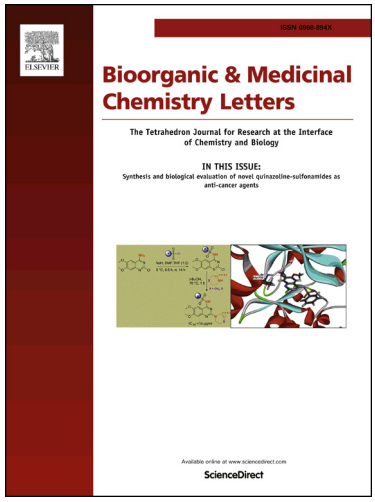

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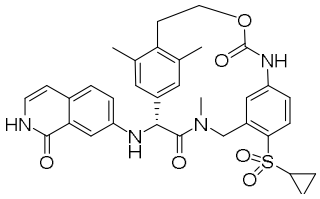

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ABSTRACT

Factor VIIa (FVIIa) inhibitors have shown strong antithrombotic efficacy in preclinical thrombosis models with limited bleeding liabilities. Discovery of potent, orally active FVIIa inhibitors has been largely unsuccessful due to the requirement of a basic P1 group to interact with Asp189 in the S1 binding pocket, limiting their membrane permeability. We have combined recently reported neutral P1 binding substituents with a highly optimized macrocyclic chemotype to produce FVIIa inhibitors with low nanomolar potency and enhanced permeability.

Vitamin K antagonists and heparins have been part of the standard of care for thromboembolic disorders for many years, but these antithrombotic therapies possess significant limitations. Vitamin K antagonists, such as warfarin, have significant drug and food interactions, which create a challenge for maintenance of the desired therapeutic effect without excessive bleeding.[1] Heparins are parenterally administered, which prevents their widespread use in long-term therapy. Several decades of research have produced anticoagulants targeting thrombin or FXa that demonstrate improved efficacy with decreased bleeding in clinical studies.[2,3] Even though these new therapies are replacing the older antithrombotics, research to find safe, effective anticoagulants to complement existing therapies continues.

The extrinsic pathway in the clotting cascade is initiated by the tissue factor-Factor VIIa complex (TF-FVIIa) that activates Factors IX and X.[4] This protein complex has attracted significant interest because small molecule FVIIa inhibitors have strong efficacy in preclinical models of thrombosis with minimal effect on provoked bleeding at antithrombotic doses.[5] Inhibition of the TF-FVIIa complex may also have therapeutic potential in other areas such as cancer or inflammation due both to its antithrombotic effect as well as impact on other signaling pathways.[6-8] Despite years of study, the discovery of a potent, orally bioavailable FVIIa inhibitor clinical candidate remains elusive.[9]

Recently we have reported the discovery of potent phenylpyrrolidine FVIIa inhibitors with a series of neutral P1[10]

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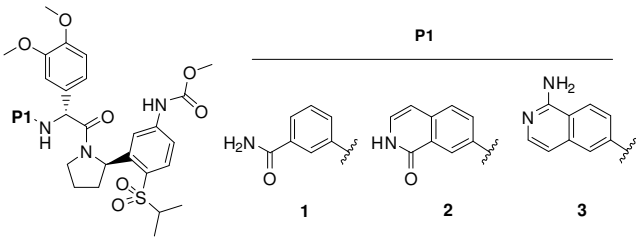


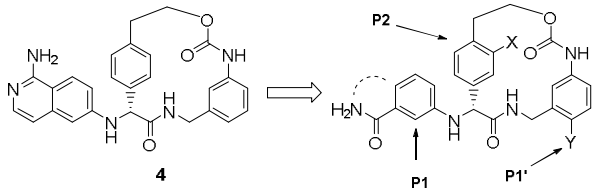
	1	2	3
FVIIa K_i, nM[a]	190	13	1.8
FVII def PT, μM[a]	8.6	7.3	3.5
PAMPA, nm/s, pH 7.4	24	87	2
tissue kallikrein K_i, nM	>10000	>10000	110

[a] FVIIa enzyme assays and the FVIIa-deficient prothrombin time assay were performed according to established protocols.[4]

