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Synthesis, spectroscopic characterization, DFT studies, and antibacterial and antitumor activities of a novel water soluble Pd(II) complex with L-alliin

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HIGHLIGHTS

- A novel water soluble Pd(II) complex with L-alliin.
- The coordination was determined by IR, ^{1}H, ^{13}C, ^{15}N NMR, mass spectrometry and DFT studies.
- The compound shows antibacterial activity against Gram positive and Gram negative bacterial strains.
- The complex also exhibits cytotoxic activities against HeLa tumorigenic cells.

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ABSTRACT

A new water soluble Pd(II) complex with L-alliin (S-allyl-L-cysteine sulfoxide) was obtained and characterized by a set of chemical and spectroscopic measurements. Elemental and mass spectrometric data are consistent with the formula $[Pd(C_6H_{10}NO_3S)_2]$. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) data, [^{1}H–^{15}N] two dimensional (2D) NMR and infrared spectroscopic measurements indicate coordination of the ligand to Pd(II) through N and O atoms. DFT studies showed that the trans isomer is the most stable and preferred geometry for the complex. The complex is soluble in water and dimethylsulfoxide. An antibiogram assay revealed that the complex possess antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacterial strains in the range 125–500 μg mL^{-1}. Antitumor assays revealed that the complex presents cytotoxic activity over HeLa cells with an estimated IC_{50} of 20 μmol L^{-1}.

1. Introduction

Metal complexes have been considered as therapeutic agents for a long time. Cisplatin, or cis-diamminedichloroplatinum (II), and second-generation drugs based on the cisplatin structure, such as carboplatin and oxaliplatin, are the best examples of metal-based compounds in clinical use. Cisplatin is a chemoterapeutic agent particularly effective against cervical, head, neck, bladder and testicular cancer. In spite of its anticancer activity, cisplatin has been shown to possess toxic side effects such as nephro-, neuro- and hematotoxicity. Continuous efforts have been made to alleviate these side effects and improve its solubility, including changes in the cisplatin structure and also the synthesis of new anticancer compounds with other metal ions [1]. Palladium(II), as a soft Lewis acid, has the ability to form stable chelated rings with N,S-donor ligands [2]. Based on the similarities of coordination geometry and thermodynamic parameters of the palladium(II) complexes when compared to the platinum(II) analogues, synthesis and application of palladium-based compounds in medicinal

Abbreviations: Ali, S-Allyl-L-cysteine sulfoxide; ATCC, American type collection cell; BEC, Brazilian endemic clone; CLSI, Clinical and Laboratory Standards Institute; DFT, density functional theory; DMEM, Dulbecco's modified eagle medium; DMSO, dimethylsulfoxide; D_2O, deuterium oxide; ESI-MS, electrospray mass spectrometry; FCS, fetal calf serum; HMBC, heteronuclear multiple bond correlation; IR, infrared spectroscopy; MIC, minimal inhibitory concentration; MTT, (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMR, nuclear magnetic ressonance; PBS, phosphate buffer saline; PES, potential energy surface; Pd-Ali, palladium(II) complex with alliin; ZPE, zero point energies.

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chemistry started to be considered. However, the palladium compounds were reported to be kinetically more reactive than the platinum analogues [3].

Due to the growth of multi-resistant bacterial strains, syntheses of new antibacterial agents of silver(I), gold(I) and also platinum(II) and palladium(II) for the treatment of infectious diseases have also been evaluated. Kazachenko et al. [4] investigated the synthesis and antibacterial activities of silver complexes with the amino acids histidine and tryptophan. Both compounds showed a good antibacterial activity against Gram-negative and Gram-positive bacterial strains and low toxicity. In addition, the antibacterial activities of palladium(II) complexes of tetracyclines (tetracycline, doxycycline and chlortetracycline) have been reported [5]. The palladium(II) complex of tetracycline is practically as efficient as free tetracycline in inhibiting the growth of two *Escherichia coli* sensitive bacterial strains and 16 times more potent than free tetracycline against *E. coli* HB101/pBR322, a bacterial strain resistant to tetracycline. A palladium(II) complex with benzimidazole showing significative antibacterial activity against Gram-negative strains, and also antitumor activities against breast cancer (MCF7), colon carcinoma (HCT) and human heptacellular carcinoma (Hep-G2) has been recently described [6]. Budige et al. also described the synthesis of Pd(II) complexes with Schiff bases with a pronounced antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* [7].

