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The electrochemical behavior's character of a potential antiviral drug 3-nitro-4-hydroxy-7-methylthio-4H-[1,2,4]triazolo[5,1-c][1,2,4]triazinide monohydrate

Polina N. Mozharovskaia [a*], Alexandra V. Ivoilova [a], Roman A. Drokin [a], Alla V. Ivanova [a], Alisa N. Kozitsina [a], Vladimir L. Rusinov [ab]

a: Institute of Chemical Engineering, Ural Federal University, Ekaterinburg 620009, Russia
b: Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences, Ekaterinburg 620137, Russia
* Corresponding author: pnmozharovskaia@urfu.ru

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Abstract

The results of this study of the electrochemical transformation of 3-R-4-hydroxy-1,4-dihydro-7-X-1,2,4-triazolo[5,1-c][1,2,4] obtained by voltammetry are presented. It was found that 3-R-4-hydroxy-1,4-dihydro-7-X-1,2,4-triazolo[5,1-c][1,2,4] derivatives are capable of electrochemical reduction in the potential range of –0.28 to –0.33 V (relative to Ag/AgCl) in Britton–Robinson buffer at pH = 2. The electrochemical behavior of the sodium salt of 3-nitro-4-hydroxy-7-methylthio-4H-[1,2,4]triazolo[5,1-c][1,2,4]triazinide monohydrate (**compound 1**), which *in silico* modeling predicted possible biological activity against various tick-borne encephalitis and Coxsackie B3 viruses. At the potentials of the first stage of electroreduction at pH = 2, the main transformation process is the three-electron reduction scheme of the nitro group of **compound 1**. It was established that **compound 1** in an aprotic medium is reduced in ionic form, most likely in the form of an ion pair with the Na^+ cation, and in an aqueous medium in the form of a protonated particle. Based on this, a scheme was proposed for the probable electrochemical transformation of the studied compound.

Keywords

nitroheterocyclic compounds
antiviral activity
cyclic voltammetry
triazolotriazines
electrotransformations

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1. Introduction

Because of the constant variability of viruses and their increasing resistance to existing drugs it becomes relevant to create original antiviral drugs with low toxicity and high biological activity. Medicines whose active ingredients contain a nitro group in their structure are of great interest due to the fact that they exhibit a wide range of biological activity, including against various strains of viruses [1, 2]. Wardman [3] associated the biological activity of such drugs with the formation of radial particles, primarily, of the radical anion $ArNO_2^{\bullet-}$, during the reduction of aromatic nitro compounds *in vivo*. However, the mechanism of action of many pharmaceutical preparations containing nitroheterocyclic compounds is currently not fully understood. Therefore, the development and study of models that can describe the redox transformation of new, original nitro compounds is an urgent task. Its solution will help to advance the understanding of the biological effects of drugs in living organisms.

Currently, there is a rapidly increasing interest in a number of azoloazines, which is primarily due to their biological activity [4, 5]. Due to their structural similarity to nucleic bases, they can be effective antiviral agents [6–9]. On the basis of nitro-containing azoloazinium compounds, employees of Ural Federal University, Institute of Organic Synthesis of the Ural Branch of the Russian Academy of Sciences, and the Research Institute of Influenza of the Ministry of Health of Russia developed a new class of substances – potential drugs with a wide range of antiviral activity [8]. The main representative of this class of compounds is a drug Triazavirin® (Riamilovir), which is registered in the Russian Federation and successfully used in the treatment of influenza, SARS, COVID-19 [10–13]. The sodium salt of 3-nitro-4-hydroxy-7-methylthio-4H-[1,2,4]triazolo[5,1-c][1,2,4]triazinide monohydrate (**compound 1**) is the closest, in

structure, compound to the antiviral drug Triazavirin® and is currently under development as a drug. It is possible, using electrochemical methods, to study the transformation of the drug *in vitro* in conditions as close as possible to those of the processes occurring with the drug *in vivo* [14–17]. Therefore, the obtained data on the study of the electroconversion of **compound 1** can provide very important information on the interpretation of the mechanism of antiviral action.

Previously, we studied the redox transformations of substances of a number of triazolotriazines (Triazavirin® and Triazide) [18, 19]. The studies demonstrated that the electrochemical behavior of a structural analog may differ due to the difference in the structure and requires an individual approach to the investigation of its redox mechanism.