Figure 1. Comparison of phenylpyrrolidine FVIIa inhibitors with different P1 groups

substituents (Figure 1).[11] Compound **1** with a *m*-aminobenzamide P1 group is moderately potent with good selectivity and served as a starting point for optimization. A FVIIa fragment screening effort lead to the identification of a series of bicyclic amide P1 binding groups, such as the isoquinolinone, which were incorporated into the phenylpyrrolidine scaffold to afford compounds such as **2**, which resulted in a significant increase in potency and permeability.[11] These neutral P1 groups have potential advantages over the more commonly reported basic P1 groups such as amidines which typically lack permeability since they exist as charged species at physiological pH. Additionally,

neutral P1 substituents often have improved selectivity because they lack the dominant salt bridge formed between cationic P1 groups, such as amidines, and Asp189, which is located at the base of the S1 pocket of many serine proteases. Weakly basic P1 groups, such as the aminoisoquinoline shown in compound **3**, are a significant advance,[12-15] but the discovery of orally active FXa inhibitors demonstrates the potential advantages of utilizing neutral groups that bind in the S1 pocket.[2] While the installation of neutral P1 groups in the phenylpyrrolidine chemotype demonstrated improved permeability and selectivity over the weakly basic aminoisoquinoline, the molecules still do not reach sufficient potency and clotting activity for the desired antithrombotic effect.

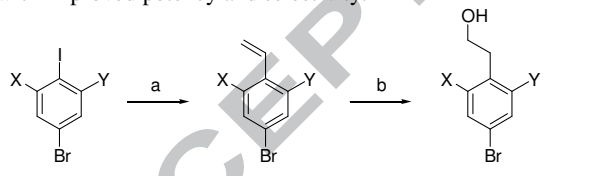


FVIIa K_i, nM[a]	140
FVII def PT, μM[a]	45.5
PAMPA, nm/s, pH 7.4	0
tissue kal K_i, nM	14

[a] FVIIa enzyme assays and the FVIIa-deficient prothrombin assay were performed according to established protocols.[4]

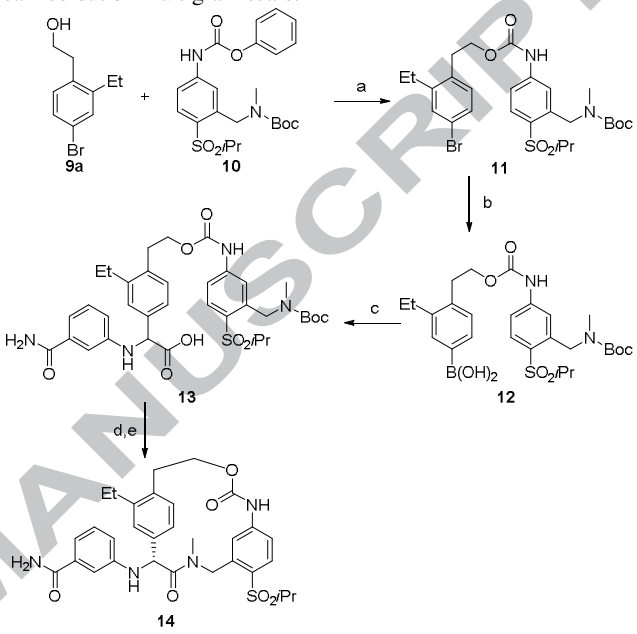
Figure 2. Introduction of the *m*-aminobenzamide P1 into the macrocyclic FVIIa chemotype.

Macrocyclic inhibitors of FVIIa[16-18] (e.g., compound **4**, Figure 2) provide an alternative to the phenylpyrrolidines as a way to restrict the bioactive conformation of phenylglycine-based inhibitors. The rigid macrocyclic scaffold efficiently displays P1 groups as well as provides access to key hydrophobic S2 and S1' binding pockets necessary for high affinity binding. Initially reported with the weakly basic aminoisoquinoline, the macrocycles are very potent compounds with little permeability and poor selectivity vs. tissue kallikrein. Incorporation of the neutral P1 groups into the macrocycle chemotype was undertaken to determine if the resulting FVIIa inhibitors would be permeable with improved potency and selectivity.



Scheme 1. Synthesis of substituted *p*-bromophenethylalcohols. (a) trimethyl(vinyl)silane (4 eq), KF (3 eq), *n*-Bu_4NCl (2 eq), $Pd(dba)_2$ (0.1 eq), toluene (0.5 M), molecular sieves, 170 °C, 45 min; (b) 9-BBN (10 eq), THF, 120 °C; NaOH, H_2O_2.

