Sparsomycin Analogs. VI.[1] Synthesis and Antitumor Activity of Octylsparsomycin Analogs

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Five sparsomycin analogs (9—13) were prepared and examined for their ability to inhibit deoxyribonucleic acid (DNA) synthesis in L5178Y lymphoma cells. All of the compounds showed significant activity in the DNA synthesis assay. The compounds having R_c configuration exhibited almost the same activities independently of the configuration at the sulfoxide sulfur atom. Among the S_c isomers, the R_s configuration was advantageous for the appearance of activity.

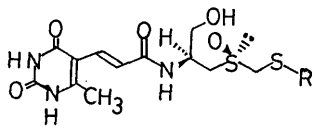
Keywords sparsomycin; octylsparsomycin analog; sparsomycin analog; antitumor activity; configuration

Sparsomycin (**1**), a metabolite of *Streptomyces sparsogenes*[2] is an antitumor antibiotic having a wide range of biological activities[3] and a unique structure (Fig. 1).[4,5] The biological activity of this antibiotic is due to its ability to inhibit protein synthesis at the ribosomal level.[6] Its antitumor activity has attracted great interest, and sparsomycin was subjected to clinical studies, but was found unsuitable because of its eye toxicity.[7]

The total synthesis of sparsomycin was first accomplished in 1981,[5] while structure–activity relationship studies of this antibiotic have been made for years.[8-13] Those studies have provided useful information on the sppearance of activity, and analogs with a certain degree of activity have been reported. Among these analogs, octylsparsomycin (**2**) is noteworthy.[13a] This analog has an octylthio group instead of a methylthio group in the structure of sparsomycin and exhibits three times greater activity than sparsomycin in the clonogenic leukemia L1210 assay *in vitro*.

We have also synthesized many analogs of sparsomycin and examined their biological activities,[1,14] and found that the methylation of both oxygen atoms on the pyrimidine ring enhanced the biological activity remarkably. This result suggests that an increase of lipophilicity of analogs increases the ability to permeate into cells, leading to higher activity.

In this paper, we describe the synthesis of new octylsparsomycin analogs together with the result on their antitumor activity *in vitro*.



1: R = CH_3 sparsomycin (S_C–R_S)
2: R = $(CH_2)_7CH_3$ octylsparsomycin (S_C–R_S)

Fig. 1

Results and Discussion

The N-protected alcohol parts (**3**—**6**) were prepared from L-and D-cystine by the reported method[13a] as outlined in Chart 1. Compound **7** was a by-product in the preparation of **6**; extraction of **6** with ethyl acetate induced ester exchange to afford **6** and **7** in 30 and 47% yields, respectively.

Removal of the *tert*-butyloxycarbonyl group was carried out by using trifluoroacetic acid. The condensations of acid (**8**)[14c] with amino alcohols were carried out by the mixed anhydride (MA) method using isobutyl chlorocarbonate (BCC) to afford octylsparsomycin analogs (**9**—**13**). Compounds **9** and **12**, **10** and **11** are enantiomers of one another. Thus, the two compounds in each pair showed identical melting points and absolute values of specific rotation. Treatment of **10** with trimethylsilyl chloride and sodium iodide afforded the *S*-deoxo analog (**13**) in 34% yield (Chart 2).

