The Journal of Antibiotics (2012) 65, 579–581

www.nature.com/ja

NOTE

A new furaquinocin derivative, JBIR-136, from *Streptomyces* sp. 4963H2

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The Journal of Antibiotics (2012) **65,** 579–581; doi:10.1038/ja.2012.72; published online 29 August 2012

Keywords: cytotoxicity; furaquinocin; *Streptomyces*

Natural products are considered to be good sources for the screening of lead compounds of clinical drugs. We performed a large number of drug screenings by using a variety of assay systems with crude extracts of microbial cultures as a traditional natural product library. In some assay systems, our crude extract library could not work effectively. With this in mind, we started to construct a purified natural compound library possessing various skeletons from cultures of microorganisms. As actinomycetes are known to produce pharmaceutically useful compounds,[1,2] we focused mainly on secondary metabolites from cultures of actinomycetes. To achieve this, we established a high-throughput system for the detection of secondary metabolites from the cultures of actinomycetes with the retention-time and HR-MS data of known compounds by using a UPLC-TOF-MS system (Waters, Milford, MA, USA).[3] We analyzed the secondary metabolites, including potential novel compounds, present in the cultures of the strains isolated from a variety of resources by this system.[3] In the course of our chemical screening program, a new furaquinocin derivative named JBIR-136 (**1**, Figure 1a), together with the known compound kujimycin A,[4] was isolated from a culture broth of *Streptomyces* sp. 4963H2. This paper describes the fermentation, isolation, structural elucidation and, in brief, the biological activity of **1**.

Streptomyces sp. 4963H2 was isolated from a soil sample collected in Kimitsu, Chiba Prefecture, Japan. The strain was cultivated in 50-ml test tubes, each containing 15 ml of a seed medium consisting of 1.0% starch (Kosokagaku, Tokyo, Japan), 1.0% polypeptone (Nihon Pharmaceutical, Tokyo, Japan), 1.0% molasses (Dai-Nippon Meiji Sugar, Tokyo, Japan) and 1.0% meat extract (Extract Ehlrich, Wako Pure Chemical Industry, Osaka, Japan) at pH 7.2 (adjusted before sterilization). The test tubes were shaken on a reciprocal shaker (320 r.p.m.) at 27 °C for 2 days. Aliquots (2.5 ml) of the broth were transferred to 500-ml baffled Erlenmeyer flasks containing 100 ml of a production medium consisting of 2.0% glycerol (Nacalai Tesque, Kyoto, Japan), 1.0% molasses (Dai-Nippon Meiji Sugar), 0.5% casein (Kanto Chemical, Tokyo, Japan), 0.1% polypeptone (Nihon Pharmaceutical) and 0.4% $CaCO_3$ (Kozaki Pharmaceutical, Tokyo, Japan) at pH 7.2 (adjusted before sterilization), and were cultured on a rotary shaker (180 r.p.m.) at 27 °C for 5 days.

The fermentation broth (2 l) was separated by centrifugation. The supernatant was partitioned between EtOAc and H_2O (2 l × 3), whereas the mycelial cake was extracted with acetone (50 ml) and filtered, and the filtrate was concentrated *in vacuo*. The residual aqueous concentrate was extracted with EtOAc (equal volume × 3). The combined EtOAc layers were dried over Na_2SO_4, and then evaporated to dryness. The residue (733 mg) was subjected to normal-phase medium pressure liquid chromatography (Purif-Pack SI-30, Shoko Scientific, Yokohama, Japan) and developed successively with a gradient system of *n*-hexane–EtOAc (0–15% EtOAc) followed by the stepwise solvent system of $CHCl_3$–MeOH (0, 2, 5, 10, 20, 30 and 100% MeOH). The 5% MeOH-eluted fraction (91.3 mg) was then subjected to preparative reversed-phase HPLC on a CAPCELL PAK C18 MGII column (5.0 μm, 20 i.d. × 150 mm; Shiseido, Tokyo, Japan) with 60% aqueous MeOH containing 0.1% formic acid (flow rate: 10 ml min^{-1}) to give **1** (7.2 mg; retention time: 10.6 min).

