POLITEHNICA UNIVERSITY OF BUCHAREST
Faculty of Applied Chemistry and Materials Science
Department of Inorganic Chemistry, Physical Chemistry and Electrochemistry

Summary of PhD Thesis

COMPLEX COMBINATIONS OF TRANSITIONAL METALS WITH MIXED LIGANDS (POLYAMINES, POLYACIDS)

Thesis Supervisor:

Prof.PhD.Emeritus Aurelia Meghea

PhD Student:

Chem. Mădălina Mihalache

BUCHAREST
2019

CONTENTS

(Corresponding to the doctoral thesis)

(The numbering of the figures and references is that from the PhD thesis)

Abbreviations

H_2A	α-ketoglutaric acid
HA	deprotonated α-ketoglutaric acid
A	deprotonated double α-ketoglutaric acid
H_3AU	usnic acid
H_2AU	deprotonated usnic acid
TB	1-(*o*-tolyl)biguanide
DMSO	dimethylsulfoxide
MIC	Minimum Inhibitory Concentration
MBEC	Minimal Biofilm Eradication Concentration

INTRODUCTION

Inorganic biochemistry has seen steady and rapid progress in recent decades, primarily due to the emergence of new chemical species (complex combinations or inorganic materials) with spectacular biological properties at times.

Many of the essential biological mechanisms could be explained using working tools specific to coordinative chemistry, which drove the development of this branch by obtaining new coordinating compounds, but also by identifying biological properties/applications.

A large number of metal compounds and complex combinations are used in medicine due to their anti-inflammatory, antibacterial, antitumor, antioxidant, antihypertensive properties [4-10].

The involvement of the coordination compounds in these phenomena is determined by the possibility of their interaction with the microorganisms (bacteria and fungi) that cause different infectious diseases. Metal ions can bind to certain parts of the microorganism, or the complex combination can produce hydrogen bonds or weak physical bonds through the ligand that can lead to alterations that reduce or eliminate their ability to cause disease. It has been known and demonstrated in numerous scientific works that the process of coordination of organic ligands with biological activity to a metal ion intensifies this activity, the mode of action of the complex combination being different from that of the free organic species [11-13]. The literature data on the complex combinations containing both types of ligands (from the class of biguanides and polyacids) are limited and of these very few study their biological activity; Most are structurally characterized with no emphasis on the possibility of their therapeutic use.

Given that in the literature there is no information on the existence of complex combinations containing 1-(*o*-tolyl) biguanide and polyacids, as well as the biological aspects of these types of organic compounds, the PhD thesis **Complex combinations of some Transitional metals with mixed ligands (polyamines, polyacids)** approach the synthesis and characterization of complexes containing ligands in these classes.

The doctoral thesis is divided into two parts. In the first part, data from the specialized literature regarding compounds in the class of biguanides and polyacids (general properties, biological activity and complexes in which they are ligands) are systematized.

The second part contains original contributions regarding the synthesis and characterization

of 22 new complex combinations of Cu(II), Co(II), Ni(II), Zn(II), Mn(II), Mn(III), Pd(II), Pt(II) and Pt(IV) with 1-(*o*-tolyl) biguanide and α-ketoglutaric acid as ligands.

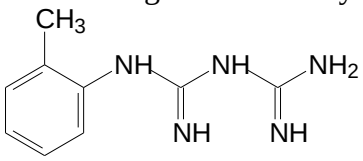
Also in this part are presented the original contributions regarding the obtaining and characterization of new complexes (15) of Cu(II), Co(II), Ni(II), Pd(II), Pt(II) and Pt(IV) having 1-(*o*-tolyl) biguanide ligands and usnic acid.

The formulas for the synthesized complex combinations were proposed following the interpretation of the results obtained by: elementary chemical analysis, UV-Vis-NIR electron spectroscopy, IR, EPR, thermogravimetric analysis, magnetic properties and molar electric conductivity. The interest for these complexes is that they can exhibit biological activity near or better than free ligands.

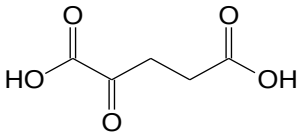
The thesis presents the results of testing the antimicrobial activity of the complexes and ligands against some Gram positive - *Pseudomonas aeruginosa* and Gram negative - *Staphylococcus aureus* strains. Following the research, it was found that most complex combinations have antimicrobial activity, and the minimum inhibitory concentrations have low values, which recommends the use of these compounds as antimicrobial agents.

Analyzing the influence of the synthesized complexes on the development of microbial biofilms on the inert substrate, it was observed that some of them inhibit the adhesion to the inert substrate of the studied strains to a large extent over a wide range of concentrations. The antifungal activity of some synthesized complexes and ligands on *Candida albicans* and antitumor activity on HeLa cells was also tested.

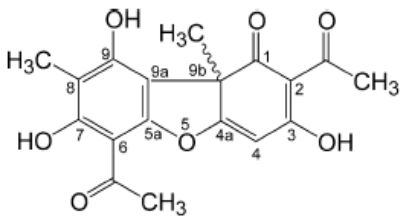
The formulas of ligands used in synthesis:



1-(*o*-tolyl) biguanide



α-ketoglutaric acid



usnic acid

The objectives of the doctoral thesis are:

- obtaining complex combinations of Cu(II), Co(II), Ni(II), Zn(II), Mn(II), Mn(III), Pd(II), Pt(II) and Pt(IV) with 1-(*o*-tolyl) biguanide and α-ketoglutaric acid as mixed ligands;

- obtaining complex combinations of Cu(II), Co(II), Ni(II), Pd(II), Pt(II) and Pt(IV) having 1-(*o*-tolyl) biguanide ligands and usnic acid;

- characterization of the compounds obtained in order to establish their structure by:

  • elemental analysis,

  • UV-Vis-NIR, FT-IR, EPR spectra

  • thermogravimetric analysis,

  • molar electrical conductivity,

  • magnetic measurements,

- biological activity testing:

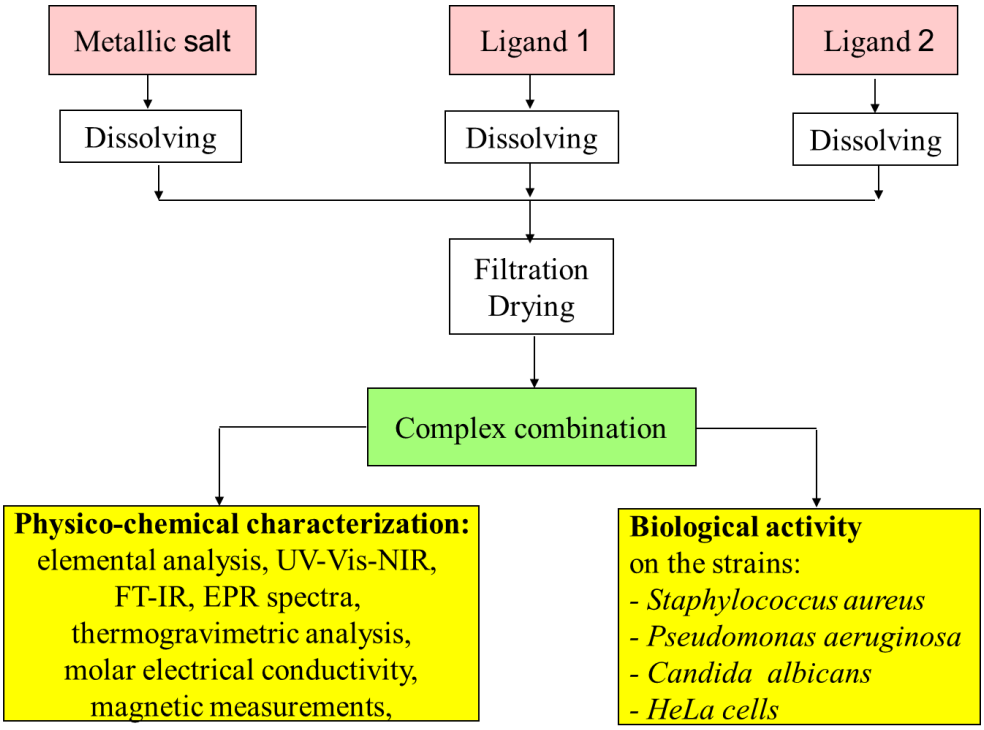
  • antibacterial activity,

  • antifungal activity,

  • antitumor activity,

  • influence of complex combinations on substrate adhesion of bacterial strains.

In principle, the working scheme used for the synthesis of the 37 complex combinations is as follows:



II.1.1. Synthesis and characterization of complex combinations of Ni(II) with α-ketoglutaric acid and 1-(*o*-tolyl) biguanide ligands

Four Ni (II) complexes were synthesized starting from its salts (chloride, bromide, nitrate, acetate) and the two ligands (α-ketoglutaric acid and 1-(*o*-tolyl) biguanide) [221]. The molar ratio of metal salt: α-ketoglutaric acid: 1-(*o*-tolyl) biguanide of 1:1:1 was worked. This report was also found in the complexes obtained.

The complex combinations obtained were characterized based on data provided by elemental analysis, molar electric conductance, thermal analysis, UV-Vis-NIR, FT-IR spectra and magnetic susceptibility.

The formulas proposed for these complexes are:

$[Ni(TB)(HA)(H_2O)_2]Cl$	light green	**(C1)**
$[Ni(TB)(HA)(H_2O)_2]Br$	dark green	**(C2)**
$[Ni(TB)(HA)]NO_3 \bullet H_2O$	orange	**(C3)**
$[Ni(TB)(HA)]CH_3COO$	beige	**(C4)**

Electric molar conductance

It was determined in solution of N,N-dimethylformamide, concentration 10^{-3}M at 25°C. For the C1 complex a molar conductance of 79.5 $S \bullet cm^2mol^{-1}$ was obtained, for C2 $S \bullet cm^2mol^{-1}$, for C3 72.6 $S \bullet cm^2mol^{-1}$, and for C4 77.2 $S \bullet cm^2mol^{-1}$. The obtained values indicate a 1:1 type electrolyte for all four complexes [222].

II.1.1.2. Thermal analysis

The interpretation of the results of the thermogravimetric analysis confirmed the presence of water molecules in the composition of complex combinations C1-C3.

For all four complexes analyzed the residue obtained is NiO (green), from which the percentage of nickel can be determined, namely: for C1 (12.73% experimental, 12.58% calculated), for C2 (11.28% experimental, 11.49% calculated), for C3 (11.98% experimental, 12.36% calculated), and for C4 (13.42% experimental, 13.00% calculated).

II.1.1.3. UV-Vis-NIR spectra and magnetic measurements

UV-Vis-NIR Spectra

The stereochemistry of the synthesized complex combinations was assessed using the near-ultraviolet-visible-infrared spectra, as compared to those of the ligands (α-ketoglutaric acid and 1-(*o*-tolyl) biguanide).

Magnetic susceptibility

Magnetic susceptibility determinations led to the following magnetic moment values for C1-C4 complexes: 3.19 μB for C1, 3.27 μB for C2 and zero for C3 and C4. In the case of complexes C3 and C4, the magnetic moment does not assume that they are diamagnetic, while the first two complexes are paramagnetic.

The proposed stereochemistry for the 4 complexes are:

(C1) $[Ni(TB)(HA)(H_2O)_2]Cl$	Rhombic distorted octahedral
(C2) $[Ni(TB)(HA)(H_2O)_2]Br$	Rhombic distorted octahedral
(C3) $[Ni(TB)(HA)]NO_3 \bullet H_2O$	Square planar
(C4) $[Ni(TB)(HA)]CH_3COO$	Square planar

II.1.1.4. FT-IR Spectra

In order to determine the coordination mode of the ligands to the metal ion, the FT-IR spectra of the C1-C4 complexes were analyzed in comparison with the H_2A and TB ligands.

In the spectra of the four complex combinations, a band shift is observed due to the valence vibration of the imine group, ν(C=N), from 1610 cm^{-1} to higher values. This is in agreement with the coordination of the TB ligand to the metal ions through the non-participating electron pair of imine nitrogen, and the displacement of this band to larger wave numbers is explained by the destruction of the π electron delocalization [231]. Coordination of 1-(*o*-tolyl) biguanide by the imminent nitrogen atoms is also supported by the displacement of the bands due to δ(NH)+ν(C-N) coupled vibrations.

The keto group at the α-position of the H_2A ligand is involved in coordination in all the analyzed complexes, confirmed by the movement of the corresponding band, ν(C=O), below 1720

cm^{-1}. It coordinates in the deprotonated form HA^- through the oxygen atom in the ketone group at the alpha position and the oxygen in the hydroxyl of the adjacent carboxyl group.

II.1.1.5.1. Antimicrobial activity

The evaluation of the antimicrobial activity of the synthesized ligands and complexes was performed on the species *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. It was observed that DMSO (the solvent used in the dilutions) does not influence the antimicrobial activity of the tested compounds at the working concentrations at the working intervals 5.00 - 0.039 mg/mL.

Determination of the minimum concentration of inhibitors

For the quantitative determination of the antimicrobial activity of the compounds tested against the two strains, the minimum inhibitory concentration, MIC, was determined. In the case of the Gram-positive *Staphylococcus aureus* species, complex C2 exhibited the same activity as ligands and complexes C1, C3 and C4 had better activity. Thus, the best activity is the complex combination C4, whose MIC value is 0.312 mg/mL.

The activity of the synthesized complexes against Gram negative bacterium *Pseudomonas aeruginosa* is better for the synthesized complexes than for the H_2A ligand, but similar to the TB ligand. Complex C4 has the best activity of the substances tested against this bacterium (MIC is 0.625 mg/mL).

Study of the influence of complex synthesized combinations on the development of microbial biofilms on an inert substrate

The synthesized complexes and ligands used to obtain them were investigated in terms of the ability to inhibit the adhesion of the microbial biofilm to the inert substrate.

All tested compounds inhibit this process in a dose-dependent manner up to a minimum biofilm eradication concentration (MBEC) of 0.039 mg/mL (C3, C4), 0.078 mg/mL (TB, H_2A, C2) and 0.156 mg/mL (C1), in the case of *Staphylococcus aureus*. For *Pseudomonas aeruginosa* all compounds have inhibition capacity up to a minimum biofilm eradication concentration of 0.039 mg/mL (TB, C1-C4) and for H_2A of 0.078 mg/mL.

II.1.1.5.2. Antitumor activity

The metabolism of HeLa cells varies depending on the type of material used. Thus, C1, C2 ligands and complexes have a very weak effect on HeLa tumor cells under the tested conditions, decreasing their viability by 12% and 13%, while complex combinations C3 and C4 demonstrated a moderate cytotoxic effect on them (decreasing them). Viability with 17% and 24.3% respectively). For the analyzed samples (at a concentration of 500 μg/mL for 24h incubation at 37^{o}C) and the untreated control sample, absorbances at 570 nm were read, from which the percentages of viability of HeLa cells were calculated in the presence of the compounds tested. For the untreated control sample, the viability of these cells was 100% and based on this value and the read absorbances, the viability was calculated for compounds C1-C4, H_2A and TB.

