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RUSNAC ANNA

SYNTHESIS AND BIOLOGICAL PROPERTIES OF COORDINATION COMPOUNDS WITH SOME BIOMETALS BASED ON THIOSEMICARBAZONES DERIVED FROM ETHYL 4-AMINOBENZOATE

141.02 COORDINATION CHEMISTRY

Abstract of the doctoral thesis in chemical sciences

CHISINAU, 2023

The thesis was developed at the Doctoral School of Natural Sciences of the Moldova State University, at the Scientific Research Laboratory „Advanced Biopharmaceutical and Technical Materials"

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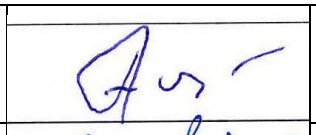
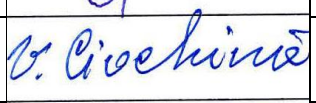
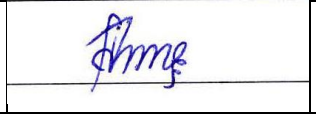
Scientific leader		**GULEA Aurelian**, doctor habilitat in chemical sciences, university professor, academician
Scientific secretary		**CIOCHINĂ Valentina** doctor in biological sciences, research associate
Author		**RUSNAC Anna**

SUMMARY

CONCEPTUAL GUIDELINES OF RESEARCH

The topicality and importance of the topic addressed

Cancer is a major public health problem worldwide and is the second leading cause of death. The cancer death rate has declined continuously since 1991, resulting in an overall decline of 33% and approximately 3.8 million cancer deaths averted. This steady progress is due to the reduction in smoking, the adoption of breast, colorectal, and prostate cancer screening, and treatment improvements such as adjuvant chemotherapy for colon and breast cancers [1].

Over several decades and until today, thiosemicarbazone derivatives, having the general formula $R^1R^2C{=}N{-}NH{-}C(=S){-}NR^3R^4$, represent an interesting category of organic compounds due to their variable donor properties, structural diversity, diverse synthesis, and biological, medicinal, and pharmaceutical applications [2]. They possess a wide spectrum of biological properties, such as antioxidant [3], antibacterial [4], antifungal [5], antidiabetic [6], antiproliferative [7], antitumor [8], anticancer [9], and anti-inflammatory activities [10]. Surprisingly, the coordination of thiosemicarbazones with metal ions proved beneficial in terms of improving the activity and considerably decreasing the side effects of the ligands [11], [12]. Coordinative compounds are molecules that have one or more metal centers that are linked to ligands (atoms, ions, or organic molecules that donate electrons to the metal), forming coordinative (covalent) bonds. Metals play many different roles in the biological world, either through their participation in essential biological processes or as indispensable diagnostic and therapeutic agents in human medicine. In the research given in the 3*d* metal series, the following biometals were chosen to obtain the coordination combinations: Zn(II), Cu(II), Ni(II), Co(III), Fe(III) and Mn(II) with the purpose of obtaining compounds with less toxicity and for their easier metabolism in the body.

The aim of the work is the synthesis, characterization, and research of the biological properties of the coordination compounds with some biometals based on the thiosemicarbazones of ethyl 4-aminobenzoate.

Research objectives:

- synthesis of (*p*-ethyl benzoate)thiosemicarbazones of 2-formylpyridine/salicylic aldehyde and their derivatives;
- synthesis of the coordination compounds of Zn(II), Cu(II), Ni(II), Co(III), Fe(III) and Mn(II) based on the obtained thiosemicarbazones;

- determination of the composition and structure of the synthesized compounds using IR spectroscopy, ^{1}H-NMR, ^{13}C-NMR spectroscopy, elemental analysis, and single crystal X-ray analysis;
- research of antioxidant, antibacterial, antifungal, and antitumor properties.

The research hypothesis involves the synthesis of coordination compounds of biometals with thiosemicarbazonium class ligands based on the functionalization of ethyl 4-aminobenzoate and its derivatives as an active principle, which will play a key role in improving biological activities. Coordination compounds will increase cellular permeability, antioxidant capacity, antimicrobial-antifungal action, and antitumor potential.

Synthesis of the research methodology and justification of the chosen research methods. The research methodology involves the synthesis of coordination compounds and organic ligands from the thiosemicarbazone class based on ethyl 4-aminobenzoate by known methods with some improvements. For the synthesis of thiosemicarbazones based on ethyl 4-aminobenzoate and its derivatives, methods such as organic synthesis, purification, and recrystallization from organic solvents were applied; separation of solvents from research samples by evaporation and distillation; and to determine the purity of the organic substances, the thin layer and column chromatography method was applied. All synthesized compounds were analyzed by FTIR spectroscopy. The investigation of structural forms and tautomers in solution used nuclear resonance spectroscopy (^{1}H and ^{13}C-NMR) as well as the techniques DEPT-135 (Institute of Chemistry). To confirm the crystalline structures of some crystalline compounds, single crystal X-ray diffraction was applied, performed at the "Petru Poni" Institute of Macromolecular Chemistry, Iaşi, Romania. For some studied compounds, elemental analysis was performed with the help of the "PERKIN ELMER 2400" analyzer in the BioCIS laboratory in Chatenay-Malabry, France. Antimicrobial and antifungal activity was performed in the Microbiology Laboratory of the National Public Health Agency. The anticancer properties were carried out in the Biochemistry Laboratory of the "Nicolae Testemițanu" State University of Medicine and Pharmacy. The antioxidant properties were performed in the Systematics and Molecular Phylogeny Laboratory, Biological Invasion Research Center of the Institute of Zoology.

Scientific novelty and originality

For the first time, 15 thiosemicarbazones functionalized with the ethyl 4-aminobenzoate fragment were obtained, and the optimal synthesis conditions were established to obtain 33 new coordination compounds of Zn(II), Cu(II), Ni(II), Co(III), Fe(III) and Mn(II) in high yields. Single crystals were obtained for 14 compounds and were investigated by single crystal X-ray analysis. The structural study demonstrated that the ligands derived from 2-formylpyridine are tridentate

and coordinate to the metal ions via the pyridinic, azomethine, and thionic sulfur atoms, forming two metallocycles of 5 atoms. A number of coordination compounds have been investigated for potential: antioxidant, antimicrobial, antifungal, and anticancer. An organic substance of the thiosemicarbazone class HL^1 has been patented which shows increased application potential due to the inhibitory properties of the proliferation of HL-60 human myeloid leukemia cells with high cytostatic activity.

The scientific problem that was solved consisted of determining the optimal conditions for the synthesis of a series of coordination compounds based on biometals with different functionalized thiosemicarbazones containing the ethyl 4-aminobenzoate fragment. Antimicrobial, antifungal, anticancer, and antioxidant activities were screened depending on the following factors: the nature of the central atom within the coordination compound; the rest of the acid; the position of the ester group in the aromatic ring in the composition of thiosemicarbazones; and the carbonyl component.

Applicative value

The most pronounced antioxidant properties among coordination compounds are possessed nickel-based complexes. The best results of antibacterial activity were recorded on Staphylococcus aureus and Candida krusei by copper complexes. The anticancer properties of human myeloid leukemia HL-60 cells were investigated. Some thiosemicarbazones, after anticancer activity, are three times more effective than doxorubicin, used in medicine, and 250 times more active than *cis*-platinum.

Implementation of scientific results

An organic compound of the thiosemicarbazone type (**HL^1**) was patented, which shows increased application potential due to the half-maximal inhibition concentration of the order of $1 \cdot 10^{-7}$ mol/L when inhibiting the growth of HL-60 cancer cells.

Publications on the topic of the thesis

As part of the doctoral thesis topic, **five** articles were published in national peer-reviewed journals, **one** in the journal of national collections, and **13** summaries of communications at various national and international scientific events; **a patent** was obtained.

The volume and structure of the work

The thesis material is presented on 205 pages, including 71 figures and 13 tables. The work is structured in 4 chapters, introduction, summary of the thesis in 3 languages, list of abbreviations, synthesis of specialized literature, 3 basic chapters, general conclusions and recommendations, 164 bibliographic references, statement regarding the assumption of responsibility, and the candidate's CV.

THESIS CONTENT

The introduction includes the actuality and importance of the topic addressed, the purpose of the paper, the research objectives, the research hypothesis, the synthesis of the research methodology and the justification of the chosen research methods, the summary of the thesis chapters.

1. COORDINATION COMPOUNDS OF SOME BIOMETALS BASED ON THIOSEMICARBAZONES

Thiosemicarbazones have important functions in organic and medicinal chemistry, they are obtained by the condensation reaction between thiosemicarbazide and aldehydes or ketones [13].

Thiosemicarbazones [$R^1R^2C^2=N^3-N^2H-C^1(=S)-N^1HR^3$] represent an interesting class of N, S donor ligands due to their variable donor capacity and structural diversity. Salicylaldene-thiosemicarbazones with O, N, S donor atoms are of considerable interest due to their remarkable structural and biological properties. The interactions between the aromatic rings of the salicylaldehyde thiosemicarbazone ligands are very important in proteins and protein-DNA systems for protein stabilization and various regulatory processes. A number of thiosemicarbazones and copper(II) complexes have been shown to be active in killing tumor cells as well as inhibiting their DNA synthesis. The copper(II) ion is particularly attractive for study because of its rich spectroscopic and magnetic properties, which often change during enzyme catalysis. The thiosemicarbazones of 2-formylpyridine derivatives are also of considerable interest due to their structural (coordinated through the set of NNS donor atoms) and antiproliferative properties. Coordination of thiosemicarbazones to copper(II) is beneficial because it lowers the dose of the minimum inhibitory concentration compared to uncoordinated thiosemicarbazones. Thiosemicarbazones derived from 2-formylpyridine behave as NNS-type donor ligands, they are usually monodeprotonated but can also be neutral. Regardless of the substituent at the terminal nitrogen position $N^{(4)}$ of thiosemicarbazide, the coordination mode of the central atom remains unchanged. The coordination compounds in most cases with 3*d* metals are monomers, and in some cases they dimerize by means of uncoupled electron pairs from donor atoms such as O, N, and S, as in the case of halide atoms (I^-, Br^-, Cl^-). Given the fact that interest in thiosemicarbazones derived from 2-formylpyride is a research objective, their research is of interest regarding the close connection between their biological activity and structure.

According to the study of the specialized literature, thiosemicarbazones with derivatives of 2-formylpyridine or salicylic aldehyde based on ethyl 4-aminobenzoate and their coordination

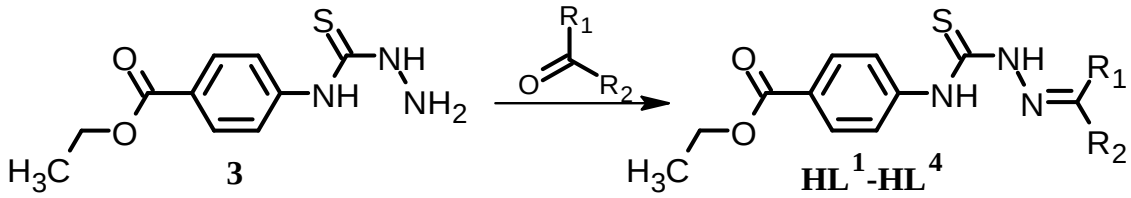
compounds are not described in the literature to date. And so the synthesis and study of their antiproliferative properties are of interest.

