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Studies on Antitumor-active 2,3-Dioxopiperazine Derivatives. III.¹⁾ Synthesis and Structure-Antitumor Activity Relationship of 1-(4-Aminobenzyl)-2,3-dioxopiperazine Derivatives

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The present investigation was undertaken to find more effective antitumor agents than 1-(4-diethylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (**1**), which was found in our laboratory in previous studies.

1-(4-Diethylamino-3- or 2-substituted benzyl)-2,3-dioxopiperazine derivatives and 1-(4-substituted aminobenzyl)-2,3-dioxopiperazine derivatives were designed and synthesized with the aim of suppressing the metabolism of the Et₂N- group of **1**. The structure-activity relationships and metabolism of these compounds were studied.

It was found that 1-benzyl-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (**17c**) possessed the highest *in vitro* and *in vivo* activities.

Keywords—new type of antitumor agent; 1-(4-aminobenzyl)-2,3-dioxopiperazine derivatives; structure-activity relationship; Ehrlich ascites carcinoma; L1210; HeLa S3 cell; metabolism; 1-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine derivatives

Antitumor agents containing a dioxopiperazine ring (for example ICRF 159²⁾ among 2,6-dioxopiperazine derivatives and 593A(NSC-135768)³⁾ or albonoursin⁴⁾ among 2,5-dioxopiperazine derivatives) have been reported, but our work is the first to describe antitumor agents having a 2,3-dioxopiperazine moiety.

In our preceding paper¹⁾ dealing with a new type of synthetic antitumor agent having the 2,3-dioxopiperazine moiety, we reported that 1-(4-diethylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (**1**) possessed antitumor activity but was easily metabolized into the inactive AcNH compound (**11j**). We also suggested that a more effective antitumor agent might be obtained by suppressing the metabolism of the Et₂N- group of **1**. In the present investigation, it was found that compounds of the type **2**, which carry a heterocyclic amino group instead of the Et₂N- group at the 4-position of the benzene ring, possessed high antitumor activity, and 1-benzyl-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (**17c**) in particular possessed excellent activity. This paper describes the development of the compounds **2** (Chart 1).

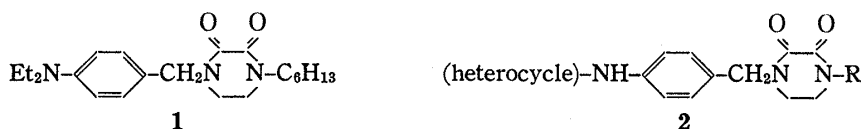
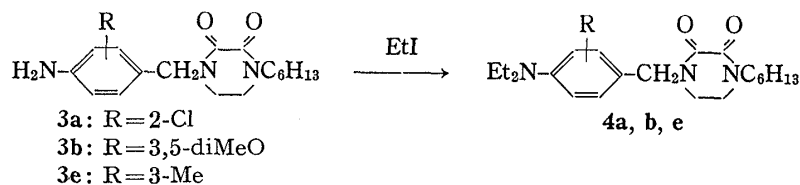


Chart 1

This work was undertaken in order to develop a more potent antitumor agent than **1** by suppressing the metabolism of the Et₂N-group of **1**. One approach was to introduce a substituent on the benzene ring in an attempt to prevent enzymatic attack on the Et₂N- group of **1** by introducing steric hindrance. The other was to exchange the Et₂N- group for some other substituted amino group which is less easily metabolized.

method A



method B

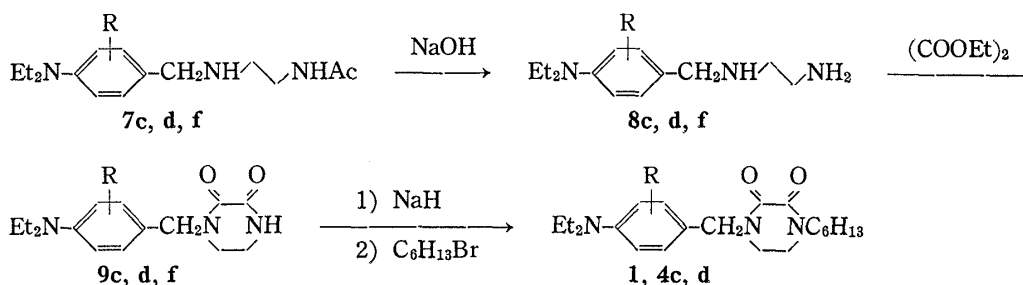
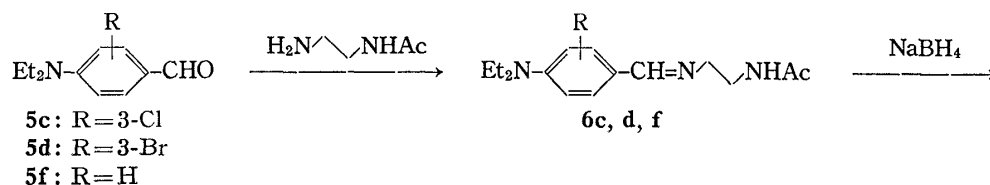
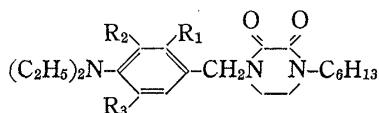


Chart 2

TABLE I. Structure-Antitumor Activity Relationship of 1-(4-Diethylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine Derivatives

Compd. No.	R ₁	R ₂	R ₃	<i>In vitro</i> MIC ^{a)} (μg/ml) HeLa S3	<i>In vivo</i>		
					LD ₅₀ ^{b)} (mg/kg)	EAC(<i>i.p.</i> - <i>i.p.</i>) ^{c)} Dose (mg/kg)	T/C (%)
1	H	H	H	3.13	100	40 × 7	157
4a	Cl	H	H	3.13	100	100 × 1	152.1
4b	H	CH ₃ O	CH ₃ O	50	—	—	—
4c	H	Cl	H	12.5	100	40 × 7 100 × 1	>170 (1/4) ^{d)} 165
4d	H	Br	H	6.25	100	50 × 7	147.1
4e	H	CH ₃	H	12.5	100	100 × 1	184

a) Microplate method

Incubation medium: Eagle's MEM containing 10% calf serum.

Inoculum size: 2 × 10⁴ cells/ml.

Incubation period: 4 days.

Determination: Giemsa staining.

b) Animal: SLC-ICR (♀), 6 weeks old, 2 mice/group.

Treatment: *i.p.*

Observation period: 1 week.

c) Animal: SLC-ICR (♀), 6 weeks old, 4 or 5 mice/group.

Inoculum size: EAC 1 × 10⁶ cells/mouse, *i.p.*Treatment: from Day 1, *i.p.*

$$T/C(\%) = \frac{\text{mean survival time (days) of treated}}{\text{mean survival time (days) of control}} \times 100$$

d) 43-day survivor.

First, **4a–e**, in which a Cl, Br, CH₃, or OCH₃ group is present on the benzene ring of **1**, were studied. These compounds were synthesized by diethylation of the 4-NH₂ group on the benzene ring (method A) or by starting from 4-diethylaminobenzaldehyde derivatives (method B) as shown in Chart 2.

Table I shows the cytotoxicities of **4a–e** against HeLa S3 cells and the antitumor activities against Ehrlich ascites carcinoma (EAC) (*i.p.–i.p.*). The compounds **4c**, **e** which carry Cl or CH₃ adjacent to the 4-Et₂N- group on the benzene ring of **1**, showed *in vitro* activities slightly inferior to that of **1**, but their *in vivo* activities were rather better than that of **1**. However, the improvement of the *in vivo* activities was not substantial. Furthermore, **4a** which has Cl at the 2-position on the benzene ring of **1**, showed nearly the same *in vitro* and *in vivo* activities as **1** and no substituent effect was observed. The 2,5-dimethoxy compound **4b** showed very low *in vitro* cytotoxicity.

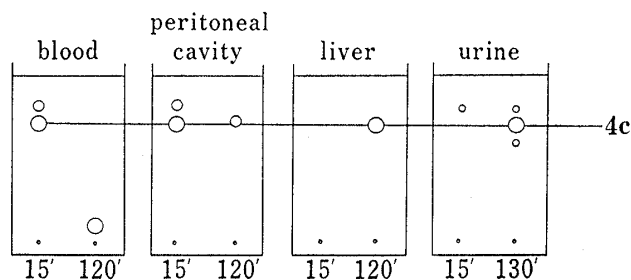


Fig. 1. Metabolism of **4c** in Mice
TLC (silica gel, CHCl₃: EtOH=15: 1).

