CZU: 542.913:546.562:547.497.1 https://doi.org/10.59295/mabt2026.15

SYNTHESIS AND BIOLOGICAL PROPERTIES OF COORDINATION COMPOUNDS OF Cu(II) BASED ON N^4-CYCLOHEXYLTHIOSEMICARBAZONE 2-ACETYLPYRIDINE

Iulia GRIBANOVA, Andrei CIURSIN, Roman RUSNAC, Aurelian GULEA

Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, Chisinau, MD 2009, Republic of Moldova

Abstract

Thiosemicarbazones are a class of organic compounds with versatile biological properties. In addition to this, they are also known as efficient ligands for the coordination of 3*d* metals. Coordination compounds of thiosemicarbazones with Cu(II) are of major interest, as they often exhibit better biological activities than the free ligand. In this work, the N^4-cyclohexylthiocarbazone of 2-acetylpyridine and four coordination compounds based on it with the salts $CuCl_2$, $CuBr_2$, $Cu(Ac)_2$ and $Cu(NO_3)_2$ were synthesised. The synthesised compounds were characterised by physico-chemical methods and subjected to the evaluation of their antioxidant, antimicrobial and antifungal activities.

Keywords: coordination compounds, thiosemicarbazones, copper(II), antimicrobial and antifungal activity, antioxidant properties.

SINTEZA ȘI ANALIZA BIOLOGICĂ A COMPUSILOR COORDINATIVI AI CU(II) ÎN BAZA N^4-CICLOHEXILTIOSEMICARBAZONEI 2-ACETILPIRIDINEI

Rezumat

Tiosemicarbazonele constituie o clasă de compuși organici cu proprietățile biologice versatile. Pe lângă acestui fapt, ei sunt cunoscuți și ca liganzi eficienți pentru coordinarea metalelor 3*d*. Compuși coordinativi ai Cu(II) cu tiosemicarbazone cu sunt de interes major, întrucât prezintă adesea activități biologice mai bune față de ligandul necoordinat. În această lucrare au fost sintetizați: patru compuși coordinativi în baza sărurilor de cupru - $CuCl_2$, $CuBr_2$, $Cu(Ac)_2$ și $Cu(NO_3)_2$ și N^4-ciclohexiltiocarbazona 2-acetilpiridinei. Compuși sintetizați au fost caracterizați prin metodele fizico-chimice și supuși evaluării activităților antioxidante, antimicrobiene și antifungice.

Cuvinte-cheie: compuși coordinativi, tiosemicarbazonele, cupru(II), activitate antimicrobiană și antifungică, proprietăți antioxidative.

Introduction

In recent decades, humanity has been in a continuous search for new medicines capable of effectively combating numerous diseases that threaten the health of the population. The development of resistance of microorganisms to conventional treatments, the increase in cancer cases and in deaths related to oncological diseases, as well as the necessity of more selective and less toxic therapies present a particular interest for scientists from all over the world. The International Agency for Research on Cancer founded by the World Health Organization forecasts that the number of new cancer cases will increase significantly in all regions, the largest absolute share being in Asia (+64.5% of the number of cases from the year 2022), and Africa stands out by the largest relative increase, of over 99%, which indicates a doubling of the number of cases, up to approximately 2.45 million (Figure 1.) [1]. This trend underlines the necessity of intensifying research in the field of the synthesis of new compounds with potential biological activity.

