The synthesized compounds are slightly toxic, and their $LD_{50}$ is about 400-600 mg/kg. The $LD_{50}$ was determined on nonpedigreed white mice by intraperitoneal administration according to the V. B. Prozorovskii method.

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# SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF BENZIMIDAZOLYL DERIVATIVES OF PENICILLIN AND CEPHALOSPORIN


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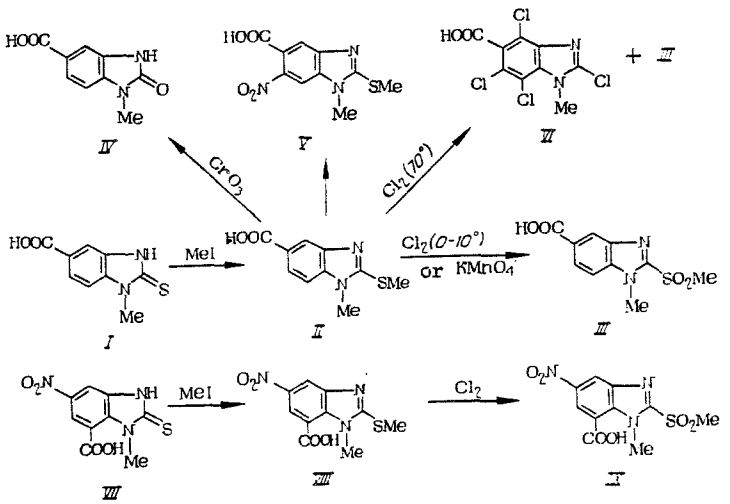
In the last ten years intensive investigations in the field of semisynthetic β-lactam antibiotics have been carried out. In particular, for the modification of antibiotics of the penicillin and cephalosporin series successful use has been made of derivatives of 4-, 5-, and 6-benzimidazolylcarboxylic acids having at the 2-position hydrogen, substituted aryl, or a heterocyclic group: furan or thiophene. The new antibiotics have a broad spectrum of antibacterial activity, in particular with regard to the Gram-negative microorganisms Pseudomonas and Proteus [2, 3].




For the preparation of structural analogs of penicillin and cephalosporin we have synthesized 5- and 7-benzimidazolylcarboxylic acids having at the 2-position methylthio and methylsulfonyl groups: 1-methyl-2-methylthiobenzimidazolyl-5-carboxylic acid (II), 1-methyl-2-methylsulfonylbenzimidazolyl-5-carboxylic acid (III), 1-methyl-2-methylthio-5-nitrobenzimidazolyl-7-carboxylic acid (VIII), and 1-methyl-2-methylsulfonyl-5-nitrobenzimidazolyl-7-carboxylic acid


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TABLE 1. PMR Spectra of Compounds II-IX

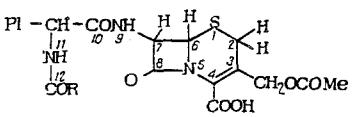
| Compound | Proton signals, ppm | | | | | | J, Hz |
|---|---|---|---|---|---|---|---|
| | $NCH_3S$ | $SCH_3S$ | $SO_2CH_3S$ | 4-H | 6-H | 7-H | |
| II | 3,68 | 2,73 | — | 8,07 (dd) | 7,82 (dd) | 7,48 (dd) | $J_{46}=1,5$; $J_{67}=8,5$ $J_{47}=0,5$ |
| III | 3,60 | — | 4,13 | 8,38 (dd) | 8,09 (dd) | 7,86 (dd) | $J_{46}=1,4$; $J_{67}=8,7$ $J_{47}=0,5$ |
| V | 3,76 | 2,78 | | 8,23 (s) | — | 7,83 (s) | |
| VI | 3,52 | — | — | — | — | — | |
| VII | 3,80 | — | — | 8,27 (d) | 7,93 (d) | — | $J_{46}=2,7$ |
| VIII | 3,81 | 2,80 | — | 8,43 (d) | 8,35 (d) | — | $J_{46}=2,4$ |
| IX | 3,72 | — | 4,24 | 8,86 (d) | 8,58 (d) | — | $J_{46}=2,3$ |