II.1.1.6. Partial conclusions

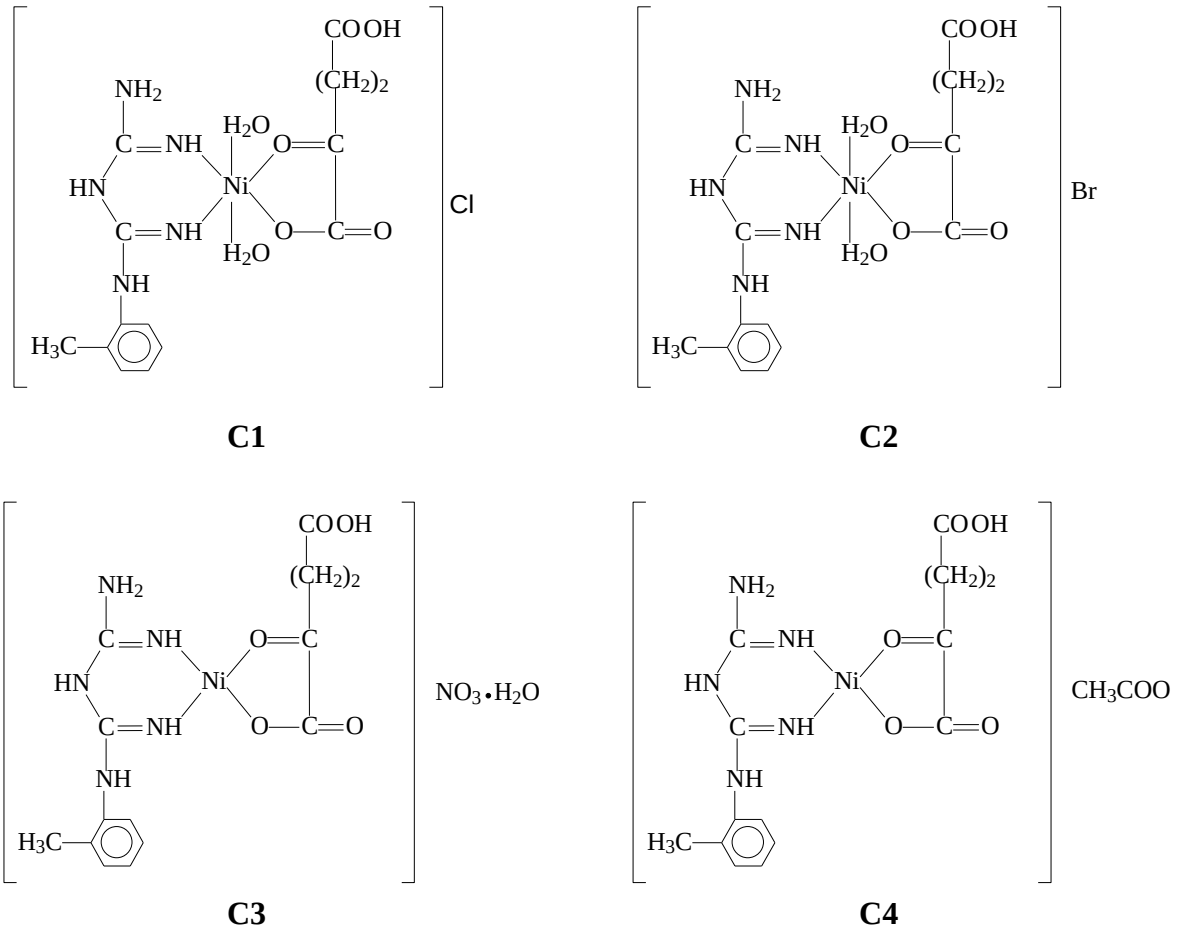
In this subchapter, the synthesis and characterization of four new complex combinations of nickel having α-ketoglutaric acid and 1-(*o*-tolyl) biguanide ligands have been described [221].

Based on the analyses performed (elemental analysis, UV-Vis-NIR spectra, FT-IR, thermal analysis, molar electric conductance, magnetic susceptibility), the formulas of C1-C4 complexes were proposed.

$[Ni(TB)(HA)(H_2O)_2]Cl$ and $[Ni(TB)(HA)(H_2O)_2]Br$ complexes have rhombic distorted octahedral stereochemistry while $[Ni(TB)(HA)]NO_3 \bullet H_2O$ and $[Ni(TB)(HA)]CH_3COO$ adopt square planar stereochemistry.

Both α-ketoglutaric acid and 1-(*o*-tolyl) biguanide function as bidentate ligands in the four complexes. The α-ketoglutaric acid participates in the deprotonated form HA^- and coordinates to the metal ion through the oxygen atom in the ketone group at the alpha position and the oxygen in the adjacent carboxyl group hydroxyl, while the 1-(*o*-tolyl) biguanide is in neutral form and coordinates with nickel ion through imminent nitrogen atoms.

For the synthesized complex combinations, the following formulas have been proposed (Figure II.1.14):



C1 **C2**

C3 **C4**

Fig. II.1.14. Proposed formulas for complex combinations C1 - C4

Antitumor activity on HeLa tumor cells of these complexes and ligands used in synthesis was tested. The best cytotoxic effect on this type of cell has the C4 complex. Regarding the antibacterial activity of the complexes obtained against the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*, this is comparable or better than that of the ligands.

Because C1-C4 complexes are electrolyte-like, their biological activity may come from the electrostatic interaction of the complex cation of these species with the negatively charged components of the membrane and their inactivation. On the other hand, antimicrobial activity may also be associated with stereochemistry, as well as the combined effect of the metal ligand and ion to inactivate a particular component involved in the pathogenesis of the microorganism.

II.1.2. Synthesis of complex combinations of Cu(II) with α-ketoglutaric acid and 1-(*o*-tolyl) biguanide ligands

For the synthesis of complexes of divalent copper were used its salts (chloride, nitrate, acetate, chlorate), and as ligands α-ketoglutaric acid and 1-(*o*-tolyl) biguanide [236-237]. Following the analyzes performed on these complexes, the following formulas have been proposed:

[Cu(TB)(HA)]Cl	light blue	**(C5)**
[Cu(TB)(HA)]NO_3	purple	**(C6)**
[Cu(TB)(HA)CH_3COO]•H_2O	light blue	**(C7)**
[Cu(TB)(HA)ClO_4]•$5H_2O$	green	**(C8)**

Electric molar conductance

For C5-C8 complexes, the molar electrical conductance at 25°C in N,N-dimethylformamide solution of 0.001 mol/L concentration was determined. Thus, for the C5 complex, molar electric conductance was obtained 72.1 S•cm2mol^{-1}, 81.1 S•cm^{2}mol^{-1} for C6, 36.5 S•cm^{2}mol^{-1} for C7, and for C8 S•cm^{2}mol^{-1}. The obtained values indicate a 1:1 type electrolyte for complexes C5 and C6, while complexes C7 and C8 are of non-electrolyte type [222].

II.1.2.2. Thermal analysis

Because there is a perchlorate anion in the composition of complex C8, its thermal analysis could not be performed as it was explosion hazard.

In the case of the C7 complex it is observed that below 160^{o}C there is a mass loss corresponding to a crystallization water molecule (experimental loss 3.72%, calculated 3.77%).

The residue obtained from the thermal analysis is CuO, from which the percentage of copper can be determined, namely: for C5 (14.81% experimental, 14.60% calculated), for C6 (13.58% experimental, 13.76% calculated, and for C7 (13.05% experimentally, 13.32% calculated).

II.1.2.3. UV-Vis-NIR spectra and EPR spectra

The correlation of the results obtained on the basis of the electronic paramagnetic resonance (EPR) spectra and the bands observed in the UV-Vis-NIR electronic spectra led to important

information regarding the stereochemistry of the obtained complexes and the character of the metal-ligand bonds in the analyzed complexes [238-246].

From the EPR spectrum of the C5 complex for the coefficients α^2, β^2_1, β^2 the following values were obtained: $\alpha^2 = 0.61$ which means that the sigma bonds in the plane are strong having predominantly covalent character; $\beta^2_1 = 0.57$ implies strong π bonds in the xoy plane and $\beta 2 = 0.93$ indicates weak π bonds outside the plane.

The values for the parameter $K_\perp$ for complexes C6 and C8 (0.62, respectively 0.64) indicate a predominantly covalent character of the ML bond, while for C7 the value $K_\perp = 0.85$ implies almost ionic metal-ligand bonds.

The proposed stereochemistry for the C5-C8 combinations are:

(C5) [Cu(TB)(HA)]Cl	Square planar
(C6) [Cu(TB)(HA)]NO_3	Square planar
(C7) [Cu(TB)(HA)CH_3COO]•H_2O	Square pyramid
(C8) [Cu(TB)(HA)ClO_4]•$5H_2O$	Distorted octahedral

II.1.2.4. FT-IR Spectra

From the analysis of the FT-IR spectra of the C5-C8 complexes and their comparison with those of the ligands used in the synthesis, it was possible to determine the coordination of the two ligands to the copper ion.

The keto group from the α-position of the H_2A ligand is involved in coordination in all complexes, which is confirmed by moving the ν(C=O) band below 1720 cm^{-1} in the complex spectrum from its position in the α-ketoglutaric acid spectrum.

In the spectra of the four complex combinations, a band shift due to the valence vibration of the imine group, ν(C=N), from 1610 cm^{-1} is observed. This shift is in agreement with the coordination of 1-(*o*-tolyl) biguanide to the copper ion through the pair of non-participating electrons of imine nitrogen.

II.1.2.5.1. Antimicrobial activity

Determination of the minimum concentration of inhibitors

In order to determine the antimicrobial activity for ligands and complexes, the minimum inhibitory concentration was determined on the *Staphylococcus aureus* strains ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. The working solutions had concentrations between 5.00 and 0.039 mg/mL.

Following the test on the species Staphylococcus aureus ATCC 25923, it was found that the best antimicrobial activity was C5 and C6 complexes with a MIC of 0.312 mg/mL. Complex C7 has slightly lower activity than C5 and C6, but better than ligands and C8.

The activity of H_2A ligand against *Pseudomonas aeruginosa* ATCC 27853 is lower than that of the other ligand, TB and the four complexes. The best activity against this strain is C5 and C6 complexes (MIC is 0.625 mg/mL), while C7 and C8 complexes have the same TB ligand.

Study of the influence of complex synthesized combinations on the development of microbial biofilms on an inert substrate

In terms of the ability to inhibit the adhesion of the microbial biofilm to the inert substrate, all tested compounds inhibit this process depending on the dose up to a minimum biofilm eradication concentration (MBEC). All complexes have a MBEC of 0.039 mg/mL, lower than the TB and H_2A ligands (0.078 mg/mL), against the *Staphylococcus aureus* species.

In the case of *Pseudomonas aeruginosa* strain, all complexes and TB ligands have the ability to inhibit the biofilm to a minimum biofilm eradication concentration of 0.039 mg/mL minus the H_2A ligand having MBEC 0.078 mg/mL.

II.1.2.5.2. Antifungal activity

Antifungal activity was determined on *Candida albicans* ATCC 10231. The working solutions had concentrations in the range 1.00-0.0019 mg/mL. Ligands have the lowest activity, C5 and C6 complexes have moderate activity, and the best C7 and C8 complexes have a MIC of 0.125 mg/mL.

II.1.2.5.3. Antitumor activity

The antitumor activity of the ligands and the four complexes on HeLa cells was tested.

500 μg/mL concentration solutions were used for testing, and incubation was carried out for twenty-four hours at 37°C.

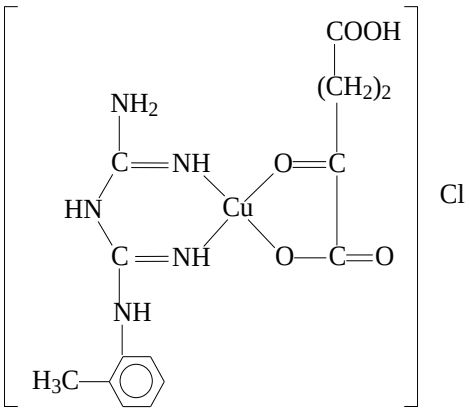
H_2A ligand has a very weak effect on HeLa tumor cells under the conditions tested, while TB ligand and C7-C8 complexes demonstrated a moderate cytotoxic effect on them, reducing their viability by 18% and 20%, respectively. C5 and C6 complexes have a better cytotoxic effect against HeLa cells, reducing their viability by 27% and 23%, respectively.

II.1.2.6. Partial conclusions

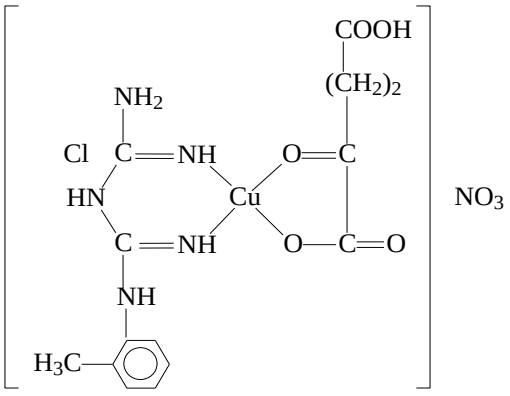
Based on various copper salts, four complex combinations of Cu (II) were synthesized, characterized and tested in terms of biological activity [236-237]. The ligands used in their synthesis were α-ketoglutaric acid and 1-(*o*-tolyl) biguanide.

The proposed formulations for the synthesized combinations were based on the analyses performed (elemental analysis, UV-Vis-NIR, FT-IR, EPR spectra, thermal analysis, molar electric conductivity).

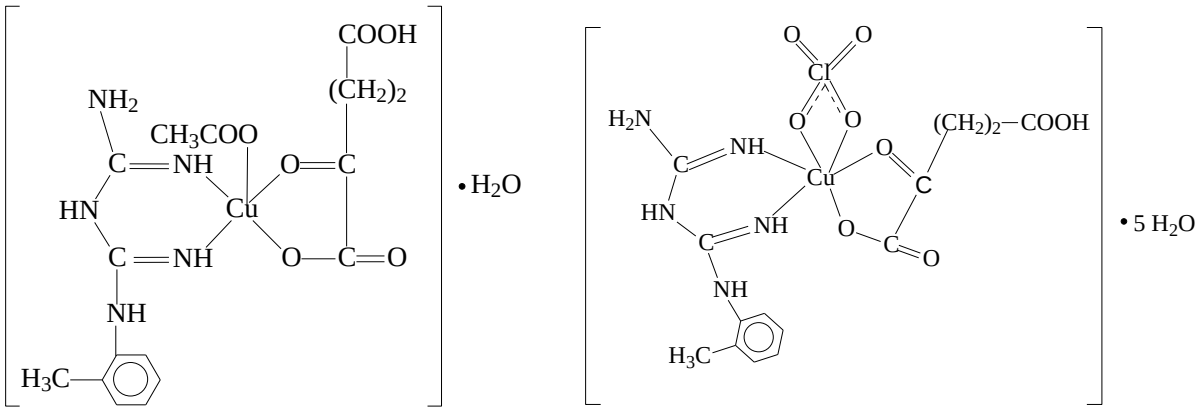
For the synthesized complexes, the following formulas have been proposed (Figure II.1.28):



C5



C6



C7 **C8**

Fig. II.1.28. Proposed structures for the synthesized complexes

The molar ratio used in metal salt synthesis: ligand 1: ligand 2 of 1:1:1 is also found in the complexes obtained.

The α-ketoglutaric acid functions as a bidentate ligand in deprotonated form in all four synthesized complexes. It coordinates to the copper ion through the oxygen atom in the ketone group at the alpha position and the oxygen in the hydroxyl of the adjacent carboxyl group.

1-(*o*-tolyl) biguanide also functions as a bidentate ligand in C5-C8 complexes, the imminent nitrogen atoms being involved in coordination with the metal ion.

For the two strains tested (*Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853) the best antimicrobial activity is C5 and C6 complexes.

All synthesized complexes have better antifungal activity (tested on *Candida albicans* strain ATCC 10231) than ligands.

C5 and C6 complexes have the best antitumor activity on HeLa cells.

The different biological activity for the four complexes is due to their different stereochemistry (square planar C5 and C6, square pyramid C7, distorted octahedral C8), as well as the inactivation of certain components involved in the pathogenesis of the microorganism by the metal ion and ligands.

II.1.3. Synthesis of complex combinations of Mn(II) and Mn(III) with α-ketoglutaric acid and 1-(*o*-tolyl) biguanide ligands

Using α-ketoglutaric acid (H_2A) and 1-(*o*-tolyl) biguanide (TB) as ligands and Mn(II) salts four manganese complexes were synthesized [237,248]. Three of these are Mn(III) and one of Mn(II), which means that during the synthesis reactions the manganese was oxidized to a higher oxidation state when C10-C12 complexes were obtained. Although metal salt: H_2A: TB of 1:1:1 molar ratio has been worked, it is not found in any complex.

The proposed formulas for complex syntheses are:

$[Mn(TB)_2(H_2A)]Cl_2 \bullet 0{,}5C_2H_5OH$	pink	**(C9)**
$[Mn_2(TB)(A)(H_2A)_2(H_2O)_2(NO_3)_2](NO_3)_2 \bullet C_2H_5OH$	beige	**(C10)**
$[Mn_2(TB)(A)(H_2A)_2(H_2O)_2(CH_3COO)_2](CH_3COO)_2 \bullet 3H_2O$	brown	**(C11)**
$[Mn_2(TB)(HA)_2(OH)(H_2O)_4](ClO_4)_3$	light brown	**(C12)**

II.1.3.2. Thermal analysis

Thermogravimetric analysis provided information that confirmed the proposed formulations for the complex combinations, the presence of water molecules and ethyl alcohol and the thermal effects that accompany the mass loss processes. For the C12 complex containing perchlorate anions, this analysis could not be performed for security reasons.

