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Microbial metabolites affecting lipid biosynthesis

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Abstract: Microbial inhibitors for two enzymes involved in lipid metabolism, acyl-CoA:cholesterol acyltransferase (ACAT) and farnesyl-protein transferase (FTase), were screened with assay systems using partially purified enzymes. ACAT inhibitors are expected to work as cholesterol-lowering or anti-atherosclerotic agents, and FTase inhibitors are as anticancer drugs. Three kinds of new ACAT inhibitors were discovered as fungal metabolites; glisoprenins, pyripyropenes and terpendoles. All these inhibitors have a terpene moiety in the structures. Pyripyropenes exhibited very potent ACAT inhibition with nanomolar level of IC50 values and *in vivo* efficacy in a hamster model. Terpendole D showed high specificity for ACAT inhibition in an intact cell assay using J774 macrophages. A series of new FTase inhibitors, pepticinnamins, were isolated from the culture broth of *Streptomyces* sp. OH-4652. Pepticinnamin E, composed of five amino acids, showed potent FTase inhibition with an IC50 value of 0.1 μM. The compound inhibited FTase competitively with respect to the substrate *ras* p21 protein.

Introduction

Our research group has been interested in microbial metabolites affecting lipid biosynthesis and has discovered various useful compounds (1). Many essential products and substrates such as cholesterol, farnesyl pyrophosphate and isopentenyl pyrophosphate are produced via mevalonate biosynthetic pathway. Enzymes involved in the pathway are focused on as potential therapeutic targets for anti-atherosclerotic, antifungal and anticancer drugs. Acyl-CoA:cholesterol acyltransferase (ACAT) and farnesyl-protein transferase (FTase) utilize cholesterol and farnesyl pyrophosphate as substrate, respectively, both of which are synthesized via the mevalonate pathway. In this paper, microbial metabolites inhibiting the two enzymes will be described.

Inhibitors of ACAT

Hypercholesterolemia is a serious risk factor of atherosclerosis developing into coronary heart disease or myocardial infarction. Several therapeutic means have been proposed to lower cholesterol levels in plasma. One successful example is to inhibit *de novo* cholesterol biosynthesis. Lovastatin and its analogs (pravastatin and simvastatin), potent inhibitiors of HMG-CoA reductase, the rate-limiting enzyme in the biosynthetic pathway, are now clinically used (2). Another possible therapy is the prevention of the dietary cholesterol absorption. ACAT utilizes long-chain fatty acyl-CoA and cholesterol as substrates to catalize the intracellular formation of cholesteryl ester. ACAT plays important roles in cholesterol metabolism in human body; 1) Dietary cholesterol is absorbed at intestines as cholesteryl ester by the reaction of ACAT, 2) cholesterol in liver supplied by both factors of taking diet and *de novo* synthesis is acylated by ACAT to be secreted as VLDL, and 3)

macrophages and smooth muscle cells in arterial wall accumulate cholesterol as cholesteryl ester in oil droplets by the reaction of ACAT, leading to foam cells in atherosclerotic lesions. Therefore, ACAT inhibitors are expected not only to lower plasma cholesterol levels but also to have a direct effect on the arterial wall (3).

A large number of synthetic ACAT inhibitors have been reported (4), but not used clinically. Microbial ACAT inhibitors had been rarely known at that time when we started screening inhibitors in 1988. Culture broths of over 10,000 microorganisms including actinomycetes, fungi and bacteria were screened with an *in vitro* assay using rat liver microsomes as the enzyme source. Consequently, we discovered five kinds of new ACAT inhibitors which were all produced by fungal strains. Among them, glisoprenins, pyripyropenes and terpendoles having isoprene units in their structures are described below.

Glisoprenins A and B (Fig. 1) were isolated from the culture broth of *Gliocladium* sp. FO-1513 (5,6). They are composed of nine isoprene units. The IC50 (a drug concentration causing 50% inhibition of ACAT activity) values were 46 μM for glisoprenin A and 61 μM for glisoprenin B. The *in vivo* test showed about 25% inhibition of cholesterol absorption from intestines when glisorprenin B was orally administered to hamsters at 50 mg/kg.

