TABLE 5. 2-Aminomethyl Derivatives of Benzofuran (XI)-(XIX)

Compound	Yield, %	Mp, °C	Found, %				Formula	Calculated, %			
			C	H	Hal	N		C	H	Hal	N
XI	76	119—20	81,21	6,51	—	7,79	$C_{25}H_{24}N_2O$	81,49	6,56	—	7,60
	82,0	177—80	67,66	6,11	—	—	$C_{25}H_{24}N_2O \cdot 2HCl$	68,02	5,94	—	—
XII	72	93—4	77,57	6,58	—	—	$C_{19}H_{19}NO_2$	77,79	6,53	—	—
	100	212—4	69,80	6,20	10,50	—	$C_{19}H_{19}NO_2 \cdot HCl$	69,19	6,11	10,75	—
XIII	74	233—5	69,05	5,11	28,36	—	$C_{20}H_{21}BrNO \cdot HCl$	69,05	5,20	28,36	—
XIV	76	176—8	57,35	5,30	8,76	—	$C_{17}H_{18}BrNO \cdot HCl$	57,81	5,36	8,98	—
XV	93	132—4	74,64	5,72	8,91	6,97	$C_{25}H_{23}ClN_2O$	74,53	5,75	8,80	6,95
	74	224—6	63,48	5,32	27,20	—	$C_{25}H_{23}ClN_2O \cdot 2HCl$	63,10	5,30	22,35	5,89
XVI	86	193—4	65,07	6,12	20,89	—	$C_{19}H_{20}ClNO \cdot HCl$	65,14	6,04	20,24	—
XVII	98	113—5	78,00	6,64	—	7,08	$C_{26}H_{24}N_2O_2$	78,46	6,58	—	7,03
	59	158	64,17	6,10	14,66	—	$C_{26}H_{24}N_2O_2 \cdot 2HCl$	63,80	6,18	—	14,50
XVIII	47,2	105—7	81,51	6,95	—	7,37	$C_{26}H_{24}N_2O$	81,64	6,85	—	7,32
XIX	44,3	116—8	79,55	6,95	—	10,21	$C_{36}H_{38}N_4O$	79,67	7,06	—	10,32

2,5-Bis(N-phenylpiperazinomethyl)-3-phenylbenzofuran (XIX) was prepared in the same way but by the reaction of 2,5-bis(bromomethyl)-3-phenylbenzofuran (0.01 mole) with the secondary amine (0.04 mole).

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DERIVATIVES OF ACRIDINE ORANGE AND THEIR BIOLOGICAL ACTIVITY

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Among acridine derivatives 3,6-diaminoacridines (acridine orange, AO; proflavin; etc.) occupy a special place by virtue of their remarkable biological activity (antimitotic, mutagenic, the ability to inhibit enzymes), which stems from their high affinity for nucleic acids [1, 2].

By penetrating into the living cell, many acridine derivatives, notably 3,6-bis(dimethylamino)acridine (AD), cause the cell structures to become luminscent [3]. Moreover AO suppresses the formation of Yoshida ascites tumor [4]. These facts have motivated the synthesis and study of derivatives of AO.

We have previously examined the nitration of, reduction of the nitro group in, and attachment of an aldehyde group to AO [5].

Our intention in the work reported here was to synthesize derivatives of AO 4,5-diamine and 4,5-dicarbaldehyde and examine their biological activity.

We used the reaction of 3,6-bis(dimethylamino)-4,5-diaminoacridine with the azlactone of N-acetylsarcolysine, prepared from N-acetylsarcolysine and dicyclohexylcarbodiimide (DCC) in DMF following Siemion [6], to

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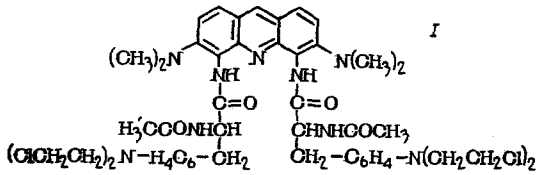
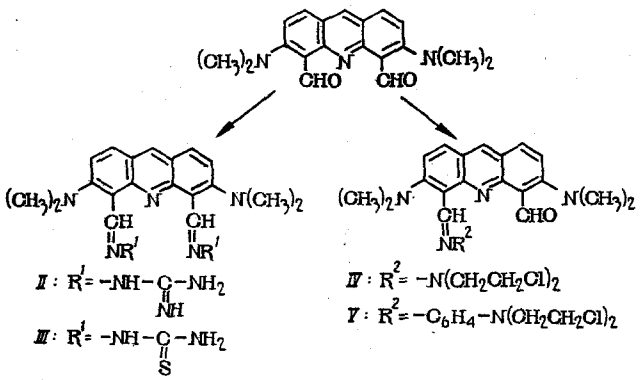
synthesize diamide (I). Our intention here was to evaluate its antitumor action, since sarcolysine amides are known to have a specific antitumor action and to be less toxic than sarcolysine [7, 8].



