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Myxochelin A, a cytotoxic antibiotic from the myxobacterium *Angiococcus disciformis*

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SUMMARY

In the course of screening for new anticancer antibiotics from myxobacteria, strain JW357 was found to produce an antibiotic that was active against several human cancer cell lines. This strain was identified as *Angiococcus disciformis* by morphological and cultural characteristics. The antibiotic produced was identified as myxochelin A. It demonstrated significant cytotoxicity against certain human cancer cells with IC_{50} values ranging 1.15 to 2.36 μg/ml. Myxochelin A was interestingly as active against multidrug-resistant CL02 cells as against the sensitive parental cells (HCT15).

Key words: Myxochelin A; *Angiococcus disciformis;* Myxobacteria; Cytotoxic; Human cancer cells

INTRODUCTION

Myxobacteria have proved to be a promising source of biologically active compounds. For example, new active substances that influence the growth of microorganisms (Gerth *et al.*, 1995; Takayama *et al.*, 1988), cancer cells (Kim *et al.*, 1991), and the replication of viruses (Trowitzsch-Kienast *et al.*, 1992) have been isolated from these organisms. One of the most significant examples is epothilones (Gerth *et al.*, 1996), which have already attracted the attention of many researchers (Cameron and Paterson, 1997; Wessjohann, 1977). The current authors previously conducted a search for new compounds from myxobacteria using chemical and biological screening, and reported on a novel bithiazole-type compound, KR-025 from the myxobacterium *Myxococcus fulvus* as a new cytotoxic compound (Ahn *et al.*, 1999).

During further search for cytotoxic compounds from myxobacteria, the authors encountered a strain of *Angiococcus* from a soil sample collected at vineyard in Gyugsan-si, Kyungpook province which exhibited a strong activity in SRB assay using HCT human colon cancer cell. Cytotoxicity-guided chromatographic fractionation then led to the isolation of a phenolic compound, myxochelin A.

The present paper now shows that myxochelin A has a significant cytotoxic activity against certain human cancer cells.

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MATERIALS AND METHODS

Microorganism and culture conditions

The producing strain JW357 was originally isolated from a soil sample collected at vineyard in Gyugsan-si, Kyungpook province, Korea, and identified as *Angiococcus disciformis* according to Bergeys Manual of Determinative Bacteriology 9th edition. The isolated strain exhibited fruiting bodies with small sporangioles and myxospores were ovoid (Fig. 1). Stock cultures were kept on yeast agar plate (VY/2 agar; Reichenbach *et al.*, 1992). The organism was inoculated from the yeast agar plates into 300-ml Erlenmeyer flasks containing 50 ml of a seed medium [Casitone (Difco) 0.3%, soluble

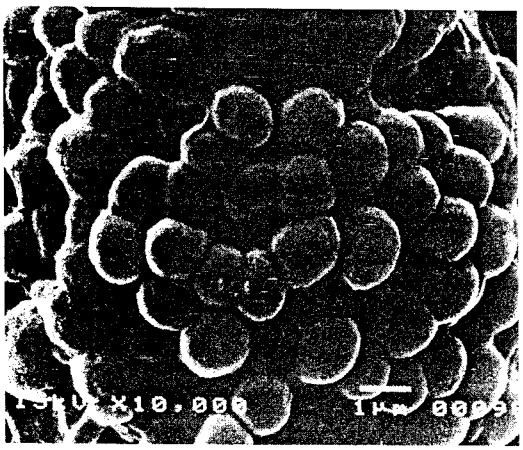

Fig. 1. Scanning electron micrograph of *Angiococcus disciformis* JW357 cultured in VY/2 agar medium.

starch 0.1%, $CaCl_2 2H_2O$ 0.07%, $MgSO_4 7H_2O$ 0.2%, cyanocobalamin 0.5 mg/L, agar 1.5%, pH 7.2]. The flasks were shaken on a rotary shaker (150 rpm) for 2 days at 30°C. 20 ml of the seed culture thus obtained was inoculated into a 2-L Erlenmeyer flask containing of a production medium. The production medium was the same as the seed medium except that 1.5% (w/v) adsorber resin Amberlite XAD-16 (Aldrich Co.) was added to promote the productivity of the active substances. The fermentation was performed on a rotary shaker (150 rpm) at 30°C for 5 days.

