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SW-163A and B, Novel Immunosuppressants Produced by *Streptomyces* sp.

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SW-163A and B, novel immunosuppressants, were isolated from the culture broth of *Streptomyces* sp. SNA15896. The molecular formulas of SW-163A and B were determined as $C_{34}H_{42}N_2O_{12}$ and $C_{33}H_{40}N_2O_{12}$ based on the HRFAB-MS analysis, respectively. Both compounds were elucidated to be a large ring ester structure through spectroscopic analyses including various NMR measurements. SW-163A and B showed immunosuppressive and antimicrobial activities *in vitro*.

Cyclosporin A[1] and FK-506[2], the most potent immunosuppressants, are widely used for the prevention against the rejection of organ transplants and autoimmune diseases. Cyclosporin A and FK-506 exert immunosuppressive activity by blocking the signal transduction pathway for T cell activation[3,4]. However, both agents have the adverse reactions such as nephrotoxicity, neurotoxicity, and gastric toxicity[5~7]. Accordingly, the development of novel immunosuppressive agents with more safety has been anticipated. During our screening of microbial products for new immunosuppressants, SW-163A (**1**) and B (**2**) were found in the culture broth of *Streptomyces* sp. SNA15896 originally isolated from a soil sample collected in Ibaraki Prefecture, Japan. Compounds **1** and **2** showed inhibitory activity on mixed lymphocyte reaction (MLR) and mitogen stimulated proliferation of splenocytes. On the other hand, *Streptomyces* sp. SNA15896 produces not only compounds **1** and **2**, but also novel antitumor depsipeptides, SW-163C and E[8,9]. The taxonomy of this strain was recently reported in a previous paper[8]. In this publication, we describe the structural elucidation and biological activities of **1** and **2**.

Materials and Methods

Microorganism

The producing strain SNA15896 was isolated from a soil sample collected in Yuuki, Ibaraki Prefecture, Japan by a dilution agar plating method using HV agar[10]. The stock culture has been maintained on oatmeal agar (ISP No. 3) slopes or in 20% (W/V) glycerol solution at −80°C. The taxonomy studies of this strain were described in a previous paper[8]. The strain SNA15896 has been deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan under the accession number of FERM BP-6735.

Fermentation

A slant culture of the strain SNA15896 grown on glucose starch asparagine agar was inoculated into two 500-ml Erlenmeyer flasks containing 70 ml of seed medium composed of $(NH_4)_2SO_4$ 0.14%, KH_2PO_4 0.2%, $CaCl_2$ 0.03%, $MgSO_4 \cdot 7H_2O$ 0.03%, urea 0.03%, Polypepton 0.5%, yeast extract 0.1%, soybean meal 3%, glucose 1%, soluble starch 0.5%, $FeSO_4 \cdot 7H_2O$ 0.0005%, $MnSO_4 \cdot 4\sim6H_2O$ 0.00016%, $ZnSO_4 \cdot 7H_2O$ 0.00014%, and $CoCl_2$ 0.0002% (pH 7.0 before sterilization). The flasks were shaken at 27°C for 4 days on a rotary shaker (230 rpm). The seed culture (140 ml) was transferred into a 10-liter fermentor containing 5 liters of the culture medium with the same composition as the seed culture. The fermentation was carried out at 27°C for 5 days under aeration of 5 liters/minute and agitation at 300 rpm.

Mixed Lymphocyte Reaction (MLR)

