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Prodrug-based design, synthesis, and biological evaluation of *N*-benzenesulfonylpiperidine derivatives as novel, orally active factor Xa inhibitors

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Abstract—We describe here our investigation of a new series of orally active fXa inhibitors based on a prodrug strategy. Solid-phase parallel synthesis identified a unique series of fXa inhibitors with a substituted benzenesulfonyl group as a novel S4 binding element. This series resulted in compound **39**, which exhibited potent inhibitory activity against fXa (IC_{50} = 13 nM) and excellent selectivity over thrombin (>7000-fold). The masking of its highly hydrophilic groups led to the creation of related prodrug **28**, which demonstrated an anticoagulant effect after oral dosing.

1. Introduction

Intravascular clot formation results in thromboembolic diseases including ischemic stroke, deep vein thrombosis, myocardial infarction, unstable angina, and pulmonary embolism, which are major cause of morbidity and mortality in the industrialized world. Up to this point, the primary medical strategy for treating and preventing such diseases has been the use of the anticoagulants heparin, low molecular weight heparins, and vitamin K antagonist warfarin. However, all these drugs have therapeutic limitations. For example, neither heparin nor low molecular weight heparins can be administered orally.[1] The oral anticoagulant agent warfarin is being used for a growing number of antithrombotic indications; however, it has a slow onset of action and also requires individual dose titration and periodic monitoring due to its indirect mechanism of action.[2] Therefore, the discovery of novel, orally active anticoagulants that directly inhibit blood coagulation enzymes has emerged as one of the most active areas of current research in the drug discovery field.

Recent understanding of the mechanisms of blood coagulation has increased rapidly. Thrombin, which promotes blood clot formation by catalyzing the conversion of fibrinogen to insoluble fibrin as well as strongly inducing platelet aggregation,[3] plays a central role in thrombosis. Therefore, thrombin inhibitors have been extensively studied as potential anticoagulant agents. The direct blocking of thrombin, however, shows a tendency to prolong bleeding at levels approaching the effective dose.[4]

An attractive alternative to direct thrombin inhibition is blocking the biosynthesis of thrombin itself. Thrombin formation from prothrombin in the prothrombinase complex is catalyzed by factor Xa (fXa). The generation of thrombin by fXa is a highly amplified process,[5] suggesting that inhibition of fXa may be more efficacious than direct inhibition of thrombin in interrupting the blood coagulation cascade. Moreover, since fXa inhibitors affect coagulation specifically and do not affect platelet function, they theoretically have less potential to increase the risk of bleeding.[6] Consequently, fXa

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has emerged as an attractive target for the development of new therapeutic agents with potential for the treatment of arterial and venous thrombosis.

We have already reported some potent amidine-based fXa inhibitors, such as compound **1**,[7] which originated from Daiichi's DX-9065a.[8] fXa inhibitors with amidine groups are highly efficacious in animal thrombosis models,[6,9] yet they do not have an appropriate pharmacokinetic profile for use as oral antithrombotic agents due to the presence of highly basic and hydrophilic amidine units.[10] One methodology employed to improve the oral bioavailability of amidine derivatives is to mask the polar groups, that is, a prodrug strategy. Herein, we report on our discovery of a novel class of potent and selective amidine-based fXa inhibitors and also describe the oral anticoagulant activity of the related prodrugs.

2. Inhibitor design

In order to discover potent and orally active fXa inhibitors, we adopted a strategy based on the combination of amidine derivatives and their prodrugs because of the following three reasons: (1) an amidine derivative is known to demonstrate potent anti-fXa activity due to the bidentate salt bridge interaction between its amidine moiety and the carboxylic acid of Asp189 in the S1 site of the enzyme, (2) masking the high hydrophilicity of the polarizable groups on the inhibitor reportedly enhances its membrane permeability, improves its activity via oral administration, and reduces drug–food interaction,[11] and (3) a prodrug with low inhibitory activity does not interact with fXa in the gastrointestinal tract, which will provide a potential advantage for patients with silent gastrointestinal bleeding.

Our previous studies demonstrated that diamidine derivative **1** was a potent fXa inhibitor with an IC_{50} value of 0.0038 μM (Fig. 1).[7] In this research program, we selected compound **1** as a starting point and applied the following two-step design strategy (Step A and B) to generate new scaffold fXa inhibitors (Fig. 2).

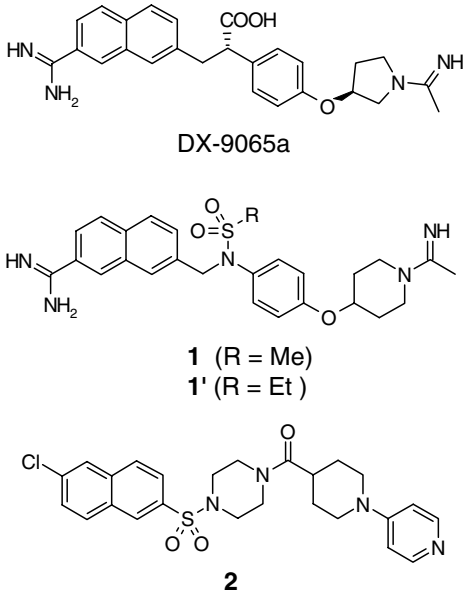


Figure 1. Structures of fXa inhibitors.

Compound **1** has two amidino groups. One is a benzamidine group, and the other is an acetimidoyl group. *N*-Hydroxybenzamidine is the well-established bioprecursor of benzamidine, for example, the thrombin inhibitor ximelagatran,[11] the GP IIb-IIIa receptor antagonist sibrafiban,[12] so we planned that the benzamidine group of compound **1** would be transformed into *N*-hydroxybenzamidine based on prodrug strategy. To the contrary, no promoieties for an acetimidoyl group have been reported to date. These backgrounds led us to eliminate the *N*-acetimidoylpiperidine moiety of compound **1** (Step A). Exploring surrogates for this group, we focused on compound **2**, a member of the first class of nonamidine fXa inhibitors (Fig. 1).[13] Our molecular modeling study based on the reported X-ray crystal structure of compound **2**[14] indicated that this inhibitor was bound to the active site of fXa, with its pyridine moiety in the S4 pocket bordered by the residues of Phe174, Tyr99, and Trp215. Similarly, the acetimidoylpiperidine unit of the ethanesulfonamide derivative **1′** lay in the S4 binding region.[7] We expected that the replacement of the acetimidoylpiperidine moiety of analogue **1** with a pyridine ring would afford a potent fXa inhibitor. Thus we designed compound **X**, and undertook further structural modifications using a parallel synthesis methodology to obtain suitable S4 binding elements (compound **Y**).

The second round consisted of the installation of a carboxyl group into the amidine-based inhibitor and optimization of the compound involving the prodrug approach (Step B). Compounds with amidine groups sometimes cause cardiovascular side effects in terms of reduced blood pressure and heart rate, probably because of the high basicity of the amidine group.[15] The introduction of an acidic carboxyl group into the amidine derivative and the consequent reduction of the basicity of the whole chemical structure are important for the avoidance of these side effects.[16] Moreover, we found that the installation of a carboxyl group into a prodrug with a masked amidine moiety was important for the biotransformation of the masked amidine into amidine.[17] Therefore, we decided to construct a molecule with a carboxyl group. The appropriate position for the introduction of the carboxyl group was determined based on computational modeling studies. We also explored the effects of naphthamidine replacements to enhance the fXa selectivity over other serine proteases and to improve the oral anticoagulant activity (compound **Z**).

3. Chemistry

The library of piperidine derivatives was prepared using solid-phase parallel synthesis as outlined in Scheme 1. Conversion of 7-bromomethylnaphthalene-2-carbonitrile (**3**)[18] into the corresponding amine **5** was accomplished through a two-step sequence involving a

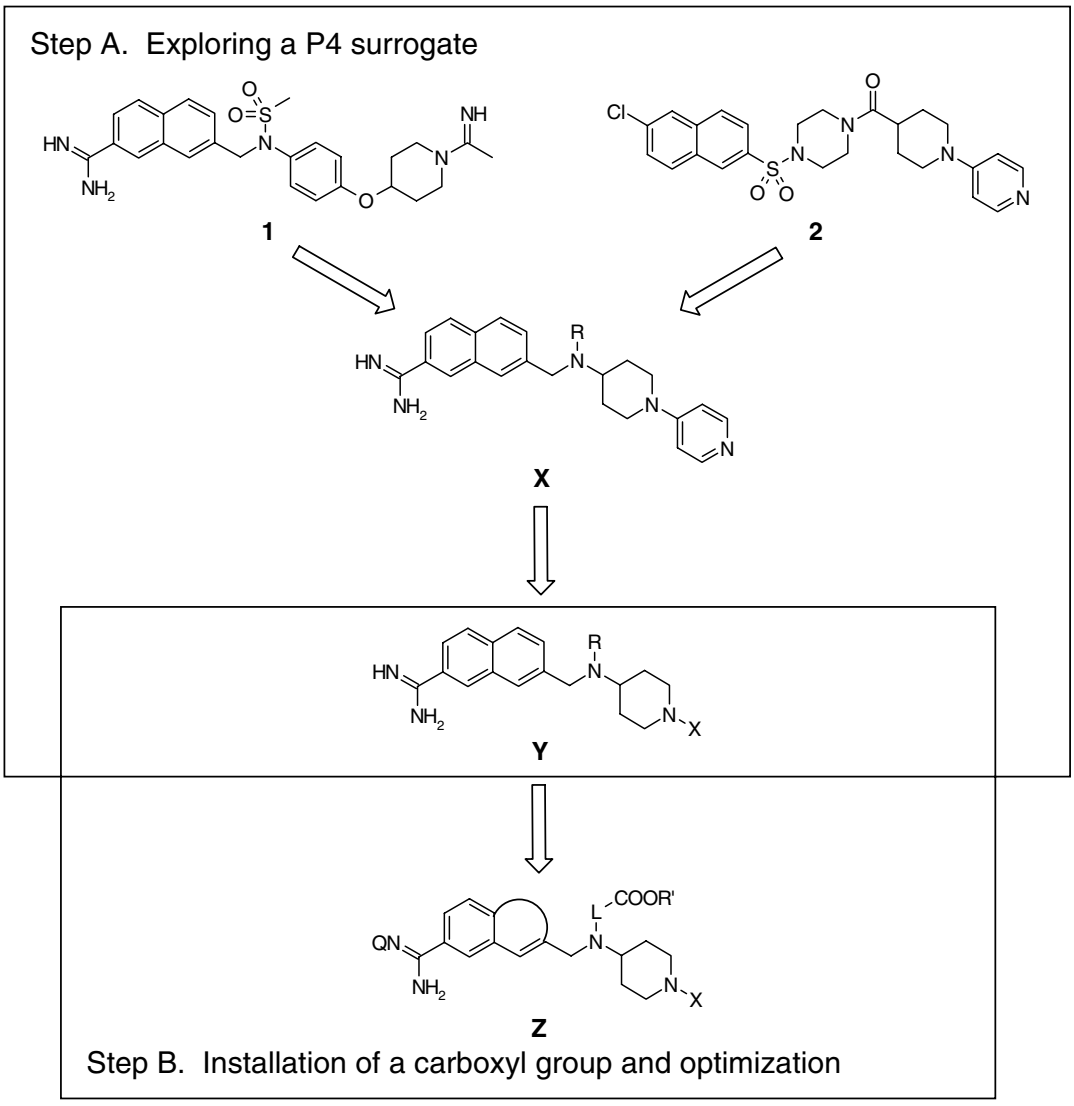
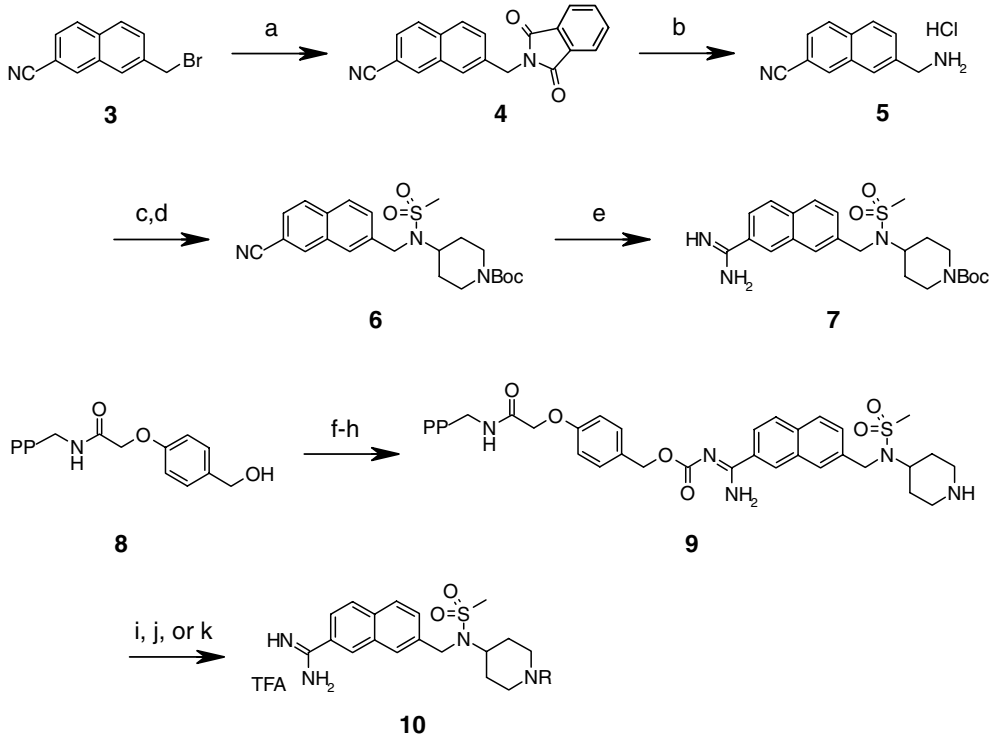


