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Sparsomycin Analogs. V.[1] Synthesis and Antitumor Activity of (*E*)-β-(Pyrimidin-5-yl)acrylamides

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Various (*E*)-β-(pyrimidin-5-yl)acrylamides (**4** and **5**) were synthesized as sparsomycin analogs, and the relationship between chemical structure and antitumor activity was examined. Synthesis involved condensation of appropriate acids (**3**) and methyl methioninate (L and D isomers) by the mixed anhydride method using isobutyl chlorocarbonate. The antitumor activity was studied by [methyl-^{3}H]thymidine incorporation assay with mouse leukemia L5178Y cells *in vitro*. The concentrations in μg/ml required for 50% inhibition of incorporation by compounds **4b**, **4f, g**, **5b**, and **5f, g**, which have no substituent at the 2 or 6 position on pyrimidine ring, were particularly high. Thus, such substituents enhanced antitumor activity, although the activity was almost uninfluenced by changes of the alkoxy group as a substituent.

Keywords——sparsomycin; sparsomycin analog; (*E*)-β-(pyrimidin-5-yl)acrylamide; antitumor activity

Sparsomycin (**1**) is a metabolite of *Streptomyces sparsogenes*[2] and *S. cuspidosporus*.[3] Many years were required for its structure determination because of some unique features (Fig. 1).[4] Although this compound exhibits a wide range of biological activities,[3,5] the most interesting biological activity was its antitumor activity, which appears to be primarily due to inhibition of protein synthesis by blocking the peptidyl transferase function of the larger ribosomal subunit.[6] Sparsomycin has attracted widespread attention from the viewpoint of possible clinical application, but its severe eye toxicity[7] has limited its usefulness in cancer chemotherapy. Therefore several studies dealing with the synthesis of sparsomycin analogs have been made[8–13] with the aim of eliminating its toxicity and clarifying the structure–activity relationship of sparsomycin.

We have also synthesized many analogs of sparsomycin and tested their biological activities.[1,14] In a preliminary report,[1] we showed that O-methylation of the pyrimidine ring remarkably enhanced the antitumor activity *in vitro* of these analogs. The present paper describes the preparation of O-alkoxy derivatives of (*E*)-β-(pyrimidin-5-yl)acrylamides, which have various substitution patterns on the pyrimidine ring, and also an extended study of the structural and stereochemical requirements for their antitumor activity against mouse leukemia cells *in vitro*.

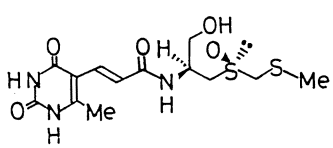


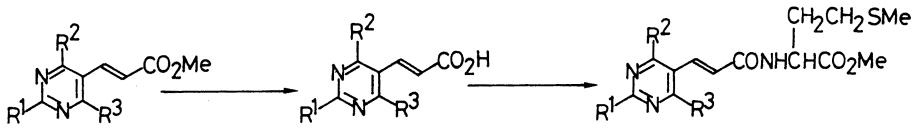
Fig. 1. Structure of Sparsomycin (S_c–R_s) (**1**)

Results and Discussion

The synthetic routes employed for the preparation of analogs are outlined in Chart 1. Methyl (*E*)-β-(pyrimidin-5-yl)acrylates (**2a**—**g**) were prepared according to the previously reported method.[15] Hydrolyses were carried out by using potassium hydroxide in aqueous methanol at room temperature to afford the corresponding acids in excellent yields, and these results are summarized in Table I. The condensations of **3a**—**g** with both (L)- and (D)-methyl methioninates were performed by the mixed anhydride (MA) method using isobutyl chlorocarbonate (BCC) to afford sparsomycin analogs (**4** and **5**) in moderate to excellent yields. These results are summarized in Table II.

