THIOUREA AND THIOSEMICARBAZIDE DERIVATIVES: STRUCTURE, TRANSFORMATIONS, AND PHARMACOLOGICAL ACTIVITY. PART III. ANTIHYPOXIC AND ANTIINFLAMMATORY ACTIVITY OF 1,2,4-TRIAZINO[6,5-b]INDOLE DERIVATIVES

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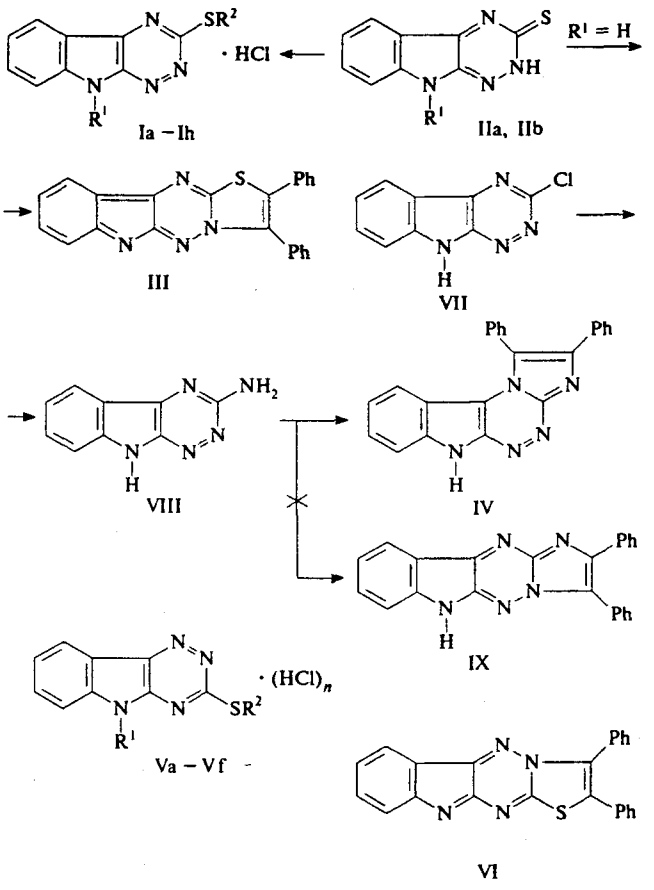
In the previous paper [1] we reported on the antihypoxic activity of compounds of the 1,2,4-triazino[5,6-b]indole series. The purpose of this work was to find antihypoxic agents in the isomer series of 1,2,4-triazino[6,5-b]indole derivatives differing from the compounds studied in [1] by the site of condensation of the indole and triazine rings. We have tried to compare the activities of both isomers and studied the possibility of extending the activity spectrum [2]. In continuation of the work [3] showing evidence of an antiinflammatory activity of two derivatives of the former series (Ia, Ib), we have also studied this type of activity in the isomer series synthesized. Note that, since 1,2,4-triazino[6,5-b]indoles are synthetically less available than derivatives of the isomer system, their properties are less known.

The tests were performed with 3-aminoalkylthio 1,2,4-triazino[6,5-b]indole derivatives (Ia – Ih) synthesized in the form of hydrochlorides by aminoalkylation of the corresponding thiones (IIa, IIb). For comparison, we have also synthesized insoluble condensed derivatives of this heterocyclic system, 2,3-diphenylthiazolo[3′,2′ : 2,3]-1,2,4-triazino[6,5-b]indole (III) and 1,2-diphenylimidazo[2′,3′ : 3,4]-1,2,4-triazino[6,5-b]indole (IV), and the corresponding derivatives of the isomer series (Va – Vf, VI) [1]. Compound IV was obtained through amination of the chloro derivative VII [4] followed by condensation of the product VIII with α-bromo-α-phenylacetophenone. By analogy with reactions observed in the isomer series, we could also expect the formation of another cyclization product (IX) having a considerable bathochromic shift of a longwave band in the electronic absorption spectrum [5]. The position of this band in the spectrum of compound IV is strongly dependent of the nature of the solvent, which is probably related to the tautomeric transformations involving a hydrogen atom at the indole nitrogen.

