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Stereoselective synthesis and biological evaluation of 3,4-diaminocyclohexanecarboxylic acid derivatives as factor Xa inhibitors

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ABSTRACT

There have been few reports on synthetic methods for *cis*-1,2-diaminocyclohexane bearing a third ring substituent. Starting from 3-cyclohexenecarboxylic acid, we developed efficient methods for synthesizing the 3,4-diaminocyclohexanecarboxylic acid derivatives **2**–**5**. We also evaluated their anti-Xa and anticoagulant activities. Among the compounds, acid **2a** and amide **2b** exhibited the most potent in vitro anti-fXa activity, indicating that the position and stereochemistry of a polar functional group on the cyclohexane ring greatly affected the in vitro anti-fXa activity.

The extrinsic and intrinsic coagulation systems converge at the activation of factor X to Xa. Activated factor X (fXa) has an important role in the conversion of prothrombin to thrombin, which produces blood clots.[1] Thus, fXa is a key enzyme in the coagulation cascade and is also an attractive target enzyme for the therapy of thrombosis and related diseases.

In the previous paper,[2] we reported that the synthesis of *cis*-1,2-diaminocyclohexane derivative (−)-**1** has a potent inhibitory activity for blood coagulation factor Xa (Fig. 1). However, compound (−)-**1** showed poor oral bioavailability ($F = 6.1\%$) in monkeys. The reason for such poor bioavailability of (−)-**1** can be explained by its low remaining rate (46%) in a human liver microsome test. Consequently, we speculated that the introduction of a polar functional group into the chemical structure would decrease the lipophilicity, and hence improve the metabolic stability. Based on this hypothesis, we planned the synthesis and pharmacological evaluation of four regio- and stereoisomers **2**–**5** as racemates, with a carboxylic acid group or dimethylcarbomoyl group on the central cyclohexane ring (Fig. 1).

In the synthesis of **2**–**5**, there have been few reports on synthetic methods for *cis*-1,2-diaminocyclohexane bearing a third ring substituent.[3] Therefore, we first examined the conventional synthetic method described in Scheme 1.

Commercially available ester **6** was oxidized utilizing microencapsulated osmium (VIII) oxide ($MC.OsO_4$) to give a mixture of *cis*-diols. Although we expected that the electrophilic reagent could exhibit a certain preference for attack *anti* to the methyl ester group, no stereoselectivity was observed in this oxidation step. The mixture was subsequently treated with 2,2-dimethoxypropane and separated by silica gel chromatography to give acetonide **7** (41%) and its stereoisomer **8** (35%), respectively. Acidic hydrolysis of the acetonide **7** afforded the diol, which was successively mesylated and treated with sodium azide to give azide **9**. Reduction and deprotection were followed by acylation with commercially available 5-chloroindole-2-carboxylic acid to give a separable 1:1 mixture of amides **10a** and **10b**, showing no regioselectivity in this acylation step. Amides **12a** and **12b** were also prepared from **8** by the procedures described above. Finally, amides **10a**, **10b**, **12a**, and **12b** were condensed with thiazolopyridinecarboxylic acid, followed by ester hydrolysis to give the carboxylic acids **2a**–**5a**.

Next, we examined a regio- and stereoselective synthesis of **2**–**5** for further evaluation. Our strategy is summarized in Figure 2. In general, 1-substituted-3,4-epoxycyclohexanes undergo a ring opening with nucleophiles to give *trans*-diaxial products with high regio- and stereoselectivity (Fürst-Plattner rule).[4] Therefore, to take advantage of this rule, we employed *cis*- and *trans*-3,4-epoxycyclohexanecarboxylates **A** and **D** as synthetic precursors. Treatment with sodium azide would afford azides **B** and **E**, respectively, which could be subsequently converted to diamino derivatives **C** and **F** in several steps. Compounds **C** and **F** have not only the desired relative stereochemistry for compounds **2**–**5**, but also independently removal protecting groups on each functional group (the azido functionality serves as an amine protecting