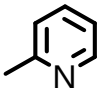
2. METHODS OF SYNTHESIS AND RESEARCH OF COORDINATION COMPOUNDS WITH SOME BIOMETALALS BASED ON THIOSEMICARBAZONES DERIVED FROM ETHYL 4-AMINOBENZOATE

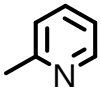
The research took place within the Scientific Research Laboratory "Advanced Biopharmaceutical and Technical Materials" of the Moldova State University. All the reagents and solvents used were of analytical purity, purchased from the following companies: Sigma-Aldrich®, Acros Organics®, Alfa Aesar®, etc.

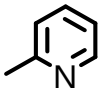
2.1 Synthesis and research of (*p*-ethyl benzoate)thiosemicarbazones of 2-formylpyridine/salicylic aldehyde and their derivatives

The general method for the synthesis of thiosemicarbazones follows the condensation reaction between thiosemicarbazide and the corresponding aldehydes or ketones in ethanol and two to three drops of glacial acetic acid as a catalyst under reflux for one to six hours, according to the reactions in Figures 2.1, 2.2, 2.3, and 2.5.



R_1 = H, R_2 =  noted by **HL**^{1}

R_1 = CH_3, R_2 =  noted by **HL**^{2}

R_1 = C_6H_5, R_2 =  noted by **HL**^{3}

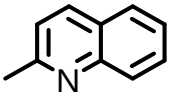
R_1 = H, R_2 =  noted by **HL**^{4}

Figure 2.1. Synthesis scheme of (*p*-ethyl benzoate)thiosemicarbazones of 2-formylpyridine and their derivatives

The total consumption of thiosemicarbazides is verified by thin-layer chromatography. The products obtained are filtered, recrystallized from ethanol, and dried. Thiosesemicarbazones are obtained with yields between 75 and 95%.

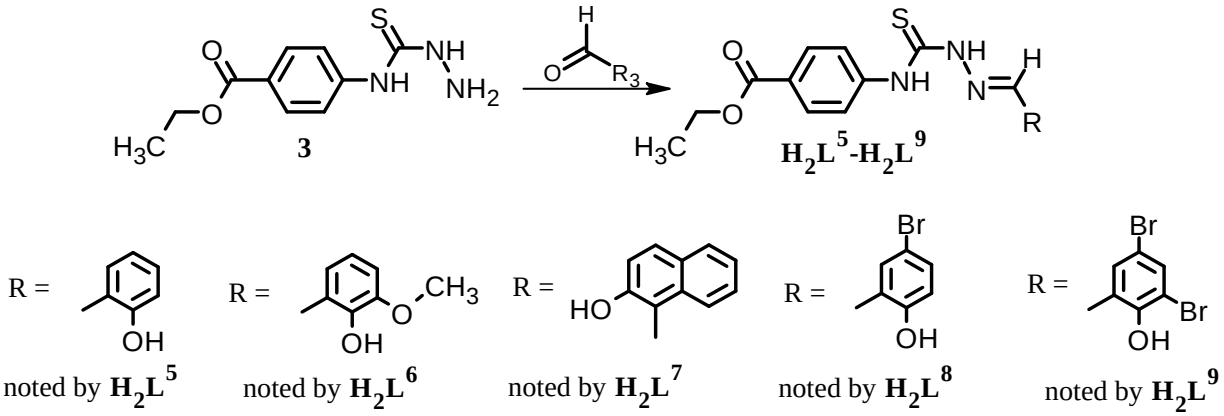


Figure 2.2. Scheme of the synthesis of (*p*-ethyl benzoate)thiosemicarbazones of salicylic aldehyde and their derivatives

2.2 Synthesis, research of (*o*/*m*)-benzoate of ethyl)thiosemicarbazones of 2-formylpyridine and their derivatives

To investigate the structure-activity relationship with respect to the position of the substituents in the benzene ring, the (*o*/*m*)-ethyl benzoate)thiosemicarbazones of 2-formyl(2-acetyl and 2-benzoyl)pyridine were synthesized according to the scheme (Figures 2.5, 2.7).

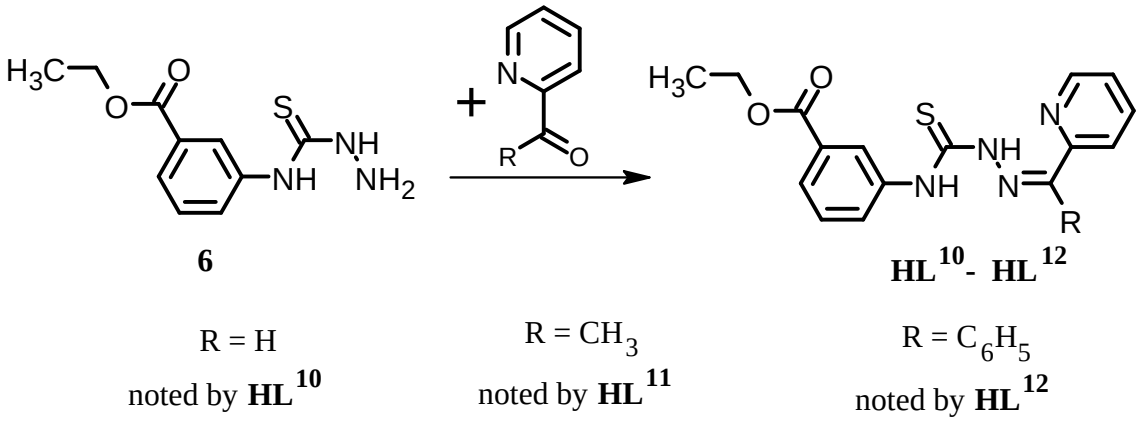
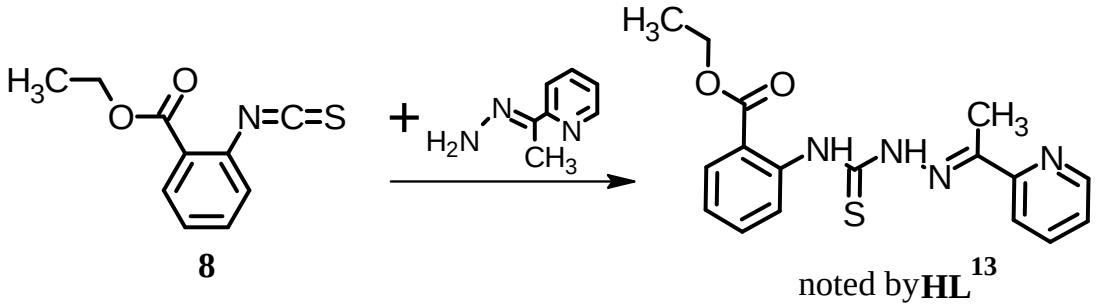


Figure 2.3. Synthesis scheme of (*m*-ethyl benzoate)thiosemicarbazone of 2-formylpyridine and their derivatives

From **ethyl 2-isothiocyanatobenzoate (8)** in the reaction with hydrazine hydrate, it is not possible to obtain thiosemicarbazone due to steric difficulty and heterocyclization of the compound to obtain **3-amino-2-sulfanilidene-2,3-dihydroquinazolin-4(1*H*)-one**. To obtain thiosemicarbazone **HL**[13], the following synthesis method was used: the reaction between isothiocyanate **8** and 2-acetylpyridine hydrazone (Figure 2.4).

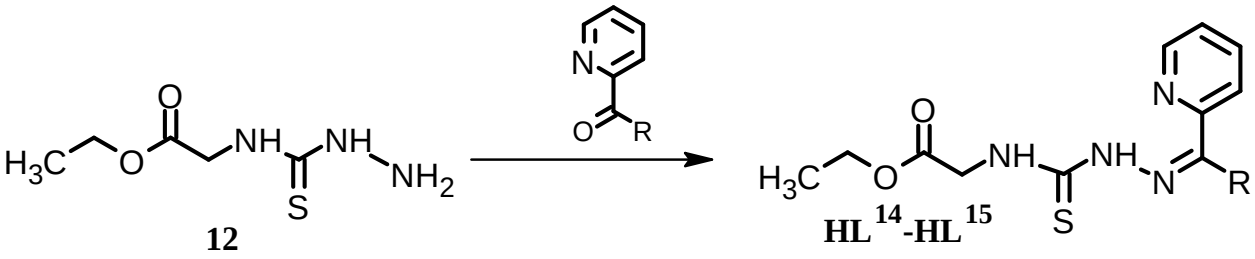


8 noted by **HL**[13]

Figure 2.4. Synthesis scheme of (*o*-ethyl benzoate)thiosemicarbazone of 2-acetylpyridine

2.3 Synthesis and research of the (ethyl acetate)thiosemicarbazones of 2-formylpyridine and 2-acetylpyridine

To observe the influence of the aromatic ring in position 4 of thiosemicarbazones on the biological activity, it was replaced by the –CH_2– group (Figure 2.5).



12 **HL**[14]**-HL**[15]

R = H noted by **HL**[14]

R = CH_3 noted by **HL**[15]

Figure 2.5. Synthesis scheme of ethyl acetate thiosemicarbazones of 2-formylpyridine and 2-acetylpyridine

2.4 Synthesis of coordination compounds of 3*d* metals: Zn(II), Cu(II), Ni(II), Co(III), Fe(III) and Mn(II) with thiosemicarbazones HL^1 – HL^{15}

In order to synthesize the coordination compaunds of Cu(II), Mn(II), Fe(III), Co(III), Ni(II) and Zn(II) as a ligand, we used thiosemicarbazones **HL**[1] – **HL**[15].

The general method of synthesizing coordination compounds. To the mixture consisting of 1 mmol (or 2 mmol) ligand and 20 mL of ethyl alcohol, the mixture consisting of 1 mmol of metal salt and 5 mL of ethyl alcohol is added while heating and stirring. After mixing the solutions, the reaction mixture immediately changes color. Leave to stir and reflux for 0.5 h (T=70-80 ^oC). A precipitate gradually forms. It is then cooled to room temperature, filtered, and dried. The equations of the reactions to obtain the coordination compounds are given below.

$$\mathbf{Cu}X_2 + HL^{1\text{-}15} = [CuL^{1\text{-}15}X] + HX$$

Where X= Cl^-, Br^-, NO_3^-, CH_3COO^-, ClO_4^-

$$\mathbf{M}Cl_2+HL^{1-2} = [MiL^{1-2}Cl] + HCl$$

Unde M= Ni(II), Mn(II), Zn(II)

$$4\mathbf{Co}Cl_2\cdot 6H_2O+8HL^{1-2} +O_2 = 4[Co(L^{1-2})_2Cl] + 4HCl + 26H_2O$$

$$\mathbf{Fe}Cl_3\cdot 6H_2O+2HL^{1-2} = [Fe(L^{1-2})_2Cl] + 2HCl + 6H_2O$$

As we observe following the reactions to obtain the coordination compounds, acid is formed, and its presence was experimentally proven with the help of the pH meter. The solutions obtained after the complexation showed pH values of 1-2 in the case of HCl, HBr, HNO_3, $HClO_4$, and 4-5 in the presence of CH_3COOH.

