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Studies on Antitumor-active 2,3-Dioxopiperazine Derivatives. IV.[1] Synthesis and Structure-Antitumor Activity Relationship of 1-[4-(2-Pyridylamino)benzyl]-2,3-dioxopiperazine Derivatives

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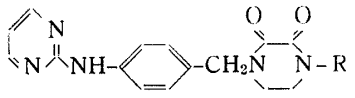
1-Benzyl-4-[4-(2-pyridylamino)benzyl]-2,3-dioxopiperazine derivatives, which are antitumor agents of a new type, were synthesized and the structure-activity relationships were investigated. Furthermore, the antitumor activities of 2,3-dioxopiperazine derivatives were compared with those of 2,5- or 2,6-dioxopiperazines. 1-[4-(5-Amino-6-chloro-2-pyridyl)aminobenzyl]-4-benzyl-2,3-dioxopiperazine (**3a**) showed excellent *in vitro* and *in vivo* antitumor activities. The compound **3a** was obtained by reduction of 1-benzyl-4-[4-(5-nitro-2-pyridyl)aminobenzyl]-2,3-dioxopiperazine (**2d**) with Sn–conc. HCl or $SnCl_2$–hydrogen chloride–MeOH. Reduction products of **2d** obtained under different conditions are discussed.

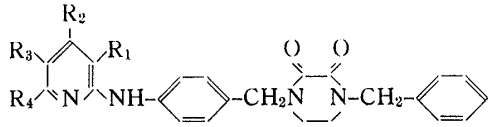
Keywords——new type of antitumor agents; 1-[4-(2-pyridylamino)benzyl]-2,3-dioxopiperazine derivatives; reduction; structure-activity relationship; metabolism; HeLa S3 cells; L1210; Ehrlich ascites carcinoma; 2,5-dioxopiperazine derivatives; 2,6-dioxopiperazine derivative

In the previous paper[1] of a developing study on a new type of antitumor agents having a 2,3-dioxopiperazine moiety, we reported that the compounds **1a**, **b** (shown in Chart 1) possessed excellent antitumor activities, that the 4-(2-pyrimidinylamino)benzyl group of **1** was a significant functional group for antitumor activity, and that the 4-benzyl group of **1a** was essential as a lipophilic functional group. This work was undertaken in order to find more potent antitumor agents than **1**. Compounds **2**, **3** carrying a pyridine ring in place of the pyrimidine ring of **1a** were synthesized and their structure-antitumor activity relationships were investigated.



1a: R = -CH_2- 

1b: R = *n*-C_6H_{13}

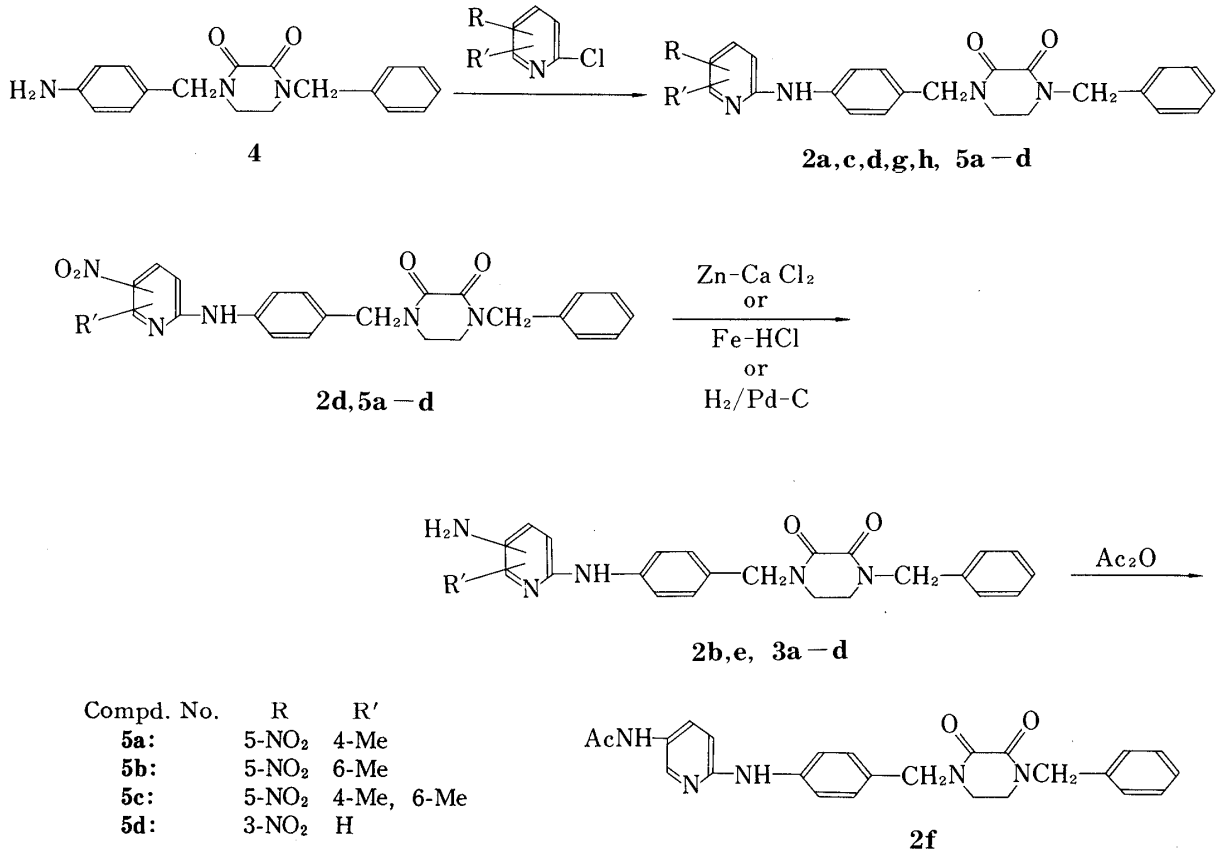


2a—h, 3a—d

Compd. No.	R_1	R_2	R_3	R_4
2a	H	H	H	H
2b	NH_2	H	H	H
2c	Me	H	H	H
2d	H	H	NO_2	H
2e	H	H	NH_2	H
2f	H	H	AcNH	H
2g	H	H	Me	H
2h	H	H	Cl	H
3a	H	H	NH_2	Cl
3b	H	H	NH_2	Me
3c	H	Me	NH_2	H
3d	H	Me	NH_2	Me

Chart 1

First, **2a—h** having a substituted pyridine ring were synthesized by the method shown in Chart 2 and the structure-antitumor activity relationship was studied *in vitro* and *in vivo* (Table I). The results of these investigations of cytotoxic effect on HeLa cells and antitumor activity against Ehrlich ascites carcinoma (EAC) (*i.p.*—*i.p.*) are as follows. 1) Compounds **2b**, **c** which have a CH_3 or NH_2 group at the 3-position on the pyridine ring showed lower



Compd. No.	R	R′
5a:	5-NO_2	4-Me
5b:	5-NO_2	6-Me
5c:	5-NO_2	4-Me, 6-Me
5d:	3-NO_2	H

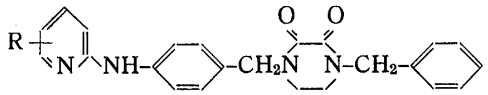
Chart 2

cytotoxicity than the unsubstituted pyridine derivative (**2a**). 2) Compounds **2d**, **f** which have a NO_2 or AcNH group at the 5-position on the pyridine ring also showed lower cytotoxicity than **2a**. 3) Compound **2e** which has an NH_2 group at the 5-position on the pyridine ring showed the highest *in vitro* cytotoxicity [minimum inhibitory concentration (MIC): <0.05 μg/ml against HeLa cells], but no activity was found against *in vivo* Ehrlich ascites carcinoma (EAC) (*i.p.*—*i.p.*). 4) Compound **2g**, in which a CH_3 group (an electron donating substituent, like NH_2) is substituted at the 5-position on the pyridine ring, possessed nearly the same *in vitro* cytotoxicity and *in vivo* antitumor activity against EAC (*i.p.*—*i.p.*) as **2a**. 5) Compound **2h** which has Cl instead of the CH_3 group of **2g** showed *in vitro* activity almost equal to that of **2a**, but no *in vivo* activity was found. Among these compounds, **2a**, **e**, **g** showed moderate antitumor activites against L1210 (*i.p.*—*i.p.*) in a second screening. The high *in vitro* cytotoxicity of **2e** was not reflected in the *in vivo* antitumor activity. This might be due to pharmacodynamic factors, so the metabolism of **2e** in rats was examined by high performance liquid chromatography (HPLC) (Fig. 1). It was found that the 5-NH_2 group on the pyridine ring of **2e** was easily metabolized to the AcNH group, and the acetamide (**2f**) was observed in the blood of rats at 15 min after administration of **2e**.

