STRUCTURE-ANTITUMOR ACTIVITY RELATIONSHIP OF SEMI-SYNTHETIC SPICAMYCIN ANALOGUES

MASARU KAMISHOHARA*[,a], HIROYUKI KAWAI[a], ATSUO ODAGAWA[a], TOSHIYUKI ISOE[a], JUN-ICHIRO MOCHIZUKI[a], TAKESHI UCHIDA[a], YOICHI HAYAKAWA[b], HARUO SETO[b], TAKASHI TSURUO[b] and NOBORU ŌTAKE[c]

[a]Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., 3 Miyahara-cho, Takasaki-shi, Gunma 370-12, Japan
[b]Institute of Applied Microbiology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan
[c]Department of Biosciences, Teikyo University, 1-1-1 Toyosatodai, Utsunomiya-shi, Tochigi 320, Japan

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Spicamycin, a nucleoside antibiotic containing fatty acids with a variety of chain lengths (C_{12}~C_{18}), showed potent antitumor activity against human gastric cancer SC-9 and human breast cancer MX-1 in a xenograft model. We have made several semi-synthetic spicamycin analogues (SPMs) which differed in the chain length of the fatty acid moiety, and examined their structure-antitumor activity relationship.

The cytotoxic activities of SPMs depended on the chain length of the fatty acid moiety, with dodecanoyl, tetradecanoyl, hexadecanoyl and icosanoyl analogues (SPM VIII, SPM X, SPM XII and SPM XVI) exhibiting the most potent cytotoxic activity against P388 murine leukemia cells.

SPM VIII showed the most activity against SC-9 in the human tumor xenograft model with the highest therapeutic index among SPMs. The antitumor activity of SPM VIII was superior to that of mitomycin C.

Spicamycin (Fig. 1), a mixture of several related components that differ only in the nature of their fatty acid moieties produced by *Streptomyces alanosinicus* 879-MT_3, was isolated as a differentiation inducer of HL60 and M1 myeloid leukemic cells. It also exhibited antitumor activity against P388 murine leukemia[1,2].

Septacidin, a compound similar to spicamycin, was reported to have antitumor activity against several murine tumors[3]. However, its effect on human tumor xenografts has not been reported yet.

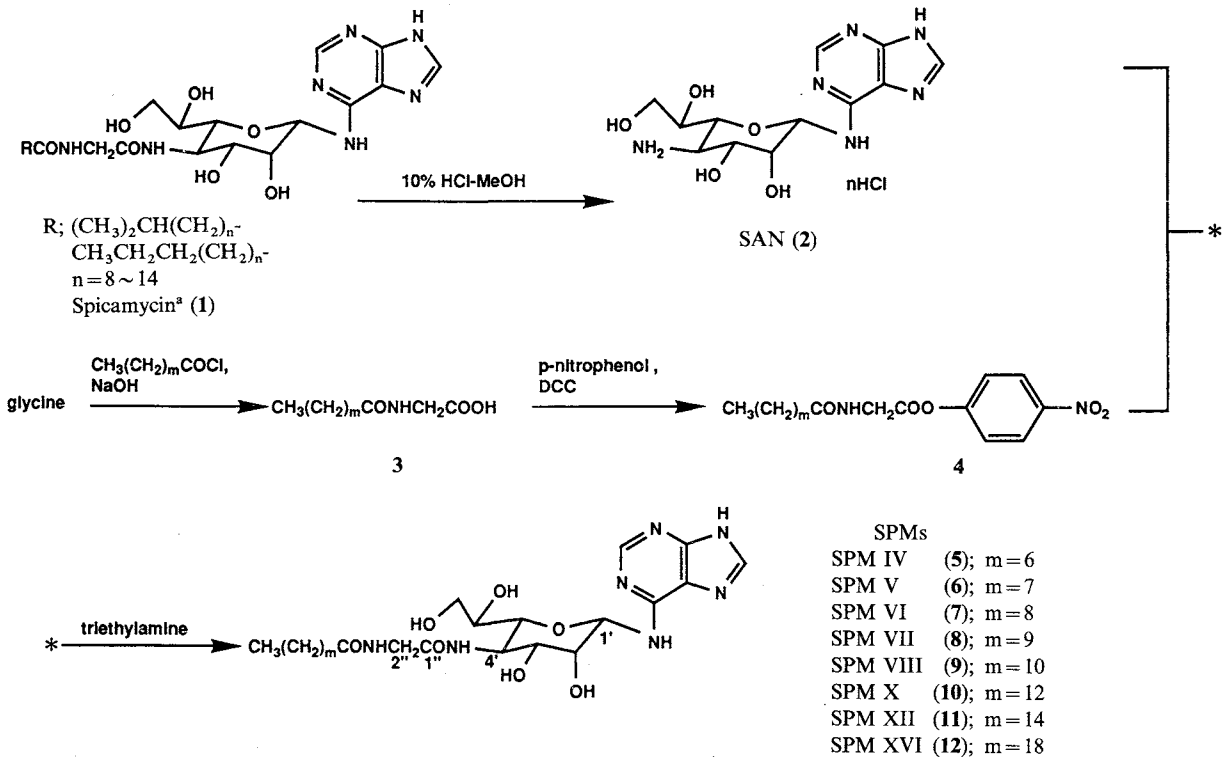
In this study we have examined the antitumor activity of spicamycin against several human tumors using the nude mice human tumor xenograft model[4~7].

