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YM-216391, a Novel Cytotoxic Cyclic Peptide from *Streptomyces nobilis*

I. Fermentation, Isolation and Biological Activities

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Abstract YM-216391, a novel cyclic peptide, was isolated from the cultured mycelium of *Streptomyces nobilis* JCM 4274. It was purified by solvent extraction, silica gel and ODS flash column chromatographies, followed by preparative HPLC. YM-216391 dose-dependently inhibited the growth of human cervical cancer HeLa S3 cells with an IC_{50} value of 14 nM. YM-216391 also showed potent cytotoxic activity against a human cancer cell line panel.

Keywords YM-216391, cyclic peptide, trisoxazole, cytotoxic

Introduction

In the course of screening for new anticancer drugs, *Streptomyces nobilis* JCM 4274 [1] was found to produce a novel active substance, YM-216391 (**1**, Fig. 1), which was determined using spectral analyses and chemical degradation to be a unique cyclic peptide. It possesses potent cytotoxicity against human cancer cell lines *in vitro*, and is structurally related to telomestatin (**2**, Fig. 1), which is a potent telomerase inhibitor isolated from *Streptomyces anulatus* [2], and shown to induce telomere shortening with apoptosis in myeloma and leukemia [3～5]. Additionally, some cytotoxic trisoxazole-containing macrolides that were discovered from marine organisms [6～10] have shown to target actin with high affinity and specificity [11, 12], and have the potential to function as anticancer drugs [13～15]. In this paper, we describe the fermentation, isolation, and biological activities of **1**. To evaluate the profile of its anticancer activity *in vitro*, we used a human cancer cell line panel combined with database analysis [16, 17]. The physico-chemical properties and structure elucidation of **1** are described in the following paper [18].

Materials and Methods

Microorganism

The organism was obtained from the RIKEN BioResource Center.

Cell Proliferation Assay

HeLa S3 cells were seeded in a 96-well plate at a density of

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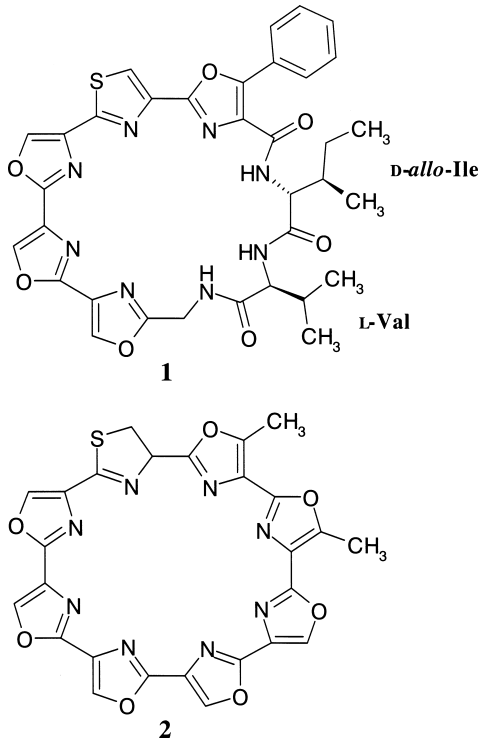

Fig. 1 Structures of YM-216391 (**1**) and telomestatin (**2**).

1～1.4×10^4 cells/well in 200 μl of MEM medium complemented with 10% fetal bovine serum. Serial dilutions of **1** were then added to wells. Control wells were treated with the vehicle (DMSO). After 72 hours of incubation at 37°C in a 5% CO_2 atmosphere, the medium in each well was removed and replaced with 100 μl of fresh medium. Cell viability was measured as mitochondrial NADH-dependent dehydrogenase activity with a Cell Counting Kit (Dojindo) using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2-disulfophenyl)-2*H*-tetrazolium monosodium salt (WST-1) [19]. Ten microliters of 5 mM WST-1 solution containing 0.2 mM 1-methoxy-5-methyl-phenazinium methosulfate (1-methoxy PMS) was added to each well. The cells were then allowed to incubate for 4 hours under the same condition as above. The absorbance of each well was measured at 415 nm, with a reference wavelength of 630 nm, using a MTP-32 microplate reader (Corona Electric).

Human Cancer Cell Line Panel

This experiment was carried out at the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research. The screening panel consisted of the following 39 human cancer cell lines: breast cancer HBC-4, BSY-1, HBC-5, MCF-7, and MDA-MB-231; brain cancer U251, SF-268, SF-295, SF-539, SNB-75, and SNB-78; colon cancer HCC2998, KM-12, HT-29, HCT-15, and HCT-116; lung cancer NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273, and DMS114; melanoma LOX-IMVI; ovarian cancer OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; renal cancer RXF-631L and ACHN; stomach cancer St-4, MKN1, MKN7, MKN28, MKN45, and MKN74; and prostate cancer DU-145 and PC-3. The GI_{50} (50% growth inhibition), TGI (total growth inhibition), and LC_{50} values for these cell lines were determined using the sulforhodamine B (SRB) colorimetric method. Computer processing of these values produced differential activity patterns against the cell lines (mean graphs). The mean graph was compared with those of standard compounds, including various anticancer drugs, by using COMPARE analysis. The detailed methods are described elsewhere [20].

