Complexes of Cu (II) with α-Ketoglutaric Acid and 1- (*o*-tolyl) Biguanide

Synthesis, characterization and biological Activity

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*The three copper complexes having α-ketoglutaric acid (H_2A) and 1- (o-tolyl) biguanide (TB) ligands have been synthesized and characterized. The proposed formulas for these complexes are: [Cu(TB)(HA)]Cl **(C1)**, [Cu(TB)(HA)CH_3COO]•H_2O **(C2)** and [Cu(TB)(HA)](NO_3) **(C3)** where HA represents deprotonated H_2A. The complexes obtained were tested for antibacterial activity against Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853, antifungal activity on Candida albicans ATCC 10231 and antitumor activity on HeLa tumor cells. Due to the antitumor, antifungal, antimicrobial activity and inhibition of inert substrate adhesion, complexes synthesized could be used for potential therapeutic applications.*

Keywords: 1-(o-tolyl)biguanide, α-ketoglutaric acid, copper complexes, antibacterial activity, celule HeLa, antifungal activity.

a-ketoacids are very important agents in the synthesis and degradation of amino acids and proteins in the metabolism of lipids and carbohydrates [1]. Studies on a-ketoacids with biological activity have shown that they can produce different effects on the biological tissue, interacting at different cellular levels at different *p*H, temperatures enzyme-controlled and environment [2-4]. α-ketoglutaric acid plays an essential role in the Krebs cycle; it antagonizes the toxic effects against both HCN and NaCN [5-6]. It has also a neuroprotective effect, helping to heal and regenerate tissues and to set up gastrointestinal tract, in lung disorders and some types of cancer [1].

Several complexes of lanthanides containing a-ketoglutaric acid: $[M_2^{III}M^{II}L_6(NO_3)_6(OH_2)_6](NO_3)$ with antibacterial and antioxidant activity, have been synthesized; where M^{III} = Ce and M^{II} = Cu, Co, Ni , and L is α-ketoglutaric acid [7-8].

Biguanides are compounds exhibiting important biological properties, e.g. antibacterial, antifungal, hypoglycaemic, antimalaric and antitumor activity [9-16]. Many of the complex combinations in which the ligand is part of this class of compounds have biological properties, which explains the particular interest in their study.

Complexes of Cu (II), Fe (III) and Ni (II) with N, N-dimethylbiguanide ligands and derivatives thereof [17-19], $[Cu(HTBG)_2]Cl_2$, $[Cu(TBG)_2]\cdot 3H_2O$ where HTBG is 2-tolylbiguanide [20] are known. Complexes of Cu (II) and Zn (II) having chlorhexidine (CHX) as ligand: $[CuZn(CHX)(NO_3)_2Cl_2]$. $2C_2H_5OH$, $[Cu(CHX)SO_4]$. C_2H_5OH,$[Cu_2(CHX)(NO_3)_4]$, $[CuZn(CHX)(CH_3COO)_2]Cl_2$, have antibacterial and anti-fungal properties [21-23]. Spectral, biological and molecular docking studies have demonstrated the antimicrobial activity of 1-*o*-tolyl biguanide [24].

Given the biochemical role of copper and the biological properties of á-ketoglutaric acid and 1- (o-tolyl) biguanide, we have proposed to synthesize and characterize new complex combinations of Cu (II) with these ligands.

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Experimental part

Synthesis of copper complexes

For the synthesis of the three complexes, high purity substances has been used: α-ketoglutaric acid - $C_5H_6O_5$ (Alfa Aesar), 1- (*o*-tolyl) biguanide - $C_9H_{13}N_5$, $CuCl_2\cdot 2H_2O$ $Cu(NO_3)_2\cdot 6H_2O$, $Cu(CH_3COO)_2\cdot H_2O$ and C_2H_5OH (Sigma Aldrich).In the first step, the metal salts and the two ligands were dissolved in ethanol.

For all three complexes the metal salt molar ratio: α-ketoglutaric acid: 1- (*o*-tolyl) biguanide was 1:1:1, using 1mmol of each substance (0.1461g $C_5H_6O_5$, 0.1913g $C_9H_{13}N_5$, 0.1705g $CuCl_2\cdot 2H_2O$, 0.2956g $Cu(NO_3)_2\cdot 6H_2O$ and 0.1997g $Cu(CH_3COO)_2\cdot H_2O$.

The resulted metallic complexes have been further washed with ethanol and ethyl ether. The compounds obtained were pure and therefore no further purification was required.

Chemical and spectral analysis

Elemental analysis to determine the carbon, nitrogen and hydrogen content has been done by microcombustion using an Organic Elemental Flash 2000 Analyzer. For copper determination atomic absorption spectrometry using a Perkin Elmer Aanalyst 400 Atomic Absorption Spectrophotometer have been used.

The presence of chlorine in the C1 complex was marked with $AgNO_3$.

