Bacterial enoyl-acyl carrier protein (ACP) reductase has been confirmed as a novel target for antibacterial drug development. In the screening of inhibitors of Staphylococcus aureus enoyl-ACP reductase (FabI), complestatin was isolated as a potent inhibitor of S. aureus FabI together with neuroprotectin A and chloropeptin I from Streptomyces chartreusis AN1542. Completstatin and related compounds inhibited S. aureus FabI with IC_{50} of 0.3–0.6 µM. They also prevented the growth of S. aureus as well as methicillin-resistant S. aureus (MRSA) and quinolone-resistant S. aureus (QRSA), with minimum inhibitory concentrations (MICs) of 2–4 µg/mL. Consistent with its FabI-inhibition, complestatin selectively inhibited the intracellular fatty acid synthesis in S. aureus, whereas it did not affect the macromolecular biosynthesis of other cellular components, such as DNA, RNA, proteins, and the cell wall. Additionally, supplementation with exogenous fatty acids reversed the antibacterial effect of complestatin, demonstrating that it targets fatty acid synthesis. In this study, we reported that complestatin and related compounds showed potent antibacterial activity via inhibiting fatty acid synthesis.

**Key words** complestatin; enoyl-acyl carrier protein reductase; antibacterial; fatty acid synthesis; *Staphylococcus aureus*

Bacterial fatty acid synthesis (FAS) is an attractive antibacterial target, since FAS is organized differently in bacteria and mammals. Fatty acid biosynthesis in bacteria is crucial for the production of a number of lipid-containing components, including the cell membrane. Bacterial enoyl-acyl carrier protein (ACP) reductase, which catalyzes the final and rate-limiting step in bacterial fatty acid synthesis, has been validated as a novel target for the development of antibacterial drugs. Four isoforms, FabI, FabK, FabL, and FabV have been detected in enoyl-ACP reductase. FabI is distributed broadly throughout the majority of bacteria including *S. aureus*, while *Streptococcus pneumoniae* contains only FabK, *Enterococcus faecalis* and *Pseudomonas aeruginosa* contain both FabI and FabK, and *Bacillus subtilis* contain both FabI and FabL. Indeed, FabI has been identified as the antibacterial target of both triclosan, a broad spectrum biocide used in a wide range of consumer goods, and isoniazid, which has been utilized for 50 years in the treatment of tuberculosis. Therefore, inhibitors of *S. aureus* FabI may prove to be interesting lead compounds for the development of effective antibacterial drugs.

In our screening for *S. aureus* FabI inhibitors from microbial metabolites, we isolated complestatin (1) together with neuroprotectin A (2) and chloropeptin I (3) from the mycelium of *Streptomyces chartreusis* AN1542 as potent inhibitors of FabI (Fig. 1). Complestatin has previously been isolated from *S. lavendulae* SANK 60477 as an anti-complement substance, from *Streptomyces* sp. MA7234 as potential complement inhibitory virus-1 (HAV-1) integrase inhibitor, and from *Streptomyces* sp. WK-3419 as an inhibitor of gpl20-CD4 binding. Chloropeptin I, a structural isomer of complestatin, was isolated along with complestatin (Chloropeptin II). Neuroprotectins A and B, analogs with an oxindole-alanine in place of the tryptophan, have been isolated together with complestatin from *Streptomycyes* sp. Q27107 as neuroprotective agents. An antimicrobial activity of complestatin and related compounds, however, has not yet been reported. Here, we describe the isolation, FabI-inhibitory, and antibacterial activity of 1–3.

**MATERIALS AND METHODS**

**General Experimental Methods** NMR spectra were recorded on a Bruker 300 and 500 spectrometer. The electrospray ionization (ESI)–MS data were recorded with a Jeol JMS-HX110/110 A mass spectrometer. Column chromatography on silica gel (Kieselgel 60, 70–230 mesh, Merck) and Sephadex LH-20 (Amersham Biosciences) were conducted. All chemicals utilized in the study, including methanol (MeOH), ethyl acetate (EtOAc), chloroform (CHCl₃), butanol (BuOH), acetonitrile (ACN), and hexane, were of analytical grade. Triclosan, rifampin, norfloxacain, chloramphenicol, vancomycin, trfluoroacetic acid (TFA), and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, U.S.A.).

