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Original Scientific Article

A New Complex of Palladium(II) With 2-Furoic Hydrazide: Synthesis, Characterization, Theoretical Calculations and Biological Studies

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Abstract. A new complex of palladium was isolated with 2-furoic hydrazide (FH) and characterized by spectroscopic methods. The results show that the ligand is coordinated to palladium by the basic nitrogen of NH_2 group and has a general structure of type *cis*-$[Pd(FH)_2Cl_2]$. The structure of palladium(II) complex was optimized and theoretical data show good agreement with the experimental results. The cytotoxic activity was evaluated in a chronic myelogenous leukemia cell line, which revealed that the compound is less active than cisplatin or carboplatin. At 300 μg mL^{-1}, the complex presented antimicrobial activity more efficient than ampicillin, chloranfenicol and kanamicyn. (doi: 10.5562/cca2151)

Keywords: palladium complexes, hydrazides, hydrazones, DFT studies, antibacterial activity

INTRODUCTION

The hydrazide properties are of major interest due to their biological activities. Hydrazides have been demonstrated to possess, among other, antibacterial, antifungical, and antitumoral activities. For example, isonicotinic acid hydrazide (isoniazid) exhibited high *in vivo* inhibitory activity towards *M. tuberculosis* H37Rv.[1]

The formation of metallic complexes plays an important role in the growth of their biological activity[1–4] and, therefore, many complexes of hydrazides have been synthesized and characterized.[5–7] Some of these metallic complexes also exhibit good fungicide, antitumoral and antibacterial activity.[8–11] As example, platinum complexes containing hydrazides derived from benzoic acid showed strong growth inhibitory effect in leukemia cells *in vitro*, not verified with the free ligands.[9]

On the other hand, palladium complexes have been obtained aiming to produce drugs, due to its chemical similarity with platinum, and the fact that they showed excellent antitumoral and anti infective activities *in vitro* and *in vivo*.[12] For example, our research group showed that the palladium(II) complex of tetracycline is 16 times more potent than free tetracycline against *E. coli HB101/pBR322*, a bacterial strain resistant to tetracycline.[13] In addition, this complex displays a significant cytotoxic activity.[14] These observations encouraged us to synthesize new palladium complexes as possible antitumoral and antibacterial agents. In this work, we report the synthesis and characterization of a new palladium(II) compound with 2-furoic hydrazide. The compound was characterized by ^1H NMR, IR, UV-Vis, thermal (TG/DTA), elemental and theoretical analysis. The cytotoxic and antibacterial activity of the synthesized compound was evaluated.

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EXPERIMENTAL

Physical Measurements

Conductivity study was carried out with a Digimed DM 32 conductivity meter using a cell of constant 1.00 cm^{-1}, spectroscopic grade dimethylformamide (Merck) (Λ_M = 1.20 μs cm^{-1}) and tetraethylammonium bromide (Λ_M = 78.39 μs cm^{-1}) as a standard.

Elemental analyses were performed at the Analytical Central of the University of São Paulo, using a Perkin-Elmer 2400 CHN Elemental Analyser.

IR spectra were registered in KBr pellets on a Shimadzu FTIR-Irprestige-21 spectrometer.

A spectrophotometer UV-2501 PC Shimadzu was used for UV and visible absorption measurements.

NMR spectra were obtained using a Bruker Avance DPX 200 spectrometer with tetramethylsilane as an internal standard.

Thermogravimetric analyses (TG/DTA) were performed on a TGA-50 Shimadzu, using 6.0 mg sample packed in aluminum crucible. The samples were heated at 10 °C min^{-1} from room temperature to 500 °C, in a dynamic nitrogen atmosphere (flow rate = 200 mL min^{-1}).

Theoretical Details

Geometry optimization and vibrational frequency calculations were performed at MP2 and DFT level of theory in Gaussian 03,[15] while TD-DFT/B3LYP UV-visible spectra were computed in Orca program package.[16] For optimization and frequency calculations, the LANL2DZ atomic basis set and effective core potential[17] were used for the metal center, while the standard 6-311++G(d,p) basis sets were employed for the other atoms.[18] The electronic spectrum was computed at B3LYP level, with def2-SVP basis set for all atoms.[19] No symmetry restrictions have been imposed during the geometry optimizations.