Male BALB/c and C57BL/6 mice (6~8 weeks old,

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Charles River Japan) were sacrificed, and the spleens were excised and teased into a single cell suspension in phosphate-buffer saline (PBS) using a scalpel and forceps. The cell suspension was filtered through a nylon mesh to remove clumps. The cells were freed of red blood cells by treatment with ammonium chloride buffer (0.15 M NH_4Cl, 0.01 M $KHCO_3$, and 0.1 mM Na_2EDTA, pH 7.4) and washed three times with PBS. The splenocytes were resuspended in the culture medium, RPMI 1640 supplemented with 10% fetal bovine serum (FBS, Gibco), 100 IU/ml penicillin (Meiji Seika), and 100 μg/ml streptomycin (Meiji Seika). Cell viability was determined by nigrosin dye exclusion method[11]. The C57BL/6 splenocytes treated with 25 μg/ml mitomycin C (Wako) at 37°C for 30 minutes and the untreated BALB/c splenocytes were prepared for the stimulator and the responder cells, respectively. The test sample was dissolved in dimethyl sulfoxide and serially diluted with the culture medium. Both the 5×10^5 stimulator and responder cells in 100 μl of the culture medium, and 100 μl of the test sample solution were added into each well in 96-well microplates. The plates were incubated at 37°C for 72 hours in a humidified 5% CO_2 atmosphere. During the last 4 hours, the cells were pulse-labeled with 1.0 μCi of [^{3}H]thymidine (Amersham) and harvested with a cell harvester (MICROMATE196, Packerd). The incorporation of [^{3}H]thymidine into the cells was determined with a liquid scintillation counter (MATRIX96, Packerd). The inhibitory activity of the test sample was calculated as percent inhibition of [^{3}H] thymidine uptake into the cells.

Mitogen Induced Lymphocyte Blastogenesis

The splenocytes of male BALB/c mice (6~8 weeks old, Charles River Japan) prepared as described above were further purified by density gradient centrifugation on Lymphoprep (Nycomed), washed twice with PBS, and then suspended in RPMI 1640 medium supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Cell viability was determined by nigrosin dye exclusion method[11]. The test sample was dissolved in dimethyl sulfoxide and serially diluted with the medium. The medium used for suspension of the splenocytes (the suspension medium) was supplemented with either 2 μg/ml concanavalin A (Con A, Sigma) or 10 μg/ml lipopolysaccharide (LPS, from *E. coli*, Difco). The 10^6 splenocytes in 100 μl of the suspension medium containing either Con A or LPS, and 100 μl of the serially diluted test sample solution were added into each well in 96-well microplates. The plates were incubated at 37°C under a humidified 5% CO_2 atmosphere. The measurement of [^{3}H]thymidine incorporation into the cells and the calculation of the inhibitory activity were done as described in MLR.

Cytotoxicity

K562 (human myelogenous leukemia) and KB (human epidermoid carcinoma, oral) cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37°C under a humidified 5% CO_2 atmosphere. The K562 and KB cells (2×10^3 cells) suspended in 200 μl of the culture medium was inoculated into each well in 96-well microtiter plates, and preincubated for 6 hours and 24 hours under the above conditions, respectively. The test sample dissolved in dimethyl sulfoxide was serially diluted with the culture medium and added to each well in the microtiter plate. After 48 hours culture, inhibition of the cell growth was measured colorimetrically at 550 nm by the MTT method[12].

Antimicrobial Activity

Bacteria were grown on nutrient agar medium, and fungi and yeasts were grown on Sabouraud agar medium. A paper disk (8 mm in diameter, Advantec) containing 100 μg of the test sample was placed on the agar plates. The antimicrobial activity was evaluated by measuring the diameter of inhibitory zone after 24 hours incubation at 37°C for bacteria and 48 hours incubation at 27°C for fungi and yeasts.

Amino Acid Analysis

The sample (1 mg) was hydrolyzed in a gas phase of 6 M HCl (990 μl) and phenol (10 μl) at 110°C for 24 hours using the PICO-TAG™ work station. The product of hydrolysis was dissolved in 100 μl of 20 mM HCl and analyzed for the amino acid components with an amino acid analyzer (L-8500, Hitachi).

General Procedures

UV spectra were recorded on a Hitachi U-3210 spectrophotometer. IR spectra were measured on a Jasco IR-810 infrared spectrophotometer. FAB-MS were obtained with a Jeol JMS-HX110 mass spectrometer. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. NMR spectra were recorded on a Jeol JNM-A500 NMR spectrometer.

