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Synthesis and Biological Properties of New Nitrobenzoxadiazole Derivatives

E.V. Prazdnova*, E.Y. Kharchenko, V.A. Chistyakov, Yu.P. Semenyuk, P.G. Morozov, S.V. Kurbatov, V.K. Chmyhalo

Southern Federal University, 194/1, Stachki Avenue, Rostov-on-Don, Russian Federation

Abstract

This paper is devoted to the synthesis, structure and biological properties of nitrobenzoxadiazole derivatives, which are potential exogenous sources of nitric oxide (II). Using the methods of nucleophilic aromatic substitution and polar cycloaddition, new dihetaryls, including superelectrophilic and π-excessive carbo and heterocycles have been synthesized. Using the genetically engineered Lux-biosensors based on *Escherichia coli—E. coli* MG 1655 (pSoxS-lux), *E. coli* MG 1655 (pXen7-lux), *E. coli* MG 1655 (pRecA-lux)—their ability to cause SOX-induction has been quantitatively determined, which may be a result of nitrogen oxide (II) generation *in vivo*. It has been found that 4-(4-dimethylaminophenyl)-7,8-dihydro-7-methyl-5-nitro-6H-[1,2,5]oxadiazolo[3,4-e]isoindol is a more effective inducer of SOX-response than compared preparations, such as nitroglycerin and NOC-5. The use of biosensor *E. coli* MG 1655 (pXen7-lux) showed the absence of nonspecific toxicity in all synthesized compounds. A significant DNA-protective effect has been detected together with the absence of genotoxicity in N,N-dimethyl-4-(7-methyl-5-nitro-7,8-dihydro-6H -[1,2,5]oxadiazolo[3,4-e]isoindol-4-yl)aniline.

Keywords

Dinitrobenzofuroxan; Dinitrobenzofurazan; Pyrrole; Indole; 1,3-N-oxidic rearrangement; Cycloaddition; NO donor; Lux-biosensor; SOX-induction; Genotoxicity, DNA-protective activity

Introduction

It is known that furoxans and furozans feature a broad spectrum of biological activity, e.g., antibacterial [1,2], antifungal [1,2], algicidal [3], vasodilatory, and antiaggregating [4]. The last two effects are associated with their ability to be sources of nitrogen oxide (II), which is a multimodal regulator of many physiological processes (blood vessels relaxation, inhibition of platelets aggregation, operation of the immune and nervous systems) as well as pathological conditions (infectious, inflammatory, neoplastic diseases) in humans [5-7].

There are two basic approaches to increasing the levels of NO *in vivo*: stimulating NO synthase (NOS) [8-10] and using exogenous sources of NO that spontaneously or enzymatically release nitric oxide in an organism [5,11]. Condensed furoxans are among major NO donors [5,10,11].

Materials and Methods

^{1}H NMR spectra were recorded on the "Bruker DPX-250 (250 MHz) spectrometer in $CDCl_3$. The melting temperatures of substances were determined in glass capillaries on the PTP-M device. 4-Chloro-5,7-dinitrobenzofurazan (**1**) was synthesized using method [12], 4,6-dinitro-7-chlorobenzofuroxan (**2**)—using method [13], compound **4**—using method [14], compounds **5** and **17**—using method [15], compound **6**—using method [16], and compound **19**—using method [17].

Synthesis of compound (**3**)—**4-(5,7-dinitro-2,1,3-benzoxadiazol-4-yl)-N,N-dimethylaniline**: to 50 mg (0.2 μmol) of 5,7-dinitro-4-chlorobenzofurazan (**1**) in 3 ml of warm chloroform, a solution of 50 mkl (0.4 μmol) of N,N-dimethylaniline in 1 ml of chloroform was added. And 24 h later, the solution was rinsed with 10% hydrochloric acid and water, dried with anhydrous sodium sulfate, and boiled out in the air. The residue was purified by column chromatography (SiO_2, eluent—$CHCl_3$). The yield was 60 mg (88%), dark blue crystals, melting temperature—172-174°C. The ^{1}H NMR spectrum (ppm, *J*/Hz): 3.13 (s, 6H, $N(Me)_2$); 6.79 (d, 2H, N(3′, 5′), J = 9.1); 7.66 (d, 2H, N(2′, 6′), J = 9.1); 8.77 (s, 1H, H(6)). $C_{24}H_{17}N_5O_5$. Found, %: C, 51.09; H, 3.35; N, 21.30. Calculated, %: C, 51.07; H, 3.37; N, 21.27.

