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Synthesis and Structure of COPPER (II) Coordination Compounds with 4-N-SUBSTITUTE-THIOSEMICARBAZONE OF 4-BENZOIL-5-METHYL-2-PHENYL-2,4-DIHIDRO-3H-PYRAZOL-3-ONE. Antioxidant, Antimicrobial and Antitumor Properties

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Abstract: *In this paper methods of synthesis of four Cu (II) coordination compounds with two thiosemicarbazones tridentate bibasic ligands are described. Ligands are coordinated through the atoms of enolic oxygen, aomethine nitrogen and thionic sulfur. The synthesis of the coordination compounds was performed at the interaction of the ethanol solutions of copper (II) salt, respectively chloride or bromide, with the ethanol solution of H2L1 or H2L2 ligand, and refluxing for 4 hours in the ethanol. The solution results in dark green microcrystalline powders, which are recrystallized from ethanol or N, N-dimethylformamide. The obtained single crystals were investigated by X-ray diffraction on the single crystal, elucidating their structures. In the case of the coordination compound [Cu(HL1)Cl]C2H5OH and [Cu(HL1)Cl]DMF have the same set of coordinating atoms through the atoms of enolic oxygen, aomethine nitrogen and thionic sulfur at the central atom, only the solvent from the outer- sphere differs. In the case of copper (II) bromide and chloride with H2L1 the isostructural Cu (II) coordination compound is obtained. The copper (II) coordination compounds of H2L2 form dimers by oxygen bonding of the phenolic oxygen atom. Only in the case of copper (II) bromide was it possible to determine the structures by X-ray diffraction on a single crystal, showing the dimer [Cu2(HL2)2Br2] DMF, the molecule of N, N-dimethylformamide as a solvent that entered the crystalline network of the coordination compound. Antiproliferative activity of [Cu(HL1)Cl]C2H5OH, [Cu(HL1)Br]C2H5OH and H2L1 against HEp-2, BxPC-3, RD, L20B is moderate. The best antimicrobial activity results for [Cu2(HL2)2Br2]DMF with a MIC - 0.031 mg/mL.*
Keywords: *coordination compounds, thiosemicarbazone, antimicrobial, anticancer, antioxidants*

Introduction

Coordination compounds of 3*d* metals have lately taken the news in the field of chemistry. Relating the activity structure relationship and the assumption of potential drugs based on their coordinating compounds is the aim of the researchers in the investigated literature. The tendency to create new molecules of biological character based on thiosemicarbazones puts many in the situation of proposing new ideas and methods, as well as the synthesis of the precursors that lead to the production of thiosemicarbazones. In world practice, the design of molecules with anticancer properties has

developed predominantly in the direction of the synthesis of *cis-platinum* combinations and steroid compounds. The application of platinum compounds to the treatment of cancer today has multiple adverse effects. The problem of assembling new agents to inhibit the proliferation of cancer cells, which are efficient and slightly toxic, remains a current one [1]. Pyrazolonic skelton is the basis of many drug products such as: CELECOXIB a strong anti-inflammatory, anti-psychotic CDPPB, anti-obesity REMONABANT, DIFENAMIZOL an analgesic, BETAZOL an H_2-receptor agonist and FEZOLAMIDA antidepressant agent, which have been shown to be pharmacologically active containing the pyrazole moiety. Pyrazole and its derivatives are considered a pharmacologically active scaffold that possesses almost all types of pharmacological activities [4]. Due to this diversity in the biological field, this nucleus has attracted the attention of many researchers to study its chemical and biological skeleton [4]. Antipyrine or 1,2-dihydro-1,5-dimethyl-2-phenylpyrazole-3-one in a structural frame consists of a five-membered lactam pyrazolone heterocyclic ring as a pharmacophore moiety [3]. Commercially available pyrazolone derivatives as drugs, analgin and metamizol are an established chemical class of analgesics. Indeed, the synthesized derivatives possess antipyrine moiety having versatile biological properties, antimicrobial, antitubercular, anthelmintic, antioxidant, analgesic, antiinflammatory, cytotoxic and antiviral activities [3]. In the last three decades such ligands have been used to coordinate most of 3*d*-metal ions, and many metal complexes have been investigated as pharmacophore moiety [11]. Thiosemicarbazones are important precursors for the synthesis of biologically active coordinating compounds. The thiosemicarbazones derived from 4-benzoyl-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazole-3-one have been highlighted as a result of their particular structural and biological properties [2]. The generator of a complex is held by a place no less main attraction due to its theoretical and practical significance, including catalytic, photochromic properties and biological activity [2]. The antimicrobial properties of some Schiff bases are 10 times better than some of the coordinating compounds of copper [5], but these are usually exceptional because in most cases the coordinating compounds of the traction metals are more active than their precursors (thiosemicarbazones/schiff base) [5-6].

