Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1253–1256

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Activity Studies of Conformationally Restricted α-Ketoamide Factor Xa Inhibitors

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Received 24 February 2000; accepted 29 March 2000

Abstract—Conformationally restricted borolysine compounds containing a 2-(2-cyanophenylthio) benzoyl in the P3 position unexpectedly led to enhanced factor Xa inhibition. In an effort to improve both the potency and selectivity of this series by extending into the S′ domain, we have replaced the boronic acid with α-ketoamides, utilizing a novel process that was developed in our labs.

Factor Xa (fXa) plays a crucial role in the coagulation cascade by occupying the juncture of the intrinsic and extrinsic clotting pathways. The physiological role of fXa is the proteolytic cleavage of prothrombin to thrombin, which once generated can proceed to cleave fibrinogen to fibrin. Fibrin can then proceed to crosslink with activated platelets to form a fibrin blood clot. Imbalances in the coagulation cascade can lead to excessive thrombotic conditions such as pulmonary embolism, myocardial infarction, deep vein thrombosis and stroke. Current antithrombotic therapies all suffer from drawbacks associated with inconvenient dosing regimens, the need for careful patient monitoring, or undesirable side effects. Due to the central role of fXa in the coagulation cascade, its inhibition is an attractive method of thrombus prevention. During the screening of a series of conformationally restricted boropeptide thrombin inhibitors,[1] it was observed that placement of a nitrile moiety in the 2′ position (compound **3**) of the diaryl thioether unexpectedly led to enhanced fXa inhibition while lowering the corresponding thrombin activity (Table 1).

In order to determine the role of the nitrile in influencing the fXa/thrombin activity, a crystal structure of **3** in the thrombin active site was analyzed. Unfortunately, due to disorder in the P3 diaryl region, the crystal structure failed to yield any information that could be used to improve the fXa affinity. In an effort to engineer both greater potency and selectivity into this series, it was decided to retain the 2-cyanophenylthio P3 fragment while replacing the boronic acid with lysine α-ketoamides to extend the inhibitors into the S′ site. This method of extending into the S′ site has proven to be an effective means of producing potent α-ketoamides at both Merck[3] and Corvas.[4] This substitution could be readily accomplished by utilizing a methodology developed recently in our laboratories.[5] This method, shown in Scheme 1,[6] involves the use of azide ion to undergo a regioselective C3 epoxy amide opening to give the azido alcohol **10**, which can then be rapidly converted to the desired α-ketoamide.

Initially, simple alkyl and aryl substituted α-ketoamides were prepared to probe the S′ site. As can be seen in Table 2, an aryl substituent not only imparts greater activity toward fXa, but also elicits a ~10-fold increase in thrombin affinity. The crystal structure of **18** in the thrombin active site was determined to guide our efforts deeper into the S′ pocket.[7]

Clear density is seen for most of the inhibitor, however, a portion of the P2-P3 part of the inhibitor is characterized by poor electron density (Fig. 1). The phenyl ring of the inhibitor that resides in the S′ pocket is well ordered and is observed packing against the main chain residue of Gly-193. Continuous electron density is observed between the side chain of Ser-195 and the α-carbonyl carbon of the inhibitor (1.3 Å), the corresponding oxygen also forms a hydrogen bond with His-57 (2.6 Å) . The oxygen of the phenyl amide occupies the oxyanion hole and forms hydrogen bonds with Ser-195 (2.9 Å), Asp-194 (3.0 Å),

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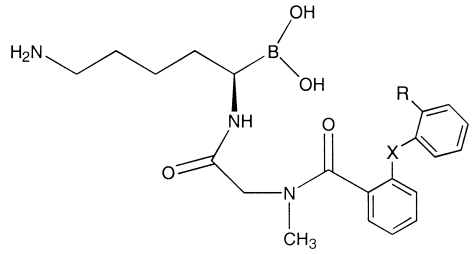
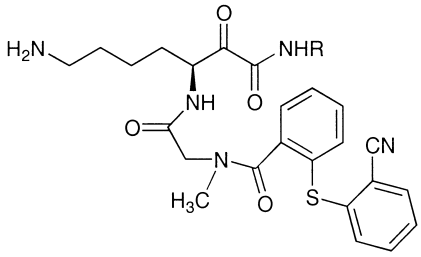
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00215-8

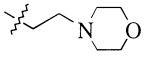
Table 1.



