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Targeting Thrombin and Factor VIIa: Design, Synthesis, and Inhibitory Activity of Functionally Relevant Indolizidinones

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Abstract—Guided by molecular modeling, docking experiments, and available X-ray crystal structure data on the serine protease Factor VIIa and thrombin, a series of indolizidinone derivatives was designed and synthesized having diverse functionality at the P_1, P_2, and P_3 sites.

Thrombin [Factor IIa (FIIa)] is the ultimate enzyme in the blood coagulation cascade, and is capable of cleaving fibrinogen to soluble fibrin, which in turn can polymerize to form a fibrin plug or to stabilize a clot.[1] Although *direct* inhibition of FIIa has been well studied, and has resulted in promising clinical drug candidates,[2] other enzymes in the serine protease family have become targets for intensive research in response to the medical need for new anticoagulants with improved safety profiles over existing antithrombotic therapies.[3] Attention has been drawn most recently to Factor VIIa (FVIIa)[4] because of its role as initiator of the cascade in complex with tissue factor (TF) upon blood vessel damage. Other important targets are FXa as the ultimate step in FIIa generation, FIXa and FXIa for their roles in maintaining the coagulation process after intiation.[4]

The first X-ray structure of a TF/FVIIa complex with D-Phe-Phe-Arg-chloromethyl ketone (PPACK II),[5] (Fig. 1), has been instrumental in the design and synthesis of prototypical inhibitors.[6] Generally, such inhibitors incorporate a basic P_1 guanidino, amidino, or amino group which interact with Asp 189 in the S_1 pocket. Irreversible inhibitors such as PPACK II, an electrophilic substrate that mimics the oxyanion hole, are susceptible to an attack by Ser 195.[7] Potency is gained by the incorporation of complimentary lipophilic residues at P_2 and P_3 in inhibitors.

While a major part of the synthetic effort has been directed at inhibitors of FIIa, little is known about molecules that may interact with other enzymes in the cascade such as FVIIa.[6] We recently reported the design, synthesis, and X-ray co-crystal structure with FIIa of a $IC_{50} = 20$ nM inhibitor, **2**.[8] The validity of molecular modeling prior to performing synthesis was evident when it was predicted that the presence of a benzyl group as in **2** rather than a methyl (**22**, FIIa $IC_{50} = 270$ nM, Table 1, entry 1) was beneficial for the P_3–S_3 interaction. Furthermore, a tertiary hydroxyl group at C-3, and the lactam carbonyl appeared to provide the requisite interaction with Gly 216.

Molecular modeling[9] of the indolizidinone motif in **2** with FVIIa indicated that **2** will bind in a similar conformation as observed in the X-ray of **2** and FIIa and that productive interactions could be had by further functionalization with specific groups in the P_2 region (Fig. 2, left panel). Comparison of the S_2 proximal pocket in FIIa and FVIIa indicates that whereas a small cavity exists in the latter, the same volume is partially occupied by Leu 99 in FIIa.[8] Also, the transition between S_2 and S_3 is clearer in FVIIa compared to FIIa, with a canyon that leads to S_4, having Thr 99 at the bottom and Thr 98 and Pro 170I on the sides. As seen in Figure 2 (yellow capped sticks), the P_2-Phe group of PPACK II partly occupies the small S_2 cavity in FVIIa. We argued that suitable substituents in the 7-position of the indolizidinone could fill this cavity and provide selectivity over FIIa. Modeling also indicated that a C-5 substituent should have an effect on the location of the