This work is the result of our systematic studies in this field, such as the research of the structure-activity relationship. The synthesis and characterization of two new N4-substituents-thiosemicarbazones derived from 4-benzoyl-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazole-3-one (H_2L_1, H_2L_2) are reported the chelating behavior of these ligands relative to copper (II). The molecular structures of the coordinating compounds 1, 2, 3 have been demonstrated by single-crystal X-ray diffraction. Given their possible biological properties, we tested ligands and coordination compounds for their *in vitro* antiproliferative activity on HEp-2, BxPC-3, RD, MDCK, L20B cells at three concentrations. But for a deeper study, the antimicrobial properties of the copper (II) coordination compounds on gram-positive organisms were also investigated.

1. Experimental

1.1 Materials

4-Benzoil-5-methyl-2-phenyl-2,4-dihidro-3H-pyrazol-3-one and cyclohexylamine, carbon disulfide, triethylamine, ethyl chloroformate, hydrazine hydrate, TMDT (Sigma Aldrich, Munich, Germany) were used as received. CuBr2, CuCl2□2H2O (Merck, Darmstadt, Germany) were used as supplied. Solvents used for the reactions were purified and dried by conventional methods [12].

1.2. Synthesis of Ligands

The synthesis of the thiosemicarbazone H_2L_1 took place according to the scheme in figure no.1.

The mixture consisting of 1.98 g (20 mmol) cyclohexylamine and 2.02 g (20 mmol) triethylamine ($(Et)_3N$) to which 1.52 g (20 mmol) carbon disulfide (CS_2) was added for 10 min. at 0 °C, then stirred at room temperature for one hour. The desulfurization reagent is added to the formed product which according to the scheme of figure 1, can be: a) iodine (I_2); b) ethyl chlorformate (ClCOOEt); or c) 2,4,6-trichloro-1,3,5-triazine (TCT) with a 1:1 molar ratio in benzene. The organic residue was refluxed for 25 hours with 25 mL of benzene, then cooled to 25 °C. After regeneration of benzene by distillation, a light yellow oil is obtained. The synthesis of cyclohexyl-thiosemicarbazide is obtained at the interaction of cyclohexyl-isothiocyanate (corresponds to the literature data [10]) with 1 g (20

mmol) of hydrazine monohydrate in stoichiometric ratio. Condensation of cyclohexyl-thiosemicarbazide with 5.56 g (20 mmol) of 4-benzoyl-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazole-3-one occurs at ethanol reflux for 12 hours. Yield: 5.21 g (60%), R_f= 0.55 (benzene-isopropanol 1:3), m.p. = 208-209 ^{0}C, yellow crystalline crystals, corresponds to the literature data [8]. The purity was confirmed by layer chromatography as well as ^{1}H and ^{13}C-NMR, FTIR.

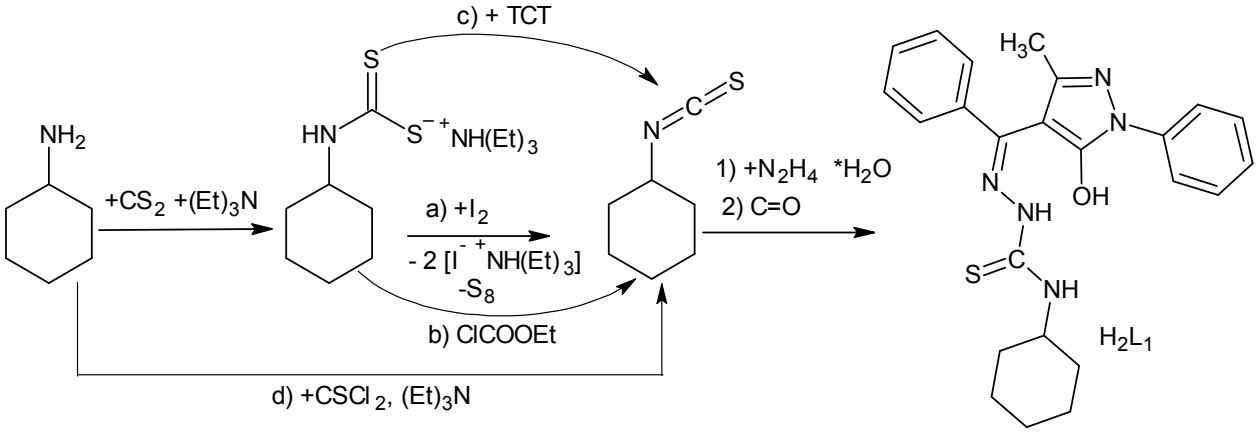

Figure no. 1. Scheme of synthesis H_2L_1.
N-cyclohexyl-2-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(phenyl)methylene)-hydrazinecarbothioamide (H_2L_1)

