Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Discovery of *N*-[2-Hydroxy-6-(4-methoxybenzamido)phenyl]-4-(4-methyl-1,4-diazepan-1-yl)benzamide (Darexaban, YM150) as a Potent and Orally Available Factor Xa Inhibitor

Fukushi Hirayama,*[,†] Hiroyuki Koshio,[†] Tsukasa Ishihara,[†] Shunichiro Hachiya,[†] Keizo Sugasawa,[†] Yuji Koga,[†] Norio Seki,[†] Ryouta Shiraki,[†] Takeshi Shigenaga,[†] Yoshiyuki Iwatsuki,[†] Yumiko Moritani,[†] Kenichi Mori,[†] Takeshi Kadokura,[‡] Tomihisa Kawasaki,[‡] Yuzo Matsumoto,[§] Shuichi Sakamoto,[∥] and Shin-ichi Tsukamoto[†]

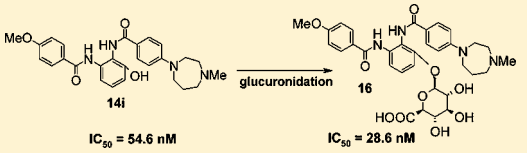
[†]Drug Discovery Research, Astellas Pharma Inc., 21, Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan

[‡]Development, Astellas Pharma Inc., 17-1, Hasune 3-chome, Itabashi-ku, Tokyo 174-8612, Japan

[§]Drug Discovery Research, Astellas Pharma Inc., 2-3-11, Nihonbashi-Honcho, Chuo-ku, Tokyo 103-8411, Japan

[∥]Technology Supply Chain & Manufacturing, Astellas Pharma Europe B.V., Elisabethhof 19, 2350 AC Leiderdorp, The Netherlands

ABSTRACT: Inhibitors of factor Xa (FXa), a crucial serine protease in the coagulation cascade, have attracted a great deal of attention as a target for developing antithrombotic agents. We previously reported findings from our optimization study of a high-throughput screening (HTS) derived lead compound **1a** that resulted in the discovery of potent amidine-containing FXa inhibitors represented by compound **2**. We also conducted an alternative optimization study of **1a** without incorporating a strong basic amidine group, which generally has an adverse effect on the pharmacokinetic profile after oral administration. Replacement of 4-methoxybenzene with a 1,4-benzodiazepine structure and introduction of a hydroxy group at the central benzene led to the discovery of the potent and orally effective factor Xa inhibitor **14i** (darexaban, YM150). Subsequent extensive study revealed a unique aspect to the pharmacokinetic profile of this compound, wherein the hydroxy moiety of **14i** is rapidly transformed into its glucuronide conjugate **16** (YM-222714) as an active metabolite after oral administration and it plays a major role in expression of potent anticoagulant activity in plasma. The distinctive, potent activity of inhibitor **14i** after oral dosing was explained by this unique pharmacokinetic profile and its favorable membrane permeability. Compound **14i** is currently undergoing clinical development for prevention and treatment of thromboembolic diseases.



INTRODUCTION

Intravascular clot formation followed by blood flow blockage in the vital organs causes thromboembolic disorders such as myocardial infarction, unstable angina, deep vein thrombosis, pulmonary embolism, and ischemic stroke. One of the most effective methods for treating and preventing these life threatening conditions has been the use of anticoagulants including unfractionated and low-molecular-weight heparins and warfarin. While warfarin has historically been the sole orally effective anticoagulant used worldwide, its many clinical limitations, such as slow onset and offset of action, narrow therapeutic window, and interaction with many foods and drugs resulting in a need for coagulation monitoring, have long prompted physicians to limit its use.[1] Several of these drawbacks are caused by its mechanism of inhibiting the biosynthesis of vitamin K dependent coagulation factors, highlighting the clear unmet need for anticoagulants with alternative mechanisms that are orally active, clinically safe, and require less monitoring.[2]

Numerous efforts to identify such a novel anticoagulant have been made over the past several decades, and factor Xa (FXa) has attracted substantial attention as a target enzyme. FXa is a trypsin-like serine protease that plays a central role in the blood coagulation cascade. Located at the convergence point of the intrinsic and extrinsic coagulation cascades, FXa is responsible for the proteolysis of prothrombin to catalytically active thrombin. Thrombin has several thrombotic functions, including the proteolytic activation of fibrinogen to fibrin, activation of platelets, and feedback activation of several coagulation factors.[3] As such, inhibition of thrombin generation by a FXa inhibitor is recognized as an attractive target for anticoagulant development. Many small-molecule FXa inhibitors have been reported to date,[4] and the excellent anticoagulant effects of FXa inhibitors without significant hemorrhagic adverse reactions have been demonstrated in animal models[5] as well as recent clinical trial data obtained regarding advanced oral FXa inhibitors such as rivaroxaban,[6] apixaban,[7] betrixaban,[8] and edoxaban.[9] Of these, rivaroxaban was the first to be approved in U.S., EU, Canada, and several other countries for the prevention of venous thromboembolism.

Received: July 3, 2011
Published: October 13, 2011

dx.doi.org/10.1021/jm200868m | *J. Med. Chem.* 2011, 54, 8051–8065

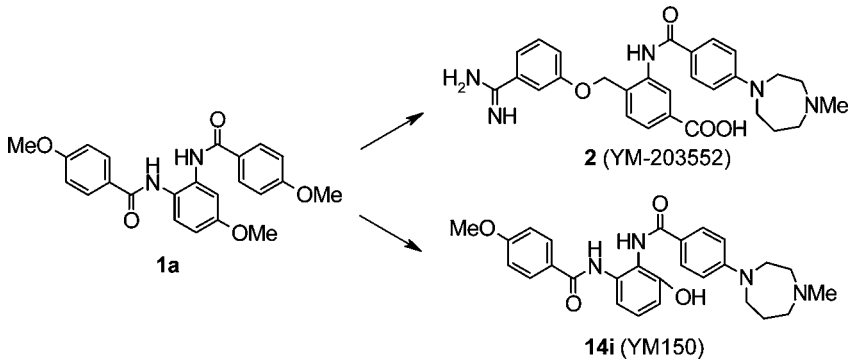
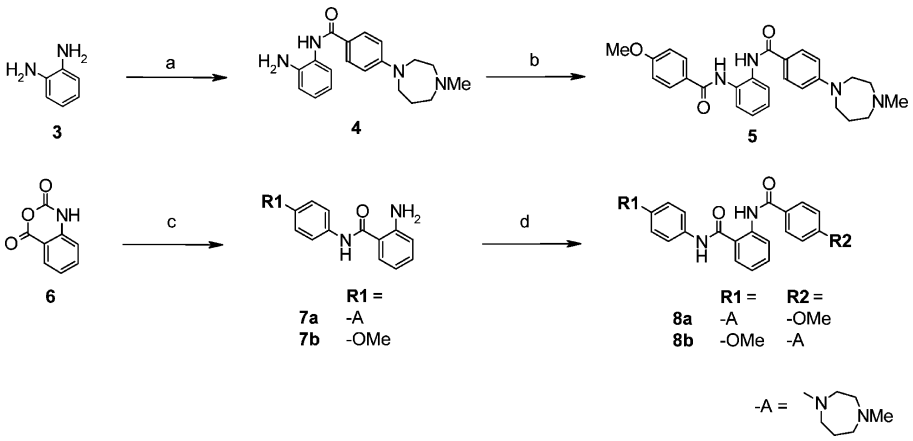


Figure 1. Optimization from HTS hit compound **1a**.

Scheme 1. Synthesis of 1,2-Phenylenediamide and Anthranilic Diamide Derivatives[a]



[a]Reagents and conditions: (a) 4-(4-methyl-1,4-diazepan-1-yl)benzoic acid, WSC, HOBt, DMF, room temperature; (b) 4-methoxybenzoyl chloride, pyridine, room temperature; (c) 4-(4-methyl-1,4-diazepan-1-yl)aniline or 4-methoxyaniline, toluene, reflux; (d) 4-methoxybenzoyl chloride or 4-(4-methyl-1,4-diazepan-1-yl)benzoyl chloride, pyridine, room temperature.

In our search for orally bioavailable FXa inhibitors, we conducted high-throughput screening (HTS) of the library of compounds in the collection of Yamanouchi Pharmaceutical Co., Ltd. (now Astellas Pharma Inc.) and identified compound **1a** as a novel lead compound[10] with a unique 1,2-phenylenediamide scaffold despite its limited activity (Figure 1). We previously reported on the structural optimization of compound **1a** by way of the introduction of an amidine group leading to the potent and selective series of novel amidine FXa inhibitors represented by compound **2** (YM-203552).[11,12] Learning from setbacks experienced in clinical development of amidine-containing FXa inhibitors,[4] due to the low oral absorption caused by high polarity, we attempted to identify another type of FXa inhibitor from the same lead compound **1a**. Here, we describe the results of this optimization study without incorporation of an amidine moiety, thereby leading to discovery of the novel FXa inhibitor **14i** (darexaban, YM150).[12] We also report on its unique property of exerting activity after oral administration mainly via its active metabolite **16** (YM-222714),[13] the glucuronide conjugate of compound **14i**.

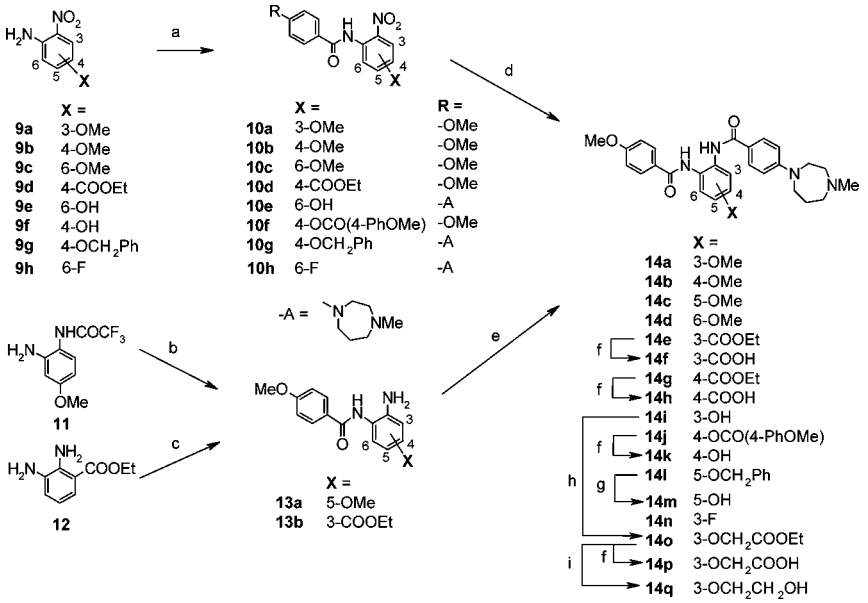
CHEMISTRY

1,2-Phenylenediamide derivative **5** and anthranilic diamide derivatives **8a** and **8b** were synthesized as shown in Scheme 1. Compound **5** was obtained from condensation between 4-methoxybenzoyl chloride and aniline **4**.[11] Anthranilic diamide derivatives **8a** and **8b** were prepared by treatment of commercially available benzoxazine derivative **6** and the corresponding anilines under reflux in toluene followed by acylation with benzoyl chlorides.

The synthesis of substituted 1,2-phenylenediamide derivatives **14a–q** is shown in Scheme 2. Condensation of 2-nitroaniline derivatives (**9a–h**) with 4-methoxybenzoyl chloride or 4-(4-methyl-1,4-diazepane-1-yl)benzoyl chloride[11] gave the intermediate nitro derivatives **10a–h**. Reaction of **9e** with 4-(4-methyl-1,4-diazepane-1-yl)benzoyl chloride generated a phenyl ester intermediate by acylation of the hydroxy group in **9e**. The desired amide intermediate **10e** was obtained by acyl transformation under basic conditions (NEt_3, CH_3CN). Under the same conditions, the hydroxyl group in **9f** was also acylated by treatment of 2.2 equiv of benzoyl chloride and afforded diacyl derivative **10f**. Catalytic hydrogenation of the nitro group of the intermediates **10a–h** gave the corresponding aniline derivatives, followed by condensation with 4-(4-methyl-1,4-diazepane-1-yl)benzoyl chloride[11] or 4-methoxybenzoyl chloride to give the 1,2-phenylenediamide derivatives **14a**, **14b**, **14d**, and **14g–n**. The compounds containing carboxyl (**14h**) or hydroxyl groups (**14k** and **14m**) were prepared by conversion from the protected precursors **14g**, **14j**, and **14l**, respectively. Compound **14o** was prepared from **14i** via benzylammonium intermediate to avoid reaction of **14o** with bromoacetate at the terminal amine. The ethyl ester derivative **14o** was then transformed to the corresponding acid (**14p**) and alcohol

dx.doi.org/10.1021/jm200868m | J. Med. Chem. 2011, 54, 8051–8065

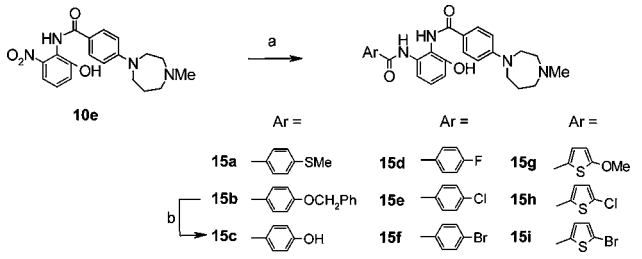
Scheme 2. Synthesis of Substituted 1,2-Phenylenediamide Derivatives[a]



[a]Reagents and conditions: (a) (1) 4-methoxybenzoyl chloride or 4-(4-methyl-1,4-diazepan-1-yl)benzoyl chloride, pyridine, room temperature; (2) for **10e**, NEt_3, CH_3CN, 70 °C; (b) 4-methoxybenzoyl chloride, NEt_3, 1,2-dichloroethane, room temperature; (c) 4-methoxybenzoyl chloride, pyridine, dichloromethane, −78 to 0 °C; (d) (1) H_2, Pd−C, MeOH, or for **14l**, ammonium chloride, iron powder, EtOH, H_2O, reflux; (2) 4-methoxybenzoyl chloride or 4-(4-methyl-1,4-diazepan-1-yl)benzoyl chloride, pyridine, room temperature; (e) 4-methoxybenzoyl chloride, pyridine, room temperature; (f) aq NaOH, MeOH, room temperature; (g) H_2, Pd−C, AcOH; (h) (1) benzyl bromide, MeOH, $CHCl_3$ room temperature; (2) bromoacetate, K_2CO_3, DMF, 100 °C; (3) H_2, Pd−C, AcOH; (i) $NaBH_4$, MeOH, THF, 60 °C.