The macrocycle chemotype can be assembled from three key fragments containing the P1, P2 and P1' binding units. We set out to make a variety of substituents on the internal phenyl ethyl alcohol to access the hydrophobic P2 binding pocket. *p*-Bromophenylethanols, the key intermediates for this route, proved difficult to synthesize when symmetrically disubstituted. Using 1,3-substituted 5-bromo-2-iodobenzenes **7a-c** as starting materials, the aryliodide could be selectively exchanged in preference to the arylbromide to form the corresponding organolithium or Grignard species, but efforts to find a suitable electrophile that did not require a multistep homologation were unsuccessful. Palladium coupling with a zinc enolate occurred selectively at the less hindered bromide, providing an undesired product. After trying several vinylation protocols, we discovered that coupling trimethyl(vinyl)silane in the presence of KF, *n*-Bu_4NCl and $Pd(dba)_2$[19] was a robust, high yielding solution that worked for both electron rich and electron poor substituents (Scheme 1). After the Pd-mediated vinylation, the resulting stilbenes could be converted to the desired phenethyl alcohol by hydroborylation with 9-BBN. This sequence was routinely carried out on multigram scale.



Scheme 2. Example of synthetic route for macrocycles. (a) NaH (2.5 eq), THF, rt, 92%; (b) Bis(neopentyl glycolato)diboron, $PdCl_2$(dppf), KOAc, 80 °C, 4 h; prep HPLC (0.1% TFA, MeOH/water), 59%; (c) 3-aminobenzamide, glyoxylic acid, acetonitrile/DMF, μwave, 100 °C, 600 s, 86%; (d) HCl; (e) PyBOP, DMAP, DIEA, 26% over 2 steps.

The synthesis of macrocycle **14** is shown as an example of assembly of the key fragments (Scheme 2). *p*-Bromophenethanol **9a** was coupled with the phenyl carbamate **10** in the presence of sodium hydride in good yield. As previously reported,[16] bromide **11** was converted into boronic acid **12** via Suzuki-Miyaura conditions and the hydrolysis of the resulting boronic ester during purification by preparative HPLC. A mixture of the P1 group, in this case *m*-aminobenzamide, glyoxylic acid and the boronic acid was heated in the microwave to yield the desired phenylglycine via a Petasis reaction.[12,20] Deprotection of the Boc-group followed by PyBOP or BOP mediated cyclization using slow addition of the starting material to the coupling reagents over several hours provided the desired macrocycle. A mixture of atropisomers (2:1 inactive:active) was observed after cyclization. Atropisomers could be separated by preparative HPLC and the enantiomers could be separated by chiral HPLC either pre- or post- cyclization. When the atropisomers did not readily interconvert (e.g., compound **14**), the inactive isomer could be recycled by heating in DMSO at 100 °C for 2 days to again reach the thermal dynamic equilibrium (2:1 mixture) and repurified. Multiple inhibitor-FVIIa co-crystal structures have established that the R stereochemistry is the active enantiomer for phenylglycine inhibitors of FVIIa.[16-18]

Table 1. Benzamide macrocycle SAR.

Compd #	FVIIa K_i (nM)	FVIIa Def PT (μM)[a]
15	>10,000	
16	9200	
17	2100	
18	940	78
14 (atropiso 1)	23	2.9
19 (atropiso 2)	610	
20	91	42

[a] FVIIa enzyme assays and the FVIIa-deficient prothrombin assay were performed according to established protocols.[5]

