# KR025, a New Cytotoxic Compound from *Myxococcus fulvus*

Jong-Woong Ahn,*,† Sung-Hee Woo,† Chong-Ock Lee,† Kwang-Yun Cho,† and Byung-Sup Kim‡

*Korea Research Institute of Chemical Technology, P.O. Box 107, Taejon 305-600, Korea, and Department of Horticulture, Kangnung National University, Kangnung 201-702, Korea*

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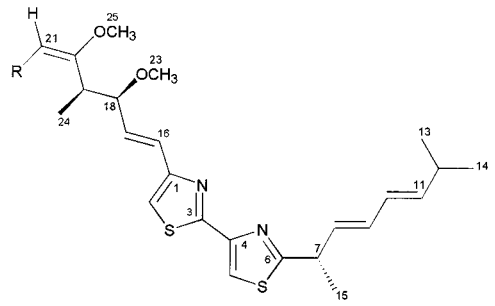
A new bithiazole, KR-025 (**1**), was isolated from *Myxococcus fulvus*. Its structure was elucidated by spectroscopic analysis. In addition to **1**, the strain produced relatively large quantities of a second, closely related antibiotic, myxothiazol. These compounds demonstrated potent cytotoxicity against human tumor cells.

The myxobacteria have proved to be a rich source of novel natural products, and a variety of biologically active substances are produced by different myxobacterial species.[1] For example, new active substances that influence the growth of microorganisms,[2−4] tumor cells[5,6] and the replication of viruses have been isolated from these organisms.[7]

In the course of screening for new antitumor antibiotics from myxobacteria, strain JW025 was found to produce two closely related antibiotics that were active against several human tumor cell lines. This strain was identified as *Myxococcus fulvus* (Myxococcaceae) by morphological and cultural characteristics.[8] The major component of the antibiotics produced was identified as myxothiazol,[9,10] while the other component, KR025 (**1**), was structurally related to myxothiazol. In this paper, we describe the isolation and characterization of **1**, as well as the cytotoxicities of these compounds to human tumor cell lines.



**1.** R=$CO_2CH_3$

Myxothiazol. R=$CO_2NH_2$

Isolation and culture of the myxobacterium was carried out by a general procedure that has been described elsewhere.[11] The cytotoxic activity was observed in acetone extracts of the cell mass. After partitioning the acetone extract between *n*-BuOH and water, the cytotoxic *n*-BuOH fraction was purified by repeated Si gel column chromatography followed by Sephadex LH-20 column chromatography to give **1** and myxothiazol. These active compounds were finally purified by recycling preparative HPLC. The molecular formula of **1** was determined to be $C_{26}H_{34}N_2O_4S_2$ by HREIMS. This molecular formula was also supported by NMR data. The IR spectrum showed the presence of an $\alpha,\beta$-unsaturated ester (1715 $cm^{-1}$).

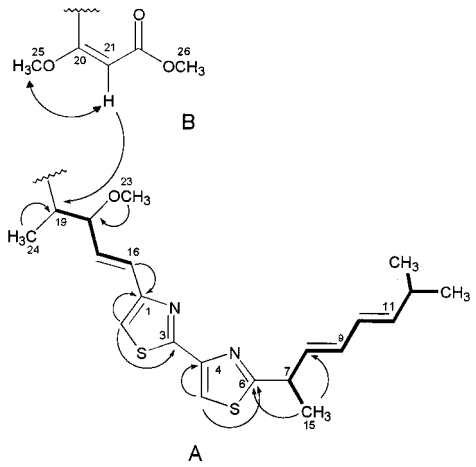


**Figure 1.** Partial structures A and B of **1**. Solid lines indicate $^1H$ spin systems identified from COSY data, and arrows indicate selected, diagnostic $^1H-^{13}C$ long-range correlations observed in the HMBC spectrum. Double headed arrow indicates ROE.