**Bacterial Strains** The actinomycetal strain AN1542 was isolated from soil collected near Gunju city, Chungcheongnam-do, Korea. The strain was identified as *S. chartreusis* based on the 16S ribosomal RNA (rRNA) sequence. The bacterial strains used in antibacterial activity were obtained from the Culture Collection of Antimicrobial Resistant Microbes of Korea (CCARM) and the Korean Collection for Type Cultures (KCTC).

**Fermentation and Isolation** Fermentation was carried out in a liquid culture medium containing soluble starch 1%, glucose 2%, soybean meal 2.5%, beef extract 0.1%, yeast extract 0.4%, NaCl 0.2%, K₂HPO₄ 0.025%, and CaCO₃ 0.2% (adjusted to pH 7.2 before sterilization). A sample of the strain from a mature plate culture was inoculated into a 500-mL Erlenmeyer flask containing 80 mL of the above sterile seed
liquid medium and cultured on a rotary shaker (150 rpm) at 28°C for 3d. For the production of the active compounds, 5mL of the seed culture was transferred into 500-mL Erlenmeyer flasks 60 flasks containing 100mL of the same medium, then cultivated for 7d at 28°C. The fermented whole medium (6L) was centrifuged at 6000rpm for 10min and then the resultant mycelium was extracted twice with 80% acetone. The extract was concentrated in vacuo to an aqueous solution, which was adjusted to pH 3.0 with 1N HCl and then extracted with an equal volume of ethyl acetate (EtOAc) twice. The EtOAc extract was concentrated to a dryness. The crude extract was subjected to SiO2 (Merck Art No. 7734.9025) column chromatography followed by stepwise elution with CHCl3–methanol (MeOH) (20:1–1:1) to give two active fractions (I and II). The active fractions (I) eluted with CHCl3–MeOH (1:1). The residue (70 mg) dissolved in CHCl3 was further purified by HPLC column (100×250mm, YMC C18) chromatography. The column was eluted with CHCl3–MeOH (20:1–1:1) to give two active fractions (I and II). The active fractions (II) eluted with CHCl3–MeOH. The residue (24 mg) dissolved in CHCl3 was further purified by HPLC column (100×250mm, YMC C18) chromatography. The column was eluted with CHCl3–MeOH (20:1–1:1) to give two active fractions (I and II). The active fractions (I) eluted with CHCl3–MeOH (1:1) were pooled and concentrated in vacuo to afford 1 (2.2 mg), 2 (1.9 mg), and 3 (1.8 mg) with retention times of 34.8, 15.8, and 50.3 min, respectively, as yellow powders.

The second active fraction (II) eluted with CHCl3–MeOH (1:1) were pooled and concentrated in vacuo. The residue (80 mg) was applied to the Sephadex LH-20 column chromatography and eluted with MeOH to give 1 (5 mg) as a yellow powder.

