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Clavatadine A, A Natural Product with Selective Recognition and Irreversible Inhibition of Factor XIa[†]

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Bioassay-guided fractionation of a CH_2Cl_2/MeOH extract of the sponge *Suberea clavata* using the serine protease factor XIa to detect antithrombotic activity led to the isolation of the new marine natural products, clavatadines A and B. Clavatadines A and B inhibited factor XIa with IC_{50}'s of 1.3 and 27 μM, respectively. A crystal structure of protein−inhibitor (clavatadine A) complex was obtained and revealed interesting selective binding and irreversible inhibition of factor XIa. The cocrystal structure provides guidance for the design and synthesis of future factor XIa inhibitors as antithrombotic agents.

Introduction

The blood coagulation pathway is implicated in a number of thrombotic disorders such as myocardial infarction, angina, pulmonary embolism, and cerebrovascular events.[1] Antithrombotics are also useful for postoperative thromboembolism prevention such as orthopedic and abdominal surgeries. The traditional antithrombotic therapy is by intravenous administration of heparin followed by oral treatment with warfarin.[1] Both of these drugs are indirect and nonspecific inhibitors of coagulation serine proteases and require very careful and costly monitoring to ensure safe therapeutic drug levels for the duration of treatment because of the high risk of bleeding. Clearly, there is a medical need for new antithrombotic agents to replace heparin and warfarin. For a long time now, the pharmaceutical industry has focused on the development of new potent and selective anticoagulants to target specific enzymes in the coagulation pathway. Thrombin (factor IIa) and factor Xa (FXa)[a] have received most attention; however, although these new thrombin and FXa inhibitors have advantages over heparin therapy, they still have bleeding problems and no significant improvement in efficacy-to-safety index compared to heparin and warfarin.[1] Factor XIa (FXIa) is a trypsin-like serine protease that plays a major role in the amplification phase of the coagulation cascade and in maintaining clot integrity. It is a unique target where specific inhibitors of FXIa might inhibit thrombosis without completely interrupting the function of normal hemostasis and, thus, might prevent or minimize the risk of hemostasis complication.

Previously, we have reported dysinosins A−D as inhibitors of the serine proteases factor VIIa (FVIIa) and thrombin.[2,3] In our continuing search to find novel lead compounds for development into antithrombotic agents, 38252 biota extracts were screened against FXIa. An extract of the marine sponge *Suberea clavata* Pulitzer−Finali, 1982 (Aplysinellidae) was investigated further because it showed inhibition of FXIa with an approximate IC_{50} of 0.4 μg/μL. Bioassay-guided fractionation of the active extract led to the isolation of two new bioactive bromophenol alkaloids, named clavatadines A (**1**) and B (**2**). This is the first reported chemical investigation of *S. clavata*.
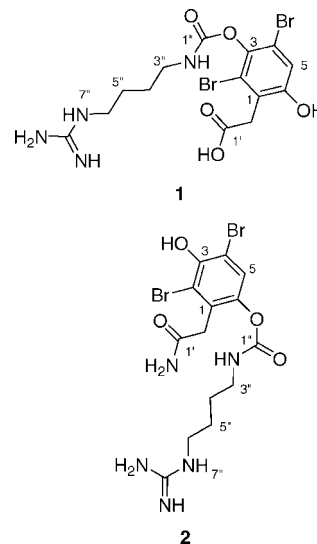
Results and Discussion

Compound **1** was obtained as an optically inactive amorphous solid. The (+)-LRESIMS of **1** displayed three pseudomolecular ion peaks at *m/z* 481, 483, and 485 [M + H]$^+$, which indicated the molecule contained two bromines. The molecular formula of **1** was determined to be $C_{14}H_{18}Br_2N_4O_5$ by (+)-HRESIMS of the [M + H]$^+$ ion at *m/z* 480.9718 (δ 0.2 ppm). Absorptions