The UV and NMR spectra of **1** and myxothiazol were very similar. Detailed analyses of $^1H$ and $^{13}C$ NMR, $^1H-^1H$ COSY, and HSQC spectra, as well as an HMBC spectrum, indicated that C-1−C-19 in **1** was the same as myxothiazol. This identity in **1** was confirmed by the ion ($[M]^+$, $C_7H_{11}O_3$) at *m/z* 359 in the EIMS and was also supported by the UV absorption maxima at 231 nm (log $\epsilon$ 4.69) and 311 nm (log $\epsilon$ 4.08). Assignment of the carbons (C-20−C-26) was based on extensive HSQC and HMBC data. The individual spin systems were connected (C-19−C-20) based on HMBC correlations from $H_3$-24 to C-19 and C-20, and by correlation from H-21 to C-19. The ROE observed between H-21 and $H_3$-25 established the geometry as 20-*E*, as illustrated in Figure 1.

The relative configurations at positions 7, 18, and 19 of **1** are likely to be the same as those of myxothiazol, because the $^1H$ coupling patterns of H-7, H-18, and H-19 were identical with those of myxothiazol.

Myxothiazol and **1** were tested for *in vitro* cytotoxicity against several human tumor cells using antimycin A as a reference. The results were summarized in Table 1. Both myxothiazol and **1** demonstrated potent cytotoxicity against human tumor cells, having $IC_{50}$ values ranging from 0.01 to 9.7 ng/mL. Against human tumor cells such as SK-OV-3, the activity of **1** was more than 100 000 times stronger than that of antimycin A in terms of $IC_{50}$.

## Experimental Section

**General Experimental Procedures.** Optical rotations were determined on a AUTOPOL III automatic polarimeter.

* To whom correspondence should be addressed. Tel.: 82-42-860-7164. Fax: 82-42-860-7160. E-mail: jwahn@pado.krict.re.kr.
† Korea Research Institute of Chemical Technology.
‡ Kangnung National University.

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**Table 1.** Cytotoxicities of **1**, Myxothiazol, and Antimycin A (reference)

| | $IC_{50}$ (ng/mL) | | | | |
|---|---|---|---|---|---|
| compound | A-549 | SK−OV-3 | SK-MEL-2 | XF-498 | HCT-15 |
| **1** | 0.02 | 0.04 | 8.5 | 0.03 | 0.06 |
| myxothiazol | 0.01 | 0.01 | 9.7 | 0.03 | 0.03 |
| antimycin A | 480 | 5500 | 270 | 390 | 410 |

UV spectra were recorded on a Shimadzu UV 265 spectrophotometer; and IR spectra, on a Genesis II FTIR spectrometer. NMR data, including COSY, ROE, DEPT, HMBC, and HSQC, were taken on a Varian UNITY 500 spectrometer ($^1$H, 500 MHz; $^{13}$C, 125 MHz). Chemical shifts are reported in parts per million relative to the solvent ($CDCl_3$, $\delta_H$ 7.24, $\delta_C$ 77.0). EIMS (low and high resolution) were recorded on a Micromass AutoSpec spectrometer equipped with a OPUS(V 3.5X) data system. A recycling preparative HPLC was used for separation of the mixture. The column employed was a Jaigel-ODS (2.2 cm i.d. × 20 cm), and solvents were HPLC grade. UV detection was at 254 nm.

**Organism and Culture Conditions.** *M. fulvus*, designated strain JW025, was isolated from a soil sample collected at Taejon, Korea. The culture is deposited in the Korean Collection for Type Culture with the accession number KCTC 0337. This organism was cultivated in 2-L Erlenmeyer flasks containing 600 mL of a medium consisting of Casitone (Difco) 0.3%, $MgSO_4 \cdot 7H_2O$ 0.2%, and $CaCl_2 \cdot 2H_2O$ 0.05%. Prior to autoclaving, the pH of the medium was adjusted to 7.2 with KOH. The flasks were incubated at 30 °C for 3 days on a rotary shaker at 160 rpm.