Cells and Culture

The K562 cell line was purchased from the Rio de Janeiro Cell Bank (number CR083 of the RJCB collection). This cell line was established from pleural effusion of a 53 year-old female with chronic myelogenous leukemia in terminal blast crisis. Cells were cultured in RPMI 1640 (Sigma Chemical Co.) medium supplemented with 10 % fetal calf serum (CULTILAB, São Paulo, Brazil) at 37 °C in a humidified 5 % CO_2 atmosphere. Cultures grow exponentially from 10^5 cells mL^{-1} to about 8×10^5 cells mL^{-1} in three days. Cell viability was checked by Trypan Blue exclusion. The cell number was determined by Coulter counter analysis.

The cytotoxic effect was evaluated by incubating 1×10^5 cells mL^{-1} in the absence and the presence of a range of concentrations of tested compounds for 72 h. Afterwards, cells were counted and the concentration required to inhibit cellular growth by 50 %, the IC_{50}, was calculated. Stock solutions of the compounds were prepared in DMSO (2 %) and H_2O.

Antimicrobial Activity

An oxaciclin-resistant *Escherichia coli* strain and a methicilin-resistant *Staphylococcus aureus* strain were used to evaluate the antimicrobial activity of the complex. Both strains were isolated from Uberlândia Federal University Hospital (HCU/UFU, Uberlândia, MG, Brazil). 100 μL of a suspension of each strain previously cultured at 37 °C in LB (NaCl 10 g L^{-1}; Yeast extract 5 g L^{-1}; Triptone 10 g L^{-1}) were used to obtain an OD600 = 0,35 LB culture. The complex was added to each culture and the bacterial growth was monitored by optical density observation at 0; 6; 12; 24; 48; and 72 hours. The complex was tested at the concentrations of 100 μg mL^{-1}; 200 μg mL^{-1}; 300 μg mL^{-1}; 500 μg mL^{-1} and 1000 μg mL^{-1}. Each test was replicated 4 times.

Starting Materials

The reagents K_2PdCl_4 and 2-furoic hydrazide (FH) are commercially available (Aldrich). All other reagents chemicals were of analytical grade, purchased from different sources, and used without further purification.

Preparation of the Complex

0.1632 g of K_2PdCl_4 (0.5 mmol) was added to 5 mL of an aqueous solution of FH (1.0 mmol) and the mixture was stirred for 24 h. The solid formed was separated by filtration, washed with water, and dried under vacuum.

Yield: 91 %. Color: Yellow. Anal. Calcd. for $[Pd(C_5N_2O_2H_5)_2Cl_2]$: C, 27.96; H, 2.82; N, 13.04 %; Found: C, 27.92; H, 2.78; N, 12.98 %. IR spectra in KBr, ν (cm^{-1}): 3295, 3256, 3197, 3086, 1661, 1596, 1562, 1526, 1469, 1316, 1253, 1234, 1180, 1144, 1016, 953, 884, 865, 773, 751, 640, 541, 505, 498, 417. RMN de ^1H (200 MHz; DMSO-d_6): δ 6.91 (s, 2H, -NH_2); δ 6.63−7.80 (3d, 3H, HAr); δ 9.60 (s, H, -NH).

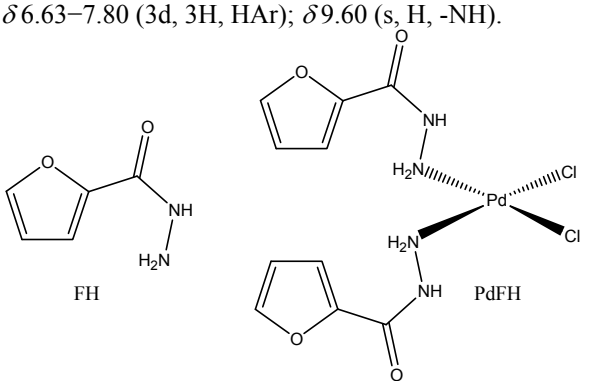

Figure 1. 2-furoic hydrazide and its palladium complex.

RESULTS AND DISCUSSION

A new complex containing 2-furoic hydrazide (FH) as ligand was synthesized by the slow addition of the corresponding ligand to K_2PdCl_4 dissolved in water. After 24 hours, the compound was isolated by simple filtration. The complex was characterized by elemental analysis, thermal analysis, IR, UV-Vis and ^{1}H NMR.

In this work, the ligand acts as a monodentate ligand replacing two Cl ligands from the precursor. The palladium complex is soluble in organic solvents such as DMSO and DMF. The chemical structures of the ligand (FH) and its complex (PdFH) are presented in Figure 1.