Figure 2. Inhibitor design.

substitution reaction with potassium phthalimide followed by a methylamine-promoted deprotection. Reductive amination of *tert*-butyl 4-oxopiperidine-1-carboxylate with the amine **5** and sodium triacetoxyborohydride as the reducing agent followed by mesylation afforded intermediate nitrile **6**. Compound **6** was then converted into amidine **7** using the thio-Pinner procedure[19] followed by treatment with ammonium acetate. Amidine **7** was loaded onto resin **8**,[20] followed by acidic removal of its Boc group to yield a polymer-bound piperidine precursor **9**. Treatment of the polymer-bound piperidine **9** with a collection of sulfonyl chlorides followed by TFA-mediated cleavage from the polymer afforded a set of 74 *N*-sulfonylpiperidine derivatives **10**. *N*-Acyl piperidine derivatives were prepared by the condensation of the polymer-bound piperidine **9** with a library of carboxylic acids using PyBOP (benztriazol-1-yl-oxytris(pyrrolidino)phosphonium hexafluorophosphate)[21] followed by cleavage from the polymer. Two dozen compounds were synthesized in this fashion. Preparation of a set of 37 *N*-alkylpiperidine analogues **10** was accomplished in two steps via reductive amination of the polymer-bound piperidine **9** with various aldehydes, and subsequent acidic cleavage from the resin.

Scheme 2 depicts the stepwise processes that were employed to elaborate nitriles **17**–**24**, the precursors of target amidine derivatives. The sulfonylation of 4-piperidone hydrate (**12**) with 2,5-dimethoxyphenylsulfonyl chloride yielded ketone **13**. The ketones **11**[22] or **13** were each coupled with 7-aminomethylnaphthalene-2-carbonitrile (**5**) or (*E*)-3-(3-aminopropenyl)benzonitrile[23] in the presence of sodium triacetoxyborohydride to provide intermediate amines **14**–**16**. The secondary amines **14**–**16** were treated with appropriate sulfonyl chlorides to yield sulfonamides **17**, **18**, **21**, and **23**. Amide analogues **19**, **22**, and **24** were synthesized by acylating the intermediate amines **15** and **16** with appropriately substituted acid chlorides. Tertiary amine **20** was obtained via reductive amination of the secondary amine **15** with formaldehyde. All samples prepared by the solid-phase techniques were analyzed by LC–MS to confirm purity and identity of the compounds.



Scheme 1. Reagents and conditions: (a) potassium phthalimide, DMF, 90 °C; (b) i—$MeNH_2$, MeOH; ii—HCl, EtOAc; (c) *tert*-butyl 4-oxopiperidine-1-carboxylate, $NaBH(OAc)_3$, Et_3N, AcOH, 1,2-dichloroethane; (d) methanesulfonyl chloride, Et_3N, 1,2-dichloroethane, 0 °C; (e) i—H_2S, Et_3N, pyridine; ii—MeI, acetone, reflux; iii—$AcONH_4$, MeOH, reflux; (f) 4-nitrophenylchloroformate, Py, CH_2Cl_2; (g) **7**, Et_3N, NMP; (h) HCl/EtOAc, MeOH; (i) i—sulfonyl chloride, *i*-Pr_2EtN, dioxane; ii—TFA, CH_2Cl_2; (j) i—carboxylic acid, PyBOP, *i*-Pr_2EtN, DMF; ii—TFA, CH_2Cl_2; (k) i—aldehyde, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane; ii—TFA, CH_2Cl_2.

The preparation of the amidine derivatives **10x** and **25**–**39** is shown in Scheme 3. Conversion of the nitrile groups to amidines was performed with two general procedures. The precursor nitriles **21**–**24** were transformed into *N*-hydroxyamidine derivatives **25**–**28** by treatment with hydroxylamine. Subsequent in situ activation of the *N*-hydroxyamidine groups by formation of the acetate ester and N–O bond cleavage by catalytic hydrogenation afforded the corresponding amidine derivatives **32**–**33**.[24] Alternatively, the treatment of nitriles**17**–**20**, **23**, and **24** under the Pinner conditions (HCl/EtOH) afforded imidates,[25] which were immediately reacted with ammonium acetate to yield amidines **10x**, **29**–**31**, **34**, and **35**. Finally, the ester groups of compounds **32**–**35** were hydrolyzed under basic conditions to provide the desired carboxylic acids **36**–**39**.

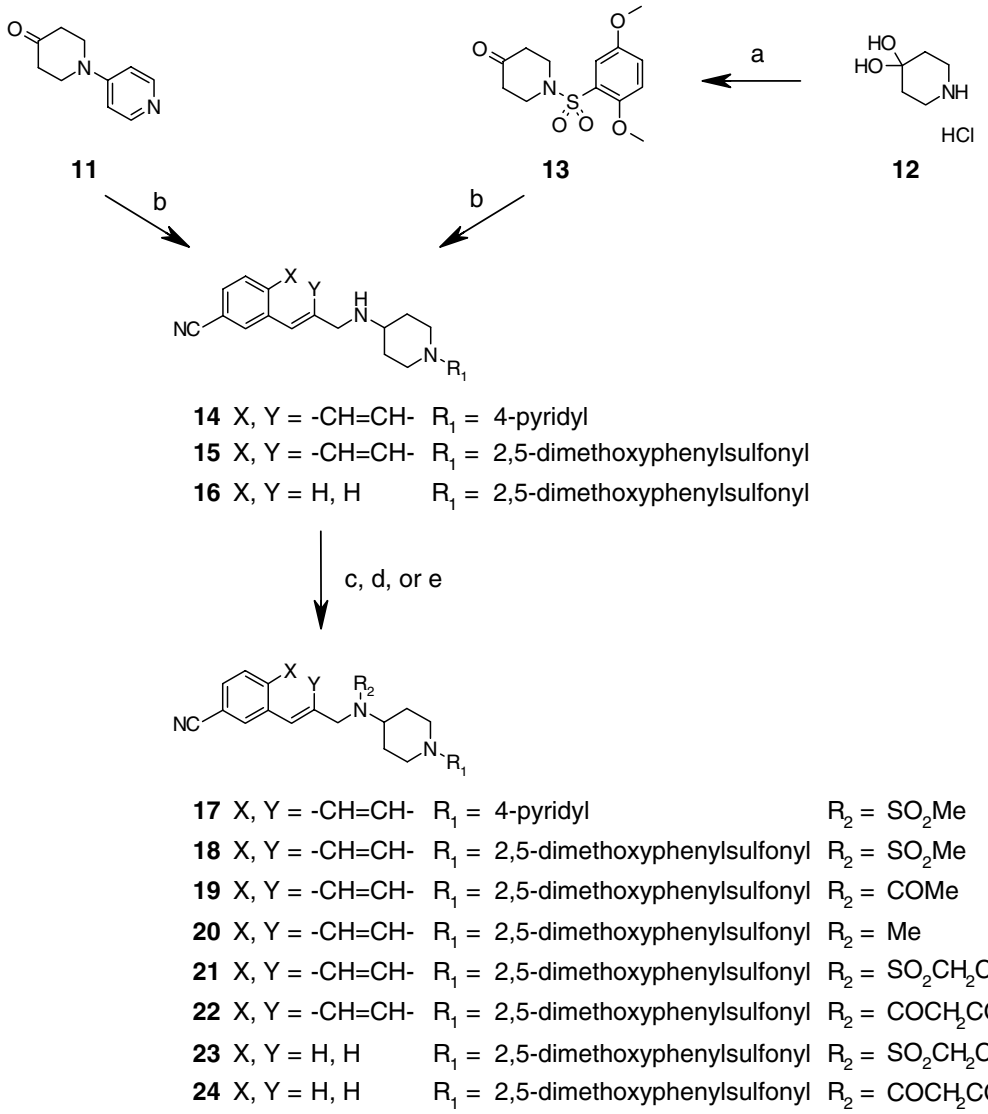
4. Pharmacology

All compounds synthesized were tested for their potency to inhibit human fXa in a purified enzyme system. Subsequent screening against human thrombin and human trypsin was carried out for further selected compounds. The anticoagulant activity for selected compounds was evaluated by measuring the prolongation of prothrombin time (PT) in mouse and human plasma. Selected compounds were evaluated for their PT-prolongation effects after oral administration in mice. Each test drug was suspended in a 0.5% methylcellulose solution prior to use and was orally administered to male ICR mice using a gastric tube. After oral administration of compounds, citrated blood was collected and platelet poor plasma was prepared to measure PT at a predetermined time.

5. Results and discussion

Table 1 shows the result for the replacement of the acetimidoylpiperidinyloxyphenyl moiety in lead compound **1** with a 4-(piperidino)pyridine group. The resulting compound **29** exhibited good inhibitory activity against fXa, which was only twofold less potent than the clinical candidate DX-9065a. This result led us to extensive modification of compound **29**. The pK_a value of highly basic benzamidine is 11.6, and 4-(piperidino)pyridine is also highly basic comparable to benzamidine.[26] This might cause the 4-(piperidino)pyridine derivatives to have poor oral absorption. In addition to that, the 4-(piperidino)pyridine derivatives were reported to be rapidly metabolized by *N*-oxide formation.[27] Based on these data, we next turned our attention to the investigation of alternative fragments to the pyridine moiety.

