Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 957–961

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design, Synthesis, and In Vitro Biological Activity of Indole-based Factor Xa Inhibitors

Damian O. Arnaiz,* Zuchun (Spring) Zhao, Amy Liang, Lan Trinh, Marc Whitlow, Sunil K. Koovakkat and Kenneth J. Shaw

Discovery Research, Berlex Biosciences 15049 San Pablo Ave. PO Box 4099, Richmond, CA 94804, USA

Received 13 December 1999; accepted 22 February 2000

Abstract—A series of indole and carbazole based inhibitors of factor Xa (FXa) has been investigated. The most potent compound inhibits FXa with a K_i of 0.2 nM and has 900- and 750-fold selectivity over thrombin and trypsin, respectively.

Introduction

The interruption of the coagulation cascade is of primary importance in the prevention and treatment of thrombotic disorders. The serine protease factor Xa (FXa) links the intrinsic and extrinsic pathways of the coagulation cascade. The prothrombinase complex, formed by the combination of FXa with factor Va, Ca^{2+} and phospholipids, catalyzes the conversion of prothrombin to thrombin. Thrombin causes blood clotting by induction of platelet aggregation and conversion of fibrinogen to insoluble fibrin. Thrombin inhibitors have been extensively studied as anticoagulants, but have shown a tendency to prolong bleeding at plasma concentrations close to the effective dose.[1] Since FXa inhibitors do not affect platelet function, they may have a wider therapeutic index than thrombin inhibitors have. This is supported by studies with the naturally occurring proteinaceous FXa inhibitor tick anticoagulant peptide (TAP), which has been shown to be efficacious in several animal models of thrombosis with less bleeding when compared to other antithrombotic agents.[2]

Early studies in our laboratory led to the identification of *Z*,*Z*-2,7-bis-(4-amidinobenzylidene) cycloheptanone (*Z*,*Z*-BABCH, **1**) as the active isomer in a series of conformationally rigid bis-benzamidine inhibitors (Fig. 1).[3] *Z*,*Z*-BABCH inhibits human FXa with a K_i of 0.66 nM, but has limited potential for development due to its photochemical instability. We sought to replace the cycloheptanone core of **1** with stable scaffolds that could maintain the U-shaped conformation of this molecule. This work ultimately led to the discovery of ZK 807834 (CI 1031) (**2**), a highly potent, selective, efficacious and orally active inhibitor of FXa.[4] Molecular modeling studies suggested that a bis-aryl substituted indole such as **3** could bind to FXa in a similar manner as **1**, and in this paper we describe the development of a series of potent FXa inhibitors based on this template.

Chemistry

The indole analogues in Table 1 (**7a**–**f**) were prepared from commercially available 6-nitroindole (**4**), and a representative synthesis is illustrated in Figure 2, route A. Arylation of the indole nitrogen with sodium hydride and 4-fluorobenzonitrile at 60 °C followed by reduction of the nitro group afforded **5**.[5] Alkylation with 7-(bromomethyl)-2-naphthalenecarbonitrile[6] then afforded **6**. Finally, the nitrile groups were converted to amidines by the Pinner[7] method to afford indole **7f**.

The carbazole analogues in Tables 2 and 3 (**11a**–**d**, **14a**–**b**) were synthesized from 2-hydroxycarbazole (**8**) in a similar manner as the indole analogues. A representative synthesis of **11c** is shown in Figure 2, route B. Selective arylation of the carbazole oxygen with 3-fluorobenzonitrile followed by alkylation of the indole nitrogen with 7-(bromomethyl)-2-naphthalenecarbonitrile afforded **10**. Pinner reaction then afforded **11c**. Alternatively, **8** was treated with hydroxypiperidine under Mitsunobu[8] conditions (route C) to afford **12**,

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

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00138-4

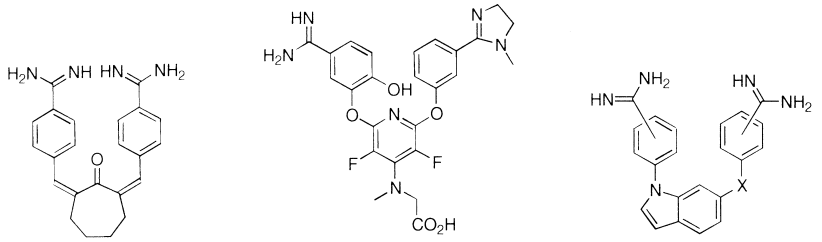


Figure 1.

