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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 autitumor agents than 1-(4-diethylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (**1**), which was found in our laboratory in previous studies.

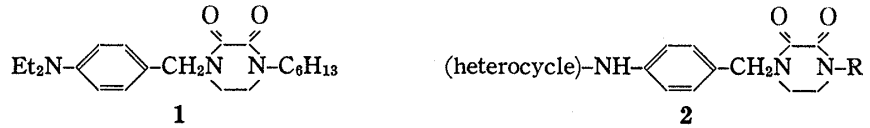
1-(4-Diethylamino-3- or 2-substituted benzyl)-2,3-dioxopiperazine derivatives and 1-(4-substituted aminobenzyl)-2,3-dioxopiperazine derivatives were designed and synthesized with the aim of suppressing the metabolism of the Et_2N- group of **1**. The structure–activity relationships and metabolism of 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 159[2] among 2,6-dioxopiperazine derivatives and 593A(NSC-135768)[3] or albonoursin[4] 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 paper[1] 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 Et_2N- 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 Et_2N- 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).

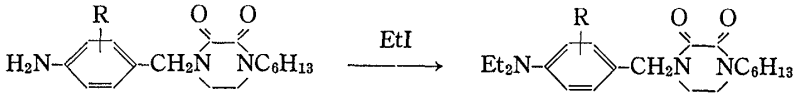


1 **2**

Chart 1

This work was undertaken in order to develop a more potent antitumor agent than **1** by suppressing the metabolism of the Et_2N-group of **1**. One approach was to introduce a substituent on the benzene ring in an attempt to prevent enzymatic attack on the Et_2N- group of **1** by introducing steric hindrance. The other was to exchange the Et_2N- group for some other substituted amino group which is less easily metabolized.

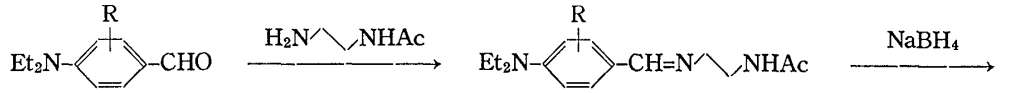
method A



3a: R=2-Cl
3b: R=3,5-diMeO
3e: R=3-Me

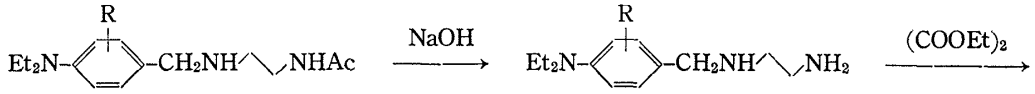
4a, b, e

method B



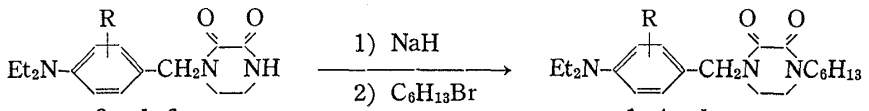
5c: R=3-Cl
5d: R=3-Br
5f: R=H

6c, d, f



7c, d, f

8c, d, f

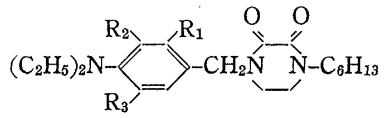


9c, d, f

1, 4c, d

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	*In vitro* MIC[a] (μg/ml) HeLa S3	*In vivo* LD_{50}[b] (mg/kg)	*In vivo* EAC(*i.p.*–*i.p.*)[c] Dose (mg/kg)	*In vivo* EAC(*i.p.*–*i.p.*)[c] 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_3O	CH_3O	50	—	—	—
4c	H	Cl	H	12.5	100	40×7	>170(1/4)[d]
						100×1	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: 2×10^4 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 1×10^6 cells/mouse, *i.p.*
Treatment: from Day 1, *i.p.*

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

d) 43-day survivor.

First, **4a**—**e**, in which a Cl, Br, CH_3, or OCH_3 group is present on the benzene ring of **1**, were studied. These compounds were synthesized by diethylation of the 4-NH_2 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 CH_3 adjacent to the 4-Et_2N- 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.

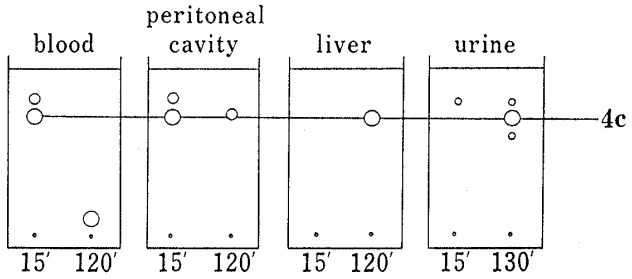


Fig. 1. Metabolism of **4c** in Mice
TLC (silica gel, $CHCl_3$: EtOH=15: 1).

