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Synthesis, SAR exploration, and X-ray crystal structures of factor XIa inhibitors containing an α-ketothiazole arginine

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Abstract—Using an α-ketothiazole arginine moiety as a key recognition element, a series of small peptidomimetic molecules was designed and synthesized, and their co-crystal structures with factor XIa were studied in an effort to develop smaller, less peptidic inhibitors as antithrombotic agents.

Thromboembolic diseases are the leading causes of morbidity and mortality in the developed countries.[1] Conventional antithrombotic therapy includes intravenous administration of heparin followed by oral treatment with warfarin.[2] Since both agents are indirect and non-specific inhibitors of the coagulation serine proteases, this treatment carries high risk of excessive bleeding and requires routine monitoring. To overcome these limitations, new antithrombotic agents have been designed to target specific enzymes in the coagulation pathway, such as thrombin and factor Xa (FXa). Although these newer anticoagulants offer some advantages over heparin therapy, some undesirable features such as bleeding and a narrow therapeutic window have been demonstrated and their safety profiles have yet to be established in clinical trials.[3]

Coagulation factor XIa (FXIa) is a trypsin-like serine protease that plays a major role in amplification of the thrombotic response and in maintaining clot integrity.[4] The inhibition of FXIa in vivo may result in the formation of smaller thrombi without affecting the initiation of clot formation. The lack of an effect upon initiation may result in a lesser bleeding liability for FXIa inhibitors than anticoagulants that do suppress clot initiation. Supporting evidence for this hypothesis includes the observation that administration of an anti-human FXIa antibody significantly reduces intraluminal thrombus growth in a baboon thrombosis model without preventing thrombus initiation.[5] The anti-FXIa antibody prolongs the partial thromboplastin time (a measure of the coagulation function involving FXIa) without affecting prothrombin time (a measure of the coagulation function involving FVIIa and tissue factor). Most significantly, the anti-FXIa antibody does not prolong bleeding time. Also supporting the FXIa lowered bleeding risk hypothesis is the finding that individuals with a natural FXIa deficiency do not show severe bleeding problems.[6]

Keywords: Factor XIa inhibitor; α-Ketothiazole arginine; Anticoagulant; X-ray crystal structure.
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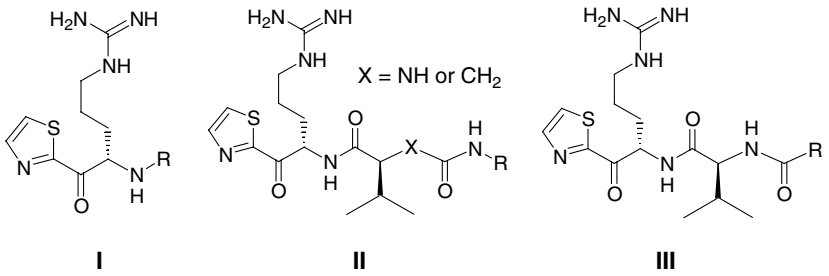
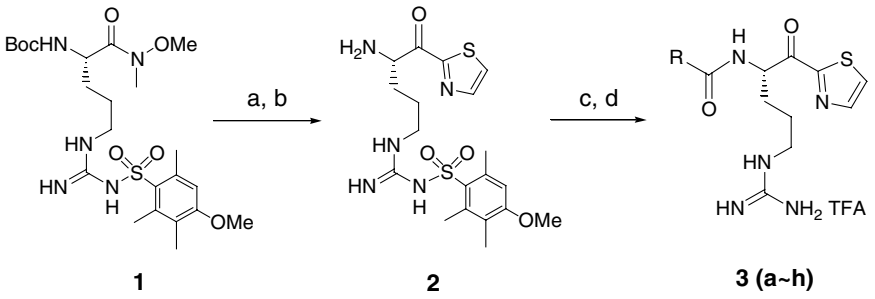
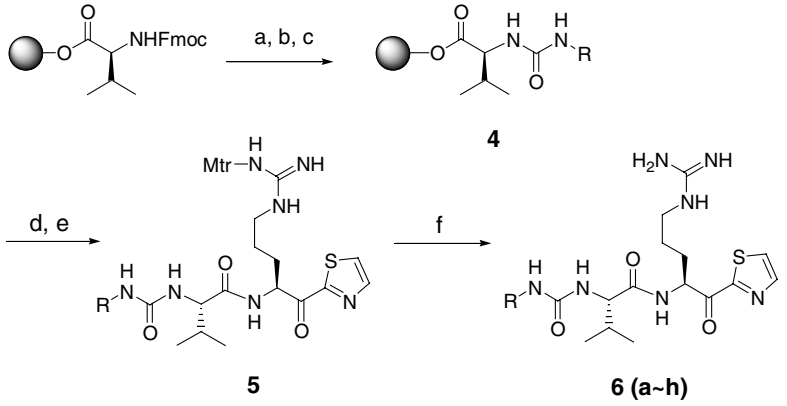