Synthesis of compound (7)—**7-ethoxy-4,6-dinitro-2,1,3-benzoxadiazole 1-oxide**: to 75 mg (0.6 μmol) of salicylic aldehyde **1** in ethanol, 62 mg (0.6 μmol) of Et_3N and 160 mg (0.6 μmol) of 4,6-dinitro-7-chlorobenzofuroxan (2) were added. The mixture was heated until dissolution of reactants. The precipitate was separated and rinsed with Et_2O. The yield was 130 mg (78%), orange crystals, melting temperature—188-190°C. The ^{1}H NMR spectrum (ppm, J/Hz): 1.37 (t, 3H, CH_3, J = 7.2); 3.30 (q, 2H, CH_2, J = 7.2, J = 4.1); 9.42 (s, 1H, H-6). $C_8H_6N_4O_7$. Found, %: C, 35.59; H, 2.27; N, 20.71. Calculated, %: S, 35.57; H, 2.24; N, 20.74.

Synthesis of compound (**11**)—**4-(5,8-dimethyl-6a,9a-dinitro-3-oxido-5,6,6a,6b,7,8,9,9a-octahydro[1,2,5]oxadiazolo[3,4-*e*]pyrrolo[3,4-*g*]isoindole-3b(4*H*)-yl)-N,N-dimethylaniline**: to the suspension of 52 mg (0.58 μmol) of finely powdered sarcosine and 17 mg (0.58 μmol) of paraformaldehyde in 3 ml of anhydrous benzene, 100 mg (0.29 μmol) of the compound **4** were added. The reaction mixture was boiled for 1 h until complete discoloration, after which the solution was cooled and filtered; the filtrate was concentrated and purified using column chromatography, and the second fraction (SiO_2, eluent—ethyl acetate:chloroform, 1:10) was collected. The yield was 27 mg (20%), brown crystals, melting temperature—238-240°C. The ^{1}H NMR spectrum (δ, ppm, *J*/Hz): 2.45 (s, 3H, C(5)NCH_3); 2.50 (s, 3H, C(8)NCH_3); 2.42-2.59 (m, 1H, H(6)); 2.91 (s, 6H, $N(CH_3)_2$); 3.25 (dd, 1H, H(6), J = 7.5, J = 10.5); 3.40 (d, 1H, H(7), J = 13.2); 3.49 (d, 1H, H(4), J = 12.2); 3.62 (d, 1H, H(9), J = 9.9); 3.66 (d, 1H, H(7), J = 13.2); 3.95 (d, 1H, H(9), J = 9.9); 4.15 (d, 1H, H(4), J = 12.2); 4.64 (dd, 1H, H(6a), J = 3.0, J = 7.5); 6.58 (d, 2H, H(3′, 5′), J = 9.0); 6.90 (d, 2H, H(2′, 6′), J = 9.0). $C_{20}H_{25}N_7O_6$. Found, %: C, 52.32; H, 5.51; N, 21.31. Calculated, %: C, 52.28; H, 5.48; N, 21.34.