Results

Extraction and Isolation

Five liters of the culture broth of *Streptomyces* sp. SNA15896 was centrifuged to separate mycelia and supernatant. The mycelia were extracted with acetone. The extract was concentrated *in vacuo* to give aqueous residue. The residue was extracted with EtOAc and the EtOAc soluble portion was evaporated to obtain a brown residue (3.0 g). The residue was subjected to silica gel column chromatography (Wakogel C-200, Wako) using $CHCl_3$ as a mobile phase. The active fractions were combined and further subjected to silica gel column chromatography using a mixture of *n*-hexane and EtOAc (7 : 3) as a mobile phase. The fractions containing active substances were combined and concentrated *in vacuo*. The obtained crude material (52 mg) was dissolved in 500 μl of MeOH and subjected to reverse phase HPLC (TSK gel ODS-80TM column, 20×250 mm, Tosoh) using a mixture of CH_3CN and H_2O (7 : 3) as a mobile phase at a flow rate 10 ml/minute. SW-163A (30 mg) and B (8 mg) were eluted at 28 minutes and 23 minutes, respectively.

Structural Elucidation of SW-163A (1)

The physico-chemical properties of SW-163A (**1**) are summarized in Table 1. The IR spectrum indicated the presence of an ester group at 1760 cm^{-1} and an amide group at 1650 and 1520 cm^{-1}. The molecular formula was established as $C_{34}H_{42}N_2O_{12}$ on the basis of HRFAB-MS analysis. The ^1H and ^{13}C NMR spectral data for **1** are shown in Table 2. The ^1H NMR spectrum of **1** showed 42 proton signals. The ^{13}C NMR and DEPT spectra of **1** revealed 32 signals that were equivalent to 34 carbons classified into six methyl, two methylene, sixteen methyne, and ten quaternary carbons. The amino acid analysis of **1** indicated the presence of a threonine in the molecule. The direct connectivity of protons and carbons was established by the HMQC spectrum. The COSY and HMBC spectra showed the presence of five partial structures (Fig. 2). For partial structure A, the COSY correlations of H-1 with H-2 and H-12, and of H-2 with 2-NH were observed. The HMBC correlation was observed between H-2 and C-3. These correlations and the result of the amino acid analysis of **1** indicated that the partial structure A was a threonine moiety. The partial structure B was shown to be a 2-hydroxypropionic acid moiety by the HMBC correlations from H-21 to C-4 and C-5, and from H-4 to C-5. The

Fig. 1. Structures of SW-163A (**1**) and B (**2**).

