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The De Novo Design and Synthesis of Cyclic Urea Inhibitors of Factor Xa: Optimization of the S4 Ligand†

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Abstract—In this report refinements to the S4 ligand group leads to compound **19**, an inhibitor of fXa with good potency in vitro and an improved pharmacokinetic profile in rabbit. The X-ray crystallographic study of a representative analogue confirms our binding model for this series.

Our effort to develop oral anticoagulants has focused on inhibitors of the blood coagulation protease Factor Xa (fXa)[1–6] because of its role as the chief agent of thrombin generation in the common pathway of coagulation[7,8] and reports suggesting that inhibitors of fXa exert effective control of thrombogenesis with a minimal effect upon bleeding.[9,10] The current drug of choice for oral anticoagulant therapy, Coumadin®¶, is highly effective and being utilized in a growing number of antithrombotic indications.[11,12] However, it is a narrow therapeutic index drug that requires careful dose titration, stabilization, and periodic monitoring for each patient.[13] Our objective has been to discover an orally bioavailable drug with an optimal pharmacokinetic profile amenable to once- or twice-daily administration.

In a previous report,[14] we disclosed the design and synthesis of a new class of fXa inhibitors utilizing a cyclic urea[15] 2,4-diazepin-3-one core. The initial compound of this series, **1**, utilized the 2,4-diazepin-3-one ring as a rigid platform upon which to tether ligand groups: a *meta*-benzamidine to interact with the S1 pocket and a *para*-benzamidine to interact with the S4 pocket of the fXa enzyme. It was anticipated that a dicationic species such as **1** would be poorly adsorbed in vivo, hence the discovery of **2**, which has the substantially less basic *N*-benzylpiperidine as a ligand for the S4 pocket, was considered an important advance and used as a lead for the development of this series. In this paper, we report on further refinements to the S4 ligand group which gives a 10-fold increase of in vitro activity over **2**, the in vivo pharmacokinetic profile of selected analogues, and an X-ray crystallographic study that confirms our binding model for these compounds.

Synthesis

The compounds for this study **6**–**19** were prepared according to the methods outlined in Scheme 1.[16] Intermediate **4** is prepared in good (75%) yield by stirring the protected 4-aminopiperidine **3**[17] with distilled 3-cyanophenylisocyanate in DMF at ambient temperature. The 2,4-diazepin-3-one ring is formed by di-*N*-alkylation of the urea functionality of **4** with 1,4-dibromobutane and sodium hydride in hot DMF in 65% yield; compound **5** is obtained by Boc deprotection

†A portion of this work was reported in preliminary form: Galemmo, Jr., R. A.; Maduskuie, T. P; Dominguez, C.; Stouten, P. F. W.; Alexander, R. S; Wells, B. L; Rossi, K. A; Drummond, S.; Hausner, E.; Wright, M. R.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. The design and synthesis of cyclic urea inhibitors of Factor Xa; In *XVth EFMC International Symposium on Medicinal Chemistry*, September 1998, p 46.

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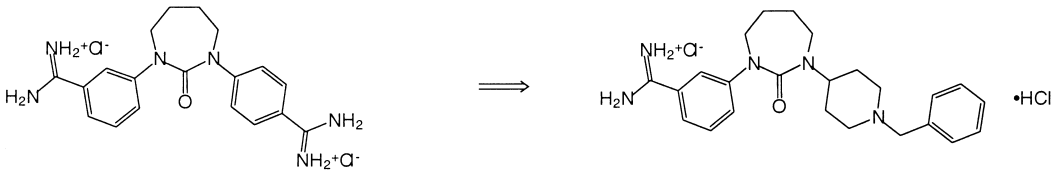


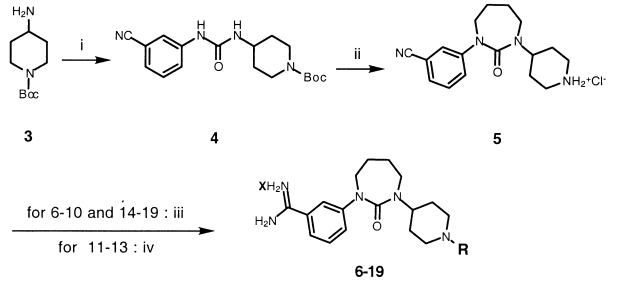
1 : fXa Ki = 800 nM

2 : fXa Ki = 102 nM

of the dialkylation product with HCl in ether. Compounds **6**, **14**–**16** and **18** were prepared by reaction with the appropriate alkylchloride in THF with triethylamine as base, then the amidine was formed using Pinner's method[18] by reaction of the nitrile substituent 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. The amides **7** and **8** were obtained by acylation with benzoyl or phenylacetyl chloride in triethylamine/THF solution followed by amidine formation as described above. Sulfonamides **9**, **10**, **17** and **19** were derived from **5** by reaction with the corresponding sulfonylchloride in triethylamine/THF then amidine formation. Compounds **11**–**13** were prepared by sulfonylation of **5** with 2-, 3- or 4-nitrophenyl sulfonylchloride followed by amidine formation, then the nitro group was reduced by catalytic hydrogenation to give the amines.