***Corresponding author:** Prazdnova EV, Southern Federal University, 194/1, Stachki Avenue, Rostov-on-Don, Russian Federation

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Synthesis of compound (**14**)—***N,N*-dimethyl-4-(7-methyl-5-nitro-7,8-dihydro-6H-[1,2,5]oxadiazolo[3,4-e]isoindol-4-yl)aniline**: to the suspension of 134 mg (1.50 μmol) of finely powdered sarcosine and 54 mg (1.80 μmol) of paraformaldehyde in 3 ml of anhydrous acetonitrile, 100 mg (0.30 μmol) of compound **3** were added. The reaction mixture was boiled until complete discoloration for 1 h, and then the solution was cooled and filtered; the filtrate was concentrated and purified using column chromatography, and the second fraction (SiO_2, eluent—ethyl acetate:chloroform, 1:3) was collected. The yield was 35 mg (34%), brown crystals, melting temperature—196-198°C. The ^{1}H NMR spectrum (δ, ppm, *J*/Hz): 2.65 (s, 3H, NCH_3); 3.03 (s, 6H, $N(CH_3)_2$); 4.09 (t, 2H, H(8), J = 3.7); 4.35 (t, 2H, H(6), J = 3.7); 6.76 (d, 2H, H(3′, 5′), J = 9.0); 7.48 (d, 2H, H(2′, 6′), J = 9.0). $C_{17}H_{17}N_5O_3$. Found, %: C, 60.21; H, 5.06; N, 20.69. Calculated, %: C, 60.17; H, 5.05; N, 20.64.

Synthesis of compound **(15)—*N,N*-dimethyl-4-(7-methyl-5-nitro-3-oxido-7,8-dihydro-6*H*-[1,2,5] oxadiazolo[3,4-*e*]isoindol-4-yl)aniline:** to the suspension of 129 mg (1.45 μmol) of finely powdered sarcosine and 52 mg (1.73 μmol) of paraformaldehyde in 3 ml of anhydrous acetonitrile, 100 mg (0.29 μmol) of compound **4** were added. The reaction mixture was boiled until complete discoloration for 1 h, and then the solution was cooled and filtered; the filtrate was concentrated and purified using column chromatography, and the second fraction (SiO_2, eluent—ethyl acetate:chloroform, 3:2) was collected.

Mixture of isomers 15 and 15′. The yield was 20 mg (34%), violet crystals, melting temperature—170-180°C. The ^{1}H NMR spectrum (δ, ppm, *J*/Hz): (**15**:**15′** = 3:2) 2.60 (**15′**) (s, 3H, C(7)NCH_3); 2.65 (**15**) (s, 3H, C(7)NCH_3); 3.02 (**15′**) (s, 6H, $N(CH_3)_2$); 3.03 (**15**) (s, 6H, $N(CH_3)_2$); 3.99 (**15′**) (t, 2H, H(8), J = 3.3); 4.09 (**15**) (t, 2H, H(8), J = 3.7); 4,28 (**15′**) (t, 2H, H(6), J = 3.5); 4.35 (**15**) (t, 2H, H(6), J = 3.7); 6.70-6.80 (**15′**, **15**) (m, each 2H, H(3′, 5′)); 7.47 (**15**) (d, 2H, H(2′, 6′), J = 2.1); 7.50 (**15′**) (d, 2H, H(2′, 6′), J = 2.1). $C_{17}H_{17}N_5O_4$. Found, %: C, 57.42; H, 4.85; N, 19.69. Calculated, %: C, 57.46; H, 4.82; N, 19.71.

Synthesis of compound (**20**)—**4-(5,9a-dinitro-3-oxido-5a,6,9,9a-tetrahydro-6,9-methanonaphtho[1,2-*c*][1,2,5]oxadiazol-4-yl)-*N,N*-dimethylaniline**: To the solution of 200 mg (0.59 μmol) of compound **4** in 7 ml of ethanol, 116 mg (1.76 μmol) of freshly distilled cyclopentadiene were added. The reaction mixture was boiled for 24 h, and the precipitate was filtered.

