d) phthalimide, PPh_3, DEAD, THF; (e) BH_3–THF, THF, (f) Fe, NH_4Cl, EtOH, H_2O; (g) $NH_2NH_2{\cdot}H_2O$, EtOH; (h) sulfamide, pyridine; (i) 3-((*E*)-3-Hydroxypropenyl)benzonitrile, PPh_3, DEAD, THF; (j) MeI, K_2CO_3, CH_3CN; (k) HCl, EtOH; (l) NH_4OAc, EtOH; (m) ethyl acetimidate hydrochloride, Et_3N, EtOH.

followed by cyclization under basic conditions (NaOEt, EtOH). Bis-amidines **6f** and **6g** were prepared using the same procedure as described above. Phenylpropyl derivative **6h** was prepared through intermediate **14c** which was obtained following reduction of the phenylpropenyl intermediate **14b**. Compound **4c**, an intermediate of phenoxyethyl derivative **6i**, was synthesized by the reductive coupling of aniline **3** and phenoxyacetaldehyde **17** which was prepared by the oxidative cleavage of diol **16**.[8] Different routes were employed to synthesize phenylcarbamoylmethyl derivative **6j** and its methyl substituted analogue **6k**. *N*-Methyl substituted phenylcarbamoylmethyl intermediate **4d** was readily synthesized by the coupling of alkylbromide **21** with aniline **3**. However, the corresponding reaction for the *N*-demethyl analogue provided a complex mixture. Thus the trifluoroacetylated derivative of **3** was alkylated with benzyl 2-bromoacetate in the presence of sodium hydride to afford intermediate **18**. Reductive cleavage of the benzyl ester of **18** followed by condensation of aminobenzonitrile gave phenylcarbamoylmethyl intermediate **19**. **6j** and **6k** were obtained readily from **19** and **4d** respectively by an identical procedure to that described above.

Results and Discussion

As a preliminary investigation, cyclic templates which could potentially exhibit the L-shaped conformation as observed for modeling study of YM-60828[7] in the active site of FXa, were initially screened in the Cambridge Structural Database based on the distance and the angle between the two benzene rings (A and B rings). Among this database search results, a benzodiazepine derivative BEDZPN10, which possessed a similar L-shape conformation to YM-60828, as shown in Fig. 2, was selected as an appropriate scaffold. Next, benzodiazepine derivative **6a** designed from BEDZPN10 was synthesized and evaluated for its inhibitory activities against FXa and selectivities against related serine proteases thrombin and trypsin. As shown in Table 1, **6a** displayed a moderate activity and selectivity for FXa ($IC_{50} = 24.0$ nM, 29-fold against trypsin). These results indicated that cyclization of YM-60828 at this position was tolerable, and optimization of this series of compound could lead to some novel series of potent and selective FXa inhibitors. Since styrene analogue **6b**, which was a readily synthesized and accessible analogue of **6a**, retained the activity and selectivity ($IC_{50} = 27.4$ nM, 39-fold against trypsin), we commenced optimization of the ring structure of **6b**. Introduction of the sulfonamide structure, which was also contained in YM-60828, resulted in a decrease in FXa inhibitory activity (**6c**). However, the six-membered ring analogue **6d** enhanced FXa potency. Methylation at the ring nitrogen (**6e**) did not affect the activity. The most potent derivative from this set was compound **6g** containing a benzothiadiazine-4-one scaffold, which showed an 10- and 2-fold enhancement in FXa inhibitory activity ($IC_{50} = 2.8$ nM) compared to lead compounds **6b** and YM-60828, respectively.

Further modification of the styrene moiety led to identification of other dispensable P1 groups as shown in Table 2. Replacement of the styrene with the initial naphthalene structure retained the activity (**6f**, $IC_{50} = 2.1$ nM), however conformationally unrestricted phenethyl (**6h**) and phenoxymethyl (**6i**) derivatives resulted in a decreased FXa inhibitory activity. The anilide derivative **6j** showed potent activity ($IC_{50} = 2.5$ nM) equal to that of styrene and naphthalene derivatives **6g** and **6f** and exhibited an improved selectivity against trypsin (248-fold for **6j** vs 95- and 33-fold for **6g** and **6f**). In contrast, the same translation was not tolerated for the uncyclized derivatives of YM-60828.[5] *N*-Methylation of anilide derivative **6j** resulted in a drop in potency (**6k**).



Scheme 3. Synthesis of benzothiadiazine-4-one derivatives: (a) *tert*-butyl chlorosulfonylcarbamate, pyridine; (b) trifluoroacetic acid, CH_2Cl_2; (c) NaOEt, EtOH; (d) HCl, EtOH; (e) NH_4OAc, EtOH; (f) ethyl acetimidate hydrochloride, Et_3N, EtOH; (g) H_2, $PdO/BaSO_4$, EtOH; (h) $NaIO_4$, CH_2Cl_2, H_2O; (i) **3**, $NaB(OAc)_3H$, AcOH, 1,2-dichloroethane; (j) trifluoroacetic anhydride, pyridine, 1,2-dichloroethane; (k) NaH, benzyl 2-bromoacetate, DMF; (l) H_2, Pd/C, MeOH; (m) 3-aminobenzonitrile, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole DMF; (n) K_2CO_3, MeOH, H_2O; (o) bromoacetylbromide, $NaHCO_3$, EtOAc, H_2O; (p) **3**, K_2CO_3, CH_3CN.

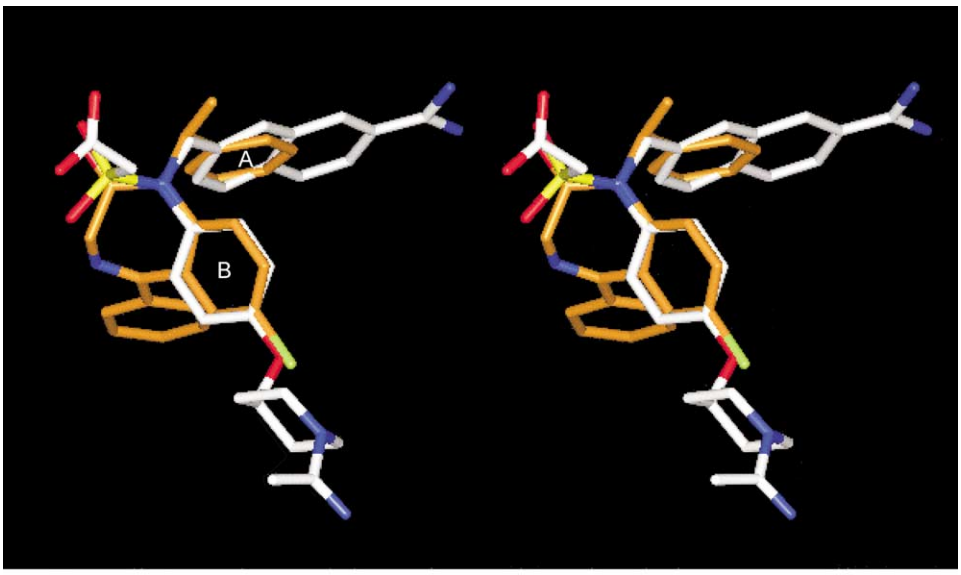
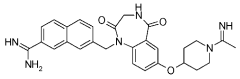
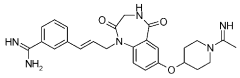
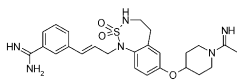
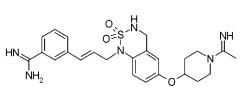
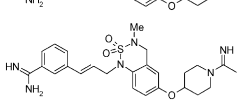
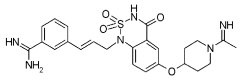


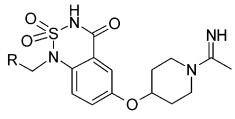
Figure 2. Overlay of FXa-binding conformation of YM-60828 (with white carbons) and crystal structure of BENZEPIN10 (brown).

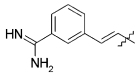
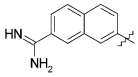
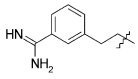
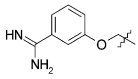
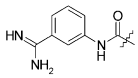
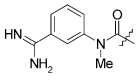
Table 1. In vitro activity of inhibitors based on benzene fused cyclic templates

Compd	R	IC_{50} (nM)[a]		
		Factor Xa	Thrombin	Trypsin
6a		24.0	> 100,000	693.3
6b		27.4	> 100,000	1075.4
6c		55.9	> 100,000	1256.3
6d		16.0	> 100,000	279.3
6e		16.9	> 100,000	212.9
6g		2.8	> 100,000	266.2
	YM-60828	6.0	> 100,000	159.0

[a]Human purified enzymes were used. IC_{50} values represent the averaged of three or more determinations with the average standard error of the mean < 25%.

Table 2. Replacement of styrylamidine



Compd.	R	IC_{50} (nM)[a]		
		Factor Xa	Thrombin	Trypsin
6g		2.8	> 100,000	266.2
6f		2.1	> 100,000	68.4
6h		74.1	> 100,000	7169.6
6i		137.6	> 100,000	13,699.5
6j		2.5	> 100,000	619.3
6k		456.1	> 100,000	5002.7

[a]Refer to Table 1.

