TALLYSOMYCIN*, A NEW ANTITUMOR ANTIBIOTIC COMPLEX RELATED TO BLEOMYCIN

V. PRODUCTION, CHARACTERIZATION AND ANTITUMOR ACTIVITY OF TALLYSOMYCIN S_{10b}, A NEW BIOSYNTHETIC TALLYSOMYCIN DERIVATIVE

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Tallysomycin S_{10b} is a biosynthetic derivative of tallysomycin B obtained by precursor amine-feeding fermentation. Tallysomycin S_{10b} contains 1,4-diaminobutane as the terminal amine moiety in place of spermidine of tallysomycin B, and its assigned structure was verified by carbon-13 NMR spectrum. The antitumor activity of tallysomycin S_{10b} was comparable to that of tallysomycin A against Lewis lung carcinoma and B16 melanoma. Tallysomycin S_{10b} was more active than tallysomycin A against sarcoma 180 but less active than the latter against leukemia P388. Tallysomycin S_{10b} was less toxic than tallysomycin A in terms of acute and subacute LD_{50} values. The nephrotoxic potential of tallysomycin S_{10b} in rats was less than that of tallysomycin A.

In a preceding paper[1], we reported that certain diamine compounds added to the fermentation medium were incorporated into the terminal amine moiety of tallysomycin molecule, affording two series of biosynthetic derivatives with or without a β-lysine unit in the subterminal position. Among a number of new biosynthetic derivatives prepared and examined, tallysomycin S_{10b} was selected for further study in view of its promising antitumor activity and reduced toxicity.

The present paper describes the fermentation, isolation and structure verification of tallysomycin S_{10b}. The antitumor activities of tallysomycin S_{10b} in several experimental tumor systems as well as preliminary toxicological evaluation are also reported in this paper.

Fermentation Studies

The antibiotic productivity of the original isolate, *Streptoalloteichus hindustanus* strain No. E465-94[2], was improved by a combination of mutational procedures (UV and N-methyl-N′-nitro-N-nitrosoguanidine treatments) and a monospore isolation process, which afforded mutant strain No. AC-10570. This strain was used for the studies of tallysomycin S_{10b} fermentation.

Strain No. AC-10570 was grown and maintained on an agar slant containing 1.0% malt extract, 0.4% yeast extract, 0.4% glucose, 0.05% $CaCO_3$ and 1.6% agar. An agar culture was used to inoculate 100 ml of vegetative medium comprised of 1.5% glucose, 0.2% yeast extract, 0.5% peptone, 0.05% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.5% $CaCO_3$ (pH 7.2 before sterilization) in a 500-ml Erlenmeyer flask. The seed culture was incubated at 33°C for 48 hours on a rotary shaker (250 rpm), and 5 ml of

* The USAN generic name "Talisomycin" has been assigned to tallysomycin A.

Table 1. Effect of nitrogen source on tallysomycin production.

Nitrogen source	Maximum potency* (mcg/ml)		
	4%	3%	2%
Cottonseed meal	323	375	241
Cornsteep liquor	161	305	155
Soybean meal	155	241	155
Fish meal	299	180	99
Linseed meal	229	307	307
Distiller's solubles	219	583	375

Basal medium: 2% glycerol, 0.3% $(NH_4)_2SO_4$, 0.003% $ZnSO_4 \cdot 7H_2O$, 0.01% $CuSO_4 \cdot 5H_2O$ and 0.4% $CaCO_3$, pH 7.2 before sterilization. Cultured for 7 days at 28°C.

* Potency: Total tallysomycin activity (assay standard: tallysomycin A).

Table 2. Effect of DAB concentration on tallysomycin S_{10b} production.

Concentration of DAB (%)	Potency (mcg/ml)*	Relative production of tallysomycin components (%)**		
		S_{10b}	S_{10a}+B	A
0.8	50	70	21	9
0.4	105	63	24	13
0.2	150	42	38	20
0.1	210	35	45	20
0.05	250	23	52	25
0.025	300	13	60	27

* Overall potency determined by paper disc-agar plate assay using tallysomycin A (copper-free base, 1,000 mcg/ml) as a standard.