Thermal analyses were performed with a simultaneous thermogravimetric analysis/differential scanning calorimetry (STA/DSC) 449 F1 Jupiter working in a dynamic air atmosphere at a flow rate of 20 mL/min and at a heating rate of 10 °C/min. From RT to 900°C.

The electron spectra of complexes C1-C3 and H_2A and TB ligands were recorded with a Jasco V670 spectrometer by diffuse reflection, in the range of 200-1500nm using MgO standard.

The EPR spectra for complexes synthesys were determined on powders at room temperature in the X band (frequency = 9.4 GHz) using a ADANI CMS 8400 spectrometer. The experimental set up was chosen to obtain the best ratio signal/noise.

For spectra FT-IR, 4000-200 cm^{-1} was used using a Nicolet IS 50 FT-IR spectrophotometer. The molar electrical conductivity was determined with a CybernScan PCD 6500 conductivity meter at 25°C in a solution of *N,N*-dimethylformamide at a concentration of 0.001 mol/L.

Biological activity

Antimicrobial activity was determined for both C1-C3 and H_2A and TB ligands. The tests were performed *in vitro* and the bacterial strains were *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. Antifungal activity was tested on *Candida albicans* ATCC 10231.

To determine the minimal inhibitory concentration, (MIC), a quantitative method based on binary serials microdilutes in a broth, sterilely distributed into 96-well plates, was used. For each tested compound, working solutions of 10 mg/mL in DMSO were prepared.

By serial binary dilutions, eight solutions were prepared with concentrations ranging from 5 mg/mL to 0.0391 mg/mL. Eight wells were pipetted with 150 µL of liquid medium (broth). Into the first well were added 150µL of 10 mg/mL compound, into the second 150 µL from the first well, into the third 150 µL from the second, and so on to the eight well.

After microdilutions, 20 µL of 0.5 McFarland microbial suspension was added to each well. The same was done for the solvent used in the dilutions, (DMSO). A microbial culture witness (a well of medium containing culture medium inoculated with microbial suspension) and an environmental sterility test for each assay were also run. The so-seeded plates were incubated at 37 °C for 24h.

After incubation for each compound, the macroscopic MIC value was determined as the latter concentration at which the environmental turbidity (microbial growth) was not observed, but also by spectrophotometric reading of the microbial culture absorbance developed in the liquid medium at a length of 620 nm wave.

In the following wells, including the growth control well, the medium was turbidity due to microbial growth. In the sterility control well, the liquid content remained transparent because there was no bacterial growth.

To determine the influence of the tested compounds on the adhesion of microbial biofilm to the inert substrate, microbial cells were instilled in 96-well plate with nutrient broth in their presence. The preparation of solutions of each compound (H_2A, TB, C1-C3, DMSO) is the same as for MIC determination.

The seedlings were also incubated at 37 °C for 24 h. After incubation the plates were emptied and washed with physiological sterile water. Fixation of the adherent cells was done with 0.1 mL of 80% methanol for 5 min. The methanol solution was removed by overturning and the adherent cells were stained with 1% (0.1 mL/well) purple crystallium (hexamethyl-parahydrochloride) for 15 min.

The staining solution was removed by washing the plates with water; platelet microbial biofilms were resuspended by bubbling in a 33% acetic acid solution. The intensity of the suspension color was evaluated by measuring the absorbance at 492 nm with a Max F5 Multi-Mode Microplate Reader Spectrophotometer.

Antitumor activity for C1-C3 complexes and H_2A and TB ligands was performed on HeLa cells (MTT assay). The concentration of each compound was 500µg/mL in DMSO, the incubation time was 24h at 37°C in a 5% CO_2 atmosphere. For incubation, Heratherm Microbiological Incubator - Thermo Scientific - static incubator for microorganisms has been was used.

The test was also performed for the solvent used dilutions (HeLa control). MTT Method Principle: The MTT cell viability assay is based on the ability of viable cell-dependent NAD (P) H oxidoreductases to reduce MTT formazan, a violet insoluble compound, of whom the absorbance can be spectro-photometrically determined (Scheme 1).

To assess the color intensity of formazan, optical density was measured with a Max F5 Multi-Mode Microplate Reader Spectrophotometer at 570 nm. This method is often used to measure the cytotoxicity of some compounds and cell proliferation. The reaction is carried out in the dark because the MTT reagent is light sensitive.

Results and discussions

Elemental analysis

For C1-C3 complex combinations, elemental analysis was performed to determine the corresponding formulations. The percentages of experimentally determined carbon, nitrogen, hydrogen and copper is in good concordance with the calculated ones.

Found: C, 38.81; H, 4.06; N, 15.98; Cu, 14.39%. Calculated for [Cu(TB)(HA)]Cl: C, 38.63; H, 4.17; N, 16.09; Cu, 14.60%.