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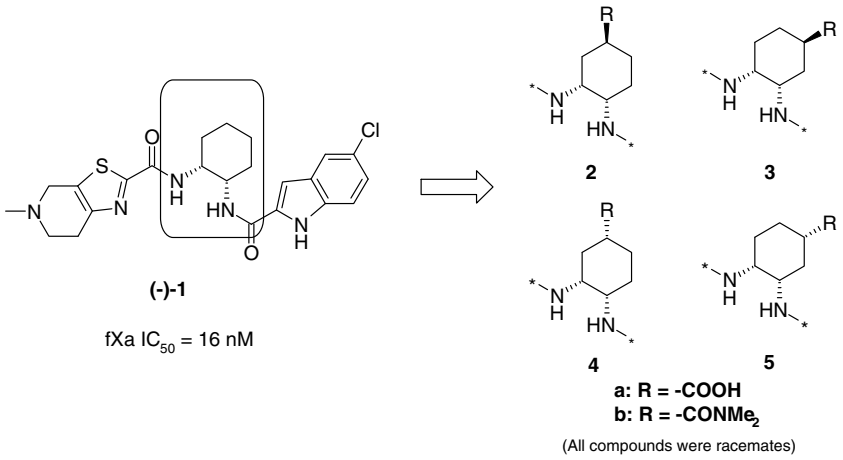
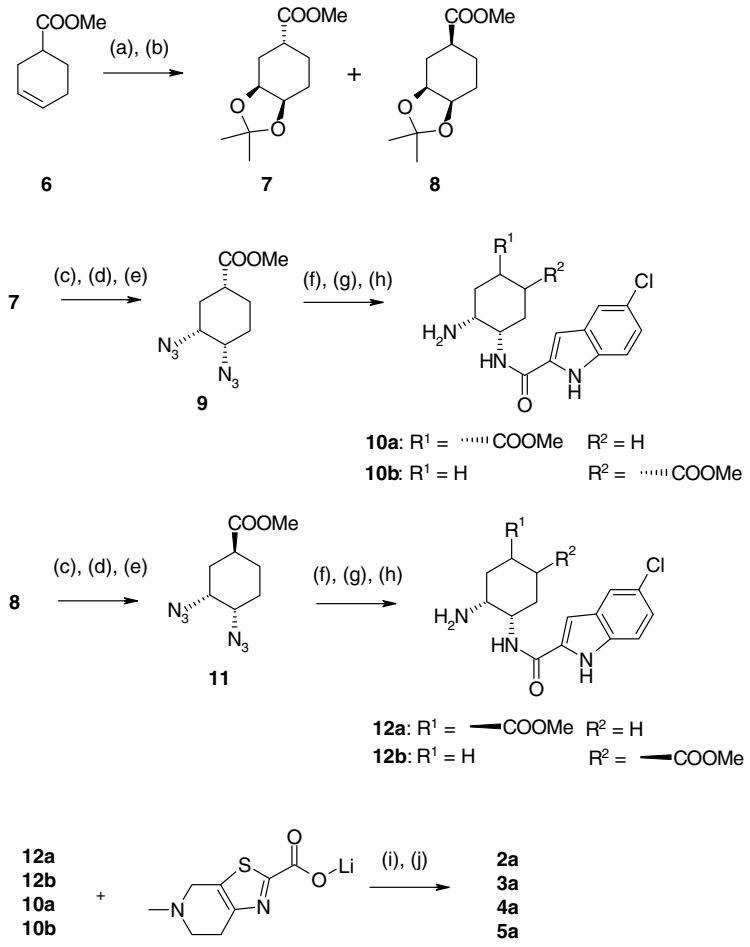


Figure 1. 3,4-Diaminocyclohexanecarboxylic acid derivatives.