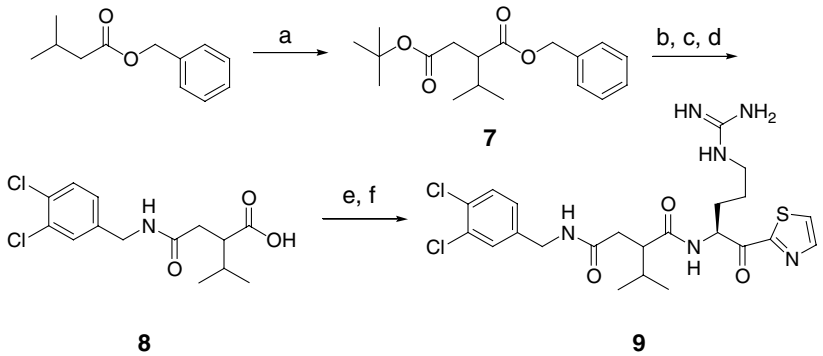
Figure 1.



Scheme 1. Reagents and conditions: (a) thiazole (4.6 equiv), *n*-BuLi (4.16 equiv), TMEDA (4.6 equiv), THF, −78 °C to −41 °C then −78 °C, 75%; (b) 25% TFA–DCM, rt; (c) RCOOH, EDC (1.24 equiv), HOBt (1.24 equiv), DIEA (4.0 equiv), THF, rt; or RCOCl (RSO_2Cl), TEA, DCM; (d) 95% TFA, 2.5% thioanisole, 2.5% H_2O, 30–45% for three steps.



Scheme 2. Reagents and conditions: (a) 20% piperidine–DMF, 2 h; (b) 4-nitrophenyl chloroformate, DIEA, THF–DCM (1/1), 1 h; (c) RNH_2, TEA, DMF, 2 h; (d) 50% TFA–DCM, 30 min; 28–40% for four steps; (e) **2**, EDC, HOBt, DIEA, DCM–THF (1/1), 1 h; (f) 95% TFA, 2.5% H_2O, 2.5% thioanisole, 3 h, 30–45% for two steps.



Scheme 3. Reagents and conditions: (a) LDA (1.2 equiv), THF, −78 °C to 0 °C; *tert*-butyl 2-bromoacetate, THF, 0 °C to rt, 48%; (b) 50% TFA–DCM, 30%; (c) 3,4-dichlorobenzylamine, EDC, HOBt, DIEA, THF, 99%; (d) H_2, Pd–C (5%), MeOH, 70%; (e) compd **2**, EDC, HOBt, DIEA, THF; (f) 95% TFA, 2.5% H_2O, 2.5% thioanisole, DCM, 3 h, 36% for two steps.

We recently reported a series of specific tripeptidomimetic FXIa inhibitors.[7] In vivo pharmacological studies revealed that these inhibitors effectively reduce the size of clot without altering bleeding times and other observable side effects. In this paper, we report the design and synthesis of smaller, less peptidic FXIa inhibitors, represented by the generic structures I, II, and III (Fig. 1).

The synthesis of type I compounds is shown in Scheme 1. The Mtr-protected α-ketothiazole arginine (**2**) was prepared from Weinreb amide (**1**) using a modified procedure in which lithium thiazole, generated in situ, was added by cannula to pre-cooled Weinreb amide at −78 °C.[8] After workup, the isolated product was carefully treated with 25% TFA-DCM to afford the desired amine **2**. Direct coupling of **2** with selected acids, acid chlorides, or sulfonyl chlorides, followed by Mtr-deprotection provided compounds **3** (**a**–**h**).

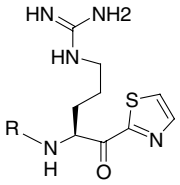
A combination of solid-phase and solution-phase synthesis was used to prepare urea type II compounds (X = NH) (Scheme 2). Pre-loaded Fmoc Wang resin was treated with 20% piperidine in DMF followed by *para*-nitrophenyl chloroformate to form the carbamate intermediate, which was then treated with various selected amines to form the N-terminal ureas (**4**). Cleavage from the resin followed by standard EDC-HOBt coupling with the aminoketone (**2**) yielded compounds **6** (**a**–**h**) after Mtr-deprotection.

