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Design, Synthesis, and Structure–Activity Relationships of Substituted Piperazinone-Based Transition State Factor Xa Inhibitors

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Abstract—The structure–activity relationship of a novel series of substituted piperazinone-based factor Xa inhibitors is described. The most potent compound **34** displays IC_{50} of 0.9 nM.

Factor Xa is a serine protease, which plays a pivotal role in the blood coagulation cascade. Early animal model studies of selective and potent factor Xa inhibitors suggest they are effective in preventing venous and arterial thrombosis formation and may have a much wider therapeutic index (efficacy/bleeding) than thrombin inhibitors. Therefore, factor Xa has emerged as an attractive biological target for the development of either parenteral or oral anticoagulants.[1]

We previously presented the discovery of a class of tri-peptide ketoheterocyclic transition state factor Xa inhibitors represented by $BnSO_2$-D-Arg-Gly-Arg-thiazole **1**[2] and $BnSO_2$-D-Phe-Gly-Arg-thiazole **2**[3] (Fig. 1). Compound **1** inhibits factor Xa with an IC_{50} of 0.65 nM. More importantly, it shows strong in vivo antithrombotic efficacy (in vivo $EC_{50} = 30–50$ nM) without significant bleeding complications in our rabbit deep vein thrombosis model.[4] To potentially improve the pharmacokinetic properties of tri-peptide based factor Xa inhibitors, we incorporated the conformationally restricted piperazinone template into these inhibitors. In the preceding publication,[5] the initial unsubstituted piperazinone compound **3** prepared displayed potent fXa inhibitory activity (IC_{50} of 4 nM). To explore this further, we envisioned that incorporation of various side chains at the 3-position of the piperazinone ring system and hydrophobic sulfonamide functionality at the 4-position might result in chemical entities with a better pharmacological profile. In this report, the design, synthesis and in vitro structure–activity relationships of a series of substituted piperazinone-based transition state factor Xa inhibitors with high potency and specificity will be discussed.

The synthesis of our key intermediate Arg(Tos)-thiazole **6** is outlined in Scheme 1. Coupling of Boc-Arg(Tos)-OH **4** and *N,O*-dimethylhydroxylamine hydrochloride generated Weinreb amide **5**. Treatment of **5** with excess 2-lithiothiazole at −78 °C[6] in THF, followed by Boc deprotection with 50%TFA in DCM yielded the Arg(Tos)-thiazole **6**. The preparation of compounds **11**, **12**, and **15–34** is outlined in Scheme 2. Treatment of 2,2-dimethoxyethylamine with *tert*-butyl chloroacetate and potassium carbonate, followed by coupling with various amino acids yielded intermediate **7**. The key cyclic intermediate **8** was obtained when dimethyl acetal **7** was treated with 70% aqueous trifluoroacetic acid or heated in toluene containing catalytic amount of *p*-TsOH.[7,8] Hydrogenation of the double bond of **8**, esterification of the acid, methyl or benzyl sulfonylation at the 4 position, hydrolysis of the ester, and coupling to Arg(Tos)-thiazole **6** followed by cleavage of the Tos group afforded compounds **15–28** and **31–34**. The synthesis of compound **13** is shown in Scheme 3. Coupling of Boc-Arg(Tos)-OH with diethyl iminodiacetate,

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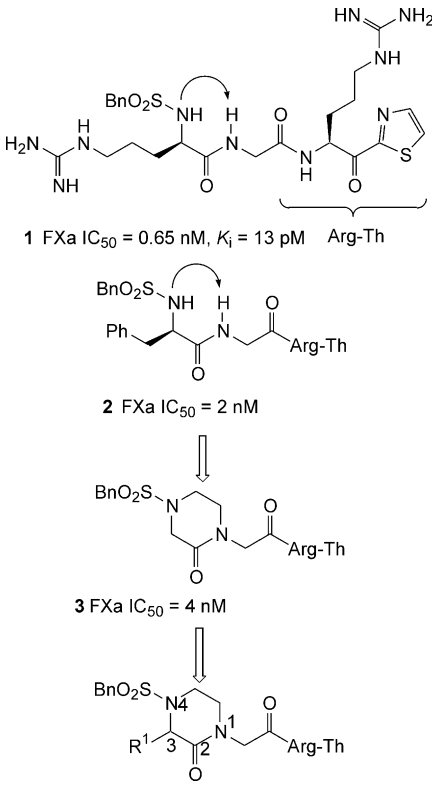
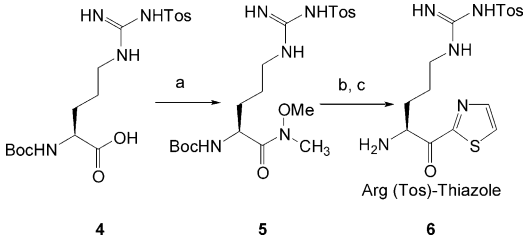


Figure 1. Design of substituted piperazinone based factor Xa inhibitors.