The proposed structures of compounds Ic – Ih were confirmed by data of the electronic absorption spectroscopy and potentiometric titration, and the structures of compounds IV and VIII were consistent with the mass-spectrometric data. Some of the compounds were reported earlier: Ia, Ib [3], IIa, IIb [6], III, Vb, VI [5], Va [7], and Vc – Vf [1].



I: R^1 = Me, R^2 = $(CH_2)_2NMe_2$ (a);
R^1 = Me, R^2 = 2-piperidinoethyl (b);
R^1 = H, R^2 = $(CH_2)_2NMe_2$ (c);
R^1 = H, R^2 = $(CH_2)_2NEt_2$ (d);
R^1 = H, R^2 = $(CH_2)_2NPr_2$ (e);

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R^1 = H, R^2 = 2-piperidinoethyl (f);
R^1 = H, R^2 = 2-morpholinoethyl (g);
R^1 = Me, R^2 = $(CH_2)_2NEt_2$ (h);
II: R^1 = H (a), R^1 = Me (b);
V: R^1 = H, R^2 = $CH_2CH{=}CH_2$, n = 0 (a);
R^1 = H, R^2 = CH(Ph)COPh, n = 0 (b);
R^1 = H, R^2 = $(CH_2)_2NMe_2$, n = 1 (c);
R^1 = H, R^2 = $(CH_2)_2NEt_2$, n = 1 (d);
R^1 = $(CH_2)_2NEt_2$, R^2 = Et, n = 2 (e);
R^1 = 2-morpholinoethyl, R^2 = Et, n = 1 (f).

The yields and physicochemical characteristics of the synthesized compounds are presented in Table 1. The products are stable crystalline substances of yellow color. The aminoalkylthio 1,2,4-triazino[6,5-b]indole derivatives were also characterized by ionization constants pK_a and distribution coefficients in the system octanol – buffer solution (pH 7.36) [1]. It was found that these values could be related to the corresponding characteristics of the isomer derivatives of

TABLE 1. Yields and Physicochemical Characteristics of 1,2,4-Triazino[6,5-b]indole Derivatives

Compound	Yield, %[a]	M.p., °C (solvent for crystallization)	TLC (Al_2O_2, activity order II)			log P_{appl}	log P	pK_a	Empirical formula	UV spectrum in H_2O: λ_{max}, nm (log ε)
			solvent for application	eluent	R_f					
Ic	74.1 66.6	196 (isopropyl alcohol – 0.2 N HCl – water, 30.2 : 2.19 : 1)	methyl alcohol	methyl alcohol – 3 N aqueous ammonia, 10 : 1	0.43[b]	1.76	2.59	8.12	$C_{13}H_{15}N_5S \cdot HCl$	216 (4.42), 266 (4.50), 299 (3.99), 335 (4.06), 410 (3.38)
Id	87.2	243 (methyl alcohol – water, 8.2 : 1)	acetone	benzene – ethanol, 7.5 : 1	0.46	1.94	3.11	8.50	$C_{15}H_{19}N_5S \cdot HCl$	265 (4.51), 300 (3.80), 335 (4.01), 410 (3.29)
			methyl alcohol	methyl alcohol – 3 N aqueous ammonia, 10 : 1	0.46[b]					
Ie	81.6 70	240 (benzene – hexane, 1 : 1)[c]	methyl alcohol	methyl alcohol – 3 N aqueous ammonia, 10 : 1	0.67[b]	3.40	4.31	8.22	$C_{17}H_{23}N_5S \cdot HCl$	268 (4.51), 297 sh (3.85), 337 (3.91), 409.5 (3.21)[d]
If	75.7	243 (isopropyl alcohol – water, 8 : 1)	methyl alcohol	benzene – ethanol, 10 : 1	0.41	2.80	3.97	8.53	$C_{16}H_{19}N_5S \cdot HCl$	215 (4.25), 265 (4.44), 300 (3.75), 334 (3.96), 412 (3.17)
				benzene – acetone – ether – 18% aqueous ammonia, 15.3 : 6 : 1 : 0.3[e]	0.32[b]					
Ig	77 93.3	246 (isopropyl alcohol – water, 8.33 : 1)	methyl alcohol	benzene – ethanol, 7.5 : 1	0.39	2.55	2.57	6.00	$C_{15}H_{17}N_5OS \cdot HCl$	213.5 (4.38), 266 (4.46), 302 (3.79), 336 (3.94), 414 (3.22)
Ih	96 92.5	266 (methyl alcohol)	chloroform	benzene – ethanol, 7.5 : 1	0.78	1.81	3.01	8.56	$C_{16}H_{21}N_5S \cdot HCl$	217 (4.34), 270 (4.55), 302 sh (3.77), 336 (3.99), 430 (3.29)
			methyl alcohol	methyl alcohol – 3 N aqueous ammonia, 10 : 1	0.44[b]					
IV	76	334 (DMF – butyl alcohol, 1 : 2.39)	acetone	Chloroform – methyl alcohol, 9 : 1	0.80	...	...	...	$C_{23}H_{15}N_5 \cdot C_4H_{10}O$	272.5 (4.63), 426 (4.28)[d], 275 (4.57), 450 (4.27)[f]
VIII	75.5	319 (pyridine – water, 1 : 1)	tetrahydrofuran	tetrahydrofuran	0.16	...	...	...	$C_9H_7N_5 \cdot H_2O$	260.5 (4.24), 290 (3.66), 334 (4.04), 429 (3.40)[d]