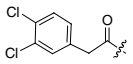
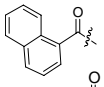
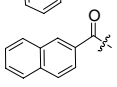
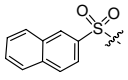
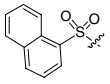
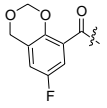
The synthesis of a reverse amide type II compound ($X = CH_2$) is shown in Scheme 3. 3-Methyl butyric acid benzyl ester was treated with LDA followed by *tert*-butyl α-bromoacetate to give the 2-isopropyl succinic acid derivative **7**. After removal of the *tert*-butyl ester, the acid was coupled with 3,4-dichlorobenzylamine and then hydrogenolysis afforded the acid **8**. Coupling of **8** with aminoketone **2** provided compound **9** after standard Mtr-deprotection.

The synthesis of type III compounds is shown in Scheme 4. Standard EDC–HOBt coupling of **2** with CBz-Val-OH followed by hydrogenolysis yielded **10**. EDC–HOBt coupling with various carboxylic acids and standard Mtr-deprotection provided the N-acylated Val-Arg-ketothiazoles **11** (**a**–**h**).

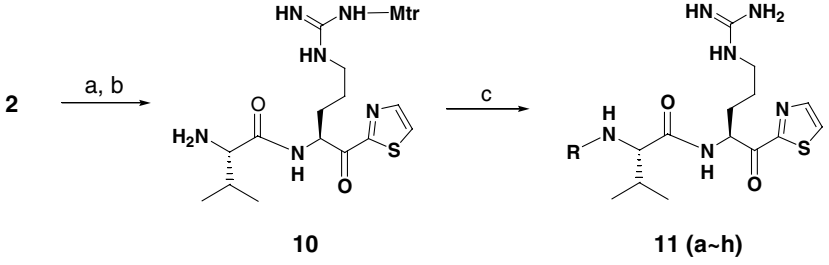
Peptidolytic activities for FXIa were measured using a fluorogenic reporter group, 7-methyl-4-aminocoumarin (AMC), attached via an amide linkage to the tripeptide pyroglutamyl-prolyl-arginyl (Glu-Pro-Arg). The assay was carried out in a buffer containing 20 mM Tris–HCl, pH 7.4, 150 mM NaCl, and 0.02% Tween 20. Enzyme and substrate concentrations were 0.25 nM and 50 μM, respectively. The assay was incubated at 30 °C for 15–30 min and then released AMC was measured (emission 460 nm, excitation 355 nm) on a Wallac 1420 Multilabel Counter. The data were fit to a one-site dose–response curve using non-linear least squares to determine the IC_{50} value.

Table 1. SAR of ketothiazole arginine analogs with one amide bond



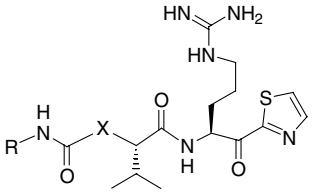
Compound	R	MH^+ (*m*/*z*)	IC_{50}[a] (μM) FXIa
3a		326.3	123
3b		410.2	>250
3c		428.3	37.9
3d		396.3	2.25
3e		396.2	>200
3f		432.2	86.0
3g		432.2	250
3h		422.2	5.90

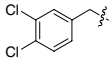
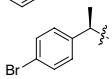
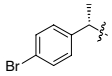
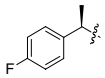
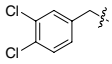
[a] IC_{50}s were derived from triplicate measurements whose standard errors were normally <5% in a given assay. Assay-to-assay variability was within ±2-fold based on the results of a standard compound, benzamidine.



Scheme 4. Reagents and conditions: (a) CBz-Val-OH, EDC, HOBt, DIEA, DMF, 85%; (b) H_2, Pd–C (10%), EtOAc, 95%; (c) i—RCO_2H, EDC, HOBt, DIEA, DMF, 60–80%; ii—95% TFA, 2.5% H_2O, 2.5% thioanisole, DCM, 3 h, 55–70%.