Table 1. ^{1}H (600 MHz), ^{13}C (125 MHz), gCOSY, and gHMBC NMR Data for Clavatadine A (**1**) in DMSO-d_6

position	δ_C	δ_H (mult, J Hz)	COSY (H no.)	${}^{2,3}J_{CH}$ HMBC (C no.)
1	123.2 qC			
2	121.8, qC			
3	138.0 qC			
4	115.6 qC			
5	117.1 CH	7.08 (s)		1, 3, 4, 6
6	154.1 qC			
6-OH		10.42 (brs)		
1′	170.7 qC			
2′	35.4 CH$_2$	3.66 (s, 2H)		1′, 2, 6
COOH		12.38 (brs)		
1″	152.6 qC			
2″ (N)		7.99 (t, 6.0)	3″	3″
3″	40.0 CH$_2$^{a}	3.08 (dt, 6.0, 6.0, 2H)	2″, 4″	1″
4″	26.4 CH$_2$	1.49 (m, 2H)	3″	3″, 5″
5″	25.7 CH$_2$	1.52 (m, 2H)	6″	3″, 4″
6″	40.4 CH$_2$	3.11 (dt, 6.0, 6.0 2H)	5″, 7″	8″
7″ (N)		7.45 (t, 6.0)	6″	6″
8″	156.6 qC			

^{a} Chemical shift obtained from 2D NMR data as signal obscured by DMSO peak.

Table 2. ^{1}H (600 MHz), ^{13}C (125 MHz), gCOSY, and gHMBC NMR data for Clavatadine B (**2**) in DMSO-d_6

position	δ_C	δ_H (mult, J Hz)	COSY (H no.)	${}^{2,3}J_{CH}$ HMBC (C no.)
1	129.9 qC^a			
2	116.0 qC			
3	148.2 qC^a			
4	109.2 qC			
5	125.5 CH	7.33 (s)		1, 3, 4, 6
6	143.1 qC			
3-OH		9.72 (s)		2, 3, 4
1′	169.5 qC			
2′	37.0 CH$_2$	3.48 (s, 2H)		1′, 1, 2, 6
NH$_2$		7.26 (brs)a	NH$_2$b	
		6.92 (brs)b	NH$_2$a	2′
1″	154.1 qC^a			
2″ (N)		7.88 (t, 6.0)	3″	3″
3″	40.0 CH$_2$^{b}	3.06 (dt, 6.0, 6.0 2H)	2″, 4″	1″
4″	26.3 CH$_2$	1.48 (m, 2H)	3″	5″
5″	25.8 CH$_2$	1.49 (m, 2H)	6″	4″
6″	40.4 CH$_2$	3.11 (dt, 6.0, 6.0 2H)	5″, 7″	8″
7″ (N)		7.43 (t, 6.0)	6″	
8″	156.6 qC			

^{a} Chemical shifts determined from 2D NMR experiments. ^{b} Chemical shift obtained from 2D NMR data as signal obscured by DMSO peak.

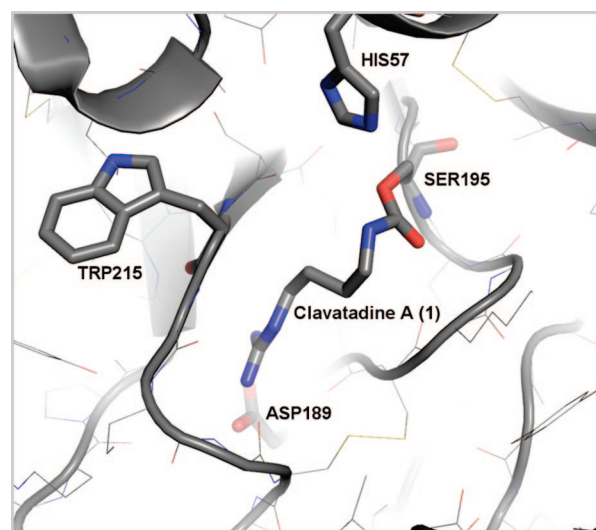**Figure 1.** Ligand−FXIa complex.

