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THE DE NOVO DESIGN AND SYNTHESIS OF CYCLIC UREA INHIBITORS OF FACTOR Xa: INITIAL SAR STUDIES

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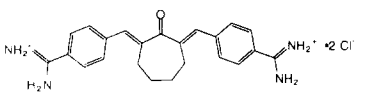
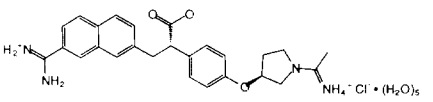
Abstract: In this report we discuss the design, synthesis, and validation of a novel series of cyclic urea inhibitors of the blood coagulation protein Factor Xa. This work culminates in compound **11**, a mono-amidine inhibitor of fXa employing a new S4 ligand that reduces the cationic character of these analogs. Compound **11** represents a lead for a series of more potent and selective inhibitors.

The blood coagulation protease Factor Xa (fXa) is a therapeutic target of considerable interest because of its role as the chief agent of thrombin generation in the common pathway of coagulation.[1] Recent reports have suggested that a specific inhibitor of fXa may allow effective control of thrombogenesis with a minimal effect upon bleeding.[2] The current drug of choice for oral anticoagulant therapy, Coumadin®, is highly effective and being utilized in a growing number of antithrombotic indications.[3] It is, however, a narrow therapeutic index drug requiring careful dose titration, stabilization, and periodic monitoring for each patient.[4] Our efforts have been directed towards the discovery of effective orally bioavailable anticoagulants with an optimal pharmacokinetic profile that will require less patient management by the prescribing physician.

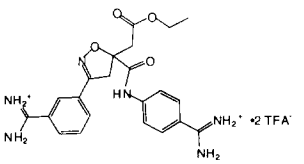
When this work was initiated there had appeared in the literature a number of nonpeptide small molecule inhibitors containing the di-cationic bis-amidine moiety such as BABCH (**1**)[5] and more recently DX-9065a (**2**).[6] Since then, we have reported two series of bis-benzamidine inhibitors, one employing an isoxazoline core structure (**3**)[7] and another utilizing an alkyl tether between the basic groups (**4**).[8] The focus in our laboratory[9] and by others,[10] has been to improve upon bioavailability by diminishing the cationic character of these inhibitors. In this work we discuss the design and initial SAR of a series of fXa inhibitors based upon a cyclic urea core structure that can utilize either a bis-benzamidine di-cationic binding motif or a single charged benzamidine/neutral group binding motif.



1: BABCH



2: DX-9065a



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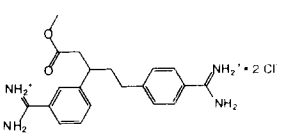

