SYNTHESIS AND ANTILEISHMANIASIS ACTIVITY OF 2-[2'-CHLOROSTYRYL]-4-[δ-DIETHYL-AMINO-α-METHYLBUTYLAMINO]-7-CHLOROQUINAZOLINE DIPHOSPHATE

L. N. Yakhontov, G. P. Zhikhareva,
L. I. Mastafanova, M. I. Evstratova,
G. N. Pershin, N. Yu. Moskalenko,
T. V. Pushkina, S. N. Kutchak,
N. I. Fadeeva, N. D. Sokolova,
T. A. Gus'kova, L. A. Chicherina,
and S. A. Sharova

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The prophylaxis and treatment of leishmaniasis, including the dermal form, is one of the six most urgent problems listed by the WHO in the chemotherapy of parasitic infections [18].

According to the WHO [13], leishmaniases have been recorded in 80 countries of the world, in which some 400,000 new cases of infection are noted every day. In the Soviet Union, dermal leishmaniasis is a serious problem in the mid-Asiatic republics, in many regions of which morbidity occurs in the nonimmune population.

Four drugs are currently used in the USSR for the treatment of dermal leishmaniasis, these having specific effects on the causative agents of the disease [3]. These are acriquin, solusurmin, monomycin, and aminoquinol. Systemic treatment of visceral and dermal leishmaniases abroad is usually carried out with pentavalent antimony compounds, namely an analog of solusurmin (pentostam) and glucanthim [10, 14, 15]. However, acriquin is suitable only for use in the early stages of leishmaniasis, as a local treatment [11]. The use of aminoquinol ointment has an irritant effect on the skin in many cases, and when taken internally aminoquinol has no effect on secondary infection accompanying the principal disease in the later stages. Solusurmin is effective only on extended (3-4 weeks) daily intravenous administration [7]. Like acriquin, it gives rise to side effects, and does not prevent relapse. The antimony derivatives glucanthim and pentostam, although having high therapeutic activity, adversely affect liver and kidney function and the cardiovascular system [10, 14, 15], and the usefulness of the highly active monomycin, which is used parenterally in two types of dermal leishmaniasis, is limited by its possession of nephro- and ototoxicity, like all the aminoglycoside antibiotics.

These deficiencies limit the use of existing drugs in dermal leishmaniasis, making the search for novel antileishmaniasis drugs most urgent.

For this reason, the All-Union Research Institute for Pharmaceutical Chemistry, with the financial support of the WHO (Special Program for Research and Training in Tropical Diseases ID770258) carried out a search for such drugs in a novel series of 2-styryl-4-aminoquinazolines. As reported in earlier publications [1, 2, 5], compounds of this type display high activity in experimental models against the causative agents of dermal leishmaniasis.

The most active compound, 2-(2'-chlorostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline diphosphate (I), known provisionally as aminazoline, was the subject of further intensive study in comparison with its des-aza-analog aminoquinol 2-(2'-chlorostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinoline triphosphate (II), which is produced by the chemical industry [8] and is used in the USSR for the treatment of dermal leishmaniasis [4].

S. Ordzhonikidze All-Union Scientific Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 21, No. 1, pp. 38-49, January, 1987. Original article submitted July 18, 1985.

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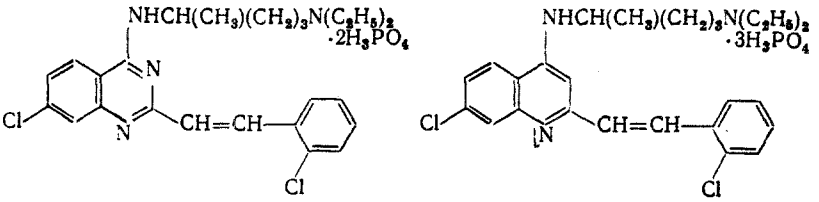
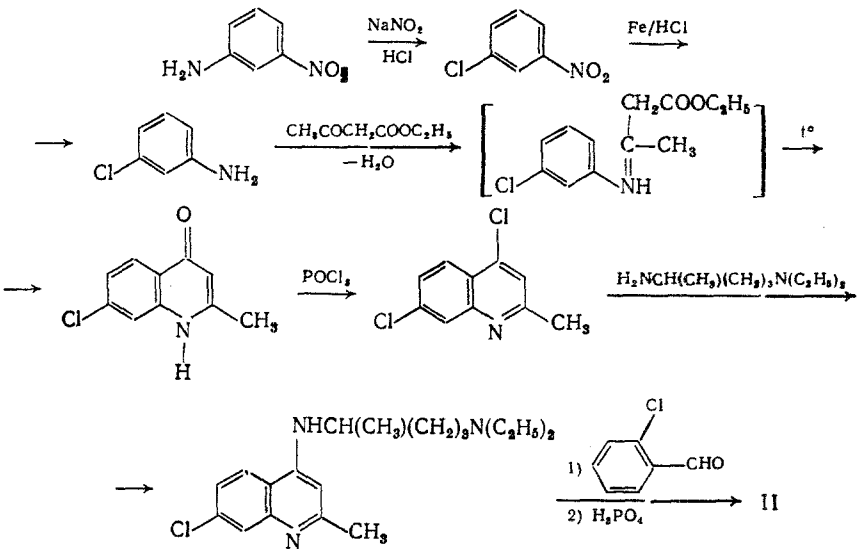