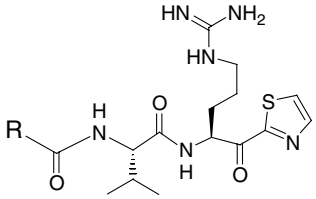
Table 2. SAR of ketothiazole arginine analogs with two amide bonds and a urea moiety at the N-terminus

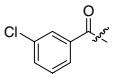
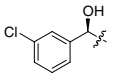
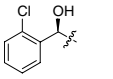
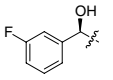
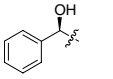
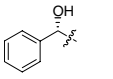
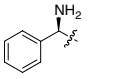


Compound	X	R	MH$^+$ (*m/z*)	IC_{50}[a] (μM) FXIa
6a	NH	H	384.3	0.502
6b	NH		542.1 544.1	0.480
6c	NH		488.3	1.72
6d	NH		566.6 568.2	1.20
6e	NH		566.6 568.2	12.5
6f	NH		506.4	2.32
6g	NH		520.4	0.713
6h	NH		520.4	3.31
9	CH_2		541.5 543.3	6.22

[a] IC_{50}s were derived from triplicate measurements whose standard errors were normally <5% in a given assay. Assay-to-assay variability was within ±2-fold based on the results of a standard compound, benzamidine.

Table 3. SAR of ketothiazole arginine analogs with two amide bonds and an acyl moiety at the N-terminus



Compound	R	MH$^+$ (*m/z*)	IC_{50}[a] (μM) FXIa
11a		439.3	0.850
11b		507.2	0.254
11c		509.2	0.116
11d		509.2	0.790
11e		493.2	0.956
11f		475.2	0.425
11g		475.2	0.431
11h		474.2	1.75

[a] IC_{50}s were derived from triplicate measurements whose standard errors were normally <5% in a given assay. Assay-to-assay variability was within ±2-fold based on the results of a standard compound, benzamidine.

Table 1 shows selected SAR of type I compounds with the ketothiazole arginine moiety directly coupled to a variety of different non-amino acid fragments through either an amide or a sulfonamide bond. In general, such compounds have very weak to moderate potency. The most potent analog in the series has R = naphthalene-1-carbonyl (**3d**), with an IC_{50} (FXIa) of 2.25 μM. When R = naphthalene-2-carbonyl (**3e**), the potency is 100-fold less. The same diminished affinity is seen for sulfonamide analog **3g**. It seems that the 6-fluoro-benzo[1,3]-dioxane ring (**3h**) is a good bioisostere of a 1-naphthyl ring since **3d** has about the same potency as **3h**. Due to the overall low affinity for FXIa, no further optimization effort was pursued for type I compounds.

Table 2 shows the SAR of type II compounds having the ketothiazole arginine linked to a urea derivative through a Val residue.[9] The addition of the Val residue as the P2 recognition element and the urea unit as an N-terminal cap further increased FXIa inhibition, as demonstrated by inhibitor **6a**. 3,4-Dichlorobenzyl substitution of the urea maintained the FXIa potency (**6b**). While the chirality of *R* groups attached to the urea appears to significantly affect potency of these analogs (e.g., **6d** vs **6e** and **6g** vs **6h**), different substituents on the *para*-position of the phenyl ring, such as, hydrogen (**6c**), bromo (**6d**), or fluoro (**6f**), cause little change in affinity. Notably, replacement of the urea NH of **6b** with a methylene moiety (**9**) significantly decreases potency, suggesting that this NH is important for FXIa inhibition.

We then explored whether the external urea NH group is necessary for inhibition. The results of this study are summarized in Table 3. Compound **11a**, with an isopentyl group replacing the external urea NH_2 of **6a**, has similar potency, an indication that this NH is dispensable. When R is 3-chlorobenzoyl (**11b**), the potency increases ~3-fold. Changing the carbonyl to a hydroxyl group further improves potency (**11c**). The stereochemistry of this hydroxyl group seems to be not important, since both *R*

