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INVESTIGATION OF THE SOLUTION PHASE CHEMI-LUMINESCENCE OF 1,2,4-TRIAZOLE DERIVATIVES

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We discovered and studied the chemiluminescence occurring during the oxidation of 3-bromo-5-hydrazino-1-(thietan-3-yl)-1H-1,2,4-triazole with superoxide anion. The chemiluminescence wavelength interval was 400-590 nm with the maxima (at 436 and 540 nm) matching the peak fluorescence wavelengths of the reaction products. The possible reaction products were determined by NMR spectroscopy, mass spectrometry, IR and UV spectroscopy. The quantum yield of chemiluminescence was 10^{-9} E/mol.

Keywords: superoxide, 1,2,4-triazole derivatives, mass spectrometry, fluorescence, chemiluminescence.

N-Substituted 1,2,4-triazoles are characterized by a broad spectrum of pharmacological effects, such as antifungal, antibacterial, hypotensive, and other activity. Some derivatives of 1,2,4-triazole are currently used as drugs, for example, ribavirin, fluconazole, trazodone [1-3]. The presence of various substituents in the 1,2,4-triazole ring allow to diversify both the biological mechanisms of action, as well as the spectral and luminescent properties of these compounds. We have synthesized a new series of 1,2,4-triazole derivatives with thietane ring as substituent, because thietane-containing benzimidazoles and xanthines are known to possess antiviral, antimicrobial, and immunotropic properties [4]. Besides that, triazoles are perspective compounds for optoelectronics [5, 6]. 1,2,4-Triazole is used also as peroxalate chemiluminescence catalyst [7]. We have previously reported the study results about the luminescent properties of thietane-containing 1,2,4-triazoles: 3,5-dibromo-1-(thietan-3-yl)-1*H*-1,2,4-triazole, 3-bromo-5-hydrazino-1-(thietan-3-yl)-1*H*-1,2,4-triazole (**1**), 3-bromo-5-{2-[1-(4-nitrophenyl)ethylidene]hydrazino}-1-(thietan-3-yl)-1*H*-1,2,4-triazole, 3-bromo-5-{2-[1-(4-nitrophenyl)ethylidene]hydrazino}-1-(1-oxidothietan-3-yl)-1*H*-1,2,4-triazole, and 3-bromo-1-(1,1-dioxidothietan-3-yl)-5-{2-[1-(4-nitrophenyl)ethylidene]hydrazino}-1*H*-1,2,4-triazole [8]. The observed luminescent properties motivated our further study of triazole excitation during redox reactions. In the current work, we studied the oxidation of compound **1** (Fig. 1) with superoxide ion ($O_2^{-\cdot}$), an important intermediate in many biological processes.

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Superoxide ion is synthesized in the human body by phagocytes (granulocytes and monocytes in the blood), as well as macrophages in the tissues [9]. Reactive oxygen species, including superoxide, can have both positive effects (the maintenance of redox homeostasis, antibacterial activity), as well as destructive influence on the cells (cellular aging and induction of apoptosis) [10].

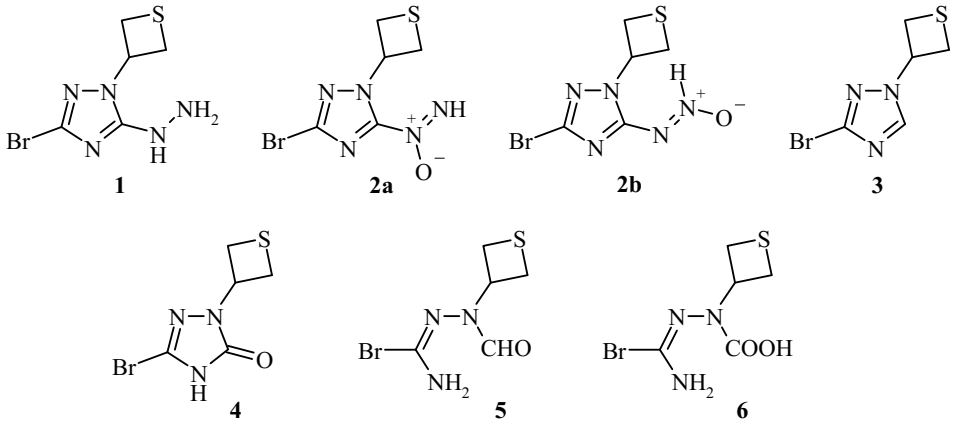


Fig. 1. The structure of the studied compound **1** and the possible oxidation products **2a**,**b**, **3**-**6**.