Mixture of isomers 20 and 20′. The yield was 70 mg (30%), brown crystals, melting temperature—168-172°C. The ^{1}H NMR spectrum (δ, ppm, *J*/Hz): (**20**: **20′** = 5:1) 1.53 (**20**) (d, 1H, H(10), J = 10.4); 1.82 (**20**) (d, 1H, H(10), J = 10.4); 1.96 (**20′**) (d, 1H, H(10), J = 10.4); 2.21 (**20′**) (d, 1H, H-10, J = 10.4); 2.99 (**20′**) (s, 6H, NMe_2); 3.00 (**20**) (s, 6H, NMe_2); 3.30-3.40 (**20**) (m, 1H, H(6)); 3.42-3.51 (**20′**) (m, 1H, H(6)); 3.87 (**20**) (d, 1H, H(5a), J = 2.1); 4.14-4.23 (**20**) (m, 1H, H(9)); 4.22-4.30 (**20′**) (m, 2H, H(9), H(5a)); 6.00-6.08 (**20′**) (m, 1H, H(8)); 6.28 (**20**) (dd, 1H, H(8), J = 5.5, J = 2.8); 6.46 (**20′**) (dd, 1H, H(7), J = 5.6, J = 2.8); 6.58 (**20**) (dd, 1H, H(7), J = 5.5, J = 2.8); 6.65-6.73 (**20′**) (m, 2H, H(3′, 5′)); 6.70 (**20**) (d, 2H, H(3′, 5′), J = 8.8); 7.24 (**20′**) (d, 2H, H(2′, 6′), J = 8.7); 7.30 (**20**) (d, 2H, H(2′, 6′), J = 8.8). $C_{19}H_{17}N_5O_6$. Found, %: C, 55.51; H, 4.14; N, 17.04. Calculated, %: C, 55.48; H, 4.17; N, 17.02.

Biosensing

The test objects of this study were the following bacterial strains: *Escherichia coli* MG 1655 (pSoxS-lux), *E. coli* MG 1655 (pXen7-lux), *E. coli* MG 1655 (pRecA-lux), kindly provided by I.V. Manukov (GosNIIgenetika, Moscow). Cell cultures of *E. coli* were grown on rich Luria-Bertani (LB) medium [18]. The bacteria in the liquid medium were cultivated with constant aeration in an orbital shaker at 30°C. For cultivation on solid medium, LB-agar was used (LB with an addition of 20 g/liter of microbiological agar). Antibiotic ampicillin was added to both liquid and solid media (100 mg/l). The bacteria were cultivated in liquid medium at 37°C until early or mid-logarithmic phase. The night culture was diluted with a fresh medium to the density of 0.01, 0.1, and 1 Mac-Farland units (density: 3·108-3·106 cells/ml) for the strain *E. coli* MG 1655 (pSoxS-lux).

Measurements were taken with DEN-1B densitometer (Biosan, Lithuania). After that, the suspension was left to grow for 2 h until early logarithmic phase. Aliquots of the culture (90 μl) were transferred into the wells of a sterile 96-well plate for the luminometer, and were added with 10 μl of the tested compound solution (when measuring protective activity, 10 μl of the inductor and 10 μl of the protector were added). Further, 10 μl of deionized water were added to the reference wells.

Measurement of Luminescence

After treatment, the plate with samples was placed into the luminometer and incubated at 30°C. Intensity of bioluminescence was measured every 15 min. Microplate luminometer LM-01T (Immunotech, Czech Republic) was used for measuring luminescence. The factor of bioluminescent response induction (I) was calculated using the following formula:

$$I = L_e/L_k - 1,$$

where L_k is the luminescence intensity of the reference sample (in relative light units); L_e is the intensity of luminescence of the test sample (in relative light units).

A statistically significant ($p < 0.05$) excess of L_e over L_k, as measured by the t-criterion, was considered as the attribute of reliability of the SOX-induction effect. The RMS deviation of the induction factor was calculated using the following formula:

$$S_I = \bar{I} \cdot ((S_{L_e}/\bar{L}_e)^2 + (S_{L_k}/\bar{L}_k)^2)^{1/2},$$

where S_{L_e} is the standard deviation of the intensity of luminescence of the test sample; S_{L_k} is the RMS deviation of reference sample's luminescence intensity; $\bar{L}_k$ is the mean value of the reference sample's luminescence intensity; $\bar{L}_e$ is the mean value of the test sample's luminescence intensity; and $\bar{I}$ is the mean value of the induction factor.