Initial incorporation of the *m*-aminobenzamide into the macrocycle scaffold produced compound **5** with FVIIa K_i >10,000 nM, which was not surprising considering the 100-fold loss of potency observed in the phenylpyrrolidine series relative to an aminoisoquinoline P1 (Cf. Figure 1 and Table 1). Despite the poor activity of the initial neutral macrocycle 1**5**, we decided to build in functionality that had been shown to increase potency in related FVIIa inhibitors. Ethyl substitution at the P2 position produced compound **16** with FVIIa Ki = 9200 nM, which existed as a mixture of atropisomers that readily isomerized at room temperature after isolation. In order to avoid rotamers, we installed a symmetrical P2 group which locks one ethyl group into the bioactive conformation in compound **17**, resulting in a 4-fold increase in potency. Separately, an isopropyl sulfone that reaches the C42-C58 disulfide bridge in the S1' pocket was added to produce **18**, with comparable potency to the diethyl analogue **17**. Adding one ethyl group to this compound produced atropisomers **14** and **19**. Each isomer was isolated by preparatory HPLC and was stable at room temperature in both organic and aqueous solvents for days. Interestingly, the presence of the internal methyl group on the amide apparently locks the ethyl group on one side of the ring and prevents interconversion. Atropisomer **14** was 30-fold more potent than the less active rotomer **19**, which had similar potency to unsubstituted **18**. The crystal structure of compound **14** (Figure 3) shows the ethyl efficiently filling the S2 binding pocket of FVIIa, whereas in the less active conformer, the Et group must be projected into solution, where it does not participate in favorable binding interactions.[22] The crystal structure also shows the benzamide binding in the narrow P1 pocket and making key hydrogen bonds with Asp189 and Gly219, and the isopropyl sulfone contacting the C42-C58 disulfide bridge.

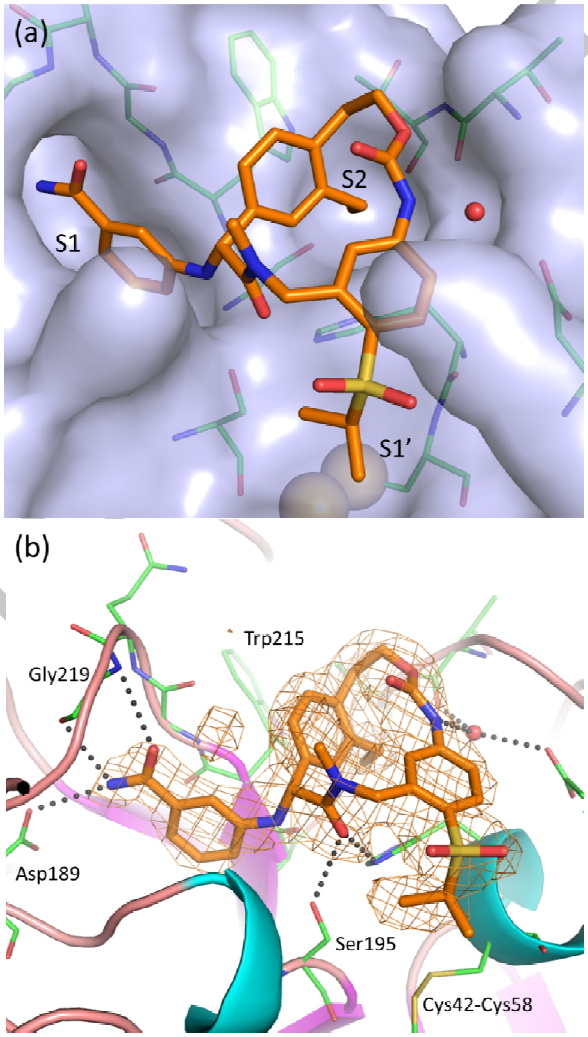
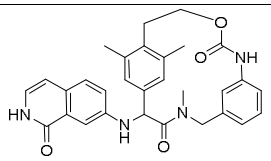
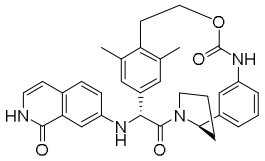
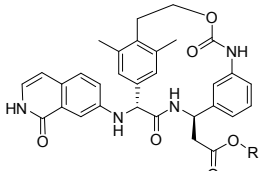
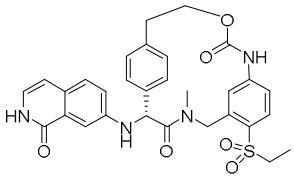
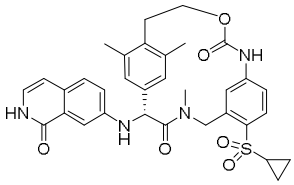


Figure 3. Crystal structure of macrocycle **14** bound to FVIIa at 2.30 Å resolution. The X-ray structure (5U6J) has been deposited in the PDB (www.pdb.org). (a) Crystal structure depicted with surface rendering of the FVIIa binding site. (b) Electron density for **14** is depicted with the omit Fc-Fo map at 3 rmsd. Graphics were generated with the software program PyMOL.[23]