** Ratio of each component in fermentation broth determined by HPLC[1]; retention time (Rt) for tallysomycin S_{10b} 4'47", S_{10a} 6'20", B 6'30", and A 8'30".

the seed was transferred to a 500-ml Erlenmeyer flask containing 100 ml of various fermentation media to be examined. The production cultures were fermented at 28°C on a rotary shaker. The assay of antibiotic activity in fermentation broth was made by a paper disc-agar plate method using *Mycobacterium smegmatis* strain M6-3 as the test organism[3]. The relative productivity of tallysomycin S_{10b} and other co-produced components (tallysomycins A, B and S_{10a}) was determined by HPLC or UV-scanning TLC as described in the preceding paper[1].

Various nitrogen sources were tested to determine their effects on the production of tallysomycin components (Table 1). Distiller's solubles was found to be a suitable nitrogen source for overall tallysomycin production. A combination of distiller's solubles, cottonseed meal and ammonium sulfate gave a relatively high yield of the tallysomycin S_{10b} component, while replacement of distiller's solubles with fish meal increased the relative production of tallysomycin A and S_{10a} components. A combination of glucose and sucrose was selected as the preferred carbon source.

The optimum concentration of 1,4-diaminobutane hydrochloride (DAB) to be added to the fermentation medium was investigated. As shown in Table 2, the formation of tallysomycin S_{10b} increased with the increase in DAB concentration in the medium. However the presence of a high concentration of DAB showed a tendency to suppress cell growth and overall tallysomycin production. The DAB concentration in the fermentation medium finally selected for the production of tallysomycin S_{10b} was 0.2%. The optimal time for the addition of DAB to the fermentation medium was also tested. The precursor amine was well incorporated when it was added to the medium within 24 hours of the start of the fermentation. Incorporation was markedly decreased when added thereafter.

Based on the results of fermentation studies described above, a fermentation medium having the following composition was selected for the production of tallysomycin S_{10b}: 2.5% sucrose, 0.5% glucose, 3.0% distiller's solubles, 3.0% cottonseed meal, 0.3% $(NH_4)_2SO_4$, 0.003% $ZnSO_4 \cdot 7H_2O$, 0.4% $CaCO_3$, 0.01% $CuSO_4 \cdot 5H_2O$ and 0.2% DAB. In one experimental fermentation, a seed culture was shaken for 48 hours at 33°C in Erlenmeyer flasks and used to inoculate 130 liters of germination medium (1.5%

glucose, 0.5% peptone, 0.25% dry yeast, 0.05% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.3% $CaCO_3$) in a 200-liter seed tank which was stirred at 190 rpm for 32 hours at 28°C. This seed culture was transferred to a 4,500-liter fermentation tank containing 3,000 liters of the above-described production medium. The fermentor was operated at 190 rpm with aeration at 2,000 liters per minute. The time course of the fermentation is shown in Table 3.

Table 3. Fermentation time course.

	Fermentation hours					
	24	48	72	96	120	144
Potency (mcg/ml)	—	126	162	321	392	393
pH	7.5	7.4	7.2	7.4	6.7	6.1
PCV* (%)	39	60	78	68	70	70

* Packed cell volume.

Isolation and Purification

The harvested broth (3,000 liters) was adjusted to pH 7.0 with 3 N H_2SO_4 and filtered with filter aid. The filtrate was applied on a column of Amberlite IRC-50 (NH_4^+ form, 300 liters) which was washed with water (600 liters) and 0.1 N NH_4OH (1,000 liters) to elute the co-produced nebramycin factors. The column was washed again with water and tallysomycin components were eluted with 1 N HCl. The active fractions were combined, adjusted to pH 7.3 with 28% NH_4OH and concentrated *in vacuo*. The resulting solution (50 liters) was charged on a column of Diaion HP-20 (48 liters) which was then developed with water (30 liters) and 0.01 N HCl (100 liters). The active eluates were neutralized with Amberlite IR-45 (OH^- form) and concentrated *in vacuo* to afford a blue-colored powder of tallysomycin complex (652 g) which was shown by TLC to be comprised of four components, A, B, S_{10a} and S_{10b}.