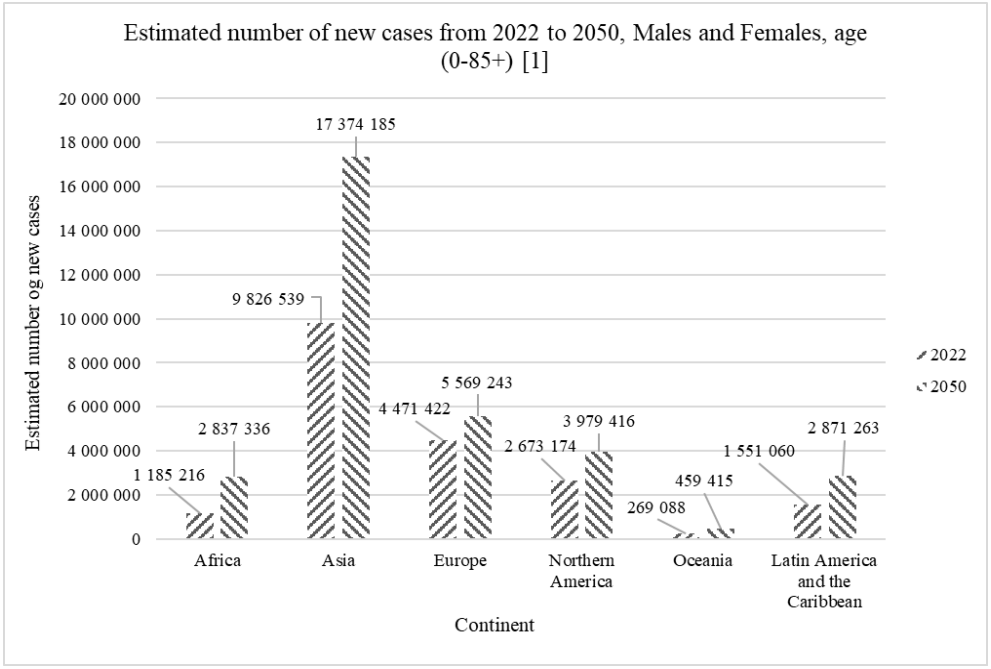
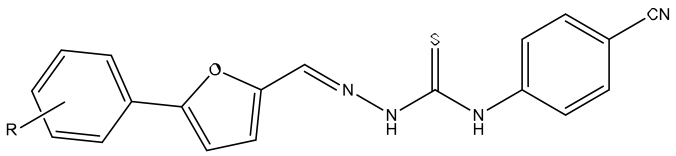


Figure 1. Estimated number of new cases from 2022 to 2050 [1]

In this context, the chemistry of coordination compounds acquires a particular importance, being capable of forming innovative molecular systems with promising pharmacological properties. Metal complexes present physico-chemical and biological properties different from the free ligand, sometimes even considerably surpassing its biological activity [2]. They, especially those of transition metals, have the capacity to interact with various essential biomolecules, such as DNA or proteins, and to induce oxidative processes at the cellular level [3]. Due to these characteristics, complexes with organic ligands represent a promising direction in the development of anticancer and antimicrobial agents.

Copper(II) presents an important role in chemistry due to its essential role in biological processes and its redox properties. This metal ion is involved in numerous enzymatic reactions and electron transfer processes in living processes. Due to its capacity to alternate between different oxidation states, Cu(II) can generate reactive oxygen species (ROS), which induce oxidative stress at the level of tumour cells [3]. In addition, it can interact with DNA, inhibiting the processes of replication and transcription, which contributes to their cytotoxic effect [4]. Thus, coordination compounds of Cu(II) have a particular potential in the development of new antitumoral and antimicrobial agents.

Another important aspect is manifested by the choice of the ligand. A particular interest is given to thiosemicarbazones. They are generally obtained through the condensation reaction between thiosemicarbazide or derivatives of it and carbonyl compounds, such as aldehydes or ketones. Due to their diverse biological activities, such as antioxidant [5-6], antiviral [7], antimicrobial [8], anti-inflammatory [9], antifungal [10], antitumoral [11], they present a high interest in the field of organic and medicinal chemistry. Recent studies have also demonstrated high anticancer activity against different types of cancer. For example, they have manifested the capacity to inhibit tumour cells of lung cancer (A549) and cervical cancer (HeLa) [12].



