Synthesis of 3,6-Bis(aminoalkoxy)acridines and Their Effect on the Immune System

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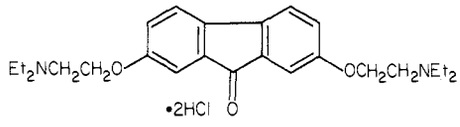
A series of 3,6-bis(aminoalkoxy)acridines (2) was prepared and shown to have a protective antiviral effect against an interferon-sensitive virus (Columbia SK) and to partially restore an antibody response to a T-cell-dependent antigen in leukemic immunosuppressed mice. The presence of circulating interferon and the stimulation of natural killer cell activity in mice was observed for 21.

Experimental studies in animals have demonstrated the antitumor potential of a number of immunostimulants, including live organisms of bacillus Calmette-Guerin (BCG), interferon, tilorone (1),[2] heat-killed cells of *Corynebacterium parvum*, polynucleotides, and the anthelmintic drug levamisole. These substances have been shown to stimulate cellular immunity and to produce tumor regressions. Some successes have been claimed in early clinical trials with BCG against malignant melanoma and acute leukemia, with levamisole against lung cancer and breast cancer, and with interferon in osteosarcoma. Although the antitumor effects produced by these agents have been promising, significant therapeutic benefits have yet to be realized. Since this is a new therapeutic approach, new drugs and methods of treatment must receive careful clinical evaluation in order to reveal the full potential of these drugs.



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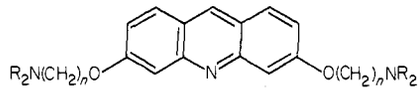

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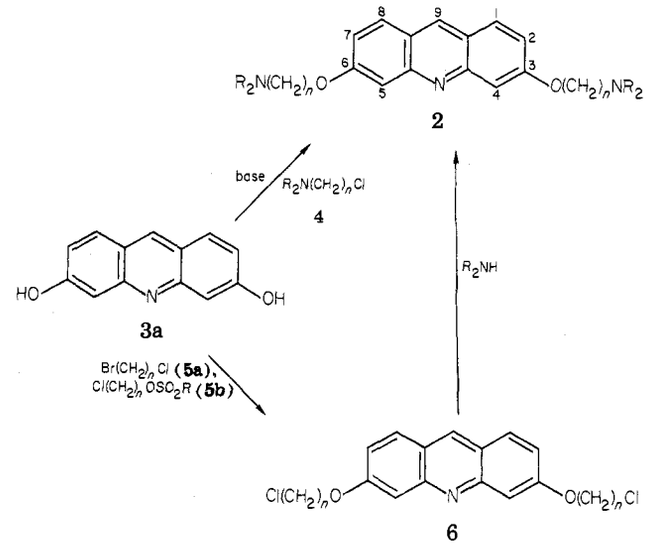
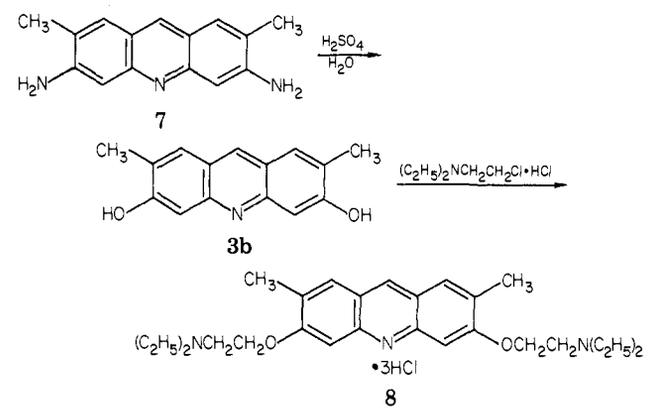
A limited series of 3,6-bis[(dialkylamino)ethoxy]acridines and 3,6-bis[(dialkylamino)propoxy]acridines was synthesized earlier as distant analogues of tilorone. Several of these compounds were shown to have antiviral activity against a lethal Columbia SK virus infection in mice.[3] The Columbia SK virus is an interferon-sensitive virus, and the ability of these compounds to induce circulating interferon indirectly was demonstrated. Subsequent testing showed a much broader effect on the immune system. In normal mice, oral administration of **21** induced significant increases in NK cell activity of splenic cell suspensions, as evidenced by in vitro lysis of xenogeneic human Molt-4 tumor target cells in ^{51}Cr release assays. Partial restoration of the antibody response to a T-cell-dependent antigen [sheep red blood cells (SRBC)] was demonstrated in leukemic, immunosuppressed mice. Protective levels of circulating interferon were induced for **21** in mice. Although there was some reduction of splenomegaly in mice infected with the Rauscher virus and some activity against P-388 leukemia in mice, most of the compounds, in general, were not substantially cytotoxic in in vivo leukemia test systems.