Compound 1: C_{61}H_{45}N_{7}O_{16}Cl_{6}; a yellow powder: [α]_{D}^{25} 22.6° (c=0.13, dimethyl sulfoxide (DMSO)); 1H-NMR (500MHz, DMSO-δ_{6}) δ: 6.86 (2H, d, J=8.0Hz, A-2, 6), 6.63 (2H, d, J=8.0Hz, A-3, 5), 4.67 (1H, brs, A-αCH), 7.88 (1H, d, J=9.0Hz, A-NH), 7.17 (1H, dd, J=2.0, 8.5Hz, B-2), 7.08 (1H, dd, J=2.0, 8.5Hz, B-3), 6.84 (1H, dd, J=2.0, 9.0Hz, B-5), 7.86 (1H, dd, J=2.0, 9.0Hz, B-6), 5.01 (1H, d, J=10.6Hz, B-αCH), 2.98 (1H, t, J=12.5Hz, B-βCH), 3.24 (1H, d, J=11.9Hz, B-βCH), 2.90 (3H, s, B-NCH3), 7.33 (2H, s, C-2, 6), 5.06 (1H, d, J=5.4Hz, C-αCH), 8.62 (1H, d, J=5.4Hz, C-NH), 5.49 (1H, d, J=2.5Hz, D-2), 5.07 (1H, d, J=2.5Hz, D-6), 5.53 (1H, d, J=9.0Hz, D-αCH), 8.21 (1H, d, J=9.0Hz, D-NH), 7.26 (2H, s, E-2, 6), 5.55 (1H, d, J=8.5Hz, E-αCH), 7.74 (1H, d, J=8.5Hz, E-NH), 10.88 (1H, s, F-1), 7.27 (1H, s, F-2), 7.45 (1H, d, J=8.5Hz, F-4), 6.81 (1H, d, J=8.5Hz, F-5), 7.24 (1H, s, F-7), 4.11 (1H, m, F-αCH), 2.86 (1H, d, J=11.7Hz, F-βCH), 3.39 (1H, t, J=12.5Hz, F-βCH), 8.27 (1H, d, J=7.0Hz, F-NH), 7.78 (2H, s, G-2, 6). 13C-NMR (125 MHz, DMSO-δ_{6}) δ: 131.4 (A-1), 127.5 (A-2, 6), 114.5 (A-3, 5), 155.6 (A-4), 171.2 (A-CO), 136.4 (B-1), 130.4 (B-2, 6), 121.7 (B-3), 125.1 (B-4), 123.4 (B-5), 123.1 (B-5), 121.6 (B-6), 168.5 (B-CO), 61.9 (B-αCH), 134.3 (B-βCH), 31.0 (B-NCH3), 131.1 (C-1), 127.2 (C-2, 6), 122.1 (C-3, 5), 149.8 (C-4), 169.2 (C-CO), 51.9 (C-αCH), 126.3 (D-1), 110.7 (D-2), 149.0 (D-3), 139.0 (D-4), 131.0 (D-5), 129.4 (D-6), 167.5 (D-CO), 54.9 (D-αCH), 131.9 (E-1), 267.2 (E-2, 6), 121.7 (E-3, 5), 149.0 (E-4), 169.5 (E-CO), 54.7 (E-αCH), 123.5 (F-2), 111.7 (F-3), 126.0 (F-4), 118.4 (F-4), 123.6 (F-5), 134.6 (F-6), 114.4 (F-7), 136.1 (F-7), 137.0 (F-CO), 56.9 (F-αCH), 28.4 (F-βCH), 127.2 (G-1), 130.6 (G-2, 6), 122.5 (G-3, 5), 166.7 (G-4), 181.6 (G-CO), 164.5 (G-αCO); high resolution-electrospray ionization mass spectrometry (HR-ESI-MS): m/z 661.5490 [M−2H]^{2+}, C_{61}H_{45}N_{7}O_{15}Cl_{6} requires 661.5480.