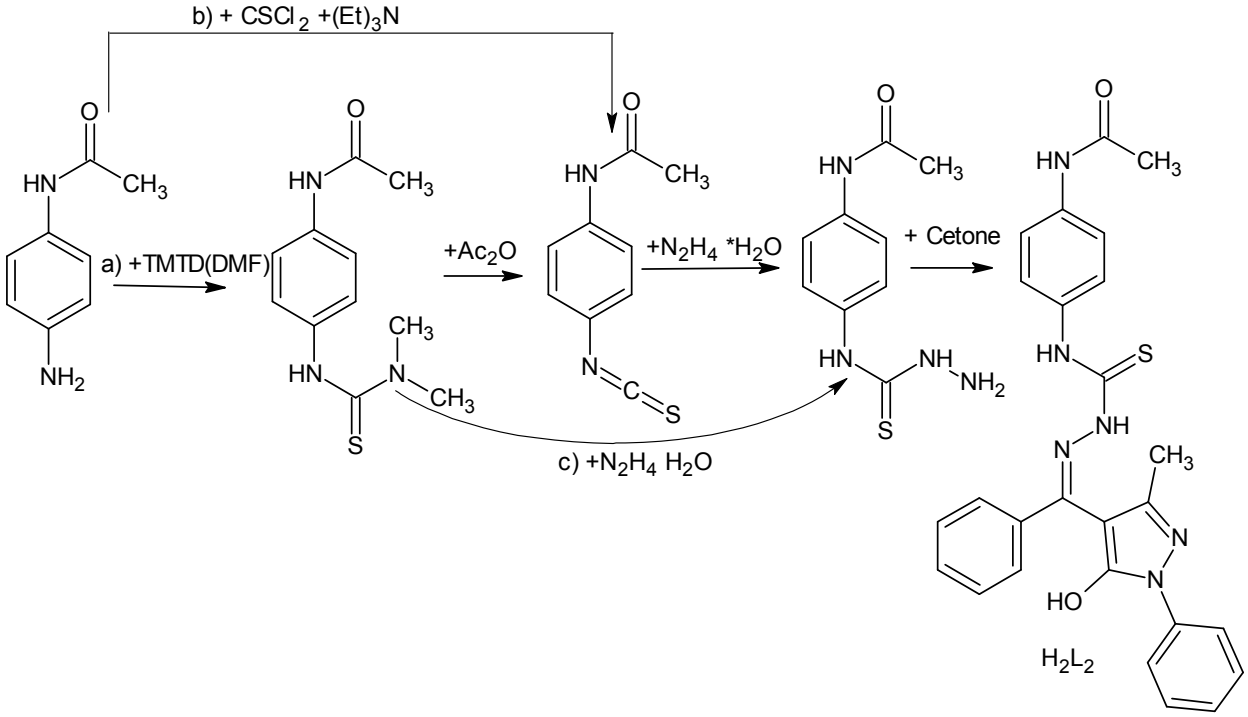

Figure no. 2. Scheme of synthesis H_2L_2.
N-(4-(2-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(phenyl)methylene)hydrazine-carbothioamido)phenyl)acetamide (H_2L_2)

The mixture of 3.0 g (20 mmol) N-(4-aminophenyl) acetamide and 2.4 g (10 mmol) tetra-methylthiuram disulfide (DTMT) and 5 mL of dimethylformamide (DMF) was heated for one hour at 100^{0}C, then the solvent is distilled under reduced pressure. The organic residue was refluxed for 25 minutes with 25 mL of benzene, then cooled to 25^{0}C. Sulfur remains in solution, and the solid is recrystallized from butanol. At the interaction of the reaction product with 2.04 g (20 mmol) acetic anhydride and 30 mL of 1,4-dioxane obsolut is heated for one hour at 100 ^{0}C. Then part of the dioxane

is distilled and the rest is diluted with water and cooled. The precipitate was filtered and recrystallized from ethylacetate. The next step is the synthesis of N-{4-[(hydrazinylcarbonothioyl)amino]phenyl} acetamide which occurs at the interaction of 1 g (20 mmol) hydrazine hydrate with the isothiocyanate (corresponds to the literature data [10]) of the preceding step in 15 mL of dioxane, then heated to 90-95^{0}C for two hours, then cool to room temperature. The reaction product is purified by recrystallization from ethanol and then condensation with 5.56 g of 4-benzoyl-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazole-3-one is carried out for 16 hours under reflux of ethanol. Yield: 6.29 g (65%), R_f = 0.36 (benzene-isopropanol 1:3), m.p. = 183-185 ^{0}C, matte yellow crystals. It corresponds to the literature data [7,9]. The purity was confirmed by layer chromatography as well as ^{1}H and ^{13}C-NMR, FTIR.

1.3. General Procedure of Preparation of Metal Complexes.

1.3.1 Synthesis of $[Cu(HL_1)Cl]C_2H_5OH$

An ethanol solution (10 mL) of $CuCl_2 \cdot 2H_2O$ (0.1705 g, 1 mmol) was added to stirred ethanol solution (25 mL) of H_2L_1 (0.4336 g, 1 mmol). The resulting solution was stirred for 2 h at reflux. After a few days, the brown-coloured solid cristaline was filtred, washed with hot ethanol, then diethyl ether and dried *in vacuo* in the presence of anhydrous $CaCl_2$. Yield 82 %; m.p. >350 ^{0}C; Molecular weight: 578.06 g/mol. Anal. Calcd. For $C_{26}H_{32}ClCuN_5O_2S$ (%): Cu 11.0 . Found (%): 11.03. Selected FT-IR peaks (Bruker ALPHA), cm^{-1}: ν(N-H, Ar-H) 3100-3033; ν(C-$H_{as/sy}$ from Cy)2930, 2850; ν(C=N) 1589; ν(C-OH_{enol})-1127; ν(Cu-$N_{azom.}$)523; ν(Cu-O)499; ν(Cu-S)415.

