Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2253–2256

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Optimization of a Screening Lead for Factor VIIa/TF

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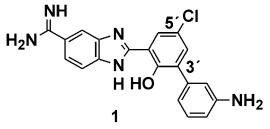
Received 2 April 2001; accepted 18 May 2001

Abstract—The structure-based design and progression of a screening lead to a 3 nM factor VIIa/TF inhibitor with improved selectivity versus related enzymes is described.

Introduction

Factor VIIa, when complexed with Tissue Factor (TF), rapidly activates coagulation factors IX and X to factors IXa and Xa, respectively, which in turn are then responsible for the activation of prothrombin to thrombin.[1] Active thrombin then cleaves fibrinogen to fibrin which forms the structural basis of all blood clots. Much research has focused on the generation of potent and selective inhibitors of factor Xa or thrombin as potentially valuable therapeutic agents for the treatment of thromboembolic disorders.[2–4] More recently, the fVIIa/TF complex has been deemed a worthwhile point of intervention.[5] Studies using anti-factor VIIa antibodies,[6] active-site-inhibited factor VIIa,[7] and recombinant anticoagulant tissue factor pathway inhibitor (TFPI)[8] have shown promising results and prompted the attention of many research groups. Accordingly, there is increasing interest in the generation of potent and selective small molecule factor VIIa inhibitors in the search for novel antithrombotics.[9–11] Herein, we describe our efforts in the design and synthesis of a series of novel, potent and selective factor VIIa inhibitors.

Since factor VIIa is a trypsin-like serine protease we began our search by screening our collection of proprietary serine protease inhibitors.[12–15] From this library, we identified **1**, 2-(3′-amino-5-chloro-2-hydroxybiphenyl-3-yl)-1*H*-benzoimidazole-5-carboxamidine, as a potent inhibitor of factor VIIa/TF and several serine proteases.[16] This compound showed good inhibition for factor VIIa/TF ($K_{i'} = 78$ nM), although also demonstrated submicromolar inhibition for factor Xa and urokinase-type plasminogen activator (uPa) and low micromolar inhibition versus thrombin (fIIa), plasmin, and trypsin.[17] The structure-based design and progression of **1** to a 3 nM inhibitor of factor VIIa/TF with improved selectivity versus relevant anti-targets is described.



1

	$K_{i'}$ (μM)
fVIIa/TF	0.078
fIIa	4.2
fXa	0.50
plasmin	3.2
trypsin	4.7
uPa	0.46

Utilizing the crystal structures of related compounds in uPa[16,18] as well as other serine proteases, a model of **1** bound to factor VIIa was constructed (Fig. 1a). The binding mode, which is consistent between all these structures, involves a network of short and normal hydrogen bonds between the phenol group, the Ser195 and His57 residues, and a water molecule bound in the oxyanion hole as depicted in Figure 1b.[16] The 5-amidinobenzimidazole binds as expected in the S1 pocket with a salt-bridge between the amidine and Asp189. Occasionally, a water molecule spans the two moieties instead of through direct hydrogen bonds. Due to the smaller size of the factor VIIa S1 pocket (as compared to uPa), we built the model with direct hydrogen bonds between

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0960-894X/01/$ - see front matter
PII: S0960-894X(01)00420-6

Asp189 and **1**. The model indicates that the C3′ phenyl ring is bound in the S1′ pocket of factor VIIa and, in addition to hydrophobic interactions with the Cys40-Cys58 disulfide, appears to be involved in edge to face β-stacking with the catalytic imidazole ring of His57. The model also indicates that the methylene chain of Lys60A lies immediately above this ring and is directed towards the central phenolic ring of the inhibitor. The central phenolic ring is packed against residue 192, which is a lysine in factor VIIa. Interestingly, most serine proteases have a glutamine at this site with the notable exception of thrombin which has a glutamic acid. Interaction between this residue and an acidic functionality might be expected to afford a degree of selectivity versus thrombin.

Based on this model and previous work with this class of inhibitors,[16,18] we believed that immediate improvement of the inhibition for factor VIIa by **1** could be realized in two ways. First, we transformed the benzimidazole ring of **1** into an indole. Earlier results from our proprietary serine protease inhibitor library clearly indicate that indole rings provide improved inhibition for factor VIIa as compared to benzimidazole rings. Secondly, we exchanged the chlorine at C5′ of **1** with an acetic acid moiety to interact with Lys192 and, potentially, Lys60A as well. Additionally, we anticipated that an anionic charge here would repel the Glu192 of thrombin imparting target selectivity. These changes are represented in compound **2** as depicted in Table 1. Compound **2** did prove to have a stronger binding affinity toward factor VIIa/TF ($K_{i'} = 12$ nM) and, as expected, improved selectivity versus thrombin. The selectivity versus the other anti-targets studied remained fairly consistent.