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

Comparison of the IC_{50} value of **4a** (having three substituents) with those of **4b** and **4g** (having two substituents) demonstrates that all substituents on the pyrimidine ring were necessary for biological activity. The same results were obtained for sulfur-containing analogs. Thus, the compound having a methyl group at the C-6 position (**4e**) has a lower IC_{50} value than that of the demethylated derivative (**4f**). The replacement of a methoxy group with an ethoxy (**4a**→**4c**), propoxy (**4a**→**4d**), or methylthio (**4a**→**4e**) group had little effect on the



a: R^1=OMe, R^2=OMe, R^3=Me
b: R^1=OMe, R^2=OMe, R^3=H
c: R^1=OEt, R^2=OEt, R^3=Me
d: R^1=OPr, R^2=OPr, R^3=Me
e: R^1=SMe, R^2=OMe, R^3=Me
f: R^1=SMe, R^2=OMe, R^3=H
g: R^1=H, R^2=OMe, R^3=Me

Chart 1

TABLE I. Yields of (*E*)-β-Pyrimidinylacrylic Acids (**3**)

Compound No.	Substituent			Reaction time (h)	Product No.	Yield (%)
	R^1	R^2	R^3			
2a	OMe	OMe	Me	2	**3a**	90
2b	OMe	OMe	H	1	**3b**	84
2c	OEt	OEt	Me	1	**3c**	86
2d	OPr	OPr	Me	1	**3d**	85
2e	SMe	OMe	Me	1	**3e**	82
2f	SMe	OMe	H	2	**3f**	92
2g	H	OMe	Me	1	**3g**	86

TABLE II. Physical and Chemical Data for Analogs (**4** and **5**)

Compound No.	Yield (%)	Optical rotation[a]	mp (°C) (Solvent)	Molecular formula	Analysis (%) Calcd (Found)		
					C	H	N
4a	59	−31.9	152—153 (*n*-Hexane–MeOH)	$C_{16}H_{23}N_3O_5S$	52.02 (52.03)	6.28 (6.08)	11.38 (11.18)
5a	59	+32.1	148—149 (*n*-Hexane–MeOH)	$C_{16}H_{23}N_3O_5S$	52.02 (52.55)	6.28 (6.31)	11.38 (11.40)
4b	61	−28.3	101—102 (*n*-Hexane–MeOH)	$C_{15}H_{21}N_3O_5S$	50.70 (50.63)	5.96 (6.12)	11.83 (11.65)
5b	46	+29.1	100—103 (*n*-Hexane–MeOH)	$C_{15}H_{21}N_3O_5S$	50.70 (50.62)	5.96 (6.01)	11.83 (11.73)
4c	74	−31.5	136—137 (Pet. ether–MeOH)	$C_{18}H_{27}N_3O_5S$	54.40 (54.20)	6.85 (6.81)	10.57 (10.51)
5c	88	+33.1	133—134 (Pet. ether–MeOH)	$C_{18}H_{27}N_3O_5S$	54.40 (54.22)	6.85 (6.91)	10.57 (10.70)
4d	65	−30.8	74—75 (Pet. ether–MeOH)	$C_{20}H_{31}N_3O_5S$	56.46 (56.07)	7.34 (7.35)	9.88 (9.95)
5d	68	+31.5	73—74 (Pet. ether–MeOH)	$C_{20}H_{31}N_3O_5S$	56.46 (55.97)	7.34 (7.33)	9.88 (10.13)
4e	60	−30.6	121—122 (*n*-Hexane–MeOH)	$C_{16}H_{23}N_3O_4S_2$	49.86 (49.85)	6.02 (5.91)	10.90 (10.94)
5e	76	+29.6	121—122 (*n*-Hexane–MeOH)	$C_{16}H_{23}N_3O_4S_2$	49.86 (49.65)	6.02 (6.01)	10.90 (10.96)
4f	64	−25.8	112—114 (Pet. ether–$CHCl_3$)	$C_{15}H_{21}N_3O_4S_2$	48.51 (48.29)	5.70 (5.50)	11.32 (11.17)
5f	79	+26.8	111—113 (Pet. ether–$CHCl_3$)	$C_{15}H_{21}N_3O_4S_2$	48.51 (48.74)	5.70 (5.66)	11.32 (11.13)
4g	46	−31.7	120—121 (Pet. ether–MeOH)	$C_{15}H_{21}N_3O_4S$	53.09 (53.55)	6.24 (6.47)	12.38 (12.63)
5g	44	+30.5	121—121.5 (Pet. ether–MeOH)	$C_{15}H_{21}N_3O_4S$	53.09 (53.49)	6.24 (6.77)	12.38 (12.63)

a) $[\alpha]_D^{21}$ (c=1.0, MeOH).

