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Discovery of sulfonylalkylamides: A new class of orally active factor Xa inhibitors

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Abstract—Factor Xa (FXa) is a trypsin-like serine protease involved in the coagulation cascade and has received great interest as a potential target for the development of new antithrombotic agents. Most of amidine-type FXa inhibitors reported have been found to show extremely poor oral bioavailability. Compound **1** is one of the first reported non-amidine type FXa inhibitors. To discover novel and orally active FXa inhibitors, we investigated flexible linear linkers between the 6-chloronaphthalene ring and the 1-(pyridin-4-yl)piperidine moiety of **1** and found the orally active sulfonylalkylamide **2f** with an FXa IC_{50} of 0.05 μM, comparable with that of **1**. Further modification to reduce the CYP3A4 inhibitory activity of **2f** resulted in the potent, selective, and orally active 2-methylpyridine analogue **2s** (FXa IC_{50} of 0.061 μM), for which the liability of CYP3A4 inhibition was significantly weakened compared to **2f**. Compound **2s** also showed long lasting anticoagulant activity in cynomolgus monkeys.

1. Introduction

Abnormal blood clotting is responsible for a number of thromboembolic diseases, including myocardial infarction, unstable angina, deep vein thrombosis, pulmonary embolism, and ischemic stroke. While several anticoagulants, such as warfarin (Coumadin), heparins, hirudin, hirulog, and argatroban, are currently available for the treatment and prevention of thrombus formation, some of these lack oral bioavailability.[1] Furthermore, the normal protocol for these therapies requires careful monitoring of clotting times to achieve efficacy and individual dose titration to minimize excessive bleeding.[2] Therefore, there is a clear need to develop improved anticoagulants which are orally active and have long half-lives, but which do not cause unpredictable or serious bleeding complications.

Factor Xa (FXa), a trypsin-like serine protease converting prothrombin to thrombin, is located at the central point linking the intrinsic and the extrinsic coagulation pathways.[3] Since this process involves signal amplification, with one molecule of FXa activating many molecules of prothrombin to thrombin,[4] FXa inhibitors are expected to be more efficacious in interrupting the coagulation cascade than direct thrombin inhibitors. Moreover, it was also shown recently in in vivo studies that FXa inhibitors may have less bleeding risk than thrombin inhibitors.[5] Accordingly, FXa has emerged as an attractive target enzyme for the development of new antithrombotic agents.[6]

A variety of low-molecular-weight FXa inhibitors have been described. However, first generation FXa inhibitors[7,8] (e.g., DX-9065a[8a] shown in Fig. 1) incorporated highly basic groups, such as amidine and/or guanidine groups,[8] which imparted poor pharmacokinetic properties after oral administration.[7b] Therefore, a range of potent non-amidine (non-guanidine) type FXa inhibitors have been extensively explored. Compound **1**[9] is one of the first reported non-amidine type FXa inhibitors (Fig. 1), and currently some non-amidine type FXa inhibitors such as rivaroxaban[10] and apixaban[11] are being investigated in clinical trials.

A primary objective in our FXa inhibitor program is to identify novel non-amidine type FXa inhibitors, bearing new chemical frameworks, with selectivity and pharmacokinetic properties sufficient to demonstrate antithrombotic efficacy in animals, especially in primates. To

Keywords: Factor Xa inhibitor; Anticoagulant; Sulfonylalkylamide; CYP3A4.
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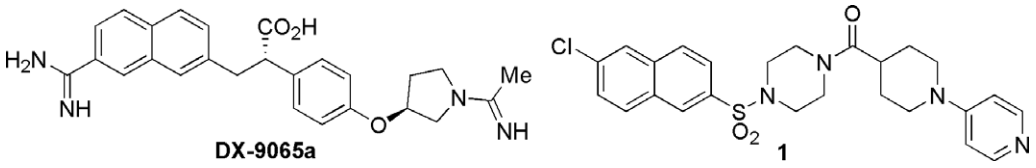


Figure 1. Structure of DX-9065a and compound **1**.

achieve this objective, we focused our attention on non-amidine FXa inhibitor **1** as a starting point. Molecular modeling studies on the binding mode of compound **1** with FXa have already been reported and showed the following features: (1) the 6-chloronaphthalene moiety takes the place of the amidinobenzene or the amidinonaphthalene moieties of amidine type FXa inhibitors as the S1 binding element in FXa,[7b,12] (2) the basic 1-(pyridin-4-yl)piperidine moiety is located in the S4 site,[7b,12] (3) the sulfonyl group is critical for the restricted L-shaped conformation, which is essential for high affinity to FXa,[7b,11b] causing the aryl ring to turn 90° into the S1 site and allowing the sulfonyl group to form a hydrogen bond with Ser195 or Gln192 in FXa,[7b] (4) the piperazine ring adopts a chair conformation[7b] playing an important role in presenting the S1 and S4 binding elements in favorable positions, and is not a mere spacer.[12] In contrast, the molecular conformation of compound **1** appears to be highly rigid because the chemical structure has four rings connected linearly by a direct bond or one-atom spacer. The rigid conformation of **1** might be expected to influence its pharmacokinetic and physicochemical properties.[13] Therefore, we applied the following design strategy to generate new lead compounds based on information gained from these molecular modeling studies. We focused our synthetic efforts on the possibility of replacing the piperazine ring of **1** with a flexible linear linker and the sulfonamide bond with the sulfonylmethylene bond, hoping to provide more desirable properties for an orally available drug (Fig. 2). In this paper, we describe the synthesis, structure–activity relationships, ex vivo anticoagulant activities, selectivity, and pharmacokinetics of sulfonylalkylamides **2** as novel non-amidine FXa inhibitors.[14]

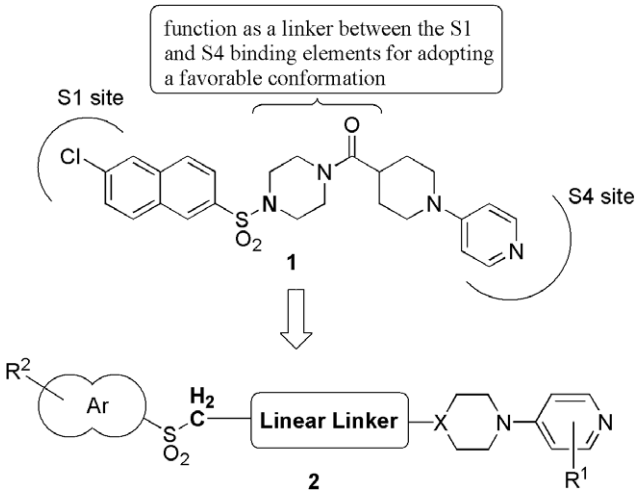


Figure 2. Sulfonylalkylamides **2**.

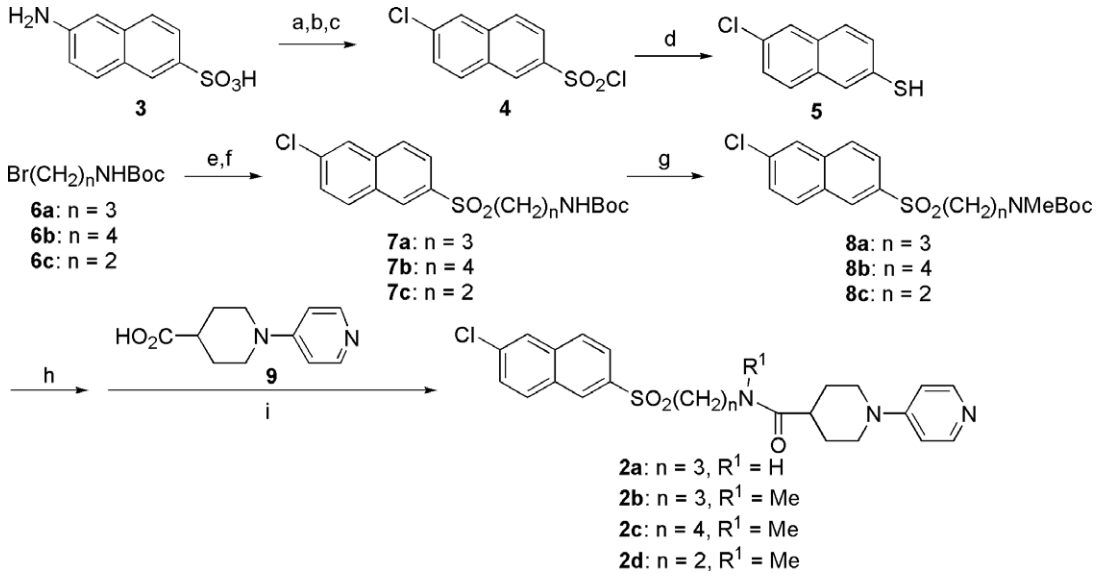
2. Chemistry

The synthesis of sulfonylalkylamides **2a–d** is depicted in Scheme 1. Preparation of **4** was performed by diazotization of 6-amino-2-naphthalenesulfonic acid (**3**) with 6 M HCl and sodium nitrite followed by Sandmeyer reaction to introduce a chlorine atom and sulfonylchlorination with phosphorus pentachloride. Reduction of **4** with lithium aluminum hydride (LAH) gave thiol **5**. Alkylation of thiol **5** with *N*-Boc-bromoalkylamines **6a–c**[15] followed by oxidation with *m*-chloroperbenzoic acid (mCPBA) afforded alkylsulfones **7a–c**. The resulting compounds **7a–c** were methylated with iodomethane to yield corresponding *N*-methylcarbamates **8a–c**. Deprotection of *N*-Boc-alkylamines **7a** and **8a–c** with trifluoroacetic acid (TFA) followed by condensation with carboxylic acid **9**[16] via the corresponding acyl chloride afforded the desired amides **2a–d**.

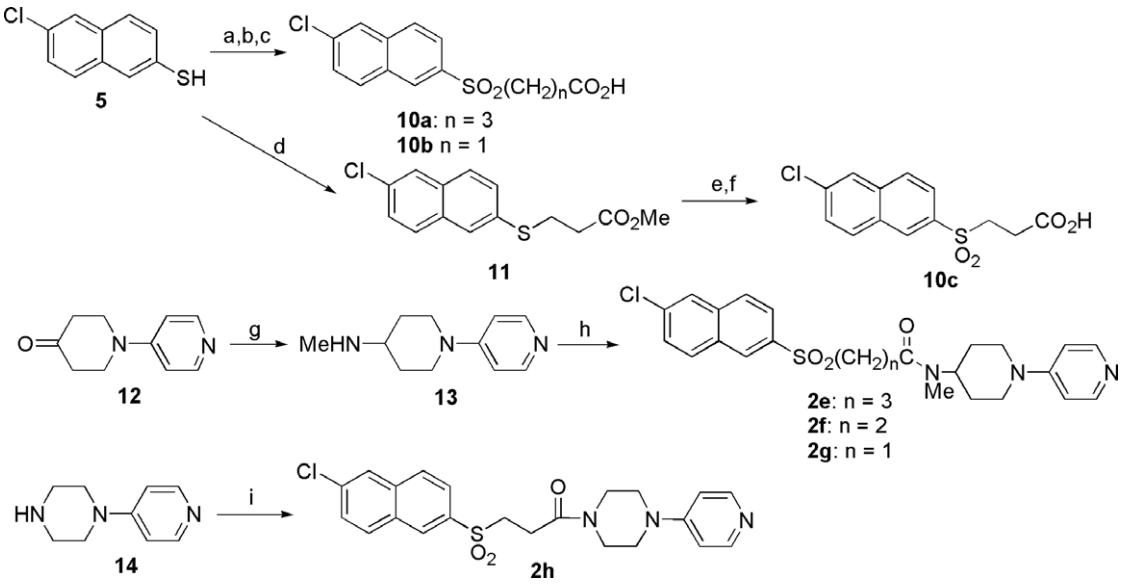
Sulfonylalkylamides **2e–h** were synthesized as shown in Scheme 2. The required carboxylic acids **10a** and **10b** were prepared from **5** by alkylation with ethyl 4-bromobutanoate or ethyl bromoacetate in the presence of sodium ethoxide followed by oxidation with mCPBA and alkali hydrolysis. Michael addition of thiol **5** to methyl acrylate, oxidation with mCPBA, and sequential acid hydrolysis of the ester group afforded 3-sulfonylpropionic acid **10c**. The requisite amine **13** was synthesized by reductive amination of ketone **12**[17] with methylamine. Amine **13** or **14** was condensed with carboxylic acids **10a–c** using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC) and 1-hydroxybenzotriazole hydrate (HOBt) to afford the desired amides **2e–h**.

The synthesis of sulfonylalkylamides **2i–n** is shown in Scheme 3. Alkylation of thiol **5** with 3-bromopropanol followed by oxidation with mCPBA afforded alcohol **15**. Amines **16a–c** were sulfonylated with 2,4-dinitrobenzenesulfonyl chloride to sulfonamides **17a–c**, which were alkylated with alcohol **15** by the Mitsunobu reaction[18] and then desulfonylation with propylamine to give amines **18a–c**.[18] Condensation of amines **18a–c** with carboxylic acid **9** via the corresponding acyl chloride afforded the desired amides **2i–k**. Ester **2k** was hydrolyzed to carboxylic acid **2l**, which was condensed with ammonia or morpholine to afford amides **2m** and **2n**.