(**14q**) by alkaline hydrolysis and reduction with $NaBH_4$−MeOH, respectively. Some compounds (**14c** and **14f**) were prepared via intermediate aniline compounds (**13a** and **13b**) synthesized from protected phenilendiamine derivatives **11**[21] or by selective amide formation of **12**. The 3-hydroxy-1,2-phenylenediamide derivatives **15a−i** in Scheme 3 were also

Scheme 3. Synthesis of 3-Hydroxy-1,2-phenylenediamide Derivatives[a]

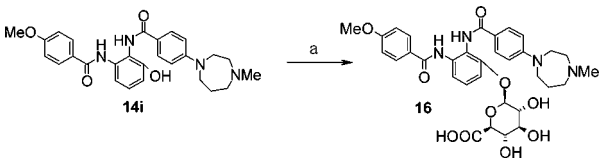


[a]Reagents and conditions: (a) (1) H_2, Pd−C, MeOH; (2) acid chloride, pyridine, room temperature; (b) H_2, Pd−C, AcOH.

prepared from the common intermediate **10e** in the same manner.

Synthesis of **16**, the glucuronide conjugate of **14i**, was accomplished as follows (Scheme 4): Glycosylation of **14i** was accomplished using methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in a mixed solvent of MeOH and $CHCl_3$ at room temperature with simultaneous deacetylation of the sugar moiety. Although significant amounts of **14i** remained in this glycosylation step, despite using excess equivalents of reagents, **14i** was easily removed by extraction with organic solvent after

Scheme 4. Chemical Conversion of Compound 14i into Glucuronide Conjugate 16[a]



[a]Reagents and conditions: (a) (1) methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide, DBU, MeOH, $CHCl_3$, room temperature; (2) Na_2CO_3, MeOH, H_2O, room temperature

hydrolysis of the methyl ester of the product by Na_2CO_3 in MeOH and H_2O. Compound **16** was then purified using chromatography on ODS as a sodium salt, and subsequent precipitation from weakly acidic water yielded the compound in free form.

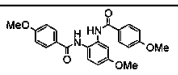
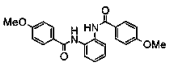
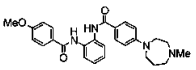
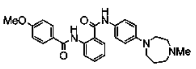
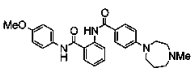
RESULT AND DISCUSSION

Optimization of Lead Compound 1a. Compounds in this report were evaluated based on IC_{50} values for inhibition of human FXa enzymatic activity. Selected compounds were further evaluated based on CT_2 values for the prolongation of prothrombin time (PT) using mouse plasma as an indicator of in vitro anticoagulant activity. CT_2 values were defined as the concentration required to double clotting time. To validate oral efficacy, ex vivo anticoagulant activities after oral administration in mice (100 mg/kg) were also determined based on PT-prolonging effects and expressed as ratios of the PT of the compound-treated mice plasma with those in the vehicle treated group.

Table 1 shows the results of an initial structure−activity relationship (SAR) study for lead compound **1a**. We previously

dx.doi.org/10.1021/jm200868m | J. Med. Chem. 2011, 54, 8051−8065

Table 1. Initial SAR for HTS Hit Compound 1a

compd.	Structure	IC_{50} (nM)[a] factor Xa	CT_2(μM)[b] PT[c]	PT/control PT[d] 0.5 h	2.0 h
1a		6216[e]	ND[f]	ND[f]	ND[f]
1b		4224[e]	ND[f]	ND[f]	*ND*[f]
5		103	2.8	1.38	1.39
8a		539	ND[f]	ND[f]	ND[f]
8b		140	5.9	1.02	0.98

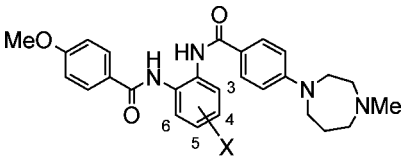
[a]Inhibitory activity against human purified FXa. IC_{50} values are represented by the average of three or more separate determinations with an average standard error of the mean of <15%. [b]CT_2 values are defined as the concentration required to double clotting time and represent the average of three or more separate determinations with the average standard error of the mean being <10%. [c]Prothrombin time using mouse plasma. [d]The relative prothrombin time compared with that measured using normal mice plasma at 0.5 and 2.0 h after oral administration (100 mg/kg, n = 3). [e]See ref 11. [f]ND: not determined.

reported on the 1,4-benzodiazepane structure as an excellent template for docking in the S4 pocket of active site of FXa.[11,14] We therefore adapted this template to non-amidine compound **1b**, which was derived from lead compound **1a** by removing the methoxy moiety on the central benzene,[11] and the resulting compound **5** bearing a 1,4-benzodiazepane instead of a methoxy group showed a 40-fold increase in FXa inhibitory activity (IC_{50} = 4224 nM for **1b** vs IC_{50} = 103 nM for **5**).

We then explored compounds containing the reversed amide linkage in compound **5**. Transformation of the amide linkage attached to the 4-(1,4-diazepane)benzene resulted in a 5-fold decrease in potency (IC_{50} = 539 nM for **8a**), whereas transformation of the amide linkage attached to the 4-methoxybenzene showed only slightly reduced activity (IC_{50} = 140 nM for **8b**). 1,2-Phenylenediamide **5** and anthranilic diamide **8b**, both showing preferable FXa inhibitory activity, were further evaluated in vitro and for ex vivo anticoagulant activity. Compound **5** showed 2-fold more potent in vitro anticoagulant activity than compound **8b** (2.8 μM for **5** vs 5.9 μM for **8b**) and exhibited approximately 1.4-fold PT-prolonging effects at both 0.5 and 2.0 h after oral administration, whereas **8b** lacked any oral efficacy ex vivo. On the basis of these results, we selected 1,2-phenylenediamide **5** for further structural optimization.

We first attempted to introduce substituents to the central phenyl moiety of compound **5** (Table 2). To explore the tolerability of the substituents, a methoxy moiety was introduced at each position. Results showed that the compounds bearing the methoxy moiety at the 3- and 4-position (**14a** and **14b**) had slightly improved FXa inhibitory activity (IC_{50} of 62.6 and 73.4 nM, respectively) compared with compound **5**, whereas compounds modified at the 5- and 6-position (**14c** and **14d**) exhibited diminishing FXa inhibitory activity (IC_{50} of 118 and 213 nM, respectively). Introduction of the methoxy moiety exerted no positive effect on in vitro and ex vivo anticoagulant activity (**14d** was not tested). Taken together, these observations indicated that the 3- and 4-position of the central benzene were likely to be preferable to others when introducing substituents.

Table 2. Effects of Substituents on Central Phenyl Moiety of Compound 5

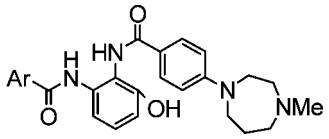


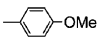
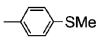
compd	X	IC_{50} (nM),[a] factor Xa	CT_2 (μM),[b] PT[c]	PT/control PT[d] 0.5 h	2.0 h
5	H	103	2.8	1.38	1.39
14a	3-OMe	62.6	3.7	1.50	1.32
14b	4-OMe	73.4	7.5	1.35	1.38
14c	5-OMe	118	7.1	1.25	1.34
14d	6-OMe	213	13	ND[e]	ND[e]
14f	3-COOH	99.2[f]	9.0	1.05	1.08
14h	4-COOH	31.1	1.7	1.00	1.00
14i	3-OH	54.6	4.1	3.49	2.09
14k	4-OH	50.3	1.4	1.04	1.00
14m	5-OH	157	4.7	1.02	1.01
14n	3-F	155	4.3	1.43	1.22
14p	3-OCH2COOH	123	2.8	0.99	0.98
14q	3-OCH_2CH_2OH	106[g]	3.0	1.36	1.31

[a]Inhibitory activity against human purified FXa. IC_{50} values are represented by the average of three or more separate determinations with an average standard error of the mean of <15%. [b]CT_2 values are defined as the concentration required to double clotting time and represent the average of three or more separate determinations with the average standard error of the mean being <10%. [c]Prothrombin time using mouse plasma. [d]The relative prothrombin time compared with that measured using normal mice plasma at 0.5 and 2.0 h after oral administration (100 mg/kg, n = 3). [e]ND: not determined. [f]SEM = ±23.4. [g]SEM = ±18.2.

Given our previous findings that the carboxyl group in certain FXa inhibitors provides favorable effects on activity after oral dosing,[11,14,15] we attempted to introduce a carboxyl group

Table 3. Effects of the Distal Phenyl Moiety



compound	Ar	IC_{50} (nM)[a] factor Xa	CT_2(μM)[b] PT[c]	PT/control PT[d] 0.5 h	2.0 h
14i		54.6	4.1	3.49	2.09
15a		1438	ND[e]	ND[e]	ND[e]
15c		6143	ND[e]	ND[e]	ND[e]
15d		349	ND[e]	ND[e]	ND[e]
15e		64.2	5.3	1.42	1.32
15f		59.8	6.7	1.41	1.62
15g		86.1	3.8	2.17	1.90
15h		41.3	5.8	1.48	1.27
15i		41.4	7.1	1.53	1.22

[a]Inhibitory activity against human purified FXa. IC_{50} values are represented by the average of three or more separate determinations with an average standard error of the mean of <15%. [b]CT_2 values are defined as the concentration required to double clotting time and represent the average of three or more separate determinations with the average standard error of the mean being <10%. [c]Prothrombin time using mouse plasma. [d]The relative prothrombin time compared with that measured using normal mice plasma at 0.5 and 2.0 h after oral administration (100 mg/kg, n = 3). [e]ND: not determined.

to the central benzene ring of compound **5**. While introduction at the 3-position (**14f**) had no effect on FXa inhibitory activity (IC_{50} = 99.2 nM), in vitro anticoagulant activity was decreased (CT_2 = 9.0 μM). In contrast, introduction at the 4-position (**14h**) increased the FXa inhibitory activity 3-fold (IC_{50} = 31.1 nM) and slightly improved the in vitro anticoagulant activity (CT_2 = 1.7 μM). However, both compounds lost their PT prolongation effect after oral dosing.

We then attempted to introduce a phenolic hydroxy group to the benzene ring as a similar acidic substituent. Addition at the 3-position (**14i**) led to modest improvement in the FXa inhibitory activity but decreased anticoagulant activity in vitro (IC_{50} = 54.6 nM, CT_2 = 4.1 μM). However, despite these marginal effects, compound **14i** was found to have a surprisingly potent PT prolongation effect after oral administration in mice (3.49-fold at 0.5 h and 2.09-fold at 2.0 h). In contrast, 4-hydroxy regioisomers **14k** lost all activity after oral administration in mice, despite showing the more potent PT prolongation effects in vitro. Then 5-hydroxy regioisomer **14m** was explored for confirmation, and it gave the same ex vivo results as compound **14k**. While other compounds substituted at the 3-position with introduction of several functional moieties (**14n**, **14p** and **14q**) retained in vitro activity, they did not produce a similar remarkable effect ex vivo as that achieved with **14i**. These results indicate that the introduction of a phenolic hydroxyl moiety at the 3-position on the central benzene improves ex vivo activity after oral dosing over other substituents, an extremely potent effect that is presumed to reflect its preferable pharmacokinetic profile given that the in vitro potency for **14i** was not remarkably high compared with other analogues evaluated in this study.

Table 3 shows the results of structural modification of the distal benzene for the S1 ligand of **14i**. Given the findings from an initial combinatorial modification study in which 4-substituted benzenes showed potent factor Xa inhibitory activity compared to the 2- and 3-substituted regioisomer,[16] optimization was focused on the 4-position. Replacement of the methoxy with a methylthio (**15a**) or hydroxy (**15c**) group reduced the potency of factor Xa inhibitory activity. While halogen substituents such as chloro (**15e**) and bromo (**15f**) displayed in vitro activities (IC_{50} of 64.2 and 59.8 nM and CT_2 of 5.3 and 6.7 μM, respectively) similar to that of the methoxy analogue **14i**, the fluoro substituent (**15d**) had reduced activity (IC_{50} = 349 nM). However, despite demonstration of acceptable activity, both the chloro and bromo analogues showed diminished activity after oral administration and expressed no activity comparable to that of **14i**.

We then transformed benzene into thiophene, a well-known bioisostere for benzene,[17] and introduced methoxy (**15g**), chloro (**15h**), or bromo (**15i**) substituents. These thiophene derivatives also showed similar in vitro activities (IC_{50} of 86.1, 41.3, 41.4 nM and CT_2 of 3.8, 5.8, 7.1 μM, respectively) to those noted with the benzene analogue, and just as was observed with the benzene analogues, the methoxy derivative

dx.doi.org/10.1021/jm200868m|J. Med. Chem. 2011, 54, 8051–8065

15g exerted enhanced ex vivo anticoagulant activity after oral dosing (2.17-fold at 0.5 h and 1.90-fold at 2.0 h). Taken together, these data suggested that both a methoxy moiety at the S1 aromatic ligand and a hydroxy moiety on the central benzene in this series are essential for expression of excellent activity after oral administration. In this way, we successfully identified compound **14i** as the most potent and orally effective FXa inhibitor in this series.

Profiles of Selected Compound 14i. Given that the observed in vitro and ex vivo activity relationships implied favorable pharmacokinetic properties for compound **14i**, we next evaluated plasma concentration after oral dosing to rats. The surprisingly low concentrations of **14i** in the collected plasma samples at all time points measured suggested the potential generation of active metabolites. After intensive examination, we identified glucuronide conjugate **16** as a major metabolite of **14i** in much higher concentration than its parent compound (approximately 50- and 30-fold higher in C_{max} and AUC, respectively, Table 4, Figure 2). To confirm the

Table 4. Pharmacokinetic Parameters of 14i and 16 in Rats after Oral Administration of 100 mg/kg 14i[a]

	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)
14i	2.0	174.8	1291.8
16	0.5	9439.5	38539.2

[a]Monomaeate salt of **14i** was used.