The results of the elemental analysis (C, N, and H) are in accordance with the proposed structure.

The freshly prepared DMF solution of the complex exhibited molar electric conductivity value far below that of the 1 : 1 standard electrolyte indicating that the complex is not charged.[20]

Thermal Analysis

The thermal stability of the complex was followed in the temperature range 25–500 ºC. The TG/DTA curve for complex (Figure 2) shows a series of events weight loss at 170–408 °C range that is attributed to thermal decomposition of the complex. At 420 °C there is a residue (elemental palladium) corresponding to 24.90 % (Calculated: 24.77 %), confirming the structure proposed since the percentage of metal fits well with the proposed formula.

IR Spectra

The IR spectrum of the free ligand was performed just for comparison to the corresponding complex isolated. The characteristic absorptions in the 3400 to 3032 cm^{-1} region were observed, corresponding to ν(NH$_2$), ν(NH), and ν(CH). The spectrum also exhibited bands at 1657 and 1633 cm^{-1}, assigned to ν(C=O) and ν(C=C), respectively.[7]

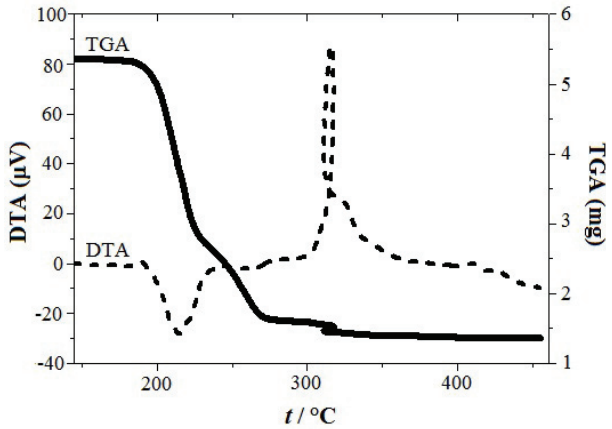

Figure 2. TG/DTA curve for complex PdFH.

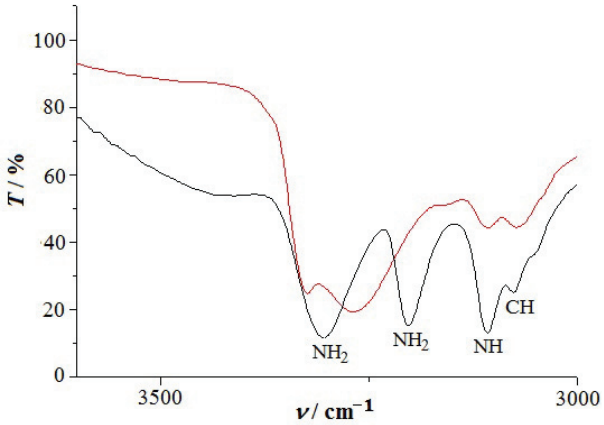

Figure 3. IR spectra (3600 – 3000 cm^{-1}) of FH (in red) and its complex.

In the IR spectrum of the complex, the bands due ν(NH$_2$) were found to be shifted towards lower wavenumbers (Figure 3). This shift can be attributed to participation of the NH$_2$ group in the coordination to the palladium ion. In addition, in the IR spectrum of the complex, a new peak assigned to ν(M–N) appeared at 541 cm^{-1}, indicating formation of the M–N bond (Figure 4). Another observation is that the ν(C=O) of the carbonyl group appears almost in the same wavenumber of the ligand, therefore, we ruled out an involvement of this group in the coordination to the metallic ion.

^{1}H NMR

The ^{1}H NMR spectra of the complex and of the 2-furoic hydrazide ligand were recorded in d_6-DMSO.

In the spectra of the free ligand, the NH$_2$ protons appear as a singlet at δ 4.42. This signal has been shifted downfield in the complex (δ 6.91) and this result suggests that the nitrogen of the NH$_2$ group is involved in the coordination sphere.[8]

The complex shows a singlet near at δ 9.60 ppm due to the NH proton. The signal of the NH proton was much less affected when compared to the group NH$_2$