The study of chemiluminescence (CL), fluorescence (FL), FL excitation, and absorption spectra. CL was observed upon mixing the compound **1** solution in DMSO (c 10^{-2} M) with aerated aqueous alkaline DMSO solution, where the concentration of superoxide ion was $3 \cdot 10^{-3}$ M. The kinetics of CL were complex: a sharp increase of CL was observed at the moment of mixing the solutions, followed by exponential decay, then a new increase was observed after 2 min induction period, reaching the peak intensity after 40 s (Fig. 2).

The flash observed at the moment of mixing the solutions (about 5 s) could have a physical rather than chemical cause. Analogous flashes have been described before [11, 12] and explained by specific physical interactions of droplets with the surface layer of reaction solution at the moment of mixing.

The CL spectrum of the reaction, presented in Fig. 3 (curve *3*), was in the region of 400-590 nm with two maxima at about 436 and 540 nm (identified by using optical interference filters). This spectrum mostly corresponded to the second peak of CL intensity (150 s from the start of the reaction) because the emission intensity at that time was many times greater than initially.

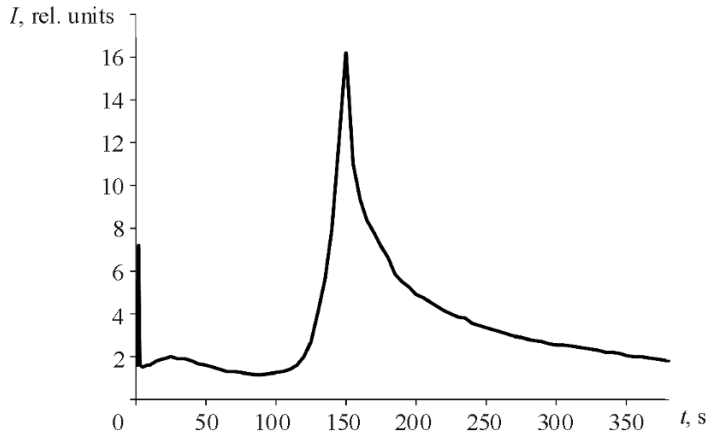


Fig. 2. The changes in CL intensity depending on time during the oxidation reaction of compound **1** (c 10^{-2} M) with superoxide ion $O_2^{-\cdot}$ (c $3 \cdot 10^{-4}$ M), solvent DMSO, 20°C.

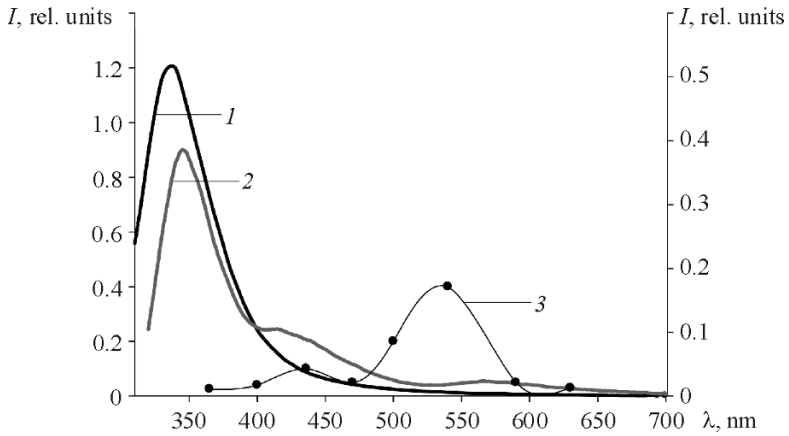


Fig. 3. FL spectra: *1* – compound **1** (c 10^{-2} M), *2* – oxidation products after excitation at 270 nm wavelength (slit width 10/10 nm), *3* – CL spectrum of the oxidation reaction of compound **1** (c 10^{-2} M) with superoxide ion $O_2^{-\cdot}$ (c $3 \cdot 10^{-4}$ M), solvent DMSO, 20°C. Curves *1*, *3* correspond to vertical axis on the left side, curve *2* – to vertical axis on the right side.

In order to establish the emitting species, the FL spectra were obtained for compound **1** in DMSO (Fig. 3, curve *1*) and the final reaction products (the reaction mixture 4 h after the mixing of compound **1** with superoxide ion, Fig. 3, curve *2*).