Highly potent ACAT inhibitors were found to be produced by *Aspergillus fumigatus* FO-1289 (7). Four active compounds, consisting of pyridine, α-pyrone and sesquiterpene moieties in common, were named pyripyropenes (Fig. 1). All the compounds inhibited ACAT activity in microsomes with nanomolar level of IC50 values (58, 120, 53 and 270 nM for pyripyropenes A, B, C and D, respectively), indicating that pyripyropenes are the most potent ACAT inhibitors of microbial origin. Since the general structures look similar to cholesterol, they might inhibit competitively the enzyme with respect to the substrate. The *in vivo* efficacy of pyripyropene A was observed in hamsters. The cholesterol absorption was reproducibly inhibited in a dose dependent fashion. Pyripyropenes are expected as a new type of lead compounds for cholesterol-lowering agents.

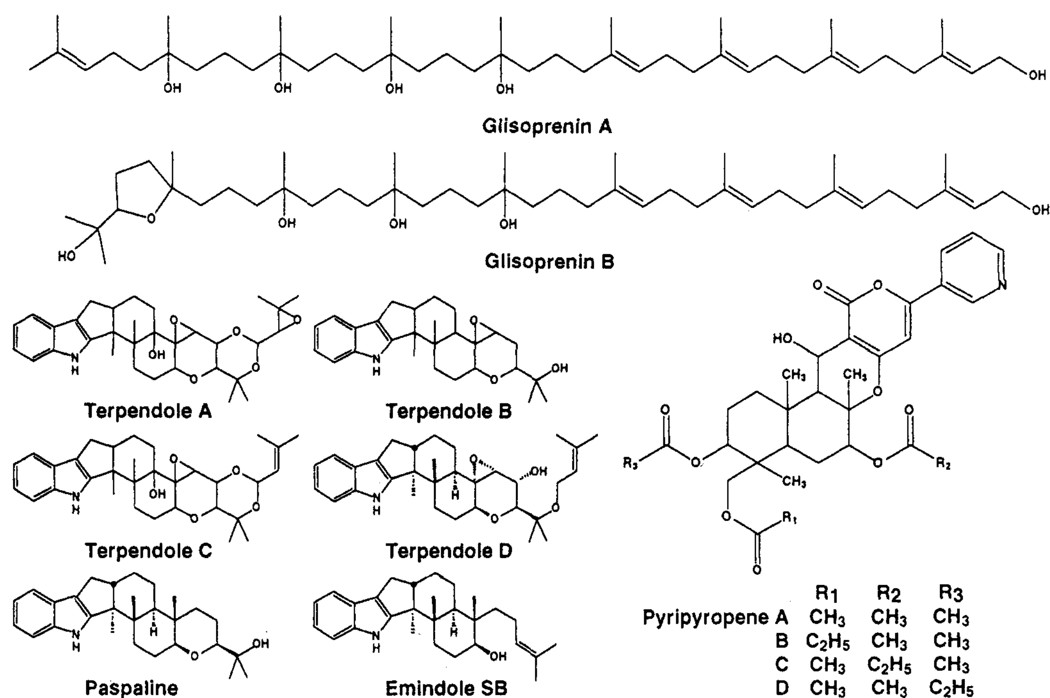

Fig. 1 Structure of ACAT inhibitors

Very recently, another series of new ACAT inhibitors named terpendoles were isolated from the culture broth of a fungal strain FO-2546, which was proposed to belong to a new genus designated as *Albophoma yamanashiensis* (8). Four terpendoles have common indole and diterpene moieties to form steroid-like structures (Fig. 1). The relative stereochemistry of terpendole D was determined by X-ray crystalographic analysis. The ACAT inhibitory activity is summarized in TABLE 1. Among them terpendole C showed the most potent inhibitory activity in microsomes with an IC50 value of 2.1 μM. Terpendole D was the second potent (IC50: 32 μM), followed by terpendoles A (15.1 μM) and B (26.8 μM). Structurally related emindole SB (41 μM) and paspaline (134 μM), known fungal metabolites (9), which were co-produced by the terpendole-producing fungus, were less potent than terpendoles. These data indicated that additional isoprene unit with a double bond at the diterpene moiety is responsible for potent ACAT inhibition. ACAT inhibition by terpendoles were evaluated in an intact cell assay using J774 macrophages according to the established method (10). The ratio of a CD50 (a drug concentration causing 50% cell damage) value to an IC50 value for cholesteryl ester formation was defined as specificity for ACAT inhibition. Terpendole D showed the ratio of over 500, indicating the highest specificity for ACAT inhibition among the microbial ACAT inhibitors discovered. Therefore, terpendole D is also expected as a new lead compound for anti-atherosclerotic agents.