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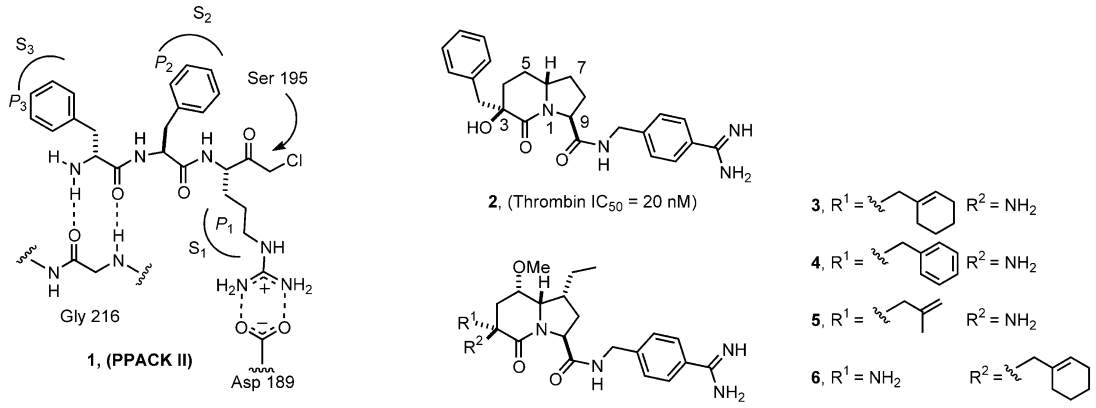
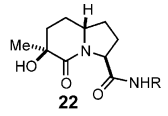
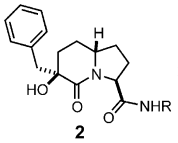
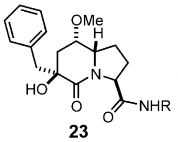
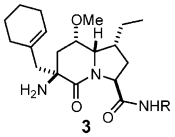
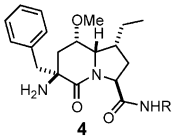
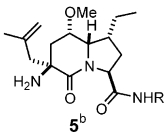
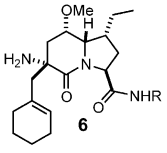
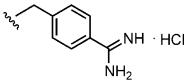


Figure 1.

Table 1. Inhibition of FIIa, FVIIa, Plasmin, and FXIa, measured as IC_{50} (μM) unless noted[b]

Entry	Compd[a]	Factor IIa	Factor VIIa	Plasmin	Factor XIa
1	 ref 10	0.27	>10	—	20% (Inhib. at 8 μM)
2	 ref 10	0.020	1.1	—	78% (Inhib. at 8 μM)
3	 ref 10	0.11	0.52	71	35
4		0.17	4.6	141	>133
5		0.21	0.42	11	>133
6		0.38	1.6	37	>133
7		7.2	109	—	—

[a]R = 

[b]Formate salt.