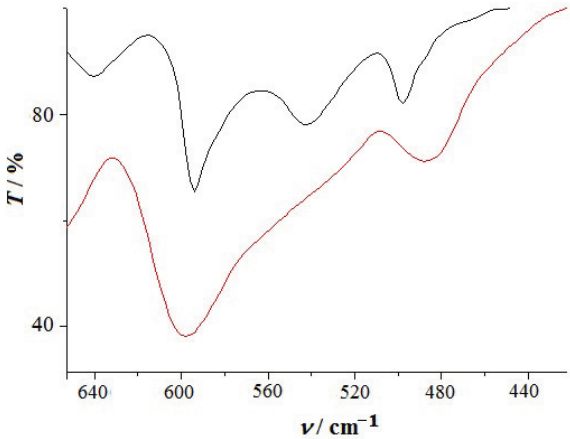

Figure 4. IR spectra (640–440 cm^{-1}) of FH (in red) and its complex.

protons excluding the participation this group in the coordination. Thus, considering ours spectral results, we propose the coordination of metallic ion *via* NH_2 group. The same coordination mode here proposed was observed in previous works for some complexes containing hydrazides as ligands.[9,21–25]

Computational Results

The MP2 and B3LYP bond lengths and bond angles of the palladium(II) complex optimized are collected in Table 1. The optimized structure of complex is shown in Figure 5. The results indicate that the coordination sphere of the complex around metal center is a distorted square-planar. The molecular distortion of palladium(II) complex can be visualized by the bite Cl−Pd−Cl and N−Pd−N angles, which are computed to be 99° (97°) and 89° (89°) at MP2(B3LYP) theory levels, respectively. A rather small difference can be observed between results of the bond lengths and bond angles at MP2 and B3LYP levels. The largest deviation of 0.1 Å is observed in Pd−N bond length, while the value of 1.8 degree is observed in the Cl−Pd−Cl bond angle.

The experimental and computed Td-DFT absorption spectra of 2-furoic hydrazide ligand and its palladium(II) complexes are displayed in the Figure 6. The experimental UV-visible absorption spectrum of the ligand (Figure 6) exhibit two bands centered at 220 nm and 251 nm, while the spectrum of the complex exhibited three peaks in the ultraviolet region. As shown in computed spectrum of the ligand, Td-DFT/B3LYP calculations in the gas-phase provide a very good description of the spectral profile and the absorption positions of the excitation electronic energies of the hydrazide ligand. Two more intense bands are calculated to be at 227 nm and 248 nm, in good agreement with the experimental measurements (220 nm and 251 nm). The computed spectral profile of the palladium complex shows a good agreement with experimental spectrum. However, regarding the absorption positions, the experimental bands are localized at 255, 308 and 344 nm, while the computed positions are at 409, 448 and 483 nm, respectively. These large solvatochromic shifts should be owing to the solvent effects, which are not taken into account in our calculations. It is worth noting

Table 1. The MP2 and B3lYP optimized bond lengths (Å) and bond angles (degree)

Distances	B3LYP	MP2	Angles	B3LYP	MP2
Pd−Cl	2.266	2.279	Cl−Pd−Cl	97.4	99.2
Pd−N	1.855	1.986	N−Pd−N	89.4	89.6
N−C	1.501	1.523	Cl−Pd−N	83.3	83.2
N−N	1.606	1.543	Pd−N−N	117.6	117.9
C−O	1.259	1.260	N−N−C	112.8	111.5
C−C	1.519	1.529	N−C−C	113.9	114.3
$C-O_{ring}$	1.426	1.413			
$C-C_{ring}$	1.446	1.453			

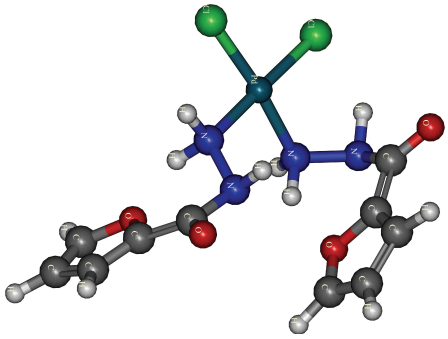

Figure 5. The optimized structure of the palladium (II) complex. Molecule displayed in a ball-and-sick model (atoms are color coded as gray: carbon; blue: nitrogen, red: oxygen; green: chlorine, and white: hydrogen).

that the experimental measurements (Figure 6) were done in the methanol solution and large solvatochromic shifts are verified for absorption spectra varying the basicities of solvents. It is important to note that similar large solvatocromic shifts have been already observed in some absorption spectra of the other planar TM complexes when solvents with different basicities are taken into account.[26] Regarding the assignment of the electronic spectrum of the palladium (II) complex the theoretical results confirm that the experimental band at 255 nm is assignable to intraligand $\pi\rightarrow\pi^*$ transitions, while the band centered at 344 nm is assigned to metal-to-ligand charge-transfer. The Figure 7 shows the $HOMO^{-1}$ and $LUMO^{+1}$ molecular orbitals which are involved in the latter electronic transition. Overall, the computational results show a good agreement with

