4-AMINO-2-STYRYLQUINAZOLINES, A NEW CLASS OF ANTIPROTOZOAL DRUGS

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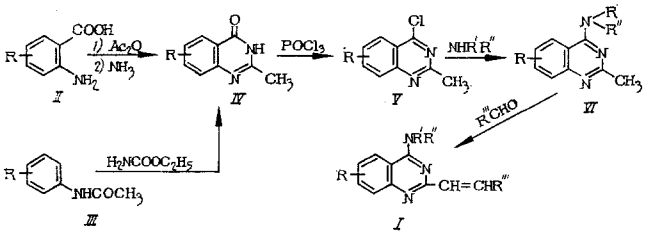
Earlier research by Soviet scientists [11, 14, 18] showed that 4-amino-2-styrylquinolines display considerable and varied biological activity.

The novel Soviet drugs trichomonacid [2-(p-nitrostyryl)-4-(δ-diethylamino-α-methylbutylamino)-6-methoxyquinoline triphosphate] [12, 18] and aminoquinol (2-(o-chlorostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinoline triphosphate [11, 18], which have found application for the treatment of trichomonad infections and lambliasis, are produced by the pharmaceutical chemical industry in the USSR [13].

Pursuing this novel direction of research, some ten years ago we developed general methods for the synthesis of previously unknown aza-analogs of styrylquinolines, namely 4-amino-2-styrylquinazolines (I) [15, 16]. These methods were based on the reaction of 2-methyl-4-chloroquinazolines (V), obtained from the anthranilic acids (II) or acetanilides (III) via the 2-methyl-4-quinazolones (IV) with amines, followed by condensation of the resulting 4-amino-2-methylquinazolines (VI) with aromatic or heterocyclic aldehydes.



The use of accessible starting materials, relatively mild reaction conditions, and the usually satisfactory yields of final products enabled a large number of 4-amino-2-styrylquinazolines to be synthesized [1-7], the biological examination of which revealed high bacteriostatic [1, 6, 8, 17], antitubercular [1, 6, 8, 17], antiviral [3, 6], antiprotozoal [1, 5, 7], and antiinflammatory activity [2, 4, 7, 17].

Of greatest practical interest was the high antiprotozoal activity of these compounds, and we therefore undertook a more systematic examination of their activity against a number of pathogenic protozoa, using experimental models of dermal leishmaniasis (*Leishmania major*),* trypanosomiasis (*Trypanosoma equiperdum*), lambliosis (*Lamblia muris*), and malaria (*Plasmodium berghei*) in mongrel white mice, and *in vitro* against *Trichomonas vaginalis* and *Entamoeba histolytica*.

The compounds tested were novel 4-aminostyrylquinazolines synthesized as described above (I-1, I-7, I-9, I-10, I-20, I-26, Table 1), the preparation of which is described here, and other compounds (I) obtained by methods which we have described previously.

Several of the compounds were obtained by modifying the substituents in available 4-amino-2-styrylquinazolines.

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The principal chemical characteristics of the compounds synthesized and examined in this study are given in Table 1. According to their PMR spectra (obtained on an INM-4H-100 (100 MHz) instrument in various solvents [CD_3OD, CD_3COCD_3, $(CD_3)_2SO$, internal standard TMS]), all the compounds (I) have the trans-configuration of the substituent at the double bond in the styryl moiety of the molecule (J = 16 Hz for the relevant protons).

The results of studies of various types of antiprotozoal activity of the test compounds are shown in Tables 2-4.

In studies of antileishmaniasis activity, the test compounds, as shown in Table 2, were of relatively low toxicity, the maximum tolerated dose by the oral route in animals being 100-500 mg/kg.

Compounds I-22, I-19, I-3, I-14, and I-25 showed high chemotherapeutic activity in experimental dermal leishmaniasis in white mice, the disease process being suppressed to the extent of 57-70%. During treatment, a reduction in the infective process was noted, which was limited by infiltrate. In the control group of animals, widespread leishmaniomas were present which showed no tendency to heal. Parasitological examination of the exudate from the ulcers showed a reduction in the number, or a total absence of amstygotes in the treated mice, the causative agents in the ulcer exudate from the control animals always being present.

High chemotherapeutic activity was only apparent in the compounds at the maximum tolerated doses. Treatment of the animals with these drugs in lower doses resulted in a decrease in chemotherapeutic activity.

It will be seen from Table 3 that none of the test compounds gave a significant antitrypanosome effect either in therapeutic or prophylactic doses. The mean lifespan of the treated mice did not differ from that in the control groups. The duration of life was determined by the type of current infection; in mice the infection produced by this causative agent is of the acute type, resulting in the deaths of the animals, in which large numbers of microorganisms were present in the blood.

