Synthesis and Antitumor Properties of N^1-Acyloxymethyl Derivatives of Bis(2,6-dioxopiperazines)[1)]

Jun Chao CAI,[a] Han Li SHU,[a] Cai Fang TANG,[a] Toshihiko KOMATSU,*[,b] Toshiyuki MATSUNO,[b] Toshiharu NARITA,[b] Shin-ichi YAGUCHI,[b] Yuji KOIDE,[b] and Muneaki TAKASE[b]

Shanghai Institute of Materia Medica,[a] Academia Sinica, Shanghai, China and Research Laboratory, Zenyaku Kogyo Co., Ltd.,[b] 33-7, Ohizumi-machi 2-chome, Nerima-ku, Tokyo 178, Japan. *Received March 31, 1989*

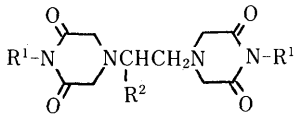
Many N^1-acyloxymethyl derivatives VI of bis(2,6-dioxopiperazine) I, ICRF-154, were prepared and tested for antitumor activity. The treatment of I with formaldehyde gave a crystalline bis(N^1-hydroxymethyl) derivative VII, which was acylated under various conditions to give bis(N^1-acyloxymethyl) derivatives VI. Antitumor activity of VI against P388 leukemia in mice was studied.

Several bis(N^1-acyloxymethyl) compounds such as phenylacetyloxymethyl VI-6, methoxycarbonyloxymethyl VI-41, isobutoxycarbonyloxymethyl VI-44, and furancarboxymethyl VI-38 compounds were found to have potent antitumor activities. On the other hand, water-soluble esters having an amine or a carboxylic acid function in their acyl groups showed rather reduced activity.

These bis(N^1-acyloxymethyl) derivatives VI were presumably hydrolyzed into the parent bis(2,6-dioxopiperazine) I by nonspecific esterase in the body to exhibit their antitumor activity.

Keywords acyloxymethylation; bis(dioxopiperazine); antitumor activity; hydroxymethyl imide; ICRF-154; enzymatic hydrolysis; esterase; P388

The class of antineoplastic agents broadly referred to as bis(2,6-dioxopiperazines), such as ICRF-154 (I) and ICRF-159 (II), has been studied extensively over the past several years[2)] and their chemical, biochemical and pharmacological properties have recently been reviewed.[3)] Their unique antimetastatic properties and their actions ameliorating anthracycline-induced toxicity in animals have aroused considerable interest,[4)] and some work on structural modifications has been carried out with the aim of finding derivatives with an improved antitumor activity and spectrum.[5)]



I : $R^1=R^2=H$
II : $R^1=H$, $R^2=CH_3$
III : $R^1=CH_2N$⟨O⟩, $R^2=H$
IV : $R^1=CH_2N$⟨O⟩, $R^2=CH_3$
V : $R^1=CH_2N<^{R}_{R}$, $R^2=CH_3$
VI : $R^1=CH_2OCOR$, $R^2=H$

Recent reports from China have indicated the bis(N^1-morpholinomethyl) derivatives of I and II (namely, III[6)] and IV[7)]) to be active against various experimental tumor models. In addition, the results of clinical investigation of III in China indicated that III may be useful in the treatment of malignant lymphoma, uveitis, sympathetic ophthalmitis, and psoriasis.

In our initial study, aminomethylation of the imide proton of ICRF-159 (II) was carried out to give IV and some analogous compounds V which showed a significant antitumor activity against P388 leukemia in mice,[7)] suggesting that the introduction of these aminomethyl groups into II did not affect the original antitumor activity of II. However, it was also found that these derivatives (IV and V) are fairly unstable in aqueous solution at room temperature in spite of their stability in the crystalline state. These promising biological results with IV and V obtained in animal tumor screening prompted us to carry out further N^1-chemical modification of bis(2,6-dioxopiperazines), I and II.

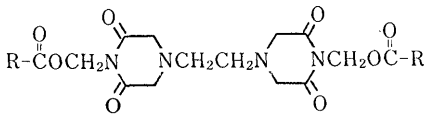
Synthesis of acyloxymethyl derivatives of penicillins,[8)] cephalosporins,[9)] and so on and their biological properties as pro-drugs have attracted the attention of medicinal chemists. These compounds after absorption in the animal body are presumably enzymatically hydrolyzed by nonspecific esterase to the corresponding hydroxymethyl derivative, which subsequently decomposes spontaneously to the original active compound and formaldehyde, showing the biological activity. Bis(2,6-dioxopiperazines), I and II, are orally absorbed very poorly, probably due to their reduced solubility in water and organic solvents.[10)] Substitution of the imide proton with an acyloxymethyl group would be expected to increase their solubility and to improve their bioavailability.

In the present paper, we report the synthesis of N^1-acyloxymethyl derivatives of bis(2,6-dioxopiperazine) I (namely, VI) and we describe their chemical stabilities, antitumor activities, and susceptibility to enzymatic hydrolysis.

Synthesis Two pathways were employed for the synthesis of N^1-acyloxymethyl derivatives VI of bis(2,6-dioxopiperazine) I. In the first one, the starting material was the sodium salt of I, which was treated in *N,N*-dimethylformamide (DMF) with a slight excess of a halomethyl ester[8a,c)] such as $ClCH_2OCOR$ to form the corresponding acyloxymethyl derivative VI. However, it was difficult to prepare some halomethyl esters, many of which are quite unstable to moisture.

As the second pathway, treatment of I with an excess amount of formalin gave the bis(N^1-hydroxymethyl) derivative VII, mp 170—172 °C, in a good yield. The nuclear magnetic resonance (NMR) absorption of VII at δ 5.02 was

TABLE I. 4,4′-(1,2-Ethanediyl)bis(1-acyloxymethyl-2,6-piperazinediones) (VI) and Their Antitumor Activities against P388 (i.p.–i.p.) in Mice



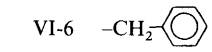
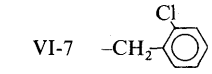
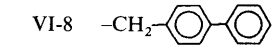
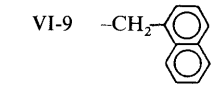
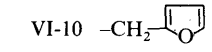
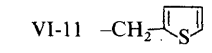
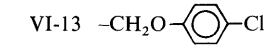
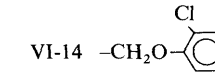
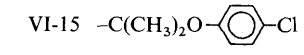
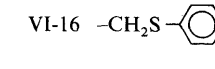
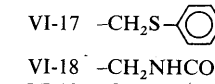
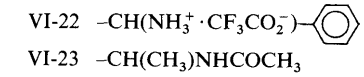
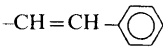
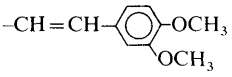
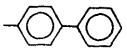
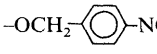
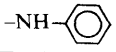
No.	R	Solubility[a] in water (g)	Dose (mg/kg/d)	T/C (%)
I (ICRF-154)		Ins	60	213
			89	231
			133	255
			200	113
VI-1	–H	Ins (0.05)	200	Toxic[b]
VI-2	$-CH_3$	Ins (0.015)	100	122
			200	145
			400	201
			500	240
VI-3	$-CH_2CH(CH_3)_2$	Ins	200	115
VI-4	$-C(CH_3)_3$	Ins	100	128
			200	166
			300	131
			400	142
VI-5	$-(CH_2)_{14}CH_3$	Ins	200	125
VI-6		Ins	25	146
			50	164
			150	217
			200	303
			250	247
			300	245
VI-7		Ins	50	159
			100	184
			200	182
			250	150
			300	101
VI-8		Ins	200	126
VI-9		Ins	200	96
VI-10		Ins	200	193
VI-11		Ins	200	227
VI-12	$-CH_2OCH_3$	Ins	200	163
VI-13		Ins	100	153
			200	194
			300	113
			400	101
VI-14		Ins	200	172
VI-15		Ins	50	123
			100	154
			200	214
			300	199
			400	190
VI-16		Ins	50	154
			100	176
			200	220
			250	215
VI-17		Ins	200	208
VI-18	$-CH_2NHCO_2C(CH_3)_3$	Ins	200	224
VI-19	$-CH_2NH_3^+ \cdot CF_3CO_2^-$	Vsol	200	100
VI-20	$-CH_2NHCO_2CH_3$	Ins	200	139
VI-21		Ins	200	176
VI-22		Vsol	200	126
VI-23	$-CH(CH_3)NHCOCH_3$	3	200	117

