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**SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT ACTIVITY EVALUATION OF
3d METALS COMPLEXES WITH N(4)-((3)-ETHYL BENZOATE)
THIOSEMICARBAZONES OF 2-FORMYL(2-ACETYL, 2-BENZOIL)PYRIDINE**

Anna RUSNAC¹, Olga GARBUZ^{1,2}, Sergiu SHOVA^{1,3}, Aurelian GULEA¹

¹Moldova State University

²Institute of Zoology

³Institute of Macromolecular Chemistry "Petru Poni": Iasi, Romania

The paper presents the synthesis of new coordination compounds of Cu(II), Mn(II), Fe(III) Co(III), Ni(II) and Zn(II) with N(4)-((3)-ethyl benzoate)thiosemicarbazones of 2-formyl pyridine derivatives obtained through the condensation of N(4)-((m)-ethyl benzoate) thiosemicarbazide with 2-pyridinecarboxaldehyde, 2-acetylpyridine and 2-benzoylpyridine. The characterization of new compounds was done by ¹H NMR, ¹³C NMR, IR spectroscopy, conductivity and elemental analysis. In addition, the structures of the ligands (HL^{1,3}) were determined by single-crystal X-ray diffraction. The effect of these complexes on antioxidant activity were studied and compared with those of free ligands. The best result was found to be on coordination compound [Zn(L¹)Cl], with IC₅₀ = 11.45 μM, it is better than the free ligand HL¹, IC₅₀ = 13.15 μM and is better than the substance Trolox, IC₅₀ = 15.86 μM (it is a pronounced antioxidant taken as a control substance).

Keywords: synthesis, characterization, thiosemicarbazones, coordination compounds, antioxidant activity.

**SINTEZA, CARACTERIZAREA, CERCETAREA ACTIVITĂȚII ANTIOXIDATIVE A COMPLECȘILOR
METALELOR 3d CU N(4)-((3)-BENZOAT DE ETIL) TIOSEMICARBAZONE 2-FORMIL(2-ACETIL,
2-BENZOIL) PIRIDINEI**

Lucrarea prezintă sinteza combinațiilor coordinative noi ale Cu(II), Mn(II), Fe(III) Co(III), Ni(II) și Zn(II) cu N(4)-((3)-benzoat de etil) tiosemicarbazone ale derivaților de 2-formilpiridinei obținuți prin condensarea N(4)-((3)-benzoat de etil) tiosemicarbazida cu 2-formilpiridina, 2-acetilpiridina și 2-benzoilpiridina. Caracterizarea compușilor noi obținuți a fost efectuată cu ajutorul spectroscopiilor ¹H RMN, ¹³C RMN, IR; conductivitate în soluție și analiza elementală. În plus, structurile moleculare ale liganzilor (HL^{1,3}) au fost stabilite utilizând difracția cu raze X pe monocristal. A fost studiată activitatea antioxidantă a combinațiilor coordinative în comparație cu cea a liganzilor liberi. Cel mai bun antioxidant s-a dovedit a fi complexul [Zn(L¹)Cl], cu IC₅₀ = 11,45 μM, care prezintă potențial antioxidant, este mai bun decât al ligandului liber HL¹, cu IC₅₀ = 13,15 μM și este mai bun decât al troloxului, IC₅₀ = 15,86 μM (este un antioxidant pronunțat luat în calitate de substanță de referință).

Cuvinte-cheie: sinteză, caracterizare, tiosemicarbazone, combinații coordinative, activitate antioxidantă.

Introduction

Thiosemicarbazones and their metal complexes exhibit a wide range of biological applications. Owing to the interest they generate in pharmacology, thiosemicarbazones and their metal complexes have been extensively studied. The biological activity of thiosemicarbazones results from their ability to form chelates with metal ions. Thiosemicarbazones exist in the two tautomeric forms: thione and thiol. The thione form acts as a neutral bidentate ligand while the thiol form deprotonates and acts as an anionic ligand [1].

There are some reports on the studies of N(4)-substituted thiosemicarbazones of 2-formylpyridine derivatives and their metal complexes, where spectral and structural studies of copper(II) and iron(III) complexes with thiosemicarbazones are reported, which demonstrated a high antioxidant effect against ABTS⁺ radical cations, as well as low toxicity on *Daphnia magna*, which have a lower toxicity than DOXO [2,3]. The synthesized complexes exhibit antimicrobial, antifungal, antioxidant, and anticancer activities [4].

Among the metals, copper and zinc are the essential trace metals required for organisms to perform several important biological processes. Their chelates have been studied very effectively as less toxic alternatives and are very selective in anticancer treatments [5].