The binding models of compounds **6a** and **6f** in the active site of FXa was proposed on the basis of X-ray crystallographic analysis of these compounds complexed to the related enzyme trypsin (Fig. 3). This study revealed that naphthalene and piperidine moieties of both compounds docked into S1 and S4 specific pockets, respectively, similar to that of YM-60828. Interestingly, however, the conformations of the benzene fused ring and the piperidine ring of compound **6f** were different from that of YM-60828.

The diazepine ring of **6a** directs solvent away from the enzyme following a similar trend to that shown by the sulfamoylacetic acid of YM-60828, and the carbonyl oxygen attached to the aniline moiety forms a hydrogen bond to the side chain amide nitrogen of Gln-192. Moreover, a further carbonyl oxygen at the diazepine ring is engaged in hydrogen bonding to the side chain phenol oxygen of Tyr-99. The piperidine ring of **6a** also has the same conformation as that of YM-60828 and the nitrogen of the acetimidoyl group forms a hydrogen bond to the carbonyl oxygen of Thr-98 and Ile-175 through a bridging water molecule. On the other hand, unlike the sulfamoylacetic acid of YM-60828, the thiadiazine ring of **6f** is distributed along the enzyme surface,

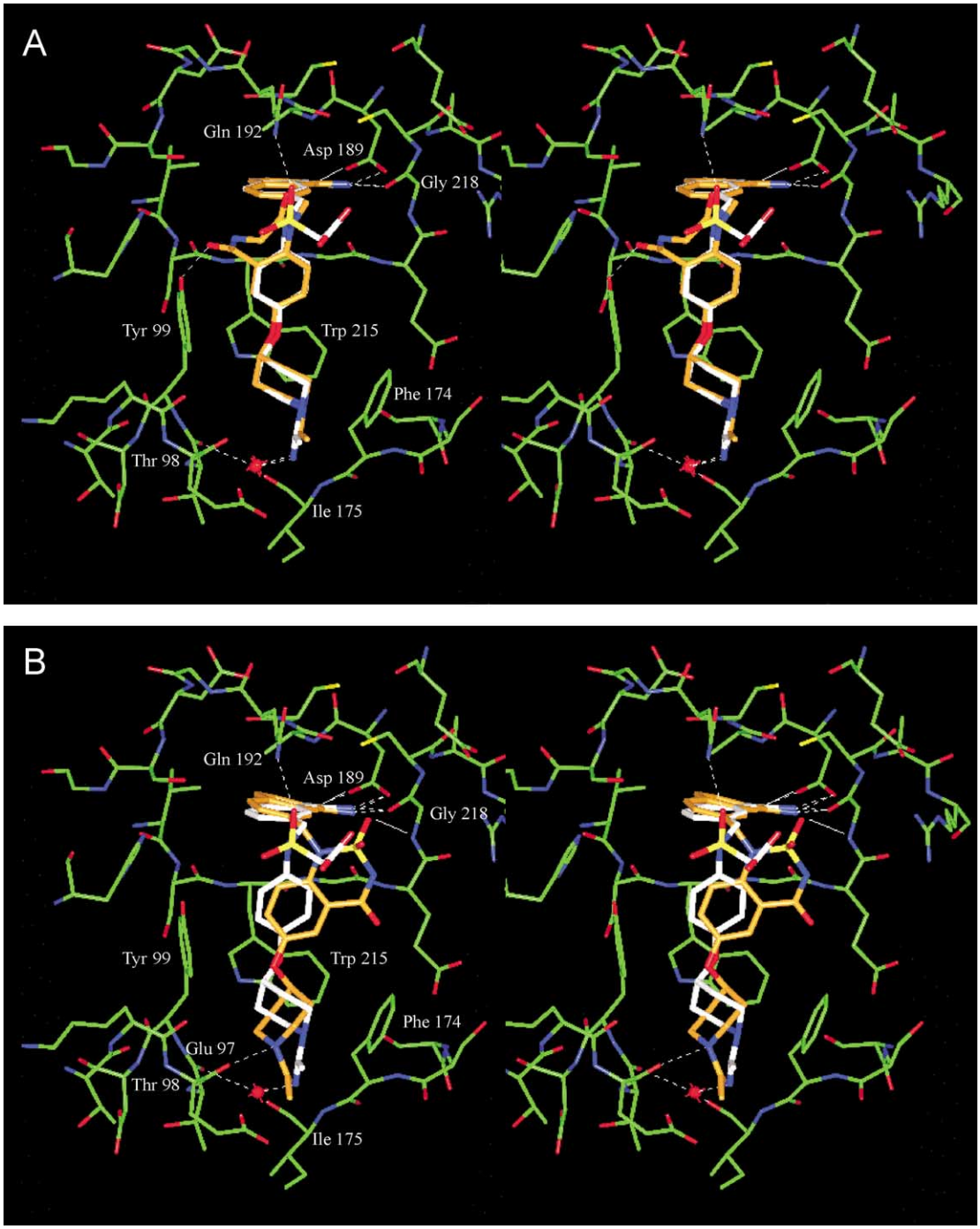


Figure 3. Stereoviews of the binding models in FXa: (A) overlay of inhibitor **6a** (with brown carbons) and YM-60828 (white); (B) overlay of inhibitor **6f** (with brown carbons) and YM-60828 (white).

Table 3. Anticoagulant activities in vitro and ex vivo

Compd	CT_2 [a] (μM)	PT/control PT[c]			
	PT[b]	Mice		Squirrel monkeys	
		0.5 h	2.0 h	1.0 h	4.0 h
6f	0.081	2.6	2.0	3.7	1.8
6g	0.085	3.7	1.9	7.1	1.6
6j	0.12	4.0	3.3	8.3	3.5
YM-60828	0.21[d]	2.6	1.7	4.3[e]	2.0[e]

[a]Values represent the concentration required to double clotting time and represent the average of four determinations with the average standard error of the mean <10%.
[b]Prothrombin time using human plasma.
[c]The relative prothrombin time compared with that measured using normal plasma were determined in blood samples taken at the indicated time points after oral administration at 100 mg/kg in mice, and at 3 mg/kg in squirrel monkeys ($n=3$).
[d]$n=3$.
[e]The data for YM-75466 (methansulfonate salt of YM-60828).

and the carbonyl oxygen of the sulfon forms a novel hydrogen bond to the backbone amide nitrogen of Gly-218. Further, the conformational change of the piperidine ring in **6f** affords an additional hydrogen bond between the piperidine nitrogen and the backbone carbonyl oxygen of Glu-97, and the water-mediated hydrogen bonds of the nitrogen of acetimidoyl group, as mentioned above, are retained. These different binding conformations might, in part, explain one order of magnitude difference of potencies observed for **6a** and **6f**.

Potent FXa inhibitors **6f**, **6g** and **6j** were further evaluated for both their anticoagulant activity in vitro and their oral anticoagulant efficacy ex vivo based on the prolongation of prothrombin time (PT). All of these compounds showed significantly potent anticoagulant activities in vitro as shown in Table 3 (CT_2, PT = 0.081–0.12 μM), reflecting their potent FXa inhibitory activities. In the ex vivo oral test, benzamidine derivatives **6g** and **6j** demonstrated potent oral efficacies in both mice and squirrel monkeys. In particular, compound **6j** (YM-169920) effectively prolonged the PT by more than 3-fold, at all time points investigated after oral administration, in both mice and squirrel monkeys.

Conclusion

We have designed and synthesized a novel series of FXa inhibitors based on a benzothiadiazine-4-one template. The docking study of the series of compound **6f** in the active site of FXa revealed that the conformation at the benzene fused ring and piperidine ring differed from that of YM-60828. This conformational change afforded novel hydrogen bonding of inhibitor **6f** to FXa. In this series of compound, three derivatives, **6f**, **6g** and **6j**, showed potent FXa inhibitory activities and anticoagulant activities in vitro. Among them, compound **6j** (YM-169920) exhibited excellent anticoagulant efficacies in both mice and squirrel monkeys after oral administration ex vivo. Further optimization studies based on compound YM-60828 will be reported in future publications.

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, 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).