Scheme I



Scheme II



Chemistry. Two synthetic pathways were followed to obtain the compounds (Scheme I). Both proceeded via a common intermediate, 3,6-acridinediol (**3a**).[4] The desired compounds (**2**) were obtained from 3,6-acridinediol (**3a**) either by direct reaction with a (dialkylamino)alkyl chloride (**4**) or in two steps by reaction with a bromochloralkane (**5a**) or a chloroalkyl sulfonate (**5b**) to give an intermediate 3,6-bis(chloroalkoxy)acridine (**6**), which subsequently reacted with an amine. The latter method was the sole method for the preparation of the unsubsti-

(1) 1979 Summer Intern from Mount Holyoke College.
(2) (a) R. H. Levin, *Aldrichim. Acta*, 12(4), 77–82 (1979). (b) W. L. Albrecht, R. W. Flemming, S. W. Horgan, and G. D. Mayer, *J. Med. Chem.* **20**(3), 364 (1977).
(3) K. C. Murdock, U.S. Patent, 3 740 403, 1973.
(4) L. Benda, *Ber. Dtsch. Chem. Ges.*, **45**, 1787 (1912).

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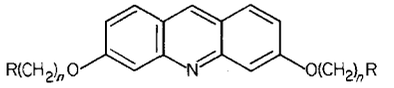
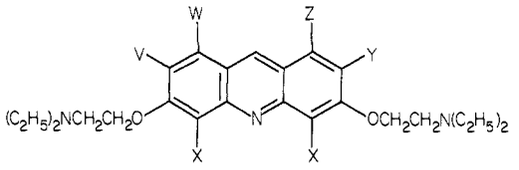
Table I. Acridines Prepared[a]



no.	n	R	yield, %	mp, °C	method of preparation	% reduction in splenomegaly,[b,c] mg/kg	antibody stimulation[c,d]	antiviral act.[c,e]
2a	2	$N(C_2H_5)_2 \cdot 3HCl \cdot H_2O$	41	225–227[g]	A	70 (100)	256/32 (50)	15/15[f]
2b	2	$N(CH_3)_2$	84	103–104[h]	A	101[i] (50)	256/32 (100)	15/15
2c	3[l]	$N(CH_3)_2$	13	202–204[l]	A	NT[k]	NT[k]	5/15
2d	2	$NHCH_3 \cdot 3HCl$	68	267–268	B	86 (100)	128/16 (50)	1/15[j]
2e	2	$NH_2 \cdot 3HCl \cdot 2H_2O$	42	274–275	B	40 (100)[j]	32/16 (25)[j]	1/15[j]
2f	2	$NHC_2H_5 \cdot 3HCl \cdot H_2O$	71	253–255	B	89 (100)	32/16 (25)[j]	7/15
2g	2	$NH\text{-}n\text{-}C_3H_7 \cdot 3HCl \cdot H_2O$	61	250–252	B	101 (100)	64/16 (200)	10/15
2h	2	$NH\text{-}i\text{-}C_3H_7 \cdot 3HCl$	68	254–256	B	96 (200)	512/32 (100)	15/15
2i	2	$NH\text{-}n\text{-}C_4H_9 \cdot 3HCl \cdot 2H_2O$	70	254–256	B	92 (100)	128/16 (200)	7/15
2j	2	$NHCH_2CH_2OH \cdot 3HCl$	10	256–258	B	35 (50)[j]	32/16 (100)[j]	3/15[j]
2k	2	$c\text{-}NC_4H_8$	85	95–98	B	82 (50)	64/16 (50)	5/15[j]
2l	2	$c\text{-}NC_5H_{10} \cdot 3HCl$	66	236–238	B	62 (100)	128/16 (100)	5/15
2m	2	$c\text{-}N(CH_2CH_2)_2N\text{-}CH_3$	82	142–145	B	75 (50)	32/16 (50)[j]	1/15[j]
2n	3	$N(CH_3)_2 \cdot 3HCl \cdot 2.5H_2O$	57	248–250[m]	A	54 (200)	16/16 (25)[j]	9/15
2o	3	$N(C_2H_5)_2 \cdot 3HCl \cdot H_2O$	53	235–236	B	72 (100)	128/16 (25)	13/15
2p	3	$NH\text{-}n\text{-}C_3H_7 \cdot 3HCl \cdot H_2O$	58	260–262	B	96 (200)	NT[k]	13/15
2q	3	$NHC_2H_5 \cdot HCl \cdot H_2O$	53	265–266	B	78 (200)	32/16 (200)[j]	7/15
2r	3	$NHCH_3 \cdot 3HCl \cdot H_2O$	57	248–250	B	18[m] (100)	32/16 (25)[j]	8/15
2s	3	$c\text{-}NC_5H_{10} \cdot 3HCl \cdot H_2O$	60	264–266	B	74 (50)	1024/32 (50)	9/15
2t	3	$NHCH(C_2H_5)CH_2OH \cdot 3HCl \cdot 3H_2O$	68	228 dec		74 (100)	NA[j]	NT[k]
2u	4	$N(CH_3)_2 \cdot 3HCl \cdot H_2O$	49	218–220		52 (50)	NA[j]	2/15[j]
						cyclophosphamide 102 (25)	poly IC, ip, 1024/128 (10)	tilorone 5/20 (300)
								placebo controls 0/20