The antiproliferative properties of some copper, nickel, cobalt, and zinc complexes with 2-formylpyridine N(4)-allylthiosemicarbazone toward a series of cancer cell lines (HL-60, HeLa, BxPC-3, RD) and a normal cell line (MDCK) have been investigated [6]. The nickel complex shows high selectivity (SI > 1000) toward HL-60 cell line and is the least toxic. The zinc complex shows the highest selectivity toward RD cell line (SI - 640). The copper complexes are the most active molecular inhibitors of proliferation of cancer cells, but

exhibit not such a high selectivity and are significantly more toxic. Zinc and copper complexes manifest high antibacterial activity.

This paper presents the synthesis and characterization of newly ligands: N(4)-((3)-ethyl benzoate)thiosemicarbazones of 2-formylpyridine derivatives and complexes of 3d metals with this ligands HL¹, HL², HL³ using salts of Cu(II), Mn(II), Fe(III) Co(III), Ni(II) and Zn(II). The biochemical effects involved in the antioxidant activity of ligands and synthesized complexes were investigated.

Materials and methods

1) The chemical elemental analysis, melting points of the thiosemicarbazones, was determined by specific methods [7].

2) IR spectra were recorded on the Bruker Alpha spectrometer, 4000-400 cm⁻¹.

3) ¹H and ¹³C Nuclear Magnetic Resonance was performed by Bruker DRX-400 spectrometer. Chemical shifts are shown in ppm relative to Si(Me)₄; DMSO-d₆ was used as solvent.

4) Study of single crystal X-ray diffraction. Crystallographic measurements were performed using an Oxford-Diffraction Xcalibur-Gemini X-ray diffractometer with a graphite monochromator equipped with a Mo-K_α X-ray source. The crystals were placed at a distance of 40 mm from the CCD detector. The procedures for determining the parameters of the elementary cell and for integrating the experimental data were performed using the "CrysAlis package Oxford Diffraction" program set. To obtain the structure, the solutions were determined by the direct method using the SHELXS-97 program [8] and fitted by the Fo²-based least squares method in the SHELXL-97 program in the anisotropic variant for all atoms with a molar mass greater than the atom of hydrogen.

5) Conductometry in solution. Most substances dissolved in water/protic organic solvent dissociate into ions that can conduct electricity. Electrical conductivity indicates the amount of material in which water expressed in μS/cm at 25 °C is dissolved. Conductometric analysis was measured on the ADWA AD8000 (pH/mV/EC/TDS & Temperature Meter). The calibration of the electrode (AD 76309) was performed with standard solutions of 1430.0 μS/cm and 12,880.0 μS/cm. The samples were solubilized in H₂O/DMF/DMSO/EtOH or mixtures of the listed solvents. The concentration of the investigated samples was 1·10⁻³M [9,10].

6) Method of studying antioxidant activities: To determine the antioxidant activity, the spectrophotometric method was used, where to the solutions containing specific free radicals (ABTS^{•+} cation radical (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate) the substance is added in different concentrations and the absorbance is determined. These measures show the ability of substances to interact with ABTS^{•+} cation radicals [11,12].

Experimental

All reagents and solvents used were of analytical purity, purchased from the following companies: Sigma-Aldrich®, Acros Organics®, Alfa Aesar®, etc.

Synthesis of N(4)-((m)-ethyl benzoate)thiosemicarbazones of 2-formylpyridine derivatives was performed according to the following steps:

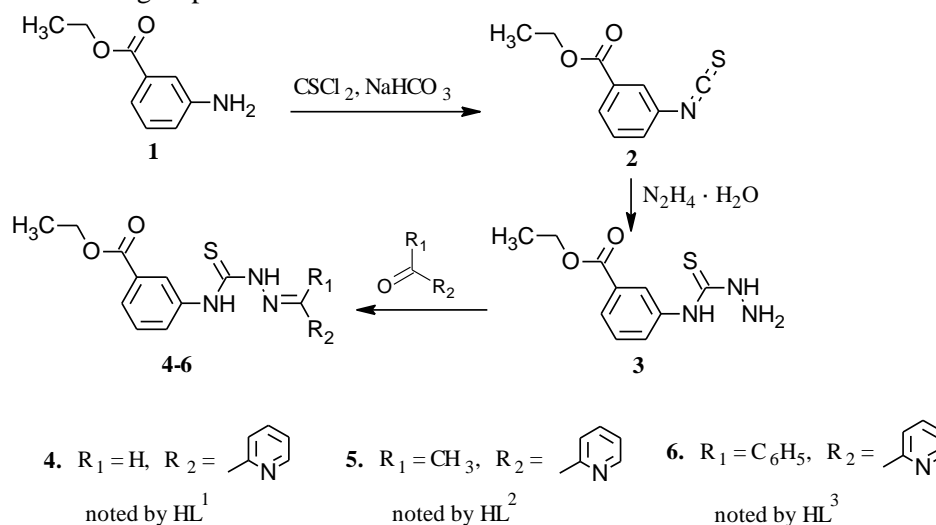


Fig.1. Synthesis of N(4)-((m)-ethyl benzoate) thiosemicarbazones of 2-formylpyridine derivatives (noted by HL¹⁻³).