Scheme 1. Reagents and conditions: (a) MC.OsO_4, 4-methylmorpholine 4-oxide, CH_3CN, 60 °C, 19 h (91%); (b) 2,2-dimethoxypropane, pyridinium *p*-toluenesulfonate, CH_2Cl_2, rt, 1d (**7**: 41%, **8**: 35%); (c) *p*-toluenesulfonic acid, MeOH/THF, rt, 13 h (91–97%); (d) MsCl, Et_3N CH_2Cl_2, −78 °C → 0 °C, 2 h (77–88%); (e) NaN_3, DMF, 100 °C, 1d (82–85%); (f) H_2, Pd-C, Boc_2O, THF, rt, 1d, (46–68%); (g) HCl/EtOH, CH_2Cl_2, 1d; (h) 5-chloroindole-2-carboxylic acid, WSCI, HOBt, 4-methylmorpholine, DMF, rt, 1d (**10a**: 22%, **10b**: 20%; **12a**: 22%, **12b**: 23%); (i) WSCI, HOBt, DMF, rt, 1-4d (41–52%); (j) LiOH, THF/H_2O, 1–21 h (76–82%).

group). Finally, the selective deprotection and acylation of **C** and **F** would readily afford target compounds **2**–**5**.

The synthetic pathway of key intermediate **18** (compound **C**, R = ethyl) is detailed in Scheme 2. Commercially available acid **13** was treated with I_2-KI to afford 4-iodo-6-oxabicyclo[3.2.1]octan-7-one (**14**).[5] The conversion of **14** to *cis*-epoxide **15** was accomplished by ethanolysis and successive treatment under an alkaline condition (1.2 equivalents of NaOH). In this reaction, an excess or lower amount of NaOH resulted in a decrease in the yield of **15**. *cis*-Epoxide **15** was treated with sodium azide in the presence of

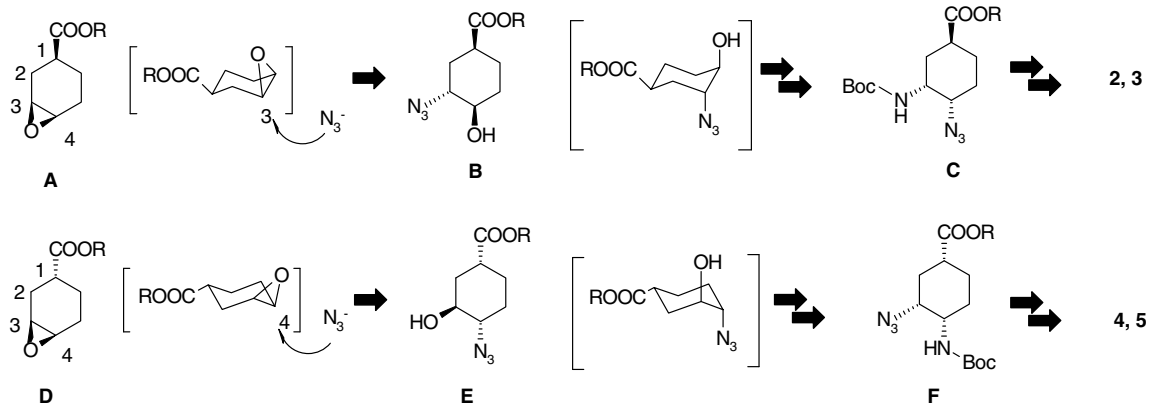
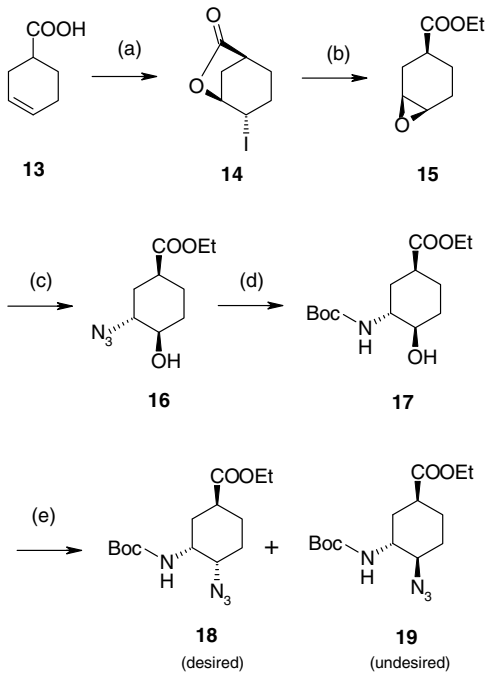


