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Research Letter

Synthesis of New 'Hybrid' Compounds Based on Benzofuroxans and Aminoalkylnaphthalimides

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Pathogenic bacteria and fungi eventually develop resistance to existing drugs, and therefore, we need constant development of new drugs. The research is aimed at addressing fundamental scientific problems —the search for new biologically active compounds among several benzofuroxan-containing 'hybrid' products. *N*-substituted naphthalimides were chosen as a second pharmacophore. Benzofuroxanes biological effects were studied by means of bacterial lux-biosensors. Compounds IIIa, IVa, IIIc, and IVc displayed more expressed bacteriotoxic action in comparison with the initial substances Ia-c and represent a certain interest for using as antibacterial substances.

Key words: aminoalkylnaphthalimide, bacterial lux-biosensor, benzofuroxan, 'hybrid' compound

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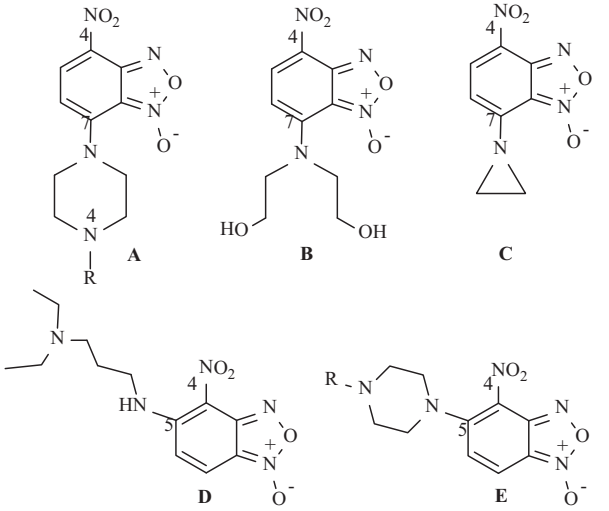
One of the directions in medical chemistry is the concept of 'hybrid multipurpose drugs', that is combination of two and more pharmacophores in one molecule. Compounds of this kind possess a wide range of pharmacological properties and the ability of medicinal substance to interact with several targets (1).

Benzofuroxans are a convenient platform for creation of multipurpose drugs. They are heterocyclic thermally stable compounds capable to generate NO slowly and for long time and do not cause development of nitrate tolerance (2). Besides, these compounds possess a wide range of biological activity properties (3).

Amino-substituted 4-nitrobenzofuroxans are known to display a wide range of biological activities. For example, a number of nitrobenzofuroxans have been developed as anticancer agents according to their mutagenicity (4–6). Compounds of type **A** sharing a piperazinyl residue with substitution at the N4 position have been shown to have a significant antileukemic activity in mice. This activity is also displayed by two further benzofuroxans in which the piperazinyl residue is replaced by 7-di(hydroxyethyl)amino **B** and aziridino **C** moieties, respectively. Similarly, benzofuroxan derivatives **D** and **E**, bearing at the 5 position a substituted amine moiety, have been tested for mutagenicity (7).



Benzofuroxan derivatives display typical NO-dependent activities both *in vitro* and *in vivo*, and the possibility of modulating NO release by changing the substituent on the ring makes them versatile tools in designing NO donor/drug hybrids (8). Actually, the combination of a benzofuroxanyl moiety with another biologically active substructure in a single molecule has recently received particular attention. For example, the 1-oxy-benzo[1,2,5]oxadiazol-5-ylmethyl[2-(2,6-dichloro-phenylamino)-phenyl]-acetate is a

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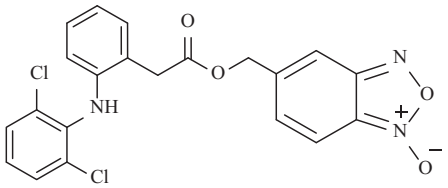
diclofenac derivative bearing a benzofuroxan moiety in its structure that showed anti-inflammatory activity and with better gastric tolerability with respect to that of native diclofenac, probably related to nitric oxide release ability (9).



As a second pharmacophore in this work we have chosen some N-substituted naphthalimides (**Ia-c**) (10). Benzo[de]isoquinoline-1,3-dione's derivatives (naphthalimides) are well known as a potent DNA intercalators and anticancer drugs (11). Besides, recently was shown their antibacterial (12) and antiviral (10) properties, which may be caused by DNA intercalators and topoII inhibition (13). Interferonogenic activity of **Ia-c**, shown in (10), may be explained with derepression of interferon gene or with specific naphthalimide receptor interactions.

Taking into consideration the above-described beneficial effects of the nitric oxide, we realized that it would be of interest to synthesize novel structural hybrids containing both heterocyclic ring systems, benzofuroxan, able to release NO, and naphthalimide owing to the biological effects related to its structure. We expected that presence of naphthalimide moiety will increase the affinity of target compounds to the nucleus, which increase benzofuroxan's effects.

To identify pharmacologically valuable properties of the newly synthesized compounds (antimicrobial, antioxidant, antimutagenic activity, etc.), the use of rapid methods is required. Express methods based on microbial whole-cell bioreporters are the most promising among them for use in drug toxicology. Microbial whole-cell bioreporters are genetically modified micro-organisms that produce a quantifiable output in response to the presence of toxic chemicals or other stress factors (14).

The optimal tool for routine screening of test chemicals is microbial whole-cell bioreporters on the basis of luminescent micro-organisms (15). Luminescence-based bacterial biosensors had several practical advantages over the traditional assays including procedural simplicity, sensitivity, a rapid result, ease of measurement and *in vivo* analysis without cell disruption (15,16).