R= 2-Cl; 3-Cl; 4-Cl; 2-NO2; 3-NO2; 4-NO2; 2,4-Cl2; 3,4-Cl2; 2,5-Cl2; 2-NO2-4-Cl.

Figure 2. Structure of the thiosemicarbazone [12].

Due to the presence of donor atoms of nitrogen and sulphur, thiosemicarbazones can form stable complexes with metal ions, present a wide range of biological activities identical to those of the free ligand [14–15]. The structural modifications of the ligand, especially substitution in the N4 position, can significantly influence the physico-chemical and biological properties of the resulting compounds. For example, the introduction of the cyclohexyl group in the N4 position of the thiosemicarbazone leads to the increase of the hydrophobic character of the molecule, which can facilitate the penetration of lipid cellular membranes [13]. This capacity is essential for the efficiency of bioactive compounds, as it allows the penetration of the compounds into the target cells and can lead to an improved biological activity.

In this article will be described the synthesis and physico-chemical characterisation of coordination compounds of Cu(II) with N^4-cyclohexylthiosemicarbazone of 2-acetylpyridine. Also, synthesised compounds were analysed for their antioxidant, antimicrobial and antifungal activity.

Materials and methods

Synthesis of precursors

Cyclohexyl isothiocyanate

To a mixture consisting of (an equivalent) cyclohexylamine, (an equivalent) triethylamine and benzene were added (an equivalent) thiophosgene. The reaction mixture was stirred for 3 hours at room temperature, followed by additional heating for 30 minutes at 40–55 °C. After a short time, the formation of a white-yellowish precipitate was observed. The solvent was distilled at reduced pressure up to approximately 70% of the initial volume. The formed precipitate was separated at reduced pressure by filtration using a Büchner funnel. The resulting solution was concentrated and purified by column chromatography on silica gel (63–200 μm). Elution with hexane was carried out until the chromatographic confirmation of the disappearance of the reaction product. Finally, the solvent was removed by distillation, obtaining a pale-yellow oil, corresponding to cyclohexyl isothiocyanate.

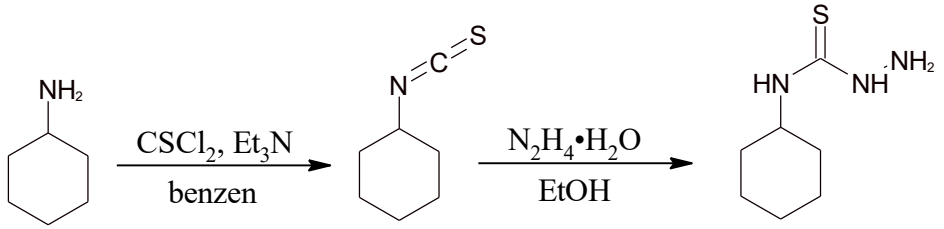


Figure 3. Synthesis scheme of N-cyclohexylhydrazinecarbothioamide from amine

N-cyclohexylhydrazinecarbothioamide

Into a conical flask were introduced (an equivalent) hydrazine monohydrate and 25 mL ethyl alcohol. The mixture was cooled in an ice-water bath. Subsequently, under stirring, were gradually added (an equivalent) cyclohexyl isothiocyanate, dissolved in a minimal amount of

ethyl alcohol. The reaction mixture was maintained under stirring for one hour at room temperature, the evolution of the reaction being monitored chromatographically.

N-cyclohexyl-2-[1-(pyridin-2-yl)ethylidene]hydrazine-1-carbothioamide or N^4-cyclohexiltiosemicarbazone of 2-acetylpyridine (HL)

A mixture consisting of (an equivalent) N-cyclohexylhydrazinecarbothioamide, (an equivalent) 1-(pyridin-2-yl)ethan-1-one, 0.5 mL glacial acetic acid and 20 mL ethanol was subjected to reflux for 7 hours, the evolution of the reaction being monitored chromatographically. After the N-cyclohexylhydrazinecarbothioamide was consumed, the solvent is partially distilled, up to approximately 70% of the initial volume. The obtained sediment being then filtered, washed with distilled water and recrystallised from ethanol. Obtained structural data coincide with the source [16].