Sulfonylalkylamides **2o–v** were synthesized as shown in Scheme 4. Arylation of 1,4-dioxa-8-azaspiro[4.5]decane



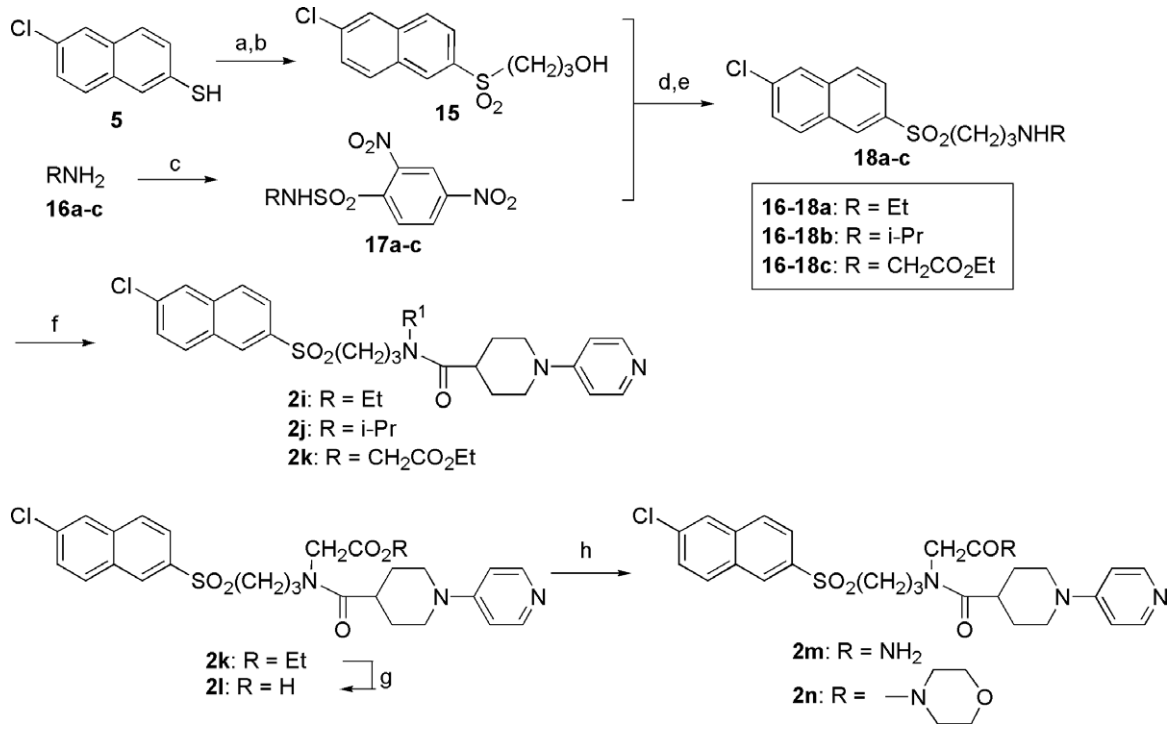
Scheme 1. Synthesis of sulfonylakylamides **2a**–**d**. Reagents (a) 6 M HCl, $NaNO_2$; (b) 6 M HCl, CuCl; (c) PCl_5, (d) LAH; (e) **5**, Et_3N; (f) mCPBA; (g) MeI, NaH; (h) TFA; (i) $SOCl_2$, then Et_3N.



Scheme 2. Synthesis of sulfonylakylamides **2e**–**h**. Reagents (a) $Br(CH_2)_3CO_2Et$ or $BrCH_2CO_2Et$, NaOH; (b) mCPBA; (c) NaOH, H_2O; (d) methyl acrylate, Et_3N; (e) H_2O_2, AcOH; (f) H_2SO_4; (g) $MeNH_2$-HCl, $NaBH_3CN$, AcOH; (h) **10a**–**c**, WSC, HOBt; (i) **10c**, WSC, HOBt.

with 4-chloropyridines **19a**–**d**[19–21] in the presence of triethylamine in a sealed tube followed by hydrolysis of the ketal groups with 4 M HCl produced piperidones **20a**–**d**. Reductive amination of **12** or **20a**–**d** with methylamine, glycine ethyl ester, ethyl 3-aminopropionate, or *tert*-butyl (2-aminoethyl)carbamate afforded the requisite secondary amines **21a**–**g**. Condensations of carboxylic acid **10c** with amines **21a**–**g** were conducted under several conditions. Amine **21a** was condensed with carboxylic acid **10c** using HOBt and WSC to afford amide **2o** in low yield. Carboxylic acid **10c** was converted to acyl chloride with thionyl chloride, which was coupled with amine **21b** to afford amide **2p** in low yield. The coupling of carboxylic acid **10c** and amines **21c**–**g** using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM)[22] as a dehydrating agent gave amides **2q** and **2s**–**v**, respectively, in moderate to high yields. Removal of the Boc group from **2q** with TFA provided amine **2r**.

Sulfonylalkylamides **2w**–**aa** were prepared as shown in Scheme 5. Arylsulfonyl chlorides **22a**–**e**[23–25] were reduced with sodium sulfite to give corresponding sodium arylsulfinates **23a**–**e**, which were then alkylated with bromosuccinic acid and subsequently decarboxylated by heat under basic conditions to afford arylsulfonylpropionic acids **24a**–**e**. The coupling of carboxylic acids **24a**–**e** and amine **21d** using DMTMM gave amides **2w**–**aa**.



Scheme 3. Synthesis of sulfonylalkylamides **2i**–**n**. Reagents (a) 3-bromopropanol, Et_3N; (b) mCPBA; (c) 2,4-dinitrobenzenesulfonyl chloride, pyridine; (d) DEAD, PPh_3; (e) *n*-$PrNH_2$; (f) (1) **9**, $SOCl_2$, (2) **18a**–**c**, *i*-Pr_2NEt; (g) 2 M NaOH; (h) NH_4OH or morpholine, WSC, HOBt.

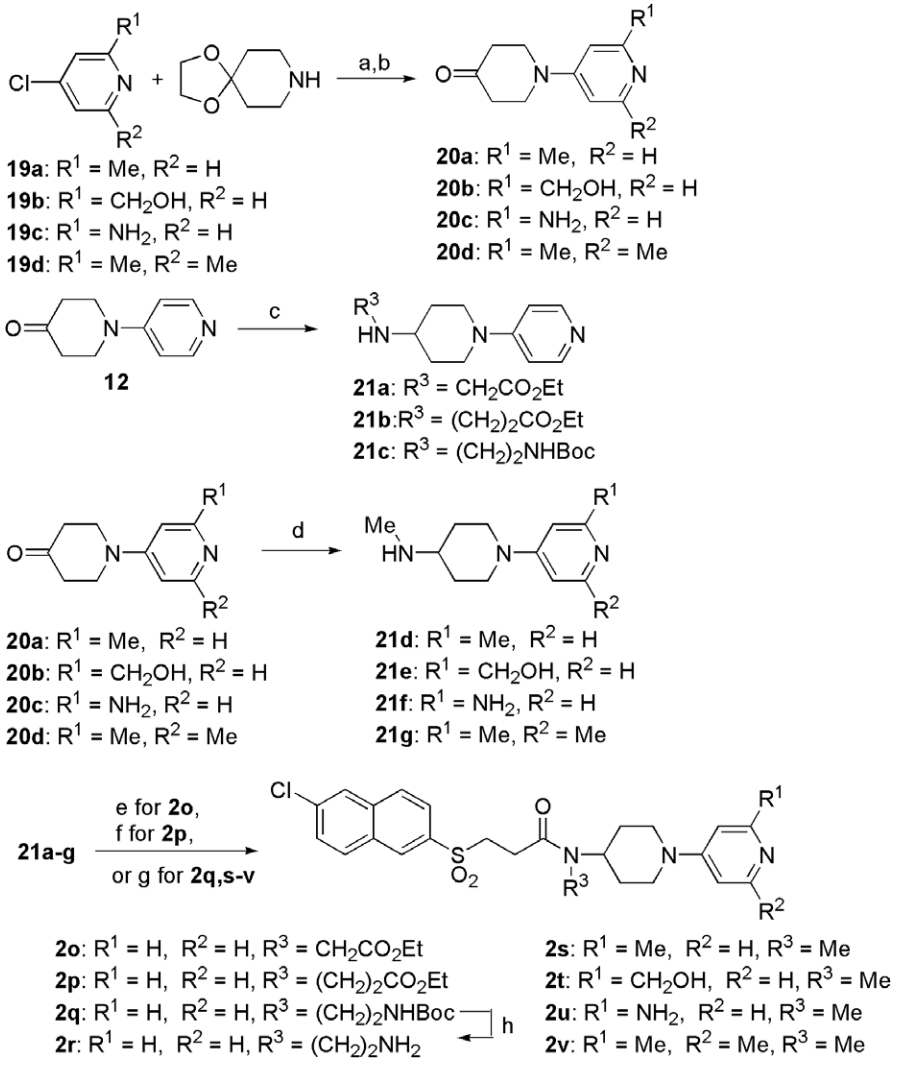
3. Results and discussion

The compounds thus synthesized were evaluated for in vitro inhibitory potency against human FXa, expressed as IC_{50} values, and their activity in the prolongation of human prothrombin time (PT), expressed as the concentration of compound required to double the clotting time (PT_2) in the PT assay. To estimate the oral bioavailability of these compounds, the ex vivo PT prolonging activity was also determined 1 h after oral administration to mice at a dose of 30 mg/kg and expressed as ratios of the PT of the compound-treated animals with that of control group.

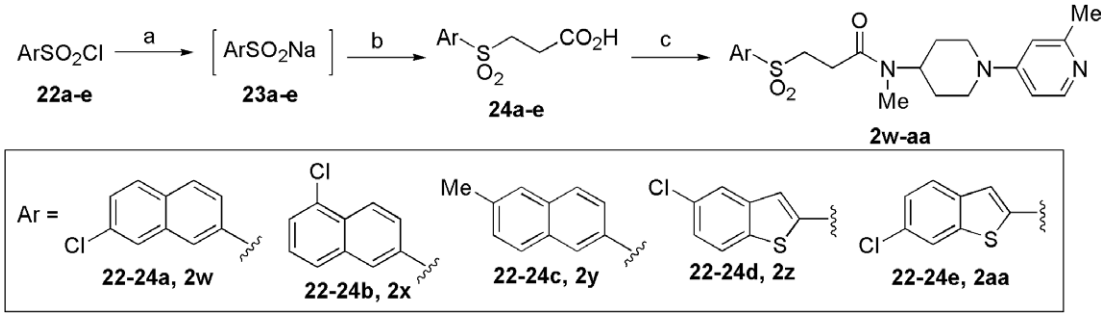
In vitro FXa inhibitory activities of the compounds having varied linear linkers between the 6-chloronaphthalene ring and the 1-(pyridin-4-yl)piperidine moiety are listed in Table 1. The cleavage of the ethylene bond in the piperazine ring of **1** and the replacement of the sulfonamide bond in **1** with a sulfonylmethylene bond led to open chain *N*-(3-sulfonylpropyl)amide **2a**, which was found to be a less potent FXa inhibitor than **1**. Interestingly, *N*-methylation of the amide functionality in **2a** gave **2b** with slightly increased FXa inhibitory activity compared to **2a**. Extension of the propylene linkage of **2b** by one atom, as in *N*-(3-sulfonylbutyl)amide **2c**, led to remarkably decreased activity, whereas deletion of the methylene group in the linker of **2b**, as in **2d**, afforded decreased FXa inhibitory activity. Further investigation of the linker was performed by inversion of the amide bond in **2b** to lead to the 3-sulfonylbutanamide **2e** with comparable FXa inhibitory activity to that of **2b**. Surprisingly, 3-sulfonylpropanamide **2f**, in which the methylene group of **2e** was deleted from the linker, was found to exhibit comparable FXa inhibitory activity to that of piperazine **1**. Reduction of the alkylene length in the linker of **2f** resulted in decreased activity (**2g**). Piperazinamide **2h** afforded slightly less potent FXa inhibitory activity than **2f**. These results suggest the importance of the distance between the 6-chloronaphthalene ring and the 1-(pyridin-4-yl)piperidine moiety for these activities. It is interesting that the orientation of the amide bond affects the optimal length in the linker (**2b** and **2f**).

To further improve the activities in this series, we investigated the effect of substitution on the amide nitrogen atoms in *N*-(3-sulfonylpropyl)amide **2b** and 3-sulfonylpropanamide **2f**. The results of in vitro FXa inhibition and PT assays are shown in Table 2. The compounds bearing alkyl groups larger than methyl (such as an ethyl (**2i**) and an isopropyl (**2j**) group) exhibited slightly reduced FXa inhibitory potencies and diminished PT prolonging activities. Introduction of polar functionalities such as ethoxycarbonylalkyl (**2k**, **2o**, and **2p**), carboxymethyl (**2l**), carbamoylmethyl (**2m** and **2n**), or aminoethyl (**2r**) provided comparable FXa inhibitory activity to **2b** or **2f**.

