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A-503083 A, B, E and F, Novel Inhibitors of Bacterial Translocase I, Produced by *Streptomyces* sp. SANK 62799

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Novel nucleoside antibiotics were isolated from the cultured broth of the strain classified as *Streptomyces* sp. SANK 62799. The strain produced four novel capuramycin derivatives designated as A-503083 A, B, E and F. Their structures were elucidated as 2′-*O*-carbamoyl derivatives of A-500359 A, B (capuramycin), E and F, respectively. A-503083 A, B, E and F inhibited bacterial phospho-*N*-acetylmuramyl-pentapeptide-translocase (translocase I: EC 2.7.8.13) with IC_{50} values of 0.024, 0.038, 0.135 and 17.9 μM, respectively.

Bacterial phospho-*N*-acetylmuramyl-pentapeptide-translocase (translocase I) is an enzyme that is essential and specific for bacteria. It catalyzes the first step in the lipid cycle of peptidoglycan biosynthesis. Translocase I inhibitors such as A-500359 A[1], capuramycin[2,3] and mureidomycins[4,5] exhibit antimicrobial activity without any toxicities.

In the course of our screening for bacterial translocase I inhibitors, we found inhibitory activity in the cultured broth of *Streptomyces* sp. SANK 62799. The strain produced four novel capuramycin derivatives designated as A-503083 A, B, E and F (Fig. 1). In this paper, we describe the taxonomy and fermentation of the producing microorganism, isolation, physico-chemical properties, structure elucidation and biological activities of A-503083 A, B, E and F.

Materials and Methods

Materials

Undecaprenylphosphate was purchased from Larodan Fine Chemicals. Preparation methods for translocase I and a fluorescent substrate (UDP-MurNAc-L-Ala-γ-D-Glu-*m*-DAP-[N^{ε}-dansyl]-D-Ala-D-Ala) were previously reported.[1]

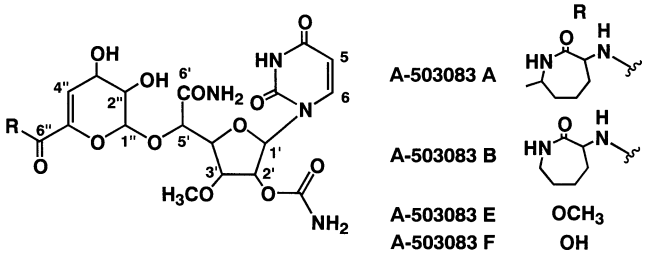
Taxonomy of the Producing Organism

The producing organism, strain SANK 62799, was isolated from a soil sample collected in Naha city, Okinawa prefecture, Japan. Methods and media described by the International Streptomyces Project (ISP)[6] and WAKSMAN[7] were used to determine the morphological and physiological properties of the producing organism. The cell wall and whole-cell hydrolysates were analyzed by the methods of HASEGAWA *et al.*[8]

Fermentation of Strain SANK 62799

Two loopfuls of spores from a culture of strain SANK 62799 were inoculated into each of two 2-liter Erlenmeyer

Fig. 1. Structures of A-503083 A, B, E and F.



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flasks containing 500 ml of sterilized seed medium consisting of glucose 2%, soluble starch 1%, pressed yeast 0.9%, Polypepton (Nihon Seiyaku) 0.5%, meat extract (Kyokuto) 0.5%, NaCl 0.5%, $CaCO_3$ 0.3% and CB-442 (NOF Co., Ltd.) 0.01%, pH 7.4. The inoculated flasks were incubated on a rotary shaker (210 rpm) at 28°C for 4 days. Then a 450-ml aliquot of the culture was transferred into each of two 30-liter jar fermentors containing 15 liters of a sterilized culture medium consisting of glucose 5%, soy bean meal 1%, Polypepton 0.4%, meat extract 0.4%, yeast extract (Difco) 0.1%, NaCl 0.25%, $CaCO_3$ 0.5% and CB442 0.02%, pH 7.2. Fermentation was carried out at 28°C for 7 days with an air-flow rate of 15 liters/minute and agitation rate of 150 rpm.

