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Coordination Compounds of Platinum and Palladium with Mixed Ligands (Usnic Acid and 1-(*o*-Tolyl) Biguanide) – Synthesis, Spectral Characterization and Biological Activity

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***Abstract**: Four coordination compounds of Pd(II), Pt(II) and Pt(IV) with usnic acid (H_3AU) and 1-(o-tolyl)biguanide (TB) as ligands have been synthesized in view of their potential as antimicrobial, antifungal and antitumor agents. The metal complexes have been characterized by elemental and thermogravimetrical analyses, infrared and electronic spectra. Based on these studies, the following formulas have been proposed for the complexes: $[Pd(TB)(H_3AU)][PdCl_4]$ **(C1)**, $[Pd(TB)(H_2AU)]CH_3COO$ **(C2)**, $[Pt(TB)(H_2AU)Cl_2]Cl$ **(C3)** and $[Pt(TB)(H_2AU)]Cl$ **(C4)**, where H_2AU is deprotonated usnic acid. The in vitro biological activities of the new complexes were tested against Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 10231 and HeLa tumor cells. All complexes were found to have good biological properties and therefore they can be further explored in therapeutic applications.*

***Keywords**: biguanides, usnic acid, coordination compounds, biological activity*

1. Introduction

Biguanides and related compounds have remained an important area of research, especially for their wide applications, due to their antibacterial, antifungal, antimalarial, hypoglycemic or antitumor activities [1-8]. Biguanides have also a remarkable coordination capability to various metal ions and the resulting complexes have often higher biological activity than ligands [9-11].

Thus, some coordination compounds of palladium and platinum with biguanides, such as $[Pd(C_2H_6N_5)_2]\cdot H_2O$, $[Pd(HDMBG)_2]Cl_2\cdot H_2O$, $[PdL^1]Cl_2\cdot 0.5H_2O$ and $[PdL^2]Cl_2\cdot 1.5H_2O$ (where HDMBG: dimethylbiguanide, L^1: $C_{12}H_{28}N_{12}$, L^2: $C_{12}H_{30}N_{14}$), $[Pd(CHX)][PdCl_4]\cdot 2H_2O$, $[Pd(CHX)](CH_3COO)_2$, $[PtC1_4(C_4H_{11}N_5)]\cdot C_2H_6OS$ (where CHX: chlorhexidine), have been synthesized and tested for their biological activity [12-15].

On the other hand, the usnic acid shows also a wide range of biological properties, such as antibiotic, antiviral, antibacterial, apoptotic, analgesic and anti-inflammatory activities [16-27]. The usnic acid was the most effective antibiotic in lichens and appears to be an exclusive lichen product. Up to now, no synthetic derivative more effective than the natural form is known [28-29]. Literature survey revealed some metal complexes with usnic acid or its derivatives as ligands [30-34].

Lauterwein et al. (1995) stated that usnic acid is effective in vitro against staphylococci, enterococci and anaerobic bacteria strains from American Type Culture Collection (ATCC) [35]. The two usnic acid enantiomers tested against *Enterococcus faecalis, Enterococcus faecium* and *Staphylococcus aureus* strains showed a high level of antimicrobial activity [35].

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In addition, the compound obtained from acyl hydrazone and usnic acid has good activity against *Mycobacterium tuberculosis* (Koch's bacillus) [36].

Usnic acid (-) showed moderate activity in the P388 (murine leukemia) assay and *in vitro* cytotoxic activity against L1210 cell lines [37].

Copper(II) complexes synthesized with ligands obtained by condensation of the usnic acid with acyl hydrazides of α-naphthoic, caprylic and oxamic acids, as well as thiosemicarbazone derivatives were tested against Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*). The antibacterial activity proved to be higher for the complexes than that for the ligands and the Cu(II) ion [33].

Moreover, new synthesized Pd(II) and Cu(II) complexes with ligands obtained by condensation of usnic acid and hydrazide showed significant biological activity against *Aspergillus niger*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* strains as well as cytotoxic activity against HeLa cells of human cervical carcinoma [32].

Most bacteria are very resistant and adaptive microorganisms to environmental changes mainly due to their defense mechanism based on the efflux pumps which eliminate the toxic compounds from inside cells. Bacteria could have different defense response based on the presence of harmful compounds in the environment [38,39]. Various bacterial strains (*Pseudomonas aeruginosa*, *Staphylococcus warneri* and *Pseudomonas aurantica*) proved to be very effective in Reldan EC40 pesticide (Nita-Lazar et al. [40]) and pharmaceutical compounds metabolisation (Nita-Lazar et al. [41]).