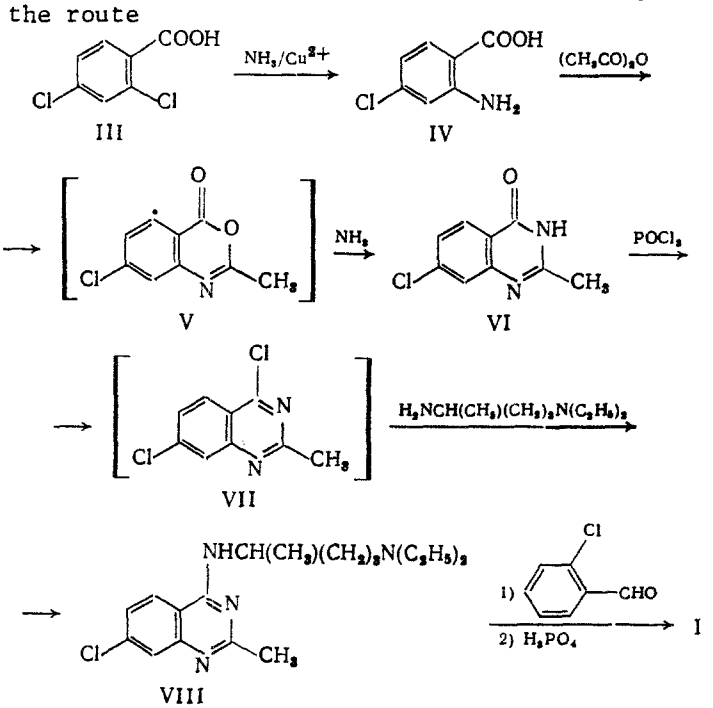
I II

As would be expected from the general features of the synthesis of quinolines and quinazolines, substituted 2-stryl-4-aminoquinazolines are more readily accessible than the analogous 2-styryl-4-aminoquinolines.

From the practical point of view, whereas the manufacture of aminoquinol requires seven chemical



steps, the yields at each step being 54, 86, 44, 60, and 76%, the closure of the hydroxyquinoline ring requiring a temperature of 275-300°C, introduction of the amino-side chain 145-150°C for 22 h, and the condensation with o-chlorobenzaldehyde 5h at 168-173°C, the synthesis of (I) by the route



requires six chemical steps with stage yields of 68, 73, 63, and 43%, i.e., the overall yield is greater by a factor of 1.5 than in the case of aminoquinol, closure of the aminoquinazoline ring requiring temperatures of only 120°C, introduction of the amino-side chain 6 h at 83-87°C, and condensation with o-chlorobenzaldehyde 6 h at 134-142°C.

In the development of the technology of manufacture of aminazoline, two variants of the conversion of 2,4-dichlorobenzoic acid (III) into 4-chloroanthranilic acid (IV) were tried, namely, charging the dry acid (III) in the autoclave, and charging the acid (III), in accordance with technical safety rules, as an aqueous solution of its potassium salt, which is the most soluble salt (at 80-85°C, the water solubility of the potassium salt is 1:1.6, ammonium salt 1:2.4, and sodium 1:3.3). In the first variant, ammonolysis was complete in 6 h (followed by TLC), and the yield of (IV) was 88%. In the second variant, the lower initial concentration of ammonia increased the time required for ammonolysis to 10 h, and as a result of the higher pH (7.5-9.0), in addition to the primary reaction, hydrolysis to give 4-chlorosalicylic acid occurred, but this remained in solution on acidification to pH 6.0-5.6, separating as a solid only when the pH was reduced further. The yield of (IV) in this variant was 68%.

Blowing off the ammonia enabled reprecipitation of the acid (IV) to be avoided, a product suitable for subsequent use being obtained without the need for further purification.

The preparation of 2-methyl-7-chloro-4-quinazolone (VI) was effected in two stages. First, the acid (IV) was reacted with acetic anhydride to give 2-methyl-7-chloro-3,1-benzoxazin-4-one (V) [12], which was then heated in the crude state with aqueous ammonia to give the quinazolone (VI). When developing a technological method, it was necessary to separate these two steps, since they differ considerably in corrosive effects. The use of toluene as solvent in the first stage, with a decrease in the amount of acetic anhydride, proved undesirable, since it hindered the transport of the product (V), which is readily soluble in toluene only at temperatures above 85°C. In the absence of toluene, further workup of the cooled reaction mixture with water resulted in considerable resinification, and treatment with 25% ammonia gave material which could not be handled. Better results were obtained by pouring the hot reaction mixture into 25% aqueous ammonia cooled to 0-5°C.

In the second stage, the best results were obtained by using (V) which had been dried to constant weight, and heating it with fresh 25% aqueous ammonia for 10 h at 48-50°C. Raising the temperature to 60°C, or the use of undried product at 48-50°C resulted in considerable foaming and determination in the quality of (VI). Attempts to use hermetically sealed vessels to prevent this foaming gave unsatisfactory results, and any departure from the conditions worked out resulted in a decrease in the quality of the (VI) obtained.

The preparation of 2-methyl-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline (VIII) is also carried out in two stages, without the isolation in the pure state of the highly reactive 2-methyl-4,7-dichloro-quinazoline (VII).

In the first stage (preparation of (VII)), attempts to carry out the reaction as in the preparation of chloropurines and 4,6-dichloropyrimidine (an intermediate in the production of sulfomonomethoxin), by boiling the quinazoline (VI) with 15-20 volumes of phosphoryl chloride or with a tenfold amount of this reagent in the presence of triethylamine hydrochloride, resulted in resinification of the mixture. A more successful method, reported in the literature for the preparation of 2-methyl-4-chloroquinazoline [17], was by reacting the quinazolone (VI) with phosphoryl chloride in the presence of dimethylaniline. The proportions of the reagents, solvent, and reaction time for the process were found experimentally so as to provide the maximum yields of (VII) with the most complete separation of unreacted (VI). The second stage of the synthesis was desirably carried out in the same toluene solution following removal of water-soluble products without removal of excess dimethylaniline. This considerably simplified the technology, reduced by nearly a half the consumption of the amine side chain (1.2 moles per mole of chloro-compound (VII)), shortened the reaction time from ten to six hours, and avoided the need to recrystallize the amine (VIII) from light petroleum.

