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SYNTHESIS AND ANTIPROTOZOAL AND ANTIINFLAMMATORY ACTION OF 2-(3',4'-DIMETHYOXYSTYRYL)-4-(δ-DIETHYLAMINO-α-METHYLBUTYLAMINO)-QUINAZOLINE AND ITS CHLORO DERIVATIVES

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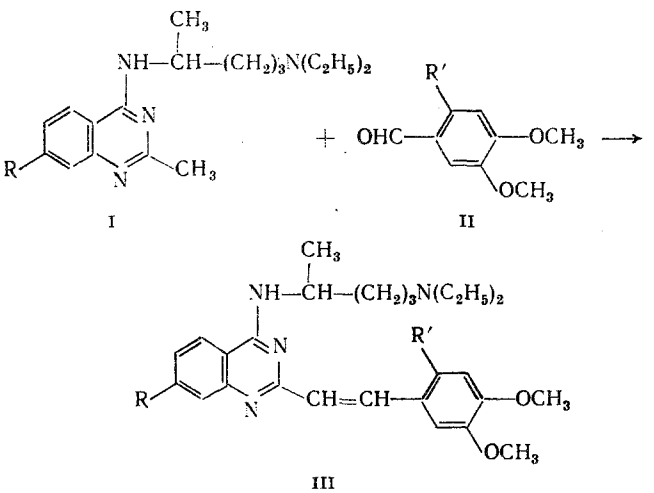
In previous comuunications [3-5, 7] a descriptionwas given of the high antiprotozoal and antiinflammatory activity of various substances belonging to an original class of compounds, derived from 2-styryl-4-aminoquinazolines, which has been developed in VNIKhFI. With the aim of searching for new antiiflammatory and chemotherapeutic agents we have synthesized new derivatives by the general method for this type of substance [1, 2], viz., by the condensation of 2-methyl-4-(δ-diethylamino-α-methylbutylamino)quinazoline (Ia) and its 7-chloro derivative (Ib) with veratraldehyde (IIa) and with 6-chloroveratraldehyde (IIb) [12].



Ia: R = H, b: R = Cl; IIa: R′ = H, b: R′ = Cl; IIIa: R = R′ = H, b: R = H, R′ = Cl, c: R = Cl, R′ = H.

Boiling acetic anhydride was used as condensing agent. The reaction time in each case was determined by checking the course of the process by TLC (from the absence on the chromatogram of the spot for the initial 2-methylquinazoline Ia, b. It was from 8 to 18 h, depending on the reactivity of the 2-methyl group in compounds (I) and the aldehyde group in compounds (II). The synthesized 2-(3',4'-dimethoxystyryl)quinazolines (IIIa-c) were subjected to chemotherapeutic and pharmacological study as the dihydrochlorides, readily soluble in water (Table 1).

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TABLE 1. 2-(3',4'-Dimethoxystyryl)quinazolines (IIIa-c)

Compound	mp, °C	Yield, %	R_f	Found, %				Empirical formula
				C	H	Cl	N	
IIIa	125	53	0,38	72,33	8,03	—	12,30	$C_{27}H_{26}N_4O_2$
IIIa·2HCl	251	—	—	...	...	13,80	10,77	$C_{27}H_{26}N_4O_2 \cdot 2HCl$
IIIb	133	41	0,45	66,99	7,30	7,53	11,82	$C_{27}H_{25}ClN_4O_2$
IIIb·2HCl	255	—	—	...	...	18,95	10,00	$C_{27}H_{25}ClN_4O_2 \cdot 2HCl$
IIIc	126	57	0,45	66,97	7,32	7,25	11,90	$C_{27}H_{25}ClN_4O_2$
IIIc·2HCl	171	—	—	...	...	19,16	10,00	$C_{27}H_{25}ClN_4O_2 \cdot 2HCl$

TABLE 2. Pharmacological Properties of 2-(3',4'-Dimethoxystyryl)quinazolines

Compound	LD_{50}, mg/kg	AIA, † % suppresion of edema	Analgesic action		Antipyresis, ‡ % reduction of hyperthermia
			% increase of TPS†	% suppression of spam **	
IIIa·2HCl	1000	5†	22 ($P>0,1$)	0	3
IIIb·2HCl	1500	10†	33* ($P<0,05$)	7	—3
IIIc·2HCl	1500	13†	77 ($P=0,001$)	49	—9
ASA	1500	43	77* ($P<0,001$)	45	69

*Difference in action of ASA and IIIb•2HCl was significant ($P < 0.05$).
†Difference from control was not significant ($P > 0.05$).
‡At a dose of 100 mg/kg orally.
**At a dose of 200 mg/kg orally.