For an evaluation of the relationship between the chem-

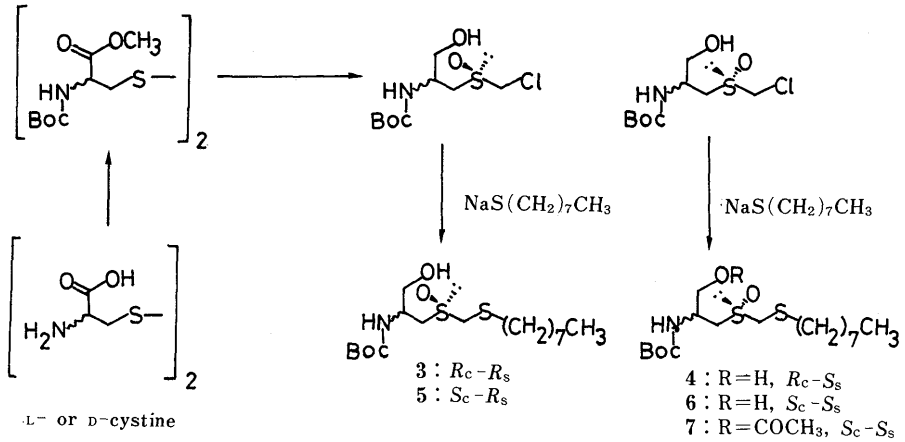


Chart 1

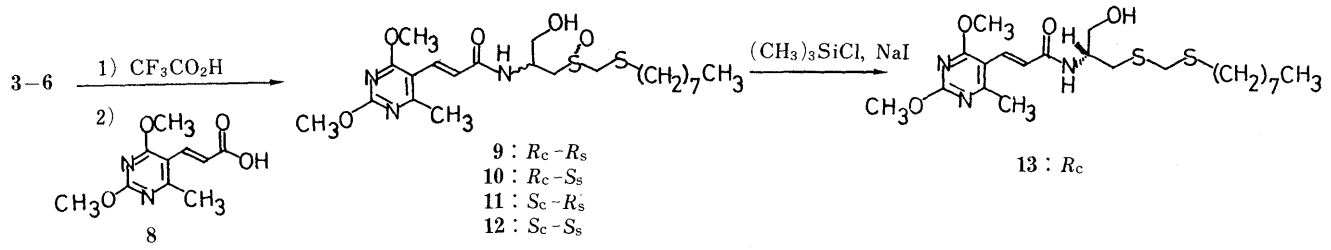
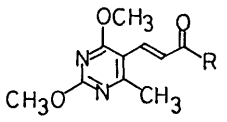
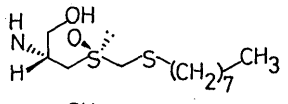
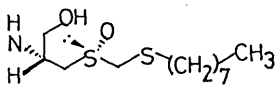
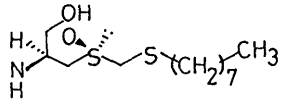
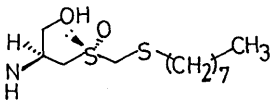
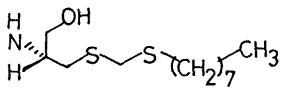


Chart 2

TABLE I. Biological Activity of Octylsparsomycin Analogs



R		Chirality	IC_{50}[a] (μg/ml)
	9	R_c–R_s	1.76
	10	R_c–S_s	1.67
	11	S_c–R_s	1.75
	12	S_c–S_s	2.72
	13	R_c	1.80

a) Under the same conditions, the IC_{50} value of sparsomycin was 0.07 μg/ml.

ical structure and antitumor activity of newly synthesized sparsomycin analogs, we used an *in-vitro* [methyl-^{3}H]thymidine incorporation assay of L5178Y murine lymphoma cells. The antitumor activity was evaluated as IC_{50} (the concentration in μg/ml required for 50% inhibition of incorporation). The IC_{50} value of sparsomycin, the reference compound, was 0.07 μg/ml. These results are summarized in Table I.

Comparison of the IC_{50} value of **9** with that of **10**, both of which have *R* configuration at the chiral carbon atom, demonstrates that the biological activity is independent of the configuration of the chiral sulfur atom. This result is further supported by the fact that the IC_{50} value of **13** is similar to those of **9** and **10**. It indicates that the sulfoxide function is not essential for the expression of the antitumor activity.

