Research Article

Synthesis and Antimicrobial Activity of Bis-4,6-sulfonamidated 5,7-Dinitrobenzofuroxans

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A new series of bis-4,6-sulfonamidated 5,7-dinitrobenzofuroxans 7–11 had been synthesized and tested for antimicrobial activity. The structures of new sulfanilamide derivatives were characterized by elemental analysis, IR spectroscopy, and mass spectrometry (MALDITOF). The synthesized compounds were tested for their *in vitro* antimicrobial activity using the disk diffusion method against Gram-positive bacteria *Staphylococcus aureus*; the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*; the fungal strain *Aspergillus niger*; and the yeast-like pathogenic fungus *Candida albicans*. Our results indicate that the compounds 7–11 exhibit potent antimicrobial activity. The stability of the compounds was evaluated by TG and DSC methods.

1. Introduction

A need for new antimicrobial agents is justified as more microorganisms develop resistance to the present drugs available in the market. Resistance of pathogenic bacteria to antibiotics is quickly becoming a major problem in the community and hospital-based healthcare settings. The search for novel agents to combat resistant bacteria has become one of the most important areas of antibacterial research today [1]. Some microorganisms are resistant to all approved antibiotics and can only be treated with potentially toxic drugs. Pharmaceutical and organic chemists are trying to synthesize new drugs with better pharmacokinetic and dynamic properties.

Since the discovery of antibacterial properties of the synthetic dye Prontosil over 70 years ago, sulfa drugs have been widely used to treat a broad spectrum of microbial diseases [2–5]. Sulfa drugs kill bacteria and fungi by interfering with cell metabolism. They exert their effect by targeting the synthase dihydropteroate (DHPS) enzyme, which catalyzes folic acid pathway in bacteria and some eukaryotic cells [6] but is not present in human cells [4].

This is the basis for the selective effect of sulfonamides on bacteria and for their broad spectrum of antibacterial activity. A free amino group (only in the *para*-position) is required for antibacterial activity. The sulfonamide family includes sulfadiazine, sulfamethizole (brand name Thiosulfil Forte), sulfaethylthiadiazole, sulfamethoxazole (Gantanol), sulfathiazole, sulfasalazine (Azulfidine), sulfametopyrazine, sulfisoxazole (Gantrisin), sulfadimethoxine, and various high-strength combinations of three sulfonamides. Before penicillin G, these antimicrobials were standard therapies and are still in use today [7].

The benzofuroxans and their chloro-nitro-substituted derivatives have been shown to exhibit a wide spectrum of biological activities [8] and the interest of medicinal chemists in them has grown over the last two decades. Perhaps the most relevant finding reported so far is related to their capability to release nitric oxide; in addition to that, other significant biological activities have been identified, such as antibacterial, antifungal, antiparasitic, cytotoxic, and herbicidal properties [9–12]. Recently, research and development in the medicinal chemistry field have produced hybrid compounds in which benzofuroxanyl moieties are present in

a single molecule together with classical drug moieties. Consequently, new antiulcer drugs, calcium channel modulators, vasodilator derivatives, antioxidants, and others have been described and are currently under study [13–19]. On the other hand, these kinds of compound have also been reported as components in primary explosives, polymers, and propellants [8, 20–22].

In this paper, we present the synthesis and antimicrobial activity of a series of substituted 5,7-dichloro-4,6-dinitrobenzofuroxan **1** by sulfonamides **2–6**, whose chemical structure has been confirmed by IR spectroscopy, mass spectrometry, and elemental analysis.

2. Experimental

2.1. Chemistry. All drugs used in this study were purchased from commercial suppliers and satisfy the corresponding pharmacopoeia purity grades. Compound **1** (5,7-dichloro-4,6-dinitrobenzofuroxan) was previously synthesized in our laboratory [23]. Compound **2** (sulfanilamide) was purchased from JSC Irbit Pharmaceutical chemistry production plant. Compounds **3–6** (sulfadimethoxine, sulfametopyrazine, sulfathiazole, and sulfaethylthiadiazole) were purchased, respectively, from OJSC “Organica,” OJSC “Usolie Siberian Chemical Pharmaceutical Plant,” LLC “Asfarma,” and OJSC “Centre of the Chemistry for Therapeutic Agents” (all Russia). Penicillin G (sodium salt) and gentamicin (sulfate salt) were purchased from Kurgan JSC “Sintez” (Russia). Rifampicin, chloramphenicol (sodium succinate), nystatin, and ketoconazole were purchased, respectively, from CJSC “Bryntsalov A,” OJSC “Organica,” OJSC “Biosintez” (all Russia), and Ultratech India Ltd.

