Heterocyclic Quinones. 17. A New in Vivo Active Antineoplastic Drug: 6,7-Bis(1-aziridinyl)-4-[[3-(*N*,*N*-dimethylamino)propyl]amino]-5,8-quinazolinedione

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A series of heterocyclic quinones, 6-substituted and 6,7-disubstituted 4-(alkylamino)-5,8-quinazolinediones, have been synthesized in order to evaluate their in vitro cytotoxicity on L1210 leukemia cells. Among 14 derivatives that have been prepared and studied for the structure–activity relationship, the most potent cytotoxic compound on L1210 leukemia cells was the 6,7-bis(1-aziridinyl)-4-[[3-(*N*,*N*-dimethylamino)propyl]amino]-5,8-quinazolinedione (**24**). This compound has been tested with the use of a cell-image processor on MCF-7 human mammary and HBL human melanoma cell lines. The results show that compound **24** influences cell proliferation and blocks both cells lines in the S phase. In vivo antineoplastic activity of compound **24** has been demonstrated on a broad spectrum of murine experimental models, but it was found highly toxic and produced long-delayed deaths.

Streptonigrin (**1**; Chart I) is a heterocyclic quinone that exhibits pronounced antitumor effects. Its activity seems to be due to the 5,8-quinolinedione moiety. According to Lown, single-strand cleavage of covalently closed circular DNA was due to free OH radicals generated from reduced quinone and oxygen.[1] Unfortunately this polyvalent antitumoral compound is too toxic for clinical use.[2]

In connection with our research on the role of the nitrogen heterocyclic quinone nucleus on the antitumoral activity, we have previously described 6,7-bis(1-aziridinyl)-5,8-quinazolinedione (**3**). This molecule is very cytotoxic to L1210 leukemia cells and is active in vivo against P388 lymphocytic leukemia only when the drug is given intraperitoneally (ip) or intravenously (iv), and no activity is detected when the drug is given orally (po). Poor in vivo activity was noted against melanocarcinoma B16, and no significant effect was observed with L1210 leukemia and sarcoma 180.[3] The benzoquinone AZQ (NSC 182986) (**4**) is well known to be a bifunctional alkylating agent which may produce DNA cross-links because of the presence of para aziridinyl groups.[4] The poor in vivo activity of the quinone **3** could be explained by the impossibility of DNA interstrand cross-links due to the short distance between the two aziridinyls groups which are ortho. The low water solubility of **3** or its weak DNA affinity should also be taken into consideration. Recently, we described the synthesis and the antitumor properties of dimers of the 6,7-bis(1-aziridinyl)-5,8-quinazolinedione (bis compounds) in which the two heterocycles are linked in the 4-position by a simple or a substituted α,ω-diaminopolymethylenic chain.[5] These compounds could react as bis-alkylating agents. The more cytotoxic compound on L1210 leukemia cells which is also the more potent in vivo on P388 leukemia in mouse is the dimer **5**, which possesses a tertiary amino function on the aliphatic chain.

We intended to elucidate whether the dimerization or the presence of an α,ω-diamino chain in the 4-position is responsible for the activity. In comparison with **3**, a quinone with such a chain in the 4-position should have the following advantages: (i) at physiological pH, the amino group is protonated and then can react with the DNA phosphate groups involving an increase in DNA affinity;[6] (ii) because of the presence of a cationic chain and of an electron-deficient quinone nucleus, these quinones can be compared with the electron-deficient nitrobenzenoid compounds such as **7**, which has been shown to bind to DNA by charge-transfer interactions;[7] (iii) in addition, Ambrose and co-workers have shown that tumor cells apparently have a higher negative surface charge, suggesting that basic compounds should be concentrated in such cells.[8] The favorable influence on antitumoral activity of the presence of a [(dialkylamino)alkyl]amino side chain was pointed out in several series of molecules with three or four nuclei and less often with two nuclei compounds. According to Bisagni and co-workers, pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinolines with a [(dialkylamino)alkyl]amino side chain at the 1-position such as **8** display a higher antitumoral activity on L1210 leukemia systems than the ellipticine analogue (**9**).[9] The activity appears closely dependent upon the number of methylenes in the side chain and upon the nature of the tertiary amino alkyl substituents.[10] Thioxanthen-9-one derivates with a [(dialkylamino)alkyl]amino side chain, Miracil D (**10**) and Hycanthone (**11**), inhibit the growth of a wide spectrum of experimental tumors.[11] The presence of a diamino chain is essential to the antitumor activity.[12] It is of interest to notice reported antineoplastic activity of Mitonafide (**12**) in which the basic nitrogen atom on the

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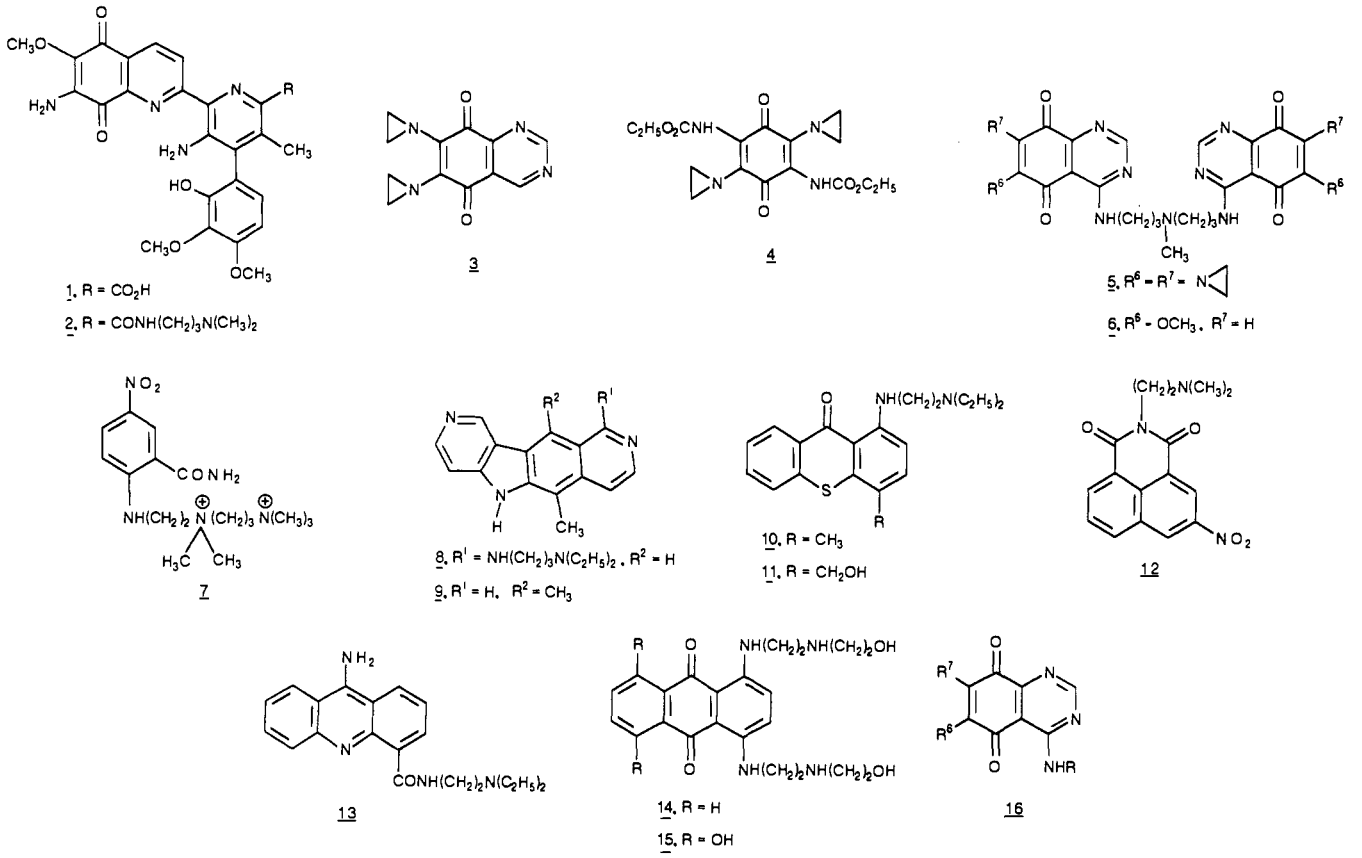
Chart I



