Bithiazole Metabolites from the Myxobacterium *Myxococcus fulvus*

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Received October 11, 2006; accepted November 24, 2006

Two new bithiazole metabolites (3, 4) along with the previously reported compounds (1, 2) were isolated from the culture broth of the gliding bacterium *Myxococcus fulvus* collected from a Korean soil. The structures of these compounds were determined to be analogous to myxothiazole A on the basis of combined spectroscopic analyses. The new compounds exhibited significant cytotoxicity and moderate antifungal activity against the mouse fibloblast cell-line L929 and *Candida albicans*, respectively. These data were consistent with the previous finding that the bioactivity of myxothiazoles was highly dependent on the presence of terminal methoxy enol functionality.

Key words myxobacterium; myxothiazole; antifungal activity; cytotoxicity

The myxobacteria have produced a wide variety of structurally unique secondary metabolites.[1)] Of the myxobacteria-derived metabolites, bithiazole-containing compounds *e.g.* myxothiazoles,[2—4)] cystothiazoles,[5,6)] and melithiazoles,[7)] exhibit potent antimicrobial activity, cytotoxicity and inhibition of mitochondrial respiration which have attracted considerable attention for synthetic, biochemical, and related studies.[8—13)]

During the course of our search for bioactive compounds from myxobacteria of Korean soil, we collected a strain of *Myxococcus fulvus* (strain number JW484) whose crude organic extract exhibited significant cytotoxicity toward the mouse fibroblast cell-line L929. The culture broth of this strain contained several compounds containing a bithiazole moiety as a common structural feature. Herein we describe the isolation, structure determination, and bioactivity of two new compounds of the myxothiazole class.

Isolation and fermentation of the microbial strain was carried out following the procedure reported elsewhere.[14)] The organic extracts from the culture broth and cell mass were prepared by solvent partitioning. Guided by the results of cytotoxicity test and ^{1}H-NMR analyses, the moderately polar fractions were separated by silica and ODS vacuum flash chromatography followed by ODS HPLC to yield compounds **1**—**4** as white amorphous solids.

Compound **1**, the major component, was elucidated to be myxothiazole A, previously isolated from *Myxococcus fulvus* collected from a tropical soil sample.[2,3)] The ^{1}H- and ^{13}C-NMR data of this compound were in good agreement with those reported previously.

The structure of a minor component, compound **2**, was also identified to be desmethylmyxothiazole, a derivative of myxothiazole A having a β-diketo functionality, on the basis of combined spectroscopic methods. This compound was previously reported as a synthetic analog of myxothiazole.[3)]

The molecular formula of compound **3** was deduced as $C_{25}H_{33}N_3O_3S_2$ by combined HR-FAB-MS and ^{13}C-NMR analyses. The spectral data for this compound were very similar to those obtained for **1**. However, detailed examination of the ^{13}C-NMR data revealed that carbon signals at C-17 and vicinity were considerably shifted from those of **1** (Table 1); δ 132.5, 131.8, 126.5, 142.4, and 31.1 for C-15—C-19, respectively, in **1**. Corresponding differences were also observed in the ^{1}H-NMR spectra in which the chemical shifts of H-15—H-19 as well as the coupling constant between H-17 and H-18 were changed noticeably; δ 5.80, 6.19, 6.03, 5.69, and 2.35 for H-15—H-19, respectively, $J_{17,18}$=15.2 Hz in **1**. These differences were readily accommodated by the configurational change of the C-17 double bond from *E* to *Z* that was supported by HMBC experiments. The relative configurations at the C-4, C-5, and C-14 of **2** were assigned to be identical to those of **1** as previously determined by chemical reaction and spectroscopic methods because the chemical shifts of carbons and protons at these asymmetric centers and vicinity of **3** were very similar to those of **1**.[15)]

The molecular formula of compound **4** was established as $C_{25}H_{33}N_3O_4S_2$ on the basis of HR-FAB-MS and ^{13}C-NMR analyses. Although the NMR data for this compound were reminiscent of those obtained for **1** and **2**, the ^{13}C-NMR spectrum indicated replacement of a double bond of **1** and **2** with an epoxide in **3**; δ_C 65.9 (CH), 57.1 (CH). A combination of ^{1}H COSY and HSQC experiments placed the epoxide

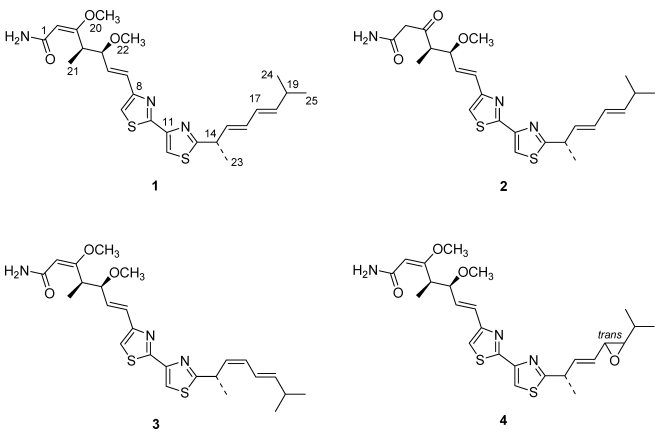

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Table 1. Proton and Carbon NMR Assignments for Compounds **3** and **4**