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]anthranilate (3). To a stirred solution of methyl 2-amino-5-hydroxybenzoate **1** (0.83 g, 5.0 mmol) and *tert*-butyl 4-hydroxypiperidine-1-carboxylate **2** (0.86 g, 5.5 mmol) in tetrahydrofuran (THF) (10 mL) at ambient temperature was added triphenylphosphine (PPh_3) (1.57 mg, 6.0 mmol), and diethyl azodicarboxylate (DEAD) (0.94 mL, 6.0 mmol). After stirring at ambient temperature for 4 days, the reaction mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate (EtOAc) and the solution was washed with saturated aqueous $NaHCO_3$ and 10% aqueous citric acid, dried over Na_2SO_4 and then concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/*n*-hexane (Hex) (15:85) to give **3** (716 mg, 41%) as a pale brown solid: mp 96–97 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.63–1.76 (2H, m), 1.80–1.93 (2H, m), 3.22–3.32 (2H, m), 3.62–3.75 (2H, m), 3.87 (3H, s), 4.23–4.31 (1H, m), 5.47 (2H, bs), 6.63 (1H, d, $J=8.5$ Hz), 6.96 (1H, dd, $J=3.1$, 8.5 Hz), 7.41 (1H, d, $J=3.1$ Hz); FAB MS *m*/*e* $(M)^+$ 350.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-[(7-cyano-2-naphthyl)methyl]anthranilate (4a). To a stirred solution of **3** (2.70 g, 7.7 mmol) and 7-formylnaphthalene-2-carbonitril[5] (1.39 g, 7.7 mmol) in CH_2Cl_2 (40 mL) and acetic acid (AcOH) (11 mL) at ambient temperature was added sodium triacetoxyborohydride (3.27 g 15.4 mmol). After 14 h, the reaction mixture was washed with saturated aqueous $NaHCO_3$

and H_2O. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/Hex (10:90–80:20) to give **4a** (3.93 g, 99%) as a pale yellow solid: mp 125–126 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.61–1.73 (2H, m), 1.79–1.91 (2H, m), 3.21–3.31 (2H, m), 3.61–3.73 (2H, m), 3.89 (3H, s), 4.21–4.30 (1H, m), 4.62 (2H, s), 6.55 (1H, d, J = 9.1 Hz), 6.95 (1H, dd, J = 3.1, 9.1 Hz), 7.52 (1H, d, J = 3.0 Hz), 7.58 (1H, d, J = 8.2 Hz), 7.64 (1H, d, J = 8.5 Hz), 7.83 (1H, s), 7.87 (1H, d, J = 8.5 Hz), 7.89 (1H, d, J = 8.5 Hz), 8.05 (1H, bs), 8.18 (1H, s); FAB MS m/e $(M)^+$ 515.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-[(*E*)-3-cyanocinnamyl]anthranilate (4b). Compound **4b** was synthesized from **3** and (*E*)-3-cyanocinnamaldehyde[5] according to the same procedure as that for **4a**. Compound **4b** was obtained as a brown amorphous powder (99% yield): ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.62–1.77 (2H, m), 1.82–1.94 (2H, m), 3.23–3.35 (2H, m), 3.65–3.77 (2H, m), 3.88 (3H, s), 4.05 (2H, d, J = 5.0 Hz), 4.22–4.32 (1H, m), 6.37 (1H, dt, J = 5.0, 16.1 Hz), 6.58 (1H, d, J = 16.1 Hz), 6.65 (1H, d, J = 9.1 Hz), 7.04 (1H, dd, J = 2.7, 9.1 Hz), 7.39 (1H, t, J = 7.8 Hz), 7.45–7.83 (5H, m); FAB MS m/e $(M)^+$ 491.

7-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]-1-[(7-cyano-2-naphthyl)methyl]-1*H*-1,4-benzodiazepine-2,5(3*H*,4*H*)-dione (5a). To a stirred solution of **4a** (0.90 g, 1.8 mmol) in diethylether (20 mL) at ambient temperature was added bromoacetylbromide (0.23 mL, 2.6 mmol) and pyridine (0.21 mL, 2.6 mmol). After 1 h, the reaction mixture was filtered. The filtrate was evaporated and dried in vacuo. The resulting residue was dissolved in methanol and ammonia gas was bubbled through the solution at ambient temperature until the solution was saturated. The reaction mixture was stirred for 5 h at ambient temperature, and evaporated in vacuo. The resulting residue was chromatographed on silica gel eluting with MeOH/$CHCl_3$ (0:100–2:98) to give **5a** (885 mg, 94%) as a white amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.63–1.76 (2H, m), 1.83–1.96 (2H, m), 3.25–3.35 (2H, m), 3.60–3.73 (2H, m), 3.79–4.10 (2H, m), 4.44–4.51 (1H, m), 5.13 (1H, d, J = 15.8 Hz), 5.34 (1H, d, J = 15.8 Hz), 6.69 (1H, t, J = 6.4 Hz), 6.99 (1H, dd, J = 3.1, 9.1 Hz), 7.17 (1H, d, J = 9.1 Hz), 7.32 (1H, d, J = 3.1 Hz), 7.44 (1H, d, J = 8.5 Hz), 7.58 (1H, d, J = 8.5 Hz), 7.66 (1H, s), 7.83 (1H, d, J = 8.5 Hz), 7.86 (1H, d, J = 8.5 Hz), 8.14 (1H, s); FAB MS m/e $(M)^+$ 541.

7-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]-1-[(*E*)-3-cyanocinnamyl]-1*H*-1,4-benzodiazepine-2,5(3*H*,4*H*)-dione (5b). Compound **5b** was synthesized from **4b** according to the same procedure as that for **5a**. Compound **5b** was obtained as a pale yellow amorphous powder (55% yield): ^{1}H NMR (DMSO-d_6) δ 1.40 (9H, s), 1.43–1.59 (2H, m), 1.82–1.95 (2H, m), 3.01–3.25 (2H, m), 3.44–3.90 (4H, m), 4.56–4.65 (3H, m), 6.46–6.50 (2H, m), 7.16–7.22 (2H, m), 7.39–7.44 (1H, m), 7.52 (1H, t, J = 7.8 Hz), 7.62–7.73 (2H, m), 7.84–7.88 (1H, m), 8.79 (1H, t, J = 6.1 Hz); FAB MS m/e $(M)^-$ 515.

General procedure for synthesis of bis-amidine derivatives 6

7-[(1-Acetimidoyl-4-piperidyl)oxy]-1-[(7-amidino-2-naphthyl)methyl]-1*H*-1,4-benzodiazepine-2,5(3*H*,4*H*)-dione (6a). HCl gas was bubbled through a solution of **5a** (0.47 g, 0.86 mmol) in ethanol (EtOH) (30 mL) under −20 °C for 20 min. The mixture was allowed to stir for 29 h at 5 °C, and then concentrated in vacuo. To the crude imidate dissolved in EtOH (20 mL) and MeOH (30 mL) was added ammonium acetate (1.99 g, 25.8 mmol) at ambient temperature. The reaction mixture was stirred at ambient temperature for 72 h and concentrated in vacuo. The resulting residue was chromatographed on ODS gel eluting with MeOH/H_2O (0:100–2:98). MeOH was removed in vacuo, and the aqueous solution was lyophilized after acidification with 1 N HCl. 7-[(4-piperidyl)oxy]-1-[(7-amidino-2-naphthyl)methyl]-1*H*-1,4-benzodiazepine-2,5(3*H*,4*H*)-dione (369 mg, 81%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.74–1.86 (2H, m), 2.01–2.09 (2H, m), 2.98–3.07 (2H, m), 3.14–3.22 (2H, m), 3.55–3.64 (1H, m), 3.87–3.96 (1H, m), 4.62–4.70 (1H, m), 5.17 (1H, d, J = 16.2 Hz), 5.41 (1H, d, J = 16.2 Hz), 7.15 (1H, dd, J = 3.1, 9.2 Hz), 7.19 (1H, d, J = 3.1 Hz), 7.38–7.48, (2H, m), 7.79 (1H, dd, J = 1.8, 8.6 Hz), 7.83 (1H, s), 7.99 (1H, d, J = 8.5 Hz), 8.10 (1H, d, J = 9.2 Hz), 8.36 (1H s), 8.81–8.87 (1H, m), 9.22 (1H, br-s), 9.49 (2H, br-s); FAB MS m/e $(M+1)^+$ 458.

To a stirred solution of the mono-amidine intermediate (0.30 g, 0.56 mmol) in EtOH (4 mL) at ambient temperature was added ethyl acetimidate hydrochloride (0.69 g, 5.6 mmol) and triethylamine (Et_3N) (0.78 mL, 5.6 mmol). The mixture was allowed to stir for 14 h at ambient temperature, and then concentrated in vacuo. The resulting residue was chromatographed on ODS gel eluting with MeOH/H_2O (0:100–2:98). MeOH was removed in vacuo, and the aqueous solution was lyophilized after acidification with 1 N HCl. **6a** (0.17 g 46%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.63–1.80 (2H, m), 1.93–2.09 (2H, m), 2.28 (3H, s), 3.45–4.00 (6H, m), 4.67–4.78 (1H, m), 5.10–5.47 (2H, m), 7.15 (1H, dd, J = 3.1, 9.2 Hz), 7.20 (1H, d, J = 3.1 Hz), 7.42 (1H, d, J = 9.2 Hz), 7.46 (1H, d, J = 8.6 Hz), 7.80 (1H, d, J = 8.6 Hz), 7.83 (1H, s), 8.0 (1H, d, J = 8.6 Hz), 8.10 (1H, d, J = 8.6 Hz), 8.37 (1H, s), 8.77 (1H, s), 8.85 (1H, t, J = 6.1 Hz), 9.17–9.36 (3H, m), 9.50 (2H, s); FAB MS m/e $(M+1)^+$ 499. Anal. calcd for $C_{28}H_{30}N_6O_3 \cdot 2.1HCl \cdot 3.3H_2O$: C, 53.00; H, 6.15; N, 13.24; Cl, 11.73. Found: C, 52.79; H, 5.92; N, 13.17; Cl, 11.78.