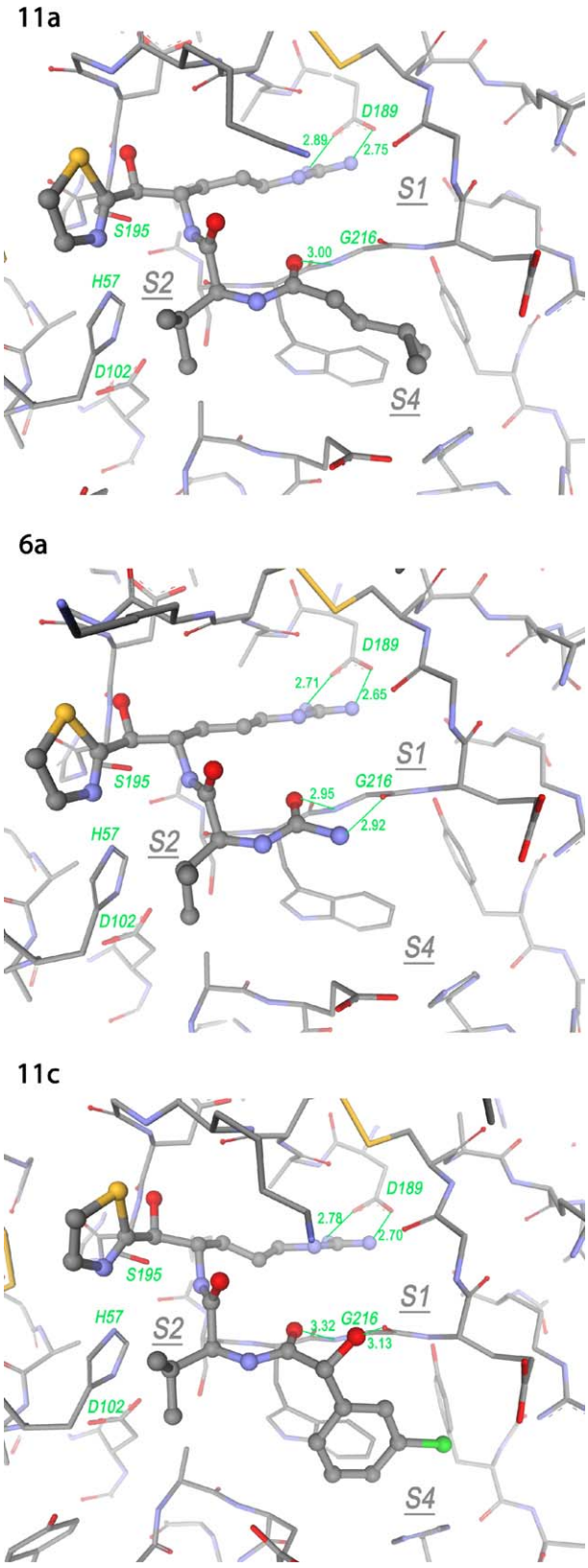


Figure 2. X-ray crystal structures of **11a**, **6a**, and **11c** with FXIac. FXIac is in stick representation and the ligands are in ball-and-stick representation. H-bond distances are labeled in angstrom. Substrate-binding sites are labeled in gray letters. The crystallization and ligand-soaking conditions have been published.[11]

and *S* are equally potent (**11f** and **11g**). Replacing this hydroxyl with an amino group is detrimental for affinity (**11h**). The chloro group and its position on the phenyl ring of **11c** are important for the activity. Compounds where it is switched from *meta*-position (**11c**) to the *ortho*-position (**11d**), replaced with a fluoro group (**11e**), or completely removed (**11f**) all have decreased potency.

Figure 2 shows the X-ray crystal structures of **11a** (PDB code: 1ZPB), **6a** (PDB code: 1ZPC), and **11c** (PDB code: 2FDA) in co-complex with FXIa catalytic domain (FXIac). All three structures show salt bridge interaction between the P1 arginine and D189 in the S1 pocket, while the keto group is covalently attached to S195 in the enzyme active site.[10] The P2 Val side chain occupies the S2 pocket. The N-terminal carbonyl groups in **11a**, **6a**, and **11c** form an H-bond interaction with the backbone NH of G216. The differences among these structures include: (1) the isopentyl group in **11a** forms a hydrophobic interaction with residues that form the S4 pocket; (2) the NH_2 of the urea in **6a** forms an additional H-bond interaction with the backbone carbonyl group of G216; and (3) in **11c**, the above two interactions, a hydrophobic S4 interaction and a hydrogen bond with the hydroxyl group, are combined together. Since the hydrogen bonds between the hydroxyl group of **11c** and G216 are weak, the enhanced hydrophobic interactions of the chlorophenyl moiety in the S4 pocket might contribute to the higher affinity of **11c**.

In conclusion, three types of FXIa inhibitors containing fewer peptide bonds than those previously reported are disclosed. Several compounds from these series were found to have submicromolar potency. The SAR for these compound series and the analysis of their co-crystal structures provide guidance for the design of future FXIa inhibitors as antithrombotic agents.

References and notes

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9. Compounds in Tables 2 and 3 were also assayed against thrombin and trypsin. In general, the affinity for these enzymes was similar to that for FXIa, that is, within 100-fold.

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