# SYNTHESIS AND IMMUNOCORRECTOR ACTIVITY OF AROMATIC SULFONIC ACID AZOLIDES

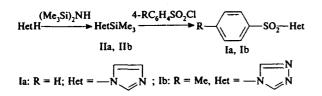
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Previously we have established that some imidazole and benzimidazole derivatives exhibit cytotoxic and mutagenic action with respect to *Drosophila melanogaster* larvae [1]. Therefore, it was of interest to study the immunocorrector activity of azolides with respect to the human blood cells.

For this purpose, we have synthesized aromatic sulfoacid azolides (Ia, Ib) using the following reaction scheme:



#### EXPERIMENTAL CHEMICAL PART

The IR absorption spectra were measured on an IKS-29 spectrophotometer (LOMO company, Russia) using samples pelletized with KBr. The UV spectra were recorded on an SF-46 spectrophotometer (LOMO, Russia). The melting temperatures were determined using a PTP instrument (Khimlabpribor plant, Russia). The yields and physicochemical characteristics of compounds Ia and Ib are given in Table 1.

1-Benzenesulfonylimidazole (Ia). To 0.95 g (5 mmole) of benzenesulfochloride dissolved in 25 ml of absolute benzene was added 0.90 ml (5 mmole) of N-trimethylsilylimidazole (IIa) obtained as described in [2], and the mixture was boiled for 3.5 h. After termination of the reaction, the reaction mass was evaporated to dryness on a rotor evaporator and the residue recrystallized from a benzene – cyclohexane . (1:3) mixture.

**4-(4-Toluenesulfonyl)-1,2,4-triazole** (Ib). To 0.63 g (4.5 mmole) of N-trimethylsilyltriazole (IIb) obtained as described in [2] was added 0.85 g (4.5 mmole) of 4-toluenesulfochloride and the mixture was heated for 2.5 h at 80°C. The resulting triazolide was recrystallized from methanol.

#### EXPERIMENTAL BIOLOGICAL PART

Experiments were performed on lymphocytes and polymorphonuclear granular leukocytes (PGL) isolated from the blood of 15 healthy donors and 20 patients with a bronchial asthma diagnosis in the remittance state. We have estimated the cytotoxic action of compounds Ia and Ib on leukocytes, on the phagocytic activity of PGL, and on the ability of blood lymphocytes to spontaneous E-rosette formation (E-RF) with goat erythrocytes; for this purpose, the test compounds were incubated with these objects.

Lymphocytes were isolated by the ficoll – verografin gradient centrifugation technique  $(1.077 \text{ g}/\text{cm}^3)$ .

The cytotoxicity of compounds Ia and Ib was evaluated by counting viable and lost cells upon the ethidium bromide and acridine orange staining [3].

The effect of compounds Ia and Ib on the E-RF ability of lymphocytes were assessed using the spontaneous rosette formation tests with lymphocytes preincubated for 30 min with Ia and Ib or with a physiological solution (control) [4].

The phagocytic activity of leukocytes (PAL) was estimated by their ability to produce phagocytosis of polystyrene latex upon a 30 min incubation at 37°C with compounds Ia

TABLE 1. Yields and Physicochemical Characteristics of Compounds Ia and Ib

Com- pound		М.р., °С	Elemental analysis, %	UV spectrum: $\lambda_{max}$ , nm (log $\epsilon$ )	IR spectrum: v, cm <sup>-1</sup>
Ia	99	83 – 84	Found: C, 51.88; H, 3.69; N, 13.55. For C <sub>9</sub> H <sub>8</sub> N <sub>2</sub> O <sub>2</sub> S anal. calcd.: C, 51.91; H, 3.87; N, 13.45	210 (3.82) 224 (3.95)	3150, 3120 (C H <sub>imidazole</sub> ), 3100, 3000 (CH <sub>phenyl</sub> ), 1375, 1040 (-SO <sub>2</sub> )
њ	- 75	106	Found: C, 48.17; H, 4.39; N, 18.80. For $C_9H_9N_3O_3S$ anal. calcd.: C, 48.21; H, 4.46; N, 18.75	204 (4.14) 235 (3.91)	3160, 3100 (C- H <sub>triazole</sub> ), 3070 (C- H <sub>phenyl</sub> ), 2920, 2860 (C-H <sub>methyl</sub> ), 1380, 1050 (-SO <sub>2</sub> )

