Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2935–2939
Bioorganic & Medicinal Chemistry Letters

Orally active factor Xa inhibitors: 4,5,6,7-tetrahydrothiazolo-[5,4-*c*]pyridine derivatives

Noriyasu Haginoya,[a,*] Syozo Kobayashi,[a] Satoshi Komoriya,[a] Yumiko Hirokawa,[b] Taketoshi Furugori[b] and Takayasu Nagahara[b]

[a]*Medicinal Chemistry Research Laboratory, Daiichi Pharmaceutical Co. Ltd, 1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan*
[b]*New Product Research Laboratory 2, Daiichi Pharmaceutical Co. Ltd, 1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan*

Received 13 February 2004; revised 11 March 2004; accepted 11 March 2004

Abstract—In our investigation of factor Xa inhibitors, a series of 1-(6-chloronaphthalen-2-yl)sulfonyl-4-(4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carbonyl)piperazines **3a**–**i** were synthesized. In vitro inhibitory activities of the compounds against factor Xa and coagulation are summarized. Among the compounds, **3c** and **3d**, possessing a carbamoyl or *N*-methylcarbamoyl moiety, showed potent inhibitory activities when administered orally to rats.

1. Introduction

Anti-coagulants have been prescribed for the treatment of thromboembolism, which is a major cause of such ischemic diseases as cardiac infarction, cerebral infarction, deep vein thrombosis and unstable angina. Heparin and warfarin are standard anti-coagulants. However, heparin is not suitable for long-term treatment of the above diseases because of parenteral administration and side effects such as bleeding tendency.[1] Although warfarin is administered orally and is able to be used for long-term administration, the drug needs continuous administration for several days to exhibit anti-coagulation activity and also needs frequent monitoring to maintain that activity at appropriate levels.[2] Therefore, a novel anti-coagulant should satisfy the following requirements: a good absorption rate in intestinal tracts, a fast onset of action and a low risk of bleeding.

In order to develop such an anti-coagulant, we have studied factor Xa (fXa) inhibitors. FXa is a serine protease that cleaves prothrombin to produce thrombin, a pivotal serine protease in the blood coagulation cascade. In addition, since fXa is situated at the confluent position where the intrinsic and extrinsic pathways in the coagulation cascade meets one another, fXa inhibitors theoretically block the coagulation cascade evoked in either pathway.[3] Up to the present, a lot of fXa inhibitors have been synthesized. Such fXa inhibitors can be classified into two groups, that is, amidine derivatives exemplified by DX-9065a (**1**)[4] and nonamidine derivatives such as compound **2**.[5] As already described in many reports, most of the amidine inhibitors are not sufficiently absorbed in intestinal tracts.[6] Therefore, a trend in the synthetic study of fXa inhibitors seems to be shifted to nonamidine derivatives from amidine derivatives (Fig. 1).[7]

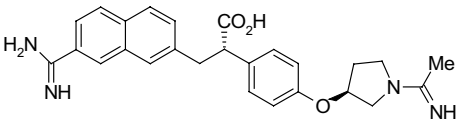
In our synthetic study of nonamidine type fXa inhibitors, we surveyed bicyclic heterocycle structures that can satisfy the three-dimensional requirements in the active site of fXa as well as does the 4-(piperidino)pyridine moiety of **2**. In this line of investigation, we have found 4,5,6,7-tetrahydrothiazolopyridine derivatives **3** that show potent fXa inhibitory activity in both in vitro and ex vivo assays.

2. Chemistry

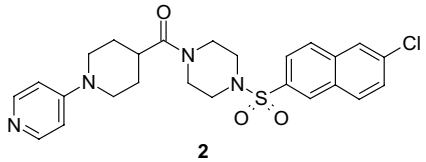
Synthetic routes to compounds **3a**–**i** and **10** listed in Table 1 were shown in Scheme 1. Reaction of 3-chloro-1-ethoxycarbonyl-4-piperidone (**4**)[8] with thioformamide

Keywords: Factor Xa inhibitor; 4,5,6,7-Tetrahydrothiazolo[5,4-*c*]pyridine.
* Corresponding author. Tel.: +81-3569-67435; fax: +81-3569-68723; e-mail: hagin9kg@daiichipharm.co.jp

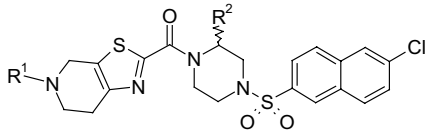
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.03.036



