to be considered active at a given dose. The results appear in Tables I and II.

Enhancement of NK-Cell Activity in Mice. Duplicate groups of six normal BDF_1 mice were given a single oral dose of **21** at 150 to 800 mg/kg or a single intraperitoneal dose of poly IC at 10 mg/kg. Eighteen hours later, mice were bled from the retro-orbital sinus, and the serum from each group was pooled. Assays of serum interferon were carried out as described in ref 7. Spleens from these same mice were removed and pooled, and erythrocyte-free spleen cell suspensions were prepared. Each spleen cell suspension was assayed for NK-cell cytotoxicity, in triplicate. Briefly, spleen cells (5×10^6) and Molt-4 human tumor target cells (1×10^5 cells labeled with chromium-51) were cocultured in 1.0 mL of Eagles minimum essential medium, supplemented with 10% fetal calf serum, for 4 h at 37 °C. After incubation, the cultures were chilled to 4 °C and centrifuged to pellet cells and cell debris, and aliquots of cell-free supernatant were removed for counting of chromium-51 released by lysed Molt-4 cells. The percent cytotoxicity of each spleen assay was calculated in the manner shown by eq 1. The spontaneous release

$$\% \text{ cytotoxicity} = (\text{cpm of } ^{51}\text{Cr in test} - \text{cpm of } ^{51}\text{Cr spontaneously released in supernatant}) / (\text{total cpm of } ^{51}\text{Cr per } 10^5 \text{ Molt-4 cells} - \text{cpm of } ^{51}\text{Cr spontaneously released in supernatant}) \times 100 \quad (1)$$

of chromium-51 into the supernatant during the 4-h incubation period was determined in separate assays of Molt-4 cells cultured in the absence of added spleen cells.

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Oral administration of 150–300 mg of **21** to normal hybrid BDF_1 mice produced an increase in NK-cell activity and an increase in serum interferon levels, in relation to the placebo-treated controls (Table III).

Acknowledgment. We thank Dr. W. Gore, G. Morton, and colleagues for spectral data and L. Brancone and staff for microanalyses.

Registry No. **2a**, 79940-05-9; **2a**·3HCl, 43129-68-6; **2b**, 43129-67-5; **2c**, 87040-55-9; **2d**, 87040-56-0; **2d**·3HCl, 79940-07-1; **2e**, 79940-00-4; **2e**·3HCl, 79939-92-7; **2f**, 87040-57-1; **2f**·3HCl, 79939-88-1; **2g**, 87040-58-2; **2g**·3HCl, 79939-87-0; **2h**, 87040-59-3; **2h**·3HCl, 79939-93-8; **2i**, 87040-60-6; **2i**·3HCl, 79939-89-2; **2j**, 79939-90-5; **2j**·3HCl, 79939-91-6; **2k**, 87040-61-7; **2l**, 81541-32-4; **2l**·3HCl, 81541-26-6; **2m**, 81541-35-7; **2n**, 43129-69-7; **2n**·3HCl, 87040-62-8; **2o**, 79940-01-5; **2o**·3HCl, 79939-95-0; **2p**, 87050-16-6; **2p**·3HCl, 79939-96-1; **2q**, 87040-63-9; **2q**·HCl, 87040-64-0; **2r**, 87040-65-1; **2r**·3HCl, 79939-99-4; **2s**, 87040-66-2; **2s**·3HCl, 81541-25-5; **2t**, 87040-67-3; **2t**·HCl, 87040-68-4; **2u**, 87040-69-5; **2u**·3HCl, 87040-70-8; **3a**, 43129-74-4; **3a** hemisulfate, 87040-75-3; **3b** hemisulfate, 87040-77-5; **6a**, 87040-78-6; **6a**·HCl, 79940-06-0; **6b**·HCl, 79939-94-9; **7**, 135-49-9; **8**, 87050-17-7; **8**·3HCl, 87050-18-8; **9a**, 79939-85-8; **9b**, 79939-86-9; **9c**, 79939-84-7; **9d**, 79939-83-6; **9e**, 87040-71-9; **9f**, 87040-72-0; **9f**·4HCl, 87040-73-1; **9g**, 87040-74-2; 2-(dimethylamino)ethyl chloride hydrochloride, 4584-46-7; 2-(diethylamino)ethyl chloride hydrochloride, 869-24-9; 2-chloro-1,1,*N*,*N*-tetramethylethylamine hydrochloride, 1484-36-2; methanamine, 74-89-5; 2-chloroethyl *p*-toluenesulfonate, 80-41-1; 3-chloropropyl *p*-toluenesulfonate, 632-02-0; ethanamine, 75-04-7; 1-propanamine, 107-10-8; 2-propanamine, 75-31-0; 1-butanamine, 109-73-9; 2-aminoethanol, 141-43-5; pyrrolidine, 123-75-1; piperidine, 110-89-4; 1-methylpiperazine, 109-01-3; diethylamine, 109-89-7; dimethylamine, 124-40-3; 2-amino-1-butanol, 96-20-8.