A part of the crude tallysomycin complex (326 g) was dissolved in 2% $HCOONH_4$ solution (1.5 liters) and a small amount of $CuCl_2$ (7.0 g) was added to the solution to assure that there was complete copper chelation. The solution was chromatographed on a column of CM-Sephadex C-25 (4.8 liters) which had been equilibrated with 2% $HCOONH_4$. Elution was carried out with increasing concentrations of $HCOONH_4$ solution (2%~5%) and the eluates were monitored by UV absorption at 290 nm. Tallysomycin S_{10b} was eluted first with 3% $HCOONH_4$ solution followed by a mixture of tallysomycins S_{10a} and B. Tallysomycin A was eluted last with 5% $HCOONH_4$. The fractions containing tallysomycin S_{10b} were pooled and passed through a column of Diaion HP-20 for desalting, and the column then developed with water. The active eluates were concentrated *in vacuo* and lyophilized to afford pure copper-chelated tallysomycin S_{10b} formate (35.7 g). A mixture of tallysomycins S_{10a} and B (62.7 g) and tallysomycin A (35.0 g) were also obtained by similar work-up of respective fractions from the above CM-Sephadex chromatography.

Copper-free tallysomycin S_{10b} was prepared by a treatment with hydrogen sulfide in acidic methanol. A solution of copper-chelated tallysomycin S_{10b} (24 g) in anhydrous methanol (1.2 liters) was acidified to pH 3.0 with 1 N methanolic HCl solution. Dry H_2S gas was bubbled into the solution for 15 minutes under stirring. Cupric sulfide precipitate was removed by filtration using a small amount of active carbon (pre-washed with EDTA solution). The filtrate was concentrated *in vacuo* to a syrup which was dissolved in water. The aqueous solution was again evaporated azeotropically with *n*-butanol to remove excess HCl and solvated methanol. Lyophilization of the residual solution yielded copper-free tallysomycin S_{10b} hydrochloride (23.2 g).

Physico-chemical Properties

The copper-chelated tallysomycin S_{10b} was obtained as an amorphous blue powder, while the copper-free preparation was a white powder. Both forms of the antibiotic did not have a definite melting point

Table 4. Physico-chemical properties of tallysomycin S_{10b}.

		Copper-chelated tallysomycin S_{10b}	Copper-free tallysomycin S_{10b}
Appearance		Blue powder	White powder
M.p.		>210°C (dec.)	>210°C (dec.)
Analysis	Calcd for	$C_{59}H_{91}N_{19}O_{26}S_2 \cdot Cu \cdot 3HCl \cdot 6H_2O$	$C_{59}H_{91}N_{19}O_{26}S_2 \cdot 5HCl \cdot 8H_2O$
	C:	38.77	37.83
	H:	5.84	6.02
	N:	14.56	14.20
	S:	3.50	3.40
	Cl:	5.82	9.46
	Found C:	38.81	37.58
	H:	5.58	5.59
	N:	14.74	14.21
	S:	3.45	3.42
	Cl:	5.55	9.53
$[\alpha]_D^{26}$ (c 1.0, H_2O)		+137°	−38°
UV $\lambda_{max}^{H_2O}$ nm ($E_{1cm}^{1\%}$)		243.5 (141) 292 (114)	235 (inflexion) 290 (87)
TLC S-102 (SiO_2, MeOH - 10 % $AcONH_4$=1 : 1)		Rf 0.60	0.51

Fig. 1. IR spectrum of tallysomycin S_{10b} (Cu-free hydrochloride).