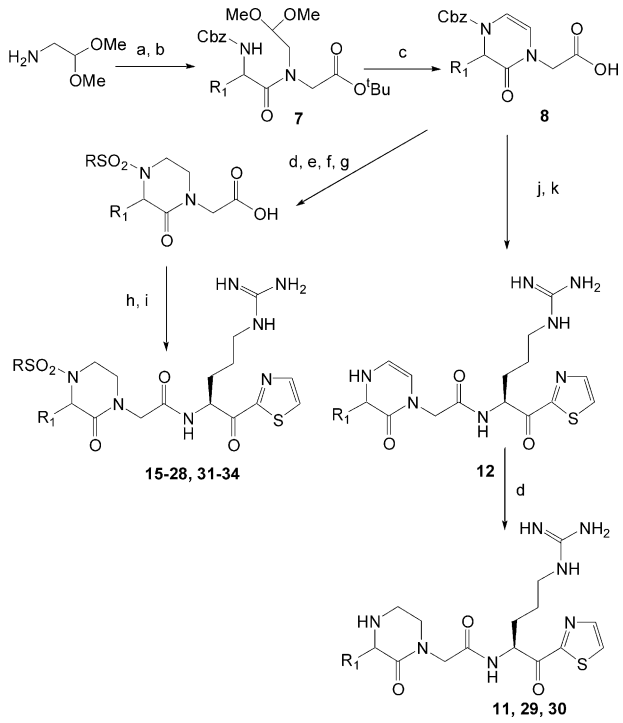


Scheme 1. Synthesis of compound **6**: (a) CH_3NHOCH_3·HCl, BOP, DIEA, DMF, rt; (b) *n*BuLi, thiazole, THF; (c) 50% TFA in DCM.

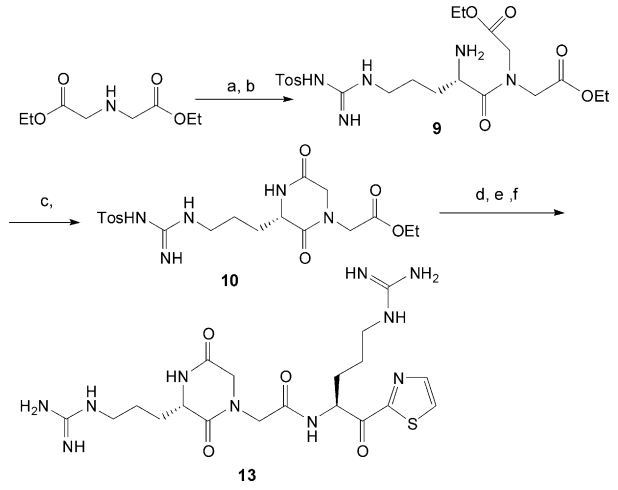
followed by Boc deprotection yielded **9**. Piperazinone **10** was formed in EtOH upon neutralization of **9** with DIEA and stirring at rt for 5 h. Hydrolysis of the ester, coupling to Arg(Tos)-thiazole **6** and subsequent cleavage of Tos group afforded analogue **13**. Analogue **14** was synthesized employing a similar procedure as **13** using Boc-D-Arg(Tos)-OH.

The biological activity for compounds **11**–**34** against fXa and the structurally related serine protease thrombin are summarized in Table 1.[4]

The basic 3-guanidinopropyl side chain was initially chosen as the substitute at 3-position of the piperazinone ring (**11**–**16**) based on the excellent fXa inhibitory activity of its tri-peptide counterpart $BnSO_2$-D-Arg-Gly-Arg-thiazole **1** (Fig. 1). Fully saturated piperazinone ring-containing analogue **11** is 15-fold more potent in fXa inhibitory activity than the unsaturated analogue **12**. This suggests the preference for a relatively flexible ring at the central piperazinone unit.