Amino acids are molecules of high interest for pharmacological applications. In general, amino acids have been shown to possess low toxicity and high affinity to specific sites in the body. In addition, amino acids contain at least two coordination sites, the amino group and the carboxylic group. They can also have a third coordination site when a sulfur atom is present, as observed with cysteine, methionine and their derivatives. In our group palladium(II) complexes of methionine sulfoxide and deoxyallin were synthesized, characterized and their antitumor activities evaluated against HeLa, tumorigenic cells, with promising results [8,9]. The Pd(II) complex with deoxyalliin was also shown to posses antibacterial activities against pathogenic bacterial strains of *S. aureus* and *E. coli* [10]. More recently, Carvalho et al. published the antibacterial activities of Ag(I) and Pd(II) complexes with tryptophan. The Ag(I) complex was also cytotoxic against Panc-1 (human pancreatic carcinoma) and SK-Mel 103 (human melanoma) cells [11,12].

S-allyl-L-cysteine sulfoxide ($C_6H_{11}NO_3S$, Ali) is a sulfur containing amino acid present in garlic and onion bulbs. The medicinal properties of *Allium* species have been studied for centuries. However the mode of action of the garlic components is still uncertain. It has been found that *Allium* compounds exhibit immune system improvement, and antibacterial and antifungal actions [13,14]. The major components of garlic oil were isolated in 1944 [15,16]. The most abundant compounds were diallyl disulfide and allicin. Alliin and the product of its enzymatic decomposition (allicin) were isolated in 1948 [13]. Extracts containing allicin displayed the most prominent antimicrobial properties. Other cysteine sulfoxides and their corresponding thiosulfinates were isolated latter. Nevertheless, the antibacterial and antifungal properties are still attributed to allicin. More recently, garlic preparations were evaluated against human tumor cells with promising results [17]. Alliin was found to be inactive against bacteria and tumor cells, but it acts as allicin generator *in situ* [13].

The first platinum(II) complex with alliin was initially synthesized in our laboratories. Preliminary cytotoxic studies indicate moderated activity of the complex toward HeLa cells [18]. Here, we report the synthesis, spectroscopic characterization, DFT studies and antibacterial and antitumor activities of a new water soluble Pd(II) complex with L-alliin.

2. Experimental

2.1. Materials and methods

L-Deoxyalliin and lithium tetrachloropalladate(II) hydrate of analytical grade were purchased from LKT and Sigma–Aldrich laboratories, respectively. Hydrogen peroxide was obtained from Synth and potassium hydroxide was purchased from Sigma. The methanol used in the synthesis was previously treated and kept over 100 g L^{-1} 5 Å molecular sieves. Elemental analyses for carbon, hydrogen and nitrogen were performed using a Perkin-Elmer 2400 CHNS-O analyser. Electrospray mass spectrometric (ESI-MS) measurements were carried out using a Waters Quattro Micro API. Samples were evaluated in the positive mode in an 1:1 acetonitrile:water solution with addition of 0.10% (v/v) formic acid. The infrared (IR) spectra were measured using a Bomem MB-Series Model B100 FT-IR spectrophotometer in the range 4000–400 cm^{-1} with resolution of 4 cm^{-1}. Samples were prepared as KBr pellets. The ^1H, ^{13}C and [^1H–^{15}N] NMR spectra were recorded on a Bruker 400 MHz Avance II (9.395 T). The ^1H NMR spectra were acquired at 400 MHz while the ^{13}C were acquired at 100 MHz. Two dimensional [^1H–^{15}N] NMR data were acquired at 40.55 MHz for ^{15}N and 400 MHz for ^1H. Samples were prepared in deuterium oxide (D_2O).