Results

Fermentation

The seed medium consisted of glucose 1%, potato starch 2%, Polypeptone (Nihon Pharmaceutical) 0.5%, yeast extract 0.5%, and $CaCO_3$ 0.4% (pH 7.0). A 500-ml Erlenmeyer flask containing 100 ml of the seed medium was inoculated with a stock culture of the producing strain maintained on a Bennet's agar slant. After incubation at 28°C for 4 days on a rotary shaker set at 200 rpm, 2 ml of the seed culture was transferred to each of twenty five 500-ml Erlenmeyer flasks containing 100 ml of the production medium which consisted of potato starch 3%, yeast extract 1.5%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, and $CaCO_3$ 0.2% (pH 7.0). The fermentation was carried out at 28°C for 7 days on a rotary shaker set at 200 rpm.

Isolation

The isolation scheme for **1** is shown in Fig. 2. A mycelial cake obtained from the fermentation broth (2.5 liters) was extracted with Me_2CO. After concentrating to remove the Me_2CO, water was added to dilute any residual Me_2CO. The solution was then extracted twice with EtOAc. The organic layer was concentrated *in vacuo* and the residue was defatted by partitioning between MeOH and hexane. The MeOH layer was concentrated *in vacuo* and subjected to silica gel flash column chromatography (Kieselgel 60, 70～230 mesh, Merck) with hexane - EtOAc (1 : 1), $CHCl_3$ -