In this paper we report the synthesis of four coordination compounds of palladium and platinum with mixed ligands: usnic acid and 1-(*o*-tolyl)biguanide, as well as their analytical and spectral characterization and biological activity screening.

2. Materials and methods

2.1. Synthesis of the complexes

The following chemical reagents were used for the synthesis of the four complexes: usnic acid,1-(*o*-tolyl)biguanide, $PdCl_2$, $Pd(CH_3COO)_2$, H_2PtCl_6, $PtCl_2$, C_2H_5OH and $(C_2H_5)_2O$. All the chemicals used were of reagent grade and were purchased from Merck, Alfa Aesar and Sigma-Aldrich. The molar ratio metallic salt: usnic acid: 1-(*o*-tolyl)biguanide was 2:1:1, this ratio being subsequentely found only in the C1 complex.

For the synthesis of complexes $[Pd(TB)(H_3AU)][PdCl_4]$ (C1) and $[Pt(TB)(H_2AU)]Cl$ (C4), palladium chloride and platinum chloride, respectively, were refluxed with acetonitrile for 5 h at 50 - 60°C, to obtain more reactive compounds, according to the reactions:

$$PdCl_2 + 2\ CH_3CN \rightarrow PdCl_2(CH_3CN)_2$$
$$PtCl_2 + 2\ CH_3CN \rightarrow PtCl_2(CH_3CN)_2$$

To these solutions, an ethanolic solution of the two ligands was added dropwise, with stirring. The resulting mixtures were stirred for 1 h, when solid products were separated out.

Synthesis of complexes $[Pd(TB)(H_2AU)]CH_3COO$ (C2) and $[Pt(TB)(H_2AU)Cl_2]Cl$ (C3): the ligands were dissolved in ethanol, under slight heating; the required metal salt, *i.e.*, $Pd(CH_3COO)_2$ and H_2PtCl_6, respectively, dissolved in a minimal amount of ethanol, was added slowly under stirring, to the solution of the ligands. After two hours of stirring, the complexes were obtained as a precipitate.

The synthesized complexes were filtered off, washed with ethyl ether and dried under air. The compounds had a high degree of purity, so they did not require further purification.

2.2. Physical-chemical characterization of the complexes

The percentages of C, N and H in the composition of the four complexes were analyzed using Flash 2000 Elemental Analyzer. Palladium and platinum content was determined using a Perkin Elmer Aanalyst 400 atomic absorption spectrophotometer.

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 up to 900°C.

The UV-Vis diffuse reflectance spectra were recorded on a Jasco V670 spectrometer, in the range of 200-800 nm, using MgO as standard.

The infrared spectra were recorded on a Nicolet IS 50 FT-IR spectrophotometer, in the range 4000–200 cm^{-1}.

2.3. Biological activity

The biological activity of the ligands and C1-C4 complexes was tested *in vitro* against two Gram-positive and Gram-negative bacterial strains (*Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853), one fungal strain (*Candida albicans* ATCC 10231) and HeLa cells.

The antimicrobial and antifungal activities were quantified based on bacterial and fungal growth inhibition monitored by spectrophotometric determination at 620 nm. A quantitative method was used, based on the production of binary serial microdilutions. The working range was 1.00 – 0.0019 mg/mL and the solvent used was DMSO.

Antitumor activity of newly synthesized complexes and ligands was determined by MTT assay (MTT tetrazolium reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium)). The cellular metabolic activity was linked to the reduction of MTT tetrazolium reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) to an insoluble formazan compound.

This process was NAD(P)H-dependent cellular oxidoreductases and spectrophotometrically monitored at a wavelength of 570 nm.

The detection of biological activity was performed according with the methods described by Mihalache et al. [42].

3. Results and discussions

3.1. Elemental analysis

To determine the molecular formulas of the four complexes, the percentages of carbon, nitrogen, hydrogen were determined by elemental analysis and the metal content by molecular absorption spectrometry. The differences between the percentages obtained experimentally and those calculated were very small.

$[Pd(TB)(H_3AU)][PdCl_4]$ Found (%): C, 36.26; H, 3.19; N, 7.45; Pd, 23.77.
Calculated (%): C, 36.43; H, 3.28; N, 7.87; Pd, 23.91.

$[Pd(TB)(H_2AU)]CH_3COO$ Found (%): C, 49.93; H, 4.38; N, 10.11; Pd, 15.31.
Calculated (%): C, 49.76; H, 4.46; N, 10.00; Pd, 15.20.