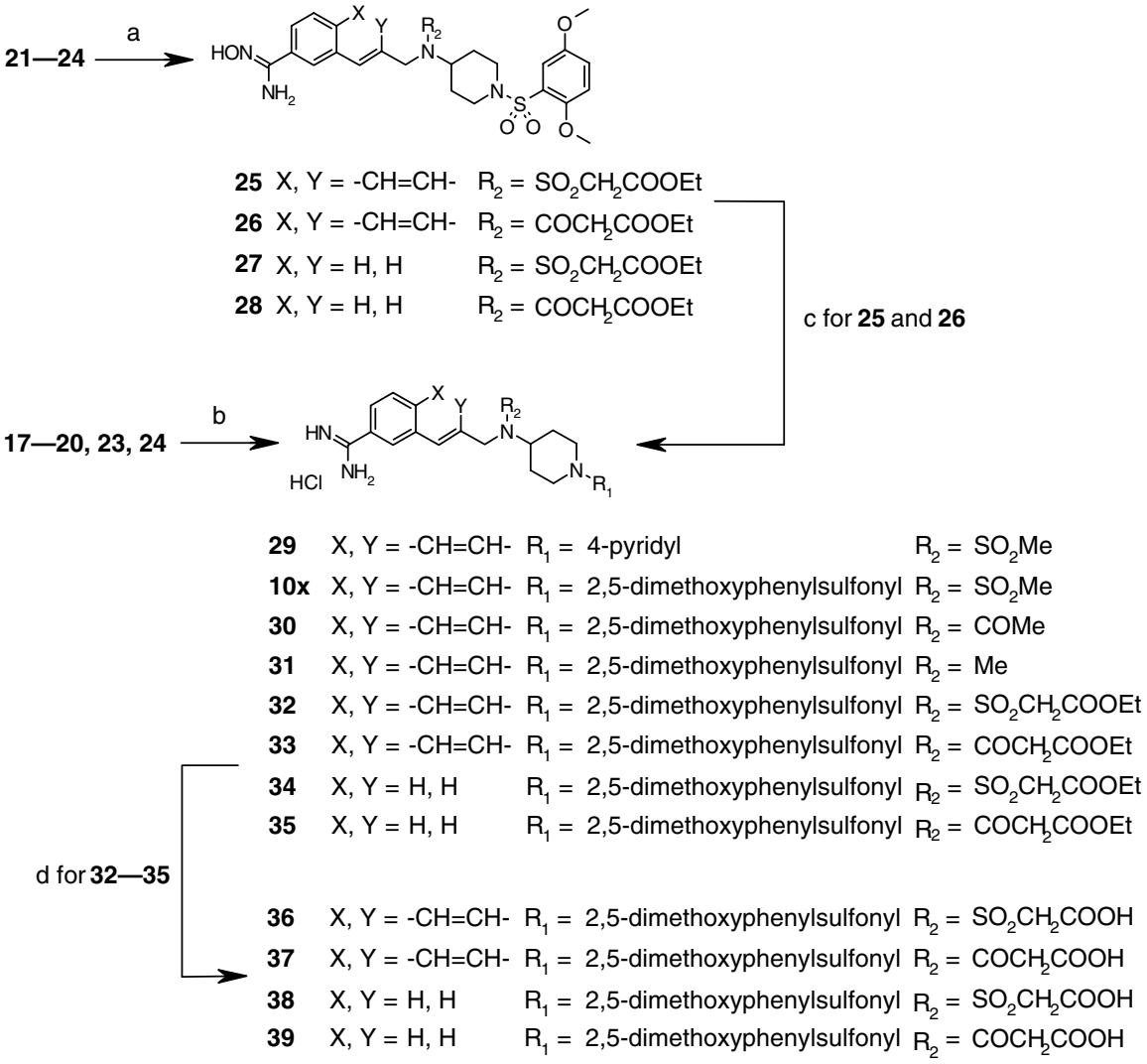
Over 100 compounds were prepared to scan new P4 ligands by means of parallel solid-phase synthesis, and the results for a representative set of compounds are shown in Table 2. *N*-Alkylsulfonyl derivatives, such as compound **10b**, did not show inhibitory activity, and *N*-benzenesulfonyl derivative **10c** was found to be a moderate but novel fXa inhibitor. The introduction of



Scheme 2. Reagents and conditions: (a) 2,5-dimethoxybenzenesulfonyl chloride, Et_3N, 1,2-dichloroethane; (b) **5** or (*E*)-3-(3-aminopropenyl)benzonitrile, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane; (c) sulfonyl chloride, Et_3N, 1,2-dichloroethane, 0 °C; (d) acid chloride, Et_3N, 1,2-dichloroethane, 0 °C; (e) HCHOaq, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane.

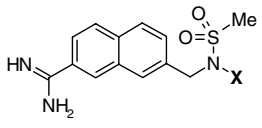
a methyl or chloro group at the 2-position on the phenyl ring did not cause a reduction of potency (**10d** and **10e**), and their incorporation at the 3-position served more suitably as S4 binding elements (**10f** and **10g**). Substitution at the 4-position was, however, less well tolerated (**10h** and **10i**). The 3-position on the phenyl ring tolerated various substituents without deleterious effects on potency (**10f**, **10g**, **10j**–**10l**). In this series, 3-chloro derivative **10g** showed potent inhibitory activity with an IC_{50} value of 0.049 μM. In contrast, 3-substituted benzyl or benzoyl analogues were found to be poor inhibitors (**10m**–**10p**). In disubstituted benzenesulfonyl derivatives, compound with a 2,5-disubstitution pattern maintained high inhibitory activity (**10t**). The 2,5-dichlorothiophenesulfonyl analogue **10u** exhibited comparable activity to the phenyl derivative **10t**, indicating that the thiophene core would become the isostere of a benzene ring for an S4 binding element. Analogues incorporating a variety of 2,5-disubstituted benzenesulfonyl groups were well tolerated (**10v**–**10x**), and the 2,5-dimethoxybenzenesulfonyl derivative **10x** was found to be a highly potent fXa inhibitor with an IC_{50} value of 0.011 μM.

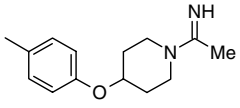
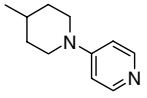
Figure 3 shows the proposed binding model of compound **10x** docked into the active site of fXa, and the important interactions of the inhibitor with the enzyme are schematically depicted in Figure 4. In this model, compound **10x** is bound in an extended L-shaped conformation similar to the reported binding mode of DX-9065a.[28] The naphthamidine moiety of compound **10x** occupies the S1 pocket of fXa, and its amidine group binds via a bidentate charge interaction with Asp189 at the bottom of the S1 pocket. The 2,5-dimethoxybenzenesulfonyl unit fills the S4 aryl binding pocket with its *m*-methoxy substituent stretching along the cleft in the S4 pocket. Our docking study suggests that *o*-methoxy group occupies the cavity of S4 binding pocket and interacts through hydrophobic contacts. This binding model also indicates that the central



Scheme 3. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, Et_3N, EtOH, reflux; (b) i–HCl, EtOH, $CHCl_3$, 0 °C; ii—$AcONH_4$, EtOH; (c) H_2 (1 kgf/cm^2), 10% Pd/C, Ac_2O, AcOH; (d) 1 N aq NaOH, dioxane, 0 °C.

Table 1. Effect of modification of P4 element



Compound	X	IC_{50}[a] (μM) fXa
1		0.0038[b]
29		0.090
	DX-9065a	0.040

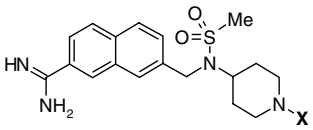
[a] Human purified enzyme was used. The IC_{50} value represents the mean of three separate experiments, and the average standard error is less than 15% of the mean.

[b] See Ref. 7.

methanesulfonyl group extends into the solvent and that no direct interaction is observed between this group and the enzyme (Fig. 3, right panel). This points toward our prediction that an additional substitution in the methanesulfonyl group would be tolerated.

Next we modified the central hinge and the P1 ligand of compound **10x**. The structure–activity relationships (SAR) for fXa binding are summarized in Table 3. Replacement of the methanesulfonyl group with an acetyl group afforded compound **30**, which maintained potent anti-fXa activity. On the other hand, tertiary amine analogue **31** suffered a 50–fold loss in activity compared to the methanesulfonyl derivative **10x**. Here, we directed our efforts toward the introduction of a carboxyl group into the inhibitor. Our modeling study of compound **10x** within fXa indicated that its analogue, with an additional substituent on the methanesulfonyl group, would retain potent inhibitory activity. This prompted us to apply the installation of carboxyl groups onto the limbs of the central elements of compounds **10x**

Table 2. Effect of modification of P4 element



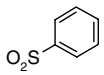
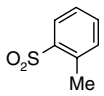
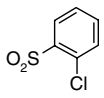
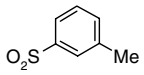
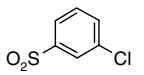
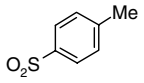
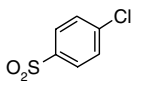
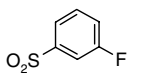
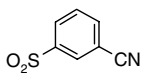
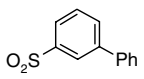
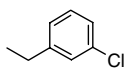
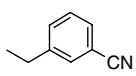
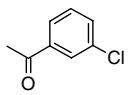
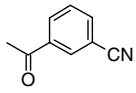
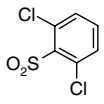
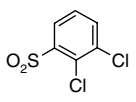
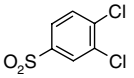
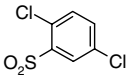
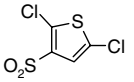
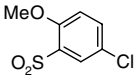
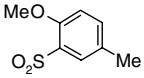
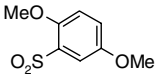
Compound	X	IC_{50}[a] (μM) fXa
10a	H	>100
10b	SO_2Me	>100
10c		0.85
10d		0.95
10e		0.35
10f		0.25
10g		0.049
10h		>100
10i		1.6
10j		0.80
10k		0.17
10l		0.20
10m		1.7
10n		2.3
10o		>100
10p		>100
10q		>100
10r		0.56

Table 2 (*continued*)

Compound	X	IC_{50}[a] (μM) fXa
10s		0.19
10t		0.065
10u		0.10
10v		0.039
10w		0.058
10x		0.011[b]

[a] Human purified enzyme was used. IC_{50} values are determined by a single experimental run.

[b] Activity of resynthesized sample. The IC_{50} value represents the mean of three separate experiments, and the average standard deviation is less than 10% of the mean.

and **30**. As anticipated, these modifications maintained anti-fXa activity, and carboxylic acid derivatives **36** and **37** were revealed as potent inhibitors with IC_{50} values of 0.013 and 0.0081 μM, respectively. We also evaluated the related styrylamidine analogues **38** and **39** which would have stereochemical geometry similar to naphthamidines. Compounds **38** and **39** were potent inhibitors, comparable to naphthamidine derivatives **36** and **37**, respectively, which led us to conclude that a styrene unit functioned as the isostere of the naphthalene ring system.

Subsequent screening against human thrombin for selectivity within the coagulation cascade and human trypsin for general specificity against serine proteases was carried out for the potent fXa inhibitors. The results are also listed in Table 3. All compounds tested demonstrated excellent selectivity against thrombin (>7000-fold) and trypsin (>100-fold). The styrylamidine-based inhibitors **38** and **39** were more selective versus trypsin than the naphthamidine analogues **36** and **37**.

The installation of a carboxyl group into a fXa inhibitor reportedly leads to an increase of selectivity over thrombin.[29] The novel fXa inhibitors reported here, however, demonstrated high selectivity versus thrombin, even if they had no carboxyl groups. This selectivity profile can be explained by our molecular modeling studies. Figure 5 shows the proposed binding model of compound **10x** docked into the active site of fXa and its subsequent superimposition onto that of thrombin. These docking simulations revealed that the central methanesulfonyl group would hit the

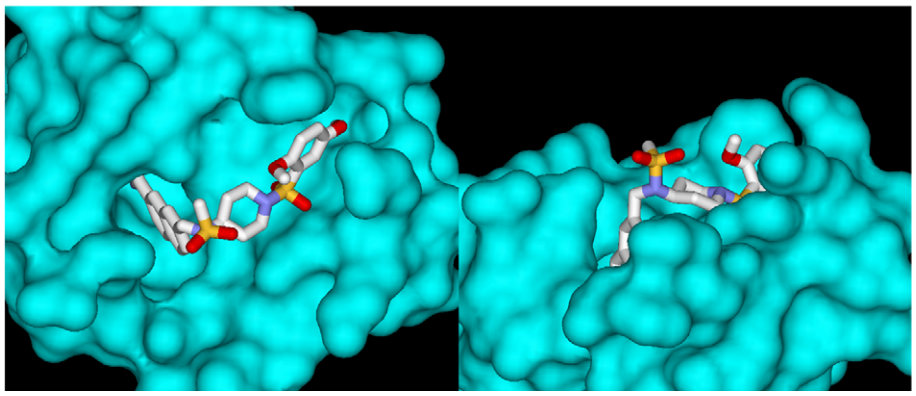


Figure 3. Proposed binding model of compound **10x** within the active site of fXa. Left and right panels correspond to top and side views of the complex, respectively. The inhibitor is colored by atom type and the protein surface is colored cyan. Coordinates of fXa are available on the Brookhaven database (Accession code: 1FAX).

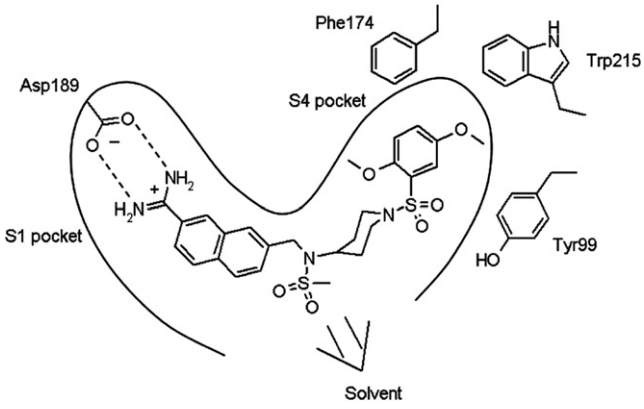


Figure 4. Diagram representing the key interactions between compound **10x** and fXa.