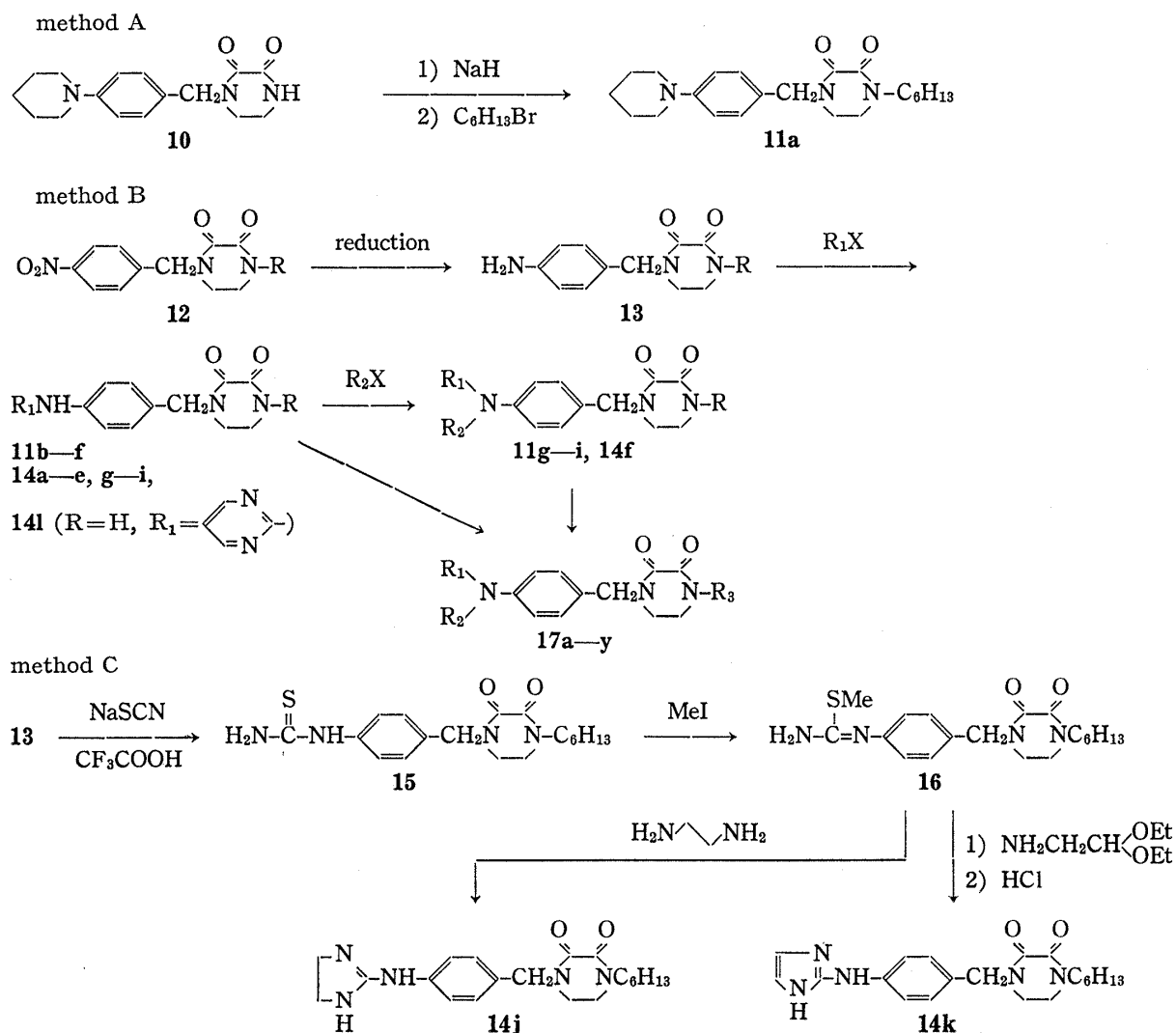


Chart 3

On the other hand, it was found that the metabolism of **4c** was suppressed compared with that of **1** (Fig. 1). At 2 hr after intraperitoneal administration of **4c**, **4c** was observed in the liver and urine. This suppression of the metabolism of **4c** may have led to the improved *in vivo* activity.

Next, we introduced other substituents, such as branched alkylamino groups, cyclic amino groups, or acyl amino groups, in place of the Et₂N- group on the benzene ring of **1**. Compounds

TABLE II. Structure-Antitumor Activity Relationship of 1-(4-Aminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine Derivatives

$$\begin{array}{c} \text{R}_1 \diagup \text{N} \text{---} \text{C}_6\text{H}_4 \text{---} \text{CH}_2 \text{---} \text{N} \begin{array}{c} \text{O} \diagup \text{C} \diagdown \text{O} \\ \text{O} \diagdown \text{C} \diagup \text{O} \end{array} \text{---} \text{N} \text{---} \text{C}_6\text{H}_{13} \\ \text{R}_2 \diagdown \end{array}$$

Compd. No.	R ₁ \ N- R ₂ \	<i>In vitro</i> MIC (μg/ml) ^{a)} HeLa S3	<i>In vivo</i>		
			LD ₅₀ ^{b)} (mg/kg)	EAC (<i>i.p.</i> - <i>i.p.</i>) ^{c)} Dose (mg/kg)	T/C (%)
1	(C ₂ H ₅) ₂ N-	3.13	100	40 × 7	157
11a	 N-	6.25	150	40 × 7	138
11b	 CHNH-	0.78—1.56	100	40 × 7	130
11c	HOOCCH ₂ NH-	100	—	—	—
11d	ClCH ₂ CONH-	0.39—0.78	>400	100 × 7 200 × 7	>142 (1/4) ^{d)} 156.8
11e	FCH ₂ CONH-	25	—	—	—
11f	ClCH ₂ CH ₂ CONH-	6.25	>600	50 × 7 100 × 7	84.1 95.2
11g	ClCH ₂ CONH- C ₂ H ₅	1.56	300	50 × 7 100 × 7	121.5 135.1
11h	(CH ₃ CO) ₂ N-	12.5	>500	100 × 7 200 × 7	91 160
11i	 N-	100	—	—	—

a), b), c) See the legend to Table I. d) 36-day survivor.

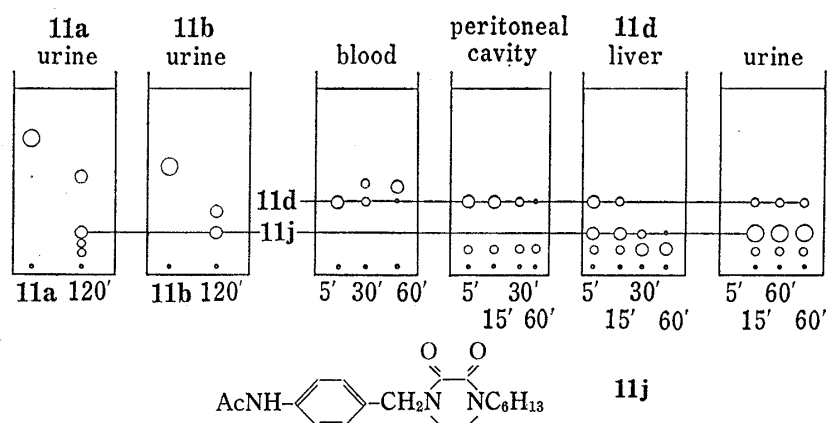


Fig. 2. Metabolism of **11a**, **11b**, and **11d** in Mice

TLC (silica gel, CHCl₃: EtOH=15: 1).

11a—i were synthesized by method A or method B as shown in Chart 3. Table II shows the structure-antitumor activity relationship. These results showed that **11b, d, g** possessed higher *in vitro* cytotoxicity against HeLa S3 cells than **1**, but their *in vivo* antitumor activities against EAC (*i.p.-i.p.*) were not superior to that of **1**.

The metabolism of **11a, b, d** in mice was qualitatively studied by thin layer chromatography and it was found that they were readily metabolized to the 4-AcNH compound (**11j**) in the same way as **1**. Again, the low *in vivo* activities appear to correlate with the metabolism (Fig. 2). Since heterocyclic amino groups are less easily metabolized than the above

TABLE III. Structure-Antitumor Activity Relationship of 1-(4-Aminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine Derivatives

Compd. No.		<i>In vitro</i> MIC (μg/ml) ^{a)} HeLa S3	LD ₅₀ ^{b)} (mg/kg)	<i>In vivo</i>			
				EAC (<i>i.p.-i.p.</i>) ^{c)}		L1210 (<i>i.p.-i.p.</i>) ^{d)}	
				Dose (mg/kg)	T/C (%)	Dose (mg/kg)	T/C (%)
14a		0.2—0.39	>500	100×7 200×7	123 >221.4 (2/4) ^{e)}	—	—
14b		100	—	—	—	—	—
14c		3.13	100	20×7	93.3	—	—
14d		6.25	200	50×7 100×7	145.6 >186.7 (1/4) ^{f)}	100×7	106.7
14e		0.39—0.78	>1000	50×7 100×7	>186.7 (1/4) ^{f)} >206.7 (1/4) ^{f)}	50×9 100×7 200×3	181.0 168.7 159.5
14f		50	—	—	—	—	—
14g		1.56	—	—	—	—	—
14h		1.56	—	—	—	—	—
14i		0.78—1.56	>500	100×7 200×7	96.6 103.3	—	—
14j		100	—	—	—	—	—
14k		50	—	—	—	—	—

a), b), c) See the legend to Table I.

d) Animal: BDF₁ (♂) mice, 6 weeks old, 4 or 5 mice/group.

Inoculum size: L12101 × 10⁵ cells/mouse, *i.p.*

Treatment: days 1—7, days 1—9 or days 1,5,9, *i.p.*

T/C(%) = $\frac{\text{mean survival time (days) of treated}}{\text{mean survival time (days) of control}} \times 100$

e) 34-day survivor.

f) 44-day survivor.

TABLE IV. Structure-Antitumor Activity Relationship of 1-[4-(2-Pyrimidinyl-amino)benzyl]-2, 3-dioxopiperazine Derivatives

Compd. No.	R	<i>In vitro</i> MIC ($\mu\text{g/ml}$) ^{a)} HeLa S3	<i>In vivo</i>				
			LD ₅₀ ^{b)} (mg/kg)	EAC (<i>i.p.-i.p.</i>) ^{c)}		L1210 (<i>i.p.-i.p.</i>) ^{d)}	
				Dose (mg/kg)	T/C (%)	Dose (mg/kg)	T/C (%)
14e	C ₆ H ₁₃	0.39—0.78	>1000	{ 50×7 100×7	{ >186.7 (1/4) ^{e)} >206.7 (1/4) ^{e)}	{ 50×9 100×7 200×3	{ 181.0 168.7 159.5
17a	—CH ₂ —	1.56	>500	100×7	117.2	—	—
17b	—	0.78	200—500	100×7	124.1	—	—
17c	—CH ₂ —	0.1	>1000	{ 20×7 50×4 100×7	{ 217.9 >226.9 (2/5) ^{f)} >176.7 (1/4) ^{f)}	{ 20×7 50×7 100×6 200×3	{ 181.4 >262 (1/5) ^{g)} 150.0 229.0
17d	—(CH ₂) ₂ —	0.39	>500	100×7	>178.7 (1/4) ^{f)}	100×6	108.8
17e	—(CH ₂) ₃ —	1.56—3.13	>500	100×7	106.9	—	—
17f	—CH— CH ₃	1.33	>500	100×7	108.1	—	—

a), b), c) See the legend to Table I.

d) See the legend to Table III.

e) 44-day survivor.

f) 35-day survivor.

g) 30-day survivor.

