Alutacenoic Acids A and B, Rare Naturally Occurring Cyclopropenone Derivatives Isolated from Fungi: Potent Non-Peptide Factor XIIIa Inhibitors

Hiroshi Kogen,*[,†] Toshihiro Kiho,[†] Keiko Tago,[†] Shuichi Miyamoto,[†] Tomoyuki Fujioka,[‡] Noriko Otsuka,[‡] Keiko Suzuki-Konagai,[§] and Takeshi Ogita*[,§]

Exploratory Chemistry Research Laboratories Pharmacological and Molecular Biological Research Laboratories, and Biomedical Research Laboratories Sankyo Co., Ltd., 2-58, Hiromachi 1-chome Shinagawa-ku Tokyo, 140-8710 Japan

Received July 7, 1999

Factor XIII is a plasma transglutaminase which acts on the final step in the blood coagulation cascade.[1] Factor XIII exists in plasma as a zymogen and is activated to factor XIIIa by thrombin in the presence of Ca^{2+} in a process that reveals a cysteine active center.[2] The enzyme covalently cross-links fibrin monomers and converts soft fibrin clots to hard clots.[3] These clots are less rapidly lysed by agents such as plasmin or tissue plasminogen activator. In addition, factor XIIIa is also known to cross-link fibrin to extracellular matrixes, such as vitronectin, fibronectin, and collagen, thereby forming additional clots to the vessel wall. Specific inhibitors of factor XIIIa are therefore thought to offer possibilities in the therapy for thrombosis,[1a] atherosclerosis, and coronary heart disease,[4] and a few such inhibitors have already been reported. In addition to several peptides[5] and the synthetic compound L-722,151,[6] a known antimicrobial agent, cerulenin,[7] has also been reported by Tymiak to show inhibitory activity (10 μM)[8] against factor XIIIa. To our knowledge, cerulenin is the only naturally occurring non-peptide factor XIIIa inhibitor. Here we report the isolation of novel specific non-peptide inhibitors of factor XIIIa, alutacenoic acids A (**1a**) and B (**1b**), from fungi. Both compounds have an extremely rare cyclopropenone ring isolated from natural sources.

Our recent screening efforts have concentrated on the search for specific inhibitors of factor XIIIa. As a result of our efforts, we have isolated alutacenoic acids A (**1a**) and B (**1b**)[9] (**1a**, IC_{50} = 1.9 μM; **1b**, IC_{50} = 0.61 μM),[10] a pair of fungal metabolites from *Eupenicillium alutaceum* Scott,[11] and found them to be

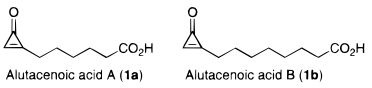


Figure 1.

potent specific inhibitors of factor XIIIa (see Supporting Information). Antibacterial activities are absent in both the **1a** and **1b**. Surprisingly, structure determination by spectroscopic methods (IR, MS, ^{1}H,^{13}C NMR, DQFCOSY, HOHAHA, and HMBC)[12] revealed that these two compounds are monosubstituted cyclopropenone derivatives with aliphatic tethers which attach to a terminal carboxylic acid (Figure 1). Cyclopropenones have attracted considerable attention from chemists during the past four decades. In fact, there have been many efforts toward the synthesis of cyclopropenone derivatives.[13−15] However, only three cyclopropenone derivatives, penitricin[16] and two sesquiterpenes,[17] have been reported to be isolated from natural sources. Among them, penitricin is the only naturally occurring cyclopropenone which shows biological activities as an antibiotic. It is particularly interesting that these cyclopropenone derivatives **1a** and **1b** are isolated from a common fungus, *Eupenicillium*. We applied the synthesis of **1a**, **1b**, and their derivatives to confirm these unique ring systems and study the structure−activity relationships for chain length between the ring and carboxyl group.

Total syntheses of cyclopropenone derivatives **1** (n = 5−11) were accomplished according to the method of Nakamura,[15] establishing the construction of substituted cyclopropenone rings as shown in Scheme 1. Readily available cyclopropenone acetal

† Exploratory Chemistry Research Laboratories.
‡ Pharmacological and Molecular Biological Research Laboratories.
§ Biomedical Research Laboratories.