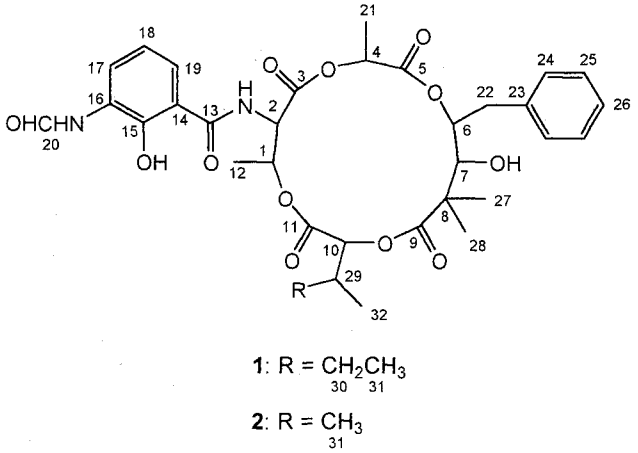
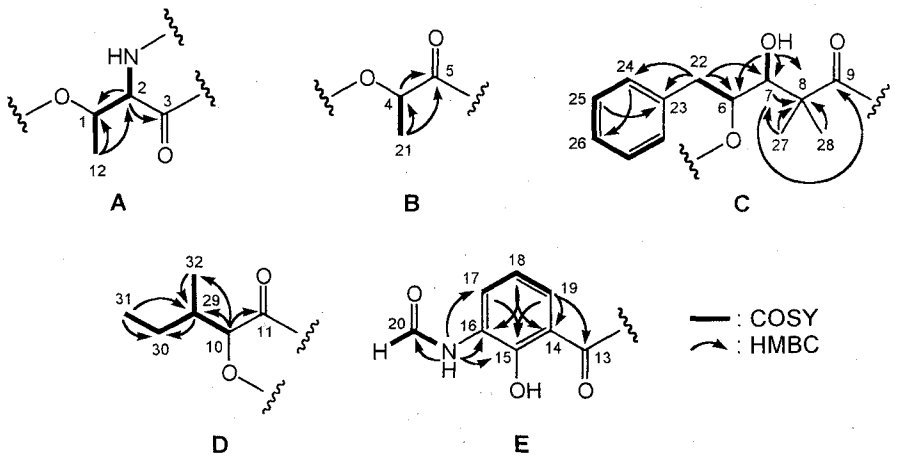
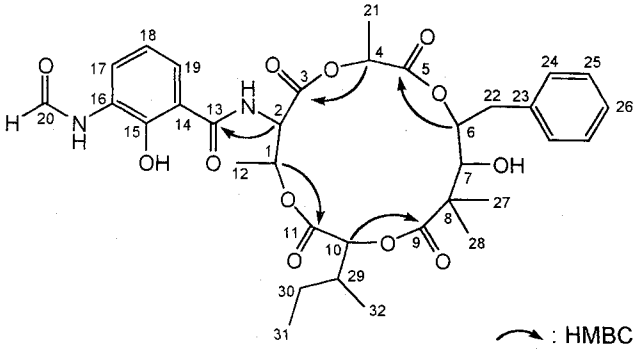


Fig. 2. Partial structures A~E of SW-163A (**1**).



presence of a phenyl group was shown by five aromatic proton signals (δ 7.19, δ 7.22, and δ 7.28) and four related sp^2 hybridized carbon signals (δ 126.3, δ 128.2, δ 129.1, and δ 137.6) corresponding to six aromatic carbons. H-22 had the long-range couplings with C-6, C-7, C-23, and C-24. Moreover, the HMBC correlations from 7-OH to C-6, C-7, and C-8, from H-7 to C-8 and C-9, and from H-27 and H-28 to C-8 showed that the partial structure C was a 3,4-dihydroxy-2,2-dimethyl-5-phenylvaleric acid moiety. The partial structure D was identified as a 2-hydroxy-3-methylvaleric acid moiety by the COSY corrrelations of H-29 with H-10, H-30, and H-32, and of H-30 with H-31 and the HMBC correlation between H-10 and C-11. For the partial structure E, the COSY correlations of H-18 with H-17 and H-19 suggested the presence of three vicinal protons on aromatic ring. A hydroxyl group substituted on C-15 was suggested by the chemical shifts of 15-OH (δ 12.80) that was shifted downfield by a hydrogen bond and C-15 (δ 150.7). The HMBC correlations from H-17 and H-19 to C-15 showed that C-15 was placed in *meta* position to C-17 and C-19. The connectivity of C-13 and C-14 was indicated by the HMBC correlations of H-19 with C-13 and C-14. The long-range couplings of 16-NH with C-15, C-16, C-17, and C-20 were inductive of a formylamino group that was subsituted on C-16. These 2D NMR correlations verified that the partial structure E was a 3-formylamino-2-hydroxybenzoic acid moiety. The cyclic structure comprised of the partial structure A~D was established by the HMBC correlations from H-1 to C-11, from H-4 to C-3, from H-6 to C-5, and from H-10 to C-9 (Fig. 3). Furthermore, the attachment of the threonine and the 3-formylamino-2-hydroxybenzoic acid moieties through an amide linkage was confirmed by the HMBC correlation between H-2 and C-13 (Fig. 3). Therefore, the structure of **1** was determined to be that shown in Fig. 1.

