Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 327 (2005) 589–596
BBRC
www.elsevier.com/locate/ybbrc

Structure-based design of P3 moieties in the peptide mimetic factor VIIa inhibitor☆

Shojiro Kadono*, Akihisa Sakamoto, Yasufumi Kikuchi, Masayoshi Oh-eda, Naohiro Yabuta, Kazutaka Yoshihashi, Takehisa Kitazawa, Tsukasa Suzuki, Takaki Koga, Kunihiro Hattori, Takuya Shiraishi, Masayuki Haramura, Hirofumi Kodama, Yoshiyuki Ono, Toru Esaki, Haruhiko Sato, Yoshiaki Watanabe, Susumu Itoh, Masateru Ohta, Toshiro Kozono

Fuji Gotemba Research Labs, Chugai Pharmaceutical Co., Ltd., 1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan

Received 26 November 2004
Available online 18 December 2004

Abstract

Selective factor VIIa-tissue factor complex (FVIIa/TF) inhibition is seen as a promising target for developing new anticoagulant drugs. Structure-based designs of the P3 moiety in the peptide mimetic factor VIIa inhibitor successfully lead to novel inhibitors with selectivity for FVIIa/TF and extrinsic coagulation the same as or even higher than those of previously reported peptide mimetic factor VIIa inhibitors. X-ray crystal structure analysis reveals that one of the novel inhibitors shows improved selectivity by forming interactions between the inhibitor and FVIIa as expected. Another of the novel inhibitors achieves improved selectivity through an unexpected hydrogen bond with Gln217, with a unique bent conformation in FVIIa/TF accompanied by conformational changes of the inhibitor and the protein.

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 cause 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 that compound **1** (Fig. 1) with the large P3 moiety D-biphenylalanine showed relatively high selectivity against thrombin (101-fold) [8] unlike the previously reported FVIIa/TF selective inhibitors [7,9] and a relatively large concentration ratio for 2-fold prolongation of the prothrombin time and an activated

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

* Corresponding author. Fax: +81 550 87 5326.
E-mail address: kadonosuj@chugai-pharm.co.jp (S. Kadono).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.12.042

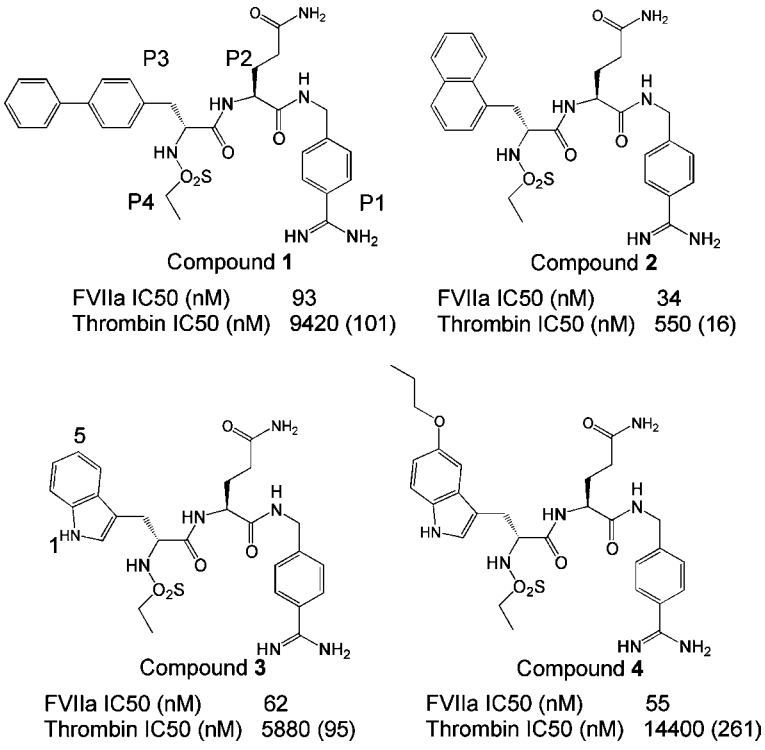

Fig. 1. Chemical structures of peptide mimetic factor VIIa inhibitors.

partial thromboplastin time (2× APTT/2× PT) (6.2) [9] compared to the thrombin selective inhibitor Argatroban [10] (2× APTT/2× PT = 0.4, in-house data), or the factor Xa selective inhibitor DX-9065a [11] (2× APTT/2× PT = 2.2, in-house data). These data show that compound **1** achieves selective inhibition for extrinsic coagulation by selective inhibition for FVIIa/TF because specific inhibition for FVIIa/TF, which blocks only extrinsic coagulation, should theoretically result in prolongation of PT without APTT, thus making 2× APTT/2× PT infinitely large. X-ray crystal structure analysis of compound **1** bound to FVIIa and the soluble domain of tissue factor (sTF) complex revealed characteristic interactions between compound **1** and FVIIa/sTF [8]: (a) hydrogen bonds between the glutamine side chain in P2 and a hydrophilic pocket consisting of Asp60, Tyr94, and Thr98 at the S2 site, (b) a charge–dipole interaction between the sulfonamide group in P4 and Lys192 at the S1-sub-site adopting a unique conformation of the ethylsulfonamide moiety, and (c) good hydrophobic interactions of the D-biphenylalanine side chain in P4 with an additional site consisting of residues in the 170-loop accompanying ligand-induced conformational change of the 170-loop and Gln217 (Fig. 2A). Focusing on the importance of the 170-loop to the specific binding for FVIIa/TF and the ligand-induced fitting of the 170-loop, we continued the modification of the P3 moiety in the peptide mimetic inhibitor and achieved more selective inhibition for extrinsic coagulation by improving selectivity for FVIIa/TF. Herein, we report the structure-based design of the P3 moieties based on the crystal structures of FVIIa/sTF. Consequently, peptide mimetic FVIIa inhibitors with selectivity for FVIIa/TF the same as or higher than that of compound **1** were successfully obtained. X-ray crystal structure analysis of these inhibitors bound to FVIIa/sTF revealed that one of the inhibitors achieves improved selectivity by forming interactions between FVIIa and the inhibitor as expected; another inhibitor improves selectivity by forming an unexpected hydrogen bond.

Materials and methods

Compound synthesis. The peptide mimetic FVIIa/TF inhibitors used in this study were synthesized in our laboratory. The details of the synthesis and structure–activity relationship will be reported elsewhere.

Inhibition assays. Inhibition activities for human FVIIa/TF, thrombin, FXa, factor XIa (FXIa), and activated protein C (APC) were measured using chromogenic substrates as described previously [8].

Coagulation assays. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using human plasma (Dade Behring) as described previously [8].

Computational modeling. Computational models of compound **2** bound to thrombin and FVIIa/TF were built based on X-ray crystal structures of D-Phe-Pro-Arg chloromethyl ketone bound to thrombin (PDB code 1PPB) and compound **1** bound to FVIIa/sTF (PDB code 1WTG), respectively. After the modification of the P1, P2, P3, and P4 moieties, and the manual docking of compound **2** to the thrombin or FVIIa/sTF active site using QUANTA (Accelrys), energy minimization was performed using CHARMm (Accelrys).