The results of the investigations are given in Tables 2 and 3. Analysis of the data of biological testing of the synthesized compounds (IIIa-c) made it possible to draw the following conclusions.

The derivatives of 2-(3',4'-dimethoxystyryl)quinazoline (IIIa-c) were devoid of antiinflammatory and antipyretic properties; they had low toxicity and in analgesic activity they were superseded somewhat by acetylsalicylic acid (ASA). Introduction of a chlorine atom at position 7 of the quinazoline nucleus or at position 6' of the styrene portion of the molecule aided the development of analgesic properties which were more marked for the 7-chloroquinazoline derivatives.

The indicated compounds were devoid of antitrichomonal and trypanosomal activity and weakly suppressed the growth of *Entamoeba histolytica in vitro* but possessed high antilamblial activity in experiments in animals, (compounds IIIb•2HCl and IIIc•2HCl). This was close to the activity of aminoquinol, the therapeutic preparation of the styrylquinoline series which is used in medical practice. In addition, the dihydrochlorides of compounds (IIIa-c) inhibited statistically significantly the development of the leishmanial process in experiments in animals and may be assigned to the group of moderately active antileishmanial agents.

It is interesting to note that in relation to antiprotozoal (antilambial and antileishmanial) activity the same rules linking the structure of substances and their biological activity are retained as in the development of the analgesic properties of these substances. The introduction of a chlorine atom into position 7 of the quinazoline 2-(3',4'-dimethoxystyryl-4-(δ-diethylamino-α-methylbutylamino)quinazoline or into position 6' of the styrene portion of this molecule strengthens the biological antivity, although in relation to protozoal infections a more marked effect took place on introducing the chlorine atom into the quinazoline nucleus.

The effects shown were of definite theoretical interest and deserve further study.

TABLE 3. Chemotherapeutic Activity of Compounds (IIIa-c) in Relation to Protozoa

Compound	Maximum tolerated dose	Antileishmanial activity				Antitrypanosomal activity				Antilambial activity				Antiamoebic activity
		dose mg/kg	average extent of leishmanial lesion (M ± m)	P	degree of effectiveness	dose mg/kg	mean lifespan of mice, days	P	degree of effectiveness	dose mg/kg	mean extent of lesion (M ± m)	P	degree of effectiveness	minimum concentration suppressing growth of Entamoeba histolytica, mg/ml
IIIa·2HCl	500×10	500×10	1,1±0,2	<0,001	45	500×8 (p) 500×2 (t)	6,3±0,3 (p) 4,5±0,2 (t)	>0,1 >0,2	0 0	400×5	2,5±0,6	>0,5	17	50
IIIb·2HCl	400×10	400×10	1,2±0,2	<0,01	40	500×8 (p) 500×2 (t)	7,4±0,4 (p) 4,2±0,2 (t)	>0,1 >0,2	0 0	400×5	1,2+0,6	<0,05	60	25
IIIc 2HCl	400×10	400×10 300×10	0,8±0,1 0,9±0,1	<0,001 <0,001	60 55	500×8 (t) 500×2 (t)	7,0±0,3 (p) 4,2±0,1 (t)	>0,1 >0,2	0 0	400×5	1,0±0,5	<0,02	66	125
Aminoquinol		400×10 200×10	1,5±0,2 1,2±0,2	<0,001 <0,001	35 48					400×5	0,7±0,3	<0.01	77	
Control							6,2±0,4 (p) 4,5±0,2 (t)				3,0±0,5			

Note. p is a prophylactic experiment, t a therapeutic experiment.