2.5 Research methods

Within the doctoral thesis, a series of research methods were selected, such as: Nuclear Magnetic Resonance Spectroscopy (NMR) ^1H and ^{13}C; FTIR spectroscopy; Single-crystal X-ray diffraction was applied to determine the structural parameters. The laboratory studies also included analytical analyses, such as: titrimetric analysis of Cu(II), Mn(II), Co(III), Zn(II) and Ni(II); conductometry in solution; determination of melting point; and elemental analysis. In order to determine the applicative potential, some biological properties were researched, such as: the research method of the antiproliferative properties of antitumor cells; the method of studying antimicrobial activities; the method of studying antifungal activities; and the antioxidant activity studies method (ABTS).

3. PHYSICAL-CHEMICAL ANALYSIS OF THE COORDINATION COMPOUNDS BASED ON SOME BIOMETALS WITH THIOSEMICARBAZONES DERIVED FROM ETHYL 4-AMINOBENZOATE

3.1 Investigation of the structure of thiosemicarbazones HL^1-HL^{15} using Nuclear Magnetic Resonance Spectroscopy ^1H-RMN, ^{13}C-RMN

NMR spectroscopy of the thiosemicarbazones HL^1-HL^{15} helps us confirm their structure and composition. In the ^1H NMR spectra, upon integration, we can find a fixed number of protons in the molecule. In the ^{13}C NMR spectra, we can detect the presence of all carbon atoms in the molecule. The displacement of the peaks relative to tetramethylsilam helps us understand how shielded it is and in which place of the molecule it is. The possible isomeric forms (cis, trans) can

be highlighted, such as the tautomeric forms thione and thiol. The ^{13}C spectra (DEPT-135) highlight the carbons bound to two protons.

In most of the thiosemicarbazones obtained in the ^{1}H NMR spectra, solvent ***DMSO-d$_6$***, two tautomeric forms are observed. When integrating the peaks at 14.08 ppm, ^{1}H(S-H), they are 1-50% thiol tautomeric form, and when integrating the peak at 10.90 ppm, s, $^{1}H(N^{2}$-H), 50-99% form thione tautomer. Examples of tautomeric forms of HL^1 and HL^2 ligands are given below (Figure 3.1).

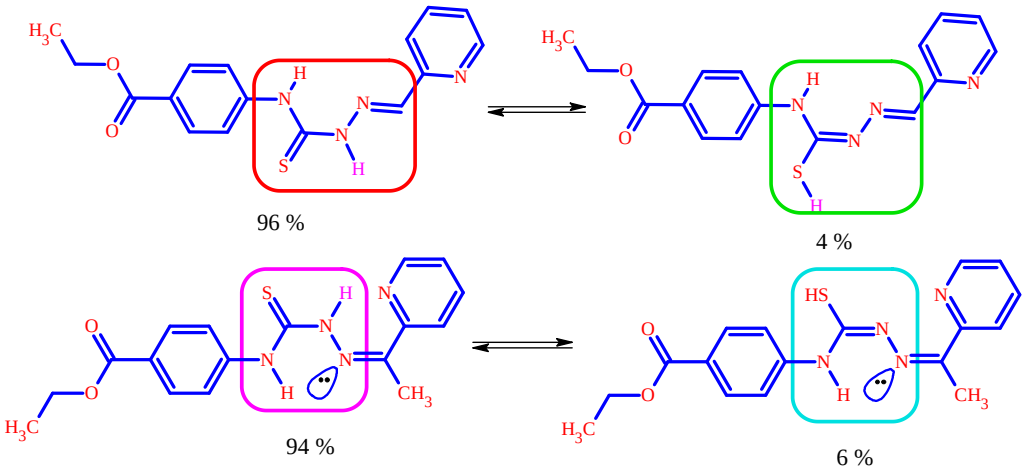


Figure 3.1. Tautomeric forms of thiosemicarbazones HL^1 și HL^2

3.2 IR investigation of coordination compounds and thiosemicarbazones HL^1-HL^{15}

The IR spectroscopy of thiosemicarbazones completes the NMR spectroscopy, where we can identify moments such as substitution in the benzene ring and the presence of functional groups such as the carbonyl and acetyl groups.

IR spectroscopy of the complexes synthesized based on thiosemicarbazones HL^1-HL^{15}, helps us understand the formation of new bonds by superimposing (comparing) the spectra of thiosemicarbazones and their coordination compounds. We can also detect the displacement or disappearance of some groups following coordination.

Table 3.1. Data of the analysis in the field of IR of the coordination compound in the base (*p*-ethyl benzoate) thiosemicarbazones of 2-formylpyridine and 2-acetylpyridine (HL^1, HL^2)

Nr.	Compound	IR(cm^{-1})					
		ν $N^{(Py/N1)}\rightarrow Cu$ / (S-Cu)	ρ (Py)	ν (C=S)	ν (C-S)	ν (=N-N=)	ν (C=N) azometin
HL^1		-/-	625	1310	-	-	1592
C1	$[Cu(L^1)Cl]$	546/413	621	-	899	1027	1605
C2	$[Cu(L^1)Br]$	456/410	615	-	892	1026	1606
C3	$[Cu(L^1)NO_3(H_2O)]$	557/416	617	-	895	1025	1607

Continuation Table 3.1)							
C4	$[Cu(L^1)CH_3COO(H_2O)]$	456/413	620	-	899	1029	1598
C5	$[Cu(L^1)(H_2O)]ClO_4$	449/416	618	-	893	1030	1602
C6	$[Ni(L^1)_2]$	513/410	635	-	903	1062	1598
C7	$[Co(L^1)_2Cl]$	469/430	645	-	867	1049	1593
C8	$[Fe(L^1)_2Cl]$	498/410	621	-	839	1054	1584
C9	$[Mn(L^1)Cl]$	517/431	634	-	879	1063	1571
C10	$[Zn(L^1)Cl]$	521/439	632	-	831	1064	1563
HL^2		-/-	567	1313	-	-	1606
C11	$[Cu(L^2)Cl]$	517/418	632	-	827	1046	1592
C12	$[Cu(L^2)Br]$	489/410	630	-	829	1049	1595
C13	$[Cu(L^2)NO_3]$	518/419	628	-	828	1045	1593
C14	$[Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O$	507/421	631	-	827	1047	1588
C15	$[Cu(H_2O)(L^2)]ClO_4$	512/414	633	-	826	1045	1593

The absorption bands in the IR range of the complexes synthesized based on thiosemicarbazones of 2-formylpyridine and its derivatives (**C1-C22**, **C28-C33**) suggest that the azomethine functional group ν(C=N, 1600 cm^{-1}) moves to wave numbers higher compared to the non-coordinated ligand ν(C=N, 1592 cm^{-1}), new bands appear ν(N→Cu, S-Cu, C-S, =N-N=, 470, 419, 830, 1026 cm^{-1}), missing in the ligand spectrum, ν(pyridine ring, 624, cm^{-1}) shifts to lower or higher wavenumbers. According to the data obtained, we can assume that the ligands coordinate tridentately to the metal, coordinating to the central atom through the pyridinic nitrogen, azomethine nitrogen, and thiolic sulfur atoms, forming two metallocycles of 5 atoms.

The absorption bands in the IR range of the complexes synthesized based on the thiosemicarbazones of salicylic aldehyde and its derivatives (**C23-C27**) suggest that the azomethine functional group ν(C=N, 1598 cm^{-1}) moves to higher wave numbers compared to the uncoordinated ligand ν(C=N, 1585 cm^{-1}), new bands ν(O-Cu, N→Cu, S-Cu at 545, 472, 436 cm^{-1}) appear that are missing in the spectrum of the ligand, ν(C= S, 1250, cm^{-1}) shifts to lower wavenumbers compared to the uncoordinated ligand ν(C=S, 1259, cm^{-1}). The absorption band ν(O-H, 3383 cm^{-1}) disappears from the spectra of the coordination combinations, which speaks of its deprotonation. According to the data obtained, we can assume that the ligand coordinates tridentately to the metal, through the sulfur thiolic atom, the azomethine nitrogen atom and the oxygen phenolic atom. forming two metallocycles one of 5 atoms and one of 6 atoms.

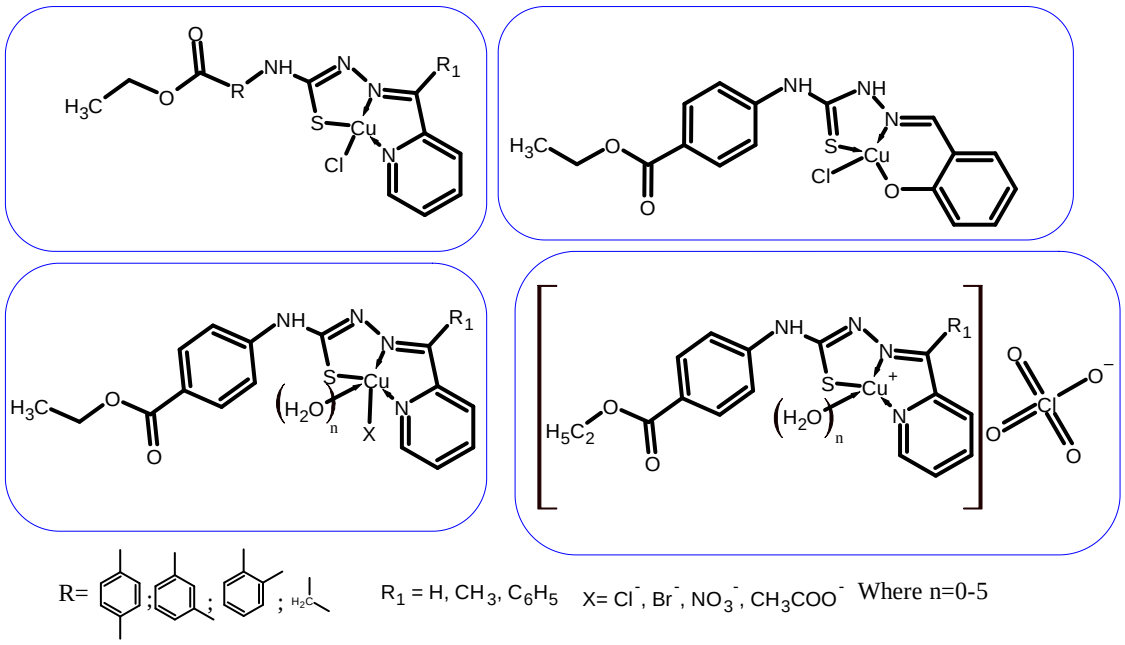
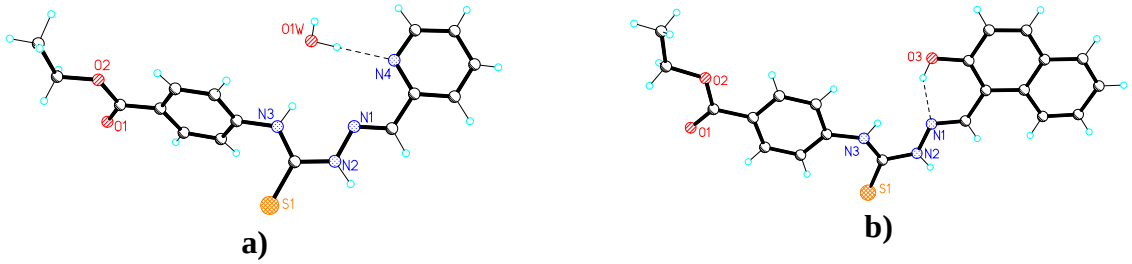