Ethyl 3-isothiocyanatobenzoate (2). To the solution of 8.25 g (50 mmol) of ethyl 3-aminobenzoate (**1**) and 20 mL of hexane was added 4.20 g (50 mmol) of NaHCO₃ dissolved in 60 ml of water; the mixture is placed in the ice bath on a magnetic stirrer. To the mixture obtained, it was added dropwise the solution consisting of 5.75 g (50 mmol) of CCl₄ and 10 ml of hexane (precipitation of the intermediate ethyl 3-[(chlorocarbonothioyl)amino]benzoate was observed). After the total addition of the CCl₄ solution, 4.20 g (50 mmol) of NaHCO₃ are added, stirring continuously for 2 hours at 0-10°C. For the total transformation of the intermediate into isothiocyanate **3** the temperature rises to 60 °C for one hour. The organic layer was separated from the aqueous layer and dried over anhydrous CaCl₂. The solution is passed through a silica gel column and the reaction product is purified by chromatography. 9.34 g (90%) of isothiocyanate **2** are obtained, m.p. 50-52°C. R_f = 0.81 (eluent: benzene - ethyl acetate, 2: 1). The physico-chemical and spectral characteristics corresponds to literature [13].

Ethyl 3-[(hydrazinecarbothioyl)amino]benzoate (3). Dissolve 9.34 g (45 mmol) of ethyl 3-isothiocyanatobenzoate **2** in 10 ml of ethanol and add dropwise to the ethanolic solution of 2.5 g (50 mmol) hydrazine monohydrate at room temperature with magnetic stirring for 30 minutes. The reaction mixture was further stirred at 50°C for 30 minutes (total consumption of isothiocyanate **2** was confirmed by chromatography) and cooled to 0°C. The product obtained is filtered and recrystallized from ethanol. 12.52 g of thiosemicarbazide **3** are obtained in a yield of 86%, m.p. = 109-110°C. R_f = 0.18 (eluent: benzene-ethyl acetate, 2:1). The physico-chemical and spectral characteristics corresponds to literature [14].

Ethyl 3-[(2-[(pyridin-2-yl)methylidene]hydrazinecarbothioyl)amino]benzoate (4) (denoted by HL¹). The mixture of 2.86 g (12 mmol) ethyl 3-[(hydrazinecarbothioyl)amino]benzoate **3**, 1.28 g (12 mmol) pyridine-2-carbaldehyde, 10 mL ethanol and 4 drops of glacial acetic acid is refluxed for 2 hours (total consumption of thiosemicarbazide **3** was confirmed by chromatography). The reaction mixture is diluted with a 3 ml of water and cooled to 0°C. The product obtained is filtered, recrystallized from ethanol and dried. Obtained: 3.51 g (89%) HL¹, mp = 170-171°C, white crystals, R_f = 0.25 (benzene: ethyl acetate, 2:1).

Elemental analysis for: C₁₆H₁₆N₄O₂S, (%): C, 58.5; N, 17.1; Found, (%): C, 58.4; N, 17.0. **FTIR**(ν_{max}, cm⁻¹): 3333, m, (N-H); 3113, w, (C-H, Ar); 2916, m, as, (C-H, CH₂); 2849, m, sy, (C-H, CH₃); 1722, s, (C=O, ester); 1531, s, (C=C, Ar); 1271, s, as, (C-O); 1193, s, sy, (C-O); 746, 707, s, (C-H, meta-Aryl). **RMN (DMSO-d₆) δ (ppm) ¹H, 400 MHz:** 12.17, (s, 1H, N-H); 10.44, (s, 1H, N-H); 8.61, (d, 1H, C-H); 8.46, (d, 1H); 8.22, (s, 1H, C-H); 8.16, (s, 1H, C-H); 7.89, (d, 2H, C-H); 7.83, (d, 1H, C-H); 7.54, (t, 1H, C-H); 7.41, (m, 1H, C-H); 4.35, (q, 2H, CH₂); 1.34, (t, 3H, CH₃). **RMN (DMSO-d₆) δ (ppm) ¹³C 100 MHz:** 177.1, (C=S); 165.9, (C=O); 153.5, (C=N); 149.8, 143.9, 139.8, 137.0, 131.3, 130.4, 128.9, 126.9, 126.5, 124.8, 121.2, (C-Ar); 61.3, (CH₂); 14.6 (CH₃).

Ethyl 3-[(2-[1-(pyridin-2-yl)ethylidene]hydrazinecarbothioyl)amino]benzoate (5) (denoted by HL²). Mixture of 2.86 g (12 mmol) ethyl 3-[(hydrazinecarbothioyl)amino]benzoate **3**, 1.45 g (12 mmol) 2-acetylpyridine, 10 ml of ethanol and 4 drops of glacial acetic acid, reflux for 4 hours (check the end of the reaction by chromatography). The reaction mixture is diluted with 3 ml of water and cooled to 0°C. The product obtained is filtered and recrystallized from ethanol. It was obtained: 3.21 g (78%) HL², mp = 119-120°C, white crystals, R_f = 0.35 (benzene: ethyl acetate, 2:1).