TABLE V. Structure-Antitumor Activity Relationship of 1-Benzyl-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine Derivatives

Compd. No.	R ₁	R ₂	<i>In vitro</i> MIC ($\mu\text{g/ml}$) ^{a)} HeLa S3	<i>In vivo</i>		
				LD ₅₀ ^{b)} (mg/kg)	EAC (<i>i.p.-i.p.</i>) ^{c)}	
					Dose (mg/kg)	T/C (%)
14e	H	H	0.1	>1000	100×7	>176.7 (1/4) ^{d)}
17g	NH ₂	H	3.13	>500	100×7	124.3
17h	N(C ₂ H ₅) ₂	H	12.5	—	—	—
17i	NO ₂	H	3.13	>500	100×7	118.9
17j	Cl	H	1.56	200—500	100×7	137.9
17k	COOH	H	>100	—	—	—
17l	COOCH ₃	H	6.25	>500	100×7	98.7
17m	CH ₃ O	CH ₃ O	3.13	>500	100×7	95.9
17n	—NH—	H	6.25	>500	100×7	159

a), b), c) See the legend to Table I.

d) 32-day survivor.

substituents of this series, we next designed compounds of the type 2. First, as shown in Chart 3, **14a**—**i** were synthesized by method B, in which a heterocyclic group was directly introduced at the 4-NH₂ group on the benzene ring, and **14j**, **k** were prepared by method C, in which the heterocyclic groups were synthesized by cyclization in the last step. Structure-activity relationships of **14a**—**k** are summarized in Table III. The following findings were obtained. 1) Compounds **14a**, **e**, which have a pyridine or pyrimidine ring, show very high *in vitro* cytotoxicity against HeLa S3 cells (MIC: <1 µg/ml) and high *in vivo* activity against EAC (*i.p.*—*i.p.*). 2) The NH moiety of an amino group carrying a heterocyclic group is essential for activity. 3) The heterocyclic ring should be linked to the 4-NH₂ group on the benzene ring at the 2-position of the heterocyclic ring. 4) These compounds show lower toxicity than 1.

The antitumor activities against L1210 of **14d** and **14e**, which showed high activity against EAC at 100 mg/kg/day dosage, were tested. Compound **14d** did not show any antitumor activity against L1210 but **14e** showed antitumor activity (*T/C*% of **14e**: 181% at 50 mg/kg/day dosage for 9 successive days). This antitumor activity supported the view that the metabolism of **14e** was strongly suppressed.

TABLE VI. Structure-Antitumor Activity Relationship of 1-[4-(2-Pyrimidinyl-amino)benzyl]-2,3-dioxopiperazine Derivatives

Compd. No.	R	<i>In vitro</i> MIC (µg/ml) ^{a)} HeLa S3	<i>In vivo</i>		
			LD ₅₀ ^{b)} (mg/kg)	EAC (<i>i.p.</i> — <i>i.p.</i>) ^{c)}	
				Dose (mg/kg)	<i>T/C</i> (%)
17o		6.25	>500	100×7	93.3
17p		>100	—	—	—
17q		3.13	200—500	100×7	92.2
17r		1.56—3.13	>400	100×7	96.7
17s		25	—	—	—
17t		>100	—	—	—
17u		3.13	>500	100×7	79.2
17v		0.78	>500	100×7	117.9
17w		100	—	—	—
17x		50	—	—	—
17y		>100	—	—	—

a), b), c): See the legend to Table I.

Next, other lipophilic groups, in place of $n\text{-C}_6\text{H}_{13}$, were investigated to find compounds more effective than **14e**. Compounds **17a–f** were synthesized by method B, as shown in Chart 3, and their structure–activity relationships were examined (Table IV). It was found that **17c** in which the lipophilic group was benzyl possessed significant cytotoxicity against HeLa S3 cells (MIC: 0.1 $\mu\text{g}/\text{ml}$), and the relationship between the substituent at the 4-position on the 2,3-dioxopiperazine ring and the cytotoxicity against HeLa S3 cells decreased in the following order: $-\text{CH}_2-\text{C}_6\text{H}_5 > -(\text{CH}_2)_2-\text{C}_6\text{H}_5 \cong n\text{-C}_6\text{H}_{13} \cong -\text{C}_6\text{H}_5 > -\text{CH}_2-\text{C}_6\text{H}_4 \cong -(\text{CH}_2)_3-\text{C}_6\text{H}_5 \cong -\text{CH}(\text{CH}_3)-\text{C}_6\text{H}_5$. It is of interest that **14e** (R: $n\text{-C}_6\text{H}_{13}$), **17c** (R: $-\text{CH}_2-\text{C}_6\text{H}_5$),

and **17d** (R: $-(\text{CH}_2)_2-\text{C}_6\text{H}_5$) showed high antitumor activity against EAC (*i.p.*–*i.p.*) while **17b** (R: $-\text{C}_6\text{H}_5$), **17e** (R: $-(\text{CH}_2)_3-\text{C}_6\text{H}_5$), and **17f** (R: $-\text{CH}(\text{CH}_3)-\text{C}_6\text{H}_5$) showed no antitumor activity

at all. The antitumor activities against L1210 (*i.p.*–*i.p.*) of the above three compounds having *in vivo* antitumor activity against EAC were examined. It was found that the order of antitumor activities against L1210 was **17c** > **14e** > **17d**; it is noteworthy that **17c** showed excellent antitumor activity at low dosage ($T/C\%$: 181.4% at 20 mg/kg/day dosage for 7 successive days and >262% at 50 mg/kg/days for 7 successive days), but **17d** showed no antitumor activity against L1210 (*i.p.*–*i.p.*). Further, **17c** showed low toxicity (LD_{50} value of **17c** was over 1000 mg/kg). Compound **17c** was not as easily metabolized in mice and rats as **1**, and this may be related to the antitumor activity.

Next, introduction of substituents on the benzyl group of **17c** was studied. Compounds **17g–n** were synthesized by method B as shown in Chart 3. These structure–antitumor activity relationships are summarized in Table V. It was found that the *in vitro* and *in vivo* activities of these compounds were inferior to those of **17c**.

Further, **17o–y**, which had heterocyclic groups instead of the benzyl group of **17c**, were designed and synthesized by method B, as shown in Chart 3, and their antitumor activities were tested. No compounds having antitumor activity were found (Table VI). From the results of these investigations, it is suggested that the benzyl group plays a significant role as a lipophilic functional group. Among these synthetic compounds, **17c** was found to be the most potent antitumor agent.

Further studies on this series of compounds are in progress.

Experimental⁵⁾

1-(3-Chloro-4-diethylamino)benzyl-4-*n*-hexyl-2,3-dioxopiperazine (4c)—Compound **4c** was obtained by hexylation of **9c**¹⁾ as shown in Chart 2. mp 62–63° (AcOEt–iso-Pr₂O). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1660 (C=O). NMR (CDCl_3) δ : 0.86 (3H, m, CH_3), 1.04 (6H, t, $J=6.5$ Hz, $2 \times \text{CH}_3$), 1.00–1.85 (8H, m, $4 \times \text{CH}_2$), 3.00–3.60 (10H, m, $5 \times \text{CH}_2$), 4.54 (2H, s, CH_2), 7.00–7.18 (3H, m, benzene ring $3 \times \text{CH}$). Anal. Calcd for $\text{C}_{21}\text{H}_{32}\text{ClN}_3\text{O}_2$: C, 64.03; H, 8.19; N, 10.67. Found: C, 64.21; H, 8.41; N, 10.87. The following compound was similarly obtained. (**4d**): mp 176° (iso-PrOH–iso-Pr₂O). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1660 (C=O). NMR (CDCl_3) δ : 0.92 (3H, m, CH_3), 1.18 (6H, t, $J=7.5$ Hz, $2 \times \text{CH}_3$), 1.00–1.80 (8H, m, $4 \times \text{CH}_2$), 3.14 (4H, q, $J=7.5$ Hz, $2 \times \text{CH}_2$), 3.30–3.75 (6H, m, $3 \times \text{CH}_2$), 4.61 (2H, s, CH_2), 6.60 (1H, d, $J=7$ Hz, benzene ring CH), 7.06–7.28 (2H, m, benzene ring $2 \times \text{CH}$). Anal. Calcd for $\text{C}_{21}\text{H}_{32}\text{BrN}_3\text{O}_2 \cdot \text{HCl}$: C, 53.12; H, 7.00; N, 8.85. Found: C, 53.43; H, 7.21; N, 9.02.