In the previous paper,[1] we reported that substitution by a Cl or CH_3 group adjacent to the Et_2N group of 1-(4-diethylamino)benzyl-4-*n*-hexyl-2,3-dioxopiperazine (**22**) suppressed the metabolism of the Et_2N group to the AcNH group.

In order to prevent acetylation of **2e**, substituents which would cause steric hindrance to acetylation *in vivo* were introduced at a position adjacent to the 5-NH_2 group on the pyridine ring of **2e**. Compounds **3a**—**d** were synthesized and the structure-activity relationship was investigated (Table II). The compounds **3a**—**c** showed cytotoxicity as high as that of **2e** (MIC values: <0.05 μg/ml.) The 5-amino-6-chloro compound (**3a**) possessed high *in vivo*

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



Compd. No.	*In vitro* MIC (μg/ml)[a] HeLaS3	*In vivo* LD_{50}[b] (mg/kg)	EAC(*i.p.*—*i.p.*)[c] Dose (mg/kg)	EAC(*i.p.*—*i.p.*)[c] T/C (%)	L1210 (*i.p.*—*i.p.*)[d] Dose (mg/kg)	L1210 (*i.p.*—*i.p.*)[d] T/C (%)
2a	0.39—0.78	200	100×6	>213.5 (3/5)[e]	50×2 100×2	136.4 >179.5 (1/5)[e]
2b	25	—	—	—	—	—
2c	6.25	200—500	100×7 200×7	101.8 125	—	—
2d	3.13—6.25	—	—	—	—	—
2e	<0.05	200—500	50×7	93.1	50×9 100×3	164.3 161.9
2f	3.13—6.25	—	—	—	—	—
2g	0.39—0.78	200—500	50×7	>183.5 (1/5)[e]	20×7 100×7	150.7 131.0
2h	0.78	>500	50×7	87.5	—	—

a) Microplate method. Inoculation medium: Eagle's MEM containing 10% calf serum, inoculum size: 2×10^4 cells/ml, 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.*,

$$T/C(\%)=\frac{\text{mean survival time (days) of treated}}{\text{mean survival time (days) of control}}\times100.$$

d) Animal: BDF_1(♂) mice, 6 weeks old, 4 or 5 mice/group. Inoculum size: L1210, 1×10^5 cells/mouse, *i.p.* Treatment: days 1—7, days 1—9 or days 1, 5, 9 *i.p.*
e) 35-day survivors.

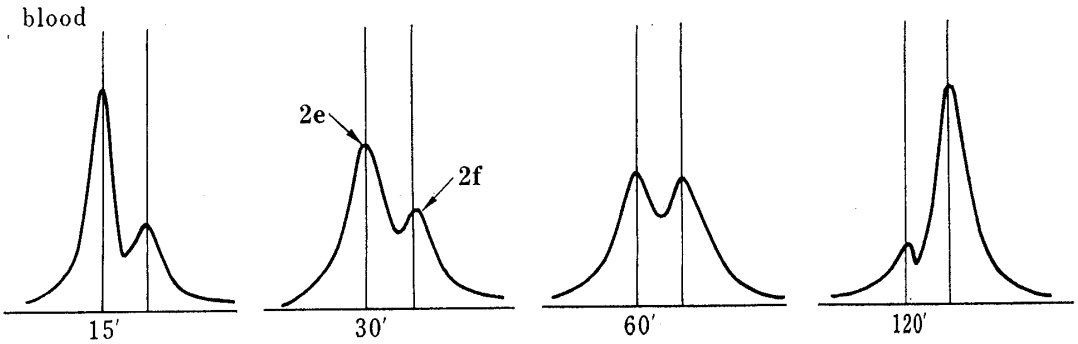


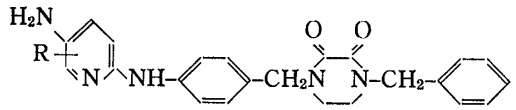
Fig. 1. Metabolism of **2e** in Rats

HPLC [column: LiChrosorb RP-18, (4 mm×25 cm); mobile phase: 30% CH_3CN in pH 8 H_3BO_4-NaOH buffer; detection: OD_{280}]

antitumor activity (against EAC (*i.p.*—*i.p.*), T/C%=>249.2% at 20 mg/kg/day dosage for 7 successive days; against L1210 (*i.p.*—*i.p.*), T/C%=236% at 20 mg/kg/day dosage for 7 successive days). However, the 5-amino-6- or 4-methyl compound (**3b**, **c**) showed no *in vivo* antitumor activity against EAC (*i.p.*—*i.p.*).

Thus, only **3a** showed higher activity than **2e**. In order to clarify the differences between the *in vitro* and *in vivo* activities of **3b**, **c**, the metabolism of **3a**, **c** in mice was studied by thin layer chromatography (TLC) (Fig. 2). The two compounds **3a**, **c** were not as easily metabolized as **2e**. Accordingly, it is suggested that the lower *in vivo* activity of **3c** is a result of factors other than metabolism.

TABLE II. Structure-Antitumor Activity Relationship of 1-[4-(5-Amino- 2-pyridyl)-aminobenzyl]-4-benzyl-2,3-dioxopiperazine Derivatives



Compd. No.	*In vitro* MIC (μg/ml)[a] HeLaS3	*In vivo* LD_{50}[b] (mg/kg)	EAC (*i.p.*—*i.p.*)[c] Dose (mg/kg)	EAC (*i.p.*—*i.p.*)[c] T/C (%)	L1210 (*i.p.*—*i.p.*)[d] Dose (mg/kg)	L1210 (*i.p.*—*i.p.*)[d] T/C (%)
2e	<0.05	200—500	50×7	93.1	50×9	164.3
					100×3	161.9
			5×7	189.6		
3a	<0.05	200—500	20×7	>249.2 (4/5)[e]	20×7	236
			50×4	195		
3b	<0.05	200—500	50×7	108	50×7	148
3c	<0.05	200—500	50×7	109	50×7	143
3d	0.78	200—500	—	—	—	—

a), *b*), *c*), *d*),: See the legend to Table I.
e) 35-day survivors.

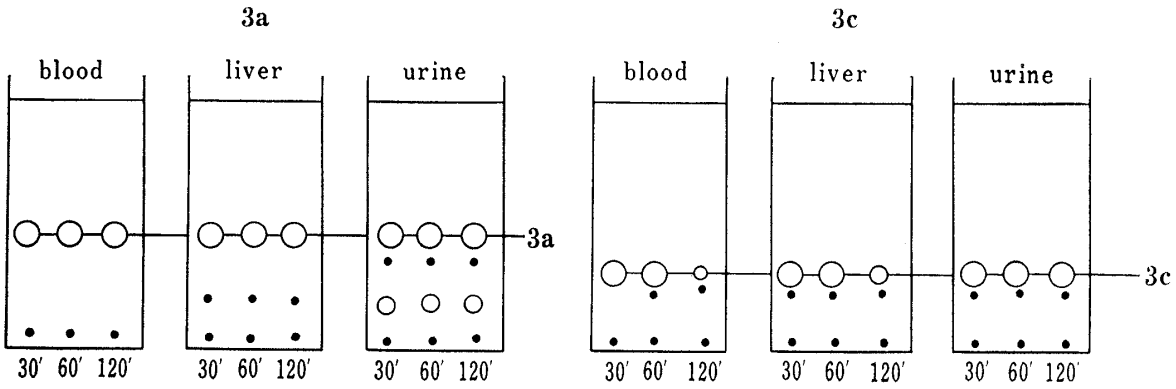


Fig. 2. Metabolism of **3a** and **3c** in Mice
TLC (silica gel, EtOH: $CHCl_3$=1: 9).

