SYNTHESIS AND BIOLOGICAL ACTIVITY OF ALIPHATIC AND AROMATIC SULFONIC ACID AZOLIDES

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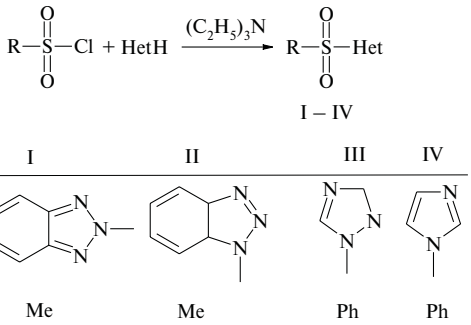
Previously [1, 2] we synthesized a series of azolides of aliphatic and aromatic sulfonic acids and studied their immunomodulant and cytoprotector properties. In continuation of that work, we have synthesized a series of new aliphatic and aromatic sulfonic acid azolides and evaluated the products with respect to biological activity. The new series includes 2-benzotriazolide (I) and benzimidazolide (II) of methanesulfonic acid and 1,2,4-triazolide (III) and imidazolide (IV) of benzenesulfonic acid.

The initial compounds were chloroanhydrides of the sulfonic acids and the corresponding nitrogen-containing heterocycles: imidazole, 1,2,4-triazole, benzimidazole, and benzotriazole. The reactions were conducted at room temperature in anhydrous ether or anhydrous chloroform in the presence of triethylamine.



EXPERIMENTAL CHEMICAL PART

The IR absorption spectra were measured on an IKS-29 spectrophotometer (LOMO Company, Russia) using samples pelletized with KBr. The ^{1}H NMR spectra were recorded on a Bruker WP-270SY spectrometer (Germany) operating at a working frequency of 270.12 MHz. The samples were dissolved in DMSO-d_6. The yields and physicochemical characteristics of the synthesized compounds (I – IV) are listed in Table 1.

Methanesulfonic acid 2-benzotriazolide and benzimidazolide (I, II). To a solution of 0.495 g (5 mmole) 2-benzotriazole (or 0.590 g benzimidazole) in 15 ml of diethyl ether was added 0.607 g (6 mmole) of triethylamine. To this mixture was added dropwise over 30 min a solution of 0.573 g (5 mmole) of methanesulfonic acid chloroanhydride in 10 ml of anhydrous ether, and the reaction mixture was kept for 3 h at room temperature. The precipitate of triethylamine hydrochloride was separated by filtration and the filtrate was evaporated in vacuum to obtain compounds I and II in the form of yellowish oils.

Benzenesulfonic acid 1,2,4-triazolide and imidazolide (III, IV). To a solution of 0.345 g (5 mmole) 1,2,4-triazole (or 0.340 g imidazole) in 20 ml of anhydrous chloroform was added 0.607 g (6 mmole) of triethylamine. To this mixture was added dropwise over 40 min a solution of 0.885 g (5 mmole) of benzenesulfochloride in 15 ml of anhydrous chloroform, and the reaction mixture was kept for 4 h at room temperature. The precipitate of triethylamine hydrochloride was separated by filtration and the filtrate was evaporated in vacuum to obtain compounds III and IV in the form of white crystals.

EXPERIMENTAL BIOLOGICAL PART

Investigation of the pharmacological properties of new compounds must be preceded by studying their mutagenicity and cytotoxicity aimed at roughly estimating the prospects for practical use of these compound as drugs. Genotoxicity was evaluated by the standard tests on *Drosophila menogaster*. The recessive lethal mutation analysis involved 500 to 900 chromosomes, while the dominant lethals were analyzed for 1500 chromosomes. All sulfonic acid azolides were studied at LD_{50} for the test fly species.