Compound	X	R	Thrombin K_i (nM)[2]	fXa K_i (nM)[2]
1	CH_2	H	4.9	100
2	S	H	1.6	17
3	S	CN	8.8	1.1
4	SO_2	H	97	5800

Table 2.

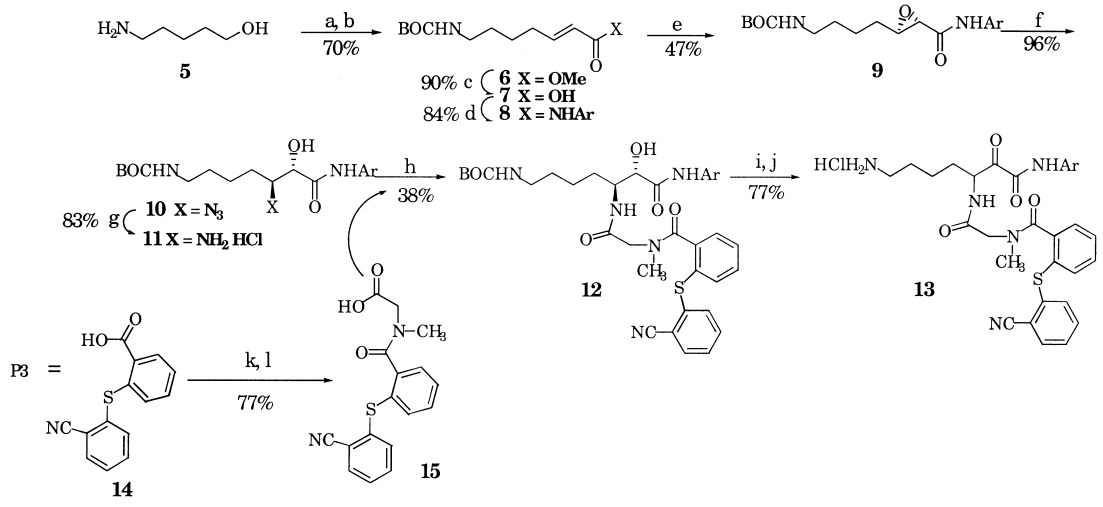


Compound	R	fXa K_i (nM)[2]	Thrombin K_i (nM)[2]
16	CH_3	110	570
17		200	8700
18		54	60

and Gly-193 (2.6 Å). The overall binding of this compound is interesting in that it has a different binding mode compared to the structure of APPA in trypsin.[8] The keto group in the thrombin:**18** structure is interacting with Ser-195, while in the trypsin:APPA structure (and in some recently reported α-ketoamide compounds complexed to thrombin[9]) the analogous keto group is interacting with the oxyanion hole. In our structure, it is the amide oxygen that is interacting with the oxyanion hole, making several hydrogen bonds. This interaction has been observed previously in a series of α-ketoamide thrombin inhibitors.[10] The amine group in the S′ pocket is interacting with Asp-189 (3.3Å) and solvent molecules as observed previously.[11] Unfortunately, there is ambiguous density for the nitrile group of the inhibitor in the P3 aryl pocket preventing us from determining the exact role of the nitrile.

Based on the coordinates of **18** in human thrombin, a series of modeling experiments were conducted to maximize the interactions between the S′ site of fXa and the inhibitor (Fig. 2). The modeling study suggested that a substituted aryl or diaryl scaffold was expected to provide both a semi rigid framework and the proper orientation for the inhibitor to interact with several backbone carbonyls and acidic residues deep in the S′ pocket of fXa.

The P_1' phenyl ring of compound **19** (Table 3) was intended to lie on a low ridge formed by Gly-193 in a shallow pocket while the positively charged nitrogen substituents of aniline **19** and pyridine **20** were intended to form hydrogen bonds with two backbone carbonyl groups, Gly-40 and Phe-41. Modeling also suggested that a basic substituent placed deep within the pocket could potentially form strong ionic bonds with acidic side chain of Glu-39. A diaryl ether containing an



Scheme 1. (a) $(BOC)_2O$, Ch_2Cl_2; (b) $(COC)_2$, DMSO, TEA; then $Ph_3P{=}CHCO_2Me$, CH_2Cl_2; (c) LiOH, $MeOH/H_2O$; (d) IBCF, NMM, $ArNH_2$, CH_2Cl_2; (e) *t*-BuOOH, *n*-BuLi, THF, −78→25 °C; (f) $MgSO_4$, NaN_3, MeOH, reflux; (g) H_2, Pd/C, MeOH, HCl; (h) EDC, HOBT, NMM, P3, Me_2NCOMe; (i) $(COCl)_2$, DMSO, TEA, CH_2Cl_2; (j) TFA or 4 M HCl in dioxane; (k) DCC, HOBT, Sar OMe; (l) LiOH, $MeOH/H_2O$.