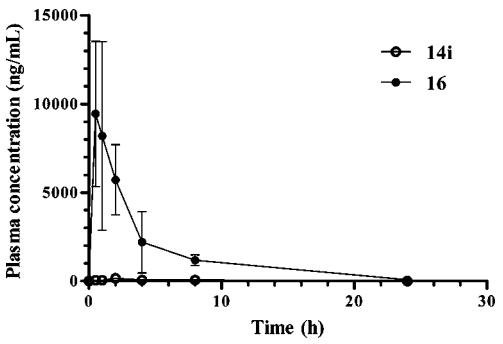


Figure 2. Plasma concentration−time profiles of **14i** and **16** in rats following oral administration of 100 mg/kg **14i** (mean ± SD, n = 3). Monomaleate salt of **14i** was used in this study.

activity of metabolite **16**, we evaluated the chemically synthesized compound **16** and found that glucuronide conjugate **16** surprisingly maintained slightly more potent in vitro activity relative to parent compound **14i** (IC_{50} = 28.6 nM for **16** vs IC_{50} = 54.6 nM for **14i**; CT_2 = 2.5 μM for **16** vs CT_2 = 4.1 μM for **14i**). In addition, the high selectivities of **14i** and **16** were confirmed against other related serine proteases, such as trypsin, thrombin, and kallikrein, and potent anticoagulant activities of **14i** and **16** were also confirmed using human plasma. Detailed in vitro activities are summarized in Table 5.[18]

The above observations clarified the unique behavior of **14i** after oral administration, wherein the compound is immediately converted to its glucuronide conjugate **16** after administration and anticoagulant activity in the blood is mainly exerted by this active metabolite. This pharmacokinetic profile was confirmed in a clinical trial that also identified the glucuronide conjugate **16** as the main active metabolite in human subjects.[19]

To further understand the SAR, in which **14i** shows distinctive, potent activity after oral administration compared

Table 5. In Vitro Activities of Selected Compound 14i and Its Active Metabolite 16

	K_i (μM)[a]				CT_2[b] (μM), PT[c]	
	FXa	thrombin	trypsin	plasma kallikrein	mouse	human
14i	0.031	>100	>100	11	4.1	1.2
16	0.020	>100	>100	17	2.5	0.95

[a]Inhibitory constant for human enzymes. K_i values are the average of four separate determinations with the average standard error of the mean being <10%. [b]CT_2 values are defined as the concentration required to double clotting time and represent the average of three or more separate determinations with the average standard error of the mean being <10%. [c]Prothrombin time using mouse and human plasma.

to related compounds with similar in vitro activity, we evaluated several biological and physiological parameters of the representative compounds (Table 6). Interestingly, values in

Table 6. Comparisons of 14i and Related Compounds in MLM Stability, Membrane Permeability, and Lipophilicity

compd	PAMPA at pH 6.5,[a] P_e (10^{-6} cm/s)	MLM stability,[b] CL_{int} (mL min^{-1} kg^{-1})	CLogP[c]
14i	30.0	1907	2.30
16	<0.2	90.6	−1.03
5	42.0	2047	1.95
14a	47.5	2205	2.84
14k	<0.3	1705	1.41

[a]*p*ION membrane lipid was used at donor buffer, pH 6.5 (n = 2). [b]In vitro metabolism with mouse liver microsomes in the presence of the NADPH-generating system (n = 2). [c]CLogP values were calculated using the software ACD/LogP, version 9.0.

the parallel artificial membrane permeability assay (PAMPA)[20] differed starkly between compounds **14i** and **14k**. Despite both containing hydroxy moieties, the 3-hydroxy derivative **14i** demonstrated favorable membrane permeability (P_e = 30.0 × 10^{-6} cm/s at pH 6.5), while the 4-hydroxy derivative **14k** showed a low value (P_e < 0.3 × 10^{-6} cm/s at pH 6.5). The considerable discrepancy between **14i** and **14k** in activity after oral administration may be attributed to the differing PAMPA values. In terms of metabolic stability to mouse liver microsomes (MLMs), only glucuronide conjugate **16** showed adequate stability (CL_{int} = 90.6 mL min^{-1} kg^{-1}), with the other compounds liable to be metabolized (CL_{int} > 1700 mL min^{-1} kg^{-1}). Taken together, these findings suggest that the remarkable oral activity of compound **14i** may be due to its rapid conversion to the metabolically stable glucuronide conjugate **16** after oral absorption, because of its favorable permeability, subsequently exerting steady anticoagulant activity. Further, as many previous reports have mentioned,[15,21] the markedly low lipophilicity of compound **16** (CLogP = −1.03) may contribute to its excellent anticoagulant activity after oral dosing due to factors including low plasma protein binding. Additionally, glucuronidation is generally recognized as a detoxication process, and subsequently generated glucuronide conjugates are biologically nontoxic;[22] therefore, some measure of safety may be achieved with **14i** given that it exerts its in vivo activity as the glucuronide conjugate **16**.

Molecular Modeling Study of Compounds 14i and 16 in Factor Xa. Figures 3 and 4 show inhibitors **14i** and **16**

dx.doi.org/10.1021/jm200868m | J. Med. Chem. 2011, 54, 8051−8065

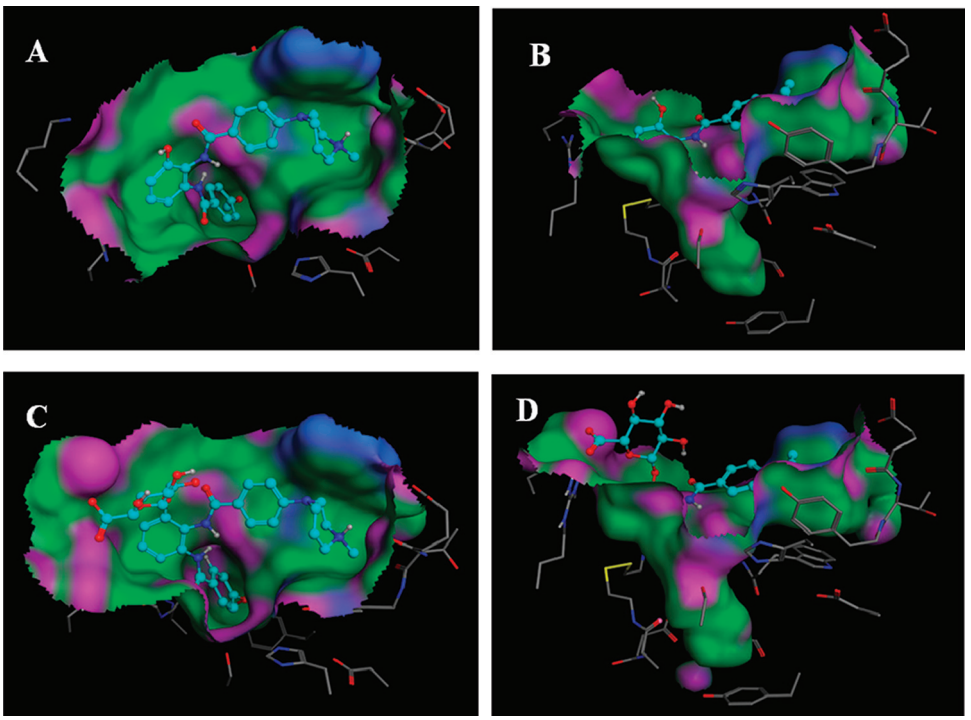


Figure 3. Docking study of **14i** (A, top view; B, side view) and **16** (C, top view; D, side view) in the active site of FXa depicting the surface of FXa. Surface color codes are as follows: magenta, hydrogen bond potential; green, hydrophobic; blue, mildly polar. The hydroxy group of **14i** at the

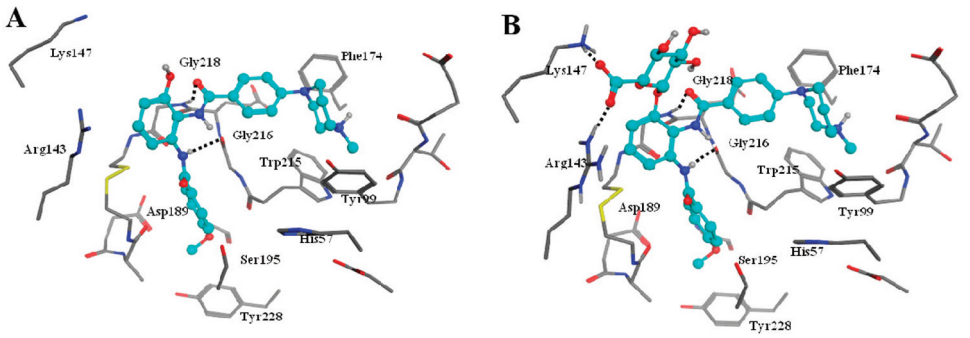


Figure 4. Docking study of **14i** (A) and **16** (B) in active site of FXa. Essential amino acids are depicted with hydrogen bonds shown as dotted lines.

docked in the active site of FXa. The proposed binding model indicated that the 4-methoxyphenyl moiety deeply occupied the S1 pocket, while the 1,4-diazepane moiety fits in the S4 pocket formed by the residues Phe174, Trp215, and Tyr99. The model further suggested that the NH group of the amide bond linked to the methoxyphenyl moiety was within an H-bond distance to the backbone carbonyl group of Gly216. Likewise, the carbonyl group of the amide bond connected to the 4-(1,4-diazepane)benzene was located within an H-bond distance to the backbone NH group of Gly218. These binding elements shape inhibitors **14i** and **16** into an L conformation, with the hydroxy group at the central benzene of **14i** pointing away from the protein surface and facing out toward the wide space of the solvent. As a result, glucuronidation of **14i** at the hydroxy moiety forms without colliding with any residual portions of FXa, and the glucuronic acid moiety in **16** is exposed to the solvent, thereby stabilizing the moiety by hydration. In addition, the docking mode indicated that the carboxyl group of the glucuronic acid moiety forms hydrogen-bonding and ionic interaction with basic amino acid residues Arg143 and Lys147. These docking conformational features explain FXa inhibitory activity expression of the glucuronide conjugate **16**.

CONCLUSION

Optimization of the lead compound **1a** by incorporation of 1,4-benzodiazepane led to compound **5**, with 60-fold enhancement in FXa inhibitory activity. We further optimized its ex vivo activity by modifying the central and distal benzenes, leading to the discovery of **14i**, which demonstrated excellent anticoagulant activity after oral administration. Subsequent extensive studies on **14i** revealed a unique pharmacokinetic feature in the rapid generation of glucuronide conjugate **16** as an active metabolite after oral administration of **14i**, with the activity in the blood predominantly attributed to **16**. The favorable membrane permeability of **14i** and high metabolic stability of active metabolite **16** may contribute to potent activity expression after oral administration of **14i**. The maleate salt of **14i** is currently under clinical development for treatment and prevention of thromboembolic diseases.

dx.doi.org/10.1021/jm200868m | J. Med. Chem. 2011, 54, 8051–8065

EXPERIMENTAL SECTION

General Methods. All reagents and solvents were purchased from commercial sources and used without further purification. ^{1}H NMR spectra were measured using a JEOL JMN-LA-300 or JEOL JMN-EX-400 spectrometer, and chemical shifts were expressed in δ (ppm) units using tetramethylsilane as an internal standard (in ^{1}H NMR description, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). Mass spectra were recorded with a Hitachi M-80 or JEOL JMS-DX300 spectrometer. Elemental analysis was performed on a Yanaco MT-5 microanalyzer (C, H, N) and Yokogawa IC-7000S ion chromatographic analyzer (S, halogens). Melting points were measured with a Yanaco MP-500P melting point apparatus without correction. Purification with ODS column chromatography was performed on YMC gel (ODS-A 120-230/70). For elemental analysis, all tested compounds were within ±0.4% of the theoretical value unless otherwise noted. Purity values for all tested compounds were found to be above 95% from the high-performance liquid chromatography (HPLC) analyses (column, TSKgel ODS-80TM; UV detection, 254 nm; eluent, CH_3CN/0.01 M aq KH_2PO_4 or CH_3CN/0.01 M aq $HClO_4$, with appropriate isocratic conditions selected for each compound; flow rate, 1 mL/min).

4-Methoxy-*N*-(2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]-amino}phenyl)benzamide Hydrochloride (5). A solution of *N*-(2-aminophenyl)-4-(4-methyl-1,4-diazepan-1-yl)benzamide (**4**)[11] (810 mg, 2.50 mmol) and 4-methoxybenzoyl chloride (470 mg, 2.80 mmol) in pyridine (20 mL) was stirred at room temperature for 26 h. The reaction mixture was concentrated under reduced pressure, and the residue was chromatographed on silica gel, eluting with MeOH/$CHCl_3$ (1/10). The solution containing purified compound was acidified with 4 N HCl/EtOAc and concentrated under reduced pressure. The solid was dissolved in H_2O and lyophilized to give **5** (1.18 g, 86%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ:2.13−2.21 (1H, m), 2.24−2.34 (1H, m), 2.78 (3H, d, J = 3.9 Hz), 3.04−3.21 (2H, m), 3.40−3.54 (4H, m), 3.68−3.75 (1H, m), 3.84 (3H, s), 3.86−3.96 (1H, m), 6.85 (2H, d, J = 8.8 Hz), 7.06 (2H, d, J = 8.8 Hz), 7.24−7.27 (2H, m), 7.57−7.66 (2H, m), 7.87 (2H, d, J = 8.8 Hz), 7.96 (2H, d, J = 8.8 Hz), 9.89 (1H, s), 10.14 (1H, s), 10.57 (1H, br). FAB MS m/z 459 (M + H)$^+$. Anal. ($C_{27}H_{30}N_4O_3$·1.3HCl·2.2H_2O) C, H, N, Cl.

2-Amino-*N*-[4-(4-methyl-1,4-diazepan-1-yl)phenyl]-benzamide (7a). A solution of 4-(4-methyl-1,4-diazepan-1-yl)-aniline[23] (1.00 g, 4.90 mmol) and 2*H*-3,1-benzoxazine-2,4(1*H*)-dione (**6**, 0.80 g, 4.90 mmol) in toluene (10 mL) was refluxed for 5 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with 25% aqueous ammonia/MeOH/$CHCl_3$ (0.1/1/10) to give **12** (816 mg, 52%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.96−2.06 (2H, m), 2.38 (3H, s), 2.54−2.59 (2H, m), 2.69−2.73 (2H, m), 3.49 (2H, t, J = 6.2 Hz), 3.55−3.60 (2H, m), 5.49 (2H, s), 6.66−6.73 (4H, m), 7.20−7.24 (1H, m), 7.34−7.38 (2H, m), 7.43−7.47 (1H, m), 7.57 (1H, s). FAB MS m/z 325 (M + H)$^+$

2-[(4-Methoxybenzoyl)amino]-*N*-[4-(4-methyl-1,4-diazepan-1-yl)phenyl]benzamide Hydrochloride (8a). Compound **8a** was prepared from **7a** and 4-methoxybenzoyl chloride according to the procedure for the preparation of **5** in 81% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.11−2.22 (1H, m), 2.26−2.34 (1H, m), 2.79 (3H, d, J = 4.9 Hz), 3.05−3.23 (2H, m), 3.37−3.51 (4H, m), 3.65−3.75 (1H, m), 3.77−3.83 (1H, m), 3.84 (3H, s), 6.80 (2H, d, J = 8.8 Hz), 7.11 (2H, d, J = 8.8 Hz), 7.22−7.28 (1H, m), 7.54 (2H, d, J = 8.8 Hz), 7.56−7.62 (1H, m), 7.88 (2H, d, J = 8.8 Hz), 7.95 (1H, d, J = 7.9 Hz), 8.56 (1H, d, J = 7.9 Hz), 10.38 (1H, s), 10.88 (1H, s), 11.99 (1H, s). FAB MS m/z 459 (M + H)$^+$. Anal. ($C_{27}H_{30}N_4O_3$·1.8HCl·2.2H_2O) C, H, N, Cl.