at 209 and 293 nm in the UV spectrum suggested that **1** contained a 1,4-oxygen-substituted benzene ring.[4] Analysis of the ^{1}H NMR and gCOSY NMR spectra (Table 1) for **1** indicated that the molecule contained a pentasubstituted aromatic ring (δ_H 7.08), an isolated methylene (δ_H 3.66), a NH(CH$_2$)$_4$NH spin system, and a phenolic hydroxyl (δ_H 10.42). Analysis of the ^{13}C and DEPT NMR spectra (Table 1) indicated that the molecule contained eight downfield quaternary carbons, one downfield methine carbon, and five upfield methylene carbons. A gHSQC spectrum allowed all of the protonated carbons to be assigned. The correlations from a gHMBC spectrum (Table 1) allowed the structure to be assigned. The isolated methylene singlet δ_H 3.66 showed correlations to a carboxyl carbon C-1′ (δ_C 170.7), an oxygen bearing quaternary aromatic carbon C-6 (δ_C 154.1), and one other quaternary aromatic carbon C-2 (δ_C 121.8). This indicated a phenylacetic acid moiety. It was deduced that the chain of four methylenes had a guanidine group at one end due to the correlation between the methylene δ_H 3.11 and the quaternary carbon δ_C 156.6.[5] The other end of the chain of four methylenes contained a carbamate group. This was inferred by the correlation between the methylene at δ_H 3.08 and the carbamate carbon at δ_C 152.6. An aromatic ring with this carbamate side chain, an acetic acid moiety, two bromines, and a hydroxyl (δ_H 10.42) accounted for the molecular formula determined by HRESIMS. The substitution pattern of the aromatic ring was revealed by the HMBC correlations from the isolated methylene, discussed above, and the aromatic methine. Thus, the singlet δ_H 7.08 showed correlations to two oxygenated aromatic carbons C-3 (δ_C 138.0) and C-6 (δ_C 154.1) and two other quaternary aromatic carbons C-1 (δ_C 123.2) and C-4 (δ_C 115.6). The carbamate side-chain was attached at C-3 (δ_C 138.0) as a ^{13}C NMR chemical shift further downfield would have been expected if it was the hydroxyl.[6,7] Thus, clavatadine A was assigned structure **1**.

The (+)-HRESIMS of compound **2** established the molecular formula C$_{14}$H$_{19}$Br$_2$N$_5$O$_4$ (m/z 479.9880, (M + H)${}^+$, δ 0.6 ppm). The ^{1}H NMR spectra of **1** (Table 1) and **2** (Table 2) were very similar except for two additional mutually coupled signals at δ_H 7.26 (brs) and 6.92 (brs) in the spectrum of **2**. This data, together with the loss of one mass unit in **2** compared with **1**, indicated that we had an acetamide moiety in **2** compared with an acetic acid moiety in **1**. The other aromatic substituents in **1** and **2** were clearly the same. The HMBC correlations (Table

2) from the hydroxyl (δ_H 9.72), isolated methylene (δ_H 3.48), and aromatic methine (δ_H 7.33) revealed the substitution pattern on the aromatic ring of **2**. The substitution patterns of **1** and **2** were similar except for the reversal in positions of the hydroxyl and the carbamate side chain. Clavatadine B therefore had structure **2**.

Compounds **1** and **2** inhibited FXIa with IC$_{50}$'s of 1.3 and 27 μM, respectively, and did not inhibit FIXa (<5% at 222 μM). Compound **1** was soaked into a single crystal of FXIa and a crystal structure obtained (Figure 1). The crystal structure showed only the carbamate side chain of **1** covalently bonded to the active site serine residue (Ser 195). Figure 2 shows detailed hydrogen bonding between the ligand and FXIa protein. The guanidine binds to Asp 189 (bidentate H-bonds) and Gly 218 (H-bond to the amide O). The carbamate O was H-bonded to Gly 193 (amide N), while the carbamate N was close to a water molecule.