TABLE 2. PMR Spectra of Antibiotics X-XV

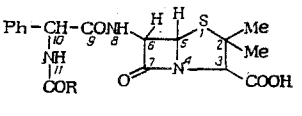
| Compound | Proton signals, ppm ; J, Hz | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $HN_9$ (8)* d | $HN_{12}$ (11) d | $C_6H_5$ m | $H_{11}$ (10) d | $H_7$ (6) dd | $H_6$ (5) d | H-2 (AB) | $CH_2O$(AB) | $CH_2CO$ s | $H_3$ s | $CH_3(C_4)$ s |
| X | 9,27 J=8,2 | 8,75 J=8,2 | 7,3—7,7 | 5,89 J=8,2 | 5,77 J=4,9 J=8,2 | 5,06 J=4,9 | 3,67 3,65 J=18,0 | 4,98 4,65 J=12,9 | 2,02 | — | — |
| XI | 9,31 J=8,2 | 8,98 J=7,9 | 7,3—7,6 | 5,90 J=7,9 | 5,77 J=4,7 J=8,2 | 5,07 J=4,7 | 3,68 3,41 J=18,1 | 4,98 4,65 J=12,7 | 2,02 | — | — |
| XII | 9,38 J=7,7 | 9,57 J=7,7 | 7,2—7,6 | 5,87 J=7,7 | 5,78 J=4,7 J=7,7 | 5,07 J=4,7 | 3,59 3,35 J=18,4 | 4,98 4,66 J=13,0 | 2,02 | — | — |
| XIII | 9,40 J=8,4 | 9,68 J=7,1 | 7,3—7,7 | 5,89 J=7,1 | 5,79 J=4,6 J=8,4 | 5,07 J=4,6 | 3,59 3,36 J=18,4 | 4,98 4,66 J=13,1 | 2,02 | | |
| XIV | 9,05 J=7,5 | 8,81 J=8,1 | 7,2—7,7 | 5,94 J=8,1 | 5,57 J=4,0 J=7,5 | 5,44 J=4,0 | — | — | — | 4,23 | 1,54 1,42 |
| XV | 9,08 J=7,1 | 9,00 J=8,1 | 7,2—7,7 | 5,94 J=8,1 | 5,57 J=4,0 J=7,5 | 5,44 J=4,0 | — | — | — | 4,23 | 1,54 1,42 |

*The numbers of the atoms of compounds XIV and XV are given in parentheses. The signals and coupling constants of the benzimidazole protons completely correspond with those listed in Table 1.

(IX). Compounds II, III, VIII, and IX were prepared from the corresponding thiones (I) and (VII).



X-XIII



XIV, XV

R = 1-methyl-2-methylthiobenzimidazolyl-5 (X, XIV), 1-methyl-2-methylsulfonylbenzimidazolyl-5 (XI, XV), 1-methyl-2-methylthio-5-nitrobenzimidazolyl-7 (XII), 1-methyl-2-methylsulfonyl-5-nitrobenzimidazolyl-7 (XIII).

Methylation of 1-methyl-2-thiobenzimidazolyl-5-carboxylic acid I with methyl iodide in alkaline medium yielded acid II. Oxidation of the methylthio derivatives to the sulfones may be carried out by means of $CrO_3$, $HNO_3$, $KMnO_4$, or $Cl_2$. Acid III was prepared in 60-70% yield by oxidation of methylthio derivative II with chlorine at 0-10°C. Increasing the reaction temperature to 70°C leads to a mixture of compounds from which we could isolate sulfone III and 1-methyl-2,4,6,7-tetrachlorobenzimidazolyl-5-carboxylic acid (VI). In case of oxidation with $KMnO_4$, both at room temperature and at increased temperature, the yield of sulfone III did not exceed 35%. Reaction with $CrO_3$ causes hydrolysis of the methylthio group and gives benzimidazolone (IV), the IR spectrum of which is identical with that of a sample prepared by melting 3-amino-4-methylamino-benzoic acid with urea. Oxidation of the methylthio group with nitric acid failed; in that case nitration at the 6-position takes place and the methylthio group remains unchanged (compound V).