2.2. Synthesis of L-alliin

L-alliin was obtained from the amino acid L-deoxyalliin by an oxidative process with hydrogen peroxide in a procedure similar to that described in the literature [18,19]. Anal. Calcd for $C_6H_{11}NO_3S \times 0.5\ H_2O$ (%): C 38.7 H 6.51 N 7.52. Found (%): C 38.1 H 6.29 N 7.49.

2.3. Synthesis of the Pd-alliin complex

The palladium(II) complex with alliin (Pd-Ali) was synthesized by the reaction of 0.50×10^{-3} mol of lithium tetrachloropalladate(II) hydrate, $Li_2[PdCl_4]\cdot xH_2O$, in methanolic solution (4.0 mL) with the freshly prepared potassium salt of alliin containing 1.0×10^{-3} mol of the ligand, also in methanol (10 mL). The synthesis of the complex was carried out with stirring. A pale yellowish solid of the complex was slowly precipitated. After 2 h of constant stirring, the precipitate was filtered, washed with cold water and dried in a desiccator over P_4O_{10}. Anal. Calcd for Pd $(C_6H_{10}NO_3S)_2\cdot 2H_2O$ (%): C 29.1 N 5.66 H 4.90. Found (%): C 28.4 N 5.62 H 4.54. The complex is soluble in water and dimethylsulfoxide (DMSO).

2.4. Molecular modeling

Geometric optimizations were carried out using GAMESS software [20] with a convergence criterion of 10^{-4} a.u. in a conjugated gradient algorithm. The LANL2DZ effective core potential was used for the palladium atom and the atomic 6-31G(d) basis set [21–25] for all other atoms. Density functional theory (DFT) calculations were carried out using B3LYP [26,27] gradient-corrected hybrid to solve the Kohn–Sham equations with a 10^{-5} a.u. convergence criterion for the density change. The bidentated coordination through carboxylate and amino groups was confirmed as minimum of the potential energy surface (PES) with calculations of the Hessians showing no imaginary frequencies.

2.5. In vitro assays with tumor cells

HeLa cells (ATCC CCL-2) were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% of fetal calf

serum (FCS), using streptomycin and penicillin as antibiotics, in an atmosphere of 5% CO_2 at 37 °C. All cell culture reagents were purchased from Costar (Corning Inc., NY). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium salt (MTT) was acquired from Sigma. Cells were placed in a 48-well plate (4×10^4 cells/well) 24 h prior to the beginning of the experiment. A stock solution of the Pd-Ali complex was prepared in phosphate buffer saline (PBS), which was diluted into the cells medium in order to achieve different concentrations. Forty-eight hours after addition of the complex, MTT salt was added (aiming for a final concentration of 0.50 mg mL^{-1}) and the cells were incubated with this reagent for a period of 3 h [28]. After the incubation period, cells were washed with PBS and isopropanol was added. The cell viability was determined by absorbance measurements at 570 nm.

2.6. Antibacterial assays

An antibiogram assay was carried out in order to evaluate the antibacterial activities of the Pd-Ali complex. Six pathogenic bacterial strains, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* 31NM, *S. aureus* ATCC 25923, *S. aureus* BEC9393 and *S. aureus* Rib1, were selected. Bacterial susceptibilities were performed by the well diffusion method in accordance with Bauer et al. and confirmed by determination of minimal inhibitory concentration (MIC) as recomended by the Clinical and Laboratory Standards Institute (CLSI) [29,30]. Commercial antibiotic discs of ofloxacin and imipenen were used as positive controls. The MIC values were determined by the following methodology. Stock solutions (10.0 mg mL^{-1}) of Pd-Ali in water were prepared before the experiments. Samples were submitted to serial dilutions (1:1) in a 96 well multiplate with 100.0 μL of each Pd-Ali dilution and then transferred to the plates containing the respective bacterial strain in seven decreasing concentrations. The plates were submitted to 37 °C for 24 h.

3. Results and discussion

3.1. Mass spectrometric data

The most significant *m/z* peaks in electrospray mass spectrum of alliin and Pd-Ali complex are described in Table 1.