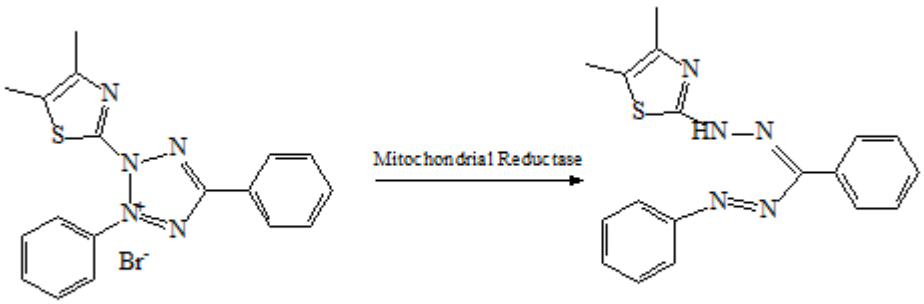
Found: C, 40.13; H, 4.72; N, 14.81; Cu, 13.51%.

Calculated for $[Cu(TB)(HA)CH_3COO]\cdot H_2O$: C, 40.29; H, 4.86; N, 14.68; Cu, 13.32%.

Found C, 36.21; H, 3.78; N, 18.37; Cu, 13.84%. Calculated for $[Cu(TB)(HA)](NO_3)$: C, 36.40; H, 3.93; N, 18.20; Cu, 13.76%.

Molar conductivity

For C1-C3 complexes molar conductivity at 25 °C was determined in *N,N*-dimethylformamide solution at a concentration of 0.001 mol/L. The molar conductivity for the complexes are: C1- 72.1 $S\cdot cm^2\cdot mol^{-1}$, C2 36.5 $S\cdot cm^2\cdot mol^{-1}$ and C3 81.1 $S\cdot cm^2\cdot mol^{-1}$. The values obtained indicate a 1:1 type electrolyte for C1 and C3 complexes, while C2 is a non-electrolyte [25].



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

(*E,Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan (Formazan)

Scheme1. Reduction of MTT to formazan

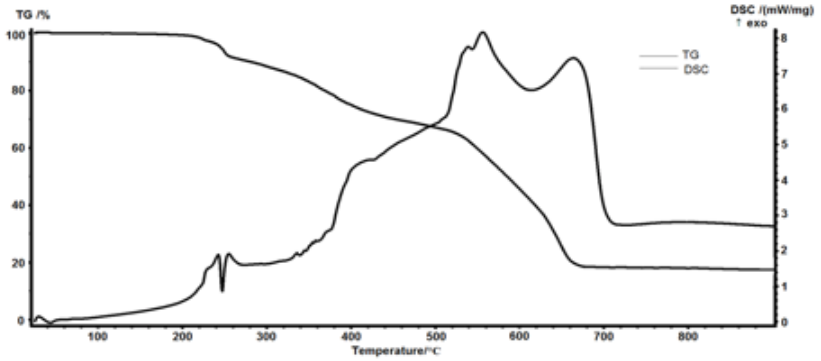


Fig. 1a. TG and DSC curves of complex [Cu(TB)(HA)]Cl

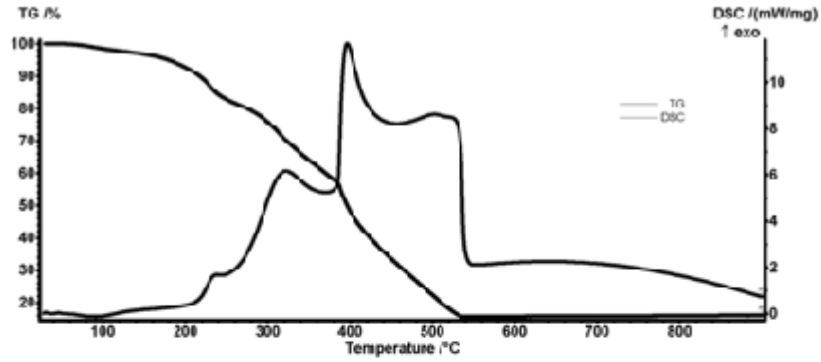


Fig. 1b. TG and DSC curves of complex $[Cu(TB)(HA)CH_3COO]\cdot H_2O$

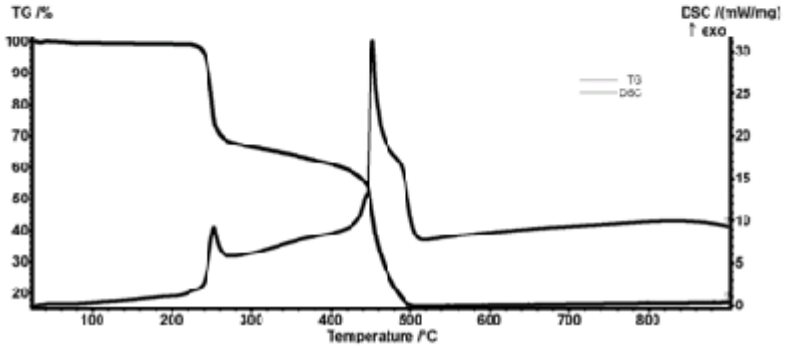


Fig. 1c. TG and DSC curves of complex $[Cu(TB)(HA)](NO_3)$

Thermal analysis

From the results of the thermogravimetric analysis, the information obtained contributed of the three proposed complexes formulations, namely the presence of water molecules and the thermal effects accompanying the mass loss processes.