The aim of this work is to study the nature of the electrochemical transformations of the sodium salt of 3-Nitro-4-hydroxy-7-methylthio-4H-[1,2,4]triazolo[5,1-c][1,2,4]triazinide monohydrate by electrochemical methods.

2. Experimental

2.1. Materials

A 3-nitro-4-hydroxy-7-methylthio-4H-[1,2,4]triazolo[5,1-c][1,2,4]triazinide monohydrate sodium salt (**1**); 3-nitro-4-hydroxy-1,4-dihydro-7-ethylthio-[1,2,4]triazolo[5,1-c][1,2,4]triazine (**2**); 3-nitro-4-hydroxy-1,4-dihydro-7-propargylthio-[1,2,4]triazolo[5,1-c][1,2,4]triazine (**3**); 3-ethoxycarbonyl-4-hydroxy-1,4-dihydro-1,2,4-triazolo[5,1-c][1,2,4]triazine (**4**) were synthesized at the Department of Organic and Biomolecular Chemistry, Ural Federal University. Structural formulas of compounds **1–4** are given in Figure 1.

Aqueous buffer solutions of Britton-Robinson (BRB), which were prepared according to the recommendations [20], were used as supporting electrolytes. The choice of this buffer is due to its high buffer capacity in a wide pH range (2–12), which also makes it possible to exclude acidification or alkalization of the medium. To prepare the solutions, deionized water was used, which was obtained on a DVS-M/1NA (18)-N unit from Mediana-Filter, Moscow, Russia.

To carry out the study in aprotic solutions, we used dimethylformamide (DMF) of the extra-pure grade from Sigma-Aldrich (US) with preliminary distillation in the presence of nanoparticles. We used tetrabutylammonium tetrafluoroborate (extra-pure grade) from Sigma-Aldrich (US).

2.2. Electrochemical devices and methods

A μAutolab Type III potentiostat/galvanostat (Metrohm, Switzerland) was used to record cyclic voltammograms (CV curves) and chronoamperograms (CA). The working electrodes were glassy carbon disks (GCE S = 7.065 mm²) with a surface diameter of 3 mm for stationary and 5 mm for rotating (GCE S = 19.625 mm²) (Metrohm, Switzerland). To polish the surface of the glassy carbon electrode, kit 6.2802.010 (Metrohm, Switzerland) was used, which included aluminum oxide with a particle size of 0.3 μm and a fabric substrate. For experiments with a rotating electrode, an Ametek Model 616A (USA) setup was used. A graphite electrode (Metrohm, Switzerland) was used as an auxiliary electrode. A silver chloride electrode Ag/AgCl/KCl_{sat} (Metrohm, Switzerland) used as a reference electrode in aqueous solutions. The potentials of the working electrode in an aprotic DMF solution were measured relative to a silver chloride reference electrode with two Ag/AgCl/KCL_{sat}/DMF membranes (the inner part of the electrode was filled with 0.1 mol·L^{-1} KCl aqueous solution, the outer part was filled with 0.1 mol·L^{-1} Bu_4NBF_4in DMF) Before each measurement for 10 min, the solutions were purged with argon (purity 99.9%).

3. Results and Discussion

Electrochemical reduction (ECR) of 3-R-4-hydroxy-1,4-dihydro-7-X-1,2,4-triazolo[5,1-c][1,2,4]triazines with various substituents was carried out in BRB solution by cyclic voltammetry with linear potential sweep. The presented CV curves of **compounds 1–4**, recorded in an aqueous medium at pH = 2, are shown in Figure 2. It is seen that the reduction of these compounds occurs in one stage. The presented voltammograms show that the peaks of ECR in the range of potential values from –0.28 to –0.33 V belong to compounds 1–3 having a nitro group in the structure, while for compound 4 the reduction peaks in this range of potential values are not visible. Apparently, the elongation of the thiol bond in the –SMe substituent does not noticeably affect the ECR potential of heterocyclic nitro compounds and, probably, the electroconversion mechanism. It is possible that the peak on the CV curves for compounds 1–3 can be attributed to the reduction of the nitro group associated with the heterocyclic system.

The effect of proton donors on the ECR process of compound 1 was considered. Figure 3a show that an increase in the pH of the solution has little effect on the current value.

