ORGANIC LETTERS

2010
Vol. 12, No. 20
4664−4666

Anti-Influenza Virus Compound from *Streptomyces* sp. RI18

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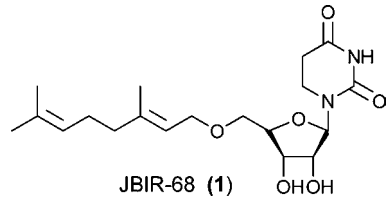
Received August 25, 2010

ABSTRACT



JBIR-68 (**1**)

Screening for anti-influenza virus activity in compounds isolated from *Streptomyces* sp. RI18, which was isolated using the membrane filter method, uncovered a novel compound, JBIR-68 (1), which contains a unique skeleton. Its structure was established by extensive NMR and MS analyses. In addition, 1 was synthesized to confirm the configuration of its sugar moiety. Compound 1 inhibited influenza virus growth in plaque assays.

The influenza virus is a major human and animal pathogen with the potential to cause catastrophic mortality. The virus reproduces rapidly, mutates frequently, and occasionally crosses species barriers. The recent emergence in Asia of the avian influenza virus related to a highly pathogenic form of the human virus has highlighted the strong need for effective new treatments.[1−3] Moreover, the emergence and subsequent swift, worldwide spread of the swine-origin influenza virus A (H1N1) in 2009 once again emphasized the urgency for rapidly developing safe, effective vaccines.[4] Potent anti-influenza agents are urgently required in our ongoing fights against seasonal outbreaks and to address the

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10.1021/ol102007d
Published on Web 09/15/2010

risk of future pandemics, given the low probability of developing a universally effective influenza virus vaccine in the near future.[5,6] Adamantane derivatives, amantadine and rimantadine, which target M2 ion channels, were the first antiviral drugs used to treat influenza.[7,8] Recently, orally administered oseltamivir and inhaled zanamivir, which target neuraminidase (NA), were also approved for use in treatments.[7,8] However, alternative therapeutics are needed because the appearance of influenza strains resistant to drugs targeting NA, particularly oseltamivir, is an increasing clinical problem.[9−11] Thus, new anti-influenza agents are very necessary.

Members of the class *Actinobacteria* have been extensively studied for their tendency to produce pharmaceutically useful compounds. However, the discovery rate of novel compounds from these strains has decreased significantly. Therefore, we isolated rare *Actinobacteria* using the membrane filter (MF) method,[12] which allows for the selection of *Actinobacteria* without antibiotics, unlike traditional selection methods. In fact, we have already isolated a new *Streptomyces* sp. using this method[13] and have described new promothiocin derivatives from the isolated *Streptomyces*.[14] In the course of screening for compounds with anti-influenza virus activity from our *Actinobacteria* cultures, we identified a novel compound from a culture of *Streptomyces* sp. RI18 (Figure 1). This compound possesses a unique skeleton and was named JBIR-68 (**1**).

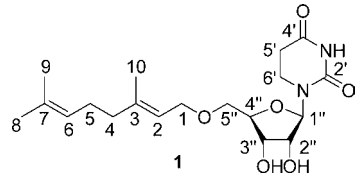

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Figure 1. Structure of JBIR-68 (**1**).

Streptomyces sp. RI18 was cultured for 5 days at 27 °C, with rotary shaking in 500 mL baffled Erlenmeyer flasks each containing 100 mL of a production medium. The mycelial cake was extracted with 80% acetone and concentrated in vacuo. The aqueous concentrate was extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4, concentrated in vacuo, and subjected to sequential silica gel flash column chromatography. The pure form of **1** was obtained by reversed-phase HPLC (see Supporting Information).