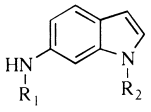
which was subsequently alkylated to afford **13**. Amidine formation resulted in concomitant cleavage of the Boc-group affording **14a**. Finally, treatment of **14a** with ethyl acetimidate afforded **14b**.

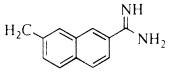
The indole analogues in Table 4 (**18a**–**e**) were synthesized from 2-carbomethoxy-6-hydroxyindole[9] (**15**) in a manner similar to that described for **14b**, and a representative synthesis of **18a** is illustrated in Figure 2, route D. Indole **18b** was prepared by a transesterification reaction by forming the imidate in ethanol. The amide analogues (**18c,d**) were formed by hydrolysis of **16** followed by a standard coupling reaction with the appropriate amines and subsequent amidine formation. The corresponding carboxylate analogue **18e** was prepared by hydrolysis of **18a**.

Results and Discussion

A series of bis-amidine substituted indoles was prepared and evaluated (Table 1). The initial compounds in this

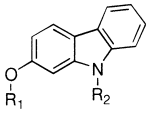
Table 1. 1,6-Disubstituted indoles[a]

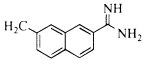
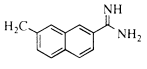
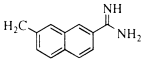


	R_1	R_2	K_i hFXa (nM)	K_i hFIIa (nM)	K_i bTrp (nM)
7a	CH_2Ph-4-Amidine	CH_2Ph-4-Amidine	1050	3530	670
7b	CH_2Ph-4-Amidine	Ph-4-Amidine	840	4530	690
7c	CH_2Ph-3-Amidine	Ph-4-Amidine	340	3780	1620
7d	CH_2Ph-3-Amidine	Ph-3-Amidine	190	4000	410
7e	C(O)Ph-3-Amidine	Ph-4-Amidine	160	2320	410
7f		Ph-4-Amidine	30	570	300

[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. See ref 4 for assay conditions.

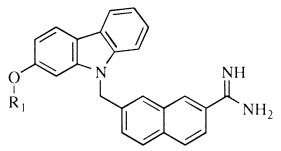
Table 2. Carbazoles[a]

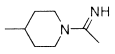


	R_1	R_2	K_i hFXa (nM)	K_i hFIIa (nM)	K_i bTrp (nM)
11a		Ph-4-Amidine	60	1990	510
11b	Ph-4-Amidine		6.0	210	250
11c	Ph-3-Amidine		0.90	170	80

[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. See ref 4 for assay conditions.

Table 3. Amidine replacement[a]



	R_1	K_i hFXa (nM)	K_i hFIIa (nM)	K_i bTrp (nM)
11d	$CH_2C(O)N(CH_3)_2$	5.0	50	90
14a		4.0	170	30
14b		1.6	40	40

[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. See ref 4 for assay conditions.

series (**7a**,**b**) inhibited FXa with a K_i of approximately 1 μM with a 3- to 5-fold selectivity over thrombin, and no selectivity over trypsin. Similar to the results observed with our diphenoxypyridine FXa inhibitors,[4] changing the R_1-amidine from the *para*- to the *meta*-position gave an increase in potency (**7c** versus **7b**), and a further increase in potency was observed by a similar transformation of the R_2-amidine (**7d**). Comparison of **7c** and **7e** demonstrates that a 2-fold increase in potency was achieved by introduction of an amide linker. Naphthylamidine is a common substituent in many known FXa inhibitors,[6,10] and studies in our laboratories have shown that 7-methyl-2-naphthylamidine is 10-fold more potent than benzamidine against FXa.[11] Consistent with these results, introduction of a naphthylamidine gave a 10-fold increase in FXa inhibitory activity (**7f** versus **7c**).