Measurement of Translocase I Inhibitory Activity

The method used to measure translocase I inhibitory activity was previously reported.[1] Briefly, 50 μl of 200 mM Tris-HCl buffer (pH 7.5) containing 100 mM KCl and 50 mM $MgCl_2$ were added to each well of a 96-well microtitre polystyrene plate (Corning Coaster, #3694) followed by the addition of 12 μl of water and 10 μl of 3.15 mg/ml UDP-MurNAc-L-Ala-γ-D-Glu-*m*-DAP-(N^{ε}-dansyl)-D-Ala-D-Ala (UDP-MurNAc-[N^{ε}-Dns]pentapeptide). Then, 8 μl of lipid solution containing 2.5 mg/ml phosphatidylglycerol, 3.65 mg/ml undecaprenylphosphate and 10% (v/v) Triton X-100 were added to the assay mixture followed by the addition of the test sample. The reaction was started by the addition of 20 μl of the stored enzyme solution (0.625~2.5 μg protein). The enzyme activity was monitored by measuring the increase in fluorescence at 538 nm (excitation at 355 nm).

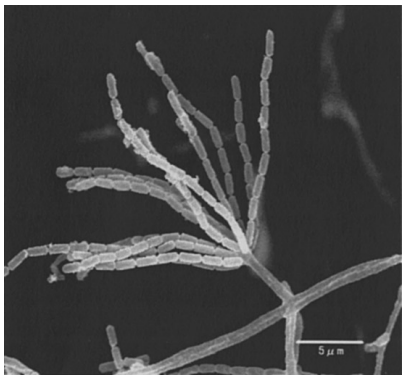
Antimicrobial Activities

MICs were determined by the agar dilution method using Mueller Hinton agar (Becton Dickinson and Company).

General Experimental Procedures

The various NMR spectra were obtained on a Brucker AMX 360 or AVANCE 500 spectrometer. FAB-MS spectra were recorded on a JEOL JMS-700QQ mass spectrometer. LC-MS/MS spectrum was obtained on a Thermoquest LCQ mass spectrometer. Optical rotations were recorded on a JASCO DIP-370 spectropolarimeter. UV spectra were obtained on a Shimadzu UV-265FW spectrometer. Fluorescence was measured at room temperature on a fluorescence spectrophotometer, Fluoroskan Ascent (Labsystems).

Fig. 2. Scanning electron micrograph of the strain SANK 62799 on humic acid-vitamin agar at 28°C for 7 days.



Results

Taxonomy of Strain SANK 62799

Strain SANK 62799 formed primary or secondary verticils of straight to flexuous spore chains. Most spores were oblong and 0.6~0.9×0.9~1.4 μm in size with a smooth surface (Fig. 2). The cultural characteristics of the various agar media at 28°C for 14 days are presented in Table 1. The physiological properties of the strain and the type of carbon source utilized are summarized in Table 2. The vegetative mycelium was pale yellowish brown to light olive gray and the aerial mycelium was yellowish gray.

The whole-cell hydrolysates of the strain contained LL-diaminopimelic acid. Based on the taxonomic properties described above, the strain was classified as *Streptomyces* and was named *Streptomyces* sp. SANK 62799. The strain SANK 62799 was deposited in the International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, Ibaraki Prefecture, Japan with the accession number FERM BP-7201.

Isolation of A-503083 A, B, E and F

The isolation procedure for A-503083 A, B, E and F is shown schematically in Fig. 3. First, the culture filtrate (22 liters) was adjusted to pH 2.0 with 1N HCl and adsorbed onto a SEPABEADS SP207 column (4 liters, Mitsubishi Chemical Corporation). The column was washed successively with 0.04% aqueous trifluoroacetic acid (9 liters) and water (8 liters). The active substances were eluted stepwise with 15% aqueous MeOH (fraction 1: 12 liters), 20% aqueous MeOH (fraction 2: 16 liters), 30%

Table 1. Cultural characteristics of the strain SANK 62799.