Notes: [a] Here and below the first value corresponds to the yield of base, and the second value indicates the yield of salt relative to the base; [b] adsorbent Silufol UV-254; [c] Solvent for recrystallization of the base; [d] in ethanol; [e] bottom layer; [f] in chloroform.

TABLE 2. Antihypoxic Activity and Acute Toxicity of 1,2,4-Triazino[6,5-b]-indole and 1,2,4-Triazino[5,6-b]indole Derivatives

Compound	Dose, mg/kg (i.p.)	PC in hypoxia			LD_{50}, mg/kg (i.p.)	TI (hypobaric hypoxia)
		hypobaric	hemic	histotoxic		
Id	25	1.56*	1.65*	1.40*	246 ± 7.9	41
Ig	25	1.57*	1.43*	1.50*	325 ± 21.6	36
Ih	12	1.59*	1.26	1.60*	156 ± 8.2	24
Vb	50	1.72**	1.60*	1.60*	1450 ± 83	392
Vc	50	1.80**	1.80**	1.20	384 ± 1.2	26.7
Vd	50	1.51*	1.50*	1.60*	250 ± 9.3	15.9
Ve	50	1.40*	1.21	1.30	156 ± 7.7	23.6
Gutimine	100	1.40*	1.14	1.30	1325 ± 78	130
Amtizole	50	1.27	1.20	1.30	160 ± 13.5	8.0

* $p < 0.05$.
** $p < 0.01$ with respect to control (unprotected animals) according to the exact Fisher method.

the 1,2,4-triazino[5,6-b]indole series by linear correlations of the following type:

$$pK_{a[6,5\text{-}b]} = 1.012\ pK_{a[5,6\text{-}b]} - 0.047\ (r = 0.997),$$

$$\log P_{app[6,5\text{-}b]} = 1.035 \log P_{app[5,6\text{-}b]} + 0.087\ (r = 0.977),$$

$$\log P_{[6,5\text{-}b]} = 1.179 \log P_{[5,6\text{-}b]} - 0.325\ (r = 0.981).$$

Thus, the change in the site of condensation of the indole and triazine rings leads to insignificant increase in the basicity and hydrophobicity of the compounds studied.

As is seen from the data of Table 2, all the synthesized compounds (except Ve) exhibited comparable activity with respect to the model of hypobaric hypoxia in mice, exceeding the activity of the reference agents gutimine and amtizole. Generally similar results were obtained for hemic hypoxia, except for a somewhat lower activity of compounds Ih and Ve. The maximum activity with respect to both hypoxia models is observed for compound Vc. At the same time, compounds Vc and Ve were inefficient with respect to the histotoxic hypoxia model where all other compounds exhibited comparable effects exceeding those of the reference drugs. Judging by the therapeutic index, the most safe compound is Vb.

