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BRIEF COMMUNICATION

Pyrizomicin A and B: structure and bioactivity of new thiazolyl pyridines from *Lechevalieria aerocolonigenes* K10-0216

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Abstract

Two new antibiotics, designated pyrizomicin A and B, were isolated from the cultured broth of a rare actinomycete strain, *Lechevalieria aerocolonigenes* K10-0216, by silica gel and HPLC purification. The chemical structures of pyrizomicin A and B were elucidated as new thiazolyl pyridine compounds by nuclear magnetic resonance and mass spectrometry. Pyrizomicin A and B both showed antimicrobial activity.

Our group has reported that an actinomycete strain, *Lechevalieria aerocolonigenes* K10-0216, produces new natural products, such as mangromicin A–I [1–3] and K10-0216 KA and KB [4]. Mangromicins have a unique structure, a macrocyclic pentadecane framework with a tetrahydrofuran unit and a 5, 6-dihydro-4-hydroxy-2-pyrone moiety. The mangromicins possess antitrypanosomal and radical scavenging activities. The total synthesis of mangromicin A has already been accomplished by Takada et al. [5]. K10-0216 KA and KB are compounds with a steroid skeleton moiety. K10-0216 KB shows stronger inhibition of lipid accumulation in 3T3-L1 adipocytes than that of testosterone. All these compounds were discovered from a rare actinomycete, *L. aerocolonigenes* K10-0216, by physicochemical (PC) screening. [6] Furthermore, new two compounds, designated as pyrizomicin A (**1**) and B (**2**), have now been discovered from a cultured broth of *L. aerocolonigenes* K10-0216 by further PC screening. This paper describes the fermentation, isolation, structure determination, and some biological activities of **1** and **2**.

A loop of glycerol stock of the strain K10-0216 was inoculated into 100 mL of seed medium, consisting of 2.4% starch (Wako Pure Chemical Industries Ltd., Osaka, Japan), 0.1% glucose (Wako), 0.3% peptone (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan), 0.3% meat extract (Kyokuto Pharmaceutical Industrial Co), 0.5% yeast

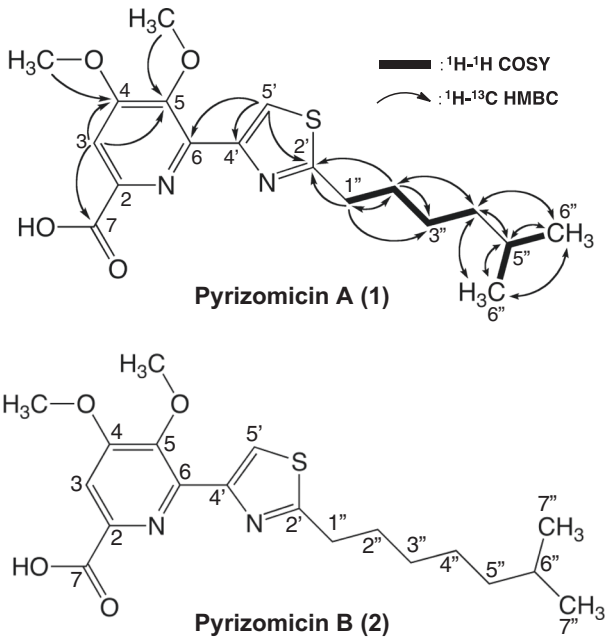

Fig. 1 Chemical structures of pyrizomicin A (**1**) and B (**2**) and 2D NMR analyses of compound **1**

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Table 1 ^{1}H and ^{13}C NMR spectroscopic data of pyrizomicin A (**1**), pyrizomicin B (**2**), and WS75624 A in CD_3OD

