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Synthesis and biological evaluation of a focused library of beauveriolides

Kenichiro Nagai [a], Takayuki Doi [b], Taichi Ohshiro [c], Toshiaki Sunazuka [a], Hiroshi Tomoda [c], Takashi Takahashi [b], Satoshi Ōmura [a,*]

[a] *Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University and The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan*
[b] *Department of Applied Chemistry, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro, Tokyo 152-8552, Japan*
[c] *School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan*

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ABSTRACT

Fungal beauveriolide III (**1b**), discovered as an inhibitor of lipid droplet accumulation in mouse macrophages and showing antiatherogenic activity in mouse model, consists of L-Phe, L-Ala, D-*allo*-Ile, and (3*S*, 4*S*)-3-hydroxy-4-methyloctanoic acid moieties. A combinatorial library of beauveriolide analogues focusing on L-Ala and D-*allo*-Ile of **1b** was synthesized by combinatorial synthesis. Among them, D-Ala analogues consisting of A{*2*} improved their solubility, while those with **7**{*1,3,2*},**7**{*2,3,1*}, and **7**{*2,3,2*} were 20 times more potent than **1b**.

Lipid droplet accumulation in macrophages is a critical stage for plaque formation, which limits blood flow and rupture of blood vessels, ultimately leading to the development of atherosclerosis in the arterial wall. Thus, inhibitors for lipid droplet accumulation in macrophages would be useful for the treatment of atherosclerosis.[1] Beauveriolides are a family of cyclic depsipeptides isolated from a culture broth of *Beauveria* sp. FO-6979 during the course of our screening program for inhibitors of lipid droplet accumulation in mouse macrophages. In particular, beauveriolides I (**1a**) and III (**1b**) were found to dose-dependently reduce the number and size of lipid droplets in mouse macrophages without cytotoxicity, and to inhibit cholesteryl ester (CE) synthesis with IC_{50} values of 0.78 and 0.41 μM, respectively (Fig. 1).[2a,b,3] In addition, beauveriolides decreased atherogenic lesions in the aorta and heart when administrated orally to apoE and LDL receptor knockout mice. This mode of action has been examined indicating that **1a** and **1b** inhibit CE synthesis by blocking acyl-CoA:cholesterol acyltransferase (ACAT) activity to suppress foam cell formation.[2c]

Synthetic ACAT inhibitors including amides, ureas, and imidazoles do not have structural similarity to beauveriolides, which are expected to be promising lead compounds for the treatment of atherosclerosis.[4] We established a method for combinatorial synthesis of beauveriolide analogues using a 2-chlorotrityl chloride linker and evaluated their inhibitory activity against CE synthesis in mouse macrophages to elucidate partial structure–activity relationships.[5a] In the 3-hydroxy-4-methyloctanoic acid moiety, the importance of stereochemistry of the 3-hydroxyl and 4-methyl groups has been investigated. The configuration of the 3*S* hydroxyl group is essential for the activity because isomers with an inverted hydroxyl group at C-3 were inactive, while the stereochemistry of the methyl group at C-4 did not affect the inhibition of CE synthesis.[5b] Naturally occurring beauveriolides IV, V, and VI in which L-Phe is replaced with L-Val show remarkably decreased activity.[2d] With regard to the L-Phe moiety, *p*- and *m*-chloro substituents on the phenyl ring enhanced the activity. Furthermore, the diphenyl alanine analogue was 10-fold more potent than **1b**.[5] These results indicate that the phenyl group can be replaced by aromatic rings to increase the activity. To date, the effects of substituents on L-Ala and D-Leu parts in **1a** (or D-*allo*-Ile in **1b**) are unclear. Herein, we report on the synthesis of beauveriolide analogues focusing on the L-Ala and D-Leu (or D-*allo*-Ile) parts and evaluation of their ACAT inhibitory activity in cell- and enzyme-based assays.