A comparative study of the mechanism of the antihypoxic action of triazinoindole derivatives Id, Ig, IVd, 8-methoxy-3-(2-morpholinoethylthio)-1,2,4-triazino[5,6-b]indole dihydrochloride (X) [1], gutimine, amtizole, and 2-(2-morpholinoethylthio)-4,5-diphenylimidazole dihydrochloride (XI) showed that all these antihypoxic agents at small concentrations (5 – 10 times the threshold values) are capable of reducing the flavine nucleotides of mitochondria. The 1,2,4-triazino[5,6-b]indole derivatives Vd and X at all concentrations oxidize the pyridine nucleotides, and at sufficiently high concentrations (2 – 3 orders above the threshold) decrease the reducing effect of the breathing chain substrate sodium succinate. At the same time, the 1,2,4-triazino[6,5-b]indole derivatives Id and Ig (as well as amtizole) at small concentrations reduce both the flavine and pyridine nucleotides, while completely retaining the reducing effect of sodium succinate. At higher concentrations (1 – 1.5 orders above the threshold) these compounds reduce the flavine nucleotides and oxidize the pyridine ones, while the further increase in their concentration changes the sign of redox reaction of the flavine nucleotide: their oxidation increases simultaneously with the oxidation of pyridine nucleotides, while mitochondria are no more sensitive to sodium succinate. These effects are not related to the extent of antihypoxic action, which is approximately the same in both groups of the triazinoindole derivatives.

The antihypoxic activity of compounds Id, Ig, Vd, X, and XI, as judged by the protection coefficient (PC) values, is correlated with the threshold concentrations determined by the effect on the fluorescence intensity of nucleotides: $PC = 0.35 - 0.18 \log C$ ($r = 0.94$ for $p < 0.001$).

Compounds Ia, Ib, Id, If, Ig, III, Va, Vb, Ve, Vf, and VI showed reliable antiexudative and antiproliferative action with respect to the cotton-induced granuloma model (Table 3). The maximum activity was observed for compounds Id and VI, which (like Ia, Ib, If, Ig, III, and Ve) were more effective than indomethacin.

All the triazinoindole derivatives studied also exceeded indomethacin with respect to the antiinflammatory activity in a thermal burn model. The maximum effect was observed for compounds Ig and If.

Equal protective effects of the triazinoindole derivatives was observed with respect to the adrenaline- induced model of lung edema as well.

Thus, both isomer series of triazinoindoles contain active compounds (Ia, Ib, Id, If, Ig, III, Ve, and VI) whose antiinflammatory effect exceeds that of indomethacin. This type of activity, in contrast to the antihypoxic effect, is significantly dependent on the site of condensation of the indole and triazine rings. The probability of finding a compound possessing antiinflammatory activity is higher in the series of 1,2,4-triazino[6,5-b]indole derivatives, and the most active triazinoindoles belong to this very series of compounds. In contrast, some of the 1,2,4-triazino[5,6-b]indole derivatives even favored the development of inflammation.

As is seen from Table 3, all compounds (except for the high-toxicity compound Ib) show antiinflammatory activity at doses not exceeding 1/10 LD_{50} and, hence, possess a sufficiently broad therapeutic range. Moreover, a 6-day introduction of triazinoindole derivatives intraperitoneally at a dose of 10 – 15 mg/kg (i.e., about 1/10 LD_{50}) was not accompanied, in contrast to the case of indomethacin administration, by any observable toxicity manifestations on the side of the gastrointestinal tract. Therefore, the triazinoindole derivatives possess a lower toxicity as compared to that of indomethacin.

The results of this work allow us to conclude that the series of 1,2,4-triazino[6,5-b]indole derivatives is close to the 1,2,4-triazino[5,6-b]indole isomer series with respect to ionization, lipophilicity, antihypoxic activity, and acute toxicity, but exhibits a generally higher antiinflammatory activity.

The former series of compounds is promising from the standpoint of searching for the new antiinflammatory and antihypoxic agents.