TABLE I. (continued)

No.	R	Solubility[a] in water (g)	Dose (mg/kg/d)	T/C (%)
VI-24	$-CH(CH_3)N(CH_3)COCH_3$	Vsol	200	102
VI-25	$-(CH_2)_2CO_2H$	1	100	109
			200	108
			400	112
VI-26	$-(CH_2)_2CO_2Na$	Vsol	200	112
VI-27		Ins	200	206
VI-28		Ins	200	152
VI-29		Ins	200	142
VI-30		Ins	200	199
VI-31		Ins	200	112
VI-32		Ins	200	213
VI-33		Ins	100	155
			200	190
			300	182
VI-34		Ins	200	134
VI-35		Ins	200	113
VI-36		Ins	200	104
VI-37		Ins	200	101
VI-38		Ins	50	166
			100	212
			200	300
			250	280
VI-39		Ins	200	195
VI-40		Ins	200	218
VI-41	$-OCH_3$	Ins (0.01)	50	129
			100	170
			200	203
			300	210
VI-42	$-OCH_2CH_3$	Ins	200	145
VI-43	$-O(CH_2)_3CH_3$	Ins	200	112
VI-44	$-OCH_2CH(CH_3)_2$	Ins	50	179
			100	203
			200	238
			250	254
			300	109
VI-45	$-OCH_2CH(C_2H_5)-(CH_2)_3CH_3$	Ins	200	138
VI-46		Ins	200	150
VI-47		Ins	200	163
VI-48	$-NH_2$	Ins	200	137
VI-49		Ins	200	107

a) Maximum amount soluble in 100 ml of water at room temperature; Ins, less than 0.1; Vsol, greater than 5. b) Some deaths occurred in the animals given this dose.

considered to be due to the methylene protons of newly introduced hydroxymethyl groups, reflecting the structure of VII. The derivative VII was sufficiently stable in the crystalline state to be recrystallized from dioxane, but was still unstable in solution in protic solvents such as water or methanol, immediately decomposing into the parent compound I. Acylation of VII was carried out with acid anhydride (method A), mixed anhydride (method B), acid chloride (method C), acid in the presence of dicyclohexylcarbodiimide (DCC) and *N*,*N*-dimethylaminopyridine (method D). The derivatives VI thus prepared are shown in Table I.

The acetate VI-2 and formate VI-1 were prepared by methods A and B, respectively. Esterification with alkyl- or aryl-carboxylic acid was done by method C *via* the corresponding acid chloride, while esterification with arylacetic acids such as phenyl-, phenoxy-, phenylthio-, furan-, thiophene-acetic acid was done by method D. Synthesis of arylcarboxylates VI was attempted *via* method D, but without success. The yield of the desired acyloxymethyl derivatives was low and acylurea formation, probably *via* the reaction of the acid with DCC followed by Chapman rearrangement, was mostly observed.[11]

Acylation of VII with *N*-*tert*-butoxycarbonyl protected glycine or phenylglycine followed by removal of the protecting group with trifluoroacetic acid gave the trifluoroacetate of the corresponding ester, VI-19 or VI-22, as a powder which was submitted to biological testing. Removal of trifluoroacetic acid from VI-19 or VI-22 was attempted by treatment with Amberlite IR-45 in water, but the resulting free amine was too unstable to collect, and decomposed.

The succinate half ester VI-25 was prepared by esterification of VII with mono-benzyl succinate by method D, followed by removal of the benzyl group by hydrogenation over palladium-charcoal. The sodium salt VI-26 of the succinate VI-25 was obtained by treatment of the succinate VI-25 with an equivalent amount of sodium carbonate followed by lyophilization.

Treatment of the bis(N^1-hydroxymethyl) derivative VII with alkyl and aryl chloroformates afforded the corresponding carbonates, such as VI-41 and VI-47, respectively, in good yields. The carbamate VI-48 was synthesized by treatment of VII with trichloroacetyl isocyanate followed by removal of the formed trichloroacetyl group with a base. Treatment of VII with phenyl isocyanate in the presence of triethylamine afforded the phenyl carbamate VI-49 as a stable colorless oil.

Chemical Stability Though the acyloxymethyl derivatives VI thus obtained have a hydrolyzable ester bond along with an amine function in the molecule, they are quite stable in crystalline form and hardly decomposed even on standing at room temperature for several months.

The stability of some acyloxymethyl derivatives VI such as acetyloxymethyl and methoxycarbonyloxymethyl derivatives, VI-2 and VI-41, was examined in water, or acidic or basic solution by collecting aliquots periodically. The amount of remaining material was determined by work-up and high-pressure liquid chromatography (HPLC). The results are shown in Table II. These compounds are fairly stable in water. It was also found that the acetyloxymethyl derivative VI-2 is more stable in water than in a buffer solution, in which increasing pH reduces its stability. The methoxycarbonyloxymethyl derivative VI-41 seems to be more stable than the acetyloxymethyl derivative VI-2.

TABLE II. Stabilities of 4,4′-(1,2-Ethanediyl)-bis(1-acetyloxymethyl-2,6-piperazinedione) (VI-2), and -bis(1-methoxycarbonyloxymethyl-2,6-piperazinedione) (VI-41) in Aqueous Solution

Time (h)	Recovery (%) of VI-2					VI-41
	Water	Phosphate buffer[a]			0.1 N HCl	Water
		pH 8.0	pH 7.0	pH 5.6		
0	100.0	100.0	100.0	100.0	100.0	100.0
1	99.2	—	95.0	—	87.9	95.9
2	96.9	—	88.0	—	77.8	93.1
3	95.8	—	85.5	—	68.2	93.1
4	93.9	—	79.3	—	61.1	92.4
5	92.0	—	74.8	—	53.7	92.4
6	90.0	—	69.4	—	—	—
7	90.0	—	65.3	—	—	—
24	67.4	3.3	36.4	53.2	7.2	62.8
48	39.7	0.0	6.6	27.9	0.0	35.9
72	—	—	2.1	20.1	—	—

a) Prepared by mixing with Na_2HPO_4 (1/15 mol/l) and KH_2PO_4 (1/15 mol/l) in the following proportions by volume; 1.5 : 28.5 (for pH 5.6), 18 : 12 (for pH 7.0), 28.5 : 1.5 (for pH 8.0).

