UNIVERSITY OF BUCHAREST
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ABSTRACT OF THE PhD THESIS

COMPLEXES OF SOME 3d METAL IONS WITH LIGANDS BEARING 1,2,4-TRIAZOLE UNIT WITH VARIOUS TYPES OF BIOLOGICAL ACTIVITY

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The PhD thesis is divided into two main parts: the theoretical part, which contains data form literature regarding the research topics approached and the original part which presents the results of research performed in the PhD theme.

The theoretical part contains the most important literature data concerning Schiff base ligands which contain triazole unit or triazolopyrimidine derivatives. Schiff base ligands derived from triazole unit and their complexes are recognized for antimicrobial, antiviral, anti-inflammatory and antitumoral activity.

In the same section is presented also an interesting and important class of condensed heterocycles which contain the 1,2,4-triazole unit, namely triazolopyrimidine class. These compounds are important due to the number and the arrangement of the nitrogen atoms from the aromatic cycle which is similar with the purinic cycle from the nucleic acids structure. Triazolopyrimidine derivatives and their complexes are presented from the point of view of coordination mode, of physicochemical properties as well as of the antimicrobial activity observed for this species. The study of complexes and triazolopyrimidine derivatives represents nowadays an interesting topic due to antipyretic, anti-inflammatory, antitumor and antiparasitic properties which have already led to drugs development based on this compounds.

In the second part of the paper are presented the original results of the PhD thesis „Complexes of some d metal ions with ligands bearing 1,2,4-triazole unit with various types of biological activity" concerning the synthesis and characterization of new complexes with ligands which contain 1,2,4-triazole unit and a new organic compound 5-phenyl-7-methyl-1,2,4-triazolo[1,5-*a*]pyrimidine by specific methods (elemental chemical analysis, thermal analysis, magnetic moments, ESI-MS, IR, electronic spectra, EPR, ^{1}H NMR and ^{13}C NMR, cyclic voltammetry and in some cases single crystal X-ray diffraction).

The new obtained compounds were tested in order to establish the antimicrobial activity against pathogenic bacterial strains, standard or isolated from the clinic, Gram negative, Gram positive strains and against the *C. albicans* fungal strain by determining the minimum inhibitory concentration (MIC). The species have also been studied concerning their efficiency against adherent cells to inert material in order to highlight the differences between the physiology and the antibiotics sensibility of microorganisms embedded in biofilms.

Some selected complexes and ligands were tested in order to determine the cytotoxic activity on tumor cell lines HEp 2 (human laryngeal carcinoma), HCT 8 (human ileocecal adenocarcinoma) and HT 29 (human colorectal adenocarcinoma).

II.1. New complexes of Co(II), Ni(II), Cu(II) and Zn(II) with 2-[(E)-(1H-1,2,4-triazol-3-ylimino)methyl]phenol

In this subchapter are presented aspects concerning the synthesis and characterization of the first complexes series (**1**)-(**16**) which contain 2-[(E)-(1H-1,2,4-triazol-3-ylimino)methyl]phenol ($L^{\mathbf{1}}$) as Schiff base ligand, derived from salicylaldehyde and 3-amino-4H-1,2,4-triazole.

Complexes were formulated based on data provided by elemental chemical analysis and by correlating these data with other physicochemical methods used to characterize the compounds, as follows:

$[Co_2(L^{\mathbf{1}})_2Cl_2]\cdot H_2O$ (**1**)
$[Ni_2(L^{\mathbf{1}})_2Cl_2(OH_2)_2]$ (**2**)
$[Cu_2(L^{\mathbf{1}})_2Cl_2]$ (**3**)
$[Zn_2(L^{\mathbf{1}})_2Cl_2]\cdot H_2O$ (**4**)
$[CoL^{\mathbf{1}}(OH_2)_4](ClO_4)$ (**5**)
$[NiL^{\mathbf{1}}(ClO_4)(OH_2)_3]\cdot 3H_2O$ (**6**)
$[CuL^{\mathbf{1}}(ClO_4)(OH_2)_2]$ (**7**)
$[ZnL^{\mathbf{1}}(OH_2)_2](ClO_4)$ (**8**)
$[CoL^{\mathbf{1}}(CH_3COO)(OH_2)_2]$ (**9**)
$[NiL^{\mathbf{1}}(CH_3COO)(OH_2)_2]$ (**10**)
$[Cu_2(L^{\mathbf{1}})_2(CH_3COO)_2(OH_2)_2]$ (**11**)
$[Zn_2(L^{\mathbf{1}})_2(CH_3COO)_2]$ (**12**)
$[Co(L^{\mathbf{1}})_2]\cdot 0{,}5H_2O$ (**13**)
$[Ni(L^{\mathbf{1}})_2(OH_2)_2]\cdot H_2O$ (**14**)
$[Cu(L^{\mathbf{1}})_2]\cdot 2H_2O$ (**15**)
$[Zn(L^{\mathbf{1}})_2]$ (**16**)

$HL^{\mathbf{1}}\cdot H_2O$: 2-[(E)-(1H-1,2,4-triazol-3-ylimino)methyl]phenol; $C_2H_4N_4$: 3-amino-4H-1,2,4-triazole

The nature of the acetate complexes, as monomeric or dimeric species, has been confirmed by mass spectrometry using electrospray ionization techniques, in positive and in negative mode. In the positive mode the pseudomolecular ions were found as $[CoL(CH_3COO)+CH_3CN+4H]^+$ (m/z: 350.10), $[NiL(CH_3COO)(OH_2)_2\text{-}2H]^+$ (m/z: 338.40), $[Cu_2L_2(CH_3COO)_2+2CH_3\text{-}H]^+$ (m/z: 648.40) and $[Zn_2L_2(CH_3COO)+H]^+$ (m/z: 565.00) respectively. Moreover, in the negative mode the pseudomolecular ions $[Cu_2L_2(CH_3COO)_2+4H+OH]^-$ (m/z: 640.30) and $[Zn_2L_2(CH_3COO)_2+OH]^-$ (m/z: 640.40) represents a proof for the dimeric structure of complexes (**11**) and (**12**).

The data provided by thermal analysis have confirmed the composition of the ligand and complexes, have allowed the establishment of the number and the role of water molecules (crystallization or coordination), established the steps number of decomposition and the effect that accompany this steps. For all species, the final product of thermal degradation is the most stable metal oxide as has been demonstrated by powder X-ray diffraction, in some cases non-stoichiometric oxide species is formed.

IR spectra confirmed that the condensation process has occurred and also provided information concerning the coordination mode of Schiff base or of other potential ligands (perchlorate, acetate). Schiff base formation is confirmed by the absence of characteristic bands for the stretching vibrations ν(CHO) for aldehyde group and for primary amine ν(NH_2) and new bands assigned to vibration mode ν(HC=N) for azomethine group appear at 1615 cm^{-1}, this band is shifted with 10-50 cm^{-1} to higher wave number in (**1**)-(**16**) complexes spectra as indication of the azomethine nitrogen coordination to metal ions [81].