1.3.2. Synthesis of $[Cu(HL_1)Br]C_2H_5OH$

An ethanol solution (10 mL) of $CuBr_2$ (0.2234 g, 1 mmol) was added to stirred ethanol solution (25 mL) of H_2L_1 (0.4336 g, 1 mmol). The resulting solution was stirred for 2 h at reflux. After a few days, the brown-colored solid crystalline was filtered, washed with hot ethanol, then diethyl ether and dried *in vacuo* in the presence of anhydrous $CaCl_2$. Yield 85 %; m.p. >350 ^{0}C; Molecular weight: 622.07 g/mol. Anal. Calcd. For $C_{26}H_{32}BrCuN_5O_2S$ (%): Cu 10.22 . Found (%): 10.19. Selected FT-IR peaks (Bruker ALPHA), cm^{-1}: ν(N-H, Ar-H) 3378-3049; ν(C-$H_{as/sy}$ from Cy)2929, 2845; ν(C=N) 1588; ν(C-OH_{enol})-1150; ν(Cu-$N_{azom.}$)551; ν(Cu-O)485; ν(Cu-S)396.

1.3.3 Synthesis of $[Cu_2(HL_2)_2Br_2]DMF$

An ethanol solution (10 mL) of $CuBr_2$ (0.2234 g, 1 mmol) was added to stirred ethanol solution (35 mL) of H_2L_1 (0.4846 g, 1 mmol). The resulting solution was stirred for 3 h at reflux. After two days, the brown-colored solid crystalline was filtered, washed with hot ethanol, then diethyl ether and dried *in vacuo* in the presence of anhydrous $CaCl_2$. Yield 95 %; m.p. >350 ^{0}C; Molecular weight: 1327.02 g/mol. Anal. Calcd. For $C_{55}H_{53}Br_2Cu_2N_{13}O_5S_2$ (%): Cu 9.64 . Found (%): 9.70. Selected FT-IR peaks (Bruker ALPHA), cm^{-1}: ν(N-H, Ar-H) 3356-3026; ν(C-$H_{as/sy}$ from Cy)2956, 2850; ν(C=N) 1580; ν(C-OH_{enol})-1130; ν(Cu-$N_{azom.}$)541; ν(Cu-O)482; ν(Cu-S)391.

1.3.4 Synthesis of $\{Cu_2(HL_2)_2Cl_2\}DMF$

An ethanol solution (10 mL) of $CuCl_2 \cdot 2H_2O$(0.1705 g, 1 mmol) was added to stirred ethanol solution (35 mL) of H_2L_1 (0.4846 g, 1 mmol). The resulting solution was stirred for 3 h at reflux. After two days, the brown-colored solid crystalline was filtered, washed with hot ethanol, then diethyl ether and dried *in vacuo* in the presence of anhydrous $CaCl_2$. Yield 84 %; m.p. >350 ^{0}C; Molecular weight: 1238.12 g/mol. Anal. Calcd. For $C_{55}H_{53}Cl_2Cu_2N_{13}O_5S_2$ (%): Cu 10.33. Found (%): 10.39. Selected FT-IR peaks (Bruker ALPHA), cm^{-1}: ν(N-H, Ar-H) 3321-3041; ν(C-$H_{as/sy}$ from Cy)2960, 2867; ν(C=N) 1575; ν(C-OH_{enol})-1164; ν(Cu-$N_{azom.}$)565; ν(Cu-O)479; ν(Cu-S)402.

1.4 Physical Measurements

The chemical elemental analysis of metal-titrimetric method. NMR spectra: Bruker 400MHz (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz), the spectra were recorded in DMSO-*d6* using TMS as internal standard and are reported in ppm. ^{1}H NMR data are reported as: (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, ArH = aromatic, integration, proton assignment). In the ^{1}H NMR spectra, signal positions (δ) are given in parts per million (ppm) from tetramethylsilane (δ = 0) and were measured relative to residual solvent signal (DMSO-*d6* = 2.50 ppm, NMR water signals = 3.3 ppm).

^{13}C NMR spectra were recorded using the same spectrometers. Signal positions (δ) are given in parts per million (ppm) from tetramethylsilane (δ = 0) and were measured relative to the signal of DMSO-d6 (δ= 39.5 ppm). Reagents were purchased and used without further purification. All significant resonances (carbon skeleton) were assigned by DEPT-135 correlations. FTIR-ATR spectra were recorded in powder form on the Bruker ALPHA apparatus in the wavelength range 4600-360 cm^{-1}. The spectral results were interpreted using OPUS version 7.5. The multiplicities of signals are given as strength/shape s = strong, m = medium, w = weak. All other commercially available solvents were used without purification. Thin-layer chromatography was carried out on silica gel 60 F254 (Merck). Column chromatography was performed on silica gel 60 (70–230 mesh, Merck). The organic solutions were dried over $MgSO_4$ or Na_2SO_4.

