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Discovery and Combinatorial Synthesis of Fungal Metabolites Beauveriolides, Novel Antiatherosclerotic Agents

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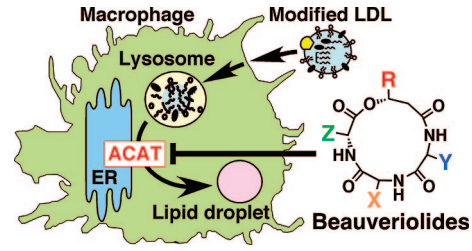
CONSPECTUS

For discovery of a new type of antiatherosclerotic agents, a cell-based assay of lipid droplet accumulation using primary mouse peritoneal macrophages was conducted as a model of macrophage-derived foam cell accumulation, which occurs in the early stage of atherosclerogenesis. During the screening of microbial metabolites for inhibitors of lipid droplet accumulation, 13-membered cyclodepsipeptides, known beauveriolide I and new beauveriolide III, were isolated from the culture broth of fungal *Beauveria* sp. FO-6979, a soil isolate, by solvent extraction, ODS column chromatography, silica gel column chromatography, and preparative HPLC. The structure including the absolute stereochemistry of beauveriolide III was elucidated as cyclo-[(3*S*,4*S*)-3-hydroxy-4-methyloctanoyl-L-phenylalanyl-L-alanyl-D-alloisoleucyl] by spectral analyses, amino acid analyses, and synthetic methods. Furthermore, the absolute stereochemistry was confirmed by the total synthesis of beauveriolides. Study on the mechanism of action revealed that beauveriolides inhibited macrophage acyl-CoA:cholesterol acyltransferase (ACAT) activity to block the synthesis of cholesteryl ester (CE), leading to a reduction of lipid droplets in macrophages. There are two ACAT isozymes in mammals, ACAT1 and ACAT2. ACAT1 is ubiquitously expressed in most tissues and cells including macrophages, while ACAT2 is expressed predominantly in the liver (hepatocytes) and the intestine (enterocytes). Interestingly, beauveriolides inhibited both ACAT1 and ACAT2 to a similar extent in an enzyme assay that utilized microsomes but inhibited ACAT1 selectively in intact cell-based assays. Beauveriolides proved orally active in both low-density lipoprotein receptor and apolipoprotein E knockout mice, reducing the atheroma lesion of heart and aorta without any side effects such as diarrhea or cytotoxicity to adrenal tissues as observed for many synthetic ACAT inhibitors. To obtain more potent inhibitors, a focused library of beauveriolide analogues was prepared by combinatorial chemistry in which solid-phase assembly of linear depsipeptides was carried out using a 2-chlorotrityl linker, followed by solution-phase cyclization, yielding 104 beauveriolide analogues. Among them, diphenyl derivatives were found to show 10 times more potent inhibition of CE synthesis in macrophages than beauveriolide III. Furthermore, most analogues showed selective ACAT1 inhibition or inhibition of both ACAT1 and ACAT2, but interestingly certain analogues gave selective ACAT2 inhibition. These data indicated that subtle structural differences of the inhibitors could discriminate the active sites of the ACAT1 and ACAT2 isozymes. Efforts of further analogue synthesis would make it possible to obtain highly selective ACAT1/ACAT2 inhibitors.



Introduction

Hypercholesterolemia consists of heterogeneous disorders of lipid metabolism characterized by elevated levels of plasma total cholesterol and low-density lipoprotein (LDL)-derived cholesterol. It is definitively linked to increased morbidity and mortality because of myocardial infarction. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, one of the rate-limiting enzymes in the cholesterol biosynthetic pathway, proved to be an effective target for inhibition for the treatment of hypercholesterolemia, and derivatives of fungal compactin (ML236B) and mevinolin (monacolin K) and synthetic atrovastatin, inhibitors of this

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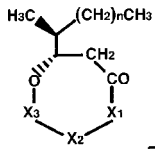