Compound 2: C_{61}H_{45}N_{7}O_{15}Cl_{6}; a yellow powder: [α]_{D}^{25} 11.6° (c=0.14, MeOH); 1H-NMR (500MHz, DMSO-δ_{6}) δ: 7.10 (2H, d, J=8.5Hz, A-2, 6), 6.77 (2H, d, J=8.5Hz, A-3, 5), 5.06 (1H, d, J=6.5Hz, A-αCH), 8.44 (1H, d, J=6.5Hz, A-NH), 7.19 (1H,
dd, J=2.0, 8.0 Hz, B-2), 7.15 (1H, dd, J=2.5, 8.0 Hz, B-3), 6.78 (1H, dd, J=2.0, 9.0 Hz, B-5), 7.79 (1H, dd, J=2.0, 9.0 Hz, B-6), 5.08 (1H, m, B-αCH), 3.05 (2H, m, B-βCH2), 2.99 (3H, s, B-NCH3), 7.37 (2H, s, C-2, 6), 5.18 (1H, d, J=6.0 Hz, C-αCH), 8.91 (1H, d, J=6.0 Hz, C-NH), 5.77 (H, d, J=2.5 Hz, D-2), 5.69 (1H, d, J=2.5 Hz, D-6), 5.71 (1H, d, J=9.5 Hz, D-αCH), 8.56 (1H, d, J=9.5 Hz, D-NH), 7.02 (2H, s, E-2, 6), 5.56 (1H, d, J=8.5 Hz, E-αCH), 7.10 (1H, d, J=8.5 Hz, E-NH), 10.61 (1H, s, F-1), 3.70 (1H, t, J=3.7 Hz, F-3), 7.11 (1H, d, J=8.0 Hz, F-4), 6.74 (1H, d, J=8.0 Hz, F-5), 7.65 (1H, s, F-7), 3.47 (1H, m, F-αCH), 3.11 (1H, d, J=5.0, 13.5 Hz, F-βCH2), 1.98 (1H, brd, 13.5 Hz, F-βCH3), 9.63 (1H, d, J=7.5 Hz, F-NH), 7.94 (2H, s, G-2, 6); HR-ESI-MS: m/z 669.5454 [M−2H]2−.

Compound 3: C61H45N7O15Cl6; a yellow powder; [α]D25 = −16.4° (c=0.17, DMSO); 1H-NMR (500 MHz, DMSO-d6) δ: 7.08 (2H, d, J=8.5 Hz, A-2, 6), 6.75 (2H, d, J=8.5 Hz, A-3, 5), 5.04 (1H, d, J=6.5 Hz, A-αCH), 8.40 (1H, d, J=6.5 Hz, A-NH), 7.19 (1H, d, J=8.0 Hz, B-2), 7.15 (1H, dd, J=2.5, 8.0 Hz, B-3), 6.78 (1H, dd, J=2.5, 8.5 Hz, B-5), 7.81 (1H, d, J=8.5 Hz, B-6), 5.06 (1H, m, B-αCH), 3.05 (2H, m, B-βCH2), 2.99 (3H, s, B-NCH3), 7.39 (2H, s, C-2, 6), 5.16 (1H, d, J=6.5 Hz, C-αCH), 8.79 (1H, d, J=6.5 Hz, C-NH), 5.71 (H, dd, J=2.0 Hz, D-2, D-6), 5.94 (1H, d, J=9.0 Hz, E-αCH), 8.20 (1H, d, J=9.0 Hz, E-NH), 10.58 (1H, s, F-1), 7.65 (1H, s, F-2), 7.23 (1H, d, J=8.0 Hz, F-4), 6.92 (1H, t, J=7.5 Hz, F-5), 7.07 (1H, d, J=8.0 Hz, F-6), 5.08 (1H, m, F-αCH), 3.02 (2H, m, F-βCH2), 9.05 (1H, d, J=16.0 Hz, F-NH), 7.86 (2H, s, G-2, 6); HR-ESI-MS: m/z 1326.1196 [M+H]2+. C62H59N7O15Cl6 requires 1326.1183.

Assay of FabI and FabK S. aureus FabI and S. pneumoniae FabK enzymes were cloned, overexpressed and purified as described previously.13) Assays were carried out in half-volume, 96-well microtiter plates. Compounds were evaluated in 100 µL assay mixtures containing components specific for each enzyme (see below). Reduction of the trans-2-octeno- yl N-acetylcyesteine (t-o-NAC thioester) substrate analog was measured spectrophotometrically by following the utilization of reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm at 30°C for the linear period of the assay. S. aureus FabI assays contained 50 mM sodium acetate, pH 6.5, 400 µM t-o-NAC thioester, 200 µM NADPH, and 150 nM S. aureus FabI. The rate of decrease in the amount of NADPH or reduced nicotinamide adenine dinucleotide (NADH) at 340 nm at 30°C for the linear period of the assay. S. aureus FabK assays contained 50 mM sodium acetate, pH 6.5, 400 µM t-o-NAC thioester, 200 µM NADPH, and 150 nM S. pneumoniae FabK.