^1H NMR spectrum of Schiff base 2-[(E)-(1H-1,2,4-triazol-3-ylimino)methyl]phenol presents a multiplet in the domain 7.04-7.81 ppm which is assigned to benzene ring protons. The proton of the OH group appears as a singlet at 12.40 ppm, while the signal from 8.46 ppm is characteristic for azomethine proton group [83]. For Zn(II) complexes was not possible to record ^1H NMR spectra due to their low solubility in all deuterated solvents.

In the electronic spectra of Co(II) complexes with octahedral stereochemistry, appear bands due to d-d transitions at wavelengths of approximately 525 and 1180 nm, while, in the case of tetrahedral complexes bands are situated at 660 and 1465 nm [113]. Electronic spectra of Ni(II) complexes indicates an octahedral stereochemistry by the presence of three bands which are assigned to spin allowed d-d transitions [113]. Electronic spectra of Cu(II) complexes shows a band with the maximum in the domain 700-770 nm which can indicate a square planar stereochemistry. In the case of square pyramidal stereochemistry, is observed a broad band with a maximum around 625 nm. The stereochemistry is confirmed also by the magnetic susceptibility data at room temperature for Co(II) and Ni(II) species.

The powder EPR spectra for Cu(II) complexes at room temperature and in frozen DMSO have given important information about the surrounding metal ions, the degree of distortion from the perfect stereochemistry, the bonds nature of metal ion-ligands and the interaction between paramagnetic ions for dimeric species.

The EPR spectrum of $[Cu_2(L^{\mathbf{1}})_2Cl_2]$ (**3**) presents a broad signal with a width of approximately 380 G which suggests interactions between the paramagnetic centers (Figure II.1.1.5.1) [116]. This aspect is characteristic for species with square pyramidal or square planar stereochemistry and this fact may be due to a network arrangement as dimeric species for the ion pairs which can be antiferromagnetic coupled along the z-axis in both cases of stereochemistry.

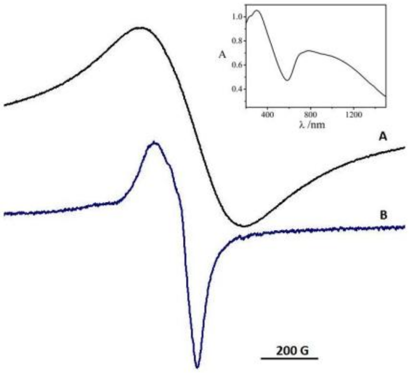

Figure II.1.1.5.1. EPR spectrum of complex (**3**) at room temperature on powder (A), in DMSO (B) and electronic spectrum (onset)

EPR spectra for complexes (**7**), (**11**) and (**15**) presents a weak, broad signal and with low intensity with g_{av}=2.148, characteristic for species with square pyramidal or square planar stereochemistry, this aspect may be due to the dimeric species [96].

In the quantitative *screening* step were assayed the antimicrobial activity of complexes on pathogenic microbial strains Gram negative (*E. coli* ATCC 25922, *E. coli* 832, *K. pneumoniae* ATCC 134202, *K. pneumoniae* 806, *P. aeurginosa* ATCC 27853, *P. aeruginosa* 392), Gram positive (*S. aureus MRSA* 1263, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633) and also on fungal strain *C. albicans* 22. Important to mention is that the bacterial strains of *E. coli* 832, *K. pneumonia*e 806 and *P. aeruginosa* 392 were isolated from urine and wound secretion respectively, showing a resistance phenotype to multiple antibiotics, defined as non susceptibility acquiring agent at least three or more categories of antimicrobial drugs. Were obtained different values of MIC depending on the bacteria strain assayed, the MIC values were in the range 3.91 and 1000 μg mL^{-1}. In the case of *E. coli* ATCC 25922 bacterial strain the tests revealed a very good activity for complex $[Cu_2(L^{\mathbf{1}})_2Cl_2]$ (**3**) (MIC of 3.91 μg mL^{-1}) and for

$[Cu_2(L^1)_2(CH_3COO)_2(OH_2)_2]$ (**11**) (MIC of 31.25 μg mL^{-1}) while the complexes (**5**) and (**9**) exibited a MIC value of 62.50 μg mL^{-1} against this Gram negative strain.

Complexes of $Cu_2(L^1)_2Cl_2]$ **(3)** and $[Cu(L^1)_2]\cdot 2H_2O$ (**15**) exhibited the most evident inhibitory effect upon adherence ability of *E. coli* ATCC 25922 up to the minimum biofilm eradication concentration (MBEC) of 3.91 μg mL^{-1} and in the case of complex $[Zn_2(L^1)_2Cl_2]\cdot H_2O$ **(4)** the inhibitory effect upon de adherence ability of biofilm was up to 7.81 μg mL^{-1} for the same strain.

The cytotoxic activity of the L^1 ligand, complexes, compounds $[Co_2(L^1)_2Cl_2]\cdot H_2O$ (**1**), $[Ni_2(L^1)_2Cl_2(OH_2)_2]$ (**2**), $[Cu_2(L^1)_2Cl_2]$ (**3**), $[Zn_2(L^1)_2Cl_2]\cdot H_2O$ (**4**) and the other species of Cu(II) were assayed on tumor cell lines HEp 2, HCT 8 and HT 29.

An increased cytotoxicity was observed in the case of Cu(II) compounds, behavior associated with the generation of reactive oxygen species (ROS) or by changing the proteins functions which have cysteine residues in the composition. The study of cellular cycle phases indicated for $[Cu_2(L^1)_2Cl_2]$ (**3**), $[CuL^1(ClO_4)(OH_2)_2]$ (**7**), $[Cu_2(L^1)_2(CH_3COO)_2(OH_2)_2]$ (**11**) and $[Cu(L^1)_2]\cdot 2H_2O$ (**15**) complexes a higher number of cells blocked in the G2 phase, aspect that leads to the reduction of the DNA replication.