We prepared several semi-synthetic spicamycin analogues (SPMs) which differed in the length of the fatty acid moiety and examined their *in vitro* and *in vivo* structure-activity relationship in the hope of finding compounds with higher antitumor activity and improved therapeutic effects on human tumor xenografts.

Chemistry

Spicamycin (**1**) was obtained from the cultured broth of *Streptomyces alanosinicus* 879-MT_3 by the method of HAYAKAWA *et al.*[2]. Modification of the fatty acid moiety of spicamycin was carried out by the methods described by ASZALOS *et al.*[3] (Fig. 1). The amino nucleoside moiety of spicamycin (SAN, **2**) was prepared by hydrolysis of spicamycin with 10% HCl-MeOH. Coupling of appropriate fatty acids with glycine yielded acyl-glycines, which were condensed with **2** to afford fatty acid derivatives (SPMs).

Fig. 1. Syntheses and structures of SAN, spicamycin and SPMs.



[a] The stereochemistry of 6′ position and the absolute configuration have not been determined yet.

Results

Antitumor Activity of Spicamycin against Human Tumors

The antitumor activity of spicamycin against 3 human gastric cancers, one human breast cancer and one human lung cancer was tested using a nude mice human tumor xenograft model. The dose used in these experiments was the maximum tolerated dose in each tumor bearing mice (Table 1). The most dramatic effect of spicamycin was apparent in the experiment on SC-9. Spicamycin also exhibited antitumor activity against MX-1 but had no activity against three other tumors.

Cytotoxicity of SAN, SPMs and Spicamycin against P388 Murine Leukemia Cells *In Vitro*

The cytotoxicity of SAN, SPMs and spicamycin against P388 cells is summarized in Table 2. SAN had no cytotoxic activity. The activities of SPMs had clear relationship with the length of fatty acid chain. That is, the activities increased with the increase of chain length, reaching a maximum with the C_{12} acid of SPM VIII. The activities of SPM VIII, SPM X, SPM XII and SPM XVI appeared to be stronger than that of the parent compound, spicamycin. This result indicates that the fatty acid chain moiety is essential for the cytocidal activity of SPMs, whose potency depends on the length of their fatty acids.