In the final stage of the synthesis (condensation of (VIII) with o-chlorobenzaldehyde), the reaction was preferably carried out in acetic anhydride in the presence of anhydrous sodium acetate. The optimum reaction conditions were found experimentally by withdrawing samples for TLC analysis. The presence in the styryl compound (I) of by-products resulting from insufficiently pure amine component in the preceding stage, and the formation of other products in the condensation with o-chlorobenzaldehyde, demanded special attention to the methods of purification and isolation of (I). The direct isolation of the end-product from

the reaction mixture as the diphosphate using a variety of solvents (methanol, ethanol, and isopropyl alcohol) did not give a satisfactorily pure product. Nor was substantial purification obtained by converting the diphosphate into the free base followed by reconversion to the phosphate salt. The best results were obtained by utilizing the differing solubilities of the disulfate of the styryl compound in water and acetone, this being readily (1:10) soluble in hot water, less so in cold water, and sparingly soluble in acetone. Treatment of the condensation product with 5% sulfuric acid with heating, followed by filtration of the hot solution, removed the bulk of the nonbasic impurities. Further amounts of these impurities were removed by filtration of the sulfuric acid solution after cooling to room temperature. Subsequent treatment of the solution with an equal volume of acetone followed by keeping for three hours at room temperature resulted in the separation of the disulfate of the required product which, however, according to TLC still contained a certain amount of resinous material together with some by-products.

It was therefore necessary to further purify the styryl derivative by recrystallization of the free base, which can be carried out either from acetone (1:2, yield 70%), toluene (1:1.4, yield 82%), benzene (1:1.3, yield 73%), ethyl acetate (1:1.3, yield 68%), heptane (1:1.9, yield 82%), or a mixture of equal amounts of heptane and benzene (1:2, yield 92%).

The most consistent results in terms of the color of the product obtained and the completeness of separation of by-products were obtained by recrystallization from acetone. When the product was converted into the diphosphate, the use of methanol and 20% methanolic phosphoric acid gave a lighter colored product than when acetone or isopropyl alcohol were used. Since the (I) obtained readily adsorbs additional amounts of phosphoric acid, when isolating the diphosphate it is necessary to use methanolic solutions of phosphoric acid of no more than 20% concentration, and to carry out the process with heating and with strict control of the acidity of the medium using a pH-meter, keeping it at pH 4.5—6.0. The yields of (I) were 42—45%, color pale yellow to yellow, active constituent content not less than 99%. The drug is of low solubility in water and methanol, and is insoluble in ethanol, ether, and chloroform. It is nonhygroscopic. The shelf life is at least two years.

Biological studies showed that internal administration of (I) gave a good chemotherapeutic effect in model dermal leishmaniasis in mice induced by *Leishmania major*. Comparative studies of the antileishmaniasis activity of (I) and aminoquinol showed that (I) was to be preferred. The latter suppressed the leishmaniasis process in animals over a wide range of doses (200, 300, 400, 500, 600, 700, and 800 mg/kg), had a satisfactory chemotherapeutic index of 3.5, and displayed both prophylactic and therapeutic activity. Comparative studies of (I) and glucanthim showed (I) to be as active as glucanthim.

The drug is of relatively low toxicity, and is well tolerated in mice and rats in a single dose. It is considerably less toxic than aminoquinol by the intraperitoneal route, and is of equal toxicity when administered internally in a single dose.

Comparative studies of tolerance, carried out in view of the chronic course of dermal leishmaniasis in mice, showed that tenfold administrations of (I) in doses of from 200 to 700 mg/kg were well tolerated by intact mice, and resulted in no appreciable pathomorphological changes under the experimental conditions. In rats, administration of the drug internally in doses of 10 and 50 mg/kg for one month did not affect homeostasis, and resulted in no pathological changes. Increasing the dose to 200 mg/kg and the duration of treatment to 1.5-2 months resulted in the development of pathological processes in the internal organs. These changes, however, were less pronounced than in the case of aminoquinol.

In a study of the molecular mechanism of the action of (I) as compared with acriquin, with which stacking takes place (reaction with DNA [16]), we examined the interaction of (I) and some other 2-styryl-4-aminoquinazolines with DNA. It was found that 2-styryl-4-aminoquinazolines, including (I), have no appreciable effects on the melting point parameters of native bacterial DNA (the melting points of the complexes are raised by 0.5—2.5°C, and the melting range by 1-2°C), indicating that the mechanism of interaction of (I) with DNA is very different from that of acriquin, which increases the melting point of the complex by 10.5°C, and the melting range by 6°C.

Spectral studies have shown that increasing the concentration of DNA in solution results in a decrease in absorption in spectra of (I), accompanied by a bathochromic shift in the absorption maxima, indicating the formation of a complex between DNA and (I). To elucidate the nature of this complex formation, the thermostability of the complex was examined at different ionic strengths of the solution. When the concentration of sodium chloride in the

solution was raised from 10^{-3} to 10^{-1} M, dissociation of the DNA–(I) complex occurred (the melting point of the complex as compared with that of native DNA changed from 16 to 0°C). These results show that the complex is stabilized by electrostatic interactions only, these being nonspecific and forming only the initial contact between the drug and DNA.

The melting points of complexes of (I) with polynucleotides of different GC contents (an index of the presence of the guanine–cytosine pair) (from 0 to 48%) showed a slight increase (of 2–3.5°C) which was independent of the GC content of the polynucleotides. This leads to the conclusion that (I), unlike acriquin, is not a true intercalator, but forms electrostatic complexes with DNA.

EXPERIMENTAL —CHEMICAL

4-Chloroanthranilic Acid (IV). A. A rotatory stainless steel autoclave of capacity 0.5 liter, equipped with electrical heating and an ammonia manometer reading up to 25 atm was charged with 122 ml of an aqueous solution of potassium 2,4-dichlorobenzoate (prepared from 62 g of 98.8% acid (III) and 24 ml of 50% aqueous potassium hydroxide) at 80°C, 3.1 g of cupric acetate, and 140 ml of 25% aqueous ammonia. The autoclave was sealed, and heated with stirring for 10 h at 126-129°C. After cooling, completion of the reaction was established by TLC (not more than 2% of the total of 2,4-dichlorobenzoic and 4-chlorosalicylic acids should remain). Excess ammonia was removed at the water pump at 40-50°C for 6 h (not more than 2.5% of ammonia should remain), 370 ml of water added followed by 6.2 g of charcoal, heated for 20 min at 40-50°C, the mixture filtered, and the filtrate acidified to pH 6.0–5.6 (meter) with 20% sulfuric acid (∿44 ml). The solid was filtered off, washed with 4 × 50 ml of water, and dried at 70-80°C to give 39 g of (IV), of 96% purity. Yield 68%.