Fig. 3. Connectivity of the partial structures A~E of SW-163A (**1**).



Structural Elucidation of SW-163B (**2**)

The molecular formula of **2** was determined to be $C_{33}H_{40}N_2O_{12}$ through HRFAB-MS analysis and differed from that of **1** by a methylene group. The other physico-chemical properties of **2** had high similarity to those of **1** (Table 1). The amino acid analysis of **2** indicated the presence of a threonine in the molecule. Careful invesigation of the ^{1}H NMR spectrum for **2** revealed the missing

Table 1. Physico-chemical properties of SW-163A (**1**) and B (**2**).

	1	**2**
Appearance	White powder	White powder
$[\alpha]^{27}_D$ (c 0.2, $CHCl_3$)	+47.8°	+49.1°
Molecular weight	670	656
Molecular formula	$C_{34}H_{42}N_2O_{12}$	$C_{33}H_{40}N_2O_{12}$
FAB-MS (m/z)	671 $(M+H)^+$	657 $(M+H)^+$
HRFAB-MS (m/z)		
Found:	671.2819 $(M+H)^+$	657.2670 $(M+H)^+$
Calcd.:	671.2816 for $C_{34}H_{43}N_2O_{12}$	657.2659 for $C_{33}H_{41}N_2O_{12}$
UV λ_{max}^{MeOH} nm (ε)	223 (37000), 341 (7700)	223 (34300), 341 (6900)
IR ν_{max} (KBr) cm^{-1}	3400, 2970, 1760, 1700, 1650, 1520	3400, 2970, 1760, 1700, 1650, 1520
Solubility		
soluble	MeOH, DMSO, $CHCl_3$	MeOH, DMSO, $CHCl_3$
insoluble	H_2O, n-Hexane	H_2O, n-Hexane

Table 2. ^{1}H and ^{13}C NMR data for SW-163A (**1**) and B (**2**) in DMSO-d_6.