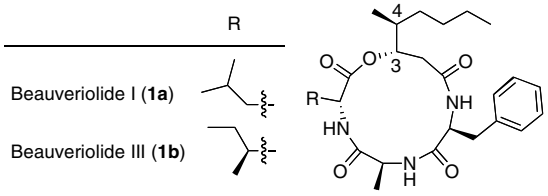

Figure 1. Structures of beauveriolides I and III.

* Corresponding author. Fax: +81 3 3444 8360.
E-mail address: omura-s@kitasato.or.jp (S. Ōmura).

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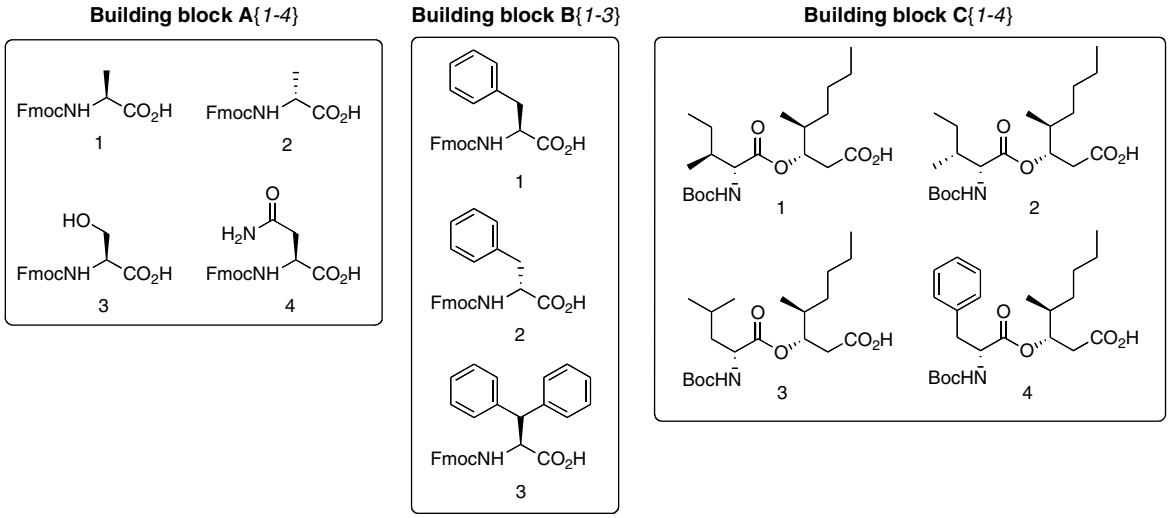
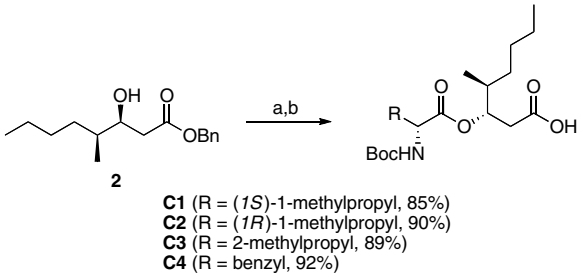


Figure 2. Building blocks **A**, **B**, and **C** for a combinatorial library of beauveriolides.