B. The reaction was carried out similarly, the autoclave being charged with 2,4-dichlorobenzoic acid (III) as 62 g of the dry powder. Ammonolysis was carried out at 126-129°C for 6 h, the pressure in the autoclave falling from 8-12 to 5 atm. Workup as in A gave 88% of (IV).

2-Methyl-7-chloro-4-quinazolone (VI). A mixture of 44 g of 91% (IV) and 57.5 ml of 96% acetic anhydride was heated for 3 h at 110-120°C, the completeness of the conversion of (IV) into 2-methyl-7-chloro-3,1-benzoxazin-4-one (V) being followed by TLC. The mixture was then poured slowly with stirring and cooling to 0-5°C into 150 ml of 25% aqueous ammonia. The resulting suspension was filtered, the solid washed with 4 × 20 ml of 25% aqueous ammonia, and dried to constant weight at 70-80°C to give 41 g of technical (V), which was added to 230 ml of 25% aqueous ammonia. The mixture was stirred for 10 h at 48-50°C, the completion of the conversion of (V) to (VI) checked by TLC, and cooled to room temperature. The solid was filtered off, washed with 4 × 20 ml of water, and dried at 70-80°C to give 36.9 g of (VI), purity 96.5%. Yield 78.4%.

2-Methyl-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline (VIII). A mixture of 29.2 g of 97.3% (VI), 450 ml of toluene, 22.6 ml of 99% dimethylaniline, and 9 ml of freshly distilled phosphoryl chloride was beaten at 83-87°C with vigorous stirring for 6 h. The mixture was cooled to room temperature, 200 ml of water added, and stirred for 30 min until a homogeneous suspension was obtained. The mixture was filtered, the solid washed with 2 × 30 ml of toluene, and the toluene layer separated and washed with 2 × 100 of 10% aqueous sodium hydroxide. The solid and the aqueous alkaline solution were used to recover unreacted (VI), by bringing a mixture of the undissolved salts, acidic aqueous mother liquors, and basic wash liquors to pH 7.0-8.0, and adding 100 ml toluene. The solid (VI) which separated was filtered off, washed twice with a mixture of 20 ml of water and 20 ml of toluene, and dried at 70-80°C to give 9 g (31%) of (VI), which was subsequently used for the preparation of more (VII). The toluene solution of 2-methyl-4,7-dichloroquinazoline (VII)* was washed with 2 × 100 ml of water, and used without further purification of (VIII). For this purpose, it was treated with 40 ml (31.66 g of 100% δ-diethylamino-α-methylbutylamine, and the mixture

*For characterization, a sample of the solution of (VII) was dried over magnesium sulfate and evaporated to dryness. The residue was recrystallized from light petroleum, to give a colorless, crystalline solid which was sparingly soluble in light petroleum, but readily soluble in the usual organic solvents, and insoluble in water. Its melting point was 95-96°C. Found,%: C 51.00; H 3.06; Cl 32.92; N 13.09; $C_9H_6Cl_2N_2$. Calculated %: C 50.75; H 2.85; Cl 33.24; N 13.16.

stirred 83-87°C for 6 h. Heating was then terminated, and completion of the reaction checked by TLC. There were then added at room temperature 60 ml of 10% sodium hydroxide solution, and the mixture stirred. The toluene layer was separated and washed with 2 × 100 ml of water. The toluene was removed in vacuo, and the residue recrystallized from 290 ml of light petroleum with the addition of 2.5 g of charcoal. The decolorized and filtered solution was kept for 6 h at room temperature, and the (VIII) which crystallized was filtered off, washed with 3 × 20 ml of light petroleum, and dried at 70-80°C to give 22.07 g of (VIII), purity 97.99%, yield 44.5% on (VII) taken, or 63.1% taking into account recovered (VI). Colorless, crystalline compound, insoluble in water, sparingly soluble in light petroleum, readily soluble in the usual organic solvents, melting point 140-141°C. Found, %: C 64.72; H 8.02; Cl 10.30; N 17.25. $C_{18}H_{27}ClN_4$. Calculated, %: C 64.75; H 7.84; Cl 10.62; N 16.79.

2-(2'-Chlorostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline Diphosphate (Aminazolin, I). A mixture of 18 g of 97.99% (VIII), obtained in the preceding stage (17.65 g, 100%), 6.56 g of anhydrous sodium acetate, 22.6 g of o-chlorobenzaldehyde, and 90 ml of acetic anhydride was heated to boiling.. The temperature rose during the course of the reaction from 134 to 142°C. After boiling for 6 h, the completion of the reaction was checked by TLC, and the mixture stirred at 50-60°C and poured into 360 ml of 5% sulfuric acid. The resulting reddish suspension was treated with 9 g of charcoal, and the mixture boiled for 15 min, filtered hot, and the charcoal washed on the filter with 60 ml of boiling water. The combined filtrate and wash water was cooled to room temperature, kept for 1 h, diluted with 360 ml of acetone, and kept for 3 h at room temperature. The solid which separated [2-(2'-chlorostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline disulfate] was filtered off and washed on the filter with 3 × 50 ml of acetone, following which it was mixed with 180 ml of water and 100 ml of toluene, and basified with vigorous stirring with 55 ml of 10% sodium hydroxide to pH 9.0–10.0. The toluene layer was separated and the aqueous layer extracted with 100 ml followed by 2 × 50 ml toluene. The combined toluene solutions were washed with 2 × 50 ml of water, and evaporated in vacuo. The residue was recrystallized from 28 ml of acetone with the addition of 1.4 g of charcoal to give 10.55 g of 2-(2'-chlorostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline of purity† 98.61%. This was dissolved in 80 ml of methanol, purified with 1 g of charcoal, and treated at 50-55°C with 22.4 ml of 20% methanolic orthophosphoric acid over 10 min, completeness of separation of (I) being checked by a pH-meter (to pH 4.5–6.0). The solid was filtered off, washed with 10 ml of methanol and 3 × 10 ml of acetone, and dried at 70-80°C to constant weight. There were obtained 4.91 g of (I), mp 237-238°C, purity 99.2%, yield 42.94%. Found, %: C 45.80; H 5.69; P 9.60. $C_{25}H_{30}Cl_2N_4 \times 2H_3PO_4$. Calculated, %: C 45.96; H 5.55; P 9.48.