$[Pt(TB)(H_2AU)Cl_2]Cl$ Found (%): C, 38.91; H, 3.51; N, 8.59; Pt, 23.42.
Calculated (%): C, 38.79; H, 3.38; N, 8.38; Pt, 23.34.

$[Pt(TB)(H_2AU)]Cl$ Found (%): C, 42.13; H, 3.96; N, 9.21; Pt, 25.67.
Calculated (%): C, 42.33; H, 3.82; N, 9.14; Pt, 25.46.

3.2. Thermal analysis

The thermograms obtained from the thermal decomposition of complexes C1-C4 gave information's regarding the mass losses and the thermal effects that accompany them.

The TG curves of the palladium (II) complexes indicate that these compounds are stable to 220°C, when it starts their decomposition. This indicates 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 [43].

As for the previous two compounds, the C3 and C4 complexes are stable over a large temperature range (30–200ºC), which confirms that they do not contain water or alcohol. Above this temperature the complexes undergo decomposition. For the two complexes, the slightly endothermic process,

without mass loss, at 210°C, could be attributed to their melting. The large, highly exothermic process, occurring between 400 and 500°C is due to oxidative degradation and elimination of the organic ligands. The final residue is platinum [44].

3.3. Ultraviolet-visible spectra

Based on the UV-Vis spectra of the complexes compared to the ligands used in the synthesis, their stereochemistry was established. The electronic d–d transitions observed in the spectra of the complexes with their assignments and the corresponding symmetry are given in Table1.

The electronic spectra of the complexes C1 and C2 exhibited a strong absorption band, with maximum at 25975 cm^{-1} and 26315 cm^{-1}, respectively, that can be attributed to $^1A_{1g} \rightarrow {}^1B_{1g}$ transition.

In the case of C1 complex the band at 19230 cm^{-1} corresponds to the transition $^1A_{1g} \rightarrow {}^1A_{2g}$. The same assignment corresponds to the shoulder in the spectrum of the C2 complex located at 19610 cm^{-1}.

According to the experimental data and to the literature reports, the symmetry proposed for these two complexes is square planar [45, 46].

C3 and C4 complexes contain platinum in different oxidation states, namely +IV in C3 and +II in C4. It was found that platinum maintained its valence in the substances used in synthesis: H_2PtCl_6 for C3 and $PtCl_2$ in C4.

In the UV-Vis spectrum of the C3 complex the three bands observed at 17095 cm^{-1}, 22990 cm^{-1} and 28570 cm^{-1} can be attributed to the d-d transitions from the ground state $^1A_{1g}$ to $^1T_{1g}$, $^1T_{2g}$ and $^3T_{1g}$, respectively, according to an octahedral symmetry.

For the C4 complex the symmetry proposed is square-planar, as confirmed by the bands at 20830 cm^{-1} and 25640 cm^{-1} which are assigned to $^1A_{1g} \rightarrow {}^1A_{2g}$ and $^1A_{1g} \rightarrow {}^1B_{1g}$ transitions.

Table 1. Maxima of *d-d* absorption bands for C1 – C4 complexes

Complex	Observed bands λ_{max}(nm)	υ(cm^{-1})	Assignments	Proposed stereochemistry
[Pd(TB)(H_3AU)][$PdCl_4$]	520	19230	$^1A_{1g} \rightarrow {}^1A_{2g}$	square-planar
	385	25975	$^1A_{1g} \rightarrow {}^1B_{1g}$	
[Pd(TB)(H_2AU)]CH_3COO	510	19610	$^1A_{1g} \rightarrow {}^1A_{2g}$	square-planar
	380	26315	$^1A_{1g} \rightarrow {}^1B_{1g}$	
[Pt(TB)(H_2AU)Cl_2]Cl	585	17095	$^1A_{1g} \rightarrow {}^1T_{1g}$	octahedral
	435	22990	$^1A_{1g} \rightarrow {}^1T_{2g}$	
	350	28570	$^1A_{1g} \rightarrow {}^3T_{1g}$	
[Pt(TB)(H_2AU)]Cl	480	20830	$^1A_{1g} \rightarrow {}^1A_{2g}$	square-planar
	390	25640	$^1A_{1g} \rightarrow {}^1B_{1g}$	

3.4. FT-IR Spectra

Comparative analysis of the IR spectra of the usnic acid and 1-(*o*-tolyl)biguanide and their metal complexes allows to establish the donor atoms of the ligands in coordination to the metal ions. The assignments of the spectral bands characteristic of the ligands and the complexes C1-C4 are presented in Table 2.

