SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF 5-PYRAZOLONE DERIVATIVES AND THERE CU(II), NI(II), CO(II) AND V(V) COMPLEXES

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Summary. Complex combinations of Cu(II), Ni(II), Co(II) and V(V) with thiosemicarbazone derivatives of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone-4-R-thiosemicarbazone (where R = CH3, C2H5, C6H5, C5H5N) were synthesized. The characterization of the newly formed compounds was done by 1H NMR, 13C NMR, IR, UV–Vis spectroscopy, elemental analysis, molar electric conductibility, magnetic susceptibility measurements and thermal analysis. For the copper(II) complexes metal–ligand bonding parameters have been evaluated from the EPR spectra. In addition, the structures of the ligands (HL1-3) and its copper(II) (1, 6, 8, 12), nickel(II) (3, 13) and vanadium(V) (9) complexes were determined by single-crystal X-ray diffraction. The composition of the coordination polyhedron of the central atom in complexes is different. The complexes and ligands were tested for their in vitro antiproliferative activity on human leukemia HL-60. Antiproliferative activity of copper(II) complexes (1, 2, 5 - 8, 11, 12, 15, 16) at 10 μM is similar to doxorubicin, used in medical practice as antileukemia drug. The IC50 values were found to be 0.24 μM for complex 2, 0.3 μM for complex 6 and 0.36 μM for complex 11 reveal the potential antiproliferative of these compounds.

Keywords: 5-pyrazolones, Thiosemicarbazones, Metal complexes, Antiproliferative agents, HL-60 leukemia cells.

SINTEZA ȘI ACTIVITATEA ANTIPROLIFERATIVĂ A DERIVAȚILOR 5-PYRAZOLONEI ȘI A COMPUȘILOR COMPLECȘI AI CU(II), NI(II), CO(II) ȘI V(V)

Rezumat: Au fost sintetizate combinațiile complexe ale Cu(II), Ni(II), Co(II) și V(V) cu derivații thiosemicarbazonelor 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone-4-R (unde R = CH3, C2H5, C6H5, C5H5N). Caracterizarea compușilor noi formați a fost efectuată cu ajutorul spectroscopiilor 1H NMR, 13C NMR, IR, UV–Vis, analiza elementală, conductibilitatea electrică molară, măsurătorilor suscibilității magnetice și analizei termice. Pentru complecșii cuprului(II) parametrii legăturii metal-ligand au fost estimate din spectrele RES. În continuare au fost identificate structurile pe monocristal ai liganzilor(HL1-3) și ai complecșilor de cupru (1, 6, 8, 12), nichel (3, 13) și vanadiu(V) (9) utilizând difracția cu raze X. Compoziția poliedrului de coordinare a atomului central în complecși este diferită. Complecșii și liganzii au fost testați la activitatea antiproliferativă in vitro pe celule de leucemie mieloidă umană HL-60. Activitatea antiproliferativă a complecșilor de cupru(II) (1, 2, 5 - 8, 11, 12, 15, 16) la 10μM este similară cu cea a doxorubicinei, utilizată în practică ca medicament la tratamentul leucemiei. Valorile IC50 variază de la 0,24 μM pentru complexul 2 la 0,30 μM pentru complexul 6 si la 0,36 μM pentru complexul 11 punând astfel în valoare perspectiva de utilizare a acestora în practica medicinală.

Cuvinte-cheie: 5-pyrazolone, Thiosemicarbazone, complexe metalice, agenți antiproliferativi, HL-60 celule de leucemie.

1.INTRODUCTION

Cancer is one of the most aggressive diseases of mankind [1]. The uncontrolled proliferation of the tumour cells is their specific feature [2]. The present strategies of antitumour treatment involve several techniques: surgery, radiotherapy and chimotherapy. Despite many decades of research, the long term perspectives for the patients with aggressive cancer are still discouraging. Thus, there is the necessity to discover new antitumour inhibitors, with therapeutic effectiveness and reduced toxicity [3, 4].

The thiosemicarbazones and their complex combinations drew the attention of researchers in the fields of chemistry and medicine [5-7].

From all the antiviral agents synthesized in the last two decades, thiosemicarbazones are, certainly, one of the most interesting classes of organic compounds [8].

The research carried out on these compounds [9-11] have proven their antitumoral action as well as the link between viruses and malign tumours. The pharmaceological activity of thiosemicarbazones is accounted for through their ability to form chelates with the metals present in the lifecells.

The interest for thiosemicarbazones as well as for their complex combinations is due to the wide range of biological properties manifested by these compounds, such as the antitumour, antibacterial, antifungic, antioxidant, antituberculosis and anti HIV activity [12-18]. Also, pyrazolone derivatives represent a class of organic compounds that has been extensively studied due to a broad spectrum of biological activities [19-21]. In the field of anticancer research, certain pyrazolones exhibited the promising antiproliferative activity against human myelogenous leukemia HL-60 [22] and human ovarian carcinoma OVCAR3 cell lines [23]. The metal complexes derived from 5-pyrazolone have stood out through their antitumour activity (HL-60, B16, Eca-109, A2780cisR) [24, 25].

This work is a presentation of the synthesis of some complex combinations with thiosemicarbazones derived from 5-pyrazolone and *in vitro* research of their antiproliferative activity on human leukemia HL-60.

2. EXPERIMENTAL

2.1. Thiosemicarbazones derived from 5-pyrazolone: Structure and synthesis.

The thiosemicarbazones is an important class of ligands cu atoms which are donors of *N* and *S*. The importance of thiosemicarbazones within coordinative chemistry consists in the ability of these organic compounds to function as multidentate ligands.

The study of thiosemicarbazones derived from 5-pyrazolone and of their complex combinations with different metal ions stirred the interest of many researchers.