DX-9065a (1)



2



3

Figure 1. Structures of DX-9065a (**1**), **2** and **3**.

gave 5-ethoxycarbonyl-4,5,6,7-tetrahydro-thiazolo[5,4-*c*]-pyridine (**5**). After removal of the ethoxycarbonyl group of **5** by alkali hydrolysis, the resulting 4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine was protected with di-*tert*-butyl dicarbonate then treated with *n*-BuLi and CO_2 to give lithium 5-Boc-4,5,6,7-tetrahydrothiazolo[5,4-*c*]-pyridine-2-carboxylate (**6a**). Compound **5** was reduced with $LiAlH_4$ and then treated with *n*-BuLi and CO_2 to give lithium 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]-pyridin-2-carboxylate (**6b**).

(6-Chloronaphthalen-2-yl)sulfonylchloride (**8**) was synthesized via β-sulfonylation and successive chlorination of 2-chloronaphthalene (**7**). Compound **8** was treated with *tert*-butyl 1-piperazinecarboxylate and the resulting compound was deprotected to give 1-(6-chloronaphthalene-2-sulfonyl)piperazine (**9a**). On the other hand, compound **8** was treated with 2-ethoxy-carbonylpiperazine to obtain 1-Boc-4-(6-chloronaphthalen-2-yl)sulfonylpiperazine-2-carboxylic acid (**9b**). Compound **9b** was converted to 4-(6-chloronaphthalen-2-yl)sulfonyl-2-carbamoylpiperazines **9c**–**i** by reactions with a series of amines.

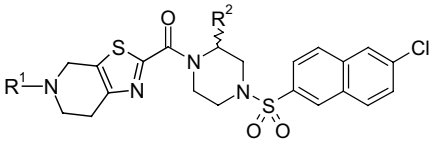
Piperazine derivative **9a** was condensed with carboxylic acid lithium salt **6a**. Following treatment of the product with hydrochloric acid afforded 1-(6-chloronaphthalen-2-yl)sulfonyl-4-(4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonylpiperazine (**3a**), which was converted to **3b** by reductive methylation. Compound **3b** was alkylated further with methyl iodide to give quaternalized compound **10**.

Condensation of a series of 3-carbamoylpiperazines **9c**–**i** with carboxylic acid **6b** afforded 1-(6-chloronaphthalen-2-yl)sulfonyl-4-(4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonyl-3-carbamoylpiperazines **3c**–**i**.

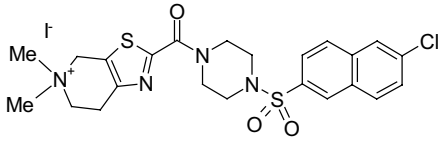
3. Results and discussion

In Table 1, in vitro anti-fXa, anti-thrombin and anti-coagulant activities are summarized. All compounds showed selective anti-fXa activity comparing with anti-thrombin activity. Compounds **3a** ($R^1 = H$, $R^2 = H$) showed IC_{50} value of 60 nM as anti-fXa activity. *N*-Methyl derivative **3b** showed approximately 3-fold

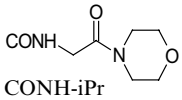
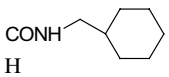
Table 1. In vitro anti-fXa, anti-thrombin and anti-coagulant activities of the synthesized compounds