EXPERIMENTAL – BIOLOGICAL

Materials and Methods. Antileishmaniasis activity was examined in a model of dermal leishmaniasis in mongrel white mice developed in the All-Union Research Institute for Pharmaceutical Chemistry [6]. This model enables dermal leishmaniasis in experimental animals to be reproduced which is identical in its clinical features to the disease in man. The animals were infected intradermally in a volume of 0.05 ml in the tail root. The infective dose was $5 \cdot 10^5$-$5 \cdot 10^6$ promastigotes. The compounds were administered internally once daily for ten days in isotonic sodium chloride in a volume of 0.02 ml per 1 g body weight. In prophylactic experiments, the drug was introduced one hour before infection. In therapeutic experiments, treatment with the drug was commenced 5-10 weeks following infection. Several doses of the drug were used simultaneously in the tests, the values of which were determined from studies of their tolerance in intact mice. The indices of tolerance were changes in body weight, the survival rate over a period of 20 days, and the results of pathomorphological studies of the internal organs (liver, kidneys, myocardium, stomach, small and large intestines, pancreas, and adrenals). The duration of treatment (ten days) corresponded to the optimum plan of the chemotherapeutic experiment. Treatment of the animals was commenced at the maximum tolerated doses. The effectiveness of treatment was assessed from the extent of local leishmaniasis lesions, which are an indication of all the stages of development of the infection, the size of the leishmaniomas, data from parasitological studies of individual ulcers, and pathomorphological studies of the skin and internal organs in the treated and

†After recrystallization from cyclohexane, the mp was 149-150°C. Found, %: Cl 15.23; N 12.29; $C_{25}H_{30}Cl_2N_4$. Calculated, %: Cl 15.50; N 12.25.

control animals. Observations and calculation of results were carried out weekly. The experiments were continued for 15–25 weeks. For the quantitative comparison of the chemotherapeutic activity of the test compounds, the index of effectiveness was used, expressed as a percentage calculated from the formula $(A - B)100/A$, where A is the mean extent of local leishmaniasis lesions over the whole period of the experiment in the control mice, and B is the same factor for the treated mice. More than 1100 mice were used. The results given are statistically significant.

In its activity and toxicity, (I) was first compared with aminoquinol. The toxicity following a single dose was examined in mongrel white mice weighing 17-18 g, and white rats weighing 120-150 g. The drugs were administered internally and intraperitoneally, and the condition of the animals assessed over seven days. The LD_{50} values were calculated by the method of Litchfield and Wilcoxon.

Chronic toxicity was determined in white rats of both sexes. The drugs were administered internally, daily, seven times per week in doses of 10, 50, and 200 mg/kg for 1, 1.5, and 2 months. When administered for two months, there was a gap of one week between the first and second months. 420 rats were used. The state of the animals was assessed from physiological, biochemical, histological, and histochemical studies. The results were treated statistically [9]. The differences in the values compared were significant at the $P = 0.05$ level.

Results of Studies. 1. Chemotherapeutic Activity. Following ten doses of 200 mg/kg internally, (I) showed high chemotherapeutic activity, suppressing the development of leishmaniasis in mice by 44% (Table 1). When treated with (I) in doses of 300, 400, 500, 600, 700, and 800 mg/kg under the same experimental conditions, in most of the animals leishmaniasis did not develop, and in the remaining animals development was less rapid than in then in the controls.

In contrast to aminoquinol, the chemotherapeutic effectiveness of (I) increased with increasing doses.

In a dose of 200 mg/kg, (I) and aminoquinol on administration to infected animals have equal chemotherapeutic effects, suppressing the development of leishmaniasis by 47-44%. As the dose was increased, the superiority of (I) became apparent. At 300 mg/kg, (I) had a greater antileishmaniasis effect than aminoquinol in the same dose. The effectiveness indices were 56 and 48%, respectively. At doses of 400-800 mg/kg, the values of the index increased. The greatest chemotherapeutic effect (66%) was achieved in the groups of mice treated with (I) in a dose of 600 mg/kg. The effectiveness of aminoquinol at a dose of 400 mg/kg decreased, apparently as a result of the toxicity of the drug. It was not possible to determine the chemotherapeutic activity of aminoquinol in doses of 500-800 mg/kg, since these doses resulted in the early deaths of the animals.

Studies of the therapeutic effectiveness of (I) in comparison with aminoquinol also showed the superiority of (I) (Table 2). It was found that (I) on administration to mice five and ten weeks after infection in doses of 200, 350, 500, 600, and 750 mg/kg had antileishmaniasis activity, as shown by a reduction in the extent of leishmaniasis lesions as compared with the controls, and a decrease in the numbers of causative agents from the skin lesions and healing of the ulcers.

Under the same experimental conditions, aminoquinol had no antileishmaniasis activity, and was therapeutically ineffective.

Data from a comparative study of the antileishmaniasis activity of (I), aminoquinol, and glucanthim, which is used extensively abroad as a 33% solution of Sb^{5+} and N-methylglucamic acid in the extended parenteral treatment by intramuscular injection of patients for the most part suffering from visceral leishmaniasis, are given in Table 3.