The mass spectrum of alliin shows the presence of the molecular ion at m/z 178. The dimeric species was observed at m/z 355. The spectrum of the Pd-Ali complex shows one peak with m/z 459 corresponding to the $[Pd\ (C_6H_{10}NO_3S)_2+H]^+$ molecular ion. Other fragment species are also observed. The mass spectrometric data confirmed the palladium/alliin composition of 1:2.

3.2. NMR spectroscopic measurements

Solution state ^{13}C and [^1H–^{15}N] NMR spectra of the Pd-Ali complex were analyzed by comparison with the NMR spectra of pure Ali. The structure of alliin with carbon and hydrogen atoms numbering is presented in Fig. 1.

Table 1
Electrospray mass signals *m/z* and relative abundances of Ali and Pd-Ali (ESI+).

Compounds	Ion assignment	*m/z*	Rel. Abundance (%)
Ali	$[C_6H_{11}NO_3S + H]^+$	178	100
	$[(C_6H_{11}NO_3S)_2 + H]^+$	355	30
	$[C_6H_{11}NO_2S + H]^+$	161	25
Pd-Ali	$[Pd(C_6H_{11}NO_3S)_2 + H]^+$	459	75
	$[Pd(C_6H_{10}NO_3S) + H]^+$	282	65
	$[Pd(C_4H_7NO_3S) + H]^+$	238	28

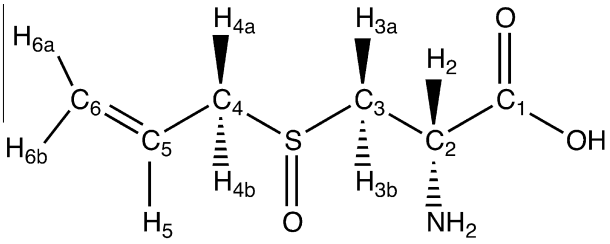

Fig. 1. Structure of alliin with carbon and hydrogen atoms numbered.

The ^{13}C NMR spectrum of the alliin and Pd-Ali are shown in Fig. 2. The ^{13}C NMR spectrum of alliin and Pd-Ali consists of two defined sets of resonances for the carbons C_2, C_3, C_4 and C_5. The occurrence of two sets of resonance frequencies is due to the presence of an asymmetric center on the sulfur atom, which was not observed in the case of L-deoxyalliin [18]. A comparison between the NMR spectra of the ligand and the complex permit us to confirm the coordination of the ligand to the metal through the N atom of amino group and the O atom of carboxylate group. In the free ligand the chemical shift at 171.4 ppm is assigned to the carbon of COOH group (C_1 in Fig. 1). In the spectrum of the complex this signal is shifted downfield by 11 ppm, being observed at 182.4 ppm. This shift shows the coordination through the oxygen atom of the carboxylate group [31,32]. A significative change is also observed for the chemical shift assigned to C_2 when the spectra of the complex and the ligand are compared. These signals are observed at 50.2 ppm and 54.0 ppm in ligand and the complex spectra, respectively, indicating coordination of the ligand to the metal through the nitrogen atom. The spectra also show a shift of 2.9 ppm for carbon C_3. No change is observed in the C_4 and C_5 chemical shifts, which demonstrate that alliin is not coordinate to the metal through the sulfur or oxygen atoms of the sulfoxide group. The ^{13}C chemical shifts for Ali and Pd-Ali are given in Table 2.

The coordination through the nitrogen atom of the NH_2 was evaluated by ^{15}N NMR. The ^{15}N chemical shifts for Ali and Pd-Ali were indirectly obtained from the 2D spectra *via* the heteronuclear

