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Bioorganic & Medicinal Chemistry Letters 14 (2004) 4281–4286
Bioorganic & Medicinal Chemistry Letters

Structure–activity relationships of potent and selective factor Xa inhibitors: benzimidazole derivatives with the side chain oriented to the prime site of factor Xa

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Received 26 April 2004; accepted 31 May 2004

Abstract—A series of benzimidazole derivatives with the side chain on the nitrogen atom oriented to the prime site of factor Xa (FXa) were designed and synthesized. Compounds with substituted aminocarbonylmethyl groups as the side chain showed potent FXa inhibitory activity. Compounds **1** and **2** exhibited most potent inhibitory activity and were effective as anticoagulants in a DIC model.
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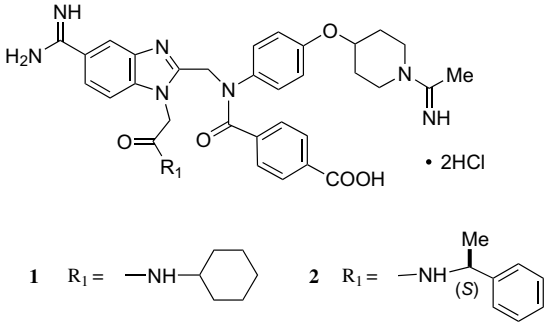
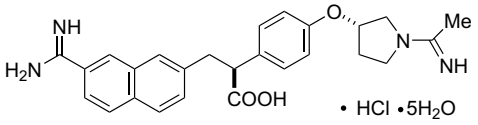
Factor Xa (FXa) plays a crucial role in the coagulation cascade, since it is the point of convergence of the intrinsic and extrinsic pathways. Together with nonenzymatic cofactor Va and Ca^{2+} on the surface phospholipids of platelets and endothelial cells, factor Xa forms the prothrombinase complex that is responsible for

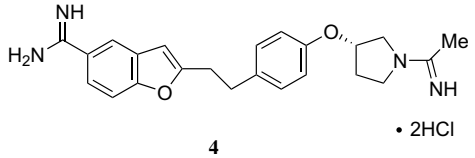


1 R_1 = —NH—

2 R_1 = —NH—



3 (DX-9065a)



4

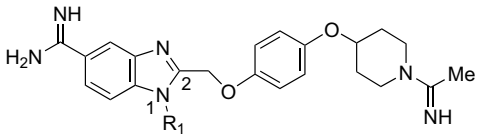


Figure 1. Skeleton A.

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doi:10.1016/j.bmcl.2004.05.092

the proteolysis of prothrombin to thrombin. Then, thrombin catalyzes the cleavage of fibrinogen to fibrin, initiating a process that ultimately leads to clot formation.

