PHARMACOLOGICAL PROPERTIES OF PYRIDINIUM SALTS, PYRAZOLINES, AND PYRAZOLES

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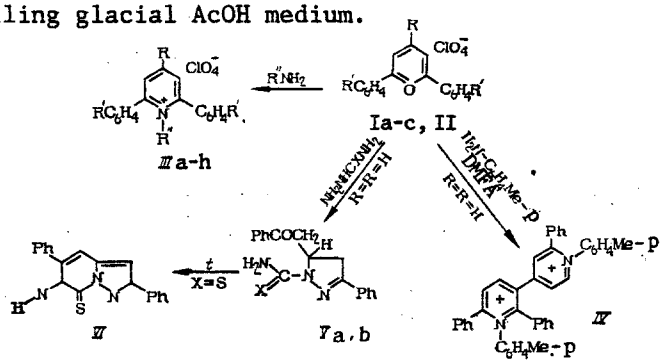
We studied the antimicrobial properties of pyridinium salts, as well as some derivatives of pyrazolines and pyrazoles, obtained in the interaction of 2,4,6-triarylpyrilium perchlorates with various nitrogen-containing nucleophiles [2, 4].

According to the literature data, pyridinium salts possess a broad spectrum of antimicrobial action [3, 6]; drugs have also been found among pyrazoline and pyrazole derivatives [8]. This work presents information on the investigation of the antimicrobial, neurotropic, and fungicidal action of pyridinium salts (III), bis-pyridinium salts (IV), pyrazolines (V), and pyrazolo[1,5-c]pyrimidine (VI), produced in the recyclization of 2,6-diarylpyrilium perchlorates (I) under the action of amines, semi-, and thiosemicarbazides.

The methods of synthesis of the pyridinium salts (IIIa-e) and bis-pyridinium diperchlorate (IV) were described in our study [5]. 1-p-Aminophenyl-2,6-diarylpyridinium perchlorates (IIIf, g) were produced for the first time by the reaction of pyrilium salts (Ib, c) with p-phenylenediamine in boiling glacial AcOH medium.



R = H (Ia—c, IIIa—g); C_6H_4OMe-p (II, IIIh); R′ = H (Ia; II; IIIa—e, h); OMe-p (Ib, IIIf); Br-p (Ic, IIIg); R″ = Me (IIIa); Ph (IIIb); C_6H_4Me-p (IIIc); C_6H_4OMe-p (IIId); C_6H_4OH-p (IIIe); $C_6H_4NH_2$-p (IIIf—h) X = O (Va); S (Vb)

For a comparison of the influence of structural factors on the pharmacological activity we synthesized the pyridinium salt IIIh with an aryl substituent in the 4-position in the reaction of the perchlorate II with p-phenylenediamine. Pyrazolines (V) are formed when the pyrilium salt Ia is boiled with semi- or thiosemicarbazides in ethanol. The pyrazoline (Vb) undergoes intramolecular condensation during thermolysis, forming 7-thio-2,5-diphenylpyrazolol[1,5-c]pyrimidine (VI).

EXPERIMENTAL — CHEMICAL

The IR spectra of the compounds synthesized were recorded on a Specord 71-IR spectrometer (German Democratic Republic) in liquid petrolatum; the PMR spectra were recorded on a Tesla BS 467C instrument (Czechoslovakia) with HMDS as an internal standard. The purity of the compounds synthesized was monitored by thin-layer chromatography on plates with SiO_2, eluent $CHCl_3$.

1-(p-Aminophenyl)-2,6-di-p-methoxyphenylpyridinium Perchlorate (IIIf). A mixture of 0.79 g (2 mmoles) 2,6-di-p-methoxyphenylpyrilium perchlorate and 0.26 g (2.4 mmoles) p-phenylenediamine in 35 ml AcOH was boiled for 12 h. Then half the solvent was distilled

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TABLE 1. Antimicrobial Activity (MBC, μg/ml) of the Synthesized Compounds

Microorganism	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	IIIg	IIIh	IV	Va	Vb	VI
Staph. aureus 209P	500	100	50	100	100	250	50	6,25	25	—	—	—
Staph. Buomko	500	100	50	100	100	100	125	12,5	25	—	—	—
Sh. flexneri 2a	—	100	100	200	200	—	500	500	—	500	—	500
Bacillus subtilis	—	—	—	—	—	50	500	12,5	—	—	—	—
Salmonella paratyphi A	—	500	500	500	500	500	500	500	—	500	—	500
Ps. aeruginosa	—	—	—	—	—	—	500	500	—	—	—	—
Serratia marcesceus	—	—	—	—	=	—	125	500	—	500	—	500
Klebsiella pneumonieae	—	—	—	—	=	125	—	500	—	500	—	500

Note. —) The compounds are inactive toward the indicated microorganisms.

off, and the gradually crystallizing oil was precipitated from the residue after cooling. Yield 0.93 g (96%) of the salt IIIf, mp 145-147°C (from isobutanol). Found, %: C 62.05; H 5.01; Cl 7.04; N 5.98. $C_{25}H_{23}ClN_2O_6$. Calculated, %: C 62.18; H 4.80; Cl 7.34; N 5.80. IR spectrum, ν_{max}, cm^{-1}: 1100, 1235, 1255, 1530, 1570, 1600, 1620, 1690, 3360.

