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Design, Synthesis, and Structure–Activity and Structure–Pharmacokinetic Relationship Studies of Novel [6,6,5] Tricyclic Fused Oxazolidinones Leading to the Discovery of a Potent, Selective, and Orally Bioavailable FXa Inhibitor

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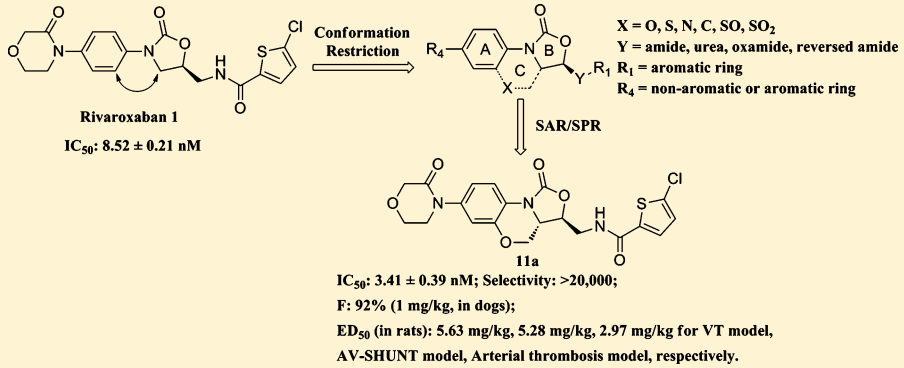
Supporting Information



ABSTRACT: The blood coagulation enzyme factor Xa (FXa) is a particularly promising target for anticoagulant therapy, and identification of oral small-molecule inhibitors of FXa remains a research focus. On the basis of the X-ray crystal structure of FXa and its inhibitor rivaroxaban, we designed and synthesized a series of conformationally restricted mimics containing a novel [6,6,5] tricyclic fused oxazolidinone scaffold. Intensive structure–activity relationship (SAR) and structure–pharmacokinetic relationship (SPR) studies on this new series led to the discovery of compound **11a**: a highly potent, selective, direct, and orally bioavailable FXa inhibitor with excellent in vivo antithrombotic efficacy and preferable pharmacokinetic profiles. Druggability evaluation of compound **11a** was undertaken and elicited positive outcomes. All results indicate that compound **11a** is a promising drug candidate for the prevention and treatment of thromboembolic diseases in venous and arterial systems.

INTRODUCTION

Thromboembolic disorders such as deep venous thrombosis (DVT), pulmonary embolism (PE), ischemic stroke, myocardial infarction, and unstable angina remain the leading cause of morbidity and mortality worldwide.[1−3] Several anticoagulants such as heparins (unfractionated and low-molecular-weight heparins) and vitamin K antagonists (VKAs; e.g., warfarin) have proved to be effective in the prevention and treatment of these thrombotic diseases, but considerable shortcomings (e.g., inconvenient drug administration and unneglectable side effects for heparins and extensive drug and food interactions for VKAs) restrict their clinical use.[4] Thus, identifying novel oral anticoagulants with improved efficacy and safety has become increasingly important.

After extensive studies, the discovery and development of inhibitors that selectively target specific enzymes within the blood coagulation cascade has attracted great interest. Factor Xa and thrombin (FIIa) are particularly promising targets. FXa is located at the junction of the intrinsic and extrinsic pathways of coagulation and catalyzes the conversion of prothrombin to thrombin. Being different from thrombin inhibitors, selective FXa inhibitors exhibit their antithrombotic effects by reducing the further generation of thrombin while exerting less influence on existing thrombin levels, which may result in a decreased risk of bleeding and provide a much more favorable safety profile.[5]

Recently, three novel oral FXa inhibitors (Figure 1) have been approved: rivaroxaban[6−8] (**1**, Bayer) and apixaban[9,10] (**2**, Bristol-Myers Squibb/Pfizer) by the U.S. Food and Drug

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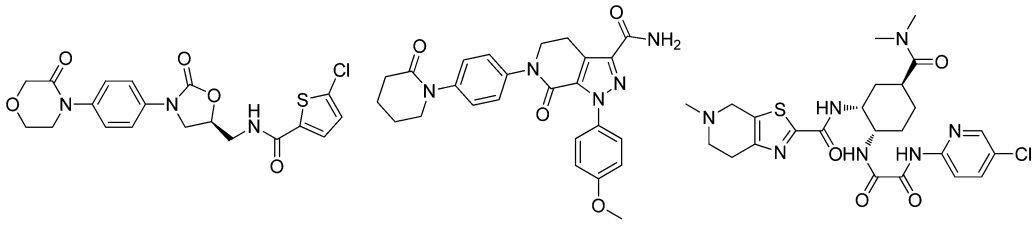

Figure 1. Currently approved FXa inhibitors.

Administration (FDA) and edoxaban[11] (**3**, Daiichi-Sankyo) in Japan. In July 2011, rivaroxaban was the first FXa inhibitor to be approved by FDA for prophylaxis of DVT in adults undergoing hip and knee replacement surgery. Subsequently, in November 2011, new indications for reducing the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation were approved by the FDA.

The X-ray crystal structure of rivaroxaban in complex with human FXa demonstrated that the formation of two hydrogen bonds between rivaroxaban and Gly219 has a crucial role in its high affinity.[6,12] Additionally, the co-planar stereo conformation between the aryl ring and oxazolidinone core also has an indispensable effect on maintaining its high binding affinity. If the co-planar conformation is disturbed into an unfavorable twist arrangement by introducing a substitution at the 2-position of the aryl ring, then the activity decreases sharply.[6] In light of the findings noted above, we bridged the benzene ring and oxazolidinone ring by an additional linker to form a novel [6,6,5] tricyclic fused oxazolidinone scaffold to maintain this preferred conformation (Figure 2). With the intention of

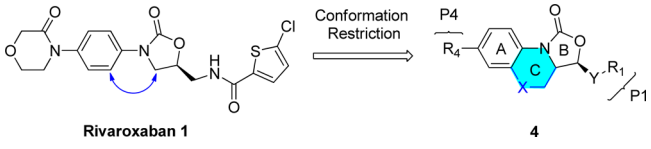

Figure 2. Design of [6,6,5] tricyclic fused oxazolidinones.

ascertaining if this new scaffold is a favorable framework for FXa inhibitors, comprehensive structure−activity relationship (SAR) studies were carried out. Aside from the traditional P1 and P4 surrogate optimizations, we were interested in modification of the bridge linkers in the C ring, which provides an opportunity to increase the activity and improve PK properties. Herein, we describe the design, synthesis, and SAR and structure−pharmacokinetic (SPR) studies of novel [6,6,5] tricyclic fused oxazolidinone FXa inhibitors, which resulted in the discovery of a promising anticoagulant candidate: **11a**.

CHEMISTRY

The synthesis of the nonaromatic P4 moiety-modified benzoxazinyl-oxazolidinones **11a−g**, **11k−n**, and **11o−u** is illustrated in Scheme 1. Key intermediate **5** was prepared using published procedures with minor modifications.[13] A Buchwald−Hartwig coupling reaction between compound **5** and a series of amide analogues yielded compounds **6a**, **6b**, and **6d−j**. Because of the ineffectiveness of direct coupling of *N*-methylacetamide with **5**, acetamide was used, and then *N*-methylation was undertaken to give the desired product **6c**. Cleavage of the *O*-TBS protective group gave products **7a−j**. Alcohol intermediates **7a−j** were converted to their mesylates and then reacted with potassium phthalimide to obtain compounds **9a−j**. Ammonolysis of the resulting compounds **9a−j** using methylamine in ethanol afforded amines **10a−j**. A condensation reaction was carried out between **10a−j** and 5-chlorothiophene-2-carboxylic acid to yield compounds **11a−j**. TBDPS deprotection of **11h** and **11i** using tetrabutylammonium fluoride (TBAF) gave alcohols **11k** and **11m**, which were then converted to their corresponding fluorides **11l** and **11n** using DAST as the fluorination reagent. Deprotection of the Cbz group in **11j** provided **11o**. Subsequent reductive amination with formaldehyde and acetaldehyde afforded **11p** and **11q**, respectively. The benzyl analogue **11r** was obtained by a nucleophilic substitution reaction between **11o** and benzyl bromide. **11o** was reacted with acetylchloride, mesyl chloride, and benzenesulfonyl chloride to yield the corresponding compounds **11s**, **11t**, and **11u**, respectively.

The synthetic route for the preparation of compound **18**, the diastereomer of **11a**, is depicted in Scheme 2, which is similar to that described in Scheme 1. The starting material **12** was obtained via published procedures with slight modifications.[14]

The synthesis of aromatic P4 moiety-modified benzoxazinyl-oxazolidinones is outlined in Scheme 3. The first five steps in this procedure for the synthesis of compound **23** are similar to those in Scheme 1. Next, the Miyaura coupling reaction between compound **23** and bis(pinacolato)diboron yielded boric ester **24**, which was then converted to biaryl compounds **25a** and **25b** by the Suzuki coupling reaction. Cleavage of the *N*-*tert*-butyl group in compound **25b** using trifluoroacetic acid (TFA) afforded **25c**.

Preparation of P1 moiety-modified benzoxazinyl-oxazolidinones is depicted in Scheme 4. Compounds **26a−l** or compounds **28a−c** were assembled by a condensation reaction between intermediate **10a** and various carboxylic acids or *N*-substituted oxamic acids. **10a** was reacted with different isocyanates to afford compounds **27a** and **27b**. Dess−Martin oxidation of intermediate **7a** provided the corresponding aldehyde, which, without further purification, was oxidized to carboxylic acid **29** by $NaClO_2/H_2O_2$. Compound **29** was then converted to the reversed amide compounds **30a** and **30b** via a condensation reaction.

As shown in Scheme 5, the tricyclic S-containing key intermediate **41** was synthesized through a convergent process. Nucleophilic substitution of 4-bromo-2-fluoro-1-nitrobenzene **31** with *tert*-butyl mercaptan afforded compound **32**. Cleavage of the *t*-butyl group by TFA and then reduction of the nitro group using hydrazine hydrate/$FeCl_3$ in MeOH provided disulphide **34**. Given the discrepant influence of different substitutions in *cis*-2-butene-1,4-diol on the ee value of the Sharpless asymmetric epoxidation reaction, trityl was chosen as the protective group with an ee value ≥96% for compound **37**. Compound **37** was then converted to its corresponding

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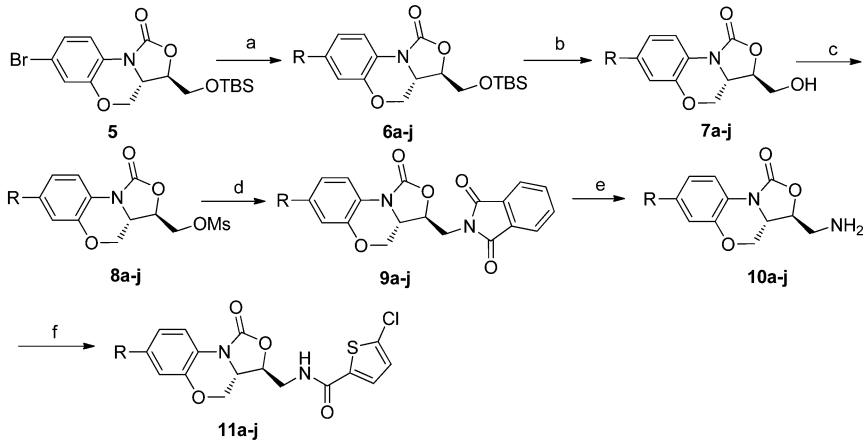
Scheme 1. Synthesis of Nonaromatic P4 Moiety-Modified Benzoxazinyl-oxazolidinones[a]



6-10	R
a	morpholin-3-one-4-yl
b	piperidin-2-one-1-yl
c	N-methyl-acetamido
d	oxazolidin-2-one-3-yl
e	1,4-dioxa-8-azaspiro[4.5]decan-7-one-8-yl
f	(4*R*)-4-methoxypiperidin-2-one-1-yl
g	(4*S*)-4-methoxypiperidin-2-one-1-yl
h	(4*R*)-4-TBDPSO-piperidin-2-one-1-yl
i	(4*S*)-4-TBDPSO-piperidin-2-one-1-yl
j	4-Cbz-2-oxopiperazine-1-yl

11	R
a	morpholin-3-one-4-yl
b	piperidin-2-one-1-yl
c	N-methyl-acetamido
d	oxazolidin-2-one-3-yl
e	1,4-dioxa-8-azaspiro[4.5]decan-7-one-8-yl
f	(4*R*)-4-methoxypiperidin-2-one-1-yl
g	(4*S*)-4-methoxypiperidin-2-one-1-yl
h	(4*R*)-4-TBDPSO-piperidin-2-one-1-yl
i	(4*S*)-4-TBDPSO-piperidin-2-one-1-yl
k	(4*R*)-4-hydroxypiperidin-2-one-1-yl
l	(4*S*)-4-fluoropiperidin-2-one-1-yl
m	(4*S*)-4-hydroxypiperidin-2-one-1-yl
n	(4*R*)-4-fluoropiperidin-2-one-1-yl
j	4-Cbz-piperazin-2-one-1-yl
o	piperazin-2-one-1-yl
p	4-methyl-piperazin-2-one-1-yl
q	4-ethty-piperazin-2-one-1-yl
r	4-benzyl-piperazin-2-one-1-yl
s	4-acetyl-piperazin-2-one-1-yl
t	4-mesyl-piperazin-2-one-1-yl
u	4-phenylsulfonyl-piperazin-2-one-1-yl

[a]Reagents and conditions: (a) for **6c**: (i) acetamide, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, reflux, 72.8%; (ii) CH_3I, NaH, THF, 0 °C to room temp, 86.8%; for **6a**, **6b**, and **6d**–**j**: amides, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, reflux, 51.7–76.2%; (b) TBAF, THF, 0 °C to room temp, 74.2–97.6%; (c) MsCl, Et_3N, 0 °C to room temp, 82.3–95.9%; (d) potassium phthalimide, DMF, 80 °C, 63.8–87.8%; (e) $MeNH_2$, EtOH, reflux; (f) 5-chlorothiophene-2-carboxylic acid, HATU, Et_3N, DMF, 0 °C to room temp, 61.7–84.2% (for two steps); (g) TBAF, THF, 0 °C to room temp, 83.9% for **11k** and 86.0% for **11m**; (h) DAST, CH_2Cl_2, 0 °C to room temp, 79.7% for **11l** and 85.6% for **11n**; (i) $BF_3{\cdot}Et_2O$, Me_2S, CH_2Cl_2, 0 °C to room temp, 82.5%; (j) aldehyde, $NaBH(OAc)_3$, MeOH, 0 °C to room temp, 69.7% for **11p** and 61.3% for **11q**; (k) benzyl bromide, Et_3N, DMF, 0 °C to room temp, 78.1%; (l) acetyl chloride or sulfonyl chloride, Et_3N, DMF, 0 °C to room temp, 61.9% for **11s**, 85.6% for **11t**, and 57.6% for **11u**.

mesylate **38**. Cleavage of the disulfide bond by 1,4-dithiothreitol (DTT)[15] and in situ alkylation with mesylate **38** afforded epoxide **39**, which was protected further by Cbz to yield compound **40**. Next, treatment of compound **40** with ^{n}BuLi afforded the desired intermediate **41**.

The synthetic strategy toward compounds **47a**–**c**, **48**, **49a**, and **49b** is summarized in Scheme 6. A Buchwald–Hartwig coupling reaction between intermediate **41** and 3-morpholinone or 2-piperidinone yielded **42a** or **42b**, respectively. Deprotection of the trityl group with TFA transformed compounds **42a** and **42b** into alcohol **43a** and **43b**, respectively. The subsequent four steps in this procedure for the synthesis of **47a**–**c** are similar to those depicted in Scheme 1. Finally, oxidation of the S-containing analogues **47a** and **47b** proceeded smoothly upon exposure to 1 or 2 equiv of *m*CPBA to give the corresponding sulfoxide **48** and sulphones **49a** and **49b**.

The preparation route for compounds **56a** and **56b** is outlined in Scheme 7, and the reaction conditions were almost the same as those used in Schemes 3 and 6.

Preparation of compounds **72a** and **72b** is outlined in Scheme 8. Nucleophilic substitution of 4-bromo-2-fluoro-1-nitrobenzene **31** with diethyl malonate followed by ester hydrolyzation and then decarboxylation provided the single ester **58**. Compound **59**[16] was reacted with **58** to construct intermediate **60**, which was subjected to hydrolyzation and decarboxylation to yield compound **61**. Reduction of the nitro group and then Cbz protection of the unpurified aniline followed by *O*-TBS deprotection gave alcohol **63**. A Sharpless asymmetric epoxidation reaction was carried out to convert **63** into epoxide **64**, and then the hydroxyl group was protected with TBSCl to yield compound **65**. Next, treatment of compound **65** with ^{n}BuLi afforded the key tricyclic intermediate **66**. The subsequent several steps for the synthesis of compounds **72a** and **72b** were similar to the procedures described in Scheme 1.

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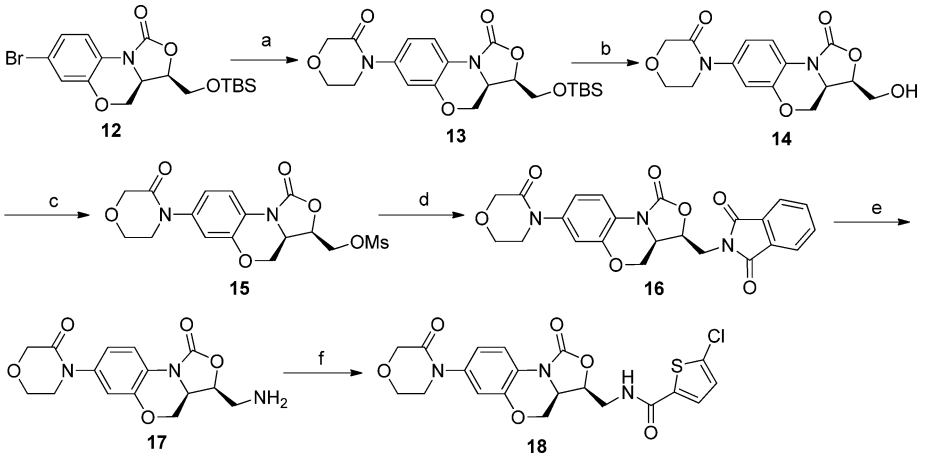
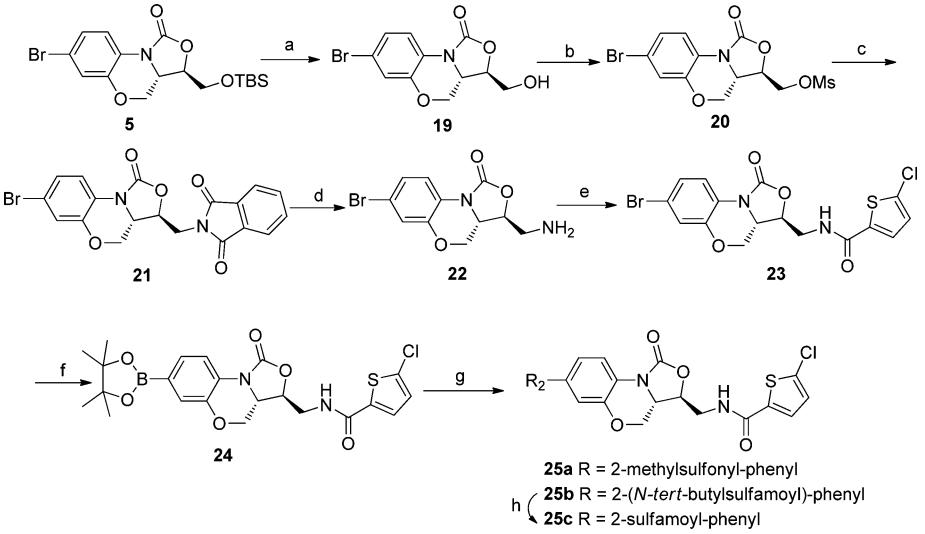
Scheme 2. Synthesis of Compound 18[a]



[a]Reagents and conditions: (a) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, reflux, 73.1%; (b) TBAF, THF, 0 °C to room temp, 92.5%; (c) MsCl, Et_3N, 0 °C to room temp, 97.6%; (d) potassium phthalimide, DMF, 80 °C, 83.1%; (e) $MeNH_2$, EtOH, reflux, 85.4%; (f) 5-chlorothiophene-2-carboxylic acid, HATU, Et_3N, DMF, 0 °C to room temp, 82.4%.

