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Antimicrobial activity of novel isothiuronium salts with 7-chloro-4,6-dinitrobenzofuroxan-5-olate anion

Irina V. Galkina,*[a] Dmitriy I. Bakhtiyarov,[a] Luiza M. Usupova,[b] Alexander V. Gerasimov,[a] Marina P. Shulaeva,[c] Oskar K. Pozdeev,[c] Ahat V. Ilyasov,[d] Daut R. Islamov,[a] Konstantin S. Usachev,[e] Yulia V. Bakhtiyarova[a] and Vladimir I. Galkin[a]

[a] *A. M. Butlerov Institute of Chemistry, Kazan Federal University, 420008 Kazan, Russian Federation. E-mail: vig54@mail.ru*
[b] *Kazan National Research Technological University, 420015 Kazan, Russian Federation. E-mail: luizamagdanurovna@yandex.ru*
[c] *Kazan State Medical University, 420012 Kazan, Russian Federation. E-mail: shulaeva.m@mail.ru*
[d] *Tatarstan Academy of Sciences, 420011 Kazan, Russian Federation. E-mail: ilyasov_ahv@mail.ru*
[e] *A. E. Arbuzov Institute of Organic and Physical Chemistry, FRC Kazan Scientific Center of the Russian Academy of Sciences, 420088 Kazan, Russian Federation. E-mail: k.usachev@mail.ru*

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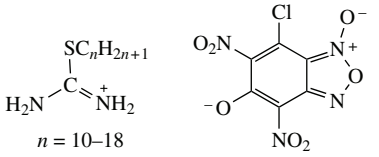
New thermally stable long-chained 2-alkylisothiuronium 7-chloro-4,6-dinitrobenzofuroxan-5-olates were obtained from the corresponding bromides and 5,7-dichloro-4,6-dinitrobenzofuroxan, the hydrolysis of the C(5)–Cl bond to produce phenolic function having occurred in the course of the process. The compound structure was determined by IR spectroscopy, elemental analysis and X-ray single crystal study. Salts with C_{14}–C_{18} alkyl groups revealed moderate antibacterial and antifungal activities.

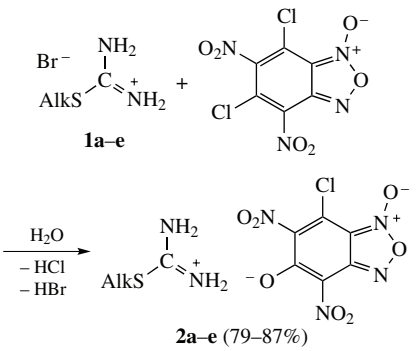


Keywords: benzofuroxan-5-olates, nitroarenes, isothiuronium salts, hydrolysis, antimicrobial activity, X-ray diffraction.

5,7-Dichloro-4,6-dinitrobenzofuroxan and its derivatives exhibit a broad spectrum of biological activity[1–13] and can serve as donors of nitric oxide.[5–9] They can be considered as prodrugs whose biological activity develops through a series of intermediates products within cells.[6–14] *S*-Alkylisothioureas and their derivatives, due to their unique reactivity, play an important role in the creation of various multifunctional compounds with a wide spectrum of biological activity.[15–23] However, the reactions of isothiuronium salts bearing higher alkyl groups with dichlorodinitrobenzofuroxan have not been documented.

This article describes our study on the reactions of 4,6-dichloro-5,7-dinitrobenzofuroxan with avalilable[24] isothiuronium bromides **1a–e** in ethanol. The gradual saturation of the reaction mixture with atmospheric water leads to the hydrolysis of the C(5)–Cl bond within the nitrogen heterocyclic moiety affording salts **2a–e** (Scheme 1).† This hydrolysis gives rise to formation of the phenolate function in 7-chloro-4,6-dinitrobenzofuroxan-5-olate anion. The reaction proceeded for a week to furnish bright yellow crystals of final products **2a–e**.