1-(p-Aminophenyl-2,6-di-p-bromophenylpyridinium Perchlorate (IIIg) was produced analogously from 1.25 g (2.5 mmoles) 2,6-di-p-bromophenylpyrilium perchlorate and 0.33 g (3 mmoles) p-phenylenediamine; however, the reaction ends in 3 h. The precipitate that formed upon cooling was filtered off. Yield 0.93 g (64%) of the perchlorate IIIg, mp 288-289°C (from glacial AcOH). Found, %: C 47.81; H 2.72; Cl + Br 33.28. $C_{23}H_{17}Br_2ClN_2O_4$. Calculated, %: C 47.58; H 2.95; Br 27.52; Cl 6.11. IR spectrum, ν_{max}, cm^{-1}: 1090, 1315, 1530, 1560, 1590, 1615, 1690, 3355.

1-p-Aminophenyl-2,6-diphenyl-4-p-methoxyphenylpyridinium Perchlorate (IIIh). A mixture of 2,6-diphenyl-4-p-methoxyphenylpyrilium perchlorate (0.87 g, 2 mmoles) and p-phenylenediamines (0.25 g, 2.4 mmoles) in 5 ml DMFA was boiled for 1 h. Then it was cooled, and the pyridinium salt IIIh precipitated with ether. Yield 1.02 g (96%), mp 197.5-198°C (from 80% aqueous ethanol). Found, %: C 68.07; H 4.54; Cl 6.51; N 5.42. $C_{30}H_{25}ClN_2O_5$. Calculated, %: C 68.12; H 4.76; Cl 6.70; N 5.30. IR spectrum, ν_{max}, cm^{-1}: 1100, 1185, 1250, 1280, 1315, 1525, 1570, 1600, 1627, 3380, 3470.

1-Carbamoyl-3-phenyl-5-phenacyl-4,5-dihydropyrazole (Va). To a suspension of 0.7 g (6 mmoles) of the semicarbazide hydrochloride in 12.5 ml of ethanol we added 2.5 ml of an aqueous solution of 0.24 g (6 mmoles) NaOH, and after 10 min, 1.66 g (5 mmoles) of the perchlorate Ia. The reaction mixture was boiled for 1 h 20 min, cooled, and the precipitate formed was fittered off. Yield 1.3 g of compound Va (85%), mp 182-183°C (from 80% aqueous ethanol). Found, %: C 70.05; H 5.81; N 1373. $C_{18}H_{17}N_3O_2$. Calculated %: C 70.34; H 5.58; N 13, 67. IR spectrum, ν_{max}, cm^{-1}: 1355, 1440, 1500, 1590, (C=C:C=N), 1670 (C=O), 3140, 3250, 3320, 3460 (NH_2). PMR spectrum in CCl_3, δ, ppm: 2.75-4.10 (m, 4H, $2CH_2$), 4.6-5.0 (m, 1H, CH), 5.6 (s, 2H, NH_2), 7.1-8.0 (m, 10H, 2Ph).

1-Thiocarbamoyl-3-phenyl-5-phenacyl-4,5-dihydropyrazole (Vb) was produced analogously from the salt Ia and thiosemicarbazide. After cooling of the reaction mixture, the pyrazoline Vb was filtered off, with a yield of 73%, mp 163°C (from benzene). Found, %: C 66.61; H 5.39; N 12.84; S 9.62. $C_{18}H_{17}N_3O$. Calculated %: C 66.85; H 5.30; N 12.99; S 9.91. IR spectrum, ν_{max}, cm^{-1}: 1215 (C=S), 1350, 1440, 1500, 1585 (C=C, C=N), 1670 (C=O), 3055, 3160, 3270, 3350 (NH_2). PMR spectrum in CCl_3, δ, ppm: 2.9-4.45 (m, 4H, $2CH_2$), 5.15-5.5 (m, 1H, CH), 6.5 (s, 2H, NH_2), 7.25-7.95 (m, 10H, 2Ph).

2,5-Diphenyl-7-thiopyrazolo[1,5-c]pyrimidine (VI). The pyrazoline Vb (1.5 g) was exposed at the temperature 200-215°C for 1.5 h, the melt was cooled, ground, treated with 3-5 ml of acetone, and 0.82 g (58%) of compound VI was filtered off. mp 215°C (from a mixture of ethanol with $CHCl_3$, 3:5). Found, %: C 71.08; H 4.62; N 13.60; S 10.31. $C_{18}H_{13}N_3S$. Calculated, %: C 71.26, H 4.32; N 13.85; S 10.57. IR spectrum, ν_{max}, cm^{-1}: 1260 (C=S), 1330, 1435, 1515, 1630 (C=C, C=N), 3120, 3170 (NH). PMR spectrum in DMFO-D_6, δ, ppm: 7.0-8.0 (m, 12H arom. and heteroarom.), 8.3 (s, NH).