Position	**3**[a]		**4**[b]	
	δ_H	δ_C	δ_H	δ_C
1		169.2 (C)		168.8 (C)
2	4.94, s	94.3 (CH)	5.11, s	94.6 (CH)
3		171.7 (C)		173.0 (C)
4	4.09, dq (6.9, 6.9)	39.6 (CH)	4.47, dq (8.3, 6.8)	39.6 (CH)
5	3.81, dd (8.1, 6.9)	85.2 (CH)	3.75, dd (8.3, 8.3)	85.5 (CH)
6	6.42, dd (15.8, 8.1)	131.2 (CH)	6.43, dd (15.6, 8.3)	133.0 (CH)
7	6.57, d (15.8)	126.0 (CH)	6.58, d (15.6)	126.3 (CH)
8		154.4 (C)		155.6 (C)
9	7.12, s	115.2 (CH)	7.40, s	116.6 (CH)
10		162.6 (C)		163.1 (C)
11		148.9 (C)		150.0 (C)
12	7.86, s	115.5 (CH)	8.06, s	116.7 (CH)
13		176.2 (C)		176.0 (C)
14	3.98, dq (7.4, 7.1)	41.5 (CH)	4.00, dq (7.3, 6.8)	41.5 (CH)
15	5.86, dd (15.2, 7.4)	134.5 (CH)	6.17, dd (15.6, 7.3)	136.8 (CH)
16	6.51, dd (15.2, 11.2)	126.9 (CH)	5.49, dd (15.6, 7.8)	130.9 (CH)
17	5.89, dd (11.2, 10.2)	125.5 (CH)	3.23, dd (7.8, 2.4)	57.1 (CH)
18	5.29, dd (10.2, 10.0)	140.3 (CH)	2.62, dd (6.9, 2.4)	65.9 (CH)
19	2.78, m	27.1 (CH)	1.53, m	31.2 (CH)
20	3.58, s	55.1 (CH_3)	3.52, s	55.3 (CH_3)
21	1.18, d (6.9)	14.3 (CH_3)	1.16, d (6.8)	15.2 (CH_3)
22	3.34, s	56.7 (CH_3)	3.27, s	56.8 (CH_3)
23	1.57, d (7.1)	21.0 (CH_3)	1.52, d (6.8)	20.2 (CH_3)
24	1.00, d (6.4)	23.1 (CH_3)	0.97, d (6.4)	19.1 (CH_3)
25	0.99, d (6.5)	23.1 (CH_3)	0.94, d (6.8)	18.5 (CH_3)

a, *b*) Measured in chloroform-*d* and acetone-d_6, respectively. Assignments were aided by ^{1}H COSY, TOCSY, HSQC, and HMBC experiments.

at C-17, a conclusion that was supported by HMBC correlations of the epoxide-bearing carbons with neighboring protons; H-15/C-17, H-16/C-18, H-24/C-18, H-25/C-18. The small coupling constant ($J_{17,18}$=2.4 Hz) between the epoxide protons as well as NOE correlations H-17/H-24 and H-17/H-25 in conjunction with the lack of NOE between the epoxide protons indicated *trans* orientation for the epoxide. However, the large spatial distance between the epoxide and the nearby asymmetric center at C-14 prohibited the NOE based assignment of the relative configuration of the epoxide.

Bithiazole and related metabolites derived from myxobacteria exhibit potent cytotoxicity and antifungal activity.[16] In particular, myxothiazoles A and Z displayed LC_{50} values of sub-nanogram levels against various human tumor cell-lines that may be attributed to inhibition of cytochrome *b* in the respiratory chain.[4,8] In our measurement of cytotoxicity, compounds **1**, **3**, and **4** exhibited LC_{50} values of 3.90, 1.54×10^2, and 37.8 nM against the mouse fibroblast cell-line L929, respectively, while **2** was inactive even at the concentration of 10 μM. A similar trend was also found in the antifungal activity test in which compounds **1**—**4** displayed inhibition against *Candida albicans* (ATCC 10231) with the MIC values of 0.64, >20, 5.13, and 2.49 μM, respectively. These data are consistent with the previous finding that the bioactivities of myxobacteria-derived bithiazoles are mainly contributed from the presence of a methoxy enol functionality at the terminus that was based on the cytochrome *b* inhibition assay.[8]

Experimental

General Experimental Procedures Optical rotation was measured on a JASCO digital polarimeter using a 5 cm cell. IR spectra were recorded on a Mattson GALAXY spectrophotometer. UV spectra were recorded on a Hitachi U-3210 spectrophotometer. NMR spectra were recorded in $CDCl_3$ and acetone-d_6 solutions containing Me_4Si as internal standard, on a Varian Unity 500 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectral data were provided by the Korea Basic Science Institute, Seoul, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

Collection and Taxonomic Identification The bacterial strain JW484 was isolated from a soil sample collected at Yongjin, Korea. The strain was identified as *Myxococcus fulvus* by morphological characteristics such as the presence of deliquescent fruiting body with refractile spherical microcysts (1.5 μm or less), staining with Congo red as well as SEM imaging.[17] The strain is currently on deposit in the Microbial Collection, Korea Maritime University, under the curatorship of J.-W.A.