Therefore, for the research of benzofuroxans mode action and determination of the molecular target in a cell that is damaged in their presence, we used bacterial lux-biosensors.

Materials and Methods

Chemistry

General comments

Melting points were determined on a melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on Bruker AVANCE 400 (Bruker BioSpin, Rheinstetten, Germany) NMR spectrometer (400.13 MHz) using tetramethylsilane as an internal standard, and the chemical shifts are reported in δ units. The IR spectra were recorded on a Bruker TENSOR 27 Fourier spectrometer (Bruker Optik GmbH, Karlsruhe, Germany) in the range 400–4000 cm^{-1}. Crystalline samples were studied as emulsions in Vaseline oil. Elemental analyses were performed on a CHNS-O Elemental Analyser EuroEA3028-HT-OM (EuroVector S.p.A., Milan, Italy) with an accuracy ±0.4% for C, H, Cl, and N. Most chemicals were purchased from commercial sources. Solvents were of analytical grade and were used without further purification unless otherwise stated. X-Ray analysis was performed in laboratory of X-ray analysis of Arbuzov Institute of Organic and Physical Chemistry of Kazan Scientific Centre of RAS. The crystallographic data of compound **IIIa** are as follows: ($C_{22}H_{16}ClN_5O_6$, M = 481.85) monoclinic, at 296 K a = 16.368(7), b = 7.238(3), c = 17.351(7) Å, β = 100.099 (6)°, V = 2023.8(15) Å^3, Z = 4, space group P21/c, 1.582 г·см$^{-3}$, μ = 0.244 мм$^{-1}$, F(000) = 992. The cell parameters and the experimental data were obtained on an automatic Bruker Smart APEX II CCD diffractometer [λ (MoK_α) = 0.71073 Å, ω-scanning], $2\theta < 54°$, R_{int} = 0.094. 16507 reflections were collected, 4401 of them were independent; the number of the observed reflections with $I > 2\sigma(I)$ was 2194. The absorption correction was applied using the SADABS program (17). The structure was solved by the direct method using the SIR program (18), and it was refined by the full-matrix least-squares method using the SHELXL97 program package (19). The hydrogen atoms of the hydroxyl groups were revealed by means of the difference electron density maps and refined in the isotropic approximation. The co-ordinates of the other hydrogen atoms were calculated geometrically and refined in a riding model. All the calculations were carried out using the WINGX (20) and APEX2 (21) programs; the final values of the divergence factors were R 0.0587, $_wR_2$ 0.1462, GOF = 1.00; the number of parameters to be refined was 308. The crystallographic data of the structure of **IIIa** have been deposited with the Cambridge Crystallographic Data Centre (http://www.ccdc.cam.ac.uk; the deposition code is CCDC 1423344).

The synthesis of compounds IIIa-c, IVa-c, Va-c:

The benzofuroxan derivatives **IIa-c** were prepared according to the published procedures (22–24).

6-chloro-4-((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2 (3H)-yl)propyl)(methyl)amino)-5-nitrobenzo[c][1,2,5] oxadiazole 1-oxide) (IIIa). To a solution of 4,6-dichloro-

5-nitrobenzofuroxan **IIa** (0. 25 g, 0.001 mole) in 3 mL of DMSO at room temperature a solution of amine **Ia** (0.27 g, 0.001 mole) in 3 mL of DMSO was added. The reaction mixture was stirred for 30 min. The crude mixture was precipitated in distilled water and the purple solid was filtered off, washed with cold water, diethyl ether and dried under vacuum (0.06 mm Hg) at 40 °C temperature to constant weight. Yield: 0.23 g (60%); Mp: 130 °C; IR (KBr, cm^{-1}): 1559 (NO_2), 1613 (furoxan ring), 1697 (CO); ^{1}H NMR (DMSO-d_6), δ (ppm): 2.12 (m, 2H, $CH_2C\underline{H}_2CH_2$); 3.04 (s, 3H, CH_3); 3.85 (t, 2H, $CH_2CH_2C\underline{H}_2N$, J = 7.0); 4.13 (t, 2H, $CH_2CH_2C\underline{H}_2N$, J = 6.6); 7.17 (s, 1H, Ar); 7.85 (t, 2H, Ar, J = 7.6); 8.42 (d, 2H, Ar, J = 8.0), 8.45 (d, 2H, Ar, J = 7.6). ^{13}C NMR (DMSO-d_6), δ (ppm): 26.37, 37.65, 39.50, 53.40, 101.36, 114.41, 122.55, 127.37, 127.66, 127.89, 131.10, 131.78, 134.48, 134.82, 136.66, 149.95, 164.11. Anal. calcd. for $C_{21}H_{14}ClN_5O_6$: C 53.92; H 3.02; Cl 7.58; N 14.97; Found: C 53.81; H 3.30; Cl 7.25; N 14.69.

Compounds **IIIb** and **IIIc** are prepared as above using 0.25 g (0.001 mole) of 4,6-dichloro-5-nitrobenzofuroxan **IIa** in 3 mL of DMSO and a solution of amine **I** (0.001 mole) in 3 mL of DMSO to give orange solids.