On the other hand, the IC_{50} value of the analogs having *S* configuration at the chiral carbon atom was affected by the configuration at the chiral sulfur atom. Thus, the IC_{50} value of **11** (R_s configuration) is about one-half that of **12** (S_s configuration). But, this effect was rather smaller than we had expected. It is very surprising that compound **11**, which has the same configuration as sparsomycin, is not the most effective analog, and its IC_{50} value is close to those of compounds **9**, **10**, and **13**.

These results are different from the previous findings that the *S* and *R* configurations on the carbon or the sulfur atom both play important roles and have a significant effect on the antitumor activity.[8,9,13] However, it is noteworthy that the antitumor activities *in vitro* of these analogs are 10 times higher than those of the analogs reported in the previous paper.[1]

In summary, from our observations on the relationship between the structure of sparsomycin analogs and their antitumor activities, it is clear that an increase of lipophilicity is favorable for enhancement of the activity, but there is no correlation between the antitumor activity and the combination of the configurations of chiral atoms in the modified analogs. These considerations should facilitate the design of compounds that are more effective against human neoplasms.

Experimental

Chemicals All melting points are uncorrected. Optical rotations were obtained with a JASCO DIP-4 digital polarimeter. Infrared (IR) absorption spectra were recorded on a JASCO IRA-2 spectrometer, and nuclear magnetic resonance (NMR) spectra on a JEOL JNM-MH-100 spectrometer (with tetramethylsilane as an internal standard). The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Thin layer chromatography (TLC) was performed on Silica gel GF_{254} (Merck). For column chromatography, Silica gel 60 (Merck) was used.

***N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-(octylthio)methyl-cysteinols (3—7)** These were prepared from the corresponding cystine by the previous reported methods.[13] The physical and chemical data for the compounds are as follows.

(R_s)-*N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-(octylthio)methyl-L-cysteinol (3) The residue of the reaction mixture was triturated with *n*-hexane and filtered off with suction to afford **3** (83%) as a white powder. mp 64—67 °C. $[\alpha]_D^{23}$: +90° (c=1.03, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1680, 1520. NMR ($CDCl_3$) δ: 0.88 (3H, br t, J=6 Hz, Me), 1.1—1.9 (12H, m, $CH_2\times6$), 1.45 (9H, s, CMe_3), 2.71 (2H, t, J=7 Hz, SCH_2), 2.94 and 3.43 (2H, AB in ABX, J_{AX}=4 Hz, J_{BX}=5 Hz, J_{AB}=14 Hz, $CHCH_2S$), 3.77 and 3.86 (2H, AB, J=14 Hz, $SOCH_2S$), 3.6—3.9 (3H, br, CH_2OH), 3.9—4.1 (1H, m, CH), 5.54 (1H, br d, J=7.5 Hz, NH). MS m/z: 382 (M+1). *Anal.* Calcd for $C_{17}H_{35}NO_4S_2$: C, 53.51; H, 9.24; N, 3.67. Found: C, 53.28; H, 9.38; N, 3.83.

(S_s)-*N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-(octylthio)methyl-L-cysteinol (4) The residue of the reaction mixture was triturated with ether and filtered off with suction to afford **4** (86%) as a white powder. mp 97—100 °C. $[\alpha]_D^{23}$: −74° (c=1.02, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1680, 1520. NMR ($CDCl_3$) δ: 0.88 (3H, br t, J=6.5 Hz, Me), 1.1—2.1 (12H, m, $CH_2\times6$), 1.45 (9H, s, CMe_3), 2.71 (2H, t, J=7 Hz, SCH_2), 2.97 and 3.26 (2H, AB in ABX, J_{AX}=5 Hz, J_{BX}=6 Hz, J_{AB}=14 Hz, $CHCH_2S$), 3.4—3.9 (5H, m, $SOCH_2S$ and CH_2OH), 3.9—4.3 (1H, m, CH), 5.57 (1H, br d, J=7.5 Hz, NH). MS m/z: 382 (M+1). *Anal.* Calcd for $C_{17}H_{35}NO_4S_2$: C, 53.51; H, 9.24; N, 3.67. Found: C, 53.61; H, 9.36; N, 3.50.