2-(dimethylamino)ethyl side chain is essential to the activity.[13] In connection with this, Atwell has shown that dibasic 9-aminoacridine-4-carboxamides such as **13** are active in vitro against L1210 leukemia and HCT-8 human colon adenocarcinoma cells and in vivo against P388 leukemia.[14] In the quinonic series, replacement of the alkylamino groups by [[(hydroxyalkyl)amino]alkyl]amino groups in 1,4-bis(alkylamino)anthraquinone affords Ametantrone (NSC-196473) (**14**), which exerts antineoplastic activity.[15] Its 5,8-dihydroxy analogue, Mitoxantrone (NSC-279836) (**15**), possesses outstanding antineoplastic activity in many experimental animal systems.[15] In contrast, replacement of the carboxylic function by a [3-(dimethylamino)propyl]carbamoyl (**2**) in streptonigrin decreases the cytotoxic activity on various tumor cells.[16]

On the basis of these considerations, we describe in this paper the synthesis and cytotoxicity to L1210 cells of a series of 6-substituted and 6,7-disubstituted 4-(alkylamino)-5,8-quinazolinediones of type **16** with various functional groups on the alkyl side chain R. Among 14 tested derivatives, the most cytotoxic was studied in human tumor cell lines with use of a cell image processor and evaluated in a broad spectrum of murine tumors.

Results and Discussion

Chemistry. The methoxy group in the para position relative to the nitrogen of quinoline nucleus was important for biological activity of streptonigrine (**1**).[17] Such a group has been preserved in the 6-position of the 5,8-quinazolinediones nucleus. The 5-(benzyloxy)-4-chloro-6-methoxyquinazoline (**17**) (Scheme I), key compound of the 4-amino-5,8-quinazolinediones synthesis, was obtained from commercial 2-hydroxy-3-methoxybenzaldehyde according to the method described in our previous paper.[5] The amine AH **18** reacted with the chloro derivate **17** in ethanol in the presence of triethylamine at room temperature. The resulting 4-amino-5-(benzyloxy)-6-methoxyquinazoline (**19**) was specifically debenzylated into the methoxyphenol **20** either by heating in trifluoroacetic acid (TFA) or by hydrolysis in the presence of palladium on activated charcoal. The unstable methoxyphenol **20** could not be purified; the crude compound was oxidized to quinone **21** by potassium nitrosodisulfonate (Fremy's salt).[18]

In order to establish structure–activity relationships, various amines have been used. In the first, 3-(*N*,*N*-dimethylamino)propylamine (**18h**) was used because it is the bis(aminoquinazolinedione) **5** chain reduced by half. Then, the number of methylenes between the two nitrogen atoms and in the tertiary nitrogen alkyl substitutents was modified in quinones **21f**, **21g**, **21h**, **21i**, and **21j**. The aliphatic tertiary amino function was replaced by an aden-9-yl group, a biological heterocycle, by use of 9-(3-aminopropyl)adenine (**18l**), prepared according to Leonard.[19] The quinone **21l** was obtained via compounds **19l** and **20l**. In view of studying the influence of the secondary amino function on biological activity, we attempted to synthesize

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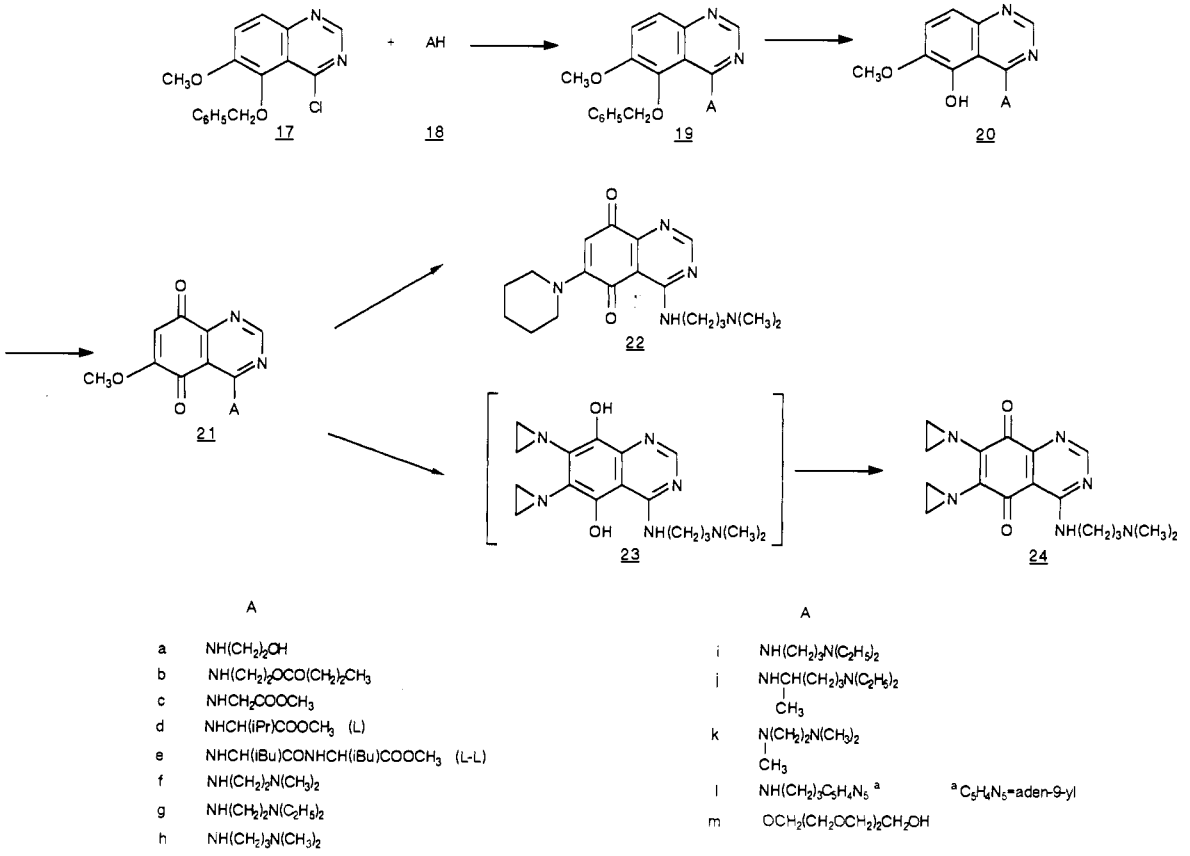
Scheme I



the quinone **21k**. Reaction of the *N,N,N'*-trimethylethylenediamine with the chlorinated compound **17** gave the aminomethoxyquinazoline **19k** in 90% yield. Unfortunately, debenzylation either by TFA or by catalytic hydrogenation afforded several undefined products. In order to increase water solubility, we substituted the aminoethanol chain on the 6-methoxy-5,8-quinazolinedione nucleus to give the quinone **21a**. Esterification of the OH function by *n*-butyryl chloride produced the lipophilic quinone **21b**.

An amino acid was introduced as biological vector in the 4-position by reaction of **17** and glycine methyl ester to give the quinone **21c**. As ramified amino acids are well known to facilitate diffusion across cell membranes,[20] we prepared the quinones **21d** and **21e** respectively substituted by L-valine methyl ester and L-leucyl-L-leucine methyl ester. Because of methoxy group mobility in quinones in the presence of acids or bases, ester hydrolysis was impossible. An attempt to hydrolyze the ester function after reduction of the quinone to the hydroquinol according to Rao was unsuccessful.[21] Indeed, after reduction of the quinone by hydrogen sulfide, neither 1 N sodium hydroxide nor saturated barium hydroxide solution nor 1 N sulfuric acid hydrolyzed the ester function at 20 or 40 °C. Under more drastic conditions, TLC showed the presence of several inseparable compounds. Hydrolyses of 5-hydroxy-6-methoxyquinazoline (**20c**) and subsequent oxidation gave only tars.

