Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 782–787
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and biological activity of piperidine diamine derivatives as factor Xa inhibitor

Akiyoshi Mochizuki,[a,*] Yumi Nakamoto,[a] Hiroyuki Naito,[b] Kouichi Uoto[b] and Toshiharu Ohta[c]

[a]*Medicinal Chemistry Research Laboratory I, Daiichi Sankyo Co., Ltd, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*
[b]*Medicinal Chemistry Research Laboratory II, Daiichi Sankyo Co., Ltd, 1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan*
[c]*Biological Research Laboratories I, Daiichi Sankyo Co., Ltd, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

Received 4 July 2007; revised 22 October 2007; accepted 12 November 2007
Available online 17 November 2007

Abstract—Previously, we identified cyclohexane diamine derivative **1** as orally bioavailable factor Xa inhibitor. We have investigated two racemic *cis*-piperidine diamine derivatives **2** and **3** based on **1**. Compounds **2a**–**e** showed higher fXa inhibitory activity, anticoagulant activity, and aqueous solubility than **3a**–**e** having same substituent. Compounds **2a**, **2c**, **2e**, and **2g**–**m** having sp2 nitrogen, especially amide and urea derivatives, showed potent anticoagulant activity. Compounds **2h** and **2k** showed high oral activities in rats.

Thrombosis-related diseases, such as myocardial infarction, deep vein thrombosis, and unstable angina, are major causes of mortality in the industrialized world. Current therapies, using heparin and warfarin, are based on indirect inhibition of thrombin, the terminal enzyme in the coagulation cascade. These therapies are limited by side effect, mode of administration, careful monitoring of drug level, slow onset of action, and excessive bleeding.[1] Therefore, safer and more effective orally administered anticoagulant agents to combat these diseases are required in clinical treatment.

Factor Xa (fXa) is a serine protease that is situated in convergence of the intrinsic and the extrinsic coagulation pathways. This enzyme plays an important role in the conversion of prothrombin to thrombin, which is a highly amplified process.[2] It is thought that the inhibition of fXa may be more effective and involve a lower risk of abnormal bleeding.[3]

DX-9065a is a potent fXa inhibitor and exerts antithrombotic effects in animal models.[4] But DX-9065a is not sufficiently absorbed in oral administration.[5] This insufficient absorption is due to its strongly basic amidine groups,[6] so it is only used as an injectable formulation in clinical studies. Therefore, non-amidine type fXa inhibitors were continuously studied by us[7,8] and other groups.[9] Recently cyclicdiamine derivatives were reported by our group[8] and Qiao et al.[9a,b] We reported that the (−)-(1*R*,2*S*)-cyclohexanediamine derivative **1** showed selective fXa inhibition activity (Fig. 1).[8] Its in vivo activity and anticoagulant activity were inadequate, we thought that its weak in vivo activity was due to its low solubility in neutral water and the replacement of lipophilic cyclohexane into piperidine would increase the solubility. Then we designed two types of *cis*-piperidine diamine derivatives **2** and **3**, and their various *N*-substituted derivatives were synthesized as *cis* racemic compounds.

Piperidine diamine derivatives were prepared from common intermediate **9**. The synthesis of intermediates is shown in Scheme 1. Commercially available tetrahydropyridine (**4**) was protected with Boc group. Compound **5** was oxidized with OsO_4 to give diol **6**. Diol **6** was transformed to diamine **9** via mesylate **7** and azide **8**. To introduce 5-chloroindole as S1 ligand and 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine as S4 ligand into diamine **9**, nitrophenol esters **11** and **13** were prepared from each corresponding carboxylic acid.[7b,10]

Keywords: Factor Xa inhibitor; Anticoagulant; *cis*-Piperidine diamine derivative.
* Corresponding author. E-mail: mochizuki.akiyoshi.yk@daiichisankyo.co.jp