Extraction and isolation

The bacterial cells and adsorber resin were collected from a 7 liter culture broth and extracted with acetone. After removing the solvent, the aqueous solution was extracted with ethyl acetate (EtOAc, 200 ml). The EtOAc layer was evaporated *in vacuo*, and the residue applied to a silica gel column (2.5 i.d. ×35 cm), eluted with CH_2Cl_2 (800 ml), CH_2Cl_2-acetone (90:10, 800 cm), and CH_2Cl_2-acetone-MeOH (90:5:5, 800 ml). The fraction eluted with CH_2Cl_2-acetone-MeOH (90:5:5) was chromatographed on a RP-18 column (2.5 i.d. ×18 cm) with 50% aq. MeOH followed by purification in a recycling preparative HPLC (column: JAIGEL-ODS, mobile phase: MeOH, flow rate: 4 ml/min) to yield a pure active compound (**1**).

Identification of the active compound

The optical rotation was determined using an AUTOPOL III automatic polarimeter. The UV spectra were recording using a Shimadzu UV265 spectrophotometer, and IR spectra, by a Genesis II FTIR spectrometer. The NMR data, including COSY, NOESY, DEPT, HMBC and HMQC were taken using a Bruker AMX500 FT-NMR spectrometer (^1H, 500MHz; ^{13}C, 125MHz). The chemical shifts are reported in ppm using TMS as the internal standard. The MS (low and high resolution) were recorded using a Micromass AutoSpec spectrometer equipped with a OPUS (v 3.5X) data system.

The compound (**1**): brownish oil; $[\alpha]_D$ 25.8° (*c* 0.6, MeOH); UV(MeOH) λ_{max} (log ε): 210 (4.9), 249(4.5), 313(4.1); IR(film) ν_{max}: 3352(br.), 1638, 1586, 1545, 1262 cm^{-1}; EIMS m/z 404[M^+], 386, 372, 342, 268, 137, 105; ^1H NMR (acetone-d_6, 500 MHz): δ 8.21(1H, br t, -NH), 7.81(1H, br d, -NH), 7.29(1H, br d, J=8.5 Hz), 7.21(1H, br d, J=8.5 Hz), 6.95(2H, dd, J=8.5, 2.0 Hz), 6.69(2H, t, J=8.5 Hz), 4.19(1H, m), 3.66(2H, d, J=5.0 Hz), 3.43(2H, m), 1.70(2H, m), 1.69(2H, m), 1.51(2H, m); ^{13}C NMR (acetone-d_6, 125 MHz): δ171.2(s), 171.1(s), 150.9(s), 150.8(s), 147.4(s), 147.2(s), 118.8(d), 118.7(d), 118.6(d), 118.5(d), 117.6(d), 117.3(d), 115.3(s), 115.2(s), 64.5(t), 52.3(d), 39.7(t), 31.0(t), 29.6(t), 23.9(t)

Cytotoxicity assay

13 human cancer cell lines except CL02 cell were kindly provided by the US National Cancer Institute (NCI) and maintained as stocks in RPMI1640 medium. CL02 (a resistant subline of HCT15 to doxorubicin) was established by one of the current authors (Choi *et al.*, 1996). The cytotoxicity was assessed using a sulforhodamine B (SRB)-assay (Skehan *et al.*, 1990). The cells were cultured with an RPMI1640 medium containing 5% fetal bovine serum under 5% CO_2 at 37°C for 72 hr, at which time the SRB was added. The results are expressed as the IC_{50}, which was the drug concentration required to inhibit cell proliferation (absorbance at 520 nm) to 50% of that of the untreated control cells. Etoposide (Sigma) was used as a reference compound.

RESULTS AND DISCUSSION

Isolation and identification of active substance

The EtOAc and water partition of the acetone extract of the bacterial cells and the adsorber resin

were tested for their cytotoxic activity against HCT human colon cancer cell. The active EtOAc fraction was subjected to silica gel and then RP-18 column chromatography to afford an active compound. The compound was finally purified using a recycling preparative HPLC and obtained as an oil (**1**, 18 mg). The SRB assay indicated that this active compound also had a significant cytotoxicity against several human cancer cell lines.