[a] All new compounds have correct supporting analytical and spectral data. [b] Percent reduction in spleen weight as compared to infected nontreated controls; a reduction of >50% is considered active. [c] Oral dosing. [d] Serum hemagglutinin titer (reciprocal of the highest dilution of serum prducing a ≥50% agglutination of sheep red blood cells as visualized on the bottom of plastic microwells) of test compound over the titer for the infected placebo-treated controls. The dosage is in parentheses and is in milligrams per kilogram. A restoration 4-fold is considered necessary for activity. [e] Efficacy of drugs against a lethal virus challenge with an interferon-sensitive virus, Columbia SK. Test evaluated 14 days postinfection. Active drugs have a S/T of ≥5/15 when the controls were 0/15. [f] S/T = survivors/treated. [g] Literature[3] mp 225–227 °C. [h] Literature[3] mp 103–104 °C. [i] Intraperitoneal drug administration. [j] NA = not active. [k] NT = not tested. [l] Side chain is $CH_2CH_2C(CH_3)$; literature[3] mp 202–204 °C. [m] Literature[3] mp of the free base is 90–91 °C.

Table II. Ring-Substituted Acridines Prepared[a]



no.	V	W	X	Y	Z	salts or other	yield, %	mp, °C	reduction in splenomegaly,[b,c] mg/kg	antibody stimulation[c,d]	antiviral act.[c,e,f]
8	CH_3	H	H	CH_3	H	$3HCl \cdot 2H_2O$	35	180-185[g]	21[h] (100)	256/64 (25)	4/15[h]
9a	H	H	Cl	H	H		86	81-83	86 (50)	128/16 (100)	7/15
9b	H	H	Br	H	H		74	66-68	92 (200)	64/16 (100)	6/15
9c	H	H	NO_2	H	H		82	138-140	108 (100)	64/16 (100)	1/15[h]
9d	H	H	Cl	NO_2	H		53	95-97	61 (200)	64/16 (50)	1/15[h]
9e	H	NO_2	Cl	NO_2	H		11	110 dec	46[h] (50)	32/16[h] (100)	3/15[h]
9f	H	H	NH_2	H	H	$4HCl \cdot 3H_2O$		190-195	55 (25)	128/16	3/15[h]
9g	H	H	Br	NO_2	H		46	98-101	NA[h]	NA[h]	5/15
									cyclophosphamide 102 (25)	poly IC, ip 1024/128 (10)	tilorone 5/20 (100)

[a] All new compounds have correct supporting analytical and spectral data. [b] Percent reduction in spleen weight as compared to infected nontreated controls; a reduction of ≥50% is considered active. [c] Oral dosing. [d] Serum hemagglutinin titer (reciprocal of the highest dilution of serum producing a ≥50% agglutination of sheep red blood cells as visualized on the bottom of plasma microwells) of test compound over the titer for the infected nontreated controls. A restoration of ≥4-fold is considered necessary for activity. [e] Efficacy of drugs against a lethal virus challenge with an interferon-sensitive virus, Columbia SK. Test evaluated 14 days postinfection. Active drugs have a S/T of >.5/15 when the controls were 0/15. [f] S/T = survivors/treated. [g] Literature[5,6] mp of the free base 108 °C. [h] Not active.