The remaining residue is Mn_2O_3 for all complexes, from which the percentage of manganese can be determined; 8.04% experimental, 8.12% calculated for C9,10.37% experimental, 10.30% calculated for C10, 10.61% experimental, 10.34% calculated for C11.

II.1.3.3. UV-VIS-NIR Spectra

The stereochemistry of the complex combinations C9 - C12 proposed based on the near ultraviolet-visible-infrared spectra are:

(C9) $[Mn(TB)_2(H_2A)]Cl_2 \bullet 0{,}5C_2H_5OH$	Octahedral
(C10) $[Mn_2(TB)(A)(H_2A)_2(H_2O)_2(NO_3)_2](NO_3)_2 \bullet C_2H_5OH$	Tetragonal distorted octahedral
(C11) $[Mn_2(TB)(A)(H_2A)_2(H_2O)_2(CH_3COO)_2](CH_3COO)_2 \bullet 3H_2O$	Tetragonal distorted octahedral
(C12) $[Mn_2(TB)(HA)_2(OH)(H_2O)_4](ClO_4)_3$	Tetragonal distorted octahedral

II.1.3.4. FT-IR Spectra

By analyzing the FT-IR spectra of the four complexes and comparing them with those of ligands, the coordination of ligands to the metal ion was established. The H_2A ligand coordinates in the form of HA^- (deprotonated) through the ketone oxygen atom at the alpha position and the oxygen in the hydroxyl of the adjacent carboxyl group (in the C12 complex).

In complex C9 the ketoglutaric acid coordinates to the metal ion in the form of H_2A while in complexes C10 and C11 one acid is bridged as A2-, the other two coordinating as H_2A.

1-(*o*-tolyl) biguanide functions as bidentate ligand coordinating to the metal ion through non-participating electrons of imine nitrogen [231].

II.1.3.5.1. Antimicrobial activity

Determination of the minimum concentration of inhibitors

The evaluation of the antimicrobial activity of the new complexes and of the ligands used was performed on the species *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. Work was carried out at concentrations between 5.00 mg/mL and 0.039 mg/mL.

In the case of the species *Staphylococcus aureus*, the complex combinations showed a similar activity to the ligands (complexes C10 and C11) and better than theirs for C9 and C12, having a MIC value of 0.625 mg/mL and 0.312 mg/mL, respectively. The best antimicrobial activity against *Pseudomonas aeruginosa* is the C12 complex, while the C9-C11 complexes are better than for the H_2A ligand, but weaker than the TB ligand.

Study of the influence of complex synthesized combinations on the development of microbial biofilms on an inert substrate

C10 - C12 complexes have the ability to inhibit the adhesion of the microbial biofilm to the inert substrate of the *Staphylococcus aureus* strain better than the ligands and the C9 complex, having a MBEC of 0.039 mg/mL.

For *Pseudomonas aeruginosa* strain C11, C12 and TB have the lowest biofilm eradication concentration of 0.039 mg/mL compared to C9 and H2A (0.078 mg/mL) and C10 (0.156 m/mL).

II.1.3.5.2. Antitumor activity

Following the tests performed for C9-C12 complexes and the two ligands on HeLa cells, a moderate cytotoxic effect was observed for C10 and C11, which decreased the viability of these cells by 26% and a weak effect of the other two complexes.

II.1.3.6. Partial conclusions

Four complex combinations of manganese were synthesized and characterized as α-ketoglutaric acid and 1-(*o*-tolyl) biguanide ligands [237,248].

Based on the interpretation of the analyses performed for these complexes (elemental analysis, UV-Vis-NIR, IR spectra, thermal analysis, molar electric conductance), their formulas have been proposed.

Although divalent manganese salts have been used in synthesis, only C9 complex contains manganese with valence two, which means that in the other syntheses manganese has been oxidized so that in the complex combination it is found in the three valence state.

Both ligands function bidentate in the four complexes, TB coordinating to the metal ion through the imminent nitrogen atoms, while H_2A through an oxygen atom in the ketone group at the alpha position and one in the adjacent carboxyl group.

C10-C12 complexes are dinuclear and have a bridging ligand, namely the double-deprotonated α-ketoglutaric acid in C10 and C11, while in C12 the hydroxyl ion forms a bridge between two trivalent manganese ions.

The proposed stereochemistry for the synthesized complexes are: octahedral for C9 and tetragonally distorted octahedral for C10-C12.

Complexes C9 and C12 have the best antibacterial activity on the *S. aureus* strain, while *P. aeruginosa* has C12. The best ability to inhibit the adhesion of the microbial biofilm to the inert substrate of the two tested strains is C11 and C12 complexes.

A moderate cytotoxic effect on HeLa cells has C10 and C11 complexes.

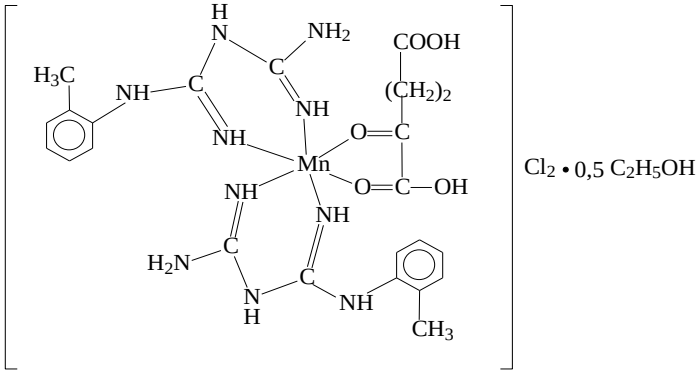
The biological activity of the complexes and the differences that appear for the four complexes can be accounted for by several factors:

- all are electrolyte type
- their stereochemistry
- manganese valence

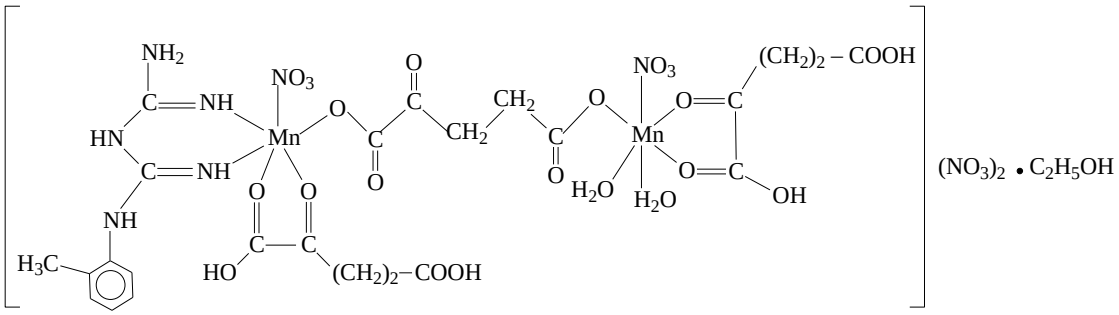
- mono- or dinuclear complexes

- the nature of the anions in the composition of the complexes.

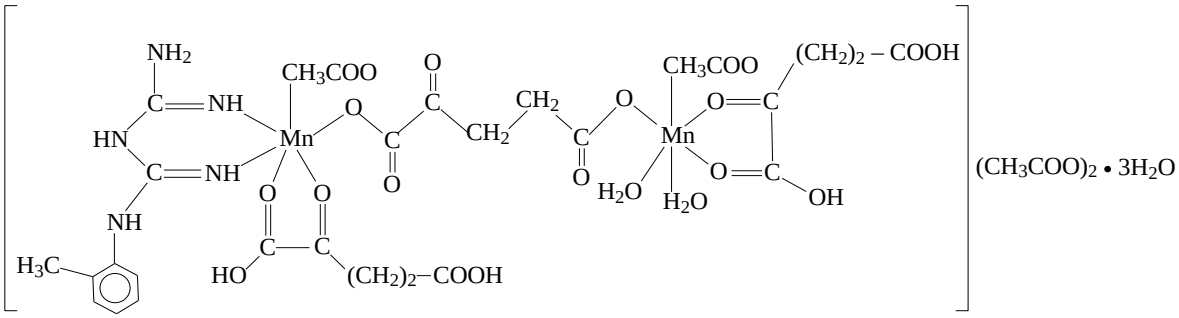
The proposed structures for complexes C9-C12 are shown in Figure II.1.41.



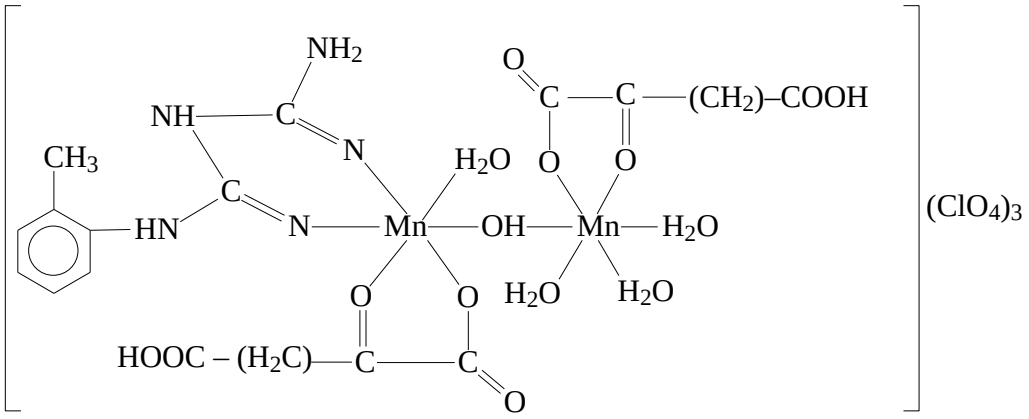
C9



C10



C11



C12

Fig.II.1.41. Proposed formulas for complexes C9-C12

II.1.4. Synthesis of complex combinations of Zn(II) with α-ketoglutaric acid and 1-(*o*-tolyl) biguanide ligands

Three novel complex combinations of Zn (II) were synthesized using chloride, nitrate, zinc acetate and α-ketoglutaric acid and 1-(*o*-tolyl) biguanide as ligands. Metal salt: α-ketoglutaric acid: 1-(*o*-tolyl) biguanide of 1:1:1 was worked for all complexes. This ratio was maintained only in complexes C13 and C14.

Following the analyses performed for these complexes (elemental analysis, FT-IR and UV Vis spectra, molar electric conductance and thermal analysis), the following formulas have been proposed:

$[Zn(TB)(HA)(H_2O)_2]Cl$	light yellow	**(C13)**
$[Zn(TB)(HA)(H_2O)_2]NO_3$	light yellow	**(C14)**
$[Zn_2(TB)_2(A)(CH_3COO)_2] \cdot H_2O$	light yellow	**(C15)**

Electric molar conductance

From the values obtained for the molar electric conductance for the synthesized complexes, the following conclusions are drawn: complexes C13 and C14 are electrolytes of type 1:1, while complex C15 is non-electrolyte [222].

II.1.4.2. Thermal analysis

The presence of water molecules in the composition of complex combinations C13-C15 was revealed following the thermogravimetric analysis.

For all analyzed complexes the obtained residue is ZnO. From this one can determine the percentage of zinc, namely: for C13 (13.54% experimental, 13.82% calculated), for C14 (13.38% experimental, 13.09% calculated, and for C15 (16.85% Experimental, 16.49% calculated).

II.1.4.4. FT-IR Spectra

In all complexes, the keto group at position α of the H_2A ligand is involved in coordination, which is confirmed by the displacement of the corresponding band, ν(C=O), below 1720 cm^{-1}. This ligand coordinates to the bidentate metal ion, in the form deprotonated HA^-, through the oxygen atom in the ketone group at the alpha position and the oxygen in the hydroxyl of the adjacent carboxyl group in the C13 and C14 complexes. In the C15 complex, he coordinates all the bidentate, in the bridge, as dianion.

In the spectra of the three complexes, a band shift is observed due to the valence vibration of the imine group, ν(C=N), from 1610 cm^{-1} to higher values. This is in agreement with the coordination of the TB ligand to the metal ions by the pair of non-participating electrons of imine nitrogen.

II.1.4.5. Testing of biological activity

After testing the complexes synthesized on HeLa cells it was found that they do not exhibit antitumor activity.

II.1.4.5.1. Antimicrobial activity

Determination of the minimum concentration of inhibitors

Evaluation of antimicrobial activity was performed in *vitro* on *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 strains for synthesized complexes and

ligands used in synthesis. For all tested compounds the concentration range was worked from 1.00 to 0.0019 mg/mL.

Analyzing the results obtained from the tests performed, it was found that the best antimicrobial activity against *S. aureus* has the C15 complex having the lowest inhibitory concentration of 0.062 mg/mL. The other two complexes and ligands have slightly lower activity than C15, but good, with MIC of 0.125 mg/L.

On the *P. aeruginosa* strain, all the complexes have much better antimicrobial activity than the ligands, with the lowest MIC having C13 and C14 (0.125 mg/mL).

Following the study of the influence of the synthesized complex combinations on the development of microbial biofilms on the inert substrate, it was found that none of the three complexes has the capacity to inhibit the adhesion of the microbial biofilm to the inert substrate on the tested strains (*S. aureus* and *P. aeruginosa*).

II.1.4.5.2. Antifungal activity

Antifungal activity for ligands and complexes was tested in *vitro* on Candida albicans ATCC 10231, in the concentration range 1.00-0.0019 mg/mL. C13 and C14 complexes have a better activity against this strain having a MIC of 0.5 mg/mL. The two ligands and C15 have the same antifungal activity; they have a MIC of 1.00 mg/mL.

II.1.4.6. Partial conclusions

Three complex combinations of Zn(II) with α-ketoglutaric acid and 1-(*o*-tolyl) biguanide were synthesized and characterized.

Based on the analyses performed, the formulas of complexes C13-C15 were proposed. Both ligands function as bidentate ligands in the three complexes. In complex C15 the α-ketoglutaric acid is bridged, as a dianion, and in C13 and C14 it is bidentate in anionic form.

1-(*o*-tolyl) biguanide coordinates to the metal ion through imine nitrogen atoms.

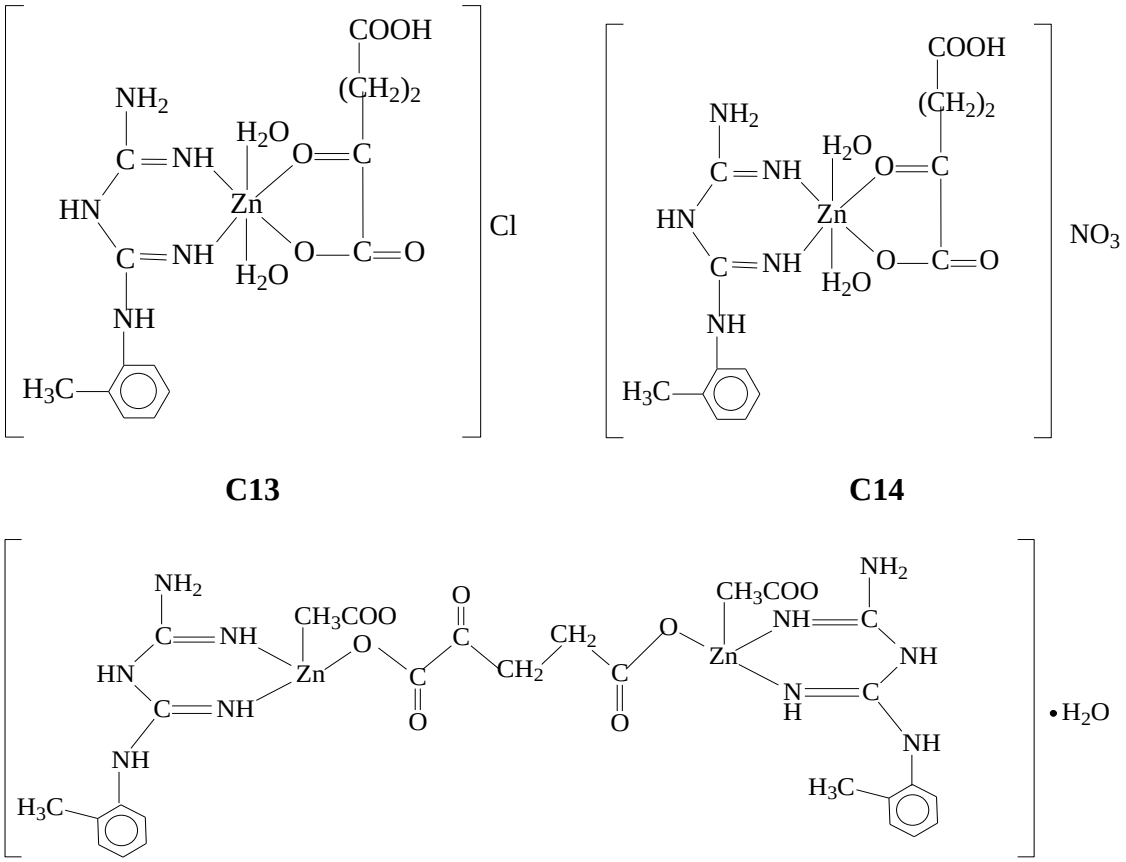
Complexes C13 and C14 have octahedral stereochemistry, and complex C15 is square; C13 and C14 contain a single zinc ion, while C15 has two zinc ions linked together by α-ketoglutaric acid in bridges.