The ex vivo PT prolonging activities in mice of a select set of the potent inhibitors were determined and the results are shown in Table 2. The *N*-methyl derivatives **2b** and **2f** significantly prolonged mouse PT by 1.3- and 1.6-fold, respectively, whereas the compounds with polar functionalities exhibited little to undetectable amounts of prolongation in the assay (**2k**, **2l**, **2n**–**p**, and **2r**). Although each of the compounds had a different in vitro mouse PT prolonging activity, these



Scheme 4. Synthesis of sulfonylakylamides **2o**–**v**. Reagents: (a) Et_3N; (b) 4 M HCl; (c) R^3NH_2, $NaBH_3CN$, AcOH; (d) $MeNH_2$-HCl, $NaBH_3CN$, AcOH; (e) **10c**, WSC, HOBt; (f) **10c**, $SOCl_2$, $(i\text{-}Pr)_2NEt$; (g) **10c**, DMTMM; (h) TFA.

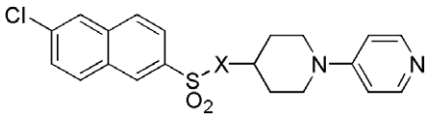


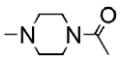
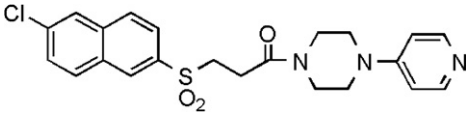
Scheme 5. Synthesis of sulfonylakylamides **2w**–**aa**. Reagents: (a) Na_2SO_3, $NaHCO_3$; (b) bromosuccinic acid, NaOH; (c) **21d**, DMTMM.

results suggested that **2b** and **2f** would show good oral bioavailability in mice. The *N*-methylamide **2f** was found to be the most potent human FXa inhibitor with good oral bioavailability. Our strategy to discover novel and orally active FXa inhibitors by increasing the flexibility and converting the sulfonamide linkage to a sulfonyl group did not prove entirely correct (for example **2f** vs **2o**).

In the course of further evaluation of **2f**, it was found to inhibit the human cytochrome P450 enzyme CYP3A4 (81% inhibition at 1 μM) which is known

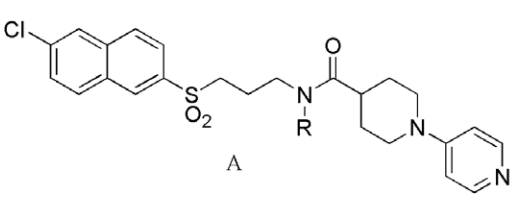
Table 1. In vitro activites of sulfonylalkylamides **2a–h**



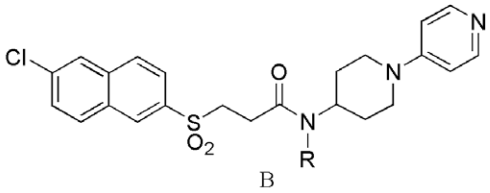
Compound	X	Human FXa, IC_{50}[a] (μM)
1		0.033
2a	$CH_2CH_2CH_2NHCO$	0.47
2b	$CH_2CH_2CH_2NMeCO$	0.095
2c	$CH_2CH_2CH_2CH_2NMeCO$	
2d	CH_2CH_2NMeCO	0.84
2e	$CH_2CH_2CH_2CONMe$	0.14
2f	CH_2CH_2CONMe	0.050
2g	CH_2CONMe	0.72
2h		0.16

[a] Inhibitory activity against human FXa. IC_{50} values shown are the means of duplicate measurement.

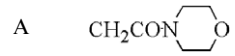
Table 2. In vitro and ex vivo activites of sulfonylalkylamides **2b**, **2f**, **2i–p**, and **2r**



A



B

Compound	A/B	R	In vitro			Ex vivo
			Human FXa, IC_{50}[a] (μM)	Human PT, PT_2[b] (μM)	Mouse, PT_2[b] (μM)	Mouse PT, ratio[c]
2b	A	Me	0.095	1.7	17	1.3
2i	A	Et	0.12	3.2	NT[d]	NT[d]
2j	A	*i*-Pr	0.25	8.4	NT[d]	NT[d]
2k	A	CH_2CO_2Et	0.064	1.9	11	1.0
2l	A	CH_2CO_2H	0.054	1.8	14	1.0
2m	A	$CH_2CO_2NH_2$	0.12	2.1	NT[d]	NT[d]
2n	A		0.085	1.6	26	1.0
2f	B	Me	0.050	1.0	8.0	1.6
2o	B	CH_2CO_2Et	0.060	1.1	10	1.1
2p	B	$CH_2CH_2CO_2Et$	0.037	0.85	14	1.0
2r	B	$CH_2CH_2NH_2$	0.095	0.63	8.6	1.0
DX-9065a			0.13	0.69	6.8	1.1[e](1.4)[f]

[a] See corresponding footnote in Table 1.

[b] PT (prothrombin time) is defined as the concentration of compound required to double the time to clot formation in the PT assay. PT_2 values shown are means of duplicate measurement.

[c] Ex vivo mouse PT prolonging activity was determined 1 h after oral administration of the PT of the compound at a dose of 30 mg/kg and expressed as ratios of the compound-treated mice to that of control group (n = 3–5).

[d] NT means 'not tested'.

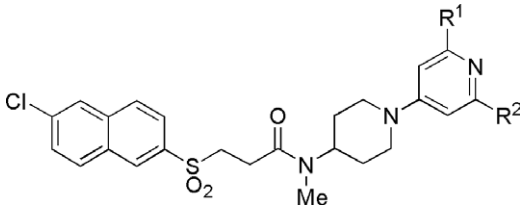
[e] 30 mg/kg po (n = 5).

[f] 100 mg/kg po (n = 5).

to be a primary factor responsible for the metabolism of most drugs.[26] Drugs that show CYP inhibition influence the pharmacokinetics of other co-administered drugs and can cause undesired effects in patients.

It might be reasoned that the inhibitory potency toward CYP3A4 is attributed to coordination of the pyridine ring to the heme.[27] We thought that the introduction of a substituent at the 2- or 6-position of the pyridine ring might prevent it from coordinating and reduce the

Table 3. In vitro and ex vivo activites of sulfonylalkylamides **2f** and **2s–v**



Compound	R^1	R^2	In vitro				Ex vivo
			Human FXa, IC_{50}[a] (μM)	Human PT, PT_2[b] (μM)	CYP3A4, % inhibition at 1 μM[c]	Mouse PT, PT_2[b] (μM)	Mouse PT, ratio[d]
2f	H	H	0.05	1.0	81	8.0	1.6
2s	Me	H	0.061	1.6	40	4.8	1.6
2t	CH_2OH	H	0.11	1.5	21	6.4	1.1
2u	NH_2	H	0.079	3.6	49	NT[e]	NT[e]
2v	Me	Me	0.063	1.6	40	4.1	1.3

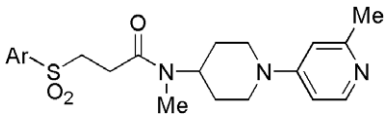
[a,b] See corresponding footnotes in Tables 1 and 2.
[c] Inhibitory activity against human CYP3A4.
[d,e] See corresponding footnotes in Table 2.

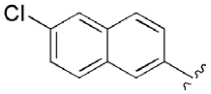
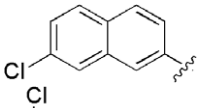
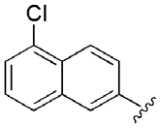
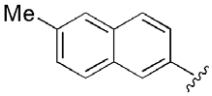
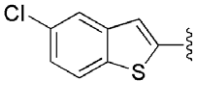
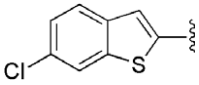
CYP3A4 inhibitory potency.[28] Some 2- and 6-substituted pyridine analogues such as 2-methyl (**2s**), 2-hydroxymethyl (**2t**), 2-amino (**2u**), and 2,6-dimethyl (**2v**) pyridines were examined and the results are shown in Table 3. The inhibitory potencies of these compounds against CYP3A4 were successfully lowered with little effect on FXa inhibitory potencies and the human PT prolonging activities. Among these compounds, **2s** was the most potent and orally active inhibitor with reduced CYP3A4 inhibition.

To understand the influence of the 6-chloronaphthalene moiety on the FXa inhibitory activity in this series, we investigated the effect of 6-chloronaphthalene replacements in compound **2s** (Table 4). 7- and 5-chloronaphthalene analogues **2w** and **2x** displayed significant drops in activity. Replacement of the chlorine atom with the isosteric methyl group resulted in a slight loss in activity (**2y** vs. **2s**). Surprisingly, 5- and 6-chlorobenzothiophene analogues **2z** and **2aa** showed remarkably decreased activity, while some potent FXa inhibitors bearing the 5- and 6-chlorobenzothiophene moieties have been reported.[25,29] The difference in the binding mode between this series and the other FXa inhibitors in the S1 region might be responsible for this result. These findings confirm the importance of the 6-chloronaphthalene moiety for the FXa inhibitory activity in our sulfonylalkylamide series.

Since many trypsin-like proteases have essential physiological functions,[30] an important consideration for the development of FXa inhibitors is the ability of the molecule to bind selectively. In several enzyme inhibition assays, compound **2s** displayed >200-fold selectivity for FXa versus several coagulation (thrombin, kallikrein) and fibrinolytic enzymes (plasmin, tissue plasminogen activator (t-PA)) (Table 5). Furthermore, **2s** showed no significant binding to trypsin.

Table 4. In vitro activities of sulfonylalkylamides **2w–2aa**



Compound	Ar	Human FXa, IC_{50}[a] (μM)
2s		0.061
2w		>5.0
2x		>5.0
2y		0.72
2z		>5.0
2aa		>5.0

[a] See corresponding footnotes in Table 1.

The pharmacokinetic profile of compound **2s** was evaluated in cynomolgus monkeys. As illustrated in Table 6, **2s** displayed good oral bioavailability (66.7%) after oral administration at a dose of 1 mg/kg in fasted monkeys. Recently, several FXa inhibitors were reported to have good oral bioavailability in monkeys.[17,31]

Table 5. Selectivity profile for compound **2s**

Serine protease	FXa	Thrombin	Kallikrein	Trypsin	Plasmin	t-PA
K_i (μM)	0.015	7.5	3.0	>200	>200	67

Data are expressed as the means of three determinations.

Table 6. In vivo cynomolgus monkey pharmacokinetics of compound **2s** ($n = 3$, fasted)[a]

iv dose	C_{5min} (μg/mL)	AUC (μg h/mL)	MRT (h)	$V_{d(ss)}$ (L/kg)	CL_{total} (L h/kg)
1 mg/kg	0.565 ± 0.283	1.579 ± 0.799	4.54 ± 0.32	3.285 ± 1.270	0.734 ± 0.303
po dose	C_{max} (μg/mL)	T_{max} (h)	AUC (μg h/mL)	MRT (h)	B.A. (%)
1 mg/kg	0.134 ± 0.032	2.00 ± 1.73	1.030 ± 0.449	6.60 ± 0.97	66.7 ± 4.2

[a] Values are expressed as means ± SD of three determinations.

Table 7. Ex vivo PT prolonging activities of compound **2s** in cynomolgus monkeys at 3 mg/kg, po ($n = 3$, fasted)

Dose	PT (% of basal value)[a]				
	0.5 h	1 h	2 h	4 h	8 h
3 mg/kg	142.0 ± 41.22	133.6 ± 30.48	146.9 ± 18.71	144.6 ± 5.20	117.7 ± 0.64

[a] Values are expressed as means ± SEM of three determinations.

The ex vivo PT prolonging activity of **2s** was evaluated in cynomolgus monkeys, since the in vitro concentration response of PT in cynomolgus monkey plasma was found to be similar to that in human plasma (the PT_2 in cynomolgus monkey plasma was 1.1 μM). As shown in Table 7, **2s** displayed potent and long lasting PT prolonging activity after oral administration at a dose of 3 mg/kg.

To predict the binding mode of these inhibitors, a molecular modeling study of **2s** was carried out using the program GOLD and the X-ray structure of FXa complexed with the inhibitor RPR128515, reported by Sanofi-Aventis[32] (Fig. 3). In the model, compound **2s** adopted nicely the L-shape conformation needed for FXa binding. There is no interaction between the strongly basic pyridine ring and the carboxyl group of Asp189 at the bottom of the S1 pocket, while the 6-chloronaphthyl group is situated in the hydrophobic S1 region and a close contact is observed between the chlorine atom and the benzene ring of Tyr228.[10,12,33] Compound **2s** represents a parallel example in FXa inhibition wherein a basic group, thought to be essential in forming the salt bridge with Asp189, can be eliminated and replaced by a neutral group capable of contributing enough favorable interaction to produce good affinity to the enzyme. The pyridine ring is located in the hydrophobic S4 site and makes hydrophobic contacts with the aromatic rings of Tyr99, Phe174, and Trp215. The sulfone oxygen is involved in a hydrogen bond with the Gln192 main chain nitrogen. The linker in **2s** seems to control the conformation to fit the FXa binding sites without direct interaction with FXa. Recently, other researchers have reported that the important interaction for high affinity to FXa is not an ionic interaction with Asp189, but hydrogen bonding interactions with the carbonyl groups in the amide linkages of Gly216 and/or Gly219.[12,32,33c,34] It is interesting to note that a hydrogen bond could not be observed between **2s** and Gly216 and/or Gly219.