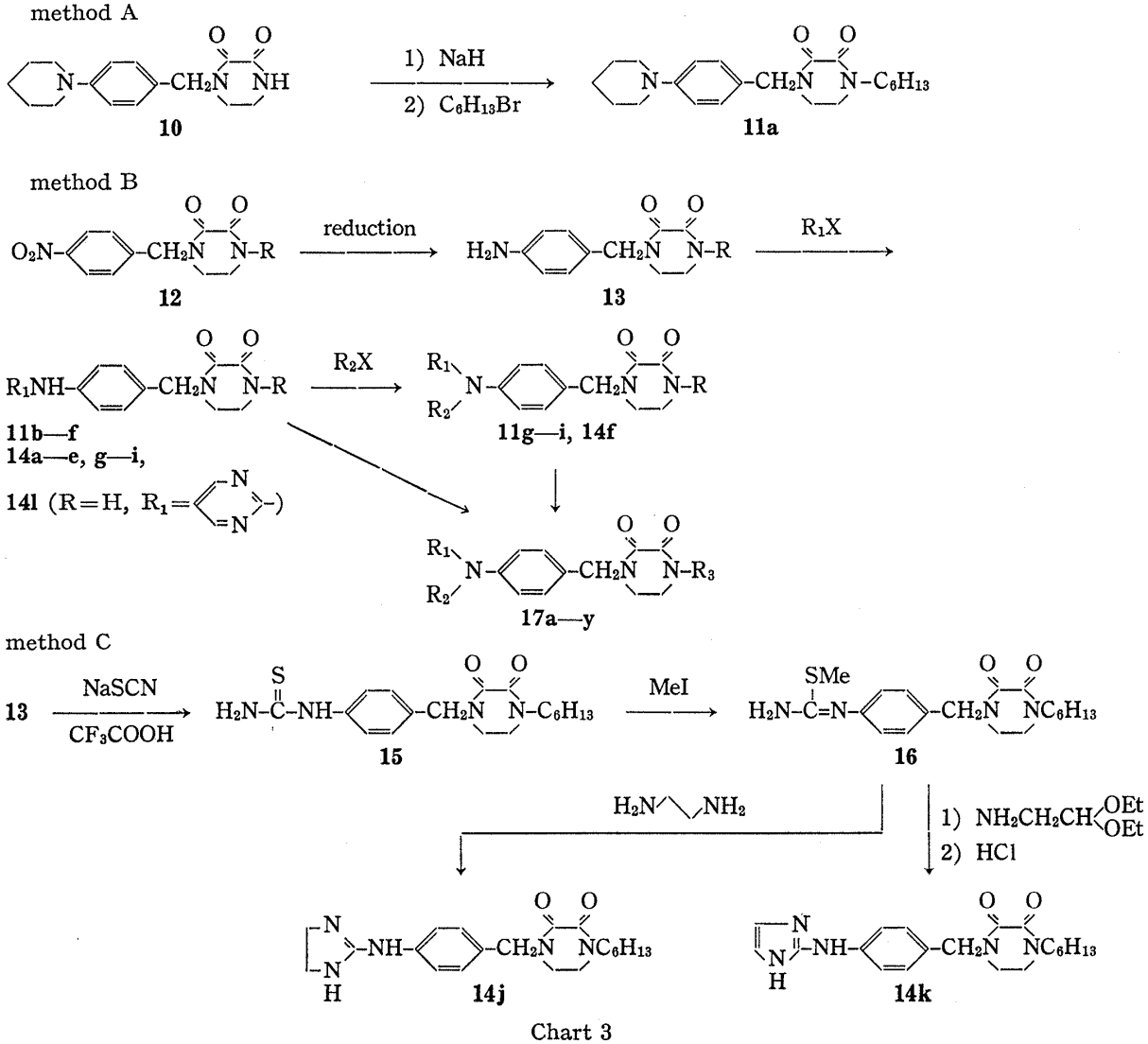
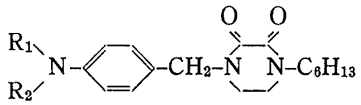


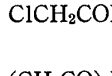
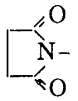
Chart 3

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 Et_2N- 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



Compd. No.	R_1, R_2	*In vitro* MIC (μg/ml)[a] HeLa S3	*In vivo* LD_{50}[b] (mg/kg)	*In vivo* EAC (*i.p.*–*i.p.*)[c] Dose (mg/kg)	*In vivo* EAC (*i.p.*–*i.p.*)[c] *T/C* (%)
1	$(C_2H_5)_2N$-	3.13	100	40×7	157
11a		6.25	150	40×7	138
11b		0.78—1.56	100	40×7	130
11c	$HOOCCH_2NH$-	100	—	—	—
11d	$ClCH_2CONH$-	0.39—0.78	>400	100×7 200×7	>142 (1/4)[d] 156.8
11e	FCH_2CONH-	25	—	—	—
11f	$ClCH_2CH_2CONH$-	6.25	>600	50×7 100×7	84.1 95.2
11g		1.56	300	50×7 100×7	121.5 135.1
11h	$(CH_3CO)_2N$-	12.5	>500	100×7 200×7	91 160
11i		100	—	—	—

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

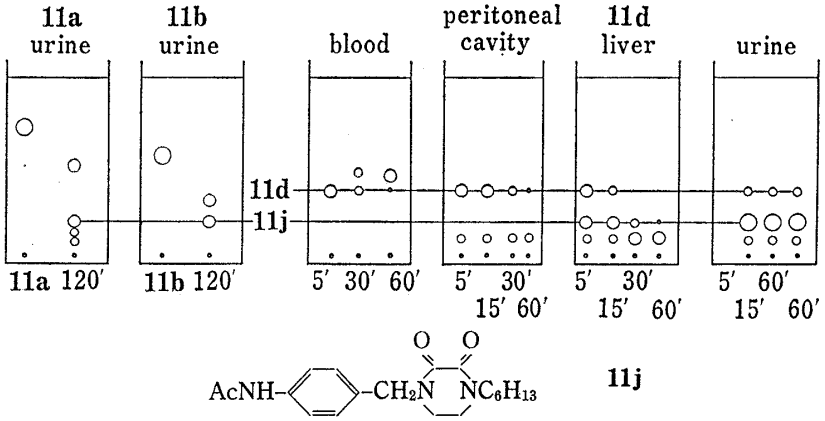


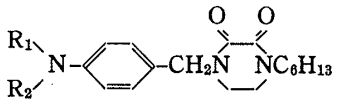
Fig. 2. Metabolism of **11a**, **11b**, and **11d** in Mice