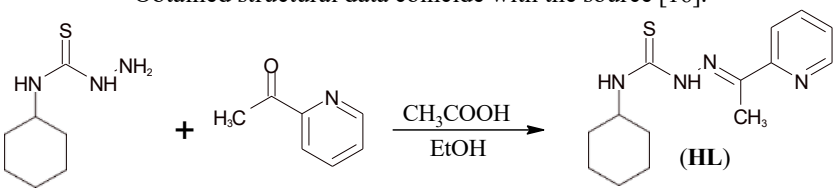


Figure 4. Synthesis scheme of thiosemicarbazone HL

Synthesis of coordination compounds

Synthesis of [Cu(HL)Br]Br (1)

The coordination compound (1) was synthesised following the general method with a 1:1 molar ratio. In a first step (1 mmol) of $CuBr_2$ were dissolved in 2 mL of ethanol, after which (1 mmol) of HL, dissolved in 3 mL of ethanol, were added with gentle heating in a water bath. The resulting mixture was homogenised and then refluxed for one hour. As a result of the reaction, a light green microcrystalline solid was formed. Obtained structural data coincide with the source [16].

Synthesis of [Cu(L)Cl] (2)

The coordination compound (2) was synthesised according to the general method with a 1:1 molar ratio. Thus, (1 mmol) of $CuCl_2 \cdot 2H_2O$ were dissolved in 2 mL of ethanol, after which (1 mmol) of HL, dissolved in 3 mL of ethanol, were added with gentle heating in a water bath. The resulting mixture was homogenised and refluxed for one hour. As a result of the reaction, a green microcrystalline solid was formed. Obtained structural data coincide with the source [16].

Synthesis of [Cu(L)CH_3COO] (3)

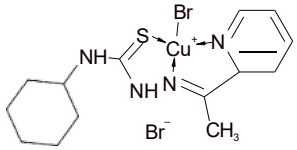
The coordination compound (3) was synthesised following the general method with a 1:1 molar ratio. For this purpose, (1 mmol) of $Cu(CH_3COO)_2 \cdot H_2O$ were dissolved in a mixture of 6 mL ethanol and 4 mL water, after which (1 mmol) of HL, dissolved in 3 mL ethanol, were added with gentle heating in a water bath. The resulting mixture was homogenised and refluxed for one hour. As a result of the reaction, a green microcrystalline solid was formed. Obtained structural data coincide with the source [16].

Synthesis of [Cu(L)NO_3] (4)

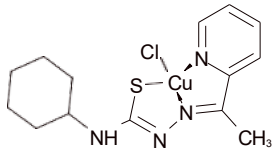
The coordination compound (4) was synthesised according to the general method with a 1:1 molar ratio. Thus, (1 mmol) of $Cu(NO_3)_2 \cdot 3H_2O$ were dissolved in 2 mL of ethanol, after which (1 mmol) of HL, dissolved in 3 mL of ethanol, were added with gentle heating in a water bath. The resulting mixture was homogenised and refluxed for one hour. As a result of the reaction, a green microcrystalline solid was precipitated. Obtained structural data coincide with the source [16].

Results and discusion

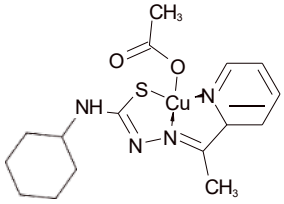
In total, one thiosemicarbazone and its precursors were obtained. On the basis of the obtained ligand, coordination compounds with different salts of Cu(II) were synthesised. The syntheses of the coordination compounds were carried out according to a standard procedure, without special synthesis conditions. Obtained structural data perfectly fits data demonstrated in source [16]. In Figure 5 are demonstrated the proposed chemical structures for the synthesised coordination compounds (**1-4**).