Antitumor Activity These acyloxymethyl derivatives VI were submitted to antitumor testing against P388 leukemia and many of them exhibited significant activities as shown in Table I. Although a comparison of activity between them was not carried out under optimal dosing, but under single dosing of 200 mg/kg, the following tentative conclusions on the structure–activity relationship were obtained.

a) The most active compound among the alkylcarboxylates is the acetate VI-2. The formate VI-1 is slightly soluble in water but shows reduced activity. Administration of 200 mg of VI-1 resulted in signs of toxicity. Elongation of the alkyl chain in alkylcarboxylates leads to lower activity, while branching of the alkyl chain at the 2-position does not reduced the activity.

b) Phenylacetates, VI-6 and VI-7, are active and VI-6 is the most active (T/C 303%), while the biphenylacetate VI-8 and naphthylacetate VI-9 are less active.

c) Activity of the alkoxyacetate VI-12 is marginal, while the aryloxyacetates, VI-13, VI-14, and VI-15, are active.

d) Compounds having a water-soluble amine function in the acyl moiety, VI-19 and VI-22, are not active but their *N*-protected derivatives, VI-18 and VI-21, are active. The *N*-acetylalanine derivatives, VI-23 and VI-24, have moderate solubilities of 2.5% and 10%, respectively, but with less activity. The alkyl ester with a carboxylic group in the alkyl chain and its sodium salt, VI-25 and VI-26, show poor activity.

e) Most of the arylcarboxylate derivatives exhibit good activities; for example, the T/C value of the furancarboxylate VI-38 is 300%. On the other hand, the piperonylate VI-31 is less active.

f) Cinnamates, VI-34 and VI-35, show reduced activities.

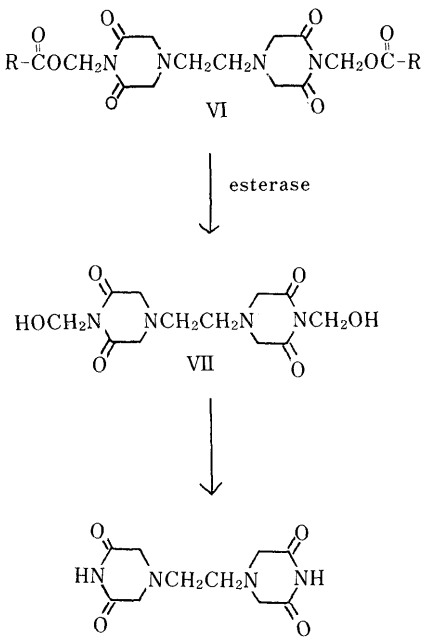
g) Some carbonates, VI-41 and VI-44, have good activities.

h) Urethanes, VI-48 and VI-49, are not active, presumably due to a poor susceptibility of the ester bond to cleavage by esterase in the body.

Hydrolysis in the Presence of Esterase It was found that

the bis(N^1-acyloxymethyl) derivatives VI described above were hydrolyzed by enzymes present in porcine liver or rat tissue (small intestine) homogenate, liberating bis(2,6-dioxopiperazine) I. Although the mechanism of this reaction has not been elucidated, it might be assumed that enzymatic hydrolysis of VI to the bis(hydroxymethyl) derivative VII occurs initially and is followed by spontaneous decomposition to I as shown below in the Chart 1.

Hydrolysis rates of acyloxymethyl derivatives VI in the presence of esterase were determined at pH 8.0 at 30 °C by HPLC of aliquots of the reaction mixture during the reaction to determine I.



ICRF-154 (I)

Chart 1

TABLE III. Relative Rates of Hydrolysis of 4,4′-(1,2-Ethanediyl)bis(1-acyloxymethyl-2,6-piperazinedione) VI by Esterase Present in Animal Tissue (*in Vitro*)

Compound	Porcine liver esterase	Rat small intestine esterase
VI-1	<3	<3
VI-2	<3	<3
VI-6	47.6	26.5
VI-7	93.2	12.3
VI-11	145.5	317.5
VI-12	<3	62.0
VI-14	151.5	364.9
VI-15	76.7	16.9
VI-18	<3	<3
VI-19	<3	6.2
VI-20	<3	<3
VI-21	<3	<3
VI-23	<3	<3
VI-25	<3	<3
VI-27	19.6	426.6
VI-30	8.7	12.3
VI-33	36.2	140.4
VI-34	95.7	190.4
VI-36	<3	9.6
VI-40	53.7	259.5
VI-41	<3	<3
VI-43	127.8	159.7
VI-44	100	100
VI-46	27.3	39.0
VI-48	<3	<3

Relative rates of hydrolysis (the rate of hydrolysis of the isobutoxycarbonyloxymethyl derivative VI-44 is taken as 100) after incubation for 10 min are shown in Table III, indicating a rough correlation between the value and the structure of the side chain in the following respects.

a) Small esters such as formate VI-1 and acetate VI-2 are hardly hydrolyzed, while medium- or larger-size esters such as thiopheneacetate VI-11, phenoxyacetate VI-14, and cinnamate VI-34 are hydrolyzed more rapidly.

b) Alkylcarboxylic acid esters which have an ionic function such as an amino or a carboxylic group in the alkyl chain are less rapidly hydrolyzed.

c) Medium-size carbonates VI-43, VI-44, and VI-46 show rapid hydrolysis, while the carbamate VI-48 does not.

Most of the bis(N^1-acyloxymethyl) derivatives VI thus synthesized are soluble in organic solvents such as alcohol, ethyl acetate, chloroform, and so on. This result encourages us to expect good efficacy as antitumor drugs *via* enhanced tissue transfer as compared with the parent compound I. Some of compounds VI were selected as candidate anticancer drugs, and their activities against several kinds of tumor models, along with their toxicities and bioavailabilities were further studied. These results will be reported in the following papers.

Experimental

Melting points were determined in capillary tubes (Yamato melting point apparatus) and are not corrected. Infrared spectra (IR) were taken on a Hitachi 260-10 spectrometer. Proton nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on a JEOL FX-270 spectrometer with tetramethylsilane in $CDCl_3$ and DMSO-d_6, or with sodium 3-(trimethylsilyl)-1-propanesulfonate in D_2O as an internal standard. HPLC analysis was carried out by a Hitachi 635 or 655 liquid chromatograph equipped with a UV recorder.

N,*N*-Dimethylformamide (DMF) and pyridine used as a reaction solvent were dried over Molecular Sieves (type 4A), and dichloromethane and chloroform were washed with water and dried over anhydrous calcium chloride.

4,4′-(1,2-Ethanediyl)bis(1-hydroxymethyl-2,6-piperazinedione) (VII) A mixture of 4,4′-(1,2-ethanediyl)bis(2,6-piperazinedione) (I) (2.0 g, 7.9 mmol) and DMF (10 ml) was heated at 130 °C for 10 min with stirring. Then 2 ml of 37% aqueous formaldehyde solution was gradually added and the mixture was successively heated at the same temperature for 1.5 h with stirring. The reaction mixture was cooled and left to stand overnight in a refrigerator. The resulting precipitates were collected, washed with ethyl acetate, and dried *in vacuo* to give 1.6 g (64.7%) of VII, which was recrystallized from dioxane as a colorless powder, mp 170—172 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.61 (4H,s), 3.49 (8H, s), 5.02 (4H, d, J=7.2 Hz), 6.20 (2H, t, J=7.2 Hz), IR (KBr) cm^{-1}: 1735, 1690, 1670.