Molecular docking gave Figure 3. It is most likely that the free carboxylate from **1** was involved in the selective recognition because its position can be in close proximity to the side chain of either Arg 37D or Lys 192 when overlaying the carbamate side chain in the bound conformation. Thus, the dibromophenol
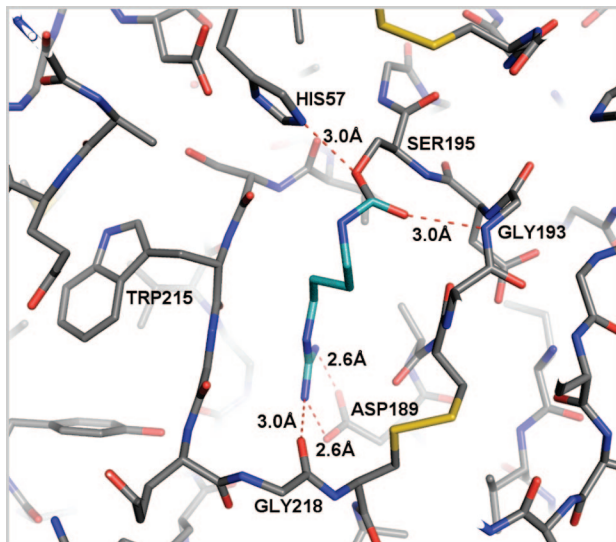
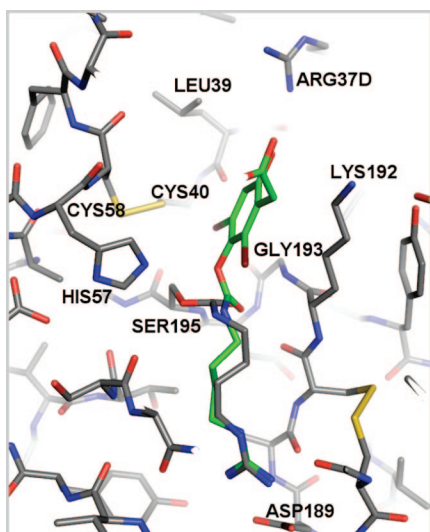**Figure 2.** Hydrogen bonding pattern of ligand—FXIa complex.

Figure 3. Docking of Clavatadine A in FXIa.

ring would occupy a pocket defined by Arg 37D, Lys 192, and Gly 193 on one side and Leu 39, Cys 40—Cys 58, and His 57 on the other side. Overall, **1** can approach/bind in the S1—S1′ pocket of FXIa by favorable interactions with Asp 189 from its guanidine group on one end and the free carboxylate to either Arg 37D or Lys 192 on the other end. This would result in a close contact between the side chain of Ser 195 and the carbamate group of **1**, which eventually led to the covalent bond between **1** and FXIa. Compound **2** was more than an order of magnitude less potent than compound **1**, presumably due to weaker interactions between the amide in **2** and either Arg 37D or Lys 192, compared to the carboxylate in **1** and Arg 37D or Lys 192.

Compound **1** has specific recognition for FXIa and is cleaved by a nucleophilic serine at the carbamate bond (Figure 4). Replacement of the carbamate by a noncleavable bond may lead to reversible noncovalent inhibitors.