II. 2. Complexes of Co(II), Ni (II), Cu(II) and Zn(II) with 5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine

In this subchapter are presented aspects regarding the synthesis and characterization of the second series of complexes (**17**)-(**33**) which contain the 5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine (L^2) as ligand, resulted by [1+1] condensation of acetylacetone with 3-amino-4H-1,2,4-triazole. The compounds were formulated based on data provided by elemental chemical analysis and their correlation with data provided by physicochemical methods, as follows:

$[CoL^2(OH_2)_4][CoCl_4]$ (**17**)
$[Ni_2(L^2)_2Cl_4(OH_2)_4]$ (**18**)
$[Cu_2(L^2)_2Cl_4]\cdot H_2O$ (**19**)
$[Zn_2(L^2)_2Cl_4]$ (**20**)
$[CoL^2(OH_2)5](ClO_4)_2\cdot H_2O$ (**21**)
$[CoL^2(CH_3COO)_2(OH_2)]\cdot 0{,}5H_2O$ (**25**)
$[NiL^2(CH_3COO)_2(OH_2)]\cdot H_2O$ (**26**)
$[CuL^2(CH_3COO)_2]$ (**27**)
$[ZnL^2(CH_3COO)_2(OH_2)]\cdot 1{,}5H_2O$ (**28**)
$[Co(L^2)_2Cl_2]$ (**29**)

$[NiL^2(OH_2)_5](ClO_4)_2 \cdot H_2O$ (**22**)
$[CuL^2(OH_2)_2(ClO_4)_2] \cdot 3H_2O$ (**23**)
$[ZnL^2(OH_2)_3](ClO_4)_2$ (**24**)
$[Co(L^2)_2(OH_2)_4]Cl_2 \cdot 2H_2O$ (**30**)
$[Ni(L^2)_2Cl_2(OH_2)_2] \cdot 2{,}5H_2O$ (**31**)
$[Cu(L^2)_2Cl_2] \cdot H_2O$ (**32**)
$[Zn(L^2)_2Cl_2]$ (**33**)

L^2: 5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine

Thermal analysis data confirmed the presence or the absence of crystallization and/or coordination of water molecules. For the chloride species appear a supplementary step of chloride anion elimination as hydrochloric acid and for acetate species appear in addition a step which corresponds to the transformation of acetate into carbonate, and sometimes, the overlapping takes place with oxidative degradation of triazolopyrimidine derivative which is usually decomposed in two steps.

The IR spectra of complexes confirmed the formation of triazolopyrimidine derivative, its coordination mode as unidentate through the nitrogen atom N3 and provided information about the presence of perchlorate as unidentate or in ionization sphere and of acetate chelate ligand. In the IR spectra of triazolopyrimidine ligand appears two bands, one intense at 1635 cm^{-1} which is attributed to stretching vibration mode ν_{tp} of triazolopyrimidine unit and the second one is attributed to stretching vibration mode ν_{py} for pyrimidine cycle which appears at 1551 cm^{-1}; in the IR spectra of complexes (**17**)-(**33**) the band which corresponds to ν_{tp} is shifted with 5-55 cm^{-1} to lower wave numbers as a sign of coordination through nitrogen atom N3 [169].

^1H RMN and ^{13}C RMN spectra for ligand, $[ZnL^2(OH_2)_3](ClO_4)_2$ (**24**) and $[ZnL^2(CH_3COO)_2(OH_2)] \cdot 1{,}5H_2O$ (**28**) complexes confirmed that the condensation process occurred by the appearance of the characteristic peaks of the bonds atoms -C=N- from triazolopyrimidine unit [170].

Studies on single crystal X-ray diffraction indicated the fact that $[Co(L^2)_2(OH_2)_4][CoCl_4]$ (**17**) crystallizes in the monoclinic C2/c space group and its crystal structure is formed of two discrete units of cation complex $[Co(dmtp)_2(OH_2)_4]^{2+}$ and anion complex $[CoCl_4]^{2-}$ (Figure II.2.1.4.2.1).

The Co(1) metal atom from first unit adopts an octahedral stereochemistry being surrounded by four water molecules and two L^2 moieties in a *cis* arrangement. The organic ligand is coordinated in an unidentate fashion through N(3), this mode being observed also for other complexes described in literature [173]. The bonds length and angles are similar with those reported for the free ligand [165] except for the bond N(3)-C(2) that becomes slightly longer (1.356(3) over 1.337(3) Å) in complex as result of nitrogen coordination. The bond lengths Co(1)-N(3) of 2.154(2) Å are in the usually range observed for $[Co(L^2)_2(H_2O)_4]Br_2\cdot 2H_2O$ of 2.116(6) Å [155] and for $[Co(L^2)_2(H_2O)_4](NO_3)_2$ of 2.170(3) Å [154], species containing the same complex cation.

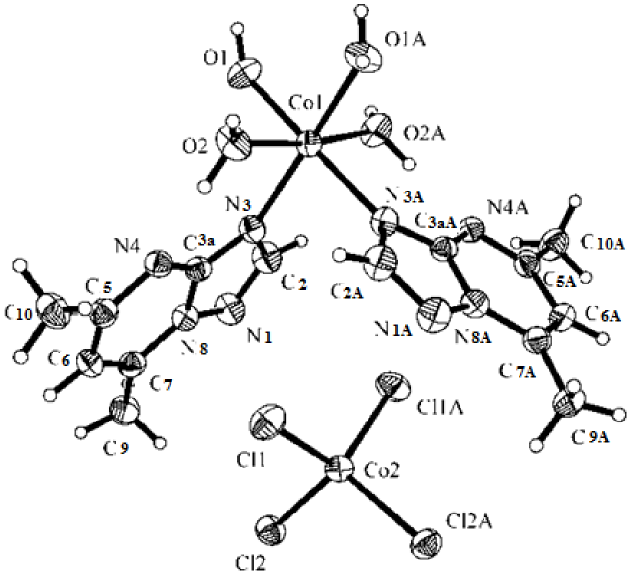

Figure II.2.1.4.2.1. Crystal structure of $[CoL^2(OH_2)_4][CoCl_4]$ (**17**) with the labeling of different atoms for the non-hydrogen (50% probability thermals ellipsoids; H atoms are represented as spheres of arbitrary radii)

The second Co(2) metal ion is surrounded by four chloride ions with Cl-Co(2)-Cl angles varying from 108.29(3) to 113.63(4)° and Co(2)-Cl(1A) bonds lengths ranging from 2.2757(7) to 2.2778(7) Å, typical for the tetrahedral stereochemistry.

As can be seen in the packing diagram presented in the Figure II.2.1.4.2.2, the complex cations adopt a layered arrangement through π-π stacking interaction at 3,45 and 3,49 Å respectively, these interactions are responsible for *cis* arrangement of two organic ligands. The packing is completed by hydrogen bonds realized with complex anions. The coordinated water molecules are involved in different hydrogen bonding interactions generating chains. Two of the water molecule are involved in intramolecular interaction with nitrogen atom (N3) having the O(2)-H(03)···N(3) bond length of 2,730(3) Å while the other two water molecules link complex cations with complex anions by O-H···Cl interaction with O(1)-H(01)···Cl(2) bond length of 3,105(2) Å and O(1)-H(02)···Cl(1) bond length of 3,182 Å.

The $[Co(L^2)_2Cl_2]$ (**29**) species crystallizes in the orthorhombic Pbca space group and the Co(1) central metal atom exhibits a tetrahedral stereochemistry realized by coordination of two chloride anions and of the N3 atom from two triazolopyrimidine molecules (Figure II.2.1.4.3.1).