Agar Media		Strain SANK 62799
Yeast extract-malt extract agar (ISP-2)	G[a]:	Abundant, raised, light olive gray (5Y 8/4)
	AM:	Good, velvety, yellowish gray (5Y 9/1)
	R:	Light olive gray (5Y 8/4)
	SP:	None
Oatmeal agar (ISP-3)	G:	Good, raised, pale yellowish brown (2.5Y 7/3)
	AM:	Poor, velvety, yellowish gray (5Y 9/1)
	R:	Pale yellowish brown (2.5Y 7/3)
	SP:	None
Inorganic salts-starch agar (ISP-4)	G:	Good, raised, light olive gray (5Y 8/4)
	AM:	Abundant, velvety, pale brown (2.5Y 8/2)
	R:	Light olive gray (5Y 8/4)
	SP:	None
Glycerol-asparagine agar (ISP-5)	G:	Good, raised, pale yellowish brown (2.5Y 7/6)
	AM:	Abundant, velvety, yellowish gray (5Y 9/1)
	R:	Dark brown (5YR 3/3)
	SP:	None
Peptone-yeast extract-iron agar (ISP-6)	G:	Moderate, raised, dark gray (7.5Y 3/N)
	AM:	None
	R:	Dark gray (7.5Y 3/N)
	SP:	None
Tyrosine agar (ISP-7)	G:	Abundant, raised, yellowish brown (2.5Y 5/6)
	AM:	Abundant, velvety, pale pink (7.5R 7/3)
	R:	Dark brown (5YR 3/3)
	SP:	None
Sucrose-nitrate agar	G:	Moderate, raised, yellowish gray (5Y 9/1)
	AM:	Good, velvety, pale brown (7.5YR 8/3)
	R:	Yellowish gray (5Y 9/1)
	SP:	None
Glucose-asparagine agar	G:	Poor, flat, dull yellow (5Y 8/8)
	AM:	Poor, yellowish gray (5Y 9/1)
	R:	Dull yellow (5Y 8/10)
	SP:	None
Nutrient agar (Difco)	G:	Moderate, flat, light olive gray (5Y 8/4)
	AM:	Poor, velvety, yellowish gray (5Y 9/1)
	R:	Light olive gray (5Y 8/4)
	SP:	None
Potato extract-carrot extract agar	G:	Good, flat, yellowish gray (7.5Y 9/2)
	AM:	Abundant, velvety, light brownish gray (10YR 8/2
	R:	Yellowish gray (7.5Y 9/2)
	SP:	None
Water agar	G:	Poor, flat, yellowish gray (5Y 9/1)
	AM:	Moderate, velvety, pale brown (7.5YR 8/3)
	R:	Pale yellow (5Y 9/3)
	SP:	None

[a]G: Growth, AM: Aerial mycelium, R: Reverse, SP: Soluble pigment.

aqueous MeOH (fraction 3: 16 liters) and 50% aqueous MeOH (fraction 4: 24 liters).

Isolation of A-503083 F: Fractions 1, 2 and 3 of the first column eluate were combined, concentrated *in vacuo* and lyophilized to give a crude powder (27.7 g). Then, a 25-mg sample of the powder was dissolved in water and further purified by preparative HPLC using a Pegasil ODS column (20 i.d.×250 mm, Senshu Scientific) with acetonitrile and 0.04% aqueous trifluoroacetic acid (1.8 : 98.2) as a mobile phase (flow rate: 10 ml/minute). An active material was eluted at 17.1 minutes as a single peak, and thus the peak fraction was collected. This procedure was repeated 20 times. The eluates were combined and concentrated *in vacuo* to 10 ml. Then, the solution was applied on a TOYOPEARL HW40-F column (100 ml, TOSOH Corporation). After the column was developed with

Table 2. Physiological properties of the strain SANK 62799.