1-(4-Diethylamino-3-methyl)benzyl-4-*n*-hexyl-2,3-dioxopiperazine (4e)—A mixture of 1-(4-amino-3-methyl)benzyl-4-*n*-hexyl-2,3-dioxopiperazine¹⁾ (1.5 g), K_2CO_3 (1.4 g), EtI (3.0 g) and dimethylformamide (DMF) (2.0 ml) was heated at 90° for 10 min and was then evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel with CHCl_3 to afford **4e** (1.28 g, 72.2%) as an oil. IR $\nu_{\text{max}}^{\text{NaCl}}$ cm^{-1} : 1665 (C=O). NMR (CDCl_3) δ : 0.87 (3H, m, CH_3), 0.96 (6H, t, $J=6.5$ Hz, $2 \times \text{CH}_3$), 1.00–1.80 (8H, m, $4 \times \text{CH}_2$), 2.24 (3H, s, CH_3), 2.96 (4H, q, $J=6.5$ Hz, $2 \times \text{CH}_2\text{CH}_2$), 3.25–3.65 (6H, m, piperazine ring 5 and 6 CH_2 , and $-\text{NCH}_2-$), 4.57 (2H, s, CH_2), 7.00–7.20 (3H, m, benzene ring $3 \times \text{CH}$). Anal. Calcd for $\text{C}_{22}\text{H}_{35}\text{N}_3\text{O}_2$: C, 68.12; H, 8.85; N, 10.67. Found: C, 68.12; H, 8.85; N, 10.67.

HCl: C, 64.45; H, 8.85; N, 10.25. Found: C, 64.72; H, 8.94; N, 9.98.

The following compounds were similarly obtained. (4a): mp 92–93° (AcOEt–iso-Pr₂O). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1675 (C=O). NMR (CDCl₃) δ : 0.88 (3H, m, CH₃), 1.17 (6H, t, J =6.5 Hz, 2×CH₂), 1.00–1.80 (8H, m, 4×CH₂), 3.15–3.60 (10H, m, 5×CH₂), 4.67 (2H, s, CH₂), 6.58 (1H, s, benzene ring CH), 7.19 (2H, m, benzene ring 2×CH). Anal. Calcd for C₂₁H₃₂ClN₃O₂: C, 64.03; H, 8.19; N, 10.67. Found: C, 63.92; H, 8.31; N, 10.79. (4b): amorphous solid. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1670 (C=O). NMR (CDCl₃) δ : 0.85 (3H, m, CH₃), 1.00–1.80 (8H, m, 4×CH₂), 1.12 (6H, t, J =7.5 Hz, 2×CH₂), 3.10–3.62 (10H, m, 5×CH₂), 3.80 (6H, s, 2×OCH₃), 4.49 (2H, s, CH₂), 6.46 (2H, s, benzene ring 2×CH). Anal. Calcd for C₂₃H₃₇N₃O₄: C, 65.84; H, 8.89; N, 10.02. Found: C, 65.49; H, 8.68; N, 9.76.

1-[4-(Chloroacetyl)aminobenzyl]-4-*n*-hexyl-2,3-dioxopiperazine (11d)—Compound 11d was obtained from 1-(4-aminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine,¹⁾ chloroacetyl chloride, and triethylamine in CHCl₃. mp 178° (MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3285 (NH), 1680, 1665 (C=O). NMR (CDCl₃) δ : 0.87 (3H, m, CH₃), 1.00–1.90 (8H, m, 4×CH₂), 3.20–3.60 (6H, m, piperazine ring 5 and 6 CH₂ and $\text{NCH}_2\text{--}$), 4.19 (2H, s, CH₂), 4.57 (2H, s, CH₂), 7.14 (2H, d, J =8.5 Hz, benzene ring 2×CH), 7.54 (2H, d, J =8.5 Hz, benzene ring 2×CH), 9.37 (1H, bs, NH). Anal. Calcd for C₁₉H₂₆ClN₃O₃: C, 60.07; H, 6.70; N, 11.06. Found: C, 60.04; H, 7.03; N, 11.21.

The following compounds were similarly obtained. (11b): mp 116–117° (AcOEt–iso-Pr₂O). NMR (CDCl₃) δ : 0.87 (3H, m, CH₃), 1.00–1.80 (8H, m, 4×CH₂), 1.20 (6H, d, J =6.0 Hz, 2×CH₃), 3.39 (4H, bs, 2×CH₂), 3.20–3.90 (4H, m, CH₂, CH, and NH), 4.49 (2H, s, CH₂), 6.46 (2H, d, J =8.5 Hz, benzene ring 2×CH), 7.02 (2H, d, J =8.5 Hz, benzene ring 2×CH). Anal. Calcd for C₂₀H₃₁N₃O₂: C, 69.53; H, 9.04; N, 12.16. Found: C, 69.59; H, 9.12; N, 12.12. (11c): mp 167° (EtOH). NMR (DMSO-*d*₆) δ : 0.86 (3H, m, CH₃), 1.00–1.80 (8H, m, 4×CH₂), 3.40 (4H, bs, 2×CH₂), 3.20–3.50 (2H, m, CH₂), 3.77 (2H, s, CH₂), 4.37 (2H, s, CH₂), 6.46 (2H, d, J =8.5 Hz, benzene ring 2×CH), 6.96 (2H, d, J =8.5 Hz, benzene ring 2×CH). Anal. Calcd for C₁₉H₂₇N₃O₄: C, 63.14; H, 7.53; N, 11.63. Found: C, 63.01; H, 7.50; N, 11.57. (11e): mp 147–149° (iso-PrOH). NMR (CDCl₃) δ : 0.87 (3H, m, CH₃), 1.04–1.74 (8H, m, 4×CH₂), 3.26–3.62 (6H, m, 3×CH₂), 4.48 (2H, s, CH₂), 4.60 (2H, s, CH₂), 7.20 (2H, d, J =9 Hz, benzene ring 2×CH), 7.53 (2H, d, J =9 Hz, benzene ring 2×CH), 8.32 (1H, bs, NH). Anal. Calcd for C₁₉H₂₆FN₃O₃: C, 62.79; H, 7.21; N, 11.56. Found: C, 62.79; H, 7.29; N, 11.44. (11f): mp 173–176° (MeOH). NMR (CDCl₃) δ : 0.86 (3H, m, CH₃), 1.00–1.80 (8H, m, 4×CH₂), 2.94 (2H, t, J =6 Hz, CH₂), 3.20–3.60 (6H, m, 3×CH₂), 3.84 (2H, m, CH₂), 4.61 (2H, s, CH₂), 7.16 (2H, d, J =8.5 Hz, benzene ring 2×CH), 7.59 (2H, d, J =8.5 Hz, benzene ring 2×CH), 9.34 (1H, bs, NH). Anal. Calcd for C₂₀H₂₈ClN₃O₃: C, 60.95; H, 7.16; N, 10.67. Found: C, 60.85; H, 7.43; N, 10.64. (11g): oil (purified by silica gel column chromatography). NMR (CDCl₃) δ : 0.84 (3H, m, CH₃), 1.02–1.82 (8H, m, 4×CH₂), 1.09 (3H, t, J =7.5 Hz, CH₃), 3.22–4.77 (6H, m, 3×CH₂), 3.75 (2H, s, CH₂), 4.05 (2H, q, J =7.5 Hz, CH₂), 4.64 (2H, s, CH₂), 7.12 (2H, d, J =8 Hz, benzene ring 2×CH), 7.36 (2H, d, J =8 Hz, benzene ring 2×CH).

1-(4-Diacetylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (11h)—A mixture of 13 (R=*n*-C₆H₁₃) (0.5 g) and Ac₂O (10 ml) was refluxed for 6 hr and was then evaporated to dryness to give 11h (0.53 g, 98%) as an oil. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1690 (C=O). NMR (CDCl₃) δ : 0.88 (3H, m, CH₃), 1.08–1.83 (8H, m, 4×CH₂), 2.28 (6H, s, 2×COCH₃), 3.33–3.65 (6H, m, piperazine ring 5 and 6 CH₂ and $\text{NCH}_2\text{--}$), 4.64 (2H, s, CH₂), 7.04 (2H, d, J =8 Hz, benzene ring 2×CH), 7.33 (2H, d, J =8 Hz, benzene ring 2×CH).

1-*n*-Hexyl-4-(4-piperidinobenzyl)-2,3-dioxopiperazine Hydrochloride (11a·HCl)—Compound 11a·HCl was obtained from 4-piperidinobenzaldehyde by the method used to prepare 1 in the preceding paper.¹⁾ mp 234–236° (iso-PrOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1650 (C=O). NMR (CDCl₃) δ : 0.86 (3H, m, CH₃), 1.00–1.85 (14H, m, 7×CH₂), 2.90–3.60 (10H, m, 5×CH₂), 4.54 (2H, s, CH₂), 6.87 (2H, d, J =9.5 Hz, benzene ring 2×CH), 7.17 (2H, d, J =9.5 Hz, benzene ring 2×CH). Anal. Calcd for C₂₂H₃₃N₃O₂·HCl: C, 64.77; H, 8.40; N, 10.30. Found: C, 63.11; H, 8.34; N, 10.14.

1-*n*-Hexyl-4-(4-succinimidobenzyl)-2,3-dioxopiperazine (11i)—A mixture of 13 (R=*n*-C₆H₁₃) (2.0 g), succinic anhydride (0.66 g) and toluene (20 ml) was refluxed for 3 hr. Precipitated crystals (2.2 g) were collected. Toluene (15 ml) and Ac₂O (0.6 ml) were added to the crystals and the whole was refluxed for 1 hr. The solvent was removed under reduced pressure and the residue was extracted with CHCl₃. The extract was washed with H₂O and dried over MgSO₄. Removal of the solvent *in vacuo* afforded crude crystals, and recrystallization from MeOH gave 11i (15 g, 59%) as white needles of mp 196°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1705, 1670 (C=O). Anal. Calcd for C₂₁H₂₇N₃O₄: C, 65.43; H, 7.06; N, 10.90. Found: C, 65.42; H, 7.19; N, 10.77.