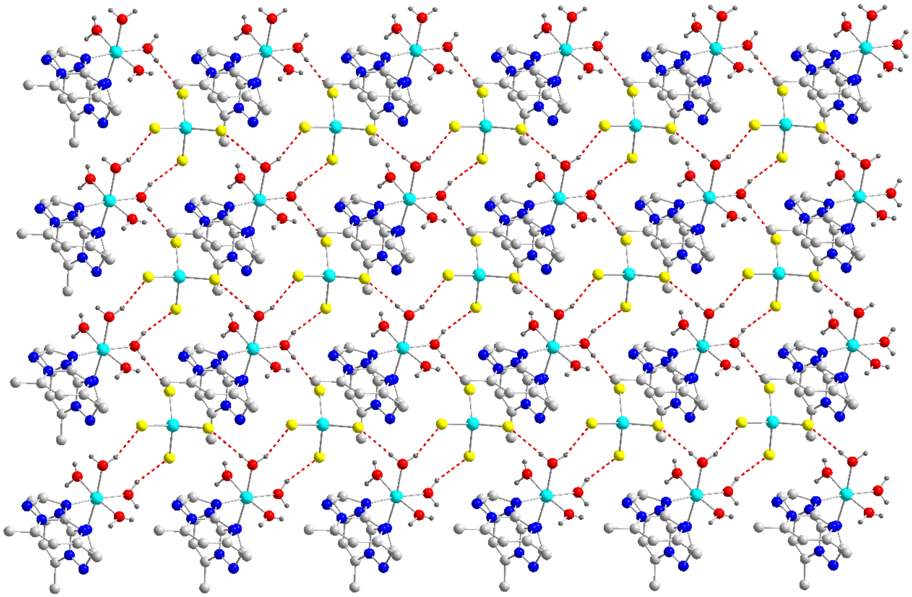

Figure II.2.1.4.2.2. Packing diagram of $[Co(L^2)_2(OH_2)_4][CoCl_4]$ (**17**) showing O-H···Cl hydrogen bonds

The bond angles vary from 103.59(6) to 116.11(2)°, being characteristic for tetrahedral stereochemistry. The C(3aB)-N(3B) bond length of 1.343(2) Å and C(3aA)-N(3A) of 1.344(2) Å, both are longer than the free ligand (1.337(3) Å) as a result of the coordination of the nitrogen atom from this bond to the metal ion.

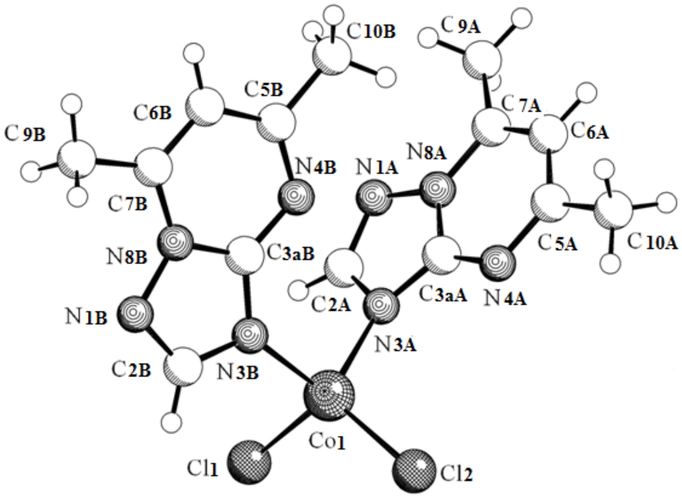

Figure II.2.1.4.3.1. Crystal structure of complex $[Co(L^2)_2Cl_2]$ (**29**)

As can be seen in Figure II.2.1.4.3.2, $[Co(L^2)_2Cl_2]$ (**29**) molecules are arranged as supramolecular dimers through the π-π stacking interactions between the pyrimidine units which generate an "herringbone" arrangement.

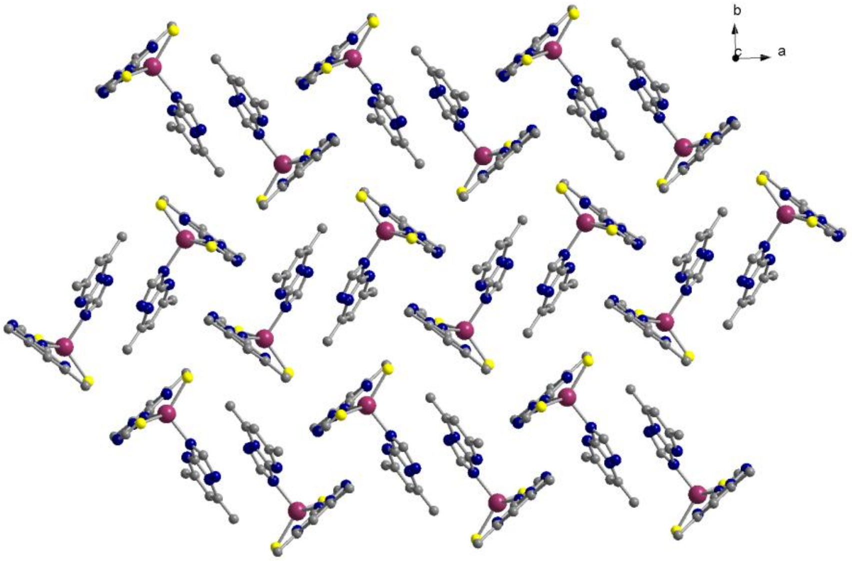

Figure II.2.1.4.3.2. Packing diagram of complex $[Co(L^2)_2Cl_2]$ (**29**)

The $[Co(L^2)_2(OH_2)_4]Cl_2 \cdot 2H_2O$ (**30**) complex crystallizes in triclinic P-1 spatial group. The structure of this compound consists from discrete cations of $[Co(L^2)_2(H_2O)_4]^{2+}$, two molecules of crystallizations water and two chloride anions which neutralize the charge of the complex species. The Co(1) central metal ion is in the center of crystallographic symmetry and exhibits an octahedral stereochemistry surrounded by two nitrogen atoms N3 from triazolopyrimidine units and four water molecules (Figure II.2.1.4.4.1), with the mention that in this case the triazolopyrimidine ligands adopt a "*trans*" arrangement, this aspect was observed to other cobalt species analyzed by single crystal X-ray diffraction containing the same cationic units [154, 155]. In the grid are two molecules of crystallization water and two chloride per unit.

The Co(1)-N(3) bond length of 2.151(3) Å is similar to that which was observed for $[Co(dmtp)_2(H_2O)_4]Br_2 \cdot 2H_2O$ of 2.116(6) Å [155] and $[Co(dmtp)_2(H_2O)_4](NO_3)_2$ of 2.170(3) Å [154].

The angles around the cobalt ion (II) are slightly deviated which shows that the octahedron is slightly distorted (~ 4°) from 90° and the Co(1)-O(1) bond length of 2.059(3) Å is slightly lower than of the Co(1)-O(2) bond length of 2.096(2) Å indicating that oxygen atoms are coordinated and they are involved in different hydrogen bonds interactions, some with chloride anions and some with the crystallization water molecules (Figure II.2.1.4.4.2).