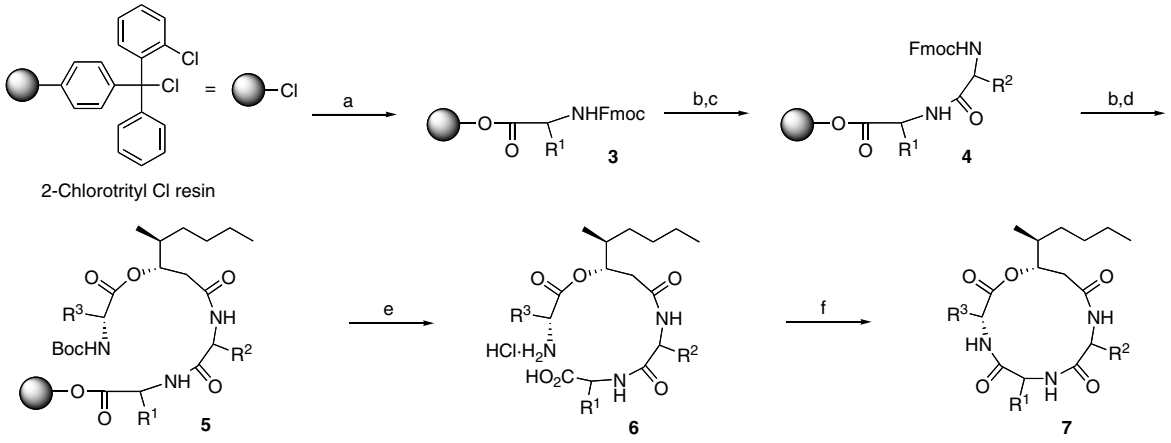
To examine the effect of the L-Ala and D-Leu parts, we designed a 48-member library of beauveriolides consisting of building blocks **A**{*1–4*}, **B**{*1–3*}, and **C**{*1–4*} as shown in Figure 2. The library includes **1a** and **1b** as standards. Building block **C**{*1–4*} was prepared in good yield by condensation of a known benzyl ester **2** and Boc-D-amino acids under DCC-DMAP conditions followed by the removal of the benzyl group (Scheme 1).[6]

Cyclic products **7** were prepared in the same way as the first generation beauveriolide library (Scheme 2). Building block **A** was loaded onto 2-chlorotrityl chloride resin. Next, deprotection of the Fmoc group in **3**, followed by coupling with building block **B** afforded dipeptide **4**. After subsequent deprotection, depsipeptide **5** was obtained by coupling of the resulting amine and building block **C** using PyBrop. Treatment of **5** with 4 M HCl in 1,4-dioxane cleaved the C–O bond between the carboxylic acid and linker concomitantly with the removal of the Boc group to release depsipeptide **6**, which was subjected to cyclization with EDCI and *i*-Pr_2NEt under high dilution conditions to provide cyclic depsipeptide **7**. Each compound was identified by LCMS analysis and purified by preparative reversed-phase HPLC.

We tested the effect of beauveriolide analogue **7** on ACAT activity by measuring the CE synthesis of lipid droplets in a cell-based assay using mouse macrophages and in an enzyme assay using mouse liver microsomes.[2] These results are summarized in Table 1. Inhibition of ACAT activity in the macrophage assay was in good agreement with that in the enzyme assay. In the building block **A**, D-Ala analogues consisting of **A**{*2*} showed a similar activity compared to L-Ala analogues consisting of **A**{*1*} and improved their solubility in assay media. Cyclic compounds from **A**{*3*} and **A**{*4*} tend to decrease the inhibitory activity. However, **7**{*3,2,4*}, **7**{*3,3,1*}, **7**{*3,3,2*}, and **7**{*3,3,4*} inhibited CE synthesis in macrophage at IC_{50} values of 0.11–0.16 μM. These data indicate that fluorescent or biotin labeled groups could be introduced via the hydroxyl



Scheme 1. Synthesis of building block **C**. Reagents and conditions: (a) Boc-D-amino acid-OH, DCC, DMAP, CH_2Cl_2, 0 °C to rt, 22 h; (b) 10% Pd/C, H_2, EtOH, rt, 12 h.



Scheme 2. Synthesis of beauveriolide analogues. Reagents and conditions: (a) Block **A**{*1–4*}, *i*-Pr_2NEt, CH_2Cl_2, rt, 2 h; (b) 20% piperidine/DMF, rt, 1 h; (c) Block **B**{*1–3*}, DIPCI, HOBt, CH_2Cl_2/DMF (4:1), rt, 2 h; (d) Block **C**{*1–4*}, PyBrop, *i*-Pr_2NEt, CH_2Cl_2/DMF (4:1), rt, 1.5 h; (e) 4 M HCl in 1,4-dioxane, rt, 2 h; (f) EDCI·HCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 2 h.

