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NOTE

Pseudopyronine B, an inhibitor of sterol *O*-acyltransferase, produced by *Pseudomonas* sp. BYK11209

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The enzyme sterol *O*-acyltransferase (SOAT, also known as acyl-CoA: cholesterol acyltransferase (ACAT)), an endoplasmic reticulum membrane protein, has important roles in cholesterol regulation in humans.[1] SOAT is expected to be a promising target for the development of new anti-atherosclerotic agents. Pharmaceutical laboratories have reported a number of synthetic SOAT inhibitors; however, none have been successfully developed to date because of their associated side effects or low/no efficacy in clinical trials.[2,3] Recent molecular biological studies revealed the existence of two SOAT isozymes, SOAT1 and SOAT2, with distinct functions. SOAT1 is ubiquitously expressed in all tissues and cells, while SOAT2 is expressed predominantly in the liver (hepatocytes) and small intestine.[1,4] Therefore, it is important to determine the selectivities of inhibitors to the two SOAT isozymes for their development as new anti-atherosclerotic reagents.[2,3] Our group has extensive experience of discovering SOAT inhibitors of microbial origins using an enzyme assay with rat liver microsomes.[5] After the discovery of two SOAT isozymes, we established cell-based and enzyme assays utilizing Chinese hamster ovary (CHO) cells expressing African green monkey SOAT1 (SOAT1-CHO cells) and SOAT2 (SOAT2-CHO cells).[6] In the two assays, the IC_{50} values of a compound were measured to calculate the selectivity index (SI, log [IC_{50} for SOAT1]/[IC_{50} for SOAT2]). We defined compounds into three types of SOAT inhibitors from the SI values; dual-type inhibitors with $-1.00 \leqq SI \leqq +1.00$, SOAT1-selective inhibitors with $SI < -1.00$ and SOAT2-selective inhibitors with $+1.00 < SI$. Using these assay systems,[7] we have discovered known and novel compounds such as flavasperone, sterigmatocystin, verticilides, clonoamide and two diketopiperazines.[8–11] During our continuous screening for SOAT inhibitors using these assay systems, a known compound having a pyrone ring, pseudopyronine B[12] (**1**), was isolated from a culture broth of *Pseudomonas* sp. BYK11209.

The bacterium *Pseudomonas* sp. BYK11209 was isolated from soil collected at Kamakura in Kanagawa prefecture, Japan, in 2013. The strain was inoculated into a 500 ml Erlenmeyer flask containing 100 ml seed medium (1.0% glucose, 0.40% yeast extract, 0.050% $MgSO_4 \cdot 7H_2O$, 0.40% polypeptone and 0.10% KH_2PO_4). The flask was shaken on a rotary shaker at 27 °C for 3 days. The seed culture (1.0 ml) was transferred to a 500 ml Erlenmeyer flask containing 100 ml production medium (3.0% mannitol, 1.0% glucose, 0.50% yeast extract, 0.50% ammonium succinate, 0.10% K_2HPO_4, 0.10% $MgSO_4 \cdot 7H_2O$ and 0.10% trace solution, pH 7.0). Fermentation was carried out at 27°C for 7 days under shaking conditions (200 r.p.m.). The culture broth (100 ml × 5) was centrifuged and the supernatant collected. This supernatant was lyophilized to yield 3.4 g of a yellow solid material. This material was dissolved in a small volume of H_2O and MeOH, applied to an ODS column (45 g, 3.0 × 20 cm, 100–200 mesh; Fuji Silysia Chemical LTD, Aichi, Japan), and eluted stepwise with 100% H_2O, 20, 40, 60, 80, and 100% CH_3CN with 0.05% H_3PO_4 (300 ml each). This 80% CH_3CN fraction was evaporated *in vacuo* to remove the organic layer and extracted with EtOAc. This extract was concentrated to yield 625 mg of active material. This material was further purified by reversed-phase C18 HPLC (20 × 250 mm; PEGASIL ODS, Senshu Scientific Co., Tokyo, Japan) under the following conditions: solvent, a 20 min linear gradient from 80 to 100% CH_3CN with 0.05% H_3PO_4; flow rate, 8.0 ml min^{-1}; detection, UV detection at 210 nm. Under these conditions, an active compound was eluted as a peak with a retention time of 16.0 min. This fraction was concentrated and extracted with EtOAc to yield a pure compound (18.8 mg) as a pale yellow solid. The structure of the compound was identified as pseudopyronine B (**1**)[12] (Figure 1) by an analysis of spectroscopic data, including MS, UV, IR and NMR, particularly HSQC and TOCSY experiments.

In the cell-based assay,[6,7] pseudopyronine B (**1**) inhibited cholesteryl ester synthesis with IC_{50} values of 20.0 and 3.70 μM in SOAT1- and SOAT2-CHO cells, respectively, giving an SI of +0.73 (dual-type inhibition). Compound **1** showed almost no effects of other lipids (triglyceride and phospholipid) synthesis and morphology even at the highest dose of 165 μM in SOAT1- and SOAT2-CHO cells. The compound showed no cytotoxicity on mouse peritoneal macrophages[13] up to 34 μM and Jurkat cells,[14] a human T-cell

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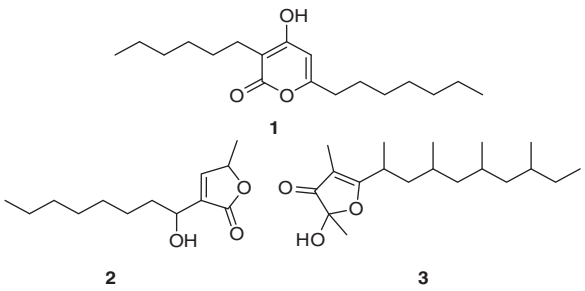

Figure 1 Structures of pseudopyronine B (**1**), acaterin (**2**) and AS-183 (**3**).

leukemia-derived cell line, up to 165 μM. The inhibition of SOAT was confirmed in the enzyme assay using microsomes prepared from SOAT1- and SOAT2-CHO cells.[7] Compound **1** inhibited both SOAT1 and SOAT2 with IC_{50} values of 28.0 and 15.0 μM, respectively, calculating the SI of +0.27 (dual-type inhibition). Although there are subtle differences in IC_{50} values between cell-based and enzyme assay, we concluded that **1** was a dual-type SOAT inhibitor.

From a chemical point of view, synthetic SOAT inhibitors have been classified into three types: amides, ureas and imidazoles, while natural SOAT inhibitors show diverse structures.[2,3] Bacterial pseudopyronine B has structural characteristics with a core pyran ring attaching two alkyl chains, pentane and heptane. Acaterin (**2**)[15] and AS-183 (**3**)[16] have been listed as structurally close SOAT inhibitors of a bacterial origin, if any. They were isolated from the culture broths of *Pseudomonas* sp. A92 and *Scedosporium* sp. SPC-15549, respectively (Figure 1). Both compounds have characteristics with a five-membered ring attaching one long alkyl chain. Therefore, even known compound, pseudopyronine B is a structurally rare SOAT inhibitor with one core ring attaching two alkyl chains.

Regarding biological activity, **1** was reported to exhibit several activities. For example, **1** showed antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.[17] In addition, **1** was reported to be an inhibitor of fatty acid biosynthesis in *Bacillus subtilis*.[18] In bacteria, the different target molecule(s) of **1** may be involved in the expression of these activities. In this study, we discovered **1** from *Pseudomonas* sp. BYK11209 as a structurally rare dual-type SOAT inhibitor.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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