The strong band appearing at 1676 cm^{-1} in the IR spectrum of the usnic acid may be assigned to the stretching vibration ν(C=O) of the ketone function [47,48]. This band shifts to lower wave numbers in the IR spectra of all complexes, indicating the coordination of the usnic acid through the ketone oxygen atoms.

The very strong band, with maximum at 1191 cm^{-1}, may be assigned to the stretching vibration of $C\text{-}O_{phenolic}$ [49]. Its position is practically unchanged in the spectrum of the complex C1, but it is shifted to higher wavenumbers in the spectra of the complexes C2-C4. These observations suggest the deprotonation of the usnic acid in the complexes C2-C4 and coordination through phenolic oxygen linked to carbon 3. Thus, the usnic acid acts as neutre bidentate OO donor in the complex C1 and as a monobasic bidentate OO donor in the complexes C2-C4.

The band due to the stretching vibration of the imine group, ν(C=N), appearing at 1610 cm^{-1} in the IR spectrum of 1-(*o*-tolyl)biguanide, shows a downward shift in the spectra of the complexes C2-C4

and an upward shift in the spectrum of the complex C1. These observations are in according to the involvement of imine nitrogen atoms in coordination to the metal ions [49].

In the IR spectrum of the complex C2 a new strong absorption band at 1395 cm^{-1} may be assigned to ν_{sym}(COO) of acetate group. The strong absorption band at 1552 cm^{-1} in the spectrum of the same complex is due to ν_{asym}(COO), the value of $\Delta = \nu_{asym}(COO) - \nu_{sym}(COO) = 157$ cm^{-1} being in the range of ionic acetate [50].

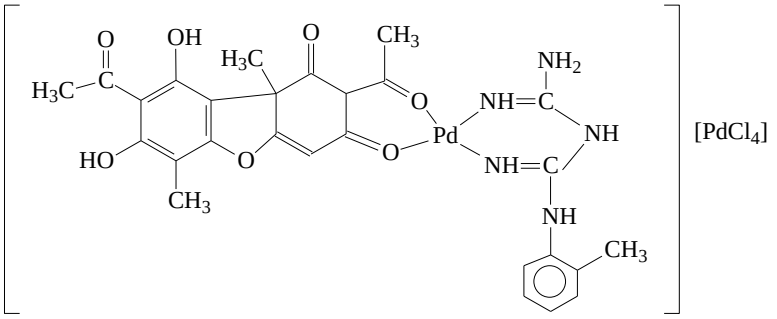
Table 2. IR bands and their assignments for the synthesized complexes and ligands ($\bar{\upsilon}_{max,}$ cm^{-1})

Assignments	1-(*o*-tolyl) biguanide	usnic acid	C1	C2	C3	C4
ν(C=O) keto of metyl ketone		1676 s	1662 s	1635 s	1641 s	1653 s
ν(C=N)	1610 vs		1645 vs	1602 vs	1618 vs	1624 vs
δ(NH)+ν(C-N)	1577 m 1270 w		1590 m 1231 w	1589m 1246w	1557m 1240m	1590m 1265w
ν(CO) phenolic		1191 vs	1197 vs	1220 vs	1217 vs	1209 vs
δ(OH) phenolic outside the plan		700 m	708 m	716 m	722 m	729 m
M-Cl			303 w		279 w	
ν_{asym}(C=O)$_{acetate}$				1552 s		
ν_{sym}(C-O)$_{acetate}$				1395 s		
ν (M-N)			503m	532m	490m	521m
ν (M-O)			597m	602w	595w	580m

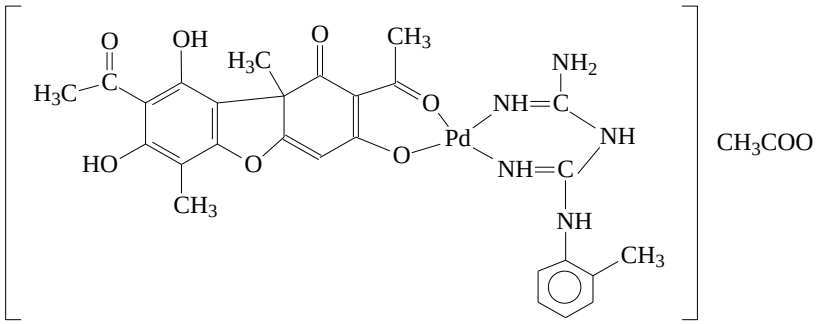
s – strong, vs – very strong, m – medium, w – weak

Supplementary bands appearing in the IR spectra of the complexes at low wave numbers may be assigned to the vibrations metal-donor atom: ν(M-O) (580-602 cm^{-1}) and ν(M-N) (490-521 cm^{-1}) [50].