TABLE III. IC_{50} Values of Sparsomycin and Its Analogs on L5178Y Cell Growth *in Vitro*

Substituent			Compound No. (L-Isomer)	IC_{50} (μg/ml)	Compound No. (D-Isomer)	IC_{50} (μg/ml)
R^1	R^2	R^3				
OMe	OMe	Me	**4a**	11.3	**5a**	16.5
OMe	OMe	H	**4b**	59	**5b**	54
OEt	OEt	Me	**4c**	19.5	**5c**	11.6
OPr	OPr	Me	**4d**	10.0	**5d**	9.0
SMe	OMe	Me	**4e**	11.5	**5e**	13.6
SMe	OMe	H	**4f**	34.0	**5f**	13.2
H	OMe	Me	**4g**	74.0	**5g**	36.0
Sparsomycin				0.07		

IC_{50} value. Similar results were obtained in the compounds **5** series, which only differ from **4** in the configuration on the chiral carbon atom.

In the comparison between the **4** and **5** series of compounds with three substituents, it is very surprising that the chirality of the carbon atom has no important role in the antitumor

activity. Thus, **4a**, **4c**—**e** and the corresponding enantiomers (**5a**, **5c**—**e**) showed almost the same antitumor activities. It has been mentioned earlier that among the analogs derived from the chemical modification of the aminoalcohol moiety in sparsomycin, the compound having *S* configuration of the chiral carbon atom has a significantly lower IC_{50} value than that of the corresponding compound with *R* configuration.[13] Our observation is not in accordance with the previous findings, presumably because the acid moiety was changed in our tested analogs. Also, it appears that the configuration of the sulfur atom is more important for the expression of antitumor activity in this type of sparsomycin analog. In order to evaluate this speculation, further investigation is in progress in our laboratory.

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). All new compounds were identified by IR and NMR spectroscopy.

General Procedure for Preparation of Pyrimidinylacrylic Acids (3)——These acids, listed in Table I, were prepared by the hydrolysis of the corresponding esters[15] according to the reported method for **3a**.[1] The physical and chemical data for the products are as follows.

(*E*)-β-(2,4-Dimethoxypyrimidin-5-yl)acrylic Acid (**3b**): mp 198—199 °C (MeCN–MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1680, 1625, 1585. NMR (DMSO-d_6) δ: 7.95 (1H, s, ArH), 7.10 (1H, d, J=15.5 Hz, =CH), 6.16 (1H, d, J=15.5 Hz, =CH), 3.82 (3H, s, OMe), 3.75 (3H, s, OMe), and COOH is absent. MS *m/z*: 210 (M^+). *Anal.* Calcd for $C_9H_{10}N_2O_4$: C, 51.42; H, 4.82; N, 13.33. Found: C, 51.29; H, 4.80; N, 13.31.

(*E*)-β-(2,4-Diethoxy-6-methylpyrimidin-5-yl)acrylic Acid (**3c**): mp 132—133 °C (MeCN). IR ν_{max}^{Nujol} cm^{-1}: 1685, 1620, 1575. NMR (DMSO-d_6) δ: 10.60 (1H, s, COOH), 7.84 (1H, d, J=16 Hz, =CH), 6.66 (1H, d, J=16 Hz, =CH), 4.6—4.3 (4H, m, CH_2 × 2), 2.54 (3H, s, Me), 1.46 (3H, t, J=7 Hz, Me), 1.40 (3H, t, J=7 Hz, Me). MS *m/z*: 252 (M^+). *Anal.* Calcd for $C_{12}H_{16}N_2O_4$: C, 55.16; H, 6.56; N, 10.72. Found: C, 54.96; H, 6.44; N, 10.81.

(*E*)-β-(2,4-Dipropoxy-6-methylpyrimidin-5-yl)acrylic Acid (**3d**): mp 134—134.5 °C (MeCN). IR ν_{max}^{Nujol} cm^{-1}: 1685, 1630, 1575. NMR (DMSO-d_6) δ: 10.01 (1H, s, COOH), 7.86 (1H, d, J=16 Hz, =CH), 6.67 (1H, d, J=16 Hz, =CH), 4.51 (2H, t, J=7 Hz, OCH_2), 4.44 (2H, t, J=7 Hz, OCH_2), 2.54 (3H, s, Me), 2.0—1.6 (4H, m, CH_2 × 2), 1.07 (3H, t, J=6.5 Hz, Me), 1.03 (3H, t, J=6.5 Hz, Me). MS *m/z*: 280 (M^+). *Anal.* Calcd for $C_{14}H_{20}N_2O_4$: C, 59.98; H, 7.19; N, 9.99. Found: C, 59.69; H, 6.85; N, 10.27.