Beauveriolide	n	Amino acid X1	Amino acid X2	Amino acid X3	IC_{50} (μM) CE synthesis	IC_{50} (μM) ACAT
I	3	*L*-Phe	*L*-Ala	*D*-Leu	0.78	6.0
III	3	*L*-Phe	*L*-Ala	*D*-*allo*-Ile	0.41	5.5
IV	3	*L*-Val	*L*-Ala	*D*-Val	>25	100
V	3	*L*-Val	*L*-Ala	*D*-*allo*-Ile	>45	>100
VI	3	*L*-Val	*L*-Ala	*D*-Leu	>25	>100
VII	3	*L*-Phe	*L*-Ala	*D*-Val	21	>100
VIII	5	*L*-Val	*L*-Ala	*D*-*allo*-Ile	>25	≒100
IX	3	*L*-Phe	*L*-Phe	*D*-*allo*-Ile	4.4	1.0

FIGURE 1. Structures of beauveriolides produced by *Beauveria* sp. FO-6979 and their effects on the synthesis of CE by macrophages and ACAT activity in macrophage microsomes.

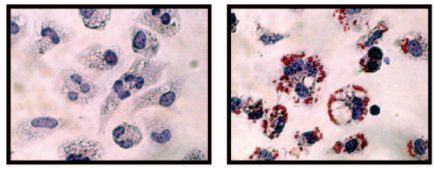


FIGURE 2. Lipid droplet formation in mouse macrophages. macrophage monolayers grown in a tissue culture chamber were incubated without (A) or with (B) liposomes. Lipid droplets were stained in red by oil red O.

enzyme, have been used clinically as cholesterol-lowering or antiatherosclerotic agents.[1−6]

We focused on the role of macrophages in the atherosclerogenic process. In the early stage of the process, macrophages penetrate into the intima, efficiently take up modified LDL, store cholesterol and fatty acids as a form of neutral lipids in the cytosolic lipid droplets, and are converted into foam cells, leading to the development of atherosclerosis in the arterial wall.[7−9] Therefore, inhibitors of the macrophage-derived foam cell formation would be expected to retard the progression of atherosclerosis.

We established a cell-based assay system of lipid droplet synthesis using mouse macrophages as a model of macrophage-derived foam cell formation.[10] Screening for inhibitors with this system led to the discovery of fungal cyclodepsipeptides called beauveriolides (Figure 1) in 1999.[11−13] In this Account, we summarize the discovery, structures, molecular targets, antiatherogenic activity, and total synthesis of beauveriolides and the combinatorial synthesis of beauveriolide analogues.

Establishment of the Cell-Based Assay for Lipid Droplet Formation

Nishikawa et al.[14] reported that, when mouse peritoneal macrophages are cultured in the presence of negatively charged liposomes, they take up the liposomes via the scavenger receptors and metabolize their components (such as phospholipids and cholesterol) to form cytosolic lipid droplets containing neutral lipids of cholestryl ester (CE) and triacylglycerol (TG). Thus, the liposomes are metabolized in a manner similar to natural ligand-modified LDL in macrophages. On the basis of their observations, we have developed cell-based assays[10] using microscopic observation of oil red O-stained lipid droplets (morphological assay) and also using the measurement of [^{14}C]CE and [^{14}C]TG synthesized from [^{14}C]oleic acid (biochemical assay). When macrophages are cultured in the presence of liposomes, a number of lipid droplets are formed in the cytosol of macrophages in the morphological assay (Figure 2).

Discovery and Absolute Structure of Beauveriolides

For the primary screen, culture broths of actinomycetes, fungi, and bacteria were evaluated with the morphological assay and the culture broths that caused a reduction of the size and/or the number of lipid droplets without having a cytotoxic effect on macrophages were selected. Then, the inhibition was confirmed in the biochemical assay. From about 20 000 samples, we discovered beauveriolides I[15] and III from the culture broth of *Beauveria* sp. FO-6979,[11−13] and phenochalasins,[16] sespendole,[17] spylidone,[18] and isobisvertinol[19] from fungal strains, and finally, K97−0239s[20] from an actinomycete strain.