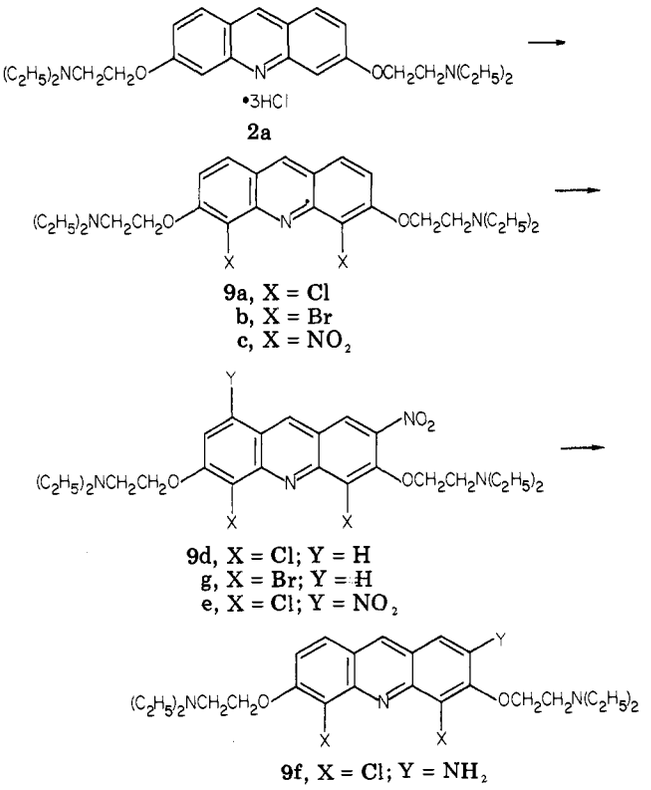
tuted and monoalkyl analogues listed in Table I.

2,7-Dimethyl-3,6-acridinediol (**3b**) was prepared from Acridine Yellow G (**7**) by the method described for the synthesis of 3,6-acridinediol.[4] Reaction of this intermediate with (diethylamino)ethyl chloride hydrochloride gave the 2,7-dimethyl analogue (**8**) (Scheme II) in low yield. Other ring-substituted derivatives were prepared by electrophilic aromatic substitution (Scheme III). The compounds synthesized are listed in Table II.

Reaction of **2a** with *N*-chlorosuccinimide or *N*-bromosuccinimide gave, respectively, 4,5-dichloro (**9a**) or 4,5-dibromo (**9b**) analogues (Scheme III). Similarly, nitration gave the 4,5-dinitro derivative (**9c**). The NMR in the aromatic region of these compounds showed a singlet and two doublets, consistent with symmetrical substitution at the 3,6- and 4,5-positions. Subsequent nitration of **9a** or **9b** under mild conditions gave 2-nitro-4,5-dihalo derivatives (**9d,g**) as the major product, accompanied by minor amounts of an isomeric mononitro material. Nitration under more forcing conditions gave the 1,7-dinitro-4,5-dichloro analogue (**9e**). The nitro group in **9d** was reduced catalytically to give the corresponding amino analogue (**9f**). The C-9 proton of one of the mononitro derivatives shifted from δ 8.52 to 8.83. The C-9 proton in the other mononitro derivative shifted from δ 8.52 to 9.56. The larger downfield shift of the C-9 proton would be expected from a nitro group peri to the C-9 position, so this material was assigned the 1-nitro-4,5-dichloro structure. The other compound was presumed to be the 2-nitro-4,5-dichloro isomer (**9d**). The 2-nitro compound also showed considerable change in the side-chain proton spectra due to the steric effect of the nitro group, thus strengthening this structural assignment. The dinitro derivative showed three singlets in the aromatic region. The only structure compatible with this pattern is the 1,7-dinitro-4,5-dichloro isomer (**9e**).

Pharmacology. Compounds similar to, but more potent than, known immunostimulants, such as levamisole and tilorone, could be effective in the eradication of tumor cells when used in conjunction with standard therapeutic measures. Stimulators of host resistance may be detected in animal models that can, in fact, detect both immunostimulators and anticancer agents. Mice are put in a

Scheme III



condition stimulating the immunodepression common to cancer patients. This was accomplished by infecting mice with a leukemia virus that produces both leukemia and a disease-related immunodepression. Effective compounds are recognized by their ability to stimulate the antibody response in the experimental mice. The anticancer effect is monitored by a reduction in splenomegaly associated with the leukemia (Tables I and II). Antiviral effects were measured as survival against a lethal infection of an interferon-sensitive virus (Columbia SK), and results are shown in Tables I and II. The mechanism for the antiviral action is presumed to be related to the in vivo generation

Table III. Effect of a Single Oral Dose of 21 on Induction of Interferon and Stimulation of Natural Killer Lymphocyte (NK-Cell) Activity in BDF_1 Mice

drug[a]	dose, mg/kg	av NK-cell cytotoxicity,[b] %	range of serum interferon titers,[c] units/mL
placebo	saline	25.3 (control)	10-10
21	800	8.1[d] ($p = 0.01$)	1000-1250
(test 1)	600	23.1[e]	1300-1500
	300	34.4[f] ($p = 0.01$)	2000-3200
	150	41.5[f] ($p = 0.01$)	1600-2000
placebo	saline	14.4 control	10-10
21	600	18.6[e]	3200-3200
(test 2)	300	31.7[f] ($p = 0.01$)	3200-5000
	150	39.6[f] ($p = 0.01$)	3200-5000
poly IC	10	37.9[f] ($p = 0.01$)	1600-1600