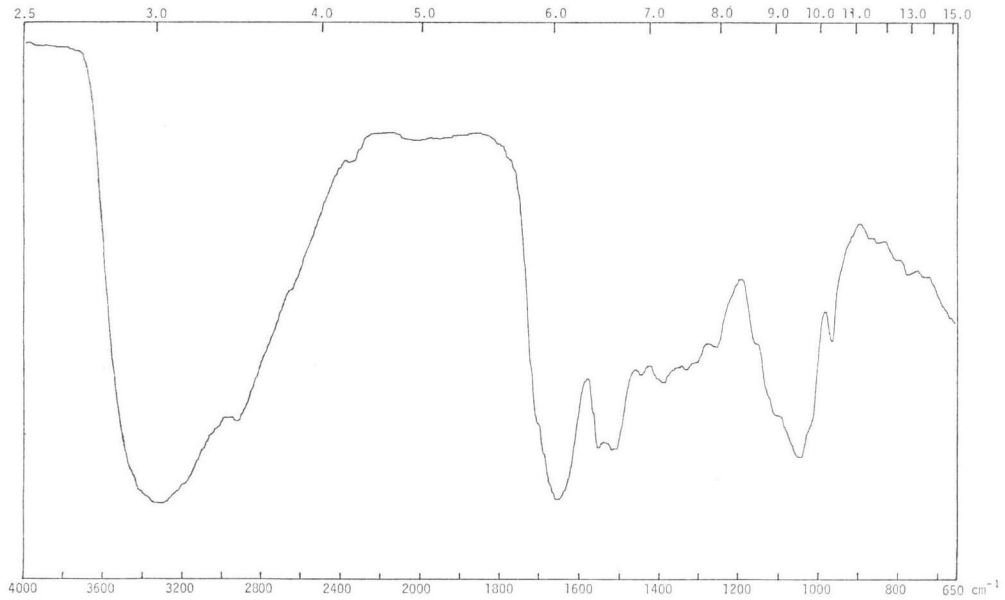


Fig. 2. NMR spectrum of tallysomycin S_{10b} (Cu-free hydrochloride).

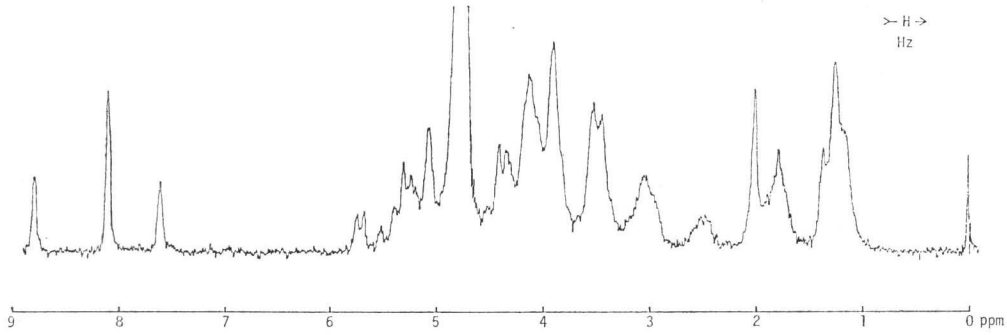
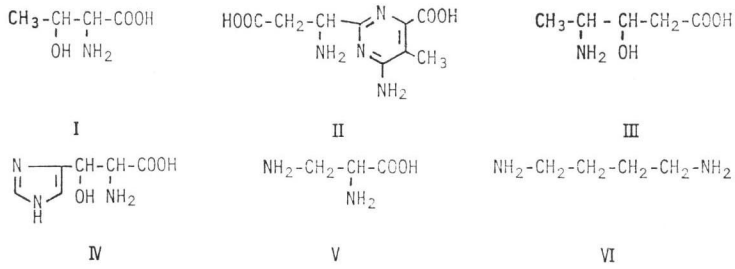


Fig. 3. Constituent amino acids and amine of tallysomycin S_{10b}.



but gradually decomposed above 210°C. Tallysomycin S_{10b} was readily soluble in water, methanol and dimethylformamide, slightly soluble in ethanol but insoluble in other organic solvents. The antibiotic showed positive responses to ninhydrin, anthrone and RIMINI[4] reagents but was negative in FEHLING, TOLLENS and SAKAGUCHI reactions. The copper-chelated tallysomycin S_{10b} formed a green precipitate with rubeanic acid reagent[5]. The physico-chemical properties of tallysomycin S_{10b} are shown in Table 4. The microanalysis of copper-chelated and copper-free tallysomycin S_{10b} hydrochloride agreed with the molecular formula of $C_{59}H_{91}N_{19}O_{26}S_2$ for a free base. Tallysomycin S_{10b} showed a UV spectrum similar to that of natural tallysomycins A and B. The IR and ^{1}H-NMR spectra of copper-free tallysomycin S_{10b} are shown in Figs. 1 and 2, respectively. The ^{1}H-NMR spectrum of tallysomycin S_{10b} differs from those of tallysomycins A and B in the methylene proton region (δ 1.5~3.2 ppm).