In Vivo Antitumor Activity of SAN, SPMs and Spicamycin against SC-9 Human Gastric Cancer

The antitumor activity of SAN, SPMs and spicamycin against SC-9 was evaluated by tumor growth inhibition rate (T.G.I.R.) at each dose and the therapeutic index (T.I.) of each SPM was calculated (Table 3). SAN had no activity or toxicity even at the highest dose. SPM IV and SPM V with short-chain fatty acids (C_8 and C_9) showed toxicities at doses of more than 12 mg/kg/day, but had no antitumor activity. SPM VI, SPM VII, SPM VIII and SPM X which have intermediate-chain fatty acids (C_{10}, C_{11}, C_{12} and C_{14}) exhibited inhibitory effects on the growth of SC-9. On the other hand, SPM XII and SPM XVI with long-chain fatty acid moieties (C_{16} and C_{20}) showed no therapeutic effect but had toxicity. Among the active SPMs, SPM VIII showed a considerable reduction of toxicity without loss of the antitumor activity, resulting in the highest T.I. which was 4-times higher than that of spicamycin (Table 3).

Table 1. Antitumor activities of spicamycin against human tumors.

Tumor	Intravenous dose	T.G.I.R. (%)[a]
SC-7 stomach	3 mg/kg/day	38
SC-9 stomach	3 mg/kg/day	**95**
4-1ST stomach	3 mg/kg/day	3
MX-1 breast	5 mg/kg/day	**61**
LX-1 lung	5 mg/kg/day	24

Fragments of human tumor were implanted subcutaneously into athymic nude mice. When the tumor size reached 100~300 mm^3, spicamycin was given intravenously daily for five days.

[a] Maximum tumor growth inhibition rate (T.G.I.R.) values through the experimental span were expressed. Bold figures mean that spicamycin was evaluated as "effective" according to the criteria described in "Experimental."

Table 2. Cytotoxic effects of SAN, spicamycin and SPMs on P388 murine leukemia cells.

Compound	Carbon number of fatty acid	IC_{50} (μg/ml)
SAN	—	>100
SPM IV	C_8	12.0
SPM V	C_9	4.5
SPM VI	C_{10}	3.0
SPM VII	C_{11}	0.34
SPM VIII	C_{12}	0.012
SPM X	C_{14}	0.016
SPM XII	C_{16}	0.007
SPM XVI	C_{20}	0.020
Spicamycin	C_{12}~C_{18}	0.063

P388 cells were exposed to SAN, spicamycin or SPMs for 3 days and the cell number was counted.

Table 3. Antitumor activities of SAN, SPMs and spicamycin against SC-9 human gastric cancer.

Compound	Intravenous dose (mg/kg/day)							
	24	12	6	3	1.5	0.75	0.375	T.I.[a]
SAN	30[b]			4	− 4			
SPM IV	Toxic[c]	Toxic	22					
SPM V		Toxic	44					
SPM VI	Toxic	Toxic	**78**[d]	**71**	−12			2
SPM VII	Toxic	**83**		**62**	16			4
SPM VIII	Toxic	**99**	**91**	**75**	**56**	14		8
SPM X		Toxic	Toxic	**89**	18			1
SPM XII				Toxic	41			
SPM XVI				Toxic	Toxic	Toxic	17	
Spicamycin			Toxic	**95**	**85**	42		2

Fragments of SC-9 human gastric cancer were implanted subcutaneously into athymic nude mice. When the tumor size reached 100~300 mm^3, SAN, SPMs or spicamycin were given iv daily for five days.

[a] Therapeutic index (T.I.) = maximum tolerated dose/minimum effective dose

[b] Results were expressed as the values of maximum tumor growth inhibition rate (T.G.I.R.) through the exprimental span; five mice per group.