The structure of the isolated compounds was determined by IR spectroscopy, elemental and X-ray diffraction analysis (for details, see Online Supplementary Materials). ^{1}H NMR studies cannot be informative as the chlorodinitrobenzofuroxanolate anion is deprived of hydrogen atoms, while ^{13}C NMR spectra were not recorded due to poor solubility of salts **2a–e**. The IR studies provided some information, for example, the spectrum of salt **2e** contained the following absorption bands (cm^{-1}): 3300–3000 (NH), 2918–2849 (CH), 1654 (C=N), 1607



a Alk = *n*-$C_{10}H_{21}$ **d** Alk = *n*-$C_{16}H_{33}$
b Alk = *n*-$C_{12}H_{25}$ **e** Alk = *n*-$C_{18}H_{37}$
c Alk = *n*-$C_{14}H_{29}$

Scheme 1 *Reagents and conditions*: EtOH, reflux, 2 h; then storage (–6 °C) in contact with air moisture.

† *Alkylisothiouronium 7-chloro-4,6-dinitro-1-oxido-2,1,3-benzoxadiazol-5-olates* **2a–e** *(general procedure).* To a magnetically stirred solution of alkylisothiouronium bromide **1a–e** in anhydrous ethanol (25 ml), an equimolar amount of 5,7-dichloro-4,6-dinitrobenzofuroxan in the same solvent (25 ml) was added. The mixture was heated to reflux under nitrogen atmosphere for 2 h, then kept for 24 h at room temperature, cooled, and stored in refrigerator at –6 °C until yellow flakes consisting of single crystals appeared. The solvent was removed, and fine yellow crystals were left for aging for 48 h or more. The crystals were filtered off on a Schott filter and washed with ethanol and diethyl ether to remove unreacted initial compounds.

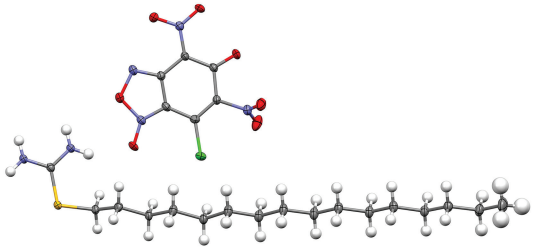


Figure 1 Molecular structure of 2-hexadecylisothiouronium 7-chloro-4,6-dinitro-1-oxido-2,1,3-benzoxadiazol-5-olate **2d** in the crystal according to the X-ray diffraction data.

(C=N–O), 1547 (NO_2), 1358 (NO_2), and 1482 (C–S). Luckily, due to low solubility single crystals of compound **2d** suitable for X-ray diffraction analysis were grown form ethanol at –6 °C (Figure 1), which allowed us to ultimately establish its structure.‡

Thermogravimetry and differential scanning calorimetry (TG-DSC) were used to explore the thermal stability of compounds **2a–e**, to determine their temperatures of the beginning and end of melting as well as the decomposition temperatures. The TG-DSC curve for compound **2e** (Figure 2) reveals no weight loss in the 40–135 °C temperature range, and thermal decomposition starts only above 140 °C as the exothermic peak (146 °C, 513.4 J g^{-1}) would appear. At 500 °C, the total weight loss approaches 74.5%. Such a high stability of salts **2a–e** makes them promising for further study as antiseptics. Importantly, the thermal sterilization of medicinal forms of antiseptics is carried out at 100 °C for 30 min and at 120 °C for 8 min. Apparently, salts **2a–e** can sustain the sterilization conditions without degradation and weight loss.

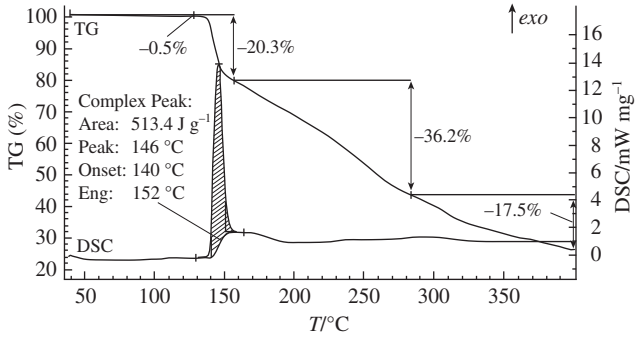


Figure 2 TG/DSC analysis of compound **2e** in dynamic argon atmosphere 75 ml min^{-1} in the temperature range 40–500 °C at heating rate 10 K min^{-1}.