Compounds **3b—d** were synthesized by the method shown in Chart 2, but **3a** could not be obtained by the usual method. During the course of studies on the synthesis of **2e**, it was found that reduction of **2d** with Sn–conc. HCl afforded **3a** and **2e**. In general, aromatic nitro compounds give amino compounds on reduction with Zn, Sn, Fe, *etc.* under acidic conditions, but the nitro compound (**2d**) afforded unexpected products besides the normal product under various reduction conditions.

Compound **2d** afforded the amino compound (**2e**) in high yield on catalytic hydrogenation or reduction with Fe–conc. HCl or Zn–$CaCl_2$, but reduction with Sn–conc. HCl gave **2e** and **3a** in the ratio of 1: 1. The structure of **3a** was confirmed by elemental analysis, and infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. The selective synthesis of **3a** was investigated in order to elucidate the reaction mechanism. First, **2e** was treated with Sn–conc. HCl, but **2e** was quantitatively recovered, and **3a** was not obtained. This finding shows that the chlorination does not proceed after reduction of the NO_2 group of **2d**.

Kiewiet *et al.* had reported that reduction of 2-chloronitrobenzene with $SnCl_2$–Ac_2O gave 2,4-dichloroacetanilide.[2] Thus, **2d** was treated with $SnCl_2$–conc. HCl instead of Sn–conc. HCl. Only **2e** was obtained without any **3a**. As a result of some investigations on reduction condi-

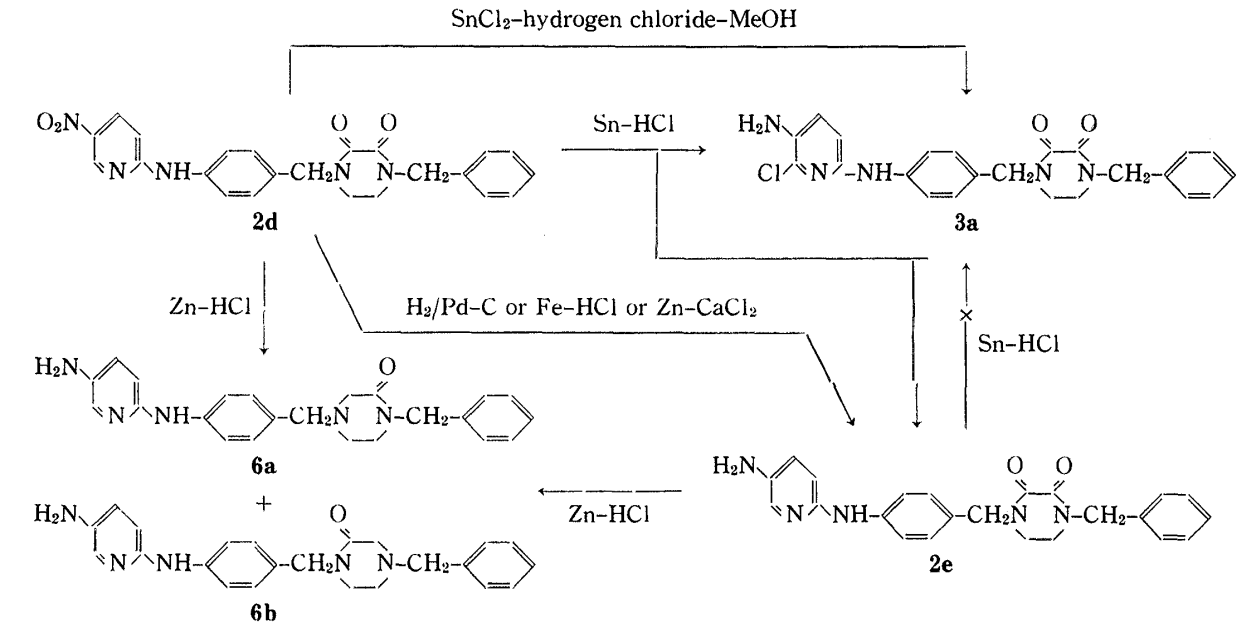


Chart 3

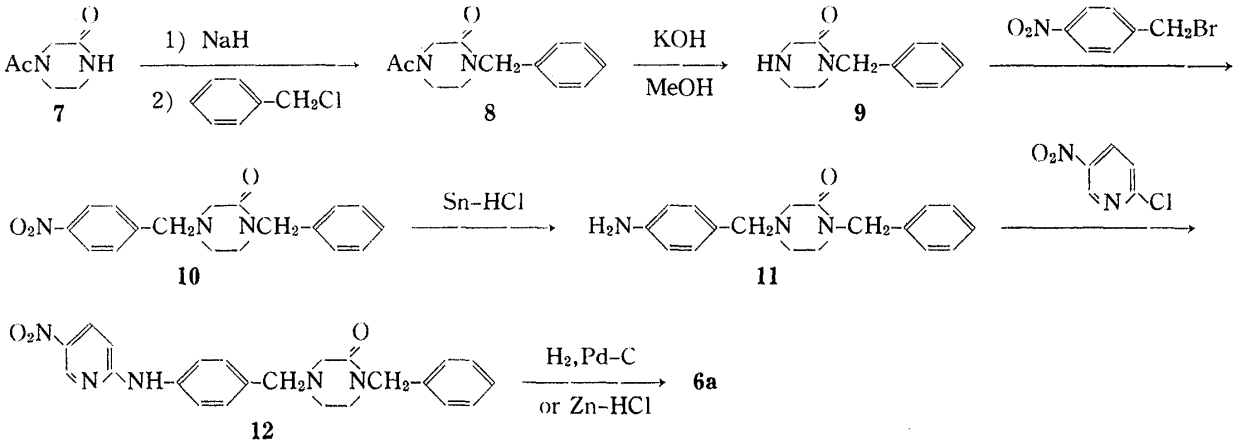


Chart 4

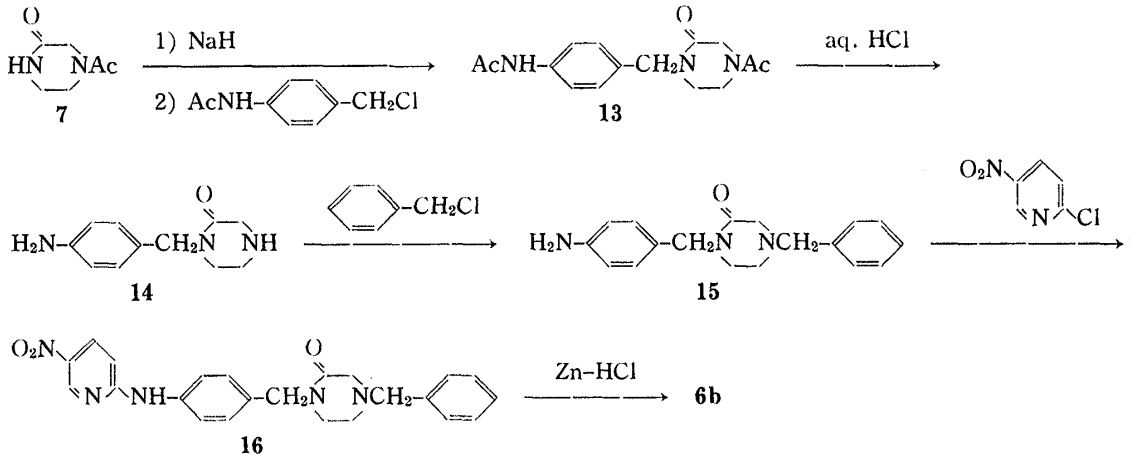


Chart 5

tions, it was found that reduction of **2d** with $SnCl_2$–hydrogen chloride in MeOH gave **3a** selectively. However, the reaction mechanism affording **3a** is still unclear.

