Introduction of a longer aliphatic chain and a second acetylenic group (compound V) had little effect on antiinflammatory activity, but decreased analgesic activity in both types of pain test.

It is noteworthy that the carboxyl-analog of (II), undecynoic acid, has a similar antiexudative and analgesic effect in the chemical test, whereas with chemical stimulation this compound has analgesic activity.

Antiexudative activity therefore requires the presence of an acetylenic and an NHOH group, and decreased activity consequent upon introduction of an alkyl radical may be compensated for by an additional acetylenic group.

The presence of an acetylenic group is also essential for the possession of analgesic activity in respect of chemical stimulus. Unlike antiexudative activity, analgesic activity is shown by both the free acids and their salts, the nature of the cation having a considerable influence on the magnitude of this activity.

These findings may be of interest in designing antiinflammatory mono- and diacetylenic hydroxamic acids.

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SYNTHESIS AND ANTIMALARIAL ACTIVITY OF 2-STYRYL-4-(δ-DIETHYLAMINO-α-METHYLBUTYLAMINO)-7-CHLOROQUINAZOLINES

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The search for new antimalarial drugs has not lost its urgency, in view of the need for backup drugs which are active when resistance to those in current use develops, or which potentiate their effects, are better tolerated, or possess other useful properties.

Previous reports [2-7, 12, 13] have described the antiprotozoal activity of a novel class of chemotherapeutants, namely 4-aminostyrylquinazolines. Developing our earlier work on the antimalarial activity of 4-amino-2-styrylquinazolines [7], we examined in greater detail the activity of some compounds of this type in a model infection of mice with *Plasmodium berghei*. These studies showed that the compounds of greatest interest were the dihydrochlorides of 2-(4′-bromostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline (I), 2-(2′,4′-

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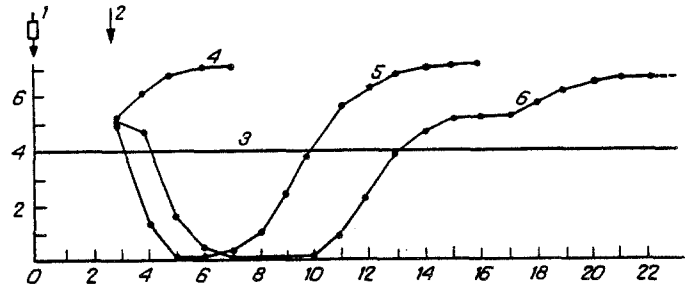


Fig. 1

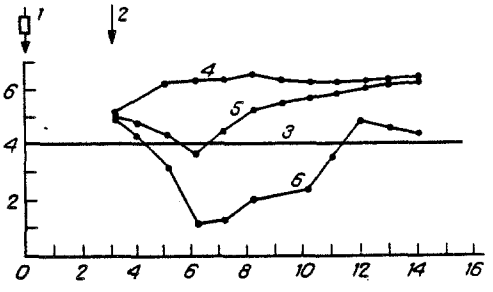


Fig. 2

Fig. 1. Activity of (I) in comparison with chloroquin against the drug-sensitive isolate N. 1) Infection of animals; 2) treatment with drug; 3) arbitrary level of parasitemia (four points); 4) control; 5) chloroquin, 50 mg/kg; 6) compound (I), 60 mg/kg.

Fig. 2. Activity of (I) in comparison with chloroquin against chloroquin-resistant isolate LNK65. 1) Infection of animals; 2) treatment with compounds; 3) arbitrary level of parasitemia (four points); 4) control; 5) chloroquin, 150 mg/kg; 6) compound (I), 50 mg/kg.