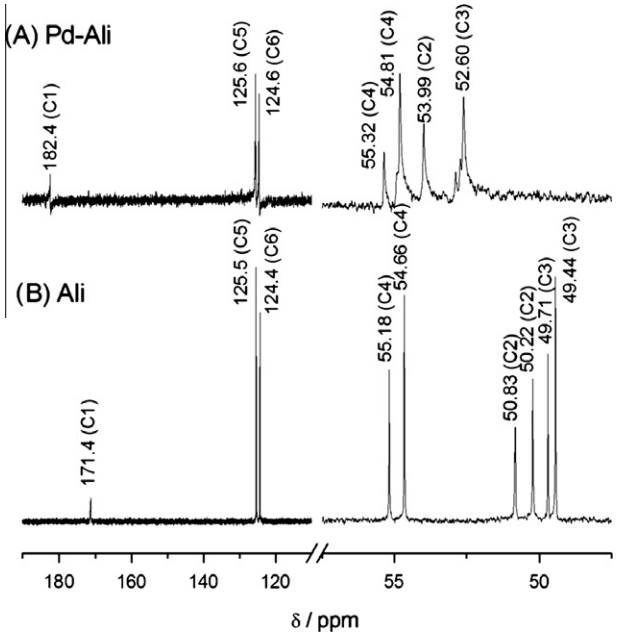

Fig. 2. ^{13}C NMR of Ali and Pd-Ali. Assignment of the carbons are presented according to Fig. 1.

Table 2
^{13}C chemical shifts for Ali and Pd-Ali.

Compounds	Chemical shifts (ppm)					
	C_1	C_2	C_3	C_4	C_5	C_6
Ali	171.4	50.22	49.71	54.66	125.5	124.4
Pd-Ali	182.4	53.99	52.60	54.81	125.6	124.6

[^{1}H–^{15}N] multiple bond coherence technique (HMBC), as decribed for other metal complexes with N donor ligands [31–33]. The ^{15}N spectra are provided in Fig. 3. The nitrogen signal was observed by its correlation with hydrogens H3a and H3b in Fig. 1. The chemical shift for nitrogen in the ligand was observed at 40 ppm and for the complex at −20 ppm. The $\Delta\delta$ of 60 ppm reinforces the coordination of alliin to palladium through the nitrogen atom of NH_2 group.

3.3. Infrared absorption spectroscopy

The infrared spectrum of the Pd-Ali complex was analyzed in comparison to the spectrum of the free ligand (Ali). Both spectra are presented in Fig. 4.

According to the literature, the presence of two resolved bands in the region of 3000–3400 cm^{-1} is an indication of coordination of the amino group to metal ions in amino acids [34,35]. The IR spectrum of alliin has a broad band in the range 3100–2700 cm^{-1}, which is assigned to NH_2 and CH_2 stretching modes. Enlargement of this band is due to the presence of hydrogen bonding. In the spectrum of the complex two bands can be identified at 3215 and 3103 cm^{-1} corresponding to NH_2 asymmetric and symmetric stretching modes, respectively. In addition two bands at 1516 cm^{-1} and 1538 cm^{-1}, assigned to NH_2 deformation, are present in the spectrum of the free ligand. The absence of these bands in the IR spectrum of the complex is another valuable proof of coordination of the ligand to Pd(II) through the NH_2 group. The IR spectrum of the Pd-Ali complex shows the asymmetric and symmetric stretching modes of carboxylate group at 1651 cm^{-1} and at 1379 cm^{-1}. In the free ligand spectrum, these bands are observed at 1650 cm^{-1} and at 1394 cm^{-1}. Since the difference between the asymmetric and symmetric stretching modes, Δ, is larger in the spectrum of the complex when compared to that of the ligand, a monodentate coordination of the carboxylate group through the oxygen atom is proposed. In this case, $\Delta(COO^-)$ for the complex is 272 cm^{-1}, while for the ligand, this value is 257 cm^{-1}.

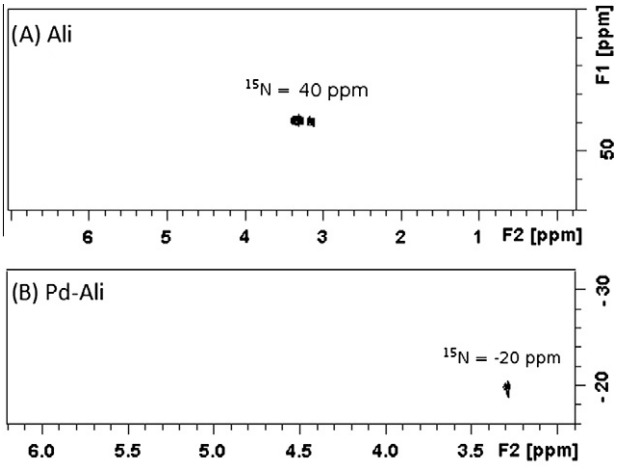

Fig. 3. [^{1}H–^{15}N] HMBC spectra of Ali and Pd-Ali.