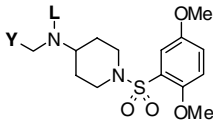
thrombin-specific insertion loop (Tyr60A-Pro60B-Pro60C-Trp60D), thus conferring high selectivity against thrombin.[30] Extensive modeling studies indicated that analogous inhibitors bound to the active site of fXa in a similar fashion as compound **10x**, so we propose that this novel class of fXa inhibitors demonstrates high selectivity over thrombin due to steric repulsion of their central moieties and the residues of the 60-insertion loop of thrombin.

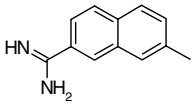
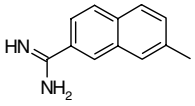
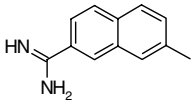
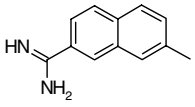
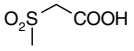
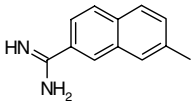
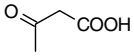
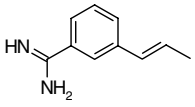
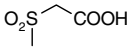
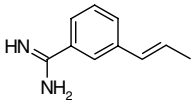
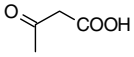
The four potent and selective carboxylic acid derivatives **36**–**39** were selected for evaluation in our advanced profiling assays. To evaluate the potential efficacy of these compounds, in vitro anticoagulation tests were conducted using human and mouse plasma. Table 4 shows the concentrations of inhibitors required to double the prothrombin time (PT) in plasma clotting. All compounds indicated potent anticoagulant activities, which were comparable to those of DX-9065a. We finally examined the anticoagulant potency after oral dosing of their related *N*-hydroxyamidine prodrugs **25**–**28** to assess their ability as orally active anticoagulants. Confirmatory in vitro tests showed that none of the *N*-hydroxyamidine prodrugs had any fXa inhibitory activity or anticoagulant potency ($IC_{50} > 10$ μM, $CT_2 > 100$ μM). Although compounds **25**–**27** did not exhibit anticoagulant activity after oral administration in mice, compound **28** did display evident prolongation of PT (Table 5). Figure 6 depicts the time course for the PT-prolongation effect of compound **28**, which indicated the anticoagulant effects peaked at 30 min after oral dosing. Its parent amidine **39** showed no anticoagulant effect when dosed orally. These results demonstrated that prodrug **28** was absorbed after oral dosing and rapidly converted into the corresponding active compound in vivo.

6. Conclusion

In this paper, we have designed and synthesized a new class of potent, selective, and orally active fXa inhibitors based on prodrug exploration. Replacement of the S4 binding element in lead compound **1** with a 4-(piperidino)pyridine and extensive structural modifications using solid-phase parallel synthesis methodology led to the identification of novel piperidine derivatives with substituted benzenesulfonyl groups as P4 ligands (e.g., compound **10x**, fXa $IC_{50} = 0.011$ μM). Subsequent SAR studies demonstrated that the methanesulfonyl to acetyl transformation at the center of the molecule or the naphthamidine to styrylamidine conversion as the P1 ligand yielded equipotent inhibitors. We installed a carboxyl group on either the central methanesulfonyl or the acetyl group based on molecular modeling studies which indicated that the central units were directed out of the fXa binding site into the solvent environment. These modifications did not cause any reduction of anti-fXa activity. The modeling studies also demonstrated that the central groups would hit the thrombin-specific 60-insertion loop, which is consistent with high selectivity over thrombin (>7000-fold). The related *N*-hydroxyamidine prodrug **28**, which had no in vitro activity, demonstrated an evident PT-prolongation effect in mice when administered orally, while its parent amidine **39** showed no oral anticoagulant potency. These results indicated that the prodrug **28** was absorbed via oral dosing and bioconverted into the corresponding active compound. We anticipate that further structural

Table 3. Effects of modifications of the central moiety and P1-element



Compound	Y	L	IC_{50}[a] (μM)		
			fXa	Thrombin	Trypsin
10x		Ms	0.011	>100	1.1
30		Ac	0.012	>100	2.5
31		Me	0.59	NT	NT
36			0.013	>100	2.7
37			0.0081	>100	3.7
38			0.010	>100	5.3
39			0.013	>100	6.0
	DX-9065a		0.040	>100	3.2

[a] See the corresponding footnotes in Table 1.

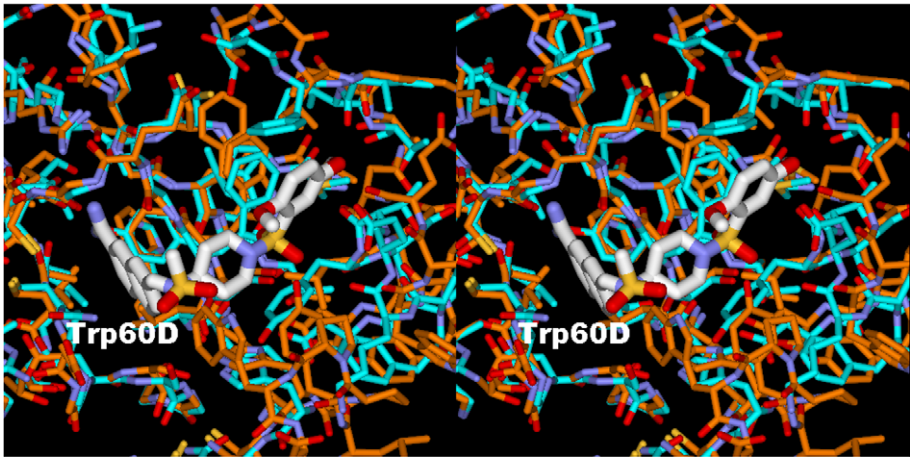


Figure 5. Stereographical representation of the basis for the selectivity profile of compound **10x**. Compound **10x** is docked in the active site of fXa (PDB Accession code: 1FAX) and transferred onto that of thrombin (PDB Accession code: 1KTT). All molecules are colored by atom type, except the carbon atoms of fXa, which are cyan, and those of thrombin, which are orange.

Table 4. In vitro anticoagulant activities

Compound	CT_2[a] (μM) PT	
	Human	Mouse
36	0.55	1.7
37	0.56	1.6
38	0.47	1.2
39	0.55	1.4
DX-9065a	0.55	4.9

[a] CT_2 is defined as the concentration of test compound required to double PT. The value represents the mean of three separate experiments, and the average standard errors are less than 10% of the mean.

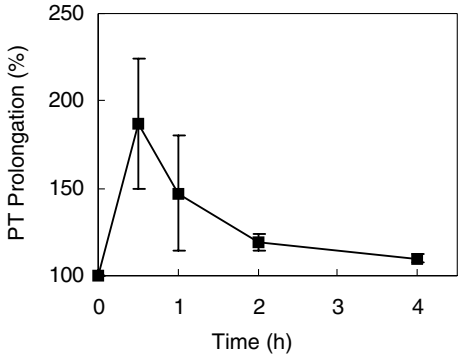


Figure 6. Anticoagulant activity of compound **28** after oral dosing in mice. Animals were dosed via oral gavage at 100 mg/kg. Values are expressed as means ± SEM ($n = 3$).

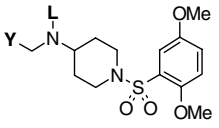
modifications of amidine **39** and its prodrug **28** will lead to more potent and orally active fXa inhibitors. Our continuing efforts to improve oral anticoagulant activity will be the subject of future publications.

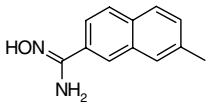
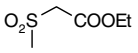
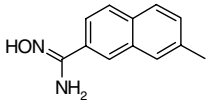
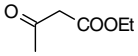
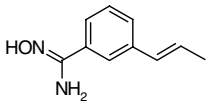
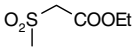
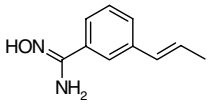
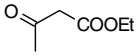
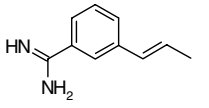
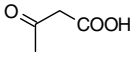
7. Experimental

7.1. Chemistry

^{1}H NMR spectra were recorded on a JEOL JNM-LA300 or a JEOL JNM-EX400 spectrometer and the chemical shifts are expressed in δ (ppm) values with tetramethylsilane as an internal standard (in the NMR description, s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad peak). Mass spectra were recorded on a JEOL JMS-LX2000 spectrometer. For salts, assignments of ion peaks are based on the basic component. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, and N) and a Yokogawa IC-7000S ion chromatographic analyzer (S and Cl), and were within ±0.4% of theoretical values unless otherwise indicated. Melting points were measured with a Yanaco MP-500D melting point apparatus without correction. ODS column chromatography

Table 5. Ex vivo anticoagulant activity after oral dosing in mice



Compound	Y	L	Prolongation effect PT[a] (%)
25			101 ± 0
26			109 ± 5
27			116 ± 4
28			187 ± 37
39			104 ± 4
	DX-9065a		151 ± 16

[a] Mean percent change of PT at 0.5 h after oral administration compared to that measured using normal mice plasma. Animals were dosed via oral gavage at 100 mg/kg. Each value is expressed as the mean ± SEM ($n = 3$). All *N*-hydroxyamidine derivatives prepared had little inhibitory activity against factor Xa ($IC_{50} > 10$ μM) and a low in vitro anticoagulant effect (PT $CT_2 > 100$ μM).

was performed on YMC gel (ODS-A 120-230/70). All reagents purchased were used without further purification.

7.1.1. *N*-(7-Cyanonaphthalen-2-ylmethyl)phthalimide (4). A mixture of 7-bromomethylnaphthalene-2-carbonitrile (**3**)[18] (40.1 g, 163 mmol), potassium phthalimide (30.2 g, 163 mmol), and DMF (200 mL) was stirred at 90 °C for 15 h. The reaction mixture was cooled to ambient temperature. To the mixture was then added H_2O (40 mL) and the whole was stirred for 0.5 h. The resulting precipitate was filtered off. A mixture of this material and EtOH (750 mL) was stirred under reflux for 10 min. The mixture was cooled to ambient temperature, and the precipitate was filtered off and dried in vacuo to yield the title compound as a colorless amorphous powder (9.71 g, 19%): ^{1}H NMR (DMSO-d_6) δ 5.03 (2H, s), 7.58 (1H, d, J = 8.4 Hz), 7.69–7.76 (3H, m), 7.83–7.89 (4H, m), 7.93 (1H, s), 8.21 (1H, s); MS (FAB) m/z 313 $[M+H]^+$.

7.1.2. 7-(Aminomethyl)-2-naphthonitrile hydrochloride (5). A mixture of compound **4** (15.6 g, 50.0 mmol), MeOH (200 mL), and methylamine in MeOH (40%, 60 mL) was stirred at ambient temperature for 21 h. The mixture was diluted with H_2O (200 mL) and extracted with ethyl acetate (200 mL). The organic layer was dried over anhydrous sodium sulfate. To this solution was added 4 N hydrogen chloride in ethyl acetate (15 mL) and the whole was stirred for 0.5 h. The resulting precipitate was filtered off and dried in vacuo to yield the title compound as a colorless solid (8.05 g, 83%): mp 266–268 °C; ^{1}H NMR (DMSO-d_6) δ 4.23 (2H, s), 7.82–7.88 (2H, m), 8.11–8.17 (3H, m), 8.55 (3H, br s), 8.60 (1H, s); MS (FAB) m/z 183 $[M+H]^+$.

7.1.3. *tert*-Butyl 4-[[(7-Cyano-2-naphthyl)methyl](methylsulfonyl)amino]piperidine-1-carboxylate (6). A mixture of compound **5** (4.64 g, 21.2 mmol), *tert*-butyl 4-oxopiperidine-1-carboxylate (4.23 g, 21.2 mmol), triethylamine (4.35 g, 43.0 mmol), AcOH (12.7 g, 212 mmol), and 1,2-dichloroethane (100 mL) was stirred at ambient temperature for 0.5 h. To this mixture was added $NaBH(OAc)_3$ (6.75 g, 31.8 mmol) at ambient temperature, and the whole was stirred for 24 h. To the reaction mixture were added 10% aqueous sodium bicarbonate (200 mL) and 1 N aqueous NaOH solution (100 mL), and the mixture was extracted with $CHCl_3$ (100 mL). The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The resulting material was dissolved in 1,2-dichloroethane (80 mL) and combined with triethylamine (6.44 g, 63.6 mmol). To this solution was added methanesulfonyl chloride (4.86 g, 42.4 mmol) at 0 °C and the whole was stirred for 4 h. To the reaction mixture was added H_2O (150 mL) and extracted with ethyl acetate (150 mL). The organic layer was washed with 10% aqueous sodium bicarbonate followed by 10% citric acid and then brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was triturated with MeOH and the resulting precipitate was filtered off to yield the title compound as a colorless solid (4.13 g, 44% from compound **5**): mp 204–206 °C; ^{1}H NMR ($CDCl_3$) δ 1.39 (9H, s), 1.56–1.62 (2H, m), 1.67–1.77 (2H, m), 2.63–2.74 (2H, m), 2.91 (3H, s), 3.86–3.97 (1H, m), 4.02–4.20 (2H, m), 4.56 (2H, s), 7.62 (1H, d, J = 8.4 Hz), 7.72 (1H, d, J = 8.4 Hz), 7.85–7.94 (3H, m), 8.22 (1H, s); MS (FAB) m/z 444 $[M+H]^+$.