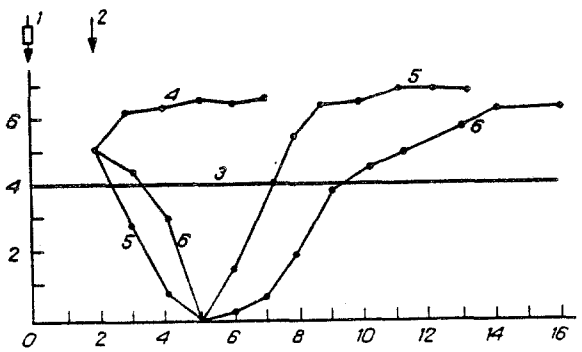


Fig. 3

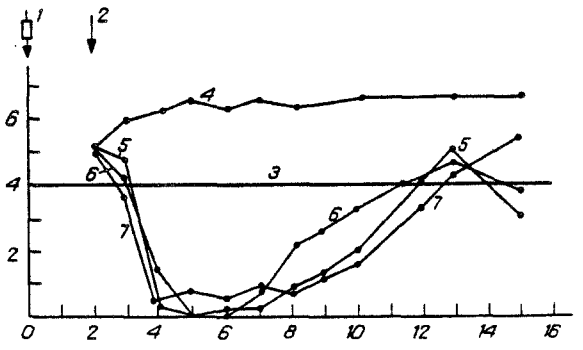


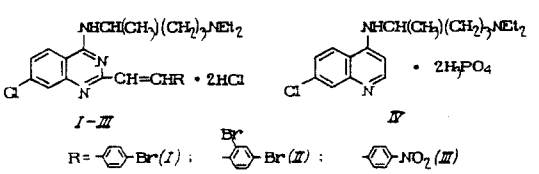
Fig. 4

Fig. 3. Activity of (I) in comparison with chloroquin against the fansidar-resistant isolate FRLNK65. 1) Infection of animals; 2) treatment with compounds; 3) arbitrary level of parasitemia (four points); 4) control; 5) chloroquin, 50 mg/kg; 6) (I), 50 mg/kg.

Fig. 4. Effect of (I) in combinations. 1) Infection of animals; 2) treatment with compounds; 3) arbitrary level of parasitemia (four points); 4) control; 5) (I), 50 mg/kg + sulfalen, 0.5 mg/kg + pyrimethamine, 0.025 mg/kg; 6) chloroquin, 150 mg/kg + sulfalen, 0.5 mg/kg + pyrimethamine, 0.025 mg/kg; 7) (I), 50 mg/kg + sulfalen, 0.5 mg/kg.

dibromostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline (II), and 2-(4′-nitrostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline (III), which were superior to varying degrees in their breadth of therapeutic activity to chloroquin (Delagid), 4-(δ-diethylamino-α-methylbutylamino)-7-chloroquionoline diphosphate (IV) used as a standard.

We here describe the synthesis and chemotherapeutic properties of the most promising of the styrylquinazoline series, (I) dihydrochloride.