4. Conclusion

To discover orally active FXa inhibitors in primates, increasing the conformational flexibility of the linker between the 6-chloronaphthalene ring and the 1-(pyridin-4-yl)piperidine moiety of **1** led to orally active sulfonylalkylamides **2b** and **2f**. Further modification to diminish the interaction with CYP3A4 resulted in 2-methylpyridine analogue **2s**, which is a potent, selective, and orally bioavailable FXa inhibitor with reduced CYP3A4 inhibitory activity, which demonstrated a long duration of anticoagulant activity in cynomolgus monkeys. Various other optimization strategies using this sulfonylalkylamide series will be reported in due course.

5. Experimental

5.1. Chemistry

Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini-200 spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. Reactions were followed by TLC on Silica gel 60 F 254 precoated TLC plates (E. Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). Chromatographic separations were carried out on silica gel 60 (0.063–0.200 or 0.040–0.063 mm, E. Merck) or basic silica gel (Chromatorex® NH, 100–200 mesh, Fuji Silysia Chemical Ltd.) using the indicated eluents. Compounds **3**, **6a**, **6c**, **14**, **16a–c**, **19a** and solvents were commercially available and used as received. Yields

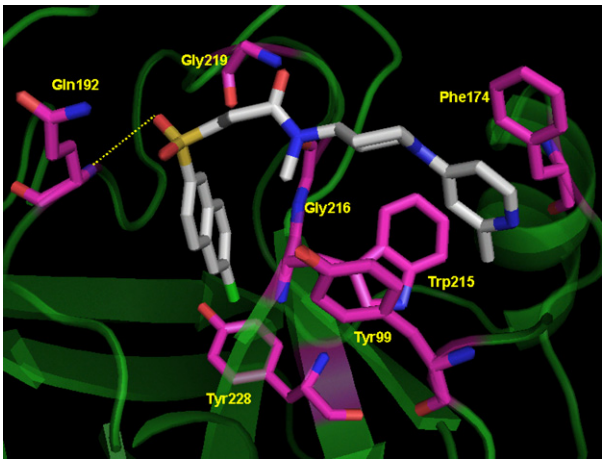


Figure 3. Binding model of compound **2s** in FXa.

are unoptimized. Chemical intermediates were characterized by ^{1}H NMR.

5.1.1. 6-Chloro-2-naphthalenesulfonyl chloride (4). To a suspension of **3** (44.6 g, 200 mmol) in water (200 mL) was added Na_2CO_3 (11.1 g, 10.4 mmol) portionwise under reflux to give a clear solution. The solution was cooled in an ice-bath to form a fine precipitate. Concentrated HCl (43 mL) was slowly added followed by dropwise addition of a solution of $NaNO_2$ (16.6 g, 240 mmol) in water (100 mL) at $\leqslant$5 °C over 30 min. The reaction mixture was stirred at 0–5 °C for a further 1 h and the pale orange precipitate was collected by filtration. The diazonium salt was added to an ice-cooled solution of CuCl (23.8 g, 240 mmol) in 28% HCl (100 mL). The resulting mixture was stirred at room temperature for 1 h and then at 60 °C for 30 min. After the reaction mixture was cooled to 0 °C, it was adjusted to pH 1–2 with 50% aqueous KOH. The precipitate was collected by filtration and the solid was suspended in hot water (200 mL). The mixture was basified with 50% aqueous KOH. The insoluble material was collected by filtration and dried to give a solid (16.8 g). The filtrate was concentrated in vacuo to give a solid (19.7 g).

The second solid (19.7 g) was heated to 100–110 °C for 1.5 h with PCl_5 (40.3 g). The reaction mixture was cooled to room temperature and ice (19.7 g) was added portionwise. The precipitate was collected by filtration and purified by silica gel chromatography ($CHCl_3$/hexane = 1/3) to give **4** (3.07 g, 6%) as a light-brown powder.

The first solid (16.8 g) was heated to 100–110 °C for 4 h with PCl_5 (33.8 g). The reaction mixture was cooled to room temperature and ice was added portionwise. The precipitate was collected by filtration and purified by silica gel chromatography ($CHCl_3$/hexane = 1/3) to give **4** (9.05 g, 17%). ^{1}H NMR ($CDCl_3$) δ: 7.66 (1H, dd, J = 2.1 and 8.7 Hz), 7.97–8.07 (4H, m), 8.59 (1H, s).

5.1.2. 6-Chloro-2-naphthalenethiol (5). To a solution of **4** (60.0 g, 230 mmol) in THF (700 mL) was added $LiAlH_4$ (17.4 g, 460 mmol) and the resulting mixture was stirred at room temperature for 10 min and then refluxed for 3 h. The reaction mixture was cooled to 0 °C and cautiously quenched with EtOAc (50 mL) and 5.5 M HCl (400 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the residue was purified by silica gel chromatography (EtOAc) to give **5** (38.5 g, 86%) as a beige powder. ^{1}H NMR ($CDCl_3$) δ: 3.60 (1H, s), 7.34–7.48 (2H, m), 7.61 (1H, s), 7.66 (1H, s), 7.68–7.81 (2H, m).

5.1.3. *tert*-Butyl{3-[(6-chloronaphthalen-2-yl)sulfonyl]propyl}carbamate (7a). To a solution of **5** (0.73 g, 3.75 mmol) and Et_3N (1.05 mL, 7.50 mmol) in DMF (20 mL) was added **6a** (0.85 g, 3.57 mmol). The resulting mixture was stirred at 100 °C for 5 h under argon atmosphere and then cooled to room temperature. The reaction mixture was poured into water (50 mL) and stirred at room temperature overnight. The pale yellow precipitate was collected by filtration. The precipitate was partitioned between brine and EtOAc, and filtered through a Celite pad. The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue was suspended in EtOAc (50 mL). mCPBA (70%, 2.19 g, 8.90 mmol) was added to the solution and the resulting mixture was stirred at room temperature overnight. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the residue was crystallized from Et_2O to give **7a** (0.80 g, 58%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 1.40 (9H, s), 1.85–2.05 (2H, m), 3.15–3.35 (4H, m), 4.68 (1H, br s), 7.59 (1H, dd, J = 2.2 and 8.4 Hz), 7.85–8.00 (4H, m), 8.46 (1H, s).

The following compounds **7b** and **7c** were prepared in a manner similar to that described for **7a**.

5.1.4. *tert*-Butyl{4-[(6-chloronaphthalen-2-yl)sulfonyl]butyl}carbamate (7b). ^{1}H NMR ($CDCl_3$) δ: 1.39 (9H, s), 1.48–1.90 (4H, m), 3.00–3.28 (4H, m), 4.54 (1H, br s), 7.59 (1H, dd, J = 1.8 and 8.8 Hz), 7.82–8.00 (4H, m), 8.46 (1H, s).

5.1.5. *tert*-Butyl{2-[(6-chloronaphthalen-2-yl)sulfonyl]ethyl}carbamate (7c). ^{1}H NMR ($CDCl_3$) δ: 1.37 (9H, s), 3.39 (2H, t, J = 5.8 Hz), 3.57 (2H, t, J = 5.8 Hz), 5.19 (1H, br s), 7.60 (1H, dd, J = 1.9 and 8.9 Hz), 7.88–7.97 (4H, m), 8.47 (1H, d, J = 1.2 Hz).

5.1.6. *tert*-Butyl{3-[(6-chloronaphthalen-2-yl)sulfonyl]propyl}methylcarbamate (8a). To an ice-cooled solution of 7a (4.91 g, 12.8 mmol) in THF (50 mL) was added NaH (60% in oil; 0.56 g, 14.0 mmol). After the mixture was stirred at room temperature for 2 h, iodomethane (4.00 mL, 64.2 mmol) was added. The resulting mixture was stirred at room temperature for 36 h and then concentrated in vacuo. The residue was diluted with water. The precipitate was collected by filtration, dried, and recrystallized from EtOAc–hexane to give **8a** (4.79 g, 94%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 1.39 (9H, s), 1.90–2.04 (2H, m), 2.80 (3H, s), 3.12–3.20 (2H, m), 3.31 (2H, t, J = 6.7 Hz), 7.60 (1H, dd, J = 1.8 and 9.0 Hz), 7.86–7.97 (4H, m), 8.46 (1H, s).

The following compounds **8b** and **8c** were prepared in a manner similar to that described for **8a**.

5.1.7. *tert*-Butyl{4-[(6-chloronaphthalen-2-yl)sulfonyl]butyl}-methylcarbamate (8b). ^{1}H NMR ($CDCl_3$) δ: 1.39 (9H, s), 1.45–1.85 (4H, m), 2.78 (3H, s), 3.18 (4H, t, J = 6.6 Hz), 7.59 (1H, dd, J = 1.8 and 8.8 Hz), 7.85–8.00 (4H, m), 8.46 (1H, s).

5.1.8. *tert*-Butyl{2-[(6-chloronaphthalen-2-yl)sulfonyl]ethyl}-methylcarbamate (8c). ^{1}H NMR ($CDCl_3$) δ: 1.35 (9H, s), 2.85 (3H, s), 3.38–3.46 (2H, m), 3.59 (2H, br s), 7.58 (1H, d, J = 8.7 Hz), 7.87–7.95 (4H, m), 8.46 (1H, s).

5.1.9. *N*-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propyl}-*N*-methyl-1-(pyridin-4-yl)piperidine-4-carboxamide (2b). A mixture of **8a** (0.33 g, 0.83 mmol) in toluene (2 mL) and TFA (2 mL) was stirred at room temperature for 30 min. The solvent was evaporated in vacuo and the residue was diluted in CH_2Cl_2 (20 mL). A mixture of **9**[16] (0.35 g, 1.70 mmol) and thionyl chloride (2 mL) was refluxed for 30 min and then concentrated in vacuo. The residue and triethylamine (4 mL) were added to the solution and the resulting mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated in vacuo and the residue was partitioned between EtOAc and water. The organic layer was separated, washed with water, aqueous $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc to 20/1 EtOAc/MeOH) to give **2b** (60 mg, 14%) as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.70–2.20 (5H, m), 2.60–3.00 (4H, m), 3.09 (3H, s), 3.09–3.22 (2H, m), 3.40–3.65 (2H, m), 3.80–4.00 (2H, m), 6.65 (2H, d, J = 6.6 Hz), 7.55–7.68 (1H, m), 7.82–8.00 (4H, m), 8.24 (2H, d, J = 6.6 Hz), 8.46 (1H, s). Anal. Calcd for $C_{25}H_{28}ClN_3O_3S{\cdot}0.1H_2O$: C, 61.55; H, 5.83; N, 8.61. Found: C, 61.29; H, 5.85; N, 8.54.

The following compounds **2a**, **2c**, and **2d** were prepared in a manner similar to that described for **2b**.

5.1.10. *N*-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propyl}-1-(pyridin-4-yl)piperidine-4-carboxamide (2a). Yield 85%, colorless powder. Mp 202–205 °C. ^{1}H NMR ($CDCl_3$) δ: 1.28–1.78 (5H, m), 2.15–2.40 (1H, m), 2.68–2.90 (2H, m), 2.98–3.18 (2H, m), 3.20–3.48 (3H, m), 3.78–3.98 (2H, m), 6.76 (2H, d, J = 6.6 Hz), 7.70–8.00 (3H, m), 8.05–8.35 (5H, m), 8.62 (1H, s). Anal. Calcd for $C_{24}H_{26}ClN_3O_3S{\cdot}0.4H_2O$: C, 60.15; H, 5.64; N, 8.77. Found: C, 60.11; H, 5.43; N, 8.57.

5.1.11. *N*-{4-[(6-Chloronaphthalen-2-yl)sulfonyl]butyl}-*N*-methyl-1-(pyridin-4-yl)piperidine-4-carboxamide (2c). Yield 88%, colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.55–1.90 (8H, m), 2.54–3.00 (3H, m), 3.02 (3H, s), 3.25 (2H, t, J = 7.5 Hz), 3.36 (2H, t, J = 6.4 Hz), 3.74–3.98 (2H, m), 6.65 (2H, d, J = 6.6 Hz), 7.58 (1H, dd, J = 2.2 and 8.8 Hz), 7.82–8.00 (4H, m), 8.25 (2H, d, J = 6.5 Hz), 8.45 (1H, s). Anal. Calcd for $C_{26}H_{30}ClN_3O_3S$: C, 62.54; H, 6.05; N, 8.40. Found: C, 62.27; H, 6.05; N, 8.49.

