J. Antibiot. 61(5): 326–329, 2008

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THE JOURNAL OF ANTIBIOTICS

Stawamycin Analog, JBIR-11 from *Streptomyces viridochromogenes* subsp. *sulfomycini* NBRC 13830

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Received: April 14, 2008 / Accepted: May 2, 2008

Abstract A stawamycin analog, JBIR-11 (**1**) was isolated from mycelium of *Streptomyces viridochromogenes* subsp. *sulfomycini* NBRC 13830. The structure was determined on the basis of the spectroscopic data. Compound **1** exhibited growth inhibitory effect against human fibrosarcoma HT1080 cells with an IC_{50} value of 25 μM.

Keywords stawamycin, JBIR-11, *Streptomyces viridochromogenes*, cytotoxic

Streptomyces viridochromogenes subsp. *sulfomycini* NBRC 13830 has been shown to produce a sulfur-containing peptide antibiotic, sulfomycin [1～3]. In the course of our screening program for novel polyketide synthase (PKS) genes that synthesize macrolides, polyethers and aromatics, we have already demonstrated that *S. viridochromogenes* NBRC 13830 possesses novel PKS genes [4]. Therefore, *S. viridochromogenes* NBRC 13830 has a potential for production of novel secondary metabolites. Searching for metabolites from culture of *S. viridochromogenes* NBRC 13830 resulted in the isolation of a novel compound JBIR-11 (**1**, Fig. 1) as an analogue of stawamycin [5]. In this paper, we report the isolation, structure elucidation and brief bioactivity of **1**. The trace amount of stawamycin was also identified in the mycelial extract of *S. viridochromogenes* NBRC 13830.

S viridochromogenes NBRC 13830 was cultured at 25°C for 5 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of 2.5% starch, 1.5% soy bean meal, 0.2% dry yeast and 0.4% $CaCO_3$ (pH 6.2 before sterilization) on a rotary shaker (120 rpm). The collected mycelial cake from a fermented whole broth (2.0 liters) was extracted with Me_2CO (500 ml). The extract was evaporated *in vacuo*, and the residual aqueous concentrate was extracted with EtOAc and evaporated to dryness. The organic layer (210 mg) was chromatographed on a silica gel flash column (Purif-Pack SI-60, Moritex) with a $CHCl_3$-MeOH gradient system (0～100% MeOH), and fractions

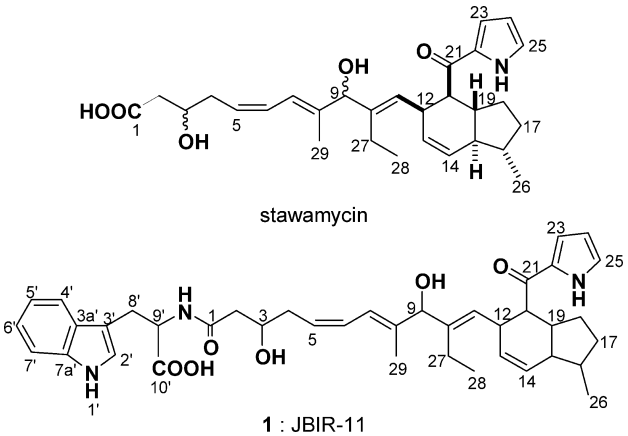

Fig. 1 Structures of stawamycin and JBIR-11 (**1**).

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Table 1 Physico-chemical properties of JBIR-11 (**1**)

Appearance	Colorless, amorphous powder
Melting point[a]	106～109°C
$[\alpha]_D^{25}$ (MeOH)[b]	−65.0° (*c* 0.6)
Molecular formula	$C_{40}H_{49}N_3O_6$
HR-ESI-MS (*m/z*)[c]	found: 690.3527 $[M+Na]^+$
	calcd: 690.3519 for $C_{40}H_{49}N_3O_6Na$
UV λ_{max} (MeOH) nm (ε)[d]	220 (58,900), 244 (38,500), 290 (25,200)
IR ν_{max} (KBr) cm^{-1} [e]	3400, 1635

[a] Melting point was determined with a Yanagimoto micro melting point apparatus. [b] Optical rotation was operated on a HORIBA SEPA-300 polarimeter. [c] HR-ESI-MS data were recorded on a Waters LCT-Premier XE. [d] UV spectrum was measured on a HITACHI U-3200 spectrophotometer. [e] IR spectrum was obtained using a HORIBA FT-720 spectrophotometer.

including major metabolites were collected by LC-MS monitoring. The 20～40% eluate (7.0 mg) was further purified by preparative HPLC using PEGASIL-ODS (Senshu Pak, 20 i.d.×150 mm) developed with 75% aq MeOH with 0.125% formic acid to yield sulfomycin (Rt=6.3 minutes, 1.23 mg). The 50～100% eluate (42 mg) was further purified by preparative HPLC developed with 85% aq MeOH with 0.075% formic acid to give two pure compounds stawamycin (Rt=8.8 minutes, 0.51 mg) and JBIR-11 (**1**, Rt=10.9 minutes, 1.86 mg). Their structures were elucidated mainly by spectroscopic methods, including 2D NMR techniques.