EXPERIMENTAL CHEMICAL PART

The homogeneity of all compounds was checked, besides the other methods, by TLC. The mass spectra were recorded on a Hewlett-Packard Model 5985A mass spectrometer with direct sample injection into the ion source operated at an ionizing electron energy of 70 eV and a temperature of 200°C. Methods used in measuring the electronic absorption spectra [3, 5] and in determining the equivalent masses, ionizations constants pK_a, and the distribution coefficients log P_{app} and log P were described previously [1]. A group of selected model compounds, including 16 water-soluble amines with similar structures (including hydrochlorides of aniline and 2-dimethylaminoethyl chloride, 2-morpholinoethyl bromide, *p*-toluidine, 2-aminopyridine, morpholine, N-methylmorpholine, diethylamine, 2-dimethylaminomethyl and 2-diethylaminoethyl alcohols, compound Ic, and dihydrochlorides of 3-[2-(4-methylpiperazino)ethylthio]-, 8-methoxy-3-(2-morpholinoethylthio)-, and 5-(2,3-dihydroxypropyl)-3-(2-morpholinoethylthio)-1,2,4-triazino[5,6-b]indoles) showed a linear correlation between the pK_a values in water and 70 % aqueous ethanol. This relationship allowed us to calculate the values of pK_a in water for insufficiently soluble 1,2,4-triazino[6,5-b]indole derivatives (Table 1). The data of elemental analyses for all compounds and the equivalent masses measured for compounds Ia – Ih agree with the results of calculations according to the empirical formulas.

3-(2-Dimethylaminoethylthio)-1,2,4-triazino[6,5-b]indole hydrochloride (Ic). To a solution of 7 g (34.65 mmole) of compound IIa [6] in 87 ml of 1 N aqueous sodium hydroxide was added a solution of 5.29 g (36.74 mmole) of 2-dimethylaminoethyl chloride hydrochloride in 14 ml of water, and the mixture was allowed to stand overnight. The reaction was completed by heating for 20 min on a boiling water bath. Upon cooling, 31.5 g of sodium chloride was added and the mixture was stirred until complete dissolution of the salt. The solution was extracted with chloroform (4 × 40 ml), and the extract dried over anhydrous sodium sulfate and filtered. The precipitate was washed with anhydrous chloroform (100 ml) and the washing liquid added to the previous filtrate. The solvent was distilled off in vacuum to obtain 8.23 g of the base of Ic in the form of a red-brown oil. The base was mixed with 35 ml of isopropyl alcohol and 4.1 ml of concentrated HCl and heated to boiling, which resulted in crystallization of the hydrochloride of Ic. The reaction mixture was cooled and allowed to stand for 10 h at – 15°C. Then the precipitate was filtered, washed with ether (2 × 5 ml), and dried in vacuum over phosphorus anhydride to obtain compound Ic in the form of orange crystals readily soluble in water.

3-(2-Diethylaminoethylthio)-1,2,4-triazino[6,5-b]indole hydrochloride (Id). To a solution of 50 g (0.247 mole) of compound IIa in 570 ml of 1 N aqueous sodium hydroxide was added a solution of 51 g (0.296 mole) of 2-diethylaminoethyl chloride hydrochloride in 30 ml of water, and the

TABLE 3. Antiinflammatory Activity and Acute Toxicity of 1,2,4-Triazino[6,5-b]indole and 1,2,4-Triazino[5,6-b]indole Derivatives