The reaction of piperidine with the quinone **21h** in the presence or in the absence of methanol produced only the 4-[[3-(*N,N*-dimethylamino)propyl]amino]-6-piperidino-5,8-quinazolinedione (**22**). When aziridine was used without solvent, the 6,7-bis(1-aziridinyl)-4-[[3-(*N,N*-dimethylamino)propyl]amino]-5,8-quinazolinedione (**24**) was obtained by methoxy substitution with one molecule of aziridine and subsequent 1,4-addition by a second molecule of aziridine to give **23** spontaneously oxidized by air. Even in the presence of solvent (such as methanol, THF, dioxane), TLC did not show the formation of monoaziridinyl compound. That reaction could not be attempted with the quinones **21b–e** because of the instability toward alkali of the ester function.

Inhibition of L1210 Cell Growth Rate by a Series of Quinazolinediones. Dose–effect relationships of 14 compounds tested were determined from the regression line of data. The drug concentration which reduces the growth rate of L1210 cells by 50% after 48-h exposure as compared to the controls was deduced from the equations. For each drug, results of one experiment are reported in Table I. They confirm one or two previous assays. As for the dimer **6**[5] (IC50 = 1.25 μM), introduction of an ω-[(*N,N*-dialkylamino)alkyl]amino chain in the 4-position of the 6-methoxy-5,8-quinazolinedione[3] (IC50 = 3.12 μM) increases the cytotoxicity to L1210 leukemia cells (quinones **21f–j**). Modifications of the side chain (number of methylenes and nature of the tertiary nitrogen alkyl substituents) do not significantly improve the biological properties. However, in the cases of the pyrido [3′,4′:4,5]pyrrolo[2,3-*g*]isoquinoline derivatives, such modifications have resulted in a 10-fold increase of the cytotoxicity on Friend tumor cells.[9] Replacement of the alkylamino group by the aden-9-yl group leads to the inactive quinone **21l**. The quinone **21a** substituted by the (hydroxyethyl)amino chain is not cytotoxic. Alcohol esterification (quinone **21b**) obviously restores the cytotoxicity. When the methoxy quinone is substituted by an amino acid methyl ester (**21c,d**) or by a peptide methyl ester (**21e**), the derivatives exhibit good cytotoxicity except

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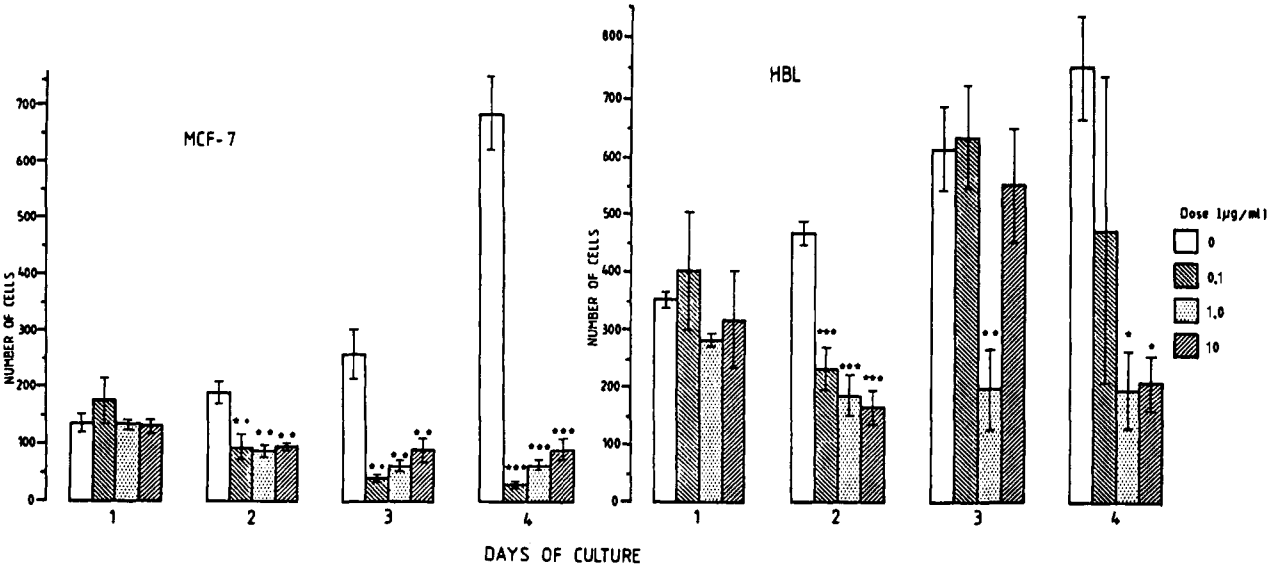

Figure 1. Influence of compound **24** on the cell proliferation of the MCF-7 human mammary and the HBL human melanoma cancer cells. The cells were cultured for various periods of time (1–4 days) with different doses of drug. The mean percent values (±SEM) of treated cells were compared (Fisher F test) to the control values (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

for quinone **21c**. The quinone **21m** with ether functions is inactive. Substitution of the methoxy group by the piperidino group does not modify the biological activity (compare **21h** to **22**). On the other hand, the introduction of two aziridinyl groups on the quinone moiety results in a striking increase of the cytotoxicity, which is weakly higher than that of the dimer **5** (IC50 = 0.0048 µM)[5] but up to 29 times higher than that of quinone without any substitution in the 4-position (**3**, IC50 = 0.08 µM).[3] Doxorubicin used as a reference has a IC50 = 0.048 µM. The quinone **24** is one of the most cytotoxic compounds which have ever been tested toward L1210 leukemia cells.

Those data demonstrate that the high cytotoxicity of the bis(aziridinyl)quinones such as **24** is due to the presence of a polyamino chain in the 4-position and not to the dimerization. Compound **24**, the most interesting in the series, was studied on different antitumor models in vitro and in vivo.

Cytotoxic effects of compound 24 were studied for 1–4 days on MCF-7 human mammary and HBL human melanoma cell lines with a cell processor image, i.e., the System for Analytical Microscopic Biomedical Applications. The results of cell growth and cell kinetics are reported in Figure 1 and Table II. Compound **24** influences the cell proliferation in a dose-independent manner for MCF-7 cells and the high dose decreases weakly the cell growth for HBL cells. For both cell lines the most pronounced effect exerted by compound **24** on the cell cycle kinetic parameters seemed to be a blockage of cells in the S phase.

Toxicity studies of compound 24 were performed by two routes of administration.

By the iv route, the maximum tolerated dosage was 0.36 mg/kg per injection given day 3, 6, 9 (total dose 1.08 mg/kg). At this dosage the agent did not cause any weight loss. At frankly toxic dosages, i.e., 0.6 mg/kg per injection or 1 mg/kg per injection, given day 3, 6, 9 frank toxicity was reached with long-delayed death occurring as late as 80 days after the start of therapy.

By the ip route the agent presented the same type of toxicity with delayed deaths occurring with the three different schedules of administration. The highest nontoxic dosages were 1 mg/kg as a single injection, 2 mg/kg on an intermittent schedules Q3D×4 and 1.6 mg/kg as a daily injection day 1–4. Necropsy of the dead animals revealed sterile peritonites with an enormous production of fluid in the peritoneal cavity and shrinkage of the organs compressed to the diaphragm. Histology studies revealed damage to the kidneys.

Solid tumor sensitivity to compound 24 is shown in Table II.

On B16 melanoma, two experiments were performed on early-stage (day 1 postimplantation) tumors implanted as an ip brei. The agent was given ip on a daily schedule; it was found active with respectively 76% ILS and 53% ILS plus 2/10 tumor-free survivors on day 60. The same agent given iv Q3D×3 on a more advanced sc tumor (day 6) was found to have minimal activity with a 41% T/C.