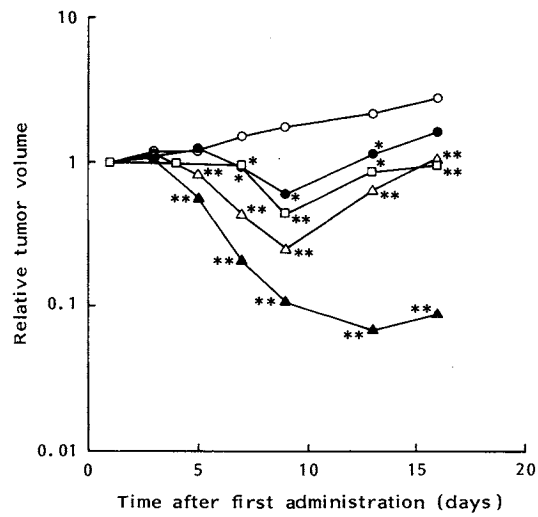
[c] One or more animals died.

[d] Bold figures mean that SPMs or spicamycin were evaluated as "effective" according to the criteria described in "Experimental".

The growth curves of SC-9 treated with SPM VIII or mitomycin C (MMC) are shown in Fig. 2. Remarkable remission of the tumor by SPM VIII was observed at the dose of 12 mg/kg/day. The minimum relative tumor volumes (RVs) of each treatment were 0.09 (SPM VIII 12 mg/kg/day), 0.25 (SPM VIII 6 mg/kg/day), 0.60 (SPM VIII 3 mg/kg/day) and 0.86 (MMC). The effect of SPM VIII at all three doses in reducing the tumor mass of SC-9 was greater than that of MMC.

Fig. 2. Changes in relative tumor (SC-9) volume implanted into nude mice after treatment of SPM VIII or mitomycin C (MMC).

○ Control, ● SPM VIII 3 mg/kg/day, △ SPM VIII 6 mg/kg/day, ▲ SPM VIII 12 mg/kg/day, □ MMC 6.7 mg/kg/day.



SPM VIII was given intravenously at each dose indicated from day 1 to 5 or MMC was given intravenously at the dose of 6.7 mg/kg/day on day 1. The relative tumor volume was measured once or twice a week. Points are the means of five determinations. * $P<0.05$ compared to control treated with vehicle. ** $P<0.01$.

Discussion

In this study we found that spicamycin had *in vivo* antitumor activity against several human tumors, especially high activity was seen against SC-9 human gastric cancer. The structure-antitumor activity relationship indicated that the *N*-acylated glycine moiety was essential for SPMs to have antitumor activity because SAN showed no *in vitro* and *in vivo* activity. The structure activity study also indicated that there was a certain relationship between the length of the fatty acid chain and antitumor activity or toxicity.

On the whole, both *in vitro* cytotoxicity and *in vivo* toxicity increased with an increase in the number of carbons in the fatty acid moiety of SPMs. However, there were some disagreements between

in vitro cytotoxicity and *in vivo* toxicity findings. Although the cytotoxicity of SPM IV, SPM V, SPM VI, SPM VII and SPM VIII increased as the number of carbons in the fatty acid moiety increased, almost all these SPMs showed the same toxicity against nude mice. SPM VIII, SPM X, SPM XII and SPM XVI showed similar cytotoxicity but the *in vivo* toxicity of these compounds increased with an increase in the length of the fatty acid chain. These differences may depend on the difference of SPMs in their selective toxicity against tumor cells *in vivo*. The fact that SPM VIII showed the maximum T.I. suggests that SPM VIII has the highest specificity towards tumor cells among the SPMs.

In addition to spicamycin and septacidin, glidobactins[8,9], 13-*O*-acyl derivatives of rhizoxin[10,11] and N^4-acyl derivatives of 1-β-D-arabinofuranosylcytosine(ara-C)[12] are known as antitumor agents which have a fatty acid moiety in their structures. It was reported that their activities depended on the length of their fatty acid chains. Although their modes of action may be different from that of spicamycin, the fatty acid moieties of these compounds may play an important role, possibly in the permeability into the tumor cells.

SPM VIII showed a maximum T.I. among SPMs and significant tumor remission which surpassed that by MMC. The T.G.I.R. value of SPM VIII on day 14 was 98% and this value was superior to those of other well-known antitumor drugs reported by INABA *et al.*[13]. These results prompt us to study SPM VIII in further detail.