The good antimicrobial activity of the complexes observed after testing on *S. aureus* is largely due to the ligands they contain, and in the case of C15 a better activity can be explained by the presence of two zinc ions. On the other hand, antimicrobial activity may also be associated with stereochemistry, electrolyte type, as well as the combined effect of metal ligand and ion.

The better antifungal activity on *C. albicans* for C13 and C14 complexes may be due to the octahedral stereochemistry they have.

The C13-C15 complexes have no antitumor activity on the tested cells (HeLa) nor inhibit the adhesion of the microbial biofilm to the inert substrate on the two strains (*S. aureus* and *P. aeruginosa*).

The proposed formulas for the three Zn (II) complexes are shown in Figure II.1.53.



C13 **C14**

C15

Fig. II.1.53. Proposed formulas for C13-C15 complexes

II.1.5. Synthesis of complex combinations of Pd(II), Pt(II) and Pt(IV) with α-ketoglutaric acid and 1-(*o*-tolyl) biguanide ligands

Using α-ketoglutaric acid and 1-(*o*-tolyl) biguanide as ligands, two complexes were synthesized with Pd(II) and two with Pt(II) and Pt(IV). The metal salts used in the synthesis are $PdCl_2$, $Pd(CH_3COO)_2$,, respectively H_2PtCl_6 şi $PtCl_2$.

Although metal salt: α-ketoglutaric acid: 1-(*o*-tolyl) biguanide of 2:1:1 has been worked for all complexes, this ratio is found only in complex combinations C16 and C19, in the other two being 1:1:1.

The proposed formulas for the four synthesized complexes are:

$[Pd(TB)(H_2A)][PdCl_4]$	dark orange	**(C16)**
$[Pd(TB)(HA)]CH_3COO•H_2O$	orange	**(C17)**
$[Pt(TB)(HA)Cl_2]Cl$	orange	**(C18)**
$[Pt(TB)(H_2A)][PtCl_4]•H_2O$	brown	**(C19)**

II.1.5.2. Thermal analysis

The presence of a crystallization water molecule in the composition of C17 and C19 complexes was also confirmed by their thermal analysis.

For complexes C16 and C17, the residue obtained by thermal decomposition was palladium (over 700°C palladium oxide passes into metallic palladium) [250].

In the case of complexes C18 and C19 at a temperature above 500 ° C the residue formed is platinum, (at 450-480°C $PtCl_2$ decomposes into platinum and chlorine) [251].

II.1.5.3. UV-Vis-NIR Spectra

Based on the comparison of the electronic spectra of the synthesized complexes with those of the ligands, their stereochemistry could be attributed.

(C16) $[Pd(TB)(H_2A)][PdCl_4]$	Square planar
(C17) $[Pd(TB)(HA)]CH_3COO •H_2O$	Square planar
(C18) $[Pt(TB)(HA)Cl_2]Cl$	Octaehedral
(C19) $[Pt(TB)(H_2A)][PtCl_4]•H_2O$	Square planar

II.1.5.4. FT-IR Spectra

In all the analyzed complexes, the keto group from the α-position of the H_2A ligand is involved in coordination, the displacement of the corresponding band, ν(C=O) at lower values than in the α-ketoglutaric acid being a proof in this respect.

The coordination of the TB ligand to the metal ions is done through the pair of non-participating electrons of imine nitrogen. By coordination, the delocalization of the π electrons is destroyed, which explains the displacement of this band to larger wave numbers [231].

II.1.5.5.1. Antimicrobial activity

In vitro antimicrobial activity assays were performed on *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 strains for synthesized complexes and ligands used in synthesis. It was worked in the concentration range 1.00 - 0.0019 mg/mL for all compounds tested.

Antimicrobial activity ~~against~~ for Gram-positive *Staphylococcus aureus* is much ~~better~~ (efficient) for the four complexes than for ligands. The lowest value of the minimum inhibitory concentration is for C16, C18 and C19 complexes, 0.0039 mg/mL.

The activity against Gram negative bacterium *Pseudomonas aeruginosa* is the same for both ligands. And in the case of this strain, all the complexes have good antimicrobial activity, C18 having MIC of 0.0312 mg/mL and C19 of 0.0625 mg/mL.

II.1.5.5.2. Antifungal activity

C16, C18 and C19 complexes have the best activity against this strain having a MIC of 0.125 mg/mL. Ligands have a much lower antifungal activity than complexes, with MIC of 1 mg/mL. The working concentration range for the tested substances was 1.00-0.0019 mg/mL.

II.1.5.5.3. Antitumor activity

Antitumor activity was tested for all compounds on HeLa cells. 500 µg/mL concentration solutions were used for testing, and incubation was done at 37°C for 24 hours.

Under the conditions tested, complexes C18 and C19 have a good cytotoxic effect on HeLa cells, reducing their viability by 41% and 38%, respectively. The other two complexes have a moderate cytotoxic effect, and the two ligands have a weak effect on these cells.

II.1.5.6. Partial conclusions

Four complex combinations of Pd(II), Pt(II) and Pt(IV) were synthesized with 1-(*o*-tolyl) biguanide and α-ketoglutaric acid and as ligands.

Their formulas were proposed based on the interpretations of the analyzes performed, namely: elemental analysis, UV-Vis-NIR, IR spectra, thermal analysis.

Both ligands are bidentate in all the complexes obtained. The TB ligand coordinates to the metal ion through the imminent nitrogen atoms and the H_2A ligand through the oxygen atoms from the ketone groups in the alpha position and in the adjacent carboxyl group. In complexes C16 and C19 the α-ketoglutaric acid is nonprotonated while in the other two complexes it is in the form of an HA^-.

The proposed stereochemistry for the synthesized complexes are: octahedral for C18 and square plane for C16, C17 and C19.

In complex C18 platinum is tetravalent and in C19 divalent, exactly as in the salts used in synthesis. The C16 and C19 complexes are of the Vauquelin type, having a complex anion $[PdCI_4]^{2-}$, respectively $[PtCI_4]^{2-}$.

Biological activity is influenced by several factors:

- the nature of the metal
- the oxidation state of the metal
- number of metal ions in the complex
- the stereochemistry of the complex.

The fact that all complexes have better biological activity than ligands, can be accounted by coordination of ligands to the metal ion.

Platinum complexes have the best antitumor activity, as expected.

The proposed structures for complexes C16-C19 are shown in Figure II.1.66.

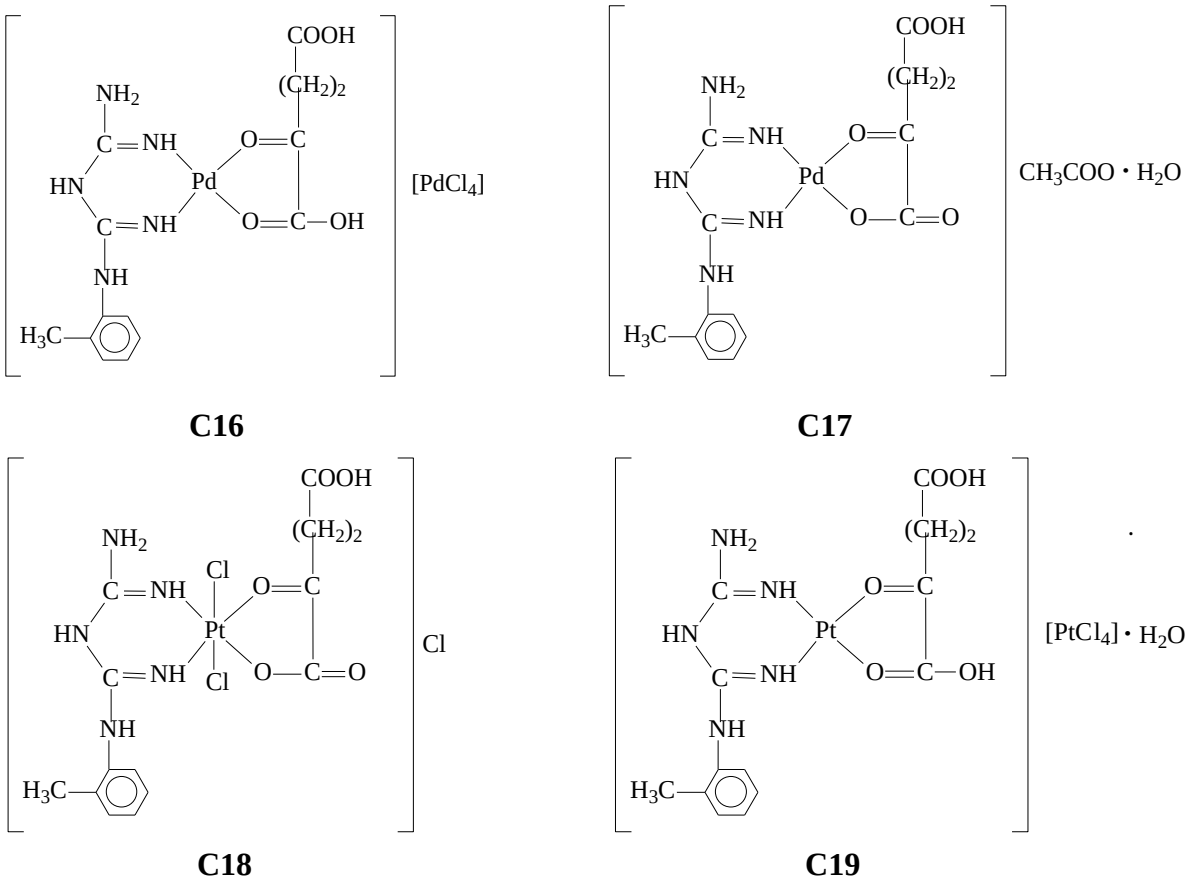


Fig. II.1.66. Formulas of complex combinations C16-C19

II.1.6. Synthesis of complex combinations of Co(II) with α-ketoglutaric acid and 1-(*o*-tolyl) biguanide ligands

Three new complexes of divalent cobalt were obtained from the reaction of salts of this metal, α-ketoglutaric acid and 1-(*o*-tolyl) biguanide (as ligands). The synthesized complex combinations have been analyzed from a physico-chemical point of view and the proposed formulas for these complexes are:

$[Co(HA)_2(TB)(H_2O)]$	light gray	**(C20)**
$[Co(HA)(TB)(H_2O)(NO_3)]\bullet C_2H_5OH$	intense pink	**(C21)**
$[Co_2(HA)(TB)(H_2O)_3(CH_3COO)_3]$	violet	**(C22)**

Electric molar conductance

Following the determination of the molar electrical conductance, in solution of N,N-dimethylformamide, of concentration 10^{-3}M at 25°C for all the complexes, values were obtained that fit them in the category of non-electrolytes: 37.5 S•cm^{2}mol^{-1} for C20, 41.4 S•cm^{2}mol^{-1} for C21 and 39.6 S•cm^{2}mol^{-1} for C22 [222].

II.1.6.2. Thermal analysis

For C20-C22 complexes, thermal analysis was performed in static air atmosphere, from which the presence of water and ethanol molecules in their composition was established.

For all complexes, the final residue is Co_3O_4. From this one it can be determine the percentage of cobalt from each complex, 10.91% for C20, 11.17% from C21, respectively 17.35% in C22.

II.1.6.3. UV-Vis-NIR Spectra

By analyzing the electronic spectra of the complexes and comparing them with the ligands, the stereochemistry for C20-C22 was proposed:

(C20) $[Co(HA)_2(TB)(H_2O)]$	Trigonal bipyramid
(C21) $[Co(HA)(TB)(H_2O)(NO_3)]•C_2H_5OH$	Tetragonal deformed octahedral
(C22) $[Co_2(HA)(TB)(H_2O)_3(CH_3COO)_3]$	Octahedral

II.1.6.4. FT-IR Spectra

The keto group located at the α-position of the H_2A ligand appears displaced in the complex spectrum to the free ligand because it is involved in coordination.

A band that have a great importance in coordinating the 1-(*o*-tolyl) biguanide to the cobalt ion is that due to the valence vibration of the imine group, ν(C=N), from 1610 cm^{-1}. In the spectra of the complexes, a displacement of this band is observed, a displacement that is in agreement with the coordination of the 1-(*o*-tolyl) biguanide to the copper ion through the pair of non-participating electrons of the imine nitrogen.

II.1.6.5.1. Antimicrobial activity

For the synthesized complexes and ligands antimicrobial activity was tested *in vitro* on the species *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. The working range for these assays was 1.00 - 0.0019 mg/mL.

In the case of *Staphylococcus aureus*, complex combinations showed better antimicrobial activity than ligands. The lowest MIC for this strain is C21 and C22 complexes of 0.0156 mg/mL.

All the complexes have better antimicrobial activity than the ligands against the *Pseudomonas aeruginosa* bacterium, the C22 complex having the lowest MIC value of 0.0313 mg/mL.

II.1.6.5.2 Antifungal activity

All compounds were tested for antifungal activity *in vitro* on *Candida albicans* ATCC 10231. It was worked with solutions ranging from 1.00 to 0.0019 mg/mL.

And for this strain, it is found that antifungal activity is better for complexes than for the two ligands. Complex C22 has the best activity with a MIC value of 0.25 mg/mL.

II.1.6.5.3. Antitumor activity

Assays performed on HeLa cells for complexes and ligands were performed on 500 μg/mL concentration solutions for an incubation time of 24 hours at 37°C. C21 and C22 complexes have a good cytotoxic effect on HeLa cells, reducing their viability by 25% and 21%, respectively. The C20 complex and the two ligands have a weak effect on these cells.

II.1.6.6. Partial conclusions

Three new divalent cobalt complexes were obtained with 1-(*o*-tolyl) biguanide ligands and α-ketoglutaric acid. Following the analyzes performed on these complexes, the appropriate formulas could be established (Figure II.1.77).

The 1-(*o*-tolyl) biguanide ligand coordinates bidentate through the imminent nitrogen atoms and the α-ketoglutaric acid monodentates into the C20 complex through the hydroxyl oxygen of

the carboxyl group. In complexes C21 and C22, however, this ligand coordinates bidentate, through the oxygen atoms in the ketone groups in the alpha position and in the adjacent carboxyl group.

The proposed stereochemistry for the four complexes are:

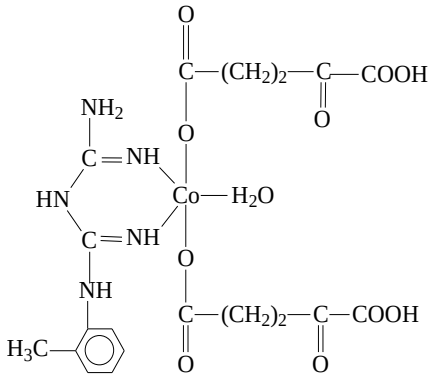
- trigonal bipyramid for C20
- tetragonal distorted octahedral for C21

The first two complexes have a single cobalt ion in their structure and the third has two cobalt ions bound by acetate bridges.