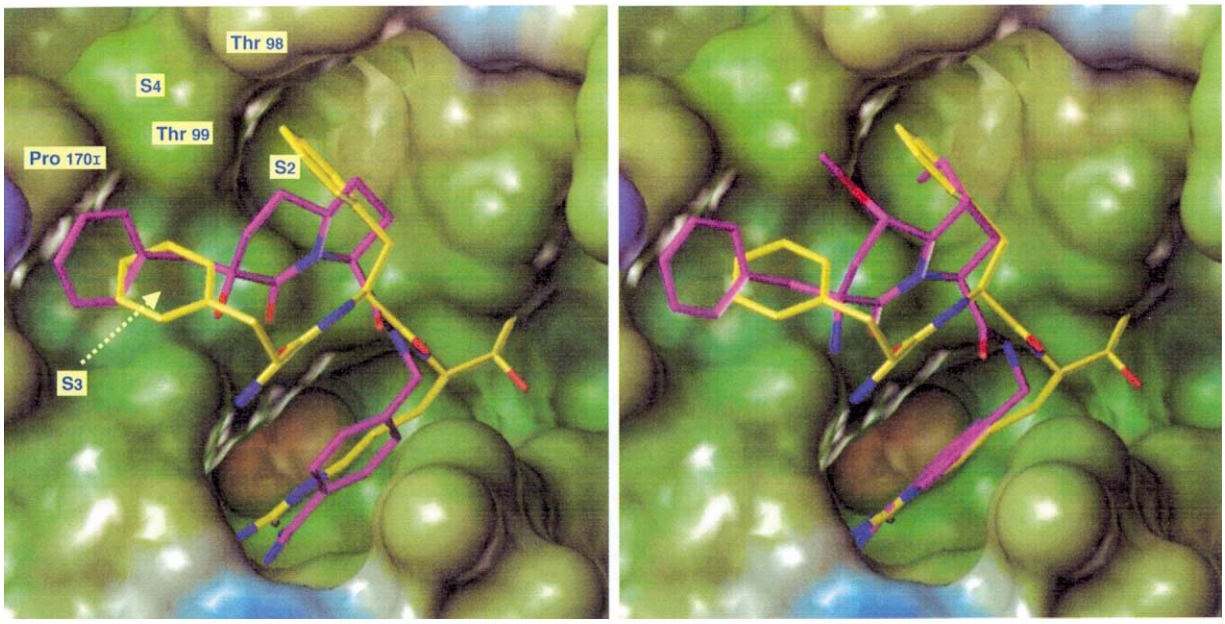
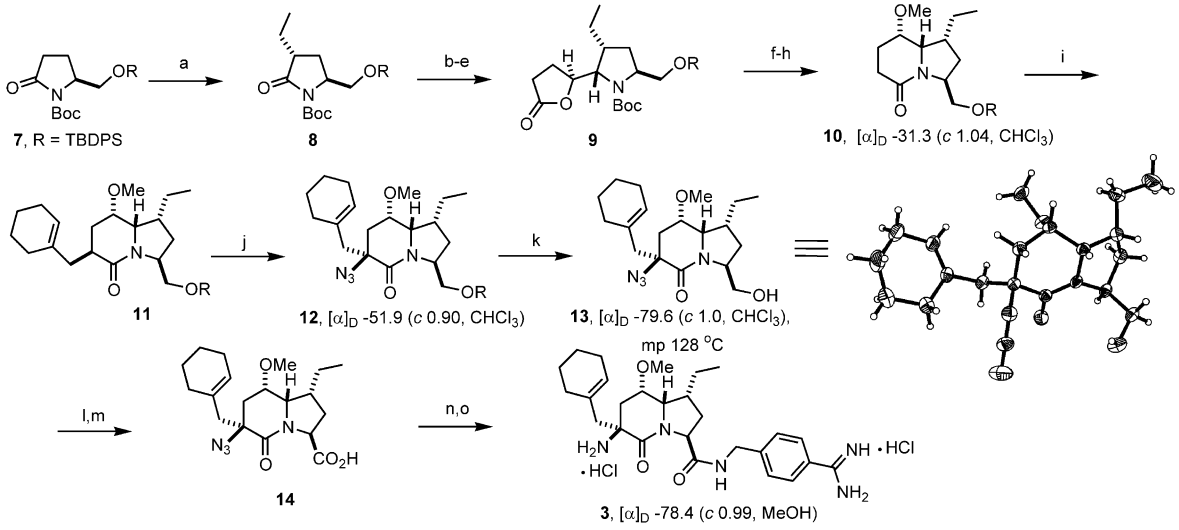


Figure 2. Left—Connolly surface map of the X-ray structure of Factor VIIa with PPACK II (yellow)[7] and docked inhibitor **2** (purple); right—proposed inhibitor **4** (purple) with PPACK II (yellow).

indolizidinone in its interaction with FIIa. On the other hand a C-(5*R*)-methoxy group nicely fits into the S_3–S_4 canyon of FVIIa without moving the indolizidinone ring. We thus chose to synthesize a series of 3-alkyl-(3*S*)-amino-(5*R*)-methoxy-(7*R*)-ethyl indolizidinones with variation in the 3-substituents (Fig. 1), to evaluate their inhibitory effect on FVIIa in comparison to FIIa, plasmin, and FXIa. Figure 2 (right panel) shows the superposition of the docked analogue **4** over the co-crystal structure of PPACK II with FVIIa.

The readily available L-pyroglutamic acid was transformed in four high yielding steps into **7**[10] which was stereoselectively ethylated with ethyl triflate to **8**. Iminium ion chemistry[11] in conjunction with 2-trimethylsilyloxy furan[8,12] afforded **9** as the major isomer (Scheme 1). Cleavage of the *N*-Boc group,[13] ring expansion from lactone to lactam and *O*-methylation gave **10**. We were now faced with the challenge of introducing *C*- and *N*-branching corresponding to the P_3 subsite with high stereocontrol. The Li lactam enolate prepared with *t*-BuLi was first alkylated with 1-bromomethyl cyclohexene to afford **11** as a major isomer. Reformation of a Li lactam enolate and treatment with trisyl azide[14] afforded the anticipated product **12**. Desilylation gave X-ray quality crystalline alcohol **13**, thus corroborating