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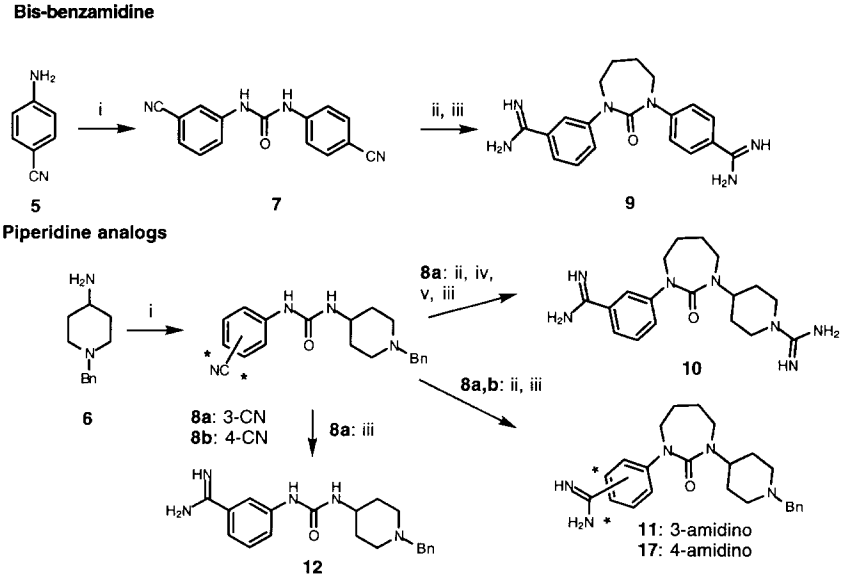
Synthesis: The preparative routes to the compounds designed for this study are outlined in Schemes 1 and 2. The synthesis of the urea bis-benzamidine and piperidine targets **9**- **12**, **17** (Scheme 1) began with the reaction of amines **5** and **6** with freshly distilled 3- or 4-cyanophenylisocyanate in DMF to give acyclic ureas **7** and **8a**,**b** in 70- 80% yield. Alkylation of the sodium salts of **7** and **8a**,**b** with 2 equiv of 1,4-dibromobutane in hot DMF gave the corresponding 2,4-diazepin-3-ones in yields ranging from 30 to 50%. For targets **9**, **11**, and **17** the amidine group was formed by Pinner's method[11] from the nitrile substituent by reaction with anhydrous HCl gas saturated in methyl acetate:methanol (5:1) at 0 °C followed by treatment with excess ammonium carbonate in methanol at ambient temperature. Compound **10** was prepared by alkylation of **8a** then catalytic hydrogenolysis to give the N-debenzylated piperidine analog. The N-deprotected piperidine was treated with a mixture of formamidine sulfonic acid and DMAP in refluxing ethanol to form the N-amidinopiperidine, subsequent amidine formation at the phenylnitrile by the Pinner reaction gave **10**. Amidine formation with **8a** gave the acyclic urea **12**.

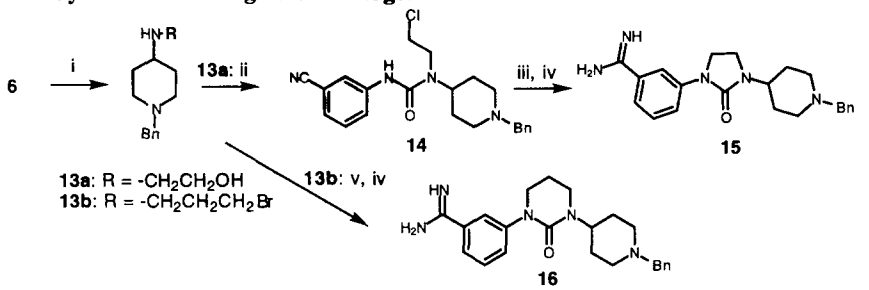
The 2-imidazolidinone and 2-pyrimidinone analogs **15** and **16** were prepared by an intramolecular annulation strategy (Scheme 2). The precursor to **15** was obtained by N-alkylation of **6** with 1 equiv of neat 2-bromoethanol to give **13a**, this material was treated with 3-cyanophenylisocyanate in DMF at 60 °C for 3 h, then the ethyl alcohol side-chain was transformed to the chloroethyl group with thionyl chloride to give intermediate **14**. Ring closure of **14** to the 2-imidazolidinone was effected with refluxing ethanolic KOH; compound **15** was obtained from the cyclized product by amidine formation. Treatment of **13b** with 3-cyanophenylisocyanate and triethylamine in DMF at 70 °C gave a cyclized product which, following the Pinner reaction, yielded 2-pyrimidinone **16**.

Scheme 1. Synthesis of 2,4-diazepin-3-one and acyclic target compounds.



Reagents: (i) 3- or 4-cyanophenylisocyanate, DMF,18 h; (ii) 3 equiv NaH, 2 equiv $BrCH_2CH_2CH_2CH_2Br$, DMF, 70 °C, 3 h, then 18 h; (iii) HCl(g), $CH_3CO_2CH_3/CH_3OH$ (5:1), 0-10 °C, 18 h, then 5 equiv NH_4CO_3, CH_3OH, 18 h; (iv) H_2, 50 psi, 10% Pd-C; (v) $H_2NC(=NH)SO_3H$, DMAP, C_2H_5OH.