(1) (a) Leidy, E. M.; Stern, A. M.; Friedman, P. A.; Bush, L. R. *Thromb. Res.* **1990**, *59*, 15−26. (b) Greenberg, C. S.; Birckbichler, P. J.; Rice, R. H. *FASEB J.* **1991**, *5*, 3071−3077. (c) Aeschliman, D.; Paulsson, M. *Thromb. Haemostasis* **1994**, *71*, 402−415. (d) Yee, V. C.; Pedersen, L. C.; Trong, I. L.; Bishop, P. D.; Stenkamp, R. E.; Teller, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7296−7300.

(2) Curtis, C. G.; Brown, K.; Credo, R. B.; Domanik, R. A.; Gray, A.; Stenberg, P.; Lorand, L. *Biochemistry* **1974**, *13*, 3774−3780.

(3) (a) Bruner-Lorand, J.; Pilkington, T. R. E.; Lorand, L. *Nature (London)* **1966**, *210*, 1273−1274. (b) Gladner, J. A.; Nosssal, R. *Thromb. Res.* **1983**, *30*, 273−288. (c) Shen, L.; Lorand, L. *J. Clin. Invest.* **1983**, *71*, 1336−1341.

(4) (a) Kloczko, J.; Wojtukiewicz, M.; Bielawiec, M.; Zarzycka, B.; Kinalska, I. *Acta Haematol.* **1986**, *76*, 81−85. (b) Francis, C. W.; Connaghan, D. G.; Scott, W. L.; Marder, V. J. *Circulation* **1987**, *75*, 1170−1177. (c) Kloczko, J.; Wojtukiewicz, M.; Bielawiec, M.; Zuch, A. *Thromb. Res.* **1988**, *51*, 575−581.

(5) Synthetic peptides: Achyuthan, K. E.; Slaughter, T. F.; Santiago, M. A.; Enghild, J. J.; Greenberg, C. S. *J. Biol. Chem.* **1993**, *268*, 21284−21292. Natural peptide: Finney, S.; Seale, L.; Sawyer, R. T.; Wallis, R. B. *Biochem. J.* **1997**, *324*, 797−805.

(6) (a) Leidy, E. M.; Stern, A. M. *Thromb. Res.* **1990**, *59*, 15−26. (b) Shebuski, R. J.; Sitko, G. R.; Claremon, D. A.; Baldwin, J. J.; Remy, D. C.; Stern, A. M. *Blood* **1990**, *75*, 1455−1459. (c) Freund, K. F.; Doshi, K. P.; Gaul, S. L.; Claremon, D. A.; Remy, D. C.; Baldwin, J. J.; Pitzenberger, S. M.; Stern, A. M. *Biochemistry* **1994**, *33*, 10109−10119.

(7) (a) Matsumae, A.; Kamio, Y.; Hata, T. J. *J. Antibiot. Ser. A* **1963**, *16*, 236−238. (b) Sano, Y.; Nomura, S.; Kamio, Y.; Omura, S.; Hata, T. *J. Antibiot.* **1967**, *20*, 344−348. (c) Arison, B. H.; Omura, S. *J. Antibiot.* **1974**, *27*, 28−30. (d) Omura, S. *Methods Enzymol.* **1981**, *72*, 520−532.

(8) Tymiak, A. A.; Tuttle, J. G.; Kimball, S. D.; Wang, T.; Lee, V. G. *J. Antibiot.* **1993**, *46*, 204−206.