3a-i



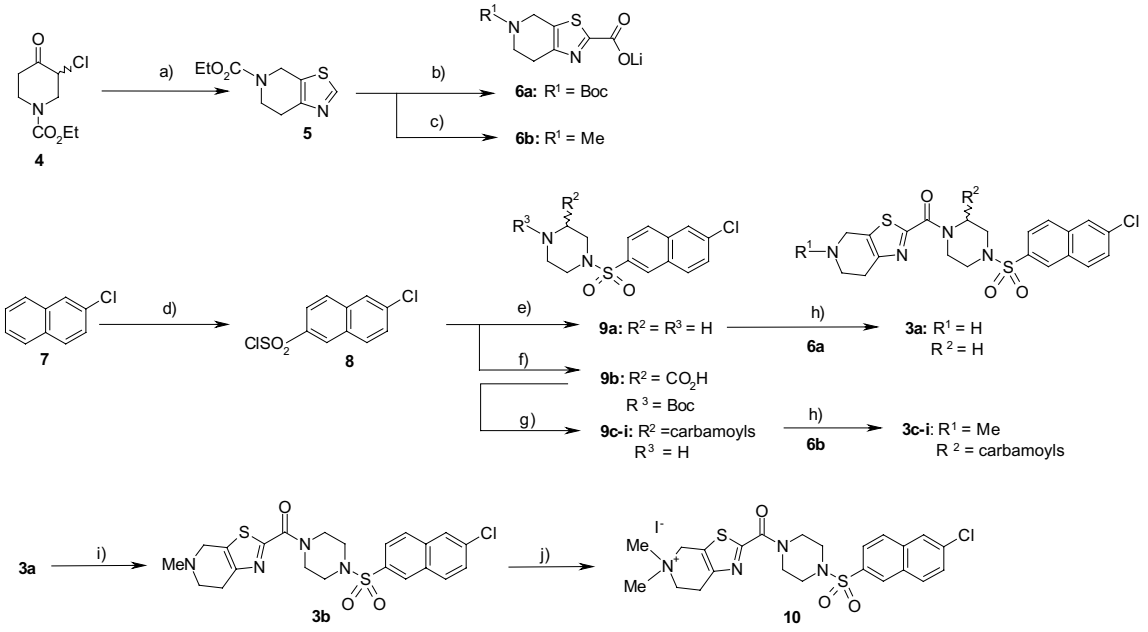
10

Compound	R^1	R^2	Anti-fXa IC_{50} (nM)[a]	Anti-thrombin IC_{50} (nM)[a]	$PTCT_2$ in human plasma (μM)[b]	$PTCT_2$ in rat plasma (μM)[b]
3a	H	H	60	2900	11.2	16.1
3b	Me	H	22	560	6.0	10.0
3c	Me	$CONH_2$	24	1000	4.4	14.0
3d	Me	CONHMe	27	1350	3.0	6.3
3e	Me	$CONHCH_2CONH_2$	23	880	3.8	8.5
3f	Me		25	1000	2.1	4.3
3g	Me	CONH-iPr	45	820	8.8	8.0
3h	Me	$CONMe_2$	130	6000	7.0	11.4
3i	Me		640	1300	NT[c]	NT[c]
10	*N*,*N*-Dimethyl	H	7.8	3200	0.8	1.6

[a] The measuring methods of anti-fXa and anti-thrombin activity were described in Refs. 9,10.

[b] Anti-coagulant activities in human and rat plasma were evaluated with the plasma clotting time doubling concentration ($PTCT_2$).[11]

[c] NT = not tested because of the weak anti-Xa and anti-thrombin activity.



Scheme 1. Syntheses of **3a**–**i** and **10**. Reagents and conditions: (a) thioformamide, molecular seaves 4 Å/EtOH, 51%; (b) (i) 5 N NaOH, then $(Boc)_2O$, 55%, (ii) *n*-BuLi/ether, then CO_2 gas, 85%; (c) (i) $LiAlH_4$/ether, 40%, (ii) *n*-BuLi/ether, then CO_2 gas, quant; (d) (i) *c*.H_2SO_4, (ii) $SOCl_2$/DMF, two steps 17%; (e) (i) *tert*-butyl 1-piperazinecarboxylate, Et_3N/CH_2Cl_2, (ii) satd HCl/EtOH, two steps quant; (f) (i) 2-ethoxycarbonylpiperazine, Et_3N/CH_2Cl_2, (ii) $(Boc)_2O$/THF, (iii) aq NaOH/EtOH, three steps 34%; (g) a series of amines, WSCI, HOBt/DMF, (ii) satd HCl/EtOH, two steps 71–99%; (h) WSCI, HOBt/DMF, 30–61%; (i) HCHO, AcOH, Et_3N/CH_2Cl_2, 71%; (j) CH_3I/DMF, 56%.

higher anti-fXa activity and 2-fold higher $PTCT_2$ activity when compared with **3a**.

We synthesized a series of carbamoyl derivatives **3c**–**i** based on the following assumption. Since the piperazine ring of compound **3b** may be a linker connecting the 5-chloronaphthalene and tetrahydrothiazolopyridine moieties, introduction of a carbamoyl group on the piperazine ring may not produce a new steric problem in the inhibitor–fXa interactions. We therefore expected that the hydrophilic carbamoyl group might associate with water molecules surrounding the complex of the inhibitor and fXa. Alternatively, the carbamoyl group might make hydrogen bonds with fXa. In either case, such a contribution of the carbamoyl group would stabilize the inhibitor–fXa interactions, leading to an elevation in potency of anti-fXa activity. As a result, carbamoyl and *N*-methylcarbamoyl derivatives **3c** and **3d** exhibited almost equipotent anti-fXa activity in comparison with **3b**. *N*-(Carbamoylmethyl)- and *N*-(morpholinecarbonymethyl)carbamoyl derivatives **3e** and **3f** also showed similar potency to that of **3b**.