Scheme 3. Synthesis of Aromatic P4 Moiety-Modified Benzoxazinyl-oxazolidinones[a]



[a]Reagents and conditions: (a) TBAF, THF, 0 °C to room temp, 95.3%; (b) MsCl, Et_3N, 0 °C to room temp, 94.4%; (c) potassium phthalimide, DMF, 80 °C, 81.5%; (d) $MeNH_2$, EtOH, reflux; (e) 5-chlorothiophene-2-carboxylic acid, HATU, Et_3N, DMF, 0 °C to room temp, 61.3% (for two steps); (f) bis(pinacolato)diboron, Pd(dppf)Cl_2·CH_2Cl_2, KOAc, DMSO, 80 °C, 38.6%; (g) aryl bromide, $Pd(PPh_3)_4$, Cs_2CO_3, dioxane/H_2O, 80 °C, 35.9% for **25a** and 35.5% for **25b**; (h) TFA, 40 °C, 84.0%.

The synthesis of compounds **85a** and **85b** was carried out according to Scheme 9. Nucleophilic substitution between 4-bromo-2-fluoro-1-nitrobenzene **31** and intermediate **73**[17] yielded compound **74**. Boc-protection of **74** followed by epoxidation with *m*CPBA as the oxidant gave epoxide **76**. A tandem reduction/ring-opening reaction was adopted to provide bicyclic product **77**. Cyclization of **77** yielded the key intermediate **78**. The following six steps employed for the synthesis of **84a** and **84b** were similar to those depicted in Scheme 1. Finally, Boc-deprotection of compounds **84a** and **84b** using TFA afforded the target compounds **85a** and **85b**, respectively.

RESULTS AND DISCUSSION

In Vitro FXa Inhibitory Activity and Selectivity. Inspired by the observation of the X-ray crystal structure of rivaroxaban in complex with human FXa in which the benzene ring and oxazolidinone core are in a coplanar conformation, we hypothesized that maintaining this preferred conformation by introducing a C ring may enforce its good inhibitory activity against FXa. Moreover, the additional fused C ring would reduce the number of rotatable bonds and increase the rigidity of the molecule, which would probably improve the PK/PD profiles of these molecules.[18−20] As an early exploration, **11a** and its diastereomer **18** were prepared to validate our tentative idea and concurrently to probe the impact of chirality on FXa inhibitory activity. To our delight, as shown in Table 1, (3*S*,3a*S*) compound **11a** exhibited activity superior to that of rivaroxaban, whereas the (3*S*,3a*R*) diastereomer compound **18** displayed activity that was almost 20-fold less potent than that seen for **11a**. This result confirmed our hypothesis and indicated that the (3*S*,3a*S*) absolute configuration was essential for good in vitro potency for these new tricyclic FXa inhibitors.

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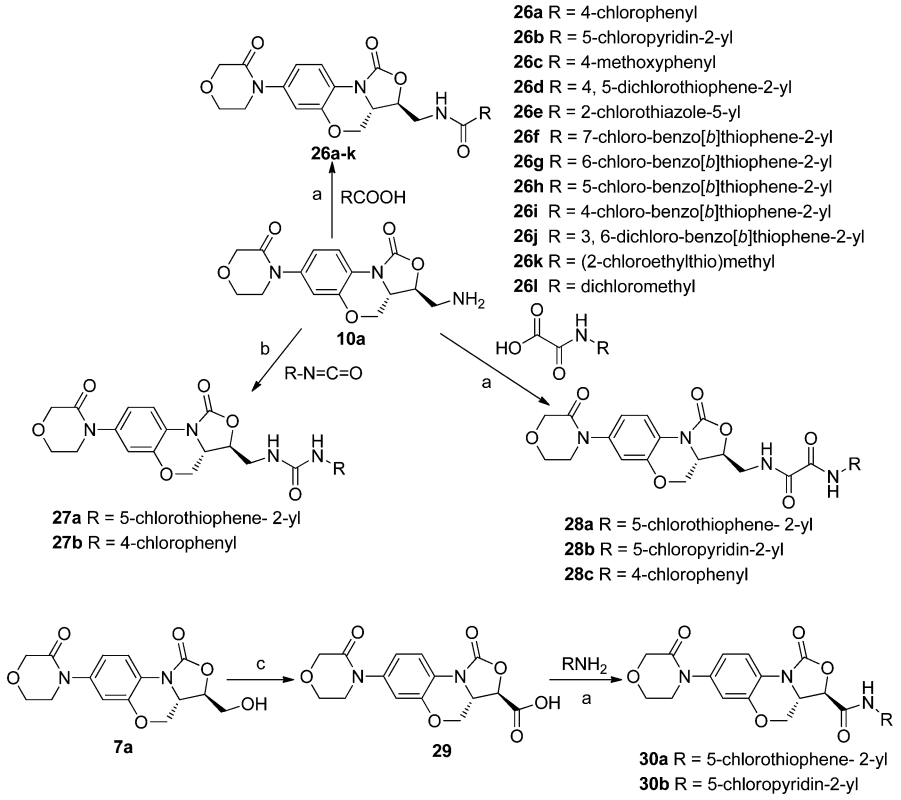
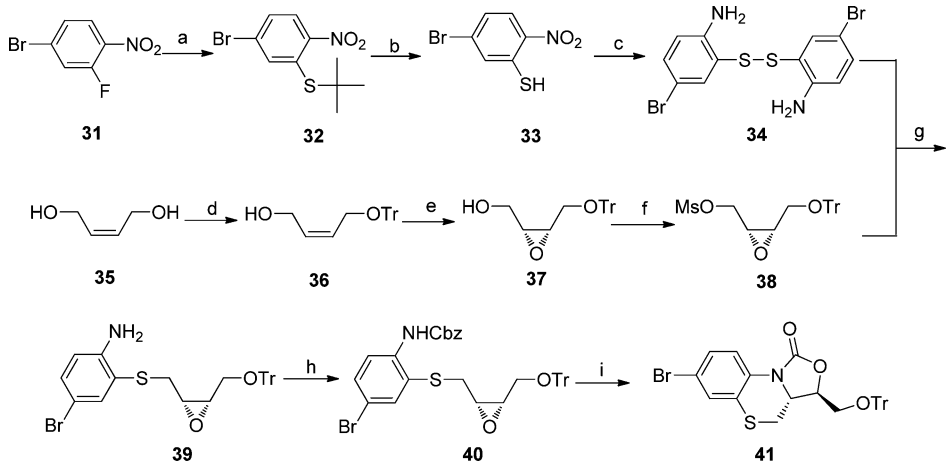
Scheme 4. Synthesis of P1 Moiety-Modified Benzoxazinyl-oxazolidinones[a]



[a]Reagents and conditions: (a) HATU, Et_3N, DMF, 0 °C to room temp, 71.4−85.9% for **26a−l**, 72.2−80.6% for **28a−c**, 60.3% for **30a**, and 52.6% for **30b**; (b) Et_3N, DMF, room temp, 59.2% for **27a** and 84.4% for **27b**; (c) (i) DMP, DMSO, 0 °C to room temp; (ii) $NaClO_2$, H_2O_2, NaH_2PO_4, CH_3CN/H_2O, room temp, 42.9% for two steps.

Scheme 5. Synthesis of S-Containing Key Intermediate 41[a]



[a]Reagents and conditions: (a) *t*-butyl mercaptan, Cs_2CO_3, DMF, room temp, 94.2%; (b) TFA, CH_2Cl_2, room temp, 92.3%; (c) hydrazine hydrate, $FeCl_3$, MeOH, room temp, 87.3%; (d) trityl chloride, TEA, DMAP, CH_2Cl_2, room temp, 61.4%; (e) D-(−)DET, $Ti(O^iPr)_4$, TBHP, 4 Å molecular sieves, extra dry CH_2Cl_2, −25 °C, 80.6%; (f) MsCl, TEA, THF, 0 °C to room temp, 97.9%; (g) DTT, THF, DMF, room temp, 60.7%; (h) CbzCl, $NaHCO_3$, THF/H_2O = 2:1, 0 °C to room temp, 68.7%; (i) ^{n}BuLi, THF, −78 °C to room temp, 75.9%.

As a result of the encouraging outcomes outlined above, a systematic and comprehensive investigation was carried out to explore the optimal P1 and P4 substituents in our new tricyclic scaffold. Initially, replacement of the morpholinone ring of the P4 group with various lactam-like moieties was explored. All analogues showed decreased in vitro FXa inhibitory activity compared with that of **11a** (Table 2). Exchanging the morpholinone to piperidinone resulted in a 6-fold loss of potency (**11b** vs **11a**), whereas ring-opening derivative **11c** lost potency by about 25-fold. Hydroxylation at position 2 of the morpholinone ring in rivaroxaban is the main metabolic pathway in humans,[21] so compounds that could remove this readily metabolized carbon atom were designed and assessed. Unfortunately, this resulted in a significant loss of potency (**11d**

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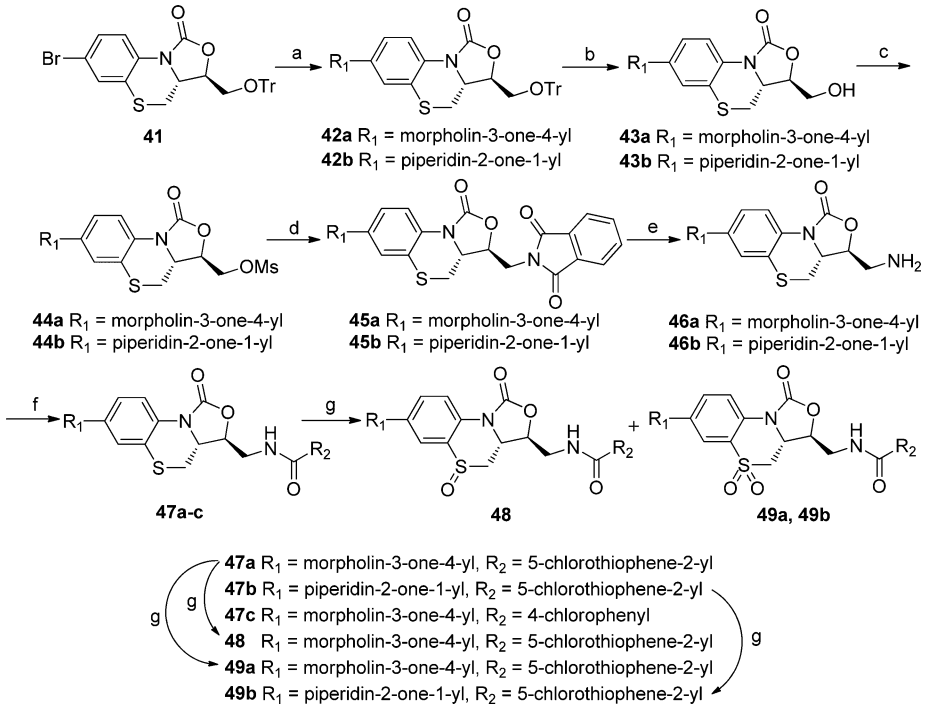
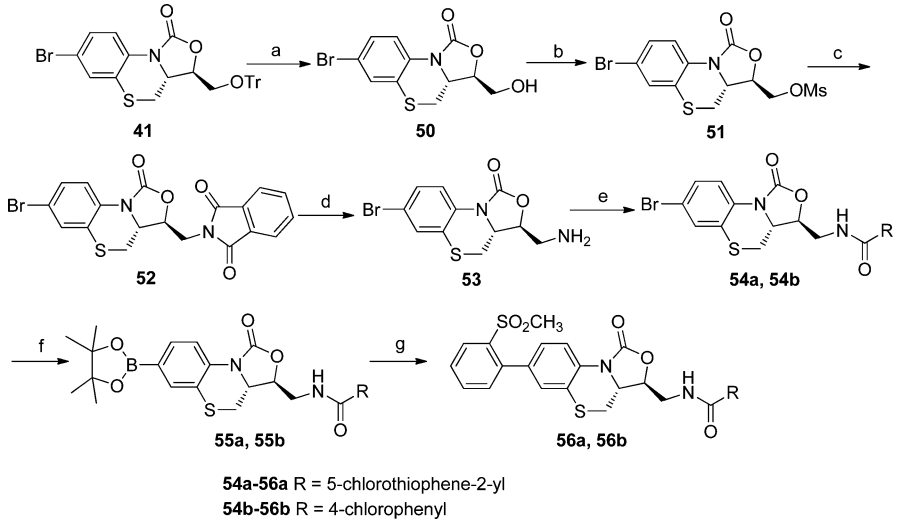
Scheme 6. Synthesis of S-Containing Compounds 47a–c, 48, 49a, and 49b[a]



[a]Reagents and conditions: (a) 3-morpholinone or 2-piperidinone, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, reflux, 66.6% for **42a** and 44.5% for **42b**; (b) TFA, CH_2Cl_2, room temp, 89.8% for **43a** and 62.6% for **43b**; (c) MsCl, Et_3N, 0 °C to room temp, 96.2% for **44a** and 94.6% for **44b**; (d) potassium phthalimide, DMF, 80 °C, 89.0% for **45a** and 93.0% for **45b**; (e) $MeNH_2$, EtOH, reflux, 83.3% for **46a** and 74.5% for **46b**; (f) carboxylic acid, HATU, Et_3N, DMF, 0 °C to room temp, 86.6% for **47a**, 82.3% for **47b** and 89.2% for **47c**; (g) *m*CPBA (1 equiv for **48** and 2 equiv for **49a** and **49b**), THF, 0 °C, 91.9% for **48**, 96.9% for **49a** and 93.7% for **49b**.

Scheme 7. Synthesis of S-Containing Compounds 56a and 56b[a]



[a]Reagents and conditions: (a) TFA, CH_2Cl_2, room temp, 87.1%; (b) MsCl, Et_3N, 0 °C to room temp, 96.4%; (c) potassium phthalimide, DMF, 80 °C, 90.4%; (d) $MeNH_2$, EtOH, reflux, 89.8%; (e) carboxylic acid, HATU, Et_3N, DMF, 0 °C to room temp, 62.5% for **54a**, 77.9% for **54b**; (f) bis(pinacolato)diboron, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, KOAc, DMSO, 80 °C, 68.0% for **55a**, 79.9% for **55b**; (g) 2-bromo(methylsulfonyl)benzene, $Pd(PPh_3)_4$, Cs_2CO_3, dioxane/H_2O, 80 °C, 66.3% for **56a**, 71.0% for **56b**.

vs **11a**). When an additional substituent at position 4 of the piperidinone ring was introduced, a further loss of activity was observed (**11e–g** and **11k–n** vs **11b**). The effect of the absolute configuration at C4 was also explored. In general, compounds with the *R*-configuration exhibited greater activity than that of their *S*-configuration counterparts (**11f** vs **11g**, **11k** vs **11m**, and **11n** vs **11l**). Most of the unsubstituted or *N*-substituted piperazinone derivatives (**11o–u**) showed similar very low activity to that of piperidinone derivatives except the *N*-acetyl-substituted piperazinone derivative **11s**, which displayed moderate potency. It is well-demonstrated that three aromatic amino-acid residues (Phe178, Trp215, and Tyr99)

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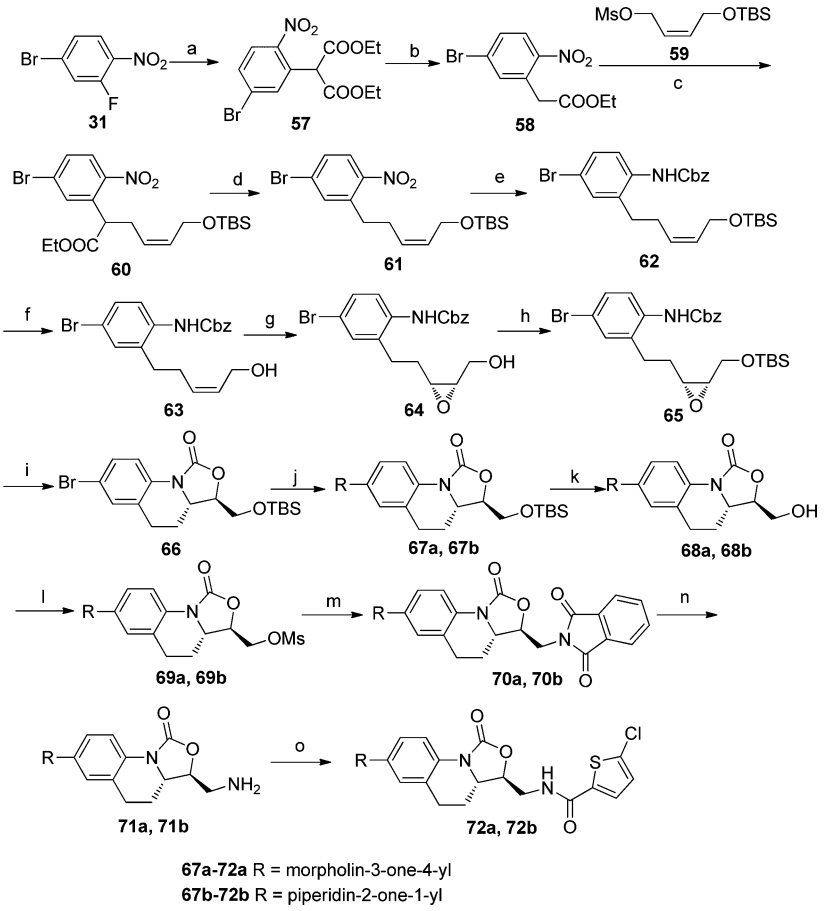
Scheme 8. Synthesis of C-Containing Compounds 72a and 72b[a]



[a]Reagents and conditions: (a) K_2CO_3, DMF, 45 °C, 67.2%; (b) LiCl, DMSO/H_2O = 10:1, 110 °C, 82.5%; (c) Cs_2CO_3, DMF, 60 °C, 70.1%; (d) (i) KOH, MeOH/H_2O = 4:1, room temp, (ii) K_2CO_3, DMF, 60 °C, 87.0% for two steps; (e) (i) Zn powder, NH_4Cl, THF, 0 °C to room temp, (ii) CbzCl, $NaHCO_3$, THF/H_2O = 2:1, 0 °C to room temp, 66.7%; (f) TBAF, THF, room temp, 89.3%; (g) L-(+)DET, $Ti(O^iPr)_4$, TBHP, 4 Å molecular sieves, extra-dry CH_2Cl_2, −25 °C, 83.2%; (h) TBSCl, imidazole, DMF, room temp, 93.1%; (i) ^{n}BuLi, THF, −78 °C to room temp, 78.2%; (j) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, reflux, 63.9% for **67a**, 52.7% for **67b**; (k) TBAF, THF, 0 °C to room temp, 94.7% for **68a**, 98.3% for **68b**; (l) MsCl, Et_3N, 0 °C to room temp, 82.1% for **69a**, 98.7% for **69b**; (m) potassium phthalimide, DMF, 80 °C, 83.9% for **70a**, 84.9% for **70b**; (n) $MeNH_2$, EtOH, reflux, 95.8% for **71a**, 76.8% for **71b**; (o) 5-chlorothiophene-2-carboxylic acid, HATU, Et_3N, DMF, 0 °C to room temp, 80.6% for **72a**, 82.3% for **72b**.

endow the S4 pocket of FXa with an aromatic environment, which would preferably accommodate an aromatic ligand by face-to-face or edge-to-face π interactions.[6,22,23] Thus, analogues featuring a P4 aromatic ring were evaluated. Unfortunately, neither the phenylsulfone derivative **25a** nor the benzsulfamide derivative **25c** retained the excellent in vitro activity of **11a**. According to the investigation stated above, morpholinone was found to be the optimal P4 pharmacophore for our new tricyclic scaffold.