4′-Methoxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]-amino}benzanilide Hydrochloride (8b). A solution of 4-(4-methyl-1,4-diazepan-1-yl)benzoic acid[11] (1.10 g, 4.06 mmol) in thionyl chloride (10 mL) was stirred at 60 °C for 2 h. The reaction mixture was concentrated under reduced pressure. To the stirred solution of **7b**[24] (930 mg, 3.84 mmol) in pyridine (10 mL,) was added a solution of the residue in 1,2-dichloroethane (10 mL), and the mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure, and the residue was chromatographed on silica gel, eluting with MeOH/$CHCl_3$ (1/8). The solution containing purified compound was acidified with 4 N HCl/EtOAc and concentrated under reduced pressure. The solid was suspended with EtOH and filtered to give **8b** (626 mg, 32%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.11−2.23 (1H, m), 2.29−2.40 (1H, m), 2.78 (3H, s), 3.03−3.24 (2H, m), 3.38−3.49 (2H, m), 3.50−3.59 (2H, m), 3.76 (3H, s), 3.79−3.82 (2H, m), 3.89−3.98 (2H, m), 6.91 (2H, d, J = 8.8 Hz), 6.97 (2H, d, J = 8.8 Hz), 7.22 (1H, t, J = 8.3 Hz), 7.58 (1H, t, J = 8.3 Hz), 7.62 (2H, d, J = 8.8 Hz), 7.79 (2H, d, J = 8.8 Hz), 7.95 (1H, d, J = 8.3 Hz), 8.61 (1H, d, J = 8.3 Hz), 10.47 (1H, s), 10.84 (1H, s), 11.84 (1H, s). FAB MS m/z 459 (M + H)$^+$. Anal. ($C_{27}H_{30}N_4O_3$·HCl·0.8H_2O) C, H, N, Cl.

4-Methoxy-*N*-(3-methoxy-2-nitrophenyl)benzamide (10a). To a stirred solution of 3-methoxy-2-nitroaniline **9a** (900 mg, 5.35 mmol) in pyridine (30 mL) at room temperature was added 4-methoxybenzoyl chloride (1.11 g, 6.51 mmol). After the mixture was stirred at room temperature for 18 h, the solvents were removed under reduced pressure. The residue was dissolved in EtOAc, and the solution was washed with 1% aqueous $NaHCO_3$ and 0.1 M aqueous HCl, dried over Na_2SO_4, and then concentrated under reduced pressure. The resulting residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH (100:1) to give **10a** (0.70 g, 43%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 3.84 (3H, s), 3.90 (3H, s), 7.02−7.13 (3H, m), 7.20 (1H, d, J = 8.4 Hz), 7.57 (1H, t, J = 8.8 Hz), 7.89 (2H, d, J = 9.2 Hz), 10.26 (1H, s). FAB MS m/z 303 (M + H)$^+$.

4-Methoxy-*N*-(4-methoxy-2-nitrophenyl)benzamide (10b). Compound **10b** was prepared from 4-methoxy-2-nitroaniline **9b** and 4-methoxybenzoyl chloride according to the procedure for the preparation of **10a** in 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.88 (3H, s), 3.89 (3H, s), 7.02 (2H, d, J = 8.8 Hz), 7.30 (1H, dd, J = 2.9, 9.3 Hz), 7.73 (1H, d, J = 2.9 Hz), 7.95 (2H, d, J = 8.8 Hz), 8.89 (1H, d, J = 9.3 Hz), 11.05 (1H, s). FAB MS m/z 303 (M + H)$^+$.

4-Methoxy-*N*-(2-methoxy-6-nitrophenyl)benzamide (10c). Compound **10c** was prepared from **9c** and 4-methoxybenzoyl chloride according to the procedure for the preparation of **10a** in 81% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.88 (3H, s), 3.95 (3H, s), 6.98 (2H, d, J = 8.8 Hz), 7.18 (1H, d, J = 8.2 Hz), 7.28 (1H, t, J = 8.2 Hz), 7.58 (1H, d, J = 8.2 Hz), 7.91 (2H, d, J = 8.8 Hz), 8.38 (1H, s). FAB MS m/z 303 (M + H)$^+$.

Ethyl 4-[(4-Methoxybenzoyl)amino]-3-nitrobenzoate (10d). Compound **10d** was prepared from **9d** and 4-methoxybenzoyl chloride according to the procedure for the preparation of **10a** in 8% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.35 (3H, t, J = 7.1 Hz), 3.86 (3H, s), 4.37 (2H, q, J = 7.1 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.96 (2H, d, J = 8.8 Hz), 8.07 (1H, d, J = 8.5 Hz), 8.28 (1H, d, J = 8.5 Hz), 8.47 (1H, s), 10.92 (1H, s). FAB MS m/z 345 (M + H)$^+$.

***N*-(2-Hydroxy-6-nitrophenyl)-4-(4-methyl-1,4-diazepan-1-yl)benzamide (10e).** To a stirred solution of 4-(4-methyl-1,4-diazepan-1-yl)benzoic acid[11] hydrochloride (16.30 g, 60.2 mmol) in EtOAc (160 mL) was added thionyl chloride (14.30 g, 120.2 mmol) and DMF (0.88 g 12.0 mmol). After being stirred at 40 °C for 3 h, the reaction mixture was concentrated under reduced pressure. To the solution of the residue in acetonitrile (130 mL) at 5 °C was added a solution of 2-amino-3-nitorophenol **9e** (8.35 g, 54.2 mmol) and pyridine (9.73 mL, 120.4 mmol) in acetonitrile (60 mL), and the mixture was stirred at 5 °C for 21 h. The resulting precipitate was filtered and dried under reduced pressure to give 2-amino-3-nitrophenyl 4-(4-methyl-1,4-diazepan-1-yl)benzoate hydrochloride (21.4 g, 87.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.15−2.22 (1H, m), 2.34−2.45 (1H, m), 2.79 (3H, d, J = 5.0 Hz), 3.05−3.22 (2H, m), 3.40−3.61 (4H, m), 3.79−3.88 (1H, m), 3.95−4.03 (1H, m), 6.69−6.75 (1H, m), 6.93 (2H, d, J = 9.0 Hz), 7.05 (2H, br), 8.00 (2H, d, J = 9.0 Hz), 11.12 (1H, br)

To a stirred solution of the resulting ester (2.0 g, 4.92 mmol) in acetonitrile (20 mL) was added triethylamine (1.37 mL, 9.84 mmol). After the mixture was stirred at 70 °C for 6 h, a solution of sodium hydroxide (197 mg, 4.92 mmol) in water (2 mL) and water (20 mL) was added to the solution. The acetonitrile was removed by heating,

followed by addition of more water (10 mL), and the mixture was stirred at room temperature for 14 h. The resulting precipitate was filtered to give **10e** (1.57 g, 82.6%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.86−1.95 (2H, m), 2.29 (3H, s), 2.45−2.52 (2H, m), 2.65 (2H, t, J = 4.4 Hz), 3.51 (2H, t, J = 6.0 Hz), 3.60 (2H, t, J = 4.4 Hz), 6.76 (2H, d, J = 9.2 Hz), 7.21−7.28 (2H, m), 7.35 (1H, dd, J = 6.8 Hz, 2.4 Hz), 7.84 (2H, d, J = 9.2 Hz), 9.53 (1H, br). FAB MS m/z 371 (M + H)$^+$.

4-[(4-Methoxybenzoyl)amino]-3-nitrophenyl 4-Methoxybenzoate (10f). Compound **10f** was prepared from **9f** and 2.2 equiv of 4-methoxybenzoyl chloride according to the procedure for the preparation of **10a** in 62% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.91 (3H, s), 3.92 (3H, s), 7.01 (2H, d, J = 8.8 Hz), 7.04 (2H, d, J = 8.8 Hz), 7.59 (1H, dd, J = 2.5, 9.3 Hz), 7.98 (2H, d, J = 8.8 Hz), 8.16 (2H, d, J = 8.8 Hz), 8.18 (1H, d, J = 2.9 Hz), 9.10, (1H, d, J = 9.3 Hz), 11.27 (1H, s). FAB MS m/z 423 (M + H)$^+$.

***N*-[4-(Benzyloxy)-2-nitrophenyl]-4-(4-methyl-1,4-diazepan-1-yl)benzamide (10g).** A solution of 4-(4-methyl-1,4-diazepan-1-yl)benzoic acid[11] hydrochloride (3.25 g, 12.0 mmol) in thionyl chloride (17 mL, 233 mmol) was stirred at 60 °C for 0.5 h and concentrated under reduced pressure. To the residue was added a solution of 4-benzyloxy-2-nitroaniline **9g** (2.47 g, 10.1 mmol) in pyridine (100 mL). After the mixture was stirred at room temperature for 14 h, the resulted precipitate was collected by filtration and washed with pyridine. The solid was dissolved in $CHCl_3$, washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_3, and concentrated under reduced pressure to give **10g** (2.23 g, 48%) as a red-brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.99−2.09 (2H, m), 2.39 (3H, s), 2.53−2.60 (2H, m), 2.70−2.75 (2H, m), 3.57 (2H, t, J = 6.3 Hz), 3.62−3.68 (2H, m), 5.12 (2H, s), 6.74 (2H, d, J = 9.0 Hz), 7.29−7.47 (6H, m), 7.82 (1H, d, J = 2.9 Hz), 7.86 (2H, d, J = 9.0 Hz), 8.93 (1H, d, J = 9.5 Hz), 11.03 (1H, s). FAB MS m/z 461 (M + H)$^+$

***N*-(2-Fluoro-6-nitrophenyl)-4-(4-methyl-1,4-diazepan-1-yl)-benzamide (10h).** To a stirred solution of 4-(4-methyl-1,4-diazepan-1-yl)benzoic acid[11] hydrochloride (4.62 g, 17.1 mmol) was added thionyl chloride (17 mL). After being stirred at 60 °C for 70 min, the reaction mixture was concentrated under reduced pressure. To the residue was added a solution of 2-fluoro-6-nitroaniline **9h** (890 mg, 5.70 mmol) and *N*,*N*-dimethylpyridin-4-amine (700 mg, 5.70 mmol) in pyridine (60 mL), and the mixture was stirred at 80 °C for 4 days. The reaction mixture was concentrated under reduced pressure, and the resulting residue was dissolved with $CHCl_3$ followed by washing with 5% aqueous $NaHCO_3$ and concentration under reduced pressure. The residue was chromatographed on silica gel, eluting with MeOH/$CHCl_3$ (1/99 to 10/90) to give **10h** (400 mg, 6.3%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.98−2.09 (2H, m), 2.38 (3H, s), 2.53−2.59 (2H, m), 2.68−2.75 (2H, m), 3.50−3.68 (4H, m), 6.72 (2H, d, J = 9.2 Hz), 7.29 (1H, dt, J = 5.0, 8.4 Hz), 7.46 (1H, ddd, J = 1.4, 8.4, 9.7 Hz), 7.80−7.92 (3H, m), 9.00 (1H, s). FAB MS m/z 373 (M + H)$^+$

4-Methoxy-*N*-(2-amino-5-methoxy)benzamide (13a). To a stirred solution of *N*-(2-amino-4-methoxyphenyl)-2,2,2-trifluoroacetoamide **11**[25] (1.21 g, 5.18 mmol) in 1,2-dichloroethane (52 mL) at 0 °C was added triethylamine (1.57 g, 15.5 mmol) and 4-methoxybenzoyl chloride (1.33 g, 7.80 mmol). After the mixture was stirred at room temperature for 13 h, ethanol (3 mL) and brine were added. The organic layer was separated and concentrated under reduced pressure. To a solution of the residue in methanol (52 mL) and H_2O (13 mL) at room temperature was added K_2CO_3, and the mixture was stirred at 60 °C for 5 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. H_2O was added to the residue, and the solution was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was chromatographed on silica gel, eluting with MeOH/$CHCl_3$ (0/100 to 5/100) to give **13a** (0.89 g, 63%) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.40 (2H, s), 3.76 (3H, s), 3.87 (3H, s), 6.63 (1H, dd, J = 2.9, 8.6 Hz), 6.81 (1H, d, J = 8.6 Hz), 6.96 (2H, d, J = 8.8 Hz), 7.32 (1H, d, J = 2.9 Hz), 7.87 (2H, d, J = 8.8 Hz), 8.18 (1H, s). FAB MS m/z 273 (M + H)$^+$.

Ethyl 2-Amino-3-[(4-methoxybenzoyl)amino)benzoate (13b). To a stirred solution of ethyl 2,3-diaminobenzoate **12** (6.07 g, 33.7 mmol) in pyridine (17 mL) and dichloromethane (17 mL) was added a solution of 4-methoxybenzoyl chloride (6.04 g, 35.4 mmol) in dichloromethane (35 mL) at −70 °C. The reaction mixture was allowed to stir at 0 °C for 2 h and concentrated under reduced pressure. The residue was partitioned between $CHCl_3$ and 5% aqueous $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was chromatographed on silica gel, eluting with MeOH/$CHCl_3$ (1/100) to give **13b** (7.35 g, 88%) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.39 (3H, t, J = 7.1 Hz), 3.88 (3H, s), 4.35 (2H, q, J = 7.1 Hz), 5,92 (2H, s), 6.72 (1H, t, J = 7.9 Hz), 6.97 (2H, d, J = 9.0 Hz), 7.42−7.56 (2H, m), 7.81−7.91 (3H, m).

4-Methoxy-*N*-(3-methoxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}phenyl)benzamide Hydrochloride (14a). To a solution of **10a** (500 mg, 1.65 mmol) in MeOH (35 mL) at room temperature was added 10% Pd−C powder (50 mg), and the mixture was treated with hydrogen at 1 atm for 16 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give crude *N*-(2-amino-3-methoxyphenyl)-4-methoxybenzamide (420 mg) which was used without further purification. To a stirred solution of 4-(4-methyl-1,4-diazepan-1-yl)benzoic acid[11] hydrochloride (570 mg, 2.11 mmol) in thionyl chloride was added DMF (0.1 mL). After the solution was stirred at 50 °C for 0.5 h, the solvents were removed under reduced pressure. A mixture of the resulting residue and crude *N*-(2-amino-3-methoxyphenyl)-4-methoxybenzamide (420 mg) prepared above in pyridine (15 mL) was stirred for 19 h at room temperature, and the reaction mixture was concentrated under reduced pressure. The residue was dissolved with $CHCl_3$, washed with 5% aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was chromatographed on silica gel, eluting with 25% aqueous ammonia/MeOH/$CHCl_3$ (1/10/100), and lyophilized after being dissolved with dilute aqueous HCl to give **14a** (170 mg, 17%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.11−2.40 (2H, m), 2.79 (3H, d, J = 4.9 Hz), 3.04−3.22 (2H, m), 3.38−3.97 (12H, m), 6.85(2H, d, J = 8.8 Hz), 6.95 (1H, d, J = 8.3 Hz), 7.02 (2H, d, J = 8.7 Hz), 7.29 (1H, t, J = 8.3 Hz), 7.42 (1H, d, J = 8.3 Hz), 7.84 (2H, d, J = 8.8 Hz), 7.92 (2H, d, J = 8.8 Hz), 9.48 (1H, s), 9.73 (1H, s), 10.58 (1H, s). FAB MS m/z 489 (M + H)$^+$. Anal. ($C_{28}H_{32}N_4O_4$·1.8HCl·3.8H_2O) C, H, N, Cl.