Copper-free tallysomycin S_{10b} was hydrolyzed with 6N HCl at 115°C for 21 hours in a sealed tube. TLC and high voltage electrophoresis of the hydrolyzate indicated the presence of five amino acids (**I**, **II**, **III**, **IV** and **V** shown in Fig. 3) which were also found in the hydrolyzate of natural tallysomycins A and B[6]. The amine component (**VI**) of tallysomycin S_{10b} was identified as 1,4-diaminobutane. β-Lysine and spermidine were not found in the acid hydrolyzate of tallysomycin S_{10b}. Thus, it was concluded that the precursor amine, 1,4-diaminobutane, was incorporated in tallysomycin S_{10b} as the terminal amine moiety.

The structure of tallysomycin S_{10b} assigned above was further verified by ^{13}C-NMR studies on the antibiotic. The ^{13}C-NMR spectrum of copper-free tallysomycin S_{10b} was recorded with a Varian FT 80A spectrometer (20 MHz, in D_2O) at various pD's (pD 1.5~8.5). The chemical shift data obtained at pD 6.5 using tetramethylsilane as an external reference are tabulated in Table 5 in comparison with those of tallysomycins A and B. The signal assignments were based on the literature data for tallyso-

Table 5. ^{13}C-NMR data for tallysomycins S_{10b}, B and A (δ in ppm at pD 6.5, external standard: tetramethylsilane).

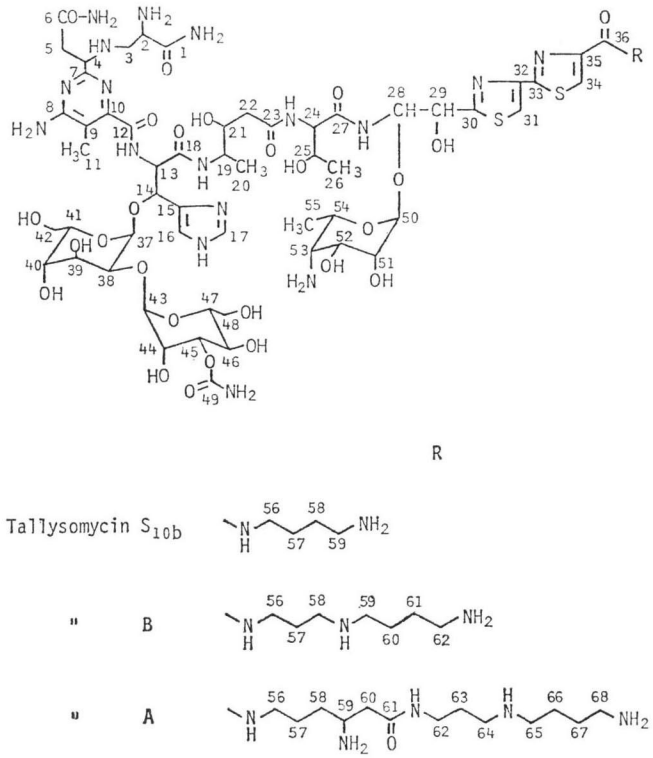
Carbon No.*	Tallysomycin			Carbon No.	Tallysomycin		
	S_{10b}	B	A		S_{10b}	B	A
1	172.1	172.2	172.2	35	149.6	149.4	149.6
2	60.3	60.3	60.3	36	172.4	172.2	172.3
3	47.8	47.6	47.5	37	98.5	98.5	98.5
4	53.1	53.2	53.4	38	74.2	74.6	74.2
5	40.7	40.6	40.6	39	71.0	70.9	70.7
6	176.7	176.5	176.5	40	69.6	69.7	69.7
7	165.9	165.8	165.9	41	67.8	68.0	67.9
8	165.0	165.0	165.0	42	60.7	60.8	60.7
9	112.4	112.5	112.4	43	99.0	98.9	98.9
10	153.1	153.1	153.1	44	68.9	68.9	68.9
11	11.2	11.2	11.2	45	75.0	74.9	74.9
12	168.2	168.1	168.1	46	65.4	65.3	65.3
13	57.3	57.4	57.3	47	74.0	74.0	74.0
14	68.6	68.3	68.4	48	61.5	61.5	61.5
15	135.4	135.3	135.4	49	158.5	158.4	158.4
16	118.4	118.5	118.5	50	100.7	100.6	100.7
17	137.5	137.4	137.4	51**	68.4	68.1	68.3
18	170.2	170.0	170.1	52**	64.8	64.7	64.8
19	50.4	50.3	50.3	53	54.9	54.9	54.9
20	15.0	14.9	14.9	54**	63.6	63.6	63.6
21	71.9	71.8	71.8	55	16.6	16.5	16.6
22	40.3	40.3	40.2	56	39.8	37.0	39.5
23	174.7	174.5	174.5	57	26.3	26.3	25.2
24	59.4	59.4	59.4	58	24.9	45.8	30.0
25	67.4	67.3	67.4	59	39.4	47.5	49.3
26	19.5	19.5	19.5	60		23.4	37.3
27	173.1	172.9	172.9	61		24.5	172.6
28	81.3	81.2	81.3	62		39.5	36.8
29	71.5	71.3	71.3	63			26.0
30	163.2	163.1	163.1	64			45.8
31	125.6	125.8	125.6	65			47.6
32	148.1	147.9	148.0	66			23.3
33	163.6	163.7	163.3	67			24.5
34	120.0	120.0	120.1	68			39.5

