SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5,6-DINITRO DERIVATIVES OF BENZIMIDAZOLE

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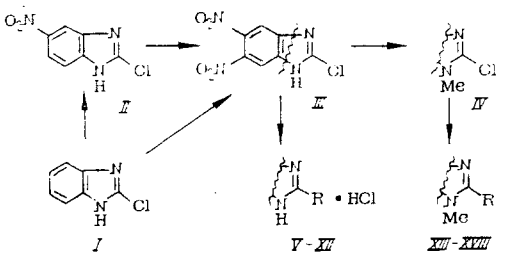
We have shown by mathematical analysis of the links between structure and anti-influenza activity that a number of benzimidazole derivatives, containing substitutions as position 2 are portentially useful agents, especially those containing amino- and alkyl(cycloalky)amino groups [4, 6]. We report here the synthesis and studies of the antiviral and antimicrobial activities of 5,6-dinitro derivatives of benzimidazole, containing amino residues in position 2 (compounds V-XVIII).



The starting material for the synthesis of V-XVIII was 5,6-dinitro-2-chlorobenzimidazole (III). This was prepared by addition of a nitro group to 2-chlorobenzimidazole, a reaction which produces either the mono- or dinitro derivatives II and III depending on the conditions. Addition of the nitro group using nitric acid (d 1.5) at 60-70°C produces 5(6)-nitro-2-chlorobenzimidazole (II) with a yield of 70-75%; reducing the nitric and concentration (d 1.4) reduces the yield of II to 20-25%. In more extreme conditions (boiling in a mixture of nitric (d 1.5) and concentrated sulfuric acids), the reaction produces 5,6-dinitro-2-chlorobenzimidazole (III) with a yield of 75-80%. 1-Methyl-5,6-dinitro-2-chlorobenzimidazole (IV) was prepared methylation of compound III with methyl iodide. Short periods of boiling 2-chloro derivatives of compounds III and IV with amines and hydrazine hydrate results in the formation of compounds V-XVIII. The structures of the newly synthesized compounds were confirmed by paramagnetic resonance (PMR) spectroscopy. The spectra of compounds V-XII contained singlet bands at 8.0 ppm, corresponding to signals from protons 4-H and 7-H, while the spectra of compounds XIII-XVIII contained two singlets at 7.8-8.0 and 8.1-8.3 ppm from protons 4-H and 7-H and a singlet at 3.7-3.9 ppm from protons of the methyl groups. Signals from substituents position 2 fully corresponded to the expected structures in terms of their singlet structure and chemical shifts (Table 1).

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TABLE 1. Properties of Compounds V-XVII

Compound	Yield, %	Melting point °C (solvent for recrystallization)	R_f (solvent system)	Molecular formula
V	70	175—177 (water)	0,58 (b)	$C_{14}H_{12}ClN_5O_4$
VI	75	178—179 (water)	0,44 (b)	$C_{11}H_{12}ClN_5O_4$
VII	65	330—335 (50% dimethylformamide)	0,64 (a)	$C_{11}H_{11}ClN_5O_5$
VIII	70	163—164 (water)	0,56 (b)	$C_{12}H_{14}ClN_5O_4$
IX	70	166—169 (water)	0,42 (a)	$C_{13}H_{16}ClN_5O_5$
X	72	193—195 (water)	0,27 (b)	$C_{13}H_{13}ClN_5O_5$
XI	68	213—215 (water)	0,60 (a)	$C_9H_{10}ClN_5O_5$
XII	75	163—165 (isopropanol)	0,20 (a)	$C_{15}H_{22}ClN_5O_4$
XIII	70	170—172 (water)	0,37 (a)	$C_{15}H_{13}N_5O_4$
XIV	68	230—232 (dioxane)	0,48 (b)	$C_{12}H_{13}N_5O_4$
XV	72	218—220 (70% dimethylformamide)	0,47 (a)	$C_{12}H_{13}N_5O_5$
XVI	70	152—154 (dioxane)	0,49 (a)	$C_{13}H_{15}N_5O_4$
XVII	65	175—177 (50% dimethylformamide)	0,48 (a)	$C_{14}H_{16}N_5O_4$

Antiviral activity was studied using herpes simplex virus type I, vaccinia, respiratory syncytial vesicular stomatitis, classical avian distemper, Venezuelan equine encephalomyelitis, and ECHO-6 viruses in tissue cultures. 1-Methyl-2-morpholino-5,6-dinitrobenzimidazole (XV) was active against vaccinia (+++). These results were also supported by animal experiments (Table 2). Treatment of guinea pigs with experimental vaccinia keratoconjunctivitis significantly reduced the intensity of clinical sings of disease (on the fourth and later days of treatment). This therapeutic effect was supported by a significant reduction in virus reproduction in the eye tissues in treated animals, from > 5.5 to <3.0 log $TCIC_{50}$ per 0.1 ml.