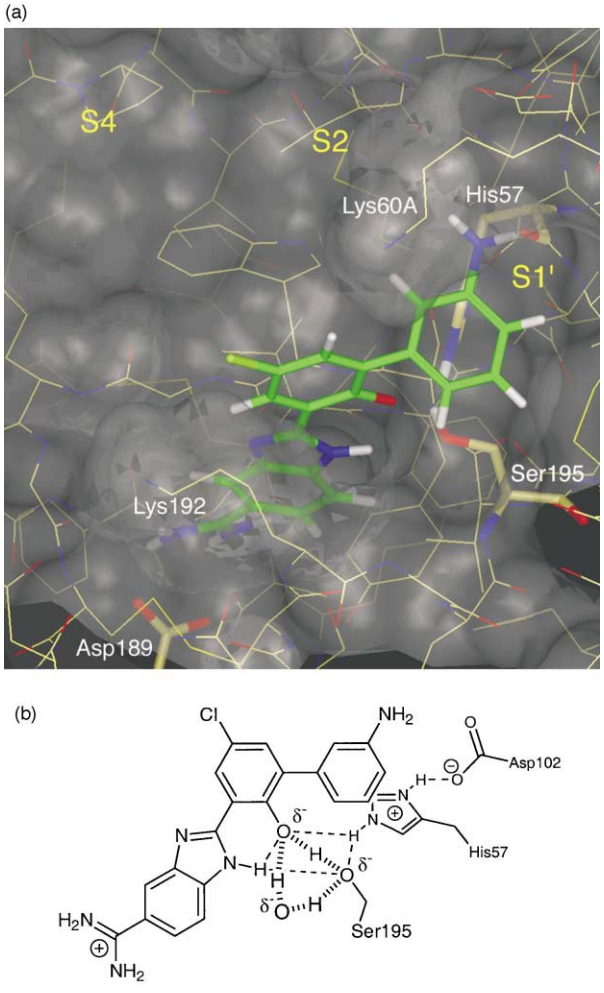
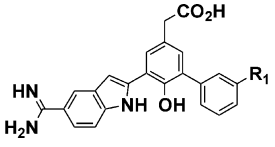


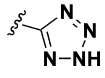
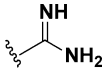
Figure 1. (a) Model of **1** bound in factor VIIa; (b) H-bonding array displayed by **1** with the catalytic residues of uPa in the crystal structure.

In an attempt to further improve the overall binding affinity of these factor VIIa inhibitors, we surveyed the effect of *meta* substitution on either affecting the strength of the β-stacking interaction of His57 or increasing S1′ pocket complementarity. Substitution at the *ortho* position might be expected to adversely affect the dihedral angle between the two phenyl rings while the model indicated that there was very little room for *para* substitution. Hence, a small set of *meta* substituted analogues was generated. Of these compounds, the 3-nitroaryl analogue, **6**, showed the strongest binding affinity to factor VIIa/TF ($K_{i'} = 3$ nM) and the best selectivity versus thrombin, plasmin, trypsin, and uPa. Acidic (**7**–**9**) and basic (**10**) analogues show decreased factor VIIa/TF inhibition most likely due to the hydrophobic nature of the site created by the alkyl chain of Lys60A and the Cys40-Cys58 disulfide.

Having identified **6**, which has a C3′ moiety that fits well into the S1′ pocket and affords increased potency for factor VIIa/TF and enhanced selectivity for relevant anti-targets, we further probed the interactions with the Lys192 of factor VIIa. Anticipating that optimization of an anionic charge near Lys192 would further enhance the selectivity profile, a series of derivatives that incorporate anionic groups attached to the C5′ position of **6** were generated (Table 2). Compounds **6**, **11**, and **13** maintain similar inhibition for factor VIIa/TF, while **12**, **14**–**15** are less effective inhibitors. These results would indicate that Lys192 can accommodate acid tethers

Table 1. C3′ modifications

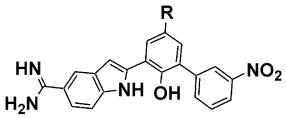


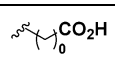
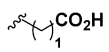
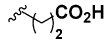
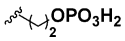
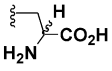
Compd	R_1	$K_{i'}$ (nM) fVIIa/TF	Selectivity ratio				
			fXa	fIIa	Plasmin	Trypsin	uPa
2	NH_2	12	26	550	27	83	6
3	H	41	4	49	6	1	1
4	Cl	33	5	112	17	3	4
6	NO_2	3	20	960	90	160	64
7	CO_2H	320	0.5	375	5	1	6
8	OPO_3H_2	950	1	87	4	Na	4
9		480	0.5	100	2	3	2
10		430	4	140	3	2	0.6