Procedure for the Preparation of 4,4′-(1,2-Ethanediyl)bis(1-acyloxymethyl-2,6-piperazinedione) (VI) Method A: A mixture of the bis(hydroxymethyl) derivative VII, an excess amount of acid anhydride and pyridine was stirred for 12 h at room temperature. The mixture was concentrated *in vacuo* and the resultant residue was purified by recrystallization and/or column chromatography on silica gel to give VI.

Method B: Acid (138 mg, 3 mmol in case of formic acid) was added to 306 mg of acetic anhydride (3 mmol) at 0 °C. The mixed anhydride solution thus obtained was dropped into a solution of 314 mg of VII (1 mmol) in 10 ml of pyridine at −20 °C with stirring. After stirred for 2 h at −20 °C, the mixture was stored for 2 d in a refrigerator. The resultant colorless crystals were collected, washed with ethyl ether, and dried over phosphorus pentoxide *in vacuo*, giving VI (305 mg in the case of formate VI-1, 82%).

Method C: Two equivalents of acid chloride or chloroformate was gradually added dropwise to a solution VII in pyridine or a pyridine–dichloromethane mixture at 0 °C, and the mixture was stirred overnight at

TABLE IV. Physical Data for 4,4′-(1,2-Ethanediyl)bis(1-acyloxymethyl-2,6-piperazinediones) (VI)

Compound	Yield % (Method)	mp (°C) (Recryst. solv.)	Formula	Analysis (%) Calcd (Found) C	H	N	IR (C=O) (KBr, cm^{-1})	^{1}H-NMR Chemical shift (δ)	Solv.
VI-1	82 (B)	154—156 (EtOH–CH_2Cl_2)	$C_{14}H_{18}N_4O_8$	45.40 (45.43)	4.89 (4.95)	15.13 (15.04)	1690, 1740	2.70 (4H, s), 3.55 (8H, s), 5.87 (4H, s), 8.03 (2H, s)	$CDCl_3$
VI-2	71 (A)	178—181 (Dioxane)	$C_{16}H_{22}N_4O_8$	48.24 (48.02)	5.57 (5.48)	14.06 (13.88)	1700, 1740	2.01 (6H, s), 2.64 (4H, s), 3.58 (8H, s), 5.60 (4H, s)	$CDCl_3$
VI-3	62 (C)	72—75.5 (MeOH)	$C_{22}H_{34}N_4O_8$	54.76 (54.51)	7.10 (7.05)	11.61 (11.67)	1695, 1740	0.94 (12H, d, J=7 Hz), 2.05 (2H, m), 2.18 (4H, d, J=7 Hz), 2.72 (4H, s), 3.56 (8H, s), 5.75 (4H, s)	$CDCl_3$
VI-4	52 (C)	116—119 (AcOEt–*n*-Hex)	$C_{22}H_{34}N_4O_8$	54.76 (54.76)	7.10 (7.26)	11.61 (11.38)	1695, 1740	1.18 (18H, s), 2.70 (4H, s), 3.54 (8H, s), 5.76 (4H, s)	$CDCl_3$
VI-5	30 (C)	94—95 (EtOH)	$C_{44}H_{78}N_4O_8$	66.80 (66.93)	9.93 (10.06)	7.08 (7.10)	1690, 1740	0.80 (6H, t, J=7 Hz), 1.25 (48H, br s), 1.6 (4H, m), 2.30 (4H, t, J=7 Hz), 2.68 (4H, s), 3.53 (8H, s), 5.77 (4H, s)	$CDCl_3$
VI-6	85 (D)	135—137 (2-Methoxyethanol)	$C_{28}H_{30}N_4O_8$	61.08 (61.15)	5.49 (5.69)	10.18 (10.49)	1690, 1740	2.62 (4H, s), 3.51 (8H, s), 3.63 (4H, s), 5.81 (4H, s), 7.3 (10H, m)	$CDCl_3$
VI-7	89 (D)	170—173 (2-Methoxyethanol)	$C_{28}H_{28}Cl_2N_4O_8$	54.28 (54.16)	4.55 (4.62)	9.04 (8.98)	1700, 1740	2.66 (4H, s), 3.53 (8H, s), 3.78 (4H, s), 5.84 (4H, s), 7.2—7.3 (6H, m), 7.4 (2H, m)	$CDCl_3$
VI-8	68 (C)	153—155 (2-Methoxyethanol)	$C_{40}H_{38}N_4O_8$	68.36 (68.35)	5.45 (5.52)	7.96 (7.69)	1710, 1720, 1755	2.58 (4H, s), 3.48 (8H, s), 3.67 (4H, s), 5.82 (4H, s), 7.3—7.6 (18H, m)	$CDCl_3$
VI-9	45 (D)	223—224 (2-Methoxyethanol)	$C_{36}H_{34}N_4O_8 \cdot 1/2\,H_2O$	65.54 (65.60)	5.34 (5.15)	8.49 (8.39)	1700, 1740	2.44 (4H, s), 3.43 (8H, s), 4.08 (4H, s), 5.82 (4H, s), 7.4—7.6 (8H, m), 7.7—8.0 (6H, m)	$CDCl_3$
VI-10	37 (D)	143—146 (EtOH)	$C_{24}H_{26}N_4O_{10}$	54.33 (54.28)	4.94 (5.10)	10.56 (10.38)	1690, 1700, 1740, 1760	2.68 (4H, s), 3.54 (8H, s), 3.70 (4H, s), 5.83 (4H, s), 6.23 (2H, dd, J=0.6, 3.2 Hz), 6.32 (2H, dd, J=2.0, 3.2 Hz), 7.35 (2H, dd, J=0.6, 2.0 Hz)	$CDCl_3$
VI-11	39 (D)	154—156 (2-Methoxyethanol)	$C_{24}H_{26}N_4O_8S_2$	51.23 (51.35)	4.65 (4.74)	9.96 (9.70)	1705, 1750, 1760	2.66 (4H, s), 3.53 (8H, s), 3.85 (4H, s), 5.83 (4H, s), 6.9 (4H, m), 7.2 (2H, m)	$CDCl_3$
VI-12	74 (C)	117—120 (EtOH)	$C_{18}H_{26}N_4O_{10}$	47.16 (47.14)	5.71 (5.83)	12.22 (11.97)	1700, 1740	2.71 (4H, s), 3.43 (6H, s), 3.55 (8H, s), 4.03 (4H, s), 5.84 (4H, s)	$CDCl_3$
VI-13	50 (C)	180—183 (2-Methoxyethanol)	$C_{28}H_{28}Cl_2N_4O_{10}$	51.62 (51.45)	4.33 (4.46)	8.60 (8.78)	1700, 1760, 1780	2.66 (4H, s), 3.52 (8H, s), 4.62 (4H, s), 5.89 (4H, s), 6.82 (4H, d, J=8.9 Hz), 7.23 (4H, J=8.9 Hz)	$CDCl_3$
VI-14	43 (C)	153—155 (2-Methoxyethanol)	$C_{28}H_{26}Cl_4N_4O_{10}$	46.68 (46.41)	3.63 (3.94)	7.78 (7.75)	1690, 1750	2.65 (4H, s), 3.59 (8H, s), 4.95 (4H, s), 5.75 (4H, s), 7.07 (2H, d, J=8.9 Hz), 7.33 (2H, dd, J=2.6, 8.9 Hz), 7.59 (2H, d, J=2.6 Hz)	DMSO-d_6
VI-15	82 (C)	113—117 (EtOH)	$C_{32}H_{36}Cl_2N_4O_{10}$	54.32 (54.07)	5.13 (5.01)	7.92 (7.76)	1705, 1755	1.48 (12H, s), 2.60 (4H, s), 3.58 (8H, s), 5.76 (4H, s), 6.81 (4H, d, J=10.2 Hz), 7.29 (4H, d, J=10.2 Hz)	DMSO-d_6
VI-16	55 (C)	103—104 (2-Methoxyethanol)	$C_{28}H_{28}F_2N_4O_8S_2$	51.68 (51.76)	4.33 (4.38)	8.61 (8.64)	1690, 1740	2.66 (4H,s), 3.51 (8H, s), 3.53 (4H, s), 5.78 (4H, s), 7.00 (4H, t, J=9.0 Hz), 7.44 (4H, dd, J=5.1, 9.0 Hz)	$CDCl_3$
VI-17	67 (C)	139—141 (2-Methoxyethanol)	$C_{28}H_{28}Br_2N_4O_8S_2$	43.53 (43.66)	3.65 (3.82)	7.25 (7.21)	1695, 1735	2.65 (4H, s), 3.49 (8H, s), 3.59 (4H, s), 5.79 (4H, s), 7.28 (4H, d, J=8.9 Hz), 7.41 (4H, d, J=8.9 Hz)	$CDCl_3$
VI-18	64 (D)	161—164 (EtOH)	$C_{26}H_{40}N_6O_{12}$	49.67 (49.79)	6.41 (6.65)	13.37 (13.33)	1690, 1740	1.38 (18H, s), 2.65 (4H, s), 3.59 (8H, s), 3.65 (4H, d, J=5.9 Hz), 5.65 (4H, s), 7.23 (2H, t, J=5.9 Hz)	DMSO-d_6
VI-19	95[a]	68—72 (dec.) (MeOH–ethyl ether)	No data[b]				1670, 1700, 1760	2.68 (4H, s), 3.65 (8H, s), 3.85 (4H, br s), 5.77 (4H, s), 8.38 (6H, s)	DMSO-d_6
VI-20	24 (C)	188—189 (2-Methoxyethanol)	$C_{20}H_{28}N_6O_{12}$	44.11 (44.02)	5.18 (5.23)	15.43 (15.18)	1700, 1720, 1750	2.65 (4H, s), 3.55 (6H, s), 3.58 (8H, s), 3.73 (4H, d, J=6.2 Hz), 5.66 (4H, s), 7.56 (2H, t, J=6.2 Hz)	DMSO-d_6
VI-21	68 (D)	72—74 (MeOH)	$C_{38}H_{48}N_6O_{12}$	58.45 (58.39)	6.19 (6.32)	10.76 (10.45)	1700, 1710, 1760	1.42 (18H, s), 2.52 (4H, s), 3.43 (8H, s), 5.31 (2H, br d, J=7.0 Hz), 5.52 (2H, br d, J=7.0 Hz, 5.83 (4H, s), 7.31 (10H, m)	$CDCl_3$
VI-22	94[a]	102—105 (MeOH–ethyl ether)	$C_{32}H_{34}F_6N_6O_{12} \cdot H_2O$	46.49 (46.03)	4.39 (4.52)	10.16 (9.87)	1670, 1690, 1755	2.54 (4H, s), 3.47 (8H, s), 5.20 (2H, s), 5.83 and 5.96 (4H, AB, J=9.9 Hz), 7.5 (10H, m)	D_2O
VI-23	20 (D)	186—188 (EtOH)	$C_{22}H_{32}N_6O_{10}$	48.88 (48.77)	5.96 (6.06)	15.55 (15.18)	1650, 1700, 1740	1.20 (6H, d, J=7.3 Hz), 1.82 (6H, s), 2.65 (4H, s), 3.59 (8H, s), 4.2 (2H, m), 5.60 and 5.66 (4H, AB, J=9.8 Hz), 8.28 (2H, d, J=6.9 Hz)	DMSO-d_6