7.1.4. *tert*-Butyl 4-[({7-[Amino(imino)methyl]-2-naphthyl}methyl)(methylsulfonyl)amino]piperidine-1-carboxylate (7). Hydrogen sulfide was bubbled through a solution of compound **6** (4.53 g, 10.2 mmol) in triethylamine (14 mL) and pyridine (80 mL) for 0.5 h, and the whole was then stirred at ambient temperature for 15 h. The solvents were removed in vacuo and the residue was redissolved in acetone (150 mL). To the solution was added iodomethane (12.7 mL, 204 mmol) at ambient temperature and the whole was heated to reflux for 3 h. The reaction mixture was allowed to cool to ambient temperature and the solvent removed in vacuo. The residue was then redissolved in MeOH (150 mL), and to the solution was added ammonium acetate (3.93 g, 51.0 mmol). This mixture was heated to reflux for 40 min. The reaction mixture was allowed to cool to ambient temperature and the solvent removed in vacuo. The residue was dissolved in H_2O (100 mL), made alkaline with 1 N aqueous NaOH solution at 0 °C, and then extracted with $CHCl_3$ (100 mL). The organic layer was dried over magnesium sulfate, and concentrated in vacuo. The residue was purified using column chromatography on silica gel (eluent: $CHCl_3$/MeOH = 70:30 by volume) to yield the title compound as a beige amorphous powder (3.24 g, 69%): ^{1}H NMR ($CDCl_3$) δ 1.39 (9H, s), 1.52–1.69 (2H, m), 1.71–1.79 (2H, m), 2.64–2.73 (2H, m), 2.88 (3H, s), 3.86–3.96 (1H, m), 3.99–4.23 (2H, m), 4.56 (2H, s), 7.60 (1H, d, J = 8.3 Hz), 7.71 (1H, d, J = 8.8 Hz), 7.85–7.89 (3H, m), 8.09 (1H, s); MS (FAB) m/z 461 $[M+H]^+$.

7.1.5. Polymer-bound 7-{[(Methylsulfonyl)(piperidin-4-yl)amino]methyl}naphthalene-2-carboximidamide (9). To a mixture of polymer-bound benzyl alcohol **8**[20] (1.63 g, 1.95 mmol; substitution 1.20 mmol/g), pyridine (10 mL), and CH_2Cl_2 (30 mL) was added 4-nitrophenylchloroformate (1.97 g, 9.75 mmol) at 0 °C, and the whole was shaken at ambient temperature for 12 h. The mixture was filtered, washed sequentially with CH_2Cl_2 and pyridine, and then dried to yield polymer-bound nitrophenylcarbonate. A mixture of this resin, compound **7** (1.80 g, 3.91 mmol), triethylamine (1.98 g, 19.6 mmol), and NMP (20 mL) was agitated at ambient temperature for 12 h. The mixture was filtered off, washed sequentially with NMP, DMF, CH_2Cl_2, and MeOH, and then dried to yield polymer-bound *N*-Boc piperidine precursor. To a mixture of this resin and MeOH (15 mL) was added 4 N hydrogen chloride in ethyl acetate (15 mL) at ambient temperature, and the whole was shaken for 2 h. The mixture was filtered off, washed sequentially with MeOH and CH_2Cl_2, and dried to yield polymer-bound piperidine intermediate **9**.

7.1.6. Solid-phase synthesis of library compounds 10: *N*-sulfonylpiperidine derivatives. Polymer **9** was partitioned into reaction vials that each contained polymer (30 mg, 0.030 mmol), and treated with sulfonyl chloride

(0.10 mmol) and diisopropylethylamine (0.026 mL, 0.15 mmol) in dioxane (1.0 mL) at ambient temperature. The reaction mixture was agitated for 12 h and washed sequentially with MeOH, pyridine, and CH_2Cl_2. The reaction mixture in each vessel was then treated with 2.0 mL of 90% aqueous trifluoroacetic acid at ambient temperature for 4 h. The cleavage mixture was collected in vials. The resin was washed with MeOH, and each eluent in the respective vials was combined. The solutions were evaporated in vacuo to afford *N*-sulfonylpiperidine derivatives **10**, which were analyzed for purity using reversed-phase HPLC and characterized by MS.

7.1.7. Solid-phase synthesis of library compounds 10: *N*-acylpiperidine derivatives. Polymer **9** was partitioned into reaction vials that each contained polymer (20 mg, 0.020 mmol), and treated with carboxylic acid (0.10 mmol), PyBOP (52 mg, 0.10 mmol), and diisopropylethylamine (0.020 mL, 0.10 mmol) in DMF (1.0 mL) at ambient temperature. The reaction mixture was agitated for 12 h and washed sequentially with DMF, MeOH, and CH_2Cl_2. The reaction mixture in each vessel was then treated with 2.0 mL of 90% aqueous trifluoroacetic acid at ambient temperature for 4 h. The cleavage mixture was collected in vials. The resin was washed with MeOH, and each eluent in the respective vials was combined. The solutions were evaporated in vacuo to afford *N*-acylpiperidine derivatives **10**, which were analyzed for purity using reversed-phase HPLC and characterized by MS.

7.1.8. Solid-phase synthesis of library compounds 10: *N*-alkylpiperidine derivatives. The polymer **9** was partitioned into reaction vials that each contained polymer (30 mg, 0.030 mmol), and treated with aldehyde (0.60 mmol), $NaBH(OAc)_3$ (64 mg, 0.60 mmol), and acetic acid (0.20 mL) in CH_2Cl_2 (2.0 mL) at ambient temperature. The reaction mixture was agitated for 12 h and washed sequentially with CH_2Cl_2 and MeOH. The reaction mixture in each vessel was then treated with 3.0 mL of 90% aqueous trifluoroacetic acid at ambient temperature for 4 h. The cleavage mixture was collected in vials. The resin was washed with MeOH, and each eluent in the respective vials was combined. The solutions were evaporated in vacuo to afford *N*-alkylpiperidine derivatives **10**, which were analyzed for purity using reversed-phase HPLC and characterized by MS.

7.1.9. 7-{[{1-[(2,5-Dimethoxyphenyl)sulfonyl]piperidin-4-yl}(methylsulfonyl)amino]methyl}naphthalene-2-carboximidamide hydrochloride (10x). Gaseous anhydrous hydrogen chloride was bubbled through a solution of compound **18** (300 mg, 0.552 mmol) in EtOH/$CHCl_3$ (11 mL, 1:1 by volume) at −20 °C for 20 min. The reaction mixture was allowed to warm to 5 °C and stirred for 22 h. The solvent was removed under reduced pressure, and the residue was redissolved in EtOH (11 mL). To this solution was added ammonium acetate (424 mg, 5.5 mmol) at ambient temperature, and the whole was stirred for 24 h. The reaction mixture was concentrated in vacuo, and the residue was purified using column chromatography on ODS gel (eluent: CH_3CN/0.001 N hydrochloric acid = 90:10 by volume). The organic solvent was removed by evaporation, and freeze-drying of the aqueous product solution yielded the title compound as a colorless amorphous powder (149 mg, 45%): ^{1}H NMR (DMSO-d_6) δ 1.48–1.59 (2H, m), 1.69–1.76 (2H, m), 2.57–2.65 (2H, m), 3.08 (3H, s), 3.63–3.67 (2H, m), 3.72 (3H, s), 3.74 (3H, s), 3.76–3.82 (1H, m), 4.57 (2H, s), 7.13–7.20 (3H, m), 7.72 (1H, d, J = 8.3 Hz), 7.83 (1H, d, J = 8.8 Hz), 8.04–8.07 (2H, m), 8.14 (1H, d, J = 8.3 Hz), 8.50 (1H, s), 9.25 (2H, s), 9.50 (2H, s); MS (FAB) m/z 561 $[M+H]^+$; Anal. Calcd for $C_{26}H_{32}N_4O_6S_2 \cdot HCl \cdot 0.6H_2O$: C, 51.37; H, 5.67; N, 9.22; Cl, 5.83; S, 10.55. Found: C, 51.04; H, 5.88; N, 9.19; Cl, 5.79; S, 10.46.

7.1.10. 1-[(2,5-Dimethoxyphenyl)sulfonyl]piperidin-4-one (13). To a stirred mixture of 4-piperidone hydrochloride hydrate (**12**) (520 mg, 3.84 mmol), 2,5-(dimethoxyphenyl)sulfonyl chloride (820 mg, 3.46 mmol), and 1,2-dichloroethane (38 mL) was added triethylamine (1.16 g, 11.5 mmol) at ambient temperature, and the whole was stirred for 20 h. The reaction mixture was washed with 10% citric acid followed by brine, dried over magnesium sulfate, and then concentrated in vacuo to yield the title compound as a colorless amorphous powder (890 mg, 76%): ^{1}H NMR ($CDCl_3$) δ 2.53 (4H, t, J = 6.0 Hz), 3.59 (4H, t, J = 6.0 Hz), 3.81 (3H, s), 3.89 (3H, s), 6.97 (1H, d, J = 9.0 Hz), 7.08 (1H, dd, J = 3.3 and 9.0 Hz), 7.48 (1H, d, J = 3.3 Hz); MS (FAB) m/z 300 $[M+H]^+$.

7.1.11. 7-{[(1-Pyridin-4-ylpiperidin-4-yl)amino]methyl}-2-naphthonitrile (14). Compound **5** (7.13 g, 32.6 mmol) was suspended in $CHCl_3$ (100 mL) and washed with 10% aqueous potassium carbonate. The organic layer was dried over magnesium sulfate and concentrated in vacuo to yield 7-(aminomethyl)-2-naphthonitrile (5.94 g, quant). To a stirred mixture of 4-(4-oxopiperidin-1-yl)pyridine (**11**)[22] (270 mg, 1.53 mmol), 7-(aminomethyl)-2-naphthonitrile (279 mg, 1.53 mmol), AcOH (0.90 mL), and 1,2-dichloroethane (15 mL) was added $NaBH(OAc)_3$ (634 mg, 3.00 mmol) at ambient temperature, and the whole was stirred for 1 h. To the reaction mixture was added 10% aqueous potassium carbonate (50 mL), and the mixture was extracted with $CHCl_3$ (50 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo to yield the title compound as a colorless amorphous powder (350 mg, 67%): ^{1}H NMR ($CDCl_3$) δ 1.30–1.75 (2H, m), 1.94–2.18 (2H, m), 2.72–3.15 (3H, m), 3.76–4.06 (2H, m), 4.10 (2H, s), 6.71 (2H, d, J = 6.6 Hz), 7.56–8.00 (5H, m), 8.26–8.33 (3H, m); MS (FAB) m/z 343 $[M+H]^+$.

7.1.12. 7-[({1-[(2,5-Dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)methyl]-2-naphthonitrile (15). To a stirred mixture of compound **13** (1.02 g, 3.41 mmol), 7-(aminomethyl)-2-naphthonitrile (621 mg, 3.41 mmol), AcOH (2.0 mL), and 1,2-dichloroethane (34 mL) was added $NaBH(OAc)_3$ (1.44 g, 6.80 mmol) at ambient temperature, and the whole was stirred for 1 h. To the reaction mixture was added 10% aqueous potassium carbonate (50 mL), and the resulting mixture was extracted with

$CHCl_3$ (50 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo to yield the title compound as a colorless amorphous powder (1.59 g, quant): ^{1}H NMR ($CDCl_3$) δ 1.41–1.56 (4H, m), 1.92–2.01 (2H, m), 2.57–2.66 (1H, m), 2.71–2.82 (2H, m), 3.79 (3H, s), 3.86 (3H, s), 3.99 (2H, s), 6.94 (1H, d, J = 9.0 Hz), 7.04 (1H, dd, J = 3.0 and 9.0 Hz), 7.43 (1H, d, J = 3.0 Hz), 7.58 (1H, d, J = 8.7 Hz), 7.62 (1H, d, J = 8.4 Hz), 7.79 (1H, s), 7.85 (1H, d, J = 8.4 Hz), 7.89 (1H, d, J = 8.7 Hz), 8.18 (1H, s); MS (FAB) m/z 466 $[M+H]^+$.