Experimental

General

MP's were determined with a Yanagimoto micro hot-stage apparatus. Specific rotations were obtained with a Jasco DIP-140 spectropolarimeter. IR spectra were recorded on a Jasco A-3 spectrophotometer. UV spectra were recorded on a Hitachi U-3200 in MeOH solution and NMR spectra were recorded on a Jeol GX-500 spectrometer. The FD mass spectra were obtained with a Hitachi M80 mass spectrometer.

Spicamycin Amino Nucleoside (2)

A solution of 1.0 g of **1** in 100 ml of 10% HCl-MeOH was allowed to stir at 30°C for 100 hours. The solution was concentrated *in vacuo* to the minimum volume and diethyl ether was added. The resulting precipitate was centrifuged and washed with diethyl ether to give a white powder. This powder was dissolved in water and washed with *n*-butanol. The aqueous layer was neutralized with silver carbonate and the precipitate formed was filtered off. The filtrate was concentrated and the residue was further purified by column chromatography on silica gel ($CHCl_3$-MeOH, 2:1) to give **2** (344 mg, 68% yield); MP 180~183°C (decomp.), $[\alpha]_D^{25} = +1.2°$ (*c* 0.25, MeOH), UV λ_{max} 264 nm, IR ν_{max} 3300, 1640 cm^{-1}, FD-MS 327 (M+H), ^1H NMR (CD_3OD) δ_H 3.04 (1H, dd, J=10.0, 10.0 Hz, 4'-H), 3.43 (1H, dd, J=6.3, 10.0 Hz, 5'-H), 3.53 (1H, dd, J=3.1, 10.0 Hz, 3'-H), 3.61 (2H, br d, 7'-H), 3.78 (1H, m, 6'-H), 3.95 (1H, dd, J=3.1, <1 Hz, 2'-H), 5.64 (1H, br s, 1'-H), 8.13 (1H, s, 8-H), 8.30 (1H, s, 2-H), ^1H NMR (CD_3OD+DCl) δ_H 3.43 (1H, dd, J=10.0, 10.0 Hz, 4'-H), 3.62 (2H, m, 7'-H), 3.72 (1H, dd, J=6.3, 10.0 Hz, 5'-H), 3.80 (1H, m, 6'-H), 3.89 (1H, dd, J=3.1, 10.0 Hz, 3'-H), 4.07 (1H, dd, J=<1, 3.1 Hz, 2'-H), 5.72 (1H, br s, 1'-H), 8.20 (1H, s, 8-H), 8.40 (1H, s, 2-H).

Dodecanoylglycine (3; m=10)

To a stirred solution of glycine (10.0 g, 0.13 mol) in NaOH (5.2 g, 0.13 mol) aqueous solution (100 ml) was added dodecanoyl chloride (2.84 g, 0.013 mol) and the mixture was stirred for 60 minutes at room temperature. The reaction mixture was adjusted to pH 3 with conc HCl. The resulting precipitate was collected and washed with water and diethyl ether to give **3** (3.27 g, 98% yield) as a white powder.

Other acyl-glycines were prepared by a similar procedure.

p-Nitrophenyl Ester of Dodecanoylglycine (4; m=10)

To a stirred solution of **3** (3.27 g, 0.013 mol) in DMF was added *p*-nitorophenol (1.76 g, 0.013 mol) and *N*,*N*'-dicyclohexylcarbodiimide (2.62 g, 0.013 mol). After 12 hours, the reaction mixture was filtered. The filtrate was concentrated and recrystallized from ethyl acetate three times to give **4** (4.17 g, 85% yield) as white needles.

Other *p*-nitrophenyl esters of acyl-glycine were prepared by a similar procedure.