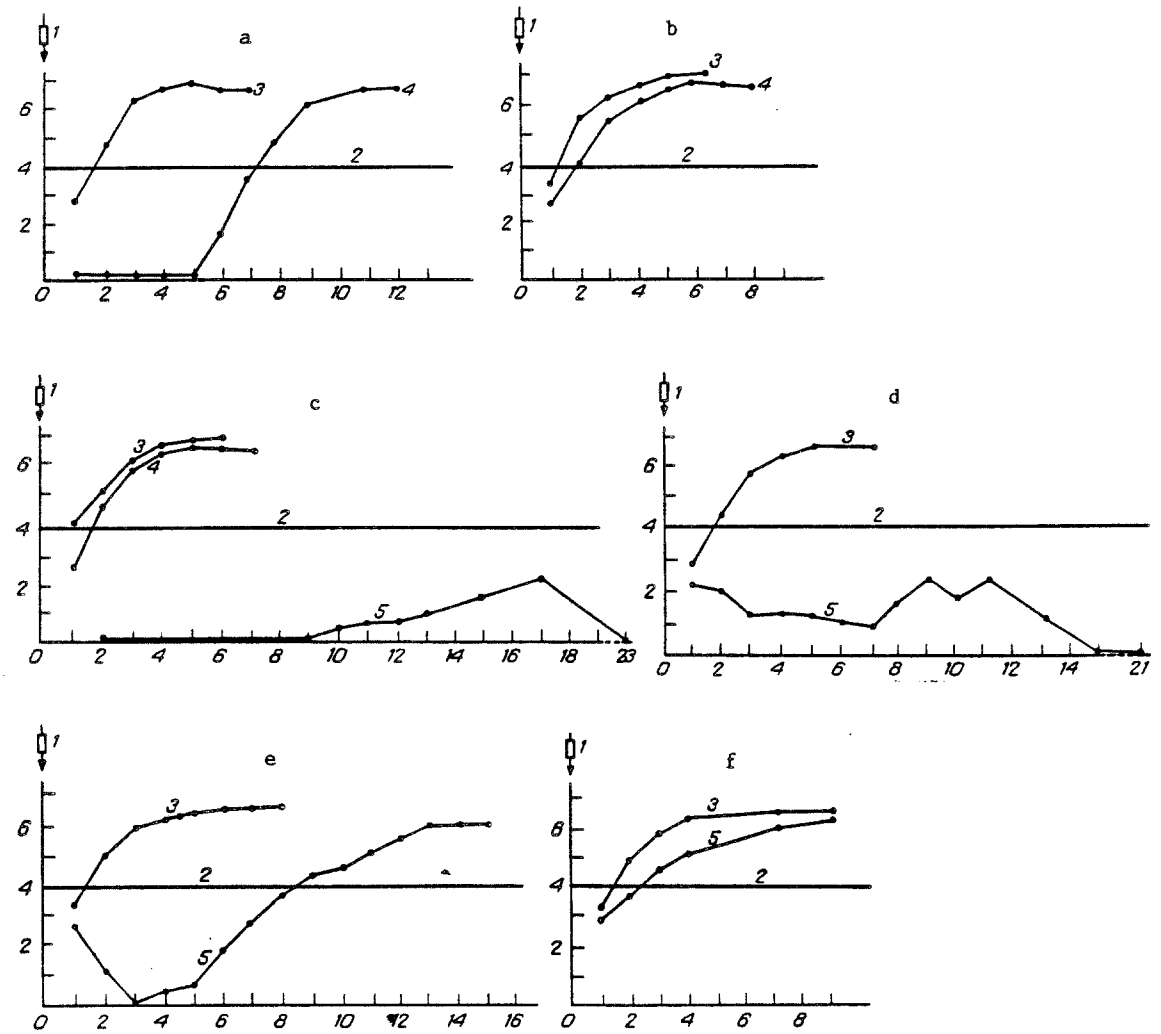
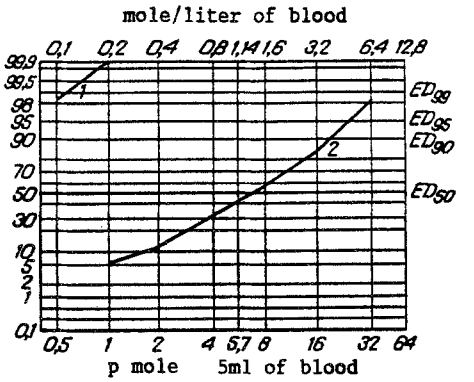


Fig. 5, a-f. Protectant effects of (I). 1) Infection of animals; 2) arbitrary level of parasitemia (four points); 3) control; 4) chloroquin, 150 mg/kg; 5) (I), 150 mg/kg. a) Infected one day following administration of compound; b) ditto after two days; c) ditto after three days; d) ditto after five days; e) ditto after seven days; f) ditto after 11 days.

This is obtained by a method similar to that described in the literature [3, 12], by condensing 4-(δ-diethylamino-α-methylbutylamino)-2-methyl-7-chloroquinazoline (V) with 4-bromobenzaldehyde (VI) in acetic anhydride in the presence of anhydrous sodium acetate.

The antimalarial activity of (I) was examined in various experimental modes bering in mind the aims of using specific compounds in the chemotherapy of malaria.

Activity against asexual erythrocytic forms was assessed *in vivo* against malaria in rodents induced by a range of isolates of *Plasmodium berghei* differing in their sensitivity to the most commonly used drugs, and *in vitro* against chloroquin-resistant isolates of *P. falciparum*. In addition, activity against pre-erythrocytic forms was examined in a test system with *P. berghei*, ANKA isolate, hamsters — *Anopheles stephensi*. Apart from the activity of (I) per se, of particular interest is its activity in conjunction with other antimalarial drugs as potentially more effective drugs for the treatment of drug-resistant plasmodia.



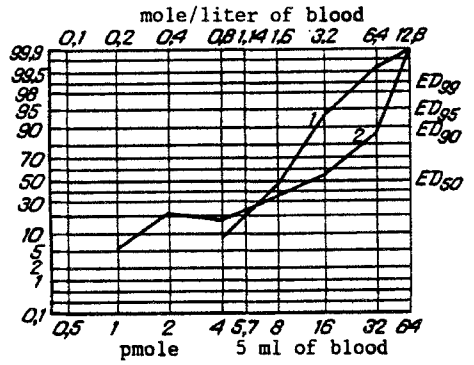


Fig. 6. Activity of (I) in comparison with chloroquin *in vitro* against *Plasmodium falciparum*, drug-resistant isolate from southern Vietnam. (a) 1987; b) 1988. 1) Compound (I); 2) chloroquin.