[a] Duplicate groups of six normal BDF_1 male mice received a single oral dose of 21 or placebo, or a single ip dose of poly IC, 18 h prior to sacrifice of mice. [b] Average percent of cytotoxicity of two pooled spleen cell suspensions prepared from duplicate groups of six male mice, for each drug level. Spleen cells/^{51}Cr-labeled Molt 4 cells ratio was 50:1. [c] Range of interferon titer observed for two pooled sera obtained from duplicate groups of six mice. [d] Indicates significant suppression of activity, relative to control. [e] No significant stimulation of activity, relative to control (p values obtained by Student's t test, using pooled standard deviation derived from analysis of variance). [f] Significant activity by Student's t test.

of interferon (see Pharmacology under Experimental Section), and **21** was shown to induce appreciable interferon titers (Table III).

Natural-killer cells represent an endogenous population of lymphocytes that mediate the direct destruction of a wide variety of syngeneic, allogeneic, and xenogeneic tumor cells, as well as virus-infected cells, when tested in vitro. These natural-killer lymphocytes (NK cells) are present in normal animals and are believed to play an important role in immune surveillance. NK-cell activity in animals increases after infection with a variety of viruses and bacteria or following the implantation of tumor cells. NK-cell activity can also be increased by injecting animals with a variety of interferon inducers (poly IC, pyran copolymer, LPS, *C. parvum*) or by injecting interferon itself. The results with **21** in stimulating NK-cell activity are shown in Table III.

Discussion

Substructure programs used to analyze the immune restoration data did not reveal any substructures that correlated well with activity within this series of compounds. Another program that uses a quantitative measure of structural similarity gave similar results.

The data in Tables I and II indicate that the highest degree of activity is present when the n in **2** is 2 or 3 and R_2 is a lower alkyl or joined to form a ring as in **2a,b** or **2k,s**. Similar activity was present when one R_2 was H and the other was i-C_3H_7. Halogen or nitro substitution gave essentially unchanged activities in the immune restoration screen but substantially decresed the activities in the remaining two tests.

Members of this series possessed an interesting spectrum of activities, and evaluation studies are in progress.

Experimental Section

All melting points were observed on a Mel-Temp apparatus. ^{1}H NMR's were determined with a Varian HA-100 spectrometer, and the chemical shifts are reported in parts per million relative to internal Me_4Si. The 3,6-acridinediol (**3a**) was essentially prepared as described in the literature.[4] The 2,7-dimethyl-3,6-acridinediol (**3b**) was prepared from 3,6-diamino-2,7-dimethylacridine (**7**) as described for 3,6-acridinediol.[4]

2,7-Dimethyl-3,6-acridinediol Hemisulfate Dihydrate (3b). The material was prepared from Acridine Yellow G (Aldrich Chemical Co.) in 68% crude yield (from an alkaline solution) by the method described for the 3,6-acridinediol hemisulfate.[4] Reprecipitation from an aqueous alkaline solution after treatment with Darco gave a yellow-green solid with a melting point over 360 °C (lit.[5,6] mp >300).

General Preparation of 3,6-Bis(aminoalkoxy)acridines. Method A. The reaction mixture from 4.65 g (0.022 mol) of 3,6-acridinediol (**3a**), 0.088 mol of NaH, and 0.044 mol of (dialkylamino)alkyl chloride hydrochloride in 50 mL of dimethylformamide was stirred at 100 ± 2 °C for 1.0 h, cooled, and then poured into 300 mL of water. The mixture was extracted with 3 × 100 mL of ether, the dried ($MgSO_4$) combined extracts were evaporated to dryness, and the residue was extracted with 50 mL of petroleum ether (bp 30–60 °C). Evaporation of this extract left a crystalline residue (5.08 g), which was dissolved in 50 mL of absolute ethanol and treated with 3.31 mL of 7.5 M ethanolic hydrogen chloride. The resulting crystals were collected and washed first with a minimum of cold, absolute ethanol and then with acetone. This material crystallized slowly from 1-propanol to give the desired product.

