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Ulbactins F and G, Polycyclic Thiazoline Derivatives with Tumor Cell Migration Inhibitory Activity from *Brevibacillus* sp.

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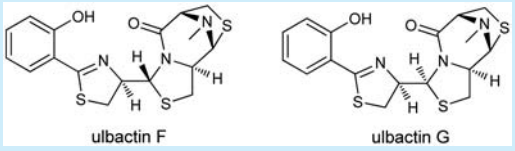
Supporting Information

ABSTRACT: Two new structurally unique compounds bearing a nitrogen- and sulfur-containing tricyclic ring system, ulbactin F (**1**) and its diastereomeric isomer ulbactin G (**2**), were isolated from the culture extract of a sponge-derived *Brevibacillus* sp. The structures and absolute configurations of **1** and **2** were determined by NMR analysis and X-ray crystallographic analysis. These compounds inhibit the migration of tumor cells in the submicromolar to micromolar range.



Despite declined interest in natural products in the pharmaceutical industry, natural products still remain a core source for the identification of novel scaffold structures that can serve as the basis for drug development.[1] Natural products are more similar to drugs than compounds obtained from combinatorial synthesis.[2] This is in part because natural products are synthesized by biological systems to specifically interact with protein targets. In addition, such drug targets may have the same or similar structural motifs as the biosynthetic proteins since the structural space of protein folds is limited.[3]

In our continuing search for novel bioactive compounds from marine bacteria,[4] we isolated ulbactins F (**1**) and G (**2**) from the culture broth of a bacterial strain of the genus *Brevibacillus* (Figure 1). The new compounds belong to the members of 2-(2-hydroxyphenyl)-2-thiazoline derivatives (Figure 1). Aerugine, a metabolite of *Pseudomonas aeruginosa*, is the smallest member of this family and is derived from the condensation of one molecule of salicylic acid and cysteine.[5] Pyochelin, a *Pseudomonas* siderophore, is an extended derivative of aerugine in which a thiazoline and a thiazolidine are tandemly connected.[6] Furthermore, ulbactin C, a derivative bearing a tandemly connected thiazoline/thiazolidine/thiazolidine ring system (see Figure 5), has been also reported from *Alteromonas*.[7] Among these thiazoline-containing metabolites, **1** and **2** are highlighted especially by their unusual heterocyclic structure in which two thiazolidine rings are fused to construct a tricyclic ring system. This unique ring system has been described only once for a bacterial metabolite, ulbactin D (see Figure 5).[7] Herein we describe the isolation, structure determination, and biological properties of **1** and **2**.

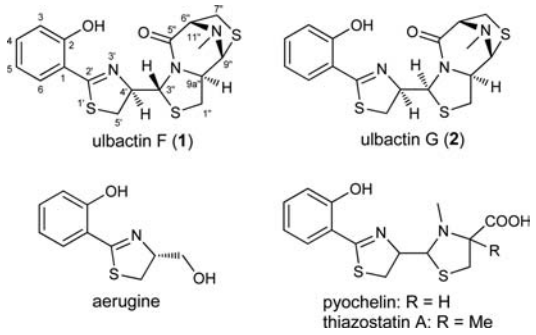


Figure 1. Structures of ulbactin F (**1**), ulbactin G (**2**), and related metabolites.

The producing strain TP-B0800 was isolated from an unidentified sponge collected in Iwate, Japan, and identified as a *Brevibacillus* sp. on the basis of 16S rRNA gene sequence similarity. Secondary metabolite production by this strain was examined by culturing in A-3M, A-11M, and A-16 media, which are routinely used for production screening in our laboratory. After cultivation, the whole culture broth was extracted with 1-butanol, and the extract was subjected to HPLC/DAD analysis. A subsequent database search using our in-house secondary metabolite library indicated the production of unknown compounds in A-3M medium that showed UV absorption maxima at 204, 251, and 316 nm characteristic of the 2-(2-hydroxyphenyl)-2-thiazoline chromophore, a ring system often seen in microbial siderophores.[8,9] Guided by HPLC/DAD analysis, several steps of chromatographic purification led to the isolation of ulbactins F (**1**) and G (**2**).