(*E*)-β-(4-Methoxy-2-methylthio-6-methylpyrimidin-5-yl)acrylic Acid (**3e**): mp 205—206 °C (MeCN). IR ν_{max}^{Nujol} cm^{-1}: 1685, 1620, 1565. NMR (DMSO-d_6) δ: 7.73 (1H, d, J=16 Hz, =CH), 6.61 (1H, d, J=16 Hz, =CH), 4.03 (3H, s, OMe), 2.55 (6H, s, Me and SMe), and COOH is absent. MS *m/z*: 240 (M^+). *Anal.* Calcd for $C_{10}H_{12}N_2O_3S$: C, 50.00; H, 5.04; N, 11.66. Found: C, 49.81; H, 5.00; N, 11.65.

(*E*)-β-(4-Methoxy-2-methylthiopyrimidin-5-yl)acrylic Acid (**3f**): mp 200—201.5 °C (MeCN). IR ν_{max}^{Nujol} cm^{-1}: 1690, 1640, 1580. NMR (DMSO-d_6) δ: 8.88 (1H, s, ArH), 7.69 (1H, d, J=16 Hz, =CH), 6.78 (1H, d, J=16 Hz, =CH), 4.15 (3H, s, OMe), 2.62 (3H, s, SMe), and COOH is absent. MS *m/z*: 226 (M^+). *Anal.* Calcd for $C_9H_{10}N_2O_3S$: C, 47.79; H, 4.46; N, 12.39. Found: C, 47.85; H, 4.61; N, 12.31.

(*E*)-β-(4-Methoxy-6-methylpyrimidin-5-yl)acrylic Acid (**3f**): mp 199—200 °C (MeCN–MeOH)). IR ν_{max}^{Nujol} cm^{-1}: 1685, 1625, 1580. NMR (DMSO-d_6) δ: 8.51 (1H, s, ArH), 7.70 (1H, d, J=16 Hz, =CH), 6.78 (1H, d, J=16 Hz, =CH), 4.04 (3H, s, OMe), 2.58 (3H, s, Me), and COOH is absent. MS *m/z*: 194 (M^+). *Anal.* Calcd for $C_9H_{10}N_2O_4$: C, 55.66; H, 5.19; N. 14.43. Found: C, 55.72; H, 5.28; N, 14.61.

General Procedure for Condensation of Acids (3) with Methyl Methioninate Hydrochloride (4 and 5)——BCC (1.5 g, 11 mmol) and *N*-methylmorpholine (1.1 g, 11 mmol) were added to a stirred solution of an acid (**3**, 10 mmol) in dimethylformamide (DMF) (20 ml) at 0 °C. The resulting mixture was stirred for 15 min at 0 °C, then a precooled solution of methyl methioniate hydrochloride (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 on silica gel ($CHCl_3$:AcOEt=9:1 as an eluent) to yield the condensation product. The results of these procedures are summarized in Table II. An example is described below.

Methyl *N*-[(*E*)-β-(2,4-Dimethoxy-6-methyl-5-pyrimidinyl)acryloyl-L-methioninate (4a)——This was prepared from **3a** (2.0 g, 8.9 mmol) and methyl (L)-methioninate hydrochloride (1.9 g, 9.8 mmol). Recrystallization from *n*-hexane and methanol gave pure **4a** (1.93 g, 59%). mp 152.5—153 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3390, 1730, 1655, 1590. MS *m/z*: 369 (M^+). NMR ($CDCl_3$) δ: 7.69 (1H, d, J=16 Hz, =CH), 6.73 (1H, d, J=16 Hz, =CH), 6.42 (1H, d, J=7 Hz, NH), 5.0—4.8 (1H, m, CH), 4.03 (3H, s, OMe), 3.99 (3H, s, OMe), 2.60 (2H, t, J=7 Hz, CH_2), 2.51 (3H, s, Me), 2.3—2.0

(2H, m, CH_2), 2.10 (3H, s, Me). *Anal.* Calcd for $C_{16}H_{23}N_3O_5S$: C, 52.02; H, 6.28; N, 11.38. Found: C, 52.03; H, 6.08; N, 11.18.

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 group, 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 acid (DNA) synthesis to each well and 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.

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