Thermogravimetric (TG) and differential scanning calorimetry (DSC) curves of the three complexes are presented in Figure 1(a-c).

From the analysis of the C1 complex TG and DSC curves, the first decomposition step (220-260 °C) indicates the loss of chlorine anion in the form of HCl (exp. loss 8.07%, calc. loss 8.38%). The ligand decomposition takes place in the range 260-660°C. In the C2 complex it is observed that below 160°C there is a mass loss corresponding to a crystallization water molecule (3.72% exp. loss, 3.77% calc. loss). The acetate ion is removed as CO_2 and H_2O to about 250 °C (12.61% exp. loss, 12.37% calc. loss) and ligand degradation occurs up to 540 °C. Analyzing the C1 complex TG and DSC curves, it is observed that above 250 °C takes place the elimination of nitrate ions as nitrogen oxides and the oxidative degradation of the ligand. Ligand degradation for all three complexes is accompanied by strong exothermic effects. For all analyzed complexes, the residue obtained is CuO. The percentage of the determined copper is: for C1 (14.81% exp., 14.60% calc.) for C2 (13.05% exp., 13.32% calc.), and for C3 (13.58% exp., 13.76% calc.).

Between the spectrophotometrically and the residue from termogravimetric analysis the metal content is in a good agreement.

Ultraviolet-visible-near-infrared spectra

The correlation of the results obtained on the basis of the EPR spectra and the bands observed in the UV-Vis NIR electron spectra led to important information regarding the stereochemistry of the complexes obtained and the character of the metal-ligand bonds [26-34]. The spectra corresponding to the C1-C3 complexes are shown in Figures 2-4, and the assignments of the bands corresponding to the *d-d* transitions are shown in Table 1.

The three complexes have absorption bands in the range of 240-370 nm that may me atributed to the π- π* and n-π* transitions of organic ligands H_2A and TB, slightly displaced by coordination of the ligand to metal ions.

In the case of C1 complex, the values of the giromagnetic factor, in the presence and in parallel (g_{II}) or perpendicular ($g_{\perp}$) direction to the external field, are g_{II} = 2.1960 and $g_{\perp}$ = 2.0669. Using the g_{II} value and the energy of transition

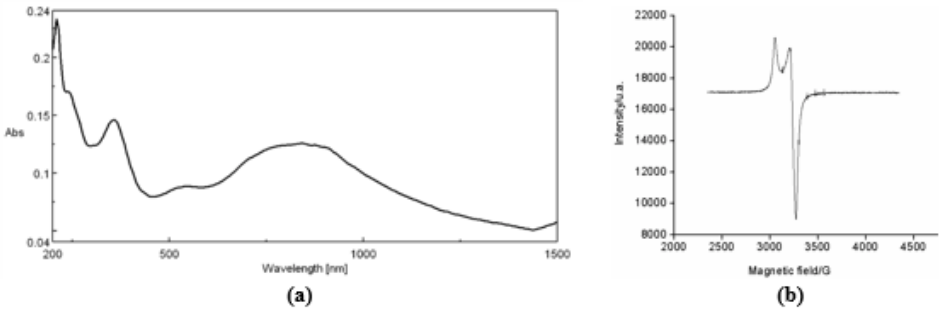


Fig. 2. The electronic spectrum (a) and EPR (b) of [Cu(TB)(HA)]Cl

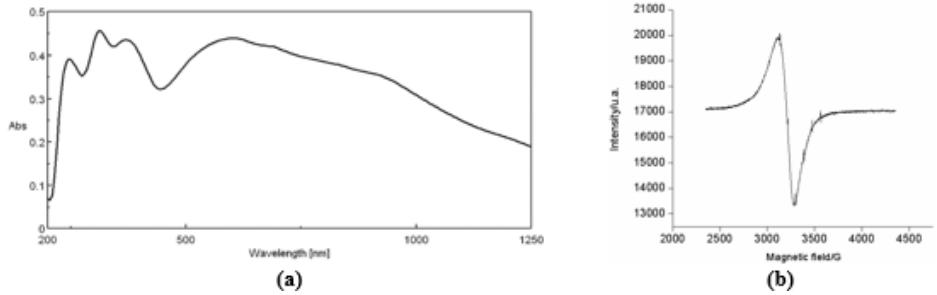


Fig. 3. The electronic spectrum (a) and EPR (b) of $[Cu(TB)(HA)CH_3COO]\cdot H_2O$