Activity *in vivo* against *P. berghei*

Therapeutic Tests. Drug-sensitive Isolate N (Fig. 1). The activity of (I) in a dose of 60 mg/kg was greater than that of (IV) in a dose of 50 mg/kg in respect of the duration of the period of remission and the severity of parasitemia during recurrence. The much greater tolerance of (I) as compared with (IV) (the maximum nonlethal dose, MND, was 3286.4 mg/kg as compared with 192.6 mg/kg) was due to its high chemotherapeutic index (CTI) of 14.3, more than ten times greater than that of (IV) (taken as unity). This indicates the considerable breadth of therapeutic effectiveness of (I), and the possibility of increasing the dosage if necessary.

Isolate LNK65, with Naturally Reduced Sensitivity to Chloroquin (Fig. 2). In a dose of (I) one third that of chloroquin (50 and 150 mg/kg respectively), it showed greater activity, as shown by the suppressive effect on the severity of parasitemia. However, remission (arbitrary effect) did not occur, indicating the occurrence of a degree of cross-resistance of (I) and chloroquin.

Isolate FR LNK65, a Strain of LNK65 with Acquired Resistance to Fansidar (a combination of sulfadioxin and pyrimethamine) (Fig. 3). In a dose the same as that of chloroquin (50 mg/kg), (I) was somewhat superior, as shown by the severity of parasitemia. After the numbers of parasites had fallen to submicroscopic levels, treatment with (I) resulted in the less rapid development of parasitemia.

Activity in Combination. Isolate LNK65 (Fig. 4). Like (IV), (I) had a potentiating effect on a combination of sulfalen (kelfizin) and tindurin (pyrimethamine, chloroquin) in a dose of one third that of (IV) (50 and 150 mg/kg respectively). The potentiating effect of (I) was also apparent in a more "economic" double combination with sulfalen, in which it essentially compensated for the activity of pyrimethamine when compared with the triple combination, and their activity coefficients (AC) were similar at 6.22 and 6.05 arb. units.

Prophylactic (Protectant) Test. Isolate N (Fig. 5, a-f). Following single doses (150 mg/kg of each) of (I) and (IV) to mice and infection of successive groups at intervals of 24 h over 12 days, the effects of chloroquin ceased after

the first few days, and during this time it only limited the rate of development of the infection. The protectant effects of (I), which were apparent to varying extents, were evident for 11 days from the time of treatment. Development of infection was completely prevented in all the animals infected within 1-2 days, and in some of the animals infected 3-5 days after treatment. In the infected mice, the effects were shown by retardation of the rate of development of the infection. These findings show that (I) is markedly superior in the retention of specific activity in the animal body.

Activity against Pre-erythrocytic Forms

ANKA Isolate, Guinea Pigs, *Anopheles stephensi*. When (I) was given once or twice 24-2 h before infection by means of a bite by *A. stephensi*, or 24 h following infection (the time required for the development of pre-erythrocytic forms of *P. berghei* in the liver is 48 h), the erythrocytic form of the infection failed to develop. Since the activity of (I) is known to persist for relatively extended periods in the body, the observed activity could be due not only to a direct effect on the pre-erythrocytic forms, but also to effects on the erythrocytic forms at the very earliest stage of their development. In order to exclude this possibility, it was necessary to examine histologically sections of the liver following treatment with (I) during the period of development of pre-erythrocytic forms.

Activity *in vitro* on *P. falciparum*

WHO Micro-test (Fig. 6, a-b). In studies carried out in 1987, 100% inhibition of the development of schizonts was obtained at a concentration of (I) of $0.02 \cdot 10^{-6}$ mole/liter, whereas a concentration of (IV) of $6.4 \cdot 10^{-6}$ mole/liter gave only 98.27% inhibition. In 1988, however, the inhibitory effect of (I) fell, becoming comparable with that of chloroquin, perhaps as a result of increased resistance of *P. falciparum* to chloroquin, although the limiting level of resistance at this time was not definitively established.