Reduction of **2d** with Zn–conc. HCl gave monooxopiperazine derivatives (**6a, b**) in the ratio of 1:1. They were separated by column chromatography. The structures of **6a, b** were assigned by elemental analysis and NMR and IR spectroscopy as two monooxopiperazine isomers having an NH_2 group on the pyridine ring. Each isomer was identified by independent synthesis, as shown in Chart 4 and 5. The monooxopiperazinens (**6a, b**) were not further reduced to piperazines. It was found treatment of **2e** with Zn-conc. HCl similarly gave **6a, b** in the ratio of 1:1, as did the reduction of **2d**. The monooxopiperaznie derivatives (**6a, b**) did not show any antitumor activity.

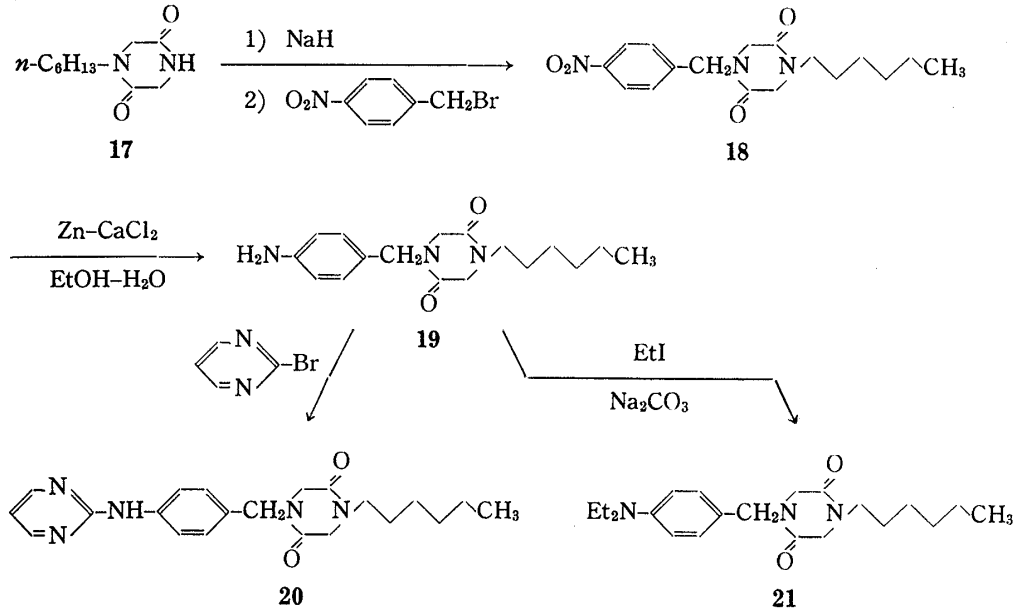
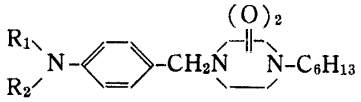


Chart 6

TABLE III. Structure–Antitumor Activity Relationship of 2,3- and 2,5-Dioxopiperazine Derivatives



Compd. No.	R_1, R_2	Dioxo-piperazine	*In vitro* MIC (μg/ml)[a] HeLaS3	*In vivo* LD50[b] (mg/kg)	*In vivo* EAC (*i.p.*–*i.p.*)[c] Dose (mg/kg)	*In vivo* EAC (*i.p.*–*i.p.*)[c] T/C (%)
1b	2-NH-pyrimidin-4-yl	2,3-	0.78—0.39	>1000	50×7	>186.7 (1/4)[e]
					100×7	>206.7 (1/4)[e]
20	2-NH-pyrimidin-4-yl	2,5-	6.25	>500	100×7	102.1
21	$(C_2H_5)_2N$–	2,5-	12.5	—	40×7	104.5
22	$(C_2H_5)_2N$–	2,3-	3.13	100	40×7	157

a), *b*), *c*),: See the legend to Table I,
e) 44-day survivor.

On the other hand, there are many 2,5-dioxopiperazine compounds in nature as amino acid dehydrates, and they have been studied by many workers. Among 2,5-dioxopiperazine compounds, albonoursin[3] and 593A[4] have been reported to possess antitumor activities. Thus, the 2,5-dioxopiperazine derivatives (**20**), (**21**), of which the former corresponds to **1b** and the latter to **22**, were synthesized and their antitumor activities were tested. Compounds **20**, **21** showed no antitumor activity. Among 2,6-dioxopiperazine derivatives, ICRF 159[5] is on the market as an antitumor drug in England. ICRF 159 was compared with **1a** and **3a** which possessed potent antitumor activities in this series. The antitumor activity of ICRF 159 was inferior to those of **1a** and **3a** (Table IV).

Table IV. Antitumor Activity of **1a**, **3a**, and ICRF 159

Compd. No.	*In vitro* MIC (μg/ml)[a] HeLaS3	*In vivo* LD_{50}[b] (mg/kg)	EAC (*i.p.*−*i.p.*)[c] Dose (mg/kg)	EAC (*i.p.*−*i.p.*)[c] T/C (%)	L1210 (*i.p.*−*i.p.*)[d] Dose (mg/kg)	L1210 (*i.p.*−*i.p.*)[d] T/C (%)
1a	0.1	>1000	20×7	217.9	20×7	181.4
			50×7	>226.9 (2/5)[f]	50×7	>262 (1/5)[e]
			100×7	>176.6 (1/4)[f]	100×6	150
					200×3	229
3a	<0.05	200—500	5×7	189.6	20×7	236
			20×7	>249.2 (4/5)[f]		
			50×4	195		
ICRF 159	6.25	500—1000	50×10	132	50×9	169
			100×5	102	200×3	183

a), b), c), d) See the legend to Table I,
e) 30-day survivor,
f) 35-day survivors.

These results suggest that 2,3-dioxopiperazine derivatives have more interesting characteristics than 2,5- or 2,6-dioxopiperazines.

From these studies, it was found that **3a** was the most effective compound among the tested 1-[4-(2-pyridylamino)benzyl]-2,3-dioxopiperazine derivatives. Further studies are in progress.

Experimental[6]

1-Benzyl-4-[4-(2-pyridylamino)benzyl]-2,3-dioxopiperazine (2a)——Compound **2a** was obtained from 1-(4-aminobenzyl)-4-benzyl-2,3-dioxopiperazine (3.10 g) and 2-bromopyridine (1.74 g) by the method described in the previous paper.[1] Yield 3.1 g (80.3%). mp 207—209° (MeOH–$CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3320 (NH), 1660 (C=O). NMR (DMSO-d_6) δ: 3.39 (4H, bs, piperazine ring 5 and 6 CH_2), 4.48 (2H, s, CH_2), 4.54 (2H, s, CH_2), 6.56—6.85 (2H, m, pyridine ring 3 and 5 CH), 7.13—7.37 (1H, m, pyridine ring 4 CH), 7.10 (2H, d, J=8.5 Hz, benzene ring 2×CH), 7.23 (5H, s, C_6H_5), 7.60 (2H, d, J=8.5 Hz, benzene ring 2×CH), 7.98—8.16 (1H, m, pyridine ring 6 CH), 8.94 (1H, s, NH). *Anal.* Calcd for $C_{23}H_{22}N_4O_2$: C, 71.48; H, 5.74; N, 14.50. Found: C, 71.21; H, 5.70; N, 14.40.

Compounds **2c**, **d**, **g**, **h**, **5a**—**d** were similarly obtained. Their physical and analytical data are shown in Table V.