The synthesis methods are based on the condensation reaction, according to the scheme 1.

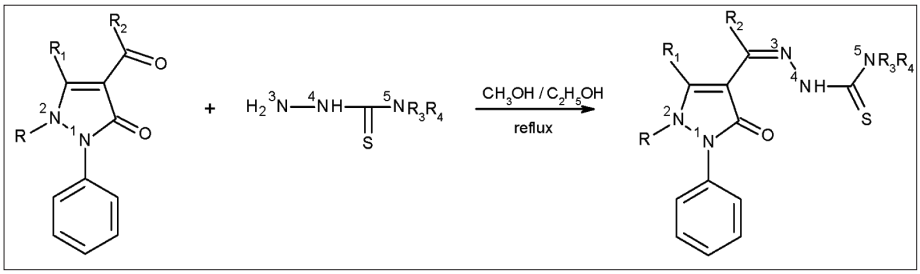
The compounds (table 1) were characterized through the following: elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.The molecular geometry of one of these ligands has been determined by single crystal X-ray study.

For the study of the molecular properties of four tautomers and their deprotonated species of thiosemicarbazone, the DFT method has been used [41].

The investigations have shown form II to be the most stable form in the gas phase, while form I becomes the most stable one in ethanol solution. However, because the difference between the relative energies of the four forms is small, it is expected that the interconversion between them easily occurs.

The condensed Fukui functions have been calculated to assess the relative reactivity of different sites in the ligands. Thus, these predictions can be made:

- the most probable reactive sites for electrophilic attack are the sulfur atoms;
- the N^{2} and N^{4} atoms, in some forms, tend to form intermolecular interaction or act as bridge linking;
- the N^{5} atoms that show higher reactivity in the form III and IV are predicted as probable sites for intermolecular interactions, especially for hydrogen bonding;
- the S, O, and N^{3} atoms, due to the high reactivity, have the greatest capacity for coordination to metal ions.



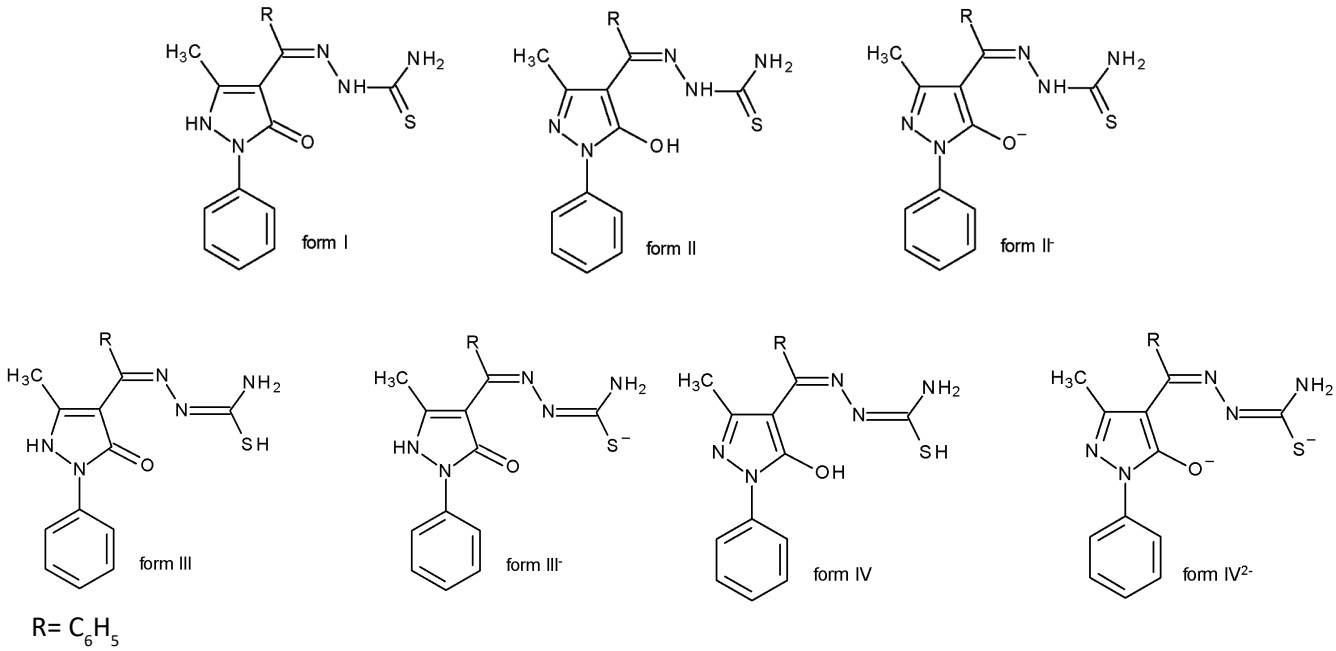
Scheme 1. Synthesis of thiosemicarbazones.