1-*n*-Hexyl-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (14e)—A solution of 13 (R=*n*-C₆H₁₃) (2.86 g) and 2-bromopyrimidine (1.5 g) in DMF (10 ml) was refluxed for 2 hr, then evaporated to dryness *in vacuo*. The residue was extracted with CHCl₃ and the extract was washed with saturated aqueous NaHCO₃ and then H₂O and dried over MgSO₄. Removal of the solvent *in vacuo* gave a crude solid. Recrystallization from EtOH afforded 14e as white crystals of mp 159–160°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3350 (NH), 1675 (C=O). NMR (DMSO-*d*₆) δ : 0.83 (3H, m, CH₃), 1.08–1.76 (8H, m, 4×CH₂), 3.23–3.63 (6H, m, piperazine ring 5 and 6

CH₂ and $\begin{array}{c} \diagup \\ \text{NCH}_2- \\ \diagdown \\ \text{O} \end{array}$, 4.53 (2H, s, CH₂), 6.75 (1H, t, $J=4.5$ Hz, pyrimidine ring 5 CH), 7.16 (2H, d, $J=8.5$

Hz, benzene ring 2 $\times$ CH), 7.72 (2H, d, $J=8.5$ Hz, benzene ring 2 $\times$ CH), 8.39 (2H, d, $J=4.5$ Hz, pyrimidine ring 4 and 6 CH), 9.50 (1H, s, NH). *Anal.* Calcd for C₂₁H₂₇N₅O₂: C, 66.12; H, 7.13; N, 18.36. Found: C, 65.93; H, 7.07; N, 18.12.

The following compounds were similarly obtained. (14a): mp 157° (iso-PrOH). NMR (CDCl₃) δ : 0.83 (3H, m, CH₃), 1.08—1.78 (8H, m, 4 $\times$ CH₂), 3.40 (6H, bs, 3 $\times$ CH₂), 4.55 (2H, s, CH₂), 6.50—6.95 (2H, m, CH₂), 7.00—7.90 (6H, m, benzene ring 4 $\times$ CH and pyridine ring CH and NH). *Anal.* Calcd for C₂₂H₂₈N₄O₂: C, 69.44; H, 7.42; N, 14.73. Found: C, 69.55; H, 7.40; N, 14.64. (14b): mp 86—89° (AcOEt). NMR (CDCl₃) δ : 0.86 (3H, m, CH₃), 1.00—1.80 (8H, m, 4 $\times$ CH₂), 3.37 (4H, bs, 2 $\times$ CH₂), 3.20—3.60 (2H, m, CH₂), 4.03 (2H, s, CH₂), 4.05 (2H, s, CH₂), 5.88 (1H, s, NH), 6.59 (2H, d, $J=8.5$ Hz, benzene ring 2 $\times$ CH), 7.03 (2H, d, $J=8.5$ Hz, benzene ring 2 $\times$ CH), 7.21 (2H, m, pyridine ring 2 $\times$ CH), 7.65 (1H, dt, $J_o=6$ Hz, $J_m=2$ Hz, pyridine ring 4 CH), 8.53 (1H, dd, $J_o=6$ Hz, $J_m=2$ Hz, pyridine ring 6 CH). *Anal.* Calcd for C₂₃H₃₀N₄O₂: C, 70.02; H, 7.67; N, 14.20. Found: C, 70.40; H, 7.72; N, 14.39. (14c): mp 159—160° (AcOEt). NMR (DMSO-*d*₆) δ : 0.82 (3H, m, CH₃), 1.00—1.80 (8H, m, 4 $\times$ CH₂), 3.48 (6H, bs, 3 $\times$ CH₂), 4.54 (2H, s, CH₂), 6.85 (2H, d, $J=7.0$ Hz, pyridine ring 2 $\times$ CH), 7.19 (4H, s, benzene ring 4 $\times$ CH), 8.12 (2H, d, $J=7.0$ Hz, pyridine ring 2 $\times$ CH), 8.80 (1H, s, NH). *Anal.* Calcd for C₂₂H₂₈N₄O₂: C, 69.44; H, 7.42; N, 14.73. Found: C, 69.42; H, 7.46; N, 14.62. (14d·HCl): mp 177° (iso-PrOH-H₂O). NMR (DMSO-*d*₆) δ : 0.85 (3H, m, CH₃), 1.00—1.80 (8H, m, 4 $\times$ CH₂), 3.10—3.70 (6H, m, 3 $\times$ CH₂), 4.43 (2H, s, CH₂), 4.68 (2H, s, CH₂), 6.74 (2H, d, $J=8.5$ Hz, benzene ring 2 $\times$ CH), 7.05 (2H, d, $J=8.5$ Hz, benzene ring 2 $\times$ CH), 8.07 (2H, d, $J=6$ Hz, pyridine ring 2 $\times$ CH), 8.80 (2H, d, $J=6$ Hz, pyridine ring 2 $\times$ CH), 8.90 (1H, m, NH). *Anal.* Calcd for C₂₃H₃₀N₄O₂·2HCl·H₂O: C, 56.91; H, 7.06; N, 11.54. Found: C, 56.80; H, 7.13; N, 11.43. (14f): mp 79—81° (Et₂O). NMR (CDCl₃) δ : 0.87 (3H, m, CH₃), 1.21 (3H, t, $J=7$ Hz, CH₃), 1.03—1.87 (8H, m, 4 $\times$ CH₂), 3.46 (6H, bs, 3 $\times$ CH₂), 3.98 (2H, q, $J=7$ Hz, CH₂), 4.64 (2H, s, CH₂), 6.48 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.21 (4H, s, benzene ring 4 $\times$ CH), 8.24 (2H, d, $J=4.5$ Hz, pyrimidine ring 2 $\times$ CH). *Anal.* Calcd for C₂₃H₃₁N₅O₂: C, 67.45; H, 7.63; N, 17.10. Found: C, 67.37; H, 7.47; N, 16.98. (14g): mp 148° (EtOH). NMR (DMSO-*d*₆) δ : 0.86 (3H, m, CH₃), 0.94—1.84 (8H, m, 4 $\times$ CH₂), 3.07—3.64 (6H, m, 3 $\times$ CH₂), 4.51 (2H, s, CH₂), 6.73 (1H, t, $J=5.4$ Hz, pyrimidine ring CH), 7.18 (2H, d, $J=8.5$ Hz, benzene ring 2 $\times$ CH), 7.61 (2H, d, $J=8.5$ Hz, benzene ring 2 $\times$ CH), 8.17 (1H, d, $J=5.4$ Hz, pyrimidine ring CH), 8.54 (1H, s, pyrimidine ring CH), 9.54 (1H, s, NH). *Anal.* Calcd for C₂₁H₂₇N₅O₂: C, 66.12; H, 7.13; N, 18.36. Found: C, 66.09; H, 7.13; N, 18.13. (14h): mp 180—181° (iso-PrOH). NMR (CDCl₃) δ : 0.85 (3H, m, CH₃), 1.00—1.80 (8H, m, 4 $\times$ CH₂), 3.42 (6H, bs, 3 $\times$ CH₂), 4.58 (2H, s, CH₂), 7.12 (2H, d, $J=8.5$ Hz, benzene ring 2 $\times$ CH), 7.56 (2H, d, $J=8.5$ Hz, benzene ring 2 $\times$ CH), 7.84 (1H, d, $J=3$ Hz, pyrazine ring CH), 8.00 (1H, q, $J_o=3$ Hz, $J_m=1.5$ Hz, pyrazine ring CH), 8.28 (1H, d, $J=1.5$ Hz, pyrazine ring CH), 8.28 (1H, s, NH). *Anal.* Calcd for C₂₁H₂₇N₅O₂: C, 66.12; H, 7.13; N, 18.36. Found: C, 66.09; H, 7.18; N, 18.23. (14i): mp 221° (EtOH). NMR (DMSO-*d*₆) δ : 0.86 (3H, m, CH₃), 1.00—1.70 (8H, m, 4 $\times$ CH₂), 3.42 (4H, bs, 2 $\times$ CH₂), 3.20—3.50 (2H, m, CH₂), 4.45 (2H, s, CH₂), 6.75 (1H, d, $J=3.5$ Hz, thiazole ring 5 CH), 7.08 (2H, d, $J=9.0$ Hz, benzene ring 2 $\times$ CH), 7.12 (1H, d, $J=3.5$ Hz, thiazole ring 4 CH), 7.49 (2H, d, $J=9.0$ Hz, benzene ring 2 $\times$ CH), 10.18 (1H, bs, NH). *Anal.* Calcd for C₂₀H₂₆N₄O₂S: C, 62.16; H, 6.78; N, 14.50. Found: C, 61.98; H, 6.75; N, 14.38.

1-*n*-Hexyl-4-[4-(*N*-thiocarbamoyl)aminobenzyl]-2,3-dioxopiperazine (15)—A suspension of 13 (5.09 g) and NaSCN (2.68 g) in toluene (50 ml) was treated dropwise with CF₃COOH (1.54 ml) over a period of 2 hr. After the addition, the whole was refluxed for 1 hr. After removal of the solvent by decantation, the resulting oil was washed with hot water and then EtOH to afford a pale yellow solid (5.0 g, 83.6%). Recrystallization from MeOH-EtOH gave yellow crystals of mp 197—198°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1670 (C=O). *Anal.* Calcd for C₁₈H₂₆N₄O₂S: C, 59.64; H, 7.23; N, 15.46. Found: C, 59.67; H, 7.45; N, 15.04.

1-*n*-Hexyl-4-[4-(*S*-methylisothiocarbamoyl)aminobenzyl]-2,3-dioxopiperazine Hydroiodide (16·HI)—MeI (0.17 ml) was added to a suspension of 15 (940 mg) in MeOH (10 ml) at room temperature. The mixture was kept for 24 hr and refluxed for 30 min. Removal of the solvent gave 16·HI as a yellow oil almost quantitatively. IR $\nu_{\text{max}}^{\text{neat}}$ cm⁻¹: 1670 (C=O). NMR (DMSO-*d*₆) δ : 0.84 (3H, m, CH₃), 1.00—1.80 (8H, m, 4 $\times$ CH₂), 2.70 (3H, s, SCH₃), 3.50 (6H, bs, piperazine ring 5 and 6 CH₂ and $\begin{array}{c} \diagup \\ \text{NCH}_2- \\ \diagdown \\ \text{O} \end{array}$), 4.60 (2H, s, CH₂),

7.32 (4H, bs, benzene ring 4 $\times$ CH), 9.30 (2H, m, 2 $\times$ NH).