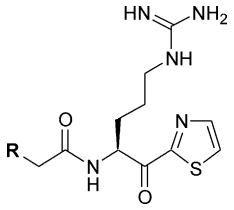
Scheme 2. Synthesis of compounds **11**, **12**, and **15**–**34**: (a) K_2CO_3, $ClCH_2CO_2tBu$, DMF; (b) Z-AA-OH·HCl, BOP, DIEA, DMF; (c) 70% aq TFA; (d) H_2, $Pd(OH)_2$/C, EtOH, cat. Amount concd HCl; (e) $SOCl_2$, MeOH; (f) RSO_2Cl, DIEA, CH_2Cl_2; (g) LiOH, THF/H_2O; (h), (j) Arg(Tos)-thiazole **6**, BOP, DIEA, DMF; (i) , (k) HF.



Scheme 3. Synthesis of compounds **13**: (a) Boc-Arg(Tos)-OH, BOP, DIEA, DMF; (b) 50% TFA/DCM; (c) EtOH, DIEA; (d) LiOH, THF/H_2O; (e) Arg(Tos)-thiazole **6**, BOP, DIEA, DMF; (f) HF.

Although diketopiperazine derivatives **13** and **14** were comparable to **11** in fXa inhibitory activity, they are the most selective inhibitors (Xa vs thrombin) in this series of analogues. Introduction of benzylsulfonamide at the 4-position of **11** afforded **15** with 7-fold enhancement in the potency, suggesting a hydrophobic group is preferred at this position. The 4-fold difference in potency between **15** and **16** indicates a fXa preference for *S*-configuration at the 3-position in 3-guanidinopropyl-substituted inhibitors. The potency and selectivity of compound **15** encouraged us to explore a variety of different substituents at the 3-position of piperazinone

Table 1. In vitro activity for compounds **10**–**33**



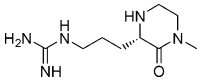
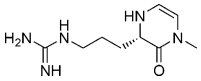
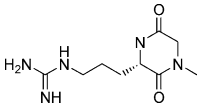
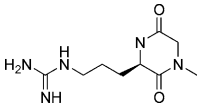
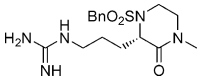
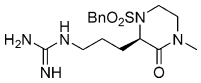
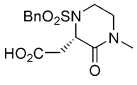
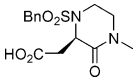
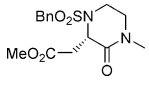
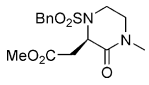
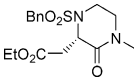
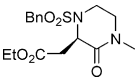
Compd	R	Factor Xa IC_{50}, μM	Thrombin IC_{50}, μM
11		0.02	12
12		0.307	140
13		0.011	150
14		0.021	200
15		0.003	12
16		0.013	3
17		0.37	325
18		0.031	40
19		0.085	1
20		0.008	5
21		0.032	4
22		0.007	3

Table 1 (*continued*)

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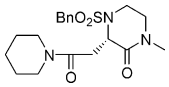
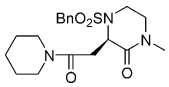
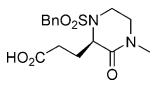
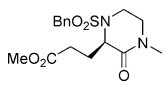
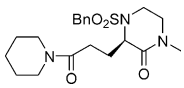
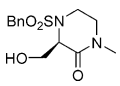
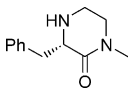
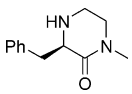
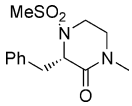
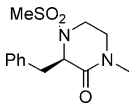
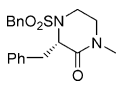
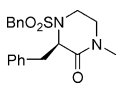
23		0.514	60
24		0.037	8
25		0.004	5
26		0.001	2
27		0.004	3
28		0.006	2
29		0.888	2
30		5	22
31		0.144	2
32		0.459	0.642
33		0.059	2
34		0.0009	0.507

ring. The acid, ester, amide and hydroxymethyl-containing side chain substituted piperazinone analogues were synthesized next (**17**–**28**). These substituents were chosen based on the reported substrates for Factor Xa [Bz-Ile-Glu-Gly-Arg-pNA, Bz-Ile-Glu(γ-Pip)-Gly-Arg-pNA][1g] and the cleavage site sequences recognized by factor Xa in the activation of prothrombin (Ile-Glu-Gly-Arg, Ile-Asp-Gly-Arg).[1f] The differences in fXa inhibitory activity between *S* and *R* analogue in analogues