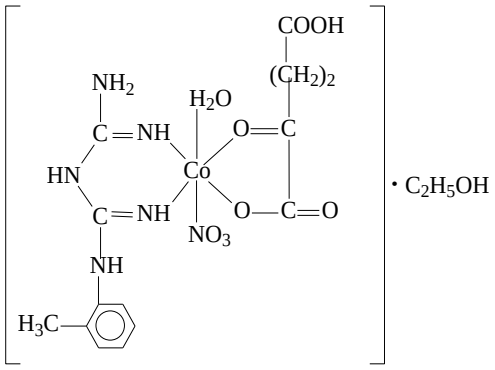
The biological activity of the complexes is influenced by:

- the stereochemistry of the complex
- surrounding the metal ion
- number of metal ions

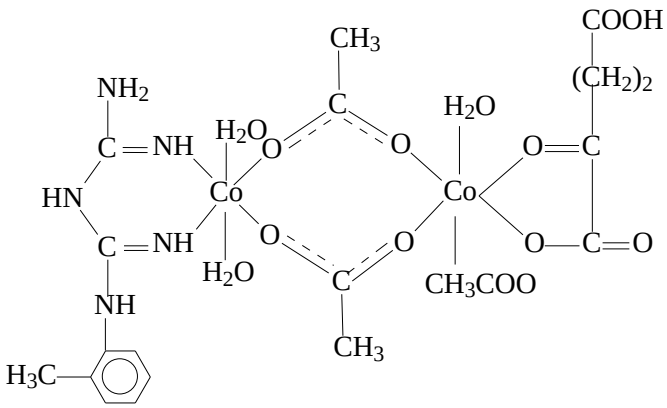
The best activity of the C22 complex for all three strains can be explained by the existence of two metal centers in its molecule, compared to the other two complexes having only one.



C20



C21



C22

Fig. II.1.77. Formulas of complex combinations C20-C22

II.2.1. Synthesis of complex combinations of Ni (II) with usnic acid and 1-(*o*-tolyl) biguanide ligands

Starting from nickel salts and using ligands as the usnic acid and 1-(*o*-tolyl) biguanide, four complexes were synthesized. The molar ratio of metal salt: usnic acid: 1-(*o*-tolyl) biguanide of 1: 1: 1 was worked. Based on the data provided by elemental analysis, molar electrical conductance, thermal analysis, UV-Vis-NIR, FT-IR spectra and magnetic susceptibility the proposed formulas for C23-C26 complexes are:

$[Ni(TB)(H_2AU)(H_2O)_2]Cl•3H_2O$	green	**(C23)**
$[Ni(TB)(H_2AU)(H_2O)_2]Br$	dark green	**(C24)**
$[Ni(TB)(H_2AU)]NO_3•2H_2O$	dark orange	**(C25)**
$[Ni(TB)(H_2AU)]CH_3COO$	dark orange	**(C26)**

The molar ratio of the substances used in synthesis is also found in the complexes obtained.

Electric molar conductance

The results of molar electric conductance for the four complexes place them in the 1:1 electrolyte type [222]. The measurements were made in solution of N,N-dimethylformamide, concentration 10^{-3}M at 25°C. For the C23 complex, a molar conductance of 69.2 3 $S•cm^2mol^{-1}$ was obtained, for C24 73.3 $S•cm^2mol^{-1}$, for C25 79.1 $S•cm^2mol^{-1}$, and for C26 81.4 $S•cm^2mol^{-1}$.

II.2.1.2. Thermal analysis

Analyzing the thermogram of C23 complex, in the first step of decomposition, it is found that at a temperature below 180°C there is a loss of mass corresponding to the 5 water molecules in its composition, namely, 3 crystallizations and 2 coordination.

In the case of the C24 complex, the first mass loss occurs below 190°C, and corresponds to each of the coordination water molecules.

From the analysis of the thermogram for the C25 complex it is observed that in the first stage (<170°C) the two water molecules are lost.

The residue obtained is NiO for all complexes, from which the percentage of nickel can be determined.

II.2.1.3. UV-Vis-NIR spectra and magnetic measurements

Magnetic susceptibility

Following the magnetic susceptibility determinations, the following magnetic moment values were obtained for the C23-C24 complexes: 3.37 μB for C23, 3.12 μB for C24 and zero for C25 and C26. The magnetic moment values for C23 and C24 complexes assume that they are paramagnetic, while C25 and C26 are diamagnetic.

UV-Vis-NIR Spectra

The data provided by the electronic spectra correlated with the magnetic moments at room temperature provide valuable information regarding the stereochemistry of the synthesized complex combinations. The proposed stereochemistry for C23-C26 complexes are:

(C23) $[Ni(TB)(H_2AU)(H_2O)_2]Cl•3H_2O$	Rhombic distorted octahedral
(C24) $[Ni(TB)(H_2AU)(H_2O)_2]Br$	Rhombic distorted octahedral
(C25) $[Ni(TB)(H_2AU)]NO_3•2H_2O$	Square planar
(C26) $[Ni(TB)(H_2AU)]CH_3COO$	Square planar

II.2.1.4. FT-IR Spectra

In the spectra of the four complexes, the presence of a very intense band at approx. 1640 cm^{-1}. This can be attributed to the valence vibration of the imine group, ν(C=N), which implies the coordination of the TB ligand to the metal ions through the pair of non-participating nitrogen electrons [231].

The presence of an intense band at approx. 1650 cm^{-1} in the spectra of the 4 complexes explains the involvement of ketone oxygen from the methyl ketonic group from the usnic acid. The displacement of the bands characteristic of the phenolic deformation vibration (in plane and outside it), at higher wavelengths, can be attributed to the coordination of this ligand by the phenolic OH grouping [262].

Since the OH group with the most acidic character is the one linked with carbon 3 of the usnic acid, it means that it participates in coordination with the oxygen in the methyl ketonic group linked with the carbon 2 and this phenolic oxygen.

II.2.1.5.1. Antimicrobial activity

Determination of the minimum concentration of inhibitors for complex combinations

Antimicrobial activity of synthesized ligands and complexes was tested on *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 strains. At working concentrations (1.00-0.0019 mg/mL), the diluent solvent, DMSO, did not influence activity antimicrobial properties of the tested compounds.

C23-C26 complexes have very good antimicrobial activity against the *S. aureus* strain, with a minimum inhibitory concentration of 0.0039 mg/mL. The TB ligand has the weakest activity for this strain, the MIC being 0.125 mg/mL, and the other ligand has a good activity, with the MIC being by 0.0078 mg/mL.

C23 and C24 complexes have a good activity against Gram negative *P. aeruginosa* bacteria, while ligands and the other two complexes have poor activity (MIC is 0.5 mg/mL for C23 and C24, and 1 mg/mL for C25, C26, TB and H_3AU).

Study of the influence of complex synthesized combinations on the development of microbial biofilms on an inert substrate

In terms of the ability to inhibit the adhesion of the microbial biofilm to the inert substrate, the complexes and ligands inhibit this process depending by the dose up to a minimum concentration of eradicating the biofilm.

Against the species *Staphylococcus aureus* complexes C23 and C24 have a MBEC of 0.0019 mg/mL, H_3AU, C25 and C26 0.0039 mg/mL. The highest MBEC has the TB ligand, 0.0312 mg/mL. In the case of *Pseudomonas aeruginosa* strain, also complexes C23 and C24 have the lowest CMEB (0.0019 mg/mL), and the highest has H_3AU (0.0078 mg/mL). The other ligand and the other two complexes have the same MBEC of 0.0039 mg/mL.

II.2.1.5.2. Antifungal activity

Antifungal activity was determined for ligands and complexes on *Candida albicans* ATCC 10231, the MIC being presented in figure II.2.13. The working concentration range was 1.00-0.0019 mg/mL. C25-C26 ligands and complexes have the lowest activity, and the best C23 and C24 have MIC 0.5 mg / mL.

II.2.1.5.3. Antitumor activity

For C23-C26 complexes and for the ligands used for obtaining them, antitumor activity on HeLa cells was tested. 500 μg/mL concentration solutions were used for testing, and incubation was done at 37°C for 24 hours.

Under the conditions tested C25 and C26 complexes have a moderate cytotoxic effect on HeLA cells, reducing their viability by 21% and 23%, respectively. C23 and C24 ligands and complexes have a weak effect on this type of cell.

II.2.1.6. Partial conclusions

Four new complex combinations of nickel with ligands, usnic acid and 1-(*o*-tolyl) biguanide, were synthesized and characterized. The formulas of the four complexes were proposed based on the interpretations of the analyzes performed (elemental analysis, UV-Vis-NIR, IR spectra, thermal analysis, molar electric conductance, magnetic susceptibility). Both ligands are bidentate in all the complexes obtained. The TB ligand coordinates to the metal ion through the imminent nitrogen atoms and the H_3AU ligand through the oxygen atom in the methyl ketonic group bound to carbon 2 and the oxygen atom in the phenolic hydroxyl linked to with carbon 3 of the usnic acid.

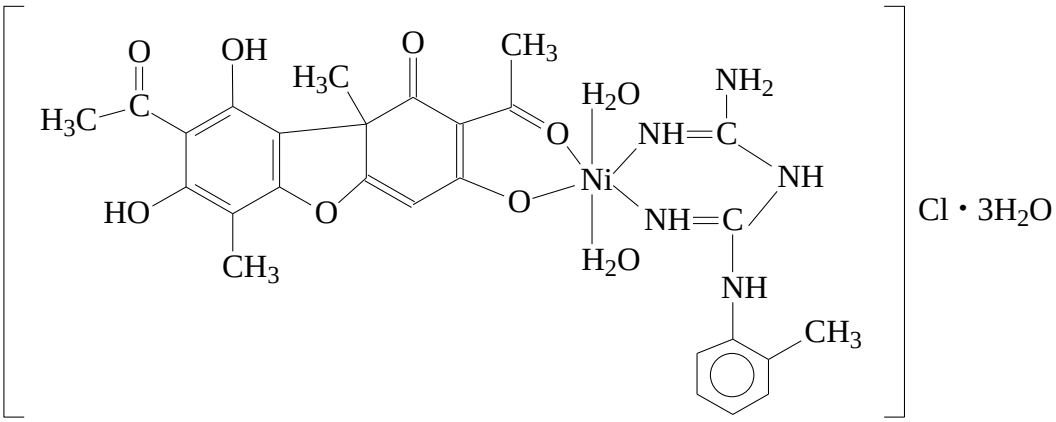
While biguanide coordinates in neutral form, usnic acid participates in deprotonated coordination, H_2AU^-.

The proposed stereochemistry for the synthesized complexes are: rhombic distorted octahedral for C23 and C24 and square plane for C25-C26, as confirmed by the electronic spectra and magnetic susceptibility values.

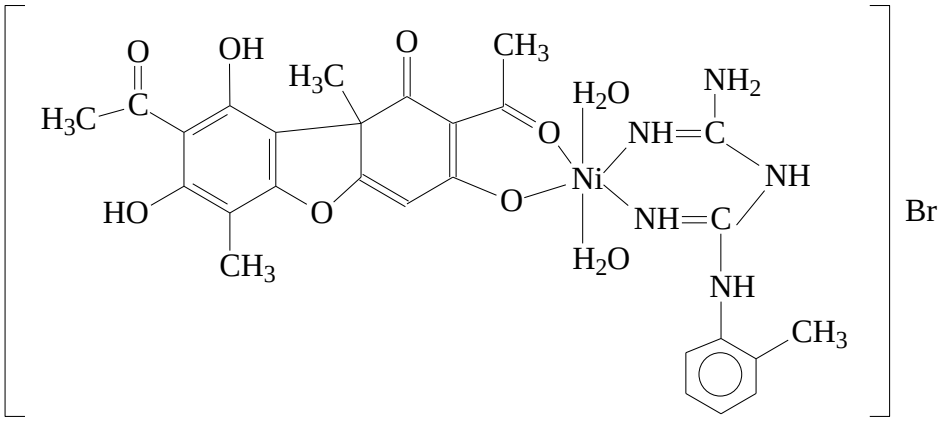
All the complexes have a good antibacterial activity on the *S. aureus* strain, which is due to the usnic acid in their composition. The best antimicrobial and antifungal activity against *P. aeruginosa* and *C. albicans* has complexes C23 and C24, which is due to their stereochemistry (rhombic distorted octahedral). The best ability to inhibit the adhesion of the microbial biofilm to the inert substrate of the two tested strains is also complexes C23 and C24. Instead, a moderate cytotoxic effect is observed on HeLa cells of complex combinations C25 and C26 that have square plane stereochemistry.

The biological activity of the complexes is comparable or better than that of the ligands and the differences that appear for the four complexes can be accounted for by their stereochemistry.

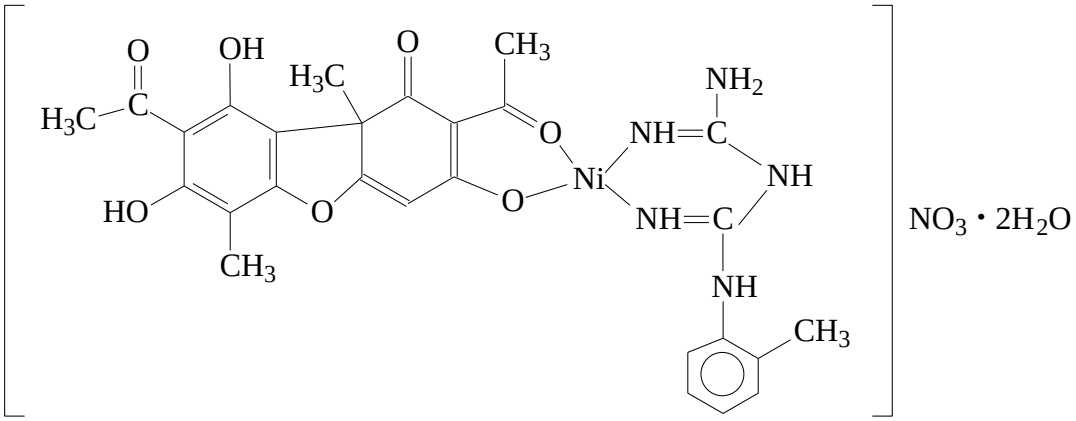
The proposed structures for complexes C23-C26 are shown in figure II.2.15.



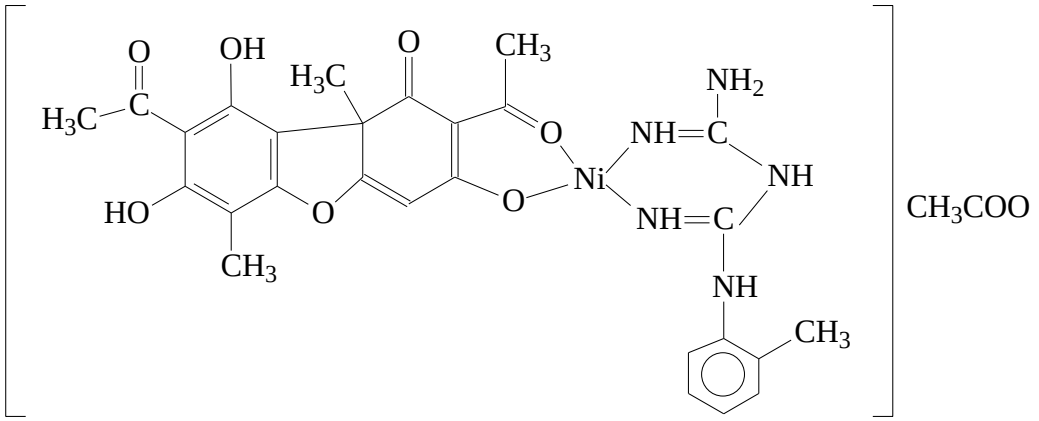
C23



C24



C25



C26

Fig. II.2.15. Proposed structures for complex combinations C23-C26

II.2.2. Synthesis of complex combinations of Cu(II) with usnic acid and 1-(*o*-tolyl) biguanide ligands

Four complex combinations were synthesized using chloride, nitrate, acetate and copper chlorate and ligands as usnic acid and 1- (*o*-tolyl) biguanide.

Following the analyzes performed (elemental analysis, UV-Vis-NIR, IR, EPR, spectra, atomic absorption spectrometry) for these complexes the proposed molecular formulas are:

[Cu(TB)(H_2AU)]Cl	blue	**(C27)**
[Cu(TB)(H_2AU)(H_2O)$_2$](NO_3)	gray blue	**(C28)**

$[Cu(TB)(H_2AU)CH_3COO]\bullet 2C_2H_5OH$ green **(C29)**

$[Cu(TB)(H_2AU)(ClO_4)]\bullet 2H_2O$ green **(C30)**

In the four syntheses, the substances used were in the molar ratio metal salt: usnic acid: 1-(*o*-tolyl) biguanide of 1:1:1, ratio found also in the formulas proposed for them.