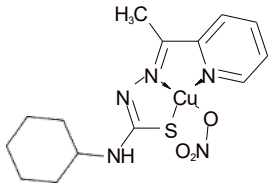
[Cu(HL)Br]Br (1)



[Cu(L)Cl] (2)



[Cu(L)CH_3COO] (3)



[Cu(L)NO_3] (4)

Figure 5. Proposed chemical structures of coordination compounds involving copper (1-4)

Antioxidant assay

Figure 6 presents the antioxidant activity of the thiosemicarbazone HL and its coordination compounds. The thiosemicarbazone HL exhibits a moderate antioxidant activity (IC_{50} = 27.4 μM), comparable to that of the standard compound Trolox (IC_{50} = 33.3 μM), whereas its complexes do not show considerable antioxidant activity under the experimental conditions.

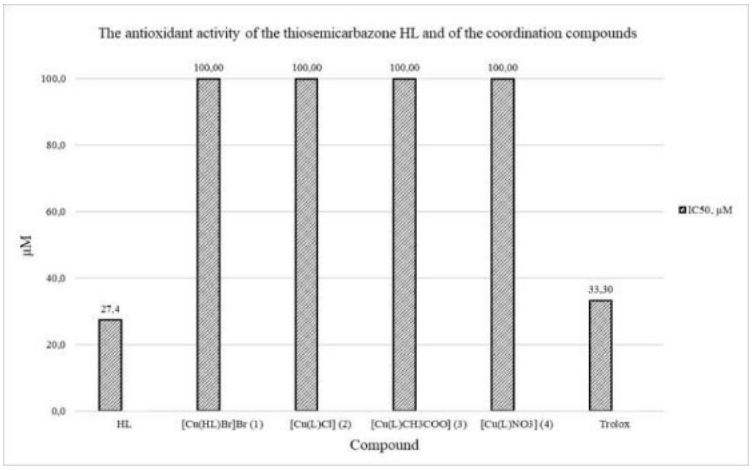


Figure 6. The antioxidant activity of the thiosemicarbazone HL and of the coordination compounds (1-4)

Antimicrobial and antifungal analysis

The antimicrobial and antifungal analysis demonstrated that the synthesised thiosemicarbazone exhibits antimicrobial activity approximately 76 times higher than the standard compound Furacilin against *S. aureus* (MIC = 0.06 μg/mL), and 20 times higher against *B. cereus* (MIC = 0.24 μg/mL) (Figure 7). Also, the thiosemicarbazone also shows moderate antifungal activity against *C. albicans* (MIC = 0.98 μg/mL). The coordination compounds of the thiosemicarbazone have lower antibacterial activity than the free ligand. However, some of them are effective against *A. baumannii*, where HL does not show activity. In general, the complexes exhibit the best activity against *S. aureus* and *C. albicans*.