1-[4-(5-Amino-2-pyridyl)aminobenzyl]-4-benzyl-2,3-dioxopiperazine (2e)——By Hydrogenation of **2d**: A suspension of **2d** (7.0 g) and 5% Pd-C (500 mg) in AcOH (150 ml) was shaken at room temperature under atmospheric pressure of H_2. After the absorption of H_2 had ceased, the reaction mixture was filtered through celite and the filtrate was evaporated to dryness. The residue in $CHCl_3$ (100 ml) was extracted with 2 N HCl (100 ml). The extract was adjusted to pH 8 with $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with H_2O and dried over anhydrous $MgSO_4$. Removal of the solvent gave pale reddish-purple crystals (5.6 g, 86%). Recrystallization from EtOH–iso-Pr_2O afforded **2e** of mp 154—155°. IR ν_{max}^{KBr} cm^{-1}: 3340 (NH), 1670 (C=O). NMR (DMSO-d_6) δ: 3.39 (4H, bs, piperazine ring 5 and 6 CH_2), 4.15 (2H, bs, NH),

Table V. Physical and Analytical Data

Compd. No.	mp °C (recryst. solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	NMR (solvent) δ (ppm)
2c	167.5° (iso-PrOH)	$C_{24}H_{24}N_4O_2$	71.98 (71.87	6.04 5.98	13.99 13.75)	($CDCl_3$): 2.20 (3H, s), 3.30 (4H, s), 4.50 (2H, s), 4.55 (2H, s), 6.42—6.70 (2H, m), 7.00—7.56 (10H, m), 7.98 (1H, d)
2d	202—204° (AcOH)	$C_{23}H_{21}N_5O_4$	64.40 (64.55	4.91 4.89	16.23 15.66)	(DMSO-d_6): 3.45 (4H, bs), 4.56 (4H, s), 6.93 (1H, d), 7.20 (2H, d), 7.24 (5H, s), 7.86 (2H, d), 8.16 (1H, dd), 8.90 (1H, d), 10.15 (1H, s)
2g	201—202° (EtOH)	$C_{24}H_{24}N_4O_2$	71.98 (71.80	6.04 6.01	13.99 13.91)	(DMSO-d_6): 2.15 (3H, s), 3.35 (4H, bs), 4.49 (2H, s), 4.56 (2H, s), 6.69 (1H, d), 6.99—7.35 (8H, m), 7.52 (2H, d), 7.89 (1H, m), 8.38 (1H, s)
2h	237—238° (EtOH)	$C_{23}H_{21}ClN_4O_2$	65.63 (65.15	5.03 4.96	13.31 12.91)	(DMSO-d_6): 3.48 (4H, bs), 4.56 (2H, s), 4.62 (2H, s), 6.92 (1H, d,), 7.26 (2H, d), 7.37 (5H, bs), 7.67 (1H, dd), 7.68 (2H, d), 8.16 (1H, d), 9.16 (1H, bs)
5a	215.5—216° (MeOH)	$C_{24}H_{23}N_5O_4$	64.71 (64.59	5.20 5.13	15.72 15.61)	(DMSO-d_6): 2.53 (3H, s), 3.46 (4H, bs), 4.61 (4H, s), 6.66 (1H, s), 7.19 (2H, d), 7.26 (5H, bs), 7.61 (2H, d), 8.84 (1H, s), 9.87 (1H, s)
5b	210—212° (DMSO)	$C_{24}H_{23}N_5O_4$	64.71 (64.60	5.20 5.13	15.72 15.84)	(DMSO-d_6): 2.70 (3H, s), 3.50 (4H, bs), 4.56 (4H, s), 6.73 (1H, d), 7.24 (2H, d), 7.26 (5H, bs), 7.70 (2H, d), 8.11 (1H, d), 9.91 (1H, s)
5c	251—253° (DMSO)	$C_{25}H_{25}N_5O_4$	65.35 (65.06	5.48 5.40	15.24 15.40)	(DMSO-d_6): 2.27 (3H, s), 2.44 (3H, s), 3.44 (4H, bs), 4.51 (2H, s), 4.55 (2H, s), 6.55 (1H, s), 7.18 (2H, d), 7.24 (5H, bs), 7.62 (2H, d), 9.51 (1H, s)
5d	204—205° (DMSO–H_2O)	$C_{23}H_{21}N_5O_4$	64.40 (64.11	4.91 4.80	16.23 16.60)	(DMSO-d_6): 3.55 (4H, bs), 4.69 (4H, s), 6.93—7.08 (1H, m), 7.37 (2H, d), 7.40 (5H, bs), 7.72 (2H, d), 8.53—8.66 (2H, m), 9.91 (1H, s)

4.45 (2H, s, CH_2), 4.54 (2H, s, CH_2), 6.63 (1H, d, J=9.5 Hz, pyridine ring 3 CH), 6.95 (1H, dd, Jo=9.5 Hz, Jm=3 Hz, pyridine ring 4 CH), 7.04 (2H, d, J=9.5 Hz, benzene ring 2×CH), 7.24 (5H, s, C_6H_5), 7.44 (2H, d, J=9.5 Hz, benzene ring 2×CH), 7.60 (1H, d, J=3 Hz, pyridine ring 6 CH), 8.45 (1H, s, NH). *Anal.* Calcd for $C_{23}H_{23}N_5O_2$: C, 68.81; H, 5.77; N, 17.44. Found: C, 68.64; H, 5.83; N, 17.09.

By Reduction of **2d** with Fe-conc. HCl: Fe powder (0.65 g) was added to a solution of **2d** (1.0 g) in conc. HCl (6 ml) in small portions under ice-cooling over a period of 15 min. After the whole had been stirred at 30—40° for 1 hr, the reaction mixture was filtered and the filtrate was diluted with H_2O (30 ml). The solution was adjusted to pH 7.5 with $NaHCO_3$ and extracted twice with $CHCl_3$ (30 ml). The extract was washed with H_2O (30 ml) and dried over Na_2SO_4. Removal of the solvent gave **2e** (0.84 g, 90.3%). mp 154—155° (iso-PrOH). The IR and NMR spectra were identical with those of the compound obtained by catalytic hydrogenation of **2d**.

By Reduction of **2d** with Zn–$CaCl_2$: Zn powder (7.0 g) and $CaCl_2$ (7.0 g) in H_2O (20 ml) were added to a solution of **2d** (7.0 g) in DMF (100 ml) and H_2O (20 ml) at room temperature. After being stirred at 70—80° for 1 hr, the whole was cooled to room temperature and filtered. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ (50 ml) and extracted with 2 N HCl. The extract was neutralized with $NaHCO_3$ and extracted twice with $CHCl_3$ (50 ml). The extract was dried over Na_2SO_4. Removal of the solvent gave **2e** (4.2 g, 64.6%). The IR and NMR spectra were identical with those of the compound obtained by hydrogenation of **2d**.

By Reduction of **2d** with $SnCl_2$–conc. HCl: $SnCl_2 \cdot 2HCl$ (10.5 g) was added to a solution of **2d** (1.0 g) in conc. HCl (30 ml) under ice-cooling. After the whole had been stirred at 50—60° for 2 hr, ice-water (30 ml) and $CHCl_3$ (100 ml) were added to the reaction mixture. The reaction mixture was adjusted to pH 7.5 with 20% NaOH under ice-cooling and the insoluble materials were removed by filtration. The organic layer was separated from the filtrate and dried over Na_2SO_4. Removal of the solvent gave **2e** (0.4 g, 43%). The IR and NMR spectra were identical with those of the compound obtained by hydrogenation of **2d**.

The compounds **2b, 3b—d** were similarly synthesized. Their physical and analytical data are shown in Table VI.

1-[4-(5-Acetamido-2-pyridyl)aminobenzyl]-4-benzyl-2,3-dioxopiperazine (2f)——The amino compound (**2e**) (0.4 g) was acetylated with Ac_2O (2.0 ml) by the usual method. Yield 0.38 g (86%). mp 200—202°

Table VI. Physical and Analytical Data

Compd. No.	mp °C (purification)	Formula	Analysis (%) Calcd (Found) C	H	N	NMR (solvent) δ (ppm)
2b	88—89° (silica gel column)	$C_{23}H_{23}N_5O_2$	68.81 (68.68	5.77 5.85	17.44 17.32)	(DMSO-d_6): 3.41 (4H, bs), 4.53 (2H, s), 4.59 2H, s), 4.50—5.30 (2H, bs), 6.64 (1H, dd), 6.99 (1H, d), 7.18 (2H, d), 7.41 (5H, bs), 7.55 (1H, d), 7.68 (2H, d), 7.88 (1H, s)
3b	176—178° (silica gel column)	$C_{24}H_{25}N_5O_2$	69.38 (69.28	6.06 5.92	16.86 16.75)	(DMSO-d_6): 2.32 (3H, s), 3.21 (4H, bs), 3.30 (2H, bs), 4.44 (2H, s), 4.51 (2H, s), 6.54 (1H, d), 6.71 (1H, s), 6.83 (1H, d), 7.07 (2H, d), 7.14 (5H, bs), 7.20 (2H, d)
3c	160—163° (silica gel column)	$C_{24}H_{25}N_5O_2$	69.38 (69.42	6.06 6.18	16.86 16.73)	($CDCl_3$): 2.05 (3H, s), 3.24 (4H, bs), 3.36 (2H, bs), 4.46 (2H, s), 4.53 (2H, s), 6.62 (1H, s), 6.93—7.30 (10H, m), 7.61 (1H, s)
3d	223—224° (recrys-tallized from MeOH)	$C_{25}H_{27}N_5O_2$	69.91 (69.44	6.34 6.37	16.30 16.06)	(DMSO-d_6): 2.09 (3H, s), 2.29 (3H, s), 3.34 (4H, bs), 3.59 (2H, bs), 4.46 (2H, s), 4.54 (2H, s), 6.46 (1H, s), 7.04 (2H, d), 7.23 (5H, bs), 7.44 (2H, d), 7.99 (1H, s)