Elemental analysis for: C₁₇H₁₈N₄O₂S, (%): C, 59.6; N, 16.4; Found, (%): C, 59.4; N, 16.3. **FTIR**(ν_{max}, cm⁻¹): 3332, w, (N-H); 3194, w, (C-H, Ar); 2966, m, as, (C-H, CH₂); 2906, m, sy, (C-H, CH₃); 1714, s, (C=O, ester); 1537, s, (C=C, Ar); 1282, s, as, (C-O); 1186, s, sy, (C-O); 751, 679, s, (C-H, meta-Aryl). **RMN (DMSO-d₆) δ (ppm) ¹H, 400 MHz:** 10.84, (s, 1H, N-H); 10.35, (s, 1H, N-H); 8.62, (d, 1H, C-H); 8.55, (d, 1H, C-H); 8.16, (s, 1H, C-H); 7.84, (t, 3H, C-H); 7.54, (t, 1H, C-H); 7.42, (m, 1H, C-H); 4.35, (q, 2H, CH₂); 2.49, (s, 3H, CH₃); 1.34, (t, 3H, CH₃). **RMN (DMSO-d₆) δ (ppm) ¹³C 100 MHz:** 177.9, (C=S); 165.9, (C=O); 154.9, (C=N); 150.1, 148.9, 140.0, 136.8, 131.6, 130.3, 128.9, 127.2, 126.6, 124.6, 121.7, (C-Ar); 61.3, (CH₂); 14.6, (CH₃-C=N); 13.0, (CH₃).

Ethyl 3-(2-[phenyl(pyridin-2-yl)methylidene]hydrazinecarbothioyl)amino]benzoate (6) (denoted by HL³). The mixture of 2.82 g (11 mmol) ethyl 3-[(hydrazinecarbothioyl)amino]benzoate **3**, 2.01 g (11 mmol) 2-benzoylpyridine, 10 ml of ethanol and 4 drops of glacial acetic acid, is refluxed for 7 hours (thiosemicarbazide **3** consumption is checked by chromatography). After confirmation of the end of the reaction, the reaction mixture is diluted with a 3 ml of water and cooled to 0°C. The product obtained is filtered, recrystallized from ethanol and dried. Obtained: 3.40 g (83 %) HL³, m.p. = 129-130°C, pale yellow crystals, R_f = 0.75 (benzene: ethyl acetate, 1: 1).

Elemental analysis for: $C_{22}H_{20}N_4O_2S$, (%): C, 65.3; N, 13.9; Found, (%): C, 65.3; N, 13.8. **FTIR**(ν_{max} , cm^{-1}): 3286, m, (N-H); 3056, w, (C-H, Ar); 2976, m, as, (C-H, CH_2); 2901, m, sy, (C-H, CH_3); 1714, s, (C=O, ester); 1536, s, (C=C, Ar); 1285, s, as, (C-O); 1199, s, sy, (C-O); 755, 705, s, (C-H, *meta*-Aryl). **RMN (DMSO $_d$) δ (ppm) 1H , 400 MHz:** 13.25, (s, 1H, N-H); 10.52, (s, 1H, N-H); 8.90, (d, 1H, C-H); 8.18, (d, 1H, C-H); 8.06, (t, 1H, C-H); 7.87, (t, 1H, C-H); 7.83, (d, 1H, C-H); 7.75, (d, 1H, C-H); 7.65, (d, 1H, C-H); 7.55, (d, 1H, C-H); 7.50, (m, 3H, C-H); 7.41, (d, 1H, C-H); 4.34, (q, 2H, CH_2); 1.33, (t, 3H, CH_3). **RMN (DMSO $_d$) δ (ppm) ^{13}C 100 MHz:** 177.2, (C=S); 165.8, (C=O); 151.8, (C=N); 149.3, 139.8, 138.8, 137.1, 130.4, 129.9, 129.6, 129.0, 128.8, 126.8, 125.6, (C-Ar); 61.3, (CH_2); 14.6, (CH_3).

The synthesis and characterization of ligands was discussed at the conference [15]. The structures of these formed ligands were established by IR, 1H NMR and ^{13}C NMR spectroscopies. In the 1H NMR all the protons were found to be in the expected regions, only the characteristic peaks of the investigated compounds are present, which confirms their purity. For the HL¹ and HL³ compounds, single crystals were obtained by recrystallization from ethanol, which were investigated using x-ray diffraction (Fig. 2).