* See Fig. 4 for numbering of carbon atoms.

** Assignments for C-51, C-52 and C-54 signals in the 4-amino-4,6-dideoxy-L-talose moiety are revised from that described in ref. 7.

mycin A[7] and bleomycin A_2[8] and also by direct comparison with the resonance peaks of methyl 4-amino-4,6-dideoxy-α-L-talopyranoside, β-lysine, spermidine and 1,4-diaminobutane. The ^{13}C-NMR spectrum of tallysomycin S_{10b} indicated the presence of 8 carbonyl carbons (7 at around δ 168~177 ppm and 1 carbamoyl carbon at δ 158.5 ppm), 13 other sp_2-carbons (δ 112~166 ppm) and 38 sp_3-carbons (δ 11~101 ppm). Of the total of 59 carbons in tallysomycin S_{10b}, 55 signals (C-1 through C-55 in Fig. 4), which constitute the tallysomycin skeleton, are consistent with those of tallysomycins A and B. Four

Fig. 4. Structure of tallysomycin S_{10b}.



sp_3-carbon signals (C-56 through C-59) of tallysomycin S_{10b} were assigned to the terminal amine moiety from the spectrum of 1,4-diaminobutane by utilizing protonation shift. The structure of tallysomycin S_{10b} is shown in Fig. 4.

Antimicrobial Activity

The antimicrobial activity of tallysomycin S_{10b} (copper-free) is shown in Table 6 in comparison with tallysomycins A and B. The minimum inhibitory concentrations (MIC) against Gram-positive, Gram-negative and acid-fast bacteria were determined in nutrient agar, and antifungal activities in SABOURAUD agar. Tallysomycin S_{10b} is less active than tallysomycins A and B against most bacteria and fungi, except for acid-fast bacteria which are comparably sensitive to tallysomycins S_{10b}, A and B.

Table 6. Antimicrobial activity of tallysomycin S_{10b}.

Organism	MIC (mcg/ml)		
	Tallysomycin		
	S_{10b}	A	B
Staphylococcus aureus 209P	3.1	0.05	0.2
" Smith	6.3	0.1	0.4
Sarcina lutea PCI 1001	3.1	0.05	0.4
Bacillus subtilis PCI 219	0.2	0.003	0.006
Escherichia coli Juhl A 15119	0.8	0.05	0.2
Klebsiella pneumoniae D 11	0.2	0.013	0.05
Pseudomonas aeruginosa A 9930	1.6	0.1	0.4
Proteus vulgaris A 9436	0.4	0.4	0.2
Proteus mirabilis A 9554	3.1	0.4	0.2
Mycobacterium smegmatis ATCC 607	0.2	0.2	0.2
Mycobacterium phlei	0.2	0.1	0.1
Candida albicans IAM 4888	12.5	12.5	6.3
Cryptococcus neoformans	6.3	0.8	0.4
Aspergillus fumigatus IAM 2503	6.3	1.6	0.8
Trichophyton mentagrophytes D 155	12.5	12.5	3.1