Electric molar conductance

For the synthesized complexes the molar electrical conductance at 25oC in the N,N-dimethylformamide solution with the concentration of 0.001 mol/L was determined. For complex combinations C29 and C30, values corresponding to non-electrolyte compounds were obtained, 37.1 $S\bullet cm^2mol^{-1}$ and 41.1 $S\bullet cm^2mol^{-1}$, respectively. For the C27 complex a molar conductance of 79.7 $S\bullet cm^2mol^{-1}$ was obtained and for C28, 72.4 $S\bullet cm^2mol^{-1}$ which indicates a 1:1 type electrolyte [222].

II.2.2.2. Thermal analysis

Complex C30 was not subjected to thermal analysis because of the perchlorate anion in its molecule (danger of explosion during thermal decomposition).

The presence of water/alcohol in the composition of complexes C28 and C29 was confirmed in the first stage of their thermal decomposition.

For the analyzed complexes the same residue, CuO, is obtained, from which the percentage of copper can be determined, namely: for C27 (9.81% experimental, 10.03% calculated), for C28 (9.24% experimental, 9.13% For C29 (8.59% experimentally, 8.48% calculated).

II.2.2.3. UV-Vis-NIR spectra and EPR spectra

By correlating the bands observed in the UV-Vis-NIR electronic spectra and the electronic paramagnetic resonance spectra of the four complexes, their stereochemistry could be proposed and the character of the metal-ligand bonds was appreciated [238-246].

For the C27 complex, the parameters $K_{\parallel}$= 0.341 were calculated from the analysis of the EPR spectrum and $K_{\perp}$ = 0.637, which means that the π bonds in the plane are strong. For the coefficients α^2, β^2_1, β^2 the following values were obtained: α^2 = 0.64 which means that the sigma

bonds in the plane are strong having predominantly covalent character; β^2_1= 0.53 implies strong π bonds in the xoy plane and β2 = 0.99 indicates weak π bonds outside the plane.

The values obtained for the spectral parameters in the case of complex C28 are $K_{\parallel}$ = 0.53 and $K_{\perp}$ = 0.70 which implies strong π connections in the plane. α^2 = 0.86 which implies that the sigma bonds in the plane are predominantly ionic in character ; β^2_1 = 0.62 implies strong π bonds in the xoy plane and β2 = 0.81 indicates weak π bonds outside the plane.

For the C29 complex $K_{\parallel} \cong K_{\perp}$, which means that the metal-ligand bond is a pure σ bond. The coefficient α^2 is 0.85 which implies that the sigma bonds in the plane are predominantly ionic. The coefficients β^2_1 and β^2 have close values, 0.67 respectively 0.66, which is why both the π bonds in the xoy plane and those outside it are relatively strong.

The value of $K_{\perp}$ for complex C30 (0.85) indicates almost ionic copper-ligand bonds.

The proposed stereochemistry for the synthesized complexes are:

(C27) [Cu(TB)(H_2AU)]Cl	Square planar
(C28) [Cu(TB)(H_2AU)(H_2O)$_2$](NO_3)	Tetragonal distorted octahedral
(C29) [Cu(TB)(H_2AU)CH_3COO]•2C_2H_5OH	Square pyramid
(C30) [Cu(TB)(H_2AU)(ClO_4)]•2H_2O	Tetragonal distorted octahedral

II.2.2.4. FT-IR Spectra

In the spectra of C27-C30 complexes, a shift of the intense band from 1610 cm^{-1} is observed due to the valence vibration of the imine group, ν(C=N), a displacement that is explained by the coordination of the 1-(*o*-tolyl) biguanide to the ion. Of copper by the pair of non-participating electrons of imine nitrogen [231]. And the displacement of the bands due to the vibrations coupled δ(NH)+ν(C-N) supports the coordination of TB through the imminent nitrogen atoms.

The intense bands presented in the spectra of the four complexes analyzed at values between 1637-1662 cm^{-1} are at wavelengths smaller than that of the usnic acid and can be attributed to the coordination of this ligand to the metal ion by the carbonyl oxygen from the methyl ketonic group.

The involvement of phenolic oxygen in coordination is explained by the existence of bands in the complex spectra at 1205-1239 cm^{-1} and 709-734 cm^{-1}.

II.2.2.5.1. Antimicrobial activity - Determination of the minimum inhibitory concentration

Antimicrobial activity was tested *in vitro* on *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 strains for C27-C30 complexes and ligands used in synthesis. The range of working concentrations was from 1.00 mg/mL to 0.0019 mg/mL.

The lowest minimum inhibitory concentration, so the best antimicrobial activity against *S. aureus* is C28 and C30 complexes (0.0039 mg/mL). Complex C29 has the same activity as one of the ligands, 1-(*o*-tolyl) biguanide, having a MIC of 0.125 mg/mL.

On *P. aeruginosa* strain, C27 and C29 complexes have the best antimicrobial activity, with MIC = 0.125 mg/mL, and the C28 complex slightly lower activity. Complex C30 has the same activity against this strain as the ligands (MIC of 1 mg/mL).

II.2.2.5.2. Antifungal activity - Determination of the minimum concentration of inhibitors

Antifungal activity was tested *in vitro* on *Candida albicans* ATCC 10231 for both C27-C30 complexes and ligands used in synthesis. Worked in the concentration range 1.00-0.0019 mg/mL. All the complexes have better antifungal activity than the ligands, the smallest MIC having a C29 complex of 0.125 mg/mL.

II.2.2.5.3. Antitumor activity

After testing the complexes and ligands used in HeLa cell synthesis, it was found that the best cytotoxic effect is complex C27, which reduces the viability of these cells by 32%. C28 and C30 complexes reduce tumor cell viability by 28% and 26% respectively.

For the tests, 500 µg/mL concentration solutions were used and incubation was carried out for twenty-four hours at 37°C.

II.2.2.6. Partial conclusions

Four new complex combinations of Cu(II) were obtained following the synthesis of copper salts, usnic acid and 1-(*o*-tolyl) biguanide. These were analyzed by various methods in order to establish the appropriate formulas.

In all the complexes the ligands coordinate bidentate to the metal ion. The 1-(*o*-tolyl) biguanide coordinates through the imminent nitrogen atoms while the usnic acid through an oxygen atom in the methyl ketonic group bound to carbon 2 and a phenolic type oxygen linked to the carbon 3 of this ligand.

The proposed stereochemistry for the four complexes are:

- square plan C27
- tetragonal distorted octahedral C28
- square pyramid C29
- tetragonal distorted octahedral C30

The good antimicrobial activity against *S. aureus* for complexes C27, C28 and C30 can be largely attributed to the usnic acid that is ligand. The weaker activity of the C29 complex could be due mainly to its stereochemistry and less to the usnic acid in its composition.

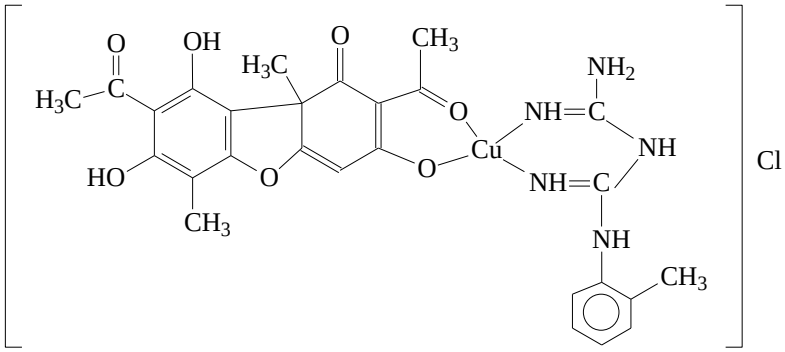
And in the case of the other strains tested, the biological activity is influenced by:

- the stereochemistry of the complex
- surrounding the metal ion
- the type of electrolyte.

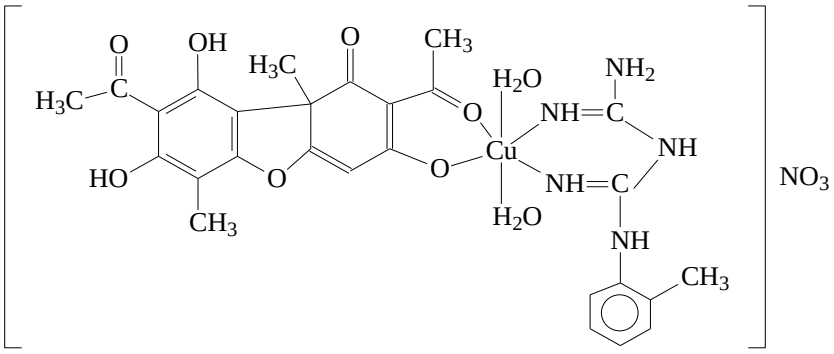
Thus C27 and C29 complexes have the best antimicrobial activity on the *P. aeruginosa* strain. The lowest MIC on *C. albicans*, so the best antifungal activity is the C29 complex.

Regarding the antitumor activity on HeLa cells, the best cytotoxic effect is the complex with square planar stereochemistry.

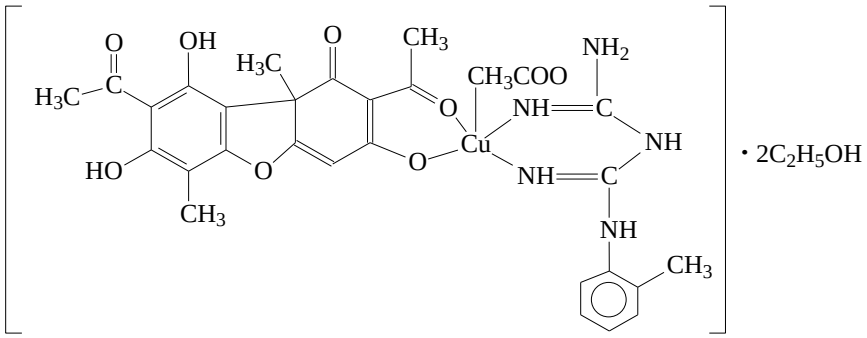
Based on the analyzes performed for the four complexes, the following structures were proposed (Figure II.2.27):



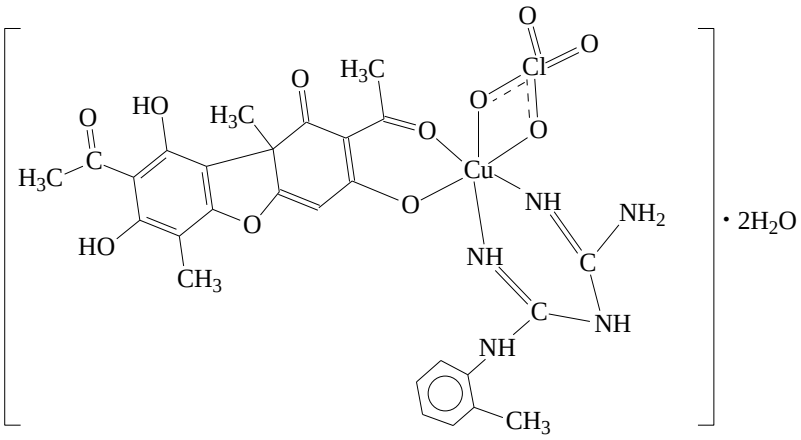
C 27



C28



C29



C30

Fig. II.2.27. Proposed formulas for complex combinations C27-C30

II.2.3. Synthesis and characterization of complex combinations of Pd(II), Pt(II) and Pt(IV) with usnic acid and 1-(*o*-tolyl) biguanide ligands

Four complexes of Pd(II), Pt(II) and Pt(IV) were synthesized from salts of these metals and two ligands: usnic acid and 1-(*o*-tolyl) biguanide. In synthesis, $PdCl_2$, $Pd(CH_3COO)_2$, H_2PtCl_6 and $PtCl_2$ were used. The molar ratio of the metal salt: usnic acid: 1-(*o*-tolyl) biguanide used in the synthesis was 2:1:1. This ratio was maintained for complex C31, while for the other three complexes the ratio of combination was 1:1:1.

The data provided by elemental analysis, thermal analysis, atomic absorption spectrometry, UV-Vis-NIR and FT-IR spectra were used to establish the structures of these complexes.

The proposed formulas for complex syntheses are:

$[Pd(TB)(H_3AU)][PdCl_4]$	dark orange	**(C31)**
$[Pd(TB)(H_2AU)]CH_3COO$	orange	**(C32)**
$[Pt(TB)(H_2AU)Cl_2]Cl$	dark brown	**(C33)**
$[Pt(TB)(H_2AU)]Cl$	orange	**(C34)**

II.2.3.2. Thermal analysis

From the analysis of the TG curves of palladium (II) complexes it is observed that up to 220°C no mass ~~stones~~ loss are recorded. This means that neither of the two complexes has water or alcohol in the molecule. Above this temperature, oxidative degradation of the complexes takes place. The final residue resulting from the thermal decomposition of the two complexes is palladium [250]. We observed a concordance between the percentage of palladium determined from the thermal analysis and the one resulting from the elemental analysis: 24.22% experimental, 23.91% calculated for C31, respectively 15.42% exp., 15.20% calc. for C32.

Neither complexes C33 and C34 contain water or alcohol, confirmed by the thermal analysis in which, up to 200°C, no loss of mass is observed. The final residue is platinum [251], 23.23% for C33, respectively 26.15% for C34 (values ~~close~~ similar to those calculated based on the molecular formula, 23.34% - C33 and 25.46% - C34).

II.2.3.3. UV-Vis-NIR Spectra

The proposed stereochemistry for C31-C34 complexes following the interpretation of UV-Vis-NIR spectra are:

(C31) $[Pd(TB)(H_3AU)][PdCl_4]$ Square planar

(C32) $[Pd(TB)(H_2AU)]CH_3COO$ Square planar

(C33) $[Pt(TB)(H_2AU)Cl_2]Cl$ Octahedral

(C34) $[Pt(TB)(H_2AU)]Cl$ Square planar

II.2.3.4. FT-IR Spectra

The usnic acid works in these complex combinations as a bidentate ligand, in the deprotonated form H_2AU^-. This is supported by the presence of the bands located at:

- 1635-1662 cm^{-1}, attributed to the involvement of oxygen in methyl ketone linked to carbon 2 of usnic acid
- 1197-1220 cm^{-1}, respectively 708-729 cm^{-1}, bands that are attributed to the involvement of phenolic type oxygen linked to carbon 3 of the ligand.

The band due to the valence vibration of the imine group, ν(C=N), from 1610 cm^{-1} of the TB ligand spectrum is found in the spectra of complex, in slightly displaced combinations. The presence of this band displaced to that of the TB ligand explains the involvement of imine nitrogen in coordination by the pair of non-participating electrons it has [231]. This coordination is also supported by the existence of bands due to the vibrations coupled δ(NH)+ν(C-N).

II.2.3.5.1. Antimicrobial activity

C31-C34, H_3AU and TB complexes were tested *in vitro* on *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 strains to determine antibacterial activity. The chosen working range was 1.00 - 0.0019 mg/mL, and the solvent used was DMSO.

Following the tests performed it was observed that the antimicrobial activity against the Gram positive species *Staphylococcus aureus* is very good for complexes and the usnic acid while

for 1-(*o*-tolyl) biguanide is much weaker. Platinum complexes have the lowest minimum inhibitory concentration, 0.0019 mg/mL, four times lower than that of usnic acid.

In the case of Gram negative bacterium *Pseudomonas aeruginosa* both ligands have low antimicrobian activity. All complexes have a good activity on this strain, the tetravalent platinum complex having the lowest MIC of 0.0625 mg/mL. The solvent used in dilutions does not influence the antimicrobial activity of the tested compounds at working concentrations.

II.2.3.5.2. Antifungal activity

After testing the antifungal activity on *Candida albicans* ATCC 10231 it was found that the ligands have a poor activity, the C31 and C32 complexes a moderate one whereas the platinum complexes have the best activity. The latter have a MIC of 0.125 mg/mL.