Results and Discussion

Table 1 illustrates the effect of varying the S4 ligand upon in vitro biological activity. The fXa inhibition of this series shows a dependence on the type of bond used to link the piperidine group to the terminal aryl ring. Compared to the *N*-alkylpiperidines **2** and **6**, the *N*-acylpiperidine analogues **7** and **8** are substantially less potent while the *N*-sulfonylpiperidines **9** and **10** are 4- and 22-fold more active than their related alkyl homologues. The sulfonyl anilines **11**–**13** represent an unsuccessful attempt to develop a new hydrogen bonding interaction with the S4 subsite. The series of picolinyl



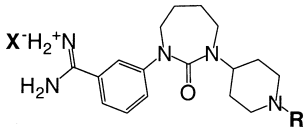
Scheme 1. Synthesis of 2,4-diazepin-3-one analogues. Reagents: (i) 3-cyanophenylisocyanate, DMF, 18 h; (ii) 3 equiv NaH, 2 equiv $BrCh_2CH_2CH_2CH_2Br$, DMF, 70 °C, 3 h, then HCl:Et_2O; (iii) **R**-Cl (see Table 1), Et_3N, THF, then HCl(g), $CH_3CO_2CH_3$:CH_3OH (5:1), 0–10 °C, 18 h, then 5 equiv NH_4CO_3, CH_3OH, 18 h; (iv) **R**-Cl (2-, 3-, 4-nitrophenylsulfonyl chlorides), Et_3N, THF, then HCl(g), CH_3CO_2-CH_3:CH_3OH (5:1), 0–10 °C, 18 h, then 5 equiv $(NH_4)_2CO_3$, CH_3OH, 18 h, then H_2, Pd-C, MeOH.

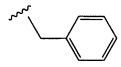
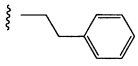
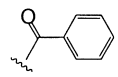
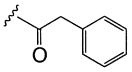
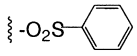
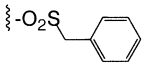
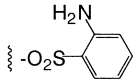
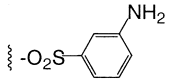
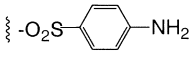
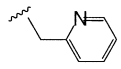
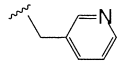
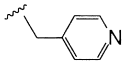
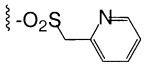
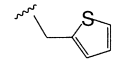
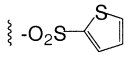
analogues **14**–**16** shows that **14** is exceptionally active, perhaps due to protonation of the pyridine nitrogen. However, as demonstrated by **17**, further improvement in inhibition is not observed when this modification is combined with a sulfonamide linkage. Thiophenes **18** and **19** follow the same trends observed for the carbocyclic analogues. Finally, while the more potent compounds **9**–**11**, **14**, **17** and **19** have 50- to 220-fold selectivity for fXa over thrombin, the selectivity against trypsin is 3- to 10-fold.

The iv pharmacokinetic profiles of **10**, **17** and **19** were determined in rabbits after a 1 h infusion of compound at 5 mg/kg (Table 2). While the benzylsulfonamide **10** and picolinylsulfonamide **17** are rapidly cleared and have half-lives of about 2 min, they do achieve an appreciable C_{max} with small apparent volumes of distribution (V_d). The thiophene sulfonamide **19** has a more encouraging profile; clearance of this compound is approximately 10-fold lower than for **10** and **17**, resulting in a half-life of 1 h, a 10-fold increase in C_{max} and little change in the volume of distribution. In a rabbit arterio-venous shunt model of thrombosis,[3,4] compound **19** was shown to inhibit thrombus formation with an $ID_{50} = 7$ μmol/kg/h upon iv administration. The potential for transport across the intestinal mucosa and in vivo absorption of **19** was evaluated by measuring permeation across Caco-2 monolayers.[21] While **19** was soluble in the aqueous vehicle used for this assay,[22] the measured permeability coefficient (2×10^{-7} cm/s) suggests poor oral absorption.