In compound **7f**, the R_2 substituent is essentially locked, and may not be optimally positioned for binding to FXa. We considered that transposition of the R_1 and R_2 substituents might improve activity by allowing more flexibility of the R_2 substituent while reducing the number of rotatable bonds of the R_1 substituent. We initially attempted this transposition on the aminoindole, however, since we were unable to introduce an aryl group onto the 6-amino substituent, we considered using commercially available 2-hydroxycarbazole as a new scaffold. Comparison of carbazole **11a** (Table 2) with indole **7f** demonstrates that the fused ring of the carbazole has minimal effect on FXa inhibitory activity. Subsequent transposition of the two substituents resulted in a 10-fold increase in activity (**11b**). Moving the R_1 amidine from the *para*- to the *meta*-position further increased the inhibitory activity, affording the first subnanomolar inhibitor in this series (**11c**). Moreover, **11c** has approximately 200-fold selectivity over thrombin and 90-fold selectivity over trypsin.

Having achieved subnanomolar FXa potency with **11c**, we next sought to replace one of the aryl amidines in an

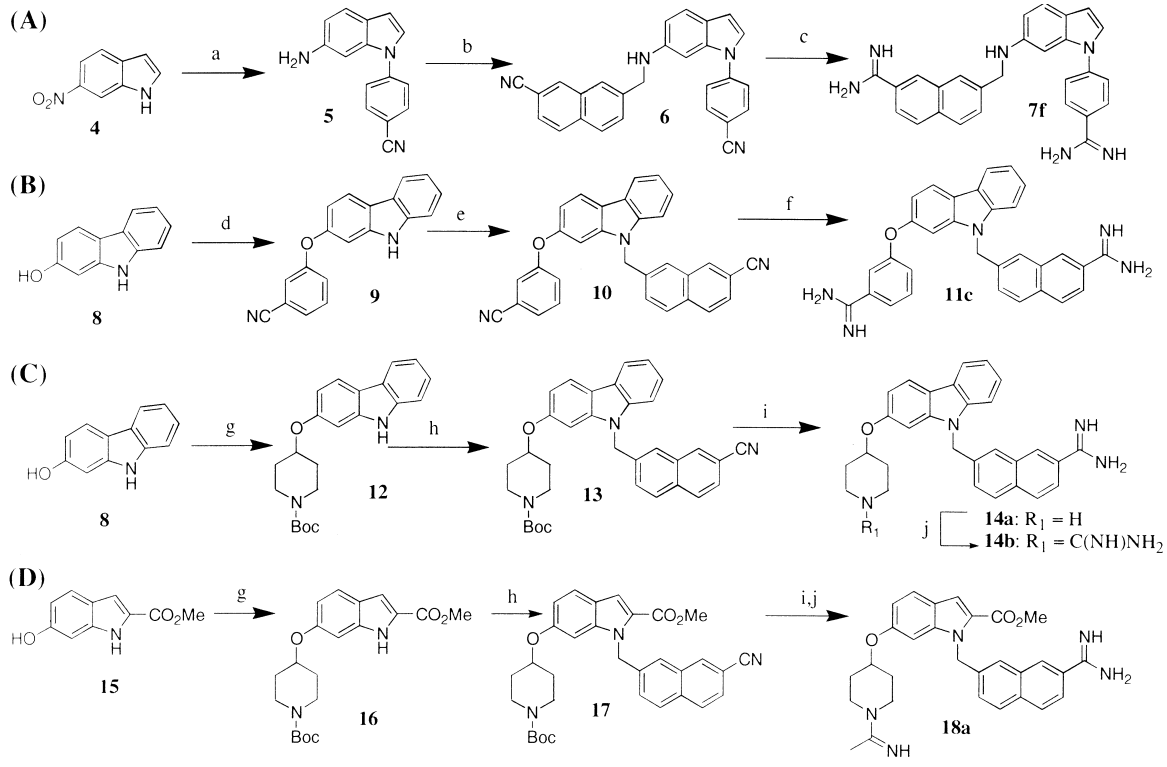
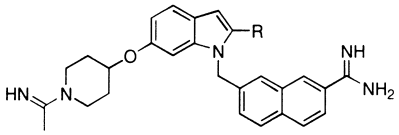


Figure 2. (a) (i) NaH, 4-FPhCN, DMF, 60 °C, (ii) $SnCl_2$, EtOAc; (b) K_2CO_3, DMF, 7-(bromomethyl)-2-naphthalenecarbonitrile; (c) (i) HCl, (ii) NH_3; (d) (i) NaH, 3-FPhCN, DMF, 60 °C; (e) NaH, DMF, 7-(bromomethyl)-2-naphthalenecarbonitrile, 25 °C; (f) (i) HCl, (ii) NH_3; (g) (i) *N*-Boc-4-hydroxypiperidine, PPh_3, DEAD, THF; (h) 7-(bromomethyl)-2-naphthalenecarbonitrile, K_2CO_3, DMF; (i) (i) HCl, (ii) NH_3; (j) ethyl acetimidate, Et_3N, MeOH.