On Glasgow osteogenic sarcoma (GOS), compound **24** given iv Q3D×3 on early-stage (day 3) tumors was found highly active with a 0% T/C and no tumor-free survivor 60 days postimplantation. Cyclophosphamide was also found very active with 5/7 tumor-free survivors on day 60.

On M5076 reticulum cell sarcoma, compound **24** given ip Q4D×4 starting day 1 was found active with a 58% ILS. The positive control, cyclophosphamide, was found highly active with a 155% ILS.

The agent was found inactive (T/C > 42%) against early-stage (day 2) colon adenocarcinoma 38, advanced-stage (day 6) mammary adenocarcinoma 16/C, and advanced-stage (day 5) Lewis lung carcinoma.

Leukemia sensitivity to compound 24 is shown in Table III.

On P388 leukemia, compound **24** was found highly active. At the maximum tolerated dosage, the increase in life span was 70% for the single bolus injection, 91% for the daily schedule day 1–4, and 108% for the intermittent schedule.

On P388/L-Pam leukemia, compound **24** was found to have marginal activity by the bolus injection schedule (36% ILS) and moderate activity by the daily schedule (43% ILS) and was found active with 61% ILS by the intermittent schedule. However the activity was lost at the next dosage down (1 mg/kg) by that same schedule. Therefore compound **24** could be considered to have only moderate activity against P388/L-Pam leukemia.

On L1210 leukemia, compound **24** was found to have very minimal activity (27% ILS). The schedule used did not make any difference.

Table I. Effect of 4-Substituted 5,8-Quinazolinediones on the Growth of L1210 Cells

compd	IC50[a] ng/mL	IC50[a] μM	correlation coefficient[b]	compd	IC50[a] ng/mL	IC50[a] μM	correlation coefficient[b]
21a	1776	7.13	0.97	**21i**	393	1.24	0.95
21b	850	2.66	0.85	**21j**	435	1.26	0.995
21c	1198	4.26	0.99	**21l**	>10000	>24.6	
21d	825	2.59	0.98	**21m**	>10000	>29.6	
21e	581	1.30	0.99	**22**	406	1.17	0.98
21f	316	1.14	0.99	**24**	1.02	0.0027	0.99
21g	281	0.898	0.99	doxorubicin[c]	28	0.048	0.99
21h	501	1.73	0.99				

[a] IC50 = drug concentration that reduces by 50% after a 48-h period the L1210 cells growth as compared to controls. [b] Correlation coefficient of the regression line (probit of the percent cell growth inhibition plotted as a function of the logarithm of the dose). [c] Doxorubicin was used as a reference.

Table II. In Vivo Antitumor Activity of Compound **24** Injected Intravenously against Murine Solid Tumors

tumor			treatment									
type	site	no. of cells	lagent, route	optimal dose, mg/kg per inj	schedule days	total dose, mg/kg	toxicity-related death	body weight change in g/mouse (day of nadir)	median tumor burden in mg (day of evaluation)	T/C % (day of evaluation)	comments	tester[a]
mammary adenocarcinoma 16/C	sc	fragment	24, iv	0.4	6,9,12	1.2	0/10	0.5 (12)	895 (14)	48 (14)	inactive	RPS
			24, iv	0.2	6,9,12	0.6	0/10	1.0 (12)	1265 (14)	68 (14)	inactive	
			CPA, iv	200.0	6	200.0	1/10	−1.8 (12)	320 (14)	17 (14)	active	
			control					+1.3 (12)	1873 (14)			
B16 melanoma	sc	fragment	24, iv	0.4	6,9,13	1.2	0/10	+0.9 (15)	2002 (15)	41 (15)	marginally active	RPS
			24, iv	0.2	6,9,13	0.6	0/10	+0.8 (15)	3807 (15)	78 (15)	inactive	
			CPA, iv	200.0	6	200.0	0/10	−1.6 (15)	444 (15)	9 (15)	active	
			control					+2.4 (15)	4882 (15)			
Glasgow osteogenic sarcoma	sc	fragment	24, iv	0.36	3,6,9	1.08	0/7	+0.6 (10)	40 (10)	0 (14)	highly active[b]	RPS
			CPA, iv	200.0	3	200.0	1/9	−2.2 (4)	0 (10)	0 (14)	highly active[b] moderately toxic	
			control					+3.2 (10)	789 (10)			
Lewis Lung carcinoma	im	10^6	24, iv	0.4	5,9,13	1.2	0/10	+0.6 (9)	7717 (19)	81 (19)[c]	inactive	RPS
			24, iv	0.2	5,9,13	0.60	0/10	+1.1 (9)	7748	81 (19)	inactive	
			CPA, iv	200.0	5	200.0	0/10	−2.8 (9)	809 (19)	11 (19)	active	
			control					+2.4 (9)	484 (19)			
colon adenocarcinoma 38	sc	fragment	24, ip	0.25	2,9	0.5	0/10		416 (20)	102 (20)	inactive	JB
			5FU, ip	70.0	2,9	140.0	0/10		60 (20)	14 (20)	active	
			control						405 (20)			
									median day of death	ILS		
B16 melanoma 1	ip	brei	24, ip	0.2	1–9	1.8	0/10		27.8	76	active	LPTF
			control						15.8			
B16 melanoma 2			24, ip	0.2	1–9	1.8	0/10		27.0	53	active	LPTF
			control						17.7			
M5076	ip	10^6	24, ip	1.0	1,5,9,13	4.0	0/10		29.0	58	active	JB
			CPA, ip	80.0	1,5,9,13	320.0	0/10		47.0	155	active	
			control						18.4			

[a] RPS = Rhône-Poulenc Santé; JB = Institut Jules Bordet; LPTF = Laboratoire de Pharmacologie et de Toxicologie Fondamentales du CNRS, Toulouse. [b] There was no tumor-free survivors on day 60. [c] The percent inhibition of metastasis was 37% for **24** 0.4 mg/kg per injection, 11% for **24** 0.2 mg/kg per injection, 99% for CPA 200 mg/kg per injection.