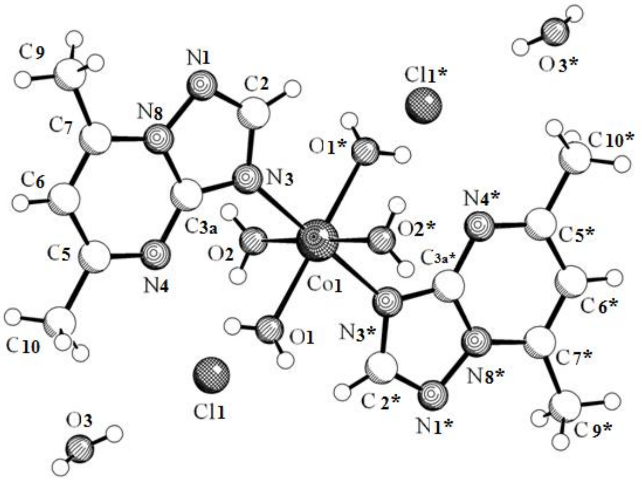

Figure II.2.1.4.4.1. Crystal structure of complex $[Co(L^2)_2(OH_2)_4]Cl_2 \cdot 2H_2O$ (**30**)

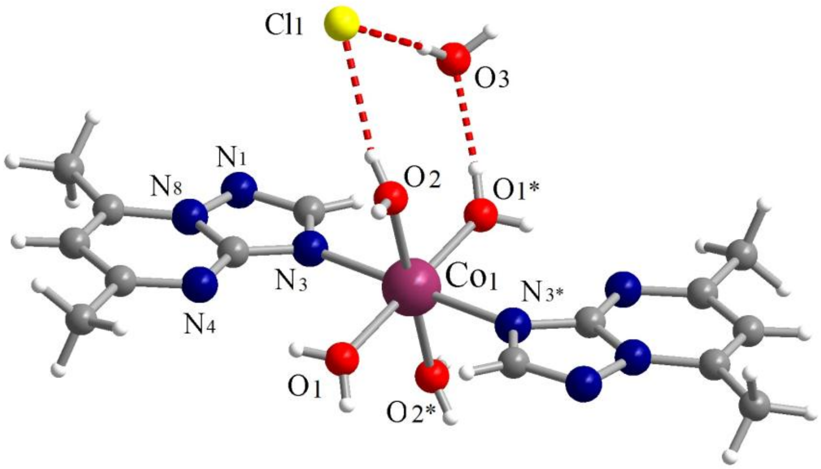

Fig. II.2.1.4.4.2. Crystal structure of $[Co(L^2)_2(OH_2)_4]Cl_2 \cdot 2H_2O$ (**30**) outlining the hydrogen bonds between coordination water molecules, chloride anions and crystallization water molecules

In the three dimensional structure are generated intercalated planes by complex cations and chloride anions which are assembled through hydrogen bonds (Fig. II.2.1.4.4.3).

The planes are arranged through weak π-π stacking interactions of 3.41-3.65 Å in to a “zipper” fashion, among pyrimidine units from parallel chains belonging to different cationic units [174].

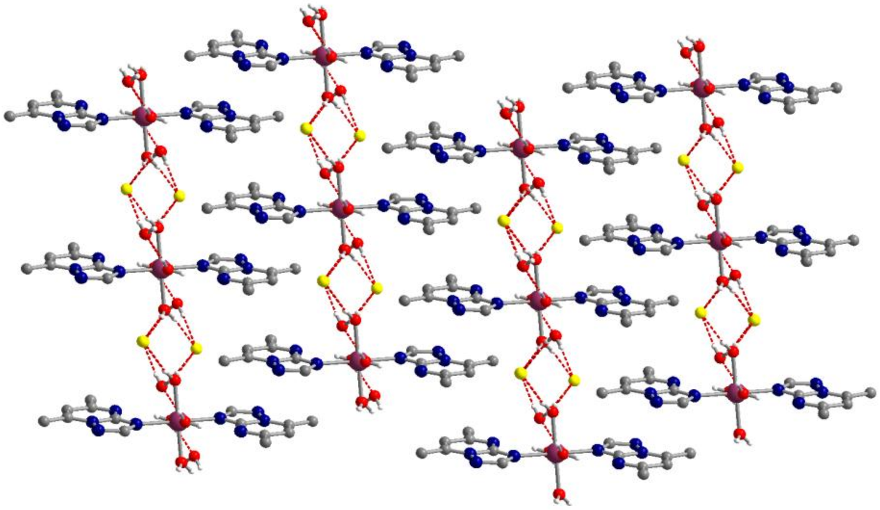

Figure II.2.1.4.4.3. Packing diagram of complex $[Co(L^2)_2(OH_2)_4]Cl_2 \cdot 2H_2O$ (**30**)

The coordinated water molecules are involved in the formation of hydrogen bonds with the crystallization water molecules and chloride anions, which are surrounded pseudotetrahedral by four water molecules which generate chloride-water chains as can be seen in Figure II.2.1.4.4.4.

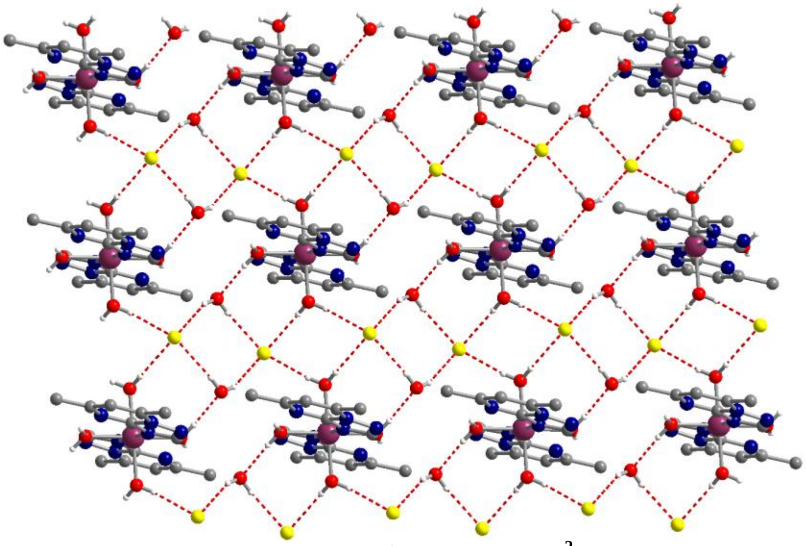

Figure II.2.1.4.4.4. Packing diagram of complex $[Co(L^2)_2(OH_2)_4]Cl_2 \cdot 2H_2O$ (**30**)

The $[Zn(L^{\mathbf{2}})_2Cl_2]$ (**33**) molecular structure is isostructural with $[Co(L^{\mathbf{2}})_2Cl_2]$ (**29**) species.