Heterocyclic Quinones. 4. A New Highly Cytotoxic Drug: 6,7-Bis(1-aziridinyl)-5,8-quinazolinedione

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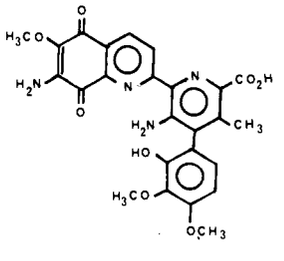
With the aim of obtaining new antitumoral agents, a series of 5,8-quinazolinediones was prepared. 5-Amino-6-methoxyquinazoline was oxidized by Fremy's salt to give 6-methoxy-5,8-quinazolinedione. Nucleophilic substitution reaction at C_6, electrophilic substitution at C_7, and synthesis of 7-amino-6-methoxy-5,8-quinazolinedione, the parent compound of streptonigrin, were studied. These compounds were tested for cytotoxic properties on L1210 leukemia cells in vitro. One of them, 6,7-bis(1-aziridinyl)-5,8-quinazolinedione, which exhibits a high cytotoxic activity (ID50 = 0.08 μM), was further screened in standard antitumor systems, including L1210 leukemia, P388 lymphocytic leukemia, sarcoma 180, and B16 melanocarcinoma. This drug gives a significant antitumoral effect on P388 leukemia but is inactive on other experimental models. Moreover, this compound was found to be highly mutagenic for *Salmonella typhimurium* TA98 and TA100 strains (Ames test), suggesting that DNA damage could be responsible for its cytotoxicity.

It has been shown that the structural element essential to the antitumor activity of streptonigrin (1) was the 7-amino-6-methoxy-5,8-quinolinedione nucleus.[1] In order to investigate the role of the heterocyclic nucleus, we performed a comparative study of its structural analogues. In previous reports, we described the nitrogen heterocyclic quinones 1,4-acridinediones,[2] 5,6- and 5,8-quinoxalinediones,[3] and 7,10-benzo[*f*]quinolinediones.[4] This paper describes the synthesis and some biological properties of 5,8-quinazolinedione derivatives.



1

Chemistry. Few 5,8-quinazolinediones have been previously reported. Malesani[5] prepared 5,8-quinazolinedione

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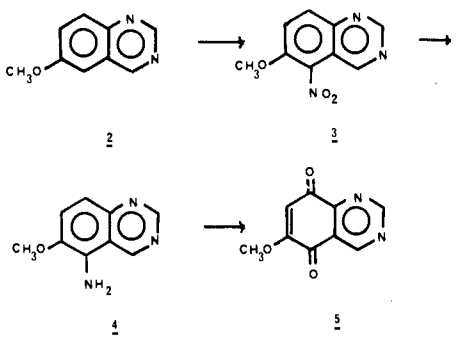
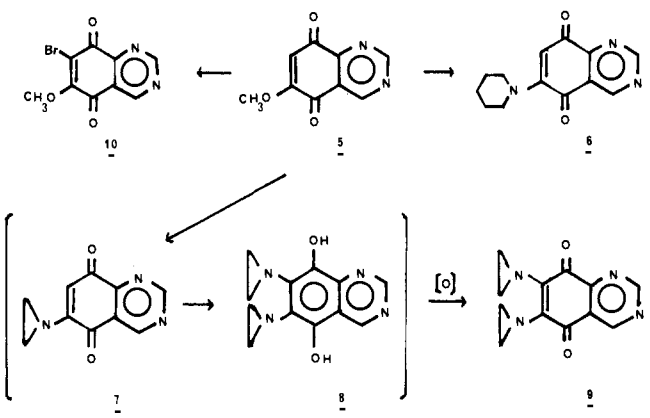
0022-2623/83/1826-1715$01.50/0