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.11.037

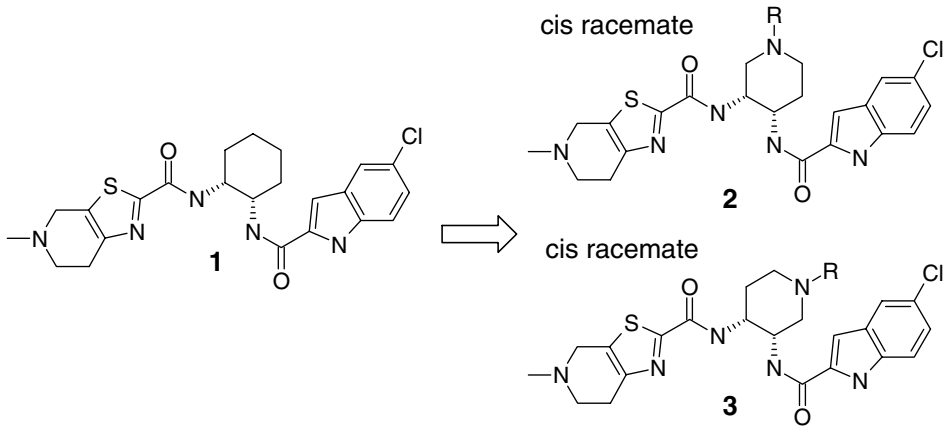
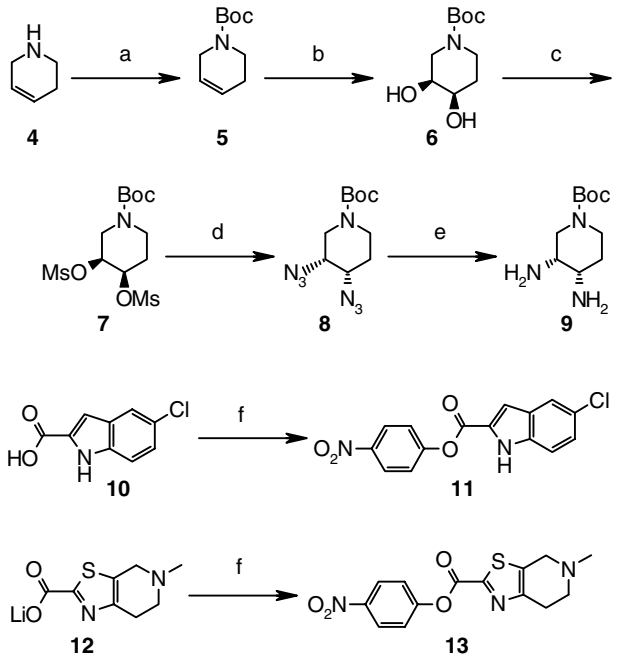


Figure 1. Structure of cyclohexane derivative **1** and *cis*-piperidine diamine derivatives **2** and **3**.



Scheme 1. Synthesis of intermediates **9**, **11**, and **13**. Reagents and conditions: (a) Boc_2O, 10% Na_2CO_3 aqueous rt 92%; (b) OsO_4, NMO, MeCN-H_2O rt 70%; (c) MsCl, Et_3N, CH_2Cl_2 rt 94%; (d) NaN_3, DMF rt 74%; (e) H_2, Pd(C), MeOH rt 89%; (f) 1—$SOCl_2$, cat. DMF, CH_2Cl_2, reflux, 2—nitrophenol, Et_3N, CH_2Cl_2 50–93%.

Diamine **9** was acylated by using nitrophenol ester **11** or **13** under basic condition to get mono acylated piperidine diamines **14** and **17** as main product, respectively.[11] Piperidine diamine derivatives **2** and **3** were prepared as shown in Scheme 2. Compounds **14** and **17** were condensed with **10** or **12** to obtain *N*-Boc piperidine derivatives **2a** and **3a**. *N*-Boc piperidine derivatives **2a** and **3a** were treated with hydrochloric acid to give NH piperidine derivatives **2b** and **3b**, respectively. *N*-acyl, *N*-sulfonyl, and *N*-carboxyl piperidines (**2c**–**f**, **2h**–**k**, **3c**–**e**) were obtained by treating **2b** or **3b** with acid chlorides. The hydrolysis of **2f** gave alcohol derivative **2g**. Urea derivatives **2k**–**m** were obtained by treating with dimethyl amino chloroformate or corresponding isocyanates. Also **2b** was *N*-alkylated with alkyl bromide in basic condition to give **2n** and **2o**, and the hydrolysis of **2o** gave acid derivative **2p**.