Position	Pyrizomicin A				Pyrizomicin B			WS75624 A		
	δ_c (ppm)	Mult.	δ_H (ppm), int., mult	HMBC	δ_c (ppm)	Mult.	δ_H (ppm), int., mult	δ_c (ppm)	Mult.	δ_H (ppm), int., mult
2	145.6	C			143.9	C		144.6	C	
3	109.2	CH	7.86, 1H, s	C-4, C-5, C-7	109.1	CH	7.84, 1H, s	109.1	CH	7.81, 1H, s
4	163.2	C			162.4	C		163.3	C	
5	147.3	C			146.8	C		147.3	C	
6	143.2	C			142.4	C		142.8	C	
7	167.2	C			166.1	C		166.5	C	
OMe on 4	57.3	CH_3	4.11, 3H, s	C-4	57.1	CH_3	4.08, 3H, s	57.4	CH_3	4.09, 3H, s
OMe on 5	61.0	CH_3	4.01, 3H, s	C-5	60.8	CH_3	4.00, 3H, s	61.0	CH_3	4.00, 3H, s
2'	173.5	C			171.6	C		173.5	C	
4'	149.1	C			148.6	C		148.6	C	
5'	123.5	CH	8.36, 1H, s	C-6, C-2', C-4'	122.6	CH	8.36, 1H, s	123.8	CH	8.32, 1H, s
1"	34.1	CH_2	3.11, 2H, t, 7.8	C-2', C-2", C-3"	34.2	CH_2	3.04, 2H, t, 7.2	34.0	CH_2	3.13, 2H, m
2"	31.6	CH_2	1.82, 2H, m	C-2', C-1", C-3", C-4"	31.8	CH_2	1.74, 2H, m	31.9	CH_2	1.85, 2H, m
3"	29.1	CH_2	1.44, 2H, m		30.7	CH_2	1.30, 2H, m	24.9	CH_2	1.54
4"	39.7	CH_2	1.26, 2H, m	C-2", C-5", C-6"	30.5	CH_2	1.30, 2H, m	44.3	CH_2	1.50, 4H, m
5"	28.0	CH	1.55, 1H, m	C-4", C-6"	40.3	CH_2	1.18, 2H, m	71.3	C	
6"	23.0	CH_3	0.89, 6H, d, 6.6	C-5", C-6"	27.6	CH	1.53, 1H, m	29.2	CH_3	1.18, 6H, s
7"					23.0	CH_3	0.88, 6H, d, 6.4			

extract (Oriental Yeast Co., Ltd., Tokyo, Japan), and 0.4% $CaCO_3$ (Wako Pure Chemical Industries) (adjusted to pH 7.0 before sterilization) in a 500 mL Erlenmeyer flask. Two flasks were incubated on a rotary shaker (210 rpm) at 27 °C for 5 days. A 1 mL portion of the seed culture was transferred to 500 mL Erlenmeyer flasks (total 120) containing 120–130 mL of defatted wheat germ medium, consisting of 2% soluble starch (Wako), 0.5% glycerol (Wako), 1.0% defatted wheat germ (Nisshin Pharma Inc., Tokyo, Japan), 0.3% meat extract, 0.3% dry yeast (JT Inc., Tokyo, Japan), and 0.3% $CaCO_3$ followed by fermentation on a rotary shaker (210 rpm) at 8 days.

An equivalent of ethanol was added to the whole cultured broth (15 L), followed by centrifugation for 10 min at 12,000 rpm. The supernatant was concentrated in vacuo to remove EtOH and then extracted with EtOAc. The EtOAc layer was concentrated in vacuo to yield 7.99 g of crude material. This material was applied on a silica gel 60 N for flash chromatography (60 i.d. ×200 mm; Kanto Chemical Co., Inc., Tokyo, Japan) and eluted with a stepwise gradient of *n*-hexane-EtOAc-$CHCl_3$-MeOH (20:1:0:0, 10:1:0:0, 5:1:0:0, 1:1:0:0, 0:0:50:1, 0:0:20:1, 0:0:10:1, 0:0:5:1, 0:0:2:1, and 0:0:0:100 (v/v), each 2.0 L) to give 10 fractions. The eluate fractions (0:0:5:1 and 0:0:2:1 fraction) were concentrated in vacuo to yield 1.38 g. A part of this material (100 mg) was purified by high-performance liquid chromatography on an Inertsil ODS-4 column (14 i.d.×250 mm; GL Sciences Inc., Tokyo, Japan) with 80% MeOH aq. containing 0.1% formic acid at 9.3 mL/min and subsequently detected at UV 220 nm. The peaks at retention time of 12.2 and 15.4 min were collected and dried in vacuo to yield **1** (5.9 mg) and **2** (1.3 mg). PC properties of **1** and **2** are summarized in Table S1.