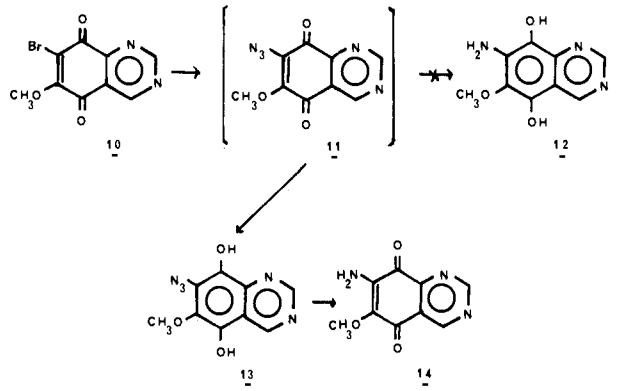
Scheme I



Scheme II



Scheme III



by oxidation of 5,8-dihydroxyquinazoline. This compound was inactive against Gram-positive or -negative bacteria, *Candida albicans*, and sarcoma 180. Tsizin[6,7] and Karpova[8], studied 5,8-quinazolinediones with phenyl or piperidine substituents in position 2, but no activity was mentioned.

In our laboratory, the quinones were prepared as indicated in Scheme I. The 6-methoxyquinazoline[9] (**2**) underwent nitration to give 6-methoxy-5-nitroquinazoline (**3**), which was identified by NMR. The catalytic reduction of nitroquinazolines has been studied.[10-12] Hydrogenation in the presence of palladium on calcium carbonate gave amino-3,4-dihydroquinazoline, which was then oxidized into aminoquinazoline. Reduction of **3** in a methanol–dioxane mixture and in the presence of Raney nickel at atmospheric pressure gave directly the 5-amino-6-methoxyquinazoline (**4**). This latter derivative was oxidized by Fremy's salt[13] (potassium nitrosodisulfonate) to yield 6-methoxy-5,8-quinazolinedione (**5**). As in previous reports,[2-4] we studied the quaternization reaction of the intracyclic nitrogen atom, the nucleophilic substitution reaction at C_6, the electrophilic substitution at C_7 and the synthesis of 7-amino-6-methoxy-5,8-quinazolinedione (**14**), the parent compound of streptonigrin. The quinone **5** did not appear to form quaternary derivatives either with methyl iodide or with methyl fluorosulfonate.

Table I. Effect of 5,8-Quinazolinediones on the Growth of L1210 Cells

	ID50[a]	
no.	ng/mL	10^{-6} M
5	593	3.12
6	359	1.48
9	20	0.08
10	889	3.31
14	215	1.05

[a] ID50 = drug concentration that decreases the growth rate of the cells by 50% after 48 h of culture. They are determinated by a least-squares plotting of the experimental data. The correlation coefficient is in all cases above 0.95, except for 14 (0.89).

Because of the ester-like properties of **5**, the methoxy group[14] could be replaced by hydroxy or amino groups. Either acidic or alkaline hydrolysis resulted only in the decomposition of **5**. Treatment with 1 equiv of piperidine in methanol readily produced 6-piperidinyl-5,8-quinazolinedione (**6**) (Scheme II). However, under the same conditions, aziridine failed to react with **5**. The use of aziridine in excess, without solvent, gave the 6,7-bis(1-aziridinyl)-5,8-quinazolinedione (**9**, NSC 346104) with **7** and **8** as intermediates.