Compound **1** was a pale yellow amorphous solid ($[\alpha]_D^{24}$ –38, *c* 0.36 in MeOH; UV λ_{max} (ε) in MeOH: 233 (17 000), 281 (12 000) and 316 (5600) nm). The IR spectrum (ν_{max} (attenuated total reflectance) 3380 and 1652 cm^{-1}) indicated the presence of hydroxy and carbonyl groups. The molecular formula was determined as $C_{21}H_{28}O_6$ by high-resolution ESI MS (*m/z* 375.1790 $[M–H]^-$, calcd for $C_{21}H_{27}O_6$ 375.1808). The direct connectivity between protons and carbons was established by a heteronuclear single quantum coherence spectrum; the tabulated ^{13}C and ^1H NMR spectroscopic data for **1** are listed in Table 1. A planar structure was elucidated through the analysis of the DQF-COSY and constant-time (CT-HMBC)[5] data, as described below.

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Received 2 April 2012; revised 2 July 2012; accepted 9 August 2012; published online 29 August 2012

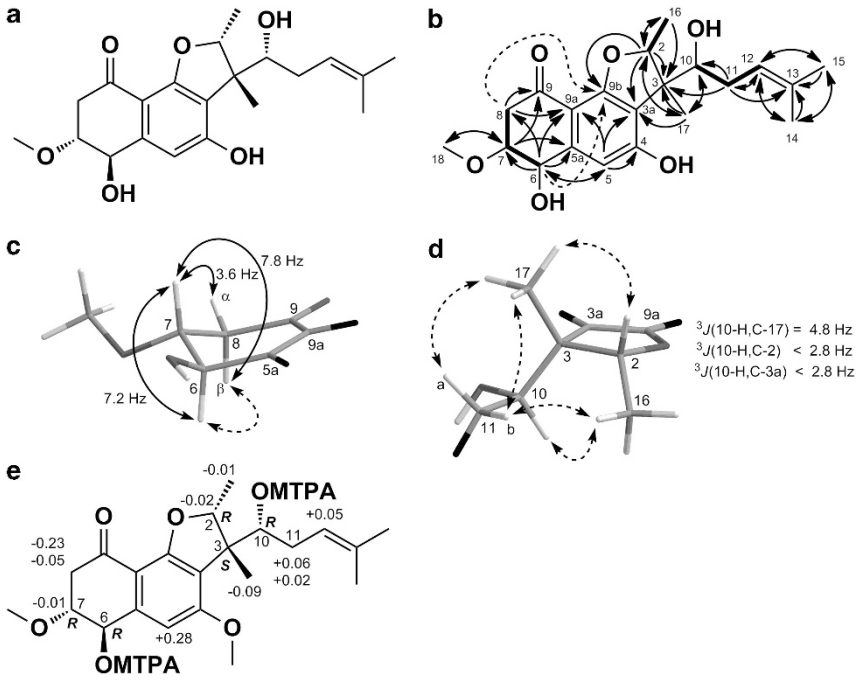

Figure 1 (**a**) Structure of JBIR-136 (**1**). (**b**) Key correlations of DQF-COSY (bold line) and HMBC (arrow; dashed arrow is four-bond coupling) for **1**. (**c**) Selected NOESY correlations and ^{1}H–^{1}H coupling constants of 4-hydroxy-5-methoxycyclohex-2-enone moiety of **1** (dashed arrow: NOESY correlation). (**d**) Relative configurations of C-2–C-3 and C-3–C-10 axes of **1** (dashed arrow: NOESY correlation). (**e**) $\Delta\delta$ values ($\Delta\delta$ (in p.p.m.) $=\delta_S-\delta_R$) obtained for the (*S*)- and (*R*)-methoxy(trifluoromethyl)phenylacetic acid (MTPA) diesters of the methyl ester derivative of **1**.