Antitumor Activity

The antitumor activity of tallysomycin S_{10b} was determined in mice using four experimental tumor systems, sarcoma 180 ascites tumor, lymphocytic leukemia P388, Lewis lung carcinoma and melanotic melanoma B16, in comparison with tallysomycin A. Bleomycin complex (major component: bleomycin A_2) was also tested as a reference compound in the latter two tumor systems. All test compounds used in the antitumor study were copper-free preparations. Treatments were given intraperitoneally once daily for 9 days (*qd* 1→9) except for the mice inoculated with Lewis lung carcinoma which were treated for 11 days (*qd* 1→11). The host animal strains and tumor inoculum size employed were the same as those described in a previous paper[9]. Death or survival of the treated and non-treated animals was recorded daily during the observation period of 45 days following the implantation of tumor cells, and the median survival time (MST) was calculated for each of the test (T) and control (C) groups. A T/C value equal to or greater than 125% indicated that a significant antitumor effect was achieved, and the lowest dose giving this effect was defined as minimum effective dose (MED). In the B16 melanoma experiment the tumor size (long axis × short axis × thickness) was measured on day 16 after subcutaneous tumor inoculation, and the dose giving tumor growth inhibition equal to or greater than 50% was defined as the MED.

As shown in Table 7, tallysomycin S_{10b} was highly active against sarcoma 180 ascites tumor, the MED being 0.03 mg/kg/day. Tallysomycin A was about 3 times less active than tallysomycin S_{10b} in this tumor system. Table 8, which shows the activity against leukemia P388, indicates that tallysomycin A was approximately 3 times more active than tallysomycin S_{10b}. Tallysomycins S_{10b} and A had similar activity against intraperitoneally-inoculated Lewis lung carcinoma, the MED of both components being 0.3 mg/kg/day (Table 9). The antitumor activity against subcutaneously implanted melanoma B16 was evaluated by the inhibition of tumor growth and by the prolongation of survival time. As shown in Table 10, tallysomycin S_{10b} was as active as tallysomycin A in both parameters; significant reduction of

Table 7. Effect of tallysomycin S_{10b} on sarcoma 180 ascites tumor.

	Dose,* i.p. (mg/kg/day)	MST (days)	T/C** (%)	Survivors/tested (day 45)
Control	—	19.0	—	1/30
Tallysomycin S_{10b}	1.0	39.5	**208**	5/12
	0.3	43.0	**226**	6/16
	0.1	32.0	**168**	0/16
	0.03	26.0	**137**	2/16
	0.01	23.0	121	2/16
Tallysomycin A	1.0	>43.5	>**229**	6/12
	0.3	38.5	**203**	7/16
	0.1	25.5	**134**	4/16
	0.03	19.5	103	1/16
	0.01	18.0	95	0/16

* Dosing schedule: *qd* 1→9.
** Boldface indicates significant antitumor effect.

Table 8. Effect of tallysomycin S_{10b} on P388 leukemia.

	Dose*, i.p. (mg/kg/day)	MST (days)	T/C** (%)	Survivors/tested (day 45)
Control	—	9.0	—	0/10
Tallysomycin S_{10b}	3.0	13.0	**144**	0/5
	1.0	14.0	**156**	0/5
	0.3	11.0	122	0/5
	0.1	9.0	100	0/5
	0.03	10.0	111	0/5
Tallysomycin A	3.0	15.0	**167**	0/5
	1.0	14.0	**156**	0/5
	0.3	12.0	**133**	0/5
	0.1	11.0	122	0/5
	0.03	10.0	111	0/5

* Dosing schedule: *qd* 1→9.
** Boldface indicates significant antitumor effect.

Table 9. Effect of tallysomycin S_{10b} on Lewis lung carcinoma.

	Dose,* i.p. (mg/kg/day)	MST (days)	T/C** (%)	Survivors/tested (day 45)
Control	—	17.5	—	0/20
Tallysomycin S_{10b}	3	28.5	**163**	1/10
	1	27.5	**157**	0/10
	0.3	23.5	**134**	0/10
	0.1	18.5	106	0/10
	0.03	17.0	97	0/10
Tallysomycin A	3	42.0	**240**	4/10
	1	27.0	**154**	0/10
	0.3	23.0	**131**	0/10
	0.1	21.0	120	0/10
	0.03	17.0	97	0/10
Bleomycin complex	3	25.0	**143**	0/5
	1	23.0	**131**	0/5
	0.3	20.0	114	0/5
	0.1	18.0	103	0/5
	0.03	17.0	97	0/5

* Dosing schedule: *qd* 1→11.
** Boldface indicates significant antitumor effect.

tumor size was seen at 0.3 mg/kg/day and significant extension of life span at 1.0 mg/kg/day. Bleomycin complex was approximately 3-fold less active than tallysomycins A and S_{10b} against Lewis lung carcinoma and B16 melanoma.