1.5. X-ray Crystallography

Crystallographic measurements of coordination compounds were carried out with an Oxford-Diffraction XCALIBUR E CCD diffractometer (Santa Clara, CA, USA) equipped with graphite-monochromated Mo K_α radiation. The unit cell determination and data integration were carried out using the CrysAlis package of Oxford Diffraction (CrysAlisProAgilentTechnologies, Version1.171.34.49 (release20-01-2011 CrysAlis171.net; compiled Jan 20 2011, 15:58:25)). All structures were solved by direct methods using SHELXS-97 and refined by full-matrix least-squares on F_o^2 with SHELXL-97. All atomic displacements for non-hydrogen, non-disordered atoms were refined using an anisotropic model. The geometric parameters were calculated and the figures were drawn with the use of the PLATON program. The hydrogen atoms that are not involved in the hydrogen bonding were omitted from the generation of the packing diagrams.

1.6. Biological Studies

1.6.1. Antimicrobial (antibacterial) Activity

The antimicrobial activity of the synthesized compounds was tested against *Staphylococcus aureus* ATCC 25923. Antibacterial activity of the synthesized compounds are assessed using the microdilution broth test, which allows determination of minimum inhibitory concentration (MIC) and minimum bactericide/fungicide concentration (MBC/MFC) [1,2]. For MIC assays a stock solution (10 mg/mL) of each test compound was prepared in dimethylsulfoxide (DMSO). This stock solution was then diluted in Muller Hinton Broth (MHB) for bacteria. Further, a serial dilution of test compounds were carried out to achieve concentrations ranging from 500 to 0,24 μg/mL. One hundred microliters of each concentration was introduced into a well (96-well microplate) containing 90 μl of RPMI or MHB, and 10 μl of inoculum (1×10^6 CFU/mL for bacteria and 1×10^5 CFU/ml for yeasts) was added. Plates were covered and incubated on the shaker at 37°C for 24 h (bacteria). MICs were assessed visually after the corresponding incubation period and were taken as the lowest sample concentration at which there was no growth or virtually no growth. For the minimum bactericidal (MMC) determination, 10 μl aliquots from each well that showed no growth of microorganism were plated on Mueller-Hinton Agar or Sabouraud Dextrose Agar and incubated at 37°C for 24 h (bacteria). The lowest concentration that yielded no growth after the subculturing was taken as the MBCs or MFCs. Furacilin for bacteria and nystatin for yeasts were used as positive controls and broth with 20 μL of DMSO was used as a negative control. All the experiments were carried out in triplicates.

1.6.2. Antiproliferative Activity

The three test substances (ligand H_2L_1 and its metal complexes)were dissolved in DMSO (Sigma□Aldrich,Ayrshire,UK) in order to obtain 5 μM stock solutions. The solutions were stored at 2–8°C. Each stock solution was formulated in various concentrations (0.1, 1, 10 or 100 μM) to obtain the half maximal inhibitory concentration (IC_{50}) values, as follows. By successive dilution of the stock solutions with the specific culture medium for each cell line, five final solutions covering a concentration range between 1 and 10 μM for all complexes were obtained.

2. Results and Discussion

The synthesis of the thiosemicarbazones H_2L_1 and H_2L_2 was carried out at the condensation of the corresponding thiosemicarbazides with 4-benzoyl-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazole-3-one in a molar ratio of 1:1, the reaction products were purify chromatographically, recrystallize and

spectrally characterize. Copper (II) coordination compounds were obtained by the interaction of the bromide/chloride copper salts with the ethanolic solution of H_2L_1 and H_2L_2 ligands in a ratio of 1:1. In the case of three coordination compounds, were obtained crystals which were investigated by X-ray diffraction on a single crystal. The structures of the coordination compounds are shown in the following figures:

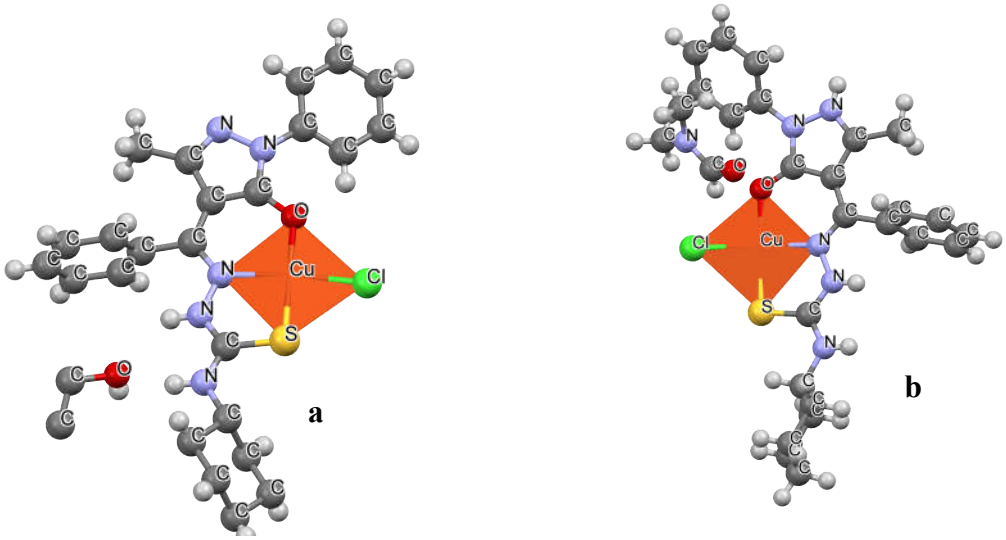

Figure no. 3. Molecular structure of a) $[Cu(HL_1)Cl]C_2H_5OH$ and b) $[Cu(HL_1)Cl]DMF$