It will also be seen from Table 3 that compounds I-9, I-3, I-2, I-14, and I-11 have high, statistically significant antilambliasis activity. In parasitological examinations of the contents of three sections of the small intestime in treated animals, there was a marked reduction in the numbers of *L. muris* organisms, showing that these compounds have a specific effect against these organisms.

Compounds I-4, I-13, I-19, I-20, I-22, and I-30 had no effect on the viability of *L. muris*, and no reduction in the numbers of this organism were found in parasitological studies of the small intestine in treated mice, no differences from the control group being present.

We have previously shown [1] that in model experimental lambliosis in white mice compound I-2 in a dose of 500 mg/kg internally for 5 days provides a complete cure of the animals, and in a dose of 200 mg/kg, partial cure.

As will be seen from Table 4, compound I-19 displays high antitrichomoniasis activity, suppressing the growth of this species of protozoan in a concentration of 2 μg/ml, compounds I-7 and I-3 giving the same effect in concentrations of 31 and 62 μg/ml, respectively.

The highest activity against *Entamoeba histolytica*, as noted previously [1], is shown by I-2 (1 μg/ml), compounds I-3 and I-44 being active in concentrations of 15.6 μg/ml, and I-7, I-19, I-20, I-21, I-22, and I-30 in concentrations of 31.2 μg/ml.

Table 4 also shows that antimalarial activity has been found for the first time in 4-amino-2-styrylquinazolines. It is clear from the results reported here that the greatest antimalarial activity is shown by I-29, compounds I-2, I-3, I-15, and I-21 being less active, and I-4 devoid of antimalarial activity. Nevertheless, these results enable 4-amino-2-styrylquinazolines to be regarded as a new class of drugs with potential antimalarial properties.

Bearing in mind that the present report completes a series of studies seeking styrylquinazoline compounds with antiprotozoic activity, we deemed it desirable, in addition to reviewing the results, also to formulate some relationships between chemical structure and antiprotozoal activity in these compounds.

We have shown previously [6] that the highest antiviral activity against the influenza virus is shown by 4-(δ-diethylamino-α-methylbutylamino)-2-styrylquinazolines containing some bromine (or chlorine) atoms in the styryl group. When the chlorine or bromine atoms are re-

TABLE 1. 4-Amino-2-styrylquinazolines

Compound	R	NR′R″	R‴	Yield, % (on VI)	mp, °C	Synthesis described in
I-1·3HCl·H_2O	H	—$NHCH(CH_3)(CH_2)_3N(C_2H_5)_2$	4-Aminophenyl	41	243—245	
I-2·2HCl	H	"	4-Nitrophenyl	53	277—278	[2]
I-3·2HCl	6-CH_3O	»	Same	34	269—270	[1]
I-4·2HCl	6-NO_2	»	»	58	264—266	[8]
I-5·2HCl	7-Cl	»	»	53	277—278	[2]
I-6·2HCl·H_2O	6-CH_3O	»	2-Nitrophenyl	34	269—270	[1]
I-7·2HCl	7-Cl	»	2-(2′-Nitrofuryl)	41	259—260	
I-8	H	»	"	27	116—118	[17]
I-9·2HCl	7-Cl	»	2-Anisyl	57	239—240	
I-10·2HCl	7-Cl	»	2-Chloro-4,5-dimethoxyphenyl	56	238—239	
I-11·2HCl	H	»	"	41	255—256	[7]
I-12·2H_3PO_4·3H_2O	7-Cl	»	3-Methoxy-4-hydroxyphenyl	28	238—240	[17]
I-13·2HCl	H	—$NHCH(CH_3)(CH_2)_3N(C_2H_5)_2$	3,4-Dimethoxyphenyl	53	251—252	[7]
I-14·2HCl	7-Cl	"	"	57	171—172	[7]
I-15·2HCl	H	»	4-Chlorophenyl	48	249—250	[17]
I-16·2HCl	6-CH_3O	»	"	30	278—279	[1]
I-17·2HCl	H	»	2-Chlorophenyl	52	237—238	[17]
I-18·2HCl·H_2O	6-CH_3O	»	"	18	266—267	[1]
I-19·2HCl	6-NO_2	»	»	61	263—264	[5]
I-20·2HCl	7-Cl	»	2,4-Dichlorophenyl	55	274—275	
I-21·2HCl	6-CH_3O	»	"	47	267—268	[3]
I-22·2HCl	6-NO_2	»	»	65	267—268	[5]
I-23·2HCl	H	»	2,4,6-Trichlorophenyl	56	176—177	[6]
I-24·2HCl	6-CH_3O	»	"	44	238—239	[3]
I-25·2HCl	7-Cl	»	»	56	212—213	[6]
I-26·2HCl·2H_2O	7-Cl	»	4-Bromophenyl	47	277—278	
I-27·2HCl·H_2O	6-CH_3O	»	"	45	277—278	[1]
I-28·2HCl	H	—$NHCH(CH)_3(CH_2)_3N(C_2H_5)_2$	2,4-Dibromophenyl	56	242—243	[3]
I-29·2HCl	6-CH_3O	"	"	55	256—257	[6]
I-30·2HCl	7-Cl	»	»	56	267—268	[3]
I-31·2HCl	6-NO_2	»	»	53	260—261	[6]
I-32·2HCl	H	»	2,4,6-Tribromophenyl	26	184—185	[6]
I-33·2HCl	7-Cl	»	"	40	202—204	[6]
I-34·2HCl·H_2O	7-Cl	»	Perfluorophenyl	48	248—249	[6]
I-35·HCl	7-Cl	N-Diethylamino	2-Chlorophenyl	66	245—246	[2]
I-36·HCl·H_2O	7-Cl	"	4-Chlorophenyl	66	246—247	[17]
I-37·HCl	6-CH_3O	»	2-Chlorophenyl	59	263—264	[1]
I-38·HCl·H_2O	6-CH_3O	»	4-Chlorophenyl	61	242—243	[1]
I-39·HCl	6-CH_3O	»	4-Bromophenyl	60	260—261	[1]
I-40·HCl	7-Cl	N-Piperidino	4-Chlorophenyl	52	271—272	[17]
I-41·HCl	7-Cl	"	4-Nitrophenyl	59	274—275	[17]
I-42·HCl	7-Cl	N-Phenylamino	4-Nitrophenyl	42	242—243	[17]
I-43·HCl	7-Cl	N-Diethylamino	»	55	267—268	[17]
I-44·HCl	6-CH_3O	"	»	57	244—245	[1]

