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Design and synthesis of macrocyclic indoles targeting blood coagulation cascade Factor XIa

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ABSTRACT

The synthesis of a series of novel macrocyclic compounds designed to target blood coagulation Factor XIa is described. The compounds were evaluated for their inhibition of a small set of serine proteases. Several compounds displayed modest activity and good selectivity for Factor XIa. Within the series, a promising lead structure for developing novel macrocyclic inhibitors of thrombin was identified.

Despite a widespread drug discovery effort, thromboembolic disease remains one of the major causes of morbidity and mortality in the world.[1] The primary strategy for its treatment and prevention is largely based on anticoagulant therapy. The only oral anticoagulant in clinical use is Warfarin, a highly efficacious vitamin K antagonist that regrettably suffers from response variation and a need for clinical monitoring.[2] Alternative treatments include various heparin analogs, and the direct thrombin inhibitor hirudin which besides the disadvantages of intravenous administration also displays problems with dosage and severe side-effects.[3] While extensive resources have been expended in the search for safer agents primarily for entities inhibiting one of the blood Factors IIa, VIIa and Xa,[4] inhibition of the blood Factor XIa has received much less attention.

Factor XIa is a trypsin-like serine protease involved in the intrinsic pathway of the blood coagulation cascade.[5] Its role as a major contributor in the amplification of the thrombotic response without affecting the initiation of clot formation has raised the hypothesis that specific inhibitors of Factor XIa may be beneficial for having an effective treatment of thrombosis without a significant risk of bleeding.[6]

Macrocyclic compounds are abundant in nature, many of which have high affinity for enzyme receptors.[7] Macrocyclic structures are often superior to their linear equivalents in terms of enhanced potency due to conformational preorganization that lowers the entropy of the molecular recognition event. In addition, the more rigid structures can potentially exhibit a better pharmacokinetic profile, such as increased metabolic stability and better membrane permeability.[7a,b]

Nature has also provided the aeruginosin chlorodysinosin A (**3** in Fig. 1),[8] as well as macrocyclic peptides like micropeptin 103 (**1**)[9a] and the cyclotheonamides **2**, a family of metabolites found in marine sponges of the genus *Theonella*, which exhibit nanomolar inhibition of Factor IIa.[9b,c] Known synthetic macrocyclic inhibitors include derivatives of the cyclotheonamides[10] and the highly potent pyrazinone **4** and proline-based **5**.[11]

Small molecule inhibitors of Factor XIa are scarcely found in the literature, and the ones that are reported sometimes suffer from weak potency and/or lack of selectivity vis-à-vis other serine proteases.[12] The known serine protease inhibitor **6**[13] was modeled into the active site of Factor XIa using FRED (OpenEye Scientific Software).[14]

The model revealed a ligand orientation having an extensive hydrogen bond network between the three amide motifs and the protein backbone (Ser214, Gly216 and Gly218), with the leucine and cyclohexyl glycine side-chains occupying S2 and S3, respectively (Fig. 2). A relatively short distance between the methylene adjacent to the sulphonamide, and the benzamidine moiety could be observed, thereby allowing the incorporation of a bridge. In light of this observation, we hypothesized that the introduction of a four-carbon bridge would lead to a macrocycle that incorporates the structural features of **6**, thereby creating a new class of potentially selective Factor XIa inhibitors. To the best of our knowledge, no macrocyclic inhibitors of Factor XIa are known to date. To further enhance the selectivity and the DMPK properties it was decided to exchange the benzamidine to a 5-chloroindole (Fig. 3),

