IMMUNOTROPIC PROPERTIES OF SYNTHETIC MACROHETEROCYCLIC COMPOUNDS AND THEIR ACYCLIC FRAGMENTS

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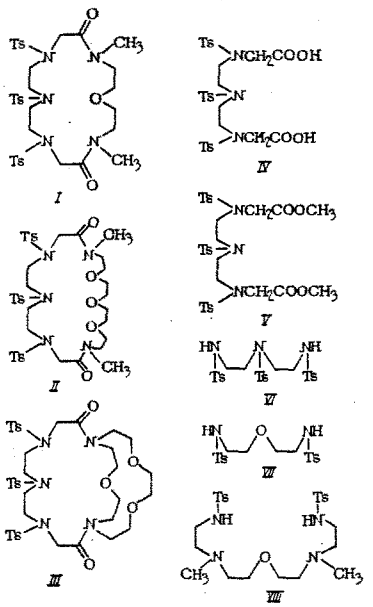
Earlier [1] we demonstrated the presence of immunotropic properties of certain synthetic macroheterocycles.

To reveal the dependence of the immunostimulating effect on the structure of the substances, we expanded our search for immunomodulators among macroheterocyclic compounds and their acylic structural fragments. Compounds I-VIII were synthesized.



EXPERIMENTAL CHEMICAL

The structure of the compounds was confirmed by the data of elementary analysis, IR spectroscopy, PMR and mass spectrometry. For an estimation of the purity and individuality of the compounds we used thin-layer chromatography.

The IR spectra were recorded on a Perkin-Elmer 580B spectrophotometer in tablets with KBr. The PMR spectra were obtained on a Tesla 497 spectrometer (Czechoslovakia) with working frequency 100 MHz; internal standard HMDS. The mass spectra were recorded on a Varian MAT CH-112 instrument (USA). Thin-layer chromatography was conducted on plates of Silufol UV-254 (Czechoslovakia); the chromatograms were developed with a soltuion of ninhydrin in ether, followed by heating to 150-200°C. For liquid chromatography we used L 100/160 silica gel.

1,13-Dimethyl-4,7,10-tris-(p-tolylsulfonyl)-16-oxa-1,4,7,10,13-penta-azacyclooctadecane-2,12-dione (I). To 350 ml of dry benzene with mixing at room temperature, solutions of 7.17 g (0.01 mole) of the dichloride of N,N',N''-tris(p-tolylsulfonyl)-3,6,9-triazaundecanoic acid and 3 g (0.01 mole) N,N'-dimethyl-4-oxa-1,7-diaminoheptane, each in 150 ml of dry benzene, were simultaneously added dropwise from separate funnels over a period of 3 h. The

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TABLE 1. Influence of Macroheterocyclic Compounds and Their Structural Fragments on the Humoral Immune Responses in Mice (M ± m, n = 8-10)

Variant	Hemolytic activity		Number of REC per 10^3 nucleated spleen cells	Number of AFC per 10^6 nucleated spleen cells	Titers	
	serum, $\cdot 10^6$	splenocytes, $\cdot 10^6$			of hemaglutinins	of hemolysins
control	12,9±5,0	19,2±2,7	70±3	923±67	4,9±0,4	6,5±0,5
Cyclophosphane (Cp)	4,5±1,7	8,8±1,7	29±2	565±53	1,9±0,6	2,9±0,6
Cp+I	8,5±0,5*	12,1±2,9	48±4*	1079±49*	2,5±0,2	3,2±0,3
Cp+II	3,1±1,0	4,9±0,7*	46±3*	1019±80*	0,5±0,1	2,3±0,6
Cp+III	6,0±0,6	10,7±1,6	56±3*	1191±88*	2,8±0,5	3,7±0,6
Cp+IV	4,1±0,5	10,5±2,0	32±4	695±65	3,8±0,4*	4,3±0,4
Cp+V	6,1±1,3	17,7±1,9*	39±4	844±170	2,7±0,4	3,5±0,5
Cp+VI	7,3±2,0	8,9±2,0	40±6	890±200	3,0±0,6	3,8±0,7
Cp+VII	7,5±2,1	12,3±2,4	38±7	915±180	2,1±0,3	3,1±0,4
Cp+VIII	4,3±0,6	7,7±0,5	38±4	634±57	3,0±0,4	3,9±0,3
Cp+IX	5,6±1,5	9,9±1,0	28±4	974±150*	1,5±0,3	2,5±0,6

Note. The hemolytic activity of the serum and splenocytes was expressed by the number of SE lysed by 0.025 ml of serum or 10^7 nucleated spleen cells. *Significance of the difference from the indices in animals that received only Cp.

mixture was mixed at room temperature for 4 h, filtered, and the solvent distilled off under vacuum. The product was isolated by chromatography on a column of silica gel. Eluent: acetone–hexane (1:1). Yield of I 2.2 g (21%), mp 112-115°C. Found, %: C 54.20; H 6.1; N 8.9. $C_{35}H_{47}N_5O_9S_3$. Calculated, %: C 54.05; H 6.05; N 9.0. M + 777.