(MeOH–iso-Pr_2O). NMR (DMSO-d_6) δ: 1.99 (3H, s, $COCH_3$), 3.39 (4H, bs, piperazine ring $2\times CH_2$), 4.49 (4H, s, $2\times CH_2$), 6.73 (1H, d, $J=7.8$ Hz, pyridine ring 3 CH), 6.99—7.21 (8H, m, benzene ring $2\times$CH, C_6H_5, and pyridine ring 6 CH), 7.53 (2H, d, $J=8.5$ Hz, benzene ring $2\times$ CH), 8.83 (1H, s, NH), 8.22 (1H, m, pyridine ring 4 CH), 9.74 (1H, s, NH). *Anal.* Calcd for $C_{25}H_{25}N_5O_3$: C, 67.71; H, 5.68; N, 15.79. Found: C, 67.52; H, 5.49; N, 15.49.

Reduction of 2d with Sn-conc. HCl——Sn pieces (30 g) were added to a solution of **2d** (5.0 g) in conc. HCl (100 ml). After being stirred at 80—90° for 1 hr, H_2O (400 ml) was added to the reaction mixture. The precipitated crystals (A) and filtrate (B) were obtained. First, a solution of (A) in MeOH (5.0 ml) was adjusted to pH 9 with 40% NaOH, then extracted with $CHCl_3$. The extract was washed with H_2O and dried over Na_2SO_4. Removal of the solvent *in vacuo* gave crude crystals, and purification by chromatography on silica gel with $CHCl_3$ afforded 1-[4-(5-amino-6-chloro-2-pyridyl)aminobenzyl]-4-benzyl-2,3-dioxopiperazine (**3a**) (2.09 g, 41.5%). mp 173—174° (MeOH–CH_2Cl_2). IR ν_{max}^{KBr} cm^{-1}: 1660 (C=O). NMR ($CDCl_3$) δ: 3.21 (4H, bs, piperazine ring 5 and 6 CH_2), 3.59 (2H, bs, NH_2), 4.44 (2H, s, CH_2), 4.51 (2H, s, CH_2), 6.61 (1H, d, $J=8.5$ Hz, pyridine ring 3 CH), 6.89—7.33 (11H, m, pyridine ring 4 CH and benzene ring $9\times$CH and NH). *Anal.* Calcd for $C_{23}H_{22}ClN_5O_2$: C, 63.37; H, 5.09; N, 16.07. Found: C, 63.62; H, 5.22; N, 16.31. The filtrate (B) was adjusted to pH 9 with 40% NaOH and $CHCl_3$ was added. The insoluble materials were filtered off and the organic layer was separated from the filtrate. Subsequently, the $CHCl_3$ layer was extracted with 2 N HCl. The extract was neutralized with 40% NaOH, and extracted with $CHCl_3$. This extract was washed with H_2O and dried over anhydrous Na_2SO_4. The residue obtained by removal of the solvent was purified by column chromatography on basic alumina with $CHCl_3$ to afford **2e** (2.1 g, 44.7%). Recrystallization from EtOH–iso-Pr_2O afforded pale reddish-purple crystals of mp 154—155°. The IR and NMR spectra were identical with those of the product obtained by catalytic hydrogenation of **2d**.

Reduction of 2d with $SnCl_2$–Hydrogen Chloride in MeOH——A suspension of **2d** (2.0 g) in MeOH (30 ml) was saturated with hydrogen chloride under reflux. The reaction mixture became homogeneous. A solution of anhydrous $SnCl_2$ (3.7 g) in MeOH (10 ml) was added to the reaction mixture over a period of 1 hr under reflux. The mixture was cooled to room temperature, then $CHCl_3$ (40 ml) and H_2O (20 ml) were added and the pH was adjusted to 1.5 with saturated aqueous $NaHCO_3$. The insoluble materials were filtered off and the filtrate was adjusted to pH 7.5 with saturated aqueous $NaHCO_3$. The insoluble materials were filtered off and the organic layer was dried over anhydrous $MgSO_4$. Removal of the solvent afforded **3a** (1.55 g, 78%). Recrystallization from 1,2-dichloroethane gave pure **3a** of mp 173.5—174°. The IR and NMR spectra were identical with those of **3a** obtained by reduction of **2d** with Sn-conc. HCl.

Reduction of 2d with Zn-conc. HCl——Zn powder (4.0 g) was added to a solution of **2d** (4.0 g) in conc. HCl (50 ml) in small portions. After being stirred at room temperature for 30 min, the reaction mixture was filtered through celite. The filtrate was neutralized with 40% NaOH under ice-cooling and extracted with $CHCl_3$. The extract was washed with saturated aqueous NaCl and dried over Na_2SO_4. The residue obtained by removal of the solvent *in vacuo* was purified by column chromatography on silica gel. Elution with $CHCl_3$–EtOH (30: 1), provided first **6b** (1.5 g) and next **6a** (1.4 g), which were isolated from the eluates as amorphous solids.

Properties of **6b**: mp 72—74°. TLC; $Rf=0.27$ (plate, silica gel; developing solvent, acetone–$CHCl_3$= 2: 1). IR ν_{max}^{KBr} cm^{-1}: 3400, 3320, 3210 (NH), 1625 (C=O), 1090, 1070, 1055, 910, 895, 750. NMR ($CDCl_3$)

δ: 2.55 (2H, m, piperazine ring CH_2), 3.19 (2H, s, piperazine ring CH_2), 3.07—3.28 (2H, m, piperazine ring CH_2), 3.23 (2H, s, NH_2), 3.48 (2H, s, $-NCH_2-C_6H_5$), 4.47 (2H, s, $-N-C_6H_4-CH_2-N-CO-$), 6.59—6.98 (3H, m, pyridine ring 2×CH and NH), 7.12 (4H, bs, benzene ring 4×CH), 7.24 (5H, bs, C_6H_5), 7.69 (1H, d, $J=2.5$ Hz, pyridine ring 6 CH). *Anal.* Calcd for $C_{23}H_{25}N_5O$: C, 71.29; H, 6.50; N, 18.07. Found: C, 70.99; H, 6.48; N, 18.50.

Properties of **6a**: mp 63—64°. TLC; $Rf=0.21$ (plate, silica gel; developing solvent, acetone–$CHCl_3$=2:1). IR ν_{max}^{KBr} cm^{-1}: 3400, 3320, 3200 (NH), 1625 (C=O), 1075, 1050, 890, 728. NMR ($CDCl_3$) δ: 2.52 (2H, m, piperazine ring CH_2), 3.04—3.28 (2H, m, piperazine ring CH_2), 3.18 (2H, s, piperazine ring CH_2), 3.40—3.80 (2H, bs, NH_2), 3.41 (2H, s, $N-C_6H_4-CH_2-N-$), 4.52 (2H, s, $-CON-CH_2-C_6H_5$), 6.58—7.01 (3H, m, pyridine ring 2×CH and NH), 7.12 (4H, bs, benzene ring 4×CH), 7.23 (5H, bs, C_6H_5), 7.65 (1H, d, $J=2.5$ Hz, pyridine ring 6 CH). *Anal.* Calcd for $C_{23}H_{25}N_5O$: C, 71.29; H, 6.50; N, 18.07. Found: C, 71.10; H, 6.44; N, 17.98.