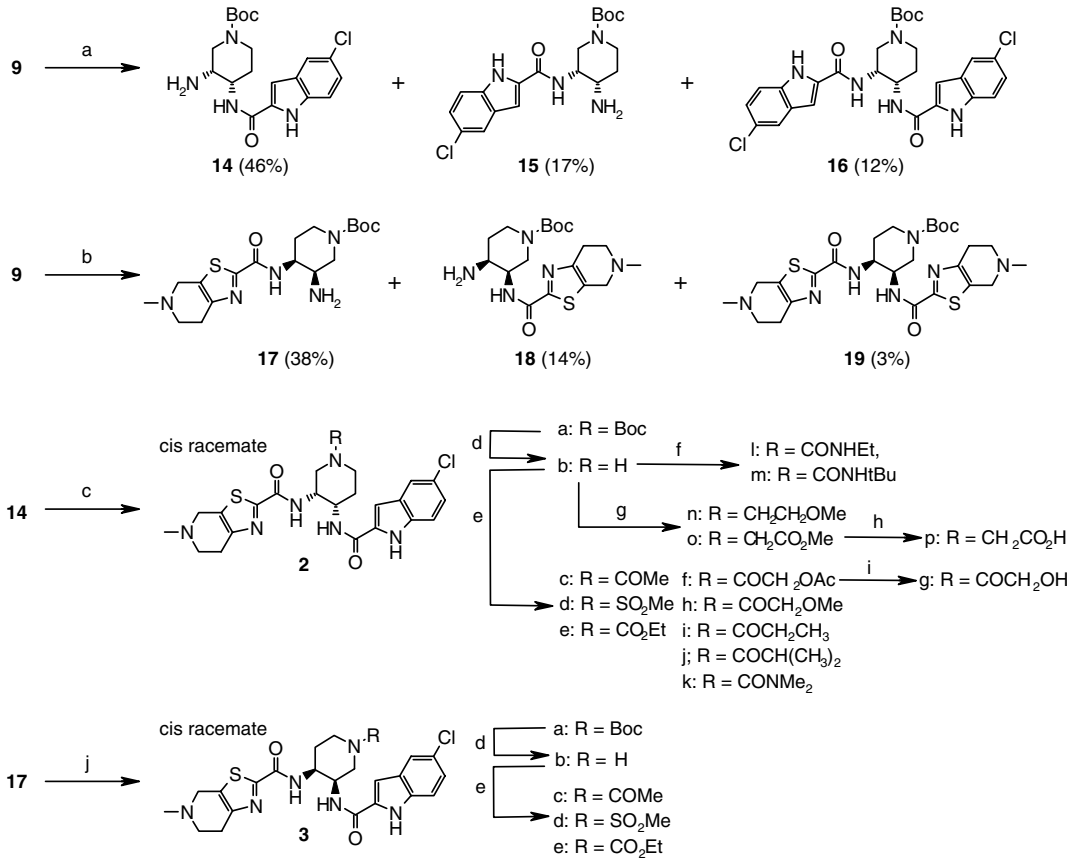
In Table 1, in vitro anti-fXa, anticoagulant activity, aqueous solubility of compounds **1**, **2a**–**e**, and **3a**–**e** are summarized. Except **2a** and **3a**, these compounds showed higher solubility in the aqueous neutral solution (JP2: Japanese Pharmacopoeia Second fluid, pH 6.8) than **1**. In general compounds **2a**–**e** showed higher anti-fXa and anticoagulant activities than **3a**–**e** for similar piperidyl substituents. Furthermore, compounds **2b**–**e** showed higher aqueous solubility. Particularly, **2c** showed exceedingly potent anticoagulant activity more than six times the activity of **1**. Additional analogs of compounds that represent structure **2** were also evaluated (Table 2).

Compounds **2g**–**p** showed higher aqueous solubility than **1**. Carbamate (**2a**, **2e**), amide (**2c**, **2g**–**j**), and urea (**2k**–**m**) derivatives showed much higher anti-fXa activity than **1**, especially amide (**2c**, **2g**–**j**), urea (**2k**–**m**) derivatives showed high anti-fXa activity with clotting activity (<1.1 μM). Unsubstituted piperidyl analog (**2b**), sulfonyl amine (**2d**), and alkyl analogs (**2n**–**p**) were comparatively weaker. A possible explanation for the high affinity could be attributable to the sp^2 hybridization of the piperidyl nitrogen.

$T_{1/2}$ in human liver microsome and intrinsic clearance (CLint) were also evaluated for active compounds (Table 3).[15,16] Carbamate derivatives **2a** and **2e** were very unstable. Many other compounds showed moderate stability at the same level as compound **1**, but, **2g** and **2h** were clearly shown to be much superior to **1**.

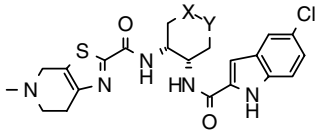
The oral activities of **2g**, **2h**, **2k**, and **2l** were examined after the administration to rats at a dose of 10 mg/kg (Table 4). Compounds **2h** and **2k** displayed potent anti-fXa activities in plasma, on the other hand, **2g** and **2l** containing hydrogen donating groups showed low anti-fXa activities in plasma.

Compound **2h** showed oral activity in rat, and the notable improvements of anti-fXa activity, anticoagulant



Scheme 2. Synthesis of *cis*-piperidine diamine derivatives **2** and **3**. Reagents and conditions: (a) **11**, NMM, DMF rt; (b) **13**, NMM, DMF rt; (c) **12**, WSCI, HOBt, NMM, DMF rt 66%; (d) HCl, EtOH rt 98–99%; (e) R-Cl, Et_3N, CH_2Cl_2; (f) RNCO, TEA, CH_2Cl_2 65–82%; (g) R-Br, K_2CO_3, DMF 38–67%; (h) LiOH, THF, H_2O rt 89%; (i) NaOH, THF, H_2O rt 65%; (j) **10**, WSCD, HOBt, NMM, DMF rt 87%.