Electronic spectra of complexes were studied to obtain information concerning stereochemistry. For Co(II) ions was proposed an octahedral and tetrahedral stereochemistry, with the mention that the electronic spectra of (**17**) compound is very complex, as also indicated the X-ray diffraction for this species which exhibited the pattern of two stereochemistrys, octahedral and tetrahedral respectively. For Ni(II) complexes is proposed an octahedral stereochemistry, this is confirmed by magnetic susceptibility values at room temperature. For Cu(II) species square planar or square pyramidal stereochemistry was confirmed by EPR spectra.

The powder EPR spectra of (**19**) and (**32**) complexes recorded at room temperature are characteristic for an axial distorted geometry. For (**19**) compound the splitting parameter values calculated are $g_{\|} = 2.271$ and $g_{\perp} = 2.063$, this values being characteristic for rhombic symmetry with aligned tetragonal axes. In frozen DMSO solution the EPR spectra of (**19**) complex also indicates an axial geometry but with changing parameters at $g_{\|} = 2.215$ and $g_{\perp} = 2.112$. EPR spectra of (**32**) complex in frozen DMSO solution looks like an isotropic spectra with g_{iso}=2.149.

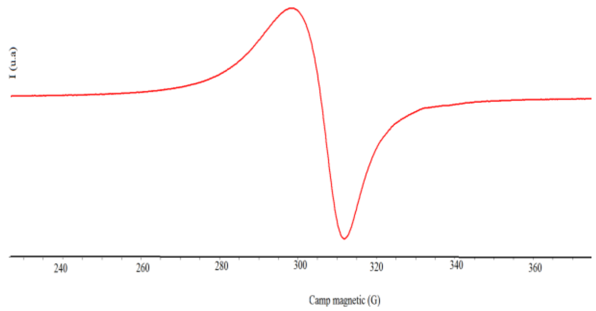

Figure II.2.1.6.1.2. EPR spectrum of complex (**23**) on powder recorded at room temperature

For (**23**) and (**27**) complexes the EPR spectra are similar, containing a broad and unsymmetrical band with a tendency to solve in components and also indicate an axial distorted stereochemistry (Figure II.2.1.6.1.2). The values of $g_{\|} = 2.160$ and $g_{\perp} = 2.068$ for (**23**) complex is usually found at species with rhombic symmetry and aligned tetragonal axes as the octahedral ligands / different donor atoms on the three coordination axes. For (**27**) complex the values of $g_{\|} = 2.167$ and $g_{\perp} = 2.069$ are similar to those of (**23**) compound and can result from a square pyramidal stereochemistry.

Complexes exhibited a good antimicrobial activity especially on Gram positive strains, their activity was higher than the 5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine ligand.

The highest antimicrobial activity showed the Cu(II) and Zn(II) complexes on the tested microbial strains, this activity can be explained based on stereochemically versatility which allow a slight interaction with the target site, and in case of Cu(II) this activity may be enhanced by an easy change of oxidation state which permit redox mediation reactions.

The cytotoxic activity of (**17**)-(**20**) compounds was assayed on the tumor cell line HCT 8 and was observed that $[Cu_2(L^2)_2Cl_4]\cdot H_2O$ (**19**) and $[Zn_2(L^2)_2Cl_4]$ (**20**) species induce cell apoptosis, and in the case of $[CuL^2(OH_2)_2(ClO_4)_2]\cdot 3H_2O$ (**23**) compound which present a good cytotoxic activity is clearly observed that it stops the cellular proliferation.

II.3. Complexes of Co(II), Ni(II), Cu(II) and Zn(II) with 5-phenyl-7-methyl-1,2,4-triazolo[1,5-*a*]pyrimidine

In order to obtain new series of complexes starting on the biological properties observed at triazolopyrimidine derivatives and their complexes, were obtained and characterized (**34**)-(**49**) complexes which contain 5-phenyl-7-methyl-1,2,4-triazolo[1,5-*a*]pyrimidine (L^3) as ligand, this new species resulting from [1+1] condensation of benzoylacetone and 3-amino-4H-1,2,4-triazole.

Compounds were formulated based on data provided by elemental chemical analysis and by correlating these data with those provided by physicochemical methods, as follows:

$[Co_2(L^3)_2Cl_4(OH_2)_4]$ (**34**)
$[Ni_2(L^3)_2Cl_4(OH_2)_4]\cdot 2H_2O$ (**35**)
$[Cu_2(L^3)_2Cl_4(OH_2)_2]$ (**36**)
$[Zn_2(L^3)_2Cl_4]$ (**37**)
$[CoL^3(OH_2)_5](ClO_4)_2\cdot H_2O$ (**38**)
$[NiL^3(OH_2)_5](ClO_4)_2\cdot H_2O$ (**39**)
$[CuL^3(OH_2)_3](ClO_4)_2$ (**40**)
$[ZnL^3(OH_2)_3](ClO_4)_2$ (**41**)
$[Co_2(L^3)_2(CH_3COO)_4(OH_2)_4]\cdot 5H_2O$ (**42**)
$[Ni_2(L^3)_2(CH_3COO)_4(OH_2)_4]\cdot 3H_2O$ (**43**)
$[Cu_2(L^3)_2(CH_3COO)_4]\cdot H_2O$ (**44**)
$[Zn_2(L^3)_2(CH_3COO)_4]$ (**45**)
$[Co(L^3)_2Cl_2]\cdot 0{,}5H_2O$ (**46**)
$[Ni(L^3)_2Cl_2(OH_2)_2]\cdot 2{,}5H_2O$ (**47**)
$[Cu_2(L^3)_4Cl_4]\cdot 2H_2O$ (**48**)
$[Zn(L^3)_2Cl_2]$ (**49**)

L^3: 5-phenyl-7-methyl-1,2,4-triazolo[1,5-*a*]pyrimidine

The ligand and complexes were characterized using thermal analysis which helped to identify the role of water molecules (crystallization and coordination) and the thermal stability ranges effects accompanying the heating process.

In the IR spectra of the triazolopyrimidine ligand appear two bands, an intense one at 1613 cm^{-1} is attributed to the stretching vibration ν_{tp} of triazolopyrimidine unit and the second band is attributed to stretching vibration mode of the pyrimidine ring ν_{py} at 1540 cm^{-1}; these bands are shifted in the IR spectra of complexes as a result of triazolopyrimidine coordination through nitrogen N3 [169].

^{1}H NMR and ^{13}C NMR spectra of the ligand and the [Zn(L$^{\mathbf{3}}$)$_2$Cl$_2$] (**49**) complex confirmed the formation of the condensation unit -C = N- from triazolopyrimidine unit [170].

Single crystal X-ray diffraction revealed that the 5-phenyl-7-methyl-1,2,4-triazolo [1,5-a]pyrimidine ligand crystallizes in monoclinic system, P21/n spatial group. The molecules are assembled as columns on the axis "a" through C-H···N intermolecular hydrogen bonds between the CH triazole cycle unit and N3 of the same cycle from another molecule (2.56-2.59 Å) and the interactions π-π staking (3.42-3.61 Å) (Figure II.3.2.3.1) [174]. The hydrogen bonds between the planes generate bidimensional networks as can be seen in Figure II.3.2.3.2.