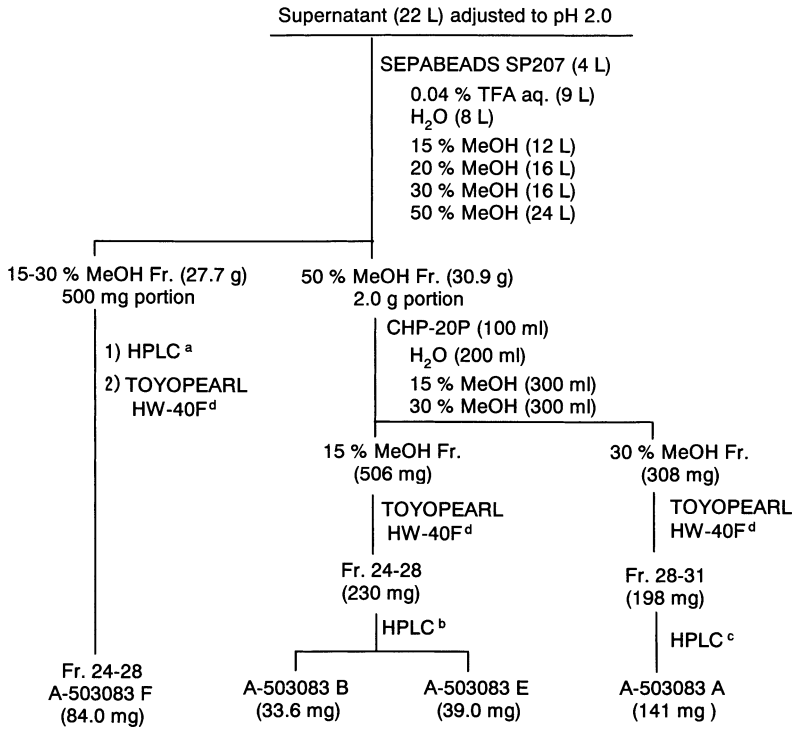
Hydrolysis of starch		+[a]
Liquefaction of gelatin		−[b]
Reduction of nitrate		−
Coagulation of milk		−
Peptonization of milk		+
Production of melanoid pigment		−
Decomposition of	Casein	−
	Tyrosine	−
	Xanthine	−
Temperature range for growth[c]		4～40℃
Optimum temperature for growth[c]		14～35℃
Sodium chloride resistance[c]		5%
Utilization of	D-Glucose	+
	L-Arabinose	−
	D-Xylose	−
	Inositol	+
	D-Mannitol	−
	D-Fructose	−
	L-Rhamnose	−
	Sucrose	−
	Raffinose	−

[a]Utilization. [b]No utilization.
[c]Observed on yeast extract-malt extract agar (ISP-2).

acetonitrile and 0.04% aqueous trifluoroacetic acid (6 : 94), the active fractions were collected, concentrated *in vacuo* and lyophilized to give A-503083 F as a colorless powder (84.0 mg).

Isolation of A-503083 B and E: Fraction 4 of the first column eluate was concentrated *in vacuo* and lyophilized to give a crude powder (30.9 g). Then, the powder (2 g) was dissolved in 10 ml of water, and the solution was adsorbed onto a MCI GEL CHP20P column (100 ml). After the column was washed with water (200 ml), active materials were eluted stepwise with 15% aqueous MeOH (fraction BE: 300 ml) and 30% aqueous MeOH (fraction A: 300 ml). Each fraction was separately concentrated *in vacuo* and lyophilized to give crude powders (506 mg and 308 mg, respectively). The powder of fraction BE was dissolved in 5 ml of acetonitrile and 0.04% aqueous trifluoroacetic acid (9 : 91), and the solution was applied on a TOYOPEARL HW40-F column (100 ml). After the column was developed with acetonitrile and 0.04% aqueous trifluoroacetic acid (6 : 94), an active fraction was collected, concentrated *in vacuo* and lyophilized to give a crude powder (230 mg). The powder was dissolved in 2 ml of acetonitrile and 0.04%

Fig. 3. Isolation procedure of A-503083 A, B, E and F.