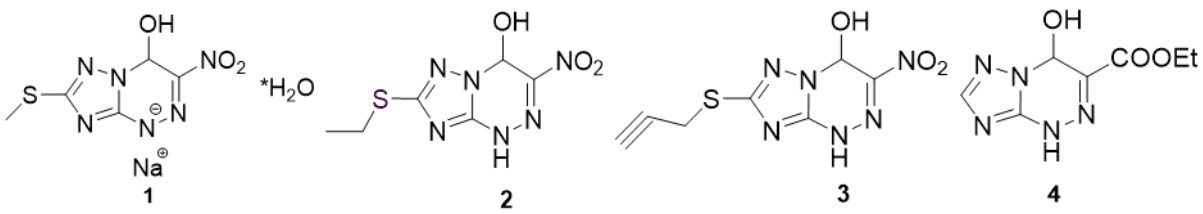

Figure 1 Structural formulas of compounds **1–4**.

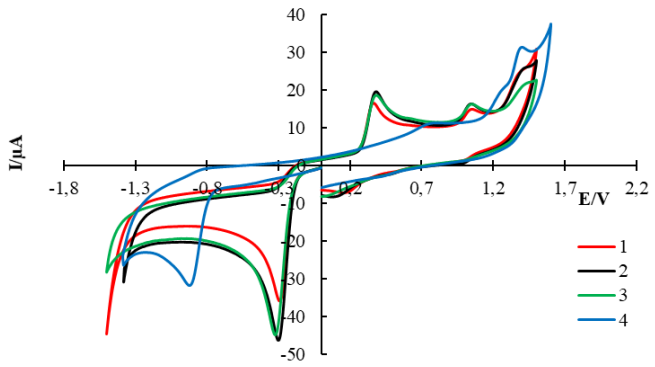

Figure 2 CV curves of 1 mM compounds **1–4**: in BRB pH = 2 using GCE at v = 0.1 V/s. Potentials were measured relative to $Ag/AgCl/KCl_{sat}$.

Moreover, the value of the cathode current imperceptibly differs from the theoretical current, which is calculated according to the Randles-Shevchik equation for irreversible systems. It can also be seen from Figure 3b that a change in pH significantly affects the potential of the EV and shifts it to the cathode region (by 400 mV). Based on the foregoing, it can be assumed that with a decrease in the acidity of the medium, the ECR becomes more difficult due to the lack of protons both for the previous protonization and for the protonation of the intermediate products of the electrochemical reaction [21]. Since the current does not depend on pH and the dependence of *E* on pH is linear, it can be assumed that the electroreduction of compound 1 in this range of pH occurs by a similar mechanism. Therefore, we can calculate thermodynamic characteristics in an acidic medium. Since the electroreduction of compound 1 under these conditions is irreversible, it is not possible to calculate the number of protons involved in the overall process from the plot of the dependence of the potential on pH. However, the dependence of the potential on pH unambiguously indicates the participation of protons in the electrochemical process both before and simultaneously with electron transfer.

It is known that nitroaromatic compounds are characterized by a diffusion process complicated by the preceding chemical reaction of anion protonation. Therefore, the experiments were carried out to study the kinetics of the electrochemical process. For this, CVs were recorded at different scanning rates of the potential and chronoamperograms. As can be seen from Figure 4a the Semerano criterion calculated from the logarithmic dependence of the current magnitude on the rate of potential application (tg = log*I*/log*v*) is 0.51. This fact, as well as the linear dependence of the peak current on the square root of the potential application rate (Figure 4b) indicates the diffusion control of the electrochemical process [22].