EXPERIMENTAL (CHEMISTRY)

Dihydrochloride 2-(4′-Bromostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinazoline (I). A mixture of 6.9 g of 2-methyl-4-(δ-diethylamino-α-butylamino)-7-chloroquinazoline [12], 11.5 g of p-bromobenzaldehyde, 2.6 g of anhydrous sodium acetate, and 40 ml of acetic anhydride was boiled with stirring, poured into 5% HCl, and heated to 85°C. After cooling, a further 40 ml of conc. HCl was added, and nonbasic material extracted with ether. The hydrochloric acid solution was basified with potassium carbonate and evaporated to dryness. The residue (9.4 g) was dissolved in 100 ml of methanol, and acidified to Congo Red with phosphoric acid. The (I) diphosphate was further purified by reconversion to the base, extraction with benzene, and recrystallization from heptane. There was obtained 4.5 g of the free base (I), mp 121-122°C [7], which on treatment with an acetone solution of alcoholic HCl to pH 3 gave the dihydrochloride (I) with mp 277-278°C [7].

EXPERIMENTAL (BIOLOGY)

The chemotherapeutic activity was assessed in a basic series of tests using mongrel white mice of both sexes weighing 14-18 g, infected with blood. The donors were mice with developing infections and a blood parasite level of around 750 thousand/μl. Citrated blood was diluted with physiological saline to a parasite level of 1.5 million per 0.1 ml, and administered intraperitoneally to the recipients, each receiving 0.1 ml of this solution. In separate series of tests, infection was transmitted via the agent *Anopheles stephensi*. Various isolates of *P. berghei* were used. ***Isolate N***, of normal drug sensitivity, was obtained in 1961 from the Gdansk Institute of Marine Medicine (Polish National Republic), and maintained in mice by blood transfusions into the peritoneum. It gave rise to acute, lethal infections. ***Isolate LNK65***, with naturally reduced sensitivity to chloroquin (6-7 times less sensitive than strain N), was obtained from the Liverpool School of Tropical Medicine in 1974, and maintained by blood transfusions into the peritoneum of 2-3 week old hamsters. It was less virulent in mice, acute infections being more prolonged, and occasionally resulting in spontaneous recovery. ***Isolate FRLNK65***, with acquired resistance to fansidar, was obtained by the remission method [11] from isolate LNK65. It was maintained in hamsters, giving rise to acute, lethal infections. The sensitivity to fansidar was reduced by a factor of at least 30. ***Isolate ANKA***, with weak natural resistance to chloroquin (1.5-2 times more resistant than strain N) was obtained in 1983 from the London School of Hygiene and Tropical Medicine, and maintained in full cycles in golden hamsters, infections being acute. It retained its ability to form gamonts.

Anopheles stephensi isolate BEECH, isolated in nature in India in 1947, and was obtained from Britain in 1983.

The test compounds were administered in accordance with the body weight of the animals via a probe into the stomach, water-soluble compounds as solutions in distilled water, and insoluble compounds as suspensions in 1% starch mucilage. The doses of the compounds were calculated on the free bases.

Antimalarial activity against asexual erythrocytic forms of the parasite was examined in therapeutic and prophylactic tests. In the therapeutic tests, the test compounds were given in a single dose when severe parasitemia was present. The blood was examined daily from the day of administration of the compound until the death of the animal or until infection abated. The effect of (I) was compared arbitrarily with that of a standard dose of the reference drug (chloroquin), chosen so as to cause only a temporary disappearance of parasites from the peripheral blood, followed by parasitic recurrence. This procedure enabled comparative results to be obtained as between the isolate N and that resistant to chloroquin. The breadth of the therapeutic effects was assessed by the chemotherapeutic index (CI) [9], using five criteria for the effectiveness of the compounds: 1) changes in the level of parasitemia on the day following treatment with the test compound (in points); 2) the time of disappearance of parasites from the blood (days); 3) the duration of remission (total absence of parasites from the blood) (in days); 4) the time required to reach an arbitrary level of parasitemia (four points on the Moshkovskii scale) in parasitic recurrence (in days); and 5) the time to death (in days). The parasite counts were obtained in thin blood smears using the scale of Moshkovskii [10].

In the therapeutic tests, the effectiveness of (I) was also assessed in combination with sulfalen and tindurin, as compared with combinations containing chloroquin. The type of combinatory effect [8] was determined using a special index of effectiveness [1].