Figure 2. Summary of regio- and stereoselective synthesis of **2**–**5**.



Scheme 2. Reagents and conditions: (a) KI, I_2, $NaHCO_3$, rt, 3 h (92%); (b) 1.2 equiv 2 N—NaOH, EtOH, rt, 3–7 h (68%); (c) 1.5 equiv NaN_3, 1.5 equiv NH_4Cl, DMF, 75 °C, 12 h (93%); (d) H_2, Pd-C, Boc_2O, EtOAc, rt, 12 h (quant.); (e) MsCl, Et_3N CH_2Cl_2, −78 °C → 0 °C → rt, 2 h; then NaN_3, DMF, 75 °C, 12 h (**18**: 39%, **19**: 7.7%).

ammonium chloride to afford azide **16** as a single isomer. In this ring-opening reaction, the addition of ammonium chloride was very effective in preventing the hydrolysis of the ester group. Catalytic hydrogenation of **16** in the presence of Boc_2O gave Boc protected amino alcohol **17** in a quantitative yield. Alcohol **17** was converted into mesylate, followed by treatment with sodium azide to afford the desired key intermediate **18**[6] (39%). In this final step, epimer **19**[6] was unexpectedly obtained as a by-product (8%), suggesting neighboring-group (*tert*-Boc) participation in forming the oxazolidine derivative, as shown in Figure 3.[7]

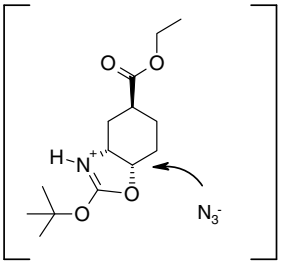


Figure 3. Neighboring-group participation.

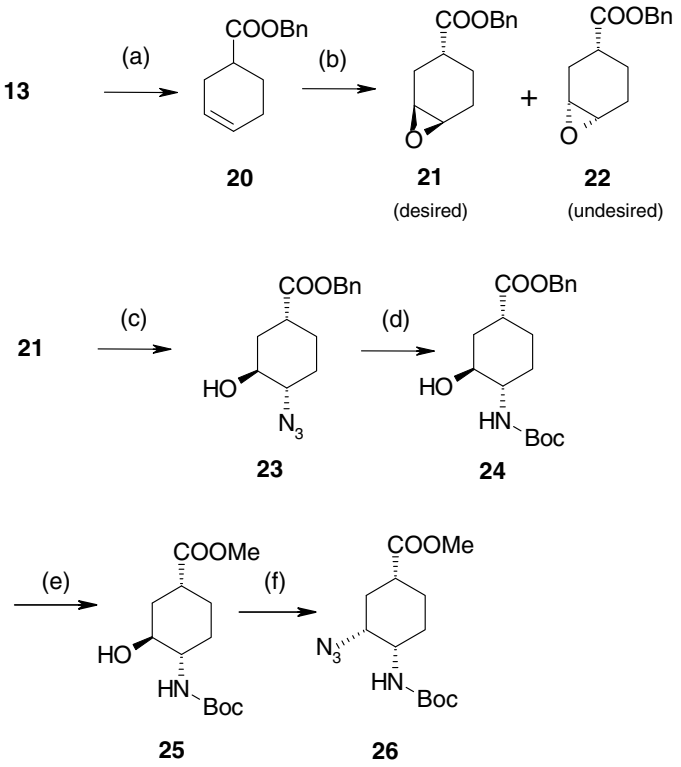
The synthetic pathway of compound **26** (compound **F**, R = methyl) is described in Scheme 3. Acid **13** was esterified with benzylbromide to afford benzyl ester **20**. Treatment of ester **20** with *m*-CPBA afforded a 2:1 mixture of epoxides **21** and **22**. This mixture was separated by silica gel chromatography to give the desired *trans*-epoxide **21**. Epoxidation of methyl 3-cyclohexenecarboxylate was also used instead of **20** to produce a mixture of its *cis*- and *trans*-epoxide.[8] However, the isolation of *trans*-epoxide from the resulting mixture was unsuccessful on a large scale because of closed Rf values. Epoxide **21** was treated with sodium azide by the procedures described above, affording azide **23** as the sole product in a quantitative yield. Azide **23** was reduced by the use of triphenylphosphine and successively protected by using Boc_2O to afford compound **24** in a good yield. Benzyl ester **24** was cleaved by hydrogenolysis, and then esterified with $TMSCHN_2$, furnishing methyl ester **25**. Ester **25** was converted into mesylate, followed by a treatment of sodium azide to afford the desired key intermediate **26**.