The cyclodepsipeptides, beauveriolides I/III composed of L-Phe, L-Ala, D-Leu/D-*allo*-Ile, and (3*S*,4*S*)-3-hydroxy-4-methyloctanoic acid, respectively, were first isolated from the fungal culture broth.[11,12] Because, for further study, it was necessary to improve the production of these compounds (original titers; ∼5−6 μg/mL for beauveriolides I and III) by fermentation, the culture conditions were studied.[21] The production of both beauveriolides was increased about 10-fold by fermentation in fermentation media containing tryptone. Further studies revealed that the addition of L-Leu (0.2%) but not D-Leu to the culture medium yielded a high and selective production of beauveriolide I (142 versus 18 μg/mL for beauveriolide III), while the addition of L-Ile (0.2%) but not D-*allo*-Ile to the culture medium yielded a high and selective production of beauveriolide III (178 versus 3.6 μg/mL for beauveriolide I). These findings suggested that a putative beauveriolide synthase can epimerase L-Leu and L-Ile to the corresponding D-amino acids to produce the cyclodepsipeptides. In fact, Kleinkauf and von Dohren reported that nonribosomal cyclodepsipeptide synthetases (e.g., enniatin synthetases) contain amino acid epimerase domains.[22−24] Despite the difficulty of separating these compounds because of the similarity of their physicochemical properties, large amounts of beauveriolide I (850 mg) and III (890 mg) were prepared from the culture broth (20 L) obtained from L-Leu- or L-Ile-supplemented

fermentation, respectively, by one-step purification using silica gel column chromatography.

Through the selective production by fermentation, we found that different beauveriolides were produced in fermentation broths supplemented with various amino acids.[13] Accordingly, eight beauveriolides (I and III–IX) were obtained as shown in Figure 1; beauveriolides IV–XIII were new compounds, while beauveriolides VI and IX were identified as beauverolides M[25] and Fa.[26] The production was changed according to the amino acids used to supplement the fermentation medium.

Absolute Stereochemistry of Beauveriolides

The structures of beauveriolides were elucidated by spectral analyses including nuclear magnetic resonance (NMR) experiments and by chemical degradation.[11,13] The structure of beauveriolide III was found to be cyclo-[(3*S*,4*S*)-3-hydroxy-4-methyloctanoyl-L-phenylalanyl-L-alanyl-D-allo-isoleucyl]. Similarly, the structures of beauveriolides IV–IX were elucidated. They all share the common 13-membered cyclodepsipeptide comprising one 3-hydroxy fatty acid, two L-amino acids, and one D-amino acid. A number of cyclodepsipeptides of this type have been reported thus far, including beauveriolides I and II[15] and beauverolides.[26–28] They were all produced by *Beauveria* sp.

The absolute stereochemistry of the amino acids in beauveriolides was firmly identified by amino acid analysis using a chiral column.[12] However, that of the 3-hydroxy-4-methyloctanoic acid moiety remained poorly understood. Mochizuki et al. previously synthesized the corresponding 3-hydroxy-4-methyloctanoic acid, compared the $[\alpha]^{20}_{D}$ values between natural-derived (−19, *c* 0.13, $CHCl_3$) and synthetic (−44, *c* 0.98, $CHCl_3$) ones,[15] and concluded that the absolute stereochemistry is 3*S*,4*S*. On the other hand, our group reported that the $[\alpha]^{20}_{D}$ value (−29, *c* 0.2, $CHCl_3$) of 3-hydroxy-4-methyloctanoic acid prepared from beauveriolide III showed good agreement with that (−27, *c* 0.3, $CHCl_3$) of 3-hydroxy-4-methyloctanoic acid from beauveriolide I, and we therefore concluded that the stereochemistry of the 3-hydroxy-4-methyloctanoic acid moiety in beauveriolide III is also 3*S*,4*S*.[12] Thus, the $[\alpha]^{20}_{D}$ values from Mochizuki's report and our report still showed a discrepancy. To clarify this point, four stereoisomers of 3-hydroxy-4-methyloctanoic acid were synthesized and labeled with (*S*)-(+)-2-(anthracene-2,3-dicarboximido)-1-propyl trifluoromethane sulfonate (AP-OTf), a chiral fluorescent reagent. The derivatives were separated by high-performance liquid chromatography (HPLC) and compared with the natural derivative. Finally, we demonstrated that the absolute stereochemistry is (3*S*,4*S*)3-hydroxy-4-methyloctanoic acid in beauveriolide III.[29]