The confidence intervals of the induction factor for $p < 0.05$ were calculated according to the standard formula as the product of multiplier 1.96, S_I, and the square root of a value inverse to the size of the sample.

The factor of protective activity (%) was calculated using the following formula:

$$A = (1 - I_a/I_p) \cdot 100\%,$$

where I_a is the factor of SOS response induction under the influence of certain exposure under study in the presence of a protector and I_p is the factor of SOS response induction under the unprotected exposure. The confidence interval

$$\bar{A} - t_{kp} \cdot S_A/n^{1/2}; \bar{A} + t_{kp} \cdot S_A/n^{1/2},$$

was calculated for the value of protective activity, where t_{kp} was taken from the Student's *t*-distribution table; $\bar{A}$ is the mean value of protective activity; and S_A is the standard deviation of the protective activity factor.

All measurements were done in triplicate. The protocol for determining toxicity is described in detail in Ref. [19] and the protocol for determining protective activity—in Ref. [20].

Results and Discussion

The synthesis and structure of the objects of study

The interest to the reactivity of benzoxadiazoles is caused by their low aromaticity and significant electron deficiency, which make them capable of engaging in reactions of nucleophilic substitution and cycloaddition even with weak, neutral C-nucleophiles.

We have shown earlier that dinitrobenzofurazan **1** and dinitrobenzofuroxan **2** when interacting with π-excessive heterocycles (indoles [21], pyrroles [16], indolizines [22]) result in products of aromatic substitution with the formation of new carbon-to-carbon bonds. Such compounds are also capable of engaging in cycloaddition reactions, resulting in the formation of new cycles, annulated to nitrobenzoxadiazole fragment of diaryl [17].

In this article we inform about the synthesis, structure, and biological properties of nitrobenzoxadiazole derivatives, which are potential exogenous sources of nitric oxide (II). It should be noted that we synthesized the five compounds studied in this article using the previously described methods [16,17]. The remaining seven compounds were synthesized for the first time.

The selected compounds for research and synthesized derivatives of nitrobenzoxazole **1** and **2** have a number of structural features that may facilitate the growth of their biological activity:

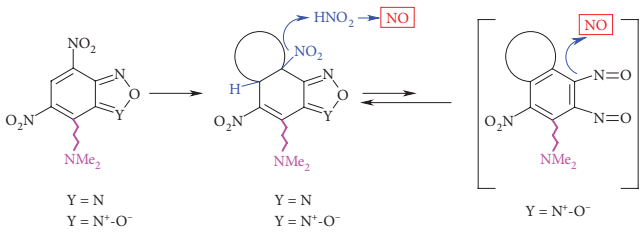
- The tertiary amino group may be protonated, and it may increase solubility in water.
- The presence of the H-C-C-NO_2 fragment that is able to eliminate HNO_2 spontaneously or under the action of alkali is an additional source of NO.
- Introduction of volume substituent at position 7 of the dinitrobenzofuroxan cycle initiates 1,3-N-oxidic rearrangement, the intermediate state of which also has the potential to eliminate NO.

These structural features are graphically summarized in Scheme 1.

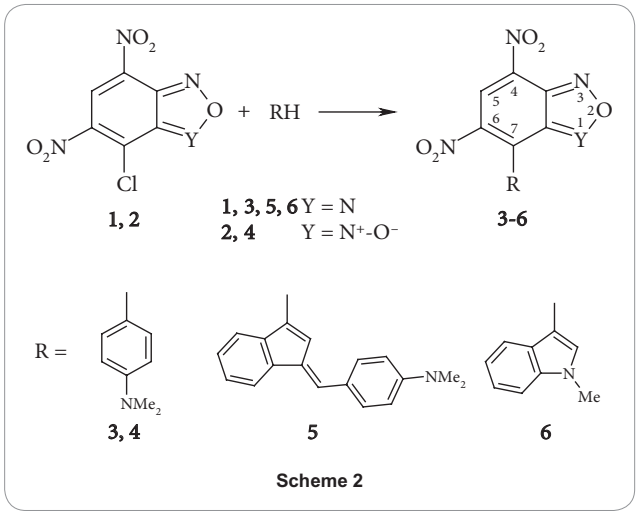
In course of the S_NAr-S_EAr reaction, the intermediate of which is a bipolar σ-complex of the Meisenheimer-Wheland type [16], compounds **3**-**6** (Scheme 2) were synthesized.