Table 1

The structures of certain substitute thiosemicarbazones, derived from 5-pyrazolone

No.	R	R_1	R_2	R_3	R_4	Reference
1.	CH_3	CH_3	H	H	CH_3	[26]
2.	CH_3	CH_3	H	CH_3	CH_3	[26]
3.	CH_3	CH_3	H	H	$C_{11}H_{12}N_2O$	[26]
4.	CH_3	CH_3	H	H	C_2H_5	[27]

5.	CH_3	CH_3	H	CH_3	CH_3	[28]
6.	H	CH_3	H	H	H	[29]
7.	H	CH_3	C_6H_5	H	H	[30]
8.	H	CH_3	C_6H_4 – p-Br	H	H	[31]
9.	H	CH_3	C_6H_4 – p-Br	H	CH_3	[32]
10.	H	C_6H_5	C_6H_4 – p-F	H	C_2H_5	[33]
11.	H	CH_3	C_6H_4 – p-F	H	CH_3	[34]
12.	H	CH_3	C_6H_4-p-CH_3	H	H	[35]
13.	H	CH_3	C_4H_3O	H	CH_3	[36]
14.	H	C_6H_5	C_6H_4 – p-CH_3	H	CH_3	[37]
15.	H	C_6H_5	C_6H_4 – p-Br	H	CH_3	[37]
16.	H	CH_3	H	H	H	[38]
17.	H	CH_3	C_6H_4 – m-CN	H	H	[39]
18.	H	CH_3	C_6H_4 – m-CN	H	CH_3	[39]
19.	H	CH_3	C_6H_4 – m-CN	H	C_2H_5	[39]
20.	H	CH_3	C_6H_4 – m-CN	H	C_6H_5	[39]
21.	H	C_6H_5	C_6H_4 – m-CN	H	H	[39]
22.	H	C_6H_5	C_6H_4 – m-CN	H	CH_3	[39]
23.	H	C_6H_5	C_6H_4 – m-CN	H	C_2H_5	[39]
24.	H	C_6H_5	C_6H_4 – m-CN	H	C_6H_5	[39]
25.	H	C_4H_3O	C_6H_4 – p-Cl	H	H	[40]
26.	H	C_4H_3O	C_6H_4 – p-Cl	H	CH_3	[40]
27.	H	C_4H_3O	C_6H_4 – p-Cl	H	C_2H_5	[40]
28.	H	C_4H_3O	C_6H_4 – o-Cl	H	CH_3	[40]
29.	H	C_4H_3O	C_6H_4 – m-Cl	H	CH_3	[40]



Scheme 2. The structures of the four isomers and their deprotonated species.

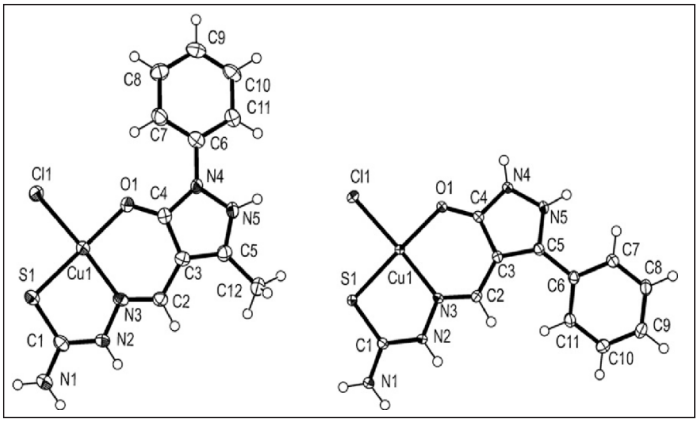


Figure 1. Atomic numbering diagram of the monomer units in polymeric Cu(II) complexes Thermal ellipsoids at 50% probability level.

2.2. Metal complexes with thiosemicarbazones derived from 5-pyrazolone

The research carried out on the complex combinations of the transitional metals with thiosemicarbazone ligands has been a real breakthrough in the development of the coordinative chemistry; thus, the stereochemistry of the metal ions has beeen cleared up as well as the antiviral, antibacterial, antifungic and antitumour properties.

The outstanding biological properties of the ligands and of their complexes are determined by the capacity of complexing the ligand to the metal ion. The nature of the substitutes from the particular azomethine carbon atom, from the atoms of N^2 and N^5 have a major influence upon the coordination way of thiosemicarbazone (form E or Z), on the complexes geometry and, implicitly, upon their biological properties [42].

Just a few of the thiosemicarbazones derived from 5-pyrazolone have been studied from this point of view. But the promising results obtained in recent years, have opened the way to a new direction of research. The research accomplished [38, 43] are just several examples in this respect.

They are studied the influence of the substitutes from the pyrazolone nucleus in the formation of the complexes of Cu(II) and their citotoxic action [38]. The characterization of the complex combinations has been carried out through spectroscopic methods as well as electrochemical means and also by X-Ray difraction. Although the two thiosemicarbazones show thione–thiol tautomerism, the Cu(II) ions coordinate in the same tautomeric form and the results are the complexes with square planar geometry. However, the geometric parameters are different as a consequence of the influence of the substitutes upon the delocalization of the π electrons from the pyrazolone ring. In the X-Ray structure, the cations of the Cu(II) complex form polymeric chains {[Cu(L)Cl]+}n having a chlorine atom in the bridge (figure 1).

The cytotoxic activity of the ligands and complex combinations was evaluated in HL60, REH, C6, L929 and B16 cell lines. One of the complex combinations has proved a strong cytotoxicity against all cell lines, especially HL60 and B16 which are resistant to cisplatin after 24 h of incubation.

Using tiosemicarbazone derived from 4-toluoyl pyrazolone and thiosemicarbazide, has synthesized two monomeric combinations of Cu(II) (figure 2) [43]. In this case, the ligands act as binegative tridentated through enol-O,thiol-S groups and azomethine nitrogen.

The spectral studies carried out have pointed to a square planar geometry for the two complex combinations. The binding properties of the complexes with pET30a DNA were examined through various techniques, and the results suggest that the complexes bind to DNA mainly through partial intercalation binding mode. Moreover, complexes show efficient "artificial nuclease activity".

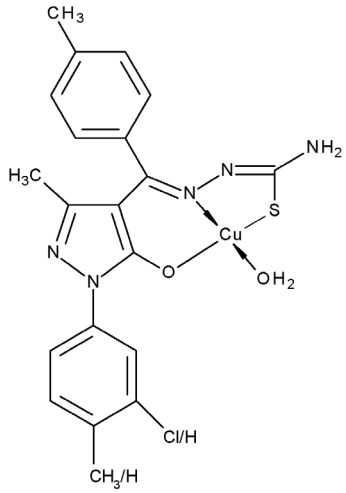


Figure 2. Structure of copper complexes.