7-[(1-Acetimidoyl-4-piperidyl)oxy]-1-[(*E*)-3-amidinocinnamyl]-1*H*-1,4-benzodiazepine-2,5(3*H*,4*H*)-dione (6b). Compound **6b** was synthesized from **5b** according to the same procedure as that for **6a**. Compound **6b** was obtained as a white amorphous powder (33% yield). ^{1}H NMR (DMSO-d_6) δ 1.64–1.85 (2H, m), 1.94–2.13 (2H, m) 2.31 (3H, s), 3.45–3.94 (6H, m), 4.53–4.70 (2H, m), 4.73–4.82 (1H, m), 6.48 (1H, dt, J = 4.8, 16.2 Hz), 6.58 (1H, d, J = 16.2 Hz), 7.21–7.27 (2H, m), 7.44–7.50 (1H, m), 7.52–7.60 (1H, m), 7.66–7.78 (2H, m), 7.87–7.93

(1H, m), 8.80–8.96 (2H, m), 9.32 (2H, s), 9.40–9.50 (3H, m); FAB MS *m/e* $(M+1)^+$ 475. Anal. calcd for $C_{26}H_{30}N_6O_3 \cdot 2.4HCl \cdot 3.1H_2O$: C, 50.54; H, 6.30; N, 13.60; Cl, 13.77. Found: C, 50.87; H, 6.85; N, 13.73; Cl, 13.80.

***tert*-Butyl 4-[(3-hydroxymethyl-4-nitro)phenoxy]piperidine-1-carboxylate (9).** To a stirred solution of 3-hydroxymethyl-4-nitrophenol **7** (4.50 g, 26.6 mmol) in *N*,*N*-dimethylformamide (DMF) (40 mL) was added potassium carbonate (5.70 g, 41.2 mmol), and *tert*-butyl 4-methanesulfonyloxypiperidine-1-carboxylate **8** (11.13 g, 39.8 mmol). After stirring at 100 °C for 2 h, the reaction mixture was cooled to ambient temperature and then concentrated in vacuo. The residue was dissolved in EtOAc and washed with 0.2 N aqueous NaOH, 10% aqueous citric acid. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/Hex (17:83) to give a solid. The solid was crystallized from toluene to give **9** (4.20 g, 45%) as a white solid: mp 141–142 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.71–1.85 (2H, m), 1.89–2.05 (2H, m), 2.56 (1H, t, $J=6.4$ Hz), 3.33–3.47 (2H, m), 3.62–3.75 (2H, m), 4.59–4.70 (1H, m), 5.00 (2H, d, $J=6.4$ Hz), 6.89 (1H, dd, $J=2.4$, 8.3 Hz), 7.23 (1H, d, $J=2.4$ Hz), 8.18 (1H, d, $J=8.3$ Hz); FAB MS *m/e* $(M+1)^+$ 353.

***tert*-Butyl 4-[(3-cyanomethyl-4-nitro)phenoxy]piperidine-1-carboxylate (10).** To a stirred solution of **9** (2.10 g, 5.97 mmol) in 1,2-dichloroethane (20 mL) at 3 °C was added Et_3N (0.92 mL, 6.57 mmol) and methanesulfonylchloride (0.49 mL, 6.27 mmol). After stirring at ambient temperature for 1 h, the reaction mixture was washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and dried over Na_2SO_4. The solution was concentrated in vacuo to give *tert*-butyl 4-[(3-methanesulfonyloxymethyl-4-nitro)phenoxy]piperidine-1-carboxylate (2.50 g, 97%) as a brown solid which was used without further purification: FAB MS *m/e* $(M+1)^+$ 431.

To a stirred solution of sodium cyanide (6.32 g, 129 mmol) in DMF (500 mL) at 3 °C was added the solution of resulting product above (5.56 g, 12.9 mmol) in DMF (200 mL) dropwise. After stirring at 3 °C for 1.5 h, the reaction mixture was diluted with H_2O and extructed with diethylether. The organic solution was dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/toluene (9:91) to give **10** (3.92 g, 84%) as a pale yellow solid: mp 121–122 °C; ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s), 1.72–1.86 (2H, m), 1.90–2.03 (2H, m), 3.34–3.46 (2H, m), 3.63–3.76 (2H, m), 4.25 (2H,s), 4.60–4.69 (1H, m), 6.97 (1H, dd, $J=2.4$, 8.8 Hz), 7.20 (1H, d, $J=2.4$ Hz), 8.25 (1H, d, $J=8.8$ Hz); FAB MS *m/e* $(M+1)^+$ 362.

***tert*-Butyl 4-[(3-phthalimidomethyl-4-nitro)phenoxy]piperidine-1-carboxylate (11).** To a stirred solution of **9** (3.97 g, 11.3 mmol) and phthalimide (1.82 g, 12.4 mmol) in THF (50 mL) at ambient temperature was added PPh_3 (3.25 g, 12.4 mmol) and DEAD (1.95 mL, 12.4 mmol). After stirring at ambient temperature for 4 h, the reaction mixture was concentrated in vacuo. H_2O was added to the residue and the solution was extracted with diethylether. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/Hex (20:80–25:75) to give **11** (4.50 g, 83%) as a yellow solid: mp 122–123 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 1.62–1.73 (2H, m), 1.78–1.91 (2H, m), 3.20–3.29 (2H, m), 3.58–3.70 (2H, m), 4.42–4.50 (1H, m), 5.33 (2H, s), 6.61 (1H, d, $J=2.5$ Hz), 6.85 (1H, dd, $J=2.5$, 9.3 Hz), 7.76–7.84 (2H, m), 7.89–7.96 (2H, m), 8.20 (1H, d, $J=9.3$ Hz); FAB MS *m/e* $(M+1)^+$ 482.

***tert*-Butyl 4-{[4-amino-3-(2-aminoethyl)]phenoxy}piperidine-1-carboxylate (12a).** To a stirred solution of **10** (7.71 g, 21.4 mmol) in THF (120 mL) was added 1.0 M solution of boran-THF complex in THF (85.4 mL, 85.4 mmol) and the mixture was refluxed for 2 h. After cooling, MeOH (24 mL) was added to the reaction mixture and it was stirred for 10 min. Then the reaction mixture was concentrated in vacuo. The resulting residue was dissolved in 15% aqueous citric acid (200 mL) and EtOAc (50 mL). After stirring for 10 min at ambient temperature, the solution was made basic with 1 N aqueous NaOH and extracted with AcOEt. It was dried over $MgSO_4$ and concentrated in vacuo to give *tert*-butyl 4-{[3-(2-aminoethyl)-4-nitro]phenoxy}piperidine-1-carboxylate (6.69 g, 86%). ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.65–1.84 (2H, m), 1.88–2.06 (4H, m), 3.07–3.22 (2H, m), 3.29–3.43 (2H, m), 3.62–3.76 (2H, m), 4.54–4.66 (1H, m), 6.79–6.90 (2H, m), 8.06 (1H, d, $J=8.7$ Hz); FAB MS *m/e* $(M+1)^+$ 366.

To a stirred solution of the resulting product above (2.19 g, 6.0 mmol) in EtOH (30 mL) and H_2O (10 mL) at ambient temperature was added ammonium chloride (0.16 g, 3 mmol) and iron powder (3.35 g, 60 mmol). The mixture was refluxed for 40 min. After cooling, the reaction mixture was filtered through a pad of Celite and concentrated in vacuo. The resulting residue was dissolved in EtOAc, and the solution was washed with 1 N aqueous NaOH. It was dried over $MgSO_4$ and concentrated. The crude product mixture was chromatographed on ODS gel eluting with MeOH/H_2O (50:50–60:40) to give **12a** (0.80 g, 40%) as a brown viscous oil. ^{1}H NMR (DMSO-d_6) δ 1.46 (9H, s), 1.65–1.76 (2H, m), 1.83–1.93 (2H, m), 2.33 (4H, br-s), 2.67 (2H, t, $J=6.8$ Hz), 3.00 (2H, t, $J=6.8$ Hz), 3.22–3.31 (2H, m), 3.63–3.75 (2H, m), 4.23–4.30 (1H, m), 6.59–6.68 (3H, m); FAB MS *m/e* $(M+1)^+$ 336.

***tert*-Butyl 4-[(4-amino-3-aminomethyl)phenoxy]piperidine-1-carboxylate (12b).** To a stirred solution of **11** (3.79 g, 7.88 mmol) in EtOH (60 mL) was added hydrazine monohydrate (1.91 mL, 39.4 mmol) and the mixture was heated at 50 °C for 17 h. After cooling, the reaction mixture was filtered and concentrated in vacuo. Resulting residue was dissolved in $CHCl_3$ and the solution was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated in vacuo to give *tert*-Butyl 4-[(3-aminomethyl-4-nitro)phenoxy]piperidine-1-carboxylate (2.74 g, 99%): ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s),

1.70–1.85 (4H, m), 1.89–2.20 (2H, m), 3.32–3.45 (2H, m), 3.62–3.76 (2H, m), 4.15 (2H, s), 4.62–4.67 (1H, m), 6.85 (1H, dd, $J=2.4$, 9.0 Hz), 7.12 (1H, d, $J=2.4$ Hz), 8.11 (1H, d, $J=9.0$ Hz), FAB MS m/e $(M+1)^+$ 352.

Compound **12b** was synthesized from the above intermediate according to the same procedure as that for **12a**. Compound **12b** was obtained as a brown viscous oil (57% yield): ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.62–1.76 (2H, m), 1.80–1.93 (2H, m), 3.21–3.32 (2H, m), 3.65–3.76 (2H, m), 3.84 (2H, s), 4.20–4.31 (1H, m), 6.57–6.63 (1H, m), 6.66–6.72 (2H, m); FAB MS m/e $(M+1)^+$ 322.