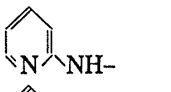
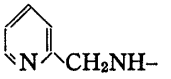
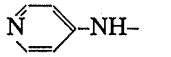
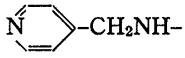
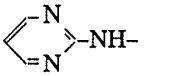
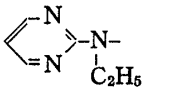
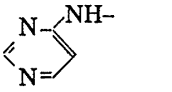
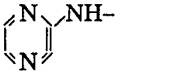
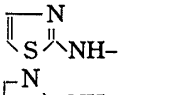
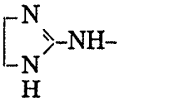
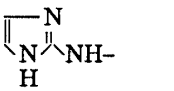
TLC (silica gel, $CHCl_3$: EtOH=15: 1).

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-dioxoipiperazine Derivatives



Compd. No.	R_1, R_2	*In vitro* MIC (μg/ml)[a] HeLa S3	*In vivo* LD_{50}[b] (mg/kg)	*In vivo* EAC (*i.p.–i.p.*)[c] Dose (mg/kg)	*In vivo* EAC (*i.p.–i.p.*)[c] *T/C* (%)	*In vivo* L1210 (*i.p.–i.p.*)[d] Dose (mg/kg)	*In vivo* L1210 (*i.p.–i.p.*)[d] *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.

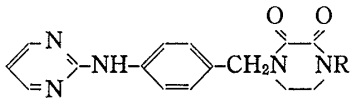
d) Animal: BDF_1 (♂) mice, 6 weeks old, 4 or 5 mice/group.
Inoculum size: L12101 $\times 10^5$ cells/mouse, *i.p.*
Treatment: days 1—7, days 1—9 or days 1,5,9, *i.p.*

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

e) 34-day survivor.

f) 44-day survivor.

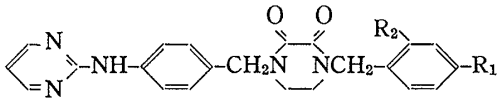
TABLE IV. Structure–Antitumor Activity Relationship of 1-[4-(2-Pyrimidinylamino)benzyl]-2, 3-dioxopiperazine Derivatives

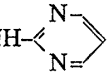


Compd. No.	R	*In vitro* MIC (μg/ml)[a] HeLa S3	*In vivo* LD_{50}[b] (mg/kg)	*In vivo* EAC (*i.p.*–*i.p.*)[c] Dose (mg/kg)	*In vivo* EAC (*i.p.*–*i.p.*)[c] *T*/*C* (%)	*In vivo* L1210 (*i.p.*–*i.p.*)[d] Dose (mg/kg)	*In vivo* L1210 (*i.p.*–*i.p.*)[d] *T*/*C* (%)
14e	C_6H_{13}	0.39—0.78	>1000	50×7	>186.7 (1/4)[e]	50×9	181.0
				100×7	>206.7 (1/4)[e]	100×7	168.7
						200×3	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	217.9	20×7	181.4
				50×4	>226.9 (2/5)[f]	50×7	>262 (1/5)[g]
				100×7	>176.7 (1/4)[f]	100×6	150.0
						200×3	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



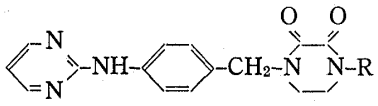
Compd. No.	R_1	R_2	*In vitro* MIC (μg/ml)[a] HeLa S3	*In vivo* LD_{50}[b] (mg/kg)	*In vivo* EAC (*i.p.*–*i.p.*)[c] Dose (mg/kg)	*In vivo* EAC (*i.p.*–*i.p.*)[c] *T*/*C* (%)
14e	H	H	0.1	>1000	100×7	>176.7 (1/4)[d]
17g	NH_2	H	3.13	>500	100×7	124.3
17h	$N(C_2H_5)_2$	H	12.5	—	—	—
17i	NO_2	H	3.13	>500	100×7	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_3O	CH_3O	3.13	>500	100×7	95.9
17n	-NH- 	H	6.25	>500	100×7	159

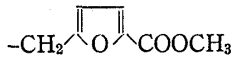
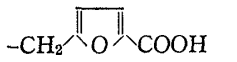
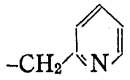
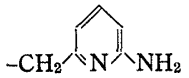
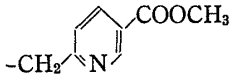
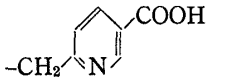
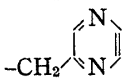
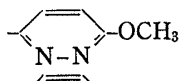
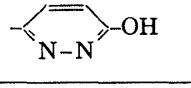
a), *b*), *c*) See the legend to Table I.
d) 32-day survivor.