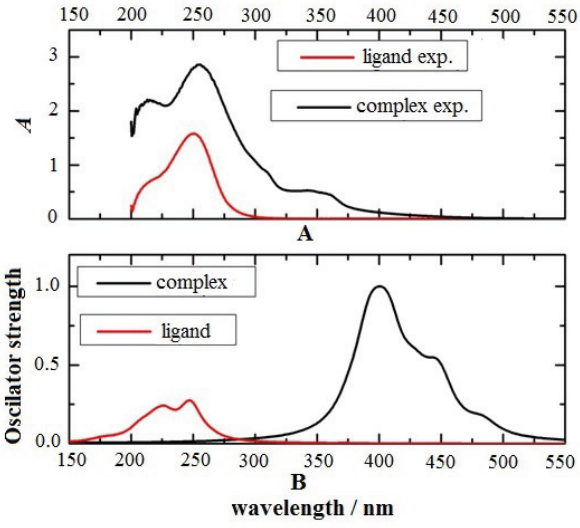

Figure 6. Experimental and computed UV-Vis spectra of the ligand and its palladium (II) complex. (A) Experimental measured in methanol (10^{-4} mol L^{-1}) and (B) The computed spectra in the gas-phase.

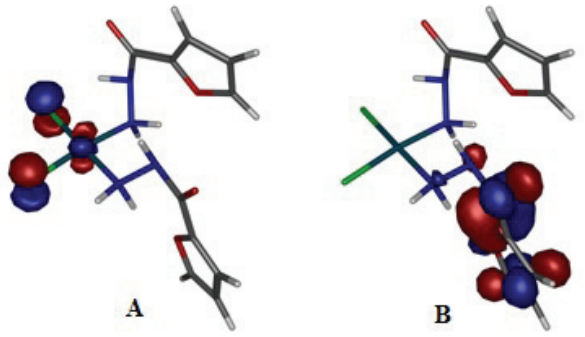

Figure 7. (A) HOMO and (B) LUMO Kohn-Sham orbitals.

experimental data, thus confirming that the ligand is coordinated to palladium by the basic nitrogen of NH_2 group and have the general structure *cis*-$[Pd(FH)_2Cl_2]$.

Cytotoxic Activity

The cytotoxic activity of the ligand (FH) and its complex was examined on K562 cells. In Table 2, IC_{50} values obtained for cisplatin, the free ligand and its complex are shown for the sake of comparison. The complex PdFH inhibits the growth of K562 cells and, its activity is 2-fold higher than of the corresponding free ligand. However, in comparison to cisplatin or carboplatin, the effect of the complex is much lower.

Table 2. IC_{50} values for the complex and free ligand

Compound	IC_{50}[(a)] (μmol L^{-1} ± s.d.)
FH	455.6 ± 20
$[Pd(FH)_2Cl_2]$	255.9 ± 13
carboplatin	60[(b)]
cisplatin	1.1 ± 0.1

[(a)] IC_{50} is the concentration required to inhibit 50 % of cell growth, after 3 days of incubation. The values are the mean of triplicate determinations.
[(b)] Value from reference 27.

Antibacterial Activity

Antimicrobial activity against two bacterial species belonged to main bacterial group was tested: the Gram-positive *Staphylococcus aureus,* and the Gram-negative *Escherichia coli.* The results showed that the complex presented antimicrobial activity against *S. aureus* from concentrations of 300 μg mL^{-1} (Figure 8A). On the other hand, this activity was not observed against *E. coli* (Figure 8B). Thus, the complex presented antimicrobial activity against Gram-positive, but was not effective against Gram-negative bacteria. However, more detailed studies have done to evaluate the action spectrum of this complex against Gram-positive bacteria. The antimicrobial activity of complex against *S. aureus* was compared to ampicillin,[28] chloranfenicol and kanamicyn.[29,30]