Cyclization of 2-methylamino-3-amino-5-nitrobenzoylbenzoic acid with $CS_2$ yielded 1-methyl-2-thio-5-nitrobenzimidazolyl-7-carboxylic acid (VII). Methylation of thione VII with MeI in

TABLE 3. Antibacterial Activity of the Cephalosporins (MIC, μg/ml)

| Strain of microorganism | Compound | | | | |
|---|---|---|---|---|---|
| | cephalexin | X | XI | XII | XIII |
| Staph. aureus 209P | 1,6 | 0,8 | 1,6 | 1,6 | 1,6 |
| Staph. epidermis 2124 | 6,2 | 25 | 12,5 | 1,6 | 6,2 |
| Staph. aureus 9597 | 12,5 | 3,1 | 3,1 | 3,1 | 12,5 |
| E. coli 675 | 25 | 25 | 50 | >200 | >200 |
| E. coli 453 | 100 | 100 | 50 | >200 | >200 |
| Enterobacter. cl. 418 | 200 | 200 | 200 | >200 | >200 |
| Pseudomonas aeruginosa 27853 | 200 | 100 | 100 | >200 | >200 |
| Pseudomonas aeruginosa 11441 M | 200 | 100 | 200 | >200 | >200 |
| Pseudomonas aeruginosa 58 | 200 | 100 | 100 | >200 | >200 |
| Enterobacter. aer. 11648 | 25 | 50 | 50 | >200 | >200 |
| Klebsiella ozaenae | 1,6 | 3,1 | 3,1 | >200 | >200 |
| Klebsiella pneumoniae 5056 | 12,5 | 25 | 25 | >200 | >200 |
| Serratia marcescens 8 | 100 | 100 | 200 | >200 | >200 |
| Proteus mirabilis 14 | 100 | 100 | 200 | >200 | >200 |
| Providentia st. 3646 | 200 | 100 | 100 | >200 | >200 |

alkaline medium gave acid VIII, which by reaction with $Cl_2$ was converted to acid IX. The structures of compounds II-IX were confirmed by their PMR spectra (Table 1).

The synthesis of the novel derivatives of cephalexin (X-XIII) and ampicillin (XIV, XV) was carried out by acylation of 7-(2-amino-2-phenylacetamido)cephalosporanic acid (CA) and 7-(2-amino-2-phenylacetamido)penicillanic acid (PA) with the chlorides of acids II, III, VIII, and IX in the presence of an acceptor for the hydrogen chloride liberated in the acylation [1]. The structures of antibiotics X-XV were confirmed by IR and PMR spectral data (Table 2).

Investigation of the antimicrobial spectrum and the antimicrobial activity of the compounds synthesized showed that introduction of benzimidazole has great influence on them. Thus, cephalosporins containing the 1-methyl-2-methylthio(methylsulfonyl)-5-nitrobenzimidazolyl-7-carboxylic acid moiety (XII, XIII) retain the antimicrobial activity of cephalexin only in the Gram-positive part of the antimicrobial spectrum. Cephalosporins X and XI, just as cephalexin, have a broad spectrum of antimicrobial activity and are active against many Gram-positive and Gram-negative microorganisms (Table 3). Penicillins XIV and XV do not only repeat the antimicrobial spectrum of the starting ampicillin, but in a number of cases also have activity against strains of microorganisms that are resistant to ampicillin (Table 4).