position	**1**		**2**	
	δ_H [a]	δ_C [b]	δ_H [a]	δ_C [b]
1	5.57 (1H, dq, J = 3.4, 6.4 Hz)	70.5 (d)	5.57 (1H, dq, J = 3.4, 6.4 Hz)	70.5 (d)
2	5.21 (1H, dd, J = 3.4, 9.2 Hz)	55.1 (d)	5.19 (1H, dd, J = 3.4, 8.9 Hz)	55.1 (d)
2-NH	9.28 (1H, br.s)		9.56 (1H, br.s)	
3		167.3 (s)		167.4 (s)
4	5.26 (1H, q, J = 7.0 Hz)	68.8 (d)	5.26 (1H, q, J = 7.0 Hz)	68.8 (d)
5		168.8 (s)		168.8 (s)
6	5.35 (1H, dd, J = 4.3, 10.4 Hz)	72.1 (d)	5.35 (1H, dd, J = 4.3, 10.4 Hz)	72.1 (d)
7	3.34 (1H, d, J = 10.4 Hz)	77.5 (d)	3.35 (1H, d, J = 10.4 Hz)	77.4 (d)
7-OH	4.45 (1H, d, J = 10.4 Hz)		4.48 (1H, d, J = 10.4 Hz)	
8		45.3 (s)		45.3 (s)
9		174.9 (s)		174.9 (s)
10	4.69 (1H, d, J = 8.2 Hz)	74.3 (d)	4.62 (1H, d, J = 7.6 Hz)	75.5 (d)
11		168.0 (s)		167.8 (s)
12	1.17 (3H, d, J = 6.4 Hz)	15.5 (q)	1.18 (3H, d, J = 6.4 Hz)	15.7 (q)
13		170.1 (s)		170.0 (s)
14		114.4 (s)		114.4 (s)
15		150.7 (s)		151.7 (s)
15-OH	12.80 (1H, br.s)		12.74 (1H, br.s)	
16		127.0 (s)		127.3 (s)
16-NH	9.80 (1H, s)		9.77 (1H, s)	
17	8.23 (1H, d, J = 7.6 Hz)	124.9 (d)	8.22 (1H, d, J = 7.6 Hz)	124.4 (d)
18	6.92 (1H, t, J = 7.6 Hz)	117.9 (d)	6.84 (1H, br.t, J = 7.6 Hz)	116.9 (d)
19	7.93 (1H, d, J = 7.6 Hz)	123.4 (d)	7.88 (1H, d, J = 7.6 Hz)	123.4 (d)
20	8.33 (1H, d, J = 1.8 Hz)	160.4 (d)	8.33 (1H, d, J = 1.5 Hz)	160.2 (d)
21	0.94 (3H, d, J = 7.0 Hz)	17.1 (q)	0.95 (3H, d, J = 7.0 Hz)	17.1 (q)
22a	2.96 (1H, dd, J = 4.3, 13.7 Hz)	38.6 (t)	2.96 (1H, dd, J = 4.3, 13.7 Hz)	38.5 (t)
22b	3.05 (1H, dd, J = 10.4, 13.7 Hz)		3.05 (1H, dd, J = 10.4, 13.7 Hz)	
23		137.6 (s)		137.6 (s)
24	7.22 (2H, d, J = 7.3 Hz)	129.1 (d)	7.22 (2H, d, J = 7.6 Hz)	129.1 (d)
25	7.28 (2H, t, J = 7.3 Hz)	128.2 (d)	7.28 (2H, t, J = 7.6 Hz)	128.2 (d)
26	7.19 (1H, d, J = 7.3 Hz)	126.3 (d)	7.19 (1H, d, J = 7.6 Hz)	126.3 (d)
27	1.26 (3H, s)	22.0 (q)	1.26 (3H, s)	22.1 (q)
28	1.33 (3H, s)	26.0 (q)	1.33 (3H, s)	26.0 (q)
29	1.85 (1H, m)	35.5 (d)	2.01 (1H, m)	29.6 (d)
30a	1.17 (1H, m)	24.2 (t)		
30b	1.49 (1H, m)			
31	0.85 (3H, t, J = 7.6 Hz)	10.2 (q)	0.90 (3H, d, J = 6.7 Hz)	17.6 (q)
32	0.86 (3H, d, J = 6.4 Hz)	14.0 (q)	0.92 (3H, d, J = 6.7 Hz)	17.9 (q)

[a] ^{1}H NMR at 500 MHz referenced to TMS.

[b] ^{13}C NMR at 125 MHz referenced to DMSO (δ 39.5).

of the methylene protons at δ 1.17 and δ 1.49 in that for **1**. Through the analyses of 2D NMR data, the COSY correlations of H-29 (δ 2.01) with H-31 (δ 0.90) and H-32 (δ 0.92) were observed and the other 2D NMR correlations for **2** were almost consistent with that for **1**. It was shown that compound **2** differs from compound **1** by the substitution of a 2-hydroxy-3-methylbutanoic acid moiety for a 2-hydroxy-3-methylvaleric acid moiety. Thus, the

structure of **2** was determined to be that shown in Fig. 1. All of the assigned proton and carbon signals are listed in Table 2.

Biological Activity

The immunosuppressive activity of **1** and **2** was assessed by one-way mixed lymphocyte reaction (MLR) and mitogen induced lymphocyte blastogenesis. The typical results are shown in Fig. 4. Compounds **1** and **2** inhibited the *in vitro* immune responses of murine splenic lymphocytes in a dose dependent manner. The IC_{50} values of **1** and **2** in MLR were 62 nM and 70 nM, respectively. In Con A and LPS induced lymphocyte blastogenesis, both the IC_{50} values of **1** were 48 nM and those of **2** were 50 nM and 55 nM, respectively. The immunosuppressive activity of **1**

Fig. 4. Inhibitory activity of SW-163A and B in MLR and mitogen induced lymphocyte blastogenesis.