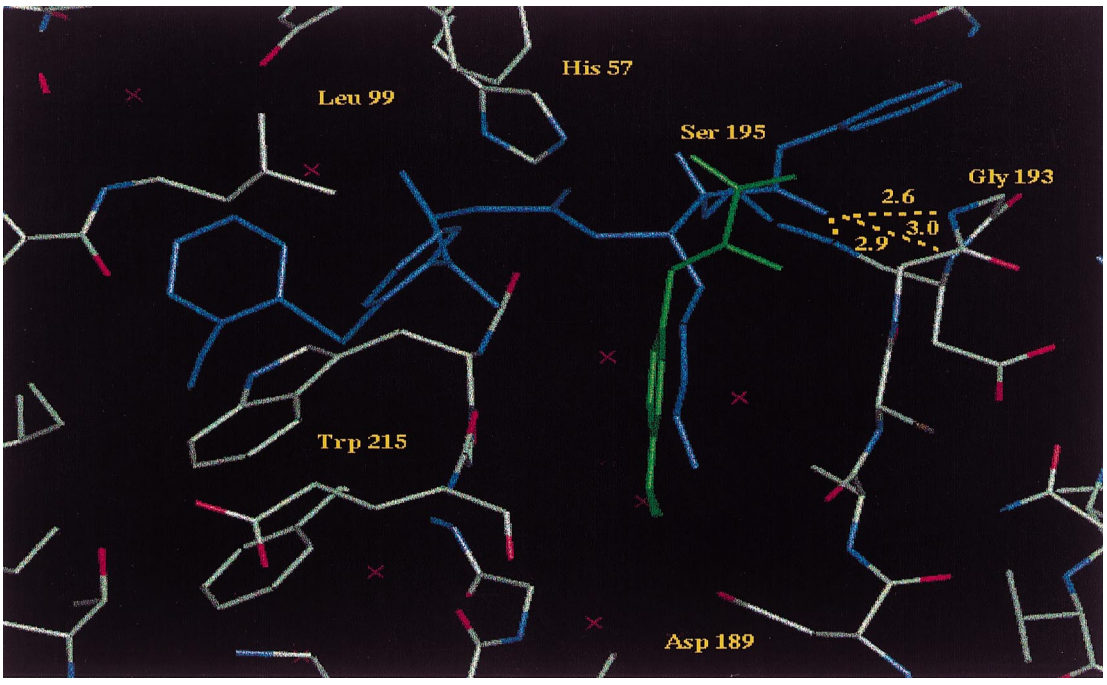


Figure 1. Superposition of thrombin-compound **18** complex and the coordinates of APPA derived from the bovine trypsin complex. Thrombin is colored by atom type, compound **18** is blue and APPA is green. Hydrogen bonds to the oxyanion hole are indicated by dashed lines.

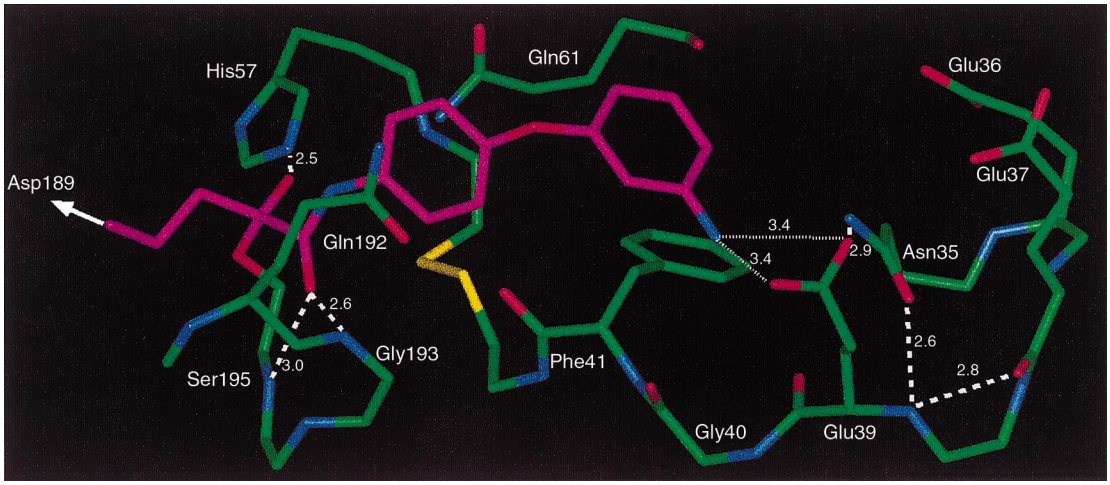
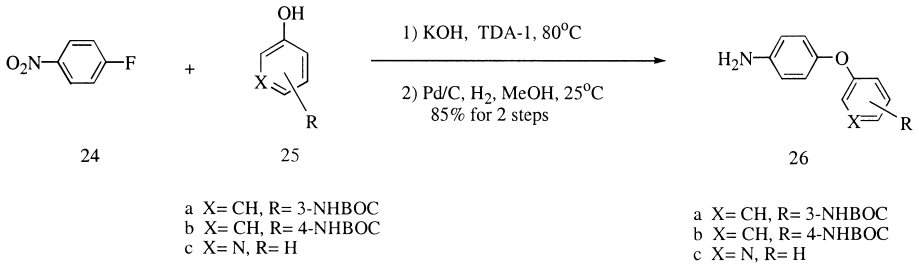