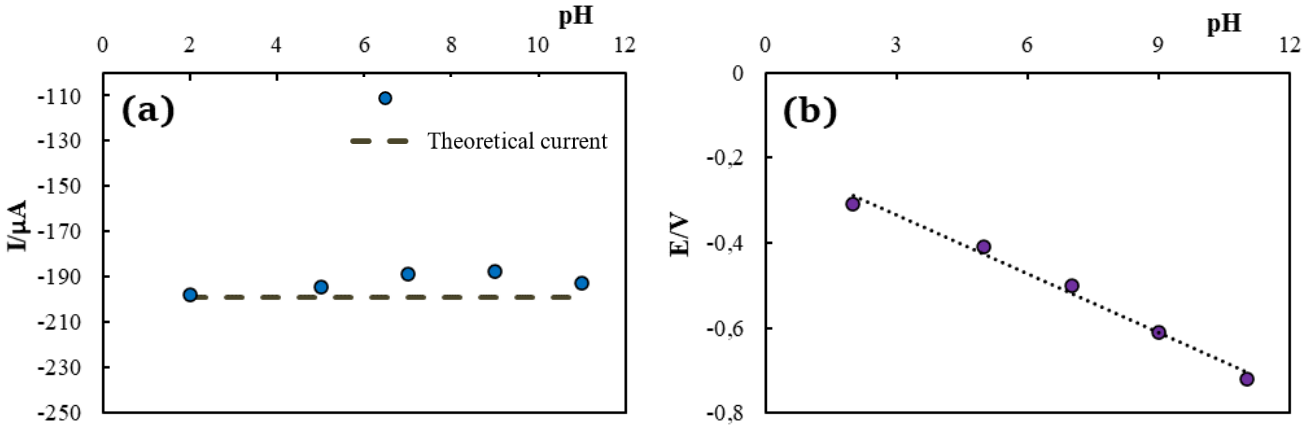

Figure 3 Values of current (A) and potential (B) of the peak of compound **1** (5 mM) with a change in the pH of the buffer solution at v = 100 mV/s.

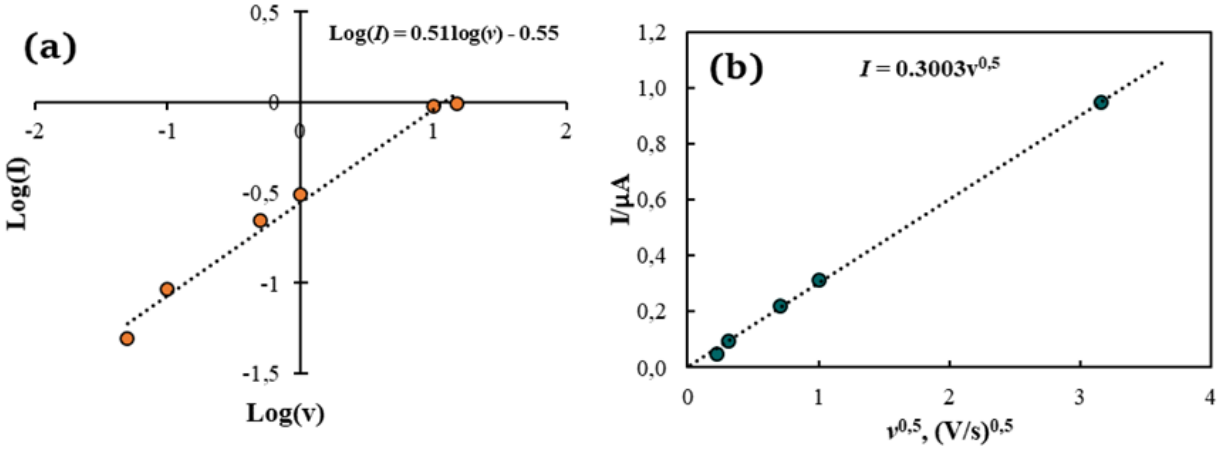

Figure 4 Logarithmic dependence of the peak current on the rate of potential application (a) and dependence of the peak current on the square root of the rate of application of potential compound 1 in the BRB pH = 2 on the GCE (b).

Note that **compound 1** is a salt formed by the Na^+ cation and heterocyclic anions; therefore, its reduction should have proceeded at more negative potential values compared to compounds 2–3. Most likely, the difference in the reduction currents of compound 1 from 2 and 3 is also associated with the influence of Na^+ ions contained in the structure of compound 1. It can be assumed that the similarity of the electrochemical behavior of compounds 1–3 in solutions at pH = 2 indicates the initial protonation of heterocyclic anions of compound 1 and further reduction at the electrode not of the heterocyclic anion, but of the corresponding protonated particle. To confirm the above assumption about the participation of protons in the ECR of compounds 1, a study was carried out in a non-aqueous medium, DMF.