In the prophylactic tests (study of the duration of maintenance of the drug in an active state), the test compounds were given in single, equal doses, following which successive groups of mice were infected with blood at intervals of 24 h. The criteria used were: 1) the number of protected (uninfected) mice; 2) the time to the appearance of parasites in infected mice (in days); 3) the time required to reach a given level of parasitemia (four points) in infected mice (in days); and 4) time to death (days). Prevention of infection was indicated by negative isodiagnostic tests (administration of the blood of the test animals to healthy individuals, followed by periodic examinations of the blood for 2-3 weeks).

These studies lead to the following conclusions.

In malaria of rodents caused by *P. berghei*, compound (I) showed a number of advantages over one of the main antimalarial drugs, chloroquin, viz.:

its breadth of chemotherapeutic activity, which enables the dose range to be extended if necessary;

its activity against an isolate with resistance to chloroquin, which can be overcome by increasing the dose;

its activity against an isolate with combined resistance to chloroquin and fansidar, in a dose no greater than that required for the drug-sensitive isolate, showing the absence of any significant cross-resistance between styrylquinazolines and antifolic drugs, such as is found in quinazolines (precursors of styrylquinazolines);

the duration of persistence of activity in the body (protectant activity);

its combined effectiveness in triple and double mixtures (in conjunction with sulfalen and tindurin, and with sulfalen).

Thus, compound (I), a styrylquinazoline, is of interest as a backup antimalarial drug.

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF 2-ANILINOMETHYL DERIVATIVES OF 5-HYDROXYINDOLE

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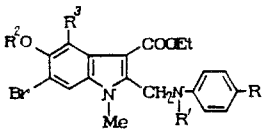
It was demonstrated earlier that 2-alkylaminomethyl derivatives of 5-acetoxy (hydroxy)indoles possessed antiviral activity [1, 2]. However, the literature holds not information on 2-arylaminomethyl derivatives of indole. In this connection it was of interest to study the antiviral activity of structures of compounds such as 2-anilinomethyl derivatives of 5-acetoxy (hydroxy)indole, the syntheses of which were brought about by alkylation of a substituted aniline with the bromomethyl derivative of 5-acetoxyindole (I), obtained earlier [2]. The condensation proceeded in benzene, both triethylamine and an excess of one of the reaction components, the corresponding aniline, may be used as acceptor of the HBr produced. The yields of the anilinomethyl derivatives of the substituted 5-acetoxyindoles (II-VI) were 60-76%. In the preparation of indole III, a 3.2% yield of N,N-bis(1-methyl-3-ethoxycarbonyl-5-acetoxy-6-bromoindole) (VII) also was isolated. The yield of compound VII could be increased to 10% if the reaction is carried out not with aniline but with excess aniline hydrochloride in aqueous dioxane with heating. Basic hydrolysis of the substituted 1-methyl-2-anilinomethyl-3-ethoxycarbonyl-5-acetoxy-6-bromoindoles II-VI lead to the formation of the corresponding substituted 5-hydroxyindoles (VIII-XI). Aminomethylation of the latter have the 4-aminomethyl indole derivatives (XII-XV).



I-VI, VIII-XV

R=H(II, III, VIII, IX, XII, XIII), OMe(IV, X), Cl(V, XI, XIV, XV) NO_2(VI); R^1=H(III—VI, IX—XII, XIV, XV), Me(II, VIII, XIII); R^2=H(VIII—XV), Ac(II—VI); R^3=H(II—VI, VIII, XI), CH_2NMe_2(XII—XIV), CH_2-morpholino (XV)

The structures of all of the compounds were verified by mass spectroscopy and by IR spectroscopy, which showed valence oscillation absorption bands characteristic of the OAc, COOEt, OH, and NH groups (cf. Table 1). Further, compounds XII-XV showed absorption bands in the 3200-3400 cm^{-1} region, characteristic of the valence oscillation of the OH group, indicating the zwitter-ionic character of these compounds, which is connected with the presence in these molecules of the phenolic hydroxyl group in position 5 and the basic dialkylaminomethyl group in position 4.

EXPERIMENTAL

IR spectra were determined on a Perkin-Elmer 599 instrument (USA) in Vaseline oil. Mass spectra were obtained with a Varian MAT-112 spectrometer (FRG). Control of the purity of the materials was maintained over the

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