Compound **1** was obtained as a yellowish oil and determined to have the molecular formula of $C_{18}H_{24}N_2O_4S$ by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) ($[M+H]^+$ ion at *m/z* 365.1525 (calculated value for $C_{18}H_{25}N_2O_4S$, 365.1535)) and nuclear magnetic resonance (NMR) spectral data.

The ^{1}H NMR and heteronuclear single quantum coherence (HSQC) data indicated the presence of one sp^3 methine, four sp^3 methylenes, four methyls, including two methoxy, and two sp^2 methines. The ^{13}C NMR spectrum and HSQC data showed the resonances of 18 carbons, which were classified into 8 olefinic carbons, 1 carbonyl carbon, 4 sp^3 methylene carbons, 1 sp^3 methine carbon, 2 methyl carbons, and 2 methoxy carbons.

The ^{1}H-^{1}H correlation spectroscopy indicated the presence of two partial structures C-1"/C-4" and C-5"/C-6", as shown in Fig. 1. Analysis of heteronuclear multiple bond correlation (HMBC) data confirmed the presence of 4, 5-dimethoxy-6-thiazolyl-picolinic acid moiety (a partial structure in pyrizomicins), based on correlations from H-3 to C-4, C-5, and C-7; from O-Me on 4 to C-4; from O-Me

on 5 to C-5; and from H-5' to C-6, C-2', and C-4'. The HMBC correlations from H-1" to C-2" and C-3"; from H-2" to C-1", C-3", and C-4"; from H-4" to C-2", C-5", and C-6"; from H-5" to C-4" and C-6"; and from H-6" to C-4", C-5", and C-6" confirmed the presence of an alkyl chain. The HMBC correlations from H-1" to C-2' and from H-2" to C-2' showed that the 4, 5-dimethoxy-6-thiazolyl-picolinic acid moiety and the alkyl chain were conjugated at the 2' position. Therefore, the structure of **1** was elucidated, as shown in Fig. 1, and it was designated as pyrizomicin A. It was considered that this compound has a structure similar to WS75624 A [7] but without a hydroxyl group on the alkyl chain. The presence of a 4, 5-dimethoxy-6-thiazolyl-picolinic acid moiety was supported by the chemical shift values of WS75624 A but a difference was found in the chemical shift at C-5" (Table 1).

Compound **2** was obtained as a yellowish oil and determined to have a molecular formula of $C_{19}H_{26}N_2O_4S$ by HR-ESI-MS $[M+H]^+$ ion at *m/z* 379.1685 (calculated value for $C_{19}H_{27}N_2O_4S$, 379.1692). It was deduced from the molecular formula that compound **2** is an analog of compound **1**, a methylene adduct. The structure of **2** was found to be similar to that of **1** from the chemical shifts of a 5-dimethoxy-6-thiazolyl-picolinic acid moiety (from position 2 to 7 and from 2' to 5') and hydroxy methyl groups (position OMe on 4 and 5) (Table 1). Additionally, **2** was determined to be one longer methylene than **1** from HR-ESI-MS and doublet (0.88 ppm, 6H) of methyl at the end of the alkyl chain moiety (Supplementary Figure S6 and S8). Therefore, compound **2** was determined to be an alkyl chain extended structure and named pyrizomicin B (Fig. 1).

Compounds **1** and **2** showed antimicrobial activity against *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* ATCC 9341, *Escherichia coli* NIHJ, *Xanthomonas campestris* pv. *oryzae* KB 88, and *Candida albicans* ATCC 64548, using a paper disk method at 10 µg per 6 mm paper disk, respectively. Compounds **1** and **2** showed no activity against *Mucor racemosus* IFO4581 even at 10 µg per 6 mm paper disk.

The broth microdilution method was carried out according to the method recommended by Japanese Society of Chemotherapy [8]. The minimum inhibitory concentrations (MICs) of **1** were 32 µg/mL against *Staphylococcus aureus* ATCC 6538P, *K. rhizophila* ATCC 9341, and *X. campestris* pv. *oryzae* KB 88 and 64 µg/mL against *E. coli* NIHJ. Compound **1** has no antibiotic activity against *B. subtilis* ATCC 6633 at 128 µg/mL. The MICs of compound **2** could not be evaluated due to the small quantity obtained from the isolation process.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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