Antiatherogenic Activity of Beauveriolides

1. Inhibition of Lipid Droplet Formation in Mouse macrophages. In the morphological assay, beauveriolides I and III caused a reduction in the number and size of cytosolic lipid droplets in macrophages at 10 μM without any cytotoxic effect, such as morphological abnormality and mitochondrial disfunction on macrophages.[11] Among the eight beauveriolides isolated from *Beauveria* sp. FO-6979, beauveriolides I and III are the most potent inhibitors of lipid droplet synthesis. Beauveriolides VII and IX also inhibited the synthesis,[13] but their potency seemed much weaker than that of beauveriolides I and III. The other beauveriolides showed only a slight effect on the formation. In the biochemical assay (Figure 1), beauveriolides I and III strongly inhibited the CE synthesis with IC_{50} values of 0.78 and 0.41 μM, respectively, without showing significant effects on the TG and phospholipids synthesis.[29] Beauveriolides VII and IX showed moderate inhibition of CE synthesis.[13] From these results, it might be that the presence of L-Phe in the X1 position and D-Leu/D-*allo*-Ile in the X3 position in the molecules is important for the inhibitory activity against lipid droplet formation and CE synthesis in macrophages (Figure 2). Furthermore, the morphological and biochemical assays using macrophages showed comparable and explainable results by various inhibitors.

These findings prompted us to study the molecular target of the beauveriolides in macrophages. Several types of inhibitors of lipid droplet accumulation in macrophages have been reported. Steroid derivatives, such as U18666A,[30] progesterone, and pregnenolone,[31] inhibit the movement of cholesterol out of the lysosome or inhibit the activity of multidrug-resistant P-glycoproteins in the plasma membrane,[32] and a large number of acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors block cholesterol esterification in the endoplasmic reticulum (ER). These compounds are known to specifically inhibit CE synthesis in macrophages.[33] On the other hand, triacsins, inhibitors of acyl-CoA synthetase, also block lipid droplet accumulation, but these compounds inhibit both CE and TG synthesis by depletion of acyl-CoA.[10] Beauveriolides inhibit CE synthesis specifically, and the inhibition occurs after cholesterol leaves lysosomes.[29] Therefore, ACAT, an ER enzyme, was tested as a target of beauveriolides.

2. Selective Inhibition of ACAT Activity in Macrophages. As expected, beauveriolides I and III were found to inhibit ACAT activity in microsomes prepared from mouse macrophages with IC_{50} values of 6.0 and 5.5 μM, respectively,

and also that in microsomes from mouse livers with IC_{50} values of 1.5 μM for both compounds.[29] Recent molecular biological studies revealed the presence of two isozymes, ACAT1 and ACAT2.[34–37] ACAT1 is ubiquitously expressed, and high-level expression is observed in sebaceous glands, steroidogenic tissues, and macrophages, while ACAT2 is expressed predominantly in the liver and intestine.[37] Therefore, it was strongly suggested that beauveriolides I and III inhibit both ACAT1 and ACAT2 to similar extents. The results for ACAT inhibition by beauveriolides are essentially comparable to those for the inhibition of lipid droplet formation in macrophages.[38] In mouse macrophage microsomes, 18-membered beauvericin with a large cyclic skeleton inhibits ACAT activity more strongly than beauveriolides, but this compound does not show specific inhibition of CE synthesis and has cytotoxic effects on macrophages.[29] Thus, among a number of 13- and 18-membered cyclodepsipeptides tested, beauveriolides I and III are the only compounds that specifically and strongly inhibit both ACAT activity and CE synthesis in macrophages, leading to a decrease in lipid droplet formation. Accordingly, the *in vivo* antiatherogenic activity of beauveriolides was studied in two mouse models.

3. Antiatherogenic Activity in Mouse Models. When apo-E knockout or LDL receptor (LDLR) knockout mice are fed high cholesterol diets, atherosclerosis develops in the aorta and heart. Beauveriolide III proved orally active in both of these knockout mice.[29] After oral administration of at least 25 mg kg^{-1} day^{-1} of this compound for 2 months, the atherosclerotic lesions of the aorta and heart were reduced over 50%. Importantly, beauveriolide III caused no side effects, such as diarrhea or cytotoxicity to adrenal tissues, during the experiments even at 100 mg kg^{-1} day^{-1}. Most synthetic ACAT inhibitors, such as CL-283,546, showed toxic effects on the adrenal gland.[30] There are no conclusive data as to whether the toxic effects on the adrenal gland are inherent in the mechanism of action of these drugs. However, certain synthetic inhibitors, such as avasimibe[39] and pactimibe,[40] proved effective *in vivo* but had no effect on the adrenal gland. Presently, the involvement of ACAT1 and ACAT2 as targets of antiatherosclerogenic drugs is a matter of controversy. Some ACAT inhibitors could reduce the development of atherosclerotic lesions independently of an effect on plasma cholesterol levels in cholesterol-fed rabbits and hamsters; however, with other inhibitors, the reduction of cholesterol levels depended upon their effect on plasma cholesterol levels.[39,40] In pharmacological and genetic studies in animals, it was shown that specific inhibition of ACAT1 might increase the lesion size because of the accumulation of free cholesterol in the lesions. Therefore, selective ACAT1 inhibition should be approached cautiously in humans.[41,22] ACAT2-deficient transgenic mice