Ulbactin F (**1**) was obtained as pale-yellow crystals. High-resolution ESI-TOF-MS analysis gave a molecular formula of $C_{17}H_{20}N_3O_2S_2$, which was consistent with the ^1H and ^{13}C NMR data. The IR spectrum of **1** showed a strong absorption

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Table 1. ^{1}H and ^{13}C NMR Data for Ulbactins F (1) and G (2) in $CDCl_3$

	ulbactin F (1)			ulbactin G (2)		
position	δ_H, mult (J in Hz)[a]	δ_C[b]	HMBC	δ_H, mult (J in Hz)[a]	δ_C[b]	HMBC
1		116.4			116.4	
2		159.3			159.2	
3	6.99, d (8.1)	117.4	1, 2, 5, 2′	6.98, d (8.1)	117.3	1, 2, 5, 2′
4	7.36, ddd (8.1, 7.6, 1.4)	133.4	2, 6	7.35, ddd (8.1, 7.8, 1.4)	133.3	2, 6
5	6.88, dd (7.6, 7.6)	119.0	1, 2, 3, 6	6.88, dd (7.8, 7.7)	119.0	1, 2, 3, 6
6	7.39, dd (7.6, 1.4)	130.9	2, 4, 2′	7.40, dd (7.7, 1.4)	131.0	2, 4, 2′
2′		173.9			172.9	
4′	4.87, ddd (8.5, 7.3, 6.0)	80.8	2′, 3″	6.25, ddd (9.3, 8.7, 4.3)	75.5	1, 2′, 5′, 3″
5′	3.45, dd (11.2, 8.5)	33.9	2′, 4′, 3″	3.54, dd (10.9, 9.3)	35.2	2′, 4′, 3″
	3.42, dd (11.2, 7.3)		2′, 4′, 3″	3.12, dd (10.9, 8.7)		2′, 4′, 3″
1″	3.01, dd (10.9, 9.5)	34.7	9″, 9″a	2.99, dd (11.3, 9.9)	32.4	9″a
	2.97, dd (9.5, 5.4)		3″, 9″a	2.71, dd (9.9, 4.6)		3″, 9″a
3″	5.76, d (6.0)	60.7	5′, 1″, 5″, 9″a	4.94, d (4.3)	66.5	5′, 5″, 9″a
5″		167.9			169.3	
6″	4.04, d (6.4)	71.3	5″, 9″, 11″	4.11, d (5.9)	72.2	5″, 9″, 11″
7″	3.32, d (10.8)	35.2	5″, 6″, 9″	3.36, d (10.7)	35.5	5″, 6″, 9″a
	3.23, dd (10.8, 6.4)		5″, 6″	3.29, dd (10.7, 6.2)		5″, 6″
9″	4.62, s	73.2	1″, 6″, 7″, 11″	4.71, d (0.9)	74.0	1″, 6″, 7″, 11″
9″a	3.83, dd (10.9, 5.4)	69.5	1″, 5″, 9″	3.94, dd (11.5, 4.6)	73.7	1″, 5″, 9″
11″	2.28, s	40.2	6″, 9″	2.39, s	39.6	6″, 9″

[a]Recorded at 500 MHz. [b]Recorded at 100 MHz.

band at 1667 cm^{-1}, suggesting the presence of a carbonyl functionality. The UV spectrum displayed high similarity to those for aerugine/pyochelin-class compounds, indicative of the presence of the 2-(2-hydroxyphenyl)-2-thiazoline substructure.[7] ^{13}C NMR and HSQC analyses confirmed the presence of 17 carbons, attributable to four quaternary sp^2 carbons including three highly deshielded carbons (δ_c 159.3, 167.9, 173.9), four sp^2 methines, five sp^3 methines, three sp^3 methylenes, and one methyl carbon.