Figure 3.2. Modes of coordination of thiosemicarbazones based on ethyl 4-aminobenzoate to the complex generator (Cu^{2+})

3.3. Study of the crystal structure of coordination compounds based on thiosemicarbazones (HL^1-HL^{15}) using single crystal X-ray diffraction analysis

X-ray diffraction on a single crystal is used for the characteristic elucidation of the molecule by identifying the interatomic distances, their valence angles, and the unit cell parameters. So, elucidating the molecular structure and determining the crystal packing of these molecules. In the case of the study of coordination compounds with paramagnetic metal ions, X-ray diffraction on a single crystal is the best and most accurate method for structural identification of the molecule. Using this method, molecular structures of 3 precursors of thiosemicarbazones were obtained for the first time: ethyl-4-[(dimethylcarbamothioyl)amino]benzoate (**2a**), ethyl-4-[(hydrazinylcarbonothioyl)amino]benzoate (**3**) and ethyl[(hydrazinecarbothioyl)amino]acetate (**24**) upon recrystallization from ethanol. The molecules are practically planar.



a) **b)**

Figure 3.2. Molecular structures of: a) ethyl 4-({2-[(pyridin-2-yl)methylidene]hydrazinecarbothioyl}amino)benzoate ($HL^1 \cdot H_2O$), b) ethyl 4-({-2-[(2-hydroxynaphthalen-1-yl)methylidene]hydrazinecarbothioyl}amino)benzoate (H_2L^7)

When recrystallization of thiosemicarbazones HL^1-HL^{15} from ethanol, it was possible to obtain single crystals in nine cases.

At (*p*-ethyl benzoate)thiosemicarbazone of 2-formylpyridine HL^1 we observe the trans configuration of the azomethine group (Figure 3.2, a). The formation of pseudo-macrocycles with the entrainment of water molecules. In (*p*-ethyl benzoate)thiosemicarbazone of 2-acetylpyridine HL^2 is the cis configuration to the azomethine group, additionally, the hydrogen atom from the hydrazinic nitrogen N^2 participates in the formation of the hydrogen bond with the pyridinic nitrogen N^4, which stabilizes this configuration. The association of the molecules of the HL^2 compound in the crystal occurs through C-H...S hydrogen bonds. In the case of (*p*-ethyl benzoate)thiosemicarbazone of 2-benzoylpyridine HL_3 we observe the cis configuration with respect to the azomethine group, additionally, the hydrogen atom from the hydrazinic nitrogen N^2 participates in the formation of the hydrogen bond with the pyridinic nitrogen N_4, which stabilizes this configuration. Formation of chains by means of C-H...O fine bonds. In the (p-ethyl benzoate)thiosemicarbazone of 2-formylquinoline HL^4, we observe the configuration trans to the azomethine group. The formation of chains by hydrogen bonds N-H…N and their association in layers by hydrogen bonds C-H…O. At the (*p*-ethyl benzoate)thiosemicarbazone of 2-hydroxynaphthaldehyde H_2L^7, we observe the trans configuration at the azomethine group stabilized by the intramolecular hydrogen bond O^3-H...N^1. The association of molecules of the compound H_2L^7 in the crystal occurs through hydrogen bonds C-H...S, N-H...S (Figure 3.2, b).

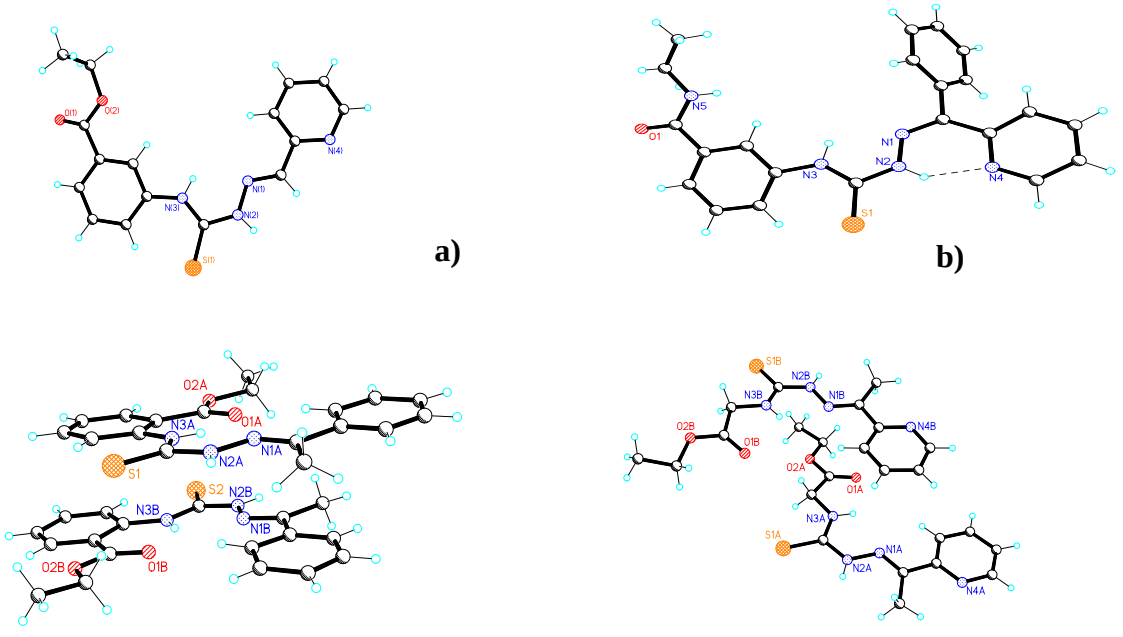


Figure 3.3. Molecular structures of: a) ethyl 3-({2-[(pyridin-2-yl)methylidene]hydrazinecarbothioyl}amino) benzoate (HL^{10}), b) ethyl 3-({2-[phenyl(pyridin-2-yl) methylidene]hydrazinecarbothioyl}amino) benzoate (HL^{12}), c) ethyl 2-({2-[1-(pyridin-2-yl)ethylidene]hydrazinecarbothioyl}amino)benzoate (HL^{13}), d) ethyl ({2-[1-(pyridin-2-yl)ethylidene]hydrazinecarbothioyl}amino)acetate (HL^{15})

In the (*m*-ethyl benzoate)thiosemicarbazone of 2-formylpyridine HL^{10} we observe the configuration trans to the azomethine group. The association of the molecules of the HL^{10} compound in the crystal occurs through N-H...N hydrogen bonds (Figure 3.3, a). At the (*m*-ethyl benzoate)thiosemicarbazone of 2-benzoylpyridine HL^{12}, we observe the cis configuration to the azomethine group, additionally, the hydrogen atom from the hydrazinic nitrogen N^2 participates in the formation of the hydrogen bond with the pyridinic nitrogen N^4, which stabilizes this configuration. In the fragment of the HL^{12} crystal structure, the formation of centrosymmetric dimers occurs through fine hydrogen bonds of the C-H...S type (Figure 3.3, b). In the (*o*-ethyl benzoate)thiosemicarbazone of 2-formylpyridine (HL^{13}), we observe the configuration trans to the azomethine group. The packing takes place by the formation of centrosymmetric dimers through N-H...S and C-H...S hydrogen bonds (Figure 3.3, c). In the (ethyl acetate)thiosemicarbazone of 2-acetylpyridine (HL^{15}) we observe the configuration trans to the azomethine group. The packing takes place by the formation of dimers of the molecules by means of hydrogen bonds N-H...S and C-H..S. Association in chains by means of fine bonds of the C-H...O type (Figure 3.3, d).

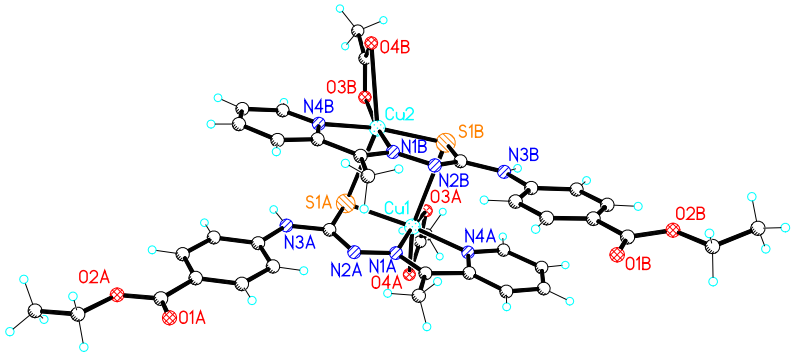


Figure 3.4. The structure of the binuclear coordination compound of $[Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O$ (C14)

Upon recrystallization of the coordination compound $[Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O$ (**C14**) from the ethanolic solution, single crystals were obtained, which were analyzed with the help of single crystal X-ray diffraction. The coordination compound **C14** represents a dimer with coordination number 6, having an octahedral geometry at the central atom. Three coordination positions are assigned to the ligand via the pyridinic nitrogen atom, the azomethine nitrogen atom, and the thiol sulfur atom. Coordination positions 4 and 5 are occupied by oxygen atoms from the acetate that coordinate bidentately to the central atom. By means of non-participating pairs of the sulfur atom of the neighboring ligand molecule, the bridge of the two complex-generating centers is formed. Association in the crystal by means of crystallization of water molecules.

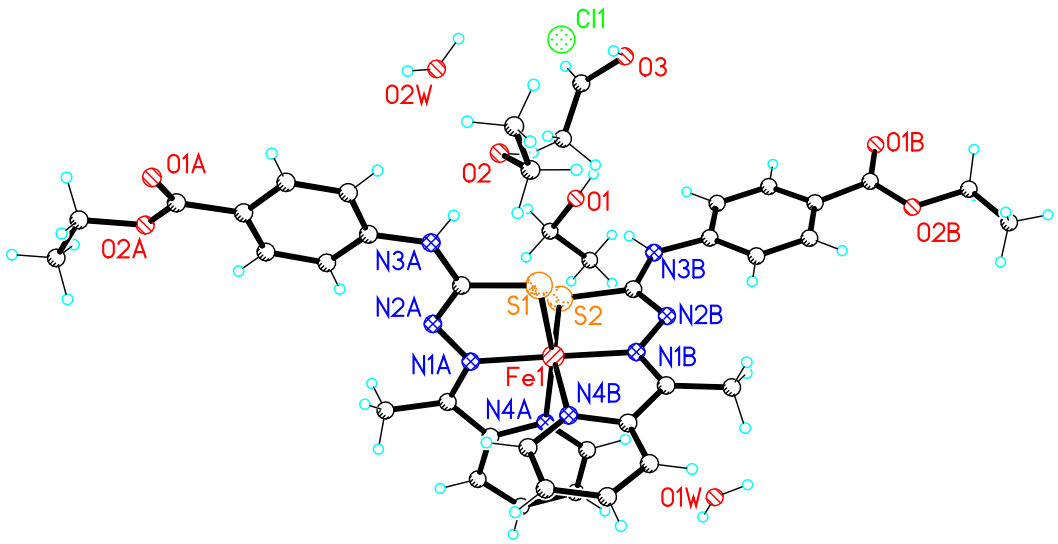


Figure 3.5. Crystal structure of the coordination compound $[Fe(L^2)_2]Cl \cdot 3C_2H_5OH \cdot 2H_2O$ (C18)

Also, upon recrystallization from the ethanolic solution of the complex $[Fe(L^2)_2]Cl \cdot 3C_2H_5OH \cdot 2H_2O$, single crystals were obtained, which were analyzed using X-ray diffraction on the single crystal. The coordination compound has coordination number 6, octahedral geometry. At the central atom, it coordinates two ligand molecules through the pyridinic nitrogen atom, the azomethine nitrogen, and the thiolic sulfur atom. The chloride anion is in the outer sphere.