Table 1. In vitro fXa inhibitory activity (IC_{50}), anticoagulant activity (PTCT2), and aqueous solubility of **1**, **2a**–**e**, and **3a**–**e**



Compound	X	Y	In vitro		Solubility JP2[c] (μg/ml)
			IC_{50}[a] (nM)	PTCT2[b] (μM)	
1	CH_2	CH_2	41	4.4	17
(−)-**1**	CH_2	CH_2	16	2.9	16
2a	N-CO_2t-Bu	CH_2	10	3.5	3.0
3a	CH_2	N-CO_2t-Bu	130	6.2	<1
2b	NH	CH_2	62	1.3	996
3b	CH_2	NH	530	>20	830
2c	$NCOCH_3$	CH_2	8.6	0.67	421
3c	CH_2	$NCOCH_3$	48	5.8	290
2d	NSO_2CH_3	CH_2	28	2.5	103
3d	CH_2	NSO_2CH_3	45	8.0	70
2e	NCO_2Et	CH_2	4.5	1.4	43
3e	CH_2	NCO_2Et	110	11	37

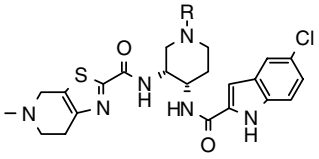
Except for (−)-**1**, these are racemic compounds.

[a] The method of measuring anti-fXa activity was described in Ref. 12.

[b] Anti-coagulant activity was evaluated with the human plasma clotting time doubling concentration (PTCT2). The measurement of PTCT2 was conducted according to Ref. 8.

[c] Aqueous neutral solution, JP2 (pH 6.8), was prepared as described in Ref. 13. The method of measuring solubility was described in Ref. 14.

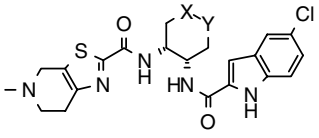
Table 2. In vitro fXa inhibitory activity (IC_{50}) and anticoagulant activity (PTCT2) of **2g–p**



Compound	R	IC_{50} fXa[a] (nM)	PTCT2[b] (μM)	Solubility JP2[c] (μg/ml)
2g	$COCH_2OH$	9.3	0.9	410
2h	$COCH_2OCH_3$	5.8	0.78	510
2i	$COCH_2CH_3$	9.0	0.78	160
2j	$COCH(CH_3)_2$	5.6	1.1	66
2k	$CONMe_2$	8.4	0.79	56
2l	CONHEt	7.8	0.80	220
2m	CONH *t*-Bu	4.8	0.78	39
2n	$CH_2CH_2OCH_3$	47	3.8	130
2o	$CH_2CO_2CH_3$	56	2.1	28
2p	CH_2CO_2H	26	1.8	723

[a] The method of measuring anti-fXa activity was described in Ref. 12.
[b] Anti-coagulant activity was evaluated with the human plasma clotting time doubling concentration (PTCT2). The measurement of PTCT2 was conducted according to Ref. 8.
[c] Aqueous neutral solution, JP2 (pH 6.8), was prepared as described in Ref. 13. The method of measuring solubility was described in Ref. 14.

Table 3. $T_{1/2}$ in human liver microsome and calculated intrinsic clearance (CLint)



Compound	X	Y	$T_{1/2}$[a] (min)	$Cl_{int,h}$[a] (ml/min/kg)
1	CH_2	CH_2	7.70	81.0
(−)-**1**	CH_2	CH_2	6.42	97.2
2a	NCO_2t-Bu	CH_2	4.44	140.4
2c	$NCOCH_3$	CH_2	6.54	95.4
2e	NCO_2Et	CH_2	4.53	137.7
2g	$NCOCH_2OH$	CH_2	15.1	41.4
2h	$NCOCH_2OCH_3$	CH_2	13.6	45.9
2i	$NCOCH_2CH_3$	CH_2	6.08	102.6
2j	$NCOCH(CH_3)_2$	CH_2	6.36	98.1
2k	$NCONMe_2$	CH_2	6.73	92.7
2l	NCONHEt	CH_2	7.22	86.4
2m	NCONH *t*-Bu	CH_2	6.24	99.9

Except for (−)-**1**, these are racemic compounds.
In vitro metabolic stability was measured in human liver microsome according to Ref. 15.
Substrate concentration, 1 μM; human liver microsomes, 1 mg of protein/ml.
[a] $T_{1/2}$ and $Cl_{int,h}$ were estimated from the remaining ratio (X%) of the substrate as described in Ref. 16.

activity, and metabolic stability were confirmed. Therefore, the pharmacokinetic properties of compound **2h** were evaluated using monkeys ($n = 3$, po 1.0 mg/kg) (Table 5).[18] Although **2h** showed higher AUC and C_{max} value than (−)-**1**, the exposure level was insufficient. We thought that further improvement of metabolic stability would be needed to raise the inhibitor exposure in oral administration.

