Secondary Metabolites Isolated From *Streptomyces* sp. MJM3055 and Their Cytotoxicity Against Jurkat Cells

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Abstract

Bacteria of the genus *Streptomyces* are used in multiple applications in the medical field owing to their ability to generate large quantities of secondary metabolites. Chromatographic purification of *Streptomyces* sp. MJM3055 led to the isolation of 1 new streptenol derivative, 1-O-acetylstreptenol A (2), along with (3E,8E)-1-hydroxydeca-3,8-dien-5-one (1), streptenol A (3), cyclo-(L-Ile-L-Pro) (4), streptazolin (5), and 7-O-acetylstreptazolin (6). The structures were elucidated by interpretation of combined mass spectrometry, circular dichroism, and 2-dimensional nuclear magnetic resonance spectroscopic data. Among these isolated compounds, compound 1 exhibited strong cytotoxic effects against Jurkat T cells.

Keywords

*Streptomyces*, secondary metabolites, streptenol derivative, jurkat T cells, cytotoxic

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Actinobacteria have been shown to be a notable source of new antibiotics and pharmaceuticals. Within the Actinobacteria phylum, *Streptomyces* is the most dominant and prolific drug-producing genus.1 The species are widely distributed in the most diverse habitats, like polar territories, deserts, highlands, insects, plant stems, and marine sediments.2-5 *Streptomyces* species have shown an outstanding capacity for the production of secondary metabolites, many of which were broadly used for the treatment of human diseases. Secondary metabolites produced by *Streptomyces* species belong to different classes of compounds; polyketides, peptides, polyketide-peptide hybrids, aminoglycosides, and polype macrolides have been characterized, with different biological activities, including antibacterial, antifungal, anticancer, and immune suppression.6-12 Based on the aforementioned properties of *Streptomyces*, in this article, we report the isolation of compounds 1-6 from *Streptomyces* sp. MJM3055, in particular, a novel streptenol derivative (2), which was isolated as a natural product for the first time; we have also elucidated their structures. In addition, we further investigated the cytotoxicity effects of these compounds.

Leukemia is a type of blood cancer that usually originates in the bone marrow and results in an abnormally high white blood cell count. Leukemia may occur in humans across different age groups, from the newborn to the elderly; however, different forms of leukemia show different age distribution patterns.13 Nevertheless, the limitations of conventional therapies for leukemia are related to their high costs and toxicity, which necessitates the development of novel biocompatible and cost-effective drugs.14 Jurkat cells, which are considered a classic acute T lymphocytic leukemia cell model, are commonly used in studies involving drug sensitivity. The antiproliferative activity of the 6 isolated compounds against Jurkat cells was analyzed using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. Among these, compound 1 exhibited a strong cytotoxic effect.

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Results and Discussion

**Phylogenetic Analysis of *Streptomyces* sp. MJM3055**

Based on the 16S rRNA sequence, *Streptomyces* sp. MJM3055 was found to be closely related to *Streptomyces chartreusis* NBRC 12753T, *Streptomyces osmaniensis* OU-63T, *Streptomyces resistomycicus* NRRL ISP-5133T, *Streptomyces chartreusis* NBRC 12753, *Streptomyces qaidamensis* S10, *Streptomyces caeruleus* NRRL B-24802, *Streptomyces aquisil* [GCR-6] MH171844, *Streptomyces cyaneus* NRRL B-2296, *Streptomyces neopeptinius* KFN 2047, *Streptomyces cytodanicus* K04-0144, *Streptomyces capoanus* JCM 4734, *Streptomyces galbus* DSM 40089, *Streptomyces longwoodensis* DSM 41677, *Streptomyces kumingensis* NBRC 14463, *Streptomyces rhizophilus* JR-41, *Streptomyces neopeptinius* KNF 2047, and *Streptomyces bungoensis* DSM 4178, with similarities of 99.38%, 99.01%, 98.83%, 98.83%, 98.78%, and 98.76%, respectively. The phylogenetic tree analysis showed that strain MJM3055 belongs to the same clade as *S. osmaniensis* OU-63T, indicating that strain MJM3055 is phylogenetically closer to *S. osmaniensis* OU-63T than to the other strains (Figure 1).