Table 1 NMR spectroscopic data (600 MHz) for JBIR-136 (1)

Position	δ_C	δ_H *(Multiplicity, J in Hz)*
2	89.2	4.58 (q, 6.6)
3	53.3	
3a	119.5	
4	160.2	
5	110.0	6.59 (s)
5a	146.9	
6	71.4	4.63 (d, 7.2)
7	81.1	3.62 (ddd, 3.6, 7.2, 7.8)
8	41.8	2.89α (dd, 3.6, 16.2); 2.42β (dd, 7.8, 16.2)
9	191.4	
9a	110.7	
9b	162.0	
10	74.9	4.00 (d, 9.0)
11	33.5	2.44a (ovl)[a]; 2.28b (dd, 7.2, 15.6)
12	121.7	5.29 (dd, 7.2, 7.2)
13	134.7	
14	18.1	1.64 (s)
15	26.0	1.69 (s)
16	16.2	1.25 (d, 6.6)
17	19.7	1.29 (s)
18	57.1	3.39 (s)

NMR spectra were taken on an NMR System 600 NB CL (Varian, Palo Alto, CA, USA) in acetone-d_6 with the residual solvent peak as an internal standard (δ_C 29.8, δ_H 2.04 p.p.m.).
[a]Overlapped with another peak.

The sequence from the oxymethine proton 6-H (δ_H 4.63) to the methylene protons 8-H$_2$ (δ_H 2.89; 2.42) via the oxymethine proton 7-H (δ_H 3.62) was observed in the DQF-COSY spectrum (Figure 1b). The CT-HMBC spectrum showed ^{1}H–^{13}C long-range correlations from 8-H$_2$ to the conjugated ketone carbonyl carbon C-9 (δ_C 191.4) and the aromatic quaternary carbon C-9a (δ_C 110.7), from 7-H to C-9 and the aromatic quaternary carbon C-5a (δ_C 146.9), from 6-H to C-5a and C-9a and from the methoxy proton 18-H$_3$ (δ_H 3.39) to the oxygenated methine carbon C-7 (δ_C 81.1), indicating the presence of a 4-hydroxy-5-methoxycyclohex-2-enone moiety. In addition, strong *m*-couplings from the aromatic methine proton 5-H (δ_H 6.59) to the aromatic quaternary carbons C-3a (δ_C 119.5) and C-9a, together with HMBC correlations to the oxygenated aromatic carbon C-4 (δ_C 160.2) and the oxymethine carbon C-6 (δ_C 71.4), and weak four-bond w-shaped couplings from 8-Hα and 6-H to the oxygenated aromatic carbon C-9b (δ_C 162.0) were observed, indicating the presence of a benzene ring substructure.

The presence of a monoterpene unit was elucidated as follows. A 3-isopropylidenepropanol moiety (from CH-10 to CH$_3$-15) was established by the spin systems from the oxymethine proton 10-H (δ_H 4.00) to the olefinic methine proton 12-H (δ_H 5.29) through the aliphatic methylene protons 11-H$_2$ (δ_H 2.44, 2.28), and ^{1}H–^{13}C long-range correlations from the two sets of allylic methyl protons 14-H$_3$ (δ_H 1.64) and 15-H$_3$ (δ_H 1.69) to the olefinic methine carbon C-12 (δ_C 121.7) and olefinic quaternary carbon C-13 (δ_C 134.7), as obtained from the DQF-COSY and CT-HMBC spectra, respectively. The assignments of C-14 and C-15 were established because of the γ-effect at C-14 (δ_C 18.1). ^{1}H–^{13}C long-range couplings from the singlet methyl proton 17-H$_3$ (δ_H 1.29) to the quaternary carbon C-3 (δ_C 53.3), the aromatic quaternary carbon C-3a, the oxymethine carbon C-10 (δ_C 74.9) and the oxymethine carbon C-2 (δ_C 89.2) revealed that the 3-isopropylidenepropanol and benzene moieties were interconnected through C-3. ^{1}H–^{1}H coupling between the oxymethine proton 2-H (δ_H 4.58) and methyl protons 16-H$_3$ (δ_H 1.25) and the ^{1}H–^{13}C long-range correlation from the methyl proton 16-H$_3$ to C-3 were evident in the DQF-COSY and CT-HMBC spectra,

respectively. In addition, the HMBC coupling from the oxymethine proton 2-H to the aromatic carbon C-9b established that these carbons were connected through an ether bond. The index of hydrogen deficiency of eight allowed the final determination of the structure of **1**, as shown in Figure 1a.