6-chloro-4-(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)-5-nitrobenzo[c][1,2,5]oxadiazole 1-oxide IIIb. Yield 0.31 g (81%). Mp 165 °C; IR (KBr, cm^{-1}): 1560 (NO_2), 1622 (furoxan ring), 1692 (CO); ^{1}H NMR (DMSO-d_6), δ (ppm): 3.05 (m, 2H, $C\underline{H}_2CH_2$); 3.41 (m, 8H, $C\underline{H}_2C\underline{H}_2NC\underline{H}_2C\underline{H}_2N$); 4.22 (m, 2H, $CH_2C\underline{H}_2$); 7.44 (s, 1H, Ar); 7.89 (t, 2H, Ar, J = 7.0); 8.46 (d, 2H, Ar, J = 8.2); 8.52 (d, 2H, Ar, J = 7.0). ^{13}C NMR (DMSO-d_6), δ (ppm): 38.23, 51.21, 53.82, 56.23, 104.84, 115.32, 123.31, 127.46, 128.60, 128.70, 132.14, 132.64, 135.73, 136.57, 137.74, 150.76, 164.83. Anal. calcd. for $C_{21}H_{14}ClN_5O_6$: C 53.92; H 3.02; Cl 7.58; N 14.97; Found: C 53.66; H 3.20; Cl 7.65; N 14.75.

6-chloro-4-((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(2-hydroxyethyl)amino)-5-nitrobenzo[c][1,2,5]oxadiazole 1-oxide IIIc. Yield 0.19 g (50%). Mp 179 °C; IR (KBr, cm^{-1}): 1546 (NO_2), 1620 (furoxan ring), 1701 (CO); ^{1}H NMR (DMSO-d_6), δ (ppm): 2.02 (m, 2H, $CH_2C\underline{H}_2CH_2$); 3.53 (t, 2H, $CH_2C\underline{H}_2OH$, J = 5.7); 3.60 (m, 4H, $CH_2CH_2C\underline{H}_2N$, $C\underline{H}_2CH_2OH$); 4.07 (t, 2H, $C\underline{H}_2CH_2CH_2N$, J = 6.7), 7.34 (s, 1H, Ar); 7.82 (t, 2H, Ar, J = 7.6); 8.37 (d, 2H, Ar, J = 7.6), 8.40 (d, 2H, Ar, J = 8.6); ^{13}C NMR (DMSO-d_6), δ (ppm): 26.56, 37.94, 50.75, 54.48, 59.39, 103.73, 114.33, 122.45, 126.78, 127.59, 127.78, 131.03, 131.71, 134.76, 136.22, 150.31, 164.01. Anal. calcd. for $C_{23}H_{18}ClN_5O_7$: C 53.97; H 3.54; Cl 6.93; N 13.68; Found: C 53.82; H 3.27; Cl 6.65; N 13.30.

Compounds **IVa-c** were prepared by the following procedure: to a solution of 7-chloro-4,6-dinitro-benzofuroxan **IIb** (0.26 g, 0.001 mole) in 3 mL of ethanol at room temperature was added a solution of amine **I** (0.001 mole) in 3 mL of ethanol. The reaction mixture was boiled for 4 h (the reaction was monitored by thin layer chromatography). After verification of the completion of the reaction by TLC, the crude mixture is precipitated in distilled water and the orange solids were filtered off, washed with cold water and dried under vacuum (0.06 mm Hg) at 40 °C temperature to constant weight.

7-((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(methyl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide IVa. Yield: 0.45 g (91%); Mp: 170 °C; IR (KBr, cm^{-1}): 1586 (NO_2), 1655 (furoxan ring), 1696 (CO); ^{1}H NMR (DMSO-d_6), δ (ppm): 2.00 (m, 2H, $CH_2C\underline{H}_2CH_2$); 2.57 (s, 3H, CH_3); 3.01 (m, 2H, $CH_2CH_2C\underline{H}_2N$); 4.12 (t, 2H, $CH_2CH_2C\underline{H}_2N$, J = 6.7); 7.86 (t, 2H, Ar, J = 7.8); 8.41 (d, 4H, Ar, J = 7.8); 8.45 (d, 4H, Ar, J = 7.8); 8.94 (s, 1H, Ar). ^{13}C NMR (DMSO-d_6), δ (ppm): 24.91, 32.98, 37.40, 46.77, 111.22, 115.04, 122.47, 127.53, 127.61, 127.86, 131.15, 131.72, 134.80, 135.07, 148.24, 161.51, 164.04. Anal. calcd. for $C_{21}H_{14}N_6O_8$: C 52.73; H 2.95; N 17.37; Found: C 52.55; H 2.72; N 17.54.

7-(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide IVb. Yield 0.47 g (95%). Mp 205 °C; IR(KBr, cm^{-1}): 1654 (furoxan ring), 1696 (CO); ^{1}H NMR (DMSO-d_6), δ (ppm): 3.42 (m, 10H, $C\underline{H}_2C\underline{H}_2NC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2$); 4.40 (m, 2H, $C\underline{H}_2$); 7.86 (t, 2H, Ar, J = 7.6); 8.48 (d, 2H, Ar, J = 7.6); 8.50 (d, 2H, Ar, J = 7.1); 8.99 (s, 1H, Ar); ^{13}C NMR (DMSO-d_6), δ (ppm): 35.17, 40.87, 49.05, 54.25, 111.32, 115.15, 122.49, 127.57, 127.73, 127.99, 131.37, 131.79, 135.10, 135.14, 148.31, 161.61, 164.38. Anal. calcd. for $C_{24}H_{19}N_7O_7$: C 55.71; H 3.70; N 18.95; Found: C 55.83; H 4.01; N 18.80.