Scheme 1. (a) LiHMDS, EtOTf, THF, −78 °C, 92%; (b) Dibal-H, toluene, −78 °C; (c) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 98% (two steps); (d) $BF_3.OEt_2$, 2-(trimethylsilyloxy)furan, CH_2Cl_2, −78 °C, 95%; (e) H_2 1 atm, 10% Pd/C, EtOAc, 99%; (f) *B*-bromocatecholborane, CH_2Cl_2; (g) toluene, reflux, 71% (two steps); (h) KH, MeI, THF, 80%; (i) *t*-BuLi, 1-bromomethyl-cyclohexene, THF, −78 °C, 72%; (j) *t*-BuLi, Trisyl-N_3, THF, −78 °C, 78%; (k) TBAF, THF, 80%; (l) Swern oxidation; (m) *t*-BuOH, $NaClO_2$, NaH_2PO_4, H_2O, 90% (two steps); (n) *N*-Cbz-protected 4-amino-methylbenzamidine, BOP reagent, *i*-Pr_2NEt, CH_2Cl_2, 50%; (o) H_2 1 atm, 10% Pd/C, MeOH, HCl 37% (1 drop), 85%.

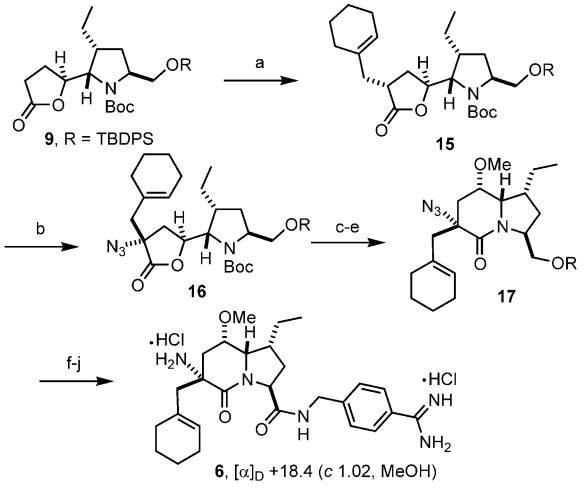
the expected stereochemistry resulting from enolate chemistry. Oxidation and further functional group manipulation led to **3** as the dihydrochloride.

We deemed it necessary to also prepare the epimeric amine **6** for comparison. In order to control the stereochemistry at the tertiary carbon atom, we capitalized on the steric bias offered by intermediate **9** (Scheme 2). Thus, sequential alkylation and azidation afforded **15** and **16**, respectively. Ring expansion to **17**, oxidation and functionalization as described in Scheme 1 afforded the epimeric amine dihydrochloride **6**.

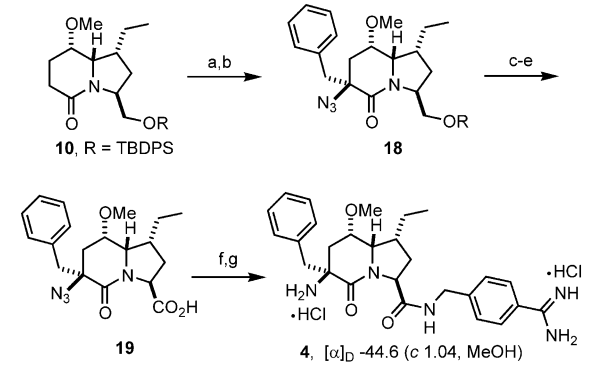
In order to compare the effect of a tertiary hydroxyl as in **2**, with the corresponding amine, we prepared analogue **4** as shown in Scheme 3, following the same protocol discussed above for **3**. The benzyl and azide groups were introduced with high stereoselectivity affording **18**, which was converted to the corresponding acid **19** and further functionalized to the intended **4**.

The synthesis of the 3-methylpropene analogue **5** presented certain operational challenges due to the presence of the double bond, thus excluding the use of catalytic reduction and strongly acidic conditions. The common bicyclic lactam intermediate **10** was converted to the 3-methylpropenyl azido analogue **20** via Li enolate chemistry as described above. Reduction of the azide group with propane 1,3-dithiol[15] followed by protection gave the corresponding *N*-Boc derivative **21** in excellent overall yield from **10**. Transformation to the carboxylic acid and amide formation took place uneventfully. However cleavage of the *N*-Boc group with TFA under normal conditions (0 °C or rt), caused migration of the *exo*-methylene group. Fortunately, cleavage without rearrangement took place with 98% formic acid to give the intended analogue **5** (Scheme 4).