## EXPERIMENTAL (CHEMICAL)

IR spectra were recorded on a Perkin-Elmer 577 spectrometer. PMR were taken from 1% solutions in DMSO-$D_6$ on a Bruker WH-90 spectrometer (FRG) operating at 90 MHz. Chemical shifts were measured relative to the internal standard tetramethylsilane (TMS; 0.00 ppm). Silica gel KSK-2 and Silufol plates (Czechoslovakia) were used for TLC. Solvent systems and visualization: 1) $CHCl_3$–MeOH–acetone 9:0.5:0.5 (UV, iodine); 2) ethyl acetate–hexane–MeOH 1:3:1 (UV, bromocresol); 3) butyl acetate–n-butanol–MeOH–water–AcOH 15:9:9:6:1,5 (UV, bromocresol); 4) ethyl acetate–hexane–MeOH 3:3:1 (UV, bromocresol). Found and calculated values of elemental analyses matched.

1-Methyl-2-methylthiobenzimidazolyl-5-carboxylic Acid (II). Six grams (0.02 mole) of 1-methyl-2-thiobenzimidazolyl-5-carboxylic acid (I) was dissolved in a mixture of 10 ml 25% KOH and 40 ml of ethanol, 8 ml of MeI was added, and the mixture was refluxed for 30 min. The solution was evaporated to dryness, the residue was dissolved in water, the solution was acidified with conc. HCl to pH 4-5, the precipitate was filtered off, and crystallized from 60% aqueous DMF. Yield 3.9 g (61%), mp 294-295°C. $C_{10}H_{10}N_2O_2S$.

1-Methyl-2-oxobenzimidazolyl-5-carboxylic acid (IV). A) To a solution of 2.2 g (0.01 mole) of compound II in 50 ml of 80% acetic acid is added in small portions a cold saturated solution of $CrO_3$ in water until the orange color persists. After 5-10 min an exothermic reaction starts, the solution turns cinnamon, and a precipitate of benzimidazolone IV is formed. mp >300°C (from EtOH or AcOH). Yield 1.2 g (62%). $C_9H_8N_2O_3$. B) 2.4 g (0.01 mole) of 3-amino-4-methylaminobenzoic acid (hydrochloride) and 1.8 g (0.03 mole) of urea are melted together at 180-190°C for 30-40 min. The melt is cooled, dissolved in 2 N NaOH, boiled with charcoal, filtered, and acidified with conc. HCl to pH 3. Benzimidazolone IV is filtered off and crystallized from ethanol. Yield 1.4 g (73%), mp >300°C.

TABLE 4. Antimicrobial Activity of the Penicillins (MIC, μg/ml)

| Strain of microorganism | Compound | | |
|---|---|---|---|
| | ampicillin | XIV | XV |
| Staph. aureus 209 P | 1 | 1 | 1 |
| Staph. epidermis 2124 | 1 | 1 | 1 |
| E. coli 104 | 100 | 200 | 200 |
| E. coli R 151 | 100 | 200 | 200 |
| Enterobacter cl. 1903 | 6,2 | 25 | 25 |
| Pseudomonas aeruginosa 11441 M | 100 | 12,5 | 50 |
| Pseudomonas aeruginosa 27853 | 100 | 12,5 | 50 |
| Klebsiella ozaenae | 25 | 3,1 | 12,5 |
| Klebsiella pneumoniae 5056 | 100 | 25 | 50 |
| Proteus mirabilis 14 | 50 | 25 | 50 |

1-Methyl-2-methylthio-6-nitrobenzimidazolyl-5-carboxylic Acid (V). A solution of 2.2 g (0.01 mole) of compound II in 40 ml of $HNO_3$ (d = 1.5) was kept at 50-60°C for 1 h. After cooling the solution was poured on 30 g of ice, alkalized to pH 5 with conc. $NH_4OH$, the light-yellow precipitated compound V was filtered off, and crystallized from AcOH. mp 310-315°C (dec.). Yield 0.4 g (33%). $C_{10}H_9N_3O_4S$.