4. BIOLOGICAL PROPERTIES OF THE COORDINATION COMPOUNDS BASED ON THIOSEMICABAZONES HL^1-HL^{15}

After the synthesis of the substances, the research and confirmation of their structure follows the last chapter with the results of the biological study of the synthesized compounds, namely the study of antioxidant, antibacterial, antifungal, and antitumor properties.

4.1 Antioxidant properties of coordination compounds based on 3d metal ions and (*p*(*m*,*o*)-ethyl benzoate) / (ethyl acetate)thiosemicarbazones of 2-formylpyridine/salicylic aldehyde and their derivatives

The antioxidant properties of 15 thiosemicarbazones (HL^1-HL^{15}) and 33 complexes synthesized using the ABTS method were investigated, the results obtained are presented in (Table 4.1).

Table 4.1. The antioxidant properties of coordination compounds based on thiosemicarbazones (HL^1, HL^2) investigated by the ABTS method

No.	Compound	IC_{50}, ±SD μM
HL^1		14.73±0.43
C 1	$[Cu(L^1)Cl]$	**6.56**±0.19
C 2	$[Cu(L^1)Br]$	**9.14**±0.25
C 3	$[Cu(L^1)NO_3]$	**8.03**±0.23
C 4	$[Cu(H_2O)(L^1)CH_3COO]$	11.35±0.33
C 5	$[Cu(H_2O)(L^1)]ClO_4$	13.39±0.40
C 6	$[Ni(L^1)Cl]$	**3.70**±0.10
C 7	$[Co(L^1)_2]Cl$	>100
C 8	$[Fe(L^1)_2]Cl$	>100
C 9	$[Mn(L^1)_2]$	14.00±0.38
C 10	$[Zn(L^1)Cl]$	23.41±0.57
HL^2		15.85±0.44
C 11	$[Cu(L^2)Cl]$	74.46±1.70
C 12	$[Cu(L^2)Br]$	33.51±1.00
C 13	$[Cu(L^2)NO_3]$	83.32±2.38
C 14	$[Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O$	≥100
C 15	$[Cu(H_2O)(L^2)]ClO_4$	>100
Trolox		**33.3±0.90**

Note: IC_{50} - half-maximal inhibitory concentration. * SD- standard deviation, (%). Data expressed as mean value of 3 measurements ± SD.

The antioxidant properties of thiosemicarbazones HL^1-HL^{15} after half-maximal inhibitory concentration (IC_{50}) of the $ABTS^{\cdot+}$ radical cation, decrease in the series:

H_2L^7>HL^{11}>HL^3>HL^{10}>HL^1>HL^2>HL^{12}>H_2L^5>H_2L^6>HL^{13}>HL^{15}>HL^{14}>H_2L^8>H_2L^9>HL^4

We observe from the series above that the most pronounced antioxidant properties are possessed by H_2L^7 (*p*-ethyl benzoate)thiosemicarbazone of 2-hydroxynaphthaldehyde, whose IC_{50} is equal to 9.38 μM. This activity is possibly due to the presence of –OH and –SH groups present in the structure of the molecule, where the hydrogen atom is more labile and can inhibit the free radicals of the oxidizing compounds. H_2L^7 is three times more pronounced an antioxidant than the comparator substance, trolox (is a hydrophilic and cell-permeable analog of vitamin E, is a potent antioxidant used in biochemical applications to reduce oxidative stress, and is the standard antioxidant used in antioxidant capacity assays), whose IC_{50} is equal to 33.3 μM.

If we change position N^1 of thiosemicarbazine (derivatives of 2-formylpyridine) and keep position N^4 (p-ethyl benzoate) unchanged, we obtain the following series of decreasing antioxidant properties: HL^3>HL^1>HL^2>>>HL^4 so correspondingly (*p*-benzoate of ethyl)thiosemicarbazone 2-benzoyl/2-formyl/2-acetyl-pyridine/2-formylquinoline, with IC_{50} 11.94, 14.73, 15.85, 100 μM. If

we follow the series of thiosemicarbazones of salicylic aldehyde derivatives and keep the N^4 position (ethyl p-benzoate) unchanged, we obtain the series H_2L^7>H_2L^5>H_2L^6>H_2L^8>H_2L^9 whose corresponding IC_{50} is 9.38, 16.74, 17.41, 81.91 μM.

If we keep the N^4 position (ethyl *m*-benzoate) of the thiosemicarbazones unchanged and change the thiosemicarbazone to the N^1 position, we obtain the following series: HL^{11}>HL^{10}>HL^{12}, corresponding to (2-acetyl, 2-formyl, 2-benzoyl)pyridine, with IC_{50} =11.53, 13.15, 16.47 μM. If we follow the N^4 position of ethyl benzoate in the series of thiosemicarbazones: ortho, meta, para, and the substitution of the aromatic ring with -CH_2-, the most active is the meta derivative (HL^{11}), followed by para (HL^2), then ortho (HL^{13}), and if the benzene ring is replaced by -CH_2-, its activity decreases even more (HL^{15}).

When complexing ligands with 3*d* metals, the antioxidant properties in some cases are more pronounced, and in others they probably disappear due to the formation of S-M, O-M coordination bonds (M, – 3*d* metal) and the disappearance of S-H, O-H groups, for example at HL^2>>>[$Cu(L^2)Cl$], H_2L^7>>>[$Cu(HL^7)Cl$].

If we follow the antioxidant properties of the coordination compounds of the HL^1 ligand (Table 4.1, **C1 - C10**) where the coordination compounds have more pronounced antioxidant activities than the free ligand, we obtain the series:

[$Ni(L^1)Cl$] > [$Cu(L^1)Cl$] > [$Cu(L^1)NO_3$] > [$Cu(L^1)Br$] > [$Cu(H_2O)(L^1)CH_3COO$]>

[$Cu(H_2O)(L^1)$]ClO_4 > [$Mn(L^1)_2$] > [$Zn(L^1)Cl$] > [$Fe(L^1)_2$]Cl > [$Co(L^1)_2$]Cl

The most active is [$Ni(L^1)Cl$] with an IC_{50} = **3.70** μM, which is 4 times more active than the HL^1 ligand and 9 times more active than trolox. The following in the series are copper(II) complexes, and depending on the anion, they have different antioxidant properties: Cl^- > NO^{3-} > Br^- > CH_3COO^- > ClO^{4-}, corresponding IC_{50} = 6.56, 8.03, 9.14, 11.35, 13.39 μM, the first is twice as active as the ligand, and the last possesses almost the same activity. Next in the series are the complexes [$Mn(L^1)_2$] > [$Zn(L^1)Cl$], which have corresponding IC_{50} = 14.00, 23.41 μM. The latter are [$Fe(L^1)_2$]Cl > [$Co(L^1)_2$]Cl, where it does not possess antioxidant activity.

So the most active coordination compounds are based on Ni(II) and Cu(II), the most suitable anion in the complex is Cl^-.

4.2. Antibacterial and antifungal properties of the coordinative compounds based on thiosemicarbazones HL^1-HL^{15}

1) Results of the study of the antibacterial properties of the synthesized compounds.

Thirty-three coordination compounds based on fifteen thiosemicarbazones (HL^1–HL^{15}) were investigated for bacteriostatic and bactericidal activities against *Staphylococcus aureus (G+), Bacillus cereus (G+), Escherichia coli (G-) și Acinetobacter bamannii (G-).*

Table 4.2. Bacteriostatic and bactericidal activities of coordination compounds based on thiosemicarbazones (HL^1, HL^2)

No.	Compound	MIC/MBC μg/mL			
		***S. aureus* ATCC 25923**	***B. cereus* ATCC 11778**	***E. coli* ATCC 25922**	***A. baumannii* BAA-747**
HL^1		>500/>500	>500/>500	>500/>500	>500/>500
C 1	$[Cu(L^1)Cl]$	**0.12/0.24**	0.24/0.49	250/500	62.50/125
C 2	$[Cu(L^1)Br]$	0.49/0.98	0.49/0.98	31.25/62.50	62.50/125
C 3	$[Cu(L^1)NO_3]$	0.24/0.49	0.24/0.49	125/250	125/250
C 4	$[Cu(H_2O)(L^1)CH_3COO]$	0.24/0.49	0.24/0.49	250/500	125/250
C 5	$[Cu(H_2O)(L^1)]ClO_4$	0.49/0.98	0.49/0.98	250/500	125/250
C 6	$[Ni(L^1)Cl]$	>500/>500	>500/>500	>500/>500	250/500
C 7	$[Co(L^1)_2]Cl$	15.63/15.63	15.63/31.25	>500/>500	>500/>500
C 8	$[Fe(L^1)_2]Cl$	>500/>500	>500/>500	>500/>500	>500/>500
C 9	$[Mn(L^1)_2]$	>500/>500	>500/>500	>500/>500	>500/>500
C 10	$[Zn(L^1)Cl]$	>500/>500	>500/>500	>500/>500	>500/>500
HL^2		125/>500	125/500	>500/>500	>500/>500
C 11	$[Cu(L^2)Cl]$	0.24/0.49	0.24/0.49	>500/>500	250/500
C 12	$[Cu(L^2)Br]$	**0.12/0.24**	0.24/0.49	>500/>500	250/500
C 13	$[Cu(L^2)NO_3]$	31.25/31.25	15.63/31.25	>500/>500	>500/>500
C 14	$[Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O$	0.24/0.49	0.24/0.49	>500/>500	250/500
C 15	$[Cu(H_2O)(L^2)]ClO_4$	3.91/15.63	7.81/15.63	>500/>500	>500/>500
Furacilin		4.67/9.35	4.67/9.35	4.67/4.67	4.67/9.35

** MIC - minimum inhibitory concentration; ** MBC - minimum bactericidal concentration.*

Uncoordinated thiosemicarbazones have almost no antimicrobial properties. Upon complexation, the antimicrobial activities of coordination compounds are much higher than those of non-coordinating ligands, for example $[Cu(L^1)Cl]$ >>>HL1 (table 4.2). *The best results were recorded on the gram-positive bacteria Staphylococcus aureus of $[Cu(L^1)Cl]$ and $[Cu(HL^2)Br]\cdot H_2O$, whose MIC is 0.12 μg/mL and is 40 times lower than that of the the medicine Furacillin* given for comparison, which is used in medicine (table 4.2). Research on the structure-activity relationship, namely the coordination compounds of Cu(II) with thiosemicarbazones of 2-formylpyridine and their derivatives, demonstrated that the most active ones are based on the HL^1 ligand (ethyl *p*-benzoate) 2-formylpyridine thiosemicarbazone.

