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Micromolecular inhibitors of superoxide radicals

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Abstract

Background: Currently, there is a growing interest in new copper (Cu^{2+}) heterocyclic coordination compounds (CC), isothiosemicarbazide derivates, which demonstrated multiple beneficial properties, but their effect on reactions with free radicals such as the superoxide radical has not been investigated.
Material and methods: The action of new micromolecular complexes of copper (Cu^{2+}) chloride and bromide with methyl n- (prop-2-en-1-yl) -2- (pyridin-2-ylmethylidene) hydrazine carbimidothioate on capturing activity of the superoxide radical was determined by the spectrophotometric method *in vitro* experiments.
Results: It was established that the micromolecular complexes of copper (II) chloride and bromide with methyl n-(prop-2-en-1-yl)-2-(pyridin-2-ylmethylidene) hydrazine carbimidothioate have been found to possess strong superoxide radical inhibitor properties when interacting with a superoxide radical. In addition to this, the IC_{50} of the studied compounds depends on the nature of the acid-ligand in the internal sphere of the complex and increases in the following sequence: Cl^-–Br^-.
Conclusions: The established property of mentioned compounds is new, because their use as micromolecular inhibitors of superoxide radicals has not been described so far. The synthesized CC expand the arsenal of superoxide radical inhibitors with high biological activity. Their possible significance for the development of new treatment strategies for diseases associated with the overproduction of superoxide radicals is discussed.
Key words: superoxide radical inhibitors, coordination compounds, isothiosemicarbazide derivates.

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Introduction

In the pathogenesis of acute and chronic degenerative diseases (the most common diseases) an important role is attributed to reactive oxygen and nitrogen species (ROS/RNS), in particular, the superoxide radical $O_2^{\cdot-}$, which from a biological point of view, can be generated from two major sources: mitochondrial respiratory chain and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase – an enzymatic complex found in the plasma membrane, as well as in the membranes of phagosomes of nucleated polymorphic leukocytes of the blood to destroy microorganisms [1-3].

The superoxide radical $O_2^{\cdot-}$ – a product of the mitochondrial respiratory chain is also a crucial component of the immune system defense. Due to the high reactivity, superoxide radicals $O_2^{\cdot-}$ through their potential to oxidize nucleic acids, proteins, lipids or carbohydrates are responsible for multiple harmful actions on the body, such as inflammation, cancer, cardiovascular disease, hypertension, ischemia / reperfusion, diabetes, neurodegenerative diseases (Alzheimer's and Parkinson's disease), rheumatoid arthritis, alcohol-induced liver disease, ulcerative colitis, senescence and atherosclerosis. The free radical scavengers presence in biological systems from a wide variety of sources endogenous and / or exogenous, limits the harmful effects of oxigen radical species (ORS), allowing the body to fight efficiently in various pathological situations, limiting the lesions and not allowing their spread [4-7].

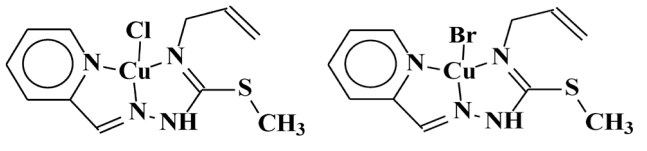
Therefore, the therapeutic inhibition of superoxide radicals is a new contribution, because the compounds with antiradical activity show a strong curative effect, thus preventing multiple harmful actions on the body.

Respectively, one of the priority directions of modern applied chemistry is the synthesis of new compounds, which capture and neutralize ORS, thus preventing the development of cell and tissue damage, including various pathologies caused by exacerbation of free radicals.

The aim of the study is to elucidate the effects of new copper (Cu^{2+}) heterocyclic coordination compounds (CC), isothiosemicarbazide derivates, on oxidation processes with free radicals, such as superoxide radical $O_2^{\cdot-}$, which can be used to prevent and treat many multifactorial acute and chronic diseases.