The compound (**1**) gave a positive color reaction on treatment with an $FeCl_3$ reagent. ^1H and ^{13}C NMR, DEPT, and mass spectroscopy established a molecular formula of $C_{20}H_{24}N_2O_7$ and molecular weight of 404. An IR absorption band at 3352 cm^{-1} and fragment ion at *m/z* 386 in EIMS indicated the presence of a hydroxyl group in the molecule. The ^{13}C NMR , DEPT and HMQC spectra showed the presence of 20 carbon signals including two amide carbonyls, one oxymethylene, and 12 aromatic carbons. In the ^1H NMR spectrum, two amide protons were clearly evident at 7.81(br. d)and 8.21 (br. t) ppm, suggesting that the nitrogens were attached to CH and CH_2 groups, respectively. ^1H-^1H COSY and HMBC experiments revealed the existence of a 1-hexanol moiety and 2,3-dihydroxybenzoyl group in the molecule, which was also supported by the UV spectrum. The structural elucidation of the active compound showed that **1** was identical to the previously known myxochelin A (Kunze *et al.*, 1989).

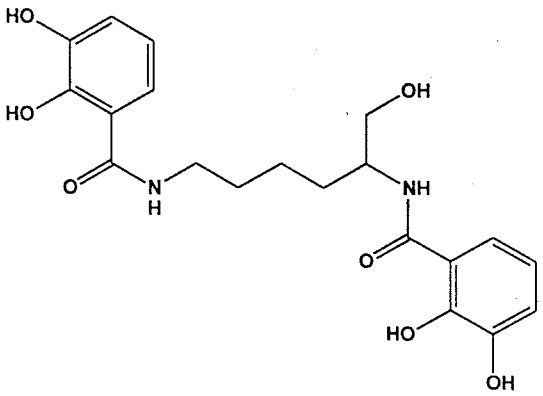

Fig. 2. Chemical structure of myxochelin A.

Cytotoxicity

Myxochelin A was tested for *in vitro* cytotoxicity against several human cancer cells using etoposide as the reference. The results are summarized in Table 1. Myxochelin A demonstrated a significant cytotoxicity against the human cancer cell lines with IC_{50} values ranging 1.15 to 2.36 μg/ml. This compound was interestingly as active against drug-resistant subline CL02 as against parental HCT15.

Myxochelin A is a phenolic compound with the structure *N,N*-bis-(2,3-dihydroxybenzoyl)-lysinol (Fig. 2). The compound was previously isolated as a siderophore (iron-chelating compound) from a culture broth of the same species of myxobacteria and shown to have a weak activity against several Gram-positive bacteria (Kunze *et al.*, 1989), however, this is the first report that myxochelin A includes cytotoxic activity against human cancer cells.

Further investigations are currently in progress as regards the possible development of a new type of anticancer agent in myxochelin A derivatives.

Table 1. Cytotoxic effects of myxochelin A(**1**) and etoposide(**2**) on human cancer cell lines

Cell lines	Origin	IC_{50}[a] [μg/ml]		Cell lines	Origin	IC_{50} [μg/ml]	
		1	2			1	2
U251	CNS carcinoma	1.53	NT	SK-MEL-5	Melanoma	1.22	NT
SNB-75	CNS carcinoma	1.84	NT	SNB-19	Melanoma	1.52	NT
SNB-78	CNS carcinoma	2.36	NT	M-14	Melanoma	1.15	NT
XF-498	CNS carcinoma	1.81	0.69	M-19-MEL	Melanoma	1.24	NT
MALME-3M	Melanoma	1.20	NT	LOXIMVI	Melanoma	1.38	NT
SK-MEL-2	Melanoma	1.27	0.97	HCT-15	Colon carcinama	2.16	2.52
SK-MEL-28	Melanoma	1.57	NT	CL02[b]	Colon carcinama	2.24	NT

[a]IC_{50}: Drug concentration inhibiting 50% of cell growth.
[b]CL02: Doxorubicin-resistant HCT-15 subline.
NT: Not tested.

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