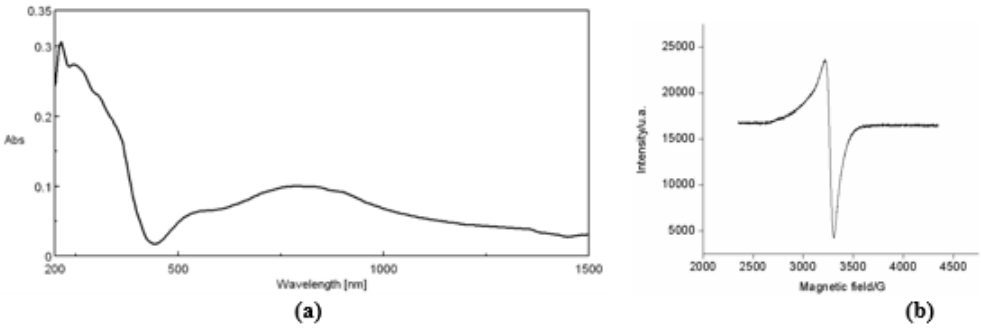


Fig. 4. The electronic spectrum (a) and EPR (b) of $[Cu(TB)(HA)](NO_3)$

Complex	Observed bands		Assignments	Symmetry
	λ_{max}(nm)	$\tilde{\nu}$(cm^{-1})		
[Cu(TB)(HA)]Cl	845	11830	$^2B_{1g}\rightarrow{}^2B_{2g}$	square-planar
	710	14085	$^2B_{1g}\rightarrow{}^2E_g$	
	545	18350	$^2B_{1g}\rightarrow{}^2A_{1g}$	
$[Cu(TB)(HA)CH_3COO]\cdot H_2O$	830	12050	$^2B_1\rightarrow{}^2B_2$	square pyramid
	695	14390	$^2B_1\rightarrow{}^2A_1$	
	605	16530	$^2B_1\rightarrow{}^2E$	
$[Cu(TB)(HA)](NO_3)$	900	11110	$^2B_{1g}\rightarrow{}^2B_{2g}$	square-planar
	790	12660	$^2B_{1g}\rightarrow{}^2E_g$	
	550	18180	$^2B_{1g}\rightarrow{}^2A_{1g}$	

Table 1
MAXIMA OF *D-D* TRANSITION BANDS FOR C1-C3 COMPLEXES

$^2B_{1g}\rightarrow{}^2B_{2g}$ results the K_{II} value (for the σ bonds from the plan); for the out of plane bonds, using $g_{\perp}$ and the energy of transition $^2B_{1g}\rightarrow{}^2E_g$ we get $K_{\wedge}$. K_{II} and $K_{\perp}$ are the orbital factors of the spin-orbit coupling constant of the free ion). The computational relationships used are:

$$g_{II}-2= -8\lambda_{I}/11830 \quad (1) \qquad K_{II}= \lambda_{I}/\lambda_0 \quad (3)$$

$$g_{\perp}-2= -2\lambda_{\perp}/14085 \quad (2) \qquad K_{\perp}= \lambda_{\perp}/\lambda_0 \quad (4)$$

$\lambda_{II}, \lambda_{\perp}$ are coupling constants for Cu^{2+} in complex in the direction parallel to the field, respectively perpendicular, λ_0 = -828 cm^{-1} and is the spin-orbit coupling constant for the Cu^{2+} free ion. The following values are obtained:

$\lambda_{||}$ = -298.8 cm^{-1}, $K_{||}$=0.36 ,$\lambda_{\perp}$ = -471cm^{-1} and $K_{\perp}$=0.57

The value of the hyperphin coupling constant is A_{II}=125.16 x10^{4}cm^{-1}.

The α^2, β^2_1, β^2 coefficients characterizing the σ bonds in the plan, the π bonds in the plan and π bonds outside the plane respectively for the Cu^{2+} metal ion are calculated with the relations 5-7:

$$\alpha^2=|A_{//}| / P + (g_{II}-2.0023) + 3/7 (g_{\perp}-2.0023) + 0.04 \quad (5)$$

where P =0.36 cm^{-1}, $K_{II}=\alpha^2 * \beta^2_1$ (6)

$K_{\perp}=\alpha^2 * \beta^2$ (7)

The following values were obtained: α^2 = 0.61 which means that the sigma bonds in the plane are strong with predominantly covalent character; β^2_1 = 0.57 implies strong π bonds in the xoy plane and β2 = 0.93 indicates weak π bonds outside the plane.