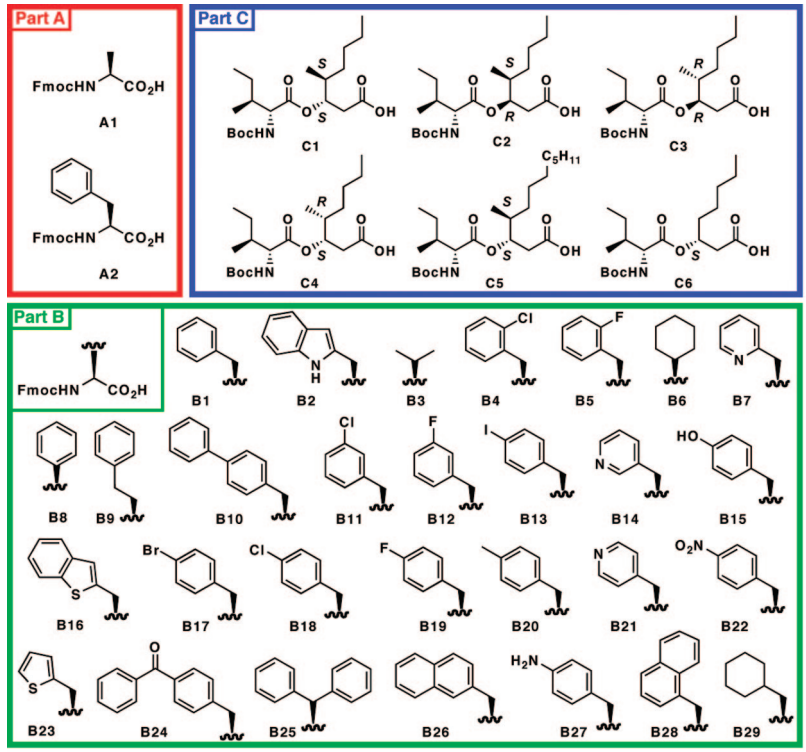
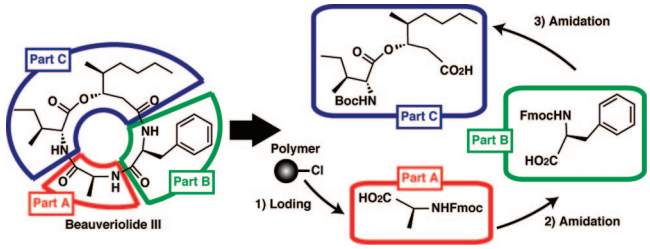


FIGURE 3. Building blocks in the synthesis of combinatorial library 1. (A) 24 analogues of beauveriolide consisted of 2 possible components for part A, 3 possible components for part B, and 4 possible components for part C. (B) 81 analogues of beauveriolide consisted of 27 possible components for part B and 3 possible components for part C. Each part is shown in Scheme 1.

SCHEME 1. Fundamental Strategy for the Combinatorial Synthesis of Beauveriolide Analogues



have a reduction in CE synthesis in the small intestine and liver, which in turn results in protection against diet-induced hypercholesterolemia and gallstone formation.[37] Furthermore, ACAT2- and apo-E-deficient mice have triglyceride-rich apo-B-containing lipoproteins and no atherogenic lesions. Selective inhibitors of ACAT2 may be useful for preventing diet-induced hypercholesterolemia,[37] but the development of such drugs has not been successful. Fungal pyripyropene A, discovered by our group, was found to be a very specific ACAT2 inhibitor.[42,43] Avasimibe and pactimibe, which inhibit both ACAT1 and ACAT2 activities, reduced atherosclerosis in several animal models[39,40] but showed no efficacy in clinical trials.[44] Our results show that beauveriolides having a different chemical structure from synthetic ACAT inhibitors, which also inhibit both ACAT1 and ACAT2 in enzyme assays, show antiatherogenic activity in both LDLR and apo-E knockout mice without any side effects. Beauveriolides I and III show promise as potential lead compounds for antiatherosclerotic agents, which prompted us to prepare a beauveriolide library.