Toxicity

The acute and subacute lethal toxicity of tallysomycin S_{10b} was determined in male *dd*Y mice by a single or multiple (*qd* 1→9) administration *via* intraperitoneal route. Death or survival of the animals was recorded for 30 or 36 days after the last dose of test compound and the LD_{50} was calculated by the method of VAN DER WAERDEN[10]. The results are shown in Table 11 along with those for tallysomycin A which was tested comparatively. Tallysomycin S_{10b} was about one-half as toxic as tallysomycin A in terms of LD_{50}'s.

The nephrotoxic potential of tallysomycin S_{10b} was tested in male SD rats comparatively

Table 10. Effect of tallysomycin S_{10b} on B16 melanoma.

	Dose,*1 i.p. (mg/kg/day)	Tumor size*2		Survival time		Survivors/tested (day 45)
		Mean tumor size (cm^3)	Inhibition*3 (%)	MST (days)	T/C*3 (%)	
Control	—	6.92	—	29.5	—	0/20
Tallysomycin S_{10b}	3	1.07	**85**	38.0	**129**	1/10
	1	1.54	**78**	40.0	**136**	0/10
	0.3	2.95	**57**	33.5	114	0/10
	0.1	4.30	38	34.5	117	0/10
	0.03	6.22	10	31.5	107	0/10
Tallysomycin A	3	0.61	**91**	38.0	**129**	0/10
	1	1.38	**80**	39.5	**134**	0/10
	0.3	2.36	**66**	35.5	120	0/10
	0.1	4.40	36	33.0	112	0/10
	0.03	5.29	24	33.0	112	0/10
Bleomycin complex	3	1.91	**72**	37.0	**125**	0/5
	1	3.84	45	36.0	122	0/5
	0.3	4.61	33	36.0	122	1/5
	0.1	7.51	−9	32.0	108	0/5
	0.03	7.05	−2	32.0	108	0/5

*1 Dosing schedule: *qd* 1→9.
*2 Measured on day 16.
*3 Boldface indicates significant antitumor effect.

Table 11. Acute and subacute toxicity of tallysomycin S_{10b}.

	LD_{50}* (i.p., mg/kg)	
	Single dose	Multiple dose**
Tallysomycin S_{10b}	123	12
Tallysomycin A	78	7.1

* Determined 30 days after single dosing or 36 days after last dose of multiple administration (×9).
** *qd* 1→9.

Table 12. Nephrotoxicity of tallysomycin S_{10b} in rats.

	Dose (mg/kg/day)	Mean score*	
		Tubular degeneration	Tubular necrosis
Tallysomycin S_{10b}	5	**3.3**	**3.0**
	2.5	**2.3**	1.6
	1.3	1.3	1.7
	0.63	1.0	1.7
Tallysomycin A	1.3	**4.0**	**3.3**
	0.63	**2.7**	**2.7**
	0.31	1.3	1.0

* Score: 1=minimal, 2=mild, 3=moderate and 4=severe. Boldface indicates significant nephrotoxic effect.

with tallysomycin A. Graded doses of the test compounds were administered intraperitoneally to a group of 3 rats for 9 consecutive days. The animals were sacrificed on day 30, the kidneys isolated and fixed with 10% formalin solution. The sections were stained by the periodic acid SCHIFF reaction for the detection of tubular degeneration and tubular necrosis. The histopathological change for each animal was scored as 0~4 according to the extent of the lesions. The mean of scores was calculated for each group, with a mean score equal to or greater than 2.0 being regarded as a significant nephrotoxic sign. As shown in Table 12, tallysomycin S_{10b} was estimated to be 4~8 times less nephrotoxic than tallysomycin A for the rat.

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