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-NH_2 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-NH_2 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-Pyrimidinylamino)benzyl]-2,3-dioxopiperazine Derivatives



Compd. No.	R	*In vitro* MIC (μg/ml)[a] HeLa S3	*In vivo* LD_{50}[b] (mg/kg)	*In vivo* EAC (*i.p.–i.p.*)[c] Dose (mg/kg)	*In vivo* EAC (*i.p.–i.p.*)[c] T/C (%)
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-C_6H_{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 μg/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: $-CH_2-\langle\bigcirc\rangle$ > $-(CH_2)_2-\langle\bigcirc\rangle$ ≧ n-C_6H_{13} ≧ $-\langle\bigcirc\rangle$ > $-CH_2-\langle\bigcirc\rangle$ ≧ $-(CH_2)_3-\langle\bigcirc\rangle$ ≧ $-CH(CH_3)-\langle\bigcirc\rangle$. It is of interest that **14e** (R: n-C_6H_{13}), **17c** (R: $-CH_2-\langle\bigcirc\rangle$), and **17d** (R: $-(CH_2)_2-\langle\bigcirc\rangle$) showed high antitumor activity against EAC (*i.p.–i.p.*) while **17b** (R: $-\langle\bigcirc\rangle$), **17e** (R: $-(CH_2)_3-\langle\bigcirc\rangle$), and **17f** (R: $-CH(CH_3)-\langle\bigcirc\rangle$) 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.

Experimental[5]

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-Pr_2O). IR ν_{max}^{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×CH_3), 1.00—1.85 (8H, m, 4×CH_2), 3.00—3.60 (10H, m, 5×CH_2), 4.54 (2H, s, CH_2), 7.00—7.18 (3H, m, benzene ring 3×CH). *Anal.* Calcd for $C_{21}H_{32}ClN_3O_2$: 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-Pr_2O). IR ν_{max}^{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×CH_3), 1.00—1.80 (8H, m, 4×CH_2), 3.14 (4H, q, J=7.5 Hz, 2×CH_2), 3.30—3.75 (6H, m, 3×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×CH). *Anal.* Calcd for $C_{21}H_{32}BrN_3O_2$·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-dioxopiperazine[1] (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 ν_{max}^{neat} cm^{-1}: 1665 (C=O). NMR ($CDCl_3$) δ: 0.87 (3H, m, CH_3), 0.96 (6H, t, J=6.5 Hz, 2×CH_3), 1.00—1.80 (8H, m, 4×CH_2), 2.24 (3H, s, CH_3), 2.96 (4H, q, J=6.5 Hz, 2×$CH_3\underline{CH_2}$), 3.25—3.65 (6H, m, piperazine ring 5 and 6 CH_2, and $\underline{NCH_2}$–), 4.57 (2H, s, CH_2), 7.00—7.20 (3H, m, benzene ring 3×CH). *Anal.* Calcd for $C_{22}H_{35}N_3O_2$·

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-Pr_2O). IR ν_{max}^{KBr} cm^{-1}: 1675 (C=O). NMR ($CDCl_3$) δ: 0.88 (3H, m, CH_3), 1.17 (6H, t, J=6.5 Hz, 2×CH_3), 1.00—1.80 (8H, m, 4×CH_2), 3.15—3.60 (10H, m, 5×CH_2), 4.67 (2H, s, CH_2), 6.58 (1H, s, benzene ring CH), 7.19 (2H, m, benzene ring 2×CH). *Anal.* Calcd for: $C_{21}H_{32}ClN_3O_2$: C, 64.03; H, 8.19; N, 10.67. Found: C, 63.92; H, 8.31; N, 10.79. (**4b**): amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1670 (C=O). NMR ($CDCl_3$) δ: 0.85 (3H, m, CH_3), 1.00—1.80 (8H, m, 4×CH_2), 1.12 (6H, t, J=7.5 Hz, 2×CH_3), 3.10—3.62 (10H, m, 5×CH_2), 3.80 (6H, s, 2×OCH_3), 4.49 (2H, s, CH_2), 6.46 (2H, s, benzene ring 2×CH). *Anal.* Calcd for $C_{23}H_{37}N_3O_4$: 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 $CHCl_3$. mp 178° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3285 (NH), 1680, 1665 (C=O). NMR ($CDCl_3$) δ: 0.87 (3H, m, CH_3), 1.00—1.90 (8H, m, 4×CH_2), 3.20—3.60 (6H, m, piperazine ring 5 and 6 CH_2 and $\underline{CH_2}$–CO–N<), 4.19 (2H, s, CH_2), 4.57 (2H, s, CH_2), 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 $C_{19}H_{26}ClN_3O_3$: 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-Pr_2O). NMR ($CDCl_3$) δ: 0.87 (3H, m, CH_3), 1.00—1.80 (8H, m, 4×CH_2), 1.20 (6H, d, J=6.0 Hz, 2×CH_3), 3.39 (4H, bs, 2×CH_2), 3.20—3.90 (4H, m, CH_2, CH, and NH), 4.49 (2H, s, CH_2), 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 $C_{20}H_{31}N_3O_2$: 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, CH_3), 1.00—1.80 (8H, m, 4×CH_2), 3.40 (4H, bs, 2×CH_2), 3.20—3.50 (2H, m, CH_2), 3.77 (2H, s, CH_2), 4.37 (2H, s, CH_2), 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 $C_{19}H_{27}N_3O_4$: 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 ($CDCl_3$) δ: 0.87 (3H, m, CH_3), 1.04—1.74 (8H, m, 4×CH_2), 3.26—3.62 (6H, m, 3×CH_2), 4.48 (2H, s, CH_2), 4.60 (2H, s, CH_2), 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 $C_{19}H_{26}FN_3O_3$: 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 ($CDCl_3$) δ: 0.86 (3H, m, CH_3), 1.00—1.80 (8H, m, 4×CH_2), 2.94 (2H, t, J=6 Hz, CH_2), 3.20—3.60 (6H, m, 3×CH_2), 3.84 (2H, m, CH_2), 4.61 (2H, s, CH_2), 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 $C_{20}H_{28}ClN_3O_3$: 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 ($CDCl_3$) δ: 0.84 (3H, m, CH_3), 1.02—1.82 (8H, m, 4×CH_2), 1.09 (3H, t, J=7.5 Hz, CH_3), 3.22—4.77 (6H, m, 3×CH_2), 3.75 (2H, s, CH_2), 4.05 (2H, q, J=7.5 Hz, CH_2), 4.64 (2H, s, CH_2), 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*-C_6H_{13}) (0.5 g) and Ac_2O (10 ml) was refluxed for 6 hr and was then evaporated to dryness to give **11h** (0.53 g, 98%) as an oil. IR ν_{max}^{neat} cm^{-1}: 1690 (C=O). NMR ($CDCl_3$) δ: 0.88 (3H, m, CH_3), 1.08—1.83 (8H, m, 4×CH_2), 2.28 (6H, s, 2×$COCH_3$), 3.33—3.65 (6H, m, piperazine ring 5 and 6 CH_2 and $\underline{CH_2}$–CO–N<), 4.64 (2H, s, CH_2), 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 ν_{max}^{KBr} cm^{-1}: 1650 (C=O). NMR ($CDCl_3$) δ: 0.86 (3H, m, CH_3), 1.00—1.85 (14H, m, 7×CH_2), 2.90—3.60 (10H, m, 5×CH_2), 4.54 (2H, s, CH_2), 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 $C_{22}H_{33}N_3O_2$·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 (IIi)——A mixture of **13** (R=*n*-C_6H_{13}) (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 Ac_2O (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 $CHCl_3$. The extract was washed with H_2O and dried over $MgSO_4$. 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 ν_{max}^{KBr} cm^{-1}: 1705, 1670 (C=O). *Anal.* Calcd for $C_{21}H_{27}N_3O_4$: 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*-C_6H_{13}) (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 $CHCl_3$ and the extract was washed with saturated aqueous $NaHCO_3$ and then H_2O and dried over $MgSO_4$. Removal of the solvent *in vacuo* gave a crude solid. Recrystallization from EtOH afforded **14e** as white crystals of mp 159—160°. IR ν_{max}^{KBr} cm^{-1}: 3350 (NH), 1675 (C=O). NMR (DMSO-d_6) δ: 0.83 (3H, m, CH_3), 1.08—1.76 (8H, m, 4×CH_2), 3.23—3.63 (6H, m, piperazine ring 5 and 6