Table III. In Vivo Antitumor Activity of Compound **24** against Murine Leukemia

implant				treatment ip				therapeutic response[a]				
tumor	no. of cells	route	agent	optimal dose, mg/kg per inj	schedule day	total dose, mg/kg	mortality range in days	body weight change in g/mouse (day of nadir)	median day of death	% ILS[b]	tumor-free survivors day 60	comments
P388 (1303)	10^6	ip	daunorubicin	1	1–4	4	(17–20)	+0.3 (7)	18	63	0/8	active
			L-PAM	5	1	5	(20–34)	+0.1 (7)	24	118	0/8	highly active
			CPA	75	1	75	(13–55)	−1.4 (14)	22.5	104	0/8	highly active
			24	0.75	1	0.75	(13–65)	−3.2 (36)	18.5	68	0/8	active
	10^6	ip	control				(9–13)	+2.9 (7)	11		0/8	
P388 (1313)	10^6	ip	adriamycin	1	1–4	4	(20–31)	−0.1 (4)	21.5	79	0/8	active
			L-PAM	5	1	5	(19–34)	−0.4 (7)	26.5	121	0/8	highly active
			24	1	1	1	(14–24)	−0.5 (7)	19	70	0/8	active
			24	0.5	1,4,7,11	2	(20–30)	0 (15)	25	108	0/8	highly active
				0.25	1,4,7,11	1	(17–27)	+2.4 (15)	19	58	0/8	active
			24	0.4	1–4	1.6	(19–39)	−0.9 (7)	23	91	1/8	active
				0.2	1–4	0.8	(16–28)	1.3 (7)	19.5	62	0/8	active
	10^6	ip	control				(11–19)	+2.9 (7)	12		0/8	
P388/L-PAM (12)	10^6	ip	daunorubicin	1	1–4	4	(16–27)	+1.4 (7)	19.5	79	0/8	active
			L-PAM	5	1	5	(13–31)	+0.6 (14)	15	15	0/8	inactive
			CPA	75	1	75	(20–62)	+0.4 (7)	22.5	73	0/8	active
			24	0.75	1	0.75	(9–19)	−1.0 (7)	15.5	19	0/8	inactive
	10^6	ip	control				(13–19)	+1.9 (7)	13		0/8	
P388/L-PAM (14)	10^6	ip	adriamycin	1	1–4	4	(20–31)	+1.1 (7)	23	64	2/8	active
			L-PAM	5	1	5	(14–19)	0 (4)	16	14	0/8	inactive
			24	1	1	1	(16–26)	−1.2 (11)	19	36	0/8	marginal activity
				0.5	1	0.5	(14–22)	+1.3 (4)	18	28	0/8	minimal activity
			24	0.5	1,4,7,11	2	(20–32)	+0.7 (15)	22.5	61	0/8	active
				0.25	1,4,7,11	1	(14–20)	0.9 (4)	16.5	18	0/8	inactive
				0.125	1,4,7,11	0.5	(13–27)	0.8 (4)	15.5	10	0/8	inactive
			24	0.4	1,2,3,4	1.6	(16–27)	0.9 (15)	20	43	0/8	some activity
				0.2	1,2,3,4	0.8	(16–20)	1.7 (7)	19	36	0/8	marginal activity
				0.1	1,2,3,4	0.4	(11–21)	0.5 (15)	14	0	0/8	inactive
	10^6	ip	control				(12–20)	+1.1 (7)	14		0/8	
L1210 (935)	10^5	ip	CPA	200	1	200	(20–48)	−3.4 (15)	22	100	2/8	highly active
				100	1	100	(14–48)	−1.8 (15)	18	64	0/8	active
			24	1	1	1	(12–16)	+0.6 (11)	14	27	0/8	minimal activity
				0.5	1	0.5	(13–16)	+0.3 (7)	14	27	0/8	minimal activity
			24	0.5	1,4,7,11	2	(13–18)	+1.1 (11)	14	27	1/8	minimal activity
				0.25	1,4,7,11	1	(12–14)	+1.6 (11)	13	18	0/8	inactive
				0.125	1,4,7,11	0.5	(12–14)	+2.7 (11)	13	18	0/8	inactive
			24	0.4	1–4	1.6	(12–20)	+0.3 (7)	14	27		minimal activity
				0.2	1–4	0.8	(13–14)	+0.4 (7)	14	27	1/8	minimal activity
				0.1	1–4	0.4	(12–14)	+0.4 (7)	14	27	1/8	minimal activity
			control				(11–13)	+1.1 (7)	11		0/8	

[a] Based on the median day of death (dying mice only). [b] Percent increase in life span (ILS) = 100 × [(median day of death of the treated group)−(median day of death of the control group)]/(median day of death of the control group).

Table IV. 5-(Benzoyloxy)-6-methoxyquinazolin-4-amines

compd	method	mp, °C	purifn proced[a]	purifn solvent	yield, %	formula	anal.
19a	A	135.5	D	CH_3CN	73	$C_{18}H_{19}N_3O_3$	C,H,N
19c	A	120	D	MeOH-H_2O	39	$C_{19}H_{19}N_3O_4$	C,H,N
19d	B	oil	E	AcOEt-ligroin (3:7)	61	$C_{22}H_{25}N_3O_4$	C,H,N
19e	B	oil	F	CH_2Cl_2-AcOEt (6:4)	88	$C_{29}H_{38}N_4O_5$[b]	
19f	B	oil	F	AcOEt-MeOH-Et_3N (8.9:1:0.1)	93	$C_{20}H_{24}N_4O_2$	C,H,N
19g	B	oil	F	CH_2Cl_2-Et_3N (9.9:0.1)	81	$C_{22}H_{28}N_4O_2$[b]	
19h	B	oil	F	MeOH-Et_3N (9.9:0.1)	95	$C_{21}H_{26}N_4O_2$	C,H,N
19i	B	oil	F	MeOH-Et_3N (9.9:0.1)	70	$C_{23}H_{30}N_4O_2$	C,H,N
19j	B	oil	F	MeOH-Et_3N (9.9:0.1)	83	$C_{25}H_{34}N_4O_2$	C,H,N
19k	B	oil	F	MeOH	90	$C_{21}H_{26}N_4O_2$ 0.25H_2O	C,H,N
19l	C	155	D	AcOEt	66	$C_{24}H_{24}N_8O_2$[b]	

[a] Purification procedure: D, recrystallization; E, flash chromatography on alumina; F, flash chromatography on silica gel. [b] Because of the instability of the amine, elemental analysis was not attempted, MS m/z 523 [$(M + H)^+$], 381 [$(M + H)^+$], 457 [$(M + H)^+$] respectively for 19e, 19g, 19l.

In conclusion, compound **24** had good antitumor activity against leukemias and early-stage solid tumors, M5076, B16 melanoma, and GOS. It was invariably inactive on advanced tumors. Although the agent had some activity, it was found highly toxic and produced long-delayed deaths.

Experimental Section

Chemistry. Melting points were determined on a Maquenne apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 157G spectrometer. NMR spectra were measured with a Bruker 270 MHz spectrometer with $(Me_3Si)_2$ as an internal reference. DCI/NH_3 mass spectra were recorded on a Nermag R10-10C instrument. Thin-layer chromatography was carried out on Merck GF 254 silica gel plates. Flash column chromatography was performed either on silica gel (Lichroprep Si60, Merck) or on alumina (N32-63, Woelm). All elemental analyses were within 0.4% of the theoretical values.

General Procedure for the Preparation of the 5-(Benzyloxy)-6-methoxyquinazolin-4-amines (19). Eleven millimoles of amino derivate **18a,f-j,k** or amino ester monohydrohalide **18c-e** was added under N_2 to a solution of 10 mmol of 5-(benzyloxy)-4-chloro-6-methoxyquinazoline (**17**)[5] and 11 or 22 mmol of Et_3N in 30 mL of dry EtOH. The mixture was stirred for 24 h at room temperature. After removal of the solvent under reduced pressure, the precipitate was filtered off and washed with H_2O (method A) or extracted with CH_2Cl_2. The organic layer was washed with H_2O until the pH was neutral and then evaporated under reduced pressure (method B). For compound **19l**, the amine **18l**[19] and 10 mL of Me_2SO were used instead of EtOH and 150 mL of H_2O was added before extraction by CH_2Cl_2 (method C). The products were purified as indicated in Table IV. IR (KBr) and NMR data were given as typical examples for compounds **19a**, **19e**, and **19h**.

19a: IR 3150 [ν(OH)], 3400 [ν(NH)] cm^{-1}; NMR (Me_2SO-d_6) δ 3.6 (t, 2 CH_2OH), 3.95 (s, 3, CH_3O), 4.4 (q, 2, CH_2NH), 4.45 (s, 1, OH), 5.15 (s, 2, CH_2O), 7.35 (d, 1, H_7), 7.3–7.45 (m, 5, ArH), 7.55 (d, 1, H_8), 8.3 (m, 2, H_2 and NH).

19e: IR 1740 [ν(CO)], 3400 [ν(NH)] cm^{-1}; NMR ($CDCl_3$) δ 0.75 (m, 12, CH_3), 1.5 (m, 6, CH_2 and CH(CH_3)$_2$), 3.6 (s, 3, CH_3OCO), 3.95 (s, 3, CH_3O), 4.6 (q, 2, CHNH), 5.1 (s, 1 H, CH_2O), 5.3 (s, 1, CH_2O), 6.95 (d, 1, NHCO), 7.45 (d, 1, H_7), 7.6 (d, 1, H_8), 7.3–7.6 (m, 5, ArH), 8.15 (d, 1, NH), 8.33 (s, 1, H_2).

19h: IR 3400 [ν(NH)] cm^{-1}; NMR (Me_2SO-d_6) δ 1.44 (quint, 2, $CH_2CH_2CH_2$), 2.00 (s, 6, N(CH_3)$_2$), 2.09 (t, 2, CH_2N), 3.33 (q, 2, CH_2NH), 3.93 (s, 3, CH_3O), 5.2 (s, 2, CH_2O), 7.29–7.64 (m, 7, ArH, H_7, H_8), 8.02 (t, 1, NH), 8.17 (s, 1, H_2).