7.1.13. 3-[(*E*)-3-({1-[(2,5-Dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)prop-1-en-1-yl]benzonitrile (16). The title compound was obtained as a colorless amorphous powder from 0.680 g (2.27 mmol) of compound **13**, 0.360 g (2.28 mmol) of (*E*)-3-(3-aminopropenyl)benzonitrile,[23] 954 mg (4.50 mmol) of $NaBH(OAc)_3$, and 1.31 mL (22.8 mmol) of AcOH in a manner identical to that described above for compound **15** (762 mg, 76%): ^{1}H NMR ($CDCl_3$) δ 1.38–1.53 (2H, m), 1.56–1.72 (2H, m), 1.87–1.99 (2H, m), 2.58–2.67 (1H, m), 2.72–2.82 (2H, m), 3.46 (2H, d, J = 6.0 Hz), 3.80 (3H, s), 3.87 (3H, s), 6.33 (1H, dt, J = 15.6 and 6.0 Hz), 6.51 (1H, d, J = 15.6 Hz), 6.94 (1H, d, J = 9.0 Hz), 7.25 (1H, dd, J = 3.3 and 9.0 Hz), 7.38–7.63 (5H, m); MS (FAB) m/z 442 $[M+H]^+$.

7.1.14. *N*-[(7-Cyano-2-naphthyl)methyl]-*N*-(1-pyridin-4-ylpiperidin-4-yl)methanesulfonamide (17). To a mixture of compound **14** (170 mg, 0.496 mmol), triethylamine (0.87 g, 8.61 mmol), and 1,2-dichloroethane (12 mL) was added methanesulfonyl chloride (986 mg, 8.61 mmol) at 0 °C and the whole was stirred for 5 h. To the reaction mixture was added 10% aqueous potassium carbonate (20 mL) followed by extraction with $CHCl_3$ (20 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo. The residue was purified using column chromatography on silica gel (eluent: $CHCl_3$/MeOH/c.NH_3 = 100:5:0.5 by volume) to yield the title compound as a colorless amorphous powder (110 mg, 53%): ^{1}H NMR ($CDCl_3$) δ 1.78–1.95 (4H, m), 2.73–2.98 (2H, m), 3.00 (3H, s), 3.78–3.93 (1H, m), 3.95–4.12 (2H, m), 4.62 (2H, s), 6.61 (2H, d, J = 6.6 Hz), 7.57–8.00 (5H, m), 8.20–8.30 (3H, m); MS (FAB) m/z 421 $[M+H]^+$.

7.1.15. *N*-[(7-Cyano-2-naphthyl)methyl]-*N*-{1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}methanesulfonamide (18). To a stirred mixture of compound **15** (500 mg, 1.07 mmol), triethylamine (325 mg, 3.21 mmol), and 1,2-dichloroethane (11 mL) was added methanesulfonyl chloride (135 mg, 1.18 mmol) at 0 °C, and the whole was stirred for 3 h. The reaction mixture was then washed with 10% citric acid, followed by brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified using column chromatography on silica gel (eluent: $CHCl_3$/MeOH = 100:5 by volume) to yield the title compound as a colorless amorphous powder (420 mg, 72%): ^{1}H NMR ($CDCl_3$) δ 1.68–1.78 (4H, m), 2.55–2.68 (2H, m), 2.90 (3H, s), 3.74–3.91 (9H, m), 4.55 (2H, s), 6.90 (1H, d, J = 8.7 Hz), 7.03 (1H, dd, J = 3.0 and 8.7 Hz), 7.34 (1H, d, J = 3.0 Hz), 7.59 (1H, d, J = 8.4 Hz), 7.69 (1H, d, J = 8.4 Hz), 7.77–7.92 (3H, m), 8.19 (1H, s); MS (FAB) m/z 544 $[M+H]^+$.

7.1.16. *N*-[(7-Cyano-2-naphthyl)methyl]-*N*-{1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}acetamide (19). The title compound was obtained as a colorless amorphous powder from 500 mg (1.07 mmol) of compound **15**, 126 mg (1.61 mmol) of acetyl chloride, and 326 mg (3.21 mmol) of triethylamine in a manner identical to that described above for compound **18** (490 mg, 90%): ^{1}H NMR ($CDCl_3$) δ 1.52–1.86 (4H, m), 2.10 (3H, s), 2.59–2.74 (2H, m), 3.69–3.94 (9H, m), 4.68 (2H, s), 6.83–7.03 (2H, m), 7.36 (1H, d, J = 3.0 Hz), 7.48 (1H, d, J = 6.9 Hz), 7.65 (1H, d, J = 9.3 Hz), 7.80–7.95 (3H, m), 8.19 (1H, s); MS (FAB) m/z 508 $[M+H]^+$.

7.1.17. 7-{[{1-[(2,5-Dimethoxyphenyl)sulfonyl]piperidin-4-yl}(methyl)amino]methyl}-2-naphthonitrile (20). The title compound was obtained as a colorless amorphous powder from 500 mg (1.07 mmol) of compound **15**, 0.550 mL (5.35 mmol) of 35% aqueous HCHO, 454 mg (2.14 mmol) of $NaBH(OAc)_3$, and 0.610 mL (10.7 mmol) of AcOH in a manner identical to that described above for compound **15** (443 mg, 86%): ^{1}H NMR ($CDCl_3$) δ 1.64–1.78 (2H, m), 1.84–1.92 (2H, m), 2.22 (3H, s), 2.49–2.68 (3H, m), 3.73 (2H, s), 3.80 (3H, s), 3.87 (3H, s), 3.91–4.00 (2H, m), 6.94 (1H, d, J = 8.7 Hz), 7.05 (1H, dd, J = 3.0 and 8.7 Hz), 7.44 (1H, d, J = 3.0 Hz), 7.56 (1H, d, J = 8.4 Hz), 7.63 (1H, d, J = 8.4 Hz), 7.76 (1H, s), 7.83 (1H, d, J = 8.4 Hz), 7.88 (1H, d, J = 8.4 Hz), 8.17 (1H, s); MS (FAB) m/z 480 $[M+H]^+$.

7.1.18. Ethyl [([(7-cyano-2-naphthyl)methyl]{1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)sulfonyl]acetate (21). The title compound was obtained as a colorless amorphous powder from 1.61 g (3.46 mmol) of compound **15**, 711 mg (3.81 mmol) of ethyl (chlorosulfonyl)acetate,[31] and 385 mg (3.81 mmol) of triethylamine in a manner identical to that described above for compound **18** (1.74 g, 82%): ^{1}H NMR ($CDCl_3$) δ 1.31 (3H, t, J = 6.9 Hz), 1.60–1.75 (2H, m), 1.85–1.93 (2H, m), 2.58–2.68 (2H, m), 2.77 (6H, s), 3.83–3.91 (5H, m), 4.24 (2H, q, J = 6.9 Hz), 4.61 (2H, s), 6.88 (1H, d, J = 9.0 Hz), 7.03 (1H, dd, J = 3.3 and 9.0 Hz), 7.35 (1H, d, J = 3.3 Hz), 7.63 (1H, d, J = 8.4 Hz), 7.70 (1H, d, J = 8.4 Hz), 7.85 (1H, s), 7.89 (1H, d, J = 8.4 Hz), 7.93 (1H, d, J = 8.4 Hz), 8.23 (1H, s); MS (FAB) m/z 616 $[M+H]^+$.

7.1.19. Ethyl 3-([(7-cyano-2-naphthyl)methyl]{1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)-3-oxopropanoate (22). The title compound was obtained as a colorless amorphous powder from 1.28 g (2.75 mmol) of compound **15**, 456 mg (3.03 mmol) of ethyl malonyl chloride, and 306 mg (3.03 mmol) of triethylamine in a manner identical to that described above for compound **18** (1.42 g, 89%): ^{1}H NMR ($CDCl_3$) δ 1.25 (2H, t, J = 7.2 Hz), 1.32 (1H, t, J = 7.2 Hz), 1.61–1.77 (4H, m), 2.57–2.75 (2H, m), 3.37 (1.3H, s), 3.62 (0.7H, s), 3.77–3.97 (9H, m), 4.16 (1.3H, q, J = 7.2 Hz), 4.26 (0.7H, q, J = 7.2 Hz), 4.69 (1.3H, s), 4.74 (0.7H, s), 6.91–6.96 (1H, m), 7.02–7.08 (1H, m), 7.38 (1H, d, J = 3.3 Hz),

7.46–7.66 (2H, m), 7.69 (0.7H, s), 7.74 (0.3H, s), 7.82–7.95 (2H, m), 8.18 (0.3H, s), 8.19 (0.7H, s); MS (FAB) *m/z* 580 $[M+H]^+$.

7.1.20. Ethyl [([(*E*)-3-(3-cyanophenyl)prop-2-en-1-yl]{1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)sulfonyl]acetate (23). The title compound was obtained as a colorless amorphous powder from 1.02 g (2.31 mmol) of compound **16**, 473 mg (2.54 mmol) of ethyl (chlorosulfonyl)acetate, and 257 mg (2.54 mmol) of triethylamine in a manner identical to that described above for compound **18** (1.01 g, 74%): ^{1}H NMR ($CDCl_3$) δ 1.29 (3H, t, J = 7.2 Hz), 1.84–1.97 (4H, m), 2.63–2.73 (2H, m), 3.75–3.82 (4H, m), 3.86 (3H, s), 3.93 (2H, s), 3.96–4.02 (2H, m), 4.04 (2H, d, J = 6.3 Hz), 4.21 (2H, q, J = 7.2 Hz), 6.22 (1H, dt, J = 15.9 and 6.3 Hz), 6.57 (1H, d, J = 15.9 Hz), 6.94 (1H, d, J = 9.0 Hz), 7.06 (1H, dd, J = 3.0 and 9.0 Hz), 7.40–7.47 (2H, m), 7.53–7.65 (2H, m), 7.64 (1H, s); MS (FAB) *m/z* 592 $[M+H]^+$.

7.1.21. Ethyl 3-([(*E*)-3-(3-cyanophenyl)prop-2-en-1-yl]{1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)-3-oxopropanoate (24). The title compound was obtained as a colorless amorphous powder from 740 mg (1.68 mmol) of compound **16**, 858 mg (5.70 mmol) of ethyl malonyl chloride, and 1.15 g (11.4 mmol) of triethylamine in a manner identical to that described above for compound **18** (490 mg, 53%): ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.2 Hz), 1.54–1.58 (2H, m), 1.74–1.81 (2H, m), 2.66–2.74 (2H, m), 3.44 (1.4H, s), 3.51 (0.6H, s), 3.80 (3H, s), 3.86 (2.1H, s), 3.88 (0.9H, s), 3.93–4.09 (5H, m), 4.17 (2H, q, J = 7.2 Hz), 6.16–6.25 (1H, m), 6.49 (1H, d, J = 15.9 Hz), 6.93–6.98 (1H, m), 7.04–7.08 (1H, m), 7.41–7.62 (5H, m); MS (FAB) *m/z* 556 $[M+H]^+$.