Further modifications were undertaken to explore favorable P1 surrogates with morpholinone as the optimal P4 substituent, but all efforts were unsuccessful (Table 3). Minor changes in the molecular structure gave rise to a considerable loss of potency. For example, closely related structures 4-chlorophenyl derivative **26a** and 3-chloropyridyl derivative **26b** were 47- and 200-fold less potent than that of **11a**, respectively. Replacement of the chlorine atom in **26a** with a methoxyl group gave compound **26c**, which exhibited a ≈30-fold loss of activity compared with that of **26a**. This finding suggested that the Cl−π interaction in the S1 pocket is essential for activity. Accordingly, the second chlorine was introduced at the 4-position of the thiophene ring. However, this additional chlorine caused a significant loss of activity (**26d** vs **11a**). Derivative **26e** with 2-chlorothiazole as the P1 surrogate also showed a sharply decreased FXa inhibitory activity compared with that of **11a**. To fine tune the spatial distance between Cl and Tyr228,[24] benzothiophene derivatives (**26f−j**) with one or two chlorine atoms attached at different positions were designed. Among compounds in this benzothiophene series, only derivative **26g**, which showed a similar orientation of the chlorine like that in the chlorothiophene in **11a**, exhibited relatively higher potency, but it remained 500-fold less potent than that of **11a**. For derivatives bearing noncyclic side chains, activity was almost completely lost (**26k** and **26l**).

Modifications on the amide linker region were also undertaken. It is well-established that the direct hydrogen-bond interaction between Gly219 and the NH group of the amide in rivaroxaban has a critical role in strong FXa inhibitory potency.[6] To mimic this interaction, urea, oxamide, and reversed amide groups were introduced into the molecule as a linker to find a more potent FXa inhibitor (Table 4). Compared with amide derivatives, a loss of potency to varying extents was observed for the urea, oxamide, and reversed amide derivatives. Interestingly, unlike the amide series, the urea and

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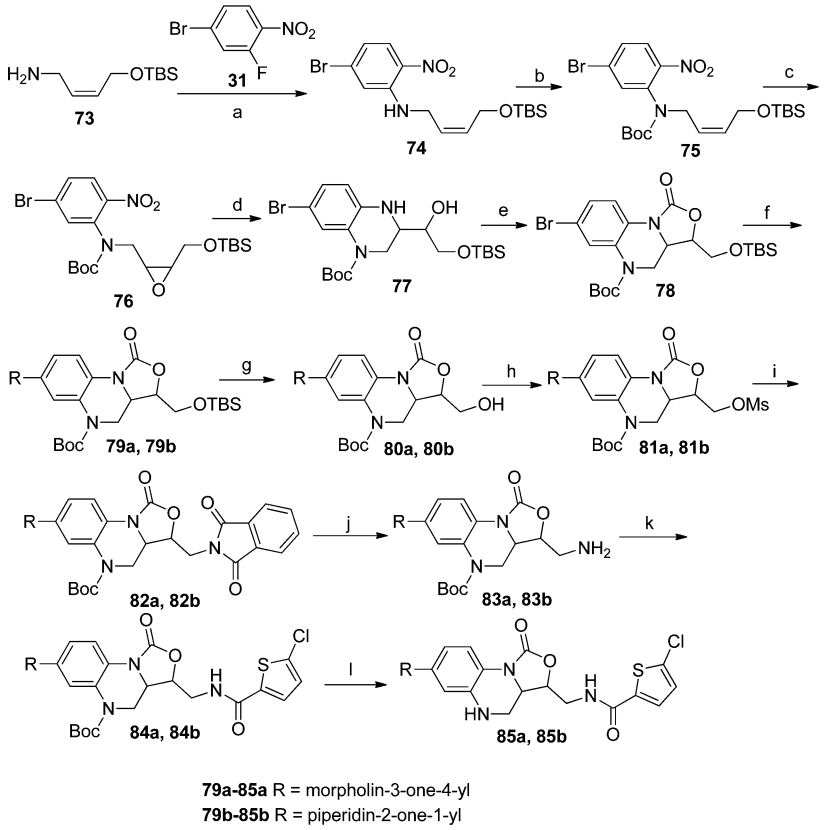
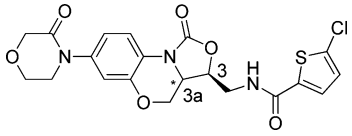
Scheme 9. Synthesis of N-Containing Compounds 85a and 85b[a]



[a]Reagents and conditions: (a) Na_2CO_3, DMF, room temp, 76.7%; (b) $(Boc)_2O$, K_2CO_3, DMF, 50 °C, 60.6%; (c) *m*CPBA, DCM, room temp, 75.6%; (d) hydrazine hydrate, $FeCl_3$, MeOH, 50 °C, 32.9%; (e) CDI, DMAP, DMF, 100 °C, 84.6%; (f) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, reflux, 58.6% for **79a**, 67.6% for **79b**; (g) TBAF, THF, 0 °C to room temp, 98.5% for **80a**, 98.2% for **80b**; (h) MsCl, Et_3N, 0 °C to room temp, 91.3% for **81a**, 95.6% for **81b**; (i) potassium phthalimide, DMF, 80 °C, 80.2% for **82a**, 82.2% for **82b**; (j) $MeNH_2$, EtOH, reflux, 88.7% for **83a**, 91.1% for **83b**; (k) 5-chlorothiophene-2-carboxylic acid, HATU, Et_3N, DMF, 0 °C to room temp, 87.5% for **84a**, 83.9% for **84b**; (l) TFA, DCM, room temp, 82.6% for **85a**, 95.3% for **85b**.

Table 1. In Vitro FXa Inhibitory Activity of Chiral Center-Changed Compounds 11a and 18



compd	ent	IC_{50} (nM)
11a	3a*S*	3.41 ± 0.39
18	3a*R*	57.81 ± 2.62
rivaroxaban (**1**)		8.52 ± 0.21

oxamide series exhibited a different SAR at P1 position. As shown in Table 4, chlorophenyl derivatives were more potent than their corresponding chlorothiophene derivatives (**27b** vs **27a** and **28c** vs **28a**). For the reversed amide series, FXa inhibitory activity was lost completely (**30a** and **30b**).

Further modification of the bridge linker in the C ring was explored to evaluate its influence on in vitro FXa inhibitory activity. Single-enantiomer analogues were accessed according to different asymmetric synthetic methods except for the N-containing series with respect to rapid preparation. Different bridge linker analogues (**47a**, **72a**, and **85a**) of compound **11a** exhibited excellent potency, with the order being S > O > C > N (Table 5). Upon replacement of the R_4 substituent morpholinone with piperidinone, a greater or lesser decrease of inhibition activity was observed for all series (**11b** vs **11a**, **47b** vs **47a**, **72b** vs **72a**, and **85b** vs **85a**). This result indicated a consistent SAR trend in the R_4 group for these four series of new tricyclic compounds, which would simplify further optimization on other positions. Given the possibility of oxidation of the S atom in **47a** to the sulfoxide or sulphone in vivo, its corresponding sulfoxide **48** and sulphone **49a** derivatives were prepared, respectively. Unlike the good tolerance seen for replacement of O, S, C, and N for retaining excellent in vitro activity, the sulfoxide **48** and sulphone **49a** displayed a 25- and 7-fold loss in potency compared with that of their parent compound **47a**, respectively. Similarly, the *N*-Boc derivative **84b** showed significantly decreased activity compared with that of **85b**. Due to **47a** having the highest in vitro activity, S-containing analogues were investigated further by optimization of P1 and P4 substituents. Unfortunately, compared with **47a**, 18- or 8-fold decreased potency was observed for the 4-chlorophenyl derivative **47c** or phenylsulfone derivative **56a**, respectively. A further decrease was displayed if the two substituents were combined in one molecule, such as **56b**.

As a result of their high potency against FXa, **11a**, **47a**, **72a**, and **85a** were selected to investigate their selectivity versus other related serine proteases. Differing from their excellent FXa inhibitory potency, all test compounds showed no

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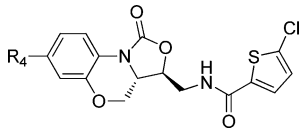
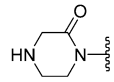
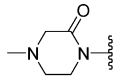
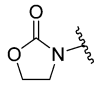
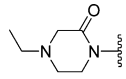
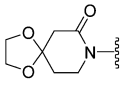
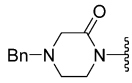
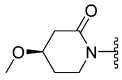
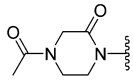
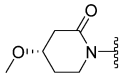
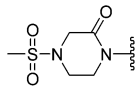
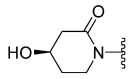
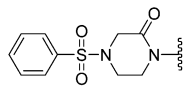
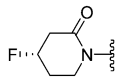
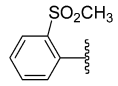
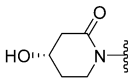
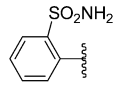
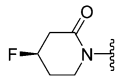
Table 2. In Vitro FXa Inhibitory Activity of P4-Substituted Derivatives



compd	R_4	IC_{50} (nM)	compd	R_4	IC_{50} (nM)
11b		20.22 ± 15.33	**11o**		190 ± 20
11c		87.17 ± 33.46	**11p**		2210 ± 1030
11d		730 ± 40	**11q**		15770 ± 3730
11e		2510 ± 330	**11r**		11360 ± 8730
11f		590 ± 30	**11s**		74.77 ± 25.31
11g		1860 ± 50	**11t**		4180 ± 2290
11k		130 ± 40	**11u**		5530 ± 4550
11l		46.64 ± 9.19	**25a**		37.65 ± 4.08
11m		1220 ± 120	**25c**		60.56 ± 16.93
11n		40.56 ± 4.34			

inhibitory activity against other related serine proteases (IC_{50} > 100 μM), with selectivity being more than 20 000-fold.

In Vitro Anticoagulant Activity. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were evaluated for selected compounds in rabbit plasma to determine their in vitro anticoagulant activity. PT measures the effect of a compound on the extrinsic pathway of coagulation, whereas aPTT represents the effect on the intrinsic pathway. Anticoagulant activity was defined as the concentration at which the plasma clotting time (CT_2) was doubled (Table 6).

All test compounds displayed moderate-to-high in vitro anticoagulant activity and showed similar sensitivity to PT and aPTT. For **11a**, PT and aPTT were doubled at 0.18 and 0.14 μM, respectively, which made it the most potent compound in these two assays. With regard to its counterparts, **11b**, **47a**, **47b**, **49a**, **72a**, and **85a** exhibited good anticoagulant activity that was comparable with that of rivaroxaban. By contrast, a higher concentration was required for **25a** and **25c** to double the PT and aPTT, which was in accordance with their relatively lower FXa inhibitory activities compared with that of **11a**.

PK Profile of Selected Compounds. As a result of the excellent in vitro profiles, some compounds were selected to evaluate their PK properties in rats and dogs, with the data for rivaroxaban included for comparison. As summarized in Tables 7 and 8, different bridge linkers in the C ring had a significant influence on the PK properties of compounds after oral administration and intravenous injection. In rats, the O-containing analogue **11a** and C-containing analogue **72a**, which exhibited the best overall PK profiles, were comparable or better than rivaroxaban (especially **11a**). The PK of **11a** in rats was outstanding, with high maximal plasma concentration (C_{max} = 11 901 ng/mL), high plasma exposure ($AUC_{0-\infty}$ = 33 044 ng·h/mL), and better oral bioavailability (F = 63.7%) after

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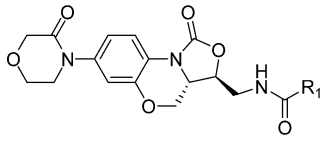
Table 3. In Vitro FXa Inhibitory Activity of P1-Substituted Derivatives



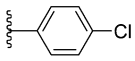
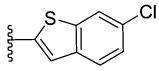
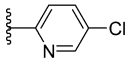
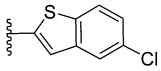
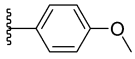
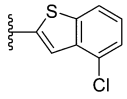
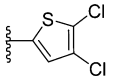
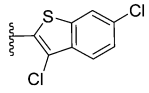
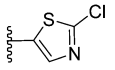
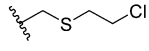
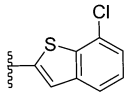
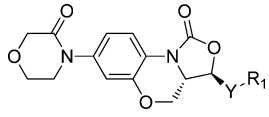
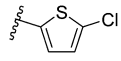
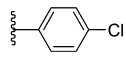
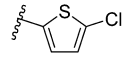
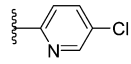
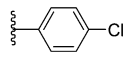
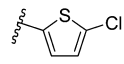
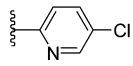
compd	R_1	IC_{50} (nM)	compd	R_1	IC_{50} (nM)
26a		160 ± 30	**26g**		1750 ± 60
26b		710 ± 60	**26h**		25880 ± 1130
26c		4780 ± 1550	**26i**		>100000
26d		8080 ± 730	**26j**		>100000
26e		7060 ± 1030	**26k**		>100000
26f		>100000	**26l**		52690 ± 8870

Table 4. In Vitro FXa Inhibitory Activity of P1 Linker-Modified Derivatives



compd	Y	R_1	IC_{50} (nM)
27a	-CH_2NHCONH-		88.53 ±16.97
27b			32.08 ±10.02
28a	-CH_2NHCOCONH-		560 ± 40
28b			1210 ± 430
28c			310 ± 50
30a	-CONH-		>100000
30b			>100000

oral administration. These values were significantly higher than those observed for rivaroxaban. When administered via the intravenous route, **11a** displayed lower clearance (CL_p = 0.201 L/h/kg) compared with that of rivaroxaban. The P4 position-modified derivatives such as **11b**, **25a**, and **25c** displayed less favorable overall PK properties compared with that of **11a**, including moderate C_{max}, moderate-to-high $AUC_{0-\infty}$, and relatively low oral bioavailability. However, **11b** showed a longer half-life ($T_{1/2}$) compared with that of **11a**. By contrast, other analogues such as **47a**, **49a**, and **85a** with S, SO_2, or NH as the bridge, respectively, showed unfavorable PK properties, such as unacceptable oral bioavailability (F < 8%).

In beagle dogs, almost all aspects of the PK properties of **11a** were superior to those of rivaroxaban (Table 8). Detailed

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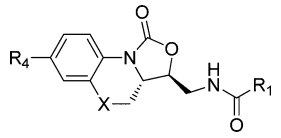
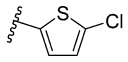
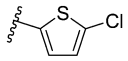
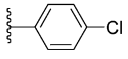
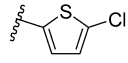
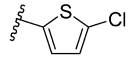
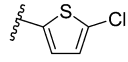
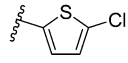
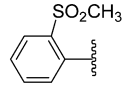
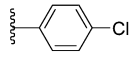
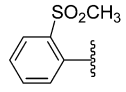
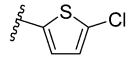
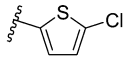
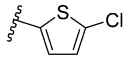
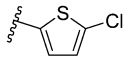
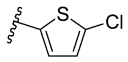
Table 5. In Vitro FXa Inhibitory Activity of Compounds Bearing Different Bridge Linkers in the C Ring



compd	X	R_1	R_4	IC_{50} (nM)
47a	S			2.51 ± 0.51
47b	S			4.89 ± 0.01
47c	S			45.22 ± 11.58
48	SO			63.96 ± 6.82
49a	SO_2			17.37 ± 0.99
49b	SO_2			50.52 ± 2.60
56a	S			20.87 ± 1.06
56b	S			1610 ± 800
72a	C			4.71 ± 2.04
72b	C			6.59 ± 0.36
84b	(Boc)N			8180 ± 857
85a	N			5.06 ± 1.78
85b	N			15.20 ± 6.22

evaluation showed that the C_{max} and $AUC_{0-\infty}$ of **11a** were 1.5-fold higher than those of rivaroxaban irrespective of the routes they were administered. In addition, considerable improvement was observed in terms of oral bioavailability (92% for **11a** vs 68% for rivaroxaban).

In Vivo Antithrombotic Efficacy and Tail-Bleeding Time Effect of 11a in Rats. Through comprehensive analyses of in vitro potency, selectivity, and PK properties, **11a** was selected to evaluate its in vivo antithrombotic efficacy in three models of thrombosis in rats: $FeCl_3$-induced venous thrombosis (VT), arteriovenous shunt (AV-SHUNT), and electrically induced rat carotid artery thrombosis. Additionally, the bleeding risk of **11a** was evaluated in a rat tail-bleeding time study. Rivaroxaban was chosen as the positive control.