5-(Benzyloxy)-6-methoxy-4-[[2-[(*n*-propylcarbonyl)oxy]ethyl]amino]quinazoline (19b). Butyryl chloride (0.245 g, 2.3 mmol) was added slowly at 5 °C and under N_2 to a solution of 0.5 g (1.5 mmol) of **19a** and 3 mL of Et_3N in 125 mL of C_6H_6. The mixture was stirred at 50 °C for 24 h. The solution was poured onto ice and then extracted with C_6H_6. The organic layer was washed with H_2O and then evaporated under reduced pressure. Flash chromatography [AcOEt–MeOH (9.8:0.2) as an eluant], gave 0.215 g (60%) of the oily compound **19b**: IR (KBr) 3250 [ν(OH)], 3400 [ν(NH)] cm^{-1}; NMR ($CDCl_3$) δ 0.85 (t, 3, CH_3), 1.5 (hex, 2, CH_2CH_3), 2.1 (t, 2, CH_2CO), 3.6 (q, 2, CH_2NH), 3.95 (s, 3, CH_3O), 4.05 (t, 2, CH_2OCO), 5.15 (s, 2, CH_2O), 7.35 (d, 1, H_7), 7.25–7.45 (m, 5, ArH), 7.5 (d, 1, H_8), 8.1 (t, 1, NH), 8.25 (s, 1, H_2). Anal. ($C_{22}H_{25}N_3O_4$) C, H, N.

5-(Benzyloxy)-4-[(8-hydroxy-3,6-dioxaoctyl)oxy]-6-methoxyquinazoline (19m). A suspension of 1 g (3.2 mmol) of **17**, 2.44 mL (18 mmol) of triethylene glycol, and 4.5 g (3.26 mmol) of K_2CO_3 in 40 mL of acetone was refluxed under N_2 for 7 h. The solvent was evaporated and the residue was extracted with CH_2Cl_2. After evaporation of the solvent, the solid residue was recrystallized from benzene: 1.38 g (82%); mp 73 °C; IR (KBr) 3250 [ν(OH)] cm^{-1}; NMR ($CDCl_3$) δ 2.15 (s, 1, OH), 3.39 (m, 6, CH_2), 3.53 (t, 2, CH_2), 3.71 (t, 2, CH_2), 3.87 (s, 3, CH_3O), 4.63 (t, 2,4-OCH_2), 5.05 (s, 2, ArCH_2O), 7.2–7.5 (m, 5, ArH), 7.55 (d, 1, H_7), 7.67 (d, 1, H_8), 8.50 (s, 1, H_2). Anal. ($C_{22}H_{26}N_2O_6$) C, H, N.

General Procedure for the Preparation of the 5-Hydroxy-6-methoxyquinazolin-4-amines (20). Method A. A solution of 3 mmol of **19** in 30 mL of TFA was refluxed under N_2 for 3 h. MeOH (20 mL) was added and the solution was evaporated under reduced pressure. The residue was triturated with H_2O and the mixture was adjusted to pH 9 with NH_4OH. The precipitate was filtered off and washed with H_2O. Compounds **20a** (80%), **20k** (20%).

Method B. Three millimoles of **19** in solution in 20 mL of dioxane–MeOH (1:1) was hydrogenated over 0.85 g of 10% palladium on activated carbon at room temperature and atmospheric pressure. After removal of the catalyst by filtration, the solvent was eliminated under reduced pressure.

The compounds **20** were not purified because of their instability in air. They were directly used in the following procedure. Compounds **20b** (90%), **20c** (93%), **20d** (100%), **20e** (100%), **20f** (92%), **20g** (100%), **20h** (90%), **20i** (100%), **20j** (100%), **20l** (95%), **20m** (97%). IR (KBr) and NMR data were given as typical examples for compounds **20a**, **20b**, **20e**, **20h**, and **20m**.

20a: IR 3250 [ν(OH)], 3400 [ν(NH)] cm^{-1}; NMR (Me_2SO-d_6) δ 3.55 (m, 4, CH_2), 4.05 (s, 3, CH_3O), 4.9 (s, 1, CH_2OH), 6.4 (s, 1, H_7), 7.15 (m, 2, H_8 and NH), 8.05 (s, 1, H_2), 11.0 (s, 1,5-OH).

20b: IR 1735 [ν(C=O)], 3325 [ν(OH)], 3420 [ν(NH)] cm^{-1}; NMR (Me_2SO-d_6) δ 0.8 (t, 3, CH_3), 1.45 (hex, 2, CH_2CH_3), 2.2 (t, 2, CH_2CO), 3.8 (s, 3, CH_3O), 3.8 (q, 2, CH_2NH), 4.2 (t, 2, CH_2OCO), 6.8 (d, 1, H_7), 7.4 (d, 1, H_8), 7.6 (t, 1, NH), 8.35 (s, 1, H_2), 11 (s, 1, OH).

20e: IR 1750 [ν(CO)], 3100–3500 [ν(OH) and ν(NH)] cm^{-1}; NMR ($CDCl_3$) δ 0.77 (m, 12, CH_3), 1.3–1.7 (m, 6, CH_2 and CH-(CH_3)$_2$), 3.7 (s, 3, CH_3O or CH_3OCO), 3.8 (s, 3, CH_3O or CH_3OCO), 4.53 (q, 1, CHN), 4.95 (q, 1, CHN), 7.2 (s, 2, H_7 and H_8), 7.50 (d, 1, NH), 7.65 (d, 1, NH), 8.15 (s, 1, H_2), 11.9 (s, 1, OH).

20h: IR 3350–3450 [ν(OH) and ν(NH)] cm^{-1}; NMR (Me_2SO-d_6) δ 1.91 (quint, 2, $CH_2CH_2CH_2$), 2.6 (s, 6, N(CH_3)$_2$), 2.88 (t, 2, CH_2N), 3.57 (q, 2, CH_2NH), 3.69 (s, 3, CH_3O), 6.4 (d, 1, H_7), 7.13 (d, 1, H_8), 8.16 (s, 1, H_2).

20m: IR 2900 and 3400 [ν(OH) and ν(NH)] cm^{-1}; NMR ($CDCl_3$) δ 2.79 (s, 1, CH_2OH), 3.56 (m, 4, CH_2), 3.62 (m, 2, CH_2), 3.68 (t, 4, CH_2), 3.96 (s, 3, CH_3O), 4.78 (t, 2, 4-OCH_2), 7.5 (s, 2, H_7 and H_8), 8.53 (s, 1, H_2), 11.40 (s, 1, 5-OH).

General Procedure for the Preparation of the 4-Amino-6-methoxy-5,8-quinazolinediones (21). To a solution of 1 mmol

Table V. 6-Methoxy-4-substituted-5,8-quinazolinediones

compd	solvent[a] (mL)	$(KSO_3)_2NO$, mmol	time, h	purifn proced[b] (solvent)	mp, °C	yield, %	formula	anal.
21a	A (55)	6.0	6	C (CH_3CN)	234	75	$C_{11}H_{11}N_3O_4$	C,H,N
21b	B (50)	2.5	3	C (*n*-PrOH)	155	80	$C_{15}H_{17}N_3O_5$	C,H,N
21c	B (60)	2.4	2	C (*n*-PrOH)	235	72	$C_{12}H_{11}N_3O_5 \cdot 0.25H_2O$	C,H,N
21d	A (65)	3.0	15	D [CH_2Cl_2-AcOEt (8.5:1.5)]	150	61	$C_{15}H_{17}N_3O_5$	C,H,N
21e	A (100)	3.0	5	D [CH_2Cl_2-AcOEt (4:6)]	98	45	$C_{22}H_{30}N_4O_6$	C,H,N
21f	A (55)	2.5	4.5	D [AcOEt-MeOH-Et_3N (8.4:1.5:0.1)]	158	31	$C_{13}H_{16}N_4O_3$	C,H,N
21g	A (60)	2.5	5	D [CH_2Cl_2-MeOH-Et_3N (9.8;0.1:0.1)]	174	25	$C_{15}H_{20}N_4O_3 \cdot 0.5H_2O$	C,H,N
21h	A (85)	3.5	4	C (AcOEt)	152	51	$C_{14}H_{18}N_4O_3$	C,H,N
21i	A (60)	5.5	24	D [MeOH-Et_3N (9.9:0.1)]	169	30	$C_{16}H_{22}N_4O_3$	C,H,N
21j	a (70)	2.5	6.5	D [MeOH-Et_3N (9.9:0.1)]	168	27	$C_{18}H_{26}N_4O_3$	C,H,N
21l[c]					206	30	$C_{17}H_{16}N_8O_3 \cdot 1.5H_2O$	C,H,N
21m[c]				C (C_6H_6)	130	36	$C_{15}H_{18}N_2O_7$	C,H,N