[a]Pegasil ODS, ϕ 20 x 250 mm, 1.8% CH_3CN/ 0.04% TFA aq., 10 ml/minute, detect: UV 260 nm.
[b]Capcell pak C18 UG 120, ϕ 20 x 250 mm, 9% MeCN/ TFA aq., 10 ml/minute, detect: UV 260 nm.
[c]Capcell pak C18 UG 120, ϕ 20 x 250 mm, 10% MeCN/ TFA aq., 10 ml/minute, detect: UV 260 nm.
[d]TOYOPEARL HW-40F, 100 ml, 6% CH_3CN/0.04 % TFA aq.

Table 3. Physico-chemical properties of A-503083 A, B, E, and F.

	A-503083 A	A-503083 B	A-503083 E	A-503083 F
Appearance	Colorless powder	Colorless powder	Colorless powder	Colorless powder
Solubility				
Soluble	H_2O, MeOH	H_2O, MeOH	H_2O, MeOH	H_2O, MeOH
Insoluble	Acetone, $CHCl_3$	Acetone, $CHCl_3$	Acetone, $CHCl_3$	Acetone, $CHCl_3$
$[\alpha]_D^{29}$	+84.0° (*c* 0.7, H_2O)	+91.0° (*c* 0.2, H_2O)	+61.0° (*c* 0.2, H_2O)	+110° (*c* 0.3, H_2O)
UV $\lambda_{max}^{H_2O}$nm (ε)	257 (11000)	257 (10900)	250 (9190)	259 (7980)
Molecular formula	$C_{25}H_{34}N_6O_{13}$	$C_{24}H_{32}N_6O_{13}$	$C_{19}H_{24}N_4O_{13}$	$C_{18}H_{22}N_4O_{13}$
FAB-MS (*m/z*)	627 $[M+H]^+$	613 $[M+H]^+$	517 $[M+H]^+$	503 $[M+H]^+$
HR-FAB-MS (*m/z*)	$C_{25}H_{35}N_6O_{13}$	$C_{24}H_{33}N_6O_{13}$	$C_{19}H_{25}N_4O_{13}$	$C_{18}H_{23}N_4O_{13}$
Calcd.:	627.2262	613.2106	517.1418	503.1262
Found:	627.2259	613.2108	517.1424	503.1255
IR (KBr) cm^{-1}	3360, 3101, 2935, 1780, 1687, 1516, 1459, 1131, 1387, 1361, 1336, 1299, 1269, 1214, 1159 1064, 1021, 976	3362, 2936, 2860, 1684, 1515, 1463, 1437, 1387, 1359, 1335, 1270, 1209, 1144, 1020, 975	3359, 3200, 2954, 2845, 1686, 1605, 1463, 1442, 1389, 1333, 1306, 1269, 1213, 1142, 1118, 1087, 1065, 1026, 977	3359, 3196, 2939, 1685, 1464, 1391, 1357, 1336, 1270, 1232, 1208, 1117, 1064, 1020, 977

aqueous trifluoroacetic acid (9 : 91). Then, a 200-μl aliquot was injected into the HPLC column [Capcell pak C18 UG 120, 20 i.d.×250 mm, SHISEIDO; mobile phase: acetonitrile and 0.04% aqueous trifluoroacetic acid (9 : 91); flow rate: 10 ml/minute], and active materials (Rt=25.2 minutes and 17.7 minutes) were collected. The same procedure was repeated 10 times. The respective fractions were combined, concentrated *in vacuo* and lyophilized to give A-503083 B (33.6 mg) and A-503083 E (39.0 mg), as colorless powders.