(R_s)-*N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-(octylthio)methyl-D-cysteinol (5) The residue of the reaction mixture was purified by column chromatography (MeOH: CH_2Cl_2 = 1 : 9 as an eluent) to afford **5** (81%) as a white powder. mp 97—100 °C. $[\alpha]_D^{22}$: +69° (c=1.03, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1680, 1520. NMR ($CDCl_3$) δ: 0.88 (3H, br t, J=6.5 Hz,

Me), 1.1—1.9 (12H, m, $CH_2 \times 6$), 1.46 (9H, s, CMe_3), 2.71 (2H, t, $J=7$ Hz, SCH_2), 2.97 and 3.24 (2H, AB in ABX, $J_{AX}=5$ Hz, $J_{BX}=6$ Hz, $J_{AB}=14$ Hz, $CHCH_2S$), 3.4—3.9 (5H, m, $SOCH_2S$ and CH_2OH), 3.9—4.3 (1H, m, CH), 5.56 (1H, br d, $J=7.5$ Hz, NH). MS *m/z*: 382 (M+1). *Anal.* Calcd for $C_{17}H_{35}NO_4S_2$: C, 53.51; H, 9.24; N, 3.67. Found: C, 53.24; H, 9.31; N, 3.66.

(S_s)-*N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-(octylthio)methyl-D-cysteinol (6) and (S_s)-*N*-[(*tert*-Butyloxy)carbonyl]-*O*-acetyl-*S*-oxo-*S*-(octylthio)methyl-D-cysteinol (7) The reaction mixture was extracted with ethyl acetate and the extract was dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by column chromatography (MeOH : CH_2Cl_2 = 1 : 40 as an eluent) to afford **6** (33%) and **7** (47%) as white powders.

6: mp 64—67 °C. $[\alpha]_D^{22}$: −92° ($c=1.00$, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1680, 1520. NMR ($CDCl_3$) δ: 0.87 (3H, br t, $J=6$ Hz, Me), 1.1—1.8 (12H, m, $CH_2 \times 6$), 1.45 (9H, s, CMe_3), 2.70 (2H, t, $J=7$ Hz, SCH_2), 2.95 and 3.39 (2H, AB in ABX, $J_{AX}=5$ Hz, $J_{BX}=6$ Hz, $J_{AB}=14$ Hz, $CHCH_2S$), 3.75 and 3.88 (2H, AB, $J=14$ Hz, $SOCH_2S$), 3.6—3.9 (3H, br, CH_2OH), 3.9—4.1 (1H, m, CH), 5.61 (1H, br d, $J=7.5$ Hz, NH). MS *m/z*: 382 (M+1). *Anal.* Calcd for $C_{17}H_{35}NO_4S_2$: C, 53.51; H, 9.24; N, 3.67. Found: C, 53.70; H, 9.22; N, 3.83.

7: mp 40—42 °C. IR ν_{max}^{Nujol} cm^{-1}: 3370, 1740, 1685, 1520. NMR ($CDCl_3$) δ: 0.87 (3H, br t, $J=6.5$ Hz, Me), 1.1—1.9 (12H, m, $CH_2 \times 6$), 1.46 (9H, s, CMe_3), 2.10 (3H, s, COMe), 2.71 (2H, t, $J=7$ Hz, SCH_2), 2.89 and 3.25 (2H, AB in ABX, $J_{AX}=5$ Hz, $J_{BX}=7.5$ Hz, $J_{AB}=14$ Hz, $CHCH_2S$), 3.72 and 3.84 (2H, AB, $J=14$ Hz, $SOCH_2S$), 4.0—4.3 (3H, m, CH_2OAc and CH), 5.15 (1H, br d, $J=6.5$ Hz, NH). MS *m/z*: 423 (M^+). *Anal.* Calcd for $C_{19}H_{37}NO_5S_2$: C, 53.87; H, 8.81; N, 3.31. Found: C, 53.75; H, 8.68; N, 3.28.