The physico-chemical properties of **1** are summarized in Table 1. The UV spectrum (MeOH, λ_{max} 220, 244, 290 nm) of **1** closely resembled those of stawamycin [5] and indanomycin [6], indicating that **1** shares a core structure similar to that of stawamycin and indanomycin. Compound **1** had the molecular formula $C_{40}H_{49}N_3O_6$ revealed by HR-ESI-MS data [*m/z* 690.3527 $(M+Na)^+$, Δ+0.8 mmu], and this molecular weight showed the difference of 186 mass units as $C_{11}H_{10}N_2O$ compared with stawamycin. The ^{1}H- and ^{13}C-NMR spectral data for **1** is shown in Table 2 and the direct connectivity of protons and carbons were established by the HSQC spectrum. The ^{13}C chemical shifts of **1** exhibited similar to those of stawamycin except for eleven carbon units.

The ^{1}H-^{1}H COSY and HMBC spectra established three partial structures (Fig. 2). The proton-proton correlations observed in the DQF-COSY including 4′-H (δ_H 7.58), 5′-H (δ_H 6.95), 6′-H (δ_H 7.02) and 7′-H (δ_H 7.27) revealed the presence of a 1,2-disubstituted benzene ring moiety. The strong ^{1}H-^{13}C *m*-coupling from 5′-H and 7′-H to C-3a′ (δ_C 129.4), and 4′-H and 6′-H to C-7a′ (δ_C 137.9) established the assignments of these carbons. A typical α-methine proton 9′-H (δ_H 4.62, δ_C 56.8) was spin-coupled to 8′-H (δ_H 3.36, 3.14), which in turn long-range coupled to C-2′ (δ_C 124.3), C-3′ (δ_C 112.0) and C-3a′ (δ_C 129.4). The ^{1}H-^{13}C long-range couplings between 2′-H and C-3′, C-3a′

Table 2 ^{13}C- (150 MHz) and ^{1}H- (600 MHz) NMR data for JBIR-11 (**1**)

Position	^{13}C	^{1}H
1	174.0	
2	44.4	2.22 (m), CH_2
3	69.9	3.92 (tt, 6.2, 6.8)
4	36.2	2.26 (m), CH_2
5	127.6	5.39 (dt, 10.5, 7.5)
6	127.5	6.23 (t, 11.3)
7	121.0	6.32 (d, 11.3)
8	139.9	
9	81.7	4.28 (s)
10	142.1	
11	127.4	5.34 (d, 10.6)
12	41.3	3.66 (m)
13	131.1	5.44 (ddd, 9.7, 3.8, 2.6)
14	129.0	5.91 (d, 9.7)
15	52.8	1.54 (m)
16	38.2	1.56 (m)
17	33.2	2.01 (m), 1.28 (m)
18	28.1	1.96 (m), 1.08 (m)
19	41.9	2.00 (m)
20	53.0	3.51 (dd, 11.3, 6.3)
21	193.0	
22	133.2	
23	118.1	7.04 (dd, 3.8, 1.2)
24	111.2	6.20 (dd, 3.8, 2.6)
25	126.5	7.01 (dd, 2.6, 1.2)
26	19.0	1.07 (d, 5.9), CH_3
27	20.6	1.66 (dd, 7.5, 6.2), 1.58 (dd, 7.5, 2.8)
28	14.5	0.50 (t, 7.5), CH_3
29	13.4	1.46 (s), CH_3
10′	178.0	
9′	56.8	4.62 (dt, 5.1, 3.8)
8′	28.9	3.36 (dd, 14.7, 4.4), 3.14 (dd, 14.7, 7.6)
2′	124.3	7.08 (s)
3′	112.0	
3a′	129.4	
4′	119.5	7.58 (d, 7.9)
5′	119.6	6.95 (ddd, 7.5, 7.1, 1.2)
6′	122.1	7.02 (ddd, 7.9, 2.9, 1.2)
7′	112.1	7.27 (d, 7.5)
7a′	137.9	

NMR spectra were recorded on a Varian NMR System 600 NB CL. The sample was dissolved in $CDCl_3$, and the solvent peak was used as an internal standard (δ_H 7.26 and δ_c 77.0).