(9) Alutacenoic acid A (**1a**): colorless oil; HRMS (FAB) calcd for $C_9H_{12}O_3$ $(M + H)^+$ 169.0865, found 169.0875; IR ν_{max} ($CHCl_3$) 3084, 2944, 2867, 1826, 1740, 1711, 1591, 1414 cm^{-1}; ^{1}H NMR (360 MHz, CD_3OD) δ (ppm) 1.43−1.53 (m, 2H), 1.67 (m, 2H), 1.77 (m, 2H), 2.32 (t, J = 7.5 Hz, 2H), 2.75 (t, J = 7.3 Hz, 2H), 8.74 (s, 1H); ^{13}C NMR (90 MHz, CD_3OD) δ (ppm) 25.7, 26.5, 27.8, 29.6, 34.9, 150.0, 161.4, 170.9, 177.8. **1a** gradually decomposed within a few months on storage at −20 °C. We stored a solution of **1a** in a mixture of benzene and *t*-BuOH at −20 °C without decomposition for at least 6 months. Alutacenoic acid B (**1b**): colorless needles; mp 66−69 °C, HRMS (FAB) calcd for $C_{11}H_{16}O_3$ $(M + H)^+$ 197.1178, found 197.1169; IR ν_{max} (KBr) 3059, 1821, 1732, 1609, 1567 cm^{-1}; ^{1}H NMR (360 MHz, CD_3OD) δ (ppm) 1.33−1.51 (m, 6H), 1.61 (m, 2H), 1.75 (m, 2H), 2.28 (t, J = 7.4 Hz, 2H), 2.74 (t, J = 7.2 Hz, 2H), 8.73 (s, 1H); ^{13}C NMR (90 MHz, CD_3OD) δ (ppm) 26.5, 27.1, 28.1, 30.2, 30.4, 30.5, 35.5, 150.2, 161.8, 171.4, 178.2. **1b** was stored in a freezer (−20 °C) without decomposition for at least 2 years.

(10) For the factor XIIIa assay utilized for this study, see: Usui, T.; Takagi, J.; Saito, Y. *J. Biol. Chem.* **1993**, *268*, 12311−12316.

(11) Alutacenoic acid B (**1b**) is also isolated from *Aspergillus fumigatus* Fresenius.

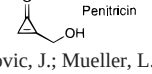
(12) For a detailed study on structure determination by NMR, see the Supporting Information.

(13) (a) Breslow, R.; Haynie, R.; Mirra, J. *J. Am. Chem. Soc.* **1959**, *81*, 247−248. (b) Greenberg, A.; Tomkins, R. P. T.; Dobrovolny, M.; Liebman, J. F. *J. Am. Chem. Soc.* **1983**, *105*, 6855−6858. (c) Staley, W. S.; Nordon, T. D.; Taylor, W. H.; Harmony, M. D. *J. Am. Chem. Soc.* **1987**, *109*, 7641−7647. (d) Wiberg, K. B.; Marquez, M. *J. Am. Chem. Soc.* **1998**, *120*, 2932−2938.

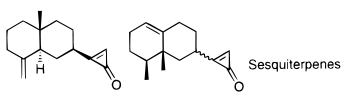
(14) Review: (a) Krebs, A. W. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 10−22. (b) Potts, K. T.; Baum, J. S. *Chem. Rev.* **1974**, *74*, 189−213. (c) Eicher, T.; Weber, J. L. *Top. Curr. Chem.* **1975**, *57*, 1−109.

(15) (a) Isaka, M.; Matsuzawa, S.; Yamago. S.; Ejiri, S.; Miyachi, Y.; Nakamura, E. *J. Org. Chem.* **1989**, *54*, 4727−4729. (b) Isaka, M.; Ando, R.; Morinaka. Y.; Nakamura, E. *Tetrahedron Lett.* **1991**, *32*, 1339−1342. (c) Isaka, M.; Ejiri, S.; Nakamura, E. *Tetrahedron* **1992**, *48*, 2045−2057. (d) Tokuyama, H.; Isaka, M.; Nakamura, E. *J. Antibiot.* **1992**, *45*, 1148−1154.

(16) (a) Okuda, T.; Yoneyama, Y.; Fujiwara, A.; Furumai, T. *J. Antibiot.* **1984**, *37*, 712−717. (b) Okuda, T.; Yoneyama, Y.; Fujiwara, A.; Furumai, T.; Maruyama, H. B. *J. Antibiot.* **1984**, *37*, 718−722. (c) Okuda, T.; Shimma, N.; Furumai, T. *J. Antibiot.* **1984**, *37*, 723−727.

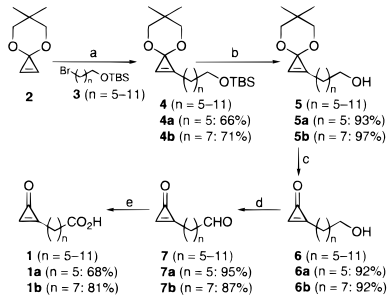


(17) Bohlmann, F.; Jakupovic, J.; Mueller, L.; Schuster, A. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 292−293.