Isolation of A-503083 A: The powder of Fraction A (308 mg) was dissolved in 2 ml of acetonitrile and 0.04% aqueous trifluoroacetic acid (6 : 94), and the solution was applied on a TOYOPEARL HW40-F column (100 ml). After the column was developed with acetonitrile - 0.04% aqueous trifluoroacetic acid (6 : 94), the active fraction was collected, concentrated *in vacuo* and lyophilized to give a crude powder (198 mg). The powder was dissolved in 0.5 ml of acetonitrile - 0.04% aqueous trifluoroacetic acid (1 : 9). A 100-μl aliquot was injected into an HPLC column [Capcell pak C18 UG 120, 20 i.d.×250 mm; mobile phase: acetonitrile - 0.04% aqueous trifluoroacetic acid (1 : 9); flow rate: 10 ml/minute], and the same procedure was repeated 10 times. Since an active material was eluted at 25.5 minutes as a single peak, the peak fraction was collected, concentrated *in vacuo* and lyophilized to give A-503083 A as a colorless powder (140 mg).

Physico-chemical Properties of A-503083 A, B, E and F

The physico-chemical properties of A-503083 A, B, E and F are summarized in Table 3. The molecular formulae of A-503083 A, B, E and F were determined by high resolution FAB-MS to be $C_{25}H_{34}N_6O_{13}$, $C_{24}H_{32}N_6O_{13}$, $C_{19}H_{24}N_4O_{13}$ and $C_{18}H_{22}N_4O_{13}$, respectively. Because of the similarity in the physico-chemical properties of these compounds to those of A-500359s,[9,10] they were considered to be A-500359-related compounds.

Elucidation of the Structures

Based on the physico-chemical properties and ^{13}C and ^{1}H NMR data (Table 4), the structure of A-503083 F was considered to be highly related to that of A-500359 F.[10] Thus, the structure of A-503083 F was elucidated based on a comparison study with A-500359 F. The observation of two exchangeable proton signals (6.59 and 6.86 ppm), the appearance of a carbonyl carbon signal (155.1 ppm) and the low field shift of the 2′ proton signal (4.38 to 5.12 ppm) were characteristic to A-503083 F. Knowing that C, H, N and O atoms were present and based on the precise analysis by ^{1}H-^{15}N HSQC and ^{1}H-^{13}C HMBC, the 6.59 and

Table 4. ^{13}C and ^{1}H signal assignments for A-503083 A, B, E and F in DMSO-d_6.