The exchange interaction parameter G is calculated by the relation (8)

$$G=(g_{||}-2)/(g_{\perp}-2) \quad (8)$$

The G value obtained is 2.93 <4 and suggests strong spin-spin interactions between Cu $^{2+}$ ions [35]. These values

Assignments	1-(o-tolyl) biguanide	α-ketoglutaric acid	C1	C2	C3
$\nu(C=O)_{keto}$		1720vs	1688vs	1690vs	1674vs
$\nu(C=N)$	1610vs		1625vs	1645vs	1637vs
$\delta(NH)+\nu(C-N)$	1577m 1270w		1582m 1257w	1599m 1265w	1590m 1244w
$\nu(COO^-)asim$			1544vs	1578vs	1558vs
$\nu(COO^-)sim$			1391s	1430s	1412s
$\Delta=\nu(COO^-)_{as}-\nu(COO^-_{sim})$			153	148	146
$\nu_3(NO_3)$					1382vs
$\nu_1(NO_3)$					1030 s
$\nu_2(NO_3)$					780m
$\nu_4(NO_3)$					700 w
$\nu(C=O)_{acetate}$				1555s	
$\nu(C-O)_{acetate}$				1365s	
$\nu(Cu-N)$			590m	588m	597m
$\nu(Cu-O)$			548m	527m	531m
$\nu(OH)_{water}$				3380m	

s – strong, vs – very strong, m – medium, w - weak

Table 2
IR BANDS AND THEIR ASSIGNMENTS FOR THE SYNTHESIZED COMPLEXES AND LIGANDS

are in agreement with axial symmetry, with the fundamental state $d_{x^2-y^2}$, D_{4h} (square planar). The appearance and position of the bands in the electronic spectrum of the C2 complex, corresponds to a C_{4v} (pentacoordinated) symmetry. From the RPE spectrum, $g_\perp=2.0856$ and with relations (2) and (4) we calculate $\lambda_\perp=-07.48cm^{-1}$ and $K_\perp=0.85$. The value of $K_\perp$ indicates almost ionic metal-ligand bonds. The symmetry of the C3 complex is the square plane being determined based on the two types of spectra. For C3 $g_\perp = 2.081$, $g_\perp-2= -2\lambda_\perp/18180$, where $\lambda_\perp$ is -512,73 cm^{-1}, $K_\perp = \lambda_\perp/\lambda_0=0.62$, which implies a predominantly covalent character of the ML bond.

FT-IR Spectra

In order to determine how copper ligands were coordinated, the FT-IR spectra of the three complexes were compared with H_2A and TB ligands [36, 37]. Spectral bands characteristic of complexes and ligands as well as their assignments are shown in Table 2.

To determine the coordination of α-ketoglutaric acid the IR spectrum of sodium α-ketoglutarate was also analyzed. The difference between the wavelengths corresponding to the $\nu(COOO^-)_{asim}$ and $\nu(COO^-)_{sim}$ bands is 181 cm^{-1}.

In the case of the H_2A ligand, the keto group in the α position is coordinated in all the complexes; this is confirmed by the displacement of the ν(C=O) band below 1720 cm^{-1}.

In the spectra of the three complex combinations, a band shift due to the valence vibration of the imine group, ν(C=N), from 1610 cm^{-1}, is observed. This displacement is in agreement with the coordination of 1-(*o*-tolyl) biguanide to the copper ion by the pair of electrons not participating in the imine nitrogen; the movement of this band to larger wavelengths is explained by the destruction of the electron delocalisation [38]. Coordination TB to copper ion by iminic nitrogen atoms is also supported by the band movement due to δ(NH)+ν(C-N) coupled vibration.

In the complex C2 the intense bands at 1555 cm^{-1} and 1365 cm^{-1} indicate monodentated coordination of the acetate and the stretched band at 3380 cm^{-1} is attributed to the crystallization water.

The presence of a single absorption band for the characteristic vibration modes ν_1 - ν_4 in the spectrum of the C3 complex indicates the presence of the nitrate anion in the ionization sphere. Bands between 527-548 cm^{-1} and 588-597 cm^{-1} were assigned to the formation of Cu-O and Cu-N bonds.

In all three complexes, both H_2A and TB function as bidentate ligands; the TB ligand coordinates to the copper ion through the imine nitrogen atoms and H_2A coordinates to the deprotonated metal ion through the oxygen atom in the ketone group at the alpha and the adjacent carboxyl group.