2.3. New compounds: Structure and antiproliferative activity.

2.3.1. Structure and synthesis of the compounds 1-17.

A series of new complexes of Cu (II), Ni (II), Co (II) and V (V) with 5-pyrazolone-derived thiosemicarbazone ligands have been synthesized and characterized in order to test their antitumoral potential. Thus, we have synthesized four ligands, with the cromophere groups ONS and NS, respectively, and different volumes of the substitutes of the nitrogen atom N^4: 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone 4-methylthiosemicarbazone(**HL**[1]); 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone 4-ethylthiosemicarbazone (**HL**[2]); 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone 4-phenylthiosemicarbazone (**HL**[3]); 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone 4-pyridinethiosemicarbazone(**HL**[4]) as well as their complex combinations with different transitional metals.

General procedure for the preparation of the ligands HL[1-4]

Methanolic solution of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (1 mmol) and 4-R-thiosemicarbazide (1 mmol) was stirred for 6 h on a water bath and kept at 4 °C for 7 days. The resulting yellow precipitate was collected by vacuum filtration. Suitable single crystals for X-ray structural determination were obtained by recrystallization from a mixture methanol-ethanol (1:2, v/v) [44, 45].

General procedure for the preparation of the metal complexes

Ethanolic solution (15 mL) of ligand was mixed with metal chloride, bromides, or sulphates or nitrate or acetate in methanol solution (15 mL). The molar ratio used was 1:2 (M:L) for the nickel(II) complexes and 1:1 (M:L) for the copper(II), cobalt(II) and vanadium(V) complexes. The following metal salts were used: $CuCl_2 \cdot 2H_2O$ for complexes **1**, **5**, **11**, **15**, $Cu(OAc)_2 \cdot H_2O$ for complexes **2**, **6**, $CuBr_2$ for complex **7**, $Cu(NO_3)_2 \cdot 3H_2O$ for complexes **8**, **12**, **16**, $NiCl_2 \cdot 6H_2O$ for complexes **3**, **10**, **13**, $CoCl_2 \cdot 6H_2O$ for complexes **4**, **14**, **17**, $VOSO_4$ $2H_2O$ for complex **9**. The mixture was stirred for 4 h at 50-60° C, after addition of 2-3 drops of glacial acetic acid. The precipitate formed was separated by filtration. By recrystallized from methanol-ethanol (1:1, v/v) brown-reddish crystals suitable for X-ray diffraction analysis were obtained [44, 45].

The methods of study used for the characterization of the complex combinations were the following: elemental analysis, IR, UV-Vis, EPR spectroscopy, molar electric conductibility, magnetic susceptibility measurements, thermogravimetric analysis and X-ray diffraction.

The single crystal X-ray study revealed that all the compounds have a molecular structure built from the neutral entities depicted in figures 3–10.

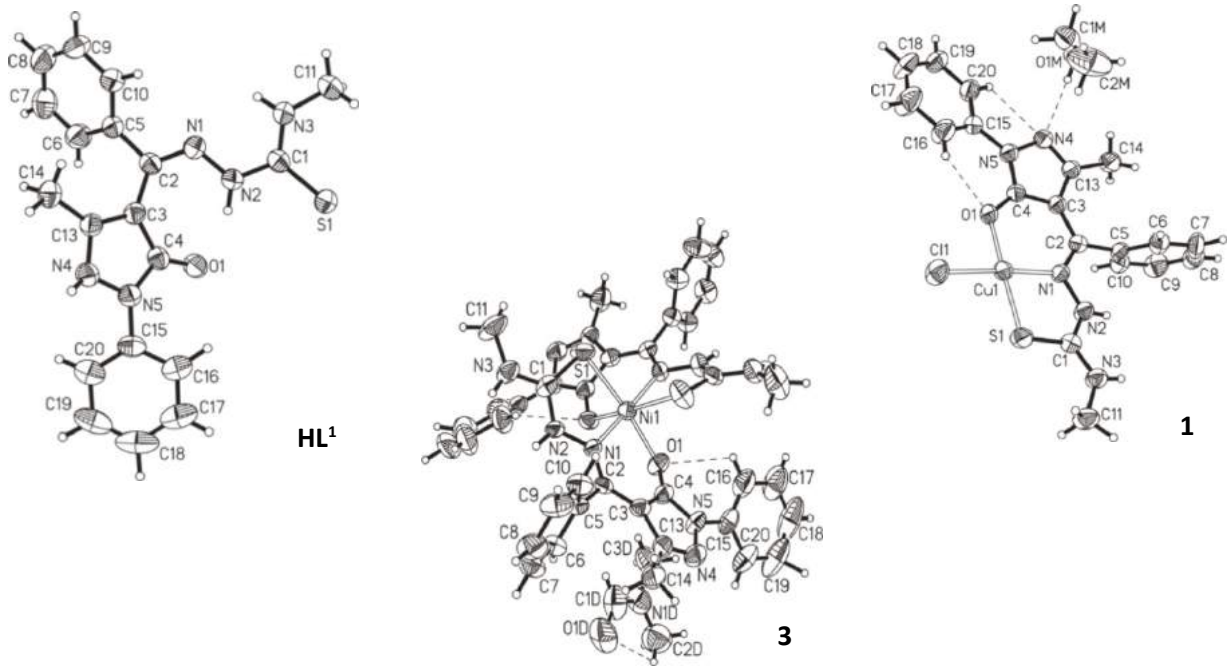


Figure 3. Perspective view of the compounds **HL**[1], **1** and **3** along with atom numbering scheme. Thermal ellipsoids are drawn at 50% probability level.