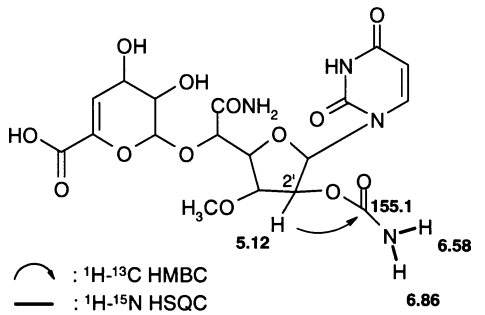
Position	A-503083 A		A-503083 B		A-503083 E		A-503083 F	
	^{13}C chemical shift (ppm) [a]	^{1}H chemical shift (ppm) [b]	^{13}C chemical shift (ppm) [a]	^{1}H chemical shift (ppm) [b]	^{13}C chemical shift (ppm) [a]	^{1}H chemical shift (ppm) [b]	^{13}C chemical shift (ppm) [a]	^{1}H chemical shift (ppm) [b]
2	150.3		150.3		150.2		150.2	
3NH		11.30 (1H, d, 1.6)		11.30 (1H, d, 2.0)		11.30 (1H, d, 2.0)		11.30 (1H, d, 2.1)
4	163.0		163.1		163.1		163.1	
5	101.8	5.62 (1H, dd, 1.6, 8.1)	101.8	5.62 (1H, dd, 2.0, 8.1)	101.6	5.62 (1H, dd, 2.0, 8.0)	101.6	5.62 (1H, dd, 8.1, 2.1)
6	139.9	7.78 (1H, d, 8.1)	139.9	7.87 (1H, d, 8.1)	139.9	7.75 (1H, d, 8.1)	139.9	7.77 (1H, d, 8.1)
1'	86.2	5.77 (1H, d, 4.6)	86.2	5.81 (1H, d, 4.5)	86.7	5.76 (1H, d, 4.1)	86.7	5.76 (1H, d, 4.1)
2'	72.9	5.13 (1H, dd, 4.6, 4.7)	72.8	5.12 (1H, dd, 4.5, 4.8)	72.9	5.11 (1H, dd, 4.1, 4.4)	72.9	5.12 (1H, dd, 4.1, 4.4)
2'$CONH_2$	155.1	6.56 (1H, br. s)	155.0	6.55 (1H, br. s)	155.1	6.57 (1H, br. s)	155.1	6.59 (1H, br. s)
		6.83 (1H, br. s)		6.86 (1H, br. s)		6.85 (1H, br. s)		6.86 (1H, br. s)
3'	78.1	3.70 (1H, t, 4.7)	78.2	3.70 (1H, t, 4.8)	77.8	3.77 (1H, dd, 5.3, 4.4)	77.7	3.77 (1H, dd, 5.2, 4.4)
3'OCH_3	58.1	3.24 (3H, s)	58.0	3.23 (3H, s)	58.0	3.26 (3H, s)	58.1	3.28 (3H, s)
4'	81.8	4.38 (1H, m)	81.8	4.38 (1H, dd, 4.8, 1.5)	81.8	4.35 (1H, m)	81.8	4.35 (1H, m)
5'	75.5	4.40 (1H, br. s)	75.4	4.43 (1H, d, 1.5)	75.5	4.34 (1H, m)	75.1	4.34 (1H, m)
6'$CONH_2$	169.9	7.69 (1H, br. s)	169.9	7.69 (1H, br. s)	169.9	7.56 (1H, br. s)	169.9	7.58 (1H, br. s)
		7.61 (1H, br. s)		7.61 (1H, br. s)		7.74 (1H, br. s)		7.70 (1H, br. s)
1''	99.2	5.10 (1H, d, 3.9)	99.2	5.11 (1H, d, 4.9)	99.4	5.07 (1H, d, 3.9)	99.0	5.04 (1H, d, 3.9)
2''	65.5	3.99 (1H, t, 3.9)	65.4	3.99 (1H, dd, 4.9, 3.7)	65.0	3.96 (1H, dd, 3.8, 3.9)	65.1	3.94 (1H, dd, 3.6, 3.9)
3''	61.4	4.28 (1H, t, 3.9)	61.4	4.28 (1H, t, 3.7)	61.4	4.31 (1H, dd, 3.8, 1.8)	61.5	4.29 (1H, dd, 3.6, 2.0)
4''	109.0	5.76 (1H, d, 2.1)	109.0	5.76 (1H, d, 3.7)	114.6	5.92 (1H, br. d, 1.8)	113.8	5.88 (1H, br. d, 2.0)
5''	141.7		141.6		139.1		139.8	
6''	159.2		159.2		161.9		162.9	
-NH		7.90 (1H, d, 6.0)		7.87 (1H, d, 6.0)				
-OCH_3					52.1	3.70 (3H, s)		
-OH								12.80 (1H, s)
1'''	172.8		173.6					
2'''	51.5	4.39 (1H, m)	51.4	4.41 (1H, m)				
3'''	30.5	1.34 (1H, m)	30.7	1.35 (1H, m)				
		1.90 (1H, m)		1.92 (1H, m)				
4'''	26.7	1.74 (1H, m)	27.4	1.66 (1H, m)				
		1.83 (1H, m)		1.90 (1H, m)				
5'''	36.6	1.16 (1H, m)	28.7	1.03 (1H, m)				
		1.67 (1H, m)		1.75 (1H, m)				
6'''	47.8	3.53 (1H, m)	40.5	3.06 (1H, m)				
				3.18 (1H, m)				
-CH_3	21.7	1.11 (3H, d, 6.5)						
- NH		7.69 (1H, d, 4.3)		7.99 (1H, t, 6.6)				

[a]The DMSO-d_6 signal (39.5 ppm) was used as a reference.
[b]The DMSO-d_6 signal (2.05 ppm) was used as a reference.