5.1.12. *N*-{2-[(6-Chloronaphthalen-2-yl)sulfonyl]ethyl}-*N*-methyl-1-(pyridin-4-yl)piperidine-4-carboxamide (2d). Yield 70%, pale yellow amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.71–1.82 (4H, m), 2.58–2.73 (1H, m), 2.80–2.94 (2H, m), 3.22 (3H, s), 3.47 (2H, t, J = 6.5 Hz), 3.79 (2H, t, J = 6.5 Hz), 3.88 (2H, m), 6.64 (2H, d, J = 6.5 Hz), 7.59 (1H, dd, J = 2.0 and 8.8 Hz), 7.90–7.97 (4H, m), 8.25 (2H, d, J = 6.5 Hz), 8.46 (1H, s). Anal. Calcd for $C_{24}H_{26}N_3O_3SCl{\cdot}0.7H_2O$: C, 59.48; H, 5.70; N, 8.67. Found: C, 59.47; H, 5.68; N, 8.53.

5.1.13. 4-[(6-Chloronaphthalen-2-yl)sulfonyl]butanoic acid (10a). To a solution of **5** (0.97 g, 4.98 mmol) and sodium ethoxide (0.48 g, 7.05 mmol) in EtOH (20 mL) was added ethyl 4-bromobutyrate (1.07 g, 5.49 mmol). The resulting mixture was stirred at 60 °C for 1 h and then concentrated in vacuo. The residue was partitioned between water and EtOAc. The organic layer was separated, washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual solid was dissolved in EtOAc (20 mL) and a solution of mCPBA (2.38 g, 13.8 mmol) in EtOAc (20 mL) was added dropwise. The resulting mixture was stirred at room temperature for 1.5 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual oil was dissolved in 1 M NaOH (10 mL) and MeOH (40 mL). The resulting mixture was stirred at room temperature for 1.5 h and then concentrated in vacuo. The residue was diluted with water and acidified with 1 M HCl. The precipitate was collected by filtration to give **10a** (1.21 g, 76%) as a colorless needle. ^{1}H NMR ($CDCl_3$) δ: 1.97–2.15 (2H, m), 2.55 (2H, t, J = 7.0 Hz), 3.27 (2H, t, J = 7.6 Hz), 7.60 (1H, dd, J = 8.7 and 1.7 Hz), 7.87–7.98 (4H, m), 8.48 (1H, s).

5.1.14. [(6-Chloronaphthalen-2-yl)sulfonyl]acetic acid (10b). Compound **10b** was prepared in a manner similar to that described for **10a**. ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 4.20 (2H, s), 7.59 (1H, dd, J = 2.2 and 8.8 Hz), 7.90–8.05 (4H, m), 8.54 (1H, s).

5.1.15. Methyl 3-[(6-chloronaphthalen-2-yl)thio]propanoate (11). A mixture of **5** (6.30 g, 32.4 mmol), methyl acrylate (2.90 g, 33.7 mmol), and Et_3N (0.90 mL, 6.46 mmol) in EtOAc (75 mL) was stirred at room temperature for 2.5 h. The solvent was evaporated in vacuo and the residue was washed with hexane to give **11** (8.88 g, 98%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 2.68 (2H, t, J = 7.4 Hz), 3.27 (2H, t, J = 7.4 Hz), 3.68 (3H, s), 7.39–7.48 (2H, m), 7.65–7.71 (2H, m), 7.74–7.77 (2H, m).

5.1.16. 3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoic acid (10c). A mixture of **11** (8.88 g, 31.6 mmol) and 30% H_2O_2 (6 mL) in AcOH (60 mL) was refluxed for 30 min. To the reaction mixture was added 98% H_2SO_4 (6 mL) and refluxing was continued for a further 1.5 h. The reaction mixture was diluted with water, and the precipitate was collected by filtration and dried. The crude product was purified by silica gel chromatography

(EtOAc). The product was recrystallized from IPE-hexane to give **10c** (8.20 g, 87%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 2.82 (2H, t, J = 7.5 Hz), 3.25 (2H, t, J = 7.5 Hz), 7.60 (1H, dd, J = 2.0 and 9.0 Hz), 7.91–7.97 (4H, m), 8.47 (1H, s).

5.1.17. *N*-Methyl-1-(pyridin-4-yl)piperidin-4-amine (13). To a solution of **12**[17] (0.88 g, 4.99 mmol), acetic acid (0.30 g, 5.00 mmol), and methylamine hydrochloride (0.37 g, 5.48 mmol) in MeOH (10 mL) was added portionwise $NaBH_3CN$ (0.34 g, 5.41 mmol). The reaction mixture was stirred at room temperature for 17 h and then concentrated in vacuo. The residue was partitioned between aqueous K_2CO_3 solution and CH_2Cl_2. The organic layer was separated, dried over anhydrous Na_2SO_4, and the solvent was evaporated in vacuo to give **13** (0.96 g, quant.) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.22–1.50 (2H, m), 1.90–2.10 (2H, m), 2.47 (3H, s), 2.51–2.70 (1H, m), 2.84–3.02 (2H, m), 3.75–3.92 (2H, m), 6.66 (2H, d, J = 6.6 Hz), 8.25 (2H, d, J = 6.6 Hz).

5.1.18. 4-[(6-Chloronaphthalen-2-yl)sulfonyl]-*N*-methyl-*N*-[1-(pyridin-4-yl)piperidin-4-yl]butanamide (2e). To a solution of **10a** (0.16 g, 0.51 mmol) and HOBt (0.08 g, 0.52 mmol) in DMF (5 mL) was added WSC (0.15 g, 0.79 mmol) and the mixture was stirred at room temperature for 1 h. **15** (0.12 g, 0.42 mmol) was added and the resulting mixture was stirred at room temperature for 14 h. The reaction mixture was concentrated in vacuo, and the residue was basified with 1 M NaOH and extracted with EtOAc. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was crystallized from Et_2O to give **2e** (0.22 g, 90%) as colorless crystals. Mp 185–186 °C. ^{1}H NMR ($CDCl_3$) δ: 1.55–1.90 (4H, m), 2.00–2.25 (2H, m), 2.45–2.74 (2H, m), 2.79 (3H, s), 2.82–3.02 (2H, m), 3.34 (2H, t, J = 7.2 Hz), 3.88–4.05 (2H, m), 4.52–4.80 (1H, m), 6.65 (2H, d, J = 6.6 Hz), 7.59 (1H, dd, J = 1.8 and 8.8 Hz), 7.80–8.00 (4H, m), 8.26 (2H, d, J = 6.6 Hz), 8.47 (1H, s). Anal. Calcd for $C_{25}H_{28}ClN_3O_3S{\cdot}0.1H_2O$: C, 61.55; H, 5.83; N, 8.61. Found: C, 61.44; H, 5.70, N, 8.76.

The following compounds **2f**–**h** were prepared in a manner similar to that described for **2e**.

5.1.19. 3-[(6-Chloronaphthalen-2-yl)sulfonyl]-*N*-methyl-*N*-[1-(pyridin-4-yl)piperidin-4-yl]propanamide (2f). Yield 60%, colorless powder. Mp 175–176 °C. ^{1}H NMR ($CDCl_3$) δ: 1.50–1.95 (4H, m), 2.70–3.08 (4H, m), 2.83 (3H, s), 3.58 (2H, t, J = 7.9 Hz), 3.80–4.10 (2H, m), 4.46–4.72 (1H, m), 6.65 (2H, d, J = 6.6 Hz), 7.60 (1H, dd, J = 1.8 and 8.8 Hz), 7.80–8.00 (4H, m), 8.26 (2H, d, J = 6.6 Hz), 8.49 (1H, s). Anal. Calcd for $C_{24}H_{26}ClN_3O_3S{\cdot}0.2H_2O{\cdot}0.1Et_2O$: C, 60.67; H, 5.75; N, 8.70. Found: C, 60.69; H, 5.72; N, 8.88.

5.1.20. 2-[(6-Chloronaphthalen-2-yl)sulfonyl]-*N*-methyl-*N*-[1-(pyridin-4-yl)piperidin-4-yl]acetamide (2g). Yield 45%, colorless powder. Mp 189–190 °C. ^{1}H NMR ($CDCl_3$) δ: 1.23–2.00 (8H, m), 2.70–3.17 (4H, m), 3.50–3.65 (2H, m), 3.70–4.10 (4H, m), 4.13–4.18 (2H, m), 6.58–6.72 (2H, m), 7.55–7.65 (1H, m), 7.80–8.00 (4H, m), 8.20–8.34 (2H, m), 8.49 (1H, s). Anal. Calcd for $C_{23}H_{24}ClN_3O_3S$: C, 60.32; H, 5.28; N, 9.18. Found: C, 60.17; H, 5.25; N, 9.19.

5.1.21. 1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(pyridin-4-yl)piperazine (2h). Yield 48%, colorless powder. Mp 216–217 °C. ^{1}H NMR ($CDCl_3$) δ: 2.85–3.00 (2H, m), 3.27 (2H, t, J = 5.4 Hz), 3.38 (2H, t, J = 5.4 Hz), 3.50–3.75 (6H, m), 6.64 (2H, d, J = 6.6 Hz), 7.58 (1H, dd, J = 2.2 and 8.8 Hz), 7.88–8.00 (4H, m), 8.32 (2H, d, J = 6.6 Hz), 8.48 (1H, s). Anal. Calcd for $C_{22}H_{22}ClN_3O_3S{\cdot}0.1H_2O$: C, 59.28; H, 5.02; N, 9.43. Found: C, 59.16; H, 5.00; N, 9.37.

5.1.22. 3-[(6-Chloronaphthalen-2-yl)sulfonyl]propan-1-ol (15). Compound **15** was prepared in a manner similar to that described for **7a**. ^{1}H NMR ($CDCl_3$) δ: 1.94–2.10 (2H, m), 3.25–3.38 (2H, m), 3.76 (2H, t, J = 6.0 Hz), 7.59 (1H, dd, J = 2.2 and 8.8 Hz), 7.80–8.00 (4H, m), 8.49 (1H, s).

5.1.23. *N*-Isopropyl-2,4-dinitrobenzenesulfonamide (17b). To a solution of **16b** (0.36 g, 6.09 mmol) and pyridine (0.53 mL, 6.55 mmol) in CH_2Cl_2 (15 mL) was added 2,4-dinitrobenzenesulfonyl chloride (1.07 g, 4.01 mmol) and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was acidified to pH 2 with 1 M HCl, concentrated in vacuo, and partitioned between water and EtOAc. The organic layer was separated, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc = 1/1) and the product was crystallized from hexane–EtOAc to give **17b** (0.46 g, 40%) as colorless needles. ^{1}H NMR ($CDCl_3$) δ: 1.19 (6H, d, J = 6.6 Hz), 3.62–3.82 (1H, m), 5.18 (1H, d, J = 7.2 Hz), 8.40 (1H, d, J = 8.8 Hz), 8.56 (1H, dd, J = 2.2 and 8.8 Hz), 8.68 (1H, d, J = 2.2 Hz).

The following compounds **17a** and **17c** were prepared in a manner similar to that described for **17b**.

5.1.24. *N*-Ethyl-2,4-dinitrobenzenesulfonamide (17a). ^{1}H NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7.0 Hz), 3.20 (2H, q, J = 7.0 Hz), 8.40 (1H, d, J = 8.8 Hz), 8.56 (1H, dd, J = 2.2 and 8.8 Hz), 8.68 (1H, d, J = 2.2 Hz).

5.1.25. Ethyl *N*-[(2,4-dinitrophenyl)sulfonyl]glycinate (17c). ^{1}H NMR ($CDCl_3$) δ: 1.19 (3H, t, J = 7.2 Hz), 4.07 (2H, q, J = 7.2 Hz), 4.08 (2H, d, J = 5.8 Hz), 6.14 (1H, t, J = 5.8 Hz), 8.31 (1H, d, J = 8.8 Hz), 8.55 (1H, dd, J = 2.2 and 8.8 Hz), 8.76 (1H, d, J = 2.2 Hz).

5.1.26. 3-[(6-Chloronaphthalen-2-yl)sulfonyl]-*N*-isopropylpropan-1-amine (18b). To a solution of **17b** (0.29 g, 1.00 mmol), **15** (0.28 g, 1.00 mmol), and triphenylphosphine (0.32 g, 1.22 mmol) in THF (5 mL) was added diethyl azodicarboxylate (DEAD; 40% toluene solution; 0.30 mL, 1.22 mmol) and the resulting mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated in vacuo and the residue was acidified to pH 2 with 1 M HCl and extracted with EtOAc. The

organic extract was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue obtained was dissolved in EtOAc (50 mL) and mCPBA (70%, 2.95 g, 12.0 mmol) was added to the solution and stirred at room temperature for 1 h. The reaction mixture was washed with aqueous $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc = 2/1) and the product was crystallized from EtOAc–Et_2O. The product was dissolved in CH_2Cl_2 (10 mL) and isopropylamine (1 mL) was added. The resulting mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo and the residue was partitioned between water and EtOAc. The organic layer was separated, washed with aqueous $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc to 30/1 EtOAc/MeOH) to give **18b** (0.16 g, 55%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.08 (6H, d, J = 6.2 Hz), 1.92–2.12 (2H, m), 2.75–3.00 (1H, m), 2.80 (2H, t, J = 7.0 Hz), 3.25–3.38 (2H, m), 7.57 (1H, dd, J = 2.0 and 8.8 Hz), 7.90–8.00 (4H, m), 8.48 (1H, s).