2. Toxicity. The results of a study of the toxicity of (I) in comparison with aminoquinol following a single administration are given in Table 4. The acute intoxication pattern was similar in mice and rats, with flaccidity and delayed reactions, some individuals displaying whole-body tremor. In rats, irritation of the mucous membranes of the nose and mouth was apparent, manifesting itself as hypersalivation following internal administration of the drug, the animals buried their noses in the sawdust, and inflammation of the mucous membranes of the nose and mouth developed. In animals which died following intraperitoneal administration of the drug, hyperemia of the peritoneum was seen, and in some cases hemorrhagic exudate was present in the peritoneal cavity. Deaths of the animals commenced within four days following administration of the drugs. By the intraperitoneal route, (I)

TABLE 1. Results of Comparative Studies of Chemotherapeutic Activity of (I) and Aminoquinol in Experimental Dermal Leishmaniasis in White Mice (by the internal route)

Therapeutic dose, mg/kg·10	Index of effectiveness of (I), %	Assessment of activity	Index of effectiveness of aminoquinol	Assessment of activity
200	44	Moderate	47	Moderate
300	56	High	48	»
400	55	»	35	Low
500	50	»	—	Toxic
600	66	»	—	—
700	61	»	—	—
800	61	»	—	—

TABLE 2. Results of a Study of the Effectiveness of Oral Administration of (I) in Developed Dermal Leishmaniasis in White Mice (arbitrary units)

Dose, mg/kg	Mean extent of local lesions 6]	Index of effectiveness, %
Treatment commenced after 5 weeks		
200	1,1±0,3	39
350	0,7±0,3	61
500	0,8±0,3	56
Treatment commenced after 10 weeks		
600	1,3±0,2	70
750	1,9±0,3	57

TABLE 3. Results of a Comparative Study of the Chemotherapeutic Activity of (I), Aminoquinol, and Glucoxanthim in Experimental Dermal Leishmaniasis in White Mice

Drug	Dose, mg/kg	Route of administration	Index of effectiveness, %
I	20	Internal	40
	40	»	77
Aminoquinol	20	»	40
	40	»	Toxic
Glucoxanthim	40	»	70
	40	Subcutaneous	70

TABLE 4. Toxicity of (I) and Aminoquinol following a Single Administration to Mice and Rats

Animal species	Mode of administration	LD_{50}, mg/kg	
		I	Aminoquinol
Mice	Internal	1200 (1090,9—1320,0)	1350 (1088,7—1674)
	Intraperitoneal	130 (106,56—158,6)	78 (64,24—90,48)
Rats	Internal	3900 (3420—4450)	3800 (2753,6—5244)
	Intraperitoneal	450 (387,9—522,0)	190 (135,7—266,0)

TABLE 5. Results of a Comparative Study of the Tolerance of (I) and Aminoquinol following Tenfold Administration Internally to Intact Mice

Dose, mg/kg	% deaths of animals by day 20	Changes in mean body weight of animals, g		
		day day 1	day 20	difference (M ± m)
		Aminozolin		
200	0	18,8	23,8	+5,0±0,5
300	0	18,2	20,7	+2,5±0,4
400	0	18,4	23,0	+4,6±0,5
500	0	17,9	20,9	+3,0±0,8
600	0	18,2	20,8	+2,6±0,6
700	0	19,4	21,4	+2,0±0,4
800	20	19,9	22,8	+2,9±0,8
		Aminoquinol		
200	0	19,2	21,8	+2,6±0,6
300	0	19,3	20,5	+1,2±0,9
400	10	18,1	16,7	−1,4±0,8
500	40	18,5	16,0	−2,5±0,6
600	80	19,0	15,0	−4,0±1,0
700	90	18,9	13,0	−6,9±0
800	70	19,0	15,7	−3,3±0

Note. Here and in Table 6, the number of mice in each group was 10.

TABLE 6. Results of a Comparative Study of the Tolerance of (I) and Aminoquinol in Mice from Histological Studies (extent of liver damage)

Dose, mg/kg	Number of doses	Damage index	
		I	Aminoquinol
200	10	0	0,3
300	10	0,2	2,8
400	10	0,3	3,2
500	10	0,5	4,0
600	10	0,5	4,0
700	10	0,5	4,0
800	7 Aminoquinol 8 I	0,8	4,0

was much less toxic than aminoquinol, whereas by the oral route it was of the same level. Mice were more sensitive than rats.

Tenfold administration of (I) internally to intact animals in doses of 200-700 mg/kg was well tolerated. Treatment with (I) in a daily dose of 800 mg/kg resulted in the deaths of individual mice from day 8 of treatment. The mean mass of the animals following administration of (I) increased by 3.2 g over the initial value by the end of the experiment (Table 5).

Aminoquinol was much less well tolerated by the experimental animals. It was toxic in doses of 400, 500, 600, 700, and 800 mg/kg, resulting in the deaths of the animals. The greatest percentage mortality was found at doses of 600, 700, and 800 mg/kg (from 70 to 90). Unlike (I), aminoquinol gave rise to a statistically significant decrease in the weights of the experimental animals as compared with the original values (Table 5).

The findings from pathomorphological studies of the internal organs of mice receiving (I) and aminoquinol were as follows. In macroscopic studies, treatment with (I) in doses of 200-800 mg/kg did not give rise to any deviations from normal, and in microscopic studies of the myocardium, lungs, and spleen of mice which had received (I) in doses of 200-800 mg/kg no changes were found. In some of the animals, following treatment with (I) in doses of 500-800 mg/kg, slight protein dystrophy of the liver was seen (Table 6).

The liver damage index in mice receiving (I) averaged 0.5-0.8.

In mice receiving aminoquinol in doses of 500,600, 700, and 800 mg/kg, the liver showed acute protein dystrophy accompanied by focal necroses of the parenchyma of varying dimensions, namely from miliary at a dose of 300 mg/kg to total necrosis of all lobes of the liver at 500-800 mg/kg.

Accordingly, the liver damage index in mice receiving aminoquinol in doses of 500-800 mg/kg was 4 (i.e., 5-8 times greater than that in mice treated with (I) in the same doses).