The antiviral activity of 5,6-dinitro derivatives of benzimidazole was studied using influenza A and B viruses in experiments on developing chick embryos and white mice. Mice received five doses, 24 and 1 h before infection, and 24, 48, and 72 h after infection. This regime combines the prophylactic and therapeutic actions of the preparations. Most of the compounds synthesized had anti-influenza activity, especially against influenza virus type B. The compounds with the greatest potential were the derivative in which position 2 was substituted with residues of benzylamine, piperidine, and morpholine, the indexes of protection for compounds VII, XIII, XV, and XVI of 56-67%. It should be noted that the activity of these compounds was lower in chick embryos than in vivo (indices of efficacy in the range 30-40%). These results support the conclusions of a mathematical analysis, which suggested that the nitro group has little positive effect on the anti-influenza A activity of benzimidazole derivatives [6]. In relation to influenza B viruses, the nitro derivatives were classed as typical structures (class II) [4], and their modification produced compounds with high level of activity.

Since the literature contains data indicating that nitrobenzimidazoles have antibacterial activity, the antimicrobial activity of the compounds synthesized here was tested using Gram positive and Gram negative opportunistic bacteria, and yeast-like and phytopathogenic fungi. The results of these studies are shown in Table 3. The 5,6-dinitro derivatives of benzimidazole had antibacterial activity against Gram positive microorganisms; the bacteriostatic dose was 10-400 μg/ml. The greatest levels of activity were found in relation to Corynebacterium diverycatum. The most active compounds lacked substituents at the N(1) atom. 5,6-Dinitrobenzimidazoles lacked antifungal activity against strains of Candida albicans and Fusarium oxysporum.

MATERIALS AND METHODS

Chemical Methods

PMR spectra were recorded using a Perkin-Elmer R-12B apparatus (60 MHz) using DMSO-d_6 as solvent and TMS as internal standard. Reaction extent and product purity were assessed by thin layer chromatography on Silufol UV-254 (Czechoslovakia) plates using the solvent systems chloroform/ethanol (10:1) (a) and chloroform/ethyl acetate (10:3) (b). Elemental analyses were supported by the calculated values.

5(6)-Nitro-2-chlorobenzimidazole (II)

2-Chlorobenzimidazole (15.3 g, 0.1 mol) was added, with mixing, to 90 ml of NHO_3 (d 1.5), heated to 70°C, and incubated for 1 h. After cooling, the reaction mix was poured onto 500 g of ice, neutralized with ammonia to pH 3-4, and the insoluble material was collected by filtration. The yield was 14.5 g (73.6%). The melting point was 222-223°C (in 50% ethanol). The molecular formula was $C_7H_4N_3O_2Cl$.

TABLE 2. Effect of 1-Methyl-2-morpholino-5,6-dinitrobenzimidazole (XV) on Experimental Vaccinia Keratoconjunctivitis in Guinea Pigs

Group of animals	Clinical symptions, points (X ± m) day of observation					Titer of virus in conjunctival scrapings, log $TCID_{50}$/0.1 ml
	first	second	fourth	seventh	tenth	
Control group (placebo)	4,5±0,63	8,8±0,24	9,4±0,62	11,7±1,7	3,1±0,66	>5,5
Treated animals	3,5±0,64	7,8±1,08	6,4±1,03	6,5±1,58	1,8±0,58	<3,0
P*	>0,05	>0,05	<0,05	<0,05	>0,05	<0,05

*Significant different between groups.

TABLE 3. Antimicrobial Activity of Compounds V-XVIII

Compound	Bacteriological dose (μg/ml) against the test cultures							
	Staph. aureus 209p	Staph. aureus AT N 23923 (F=49)	Bacillus subtillis 01011 ATCC 6633	Corynebacterium diverycatum	Mycobacterium B_s	E. coli 240533 ATCC N 25922 (F=50)	Proteus vulgaris 160111 ATCC n 6806 MXL 4636	Proteus aeruginosa 4000
V	200	200	50	50	200	400	400	400
VI	100	100	100	100	200	400	400	400
VIII	200	200	100	20	100	400	400	400
IX	200	400	50	10	200	400	400	400
X	200	200	200	50	400	400	400	400
XI	100	200	100	20	100	400	400	400
XII	200	200	100	50	400	400	400	400
XVI	400	400	200	400	400	400	400	400
XVIII	200	200	200	200	200	200	200	200

5,6-Dinitro-2-chlorobenzimidazole (III)

2-Chlorobenzimidazole (3.1 g, 0.02 mol) was added in small aliquots to a nitrating mix consisting of 15 ml NHO_3 (d 1.5) and 7 ml H_2SO_4 (d 1.84). The mixture was boiled for 3 h, cooled, and poured onto 100 g of ice. The resulting precipitate was collected by filtration, and crystallized from dioxane. The yield was 3.5 g (73%). The melting point was 199-200°C, compared with a published melting temperature of 190-191°C [7]. PMR spectrum: 8.38 ppm (c). Compound III was prepared from the mononitro derivative II in the same conditions.