Compound	Cotton granuloma			Thermal burn			Adrenaline edema of the lungs		LD_{50}, mg/kg
	Dose, mg/kg	Exudation, g	Proliferation, g	Dose, mg/kg	1 h[a]	24 h[a]	Dose, mg/kg	Lung coefficient	
Ia	10	0.1083 ± 0.0043	0.0138 ± 0.0008	10	135.8 ± 5.9	155.4 ± 4.5	15	1.15 ± 0.02	150 ± 14.5
Ib	10	0.1425 ± 0.0074	0.0191 ± 0.0018	...	...	...	...	...	33 ± 12.3
Id	10	0.1035 ± 0.0017	0.0112 ± 0.0001	...	...	...	10	0.90 ± 0.01	246 ± 7.9
If	15	0.1240 ± 0.0019	0.0107 ± 0.0002	15	84.2 ± 2.3	104.1 ± 4.8	50	0.91 ± 0.02	156 ± 7.7
Ig	15	0.1240 ± 0.0102	0.0106 ± 0.0014	15	85.1 ± 2.8	85.4 ± 2.4	50	0.81 ± 0.01	325 ± 21.6
III	10	0.0929 ± 0.0038	0.0096 ± 0.0011	...	...	...	10	0.93 ± 0.01	117 ± 9.6[b]
Va	10	0.1340 ± 0.0143	0.0130 ± 0.0015	...	...	...	10	1.0 ± 0.02	150 ± 14.5[b]
Vb	10	0.2024 ± 0.0529	0.0253 ± 0.0103	...	...	...	10	1.08 ± 0.09	1450 ± 83[c]
Ve	15	0.1367 ± 0.0022	0.0159 ± 0.0010	...	...	...	15	0.89 ± 0.02	156 ± 7.7
Vf	10	0.1775 ± 0.0136	0.0191 ± 0.0018	20	213.5 ± 4.3	163.5 ± 8.2	20	0.85 ± 0.02	767 ± 42
VI	10	0.0964 ± 0.0029	0.0096 ± 0.0010	...	...	...	10	0.88 ± 0.01	200 ± 9.1[b]
Physiological solution	0.02*	0.3318 ± 0.0275	0.0352 ± 0.0022	0.02*	280.4 ± 9.8	393.7 ± 46.4	0.01*	1.47 ± 0.14	...
DMSO	0.01*	0.2325 ± 0.0306	0.0290 ± 0.0039	0.01*	136.0 ± 8.7	144.0 ± 9.3	0.01*	1.23 ± 0.02	...
Indomethacin	1	0.2240 ± 0.0130	0.0244 ± 0.0026	1	185.2 ± 8.2	240.6 ± 9.1	1	1.43[d] ± 0.01	...
	20	0.2877[d] ± 0.0029	0.0317[d] ± 0.0029	...	...	...	20	1.21 ± 0.03	...

Notes: [a] Percentage increment of paw volume; [b] for DMSO solution; [c] for aqueous suspension with Tween-80; [d] unreliable difference against control; in all other cases $p < 0.05$ relative to control (Student's *t*-criterion).
* ml/g.

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reduce both the flavine and pyridine nucleotides, while completely retaining the reducing effect of sodium succinate. At higher concentrations (1 – 1.5 orders above the threshold) these compounds reduce the flavine nucleotides and oxidize the pyridine ones, while the further increase in their concentration changes the sign of redox reaction of the flavine nucleotide: their oxidation increases simultaneously with the oxidation of pyridine nucleotides, while mitochondria are no more sensitive to sodium succinate. These effects are not related to the extent of antihypoxic action, which is approximately the same in both groups of the triazinoindole derivatives.

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ed and washed sequentially with water (2 × 116 ml), 35 ml), and ether (2 × 35 ml). Then 36 ml of dioxadded and the mixture was boiled for 2 min. The was filtered, washed with dioxane, acetone, and dried to obtain compound 10.7 g VII in the form of en crystals; m.p., 279°C. A sample for the analytes, identical to that synthesized and purified as de- [4], was obtained by reprecipitation from dioxane

ino-1,2,4-triazino[6,5-b]indole monohydrate

3.5 g (17.1 mmole) of compound VII was added 0% aqueous ammonia, and the mixture was heated ıg for 8 h in an autoclave at 155°C. Then 5 ml of ous ammonia was added and treatment in the autonued at the same temperature for another 6 h, after precipitate was cooled and filtered. The precipitate with 150 ml 1 N hydrochloric acid, stirred, and he precipitate represented the unchanged com- The filtrate was mixed with concentrated aqueous ntil reaching pH 8 – 9 according to a universal in- ıe precipitate was filtered, washed with DMF water (3 × 5 ml) and dried in air to obtain 1.9 g compound VIII in the form of yellow crystals ble in organic solvents. Unreacted compound VII dly heated for 18 h with 50 ml of 10% aqueous olution to obtain additionally 0.74 g (21.2%) of VIII; mass spectrum, m/z (I_{rel}, %): $[M]^+$ 185 $N_2]^+$ 157 (84), $[M–N_2–HCN]^+$ 130 (32), $[M–N_2–$ 129 (49), $[M–N_2–2HCN]^+$ 103 (75), $[M–N_2–$ 102 (44).