As shown in Figure 3, **11a** exhibited strong antithrombotic activity in all three models. In VT and AV-SHUNT models,

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Table 6. In Vitro Anticoagulant Activity of Selected Compounds[a]

compd	PT CT_2 (μM)	aPTT CT_2 (μM)	compd	PT CT_2 (μM)	aPTT CT_2 (μM)
11a	0.18	0.14	**47b**	0.64	0.62
11b	0.42	1.63	**49a**	0.47	0.76
25a	5.88	4.65	**72a**	0.37	0.33
25c	4.58	4.86	**85a**	0.48	0.43
26a	1.06	0.93	rivaroxaban (**1**)	0.40	0.32
47a	0.58	0.33			

[a]PT and aPTT in vitro clotting assays were carried out in rabbit plasma.

Table 7. PK Properties of Selected Compounds in Male Rats[a]

compd	route	dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)	CL_p (L/h/kg)	V_{ss} (L/kg)	F (%)
11a	p.o.	10	11 901	0.75	1.33	33 044			63.7
	i.v.	3			1.0	15 339	0.201	0.201	
11b	p.o.	9	6927	0.67	2.10	28 527			42.0
	i.v.	4.5			1.95	33 562	0.137	0.406	
25a	p.o.	9	6763	1.0	1.43	24 547			27.3
	i.v.	4.5			0.88	43 935	0.103	0.169	
25c	p.o.	10	3626	1.0	1.65	12 277			29.0
	i.v.	3			1.38	12 226	0.253	0.350	
47a	p.o.	10	167	4.0	3.15	1480			7.4
	i.v.	3			0.89	5695	0.529	0.574	
49a	p.o.	10	917	0.38	2.14	1539			7.3
	i.v.	3			0.54	6122	0.493	0.154	
72a	p.o.	10	3020	2.3	3.54	14 200			20.4
	i.v.	3			0.74	20 450	0.149	0.144	
85a	p.o.	10	565	0.44	1.52	1349			6.8
	i.v.	3			0.86	5700	0.553	0.199	
riv (**1**)	p.o.	3	850	0.88	2.90	2264			21.3
	i.v.	3			1.27	9985	0.303	0.190	

[a]Abbreviations: C_{max}, peak plasma concentration of a drug after administration; T_{max}, time to reach C_{max}; $T_{1/2}$, elimination half-life; AUC, area under the concentration−time curve; CL_p, plasma clearance; V_{ss}, volume of distribution at steady state; F, bioavailability; p.o., per oral; i.v., intravenous; riv, rivaroxaban.

Table 8. PK Properties of 11a and Rivaroxaban in Male Beagle Dogs[a]

compd	route	dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)	CL_p (L/h/kg)	V_{ss} (L/kg)	F (%)
11a	p.o.	1	530.0	0.5	2.0	1525.8			92
	i.v.	0.5			3.1	1316.6	0.4	1.7	
riv (**1**)	p.o.	1	332.6	0.7	1.8	1001.6			68
	i.v.	0.5			2.4	915.7	0.6	2.8	

[a]Abbreviations: C_{max}, peak plasma concentration of a drug after administration; T_{max}, time to reach C_{max}; $T_{1/2}$, elimination half-life; AUC, area under the concentration−time curve; CL_p, plasma clearance; V_{ss}, volume of distribution at steady state; F, bioavailability; p.o., per oral; i.v., intravenous; riv, rivaroxaban.

11a and rivaroxaban, which were administered orally 60 min before the induction of thrombosis, reduced thrombus formation in a dose-dependent manner. The in vivo efficacy of **11a** was similar to that of rivaroxaban, with ED_{50} values of 5.63 and 5.28 mg/kg for **11a** compared with 4.86 and 3.72 mg/kg for rivaroxaban, respectively. In the model of arterial thrombosis in which **11a** and rivaroxaban were given via the oral route, a dose-dependent antithrombotic effect against thrombus formation was observed. Moreover, **11a** exhibited superior antithrombotic efficacy compared to that of rivaroxaban, with ED_{50} values of 2.97 and 4.53 mg/kg, respectively. These results suggested that **11a** would be beneficial in the prevention and treatment of thromboembolic diseases in venous and arterial systems.

To evaluate the bleeding risk of compound **11a**, a rat tail-bleeding time study was carried out. **11a** and rivaroxaban were administered via the oral route 60 min before the surgical procedure. As depicted in Figure 4, **11a** and rivaroxaban prolonged the bleeding time in a dose-dependent manner to the same extent. This outcome suggested that **11a** and rivaroxaban have a similar safety profile in terms of bleeding risk.

Liability Profiling. Parameters of liability profiling were tested to evaluate the druggability of **11a**. **11a** showed almost no inhibition of the hERG K^+ channel (IC_{50} = 79.75 μM, patch clamp assay) and various CYP450 isozymes (IC_{50} > 40 μM, summarized in Table 9). In addition, **11a** gave no genetic toxicity in the Ames mutagenicity test and micronucleus formation assay. Additionally, **11a** was administered orally once a day for 2 weeks at medium (50 mg/kg) and high (100 mg/kg) doses to evaluate its subacute toxicity in rats. We found that **11a** was well-tolerated at both doses with no obvious toxic reaction in terms of behavior, body weight, food intake, organ coefficients, and survival rate.

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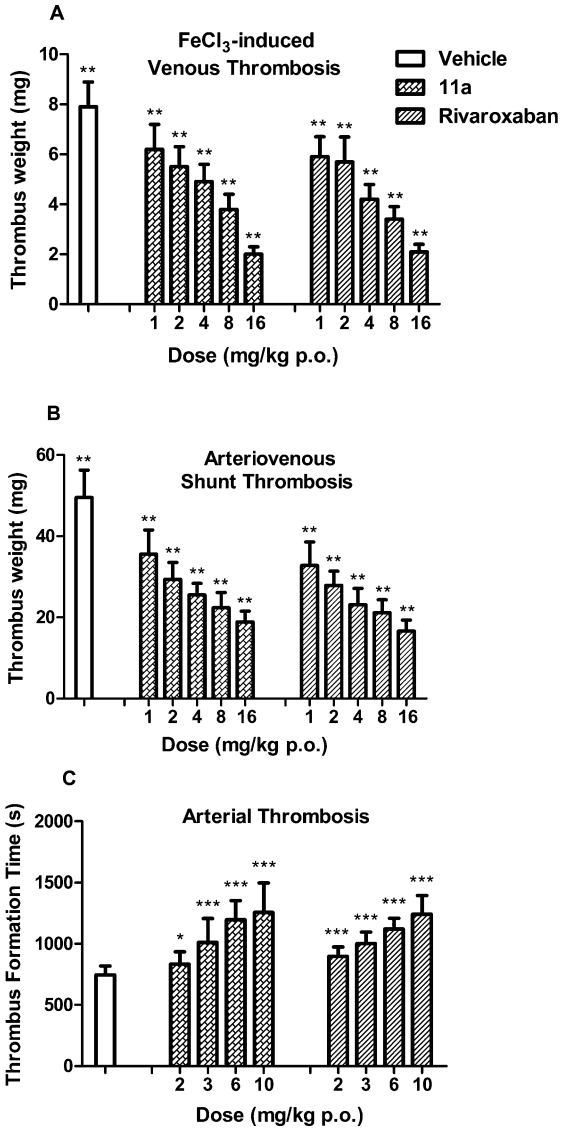

Figure 3. (A) Antithrombotic effect of **11a** and rivaroxaban in a rat $FeCl_3$-induced venous thrombosis model. (B) Antithrombotic effect of **11a** and rivaroxaban in a rat arteriovenous thrombosis model. (C) Antithrombotic effect of **11a** and rivaroxaban in a rat arterial thrombosis model. Average thrombus weight or thrombus formation time are the mean ± SD. Statistical significance compared with the vehicle group is $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

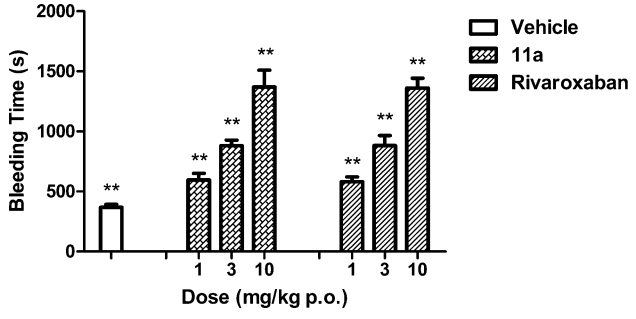

Figure 4. Influence of **11a** and rivaroxaban on rat tail-bleeding time. Results are the mean ± SD. Statistical significance compared with the vehicle group is $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

CONCLUSIONS

In an effort to find potent FXa inhibitors, we focused on the development of a series of conformationally constrained rivaroxaban analogues. Rigidification of the benzene ring and oxazolidinone moiety by introduction of an additional bridge linker yielded the [6,6,5] tricyclic fused scaffold. Extensive modifications focused on the P1 group, P4 group, and bridge linker in the C ring resulted in a novel series of FXa inhibitors showing high in vitro FXa inhibitory activity and anticoagulant potency. Further SPR studies in rats and dogs demonstrated that analogues bearing different bridges represented various PK properties. Among these, compounds carrying a methylene-oxide bridge showed the most favorable PK profiles. On the basis of excellent in vitro anticoagulant potency and favorable PK profiles, compound **11a** was selected to evaluate its in vivo antithrombotic efficacy in models of VT, AV-SHUNT, and arterial thrombosis. Importantly, compound **11a** displayed excellent efficacy in all three models, especially in the model of arterial thrombosis, with ED_{50} = 2.97 mg/kg, which is superior to that of rivaroxaban (4.53 mg/kg). Additionally, **11a** showed no significant inhibitory activity against hERG K^+ channel and various CYP450 isozymes and had no genetic toxicity in the Ames mutagenicity test and micronucleus formation assay. Compound **11a** also exhibited significant selectivity against other related serine proteases (IC_{50} > 100 μM). Furthermore, in a 2 week subacute toxicity study in rats, **11a** was well-tolerated and showed no obvious toxic reactions at medium and high doses. These data suggest that compound **11a** is a highly potent and selective FXa inhibitor and warrants further evaluation as a potential candidate for the prevention and treatment of thromboembolic disease in venous and arterial systems. Studies on **11a** are ongoing in our research team.

EXPERIMENTAL SECTION

Chemistry. Compounds not described below were purchased from commercial vendors and were used as supplied unless it is stated otherwise. Inert atmosphere operations were conducted under argon in flame-dried glassware. All reaction mixtures were monitored using thin-layer chromatography (TLC) on silica gel F-254 TLC plates. Column chromatography was carried out using silica gel (200−300 mesh). ^1H and ^{13}C NMR spectra were recorded on a Bruker 300 NMR or a Bruker 400 NMR or a Bruker 500 NMR spectrometer using solvent as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (J) are reported in Hertz (Hz). EI-MS spectra were obtained on a Finnigan MAT95 spectrometer, and ESI-MS spectra were obtained on a Krats MS 80 mass spectrometer. All final compounds were purified to >95% purity as determined by an Agilent 1100 series LC system (PLATISIL ODS 5 μm 250 × 4.6 mm) with two solvent systems (acetonitrile/water or acetonitrile/buffer (0.1% CF_3COOH in water)). Chiral LC analysis was performed on an Shimadzu LC-10AT VP LC system (Chiralpak AS-H 5 μm 250 × 4.6 mm) with a two solvent system ("hexane/2-propanol = 90:10 (v/v)), UV detection (λ = 210 nm) at 35 °C.

For detailed synthesis and characterization of all intermediates, see the Supporting Information.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-thiophene-2-carboxamide (11a). The crude amine **10a** together with 5-chlorothiophene-2-carboxylic acid (1.56 g, 9.59 mmol), TEA (1.62 g, 16.04 mmol), and HATU (4.56 g, 11.99 mmol) was dissolved in 50 mL of dry DMF. The resulting solution was stirred for 4 h at room temperature. Water was added, and it was extracted with ethyl acetate. The organic phase was washed with saturated NH_4Cl solution and brine, dried over anhydrous Na_2SO_4, and filtered, and then the filtrate was concentrated in vacuo. The residue was purified by chromatography on silica gel with dichloromethane/methanol (30:1)

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Table 9. Inhibition of hERG K^+ Channel and CYP450 Isozymes by 11a

		CYP inhibition IC_{50} (μM)					
compd	hERG K^+ inhibition IC_{50} (μM)	1A2	2C8	2C9	2C19	2D6	3A4
11a	79.75	>200	99.73	43.23	>200	>200	85.12

to give 3.13 g (84.2%, for two steps) of **11a** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (t, J = 5.8 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.71 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 7.05 (d, J = 2.3 Hz, 1H), 7.01 (dd, J = 8.7, 2.3 Hz, 1H), 4.63−4.52 (m, 2H), 4.18 (s, 2H), 4.10−4.01 (m, 2H), 3.97−3.92 (m, 2H), 3.73 (t, J = 5.5 Hz, 2H), 3.71−3.66 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.41, 161.32, 153.74, 144.52, 138.79, 138.05, 133.85, 129.06, 128.70, 122.15, 118.88, 118.81, 114.68, 75.01, 68.18, 66.24, 63.90, 53.42, 49.46, 41.76. MS (ESI) m/z: 486.3 (M + 23)$^+$. HRMS (EI): Anal. Calcd for $C_{20}H_{18}SClN_3O_6$, 463.0605; found, 463.0623.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(2-oxopiperidin-1-yl)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-thiophene-2-carboxamide (11b). Compound **11b** (0.72 g, 74.2%, for two steps) was prepared from crude **10b** and 5-chlorothiophene-2-carboxylic acid (0.41 g, 2.52 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 9.3 Hz, 1H), 7.34 (d, J = 4.0 Hz, 1H), 6.90 (d, J = 4.0 Hz, 1H), 6.87 (dt, J = 4.5, 2.3 Hz, 3H), 4.54−4.41 (m, 2H), 3.96 (ddd, J = 10.1, 7.0, 3.1 Hz, 1H), 3.90−3.70 (m, 3H), 3.59 (s, 2H), 2.55 (d, J = 6.3 Hz, 2H), 1.97−1.89 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.24, 161.31, 153.75, 144.48, 140.15, 138.79, 133.85, 129.05, 128.69, 121.83, 119.68, 118.76, 115.40, 74.96, 66.23, 53.45, 51.35, 41.78, 33.05, 23.43, 21.34. MS (EI) m/z: 461 (M$^+$). HRMS (EI): Anal. Calcd for $C_{21}H_{20}SClN_3O_5$, 461.0812; found, 461.0817.

5-Chloro-*N*-(((3*S*,3a*S*)-7-(*N*-methylacetamido)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)-methyl)thiophene-2-carboxamide (11c). Compound **11c** (0.32 g, 77.4%, for two steps) was prepared from crude **10c** and 5-chlorothiophene-2-carboxylic acid (0.19 g, 1.17 mmol) in the same manner as that described for **11a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (ddd, J = 17.8, 10.0, 7.3 Hz, 5H), 7.09−6.91 (m, 2H), 4.70 (dd, J = 18.5, 8.4 Hz, 2H), 4.21 (s, 1H), 4.09 (dd, J = 18.0, 7.4 Hz, 3H), 3.11 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.49, 161.33, 153.73, 144.97, 140.73, 138.78, 133.86, 129.07, 128.70, 123.08, 120.57, 119.39, 116.10, 75.09, 66.20, 53.30, 41.72, 36.97, 22.61. MS (EI) m/z: 435 (M$^+$). HRMS (EI): Anal. Calcd for $C_{19}H_{18}SClN_3O_5$, 435.0656; found, 435.0651.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(2-oxooxazolidin-3-yl)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)-methyl)thiophene-2-carboxamide (11d). Compound **11d** (0.17 g, 63.3%, for two steps) was prepared from crude **10d** and 5-chlorothiophene-2-carboxylic acid (0.12 g, 0.74 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 5.9 Hz, 1H), 7.83 (d, J = 8.9 Hz, 1H), 7.72 (d, J = 4.0 Hz, 1H), 7.28 (d, J = 2.4 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 7.14 (dd, J = 9.0, 2.5 Hz, 1H), 4.62−4.57 (m, 1H), 4.55 (d, J = 7.6 Hz, 1H), 4.42 (t, J = 8.0 Hz, 2H), 4.13−3.94 (m, 4H), 3.73 (t, J = 5.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.30, 155.29, 153.72, 144.72, 138.77, 135.36, 133.84, 129.04, 128.65, 119.59, 119.05, 111.53, 107.13, 74.94, 66.42, 61.87, 53.45, 45.30, 41.79. MS (ESI) m/z: 472.0 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{19}H_{16}O_6N_3ClNaS$, 472.0346; found, 472.0350.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(7-oxo-1,4-dioxa-8-azaspiro-[4.5]decan-8-yl)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d]-[1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (11e). Compound **11e** (0.15 g, 66.3%, for two steps) was prepared from crude **10e** and 5-chlorothiophene-2-carboxylic acid (0.10 g, 0.62 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (t, J = 5.9 Hz, 1H), 7.84 (d, J = 9.3 Hz, 1H), 7.72 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 4.1 Hz, 1H), 6.91−6.86 (m, 2H), 4.60 (q, J = 5.5 Hz, 1H), 4.57−4.53 (m, 1H), 4.11−4.02 (m, 2H), 3.99−3.93 (m, 4H), 3.73 (t, J = 5.6 Hz, 2H), 3.60 (t, J = 6.3 Hz, 2H), 2.64 (s, 2H), 2.05 (t, J = 6.3 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.35, 161.30, 153.71, 144.51, 139.20, 138.77, 133.83, 129.04, 128.66, 122.01, 119.51, 118.83, 115.25, 106.14, 74.97, 66.24, 64.56 (overlap), 53.43, 47.61, 43.35, 41.77, 32.11. MS (ESI) m/z: 542.0 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{23}H_{22}O_7N_3ClNaS$, 542.0765; found, 542.0752.

5-Chloro-*N*-(((3*S*,3a*S*)-7-((*R*)-4-methoxy-2-oxopiperidin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]-oxazin-3-yl)methyl)thiophene-2-carboxamide (11f). Compound **11f** (0.48 g, 75.3%) was prepared from **10f** (0.45 g, 1.30 mmol) and 5-chlorothiophene-2-carboxylic acid (0.31 g, 1.91 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 5.8 Hz, 1H), 7.83 (d, J = 9.4 Hz, 1H), 7.72 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 6.89 (s, 1H), 6.87 (d, J = 2.4 Hz, 1H), 4.59 (q, J = 5.5 Hz, 1H), 4.55 (d, J = 8.1 Hz, 1H), 4.12−4.00 (m, 2H), 3.78−3.70 (m, 3H), 3.63 (ddd, J = 12.5, 8.2, 4.8 Hz, 1H), 3.48 (dt, J = 11.6, 5.7 Hz, 1H), 3.29 (s, 3H), 2.68 (dd, J = 17.3, 4.7 Hz, 1H), 2.38 (dd, J = 17.3, 5.5 Hz, 1H), 2.09 (dt, J = 13.3, 4.0 Hz, 1H), 1.94 (dt, J = 12.6, 6.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.92, 161.31, 153.71, 144.46, 139.63, 138.77, 133.83, 129.04, 128.65, 121.84, 119.44, 118.76, 115.16, 74.96, 73.16, 66.25, 55.74, 53.46, 46.88, 41.78, 38.89, 27.40. MS (ESI) m/z: 514.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{22}H_{22}O_6N_3ClNaS$, 514.0810; found, 514.0808.