7-((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(2-hydroxyethyl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide IVc. Yield 0.28 g (58%). Mp 200 °C; IR(KBr, cm^{-1}): 1654 (furoxan ring), 1696 (CO), 3446 (OH); ^{1}H NMR (DMSO-d_6), δ (ppm): 2.05 (m, 2H, $CH_2C\underline{H}_2CH_2$); 3.00 (m, 4H, $C\underline{H}_2NC\underline{H}_2$); 3.63 (t, 2H, $CH_2C\underline{H}_2OH$, J = 5.0), 4.12 (t, 2H, $C\underline{H}_2CH_2CH_2N$, J = 5.0); 7.82 (t, 2H, Ar, J = 7.6); 8.39 (d, 2H, Ar, J = 7.1); 8.43 (d, 2H, Ar, J = 8.5); 8.94 (s, 1H, Ar); ^{13}C NMR (DMSO-d_6), δ (ppm): 24.95, 37.50, 45.37, 49.34, 56.87, 111.22, 115.04, 122.46, 127.53, 127.61, 127.85, 131.15, 131.72, 134.79, 135.08, 148.24, 161.51, 164.13. Anal. calcd. for $C_{23}H_{18}N_6O_9$: C 52.88; H 3.47; N 16.09; Found: C 52.99; H 3.20; N 16.26.

Compounds **Va-c** were prepared by the following procedure: to a solution of 5,7-dichloro-4,6-dinitro-benzofuroxan **IIc** (0.25 g, 0.0008 mole) in 3 mL of DMSO at room temperature was added a solution of amine **I** (0.0016 mole) in 3 mL of DMSO. The reaction mixture was stirred for

30 min. The crude mixture was precipitated in distilled water and the purple solid was filtered off, washed with cold water, diethyl ether and dried under vacuum (0.06 mm Hg) at 40 °C temperature to constant weight.

5,7-bis((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(methyl)amino)-4,6-dinitrobenzo[c][1,2,5] oxadiazole 1-oxide Va. Yield: 0.60 g (94%); Mp: 30 °C; IR (KBr, cm^{-1}): 1345 (NO_2), 1549 (NO_2), 1658 (furoxan ring), 1700 (CO); ^{1}H NMR (DMSO-d_6), δ (ppm): 2.01 (m, 4H, $CH_2C\underline{H}_2CH_2$); 2.57 (s, 6H, CH_3); 3.01 (m, 4H, $CH_2CH_2C\underline{H}_2N$); 4.13 (m, 4H, $NC\underline{H}_2CH_2CH_2$); 7.81 (m, 4H, Ar); 8.47 (m, 8H, Ar). ^{13}C NMR (DMSO-d_6), δ (ppm): 24.89, 32.91, 37.41, 46.71, 122.55, 127.55, 127.66, 127.82, 127.92, 131.02, 131.19, 131.69, 131.76, 134.63, 134.84, 150.31, 163.99, 164.18, 164.24. Anal. calcd. for $C_{38}H_{30}N_8O_{10}$: C 60.16; H 3.99; N 14.777; Found: C 60.28; H 4.14; N 14.97.

5,7-bis(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)-4,6-dinitrobenzo[c][1,2,5] oxadiazole 1-oxide Vb. Yield 0.48 g (75%). Mp 160 °C; IR(KBr, cm^{-1}): 1345 (NO_2), 1529 (NO_2), 1661 (furoxan ring), 1700 (CO); ^{1}H NMR (DMSO-d_6), δ (ppm): 3.06 (m, 4H, $C\underline{H}_2CH_2$); 3.41 (16H, $2C\underline{H}_2C\underline{H}_2NC\underline{H}_2C\underline{H}_2N$); 4.42 (m, 4H, $CH_2C\underline{H}_2$); 7.92 (4H, Ar, J = 7.21); 8.52 (d, 4H, Ar, J = 7.1); 8.56 (d, 4H, Ar, J = 8.1); ^{13}C NMR (DMSO-d_6), δ (ppm): 43.47, 49.88, 52.26, 54.56, 122.51, 122.59, 127.78, 127.85, 128.04, 131.31, 131.51, 131.83, 134.95, 135.15, 150.17, 163.51, 164.00, 164.48. Anal. calcd. for $C_{42}H_{36}N_{10}O_{10}$: C 60.00; H 4.32; N 16.66; Found: C 60.27; H 4.10; N 16.85.

5,7-bis((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(2-hydroxyethyl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide Vc. Yield 0.31 g (50%). Mp 185 °C; IR(KBr, cm^{-1}): 1376 (NO_2), 1589 (NO_2), 1656 (furoxan ring); ^{1}H NMR (DMSO-d_6), δ (ppm): 2.06 (m, 4H, $CH_2C\underline{H}_2CH_2N$); 2.98 (m, 4H, $CH_2C\underline{H}_2OH$); 2.99 (m, 4H, $CH_2CH_2C\underline{H}_2N$); 3.64 (t, 4H, $C\underline{H}_2CH_2CH_2N$, J = 5.2); 4.11 (t, 4H, $C\underline{H}_2CH_2OH$, J = 6.7); 4.12 (t, 4H, $NC\underline{H}_2CH_2CH_2$, J = 6.07); 7.84 (m, 4H, Ar); 8.44 (m, 8H, Ar); ^{13}C NMR (DMSO-d_6), δ (ppm): 26.56, 37.94, 50.75, 54.48, 59.32, 122.51, 122.59, 127.65, 127.82, 127.91, 131.02, 131.19, 131.62, 131.77, 134.68, 134.84, 150.17, 163.99, 164.17, 164.99. Anal. calcd. for $C_{40}H_{34}N_8O_{12}$: C 58.68; H 4.19; N 13.69; Found C 58.80; H 4.00; N 13.31.