Table 4. Ex vivo anti-fXa activity on oral administration to rats (10 mg/kg)

Compound	Anti-fXa activity (%)			
	0.5 h	1 h	2 h	4 h
2 g	0.76 ± 1.5	−2.0 ± 1.3	−2.5 ± 1.8	−7.3 ± 2.0
2h	68.0 ± 2.6	50.0 ± 2.6	38.0 ± 4.2	16.0 ± 2.2
2k	80.2 ± 1.3	78.6 ± 0.9	73.7 ± 0.3	68.1 ± 0.7
2l	16.7 ± 4.9	11.4 ± 2.8	5.3 ± 2.6	4.3 ± 2.7

The methods for measuring the ex vivo anti-fXa activities were described in Ref. 17. Values are expressed as means ± SE from three rats.

Table 5. Monkey pharmacokinetic profiles (1.0 mg/kg po)

Compound	$AUC_{0-24\ h}$ (ng h/ml)	C_{max} (ng/ml)	T_{max} (h)
(−)-**1**[a]	56.6	23.3	2.0
2h[b]	83.6	36.4	1.2

Hydrogen chloride salts were used. The method of measuring concentration was described in Ref. 18.
[a] The data of compound (−)-**1** were calculated by normalizing the data at a dose of 3.0 mg/kg as single dose.
[b] Compound **2h** was co-administered with other compounds.

In summary, two types of racemic *cis*-piperidine diamine derivatives **2** and **3** were shown to possess potent anti-fXa activity and good solubility. Also, compounds **2a–e** showed higher anti-fXa and clotting activity, solubility when compared to compounds **1** and **3**. In general, carbamate, amide, and urea derivatives showed higher anti-fXa activities than **1**. Moreover, amide and urea derivatives showed potent anticoagulant activities at less than 1 μM, even though these compounds were racemic. In addition, methoxyacetyl derivative **2h** and dimethylurea derivative **2k** showed oral activity in rats (ex vivo), and **2h** showed higher metabolic stability than **1**. On the basis of these results, the *cis*-piperidine derivative was considered a promising lead compound for exploration as a fXa inhibitor. Further efforts will be reported in separate publications.

Acknowledgments

We thank anti-coagulants group in Biological Research Laboratories I for performing the biological assays. We thank also Dr. Kanno for helpful discussions and review of this manuscript.

References and notes

1. (a) Matzsch, T.; Bergqvist, D.; Hender, U.; Østergaard, P. *Thromb. Haemost.* **1986**, *56*, 293; (b) Brieger, D. B.; Mak, K. H.; Kottke-Marchant, K.; Topol, E. J. *J. Am. Coll. Cardiol.* **1988**, *31*, 1449; (c) Stein, P. D.; Grandison, D.; Hua, T. A. *Postgrad. Med.* **1994**, *70*, S72; (d) Hirsh, J.; Poller, L. *Arch. Int. Med.* **1994**, *154*, 282.