SPM VIII (9)

To a stirred solution of **2** (500 mg, 1.53 mmol) in DMF was added **4** (578 mg, 1.53 mmol) and triethylamine (773 mg, 7.65 mmol). After 24 hours, the solvent was removed and the residue was chromatographed on silica gel with chloroform-methanol-H_2O (200:40:1) elution. Evaporation of appropriate fractions afforded **9** (567 mg, 63% yield) as a white powder; MP 232~235°C, $[\alpha]_D^{25}=+1.7°$ (*c* 0.25, MeOH), FD-MS 566 (M+H), UV λ_{max} 264 nm, IR ν_{max} 3300, 1640 cm^{-1}, ^1H NMR (CD_3OD) δ_H 0.90 (3H, t, *J*=7.0 Hz), 1.20~1.40 (16H, m), 1.60~1.70 (2H, m), 2.28 (2H, t, *J*=7.0 Hz), 3.60~3.80 (5H, 3'-H, 5'-H, 6'-H, 7'-H), 3.87 (1H, d, *J*=16.4 Hz, 2''-Ha), 3.89 (1H, d, *J*=16.4 Hz, 2''-Hb), 4.05 (1H, dd, *J*=<1, 2.1 Hz, 2'-H), 4.14 (1H, dd, *J*=10.1, 10.1 Hz, 4'-H), 5.58 (1H, brs, 1'-H), 8.10 (1H, brs, 8-H), 8.31 (1H, s, 2-H).

Preparation of compounds **5**, **6**, **7**, **8**, **10**, **11** and **12** followed a similar process as discribed above using appropriate fatty acids.

SPM IV (5)

MP 183~185°C, $[\alpha]_D^{25}+3.1°$ (*c* 0.1, MeOH), FD-MS 510 (M+H), UV λ_{max} 264 nm, IR ν_{max} 3400, 1650 cm^{-1}, ^1H NMR (CD_3OD) δ_H 0.89 (3H, t, *J*=7.0 Hz), 1.20~1.40 (8H, m), 1.60~1.70 (2H, m), 2.28 (2H, t, *J*=7.0 Hz), 3.60~3.80 (5H, 3'-H, 5'-H, 6'-H, 7'-H), 3.87 (1H, d, *J*=16.4 Hz, 2''-Ha), 3.89 (1H, d, *J*=16.4 Hz, 2''-Hb), 4.05 (1H, dd, *J*=<1, 2.1 Hz, 2'-H), 4.14 (1H, dd, *J*=10.1, 10.1 Hz, 4'-H), 5.58 (1H, brs, 1'-H), 8.10 (1H, brs, 8-H), 8.31 (1H, s, 2-H).

SPM V (6)

MP 190~192°C, $[\alpha]_D^{25}+1.3°$ (*c* 0.1, MeOH), FD-MS 524 (M+H), UV λ_{max} 264 nm, IR ν_{max} 3400, 1650 cm^{-1}, ^1H NMR (CD_3OD) δ_H 0.89 (3H, t, *J*=7.0 Hz), 1.20~1.40 (10H, m), 1.60~1.70 (2H, m), 2.28 (2H, t, *J*=7.0 Hz), 3.60~3.80 (5H, 3'-H, 5'-H, 6'-H, 7'-H), 3.87 (1H, d, *J*=16.4 Hz, 2''-Ha), 3.89 (1H, d, *J*=16.4 Hz, 2''-Hb), 4.05 (1H, dd, *J*=<1, 2.1 Hz, 2'-H), 4.14 (1H, dd, *J*=10.1, 10.1 Hz, 4'-H), 5.58 (1H, brs, 1'-H), 8.10 (1H, brs, 8-H), 8.31 (1H, s, 2-H).