1-*n*-Hexyl-4-[4-(2-imidazolyl)aminobenzyl]-2,3-dioxopiperazine (14j)—A solution of 16·HI (1.3 g) and ethylenediamine (0.39 ml) in MeOH (35 ml) was refluxed for 24 hr. After removal of the solvent, the residue was dissolved in CHCl₃ (35 ml) and washed with 2.5 *N* NaOH and then with H₂O and dried over MgSO₄. The residue obtained by removal of the solvent *in vacuo* was chromatographed on alumina with CHCl₃-AcOEt (20:1) to give a solid, and further recrystallized from CHCl₃-AcOEt to afford 14j (500 mg, 51%) as white needles of mp 170—171°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3325 (NH), 1660 (C=O). NMR (CDCl₃) δ : 0.86 (3H, m, CH₃), 1.00—1.90 (8H, m, 4 $\times$ CH₂), 3.40 (6H, bs, piperazine ring 5 and 6 CH₂ and $\begin{array}{c} \diagup \\ \text{NCH}_2- \\ \diagdown \\ \text{O} \end{array}$), 3.48 (4H, s, 2 $\times$ CH₂), 4.48 (2H, s, CH₂), 5.38 (2H, bs, 2 $\times$ NH), 6.86 (2H, d, $J=9$ Hz, benzene ring 2 $\times$ CH), 7.04

(2H, d, $J=9$ Hz, benzene ring $2 \times \text{CH}$). *Anal.* Calcd for $\text{C}_{20}\text{H}_{29}\text{N}_5\text{O}_2$: C, 64.66; H, 7.87; N, 18.85. Found: C, 64.75; H, 8.00; N, 18.72.

1-*n*-Hexyl-4-[4-(2-imidazolyl)aminobenzyl]-2,3-dioxopiperazine (14k)—A suspension of 16·HI (5.56 g) and 2-aminoacetaldehyde diethylacetal (2 ml) in iso-BuOH (50 ml) was refluxed for 5 hr, then evaporated to dryness *in vacuo*. The residue was dissolved in CHCl_3 (50 ml) and the solution was washed with 2.5 N NaOH and then with H_2O and dried over MgSO_4 . The solvent was removed under reduced pressure, and the residue was purified by chromatography on alumina to give 1-[4-(2,2-diethoxyethyl)guanizinobenzyl]-4-*n*-hexyl-2,3-dioxopiperazine (5.0 g, 96.7%) as a red oil. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1670 (C=O). This oil was dissolved in conc. HCl (10 ml). The solution was stirred at 90° for 30 min, then made alkaline with 2.5 N NaOH under ice-cooling and extracted with CHCl_3 (50 ml). The extract was washed with H_2O and dried over MgSO_4 . The solvent was removed *in vacuo* and the residue was chromatographed on alumina with MeOH. The product was recrystallized from CHCl_3 -AcOEt to give 14k (0.9 g, 22.8%) as pale yellow crystals of mp $146-147^\circ$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3280 (NH), 1680 (C=O). NMR (CDCl_3) δ : 0.88 (3H, m, CH_3), 1.00–1.90 (8H, m, $4 \times \text{CH}_2$), 3.52 (6H, bs, piperazine ring 5 and 6 CH_2 and NCH_2), 4.50 (2H, bs, $2 \times \text{NH}$), 4.65 (2H, s, CH_2),

6.65 (2H, s, imidazole ring $2 \times \text{CH}$), 7.35 (4H, s, benzene ring $4 \times \text{CH}$). *Anal.* Calcd for $\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$: C, 65.01; H, 7.37; N, 18.96. Found: C, 64.92; H, 7.41; N, 18.78.

1-[4-(2-Pyrimidinylamino)benzyl]-2,3-dioxopiperazine (14l)—A solution of 13 (R=H) (30.0 g) [obtained by reduction of 1-(4-nitrobenzyl)-2,3-dioxopiperazine with $\text{Zn}-\text{CaCl}_2/\text{H}_2\text{O}-\text{EtOH}$] and 2-bromopyrimidine (21.6 g) in DMF (50 ml) was stirred at $130-140^\circ$ for 30 min, then saturated aqueous NaHCO_3 was added to the reaction mixture. The precipitated yellow crystals were collected by filtration (28.0 g, 68.8%). Recrystallization from H_2O gave 14l of mp 253° . IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3200, 3120 (NH), 1660 (C=O). *Anal.* Calcd for $\text{C}_{15}\text{H}_{15}\text{N}_5\text{O}_2$: C, 60.59; H, 5.09; N, 23.56. Found: C, 60.25; H, 5.07; N, 23.10.

1-Benzyl-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (17c)—Compound 17c was obtained from 14l, benzylbromide, and NaH by the usual method. mp $175-176^\circ$ (EtOH- CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3320 (NH), 1670 (C=O). NMR ($\text{DMSO}-d_6$) δ : 3.42 (4H, bs, piperazine ring 5 and 6 CH_2), 4.51 (2H, s, CH_2), 4.54 (2H, s, CH_2), 6.75 (1H, t, $J=4.5$ Hz, pyrimidine ring 5 CH), 7.15 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 7.25 (5H, s, )-, 7.70 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 8.40 (2H, d, $J=4.5$ Hz, pyrimidine ring 4 and 6 CH), 9.68 (1H, s, NH). *Anal.* Calcd for $\text{C}_{22}\text{H}_{21}\text{N}_5\text{O}_2$: C, 68.20; H, 5.46; N, 18.08. Found: C, 68.24; H, 5.39; N, 17.89.

The following compounds were similarly obtained. (17a): mp $188-189^\circ$ (MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1660 (C=O). NMR ($\text{DMSO}-d_6$) δ : 1.10–1.90 (11H, m, cyclohexane ring $5 \times \text{CH}_2$ and CH), 3.08–3.57 (6H, m, $3 \times \text{CH}_2$), 4.50 (2H, s, CH_2), 6.74 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.14 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 7.70 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 8.40 (2H, d, $J=4.5$ Hz, pyrimidine ring $2 \times \text{CH}$), 9.54 (1H, s, NH). *Anal.* Calcd for $\text{C}_{22}\text{H}_{27}\text{N}_5\text{O}_2$: C, 67.15; H, 6.92; N, 17.80. Found: C, 67.15; H, 6.89; N, 17.66. (17d): mp 184° (iso-PrOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (NH), 1670 (C=O). NMR ($\text{DMSO}-d_6$ - CDCl_3) δ : 2.65–3.00 (2H, m, CH_2), 3.31 (4H, s, $2 \times \text{CH}_2$), 3.44–3.78 (2H, m, CH_2), 4.49 (2H, s, CH_2), 6.68 (1H, t, $J=5$ Hz, pyrimidine ring CH), 7.10 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 7.15 (5H, s, benzene ring $5 \times \text{CH}$), 7.69 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 8.36 (2H, d, $J=5$ Hz, pyrimidine ring $2 \times \text{CH}$), 9.43 (1H, s, NH). *Anal.* Calcd for $\text{C}_{23}\text{H}_{23}\text{N}_5\text{O}_2$: C, 68.81; H, 5.77; N, 17.44. Found: C, 68.71; H, 5.84; N, 17.22. (17e): mp $155-156^\circ$ (EtOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3290 (NH), 1675 (C=O). NMR ($\text{DMSO}-d_6$) δ : 1.54–2.09 (2H, m, CH_2), 2.34–2.74 (2H, m, CH), 3.42 (6H, bs, $3 \times \text{CH}_2$), 4.50 (2H, s, CH_2), 6.77 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.15 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 7.17 (5H, s, benzene ring $5 \times \text{CH}$), 7.70 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 8.39 (2H, d, $J=4.5$ Hz, pyrimidine ring $2 \times \text{CH}$), 9.55 (1H, s, NH). *Anal.* Calcd for $\text{C}_{24}\text{H}_{25}\text{N}_5\text{O}_2$: C, 69.38; H, 6.07; N, 16.89. Found: C, 69.15; H, 6.13; N, 16.59. (17f): mp $176-177^\circ$ (MeOH- CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3420 (NH), 1675, 1655 (C=O). NMR ($\text{DMSO}-d_6$) δ : 1.51 (3H, d, $J=7.2$ Hz, CH_3), 3.45 (4H, bs, $2 \times \text{CH}_2$), 4.58 (2H, s, CH_2), 5.78 (1H, q, $J=7.2$ Hz, CH), 6.87 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.24 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 7.37 (5H, s, benzene ring $5 \times \text{CH}$), 7.85 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 8.52 (2H, d, $J=4.5$ Hz, pyrimidine ring $2 \times \text{CH}$), 9.69 (1H, s, NH). *Anal.* Calcd for $\text{C}_{23}\text{H}_{23}\text{N}_5\text{O}_2$: C, 68.81; H, 5.78; N, 17.45. Found: C, 68.70; H, 5.76; N, 17.32. (17i): mp $214-215^\circ$ (DMF-iso-PrOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3320 (NH), 1680 (C=O), 1525, 1350 (NO_2). NMR ($\text{DMSO}-d_6$) δ : 3.51 (4H, bs, $2 \times \text{CH}_2$), 4.56 (2H, s, CH_2), 4.72 (2H, s, CH_2), 6.81 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.23 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 7.57 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 7.77 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 8.16 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 8.49 (2H, d, $J=4.5$ Hz, pyrimidine ring $2 \times \text{CH}$), 9.63 (1H, s, NH). *Anal.* Calcd for $\text{C}_{22}\text{H}_{20}\text{N}_6\text{O}_4$: C, 61.10; H, 4.66; N, 19.44. Found: C, 61.02; H, 4.56; N, 19.58. (17j): mp $216-217^\circ$ (EtOH- CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3275 (NH), 1665 (C=O). NMR ($\text{DMSO}-d_6$) δ : 3.45 (4H, s, $2 \times \text{CH}_2$), 4.50 (2H, s, CH_2), 4.53 (2H, s, CH_2), 6.76 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.13 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 7.30 (4H, s, benzene ring $4 \times \text{CH}$), 7.67 (2H, d, $J=8.5$ Hz, benzene ring $2 \times \text{CH}$), 8.38 (2H, d, $J=4.5$ Hz, pyrimidine ring $2 \times \text{CH}$), 9.53 (1H, s, NH). *Anal.* Calcd for $\text{C}_{22}\text{H}_{20}\text{ClN}_6\text{O}_2$: C, 62.63; H, 4.78; N, 16.60. Found: C, 62.59; H, 4.75; N, 16.41. (17l): mp 209° (MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3375 (NH), 1715, 1670 (C=O). NMR ($\text{DMSO}-d_6$) δ : 3.45 (4H, s, $2 \times \text{CH}_2$), 3.80 (3H, s, OCH_3),