3,6-Bis[2-(dimethylamino)ethoxy]acridine (2b). A 50% emulsion of sodium hydride in mineral oil (5.75 g, 0.12 mol) was freed of mineral oil by decantation with three portions of petroleum ether. Dry dimethylformamide (100 mL) and dried [120 °C (0.1 mm)/1.5 h/P_2O_5] 3,6-acridinediol (**3a** 6.34 g, 0.03 mol) were added, and the mixture stirred until gas evolution appeared to be complete (20 min). 2-(Dimethylamino)ethyl chloride hydrochloride (8.64 g, 0.06 mol) was added, and the mixture was stirred and heated at 100 ± 2 °C for 1 h. Solvent was removed by evaporation, finally at 100 °C (0.1 mm). The residue was washed with 4 × 100 mL of ether. These ethereal extracts were passed through a column containing 130 g of alumina. The column was developed with 300 mL of ether and then eluted first with 1.3 L of ether/ethyl acetate, 4:1, and then with 250 mL of ethyl acetate, stopping when an orange band began to be eluted. The crystalline residue from evaporation of the eluates was recrystallized from heptane to give 2.45 g of pale yellow crystals, mp 103–104 °C.

3,6-Bis[2-(diethylamino)ethoxy]acridine Trihydrochloride (2a). The reaction mixture from 4.65 g (0.022 mol) of 3,6-acridinediol (**3a**), 0.088 mol of NaH, and 7.57 g (0.044 mol) of 2-(diethylamino)ethyl chloride hydrochloride in 50 mL of dimethylformamide was stirred at 100 ± 2 °C for 1.0 h, cooled, and then poured into 300 mL of water. The mixture was extracted with 3 × 100 mL of ether, the dried ($MgSO_4$) extracts were evaporated to dryness, and the residue was extracted with 50 mL of petroleum ether (bp 30–60 °C). Evaporation of this extract left a crystalline residue (5.08 g), which was dissolved in 50 mL of absolute ethanol and treated with 3.31 mL of 7.5 M ethanolic hydrogen chloride. The resulting crystals were collected and washed first with a minimum of cold, absolute ethanol and then with acetone. This material crystallized slowly from 1-propanol to give 1.46 g of tiny, gold-colored crystals, mp 225–227 °C dec.

3,6-Bis[2-(dimethylamino)-2-methylpropoxy]acridine Trihydrochloride (2c). With 8.45 g (0.04 mol) of 3,6-acridinediol (**3a**), 0.16 mol of sodium hydride, and 13.77 g (0.08 mol) of 2-chloro-1,1,*N*,*N*-tetramethylethylamine hydrochloride, the above procedure gave an evaporated reaction residue, which was extracted with petroleum ether (bp 30–60 °C). This extract was chromatographed on alumina and eluted with CH_2Cl_2. Evaporation of the eluates left 1.11 g of a yellow syrup, which was dissolved in 15 mL of methanol and treated with 105% of the theoretical amount of 10 N methanolic HCl. Crystallization was completed after adding 30 mL of acetone. The product was collected, washed with acetone, and recrystallized from methanol/acetone: yield 1.38 g; tiny, yellow-tan needles; mp 202–204

(5) F. Mientzsch, German Patent, D.R.P. 490418 (1927); *Friedländer Fortschr. Tierfarben Fabrikation*, **16**, 2704 (1929). *Chem. Zentrabl., Sect. 2*, 2797 (1929).
(6) F. Mientzsch, U.S. Patent 1727480 (1929).

°C dec. An NMR spectrum of the product in deuterated dimethyl sulfoxide and D_2O was consistent with the assigned structure rather than the product of an ethylenimmonium rearrangement. In thin-layer chromatography on alumina using $CHCl_3$–acetone, 9:1, the product had R_f 0.53 with a bright blue fluorescence with UV light at 370 nm. A similarly fluorescent material with R_f 0.020 was eluted from the above column with CH_2Cl_2–acetone, 19:1. This fraction also contained the product of R_f 0.63 and was not investigated further.

3,6-Bis(2-chloroethoxy)acridine Hydrochloride (6a). To a mixture of NaH (11.8 g, 61.14% oil dispersion) and 3,6-acridinediol hemisulfate (**3a**, 26.0 g) was added 250 mL of dry DMF, and the mixture was added 47.0 g of 2-chloroethyl *p*-toluenesulfonate with stirring for 3.5 h and at 50 °C for 2 h. The volatile materials were removed under reduced pressure at 35–40 °C. The residue was quenched with 1200 mL of ice-cold saturated $NaHCO_3$ solution. Tan crystals thus obtained were collected and dissolved in 500 mL of $CHCl_3$:5.5 g (81%) of the brown residue was obtained. To the chloroform solution was added 30 mL of 6 N HCl solution in *i*-PrOH. Dilution of the chloroform solution with ether gave 24.0 g (65%) of yellow crystals mp 225–228 °C. Recrystallization from ethanol gave an analytical sample mp 231–233 °C dec.