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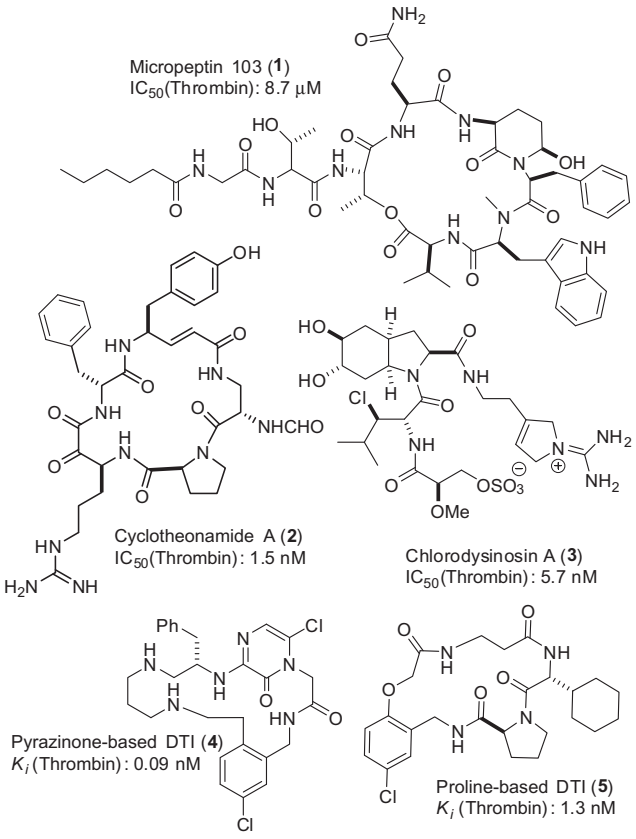


Figure 1. Natural macrocyclic, linear peptidic, and non-natural macrocyclic thrombin inhibitors.

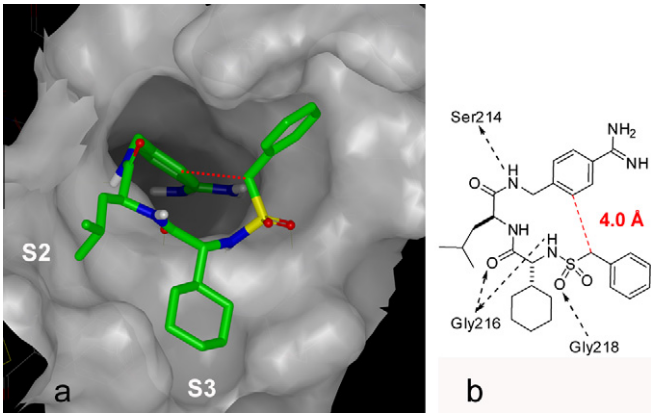


Figure 2. (a) Model of compound **6** in complex with Factor XIa. (b) Compound **6**. The hydrogen bond network to the Factor XIa backbone is shown (dotted arrows).

since Factor XIa is known to tolerate neutral P1 motifs.[12f] A small library was designed to also investigate the role and importance of the sulphonamide and the aryl moiety protruding towards a cavity formed by Tyr143, Leu146, Arg147, and Lys192. It was anticipated that a viable route to the desired structures could involve an amide coupling between building blocks **8–10** and the indole **11**, and subsequent macrocyclization by ring-closing metathesis.

Readily available *N*-tosyl 5-chloroindole[15] was halogenated and transformed to **14** via metalation, trans-metalation and allylation of the resulting 2-iodo-5-chloroindole.[16] Sequential formylation and reduction furnished **15** in excellent yields. In order to provide a traceless and straightforward deprotection of the final product,