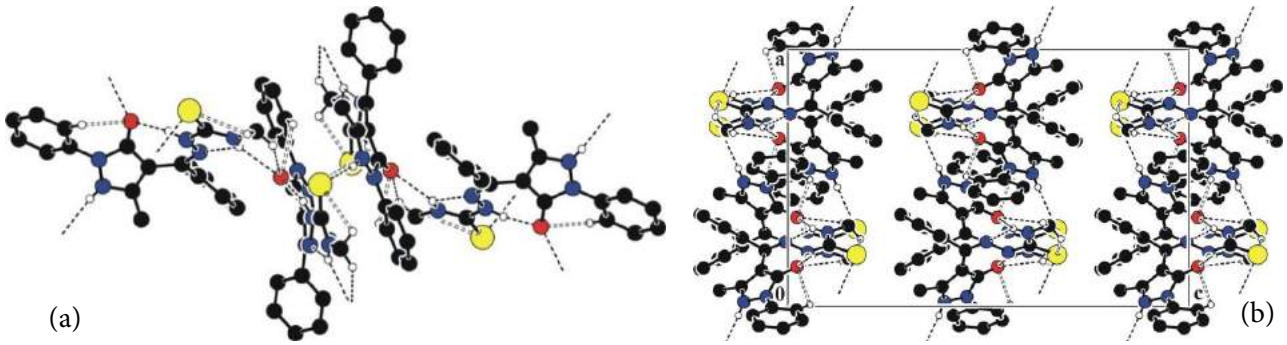


Figure 4. (a) Crystal packing of **HL**[1] representing the *2-D* layers parallel to *(001)* plane. (b) Fragment of molecular packing in the crystal of **HL**[1].

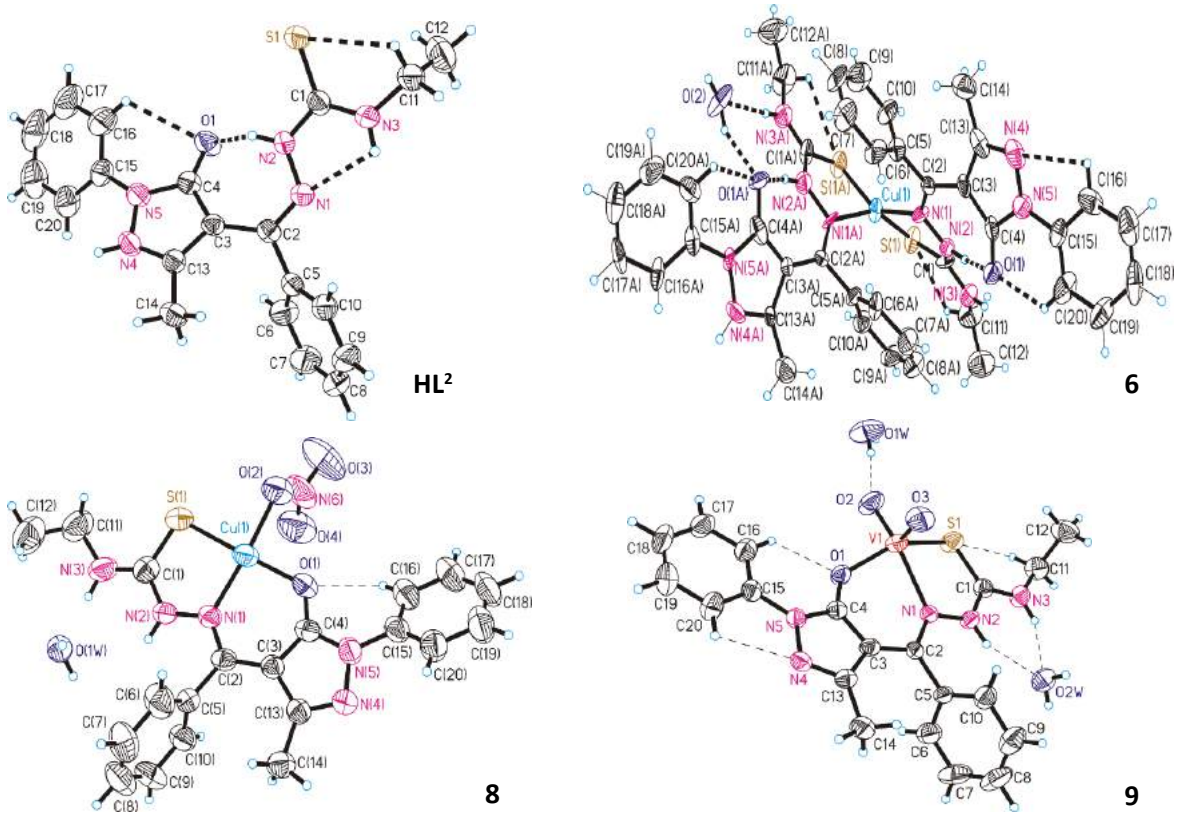


Figure 5. ORTEP drawing for compounds **HL**[2], **6**, **8**, **9** with the atomic labeling. Thermal ellipsoids are shown with the 50% probability level.

