Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 963–966

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design, Synthesis, and In Vitro Biological Activity of Benzimidazole Based Factor Xa Inhibitors

Zuchun (Spring) Zhao,* Damian O. Arnaiz, Brian Griedel, Steven Sakata, Jerry L. Dallas, Marc Whitlow, Lan Trinh, Joseph Post, Amy Liang, Michael M. Morrissey and Kenneth J. Shaw

Discovery Research, Berlex Biosciences, 15049 San Pablo Avenue, PO Box 4099, Richmond, CA 94804-0099, USA

Received 13 December 1999; accepted 22 February 2000

Abstract—Inhibitors based on the benzimidazole scaffold showed subnanomolar potency against Factor Xa with 500–1000-fold selectivity against thrombin and 50–100-fold selectivity against trypsin. The 2-substituent on the benzimidazole ring had a strong impact on the FXa inhibitory activity. Crystallography studies suggest that the 2-substituent may have a conformational effect favoring the extended binding conformation. © 2000 Elsevier Science Ltd. All rights reserved.

Factor Xa (FXa) is a trypsin-like serine protease that plays a central role in the blood coagulation cascade linking the intrinsic and extrinsic pathways to the final common pathway of coagulation. The primary role of FXa is the proteolytic activation of thrombin (FIIa), after combining with factor Va and calcium on a phospholipid membrane to form the prothrombinase complex. Prothrombinase catalyzes the formation of thrombin from prothrombin. Thrombin, in turn, promotes blood clot formation by catalyzing the formation of polymerizable fibrin from fibrinogen, as well as strongly inducing platelet aggregation.[1] Since direct inhibition of thrombin has demonstrated a tendency to undesirably prolong bleeding, the development of FXa inhibitors has emerged as a alternative focus for the treatment and prevention of thrombotic disorders.[2] This is supported by the recent reports of the low molecular weight FXa inhibitors that have been found to be efficacious in animal models of thrombosis, and offer the potential for improved treatment of a variety of thrombotic disorders.[3]

In a previous communication we identified a series of inhibitors based on indole and carbazole scaffolds.[4] These studies identified the 2-amidinonapthyl and 4-iminoethylpiperidonyl groups as optimal substituents to bind in the S1 and S4 pockets of Factor Xa, similar to the reported studies with DX9065.[3a] Compounds from these series were potent inhibitors of FXa when measured against free FXa in vitro, however were weak functional anticoagulants as measured by their prothrombin based clotting time (PT) in plasma. The poor anticoagulant activity was thought to be due to the low aqueous solubility and high lipophilicity of these inhibitors that could lead to extensive protein binding in plasma, therefore the central scaffold was modified in an attempt to increase the hydrophilicity. In this communication, we describe our initial series of inhibitors based on the benzimidazole scaffold with improved potency, selectivity and anticoagulant activity.

A general synthesis of the benzimidazole based FXa inhibitors described in Table 1 is shown in Scheme 1.[5] The commercially available 4-amino-3-nitrophenol **1** was reduced to the bis-aniline, acylated with the appropriate acid chloride and then cyclized by refluxing in concentrated hydrochloric acid to afford the various 2-substituted benzimidazole derivatives **2**. BOC protection followed by Mitsunobu[6] reaction with *N*-*t*-butoxycarbonyl-4-hydroxyl-piperidine and then selective removal of the benzimidazole BOC group with methanolic ammonia afforded intermediate **4**. Alkylation of **4** with sodium hydride and 7-cyano-2-bromomethylnapthalene **5** afforded a 1:1 mixture of 5 and 6 regioisomers (**6**). This regioisomeric mixture was transformed to the corresponding amidines by sequential treatment with HCl and ammonia gas in ethanol with concomitant deprotection of the Boc group.[7] The regioisomeric mixtures were separable by reverse-phase HPLC, but were generally carried on as a mixture and treated with ethyl acetimidoimidate and triethylamine to afford the dibasic benzimidazole analogues **7** and **8**. The regioisomers