In the case of changing the influence of anions in coordination compounds from the HL^1 ligand series on strains of *Staphylococcus aureus* microorganisms, the most active coordination

compounds are in the series $[Cu(L^1)Cl] > [Cu(L^1)NO_3] = [Cu(H_2O)(L^1)CH_3COO] > [Cu(L^1)Br] = [Cu(H_2O)(L^1)ClO_4]$, whose MIC is correspondingly 0.12, 0.24, 0.24, 0.49, 0.49 μg/mL.

On strains of gram-negative microorganisms *E. coli*, the most active coordination compound is $[Cu(L^1)Br]$. Depending on the anion in the complex, the following decreasing series is obtained: $[Cu(L^1)Br] > [Cu(L^1)NO_3] > [Cu(L^1)Cl] = [Cu(H_2O)(L^1)CH_3COO] = [Cu(H_2O)(L^1)ClO_4]$ whose MIC is corresponding to 31.25, 125, 250, 250, 250 μg/mL.

If we change the central atom in the complex from Cu(II) to Ni(II), in the case of the complexes $[Cu(L^1)Cl]>>>[Ni(L^1)Cl]$, the antibacterial activity on *Staphylococcus aureus and E. coli* disappears, and in the case of *Acinetobacter baumannii*, it decreases 4 times. In the case of $[Cu(L^2)Cl]>>>[Ni(L^2)Cl]$ complexes, the antibacterial activity on *Staphylococcus aureus* decreases 15 times. If we change the central atom in the complex from Cu(II) to Co(III) in the case of $[Cu(L^1)Cl]>>>[Co(L^1)_2]Cl$ complexes, the antibacterial activity on Staphylococcus aureus decreases 130 times. If we replace it with Fe(III), Mn(II), or Zn(II) the antibacterial activity disappears. If we compare the antibacterial results of the coordination compounds based on (p-ethyl benzoate) thiosemicarbazone 2-formylpyridine and its derivatives with (p-ethyl benzoate) thiosemicarbazone salicylic aldehyde and its derivatives on gram-positive microorganisms *Staphylococcus aureus* and *Bacillus cereus*, we notice that the coordination compounds of Cu(II) with (p-ethyl benzoate)thiosemicarbazone-2-formylpyridine and its derivatives are more active.

The change of the N^4 position (*p*-ethyl benzoate) of the thiosemicarbazone of 2-acetipyridine in coordination combinations, from *para* **C11** to *meta* **C29**, *ortho* **C31**, or the substitution of the benzene ring with the methylene group (-CH_2-) **C33**, leads to a decrease in the antibacterial activity on gram-positive microorganisms *Staphylococcus aureus*, namely in the following sequence: **C11**>**C33**>**C29**>**C31**.

2) Results of the study of the antifungal properties of the synthesized compounds.

Thirty-three coordination compounds (**C1**–**C33**) based on fifteen thiosemicarbazones (HL^1–HL^{15}) were investigated for their antifungal activities against *Candida albicans* and *Candida krusei.* The results of these studies are given in (Table 4.3).

Table 4.3. Antifungal activities of coordination compounds based on thiosemicarbazones HL^1, HL^2

No.	**Compound**	**MIC/MFC** (μg/mL)	
		***Candida albicans* ATCC 10231**	***Candida krusei* ATCC 6258**
HL^1		125/250	125/250
C 1	$[Cu(L^1)Cl]$	3.91/125	0.98/1.95
C 2	$[Cu(L^1)Br]$	3.91/15.63	7.81/15.63

(Continuation Table 4.3)			
C 3	$[Cu(L^1)NO_3]$	0.98/1.95	7.81/15.63
C 4	$[Cu(H_2O)(L^1)CH_3COO]$	1.95/3.91	3.91/7.81
C 5	$[Cu(H_2O)(L^1)]ClO_4$	3.91/7.81	31.25/62.50
C 6	$[Ni(L^1)Cl]$	>500/>500	>500/>500
C 7	$[Co(L^1)_2]Cl$	31.25/125	>500/>500
C 8	$[Fe(L^1)_2]Cl$	>500/>500	>500/>500
C 9	$[Mn(L^1)_2]$	500/>500	>500/>500
C 10	$[Zn(L^1)Cl]$	>500/>500	>500/>500
HL^2		15.63/31.25	15.63/31.25
C 11	$[Cu(L^2)Cl]$	0.98/1.95	1.95/3.91
C 12	$[Cu(L^2)Br]$	1.95/7.81	0.98/1.95
C 13	$[Cu(L^2)NO_3]$	125/250	125/250
C 14	$[Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O$	0.98/1.95	7.81/15.63
C 15	$[Cu(H_2O)(L^2)]ClO_4$	62.5/125	62.5/125
Nistatina		32/64	32/64

** MIC - minimum inhibitory concentration; ** MFC - minimum fungicidal concentration*

The antifungal properties of thiosemicarbazones are moderate, the best result was recorded by **HL^2** (ethyl *p*-benzoate)thiosemicarbazone-2-acetylpyridine, whose MIC on Candida albicans is 15.63 μg/mL, and is twice as effective as the comparison substance Nystatin (used in medicine as an antifungal drug), whose MIC is 32 μg/mL. Upon coordination, the antifungal properties, as well as the antibacterial properties, are much higher compared to the non-coordinated ligand, for example $[Cu(L^1)Cl]>>>HL^1$, whose corresponding MIC 3.91>>>125 μg/mL (Table 4.3).

The best results of antifungal activity on *Candida albicans* were recorded for the coordination compounds $[Cu(L^1)NO_3]$, $[Cu(L^2)Cl]$, and $[Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O$, whose MIC is 0.98 μg/mL and are 33 times more effective than the comparison substance Nystatin. If we follow the series of coordination compounds of Cu(II) based on the **HL^1** ligand, the influence of the anion on the antifungal properties on Candida albicans, we obtain the following series: $[Cu(L^1)NO_3] > [Cu(H_2O)(L^1)CH_3COO] > [Cu(H_2O)(L^1)]ClO_4 > [Cu(L^1)Br] > [Cu(L^1)Cl]$.

If we follow the series of coordination compounds of Cu(II) based on the **HL^2** ligand, the influence of the anion on the antifungal properties on *Candida albicans* here in the first place are the anions of Cl^- and CH_3COO^-, according to the following series of decreasing antifungal properties: $[Cu(L^2)Cl] = [Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O > [Cu(L^2)Br] > [Cu(H_2O)(L^2)]ClO_4 > [Cu(L^2)NO_3]$.

The best results of antifungal activity on *Candida krusei* were recorded for the coordination compounds $[Cu(L^1)Cl]$ and $[Cu(L^2)Br]$, whose MIC is 0.98 μg/mL and is 33 times lower in value, so it is 33 times more effective than the comparison substance Nystatin, whose

MIC is 32 µg/mL. If we follow the series of coordination compounds of Cu(II) based on the **HL^1** ligand and the influence of the anion on the antifungal properties on *Candida krusei*, we obtain the following series: [Cu(L^1)Cl] > [Cu(H$_2$O)(L^1)CH$_3$COO] > [Cu(L^1)Br] = [Cu(L^1)NO$_3$] > [Cu(H$_2$O)(L^1)]ClO$_4$. And for coordination compounds based on the **HL^2** ligand, we obtain the following series: [Cu(L^2)Br] > [Cu(L^2)Cl] > [Cu$_2$(L^2)$_2$(CH$_3$COO)$_2$]5H$_2$O > [Cu(H$_2$O)(L^2)] ClO$_4$ > [Cu(L^2)NO$_3$]. If we change the central atom in the complex, from Cu(II) to Ni(II), Co(III), Fe(III), Mn(II) or Zn(II), the antifungal activity decreases considerably.

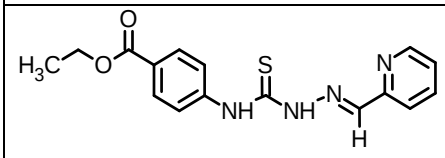
If we compare the antifungal results of the coordination compounds based on (*p*-ethyl benzoate) thiosemicarbazone of 2-formylpyridine and its derivatives with (*p*-ethyl benzoate) thiosemicarbazone of salicylic aldehyde, we notice that the coordination compounds of Cu(II) based on (*p*-benzoate of ethyl)thiosemicarbazone-2-formylpyridines possess much more pronounced antifungal properties.

If we change the fragment N^4 (p-ethyl benzoate) of the 2-acetipyridine thiosemicarbazone from coordination compounds, from *para* **C11** to *meta* **C29**, *ortho* **C31**, or the substitution of the benzene ring with the (-CH$_2$-) **C33** group, it leads to a decrease in the antifungal activity on *Candida albicans* and *Candida krusei*, namely in the following sequence: **C11**>**C33**>**C29**>**C31**.

4.3. Investigation on the anticancer activity of the coordination compounds of Cu(II) based on (*p*-ethyl benzoate)thiosemicarbazones of 2-formylpyridine and salicylic aldehyde

Anticancer properties (*p*-ethyl benzoate)thiosemicarbazone of 2-formylpyridine (**HL^1**) were investigated on human myeloid leukemia HL-60 cells.

Tabel 4.4. Data on antiproliferative activities of thiosemicarbazone HL^1 on HL-60 (human myeloid leukemia) cells

Compound	Concentration, mol/L			IC$_{50}$, µmol/L
	10^{-5}	10^{-6}	10^{-7}	
 **HL^1**	%			0.1
	100.0	85.1	78.2	

The experimental data obtained regarding the study of the anticancer properties of (ethyl *p*-benzoate) thiosemicarbazone 2-formylpyridine are presented in Table 4.4, from which it can be

seen that at a concentration of 10^{-5} M, it inhibits the growth and multiplication of 100.0%, at a concentration of 10^{-6} M, 85.1%, and at a concentration of 10^{-7} M, 78.2% of HL-60 cells of human myeloid leukemia. This compound has an IC_{50} equal to 0.1 μmol/L, according to its anticancer activity, it is three times more effective than doxorubicin (used in medicine) and 250 times more active than *cis*-platin. This data has been patented.

Six coordination compounds of Cu(II) based on (*p*-ethyl benzoate) thiosemicarbazone of 2-formylpyridine (**HL^1**) and (*p*-ethyl benzoate)thiosemicarbazone of salicylic aldehyde (**H_2L^5**) were investigated for anticancer activities on cervical cancer cells (HeLa), pancreatic cancer cells (BxPC-3), muscle cancer cells (TC-1), and totoxicity was also studied on MDCK dog kidney epidemic cells, as control was taken *cis*-platinum (cytostatic) used in medicine. The results are shown below:

Table 4.5. Anticancer activity of coordination compounds of Cu(II) with (*p*-ethyl benzoate)thiosemicarbazone of 2-formylpyridine (HL^1) and (*p*-ethyl benzoate)thiosemicarbazone of salicylic aldehyde (H_2L^5)

No.	Compound	IC_{50}, μM						
		MDCK	HeLa	SI*	BxPC-3	SI	TC-1	SI
	cis-platinum	30.9	4.0	7.7	11.2	2.8	4.6	6.7
C1	$[Cu(L^1)Cl]$	**6.5**	4.7	1.4	1.1	5.9	**0.3**	**21.7**
C2	$[Cu(L^1)Br]$	1.0	1.1	0.9	0.5	2.0	0.3	3.3
C3	$[Cu(L^1)NO_3]$	0.9	0.9	1.0	0.4	2.3	0.5	1.8
C4	$[Cu(H_2O)(L^1)CH_3COO]$	1.1	1.3	0.8	0.5	2.2	0.5	2.2
C5	$[Cu(H_2O)(L^1)]ClO_4$	**7.7**	3.8	2.0	**1.1**	**7.0**	0.6	12.8
C23	$[Cu(HL^5)Cl]$	30.9	22.9	1.3	30.2	1.0	1.6	**19.3**

**SI – selectivity index*

The results of the study of the anticancer properties of the coordinative compounds of Cu(II) with (*p*-ethyl benzoate)thiosemicarbazone of 2-formylpyridine (HL^1) show us that HeLa and BxPC-3 cells are best inhibited by $[Cu(L^1)NO_3]$, whose IC_{50} is 0.9 and correspondingly 0.4 μM, and TC-1 cells are better inhibited by $[Cu(L^1)Cl]$ and $[Cu(L^1)Br]$, whose IC_{50} is 0.3 μM. These complexes have more pronounced activity than the control substance, *cis*-platinum.