The following scale was used to assess the extent of liver protein dystrophy: 0 — no pathomorphological changes; 0.5 — occasional slight signs of protein dystrophy; 1 — constant signs of protein dystrophy, occasionally with disruption of the duct structure; 2 — pronounced protein dystrophy; 3 — in addition to acute protein dystrophy, small areas of dead liver parenchyma; 4 — focal necroses large, running together, frequently distributed over the whole lobe of the liver. The average damage index for each group of animals was calculated by adding the degrees of liver damage in the group of animals and dividing by the number of animals in the group.

The choice of the liver as the subject for pathomorphological studies in this instance was due to the fact that the liver is the most frequently damaged organ.

Comparative studies of the pathomorphology of the internal organs of mice receiving (I) or aminoquinol daily for ten days in the same doses have thus shown that under these experimental conditions (I) is tolerated by the animals much better than is aminoquinol. In doses of up to 400 mg/kg, (I) does not give rise to any pathomorphological changes, and doses of 400-800 mg/kg cause only slight protein dystrophy in the liver. However, aminoquinol in doses of greater than 200 mg/kg invariably results in the development of dry necrosis of the floor of the auricle, disappearance of fatty tissue, hypoplasia of the thymus gland, and acute protein dystrophy followed by the development of generalized necrosis in the liver.

In rats, treatment with (I) in a dose of 10 mg/kg for two months, and a dose of 50 mg/kg for one month had no deleterious effects.

Administration of the drug in a dose of 50 mg/kg for more than one month resulted in the development of fatty dystrophy in the liver. Under similar experimental conditions, aminoquinol gave rise, in addition to signs of fatty dystrophy, to focal necrosis in the animals of this group.

In a dose of 200 mg/kg, (I) showed toxic effects after treatment for one month. The liver showed hypertrophy of the Kupffer cells, and granular dystrophy developed at the periphery of the liver lobes. In the same dose, aminoquinol gave rise to changes not only in the liver, but also in other organs. The masses of the thymus and spleen were reduced, and the kidneys showed vacuolization of the epithelium of the spiral canals, and an increase in the numbers of protein bodies in their lumina.

At a dose of 200 mg/kg of (I) for 1.5 months, some of the animals died, increases in body weight were retarded, and some biochemical indices were affected. More extensive changes occurred in the liver, but in contrast to aminoquinol, no tissue necrosis was seen. Engorgement of the other parenchymatous organs was seen. During this period, 35% of the animals receiving aminoquinol died. On dissection, the liver showed focal necrosis, and the changes in the other organs were more pronounced than following treatment with (I).

Administration of (I) in a dose of 200 mg/kg for two months resulted in the development of more extensive pathological processes in various organs. In the liver, in addition to fatty dystrophy, protein dystrophy was also seen, and is the kidneys vacuolization and desquamation of the epithelium of the spiral canals was noted, with large numbers of protein bodies in their lumina, and granular cylinders were present. The vacuoles of the glomerular podocytes were markedly increased in size, and they occasionally fused. Stasis was present in some glomerules. In rats receiving aminoquinol under the same experimental conditions, in addition to the changes described above, there was a statistically significant increase in the weights of the liver and adrenals, and a decrease in the weight of the thymus. On dissection of the animals, the liver showed grayish-yellow regions measuring from 1 to 3 mm, which were found on histological examination to be necrotic foci surrounded by a zone of perifocal inflammation. In the adrenal glands, the cortex was considerably enlarged, with some narrowing of the glomerular zone. The medulla was hypertrophied, engorged, and the venous sinuses were considerably distended. In the fascicular zone there were many pale,

swollen cells, some cells being dystrophic. The reticular zone of the cortex was hypertrophied and engorged, and its appearance affected. On staining with Sudan, a considerable decrease in the number of lipid inclusions in the arenal cortex was observed.

It is noteworthy that in the group of animals receiving (I) and aminoquinol for two months with a week's interval between the first and second months, no animals died, showing that it is desirable that the drugs be used in courses of treatment with an interval between them. The pathological changes resulting from treatment with (I) or aminoquinol in high doses are reversible, and regenerative processes were seen in all the internal organs one month after cessation of treatment.

These toxicological studies of (I) and aminoquinol have thus given the following results:

1) (I) is well tolerated following a single dose. It is much less toxic than aminoquinol by the intraperitoneal route, and of equal toxicity by the oral route.

2) (I) and aminoquinol, on administration to rats in a dose of 10 mg/kg for two months, and a dose of 50 mg/kg for one month, did not give rise to toxic effects in the animals.

3) differences in the toxicity of (I) and aminoquinol were seen on extended (1.5-2 months) treatment with the drugs in high doses (50-200 mg/kg).

As compared with (I), aminoquinol gives rise to much more pronounced pathomorphological changes, particularly in the liver.

According to the Laboratory of Bacteriology, VONTs, Academy of Medical Sciences of the USSR (Yu. D. Tolcheev), *in vitro* (I) did not give rise to any mutations from auxotrophic to prototrophic in the synthesis of histidine by cells of strains of *Salmonella typhimurium* TA 98 and TA 100, and it was not activated by the nonspecific oxidase system of rat liver to products which were mutagenic with respect to these strains.

V. N. Chernov and S. M. Minakova (All-Union Research Institute for Pharmaceutical Chemistry) have arrived at the conclusion that (I) and aminoquinol in doses of 10 mg/kg do not give rise to any changes in uterine mass in ovariectomized rats as compared with the controls. In a dose of 100 mg/kg, (I) caused a significant decrease in uterine mass, aminoquinol was without effect, and phosphestrol, administrated internally in a dose of 10 mg/kg, significantly increased the uterine mass, by a factor of approximately three, indicating that in these experiments (I) and aminoquinol did not display any estrogenic activity.

Workers of the Kupavna branch of the Laboratory of Drug Toxicology of the All-Union Research Institute for Pharmaceutical Chemistry (L. F. Shashkina, V. M. Ivanova, and A. V. Morozov) have found that (I) gives rise to slight allergenization in guinea pigs.