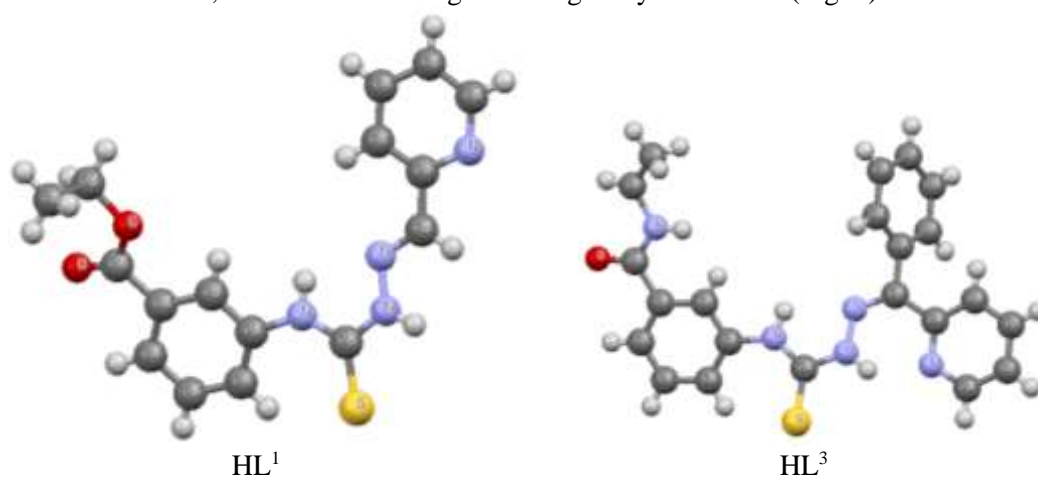
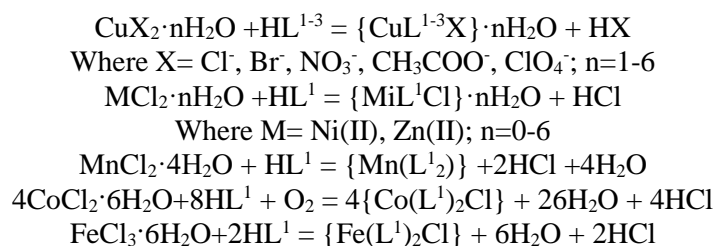


Fig.2. The structures of ligands HL¹ and HL³.

These ligands were further used for the complexation reaction with Cu^{+2} , Mn^{+2} , Fe^{+3} , Co^{+2} , Ni^{+2} and Zn^{+2} metal ions, using the following metal salts: $CuCl_2 \cdot 2H_2O$, $CuBr_2$, $Cu(NO_3)_2 \cdot 3H_2O$, $Cu(CH_3COO)_2 \cdot H_2O$, $Cu(ClO_4)_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$ and $ZnCl_2$ for HL¹; $CuCl_2 \cdot 2H_2O$ for HL² and HL³. The obtained complexes are microcrystalline solids which are stable in air and decompose above 250 °C.

The general method of synthesis of coordinative combinations: to the mixture of 1 mmol (or 2 mmol) ligand and 20 ml of ethanol, add the mixture of 1 mmol metal salt and 5 ml of ethanol to heat ($t = 70-80$ °C) and stir. After mixing the solutions, the reaction mixture immediately changes colour. Gradually a precipitate forms. It is then cooled to room temperature, filtered and dried. Below are presented equations of the reactions for obtaining the coordination compounds:



As we see in reaction written above, as a result of the reactions to obtain the coordination compounds, acid is formed, and experimentally it was proved its presence with pH-meter, which showed pH values = 1-2 of the solutions obtained after complexing in case of HCl, HBr, HNO₃, HClO₄, and pH = 4-5 in the case of the presence of CH₃COOH.

Table 1

Yield, metal analysis and conductivity of synthesized complexes

Nr.	Compound	color	Molecular formula	Yield %	Metal, Nitrogen (calculated/ determined)	Conductivity $\mu\text{S}/\text{cm}$
1.1	[Cu(L ¹)Cl]	green	C ₁₆ H ₁₅ ClCuN ₄ O ₂ S	95.8	14.9, 13.1/ 14.8, 13.0	55.4
1.2	[Cu(L ¹)Br]	green	C ₁₆ H ₁₅ BrCuN ₄ O ₂ S	82.4	13.5, 11.9/ 13.4, 11.9	47.8
1.2	[Cu(L ¹)NO ₃]	green	C ₁₆ H ₁₅ CuN ₅ O ₅ S	89.2	14.0, 15.5/ 14.0, 15.4	54.9
1.4	[Cu(L ¹)H ₂ O]CH ₃ COO	green	C ₁₈ H ₂₀ CuN ₄ O ₅ S	66.7	13.6, 12.0/ 13.5, 11.9	110.8
1.5	[Cu(L ¹)ClO ₄]H ₂ O	green	C ₁₆ H ₁₇ ClCuN ₄ O ₇ S	81.9	12.5, 11.0/ 12.5, 11.1	17.5
1.6	[Mn(L ¹) ₂]	orange	C ₃₂ H ₃₀ MnN ₈ O ₄ S ₂	96.3	7.7, 15.8/ 7.6, 15.8	11.9
1.7	[Fe(L ¹) ₂]Cl	brown	C ₃₂ H ₃₀ ClFeN ₈ O ₄ S ₂	91.9	7.5, 15.0/ 7.4, 14.9	130.2
1.8	[Co(L ¹) ₂]Cl	brown	C ₃₂ H ₃₀ ClCoN ₈ O ₄ S ₂	99.2	7.9, 15.0/ 7.8, 14.9	123.5
1.9	[Ni(L ¹)Cl]	red	C ₁₆ H ₁₅ ClNiN ₄ O ₂ S	82.9	13.9, 13.3/ 13.9, 13.2	42.3
1.10	[Zn(L ¹)(H ₂ O)Cl]	yellow	C ₁₆ H ₁₇ ClNiN ₄ O ₃ SZn	73.5	14.7, 12.6/ 14.6, 12.5	15.6
2.1	[Cu(L ²)Cl]	green	C ₁₇ H ₁₇ ClCuN ₄ O ₂ S	88.9	14.4, 12.7/ 14.3, 12.6	46.3
3.1	[Cu(L ³)Cl]	green	C ₂₂ H ₁₉ ClCuN ₄ O ₂ S	77.7	12.7, 11.2/ 12.6, 11.1	54.7