7-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]-1,3,4,5-tetrahydro-2,1,3-benzothiadiazepine 2,2-dioxide (13a). To a stirred solution of **12a** (0.77 g, 2.3 mmol) in pyridine (15 mL) was added sulfamide (1.54 g, 16.0 mmol) and the mixture was refluxed for 2 h. After cooling, the reaction mixture was concentrated in vacuo. The residual pyridine was removed by adding toluene and then evaporating to dryness. The residue was dissolved in $CHCl_3$ and the solution was washed with H_2O, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was crystallized from benzene to give **13a** (0.54 g, 60%) as a white solid: mp 165–166 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.67–1.80 (2H, m), 1.83–1.96 (2H, m), 2.95–3.03 (2H, m), 3.28–3.39 (2H, m), 3.44–3.53 (2H, m), 3.62–3.73 (2H, m), 4.37–4.46 (1H, m), 4.48–4.57 (1H, m), 6.32 (1H, s), 6.69 (1H, d, $J=2.7$ Hz), 6.75 (1H, dd, $J=2.7$, 8.4 Hz), 7.03 (1H, d, $J=8.4$ Hz); FAB MS m/e $(M)^+$ 397.

6-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]-3,4-dihydro-1*H*-2,1,3-benzothiadiazine 2,2-dioxide (13b). Compound **13b** was synthesized from **12b** according to the same procedure as that for **13a**. Compound **13b** was obtained as a pale brown solid (83% yield): mp 230–231 °C; ^{1}H NMR (DMSO-d_6) δ 1.40 (9H, s), 1.38–1.55 (2H, m), 1.80–1.92 (2H, m), 3.10–3.23 (2H, m), 3.56–3.68 (2H, m), 4.36 (2H, d, $J=7.8$ Hz), 4.33–4.46 (1H, m), 6.64 (1H, d, $J=9.0$ Hz), 6.88–6.86 (2H, m), 7.15 (1H, t, $J=7.8$ Hz), 9.81 (1H, s); FAB MS m/e $(M)^+$ 383.

7-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]-1-[(*E*)-3-cyanocinnamyl]-1,3,4,5-tetrahydro-2,1,3-benzothiadiazepine 2,2-dioxide (5c). To a stirred solution of **13a** (374 mg, 0.94 mmol) and 3-((*E*)-3-hydroxypropenyl)benzonitrile (179 mg, 1.13 mmol) in THF (6 mL) at ambient temperature was added PPh_3 (345 mg, 1.32 mmol) and DEAD (0.21 mL, 1.32 mmol). After stirring at ambient temperature for 4.5 h, the reaction mixture was diluted with EtOAc and washed with H_2O. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/Hex (40:60) to give **5c** (375 mg, 74%) as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.40 (9H, s), 1.40–1.55 (2H, m), 1.80–1.92 (2H, m), 2.80–2.92 (2H, m), 3.05–3.28 (4H, m), 3.60–3.70 (2H, m), 4.26 (2H, d, $J=6.4$ Hz), 4.42–4.55 (1H, m), 6.48 (1H, d, $J=16.4$ Hz), 6.59 (1H, dd, $J=6.4$, 16.4 Hz), 6.75–6.82 (2H, m), 7.12–7.18 (1H, m), 7.47–7.57 (2H, m), 7.69 (1H, d, $J=7.6$ Hz), 7.76 (1H, d, $J=7.6$ Hz), 7.94 (1H, s); FAB MS m/e $(M-1)^-$ 537.

6-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]-1-[(*E*)-3-cyanocinnamyl]-3,4-dihydro-1*H*-2,1,3-benzothiadiazine 2,2-dioxide (5d). Compound **5d** was synthesized from **13b** according to the same procedure as that for **5c**. Compound **5d** was obtained as a pale yellow amorphous powder (50% yield): ^{1}H NMR (DMSO-d_6) δ 1.40 (9H, s), 1.40–2.05 (2H, m), 1.77–1.92 (2H, m), 3.08–3.22 (2H, m), 3.59–3.69 (2H, m), 4.40–4.56 (5H, m), 6.55 (1H, dt, $J=5.2$, 15.6 Hz), 6.67 (1H, d, $J=15.6$ Hz), 6.84 (1H, d, $J=2.8$ Hz), 6.88 (1H, dd, $J=2.8$, 8.8 Hz), 6.97 (1H, d, $J=8.8$ Hz), 7.52 (1H, t, $J=8.0$ Hz), 7.61–7.75 (3H, m), 7.90 (1H, s) ; FAB MS m/e $(M)^+$ 524.

6-[(1-*tert*-Butoxycarbonyl-4-piperidyl)oxy]-1-[(*E*)-3-cyanocinnamyl]-3-methyl-3,4-dihydro-1*H*-2,1,3-benzothiadiazine 2,2-dioxide (5e). To a stirred solution of **5d** (0.43 g, 0.82 mmol) in CH_3CN (10 mL) at ambient temperature was added K_2CO_3 (0.34 g, 2.46 mmol) and methyliodide (0.23 g, 1.63 mmol). After stirring at ambient temperature for 22 h, the reaction mixture was filtered and dried in vacuo. The resulting residue was dissolved in EtOAc and the solution was washed with H_2O, dried over Na_2SO_4 and concentrated in vacuo to give **5e** (446 mg) as a pale yellow amorphous powder which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.63–1.76 (2H, m), 1.81–1.93 (2H, m), 2.79 (3H, s), 3.22–3.37 (2H,m), 3.62–3.73 (2H, m), 4.32–4.41 (1H, m), 4.57–4.62 (4H, m), 6.36 (1H, dt, $J=5.4$, 15.6 Hz), 6.64–6.71 (2H, m), 6.79–6.87 (2H, m), 7.41 (1H, t, $J=7.3$ Hz), 7.51 (1H, d, $J=7.3$ Hz), 7.57 (1H, d, $J=7.3$ Hz), 7.63 (1H, s); FAB MS m/e $(M)^+$ 538.

7-[(1-Acetimidoyl-4-piperidyl)oxy]-1-[(*E*)-3-amidinocinnamyl]-1,3,4,5-tetrahydro-2,1,3-benzothiadiazepine 2,2-dioxide (6c). Compound **6c** was synthesized from **5c** according to the same procedure as that for **6a**. Compound **6c** was obtained as a white amorphous powder (57% yield): ^{1}H NMR (DMSO-d_6) δ 1.62–1.80 (2H, m), 1.97–2.10 (2H, m), 2.29 (3H, s), 2.85–2.96 (2H, m), 3.14–3.23 (2H, m), 3.43–3.58 (2H, m), 3.61–3.87 (2H, m), 4.29 (2H, d, $J=6.4$ Hz), 4.61–4.69 (1H, m), 6.52 (1H, d, $J=15.7$ Hz), 6.66 (1H, dt, $J=6.4$, 15.7 Hz), 6.79–6.80 (2H, m), 7.17 (1H, d, $J=8.3$ Hz), 7.56 (1H, t, $J=7.8$ Hz), 7.64 (1H, t, $J=6.8$ Hz), 7.71 (1H, d, $J=7.8$ Hz), 7.75 (1H, d, $J=7.8$ Hz), 7.97 (1H, s), 8.82 (1H, s), 9.25 (2H, s), 9.36 (1H, s), 9.45 (2H, s); FAB MS m/e $(M+1)^+$ 497. Anal. calcd for $C_{25}H_{32}N_6O_3S\cdot2.1HCl\cdot3.0H_2O$: C, 47.87; H, 6.44; N, 13.40; S, 5.11; Cl, 11.87. Found: C, 48.00; H, 6.48; N, 13.54; S, 5.23; Cl, 12.02.

6-[(1-Acetimidoyl-4-piperidyl)oxy]-1-[(*E*)-3-amidinocinnamyl]-3,4-dihydro-1*H*-2,1,3-benzothiadiazine 2,2-dioxide (6d). Compound **6d** was synthesized from **5d** according to the same procedure as that for **6a**. Compound **6d** was obtained as a white amorphous powder (47% yield): ^{1}H NMR (DMSO-d_6) δ 1.63–1.79 (2H, m), 1.95–2.08 (2H, m), 2.29 (3H, s), 3.46–3.60 (2H, m), 3.65–3.85 (2H, m), 4.46 (2H, d, $J=7.6$ Hz), 4.54 (2H, d, $J=5.3$ Hz), 4.56–4.65 (1H, m), 6.60 (1H, dt, $J=5.3$, 16.1 Hz), 6.73 (1H, d, $J=16.1$ Hz), 6.86–6.94 (2H, m), 7.98 (1H, d, $J=9.1$ Hz),

7.56 (1H, t, $J=7.6$ Hz), 7.67–7.82 (3H, m), 7.90 (1H, s), 8.80 (1H, s), 9.20 (2H, s), 9.32 (1H, s), 9.43 (2H, s); FAB MS m/e $(M+1)^+$ 483. Anal. calcd for $C_{24}H_{30}N_6O_3S\cdot 2.0HCl\cdot 2.3H_2O$: C, 48.29; H, 6.18; N, 14.08; S, 5.37; Cl, 11.88. Found: C, 48.10; H, 5.94; N, 14.21; S, 5.29; Cl, 12.20.