2. Elodi, S.; Varadi, K. *Thromb. Res.* **1979**, *15*, 617.
3. (a) Hara, T.; Yokoyama, A.; Tanabe, K.; Ishihara, H.; Iwamoto, M. *Thromb. Haemost.* **1995**, *74*, 635; (b) Morishima, Y.; Tanabe, K.; Terada, Y.; Hara, T.; Kunitada, S. *Thromb. Haemost.* **1997**, *78*, 1366; (c) Kaiser, B. *Drugs Future* **1998**, *23*, 423.
4. (a) Nagahara, T.; Yokoyama, Y.; Inamura, K.; Komoriya, S.; Yamaguchi, H.; Hara, T.; Iwamoto, M. *J. Med. Chem.* **1994**, *37*, 1200; (b) Hara, T.; Yokoyama, A.; Ishihara, H.; Yokoyama, Y.; Nagahara, T.; Iwamoto, M. *Thromb. Haemost.* **1994**, *71*, 314.
5. Yokoyama, T.; Kelly, A. B.; Marzec, U. M.; Hanson, S. R.; Kunitada, S. *Circulation* **1995**, *92*, 485.
6. (a) Zhu, B.-Y.; Scarborough, R. M. *Annu. Rep. Med. Chem.* **2000**, *35*, 83; (b) Choi-Seledeski, Y. M.; Becker, M. R.; Green, D. M.; Davis, R.; Ewing, W. R.; Mason, H. J.; Ly, C.; Spada, A.; Liang, G.; Cheney, D.; Barton, J.; Chu, V.; Brown, K.; Colussi, D.; Bentley, R.; Leadley, R.; Dunwiddie, C.; Pauls, H. W. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2539; (c) Pinto, D. J. P.; Orwat, M. J.; Wang, S.; Fevig, J. M.; Quan, M. L.; Amparo, E.; Cacciola, J.; Rossi, K. A.; Alexander, R. S.; Smallwood, A. M.; Luettgen, J. M.; Liang, L.; Aungst, B. J.; Wright, M. R.; Knabb, R. M.; Wong, P. C.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2001**, *44*, 566; (d) Lam, P. Y. S.; Clark, C. G.; Li, C. R.; Pinto, D. J. P.; Orwat, M. J.; Galemmo, R. A.; Fevig, J. M.; Teleha, C. A.; Alexander, R. S.; Smallwood, A. M.; Rossi, K. A.; Wright, M. R.; Bai, S. A.; He, K.; Luettgen, J. M.; Wong, P. C.; Knabb, R. M.; Wexler, R. R. *J. Med. Chem.* **2003**, *46*, 4405.
7. (a) Haginoya, N.; Kobayashi, S.; Komoriya, S.; Hirokawa, Y.; Furugoori, T.; Nagahara, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2935; (b) Haginoya, N.; Kobayashi, S.; Komoriya, S.; Yoshino, T.; Suzuki, M.; Shimada, T.; Watanabe, K.; Hirokawa, Y.; Furugoori, T.; Nagahara, T. *J. Med. Chem.* **2004**, *47*, 5167; (c) Haginoya, N.; Kobayashi, S.; Komoriya, S.; Yoshino, T.; Nagata, T.; Hirokawa, T.; Nagahara, T. *Bioorg. Med. Chem.* **2004**, *12*, 5579; (d) Komoriya, S.; kanaya, N.; Nagahara, T.; Yokoyama, A.; Inamura, K.; Yokoyama, Y.; Katakura, S.; Hara, T. *Bioorg. Med. Chem.* **2004**, *12*, 2099; (e) Komoriya, S.; Haginoya, N.; Nagata, T.; Suzuki, M.; Kobayashi, S.; Mochizuki, A.; Yoshino, T.; Nagahara, T.; Suzuki, M.; Isobe, Y.; Furugoori, T. *Bioorg. Med. Chem.* **2005**, *13*, 3927; (f) Komoriya, S.; Kobayashi, S.; Osanai, K.; Yoshino, T.; Nagata, T.; Haginoya, N.; Nakamoto, Y.; Mochizuki, A.; Nagahara, T.; Suzuki, M.; Shimada, T.; Watanabe, K.; Isobe, Y.; Furugoori, T. *Bioorg. Med. Chem.* **2006**, *14*, 1309.
8. Nagata, T.; Yoshino, T.; Haginoya, N.; Yoshikawa, K.; Isobe, Y.; Furugohri, T.; Kanno, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4683.
9. There are so many reports, and recent several reports are shown below. (a) Qiao, J. X.; Chang, C.-H.; Cheney, D. L.; Morin, P. E.; King, S. R.; Wang, G. Z.; Wang, T. C.; Rendina, A. R.; Luettgen, J. M.; Knabb, R. M.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4419; (b) Qiao, J. X.; Wang, T. C.; Wang, G. Z.; Cheney, D. L.; He, K.; Rendina, A. R.; Xin, B.; Luettgen, J. M.; Knabb, R. M.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5041; (c) Li, Y.-L.; Fevig, J. M.; Cacciola, J.; Buriak, J., Jr.; Rossi, K. A.; Jona, J.; Knabb, R. M.; Luettgen, J. M.; Wong, P. C.; Bai, S. A.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5176; (d) Fevig, J. M.; Cacciola, J.; Buriak, J., Jr.; Rossi, K. A.; Knabb, R. M.; Luettgen, J. M.; Wong, P. C.; Bai, S. A.