7.1.22. Ethyl {[({7-[(amino)(hydroxyimino)methyl]-2-naphthyl}methyl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino]sulfonyl}acetate (25). A mixture of compound **21** (1.73 g, 2.81 mmol), hydroxylamine hydrochloride (389 mg, 5.60 mmol), triethylamine (627 mg, 6.20 mmol), and EtOH (28 mL) was stirred at 80 °C for 14 h. The reaction mixture was allowed to cool and the solvent was removed in vacuo. The residue was dissolved in $CHCl_3$ (30 mL) and washed with brine (20 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo. The residue was purified using column chromatography on silica gel (eluent: $CHCl_3$/MeOH/c.NH_3 = 100:3:0.3 by volume) to yield the title compound as a colorless amorphous powder (1.10 g, 60%): ^{1}H NMR (DMSO-d_6) δ 1.22 (3H, t, J = 7.3 Hz), 1.37–1.50 (2H, m), 1.69–1.76 (2H, m), 2.58–2.66 (2H, m), 3.62–3.66 (2H, m), 3.68 (3H, s), 3.71 (3H, s), 3.74–3.86 (1H, m), 4.19 (2H, q, J = 7.3 Hz), 4.44 (2H, s), 4.55 (2H, s), 5.98 (2H, br s), 7.06 (1H, d, J = 8.3 Hz), 7.10–7.17 (2H, m), 7.51 (1H, d, J = 8.3 Hz), 7.85 (1H, d, J = 8.3 Hz), 7.86 (1H, s), 7.89 (1H, d, J = 8.3 Hz), 7.93 (1H, d, J = 8.3 Hz), 8.45 (1H, s), 9.81 (1H, s); MS (FAB) *m/z* 649 $[M+H]^+$; Anal. Calcd for $C_{29}H_{36}N_4O_9S_2{\cdot}0.5H_2O$: C, 52.96; H, 5.67; N, 8.52; S, 9.75. Found: C, 52.96; H, 5.64; N, 8.36; S, 9.73.

7.1.23. Ethyl 3-[({7-[(amino)(hydroxyimino)methyl]-2-naphthyl}methyl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino]-3-oxopropanoate (26). The title compound was obtained as a colorless amorphous powder from 1.01 g (1.74 mmol) of compound **22**, 242 mg (3.48 mmol) of hydroxylamine hydrochloride, and 439 mg (4.35 mmol) of triethylamine in a manner identical to that described above for compound **25** (840 mg, 79%): ^{1}H NMR (DMSO-d_6) δ 1.17 (3H, t, J = 7.3 Hz), 1.63–1.70 (4H, m), 2.65–2.74 (2H, m), 3.55 (2H, br s), 3.70–3.74 (5H, m), 3.75 (3H, s), 4.01–4.13 (3H, m), 4.65 (2H, s), 5.87 (2H, br s), 7.10 (1H, d, J = 8.8 Hz), 7.14 (1H, dd, J = 2.9 and 8.8 Hz), 7.22 (1H, d, J = 2.9 Hz), 7.38 (1H, d, J = 8.8 Hz), 7.70 (1H, s), 7.76–7.84 (3H, m), 8.15 (1H, s), 9.46 (1H, s); MS (FAB) *m/z* 613 $[M+H]^+$; Anal. Calcd for $C_{30}H_{36}N_4O_8S{\cdot}0.6H_2O$: C, 57.79; H, 6.01; N, 8.99; S, 5.14. Found: C, 57.64; H, 5.99; N, 8.82; S, 5.07.

7.1.24. Ethyl {[((*E*)-3-{3-[(amino)(hydroxyimino)methyl]phenyl}prop-2-en-1-yl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino]sulfonyl}acetate (27). The title compound was obtained as a colorless amorphous powder from 320 mg (0.541 mmol) of compound **23**, 75 mg (1.35 mmol) of hydroxylamine hydrochloride, and 137 mg (1.35 mmol) of triethylamine in a manner identical to that described above for compound **25** (235 mg, 76%): ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, t, J = 7.3 Hz), 1.66–1.83 (4H, m), 2.61–2.70 (2H, m), 3.67–3.78 (6H, m), 3.80 (3H, s), 3.98 (2H, d, J = 6.3 Hz), 4.14 (2H, q, J = 7.3 Hz), 4.33 (2H, s), 5.88 (2H, br s), 6.24 (1H, dt, J = 16.1 and 6.3 Hz), 6.57 (1H, d, J = 16.1 Hz), 7.14–7.22 (3H, m), 7.34 (1H, t, J = 7.8 Hz), 7.42 (1H, d, J = 7.8 Hz), 7.68 (1H, d, J = 7.8 Hz), 7.73 (1H, s), 9.21 (1H, br s); MS (FAB) *m/z* 625 $[M+H]^+$; Anal. Calcd for $C_{27}H_{36}N_4O_9S_2{\cdot}0.5H_2O$: C, 51.17; H, 5.88; N, 8.84; S, 10.12. Found: C, 51.26; H, 5.91; N, 8.60; S, 9.96.

7.1.25. Ethyl 3-[((*E*)-3-{3-[(amino)(hydroxyimino)methyl]phenyl}prop-2-en-1-yl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino]-3-oxopropanoate (28). The title compound was obtained as a colorless amorphous powder from 330 mg (0.594 mmol) of compound **24**, 83 mg (1.19 mmol) of hydroxylamine hydrochloride, and 150 mg (1.49 mmol) of triethylamine in a manner identical to that described above for compound **25** (236 mg, 67%): ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 6.9 Hz), 1.67–1.81 (4H, m), 2.68–2.76 (2H, m), 3.49 (2H, s), 3.75 (3H, s), 3.76–3.83 (5H, m), 3.97–4.03 (3H, m), 4.08 (2H, q, J = 6.9 Hz), 5.50 (2H, br s), 6.36 (1H, dt, J = 16.1 and 5.4 Hz), 6.51 (1H, d, J = 16.1 Hz), 7.15 (2H, s), 7.27 (1H, s), 7.30 (1H, t, J = 7.8 Hz), 7.36 (1H, d, J = 7.8 Hz), 7.53 (1H, d, J = 7.8 Hz), 7.66 (1H, s), 9.21 (1H, br s); MS (FAB) *m/z* 589 $[M+H]^+$; Anal. Calcd for $C_{28}H_{36}N_4O_8S{\cdot}0.6H_2O$: C, 56.10; H, 6.25; N, 9.35; S, 5.35. Found: C, 56.09; H, 6.25; N, 9.17; S, 5.30.

7.1.26. 7-{[(Methylsulfonyl)(1-pyridin-4-ylpiperidin-4-yl)amino]methyl}naphthalene-2-carboximidamide hydrochloride (29). The title compound was obtained as a colorless amorphous powder from 110 mg (0.262 mmol) of compound **17** and 200 mg (2.60 mmol) of ammonium acetate in a manner identical to that described above for compound **10x** (63 mg, 47%): ^{1}H NMR (DMSO-d_6) δ 1.61–1.72 (2H, m), 1.84–1.90 (2H, m), 3.13 (3H, s),

3.16–3.22 (2H, m), 4.10–4.18 (1H, m), 4.24–4.28 (2H, m), 4.62 (2H, s), 7.14 (2H, d, J = 7.2 Hz), 7.70 (1H, d, J = 9.6 Hz), 7.84 (1H, dd, J = 1.6 and 8.4 Hz), 8.02–8.04 (2H, m), 8.11 (1H, d, J = 8.4 Hz), 8.14–8.18 (2H, m), 8.52 (1H, s), 9.39 (2H, s), 9.58 (2H, s), 13.79 (1H, br s); MS (FAB) m/z 438 $[M+H]^+$; Anal. Calcd for $C_{23}H_{27}N_5O_2S{\cdot}2.3HCl{\cdot}1.4H_2O$: C, 50.54; H, 5.92; N, 12.81; Cl, 14.92; S, 5.87. Found: C, 50.79; H, 6.24; N, 13.09; Cl, 14.96; S, 5.69.

7.1.27. *N*-({7-[Amino(imino)methyl]-2-naphthyl}methyl)-*N*-{1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}acetamide hydrochloride (30). The title compound was obtained as a colorless amorphous powder from 330 mg (0.650 mmol) of compound **19** and 500 mg (6.50 mmol) of ammonium acetate in a manner identical to that described above for compound **10x** (121 mg, 33%): ^{1}H NMR (DMSO-d_6) δ 1.59–1.70 (4H, m), 2.10 (3H, s), 2.67–2.74 (2H, m), 3.65–3.75 (5H, m), 3.77 (3H, s), 4.18–4.20 (1H, m), 4.67 (2H, s), 7.11–7.15 (2H, m), 7.22 (1H, d, J = 2.9 Hz), 7.56 (1H, d, J = 8.3 Hz), 7.80–7.84 (2H, m), 7.98 (1H, d, J = 8.7 Hz), 8.07 (1H, d, J = 8.3 Hz), 8.47 (1H, s), 9.25 (4H, s); MS (FAB) m/z 525 $[M+H]^+$; Anal. Calcd for $C_{27}H_{32}N_4O_5S{\cdot}HCl{\cdot}H_2O$: C, 56.00; H, 6.09; N, 9.67; Cl, 6.12; S, 5.54. Found: C, 55.87; H, 6.29; N, 9.64; Cl, 6.47; S, 5.49.

7.1.28. 7-{[{1-[(2,5-Dimethoxyphenyl)sulfonyl]piperidin-4-yl}(methyl)amino]methyl}naphthalene-2-carboximidamide hydrochloride (31). The title compound was obtained as a colorless amorphous powder from 400 mg (0.845 mmol) of compound **20** and 655 mg (8.50 mmol) of ammonium acetate in a manner identical to that described above for compound **10x** (242 mg, 50%): ^{1}H NMR (DMSO-d_6) δ 1.54–1.72 (2H, m), 1.94–2.18 (2H, m), 2.28–2.42 (2H, m), 2.66–2.74 (2H, m), 2.93 (3H, br s), 3.76–3.86 (9H, m), 7.16–7.19 (2H, m), 7.28 (1H, s), 7.73–7.85 (2H, m), 8.00–8.18 (3H, m), 8.46 (1H, s), 9.16 (4H, br s); MS (FAB) m/z 497 $[M+H]^+$; Anal. Calcd for $C_{26}H_{32}N_4O_4S{\cdot}1.3HCl{\cdot}2.5H_2O$: C, 53.01; H, 6.55; N, 9.51; Cl, 7.82; S, 5.44. Found: C, 52.88; H, 6.30; N, 9.30; Cl, 7.96; S, 5.92.

7.1.29. Ethyl [({7-[amino(imino)methyl]-2-naphthyl}methyl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)sulfonyl]acetate hydrochloride (32). To a mixture of compound **25** (710 mg, 1.09 mmol), acetic anhydride (224 mg, 2.19 mmol), and AcOH (11 mL) was added Pd/C (10 w/w%, 70 mg), and the mixture was stirred under hydrogen pressure (1 kg/cm^2) at ambient temperature for 8 h. The reaction mixture was filtered through a pad of Celite® and the filtrate was concentrated in vacuo. The residue was purified using column chromatography on ODS gel (eluent: EtOH/H_2O = 50:50 by volume). The solvent was removed by evaporation and the residue was dissolved in EtOH (10 mL). To this solution was added 4 N HCl/AcOEt (1.0 mL) at ambient temperature, and the whole was stirred for 10 min. The mixture was concentrated in vacuo to yield the title compound as a colorless amorphous powder (518 mg, 71%): ^{1}H NMR (DMSO-d_6) δ 1.24 (3H, t, J = 7.3 Hz), 1.39–1.51 (2H, m), 1.72–1.79 (2H, m), 2.56–2.65 (2H, m), 3.59–3.66 (2H, m), 3.71 (3H, s), 3.72 (3H, s), 3.78–3.86 (1H, m), 4.19 (2H, q, J = 7.3 Hz), 4.49 (2H, s), 4.62 (2H, s), 7.12–7.19 (3H, m), 7.71 (1H, d, J = 8.8 Hz), 7.86 (1H, d, J = 8.8 Hz), 8.05 (1H, s), 8.07 (1H, d, J = 8.8 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.50 (1H, s), 9.33 (2H, s), 9.54 (2H, s); MS (FAB) m/z 633 $[M+H]^+$; Anal. Calcd for $C_{29}H_{36}N_4O_8S_2{\cdot}HCl{\cdot}H_2O$: C, 50.68; H, 5.72; N, 8.15; Cl, 5.16; S, 9.33. Found: C, 50.86; H, 5.71; N, 8.02; Cl, 4.95; S, 9.11.

7.1.30. Ethyl 3-({{7-[amino(imino)methyl]-2-naphthyl}methyl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)-3-oxopropanoate hydrochloride (33). The title compound was obtained as a colorless amorphous powder from 0.430 g (0.702 mmol) of compound **26** and 143 mg (1.40 mmol) of acetic anhydride in a manner identical to that described above for compound **32** (321 mg, 72%): ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.3 Hz), 1.63–1.71 (4H, m), 2.66–2.74 (2H, m), 3.58 (2H, br), 3.69–3.75 (5H, m), 3.77 (3H, s), 4.03–4.14 (3H, m), 4.70 (2H, s), 7.12–7.17 (2H, m), 7.22 (1H, d, J = 2.4 Hz), 7.58 (1H, d, J = 8.8 Hz), 7.82 (1H, d, J = 8.3 Hz), 7.85 (1H, s), 7.98 (1H, d, J = 8.3 Hz), 8.07 (1H, d, J = 8.8 Hz), 8.44 (1H, s), 9.26 (4H, br s); MS (FAB) m/z 597 $[M+H]^+$; Anal. Calcd for $C_{30}H_{36}N_4O_{7}S{\cdot}HCl{\cdot}H_2O$: C, 55.34; H, 6.04; N, 8.60; Cl, 5.44; S, 4.92. Found: C, 55.13; H, 6.07; N, 8.48; Cl, 5.44; S, 4.86.