The Fig. 3 shows that the spectrum *2*, unlike the spectrum *1*, has new emission bands at 435 and 562 nm, in the same region as the CL spectrum. These data allow us to assume that the CL emitters are not the intermediates, but rather the final reaction products. The FL maximum at 338 nm wavelength, characteristic of the triazole fragment, shifted to the longer wavelengths after oxidation and the FL intensity decreased threefold, which could be explained by the consumption of compound **1** during the reaction and/or transformation of the triazole ring.

The changes in CL intensity were accompanied by changes in the absorption spectrum of oxidation products during the reaction. For this reason, we obtained the absorption spectra of the oxidation products formed during the reaction and the FL excitation spectra of the reaction mixture 4 h after the start of reaction (Fig. 4)

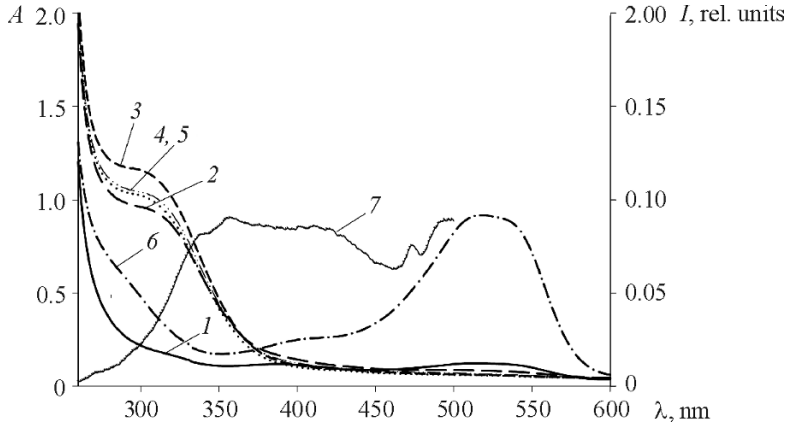


Fig. 4. The absorption spectra of oxidation products: *1* – at the moment of mixing, *2* – after 2 min, *3* – after 5 min, *4* – after 30 min, *5* – after 1 h, *6* – after 20 h, *7* – the FL excitation spectrum of oxidation products, after excitation at 550 nm (slit width 10/10 nm), solvent – DMSO, 20°C. Curves *1-6* correspond to the left side axis *A*, curve *7* – to the right side axis *I*.

A new band appeared at 300 nm in the absorption spectra obtained during the oxidation. The intensity of this band increased fivefold during the first 5 min, then slowly faded and practically disappeared after several hours. This absorption band and the FL maxima at 435 nm, as well as CL maximum at 436 nm were likely due to an intermediate carbonyl compound [13].

We performed the synthesis of 5-bromo-2-(thietan-3-yl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**4**) as a possible reaction product [14]. The absorption spectrum of compound **4** in DMSO had a maximum at 300 nm. In order to compare the spectra of reaction products and the model compound **4**, we performed a mass spectral investigation. It was found that the retention time of compound **4** in HPLC-MS experiment was 3.55 min, and matched the retention time of $[M-H]^-$ ions with *m*/*z* (I_{rel}, %) 234 (26) and 236 (20) in the sample of oxidation products. Thus, the $[M-H]^-$ ions with *m*/*z* (I_{rel}, %) 234 (26) and 236 (20) did indeed correspond to the structure of compound **4**, both by mass, as well as by retention time.

However, we can not identify this compound as the CL emitter in oxidation reaction, because the absorption spectra and FL excitation spectra of compound **4** overlap at λ_{max} 300 nm, but do not match the FL excitation spectrum of the reaction mixture (Fig. 4, curve *7*), which has its maximum at 360 nm. Besides that, the FL spectrum of compound **4** (λ_{exc} 270 nm) has a maximum at 335 nm, but lacks maxima in the region of 400-600 nm (emission in this region is characteristic to the oxidation products of compound **1**). At the same time, the oxidation of compound **4** under the same conditions leads to the appearance of new bands in the FL spectrum of products at 435 and 562 nm. For this reason, the band with the maximum at 360 nm in the FL excitation spectrum, the maxima of FL at 435 nm, and CL at 436 nm we assigned to another product, compound **5**.

Besides that, a new band appeared in the the absorption spectra of reaction products, with maximum at 520 nm, which was observed also in the FL excitation spectra of the reaction mixture. This band corresponded both to the product of hydrazine group oxidation – compound **2a** and/or **2b**, which gave the solution pink color, but did not cause FL in the long wavelength region, as well as to compound **6** – a photon emitter responsible for FL at 562 nm.