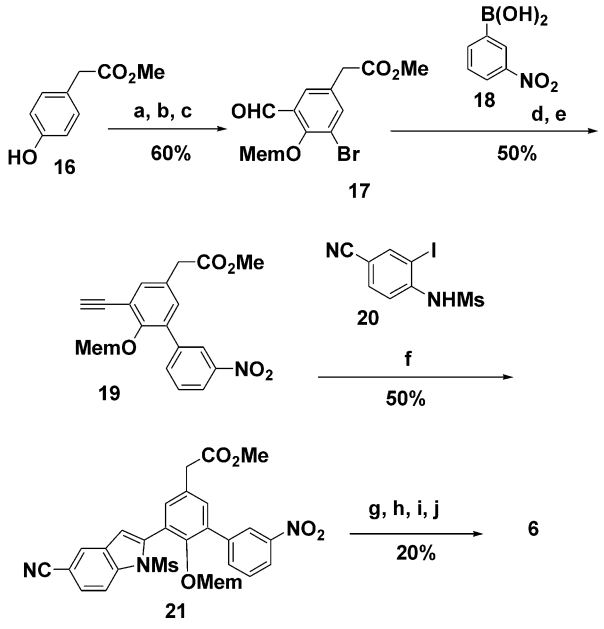
lengths of both **6** and **11**, while the length of **12** may not be optimal. The anionic charge on compound **13** is likely extended out far enough to interact with Lys60A and this may explain its improved potency.

The in vitro anticoagulant effect (prothrombin times, PTs) of several lead analogues is listed in Table 2. Compounds **6**, **11**–**12** double the PT at low micromolar levels while **14** is slightly more effective. Compound **15** was the least effective. In-house studies have indicated that by effectively decreasing the lipophilicity of such compounds (i.e., increasing water solubility/decreasing plasma protein binding) a marked improvement in the anticoagulant effect has been shown. It is also realized that the selectivity profile of the inhibitors plays a role in the overall anticoagulant effect.

Table 2. C5′ modifications



Compd	R	$K_{i'}$ (nM) FVIIa/TF	Selectivity ratio					2xPT (μM)
			fXa	fIIa	Plasmin	Trypsin	uPa	
11		5	42	420	75	70	44	4.1
6		3	20	960	90	160	64	1.7
12		27	2	59	6	37	6	2.2
13		3	5	870	33	63	21	na
14		50	0.5	40	3	5	1	0.73
15		50	0.5	140	20	40	15	16



Scheme 1. Palladium(0) catalyzed route to **6**. Reagents: (a) $MgCl_2$, $(CH_2O)_n$, MeCN; (b) NBS, DMF; (c) MemCl, Hunig's base; (d) **18**, $Pd(PPh_3)_4$ 1 M Na_2CO_3, toluene, reflux; (e) dimethyl-1-diazo-2-oxopropylphosphonate, K_2CO_3, MeOH; (f) **20**, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, MeCN, 80 °C; (g) NaOH, THF; (h) EtOH, HCl (anhyd); (i) NH_3, EtOH; (j) 1 N HCl, reflux.

The synthesis of **6**, starting from commercially available methyl 4-hydroxyphenylacetate (**16**), is illustrated in Scheme 1. Formylation of **16** with $MgCl_2$ and paraformaldehyde, bromination with NBS, and subsequent treatment with methoxyethoxymethyl chloride afforded **17**. A Suzuki coupling of **17** with the commercially available boronic acid **18** followed by treatment with dimethyl-1-diazo-2-oxopropylphosphonate[19] afforded alkyne **19**. A palladium(0) catalyzed coupling of **19** with the aryl iodo moiety **20** then gave **21**. Removal of the protecting groups and transformation of the nitrile into an amidine via standard Pinner/aminolysis conditions afforded **6**. All other analogues described were synthesized in an analogous manner to **6**, although the requisite boronic acids or a modified starting phenol moiety was utilized.[12]

The optimization of a nonselective serine protease screening lead to a 3 nM factor VIIa/TF inhibitor, which possesses improved selectivity versus relevant anti-targets, is described. Pharmacokinetics, in vivo animal efficacy, and non-amidino P1 analogue development will be the subject of a future publication.

Acknowledgements

The authors wish to thank Michael Venuti and Jochen Knolle for helpful scientific discussions, Liling Fang for analytical support and Barbara Nielsen for aid in preparing this manuscript.

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