6-[(1-Acetimidoyl-4-piperidyl)oxy]-1-[(*E*)-3-amidinocinnamyl]-3-methyl-3,4-dihydro-1*H*-2,1,3-benzothiadiazine 2,2-dioxide (6e). Compound **6e** was synthesized from **5e** according to the same procedure as that for **6a**. Compound **6e** was obtained as a white amorphous powder (54% yield): ^{1}H NMR (DMSO-d_6) δ 1.63–1.81 (2H, m), 1.96–2.09 (2H, m), 2.30 (3H, s), 2.70 (3H, s), 3.44–3.60 (2H, m), 3.66–3.88 (2H, m), 4.58–4.65 (5H, m), 6.58 (1H, dt, $J=5.4$, 16.1 Hz), 6.77 (1H, d, $J=16.1$ Hz), 6.89 (1H, d, $J=2.5$ Hz), 6.96 (1H, dd, $J=2.5$, 9.2 Hz), 7.02 (1H, d, $J=9.2$ Hz), 7.57 (1H, t, $J=7.8$ Hz), 7.72 (1H, d, $J=7.8$ Hz), 7.77 (1H, d, $J=7.8$ Hz), 7.89 (1H, s), 8.87 (1H,s), 9.27 (2H,s), 9.39 (1H, s), 9.45 (2H, s); FAB MS m/e $(M+1)^+$ 497. Anal. calcd for $C_{25}H_{32}N_6O_3S\cdot 2.0HCl\cdot 2.4H_2O$: C, 49.00; H, 6.38; N, 13.71; S, 5.23; Cl, 11.57. Found: C, 48.96; H, 6.66; N, 13.69; S, 5.18; Cl, 11.86.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-(*tert*-butoxycarbonylsulfamoyl)-*N*-[(7-cyano-2-naphthyl)-methyl]anthranilate (14a). To a stirred solution of **4a** (0.98 g, 1.89 mmol) in pyridine (15 mL) at ambient temperature was added *tert*-butyl chlorosulfonylcarbamate[5] (0.81 g, 3.76 mmol). After 1 h, the reaction mixture was concentrated in vacuo. The resulting residue was dissolved in EtOAc and the solution was washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and H_2O, then concentrated in vacuo. The residue was chromatographed on silica gel eluting with EtOAc/Hex (30:70) to give **14a** (1.07 g, 82%) as a brown amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.53 (9H, s), 1.62–1.75 (2H, m), 1.80–1.92 (2H, m), 3.26–3.39 (2H, m), 3.57–3.69 (2H, m), 3.84 (3H, s), 4.38–4.47 (1H, m), 4.92 (1H, br-s), 5.29 (1H, br-s), 6.85 (1H, dd, $J=3.0$, 8.5 Hz), 7.01 (1H, d, $J=8.5$ Hz), 7.35 (1H, d, $J=3.0$ Hz), 7.59 (1H, dd, $J=1.8$, 8.5 Hz), 7.63 (1H,s), 7.69 (1H, dd, $J=1.8$, 8.5 Hz), 7.82 (1H, d, $J=8.5$ Hz), 7.88 (1H, d, $J=8.5$ Hz), 7.92 (1H, s), 8.10 (1H, s); FAB MS m/e $(M-1)^-$ 693.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-(*tert*-butoxycarbonylsulfamoyl)-*N*-[(*E*)-3-cyanocinnamyl]-anthranilate (14b). Compound **14b** was synthesized from **4b** according to the same procedure as that for **14a**. Compound **14b** was obtained as a brown amorphous powder (99% yield): ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.53 (9H, s), 1.70–1.80 (2H, m), 1.86–1.95 (2H, m), 3.30–3.39 (2H, m), 3.60–3.71 (2H, m), 3.89–3.92 (5H, m), 4.45–4.53 (1H, m), 6.33 (1H, d, $J=15.9$ Hz), 6.46 (1H, dt, $J=6.9$, 15.9 Hz), 7.03 (1H, dd, $J=3.0$, 8.7 Hz), 7.24–7.27 (1H, m), 7.36–7.42 (2H, m), 7.48–7.55 (3H, m), 8.60 (1H, br); FAB MS m/e $(M-1)^-$ 669.

1-[(7-Cyano-2-naphthyl)methyl]-6-[(4-piperidyl)oxy]-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (15a). To a stirred solution of **14a** (1.03 g, 1.49 mmol) in CH_2Cl_2 (12 mL) at 3 °C was added trifluoroacetic acid (12 mL). After 1 h, the reaction mixture was concentrated in vacuo. To the resulting residue in EtOH (30 mL) was added sodium ethoxide (NaOEt) (0.46 g, 2.98 mmol) and the mixture was stirred for 30 min at 3 °C. Then NaOEt (0.10 g, 1.49 mmol) was added to the mixture again and the mixture was stirred at 3 °C for 50 min. To the resulting mixture was further added NaOEt (0.10 g, 1.49 mmol) and the mixture was stirred for 2 h. The mixture was concentrated in vacuo and the residue was chromatographed on ODS gel eluting with MeOH/H_2O (0:100–100:0) to give **15a** (0.60 g, 87%) as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.63–1.76 (2H, m), 1.92–2.03 (2H, m), 2.88–3.06 (2H, m), 3.10–3.21 (2H, m), 4.43–4.51 (1H, m), 5.10 (2H, s), 6.72 (1H, d, $J=9.3$ Hz), 6.91 (1H, dd, $J=2.9$, 8.8 Hz), 7.49 (1H, d, $J=3.0$ Hz), 7.74 (1H, dd, $J=1.9$, 8.8 Hz), 7.79 (1H, dd, $J=1.5$, 8.8 Hz), 7.99–8.09 (3H, m), 8.50 (1H, s); FAB MS m/e $(M+1)^+$ 463.

1-[(*E*)-3-Cyanocinnamyl]-6-[(4-piperidyl)oxy]-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (15b). Compound **15b** was synthesized from **14b** according to the same procedure as that for **15a**. Compound **15b** was obtained as a pale yellow solid (86% yield): mp 162–163 °C; ^{1}H NMR (DMSO-d_6) δ 1.45–1.60 (2H, m), 1.84–1.99 (2H, m), 2.62–2.77 (2H, m), 2.93–3.08 (2H, m), 4.29–4.40 (1H, m), 4.50 (2H, d, $J=5.0$ Hz), 6.53 (1H, dt, $J=5.0$, 16.2 Hz), 6.68 (1H, d, $J=16.2$ Hz), 6.91 (1H, d, $J=8.7$ Hz), 7.01 (1H, dd, $J=3.1$, 8.7 Hz), 7.45 (1H, d, $J=3.1$ Hz), 7.50 (1H, t, $J=7.8$ Hz), 7.67 (1H, d, $J=7.8$ Hz), 7.72 (1H, d, $J=7.8$ Hz), 7.87 (1H, s); FAB MS m/e $(M+1)^+$ 439.

6-[(1-Acetimidoyl-4-piperidyl)oxy]-1-[(7-amidino-2-naphthyl)methyl]-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (6f). Compound **6f** was synthesized from **15a** according to the same procedure as that for **6a**. Compound **6f** was obtained as a white amorphous powder (31% yield): ^{1}H NMR (DMSO-d_6) δ 1.63–1.79 (2H, m), 1.93–2.05 (2H, m), 2.27 (3H, s), 3.40–4.80 (4H, m), 4.61–4.65 (1H, m), 5.23 (2H, s), 7.02 (1H, d, $J=9.2$ Hz), 7.13 (1H, d, $J=9.2$ Hz), 7.51 (1H, s), 7.74 (1H, d, $J=8.5$ Hz). 7.82 (1H, d, $J=8.5$ Hz), 8.01 (1H, s), 8.06 (1H, d, $J=8.5$ H), 8.12 (1H, d, $J=8.5$ Hz), 8.42 (1H, s), 8.66 (1H, s), 9.15 (2H, s), 9.21 (1H, s), 9.45 (2H, s); FAB MS m/e $(M+1)^+$ 521. Anal. calcd for $C_{26}H_{28}N_6O_4S\cdot 1.8HCl\cdot 3.0H_2O$: C, 48.77; H, 5.64; N, 13.13; S, 5.01; Cl, 9.97. Found: C, 49.07; H, 5.55; N, 13.27; S, 5.07; Cl, 9.81.