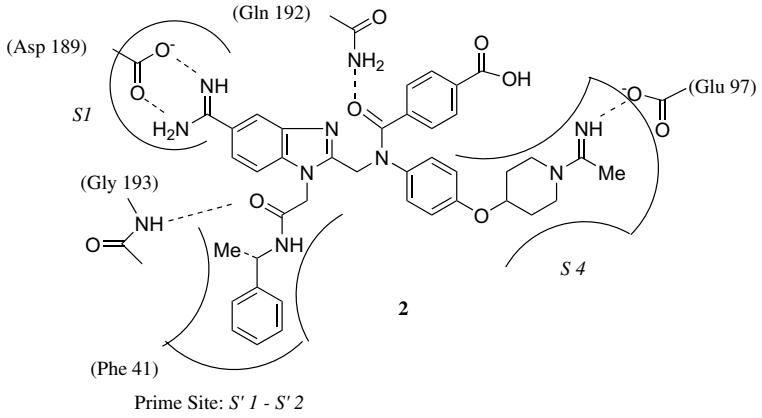
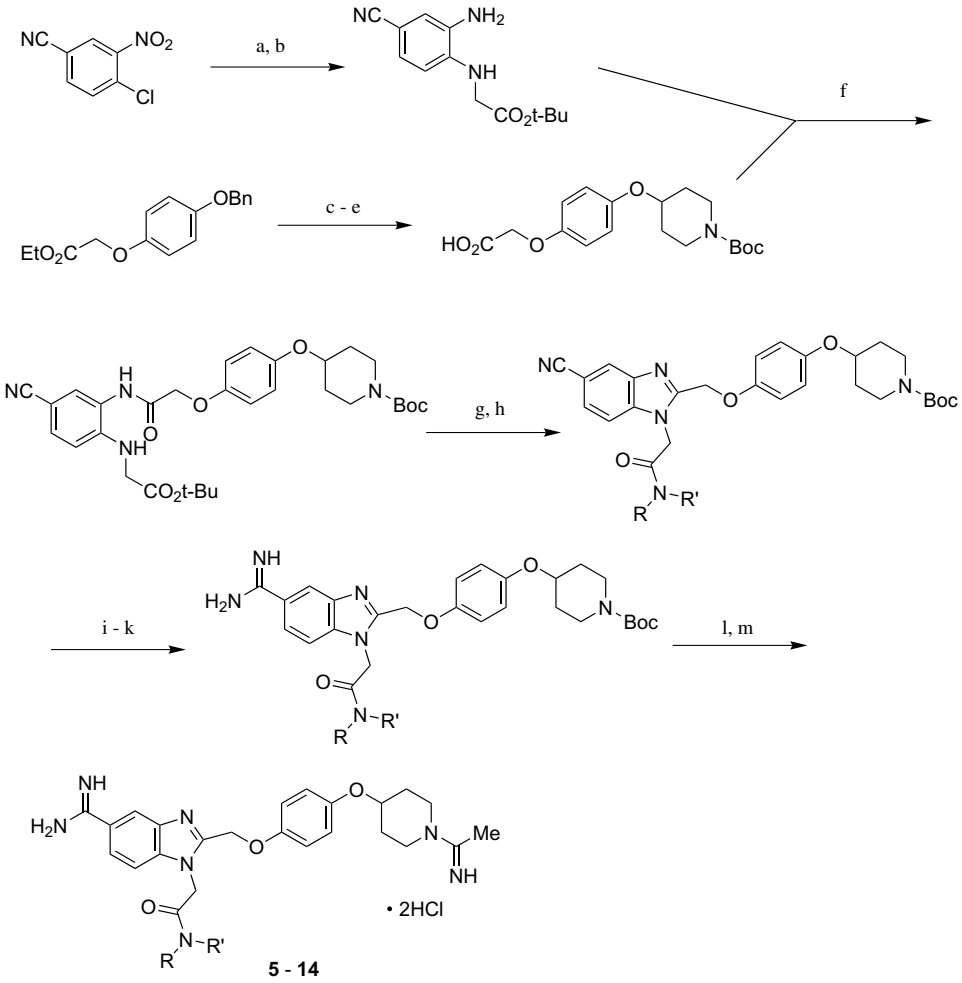


Figure 2. Computer-simulated docking of compound **2** toward FXa.



Scheme 1. Synthesis of compounds **5**–**14**. Reagents and conditions*[6]: (a) Gly-O-^{t}Bu (1.8 equiv), Et_3N (3.0 equiv), EtOH, rt −50 °C, 18 h; (b) H_2, 7.5% Pd/C, THF, 1 atm, rt, 2 h; (c) H_2, 7.5% Pd/C, THF, 3 atm, rt, 7 h; (d) *N*-Boc-4-piperidinol (1.2 equiv), DEAD (1.2 equiv), PPh_3 (1.2 equiv), THF, rt, 5 h; (e) NaOH (1.1 equiv), THF–EtOH, rt, 0.5 h; (f) 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (1.1 equiv), $CHCl_3$, rt, 3 h; (g) AcOH, 75 °C, 12 h, then Boc_2O (1.0 equiv), Na_2CO_3 (3.0 equiv), THF–H_2O, rt, 12 h; (h) RR′NH (1.0 equiv), EDC (1.1 equiv), HOBt (1.1 equiv), DMF, rt, 15 h; (i) H_2S, pyridine–Et_3N, rt, 12 h; (j) MeI (excess), acetone–MeOH, reflux, 1 h; (k) NH_4OAc (1.5 equiv), EtOH, 75 °C, 3 h; (l) TFA, $CHCl_3$, rt, 5 min; (m) ethyl acetoimidate (5.0 equiv), Et_3N (10 equiv), rt, 18 h, then HCl aq.

Since the discovery of compound **3** (DX-9065a),[1,2] a variety of compounds have been reported as FXa inhibitors.[3] Release of fondaparin sodium in 2002, which is a synthetic pentasaccharide, has proven the clinical effectiveness of FXa inhibitors as anticoagulants.[4] These situations prompted us to disclose our experimental results on novel FXa inhibitors (Fig. 1).