General Procedure for the Preparation of Octylsparsomycin Analogs (9—12) 1) Deprotection of Boc Group: According to the reported method,[13] 3 ml of trifluoroacetic acid (TFA) and 0.25 ml of anisole per 1 mmol of the N-protected compound (**3—6**) were used. After concentration of the reaction mixture, the residue was washed with petroleum ether and dried over potassium hydroxide to give oily substances (TFA salts of cysteinols), which were used in the following reaction without further purification.

2) Condensation of Acid (**8**) with TFA Salts of Cysteinol Derivatives: BCC (1.5 g, 11 mmol) and *N*-methylmorpholine (1.1 g, 11 mmol) were added to a stirred solution of the acid (**8**, 2.3 g, 10 mmol) in dimethylformamide (DMF) (20 ml) at 0 °C. The resulting mixture was stirred for 15 min at 0 °C, then a pre-cooled solution of cysteinol derivative (11 mmol) and *N*-methylmorpholine (1.1 g, 11 mmol) in DMF (20 ml) was added. The whole mixture was further stirred for an appropriate period (5—12 h). After removal of the solvent, water (50 ml) was added to the residue, which was then extracted with $CHCl_3$ (70 ml × 3). The extract was washed successively with 10% HCl, 10% Na_2CO_3, and brine, and then dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (CH_2Cl_2 : MeOH = 20 : 1 as an eluent) to give the condensation product.

(R_s)-*N*-[(*E*)-β-(2,4-Dimethoxy-6-methyl-5-pyrimidinyl)acryloyl]-*S*-oxo-*S*-(octylthio)methyl-L-cysteinol (**9**): Yield 46%. mp 93—94 °C. $[\alpha]_D^{22}$: +23° ($c=1.02$, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3250, 1650, 1620, 1580, 1520. NMR ($CDCl_3$) δ: 0.86 (3H, br t, $J=6.5$ Hz, Me), 1.1—1.9 (12H, m, $CH_2 \times 6$), 2.52 (3H, s, Me), 2.71 (2H, t, $J=7$ Hz, SCH_2), 3.05 and 3.55 (2H, AB in ABX, $J_{AX}=5$ Hz, $J_{BX}=6$ Hz, $J_{AB}=13.5$ Hz, $CHCH_2SO$), 3.78 and 3.89 (2H, AB spectrum, $J=14$ Hz, $SOCH_2S$), 3.5—3.9 (1H, br, OH), 3.97 (3H, s, OMe), 4.03 (3H, s, OMe), 4.1—5.0 (3H, m, CH_2OH and CH), 6.70 and 7.65 (2H, AB, $J=16$ Hz, CH=CH), 6.92 (1H, br d, $J=7.5$ Hz, NH). MS *m/z*: 487 (M^+). *Anal.* Calcd for $C_{22}H_{37}N_3O_5S_2$: C, 54.18; H, 7.65; N, 8.62. Found: C, 54.10; H, 7.65; N, 8.56.

(S_s)-*N*-[(*E*)-β-(2,4-Dimethoxy-6-methyl-5-pyrimidinyl)acryloyl]-*S*-oxo-*S*-(octylthio)methyl-L-cysteinol (**10**): Yield 42%. mp 140—141 °C. $[\alpha]_D^{23}$: −59° ($c=1.02$, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3270, 1650, 1610, 1575, 1540. NMR ($CDCl_3$) δ: 0.86 (3H, br t, $J=6$ Hz, Me), 1.1—1.8 (12H, m, $CH_2 \times 6$), 2.48 (3H, s, Me), 2.69 (2H, t, $J=7$ Hz, SCH_2), 3.04 and 3.39 (2H, AB in ABX, $J_{AX}=7$ Hz, $J_{BX}=5$ Hz, $J_{AB}=13$ Hz, $CHCH_2SO$), 3.7—4.2 (4H, m, CH_2OH and $SOCH_2S$), 3.93 (3H, s, OMe), 3.98 (3H, s, OMe), 4.3—4.8 (2H, m, CH_2OH and CH), 6.61 and 7.58 (2H, AB, $J=15.5$ Hz, CH=CH), 7.15 (1H, br d, $J=7$ Hz, NH). MS *m/z*: 487 (M^+). *Anal.* Calcd for $C_{22}H_{37}N_3O_5S_2$: C, 54.18; H, 7.65; N, 8.62. Found: C, 54.51; H, 7.60; N, 8.50.