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TABLE 1. Yields and Physicochemical Characteristics of Compounds I – IV

Compound	Empirical formula	IR spectrum: ν_{max}, cm^{-1}	Yield, %	^1H NMR chemical shift: δ, ppm
I	$C_7H_7N_3O_2S$	1040 (SO_2) 1380 (SN)	85	7.91 – 7.88 (m, 2H), 7.43 – 7.39 (m, 2H), 2.57 (s, 3H, CH_3)
II	$C_8H_8N_2O_2S$	1050 (SO_2) 1350 (SN)	87	8.55 (s, 1H), 7.86 – 7.81 (m, 2H), 7.48 – 7.43 (m, 2H), 3.70 (s, 3H, CH_3)
III	$C_8H_7N_3O_2S$	3160, 3100 (C-H_{triaz}), 3070 (C-H_{phenyl})	85	10.00 (s, 2H, H-triaz.), 9.16 (s, 1H, H-2′-phenyl), 7.51 (m, 1H, H-3′-phenyl), 7.49 (m, 1H, H-3′-phenyl), 7.11 (m, 1H, H-4′-phenyl), 7.10 (s, 1H, H-5′-phenyl)
IV	$C_9H_8N_2O_2S$	3150, 3120 (C–$H_{imidaz.}$), 3070 (C–H_{phenyl})	85	7.58 (m, 1H, H-2-imidaz.), 8.84 (s, 1H, H-4-imidaz.), 7.39 (s, 1H, H-2′-phenyl), 7.29 (m, 1H, H-3′-phenyl), 7.14 (m, 1H, H-4′-phenyl), 7.12 (m, 1H, H-5′-phenyl), 7.11 (m, 1H, H-5′-phenyl)

Cytotoxicity of the synthesized compounds was studied using an endothelial cell culture grown in modified Petri dishes [3]. The funic endothelium was taken from an Rh-positive fetus with blood group 0(I) obtained from physiologically pregnant females. The cell dissociation was provided by a combined enzymatic-mechanical technique. The cytotoxicity was evaluated by counting viable and lost cells after a 96-h incubation of the cell cultures with test compounds and ethidium bromide and acridine orange staining [3].

TABLE 2. Cytotoxic and Cytoprotector Effect of Aliphatic and Aromatic Sulfonic Acid Azolides with Respect to Endothelial Cell Culture

Compound (*n*, number of tests)	Percentage cell survival	Percentage cell loss	Percentage cell survival in culture with AEABs	Percentage cell loss in culture with AEABs
I (*n* = 19)	96.2 ± 3.4	3.8 ± 1.6	53.4 ± 13.2*	46.6 ± 10.2*
II (*n* = 21)	94.3 ± 2.9	5.7 ± 2.0	20.2 ± 9.6	79.8 ± 14.3
III (*n* = 24)	95.3 ± 4.1	4.7 ± 1.9	64.3 ± 11.5*	35.7 ± 9.9*
IV (*n* = 18)	90.5 ± 6.8	9.5 ± 2.3	39.6 ± 8.7	60.4 ± 12.3
Control 1 (with AEABs) (*n* = 17)	21.3 ± 12.9	78.7 ± 14.6	–	–
Control 2 (without drugs, with AEABs) (*n* = 14)	92.1 ± 3.6	7.9 ± 2.1	–	–

Note. $P < 0.05$ relative to control 1.

The immunomodulant properties were estimated using an enzymatic test with peripheral blood monocytes (PBMs), by determining the activity of pair glycolytic enzymes α-glycerophosphatedehydrogenase (α-GPDG) and succinatedehydrogenase (SDG). The test was performed with the blood of (i) females with a physiological course of pregnancy (23 cases) possessing a relative (physiological) immunodeficiency and (ii) females with hestosis (32 cases) featuring α-GPDG and SDG activity [4]; a group of 26 healthy nonpregnant females of the corresponding age served as the control group. The α-GPDG and SDG activity in PBMs was determined by microscopic examination of peripheral blood smears after conventional cytochemical processing [5]. The PBM were isolated by the ficoll – hypaque gradient method [3]. The immunomodulant effect of the synthesized compounds was evaluated 72 h after introduction into the cultures of monocytes taken from females of both test and control groups.