The obtained intermediates **18** and **26** were reduced over Pd-C to yield amines **27** and **30**, followed by selective acylation and deprotection to afford the acids **2a**–**5a**. Dimethylcarbamoyl analogues **2b**–**5b** were derived from **2a**–**5a** using amide coupling, as shown in Scheme 4.

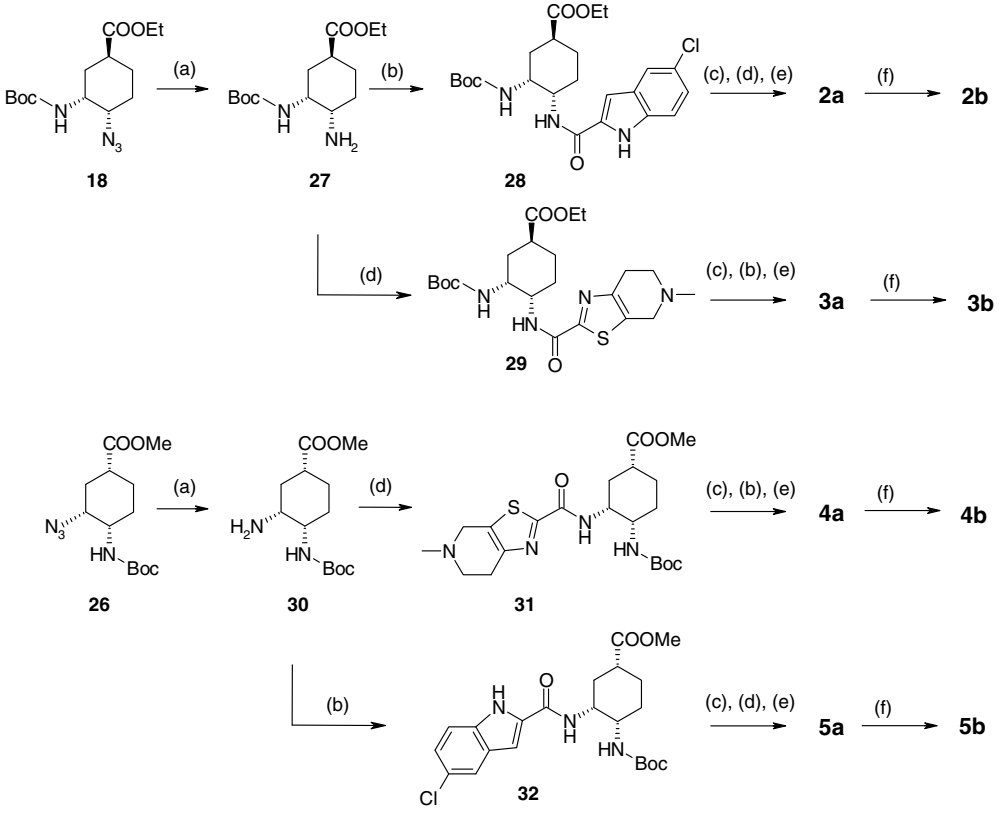
The synthesized compounds were evaluated for in vitro anti-fXa activity (IC_{50}) and anticoagulant activity (PTCT2: the concentration required to double the prothrombin time). The metabolic stability was estimated by the remaining rate of the compounds after 5 min of incubation with human liver microsomes. The results are shown in Table 1.