Table 4. 1,2,6-Trisubstituted indoles[a]



	R	K_i hFXa (nM)	K_i hFIIa (nM)	K_i bTrp (nM)
18a	CO_2CH_3	0.2	180	150
18b	$CO_2CH_2CH_3$	0.6	360	20
18c	$C(O)N(CH_3)_2$	3.5	1050	90
18d	$C(O)NHCH(CO_2H)CH_2CO_2H$	20	2350	30
18e	CO_2H	4.6	520	60

[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. See ref 4 for assay conditions.

attempt to lower the overall basicity of these inhibitors. We assumed that the naphthylamidine moiety was binding in the S1 pocket and decided to replace the benzamidine moiety. The results in Table 3 illustrate the replacement of the benzamidine with acetamide (**11d**) or piperidine (**14a**), affording only a 5-fold loss in potency relative to **11c**. Investigation of a related series of inhibitors by the Daiichi group found that the (iminoethyl)piperidine was one of the optimal substituents for binding in the S4 site.[6] Based on these studies we prepared carbazole **14b** which inhibited FXa with $K_i = 1.6$ nM.

An X-ray crystal structure of carbazole **14b** in trypsin is shown in Figure 3.[12] The inhibitor binds in an extended L-shaped conformation analogous to that observed for our series of diphenoxy-pyridine FXa inhibitors[4,13] in trypsin and for DX-9065a in both trypsin and FXa.[14] The naphthylamidine group binds in the S1 pocket, forming a salt bridge with Asp-189. The (iminoethyl)-piperidine moiety binds in the aryl binding site,[15] which is a shallow groove defined by Leu-99, Gln-175 and Trp-214. The unsubstituted phenyl ring is located near the surface of the protein and may form a hydrophobic interaction with His-57 of the active site.

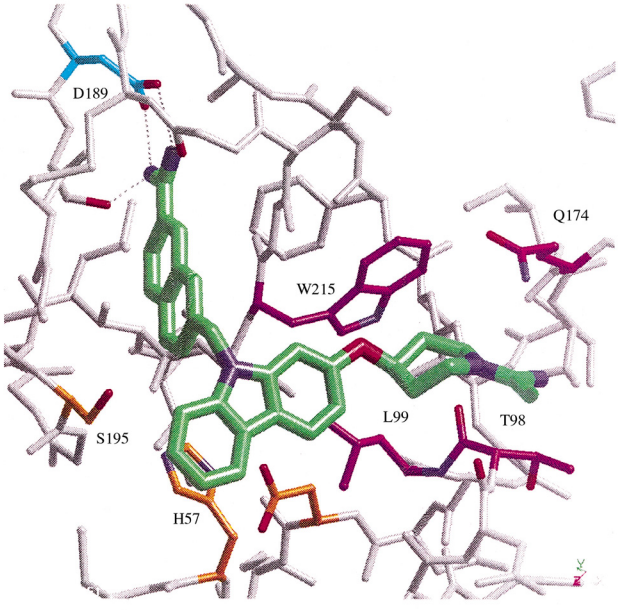


Figure 3. 1.8 Å X-ray crystal structure of **14b** in trypsin refined to an R-factor of 17.6%.

Although carbazole **14b** maintains the potency of **11c**, it is much less selective for FXa over thrombin. Furthermore, **14b** exhibited poor in vitro anticoagulant activity, causing a 2-fold prolongation of the prothrombin based clotting time (2×PT) at a concentration of 19 μM in human plasma. The poor anticoagulant activity was attributed to the limited aqueous solubility of **14b**, therefore we decided to prepare a series of indoles that maintained a substituent at the 2-position that could mimic the effect of the fused ring. The results in Table 4 show that a carbomethoxy group is the optimal substituent in this series. Compound **18a** (FXa, $K_i = 0.2$ nM) is about 10-fold more potent than carbazole **14b** and shows nearly a 1000-fold selectivity over both thrombin and trypsin. Increasing the size of the substituent (**18b–d**) causes a decrease in potency. Polar substituents also had a negative effect, decreasing potency about 20-fold (**18e** versus **18a**). While indole **18a** was significantly more potent against FXa than carbazole **14b**, the anticoagulant activity of this compound was only marginally improved (2×PT = 7 μM).