Figure 2. Modeling of compound **21** in the S' binding pocket of fXa depicting the anticipated interactions with the enzyme. fXa is coloured by atom type, compound **21** is pink and the hydrogen bonds are indicated by dashed lines.

amino functionality was chosen as a semi rigid scaffold for our molecules.

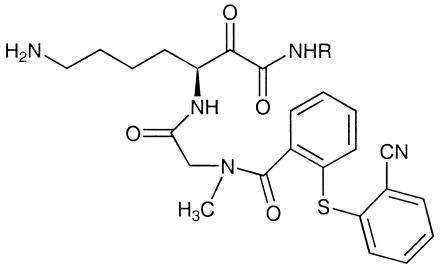
The requisite anilines **26a**, **26b**, and **26c** were prepared in a two step process, as described in Scheme 2, and converted to **21**, **22**, and **23**, respectively. Although replacing the methyl substituent of **16** by a phenyl group (**18**) improved potency, further modifications (compounds **19** through **23**) led up to 7-fold loss of fXa activity. This is most likely due to the fact that a penalty is paid to desolvate the polar groups, which is not compensated for by formation of favorable electrostatic interactions. In the case of **19** and **20**, the nitrogens may not be positioned optimally for hydrogen bond formation. The diaryl ether compounds **21**–**23** lose conformational entropy upon binding (two rotational degrees of freedom about the C–O bonds get frozen), which seems almost, but not completely, compensated for by additional interactions of the distal aryl rings with the enzyme. The unanticipated loss of thrombin selectivity observed upon placing the extended diaryl ethers into the S′ pocket may indicate that a substituted aryl ring provides a more favorable compliment to the thrombin active site than in the corresponding fXa site.

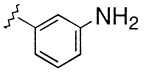
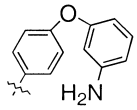
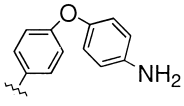
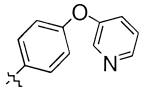
In summary, these conformationally restricted α-ketoamides represent a novel series of fXa inhibitors and that the ketoamide moiety can serve as a handle for



Scheme 2.

Table 3.



Compound	R	fXa K_i (nM)[2]	Thrombin K_i (nM)[2]
18		54	60
19		120	120
20		360	7400
21		120	59
22		120	68
23		60	50

extending into the S′ region. Interestingly, in contrast to the binding mode of APPA, it is the oxygen of the phenyl amide that occupies the oxyanion hole, which is in accord with Tulinsky's findings.[10] As yet, the extension of the amide into the S′ pocket has not produced the desired enhancement of factor Xa activity as predicted by our modeling studies.

Acknowledgements

The authors would like to thank Joseph M. Luettgen and Susan Spitz for obtaining the K_i values for the inhibitors described in this paper.

References and Notes

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6. Satisfactory spectral analysis were obtained for all new compounds described in this communication; all final compounds prepared were racemates.
7. A complex of **18** and thrombin was crystallized in space group C2 ($a=70.6$, $b=72.6$, $c=72.9$ Å, alpha = 90°, beta = 100.6°, gamma = 90°). Data were collected to 2.0 Å resolution and the structure refined to an R-value of 0.172.
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