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Novel interactions of large P3 moiety and small P4 moiety in the binding of the peptide mimetic factor VIIa inhibitor☆

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Abstract

Selective factor VIIa-tissue factor complex (FVIIa/TF) inhibition is seen as a promising target for developing new anticoagulant drugs. A novel peptide mimetic factor VIIa inhibitor, ethylsulfonamide-D-biphenylalanine-Gln-*p*-aminobenzamidine, shows 100-fold selectivity against thrombin in spite of its large P3 moiety, unlike previously reported FVIIa/TF selective inhibitors. X-ray crystal structure analysis reveals that the large P3 moiety, D-biphenylalanine, and the small P4 moiety, ethylsulfonamide, make novel interactions with the 170-loop and Lys192 of FVIIa/TF, respectively, accompanying ligand-induced conformational changes of the 170-loop, Gln217, and Lys192. Structural comparisons of FVIIa with thrombin and amino acid sequence comparisons among coagulation serine proteases suggest that these interactions play an important role in achieving selective inhibition for FVIIa/TF.

Keywords: Factor VIIa; Blood coagulation; Serine protease; X-ray crystallography; Drug design

The blood coagulation cascade is divided into extrinsic and intrinsic coagulation pathways. Factor VIIa (FVIIa) in complex with tissue factor (TF) initiates the extrinsic coagulation pathway. This complex activates factors IX to IXa (FIXa) and X to Xa (FXa), which in turn activate factor X to Xa and prothrombin to thrombin, respectively [1]. Thrombin cleaves fibrinogen to fibrin, which forms blood clots with activated platelets. Inappropriate thrombus formation in blood vessels causes cardiovascular diseases (myocardial infarction, stroke, pulmonary embolism, and so on), which are the most common causes of mortality in the industrialized world [2]. Recent studies on blood coagulation have suggested that selective inhibition of extrinsic coagulation provides effective anticoagulation and low risk of bleeding compared with other antithrombotic mechanisms [3–5]. Thus, specific FVIIa/TF complex inhibition, which blocks only extrinsic coagulation, is seen as a promising target for developing new anticoagulant drugs [6,7].

Recently, we reported on propylsulfonamide-D-Thr-Met-*p*-aminobenzamidine (compound **1**, Fig. 1), which showed submicromolar inhibition for FVIIa/TF ($IC_{50} = 130$ nM) and 26-fold selectivity against thrombin [8]. In addition, another peptide mimetic inhibitor, benzylsulfonamide-D-Ile-Gln-*p*-aminobenzamidine (compound **2**, Fig. 1), showed potent inhibition for FVIIa/TF ($IC_{50} = 25$ nM) and 6-fold selectivity against

☆ *Abbreviations:* FVIIa, factor VIIa; TF, tissue factor; sTF, soluble domain of tissue factor; FIXa, factor IXa; FXa, factor Xa; FXIa, factor XIa; FXIIa, factor XIIa; APC, activated protein C.

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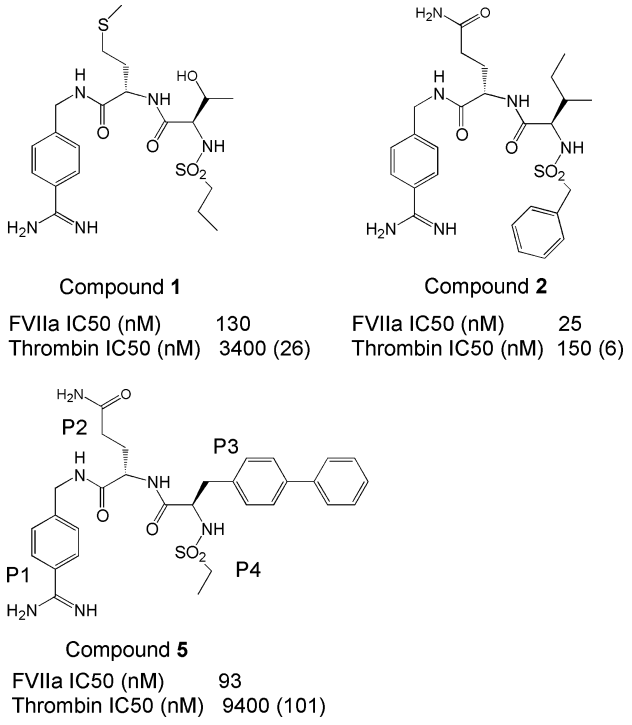

Fig. 1. Chemical structures of peptide mimetic factor VIIa inhibitors.