Table 1
A combinatorial library of beauveriolide analogues **7**

7{*A*,*B*,*C*}	Yield (%)[a]	Enzyme assay[b] % of inhibition at 20 μM	Cell-based assay[c] IC_{50} (μM)	R_t (min)[d]	MS (*m*/*z*)[e]	**7**{*A*,*B*,*C*}	Yield (%)[a]	Enzyme assay[b] % of inhibition at 20 μM	Cell-based assay[c] IC_{50} (μM)	R_t (min)[d]	MS (*m*/*z*)[e]
7{*1,1,1*}[f]	13	91	0.4	8.19	488	**7**{*3,1,1*}	10	78	12.0	7.67	504
7{*1,1,2*}	8	80	1.7	8.22	488	**7**{*3,1,2*}	10	99	7.6	7.69	504
7{*1,1,3*}[g]	24	95	0.6	8.29	488	**7**{*3,1,3*}	8	93	12.3	7.75	504
7{*1,1,4*}	18	76	7.5	8.21	522	**7**{*3,1,4*}	10	79	9.3	7.71	538
7{*1,2,1*}	16	20	>20	8.40	488	**7**{*3,2,1*}	14	12	>20	7.79	504
7{*1,2,2*}	20	70	>20	8.42	488	**7**{*3,2,2*}	6	64	>20	7.80	504
7{*1,2,3*}	15	87	>20	8.47	488	**7**{*3,2,3*}	12	40	>20	7.83	504
7{*1,2,4*}	13	47	>20	8.37	522	**7**{*3,2,4*}	9	95	0.11	7.83	538
7{*1,3,1*}	12	95	0.04	8.78	564	**7**{*3,3,1*}	19	98	0.11	8.28	580
7{*1,3,2*}	23	88	0.02	8.80	564	**7**{*3,3,2*}	20	99	0.11	8.35	580
7{*1,3,3*}	16	83	0.08	8.85	564	**7**{*3,3,3*}	17	70	>20	8.40	580
7{*1,3,4*}	12	71	0.08	8.75	598	**7**{*3,3,4*}	19	96	0.16	8.34	614
7{*2,1,1*}	12	94	0.8	8.13	488	**7**{*4,1,1*}	9	64	>20	7.28	531
7{*2,1,2*}	11	96	0.65	8.15	488	**7**{*4,1,2*}	8	52	>20	7.32	531
7{*2,1,3*}	12	98	0.48	8.22	488	**7**{*4,1,3*}	11	43	>20	7.39	531
7{*2,1,4*}	18	94	10.0	8.22	522	**7**{*4,1,4*}	8	11	>20	7.39	565
7{*2,2,1*}	11	99	0.9	7.59	488	**7**{*4,2,1*}	10	10	>20	7.43	531
7{*2,2,2*}	9	91	2.5	7.61	488	**7**{*4,2,2*}	8	27	>20	7.44	531
7{*2,2,3*}	7	96	1.0	7.69	488	**7**{*4,2,3*}	8	4	>20	7.50	531
7{*2,2,4*}	16	60	0.3	7.63	522	**7**{*4,2,4*}	6	8	>20	7.51	565
7{*2,3,1*}	16	91	0.02	8.74	564	**7**{*4,3,1*}	17	94	2.3	7.86	607
7{*2,3,2*}	21	97	0.02	8.77	564	**7**{*4,3,2*}	17	97	2.0	7.89	607
7{*2,3,3*}	21	93	0.07	8.83	564	**7**{*4,3,3*}	17	98	2.0	7.95	607
7{*2,3,4*}	14	75	0.24	8.74	598	**7**{*4,3,4*}	16	97	0.9	7.93	641

[a] Isolated yield by automated preparative HPLC (UV at 215 nm, Waters Symmetry C18 5 μm, 19 × 50 mm with a linear gradient of 10% acetonitrile containing 0.1% formic acid/aqueous 0.1% formic acid to 100% acetonitrile containing 0.1% formic acid for over 20 min at a flow rate of 10 mL/min).
[b] Assay for ACAT activity in microsomes prepared from mouse liver microsomes.
[c] Assay for CE synthesis of lipid droplet accumulation in macrophages.
[d] Retention time (UV at 215 nm, Waters Symmetry C18 5 μm, 4.6 × 50 mm with a linear gradient of 10% acetonitrile containing 0.1% formic acid/aqueous 0.1% formic acid to 100% acetonitrile containing 0.1% formic acid for over 12 min at a flow rate of 1.0 mL/min).
[e] Positive ion electrospray MS data, $[M+H]^+$ were recorded.
[f] Beauveriolide III (**1b**).
[g] Beauveriolide I (**1a**).