1,13-Dimethyl-4,7,10-tris(p-tolylsulfonyl)-16,19,22-trioxa-1,4,7,10,13-pentaazacylcotetracosane-2,12-dione (II). Produced analogously by the addition of solutions of 7.17 g (0.01 mole) of the dichloride of N,N',N"-tris(p-tolylsulfonyl)-3,6,9-triazaundecanoic acid and 4.7 g (0.01 mole) N,N'-dimethyl-4,7,10-trioxa-1,13-diaminotridecane to 350 ml of dry benzene. Yield of II 2.8 g (44%), mp 161-162°C. Found, %: C 54.10; H 6.36; N 8.09. M + 865.

4,7,10-Tris(p-tolylsulfonyl)-16,19,24-trioxa-1,4,7,10,13-pentaazabicylco-11,8,25-hexacosane-2,12-dione (III). To 300 ml of dry benzene with mixing at room temperature, solutions of 7.17 g (0.01 mole) of the dichloride of N,N',N"-tris(p-tolylsulfonyl)-3,6,9-triazaundecanoic acid and a mixture of 2.18 g (0.01 mole) diaza-15-crown-5 and 2 ml of triethylamine, each in 400 ml of dry benzene, were simultaneously added dropwise from separate funnels over a peirod of 3 h. The mixture was mixed at room temperature for 10 h, filtered, and the solvent distilled off under vacuum. The product was isolated by chromatography on a column of silica gel. Eluent: acetone–hexane (2:1). Yield of III 1.6 g (20.5%), mp 146-148°C. Found, %: C 54.20; H 6.14; N 8.10. $C_{39}H_{53}N_5O_{11}S_3$. Calculated, %: C 54.28; H 6.20; N 8.01. M + 863.

N,N',N"-Tris(p-tolylsulfonyl)-3,6,9-triazundecanoid acid (IV) and dimethyl ester of N,N',N'-tris(p-tolylsulfonyl)-3,6,9-triazaundecanoic acid (V) were produced according to the procedure of [6].

N,N',N"-Tris(p-tolylsulfonyl)diethylenetriamine (VI) and N,N'-bis(p-tolylsulfonyl)-1,5-diamino-3-oxapentane (VII) were produced according to the procedure of [3].

4,10-Dimethyl-1,13-bis(p-tolylsulfonyl)-7-oxa-1,4,10,13-tetraazatridecane (VIII). A mixture of 0.04 mole of the ditosylate of 2-aminoethanol and 0.02 mole of N,N'-dimethyl-3-oxa-1,5-pentanediamine in 50 ml of dry acetonitrile was heated to boiling, 0.05 mole of triethylamine was added with mixing, and the mixture was boiled for 3 h. Then it was evaporated, treated with water, and recrystallized from ether. Yield of the end product 62%.

p-Toluenesulfonyl chloride (IX) was a commerical reagent.

TABLE 2. Influence of Compounds I-IV and VIII on the Reaction of Cellular Immunity (M ± m, n = 8-12)

Variant	Intensity of DTH reaction	
	mm	%
Absence of sensitization	0.07±0.02	–
Control	0,19±0,02	100
I	0,17±0,02	89
II	0,30±0,03 $P<0,05$	158
III	0,18±0,02	95
IV	0,31±0,03 $P<0,05$	163
VIII	0,19±0,02	100

Note. P is the significance of the difference from the indices in the control animals.

EXPERIMENTAL PHARMACOLOGICAL

The experiments were conducted on male CBA mice weighing 16-20 g.

The immunotropic activity of compounds I-IX was evaluated according to their ability to change the intensity of the immune response to the test antigen – sheep erythorcytes (SE).