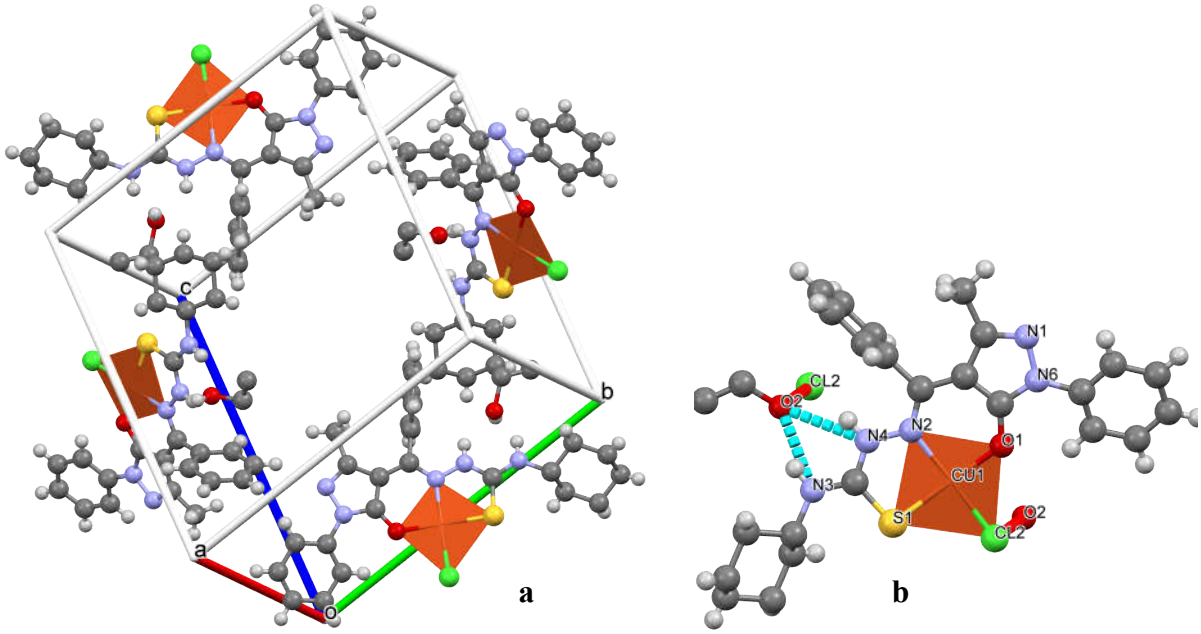

Figure no. 4. Crystallografic structure of $[Cu(HL_1)Cl]C_2H_5OH$, a) packing and b) H-bond