1-Acetyl-4-benzyl-3-oxopiperazine (8)——Compound **8** was obtained as an oil from 1-acetyl-3-oxopiperazine (**7**) and benzyl chloride by the method described in the previous paper.[1] Yield, 91.8%. IR ν_{max}^{neat} cm^{-1}: 1640 (C=O).

1-Benzyl-2-oxopiperazine (9)——Compound **8** (6.6 g) was added to a solution of KOH (1.75 g) in MeOH (20 ml) and after the whole had been refluxed for 24 hr, the reaction mixture was evaporated to dryness. H_2O (20 ml) and $CHCl_3$ (50 ml) were added to the residue and the organic layer was extracted with 2 N HCl. The extract was made alkaline with 2 N NaOH and extracted with $CHCl_3$. The extract was washed with H_2O and dried over anhydrous Na_2SO_4. Removal of the solvent *in vacuo* gave **9** as an oil (24.5 g, 83.3%). IR ν_{max}^{neat} cm^{-1}: 3270—3290 (NH), 1620—1630 (C=O).

1-Benzyl-4-(4-nitrobenzyl)-2-oxopiperazine Hydrochloride (10·HCl)——A solution of 4-nitrobenzyl bromide (5.3 g) in $CHCl_3$ (20 ml) was treated dropwise with a solution of **9** (4.5 g) and triethylamine (3.3 ml) at −20° over a period of 20 min. After being stirred for 2 hr at the same temperature, the reaction mixture was washed with H_2O (30 ml). The organic layer was separated, H_2O (10 ml) was added, and the solution was acidified with 2 N HCl. The precipitated crystals were collected by filtration and successively washed with $CHCl_3$ and iso-Pr_2O and recrystallized from MeOH. Yield 5.2 g (60.7%). mp 229—230°. IR ν_{max}^{KBr} cm^{-1}: 3320—2660 (NH^+), 1645 (C=O), 1518, 1339 (NO_2). *Anal.* Calcd for $C_{18}H_{19}N_3O_3\cdot HCl$: C, 59.57; H, 5.57; N, 11.61. Found: C, 59.60; H, 5.53; N, 11.51.

1-(4-Aminobenzyl)-4-benzyl-3-oxopiperazine (11)——Sn pieces (5.0 g) were added to **10**·HCl (5.0 g) in conc. HCl (50 ml) and the whole was refluxed for 1 hr. The unchanged Sn was removed by filtration. The filtrate was neutralized with 2 N NaOH and extracted with $CHCl_3$. The extract was washed with H_2O and dried over Na_2SO_4. The residue obtained by removal of the solvent was purified by column chromatography on silica gel with $CHCl_3$–EtOH to give an oily substance (2.4 g, 52.8%). IR ν_{max}^{neat} cm^{-1}: 3450, 3360, 3240 (NH), 1640 (C=O). NMR ($CDCl_3$) δ: 2.51 (2H, t, $J=5.4$ Hz, piperazine ring CH_2), 3.12 (2H, t, $J=5.4$ Hz, piperazine ring CH_2), 3.17 (2H, s, piperazine ring CH_2), 3.35 (2H, s, CH_2), 3.58 (2H, bs, NH_2), 4.49 (2H, s, CH_2), 6.47 (2H, d, $J=7.8$ Hz, benzene ring 2×CH), 6.93 (2H, d, $J=7.8$ Hz, benzene ring 2×CH), 7.14 (5H, bs, C_6H_5). A solution of this oily substance in EtOH was treated with hydrogen chloride to yield **11**·2HCl as crystals. mp 194—195° (MeOH–Et_2O). *Anal.* Calcd for $C_{18}H_{21}N_3O\cdot 2HCl$: C, 58.70; H, 6.29; N, 11.41. Found: C, 58.92; H, 6.33; N, 11.50.

1-Benzyl-4-[4-(5-nitro-2-pyridyl)aminobenzyl]-2-oxopiperazine (12)——Compound **12** was obtained from **11** (1.2 g) and 2-chloro-5-nitropyridine (0.71 g) as described for the preparation of **2a**. Yield 0.8 g (47.2%). mp 160° (purified by silica gel column chromatography with $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3280 (NH), 1620 (C=O), 1325 (NO_2). NMR ($CDCl_3$) δ: 2.40—2.80 (2H, m, piperazine ring CH_2), 3.10—3.40 (4H, m, piperazine ring 2×CH_2), 3.50 (2H, s, CH_2), 4.54 (2H, s, CH_2), 6.71 (1H, d, $J=9$ Hz, pyridine ring 3 CH), 6.90—7.55 (9H, m, benzene ring 4×CH and C_6H_5), 8.05 (1H, dd, $Jo=9$ Hz, $Jm=3$ Hz, pyridine ring 4 CH), 8.85—9.05 (2H, m, pyridine ring 6 CH and NH). *Anal.* Calcd for $C_{23}H_{23}N_5O_3$: C, 66.17; H, 5.55; N, 16.78. Found: C, 66.02; H, 5.48; N, 16.55.

1-[4-(5-Amino-2-pyridyl)aminobenzyl]-4-benzyl-3-oxopiperazine (6a)——Compound **6a** was obtained by catalytic hydrogenation of **12** (0.2 g) over 5% Pd–C. Yield 0.1 g (52.6%). The physico-chemical properties were identical with those of **6a** obtained by reduction of **2d** with Zn–conc. HCl.

1-(4-Acetylaminobenzyl)-4-acetyl-2-oxopiperazine (13)——Compound **13** was obtained as an amorphous solid by the procedure used to prepare **8** and was purified by silica gel column chromatography with $CHCl_3$–EtOH. Yield 83.1%. IR ν_{max}^{KBr} cm^{-1}: 3250, 3270 (NH), 1625 (C=O).

1-(4-Aminobenzyl)-2-oxopiperazine (14)——A mixture of 2 N HCl (160 ml) and **13** (8.9 g) was refluxed for 1.5 hr, then the reaction mixture was concentrated to about one-tenth of the original volume. The concentrated solution was neutralized with $NaHCO_3$ and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel column chromatography with $CHCl_3$–EtOH to afford white crystals (3.8 g, 60.3%). mp 96—99° (iso-PrOH–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 3400, 3300, 3220 (NH), 1615 (C=O). *Anal.* Calcd for $C_{11}H_{15}N_3O$: C, 64.36; H, 7.37; N, 20.47. Found: C, 64.18; H, 7.31; N, 20.19.

1-(4-Aminobenzyl)-4-benzyl-2-oxopiperazine hydrochloride (15·HCl)——The free base was obtained as an oil in 51% yield by the procedure used to prepare **10**, and a solution of **15** in EtOH was treated with hydrogen chloride to give the hydrochloride. mp 232—234° (MeOH–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O). NMR

($CDCl_3$): 2.51 (2H, t, J=5.4 Hz, piperazine ring CH_2), 3.13 (2H, t, J=5.4 Hz, piperazine ring CH_2), 3.17 (2H, s, piperazine ring CH_2), 3.46 (2H, s, CH_2), 3.61 (2H, bs, NH_2), 4.38 (2H, s, CH_2), 6.48 (2H, d, J=7.8 Hz, benzene ring 2×CH), 6.94 (2H, d, J=7.8 Hz, benzene ring 2×CH), 7.19 (5H, bs, C_6H_5). *Anal.* Calcd for $C_{18}H_{21}N_3O \cdot 2HCl$: C, 58.70; H, 6.29; N, 11.41. Found: 58.43; H, 6.36; N, 11.27.