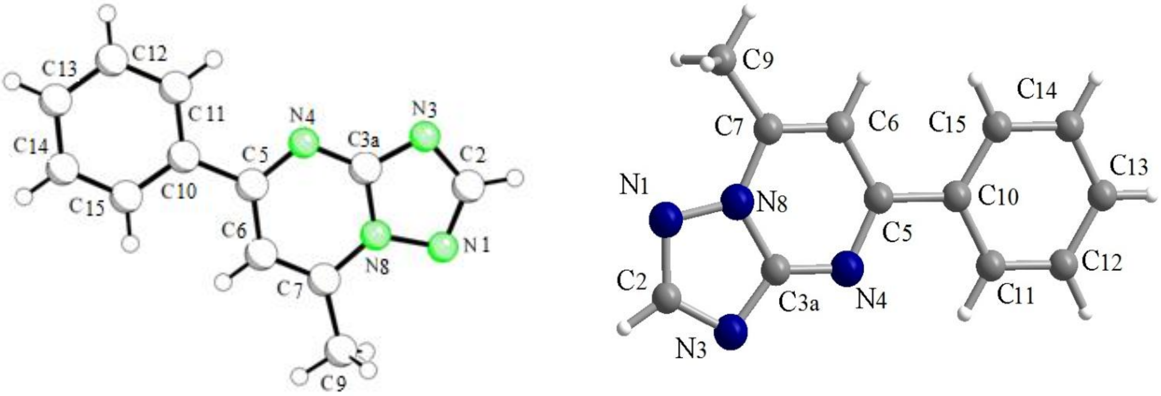

Figure II.3.2.3.1. X-ray structure of ligand 5-phenyl-7-methyl-1,2,4-triazolo[1,5-*a*]pyrimidine (L$^{\mathbf{3}}$)

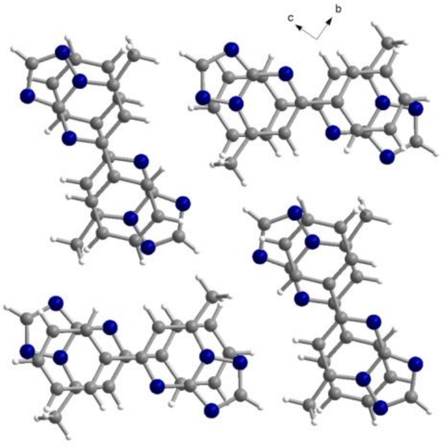

Figure II.3.2.3.2. Packing diagram of ligand 5-phenyl-7-methyl-1,2,4-triazolo[1,5-*a*]pyrimidine (L$^{\mathbf{3}}$)

For Co(II) ions was observed an octahedral and tetrahedral stereochemistry by electronic spectroscopy, for Ni(II) was proposed an octahedral stereochemistry and for Cu(II) was indicated an octahedral, square planar and square pyramidal stereochemistry; the geometry was confirmed by susceptibility magnetic data at room temperature for Co(II) and Ni(II) species.

The EPR spectra of copper (**36**) (Figure II.3.2.5.1), (**44**) and (**48**) compounds recorded on powder at room temperature present a weak, broad signal and with low intensity with g_{av} = 2.119, is characteristic for species with square pyramidal stereochemistry; the spectrum aspect may be due to a network arrangement disposed in dimeric species for the ion pairs which can be antiferromagnetic coupled by along the z-axis [96]. The aspect of spectra is preserved also in frozen DMSO solution, indicating the preservation in these conditions of dimeric units.

For copper(II) compound (**40**), the values of splitting parameter $g_{\parallel}$ = 2.066 and $g_{\perp}$ = 2.019 are characteristic for square planar stereochemistry with $G = (g_{\parallel}-2)/(g_{\perp}-2)$. The value of interaction exchange parameter of 3.47 suggests an exchange interaction between copper centers [175, 176]. The value of $A_{\parallel}$ hyperfine splitting parameter is 166 G for (**40**) compound and the value of the ratio $g_{\parallel} / A_{\parallel}$ is 125 G typically for square planar stereochemistry. The EPR spectrum in frozen DMSO solution indicates a distorted rhombic geometry as a result of solvent coordination and of the triazolopyrimidines ligand retention. In the case of this compound, the 0.57 value of the molecular orbital coefficient indicates a high degree of covalent σ bond with donor atoms in the plane [96].

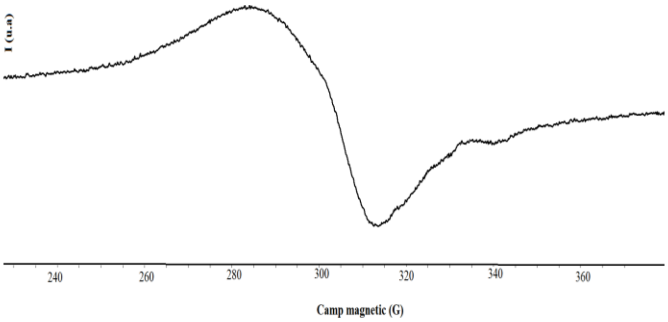

Figure II.3.2.5.1. EPR spectrum of (**36**) complex recorded
on powder at room temperature

The assays on pathogenic bacterial and fungal strains standard or isolated from clinic revealed a good activity for Co(II), Cu(II) and Zn(II) complexes.

The $[Co_2(L^3)_2(CH_3COO)_4(OH_2)_4]\cdot 5H_2O$ (**42**), $[Zn_2(L^3)_2(CH_3COO)_4]$ (**45**) and $[Zn(L^3)_2Cl_2]$ (**49**) complexes exhibited the most evident inhibitory effect upon de adherence ability of *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 134202 up to the minimum biofilm eradication concentration (MBEC) of 15.62 μg mL^{-1}.

Cytotoxic evaluation on tumor cells HT 29 revealed a very good cytotoxic activity for $[Cu_2(L^3)_2(CH_3COO)_4]\cdot H_2O$ (**44**) compound which visible stops the cell proliferation.

CONCLUSIONS

In the original part of the thesis are presented the results concerning synthesis, characterization and the establishment of antimicrobial and cytotoxic activity for new complexes of Co (II), Ni (II), Cu (II) and Zn (II) with ligands that consists 1,2,4-triazol unit. It were obtained 49 new compounds which are divided in two categories:

$MLX\cdot nH_2O$ M: Co(II), Ni(II), Cu(II) şi Zn(II),
$M(L)_2Cl_2\cdot nH_2O$ L: 2-[(E)-(1H-1,2,4-triazol-3-ylimino)methyl]phenol) ($HL^1\cdot H_2O$)
5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine (L^2)
5-phenyl-7-methyl-1,2,4-triazolo[1,5-*a*]pyrimidine (L^3)
X: Cl, ClO_4, CH_3COO

The compounds have been prepared by template condensation and were characterized by elemental chemical analysis, thermal analysis, measurement of electrical molar conductivity, IR, electronic spectra, ^{1}H NMR, ^{13}C NMR and EPR, magnetic susceptibility at room temperature, cyclic voltammetry and single crystal X-ray diffraction.