Antimicrobial and antifungal activity

Testing ligands and complexes on *Staphylococcus aureus* ATCC 25923 it has been found that the best antimicrobial activity had C1 and C3 with a MIC of 0.3125 mg/mL. Complex C2 has a slightly weaker activity than C1 and C3, but better than ligands. H_2A ligand activity against *Pseudomonas aeruginosa* ATCC 27853 is weaker than the other ligand, TB and C1-C3 complexes. The best activity

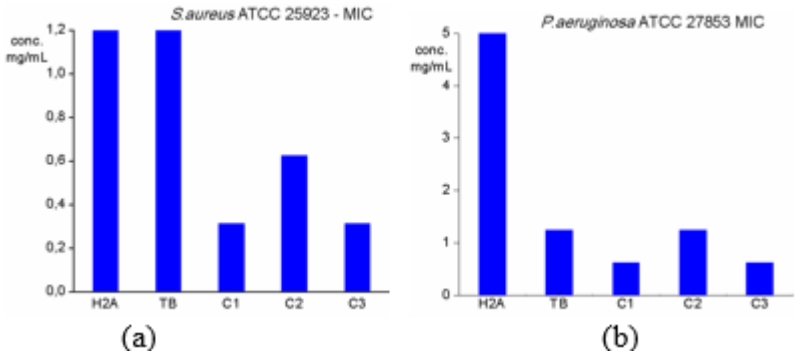


Fig. 5. MIC for C1-C3 and ligands against *S. aureus* (a) and *P. aeruginosa* (b)

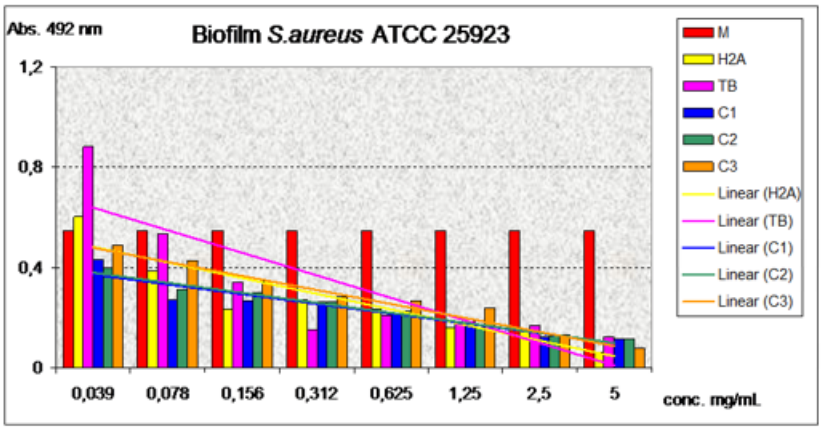


Fig. 6. Influence of H_2A, TB, C1-C3 on adhesion to the inert substrate of the strain *S. aureus*

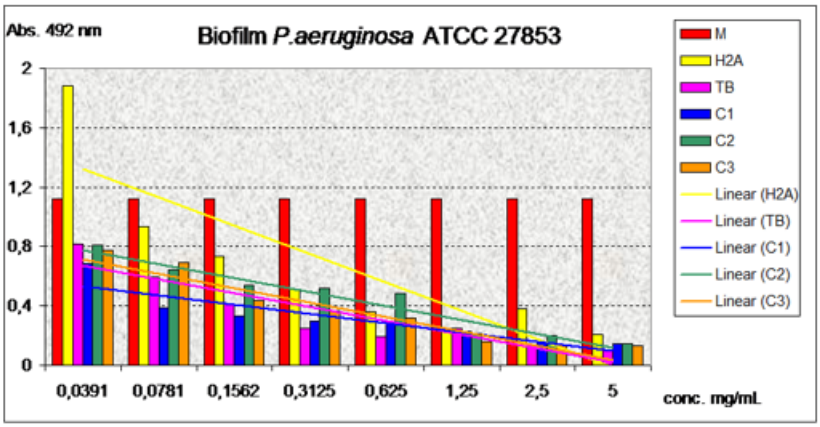


Fig. 7. Influence of H_2A, TB, C1-C3 on adhesion to the inert substrate of the strain *P. aeruginosa*

against this strain have C1 and C3 complexes (MIC is 0.625 mg / mL).

The solvent used in the dilutions does not influence the antimicrobial activity of the compounds tested at the working concentrations [39, 40]. The MIC for the compounds tested against the two bacterial strains are shown in Figures 5a and 5b.

Regarding the ability to inhibit the adhesion of microbial biofilm to inert substrate, all tested compounds inhibit this process depending on the used dose to a MBEC [41, 42]. The lowest MBEC is for C1-C3 complexes (0.0391 mg/mL), 0.1562 mg / mL for TB and 0.3125 mg / mL for H_2A against *Staphylococcus aureus.* In the *Pseudomonas aeruginosa* strain, all the complexes and the TB ligand have biofilm inhibition capacity untill a minimum biofilm eradication concentration of 0.0391 mg/mL is reached. This is not valid for H_2A ligand having a MBEC of 0.0781 mg/mL.

The influence of the tested compounds on the inert substrate adhesion capacity is shown in Figures 6 and 7.