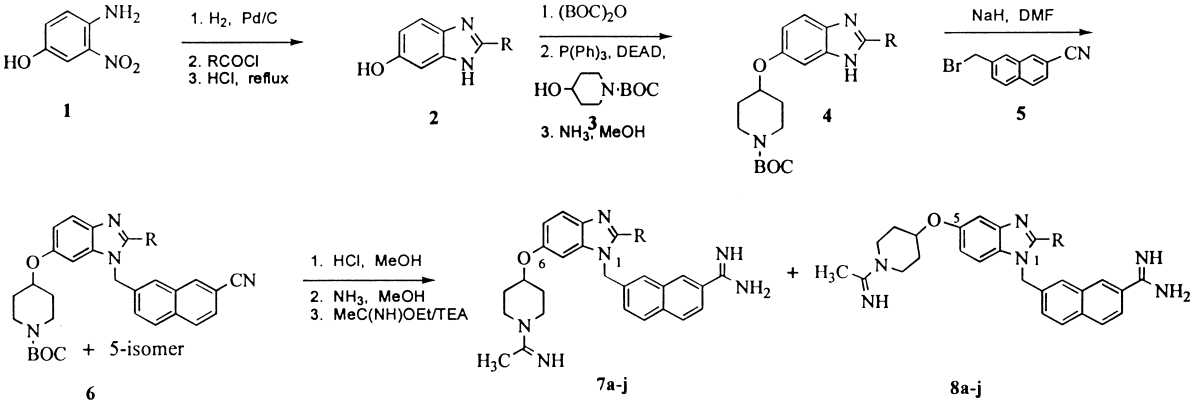
*Corresponding author. Tel.: +1-510-669-4457; fax: +1-510-669-4310; e-mail: Spring_Zhao@berlex.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00139-6

Table 1. In vitro activities of 2-substituted benzimidazole regioisomers

		6-Regioisomer, K_i (nM)[a]			2xPT(nM)[b]		5-Regioisomer, K_i (nM)		
R	No.	hFXa	hFIIa	bTrp		No.	hFXa	hFIIa	bTrp
H	**7a**	2.9	810	50	450	**8a**	6.3	>5000	8.2
Me	**7b**	0.27	230	18	350	**8b**	6.2	3900	16
Et	**7c**	0.087	60	7.7	130	**8c**	4.0	2100	17
i-Pr	**7d**	0.026	32	5.7	68	**8d**	2.0	870	16
t-Bu	**7e**	0.046	48	7.0	260	**8e**	2.0	950	18
s-Bu	**7f**	0.12	160	10	280	**8f**	3.3	2300	13
n-Pr	**7g**	0.23	70	6.8	290	**8g**	4.5	2400	16
n-Bu	**7h**	0.23	230	5.7	625	**8h**	2.8	2000	14
$(CH_2)_2COOH$	**7i**	1.3	2670	18	ND[c]	**8i**	5.9	>5000	14
$(CH_2)_2CONH_2$	**7j**	1.0	590	4.4	ND[c]	**8j**	3.6	>5000	10

[a]K_i values for these competitive inhibitors are averaged from multiple determinations ($n \geq 2$) and the standard deviations are $< 30\%$ of the mean.
[b]Concentration of inhibitor required to double the prothrombin based clotting time in human plasma.
[c]Not determined.



Scheme 1.

were separated and purified by preparative reverse-phase HPLC, and the regiochemistry of the isomers were determined by NOE studies. In the 1,5-isomers cross NOE effect was observed between the naphthyl-CH_2 and the benzimidazole H-4, whereas, in the 1,6-isomers NOE effect was observed between the CH_2 and the H-7.