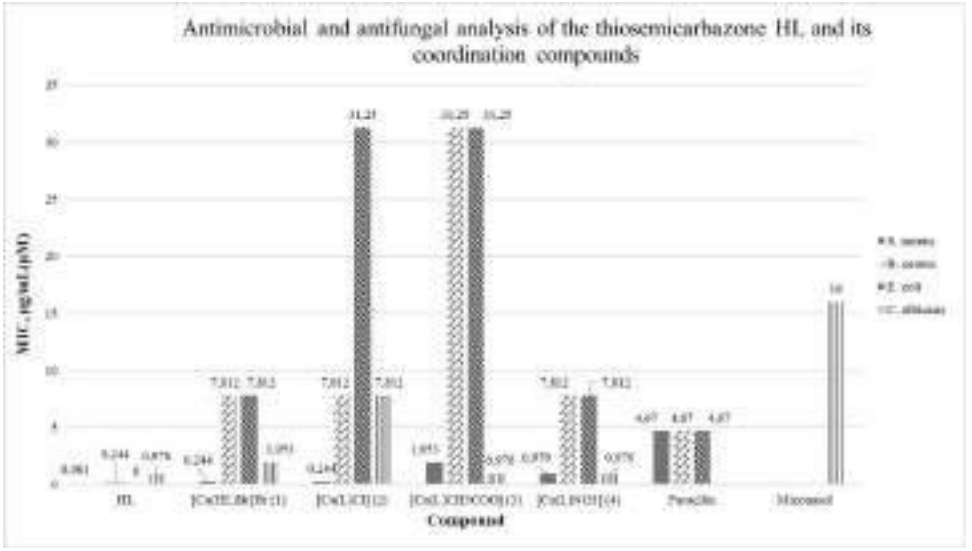


Figure 7. Antimicrobial and antifungal analysis of the thiosemicarbazone HL and its coordination compounds (1-4)

Conclusions

In total were synthesised: N^4-cyclohexylthiosemicarbazone of 2-acetylylpyridine (**HL**) and four coordination compounds based on it. The structures of the ligand and of the synthesised coordination compounds (**1-4**). Antioxidant analysis demonstrated that the coordination compounds do not exhibit considerable antioxidant activity, whereas the ligand demonstrates antioxidant properties at the level of the reference substance. Antimicrobial analysis demonstrated that the ligand HL exhibits the best activity against *S. aureus* of all investigated substances, having an MIC 75 times lower than Furacilin (Nitrofurazone). Cordination compounds **1** and **2** exhibit an MIC approximately 20 times lower than Furacilin against *S. aureus*. The ligand HL and complexes **3** and **4** exhibit similar antifungal activity against *C. albicans*, having an MIC 16 times lower than Miconazole. In the remaining cases, the investigated substances did not demonstrate a considerable effect (in comparison with reference substances).

Acknowledgment

The experimental material presented in the article was developed within the national project "Synthesis and study of new materials based on complex combinations with polyfunctional ligands and with useful properties in medicine, biology and technology" (SSMCCLP) (code: 010602); details at: https://ichem.md/SSMCCLP

References:

1. FERLAY, J., et al. Global Cancer Observatory: Cancer Tomorrow. International Agency for Research on Cancer. Available from: https://gco.iarc.who.int/tomorrow.
2. PAHONTU E., et al. Antibacterial, antifungal and in vitro antileukaemia activity of metal complexes with thiosemicarbazones. Journal of Cellular and Molecular Medicine. 2015, Vol. 19, nr. 4, p. 865–878. DOI: https://doi.org/10.1111/jcmm.12508.
3. FILOMENI, G., et al. Pro-apoptotic Activity of Novel Isatin-Schiff Base Copper(II) Complexes Depends on Oxidative Stress Induction and Organelle-selective Damage. In: Journal of biological chemistry. 2007. DOI: https://doi.org/10.1074/jbc.M610927200.
4. FEI, B., et al. Effects of copper ions on DNA binding and cytotoxic activity of a chiral salicylidene Schiff base. Journal of Photochemistry and Photobiology B: Biology. 2014, Vol. 132, p. 36–44. DOI: https://doi.org/10.1016/j.jphotobiol.2014.01.018.
5. GARBUZ, Olga et al. Thiosemicarbazone-Based Compounds: Cancer Cell Inhibitors with Antioxidant Properties. Molecules. 2025, Vol. 30, nr. 9, p. 2077. DOI: https://doi.org/10.3390/molecules30092077.
6. CIURSIN, A., RUSNAC, R., GULEA, A. Synthesis, absorption, distribution, metabolism, excretion and antioxidant assay of some N4 – substituted Thiosemicarbazones of Cinnamaldehyde. In: One Health and Risk Management. 2025, vol. 6, nr. 3, pp. 54-65. ISSN 2587-3458. DOI: https://doi.org/10.38045/ohrm.2025.3.05
7. PADMANABHAN, P., et al. Antiviral activity of Thiosemicarbazones derived from α-amino acids against Dengue virus. Journal of Medical Virology. 2016, Vol. 89, nr. 3, p. 546–552. DOI: https://doi.org/10.1002/jmv.24655.
8. MOHAMED, N., MOHAMED, R., SEOUDI, R. Synthesis and characterization of some novel antimicrobial thiosemicarbazone O-carboxymethyl chitosan derivatives. International Journal of Biological Macromolecules. 2013, Vol. 63, p. 163–169. DOI: https://doi.org/10.1016/j.ijbiomac.2013.10.044.
9. JACOB, I., et al. Anti-inflammatory activity of novel thiosemicarbazone compounds indole-based as COX inhibitors. Pharmacological Reports. 2021, Vol. 73, nr. 3, p. 907–925. DOI: https://doi.org/10.1007/s43440-021-00221-7.
10. PAIVA, R., et al. Antifungal activities of thiosemicarbazones and semicarbazones against mycotoxigenic fungi. Ciência e Agrotecnologia. 2014, Vol. 38, nr. 6, p. 531–537. DOI: https://doi.org/10.1590/s1413-70542014000600001.
11. SHAKYA, B., YADAV, P. Thiosemicarbazones as a Potent Anticancer Agents and Their Modes of Action. Mini-Reviews in Medicinal Chemistry. 2019, Vol. 19. DOI: https://doi.org/10.2174/1389557519666191029130310.
12. SEVER, B. et al. Design, synthesis and in vitro evaluation of new thiosemicarbazone derivatives as potential anticancer agents. Journal of research in pharmacy. 2018, Vol. 23, nr. 1, p. 16–24. DOI: https://doi.org/10.12991/jrp.2018.104.
13. PÎNTEA, A., CIURSIN, A., RUSNAC, R., GULEA, A. Combinații coordinative ale Cu(II) în baza n-hexil-2-[(piridin-2-il)metiliden]hidrazine-1-carbotioamidei: proiectare, sinteză, evaluarea proprietăților antimicrobiene și antifungice. In: Studia Universitatis Moldaviae (Seria Ştiinţe Reale şi ale Naturii), 2024, nr. 1(171), pp. 168-177. ISSN 1814-3237. DOI: https://doi.org/10.59295/sum1(171)2024_21
14. LIBERTA, A., și WEST, D. Antifungal and antitumor activity of heterocyclic thiosemicarbazones and their metal complexes: current status. Biometals. 1992, Vol. 5, nr. 2, p. 121–126. DOI: https://doi.org/10.1007/bf01062223.
15. AL-AMIERY, A., KADHUM, A., MOHAMAD, A. Antifungal and Antioxidant Activities of Pyrrolidone Thiosemicarbazone Complexes. Bioinorganic Chemistry and Applications. 2012, Vol. 2012, p. 1–6. DOI: https://doi.org/10.1155/2012/795812.
16. RUSNAC, R. Designul și sinteza compușilor biologic activi ai unor metale 3d cu tiosemicarbazone N(4)-substituite ale derivaților 2-formilpiridinei. Autoreferat al tezei de doctor în științe chimice. Chișinău, 2022.

Authors:



Iulia GRIBANOVA, master's student, Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, MD-2009 Chisinau, Moldova
e-mail: gribanova.iulia@usm.md



Andrei CIURSIN, PhD student, specializing in coordination chemistry, Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, MD-2009 Chisinau, Moldova
e-mail: ciursin.andrei@usm.md
ORCID: 0000-0002-3160-4582



Roman RUSNAC, Dr., senior scientific researcher/university lecturer, Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, MD-2009 Chisinau, Moldova
e-mail: roman.rusnac@usm.md
ORCID: 0000-0002-5713-5251



Aurelian GULEA, Academician, Doctor Habilitate, University Professor, Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, MD-2009 Chisinau, Moldova
e-mail: guleaaurelian@gmail.com
ORCID: 0000-0003-2010-7959