Biology

Research of benzofuroxans biological effects by means of bacterial lux-biosensors

Bacterial strains and culture conditions. Biological properties of the synthesized compounds were investigated by means of the natural strain *Vibrio* genus and bacterial lux-biosensors designed on the base of *Escherichia coli* MG1655. *Vibrio aquamarinus* VKPM B-11245 (*Vibrio aquamarinus* DSM 26054) was used for evaluation of integral toxicity. *V. aquamarinus* VKPM B-11245 is a natural *Vibrio* strain isolated from the Black Sea water (25).

A number of genetically engineered biosensor strains were used in the experiment, including *E. coli* MG1655 (pXen7), which is a biosensor with a constitutive promotor and the ones for detection of DNA-tropic effects – (*E. coli* MG1655 (pRecA-lux), *E. coli* MG1655 (pColD-lux)); of the substances causing oxidative stress (*E. coli* MG1655 (pKatG-lux), *E. coli* MG1655 (pSoxS-lux)); of protein damaging substances (*E. coli* MG1655 (pGrpE-lux), *E. coli* MG1655 (pIbpA-lux)); of membrane damaging substances (*E. coli* MG1655 (pFabA-lux)); influencing bacterial Quorum Sensing system of the 1st type (*E. coli* MG1655 (pVFR1-lux)). Biosensors contain pBR322 vector with a set of genes lux-*CDABE* from *Photorhabdus luminescens* ZM1 under control of the induced promoter. These biosensor strains are obtained by transformation of *E. coli* MG1655 with hybrid plasmids of pXen7, pRecA-lux, pColD-lux, pKatG-lux, pSoxS-lux, pGrpE-lux, pIbpA-lux, pFabA-lux, pVFR1-lux. Strains are kindly provided by I.V. Manukhov (Federal State Unitary Enterprise 'GosNIIGenetika').

The bacterial strains were cultivated in Luria–Bertani (LB) medium (26), containing 100 μg of ampicillin/mL. The cultures were grown under constant shaking to early exponential phase at 37 °C. Strain *V. aquamarinus* VKPM B-11245 was grown without addition of ampicillin to early exponential phase at 25 °C. Cells were used immediately for stress-induction tests.

Biosensors assay procedure. The detailed protocol of toxicity testing by means of a bacterial lux-biosensors battery is described in the article (27).

Calculation. The criterion of integral toxic influence is bioluminescence intensity change of the test object in the researched sample in comparison with that for the sample with the solution not containing the studied substances. The toxic influence of the studied toxicant on bacteria is evaluated according to the inhibition of their bioluminescence for 30-min exposition period.

The quantitative assessment of the test reaction parameter is reflected as a dimensionless number e the toxicity index 'T', calculated according to the Formula (1):

$$T = 100\frac{(I_c - I_e)}{I_c}, \quad (1)$$

where I_c and I_e are the intensity of bacteria luminescence in control and proof samples, respectively, at fixed exposition time of the studied solution with test object.

In some cases, a situation is possible when bioluminescence intensity of an analyzed sample is higher that of the control sample. In that case irrespective of the size of negative T value the conclusion about absence of the

sample toxicity is drawn, and the toxicity index equals zero.

The technique allows three threshold levels of the toxicity index: (i) Admissible degree of toxicity: the toxicity index is <20. (ii) The sample is toxic: the toxicity index is equal or more than 20 and less than 50. (iii) The sample is highly toxic: the toxicity index is equal or more than 50. All the experiments were carried out in three independent replications.

Toxic influence of the studied toxicant on genetically engineered biosensor strains is evaluated according to the induction of their bioluminescence for 120-min exposition period. For the assessment of the studied factors influence on the expression of operons the induction factor (I) was calculated according to the Formula (2):

$$I = \frac{L_e}{L_c}, \quad (2)$$

where L_e is the luminescence intensity of the proof sample, and L_c is the intensity of the control sample luminescence.
Difference reliability of bioluminescence in experiment from control value was estimated by t-criterion with the help of Excel program. The conclusion about sample toxicity was made at $p < 0.05$. If at significant differences from control induction factor values were less than 2, the detected toxic effect was evaluated as «weak», and if they were in the range from 2 to 10—as «medium», above 10—as «strong». All the experiments were carried out three times independently.

Results and Discussion

Chemistry

As a result of the carried out reactions of the electrophilic benzofuroxans (**IIa-c**) with various aminoalkylnaphthalimides (**Ia-c**) representatives of 'hybrid', benzofuroxans (**III**-**V**) derivatives were synthesized by us. The structure of all synthesized compounds is proved using the methods of nuclear magnetic resonance ^{1}H spectroscopy, infrared spectroscopy, data composition of the elemental analysis. Compound **IIIa** structure is proved with the method of X-ray crystal analysis (see Figure 1).