The murine splenocytes were incubated with an alloantigen (MLR, △), Con A(○), or LPS (●) in the presence of the test sample.

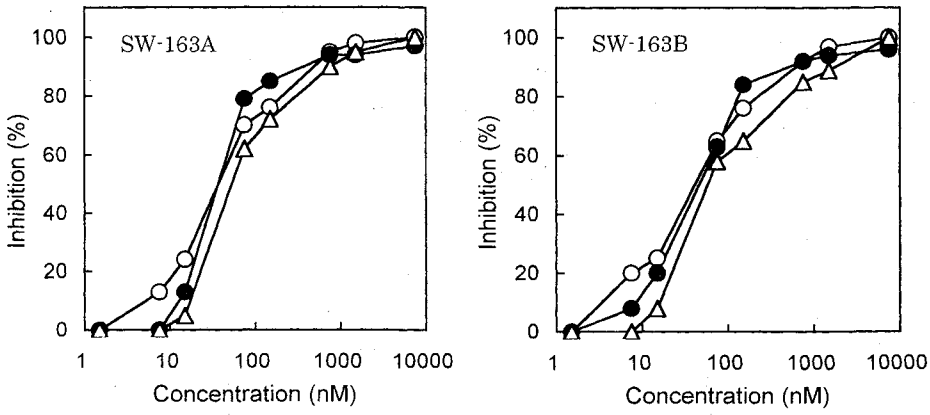


Table 3. Antimicrobial activity of SW-163A (**1**) and B (**2**).

Organisms	Diameter of inhibition zone (mm)	
	1	**2**
Escherichia coli BE 1186	0	0
Salmonella typhimurium TV 119	0	0
Pseudomonas aeruginosa IFO 13130	0	0
Xanthomonas oryzae IFO 3312	0	0
Xanthomonas citri IFO 3781	0	0
Erwinia carotovora IFO 12380	0	0
Staphylococcus aureus 209P	0	0
Bacillus subtilis H17 Rec^+	0	0
Micrococcus luteus IFO 12708	0	0
Mycobacterium phlei IFO 3158	0	0
Alternaria mali IFO 8984	20[a]	25[a]
Botryotinia fuckeliana IFO 5365	28	34
Glomerella lagenaria IFO 7513	10[a]	12[a]
Pyricularia oryzae IFO 5994	29	32
Fusarium oxysporum IFO 9761	0	0
Trichophyton rubrum IFO 6203	0	0
Aspergillus fumigatus IFO 9733	0	0
Candida albicans IFO 1594	25[a]	24[a]
Schizosaccharomyces pombe IFO 0638	0	0

[a] Hazy zone.

was almost as potent as that of **2**.

The cytotoxicity of **1** and **2** against K562 and KB cells was tested. Both compounds did not display 50% growth inhibition against the human cell lines even at 50 μM (data not shown).

The antimicrobial activity is summurized in Table 3. Compounds **1** and **2** had inhibitory activities against fungi and yeast such as *Alternaria mali*, *Botryotinia fuckeliana*, *Glomerella lagenaria*, *Pyricularia oryzae*, and *Candida albicans*. However, both compounds showed no antibacterial activity.

Discussion

SW-163A (**1**) and B (**2**) were isolated from the culture broth of *Streptomyces* sp. SNA15896. The structures of **1** and **2** were elucidated to be a large ring ester structure composed of four hydroxycarboxylic acids and a threonine that related to the structure of neoantimycin[13]. Compounds **1** and **2** showed immunosuppressive activity in MLR and mitogen induced lymphocyte blastogenesis. In both Con A and LPS induced lymphocyte proliferation assays, the dose dependent inhibition curves of these compounds were quite similar in each other. Consequently, the inhibitory activity of **1** and **2** are not selective in T and B cell activation. The mechanism for immunosuppression by **1** and **2** is suggested to be different from that of cyclosporin A and FK-506 that suppress the immune response by blocking T cell activation[3,4]. However, the mechanism involved in the action of **1** and **2** is not elucidated yet. Detailed studies on the immunosuppressive activity of **1** and **2** are in progress. We consider that **1** and **2** may be useful candidates or tools for immunosuppressive agents.