Carboxylic acids **2a**–**5a** showed significantly different anti-fXa activities, indicating that the position and stereochemistry of a carboxylic group on cyclohexane ring greatly affected the in vitro anti-fXa activity. Among them, acid **2a** was the most potent compound and was twofold more active than unsubstituted compound (−)-**1**. In contrast, the regioisomer **3a** showed a 15-fold decreased inhibition compared with that of **2a**. With respect to stereoisomers, compound **4a**, which was stereoisomer of **2a**, also exhibited 10-fold weaker potency in vitro. However, its regioisomer **5a** recovered anti-fXa activity the same as compound (−)-**1**.

Replacement of the carboxyl group with a dimethylcarbamoyl group did not affect the anti-fXa activity and showed almost the same anti-fXa inhibitory activity as the corresponding carboxylic acids. However, focusing on the anticoagulant activity, dimethyl-

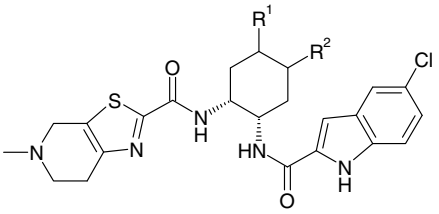


Scheme 3. Reagents and conditions: (a) BnBr, Et_3N, DMF, rt, 12 h (83%); (b) mCPBA, CH_2Cl_2, 0 °C, 2 h (**21**: 55%, **22**: 28%); (c) NaN_3, NH_4Cl, DMF, 70 °C, 24 h (quantitative yield); (d) PPh_3, THF/H_2O, rt, 20 h; then Boc_2O, rt, 2 h (93%); (e) H_2, 10% Pd-C, EtOAc, rt, 20 h; then $TMSCHN_2$, MeOH/toluene, rt, 0.5 h (92%); (f) MsCl, Et_3N CH_2Cl_2, −78 °C (0.5 h) → 0 °C (0.5 h); then NaN_3, DMF, 70 °C, 12 h (30%).



Scheme 4. Reagents and conditions: (a) H_2, 10%Pd-C, EtOH/EtOAc or EtOAc, rt (95%-quantitative yield); (b) 5-chloroindole-2-carboxylic acid, WSCI, HOBt, triethylamine, CH_2Cl_2 or DMF, rt (36–76%); (c); saturated HCl/EtOH, EtOH, rt or 4 N-HCl/dioxane, MeOH, rt (quantitative yield); (d) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid lithium salt, WSCI, HOBt, triethylamine, DMF, rt (56–78%); (e) 1 N-NaOH, EtOH/THF, rt or LiOH, THF/H_2O, rt (78–86%); (f) Me_2NH/HCl, WSCI, HOBt, triethylamine, $CHCl_3$ or DMF, rt (39–83%).