Compound **14** demonstrated good potency and clotting activity, but had only moderate permeability (PAMPA = 68 nm/sec).[21] The sulfone was replaced with a sulfide to provide compound **20** with higher permeability (PAMPA = 145 nm/sec) albeit with slightly lower potency and the potential for bioconversion to the more active species *in vivo*. Oral dosing in rat of **20** showed 5.5% oral bioavailability but only traces of conversion into the sulfone. Although the sulfide had only moderate potency, the observed oral bioavailability shows a possible path forward toward potent, orally active macrocycles.

Table 2. Macrocycles with isoquinolinone P1 groups.

Compd #		FVIIa K_i (nM)	FVIIa Def PT (μM)[a]
21		1500	
22		840	
23		360 (R = Et)	
24		9.6 (R = H)	71
25		61	17
26		4.3	3.1

[a] FVIIa enzyme assays and the FVIIa-deficient prothrombin assay were performed according to established protocols.[5]

Benzamide **14** has promising activity, but more potency is likely necessary for the desired antithrombotic efficacy. A bicyclic series of more potent neutral P1 groups were discovered while we were optimizing the neutral benzamide macrocycles. The isoquinolinone P1 was chosen for incorporation into the macrocycle scaffold since it had a good balance of permeability and potency. In the phenylpyrrolidine chemotype, the analog containing the isoquinolinone P1 was 10-fold more potent with increased permeability relative to the corresponding benzamide.[11] We chose the dimethyl P2 group to avoid atropisomer issues and to mitigate the high protein binding of diethyl substitution (data not shown). Combining these substituents produced compound **21** with a FVIIa K_i of 1500 nM. In order to determine if the phenylpyrrolidine in combination with the macrocycle would further rigidify the bioactive confirmation, we synthesized compound **22**. Since it is equipotent, it likely adopts a similar conformation and presumably does not further stabilize the bioactive confirmation. Addition of an acid adjacent to the amide afforded compound **24,** which adds a handle for a prodrug, and provided an 8-fold increase in potency over compoound **21** to 9.6 nM but had weak clotting activity, indicative of high protein binding. Sulfone **25**, devoid of P2 substitution, had 15-fold higher binding potency than the corresponding benzamide. These compounds have ethyl and isopropyl sulfone P1' binding groups that have comparable potency, as previously reported.[16] Combining the symmetrical dimethyl P2 binding group with a cyclopropylsulfone P1' group provided compound **26** with excellent potency and clotting activity, but unfortunately the compound had no oral bioavailability in mouse despite one less hydrogen bond donor and moderate permeability. A comparison of compounds **14** and **26** (Table 3) shows an increase in potency with maintenance of good selectivity over other serine proteases.

Table 3. In vitro potency and selectivity of Compounds **14** and **26**. Serine protease enzyme assays and the FVIIa-deficient prothrombin assay were performed according to established protocols.[5]

Compound	**14**	**26**
FVIIa K_i, nM	23	4.2
FVIIa def PT, μM	2.9	3.1
PAMPA, nm/s, pH 5.5	68	100
Tissue Kallikrein K_i, nM	4700	11.0
Factor Xa, K_i, nM	5800	2800
Trypsin, FIXa, thrombin, plasmin, plasma kallikrein, TPA, Urokinas, aPC K_i, nM	All > 10,000	All > 10,000

Initial replacement of the aminoisoquinoline P1 substituent in a macrocyclic FVIIa inhibitor with the *m*-aminobenzamide produced weakly active compounds. Addition of optimal P2 and P1' binding substituents produced permeable compounds that inhibit FVIIa at low nanomolar concentrations with excellent selectivity over other serine proteases. The benzamide P1 series produced a moderately active compound with measurable oral availability when the P1' sulfone substituent was changed to the corresponding sulfide. Potency in the series was further enhanced with the isoquinolinone P1 in combination with the symmetrical dimethyl P2, to afford **26**, with excellent potency and clotting activity. Despite the increased permeability, we believe that active transport limits oral bioavailability. These results serve as useful leads in the discovery of potent, orally active FVIIa inhibitors.

Acknowledgments

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