The index of cytotoxicity (IC) of the sera containing anti-endothelial antibodies (AEABs) was evaluated according to the NIAID standard (National Institute of Allergies and Infectious Diseases, USA) using the monocyte cross test based on the antigen concordance between endotheliocytes and monocytes [6]. For this analysis, monocytes used as the test cells were isolated from funic blood of healthy Rh-positive fetus with blood group 0(I). The cells were incubated for 30 min with serum, and then for 2 h with the complement. The reacted cells were fixed with formaldehyde and stained with eosin. The IC of AEABs was rated against the following scale depending on the content of damaged cells: negative result, below 10%; weak positive result, 10 – 20%; positive result, 20 – 50%; sharply positive result, 50 – 100%.

RESULTS AND DISCUSSION

According to the test results, the mutagenicity of compounds I – IV is characterized by the following values. Methanesulfonic acid benzotriazolide (I) induced 1.18% recessive lethal mutations in X-chromosomes of *Drosophila menogaster* males, while benzenesulfonic acid 1,2,4-triazolide benzimidazolide (III) induced 1.97% such lethals. Benzenesulfonic acid imidazolide (IV) induced 6.95% dominant lethal mutations, while methanesulfonic acid benzimidazolide (II) induced 21% such mutations in both

TABLE 3. Immunomodulant Effect of Aliphatic and Aromatic Sulfonic Acid Azolides with Respect to the Activity of α-Glycerophosphatedehydrogenase (α-GPDG) and Succinatedehydrogenase (SDG) in Peripheral Blood Monocytes

Compound	Physiological pregnancy		Hestosis	
	α-GPDG (granules)	SDG (granules)	α-GPDG (granules)	SDG (granules)
I	3.4 ± 0.32*	5.2 ± 0.49*	7.1 ± 0.56*	7.3 ± 0.39*
III	3.9 ± 0.24*	5.6 ± 0.51*	5.9 ± 0.42*	6.8 ± 0.44*
IV	1.1 ± 0.16	4.2 ± 0.33	18.3 ± 0.97	16.6 ± 0.81
Control	1.2 ± 0.16	4.3 ± 0.26	16.9 ± 0.71	14.2 ± 0.8

Note. $P < 0.05$ relative to normal level (average α-GPDG and SDG activity in nonpregnant females was 3.6 ± 0.62 and 5.3 ± 0.78 granules, respectively.

males and females. The difference between test and control groups was reliable to within $P > 95\%$ for both recessive and dominant lethal mutations (for drug doses on the *Drosophila menogaster* LD_{50} level). Activation of the test substances by the microsomal fraction led to an increase in the amount of dominant lethals. Thus, the lowest mutagenic activity among the studied substances was observed for compound I.

Compounds I – IV in concentrations of 1×10^{-4}, 1×10^{-6}, 1×10^{-3}, and 1×10^{-4} mole/liter, respectively, did not produce any cytotoxic action (data on the content of living cells in the cultures treated with these concentrations are summarized in Table 2). Methanesulfonic acid benzimidazolide (II) in a concentration of 1×10^{-2} mole/liter led to a 100% loss of the test cells, which was related to the manifestation of strong acidity of the medium (pH 1.62). Compounds I, III, and IV (but not II) in a concentration of 1×10^{-5} mole/liter exhibited a cytotoxic effect with respect to vascular endothelium (in the test with AEAB introduced into the endotheliocyte culture), whereby the IC in the monocyte cross test corresponded to a positive rating of 20 – 50% (Table 2).

Data on the variation of γ-GPDG and SDG activity under the action of compounds I, III, and IV in PBMs cultures of females with physiological pregnancy and hestosis are presented in Table 3. As can be seen, compounds I and III in concentrations of 1×10^{-4} and 1×10^{-5} mole/liter, respectively, produce opposite effects on the activity of pair glycolytic enzymes in the two test groups: in the former case (relative immunodeficiency), the γ-GPDG and SDG activity increases to a level observed in nonpregnant control, while in the latter case (high initial level), this activity decreases. Compound IV in a concentration 1×10^{-4} mole/liter did not produce any significant immunomodulant effect in the enzymatic test with PBMs.

Thus, the obtained experimental results show good prospects for the further study of the specific immunomodulant activity of aliphatic and aromatic sulfonic acid azolides under whole-organism conditions.

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