The reactivity of the quinone to electrophilic substitution was enhanced by the presence of a methoxy group.[15] Treatment of **5** with bromine gave the derivative **10** in good yield. Preparation of 7-amino-6-methoxy-5,8-quinazolinedione (**14**) from quinone **5** by nucleophilic addition of sodium azide in acetic acid[16] was unsuccessful. Consequently, an indirect approach to this derivative was examined (Scheme III). Brominated compound **10** reacted with sodium azide to give **11** without isolation of this highly unstable azide. When the preparation of the quinone **14** was attempted, reaction with excess sodium dithionite did not produce hydroquinol **12** but only unstable 7-azido-5,8-dihydro-6-methoxyquinazoline (**13**), which was identified by IR and NMR analysis. The internal oxidoreduction of this compound into quinone **14** was carried out

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Table II. Effect of Compound 9 (NSC 346 104) on P388 Lymphocytic Leukemia[a]

tumor site	treatment (route)	total injectn	treatment schedule	dose per injectn, mg/kg	toxicity survivors[b]	MST (control)[c]	% T/C[d]
ip	ip	1	Q01Dx01	20	10/10	9.5	134*
			Q01Dx01	10	10/10	9.5	118
		3	Q04Dx03	10	10/10	9.9	132*
		5	Q01Dx05	10	10/10	10.4	84
		5	Q01Dx05	5	10/10	10.4	138*
		9	Q01Dx09	5	10/10	10.4	148*
		9	Q01Dx09	2.5	10/10	10.4	128*
ip	iv	1	Q01Dx01	15	05/10	10.4	139*
		1	Q01Dx01	15	10/10	10.4	114
		1	Q01Dx01	5	10/10	10.4	103
		1	Q01Dx01	2.5	09/09	10.4	103
iv	po	7	Q01Dx07	20	10/10	7.6	105
		7	Q01Dx07	15	10/10	7.6	100
		7	Q01Dx07	10	10/10	7.6	100

[a] DBA/2 mice were inoculated with 10^6 cells on day 0. The first treatment was given on day 1. [b] Number of survivors on day 5/number of treated mice. [c] MST = median survival time in days. [d] % T/C = MST of treated animals over controls; *: significant if ⩾125.

Table III. Effect of Compound 9 (NSC 346 104) on Various Animal Tumors

tumor	site	route	total injectn	treatment schedule	dose per injectn, mg/kg	toxicity survivors[a]	MST (control)[b]	% T/C[c]
L1210[d]	ip	ip	1	Q01Dx01	20	10/10	9.4	115
			1	Q01Dx01	10	10/10	9.4	114
			2	Q04Dx02	15	10/10	9.0	104
			2	Q04Dx02	10	10/10	9.0	104
			5	Q01Dx05	10	10/10	9.1	69 (toxic)
			5	Q01Dx05	5	10/10	9.1	108
			9	Q01Dx09	5	10/10	9.1	109
			9	Q01Dx09	2.5	10/10	9.1	109
sarcoma 180[e]	ip	ip	1	Q01Dx01	20	10/10	19.0	110
			1	Q01Dx01	10	10/10	18.3	109
B16 melanocarcinoma[f]	sc	ip	1	Q01Dx01	10	10/10	20.8	91
	ip	ip	1	Q01Dx01	5	09/10	15.7	126
	ip	ip	1	Q01Dx01	2.5	10/10	15.7	121

[a] Number of survivors on day 5/number of treated mice. [b] MST = median survival time in days. [c] % T/C = MST of treated animals over controls; *; significant if ⩾ 125. [d] DBA/2 mice were inoculated with 10^5 cells on day 0. [e] Swiss mice were inoculated with 10^6 cells on day 0. [f] C 57 B1/6 mice were inoculated with cell suspension on day 0. The first treatment was given on day 1.

according to the method of Watanabe.[17]

Pharmacological Results

Cytotoxicity and Antitumor Activity. Data in Table I show that compounds tested are cytotoxic on L1210 cells. Two of them have an ID50 equal or lower than 1 μM, 14 (1.05) and 9 (0.08). The last one was retained for in vivo studies on various experimental tumors. Compound 9 exhibited antitumor activity on P388 leukemia when the tumor cells were intraperitoneally (ip) grafted and the product was given by the same route. Whatever the treatment schedule used, an increase in the survival of treated animals over controls resulting in a T/C greater than 125% is noted (Table II). When the drug was given intravenously (iv), a T/C of 139% was obtained, except when toxic dose was given (50% lethality before day 5). No effect was observed when the cells were given intravenously and the drug was given orally (po). No significant activity was noted on leukemia L1210 and sarcoma 180, and a poor effect was detected on melanocarcinoma B16 (Table III).