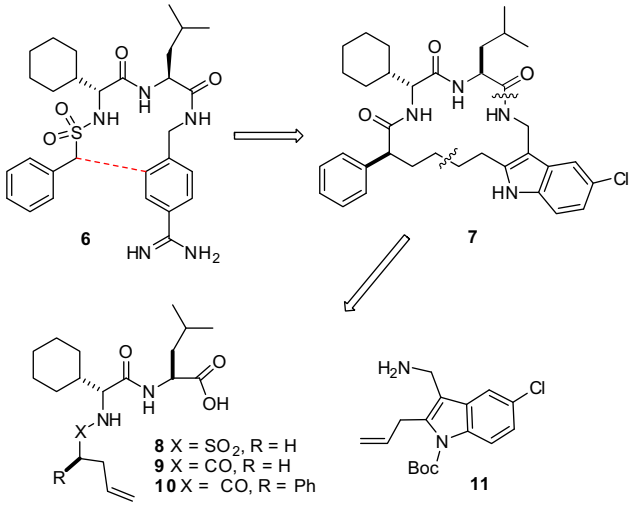
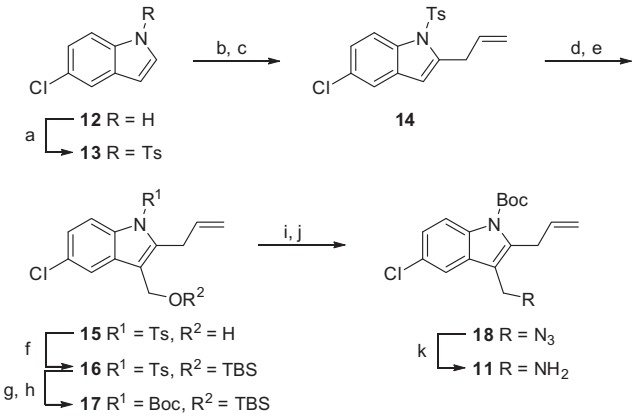


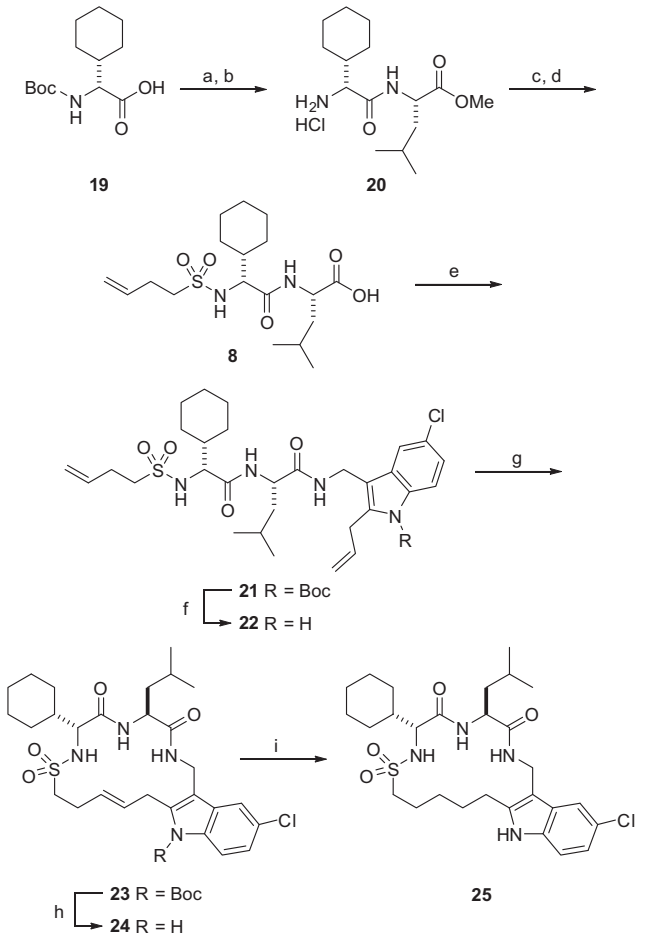
Figure 3. Retrosynthetic analysis.

we deemed it necessary to alter the *N*-Ts protection. Thus, elaboration of protecting groups as described in Scheme 1 followed by azidation using diphenyl phosphoryl azide and DBU according to Thompson's procedure[17] afforded the azide **18** in 70% yield over five-steps. A Staudinger reaction completed the synthesis of the indole building block **11**. The resulting free amine was highly unstable in neat conditions and was used as a crude compound in the following coupling reactions.