6.86 ppm proton signals and the 155.1 ppm carbon signal were assigned to the 2′-*O*-carbamoyl moiety of A-503083 F (Fig. 4). Observation of a fragment ion at *m/z* 458 assigned as the $[M-CONH_2]^-$ ion of A-503083 F in the ESI LC-MS/MS spectrum supported the conclusion that the structure of A-503083 F was a 2′-*O*-carbamoyl-derivative of A-500359 F (data not shown).

The structures of A-503083 A, B and E were also elucidated by a comparison study with A-500359 A, B and E, respectively. Since the appearance of ^{13}C and ^{1}H NMR signals derived from the carbamoyl moiety were the same as the characteristic signals of A-503083 A, B and E, their structures were elucidated as 2′-*O*-carbamoyl derivatives of A-500359 A, B and E, respectively.

Fig. 4. ^{1}H-^{13}C HMBC and ^{1}H-^{15}N HSQC data of A-503083 F.



Biological Activities of A-503083 A, B, E and F

The translocase I inhibitory activities and antimicrobial activities of A-503083 A, B, E and F are given in Table 5 and Table 6, respectively. A-503083 A, B, E and F inhibited translocase I with IC_{50} values of 0.024, 0.038, 0.135 and

17.9 μM, respectively. A-503083 A and B showed antimicrobial activity against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Mycobacterium smegmatis* and *Mollaxella catarrhalis*. A-503083 E had less potent antimicrobial activity than A-503083 A and B. A-503083 F showed no antimicrobial activity so far, in agreement with its relatively weak translocase I inhibitory activity.

Discussion

In the course of our screening for bacterial translocase I inhibitors, we discovered novel inhibitors, A-503083 A, B, E and F, and their structures were elucidated as 2′-*O*-carbamoyl derivatives of A-500359 A, B, E and F, respectively. Although the strain SANK 62799 also produced trace amounts of A-500359 A, B and E (data not shown), the taxonomic studies showed the strain was distinct from *Streptomyces griseus* SANK 60196, which was previously reported to produce capuramycin and A-500359s.[1,10] It was interesting to note that strain SANK 62799 had a greater tendency to produce *O*-carbamoyl derivatives in addition to A-500359s.

In the previous study A-500359 A, B, E and F were reported to be potent translocase I inhibitors with IC_{50} values of 0.017, 0.018, 0.057 and 2.4 μM, respectively. Comparison studies with the IC_{50} values of A-503083 A, B, E and F indicated that the addition of a 2′-*O*-carbamoyl moiety reduced the inhibitory activity 2- to 8-fold. Consistently, their antimicrobial activities were less potent compared with those of A-500359s. These results indicate that the 2′-OH in A-500359s is important for the inhibitory activity against the translocase I. However, this is the first report to show the existence of the 2′-*O*-carbamoyl derivatives among the series of A-500359s and it is interesting that various strains produce the capuramycin related compounds.

Table 5. Inhibition of translocase I by A-503083 A, B, E and F.

A-503083	IC_{50} (μM)
A	0.024
B	0.038
E	0.135
F	17.9

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Table 6. Antimicrobial activity of A-503083 A, B, E and F.

Microorganism	MIC (μg/ml) A-503083 A	B	E	F
Staphylococcus aureus FDA 209P JC-1	>100	>100	>100	>100
Streptococcus pneumoniae SANK 73201[a]	25	50	>100	>100
Streptococcus pneumoniae SANK 73301[b]	100	50	>100	>100
Streptococcus pyogenes SANK 73401	25	50	100	>100
Bacillus subtilis ATCC6633	>100	>100	>100	>100
Mycobacterium smegmatis ATCC 607	25	25	>100	>100
Moraxella catarrhalis SANK 72901	6.25	6.25	25	>100
Escherichia coli NIHJ JC-2	>100	>100	>100	>100
Klebsiella pneumoniae IID865	>100	>100	>100	>100
Enterobacter cloacae IID977	>100	>100	>100	>100
Serratia marcescens IAM 1184	>100	>100	>100	>100
Pseudomonas aeruginosa PAO1	>100	>100	>100	>100

[a]Penicillin-sensitive strain.
[b]Penicillin highly resistant strain.

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