The coordination compounds were obtained with good yields (67-99%). The metal analysis was performed by titrimetric analysis. Metal analysis showed that the combination ratio to metal and ligand in the complex of copper, nickel, zinc is 1:1, in the complex of manganese, iron and cobalt is 1:2. The determination of nitrogen in compounds (1.1-3.1) was performed by Dumas method. Conductivity value of complexes in DMF (N,N-dimethylformamide) solution greater than 100 $\mu\text{S}/\text{cm}$, suggest that the coordination compounds are ionic. The conductivity value (11-55 $\mu\text{S}/\text{cm}$) of complexes in DMF solution indicates that the complexes are non-electrolytes.

Table 2

IR data of the coordination compounds 3d metals with N(4)(ethyl 3-benzoate) thiosemicarbazone of 2-formyl (2-acetyl, 2-benzoyl)pyridine (HL¹, HL², HL³)

No	Compounds	Frequencies and assignments of IR spectra of compounds (cm ⁻¹)					
		ν (M-S)/ (M-N)	ρ (py in plane)	ν (=N-N=)	ν (C=S)	ν (C-S)	ν (C=N)
4	HL ¹	-/-	622	-	1271/807	-	1606
1.1	[Cu(L ¹)Cl]	413/508	642	1018	-	663	1586
1.2	[Cu(L ¹)Br]	410/501	647	1027	-	663	1593
1.3	[Cu(L ¹)NO ₃]	412/501	640	1019	-	654	1589
1.4	[Cu(L ¹)H ₂ O]CH ₃ COO	410/504	648	1027	-	662	1583
1.5	[Cu(L ¹)ClO ₄]H ₂ O	409/565	684	1020	-	684	1590
1.6	[Mn(L ¹) ₂]	414/508	632	1028	-	666	1585
1.7	[Fe(L ¹) ₂]Cl	419/509	637	1016	-	681	1543
1.8	[Co(L ¹) ₂]Cl	422/515	639	1016	-	667	1557
1.9	[Ni(L ¹)Cl]	418/513	641	1020	-	667	1576
1.0	[Zn(L ¹)(H ₂ O)Cl]	411/515	639	1013	-	677	1584
5	HL ²	-/-	612	-	1283/815	-	1606
2.1	[Cu(L ²)Cl]	410/506	647	1026	-	661	1592
6	HL ³	-/-	633	-	1287/813	-	1605
3.1	[Cu(L ³)Cl]	414/516	644	1010	-	657	1594

Note: ν and ρ denote stretching, bending and deformation; **py**-pyridine.

The IR absorption bands of the synthesized complexes suggest that new bands appear $\nu(\text{M-S}, \text{M-N}, 414, 508)$, which are missing in the spectrum of the ligand, $\nu(\text{py in plane}, 640, \text{cm}^{-1})$ of complexes spectra moves to higher wave numbers in comparison with uncoordinated ligand $\nu(\text{py in plane}, 622, \text{cm}^{-1})$. In spectra of

coordination compounds appear new bands $\nu(\text{C-S}, =\text{N-N}=\text{N}, 663, 1018 \text{ cm}^{-1})$, which are missing in the spectrum of the ligand. In the spectra of coordination compounds the azometin functional group $\nu(\text{C=N}, 1585 \text{ cm}^{-1})$ moves at lower wavelengths, compared to the uncoordinated ligand $\nu(\text{C=N}, 1606 \text{ cm}^{-1})$. According to the data obtained, we can assume that the ligand coordinate tridentate to the metal, coordinating at the central atom through the atoms of pyridine nitrogen, azometin nitrogen and thiol sulphur, forming two metallocycles of 5 atoms.