TABLE 2. Antileishmaniasis Activity of 4-Amino-2-styrylquinazolines

Compound	Maximum tolerated dose, mg/kg	Therapeutic dose, mg/kg	Mean extent of leishmaniasis infection (M ± m)	P	Index of effectiveness, %
I-2	250×10	250×10	0,8±0,2	<0,001	50
		200×10	0,8±0,2	<0,001	50
		100×10	0,9±0,2	<0,05	35
		75×10	0,6±0,1	<0,01	25
I-3	250×10	250×10	0,9±0,2	<0,01	44
		200×10	0,3±0,1	<0,001	62
I-4	100×10	100×10	1,2±0,3	<0,02	29
I-5	200×10	200×10	1,0±0,2	<0,001	52
		150×10	1,1±0,4	<0,05	31
I-9	300×10	300×10	1,2±0,2	>0,3	—
I-11	400×10	400×10	1,2±0,2	<0,01	40
I-13	500×10	500×10	1,1±0,2	<0,001	45
		400×10	1,7±0,2	>0,2	—
I-14	400×10	400×10	0,8±0,1	<0,001	60
		300×10	0,9±0,1	<0,001	55
I-19	500×10	500×10	0,6±0,2	<0,001	65
		400×10	1,6±0,3	>0,2	—
I-20	250×10	250×10	0,8±0,3	<0,01	50
I-22	250×10	250×10	0,4±0,1	<0,001	70
		200×10	0,6±0,1	>0,3	—
I-24	125×10	125×10	1,0±0,2	<0,01	38
		100×10	1,9±0,3	>0,2	—
I-25	100×10	100×10	0,9±0,2	<0,001	57
		75×10	1,0±0,2	<0,01	38
I-30	200×10	200×10	0,8±0,1	<0,001	50
		150×10	0,5±0,1	<0,001	38
		100×10	1,0±0,2	<0,2	—
I-33	100×10	100×10	0,9±0,1	<0,001	44
		75×10	0,8±0,2	<0,001	50

placed by fluorine, the virucidal activity of the compounds is considerably reduced, or is totally lost. Antiinflammatory activity in this series is highest in compounds with one, two, or three chlorine atoms in the styryl moiety [2, 4, 7]. Introduction of other halogens (bromine or fluorine) instead of chlorine results in the loss of antiviral activity [4]. The highest antibacterial activity is found in 4-amino-2-styrylquinalzolines containing a δ-diethylamino-α-methylbutylamino-chain in the 4-position, together with p-nitro and o-nitro or p-chloro-substituents in the styryl moiety [17]. Higher vinylogs of these compounds, viz., 2-(δ-phenylbutadienyl)quinazolines have a definitely higher chemotherapeutic effect [8].