[a] Solvent for synthesis: A, MeOH-H_2O (6:4); B, acetone-H_2O (6:4). [b] Purification procedure: C, recrystallization; D, flash chromatography on silica gel. [c] See Experimental Section.

of the phenols **20** and 0.15 g (1.1 mmol) of monobasic potassium phosphate in 50–100 mL of MeOH-H_2O (6:4) or acetone-H_2O (6:4) (see Table V) 0.67–1.61 g (2.5–6.0 mmol) of potassium nitrosodisulfonate was added. The mixture was stirred several hours under N_2. The reaction was monitored by TLC. After removal of the organic solvent under reduced pressure, the mixture was adjusted, if necessary, to pH 7 with a 10% sodium hydrogen carbonate solution and then extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give the quinones **21**, which were purified by crystallization or by flash chromatography. Yields and data are listed in Table V. IR (KBr) and NMR data were given as typical examples for compounds **21a**, **21b**, 21e, and **21h**.

21a: IR 1650 and 1672 [ν(C=O)], 3420 and 3450 [ν(OH) and ν(NH)] cm^{-1}; NMR (Me_2SO-d_6) δ 3.60 (m, 4, CH_2CH_2), 3.85 (s, 3, CH_3O), 4.85 (s, 1, OH), 6.30 (s, 1, H_7), 8.75 (s, 1, H_2), 9.15 (t, 1, NH).

21b: IR 1725 [ν(C=O ester)], 1650 and 1665 [ν(C=O quinone)], 3350 [ν(NH)] cm^{-1}; NMR ($CDCl_3$) δ 0.85 (t, 3, CH_3), 1.65 (hex, 2, CH_2CH_3), 2.3 (t, 2, CH_2CO), 3.85 (m, 5, CH_3O and CH_2NH), 4.25 (t, 2, CH_2OCO), 6.15 (s, 1, H_7), 8.85 (s, 1, H_2), 9.1 (t, 1, NH).

21e: IR 1725 [ν(C=O ester)], 1650 and 1665 [ν(C=O) quinone)], 3350 [ν(NH)] cm^{-1}; NMR ($CDCl_3$) δ 0.84 (m, 12, CH_3), 1.55–1.75 (m, 6, CH_2 and $CH(CH_3)_2$), 3.64 (s, 3, CH_3OCO), 3.88 (s, 3, CH_3O), 4.55 (q, 1, CHNH), 4.68 (q, 1, CHNH), 6.17 (s, 1, H_7), 6.33 (d, 1, NHCO), 8.8 (s, 1, H_2), 9.06 (d, 1,4-NH).

21h: IR 1650 and 1665 [ν(C=O)], 3300 [ν(NH)] cm^{-1}; NMR ($CDCl_3$) δ 1.80 (quint, 2, $CH_2CH_2CH_2$), 2.22 (s, 6, $N(CH_3)_2$), 2.35 (t, 2, $CH_2N(CH_3)_2$), 3.69 (q, 2, CH_2NH), 3.87 (s, 3, CH_3O), 6.13 (s, 1, H_7), 8.80 (s, 1, H_2), 9.36 (t, 1, NH).

4-[(3-Aden-9-ylpropyl)amino]-6-methoxy-5,8-quinazolinedione (21l). Potassium nitrosodisulfonate (0.458 mg, 1.7 mmol) was added to a solution of 0.250 g (0.68 mmol) of **20l** and 1.16 g of dibasic sodium phosphate dodecahydrate in 65 mL of MeOH-H_2O (1:1). The mixture was stirred for 5.5 h. A precipiate was then filtered off and washed with H_2O. After elimination of the MeOH-H_2O under reduced pressure, the quinone was purified by chromatography on a Servachrom XAD-2 (0.15–0.20 mm) resin column and then recrystallized from MeOH-H_2O (5:4). For data, see Table V; IR (KBr) 1650 [ν(C=O)], 3340 [ν(NH)], 3400 and 3550 [$\nu(NH_2)$] cm^{-1}; NMR ($CDCl_3$) δ 2.24 (t, 2, $CH_2CH_2CH_2$), 3.63 (q, 2, CH_2NH), 3.88 (s, 3, CH_3O), 4.27 (t, 2, CH_2N), 6.10 (s, 2, NH_2), 6.15 (s, 1, H_7), 7.81 (s, 1, H_2 or H_8 adenyl), 8.39 (s, 1, H_2 or H_8 adenyl), 8.82 (s, 1, H_2), 9.34 (t, 1, NH).

4-[(8-Hydroxy-3,6-dioxaoctyl)oxy]-6-methoxy-5,8-quinazolinedione (21m). To a solution of 0.324 g (1 mmol) of the phenol **20m** and 0.412 g (1.15 mmol) of dibasic sodium phosphate dodecahydrate in 40 mL of H_2O was added 0.94 g (3.5 mmol) of potassium nitrosodisulfonate. The mixture was stirred for 3.5 h and then extracted with CH_2Cl_2. For data, see Table V; IR (KBr) 1640 and 1685 [ν(C=O)], 3050 and 3400 [ν(OH)] cm^{-1}; NMR ($CDCl_3$) δ 2.27 (s, 1, OH), 3.52 (t, 2, CH_2), 3.60 (t, 2, CH_2), 3.73 (t, 2, CH_2), 3.82 (s, 3, CH_3O), 3.89 (t, 2, CH_2), 4.15 (t, 2, CH_2), 4.69 (t, 2, 4-OCH_2), 6.22 (s, 1, H_7), 9.0 (s, 1, H_2).

Reaction of the 4-[[3-(*N*,*N*-Dimethylamino)propyl]amino]-6-methoxy-5,8-quinazolinedione (21 h) with Amines. 4-[[3-(*N*,*N*-Dimethylamino)propyl]amino]-6-piperidino-5,8-quinazolinedione (22). Quinone 21h (0.29 g, 1 mmol) was added at 0 °C to a solution of 0.13 mL (1.3 mmol) of piperidine in 28 mL of dry MeOH. The mixture was stirred for 5 h at 0 °C and evaporated under reduced pressure. The residue was extracted with CH_2Cl_2. The organic layer was washed with H_2O and then evaporated. The quinone was purified by flash chromatography (eluent MeOH-Et_3N (99:1):0.127 g (37%); mp 105 °C; IR (KBr) 1655 [ν(C=O)], 3200 [ν(NH)] cm^{-1}; NMR ($CDCl_3$) δ 1.75 (m, 6, piperidine), 1.80 (quint, 2, $CH_2CH_2CH_2$), 2.22 (s, 6, $N(CH_3)_2$), 2.40 (t, 2, CH_2N), 3.50 (m, 4, piperidine), 3.64 (q, 2, CH_2NH), 6.0 (s, 1, H_7), 8.80 (s, 1, H_2), 9.33 (t, 1, NH). Anal. ($C_{18}H_{25}N_5O_2 \cdot 0.25H_2O$) C, H, N.