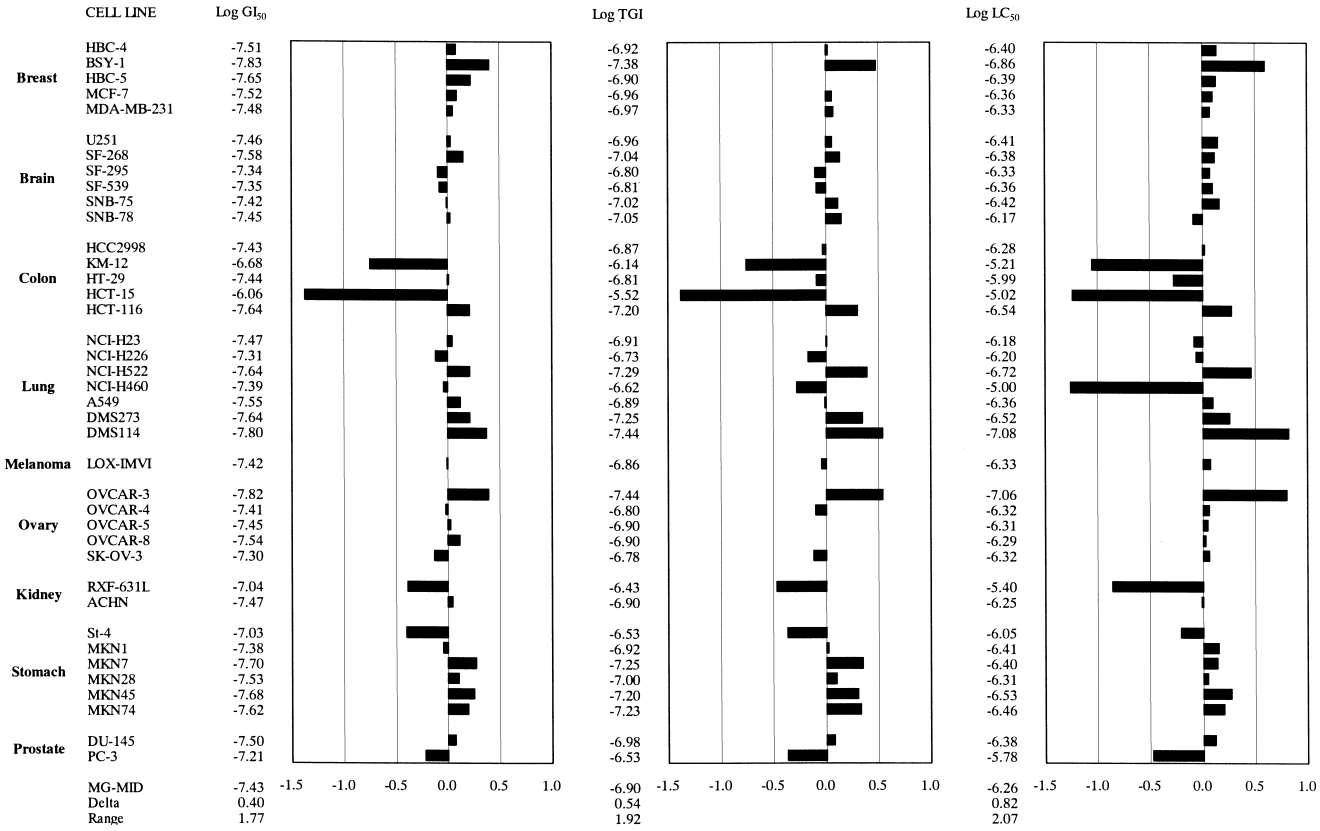

Fig. 2 Differential activity patterns for YM-216391 (**1**) against 39 human cancer cell lines. MG-MID: mean of log X values (X=GI_{50}, TGI, and LC_{50}). Delta: logarithm of the difference between the MG-MID and the log X of the most sensitive cell line. Range: logarithm of the difference between the log X of the most resistant cell line and the log X of the most sensitive cell line.

MeOH (50 : 1), and $CHCl_3$ - MeOH (20 : 1) in this order. The active fraction, eluted with $CHCl_3$ - MeOH (20 : 1), was concentrated *in vacuo* and then applied to a reverse-phase flash column (YMC * GEL ODS-A 120-230/70, YMC) with a step gradient of aqueous MeOH. The active fractions, eluted with 70% and 80% aqueous MeOH, were combined, evaporated to a small volume, and recrystallized by adding MeOH. Final purification was achieved by repeated preparative HPLC (PEGASIL ODS, 250×20 mm, Senshu Scientific) with 85% aqueous MeOH to yield 70 mg of **1** as a white powder.

Table 1 List of the compounds with growth inhibitory patterns similar to that of YM-216391 (**1**).

Rank	Compounds	Correlation coefficient with **1**	Molecular targets/ Drug type
1	Actinomycin D	0.727	DNA-dependent RNA polymerase inhibitor
2	FR901228	0.718	Histone deacetylase inhibitor
3	Epirubicin	0.617	DNA intercalater

Biological Activities

We examined the inhibitory activity of **1** against the growth of human cervical cancer HeLa S3 cells using the WST-1 colorimetric method as described in the materials and methods section. The compound dose-dependently inhibited the growth of HeLa S3 cells with an IC_{50} value of 14 nM. To investigate the profile of its anticancer activity *in vitro*, we used a human cancer cell line panel combined with COMPARE analysis. The differential activity patterns for **1** against 39 human cancer cell lines (mean graphs) are shown in Fig. 2. The compound exhibited potent cytotoxic activity, and its means for log GI_{50}, log TGI, and log LC_{50} values were −7.43 (37 nM), −6.90 (126 nM), and −6.26 (550 nM), respectively. In addition, **1** showed slightly weak differential activity, with the BSY-1, DMS114, and

OVCAR-3 cell lines being more sensitive than other cell lines. The colon cancer KM-12 and HCT-15 cell lines were more resistant than other cell lines. The COMPARE analysis of **1** revealed that its mean graph did not match that of a standard anticancer drug. The three drugs ranked in the order of their correlation coefficients are shown in Table 1.

Discussion

A human cell line panel screening can potentially produce several results. Firstly, the panel may be used to identify compounds which possess the ability to effect the growth of human cancer cell lines. The compound **1** was observed to inhibit the cell growth of these cell lines (GI_{50} and TGI values) and cause cell death (LC_{50} value). This suggests that the treatment of human cancer cells with **1** may result in apoptosis. Additionally, if the mean graph of the test compound can be matched to that of a standard anticancer drug, then the potential target or mechanism of action may be identified. The COMPARE analysis of **1** produced no strong correlation to its mean graph, but showed correlations with actinomycin D, FR901228, and epirubicin. This result indicates two alternative possibilities: first, **1** has a unique mode of action; second, **1**'s mode of action involves that of these standard anticancer drugs. In spite of the correlation with FR901228, **1** exhibited no inhibitory activity against histone deacetylase (HDAC) even at a dose of 10 μM (data not shown). Meanwhile, based on the structural relationship with telomestatin (**2**), **1** may exhibit inhibitory activity against telomerase. Interestingly, according to the report [21], **1**'s growth inhibitory effects on MCF-7 and OVCAR-3, and SK-OV-3 cell lines were 50～250 times greater than those of **2**. It is unclear whether this is due to the difference in the mode of action between **1** and **2**. Further pharmacological studies and an investigation of the mechanism of action are now underway.

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