SEARCH FOR NEW DRUGS

SYNTHESIS OF 1-CYANOBENZIMIDAZOLE AND EVALUATION OF ITS BIOLOGICAL ACTIVITY BY THE WHITE-BLOOD REACTION

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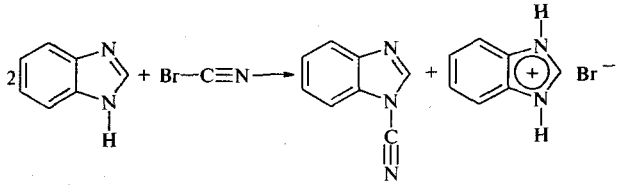
Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 34, No. 2, pp. 11 – 13, February, 2000.

Original article submitted March 18, 1999.

We have used the following scheme to synthesize 1-cyanobenzimidazole [1]:



Benzimidazole-based compounds possess a broad spectrum of action, and numerous benzimidazole derivatives are applied in various fields of medical practice. A very important feature of the action of benzimidazole is an increase in the nonspecific resistance of the organism [2]. Resistance induced by the administration of these preparations may be maintained for a prolonged time, not followed by depletion effects possible in the case of stress.

An important part in the characterization of new benzimidazole preparations is the study of their action on the hemopoietic system. Indeed, this system must react to any chemical factor – and the response reflects both the level of homeostasis maintained in the biological object and its resistance characteristics [2 – 4]. Taking into account that the resistance of the organism is largely determined by the function of formed blood elements [5, 6] it was of special interest to elucidate the character of leukocyte reaction to the introduction of 1-cyanobenzimidazole.

EXPERIMENTAL CHEMICAL PART

The IR spectra were measured with an IKS-29 spectrophotometer (LOMO, Russia) using samples pelletized with KBr. The UV spectra were recorded on an SF-26 spectrophotometer (LOMO, Russia) in aqueous solutions. The ^1H and ^{13}C NMR spectra were obtained with a Bruker WP-200SY instrument (Germany) operating at 200.13 and 50.33 MHz, respectively, using DMSO-d_6 as the solvent. The melting temperatures were determined with a PTP device (Khimlabpribor, Russia).

1-Cyanobenzimidazole. The initial benzimidazole (3.1 g, 26.25 mmole) was dissolved on heating in 200 ml of anhydrous benzene. When the major part of benzimidazole dissolved, a solution of bromocyan (1.39 g, 13.11 mmole) in 80 ml of the same solvent was added through a reflux cooler, which was accompanied by precipitation of benzimidazole hydrobromide. The reaction mixture was heated to boiling and treated at this temperature for 1 h. Then the reaction mass was allowed to cool down to 30°C and the precipitated benzimidazole hydrobromide was separated by filtration. The filtrate was immediately evaporated on a rotor evaporator at a pressure of 20 Torr and a temperature not exceeding 50°C. The product obtained upon evaporation of the solvent was purified from benzimidazole impurity by flash chtromatography on a dry column [7] filled with a silica gel (TLC chromatography grade) and eluted with anhydrous ethyl acetate (R_f = 0.95). Yield of 1-cyanobenzimidazole, 75%; m.p., 105 – 106°C; $C_8H_5N_3$; IR spectrum (ν_{max}: 2265 cm^{-1} (C≡N); UV spectrum (λ_{max}): 212 nm (log ε = 4.30); ^1H NMR spectrum (δ, ppm): 7.39 – 7.51 (m, 2H, H-1), 7.69 (g, ^1H, J 7.25 Hz), 7.81 (g, 1H, J 7.25 Hz), 8.88 (s, 1H); ^{13}C NMR (δ, ppm): 104.8 (C≡N), 111.0, 120.1, 125,4, 126.0, 132.1, 141.0, 143.4.

EXPERIMENTAL BIOLOGICAL PART

The effects of 1-cyanobenzimidazole on the morphofunctional characteristics of leukocytes were experi-

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mentally studied on a group of 20 white male rats weighing 250 – 300 g, one half used in the test and another half serving as the control. Animals in the test group received a single intraperitoneal injection of the substance studied (50 mg/kg) in 1 ml of a physiological solution for warm-blooded mammals (0.85% sodium chloride) [8]. Animals in the control group were injected with the same volume of the pure physiological solution.

The effects of 1-cyanobenzimidazole on the morphofunctional characteristics of leukocytes were judged by monitoring dynamics of the total leukocyte number, particular leukocyte forms, and morphological changes using conventional hematological techniques [9]. The total leukocyte number was determined in a Goryaev chamber using blood cells stained with Türk's reagent. The leukocyte forms were identified using blood smears stained according to the Romanowsky method.

The blood analyses in both test and control animals were carried out before and after (2.5 h, 1, 3, 7, 14, and 21 days) injections [10]. The experimental data were statistically processed using the method of indirect differences [11], after which the significance levels of leukocyte reactions were evaluated according to Student.

RESULTS AND DISCUSSION

The results of our investigation showed that a single injection of 1-cyanobenzimidazole at a dose of 50 mg/kg led to significant changes both in the total number of leukocytes and in their individual forms (Table 1).