4.50 (2H, s, CH₂), 4.63 (2H, s, CH₂), 6.78 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.00—8.02 (8H, m, benzene ring 8 × CH), 8.40 (2H, d, $J=4.5$ Hz, pyrimidine ring 2 × CH), 9.52 (1H, s, NH). *Anal.* Calcd for C₂₄H₂₃N₅O₄: C, 64.71; H, 5.20; N, 15.72. Found: C, 64.86; H, 5.18; N, 15.57. (17o): mp 190—191° (MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3315 (NH), 1730, 1685 (C=O). NMR (DMSO-*d*₆) δ : 3.49 (4H, s, 2 × CH₂), 3.78 (3H, s, OCH₃), 4.52 (2H, s, CH₂), 4.66 (2H, s, CH₂), 6.55 (1H, d, $J=4$ Hz, furan ring CH), 6.77 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.10 (1H, d, $J=4$ Hz, furan ring CH), 7.15 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.69 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 8.41 (2H, d, $J=4.5$ Hz, pyrimidine ring 2 × CH), 9.57 (1H, s, NH). *Anal.* Calcd for C₂₂H₂₁N₅O₅: C, 60.86; H, 4.86; N, 16.09. Found: C, 60.73; H, 4.84; N, 15.85. (17q): mp 160—161° (MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3300 (NH), 1650 (C=O). NMR (DMSO-*d*₆) δ : 3.52 (4H, s, 2 × CH₂), 4.54 (2H, s, CH₂), 4.66 (2H, s, CH₂), 6.78 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.20 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.05—7.42 (2H, m, pyridine ring 2 × CH), 7.71 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.55—7.85 (1H, m, pyridine ring CH), 8.42 (2H, d, $J=4.5$ Hz, pyrimidine ring 2 × CH), 8.49 (1H, m, pyridine ring CH), 9.56 (1H, s, NH). *Anal.* Calcd for C₂₁H₂₀N₆O₂: C, 64.93; H, 5.19; N, 21.64. Found: C, 64.68; H, 5.09; N, 21.68. (17s): mp 193.5° (MeOH-CHCl₃). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3225 (NH), 1720, 1675 (C=O). NMR (DMSO-*d*₆) δ : 3.56 (4H, bs, 2 × CH₂), 3.83 (3H, s, OCH₃), 4.55 (2H, s, CH₂), 4.74 (2H, s, CH₂), 6.75 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.17 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.48 (1H, d, $J=8$ Hz, pyridine ring CH), 7.70 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 8.18 (1H, dd, $J_m=1.0$ Hz, $J_o=8$ Hz, pyridine ring CH), 8.39 (2H, d, $J=4.5$ Hz, pyrimidine ring 2 × CH), 8.94 (1H, d, $J=1.0$ Hz, pyridine ring CH), 9.52 (1H, s, NH). *Anal.* Calcd for C₂₃H₂₂N₆O₄: C, 61.87; H, 4.97; N, 18.83. Found: C, 61.88; H, 4.94; N, 18.63. (17u): mp 168—170° (MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3290 (NH), 1662 (C=O). NMR (DMSO-*d*₆) δ : 3.58 (4H, bs, 2 × CH₂), 4.54 (2H, s, CH₂), 4.73 (2H, s, CH₂), 6.75 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.18 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.72 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 8.43 (2H, d, $J=4.5$ Hz, pyrimidine ring 2 × CH), 8.52 (1H, s, pyrazine ring CH), 8.59 (2H, d, $J=8$ Hz, pyrazine ring 2 × CH), 9.53 (1H, s, NH). *Anal.* Calcd for C₂₀H₁₉N₇O₂: C, 61.69; H, 4.92; N, 25.18. Found: C, 61.47; H, 4.81; N, 25.26. (17v): mp 255° (CHCl₃-MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3280 (NH), 1680 (C=O). NMR (DMSO-*d*₆) δ : 3.50—3.80 (2H, m, CH₂), 4.02—4.39 (2H, m, CH₂), 4.64 (2H, s, CH₂), 6.83 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.26 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.77 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 8.45 (2H, s, pyrazine ring 2 × CH), 8.46 (2H, d, $J=4.5$ Hz, pyrimidine ring 2 × CH), 9.29 (1H, s, pyrazine ring CH). *Anal.* Calcd for C₁₉H₁₇N₇O₂: C, 60.79; H, 4.56; N, 25.90. Found: C, 60.53; H, 4.56; N, 26.12. (17w): mp 185—186° (MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3320 (NH), 1760 (C=O). *Anal.* Calcd for C₁₉H₁₇N₇O₂ · 1/2H₂O: C, 59.37; H, 4.72; N, 25.51. Found: C, 60.21; H, 4.63; N, 25.52. (17x): mp 220° (MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3310 (NH), 1680 (C=O). *Anal.* Calcd for C₂₀H₁₉N₇O₃: C, 59.25; H, 4.72; N, 24.18. Found: C, 59.87; H, 5.05; N, 24.46. (17y): mp 185° (dec.) (DMF). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1675 (C=O). *Anal.* Calcd for C₁₉H₁₇N₇O₃: C, 58.31; H, 4.38; N, 25.05. Found: C, 58.00; H, 4.23; N, 24.76.

1-Phenyl-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (17b)—K₂CO₃ (0.5 g) and activated Cu⁶ (50 mg) were added to a solution of **14l** (1.0 g) and iodobenzene (1.5 ml) in DMF (10 ml) and the whole was refluxed for 4 hr. After removal of the solvent *in vacuo*, the residue was recrystallized from CHCl₃-iso-PrOH to afford **17b** as white needles of mp 205—206°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3300 (NH), 1675 (C=O). NMR (DMSO-*d*₆) δ : 3.47—3.74 (2H, m, piperazine ring CH₂), 3.74—4.06 (2H, m, piperazine ring CH₂), 4.58 (2H, s, CH₂), 6.77 (1H, t, $J=5$ Hz, pyrimidine ring 5 CH), 7.26 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.34 (5H, s, , 7.77 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 8.27—8.59 (2H, bs, pyrimidine ring 4 and 6 CH), 9.61 (1H, s, NH). *Anal.* Calcd for C₂₁H₁₉N₅O₂: C, 67.54; H, 5.13; N, 18.76. Found: C, 67.38; H, 5.02; N, 18.55.

1-(4-Aminobenzyl)-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (17g)—Compound **17g** was obtained by reduction of **17i** with Zn-CaCl₂/EtOH-H₂O as described for the preparation of 1-(4-aminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine in the preceding paper.¹⁾ mp 190—192° (EtOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3250 (NH), 1670 (C=O). NMR (DMSO-*d*₆) δ : 3.45 (4H, bs, 2 × CH₂), 4.38 (2H, s, CH₂), 4.49 (2H, s, CH₂), 4.81—5.16 (2H, bs, NH₂), 6.48 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 6.74 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 6.90 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.11 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.68 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 8.39 (2H, d, $J=4.5$ Hz, pyrimidine ring 2 × CH), 9.53 (1H, s, NH). *Anal.* Calcd for C₂₂H₂₂N₅O₂: C, 65.67; H, 5.51; N, 20.88. Found: C, 65.57; H, 5.48; N, 20.64.

1-(2,4-Dimethoxybenzyl)-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (17m)—Compound **17m** was obtained from 1-(4-aminobenzyl)-4-(2,4-dimethoxybenzyl)-2,3-dioxopiperazine by the method described for the preparation of **14e**. mp 186° (MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3295 (NH), 1660 (C=O). NMR (DMSO-*d*₆) δ : 3.27 (4H, bs, 2 × CH₂), 3.63 (3H, s, OCH₃), 3.65 (3H, s, OCH₃), 4.33 (2H, s, CH₂), 4.37 (2H, s, CH₂), 6.21—6.48 (2H, m, benzene ring 2 × CH), 6.63 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 6.90—7.05 (1H, m, benzene ring CH), 7.04 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 7.60 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 8.28 (2H, d, $J=4.5$ Hz, pyrimidine ring 2 × CH), 9.42 (1H, s, NH). *Anal.* Calcd for C₂₄H₂₅N₅O₄: C, 64.41; H, 5.63; N, 15.65. Found: C, 64.26; H, 5.47; N, 15.41.