The melting points were determined by TG-DSC methods on NETZSCH STA 449 C device and are uncorrected. Elemental microanalyses were performed at Microanalysis Laboratory at Kazan Institute of Organic and Physical Chemistry (Kazan Research Center), Kazan. The IR spectra were recorded on Specord M-80 spectrometer from samples dispersed in mineral oil. To determine molecular masses of compounds obtained, matrix-assisted laser desorption/ionization time of flight mass spectra (MALDI TOF Dynamo Finnigan) was used. The purity of the synthesized compounds was checked using thin layer chromatography (TLC). Evaluation of antimicrobial activities was carried out by the Department of Microbiology, Kazan State Medical Academy, Kazan, Russia.

2.2. Synthetic Procedure for 4,6-Bis-4,6-sulfonamidated-5,7-dinitrobenzofuroxans (**7–11**)

2.2.1. General Procedure. A mixture of equimolar quantities of 5,7-dichloro-4,6-dinitrobenzofuroxan **1** (0.01 mol) and appropriate sulfonamide (0.04 mol) was refluxed in dry mixture of a ratio of 1 : 1 of EtOH and $(C_2H_5)_2O$ (100 mL) for 2 h. Excess of solvent was removed under reduced pressure. The resulting compounds **7–11** were obtained as orange-red crystals and purified by diethyl ether from starting reagents.

7: Yield (74%), orange crystals, m.p. 199.9°C. IR (KBr): 3280 (N–H), 1616 (C=N–O), 1580 and 1320 (NO₂), 1310

and 1160 (SO₂) cm^{−1}. MS: *m/z* 566 M+H; C₁₈H₁₄O₁₀N₈S₂ (566.20): calcd. C 38.16%, H, 2.47%, N, 19.79%; found: C 38.45%, H 2.31%, N 19.99%.

8: Yield (83%), red crystals, m.p. 190.5°C. IR (KBr): 3227 (N–H), 1630 (C=N), 1600 (C=N–O), 1586 and 1324 (NO₂), 1311 and 1160 (SO₂) cm^{−1}. MS: *m/z* 842 M+H; C₃₀H₂₆O₁₄N₁₂S₂ (842.0): calcd. C 42.76%, H, 3.09%, N, 19.95%; found: C 42.88%, H 3.27%, N 19.34%.

9: Yield (67%), red crystals, m.p. 307.3°C. IR (KBr): 3260 (N–H), 1660 (C=N), 1616 (C=N–O), 1580 and 1320 (NO₂), 1310 and 1160 (SO₂) cm^{−1}. MS: *m/z* 782 M+H; C₂₈H₂₂O₁₂N₁₂S₂ (782.20): calcd. C 42.97%, H, 2.81%, N, 21.48%; found: C 42.71%, H 2.77%, N 21.87%.

10: Yield (73%), dark red crystals, m.p. 195.1°C. IR (KBr): 3280 (N–H), 1616 (C=N–O), 1580 and 1320 (NO₂), 1310 and 1160 (SO₂) cm^{−1}. MS: *m/z* 732 M+H; C₂₄H₁₆O₁₀N₁₀S₄ (732.20): calcd. C 39.34%, H, 2.19%, N, 19.13%; found: C 39.17%, H 2.43%, N 19.51%.

11: Yield (84%), orange crystals, m.p. 197.6°C. IR (KBr): 3280 (N–H), 1665 (C=N), 1618 (C=N–O), 1580 and 1320 (NO₂), 1310 and 1160 (SO₂) cm^{−1}. MS: *m/z* 790 M+H; C₂₈H₂₂O₁₀N₁₂S₄ (790.20): calcd. C 39.49%, H, 2.78%, N, 21.27%; found: C 39.07%, H 2.63%, N 20.97%.

2.3. Antimicrobial Screening. Bacterial and fungal strains used in the antimicrobial evaluation were obtained from the Department of Microbiology (Museum of Culture Collections) of Kazan State Medical Academy, Russia, namely, *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 12453), *Aspergillus niger* (ATCC 16620), and *Candida albicans* (ATCC 885-653). The antibacterial and antifungal activities were reported utilizing Kirby-Bauer disc diffusion method [24, 25]. Briefly, agar (pH = 7.2–7.4; Sabouraud for *Candida albicans* and Mueller-Hinton for all the others) was placed into Petri dish (100 mm size and 4 mm depth). The agar plates were then inoculated with broth cultures diluted to 0.5 McFarland turbidity ($\sim 1.5 \cdot 10^8$ cells·mL^{−1}). Discs containing known amounts of an antimicrobial agent were placed on the surface of an agar plate that has been inoculated with a standardized suspension of microorganisms to be tested. Paper discs with only dimethylsulfoxide (DMSO) or CH₂Cl₂ (tests involving Ketoconazole) were used as negative controls. The MZI for rifampicin (antibacterial), nystatin, and ketoconazole (antifungal) was used as reference values (in millimeters). All experiments were conducted in triplicate and repeated if the results differed. All compounds used having MZI larger than or equal to 19 mm were selected for MIC tests.