Because of the general difficulty in obtaining the X-ray crystal structure of fXa bound with an inhibitor, an X-ray crystal structure of **10** complexed with the related enzyme thrombin was determined (Fig. 1).[23] This thrombin structure confirms the proposed binding model[14] for these compounds: the *m*-benzamidine group binds in the S1 pocket by forming a bidentate interaction with Asp189, the *N*-(benzylsulfonyl)piperidine group reaches into the S4 pocket to form an edge-to-face interaction with Trp215 and the urea carbonyl forms an H-bond acceptor interaction with the Gly216 amide NH. Asp189, Trp215 and Gly216 have analogous residues in fXa. Minimization[24] of the thrombin-bound conformation of **10** in the crystal structure of human des(1–45) fXa[25] (Fig. 2) gives comparable distances between the amide NH of Gly216 and the urea carbonyl of **10** in fXa (2.97 Å) versus the corresponding distance in the thrombin structure (2.87 Å). Likewise, the distances observed for the Asp189 to benzamidine salt-bridge (fXa model: 2.69, 2.8 Å; thrombin structure: 2.85, 2.97 Å) and the centroid separation[26] observed for

Table 1. In vitro inhibitory activity[19]



	R	X	FXa K_i (nM)	Thrombin K_i (nM)	Trypsin K_i (nM)
2		2•Cl$^-$	102	2000	282
6		2•Cl$^-$	220	3600	1000
7		TFA$^-$[a]	7800	>21,000	—
8		TFA$^-$	900	11,800	—
9		TFA$^-$	24	1600	60
10		TFA$^-$	10	500	80
11		2•TFA$^-$	34	2400	130
12		2•TFA$^-$	200	2200	—
13		2•TFA$^-$	71	6300	190
14		2•TFA$^-$	12	2600	120
15		2•TFA$^-$	670	5300	—
16		2•TFA$^-$	200	6800	—
17		2•TFA$^-$	10	500	57
18		TFA$^-$	80	1400	170
19		Cl$^-$	12	1500	52

[a]TFA$^-$, trifluoroacetate anion.

Table 2. Pharmacokinetics of selected inhibitors[20]

	Clearance (L/h/kg)	$T_{1/2}$ (h)	C_{max} (μM)	V_d (L/kg)
10	4.1	0.04	4.8	0.23
17	7.9	0.03	2.4	0.34
19	0.68	1.1	36	1.1

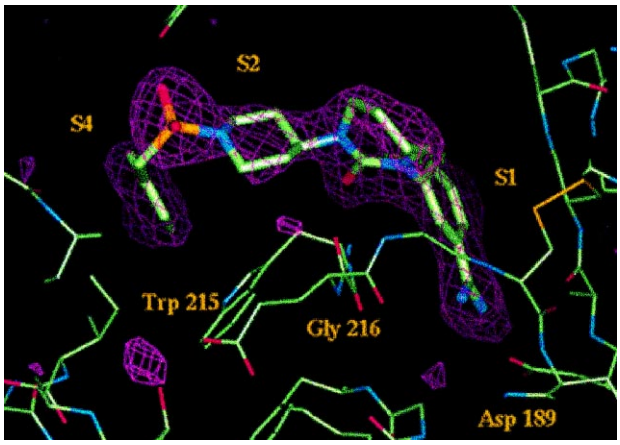


Figure 1. Electron density of **10** complexed to thrombin. Good density is observed over all of the inhibitor except for the carbon next to the sulfonamide.

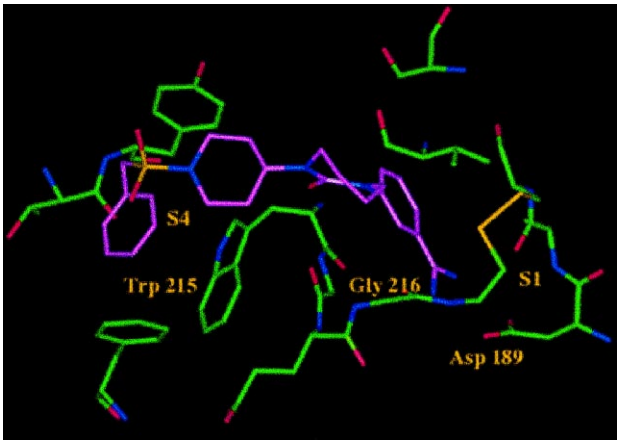


Figure 2. Model of the thrombin-bound conformation of compound **10** after minimization in the crystal structure of human des(1–45) fXa.[23]

the Trp215 to benzylsulfonyl edge-to-face interaction (fXa model: 5.28 Å; thrombin structure: 4.97 Å) are similar.

Conclusions

In our previous report,[14] we established by modeling and SAR that the seven-membered 2,3-diazepin-3-one core is the optimium ring size for this series. In this work, an X-ray crystal structure for the thrombin complex of **10** confirms the assumptions made in our binding model for these inhibitors. Furthermore, the binding affinity for fXa is increased substantially through the introduction

of an *N*-sulfonyl linkage in the S4 ligand group, while the best pharmacokinetic profile in rabbits is observed for **19**, an analogue with an *N*-(sulfonylthiophen-2-yl)-piperidine group as the S4 ligand.

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22. The aqueous vehicle was buffered at pH 7.4 and contained 2% dimethylacetamide as an adjuvant.
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24. Compound **10** was modeled into a crystal structure of Factor Xa (ref 25) using InsightII (MSI, San Diego, CA). The starting conformation of the inhibitor was from the crystal structure of **10** in thrombin. The inhibitor was minimized in a rigid protein using the CFF98 forcefield.
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