CH_2 and $\underset{\diagdown}{\overset{\diagup}{}}NC\underline{H}_2-$), 4.53 (2H, s, CH_2), 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 $C_{21}H_{27}N_5O_2$: 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 ($CDCl_3$) δ: 0.83 (3H, m, CH_3), 1.08—1.78 (8H, m, $4\times CH_2$), 3.40 (6H, bs, $3\times CH_2$), 4.55 (2H, s, CH_2), 6.50—6.95 (2H, m, CH_2), 7.00—7.90 (6H, m, benzene ring $4\times$CH and pyridine ring CH and NH). *Anal.* Calcd for $C_{22}H_{28}N_4O_2$: 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 ($CDCl_3$) δ: 0.86 (3H, m, CH_3), 1.00—1.80 (8H, m, $4\times CH_2$), 3.37 (4H, bs, $2\times CH_2$), 3.20—3.60 (2H, m, CH_2), 4.03 (2H, s, CH_2), 4.05 (2H, s, CH_2), 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, $Jo=6$ Hz, $Jm=2$ Hz, pyridine ring 4 CH), 8.53 (1H, dd, $Jo=6$ Hz, $Jm=2$ Hz, pyridine ring 6 CH). *Anal.* Calcd for $C_{23}H_{30}N_4O_2$: 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, CH_3), 1.00—1.80 (8H, m, $4\times CH_2$), 3.48 (6H, bs, $3\times CH_2$), 4.54 (2H, s, CH_2), 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 $C_{22}H_{28}N_4O_2$: 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–H_2O). NMR (DMSO-d_6): 0.85 (3H, m, CH_3), 1.00—1.80 (8H, m, $4\times CH_2$), 3.10—3.70 (6H, m, $3\times CH_2$), 4.43 (2H, s, CH_2), 4.68 (2H, s, CH_2), 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 $C_{23}H_{30}N_4O_2\cdot 2HCl\cdot H_2O$: C, 56.91; H, 7.06; N, 11.54. Found: C, 56.80; H, 7.13; N, 11.43. (**14f**): mp 79—81° (Et_2O). NMR ($CDCl_3$) δ: 0.87 (3H, m, CH_3), 1.21 (3H, t, $J=7$ Hz, CH_3), 1.03—1.87 (8H, m, $4\times CH_2$), 3.46 (6H, bs, $3\times CH_2$), 3.98 (2H, q, $J=7$ Hz, CH_2), 4.64 (2H, s, CH_2), 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 $C_{23}H_{31}N_5O_2$: 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, CH_3), 0.94—1.84 (8H, m, $4\times CH_2$), 3.07—3.64 (6H, m, $3\times CH_2$), 4.51 (2H, s, CH_2), 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 $C_{21}H_{27}N_5O_2$: 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 ($CDCl_3$) δ: 0.85 (3H, m, CH_3), 1.00—1.80 (8H, m, $4\times CH_2$), 3.42 (6H, bs, $3\times CH_2$), 4.58 (2H, s, CH_2), 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, $Jo=3$ Hz, $Jm=1.5$ Hz, pyrazine ring CH), 8.28 (1H, d, $J=1.5$ Hz, pyrazine ring CH), 8.28 (1H, s, NH). *Anal.* Calcd for $C_{21}H_{27}N_5O_2$: 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, CH_3), 1.00—1.70 (8H, m, $4\times CH_2$), 3.42 (4H, bs, $2\times CH_2$), 3.20—3.50 (2H, m, CH_2), 4.45 (2H, s, CH_2), 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 $C_{20}H_{26}N_4O_2S$: 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 CF_3COOH (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 ν_{max}^{KBr} cm^{-1}: 1670 (C=O). *Anal.* Calcd for $C_{18}H_{26}N_4O_2S$: C, 59.64; H, 7.23; N, 15.46. Found: C, 59.67; H, 7.45; N, 15.04.