6-[(1-Acetimidoyl-4-piperidyl)oxy]-1-[(*E*)-3-(3-amidinophenyl)allyl]-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (6g). Compound **6g** was synthesized from **15b** according to the same procedure as that for **6a**. Compound **6g** was obtained as a white amorphous powder (25% yield): ^{1}H NMR (DMSO-d_6) δ 1.67–1.83 (2H, m), 1.97–2.11 (2H, m), 2.31 (3H, s), 3.48–3.65 (2H, m), 3.67–3.88 (2H, m), 4.66 (2H, d, $J=5.3$ Hz), 4.74–4.82 (1H, m), 6.56 (1H, dt, $J=5.3$, 15.6 Hz), 6.72 (1H, d, $J=15.6$ Hz), 7.38–7.42 (2H, m), 7.51–7.54 (1H, m), 7.57 (1H, t, $J=7.9$ Hz), 7.70–7.76 (2H, m), 7.89 (1H, s), 8.86 (1H, s), 9.29 (2H, s), 9.41 (1H, s), 9.45 (2H, s); FAB MS

m/e $(M+1)^+$ 497. Anal. calcd for $C_{24}H_{28}N_6O_4S{\cdot}2.1HCl{\cdot}2.0H_2O$: C, 47.32; H, 5.64; N, 13.80; S, 5.26; Cl, 12.22. Found: C, 47.12; H, 5.62; N, 13.82; S, 5.31; Cl, 12.15.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-(*tert*-butoxycarbonylsulfamoyl)-*N*-[3-(3-cyanophenyl)propyl]-anthranilate (14c). To a stirred suspension of PdO/$BaSO_4$[9] (0.40 g) in EtOH (30 mL) at ambient temperature was added **14b** (1.00 g, 1.49 mmol) and the mixture was treated with hydrogen at 1 atm for 24 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with EtOAc/Hex (30:70) to give **14c** (0.90 g, 90%) as a brown amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.50 (9H, s), 1.66–2.00 (6H, m), 2.63–2.75 (2H, m), 3.29–3.45 (2H, m), 3.60–4.00 (7H, m), 4.40–4.59 (1H, m), 7.00–7.67 (7H, m); FAB MS *m/e* $(M-1)^-$ 671.

6-[(1-Acetimidoyl-4-piperidyl)oxy]-1-[3-(3-amidinophenyl)-propyl]-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (6h). Compound **6h** was synthesized from **14c** according to the same procedure as that for **6f**. Compound **6h** was obtained as a pale brown amorphous powder (16% yield): ^{1}H NMR (DMSO-d_6) δ 1.61–1.83 (2H, m), 1.83–2.18 (4H, m), 2.33 (3H, s), 2.73 (2H, t, $J=7.5$ Hz), 3.48–3.94 (6H, m), 4.74–4.86 (1H, m), 7.44 (2H, s), 7.48–7.60 (3H, m), 7.68–7.74 (2H, m), 8.98 (1H, s), 9.37 (2H, s), 9.48 (2H, s), 9.54 (1H, s); FAB MS *m/e* $(M+1)^+$ 499. Anal. calcd for $C_{24}H_{30}N_6O_4S{\cdot}2.4HCl{\cdot}2.0H_2O$: C, 46.33; H, 5.90; N, 13.51; S, 5.15; Cl, 13.68. Found: C, 46.52; H, 5.65; N, 13.11; S, 4.81; Cl, 13.68.

3-[(2-Oxo)ethoxy]benzonitrile (17). To a stirred suspension of silica gel (Kieselgel-60, 230–400 mesh, 50 g) in CH_2Cl_2 (400 mL) at ambient temperature was added sodium periodate (0.65 M aqueous solution, 50 mL, 32.5 mmol) and **16**[8] (0.31 M solution in CH_2Cl_2, 77 mL, 23.6 mmol). After stirring at ambient temperature for 20 min, the reaction mixture was chromatographed on silica gel directly eluting with $CHCl_3$/MeOH (100:0–95:5) to give a crude purified **17** (4.36 g) as a colorless viscous oil, which was used without further purification: GC MS *m/e* $(M)^+$ 161.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-[2-(3-cyanophenoxy)ethyl]anthranilate (4c). Compound **4c** was synthesized from **3** and **17** according to the same procedure as that for **4a**. Compound **4c** was obtained as a pale yellow solid (49% yield): mp 117–118 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.62–1.78 (2H, m), 1.82–1.94 (2H, m), 3.23–3.35 (2H, m), 3.59–3.78 (4H, m), 3.85 (3H, s), 4.20 (2H, t, $J=5.4$ Hz), 4.23–4.33 (1H, m), 6.72 (1H, d, $J=9.0$ Hz), 7.07 (1H, dd, $J=2.7$, 9.0 Hz), 7.13–7.19 (2H, m), 7.23–7.28 (1H, m), 7.33–7.41 (1H, m), 7.50 (1H, d, $J=2.7$ Hz), 7.60–7.80 (1H, br-s); FAB MS *m/e* $(M)^+$ 495.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-(*tert*-butoxycarbonylsulfamoyl)-*N*-[2-(3-cyanophenoxy)ethyl]anthranilate (14d). Compound **14d** was synthesized from **4c** according to the same procedure as that for **14a**. Compound **14d** was obtained as a pale yellow amorphous powder (88% yield): ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.49 (9H, s), 1.65–1.83 (2H, m), 1.86–2.00 (2H, m), 3.31–3.44 (2H, m), 3.61–3.74 (2H, m), 3.85 (3H, s), 4.10–4.35 (4H, m), 4.48–4.58 (1H, m), 6.98–7.09 (2H, m), 7.22 (1H, dt, $J=7.2$, 1.5 Hz), 7.33 (2H, d, $J=9.0$ Hz), 7.42 (1H, d, $J=3.0$ Hz), 7.59 (1H, br-s); FAB MS *m/e* $(M-1)^-$ 673.

6-[(1-Acetimidoyl-4-piperidyl)oxy]-1-[2-(3-amidinophenoxy)ethyl]-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (6i). Compound **6i** was synthesized from **14d** according to the same procedure as that for **6f**. Compound **6i** was obtained as a white amorphous powder (23% yield): ^{1}H NMR (DMSO-d_6) δ 1.68–1.86 (2H, m), 1.98–2.13 (2H, m), 2.32 (3H, s), 3.48–3.67 (2H, m), 3.67–3.89 (2H, m), 4.20–4.31 (4H, m), 4.76–4.85 (1H, m), 7.17 (1H, dd, $J=2.2$, 8.5 Hz), 7.33 (1H, s), 7.39–7.46 (2H, m), 7.46–7.52 (2H, m), 7.60 (1H, d, $J=8.8$ Hz), 8.86 (1H, s), 9.27 (2H, s), 9.39 (1H, s), 9.42 (2H, s); FAB MS *m/e* $(M+1)^+$ 501. Anal. calcd for $C_{23}H_{28}N_6O_5S{\cdot}2.1HCl{\cdot}3.0H_2O$: C, 43.77; H, 5.76; N, 13.31; S, 5.08; Cl, 11.80. Found: C, 43.52; H, 5.45; N, 13.18; S, 4.88; Cl, 12.08.

Methyl *N*-benzyloxycarbonylmethyl-5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-(2,2,2-trifluoroacetyl)anthranilate (18). To a stirred solution of **3** (7.13 g, 30.4 mmol) in 1,2-dichloroethane (150 mL) at ambient temperature was added pyridine (4.73 mL 60.5 mmol) and trifluoroacetic anhydride (4.25 mL, 30.5 mmol). After stirring at ambient temperature for 3 h, the mixture was poured into water and extracted with $CHCl_3$. The organic extracts were washed with water and dried over Na_2SO_4, and concentrated to give methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-(2,2,2-trifluoroacetyl)-anthranilate (9.43 g) which was used without further purification: FAB MS *m/e* $(M+1)^+$ 447.

To a suspension of NaH (60% in paraffin liquid, 0.24 g, 6.0 mmol) in DMF (30 mL) at 3 °C was added the above anthranilic acid derivative (2.34 g) and the mixture was stirred at ambient temperature for 50 min. Benzyl 2-bromoacetate (1.22 mL, 7.86 mmol) was then added to the mixture at 3 °C and the mixture was stirred at ambient temperature for 14 h. The reaction mixture was diluted with water and the solution was extracted with EtOAc. 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 EtOAc/Hex (15:85) to give **18** (2.42 g, 81%) as a pale yellow viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.67–1.82 (2H, m), 1.87–2.00 (2H, m), 3.32–3.44 (2H, m), 3.62–3.75 (2H, m), 3.79 (1H, d, $J=17.4$ Hz), 3.87 (3H, s), 4.49–4.58 (1H, m), 5.06 (1H, d, $J=17.4$ Hz), 5.20 (2H, d, $J=2.1$ Hz), 7.03 (1H, dd, $J=2.7$, 8.7 Hz), 7.31–7.38 (5H, m), 7.51–7.57 (2H, m); FAB MS *m/e* $(M+1)^+$ 595

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-{[(3-cyanophenyl)carbamoyl]methyl}-*N*-(2,2,2-trifluoroacetyl)-anthranilate (19). To a stirred suspension of 10% Pd/C powder (0.20 g) in MeOH (50 mL) at ambient temperature was added **18** (2.60 g, 4.37 mmol) and the mixture

was treated with hydrogen at 1 atm for 3 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo to give *N*-{2-methoxycarbonyl-4-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]}phenyl-*N*-(2,2,2-trifluoroacetyl)glycine (2.16 g) which was used without further purification: FAB MS *m*/*e* $(M-1)^-$ 503.