The following compounds **18a** and **18c** were prepared in a manner similar to that described for **18b**.

5.1.27. 3-[(6-Chloronaphthalen-2-yl)sulfonyl]-*N*-ethylpropan-1-amine (18a). ^{1}H NMR ($CDCl_3$) δ: 1.04 (3H, t, J = 7.1 Hz), 1.80–2.00 (2H, m), 2.57 (2H, q, J = 7.1 Hz), 2.69 (2H, t, J = 7.0 Hz), 3.20–3.35 (2H, m), 7.58 (1H, dd, J = 1.8 and 8.8 Hz), 7.55–8.00 (4H, m), 8.46 (1H, s).

5.1.28. Ethyl *N*-{3-[(6-chloronaphthalen-2-yl)sulfonyl]propyl}glycinate (18c). ^{1}H NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7.0 Hz), 1.80–2.00 (2H, m), 2.71 (2H, t, J = 6.6 Hz), 3.20–3.35 (2H, m), 3.31 (2H, s), 4.15 (2H, q, J = 7.0 Hz), 7.59 (1H, dd, J = 2.0 and 8.8 Hz), 7.90–8.00 (4H, m), 8.47 (1H, s).

5.1.29. *N*-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propyl}-*N*-ethyl-1-(pyridin-4-yl)piperidine-4-carboxamide (2i). Compound **2i** was prepared in a manner similar to that described for **2b** in 92% yield as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.07 (0.75H, t, J = 7.2 Hz), 1.22 (2.25H, t, J = 7.2 Hz), 1.60–2.20 (6H, m), 2.55–2.78 (1H, m), 2.78–3.00 (2H, m), 3.10–3.26 (2H, m), 3.26–3.60 (4H, m), 3.78–4.00 (2H, m), 6.64 (2H, d, J = 6.6 Hz), 7.55–7.70 (1H, m), 7.80–8.10 (4H, m), 8.24 (2H, d, J = 6.6 Hz), 8.46 (1H, s). Anal. Calcd for $C_{26}H_{30}ClN_3O_3S{\cdot}H_2O$: C, 60.28; H, 6.23; N, 8.11. Found: C, 60.41; H, 6.16; N, 8.18.

5.1.30. *N*-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propyl}-*N*-isopropyl-1-(pyridin-4-yl)piperidine-4-carboxamide (2j). Compound **2j** was prepared in a manner similar to that described for **2b** in 54% yield as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.12 (1.2H, d, J = 6.6 Hz), 1.25 (4.8 H, d, J = 6.6 Hz), 1.60–2.20 (6H, m), 2.60–2.80 (1H, m), 2.80–3.00 (2H, m), 3.21 (2H, t, J = 7.5 Hz), 3.35 (2H, t, J = 7.7 Hz), 3.80–4.00 (2H, m), 4.00–4.20 (1H, m), 6.60–6.70 (2H, m), 7.56 (1H, dd, J = 1.8 and 8.8 Hz), 7.80–8.00 (4H, m), 8.20–8.30 (2H, m), 8.47 (1H, s). Anal. Calcd for $C_{27}H_{32}ClN_3O_3S{\cdot}0.5H_2O$: C, 62.00; H, 6.36; N, 8.03. Found: C, 62.29; H, 6.34; N, 8.20.

5.1.31. Ethyl *N*-{3-[(6-chloronaphthalen-2-yl)sulfonyl]propyl}-*N*-{[1-(pyridin-4-yl)piperidin-4-yl]carbonyl}glycinate (2k). Compound **2k** was prepared in a manner similar to that described for **2b** in 61% yield as a colorless powder. Mp 169–171 °C. ^{1}H NMR ($CDCl_3$) δ: 1.23 (1.5H, t, J = 7.2 Hz), 1.29 (1.5H, t, J = 7.2 Hz), 1.65–2.15 (6H, m), 2.35–2.60 (0.5H, m), 2.70–3.02 (2.5H, m), 3.15–3.32 (2H, m), 3.54 (1H, t, J = 6.6 Hz), 3.67 (1H, t, J = 7.4 Hz), 3.80–3.96 (2H, m), 3.99 (1H, s), 4.10 (1H, s), 4.12 (1H, q, J = 7.2 Hz), 4.21 (1H, q, J = 7.2 Hz), 6.64 (2H, d, J = 4.8 Hz), 7.55–7.68 (1H, m), 7.82–8.00 (4H, m), 8.25 (2H, d, J = 5.8 Hz), 8.45 (0.5H, s), 8.47 (0.5H, s). Anal. Calcd for $C_{28}H_{32}ClN_3O_5S$: C, 60.26; H, 5.78; N, 7.53. Found: C, 60.28; H, 6.05; N, 7.63.

5.1.32. *N*-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propyl}-*N*-{[1-(pyridin-4-yl)piperidin-4-yl]carbonyl}glycine (2l). A solution of **2k** (0.44 g, 0.78 mmol) in 2 M NaOH (0.80 mL, 1.60 mmol) and MeOH (50 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and the residue was purified by CHP-20 chromatography (water to 1/9 MeOH/water) to give **2l** (0.40 g, 92%) as a colorless amorphous powder. ^{1}H NMR (CD_3OD) δ: 1.50–2.10 (6H, m), 2.75–3.75 (7H, m), 3.91 (2H, s), 4.10–4.30 (2H, m), 7.09 (2H, d, J = 7.8 Hz), 7.65 (1H, dd, J = 1.8 and 8.8 Hz), 7.90–8.20 (6H, m), 8.54 (1H, s). Anal. Calcd for $C_{26}H_{28}ClN_3O_5S{\cdot}H_2O$: C, 56.98; H, 5.52; N, 7.67. Found: C, 57.21; H, 5.29; N, 7.84.

5.1.33. *N*-{3-[(2-Amino-2-oxoethyl)sulfonyl]propyl}-*N*-(6-chloronaphthalen-2-yl)-1-(pyridin-4-yl)piperidine-4-carboxamide (2m). To a solution of **2l** (0.09 g, 0.16 mmol) and HOBt (0.03 g, 0.16 mmol) in DMF (3 mL) was added WSC (0.05 g, 0.25 mmol) and the mixture was stirred at room temperature for 1 h. Ammonia solution (25%, 0.1 mL) was added and the resulting mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated in vacuo and the residue was partitioned between water and EtOAc. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was crystallized from DMF–EtOAc to give **2m** (0.05 g, 57%) as colorless crystals. Mp 222–225 °C. ^{1}H NMR (DMSO-d_6 + D_2O) δ: 1.35–2.00 (6H, m), 2.50–3.00 (3H, m), 3.20–4.00 (6H, m), 3.78 (1H, s), 4.00 (1H, s), 6.70 (1H, d, J = 6.0 Hz), 6.76 (1H, d, J = 6.0 Hz), 7.69 (1H, dd, J = 2.0 and 8.8 Hz), 7.88–8.02 (1H, m), 8.05–8.40 (5H, m), 8.57 (0.5H, s), 8.60 (0.5H, s). Anal. Calcd for $C_{26}H_{29}ClN_4O_4S{\cdot}0.5H_2O$: C, 58.04; H, 5.62; N, 10.41. Found: C, 58.32; H, 5.59; N, 10.23.

5.1.34. *N*-(6-Chloronaphthalen-2-yl)-*N*-{3-[(2-morpholin-4-yl-2-oxoethyl)sulfonyl]propyl}-1-(pyridin-4-yl)piperidine-4-carboxamide (2n). Compound **2n** was prepared in a manner similar to that described for **2m** in 79% yield as a colorless powder. Mp 240–241 °C. ^{1}H NMR

(DMSO-d_6 + DCl) δ: 1.35–2.00 (6H, m), 2.55–4.30 (19H, m), 7.13 (2H, d, J = 6.4 Hz), 7.72 (1H, dd, J = 2.2 and 8.8 Hz), 7.95 (1H, dt, J = 1.8 and 9.6 Hz), 8.10–8.36 (5H, m), 8.61 (1H, s). Anal. Calcd for $C_{30}H_{35}ClN_4O_5S$: C, 60.14; H, 5.89; N, 9.35. Found: C, 59.86; H, 5.88; N, 9.13.

5.1.35. 1-(2-Methylpyridin-4-yl)piperidin-4-one (20a). A mixture of **19a** (15.1 g, 118 mmol), 1,4-dioxa-8-azaspiro[4.5]decane (14.0 g, 97.8 mmol), and Et_3N (30.0 g, 296 mmol) in EtOH (150 mL) was heated at 150 °C for 13 h in a sealed tube. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was basified with 4.5 M NaOH and K_2CO_3, and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was washed with hexane and the residual solid was dissolved in acetone (30 mL). To this was added 4 M HCl (52.8 mL, 211 mmol) at 50 °C and the resulting mixture was stirred at 50 °C for 5 h. The reaction mixture was concentrated in vacuo. The residue was diluted with 1 M NaOH, basified to pH 11 with K_2CO_3, and extracted with CH_2Cl_2 (3 × 100 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give **20a** (9.45 g, 51%) as a yellow oily solid. ^{1}H NMR ($CDCl_3$) δ: 2.49 (3H, s), 2.56 (4H, t, J = 6.3 Hz), 3.74 (4H, t, J = 6.3 Hz), 6.54–6.61 (2H, m), 8.23 (1H, d, J = 5.8 Hz).

The following compounds **20b**–**d** were prepared in a manner similar to that described for **20a**.

5.1.36. 1-[2-(Hydroxymethyl)pyridin-4-yl]piperidin-4-one (20b). ^{1}H NMR ($CDCl_3$) δ: 2.57 (4H, t, J = 6.1 Hz), 3.76 (4H, t, J = 6.3 Hz), 4.68 (2H, s), 6.62–6.67 (2H, m), 8.27 (1H, d, J = 5.4 Hz).

5.1.37. 1-(2-Aminopyridin-4-yl)piperidin-4-one (20c). ^{1}H NMR ($CDCl_3$) δ: 2.53 (4H, t, J = 6.0 Hz), 3.68 (4H, t, J = 6.0 Hz), 5.91 (1H, d, J = 2.2 Hz), 6.23 (1H, dd, J = 2.2 and 6.2 Hz), 7.87 (1H, d, J = 6.2 Hz).

5.1.38. 1-(2,6-Dimethylpyridin-4-yl)piperidin-4-one (20d). ^{1}H NMR ($CDCl_3$) δ: 2.45 (6H, s), 2.54 (4H, t, J = 6.2 Hz), 3.72 (4H, t, J = 6.2 Hz), 6.45 (2H, s).

5.1.39. Ethyl *N*-[1-(pyridin-4-yl)piperidin-4-yl]glycinate (21a). Compound **21a** was prepared in a manner similar to that described for **13**. ^{1}H NMR ($CDCl_3$) δ: 1.33 (3H, t, J = 7.2 Hz), 1.60–1.95 (2H, m), 2.25–2.40 (2H, m), 3.18–3.40 (2H, m), 3.55–3.80 (1H, m), 4.09 (2H, s), 4.33 (2H, q, J = 7.2 Hz), 4.35–4.55 (2H, m), 7.25 (2H, d, J = 8.0 Hz), 8.17 (2H, d, J = 8.0 Hz).

5.1.40. Ethyl *N*-[1-(pyridin-4-yl)piperidin-4-yl]-beta-alaninate (21b). Compound **21b** was prepared in a manner similar to that described for **13**. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.1 Hz), 1.28–1.55 (2H, m), 1.88–2.08 (2H, m), 2.51 (2H, t, J = 6.4 Hz), 2.64–2.85 (1H, m), 2.85–3.05 (4H, m), 3.50–4.00 (1H, br s), 3.75–3.92 (2H, m), 4.15 (2H, q, J = 7.2 Hz), 6.66 (2H, d, J = 6.4 Hz), 8.23 (2H, d, J = 6.4 Hz).

5.1.41. *tert*-Butyl (2-{[1-(pyridin-4-yl)piperidin-4-yl]amino}ethyl)carbamate (21c). Compound **21c** was prepared in a manner similar to that described for **13**. ^{1}H NMR ($CDCl_3$) δ: 1.25–1.50 (2H, m), 1.45 (9H, s), 1.90–2.05 (2H, m), 2.65–3.05 (5H, m), 3.15–3.30 (2H, m), 3.75–3.92 (2H, m), 4.91 (1H, br s), 6.60–6.72 (2H, m), 8.20–8.28 (2H, m).

5.1.42. *N*-Methyl-1-(2-methylpyridin-4-yl)piperidin-4-amine (21d). Compound **21d** was prepared in a manner similar to that described for **13**. ^{1}H NMR ($CDCl_3$) δ: 1.30–1.48 (2H, m), 1.92–2.02 (2H, m), 2.44 (3H, s), 2.47 (3H, s), 2.54–2.70 (1H, m), 2.84–2.98 (2H, m), 6.49–6.54 (2H, m), 8.14 (1H, d, J = 6.0 Hz).