Fermentation The slant culture of JW484 was cultivated in 21 Erlenmeyer flasks containing 400 ml of a medium consisting of casitone (DIFCO) 0.3%, $MgSO_4 \cdot 7H_2O$ 0.2%, $CaCl_2 \cdot 2H_2O$ 0.05%, and XAD-16 1.5% (w/v) in distilled water. Prior to autoclaving, the pH of the medium was adjusted to 7.2 with KOH. The flasks were incubated at 30 °C for 5 d on a rotary shaker at 160 rpm.

Extraction and Isolation At the end of fermentation (120 l), wet cell mass and adsorbent resin XAD-16 were harvested by centrifugation and extracted with acetone (2 l×3). The aqueous layer, after removal of acetone under vacuum, was adjusted to pH 7 by KOH and twice extracted with EtOAc (1 l×2). The organic solution was dried with Na_2SO_4 and the solvent evaporated under vacuum. The brown residue was partitioned between MeOH and *n*-heptane. The MeOH layer (3.6 g) was subjected to silica column chromatography sequentially using 100% CH_2Cl_2, 10% acetone/CH_2Cl_2, 10% MeOH/CH_2Cl_2, and 100% MeOH as eluents.

The MeOH/CH_2Cl_2 fraction (450 mg) was separated by C_{18} reversed-phase vacuum flash chromatography using gradient mixtures of MeOH and H_2O as eluents (elution order: 50%, 60%, 70%, 80%, 90% aqueous MeOH, 100% MeOH), and finally acetone. The combined fractions (123 mg) eluted with 80 and 90% aqueous MeOH were separated by C_{18} reversed-phase HPLC (YMC ODS-A column, 80% aqueous MeOH) to afford in order of elution, compounds **4**, **2**, **3**, and **1** as white amorphous solids. Final purifications were then accomplished by re-HPLC under the same chromatographic conditions to yield 48.2, 3.5, 5.1, and 1.9 mg of **1**—**4**, respectively.

Myxothiazole A (**1**): IR (KBr) cm^{-1}: 3400 (br), 2960, 2930, 1730, 1670, 1600, 1455, 1290. UV λ_{max} (MeOH) nm (ε): 312 (11700), 232 (48500). FAB-MS *m*/*z*: 488.2047 $[M+H]^+$ (Calcd for $C_{25}H_{34}N_3O_3S_2$: 488.2042). $[\alpha]_D^{25}$ +40.4° (*c*=1.0, MeOH); lit.[2] +43.4° (*c*=6.0, MeOH).

Desmethyl Myxothiazole A (**2**): IR (KBr) cm^{-1}: 3400 (br), 2960, 2930, 1725, 1690, 1460, 1290. UV λ_{max} (MeOH) nm (ε): 312 (10450), 245 (31600). FAB-MS *m*/*z*: 474.1886 $[M+H]^+$ (Calcd for $C_{24}H_{32}N_3O_3S_2$: 474.1885). $[\alpha]_D^{25}$ −58.9° (*c*=0.2, MeOH); lit.[3] −22.8 (*c* unreported, MeOH).

17(*Z*)-Myxothiazole A (**3**): ^{1}H- and ^{13}C-NMR: see Table 1. IR (KBr) cm^{-1}: 3400 (br), 2960, 2930, 1725, 1680, 1460, 1290. UV λ_{max} (MeOH) nm (ε): 312 (11480), 245 (33880). FAB-MS *m*/*z*: 488.2043 $[M+H]^+$ (Calcd for $C_{25}H_{34}N_3O_3S_2$: 488.2042). $[\alpha]_D^{25}$ +77.4° (*c*=0.2, MeOH).

17-Epoxymyxothiazole A (**4**): ^{1}H- and ^{13}C-NMR: see Table 1. IR (KBr) cm^{-1}: 3400 (br), 2960, 2925, 1730, 1600, 1285. UV λ_{max} (MeOH) nm (ε): 310 (1600), 224 (8910), 208 (9700). FAB-MS *m*/*z* 526.1810 $[M+Na]^+$ (Calcd for $C_{25}H_{33}N_3O_4S_2Na$: 526.1810). $[\alpha]_D^{25}$ +28.0° (*c*=0.1, MeOH).

Acknowledgments The authors express gratitude to the Basic Science Research Institute, Seoul, Korea, for providing mass data. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (grants KRF-2005-042-E00191).

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