II.2.3.5.3. Antitumor activity

Antitumor activity was tested on HeLa cells for complexes and ligands. The percentages of viability of these cells in the presence of the tested compounds were relative calculated to the untreated control sample. The solutions used for these assays it had a concentration of 500 μg/mL, and incubation was done at 37°C for 24 hours.

Platinum complexes have reduced the viability of HeLa cells by 35% C33 and 32% C34, respectively, thus having a good cytotoxic effect. Palladium ligands and complexes have a weaker effect on this type of tumor cells.

II.2.3.6. Partial conclusions

Four new complexes of Pd(II), Pt(II) and Pt(IV) were obtained using usnic acid and 1-(*o*-tolyl) biguanide as ligands.

In all the complexes both ligands are bidentate, 1-(*o*-tolyl) biguanide coordinating to the metal ion through the imminent nitrogen atoms, and the H_3AU ligand as an H_2AU^- anion - through the oxygen atoms of the methyl ketone group linked to carbon 2 and oxygen of the type phenolic linked to carbon 3 in the usnic acid.

Following the analyzes, the following were found:

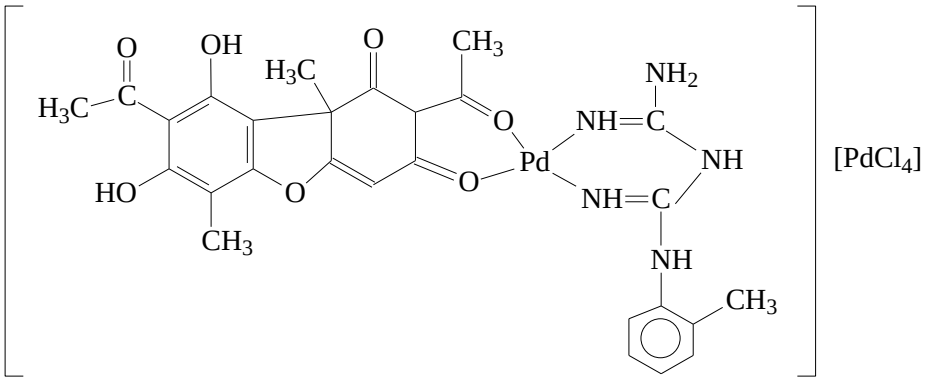
- Pd (II) complexes have square planar symmetry,

- complex C33 contains tetravalent platinum and is octahedral,

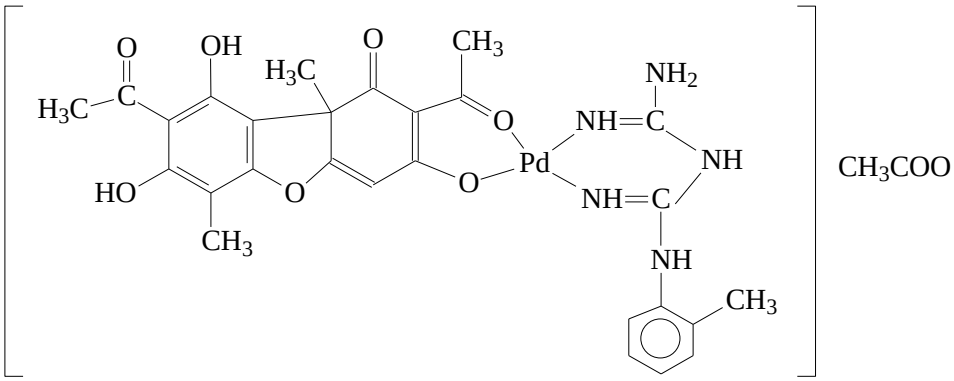
- complex C34 is square planar with divalent platinum.

Complex C31 is of the Vauquelin type, having a complex anion $[PdCl_4]^{2-}$.

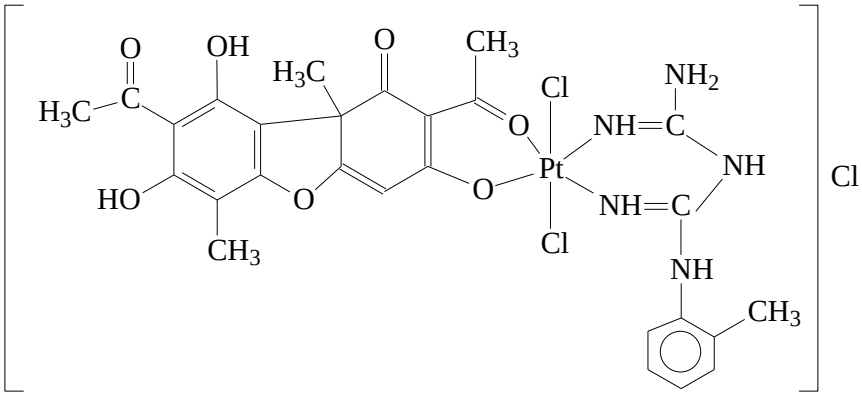
The proposed structures for complexes C31-C34 are shown in Figure II.2.40.



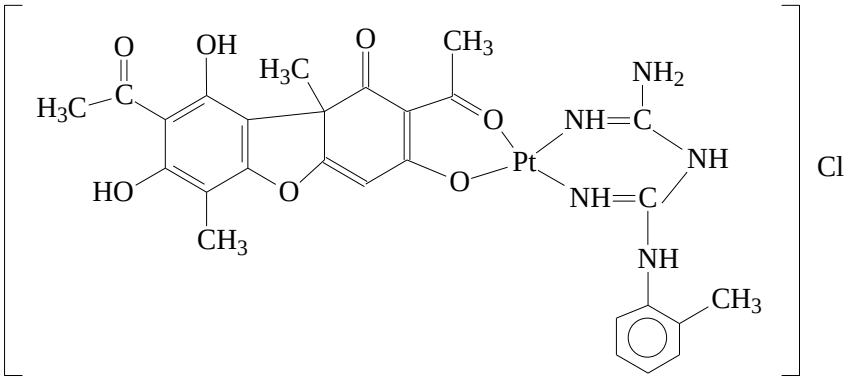
C31



C32



C 33



C34

Fig. II.2.40. Proposed formulas for C31-C34 complexes

The good antibacterial activity of the complexes against *S. aureus* is mainly due to the usnic acid found in their composition.

Platinum complexes have a better activity on all tested strains compared to palladium.

The antitumor activity of the C31 complex combination is better than the C32 and can be accounted for by the number of palladium ions in the molecule, namely, two for the first versus one for the second complex.

It is found that of following the ligands coordination ~~of~~ to the metal ion, the biological activity is improved (the complexes have a better activity than the ligands).

II.2.4. Synthesis of complex combinations of Co(II) with usnic acid and 1-(*o*-tolyl) biguanide ligands

Using as ligands, usnic acid and 1-(*o*-tolyl) biguanide and divalent cobalt salts, were synthesized three complexes for which were proposed the following molecular formulas:

$[Co(H_2AU)_2(TB)]Cl$	dark blue gray	**(C35)**
$[Co(H_2AU)(TB)(H_2O)(NO_3)]\bullet 3H_2O$	brick pink	**(C36)**
$[Co(H_2AU)(TB)_2](CH_3COO)\bullet 2H_2O$	reddish brown	**(C37)**

Electric molar conductance

For the C35 and C36 complexes, values corresponding to the non-electrolytes were obtained for the molar electrical conductivity, 37.2 $S \bullet cm^2 mol^{-1}$, respectively 40.7 $S \bullet cm^2 mol^{-1}$. Complex C37 is a 1:1 type electrolyte, confirmed by the value of λ_M = 72.3 $S \bullet cm^2 mol^{-1}$ [222]. The conductance determinations were made in solution of N,N-dimethylformamide, concentration 10^{-3}M at 25°C.

II.2.4.2. Thermal analysis

There is no loss of mass upon thermal decomposition of the C35 complex up to 220°C, which means that it does not contain water or ethyl alcohol in its composition.

From the thermogravimetric analysis of the C36 complex, a first mass loss up to 110°C is observed, which corresponds to the four water molecules in the complex composition - three crystallization and one coordination.

At the thermal decomposition of the C37 complex, the two crystallization water molecules are eliminated in a first step, below 180°C.

The final residue is Co_3O_4 for all complexes. The percentage of cobalt from each complex calculated on the basis of this residue is 9.59% for C35, 8.35% for C36, and 6.92% for C37, respectively.

II.2.4.3. UV-Vis-NIR Spectra

Stereochimiile complecşilor C35-C37 au fost propuse în urma analizei spectrelor electronice ale acestora comparativ cu ale liganzilor pe care îi conţin.

(C35) $[Co(H_2AU)_2(TB)]Cl$ Trigonal bipyramid

(C36) $[Co(H_2AU)(TB)(H_2O)(NO_3)] \bullet 3H_2O$ Octahedral

(C37) $[Co(H_2AU)(TB)_2](CH_3COO) \bullet 2H_2O$ Octahedral

II.2.4.4. FT-IR Spectra

The presence of an intense band near 1670 cm^{-1} in the spectra of all complexes indicates the involvement in the coordination of oxygen from methyl ketone from the usnic acid. Another

oxygen atom in this ligand that participates in coordination is the phenolic type. This is confirmed by the existence of bands in the spectra of complex combinations located at approx. 1200 cm^{-1} and approx. 710 cm^{-1}.

The band due to the valence vibration of the imine group, ν(C=N), from 1610 cm^{-1} in the 1-(*o*-tolyl) spectrum of biguanide is present in the spectra of the three complexes, slightly shifted to larger wave numbers, which implies the involvement of imine nitrogen atoms in coordination [231].

II.2.4.5.1. Antimicrobial activity - determination of the minimum inhibitory concentration

Antimicrobial activity for novel complexes and ligands was tested *in vitro* on *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. Experiments were made at concentrations ranging from 1.00 to 0.0019 mg/mL, obtained by successive dilutions in DMSO. The solvent did not influence the biological activity in the chosen field of work.

In the case of *Staphylococcus aureus*, complex combinations C35 and C36 showed antimicrobial activity similar to that of usnic acid, all with a MIC of 0.0078 mg/mL. The activity of the other ligand is much weaker than that of the usnic acid, with a MIC of 0.125 mg/mL.

Against *Pseudomonas aeruginosa* all complexes have better antimicrobial activity than ligands, with the C36 complex having the lowest MIC value of 0.0156 mg/mL.

II.2.4.5.2. Antifungal activity - determination of the minimum inhibitory concentration

For all compounds, antifungal activity was tested *in vitro* on *Candida albicans* ATCC 10231. Compounds solutions concentrations were between 1.00 and 0.0019 mg/mL. After testing it, was found that antifungal activity in this species was better for complexes than for the two ligands. C35 and C36 complexes have the best activity with a CMI value of 0.125 mg/mL.

II.2.4.5.3. Antitumor activity

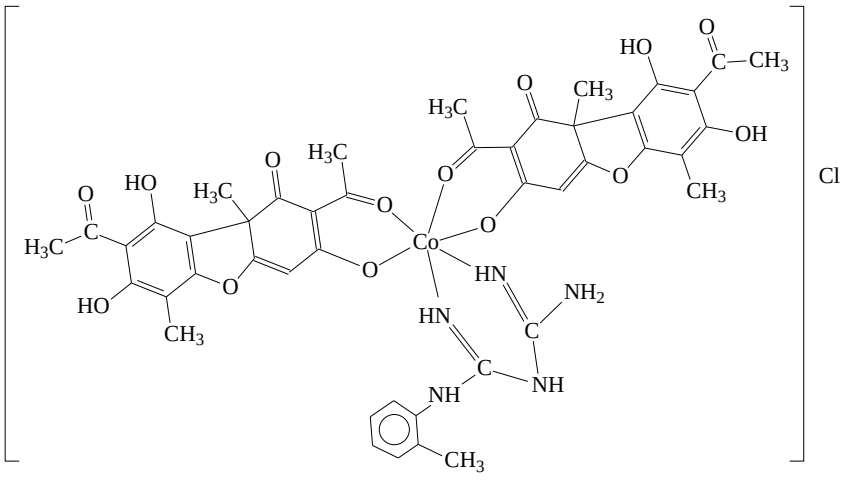
The antitumor activity of C35-C37 complexes and ligands was tested on HeLa cells. Solutions concentrations were 500 µg/mL and incubation was done at 37°C for 24 hours.

The best cytotoxic effect of all tested compounds have C37 and C35 complexes, which have reduced HeLa cell viability by 32% and 30%, respectively. Among ligands, TB has better antitumor activity than H_3AU.

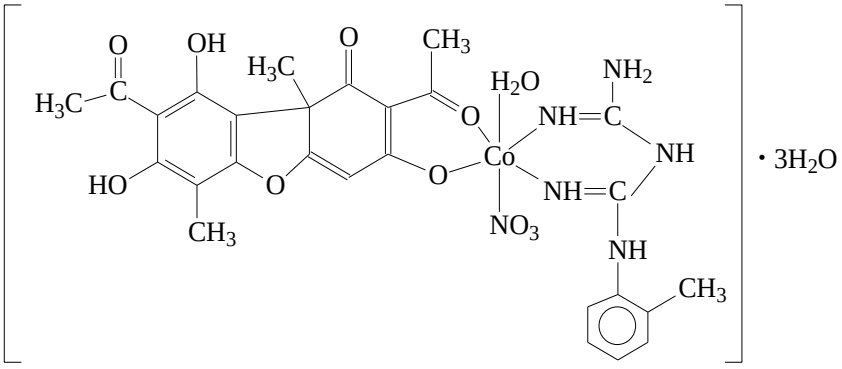
II.2.4.6. Partial conclusions

Three new complexes of divalent cobalt were obtained from the synthesis of salts of this metal (chloride, nitrate and acetate) and usnic acid and 1-(*o*-tolyl) biguanide as ligands.

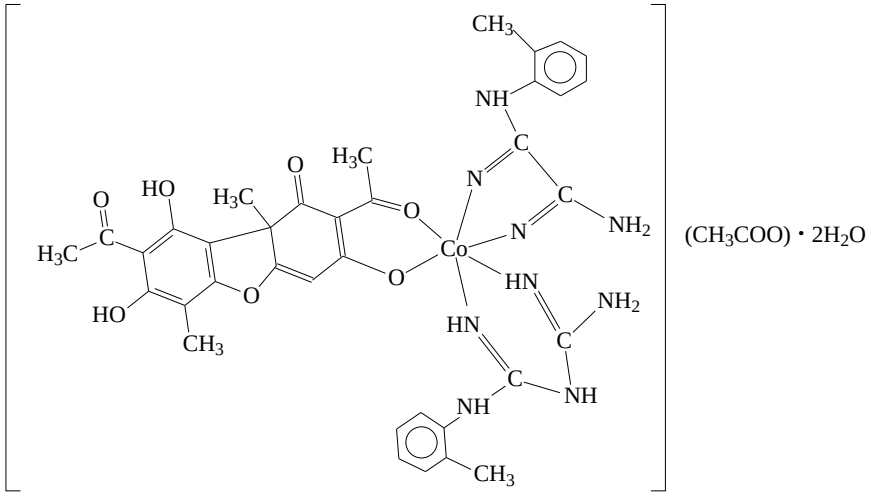
Based on the analyses performed (elemental analysis, thermal analysis, UV-Vis-NIR and FT-IR spectra) the formulas for the three complexes were proposed (fig.II.2.51).



C35



C36



C37

Fig. II.2.51. Proposed formulas for complexes C34-C37

Following the analyses performed on these complexes, it was observed that both ligands coordinate bidentate, thus:

- 1-(*o*-tolyl) biguanide coordinates bidentate through the neutral nitrogen atoms, in the neutral form TB;

- the usnic acid functions as a bidentate ligand in the deprotonated form H_2AU^- through two oxygen atoms, one of methyl ketone linked to carbon 2 and one of phenolic type linked to carbon 3 thereof.

Although the molar ratio of metal salt: TB: H_3AU was 1:1:1, it remained only in the first two complexes. In complex C37, however, two molecules of 1-(*o*-tolyl) biguanide coordinate to the cobalt ion which means that the molar ratio was 1:2:1.

For the C36 and C37 complexes, the octahedral symmetry was proposed and for the C35 trigonal bipyramid.

The good antibacterial activity of the complexes on the *S. aureus* strain is largely due to the usnic acid that is ligand in them and less to the stereochemistry of the complex.