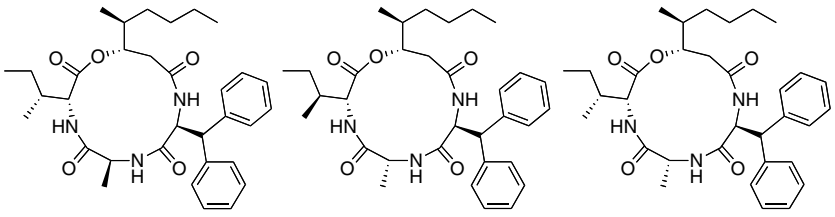


Figure 3. Structures of beauveriolide analogues **7**{*1,3,2*}, **7**{*2,3,1*}, and **7**{*2,3,2*}.

group of the serine without losing potency. Interestingly, D-Phe analogues derived from **B**{*2*} were inactive except for **7**{*2,2,1*}, **7**{*2,2,2*}, **7**{*2,2,3*}, **7**{*2,2,4*}, and **7**{*3,2,4*} in the cell-based assay. These results suggest that displacement of L-Phe with D-Phe might cause a different conformation, thus decreasing the affinity for ACAT. Diphenyl alanine analogues from **B**{*3*} enhanced the activity as previously reported.[5a] It is considered that the diphenyl group plays a critical role in inhibition of CE synthesis in macrophages. Building block **C** had little impact on the activity. Among them, **7**{*1,3,2*}, **7**{*2,3,1*}, and **7**{*2,3,2*} were the most potent inhibitors with the same IC_{50} value of 0.020 μM (Fig. 3).

In conclusion, we have synthesized a second beauveriolide library focusing on the L-Ala and D-*allo*-Ile moieties using our combinatorial strategy. Inhibitory activity of the combinatorial library was tested to elucidate structure–activity relationships. Based on these results, further new analogues have been investigated. The in vivo biological activity will be reported elsewhere.

Acknowledgments

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6. Selected spectral data. **7**{*1,1,1*} (Beauveriolide I) HRFABMS: calcd for $C_{27}H_{42}N_3O_5$ $[M+H]^+$ 488.3124, found 488.3125. ^1H NMR (300 MHz, $CDCl_3$/CD_3OD = 4:1) δ (ppm): 7.25–7.12 (m, 5H), 4.93 (dt, J = 8.5, 4.5 Hz, 1H), 4.58 (t, J = 8.0 Hz, 1H), 4.29 (t, J = 8.0 Hz, 1H), 3.82 (q, J = 7.0 Hz, 1H), 3.05 (dd, J = 13.5, 8.0 Hz, 1H), 2.94 (dd, J = 13.5, 8.0 Hz, 1H), 2.46 (dd, J = 14.5, 5.5 Hz, 1H), 2.40 (dd, J = 14.5, 8.5 Hz, 1H), 2.06 (m, 1H), 1.55–1.43 (m, 3H), 1.36–1.25 (m, 3H), 1.23 (d, J = 7.0 Hz, 3H), 1.22–1.09 (m, 2H), 1.00 (m, 1H), 0.90 (d, J = 7.0 Hz, 3H), 0.88 (d, J = 7.0 Hz, 3H), 0.84 (t, J = 7.0 Hz, 3H), 0.83 (d, J = 7.0 Hz, 3H). ^{13}C NMR (75.4 MHz, $CDCl_3$/CD_3OD = 4:1) δ (ppm): 172.2, 171.8, 171.5, 170.1, 136.6, 129.2 (2×), 128.7 (2×), 127.1, 76.4, 57.0, 52.7, 49.0, 36.1, 35.8, 35.7, 30.9, 29.5, 25.0, 23.0, 22.34, 22.26, 15.6, 15.0, 14.0.