Production of a Beauveriolide Library by Combinatorial Synthesis

1. Strategy. Our fundamental strategy for preparing a combinatorial library of beauveriolide analogues is illustrated in Scheme 1.[45] Beauveriolide III was divided into three parts A (Fmoc-L-Ala-OH), B (Fmoc-L-Phe-OH), and C ((3*S*,4*S*)-*O*-(Boc-D-*allo*-Ile)-4-hydroxy-3-methyloctanoic acid). Each part has diverse components, as shown in Figure 3. Peptide elongation of the synthetic parts A, B, and C using an Fmoc strategy can be carried out on a polymer support. Cleavage from the polymer support followed by macrolactamization will yield a beauveriolide analogue.

2. Total Synthesis of Beauveriolide III. Before a beauveriolide library was prepared, four stereoisomers of beauveriolide III were synthesized according to the strategy to determine the absolute stereochemistry of the 4-hydroxy-3-methyloctanoic acid moiety.[46] That is, four stereoisomers of *O*-(Boc-D-*allo*-Ile)-4-hydroxy-3-methyloctanoic acid (C1–C4 in Figure 3) were first synthesized.[47–49] The 2-chlorotrityl chloride resin was used for the solid-phase peptide synthesis. After Fmoc-Ala-OH (DIEA) was loaded, deprotection of the Fmoc group (20% piperidine) and coupling of Fmoc-Phe-OH (DIC/HOBt) were performed. Deprotection of the Fmoc group, coupling of each C (C1–C4) (PyBrop/DIEA), and acid cleavage (4 M HCl) from the polymer support provided cyclization precursors (93% crude yield with 97% purity). Other coupling reagents were not effective for the coupling of C because elimination of the β-acyloxy group was observed. Then, macrolactamization of the precursors was performed (EDCI/DIEA/CH_2Cl_2/2 h) under high-dilution conditions (1 mM). Finally, four stereoisomers of beauveriolide III were obtained with 58% overall yield. The use of both the HCl salt of the precursors and EDCI was essential for reducing the formation of the cyclic dimer in the cyclization. All of the spectra data of C1-derived synthetic beauveriolide III were identical to those of the natural one, which confirmed the absolute stereochemistry.

3. Beauveriolide Library. On the basis of the above solid-phase strategy, we constructed a 105-member beauveriolide library (Figure 3), namely (1) 24 analogues consisting of 2 components (A1 and A2) in part A, 3 components (B1–B3) in part B, and 4 stereoisomers (C1–C4) in part C and (2) 81 analogues consisting of 1 component (A1) in part A, 27 components (B1 and B4–B29) in part B, and 3 components (C1, C5, and C6) in part C. Cyclization precursors were synthesized by a split-and-pool method using IRORI MicroKans with radiofrequency encodings.[50,51] Cyclic products were prepared in parallel and purified by HPLC.

4. Biological Activity. The inhibitory activity of 104 beauveriolide analogues prepared by combinatorial synthesis was evaluated against CE synthesis in mouse peritoneal macrophages.[45] A smaller substitute in part A resulted in stronger inhibitory activity. With regard to part B, diphenylalanine derivatives were 10 times more potent (IC_{50} value, 0.040 μM) than beauveriolide III. Two other analogues, *p*- and *m*-chlorophenylalanine derivatives, were also very potent (IC_{50} values of 0.095 and 0.14 μM). It is interesting that the 3*S* stereochemistry at the 3 position in part C is essential for the biological activity.