(R_s)-*N*-[(*E*)-β-(2,4-Dimethoxy-6-methyl-5-pyrimidinyl)acryloyl]-*S*-oxo-*S*-(octylthio)methyl-D-cysteinol (**11**): Yield 45%. mp 140—141 °C. $[\alpha]_D^{23}$: +59° ($c=1.07$, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3270, 1650, 1610, 1575, 1540. NMR ($CDCl_3$) δ: 0.85 (3H, br t, $J=6.5$ Hz, Me), 1.1—1.8 (12H, m, $CH_2 \times 6$), 2.51 (3H, s, Me), 2.70 (2H, t, $J=7$ Hz, SCH_2), 3.06 and 3.40 (2H, AB in ABX, $J_{AX}=7$ Hz, $J_{BX}=5$ Hz, $J_{AB}=13$ Hz, $CHCH_2SO$), 3.6—4.2 (4H, m, CH_2OH and $SOCH_2S$), 3.97 (3H, s, OMe), 4.02 (3H, s, OMe), 4.3—4.9 (2H, m, CH_2OH and CH), 6.64 and 7.63 (2H, AB, $J=15.5$ Hz, CH=CH), 7.12 (1H, br d, $J=7$ Hz, NH). MS *m/z*: 487 (M^+). *Anal.* Calcd for $C_{22}H_{37}N_3O_5S_2$: C, 54.18; H, 7.65; N, 8.62. Found: C, 54.38; H, 7.70; N, 8.70.

(S_s)-*N*-[(*E*)-β-(2,4-Dimethoxy-6-methyl-5-pyrimidinyl)acryloyl]-*S*-oxo-*S*-(octylthio)methyl-D-cysteinol (**12**): Yield 42%. mp 93—94°C. $[\alpha]_D^{23}$: −23° ($c=1.00$, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3240, 1650, 1620, 1580, 1520. NMR ($CDCl_3$) δ: 0.88 (3H, br t, $J=6$ Hz, Me), 1.1—1.9 (12H, m, $CH_2 \times 6$), 2.52 (3H, s, Me), 2.71 (2H, t, $J=7$ Hz, SCH_2), 3.06 and 3.54 (2H, AB in ABX, $J_{AX}=5$ Hz, $J_{BX}=6$ Hz, $J_{AB}=13.5$ Hz, $CHCH_2SO$), 3.80 and 3.91 (2H, AB, $J=14$ Hz, $SOCH_2S$), 3.7—4.0 (1H, br, OH), 3.98 (3H, s, OMe), 4.04 (3H, s, OMe), 4.1—5.0 (3H, m, CH_2OH and CH), 6.69 and 7.67 (2H, AB, $J=16$ Hz, CH=CH), 6.95 (1H, br d, $J=7.5$ Hz, NH). MS *m/z*: 487 (M^+). *Anal.* Calcd for $C_{22}H_{37}N_3O_5S_2$: C, 54.18; H, 7.65; N, 8.62. Found: C, 53.83; H, 7.75; N, 8.48.