Changes in the leukocyte morphology were manifested only in the form of increasing cell diameters. Dynamic variations in the number of leukocytes were also observed in the peripheral blood of animals in the control group, but these changes neither exhibited pronounced trends nor followed clear laws and were most likely related to some factors permanently operative under standard nursery conditions such as the keeping regime, feeding schedule, intragroup relations, etc. [12]. Nevertheless, a comparative analysis of leukocyte reactions in the test and control groups allowed us to unambiguously separate changes caused by the presence of 1-cyanobenzimidazole, which showed evidence of the high biological activity of this compound.

In particular, statistically significant variations were observed in the numbers of leukocytes in all special groups evaluated. These changes were retained over a rather long period of time, which could be followed up to three days for the dynamics of segmented neutrophils, seven days for the

TABLE 1. Effect of 1-Cyanobenzimidazole on the Dynamics of Total Leukocyte Number and All Particular Leukocyte Forms in Peripheral Blood

Group of animals	Initial value ($M \pm m$)	Values after injection ($M \pm m$), for observation time					
		2.5 h	24 h	3 days	7 days	14 days	21 days
				Total leukocyte number			
Control, 10^9/liter	20.03 ± 1.23	22.77 ± 1.40	20.43 ± 1.24	23.00 ± 1.60	22.50 ± 1.38	22.60 ± 1.41	22.79 ± 1.75
Test, 10^9/liter	19.87 ± 1.21	18.23 ± 1.28*	16.42 ± 1.12*	18.37 ± 1.18*	19.67 ± 1.40	18.80 ± 1.36	22.10 ± 1.54
				Segmented neutrophils			
Control, %	19.17 ± 1.46	22.0 ± 1.78	26.17 ± 1.71	24.0 ± 1.78	28.67 ± 1.92	24.00 ± 2.00	23.83 ± 1.92
Test, %	20.71 ± 1.77	23.17 ± 1.91	19.33 ± 1.53*	16.50 ± 1.59*	21.00 ± 1.70*	23.67 ± 1.96	21.50 ± 1.90
				Band neutrophils			
Control, %	10.83 ± 0.51	8.00 ± 0.32	7.83 ± 0.32	7.33 ± 0.31	8.17 ± 0.39	9.67 ± 0.48	8.50 ± 0.33
Test, %	9.92 ± 0.41	9.33 ± 0.35	9.23 ± 0.34*	8.67 ± 0.34*	10.57 ± 0.39*	10.90 ± 0.38	9.00 ± 0.43
				Basophils			
Control, %	0.33 ± 0.05	0.17 ± 0.03	0.33 ± 0.03	0.17 ± 0.03	0.17 ± 0.03	0.17 ± 0.02	0.17 ± 0.03
Test, %	0.35 ± 0.03	0.50 ± 0.08*	0.67 ± 0.09*	0.67 ± 0.06*	0.50 ± 0.04*	0.33 ± 0.04*	0.33 ± 0.04*
				Eosinophils			
Control, %	0.50 ± 0.03	0.50 ± 0.02	0.50 ± 0.04	0.50 ± 0.02	0.50 ± 0.03	0.50 ± 0.02	0.50 ± 0.02
Test, %	0.50 ± 0.03	0.30 ± 0.02*	0.70 ± 0.04*	1.00 ± 0.07*	0.80 ± 0.04*	0.70 ± 0.03*	0.60 ± 0.03
				Monocytes			
Control, %	8.00 ± 1.06	4.50 ± 0.82	2.25 ± 0.41	4.33 ± 0.83	4.50 ± 0.71	4.17 ± 0.67	2.33 ± 0.33
Test, %	7.25 ± 1.01	2.50 ± 0.62	3.67 ± 0.48*	3.83 ± 0.91	1.67 ± 0.50*	1.50 ± 0.31*	3.83 ± 0.39*
				Lymphocytes			
Control, %	58.50 ± 2.18	65.33 ± 2.33	63.83 ± 2.28	57.67 ± 2.11	56.17 ± 2.04	60.07 ± 2.07	63.11 ± 2.92
Test, %	60.67 ± 2.29	63.17 ± 2.41	68.17 ± 2.39	69.50 ± 2.31*	66.50 ± 2.38*	65.17 ± 2.23*	66.57 ± 2.71

* Difference from control reliable for $p < 0.05$.

band neutrophils, two weeks for eosinophils and lymphocytes, and three weeks for basophils.

We have observed a 18 – 20% increase in the diameter of all white blood cells relative to the control ($p < 0.05$). This phenomenon is interpreted by many researchers [13 – 15] as a manifestation of enhanced trophism.

An increase in the total number of lymphocytes (20.5%, $p < 0.05$), eosinophils (100.0%, $p < 0.05$), and basophils (294.1%, $p < 0.05$) observed in all cases on the third day is evidence of activation of the specific adaptation mechanisms [16]. The fact that maximum particular leukocyte reactions were observed at different time instants (2.5 and 24 h, 3 days and later) after the administration of 1-cyanobenzimidazole indicates that these effects are realized on different levels, including circulating blood, hemopoietic organs, and humoral regulation system [15].

In concluding our analysis of the effect of 1-cyanobenzimidazole on the leukocyte reaction, it is necessary to emphasize that we did not observe changes in the cell morphology such as hypersegmentation of the neutrophil nuclei, extensive large-scale granulation, and vacuolization of the leukocyte cytoplasm. This fact is indicative of the low toxicity of 1-cyanobenzimidazole and is evidence for the possibility of using this compound in drug synthesis.

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