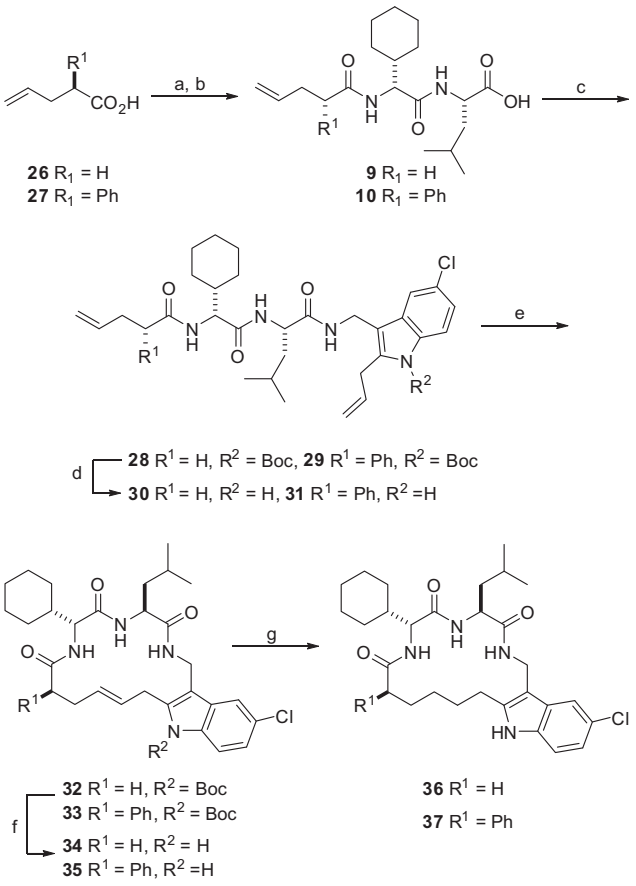
N-Boc-D-cyclohexylglycine (**19**) was converted to the dipeptide **20**, the amine was sulfonylated with but-3-en-1-sulfonyl chloride[18] and the ester hydrolyzed to afford **8** (Scheme 2). The initial couplings between the indole **11** and the dipeptide **8** showed a susceptibility to epimerization of the leucine alpha carbon when using benzotriazole-based coupling reagents (HOBt, HOAt, HATU) in combination with carbodiimidies (DIC, EDC). This could fortunately be diminished by using Goodman's phosponium based benzotriazinone reagent, DEPBT,[19] which gave **21** as a single diastereomer in good yield. Ring-closing metathesis was carried out using Grubbs' second generation catalyst and proceeded smoothly upon heating in dimethoxyethane to give macrocycle **23** in a 9:1 *E*/*Z* isomeric ratio.



Scheme 1. Synthesis of indole building block. Reagents and conditons: (a) NaOH, TsCl, *n*-Bu_4NHSO_4, CH_2Cl_2, quant.; (b) *t*-BuLi, Et_2O, −78 °C, then I_2, 79%; (c) *i*-PrMgBr, CuCN–2LiCl (cat.), THF, −30 °C, then allylBr, 90%; (d) $TiCl_4$, Cl_2CHOCH_3, CH_2Cl_2, −78 °C, 98%; (e) $NaBH_4$, EtOH/CH_2Cl_2, quant.; (f) TBS–OTf, 2,6-lutidine, CH_2Cl_2, 97%; (g) Mg, MeOH, 0 °C; (h) $(Boc)_2O$, DMAP, CH_2Cl_2, 83% (two-steps); (i) TBAF, THF, 96%; (j) DBU, DPPA, THF, 91%; (k) PPh_3, H_2O, THF, quant.



Scheme 2. Synthesis of macrocyclic sulfones. Reagents and conditons: (a) H_2N–Leu–OMe, HOAt, EDC, *i*-Pr_2NEt, CH_2Cl_2, 74%; (b) 30% v/v TFA/CH_2Cl_2, quant.; (c) but-3-en-1-sulfonyl chloride, Et_3N, DMAP, CH_2Cl_2, 68%; (d) 0.1 M LiOH aq, THF, quant.; (e) compound **11**, DEPBT, *i*-Pr_2NEt, THF, 78%; (f) *t*-$BuNH_2$, MeOH, microwave 130 °C, 90%; (g) Grubbs 2nd gen cat., DME, reflux, 90%; (h) *t*-$BuNH_2$, MeOH, microwave 130 °C, 69%; (i) H_2 1 atm, 3% PtO_2, MeOH, quant.