***N*-[(*E*)-β-(2,4-Dimethoxy-6-methyl-5-pyrimidinyl)acryloyl]-*S*-(octylthio)methyl-L-cysteinol (13)** Trimethylsilyl chloride (22 mg, 0.2 mmol) was added dropwise to a stirred suspension of **10** (49 mg, 0.1 mmol) and sodium iodide (30 mg, 0.2 mmol) in dry acetonitrile (4 ml) under nitrogen, and the reaction mixture was stirred overnight. Methanol (10 ml) was added to the reaction mixture and the solvent was evaporated off. Water (30 ml) was added to the residue, which was then extracted with ether (30 ml × 2). The combined extract was washed successively with 0.1 N sodium thiosulfate and brine, and then dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was triturated with petroleum ether to afford **13** (16 mg, 34%) as a white powder. mp 84—87 °C. $[\alpha]_D^{22}$: −43° ($c=1.00$, $CHCl_3$). NMR ($CDCl_3$) δ: 0.86 (3H, br t, $J=6.5$ Hz, Me), 1.1—1.9 (12H, m, $CH_2 \times 6$), 2.53 (3H, s, Me), 2.63 (2H, t, $J=7$ Hz, SCH_2), 2.93 (2H, d, $J=6$ Hz, $CHCH_2SO$), 3.70 (2H, s, SCH_2S), 3.83 (2H, br t, $J=6$ Hz, CH_2OH), 3.97 (3H, s, OMe), 4.03 (3H, s, OMe), 4.1—4.3 (1H, m, CH), 6.41 (1H, br d, $J=7.5$ Hz, NH), 6.65 and 7.67 (2H, AB, $J=15$ Hz, CH=CH). MS *m/z*: 471 (M^+). *Anal.* Calcd for $C_{22}H_{37}N_3O_4S_2$: C, 56.03; H, 7.91; N, 8.91. Found: C, 56.28; H, 7.75; N, 8.70.

Antitumor Assay Roswell Park Memorial Institute Medium 1640 supplemented with 10% heat-inactivated fetal calf serum and 50 μg/ml of kanamycin (RPMI-FCS) was used as the cell culture medium. A suspension of mouse L5178Y lymphoma cells (10^5) in 1 ml of RPMI-FCS was prepared. All samples were dissolved in dimethylsulfoxide (DMSO) at a concentration of 20.2 mg/ml. The cell suspension (200 μl) and a sample solution (2 μl) were mixed in a microwell tissue culture plate (Costar, Cambridge, Mass.). In this case, the final sample concentration was 200 μg/ml. As a control, the same amount of cell suspension and 2 μl of DMSO were mixed. The plate was incubated in a CO_2 incubator at 37 °C for 44 h. [Methyl-^{3}H]thymidine (0.4 μCi in 10 μl of saline: specific activity 20 Ci/mmol) purchased from New England Nuclear (Boston, Mass.) was added as a precursor of deoxyribonucleic aicd (DNA) synthesis to each well and the plate was incubated for 4 h. L5178Y cells were exposed to the sample during the assay period (48 h). Cells in each well were harvested on a glass-fiber disk (Whatman Ltd., Madison, England). The disk was successively washed with 10% ice-cold trichloroacetic acid (TCA) and water, and then dried. Radioactivity was determined with a Beckman LS9000 liquid scintillation counter (Beckman Instruments Inc., Irvine, Calif.) using toluene–PPO–POPOP counting solution [PPO, 2,5-diphenyloxazole; POPOP, 2,2-*p*-phenylenebis(5-phenyloxazole)]. Inhibition of DNA synthesis was calculated from the incorporation of ^{3}H into the TCA-insoluble fraction of cells on the disk using the following formula;

$$\text{percentage inhibition } (\%) = (A-B)/A \times 100$$

where A is the average ^{3}H count of the control group and B is that of the treated sample.

Each experiment was performed in triplicate at various concentrations, using dilutions of the initially prepared solution, and then dose-effect curves were made. From these curves, the dose causing 50% inhibition of incorporation was calculated.

References and Notes

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