Material and methods

At the State University of Moldova, in the Laboratory of Advanced Materials in the Biopharmaceutical and Technical Field was synthesized a number of new copper coordination compounds, from the class of isothiosemicarbazides, in particular, the coordinating compounds of copper (2+) - dichloro- [methyl-N-(prop-2-en-1-yl)-2-(pyridin-2-ylmethylidene)-hydrazinecarbimidothioate] copper (**compound I**) and dibromo-[methyl-N-(prop-2-en-1-yl)-2-(pyridin-2-ylmethylidene) hydrazinecarbimiothioate] copper (**compound II**) of the general formula:



I II

The synthesis of compounds I and II, their structure, physicochemical, antimicrobial and anticancer properties have been described [8-10].

Quantitative measurement of free superoxide radicals is difficult due to their exceptional reactivity and short half-life. The most commonly used in biological and chemical systems are classical methods, which are based on the generation of superoxide radicals by the phenazine metosulfate / nicotinamide adenine dinucleotide reduced system (PMS / NADH) by oxidation of NADH, and superoxide radicals reduce the tetrazolium salt – Nitro Blue Tetrazolium (NBT) in blue-purple formazane. Briefly, the capturing activity of the superoxide radical was determined by the spectrophotometric method, described in the literature [11, 12] with some modifications.

At first, the working dilutions of tested compounds in dimethylsulfoxide (DMSO) solution so that the final concentrations are equal to 0.1; 1.0; 10.0; and 100 μmol /L were prepared. Next, 20 μl of each working dilution of tested compounds was poured into the wells of the 96-well microplate and 180 μl of reagent mixture containing 0.02 M phosphate buffer (pH 7.4), 0.1 mM NADH and 0.09 mM nitro-blue tetrazolium (NBT) was added. Each dilution was poured in duplicate. The control samples (in duplicate) were mounted in the same way as the test samples, but the dilutions of the tested compounds were replaced with an equivalent volume of solution containing 0.02 M phosphate buffer (pH 7.4). The microplate was shaken for 10-15 s and the absorbance (A) was measured at 560 nm [A_0]. Then, in all wells, 20 μl of 8.0 μM solution of phenazine metosulphate (PMS) was added, and all the sample was shaken for 10-15 s and was incubated for 5 minutes at room temperature, after which the absorbance was measured again [A_1] at 560 nm. The percentage of *superoxide radicals scavenging activity* was calculated according to the formula:

Superoxide radical scavenging activity (%) = [(A_0 –A_1)/A_0 x 100];

where: A_0 is the absorbance of the control; and A_1 is the absorbance of the test compounds or of the standard and / or reference substances.

As a standard for determining the s*uperoxide radical scavenging activity* – quercetin (3,3',4,5,6-pentahydroxyflavone), which is a natural flavonol, was used [13].

Of all the chemical compounds described in the literature which contain the 4-allyl-S-alkylisothiosemicarbazide moiety and which inhibit superoxide radicals ($O_2^{\cdot-}$) the highest antiradical effect was obtained in the case of compound bis (m_2-acetate-o)-bis {[N-prop-2-en-1-yl-N'- (pyridin-2-ylmethylidene) carbamo-hydrazonothioate] copper} dihydrate (prototype and structural analogue) [14] with the formula:

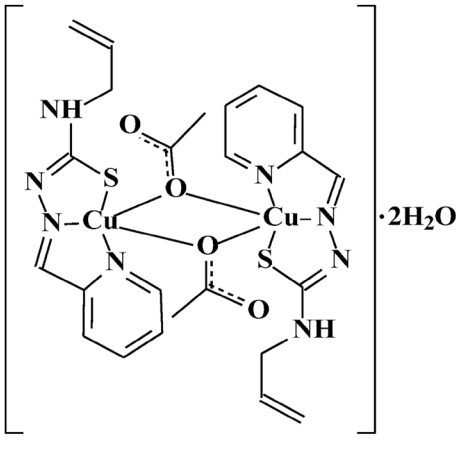


Fig. 1. Chemical structure of the compound bis (m_2-acetate-o)-bis {[N-prop-2-en-1-yl-N'-(pyridin-2-ylmethylidene) carbamo-hydrazonothioate] copper} dehydrate (structural prototype) [14]