Determination of Antibacterial Susceptibility The whole-cell antimicrobial activity was determined using broth microdilution as described previously.13) Most of the test strains were grown to mid-log phase in Mueller–Hinton broth and diluted 1000-fold in the same medium. Cells (107/mL) were inoculated into Mueller–Hinton broth and dispensed at 0.2 mL/well in 96-well microtiter plates. Enterococcus strains and Streptococcus pneumoniae were grown in Tryptic Soy Broth and Todd–Hewitt medium, respectively, instead of Mueller–Hinton broth. The minimum inhibitory concentrations (MICs) were determined in triplicate by serial two-fold dilutions of the test compounds. The MIC was defined as the concentration of a test compound that completely inhibited cell growth during a 24 h incubation at 37°C. Bacterial growth was determined by measuring the absorption at 650 nm using a microtiter ELISA reader.

Measurement of Inhibition of Macromolecular Biosynthesis To monitor the effects of a compound on lipid, DNA, RNA, protein, and cell wall biosynthesis, its effects on the incorporation of [1-14C]acetate (50 mCi/mmol), [2-14C]thymidine (59.8 mCi/mmol), [U-14C]uridine (539 mCi/mmol), 1-[U-14C]sorbose (329 mCi/mmol), and N-acetyl-o-[1-14C]glucosamine (58.1 mCi/mmol) into S. aureus RN4220 were measured as described previously.13) S. aureus was exponentially grown to an A650 of 0.2 in Mueller–Hinton broth. Test compounds were added to the 1-mL culture at concentrations of 0.25, 0.5, 1, 2, and 4 times the MIC for 10 min. An equal volume of DMSO solvent was added to the untreated control. After incubation with the radiolabeled precursors at 37°C for 1 h, followed by centrifugation, the cell pellets were washed twice with phosphate-buffered saline (PBS) buffer. After acetate incorporation, the total cellular lipids were extracted with chloroform–methanol–water. The incorporated radioactivity in the chloroform phase was measured using scintillation counting. For the other precursors, incorporation was terminated by adding 10% (v/v) trichloroacetic acid (TCA) and cooling on ice for 20 min. The precipitated material was collected on Whatman GF/C glass microfiber filters, washed with TCA and ethanol, dried, and counted using a scintillation counter. The inhibition of radiolabeled precursor incorporation was calculated using the following formula: % inhibition = 100% × [1−(radioactivity values of the treated samples/control (no antibacterial) values)]. In all experiments, known antibacterial agents were included as positive controls.

Supplementation of Exogenous Fatty Acids The effects of supplementation with exogenous fatty acids on the antibacterial activity of a compound were assessed as described previously.14) S. aureus was grown to mid-log phase in Luria broth (LB) medium and diluted 1000-fold in the same medium. A 100-µL aliquot of the diluted cell suspension (2×108 cells) was used to inoculate each well of a 96-microtiter plate containing 95 µL of LB medium with the test compound at the MICs. Subsequently, 5 µL of the serially diluted fatty acid solution was added, and the cell suspension was incubated at 37°C for 18 h. The bacterial growth was measured at 650 nm using a microtiter ELISA reader.

RESULTS AND DISCUSSION

Our continued screening of microbial extracts with the use of a combination of whole-cell and enzyme assays resulted in the identification of three FabI inhibitors (1–3) with potent antibacterial activity from S. chartreusis AN1542. Compound 1 was isolated together with 2 and 3 by activity-guided fractionation using EtOAc extraction, SiO2 column chromatography, Sephadex LH-20 chromatography, and HPLC from the
Examine the literature data except for the minor differences in chemical shifts of the same as those of complestatin in the same solvent in the formulas of $2$ and $3$ were confirmed by HR-ESI spectrum. The molecular formula of $2$ was also confirmed by HR-ESI heteronuclear multiple bond connectivity (HMBC) spectra. Additionally, the $[\alpha]_D$ value [+$22.6$ ($c=0.13$, DMSO)] of $1$ was similar to the literature value [+$16.3$ ($c=1.6$, DMSO)] for complestatin. Chloropeptin I and neuroprotectins have been coisolated in the complestatin-producing *Streptomyces* $1$ and chloropeptin I, respectively, in the literatures. The $13C$-NMR data of $2$ and $3$ were also similar with those of neuroprotectin A and chloropeptin I although the complete $13C$-NMR data of $2$ and $3$ were not obtained due to their tiny amount. The molecular formulas of $2$ and $3$ were confirmed by HR-ESI spectrum.