Mutagenesis Test. Data in Table IV show that 9 is a mutagen capable of reverting both base-pair substitution (TA100) and frame-shift mutations (TA98). The mutagenic effect occurs in the absence of metabolic activation as observed for various alkylating agents. At a concentration above 5 μM, 9 was highly toxic to the bacteria, and the appearance of the mutagenic effect was always associated with a toxic effect. The mutagenicity of 9 was also tested by the standard spot assay method,[18] and the compound was found to be highly toxic, as judged by large zones of inhibition, and highly mutagenic as judged by a

Table IV. Effect of Compound 9 on Induction of *his*$^+$ Revertants of *S. typhimurium* TA 98 and TA 100[a]

	his$^+$ revertants/plate	
	TA 98	TA 100
control	85	78
+ 0.3 μM 9	84	76
+ 0.7 μM 9	101	94
+ 4.0 μM 9	81	87
+ 10 μM 9 (T)	89	97
+ 20 μM 9 (T)	98	108
+ 40 μM 9 (T)	334	461
+ 60 μM 9 (T)	1080	501

[a] The mutagenesis tests were performed by using the liquid suspension procedures without metabolic activation as described in Experimental Section. Values indicated are the means of three different assays. T indicates toxic doses.

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large increase in colonies all around the zones of inhibition (data not shown).

Discussion

The compounds of the series presented here all possess a pyrimidine nucleus and a quinone structure. Compound **9**, the most cytotoxic, also possesses two aziridinyl substituents. The quinone group, as already shown with the parent compound streptonigrin, may undergo oxidoreduction reactions to yield oxygen-free radicals,[19] mainly $O_2^{-}\cdot$ and OH·; these oxy radicals have been reported to be cytotoxic. However, among the tested compounds, some are not cytotoxic (**14**), suggesting that the presence of a quinone group is not a sufficient condition for the cytotoxicity of the molecule. The aziridinyl substituents might therefore be considered as responsible for this effect, since they are able to covalent bind with biological nucleophiles, such as proteins and DNA. Mutagenesis data provided by the Ames test show that **9** causes, directly or indirectly, damage to DNA, whereas **14**, devoid of an aziridinyl group, does not exhibit toxic and mutagenic effects (unpublished data). Moreover, there is general agreement that frameshift mutagens, detected by the TA98 strains, react covalently with DNA. These compounds usually exhibit a planar ring structure responsible for intercalation and an electrophilic side chain responsible for covalent binding.[20] Compound **9** seems to be included in this class of compounds. The involvement of the redox cycle cannot be excluded, and compounds exhibiting quinone groups and additional electrophilic substituents could have the best chemical structure for a cytotoxic effect.

Experimental Section

Chemistry. Melting points were determined on a Maquenne apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 157G spectrometer. NMR spectra were recorded on a Bruker 80- or 270-MHz spectrometer with $(Me_3Si)_2$ as an internal standard. Thin-layer chromatography was carried out using Merck GF254 silica gel. The substituents on the quinones were kept protected from exposure to light. All elemental analyses were within 0.4% of the theoretical values.

6-Methoxy-5-nitroquinazoline (3). To 24 g (15 mmol) of 6-methoxyquinazoline (**2**) dissolved in 600 mL of sulfuric acid (*d* 1.84) was added 16.7 g (165 mmol) of potassium nitrate over a 45-min period, keeping the reaction temperature between −3 and −6 °C. The mixture was stirred at this temperature for an additional 2.5 h before being poured onto ice; ammonia (*d* 0.9) was added while cooling until a pH of 7.5 was reached. The yellow solid that separated was filtered, washed with H_2O, dried, and recrystallized from propanol to give 27 g (87%) of essentially pure **3**: mp 169 °C; IR (KBr) 1105 (CH_3O), 1320 and 1530 [$\nu(NO_2)$] cm^{-1}; NMR (Me_2SO-d_6) δ 4.1 (s, 3, CH_3O), 8.1 and 8.3 (d, 1, $J \simeq$ 10 Hz, H_7 and H_8), 9.3 (s, 1, H_2), 9.4 (s, 1, H_4). Anal. ($C_9H_7N_3O_3$) C, H, N.