On the other hand, we found that *Streptomyecs* sp. SNA 15896 produces novel antitumor compounds, SW-163C and E[8]. The structures of SW-163C and E are cyclic octadepsipeptides with 3-hydroxyquinaldic acid as a chromophore[9]. Furthermore, this strain also produces FR-900520 (ascomycin)[14] that is a potent immunosuppressant related to FK-506 (data not shown). It is interesting that *Streptomyces* sp. SNA15896 produces various structurally unrelated compounds with different biological activities.

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References

1) DREYFUSS, M.; E. HÄRRI, H. HOFFMANN, H. KOBEL, W. PACHE & H. TSCHERTER: Cyclosporin A and C. New metabolites from *Trichoderma polysporum* (Linx ex Pers.) Rifai. Eur. J. Appl. Microbiol. 3: 125~133, 1976
2) KINO, T.; H. HATANAKA, M. HASHIMOTO, M. NISHIYAMA, T. GOTO, M. OKUHARA, M. KOHSAKA, H. AOKI & H. IMANAKA: FK-506, a novel immunosuppressant isolated from a *Streptomyces*. I. Fermentation, isolation, and physico-chemical and biological characteristics. J. Antibiotics 40: 1249~1255, 1987
3) SCHREIBER, S. L.: Immunophilin-sensitive protein phosphatase action in cell signaling pathways. Cell 70: 365~368, 1992
4) SCHREIBER, S. L. & G. R. CRABTREE: The mechanism of action of cyclosporin A and FK506. Immunology Today 13: 136~142, 1992
5) MYERS, B. D.; J. ROSS, L. NEWTON, J. LUETSCHER & M. PERLROTH: Cyclosporin-associated chronic nephropathy. N. Engl. J. Med. 311: 699~705, 1984
6) The US Multicenter FK506 Liver Study Group: A comparison of tacrolimus (FK506) and cyclosporin for immunosuppression in liver transplantation. N. Engl. J. Med. 331: 1110~1115, 1994
7) ATKINSON, P.; G. JOUBERT, A. BARRON, D. GRANT, K. PARADIS, E. SEIDMAN, W. WALL, H. ROSENBERG, J. HOWARD, S. WILLIAMS & C. STILLER: Hypertrophic cardiomyopathy associated with tacrolimus in paediatric transplant patients. Lancet 345: 894~896, 1995
8) KUROSAWA, K.; K. TAKAHASHI & E. TSUDA: SW-163C and E, novel antitumor depsipeptides produced by *Streptomyces* sp. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 54: 615~621, 2001
9) TAKAHASHI, K.; H. KOSHINO, Y. ESUMI, E. TSUDA & K. KUROSAWA: SW-163C and E, novel antitumor depsipeptides produced by *Streptomyces* sp. II. Structure elucidation. J. Antibiotics 54: 622~627, 2001
10) HAYAKAWA, M. & H. NONOMURA: Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. J. Ferment. Technol. 65: 501~509, 1987
11) HUDSON, L. & F. C. HAY: Practical Immunology, pp. 93~95, 472. Blackwell Scientific Publications, Oxford, 1989
12) MOSMANN, T.: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immuno. Methods 65: 55~63, 1983
13) CAGLIOTI, L.; D. MISITI, R. MONDELLI, A. SELVA, F. ARCAMONE & G. CASSINELLI: The structure of neoantimycin. Tetrahedron 25: 2193~2221, 1969
14) HATANAKA, H.; T. KINO, S. MIYATA, N. INAMURA, A. KURODA, T. GOTO, H. TANAKA & M. OKUHARA: FR-900520 and FR-900523, novel immunosuppressants isolated from a *Streptomyces*. II. Fermentation, isolation and physico-chemical and biological characteristics. J. Antibiotics 41: 1592~1601, 1988