ENTAL BIOLOGICAL PART

ihypoxic activity of the synthesized compounds on white male mongrel mice weighing 18 – 22 g. hypoxia was induced by placing animals into an mber with controlled ventilation and "elevating" 00 m [1]. The animals were observed for 45 min entage survival in the test and control groups was

The substances were injected intraperitoneally 6.25, 12.5, 25, 50, or 100 mg/kg with an isotonic lium chloride solution or with a fine suspension -80 (0.1 – 0.4 ml per animal). The control anijected with the same volume of physiological sonjections were made 1 h before the hypoxia inelevation." Each dose was tested in a group of 10 . The antihypoxic effect was evaluated as a proicient and (for the hypoxic hypoxia model only) peutic index (TI) [2]. For the active compounds he PC values are significantly dose-dependent s the PC values corresponding to ED_{50}, i.e., imum). The hemic hypoxia was modeled by soinjections (175 mg/kg, i.p.) and the survival was e day after the injections. The histotoxic hypoxia l by introducing potassium cyanide (8 mg/kg,

i.p.) followed by the survival monitoring after a 3 h period of time. The reference drugs were represented by the well-known antihypoxic agents amtizole and gutimine.

The acute toxicity (LD_{50}) was determined by single intraperitoneal injections to a group of male mice weighing 18 – 22 g. The ED_{50} and LD_{50} values were calculated according to the Litchfield – Wilcoxon method [9]. The therapeutic index (TI) was calculated as the ratio LD_{50}/ED_{50}.

In order to elucidate the mechanism of antihypoxic activity of the synthesized compounds, we have studied the state of redox potential of the flavine and pyridine nucleotides in abdominal ganglionic neurons (Retzius cells) of the medicinal leech *Hirudo medicinalis* by the method of fluorescent microscopy. The fluorescence intensity of neurons *in situ* was measured in the green and violet spectral regions (excitation wavelengths, 436 and 385 nm; detection wavelengths, 530 and 470 nm, respectively). Variations in the intensity of emission from these nucleotides are proportional to changes in the amount of oxidized forms of the flavine nucleotides and reduced forms of the pyridine nucleotides [10]. The emission was measured with the aid of LYUMAM IUF and ML-4 microfluorimeters using a variable-area optical probe, which allowed the fluorescence of a single neuron to be observed.

The antiinflammatory activity with respect to a model of the adrenaline-induced lung edema was studied in a group of male and female white mongrel mice weighing 18 – 20 g.[2] Other models were studied on white rats weighing 180 – 200 g. Each test was performed in a group of not less than six animals. The lung edema was induced by subcutaneously injecting adrenaline hydrochloride at a dose of 5 mg/kg. The edema growth was characterized by the so-called lung coefficient (LC) determined as the percentage ratio of the lung and body weights. The compounds to be tested were introduced in the form of 0.1 or 0.25% aqueous solutions (doses 1 and 5 mg/kg or 10 and 20 mg/kg, respectively) by single intraperitoneal injections 1 h before adrenaline. Insoluble substances containing no aminoalkyl groups were injected as 1% DMSO solutions. For this reason, Table 3 also shows the results of control experiments with DMSO known to possess certain antiinflammatory activity by itself. Animals in the other control groups were injected with the corresponding solvents or physiological solution. The reference drug was indomethacin injected at a dose of 12 or 20 mg/kg in the form of a 1% solution in 2.5% aqueous sodium carbonate.

In the thermal burn model, the extent of the inflammation process was evaluated by an increase in the volume of a hind paw upon a 1-min immersion into water at 51°C. The volume increments were determined oncometrically 1, 3, 5, and 24 h after the burn. considering the volumes of intact extremities as equal to 100%. The test substances were introduced by single injections 1 h before the burn induction.

Effects of the synthesized compounds on the exudative and proliferative components of aseptic inflammation were assessed using the model of cotton-induced granuloma. The test compounds were intraperitoneally injected as 1% solutions in water or DMSO (Table 3) during three days after operation (therapeutic test) or during three days before and three days after operation (prophylactic-therapeutic test). The effect of a drug on the exudative component was judged by the weight difference between the freshly separated granuloma and that dried to a constant weight, while the effect on the proliferative component was evaluated by the weight difference between dried capsule and the ball proper.

All experimental data were mathematically processed by methods of variational statistics. The results of the control and test groups were compared using the Student *t*-criterion and correlation analysis techniques [11]. The correlation parameters were processed on an HP-1000 computer.

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