In the IR spectra it is possible to follow the anions of NO_3^- , CH_3COO^- , ClO_4^- if they coordinate monodentate or bidentate, if they form a bridge and if they are ionic [16]. In the case of complex 1.3, the NO_3^- anion is monodentate, which confirms the vibrations $\nu_5(1454)$, $\nu_1(1276)$, $\nu_2(997)$, $\Delta_{\nu_5-\nu_1}(178)$. In the case of complex 1.4, the anion CH_3COO^- is ionic, which confirms the vibrations $\nu(\text{C=O}, 1583)^a$, $\nu(\text{C-O}, 1418)^a$, $\Delta(165)$. At the complex **1.5**, the ClO_4^- anion plays the role of a monodentate ligand, which confirms the vibrations of $\nu_3(1076)$, broadband, $\nu_4(925)$. Vibrations correspond to the literature [16].

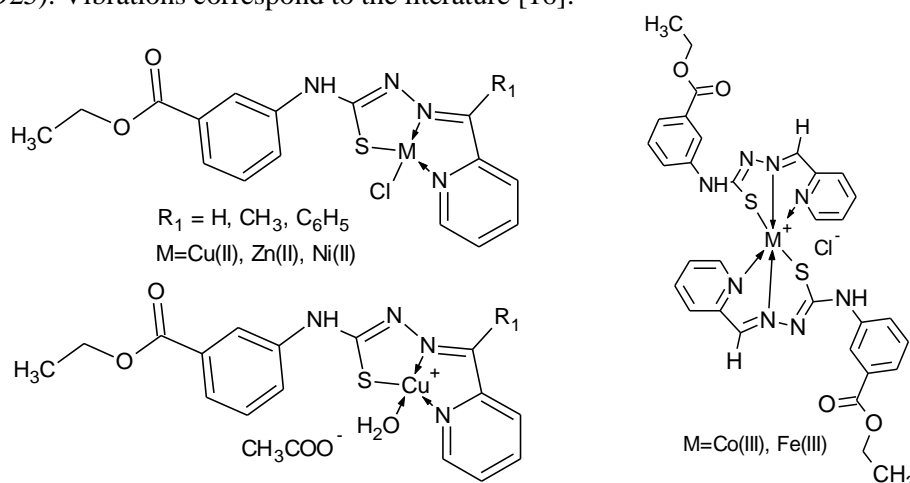


Fig.3. Ways to coordinate thiosemicarbazones based on ethyl 4-aminobenzoate.

Table 3

Antioxidant activity of compounds on $\text{ABTS}^{+\cdot}$ cation radical on concentration of 100 (μM)

No.	Compound	inhibition ($\text{ABTS}^{+\cdot}$), SD (%)
4	HL^1	96.11±0.06
1.1	$[\text{Cu}(\text{L}^1)\text{Cl}]$	51.94±0.10
1.2	$[\text{Cu}(\text{L}^1)\text{Br}]$	52.03±1.64
1.3	$[\text{Cu}(\text{L}^1)\text{NO}_3]$	47.44±0.46
1.4	$[\text{Cu}(\text{L}^1)]\text{CH}_3\text{COO}$	49.78±0.34
1.5	$[\text{Cu}(\text{L}^1)\text{ClO}_4]\text{H}_2\text{O}$	44.17±1.53
1.6	$[\text{Mn}(\text{L}^1)_2]$	77.25±0.64
1.7	$[\text{Fe}(\text{L}^1)_2]\text{Cl}$	66.39±0.05
1.8	$[\text{Co}(\text{L}^1)_2]\text{Cl}$	20.56±2.92
1.9	$[\text{Ni}(\text{L}^1)\text{Cl}]$	94.64±0.21
1.10	$[\text{Zn}(\text{L}^1)\text{Cl} \text{H}_2\text{O}]$	96.72±0.10
5	HL^2	95.69±0.06
2.1	$[\text{Cu}(\text{L}^2)\text{Cl}]$	49.69±2.94
6	HL^3	95.69±0.06
3.1	$[\text{Cu}(\text{L}^3)\text{Cl}]$	37.64±0.32
	Trolox	87.13±0.88

* SD- standard deviation, (%)

Data expressed as mean value \pm SD of triplicate measurements

Antioxidant activity was described by percentage inhibition of thiosemicarbazones HL^{1-3} (at concentration of 100 (μM)) decrease in the series: $HL^1 > HL^2 = HL^3$. So, if we follow in the series of ligands the position N(1) of (pyridin-2-yl)methylidene, the most active are based on 2-formylpyridine (HL^1) 96.11%, then follow 2-acetylpyridine and 2-benzoylpyridine ($HL^2 = HL^3$), which have the same activity 95.69 % .

If we follow the influence of the metal in coordination compounds based on the ligand HL^1 , we obtain the following sequence of decrease: $\text{Zn(II)} > \text{Ni(II)} > \text{Mn(II)} > \text{Fe(III)} > \text{Cu(II)} > \text{Co(III)}$.