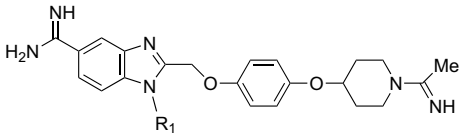
By computer associated investigation of a model of the FXa complex with compound **3**, we recognized the importance of an additional binding site around Phe 41, a prime site, in addition to the S1 site and the S4 site of FXa (Fig. 2).[5] A survey of the literature revealed no report on the synthesis of compounds with any interaction at the prime site of FXa. Therefore, we focused on the design and synthesis of novel compounds with the side chain oriented toward the prime site.

Benzofuran **4**, one of the derivatives of compound **3**, was reported to exhibit moderate enzyme inhibitory activity ($IC_{50} = 0.6\,\mu M$). From modeling studies of a complex of **4** with FXa, it was found that the oxygen atom of benzofuran was oriented to the prime site at a suitable angle. In order to introduce a substituent for the prime site, the benzofuran ring of **4** was replaced with a benzimidazole ring. After additional minor modifications from the synthetic viewpoint, we recognized that skeleton (**A**) should be the best fundamental structure for our synthesis. We initially prepared compound **5**, which showed appropriate enzyme inhibitory activity ($IC_{50} = 0.4\,\mu M$) (Scheme 1 and Table 1).[2,6] This result demonstrated that the fundamental skeleton (**A**) had sufficient potential for further studies on structure-activity relationships.

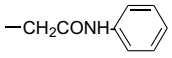
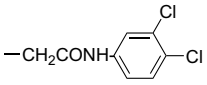
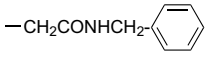
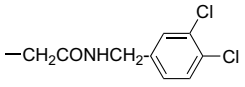
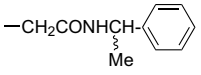
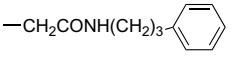
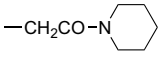
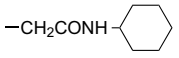
From observation of a model of complex of compound **5** with FXa, we found the following: (i) the region close to the benzimidazole ring is hydrophilic, and an oxianion hole exists in the vicinity of the ethyl group and (ii) the more distant region, where Phe 41 exists, is hydrophobic and may prefer aromatic rings or aliphatic substituents. After further investigation of a substituent closer to the prime site, we found that the oxygen of an amide could be appropriately positioned to form a hydrogen bond to the NH of Gly 193 if an ethyl group is replaced with a carbamoyl methyl group (Fig. 2).

Thus, we synthesized the compounds shown in Table 1 on the above basis (Scheme 1). Among them, compounds **7**, **9**, **10**, and **13** with appropriate side chains toward the prime site showed more potent enzyme inhibitory activity in comparison to compounds **4** and **5**.[7] In any case shown in Table 1, fatal acute toxicity was observed after intravenous administration of the compound as a bolus dose of 10 mg/kg to mice. We suspected that this toxicity might be due to strong basicity of the two amidino groups or activity for other targets. In order to counteract toxicity, we considered introducing a benzoyl group substituted with a carboxyl group with the expectation that both suspected causes might be controlled at the same time by creating acidity and increasing the size. Using compound **13**, computational simulation was undertaken to decide the position for introducing the substituted benzoyl group. As a result, it was clarified that a β-position of the side chain at position-2 of the benzimidazole ring might be replaced with a nitrogen atom, which is substituted with the benzoyl group, since this replacement was expected to be possible without much influence on the conformation of **13** or collision against FXa.

Table 1. Enzyme inhibitory activity for FXa and FIIa[2]