TABLE IV. (continued)

Compound	Yield % (Method)	mp (°C) (Recryst. solv.)	Formula	Analysis (%) Calcd (Found) C	H	N	IR (C=O) (KBr, cm^{-1})	^{1}H-NMR Chemical shift (δ)	Solv.
VI-24	64 (D)	42—45 (AcOEt–*n*-Hex)	$C_{24}H_{36}N_6O_{10}$	50.69 (50.37	6.38 6.52	14.78 14.71)	1655, 1700, 1740	1.34 (6H, d, J=7.3 Hz), 2.09 (6H, s), 2.71 (4H, s), 2.95 (6H, s), 3.56 (8H, s), 5.13 (2H, q, J=7.3 Hz), 5.76 and 5.81 (4H, AB, J=8.0 Hz)	$CDCl_3$
VI-25	37[a]	143—146 (EtOH)	$C_{20}H_{26}N_4O_{12}$	46.69 (46.60	5.09 5.21	10.89 10.60)	1690, 1710, 1740	2.45 (8H, m), 2.65 (4H, s), 3.59 (8H, s), 5.62 (4H, s)	DMSO-d_6
VI-26	—[a]	No data[b]					1690, 1740	2.10 (4H, t, J=7.2 Hz), 2.37 (4H, t, J=7.2 Hz), 2.64 (4H, s), 3.57 (8H, s), 5.59 (4H, s)	D_2O
VI-27	54 (C)	164—166 (2-Methoxyethanol)	$C_{26}H_{26}N_4O_8$	59.76 (59.81	5.01 5.06	10.72 10.69)	1710, 1730, 1750	2.71 (4H, s), 3.57 (8H, s), 6.03 (4H, s), 7.4 (4H, m), 7.55 (2H, m), 8.0 (4H, m)	$CDCl_3$
VI-28	67 (C)	156—157 (2-Methoxyethanol)	$C_{28}H_{30}N_4O_8$	61.08 (61.17	5.49 5.69	10.17 10.22)	1690, 1710, 1740	2.57 (6H, s), 2.72 (4H, s), 3.56 (8H, s), 6.00 (4H, s), 7.2 (4H, m), 7.4 (2H, m), 7.8 (2H, m)	$CDCl_3$
VI-29	64 (C)	156—158 (2-Methoxyethanol)	$C_{28}H_{30}N_4O_8 \cdot 1/2\,H_2O$	60.10 (60.34	5.58 5.44	10.01 10.03)	1700, 1710, 1740	2.38 (6H, s), 2.72 (4H, s), 3.58 (8H, s), 6.03 (4H, s), 7.3 (4H, m), 7.8 (4H, m)	$CDCl_3$
VI-30	92 (C)	146—149 (EtOH)	$C_{28}H_{30}N_4O_{10}$	57.72 (57.77	5.19 5.30	9.62 9.43)	1700, 1710, 1740	2.70 (4H, s), 3.55 (8H, s), 3.88 (6H, s), 5.99 (4H, s), 6.95 (4H, m), 7.45 (2H, m), 7.7 (2H, m)	$CDCl_3$
VI-31	61 (C)	198—199 (2-Methoxyethanol)	$C_{28}H_{26}N_4O_{12}$	55.08 (54.95	4.29 4.25	9.17 9.17)	1690, 1710, 1740	2.71 (4H, s), 3.56 (8H, s), 5.99 (4H, s), 6.03 (4H, s), 6.81 (2H, d, J=8.2 Hz), 7.40 (2H, d, J=1.7 Hz), 7.60 (2H, dd, J=1.7, 8.2 Hz)	$CDCl_3$
VI-32	75 (C)	159—160 (2-Methoxyethanol)	$C_{26}H_{24}F_2N_4O_8$	55.91 (55.75	4.33 4.35	10.03 10.00)	1695, 1740	2.73 (4H, s), 3.59 (8H, s), 6.04 (4H, s), 7.1—7.2 (4H, m), 7.5 (2H, m), 7.9 (2H, m)	$CDCl_3$
VI-33	50 (C)	164—166 (2-Methoxyethanol)	$C_{26}H_{24}Cl_2N_4O_8$	52.80 (52.55	4.09 4.18	9.47 9.22)	1695, 1720, 1740	2.72 (4H, s), 3.58 (8H, s), 6.03 (4H, s), 7.3 (2H, m), 7.4 (4H, m), 7.8 (2H, m)	$CDCl_3$
VI-34	47 (C)	151—152 (2-Methoxyethanol)	$C_{30}H_{30}N_4O_8$	62.70 (62.51	5.26 5.28	9.75 9.67)	1685, 1700, 1735	2.71 (4H, s), 3.57 (8H, s), 5.93 (4H, s), 6.40 (2H, d, J=16.0 Hz), 7.4 (6H, m), 7.5 (4H, m), 7.71 (2H, d, J=16.0 Hz)	$CDCl_3$
VI-35	54 (B)	206—209 (2-Methoxyethanol)	$C_{34}H_{38}N_4O_{12} \cdot 1/2\,H_2O$	58.03 (58.25	5.58 5.61	7.96 8.00)	1700, 1710, 1740	2.68 (4H, s), 3.61 (8H, s), 3.78 (12H, s), 5.75 (4H, s), 6.54 (2H, d, J=16 Hz), 6.95 (2H, d, J=8.6 Hz), 7.24 (2H, dd, J=2.0, 8.6 Hz), 7.35 (2H, d, J=2.0 Hz), 7.59 (2H, d, J=16 Hz)	DMSO-d_6
VI-36	50 (C)	196—198 (2-Methoxyethanol)	$C_{38}H_{34}N_4O_8$	67.64 (67.38	5.07 5.18	8.30 8.21)	1710, 1730, 1755	2.72 (4H, s), 3.59 (8H, s), 6.06 (4H, s), 7.3—7.5 (6H, m), 7.6 (8H, m), 8.05 (4H, d, J=8.6 Hz)	$CDCl_3$