5-Chloro-*N*-(((3*S*,3a*S*)-7-((*R*)-4-methoxy-2-oxopiperidin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]-oxazin-3-yl)methyl)thiophene-2-carboxamide (11g). Compound **11g** (0.50 g, 72.0%) was prepared from **10g** (0.49 g, 1.41 mmol) and 5-chlorothiophene-2-carboxylic acid (0.34 g, 2.09 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 5.6 Hz, 1H), 7.83 (d, J = 8.7 Hz, 1H), 7.72 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 3.9 Hz, 1H), 6.89 (s, 1H), 6.88 (s, 1H), 4.68−4.52 (m, 2H), 4.13−3.96 (m, 2H), 3.83−3.70 (m, 3H), 3.63 (p, J = 6.4, 5.9 Hz, 1H), 3.48 (dt, J = 11.9, 5.9 Hz, 1H), 3.29 (s, 3H), 2.68 (dd, J = 17.3, 4.7 Hz, 1H), 2.38 (dd, J = 17.1, 5.6 Hz, 1H), 2.18−2.03 (m, 1H), 1.95 (dq, J = 14.0, 6.9, 6.4 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.92, 161.30, 153.71, 144.45, 139.62, 138.77, 133.83, 129.04, 128.65, 121.85, 119.43, 118.76, 115.20, 74.96, 73.17, 66.26, 55.74, 53.46, 46.89, 41.78, 38.90, 27.42. MS (ESI) m/z: 514.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{22}H_{22}O_6N_3ClNaS$, 514.0810; found, 514.0800.

5-Chloro-*N*-(((3*S*,3a*S*)-7-((*R*)-4-hydroxy-2-oxopiperidin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (11k). To a solution of **11h** (0.25 g, 0.35 mmol) in THF (10 mL) was added ^{n}Bu$_4$NF (1 M in THF, 0.7 mL, 0.7 mmol) dropwise at 0 °C. The solution was stirred for 3 h and then concentrated in vacuo. The residue was purified by chromatography on silica gel with dichloromethane/methanol (30:1) to give 0.14 g (83.9%) of **11k** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 5.7 Hz, 1H), 7.82 (d, J = 8.9 Hz, 1H), 7.72 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 6.89 (s, 1H), 6.87 (d, J = 3.0 Hz, 1H), 5.08 (d, J = 3.7 Hz, 1H), 4.62−4.52 (m, 2H), 4.17−3.99 (m, 3H), 3.73 (t, J = 5.5 Hz, 2H), 3.66 (dd, J = 13.3, 7.2 Hz, 1H), 3.48 (dt, J = 11.7, 5.2 Hz, 1H), 2.60 (dd, J = 17.2, 4.7 Hz, 1H), 2.27 (dd, J = 17.2, 6.0 Hz, 1H), 2.08−1.94 (m, 1H), 1.80 (dt, J = 13.6, 6.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.89, 162.72, 155.14, 145.87, 141.25, 140.19, 135.24, 130.46, 130.08, 123.22, 120.95, 120.17, 116.69, 76.36, 67.66, 64.96, 54.87, 48.54, 43.45, 43.19, 32.62. MS (ESI) m/z: 500.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{21}H_{20}O_6N_3ClNaS$, 500.0654; found, 500.0647.

5-Chloro-*N*-(((3*S*,3a*S*)-7-((*S*)-4-fluoro-2-oxopiperidin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (11l). To a solution of compound **11k** (5.0 g, 32.7 mmol) in anhydrous DCM (5 mL) was added DAST (33 mg, 0.20 mmol) dropwise at 0 °C under an argon atmosphere. After addition, the reaction mixture was stirred for 3 h at room temperature and then concentrated in vacuo. The resulting residue was purified by silica gel chromatography with DCM/MeOH

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(50:1) to afford **11l** (40 mg, 79.7%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 6.0 Hz, 1H), 7.89−7.79 (m, 1H), 7.72 (d, J = 4.0 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 7.02−6.84 (m, 2H), 5.32−5.07 (m, 1H), 4.63−4.57 (m, 1H), 4.55 (d, J = 7.9 Hz, 1H), 4.18−3.96 (m, 2H), 3.79−3.67 (m, 3H), 3.54 (dt, J = 11.6, 5.4 Hz, 1H), 2.85 (ddd, J = 34.4, 17.8, 4.3 Hz, 1H), 2.61 (td, J = 17.4, 4.3 Hz, 1H), 2.32−1.93 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.69 (d, J = 4.5 Hz), 161.31, 153.72, 144.50, 139.34, 138.76, 133.83, 129.04, 128.66, 122.04, 119.50, 118.82, 115.22, 87.36 (d, J = 167.8 Hz), 74.96, 66.24, 53.44, 46.01 (d, J = 6.8 Hz), 41.78, 39.11 (d, J = 22.9 Hz), 28.39 (d, J = 19.9 Hz). MS (ESI) m/z: 502.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{21}H_{19}O_5N_3ClFNaS$, 502.0610; found, 502.0612.

5-Chloro-*N*-(((3*S*,3a*S*)-7-((*S*)-4-hydroxy-2-oxopiperidin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (11m). To a solution of **11i** (0.68 g, 0.95 mmol) in THF (10 mL) was added ^{n}Bu$_4$NF (1 M in THF, 1.8 mL, 1.8mmol) dropwise at 0 °C. The solution was stirred for 3 h and then concentrated in vacuo. The residue was purified by chromatography on silica gel with dichloromethane/methanol (30:1) to give 0.39 g (86.0%) of **11m** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 6.0 Hz, 1H), 7.82 (d, J = 9.4 Hz, 1H), 7.72 (d, J = 4.0 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 6.89 (s, 1H), 6.87 (d, J = 2.3 Hz, 1H), 5.08 (d, J = 3.7 Hz, 1H), 4.59 (q, J = 6.5, 5.9 Hz, 1H), 4.56−4.53 (m, 1H), 4.12−3.97 (m, 3H), 3.73 (t, J = 5.7 Hz, 2H), 3.67 (td, J = 7.7, 4.0 Hz, 1H), 3.52−3.44 (m, 1H), 2.60 (dd, J = 17.2, 4.7 Hz, 1H), 2.28 (dd, J = 17.4, 5.6 Hz, 1H), 2.08−1.94 (m, 1H), 1.80 (dt, J = 13.3, 6.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.02, 160.84, 153.27, 143.99, 139.38, 138.32, 133.37, 128.59, 128.22, 121.34, 119.08, 118.30, 114.80, 74.49, 65.78, 63.08, 52.99, 46.65, 41.57, 41.32, 30.72. MS (ESI) m/z: 500.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{21}H_{20}O_6N_3ClNaS$, 500.0654; found, 500.0655.

5-Chloro-*N*-(((3*S*,3a*S*)-7-((*R*)-4-fluoro-2-oxopiperidin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (11n). To a solution of compound **11m** (5.0 g, 32.7 mmol) in anhydrous DCM (5 mL) was added DAST (33 mg, 0.20 mmol) dropwise at 0 °C under an argon atmosphere. After addition, the reaction mixture was stirred for 3 h at room temperature and then concentrated in vacuo. The resulting residue was purified by silica gel chromatography with DCM/MeOH (50:1) to afford **11n** (43 mg, 85.6%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 5.8 Hz, 1H), 7.84 (d, J = 8.9 Hz, 1H), 7.72 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 6.98−6.87 (m, 2H), 5.29−5.10 (m, 1H), 4.65−4.51 (m, 2H), 4.13−4.00 (m, 2H), 3.82−3.66 (m, 3H), 3.55 (dt, J = 11.6, 5.4 Hz, 1H), 2.85 (ddd, J = 34.2, 17.8, 4.4 Hz, 1H), 2.61 (td, J = 17.8, 4.7 Hz, 1H), 2.30−1.92 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.69 (d, J = 4.9 Hz), 161.30, 153.72, 144.50, 139.34, 138.77, 133.83, 129.04, 128.66, 122.04, 119.50, 118.82, 115.25, 87.36 (d, J = 167.8 Hz), 74.97, 66.26, 53.44, 46.02 (d, J = 6.8 Hz), 41.78, 39.12 (d, J = 22.8 Hz), 28.41 (d, J = 19.9 Hz). MS (ESI) m/z: 502.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{21}H_{19}O_5N_3ClFNaS$, 502.0610; found, 502.0608.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(2-oxopiperazin-1-yl)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (11o). To a cooled (0 °C) solution of **11j** (0.86 g, 1.44 mmol) in DCM were added Me_2S (0.45 g, 7.24 mmol) and $BF_3{\cdot}Et_2O$ (1.3 g, 9.15 mmol) dropwise. The resulting solution was allowed to warm to room temperature and stirred for 8 h. The solution was concentrated in vacuo and purified on silica gel chromatography with dichloromethane/methanol (30:1) to give 0.55 g (82.5%) of **11o** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (t, J = 5.7 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 4.1 Hz, 1H), 6.99−6.91 (m, 2H), 5.76 (s, 1H), 4.65−4.50 (m, 2H), 4.13−3.98 (m, 2H), 3.72 (t, J = 5.5 Hz, 2H), 3.55 (t, J = 5.7 Hz, 2H), 3.37 (s, 2H), 3.00 (t, J = 5.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.53, 161.32, 153.74, 144.52, 138.80 (overlap), 133.84, 129.08, 128.70, 122.12, 119.21, 118.89, 114.97, 74.99, 66.24, 53.42, 50.10, 49.53, 42.54, 41.75. MS (ESI) m/z: 463.3 (M+1)$^+$. HRMS (EI): Anal. Calcd for $C_{20}H_{19}SClN_4O_5$, 462.0765; found, 462.0774.

5-Chloro-*N*-(((3*S*,3a*S*)-7-(4-methyl-2-oxopiperazin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (11p). To a solution of **11o** (78 mg, 0.17 mmol) and HCHO (37% aq., 21 mg, 0.26 mmol) in MeOH (5 mL) at room temperature was added $NaBH(OAc)_3$ (143 mg, 0.67 mmol). After being stirred at room temperature overnight, the solution was concentrated in vacuo. The residue was partitioned between 5% aq. $NaHCO_3$ and ethyl acetate. The organic phase was separated, concentrated in vacuo, and purified on silica gel chromatography with dichloromethane/methanol (50:1) to give **11p** (56 mg, 69.7%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 8.6 Hz, 1H), 7.34 (d, J = 4.0 Hz, 1H), 6.90 (dd, J = 6.2, 4.0 Hz, 3H), 6.79 (t, J = 6.1 Hz, 1H), 4.49 (ddd, J = 16.7, 10.4, 4.5 Hz, 2H), 4.04−3.94 (m, 1H), 3.92−3.74 (m, 3H), 3.69 (s, 2H), 3.31 (s, 2H), 2.84 (s, 2H), 2.44 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.42, 161.31, 153.74, 144.52, 138.79, 138.58, 133.84, 129.05, 128.70, 122.06, 119.28, 118.80, 115.00, 74.99, 66.23, 59.76, 53.42, 51.82, 49.80, 44.93, 41.77. MS (EI) m/z: 476 (M$^+$). HRMS (EI): Anal. Calcd for $C_{21}H_{21}SClN_4O_5$, 476.0921; found, 476.0932.

5-Chloro-*N*-(((3*S*,3a*S*)-7-(4-ethyl-2-oxopiperazin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (11q). To a solution of **11o** (40 mg, 0.086 mmol) and acetaldehyde (5.8 mg, 0.13 mmol) in MeOH (5 mL) at room temperature was added $NaBH(OAc)_3$ (74 mg, 0.35 mmol). After being stirred at room temperature overnight, the solution was concentrated in vacuo. The residue was partitioned between 5% aq. $NaHCO_3$ and ethyl acetate. The organic phase was separated, concentrated in vacuo, and purified on silica gel chromatography with dichloromethane/methanol (50:1) to give **11q** (26 mg, 61.3%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, J = 8.8 Hz, 1H), 7.36 (d, J = 3.4 Hz, 1H), 7.17 (s, 1H), 6.90 (d, J = 6.4 Hz, 3H), 4.45 (dd, J = 13.7, 8.2 Hz, 2H), 3.97 (s, 1H), 3.88−3.72 (m, 3H), 3.66 (s, 2H), 3.30 (s, 2H), 2.81 (s, 2H), 2.53 (d, J = 7.1 Hz, 2H), 1.14 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.56, 161.31, 153.74, 144.51, 138.79, 138.59, 133.84, 129.06, 128.70, 122.04, 119.25, 118.79, 114.97, 74.99, 66.23, 57.69, 53.42, 51.06, 49.85, 49.55, 41.77, 12.27. MS (EI) m/z: 490 (M$^+$). HRMS (EI): Anal. Calcd for $C_{22}H_{23}SClN_4O_5$, 490.1078; found, 490.1071.

***N*-(((3*S*,3a*S*)-7-(4-Benzyl-2-oxopiperazin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-5-chlorothiophene-2-carboxamide (11r).** To a solution of **11o** (30 mg, 0.065 mmol) and TEA (13 mg, 0.13 mmol) in DMF (5 mL) was added benzyl bromide (11.1 mg, 0.065 mmol). The resulting solution was stirred at room temperature for 3 h. Then, the solution was poured into 10 mL of water and extracted with ethyl acetate. The combined organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, and filtered, and then the filtrate was concentrated in vacuo. The residue was purified on silica gel chromatography with dichloromethane/methanol (50:1) to give 28 mg (78.1%) of **11r** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.4 Hz, 1H), 7.34 (t, J = 4.2 Hz, 5H), 7.32−7.27 (m, 1H), 7.04 (t, J = 5.9 Hz, 1H), 6.94−6.83 (m, 3H), 4.45 (ddd, J = 17.0, 10.9, 4.3 Hz, 2H), 4.00−3.91 (m, 1H), 3.86−3.69 (m, 3H), 3.63 (d, J = 8.7 Hz, 4H), 3.31 (s, 2H), 2.80 (t, J = 5.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.39, 161.31, 153.74, 144.51, 138.79, 138.52, 137.63, 133.84, 129.48 (overlap), 129.05, 128.80 (overlap), 128.70, 127.75, 122.05, 119.25, 118.77, 114.97, 74.99, 66.22, 61.02, 57.78, 53.42, 49.80, 49.43, 41.76. MS (EI) m/z: 552 (M$^+$). HRMS (EI): Anal. Calcd for $C_{27}H_{25}SClN_4O_5$, 552.1234; found, 552.1222.

***N*-(((3*S*,3a*S*)-7-(4-Acetyl-2-oxopiperazin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-5-chlorothiophene-2-carboxamide (11s).** To a cooled solution of **11o** (40 mg, 0.086 mmol) and TEA (13.1 mg, 0.13 mmol) in dry DMF (5 mL) was added acetyl chloride (7.1 mg, 0.090 mmol) dropwise. The resulting mixture was allowed to warm to room temperature and stirred for 3 h. Then, the reaction was quenched by addition of cold water and extracted with ethyl acetate. The organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, and filtered, and then the filtrate was concentrated in vacuo. The residue was purified on silica gel chromatography with dichloromethane/methanol (30:1) to yield **11s** (27 mg, 61.9%) as a white

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solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (t, J = 9.2 Hz, 1H), 7.35 (d, J = 3.8 Hz, 1H), 7.04−6.82 (m, 4H), 4.59−4.44 (m, 2H), 4.34 (d, J = 38.9 Hz, 2H), 4.03−3.92 (m, 2H), 3.85 (d, J = 10.5 Hz, 4H), 3.71 (dd, J = 20.5, 15.1 Hz, 2H), 2.16 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.88, 165.59, 165.02, 161.32, 153.74, 144.50, 138.79, 133.85, 129.06, 128.70, 122.20, 119.20, 119.09, 115.00, 75.00, 66.24, 53.42, 49.91, 49.18, 43.55, 41.76, 21.78. MS (EI) m/z: 504 (M^+). HRMS (EI): Anal. Calcd for $C_{22}H_{21}SClN_4O_6$, 504.0870; found, 504.0868.

5-Chloro-*N*-(((3*S*,3a*S*)-7-(4-(methylsulfonyl)-2-oxopiperazin-1-yl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]-oxazin-3-yl)methyl)thiophene-2-carboxamide (11t). To a cooled solution of **11o** (40 mg, 0.086 mmol) and TEA (13.1 mg, 0.13 mmol) in dry DMF (5 mL) was added mesyl chloride (11.9 mg, 0.10 mmol). The resulting mixture was allowed to warm to room temperature and stirred for 3 h. Then, the reaction was quenched by addition of cold water and extracted with ethyl acetate. The organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, and filtered, and then the filtrate was concentrated in vacuo. The residue was purified on silica gel chromatography with dichloromethane/methanol (50:1) to yield **11t** (40 mg, 85.6%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (t, J = 5.9 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.71 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 4.1 Hz, 1H), 7.02 (d, J = 2.3 Hz, 1H), 6.98 (dd, J = 8.7, 2.3 Hz, 1H), 4.58 (dd, J = 15.7, 7.0 Hz, 2H), 4.13−4.01 (m, 2H), 3.93 (s, 2H), 3.73 (q, J = 4.9 Hz, 4H), 3.57−3.51 (m, 2H), 3.05 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.13, 161.32, 153.74, 144.58, 138.79, 138.14, 133.85, 129.06, 128.70, 122.47, 119.45, 118.88, 115.24, 75.02, 66.23, 53.41, 49.75, 49.24, 43.10, 41.76, 35.13. MS (EI) m/z: 540 (M^+). HRMS (EI): Anal. Calcd for $C_{21}H_{21}S_2ClN_4O_7$, 540.0540; found, 540.0532.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(2-oxo-4-(phenylsulfonyl)-piperazin-1-yl)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d]-[1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (11u). To a cooled solution of **11o** (40 mg, 0.086 mmol) and TEA (16.7 mg, 0.16 mmol) in dry DMF (5 mL) was added benzenesulfonyl chloride (18.3 mg, 0.10 mmol) dropwise. The resulting mixture was allowed to warm to room temperature and stirred for 3 h. Then, water was added, and the solution was extracted with ethyl acetate. The organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, and filtered, and then the filtrate was concentrated in vacuo. The resulting residue was purified on silica gel chromatography with dichloromethane/methanol (50:1) to afford 30 mg (57.6%) of **11u** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.98 (s, 1H), 7.77 (dd, J = 27.8, 19.6 Hz, 7H), 7.19 (s, 1H), 6.80 (s, 2H), 4.53 (s, 2H), 4.02 (s, 2H), 3.73 (s, 5H), 3.60 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.55, 161.31, 153.71, 144.52, 138.78, 137.92, 135.47, 134.23, 133.84, 130.21 (overlap), 129.05, 128.69, 128.16 (overlap), 122.51, 119.41, 118.82, 115.22, 75.00, 66.19, 53.37, 49.44, 49.17, 43.47, 41.74. MS (EI) m/z: 490 (M^+). HRMS (EI): Anal. Calcd for $C_{26}H_{23}S_2ClN_4O_7$, 602.0697; found, 602.0691.