To a stirred solution of above glicine derivative (2.10 g) and 3-aminobenzonitrile (0.49 g, 4.16 mmol) in DMF (10 mL) at 3 °C was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.88 g, 4.58 mmol) and 1-hydroxybenzotriazole (0.56 g, 4.58 mmol). After stirring at ambient temperature for 17 h, The reaction mixture was diluted with water and the solution was extracted with EtOAc. 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 EtOAc/Hex (20:80–35:75) to give **19** (1.67 g, 65%) as a white amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.69–1.85 (2H, m), 1.87–2.02 (2H, m), 3.32–3.46 (2H, m), 3.60–3.75 (2H, m), 3.88 (3H, s), 4.25 (1H, d, J = 15.6 Hz), 4.49–4.68 (2H, m), 7.13 (1H, dd, J = 3.0, 9.0 Hz), 7.36–7.49 (3H, m), 7.57 (1H, d, J = 3.0 Hz), 7.74 (1H, dt, J = 6.6, 2.4 Hz), 7.99–8.03 (1H, m), 8.98 (1H, s); FAB MS *m*/*e* $(M+1)^+$ 605.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-(*tert*-butoxycarbonylsulfamoyl)-*N*-{[(3-cyanophenyl)carbamoyl]methyl}anthranilate (14e). A mixture of **19** (1.50 g, 2.48 mmol) and K_2CO_3 (0.68 g, 4.96 mmol) in MeOH (10 mL) and H_2O (10 mL) was stirred for 24 h. The mixture was concentrated to remove MeOH and to the resulting aqueous solution was added brine followed by extraction with EtOAc. The organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel eluting with MeOH/$CHCl_3$ (3:97) to give a crude purified methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-{[(3-cyanophenyl)carbamoyl]methyl}anthranilate (0.97 g, 77%) which was used without further purification: FAB MS *m*/*e* $(M)^+$ 508.

Compound **14e** was synthesized from above intermediate according to the same procedure as that for **14a**. Compound **14e** was obtained as a pale brown amorphous powder (56% yield): ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.51 (9H, s), 1.66–1.83 (2H, m), 1.83–2.00 (2H, m), 3.30–3.44 (2H, m), 3.58–3.70 (2H, m), 4.02 (3H, s), 4.37–4.56 (2H, m), 5.16 (1H, d, J = 17.4 Hz), 7.05 (1H, dd, J = 3.0, 8.7 Hz), 7.28–7.43 (4H, m), 8.08 (1H, dt, J = 7.5, 2.1 Hz), 8.11–8.15 (1H, m), 10.91 (1H, s); FAB MS *m*/*e* $(M-1)^-$ 686.

6-[(1-Acetimidoyl-4-piperidyl)oxy]-1-{[(3-amidinophenyl)-carbamoyl]methyl}-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (6j). Compound **6j** was synthesized from **14e** according to the same procedure as that for **6f**. Compound **6j** was obtained as a white amorphous powder (30% yield): ^{1}H NMR (DMSO-d_6) δ 1.68–1.85 (2H, m), 1.97–2.12 (2H, m), 2.30 (3H, s), 3.49–3.65 (2H, m), 3.68–3.86 (2H, m), 4.75–4.89 (3H, m), 7.38–7.47 (3H, m), 7.50–7.58 (2H, m), 7.78–7.84 (1H, m), 8.10 (1H, s), 8.80 (1H, s), 9.18 (2H, s), 9.32–9.42 (3H, m), 11.02 (1H, s); FAB MS *m*/*e* $(M+1)^+$ 514. Anal. calcd for $C_{23}H_{27}N_7O_5S{\cdot}2.2HCl{\cdot}1.1H_2O$: C, 45.02; H, 5.16; N, 15.98; S, 5.23; Cl, 12.71. Found: C, 45.34; H, 5.30; N, 15.77; S, 4.75; Cl, 12.83.

2-Bromo-*N*-(3-cyanophenyl)-*N*-methylacetamide (21). To a stirred solution of 3-methylaminobenzonitrile (**20**, 5.00 g, 29.6 mmol) and $NaHCO_3$ (9.20 g, 110 mmol) in EtOAc (35 mL) and H_2O (35 mL) at ambient temperature was added bromoacetyl bromide (5.90 mL, 68.2 mmol). After stirring vigorously at ambient temperature for 15 min, the reaction mixture was diluted with diethyl ether. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was recrystallized from EtOAc/Hex to give **21** (6.91 g, 92%) as a white solid: mp 115–116 °C; ^{1}H NMR ($CDCl_3$) δ 3.33 (3H, s), 3.63 (2H, s), 7.55–7.75 (4H, m),; FAB MS *m*/*e* $(M+1)^+$ 253, 255.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-{[(3-cyanophenyl)(methyl)carbamoyl]methyl}anthranilate (4d). To a stirred solution of **3** (0.80 g, 3.14 mmol) and **21** (1.00 g, 2.85 mmol) in acetonitrile (50 mL) at ambient temperature was added K_2CO_3 (0.43 g, 3.14 mmol). After the reaction mixture was refluxed for 12 h, it was filtered and concentrated in vacuo. The residue was chromatographed on silica gel eluting with EtOAc/Hex (33:77) to give **4d** (0.52 g, 35%) as a pale brown amorphous powder: ^{1}H NMR ($CDCl_3$) 1.46 (9H, s), 1.60–1.76 (2H, m), 1.80–1.93 (2H, m), 3.22–3.36 (6H, m), 3.63–3.80 (3H, m), 3.87 (3H, s), 4.21–4.31 (1H, m), 6.98 (1H, dd, J = 3.3, 8.7 Hz), 7.47–7.73 (6H, m); FAB MS *m*/*e* $(M)^+$ 522.

Methyl 5-[(1-*tert*-butoxycarbonyl-4-piperidyl)oxy]-*N*-(*tert*-butoxycarbonylsulfamoyl)-*N*-{[(3-cyanophenyl)(methyl)carbamoyl]methyl}anthranilate (14f). Compound **14f** was synthesized from **4d** according to the same procedure as that for **14a**. Compound **14f** was obtained as a brown amorphous powder (95% yield): ^{1}H NMR ($CDCl_3$) δ 1.47 (18H, s), 1.63–1.81 (2H, m), 1.82–2.00 (2H, m), 3.26–3.44 (7H, m), 3.60–3.71 (2H, m), 3.81 (3H, br-s), 4.46–4.59 (1H, m), 7.04 (1H, dd, J = 9.0, 3.0 Hz), 7.35 (1H, d, J = 2.7 Hz), 7.38–7.71 (4H, m), 7.81 (1H, d, J = 9.3 Hz); FAB MS *m*/*e* $(M+1)^+$ 702.

6-[(1-Acetimidoyl-4-piperidyl)oxy]-1-{[(3-amidinophenyl)(methyl)carbamoyl]methyl}-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (6k). Compound **6k** was synthesized from **14f** according to the same procedure as that for **6f**. Compound **6k** was obtained as a pale brown amorphous powder (9% yield): ^{1}H NMR (DMSO-d_6) δ 1.60–1.85 (2H, m), 1.92–2.12 (2H, m), 2.30 (3H, s), 3.00–4.00 (7H, m), 4.40–4.64 (2H, m), 4.73–4.82 (1H, m), 7.34 (2H, s), 7.48 (1H, s), 7.60–8.00 (4H, m), 8.73 (1H, s), 9.27 (3H, s), 9.53 (2H, s); FAB MS *m*/*e* $(M+1)^+$ 528. Anal. calcd for $C_{24}H_{29}N_7O_5S{\cdot}2.2HCl{\cdot}2.5H_2O$: C, 44.15; H, 5.59; N, 15.02; S, 4.91; Cl, 11.95. Found: C, 44.29; H, 5.98; N, 14.83; S, 4.26; Cl, 11.56.

X-ray crystallographic experiment

Crystals of the bovine pancreatic trypsin in complex with inhibitors were prepared using the same method as reported previously.[10] The crystals belonged to the space group $P2_12_12_1$ and were isomorphous to those reported for YM-60828.[7] The X-ray diffraction data were collected with the Rigaku R-AXIS IIc image-plate system and 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[11] and CNX2000 (Accelrys Inc.). Diffraction data and refinement statistics for inhibitor/trypsin complexes are as follows. **6a**: resolution range, 46.86–2.2 Å; data completeness, 74.9%; R-factor/R_{free}, 18.1%/21.0%; r.m.s.d. of bonds, 0.009 Å; r.m.s.d. of angles 1.5°. **6f**: resolution range, 46.91–2.5 Å; data completeness, 86.8%; R-factor/R_{free}, 27.8%/29.4%; r.m.s.d. of bonds, 0.006 Å; r.m.s.d. of angles 1.3°. We deposit the crystallographic data for these structures in the Protein Data Bank.

Modeling study

The ZK-807834 inhibited factor Xa X-ray coordinate (1FJS)[12] was employed in this study. Each inhibitor/trypsin complex was superimposed on to ZK-807834/factor Xa complex using coordinate of Ca atoms around active site. After manual adjustment based on the same method as reported for YM-60828,[7] energy minimization of each inhibitor/water/factor Xa complex model was performed using CFF91 force field implemented in the program DISCOVER (Accelrys Inc.). All atoms within 10 Å from inhibitor were allowed to move during the minimization.

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 portion of the enzyme solution. Enzymes and substrates were used as follows: factor Xa and S-2222; 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 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 (Hemoliance Brain Thromboplastin, Instrumentation Laboratory, Lexington, MA, USA) to initiate clot formation. The concentration required to double clotting time (CT_2) was estimated from each individual dose–response curve.

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 the staff of the Division of Analytical Science Laboratories for the elemental analysis and spectral measurements.

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