5-Amino-6-methoxyquinazoline (4). A solution of 14.35 g (70 mmol) of **3** in 700 mL of dioxane and 350 mL of MeOH was hydrogenated under catalysis of 15 g of Raney nickel until the gas uptake ceased (210 mmol). After filtration from catalyst under N_2, the mixture was charcoaled and evaporated to give 11 g (90%) of crude, essentially pure amino compound. A small sample was further purified on a thick-layer silica gel plate with 1:1 $CHCl_3$–AcOEt and AcOEt as the eluents to give a yellow, air-sensitive amine, which was crystallized from 1:1 CH_2Cl_2–ligroin: yield 61%; mp 149 °C; IR (KBr) 1120 (CH_3O), 3340 and 3430 [$\nu(NH_2)$] cm^{-1}; NMR ($CDCl_3$) δ 3.9 (s, 3, CH_3O), 4.6 (s, 2, NH_2), 7.35 and 7.5 (d, 1, $J \simeq$ 10 Hz, H_7 and H_8), 9.1 (s, 1, H_2), 9.4 (s, 1, H_4). Anal. ($C_9H_9N_3O$) C, H, N.

6-Methoxy-5,8-quinazolinedione (5). A solution of 9 g of monobasic potassium phosphate in 400 mL of water was added to 10.5 g (60 mmol) of crude amino compound **4** in 600 mL of acetone. To this mixture, strongly stirred under a N_2 atmosphere and at ambient temperature was added 40.2 g (150 mmol) of potassium nitrosodisulfonate for 1.5 h. The mixture was stirred for 2.5 h. Two more portions of the reagent [16.1 g (60 mmol) and 4 g (15 mmol)] were slowly added during this period. After the mixture was stirred overnight, the acetone was removed in vacuo. The precipitate was filtered and extracted with chloroform. The combined solvent layers were washed with water, dried, and treated with charcoal. The brown solid obtained from evaporation of the chloroform was chromatographed on a column (350 g) of Merck (0.05–0.2 mm) silica gel with chloroform as the eluant. Pure yellow quinone **5** crystallized from AcOEt: yield 4.2 g (36%); mp 233 °C; IR (KBr) 1125 (CH_3O), 1665 and 1690 [$\nu(CO)$] cm^{-1}; NMR ($CDCl_3$) δ 3.9 (s, 3, CH_3O), 6.3 (s, 1, H_7), 9.45 (s, 1, H_2), 9.6 (s, 1, H_4). Anal. ($C_9H_6N_2O_3$) C, H, N.

6-Piperidinyl-5,8-quinazolinedione (6). To a solution of 0.133 g (0.7 mmol) of **5** in 15 mL of dry MeOH there was added 0.84 mmol of piperidine (8.4 mL of a 0.1 M solution in MeOH). The mixture was stirred at 0 °C for 2 h under a N_2 atmosphere. The solvent was evaporated to give 0.144 g (85%) of **6** after crystallization form a 1:1 mixture of benzene–ligroin: mp 159 °C; IR (KBr) 1680 [$\nu(CO)$] cm^{-1}; NMR ($CDCl_3$) δ 1.7 (m, 6, piperidinyl H_β and H_γ), 3.5 (m, 4, piperidinyl H_α), 6.15 (s, 1, H_7), 9.25 (s, 1, H_2), 9.45 (s, 1, H_4). Anal. ($C_{13}H_{13}N_3O_2$) C, H, N.

6,7-Bis(1-aziridinyl)-5,8-quinazolinedione (9). Compound **5** (0.114 g, 0.6 mmol) was dissolved in 3 mL of aziridine. The reaction mixture was stirred at 0 °C under N_2 for 30 min. The excess amine was evaporated in vacuo. The black residue was dissolved in $CHCl_3$ (20 mL), washed with H_2O, dried, and evaporated to dryness to give a solid, which was then purified on a silica gel plate (solvent 1:1 $CHCl_3$–AcOEt): yield 59%; mp 188 °C; IR (KBr) 1660 [$\nu(CO)$] cm^{-1}; NMR ($CDCl_3$) δ 2.4 (s, 8, CH_2), 9.3 (s, 1, H_2), 9.5 (s, 1, H_4). Anal. ($C_{12}H_{10}N_4O_2$) C, H, N.