Scheme 3. Synthesis of amide analogs. Reagents and conditons: (a) Compound **20**, DEPBT, *i*-Pr_2NEt, THF; (b) 0.1 M LiOH aq, THF; (c) compound **11**, DEPBT, *i*-Pr_2NEt, THF; (d) *t*-$BuNH_2$, MeOH, microwave 130 °C; (e) Grubbs 2nd gen cat., DME, reflux; (f) *t*-$BuNH_2$, MeOH, microwave 130 °C; (g) H_2 1 atm, 3% PtO_2, MeOH, quant.

Unexpectedly, attempted removal of the Boc-protection using acidic conditions resulted in decomposition. Various aqueous bases and neutral conditions known for indole carbamate deprotection also gave unsatisfactory results. Gratifyingly, Boc-cleavage took place using *t*-butyl amine in MeOH[20] heated to 130 °C under microwave conditions to give the free indole macrocycle **24**. Using palladium catalysts for reduction of the double bond resulted in extensive dehalogenation of the indole moiety. In contrast, catalytic PtO_2 furnished the final saturated macrocycle **25** quantitatively.

The synthesis of the two macrocyclic amide analogs was accomplished essentially as described for **25**, utilizing the commercially available 4-pentenoic acid **26** and (*S*)-2-phenyl-4-pentenoic acid **27**,[21] respectively as coupling partners (Scheme 3).

Coupling of **26** and **27** mediated by DEPBT, afforded **9** and **10**, respectively. Further coupling with 5-chloroindole **11**, led to bis-alkenes **28** and **29**. Finally, ring-closing metathesis (giving predominantly the *E* isomer, 95:5–99:1), deprotection and hydrogenation gave macrocyclic bis-amides **36** and **37**.

The linear and macrocyclic compounds **22**, (*E*)-**24**, **25**, **30**, **31**, (*E*)-**35**, **36**, and **37**, were tested for their inhibitory activity on a small panel of serine proteases including FXIa, FIXa, and FIIa (Table 1).[22]

As anticipated, the presence of a 5-chloroindole as a P1 motif was tolerated by FXIa, with the exception of the saturated macrocycles **36** and **37**, giving K_{ic} values between 50 and 380 μM. Activity against thrombin was slightly better and limited to compounds showing low or no activity against FXIa, such as the sulphonamide **24** and the amide **36** (K_{ic} = 10.6 and 1.7 μM, respectively). The selectivity against activated protein C (APC, a down regulator of the coagulation cascade) and trypsin was also investigated. Interestingly, the sulphonamide series (**22**, (*E*)-**24**, and **25**) showed no activity against APC. The acyclic amide **30** displayed modest to low activity against Factor XIa, but with a four-fold selectivity against APC. Unfortunately, trypsin activity (K_{ic} between 20 nM and 1.2 μM) was observed for these compounds.

Although the macrocyclic compounds were designed to target Factor XIa, promising results for thrombin inhibition were observed. Compound **36** had a low micromolar activity against thrombin with a 300-fold selectivity against APC. A docking study[23] of **36** revealed a high similarity as compared to the crystal structure of the proline-based macrocycle **5** in thrombin prepared by Nantermet et al.[11] (Fig. 4).The excellent selectivity of **5** against thrombin versus trypsin (K_i 1.3 nM and 21.5 μM, respectively) was attributed in part, to the presence of heteroatoms in the tether between the P1 motif and cyclohexyl glycine residue.[11b]