4,6-Dinitro-7-ethoxybenzofuroxan (**7**) was synthesized by alcoholysis of dinitrochlorobenzofuroxan **2**.

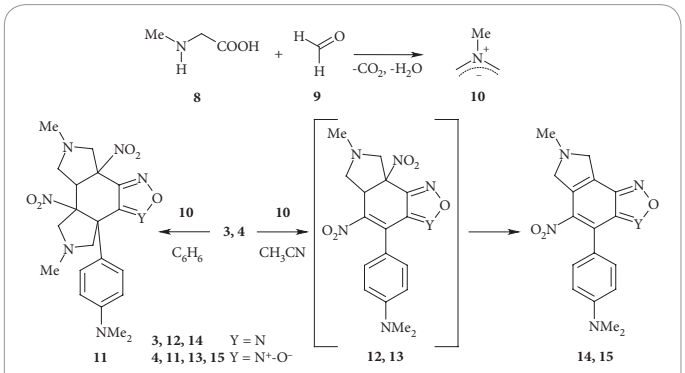
We introduced diaryls **3** and **4** in the interaction with the simplest azomethine ylide **10**, which was produced *in situ* from sarcosine (**8**) and formaldehyde (**9**) (Scheme 3).



Scheme 1



Scheme 2



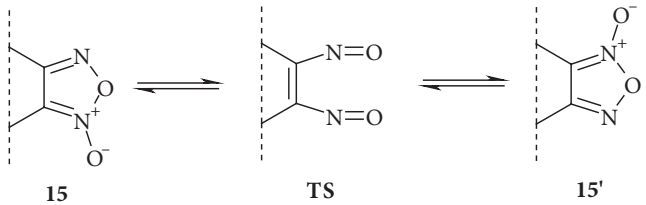
Scheme 3

In the reaction between furazan **3** and azomethine ylide **10**, we managed to extract only compound **14**, which probably formed from the product of cycloaddition **12** as a result of eliminating HNO_2.

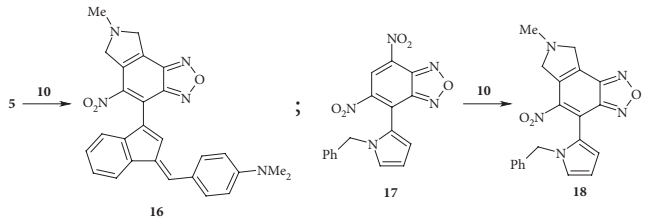
When heated in anhydrous acetonitrile of diaryl **4** with a fivefold excess of sarcosine (**8**) and a sixfold excess of paraformaldehyde (**9**), one product of addition (**15**) was also formed. In the process of cycloaddition, cycloadduct **13** is probably formed first; however, we failed to extract it preparatively. In that, in the ^{1}H NMR spectrum of compound **15**, two groups of signals of isomeric structures are observed in the ratio of 2:3. To us it seems most probable to assign these signals to 1,3-N-oxidic tautomers **15** and **15′** (Scheme 4).

Formation of such tautomeric forms that transform into each other via intermediate dinitroso form TS for benzofuroxans was proved in our previous work [23]. This process may also contribute to NO elimination from the studied compounds.