4-Methoxy-*N*-(4-methoxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}phenyl)benzamide Hydrochloride (14b). Compound **14b** was prepared from **10b** according to the procedure for the preparation of **14a** in 42% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.09−2.22 (1H, m), 2.28−2.42 (1H, m), 2.77 (3H, d, J = 4.4 Hz), 3.01−3.21 (2H, m), 3.37−3.54 (4H, m), 3.70−3.97 (8H, m), 6.80−6.88 (3H, m), 7.05 (2H, d, J = 8.8 Hz), 7.33 (1H, d, J = 2.9 Hz), 7.43 (1H, d, J = 8.8 Hz), 7.86 (2H, d, J = 8.8 Hz), 7.98 (2H, d, J = 8.8 Hz), 9.87 (1H, s), 10.07 (1H, s), 10.93 (1H, s). FAB MS m/z 489 (M + H)$^+$. Anal. ($C_{28}H_{32}N_4O_4$·1.3HCl·1.0H_2O) C, N, Cl. For H: calcd, 6.42%; found, 7.17%.

4-Methoxy-*N*-(5-methoxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl)benzamide Hydrochloride (14c). Compound **14c** was prepared from **13a** according to the procedure for the preparation of **14a** in 50% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.10−2.22 (1H, m), 2.26−2.42 (1H, m), 2.77 (3H, d, J = 4.9 Hz), 3.02−3.20 (2H, m), 3.36−3.55 (4H, m), 3.70−3.85 (7H, m), 3.87−3.97 (1H, m), 6.81−6.87 (3H, m), 7.05 (2H, d, J = 8.8 Hz), 7.28 (1H, d, J = 2.9 Hz), 7.46 (1H, d, J = 8.8 Hz), 7.88 (2H, d, J = 8.8 Hz), 7.95 (2H, d, J = 8.8 Hz), 9.83 (1H, s), 10.11 (1H, s), 10.86 (1H, s). FAB MS m/z 489 (M + H)$^+$. Anal. ($C_{28}H_{32}$-N_4O_4·1.2HCl·1.3H_2O) C, H, N, Cl.

4-Methoxy-*N*-(6-methoxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl)benzamide Hydrochloride (14d). Compound **14d** was prepared from **10c** according to the procedure for the preparation of **14a** in 48% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.08−2.22 (1H, m), 2.27−2.41 (1H, m), 2.75 (3H, d, J = 4.9 Hz), 3.00−3.20 (2H, m), 3.34−3.52 (4H, m),

3.68−4.00 (8H, m), 6.82 (2H, d, J = 8.8 Hz), 6.93 (1H, d, J = 8.3 Hz), 7.06 (2H, d, J = 8.8 Hz), 7.29 (1H, t, J = 8.3 Hz), 7.46 (1H, d, J = 8.3 Hz), 7.77 (2H, d, J = 8.8 Hz), 8.02 (2H, d, J = 8.8 Hz), 9.51 (1H, s), 9.72 (1H, s), 10.93 (1H, s). FAB MS m/z 489 $(M + H)^+$. Anal. ($C_{28}H_{32}N_4O_4 \cdot 1.2HCl \cdot 1.3H_2O$) C, H, N, Cl.

Ethyl 3-[(4-Methoxybenzoyl)amino]-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}benzoate Hydrochloride (14e). Compound **14e** was prepared from **13b** according to the procedure for the preparation of **14a** in 20% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.14 (3H, t, J = 7.3 Hz), 2.08−2.34 (2H, m), 2.80 (3H, d, J = 4.4 Hz), 3.04−3.22 (2H, m), 3.38−3.58 (4H, m), 3.66−3.77 (1H, m), 3.83 (3H, s), 3.86−3.98 (1H, m), 4.16 (2H, q, J = 7.3 Hz), 6.86 (2H, d, J = 8.8 Hz), 7.06 (2H, d, J = 8.8 Hz), 7.41 (1H, t, J = 7.8 Hz), 7.68 (1H, dd, J = 1.5 Hz, 7.8 Hz), 7.82−7.90 (3H, m), 7.94 (2H, d, J = 8.7 Hz), 10.00 (1H, s), 10.11 (1H, s), 10.30 (1H, s). FAB MS m/z 531 $(M + H)^+$.

3-[(4-Methoxybenzoyl)amino]-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}benzoic Acid Hydrochloride (14f). To a stirred suspension of **14e** (500 mg, 0.88 mmol) in MeOH (9.4 mL) was added 1 M aqueous NaOH (1.9 mL). After being stirred at room temperature for 4 h, the reaction mixture was acidified with 1 M aqueous HCl and concentrated under reduced pressure. The resulting residue was directly chromatographed on ODS gel, eluting with CH_3CN/0.01 M aqueous HCl (0/100 to 50/50) gradually and lyophilized to give **14f** (346 mg, 73%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.00−2.42 (2H, m), 2.77 (3H, d, J = 4.4 Hz), 2.97−3.25 (2H, m), 3.30−4.20 (9H, m), 6.87 (2H, d, J = 8.8 Hz), 7.05 (2H, d, J = 8.8 Hz), 7.40 (1H, t, J = 7.8 Hz), 7.75 (1H, d, J = 7.3 Hz), 7.88−7.94 (5H, m), 10.10 (1H, s), 10.33 (1H, s), 10.91 (1H, s). FAB MS m/z 503 $(M + H)^+$. Anal. ($C_{28}H_{30}N_4O_5 \cdot 1.2HCl \cdot 1.5H_2O$) C, H, N, Cl.

Ethyl 4-[(4-Methoxybenzoyl)amino]-3-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}benzoate (14g). Compound **14g** was prepared from **10d** according to the procedure for the preparation of **14a** in 82% yield as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.34 (3H, t, J = 6.9 Hz), 2.15−2.32 (2H, m), 2.76 (3H, s), 3.15−3.36 (6H, m), 3.82−3.90 (5H, m), 4.34 (2H, q, J = 6.9 Hz), 6.86 (2H, d, J = 8.8 Hz), 7.07 (2H, d, J = 8.8 Hz), 7.83−7.86 (2H, m), 7.93 (2H, d, J = 8.8 Hz), 7.99 (2H, d, J = 8.8 Hz), 8.23 (1H, s), 10.11 (1H, s), 10.32 (1H, s). FAB MS m/z 531 $(M + H)^+$.

4-[(4-Methoxybenzoyl)amino]-3-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}benzoic Acid Hydrochloride (14h). Compound **14h** was prepared from **14g** according to the procedure for the preparation of **14f** in 35% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.10−2.22 (1H, m), 2.29−2.43 (1H, m), 2.77 (3H, d, J = 4.9 Hz), 3.02−3.23 (2H, m), 3.37−3.57 (4H, m), 3.71−3.98 (5H, m), 6.86 (2H, d, J = 8.8 Hz), 7.06 (2H, d, J = 8.8 Hz), 7.82 (2H, s), 7.94 (2H, d, J = 8.8 Hz), 8.00 (2H, d, J = 8.8 Hz), 8.21 (1H, s), 10.12 (1H, s), 10.34 (1H,s), 10.94 (1H,s). FAB MS m/z 503 $(M + H)^+$. Anal. ($C_{28}H_{30}N_4O_5 \cdot 1.9HCl \cdot 2.3H_2O$) C, H, N, Cl.

***N*-[2-Hydroxy-6-(4-methoxybenzamido)phenyl]-4-(4-methyl-1,4-diazepan-1-yl)benzamide Hydrochloride (14i).** To a solution of **10e** (2.14 g, 5.77 mmol) in MeOH (43 mL) at room temperature was added 10% Pd−C powder (54.2% water wet, 0.47 g), and the mixture at 30 °C was treated with hydrogen at ambient pressure until the absorption of hydrogen had stopped. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with MeOH/$CHCl_3$ (1/20 to 1/10) to give *N*-(2-amino-6-hydroxyphenyl)-4-(4-methyl-1,4-diazepan-1-yl)-benzamide (1.61 g, 82%) as a pale brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.85−1.94 (2H, m), 2.26 (3H, s), 2.43 (2H, t, J = 5.6 Hz), 2.61 (2H, t, J = 4.8 Hz), 3.51 (2H, t, J = 6.0 Hz), 3.58 (2H, t, J = 4.8 Hz), 4.68 (2H, s), 6.16 (1H, dd, J = 7.6 Hz, 1.2 Hz), 6.24 (1H, dd, J = 8.0 Hz, 1.2 Hz), 6.70−6.81 (3H, m), 7.86 (2H, d, J = 8.8 Hz), 8.93 (1H, br), 8.94 (1H, s).

A solution of *N*-(2-amino-6-hydroxyphenyl)-4-(4-methyl-1,4-diazepan-1-yl)benzamide (2.03 g, 5.96 mmol) and 4-methoxybenzoyl chloride (1.12 g, 6.00 mmol) in pyridine (60 mL) was stirred at room temperature for 3 days. The reaction mixture was concentrated under reduced pressure. To the resulting residue was added 5% aqueous $NaHCO_3$, and the appropriate portion was extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with 25% aqueous ammonia/MeOH/$CHCl_3$ (1/10/100) and crystallized from EtOH to give the free form of **14i** (1.74 g, 62%). The HCl salt of **14i** (5.50 g, 90%) was prepared as a colorless solid by precipitation of the free form of **14i** (5.68 g) from aqueous 0.5 N HCl (100 mL). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.08−2.31 (2H, m), 2.80 (3H, s), 3.03−3.24 (2H, m), 3.36−3.60 (4H, m), 3.62−4.02 (5H, m), 6.81 (1H, d, J = 8.3 Hz), 6,85 (2H, d, J = 8.3 Hz), 7.04 (2H, d, J = 8.3 Hz), 7.14 (1H, d, J = 8.3 Hz)), 7.24 (1H, d, J = 8.3), 7.86 (2H, d, -J = 8.3 Hz), 7.92 (2H, d, J = 8.3 Hz), 9.52 (1H, s), 9.66 (1H, s), 9.80 (1H, s), 10.20 (1H, s). FAB MS m/z 475 $(M + H)^+$. Anal. ($C_{27}H_{30}N_4O_4 \cdot 1.0HCl$) C, H, N, Cl.

4-[(4-Methoxybenzoyl)amino]-3-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}phenyl 4-Methoxybenzoate (14j). Compound **14j** was prepared from **10f** according to the procedure for the preparation of **14a** in 64% yield as a colorless solid. ^{1}H NMR(300 MHz, DMSO-d_6) δ: 1.95−2.06 (2H, m), 2.37 (3H, s), 2.51−2.58 (2H, m), 2.66−2.72 (2H, m), 3.50 (2H, t, J = 6.3 Hz), 3.55−3.61 (2H, m), 3.81 (3H, s), 3.89 (3H, s), 6.67 (2H, d, J = 9.0 Hz), 6.76 (1H, dd, J = 2.6, 8.8 Hz), 6.91 (2H, d, J = 9.0 Hz), 6.95 (2H, d, J = 8.8 Hz), 7.40 (1H, d, J = 2.4 Hz), 7.43 (1H, d, J = 8.8 Hz), 7.88 (2H, d, J = 7.9 Hz), 7.91 (2H, d, J = 7.9 Hz), 8.04 (2H, d, J = 9.0 Hz), 9.20 (1H, s), 9.53 (1H, s). FAB MS m/z 609 $(M + H)^+$.

***N*-(4-Hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]-amino}phenyl)-4-methoxybenzamide Hydrochloride (14k).** Compound **14k** was prepared from **14j** according to the procedure for the preparation of compound **14f** in 46% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.06 (3H, t, J = 7.4 Hz), 2.05−2.48 (2H, m), 2.79 (3H, s), 3.00−3.60 (8H, m), 3.64−4.00 (5H,m), 4.33 (1H, s), 6.63 (1H, dd, J = 2.4, 8.8 Hz), 6.84 (2H, d, J = 8.8 Hz), 7.05 (2H, d, J = 8.8 Hz), 7.20 (1H, d, J = 2.4 Hz), 7.27 (1H, d, J = 8.8 Hz), 7.81 (2H, d, J = 8.8 Hz), 7.94 (2H, d, J = 8.8 Hz), 9.50 (1H, s), 9.68 (1H, s), 9.91 (1H, s), 10.37 (1H, s). FAB MS m/z 475 $(M + H)^+$. Anal. ($C_{28}H_{30}N_4O_5 \cdot 1.0HCl \cdot 1.0C_2H_6O \cdot 1.0H_2O$) C, N, Cl. For H: calcd, 6.84%; found, 6.24%.

***N*-[5-(Benzyloxy)-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl]-4-methoxybenzamide (14l).** A stirred suspension of **10g** (1.15 g, 2.50 mmol), ammonium chloride (0.14 g 2.62 mmol), and iron powder (0.70 g, 12.5 mmol) in EtOH (30 mL) and H_2O (15 mL) was refluxed for 1 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The suspension of the residue in saturated aqueous $NaHCO_3$ was extracted with $CHCl_3$, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give crude *N*-[2-amino-4-(benzyloxy)phenyl]-4-(4-methyl-1,4-diazepan-1-yl)benzamide (1.15 g) which was used without further purification. Compound **14l** was prepared from above aniline derivative according to the same procedure for the preparation of **14i** in 81% yield as pale brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.91−2.06 (2H, m), 2.38 (3H, s), 2.52−2.58 (2H, m), 2.67−2.73 (2H, m), 3.49 (2H, t, J = 6.2 Hz), 3.55−3 61 (2H, m), 3.82 (3H, s), 4.68 (2H, s), 6.54 (1H, dd, J = 2.8, 8.7 Hz), 6.69 (2H, d, -J = 9.0 Hz), 6.95 (2H, d, J = 8.8 Hz), 7.19−7.36 (7H, m), 7.88 (2H, d, J = 9.0 Hz), 8.00 (2H, d, J = 8.8 Hz), 8.76 (1H, s), 9.63 (1H, s).