thrombin. Crystal structures of compounds **1** [8] and **2** (S. Kadono et al., unpublished data) bound to FVIIa/sTF revealed that interactions with the S2 and S3 sites of FVIIa/sTF would play an important role in improving selectivity against thrombin. Compounds **1** and **2** have relatively small P3 moieties, D-threonine and D-isoleucine, respectively, which fit the small S3 site of FVIIa/sTF consisting of Thr99, Pro170I, Trp215, and Gln217 [8]. On the other hand, thrombin has a large and hydrophobic S3 site consisting of Leu99, Ile174, and Trp215 which fits a large P3 moiety, e.g., D-phenylalanine. Therefore, the introduction of the large P3 moiety results in good hydrophobic interactions with thrombin [7–11] and a reduction in selectivity against thrombin [7,8]. Further optimization was continued because compounds **1** and **2** have insufficient selectivity against thrombin. Consequently, as shown in Table 1, compound **3** which has small D-alanine moiety in P3 showed relatively high selectivity against thrombin (>59-fold), whereas the introduction of D-alanine led to low binding affinity for FVIIa/TF ($IC_{50} = 1700$ nM). Compound **4**, which has the D-phenylalanine moiety in P3, showed submicromolar inhibition for FVIIa/TF ($IC_{50} =$ 180 nM), whereas the introduction of D-phenylalanine led to low selectivity against thrombin (13-fold). These data are consistent with the structure–activity relationships of FVIIa/TF selective inhibitors; FVIIa/TF has a small S3 site which fits a small P3 moiety [7,8], whereas thrombin has a large S3 site which fits a large P3 moiety [9–11]. Interestingly, the introduction of the larger P3 moiety, D-biphenylalanine (compound **5**, Fig. 1), led to relatively high selectivity against thrombin. This high selectivity is not consistent with the structure–activity relationships of FVIIa/TF selective inhibitors. Therefore, the 3D structural data of compound **5** bound to FVIIa/TF help us to understand new interactions that improve specificity for FVIIa/TF. In this study, we determined the X-ray crystal structure of compound **5** bound to FVIIa/sTF in order to reveal interactions with FVIIa/sTF and the inhibitor. Based on this structure, key interactions for improving selectivity against thrombin, likely a crucial factor in reducing risk of bleeding, and other coagulation serine proteases will be discussed. These results will provide valuable information for the structure-based drug design of selective inhibitors for FVIIa/TF.

Materials and methods

Compound synthesis. The synthesis and structure–activity relationship of peptide mimetic FVIIa/TF inhibitors will be reported elsewhere.

Inhibition assays. The inhibition activities for human FVIIa/TF and thrombin were measured using chromogenic substrates as described previously [8].

Human factor Xa (FXa) inhibition was measured as follows: A 10% (v/v) DMSO solution of test compound (20 μl) was mixed with 20 μl buffer (500 mM Tris–HCl, pH 8.4, 1500 mM NaCl), 20 μl S-2222 (5 mM, Daiichi Pure Chemical), and 120 μl distilled water in a 96-well assay plate. The reaction was initiated by the addition of 20 μl human factor Xa solution (50 mU/ml, Enzyme Research Laboratories) and the absorbance at 405 nm was then monitored to measure the initial velocity of the reaction. Percent inhibition at each concentration was determined from the experimental and control samples. IC_{50} was calculated from the concentration–reaction activity curve of each test compound.

Human factor XIa (FXIa) inhibition was measured as follows: A 10% (v/v) DMSO solution of test compound (20 μl) was mixed with 100 μl buffer (200 mM Tris–HCl, pH 7.2, 300 mM NaCl), 20 μl S-2366 (2 mM, Daiichi Pure Chemical), and 40 μl distilled water in a 96-well

Table 1
IC_{50} values of peptide mimetic inhibitors (P4-P3-Gln-*p*-aminomethylbenzamidine)

	P3	P4	FVIIa/TF IC_{50} (nM)	Thrombin IC_{50} (nM)	FXa IC_{50} (nM)	FXIa IC_{50} (nM)	APC IC_{50} (nM)
2	D-Ile	SO_2Benzyl	25	150 (6)	330 (13)	35 (1.4)	83 (3.3)
3	D-Ala	SO_2Et	1700	>100,000 (>59)	—	—	—
4	D-Phe	SO_2Et	180	2400 (13)	—	—	—
5	D-BiphenylAla	SO_2Et	93	9400 (101)	74,300 (799)	460 (4.9)	2750 (30)

Values in parentheses refer to ratio against FVIIa/TF IC_{50}.