Compound **1** was isolated as a colorless oil ($[\alpha]_D^{25}$ −15.0, *c* 0.10, MeOH) that gave a $[M + H]^+$ ion at *m*/*z* 383.2189 in the HRESIMS. This was consistent with the molecular formula $C_{19}H_{30}N_2O_6$ (calcd for $C_{19}H_{31}N_2O_6$, 383.2182). **1** displayed the following IR spectrum: IR (KBr) ν_{max} 1700 cm^{-1}. The ^1H and ^{13}C NMR spectral data for **1** are shown in Table 1. The structural information for **1** was determined from a series of 2D NMR analyses, including HSQC, field-gradient $^1H-^1H$ COSY, and HMBC spectra. Two singlet methyl protons, H-8 (δ_H 1.67) and H-9 (δ_H 1.60), were $^1H-^{13}C$ long-range coupled to each other (δ_C 24.7 and 16.6, respectively), an olefinic quaternary carbon C-7 (δ_C 131.4), and an olefinic methine carbon C-6 (δ_C 123.8). Two ^1H spin systems, the sequence from H-4 to H-6 through H-5, and a correlation between H-1 and H-2, were observed by $^1H-^1H$ COSY. In addition to these correlations, $^1H-^{13}C$ long-range couplings from a singlet methyl proton H-10 (δ_H 1.68) to an olefinic methine carbon C-2 (δ_C 120.7), an olefinic quaternary carbon C-3 (δ_C 140.5), and a methylene carbon C-4 (δ_C 39.5) revealed a terpene moiety. The high-field ^{13}C chemical shift value at the methyl carbon C-10 (δ_C 15.3) determined the configuration at C-2 to be *E*. The ^{13}C chemical value at methylene carbon C-1 (δ_C 67.4) created a geraniol residue, as shown in Figure 2.

The sequence from an aminoacetal proton H-1″ (δ_H 5.86, δ_C 88.1) to oxymethylene proton H-5″ (δ_H 3.57 and 3.54) through oxymethine protons H-2″ (δ_H 4.14), H-3″ (δ_H 4.04),

Table 1. ^1H and ^{13}C NMR Spectral Data for JBIR-68 (**1**)[15]

	1	
position	^{13}C	^1H (*J* in Hz)
1	67.4	4.03[a]
2	120.7	5.31, t (7.1)
3	140.5	
4	39.5	2.05, m
5	26.2	2.11, m
6	123.8	5.10, t (6.8)
7	131.4	
8	24.7	1.67, s
9	16.6	1.60, s
10	15.3	1.68, s
2′	154.1	
4′	171.7	
5′	30.8	2.60, t (6.8)
6′	36.2	3.60, dt (13.0, 6.8), 3.45, dt (13.0, 6.8)
1″	88.1	5.86, d (6.4)
2″	71.1	4.14, t (6.4)
3″	71.4	4.04[a]
4″	83.0	3.96, ddd (6.4, 6.5, 3.2)
5″	69.9	3.57, t (6.4), 3.54, dd (6.5, 3.2)

[a] Overlapping.

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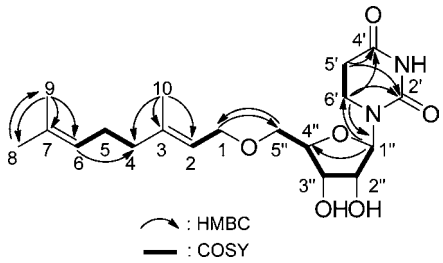


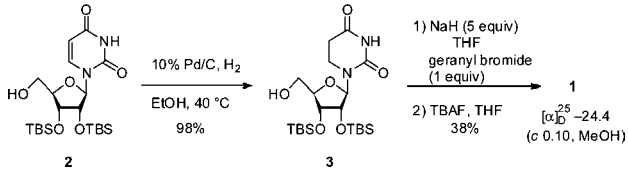
Figure 2. Key correlations observed in COSY (bold lines) and HMBC (arrows) spectra of JBIR-68 (**1**).

and H-4″ (δ_H 3.96) was confirmed by $^1H-^1H$ COSY (Figure 2). A $^1H-^{13}C$ long-range coupling from H-1″ to an oxymethylene carbon C-4″ revealed a ribosyl residue.