EXPERIMENTAL CHEMISTRY

General Method of Synthesis of 2-(3',4'-Dimethoxystyryl)-4-(δ-diethylamino-α-methylbutylamino)quinazoline (IIIa) and Its 6'- (IIIb) and 7- (IIIc) Chloro Derivatives. A mixture of (Ia) or (Ib) (118 mmoles) and (IIa) or (IIb) (360 mmoles) in acetic anhydride (180 ml) was heated at 140°C with stirring, checking the course of the reaction by TLC on Silufol plates (Silufol UV-254) in the system acetone–saturated aqueous ammonia solution (97:3).* After the end of the reaction (8-18 h for the different compounds) the mixture was evaporated in vacuum with a water-jet pump. The residue was dissolved in dry acetone (300 ml) and an alcohol solution of hydrogen chloride poured in until an acid reaction was obtained with Congo Red. The solid (III) dihydrochloride was filtered off, washed with dry acetone, dissolved in water and treated with a 10% solution of sodium hydroxide to pH 9.0. The base which separated was extracted with benzene. After distilling off the benzene, base (III) was obtained, which was recrystallized from heptane (in the case of IIIb crystallization was carried out from a mixture of heptane with acetone 5:1). Yellow crystals were obtained, readily soluble in the ususal organic solvents, soluble with difficulty in water, hexane, and heptane. The dihydrochlorides were yellow crystals, readily soluble in water.

Yields, constants, and results of analyses of the synthesized substances are given in Table 1.

EXPERIMENTAL BIOLOGY

The acute toxicity of the dihydrochlorides of compounds (IIIa-c) was studied in male mice of weight 18-20 g on peroral administration with calculation of the LD_{50} value by the graphical method of [9].

Antiinflammatory activity was studied in male rats of weight 130-140 g in the carrageenan paw edema model of [13]. An acute inflammatory reaction was caused by the subplantar injection of 1% carrageenan solution (0.1 ml) and recording with a plethysmograph (Ugo Basile, Italy) at 1, 2, and 3 h after induction of inflammation. The dihydrochlorides of (IIIa-c) were administered orally in the form of a suspension in starch paste at a dose of 100 mg/kg 1 h before the injection of carrageenan. Antiinflammatory activity was recorded as percent suppression of edema in relation to the control and was compared with the activity of ASA.

Analgesic action was assessed by the change in the threshold of pain sensitivity (TPS) of paw inflammation of male rats of weight 130-140 g [11] with the aid of an analgesiometer (Ugo Basile, Italy). Compounds were administered orally at a dose of 100 mg/kg 2 h before measuring TPS. The analgesic effect was expressed as the percentage increase of TPS in relation to a control and were compared with the action of ASA. The effect of compounds on the pain reaction (spasm) caused by intraperitoneal injection of 0.75% acetic acid solution (0.25 ml) as in [8] was studied in male mice of weight 18-20 g. Compounds were administered orally at a dose of 200 mg/kg 1 h before injecting acetic acid. The reduction in the number of spasms served to judge the presence and expression of analgesic action.

Antipyretic action was studied in the yeast fever model, caused by the subcutaneous injection of a 20% aqueous suspension of baker's yeast (1 ml per 100 g body weight) to male rats of weight 140-150 g [10]. The increase in rectal temperature measured with an electrothermometer (TPEM-1) 18 h after injection of yeast was 1.5°C. Compounds were administered orally at a dose of 100 mg/kg. Repeated measurements of temperature were carried out after 2 and 3 h. The presence of an antipyretic effect was judged by the reduction in temperature index (i.e., from the sum of the deviations of the mean temperature value after two measurements) expressed as percent of control.

Experiments were carried out in mice and rats obtained from the breeding unit of the Academy of Medical Sciences of the USSR (AMN SSSR) and were kept in the vivarium on a standard diet. Study of each index was carried out on 6-8 animals. The results were processed statistically using the Student t test.

Results of investigations are represented in Table 2, from which it is seen that (IIIa-c) dihydrochlorides had low toxicity and in this respect did not differ appreciably from ASA. All the studied compounds proved to have no significant influence on the expression of acute carrageenan paw inflammation in rats. Compounds (IIIc•2HCl) and (IIIb•2HCl) displayed analgesic activity while increasing the TPS of the inflamed tissues by 77 and 33% respectively. In the given form of activity these compounds were close to or were somewhat superseded by

*R_f of starting compounds Ia 0.17, Ib 0.28.