Table 1
Introduction of a carboxylic group or dimethylcarbamoyl group on the cyclohexane ring



Compound	R^1	R^2	Anti-fXa IC_{50}[a] (nM)	PTCT2 in human plasma[b] (μM)	PTCT2 in rat plasma a[b] (μM)	Remaining rate[c] (%)
(−)-**1**	H	H	16	2.9	9.2	46
2a	—COOH	H	**7.5**	**2.8**	2.8	**100**
2b	—$CONMe_2$	H	**5.1**	**0.9**	1.8	**63**
3a	H	—COOH	110	>20	>20	**97**
3b	H	—$CONMe_2$	840	>20	>20	36
4a	····COOH	H	75	>20	15	**98**
4b	····$CONMe_2$	H	88	6.8	19	43
5a	H	····COOH	**16**	8	3.0	**98**
5b	H	····$CONMe_2$	**12**	**1.2**	3.8	19

[a] The method for measuring anti-fXa activity is described in Ref. 9.
[b] Anticoagulant activities in human and rat plasma were evaluated by the concentration required to double the prothrombin time (PTCT2). The method is described in Ref. 10.
[c] The remaining rate of the compounds after 5 min of incubation with human liver microsomes.

Table 2
Ex vivo anti-fXa activity

Compound	Anti-fXa IC_{50} (nM)	Anti-fXa activity (%) at 1 h after oral administration[a]
(−)-**1**	16	78
2a	7.5	0
2b	5.1	39
5a	16	4

[a] The methods for measuring the ex vivo anti-fXa activity are described in Ref. 11.

carbamoyl analogues exhibited enhanced anticoagulant activity. In particular, compound **5b** showed sevenfold higher potency than the corresponding acid **5a**, suggesting that the dimethylcarbamoyl group could have better functionality for potent anticoagulant activity. In this series of compounds, **2b** showed the most potent activities (fXa IC_{50}: 5.1 nM, PTCT2: 0.9 μM) and was threefold more potent than compound (−)-**1**.

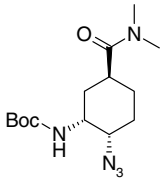
As expected, the introduction of a carboxylic group on the cyclohexane ring significantly improved the metabolic stability. Acids **2a**–**5a** showed excellent remaining rate ranging from 98% to 100%, whereas compound (−)-**1** had a low remaining rate of 46%. This stability could be mainly attributed to decreased lipophilicity. In contrast, dimethylcarbamoyl analogues **2b**–**5b** resulted in low to modest metabolic stability, ranging from 19% to 63%. Interestingly, the position and stereochemistry of a dimethylcarbamoyl group on cyclohexane ring is likely to affect metabolic stability to some extent. Among the dimethylcarbamoyl analogues, only **2b** showed an improvement in the metabolic stability compared with (−)-**1**.

Acids **2a**, **5a**, and amide **2b** were further evaluated for their oral activity in rats. Their anti-fXa activities in rat plasma after oral administration are shown in Table 2. Disappointingly, acids **2a**, **5a**, and amide **2b** were all found to have less oral activity in rats than compound (−)-**1**. Since these compounds were more metabolically stable than (−)-**1**, we reasoned that the introduction of polar functionalities might decrease the membrane permeability in rats.

Starting from 3-cyclohexenecarboxylic acid, we developed the regio- and stereoselective synthesis of 3,4-diaminocyclohexanecarboxylic acid derivatives **2**–**5** and performed a biological evaluation on them as factor Xa inhibitors. SAR for the anti-fXa study of this series revealed that the position and stereochemistry of the polar functional group was critical to the in vitro anti-fXa activity. Among the compounds, acid **2a** and amide **2b** exhibited the most potent anti-fXa activity. Furthermore, we also revealed that the dimethylcarbamoyl group could have better functionality for potent in vitro anticoagulant activity. In addition, acids **2a**–**5a** and amide **2b** with low lipophilicity showed improved metabolic stability compared with compound (−)-**1**, as we expected. However, these low lipophilic compounds also resulted in low oral activity probably due to low permeability. Therefore, to obtain more potent fXa inhibitors with improved oral bioavailability, we need to balance the lipophilicity for both metabolic stability and permeability. Further modifications of these compounds will be reported in due course.