The unrooted neighbor-joining\(^{15}\) tree was constructed using the MEGA6\(^{16}\) program. The Kimura-2 parameter was used as the nucleotide substitution model. The numbers at the nodes indicate the levels of bootstrap support (%) based on a dataset resampled 1000 times. The scale bar indicates 0.002 substitutions per nucleotide position.

**Isolation and Identification of *Streptenol* Derivatives**

After repeated column chromatography of the EtOAc extracts of *Streptomyces* sp. MJM3055, 6 compounds were isolated, 2 of which were isolated as natural products for the first time (1, 2).\(^{17,18}\) The structures were identified primarily via 1-dimensional and 2-dimensional nuclear magnetic resonance (NMR) spectroscopy (correlation spectroscopy [COSY], heteronuclear single quantum correlation, and heteronuclear multiple bond correlation [HMBC]). Five known compounds were identified as (3\(E,8\)E)-1-hydroxydeca-3,8-dien-5-one (1),\(^{17,18}\) streptenol A (3),\(^{19}\) cyclo-(L-Ile-L-Pro) (4),\(^{19}\) streptazolin (5),\(^{21}\) and 7-O-acetylstreptazolin (6),\(^{22,23}\) based on comparison of the obtained spectroscopic data with those reported in the literature (Figure 2).

Compound 2 was isolated as a colorless oil, and its molecular formula was established as C\(_{12}\)H\(_{20}\)O\(_{4}\) by high-resolution time-of-flight mass spectrometry (HR-TOF-MS) (m/z 228.1356 [M+], calcd 228.1356). Analysis of the \(^1\)H and \(^{13}\)C NMR spectra of compound 2 indicated that it was a streptenol derivative.\(^{19,21}\) The spectroscopic data of 2 closely resembled those of 3 (streptenol A) but displayed additional signals for an
acetyl group (δ_H 2.00, δ_C 21.2) (Table 1). The location of the acetyl group was confirmed by HMBC from H-1 (δ_H 4.05) to COCH₃ (δ_C 170.9). The COSY spectrum indicated 2 spin systems, 1 a sequence including H-1 through H-4 and another as H-6 through H-10, which led to the establishment of the structure fragments from C-1 to C-4 and C-6 to C-10. In the HMBC spectrum, the location of the ketone was determined to be at C-5 according to the cross-peaks from H-4, H-6, and H-7 to C-5 (Figure 3). Taking into account all the spectroscopic data, we speculate that 2 was isolated as a co-metabolite with the known streptenol A (3) from the genus *Streptomyces*. In terms of the same streptenol biogenetic pathway, we tentatively assumed that the configuration of 2 was identical to that of 3 (streptenol A). The C-C double bond was postulated as E based on the large ¹H-¹H coupling constants (15.3 Hz) in deuterated chloroform between H-8 and H-9¹,₂³ (Supplemental Figure S3). Given the high similarity of the circular dichroism spectra between 2 and 3 (Supplemental Figure S10-S13), along with similar specific optical rotations ([α]_D^20 +27, ε 0.25, methanol [MeOH]; [α]_D^20 +32, ε 0.25, MeOH) and the chemical shift differences of the corresponding Mosher’s esters (Supplemental Figure S14), we tentatively propose the absolute configuration of C-3 to be S₁,₂³. Based on the above evidence, compound 2 was assigned as (3S,8E)-3-hydroxy-5-oxodec-8-en-1-yl acetate and named 1-O-acetylstreptenol A because it differs from streptenol A only with regard to the substitution of the acetyl group.