1-*n*-Hexyl-4-[*N*-(*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 ν_{max}^{neat} cm^{-1}: 1670 (C=O). NMR (DMSO-d_6) δ: 0.84 (3H, m, CH_3), 1.00—1.80 (8H, m, $4\times CH_2$), 2.70 (3H, s, SCH_3), 3.50 (6H, bs, piperazine ring 5 and 6 CH_2 and $\underset{\diagdown}{\overset{\diagup}{}}NC\underline{H}_2-$), 4.60 (2H, s, CH_2), 7.32 (4H, bs, benzene ring $4\times$CH), 9.30 (2H, m, $2\times$NH).

1-*n*-Hexyl-4-[4-(2-imidazolinyl)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 $CHCl_3$ (35 ml) and washed with 2.5 N NaOH and then with H_2O and dried over $MgSO_4$. The residue obtained by removal of the solvent *in vacuo* was chromatographed on alumina with $CHCl_3$–AcOEt (20: 1) to give a solid, and further recrystallized from $CHCl_3$–AcOEt to afford **14j** (500 mg, 51%) as white needles of mp 170—171°. IR ν_{max}^{KBr} cm^{-1}: 3325 (NH), 1660 (C=O). NMR ($CDCl_3$) δ: 0.86 (3H, m, CH_3), 1.00—1.90 (8H, m, $4\times CH_2$), 3.40 (6H, bs, piperazine ring 5 and 6 CH_2 and $\underset{\diagdown}{\overset{\diagup}{}}NC\underline{H}_2-$), 3.48 (4H, s, $2\times CH_2$), 4.48 (2H, s, CH_2), 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 $2\times$CH). *Anal.* Calcd for $C_{20}H_{29}N_5O_2$: 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 ν_{max}^{neat} 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°. IR ν_{max}^{KBr} cm^{-1}: 3280 (NH), 1680 (C=O). NMR ($CDCl_3$) δ: 0.88 (3H, m, CH_3), 1.00—1.90 (8H, m, $4\times CH_2$), 3.52 (6H, bs, piperazine ring 5 and 6 CH_2 and $\overset{}{\underset{}{\diagdown}}NC\underline{H}_2-$), 4.50 (2H, bs, $2\times$NH), 4.65 (2H, s, CH_2), 6.65 (2H, s, imidazole ring $2\times$CH), 7.35 (4H, s, benzene ring $4\times$CH). *Anal.* Calcd for $C_{20}H_{27}N_5O_2$: 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 Zn–$CaCl_2$/H_2O–EtOH] and 2-bromopyrimidine (21.6 g) in DMF (50 ml) was stirred at 130—140° 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 ν_{max}^{KBr} cm^{-1}: 3200, 3120 (NH), 1660 (C=O). *Anal.* Calcd for $C_{15}H_{15}N_5O_2$: 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° (EtOH–$CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3320 (NH), 1670 (C=O). NMR (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 $2\times$CH), 7.25 (5H, s,  –), 7.70 (2H, d, $J=8.5$ Hz, benzene ring $2\times$CH), 8.40 (2H, d, $J=4.5$ Hz, pyrimidine ring 4 and 6 CH), 9.68 (1H, s, NH). *Anal.* Calcd for $C_{22}H_{21}N_5O_2$: 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° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 1660 (C=O). NMR (DMSO-d_6) δ: 1.10—1.90 (11H, m, cyclohexane ring $5\times CH_2$ and CH), 3.08—3.57 (6H, m, $3\times CH_2$), 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 $2\times$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), 9.54 (1H, s, NH). *Anal.* Calcd for $C_{22}H_{27}N_5O_2$: 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 ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1670 (C=O). NMR (DMSO-d_6–$CDCl_3$) δ: 2.65—3.00 (2H, m, CH_2), 3.31 (4H, s, $2\times CH_2$), 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 $2\times$CH), 7.15 (5H, s, benzene ring $5\times$CH), 7.69 (2H, d, $J=8.5$ Hz, benzene ring $2\times$CH), 8.36 (2H, d, $J=5$ Hz, pyrimidine ring $2\times$CH), 9.43 (1H, s, NH). *Anal.* Calcd for $C_{23}H_{23}N_5O_2$: C, 68.81; H, 5.77; N, 17.44. Found: C, 68.71; H,5.84; N, 17.22. (**17e**): mp 155—156° (EtOH). IR ν_{max}^{KBr} cm^{-1}: 3290 (NH), 1675 (C=O). NMR (DMSO-d_6) δ: 1.54—2.09 (2H, m, CH_2), 2.34—2.74 (2H, m, CH), 3.42 (6H, bs, $3\times CH_2$), 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 $2\times$CH), 7.17 (5H, s, benzene ring $5\times$CH), 7.70 (2H, d, $J=8.5$ Hz, benzene ring $2\times$CH), 8.39 (2H, d, $J=4.5$ Hz, pyrimidine ring $2\times$CH), 9.55 (1H, s, NH). *Anal.* Calcd for $C_{24}H_{25}N_5O_2$: C, 69.38; H, 6.07; N, 16.89. Found: C, 69.15; H, 6.13; N, 16.59. (**17f**): mp 176—177° (MeOH–$CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3420 (NH), 1675, 1655 (C=O). NMR (DMSO-d_6) δ: 1.51 (3H, d, $J=7.2$ Hz, CH_3), 3.45 (4H, bs, $2\times CH_2$), 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 $2\times$CH), 7.37 (5H, s, benzene ring $5\times$CH), 7.85 (2H, d, $J=8.5$ Hz, benzene ring $2\times$CH), 8.52 (2H, d, $J=4.5$ Hz, pyrimidine ring $2\times$CH), 9.69 (1H, s, NH). *Anal.* Calcd for $C_{23}H_{23}N_5O_2$: C, 68.81; H, 5.78; N, 17.45. Found: C, 68.70; H, 5.76; N, 17.32. (**17i**): mp 214—215° (DMF–iso-PrOH). IR ν_{max}^{KBr} cm^{-1}: 3320 (NH), 1680 (C=O), 1525, 1350 (NO_2). NMR (DMSO-d_6) δ: 3.51 (4H, bs, $2\times CH_2$), 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 $2\times$CH), 7.57 (2H, d, $J=8.5$ Hz, benzene ring $2\times$CH), 7.77 (2H, d, $J=8.5$ Hz, benzene ring $2\times$CH), 8.16 (2H, d, $J=8.5$ Hz, benzene ring $2\times$CH), 8.49 (2H, d, $J=4.5$ Hz, pyrimidine ring $2\times$CH), 9.63 (1H, s, NH). *Anal.* Calcd for $C_{22}H_{20}N_6O_4$: C, 61.10; H, 4.66; N, 19.44. Found: C, 61.02; H, 4.56; N, 19.58. (**17j**): mp 216—217° (EtOH–$CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3275 (NH), 1665 (C=O). NMR (DMSO-d_6): 3.45 (4H, s, $2\times CH_2$), 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 $2\times$CH), 7.30 (4H, s, benzene ring $4\times$CH), 7.67 (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 $C_{22}H_{20}ClN_5O_2$: C, 62.63; H, 4.78; N, 16.60. Found: C, 62.59; H, 4.75; N, 16.41. (**17l**): mp 209° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3375 (NH), 1715, 1670 (C=O). NMR (DMSO-d_6) δ: 3.45 (4H, s, $2\times CH_2$), 3.80 (3H, s, OCH_3),