Antifungal activity was determined on *Candida albicans* ATCC 10231 (Figure 8). Ligands have the weakest activity,

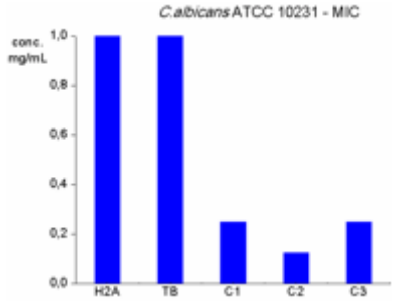


Fig. 8. Minimal inhibitory concentration for C1-C3 and ligands against *C. albicans*

C1 and C3 have moderate activity and the best one is C2 having an MIC of 0.125 mg/mL.

Antitumor activity

Metabolism of HeLa cells depends on the type of material used. Percentages of viability for the test compounds (complexes and ligands) were calculated based on the untreated control. Figure 9 shows percent viability values for the samples and untreated control, indicating the metabolic activity of HeLa cell cultures. For test purposes,

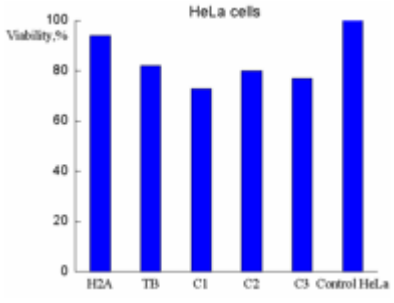


Fig. 9. Viability for ligands and complexes of HeLa cell cultures

concentrations of 500 αg/mL were used and incubation was done for twenty-four hours at 37 °C.

The H_2A ligand has a very low effect on HeLa tumor cells under the conditions tested, whereas the TB ligand and the C2 complex have demonstrated a moderate cytotoxic effect on them, reducing their viability by 18% and 20%, respectively. Complexes C1 and C3 have a better cytotoxic effect against HeLa cells, decreasing their viability by 27% and 23%.

Conclusions

Three complex combinations of Cu (II) having alpha-ketoglutaric acid ligands and 1 - (*o*-tolyl) biguanide have been synthesized, characterized and tested for their biological activity against *Staphylococcus aureus* ATCC

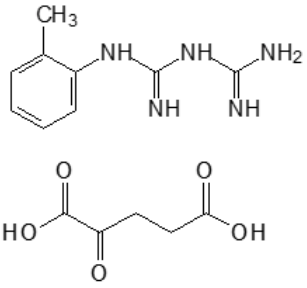


Fig. 10. Structures of TB ligand (a) and H_2A ligand (b)

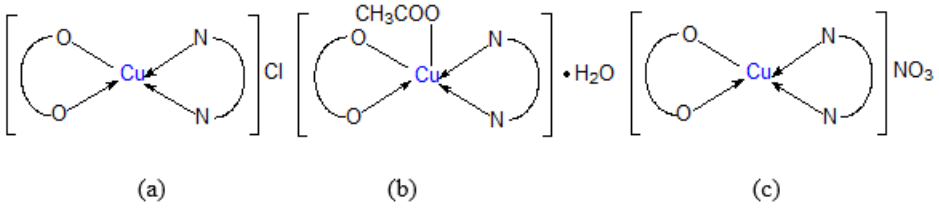


Fig. 11. Structure of the C1 complex (a), C2 complex (b), C3 complex (c)

25923, *Pseudomona aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, HeLa cells. The formulations of the C1-C3 complexes were proposed based on the analyzes performed (elemental analysis, UV-Vis-NIR spectra, IR, RPE, thermal analysis, molar conductivity).

Both α-ketoglutaric acid and 1- (*o*-tolyl) biguanide function as bidentate ligands in the three complexes (their structures are shown in Figure 10 a-b).

For the synthesized complexes, the following formulas were proposed (Figure 11 a-c):

The best cytotoxic effect against HeLa tumor cells have complexes C1 and C3. Antibacterial activity against the *Pseudomonas aeruginosa* strain ATCC 27853 is comparable or better for complexes than for ligands (the best activity have C1 and C3).

With respect to ligands and complexes testing against the *Candida Albicans* ATCC 10231 and *Staphylococcus aureus* ATCC 25923 all complexes have better antifungal activity than ligands. Since the C1 and C2 complexes are electrolyte type, their activity may result from the electrostatic interaction of the complex cation therewith with the negatively charged components of the membrane, which leads to their inactivation.

Antimicrobial/antifungal activity may also be associated with the complex combination stereochemistry but also with the combined effect of metal ion and ligand to inactivate a particular component involved in the pathogenesis of the microorganism.

The different activity of the C1-C3 complexes can be attributed to their different stereochemistry; C1 and C3 - square planar, C2 - square pyramid.

Abbreviations:

TB	1-(*o*-tolyl)biguanide
H_2A	a-ketoglutaric acid
HA	deprotonated *á*-ketoglutaric acid
DMSO	dimethylsulfoxide
NAD(P)H	nicotinamide-adenine-dinucleotide phosphate
MTT	3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide
MIC	Minimum Inhibitory Concentration
MBEC	Minimal Biofilm Eradication Concentration

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