The relative configuration of **1** was determined from NOESY and the *J* values. From the ^{1}H NMR spectrum, large coupling constants of $^3J_{6\text{-H},7\text{-H}} = 7.2$ Hz and $^3J_{7\text{-H},8\text{-H}\beta} = 7.8$ Hz and a small coupling value of $^3J_{7\text{-H},8\text{-H}\alpha} = 3.6$ Hz were obtained. The NOESY spectrum showed a correlation between 6-H and 8-Hβ. On the basis of these data, 6-H, 7-H and 8-Hβ are found to be in pseudoaxial positions, whereas 8-Hα and O-7 are in pseudoequatorial positions, as shown in Figure 1c. The NOESY correlation of 2-H/17-H implied that CH_3-16 and CH_3-17 were in the *trans* orientation. For the C-3/C-10 axis, NOESY correlations from 11-H_2 to 16-H and 17-H showed gauche orientation between C-11 and C-17. Furthermore, a strong NOESY correlation between 10-H and 16-H was seen, whereas no correlation was observed for 10-H/17-H, indicating gauche orientation between C-17 and the oxygen atom O-10. On the other hand, ^{1}H–^{13}C coupling constants of $^3J_{10\text{H–C17}} = 4.8$ Hz, $^3J_{10\text{H–C2}} < 2.8$ Hz and $^3J_{10\text{H–C3a}} < 2.8$ Hz were obtained from the analysis of the *J*-resolved HMBC-2 spectrum.[6] According to the *J*-based configuration analysis method,[7] this result strongly supported the relative configuration described above. Taken together, the relative configurations at C-2, C-3 and C-10 were elucidated to be *R**, *S** and *R**, respectively, as shown in Figure 1d.

The absolute configuration of **1** was determined using a modified Mosher's method[8] after *O*-methylation with trimethylsilyldiazomethane in MeOH to afford a methyl ester. The position of the added methoxy group was confirmed by an HMBC correlation from the emerging methoxy protons (δ_H 3.91, in Me_2CO-d_6) to the oxygenated olefinic carbon C-4 (δ_C 161.8). Treatment of the methyl ester derivative of **1** with (*R*)- and (*S*)-methoxy(trifluoromethyl) phenylacetic acid chloride in dry pyridine gave the (*S*)- and (*R*)-methoxy(trifluoromethyl)phenylacetic acid diesters, respectively. The differences in chemical shift ($\Delta\delta = \delta_S - \delta_R$) are shown in Figure 1e. From these values, it was found that C-6 and C-10 were arranged in the *R* configuration. Given this result, the absolute configurations of **1** were assigned as 2*R*, 3*S*, 6*R*, 7*R* and 10*R*.

The structure of **1** is related to those of the furaquinocins,[9–13] in particular furaquinocin D,[11,12] isolated as the secondary metabolite of *Streptomyces*. As furaquinocins show cytotoxicity against cancer cells, we evaluated the cytotoxic activity of **1** against human cervical carcinoma HeLa cells by using a (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt (WST-8) colorimetric assay (Cell Counting Kit; Dojindo, Kumamoto, Japan) for 72 h. The results showed that **1** exhibited a weak cytotoxicity, with an IC_{50} value of 44.4 μM.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the New Energy and Industrial Technology Department Organization of Japan, and a Grant-in-Aid for Scientific Research (23380067 to KS) from the Japan Society for the Promotion of Science.

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