References and notes

1. (a) Prager, N. A.; Abendschein, D. R.; McKenzie, C. R.; Eisenberg, P. R. *Circulation* **1995**, *92*, 962; (b) Kunitada, S.; Nagahara, T. *Curr. Pharm. Des.* **1996**, *2*, 531.
2. Nagata, T.; Yoshino, T.; Haginoya, N.; Yoshikawa, K.; Isobe, Y.; Furugoori, T.; Kanno, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4683.
3. Synthsis of 1-hydroxy-3,4-diaminocyclohexane Witiak, D. T.; Rotella, D. P.; Wei, Y.; Filppi, J. A.; Gallucci, J. C. *J. Med. Chem.* **1989**, *32*, 214.
4. Furst, A.; Plattner, P. A. *Helv. Chim. Acta* **1949**, *32*, 275.
5. (a) Grewe, R.; Heinke, A. *Chem. Ber.* **1956**, *89*, 1978; (b) Kato, M.; Kageyama, M.; Tanaka, R.; Kuwahara, K.; Yoshikoshi, A. *J. Org. Chem.* **1975**, *40*, 1932; (c) Bartlett, P. A.; McQuaid, L. A. *J. Am. Chem. Soc.* **1984**, *106*, 7854.
6. Configurations of compounds **18** and **19** were established by X-ray crystallographic analysis of their *N*,*N*-dimethylamide derivatives **33** and **34**, respectively. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication Nos. CCDC 637942 (**33**) and 637941 (**34**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail:deposit@ccdc.cam.ac.uk).



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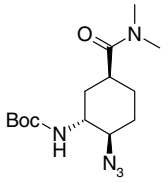

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7. (a) Qiu, J.; Silverman, R. B. *J. Med. Chem.* **2000**, *43*, 706; (b) Katagiri, N.; Matsuhashi, Y.; Kokufuda, H.; Takebayashi, M.; Kaneko, C. A. *Tetrahedron Lett.* **1997**, *38*, 1961.
8. Bellucci, G.; Marioni, F.; Marsili, A. *Tetrahedron* **1972**, *28*, 3393.
9. In vitro Anti-fXa activity was measured by using a chromogenic substrate S-2222 (Chromogenix, Inc.) and human fXa (Enzyme Research Laboratories). Aqueous DMSO (5% V/V; 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–0.2 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL). A reaction was started by the addition of 0.75 M S-2222 (40 μL). After the mixture was stirred for 10 s at rt, the increase of optical densities (OD/min) was measured at 405 nm. Anti-fXa activity (inhibition %) was calculated as follows: Anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of control]. The IC_{50} value was obtained by plotting the inhibitor concentration against the anti-fXa activity.
10. Prothrombin time (PT) was measured with an Amelung KC-10A micro coagulometer (MC Medical, Tokyo, Japan) as follows: first, 50 μL of plasma was mixed with 50 μL of inhibitor or 4% DMSO/saline and incubated for 1 min at 37 °C. Coagulation was started by the addition of 100 μL of thromboplastin C

Plus (0.5 U/mL) to the mixture and the clotting time was measured. The concentration of inhibitor required to double the clotting time (CT2) was estimated from the concentration-response curve by a regression analysis.

11. Male wister rats were fasted overnight. Synthetic compounds were dissolved in 0.5% (w/v) methylcellulose solution and administered orally to rats via a stomach tube. For the control rats, 0.5% (w/v) methylcellulose solution was administered orally. The rats were anesthetized with ravonal at several time points when blood samples were collected in the presence of trisodiumcitrate. After the blood samples were centrifuged, the platelet poor plasma samples were used for the measurement of their anti-fXa activities or anticoagulant activities. Anti-Xa activity: Plasma (5 μL) was mixed with 0.1 M Tris–0.2 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL), H_2O (5 μL) and 0.1 U/mL human fXa (10 μL). A reaction was started by the addition of 0.75 M S-2222 (40 μL). After the mixture was stirred for 10 s at rt, the increase of optical density (OD/min) was measured at 405 nm. The anti-fXa activity (inhibition %) was calculated as follows: Anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of control].