Scheme 2. Synthesis of ring-size analogs.

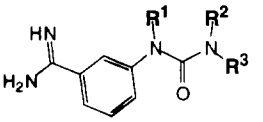


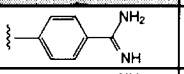
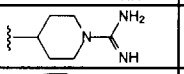
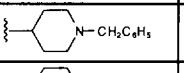
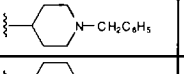
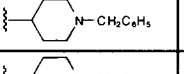
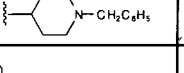
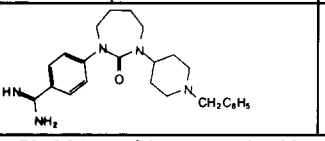
Reagents: (i) for **13a**: 1 equiv $BrCH_2CH_2OH$, neat, 48 h; for **13b**: 1 equiv $BrCH_2CH_2CH_2Br$, neat, 24 h; (ii) 3-cyanophenylisocyanate, $(C_2H_5)_3N$, DMF, 60 °C, 3 h; $SOCl_2$, $CHCl_3$, C_5H_5N (cat), -10 °C, 2 h, then reflux, 1 h; (iii) 2 equiv KOH, C_2H_5OH, reflux, 2 h; (iv) HCl(g), $CH_3CO_2CH_3/CH_3OH$ (5:1), 0-10 °C, 18 h, then 5 equiv NH_4CO_3, CH_3OH, 18 h; v) 1 equiv 3-cyanophenylisocyanate, $(C_2H_5)_3N$, DMF, 70°C, 3 h, then 18 h.

Results and Discussion: Factor Xa is a trypsin-like serine protease with a primary specificity pocket (S1) that selects for the positively charged amino acids arginine and lysine and a box-like S4 pocket defined by the side-chains of Trp215, Phe174, and Tyr99. Our design work began with the X-ray coordinates of human des(1-45) fXa[12] using an approach similar to that successful in the design of the flexible bis-benzamidine **4**.[8] A benzamidine fragment (Figure 1a) was oriented manually in the S1 pocket to achieve a symmetrical bidentate charge interaction with the carboxylate side-chain of Asp189 then refined further by energy minimization.[13] The second benzamidine fragment was minimized in the S4 pocket, resulting in a face-to-face orientation for the phenyl ring of the fragment and the indole of Trp215. This group maybe further stabilized by a cation-π interaction between the amidine and the aromatic side-chains of Phe174 and Tyr99,[14] and by a hydrogen bond with the carbonyl of Thr98. Located midway between the two fragments is the amide NH of Gly216, a functional group we wished to exploit to gain an additional interaction with the active site. Therefore, we sought a rigid scaffold that would both 'lock' the benzamidine fragments in their energy minimized positions and provide a H-bond acceptor for a new interaction with Gly216. The cyclic urea[15] was chosen for this task based upon its reputation for good bioavailability in our HIV protease inhibitor program[16] and as an excellent hydrogen-bond acceptor group.[17] The bis-benzamidine cyclic ureas were modeled with ring sizes varying from five to seven atoms with the benzamidine groups linked through the urea N to the *meta*-position of the S1 ligand and the *para*-position of the S4 ligand. Of the virtual congeners studied, the compound employing the 2,4-diazepin-3-one framework, analog **9**, yields the lowest minimization energy (Figure 1b) and gives an inhibitor with a K_i = 800 nM for fXa (Table 1).