7-Bromo-6-methoxy-5,8-quinazolinedione (10). Compound **5** (0.19 g, 1 mmol) in 10 mL of $CHCl_3$ and 0.062 mL (1.2 mmol) of Br_2 were stirred at 0 °C, under N_2 for 60 h. The oily residue obtained after evaporation of the solvent was triturated with CCl_4 to give a red solid, which was filtered and then extracted with $CHCl_3$ and H_2O, dried, and evaporated. After crystallization from MeOH, compound **10** melted at 202 °C; yield 80% (0.215 g); IR (KBr) 1120 (CH_3O), 1680 [$\nu(CO)$] cm^{-1}; NMR ($CDCl_3$) δ 4.3 (s, 3, CH_3O), 9.4 (s, 1, H_2), 9.55 (s, 1, H_4). Anal. ($C_9H_5N_2BrO_3$) C, H, N.

7-Azido-5,8-dihydro-6-methoxyquinazoline (13). Sodium azide (0.078 g, 1.1 mmol) and **10** (0.269 g, 1 mmol) in a 1:1 mixture of acetone and H_2O (135 mL) were stirred at room temperature for 2 h. The acetone was eliminated in vacuo, and the residue was extracted with $CHCl_3$, which was washed with H_2O, dried, and then evaporated. The red unstable 7-azido-6-methoxy-5,8-quinazolinedione (**11**) was a chromatographically homogeneous material (silica gel plate, 7.5:2.5 $CHCl_3$–AcOEt). In solution of a 1:1 mixture of acetone and H_2O, it was stirred for 30 min, under N_2, with 0.174 g (1 mmol) of sodium dithionite. The organic solvent was eliminated in vacuo, and the yellow solid was separated, washed with H_2O, and rapidly dried. IR and NMR analysis indicated this to be compound **13**; elementary analysis was not attempted: yield 59% (0.15 g); mp 208 °C; IR (KBr) 1105 (CH_3O), 2120 [$\nu(N_3)$], 3200 [$\nu(OH)$] cm^{-1}; NMR (DMF-d_7, 270 MHz) δ 3.85 (s, 3, CH_3O), 9.1 (s, 1, H_2), 9.5 (s, 1, H_4), 10.25 and 10.5 (s, 2, OH).

7-Amino-6-methoxy-5,8-quinazolinedione (14). A suspension of 0.14 g (0.6 mmol) of **13** in 15 mL of 1,1,2-trichloroethane was refluxed under N_2 for 4 h. The mixture was evaporated to dryness, and the residue was treated with 5 mL of $CHCl_3$ and then filtered. The filtrate was evaporated, and the violet solid was further purified on a thick-layer silica gel plate (solvent: 8:2 $CHCl_3$–AcOEt) to afford 0.038 g (31%) of **14**: mp 212 °C; IR (KBr) 1115 (CH_3O), 1690 [$\nu(CO)$], 3260 and 3460 [$\nu(NH_2)$] cm^{-1}; NMR ($CDCl_3$, 270 MHz) δ 4.05 (s, 3, CH_3O), 5.2 (s, 2, NH_2), 9.35 (s, 1, H_2), 9.5 (s, 1, H_4). Anal. ($C_9H_7N_3O_3$) C, H, N.

Growth Rate Inhibition of L1210 Cells in Culture. The experimental protocol has previously been reported.[2] The cells were exposed to increasing concentrations of drug during 48 h and incubated in a 5% CO_2 atmosphere at 37 °C. Drugs were

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dissolved in Me_2SO (1% final concentration). The growth rate inhibition of cells was determined with a ZBI Coulter Counter. Dose–effect relationship from the results obtained after 48 h of culture were calculated by a least-squares procedure. Linear regressions were determined as the percentage inhibition of the growth as a function of the logarithm of the dose. The concentration of drug that lowered control growth cell by 50% (ID50) was estimated from these equations.