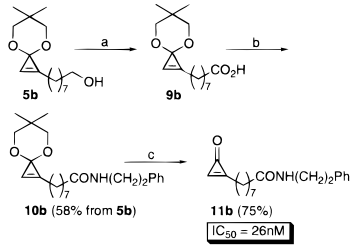
10.1021/ja992355s CCC: $19.00
Published on Web 02/10/2000

Scheme 1[a]



[a] (a) *n*-BuLi, −78 °C, HMPA, THF, then **3c**, −23 °C; (b) TBAF, AcOH, THF, 0 °C to rt; (c) Amberlyst15, THF−H_2O, rt; (d) $(COCl)_2$, Me_2SO, Et_3N, −78 to 0 °C, CH_2Cl_2; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH−H_2O, rt.

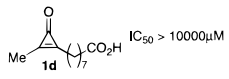
Scheme 2[a]



[a] (a) TEMPO, KBr, Aliquat336, NaClO, $NaHCO_3$, CH_2Cl_2−H_2O, 0 °C; (b) CDI, phenethylamine, CH_2Cl_2, 0 °C; (c) Amberlyst15, THF−H_2O.

2[15c] was treated with *n*-BuLi in the presence of HMPA in THF at −78 °C, and a subsequent reaction with *tert*-butyldimethylsilyloxy alkyl bromide **3** ($n = 5-11$) gave conveniently coupling product **4** ($n = 5-11$) in 60−79% yield. Deprotection of a silyl group of **4** with tetra-*n*-butylammonium fluoride (TBAF)−acetic acid in THF at 0 °C provided alcohol **5** ($n = 5-11$) in 91−97% yield. Hydrolysis of an acetal group of the alcohol **5** with an acidic ion-exchange resin, Amberlyst15, produced cyclopropenone **6** ($n = 5-11$) in 57−92% yield. Oxidation of **6** using Swern conditions provided aldehyde **7** ($n = 5-11$) in 75−95% yield.

Careful oxidation of an aldehyde group under $NaClO_2$ conditions at room temperature afforded cyclopropenone derivatives **1** ($n = 5-11$) in 63−86% yield. Synthetic **1a** and **1b** were spectroscopically identical in all respects to the natural alutacenoic acids A and B, respectively. Among all these synthetic compounds, **1c** ($n = 10$, $IC_{50} = 0.31$ μM) showed the most potent inhibitory activity against factor XIIIa. Interestingly, the inhibitory activity of disubstituted cyclopropenone derivative **1d**[18] ($n = 7$) was greatly reduced.



To further explore the inhibitory activity of this series of compounds, various carboxyl derivatives[19] were synthesized. A desirable and improved inhibitory potency of 26 nM (**11b**) was achieved by converting the terminal functional group from carboxyl to phenethyl amide. The amide **11b** was synthesized as shown in Scheme 2. The acetal derivative **5b** was used as the starting material to modify the terminal carboxyl group because of the instability of the cyclopropenone ring under even weakly basic conditions. Oxidation of **5b** with NaClO-2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO)[20] afforded carboxylic acid **9b**, and a subsequent coupling reaction with phenethylamine using the 1,1-carbonyldiimidazole (CDI) method provided amide **10b** in 58% yield from **5b**. Hydrolysis of an acetal group of the amide **10b** with an acidic ion-exchange resin, Amberlyst15, produced phenethyl amide **11b** in 75% yield.

A full kinetic analysis[21] was performed on the most potent inhibitor **11b**, which confirmed that the phenethyl amide derivative is a time-dependent (i.e., irreversible) inhibitor of factor XIIIa with a second-order inhibition rate constant (k_{inact}/K_i) of 305 000 min^{-1} M^{-1}.