We prepared several analogs to examine our model further. Replacing the rigid benzamidine S4 ligand used in **9** with a more flexible N-amidinopiperidine group gives **10**, a compound that is 3-fold less potent against fXa than **9**. It appears that the benzamidine side chain of **9** reaches deeper into the S4 pocket allowing for interaction with the backbone carbonyls of Glu97 and Thr98 (Figure 1b). However, compound **11**, a mono-amidine analog of the N-benzylpiperidine precursor to **9**, is 8-fold more potent than **9** (fXa K_i = 102 nM). This result represents a departure from the binding paradigm requiring a very basic (pK_a > 14) cationic group for an effective interaction with the S4 binding site of fXa. The model of

Table 1. Biological activity[18] of the target compounds.



	R^1	R^2	R^3	fXa K_i (nM)	Thrombin K_i (nM)	Trypsin K_i (nM)
2 DX-9065a				30 *	> 4200	1100
9	-$CH_2CH_2CH_2CH_2$-			800	9300	120
10	-$CH_2CH_2CH_2CH_2$-			2500	>21000	-
11	-$CH_2CH_2CH_2CH_2$-			102	2000	282
12	H	H		4900	> 21000	>1200
15	-CH_2CH_2-			2100	11000	-
16	-$CH_2CH_2CH_2$-			400	7300	-
17				7500	> 21000	> 1600

* Literature data for **2**: fXa IC_{50} = 70 nM (see ref 6); our sample of **2** was a diasteromeric mixture at the carboxylic acid.

Table 2. Comparison of calculated H-bond length and binding energy with fXa K_i for 11, 15, and 16.

	Ring Size	fXa K_i (nM)	H-Bond Length (Å)	Binding Energy (kcal / mol)
15	5	2100	3.66	-90.3
16	6	400	3.24	-92.2
11	7	102	3.18	-93.6

11 bound to fXa (Figure 1c) suggests that the weakly basic (pK_a < 9) N-benzylpiperidine S4 ligand contributes substantial binding energy by a lipophilic edge-to-face interaction between the benzyl group of **12** and the indole of Trp215,[19] analogous to the interaction of the D-Phe side chain of PPACK with thrombin.[20] The success of this modification represents an important step in our effort to develop inhibitors with less cationic character and enhanced bioavailability. To further evaluate **11** as a lead for a new, less cationic series of fXa inhibitors we prepared the acyclic core variant **12** and found it to be a poor inhibitor. The 2-imidazolidinone (**15**) and 2-pyrimidinone (**16**) ring-size analogs were modeled and synthesized. From the model (Figure 1c) we can estimate the hydrogen bond distance between the urea carbonyl oxygen and the Gly216 amide N and binding energy for **11**, **15**, and **16** (Table 2). We find that the trends for these values parallel the experimentally determined fXa K_i predicting that the 2,4-diazepin-3-one is the optimal ring size among the three evaluated. Finally, the poor activity for the analog of **11** with a *para*-substituted benzamidine S1 ligand, compound **17**, agrees with our earlier observations[7,8] that a *meta*-substituted benzamidine is required for effective interaction with the S1 site.