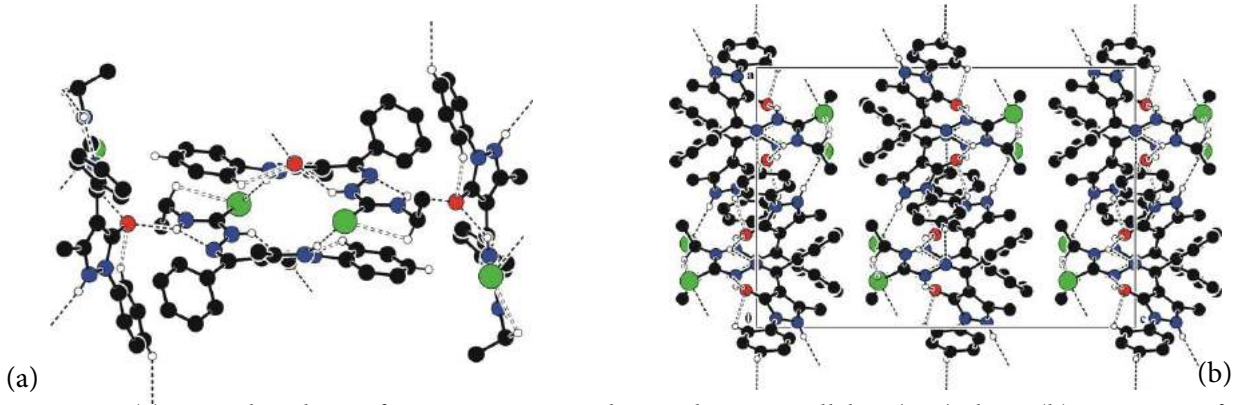
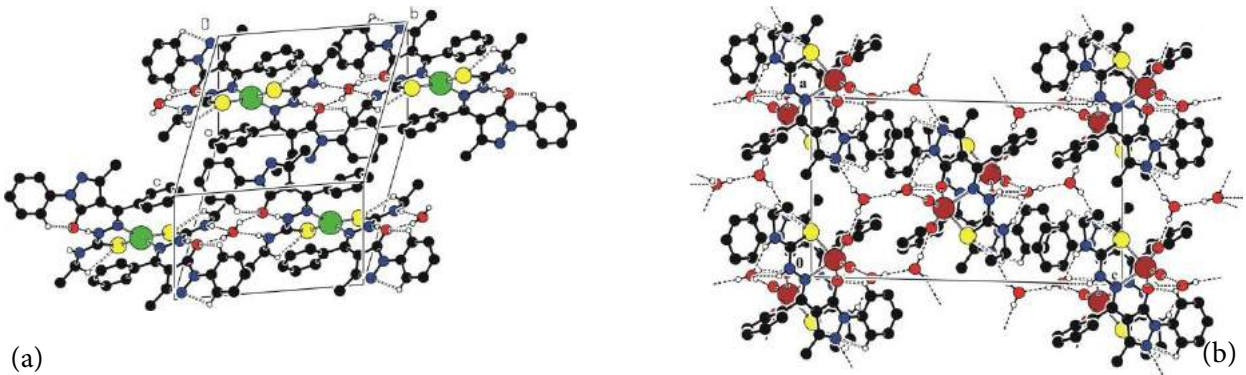
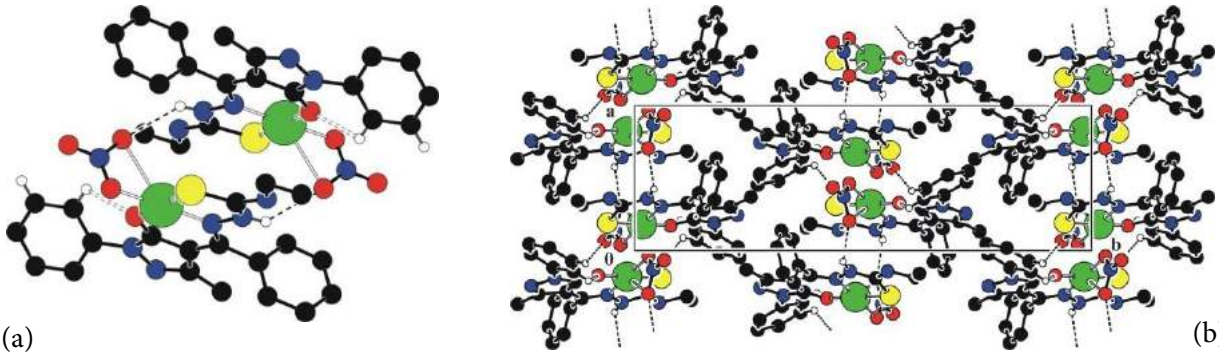


Figure 6. (a) Crystal packing of **HL**[2] representing the *2-D* layers parallel to *(001)* plane. (b) Fragment of molecular packing in the crystal of **HL**[2].



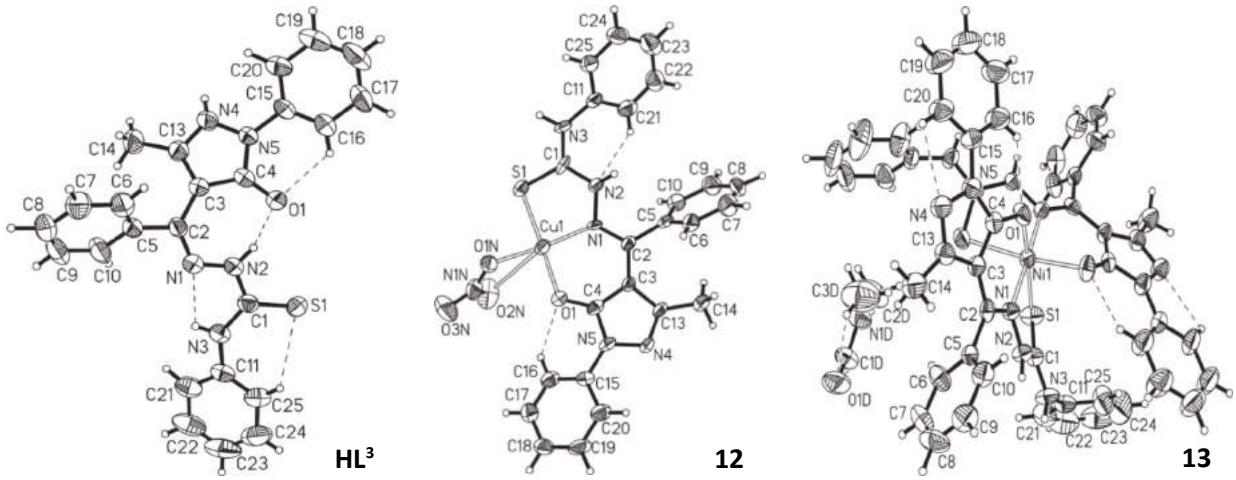
(a) (b)