1-Benzyl-4-[4-(5-nitro-2-pyridyl)aminobenzyl]-3-oxopiperazine (16)——Compound **16** was obtained by the procedure used to prepare **2a**. Recrystallization from $CHCl_3$–MeOH–AcOEt gave yellow needles of mp 119—200°. IR ν_{max}^{KBr} cm^{-1}: 1625 (C=O), 1322 (NO_2). NMR (DMSO-d_6): 2.60—2.80 (2H, m, piperazine ring CH_2), 3.07 (2H, s, piperazine ring CH_2), 3.00—3.40 (2H, m, piperazine ring CH_2), 3.50 (2H, s, CH_2), 4.45 (2H, s, CH_2), 6.83 (1H, d, J=9.0 Hz, pyridine ring 3 CH), 7.15 (2H, d, J=8.4 Hz, benzene ring 2×CH), 7.22 (5H, bs, C_6H_5), 7.61 (2H, d, J=8.4 Hz, benzene ring 2×CH), 8.16 (1H, dd, Jo=9.0 Hz, Jm=3.0 Hz, pyridine ring 4 CH), 8.92 (1H, d, J=3.0 Hz, pyridine ring 6 CH), 10.18 (1H, s, NH). *Anal.* Calcd for $C_{23}H_{23}N_5O_3$: C, 66.17; H, 5.55; N, 16.78. Found: C, 65.82; H, 5.41; N, 16.69.

1-[4-(5-Amino-2-pyridyl)aminobenzyl]-4-benzyl-2-oxopiperazine (6b)——Zn powder (0.8 g) was added to a solution of **16** (1.0 g) in 2 N HCl (30 ml). The mixture was stirred at room temperature for 30 min, then the insoluble materials were filtered off. The filtrate was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by basic alumina column chromatography with $CHCl_3$ to give a pale reddish-purple amorphous solid (0.6 g, 64.5%). This solid was identical with **6b** obtained by the reduction of **2d** with Zn-conc. HCl.

Reduction of 2e by Zn-conc. HCl——A suspension of **2e** (1.0 g) and Zn powder (1.0 g) in conc. HCl (20 ml) was treated as described for the reduction of **2d** with Zn–conc. HCl to give **6a** (0.3 g) and **6b** (0.4 g).

1-*n*-Hexyl-4-(4-nitrobenzyl)-2,5-dioxopiperazine (18)——Compound **18** was obtained from **17**[7] and 4-nitrobenzyl bromide as described in the previous paper.[1] mp 107—108° (AcOEt–iso-Pr_2O). IR ν_{max}^{KBr} cm^{-1}: 1660 (C=O), 1518, 1342 (NO_2). *Anal.* Calcd for $C_{17}H_{23}N_3O_4$: C, 61.25; H, 6.95; N, 12.60. Found: C, 60.98; H, 6.90; N, 12.45.

1-(4-Aminobenzyl)-4-*n*-hexyl-2,5-dioxopiperazine (19)——Zn powder (40 g) and anhydrous $CaCl_2$ (8.0 g) were added to **18** (8.0 g) in EtOH (80 ml) and H_2O (70 ml). After the whole had been refluxed for 2 hr, the reaction mixture was filtered through celite and the filtrate was concentrated to one-half of the original volume. The concentrated solution was extracted with $CHCl_3$ (100 ml) and the extract was washed with saturated aqueous NaCl and dried over anhydrous $MgSO_4$. The residue obtained by concentration *in vacuo* was recrystallized from AcOEt–iso-Pr_2O to give **19** of mp 86—88° (6.3 g, 86.5%). IR ν_{max}^{KBr} cm^{-1}: 3475, 3350 (NH), 1665 (C=O). NMR ($CDCl_3$): 0.85 (3H, m, CH_3), 1.10—1.80 (8H, m, 4×CH_2), 3.30 (2H, t, J=7.2 Hz, –CO–NC$\underline{H}_2$–), 3.77 (2H, s, piperazine ring CH_2), 3.92 (2H, s, piperazine ring CH_2), 3.99 (2H, s, NH_2), 4.36 (2H, s, CH_2), 6.48 (2H, d, J=9.0 Hz, benzene ring 2×CH), 6.95 (2H, d, J=9.0 Hz, benzene ring 2×CH). *Anal.* Calcd for $C_{17}H_{25}N_3O_2$: C, 67.30; H, 8.31; N, 13.85. Found: C, 67.12; H, 8.29; N, 13.67.

1-*n*-Hexyl-4-[4-(2-pyrimidinylamino)benzyl]-2,5-dioxopiperazine (20)——Compound **20** was obtained from **19** (1.0 g) and 2-chloropyrimidine (0.4 g) by the usual method for this series. Yield 1.0 g (79.5%). mp 132.5—134° (iso-PrOH). IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1655 (C=O). NMR (DMSO-d_6) δ: 0.85 (3H, m, CH_3), 1.02—1.66 (8H, m, 4×CH_2), 3.83 (2H, s, piperazine ring CH_2), 4.00 (2H, s, piperazine ring CH_2), 4.46 (2H, s, CH_2), 6.76 (1H, t, J=4.5 Hz, pyrimidine ring 5 CH), 7.14 (2H, d, J=9 Hz, benzene ring 2×CH), 7.70 (2H, d, J=9 Hz, benzene ring 2×CH), 8.40 (2H, d, J=4.5 Hz, pyrimidine ring 4 and 6 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, 66.26; H, 7.13; N, 18.22.

1-(4-Diethylaminobenzyl)-4-*n*-hexyl-2,5-dioxopiperazine (21)——After a suspension of **19** (1.3 g), EtI (3.4 g), and Na_2CO_3 (1.8 g) in EtOH (40 ml) and H_2O (30 ml) had been refluxed for 5 hr, the solvent was removed *in vacuo*. H_2O and $CHCl_3$ were added to the residue. The organic layer was washed with H_2O and dried over $MgSO_4$. The residue obtained by removal of the solvent *in vacuo* was purified by column chromatography on silica gel to afford **21**. mp 65—67° (iso-Pr_2O–pet. ether). IR ν_{max}^{KBr} cm^{-1}: 1665 (C=O). *Anal.* Calcd for $C_{21}H_{33}N_3O_2$: C, 70.16; H, 9.26; N, 11.69. Found: C, 70.40; H, 9.36; N, 11.61.

Cytotoxicity against HeLa S3 Cells (Minimum Inhibitory Concentration: MIC Value)——MIC values of test compounds were determined by the method described in an earlier paper.[8]

Acute Toxicity to Mice——Two 5-week-old SLC-ICR mice (weighing 20—21 g) per group were used. One group received test compounds suspended in 0.3% CMC (carboxymethylcellulose)—physiological saline or dissolved in physiological saline intraperitoneally once. The number of deaths was noted 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 one group consisting of five 6-week-old female ICR mice, weighing 21—22 g. Test compounds suspended in 0.3% CMC-saline or dissolved in saline were intraperitoneally administered daily from the day after transplantation for 7 successive days. The antitumor activities were evaluated in terms of the mean survival time in days compared with the controls (T/C%).

Antitumor Activity against L1210 (*i.p.-i.p.*)——L1210 cells (3×10^5 cells/head/0.2 ml physiological saline) were intraperitoneally transplanted into one group consisting of five 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 daily from the day after transplantation for 7 successive days. The antitumor activities were evaluated in terms of the mean survival time in days compared with the controls (T/C%).

Metabolism of Test Compounds in Mice——Test compounds (50 mg/kg) suspended in 0.3% CMC-saline were intraperitoneally administered to ICR female mice or rats (two per group) in which the urethral meatuses had been closed. Six groups, consisting of two mice per group, were used. At 5, 15, 30, 60, and 120 min after administration, mice or rats 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 or to high performance liquid chromatography. One group was treated in the same way without administration of test compounds; this was used as a control group.

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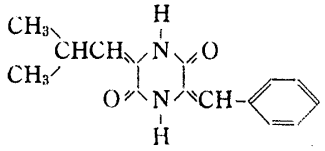

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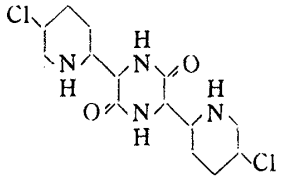

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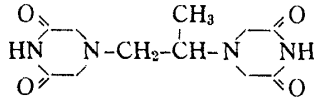

6) All melting points are uncorrected. IR spectra were recorded on a Hitachi 215 spectrometer. NMR spectra were measured with a Hitachi R24 (60 MHz) spectrometer. Chemical shift values are expressed in ppm relative to internal tetramethylsilane. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; bs, broad singlet; HPLC was run on a Shimadzu LC-1 unit with a UV detector, using a column packed with LiChrosorb RP-18 (10 μm, 4 mmϕ × 25 cm) (Merck). pH values were measured with a Toa Denpa HM-5A pH meter.
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