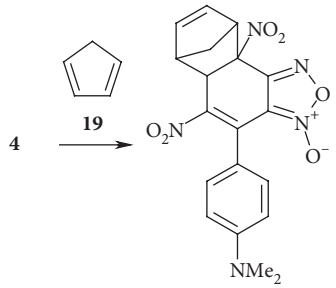
In course of the same reaction in benzene with twofold excess of sarcosine (**8**) and paraformaldehyde (**9**) in case of diaryl **4**, we managed to extract the product of double cycloaddition **11** as well. It is important to note that this compound is able to spontaneously eliminate an HNO_2 molecule, forming a structure similar to **15**. Nitrous acid is unstable and decomposes with the formation of a molecule of nitric oxide (II). Therefore, we managed to extract in a stable form the compound that has an additional NO-donor fragment in its structure.



Scheme 4



Scheme 5



Scheme 6

In course of azomethine ylide **10** reaction with 7-substituted furazans **5** and **17** in both anhydrous acetonitrile and benzene, products **16** and **18** were extracted, respectively (Scheme 5).

As a result of the interaction between cyclopentadiene (**19**) with **4** in the reaction of [4+2] cycloaddition, product **20** was formed (Scheme 6). The ^{1}H NMR spectrum confirms the existence of this compound in the form of two 1,3-N-oxidic tautomers.

The so synthesized derivatives of nitrobenzoxadiazole containing π-excessive heterocycles were studied as potential exogenous sources of nitric oxide (II) with the use of genetically engineered Lux-biosensors based on *E. coli*.

Biological activity

For detecting the ability of substances to induce the expression of SOX-operon, we used biosensor *E. coli* MG 1655 (pSoxS-lux) that was created by introducing a plasmid with operon luxCDABE of the *Photorhabdus luminescens* photobacteria placed under the control of the pSoxS promoter. This genetic structure increases bioluminescence in response to the presence of compounds that generate nitric oxide (II) or the superoxide anion radical within the cell. If the studied

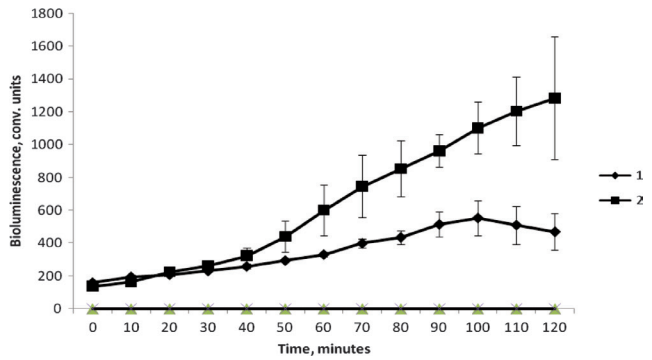


Figure 1: Luminescence of biosensor *E. coli* MG 1655 (pSoxS-lux) in the presence of nitroglycerin. 1—reference, 2—100 μg/ml of nitroglycerin

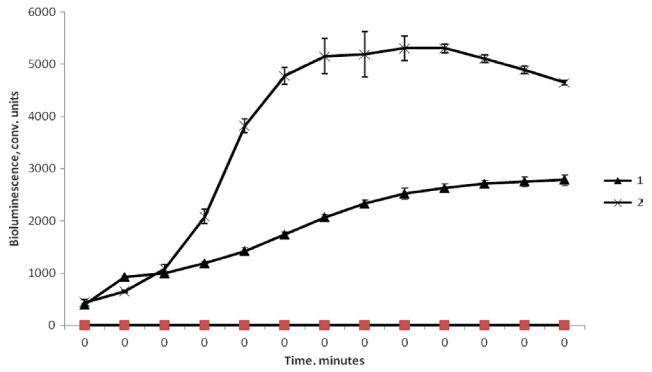


Figure 2: Luminescence of biosensor *E. coli* MG 1655 (pSoxS-lux) in the presence of NOC-5. 1—reference, 2—100 μg/ml of NOC-5