ASA. Compound (IIIc•2HCl) in addition reduced pain reactions in mice caused by chemical irritation (spasm). Compounds (IIIa•2HCl) and (IIIb•2HCl) had little activity in this respect. All the studied compounds proved to have no antipyretic action in difference to ASA.

The antiprotozoal activity of compounds (IIIa-c•2HCl) was studied in relation to a series of pathogenic *Protozoa*, leishmania (*Leishmania tropica major*), trypanosomes (*Trypanosoma equiperdum*), lamblia (*Lamblia muris*), amoeba (*Entamoeba histolytica*), and trichomonad (*Trichomonas vaginalis*). Experiments were carried out in random bred white mice of both sexes of weight 16-18 g and in experiments *in vitro* [6]. Compounds were introduced once daily orally as a suspension in isotonic solution or citrate salt.

With the aim of studying antileishmanial activity animals were infected intracutaneously in the region of the tail root with a suspension of a culture of the promastigote *Leishmania tropica major*, which is highly pathogenic in man, in a volume of 0.5 ml. Compounds were administered for 10 days. The duration of the experiment was 15 weeks. Assessment of the chemotherapeutic action of compounds was carried out by comparing the dynamics of the development of the local leishmanial process, the size of sores, and data of parasitological investigation of material separated from the sores in groups of treated and control animals. The obtained results are shown in Table 3.

The carried out investigations showed that the studied compounds proved to have a chemotherapeutic action on experimental skin leishmaniasis which was expressed as a statistically significant inhibition of the development of the leishmaniasis process in treated animals in comparison with controls. Thus for example in mice receiving compound (IIIc•2HCl) at a dose of 300 mg/kg, (IIIb•2HCl) at a dose of 400 mg/kg, and (IIIa•2HCl) at a dose of 500 mg/kg the leshmaniasis process was limited to infiltrate in a series of cases and the ulceration of leishmaniasis did not set in.

In the process of observation of reduction was recorded in the number of stimuli, and in several cases they had completely disappeared on parasitological investigation of impressions of sores of animals which indicates the specific action of the studied compounds on the stimuli of the illness. As follows from Table 3 the studied compounds suppressed the leishmaniasis process by 40-55%, which formed the basis for assigning them to the group of moderately active antileishmanial substances.

To determine antitrypanosomal activity, animals were infected subcutaneously with a suspension of *Trypanosoma equiperdum* in salt citrate at 0.5 ml per mouse. The duration of treatment was 3-7 days. The prophylactic and therapeutic effects of the compounds were studied. Chemotherapeutic effectiveness was assessed by the time needed for the appearance of trypanosomes in blood in the treated mice in comparison with control animals, from the length of the time after which trypnosomes had disappeared in the group of treated mice in comparison with controls, and from the life span of treated mice in comparison with controls.

During each experiment the blood of animals was subjected to parasitological investigation (calculation of trypanosomes was carried out in blood smears). The obtained results are given in Table 3, from which it follows that the studied compounds were ineffective in experimental trypanosomatosis in white mice. The mean life span of treated mice did not differ from that of mice of control groups.

On studying antilamblial activity animals were infected perorally with a suspension of *Lamblia muris* in isotonic solution at 0.5 ml per mouse. Compounds (IIIa-c•2HCl) were administered orally beginning from the 3rd day after infection for the next 5 days. All mice were autopsied 72 h after the end of treatment. The chemotherapeutic activity of compounds was assessed from the results of parasitological investigation of the contents of two sections of the small intestine. Calculation of the number of lamblia was carried out for groups of treated and control animals. The obtained results are given in Table 3, from which it follows that compounds (IIIb•2HCl) and (IIIc•2HCl) possessed high antilambliotial activity close to that of the drug aminoquinol.

The antitrichomonal and antiamebic activities of compounds (IIIa-c•2HCl) were investigated in experiments *in vitro*. Cultures of *Trichomonas vaginalis* and *Entamoeba histolytica* were used for this purpose. The activity of compounds was determined by serial dilution. The studied compounds (IIIa-c•2HCl) at initial concentrations of 1000 μg/ml did not suppress growth of *Trichomonas vaginalis* but possessed weak *in vitro* antiamebic activity in relation to *Entanoeba histolytica* (see Table 3).

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