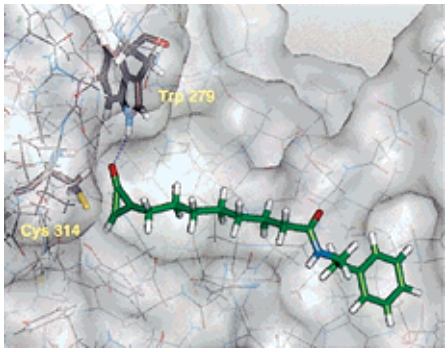


Figure 2. Complex structure model of **11b** (green) and the factor XIII binding site (black). The Connolly surface of the enzyme is shown in gray. The blue dotted line indicates the hydrogen bond between the carbonyl oxygen and the indole NH of Trp279. The docking study was performed using the QUANTA/CHARMm system[24] with the enzyme structure (1F13[22b] in the Protein Data Bank[25]) fixed.

Three-dimensional structures of factor XIII, solved recently by X-ray crystallography, suggest the movement of the β-barrel-1 domain on activation, allowing the ligands access to the active site.[1d,22] In an attempt to obtain structural insight into the inhibition mechanism for these cyclopropenone derivatives, we carried out the docking study of the inhibitors into the crystal structure of factor XIII[22b] with the amino-terminal activation peptide and the β-barrel-1 domain removed. Figure 2 shows the stable complex structure model of **11b** and the enzyme binding site thus obtained, indicating how the cyclopropenone ring fits highly complementarily into the active site located at the base of the binding cavity. The carbonyl oxygen of the cyclopropenone ring forms a hydrogen bond to the indole NH of Trp279 (the $O-N^{\epsilon 1}$ distance is 2.9 Å), and the terminal carbon atom is in close proximity to the sulfur atom of Cys314 ($C-S^{\gamma} = 2.9$ Å).[23]

In summary, we isolated novel specific non-peptide inhibitors of factor XIIIa, alutacenoic acids A and B, from fungi. Both compounds are rare cyclopropenone derivatives isolated from natural sources. Additionally, synthetic analogue **11b** shows the most potent inhibitory activity against factor XIIIa among these cyclopropenone derivatives.

Supporting Information Available: HMBC connectivities of **1a** and **1b**; experimental details of the synthesis of **1** ($n = 5-11$), **1d**, and **11** ($n = 7-10$); inhibitory activities of **1** ($n = 6, 8, 9, 11$) and **11** ($n = 8-10$) and of **1b** against several thiol enzymes; and NMR spectra (^1H and ^{13}C) of synthetic and natural **1a** and **1b** (PDF). This material is available free charge via the Internet at http://pubs.acs.org.

JA992355S

(18) Compound **1d** was derived from **4d**, which was prepared by methylation of **4b**. Further details, see the Supporting Information.

(19) We also prepared various esters, amides, ethers, and acylamines. Further detailed study will be reported elsewhere.

(20) Anelli, P. L.; Biffi, C.; Montanari, F.; Quici, S. *J. Org. Chem.* **1987**, *52*, 2559−2562.

(21) (a) Roush, W. R.; Gwaltney II, S. L.; Cheng, J.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. *J. Am. Chem. Soc.* **1998**, *120*, 10994−10995. Bieth, J. G. *Methods Enzymol.* **1995**, *248*, 59−84. Thornberry, N. A.; Peterson, E. P.; Zhao, J. J.; Howard, A. D.; Griffin, P. R.; Chapman, K. T. *Biochemistry* **1994**, *33*, 3934−3940. Progress curves were obtained with three inhibitor concentrations (0.34, 0.67, and 1.34 μM). We calculated the k_{obs} value, the rate constant for loss of enzyme activity, by using EnzFitter (Biosoft). A second-order rate constant (k_{inact}/K_i) was determined by using the program because the obtained k_{obs} varied hyperbolically with inhibitor concentration. A detailed study will be reported elsewhere.

(22) (a) Yee, V. C.; Pedersen, L. C.; Bishop, P. D.; Stenkamp, R. E.; Teller, D. C. *Thromb. Res.* **1995**, *78*, 389−397. (b) Weiss, M. S.; Metzner, H. J.; Hilgenfeld, R. *FEBS Lett.* **1998**, *423*, 291−296.

(23) Compound **1d** does not fit the active site well because of the steric repulsion of the methyl group.

(24) Molecular Simulations Inc., USA.

(25) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F., Jr.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *J. Mol. Biol.* **1977**, *112*, 535.