However, *N*-isopropylcarbamoyl analogue **3g** and *N*,*N*-dimethylcarbamoyl **3h** were less active. Furthermore, *N*-(cyclohexylmethyl)carbamoyl derivative **3i** had markedly decreased activity. Being different from our expectation, introduction of a series of carbamoyl groups did not elevate anti-fXa activity. However, it is interesting that the size of the N-substituents of the carbamoyl group did not affect the potency: for instance, carbamoyl derivatives **3c** and *N*-(morpholinecarbonymethyl)carbamoyl **3f** were equipotent. In contrast, compound **3i** with a lipophilic *N*-(cyclohexylmethyl)carbamoyl group showed markedly decreased activity as compared with carbamoyl derivative **3c**, suggesting that lipophilicity of those substituents is likely to affect the potency. These results indicate that the carbamoyl and N-substituted carbamoyl groups may direct to the outer side of fXa but not to the inner side of fXa when these inhibitors bind to fXa.

With respect to $PTCT_2$, there is some inconsistency between potency in human plasma and that in rat plasma. For instance, compound **3c** showed potent $PTCT_2$ activity in human plasma, while they did not exhibit paralleled potency in rat plasma. For the reason why such a discrepancy occurred, we thought possibly due to nonspecific plasma protein binding.

In Table 2, ex vivo anti-fXa and anti-coagulant activities were shown, compound **3c** displayed 67.4% of anti-fXa activity at 1 h after oral administration of 30 mg/kg to rats, and also showed evident prolongation of prothrombin time (PT) (1.13-fold). Compound **3d** showed higher potency in ex vivo anti-fXa activity (89.5% at 1 h) in the same condition. The activity of both compounds sustained for 6 h. On the other hand, compounds **3b**, **3e** and **3f**, which were equipotent to **3c** or **3d** in in vitro anti-fXa activity, showed only weak ex vivo anti-fXa activity in rats (data not shown). Quaternary ammonium salt **10** showed the most potent in vitro anti-fXa and $PTCT_2$ activities, however the compound **10** exhibited only 20–30% of ex vivo anti-fXa activity. The discrepancy would be attributable to its poor availability.

Table 2. Ex vivo anti-fXa and anti-coagulant activities for compounds **3c** and **3d**

Compound	At 30 mg/kg (p.o.) to rats					
	Anti-fXa activity (%)[a]			Prolongation effect of PT (fold)[a]		
	1 h	3 h	6 h	1 h	3 h	6 h
3c	67.4 ± 1.5	55.8 ± 1.5	24.7 ± 6.0	1.13 ± 0.02	1.09 ± 0.02	1.05 ± 0.00
3d	89.5 ± 0.8	73.7 ± 2.0	23.5 ± 2.6	1.35 ± 0.01	1.14 ± 0.02	1.04 ± 0.01

[a] The measuring methods of ex vivo anti-fXa and anti-coagulant activities were described in Ref. 12. Values expressed as mean ± S.E. from four rats.

In the in situ intestinal loop of rats, compounds **3c** and **3d** were shown to be absorbed in 75% and 86%, respectively.[13] The result suggested good oral absorption rates of **3c** and **3d**, which reflected on their ex vivo activity.

4. Conclusion

The 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine derivatives were synthesized. The class of compounds showed potent and highly specific inhibitory activity for factor Xa. Introduction of carbamoyl groups on the piperazine ring improved in vivo activity markedly without substantial change in anti-fXa activity. Indeed, compounds **3c** and **3d** showed anti-fXa activity and anti-coagulation activity in ex vivo test after oral administration.

Acknowledgements

The authors acknowledge Dr. J. Furukawa, Dr. S. Kunitada and Dr. Y. Watanabe for useful discussion.