The presented CV curves in Figure 3 were recorded in an aprotic solvent (DMF). The potential of the first ECR peak of compound **1** is 800 mV more negative than that of compound **2**. This can be explained by the negative charge of the reducing particles in the case of compound **1**. Adding an aqueous solution of sodium hydroxide alkali to a solution of compound **2** in DMF medium leads to a significant change in the CV curves: an increase in the values of cathodic and anodic currents is observed, the oxidation/reduction peaks are shifted to the cathode region, but the system remains irreversible (Figure 5).

In order to exclude the effect of water added together with alkali to a solution of compound **2** in DMF medium, the CV curve of compound **1** was recorded with the addition of 0.5 mM H_2O (Figure 5, red dotted line). It can be noted that not only the reduction potentials of compound **1** with the addition of water and those of compound **2** with the addition of aqueous alkali, but also the shapes of their CV curves coincide. The anodic peaks on the reverse part of voltammograms somewhat differ in the potential value, which is probably due to the influence of the additional CH_3-group in the thiol substituent. Therefore, it can be assumed that compound **1** in an aprotic medium is reduced in an ionic form, most likely in the form of an ion pair with the Na^+ cation, and in an aqueous medium in the form of a protonated particle. The participation of proton donors in the process of ECR of the nitro group of **compound 1** can also be indicated by the shift of the reduction potentials to the cathode region relative to the potentials in aqueous media.

The electrochemical behavior of compound **1** is of greatest interest; therefore, it was further studied in acidic aqueous buffer solutions. Due to the mixed acidosis of the cells [23], an acidic environment was chosen. This environment occurs with an excessive concentration of active oxygen metabolites, which, in turn, are produced in the process of viral infection [24].

The electrochemical behavior of compound **1** was studied using cyclic voltammetry, chronoamperometry, and a rotating disk electrode (RDE). The CV curves of this compound with a change in the sweep direction at the value of the potential of the limiting current of the first stage is similar to the peaks on the ECR curve of nitrobenzene in an acidic medium [18].

To approximately determine the effective number of electrons (n_e) involved in the electrochemical reduction of compound **1**, the current of the first stage in the voltammogram was compared with the current of the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ model redox pair under similar conditions. For a more accurate calculation of the number of electrons, the CA and RDE methods were used. Analysis of the chronoamperogram in the time interval from 1 to 2 s at the potential value of the limiting current of the electroreduction of compound **1** (E = –0.35 V) made it possible to calculate the amount of electricity that passed during this time and compare it with the amount of electricity passing under the same conditions at the potential value limiting ECR current (E = 0.2 V) in $K_3Fe(CN)_6$ solution. The results obtained showed that at the value of the potential of the first ECR stage of compound **1** in an acid medium, the main direction of transformations is the three-electron scheme for the reduction of its nitro group.

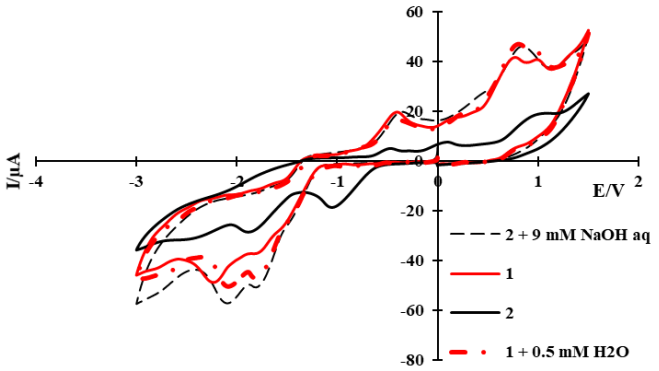

Figure 5 CV curves of 5 mM compounds 1,2 in DMF (0.1 M Bu_4NBF_4), recorded using GCE with v = 0.1 V/s: red – 1, red dotted line – 1 with the addition of 0.5 mM H_2O, black – 2, black dotted line – 2 with the addition of 9 mM aqueous NaOH.

Table 1 The values of the observed number of electrons ne participating in the ECR of compound 1 and $K_3Fe(CN)_6$ (C = 5 mM) in an aqueous buffer solution of BRB at pH = 2.