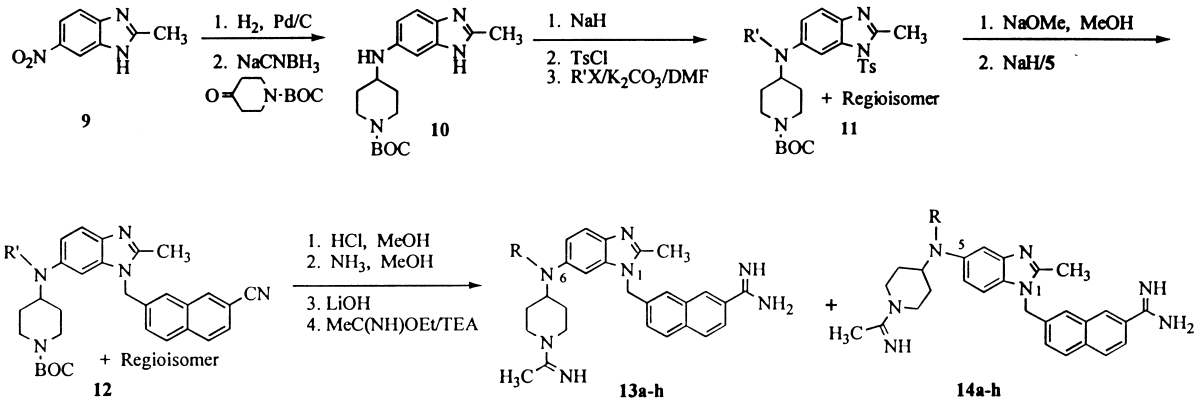
A general synthesis of the 6-aminosubstituted inhibitors described in Table 2 is illustrated in Scheme 2. Catalytic hydrogenation of commercially available 5-nitrobenzimidazole **9** followed by reductive amination with *N*-BOC-4-piperidone and sodium cyanoborohydride afforded **10**. The benzimidazole ring nitrogen was selectively protected by reaction with one equivalent of sodium hydride and *p*-toluenesulphonyl chloride, which allowed for selective alkylation or acylation of the aniline nitrogen. Selective deprotection of the benzimidazole ring nitrogen followed by alkylation with **5** afforded a 1:1 mixture of the 5 and 6 regioisomers (**12**) that were converted to **13** and **14** in a similar manner as described above.

Examination of the results in Table 1 demonstrates that the substituent at the 2-position of the benzimidazole has a large influence on the FXa inhibitory activity of the 6-substituted regioisomers (**7a**–**j**), which is consistent with our earlier studies with the indole and carbazole

Table 2. In vitro inhibitory activities of *N*-substituted benzimidazole regioisomers

		6-Regioisomer, K_i (nM)[a]				5-Regioisomer, K_i (nM)		
R	No.	hFXa	hFIIa	bTrp	No.	hFXa	hFIIa	bTrp
CH_2COOMe	**13a**	0.3	98	11	**13a**	22	2700	7.6
CH_2CONH_2	**13b**	0.46	490	28	**13b**	ND[b]	ND	ND
$CH_2CH_2CH_2COOMe$	**13c**	300	4610	260	**13c**	30	6000	60
$CH_2CH_2CH_2COOH$	**13d**	420	5000	670	**13d**	370	5000	800
$CH_2CH(Me)COOMe$	**13e**	1.1	300	41	**13e**	80	5000	160
$CH_2CH(Me)COOH$	**13f**	3.0	650	47	**13f**	80	5000	120
$COCH_2CH_2COOEt$	**13g**	300	4600	256	**13g**	370	5000	800
$COCH_2CH_2COOH$	**13h**	417	5000	670	**13h**	ND	ND	ND

[a]K_i values for these competitive inhibitors are averaged from multiple determinations ($n \geq 2$) and the standard deviations are $< 30\%$ of the mean.
[b]Not determined.



9 10 11 12 13a-h 14a-h

Scheme 2.

inhibitors.[4] Substitution of a methyl group at the 2-position (**7b**, FXa $K_i = 0.27$ nM) affords a 10-fold increase in the FXa inhibitory activity compared to the unsubstituted analogue (**7a** FXa $K_i = 2.9$ nM). An additional 3-fold increase in activity is seen with the ethyl analogue (**7c**, FXa $K_i = 0.087$ nM) and another 3-fold enhancement is seen with the isopropyl substituent (**7d**, FXa $K_i = 0.026$ nM). The isopropyl substituent appears to be the optimal alkyl substituent as the *n*-propyl substituent (**7g**) or the butyl substituents (**7e,f,h**) are less active. Introduction of a polar substituent had a negative influence on the FXa inhibitory activities as demonstrated by the propionic acid and amide analogues (**7i** and **7j**). Overall, comparison of **7a** with **7d** shows a 1000-fold increase of the inhibitory activity by substitution of an isopropyl substituent for a hydrogen atom at the 2-position of the benzimidazole. A similar but less pronounced increase in potency is also seen towards FIIa (25-fold) and trypsin (Trp) (approximately 10-fold), which improves the overall selectivity profile for this series of inhibitors. In contrast to the results with the 6-regioisomers, there is a maximum 3-fold effect of the 2-substituent on the FXa inhibitory activities for the 5-regioisomers (**8a**–**8j**).