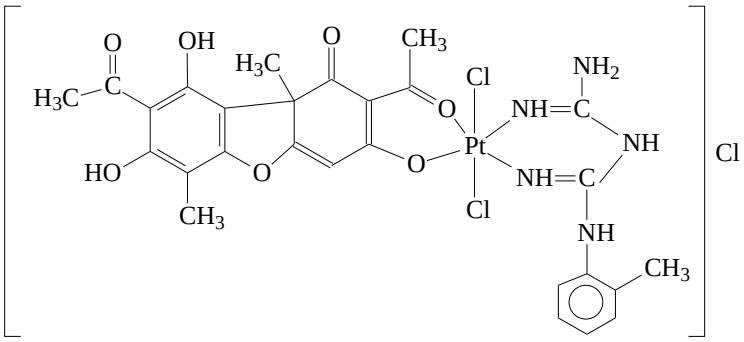
Based on these data, the four coordination complexes are proposed to have the conformations shown in Figure 1.



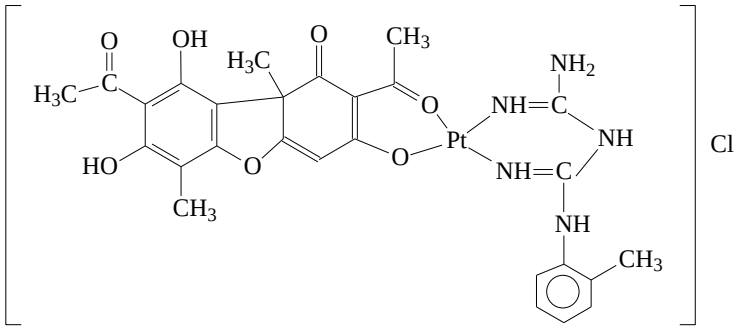
[Pd(TB)(H_3AU)][$PdCl_4$] **(C1)**



[Pd(TB)(H_2AU)]CH_3COO **(C2)**



[Pt(TB)(H_2AU)Cl_2]Cl **(C3)**



[Pt(TB)(H_2AU)]Cl **(C4)**

Figure 1. Proposed formulas for C1-C4 complexes

3.5. Antimicrobial activity

C1-C4 complexes, H_3AU and TB were tested *in vitro* against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 strains to determine antibacterial activity. Following the tests performed it was observed that the antimicrobial activity against the Gram positive species *Staphylococcus aureus* was very good for complexes and the usnic acid while for 1-(*o*-tolyl) biguanide was 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 bacteria *Pseudomonas aeruginosa* both ligands have low antimicrobial activity. All complexes have a good activity against this strain, the tetravalent platinum complex having the lowest MIC of 0.0625 mg/mL. The solvent used in dilutions did not influence the antimicrobial activity of the tested compounds at working concentrations.

The minimum inhibitory concentrations against the two bacterial strains for the usnic acid, 1-(*o*-tolyl)biguanide and the complexes synthesized are shown in Figures 2 and 3.

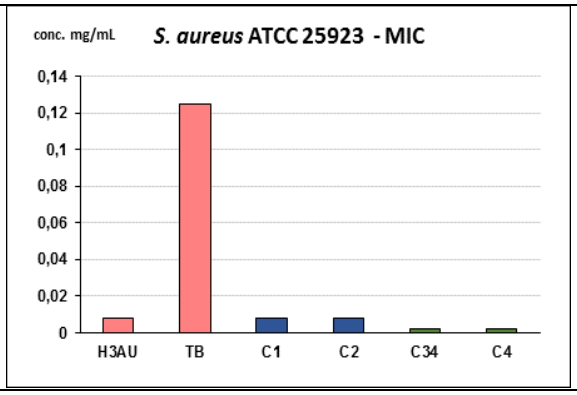


Figure 2. Minimum inhibitory concentrations for C1-C4 and ligands against *S.aureus*