The immunostimulating effect with respect to humoral immune reactions was determined according to the arrest of the immunodepressive state induced by a single injection of cyclophosphane in a dose of 50 mg per kg of body weight of the animals, together with the antigen. Compounds I-IX in a dose of 30 mg/kg were administered three times in the form of a Tween emulsion, intraperitoneally, according to the scheme −1, 0, +1, where 0 is the day of immunization ($5 \cdot 10^8$ SE per mouse intraperitoneally). The results were assessed on the fifth day after antigenic stimulation. The number of rosette-forming cells (RFC) [7] and antibody-forming cells (AFC) [4] in the spleen, the hemolytic activity of the spleen cells and serum [2], and the titers of circulating hemagglutinin and hemolysins were determined in the mice.

The influence of the compounds on the cellular immune reactions was studied on a model of delayed-type hypersensitivity (DTH) [5] on intact animals. The mice were sensitized by intraperitoneal injection of 10^7 SE (day 0). After four days a resolving dose of the antigen (10^8 SE in 40 µl of physiological saline solution subcutaneously into the pad of the right paw) was injected, and after 24 h the intensity of the DTH reaction was evaluated according to the difference in thickness of the right and left paws. The control animals received only the resolving dose of the antigen. Compounds I-IV, and VIII were injected intraperitoneally in a dose of 30 mg/kg on days 1, 2, and 3.

The results of the investigations are presented in Tables 1 and 2. From the data of Table 1 it follows that administration of the immunodepressant cyclophosphane to the animals induces the development of an immunodeficient state. All the investigated indices are decreased by more than half. Administration of macroheterocyclic compounds I and III against this background leads to a partial arrest of the state of immunodepression. Thus, the number of AFC in the spleen is restored to their level in the control animals, and the number of antigen-reactive cells is increased by 70-90%. The antibody titers and the intensity of antibody synthesis by spleenocytes increased by 30-50%.

A study of the immunotropic properties of compound II showed that it gives an immunosuppressive effect with respect to the indices of the humoral immunity. Thus, the hemolytic activity of the serum, antibody titers in the blood, and the intensity of antibody synthesis by the spleen cells were insignificantly below the level created by the administration of cyclophosphane. However, with respect to the number of RFC and AFC a stimulating effect was noted.

When the animals are given noncyclic tosyl-containing fragments of macroheterocycles (compounds IV-VIII), a negligible immunostimulating effect is observed, manifested in an increase in the numbers of RFC and AFC in the spleen by 15-40 and 10-45%, respectively.

A comparison of the immunotropic properties with the structure of the compounds studied showed that the appearance of immunostimulating activity is not associated with the presence of the tosyl group, since p-toluenesulfonyl chloride (IX) has no immunostimulating effect.

Thus, the greatest immunostimulating activity is possessed by cyclic compounds, and their noncyclic structural fragments have a substantially smaller effect.

Table 2 presents data on the influence of compounds I-IV and VIII on the reactions of cellular immunity. It was shown that compounds II and IV, which do not affect the humoral immune responses or suppress them, induce a more than 50% increase in the intensity of the DTH reaction. On the contrary, compounds I and III, the strongest stimulators of the humoral reactions, and compound VIII do not change the intensity of the cellular immune reactions.

The data cited permit us to conclude that the immunostimulating effect of the compounds studied is associated with the presence of a cyclic structure. Since the ability of macroheterocycles to form complexes with metals is known, one of the possible mechanisms of the action of such substances may be considered to be their influence on the local concentrations of ions, especially such as Ca^{2+} and Mg^{2+}. Modification of the physicochemical properties of the plasma membranes of the immunocytes also is not excluded (by analogy with polyene antibiotics).

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 6-METHOXY-1H-1,2-DIAZAPHENALENE DERIVATIVES

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To clarify the biological activity of 1H-1,2-diazaphenalene derivatives, a little studied peri-heterocyclic system, we were the first to synthesize 6-methoxy-1,3-substituted 1H-1,2-diazaphenalenes (I-IV) and to study their antioxidant and hepato-protecting activity. The prerequisite for studying the above biological properties of this class of compounds was that the π-excessive heterocyclic system, 1H-1,2-diazaphenalene, has a low ionization potential, forms stable charge transfer complexes, [2], readily enters into one-electron oxidation reactions, forms thus stable cation-radicals, and has high reactivity with respect to electrophilic reagents [1, 3]. There are reports in the literature that the reason for the pharmacological action of several compounds are their donor-acceptor properties, [4-6], and therefore it could be expected that 1H-1,2-diazphenalenes will have a biological activity that involves a transfer of electrons.

Compounds I-IV were obtained by condensing the corresponding per-hydroxyketones with methylhydrazine and phenylhydrazine at the boiling point in ethylene glycol, according to the method in [9]:

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