Compound	n^1_e	n^2_e (CC)	n^3_e	n^4_e (CA)	n^5_e (RDE)
1	2.98	3.10	3.01	3.07	3.23
$K_3Fe(CN)_6$	1	1	1	1.02	0.96

n^1_e – the ratio of current of compound 1 with current of the model redox pair $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ in the same conditions;
n^2_e – the ratio of amount of electricity of compound 1 with amount of electricity of the model redox pair $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ in the same conditions;
n_e^3 – the effective number of electrons which were calculated using Rendls–Shevchik equation for irreversible processes [25];
n^4_e – was calculated from the current value in the chronoamperogram of compound 1 at t = 1 s using the Cottrell equation, taking into account that the value of the diffusion coefficient for nitroaromatic compounds in aqueous media is ~10^{-5} $cm^2 \cdot s^{-1}$[26];
n^5_e – according to Levich's equation [27].

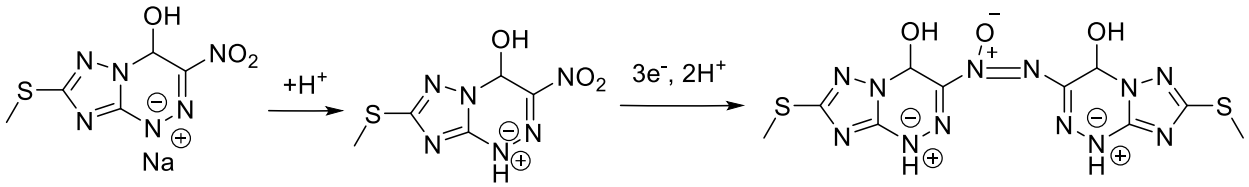

Figure 6 Probable mechanism of electrochemical reduction of compound **1** in BRB pH = 2.

On the basis of the obtained data and the literature studied [28], it can be assumed that compound **1** in an aqueous medium at pH = 2 is irreversibly reduced in the form of a protonated particle with the consumption of 3 electrons until the formation of a dimeric product (Figure 6). To more accurately establish the mechanism of electroreduction of compound **1**, it is necessary to carry out preparative electrolysis followed by detection of products by high performance liquid chromatography (HPLC) in tandem with high resolution mass spectrometry.

Conclusions

The results of this study show the electrochemical behavior of compounds 3-R-4-hydroxy-1,4-dihydro-7-X-1,2,4-triazolo[5,1-c][1,2,4] using electrochemical methods. It was established that compounds containing a nitro group undergo an irreversible reduction process in the potential range of –0.28 to –0.33 V (relative to Ag/AgCl) in a Britton–Robinson buffer solution at pH = 2. The sodium salt of 3-nitro-4-hydroxy-7-methylthio-4H-[1,2,4]triazolo[5,1-c][1,2,4]triazinide monohydrate (compound 1) in an aprotic medium is reduced in ionic form, most likely in the form of an ion pair with the Na^+ cation, and in an aqueous medium in the form of a protonated particle. The main direction of the transformations of compound 1 at the first stage at pH = 2 is the three-electron scheme for the reduction of the nitro group of the compound. Then it followed by dimerization of cathodic electrolysis products. The obtained information is very useful for further understanding connection between compound's structure, electrochemistry transformations and biological activity.

Supplementary materials

No supplementary materials are available.

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Author contributions

Conceptualization: P.N.M., A.V.I., R.A.D.
Data curation: P.N.M.
Formal Analysis: P.N.M., A.V.I., R.A.D.
Funding acquisition: P.N.M.
Investigation: P.N.M., A.Vs.I., R.A.D.
Methodology: P.N.M., A.Vs.I., R.A.D.
Project administration: A.N.K.
Resources: P.N.M., A.Vs.I., R.A.D.
Software: P.N.M.
Supervision: A.N.K., A.Vl.I., V.L.R.
Validation: A.N.K., A.Vl.I.
Visualization: P.N.M.
Writing – original draft: P.N.M., A.Vs.I.
Writing – review & editing: A.N.K., A.Vl.I., V.L.R.

Conflict of interest

The authors declare no conflict of interest.

Additional information

Author IDs:
Polina N. Mozharovskaia, Scopus ID 57232723000;
Alexandra V. Ivoilova, Scopus ID 57211981729;
Roman A. Drokin, Scopus ID 57053422300;
Alla V. Ivanova, Scopus ID 8233431000;
Alisa N. Kozitsina, Scopus ID 16432620500;
Vladimir L. Rusinov, Scopus ID 7006493788.

Websites:
Ural Federal University, https://urfu.ru/en;
Institute of Organic Synthesis, https://iosuran.ru.

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