3,6-Bis(3-chloropropoxy)acridine Hydrochloride (6b). To a mixture of NaH (11.8 g, 61.14% oil dispersion) and acridinediol hemisulfate (**3a**, 26.0 g) was added 250 mL of dry DMF with stirring at room temperature for 1.5 h. After the addition of 49.8 g of 3-chloropropyl *p*-toluenesulfonate, the mixture was stirred for 3 h and 20 min and at 50 °C for 2 h. Volatile materials were removed under reduced pressure at 35–40 °C. The residue was quenched with 1200 mL of ice-cold saturated $NaHCO_3$ solution. The tan crystals thus obtained were collected and dissolved in 500 mL of chloroform. To the chloroform solution was added 30 mL of 6 N HCl solution in *i*-PrOH. Dilution of the chloroform solution with ether gave 34.9 g of tan crystals, mp 206–208 °C. Recrystallization (2.0 g) from ethanol gave an analytical sample, mp 211–213 °C.

General Preparation of 3,6-Bis(aminoalkoxy)acridines. Method B. A mixture of 3,6-bis(2-chloroethoxy)acridine (**6**) and excess amine (100 mL) was heated in a bomb at 80 °C for 24 h. The excess amine was removed under reduced pressure. The orange residue was dissolved in 150 mL of $CHCl_3$ washed with 2 × 30 mL of saturated $NaHCO_3$ solution, dried over Na_2SO_4, and filtered. The $CHCl_3$ was removed under reduced pressure, and the residue was dissolved in 50 mL of absolute EtOH. HCl (10 mL, 6 N) in PrOH was added, and the mixture brought to boiling. Water was added until the crystals dissolved. On cooling, the solution deposited the product.

3,6-Bis[2-(methylamino)ethoxy]acridine Trihydrochloride (2d). A mixture of 3,6-bis(2-chloroethoxy)acridine (**6**) hydrochloride (1.5 g) and 100 mL of methylamine was heated in a bomb at 80 °C for 24 h. The excess methylamine was removed under reduced pressure. The orange residue was dissolved in 150 mL of $CHCl_3$, washed with 2 × 30 mL of saturated $NaHCO_3$ solution, dried over Na_2SO_4, and filtered. The $CHCl_3$ was removed under reduced pressure, and the residue was dissolved in 50 mL of absolute EtOH. HCl (10 mL, 6 N) in *i*-PrOH was added, and the mixture was brought to boiling. Water was added until the crystals dissolved. The solution deposited 1.25 g of orange crystals, mp 267–269 °C.

3,6-Bis[3-(diethylamino)propoxy]acridine Trihydrochloride (2o). The reaction mixture [2 g of 3,6-bis(3-chloropropoxy)acridine hydrochloride (**6**) and 150 mL of diethylamine] was heated in a steel bomb at 100 °C for 24 h and worked up as described in method B. Recrystallization was done in absolute ethanol: yield 1.5 g (53%) of yellow crystals, mp 235–236 °C.

4,5-Dichloro-3,6-bis[2-(diethylamino)ethoxy]acridine (9a). A solution of 3,6-bis[2-(diethylamino)ethoxy]acridine trihydrochloride monohydrate (8.0 g, 0.016 mol) in 32 mL of concentrated sulfuric acid was cooled in an ice bath and *N*-chlorosuccimide (4.7 g, 0.035 mol) was added. The mixture was stirred in an ice bath for 1 h and at room temperature overnight. The mixture was poured into 400 mL of ice and water, and the pH was adjusted to 12.5 with 10 N NaOH. The mixture was extracted thrice with methylene chloride, dried, and concentrated in vacuo to an orange solid. Recrystallization from 100 mL of hexane gave yellow crystals: mp 81–83 °C; yield 6.55 g (85%).

4,5-Dibromo-3,6-bis[2-(diethylamino)ethoxy]acridine (**9b**) and 3,6-bis[2-(diethylamino)ethoxy]-4,5-dinitroacridine (**9c**) were prepared in a similar manner using NBS and sodium nitrate, respectively.

4,5-Dichloro-3,6-bis[2-(diethylamino)ethoxy]-2-nitroacridine (9d). A solution of 4,5-dichloro-3,6-bis[2-(diethylamino)ethoxy]acridine (**9a**; 3.6 g, 0.0075 mol) in 15 mL of concentrated H_2SO_4 was cooled in an ice bath, and potassium nitrate (1.8 g, 0.018 mol) was added. The reaction mixture was stirred in an ice bath for 1 h and at room temperature overnight, and then poured into 200 mL of ice and water. The mixture was adjusted to pH 12.5 with 10 NaOH and extracted with methylene chloride. Removal of the solvent in vacuo gave a dark gummy solid. This material was extracted with 250 mL of boiling hexane. The solution was reduced to 100 mL and stirred at 5 °C overnight. The orange crystals that precipitated were collected by filtration and dried in vacuo: yield 2.07 g (53%); mp 95–97 °C.