5.1.43. {4-[4-(Methylamino)piperidin-1-yl]pyridin-2-yl}methanol (21e). Compound **21e** was prepared in a manner similar to that described for **13**. ^{1}H NMR ($CDCl_3$) δ: 1.36 (2H, m), 1.98 (2H, m), 2.47 (3H, s), 2.61 (1H, m), 2.94 (2H, m), 3.86 (2H, m), 4.63 (2H, s), 6.60 (2H, m), 8.19 (1H, d, J = 6.0 Hz).

5.1.44. 4-[4-(Methylamino)piperidin-1-yl]pyridin-2-amine (21f). Compound **21f** was prepared in a manner similar to that described for **13**. ^{1}H NMR ($CDCl_3$) δ: 1.34 (2H, m), 1.95 (2H, m), 2.46 (3H, s), 2.58 (1H, m), 2.87 (2H, m), 3.76 (2H, m), 4.19 (2H, br s), 4.63 (2H,s), 5.87 (1H, d, J = 2.4 Hz), 6.20 (1H, dd, J = 2.4 and 6.2 Hz), 7.80 (1H, d, J = 6.2 Hz).

5.1.45. 1-(2,6-Dimethylpyridin-4-yl)-*N*-methylpiperidin-4-amine (21g). Compound **21g** was prepared in a manner similar to that described for **13**. ^{1}H NMR ($CDCl_3$) δ: 1.40 (2H, m), 2.00 (2H, m), 2.47 (9H, s), 2.69 (1H, m), 2.98–3.12 (4H, m), 3.88 (1H, m), 6.43 (2H, s).

5.1.46. Ethyl *N*-{3-[(6-chloronaphthalen-2-yl)sulfonyl]propanoyl}-*N*-[1-(pyridin-4-yl)piperidin-4-yl]glycinate (2o). Compound **2o** was prepared in a manner similar to that described for **2e** in 9% yield as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 3.91 (3H, s), 7.79 (1H, d, J = 8.8 Hz), 8.13–8.17 (3H, m), 8.34 (2H, d, J = 8.4 Hz), 8.50 (1H, s). Anal. Calcd for $C_{27}H_{30}ClN_3O_5S{\cdot}0.55H_2O$: C, 58.54; H, 5.66; N, 7.59. Found: C, 58.27; H, 5.77; N, 7.87.

5.1.47. Ethyl *N*-{3-[(6-chloronaphthalen-2-yl)sulfonyl]propanoyl}-*N*-[1-(pyridin-4-yl)piperidin-4-yl]-beta-alaninate (2p). A mixture of **10c** (0.30 g, 1.00 mmol) and thionyl chloride (2 mL) was stirred at 90 °C for 1 h and then concentrated in vacuo. The residue was dissolved in THF (5 mL), and the solution was added to an ice-cooled solution of **21b** (0.28 g, 1.00 mmol) and *N,N*-diisopropylethylamine (0.40 mL, 2.30 mmol) in THF (20 mL). After the resulting mixture was stirred at room temperature for 2 h, it was concentrated in vacuo. The residue was partitioned between brine and THF. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc/MeOH = 60/1) to give **2p** (45 mg, 8%) as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.22–1.28 (3H, m), 1.50–2.00 (5H, m), 2.35–2.60 (2H, m), 2.72–

3.08 (4H, m), 3.32–3.68 (4H, m), 3.70–4.20 (4H, m), 6.58–6.74 (2H, m), 7.61 (1H, dd, J = 2.2 and 8.8 Hz), 7.80–8.05 (4H, m), 8.20–8.40 (2H, m), 8.49 (1H, s). Anal. Calcd for $C_{28}H_{32}ClN_3O_5S{\cdot}0.5H_2O$: C, 59.30; H, 5.87; N, 7.41. Found: C, 59.38; H, 5.61; N, 7.51.

5.1.48. 3-[(6-Chloronaphthalen-2-yl)sulfonyl]-*N*-methyl-*N*-[1-(2-methylpyridin-4-yl)piperidin-4-yl]propanamide (2s). To a stirred solution of **10c** (0.45 g, 1.50 mmol) and **21d** (0.31 g, 1.50 mmol) in THF (50 mL) was added DMTMM (0.56 g, 2.00 mmol) and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo and the residue was partitioned between THF and aqueous K_2CO_3. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc/MeOH = 40/1) and crystallized from EtOAc to give **2s** (0.34 g, 70%) as a colorless powder. Mp 186 °C. ^{1}H NMR ($CDCl_3$) δ: 1.52–1.95 (4H, m), 2.50 (3H, s), 2.75–3.15 (4H, m), 2.84 (3H, s), 3.50–3.65 (2H, m), 4.45–4.80 (1H, m), 6.50–6.65 (2H, m), 7.60 (1H, dd, J = 2.2 and 8.8 Hz), 7.90–8.00 (4H, m), 8.20 (1H, d, J = 6.4 Hz), 8.45 (1H, s). Anal. Calcd for $C_{25}H_{28}ClN_3O_2S$: C, 61.78; H, 5.81; N, 8.65. Found: C, 61.51; H, 5.82; N, 8.44.

The following compounds **2q** and **2t**–**v** were prepared in a manner similar to that described for **2s**.

5.1.49. *tert*-Butyl[2-({3-[(6-chloronaphthalen-2-yl)sulfonyl]-propanoyl}[1-(pyridin-4-yl)piperidin-4-yl]amino)ethyl]carbamate (2q). Yield 56%, colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.40 (4.5H, s), 1.45 (4.5H, s), 1.55–2.00 (4H, m), 2.70–3.10 (5H, m), 3.10–3.40 (4H, m), 3.50–3.70 (2H, m), 3.80–4.10 (2H, m), 4.80–5.05 (1H, m), 6.60–6.75 (2H, m), 7.55–7.65 (1H, m), 7.90–8.00 (4H, m), 8.20–8.35 (2H, m), 8.50 (1H, s).

5.1.50. 3-[(6-Chloronaphthalen-2-yl)sulfonyl]-*N*-{1-[2-(hydroxymethyl)pyridin-4-yl]piperidin-4-yl}-*N*-methylpropanamide (2t). Yield 54%, colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.56–1.79 (4H, m), 2.83 (3H, s), 2.86–2.99 (4H, m), 3.57 (2H, dd, J = 3.2 and 8.2 Hz), 3.95 (2H, m), 4.63 (2H, s), 4.65 (1H, m), 6.56–6.63 (2H, m), 7.60 (dd, J = 2.2 and 8.2 Hz), 7.93–7.97 (4H, m), 8.20 (1H, d, J = 6.6 Hz), 8.49 (1H, s). Anal. Calcd for $C_{25}H_{28}ClN_3O_4S{\cdot}0.5H_2O$: C, 58.76; H, 5.72; N, 8.22. Found: C, 58.89; H, 5.92; N, 8.02.

5.1.51. *N*-[1-(2-Aminopyridin-4-yl)piperidin-4-yl]-3-[(6-chloronaphthalen-2-yl)sulfonyl]-*N*-methylpropanamide (2u). Yield 50%, colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.56–1.75 (4H, m), 2.82 (3H, s), 2.82–2.96 (4H, m), 3.56 (2H, m), 3.81 (2H, m), 4.24 (2H, br s), 4.57 (1H, m), 5.84 (1H, d, J = 2.6 Hz), 6.16 (1H, dd, J = 2.6 and 6.6 Hz), 7.60 (1H, m), 7.78–7.94 (5H, m), 8.48 (1H, s). Anal. Calcd for $C_{24}H_{27}ClN_4O_3S{\cdot}0.5H_2O$: C, 58.11; H, 5.69; N, 11.30. Found: C, 58.38; H, 5.91; N, 11.56.

5.1.52. 3-[(6-Chloronaphthalen-2-yl)sulfonyl]-*N*-[1-(2,6-dimethylpyridin-4-yl)piperidin-4-yl]-*N*-methylpropanamide (2v). Yield 61%, colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.56–1.68 (4H, m), 2.44 (6H, s), 2.83 (3H, s), 2.88–3.04 (4H, m), 3.57 (2H, dd, J = 7.0 and 8.0 Hz), 3.94 (2H, m), 4.60 (1H, m), 6.38 (2H, s), 7.60 (1H, dd, J = 2.2 and 8.8 Hz), 7.93–7.97 (4H, m), 8.48 (1H, s). Anal. Calcd for $C_{26}H_{30}ClN_3O_3S{\cdot}1.5H_2O$: C, 59.25; H, 6.31; N, 7.97. Found: C, 59.34; H, 6.19; N, 8.33.

5.1.53. *N*-(2-Aminoethyl)-3-[(6-chloronaphthalen-2-yl)sulfonyl]-*N*-[1-(pyridin-4-yl)piperidin-4-yl]propanamide ditrifluoroacetate (2r). To a solution of **2q** (0.14 g, 0.24 mmol) in toluene (2 mL) was added trifluoroacetic acid (2 mL) at room temperature and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo and the residual solid was collected by filtration to give **2r** (0.14 g, quant.) as a colorless amorphous powder. ^{1}H NMR (CD_3OD) δ: 1.64–2.05 (4H, m), 2.97 (2H, t, 6.3 Hz), 3.05 (2H, t, J = 7.2 Hz), 3.15–3.40 (2H, m), 3.47 (2H, t, J = 6.3 Hz), 3.70 (2H, t, J = 7.2 Hz), 4.10–4.48 (3H, m), 7.18 (2H, d, J = 7.8 Hz), 7.66 (1H, dd, J = 2.2 and 8.8 Hz), 7.90–8.20 (6H, m), 8.59 (1H, s). Anal. Calcd for $C_{25}H_{29}ClN_4O_3S{\cdot}2CF_3CO_2H{\cdot}2H_2O$: C, 45.52; H, 4.61; N, 7.32. Found: C, 45.67; H, 4.60; N, 7.32.

5.1.54. 3-[(7-Chloronaphthalen-2-yl)sulfonyl]propanoic acid (24a). To a solution of sodium sulfite (0.54 g, 4.20 mmol) and sodium bicarbonate (0.66 g, 7.86 mmol) in water (20 mL) was added **22a**[23] (1.00 g, 3.83 mmol) at 75 °C and the mixture was stirred at 75 °C for 1.5 h. A solution of sodium hydroxide (0.31 g, 7.75 mmol) in water (1 mL) and bromosuccinic acid (1.54 g, 8.65 mmol) were successively added and the reaction mixture was stirred at 110 °C for 20 h. The resulting precipitate was collected by filtration, washed with water, and dried to give **24a** (0.61 g, 53%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 2.58 (2H, t, J = 7.3 Hz), 3.62 (2H, d, J = 7.3 Hz), 7.78 (1H, dd, J = 2.2 and 8.8 Hz), 7.95 (1H, dd, J = 2.0 and 8.8 Hz), 8.16 (1H, d, J = 8.8 Hz), 8.25 (1H, d, J = 8.8 Hz), 8.40 (1H, d, J = 2.0 Hz), 8.59 (1H, s).

The following compounds **24b**–**e** were prepared in a manner similar to that described for **24a**.

5.1.55. 3-[(5-Chloronaphthalen-2-yl)sulfonyl]propanoic acid (24b). ^{1}H NMR (DMSO-d_6) δ: 2.59 (2H, t, J = 7.3 Hz), 3.64 (2H, d, J = 7.3 Hz), 7.71 (1H, t, J = 8.0 Hz), 7.96 (1H, d, J = 7.8 Hz), 8.09 (1H, dd, J = 2.0 and 8.8 Hz), 8.28 (1H, d, J = 8.4 Hz), 8.42 (1H, d, J = 8.8 Hz), 8.71 (1H, d, J = 2.0 Hz).

5.1.56. 3-[(6-Methylnaphthalen-2-yl)sulfonyl]propanoic acid (24c). ^{1}H NMR (DMSO-d_6) δ: 2.54 (3H, s), 2.56 (2H, t, J = 7.4 Hz), 3.59 (2H, d, J = 7.4 Hz), 7.56 (1H, dd, J = 1.4 and 8.4 Hz), 7.86 (1H, dd, J = 2.0 and 8.6 Hz), 7.87 (1H, s), 8.08 (1H, d, J = 8.6 Hz), 8.12 (1H, d, J = 8.4 Hz), 8.52 (1H, s).

5.1.57. 3-[(5-Chloro-1-benzothien-2-yl)sulfonyl]propanoic acid (24d). ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.75 (2H, t, J = 7.7 Hz), 3.55 (2H, t, J = 7.7 Hz), 7.47 (1H, dd, J = 2.2 and 8.4 Hz), 7.87 (1H, d, J = 8.4 Hz), 8.25 (1H, d, J = 2.2 Hz), 8.42 (1H, s).