### Cytotoxic Effects of Compounds 1-6 and the EtOAc Fraction Against Jurkat Cells

The antiproliferative potential of *Streptomyces* sp. MJM3055 compounds and fractions were tested via a cell proliferation assay using the blood cancer-derived Jurkat cell line. As shown in Figure 4, compounds 1-6 showed cytotoxic effects. Among them, compound 1 exhibited the strongest cytotoxicity against Jurkat cells, in a dose-dependent manner, in comparison with the other 5 compounds. Compound 1 had a certain cytotoxic

### Table 1. ¹H (400 MHz) and ¹³C (100 MHz) Nuclear Magnetic Resonance Data for Compound 2 in Dimethylsulfoxide-d₆.

| Position | δ_C | δ_H (J in Hz) |
|----------|-----|---------------|
| 1        | 61.5| 4.05, td (7.2, 1.2) |
| 2        | 36.3| 1.67, m       |
| 3        | 64.1| 3.97, dddd (8.4, 8.4, 4.0, 4.0) |
| 4        | 50.6| 2.49, dt (7.2, 1.6) |
| 5        | 209.3|                  |
| 6        | 43.0| 2.48, t (7.8)  |
| 7        | 26.4| 2.12, qd (6.2, 1.6) |
| 8        | 130.6| 5.39, m      |
| 9        | 125.3| 5.39, m      |
| 10       | 18.2| 1.60, d (5.0) |
| 3-OH     |      |               |
| COCH₃    | 170.9|               |
| COCH₃    | 21.2| 2.00, s       |

![Figure 3. Key correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) of compound 2.](image-url)
effect at a concentration of 50 mM, but the survival rate of the Jurkat cells was reduced to less than 40% at a concentration of 100 µM. To differentiate the effect of compound 1 on selective cell death in leukemia cells from its effect on normal cells, we examined the cytotoxicity of compound 1 in human dermal fibroblasts (HDF) and CCD-1070Sk (human normal fibroblast cells). Treatment with compound 1 up to 100 µM did not lead to cell death in either of the normal cell lines (Figure 4). This observation suggested that compound 1 selectively induces apoptosis in Jurkat cells but not in normal cells. Therefore, compound 1 could be considered as a potential lead compound for the treatment of human leukemia cells.

Conclusions

In summary, this study was an extended chemical examination of Streptomyces sp. MJM3055, which led to the isolation of a new streptenol derivative (2), which further enriched the chemical diversity of the collection of secondary metabolites available from Streptomyces species. The 6 isolated compounds exhibited cytotoxic effects in Jurkat cells; in particular, compound 1 exhibited the strongest cytotoxicity at a concentration of 100 µM. The findings of this study further indicate that compound 1, isolated from Streptomyces sp. MJM3055, could be a possible lead compound for the treatment of human leukemia cells.

Materials and Methods

General Experimental Procedures

1H and 13C spectra were recorded using AVANCE-400 NMR spectrometers (Bruker, Billerica, MA, USA), and the peak positions are indicated in parts per million. In this article, 1H-NMR data are reported in the following order: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and/or multiple resonances), number of protons, and coupling constant (in Hz). HR-TOF-MS data were obtained using a JMS-T200GC AccuTOF GCxGC-TOF mass spectrometer (JEOL Ltd., Japan). Optical rotations were determined using a PerkinElmer model 343 Plus polarimeter (JASCO Corp., Japan). ECD data were obtained using a JASCO J-715 CD spectrometer (Jasco Corp., Japan), and thin-layer chromatography was conducted on Kieselgel 60 F254 plates.

Screening of Extract Library and Isolation of Streptomyces sp. MJM3055

Streptomyces sp. MJM3055 was isolated from an actinomycete metabolite natural product library using an antiproliferation assay, as described below. Among the 1000 extracts obtained from different actinomycetes, those derived from strain MJM3055 exhibited a strong antitumor activity. For the purification of active compounds, strain MJM3055 was cultured in GSS medium (Soluble starch 10g, Glucose 20g, Soybean meal 25g, Beef extract 1g, Yeast extract 4g, NaCl 2g, K2HPO4 0.25g, CaCO3 2g, Distilled water 1L, pH 7.2) to facilitate large-scale fermentation. Briefly, strain MJM3055 was activated by incubating in Bennett's agar medium for 7 days. Next, 2 agar blocks (1 cm in diameter) were cut and used to inoculate 40 mL of BN broth media (Glucose 10g, Yeast extract 1g, Bactopeptone 2g, Beef extract 1g, Distilled water 1L) contained in a 250 mL baffled flask, which was used as the seed culture. The seed culture was incubated at 28 °C with shaking at 180 rpm for 48 hours and then transferred to a 2-L baffled flask containing 500 mL of GSS medium with an inoculation size of 1%. The primary culture was incubated at 28 °C with shaking at 180 rpm for 7 days. The culture broth was harvested and centrifuged; the supernatants were subjected to ethyl acetate (EtOAc) extraction for the isolation and purification of active compounds, as described below.