5-Chloro-*N*-(((3*S*,3a*R*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-thiophene-2-carboxamide (18). Compound **18** (73 mg, 82.4%) was prepared from **17** (61 mg, 0.19 mmol) and 5-chlorothiophene-2-carboxylic acid (47 mg, 0.29 mmol) in the same manner as that described for **11a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.94 (s, 1H), 7.87 (d, J = 8.7 Hz, 1H), 7.73 (d, J = 3.6 Hz, 1H), 7.22 (d, J = 3.6 Hz, 1H), 7.06 (s, 1H), 7.02 (d, J = 8.6 Hz, 1H), 5.02 (d, J = 3.3 Hz, 1H), 4.66 (d, J = 10.4 Hz, 1H), 4.40 (t, J = 8.5 Hz, 1H), 4.17 (d, J = 14.1 Hz, 3H), 3.95 (d, J = 4.6 Hz, 2H), 3.72−3.62 (m, 3H), 3.55−3.45 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.35, 160.97, 153.64, 144.53, 138.86, 138.02, 133.70, 129.00, 128.61, 122.53, 119.00, 118.76, 114.63, 73.82, 68.12, 63.85, 63.62, 52.05, 49.42, 39.32. MS (EI) m/z: 463 (M^+). HRMS (EI): Anal. Calcd for $C_{20}H_{18}SClN_3O_6$, 463.0605; found, 463.0608.

5-Chloro-*N*-(((3*S*,3a*S*)-7-(2-(methylsulfonyl)phenyl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)-methyl)thiophene-2-carboxamide (25a). A mixture of **24** (50 mg, 0.10 mmol), cesium carbonate (83 mg, 0.26 mmol), 1-bromo-2-(methylsulfonyl)benzene (29 mg, 0.12 mmol), and tetrakis-(triphenylphosphine)palladium(0) (12 mg, 0.01 mmol) in dioxane (5 mL) and H_2O (0.5 mL) was degassed and flushed with argon. The mixture was heated at 80 °C overnight. Dichloromethane (15 mL) was added, and the mixture was filtered through Celite, washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel with petroleum/ethyl acetate (2:1) to afford 19 mg (35.9%) of **25a** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 5.8 Hz, 1H), 8.08 (dd, J = 7.9, 1.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.80−7.70 (m, 2H), 7.70−7.63 (m, 1H), 7.39 (dd, J = 7.5, 1.2 Hz, 1H), 7.22 (d, J = 4.0 Hz, 1H), 7.07−6.99 (m, 2H), 4.67−4.54 (m, 2H), 4.19−4.06 (m, 2H), 3.73 (d, J = 5.6 Hz, 2H), 2.86 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.33, 153.72, 143.66, 140.51, 139.68, 138.80, 134.85, 133.86, 133.76, 133.21, 129.08, 128.83, 128.71, 128.38, 123.73, 123.34, 118.78, 118.01, 75.09, 66.18, 53.47, 44.04, 41.73. MS (EI) m/z: 518 (M^+). HRMS (EI): Anal. Calcd for $C_{23}H_{19}S_2ClN_2O_6$, 518.0373; found, 518.0371.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(2-sulfamoylphenyl)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-thiophene-2-carboxamide (25c). Compound **25b** (0.12 g, 0.21 mmol) was dissolved in TFA (2 mL) and heated at 40 °C for 2 h. Then, the solution was evaporated, and the residue was purified by chromatography on silica gel with petroleum/ethyl acetate (2:1) to afford 91 mg (84.0%) of **25c** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.03 (t, J = 5.5 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.87 (d, J = 8.6 Hz, 1H), 7.73 (d, J = 4.1 Hz, 1H), 7.64−7.52 (m, 2H), 7.30 (d, J = 7.3 Hz, 1H), 7.27−7.18 (m, 3H), 7.00 (d, J = 7.5 Hz, 2H), 4.60 (dd, J = 16.6, 6.9 Hz, 2H), 4.20−4.01 (m, 2H), 3.75 (d, J = 5.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.34, 153.75, 143.67, 142.65, 139.45, 138.80, 136.42, 133.86, 132.83, 131.92, 129.08, 128.72, 128.12, 127.73, 123.12, 122.91, 118.22, 118.02, 75.01, 66.20, 53.51, 41.75. MS (EI) m/z: 519 (M^+). HRMS (EI): Anal. Calcd for $C_{22}H_{18}S_2ClN_3O_6$, 519.0326; found, 519.0339.

4-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-benzamide (26a). Compound **26a** (72 mg, 83.7%) was prepared from **10a** (60 mg, 0.19 mmol) and 4-chlorobenzoic acid (35 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.97 (t, J = 5.7 Hz, 1H), 7.87 (dd, J = 13.0, 8.6 Hz, 3H), 7.56 (d, J = 8.6 Hz, 2H), 7.11−6.96 (m, 2H), 4.67−4.52 (m, 2H), 4.17 (s, 2H), 4.08 (dt, J = 19.5, 6.2 Hz, 2H), 3.98−3.91 (m, 2H), 3.76 (t, J = 5.5 Hz, 2H), 3.72−3.64 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.50, 166.41, 153.82, 144.56, 138.06, 136.78, 133.10, 129.77 (overlap), 128.95 (overlap), 122.17, 118.89 (overlap), 114.69, 74.94, 68.18, 66.31, 63.90, 53.52, 49.47, 41.95. MS (ESI) m/z: 480.1 $(M + 23)^+$. HRMS (EI): Anal. Calcd for $C_{22}H_{20}N_3O_6$, 457.1041; found, 457.1032.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-picolinamide (26b). Compound **26b** (74 mg, 85.8%) was prepared from **10a** (60 mg, 0.19 mmol) and 5-chloropicolinic acid (36 mg, 0.23 mmol) in the same manner as that described for **11a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.75 (s, 1H), 8.16 (d, J = 9.1 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.09−6.99 (m, 2H), 4.66 (d, J = 5.4 Hz, 1H), 4.52 (d, J = 10.2 Hz, 1H), 4.19 (s, 3H), 4.05 (t, J = 9.9 Hz, 1H), 3.95 (d, J = 4.6 Hz, 2H), 3.79 (s, 2H), 3.70 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.40, 164.28, 153.75, 148.50, 147.70, 144.55, 138.14, 138.06, 134.66, 124.10, 122.14, 118.91, 118.86, 114.67, 74.79, 68.17, 66.32, 63.90, 53.53, 49.46, 41.72. MS (EI) m/z: 458 (M^+). HRMS (EI): Anal. Calcd for $C_{21}H_{19}N_4O_6$, 458.0993; found, 458.1000.

4-Methoxy-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)-methyl)benzamide (26c). Compound **26c** (61 mg, 85.9%) was prepared from **10a** (50 mg, 0.16 mmol) and 4-methoxybenzoic acid (29 mg, 0.19 mmol) in the same manner as that described for **11a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76 (t, J = 5.8 Hz, 1H), 7.90−7.83 (m, 3H), 7.03 (dt, J = 8.9, 2.4 Hz, 4H), 4.62 (dd, J = 12.0, 5.3 Hz, 1H), 4.54 (dd, J = 10.1, 2.8 Hz, 1H), 4.18 (s, 2H), 4.13 (ddd, J = 9.8, 6.9, 2.8 Hz, 1H), 4.06 (t, J = 10.1 Hz, 1H), 3.95 (t, J = 5.0 Hz, 2H), 3.80 (d, J = 14.1 Hz, 3H), 3.74 (dd, J = 10.6, 5.3 Hz, 2H), 3.71−3.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.95, 166.41, 162.22, 153.85, 144.53, 138.02, 129.67 (overlap), 126.49, 122.18, 118.90, 118.86, 114.69, 114.04 (overlap), 75.09, 68.16, 66.32, 63.89, 55.83,

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53.56, 49.46, 41.84. MS (EI) m/z: 453 (M^+). HRMS (EI): Anal. Calcd for $C_{23}H_{23}N_3O_7$, 453.1536; found, 453.1531.

4,5-Dichloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)thiophene-2-carboxamide (26d). Compound **26d** (76 mg, 81.2%) was prepared from **10a** (60 mg, 0.19 mmol) and 4,5-dichlorothiophene-2-carboxylic acid (44 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (t, J = 5.8 Hz, 1H), 7.91 (s, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.06 (d, J = 2.3 Hz, 1H), 7.02 (dd, J = 8.7, 2.4 Hz, 1H), 4.58 (ddd, J = 9.3, 6.4, 3.4 Hz, 2H), 4.18 (s, 2H), 4.11−4.02 (m, 2H), 3.95 (dd, J = 5.9, 4.2 Hz, 2H), 3.75 (t, J = 6.0 Hz, 2H), 3.72−3.67 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.38, 160.47, 153.66, 144.50, 138.04, 136.99, 129.10, 128.36, 123.80, 122.14, 118.85, 118.77, 114.66, 74.88, 68.18, 66.23, 63.90, 53.36, 49.46, 41.79. MS (ESI) m/z: 520.0 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{20}H_{17}O_6N_3Cl_2NaS$, 520.0107; found, 520.0112.

2-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-thiazole-5-carboxamide (26e). Compound **26e** (72 mg, 82.4%) was prepared from **10a** (60 mg, 0.19 mmol) and 2-chlorothiazole-5-carboxylic acid (37 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (t, J = 5.8 Hz, 1H), 8.31 (s, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.06 (d, J = 2.3 Hz, 1H), 7.02 (dd, J = 8.7, 2.4 Hz, 1H), 4.64−4.55 (m, 2H), 4.18 (s, 2H), 4.11−4.02 (m, 2H), 3.95 (t, J = 5.0 Hz, 2H), 3.76 (t, J = 5.2 Hz, 2H), 3.73−3.65 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.38, 159.98, 154.44, 153.67, 144.51, 142.64, 138.05, 138.01, 122.14, 118.86, 118.78, 114.67, 74.96, 68.18, 66.23, 63.90, 53.36, 49.46, 41.75. MS (ESI) m/z: 462.8 (M−1)$^-$. HRMS (ESI): Anal. Calcd for $C_{19}H_{17}O_6N_4ClNaS$, 487.0450; found, 487.0443.

7-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-benzo[b]thiophene-2-carboxamide (26f). Compound **26f** (74 mg, 76.6%) was prepared from **10a** (60 mg, 0.19 mmol) and 7-chlorobenzo[b]thiophene-2-carboxylic acid (48 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (t, J = 5.8 Hz, 1H), 8.27 (s, 1H), 7.99 (dd, J = 8.0, 1.0 Hz, 1H), 7.87 (d, J = 8.7 Hz, 1H), 7.62 (dd, J = 7.7, 0.9 Hz, 1H), 7.51 (t, J = 7.8 Hz, 1H), 7.06 (d, J = 2.3 Hz, 1H), 7.02 (dd, J = 8.7, 2.4 Hz, 1H), 4.66 (q, J = 5.6 Hz, 1H), 4.60 (dd, J = 9.9, 2.7 Hz, 1H), 4.18 (s, 2H), 4.16−4.12 (m, 1H), 4.12−4.04 (m, 1H), 3.95 (t, J = 5.0 Hz, 2H), 3.85−3.78 (m, 2H), 3.71−3.66 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.38, 162.25, 153.73, 144.53, 141.03, 140.67, 139.47, 138.04, 127.22, 126.99, 126.61, 126.39, 124.95, 122.16, 118.86, 118.81, 114.67, 74.89, 68.17, 66.28, 63.90, 53.48, 49.46, 41.99. MS (ESI) m/z: 511.7 (M − 1)$^-$. HRMS (ESI): Anal. Calcd for $C_{24}H_{20}O_6N_3ClNaS$, 536.0654; found, 536.0668.

6-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-benzo[b]thiophene-2-carboxamide (26g). Compound **26g** (81 mg, 83.9%) was prepared from **10a** (60 mg, 0.19 mmol) and 6-chlorobenzo[b]thiophene-2-carboxylic acid (48 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (t, J = 5.9 Hz, 1H), 8.22 (d, J = 1.9 Hz, 1H), 8.19 (s, 1H), 7.99 (d, J = 8.6 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.48 (dd, J = 8.6, 2.0 Hz, 1H), 7.06 (d, J = 2.3 Hz, 1H), 7.01 (dd, J = 8.7, 2.3 Hz, 1H), 4.68−4.62 (m, 1H), 4.59 (dd, J = 10.2, 2.8 Hz, 1H), 4.18 (s, 2H), 4.16−4.12 (m, 1H), 4.07 (t, J = 10.0 Hz, 1H), 3.95 (t, J = 5.0 Hz, 2H), 3.79 (t, J = 5.6 Hz, 2H), 3.69 (dd, J = 6.2, 4.1 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.38, 162.46, 153.74, 144.52, 141.96, 140.64, 138.33, 138.04, 131.65, 127.20, 126.05, 125.40, 122.88, 122.16, 118.86, 118.82, 114.66, 74.89, 68.17, 66.29, 63.90, 53.49, 49.46, 41.91. MS (ESI) m/z: 536.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{24}H_{20}O_6N_3ClNaS$, 536.0654; found, 536.0650.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-benzo[b]thiophene-2-carboxamide (26h). Compound **26h** (69 mg, 71.4%) was prepared from **10a** (60 mg, 0.19 mmol) and 5-chlorobenzo[b]thiophene-2-carboxylic acid (48 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.31−9.16 (t, J = 5.7 Hz, 1H), 8.13−8.03 (m, 3H), 7.90−7.77 (d, J = 8.7 Hz, 1H), 7.54−7.43 (dd, J = 8.7, 2.1 Hz, 1H), 7.09−7.02 (d, J = 2.3 Hz, 1H), 7.02−6.94 (dd, J = 8.7, 2.3 Hz, 1H), 4.66−4.59 (q, J = 5.9 Hz, 1H), 4.59−4.48 (dd, J = 9.4, 2.2 Hz, 1H), 4.19−4.13 (s, 2H), 4.13−4.00 (m, 2H), 3.97−3.88 (dd, J = 6.0, 4.1 Hz, 2H), 3.81−3.72 (t, J = 5.6 Hz, 2H), 3.70−3.61 (dd, J = 6.1, 4.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.40, 162.40, 153.74, 144.52, 141.88, 141.03, 139.18, 138.03, 130.42, 126.88, 126.61, 125.08, 124.97, 122.16, 118.87, 118.82, 114.68, 74.90, 68.17, 66.27, 63.90, 53.47, 49.46, 41.96. MS (ESI) m/z: 536.2 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{24}H_{20}O_6N_3ClNaS$, 536.0654; found, 536.0640.

4-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-benzo[b]thiophene-2-carboxamide (26i). Compound **26i** (76 mg, 78.7%) was prepared from **10a** (60 mg, 0.19 mmol) and 4-chlorobenzo[b]thiophene-2-carboxylic acid (48 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (t, J = 5.8 Hz, 1H), 8.34 (s, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.7 Hz, 1H), 7.56 (dd, J = 7.7, 0.9 Hz, 1H), 7.48 (t, J = 7.9 Hz, 1H), 7.06 (d, J = 2.3 Hz, 1H), 7.02 (dd, J = 8.7, 2.4 Hz, 1H), 4.66 (q, J = 5.6 Hz, 1H), 4.60 (dd, J = 10.0, 2.7 Hz, 1H), 4.18 (s, 2H), 4.16−4.12 (m, 1H), 4.12−4.05 (m, 1H), 3.95 (dd, J = 6.0, 4.1 Hz, 2H), 3.84−3.78 (m, 2H), 3.72−3.67 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.38, 162.22, 153.76, 144.53, 142.04, 141.33, 138.05, 137.64, 129.01, 127.83, 125.33, 123.19, 122.54, 122.15, 118.84-(overlap), 114.66, 74.94, 68.17, 66.28, 63.90, 53.50, 49.46, 42.02. MS (ESI) m/z: 536.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{24}H_{20}O_6N_3ClNaS$, 536.0654; found, 536.0653.

3,6-Dichloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)benzo[b]thiophene-2-carboxamide (26j). Compound **26j** (86 mg, 83.4%) was prepared from **10a** (60 mg, 0.19 mmol) and 3,6-dichlorobenzo[b]thiophene-2-carboxylic acid (56 mg, 0.23 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (t, J = 5.9 Hz, 1H), 8.34 (d, J = 1.9 Hz, 1H), 7.91 (d, J = 8.7 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.63 (dd, J = 8.7, 1.9 Hz, 1H), 7.06 (d, J = 2.3 Hz, 1H), 7.03 (dd, J = 8.7, 2.4 Hz, 1H), 4.69 (q, J = 5.2 Hz, 1H), 4.62−4.59 (m, 1H), 4.18 (s, 2H), 4.13−4.06 (m, 2H), 3.95 (t, J = 5.0 Hz, 2H), 3.83 (q, J = 5.0 Hz, 2H), 3.69 (dd, J = 6.3, 4.3 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.39, 161.24, 153.83, 144.62, 138.37, 138.13, 135.20, 133.16, 132.84, 127.14, 124.56, 123.56, 122.09, 119.44, 119.10, 118.89, 114.68, 74.64, 68.17, 66.32, 63.90, 53.28, 49.47, 41.93. MS (ESI) m/z: 570.0 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{24}H_{19}O_6N_3Cl_2NaS$, 570.0264; found, 570.0255.

2-((2-Chloroethyl)thio)-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (26k). Compound **26k** (72 mg, 84.0%) was prepared from **10a** (60 mg, 0.19 mmol) and 2-((2-chloroethyl)thio)-acetic acid (35 mg, 0.23 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (t, J = 5.9 Hz, 1H), 7.87 (d, J = 8.7 Hz, 1H), 7.06 (d, J = 2.3 Hz, 1H), 7.02 (dd, J = 8.7, 2.4 Hz, 1H), 4.57 (dd, J = 9.6, 2.4 Hz, 1H), 4.55−4.49 (m, 1H), 4.18 (s, 2H), 4.09−3.98 (m, 2H), 3.97−3.93 (m, 2H), 3.76 (t, J = 7.6 Hz, 2H), 3.69 (dd, J = 6.1, 4.1 Hz, 2H), 3.59 (dt, J = 10.1, 5.4 Hz, 2H), 3.24 (s, 2H), 2.95−2.88 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.59, 166.39, 153.69, 144.52, 138.03, 122.12, 118.86, 118.83, 114.64, 74.95, 68.18, 66.31, 63.90, 53.22, 49.47, 43.65, 41.12, 34.37, 34.32. MS (ESI) m/z: 456.1 (M + 1)$^+$. HRMS (ESI): Anal. Calcd for $C_{19}H_{22}O_6N_3ClNaS$, 478.0816; found, 478.0812.