Changes in the IR spectra, ^{13}C and ^{1}H NMR spectra, and mass spectra. In order to identify stable products, the reaction mixture composition was analyzed by IR and NMR spectroscopy, as well as by mass spectrometry.

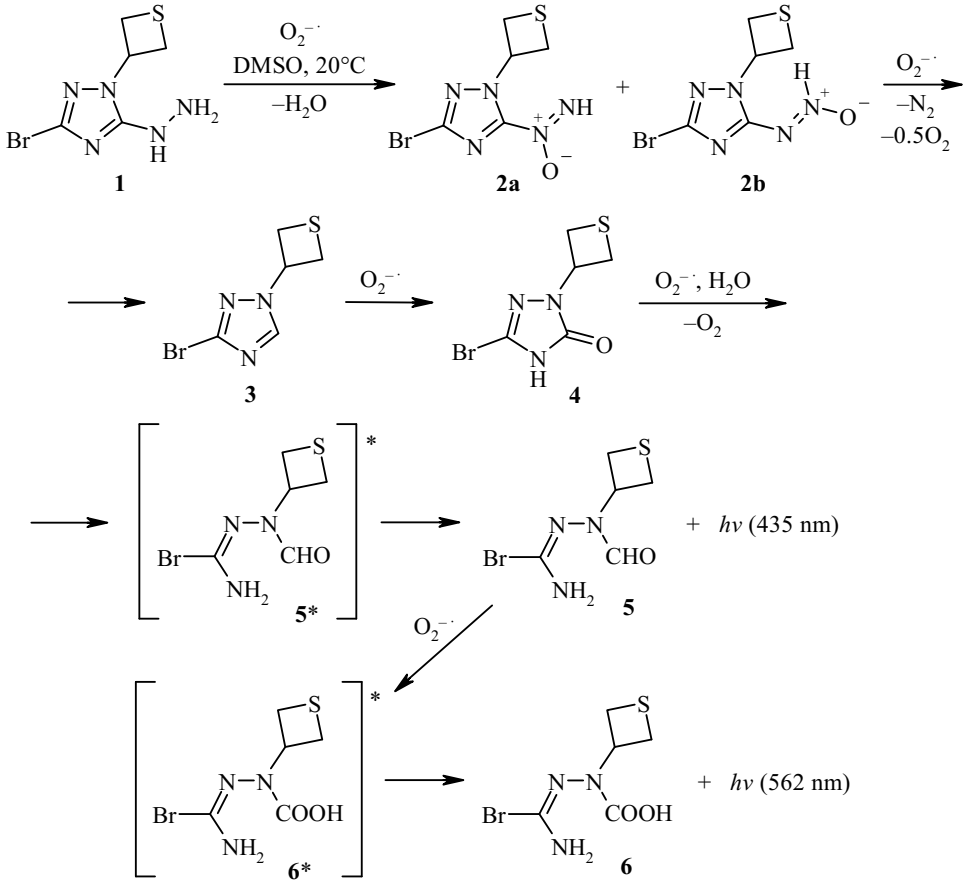
Prior to the analysis of oxidation products by NMR spectroscopy, the solvent was removed from reaction mixture by distillation. In contrast to the ^{13}C and ^{1}H NMR spectra of compound **1** in DMSO-d_6, the ^{13}C NMR spectrum of reaction products contained new signals at 145.3 and 157.6 ppm, while the ^{1}H NMR spectrum contained a singlet at 6.49 ppm and broadened downfield singlets at 7.82, 8.41, 9.50, and 10.04, apparently corresponding to unstable intermediates of hydrazine group degradation. These data were in agreement with the IR spectra, in which the characteristic –NH–NH_2 band (ν 1581 cm^{-1}) disappeared during oxidation [15]. Thus, the reaction involves an elimination of hydrazine group, and the formation of a compound with unsubstituted double bond, 3-bromo-1-(thietan-3-yl)-1,2,4-triazole (**3**) (Fig. 1). Besides that, the ^{13}C NMR spectrum of the oxidation products contains a carbonyl group signal at 198.6 ppm and a carboxy group signal at 168.7 ppm, indicating the oxidation of triazole ring C-5 atom. The chemical shifts of thietane ring protons and carbon atoms changed insignificantly, thus the thietane ring apparently did not participate in the oxidation reaction.