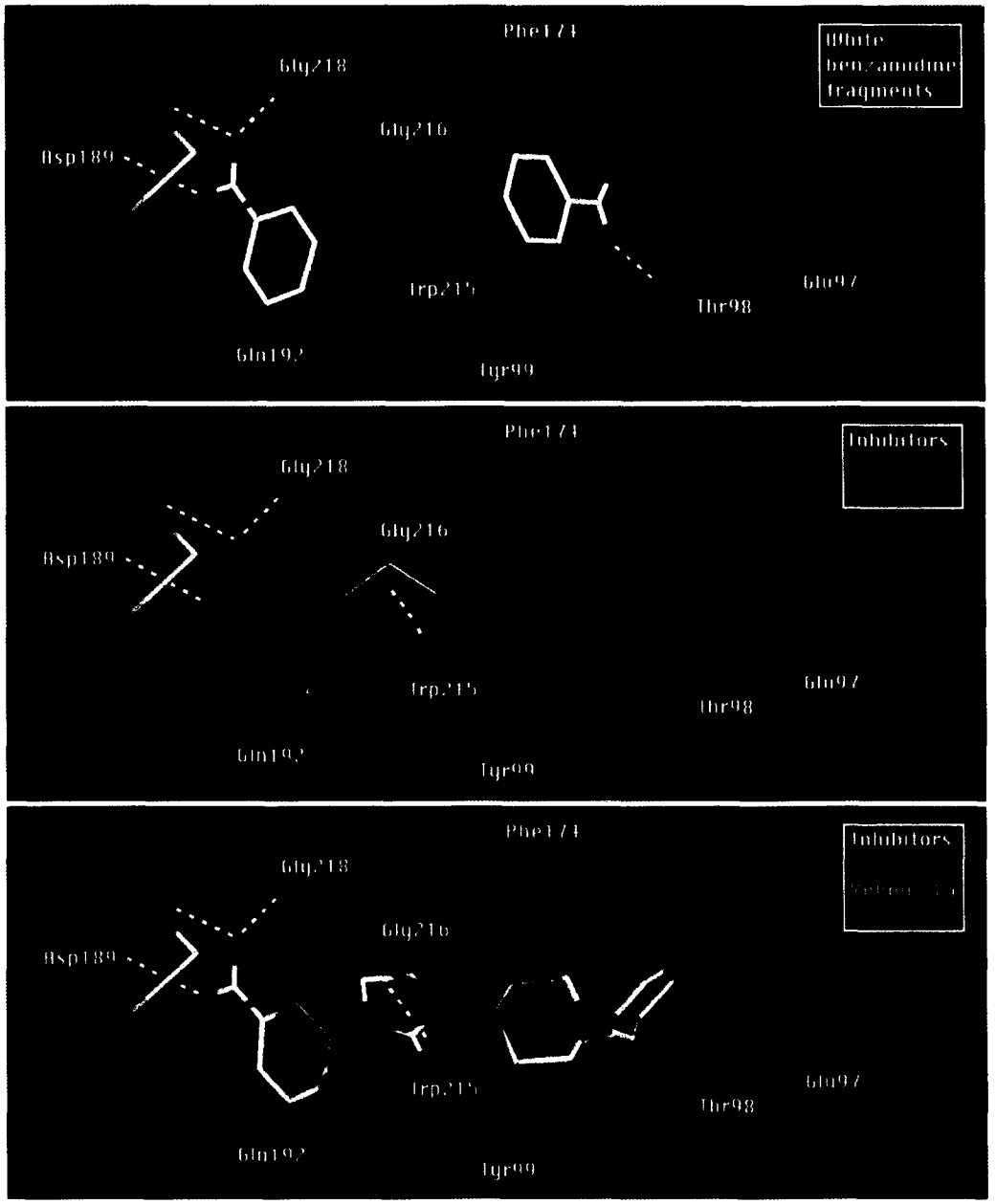


Figure 1. Ligands docked in the factor Xa active site: benzamidine fragments (Figure 1a); compounds **9** and **10** with basic aromatic and aliphatic S4 ligands, respectively (Figure 1b); compounds **11**, **15** and **16** with varying cyclic urea ring sizes (Figure 1c). Hydrogen bonds are indicated with thick dotted lines; two marginal hydrogen bonds between compound **9** and factor Xa (N...O distance 3.5- 3.6 Å) are indicated with thin dotted lines in Figure 1b.

Conclusion: While compound **11** has only a modest 2- to 3-fold selectivity over trypsin and moderate potency as an inhibitor of fXa this compound represents the point of departure for a novel series of potent and selective inhibitors of fXa. Compound **11** offers the additional advantage of requiring a single amidine for effective interaction with the target enzyme. Our efforts to develop further fXa inhibitors employing the 2,4-diazepin-3-one core structure will be published in due course.

References and Notes

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\# Current Address: Amgen Inc., 1840 DeHavilland Drive, Thousand Oaks, CA.

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