Antitumor Studies. Experiments with **9** were performed on L1210 lymphoid leukemia, P388 lymphocytic leukemia, sarcoma 180, and B16 melanocarcinoma. The protocols were already described.[21] Antitumor activity was expressed as (T/C) 100, *T* being the median survival time of treated animals and *C* the median survival time of controls. A significant tumor activity is considered for T/C > 125%. Drug was dissolved in saline solution (0.15 M NaCl).

Mutagenic Test. The mutagenic property of **9** was determined by the selection of *his*$^+$ revertants of *Salmonella typhimurium* histidine auxotroph strains TA98 and TA100 according to the procedure described by Ames et al.,[18] with minor modifications as follows: overnight cultures growing in Oxoid nutrient broth (Oxoid Ltd., England) were subcultured into fresh media of the same composition. The subcultures were allowed to grow for 2 h, subdivided into 20-mL aliquots, and reincubated during 5 h in the absence (controls) or in the presence of various concentrations of drug. After the incubation was terminated, bacteria were removed by centrifugation and washed once with the Oxoid medium. Final pellets of bacteria were resuspended in Oxoid to obtain an absorbance of 2.50 at 650 nm. Aliquots of these bacteria suspensions were diluted in the top agar (0.6% Difco agar, 0.6% NaCl) and distributed on the petri plates. Histidine revertants were counted after 48 h incubation at 37 °C.

Acknowledgment. This investigation was supported by contrat de recherche libre 78.1160-3 from INSERM. The technical assistance of Georgette François for cytotoxicity and antitumoral tests is gratefully acknowledged.

Registry No. **2**, 7556-92-5; **3**, 87039-48-3; **4**, 87039-49-4; **5**, 87039-50-7; **6**, 87039-51-8; **9**, 87039-52-9; **10**, 87039-53-0; 11, 87039-54-1; **13**, 87039-55-2; 14, 87039-56-3; aziridine, 151-56-4.

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Methotrexate Analogues. 20. Replacement of Glutamate by Longer-Chain Amino Diacids: Effects on Dihydrofolate Reductase Inhibition, Cytotoxicity, and in Vivo Antitumor Activity

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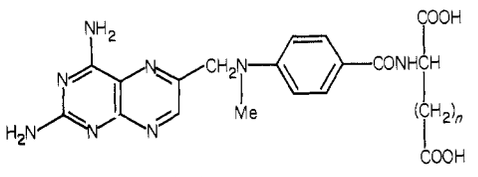
Chain-extended analogues of methotrexate were synthesized by condensation of 4-amino-4-deoxy-N^{10}-methylpteroic acid with esters of L-α-aminoadipic, L-α-aminopimelic, and L-α-aminosuberic acids, followed by ester hydrolysis with acid or base. Coupling was accomplished in up to 85% yield by the use of the peptide bond forming reagent diethyl phosphorocyanidate at room temperature. The products were found to bind bacterial (*Lactobacillus casei*) and mammalian (L1210 mouse leukemia) dihydrofolate reductase with an affinity comparable to methotrexate and were also equitoxic to L1210 cells in culture. Cytotoxicity increased up to 3-fold as the number of CH_2 groups in the amino acid side chain was extended from two to five. The α-aminoadipate and α-aminopimelate analogues were poor substrates for carboxypeptidase G_1, confirming that this enzyme has a strict requirement for a C-terminal L-glutamic acid residue. The in vivo antitumor activity of the chain-extended analogues against L1210 leukemia in mice was comparable to that of the parent drug on the qd×9 schedule, but higher doses were required to achieve the same increase in survival. The results were consistent with findings, reported separately, that these compounds are poor substrates for folate polyglutamate synthetase and therefore would not be expected to form γ-polyglutamates once they enter a cell. This distinctive property has potential therapeutic implications for the treatment of certain MTX-resistant tumors whose resistance may be associated with a lower than normal capacity to form γ-polyglutamates in comparison with proliferative tissues such as intestinal mucosa or marrow.

Previous work in this laboratory and others has led to the recognition that the terminal region of the glutamate side chain in classical antifols, such as methotrexate (MTX, 1), is amenable to structural modification with no significant loss of binding to the target enzyme dihydrofolate reductase.[1-8] As part of a continuing program of systematic molecular change aimed at the development of new or improved folate antagonists as antitumor agents,[9,10] we



1-4, n = 2-5

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