Compound **17h** was similarly obtained. mp 131° (EtOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3300 (NH), 1670 (C=O). NMR (DMSO-*d*₆) δ : 1.04 (6H, t, $J=7.0$ Hz, 2 × CH₃), 3.27 (4H, q, $J=7.0$ Hz, 2 × CH₂), 3.33 (4H, bs, 2 × CH₂), 4.40 (2H, s, CH₂), 4.49 (2H, s, CH₂), 6.55 (2H, d, $J=8.5$ Hz, benzene ring 2 × CH), 6.77 (1H, t, $J=4.5$ Hz,

pyrimidine ring CH), 7.03 (2H, d, $J=8.5$ Hz, benzene ring $2\times$ CH), 7.14 (2H, d, $J=8.5$ Hz, benzene ring $2\times$ CH), 7.70 (2H, d, $J=8.5$ Hz, benzene ring $2\times$ CH), 8.40 (2H, d, $J=4.5$ Hz, pyrimidine ring $2\times$ CH), 9.56 (1H, s, NH). *Anal.* Calcd for $C_{26}H_{30}N_6O_2$: C, 68.10; H, 6.59; N, 18.33. Found: C, 68.21; H, 6.51; N, 18.13.

1,4-Bis[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (17n)—A solution of 1,4-dibenzyl-2,3-dioxopiperazine (16.2 g) in conc. H_2SO_4 (40 ml) was treated dropwise with conc. HNO_3 (22.8 g) under ice-cooling. After being stirred for 1 hr at the same temperature and for a further 11 hr at room temperature, it was poured into ice-water in small portions and the precipitated solid was collected by filtration. Recrystallization from DMF afforded 1,4-bis(4-nitrobenzyl)-2,3-dioxopiperazine (12.9 g, 61%) as yellow crystals of mp 257–260°. IR ν_{max}^{KBr} cm^{-1} : 1670 (C=O), 1330 (NO_2). *Anal.* Calcd for $C_{18}H_{10}N_4O_6$: C, 56.25; H, 4.20; N, 14.58. Found: C, 56.22; H, 4.16; N, 14.60. The above crystals (12.9 g) and Zn powder (130 g) were added to a mixture of EtOH (130 ml) and H_2O (130 ml). Then $CaCl_2$ (26 g) in H_2O (45 ml) was added dropwise to the above suspension and the whole was refluxed for 2 hr. Insoluble materials were filtered off under heating and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and the solution was washed with H_2O and dried over $CaCO_3$. The crude crystals obtained by removal of the solvent *in vacuo* were recrystallized from EtOH to afford pure 1,4-bis(4-aminobenzyl)-2,3-dioxopiperazine as colorless crystals (5.1 g, 46.9%) of mp 193–194°. IR ν_{max}^{KBr} cm^{-1} : 1660 (C=O). *Anal.* Calcd for $C_{18}H_{20}N_4O_2$: C, 66.65; H, 6.22; N, 17.27. Found: C, 66.44; H, 6.23; N, 17.19. Treatment of the above crystals (2.3 g) and 2-bromopyrimidine (2.5 g) as described for **14e** provided **17n** (1.5 g, 44.1%). mp 226–228.5° (DMF-iso-PrOH). IR ν_{max}^{KBr} cm^{-1} : 3425 (NH), 1670 (C=O). NMR (DMSO- d_6) δ : 3.40 (4H, bs, $2\times$ CH₂), 4.51 (4H, s, $2\times$ CH₂), 6.74 (2H, t, $J=4.5$ Hz, pyrimidine ring $2\times$ CH), 7.12 (4H, d, $J=8.5$ Hz, benzene ring $4\times$ CH), 7.64 (4H, d, $J=8.5$ Hz, benzene ring $4\times$ CH), 8.39 (4H, d, $J=4.5$ Hz, pyrimidine ring $4\times$ CH), 9.53 (2H, s, $2\times$ NH). *Anal.* Calcd for $C_{26}H_{24}N_8O_2$: C, 64.98; H, 5.03; N, 23.32. Found: C, 64.78; H, 5.13; N, 23.47.

1-[2-(5-Carboxypyridyl)methyl]-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (17t)—Compound **17s** (500 mg) was added to a solution of KOH (74 mg) in 50% MeOH- H_2O (10 ml). The whole was refluxed for 15 min, then the solvent was evaporated off. The residue was dissolved in H_2O (10 ml) and the solution was neutralized with 2N HCl. Precipitated crystals were collected by filtration (300 mg, 62.5%). mp 214° (dec.) (MeOH- $CHCl_3$). IR ν_{max}^{KBr} cm^{-1} : 3280 (NH), 1660 (C=O). NMR (DMSO- d_6) δ : 3.62 (4H, bs, $2\times$ CH₂), 4.60 (2H, s, CH₂), 4.81 (2H, s, CH₂), 6.80 (1H, t, $J=4.8$ Hz, pyrimidine ring CH), 7.22 (2H, d, $J=9.0$ Hz, benzene ring $2\times$ CH), 7.50 (1H, d, $J=7.8$ Hz, pyridine ring CH), 7.78 (2H, d, $J=9.0$ Hz, benzene ring $2\times$ CH), 8.28 (1H, dd, $J_o=7.8$ Hz, $J_m=1.5$ Hz, pyridine ring CH), 8.47 (2H, d, $J=4.8$ Hz, pyrimidine ring $2\times$ CH), 9.04 (1H, d, $J=1.5$ Hz, pyridine ring CH), 9.60 (1H, s, NH). *Anal.* Calcd for $C_{22}H_{20}N_6O_4\cdot 1/2H_2O$: C, 59.86; H, 4.79; N, 19.04. Found: C, 59.92; H, 4.66; N, 18.80.

The following compounds were similarly obtained. (**17k**): mp 170° (MeOH- $CHCl_3$). IR ν_{max}^{KBr} cm^{-1} : 3500–2700 (NH, OH), 1700, 1670 (C=O). *Anal.* Calcd for $C_{23}H_{21}N_5O_4$: C, 64.03; H, 4.91; N, 16.23. Found: C, 63.88; H, 4.78; N, 16.01. (**17p**): mp 205° (MeOH). IR ν_{max}^{KBr} cm^{-1} : 3500–2730 (NH, OH), 1700, 1670 (C=O). *Anal.* Calcd for $C_{21}H_{19}N_5O_5$: C, 59.85; H, 4.54; N, 16.62. Found: C, 59.70; H, 4.46; N, 16.49.

1-[2-(6-Aminopyridyl)methyl]-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (17r)—1-[2-(6-Acetamidopyridyl)methyl]-4-[4-(2-pyrimidinylamino)benzyl]-2,3-dioxopiperazine (0.4 g), obtained by the method described for **17c**, was added to 2N HCl (6 ml). The solution was refluxed for 1.5 hr, then neutralized with $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with H_2O , and dried over $MgSO_4$. Crude crystals obtained by removal of the solvent *in vacuo* were recrystallized from $CHCl_3$ -MeOH. Yield 0.3 g (83.3%). mp 225–226°. IR ν_{max}^{KBr} cm^{-1} : 3120, 3190, 3275 (NH), 1670 (C=O). NMR (DMSO- d_6) δ : 3.49 (4H, bs, $2\times$ CH₂), 4.43 (2H, s, CH₂), 4.53 (2H, s, CH₂), 5.67–6.62 (2H, m, NH₂), 6.18–6.50 (2H, m, pyridine ring $2\times$ CH), 6.77 (1H, t, $J=4.5$ Hz, pyrimidine ring CH), 7.18 (2H, d, $J=8.5$ Hz, benzene ring $2\times$ CH), 7.19–7.31 (1H, m, pyridine ring CH), 7.73 (2H, d, $J=8.5$ Hz, benzene ring $2\times$ CH), 8.38 (2H, d, $J=4.5$ Hz, pyrimidine ring $2\times$ CH), 9.53 (1H, s, NH). *Anal.* Calcd for $C_{21}H_{21}N_7O_2$: C, 62.54; H, 5.25; N, 24.30. Found: C, 62.45; H, 5.19; N, 24.04.

Cytotoxicity against HeLa S3 (Minimum Inhibitory Concentration: MIC Values)—MIC values of test compounds were determined by the method described in the previous paper.¹⁾

Acute Toxicity against Mouse—One group consisting of two 6-week-old female SLC-ICR mice weighing 20 ± 1 g was given test compounds dissolved or suspended in saline or saline containing 0.3% CMC (carboxymethyl cellulose) intraperitoneally. At 7 days after administration, the number of deaths or survivals was checked and the LD_{50} was calculated.

Antitumor Activity against Ehrlich Ascites Carcinoma (i.p.-i.p.)—EAC cells (1×10^6 cells/head/0.2 ml physiological saline) were intraperitoneally transplanted into 6-week-old female ICR mice weighing 21 ± 1 g. Test compounds were intraperitoneally administered from day 1 to day 7 once a day for 7 successive days. Their antitumor activities were evaluated in terms of the mean survival days compared with the controls ($T/C\%$). In these experiments, five mice were used in each group.

Antitumor Activity against L1210 (i.p.-i.p.)—L1210 cells (3×10^5 cells/head/0.2 ml physiological saline) were intraperitoneally transplanted into 6-week-old male BDF₁ mice weighing 21–23 g. Test compounds dissolved in saline or suspended in 0.3% CMC-physiological saline were intraperitoneally administered from

day 1 to day 7 once a day for 7 successive days. Their antitumor activities were evaluated in terms of the mean survival days compared with the controls ($T/C\%$). In these experiments, five mice were used in each group.

Metabolism of Test Compounds in Mice—Test compounds suspended in 0.3% CMC-saline (50 mg/kg) were intraperitoneally administered to ICR female mice in which the urethral meatus had been closed. Six groups consisting of two mice per group were used. At 5, 15, 30, 60, and 120 min after administration, mice were sacrificed and the blood, peritoneal cavity, liver (homogenized) and urine were taken up with H_2O and $CHCl_3$. The $CHCl_3$ extract was subjected to thin-layer chromatography on silica gel. The control group was treated in the same way, except that no drug was administered.

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References and Notes

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