Results and discussion

The obtained experimental data presented in tab. 1 demonstrated that the compounds I and II manifest high antiradical activity in the IC_{50} range equal to 0.05-0.06 μmol/L which is 6-7.0 times higher than the antiradical activity of the prototype and the structural analogue [compound bis (m_2-acetate-o)-bis {[N-prop-2-en-1-yl-N'- (pyridin-2-ylmethylidene) carbamo-hydrazonothioate] copper} dihydrate] [14], which manifests the highest antiradical effect among all biometal complexes with ligands from the thiosemicarbazonic class, described in the literature.

In addition to this, as can be seen from the presented

data, the IC_{50} of the studied compounds depends on the nature of the acid-ligand in the internal sphere of the complex and increases in the following sequence: Cl-–Br-.

Table 1. Superoxide radical scavenging activity of tested compounds compared to prototype and structural analogue

Tested compound	IC_{50}, μmol/L
Quercetin (3,3',4,5,6-pentahydroxyflavone) [1]	61.86±2.5
Bis (μ_2-acetate-O)-bis {[N-prop-2-en-1-yl-N'- (pyridin-2-ylmethylidene) carbamo-hydrazonothioate] copper} dehydrate (prototype structural analogue) [1]	0.35±0.07
Dichloro- [methyl-N- (prop-2-en-1-yl) -2- (pyridin-2-ylmethylidene) –hydrazine-carbimidothioate] copper (**compound I**)	0.06±0.01
Dibromo - [methyl-N- (prop-2-en-1- yl) -2- (pyridin-2-ylmethylidene) hydrazinecarbimidothioate] copper (**compound II**)	0.05±0.01

The established property of above mentioned compounds I and II is new, because their use as micromolecular inhibitors of superoxide radicals has not been described yet.

Comparative analysis of compounds I and II with the prototype and the structural analogue demonstrates that they differ in that the monodeprotonated salicylidene moiety of azomethine is eventually replaced by the neutral picolinic moiety, due to which the six-atom metallocycle in the structural analogue composition changed to the five-atom metallocycle and the coordination nodes formed by isothiosemicarbazone are changed from O, N, N to N, N, N. In the compounds claimed in addition, in the internal sphere of declared compounds nitrate-ion and water are replaced by chlorine and bromine ions. Due to these changes in the internal sphere of compounds I and II, a new combination has already known chemical bonds.

Detected properties of dichloro- [methyl-N-(prop-2-en-1-yl)-2-(pyridin-2-ylmethylidene)-hydrazincarbimidothioate] copper and dibromo- [methyl-N-(prop-2-en- 1-yl)-2-(pyridin-2-ylmethylidene) hydrazine carbimidothioate] copper are of interest to medicine in terms of expanding the arsenal of highly efficient synthetic inhibitors of superoxide radicals.

After antiradical activity tested compounds are over 1030-1237 times more effective than quercetin, used as a standard for determining the activity of superoxide radical inhibition and 6-7 times more effective than the most active synthetic antiradical inhibitor described in the literature [Bis (m_2-acetate-O)-bis {[N-prop-2-en-1-yl-N'-(pyridin-2-ylmethylidene) carbamo-hydrazonothioate] copper dihydrate (prototype, structural analogue)].

The high toxicity of the superoxide anion is due to its ability to inactivate enzymes containing critical iron and sulfur groups in a wide variety of metabolic pathways, thus releasing free iron into the cell, which can undergo the Fenton reaction and generate highly reactive hydroxyl, as well as other free radicals that can damage important molecules, such as DNA, proteins, lipids and carbohydrates [15].