6,7-Bis(1-aziridinyl)-4-[[3-(*N*,*N*-dimethylamino)propyl]amino]-5,8-quinazolinedione (24). Quinone **21h** (0.500 g, 1.72 mmol) was added at 0 °C to 10 mL of aziridine. After 4.5 h of stirring at 0 °C under N_2, an excess of amine was eliminated under reduced pressure. The residue was extracted with CH_2Cl_2 which was washed with H_2O. After removal of the solvent, the solid has to be immediately purified by flash chromatography (eluent: CH_2Cl_2-MeOH, 8:2). The fractions were evaporated as fast as possible. The solid was then washed with ligroin: 0.206 g (35%); mp 100 °C; IR (KBr) 1660 [ν(C=O)], 3320 [ν(NH)] cm^{-1}; NMR ($CDCl_3$) δ 1.75 (quint, 2, $CH_2CH_2CH_2$), 2.21 (s, 6, $N(CH_3)_2$), 2.34 (t, 2, CH_2N), 2.30 (s, 4, CH_2 aziridine), 2.37 (s, 4, CH_2 aziridine), 3.62 (q, 2, CH_2NH), 8.71 (s, 1, H_2), 9.29 (t, 1, NH). Anal. ($C_{17}N_{22}N_6O_2$) C, H, N.

L1210 Inhibition Growth Rate Determination. L1210 leukemia cells were grown in nutrient medium RPMI 1640 supplemented with 2 mM L-glutamine, 200 IU/mL penicillin, 50 μg/mL streptomycin, and 20% heat inactivated horse serum. They were incubated in a 5% CO_2 atmosphere at 37 °C. For the experiments the drugs were dissolved in dimethyl sulfoxide (0.5% final) and added to the cells in exponential phase of growth at an initial concentration of 0.8×10^5 cells/mL. The cells were counted in triplicate after 48 h with a Coultronics Coulter Counter and results were expressed as the drug concentration which inhibited cell growth by 50% as compared to controls (IC50). The IC50 values were calculated from regression lines obtained from the probit of the percent cell growth inhibition plotted as a function of the logarithm of the dose.

Sensitivity of Human Cell Lines Evaluated by a Cell Image Processor. MCF-7 human breast cancer cells and HBL human melanoma tumor cells were maintained as monolayers in Eagle's minimal essential medium (MEM) supplemented with 0.6 mg/mL glutamine, 100 IU/mL penicillin, 10 μg/mL streptomycin, and 10% fetal heat-inactivated calf serum. The experimental schedule has been described.[22] Briefly, cells growing in the logarithmic phase were cultured on glass coverslips placed in 35 × 10 mm petri dishes containing 3 mL of medium with 10.1 and 0.1 μg/mL of drug. After 1–4 days the coverslips were fixed, mounted on histological slides, and stained by the Feulgen reaction.[23] These were then studied by a cell image analysis which permits determination of the effects of a drug on cell proliferation

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and cell cycle kinetic parameters.

In Vivo Murine Tumor Models. The murine tumors used for in vivo evaluation are currently passaged in our laboratories and were obtained either from the National Cancer Institute (NCI), Bethesda, MD, or from Dr. Corbett, Wayne State University, Detroit, MI.

All tumors were maintained in the mouse strain of origin: DBA/2 mice, P388, L1210 leukemias; C57BI/6 mice, B16 melanoma, Glasgow osteogenic sarcoma,[24] colon adenocarcinoma 38,[25] Lewis lung carcinoma, M5076 reticulum cell sarcoma; C3H/He mice, mammary adenocarcinoma 16/C.[26] These tumors were transplanted into the appropriate F1 hybrid or the strain of origin for therapy trials. Mice were bred at IFFA-CREDO (Lyon, France) from strains obtained from Jackson Laboratories, Bar Harbor, ME, or Charles River, France. Mice were over 18 g at the start of therapy. They were supplied food and water ad libidum.

Solid Tumors. Mammary adenocarcinoma 16/C, B16 melanoma, Glasgow osteogenic sarcoma, and Colon adenocarcinoma 38 were implanted subcutaneously (sc) with 30–60-mg tumor fragments by trocar on day 0. B16 melanoma was also implanted as an intraperitoneal 10% brei. Lewis lung carcinoma was implanted intramuscularly (im) with 10^6 cells. M5076 reticulum cell sarcoma was implanted ip with 10^6 cells.

Chemotherapy was started within 1–6 days after tumor implantation. Subcutaneously implanted tumors were measured with a caliper. Tumor weights were calculated from 2-dimensional measurements: tumor weight (mg) = $(l \times w^2)/2$, where l and w are the tumor length and width, respectively. Tumor growth inhibition (T/C value in percent) was used for the determination of activity where T and C are respectively the median tumor weight of the treated and the control groups. A T/C equal or less than 42% is considered significant antitumor activity by the NCI; a T/C value <10% is considered to indicate high antitumor activity and is the level used by NCI to justify further development. In the case in which survival was the end point for the therapeutic effectiveness (B16 melanoma ip brei, M5076 ip), the following criteria for activity was used: increase in life span, % ILS = 100 × [(median day of death of the treated group) − (median day of death of the control group)]/(median day of death of control group). Finally the percentage of metastasis inhibition was also used with the Lewis lung carcinoma: the surviving mice were killed on day 19, the lungs removed and fixed in Fekete solution, and the macroscopic lung metastasis was counted. The percentage metastasis inhibition was evaluated as follows: T/C %, where T and C are respectively the mean number of metastasis of the treated and the control groups.

Leukemias. Viable P388 (10^6) and L1210 (10^5) leukemia cells were inoculated intraperitoneally on day 0. Treatment was started on day 1 by the ip route. Animal mortality was checked daily. The antitumor activity was evaluated as follows: percent increase in host life span ILS % = 100 × [(median day of death of the treated group) − (median day of death of the control group)]/(median day of death of the control group). The NCI criteria for activity are moderate activity P388 ILS > 25%, L1210 ILS > 20% and significant activity P388 ILS > 75%, L1210 ILS > 50%.

Drug Treatment. Compound **24** was administered iv or ip. It was dissolved in 5% glucose in distilled water and injected as a solution under 0.2 mL iv and 0.5 mL ip.

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Supplementary Material Available: Tables showing iv and ip toxicity of compound **24** in BD2F1 mice and percentage repartition of the MCF-7 cells and HBL human melanoma cancer cells into cell cycle (3 pages). Ordering information is given on any current masthead page.

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On the Optimization of Hydrophobic and Hydrophilic Substituent Interactions of 2,4-Diamino-5-(substituted-benzyl)pyrimidines with Dihydrofolate Reductase

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The inhibition constants (K_{iapp}) were obtained from the action of 68 2,4-diamino-5-(substituted-benzyl)pyrimidines on dihydrofolate reductase from an *Escherichia coli* strain MB 1428. Subsequently, these results were used to formulate appropriate quantitative structure–activity relationships (QSAR). Once again these equations emphasize the paramount importance of steric/dispersion factors in enhancing antibacterial potency. Hydrophobicity also plays a role, albeit a minor one. Comparisons with the QSAR obtained versus prokaryotic dihydrofolate reductase (DHFR) demonstrate subtle differences in binding behavior between meta and para substituents which may be effectively maximized in the design of more efficacious and selective antibacterial agents. The bacterial and avian QSAR equations can be used to calculate selectivity indices for trimethoprim, tetroxoprim, and two other specially designed 2,4-diamino-5-(substituted-benzyl)pyrimidines.

In the design of more efficacious drugs, one of the most serious problems is minimizing toxicity to the host while maximizing it toward the pathogen. Adrien Albert has succinctly defined this goal as "selective toxicity".[1] In the past, one hoped to make major advances by synthesizing and testing as many compounds as possible while always relying on the "intuition" of the medicinal chemist. Although this classical approach to drug research has not been deserted, the glimmerings of the promised land of "rational drug design" loom near the horizon. Elegant techniques for the isolation of enzymes and receptors and their subsequent cloning to obtain reasonable quantities of enzymes for routine work auger well for the future. In the case of antibacterial agents, one hopes to be able to detect significant differences between the human drug

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