I

We prepared the hydrazones (II), (III), and (IV) by heating 3,6-bis(dimethylamino)acridine-4,5-dicarbaldehyde with aminoguanidine, thiosemicarbazide, and N,N-bis(2-chloroethyl)hydrazine. Reaction with N,N-bis(2-chloroethyl)-p-phenylenediamine gave azomethine (V).



Elemental analyses indicated that the reaction of AO 4,5-dicarbaldehyde with N,N-bis(2-chloroethyl)hydrazine and N,N-bis(2-chloroethyl)-p-phenylenediamine involves only one of the aldehyde groups, whereas both react with thiosemicarbazide and aminoguanidine.

We verified the structures of the synthetic compounds by examining the IR spectra of AO, the 4,5-dicarbaldehyde, and compounds (II)-(V), together with those of some model compounds.

The IR spectrum of AO 4,5-dicarbaldehyde contains an intense absorption band at 1665 cm^{-1}, which is absent from the spectrum of AO. We assign it as aldehyde–carbonyl stretching. Though aromatic aldehyde carbonyl is known to absorb in the 1710-1695 cm^{-1} region, its absorption is strongly substituent-dependent [9]. The absorption in the model compounds appears at 1685 cm^{-1} in 2-dimethylamino-5-methylbenzaldehyde [10] (1704 cm^{-1} in benzaldehyde) and at 1690 cm^{-1} in 4-acridinecarbaldehyde, i.e., the dimethylamino group has a greater effect on the position of the aldehyde–carbonyl band.

Obviously the position of the carbonyl band in AO 4,5-dicarbaldehyde is also due more to the effect of the dimethylamino group.

The carbonyl band persists in the spectra of compounds (IV) and (V), implying the formation of the monohydrazone (IV) and the monoazomethine (V). The carbonyl absorption appears at 1740 cm^{-1} in monoazomethine (V) and at 1661 cm^{-1} in the carbonyl compound (IV).

The aldehyde carbonyl band is absent from the IR spectra of compounds (II) and (III), thus supporting the assignment of their structures. Moreover the high-frequency region contains three strongly broadened bands at 3440-3360, 3250-3200, and 3185-3040 cm^{-1}, due to the NH group in compounds of the thiosemicarbazone type [11]. The IR spectrum of compound (III) also contains an absorption band at 1540 cm^{-1}, which can be assigned to a thione group attached to amino [12].

We examined the ability of compounds (II), (IV), and (V) to inhibit nucleic acid synthesis in tumor cell cultures by the method described earlier [13]. Our results are summarized in Table 1.

We also evaluated the antitumor activity of compounds (IV) and (V) in mice by intraperitoneal administration. The results also appear in Table 1. The test compounds displayed moderate in vivo activity and high in vitro activity.

We also examined the antiviral activity of bis(aminoguanylhydrazone) (II), by assaying its virucidal action against influenza A2 Hong Kong/68 virus in chick embryos and white mice. The preparation was relatively inactive.

TABLE 1. Inhibition of *in vitro* Nucleic Acid Synthesis and *in vivo* Antitumor Activity of the Synthetic Compounds

Compound	LD_{50}, μg/g	ED_{50}, μg/ml		Therapeutic dose (μg/kg) and mode of administration	Tumor	Inhibition of growth of tumor, %
		NK-LI	sarcoma 37			
II		50	50			
IV	60	1—10	0,1—0,001	10×5/24	AC-755	44
					S-37	5
					Lewis lung carcinoma	20
V	150	1—10	10—20	15×5/24	AC-755	51
					S-37	45
					Lewis lung carcinoma	44

Note. Here ED_{50} is the concentration that causes 50% inhibition of nucleic acid synthesis relative to the control.

Bis(thiosemicarbazone) (III) had antituberculosis activity in 1:64,000 dilution in the absence of blood serum.

EXPERIMENTAL PHARMACOLOGICAL PART

Antitumor activity was assayed in $C_{57}Bl/6$ hybrid mice and non-inbred mice against the transplantable tumors adenocarcinoma 755 (AC-755), sarcoma 37 (S-37), and Lewis lung carcinoma. The preparations were first administered 48 h after transplantation, and then a total of five times at 24 h intervals (intraperitoneally). Animals were killed seven days after the last administration. The tumors were weighed and the percentage inhibition of the growth of the tumor was evaluated from the equation

$$T\% = [(P_C - P_0)/P_C] \cdot 100\%,$$

where P_C and P_0 denote the average weight of the tumors in the control and the experimental group respectively.