VI-37	30 (C)	206—207 (2-Methoxyethanol)	$C_{34}H_{30}N_4O_8 \cdot 1/2\,H_2O$	64.65 (64.36	4.94 4.89	8.87 8.77)	1700, 1710, 1740	2.70 (4H, s), 3.57 (8H, s), 6.11 (4H, s), 7.4—7.6 (6H, m), 7.8 (2H, m), 8.0 (2H, m), 8.1 (2H, m), 8.9 (2H, m)	$CDCl_3$
VI-38	52 (C)	166—167 (2-Methoxyethanol)	$C_{22}H_{22}N_4O_{10} \cdot 1/2\,H_2O$	51.66 (51.79	4.53 4.41	10.95 10.89)	1695, 1710, 1740	2.71 (4H, s), 3.57 (8H, s), 6.00 (4H, s), 6.50 (2H, dd, J=1.6, 3.6 Hz), 7.20 (2H, dd, J=0.6, 3.6 Hz), 7.58 (2H, dd, J=0.6, 1.6 Hz)	$CDCl_3$
VI-39	72 (C)	181—182 (2-Methoxyethanol)	$C_{22}H_{22}N_4O_{10}$	52.59 (52.62	4.41 4.40	11.15 11.10)	1690, 1715, 1730	2.70 (4H, s), 3.56 (8H, s), 5.96 (4H, s), 6.72 (2H, dd, J=0.7, 2.0 Hz), 7.41 (2H, dd, J=1.6, 2.0 Hz), 8.01 (2H, dd, J=0.7, 1.6 Hz)	$CDCl_3$
VI-40	70 (C)	158—160 (2-Methoxyethanol)	$C_{22}H_{22}N_4O_8S_2$	49.42 (49.35	4.14 4.13	10.48 10.32)	1700, 1710, 1740	2.71 (4H, s), 3.57 (8H, s), 6.00 (4H, s), 7.08 (2H, dd, J=3.9, 4.9 Hz), 7.57 (2H, dd, J=1.3, 4.9 Hz), 7.79 (2H, dd, J=1.3, 3.9 Hz)	$CDCl_3$
VI-41	88 (C)	163—165 (EtOH)	$C_{16}H_{22}N_4O_{10}$	44.65 (44.55	5.15 5.21	13.02 12.96)	1705, 1760	2.67 (4H, s), 3.52 (8H, s), 3.80 (6H, s), 5.81 (4H, s)	$CDCl_3$
VI-42	86 (C)	106—108 (EtOH)	$C_{18}H_{26}N_4O_{10}$	47.15 (47.21	5.71 5.67	12.22 12.13)	1695, 1735	1.31 (6H, t, J=7.1 Hz), 2.67 (4H, s), 3.52 (8H, s), 4.22 (4H, q, J=7.1 Hz), 5.82 (4H, s)	$CDCl_3$
VI-43	69 (C)	87—88 (MeOH)	$C_{22}H_{34}N_4O_{10}$	51.35 (51.05	6.66 6.77	10.89 10.88)	1700, 1740	0.92 (6H, t, J=7.3 Hz), 1.4 (4H, m), 1.6 (4H, m), 2.67 (4H, s), 3.52 (8H, s), 4.16 (4H, t, J=6.8 Hz), 5.81 (4H, s)	$CDCl_3$
VI-44	88 (C)	132—133 (EtOH)	$C_{22}H_{34}N_4O_{10}$	51.35 (52.12	6.66 6.70	10.89 10.86)	1700, 1730, 1750	0.94 (12H, d, J=6.9 Hz), 2.0 (2H, m), 2.68 (4H, s), 3.53 (8H, s), 3.94 (4H, d, J=6.9 Hz), 5.81 (4H, s)	$CDCl_3$

TABLE IV. (continued)

Compound	Yield % (Method)	mp (°C) (Recryst. solv.)	Formula	Analysis (%) Calcd (Found) C	H	N	IR (C=O) (KBr, cm^{-1})	^{1}H-NMR Chemical shift (δ)	Solv.
VI-45	86 (C)	59—61 (MeOH)	$C_{30}H_{50}N_4O_{10}$	57.49 (57.32	8.04 8.18	8.94 9.12)	1700, 1740	0.9 (12H, m), 1.2—1.4 (16H, m), 1.6 (2H, m), 2.67 (4H, s), 3.53 (8H, s), 4.1 (4H, m), 5.81 (4H, s)	$CDCl_3$
VI-46	49 (C)	158—160 (2-Methoxyethanol)	$C_{28}H_{28}N_6O_{14}$	50.00 (50.18	4.19 4.19	12.49 12.31)	1700, 1750	2.68 (4H, s), 3.53 (8H, s), 5.27 (4H, s), 5.86 (4H, s), 7.54 (4H, d, J=8.6 Hz), 8.22 (4H, d, J=8.6 Hz)	$CDCl_3$
VI-47	60 (C)	140—141 (2-Methoxyethanol)	$C_{26}H_{26}N_4O_{10}\cdot 1/2H_2O$	55.41 (55.39	4.82 4.60	9.94 9.93)	1700, 1750	2.70 (4H, s), 3.56 (8H, s), 5.93 (4H, s), 7.2—7.5 (10H, m)	$CDCl_3$
VI-48	—[a]	210—212 (DMF–EtOH)	$C_{14}H_{20}N_6O_8$	41.99 (42.01	5.03 5.19	20.99 20.73)	1680, 1700, 1730, 1740	2.63 (4H, s), 3.55 (8H, s), 5.53 (4H, s), 6.7 (4H, br s)	DMSO-d_6
VI-49	58[a]	Glassy-oil	$C_{26}H_{28}N_6O_8$	56.51 (56.58	5.10 5.31	15.21 15.38)	1700, 1730, 1740	2.71 (4H, s), 3.55 (8H, s), 5.82 (4H, s), 7.05 (2H, m), 7.3 (4H, m), 7.4 (6H, m)	$CDCl_3$