**Extraction and Isolation.** The cells, which were harvested by centrifugation from the whole broth (6 L), were extracted with $Me_2CO$. The aqueous solution, after removal of $Me_2CO$, was extracted with 1 L of *n*-BuOH. The organic layer was evaporated in vacuo, and the residue was applied to a Si gel column (2.5 i.d. × 30 cm, Kieselgel 60, Merck), eluted with hexane−EtOAc (9:1, 500 mL), hexane−EtOAc (1:1, 500 mL), and EtOAc (500 mL). Two active fractions were obtained from this extraction procedure. The fraction that eluted with hexane−EtOAc (1:1) was chromatographed on a Sephadex LH-20 column (2.5 i.d. × 80 cm) with MeOH followed by purification on recycling preparative HPLC (column; Jaigel-ODS, mobile phase; MeOH, flow rate; 2.5 mL/min) to yield compound **1** (9.8 mg). Purification of the EtOAc fraction was achieved using preparative TLC (Merck, Kieselgel $60F_{254}$, 1.0 mm), which was developed with $C_6H_6$−EtOAc (3:7) to yield myxothiazol (25.5 mg).

**KR025 (1):** colorless oil; $[\alpha]^{25}_D$ +152°(*c* 0.67, MeOH); UV (MeOH) reported in text above; IR (KBr) $\nu_{max}$ 3110, 1715, 1624, 1147, 1095 cm$^{-1}$; EIMS *m*/*z* 502 $[M]^+$ (10), 487 (6), 455 (3), 359 (100), 343 (7), 279 (6); $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 7.88 (1H, s, H-5), 7.10 (1H, s, H-2), 6.57 (1H, s, H-16), 6.41 (1H, dd, $J$ = 15.5, 8.1 Hz, H-17), 6.19 (1H, dd, $J$ = 15.3, 10.2 Hz, H-9), 6.03 (1H, dd, $J$ = 15.3, 10.2 Hz, H-10), 5.80 (1H, dd, $J$ = 15.1, 7.7 Hz, H-8), 5.69 (1H, dd, $J$ = 15.3, 7.0 Hz, H-11), 4.96 (1H, s, H-21), 4.19 (1H, m, H-19), 3.94 (1H, m, H-7), 3.81 (1H, t, $J$ = 8.4 Hz, H-18), 3.67 (3H, s, OMe-26), 3.61 (3H, s, OMe-25), 3.32 (3H, s, OMe-23), 2.32 (1H, m, H-12), 1.54 (3H, d, $J$ = 7.0 Hz, Me-15), 1.20 (3H, d, $J$ = 6.8 Hz, Me-24), 1.01 (6H, d, $J$ = 6.7 Hz, Me-13, Me-14); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 176.7 (s, C-20), 176.2 (s, C-6), 167.7 (s, C-22), 162.5 (s, C-3), 154.4 (s, C-1), 148.9 (s, C-4), 142.4 (d, C-11), 132.5 (d, C-8), 131.8 (d, C-9), 131.6 (d, C-17), 126.5 (d, C-10), 125.6 (d, C-16), 115.5 (d, C-5), 115.0 (d, C-2), 91.1 (d, C-21), 84.4 (d, C-18), 57.0 (q, C-23), 55.5 (q, C-25), 50.8 (q, C-26), 41.2 (d, C-7), 39.8 (d, C-19), 31.1 (d, C-12), 22.2 (q, C-13, C-14), 20.8 (q, C-15), 14.1 (q, C-24); HREIMS *m*/*z* 502.1945 (calcd for $C_{26}H_{34}N_2O_4S_2$, 502.1960).

**Cytotoxicity Assay.** Cytotoxicity was assessed using the sulforhodamine B (SRB)-assay[12] using human tumor cell lines of A-549 (human lung carcinoma), SK-OV-3 (human ovary carcinoma), SK-MEL-2 (human melanoma), XF-498 (human CNS carcinoma), and HCT-15 (human colon carcinoma). The cells were incubated at 37 °C for 72 h, at which time the SRB was added. The results are expressed as an $IC_{50}$, which is the drug concentration required to inhibit cell proliferation (absorbance at 520 nm) to 50% of that of untreated control cells.

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