LITERATURE CITED

1. G. P. Zhikhareva, N. Yu. Moskalenko, L. I. Mastafanova, et al., Khim.-farm. Zh., No. 6, 40-42 (1980).
2. G. P. Zhikhareva, N. Yu. Moskalenko, G. Ya. Shvarts, et al., Khim.-farm. Zh., No. 12, 1469-1477 (1984).
3. I. A. Kassirskii, N. N. Plotnikov, Yu. N. Tokarev, and A. Ya. Lysenko, Manual of Tropical Diseases [in Russian], Moscow (1974), pp. 149-151.
4. M. D. Mashkovskii, Drugs (9th edn.), Moscow (1984), Vols. 1-2.
5. N. Yu. Moskalenko, L. N. Yakhontov, G. P. Zhikhareva, et al., Khim.-farm. Zh., No. 4, 437-446 (1986).
6. G. N. Pershin and N. Yu. Moskalenko, Med. Parazitol., 31, No. 6, 727-733 (1962).
7. N. F. Rodyakin, in: Dermal Leishmaniasis [in Russian], Ashkhabad (1982).
8. M. V. Rubtsov and A. G. Baichikov, Synthetic Pharmaceutical Preparations [in Russian], Moscow (1971), pp. 220-221.
9. N. N. Samsonov, Tables of Values of Mean Errors and Confidence Limits for Mean Arithmetical Values of a Variational Series [in Russian], Tomsk (1970).
10. D. N. Chistyakov, N. A. Drabkina, and L. V. Rusan, Med. Parazitol., 60, No. 4, 57-68 (1982).
11. E. P. Shuvalova, Tropical Diseases [in Russian], Moscow (1979).
12. A. Buzas and G. Hoffman, Bull. Soc. Chim. Fr., 1889-1891 (1959).
13. C. J. Marinkelle, Bull. World Health Org., 58, 807-818 (1980).
14. J. L. Pace and M. D. Takun, Arch. Derm., 118, 880 (1982).
15. W. Peters, E. R. Trotter, and B. L. Robinson, Ann. Trop. Med. Parasitol., 74, 289-298 (1980).

16. Q. M. Saucier, B. Festy, and G. B. Le Pecq, Biochimie, 53, 973-980 (1971).
17. H. C. Scarborough, B. C. Lawes, J. L. Minielli, and J. L. Compton, J. Org. Chem., 27, 957-961 (1962).
18. World Health Org. Spec. Program for Research and Training in Tropical Diseases, 1, (1976).

SYNTHESIS AND BIOLOGICAL ACTIVITY OF CERTAIN PHENYLAZOSELENAZOLES

Z. I. Popova, A. A. Tsurkan, and P. N. Steblyuk

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We have previously obtained azo products [2-4] that exhibit antibacterial and antifungal activity by the azo coupling of aromatic amines and sulfanilamide preparations with 2-arylidenhydrazino-4-phenylselenazoles [2]. As a continuation of our studies we synthesized compounds I-XXX (see Table 1). The diazo components used to obtain those compounds were aniline, p-phenetidine, sulfanilic acid, sodium p-aminosalicylate, benzocaine, novocaine, and the following sulfanilamides: Streptocid, Sulfacyl, Urosulfan, sulfaguanidine, Norsulfasol, Etasol, and Sulfadimezine.

The antimicrobial activity of the synthesized compounds was tested on five strains of bacteria and fungi: *Staphylococcus aureus, E. coli, B. pyocyaneus, B. anthracoides,* and *Candida albicans*. The test results are presented in Table 1.

The products formed by azo coupling with amines were found to be more active against staphylococci (compound X) than against the other microorganisms. Compounds XI and XIV were more active against *Candida albicans*. The most active compound of this group was the coupling product of 2-(4-methoxybenzylidene) hydrazino-4-phenylselenazole and diazotized sulfanilic acid (compound VI) which suppressed *B. anthracoides* growth at a dilution of 1:16,000 and *Candida albicans* at 1:64,000. Azocoupling with benzocaine and novocaine resulted, as expected, in compounds IX-XIV which did not exhibit any significant antimicrobial activity. The test results for 2-arylidenhydrazino-4-phenyl-5-p-R-sulfamoylphenylazoselenazole demonstrated activity against both staphylococci and *Candida albicans*.

The coupling product of diazotized urosulfan (compound XVIII) exhibited the greatest activity against *B. anthracoides*.

Thus, the results of the study demonstrated that the tested compounds characteristically exhibit antibacterial and antifungal activity.

EXPERIMENTAL — CHEMICAL

2-(4-Methoxybenzylydenhydrazino)-4-phenyl-5-p-phenylazoselenazole (II). A 1-ml (0.01 mole) portion of freshly distilled aniline was dissolved while stirring in 10 ml of HCl (1:3). The solution was placed in an ice bath. After the solution was cooled to 0°C a solution of 0.69 g (0.01 mole) of sodium nitrite in 4 ml of water was added dropwise to the mixture. A 4.4-g (0.01 mole) portion of 2-(4-methoxybenzylydenhydrazino)-4- phenylselenazole was dissolved upon heating in 40 ml of ethanol. After cooling, diazotized aniline was added to the resultant solution as the temperature was maintained at 0-5°C. This resulted in the formation of thick crimson-red colored paste. Yield 1.5 g (78%). Red plates, mp 204-206°C (from ethanol). Found, %: N 15.29; Se 15.55. $C_{23}H_{19}N_5OSe$. Calculated, %: N 15.21; Se 15.59. Compounds I, III-XXX were obtained in a similar fashion.

EXPERIMENTAL — BIOLOGICAL

The bacteriostatic and mycostatic activity of the synthesized compounds was examined by the generally accepted method [1] of series dilutions in a liquid nutrient. An aminopeptide, preliminarily diluted with water at a pH of 7.2, was used as the nutrient medium. The testing of a compound's activity against the indicated test microbes consisted of broad spectrum activity tests, beginning with a dilution of 1:2000 (500 μg/ml), on five strains of the fol-

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