For MIC assays [26, 27], a stock solution (1 mg·mL^{−1}) of each test compound was prepared in DMSO. Further, a serial dilution of test compounds was carried out to achieve concentrations ranging from 50 to 400 μg·mL^{−1}. Test compounds at various concentrations were then added to culture medium in a test tube and different strains were inoculated at 10⁸ bacteria·mL^{−1} concentration. Trypticase soy agar and nutrient agar (for antibacterial) and Sabouraud liquid medium (for antifungal) were utilized as culture media. The tubes were incubated at 37°C (antibacterial) or 30°C

SCHEME 1: Synthetic routes of bis-sulfonamidated 4,6-dinitrobenzofuroxans 7–11; reagents and conditions.

(antifungal) for 24–48 h and then examined for the growth of the tested organisms or their absence. Chloramphenicol, rifampicin, nystatin, and ketoconazole were used as antibacterial and antifungal substances, respectively. The MIC values were obtained from the lowest concentration of the test compounds where the tubes remained clear, indicating that the bacterial or fungal growth was completely inhibited at this concentration.

3. Results and Discussions

3.1. Chemistry. The series of sulfanilamide derivatives 7–11 was synthesized by a substitution reaction between the 5,7-dichloro-4,6-dinitrobenzofuroxan **1** and the substituted sulfonamides: 4-aminobenzenesulphonamide **2** (sulfanilamide), 4-(aminobenzenesulphamido)-2,4-dimethoxypyrimidine **3** (sulfadimethoxine), 2-(*para*-aminobenzenesulphamido)-3-methoxypyrazine **4** (sulfametopyrazine), 2-(*para*-aminobenzenesulphamido)-thiazol **5** (sulfathiazole), and 2-(*para*-aminobenzenesulfamido)-5-ethyl-1,3,4-thiadiazole **6** (sulfathiazole). The synthetic route for the preparation of these molecules is outlined in Scheme 1.

All reactions of 5,7-dichloro-4,6-dinitrobenzofuroxan **1** with sulphonilamides 2–6 were prepared with satisfactory yield and accompanied with intensive change in color of the reaction mixture from orange to red and dark red. On completion of the reactions the precipitated colored crystals were of the synthesized compounds 7–11 purified with ether.

The chemical structures were elucidated on the basis of elemental analysis, IR, and mass spectroscopy. The results of elemental analysis (C, H, and N estimation) were found to be within $\pm 0.4\%$ of the theoretical values. IR data also confirmed the presence of specific functional groups present

in the final synthesized compounds. The mass spectra of new compounds were in conformity with the assigned structure. The melting points and the thermal stability of the compounds were taken from TG and DSC methods.

3.2. Antimicrobial Activity. Compounds 2–6 are historically well-known sulfonamide drugs and have been extensively studied [5, 6, 21], but microorganisms now often show resistance against them [22]. Compounds 1–2 and all synthesized sulfonamide derivatives 7–11 were tested for antimicrobial activity by the disc diffusion method. In general, these results indicated good antimicrobial activities for all compounds (Table 1). However, some compounds showed significant mean zone inhibition (MZI) for both bacterial and fungal strains: **7** (21 mm, 20 mm, and 27 mm), **8** (24 mm, 25 mm, and 21 mm), **9** (18 mm, 13 mm, and 20 mm), **10** (25 mm, 21 mm, and 23 mm), and **11** (21 mm, 21 mm, and 30 mm) against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, respectively; **8** (26 mm) and **10** (25 mm) against *Pseudomonas aeruginosa*; and **10** (18 mm) against *Aspergillus niger*. Among these synthesized compounds, compounds **8**, **10**, and **11** were found to possess significant antibacterial and antifungal activity when compared to standard antibacterial and antifungal drugs, such as penicillin G, rifampicin, sulfanilamide, ketoconazole, and nystatin. Compounds **7** and **9** were found to possess antibacterial activity almost equivalent to standard drugs as well as considerable antifungal activity.

To further evaluate antimicrobial potential, we determined the minimum inhibitory concentration (MIC) for the compounds with MZI above or equal to 19 mm. The results (Table 2) show that the tested compounds (especially compounds **8**, **10**, and **11**) possess significant antibacterial and antifungal activities compared to the standard drugs

TABLE 1: The *in vitro* antimicrobial activity of the synthesized compounds and the control drugs (1 mg/mL).