‡ *Crystal data for* **2d**. $C_{23}H_{37}ClN_6O_7S$ (M = 577.09), triclinic, space group $P\bar{1}$ at 100.00(10) K: a = 5.24790(10), b = 11.1856(2) and c = 24.7646(4) Å, α = 93.506(2)°, β = 94.7520(10)°, γ = 103.048(2)°, V = 1406.52(4) Å^3, Z = 2, d_{calc} = 1.363 g cm^{-3}, μ(CuKα) = 2.342 mm^{-1}, F(000) = 612.0. Total of 16150 reflections were collected (5666 independent reflections, R_{int} = 0.0470) and used in the refinement, which converged to wR_2 = 0.1292, GOOF = 1.038 for all independent reflections [R_1 = 0.0457 was calculated for 5666 reflections with $I > 2\sigma(I)$].

Data set for single crystals was collected on a Rigaku XtaLab Synergy S instrument with a HyPix detector and a PhotonJet microfocus X-ray tube using CuKα (1.54184 Å) radiation at 100 K. Images were indexed and integrated using the CrysAlisPro data reduction package. Data were corrected for systematic errors and absorption using the ABSPACK module. The GRAL module was used for analysis of systematic absences and space group determination. Using Olex2,[25] structure was solved by direct methods with SHELXT[26] and refined by the full-matrix least-squares on F^2 using SHELXL.[27] Non-hydrogen atoms were refined anisotropically. The figures were generated using Mercury 4.1[28] program.

CCDC 1990614 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

Table 1 Antimicrobial activity (growth inhibition zone/mm) of salts **2a–e** (c = 500 μg ml^{-1}).

Compound	*Escherichia coli*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*
2a	12±0.4	17±0.3	10±0.5	27±0.5	21±0.4
2b	0	21±0.3	8±0.3	21±0.1	23±0.3
2c	0	21±0.5	8±0.1	21±0.4	27±0.5
2d	0	21±0.1	0	23±0.3	28±0.3
2e	0	22±0.3	0	26±0.5	43±0.5
Ethanol	0	0	0	0	5±0.1
Chlorhexidine	14±0.5	11±0.2	10±0.3	8±0.1	16±0.3
Miramistin	0	13±0.5	0	0	13±02
Clotrimazole	0	0	0	0	15±0.1

New compounds **2a**,**b** showed pronounced antibacterial and antifungal activity in tests on collection strains of pathogenic and opportunistic human and animal microorganisms (provided by the Microbiology Department of the Kazan State Medical Academy). The studies were performed *in vitro* against several representative pathogenic Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922), Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* ATCC 11778), and pathogenic fungi *Candida albicans* ATCC 885-653 (Table 1).§ The synthesized compounds exhibited moderate antimicrobial activities, however salts **2c–e** with longer alkyl chains showed the most potent antibacterial and antifungal activity compared to Chlorhexidine, Miramistin and Clotrimazole as reference drugs, against *Staphylococcus aureus, Escherichia coli*, *Bacillus cereus, Pseudomonas aeruginosa* and *Candida albicans* (see Table 1).

In conclusion, new thermally stable alkylisothiouronium 7-chloro-4,6-dinitrobenzofuroxan-5-olates were found to possess moderate antibacterial and antifungal activity, which makes them promising for further modification in search for other useful compounds. Salts of such chemotype are also novel for X-ray diffraction study.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.05.027.

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§ To assess the antibacterial activity, the agar plate method was used. The medium with bacteria was poured into sterilized Petri dishes under aseptic conditions. The standard formulations were Chlorhexidine and test compounds at a concentration of 500 μg ml^{-1}. Compounds **2a–e** were dissolved in DMSO immediately before use. Plates were incubated at 37 °C for 24 h. Antifungal activity was assessed by method of plates with Sabouraud's agar medium. Griseofulvin and test compounds were used at specified concentrations in ethanol–water mixtures in various ratios (1 : 10). After incubation, the mean inhibition was recorded in mm.

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