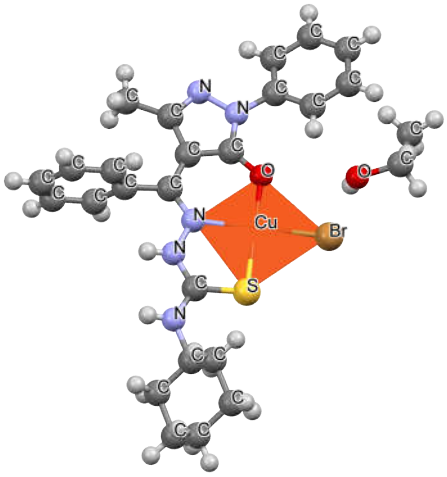

Figure no. 5. Molecular structure of $[Cu(HL_1)Br]C_2H_5OH$

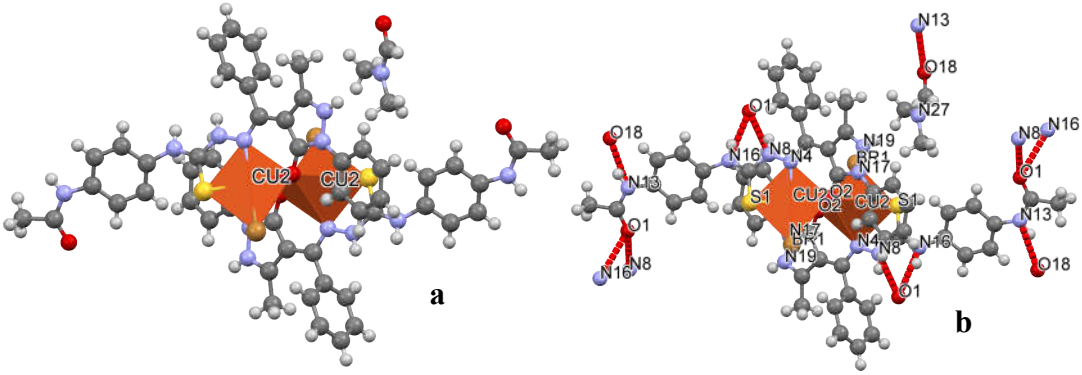

Figure no. 6. Molecular structure of a) $[Cu_2(HL_2)_2Br_2]$DMF and b) H-bond

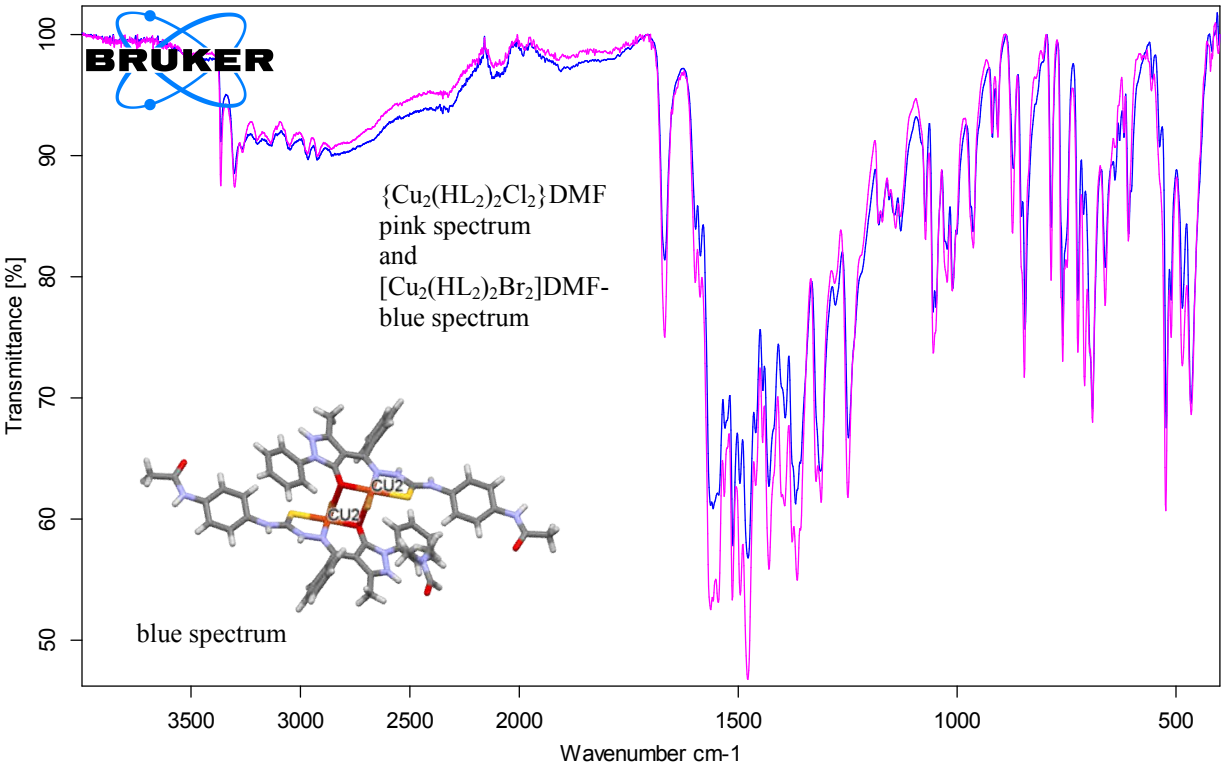

Figure no. 7. FT-IR spectra of compound $\{Cu_2(HL_2)_2Cl_2\}$DMF-pink spectrum, and $Cu_2(HL_2)_2Br_2]$DMF- blue spectrum