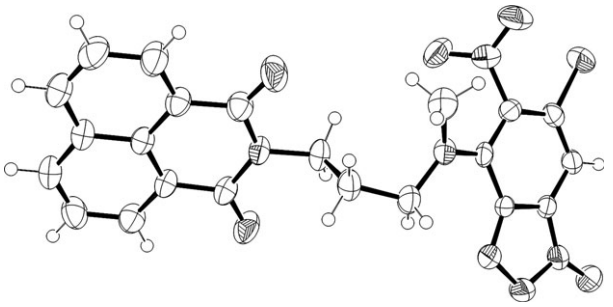


Figure 1: General view of the compound **IIIa** molecule according to the X-ray analysis data.

The crystals of compound **IIIa** have one molecule in the independent part of the crystal unit cell. Benzofuroxan and aminoalkylnaphtalimide moieties are planar and situated at an angle 46.9° to each other. The nitro-group at benzofuroxan fragment lies at an angle to the plane of the benzofuroxan (torsion angle $O^{12}N^{10}C^5C^6$ 57.8 (4)°). The bond lengths are within the standard values for this type of bonds. The crystal packing is stabilized by C–H···O intermolecular interactions and lead to the formation of a complex three-dimensional structure.

Research of benzofuroxanes biological effects by means of bacterial lux-biosensors

Integral toxicity of these compounds for bacterial cells was evaluated by means of *V. aquamarinus* VKPM B-11245 and *E. coli* MG1655 (pXen7) bacterial strains.

All the studied substances displayed toxicity concerning *V. aquamarinus* VKPM B-11245 in the concentration range up to 10^{-4} M. In the concentration of 10^{-5} M, all the compounds were also toxic. For the compounds **IIIa, IVa, Va, IIIb, IVc** high toxicity level ranging from 54 to 240 was registered. Substances **IIIb**, **IVb** and **Vb** (toxicity index varied from 27 to 42), **IIIc**, **IVc** and **Vc** (toxicity index ranged from 25 to 44) in the concentration of 10^{-6} M maintained toxicity for *Vibrio* cells, and substance **IIIa** was highly toxic (T = 72). In the concentration of 10^{-7} M, toxicity was registered only for compounds **IVc** and **Vb**.

Compounds **IIIb**, **Vb**, **III-Va**, **IIIc**, **IVc** displayed low toxicity level in the range of concentrations from 10^{-9} to 10^{-11} M (toxicity index equaled from 21 to 28) (see Figure 2). Bacteriotoxic effect for these compounds concerning the constitutive biosensor on the basis of *E. coli* MG1655 was not registered.

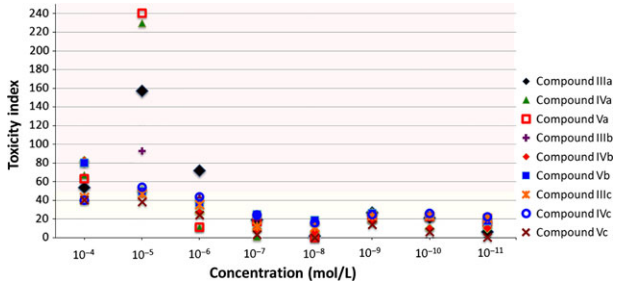


Figure 2: Integral toxicity of compounds **III**-**V**, registered with *Vibrio aquamarinus* VKPM B-11245 biosensor strain.

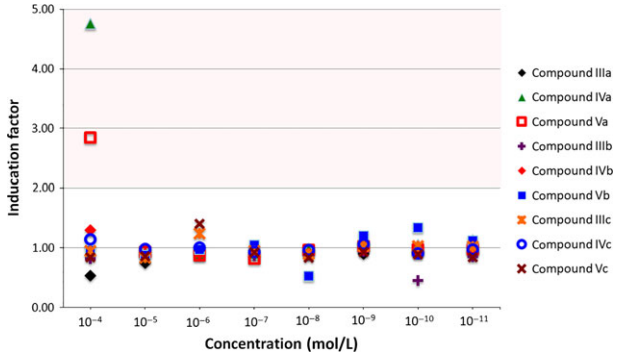


Figure 3: Genotoxicity of compounds **III**-**V**, registered with *Escherichia coli* MG1655 (pRecA-lux) biosensor strain without use of metabolic activation.

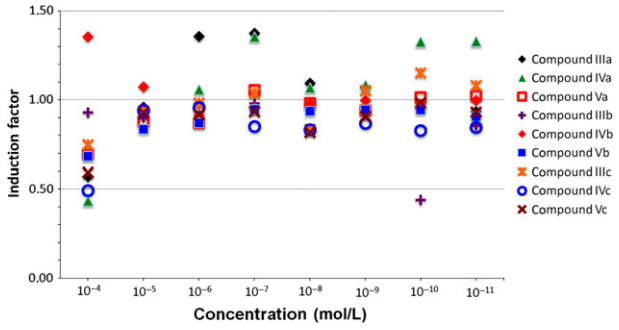


Figure 4: Genotoxicity of compounds **III**-**V**, registered with *Escherichia coli* MG1655 (pRecA-lux) biosensor with use of metabolic activation.

Compounds **IVa** and **Va** in high concentration (10^{-4} M) displayed the average genotoxicity level during the tests with *E. coli* MG1655 (pRecA-lux) biosensor without use of metabolic activation (the maximum induction factor 'I' was 4.8) (Figure 3).

Genotoxic effect was not registered with *E. coli* MG1655 (pRecA-lux) biosensor with metabolic activation (Figure 4).