Compound number	Microorganisms, zone of inhibition (diameter, in mm)					
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus mirabilis</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>
1	17	—	—	—	10	15
Sulfanilamide (2)	20	17	15	12	7	9
7	21	20	7	11	15	27
8	24	25	26	15	17	21
9	18	13	18	13	15	20
10	25	21	25	17	18	23
11	21	21	10	12	13	30
Penicillin G	21	20	8	10	—	—
Rifampicin	25	21	—	—	—	—
Ketoconazole	—	—	—	—	11	18
Nystatin	—	—	—	—	18	21
DMSO	—	—	—	—	—	—

Shown are mean values of triplicate tests; “—” indicates no significant inhibitory effect (<6 mm).

TABLE 2: Minimum inhibitory concentration (MIC) of selected compounds.

Organism	Compound	MIC ($\mu\text{g}\cdot\text{mL}^{-1}$)	MIC (μM)
<i>Staphylococcus aureus</i>	1	100	339
	7	60	106
	8	40	47
	9	120	153
	10	30	41
	11	25	32
	Chloramphenicol	45	101
	Penicillin G	70	197
	Rifampicin	50	61
	7	90	159
<i>Escherichia coli</i>	8	35	42
	9	120	153
	10	30	41
	11	25	32
	Chloramphenicol	25	56
<i>Pseudomonas aeruginosa</i>	8	30	36
	10	30	41
	Gentamicin	55	96
	1	40	136
<i>Candida albicans</i>	7	35	62
	8	25	30
	9	60	77
	10	30	41
	11	15	19
	Ketoconazole	30	56
	Nystatin	55	59

MIC: minimum inhibitory concentration (the lowest concentration that inhibited the bacterial growth).

(chloramphenicol, rifampicin, penicillin G, and gentamicin for antibacterial activity and ketoconazole and nystatin for antifungal activity).

3.3. Biological Assays. Bacterial and fungal strains used in the antimicrobial evaluation were obtained from the Department of Microbiology (Museum of Culture Collections) of Kazan State Medical Academy, Russia, namely, *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 12453), *Aspergillus niger* (ATCC 16620), and *Candida albicans* (ATCC 885-653). The antibacterial and antifungal activities were reported utilizing Kirby-Bauer disc diffusion method [24, 25]. Briefly, agar (pH = 7.2–7.4; Sabouraud for *Candida albicans* and Mueller-Hinton for all the others) was placed into Petri dish (100 mm size and 4 mm depth). The agar plates were then inoculated with broth cultures diluted to 0.5 McFarland turbidity ($\sim 1.5 \cdot 10^8$ cells·mL⁻¹). Discs containing known amounts of an antimicrobial agent were placed on the surface of an agar plate that has been inoculated with a standardized suspension of microorganisms to be tested. Paper discs with only dimethylsulfoxide (DMSO) or CH₂Cl₂ (tests involving ketoconazole) were used as negative controls. The MZI for rifampicin (antibacterial) and nystatin and ketoconazole (antifungal) was used as reference values (in millimeters). All experiments were conducted in triplicate and repeated if the results differed. All compounds used having MZI larger than or equal to 19 mm were selected for MIC tests.

For MIC assays [26, 27], a stock solution (1 mg mL⁻¹) of each test compound was prepared in DMSO. Further, a serial dilution of test compounds was carried out and the concentrations ranged from 50 to 400 µg·mL⁻¹. Test compounds at various concentrations were then added to culture medium in a test tube and different strains were inoculated at 10⁸ bacteria·mL⁻¹ concentration. Trypticase soy agar and nutrient agar (for antibacterial) and Sabouraud liquid medium (for antifungal) were utilized as culture media. The tubes were incubated at 37°C (antibacterial) or 30°C (antifungal) for 24–48 h and then examined for the presence or absence of growth organisms tested. Chloramphenicol, rifampicin, nystatin, and ketoconazole were used as antibacterial and antifungal substances, respectively. The MIC values were obtained from the lowest concentration of the test compounds where the tubes remained clear, indicating that the bacterial or fungal growth was completely inhibited at this concentration.

4. Conclusions

A novel series of bis-sulfonamidated 4,6-dinitrobenzofuroxans **7–11**, obtained from 5,7-dichloro-4,6-dinitrobenzofuroxan **1** and the substituted sulfonamides **2–6**, were synthesized and characterized based on their physical, analytical, and spectral data. The compounds **7–11** were evaluated *in vitro* against bacterial and fungal species, showing significant antimicrobial activities. These new data of the molecules **8** and **10** might be helpful in the future development of sulfanilamide analogues as novel antimicrobial agents.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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