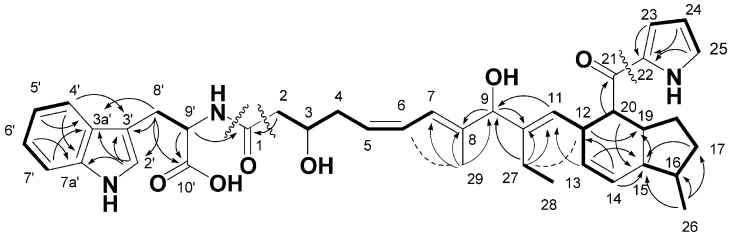

Fig. 2 NMR analyses of **1**.

Bold line and dashed line indicate ^{1}H-^{1}H spin coupling and NOE, respectively. Arrows show ^{1}H-^{13}C long-range couplings.

and C-7a′, and between 9′-H and a carbonyl carbon C-10′ (δ_C 178.0) together with the typical ^{13}C chemical shifts for an indole moiety established a tryptophan substructure as shown in Fig. 2.

The sequence from 2-H (δ_H 2.22) to 7-H (δ_H 6.32) through an oxymethine proton 3-H (δ_H 3.92), 4-H (δ_H 2.26), olefinic protons 5-H (δ_H 5.39) and 6-H (δ_H 6.23) was observed in DQF-COSY spectrum of **1**. According to the coupling constant between 5-H and 6-H (J=10.5 Hz), the geochemistry at C-5 was deduced to be *Z*. The continuous correlations in ^{1}H-^{1}H couplings created a linear sequence from 11-H (δ_H 5.34) to 14-H (δ_H 5.91) through 12-H (δ_H 3.66), 13-H (δ_H 5.44), and from 12-H to 15-H (δ_H 1.54) through 20-H (δ_H 3.51), 19-H (δ_H 2.00), 18-H (δ_H 1.96, 1.08), 17-H (δ_H 2.01, 1.28), 16-H (δ_H 1.56), which further spin-coupled with a methyl proton 26-H (δ_H 1.07). The ^{1}H-^{13}C long-range couplings between the olefinic proton 14-H and C-15 (δ_C 52.8), and between 15-H and C-19 (δ_C 41.9) established an octahydro-indene like substructure as shown in Fig. 2. A long-range coupling from 20-H to a carbonyl carbon C-21 (δ_C 193.0) revealed that the carbonyl function is substituted at the position of C-20 (δ_C 53.0).

The connectivity of these two partial structures was established by the ^{1}H-^{13}C long-range couplings observed in the HMBC spectra of **1**. A singlet methyl proton 29-H (δ_H 1.46) was long-range coupled to an olefinic methine carbon C-7 (δ_C 121.0), an olefinic quaternary carbon C-8 (δ_C 139.9) and an oxymethine carbon C-9 (δ_C 81.7). A methylene proton 27-H (δ_H 1.66, 1.58), which was spin coupled to a methyl proton H-28 (δ_H 0.50), was long-range coupled to C-9, an olefinic quaternary carbon C-10 (δ_C 142.1) and an olefinic methine carbon C-11 (δ_C 127.4). NOEs between 29-H and 6-H, and 27-H and 12-H indicated that the absolute stereochemistry at C-7 and C-10 had both *E* configurations. Thus, the main skeletal substructure which is also involved in stawamycin was established as shown in Fig. 2. The connectivity between this substructure and the tryptophan moiety was revealed by long-range couplings from 2-H and 9′-H to a carbonyl carbon C-1 (δ_C 174.0) through an amide bond.

A pyrrole moiety in **1** was successively established by coupling pattern between 23-H (δ_H 7.04), 24-H (δ_H 6.20) and 25-H (δ_H 7.01) with characteristic coupling constants ($J_{23H\text{-}24H}$=3.8 Hz, $J_{23H\text{-}25H}$=1.2 Hz, $J_{24H\text{-}25H}$=2.6 Hz) and ^{13}C chemical shifts (C-23, δ_C 118.1; C-24, δ_C 111.2; C-25, δ_C 126.5) with ^{1}H-^{13}C long-range couplings from H-23, H-24 and H-25 to C-22 (δ_C 133.2). The substituted position of the pyrrole moiety was determined by the ^{13}C chemical shifts at C-21 and C-22 indicating typical α,β-unsaturated carbonyl carbon and the acylated C-2 carbon of pyrrole moiety, respectively, which are confirmed by the comparison with ^{1}H- and ^{13}C-NMR spectra, using a commercial standard 2-acetyl pyrrole as a model. In this manner, the structure of **1** was determined as shown in Fig. 1.

Compound **1** was examined for its growth inhibitory activity toward the highly metastatic human HT-1080 fibrosarcoma cell line. Compound **1** inhibited the cell growth in a concentration-dependent manner with the IC_{50} values of 25 μM. Studies on detailed biological activity of **1** are now underway.

Acknowledgement This work was supported by the grant from the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

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