The results reported here and in earlier communications [5, 7] lead to the conclusion that for high or moderate antileishmaniasis activity, the presence in the 2-styrylquinazoline molecule of a δ-diethylamino-α-methylbutylamino-group in the 4-position is required, together with 4-nitro-, 2-chloro-, 2,4-dichloro-(or dibromo), 2,4,6-trichloro-(or tribromo-), or 3,4-dimethoxy substituents in the styryl moiety of the molecule. Preferred substituents in the benzene ring of the styryl group are 6-nitro-, 6-methoxy-, or 7-chloro-.

Antilambliosis activity is highest in 4-(δ-diethylamino-α-methylbutylamino)-2-styrylquinazolines with 2-nitro- and 2-methoxy or 3,4-dimethoxy groups in the styryl moiety of the molecule. 7-Chloro- or 6-methoxy-substituents are preferred in the benzene ring of the quinazoline moiety.

In trichomonad infections, the highest activity is displayed by 4-(δ-diethylamino-α-methylbutylamine)-2-styrylquinazolines with a p-nitro group in the styryl moiety of the molecule, or with a nitrofuryl residue instead of nitrophenyl. Introduction of halogen atoms in place of the nitro-groups reduces antitrichomonad activity, and the presence of other amine residues (diethylamine, piperidine, or aniline) in the 4-position in place of the diethylaminoisopentyl chain leads to total loss of this type of anitprotozoal activity.

Antiamoeba activity in this series is highest in the 4-nitrostyryl and 2,4-dihalostyryl compounds. As in the other cases, the compounds preferably contain the 4-(δ-diethylamino-α-methylbutylamino) groupings. However, high chemotherapeutic activity in antiamoeba compounds is found in the 6-methoxy-2-(4'-nitrostyryl)quinazoline derivative containing a diethylamino-group in the 4-position (compound (I-44).

TABLE 3. Antitrypanosomiasis and Antilambliasis Activity of 4-Amino-2-styrylquinazolines

Compound	Antitrypanosomiasis activity				Antilambliasis activity			
	dose, mg/kg	mean lifespan of animals, days (M ± m)	P	effectiveness, %	therapeutic dose, mg/kg	mean numbers of lamblia in three sections of small intestine (M ± m)	P	effectiveness, %
I-2	250×5 (p)	6,0±0,5	>0,2	None	400×5	0,3±0,3	<0,001	87
	250×5 (t)	7,2±0,5	>0,7	»	250×5	0,6±0,2	<0,001	80
I-3	250×5 (p)	5,4±0,2	>0,5	»	400×5	0,9±0,5	<0,02	96
	250×3 (t)	4,6±0,2	>0,1	»	200×5	1,3±0,6	<0,05	55
I-4	200×5 (p)	5,4±0,7	>0,1	»	200×5	2,6±0,5	>0,7	—
	200×3 (t)	8,0±0	>0,9	»				
I-5	200×5 (p)	5,0±0	>0,9	»	200×5	2,6±0,5	>0,7	—
	200×3 (t)	5,0±0	>0,9	»	500×5	3,0±0,3	>0,7	—
I-9	250×5 (p)	5,4±0,2	>0,1	»	400×5	0	<0,001	98
	250×3 (t)	5,0±0,3	>0,2	»	200×5	0,8±0,6	<0,02	72
I-11	500×8 (p)	7,4±0,4	>0,1	»	500×5	1,0±0,3	<0,01	54
	500×2 (t)	4,2±0,2	>0,2	»	400×5	1,2±0,6	<0,05	60
					250×5	1,6±0,4	<0,05	45
I-13	500×8 (p)	6,3±0,3	>0,1	»	400×5	2,5±0,6	>0,5	—
	500×2 (t)	4,5±0,2	>0,2	»				
I-14	500×8 (p)	7,0±0,3	>0,1	»	400×5	1,0±0,5	<0,02	66
	500×2 (t)	4,2±0,1	>0,2	»				
I-19	500×5 (p)	7,8±0,2	>0,1	»				
	500×3 (t)	7,8±0,2	>0,1	»				
I-20	300×5 (p)	5,2±0,2	>0,4	»	300×5	3,1±0,3	>0,6	—
I-22	250×5 (p)	6,8±0,5	>0,7	»	250×5	3,3±0,4	>0,4	—
	250×3 (t)	7,4±0,4	>0,5	»				
I-25	100×5 (p)	4,4±0,4	>0,2	»				
	100×3 (t)	4,8±0,2	>0,2					
I-30	200×5 (p)	5,2±0,2	>0,1	»	200×5	3,0±0,4	>0,7	—
	200×3 (t)	5,2±0,2	>0,1					
I-33	100×5 (p)	4,6±0,2	>0,1	»				
	100×3 (t)	5,4±0,2	>0,5					

Note. p-indicates prophylactic dose, t therapeutic dose.