Phylogenetic Analysis of MJM3055

Genomic DNA was extracted and purified using the ExgeneTM cell SV kit (GeneAll). The 16S rRNA gene was amplified using the universal primer set 27F/1492R and sequenced by the BioFact sequencing company (Daejeon, Korea). Similar sequences were searched in the EzBioCloud database, a public database for prokaryotic strains (www.ezbiocloud.net). The retrieved sequences were aligned against those of the reference type strains using CLUSTALX ver. 1.8. The phylogenetic tree was constructed via the neighbor-joining method using MEGA6 software. The robustness of individual branches was evaluated by bootstrapping 1000 replications.

Extraction and Isolation of Active Compounds

The dried EtOAc extract fractions (3.0 g) obtained from Streptomyces sp. MJM3055 were separated using C18-medium pressure liquid chromatography (10% methanol to 90% methanol) to yield 5 su-fractions (A1-A5). Subfraction A3 was subjected to preparative high-performance liquid chromatography (HPLC) (using 15%-90% acetonitrile as the mobile phase), which yielded 4 subfractions (A6-A9). Compound 5 (300.1 mg) was purified from subfraction A8 using preparative HPLC (using 20%-80% acetonitrile as the mobile phase). Compound 4 (5.6 mg) was purified from subfraction A6 via preparative HPLC using 35% methanol. Subfraction A4 was subjected to preparative HPLC (methanol-water, 7:3), which yielded 8 subfractions (A10-A17). Compound 1 (8.0 mg) was separated from subfraction A13 using 35% methanol. Subfraction A14 was further purified via HPLC using 35% methanol to generate compound 6. Compound 2 (24.2 mg) was obtained from subfraction A15 via HPLC using 55% methanol, and subfraction A15 was further purified via HPLC using 20% acetonitrile to yield compound 3 (60.0 mg).

1-O-acetylstreptenol A (2): a colorless oil. [α]20° +27 (c 0.25, MeOH). 1H NMR (400 MHz, dimethylsulfoxide [DMSO]-d6) and 13C NMR (100 MHz, DMSO-d6) data are given in Table 1.
The CD spectrum is given in Supplemental Figure S10-S11. HR-TOF-MS $m/z$ 228.1356 [M$^+$] (calc for C$_{12}$H$_{20}$O$_4$, 228.1356).

**Cell Culture**

Advanced Roswell Park Memorial Institute 1640 medium supplemented with 2% fetal bovine serum and 1% penicillin-streptomycin was used to culture Jurkat cells in a 75 cm$^2$ flask. The cells were cultured in a humidified atmosphere with 5% CO$_2$ at 37 °C for 2-3 days. The cells were subcultured at a ratio of 1:4 when the cell density reached 80-90%.

**Antiproliferation Assay**

For cytotoxicity screening assays, Jurkat cells were seeded in 96-well plates at a density of $1 \times 10^5$ cells/well. The cells were then treated with the compounds at different concentrations (0, 25, 50, and 100 µM). To estimate cell viability (%) at 48 hours post-treatment with each of the compounds, the cells were incubated with 20 µL of CellTiter 96 AQueous One solution Cell Proliferation Assay reagent at 37 °C for 2 hours. The optical density of the samples was measured at 490 nm using a microplate reader (Synergy HTX, BioTek Instruments, Inc., Winooski, VT, USA). The cell growth effect was expressed as a percentage relative to that in the control.

**Statistical Analyses**

Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA), and the data are expressed in terms of the mean ± SEM. The data were further analyzed using Student’s t-test; P values <0.001 were considered statistically significant.

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**Declaration of Conflicting Interests**

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**Supplemental Material**

Supplemental material for this article is available online.

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