***N*-(5-Hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]-amino}phenyl)-4-methoxybenzamide Hydrochloride (14m).** To a suspension of **14l** (0.98 g, 1.70 mmol) in AcOH (30 mL) at room temperature was added 10% Pd−C powder (300 mg), and the mixture was treated with hydrogen at ambient pressure for 24 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was suspended in saturated aqueous $NaHCO_3$, extracted with $CHCl_3$, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was crystallized from EtOH and the aqueous solution of the resulting crystal was lyophilized after being acidified with 1 N HCl. **14m** (558 mg, 59%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.11−2.35 (2H, m), 2.80 (3H, d, J = 5.4 Hz),

3.02−3.21 (2H, m), 3.38−3.58 (4H, m), 3.67−3.96 (5H, m), 6.66 (1H, dd, J = 2.4, 8.8 Hz), 6.84 (2H, d, J = 8.8 Hz), 7.04 (2H, d, J = 8.8 Hz), 7.15 (1H, d, J = 2.4 Hz), 7.30 (1H, d, J = 8.8 Hz), 7.85 (2H, d, J = 8.8 Hz), 7.90 (2H, d, J = 8.8 Hz), 9.52 (1H, s), 9.71 (1H, s), 9.95 (1H, s), 10.36 (1H, s). FAB MS m/z 475 $(M + H)^+$. Anal. ($C_{27}H_{30}N_4O_4$·1.0HCl·2.0 H_2O) C, H, N, Cl

***N*-(3-Fluoro-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}phenyl)-4-methoxybenzamide Hydrochloride (14n).** Compound **14n** was prepared from **10h** according to the procedure for the preparation of **14i** in 53% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.16−2.36 (2H, m), 2.79 (3H, d, J = 5.9 Hz), 3.05−3.21 (2H, m), 3.30−3.57 (4H, m), 3.66−3.78 (1H, m), 3.82 (3H, s), 3.88−3.99 (1H, s), 6.85 (2H, d, J = 9.3 Hz), 7.03 (2H, d, J = 8.8 Hz), 7.13−7.18 (1H, m), 7.31−7.37 (1H, m), 7.55−7.59 (1H, m), 7.89 (2H, d, J = 8.8 Hz), 7.94 (2H, d, J = 8.7 Hz), 9.72 (1H, s), 10.03 (1H, s), 10.42 (1H, s). FAB MS m/z 477 $(M + H)^+$. Anal. ($C_{27}H_{29}N_4O_3F$·1.0HCl·1.4H_2O) C, H, N, Cl, F

Ethyl (3-[(4-Methoxybenzoyl)amino]-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}phenoxy)acetate Hydrochloride (14o). To a stirred solution of the free form of **14i** (500 mg, 1.05 mmol) in MeOH (11 mL) and $CHCl_3$ (11 mL) at room temperature was added benzyl bromide (428 mg, 2.50 mmol). The mixture was stirred for 22 h. The resulting precipitate was filtered and washed with the MeOH/$CHCl_3$ (50/50) to give crude 1-benzyl-4-[4-({2-hydroxy-6-[(4-methoxybenzoyl)amino]phenyl}carbamoyl)phenyl]-1-methyl-1,4-diazepan-1-ium bromide (770 mg) as a solid, which was used without further purification. To the stirred solution of the solid in DMF at room temperature, ethyl bromoacetate (210 mg, 1.30 mmol) and potassium carbonate (174 mg, 1.30 mmol) were added, and the mixture was stirred at 100 °C for 1 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give crude 1-benzyl-4-[4-({2-(2-ethoxy-2-oxoethoxy)-6-[(4-methoxybenzoyl)amino]phenyl}carbamoyl)phenyl]-1-methyl-1,4-diazepan-1-ium as a solid, which was used without further purification. To the stirred solution of the solid in acetic acid (16 mL) at room temperature was added 10% Pd−C powder (100 mg), and the mixture was treated with hydrogen under 2.9 atm at room temperature for 3 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The resulting residue was chromatographed on silica gel, eluting with 25% aqueous ammonia/MeOH/$CHCl_3$ (0/0/100 to 1/10/100) and chromatographed on ODS, again eluting with 0.001 M aqueous HCl/EtOH (0/100 to 30/70). The purified solid was lyophilized after being dissolved with dilute aqueous HCl to give **14o** (350 mg, 59%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.21 (3H, t, J =7.3 Hz), 2.01−2.22 (1H, m), 2.26−2.42 (1H, m), 2.78 (3H, d, J = 4.9 Hz), 3.02−3.22 (2H, m), 3.35−4.02 (9H, m), 4.18 (2H, q, J = 7.3 Hz), 4.83 (2H, s), 6.86 (2H, d, J = 9.3 Hz), 6.92 (1H, d, J = 7.3 Hz), 7.04 (2H, d, J = 8.8 Hz), 7.27 (1H, t, J = 8.3 Hz), 7.49 (1H, d, J = 7.8 Hz), 7.86 (2H, d, J = 8.8 Hz), 7.93 (2H, d, J = 8.8 Hz), 9.57 (1H, s), 9.86 (1H, s). FAB MS m/z 561 $(M + H)^+$

(3-[(4-Methoxybenzoyl)amino]-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}phenoxy)acetic Acid Hydrochloride (14p). Compound **14p** was prepared from **14o** according to the procedure for preparation of **14f** in 76% yield as a colorless solid. This compound was obtained by crystallization from H_2O. Mp 186−188, 2.16−2.30 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.16−2.30 (2H, m), 2.78 (3H, s), 3.15−3.58 (6H, m), 3.78−3.88 (5H, m), 4.75 (2H, s), 6.86 (2H, d, J = 9.3 Hz), 6.94 (1H, d, J = 7.3 Hz), 7.04 (2H, d, J = 8.8 Hz), 7.28 (1H, t, J = 7.8 Hz), 7.50 (1H, d, J = 7.9 Hz), 7.85 (2H, d, J = 8.8 Hz), 7.95 (2H, d, J = 8.8 Hz), 9.85−9.98 (2H, m). FAB MS m/z 533 $(M + H)^+$. Anal. ($C_{29}H_{32}N_4O_6$·0.9HCl·0.5H_2O) C, H, N, Cl.

***N*-[3-(2-Hydroxyethoxy)-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl]-4-methoxybenzamide Hydrochloride (14q).** To a stirred solution of **14o** (370 mg, 0.71 mmol) in THF (14 mL) at room temperature was added $NaBH_4$ (216 mg, 5.70 mmol). The reaction mixture was heated to 60 °C, and MeOH (1.86 g, 58.0 mmol) in THF (14 mL) was added dropwise and stirred for 10 min. After the reaction was quenched with H_2O (2 mL), the mixture was concentrated under reduced pressure. The residue was dissolved with $CHCl_3$, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with 25% aqueous ammonia/MeOH/$CHCl_3$ (0/0/100 to 1/10/100). The purified product (150 mg) was dissolved with a mixtutre of EtOH (3 mL) and 1 M aqueous HCl (0.4 mL) and concentrated under reduced pressure. The residue was crystallized from H_2O to give **14q** (107 mg, 27%) as a colorless solid. Mp 174−176 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.12−2.22 (1H, m), 2.26−2.39 (1H, m), 2.79 (3H, d, J = 3.9 Hz), 3.05−3.21 (2H, m), 3.39−3.55 (4H, m), 3.66−3.79 (3H, m), 3.81 (3H, s), 3.90−3.97 (1H, m), 4.11 (2H, t, J = 4.9 Hz), 4.86 (1H, s), 6.86 (2H, d, J = 8.8 Hz), 6.97 (1H, d, J = 7.4 Hz), 7.04 (2H, d, J = 8.8 Hz), 7.27(1H, t, J = 8.3 Hz), 7.42 (1H, d, J = 8.3 Hz), 7.86 (2H, d, J = 8.7 Hz), 7.92 (2H, d, J = 8.8 Hz), 9.55 (1H, s), 9.89 (1H, s), 10.67 (1H, s). FAB MS m/z 519 $(M + H)^+$. Anal. ($C_{29}H_{34}N_4O_5$·1.0HCl·1.2H_2O) C, H, N, Cl.

***N*-(2-Hydroxy-6-{[4-(methylsulfanyl)benzoyl]amino}phenyl)-4-(4-methyl-1,4-diazepan-1-yl)benzamide Hydrochloride (15a).** Compound **15a** was prepared from **10e** and 4-(methylsulfanyl)benzoyl chloride according to the procedure for preparation of **14i** in 46% yield as a colorless solid. Mp 264−268 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.09−2.41 (2H, m), 2.52 (3H, s), 2.78 (3H, s), 3.00−3.23 (2H, m), 3.37−3.57 (4H, m), 3.67−3.82 (1H, m), 3.87−4.00 (1H, m), 6.78−6.90 (3H, m), 7.14 (1H, t, J = 8.3 Hz), 7.24 (1H, d, J = 8.3 Hz), 7.35 (2H, d, J = 8.3 Hz), 7.83 (2H, d, J = 8.3 Hz), 7.93 (2H, d, J = 8.8 Hz), 9.57 (1H, s), 9.68 (1H, s), 9.91 (1H, s), 10.72 (1H, s). FAB MS m/z 491 $(M + H)^+$. Anal. ($C_{27}H_{30}N_4O_3S$·1.0HCl·0.5 H_2O) C, H, N, S, Cl.

4-(Benzyloxy)-*N*-(3-hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}phenyl)benzamide (15b). Compound **15b** was prepared from **10e** and 4-(benzyloxy)benzoyl chloride according to the procedure for preparation of **14i** in 62% yield as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.96−2.08 (2H, m), 2.38 (3H, s), 2.53−2.59 (2H, m), 2.68−2.75 (2H, m), 3.50−3.66 (4H, m), 5.15 (2H, s), 6.73−6.75 (3H, m), 6.82−6.99 (2H, m), 7.08 (2H, d, J = 9.0 Hz), 7.30−7.50 (5H, m), 7.82 (2H, d, J = 9.0 Hz), 7.95 (2H, d, J = 8.8 Hz), 8.61 (1H, s), 9.83 (1H, s). FAB MS m/z 551 $(M + H)^+$.

4-Hydroxy-*N*-(3-hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl)benzamide Hydrochloride (15c). Compound **15c** was prepared from **15b** according to the procedure for preparation of **14m** and crystallized from EtOH−H_2O in 58% yield as a colorless solid. Mp 195−197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.06 (3H, t, J = 6.8 Hz), 2.08−2.40 (2H, m), 3.78 (3H, s), 3.04−3.57 (8H, m), 3.67−3.82 (1H, m), 3.87−3.99 (1H, m), 4.36 (1H, s), 6.77−6.90 (5H, m), 7.13 (1H, t, J = 7.9 Hz), 7.23 (1H, d, J = 7.9 Hz), 7.76 (2H, d, J = 8.8 Hz), 7.93 (2H, d, J = 8.8 Hz), 9.59 (1H, s), 9.65 (1H, s), 9.72 (1H, s), 10,21 (1H, s), 10,76 (1H, s). FAB MS m/z 461 $(M + H)^+$. Anal. ($C_{26}H_{28}N_4O_4$·1.0HCl·1.0C_2H_6O·0.3H_2O) C, H, N, Cl.

4-Fluoro-*N*-(3-hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl)benzamide Hydrochloride (15d). Compound **15d** was prepared from **10e** and 4-fluorobenzoylchloride according to the procedure for preparation of **14i** in 57% yield as a colorless solid. This compound was obtained by crystallization from EtOH−H_2O. Mp 196−198 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.06 (3H, t, J = 6.8 Hz), 2.07−2.40 (2H, m), 2.78 (3H, s), 3.00−3.22 (2H, m), 3.35−3.60 (6H, m), 3.67−3.81 (1H, m), 3.85−4.00 (1H, m), 4.35 (1H, s), 6.82−6.85 (3H, m), 7.13−7.17 (1H, m), 7.22 (1H, d, J = 7.8 Hz), 7.32−7.37 (2H, m), 7.93 (2H, d, J = 8.8 Hz), 7.95−7.99 (2H, m), 9.56 (1H, s), 9.68 (1H, s), 10.01 (1H, s), 10.65 (1H, s). FAB MS m/z 463 $(M + H)^+$. Anal. ($C_{26}H_{27}N_4O_3F$·1.0HCl·1.0C_2H_6O) C, H, N, Cl, F

4-Chloro-*N*-(3-hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl)benzamide Hydrochloride (15e). Compound **15e** was prepared from **10e** and 4-chlorobenzoyl chloride according to the procedure for preparation of **14i** in 51% yield as a colorless solid. This compound was crystallized from EtOH−H_2O. Mp 257−258 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.06 (3H, t, J = 6.8 Hz), 2.10−2.37 (2H, m), 2.79 (3H, s), 3.02−3.22 (2H, m), 3.35−3.58 (6H, m), 3.66−3.80 (1H, m), 3.85−3.99 (1H, m), 4.34 (1H, s), 6.82−6.86 (3H, m), 7.13−7.17 (1H, m), 7.22 (1H, d, J = 8.3 Hz), 7.58 (2H, d, J = 8.3 Hz), 7.89−7.93 (4H, m), 9.53 (1H, s), 9.68 (1H, s), 10.03

(1H, s), 10.45 (1H, s). FAB MS m/z 479 $(M + H)^+$. Anal. ($C_{26}H_{27}N_4O_3Cl \cdot 1.0HCl \cdot 0.3H_2O \cdot 1.0C_2H_6O$) C, H, N, Cl.

4-Bromo-*N*-(3-hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl)benzamide Hydrochloride (15f). Compound **15f** was prepared from **10e** and 4-bromobenzoyl chloride according to the procedure for preparation of **14i** in 54% yield as a colorless solid. This compound was crystallized from EtOH–H_2O. Mp 263–268 °C . ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.10–2.36 (2H, m), 2.79 (3H, s), 3.02–3.23 (2H, m), 3.37–3.58 (4H, m), 3.67–3.80 (1H, m), 3.86–3.98 (1H, m), 6.82–6.86 (3H, m), 7.13–7.17 (1H, m), 7.22 (1H, d, J = 7.8 Hz), 7.72 (2H, d, J = 8.3 Hz), 7.83 (2H, d, J = 8.3 Hz), 7.92 (2H, d, J = 8.8 Hz), 9.53 (1H, s), 9.68 (1H, s), 10.03 (1H, s), 10.49 (1H, s). FAB MS m/z 523, 525 $(M + H)^+$. Anal. ($C_{26}H_{27}N_4O_3Br \cdot 1.0HCl \cdot 0.3H_2O$) C, H, N, Br, Cl.

***N*-(3-Hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]-amino}phenyl)-5-methoxythiophene-2-carboxamide Hydrochloride (15g).** Compound **15g** was prepared from **10e** and 5-methoxythiophene-2-carbonyl chloride according to the procedure for preparation of **14i** in 39% yield as a colorless solid. This compound was crystallized from EtOH–H_2O. Mp 245–247 °C . ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.10–2.40 (2H, m), 2.80 (3H, d, J = 2.4 Hz), 3.03–3.25 (2H, m), 3.38–3.59 (4H, m), 3.68–3.82 (1H, m), 3.85–4.01 (4H, m), 6.40 (1H, d, J = 3.9 Hz), 6.80 (1H, dd, J = 1.5, 7.8 Hz), 6.86 (2H, d, J = 8.8 Hz), 7.10–7.18 (2H, m), 7.53 (1H, d, J = 3.9 Hz), 7.94 (2H, d, J = 8.8 Hz), 9.57 (1H, s), 9.63 (1H, s), 9.83 (1H, s), 10.61 (1H, s). FAB MS m/z 481 $(M + H)^+$. Anal. ($C_{25}H_{28}N_4O_4S \cdot 1.0HCl \cdot 1.0H_2O \cdot 0.5C_2H_6O$) C, H, N, S, Cl.