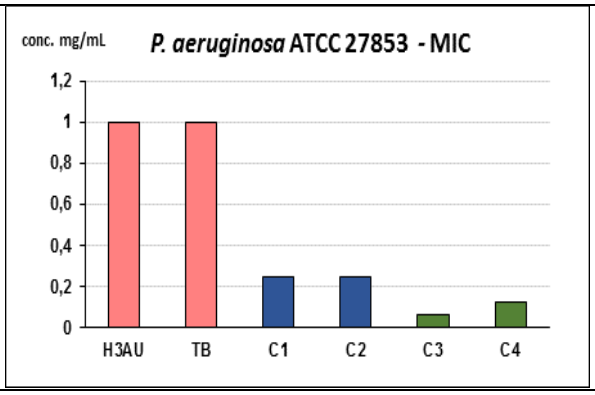


Figure 3. Minimum inhibitory concentrations for C1-C4 and ligands against *P.aeruginosa*

3.6. Antifungal activity

Antifungal activity of the ligands and the metal complexes was tested against *Candida albicans* ATCC 10231. The results indicate that the ligands have a poor activity, C1 and C2 complexes have a moderate one, while the platinum complexes have the best antifungal activity. The latter have a MIC of 0.125 mg/mL.

The minimum inhibitory concentrations for ligands and C1-C4 complexes are shown in Figure 4.

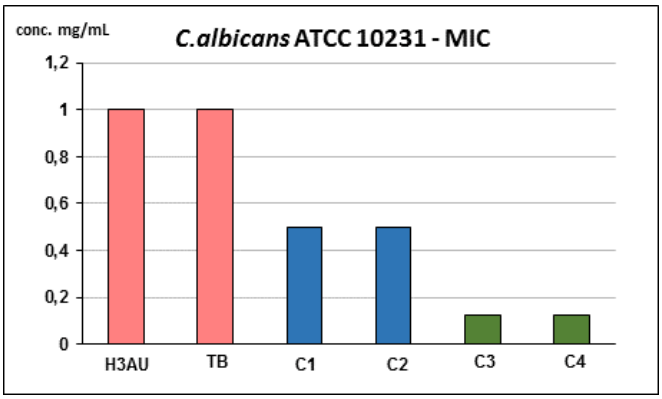


Figure 4. Minimum inhibitory concentrations for C1-C4 and ligands against *C. albicans*

3.7. Antitumor activity

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

Platinum complexes have reduced the viability of HeLa cells by 35% C3 and 32% C4, respectively, thus showing a good cytotoxic effect. The ligands and palladium complexes had a weaker effect on this type of tumor cells.

Figure 5 shows the viability of HeLa cells in the presence of ligands and complexes.

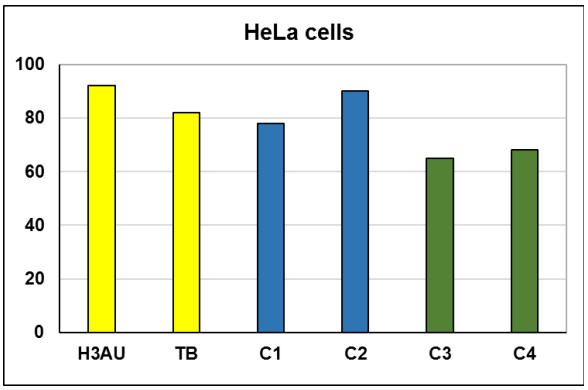


Figure 5. Viability of HeLa cell cultures in the presence of the ligands and complexes

4. Conclusions

Four new complexes of Pd(II), Pt(II) and Pt(IV) were obtained using usnic acid and 1-(*o*-tolyl)biguanide as ligands. The composition and the stereochemistry around the metal ions were established based on the analytical and spectral data. Pd(II) complexes have square planar symmetry, C3 complex contains tetravalent platinum and it has octahedral symmetry, while the complex C4 is square planar with divalent platinum. C1 is of the Vauquelin type, having a complex anion $[PdCl_4]^{2-}$.

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 against all tested strains compared to palladium complexes.

The antitumor activity of the C1 complex is better than the C2 and can be explained by the number of palladium ions in the molecule, namely, two for the first versus one for the second complex. As a general observation, the biological activity of the ligands is improved by complexation to the metal ions.

Abbreviations:

H_3AU	usnic acid
H_2AU	deprotonated usnic acid
TB	1-(*o*-tolyl)biguanide
DMSO	dimethylsulfoxide
NAD(P)H	nicotinamide-adenine-dinucleotide phosphate
MTT	3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide
MIC	Minimum Inhibitory Concentration

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