Experimental Section

General. Water was Millipore Milli-Q PF filtered, while all other solvents used were Laboratory-Scan HPLC grade. Trifluoroacetic acid (TFA) was Fluka spectroscopic grade. A Betasil C_{18} 5μm (21.2 mm × 150 mm i.d.) was used for semipreparative HPLC. A Waters 600 pump fitted with a 996 photodiode array detector and 717 Plus Autosampler was used for the semipreparative separations. C_{18} was Sepra C_{18} end-capped silica (04K-4348). NMR spectra were recorded at 30 °C on Varian Inova 500 and 600 MHz NMR spectrometers. Samples were dissolved in DMSO-d_6 (residual ^{1}H δ 2.50 and ^{13}C δ 39.5 ppm). Multiplicity determined by DEPT (s = C, d = CH, t = CH$_2$, q = CH$_3$). Standard parameters were used for the 2D experiments, which included gradient COSY, HSQC ($^1J_{CH}$ = 140 Hz), and HMBC ($^nJ_{CH}$ = 8.3 Hz). FTIR and UV spectra were recorded on a Bruker Tensor 27 FTIR spectropho-tometer and a Camspec M501 UV/vis spectrophotometer, respec-tively. HRESIMS were measured on a Bruker Daltonics Apex III 4.7e Fourier transform mass spectrometer, fitted with an Apollo API source. Assay materials: TrisHCl, NaCl, CaCl$_2$, bovine serum albumin (BSA), and ethylene glycol were purchased from Sigma. Factor IXa and factor XIa were purchased from Haematologic Technologies Inc. (Vermont). Perfachrome FIXa was purchased from Pentapharm (Switzerland), and substrate S2366 from Chro-mogenix (Italy). Clear 384-well polystyrene microtiter plates were purchased from Falcon. A Multiskan Ascent (Thermo) and a Powerwave (Biotek) were used as spectrophotmetric readers.

Animal Material. The sponge sample *Suberea clavata* (phylum Porifera, class Demospongiae, order Verongida, family Aplysinel-lidae) was collected on 8 February 2001 by scuba diving at a depth of 17 m at Swain Reefs, Great Barrier Reef, Queensland, Australia. A voucher sample, QMG317611, was lodged at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. The sponge was freeze-dried and ground (16.4 g) then extracted with CH$_2$Cl$_2$ followed by MeOH. The combined CH$_2$Cl$_2$/MeOH extract (2.88 g) was further purified. A portion of this extract (1.44 g) was preadsorbed on C$_{18}$ and loaded into a refillable preparative guard column (30 mm × 10 mm i.d.), in line with a semipreparative C$_{18}$ HPLC column. The following solvent conditions were used: a linear gradient from H$_2$O/1% TFA to MeOH/1% TFA in 35 min, then isocratic conditions for 25 min (flow rate 9 mL/min), 60 fractions were collected from time = 0 min. Bioactive fractions 15—24 were combined (223 mg) and further purified. This fraction was again preadsorbed on to C$_{18}$ and loaded into a refillable preparative guard column (30 mm × 10 mm i.d.), in line with a semipreparative C$_{18}$ HPLC column. The following solvent conditions were used: a linear gradient from H$_2$O/1% TFA to H$_2$O/1% TFA:MeOH/1% TFA (4:1) in 5 min, then to H$_2$O/1% TFA:MeOH/1% TFA (7:13) in 45 min, then finally to MeOH/1% TFA in 10 min (flow rate 10 mL/min), 60 fractions were collected from time = 0 min. Bioactive fractions 25—26 (4.5 mg) and 27—30 (12.0 mg) were purified further by C$_{18}$ HPLC. Fractions 25—26 (4.5 mg): a linear gradient from H$_2$O/1% TFA to H$_2$O/1% TFA:MeOH/1% TFA (3:1) in 5 min, followed by isocratic conditions for 45 min, then to MeOH/1% TFA in 10 min (flow rate 10 mL/min), 60 fractions were collected from time = 0 min. Fractions 35—36 were then combined (2.8 mg) for further C$_{18}$ HPLC purification: a linear gradient from H$_2$O/1% TFA to H$_2$O/1% TFA:MeOH/1% TFA (4:1) in 1 min, followed by isocratic conditions for 49 min, then to MeOH/1% TFA in 10 min (flow rate 10 mL/min), 60 fractions were collected from time = 0 min. Compound **2** (0.8 mg, 0.01% dry wt) eluted with a retention time of 57 min. Fractions 27—30 (12.0 mg): a linear gradient from H$_2$O/1% TFA to H$_2$O/1% TFA:MeOH/1% TFA (7:3) in 5 min, followed by isocratic conditions for 45 min, then to MeOH/1% TFA in 10 min (flow rate 10 mL/min), 60 fractions were collected from time = 0 min. Compound **1** (2.0 mg, 0.02% dry wt) eluted with a retention time of 44 min (fractions 43—44). Fractions 41—42 and 45 were combined (4.3 mg) and purified further by C$_{18}$ HPLC: a gradient from H$_2$O/1% TFA to H$_2$O/1% TFA:MeOH/1% TFA (7:3) in 5 min, followed by isocratic conditions for 45 min, then to MeOH/1% TFA in 10 min (flow rate 10 mL/min), 60 fractions were collected from time = 0 min. Compound **1** (1.5 mg, 0.02% dry wt) eluted with a retention time of 49 min. Compounds **1** and **2** were isolated as their TFA salts.
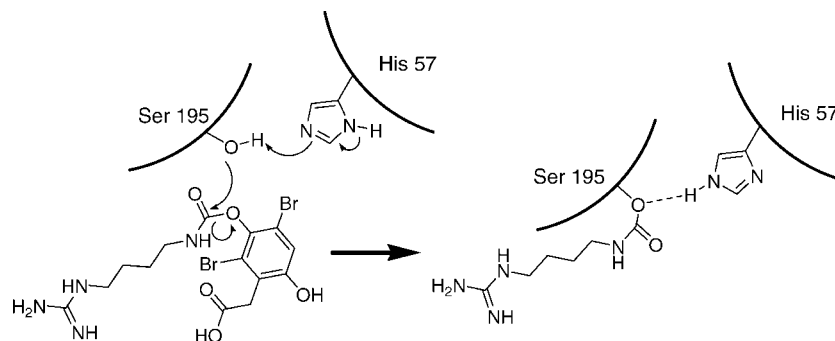
Figure 4. Clavatadine A irreversibly binding to FXIa.