1-Methyl-5,6-dinitro-2-chlorobenzimidazole (IV)

Compound III (5 g, 0.02 mol) was dissolved in sodium ethanoate (0.7 g of sodium in 100 ml ethanol), and 6 ml (0.1 mol) of methyl iodide was added, and the mixture was boiled for 1 h. The solvent was evaporated to half the initial volume, and 1-methyl-5,6-dinitro-chlorobenzimidazole was precipitated with water. The yield was 3.7 g (70%). The melting point was 197-198°C (from ethanol).

2-Benzylamino-5,6-dinitrobenzimidazole Hydrochloride (V)

2-Chloro-5,6-dinitrobenzimidazole (2.5 g, 0.01 mol) was boiled with 2.1 ml (0.02 mol) of benzylamine in 40 ml of ethanol for 30 min. The reaction mix was evaporated to dryness, and the residue was recrystallized from water. Compounds VI-XII were prepared by similar reactions.

1-Methyl-2-benzylamino-5,6-dinitrobenzimidazole (XIII)

Benzylamine (2.1 ml, 0.02 mol) was added to a solution of 1.4 g (0.005 mol) of 1-methyl-2-chloro-5,6-dinitrobenzimidazole in 60 ml of ethanol. The mix was boiled for 1 h, ethanol was evaporated to half the original volume, the mix was cooled, and a yellow precipitate was collected by filtration. Compounds XIV-XVII were prepared by similar reactions. The properties of compounds V-XVII are shown in Table 1.

1-Methyl-2-hydrazino-5,6-dinitrobenzyumidazole (XVIII)

Hydrazine hydrate (1.8 ml, 0.06 mol) in 10 ml of ethanol was added dropwise to a suspension of 2.6 g (0.01 mol) of the chloro derivative IV in 50 ml of ethanol. The solution was gradually brought to boiling point, and was then boiled for 20 min. The mix was cooled, and an orange precipitate was collected filtration. The yield was 2.0 g (78%). The melting temperature was 210-212°C (from 50% dimethylformamide).

Biological Methods

In vitro antiviral activity was assayed as described previously [1]. 1-Methyl-2-morpholino-5,6-dinitrobenzimidazole (XV) was studied in vivo in guinea pigs (200-300 g) with experimental vaccinia keratoconjunctivitis as described in [5], with prior treatment with an infecting dose of the virus. Animals received lyophilized vaccinia virus at a dose of 1000 $TCID_{50}$ in 0.1 ml. Anti-influenza activity was studied in developing chick embryos and in white mice infected with influenza A and B viruses. Each experiment included three groups: embryos (mice) receiving the compound of interest; embryos (mice) receiving control compounds with known activity against these viruses: remantadine for experiments with influenza A and adapromin in experiments with influenza B; and embryos (mice) receiving a placebo instead of the drug (usually physiological saline or water). Experiments and evaluations were carried out as described in [3].

Antimicrobial properties were studied by serial dilutions in liquid growth media suitable for the growth of the strains being used, and experiments were carried out using Gram positive and Gram negative opportunistically pathogenic bacteria, dermatophytes, and yeast-like and phytopathogenic fungi [2]. Strains of Staphylococcus aureus, Proteus vulgaris, Bacilus subtilis, Mycobacterium B_5, Escherichia coli, and Pseudomonas aeruginosa were grown on meat peptone agar and broth for 18-24 h at 37°C. A strain of C. diverycatum was grown in the same conditions at 28°C. A strain of the yeast-like fungus Candida albicans was grown at 37°C for 18-24 h on must agar. A strain of the phytopathogenic fungus Verticillium dahlias was grown on the same medium for 72-168 h at 28°C. The microbial load was 200,000 cells/ml of medium. Antimicrobial activities were determined using the minimal bacteriostatic dose producing complete inhibition of the test cultures studied.

LITERATURE CITED

1. V. I. Votyakov, E. I. Boreko, G. V. Vladyko, et al., Primary Studies of the Antiviral Properties of Synthetic and Natural Compounds: Methodological Recommendations [in Russian], Minsk (1968), pp. 8-12.
2. N. S. Egorov, Basic Studies on Antibiotics [in Russian], Moscow (1979), pp. 46-67.
3. V. I. Il'enko, Methods for the Testing and Evaluation of the Antiviral Activity of Compounts in Relation to Influenza Virus [in Russian], Leningrad (1977), p. 36.
4. S. K. Kotovskaya, L. A. Tyurina, E. Yu. Chermova, et al., Khim.-farm. Zh., No. 3, 310 (1989).
5. S. S. Marennikova, G. R. Matsevich, and É. V. Chekunova, Vopr. Virusol., No. 1, 59-63 (1989).
6. L. A. Tyurina, S. K. Kotovskaya, E. Yu. Chernova, et al., Khim.-farm. Zh., No. 1 (1991).
7. D. Harrison and J. T. Ralph, J. Chem. Soc., 236-239 (1965).