5-Chloro-*N*-(3-hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl)thiophene-2-carboxamide Hydrochloride (15h). Compound **15h** was prepared from **10e** and 5-chlorothiophene-2-carbonyl chloride according to the procedure for preparation of **14i** in 37% yield as a colorless solid. This compound was crystallized from EtOAc. Mp 247–248 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.11–2.38 (2H, m), 2.80 (3H, d, J = 3.9 Hz), 3.04–3.23 (2H, m), 3.42–3.60 (4H, m), 3.67–3.80 (1H, m), 3.87–3.98 (1H, m), 6.79–6.88 (3H, m), 7.10–7.18 (2H, m), 7.24 (1H, d, J = 3.9 Hz), 7.72 (1H, d, J = 3.9 Hz), 7.95 (2H, d, J = 8.8 Hz), 9.58 (1H, s), 9.67 (1H, s), 10.17 (1H, s), 10.49 (1H, s). FAB MS m/z 485 $(M + H)^+$. Anal. ($C_{24}H_{25}N_4O_3SCl \cdot 1.0HCl \cdot 0.5H_2O$) C, H, N, S, Cl.

5-Bromo-*N*-(3-hydroxy-2-{[4-(4-methyl-1,4-diazepan-1-yl)-benzoyl]amino}phenyl)thiophene-2-carboxamide Hydrochloride (15i). Compound **15i** was prepared from **10e** and 5-bromothiophene-2-carbonyl chloride according to the procedure for preparation of **14i** in 40% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.11–2.38 (2H, m), 2.80 (3H, d, J = 4.3 Hz), 3.05–3.23 (2H, m), 3.38–3.58 (4H, m), 3.68–3.80 (1H, m), 3.88–3.98 (1H, m), 6.81–6.86 (3H, m), 7.11–7.17 (2H, m), 7.33 (1H, d, J = 3.9 Hz), 7.66 (1H, d, J = 4.4 Hz), 7.94 (2H, d, J = 8.8 Hz), 9.55 (1H, s), 9.65 (1H, s), 10.13 (1H, s), 10.48 (1H, s). FAB MS m/z 529, 531 $(M + H)^+$. Anal. ($C_{24}H_{25}BrN_4O_3S \cdot 1.3HCl$) C, H, N, S, Cl. For Br: calcd, 13.85%; found, 12.94%.

3-[(4-Methoxybenzoyl)amino]-2-{[4-(4-methyl-1,4-diazepan-1-yl)benzoyl]amino}phenyl β-D-Glucopyranosiduronic Acid (16). To a stirred solution of the free form of **14i** (5.69 g, 12.0 mmol) in MeOH (50 mL) and $CHCl_3$ (60 mL) at room temperature were added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 7.18 mL, 48.0 mmol) and methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (14.3 g, 36.0 mmol). After the mixture was stirred at room temperature for 2 h, DBU (7.18 mL, 48.0 mmol) and methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (14.3 g, 36.0 mmol) were added again, and the mixture was stirred for 3 h. H_2O (200 mL) was added to the reaction mixture and extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. To the solution of the resulting residue in MeOH (100 mL) at room temperature was added a solution of Na_2CO_3 (12.7 g) in H_2O (200 mL), and the mixture was stirred for 1 h. Then the mixture was washed with EtOAc (200 mL) 3 times to remove unreacted starting material **14i**, and the aqueous layer was concentrated under reduced pressure. The residue was directly chromatographed on ODS gel, eluting with CH_3CN/H_2O (0/100 to 30/70) gradually, and the solutions containing purified target material was concentrated under reduced pressure to remove CH_3CN and then acidified with AcOH (pH 4–5). The resulting precipitate was filtered and washed with H_2O to give **16** (3.18 g, 38%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.75–2.00 (2H, m), 2.45 (3H, s), 2.65–3.70 (12H, m), 3.75 (1H, d, J = 9.3 Hz), 3.84 (3H, s), 4.95 (1H, d, J = 7.3 Hz), 5.21 (1H, s), 5.43 (1H, s), 6.75 (2H, d, J = 8.8 Hz), 7.02–7.11 (3H, m), 7.33 (1H, t, J = 8.3 Hz), 7.62 (1H, d, J = 8.3 Hz), 7.86 (2H, d, J = 8.3 Hz), 7.91 (2H, d, J = 8.3 Hz), 9.66 (1H, s), 9.99 (1H, s). FAB MS m/z 651 $(M + H)^+$. Anal. ($C_{33}H_{38}N_4O_{10} \cdot 2.5H_2O$) C, H, N,

In Vitro Assay for Inhibition of Factor Xa. The hydrolysis rates of synthetic substrates were assayed by continuously measuring absorbance at 405 nm at 37 °C with a microplate reader (model 3550, Bio-Rad, U.S.). Reaction mixtures (125 μL) were prepared in 96-well plates containing chromogenic substrates (S-2222) and an inhibitor in either 0.05 M Tris-HCl, pH 8.4, or 0.15 M NaCl. Reactions were initiated with 25 μL of enzyme solution. The concentration of inhibitor required to inhibit enzyme activity by 50% (IC_{50}) was calculated from dose–response curves in which the logit transformation of residual activity was plotted against the logarithm of inhibitor concentration.

Enzyme Selectivity of 14 and 16i. Reaction mixtures were prepared in 96-well plates containing the chromogenic substrate and test compound. The reaction was initiated by the addition of enzyme, and the color was continuously monitored at 405 nm using a microplate reader SpectraMax 340PC (Molecular Devices, CA, U.S.) at 37 °C. Each enzyme was used at final concentration as follows: 4.2 mU mL^{-1} FXa, 0.20 U mL^{-1} thrombin, 1.0 U mL^{-1} trypsin, and 1.7 mU mL^{-1} plasma kallikrein. The enzymatic activities were assessed by the amidolysis of the following chromogenic substrates for the corresponding protease: S-2222 for FXa and trypsin, S-2238 for thrombin, and S-2302 for plasma kallikrein. The rate of substrate hydrolysis (mOD min^{-1}) was measured at 37 °C. The mode of inhibition was estimated from a Lineweaver–Burk plot. The K_i was determined from a Dixon plot by plotting the reciprocal of the initial reaction velocities at different substrate concentrations against different inhibitor concentrations.

Prothrombin Time Assays in Vitro. After collection of citrated blood samples, platelet-poor plasma was prepared by centrifugation at 3000 rpm for 10 min and stored at −40 °C until use. Plasma clotting times were measured using a KC10A coagulometer (Amelung Co., Lehbrinsweg, Germany) at 37 °C. Prothrombin time (PT) was measured using Orthobrain thromboplastin (OrthoDiagnostic Systems Co., Tokyo, Japan), and values for each test sample were compared with coagulation times of a distilled water control. The concentration required to double the clotting time (CT_2) was estimated from each individual concentration–response curve. Each measurement was performed three times and represented as the mean value.

Ex Vivo Studies. The test drug was dissolved or suspended in 0.5% methyl cellulose and orally administered to male ICR mice (mass range: 30–37 g) at a dose of 100 mg/kg using a gastric tube. Citrated blood was collected from the inferior vena cava 0.5 and 2.0 h after oral administration, and platelet-poor plasma was prepared by centrifugation for measurement of PT. All data were expressed as relative-fold values, compared with the baseline value of vehicle-treated mice.

Pharmacokinetic Study. Monomaleate salt of **14i** suspended in 0.5% methylcellulose was orally administered to male F344/DuCrlCrlj rats (aged 6 weeks, Charles River Japan) at a dose of 100 mg/kg under nonfasted conditions. Three animals were assigned to each sampling point. Blood samples were collected at 0.5, 1, 2, 4, 8, and 24 h after administration and centrifuged to obtain the plasma fraction. The plasma samples were then deproteinized with acetonitrile containing internal standards for each analyte. After centrifugation, the supernatant was evaporated to dryness, and the residue was reconstituted in mobile phase and injected into an LC–MS/MS apparatus to determine the plasma concentrations of **14i** and **16**. Dose and plasma concentrations were expressed as free form.

PAMPA. The PAMPA Evolution instrument from *p*ION Inc. was used in this study. In PAMPA, a "sandwich" is formed from a 96-well microtiter plate (*p*ION Inc., part no. 110163) and a 96-well filter plate

(Millipore, IPVH) such that each composite well is divided into two chambers: donor at the bottom and acceptor at the top, separated by a 125 μm thick microfilter disk (0.45 μm pores) and coated with a 20% (w/v) dodecane solution of a lecithin mixture (*p*ION Inc., part no. 110669). Drug samples were introduced as 10 mM DMSO stock solutions in a 96-well polypropylene microtiter plate. The robotic liquid handling system draws a 5 μL aliquot of the DMSO stock solution and mixes it into an aqueous buffer solution including 10% (v/v) of DMSO so that the final typical sample concentration is 50 μM. The drug solutions were filtered using a 96-well filter plate (Corning, PVDF) and added to the donor compartments. The donor solutions were adjusted in pH 6.5 (NaOH-treated universal buffer, *p*ION Inc., part no. 110151), while the acceptor solution had the same pH 7.4 (*p*ION Inc., part no. 110139). The plate sandwich was formed and allowed to incubate at 25 °C for 2 h in a humidity-saturated atmosphere. On completion of the prescribed incubation time, the sandwich plates were separated and both the donor and acceptor compartments were assayed for the amount of material present by comparison with the UV spectrum (270−400 nm) obtained from reference standards. Mass balance was used to determine the amount of material remaining in the membrane barrier, and permeability (P_e) was calculated using PAMPA Evolution software (*p*ION Inc.).

Mouse Liver Microsomal Stability. For estimation of stability against mouse hepatic CYPs, compounds (0.2 μM) were incubated with male CD1 mouse liver microsomes (0.2 mg/mL) protein in the presence of NADPH (1 mM) and EDTA (0.1 mM) in phosphate buffered saline (100 mM) at 37 °C. Incubations were conducted for 0 and 30 min. Control incubations were conducted by omitting NADPH from the incubation reaction. The percentage compound remaining was determined after analysis by LCMS.

Docking of 14i to Factor Xa. For docking of **14i**, the coordinates of factor Xa with a ligand similar to **14i** was used (PDB code 1MQ5).[26] The docking was done using GOLD with default parameters. The docking mode with the highest score was employed. The methoxy group of **14i** in the S1 pocket was manually adjusted to be consistent with the reported X-ray structures of other compounds with a methoxy group in the S1 pocket (PDB codes 2BQ7,[27] 2P16,[28] 2XCO,[29] 3CS7[30]) . The coordinates of the residues with alternative charged states or flipped conformation were reassigned using Protonate3D implemented in MOE. The coordinates were further minimized using MOE under conditions where the OPLS-AA force field parameters and generalized Born implicit solvation model were employed.

Modeling of 16 in Factor Xa. Glucuronic acid was made to covalently bond with the oxygen atom in the phenol part of **14i**. The coordinate was minimized using MOE under the same conditions described in the section on docking of **14i**.

AUTHOR INFORMATION

Corresponding Author

*Phone: +81-29-863-6712. Fax: +81-29-852-5387. E-mail: fukushi.hirayama@astellas.com.

ACKNOWLEDGMENTS

We thank Dr. Tadashi Hashimoto for pharmacokinetic analysis and Dr. Katsuhiro Yamano for the useful discussion on MLM stability and PAMPA. We also express our thanks to Dr. David Barrett for proofreading the manuscript.

ABBREVIATIONS USED

FXa, factor Xa; HTS, high-throughput screening; PT, prothrombin time; SAR, structure−activity relationship; PAMPA, parallel artificial membrane permeability assay; MLM, mouse liver microsome

REFERENCES

(1) (a) Harder, S.; Thurmann, P. Clinically important drug interactions with anticoagulants. *Clin. Pharmacokinet.* **1996**, *30*, 416−444. (b) Ansell, J.; Hirsh, J.; Hylek, E.; Jacobson, A.; Crowther, M.; Palareti, G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th edition). *Chest* **2008**, *133*, 160S−198S.

(2) (a) Bennira, S.; Banda, Z. K.; Bhattacharya, V. Old versus new anticoagulant: focus on pharmacology. *Recent Pat. Cardiovasc. Drug Discovery* **2010**, *5*, 120−137. (b) Thomas, M.; Bounameaux, H. The potential role of new oral anticoagulants in the prevention and treatment of thromboembolism. *Pharmacol. Ther.* **2011**, *130*, 46−58.

(3) Kumar, R.; Beguin, S.; Hemker, H. C. The influence of fibrinogen and fibrin on thrombin generation: evidence for feedback activation of the clotting system by clot bound thrombin. *Thromb. Haemostasis* **1994**, *72*, 713−721.

(4) Pinto, D. J. P.; Smallheer, J. M.; Cheney, D. L.; Knabb, R. M.; Wexler, R. R. Factor Xa inhibitors: next-generation antithrombotic agents. *J. Med. Chem.* **2010**, *53*, 6243−6274.

(5) (a) Hara, T.; Yokoyama, A.; Tanabe, K.; Ishihara, H.; Iwamoto, M. DX-9065a, an orally active, specific inhibitor of factor Xa, inhibits thrombosis without affecting bleeding time in rats. *Thromb. Haemostasis* **1995**, *74*, 635−639. (b) Kawasaki, T.; Sato, K.; Sakai, Y.; Hirayama, F.; Koshio, H.; Taniuchi, Y.; Matsumoto, Y. Comparative studies of an orally-active factor Xa inhibitor, YM-60828, with other antithrombotic agents in a rat model of arterial thrombosis. *Thromb. Haemostasis* **1998**, *79*, 410−416. (c) Abendschein, D. R.; Baum, P. K.; Martin, D. J.; Vergona, R.; Post, J.; Rumennik, G.; Sullivan, M. E.; Eisenberg, P. R.; Light, D. R. Effects of ZK-807834, a novel inhibitor of factor Xa, on arterial and venous thrombosis in rabbits. *J. Cardiovasc. Pharmacol.* **2000**, *35*, 796−805. (d) Abboud, M. A.; Needle, S. J.; Burns-Kurtis, C. L.; Valocik, R. E.; Koster, P. F.; Amour, A. J.; Chan, C.; Brown, D.; Chaudry, L.; Zhou, P.; Patikis, A.; Patel, C.; Pateman, A. J.; Young, R. J.; Watson, N. S.; Toomey, J. R. Antithrombotic potential of GW813893: a novel, orally active, active-site directed factor Xa inhibitor. *J. Cardiovasc. Pharmacol.* **2008**, *52*, 66−71.

(6) Perzborn, E.; Roehrig, S.; Straub, A.; Kubitza, D.; Misselwitz, F. The discovery and development of rivaroxaban, an oral, direct factor Xa inhibitor. *Nat. Rev. Drug Discovery* **2011**, *10*, 61−75.

(7) Roser-Jones, C.; Becker, R. C. Apixaban: an emerging oral factor Xa inhibitor. *J. Thromb. Thrombolysis* **2010**, *29*, 141−146.