Clavatadine A (1), (2,4-Dibromo-3-{[(4-carbamimidamidobutyl)carbamoyl]oxy}-6-hydroxyphenyl)acetic acid. Isolated as an amorphous solid. UV (MeOH) λ_{max} (log ε) 208.5 (4.04), 293.0 (3.23) nm. IR ν_{max} (film) 3394, 1684, 1205 cm^{-1}. ^{1}H and ^{13}C NMR: see Table 1. (+)-HRESIMS m/z 480.9718 [C$_{14}$H$_{18}$^{79}Br$_2$N$_4$O$_5$ + H]$^+$ (calcd 480.9717, δ +0.2 ppm).

Clavatadine B (2), 2-(2-Amino-2-oxoethyl)-3,5-dibromo-4-hydroxyphenyl (4-carbamimidamidobutyl)carbamate. Isolated as an amorphous solid. UV (MeOH) λ_{max} (log ε) 205.0 (4.35), 294.5 (3.29) nm. IR ν_{max} (film) 3423, 1685, 1206, 1139 cm^{-1}. ^{1}H and ^{13}C NMR: see Table 2. (+)-HRESIMS m/z 479.9880 [C$_{14}$H$_{19}$Br^{79}Br^{81}N$_5$O$_4$ + H]$^+$ (calcd 479.9877, δ +0.6 ppm).

Factor IXa Assay. Stock solutions of factor IXa enzyme was diluted to a working concentration of 45 nM in assay buffer consisting of 50 mM Tris, 100 mM NaCl, 5 mM CaCl$_2$, 3 mg/mL BSA, and 33% ethylene glycol ("FIXa assay buffer"). The assay was performed in 384-well microtiter plates and consisted of the following: 1 μL of compound (stock 10 mM in DMSO), 4 μL of water, and 20 μL of factor IXa, followed by incubation at rt for 10 min. Then 20 μL of Pentachrome FIXa (1.125 mM in FIXa assay buffer) was added, followed by another incubation of 90 min at rt. Absorbance was then read at 405 nm. In-plate control consisted of 100% inhibition (1 μL of control compound, AZ12425961, 50 μM) and 0% inhibition (1 μL of DMSO).