Figure 7. a) The crystal packing of **6** showing the formation of chains which are aligned along [0 1 0] direction due to water molecules. (b) The crystal packing showing the *3D hydrogen*-bonded *network* built from complexes of **9**.



(a) (b)

Figure 8. (a) The dimer formation where the complexes are linked by NO_3-groups. (b) The crystal packing of **8** representing the consolidation of dimers into chains aligned along [1 0 0] direction.



HL³ **12** **13**

Figure 9. Perspective view of the compounds **HL³**, **12** and **13** along with atom numbering scheme. Thermal ellipsoids are drawn at 50% probability level.

The EPR spectra of the complexes recorded in the polycrystalline and solution state provide information about the coordination environment around copper (II). The EPR parameters g||, g, A|| and the energies of d-d transition were used to evaluate the bonding parameters. The orbital reduction factors indicate the presence of strong in-plane π-bonding for complexes **1**, **2**, **6**, **11**, **12**, **15**, **16** and of some out-of-plane π-bonding for the complexes **5**, **7**, **8**.

2.3.2. Antiproliferative activity

Cell culture

Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, USA) containing *L*-glutamine (2 nM), antibiotics (100 IU penicillin/mL, 100 μg streptomycin/mL) and supplemented with 10% (v/v) foetal bovine serum (FBS), in a 5% CO_2 humidified atmosphere at 37°C.

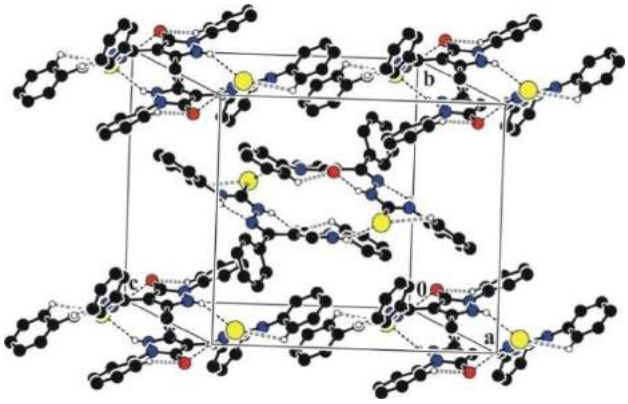


Figure 10. The crystal packing of **HL**[3] showing the formation of *centrosymmetric dimmers in the crystal.*

Cells were currently maintained in continuous exponential growth with dilution of the cells in culture medium twice a week.

Cell proliferation assay

The cell proliferation assay was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Cell Titer 96 Aqueous, Promega, USA), which allowed us to measure the number of viable cells. In brief, triplicate cultures of 1 x 10^4 cells in a total of 100 µL medium in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ, USA) were incubated at 37°C, in 5% CO_2.

Compounds were dissolved in DMSO to prepare the stock solution of 1 x 10^{-2} M. These compounds were diluted to the appropriate concentration (1 or 10 µM) with culture media, added to each well and incubated for 3 days. Following each treatment, 20 µL MTS was added to each well and incubated for 4 h. MTS is converted to water-soluble colored formazan by dehydrogenase enzymes present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA). The results were reported as the percentage of cell proliferation inhibition compared to the control (basal cell proliferation = 100%).

The complexes and ligands **HL**[1-4] were screened for their *in vitro* antiproliferative activity of on human leukaemia HL-60 cells (Table 2).

Table 2

Antiproliferative activity of ligand and metal complexes on human leukaemia HL-60 cells at three concentrations

Compounds	Inhibition of cell proliferation (%)*			IC_{50} (µM/L)
10 µM		1 µM	0.1 µM	
HL[1]	0	0	0	-
$[Cu(L^1)(Cl)]C_2H_5OH$ (**1**)	100	32.2	3.0	1.35
$[Cu(L^1)_2]$ (**2**)	100	99.3	5.0	0.24
$[Ni(L^1)_2]\cdot 2DMF$ (**3**)	6.1	0	0	-
$[Co(L^1)(Cl)(H_2O)_2]$ (**4**)	4.2	2.0	2.0	-
HL[2]	0	0	0	-
$[Cu(L^2)(Cl)]\cdot C_2H_5OH$ (**5**)	98.9	41.3	2.0	1.2
$[Cu(L^2)_2]\cdot H_2O$ (**6**)	99.9	96.0	5.0	0.3
$[Cu(L^2)(Br)]\cdot H_2O$ (**7**)	98.8	35.5	0	1.3
$[Cu(L^2)(NO_3)]\cdot 2CH_3CH_2OH$ (**8**)	96.8	45.8	4.0	1.1
$[VO_2(L^2)]\cdot 2H_2O$ (**9**)	4.0	0	0	-
$[Ni(L^2)_2]\cdot H_2O$ (**10**)	5.7	0	0	-
HL[3]	6.6	0	0	-
$[Cu(L^3)_2]$ (**11**)	100	96.0	2.0	0.36
$[Cu(L^3)(NO_3)]$ (**12**)	100	36.5	3.0	1.27
$[Ni(L^3)_2]\cdot 2DMF$ (**13**)	5.7	0	0	-
$[Co(L^3)(Cl)(H_2O)_2]$ (**14**)	3	2	2	-
HL[4]	78.5	23.1	0	3.05
$[Cu(L^4)(Cl)]DMSO$ (**15**)	100	28.5	9.8	1.52
$[Cu(L^4)(NO_3)]$ (**16**)	96.8	45.8	4.0	1.12
$[Co(L^4)(Cl)]$ (**17**)	60	16.5	14.9	6.25
$CuCl_2\cdot 2H_2O$	0	0	0	-
$Cu(CH_3COO)_2\cdot H_2O$	0	0	0	-
$Cu(NO_3)_2\cdot 3H_2O$	0	0	0	-