EXPERIMENTAL CHEMICAL PART

The IR spectra were recorded on an IR-10 spectrophotometer in CCl_4 or perfluorocarbons.

3,6-Bis(dimethylamino)-4,5-bis(N-acetylsarcolysylamido)acridine (I). A solution of N-acetylsarcolysine (0.7 g, 0.002 mole) [14] and DCC (0.41 g, 0.002 mole) in dry DMF was left at room temperature for 5 h. The precipitated dicyclohexylurea was filtered off and a solution of AO 4,5-diamine (0.3 g, 0.001 mole) in the same solvent was added to the filtrate. The mixture was left at room temperature for 24 h. Half the solvent was stripped under vacuum without heating and the newly precipitated dicyclohexylurea was filtered off. The filtrate was poured into water. The precipitate was filtered off, dried, and washed with petroleum ether and then with ether to give (I) (0.4 g, 53%), mp 103-106°C. Found, %: C 59.06; H 6.06; N 13.74; Cl 14.84. $C_{47}H_{57}N_9O_4Cl_4$. Calculated, %: C 59.2; H 6.03; N 13.25; Cl 14.9.

3,6-Bis(dimethylamino)acridine-4,5-dicarbaldehyde-bis(aminoguanylhydrazone) (II). To a solution of aminoguanidine hydrochloride (0.22 g, 0.002 mole) in dry ethanol (minimum amount) was added sodium metal (0.046 g, 0.002 mole). The precipitated sodium chloride was filtered off and a solution of 3,6-bis(dimethylamino) acridine-4,5-dicarbaldehyde (0.32 g, 0.001 mole) in ethanol (50 ml) was added to the filtrate. After 1 h reflux, the red crystalline precipitate was filtered off to give (II) (0.2 g, 46%), mp 280°C. Found, %: C 58.28; H 6.05; N 35.35; $C_{21}H_{27}N_{11}$. Calculated, %: C 58.2; H 6.29; N 35.51.

3,6-Bis(dimethylamino)acridine-4,5-dicarbaldehyde-bis(thiosemicarbazone) (III). To a solution of 3,6-bis-(dimethylamino)acridine-4,5-dicarbaldehyde (0.32 g, 0.001 mole) in dry ethanol (80 ml) acidified with acetic acid (2 drops) was added a solution of thiosemicarbazide (0.27 g, 0.002 mole) in water (1 ml). After 2 h reflux, the precipitate was filtered off and washed with ethanol and hot water to give reddish orange crystals (0.21 g, 54%), mp 210°C. Found, %: C 53.99; H 5.26; N 26.38; S 13.4; $C_{21}H_{25}N_9S_2$. Calculated, %: C 53.9; H 5.4; N 27.0; S 13.7.

3,6-Bis(dimethylamino)acridine-4,5-dicarbaldehyde-N,N-bis(2-chloroethyl)hydrazone (IV). To a stirred suspension of 3,6-bis(dimethylamino)acridine-4,5-dicarbaldehyde (0.96 g, 0.003 mole) in dry methanol (50 ml) heated to 50°C was added portionwise N,N-bis(2-chloroethyl)hydrazine hydrochloride (0.77 g, 0.004 mole) [15]. After 30 min reflux, methanol was stripped under vacuum. The oily precipitate was treated with triethylamine (TEA) (2 ml) and left for 1.5 h, whereupon the excess TEA and its hydrochloride were rinsed off with water.

The red precipitate was crystallized from ethanol to give (IV) (0.8 g, 54%), mp 142°C. Found, %: C 59.80; H 5.95; N 15.55; Cl 15.25; $C_{23}H_{27}N_5OCl_2$. Calculated, %: C 60.00; H 5.87; N 15.21; Cl 15.40.

Monoazomethine of 3,6-Bis(dimethylamino)acridine-4,5-dicarbaldehyde and N,N-Bis(2-chloroethyl)-p-phenylenediamine (V). To a suspension of N,N-bis(2-chloroethyl)-p-phenylenediamine hydrochloride (1.08 g, 0.004 mole) [16] in dry benzene (70 ml) was added TEA (0.56 ml, 0.004 mole). The mixture was shaken for several minutes and TEA hydrochloride was filtered off. A suspension of 3,6-bis(dimethylamino)acridine-4,5-dicarbaldehyde (0.62 g, 0.002 mole) in dry benzene (30 ml) containing acetic acid (2-3 drops) was added to the filtrate. After 1 h reflux, benzene was stripped. The residual oil was triturated with small portions of 25% ammonia solution and then dried and washed with ether to give (V) (0.8 g, 66.5%), mp 103°C. Found, %: C 63.13; H 6.14; N 12.81; Cl 12.82; $C_{29}H_{31}N_5OCl_2 \cdot H_2O$. Calculated, %: C 62.8; H 5.97; N 12.65; Cl 12.84.

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