In conclusion, we have described the design and synthesis of a series of macrocyclic compounds intended to display activity against Factor XIa. This goal was only partially achieved with analog **25** (Table 1). On the other hand, macrocyclic chloroindole **36** displayed a promising activity against thrombin and good selectivity against APC. The lack of selectivity versus trypsin can be addressed by an introduction of heteroatoms in the alkane bridge as for **5**. Both compounds represent good starting points for further

Table 1
Inhibition of FXIa, FIXa, FIIa, APC, and trypsin measured as IC_{50} (μM) and K_{ic} (μM) for compounds **22**, (*E*)-**24**, **25**, **30**, **31**, (*E*)-**35**, **36**, and **37**[a]

Entry	FXIa IC_{50}[b]	FXIa K_{ic}[c]	FIIa IC_{50}[b]	FIIa K_{ic}[c]	APC IC_{50}[b]	APC K_{ic}[c]	Trypsin human IC_{50}[b]	Trypsin human K_{ic}[c]
22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.16	0.142
24	767.8	383.9	67.9	10.6	n.d.	n.d.	2.69	0.328
25	175.9	87.9	n.d.	n.d.	n.d.	n.d.	2.43	0.296
30	103.1	51.6	n.d.	n.d.	284.1	227.3	2.17	0.264
31	145.9	73.0	n.d.	n.d.	103.5	82.8	0.16	0.020
35	135.8	67.9	n.d.	n.d.	144.5	115.6	0.26	0.031
36	n.d.	n.d.	10.6	1.7	395.3	316.2	10.0	1.220
37	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.31	0.281

[a] Compounds were tested in a concentration range from 300 μM to 0.015 μM. IC_{50} and K_{ic} values are reported as the average of three, four experiments ± SD.
[b] IC_{50} values were determined by non-linear fit (using Sigma Plot 8) to all data from the concentration curve.
[c] For better comparisons between the different proteases, the IC_{50} values were converted into K_{ic} using the equation: $K_{ic} = IC_{50}/(1 + [S]/K_m)$ with the respective substrate concentration and K_m values for each protease and assay. (n.d. = not determined, no inhibition detected at 300 μM).

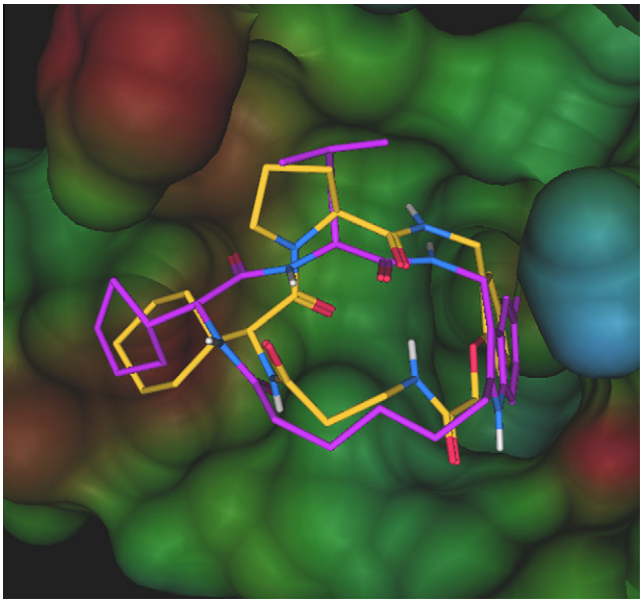


Figure 4. Model of **36** (magenta) docked in to the co-crystal structure[11b] of thrombin and **5** (yellow).

refinements towards macrocyclic compounds with higher activity and selectivity.

Acknowledgments

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22. Compound (*E*)-**34** could not be tested due to insolubility in the conditions required. No inhibition of FIXa could be detected at 300 μM.
23. A conformational ensemble generated in MOE (Molecular Operating Environment, CCG, Montréal, Canada) using a stochastic conformational search (default settings except 60 kJ energy cutoff, 0.9 RMS tolerance, and amide bond rotation allowed), was docked in the active site of thrombin (PDB code: 1NT1) using the rigid docking program FRED (OpenEye Scientific Software, New Mexico, USA).