SPM VI (7)

MP 206~208°C, $[\alpha]_D^{25}+0°$ (*c* 0.1, MeOH), FD-MS 538 (M+H), UV λ_{max} 264 nm, IR ν_{max} 3400, 1650 cm^{-1}, ^1H NMR (CD_3OD) δ_H 0.89 (3H, t, *J*=7.0 Hz), 1.20~1.40 (12H, m), 1.60~1.70 (2H, m), 2.28 (2H, t, *J*=7.0 Hz), 3.60~3.80 (5H, 3'-H, 5'-H, 6'-H, 7'-H), 3.87 (1H, d, *J*=16.4 Hz, 2''-Ha), 3.89 (1H, d, *J*=16.4 Hz, 2''-Hb), 4.05 (1H, dd, *J*=<1, 2.1 Hz, 2'-H), 4.14 (1H, dd, *J*=10.1, 10.1 Hz, 4'-H), 5.58 (1H, brs, 1'-H), 8.10 (1H, brs, 8-H), 8.31 (1H, s, 2-H).

SPM VII (8)

MP 215~217°C, $[\alpha]_D^{25}=+3.7°$ (*c* 0.1, MeOH), FD-MS 552 (M+H), UV λ_{max} 264 nm, IR ν_{max} 3400, 1650 cm^{-1}, ^1H NMR (CD_3OD) δ_H 0.89 (3H, t, *J*=7.0 Hz), 1.20~1.40 (14H, m), 1.60~1.70 (2H, m), 2.28 (2H, t, *J*=7.0 Hz), 3.60~3.80 (5H, 3'-H, 5'-H, 6'-H, 7'-H), 3.87 (1H, d, *J*=16.4 Hz, 2''-Ha), 3.89 (1H, d, *J*=16.4 Hz, 2''-Hb), 4.05 (1H, dd, *J*=<1, 2.1 Hz, 2'-H), 4.14 (1H, dd, *J*=10.1, 10.1 Hz, 4'-H), 5.58 (1H, brs, 1'-H), 8.10 (1H, brs, 8-H), 8.31 (1H, s, 2-H).

SPM X (10)

MP 226~228°C, $[\alpha]_D^{25}+1.8°$ (*c* 0.1, MeOH), FD-MS 594 (M+H), UV λ_{max} 264 nm, IR ν_{max} 3400, 1650 cm^{-1}, ^1H NMR (CD_3OD) δ_H 0.89 (3H, t, *J*=7.0 Hz), 1.20~1.40 (20H, m), 1.60~1.70 (2H, m), 2.28 (2H, t, *J*=7.0 Hz), 3.60~3.80 (5H, 3'-H, 5'-H, 6'-H, 7'-H), 3.87 (1H, d, *J*=16.4 Hz, 2''-Ha), 3.89 (1H, d, *J*=16.4 Hz, 2''-Hb), 4.05 (1H, dd, *J*=<1, 2.1 Hz, 2'-H), 4.14 (1H, dd, *J*=10.1, 10.1 Hz, 4'-H), 5.58 (1H, brs, 1'-H), 8.10 (1H, brs, 8-H), 8.31 (1H, s, 2-H).

SPM XII (11)

MP 230~232°C, $[\alpha]_D^{25}+6.8°$ (*c* 0.1, MeOH), FD-MS 622 (M+H), UV λ_{max} 264 nm, IR ν_{max} 3400, 1650 cm^{-1}, ^1H NMR (CD_3OD) δ_H 0.89 (3H, t, *J*=7.0 Hz), 1.20~1.40 (24H, m), 1.60~1.70 (2H, m), 2.28 (2H, t, *J*=7.0 Hz), 3.60~3.80 (5H, m, 3'-H, 5'-H, 6'-H, 7'-H), 3.87 (1H, d, *J*=16.4 Hz, 12''-Ha), 3.89 (1H, d, *J*=16.4 Hz, 2''-Hb), 4.05 (1H, dd, *J*=<1, 2.1 Hz, 2'-H), 4.14 (1H, dd, *J*=10.1, 10.1 Hz, 4-H), 5.58 (1H,

br s, 1'-H), 8.10 (1H, br s, 8-H), 8.31 (1H, s, 2-H).