The formation of $O_2^{-\cdot}$ ion, according to the literature [16], occurs during DMSO reaction with KOH, when the loss of one proton is followed by electron transfer to molecular oxygen, giving $O_2^{-\cdot}$ ion and the methylsulfonyl carbanion of DMSO with activated CH_3 group. This assumption was supported by the appearance of new signals in the ^{13}C NMR spectrum of DMSO, corresponding to partial deuterium exchange. The type of new signals indicated that 1-3 protons were exchanged in the methyl groups of DMSO.

The mass spectrum of products from oxidation of compound **1** had $[M-H]^-$ ion peaks with *m*/*z* (I_{rel}, %) 262 (74) and 264 (84), 234 (26) and 236 (20), 252 (16) and 254 (6), apparently corresponding to the structures of compounds **2a**,**b**, **4**, **6**, presented in Fig. 1.

In order to identify the possible photon emitter, the reaction mixture was separated by HPLC, using hexane–2-PrOH and H_2O–MeCN as mobile phase. The FL spectra indicated that the luminescent component emitting in the region of 420-600 nm with emission peak at 565 nm was present in the fraction eluted with hexane–2-PrOH. The mass spectrum of this fraction contained $[M-H]^-$ ion peaks with *m*/*z* (I_{rel}, %) 234 (100) and 236 (90), 250 (88) and 252 (100), corresponding to compounds **4** and **6** (Fig. 1).

Based on the obtained data, we can propose the following reaction scheme for the oxidation of compound **1** with superoxide ion.



The CL at the initial reaction stage (5-50 s) can be interpreted as due to the hydrazine group oxidation involving compounds **2a**,**b** as intermediates. Further, 50-120 s after the start of the reaction, hydrazine group is eliminated from the triazole ring with the formation of compound **3** and the subsequent addition of oxygen atom to the C-5 atom, forming compound **4**. The most intense emission from the reaction mixture 2 min after mixing apparently corresponds to the stage of triazole ring opening, with the formation of excited intermediates **5***, **6***, the spontaneous transition of which to the ground state is accompanied by CL.

The quantum yield of this reaction was calculated as equal to 10^{-9} E/mol. When accounting for the emission quantum yield previously determined for compound **1** (10^{-2} E/mol [8]), the quantum yield of excitation (the ratio of excited product molecules to the number of chemical interaction events) was 10^{-7}. The low quantum yield of excitation may be a consequence of side reactions, leading to the final products by non-radiative mechanism. For example, a condensation is possible with ring closure through the interaction of carbonyl and amine groups or the formation of internal salt by the reaction of carboxy and amine groups.

Thus, the source of electronically excited species in the studied chemiluminescent reaction of 1,2,4-triazole derivative oxidation with superoxide ion was generated in a number of steps, involving triazole ring opening.

EXPERIMENTAL

IR spectra were recorded on a Bruker Tensor 27 FTIR spectrometer in DMSO. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker AM-300 instrument (300 and 75 MHz, respectively) in DMSO-d_6, internal standard TMS. Mass spectra with electrospray ionization for compound **1** samples in DMSO and for the products of oxidation reaction were recorded on a Shimadzu quadrupole LCMS-2010 EV instrument (injection by syringe, sample dissolved in DMSO–H_2O–MeCN with 60 μl/min consumption rate, eluent – 95:5 MeCN–H_2O) in negative ion recording mode, at -3.5 kV capillary voltage. The interface capillary temperature was 250°C, the interface capillary voltage was -25 V. The nebulizer gas (N_2) flow rate was 0.8 l/min. The high-frequency lens (Q-array) voltage was -5 V. The HPLC analysis was performed on a Shimadzu LC-20 chromatograph with diode array detector. Exsil Silica column was used (250×4.6 mm) with Luna C18 stationary phase, 5 μm particle size (Phenomenex, USA). Elution was performed in isocratic mode (eluent 87:13 hexane–2-PrOH, 80:20 H_2O–MeCN) with 1 ml/min flow rate, detection at 254 and 300 nm.

The intensity of CL was determined with an apparatus consisting of dark chamber and a photomultiplier detector FEU-140, with stabilized high voltage power supply based on BNV 3-09 rectifier. The photomultiplier signal was transmitted to high impedance input of K 201 electronic potentiometer. The spectral region of CL was recorded with optical interference filters, positioned between the bottom of glass cuvette and photocathode of the photomultiplier tube. Integrated amounts of light determined with filters were compared to the amount of light obtained without filters. Absorption spectra were recorded on a Specord M-400 spectrometer (Carl Zeiss Jena) in 1-cm cuvette, with pure solvent as reference. Fluorescence and FL excitation spectra were recorded on a SM-2203 spectrofluorimeter (Solar, Belarus).