Table 1. Inhibitory Activity of Complestatin (1), Neuroprotectin A (2), and Chloropeptin I (3) on *S. aureus* FabI, *S. pneumoniae* FabK, and Growth of *S. aureus* RN4220 and *S. pneumoniae* KCTC 5412

| Compounds                   | IC$_{50}$ ($\mu$m) | MIC ($\mu$g/mL) |
|-----------------------------|--------------------|-----------------|
|                            | *S. aureus* FabI   | *S. pneumoniae* FabK | *S. aureus* | *S. pneumoniae* |
| 1                           | 0.5                | 10              | 2            | 16             |
| 2                           | 0.3                | N.T.            | 4            | N.T.           |
| 3                           | 0.6                | N.T.            | 4            | N.T.           |
| Triclosan                   | 0.6                | >100            | 0.01         | 64             |

N.T.: not tested.

Table 2. Minimum Inhibitory Concentrations (MICs) of Complestatin (1)

| Test organisms               | MIC (mg/L) | Completatin | Vancomycin | Oxacillin | Norfloxacin |
|------------------------------|------------|-------------|------------|-----------|-------------|
| *Staphylococcus aureus* KCTC 1916 |            | 1           | 0.5        | 0.25      | 0.25        |
| *S. aureus* RN 4220          |            | 2           | 1          | 0.25      | 1           |
| MRSA CCARM 3167              |            | 2           | 2          | 500       | 8           |
| MRSR CCARM 3506              |            | 2           | 0.5        | 500       | 2           |
| QRSR CCARM 3505              |            | 2           | 1          | 0.5       | 250         |
| QRSR CCARM 3519              |            | 4           | 1          | 0.5       | 125         |
| *Bacillus subtilis* KCTC 1021 |            | 0.5         | 0.1        | N.T.      | N.T.        |
| *Bacillus cereus* KCTC 1661  |            | 2           | 1          | N.T.      | N.T.        |
| *Streptococcus pneumoniae* KCTC 5412 |       | 16          | N.T.      | N.T.      | 4           |
| *Enterococcus faecalis* KCTC 5191 |       | 2           | 1          | 4         | 4           |
| *E. faecalis* KCTC 3511      |            | 1           | 2          | 8         | 4           |
| *Staphylococcus epidermidis* KCTC 3958 |     | 2           | 2          | >128      | 0.5         |
| *Salmonella typhimurium* KCTC 1926 |     | >128        | >128       | 128       | 2           |
| *Escherichia coli* CCARM 1356 |     | >128        | >128       | >128      | >128        |
| *E. coli* KCTC 1682          |            | >128        | >128       | >128      | 0.06        |
| *Pseudomonas aeruginosa* KCTC 2004 |     | >128        | >128       | >128      | 1           |
| *P. aeruginosa* KCTC 2742    |            | >128        | >128       | >128      | 0.5         |
| *Klebsiella aerogenes* KCTC 2619 |     | >128        | >128       | >128      | 0.25        |
| *Candida albicans* KCTC 7535 |            | >128        | 64         | 16        | 8           |

Table 3. The Cross Resistance between Triclosan and Complestatin (1)

| Compounds | S. aureus RN4220 | Triclosan-resistant S. aureus RN4220 |
|-----------|------------------|-------------------------------------|
| 1         | 2                | 16                                  |
| Triclosan | 0.01             | >1                                  |
| Norfloxacin | 1                | 1                                   |

Especially, the $[\alpha]_D$ values [+11.6 ($c=0.14$, MeOH) and −16.4 ($c=0.17$, DMSO), respectively] of $2$ and $3$ were also similar to the literature values [+18.0 ($c=0.024$, MeOH) and −18.8 ($c=1.6$, DMSO), respectively] for these compounds. Thus, compounds $1$, $2$, and $3$ were identified as complestatin, neuroprotectin A, and chloropeptin I, respectively (Fig. 1).