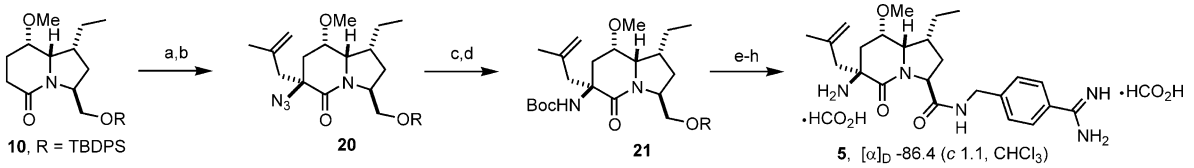
Table 1 lists the IC_{50} values of inhibition for FIIa, FVIIa, plasmin and FXIa. The 3-benzyl analogue **4** proved to be a better FVIIa inhibitor than the corresponding cyclohexenylmethyl (**3**) or 3-methyl propenyl (**5**) analogues (Table 1, entries 5, 4, and 6, respectively). The inverted stereochemistry at C-3 results, as expected in a loss of activity (Table 1, entries 4 and 7). It is of interest that the introduction of the C-5 methoxy group reduces the activity of FIIa while slightly improving the affinity for FVIIa (Table 1, compare entry 2 with entries 3 and 5). We believe this is due to a smooth fit of the C-5 methoxy in the canyon clearly present in FVIIa in contrast to FIIa. Eventually this preference for FVIIa compared to FIIa may be further improved by slightly larger hydrophobic groups. In contrast, the C-7 ethyl substituent exhibits a negligible effect on the selectivity for FVIIa versus FIIa (Table 1, entries 3 and 5). This may be due to a better hydrophobic interaction of the C-7 ethyl group in the S_2 pocket of FIIa than anticipated, which may in part compensate for the displacement of the indolizidinone in the docked model, compared to the $IC_{50} = 20$ nM FIIa inhibitor **2**.[8] Selectivity was achieved over plasmin and FXIa with this



Scheme 2. (a) LiHMDS, 1-bromomethyl-cyclohexene, THF, −78 °C, 74%; (b) *t*-BuLi, trisyl-N_3, THF, −78 °C, 72%; (c) *B*-bromocatecholborane, CH_2Cl_2; (d) toluene, reflux, 50% (two steps); (e) KH, THF, Me_2SO_4 94%; (f) HF.pyr, MeCN, 75%; (g) Dess–Martin, CH_2Cl_2; (h) *t*-BuOH, $NaClO_2$, NaH_2PO_4, H_2O, 70% (two steps); (i) *N*-Cbz-protected 4-aminomethylbenzamidine, EDC, HOBt, *i*-Pr_2NEt, DMF, 55%; (j) H_2, 40psi, 10% Pd/C, MeOH, HCl 37% (1 drop), quant.



Scheme 3. (a) *t*-BuLi, BnBr, THF, −78 °C, 78%; (b) *t*-BuLi, Trisyl-N_3, THF, −78 °C, 60%; (c) TBAF, THF, 76%; (d) TPAP, NMO, CH_2Cl_2; (e) *t*-BuOH, $NaClO_2$, NaH_2PO_4, H_2O, 60% (two steps); (f) *N*-Cbz-protected 4-aminomethylbenzamidine, EDC, HOBt, *i*-Pr_2NEt, DMF, 65%; (g) H_2, 1 atm, 10% Pd/C, MeOH, HCl 37% (1 drop),



Scheme 4. (a) *t*-BuLi, 3-bromo-2-methyl-propene, THF, −78 °C, 83%; (b) *t*-BuLi, Trisyl-N_3, THF, −78 °C, 72%; (c) $HS(CH_2)_3SH$, Et_3N, MeOH; (d) Boc_2O, Et_3N, CH_2Cl_2, 67% (two steps); (e) TBAF, THF, 98%; (f) PDC, wet DMF; (g) *N*-Boc-protected 4-aminomethylbenzamidine, EDC, HOBt, *i*-Pr_2NEt, DMF, 61% (two steps); (h) HCO_2H, quant.

class of compounds. Further optimization of the size and complimentary polarity, with respect to the S_2 pocket of FVIIa, and of the C-7 substituent could perhaps lead to the anticipated selectivity and for better inhibitory activity against FVIIa.

Acknowledgements

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