Furthermore, the selectivity of these beauveriolide analogues for inhibition of the ACAT1 and ACAT2 isozymes in the cell-based assay was tested.[38,46] Beauveriolides I and III showed rather selective ACAT1 inhibition in this intact cell assay,[38] suggesting that the accessibility of the two isozymes to the inhibitors is different between microsomes and intact cells. Interestingly, some analogues [compounds 258 (synthesized from A1, B20, and C1), 280 (from A1, B19, and C5), 274 (from A1, B11, and C5), 285 (from A1, B20, and C5), and 301

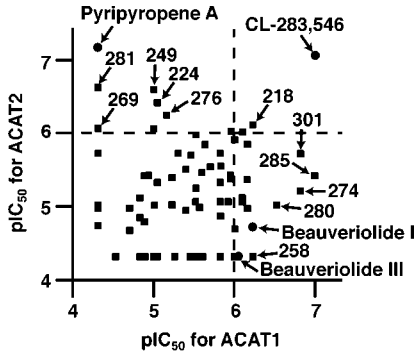


FIGURE 4. Selectivity of beauveriolide analogues toward ACAT isozymes in cell-based assays. IC_{50} values of derivatives were evaluated in ACAT1- and ACAT2-CHO cells, and their pIC_{50} values toward ACAT1 and ACAT2 were plotted on the *x* and *y* axes, respectively.

(from A1, B11, and C6)] showed ACAT1-selective inhibition, and compound 218 (from A2, B1, and C4) showed both ACAT1 and ACAT2 inhibition, while others [compounds 281 (from A1, B28, and C1), 269 (from A1, B10, and C5), 249 (from A1, B25, and C1), 224 (from A2, B2, and C1), and 276 (from A1, B25, and C5)] showed rather selective ACAT2 inhibition (Figure 4). These data indicated that subtle structural differences of the inhibitors can discriminate the active sites of the ACAT1 and ACAT2 isozymes and that it is possible to obtain highly selective ACAT2 inhibitors from beauveriolide analogues.

Concluding Remarks

We identified fungal beauveriolides as inhibitors of lipid droplet formation in mouse macrophages. Studies on the mechanism of action revealed that beauveriolides inhibited macrophage ACAT activity specifically, resulting in the blockage of the cholesteryl ester synthesis and leading to a reduction of lipid droplets in macrophages. Furthermore, beauveriolides I and III exerted antiatherogenic activity in mouse models without any side effects. A number of synthetic ACAT inhibitors have been reported, but no inhibitors have been clinically used thus far. Recent attempts to use avasimibe and pactimibe clinically to reduce atherosclerosis progression failed. There are two ACAT isozymes, ACAT1 (ubiquitously expressed) and ACAT2 (expressed in liver and intestine), which have different functions. Avasimibe and pactimibe inhibited both isozymes. Beauveriolides inhibited both isozymes in microsomal assays but rather selectively inhibited ACAT1 in the intact cell assay. A combinatorial beauveriolide library was prepared by solid-phase split-and-pool synthesis and solution-phase cyclization. We found that the 3*S* configuration in part C is essential for the inhibitory activity and that diphenylalanine analogues are 10 times more potent than beauveriolides I and III. Further studies of antiatherosclerotic agents are underway in our laboratory.

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BIOGRAPHICAL INFORMATION

Hiroshi Tomoda received his undergraduate education at University of Tokyo, Japan, and completed his Ph.D. degree in 1983. Then, he had a researcher position at The Kitasato Institute. After carrying out postdoctoral research at Johns Hopkins University, he was appointed Chief Researcher and Vice Director of the Research Center for Biological Function at The Kitasato Institute. He was Professor at Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, in 2001–2005. From 2005, he has been Professor of the School of Pharmacy, Kitasato University. His current research interest includes the discovery of new beneficial compounds from microorganisms, clarifying the mechanism of function of them, and solving the new cellular functions by using them.

Takayuki Doi received his Ph.D. degree in 1991 under the direction of Professor Takashi Takahashi at the Tokyo Institute of Technology. After spending 2 and $^1/_2$ years at Columbia University as a postdoctoral scientist with Professor G. Stork, he joined the Department of Chemical Engineering in Tokyo Institute of Technology as an Assistant Professor in 1993. He was appointed to an Associate Professor in 2001. He was a recipient of the Progress Award in Synthetic Organic Chemistry, Japan, in 2000. His research interest is in the library synthesis of natural and unnatural products, especially peptides including unnatural amino acids.

FOOTNOTES

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