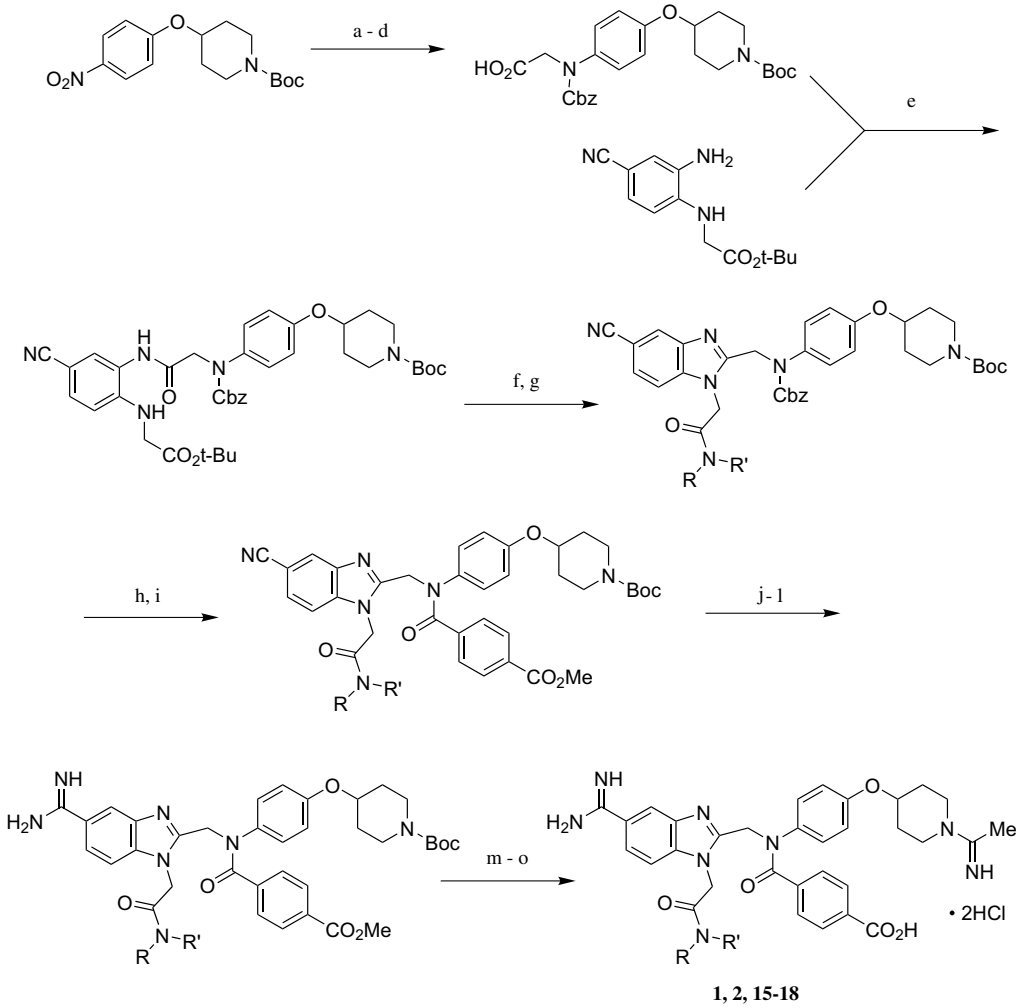


• 2HCl

Compound	R_1	FXa IC_{50} (μM)[b]	FIIa IC_{50} (μM)[b]
5	$-CH_2CH_3$	0.4	>10
6	$-CH_2CONH-$ 	0.1	>10
7	$-CH_2CONH-$ 	0.06	>10
8	$-CH_2CONHCH_2-$ 	0.1	>10
9	$-CH_2CONHCH_2-$ 	0.03	>10
10[a]	$-CH_2CONHCH(Me)-$ 	0.03	>10
11	$-CH_2CONH(CH_2)_3-$ 	0.2	>10
12	$-CH_2CO-N$ 	3.0	>10
13	$-CH_2CONH-$ 	0.04	>10
14	$-CH_2CONHCH_2-$ 	0.3	>10

[a] Racemic form.
[b] $n = 2$.

Thus, we synthesized compound **1**, which had a nitrogen atom substituted with a 4-carboxybenzoyl group (Scheme 2).[6] Compound **1** did not show fatal acute toxicity at a dose of 30 mg/kg (iv) and exhibited more potent inhibitory activity against FXa ($IC_{50} = 0.01\,\mu M$). The corresponding isomer with a *m*-carboxyl group also did not show toxicity at doses up to 30mg/kg (iv), while the methyl ester of **1** was highly toxic ($LD_{50} = <10$ mg/kg). Although the cause of the toxicity was still unclear,