Compound $1$ potently inhibited *S. aureus* FabI in a dose-dependent fashion with an IC$_{50}$ of $0.5 \mu$m (Table 1), while showed twenty-times weaker inhibition on another reductase, *S. pneumoniae* FabK, with an IC$_{50}$ of $10 \mu$m. Also compounds $2$ and $3$ showed the similar inhibitory activity against *S. aureus* FabI with IC$_{50}$ of $0.3$ and $0.6 \mu$m, respectively. In order to determine whether $1$ inhibit the bacterial growth, the antibacterial activity against the Gram-positive and Gram-negative pathogen was evaluated (Table 2). Compound $1$ showed potent antibacterial activity against Gram-positive bacteria, such as *Staphylococci*, *Enterococci*, and *Bacilli* with MICs of $2–4 \mu$g/mL, which are
comparable to those of vancomycin. No activity, however, was observed for 1 against the Gram-negative pathogens *Escherichia coli* or *Pseudomonas aeruginosa*. Consistent with its weaker inhibition on FabK, 1 showed weaker antibacterial activity on *S. pneumoniae* with an MIC of 16 M/L (Table 1). The cross resistance between triclosan, a Fabl inhibitor, and 1 was evaluated using triclosan-resistant *S. aureus* (Table 3). Indeed, triclosan-resistant *S. aureus* were resistant to 1, while were not resistant to norfloxacin, a DNA gyrase inhibitor, as a negative control. It indicates that 1 inhibits Fabl in *S. aureus*.

To investigate the frequency of mutation to complestatin resistance, the isolation of resistant mutants was carried out. *S. aureus* RN4220 (1.59×10⁷ cells) was plated onto LB plates containing complestatin at 4 times the MIC.¹³) No resistant mutants, however, were detected.

In order to determine whether the antibacterial effect of 1 is attributable to the inhibition of fatty acid synthesis, its effects on the biosynthesis of lipids, DNA, RNA, proteins, and the cell wall were examined in *S. aureus*. Compound 1 showed an MIC of 12 M/L in the macromolecular biosynthesis assay condition of an 1-mL shaking culture. Consistent with its Fabl-inhibition, 1 blocked the incorporation of [1-¹⁴C]acetate into the membrane fatty acids in a dose-dependent fashion with inhibition of 25.1% and 86.6% at 0.5 and 1 times the MIC, respectively (Fig. 2). In contrast, the incorporation of labeled thymidine, uridine, isoleucine, and N-acetylg glucosamine into DNA, RNA, proteins, and the cell wall, respectively, was almost not inhibited at the MIC (Fig. 2, Table 3). As the positive controls, antibacterials such as triclosan, norfloxacin, rifampin, chloramphenicol, and vancomycin as inhibitors of fatty acid, DNA, RNA, protein, and cell wall, respectively, selectively inhibited their corresponding macromolecular synthesis pathway (Table 4). These data clearly indicated that 1 selectively inhibited the fatty acid synthesis in *S. aureus*.

To confirm whether the antibacterial effect of 1 is due to the inhibition of fatty acid synthesis, we examined whether *S. aureus* in medium containing 1 could grow with supplementation of exogenous fatty acids. Compared to untreated control cells, *S. aureus* RN4220 in medium containing 1 at the MIC showed no growth. However, when either saturated fatty acids (stearic acid and palmitic acid) or unsaturated fatty acid (oleic acid) at sub-antibacterial concentrations were supplemented to a final concentration of 50, 100, and 200 M, the *S. aureus* cells in medium containing 1 grew well in a dose-dependent manner (Fig. 3A). Similarly, *S. aureus* cells were rescued from the growth-inhibitory effect of triclosan by the addition of exogenous fatty acids (Fig. 3B). As a negative control, *S. aureus* in medium containing chloramphenicol, a protein synthesis inhibitor, showed no growth with supplementation of the same fatty acids (Fig. 3C). This result indicates that 1 targets fatty acid synthesis.