SPM XVI (**12**)

MP 234~236°C, $[\alpha]_D^{25}$ +8.8° (*c* 0.1, MeOH), FD-MS 678 (M+H), UV λ_{max} 264 nm, IR ν_{max} 3400, 1650 cm^{-1}, ^1H NMR (CD_3OD) δ_H 0.89 (3H, t, *J*=7.0 Hz), 1.20~1.40 (32H, m), 1.60~1.70 (2H, m), 2.28 (2H, t, *J*=7.0 Hz), 3.60~3.80 (5H, 3'-H, 5'-H, 6'-H, 7'-H), 3.87 (1H, d, *J*=16.4 Hz, 2''-Ha), 3.89 (1H, d, *J*=16.4 Hz, 2''-Hb), 4.05 (1H, dd, *J*=<1, 2.1 Hz, 2'-H), 4.14 (1H, dd, *J*=10.1, 10.1 Hz, 4'-H), 5.58 (1H, br s, 1'-H), 8.10 (1H, br s, 8-H), 8.31 (1H, s, 2-H).

In Vitro Cytotoxicity

P388 murine leukemia cells were kindly provided by Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan. Tumor cells were maintained and suspended in RPMI 1640 Medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (Gibco Laboratories, Grand Island, NY), 100 units/ml of penicillin (Meiji Seika Kaisha Ltd., Tokyo, Japan), 100 μg/ml of streptomycin (Meiji Seika Kaisha Ltd., Tokyo, Japan) and 2-mercaptoethanol (5×10^{-5} M). The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. For drug treatment experiments, 5×10^3 tumor cells/well in 96-well plates (U96 Micro Well, Nunc Inter. Med., Roskilde, Denmark) containing 0.1 ml of the growth medium described above were cultured in the graded concentrations of SPMs or spicamycin. After 72 hours incubation, the number of cells in each well was counted with a Model ZM Coulter Counter (Coulter Electronics Limited, England). Four wells were used for each drug concentration. The IC_{50} was determined by plotting the logarithm of the drug concentration against the growth rate of the treated cells[14].

In Vivo Antitumor Activity

For all experiments, 6~8-week-old female, athymic nude mice (BALB/c nu/nu Slc, Japan SLC Inc., Shizuoka, Japan) were used. The animals were kept under specific pathogen free conditions using laminar air flow racks and were fed sterile food and water *ad libitum*.

Human gastric cancers SC-7, SC-9 and 4-1ST, human breast cancer MX-1 and human lung cancer LX-1 were kindly supplied by the Central Institute for Experimental Animals, Kanagawa, Japan, and maintained in athymic nude mice. Chemotherapeutic experiments were performed as described by INABA *et al.*[13]. Fragments of xenografts were implanted sc into the right subaxillary region of athymic nude mice. When the tumors had grown to a palpable size (100~300 mm^3), the mice were randomly allocated to several experimental groups consisting of five animals each and SPMs or spicamycin at each dose were given intravenously by daily injection for five days. MMC purchased from Kyowa Hakko Kogyo Co., Tokyo, Japan, was given intravenously only on day 1. Control mice were given 10 μl/g vehicle. From the start of the injections, the tumor volume (V) was calculated once or twice a week for 3 weeks as follows; V=abc/2, where a and b are the long and short diameter and c is the height of the tumor mass in mm. Relative tumor volume (RV) is expressed as $RV = V_n/V_0$, where V_n is the tumor volume on day n and V_0 is the initial tumor volume at the time treatment was commenced (day 1). T.G.I.R. was determined as follows; T.G.I.R. = $(1 - T/C) \times 100$ where T is the mean of RV in treated mice and C is the mean of RV in control mice.

Evaluation as "effective" was based on the maximum T.G.I.R. (%) for an experimental span of 50% or more showing statistical significance as determined by the MANN-WHITNEY's U-test ($P<0.05$, one sided). A toxic dose was defined as one causing the death of one or more mice in a group. The therapeutic index (T.I.) was determined as follows; T.I. = maximum tolerated dose/minimum effective dose.

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