The correlation between methylene protons H-5′ (δ_H 2.60) and H-6′ (δ_H 3.60 and 3.45) was observed in the $^1H-^1H$ COSY spectrum. The $^1H-^{13}C$ long-range couplings from H-5′ to a carbamide carbon C-2′ (δ_C 154.1), an amide carbon C-4′ (δ_C 171.7), and a methylene carbon C-6′ (δ_C 36.2), and from H-6′ to C-2′ and C-4′, were observed. The $^1H-^{13}C$ long-range coupling from H-6′ to C-1″ revealed a dihydrouridine moiety. The NMR assignment of the dihydrouridine moiety in **1** was supported by the chemical shifts observed by ^{13}C NMR in the diacetate FR-900848.[16] Finally, the connection between the geraniol and the dihydrouridine moieties was elucidated by the $^1H-^{13}C$ long-range couplings from H-1 to C-5″ and from H-5″ to C-1 (Figure 2). Thus, the structure of **1** was elucidated, as shown in Figure 1. Although FR-900848, isolated from *Streptoverticillium fevens* HP-891,[17] is the only reported natural compound that possesses the dihydrouridine moiety, **1** is the first compound in which the ribonucleoside and the geranyl residues are connected by an ether bond.

To confirm the absolute configuration of **1**, we synthesized 5′-*O*-geranyl-5,6-dihydrouridine from 2′,3′-di-*O*-(*tert*-butyldimethylsilyl)uridine (**2**; Scheme 1; also see Supporting Information).[18] Hydrogenation of **2** in the presence of 10% Pd/C provided 5,6-dihydrouridine (**3**). After formation of the dianion of **3** using NaH and THF, selective *O*-alkylation with geranyl bromide was performed at −78 °C. The subsequent removal of the TBS groups with TBAF and THF yielded 5′-*O*-geranyl-5,6-dihydrouridine, which was purified by silica gel chromatography and by gel permeation chromatography. All resulting spectral data, including the (−) signal of the optical rotation of the synthetic 5′-*O*-geranyl-5,6-dihydrouridine (**1**), were identical to those of JBIR-68 (**1**). Thus, we confirmed the stereochemistry of JBIR-68 (**1**).

Scheme 1. Synthesis of 5′-*O*-Geranyl-5,6-dihydrouridine (**1**)



The ability of **1** to inhibit influenza virus growth was evaluated using plaque assays.[19] Compound **1** (5−50 μM) reduced influenza virus plaque number and area (Figure 3; also see Supporting Information). Thus, **1** clearly inhibited the influenza virus growth. Since **1** is a nucleic acid derivative, it was expected to inhibit viral RNA synthesis. However, the mechanism of the antiviral activity of **1** was not clarified (data not shown). Therefore, the target of **1** may be distinct from that of other drugs targeting the influenza virus. A detailed investigation of the mechanism by which **1** inhibits the influenza virus is currently underway.

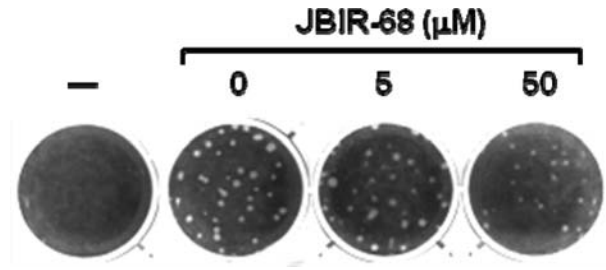


Figure 3. JBIR-68 (**1**) inhibits influenza virus plaque formation.

In conclusion, we isolated a novel compound, **1**, from *Streptomyces* sp. RI18 selected using the MF method. Compound **1** contains a unique structural skeleton and inhibits the influenza virus. We anticipate that this study will convince chemists that *Actinobacteria* species isolated using the MF method can produce bioactive compounds with unique skeletal structures. In addition, since the molecular target of **1** may be different from that of known anti-influenza drugs, it is expected to be a critical compound for developing anti-influenza drugs.

Acknowledgment. This work was supported in part by the grant from the New Energy and Industrial Technology Development Organization (NEDO) of Japan and a Grant-in-Aid for Scientific Research (20380070 to K.S.), from The Japan Society for the Promotion of Science (JSPS).

Supporting Information Available: ^1H and ^{13}C NMR, COSY, HSQC, HMBC, and HRESIMS spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL102007D

(15) NMR spectra of **1** were taken on a NMR System 600 NB CL in CD_3OD, and the solvent peak was used as an internal standard (δ_C 49.0, δ_H 3.30).

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