Complestatin was first isolated from the mycelium of *Streptomycetes lavendulae* SANK 60477 as an anticomplement agent in 1980.¹⁸) It was reisolated from the *Streptomycetes* sp. as an inhibitor of the binding of HIV gp120 to the CD4 protein and HIV replication.⁹) Complestatin is a bicyclic chlorinated hexapeptide⁵) belonging to the glycopeptide class. Glycopeptide antibiotics, such as vancomycin and teicoplanin, have unique tricyclic or tetracyclic heptapeptide aglycones, which are usually glycosylated and sometimes additionally acylated. Glycopeptide antibiotics are divided into four structural subclasses (I–IV) according to the substituents and type of residue at positions 1 and 3 of the heptapeptide backbone. Nicolaou et al. designated complestatin as a type V class of glycopeptide aglycone, which have a tryptophan in place of a phenyl group in the heptapeptide core.¹⁰) Glycopeptide antibiotics showed antibacterial activity by inhibiting the transglycosylation and/or transpeptidation steps associated with cell wall biosynthesis by binding of the heptapeptide backbone to the C-terminal 1-Lys-d-Ala-b-Ala subunit of the peptidoglycan Lipid II via five hydrogen bonds.²⁰) Aglycones of vancomycin and teicoplanin are known to retain antibacterial activity.²¹,2²) An antimicrobial activity of complestatin, however, has not yet been reported.

![Fig. 2. Dose-Dependent Effects of 1 on the Biosynthesis of DNA, RNA, Proteins, Lipids, and the Cell Wall in *S. aureus* RN4220](image)

Table 4. Comparative Effects of Complestatin (1) and Standard Antibacterials on Incorporation of Radiolabeled Precursors into *S. aureus* RN4220 at the MIC

| Compounds       | Inhibition of precursor incorporation (%) |
|-----------------|------------------------------------------|
|                 | [1-¹⁴C]Acetate | [2-¹⁴C]Thymidine | [U-¹⁴C]Uridine | 1-[U-¹⁴C]Isoleucine | N-Acetyl-d-[¹⁴C]-glucosamine |
| 1               | 87.1           | 9.8             | 27.2          | 2.9                | 25.4                        |
| Triclosan       | 88.1           | N.T.            | 9.4           | 8.6                | 1.2                         |
| Norfloxacin     | 30.1           | 74.1            | N.T.          | 9.3                | 0.7                         |
| Rifampin        | 35.4           | 3.5             | 65.4          | N.T.               | 2.6                         |
| Chloramphenicol | 7.9            | 4.5             | 3.1           | 70.9               | 18.2                        |
| Vancomycin      | 19.7           | 1.3             | 7.0           | 15.9               | 78.8                        |

N.T.: not tested.
Unlike other glycopeptide antibiotics such as vancomycin inhibiting the transglycosylation and/or transpeptidation steps involved in cell wall synthesis,\(^\text{20}\) complestatin is found to exhibit antibacterial activity by inhibiting fatty acid synthesis without affecting cell wall synthesis in this study. Interestingly, the difference in the antibacterial mechanism of complestatin from vancomycin is supported by the difference in their biosynthesis gene clusters. The biosynthesis gene clusters of vancomycin-type antibiotics contain their resistance genes (VanX, VanA, and VanH) for self-protection.\(^\text{20,23}\) However, there is no resistance gene as such in the biosynthesis gene cluster of complestatin.\(^\text{24}\)

In summary, complestatin and related compounds exhibited a potent antibacterial activity against Gram-positive bacteria including methicillin-resistance \textit{S. aureus} (MRSA) with the similar potency as vancomycin. Importantly, they showed antibacterial activity by inhibiting fatty acid synthesis which is distinct from that of vancomycin. Thus, complestatin could have potential as a useful lead compound for tackling existing drug resistance pathogens including MRSA.

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**Conflict of Interest** The authors declare no conflict of interest.

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