4.50 (2H, s, CH_2), 4.63 (2H, s, CH_2), 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 $C_{24}H_{23}N_5O_4$: 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 ν_{max}^{KBr} cm^{-1}: 3315 (NH), 1730, 1685 (C=O). NMR (DMSO-d_6) δ: 3.49 (4H, s, 2×CH_2), 3.78 (3H, s, OCH_3), 4.52 (2H, s, CH_2), 4.66 (2H, s, CH_2), 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 $C_{22}H_{21}N_5O_5$: 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 ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1650 (C=O). NMR (DMSO-d_6) δ: 3.52 (4H, s, 2×CH_2), 4.54 (2H, s, CH_2), 4.66 (2H, s, CH_2), 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 $C_{21}H_{20}N_6O_2$: C, 64.93; H, 5.19; N, 21.64. Found: C, 64.68; H, 5.09; N, 21.68. (**17s**): mp 193.5° (MeOH–$CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3225 (NH), 1720, 1675 (C=O). NMR (DMSO-d_6) δ: 3.56 (4H, bs, 2×CH_2), 3.83 (3H, s, OCH_3), 4.55 (2H, s, CH_2), 4.74 (2H, s, CH_2), 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, Jm=1.0 Hz, Jo=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 $C_{23}H_{22}N_6O_4$: 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 ν_{max}^{KBr} cm^{-1}: 3290 (NH), 1662 (C=O). NMR (DMSO-d_6) δ: 3.58 (4H, bs, 2×CH_2), 4.54 (2H, s, CH_2), 4.73 (2H, s, CH_2), 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 $C_{20}H_{19}N_7O_2$: C, 61.69; H, 4.92; N, 25.18. Found: C, 61.47; H, 4.81; N, 25.26. (**17v**): mp 255° ($CHCl_3$–MeOH). IR ν_{max}^{KBr} cm^{-1}: 3280 (NH), 1680 (C=O). NMR (DMSO-d_6) δ: 3.50—3.80 (2H, m, CH_2), 4.02—4.39 (2H, m, CH_2), 4.64 (2H, s, CH_2), 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 $C_{19}H_{17}N_7O_2$: 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 ν_{max}^{KBr} cm^{-1}: 3320 (NH), 1760 (C=O). *Anal.* Calcd for $C_{19}H_{17}N_7O_2 \cdot 1/2H_2O$: C, 59.37; H, 4.72; N, 25.51. Found: C, 60.21; H, 4.63; N, 25.52. (**17x**): mp 220° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3310 (NH), 1680 (C=O). *Anal.* Calcd for $C_{20}H_{19}N_7O_3$: 59.25; H, 4.72; N, 24.18. Found: C, 59.87; H, 5.05; N, 24.46. (**17y**): mp 185° (dec.) (DMF). IR ν_{max}^{KBr} cm^{-1}: 1675 (C=O). *Anal.* Calcd for $C_{19}H_{17}N_7O_3$: 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**)——K_2CO_3 (0.5 g) and activated Cu[6] (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 $CHCl_3$–iso-PrOH to afford **17b** as white needles of mp 205—206°. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1675 (C=O). NMR (DMSO-d_6) δ: 3.47—3.74 (2H, m, piperazine ring CH_2), 3.74—4.06 (2H, m, piperazine ring CH_2), 4.58 (2H, s, CH_2), 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 $C_{21}H_{19}N_5O_2$: 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–$CaCl_2$/EtOH–H_2O 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 ν_{max}^{KBr} cm^{-1}: 3250 (NH), 1670 (C=O). NMR (DMSO-d_6) δ: 3.45 (4H, bs, 2×CH_2), 4.38 (2H, s, CH_2), 4.49 (2H, s, CH_2), 4.81—5.16 (2H, bs, NH_2), 6.48 (2H, d, J=8.5 Hz, benzene fing 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 $C_{22}H_{22}N_6O_2$: 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 ν_{max}^{KBr} cm^{-1}: 3295 (NH), 1660 (C=O). NMR (DMSO-d_6) δ: 3.27 (4H, bs, 2×CH_2), 3.63 (3H, s, OCH_3), 3.65 (3H, s, OCH_3), 4.33 (2H, s, CH_2), 4.37 (2H, s, CH_2), 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 $C_{24}H_{25}N_5O_4$: 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 ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1670 (C=O). NMR (DMSO-d_6) δ: 1.04 (6H, t, J=7.0 Hz, 2×CH_3), 3.27 (4H, q, J=7.0 Hz, 2×CH_2), 3.33 (4H, bs, 2×CH_2), 4.40 (2H, s, CH_2), 4.49 (2H, s, CH_2), 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×CH), 7.14 (2H, d, J=8.5 Hz, benzene ring 2×CH), 7.70 (2H, d, J=8.5 Hz, benzene ring 2×CH), 8.40 (2H, d, J=4.5 Hz, pyrimidine ring 2×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×CH_2), 4.51 (4H, s, 2×CH_2), 6.74 (2H, t, J=4.5 Hz, pyrimidine ring 2×CH), 7.12 (4H, d, J=8.5 Hz, benzene ring 4×CH), 7.64 (4H, d, J=8.5 Hz, benzene ring 4×CH), 8.39 (4H, d, J=4.5 Hz, pyrimidine ring 4×CH), 9.53 (2H, s, 2×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 2 N 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×CH_2), 4.60 (2H, s, CH_2), 4.81 (2H, s, CH_2), 6.80 (1H, t, J=4.8 Hz, pyrimidine ring CH), 7.22 (2H, d, J=9.0 Hz, benzene ring 2×CH), 7.50 (1H, d, J=7.8 Hz, pyridine ring CH), 7.78 (2H, d, J=9.0 Hz, benzene ring 2×CH), 8.28 (1H, dd, Jo=7.8 Hz, Jm=1.5 Hz, pyridine ring CH), 8.47 (2H, d, J=4.8 Hz, pyrimidine ring 2×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 2 N 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×CH_2), 4.43 (2H, s, CH_2), 4.53 (2H, s, CH_2), 5.67—6.62 (2H, m, NH_2), 6.18—6.50 (2H, m, pyridine ring 2×CH), 6.77 (1H, t, J=4.5 Hz, pyrimidine ring CH), 7.18 (2H, d, J=8.5 Hz, benzene ring 2×CH), 7.19—7.31 (1H, m, pyridine ring CH), 7.73 (2H, d, J=8.5 Hz, benzene ring 2×CH), 8.38 (2H, d, J=4.5 Hz, pyrimidine ring 2×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 BDF_1 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 survial 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