The 1,6-benzimidazole based FXa inhibitors are potent functional anticoagulants as measured by the concentration needed to double the clotting fold in plasma (2×PT). All inhibitors extended the clotting time at submicromolar concentrations (2×PT = 68–450 nM) in contrast to the weak anticoagulant activity of the indole and carbazole based FXa inhibitors. The improved anticoagulant activity may be attributed to the increased FXa inhibitory activity as well as improved aqueous solubility.

X-ray crystallography of **7b** in bovine trypsin is shown in Figure 1.[8] This inhibitor is bound in the active site of trypsin in an extended L-shaped conformation, similar to the reported binding mode of DX-9065a in bovine trypsin and human FXa.[9] The naphthylamidine ring is bound in the S1 pocket, making four hydrogen bonds to Asp189, Ser 190, and Gly219. The central benzimidazole ring sits above the catalytic His57 and makes no apparent contact with the protein, and the iminoethylpiperidine ring binds in the S4 pocket, defined by Thr98, Leu99, Gln175, and Trp215. The 2-methyl group appears to make no contact with the protein but may exert a steric influence on the conformation of the naphthylamidine substituent that would favor the extended L-shape conformation of the molecule explaining the effect of this substituent.

The nitrogen substituted analogues in Table 2 were prepared in an attempt to append substituents that would either enhance the inhibitory activity, or provide a site for substitution of a carboxylate substituent that could enhance the oral activity for this series of compounds. The results in Table 2 demonstrate that while alkylation with acetate or acetamide (**13a** and **13b**) afforded no significant loss of the FXa inhibitor potency in comparison to **7b**, the larger substituents led to inhibitors with significantly lowered inhibitory activity. The corresponding acetic acid analogue was prepared in an attempt to evaluate the effect of this substituent on the oral absorption, however the compound was not stable.

In summary, we have developed a series of FXa inhibitors based on the benzimidazole scaffold. These inhibitors

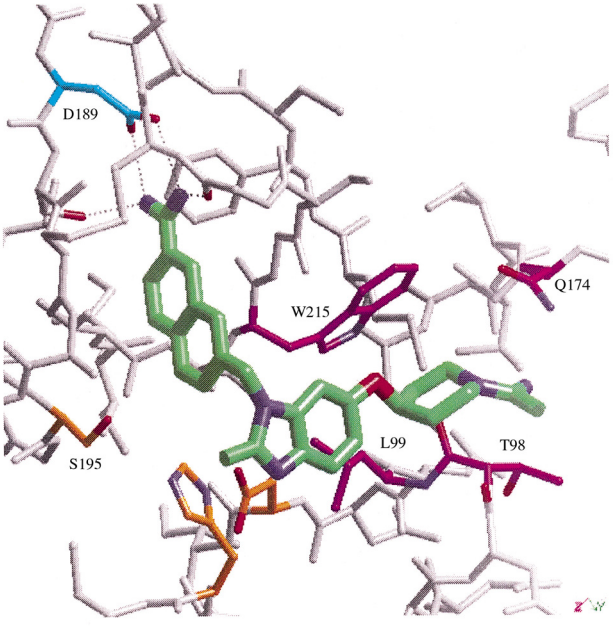


Figure 1. X-ray crystal structure of **7b** bound to the active site of bovine trypsin.

have subnanomolar inhibitory activity against FXa with 500–1000-fold selectivity for thrombin and 50–100-fold selectivity for trypsin. It was also demonstrated that the 2-substituent on the benzimidazole ring played a critical role in enhancing inhibitor potency. X-ray crystallography indicated that the inhibitor binds to trypsin in an L-shaped confirmation, and the 2-substituent on the benzimidazole may have a conformational effect favoring the extended L-shaped conformation. Substitution of an exocyclic nitrogen with carboxyl derived substituents decreased the FXa inhibitory activity. This led to a series of carboxylate analogueues derived from substitution at the 4, 5, and 7 positions of the benzimidazole ring with enhanced oral absorption. This work will be the focus of a future communication.

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