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3755; (e) Quan, M. L.; Han, Qi.; Fevig, J.; Lam, P. Y. S.; Bai, S.; Knabb, R. M.; Luettgen, J. M.; Wong, P. C.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1795; (f) Pinto, D. J. P.; Orwat, M. J.; Quan, M. L.; Han, Q.; Galemmo, R. J., Jr.; Amparo, E.; Wells, B.; Ellis, C.; He, M. Y.; Alexander, R. S.; Rossi, K. A.; Smallwood, A.; Wong, P. C.; Luettgen, J. M.; Rendina, A. R.; Knabb, R. M.; Mersinger, L.; Kettner, C.; Bai, S.; He, K.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4141; (g) Qiao, J. X.; Cheng, X.; Smallheer, J. M.; Galemmo, R. A.; Drummond, S.; Pinto, D. J. P.; Cheney, D. L.; He, K.; Wong, P. C.; Luettgen, J. M.; Knabb, R. M.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1432; (h) Fevig, J. M.; Cacciola, J.; Buriak, J., Jr.; Rossi, K. A.; Knabb, R. M.; Luettgen, J. M.; Wong, P. C.; Bai, S. A.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3755; (i) Shi, Y.; Sitkoff, D.; Zhang, J.; Han, W.; Hu, Z.; Stein, P. D.; Wang, Y.; Kennedy, L. J.; O'Connor, S. P.; Ahmad, S.; Liu, E. C.-K.; Seiler, S. M.; Lam, P. Y. S.; Robl, J. A.; Macor, J. E.; Atwal, K. S.; Zahler, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5952; (j) Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K. H.; Reinemer, P.; Perzborn, E. *J. Med. Chem.* **2005**, *48*, 5900; (k) Mendel, D.; Marquart, A. L.; Joseph, S.; Waid, P.; Yee, Y. K.; Tebbe, A. L.; Ratz, A. M.; Herron, D. K.; Goodson, T.; Masters, J. J.; Franciskovich, J. B.; Tinsley, J. M.; Wiley, M. R.; Weir, L. C.; Kyle, J. A.; Klimkowski, V. J.; Smith, G. F.; Towner, R. D.; Froelich, L. L.; Buben, J.; Craft, T. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4832; (l) Klimkowski, V. J.; Watson, B. M.; Wiley, M. R.; Liebeschuetz, J.; Franciskovich, J. B.; Marimuthu, J.; Bastian, J. A.; Sall, D. J.; Smallwood, J. K.; Chirgadze, N. Y.; Smith, G. F.; Foster, R. S.; Craft, T.; Sipes, P.; Chastain, M.; Sheehan, S. M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5801; (m) Matter, H.; Will, D. W.; Nazaré, M.; Schreuder, H.; Laux, V.; Wehner, V. *J. Med. Chem.* **2005**, *48*, 3290; (n) Nazaré, 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. *J. Med. Chem.* **2005**, *48*, 4511; (o) Kochanny, M. J.; Adler, M.; Ewing, J.; Griedel, B. D.; Ho, E.; Karanjawala, R.; Lee, W.; Lentz, D.; Liang, A. M.; Morrissey, M. M.; Phillips, G. B.; Post, J.; Sacchi, K. L.; Sakata, S. T.; Subramanyam, B.; Vergona, R.; Walters, J.; White, K. A.; Whitlow, M.; Ye, B.; Zhao, Z.; Shaw, K. J. *Bioorg. Med. Chem.* **2007**, *15*, 2127; (p) Ye, B.; Amaiz, D. O.; Chou, Y.-L.; Griedel, B. D.; Karanjawala, R.; Lee, W.; Morrissey, M. M.; Sacchi, K. L.; Sakata, S. T.; Shaw, K. J.; Wu, S. C.; Zhao, Z.; Adler, M.; Cheeseman, S.; Dole, W. P.; Ewing, J.; Fitch, R.; Lentz, D.; Liang, A.; Light, D.; Morser, J.; Post, J.; Rumennik, G.; Subramanyam, B.; Sullivan, M. E.; Vergona, R.; Walters, J.; Wang, Y.-X.; White, K. A.; Whitlow, M.; Kochanny, M. J. *J. Med. Chem.* **2007**, *50*, 2967; (q) Watson, N. S.; Brown, D.; Campbell, M.; Chan, C.; Chaudry, L.; Convery, M. A.; Fenwick, R.; Hamblin, J. N.; Haslam, C.; Kelly, H. A.; King, N. P.; Kurtis, C. L.; Leach, A. R.; Manchee, G. R.; Mason, A. M.; Mitchell, C.; Patel, C.; Patel, V. K.; Senger, S.; Shah, G. P.; Weston, H. E.; Whitwortha, C.; Young, R. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3784; (r) Senger, S.; Convery, M. A.; Chan, C.; Watson, N. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5731; (s) 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, Ping. *J. Med. Chem.* **2007**, *50*, 1546; (t) Saitoh, F.; Nishida, H.; Mukaihira, T.; Kosuga, N.; Ohkouchi, M.; Matsusue, T.; Shiromizu, I.; Hosaka,