A much more expressed genotoxic effect for a number of the studied substances was registered with the *E. coli* MG1655 (pColD-lux) biosensor. So, compounds **IIIc** and

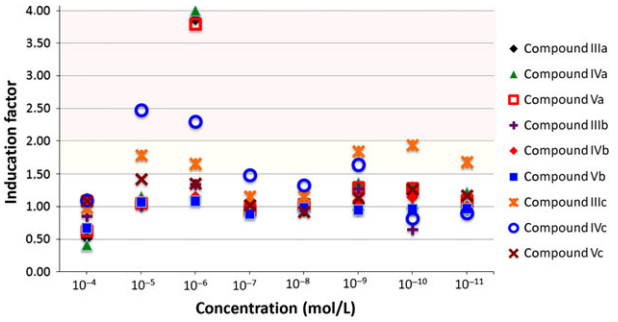


Figure 5: Genotoxicity of compounds **III**-**V**, registered with *Escherichia coli* MG1655 (pColD-lux) biosensor strain without use of metabolic activation.

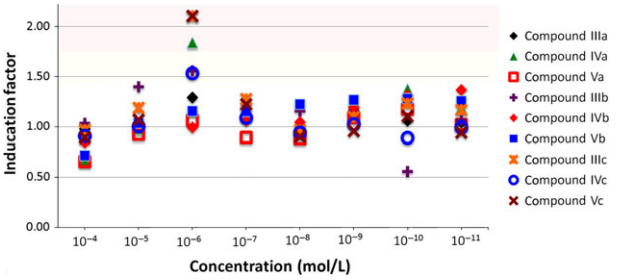


Figure 6: Genotoxicity of compounds **III**-**V**, registered with *Escherichia coli* MG1655 (pColD-lux)biosensor strain with use of metabolic activation.

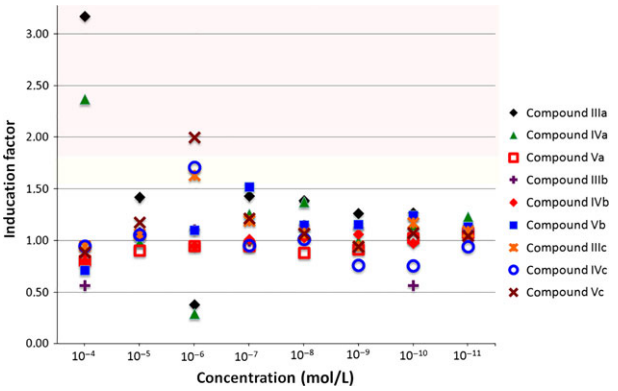


Figure 7: Proteins damage effects registered with *Escherichia coli* MG1655 (plbpA-lux) biosensor strain.

I**Vc** showed weak genotoxic effect (the maximum I equaled 1.94), and compounds **IIIa**, **IVa**, **Va**, **IVc** displayed average genotoxic effect (I ranged from 2.3 to 4.0) without application of metabolic activation in the concentration of 10^{-6} M. The substances **IVc** and **IIIc** without metabolic activation showed weak genotoxic effect in concentrations of 10^{-9} and 10^{-10} M respectively (I equaled 1.6 and 1.9) (Figure 5).

In case of application of metabolic activation, average genotoxic effect was registered for substances **IVa**, **IIIc**

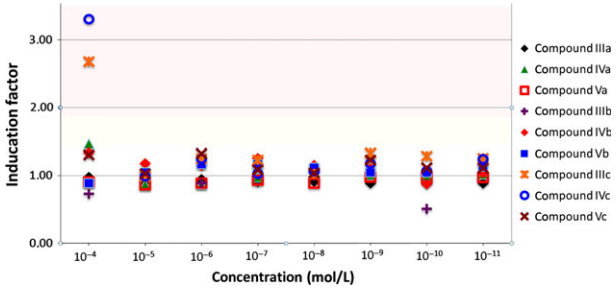


Figure 8: Proteins damage effects registered with *Escherichia coli* MG 1655 (pGrpE-lux) biosensor strain.

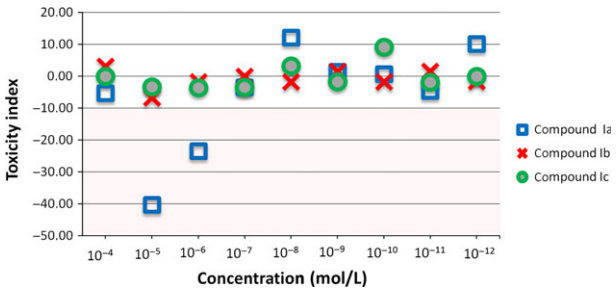


Figure 9: Integral toxicity of initial compounds **Ia**-**c**, registered with *Vibrio aquamarinus* VKPM B-11245 biosensor strain.

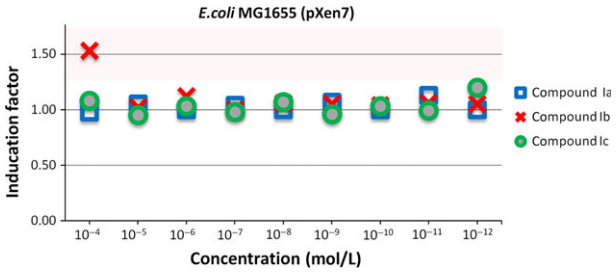


Figure 10: Integral toxicity of initial compounds **Ia**-**c**, registered with the biosensor strain *Escherichia coli* MG1655 (pXen7).

and **Vc**, and weak genotoxic effect was registered for substances **IIIb** and **IVc** in the concentration of 10^{-6} M (I ~ 1.5) (Figure 6).