$[Cu(H_2O)(L^1)]ClO_4$ (**C5**) possesses the highest selectivity index at the ratio IC_{50}(MDCK/BxPC-3) of 7, compared to the ratio of *cis*-platinum IC_{50}(MDCK/BxPC-3) of 2.8. So this SI shows us that this given substance surpasses not only the antinancer activity but also the selectivity of cis-platinum by 2.5 times.

The compound $[Cu(L^1)Cl]$ (**C1**) has the highest selectivity index at the IC_{50}(MDCK/TC-1) ratio of 21.7, compared to cis-platinum, whose IC_{50}(MDCK/TC-1) ratio is 6.7. So $[Cu(L^1)Cl]$ in the given case is 3.2 times more selective than cis-platinum.

The results of the study of the anticancer properties of the coordination compound of Cu(II) with (*p*-ethyl benzoate) thiosemicarbazone of salicylic aldehyde (**C23**) show us that the best result was recorded on TC-1 cancer cells, with this complex [Cu(HL^5)Cl] a selectivity is observed, the SI at the IC_{50}(MDCK/TC-1) ratio is 19.3, and it is 2.9 times higher (more selective) compared to the SI of cis-platinum, whose IC_{50}(MDCK/TC-1) is 6.7.

Coordination compounds based on (*p*-ethyl benzoate) thiosemicarbazone of 2-formylpyridine (**HL^1**) have more pronounced anticancer properties compared to the coordination compounds based on (*p*-ethyl benzoate) thiosemicarbazone salicylic aldehyde **(HL^5)**.

CONCLUSIONS AND RECOMMENDATIONS

1) For the first time, benzocaine was functionalized with a thiosemicarbazone fragment, thus obtaining 15 new thiosemicarbazones (**HL^1-HL^{15}**) based on ethyl 4-aminobenzoate. Upon the interaction of the synthesized thiosemicarbazones with some 3*d* metals: Cu(II), Mn(II), Fe(III), Co(III), Ni(II) and Zn(II), 33 new coordination compounds (**C1**-**C33**) were obtained.

2) The purity, composition, and structure of the obtained compounds were confirmed and determined with the help of: melting point, thin layer chromatography/silica gel column, IR spectroscopy, ^{1}H, ^{13}C/(DEPT-135) NMR spectroscopy, elemental analysis, molar conductivity, and a single-crystal X-ray diffraction.

3) ^{1}H, ^{13}C NMR spectra of thiosemicarbazones in DMSO-d_6 solution revealed the presence of two tautomeric forms: thionic (50-99%) and thiolic (1-50%).

4) Single crystals of thiourea **2a**, thiosemicarbazides **3** and **24**, nine thiosemicarbazones, and two coordination compounds were isolated, and a single-crystal X-ray diffraction study was performed. The crystal structure of organic molecules is basically planar. Different geometric configurations with respect to the double bond of the azomethine group of thiosemicarbazones were observed: *trans* in the case of HL^1, HL^4, H_2L^7, HL^{10}, HL^{13}, HL^{15}, and *cis* in the case of HL^2, HL^3, HL^{12}. In the crystalline state of thiosemicarbazones studied by X-rays, the distance between the carbon atom and the sulfur atom is 1.654-1.680Å which corresponds to the double (ionic) bond.

5) Crystallographic data of the coordination compounds $[Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O$ (**C14**) and $[Fe(L^2)_2]Cl\cdot 3C_2H_5OH\cdot 2H_2O$ (**C18**) confirms the tridentate coordination (NNS) of thiosemicarbazones derived from 2-formylpyridine in the monodeprotonated form.

Study of antioxidant properties.

1) The most pronounced antioxidant properties in the series of thiosemicarbazones HL^1-HL^{15} possess the ligand **H_2L^7** (*p*-ethyl benzoate) thiosemicarbazone 2-hydroxynaphthaldehyde, whose IC_{50} is equal to 9.38 μM and is three times more active than the standard substance Trolox. Coordination of thiosemicarbazone **H_2L^7** to Cu(II) ions leads the decreasing of an antioxidant properties.

2) The most pronounced antioxidant properties are possessed by coordination compounds based on (p-ethyl benzoate)thiosemicarbazone of 2-formylpyridine (**HL^1**). The complex $[Ni(L^1)Cl]$ (**C6**) with IC_{50} = 3.70 μM is 4 times more active than the **HL^1** ligand and 9 times more active than Trolox. The complex $[Cu(L^1)Cl]$ (**C1**) possesses an IC_{50} of 6.56 μM and is twice as active as the uncoordinated ligand. When changing the anion in the $[Cu(L^1)Cl]$ complex, the following series of decreasing antioxidant properties is obtained: **Cl^-** > NO_3^- > Br^- > CH_3COO^- > ClO_4^-. The central atom in the complex also plays an important role in the antioxidant properties of the coordination compounds, from which we obtain the following series: Ni>Cu>Mn>Zn>Fe>Co.

Study of antimicrobial properties.

1) The thiosemicarbazones **HL^1-HL^{15}** possess slightly pronounced antimicrobial properties. But upon coordination with Cu(II) ions, the amplification of these properties takes place. The best antibacterial activity results on *Staphylococcus aureus* were recorded for $[Cu(L^1)Cl]$ (**C1**) and $[Cu(L^2)Br]$ (**C12**) whose MIC is 0.12 μg/mL and which are 40 times more active than the reference substance Furacillinum.

2) The best results of antifungal activity on *Candida albicans* were recorded for the coordination compounds $[Cu(L^1)NO_3]$ (**C3**), $[Cu(L^2)Cl]$ (**C11**), and $[Cu_2(L^2)_2(CH_3COO)_2]\cdot 5H_2O$ (**C14**), whose MIC is 0.98 μg/mL and is 32 times more active than the reference substance Nystatin; in the case of *Candida krusei*, the same results were recorded for the coordination compounds $[Cu(L^1)Cl]$ (**C1**), $[Cu(L^2)Br]$ (**C12**).

3) If we change the central atom in the $[Cu(L^1)Cl]$ complex, the antimicrobial activity decreases considerably according to the following series: Cu>>>Ni>Co>Mn=Zn=Fe.

Study of anticancer properties:

1) The anticancer properties of (*p*-ethyl benzoate)thiosemicarbazone of 2-formylpyridine (**HL^1**) were investigated on HL-60 cells. Thiosemicarbazone HL^1 has an IC_{50} equal to 0.1 μM, being three times more active than Doxorubicin and 250 times more active than *cis*-platinum.

2) Complex [Cu(L^1)NO_3] (**C3**) best inhibits HeLa and BxPC-3 cells, whose IC_{50} is 0.9 and correspondingly 0.4 μM. The coordination compounds [Cu(L^1)Cl] (**C1**) and [Cu(L^1)Br] (**C2**) best inhibit TC-1 cells, whose IC_{50} is 0.3 μM, these complexes have more pronounced activity than the control substance *cis*-platinum.

3) The complex [Cu(H_2O)(L^1)]ClO_4 (**C5**) possesses the highest ***selectivity index*** on the ratio IC_{50} (MDCK/BxPC-3) is equal to 7, compared to the SI of *cis*-platinum, which is equal to 2.8. Thus, the given ratio indicates that the complex [Cu(H_2O)(L^1)]ClO_4 (**C5**) surpasses not only the anticancer activity but also the selectivity by 2.54 times. The complex [Cu(L^1)Cl] (**C1**) possesses the highest ***selectivity index*** on the ratio IC_{50} (MDCK/TC-1) is equal to 21.7, compared to the IS of *cis*-platinum is equal to 6.71, thus the complex [Cu(L^1)Cl] (**C1**) exceeds the selectivity of *cis*-platinum by 3.22 times.

Recommendations

1) The obtained data can be used as instructional and educational material in special courses in chemistry, the undergraduate cycle (Organic Chemistry II, Biopharmaceutical Chemistry), and the master's cycle.

2) In the synthesized compounds, high levels of antioxidant, antimicrobial, and anticancer properties are recorded; thus, it is recommended to expand the research on this class of compounds.

4) The compound (*p*-ethyl benzoate)thiosemicarbazone of 2-formylpyridine (**HL^1**) possesses high anticancer properties on HL-60 cells, has been patented (MD 4613), and is recommended for pharmaceutical applications to expand the arsenal of human myeloid leukemia inhibitors.

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ARTICLES

1. **RUSNAC, Anna**, GARBUZ, Olga, ŞOVA, Sergiu, GULEA, Aurelian. Sinteza combinațiilor coordinative noi ale unor metale 3*d* în baza N(4)-(acetat de etil) tiosemicarbazonelor 2-formil și 2-acetilpiridinei. Proprietăți antioxidante. In: *Revista de Ştiinţă, Inovare, Cultură şi Artă „Akademos"*, 2022, nr. 4(67), pp. 19-26. ISSN 1857-0461. DOI: 10.52673/18570461.22.4-67.02
2. GARBUZ, Olga, RAILEAN, Nadejda, GOSTEV, Igor, **RUSNAC, Anna**, TODERAŞ, Ion, GULEA, Aurelian. Evaluarea biologică a compuși lor coordinativi ai cuprului(II) cu unele tiosemicarbazone heterociclice. In: *Studia Universitatis Moldaviae (Seria Ştiinţe Reale şi ale Naturii)*, 2022, nr. 6(156), pp. 144-149. ISSN 1814-3237. DOI: 10.5281/zenodo.7445721
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11. БЭЛАН Г.Г., **РУСНАК А.Г.,** ГУЛЯ А.П. VII Ежегодная международная научно-практическая конференция «Актуальные вопросы медицины» и «Спутниковый форум по общественному здоровью и политике здравоохранения» «Симуляционный Тренинг по Лапароскопической Хирургии» 10-12 апреля **2019**, г. Баку, Азербайджан. *.Исследование антимикробных свойств координационных соединений меди(II) на основе этилового эфира 4-({[2-(2-гидроксибензилиден) гидразинил]карбонотиоил}амино)бензоата*. с.4.
12. **RUSNAC**, **Anna,** GHILEȚCHI, Natalia, GARBUZ, Olga, GULEA, Aurelian. Conferinţa ştiinţifică naţională cu participare internaţională „integrare prin cercetare şi inovare" 9-10 noiembrie **2018**. Chișinău. ISBN 978-9975-142-49-6. *Sinteza și cercetarea proprietăților anticancer ale combinațiilor coordinative în baza Cu(II) și Ni(II) cu etil 4({[2-(2-hidroxibenziliden)hidrazinil]carbonotioil}amino)benzoat*. p.228-231.
13. **RUSNAC**, **A.,** PETRENKO, P., CHUMAKOV, Yu., GULEA, A. 9th International Conference on Materials Science and Condensed Matter Physics. Chisinau, Moldova, September 25-28, **2018**. *The cristal structure and antitumor properties of ethyl 4-[(dimethylcarbamothioyl)amino]benzoate and ethyl 4-[(hydrazinylcarbonothioyl)amino]benzoate*. p.129.