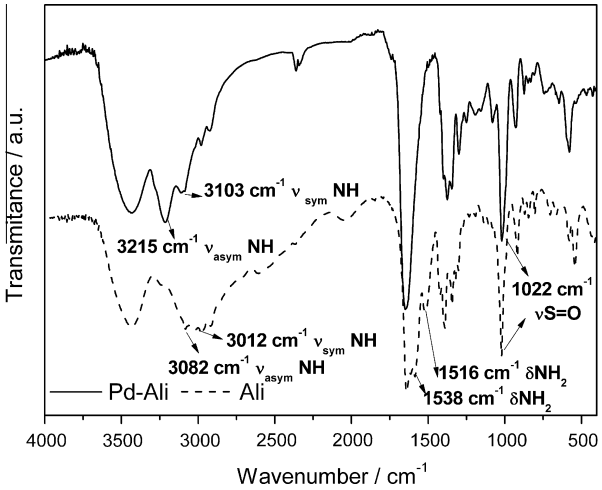

Fig. 4. Infrared spectra of Ali and Pd-Ali.

A strong and sharp absorption band at 1022 cm^{-1} is present in both spectra at the same frequency and intensity, being assigned to the S=O streching mode. The observed results reinforce the non-coordination of the sulfoxide group to Pd(II) as proposed when considering the NMR data.

3.4. Molecular modeling

The proposition of a bidentate coordination of two alliin molecules to Pd(II), through nitrogen and oxygen atoms, as evidenced by the spectroscopic evaluations, was calculated using DFT for the *cis* and *trans* isomers. Equilibrium geometries were confirmed by vibrational analysis showing no imaginary frequencies. The energies were corrected by adding zero point energies (ZPE) and they were compared for the isomers. The results demonstrated that the most stable form is the *trans* isomer, by 6.6 kcal mol^{-1}. The calculated distances and angles in the coordination sphere are described in Table 3. The optimized structure is in good agreement with the crystallographic data found in the literature for other ligands N,O- coordinated to palladium(II) [36–39]. The optimized structure can be found in Fig. 5.

The simulated IR spectrum of the free ligand is also in agreement with the experimental data. The bands related to the amino group can be observed at 3459 cm^{-1} and 3359 cm^{-1} for assymmetric and symmetric stretching modes, respectively. These bands are shown in a very low intensity, which can explain the very broad band observed in the experimental spectrum assigned to the NH_2 and CH_2 stretching modes. The NH_2 deformation mode is present at 1599 cm^{-1} and the C=O and S=O stretching modes can be seen at 1599 cm^{-1} and 1016 cm^{-1}, respectively, confirming the assignment in the experimental spectrum. The simulated spectrum of the complex shows a weak absorption at 3359 cm^{-1} and a strong absorption at 3159 cm^{-1}. The changes, mainly in the intensity of the modes can explain the appearance of well resolved bands in the experimental data, confirming the coordination of this group to the metal ion. The calculated spectrum of the complex shows that the low intensity NH_2 deformation mode has a shift to higher

Table 3
Selected distances (Å) and angles (°) for *trans*-palladium(II) complex with L-alliin as calculated by B3LYP/DFT.

	Distances (Å)		Angles (°)
Pd–O	2.025	O-Pd–N	81.76
Pd–N	2.089	O-Pd–O′	177.98
Pd–O′	2.024	O′–Pd–N′	81.59
Pd–N′	2.089	N–Pd–N′	178.38