7.1.31. Ethyl [(((*E*)-3-{3-[amino(imino)methyl]phenyl}prop-2-en-1-yl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)sulfonyl]acetate hydrochloride (34). The title compound was obtained as a colorless amorphous powder from 400 mg (0.676 mmol) of compound **23** and 524 mg (6.80 mmol) of ammonium acetate in a manner identical to that described above for compound **10x** (380 mg, 91%): ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, t, J = 7.3 Hz), 1.68–1.84 (4H, m), 2.61–2.68 (2H, m), 3.70–3.76 (6H, m), 3.81 (3H, s), 4.02 (2H, d, J = 5.9 Hz), 4.14 (2H, q, J = 7.3 Hz), 4.36 (2H, s), 6.41 (1H, dt, J = 16.2 and 5.9 Hz), 6.66 (1H, d, J = 16.2 Hz), 7.19–7.22 (3H, m), 7.59 (1H, t, J = 7.8 Hz), 7.72 (1H, d, J = 7.8 Hz), 7.78 (1H, d, J = 7.8 Hz), 7.91 (1H, s), 9.25 (2H, s), 9.44 (2H, s); MS (FAB) m/z 609 $[M+H]^+$; Anal. Calcd for $C_{27}H_{36}N_4O_8S_2{\cdot}HCl{\cdot}H_2O$: C, 48.90; H, 5.93; N, 8.45; Cl, 5.35; S, 9.67. Found: C, 48.74; H, 5.86; N, 8.23; Cl, 5.54; S, 9.49.

7.1.32. Ethyl 3-(((*E*)-3-{3-[amino(imino)methyl]phenyl}prop-2-en-1-yl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)-3-oxopropanoate hydrochloride (35). The title compound was obtained as a colorless amorphous powder from 490 mg (0.882 mmol) of compound **24** and 678 mg (8.80 mmol) of ammonium acetate in a manner identical to that described above for compound **10x** (400 mg, 74%): ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 6.8 Hz), 1.71–1.79 (4H, m), 2.67–2.76 (2H, m), 3.51 (2H, s), 3.76 (3H, s), 3.77–3.82 (2H, m), 3.83 (3H, s), 3.98–4.05 (3H, m), 4.14 (2H, q, J = 6.8 Hz), 6.36 (1H, dt, J = 16.1 and 5.4 Hz), 6.58 (1H, d, J = 16.1 Hz), 7.16–7.17 (2H, m), 7.26 (1H, d, J = 1.4 Hz), 7.55 (1H, t, J = 7.8 Hz), 7.68–7.72 (2H, m), 7.87 (1H, s), 9.23 (4H, s); MS (FAB) m/z 573 $[M+H]^+$; Anal. Calcd for $C_{28}H_{36}N_4O_7S{\cdot}HCl{\cdot}H_2O$: C, 53.62; H, 6.27; N, 8.93; Cl,

5.65; S, 5.11. Found: C, 53.89; H, 6.25; N, 8.77; Cl, 5.66; S, 5.09.

7.1.33. [({7-[Amino(imino)methyl]-2-naphthyl}methyl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)sulfonyl]acetic acid hydrochloride (36). To a stirred solution of compound **32** (270 mg, 0.403 mmol) in dioxane (4.0 mL) and H_2O (2.7 mL) was added 1 N aqueous NaOH solution (1.3 mL) at 0 °C, and the whole was stirred for 4 h. To this mixture was added 1 N hydrochloric acid (1.3 mL) at 0 °C and the whole was allowed to warm to ambient temperature. The solvent was removed in vacuo and the residue was purified using column chromatography on ODS gel (eluent: CH_3CN/0.001 N hydrochloric acid = 50:50 by volume). The organic solvent was removed by evaporation, and freeze-drying of the aqueous product solution yielded the title compound as a colorless amorphous powder (148 mg, 57%): ^{1}H NMR (DMSO-d_6) δ 1.38–1.50 (2H, m), 1.72–1.80 (2H, m), 2.55–2.63 (2H, m), 3.56–3.65 (2H, m), 3.71 (3H, s), 3.72 (3H, s), 3.75–3.85 (1H, m), 4.33 (2H, s), 4.62 (2H, s), 7.11–7.18 (3H, m), 7.72 (1H, d, J = 8.8 Hz), 7.84 (1H, d, J = 8.8 Hz), 8.05 (1H, s), 8.07 (1H, d, J = 8.8 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.49 (1H, s), 9.35 (2H, s), 9.50 (2H, s), 13.37 (1H, br s); MS (FAB) m/z 605 $[M+H]^+$; Anal. Calcd for $C_{27}H_{32}N_4O_8S_2 \cdot 0.9HCl \cdot H_2O$: C, 49.47; H, 5.37; N, 8.55; Cl, 4.87; S, 9.78. Found: C, 49.30; H, 5.36; N, 8.55; Cl, 4.93; S, 9.76.

7.1.34. 3-(({7-[Amino(imino)methyl]-2-naphthyl}methyl)-{1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}amino)-3-oxopropanoic acid hydrochloride (37). The title compound was obtained as a colorless amorphous powder from 0.240 g (0.379 mmol) of compound **33** and 1.2 mL of 1 N aqueous NaOH solution in a manner identical to that described above for compound **36** (147 mg, 64%): ^{1}H NMR (DMSO-d_6) δ 1.58–1.67 (4H, m), 2.66–2.75 (2H, m), 2.87 (2H, s), 3.69–3.76 (5H, m), 3.77 (3H, s), 4.04–4.16 (1H, m), 4.67 (2H, s), 7.11–7.17 (2H, m), 7.22 (1H, d, J = 2.5 Hz), 7.56 (1H, d, J = 8.3 Hz), 7.80–7.82 (2H, m), 7.98 (1H, d, J = 8.3 Hz), 8.07 (1H, d, J = 8.8 Hz), 8.47 (1H, s), 9.25 (4H, br s); MS (FAB) m/z 569 $[M+H]^+$; Anal. Calcd for $C_{28}H_{32}N_4O_7S \cdot HCl \cdot H_2O$: C, 53.97; H, 5.66; N, 8.99; Cl, 5.69; S, 5.15. Found: C, 54.17; H, 5.73; N, 8.72; Cl, 5.61; S, 5.02.

7.1.35. [((*E*)-3-{3-[Amino(imino)methyl]phenyl}prop-2-en-1-yl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}-amino)sulfonyl]acetic acid hydrochloride (38). The title compound was obtained as a colorless amorphous powder from 360 mg (0.558 mmol) of compound **34** and 1.2 mL of 1 N aqueous NaOH solution in a manner identical to that described above for compound **36** (233 mg, 68%): ^{1}H NMR (DMSO-d_6) δ 1.67–1.85 (4H, m), 2.59–2.67 (2H, m), 3.69–3.76 (6H, m), 3.81 (3H, s), 4.03 (2H, d, J = 5.8 Hz), 4.23 (2H, s), 6.42 (1H, dt, J = 16.2 and 5.8 Hz), 6.66 (1H, d, J = 16.2 Hz), 7.19–7.22 (3H, m), 7.59 (1H, t, J = 7.8 Hz), 7.70 (1H, d, J = 7.8 Hz), 7.78 (1H, d, J = 7.8 Hz), 7.90 (1H, s), 9.23 (2H, s), 9.42 (2H, s), 13.33 (1H, br s); MS (FAB) m/z 581 $[M+H]^+$; Anal. Calcd for $C_{25}H_{32}N_4O_8S_2 \cdot 1.1HCl \cdot 0.7\ H_2O$: C, 47.41; H, 5.49; N, 8.85; Cl, 6.16; S, 10.13. Found: C, 47.70; H, 5.55; N, 8.76; Cl, 5.95; S, 9.72.

7.1.36. 3-(((*E*)-3-{3-[Amino(imino)methyl]phenyl}prop-2-en-1-yl){1-[(2,5-dimethoxyphenyl)sulfonyl]piperidin-4-yl}-amino)-3-oxopropanoic acid hydrochloride (39). The title compound was obtained as a colorless amorphous powder from 460 mg (0.755 mmol) of compound **35** and 1.8 mL of 1 N aqueous NaOH solution in a manner identical to that described above for compound **36** (249 mg, 57%): ^{1}H NMR (DMSO-d_6) δ 1.68–1.79 (4H, m), 2.68–2.76 (2H, m), 3.75 (3H, s), 3.76–3.81 (4H, m), 3.82 (3H, s), 3.99–4.04 (3H, m), 6.34 (1H, dt, J = 16.1 and 5.3 Hz), 6.54 (1H, d, J = 16.1 Hz), 7.15–7.17 (2H, m), 7.26 (1H, s), 7.49 (1H, t, J = 7.8 Hz), 7.64–7.69 (2H, m), 7.85 (1H, s), 8.72 (4H, s); MS (FAB) m/z 545 $[M+H]^+$; Anal. Calcd for $C_{26}H_{32}N_4O_7S \cdot 0.8HCl \cdot H_2O$: C, 52.77; H, 5.93; N, 9.47; Cl, 4.79; S, 5.42. Found: C, 52.76; H, 5.94; N, 9.69; Cl, 4.46; S, 5.31.

7.2. Pharmacology

7.2.1. In vitro chromogenic assay. The hydrolysis rates of the synthetic substrates were assayed by continuously measuring absorbance at 405 nm at 37 °C with a microplate spectrophotometer (Spectramax 340PC, Molecular Device Co., California, USA). The reaction mixtures (40 μL) were prepared in 96-well plates containing chromogenic substrates and an inhibitor in 20 mM Hepes, 0.01% BSA, 5 mM $CaCl_2$, 0.15 M NaCl, pH 7.4. Reactions were initiated with 10 μL of enzyme solution. Enzymes and substrates were used as follows: fXa and S-2222; thrombin and S-2238; trypsin and S-2222. The concentration of inhibitor required to inhibit enzyme activity by 50% (IC_{50}) was calculated by regression analysis.

7.2.2. In vitro plasma clotting time assay. PT was measured using a KC10A coagulometer (Amelung Co., Lehbringsweg, Germany). Fifty microliters of pooled citrated mouse plasma was incubated for 1 min at 37 °C with 50 μL of diluted compound, followed by the addition of 50 μL of PT reagent (Hemoliance Brain Thromboplastin, Instrumentation Laboratory Company, Lexington, MA, USA) to initiate clot formation. The concentration required to double clotting time (CT_2) was estimated from each individual dose–response curve.

7.2.3. Ex vivo anticoagulant assays in mice. Male ICR mice weighing 20–35 g were fasted overnight. Inhibitors were suspended in a 0.5% methylcellulose solution and administered orally to the mice at 100 mg/kg using a gastric tube. At a predetermined time after oral administration of the inhibitor, blood (0.9 mL) was collected from the abdominal vena cava into syringes containing 0.1 mL of 3.8% citrate. Platelet poor plasma was then prepared by centrifugation to measure PT. All data were expressed as relative values compared with the vehicle group.

7.3. Molecular modeling studies

Docking of the inhibitor into the active site of fXa was performed using the GOLD program[32] (version 2.1) as follows. The X-ray crystal structure of fXa was retrieved from the Brookhaven Protein Data Bank

(http://www.rcsb.org/pdb, PDB Accession code: 1FAX)[28] and used as the target for a docking. The structure of the ligand was constructed using Sybyl 6.8 and energetically minimized with molecular mechanics using a Tripos force field.[33] The docking run was carried out using standard default settings with a population size of 100, a maximum number of 100,000 operations, and a mutation crossover rate of 95. The fitness function that was implemented in GOLD basically consisted of H-bonding, complex energy, and ligand internal energy terms. Based on the GOLD fitness score, the bound conformation with the highest fitness score was considered to be the best bound conformation. The X-ray crystal structure of thrombin was also taken from the Brookhaven Protein Data Bank (PDB Accession code: 1KTT),[16b] and the coordinates of the thrombin were transferred to those of fXa (PDB Accession code: 1FAX) through superposition of the conserved residues of thrombin and fXa.

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