Factor XIa Assay. Stock solutions of factor XIa enzyme was diluted to a working concentration of 0.225 nM in assay buffer consisting of 50 mM Tris, 100 mM NaCl, 5 mM CaCl$_2$, and 0.1 mg/mL BSA ("FXIa assay buffer"). The assay was performed in 384-well microtiter plates and consisted of the following: 1 μL of compound (stock 10 mM in DMSO), 4 μL of water, and 20 μL of factor XIa, followed by incubation at rt for 15 min. Then 20 μL of substrate S2366 (0.54 mM in FXIa assay buffer) was added, followed by another incubation of 2 h at rt. Absorbance was then read at 405 nm. In-plate controls consisted of 100% inhibition (1 μL of control compound, AZ10404570, 13.5 mM) and 0% inhibition (1 μL of DMSO).

Data Analysis. For both assays, percent inhibition for each compound was calculated as follows:

$$\% \text{ inhibition} = 100 - \{[(\text{Abs}_{compound} - 0\% \text{ control})/(100\% \text{ control} - 0\% \text{ control})]100\} \quad (1)$$

BIACORE Experiments. The Biacore HBS-EP buffer (10 mM HEPES, 150 mM NaCl, and 0.01% Tween 20 at pH 7.4 completed with 5 mM CaCl$_2$) was used as running buffer at 5 mL/min and 25 °C in all experiments. Plasma derived FXIa dimer (2 × 80 kDa) (Haematologic Technology Inc., Vermont) was amine coupled on a CM5 sensor chip (Biacore, Uppsala, Sweden) to a response of 17.8 kRU (200 μL FXIa: 0.1 g/L at pH 5.5 in 10 mM NaOAc was coupled after surface activation with 50 μL EDC/NHS (*N*-ethyl-*N*′-dimethylaminopropylcarbodiimide/*N*-hydroxysuccinimide) as recommended by Biacore followed by 0.1 M ethanol amine and extra stabilized with 10 μL of EDC/NHS). The control surface was treated similarly, but without protein. Injection of compound **1** at 10 μM in running buffer over the surface resulted in binding to approximately 110 RU (Figure S1, Supporting Information). Because

steady state was not reached, the obtained resonance response suggested initial binding of more than one-to-one ligand per subunit. A multiphase dissociation phase was observed when buffer was injected. After an initial dissociation phase, approximately 50 RU remained bound after 8 h dissociation in running buffer. The binding was apparently very strong because no further dissociation was recorded by injection of 4 M MgCl$_2$ or reversible-binding FXIa ligands. The Biacore experiments were repeated on two independent days with the same result. For more details on BIACORE instrumentation and methods, see previous report.[8]

Crystallographic Data of Clavatadine A (1)−Factor XIa Complex. Protein expression and purification was carried out as described.[9] To improve crystallizability a quadrupole mutant (S434A, K437A, T475A, and C482S) was generated. These mutations were chosen in order to alter the surface charge, prevent glycolysation, and avoid free cysteines. Purified FXIa (~20 mg/mL) was mixed with benzamidine (1 mM) and the buffer components include: ~20 mM Tris pH 7.4, 200 mM NaCl. Crystallization was set up using the hanging drop method with conditions: 1.9−2.5 M AmSO$_4$, 0.1 M Tris pH 8.5. Cubic shaped crystals appeared after a few days. To form the ligand complex in the crystal, a solid sample of the inhibitor was added directly into the crystal mother liquor. The soaking lasted about 48 h at rt before freezing for data collection. The complete data were collected in ESRF (European Synchrotron Radiation Facility) on beamline ID-23. The structure was solved by molecular replacement using the benzamidine complex as template (PDB: 1ZHR). More statistics of data collection and refinement are recorded in Table S1 of the Supporting Information.