5.1.58. 3-[(6-Chloro-1-benzothien-2-yl)sulfonyl]propanoic acid (24e). ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.74 (2H, t, J = 7.5 Hz), 3.54 (2H, t, J = 7.5 Hz), 7.52 (1H, dd, J = 1.8 and 8.8 Hz), 7.94 (1H, d, J = 1.8 Hz), 8.18 (1H, d, J = 8.8 Hz), 8.33 (1H, s).

5.1.59. 3-[(7-Chloronaphthalen-2-yl)sulfonyl]-*N*-methyl-*N*-[1-(2-methylpyridin-4-yl)piperidin-4-yl]propanamide (2w). Compound **2w** was prepared in a manner similar to that described for **2s** in 58% yield as colorless crystals. Mp 96–98 °C. ^{1}H NMR ($CDCl_3$) δ: 1.50–1.95 (4H, m), 2.45–2.48 (3H, m), 2.76–2.83 (3H, m), 2.80–3.05 (4H, m), 3.57 (2H, t, J = 7.7 Hz), 3.93 (2H, m), 4.59 (1H, m), 6.45–6.60 (2H, m), 7.64 (1H, dd, J = 2.2 and 8.8 Hz), 7.85–8.05 (4H, m), 8.15 (1H, d, J = 6.0 Hz), 8.42 (1H, s). Anal. Calcd for $C_{25}H_{28}ClN_3O_3S{\cdot}0.5H_2O$: C, 60.66; H, 5.90; N, 8.49. Found: C, 60.82; H, 5.72; N, 8.53.

5.1.60. 3-[(5-Chloronaphthalen-2-yl)sulfonyl]-*N*-methyl-*N*-[1-(2-methylpyridin-4-yl)piperidin-4-yl]propanamide (2x). Compound **2x** was prepared in a manner similar to that described for **2s** in 64% yield as a colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 1.50–1.95 (4H, m), 2.45–2.47 (3H, m), 2.76–2.82 (3H, m), 2.80–3.05 (4H, m), 3.60 (2H, t, J = 7.5 Hz), 3.93 (2H, m), 4.58 (1H, m), 6.45–6.60 (2H, m), 7.58 (1H, t, J = 8.0 Hz), 7.80 (1H, d, J = 7.6 Hz), 7.95 (1H, d, J = 8.4 Hz), 8.01 (1H, dd, J = 1.8 and 8.8 Hz), 8.15 (1H, d, J = 5.8 Hz), 8.48 (1H, d, J = 8.8 Hz), 8.53 (1H, d, J = 1.8 Hz). Anal. Calcd for $C_{25}H_{28}ClN_3O_3S{\cdot}0.5H_2O$: C, 60.66; H, 5.90; N, 8.49. Found: C, 60.54; H, 6.15; N, 8.56.

5.1.61. 3-[(6-Methylnaphthalen-2-yl)sulfonyl]-*N*-methyl-*N*-[1-(2-methylpyridin-4-yl)piperidin-4-yl]propanamide (2y). Compound **2y** was prepared in a manner similar to that described for **2s** in 58% yield as colorless crystals. Mp 129–131 °C. ^{1}H NMR ($CDCl_3$) δ: 1.50–1.95 (4H, m), 2.44–2.46 (3H, m), 2.57 (3H, s), 2.73–2.80 (3H, m), 2.80–3.03 (4H, m), 3.57 (2H, t, J = 7.7 Hz), 3.91 (2H, m), 4.58 (1H, m), 6.45–6.60 (2H, m), 7.48 (1H, dd, J = 1.6 and 8.4 Hz), 7.71 (1H, s), 7.80–7.95 (3H, m), 8.14 (1H, d, J = 6.2 Hz), 8.45 (1H, s). Anal. Calcd for $C_{26}H_{31}N_3O_3S$: C, 67.07; H, 6.71; N, 9.02. Found: C, 66.77; H, 6.64; N, 8.97.

5.1.62. 3-[(5-Chloro-1-benzothien-2-yl)sulfonyl]-*N*-methyl-*N*-[1-(2-methylpyridin-4-yl)piperidin-4-yl]propanamide (2z). Compound **2z** was prepared in a manner similar to that described for **2s** in 50% yield as colorless crystals. Mp 122–124 °C. ^{1}H NMR ($CDCl_3$) δ: 1.40–2.00 (5H, m), 2.44 (2.25H, s), 2.47 (0.75H, s), 2.76 (0.75H, s), 2.78–3.10 (4H, m), 2.84 (2.25H, s), 3.52–3.76 (2H, m), 3.72–4.10 (2.25H, m), 4.48–4.80 (0.25H, m), 6.40–6.60 (2H, m), 7.47 (1H, dd, J = 1.8 and 8.8 Hz), 7.86 (1H, d, J = 8.8 Hz), 8.16 (1H, d, J = 5.8 Hz), 8.25 (1H, d, J = 1.8 Hz), 8.40 (0.75H, s), 8.42 (0.25H, s). Anal. Calcd for $C_{23}H_{26}ClN_3O_3S_2{\cdot}0.25EtOH{\cdot}0.25H_2O$: C, 55.55; H, 5.55; N, 8.12. Found: C, 55.58; H, 5.41; N, 8.12.

5.1.63. 3-[(6-Chloro-1-benzothien-2-yl)sulfonyl]-*N*-methyl-*N*-[1-(2-methylpyridin-4-yl)piperidin-4-yl]propanamide (2aa). Compound **2aa** was prepared in a manner similar to that described for **2s** in 50% yield as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.45–2.00 (5H, m), 2.45–2.47 (3H, m), 2.70–3.10 (4H, m), 2.77–2.83 (3H, m), 3.50–3.70 (2H, m), 3.70–4.10 (2.25H, m), 4.48–4.80 (0.75H, m), 6.40–6.60 (2H, m), 7.52 (1H, dd, J = 1.8 and 8.8 Hz), 7.93 (1H, d, J = 1.8 Hz), 8.10–8.20 (1H, m), 8.19 (1H, d, J = 8.8 Hz), 8.34–8.35 (1H, m). Anal. Calcd for $C_{23}H_{26}ClN_3O_3S_2{\cdot}0.25H_2O$: C, 55.56; H, 5.38; N, 8.46. Found: C, 55.58; H, 5.41; N, 8.35.

5.2. Biology

5.2.1. In vitro assays for the inhibition of human FXa. Human factor Xa (0.3 U/mL) was obtained from Roche Diagnostics. Chromogenic substrate, S-2765 (Chromogenix-Instrumentation Laboratory), was used for the measurement of the inhibition of FXa. Anti-FXa activity was assayed in a buffer containing 50 mM Tris–HCl, 145 mM NaCl, and 2 mM $CaCl_2$ at pH 8.3. Enzyme assay was carried out in 96-well microtiter plates. Test compounds were diluted in DMSO. Compound dilutions and 10 μL of enzyme solution were added to the well containing buffer, and preincubated. The enzymatic reactions were initiated with the addition of 10 μL of 3 mM substrate and the mixture was incubated for 10 min at 37 °C. The reaction was terminated with the addition of 25 μL of 50% acetic acid. The color development from the release of *p*-nitroanilide from each chromogenic substrate was measured at 405 nm on a microtiter plate reader (MTP32, Corona Electric Co.). Each absorbance [T] was calculated by subtracting the absorbance measured without the substrate. The control [C] was performed using DMSO solution in place of test compound. Inhibitory effect (%) was calculated according to the equation $(1 - [T]/[C]) \times 100$. IC_{50} values were calculated from the regression line based on the method of least squares between inhibitory effect and concentration. Data for new compounds were compared to positive control DX-9065a (FXa IC_{50} = 0.13 μM). The 95% confidence interval for the IC_{50} of DX-9065a was 0.12–0.15 μM (duplicate).

5.2.2. In vitro PT assays. The assay of plasma clotting time was performed using an automatic coagulometer (STA Compact, Diagnostica Stago). PT was measured with PT-Test Wako (Wako Pure Chemical). Compound dilutions of 1.5 μL in DMSO were added to 48.5 μL of human normal plasma (fresh human plasma: FFP, Sekisui Chemical Co.), and the mixture was preincubated at 37 °C for 4 min. Coagulation was initiated with the addition of 100 μL of thromboplastin, and coagulation time was measured. Coagulation time prolonging ratio (%) was calculated based on coagulation time when DMSO was added instead of test compound. The plasma clotting time doubling concentration (PT_2) was calculated from the regression line based on the method of least squares. Data for new compounds were compared to positive control DX-9065a (PT_2 = 0.69 μM). The 95% confidence interval for the PT_2 of DX-9065a was 0.61–0.79 μM (n = 3).

5.2.3. Measurement of CYP inhibition activity. Inhibition activity of test compounds of CYP3A4 was evaluated by

incubating 40 μM 7-benzyloxyquinoline with microsomes derived from CYP3A4-expressing insect cell (BD Biosciences) in the presence of 1 μM test compound. The concentration of 7-benzyloxyquinoline metabolite was measured with a spectrofluorometer.

5.2.4. Measurement of ex vivo PT in mouse after oral administration. Male ICR mice (30–35 g, Clea Japan Inc.) fasting for more than 12 h were employed. Test compounds were orally administered to these animals. An hour after administration, blood (800 μL) was collected from artery abdominal aorta using 3.8% sodium citrate (whole blood: sodium citrate solution = 9:1, v/v), under anesthesia with pentobarbital (50 mg/kg, ip). The citrated blood was centrifuged at 1000*g* for 10 min to obtain platelet poor plasma (PPP). PT was measured with an automatic coagulometer. Fifty microliters of PPP was preincubated at 37 °C for 4 min. The PPP was mixed with 100 μL of thromboplastin solution, and then its coagulation time was measured. Test compounds were suspended in 0.5% methyl cellulose (Metolose cp100, Shin-Etsu Chemical), and as control, 0.5% methylcellulose was administered instead of test compounds. Activity of each test compound was shown in ratio (%) determined by comparing the clotting time of compound-treated mice with that of the control group. We have validated the ex vivo PT assay for the evaluation of oral bioavailability in mice by use of the positive control compound DX-9065a. DX-9065a prolonged mouse PT by 1.1-fold at a dose of 30 mg/kg and by 1.4-fold at a dose of 100 mg/kg.

5.2.5. Measurement of ex vivo PT in monkey after oral administration. Male cynomolgus monkeys (3.5–4 kg, NAFOVANNY) fasting for more than 12 h were employed. Test compounds were orally administered to these animals. After administration, blood (1.5 mL) was collected from femoral vein using 3.8% sodium citrate (whole blood: sodium citrate solution = 9:1, v/v) at several time points. The citrated blood was centrifuged at 1000*g* for 10 min to obtain PPP. Plasma clotting time and anticoagulating activity of drug were determined as described above.

5.2.6. Enzyme inhibition assays. Enzyme assays using chromogenic substrates were performed as follows. Human factor Xa was obtained from Roche diagnostics. Human thrombin was obtained from Sigma Chemical Co. Human trypsin was obtained from Atheus Research and Technology, Inc. Human kallikrein and plasmin were purchased from BioPur AG. Human t-PA was obtained from Kyowa Hakko Kogyo Co. The chromogenic substrates used were S-2222, S-2366, S-2222, S-2366, S-2302, and S-2288 for FXa, thrombin, trypsin, plasmin, kallikrein, and t-PA, respectively, and obtained from Chromogenix-Instrumentation Laboratory. All enzyme assays were performed at 37 °C in 96-well microtiter plates. The final enzyme concentrations were 0.024 U/mL, 0.080 U/mL, 0.040 μg/mL, 0.040 U/mL, 0.020 U/mL, and 4000 U/mL for FXa, thrombin, trypsin, plasmin, kallikrein, and t-PA, respectively. Compound dilutions were added to the wells containing buffer and enzyme, and incubated for 30 min. The enzyme reactions were initiated by the addition of substrate and the color developed from the release of *p*-nitroanilide from each chromogenic substrate was monitored continuously for 5 min at 405 nm on a microtiter plate reader. The K_i values were determined from a Lineweaver–Burk plot, when the optical densities were measured with different concentrations of substrates.

5.2.7. Pharmacokinetic analysis in cynomolgus monkeys. Test compound was administered to fasted cynomolgus monkeys (male, $n = 3$) intravenously (1 mg/kg, DMA/PEG400) or orally (1 mg/kg, 0.5% methylcellulose suspension). Before and 5, 10, 15, 30 min, 1, 2, 4, 8, 24 h after intravenous administration, or before and 15, 30 min, 1, 2, 4, 8, 24 h after oral administration, blood samples were collected from femoral vein. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile. After centrifugation, the supernatant obtained was diluted with the same volume of 0.01 M HCO_2NH_4 (adjusted to pH 3.0 with HCO_2H) and centrifuged again. The compound concentration in the supernatant was measured by LC/MS/MS with an API3000 triple quadruple mass spectrometer (Perkin-Elmer Sciex). The mass spectrometer was equipped with a turbo ionspray source and operated in positive ion mode. The HPLC conditions were as follows: column, an L-column ODS (2.1 × 150 mm); mobile phase, 0.01 M HCO_2NH_4 (adjusted to pH 3.0 with HCO_2H)/acetonitrile = 5/5; flow rate, 0.2 mL/min; column temperature, 40 °C.

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