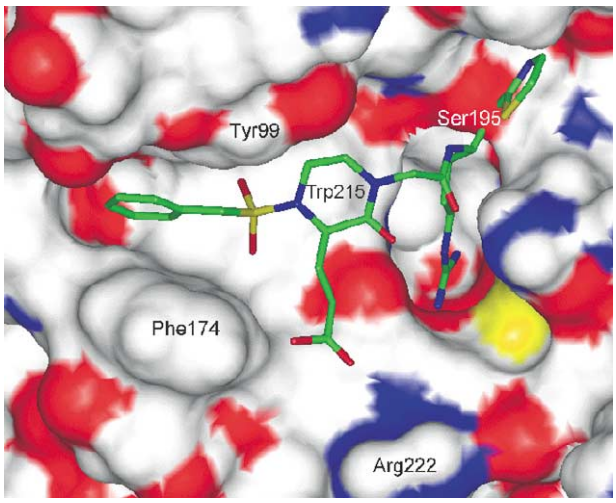


Figure 2. Proposed binding conformation of compound **25** to fXa. The Connelly surface was generated using InsightII (Accelrys, Inc.). Red, blue and yellow surface colors represent proximal oxygen, nitrogen and sulfur atoms, respectively. The carbonyl group involved in the covalant transition state interaction with SER195 is hidden by the surface.

17–**24** suggests a factor Xa preference for the *R*-configuration at the 3-position when neutral and acidic side chains are present. The homologous compounds **25**, **26**, and **27** are 7- to 9-fold more potent than compounds **18**, **20**, and **24**. Homologue acid **25** is as selective for fXa as analogue **18**, but homologue ester **26** and amide **27** are 3- to 4-fold more selective than analogues **20** and **24**. Finally, based on the excellent potency of its tri-peptide counterpart $BnSO_2$-D-Phe-Gly-Arg-thiazole **2,** a benzyl side chain was introduced into the 3-position of the piperazinone ring (**29**–**34**). This resulted in the most potent inhibitor **34** in this substituted piperazinone series. Similar to the trend in analogues **17**–**24**, analogue **34** with *R*-configuration at the 3-position is more potent than its *S*-isomer **33**.

Several compounds were also evaluated in a tissue factor mediated plasma thrombin generation assay.[5] The assay was attempted to mimic thrombin generation in the presence of components of whole blood and evaluate the effect of the specific fXa inhibitors on the rate of thrombin generation. The compound **28** was capable of producing a 2-fold extension of the lag time at a concentration of 760 nM.

Molecular modeling of compound **25** (Fig. 2) in the fXa active site shows the 3-guanidinopropyl group (Arg side chain) fitting into the S1 pocket, forming an ionic interaction with the Asp189 side chain. The benzylsulphonyl group at the N4-position of the piperazinone projects toward the S4 pocket, and is held tightly by π-stacking interactions with the Tyr99, Trp215, and Phe174 side chains. The ketone carbonyl forms a reversible covalent bond with the hydroxyl group of Ser195.

In general, enzyme selectivity shows the following trend with the following side chains: 3-guanidinopropyl > 3-carboxylethyl > 3-carboxymethyl > 3-benzyl-substituted inhibitors. This is consistent with the pattern we observed with D-Phe-Gly-Arg based ketoheterocyclic inhibitors.[3] Ester, amide, acid and hydroxymethyl groups are all tolerated as side chains at the 3-position.

Based on the unsubstituted piperazinone based factor Xa inhibitor[5] and our previously reported tripeptide ketoheterocyclic lead, we have designed, synthesized and evaluated a series of potent and selective substituted piperazinone analogues. A systematic SAR study revealed the structural basis for the potency and selectivity. The fact that various acidic, basic and neutral groups are well tolerated at the 3-position suggests that changes in this portion of the molecule may allow for modulation of PK profiles, while retaining potency and selectivity. Further SAR studies on improving pharmacokinetic properties using piperazinone as templates will be the subject of additional communications from our laboratories.

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