Scheme 2. Synthesis of compounds **1**, **2**, and **15**–**18**. Reagents and conditions*[6]: (a) H_2, 7.5% Pd/C, THF–EtOH, 3 atm, rt, 3 h; (b) CbzCl (1.0 equiv), $NaHCO_3$ (1.1 equiv), THF–H_2O, rt, 1 h; (c) NaH (1.5 equiv), $BrCH_2CO_2Et$ (1.5 equiv), DMF, rt, 2 h; (d) NaOH (1.1 equiv), THF–EtOH, 50 °C, 1 h; (e) EEDQ (1.1 equiv), $CHCl_3$, rt, 3 h; (f) AcOH, 90 °C, 4 d, then Boc_2O (1.0 equiv), Na_2CO_3 (3.0 equiv), THF–H_2O, rt, 12 h; (g) RR′NH (1.0 equiv), EDC (1.1 equiv), HOBt (1.1 equiv), DMF, rt, 15 h; (h) H_2, 7.5% Pd/C, THF, 3 atm, rt, 7 h; (i) (4-MeOCO)PhCOCl (1.0 equiv), Et_3N (1.5 equiv), $CHCl_3$, rt, 18 h; (j) H_2S, pyridine–Et_3N, rt, 12 h; (k) MeI (excess), acetone–MeOH, reflux, 2 h; (l) NH_4OAc (1.5 equiv), EtOH, 75 °C, 2 h; (m) TFA, $CHCl_3$, rt, 5 min; (n) NaOH (5.0 equiv), H_2O, rt, 5 h; (o) ethyl acetoimidate (5.0 equiv), Et_3N (10 equiv), rt, 18 h, then HCl aq.

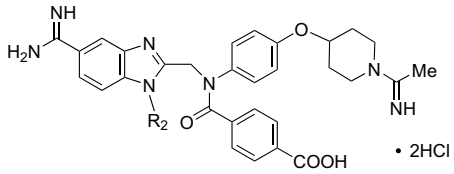
these results suggested that the toxicity was reduced by the presence of an acidic functional group instead of the steric factor. On the other hand, there was no difference in enzyme inhibitory activity between the two compounds ($IC_{50} = 0.02$ and 0.01 μM, respectively), and their activity was the same as that of compound **1**. Similarly, several derivatives corresponding to compounds showing potent activity in Table 1 were synthesized (Table 2).[6] None of the compounds in Table 2 showed fatal acute toxicity at a dose of 10 mg/kg (iv, bolus) in mice and they exhibited enhanced potency except for compound **17**.

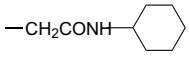
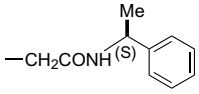
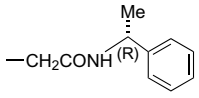
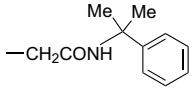
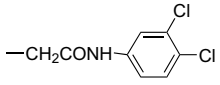
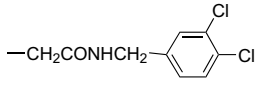
Finally, optimization of the *N*-iminoacetyl-piperidino moiety was carried out, fixing the side chain of the prime site with a cyclohexylaminocarbonylmethyl group (Table 3). In addition to the enzyme inhibitory activity shown in Table 3, pharmacokinetic evaluation[8] clarified that the compound with the *N*-iminoacetyl-piperidino group (compound **1**) was superior to other compounds with the corresponding pyrrolidino group (compound **21**), the *N*-amidino-piperidino group (compound **19**), and so on (**20**, **22**, **23**).

Pharmacological evaluation was undertaken to determine the efficacy as anticoagulants. In a model of disseminated intravascular coagulation (DIC) induced by tissue factor, compounds **1** and **2** were effective even at a low dose of 0.2 mg/kg (mouse, iv, Table 2).[9] In addition, compound **2** was shown to have potential as an orally active anticoagulant by an ex vivo inhibitory assay in mice (human FXa; Fig. 3).[8]

In conclusion, using three-dimensional computer modeling, we synthesized novel FXa inhibitors, that were benzimidazole derivatives with the side chain oriented to the prime site of FXa. Compounds **1** and **2** exhibited more potent FXa inhibitory activity than compound **3** and were effective as anticoagulants in a DIC model.

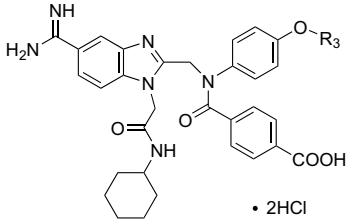
Table 2. Enzyme inhibitory activity for FXa/FIIa,[2] and effect in a model of disseminated intravascular coagulation (DIC)[9]



Compound	R_2	FXa/FIIa IC_{50} (μM)[b]	Effect in mouse DIC model ED_{50} (mg/kg)
3 (DX-9065a)	—	0.06/>10	0.5
1		0.01/>10	0.2
2		0.003/>10	0.2
15		0.08/>10	NT[a]
16		0.2/>10	NT
17		0.2/>10	0.7
18		0.006/>10	0.7

[a] NT = not tested.
[b] $n = 2$.