Supporting Information Available: BIACORE sensorgram, details of crystallography and 1D and 2D NMR spectra for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Goto, S. Factor XIa as a possible new target of antithrombotic therapy. *J. Thromb. Haemost.* **2006**, *4*, 1494–1495. (b) Lin, J.; Deng, H.; Jin, L.; Pandey, P.; Quinn, J.; Cantin, S.; Rynkiewicz, M. J.; Gorga, J. C.; Bibbins, F.; Celatka, C. A.; Nagafuji, P.; Bannister, T. D.; Meyers, H. V.; Babine, R. E.; Hayward, N. J.; Weaver, D.; Benjamin, H.; Stassen, F.; Abdel-Meguid, S. S.; Strickler, J. E. Design, synthesis, and biological evaluation of peptidomimetic inhibitors of factor XIa as novel anticoagulants. *J. Med. Chem.* **2006**, *49*, 7781–7791. (c) Deng, H.; Bannister, T. D.; Jin, L.; Babine, R. E.; Quinn, J.; Nagafuji, P.; Celatka, C. A.; Lin, J.; Lazarova, T. I.; Rynkiewicz, M. J.; Bibbins, F.; Pandey, P.; Gorga, J.; Meyers, H. V.; Abdel-Meguid, S. S.; Strickler, J. E. Synthesis, SAR exploration, and X-ray crystal structures of factor XIa inhibitors containing an α-ketothiazole arginine. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3049–3054.

(2) Carroll, A. R.; Pierens, G. K.; Fechner, G.; Leone, P. de A.; Ngo, A.; Simpson, M.; Hyde, E.; Hooper, J. N. A.; Boström, S.-L.; Musil, D.;

Quinn, R. J. Dysinosin A: a novel inhibitor of factor VIIa and thrombin from a new genus and species of Australian sponge of the family dysideidae. *J. Am. Chem. Soc.* **2002**, *124*, 13340–13341.

(3) Carroll, A. R.; Buchanan, M. S.; Edser, A.; Hyde, E.; Simpson, M.; Quinn, R. J. Dysinosins B−D, inhibitors of factor VIIa and thrombin from the Australian sponge Lamellodysidea chlorea. *J. Nat. Prod.* **2004**, *67*, 1291–1294.

(4) Shin, J.; Seo, Y.; Cho, K. W. Euplexides A−E: novel farnesylhydro-quinone glycosides from the gorgonian *Euplexaura anastomosans*. *J. Org. Chem.* **1999**, *64*, 1853–1858.

(5) Rodriguez, A. D.; Pina, I. C. The structures of aplysinamisines I, II, and III: new bromotyrosine-derived alkaloids from the Caribbean sponge *Aplysina cauliformis*. *J. Nat. Prod.* **1993**, *56*, 907–914.

(6) Kang, H.; Fenical, W. Aplidiamine, a unique zwitterionic benzyl hydroxyadenine from the western Australian marine ascidian *Aplidiopsis* sp. *Tetrahedron Lett.* **1997**, *38*, 941–944.

(7) Kobayashi, J.; Honma, K.; Tsuda, M. Lipopurealins D and E and purealidine H, new bromotyrosine alkaloids from the Okinawan marine sponge *Psammaplysilla purea*. *J. Nat. Prod.* **1995**, *58*, 467–470.

(8) Deinum, J.; Gustavsson, L.; Gyzander, E.; Kullman-Magnusson, M.; Edström, Å.; Karlsson, R. A thermodynamic characterization of the binding of thrombin inhibitors to human thrombin, combining biosensor technology, stopped-flow spectrophotometry, and microcalorimetry. *Anal. Biochem.* **2002**, *300*, 152–162.

(9) Jin, L.; Pandey, P.; Babine, R. E.; Weaver, D. T.; Abdel-Meguid, S. S.; Strickler, J. E. Mutation of surface residues to promote crystallization of activated factor XI as a complex with benzamidine: an essential step for the iterative structure-based design of factor XI inhibitors. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2005**, *61*, 1418–1425.