The human body is under constant attack by these radicals formed as a result of normal metabolic activity; therefore, there are a number of defense mechanisms against free radicals (FR), including antioxidant enzymes and non-enzymatic compounds. The involvement of FR in the etiology and progression of several acute and chronic clinical disorders has generated a growing interest in the search for substances that can eliminate FR and increase the antioxidant defense system.

There are several antioxidant tests to evaluate the antioxidant potential of different substances. For this purpose are widely used various variants of the ABTS radical cation discoloration test ($ABTS^+$) [16]. This method is based on the spectrophotometric determination of the discoloration rate of the blue-green chromophore $ABTS^+$ [(2,2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid)] when an antioxidant is added. $ABTS^+$ is added to ABTS and discolors it. $ABTS^+$ is a stable radical that has not been found in the biological systems and the human body, at the same time, the main impediment is the inability to determine / evaluate and quantitatively separate the activity of inhibition of the radical superoxide (O_2^-) is explained by the fact that the indicated method is non-specific, able to determine / evaluate the summary annihilation activity of various reactive oxygen and nitrogen species (ROS/RNS), such as the superoxide radical ($O_2^{\cdot-}$), hydroperoxyl radical (HO_2), hydroxyl radical, hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), lipid peroxyl radical (LOO^-) etc., but the rate of uptake of each of these radicals cannot be appreciated, because it depends on several factors that are difficult to take into account. Note that $ABTS^+$ radicals can be annihilated by both hydrogen and electron transfer, and the dominant reaction varies depending on antioxidant, solvent and pH, complicating the comparison of results in different systems.

Thus, ABTS is an indirect method based on the reduction of various persistent radicals, which does not allow to appreciate exactly the inhibitory activity of the superoxide radical (O_2^-).

The shortcomings of the $ABTS^+$ cationic radical method and its limitations are reflected in a number of publications and synthesis articles [17-21].

Thiosemicarbazones continue to attract the interest of researchers as potential anticancer drugs. Moreover, in recent decades, thiosemicarbazones have found wide application as effective remedies for a variety of diseases, including tuberculosis, viral infections, malaria and a number of multifactorial diseases. Regarding malignancies, the class of α-N-heterocyclic thiosemicarbazones should be considered as drugs that influence various biological pathways in a complex mode of action and with multiple cellular signaling targets [22]. Note the particularly high selectivity that normal cells have, which manifests itself on a stronger redox balance of them [23].

The antitumor activity of these compounds could relate to the disturbance of the cancer cells homeostasis, in par-

ticular the interruption of pivotal pathways for signaling cancer cells [24].

It should be noted, moreover, that highly concentrated oxygen ventilation in patients with low SpO_2 levels can produce an extremely large number of harmful superoxide free radicals, which occurs as in extremely severe patients with SARS-Cov-2 infection. In this aspect micromolecular inhibitors of superoxide radicals could counteract the negative effects of oxidative stress and inflammation; they could improve the severity of the disease and treatment outcomes, especially in patients with underlying complications of COVID-19. Further investigations are needed in this direction line to provide effective strategies for treating and preventing the complications of this terrible disease of the third millennium [25-27].

Therefore, the therapeutic inhibition of the superoxide radicals is a new contribution, because the micromolecular compounds with antiradical activity show a strong curative effect, thus preventing multiple harmful actions on the body.

Further studies have to confirm the therapeutic utility of this bioactive micromolecular compound under investigation.

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Authors' contribution
LA and VP conducted/performed the laboratory work, biochemical investigation; AG conceptualized the idea; IS, VG VM and VM designed the research, reviewed statistics and interpreted the data, wrote the first draft; VG revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
The research protocol was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 81 of 19.09.2020). No animals or human subjects were used in this work.

Conflict of Interests
The authors declare that there is no conflict of interests.

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