a) Preparation and yields are described in the experimental section. *b*) The purification and/or elemental analysis had not been done when the compound was submitted to biological testing.

room temperature. The reaction mixture was concentrated *in vacuo*, then the residue was dissolved in chloroform, washed with hydrochloric acid and water and dried over anhydrous magnesium sulfate. The solvent was removed and the residue was purified by recrystallization or silica gel column chromatography to give VI.

Method D: Two equivalents of DCC was added in portions to a solution of VII, two equivalents of alkylcarboxylic acid and a catalytic amount of *N*,*N*-dimethylaminopyridine in dichloromethane at 0 °C. The mixture was stirred overnight at room temperature. The resultant colorless crystals were filtered off and the filtrate was diluted with chloroform, washed with 5% aqueous acetic acid and successively with water, and dried over anhydrous magnesium sulfate. The solvent was removed and the residue was purified by recrystallization or silica gel column chromatography to give VI.

Trifluoroacetates of 4,4′-(1,2-Ethanediyl)-bis(1-aminoacetoxymethyl-2,6-piperazinedione) (VI-19), and -bis[1-(α-aminophenylacetoxymethyl)-2,6-piperazinedione] (VI-22) Bis[(*N*-*tert*-butoxycarbonyl)glycyl] ester VI-18 was prepared from VII by method D and removal of the protecting group was carried out as follows.

Compound VI-18 (750 mg) was added in portions to stirred trifluoroacetic acid (15 ml) at 0 °C. After being stirred for 1 h at the same temperature, the solution was concentrated *in vacuo*. Trituration of the residue with ethyl ether gave a pale yellow powder which was collected, washed with ethyl ether, and dried over phosphorus pentoxide *in vacuo* to give 740 mg (95%) of VI-19.

The trifluoroacetate of bis(phenylglycyl) ester, VI-22, was prepared analogously.

Physical data for bis(acyloxymethyl) derivatives VI-19 and VI-22 thus obtained are shown in Table IV.

4,4′-(1,2-Ethanediyl)bis[1-(β-carboxypropionyloxymethyl)-2,6-piperazinedione] (VI-25) The bis(hydroxymethyl) derivative VII was acylated with succinic acid monobenzyl ester by method D. The crude bis(benzyl succinate) thus obtained was purified by recrystallization from ethyl acetate as a colorless powder, mp 119—121 °C (66%). ^{1}H-NMR ($CDCl_3$) δ: 2.66 (4H+4H, s), 3.51 (8H, s), 5.12 (4H, s), 5.79 (4H, s), 7.3 (10H, m). IR (KBr) cm^{-1}: 1740, 1720, 1700.

A mixture of bis(benzyl succinate) (2.0 g) obtained as above, 10% palladium charcoal (500 mg), ethanol (40 ml), and ethyl acetate (20 ml) was stirred overnight under hydrogen atmosphere. The catalyst was filtered off and washed sufficiently with methanol, ethanol, and acetone successively. The filtrate and the washings were combined, and concentrated *in vacuo* to yield 1.22 g (82%) of succinate VI-25, whose physical data are shown in Table IV.

Sodium Salt of Succinate VI-25 A 0.2 N sodium carbonate solution (16.3 ml; 0.9 eq) was dropped into a suspension of succinate VI-25 (930 mg) in 10 ml of water at 0 °C with stirring. The mixture was further stirred for 10 min at 0 °C, then the remaining precipitates were removed by filtration. The filtrate was freeze-dried to give 910 mg of the sodium salt VI-26 as a colorless powder, whose physical data are shown in Table IV.

4,4′-(1,2-Ethanediyl)bis(1-carbamoyloxymethyl-2,6-piperazinedione) (VI-48) Trichloroacetyl isocyanate (3.4 g) was added dropwise to a suspension of VII (2.0 g) in 80 ml of chloroform at 0 °C with stirring. The mixture was stirred for 5 h at room temperature, then the resultant colorless precipitates were collected, washed with ethyl ether, and dried over phosphorus pentoxide to give 4.35 g (99%) of 4,4′-(1,2-ethanediyl)bis[1-(*N*-trichloroacetylcarbamoyloxymethyl)-2,6-piperazinedione], mp 134—138 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 2.67 (4H, s), 3.60 (8H, s), 5.78 (4H, s). IR (KBr) cm^{-1}: 1805, 1740, 1700.

Sodium carbonate (20 mg) was added to a suspension of 1.0 g of the bis(trichloroacetylcarbamate) obtained above in a mixture of 30 ml of methanol and 3 ml of water at room temperature with stirring. The mixture was stirred for 5 h at room temperature, and the resultant colorless needles were collected by filtration, washed with ethyl acetate and water, and dried over phosphorus pentoxide *in vacuo* to give 374 mg (65%) of VI-48, whose physical data are shown in Table IV.

4,4′-(1,2-Ethanediyl)bis[1-(*N*-phenylcarbamoyloxymethyl)-2,6-piperazinedione] (VI-49) Phenyl isocyanate (455 mg) was added to a solution of VII (500 mg) in 2 ml of DMF. After addition of a few drops of triethylamine, the mixture was stirred for 4 h at room temperature. The solvent was removed *in vacuo* and the residue was charged on a silica gel column eluted with chloroform–methanol (9:1, v/v), giving 510 mg (58%) of VI-49 as a colorless glassy oil. Physical data of VI-49 are indicated in Table IV.

Stability of 4,4′-(1,2-Ethanediyl)-bis(1-acetyloxymethyl-2,6-piperazinedione) (VI-2) and -bis(1-methoxycarbonyloxymethyl-2,6-piperazinedione) (VI-41) in Water About 20 mg of each sample was added to 20 ml of water, and the mixture was ultrasonicated for 10 min and filtered through a membrane filter (0.45 μm). Fractions of 1 ml of the filtrate was collected. After addition of 3 ml of acetonitrile, the mixture was diluted with the HPLC eluant, having the components listed below, to 20 ml and an aliquot was injected into the HPLC column.

Phosphate buffer solutions (pH 5.6, 7.0, and 8.0) and a 0.1 N HCl solution of VI-2 were also prepared and their stability was similarly examined.

HPLC conditions: column, LiChrosorb RP-18 (4×200 mm); eluant, water–acetonitrile (80:20, v/v for VI-2, 70:30, v/v for VI-41); detector, UV at 210 nm; sensitivity, 0.16 a.u.f.s.; flow rate, 1.0 ml/min; temperature, room temperature; injection volume, 100 μl.