The lower activity of the C37 complex may be due to the fact that it has two TB molecules as ligands and only one of H_3AU.

In the case of *P. aeruginosa* and *C. albicans* the biological activity is better for the complexes than for the ligands due to their coordination with the metal ion. Complex C37 has three ligands around the cobalt ion, hence a lower activity compared to C35 and C36.

The better antitumor activity of the C37 and C35 complexes is due to the presence of two TB molecules in the first and the symmetry in the second complex.

III. GENERAL CONCLUSIONS

The results obtained from the researches carried out for the purpose of elaborating the present doctoral thesis are presented in the section 'Original contributions' and have resulted in the synthesis, characterization and formulation of 37 new complex combinations.

A number of 22 complex combinations have α-ketoglutaric acid and 1-(*o*-tolyl) biguanide ligands, and 15 contain the usnic acid and 1-(*o*-tolyl) biguanide ligands.

In order to establish the structures of the synthesized compounds, they were characterized by elemental chemical analysis, molar electric conductance, IR and UV-Vis-NIR spectroscopy.

The interpretation of the results of the thermogravimetric analysis provided information that contributed to the proposed formulations for the complex combinations, namely the presence of the water/ethyl alcohol molecules and the thermal effects that accompany the mass loss processes.

The complex combinations in whose synthesis perchlorates were used (C8 and C12) were not subjected to thermal decomposition for security reasons. In most cases, the residue obtained from thermal decomposition was metal oxide. For the manganese complexes the obtained oxide was Mn_2O_3, for the cobalt oxide it was Co_3O_4, and for the platinum complexes above 500°C the residue formed was platinum which was obtained by decomposing $PtCl_2$ at 450-480°C.

For copper-containing complexes (C5-C8 and C27-C30), the EPR spectra were also analyzed and for those with nickel (C1-C4 and C23-C26), magnetic measurements were also performed.

In all complexes 1-(*o*-tolyl) biguanide functions as a bidentate ligand and coordinates to the metal ion through imminent nitrogen atoms.

α-ketoglutaric acid works as:

- monodentate ligand in deprotonated form, HA^-, in the C20 complex coordinating to the metal ion through the hydroxyl oxygen of the carboxyl group;
- as a bidentate ligand coordinating to the metal ion through the oxygen atom in the ketone group at the alpha position and the oxygen in the hydroxyl of the adjacent carboxyl group;
- in deprotonated form, HA^-, - in complexes C1-C8, C12-C14, C17, C18, C21 and C22;

- in nonprotonated form, H_2A, in complexes C9-C11, C16 and C19;

- as ligand in double-deprotonated bridge, A^{2-}, in complexes C10, C11 and C15.

The usnic acid coordinates to the metal ion bidentate through the oxygen atom of the methyl ketonic group linked to carbon 2 and the oxygen atom from the phenolic hydroxyl linked to carbon 3; It participates in deprotonated coordination, H_2AU^- except for the C31 complex in which it is in neutral form.

The complex combinations synthesized are:

- mononuclear C1-C9, C13-C14, C17-C18, C20-C21, C23-C30, C32-C37;
- dinuclear C10, C11, C12, C15, C22.

Complexes C16, C19 and C31 are of the Vauquelin type, C16 and C31 having the complex anion $[PdCI_4]^{2-}$, while C19 contains $[PtCI_4]^{2-}$.

The synthesized complex combinations have been tested in terms of biological activity:

- quantification of antimicrobial activity on *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 strains - for all complexes;
- study of the influence of complex synthesized combinations on the development of microbial biofilms on the inert substrate of the two microbial strains - complexes C1-C12, C23-C26;
- quantification of antifungal activity on *Candida albicans* ATCC 10231 - complexes C5-C8, C13-C37;
- evaluation of the antitumor activity of all complexes on HeLa cells.

The biological activity of the complex combinations tested is influenced by several factors:

- the nature of the metal
- the oxidation state of the metal
- number of metal ions in the complex
- the nature of ligands
- the stereochemistry of the complex

In the case of quantification of the antimicrobial activity on the *Staphylococcus aureus* strain ATCC 25923 complexes having the usnic acid as ligand have minimum inhibitory concentrations, much lower than the complexes containing the α-ketoglutaric acid as ligand. This difference is explained by the good antimicrobial activity that the usnic acid has on this strain.

From the series of complexes having α-ketoglutaric acid and 1-(*o*-tolyl) biguanide as ligands, the lowest MIC, of 0.0039 mg/mL, have (C16) $[Pd(TB)(H_2A)][PdCl_4]$, (C18) $[Pt(TB)(HA)Cl_2]Cl$, (C19) $[Pt(TB)(H_2A)][PtCl_4]•H_2O$.

Complexes (C33) $[Pt(TB)(H_2AU)Cl_2]Cl$, (C34) $[Pt(TB)(H_2AU)]Cl$, which have as ligands 1-(*o*-tolyl) biguanide and usnic acid have a minimum inhibitory concentration four times lower than that of usnic acid, namely 0.0019 mg/mL. Good antimicrobial activity on this strain also have complexes (C23) $[Ni(TB)(H_2AU)(H_2O)_2]Cl•3H_2O$, (C26) $[Ni(TB)(H_2AU)]CH_3COO$, (C28) $[Cu(TB)(H_2AU)(H_2O)_2]$ (NO_3) and (C30) $[Cu(TB)(\ H_2AU)(ClO_4)]•2H_2O$, having MIC = 0.0039 mg/mL.

Good antimicrobial activity against *Pseudomonas aeruginosa* strain ATCC 27853 have complexes (C36) $[Co(H_2AU)(TB)(H_2O)(NO_3)]•3H_2O$ with MIC = 0.0156 mg/mL, (C18) $[Pt(TB)(HA)Cl_2]Cl$, (C22) $[Co_2(HA)(TB)(H_2O)_3(CH_3COO)_3]$ having MIC = 0.0312 mg/mL, (C19) $[Pt(TB)(H_2A)][PtCl_4]•H_2O$ and (C33) $[Pt(TB)(H_2AU)Cl_2]Cl$ whose MIC is 0.0625 mg/mL.

After evaluating the influence of complex combinations on the inert substrate adhesion capacity of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 strains, it was found that these compounds inhibit this dose-dependent process up to a minimum biofilm eradication concentration (MBEC). The lowest MBEC value for both strains is 0.0019 mg/mL for complexes (C23) $[Ni(TB)(H_2AU)(H_2O)_2]Cl•3H_2O$ and (C24) $[Ni(TB)(H_2AU)(H_2O)_2]Br$.

When testing the antifungal activity of ligands and complexes on *Candida albicans* ATCC 10231, it was found that the lowest concentrations of inhibitors (0.125mg / mL) have the following complexes: (C7) $[Cu(TB)(HA)CH_3COO]•H_2O$, (C8) $[Cu(TB)(HA)ClO_4]•5H_2O$, (C16) $[Pd(TB)(H_2A)][PdCl_4]$, (C18) $[Pt(TB)(HA)Cl_2]Cl$, (C19) $[Pt(TB)(H_2A)][PtCl_4]•H_2O$, (C29) $[Cu(TB)(H_2AU)CH_3COO]•2C_2H_5OH$, (C33) $[Pt(TB)(H_2AU)Cl_2]Cl$, (C34) $[Pt(TB)(H_2AU)]Cl$, (35) $[Co(H_2AU)_2(TB)Cl]$ şi (C36) $[Co(H_2AU)(TB)(H_2O)(NO_3)]•3H_2O$.

The antitumor activity of all complexes as well as ligands used in synthesis was tested on HeLa cells. 500 μg/mL concentration solutions were used for testing, and incubation was carried out for twenty-four hours at 37°C. Apart from zinc complexes that had no cytotoxic effect on these tumor cells, the rest of the complexes had a weak, moderate or good effect, in the working conditions mentioned above.

Thus, (C18) $[Pt(TB)(HA)Cl_2]Cl$ and (C19) $[Pt(TB)(H_2A)][PtCl_4]$ $•H_2O$ complexes reduced the viability of HeLa cells by 41% and 38%, respectively.

A reduction in the viability of the tumor cells by over 30% was also observed in the case of complexes (C16) [Pd(TB)(H_2A)][$PdCl_4$], (C27) [Cu(TB)(H_2AU)]Cl, (C33) [Pt(TB)(H_2AU)Cl_2]Cl, (C34) [Pt(TB)(H_2AU)]Cl, (35) [Co(H_2AU)$_2$(TB)Cl] şi (C37) [Co(H_2AU)$(TB)_2$](CH_3COO)•2H_2O. As expected, platinum-containing complexes have good antitumor activity.

Further development perspectives

Given that some of the synthesized complexes have good biological properties, the following research activities are considered in the future:

- Testing of the complexes synthesized on other bacterial, fungal, and tumor strains;
- Determination of anti-inflammatory and hypoglycemic properties;
- Determination of antioxidant capacity;

Also, new complex combinations will be synthesized working in a different molar ratio (salt: L1: L2) than in the present work and determining their biological properties.

Selective Bibliography

[4] Nită, S., Andrieș, A.A., Patron, L., Albulescu, R., Rădulescu, F., Tănase, C., Vintilă, M., Analytical and Toxicological Characterization of New Co(II) Coordination Compounds with Antiinflammatory Oxicams Drugs, *Revista de Chimie* **2011,** *62* (5), 549-553.

[5] Leung, C.H., Lin, S., Zhong, H.J., Ma, D.L., Metal complexes as potential modulators of inflammatory and autoimmune responses, *Chemical Science* **2015,** *6* (2), 871-884.

[6] Knittl, E.T., Abou-Hussein, A.A., Linert, W., Syntheses, characterization, and biological activity of novel mono- and binuclear transition metal complexes with a hydrazone Schiff base derived from a coumarin derivative and oxalyldihydrazine, *Monatshefte Fur Chemie* **2018,** *149* (2), 431-443.

[7] Pahonțu, E.M., Transition Metal Complexes with Antipyrine-Derived Schiff Bases: Synthesis and Antibacterial Activity, *Descriptive Inorganic Chemistry Researches of Metal Compounds IntechOpen*, **2017**, 65-93.

[8] Liu, W.P., Su, J., Jiang, J., Li, X.Y., Ye, Q.S., Zhou, H.Y., Chen, J.L., Li, Y., Two mixed-NH3/amine platinum (II) anticancer complexes featuring a dichloroacetate moiety in the leaving group, *Scientific Reports* **2013,** *3*:2464, 1-6.

[9] Reddy, D.H.K., Lee, S.M., Seshaiah, K., Babu, K.R., Synthesis, characterization of thiosemicarbazone metal complexes and their antioxidant activity in different in vitro model systems, *Journal of the Serbian Chemical Society* **2013,** *78* (2), 229-240.

[10] Amira S. Abd-El-All, Ammar A. Labib, Hanan A. Mousa, Fatma A. Bassyouni, Khaled H. Hegab, Maher A. El-Hashash, Saad R. Atta-Allah, Wafaa H. AbdEl- Hady, Souad A. M. Osman., Synthesis of Ag(I), Cu(II), La(III) Complexes of Some Newmannich Bases Incorporating 1, 2, 4- Triazine Moiety and Studying Their Antihypertensive and Diuretic Activities, *Journal of Applied Sciences Research* **2013,** *9* (1), 469-481.

[11] Ahmed, R.M., Yousif, E.I., Hasan, H.A., Al-Jeboori, M.J., Metal Complexes of Macrocyclic Schiff-Base Ligand: Preparation, Characterisation, and Biological Activity, *The Scientific World Journal* **2013**, 1-7.

[12] Gull, P., Hashmi, A.A., Biological Activity Studies on Metal Complexes of Macrocyclic Schiff Base Ligand: Synthesis and Spectroscopic Characterization, *Journal of the Brazilian Chemical Society* **2015,** *26* (7), 1331-1337.

[13] Bendre, R. S., Tadavi, S.K., Patil, M.M., Synthesis, crystal structures and biological activities of transition metal complexes of a salen-type ligand, *Transition Metal Chemistry* **2018,** *43* (1), 83-89.

[221] Mihalache, M., Oprea, O., Guran C., Holban, A., Synthesis, characterization and biological activity of some complex combinations of nickel with α-ketoglutaric acid and 1- (*o*-tolyl) biguanide, *Comptes Rendus Chimie*, **2018,** vol. 21(1), 32-40**.**

[222] Angelici, R.J., *Synthesis and Technique in Inorganic Chemistry, 2ed*. Saunders: Philadelphia, **1977**.

[231] Singh, S., Malhotra, R., Dhindsa, K.S., *Proceedings of the National Academy of Sciences, India* **1998,** *68A*, 217.

[236] Mihalache, M., Guran, C., Meghea, A., Bercu, V., Holban, A., Complexes of Cu (II) with α-ketoglutaric acid and 1- (*o*-tolyl) biguanide - synthesis, characterization and biological activity, *Revista de Chimie – acceptat, în curs de apariţie* **– octombrie 2019.**

[237] Mihalache, M., Oprea, O., Vasile, B.S., Guran, C., Ardelean, I., Synthesis, characterization and biological activity of composite combinations of Cu(II), Fe(III) and Mn(III) with α- ketoglutaric acid and 1-(*o*-tolyl) biguanide, *Buletin Ştiinţific Universitatea Politehnica Bucureşti,* **2018,** Vol. 80, 113-126.

[238] Lever, A. B. P., *Inorganic Electronic Spectroscopy (second edition)*. Elsevier Amsterdan-Oxford-New York-Tokyo, **1984**, 554.

[239] Yokoi, H., Isobe,T., ESR and Optical Absorption Studies of the Copper(II) Complexes of Ethylenediamine and Its Alkyl Derivatives, *Bulletin of the Chemical Society of Japan* **1969,** *42* (8), 2187–2193.

[240] Yokoi, H., Sai, M., Isobe, T., ESR Studies of Bis-(N,N-diethylethylenediamine)copper(II) Perchlorate, *Bulletin of the Chemical Society of Japan* **1969,** *42* (8), 2232–2238.

[241] Kivelson, D., Neiman, R., ESR Studies on the Bonding in Copper Complexes, *The Journal of Chemical Physics* **1961,** *35*, 149-155.
[242] Hathaway, B.J., Billing, D.E., The electronic properties and stereochemistry of mono-nuclear complexes of the copper(II) ion, *Coordination Chemistry Reviews* **1970,** *5* (2), 143-207.
[243] Ray, R.K., Esr Study of Some Copper(Ii) Coordination-Compounds with Substituted Biguanidines,1, *Polyhedron* **1989,** *8* (1), 7-11.
[244] Ray, R.K.; Kauffman, G.B., An Epr Study of Copper(Ii)-Substituted Biguanide Complexes .3., *Inorganica Chimica Acta* **1990,** *174* (2), 237-244.
[245] Sreeja, P.B., Kurup, M.R.P., Kishore, A., Jasmin, C., Spectral characterization, x-ray structure and biological investigations of copper(II) ternary complexes of 2-hydroxyacetophenone 4-hydroxybenzoic acid hydrazone and heterocyclic bases, *Polyhedron* **2004,** *23* (4), 575-581.
[246] Latheef, L., Kurup, M.R.P., Spectral and structural studies of copper(II) complexes of thiosemicarbazones derived from salicylaldehyde and containing ring incorporated at N(4)-position, *Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy* **2008,** *70* (1), 86-93.
[248] Mihalache, M., Oprea, O., Guran, C., Ardelean, I., Synthesis, characterization and biological activity of the manganese complexes with α- ketoglutaric acid and 1-(*o*-tolyl) biguanide, *Revista de Chimie*, **2017,** *68*, (10), 2209-2214**.**
[250] Lemos, S.C., Franchi, S.J.S., Netto, A,V.G., Mauro, A.E., Treu-Filho, O., Frem, R.C.G., Almeida, E.T., Torres, C., , *Journal of Thermal Analysis and Calorimetry,* **2011,** *106*, 391.
[251] Hernandez, J.O.; Choren, E.A., Thermal-Stability of Some Platinum Complexes. *Thermochimica Acta* **1983,** *71* (3), 265-272.
[262] Edwards, H.G.M., Newton, E.M., Wynn-Williams, D.D., Molecular structural studies of lichen substances II: atranorin, gyrophoric acid, fumarprotocetraric acid, rhizocarpic acid, calycin, pulvinic dilactone and usnic acid, *Journal of Molecular Structure* **2003,** *651*, 27-37.