Y.; Matsumoto, M.; Yamamoto, I. *Chem. Pharm. Bull.* **2007**, *55*, 317; (u) Kohrt, J. T.; Filipski, K. J.; Cody, W. L.; Bigge, C. F.; La, F.; Welch, K.; Dahring, T.; Bryant, J. W.; Leonard, D.; Bolton, G.; Narasimhan, L.; Zhang, E.; Peterson, J. T.; Haarer, S.; Sahasrabudhe, V.; Janiczek, M.; Desiraju, S.; Hena, M.; Fiakpui, C.; Saraswat, N.; Sharma, R.; Sun, S.; Maiti, S. N.; Leadleya, R.; Edmunds, J. J. *Bioorg. Med. Chem.* **2006**, *14*, 4379.

10. Haginoya, N.; Komoriya, S.; Osanai, K.; Yoshino, T.; Nagata, T.; Nagamochi, M.; Muto, R.; Yamaguchi, M.; Kanno, H.; Nagahara, T. *Heterocycles* **2004**, *63*, 1555.
11. The structures of acylated compounds (**14** and **17**) were determined by comparison with NOE measurements between major and minor products.
12. Anti-fXa activity in vitro was measured by using a chromogenic substrate S-2222 (Chromogenix, Inc.) and human fXa (Enzyme Research Laboratories). Aqueous DMSO (5%, 10 μL) or inhibitors in aqueous DMSO (10 μL) and 0.0625 U/mL human fXa (10 μL) were mixed with 0.1 M Tris–0.3 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL). The reaction was started by the addition of 0.75 M S-2222 (40 μL). The absorbance (O.D.) at 405 nm was monitored every 10 s with a microplate spectrophotometer SPECTRAmax 340 (Molecular Devices, Sunnyvale, CA, USA) at room temperature and the reaction velocity (mO.D./min) was obtained. Anti-fXa activity (inhibition %) was calculated as follows: Anti-fXa activity = (1 − (reaction velocity of sample) ÷ (reaction velocity of control)) × 100. The IC_{50} value was obtained by plotting the inhibitor concentration against the anti-fXa activity.
13. The Japanese Pharmacopoeia Second fluid was prepared as follows. 0.2 mol/L sodium hydroxide solution (118 mL) was added to dihydrogenphosphonate potassium (6.80 g). To the mixture, water was added to make colorless clear liquid (1000 mL). The pH of this liquid was confirmed to be 6.70–6.90. Before use, the liquid was filtered by membrane filter.
14. The solubilities were determined by HPLC analysis. Ten millimolar of compound solution in DMSO (50 μl) was freeze-dried. To the residue the Japanese Pharmacopoeia Second fluid (250 μl, pH 6.8) was added, and the mixture was stirred by pipette operation. The mixture was stored under shade for 12 h. After filtration of the mixture, the filtrate was diluted by 20 times by adding aqueous DMSO solution (1:1 (v/v)) to obtain measurement sample solution. Five micromolar of compound solution in aqueous DMSO solution (1:1 (v/v)) and 100 μmol compound solution in aqueous DMSO solution (1:1 (v/v)) were prepared to make calibration curve. The measurement sample solution, 5 μmol solution, and 100 μmol solution were assayed using HPLC methodologies (Analytical Column: X Terra? MSC18 3.5 μm, 3.0× 30 mm, Waters; Mobile Phase: 10 mM ammonium acetate buffer (pH 4.5)/0.05% acetic acid in acetonitrile = 95:5–10:90 v/v; Wavelength: PDA 220–420 nm). The solubilities were analyzed using Millenium software program (Waters).
15. Obach, R. S. *Drug Metab. Dispos.* **1999**, *27*, 1350.
16. $T_{1/2}$ and CLint were calculated using the remaining ratio (X%) of the substrate after 5 min. k_{el} (/min): disappearance rate constant. C_m, concentration of microsome. We assumed that human liver microsome weight is 45 mg/g of liver and human liver weight is 20 g/kg of body weight. $X/100 = \exp(-k_{el} * 5)$. k_{el} (/min) $= -\ln(X/100)/5$. $T_{1/2} = \ln 2/k_{el} = 5 * \ln 2/\ln(100/X)$. CLint $= k_{el}/C_m = -\ln[(X/100)/5]/1.0 * 45 * 20$.
17. Anti-fXa activity in rat ex vivo. Male Wistar rats were fasted overnight. Synthetic compounds were dissolved in 0.5% methylcellulose solution and administered orally to rats with a stomach tube. For control rats, 0.5% methylcellulose solution was administered orally. The rats were anesthetized with thiopental sodium (100 mg/kg, i.v.) 15 min after the oral administration. Blood samples (450 μL) were collected from the jugular vein into syringes containing 50 μL of 3.13% trisodium citrate dihydrate 0.5, 1, 2, and 4 h after dosing. Plasma was prepared by centrifugation. Anti-fXa activity in plasma was measured as follows. Plasma sample (5 μL) was mixed with 0.1 M Tris–0.3 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL), H_2O (5 μL), and 0.1 U/mL human fXa (10 μL). The reaction was started by the addition of 0.75 M S-2222 (40 μL). The reaction velocity and anti-fXa activity (inhibition %) were obtained as mentioned above.
18. Synthetic compound was administered orally to monkey (1 mg/kg) in aqueous solution. Blood samples were collected using Labospeed tubes (Toyo-kizai Inc.) at 0.5, 1, 2, 4, 8, and 24 h after oral dosing. Respective samples at each time points were collected in $n = 3$. Serum concentrations for synthetic compound were determined by LC–MS/MS using Sciex API 365 (Sciex Inc.) coupled with Alliance 2690 HPLC system (Waters Inc.). Compound was separated on a Symmetry C18 column (Waters Inc.). The quantitation limit was 7 ng/mL. Respective pharmacokinetic parameters were measured using Top Fit ver. 2.0 (Gustav Fischer Inc.).