3-Bromo-5-hydrazino-1-(thietan-3-yl)-1*H*-1,2,4-triazole (1) was synthesized according to a published method [8]. IR spectrum, ν, cm^{-1}: 1581 (NH–NH_2), 1654 (C=N), 3028, 3142, 3208 (N–H). The NMR spectral data matched the literaure. Mass spectrum, *m*/*z* (I_{rel}, %): 248 $[M-H]^-$ (87), 328 $[M+Br]^-$ (17).

5-Bromo-2-(thietan-3-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-one (4) was synthesized according to a published method [14]. The NMR spectral data matched the literature. Mass spectrum, *m*/*z* (I_{rel}, %): 234 $[M-H]^-$ (100).

Superoxide ion ($O_2^{-\cdot}$) was generated by saturating 20 ml of KOH solution in DMSO (*c* $3\cdot10^{-3}$ M) with oxygen for 10 min [16]. The superoxide ion concentration was determined by UV spectroscopy at 256 nm wavelength (ε 780 $M^{-1}\cdot cm^{-1}$) [17].

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REFERENCES

1. M. D. Mashkovskii, *Drugs* [in Russian], Vol. 1, Novaya Volna, Moscow (2005), p. 1200.
2. A. T. Burbello, A. V. Shabrov, and P. P. Denisenko, *Modern Medicinal Preparations* [in Russian], 3rd ed., Neva, St. Petersburg (2006), p. 896.

3. A. D. M. Curtis and N. Jenning, in: A. R. Katritzky, C. A. Ramsden, E. F. V. Scriven, and R. J. K. Taylor (editors), *Comprehensive Heterocyclic Chemistry*, Vol. 5, Elsevier, Oxford (2008), p. 203.
4. V. L. Rusinov, E. N. Ulomskiy, O. N. Chupakhin, and V. N. Charushin, *Izv. Akad. Nauk*, *Ser. Khim.*, 967 (2008). [*Russ. Chem. Bull.*, **57**, 985 (2008).]
5. L.-R. Tsai and Y. Chen, *Macromolecules*, **40**, 2984 (2007).
6. X.-Y. Zhou, Y.-Q. Huang, and W.-Y. Sun, *Inorg. Chim. Acta*, **362**, 1399 (2009).
7. T. Jonsson and K. Irgum, *Anal. Chim. Acta*, **400**, 257 (1999).
8. R. R. Kayumova, S. S. Ostakhov, A. V. Mamykin, R. R. Muslukhov, G. F. Iskhakova, S. P. Ivanov, S. A. Meshcheryakova, E. E. Klen, F. A. Khaliullin, and V. P. Kazakov, *Zh. Obsch. Khim.*, **81**, 1010 (2011). [Russ. *J. Gen. Chem.*, **81**, 1203 (2011).]
9. J. A. Knight, *Ann. Clin. Lab. Sci.*, **30**, 145 (2000).
10. M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, *Int. J. Biochem. Cell Biol.*, **39**, 44 (2007).
11. R. G. Bulgakov, S. P. Kuleshov, G. A. Tolstikov, and V. P. Kazakov, *Izv. Akad. Nauk SSSR*, *Ser. Khim.*, 731 (1986).
12. N. Duran, K. Zinner, R. C. De Baptista, C. C. C. Vidigal, and G. Cilento, *Photochem. Photobiol.*, **24**, 383 (1976).
13. R. F. Vasil'ev, A. V. Trofimov, and Yu. B. Tsaplev, *Usp. Khim.*, **79**, 95 (2010).
14. E. E. Klen, I. L. Nikitina, A. G. Gil'manova, A. F. Miftakhova, O. A. Ivanova, F. A. Khaliullin, and E. K. Alekhin, RU Pat. 2459818; *Byul. Izobret.*, No. 24, 7 (2012).
15. A. J. Gordon and R. A. Ford, *The Chemist's Companion* [Russian translation], Mir, Moscow (1976), p. 233.
16. K. Hyland and C. Auclair, *Biochem. Biophys. Res. Commun.*, **102**, 531 (1981).
17. B. C. Matheson and J. Lee, *Spectrosc. Lett.*, **2**, 117 (1969).