References and notes

1. (a) Mätzsch, 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.
2. Verstrate, M.; Wessler, S. In *Thrombosis in Cardiovascular Disorders*; W.B. Saunders Co., 1992; p 141.
3. (a) Kunitada, S.; Nagahara, T. *Curr. Pharm. Des.* **1996**, *2*, 531; (b) Walenga, J. M.; Jeske, W. P.; Hoppensteadt, D.; Kaiser, B. *Curr. Opin. Cardiovascular, Pulmonary Renal Inves. Drugs* **1999**, *1*, 13; (c) Rai, R.; Sprengeler, P. A.; Elrod, K. C.; Yong, Y. B. *Curr. Med. Chem.* **2001**, *8*, 101.
4. Nagahara, T.; Yokoyama, Y.; Inamura, K.; Komoriya, S.; Yamaguchi, H.; Hara, T.; Iwamoto, M. *J. Med. Chem.* **1994**, *37*, 1200.
5. Faull, A. W.; Mayo, C. M.; Preston, J. Int. Pub. No. WO 96/10022, 4 April 1996.
6. Zhu, B. Y.; Scarborough, R. M. *Annu. Rep. Med. Chem.* **2000**, *35*, 83.
7. (a) 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; (b) 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.; Wong, J. M.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2001**, *44*, 566; (c) Jia, Z. J.; Wu, Y.; Huang, W.; Goldman, E.; Zhang, P.; Woolfrey, J.; Wong, P.; Huang, B.; Sinha, U.; Park, G.; Reed, A.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1651; (d) Song, Y.; Clizbe, L.; Bhakta, C.; Teng, W.; Li, W.; Wong, P.; Huang, B.; Sinha, U.; Park, G.; Reed, A.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2043; (e) Quan, M. L.; Ellis, C. D.; He, M. Y.; Liauw, A. Y.; Woerner, F. J.; Alexander, R. S.; Knabb, R. M.; Lam, P. Y. S.; Luettgen, J. M.; Wong, P. C.; Wright, M. R.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 369; (f) Choi-Sledeski, Y. M.; Kearney, R.; Poli, G.; Pauls, H.; Gardner, C.; Gong, Y.; Becker, M.; Davis, R.; Spada, A.; Liang, G.; Chu, V.; Brown, K.; Collussi, D.; Leadley, R., Jr.; Rebello, S.; Moxey, P.; Morgan, S.; Bntley, R.; Kasiewski, C.; Maignan, S.; Guilloteau, J.-P.; Mikol, V. *J. Med. Chem.* **2003**, *46*, 681.
8. Wender, P. A.; White, A. W. *Tetrahedron* **1983**, *39*, 3767.
9. Anti-fXa activity in vitro. In vitro anti-fXa activity was measured by using a chromogenic substrate S-2222 (Chromogenix, Inc.) and human fXa (Cosmo Bio-ERL). Aqueous DMSO (5% V/V; 10 μL) or inhibitors in aqueous DMSO (10 μL) and 0.05 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). The 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) were 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. Anti-thrombin activity in vitro. In vitro anti-thrombin activity was measured by using chromogenic substrate S-2266 (Chromogenix, Inc.) and human thrombin (Sigma Chemical, Inc.). The detail method was according to the one for anti-fXa activity.
11. Prothrombin time measurements were conducted as in the following: Hara, T.; Yokoyama, A.; Ishihara, H.; Yokoyama, Y.; Nagahara, T.; Iwamoto, M. *Thromb. Haemostasis* **1994**, *71*, 314.
12. Anti-fXa activity and anti-coagulant activity ex vivo. Male wister rats were fasted overnight. Synthetic compounds were dissolved in 0.5% (w/v) methylcellose solution and administered orally to rats with a stomach tube. For control rats, 0.5% (w/v) methylcellose solution was administered orally. Rats were anesthetized with halothane at several time points when blood samples were collected in the presence of trisodiumcitrate. After blood samples were centrifuged, the platelet poor plasma samples were used for measuring their anti-fXa activities or anti-coagulant 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). The 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) were measured at 405 nm. Anti-fXa activity (inhibition %) was calculated as follows: Anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of control]. Anti-coagulant activity: Plasma (20 μL) was mixed with inhibitors in saline (20 μL) in the process tube. The coagulation was started by the addition of SIMPLASTIN (40 μL). Anti-coagulant activity was evaluated with the prolongation rate of prothrombin time versus control.

13. Absorption rates in rat intestine were measured according to the method described in the following: Hori, R.; Okano, T.; Kato, M.; Maegawa, H.; Inui, K. *J. Pharm. Pharmacol.* **1988**, *40*, 646.