Antitumor Activity Test The activities of 4,4′-(1,2-ethanediyl)bis(1-acyloxymethyl-2,6-piperazinediones) (VI-1—VI-49) against P388 leukemia were examined as follows.

Animals: Groups of seven CDF_1 male mice, aged 6 weeks were used. The mice were maintained on a laminar air-flow shelf and were fed on ordinary feed pellets (Clea Japan Inc., Tokyo Japan) and tap water *ad libitum*.

Evaluation of Antitumor Activity: P388 tumor cells (1×10^6 cells/0.1 ml) were intraperitoneally (i.p.) inoculated into the mice on day 0. A suitable amount of drug was suspended in 0.5% aqueous carboxymethylcellulose solution and administered i.p. twice on days 1 and 5. The *T*/*C* value (%) is defined as

$$\frac{\text{median survival time of treated group}}{\text{median survival time of control}}\times 100$$

These results are shown in Table I.

Relative Rates of Hydrolysis of 4,4′-(1,2-Ethanediyl)bis(1-acyloxymethyl-2,6-piperazinediones) in the Presence of Porcine Liver Esterase or Rat Small Intestine Esterase Sample Solution: Each sample was weighed in a 50 ml flask and dissolved in 0.25 ml of dimethyl sulfoxide. After addition of 0.25 ml of Tween 80 (supplied by Kao Chemical Co., Ltd.,) as a surfactant, the mixture in the flask was mixed sufficiently, then diluted with water to 50 ml.

Porcine Liver Esterase Solution: A half ml of a dispersion of porcine liver esterase (100 U/mg protein), purchased from Sigma Chemical Co., Ltd., in 3.2 M ammonium sulfate was dissolved in a Tris–HCl buffer solution (0.01 M, pH 8.0), and the mixture was desalted by using a Centricon (Amicon Co., Ltd.).

Rat Small Intestine Esterase Solution: The small intestine (21.4 g, wet weight) obtained from two rats was washed with saline and cut to pieces. The pieces of the small intestine were homogenized in 25 ml of a Tris–HCl buffer solution (pH 8.0) by using a Waring blender. Centrifugation of the resulting homogenate at 10000 rpm gave 16 ml of a rat small intestinal esterase solution as the supernatant.

Hydrolysis by Esterase: Esterase solution (0.01 ml) after preincubation at 30 °C was added to a mixture of 1 ml of a sample solution described above and 0.99 ml of Tris–HCl buffer (0.01 M). The mixture was incubated for 10 min at the same temperature. An aliquot (100 μl) of the mixture was collected and injected into the HPLC column.

HPLC conditions: column, YMC-Pack AM312 ODS (6 × 150 mm); pre-column, LiChroprep RP-18 (25—40 μm, 4 × 40); eluant, H_2O–methanol (98 : 2, v/v); flow rate, 1.5 ml/min; detector, UV at 210 nm; sensitivity, 0.005—0.02 a.u.f.s.; temperature, room temperature.

References and Notes

1) Part of this work was presented at the 14th International Congress of Chemotherapy in Kyoto, June 1985, and the 106th Meeting of the Pharmaceutical Society of Japan in Chiba, April 1986.
2) A. M. Creighton, K. Hellmann, S. Whitecross, *Nature* (London), **222**, 384 (1969); A. M. Creighton, U. S. Patent 4275063 [*Chem. Abstr.*, **95**, 192378*k* (1981)]; Z. X. Huang, P. M. May, K. M. Quinlan, D. R. Williams, and A. M. Creighton, *Agents and Actions*, **12**, 536 (1982).
3) E. H. Herman, D. T. Witiak, K. Hellmann, and V. S. Waravdekar, *Adv. Pharmacol. Chemother.*, **19**, 249 (1982).
4) E. H. Herman, R. M. Mhatre, and D. P. Chadwick, *Toxicol. Appl. Pharmacol.*, **27**, 517 (1974); E. H. Herman, B. Ardalan, C. Bier, V. Waravdekar, and S. Krop, *Cancer Treat. Rep.*, **63**, 89 (1979); G. Wang, M. D. Finch, D. Trevan, and K. Hellmann, *Br. J. Cancer*, **43**, 871 (1981); E. H. Herman, V. J. Ferrans, R. S. K. Young, and R. L. Hamlin, *Cancer Res.*, **48**, 6918 (1988).
5) D. T. Witiak, H. J. Lee, R. W. Hart, and R. E. Gibson, *J. Med. Chem.*, **20**, 630 (1977); D. T. Witiak, H. J. Lee, H. D. Goldman, and B. S. Zwilling, *ibid.*, **21**, 1194 (1978); D. T. Witiak, B. K. Trivedi, L. B. Campolito, B. S. Zwilling, and N. A. Reiches, *ibid.*, **24**, 1329 (1981); D. T. Witiak, B. K. Trivedi, and F. A. Schmid, *ibid.*, **28**, 1111 (1985); D. T. Witiak, R. V. Nair, and F. A. Schmid, *ibid.*, **28**, 1228 (1985).
6) Y. F. Ren, H. L. Shu, T. M. Zhang, Z. Y. Chen, and C. Lin, *Kexue Tongbao*, **25**, 189 (1980).
7) Y. F. Ren, H. L. Shu, J. C. Cai, B. Xu, T. M. Zhang, T. Narita, N. Kiriki, and T. Komatsu, Abstracts of Papers, 14th International Congress of Chemotherapy, Kyoto, 1985, p. 18; T. M. Zhang, M. Y. Wang, Q. D. Wang, and Y. F. Ren, *Acta Pharmacologica Sinica*, **8**, 369 (1987); also see L. Cekuoliene, *Liet. TSR Mokslu Akad. Darb., Ser. B*, **1986**, 41 [*Chem. Abstr.*, **107**, 198247*e* (1987)].
8) *a*) A. B. A. Jansen and T. J. Russell, *J. Chem. Soc.*, **1965**, 2127; *b*) W. von Daehne, W. O. Godtfredsen, K. Roholt, and L. Tybring, *Antimicrob. Agents Chemother.*, **1970**, 431; N. O. Bodin, B. Ekstron, U. Forsgren, L. P. Jalar, L. Magni, C. H. Ramsay, and B. Sjoberg; *ibid*, **8**, 518 (1975); *c*) W. von Daehne, E. Frederiksen, E. Gundersen, F. Lund, P. Morch, H. J. Petersen, K. Roholt, L. Tybring, and W. O. Godtfredsen, *J. Med. Chem.*, **13**, 607 (1970).
9) E. Binderup, W. O. Godtfredsen, and L. Roholt, *J. Antibiot.*, **24**, 767 (1971); W. J. Wheeler, W. E. Wright, V. D. Line, and J. A. Frogge, *J. Med. Chem.*, **20**, 1159 (1977).
10) A. J. Rapta, M. J. Baltezor, and P. C. Bansal, *J. Pharm. Sci.*, **65**, 238 (1976); A. Hempel, N. Camerman, and A. Camerman, *J. Am. Chem. Soc.*, **104**, 3453 (1982).
11) D. H. R. Barton and W. D. Ollis, "Comprehensive Organic Chemistry: The Synthesis and Reactions of Organic Compounds," Vol. 2, ed. by I. O. Sutherland, Pergamon Press, Oxford, p. 634.