4,5-Dibromo-3,6-bis[2-(diethylamino)ethoxy]-2-nitroacridine (**9g**) was prepared in a similar manner.

4,5-Dichloro-3,6-bis[2-(diethylamino)ethoxy]-1,7-dinitroacridine (9e). Potassium nitrate (4.8 g, 0.048 mol) was added to a solution of 4,5-dichloro-3,6-bis[2-(diethylamino)ethoxy]-acridine (**9a**; 2.39 g, 0.005 mol) in 15 mL of concentrated sulfuric acid. The reaction mixture was heated in an oil bath at 85 °C for 16 h and then poured into 200 mL of ice and water. The pH was adjusted to 12.5 with 10 N NaOH, and the solution was extracted thrice with methylene chloride. The combined extracts were dried and concentrated in vacuo to give 650 mg of a brown solid. This material was heated in 500 mL of refluxing hexane and filtered, and the filtrate was concentrated. Upon cooling the solution in an ice bath, yellow crystals precipitated, which were collected by filtration and dried in vacuo: yield 337 mg (11%); the material gradually decomposes above 110 °C.

Pharmacology. Rauscher Leukemia Virus. The Rauscher leukemia virus was inoculated intraperitoneally into BALB/C mice. The virus inoculum was a 20% (w/v) spleen extract made from 21-day infected spleens of BALB/C mice. All mice were within a 3-g weight range, with a minimum weight of 18 g, and all mice were of the same sex, usually male. Sheep red blood cells were injected intraperitoneally on the 7th day. There were five mice per test group. The test compound was administered orally on the 6th day as 0.5 mL (in 0.2% Noble agar in saline) at a dose of 25 to 200 mg/kg of body weight and again on the 7th and 8th day in the same manner. On the 14th day, the mice were weighed and bled from the retro-orbital sinus. The blood was pooled, and the harvested serum was stored at 4 °C for 24 h. Hemagglutinin tests were performed by standard procedures by the microtiter plate technique. Acceptable hemagglutinin titer for leukemic (immunosuppressed) mice is ≤1:128. A positive control compound is poly IC (polyinosinic acid–polycytidylic acid), administered intraperitoneally on days +6, +7, and +8. Acceptable positive control hemagglutinin titers were 4-fold higher than the titers obtained in the leukemic control mice, and the results of this test appear in Tables I and II.

Mice infected with Rauscher virus rapidly developed an erythroleukemia, produced high levels of infectious virus, and developed splenomegaly, the reduction of which when compared to controls is indicative of potential antitumor activity. The results are shown in Table I, along with a positive control. Active compounds are required to produce a >50% reduction in spleen weight when compared to spleen weights of infected placebo-treated controls. The results are shown in Tables I and II.

Antiviral Activity against an Interferon-Sensitive Virus (Columbia SK). Swiss white mice were treated orally or intraperitoneally 18 h prior to subcutaneous challenge with an LD_{95} dose of an interferon-sensitive virus, Columbia SK. Survival ratios were determined 14 days postinfection.

Oral administration of the acridines protected mice from lethal infection with Columbia SK virus (Tables I and II). A protective effect produced by the administration of an agent prior (i.e., 12–24 h) to virus challenge is usually associated with the induction of interferon. An indirect measure of interferon production is reflected in the survivial of the mice. In this test where all of the infected control mice die (0/15, survivors/total), a minimum of 5 out of 15 of the drug-treated mice must survive for the drug

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

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

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.

(7) W. E. Stewart, "The Interferon System", Springer-Verlag, Wein, New York, 1979.

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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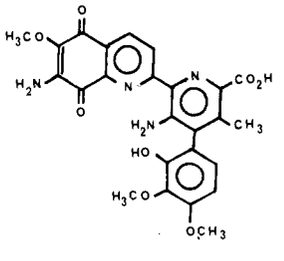
With the aim of obtaining new antitumoral agents, a series of 5,8-quinazolinediones was prepared. 5-Amino-6-methoxyquinazoline was oxidized by Fremy's salt to give 6-methoxy-5,8-quinazolinedione. Nucleophilic substitution reaction at C_6, electrophilic substitution at C_7, and synthesis of 7-amino-6-methoxy-5,8-quinazolinedione, 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.



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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