(8) Turpie, A. G. G; Bauer, K. A.; Davidson, B. L.; Fisher, W. D.; Gent, M.; Huo, M. H.; Sinha, U.; Gretler, D. D. A randomized evaluation of betrixaban, an oral factor Xa inhibitor, for prevention of thromboembolic events after total knee replacement (EXPERT). *Thromb. Haemostasis* **2009**, *101*, 68−76.

(9) Chung, N.; Jeon, H. K.; Lien, L. M.; Lai, W. T.; Tse, H. F.; Chung, W. S.; Lee, T. H.; Chen, Sh. A. Safety of edoxaban, an oral factor Xa inhibitor, in Asian patients with non-valvular atrial fibrillation. *Thromb. Haemostasis* **2011**, *105*, 535−544.

(10) Herron and co-workers have also discovered **1a** from HTS around the same time and reported several potent fXa inhibitors by modification of **1a**. (a) Herron, D. K.; Goodson, T. Jr.; Wiley, M. R.; Weir, L. C.; Kyle, J. A.; Yee, Y. K.; Tebbe, A. L.; Tinsley, J. M.; Mendel, D.; Masters, J. J.; Franciskovich, J. B.; Sawyer, J. S.; Beight, D. W.; Rats, A. M.; Milot, G.; Hall, S. E.; Klimkowski, V. J.; Wikel, J. H.; Eastwood, B. J.; Towner, R. D.; Gifford-Moore, D. S.; Craft, T. J.; Smith, G. F. 1,2-Dibenzamidobenzene inhibitors of human factor Xa. *J. Med. Chem.* **2000**, *43*, 859−872. (b) Yee, Y. K.; Tebbe, A. L.; Linebarger, J. H.; Beight, D. W.; Craft, T. J.; Gifford-Moore, D.; Goodson, T. Jr.; Herron, D. K.; Klimkowski, V. J.; Kyle, J. A.; Sawyer, J. S.; Smith, G. F.; Tinsley, J. M.; Towner, R. D.; Weir, L.; Wiley, M. R. N^2-Aroylanthranilamide inhibitors of human factor Xa. *J. Med. Chem.* **2000**, *43*, 873−882. (c) Wiley, M. R.; Weir, L. C.; Briggs, S.; Bryan, N. A.; Buben, J.; Campbell, C.; Chirgadze, N. Y.; Conrad, R. C.; Craft, T. J.; Ficorilli, J. V.; Franciskovich, J. B.; Froelich, L. L.; Gifford-Moore, D. S.; Goodson, T. Jr.; Herron, D. K.; Klimkowski, V. J.; Kurz, K. D.; Kyle, J. A.; Masters, J. J.; Ratz, A. M.; Milot, G.; Shuman, R. T.; Smith,

T.; Smith, G. F.; Tebbe, A. L.; Tinsley, J. M.; Towner, R. D.; Wilson, A.; Yee, Y. K. Structure-based design of potent, amidine-derived inhibitors of factor Xa: evaluation of selectivity, anticoagulant activity, and antithrombotic activity. *J. Med. Chem.* **2000**, *43*, 883−899. (d) Masters, J. J.; Franciskovich, J. B.; Tinsley, J. M.; Campbell, C.; Campbell, J. B.; Craft, T. J.; Froelich, L. L.; Gifford- Moore, D. S.; Hay, L. A.; Herron, D. K.; Klimkowski, V. J.; Kurz, K. D.; Metz, J. T.; Ratz, A. M.; Shuman, R. T.; Smith, G. F.; Smith, T.; Towner, R. D.; Wiley, M. R.; Wilson, A.; Yee, Y. K. Non-amidine-containing 1,2-dibenzamidobenzene inhibitors of human factor Xa with potent anticoagulant and antithrombotic activity. *J. Med. Chem.* **2000**, *43*, 2087−2092.

(11) Koshio, H.; Hirayama, F.; Ishihara, T.; Shiraki, R.; Shigenaga, T.; Taniuchi, Y.; Sato, K.; Moritani, Y.; Iwatsuki, Y.; Kaku, S.; Katayama, N.; Kawasaki, T.; Matsumoto, Y.; Sakamoto, S.; Tsukamoto, S. Synthesis and biological activity of novel 1,2-disubstituted benzene derivatives as factor Xa inhibitors. *Bioorg. Med. Chem.* **2005**, *13*, 1305−1323.

(12) Hirayama, F.; Koshio, H.; Ishihara, T.; Seki, N.; Hachiya, S.; Sugasawa, K.; Shiraki, R.; Koga, Y.; Matsumoto, Y.; Shigenaga, T.; Kawazoe, S. Preparation of Diazepane Derivatives or Salts Thereof as Inhibitors of Activated Blood Coagulation Factor X. PCT Int. Appl. WO 2001074791 A1, 2001.

(13) Ishihara, T.; Hirayama, F.; Sugasawa, K.; Koga, Y. Kadokura, T.; Shigenaga, T. Preparation of Benzanilide Derivatives as Inhibitors of Activated Blood Coagulation Factor X. PCT Int. Appl. WO 2002042270 A1, 2002.

(14) Koshio, H.; Hirayama, F.; Ishihara, T.; Taniuchi, Y.; Sato, K.; Sakai-Moritani, Y.; Kaku, S.; Kawasaki, T.; Matsumoto, Y.; Sakamoto, S.; Tsukamoto, S. Synthesis and biological activity of novel 1,4-diazepane derivatives. *Bioorg. Med. Chem.* **2004**, *12*, 2179−2191.

(15) Hirayama, F.; Koshio, H.; Katayama, N.; Kurihara, H.; Taniuchi, Y.; Sato, K.; Hisamichi, N.; Sakai-Moritani, Y.; Kawasaki, T.; Matsumoto, Y.; Yanagisawa, I. The discovery of YM-60828: a potent, selective and orally-bioavailable factor Xa inhibitor. *Bioorg. Med. Chem.* **2002**, *10*, 1509−1523.

(16) Factor Xa inhibitory activities (IC_{50}) of corresponding 2- and 3-methoxy regioisomers of compound **5** prepared by combinatorial synthesis were >10 and 2.0 μM, respectively.

(17) Burger, A. Isosterism and bioisosterism in drug design. *Prog. Drug Res.* **1991**, *37*, 287−371.

(18) (a) Iwatsuki, Y.; Shigenaga, T.; Moritani, Y.; Suzuki, M.; Ishihara, T.; Hirayama, F.; Kawasaki, T. Biochemical and pharmacological profiles of YM150, an oral direct factor Xa inhibitor. *Blood* **2006**, *108*, 911. (b) Iwatsuki, Y.; Sato, T.; Moritani, Y.; Shigenaga, T.; Suzuki, M.; Kawasaki, T.; Funatsu, T.; Kaku, S. Biochemical and pharmacological profile of darexaban, an oral direct factor Xa inhibitor. *Eur. J. Pharmacol.* [Online early access]. DOI: 10.1016/j.ejphar.2011.10.009.

(19) Groenendaal, D; Heeringa, M; Kadokura, T; Verheggen, F; Strabach, G; Heinzerling, H. YM150, an oral direct inhibitor of factor Xa, demonstrated a predictable and dose-proportional pharmacokinetic/pharmacodynamic profile after single and multiple dosing: results from three studies. *Blood* **2010**, *116*, 3323.

(20) Kansy, M.; Senner, F.; Gubernator, K. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. *J. Med. Chem.* **1998**, *41*, 1007−1010.

(21) (a) Masters, J. J.; Franciskovich, J. B.; Tinsley, J. M.; Campbell, C.; Campbell, J. B.; Craft, T. J.; Froelich, L. L.; Gifford-Moore, D. S.; Hay, L. A.; Herron, D. K.; Klimkowski, V. J.; Kurz, K. D.; Metz, J. T.; Ratz, A. M.; Shuman, R. T.; Smith, G. F.; Smith, T.; Towner, R. D.; Wiley, M. R.; Wilson, A.; Yee, Y. K. Non-amidine-containing 1,2-dibenzamidobenzene inhibitors of human factor Xa with potent anticoagulant and antithrombotic activity. *J. Med. Chem.* **2000**, *43*, 2087−2092. (b) Chou, Y.; Davey, D. D.; Eagen, K. A.; Griedel, B. D.; Karanjawala, R.; Phillips, G. B.; Sacchi, K. L.; Shaw, K. J.; Wu, S. C.; Lentz, D.; Liang, A. M.; Trinh, L.; Morrissey, M. M.; Kochanny, M. J. Structure−activity relationships of substituted benzothiopheneanthranilamide factor Xa inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 507−511. (c) Jia, Z. J.; Wu, Y.; Huang, W.; Zhang, P.; Clizbe, L. A.; Goldman, E. A.; Sinha, U.; Arfsten, A. E.; Edwards, S. T.; Alphonso, M.; Hutchaleelaha, A.; Scarborough, R. M.; Zhu, B. 1-(2-Naphthyl)-1*H*-pyrazole-5-carboxylamides as potent factor Xa inhibitors. Part 2: A survey of P4 motifs. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1221−1227. (d) Chan, C.; Borthwick, A. D.; Brown, D.; Burns-Kurtis, C. L.; Campbell, M.; Chaudry, L.; Chung, C.; Convery, M. A.; Hamblin, J. N.; Johnstone, L.; Kelly, H. A.; Kleanthous, S.; Patikis, A.; Patel, C.; Pateman, A. J.; Senger, S.; Shah, G. P.; Toomey, J. R.; Watson, N. S.; Weston, H. E.; Whitworth, C.; Young, R. J.; Zhou, P. Factor Xa inhibitors: S1 binding interactions of a series of *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}-sulfonamides. *J. Med. Chem.* **2007**, *50*, 1546−1557. (e) Nagata, T.; Yoshino, T.; Haginoya, N.; Yoshikawa, K.; Nagamochi, M.; Kobayashi, S.; Komoriya, S.; Yokomizo, A.; Muto, R.; Yamaguchi, M.; Osanai, K.; Suzuki, M.; Kanno, H. Discovery of *N*-[(1*R*,2*S*,5*S*)-2-{[(5-chloroindol-2-yl)carbonyl]amino}-5-(dimethylcarbamoyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride: a novel, potent and orally active direct inhibitor of factor Xa. *Bioorg. Med. Chem. Lett.* **2009**, *17*, 1193−1206. (f) Fujimoto, T.; Imaeda, Y.; Konishi, N.; Hiroe, K.; Kawamura, M.; Textor, G. P.; Aertgeerts, K.; Kubo, K. Discovery of a tetrahydropyrimidin-2(1*H*)-one derivative (TAK-442) as a potent, selective, and orally active factor Xa inhibitor. *J. Med. Chem.* **2010**, *53*, 3517−3531. (g) Quan, M. L.; Pinto, D. J. P.; Rossi, K. A.; Sheriff, S.; Alexander, R. S.; Amparo, E.; Kish, K.; Knabb, R. M.; Luettgen, J. M.; Morin, P.; Smallwood, A.; Woerner, F. J.; Wexler, R. R. Phenyltriazolinones as potent factor Xa inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1373−1377.

(22) (a) Boiret, M.; Marty, A. Detoxification or chemical defense by glucuronidation. An undergraduate biochemistry experiment. *J. Chem. Educ.* **1986**, *63*, 1009−1011. (b) Mulder, G. J. Glucuronidation and its role in regulation of biological activity of drugs. *Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 25−49. (c) Lucaciu, R.; Ionescu, C. Glucuronidation: major pathway in detoxication and chemical defense. *Farmacia (Bucharest, Rom.)* **2005**, *53*, 10−19.

(23) Koshio, H.; Hirayama, F.; Ishihara, T.; Kaizawa, H.; Shigenaga, T.; Taniuchi, Y.; Sato, K.; Moritani, Y.; Iwatsuki, Y.; Uemura, T.; Kaku, S.; Kawasaki, T.; Matsumoto, Y.; Sakamoto, S.; Tsukamoto, S. Orally active factor Xa inhibitor: synthesis and biological activity of masked amidines as prodrugs of novel 1,4-diazepane derivatives. *Bioorg. Med. Chem.* **2004**, *12*, 5415−5426.

(24) Coyne, W. E.; Cusic, J. W. 3,4-Dihydro-2(1*H*)-quinazolinones. *J. Med. Chem.* **1968**, *11*, 1208−1213.

(25) Joshi, K. C.; Misra, R. A.; Jain, R.; Sharma, K. Synthesis of 1-trifluoroacetyl-3-dialkylaminomethyl-5-monosubstituted benzimidazoline-2-thiones using trifluoroacetic acid as an acylating agent. *J. Heterocycl. Chem.* **1989**, *26*, 409−412.

(26) Adler, M.; Kochanny, M. J.; Ye, B.; Rumennik, G.; Light, D. R.; Biancalana, S.; Whitlow, M. Crystal structures of two potent nonamidine inhibitors bound to factor Xa. *Biochemistry* **2002**, *41*, 15514−15523.

(27) Nazare, M.; Will, D. W.; Matter, H.; Schreuder, H.; Ritter, K.; Urmann, M.; Essrich, M.; Bauer, A.; Wagner, M.; Czech, J.; Lorenz, M.; Laux, V.; Wehner, V. Probing the subpockets of factor Xa reveals two binding modes for inhibitors based on a 2-carboxyindole scaffold: a study combining structure−activity relationship and X-ray crystallography. *J. Med. Chem.* **2005**, *48*, 4511−4525.

(28) Pinto, D. J. P.; Orwat, M. J.; Koch, S.; Rossi, K. A.; Alexander, R. S.; Smallwood, A.; Wong, P. C.; Rendina, A. R.; Luettgen, J. M.; Knabb, R. M.; He, K.; Xin, B.; Wexler, R. R.; Lam, P. Y. S. Discovery of 1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (apixaban, BMS-562247), a highly potent, selective,efficacious, and orally bioavailable inhibitor of blood coagulation factor Xa. *J. Med. Chem.* **2007**, *50*, 5339−5356.

(29) Anselm, L.; Banner, D. W.; Benz, J.; Zbinden, K. G.; Himber, J.; Hilpert, H.; Huber, W.; Kuhn, B.; Mary, J. L.; Otteneder, M. B.; Panday, N; Ricklin, F; Stahl, M; Thomi, S; Haap, W. Discovery of a

factor Xa inhibitor (3*R*,4*R*)-1-(2,2-difluoro-ethyl)-pyrrolidine-3,4-dicarboxylic acid 3-[(5-chloro-pyridin-2-yl)-amide] 4-{[2-fluoro-4-(2-oxo-2*H*-pyridin-1-yl)-phenyl]-amide} as a clinical candidate. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5313−5319.

(30) Qiao, J. X.; Cheney, D. L.; Alexander, R. S.; Smallwood, A. M.; King, S. R.; He, K.; Rendina, A. R.; Luettgen, J. M.; Knabb, R. M.; Wexler, R, R.; Lam, P. Y. S. Achieving structural diversity using the perpendicular conformation of alpha-substituted phenylcyclopropanes to mimic the bioactive conformation of ortho-substituted biphenyl P4 moieties: discovery of novel, highly potent inhibitors of Factor Xa. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4118−4123.