2,2-Dichloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (26l). Compound **26l** (65 mg, 80.4%) was prepared from **10a** (60 mg, 0.19 mmol) and 2,2-dichloroacetic acid (29 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (t, J = 5.9 Hz, 1H), 7.84 (d, J = 8.7 Hz, 1H), 7.06 (d, J = 2.3 Hz, 1H), 7.02 (dd, J = 8.7, 2.4 Hz, 1H), 6.54 (s, 1H), 4.57 (d, J = 6.9 Hz, 2H), 4.18 (s, 2H), 4.08−3.99 (m, 2H), 3.95 (dd, J = 6.0, 4.2 Hz, 2H), 3.72−3.63 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.39, 164.93, 153.63, 144.54, 138.10, 122.05, 118.94, 118.88, 114.67, 74.48, 68.17, 67.06, 66.19, 63.90,

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53.22, 49.46, 41.90. MS (ESI) m/z: 452.0 $(M + 23)^+$. HRMS (ESI): Anal. Calcd for $C_{17}H_{17}O_6N_3Cl_2Na$, 452.0387; found, 452.0384.

1-(5-Chlorothiophen-2-yl)-3-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]-oxazin-3-yl)methyl)urea (27a). To a solution of **10a** (98 mg, 0.31 mmol) and TEA (47 mg, 0.46 mmol) in DCM (10 mL) was added 2-chloro-5-isocyanatothiophene (59 mg, 0.37 mmol). The solution was stirred at room temperature overnight and then concentrated. The resulting residue was purified by chromatography on silica gel with dichloromethane/methanol (100:1) to give **27a** (87 mg, 59.2%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.08−6.99 (m, 2H), 6.81−6.73 (m, 2H), 6.25 (d, J = 4.1 Hz, 1H), 4.54 (dd, J = 12.1, 6.2 Hz, 2H), 4.17 (s, 2H), 4.08−3.98 (m, 2H), 3.97−3.91 (m, 2H), 3.72−3.65 (m, 2H), 3.58 (t, J = 5.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.40, 154.89, 153.80, 144.56, 140.26, 138.07, 123.70, 122.12, 118.90, 118.86, 117.75, 114.67, 107.00, 75.35, 68.18, 66.31, 63.90, 53.14, 49.45, 42.00. MS (ESI) m/z: 501.1 $(M + 23)^+$. HRMS (ESI): Anal. Calcd for $C_{20}H_{19}O_6N_4ClNaS$, 501.0606; found, 501.0610.

1-(4-Chlorophenyl)-3-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)-methyl)urea (27b). Compound **27b** (75 mg, 84.4%) was prepared from **10a** (60 mg, 0.19 mmol) and 1-chloro-4-isocyanatobenzene (35 mg, 0.23 mmol) in the same manner as that described for **27a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (s, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.44−7.37 (m, 2H), 7.29−7.21 (m, 2H), 7.06−6.96 (m, 2H), 6.58 (s, 1H), 4.55 (d, J = 7.2 Hz, 2H), 4.16 (s, 2H), 4.06−3.98 (m, 2H), 3.96−3.89 (m, 2H), 3.70−3.62 (m, 2H), 3.57 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.41, 155.71, 153.82, 144.56, 139.64, 138.06, 128.99 (overlap), 125.25, 122.14, 119.73 (overlap), 118.87 (overlap), 114.69, 75.56, 68.18, 66.34, 63.90, 53.14, 49.46, 41.60. MS (ESI) m/z: 495.1 $(M + 23)^+$. HRMS (ESI): Anal. Calcd for $C_{22}H_{21}O_6N_4ClNa$, 495.1042; found, 495.1043.

***N*^{1}-(5-Chlorothiophen-2-yl)-*N*^{2}-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]-oxazin-3-yl)methyl)oxalamide (28a).** Compound **28a** (71 mg, 74.5%) was prepared from **10a** (60 mg, 0.19 mmol) and 2-((5-chlorothiophen-2-yl)amino)-2-oxoacetic acid (47 mg, 0.23 mmol) in the same manner as that described for **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (s, 1H), 9.41 (t, J = 6.2 Hz, 1H), 7.84 (d, J = 8.7 Hz, 1H), 7.05 (d, J = 2.3 Hz, 1H), 7.01 (dd, J = 8.7, 2.3 Hz, 1H), 6.95 (d, J = 4.2 Hz, 1H), 6.90 (d, J = 4.2 Hz, 1H), 4.63−4.52 (m, 2H), 4.18 (s, 2H), 4.16−4.11 (m, 1H), 4.03 (t, J = 10.2 Hz, 1H), 3.99−3.89 (m, 2H), 3.75−3.64 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.40, 160.01, 157.10, 153.70, 144.59, 138.07, 137.07, 124.37, 122.14, 121.43, 118.97, 118.83, 114.66, 113.57, 74.41, 68.18, 66.28, 63.90, 53.40, 49.46, 41.73. MS (ESI) m/z: 529.1 $(M + 23)^+$. HRMS (ESI): Anal. Calcd for $C_{21}H_{19}O_7N_4ClNaS$, 529.0555; found, 529.0562.

***N*^{1}-(5-Chloropyridin-2-yl)-*N*^{2}-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]-oxazin-3-yl)methyl)oxalamide (28b).** Compound **28b** (76 mg, 80.6%) was prepared from **10a** (60 mg, 0.19 mmol) and 2-((5-chloropyridin-2-yl)amino)-2-oxoacetic acid (45 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.37 (s, 1H), 9.46 (t, J = 6.1 Hz, 1H), 8.51−8.45 (m, 1H), 8.13−7.99 (m, 2H), 7.86 (dd, J = 6.0, 4.3 Hz, 1H), 7.10−6.98 (m, 2H), 4.66−4.55 (m, 2H), 4.19 (s, 2H), 4.17−4.11 (m, 1H), 4.10−4.04 (m, 1H), 3.96 (dd, J = 5.9, 4.2 Hz, 2H), 3.71 (dd, J = 11.2 Hz, 7.5, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.41, 160.66, 158.80, 153.68, 149.21, 147.34, 144.58, 138.79, 138.07, 127.14, 122.14, 118.93, 118.84, 115.70, 114.67, 74.38, 68.18, 66.28, 63.90, 53.35, 49.46, 41.85. MS (EI) m/z: 501 $(M)^+$. HRMS (EI): Anal. Calcd for $C_{22}H_{20}ClN_5O_7$, 501.1051; found, 501.1059.

***N*^{1}-(4-Chlorophenyl)-*N*^{2}-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)oxalamide (28c).** Compound **28c** (68 mg, 72.2%) was prepared from **10a** (60 mg, 0.19 mmol) and 2-((4-chlorophenyl)-amino)-2-oxoacetic acid (45 mg, 0.22 mmol) in the same manner as that described for **11a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.88 (s, 1H), 9.34 (s, 1H), 7.93−7.83 (m, 3H), 7.44 (d, J = 8.7 Hz, 2H), 7.10−7.00 (m, 2H), 4.60 (dd, J = 14.2, 8.4 Hz, 2H), 4.19 (s, 3H), 4.11−3.91 (m, 3H), 3.70 (t, J = 5.1 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.41, 161.09, 158.78, 153.71, 144.58, 138.07, 137.08, 129.11 (overlap), 128.74, 122.49 (overlap), 122.14, 118.96, 118.84, 114.67, 74.49, 68.18, 66.28, 63.90, 53.41, 49.46, 41.78. MS (EI) m/z: 500 $(M)^+$. HRMS (EI): Anal. Calcd for $C_{23}H_{21}ClN_4O_7$, 500.1099; found, 500.1098.

(3*R*,3a*S*)-*N*-(5-Chlorothiophen-2-yl)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazine-3-carboxamide (30a). Compound **30a** (73 mg, 60.3%) was prepared from **29** (90 mg, 0.27 mmol) and 5-chlorothiophen-2-amine (43 mg, 0.32 mmol) in the same manner as that described for **11a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.97 (s, 1H), 7.87 (d, J = 8.6 Hz, 1H), 7.10 (d, J = 2.1 Hz, 1H), 7.06 (dd, J = 8.6, 2.3 Hz, 1H), 6.95 (d, J = 4.2 Hz, 1H), 6.77 (d, J = 4.2 Hz, 1H), 5.15 (d, J = 6.9 Hz, 1H), 4.64 (dd, J = 10.6, 3.2 Hz, 1H), 4.49−4.34 (m, 1H), 4.30−4.13 (m, 3H), 4.04−3.91 (m, 2H), 3.80−3.65 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.44, 164.53, 153.04, 144.65, 138.37, 136.98, 124.09, 121.72, 120.71, 119.07, 118.96, 114.78, 112.28, 73.14, 68.18, 66.44, 63.91, 53.20, 49.45. MS (EI) m/z: 449 (M^+). HRMS (EI): Anal. Calcd for $C_{19}H_{16}SClN_3O_6$, 449.0448; found, 449.0452.

(3*R*,3a*S*)-*N*-(5-Chloropyridin-2-yl)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]oxazine-3-carboxamide (30b). Compound **30b** (63 mg, 52.6%) was prepared from **29** (90 mg, 0.27 mmol) and 5-chloropyridin-2-amine (41 mg, 0.32 mmol) in the same manner as that described for **11a**. ^{1}H NMR (3400 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.31 (d, J = 2.2 Hz, 1H), 8.18 (d, J = 8.9 Hz, 1H), 8.04 (d, J = 8.7 Hz, 1H), 7.73 (dd, J = 8.8, 2.5 Hz, 1H), 7.06−6.97 (m, 2H), 4.81−4.72 (m, 2H), 4.40−4.29 (m, 3H), 4.06−3.96 (m, 3H), 3.77−3.71 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.68, 166.43, 153.89, 150.31, 147.07, 144.89, 138.70, 138.51, 126.53, 121.75, 119.91, 118.84, 115.67, 114.74, 73.31, 68.18, 66.17, 63.91, 53.13, 49.46. MS (EI) m/z: 444 (M^+). HRMS (EI): Anal. Calcd for $C_{20}H_{17}ClN_4O_6$, 444.0837; found, 444.0845.

5-Chloro-*N*-(((3*S*,3a*R*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]thiazin-3-yl)methyl)-thiophene-2-carboxamide (47a). Compound **47a** (62 mg, 86.6%) was prepared from **46a** (50 mg, 0.15 mmol) and 5-chlorothiophene-2-carboxylic acid (29 mg, 0.18 mmol) in the same manner as that described for **11a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.00 (t, J = 5.9 Hz, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.70 (d, J = 4.1 Hz, 1H), 7.29 (d, J = 2.4 Hz, 1H), 7.22 (d, J = 4.1 Hz, 1H), 7.15 (dd, J = 8.9, 2.4 Hz, 1H), 4.58 (dd, J = 10.2, 5.5 Hz, 1H), 4.18 (s, 2H), 4.14−4.09 (m, 1H), 3.98−3.92 (m, 2H), 3.70 (dt, J = 10.3, 5.1 Hz, 4H), 3.34−3.27 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.46, 161.29, 154.04, 138.83, 137.84, 133.80, 130.99, 128.99, 128.70, 123.84, 122.57, 122.53, 121.02, 77.15, 68.15, 63.88, 55.56, 49.37, 41.84, 28.16. MS (EI) m/z: 479 (M^+). HRMS (EI): Anal. Calcd for $C_{20}H_{18}S_2ClN_3O_5$, 479.0376; found, 479.0356.

5-Chloro-*N*-(((3*S*,3a*R*)-1-oxo-7-(2-oxopiperidin-1-yl)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]thiazin-3-yl)-methyl)thiophene-2-carboxamide (47b). Compound **47b** (59 mg, 82.3%) was prepared from **46b** (50 mg, 0.15 mmol) and 5-chlorothiophene-2-carboxylic acid (29 mg, 0.18 mmol) in the same manner as that described for **11a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.99 (t, J = 5.9 Hz, 1H), 7.91 (d, J = 8.9 Hz, 1H), 7.70 (d, J = 4.1 Hz, 1H), 7.22 (d, J = 4.0 Hz, 1H), 7.15 (d, J = 2.3 Hz, 1H), 7.03 (dd, J = 8.9, 2.3 Hz, 1H), 4.58 (dd, J = 10.2, 5.4 Hz, 1H), 4.10 (ddd, J = 9.4, 5.4, 4.0 Hz, 1H), 3.76−3.65 (m, 2H), 3.55 (t, J = 5.5 Hz, 2H), 3.34−3.25 (m, 2H), 2.36 (t, J = 6.3 Hz, 2H), 1.89−1.77 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.31, 161.28, 154.04, 139.92, 138.83, 133.79, 130.68, 128.98, 128.70, 124.62, 123.32, 122.33, 121.03, 77.11, 55.56, 51.26, 41.85, 33.02, 28.21, 23.40, 21.33. MS (EI) m/z: 477 (M^+). HRMS (EI): Anal. Calcd for $C_{21}H_{20}S_2ClN_3O_4$, 477.0584; found, 477.0589.

4-Chloro-*N*-(((3*S*,3a*R*)-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]thiazin-3-yl)methyl)-benzamide (47c). Compound **47c** (63 mg, 89.2%) was prepared from **46a** (50 mg, 0.15 mmol) and 4-chlorobenzoic acid (28 mg, 0.18 mmol) in the same manner as that described for **11a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (t, J = 5.8 Hz, 1H), 7.93 (d, J = 8.9 Hz, 1H), 7.89−7.84 (m, 2H), 7.59−7.54 (m, 2H), 7.28 (d, J = 2.4 Hz, 1H), 7.14

(dd, J = 8.9, 2.4 Hz, 1H), 4.60 (q, J = 5.1 Hz, 1H), 4.17 (s, 2H), 4.14 (ddd, J = 9.3, 5.3, 4.0 Hz, 1H), 3.94 (dd, J = 5.7, 4.4 Hz, 2H), 3.73 (t, J = 5.4 Hz, 2H), 3.70−3.65 (m, 2H), 3.30 (dd, J = 6.4, 4.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.52, 166.46, 154.12, 137.86, 136.75, 133.17, 130.99, 129.73 (overlap), 128.95 (overlap), 123.84, 122.64, 122.52, 121.13, 77.14, 68.15, 63.88, 55.57, 49.38, 42.01, 28.25. MS (EI) m/z: 473 (M^+). HRMS (EI): Anal. Calcd for $C_{22}H_{20}SClN_3O_5$, 473.0812; found, 473.0798.

5-Chloro-*N*-(((3*S*,3a*R*)-5-oxido-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]thiazin-3-yl)methyl)thiophene-2-carboxamide (48). To a cooled (0 °C) solution of **47a** (20 mg, 0.042 mmol) in THF (5 mL) was added *m*CPBA (75%, 9.6 mg, 0.042 mmol). The solution was stirred at 0 °C for 1 h, and then ethyl acetate was added. The organics were washed with saturated sodium thiosulfate and a 10% NaOH aqueous solution, dried over anhydrous Na_2SO_4, and filtered, and then the filtrate was concentrated in vacuo. The residue was purified by chromatography on silica gel with dichloromethane/methanol (30:1) to afford **48** (19 mg, 91.9%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.05 (t, J = 5.7 Hz, 1H), 8.39 (d, J = 9.1 Hz, 1H), 7.95 (d, J = 2.5 Hz, 1H), 7.71 (d, J = 4.1 Hz, 1H), 7.67 (dd, J = 9.1, 2.5 Hz, 1H), 7.19 (d, J = 4.1 Hz, 1H), 4.72 (dt, J = 7.4, 5.0 Hz, 1H), 4.55 (ddd, J = 12.4, 7.5, 2.0 Hz, 1H), 4.21 (s, 2H), 3.97 (dd, J = 5.8, 4.3 Hz, 2H), 3.80−3.71 (m, 4H), 3.50 (dd, J = 13.9, 2.0 Hz, 1H), 3.39−3.35 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.67, 161.26, 153.23, 138.82, 137.20, 133.79, 132.08, 130.83, 129.67, 129.05, 128.66, 127.70, 119.33, 76.72, 68.15, 63.87, 49.27, 47.49, 45.33, 41.28. MS (ESI) m/z: 518.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{20}H_{18}O_6N_3ClNaS_2$, 518.0218; found, 518.0221.

5-Chloro-*N*-(((3*S*,3a*R*)-5,5-dioxido-1-oxo-7-(3-oxomorpholino)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]thiazin-3-yl)methyl)thiophene-2-carboxamide (49a). To a cooled (0 °C) solution of **47a** (30 mg, 0.062 mmol) in THF (5 mL) was added *m*CPBA (75%, 36 mg, 0.16 mmol). The solution was stirred at 0 °C for 1 h, and then ethyl acetate was added. The organics were washed with saturated sodium thiosulfate and a 10% NaOH aqueous solution, dried over anhydrous Na_2SO_4, and filtered, and then the filtrate was concentrated in vacuo. The residue was purified by chromatography on silica gel with dichloromethane/methanol (30:1) to afford **49a** (31 mg, 96.9%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.99 (t, J = 5.7 Hz, 1H), 8.31 (d, J = 9.2 Hz, 1H), 7.96 (d, J = 2.5 Hz, 1H), 7.74−7.68 (m, 2H), 7.21 (d, J = 4.1 Hz, 1H), 4.74 (ddd, J = 11.8, 7.3, 2.6 Hz, 1H), 4.71−4.65 (m, 1H), 4.22 (s, 2H), 4.20−4.08 (m, 2H), 3.98 (t, J = 5.0 Hz, 2H), 3.82−3.71 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.79, 161.31, 153.06, 138.78, 137.70, 133.81, 132.07, 130.88, 129.07, 128.66, 128.07, 120.82, 119.82, 76.41, 68.13, 63.82, 55.76, 50.62, 49.15, 40.97. MS (ESI) m/z: 534.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{20}H_{18}O_7N_3ClNaS_2$, 534.0167; found, 534.0169.

5-Chloro-*N*-(((3*S*,3a*R*)-5,5-dioxido-1-oxo-7-(2-oxopiperidin-1-yl)-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]thiazin-3-yl)methyl)thiophene-2-carboxamide (49b). Compound **49b** (30 mg, 93.7%) was prepared from **47b** (30 mg, 0.063 mmol) and *m*CPBA (75%, 32 mg, 0.14 mmol) in the same manner as that described for **49a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.01 (t, J = 5.8 Hz, 1H), 8.28 (d, J = 9.1 Hz, 1H), 7.80 (d, J = 2.5 Hz, 1H), 7.72 (d, J = 4.1 Hz, 1H), 7.60 (dd, J = 9.1, 2.5 Hz, 1H), 7.22 (d, J = 4.1 Hz, 1H), 4.74 (ddd, J = 11.9, 7.3, 2.6 Hz, 1H), 4.70−4.66 (m, 1H), 4.20−4.08 (m, 2H), 3.82−3.74 (m, 2H), 3.62 (td, J = 11.6, 5.8 Hz, 2H), 2.41 (t, J = 6.3 Hz, 2H), 1.92−1.80 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.70, 161.32, 153.09, 139.77, 138.80, 133.82, 131.95, 131.82, 129.08, 128.69, 128.00, 121.54, 119.76, 76.38, 55.76, 51.05, 50.66, 40.99, 33.03, 23.32, 21.23. MS (ESI) m/z: 532.1 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{21}H_{20}O_6N_3ClNaS_2$, 532.0374; found, 532.0379.