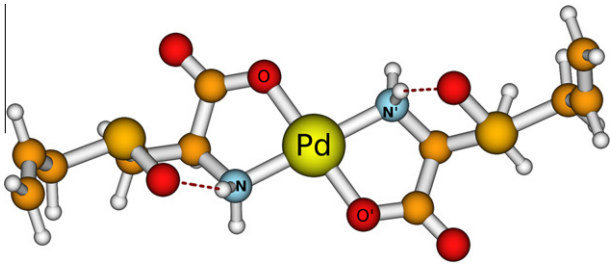

Fig. 5. Optimized structure of *trans*-Pd-Ali complex obtained by B3LYP/DFT using LANL2DZ (Pd) 6-31G (d) basis set. Pd (yellow), N (blue), O (red), C (orange) and H (white). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

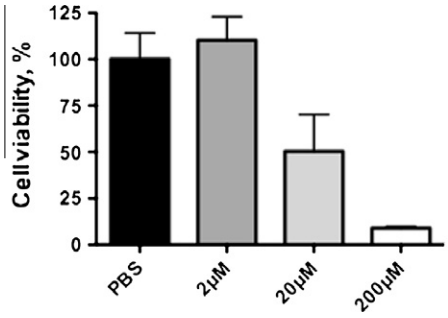

Fig. 6. Cytotoxic analyses of Pd-Ali in different concentrations (μmol L^{-1}) against HeLa cells. Data from three different experiments were normalized to the maximum values obtained for the control group and are expressed as mean ± s.e.m.

Table 4
Antibiotic sensitive profile of the Pd-Ali complex against the considered bacterial strains.

S. aureus BEC 9393	*S. aureus* Rib1	*S. aureus* ATCC25923	*P. aeruginosa* ATCC27853	*P. aeruginosa* 31NM	*E. coli* ATCC25922
Minimal inhibitory concentration (μg mL^{-1})					
500.0	250.0	500.0	500.0	250.0	125.0

energies when compared to the calculated free ligand spectrum, being observed at 1641 cm^{-1}. In addition, a strong C=O stretching mode is observed at 1712 cm^{-1}. These two absorption can be superposed in the experimental data and explain why the deformation modes are not well defined in the experimental spectrum of the complex.

3.5. In vitro assays with tumor cells

The free Ali and the palladium complex (Pd-Ali) were assayed for their cytotoxic activities *in vitro* using concentrations varying from 2.0 μmol L^{-1} to 200 μmol L^{-1}. The results show that the L-alliin has no cytotoxic effect even at the highest concentration tested (data not shown). The Pd-Ali complex has a moderate activity with an IC_{50} of ~20 μmol L^{-1} (Fig. 6), which is very close to the cisplatin IC_{50} value of 15.14 μmol L^{-1} over the same tumorigenic cell line [40]. An increase in concentration of the complex significantly increases the activity. The observed results are comparable to a series of reported Pd(II) complexes with different N-, O-, S- donor ligands [6,41–43].

3.6. Antibacterial assays

The activities of the Pd-Ali complex against the considered bacterial strains were confirmed by MIC values between 125 μg mL^{-1} and 500 μg mL^{-1}. The ligand itself did not exhibit any antibacterial activity under the same experimental conditions. Antibiotic sensitive profiles of bacterial strains are listed in Table 4. The observed results are comparable to a the Pd(II) complex with deoxyalliin [10]. As observed in that case, the activity of the Pd-Ali complex is most probably due to the presence of the Pd(II) ions since the ligand itself is inactive for the same bacterial strains in the considered conditions.

4. Conclusions

A new water soluble palladium(II) complex with L-alliin with a 1:2 M composition (metal:ligand) was obtained and structurally characterized. The ^{1}H, ^{13}C and ^{15}N NMR spectroscopies and, IR and mass spectrometric measurements support coordination of the ligand to Pd(II) *via* the nitrogen atom of the amino group and an oxygen atom of the carboxylate group, forming a five membered chelated ring. DFT studies suggest that the *trans* geometry is strongly preferred and probably the only configuration present in the complex. The calculated infrared frequencies compared with experimental data also support this structure. A schematic structure for the Pd-Ali complex is shown in Fig. 5.

The *in vitro* cytotoxic assays revealed the antitumor activity of the complex over HeLa cells while no activity was detected for L-alliin. Antimicrobial studies revealed antibacterial activity of the complex against Gram-positive and Gram-negative microorganisms in the range 125–500 μg L^{-1}.

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