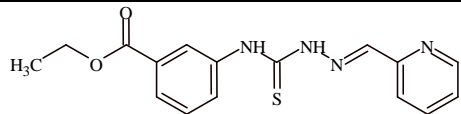
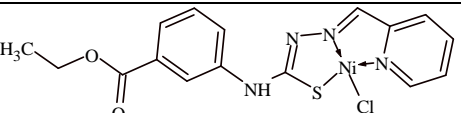
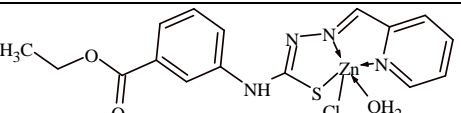
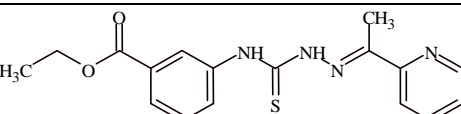
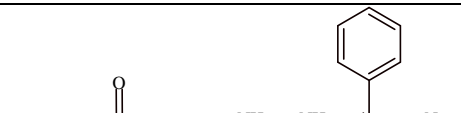
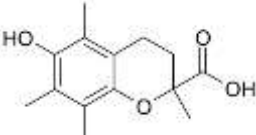
Therefore, the most active coordination compounds are based on Zn (II) 96.72% and Ni (II) 94.64 %, which is the same order compared to the ligand, the activity decreases in the case of Mn(II) 77.25%, Fe(III) 66.39%, Cu(II) 51.94% and Co(III) 20.56% complexes.

If we look at the influence of the anion in the HL^1 ligand series, the most active is $\text{Br}^- > \text{Cl}^- > \text{CH}_3\text{COO}^- > \text{NO}_3^- > \text{ClO}_4^-$.

The substances with the best inhibition of ABTS radicals at a concentration of 100 (μM) were tested for inhibition of ABTS radicals at a concentration of 10 (μM) and 1 (μM), and IC_{50} (semi-maximal inhibition concentration) was calculated.

Table 4

Antioxidant activity of compounds, data expressed in IC_{50} (μM)

No.	Compound	C (μM)	% inh (ABTS)	SD (%)	IC_{50} (μM)	SD (μM)
4		100	96.11	0.06	13.15	0.15
		10	39.79	0.36		
		1	9.34	0.30		
1.9		100	94.64	0.21	20.48	0.09
		10	22.00	0.22		
		1	8.22	0.21		
1.10		100	96.72	0.10	11.45	0.10
		10	43.72	0.29		
		1	11.42	0.22		
5		100	95.69	0.06	11.53	0.04
		10	43.96	0.18		
		1	10.21	0.36		
6		100	95.69	0.06	16.47	0.08
		10	30.52	0.10		
		1	4.79	0.45		
Trolox		100	87.13	0.88	15.86	1.53
		10	34.13	1.24		
		1	16.19	1.5		

Data expressed as mean value \pm SD of triplicate measurements.

After IC_{50} calculation, the best result was found to be in the coordinating compound $[\text{Zn}(\text{L}^1)\text{Cl}]$, whose IC_{50} is 11.45 μM and it is better than trolox 15.86 μM (used in medicine), followed by the ligand (HL^2) **5**, whose IC_{50} is 11.53 μM , then HL^1 with IC_{50} equal to 13.15 μM and HL^3 with IC_{50} – 16.47 μM .

Conclusions

1. Three new thiosemicarbazones based on ethyl 3-benzoate were synthesized;
3. Twelve coordination compounds based on these ligands (HL^{1-3}) were synthesized;

2. The purity, composition and structure of the compounds were determined using melting point, thin layer chromatography, elemental analysis, conductivity, NMR and IR spectral data and single crystal X-ray analysis;

5. The best result of antioxidant activity at coordination compound $[Zn(L^1)Cl]$, which IC_{50} is 11.45 μM , were recorded.

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Data about authors:

Anna RUSNAC, PhD student, scientific researcher in Laboratory of Advanced Materials in Biopharmaceuticals and Technics, State University of Moldova.

E-mail: ana.rusnac@usm.md

ORCID: 0000-0002-2517-8927

Olga GARBUZ, PhD, scientific researcher in Laboratory of Advanced Materials in Biopharmaceuticals and Technics, State University of Moldova and Laboratory of Systematics and Molecular Phylogeny, Institute of zoology.

E-mail: olhamos@mail.ru

ORCID: 0000-0001-8783-892X

Sergiu SHOVA, PhD, senior scientific researcher in Laboratory of Advanced Materials in Biopharmaceuticals and Technics, State University of Moldova and Institute of Macromolecular Chemistry "Petru Poni": Iasi, Romania.

E-mail: shova@icmpp.ro

ORCID: 0000-0002-1222-4373

Aurelian GULEA, academician, doctor habilitate, university professor. Laboratory of Advanced Materials in Biopharmaceuticals and Technics, State University of Moldova.

E-mail: guleaaurelian@gmail.com

ORCID: 0000-0003-2010-7959

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