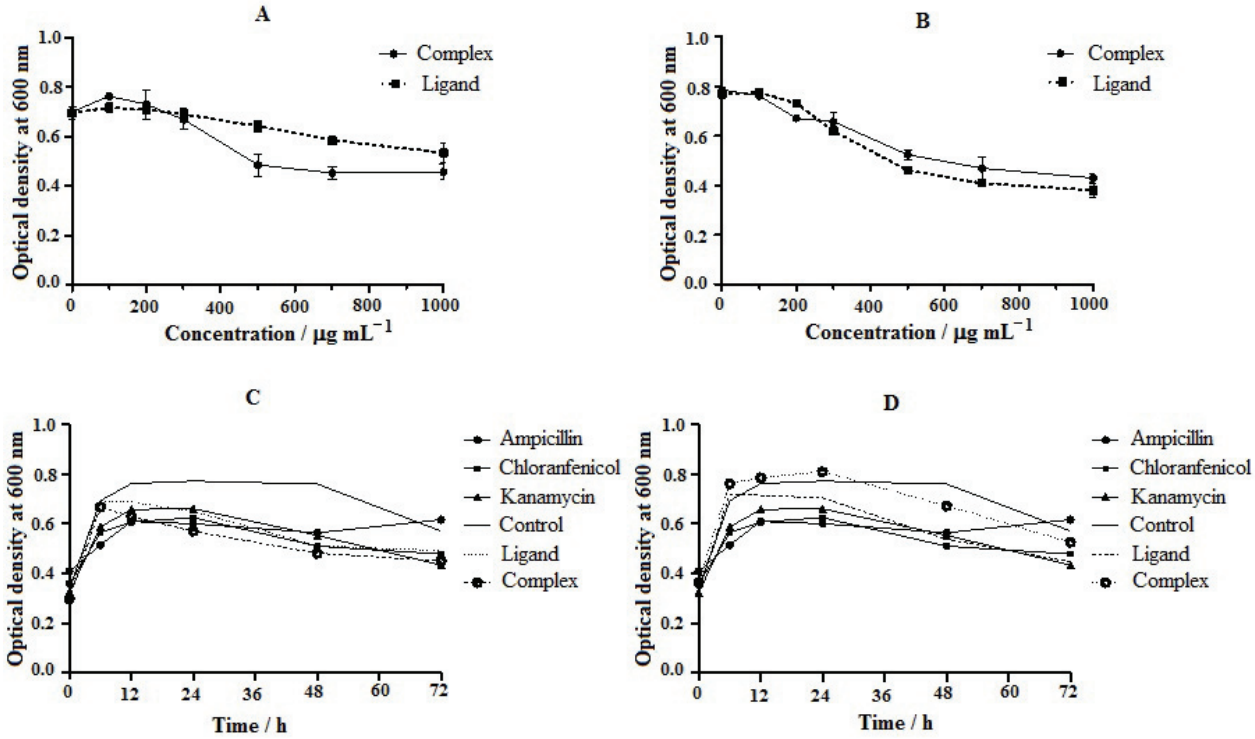

Figure 8. (A) *Staphylococcus aureus* growth after 6 hours of ligand and complex addition at 0, 100, 200, 300, 500, 700, and, 1000 μg mL^{-1}. (B) *Escherichia coli* after 6 hours of ligand and complex addition at 0, 100, 200, 300, 500, 700, and, 1000 μg mL^{-1}. (C) *Staphylococcus aureus* growth after ligand, complex, chloranfenicol, kanamicyn, and, ampicillin. Binder: 300 μg mL^{-1}; Complex: 300 μg mL^{-1}; Chloranfenicol: 100 μg mL^{-1}; Kanamicyn: 100 μg mL^{-1}; Control: *S. aureus* culture. (D) *Staphylococcus aureus* growth after binder, complex, chloranfenicol, kanamicyn, and, ampicillin at 100 μg mL^{-1}.

At 300 μg mL^{-1}, the complex presented antimicrobial activity more efficient than ampicillin, chloranfenicol and kanamicyn, and this activity was observed to 72 hours (Figure 8C). However, when the complex was used at 100 μg mL^{-1} (the same concentration of the antimicrobials) the antimicrobial activity was observed only after 24 hours (Figure 8D). The results suggested that the complex presented a stable antimicrobial activity against Gram-positive bacteria at 300 μg mL^{-1}.

CONCLUSION

A new complex containing 2-furoic hydrazide was prepared and characterized by thermal and spectroscopic techniques. The spectroscopic and theoretical techniques show that the ligand is coordinated to the palladium by the basic nitrogen of NH_2 group.

The biological studies showed that the complex has a poor cytotoxic activity against K562 cell line, but it is active against Gram-positive bacteria.

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