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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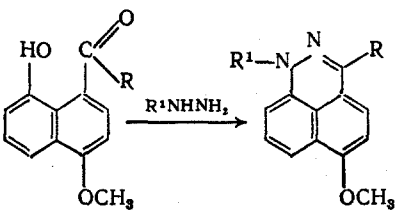
TABLE 1. Physicochemical Constants of Compounds I-IV

Compound	Yield, %	mp., °C	Found, %			Empirical formula	Calculated, %		
			C	H	N		C	H	N
I	50	152—3	79,12	5,50	9,68	$C_{18}H_{16}N_2O$	79,17	5,56	9,72
II	64	110—11	79,08	5,54	9,74	$C_{19}H_{16}N_2O$	79,17	5,56	9,72
III	60	103—4	79,34	6,08	9,32	$C_{20}H_{18}N_2O$	79,47	5,96	9,27
IV	70	78—9	82,15	5,27	7,94	$C_{24}H_{18}N_2O$	82,29	5,14	8,00



I — IV

R = Ph (I, IV); CH_3 (II); C_2H_5 (III); R^1 = CH_3 (I); Ph (II — IV).

The compounds obtained are yellow, odorless crystalline substances which are soluble in most organic solvents. The physicochemical properties and data of elemental analysis of compounds I-IV are given in Table 1.

The structure of diazaphenalenes I-IV was confirmed by the data of elemental analysis, IR and PMR spectroscopy, and the individual state by chromatography.

In the IR spectra of these compounds there are vibration bands characteristic of the C=N bonds in the 1610-1620 cm^{-1} region, C=C in the 1580-1590 cm^{-1} region, and the C—O—C bond in the 1100 cm^{-1} region. In the PMR spectrum of compound II, a singlet of methyl group protons (δ 2.09 ppm), a singlet of the methoxy group protons (δ 3.68 ppm), and a 10-proton multiplet of the methoxy group (δ 6.22-7.32 ppm), which cannot be interpreted according to the first order rules, are observed.

EXPERIMENTAL CHEMICAL

The IR spectra of compounds I-IV were run on a Specord IR-75 spectrophotometer (GDR) in mineral oil. The PMR spectrum of compound II in deuteropyridine was obtained on a Tesla BS-487C spectrometer (CSSR) (80 MHz), using HMDS as internal standard. The TLC was carried out on Silufol UV-254 plates (CSSR) and on a nonstationary layer of anhydride aluminum oxide ("analytically pure", TU [Technical Conditions] 6-09-426-70), using chloroform as the mobile phase.

6-Methoxy-1-methyl-3-phenyl-1H-1,2-diazaphenalene (I). A 7.5 g portion (0.053 mole) of methylhydrazine sulfate and 4.2 g (0.104 mole) of sodium hydroxide are mixed in 100 ml of ethylene glycol. The mixture is heated to boiling, and the precipitated sodium sulfate is filtered. A 7.2 g portion (0.026 mole) of 8-hydroxy-4-methoxy-1-naphthophenone is added to the solution obtained, and the mixture is boiled for 20 min in an argon atmosphere. The hot solution is poured into 600 ml of ice water, 5 g of sodium chloride are added, and the crude azine is filtered. It is crystallized from acetone with hot filtration.

6-Methoxy-3-methyl-1-phenyl-1H-1,2-diazaphenalene (II). A 0.55 ml portion (0.005 mole) of phenylhydrazine is added to 0.5 g (0.0025 mole) of 4-methoxy-8-hydroxyl-1-acetonaphthone in 4 ml of ethylene glycol, and the mixture is boiled for 3 h. It is then cooled, the precipitate is filtered, washed with ethanol and crystallized from ethanol.

6-Methoxy-1-Phenyl-3-ethyl-1H-1,2,diazaphenalene (III) and 6-methoxy-1,3-diphenyl-1H-1,2-diazaphenalene (IV) are obtained in the same way as compound II.

EXPERIMENTAL PHARMACOLOGICAL

The hepatoprotective activity of the compounds studied was determined from the survival of animals when tetrachloromethane was administered to them in a lethal dose (protective in-

TABLE 2. Hepatoprotective and Antioxidant Activity of Compounds I-IV during Toxic Action of Tetrachloromethane

Compound	Hepatoprotective activity		Antioxidant activity	
	ALT, mmole/(liter·h)	% of activity	MDA, nmole/h	% of activity
I	3,07±0,20	36	210,3±6,1	44
II	3,54±0,20	22	353,5±9,3	0
III	3,14±0,10	27	355,7±8,4	0
IV	2,97±0,10	41	168,3±5,3	52
Without additive (intact animals)	1,37±0,14	100	58,6±0,8	100
Without additive (nontreated animals)	4,16±0,19	0	301,3±24,7	0
Silibor	2,83±0,11	47,7	—	—
Vitamin E	—	—	218,4±5,6	29,2

dex) and from the activity of an indicator enzyme, characterizing the cytolytic process of alanine-aminotransferase (ALT) of blood serum [7, 10]. Six male rats were used in each series of experiments.

An investigation of this type of activity in the compounds studied in a dose of 25 mg/kg (in the same dose, the Soviet-produced hepatoprotector, Silibor exhibited 50% activity) showed that compound IV has the maximal hepatoprotective activity (41%), approaching that of the compound taken for comparison, compound I has the lowest activity, but the difference between the activity of these compounds and that of Silibor is statistically not reliable. Compounds II and III have a less pronounced hepatoprotective action, but the ALT activity differs statistically reliably from its activity in nontreated animals (Table 2).

The membrane-stabilizing effect, causing the hepatoprotective action, may depend on the ability of the compounds to weaken the lipoperoxidation process [8]. We therefore studied the antioxidant activity of the compounds synthesized on a model of a liver disease caused by tetrachloromethane, which is an effective prooxidant. The highest activity (1.8 times higher than that in vitamin E) was found in compound IV, and the lowest in compound I. Compounds II, III, had no such activity. The hepatoprotective activity is therefore an inherent characteristic of all the above 6-methoxy-1H-1,2-diazaphenalenes. In the most active hepatoprotectors, the anti-oxidant activity exceeds that of the reference compound, vitamin E.

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