EXPERIMENTAL (CHEMICAL)

2-(4'-Aminostyryl)-4-(δ-diethylamino-α-methylbutylamino)quinazoline Trihydrochloride Monohydrate (I = 1·3CHl·H_2O). To a solution of 3.6 g (8.3 mmoles) of I-2 [10] in a mixture of 160 ml of alcohol and 70 ml of water was added a solution of 10 g (41.7 mmoles) of sodium sulfide in 50 ml of water. The mixture was kept at room temperature for three days. Completion of the reduction was followed by TLC on plates with grade II alumina, mobile phase chloroform, detection by UV (R_f for I-2 0.77, for I-1 0.64). When the reaction was complete, the mixture was evaporated to a volume of 70 ml, basified with 50% potassium carbonate solution, and extracted with chloroform. The chloroform extract was dried over potassium carbonate and evaporated. The residue (3.5 g) was recrystallized from 600 ml of heptane to give 2 g of I-1, mp 137-138°C. This was dissolved in 60 ml of dry acetone and ethanolic hydrogen chloride added until the pH reached 2.0. The solid which separated was filtered off and washed with 3 × 20 ml of dry acetone to give 1.6 g (41%) of I·3HCl·H_2O, mp 243-245°C. The compound was yellowish-orange in color, and was readily soluble in water and alcohols, but insoluble in acetone, ether, and chloroform. Found, %: Cl 19.56; N 13.25; H_2O 3.51. $C_{25}H_{33}N_5 \cdot 3HCl \cdot H_2O$. Calculated, %: Cl 20.03; N 13.19; H_2O 3.40.

2-(5'-Nitrofuryl-2'-vinyl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline Dihydrochloride (I-7.2 HCl). A mixture of 7 g (20.9 mmoles) of 2-methyl-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline, 15.2 g (62.5 mmoles) of 5-nitrofurfural diacetate, 2.6 g (32 mmoles) of anhydrous sodium acetate, and 35 ml of acetic anhydride was heated with stirring at 125-127°C for 5 h. The mixture was then cooled to 50°C, and poured into 8% hydrochloric acid. Nonbasic products were extracted from the hydrochloric acid solution with ether, and the aqueous layer was then basified with potassium carbonate to pH 9.0-10.0, and again extracted with ether. The ether extract was dried over potassium carbonate and evaporated, and the residue (6.2 g) dissolved in 200 ml of dry acetone, followed by the addition of alcoholic hydrogen chloride to pH 2.0. The solid was filtered off, washed with dry acetone, and converted into the base, which was extracted with chloroform. The residue (5.1 g) after removal of the chloroform was recrystallized from heptane–benzene (3:1), dissolved in 100 ml of dry acetone, and the dihydrochloride isolated by adding alcoholic hydrogen chloride to pH 2.0. Yield of I-7·2HCl, 4.57 g (41%), yellow crystals, mp 259-260°C. The compound was

readily soluble in water, methanol, and ethanol, less so in propan-2-ol and chloroform, and insoluble in ether and acetone. Found, %: Cl 19.77; N 13.53. $C_{23}H_{28}ClN_5O_3 \cdot 2HCl$. Calculated, %: Cl 20.04; N 19.19.

I-7 Free Base. Yellow crystals, mp 135-136°C. Readily soluble in ether, acetone, alcohols, and ethyl acetate, sparingly in benzene, hexane, and heptane, insoluble in water. Found, %: C 59.96; H 6.03; Cl 7.83; N 15.18. $C_{23}H_{28}ClN_5O_3$. Calculated, %: C 60.33; H 6.16; Cl 7.74; N 15.29.