1-Methyl-2-methylsulfonylbenzimidazolyl-5-carboxylic Acid (III). A) A suspension of 2.2 g (0.01 mole) of compound II in 60 ml of water is cooled to 0°C and with vigorous stirring $Cl_2$ is bubbled through at 0-10°C for 1 h. Precipitated sulfone III is filtered off and crystallized from 50% aqueous DMF. Yield 1.6 g (65%), mp >300°C (dec.). $C_{10}H_{10}N_2O_4S$. B) 1.1 g (0.005 mole) of compound II is suspended in 40 ml of water, warmed to 70°C, and with vigorous stirring $Cl_2$ is bubbled through for 2 h. The mixture is cooled, the precipitate is filtered off, and boiled up in 20 ml of ethanol. The fraction that is insoluble in ethanol is crystallized from 50% aqueous DMF. Yield 0.4 g (48%) of sulfone III. The ethanolic solution is evaporated to dryness and the 1-methyl-2,4,6,7-tetrachlorobenzimidazolyl-5-carboxylic acid (VI) is crystallized from 50% aqueous ethanol. Yield 0.3 g (20%), mp 240-242°C. $C_9H_4N_2O_2Cl_4$. C) 2.2 g (0.01 mole) of compound II is dissolved in 20 ml of AcOH and at 60°C a 7% aqueous solution of $KMnO_4$ is added dropwise until the color persists. The solution is discolored with sodium hydrosulfite and the colorless precipitate is filtered off. Yield 0.9 g (35%) of III.

1-Methyl-2-thio-5-nitrobenzimidazolyl-7-carboxylic Acid (VII). To a solution of 3.6 g (0.018 mole) of $Na_2S \cdot 9H_2O$ and 0.6 g (0.02 mole) of sulfur in 15 ml of water is added a solution of 2.4 g (0.01 mole) 2-methylamino-3,5-dinitrobenzoic acid. The reaction mixture is refluxed for 2 h, evaporated to dryness, and the precipitate obtained is extracted with 200 ml of boiling ethanol. The ethanol is evaporated, the purple-colored precipitate of 1-methylamino-2-amino-4-nitrobenzoic acid is dissolved in 40 ml of pyridine, 15 ml (0.25 mole) of $CS_2$ is added, and the mixture is refluxed for 6 h. The reaction mixture is cooled, acidified to pH 7 with AcOH, and the yellow precipitate is filtered off. Yield 1.2 g (45%) of compound VII, mp 300-304°C (from AcOH). $C_9H_7N_3O_4S$.

1-Methyl-2-methylthio-5-nitrobenzimidazolyl-7-carboxylic Acid (VIII). To a solution of 2.5 g (0.01 mole) of thione VII in 50 ml of ethanol, containing 0.8 g (0.02 mole) of NaOH in 10 ml of water, is added 1.5 ml of MeI, the solution obtained is refluxed for 1 h, and evaporated to dryness. The residue is dissolved in water and the solution is acidified to pH 4-5 with conc. HCl. The precipitate is filtered off and crystallized from 50% DMF. Yield 2.2 g (84%), mp 290-294°C. $C_{10}H_9N_3O_4S$.

1-Methyl-2-methylsulfonyl-5-nitrobenzimidazolyl-7-carboxylic Acid (IX). Through a suspension of 2.6 g (0.01 mole) of methylthio derivative VIII in 100 ml of water is bubbled $Cl_2$ at room temperature for 1 h. The colorless precipitate is filtered off and crystallized from ethanol. Yield 1.9 g (65%), mp 228-230°C. $C_{10}H_9N_3O_6S$.

General Method for the Preparation of the Antibiotics. 7-[2-(1-methyl-2-methylthio(or methylsulfonyl)benzimidazolyl-5-carboxamido)-2-phenylacetamido]-3-acetoxymethylceph-3-em-4-carboxylic acids (X, XI), 7-(1-methyl-2-methylthio(or methylsulfonyl)-5-nitrobenzimidazolyl-7-carboxamido)-2-phenylacetamido]-3-acetoxymethylceph-3-em-4-carboxylic acids (XII, XIII), 6-[2-(1-methyl-2-methylthio(or methylsulfonyl)benzimidazolyl-5-carboxamido)-2-phenylacetamido]-2, 2-dimethylphenam-3-carboxylic acids (XIV, XV).