The bacteriostatic action of the 12 preparations synthesized toward a series of gram-positive [*Staphylococcus aureus* 209P, *Staphylococcus buomkol*] and gram-negative [*Sh. flexneri* 2a, *Bacillus subtilis*, *Salmonella paratuphi* A, *Pseudomonas aeruginosa*, *Serratia marcesceus*, *Klebsiella pneumonieae*] microorganisms was investigated.

The antimicrobial and antimycotic activity of the synthesized compounds was tested by the standard methods of serial dilutions in liquid and solid nutrient media [7].

Data on the antimicrobial activity of the investigated compounds are cited in Table 1. As can be seen from Table 1, almost all the investigated compounds possess one degree or another of antimicrobial properties. The pyridinium salt IIIa, containing a methyl substituent at the nitrogen atom, is active only toward the group of staphylococci, and moreover, its minimum active bacteriostatic concentration (MBC) is high. When the methyl substituent is replaced by aryl (salts IIIb-e), the spectrum of action is broadened. Compounds IIIb-e also possess activity toward *Sh. flexner-2a* and *Salmonella paratyphi*; moreover, their MBC with respect to *Staphylococcus* is 5-10 times lower than for the salt IIIa. The spectrum of action is significantly broadened by the introduction of an amino group into the N-phenyl substituent of the pyridinium salt (compounds IIIf-h). In this case the MBC with respect to *Staphylococcus* is lower than for the salts IIIa-e. The most active of the N-aminophenyl-substituted pyridinium salts is compound IIIh with a p-methoxyphenyl substituent at the C_4 atom, whereas the perchlorates IIIf, g have no substituent in the 4-position. The bis-pyridinium salt IV is active only toward *Staphylococcus*, but its MBC for it is 20 times lower than for the salt IIIa.

The pyrazoline Vb possesses no antimicrobial properties; the pyrazoline Va and pyrazolyl-[1,5-c]pyrimidine VI are active toward four species of microorganisms; however, their MBC are high.

Compounds Va, b and VI were also tested for antimycotic activity toward *Trichophyton rubrum* and *Microsporum canis*. In this case the compounds Va and VI gave negative results, but the pyrazoline Vb exhibits activity toward the indicated cultures in a dose of 12.5 μg/ml. This activity is comparable with the action of griseofulvin, which is active toward the indicated cultures in a dose of 10 μg/ml under analogous conditions.

Tests were conducted for neurotropic activity of the pyridinium salts IIIa-h and IV. The neurotropic activity of the indicated compounds was studied by the method of biochemical screening on animal (rat) brain homogenates in the presence of an injurious factor, anti-brain antibodies, present in the blood serum of schizophrenia patients, according to the method of [1]. The activity of the tested compounds was determined according to their ability to prevent intensification of processes of peroxidation of the brain lipids to malonic dialdehyde (MDA). Compounds whose preventive properties were close to that of the psychotropic drug chloropromazine, widely used for the treatment of schizophrenia, were considered neurotropic.

The investigations were conducted on noninbred white rats of both sexes. An homogenate was prepared on the basis of 1 g of brain tissue to 30 ml of Tris-HCl (pH 7.4). A 0.2 ml portion of blood serum of schizophrenic patients and 0.1 ml of guinea pig complement (dilution 1:20) were added to samples containing 0.5 ml of the homogenate. A 0.1 ml portion of the tested preparation was added to each of the experimental samples. The level of MDA was determined at the beginning and after 2 h incubation at 37°C. The reaction was stopped by adding trichloroacetic acid (TCA); 2ml of 30% TCA and 0.1 ml of a 0.75 M solution of thiobarbituric acid were added to the sample. The mixture was heated for 15 min on a water bath, then centrifuged. The optical density of the colored complex of MDA was determined on an SF-16 spectrophotometer at the wavelength 535 nm. The standard neurotropic drug chloropromazine was used as a control. The decrease in the content of MDA in the experimental sample in comparison with that in the control sample (chlorpromazine), expressed in percent, was as follows: IIIa) 37, IIIb) 62.5; IIIc) 37; IIId) 12.5; IIIe) 25; IIIf) 62.5; IIIg) 37.5; IIIh) 25; IV) 70.

From the data cited it is evident that six compounds do not protect the neuroglial elements of the brain from the injurious action of antibrain antibodies contained in the serum of schizophrenia patients. Figures approaching the control (over 50%) are given by compounds IIIb, f and IV, which indicates the presence of possible neurotropic activity for these pyridinium salts.

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