For *E. coli* MG1655 (pIbpA-lux) biosensor reacting to proteins damage average strength effects were observed for compounds **IIIa**, **IVa** in the concentration of 10^{-4} M (the maximum I equaled 3.2), and for the compound **Vc** in the concentration of 10^{-6} M (I = 2,0). The substances **IIIc** and **IVc** displayed a weak toxic effect also in the concentration of 10^{-6} M (I equaled 1.7 and 1.6, respectively) (Figure 7).

The *E. coli* MG 1655 (pGrpE-lux) biosensor registered the average strength effect for compounds **IIIc** and **IVc** and the weak effect for the compound **IVa** in the concentration of 10^{-4} M (the induction factor equaled 3.3; 2.7 and 1.5 respectively) (Figure 8).

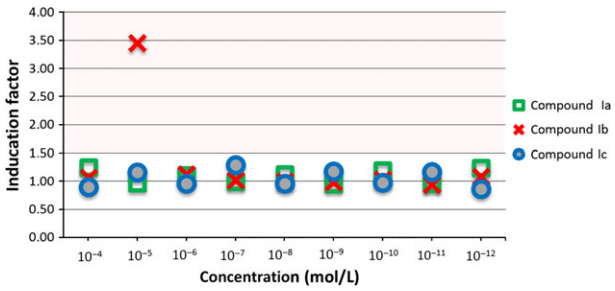


Figure 11: Genotoxicity of initial compounds **Ia**-**c**, registered with the biosensor strain *Escherichia coli* MG1655 (pColD-lux).

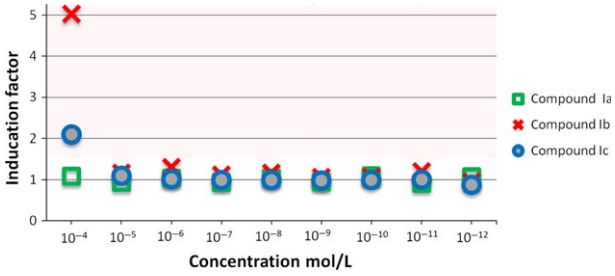


Figure 12: Prooxidant activity of initial compounds **Ia**-**c** registered with the biosensor *Escherichia coli* MG1655 (pKatG-lux).

No expressed influence of the studied compounds on bacterial lux-biosensors *E. coli* MG1655 (pSoxS-lux), *E. coli* MG1655 (pKatG-lux), *E. coli* MG1655 (pFabA-lux), and *E. coli* MG1655 (pVFR1-lux) was registered.

Initial compounds **Ia-c** were also tested by means of bacterial lux-biosensors. All substances did not display integral toxicity concerning *V. aquamarinus* VKPM B-11245 in all concentrations (Figure 9), whereas **Ib** and **Ic** substances turned out to be toxic concerning *E. coli* MG1655 (pXen7) (the toxicity index equaled 32.71 and 29.6 respectively) (Figure 10).

Compound **Ib** also displayed genotoxicity in the concentration of 10^{-5} M (*E. coli* MG1655 (pColD-lux) biosensor, I = 3.5) (Figure 11) and ability to cause oxidizing stress (*E. coli* MG1655 (pKatG-lux) biosensor, I = 5) in 10^{-4} M concentration (Figure 12). Substance **Ic** in a similar concentration also triggers hydrogen peroxide formation in a bacterial cell (I = 2.1).

Taken together, 'hybrid' benzofuroxans **(III-V)** derivatives possess toxic properties (integral toxicity, genotoxicity, proteins damage) in lower concentrations compared to initial substances **Ia**-**c**.

The maximum amount of toxic effects was registered for low concentrations of such compounds as **IIIa, IVa, IIIc,** and **IVc**, what proves their antibacterial potential.

Compounds on the basis of benzofuroxan **IIc** are not biologically active. So this scaffold is not suitable for

obtaining 'hybrid' compounds based on aminoalkylnaphthalimides. In the case of benzofuroxans substituted by naphthalimides containing cyclic amine as a linker (compounds **IIIb**, **IVb**), the activity is worse with that of benzofuroxans **IIIa**, **IVa**, **IIIc,** and **IVc**, substituted by naphthalimides containing aliphatic amines as a linker. Thus, we can conclude that the nature of the linker plays a role in the biological effect of obtained compounds and aliphatic amines are preferable.

Conclusion

Compounds **IIIa**, **IVa**, **IIIc,** and **IVc** display more expressed bacteriotoxic action in comparison with the initial substances **Ia-c** and represent a certain interest for using as antibacterial substances.

Acknowledgments

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Conflict of Interest

There is not conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. 6-chloro-4-((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(methyl)amino)-5-nitrobenzo[c][1,2,5]oxadiazole 1-oxide) **IIIa**.

Figure S2. 6-chloro-4-(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)-5-nitrobenzo[c][1,2,5]oxadiazole 1-oxide **IIIb**.

Figure S3. 6-chloro-4-((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(2-hydroxyethyl)amino)-5-nitrobenzo[c][1,2,5]oxadiazole 1-oxide **IIIc**.

Figure S4. 7-((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(methyl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide **IVa**.

Figure S5. 7-(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide **IVb**.

Figure S6. 7-((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(2-hydroxyethyl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide **IVc**.

Figure S7. 5,7-bis((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(methyl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide **Va**.

Figure S8. 5,7-bis(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide **Vb**.

Figure S9. 5,7-bis((3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)(2-hydroxyethyl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide **Vc**.