$CuBr_2$	0	0	0	-
$NiCl_2 \cdot 6H_2O$	0	0	0	-
$CoCl_2 \cdot 6H_2O$	0	0	0	-
$VOSO_4 \cdot 2H_2O$	0	0	0	-

*SEM <± 4% of a single experiment in triplicate

The tests were performed at three different concentrations: 0.1, 1.0 and 10 μM. In the case of the four ligands have an antiproliferative effect was found for the ligand **HL**[4] at 10 μM. Following to the metal ion coordination was observed an increase in the antiproliferative activity for concentrations higher than 1 μM. This fact is prove specially for copper complexes. The copper complexes (**1**, **2**, **5**-**8**, **11**, **12**, **15**, **16**), including the monodeprotonated ligand, showed an important antiproliferative activity for HL-60 leukaemia cells. On the other hand, the nickel and vanadium complexes did not reduce the cell proliferation. If we consider the three cobalt complexes, complex **17** which shows the tetrahedral geometry has a better antiproliferative activity compared to the other two complexes octahedral.

Maximum antiproliferative activity for copper complexes were observed at 10 μM. Antiproliferative activity of these complexes at 10 μM is similar to doxorubicin, used in medical practice as antileukemia drug. The percentage of cell growth inhibition was found to be 100 % for complexes **1**, **2**,**11**, **12**, **15**, 99.9 % for complex **6**, 98.9% for complexes **5**, **7** and 96.8% for complexes **8**, **16**.

The IC_{50} values were found to be 0.24 μM for complex **2**, 0.3 μM for complex **6** and 0.36 μM for complex **11** reveal the potential antiproliferative of these compounds.

3. CONCLUSION

The data obtained after the antiproliferative screening on HL-60 cells, carried out for these new classes of compounds indicate the fact that certain complex combinations have had an antiproliferative effect for concentrations higher than 1 μM. The copper complexes (**1**, **2**, **5**-**8**, **11**, **12**, **15**, **16**) demonstrate an important antiproliferative activity for HL-60 leukemia cells compared to those containing nickel, vanadium and cobalt ions. The nature of the ligand (the presence of certain substituents at the terminal nitrogen) and of the metal ion, geometry of metal complexes (the present in DMSO solution of the square planar and tetrahedral molecular species), the theory of chelation and liposolubility can influence antiproliferative effect. The geometry and the chelation may reduce the polarity of the metal ion mainly because of partial sharing of its positive charge with the donor group and possible electron delocalization over the whole chelate ring. Also, the coordination may facilitate the ability of a complex to cross the lipid layer of cell membrane and in this way may be affected the mechanisms of growth and development of the tumor cell. Our results can be useful in designing new copper(II) complexes as antiproliferative agents.

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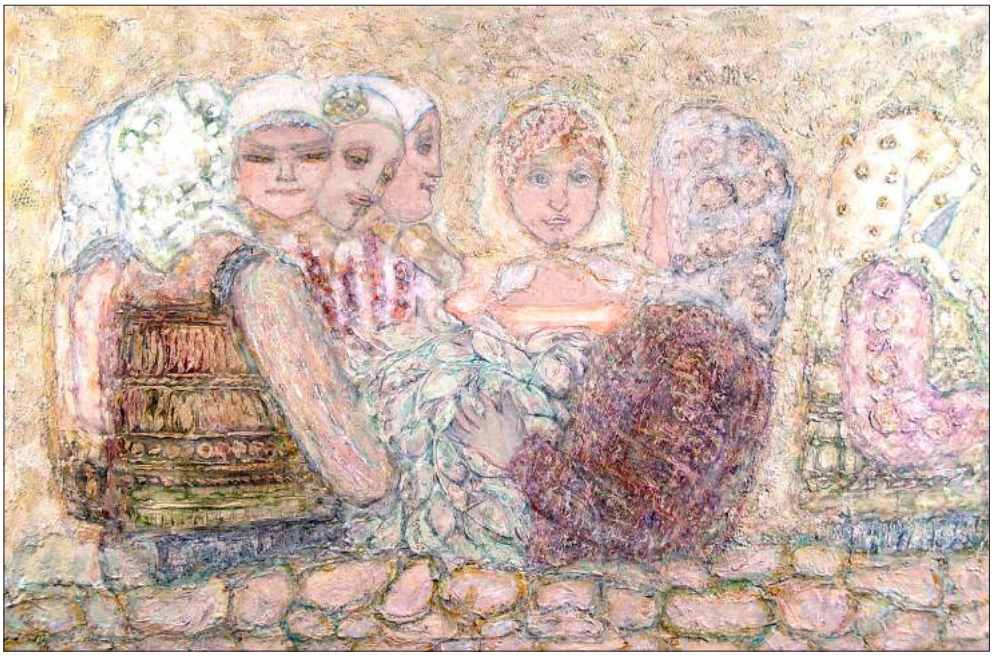

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