Substance	The concentration with the maximum value of the induction factor, μg/ml	The maximum value of the induction factor	The minimum effective concentration, μg/ml	The value of the induction factor with the minimum effective concentration
Nitroglycerine	100	1.900 ± 1.154*	1	0.048 ± 0.00857
NOC-5	100	1.75 ± 0.184	0.01	0.977 ± 0.029
3	0.01	0.221 ± 0.041	0.01	0.033 ± 0.030
4	0.1	0.507 ± 0.114	0.1	0.507 ± 0.114
5	0.01	0.459 ± 0.034	0.01	0.459 ± 0.034
6	0.01	1.040 ± 0.066	0.01	1.040 ± 0.066
7	0.1	0.303 ± 0.057	0.01	0.282 ± 0.037
11	1	0.601 ± 0.121	0.01	0.584 ± 0.093
14	0.1	2.356 ± 2.147	0.01	0.515 ± 0.092
15	0.01	0.573 ± 0.115	0.01	0.573 ± 0.115
16	1	0.759 ± 0.104	0.01	0.681 ± 0.114
18	0.1	1.008 ± 0.235	0.01	0.901 ± 0.257
20	0.001	0.530 ± 0.071	0.001	0.530 ± 0.071

*confidence interval.

Table 1: The maximum value of the induction factor and the lowest effective concentrations of the synthesized substances

compound undergoes transformations inside the cell that result in the formation of NO, it will stimulate the luminescence, intensity of which may be quantitatively compared with the luminescence caused by reference preparations—known donors of NO, so conclusions about the promising nature of the tested substance could be taken.

Figures 1 and 2 show data about the stimulation of luminescence of the used biosensor *E. coli* MG 1655 (pSoxS-lux) with the most popular nitric oxide donors known in pharmacology: nitroglycerin [24] and NOC-5 (3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazin) [25]. As can be seen in the graphs, both preparations cause statistically significant induction of SOX-operon. The maximum value of the induction factor for nitroglycerin is 1.90 and for NOC-5—1.75.

Table 1 shows the maximum value of the induction factor and the lowest effective concentrations for synthesized compounds, as well as for the two reference preparations.

As can be seen from Table 1, the maximum induction values for the majority of substances are low; they are much lower than those of nitroglycerin and NOC-5. For substance **20**, effective concentration is 0.001 μg/ml, which is an order less than the minimum valid concentration of NOC-5. Compound **14** is superior in its ability to induce SOX to the comparison preparations.

To study toxicity of the substances, their ability to inhibit luminescence of biosensor *E. coli* MG 1655 (pXen7-lux) that contains the luxCDABE operon placed under the control of a constitutive lac promoter was determined. In this case, toxicity was assessed on the basis of the inhibiting activity of luciferase [26]. No statistically significant effects of inhibiting the luminescence of the strain with a constitutive promoter were detected for any of the studied substances, which may be interpreted as absence of nonspecific toxicity.

Thus, substances **14** and **20** are of some interest for deeper studying of their mechanisms of action and for searching for pharmacological activity.

We studied genotoxicity of the leading compound **14** with the use of *E. coli* strain MG 1655 (pRecA-lux) that contains the luxCDABE operon, which is controlled by promoter recA. In the studied range of concentrations, substance **14** does not exhibit any genotoxic effects.

It is known that superoxide anion radical is one of the most active promoters of the oxidative stress. The substances that generate it feature high toxicity and genotoxicity, including those in bacteria [27]. Such generators of superoxide include dioxidine that has the ability to damage DNA.

We found that in case of joint action of dioxydine and sample **14** on bacterial cells, a statistically significant DNA-protective effect was registered in the concentration of 1,000 μg/ml (A = 41.905 + 11.8). The mechanism of protective effect may be associated with the antioxidant activity of nitric oxide [28]. Therefore we can assume that **14** is the generator of nitric oxide, rather than the superoxide anion radical. Otherwise, in case of joint action of dioxydine and sample **14**, the DNA-damaging effects of dioxydine would remain constant or increase.

Summarizing all the above, it should be noted that the use of genetically engineered Lux-biosensors based on *E. coli* is an effective *in vivo* screening tool for arrays of potential NO donors and a method for determining their toxicity.

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