2-(2'-Methoxystyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline Dihydrochloride (I-9.2HCl). A mixture of 15 g (44.8 mmole) of 2-methyl-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline, 18.3 g (134.5 mmole) of o-methoxygenzaldehyde, 5.5 g (67 mmole) of anhydrous sodium acetate, and 90 ml of acetic anhydride was boiled with stirring for 8 h. Excess acetic anhydride and the acetic acid formed were distilled off under reduced pressure. The residue was mixed with 300 ml of dry acetone, and the insoluble sodium acetate filtered off. To the filtrate was added ethanolic hydrogen chloride to pH 2.0, and the dihydrochloride which separated (I-9·2HCl) was filtered off, washed with dry acetone (3 × 15 ml), and converted into the free base by treatment with 10% sodium hydroxide solution. The free base was extracted with chloroform, the chloroform removed, and the residue crystallized from 700 ml of heptane to give 11.6 g of I-9 (57% yield), yellow crystals, mp 163-174°C. The compound was readily soluble in the usual organic solvents, but less so in heptane and hexane, and insoluble in water. Found, %: C 68.68; H 7.21; Cl 7.81; N 12.32. $C_{26}H_{33}ClN_4O$. Calculated, %: C 68.93; H 7.34; Cl 7.83; N 12.37.

The dihydrochloride I-9·2HCl was obtained as a yellow crystalline solid, mp 239-240°C, readily soluble in water, methanol, and ethanol, sparingly so in propan-2-ol, and insoluble in ether, chloroform, and acetone. Found, %: Cl 20.29; N 10,68. $C_{26}H_{33}ClN_4O \cdot 2HCl$. Calculated, %: Cl 20.22; N 10.66.

Similarly, from 12.7 g (37.9 mmole) of 2-methyl-4-(δ-diethylamino-α-methylbutylamine)-7-chloroquinazoline and 23 g (114.6 mmole) of chloroveratraldehyde in 110 ml of acetic anhydride there was obtained 11 g (56%) of 2-(4',5'-dimethoxy-2'-chlorostyryl)-4-(δ-diethylamino-α-methylbutylaminl)-7-chloroquinazoline (I-10), mp 145-146°C (from heptane). Found, %: C 62.46; H 6.70; Cl 13,71; N 10.76. $C_{27}H_{34}Cl_2N_4O_2$. Calculated, %: C 62.66; H 6.62; Cl 13.70; N

The dihydrochloride I-10·2HCl was obtained as yellow crystals, mp 238-239°C, readily soluble in alcohols and chloroform, sparingly in acetone. Found, %: Cl 11.93; N 9.19. $C_{27}H_{34}Cl_2 \cdot N_4O_2 \cdot 2HCl$. Calculated, %: Cl' 12.01; N 9.49.

2-(2',4'-Dichlorostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline Dihydrochloride (I-20·2HCl). A mixture of 5 g (15 mmoles) of 2-methyl-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline, 7.85 g (45 mmoles) of 2,4-dichlorobenzaldehyde, and 1.85 g (22.5 mmoles) of anhydrous sodium acetate in 30 ml of acetic anhydride was heated at the boil with stirring for 7 h. The mixture was then poured into 100 ml of 5% hydrochloric acid at 80–85°C. The mixture was then cooled to room temperature, and treated with a further 50 ml of concentrated hydrochloric acid. Nonbasic products were extracted with ether, and the hydrochloric acid solution basified with potassium carbonate, and the free base extracted with benzene. The residue (7.2 g) after removal of the benzene was dissolved in 150 ml of methanol, and orthophosphoric acid added to pH 2.0. The solid diphosphate (8.7 g) which separated was filtered off, washed with a small amount of methanol and dry acetone, and converted into the free base (5.88 g), which was recrystallized from 100 ml of heptane to give 4 g (55%) of I-20, colorless crystals, mp 127-128°C. The compound was readily soluble in ether, benzene, chloroform, alcohols, ethyl acetate,and acetone, sparingly in heptane, and insoluble in water. Found, %: C 60.64; H 5.90; Cl 21.64; N 11.42. $C_{25}H_{29}Cl_3N_4$. Calculated, %: C 61.04; H 5.94; Cl 21.63; N 11.39.

The dihydrochloride I-20·2HCl was obtained by adding alcoholic hydrogen chloride to an acetone solution of the free base to pH 2.0, as a colorless solid, mp 274-275°C, soluble in water, alcohols, and chloroform, but insoluble in ether and acetone. Found, %: Cl 30.96; N 9.91. $C_{25}H_{29}Cl_3N_4 \cdot 2HCl$. Calculated, %: Cl 31.39; N 9.92.

Similarly, from 6.0 g (20.6 mmoles) of 2-methyl-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline and 115 g (62.1 mmoles) of p-bromobenzaldehyde there was obtained 4.8 g (47%) of 2-(4'-bromostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline (I-26), colorless crystals, mp 121-122°C, readily soluble in the usual organic solvents, less so in heptane and hexane, and insoluble in water. Found, %: C 59.62; H 6.09; Br 15.94; Cl 7.04, N 11.06. $C_{25}H_{30}BrClN_4$. Calculated, %: C 59.63; H 6.03; Br 15.92; Cl 7.06; N 11.16.