TABLE 1. ACAT inhibitory activity (IC50) and cytotoxicity (CD50) of terpendoles

Compound	Rat liver microsomes	J774 macrophages		
	IC50 (μM)	IC50 (μM)	CD50 (μM)	CD50/IC50
Terpendole A	15.1	0.29	>23.4	>81
Terpendole B	26.8	1.80	>29.7	>17
Terpendole C	2.1	0.46	>24.1	>52
Terpendole D	3.2	0.048	>24.8	>520
Paspaline	134.0	2.85	29.0	10
Emindole SB	41.0	6.48	16.0	2.5

Inhibitors of FTase

Post-translational modification of *ras* proteins at the specific carboxy-terminal sequence CAAX (C, cysteine; A, aliphatic amino acids; X, any amino acid at carboxy-terminus) is required for the proteins to localize in the inner side of the plasma membrane and to exert transforming activity (11,12). FTase catalizes the first step of the sequential modification reactions, that is, 1) transfer of the farnesyl group of farnesylpyrophosphate to the CAAX sequence of the protein to form a thioester linkage of the cysteine residue, 2) cleavage at the third amino acid from the carboxy-terminus to release the AAX sequence, and 3) methylation of the resulting carboxy-terminal farnesyl cysteine. Inhibition of such a farnesylation would alter membrane localization and transforming activity of *ras* oncogene (13). Therefore, a possibility has been raised that FTase inhibitors can act as anti-cancer drugs (14, 15).

Culture broths of about 5,000 microorganisms were screened with an enzyme assay using partially purified FTase from THP-1 cells (16). Two strains, a fungal FO-2047 and *Streptomyces* sp. OH-4652, were found to produce FTase inhibitors.

Gliotoxin and acetylgliotoxin, known fungal toxins (17), were isolated from the culture broth of a fungal strain FO-2047 as FTase inhibitors (16). They inhibited FTase with IC50 values of 1.1 μM for gliotoxin and 4.4 μM for acetylgliotoxin. It has been reported that the disulfide bond in gliotoxin is essential for antimicrobial activity (18), but gliotoxin favors the dithio structure rather than the thiosulfinate one in the FTase reaction mixture containing 4 mM dithiothreitol. Therefore, it is likely that the inhibitory mechanism of these toxins on FTase is different from that of antimicrobial activity.

Recently, a series of new FTase inhibitors named pepticinnamins were discovered as fermentation products of *Streptomyces* sp. OH-4652 (19). Pepticinnamin E (Fig. 2), a major

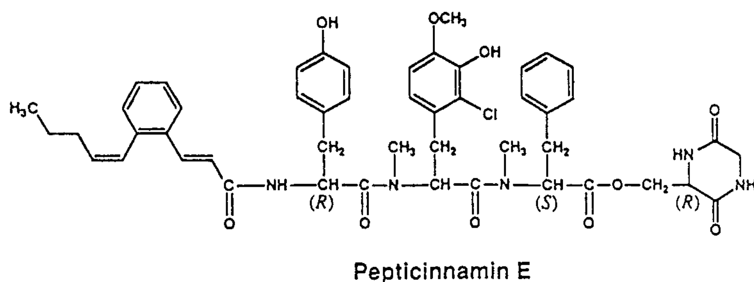

Fig. 2 Structure of FTase inhibitor

product, is composed of five amino acids having *O*-pentenylcinnamic acid at the amide-terminus and forming a diketopiperazine ring in the carboxy-terminal glycylserine moiety (20). Pepticinnamins showed rather potent inhibitory activity against FTase with IC50 values ranging from 0.1 to 1.0 μM, among which pepticinnamin C was the most potent. The kinetic analysis indicated that pepticinnamin E inhibits non-competitively with respect to the substrate farnesyl pyrophosphate (*Ki*, 1.9 μM) and competitively with p21 *ras* protein (*Ki*, 1.76 μM).

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