The full planar structure of **1** was assigned through interpretation of 1D and 2D NMR spectroscopic data (Table 1). From the $^{1}H-^{1}H$ COSY spectrum, four fragments were identified. The first fragment, containing four methine protons (H-3/H-4/H-5/H-6), showed a series of correlations with typical coupling constants for 1,2-disubstituted benzene protons (Figure 2). This fragment was expanded to include

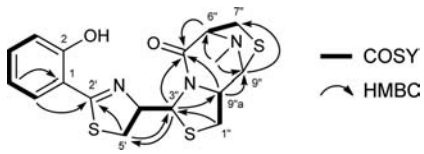

Figure 2. $^{1}H-^{1}H$ COSY and key HMBC correlations for ulbactin F (**1**).

two quaternary carbons C-1 and C-2 on the basis of HMBC correlations from H-4 and H-6 to C-2 (δ_c 159.3) and H-3 and H-5 to C-1 to complete the benzene ring and further extended to include C-2′ (δ_c 173.9) at C-1 by a long-range coupling from H-6 to C-2′, establishing a 2-substituted phenol substructure. C-2′ was also correlated to H-5′ and H-4′ in the second COSY-defined fragment (H-5′/H-4′/H-3″), indicating a connectivity of these protons to C-2′ through heteroatoms to form a five-membered heterocyclic ring. Since the carbon chemical shifts for this 2-thiazolylbenzene moiety showed close similarity to those reported for pyochelin,[6] thiazostatins,[8] and watasemycins,[9] these carbons were assigned as the thiazoline carbons, thereby establishing the 2-(2-hydroxyphenyl)-2-thiazoline substructure.

The third and the fourth COSY-defined fragments, H-1″/H-9″a and H-6″/H-7″, were assigned to constitute the tricyclic structure on the basis of the following correlations. H-1″ was long-range coupled to C-3″, and H-3″ in turn showed a correlation to C-9″a. These correlations together with chemical shift consideration allowed the determination that C-1″, C-3″, and C-9″a form a thiazolidine ring. Another thiazolidine ring was assembled starting from the HMBC correlations of the nitrogen-bonded methyl protons H-11″ to C-6″ and C-9″. HMBC correlations from H-7″ to C-9″ and H-9″ to C-7 established the sulfur bridge between C-7″ and C-9″. Further correlations from H-9″a to C-9″ and H-3″, H-6″, and H-9″a to the carbonyl carbon C-5″ allowed two thiazolidine rings to be connected between C-9″ and C-9″a and at C-6″ through C-5″ to a nitrogen bonded to C-3″ and C-9″a, completing the planar structure of **1**.

In the end, because conventional stereochemical analyses such as J-based configuration analysis and the chiral anisotropy method were not applicable, **1** was crystallized from EtOH/CH_2Cl_2 to provide orthorhombic crystals suitable for X-ray crystallographic analysis. The results confirmed the assigned planar structure of **1** and established the absolute configuration as 4′R, 3″R, 6″R, 9″R, and 9″aR (CDCC accession number 1450142; Figure 3a).

Ulbactin G (**2**), which was isolated as a pale-yellow solid, was found by interpretation of the high-resolution ESI-TOF-MS data to have a molecular formula of $C_{17}H_{20}N_3O_2S_2$, which is the same as the molecular composition of **1**, indicative of its diastereomeric relationship to **1**. The ^{1}H and ^{13}C NMR spectra were similar to those of **1** except for H-4′ and H-3″, suggesting that the difference resides around the junction part of the thiazoline and thiazolidine rings. Analysis of the COSY, HSQC, and HMBC data for **2** led to a planar structure identical to that of **1** (Figure 4 and Table 1). Crystallization of **2** was similarly

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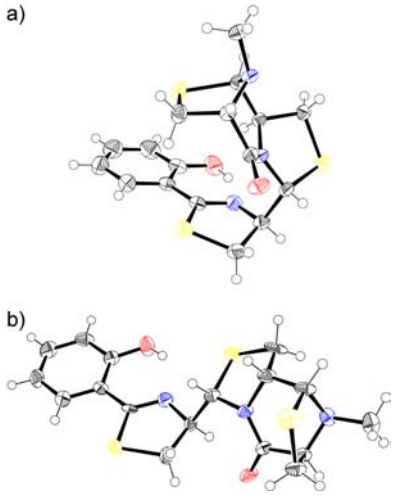

Figure 3. X-ray crystal structures of (a) ulbactin F (**1**) and (b) ulbactin G (**2**) illustrating their absolute configurations.