A) Preparation of the Acid Chloride. One hundredth mole of acid II, III, VIII, or IX is suspended in 0.03-0.05 mole of $SOCl_2$ and the mixture is refluxed for 0.5 h. The solution obtained is evaporated to dryness. To analyze the complete conversion the residue is refluxed

in absolute ethanol. Analysis is performed on Silufol plates in system 1. $R_f$ acid 0.2-0.25, $R_f$ ester 0.67-0.70.

B) Preparation of the Antibiotics. In 100 ml of dichloromethane is suspended 0.01 mole of water-free CA or PA, the suspension is cooled to 0-5°C, and 0.022 mole of $Et_3N$ is added. The mixture is stirred for 15-30 min to form a solution and then 0.01 mole of the acid chloride is added. The mixture is stirred at 0-5°C for 30 min and then for 30 min without cooling. After cooling to 0-5°C 100 ml of water is added. The aqueous layer is separated, acidified to pH 2, and the precipitate is filtered off. Yield 60-70%. $R_f$ X and XI 0.47 (3), $R_f$ XII and XIII 0.33 (4), $R_f$ XIV and XV 0.15 (2). IR spectra, $\nu_{max}$, $cm^{-1}$: 1780-1785 (CO lactam), 1640-1675 (CO, 11H-I), 1695-1730 (CO, 11H-II), 1620-1605, 1405-1420 ($COO^-$), 1730-1735 (CO, ester), 1230 (CO, acetoxymethyl), 1515-1530 (aromatic).

## EXPERIMENTAL (BIOLOGICAL)

The antibacterial activity of the ampicillin and cephalexin derivatives was studied with a selection of standard and clinical strains of Gram-positive and Gram-negative microorganisms by the method of twofold serial dilutions in liquid and solid culture mediums (beef-extract broth and beef-extract agar, pH 7.2). For the determination of the activity of the compound by the method of twofold serial dilutions on beef-extract agar the strain to be tested was seeded on the surface of the agar with a replicator strain from an 18-hours-old bouillon culture. The quantity of the seeded dose was $10^5$ cfu/ml. The results were judged 18 h after incubation of the seedings at 37°C by determining the value of the minimum inhibitory concentration (MIC) of the antibiotics for every strain studied. All investigations were carried out in comparison with ampicillin and cephalexin.

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# SYNTHESIS AND ANTIVIRAL ACTIVITY OF SPIROBROMINE AND ITS ANALOG


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The dichloride of 3,12-bis(3'-bromo-1'-oxopropyl)-3,12-diaza-6,9-diazoniadispiro[5.2.5.2] hexadecane [spirobromine, (I)] is a new preparation of original chemical structure which is applied for the treatment of acute leukemias, cervical cancer, malignant lymphomas, and cancer of the larynx [3, 5].

The preparation has low toxicity and possesses selective action toward the DNA of the tumor cells [3, 6].

Starting from recent concepts on the participation of some viruses in oncogenesis, and taking into account the spectrum of activity of spirobromine, it seemed expedient to study the action of spirobromine in relation to DNA-containing viruses [1].

In the present communication, the results of the study of the influence of spirobromine and the structurally related 3,12-bis(3'-bromo-1'-oxopropylamino)-3,12-diaza-6,9-diazoniadispiro[5.2.5.2]hexadecane dichloride (V) on the reproduction of the Herpes simplex viruses types I and II in cell culture and in animals are described. Compound (V) can be regarded as an analog of spirobromine which, as in the case of spirobromine, contains active β-bromopropionyl groupings. However, it is a dihydrazido derivative, in contrast to spirobromine.


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