5-Chloro-*N*-(((3*S*,3a*R*)-7-(2-(methylsulfonyl)phenyl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]thiazin-3-yl)methyl)thiophene-2-carboxamide (56a). Compound **56a** (56 mg, 66.3%) was prepared from **55a** (80 mg, 0.16 mmol) and 1-bromo-2-(methylsulfonyl)benzene (45 mg, 0.19 mmol) in the same manner as that described for **25a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.98 (t, J = 5.9 Hz, 1H), 8.06 (dd, J = 8.0, 1.2 Hz, 1H), 8.03 (d, J = 8.6 Hz, 1H), 7.74 (td, J = 7.5, 1.3 Hz, 1H), 7.69 (d, J = 4.1 Hz, 1H), 7.66 (dd, J = 7.8, 1.2 Hz, 1H), 7.38 (dd, J = 7.5, 1.1 Hz, 1H), 7.23 (d, J = 2.0 Hz, 1H), 7.20 (d, J = 4.0 Hz, 1H), 7.15 (dd, J = 8.5, 2.1 Hz, 1H), 4.58 (dd, J = 10.5, 5.7 Hz, 1H), 4.20−4.11 (m, 1H), 3.77−3.65 (m, 2H), 3.35 (d, J = 12.5 Hz, 2H), 2.88 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.28, 154.01, 140.23, 139.62, 138.82, 134.70, 133.78, 133.20, 132.66, 132.49, 131.90, 128.93, 128.68, 128.50, 127.98, 126.86, 121.57, 119.77, 77.15, 55.68, 44.22, 41.81, 28.09. MS (EI) m/z: 534 (M^+). HRMS (EI): Anal. Calcd for $C_{23}H_{19}S_3ClN_2O_5$, 534.0145; found, 534.0152.

4-Chloro-*N*-(((3*S*,3a*R*)-7-(2-(methylsulfonyl)phenyl)-1-oxo-1,3,3a,4-tetrahydrobenzo[b]oxazolo[3,4-d][1,4]thiazin-3-yl)methyl)benzamide (56b). Compound **56b** (75 mg, 71.0%) was prepared from **55b** (100 mg, 0.20 mmol) and 1-bromo-2-(methylsulfonyl)benzene (56 mg, 0.24 mmol) in the same manner as that described for **25a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (t, J = 5.8 Hz, 1H), 8.06 (dd, J = 8.0, 1.2 Hz, 1H), 8.01 (d, J = 8.5 Hz, 1H), 7.89−7.85 (m, 2H), 7.74 (td, J = 7.5, 1.4 Hz, 1H), 7.68−7.64 (m, 1H), 7.58−7.54 (m, 2H), 7.38 (dd, J = 7.6, 1.2 Hz, 1H), 7.23 (d, J = 2.0 Hz, 1H), 7.15 (dd, J = 8.6, 2.1 Hz, 1H), 4.61 (dd, J = 10.6, 5.3 Hz, 2H), 4.24−4.16 (m, 2H), 3.74 (dd, J = 8.7, 5.3 Hz, 2H), 2.88 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.49, 154.09, 140.24, 139.61, 136.75, 134.72, 133.78, 133.21, 133.17, 132.66, 129.73 (overlap), 128.93 (overlap), 128.50, 127.96, 126.85, 121.63, 119.89, 77.13, 55.70, 44.23, 41.99, 28.19. MS (EI) m/z: 528 (M^+). HRMS (EI): Anal. Calcd for $C_{23}H_{19}S_3ClN_2O_5$, 528.0580; found, 528.0580.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(3-oxomorpholino)-3,3a,4,5-tetrahydro-1*H*-oxazolo[3,4-a]quinolin-3-yl)methyl)thiophene-2-carboxamide (72a). Compound **72a** (88 mg, 80.6%) was prepared from **71a** (75 mg, 0.24 mmol) and 5-chlorothiophene-2-carboxylic acid (46 mg, 0.28 mmol) in the same manner as that described for **11a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.99 (t, J = 5.8 Hz, 1H), 8.10−8.05 (m, 1H), 7.70 (d, J = 4.1 Hz, 1H), 7.19 (dd, J = 7.1, 3.0 Hz, 3H), 4.47 (dt, J = 8.2, 5.2 Hz, 1H), 4.16 (s, 2H), 3.94 (t, J = 5.0 Hz, 3H), 3.66 (dt, J = 6.7, 3.3 Hz, 4H), 2.88−2.82 (m, 2H), 2.18−2.11 (m, 1H), 1.78 (dt, J = 21.9, 10.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.37, 161.15, 153.70, 138.89, 136.94, 133.76, 133.56, 128.94, 128.67, 126.94, 126.24, 124.34, 117.74, 78.18, 68.16, 63.92, 57.21, 49.57, 41.45, 26.62, 25.26. MS (EI) m/z: 461 (M^+). HRMS (EI): Anal. Calcd for $C_{21}H_{20}SClN_3O_5$, 461.0812; found, 461.0804.

5-Chloro-*N*-(((3*S*,3a*S*)-1-oxo-7-(2-oxopiperidin-1-yl)-3,3a,4,5-tetrahydro-1*H*-oxazolo[3,4-a]quinolin-3-yl)methyl)thiophene-2-carboxamide (72b). Compound **72b** (120 mg, 82.3%) was prepared from **71b** (100 mg, 0.32 mmol) and 5-chlorothiophene-2-carboxylic acid (62 mg, 0.38 mmol) in the same manner as that described for **11a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.99 (t, J = 5.8 Hz, 1H), 8.07−8.02 (m, 1H), 7.70 (d, J = 4.1 Hz, 1H), 7.19 (d, J = 4.1 Hz, 1H), 7.09−7.05 (m, 2H), 4.47 (dt, J = 8.1, 5.2 Hz, 1H), 3.94 (ddd, J = 11.1, 8.2, 2.6 Hz, 1H), 3.70−3.62 (m, 2H), 3.57−3.49 (m, 2H), 2.88−2.79 (m, 2H), 2.34 (t, J = 6.3 Hz, 2H), 2.17−2.09 (m, 1H), 1.88−1.72 (m, 5H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.19, 161.14, 153.68, 139.02, 138.90, 133.75, 133.22, 128.93, 128.66, 127.63, 126.08, 125.00, 117.70, 78.13, 57.22, 51.43, 41.47, 33.00, 26.59, 25.35, 23.46, 21.39. MS (EI) m/z: 459 (M^+). HRMS (EI): Anal. Calcd for $C_{22}H_{22}SClN_3O_4$, 459.1020; found, 459.1017.

***tert*-Butyl 3-((5-chlorothiophene-2-carboxamido)methyl)-1-oxo-7-(2-oxopiperidin −1-yl)-3a,4-dihydro-1*H*-oxazolo[3,4-a]-quinoxaline-5(3*H*)-carboxylate (84b).** Compound **84b** (0.26 g, 83.9%) was prepared from **83b** (0.23 g, 0.55 mmol) and 5-chlorothiophene-2-carboxylic acid (0.11 g, 0.68 mmol) in the same manner as that described for **11a**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.03 (t, J = 5.8 Hz, 1H), 8.10 (d, J = 8.9 Hz, 1H), 7.72 (d, J = 4.1 Hz, 1H), 7.60 (d, J = 1.5 Hz, 1H), 7.22 (d, J = 4.1 Hz, 1H), 7.04 (dd, J = 8.9, 2.4 Hz, 1H), 4.62 (dt, J = 7.6, 5.3 Hz, 1H), 4.54 (dd, J = 12.8, 3.0 Hz, 1H), 3.94 (ddd, J = 10.7, 7.7, 3.2 Hz, 1H), 3.77−3.70 (m, 2H), 3.55 (dq, J = 24.3, 5.9 Hz, 2H), 3.19 (dd, J = 12.7, 10.6 Hz, 1H), 2.37 (t, J = 6.4 Hz, 2H), 1.85 (ddd, J = 17.7, 7.5, 5.0 Hz, 4H), 1.45 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.23, 161.33, 153.81, 152.20, 138.74, 138.67, 133.88, 129.04, 128.68, 127.16, 125.26, 122.69, 122.54, 117.70, 82.05, 75.43, 55.38, 51.55, 41.39, 32.99, 31.17, 28.16 (overlap), 23.46, 21.39. MS (ESI) m/z: 583.0 (M + 23)$^+$. HRMS (ESI): Anal. Calcd for $C_{26}H_{30}SClN_4O_6$, 561.1569; found, 561.1570.

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5-Chloro-*N*-((1-oxo-7-(3-oxomorpholino)-3,3a,4,5-tetrahydro-1*H*-oxazolo[3,4-a]quinoxalin-3-yl)methyl)thiophene-2-carboxamide (85a). To a solution of **84a** (0.53 g, 0.94mmol) in DCM (5 mL) was added TFA (0.3 mL). The solution was stirred at room temperature for 2 h and then evaporated under reduced pressure. The residue was purified by chromatography on silica gel with dichloromethane/methanol (30:1) to give **85a** (0.36 g, 82.6%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.98 (t, J = 5.9 Hz, 1H), 7.70 (d, J = 4.1 Hz, 1H), 7.64 (d, J = 8.6 Hz, 1H), 7.19 (d, J = 4.0 Hz, 1H), 6.65 (d, J = 2.3 Hz, 1H), 6.56 (dd, J = 8.6, 2.3 Hz, 1H), 6.34 (d, J = 4.0 Hz, 1H), 4.50 (dd, J = 11.6, 5.3 Hz, 1H), 4.14 (s, 2H), 3.95−3.90 (m, 2H), 3.84 (ddd, J = 9.8, 6.6, 3.3 Hz, 1H), 3.71−3.65 (m, 2H), 3.63−3.59 (m, 2H), 3.53 (dt, J = 7.5, 4.0 Hz, 1H), 3.10−3.02 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.17, 161.25, 154.05, 138.83, 138.35, 136.65, 133.80, 128.96, 128.68, 120.00, 119.37, 113.49, 112.08, 76.07, 68.17, 63.92, 54.10, 49.66, 43.30, 41.87. MS (EI) m/z: 462 (M^+). HRMS (EI): Anal. Calcd for $C_{20}H_{19}SClN_4O_5$, 462.0765; found, 462.0767.

5-Chloro-*N*-((1-oxo-7-(2-oxopiperidin-1-yl)-3,3a,4,5-tetrahydro-1*H*-oxazolo[3,4-a]quinoxalin-3-yl)methyl)thiophene-2-carboxamide (85b). Compound **85b** (0.18 g, 95.3%) was prepared from **84b** (0.23 g, 0.41 mmol) in the same manner as that described for **85a**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.00 (t, J = 5.9 Hz, 1H), 7.71 (d, J = 4.1 Hz, 1H), 7.62 (d, J = 8.6 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 6.54 (d, J = 2.2 Hz, 1H), 6.47 (dd, J = 8.6, 2.2 Hz, 1H), 6.28 (d, J = 3.8 Hz, 1H), 4.52 (dd, J = 11.6, 5.4 Hz, 1H), 3.85 (ddd, J = 9.8, 6.6, 3.3 Hz, 1H), 3.69 (td, J = 5.7, 2.3 Hz, 2H), 3.54 (dt, J = 11.0, 3.6 Hz, 1H), 3.50 (t, J = 5.6 Hz, 2H), 3.10−3.05 (m, 1H), 2.34 (t, J = 6.4 Hz, 2H), 1.82 (dt, J = 10.9, 7.1 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.97, 161.26, 154.07, 140.45, 138.85, 136.61, 133.80, 128.98, 128.68, 119.75, 119.34, 114.40, 112.84, 76.03, 54.17, 51.54, 43.40, 41.91, 33.04, 23.50, 21.40. MS (EI) m/z: 460 (M^+). HRMS (EI): Anal. Calcd for $C_{21}H_{21}SClN_4O_4$, 460.0972; found, 460.0988.

In Vitro Enzyme Assays..[8,25] Enzymatic activity against human FXa and related serine proteases (FIIa, FVIIa, FIXa, FXIa, and trypsin) was measured using chromogenic substrates. Reactions were initiated by addition of different substrates (200 μM of L-pyro-Glu-Pro-Arg-pNA, S-2366 for FIIa; 2 mM of S-2366 for FVIIa and FXIa; 3.2 mM of CH_3SO_2-(D)CHG-Gly-Arg-pNA, Biophen-CS51 for FIXa; 300 μM of N-Z-D-Arg-Gly-Arg-pNA, S-2765 for FXa and trypsin) to the wells of 96-well microplates to which a premixed and preincubated enzyme/inhibitor mixture was added. Generation of pNA product was monitored continuously at 405 nm using a microplate reader (FlexStation 3; Molecular Devices, Silicon Valley, CA, USA) for 20 min at 37 °C. Percent inhibition and IC_{50} were calculated by nonlinear regression using GraphPad v5.0.

In Vitro Coagulation Assays.[8] Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured using commercially available kits. Compounds **11a**, **11b**, **25a**, **25c**, **26a**, **47a**, **47b**, **49a**, **72a**, and **85a** and rivaroxaban or DMSO (3 μL) were added to 100 μL citrated rabbit plasma [3.8% sodium citrate, 10:90 (v/v)] and preincubated for 10 min at 37 °C. Clotting times were measured using a coagulometer (C2000-1 Single Channel Coagulation Analyzer; Precil, Beijing, China) in accordance with the manufacturer's instructions. Anticoagulant activity was defined as the concentration required to double the plasma clotting times [CT_2 (μM)].

In Vivo Antithrombotic Efficacy Assays. *Venous Thrombosis Model.* Thrombosis formation was induced in anesthetized rats (n = 10 per dose group) as described previously with slight modifications.[8,26] The vena cava was isolated through a midline abdominal incision, and the surface was cleared by blunt dissection between the renal and iliolumbar veins. **11a** or rivaroxaban suspended in 0.5% carboxymethylcellulose sodium (CMC-Na) solution or vehicle was given via the oral route 60 min before thrombus formation. A strip of filter paper saturated with 20% $FeCl_3$ in water was placed on the vena cava for 1 min. Sixty minutes after application of the filter paper, the vena cava was dissected free. The thrombus was removed, cleaned in saline, blotted dry, and weighed. The protocol for this study was reviewed and approved by the Animal Care and Use Committee of China Pharmaceutical University (Nanjing, China).

AV-SHUNT Model. An arteriovenous (AV) shunt in anesthetized rats (n = 10 per dose group) was undertaken as described previously with slight modifications.[8,26] The left jugular vein and right carotid artery were each cannulated with 8 cm polyethylene tubing. A saline-filled arteriovenous shunt was assembled by inserting a 6 cm long polyethylene tube containing 4-0 silk thread (60 × 0.15 mm) between the jugular and carotid cannulas. **11a** or rivaroxaban suspended in 0.5% carboxymethylcellulose sodium (CMC-Na) solution or vehicle was given via the oral route 60 min before the shunt was opened for 15 min. The thread with its associated thrombus was withdrawn, cleaned in saline, blotted dry, and weighed. The thrombus weight formed on the thread was calculated by subtracting the average weight of the silk thread. The protocol for this study was reviewed and approved by the Animal Care and Use Committee of China Pharmaceutical University (Nanjing, China).

Arterial Thrombosis Model. Antithrombotic activity in a model of arterial thrombosis in anesthetized rats was determined as described previously with minor modifications.[27] **11a** or rivaroxaban dissolved in PEG400/ethanol/H_2O [40:10:50 (v/v/v)] or vehicle was given via the oral route 45 min before the induction of anesthesia. The left carotid artery was exposed and fitted on the proximal end with a stimulating electrode of an experimental intracorporeal thrombosis surveyor (BT87-4; BaoTou Medical College, Inner Mongolia, China). Sixty minutes after oral administration of the test compound, electrical stimulation (3 mA) was started and continued for 2 min. After the end of stimulation, we removed the electrode and fitted a temperature probe on the distal end of the artery. The time taken for thrombus formation was recorded as long as the stimulation began and lasted until the alarm started. If compounds displayed antithrombotic effects, the thrombus-formation time would be prolonged compared with that of the control. The protocol for this study was reviewed and approved by the Animal Care and Use Committee of Peking University (Beijing, China).

Tail-Bleeding Model. Bleeding risk was determined in anesthetized rats as described previously with minor modifications.[8] **11a** or rivaroxaban suspended in 0.5% carboxymethylcellulose sodium (CMC-Na) solution or vehicle was given via the oral route 60 min before the tails of anesthetized rats were transected 4 mm from the tip and immersed vertically immediately into saline at 37 °C. The time until continuous blood flow ceased for >30 s was recorded with a maximum observation time of 30 min (longer bleeding times were assigned a value of 30 min). The protocol for this study was approved by the Animal Care and Use Committee of China Pharmaceutical University (Nanjing, China).

Pharmacokinetic Studies. Compounds **11a**, **11b**, **25a**, **25c**, **47a**, **49a**, **72a**, and **85a** (5% DMSO + 5% Tween-80 in 90% saline) and rivaroxaban (10% DMSO + 10% Tween-80 + 80% PEG400 (40% solution in saline)) were subjected to PK studies on male Sprague−Dawley rats (200−220 g) with four animals in each group. Test compounds were administered via the oral route at 10 and 9 mg/kg or administered via the intravenous route at 3 and 4.5 mg/kg. Serial specimens (0.3 mL) were collected via the retrobulbar vein and quantified by liquid chromatography−mass spectrometry (LC-MS). PK parameters were calculated from the mean plasma concentration by noncompartmental analyses. The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China).

In male dogs, **11a** (5% DMSO + 5% Tween-80 in 90% saline) and rivaroxaban (10% DMSO + 10% Tween-80 + 80% PEG400) were administered via the oral route at 1 mg/kg and intravenous infusion at 0.5 mg/kg with three dogs in each group. Blood samples (1 mL) were collected via the foreleg and quantified by LC-MS. PK parameters were calculated from the mean plasma concentration by non-compartmental analyses. The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of C&O Pharmaceutical Technology (Holdings) Limited (Nanjing, China).

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of all intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

VT, venous thrombosis; AV-SHUNT, arteriovenous thrombosis; SAR, structure−activity relationship; SPR, structure−pharmacokinetic relationship; hERG, human ether-a-go-go related gene; CYP450, cytochrome P450; DTT, 1,4-dithiothreitol; TBSCl, *tert*-butyldimethylsilyl chloride; CbzCl, benzyl chloroformate; HATU, *o*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate

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