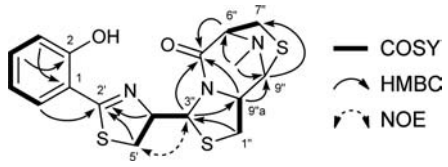

Figure 4. $^{1}H-^{1}H$ COSY and key HMBC and NOE correlations for ulbactin G (**2**).

carried out in a mixture of EtOH and CH_2Cl_2, providing orthorhombic crystals suitable for X-ray crystallographic analysis. These crystals revealed the absolute configuration of **2** with the inversed configuration at C-3″ as 4′*R*, 3″*S*, 6″*R*, 9″*R*, and 9″a*R* (CDCC accession number 1450143; Figure 3b).

The most intriguing feature of ulbactins F (**1**) and G (**2**) is the unusual 6,9-imino-1*H*,3*H*,5*H*-thiazolo[4,3-*c*][1,4]thiazepin-5-one tricyclic ring system containing two sulfur atoms and two nitrogen atoms. This ring system was previously described once (without stereochemical assignment) in a patent for ulbactin D, which lacks the *N*-methyl group (Figure 5).[7] Secondary metabolites biosynthesized from salicylic acid and cysteine(s) have been isolated from various bacterial sources, including *Pseudomonas*, *Vibrio*, and *Streptomyces*, but none have ever been reported from *Brevibacillus* or *Bacillus*, which belong to the phylum Firmicutes. Compounds **1** and **2** share the common tricyclic ring system with ulbactin D produced by *Alteromonas* in the phylum Proteobacteria. It is noteworthy that these structurally rare and complex molecules are produced by phylogenetically distant species, which may indicate gene distribution through horizontal transfer. In addition, it should be noted that all of the ulbactin producers are found in the marine environment, implying a biological function of these metabolites in their habitat.[10]

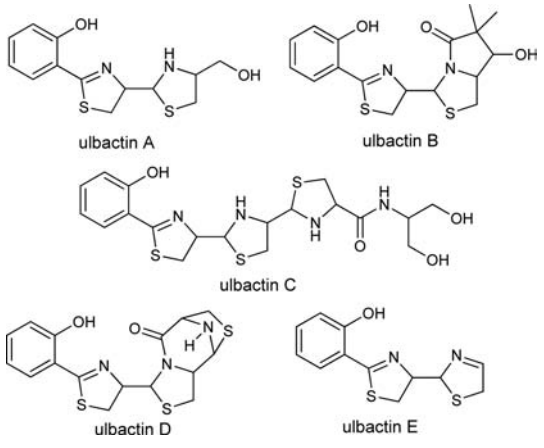

Figure 5. Structures of ulbactins A–E.

Currently the biological activity of these compounds is still under investigation. Limited testing has shown that **1** and **2** inhibit migration of epidermoid carcinoma A431 cells at non-cytotoxic concentrations with IC_{50} values of 6.4 and 6.1 μM, respectively. Further examinations of the major isomer **1** demonstrated its antimetastatic property with inhibition of cancer cell migration (IC_{50} = 2.1 μM for esophageal squamous carcinoma EC109 cells) and tumor cell invasion (IC_{50} = 1.7 μM for murine colon carcinoma 26-L5 cells). Further anticancer evaluation of **1** and **2** is in progress. Both **1** and **2** (50 μg/mL) were not active against *Escherichia coli*, *Micrococcus luteus*, and *Candida albicans*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00531.

X-ray crystallographic data for **1** (CIF)
X-ray crystallographic data for **2** (CIF)
Experimental details and 1D and 2D NMR spectra of **1** and **2** (PDF)

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Notes

The authors declare no competing financial interest.

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