1) Part II: T. Hori, C. Yoshida, S. Murakami, R. Takeno, J. Nakano, J. Nitta, H. Tsuda, S. Kishimoto, and I. Saikawa, *Chem. Pharm. Bull.*, **29**, 684 (1981).
2) A.M. Creighton, K. Hellmann, and S. Whitecross, *Nature* (London), **222**, 384 (1969); S. Tsukagoshi, *Gan to Kagakuryoho*, **4**, 1415 (1977); A.M. Creighton and Andrew M., Ger. Offen. Patent, 1910283; Landquist and Justus K., Ger. Offen. Patent, 1941564; J. R. Geigy A-G, Brit. Patent, 961065; J.R. Geigy A-G, Ger. Patent, 1180372.
3) G.R. Pettit, R.B.V. Dreele, D.L. Helald, and M.T. Edgar, *J. Am. Chem. Soc.*, **98**, 6742 (1976); F.M. Schabel, M.W. Trader, W.R. Laster, S.C. Shaddix, and R.W. Brockman, *Cancer Treatment Rep.*, **60**, 1325 (1976); T. Fukuyama, R.K. Frank, and C.F. Jewll, *J. Am. Chem. Soc.*, **102**, 2122 (1980).
4) K. Fukushima, K. Yazawa, and T. Arai, *J. Antibiot.*, **XXVI**, 175 (1973); C. Shin, M. Hayakawa, K. Mikami, and J. Yoshimura, *Tetrahedron Lett.*, **1977**, 863.
5) All melting points are uncorrected. IR spectra were recorded on a Hitachi 215 spectrometer. NMR spectra were measured with a Hitachi R24 (60 MHz) spectrometer. Chemical shift values are expressed in ppm relative to internal tetramethylsilane. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; bs, broad singlet; m, multiplet. pH values were measured with a Toa Denpa HM-5A pH meter.
6) R.Q. Brewster and T. Groening, "Organic Syntheses," Coll. Vol. II, ed. by A.H. Blatt, John Wiley and Sons Inc., New York, 1943, p. 445.