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**TABLE 2.** Effects of Compounds Ia and Ib on the E-Rosette Formation Reaction of Leukocytes in the Blood of Healthy Donors (HD) and Bronchial Asthma (BA) patients

Comment	Phagocytic activity, %		Sensitive leukocytes, %		Stimulation index, %	
Compound	HD	BA	HD	BA	HD	BA
Control	80.4 ± 3.5	76.7 ± 1.1	-	-		-
la	89.1 ± 2.9*	75.4 ± 2.5	8.7 ± 1.1	1.0 ± 1.7	11.5 ± 9.7	$1.4 \pm 2.2$
ІЪ	88.4 ± 2.3*	87.9 ± 1.0*	8.0 ± 1.5	11.1 ± 0.6	11.0 ± 2.8	14.5 ± 0.9

\* p < 0.05 relative to control.

TABLE 3. Effects of Compounds Ia and Ib on the Phagocytic Activity of Leukocytes in the Blood of Healthy Donors (HD) and Bronchial Asthma (BA) patients

	Phagocytic activity, %		Sensitive leukocytes, %		Stimulation index, %	
Compound	HD	BA	HD	BA	HD	BA
Control	81.5 ± 1.1	72.6 ± 2.5			-	
Ia	92.3 ± 1.0*	77.4 ± 2.6*	$10.8 \pm 1.1$	4.8 ± 1.1	13.4 ± 1.5	6.6 ± 1.5
Б	$93.3\pm0.6^{\ast}$	82.0 ± 2.5*	$11.8 \pm 0.7$	9.4 ± 0.4	$14.5 \pm 1.0$	$12.9\pm0.7$

p < 0.05 relative to control.</li>

and Ib or with a buffered (pH 7.2) physiological solution (control).

The results of tests were expressed as the relative numbers of rosette-forming lymphocytes and phagocytic leukocytes in the test (T) tubes with respect to the control (C) tubes. These values were used to calculate the percentage of sensitive cells (T-C) and the stimulation index  $100 \times (T-C)/K$  of the blood cells.

## **RESULTS AND DISCUSSION**

It was found that compounds Ia and Ib at a concentration of  $1 \times 10^{-5}$  or  $1 \times 10^{-7}$  M exhibited no pronounced cytotoxic effect. At the same time, compound Ib taken at a concentration of  $1 \times 10^{-3}$  M produced a 100% loss of the cells, which was related to the strong acidity of the solution (pH 1.26).

Preliminary tests showed that compound Ia exhibited the most pronounced action with respect to the E-RF reaction and

PAL manifestations at a concentration of  $1 \times 10^{-7}$  M, and compound Ib at  $1 \times 10^{-5}$  M. The subsequent experiments were conducted with these very concentrations

It was established that compounds Ia and Ib equally stimulated the E-RF reaction and PAL manifestations in the blood of both healthy donors and bronchial asthma patients (Table 2 and 3).

On the background of reduced E-RF ability and PAL observed for the bronchial asthma patients (compared to the healthy donors), the preincubation with compound Ia further decreases the content of E-rosette forming cells, whereas compound Ib stimulates the blood lymphocytes with respect to the E-RF reaction.

Study of the effect of compounds Ia and Ib on the PAL manifestations in the bronchial asthma patients showed that both compounds stimulate the absorption of polystyrene latex by leukocytes, as evidenced by an increase both in the content of active cells and in the stimulation index.

Thus, we have established that compounds Ia and Ib exhibit pronounced immunomodulating ac-

tivity *in vitro* with respect to the blood cells of both healthy donors and bronchial asthma patients. Taking into account the low cytotoxicity of these compounds, we may expect promising results from further investigations of the specific effects of aromatic sulfoacid azolides under conditions of the whole organism.

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