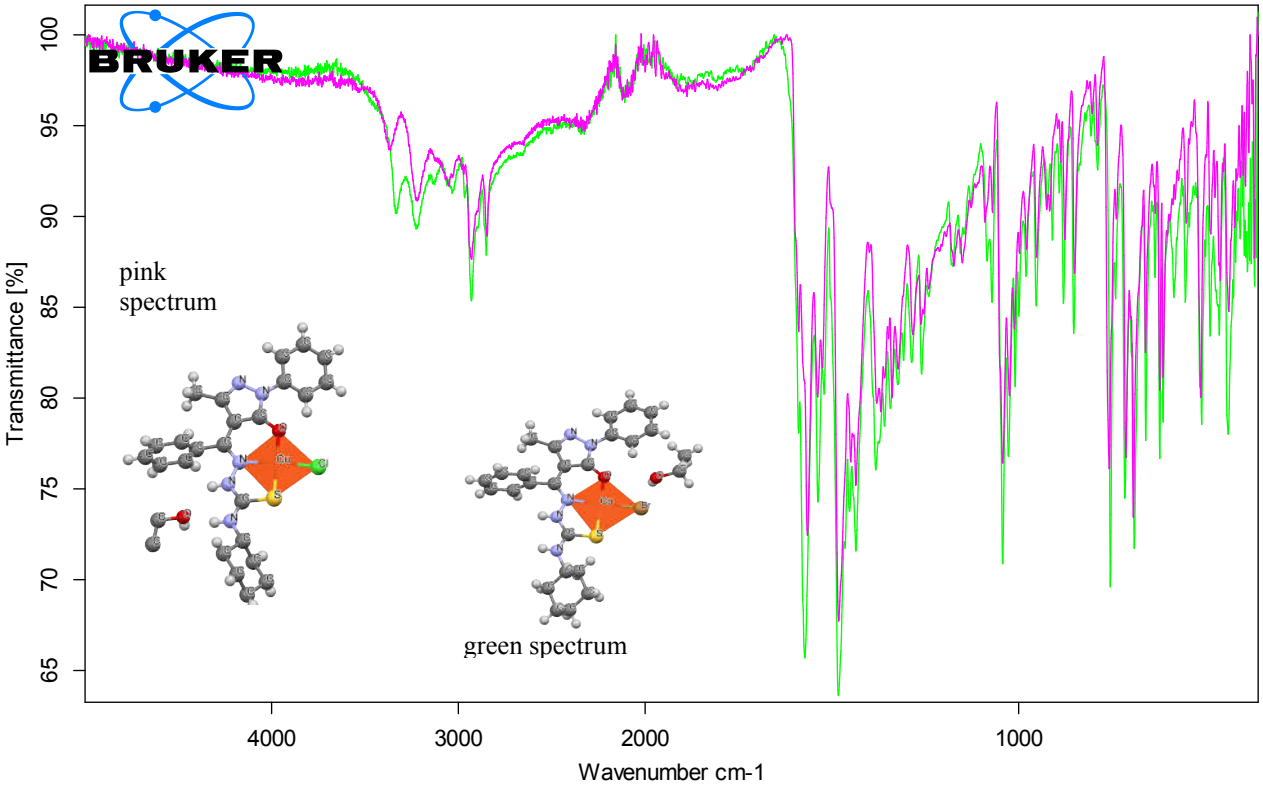

Figure no. 8. FT-IR spectra of compound $[Cu(HL_1)Br]C_2H_5OH$ -green spectrum, and $[Cu(HL_1)Cl]C_2H_5OH$ - pink spectrum

Table no. 1
Antiproliferative activity of copper (II) coordination compounds with H_2L_1 for cells HEp-2 and BxPC-3 at 100 µM

Compound / Cells	HEp-2				BxPC-3			
	% inh	SD	IC_{50}	SD	% inh	SD	IC_{50}	SD
$[Cu(HL_1)Cl]C_2H_5OH$	93,7	1,5	**24,5**	0,9	95,8	8,5	**12,7**	0,5
$[Cu(HL_1)Br]C_2H_5OH$	93,1	5,5	**8,8**	0,4	105,5	0,3	**4,7**	0,6
H_2L_1	6,8	8,1	-		24,3	6,1	-	

Table no. 2
Antiproliferative activity of copper (II) coordination compounds with H_2L_1 for cells RD, MDCK and L20B at 100 µM

Compound / Cells	RD				MDCK				L20B			
	% inh	SD	IC_{50}	SD	% inh	SD	IC_{50}	SD	% inh	SD	IC_{50}	SD
$[Cu(HL_1)Br]\ C_2H_5OH$	104,4	3,0	**13,0**	0,1	100,9	0,2	**13,5**	0,1	109,4	2,5	**4,8**	3,5
$[Cu(HL_1)Cl]\ C_2H_5OH$	102,0	1,0	**12,5**	0,5	103,6	0,4	**12,6**	0,2	110,0	3,2	**5,0**	3,0

Table no. 3
Antibacterial activity of complexes (MIC[a]/MBC[b] values) for *Staphylococcus aureus*

Coordination Compound	MIC mg/mL	MBC mg/mL
$[Cu_2(HL_2)_2Cl_2]DMF$	0.063	0.125
$[Cu_2(HL_2)_2Br_2]DMF$	0.031	>0.500
$[Cu(HL_1)Cl]C_2H_5OH$	0.250	0.500
$[Cu(HL_1)Br]C_2H_5OH$	0.250	0.500

Staphylococcus aureus (ATCC 25923), Gram-positive bacteria
[a]MIC, minimum inhibitory concentration
[b]MBC, minimum bactericidal concentration

Conclusions

The composition of the four new obtained coordination compounds was confirmed using analytical and physico-chemical analysis. Ligand H_2L_x is mononegative and tridentate. The structure of $[Cu(HL_1)Br]C_2H_5OH$, $[Cu(HL_1)Cl]C_2H_5OH$, $[Cu_2(HL_2)_2Br_2]DMF$ has been determined by single-crystal X-ray diffraction, were Cu(II) has a square-planar geometry the can of H_2L_1, and the Cu(II) has a square pyramidal core in the case of H_2L_2. Antiproliferative activity of $[Cu(HL_1)Cl]C_2H_5OH$, $[Cu(HL_1)Br]C_2H_5OH$, H_2L_1 against HEp-2, BxPC-3, RD, L20B is moderate. The best antimicrobial activity results for $[Cu_2(HL_2)_2Br_2]DMF$ with MIC - 0.031 mg/mL.

Acknowledgements

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