TABLE 4. Antitrichomonad, Antiamoeba, and Antimalarial Activity of 4-Amino-2-styrylquinazolines (I) *in vitro*

Compound	Maximum inhibitory concentration, μg/ml		CTC against Plasmodium berghei
	Trichomonas vaginalis	Entamoeba histolytica	
I-1·3HCl·H_2O	n/a	n/a	
I-2·HCl	4	1 [1]	
I-3·2HCl	62,5	15,6	
I-4·2HCl	100	n/a	
I-5·2HCl	100	62,5	~0,5
I-6·2HCl·H_2O	n/a	n/a [1]	~0,4
I-7·2HCl	31,2	31,2	n/a
I-8	n/a	n/a	
I-9·2HCl	n/a	n/a	
I-10·2HCl	n/a	n/a	
I-11·2HCl	n/a	25 [7]	0,5
I-12·2H_3PO_4·3H_2O	n/a	n/a	
I-13·2HCl	n/a	50 [7]	
I-14·2HCl	n/a	125 [7]	
I-15·2HCl	100	100 [1]	0,25
I-16·2HCl	n/a	n/a	
I-17·2HCl	n/a	n/a	
I-18·2HCl·H_2O	n/a	n/a [1]	
I-19·2HCl	2	31,2	
I-20·2HCl	250	31,2	
I-21·2HCl	n/a	31,2	0,5
I-22·2HCl	125	31,2	
I-24·2HCl	—	100	
I-25·2HCl	n/a	100	
I-26·2HCl·2H_2O	125	62,5	
I-28·2HCl	n/a	125	
I-29·2HCl	n/a	62,5	~1,04
I-30·2HCl	n/a	31,2	
I-31·2HCl	n/a	100	
I-32·2HCl	n/a	500	
I-33·2HCl	—	n/a	
I-34·2HCl·H_2O	n/a	n/a	
I-35·HCl	n/a	100	
I-36·HCl·H_2O	n/a	n/a	
I-37·HCl	n/a	100	
I-38·HCl·H_2O	n/a	n/a	
I-39·HCl	250	n/a	
I-40·HCl	n/a	n/a	
I-41·HCl	n/a	n/a	
I-42·HCl	n/a	n/a	
I-43·HCl	n/a	n/a	
I-44·HCl	n/a	15,6	

Note. n/a signifies inactive.

Dihydrochloride dihydrate (I-26·2HCl·2H_2O), colorless crystals, mp 277-278°C, soluble in water and alcohols, insoluble in ether and acetone. Found, %: Br 13.38; Cl 17.19; N 8.94; H_2O 5.52. $C_{25}H_{30}BrClN_4$·2HCl·2H_2O. Calculated, %: Br 13.09; Cl 17.41; N 9.17; H_2O 5.90.

EXPERIMENTAL (PHARMACOLOGICAL)

Antileishmaniasis Activity. White mice of both sexes weighing 16-18 g were infected intradermally in the region of tail root with 0.05 ml of suspension of *Leishmania major* promastygotes which were highly pathogenic to man. The drug was administered once daily for ten days, internally, as a suspension in isotonic saline. The antileishmaniasis activity of the compound was assessed by comparing the rate of development of local leishmaniasis lesions, the size of the ulcer, and the results of parasitological examination of the exudate from the ulcer in the control and treated animals. The course of the infection in the animals was followed for 15 weeks. The results obtained are given in Table 2.

Antitrypanosomiasis Activity. The animals were infected subcutaneously with a suspension of *Trypanosoma equiperdum* in citrate salt, in a volume of 0.5 ml per mouse. The prophylactic and therapeutic effects of the compounds following administration once daily for 8, 5, 3, and 2 days were assessed. Chemotherapeutic activity was measured in the treated group in comparison with the controls by the time elapsed before the appearance of trypanosomes in the blood, the duration of the time in which trypanosomes disappeared, and the lifespan. During the course of the experiment, the blood of the animals was subjected daily to parasitological examination (counts of trypanosomes in blood smears). The results obtained are presented in Table 3.

Antilambliosis Activity. White mice were infected orally with a suspension of *Lamblia muris*, 0.5 ml per mouse. The drugs were administered internally 72 h after infection, and daily for the following five days. Three weeks after completion of treatment, the animals were dissected, and parasitological examination of the contents of two or three segments of the small intestine for the presence of viable *Lamblia* carried out. Counts were made of *Lamblia* in untreated smears for the contents of the small intestine in the treated and control animals, and these counts were used to calculate the chemotherapeutic activity of the test compounds. The results obtained are shown in Table 3.