Conclusion

We have outlined a series of indole and carbazole inhibitors of FXa that lack the photochemical instability of Z,Z-BABCH (**1**). While initial inhibitors had only modest potency against FXa, optimization led to a dramatic increase in potency to afford compounds such as indole **18a** with subnanomolar inhibitory potency and good selectivity over other serine proteases. The relatively low anticoagulant activity of these inhibitors in plasma led to the development of other more soluble templates with improved potency and anticoagulant activity. This work will be the subject of future publications.

Acknowledgements

We thank Drs. Jerry Dallas and Baiwei Lin for help with NMR and mass spectra, respectively. We would

also like to thank Dr. Monica Kochanny for helpful discussion during the preparation of this manuscript.

References and Notes

1. For a review of Synthetic inhibitors of FXa and thrombin see Hauptman, J.; Stürzebecher, J. *Thromb. Res.* **1999**, *93*, 203 and references cited therein.
2. (a) Wong, P. C.; Crain Jr., E. J.; Nguan, O.; Watson, C. A.; Racanelli, A. *Thromb. Res.* **1996**, *83*, 117. (b) Kaiser, B.; Hauptmann, J. *Cardiovasc. Drug Rev.* **1994**, *12*, 225.
3. Shaw, K. J.; Guilford, W. J.; Dallas, J. L.; Koovakkat, S. K.; Liang, A.; Light, D. R.; Morrissey, M. M. *J. Med. Chem.* **1998**, *41*, 3551.
4. Phillips, G. B.; Buckman, B. O.; Davey, D. D.; Eagen, K. E.; Guilford, W. J.; Hinchman, J.; Ho, E.; Koovakkat, S. K.; Liang, A.; Light, D. R.; Mohan, R.; Ng, H. P.; Post, J. M.; Shaw, K. J.; Smith, D.; Subramanyam, B.; Sullivan, M. E.; Trinh, L.; Vergona, R.; Walters, J.; White, K.; Whitlow, M.; Wu, S.; Xu, W.; Morrissey, M. M. *J. Med. Chem.* **1998**, *41*, 3557.
5. Arylation with 3-fluorobenzonitrile required 100 °C.
6. Nagahara, T.; Yokoyama, Y.; Inamura, K.; Katakura, S.; Komoriya, S.; Yamaguchi, H.; Hara, T. *J. Med. Chem.* **1994**, *37*, 1200.
7. Neilson. *The Chemistry of Amidines and Imidates*; Patai, S., Ed.; Wiley: New York, 1975; pp 385–489.
8. Mitsunobu, O.; Yamada, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380.
9. Boger, D. L.; Cerbonne, L. R.; Yohannes, D. *J. Org. Chem.* **1988**, *53*, 5163.
10. (a) Kucnierz, R.; Leinert, H.; Von der Saal, W.; Neidlein R.; Kehr, C. E. P. Patent 00842941, 1998. (b) Taniuchi, Y. et al. *Thromb. Haemost.* **1998**, *79*, 543.
11. Unpublished data from this Laboratory.
12. Protein Data Bank 1qb9.
13. Whitlow, M.; Arnaiz, D. O.; Buckman, B. O.; Davey, D. D.; Griedel, B.; Guilford, W. J.; Koovakkat, S. K.; Liang, A.; Mohan, R.; Phillips, G. B.; Seto, M.; Shaw, K. J.; Xu, W.; Zhao, Z.; Light, D. R.; Morrissey, M. M. *Acta Cryst. D.* **1999**, *55*, 1395.
14. (a) For **DX-9065a** in trypsin see Stubbs, M. T.; Huber, R.; Bode, W. *FEBS Lett.* **1995**, *375*, 103. (b) For **DX-9065a** in FXa see Brandstetter, H.; Kühne, A.; Bode, W.; Huber, R.; von Der Saal, W.; Wirthensohn, K.; Engh, R. A. *J. Biol. Chem.* **1996**, *271*, 29988.
15. Bode, W.; Turk, D.; Karshikov, A. *Protein Sci.* **1992**, *1*, 426.