PATENT OF INVENTION

GULEA A., RUSNAC R., **RUSNAC A**., ȚAPCOV V. *Etil-4-{2-[(piridin-2-ilmetiliden)hidrazinocarbotioil]amino}benzoat monohidrat care manifesta proprietați de inhibitor al proliferarii celulelor HL-60 ale leucemiei mieloide umane*. Brevet de invenție 4613 (13) B1, C07C 337/08, C07C 69/78, A61K 31/175, A61P 35/02. Nr. depozit a2017 0078, Data depozit 11.09.2017. Publicat 31.01.2019. In: BOPI. **2019, nr.1,** pp. 46.

ADNOTARE

RUSNAC Anna, „Sinteza și proprietățile biologice ale combinațiilor coordinative cu unele biometale în baza tiosemicarbazonelor 4-aminobenzoatului de etil”, teza de doctor în științe chimice. Chișinău 2023.

Structura tezei: constă din introducere, patru capitole, concluzii generale și recomandări, bibliografie din 164 de titluri, 4 anexe, 114 pagini text de bază, 71 figuri, 13 tabele. Rezultatele obținute au fost publicate în 20 lucrări științifice, un brevet de invenție; 6 articole; 13 rezumate la conferințe naționale și internaționale.

Cuvinte cheie: combinații coordinative, biometale, tiosemicarbazone, 4-aminobenzoat de etil, structură, activitate antioxidantă, antibacteriană, antifungică, antitumorală.

Scopul lucrări: sinteza, caracterizarea și cercetarea proprietăților biologice ale combinațiilor coordinative cu unele biometale în baza tiosemicarbazonelor 4-aminobenzoatului de etil.

Obiectivele cercetării: sinteza 4-(p-benzoat de etil)tiosemicarbazonelor 2-formilpiridinei/aldehidei salicilice și derivaților acestora; sinteza combinațiilor coordinative ale Zn(II), Cu(II), Ni(II), Co(III), Fe(III) și Mn(II) în baza tiosemicarbazonelor (HL^1-HL^{15}); determinarea compoziției și structurii compușilor sintetizați cu ajutorul spectroscopiei IR, spectroscopiei de Rezonanță Magnetică Nucleară ^{1}H-RMN, ^{13}C-RMN, analizei elementelor și analizei cu raze X pe monocristal; cercetarea proprietăților antioxidantive, antibacteriene, antifungice și antitumorale.

Noutatea și originalitatea științifică: în premieră au fost obținute **15** tiosemicarbazone funcționalizate cu fragmentul 4-aminobenzoat de etil și derivaților lui; au fost stabilite condițiile optimale de sinteză pentru obținerea a **33** de combinații coordinative noi ale unor biometale cu randamente înalte. Au fost cercetate proprietățile biologice a unei seriei de combinații coordinative. A fost brevetată o substanță organică de tip tiosemicarbazonă HL^1 care manifestă un potențial aplicativ sporit, datorită proprietăților inhibitori ai proliferării celulelor leucemiei umane mieloide (HL-60), cu activitate citostatică înaltă.

Rezultatele obținute care contribuie la soluționarea unei probleme științifice importante: A fost efectuat *screening-ul* activităţilor antimicrobiene, antifungice, anticancer și antioxidativ în dependență de următorii factori: natura atomului central din cadrul compusului coordinativ; restul acid; poziția grupei esterice din inelul aromatic din compoziția tiosemicarbazonelor; componenta carbonilică.

Semnificația teoretică: contribuie la elaborarea unor strategii de funcționalizare eficientă a 4-aminobenzuatului de etil la obținerea combinațiilor coordinative în baza tiosemicarbazonelor.

Valoarea aplicativă: cele mai pronunțate proprietăți antibacteriene au fost înregistrate asupra *S. aureus, C. krusei,* de către complecșii de cupru(II). Unele tiosemicarbazone după activitatea anticancerigenă, sunt de trei ori mai efective decât doxorubicina, utilizată în medicină și de 250 de ori mai activ ca *cis*-platina.

Implementarea rezultatelor ştiinţifice: A fost brevetat un compus organic din clasa tiosemicarbazonelor (**HL^1**) care manifestă un potențial aplicativ sporit, datorită concetrației semimaximale de inhibiție de ordinul $1 \cdot 10^{-7}$ mol/L la inhibarea creșteri celulelor de cancer HL-60.

АННОТАЦИЯ

РУСНАК Анна, "Синтез и биологические свойства координационных соединений с некоторыми биометаллами на основе тиосемикарбазонов этил 4-аминобензоата". Диссертация на соискание ученой степени доктора химических наук. Кишинев 2023.

Структура диссертации: состоит из введения, четырех глав, общих выводов и рекомендаций, библиографии из 164 наименований, 4 приложений, 114 страниц основного текста, 71 рисунка, 13 таблиц. Полученные результаты опубликованы в 20 научных работах, в том числе: патент на изобретение; 6 статей; 13 тезисов на национальных и международных конференциях.

Ключевые слова: координационные соединения, биометаллы, тиосемикарбазоны, этил-4-аминобензоат, структура, антиоксидантная, антибактериальная, противогрибковая, противоопухолевая активность.

Цель работы: Синтез и исследование биологических свойств координационных соединений некоторых биометаллов с тиосемикарбазонами на основе этил 4-аминобензоата.

Задачи исследования: синтез 4-(*p*-этилбензоат)тиосемикарбазонов 2-формилпиридина/салицилового альдегида и их производных; синтез координационных соединений Zn(II), Cu(II), Ni(II), Co(III), Fe(III) и Mn(II) на основе тиосемикарбазонов (HL^1-HL^{15}); определение состава и строения синтезированных соединений методами ИК-спектроскопии, ^{1}H-ЯМР, ^{13}C-ЯМР-спектроскопии, элементного анализа и рентгеноструктурного анализа монокристаллов; исследования антиоксидантных, антибактериальных, противогрибковых и противоопухолевых свойств.

Научная новизна и оригинальность: впервые получены **15** тиосемикарбазонов, функционализированных этил-4-аминобензоатным фрагментом, и его производными; установлены оптимальные условия синтеза для получения **33** новых координационных соединений биометаллов с высокими выходами. Исследованы биологические свойства ряда координационных соединений. Запатентовано органическое вещество типа тиосемикарбазон HL^1, проявляющее повышенный потенциал применения, благодаря ингибирующим свойствам пролиферации клеток меланомы человека (HL-60), обладающее высокой цитостатической активностью.

Полученные результаты способствуют решению научной задачи: антимикробную, противогрибковую, противораковую и антиоксидантную активность оценивали в зависимости от следующих факторов: природы центрального атома; кислотных остатков положения сложных эфирных групп в ароматическом кольце; карбонильного компонента.

Теоретическая значимость: способствует разработке эффективных стратегий функционализации этил-4-аминобензоата для получения координационных комплексов на основе тиосемикарбазонов.

Практическая значимость: наиболее выраженные антибактериальные свойства зафиксированы на *S. aureus, C. krusei* у комплексов меди(II). Некоторые тиосемикарбазоны обладают противораковой активностью в три раза эффективнее применяемого в медицине доксорубицина и в 250 раз активнее *цис*-платины.

Внедрение научных результатов: запатентовано органическое соединение из класса тиосемикарбазонов (HL^1), которое показывает повышенный потенциал применения, за счет полумаксимальной ингибирующей концентрации порядка $1 \cdot 10^{-7}$ моль/л при торможении роста HL-60 раковых клеток.

ANNOTATION

RUSNAC Anna, „Synthesis and biological properties of coordination compounds with some biometals based on thiosemicarbazones derived from ethyl 4-aminobenzoate", PhD thesis in chemical sciences. Chisinau 2023.

Thesis structure: consists of introduction, four chapters, general conclusions and recommendations, bibliography of 164 titles, 4 annexes, 114 pages of basic text, 71 figures, 13 tables. The obtained results were published in 20 scientific papers, including: a patent; 6 articles; 13 abstracts at national and international conferences.

Keywords: coordination compounds, biometals, thiosemicarbazones, ethyl 4-aminobenzoate, structure, antioxidant, antibacterial, antifungal, antitumor activity.

The aim of the work: synthesis, characterization and investigation of biological properties of the coordination compounds with some biometals based on thiosemicarbazones derived from ethyl 4-aminobenzoate.

Research objectives: synthesis of 4-(*p*-ethyl benzoate)thiosemicarbazones of 2-formylpyridine/salicylic aldehyde and their derivatives; synthesis of coordination compounds of Zn(II), Cu(II), Ni(II), Co(III), Fe(III) and Mn(II) based on thiosemicarbazones (HL^1-HL^{15}); determining the composition and structure of the synthesized compounds using IR spectroscopy, ^{1}H-NMR, ^{13}C-NMR spectroscopy, elemental analysis and single crystal X-ray analysis; research on antioxidant, antibacterial, antifungal and antitumor properties.

Scientific novelty and originality: for the first time, **15** thiosemicarbazones functionalized with the ethyl 4-aminobenzoate fragment and its derivatives were obtained; the optimal synthesis conditions for obtaining **33** new coordinative compounds of biometals with high yields were established. The biological properties of a series of coordination compounds were investigated. An organic substance of the thiosemicarbazone type HL^1 has been patented, which shows increased application potential, due to the inhibitory properties of the proliferation of human myeloid leukemia cells (HL-60), with high cytostatic activity.

The obtained results contribute to the solution of an important scientific problem: antimicrobial, antifungal, anticancer and antioxidant activities were screened depending on the following factors: the nature of the central atom within the coordinating compound; the rest acid; the position of the ester group in the aromatic ring in the composition of thiosemicarbazones; the carbonyl component.

Theoretical significance: it contributes to the development of effective functionalization strategies of ethyl 4-aminobenzoate to obtain coordination compounds based on thiosemicarbazones.

Applicative value: the most pronounced antibacterial properties were recorded on *S. aureus, C. krusei,* by copper(II) complexes. Some thiosemicarbazones have anticancer activity three times more effective than doxorubicin, used in medicine and 250 times more active than cis-platinum.

Implementation of scientific results: an organic compound from the class of thiosemicarbazones (HL^1) was patented, which shows increased application potential, due to the half-maximal inhibition concentration of the order of $1 \cdot 10^{-7}$ mol/L when inhibiting the growth of HL-60 cancer cells.

RUSNAC ANNA

SYNTHESIS AND BIOLOGICAL PROPERTIES OF COORDINATION COMPOUNDS WITH SOME BIOMETALS BASED ON THIOSEMICARBAZONES DERIVED FROM ETHYL 4-AMINOBENZOATE

141.02 COORDINATION CHEMISTRY

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