Monomeric or dimeric structures proposed for acetate complexes with Schiff base ligand were confirmed by mass spectrometry using electrospray ionization technique in positive and negative mode (ESI-MS).

Thermal analysis of chloride and acetate complexes allowed to identify the number and the nature of the water molecules (crystallization or coordination), the intervals of thermo stability and the final products nature of thermal decomposition which were in all cases the most stable metal oxide. For the chloride species appear an additional step which corresponds to chloride anion elimination as hydrochloric acid and for acetate species appear in addition a step which corresponds to the transformation of acetate into carbonate and sometimes, the overlapping takes place with oxidative degradation of triazole derivative, which is usually decomposed in two steps.

The IR spectra confirmed that condensation process occurred and provide the information on the coordination mode of triazole derivatives or other potential ligands (perchlorate, acetate).

For Co(II) ions was indirectly observed an octahedral and tetrahedral stereochemistry, electronic spectroscopy revealed for Ni(II) an octahedral geometry and for Cu(II) an octahedral, square planar and square pyramidal stereochemistry; the stereochemistry was confirmed by magnetic susceptibility data at room temperature especially for Co(II) and Ni(II) species.

EPR spectra of Cu(II) complexes provided informations on the surrounding metal ion (octahedral, square pyramidal or square planar), distortion type (axial or rhombic), bond nature between the metal ion and plan ligands and the paramagnetic ions interaction for dimeric species with small distance between the metal ions.

^{1}H NMR and ^{13}C NMR spectra of ligands and Zn(II) complexes confirmed that the condensation process occurred with -C=N- bond formation from Schiff base or from triazolopyrimidine unit. For zinc(II) species was observed a signal shifting of C2 carbon atom, neighbor with the N3 coordination center, confirming that the ligand binds to the metal ion through this atom.

Studies of single-crystal X-ray diffraction gave detailed information about the molecular and crystalline structure of four complexes and for two triazolopyrimidine derivatives, highlighting the π-π stacking interactions between pyrimidine rings or benzene and pyrimidine rings and also highlighted the hydrogen bonds interactions.

In the case of $[Co(L^{\mathbf{2}})_2(OH_2)_4][CoCl_4]$ (**17**) compound, for complex cation was observed a special *cis* arrangement of the two 5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine organic units.

All synthesized compounds were tested for antimicrobial activity establishment on the pathogenic bacterial strains, standard or isolated from clinic, Gram negative, Gram positive and on a *C. albicans* fungal strain, in suspension and on deposited on inert surfaces as biofilms.

Complexes showed a good antimicrobial activity against the microbial strains taken in the study comparatively with the three triazole derivatives, DMSO and the corresponding metal salts.

The assays revealed good activity for Co(II), Cu(II) and Zn(II) complexes for species which contains 2-[(E)-(1H-1,2,4-triazol-3-ylimino)methyl]fenol ($HL^{\mathbf{1}}\cdot H_2O$) and 5-phenyl-7-

methyl-1,2,4-triazolo[1,5-*a*]pyrimidine (L^3) and in the case of species which contain 5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine (L^2) ligand were active only Cu(II) and Zn(II) complexes.

The improved activity of Co(II), Cu(II) and Zn(II) complexes can be explained based on their stereochemical versatility which allows an easy interaction with the target site and in the case of Co(II), Cu(II) the activity may be associated with a slight change of oxidation state, which permits also the redox mediation reactions with this targets.

Most of species with very good activity are either unsaturated coordinative species or complexes which have coordination water molecules which can easily substitute in interaction with target biomolecules.

It was observed that the obtained species interact differently with the biofilm formed by various microbial strains; the effect is either inhibition or stimulation, depending on the strain and on the concentration of the test compounds.

Complexes containing copper(II) showed the most evident inhibitory effect upon the adherence ability up to the minimum biofilm eradication concentration (MBEC) of 3.91 $\mu g\ mL^{-1}$.

Generally, the activity of Schiff base species and 5-phenyl-7-methyl-1,2,4-triazolo[1,5-a]pyrimidine was better than the 5,7-dimethyl-1,2,4-triazolo[1,5-a]pyrimidine species, especially on Gram negative strains; the activity may be due to the presence of hydrophobic aromatic ring which increases the lipophilicity.

Some species of Co(II), Cu(II) with 5-phenyl-7-methyl-1,2,4-triazolo[1,5-a]pyrimidine exhibit a very good activity on the *S. aureus* MRSA 1263 Gram positive strain, isolated from clinic, methicillin resistant bacteria is a major cause that leads to infections acquired in hospitals.

Was tested the cytotoxic activity of the three ligands and selected complexes on tumor cell lines HEp 2, HCT 8 and HT 29. The most active species were the compounds which contain copper(II), the activity might be explained based on known toxicities of copper which generates reactive oxygen species (ROS) by the Fenton or Haber-Weiss reaction and also based on a possible mechanism whereby the intranuclear DNA is cleaved into fragments by these complexes which block the cell cycle and induces the apoptosis.

Complexes which presented a very good antimicrobial activity and low cytotoxicity can be further studied to develop new materials for fighting against biofilms which is frequently involved in the etiology of chronic infections and for the treating of infections caused by strains that have acquired resistance to antibiotics.

Combinations of copper (II) which acts as mitotic blockers can be studied for the development of new anticancer agents.

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LIST OF SCIENTIFIC PAPERS

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1. Thermal study on complexes with Schiff base derived from 1,2,4-triazole as potential antimicrobial agents, **Larisa Calu**, Mihaela Badea, Denisa Falcescu, Daria Duca, Dana Marinescu, Rodica Olar, J. Therm. Anal. Calorim., 111 (2013) 1725-1730. [**FI=1,982**]
2. Thermal behaviour of some novel antimicrobials based on complexes with a Schiff base bearing 1,2,4-triazole pharmacophore, Mihaela Badea, **Larisa Calu**, Mariana Carmen Chifiriuc, Coralia Bleotu, Alexandra Marin, Sebastian Ion, Gabriela Ionita, Nicolae Stanica, Luminiţa Măruţescu, Veronica Lazăr, Dana Marinescu, Rodica Olar, J. Therm. Anal. Calorim., DOI: 10.1007/s10973-014-3821-4. [**FI=1,982**]
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2. Thermal study on new Ni(II), Cu(II) and Zn(II) complexes with a decaazabismacrocyclic ligand as biological active species, Cristina Bucur, **Larisa Calu**, Rodica Olar, Mihaela Badea, Dana Marinescu, Petre Rotaru, Carmen Chifiriuc, Luminiţa Măruţescu, Coralia Bleotu, Veronica Lazar, ICTAC15 2012, 20-24 august, Osaka, Japan, poster.
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