Antitrichomonad and Antiamoeba Activity. The activity of the compounds was assessed *in vitro* against *Trichomonas vaginalis* and *Entamoeba histolytica* by the serial dilution method. The initial concentration of the test compounds was 1000 μg/ml. The results obtained are shown in Table 4.

Antimalarial Activity. Studies were carried out using model malaria in rodents (mice) infected with *Plasmodium berghei* ("normal" drug-sensitive strain H) against the asexual erythrocyte-like form of the parasite, in therapeutic experiments. The reference drug used was Delagil (chloroquine diphosphate), manufactured in Hungary.

The experiments were carried out with mongrel white mice of both sexes weighing 12-18 g, infected with blood in the peritoneum. The test compounds were administered while the infection was developing, in a single dose in accordance with the weight of the animal, as a suspension in starch mucilage via a gastric probe.

The activity of the test compounds as compared with that of the standard was evaluated from their chemotherapeutic coefficients (CTC) [9]. The CTC (=e/t) is the ratio of the relative activity $e = DE_{st}/DE_p$ to the relative toxicity $t = DT_{st}/DT_p$ (where DE is the dose required to give an arbitrary effect, DT is the toxic dose of the drug, st is the standard reference drug, and P the test compound). The CTC of the standard, Delagil, was taken as unity.

The criteria for evaluation were as follows: 1) changes in the levels of parasitemia on the day following administration of the drug (in points); 2) the time of disappearance of the parasites from the time of administration of the drug (in days); 3) the duration of remission (in days); 4) the time to reach a given level of parasitemia (four points) in relapse (in days), and 5) the mean time of death (in days). Parasite counts were carried out daily in thin blood smears stained with Romanovskii–Gims stain, using the special scale devised by Sh. D. Moshkovskii [10].

The value of DE for Delagil was taken as 83 mg per 1 kg body weight of the animal (as the salt), which caused the disappearance of parasites from the peripheral blood for 2-3 days, followed by parasitic relapse. All the compounds examined were tested in deliberately high doses, some six times greater than the standard dose of Delagil. The results are given in Table 4.

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STUDIES OF SEMISYNTHETIC CEPHALOSPORINS.

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF CERTAIN 7-SUBSTITUTED 3-METHYL-3-CEPHEM-4-CARBOXYLIC ACIDS

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Over the last decade, intensive research has been carried out in the area of semisynthetic cephalosporins, which, similarly to penicillins, belong to the class of β-lactam antibiotics. Compared with penicillins, the cephalosporin derivatives have several advantages, so that the synthesis of new cephalosporins is a very hopeful and urgent subject.

For a directed search for new antibacterial preparations and for the clarification of the relationship between chemical structures and biological activity it was of interest to introduce into the amino group of 7-aminodesacetoxycephalosporanic acid (7-ADCA) such acyl groups, which, as has already been shown [2-4], in combination with a penicillin ring would have not only a certain antibacterial activity, but also low toxicity and pecillinase- and acid-stability.

For the synthesis of the new cephalosporin derivatives (I-XII), we chose the acid chloride method [5], consisting in direct reaction of 7-ADCA with acid chlorides in the presence of $NaHCO_3$ in acetone at low temperatures (method A). As acylating agents, acid chlorides of various carboxylic acids were used [2-4], obtained by known methods, i.e., by boiling of the acids with $SOCl_2$ in absolute benzene. In general, all the acid chlorides were distilled *in vacuo*, and in the case of resinification during vacuum distillation, they were introduced into the acylation reaction directly after multiple washing absolute benzene.

During recent years, the silyl methods [1] have been extensively used in the chemistry of β-lactam antibiotics. To increase the yields of cephalosporins I-XII, we used silyl protection of the carbonyl group in 7-ADCA, followed by the acylation of its trimethylsilyl ester by acid chlorides (method B). The convenience of this method consists in the ease of removal of the trimethylsilyl protective group during the treatment of the reaction mixture.

The desired end products I-XII were isolated in the form of sodium salts, whose purity and individual state was confirmed by the TLC method (Table 1).

The structure of the cephalosporin derivatives synthesized, characterized in the form of acids, was confirmed by elemental analysis and by spectrometry. In all the IR spectra, absorption bands were observed at 1760-1780 cm^{-1} (a β-lactam CO group), 1710-1725 cm^{-1} (a carboxylic CO group), 1640-1660 cm^{-1} (an amide CO group), 3300-3340 cm^{-1} (NH); in the mass spectra there were peaks of molecular ions and several characteristic fragmentary ions.

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