Table 3. Enzyme inhibitory activity for FXa/FIIa[2]



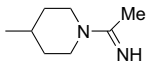
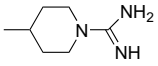
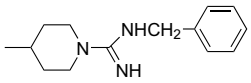
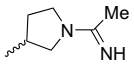
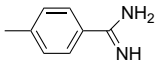
Compound	R_3	FXa IC_{50} (μM)[b]	FIIa IC_{50} (μM)[b]
1		0.01	>10
19		0.02	>10

Table 3 (*continued*)

Compound	R_3	FXa IC_{50} (μM)[b]	FIIa IC_{50} (μM)[b]
20		0.08	>10
21[a]		0.1	>10
22[a]		0.3	>10
23		2.0	>10

[a] Racemic form.
[b] $n = 2$.

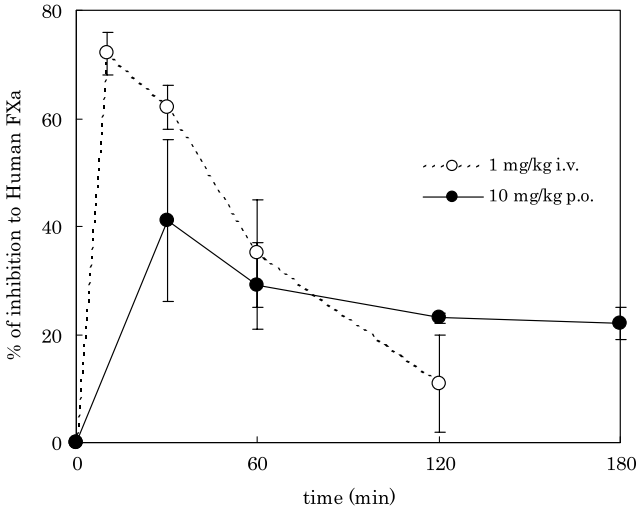


Figure 3. Percent inhibition of human FXa after intravenous and oral administration of compound **2** in mice.[8] Data represent mean ± SD ($n = 3$).

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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.
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5. The FXa-compound **3** (DX9065a) complex model was built by docking compound **3** into the S1 and aryl binding sites of the FXa crystal structure (PDB code 1hcg). The Discover and Insight II programs from Accerlys were used for energy calculation and graphical display, respectively. This model was similar to the subsequently reported crystal structure of FXa-compound **3** (PDB code 1 fax) (Ref. 5b). (a) Katakura, S.; Nagahara, T.; Hara, T.; Kunitada, S.; Iwamoto, M. *Eur. J. Med. Chem.* **1995**, *30*, 387; (b) Brandstetter, H.; Kuhne, A.; Bode, W.; Huber, R.; von der Saal, W.; Wirthensohn, K.; Engh, R. A. *J. Biol. Chem.* **1996**, *271*, 29988.
6. Katoh, S.; Yokota, K.; Hayashi, M., PCT Int. Appl.-WO9952895, 1999.
7. After pre-incubation for 2 h, enzyme inhibitory activity was unchanged. This indicated that the amide moiety was not hydrolyzed even though the carbonyl group was designed to interact with Gly 193, which formed an oxi-anion hole.
8. Pharmacokinetic evaluation was carried out by an ex vivo inhibitory assay in mice (po 10 mg/kg). Compound **1** showed 25% inhibition at 0.5 h and 15% inhibition at 1.0 h, while compounds **19** and **20** showed no inhibition. Experiment: Human factor Xa (40 μL) and diluted plasma were incubated in Tris–buffer, followed by addition of a synthetic substrate and incubation. The reaction was stopped by addition of acetic acid and the absorbance at 405 nm was measured. The control was plasma obtained prior to administration of the test compound and human factor Xa inhibitory activity was calculated as the percent inhibition relative to the control.
9. Asakura, H.; Ichino, T.; Yoshida, T.; Suga, Y.; Ontachi, Y.; Mizutani, T.; Kato, M.; Ito, T.; Yamazaki, M.; Aoshima, K.; Morishita, E.; Saito, M.; Miyamoto, K.; Nakao, S. *Blood Coagul. Fibrin.* **2002**, *3*, 233, ED_{50} values express the drug concentration required to produce 50% inhibition of tissue factor-induced platelet aggregation in comparison with the vehicle.