DEVELOPMENT OF UNPRECEDENTED PD(II) AND PT(II) COMPLEXES DERIVED FROM IMIDAZOLE

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Abstract. During this first period, the objective was to create three complexes, all containing palladium as a metallic nucleus and thus varying a radical between Chlorine, Iodine and thiocyanate, associating the known biological activity of Pyridines with palladium and platinum complexes, which also already has recognized biological activity. To date, such compounds, including the final complexes and their ligands, were synthesized and underwent the following characterization procedures: Hydrogen Nuclear Magnetic Resonance, infrared absorption spectroscopy and elemental analysis. So far, the characterization of the complex with the chlorine group has been carried out by means of infrared absorption spectroscopy, Hydrogen Nuclear Magnetic Resonance and elemental analysis, all of which favorably indicate the complexation, by the proportions of carbon, hydrogen and nitrogen composition – in in the case of elemental analysis –, by the infrared bands and finally by the NMR spectrum, which indicated the amount of expected hydrogens. In the case of the iodine ligand, the NMR spectrum and the infrared spectrum were performed, both also satisfactorily indicating the complexations, through the appearance of bands that indicate the complexation of the metallic nucleus with the Iodine radical and the NMR hydrogen count, also within expectations. Finally, the thiocyanate complex was only characterized by the IR spectrum, as it was not possible in a timely manner to carry out further characterizations, but this spectrum was faithful to what was expected by the complexation with the metallic nucleus, due to the disappearance of certain bands and the emergence of some bands that characterize the complexation of these radicals to the metallic nucleus. Biological activity has not yet been evaluated.

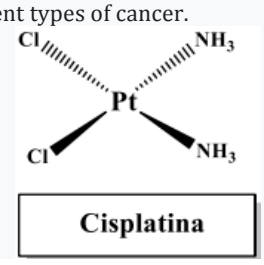
Keywords. Anticancer activity, Imidazole, Palladium(II) and Platinum(II).

1. Introduction

Within the context of bioinorganic chemistry, the development and application of inorganic ions and their respective complexes are widely used for the treatment of some pathologies. A practical example was discovery of cisplatin in 1965, whose main biological activity was antitumor, through its cytotoxicity, whose main action is the inhibition of replication, transcription and even inducing program cell death, that is, the apoptosis.

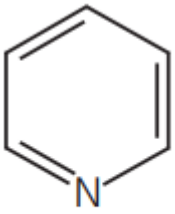
The mechanism of action consists of the entry of the cisplatin molecule into the cell, which loses its chlorine ligand, being replaced by water; such action creates a group that is reactive with nucleophilic sites. The presence of this group within of cell space has as main objective the induced arrest of the cell cycle and the induction of cellular apoptosis by connecting with strands of DNA. In this context, two major problems arise with the use of Cisplatin (**Figure 1**): the acquired resistance of tumor cells and the effects side effects caused by the continuous use of this metallopharmaceutical. One of the causes of resistance derives from the activation of intracellular protein sites, such as the synthesis metabolism of glutathione, in addition to a DNA repair with the use of cisplatin, guarantee tumor resistance to this drug.

Fig. 1 - Chemical structure of the metallopharmaceuticals cisplatin, [PtCl2(NH3)2], used to treat different types of cancer.



It is also worth noting that numerous palladium complexes have been synthesized and recorded biological activity, mainly antitumor activity, a example is 8-quinolyl methylphosphonate, in which there is the presence of a pyridine group and a palladium metallic core, in addition to being characterized as a heterocycle aromatic nitrogen, known as pyridine (**Figure 2**), which contains potential chemotherapy.

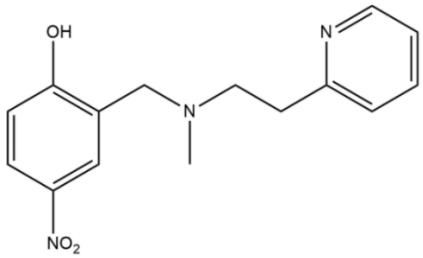
Fig. 2 - Structural formula of pyridine, structure present in the ligand.



Piridina

Thus, this work seeks to unite the coordination properties of palladium and platinum, together with the biological potential of nitrogenous aromatic heterocyclics, seeking to develop more selective, more efficient drugs with new applications. The ligand used in this work is shown in **Figure 3**:

Fig. 3 - Chemical structure of the chelating agent 2-(methyl(2-(pyridin-2-yl)ethyl)amino)-4-nitrophenol used in the work



2. Research Methods

2.1 Materials

In this study, the solvents were used without previous treatment, being all obtained commercially (Aldrich). The ligand was synthesized using anhydrous potassium carbonate and anhydrous acetonitrile, and then 2-chloromethyl-4-nitrophenol and 2-(2-methylaminoethyl)pyridine, again obtained commercially, were added.

2.2 Instruments – Vibrational spectroscopy in the infrared region

The spectra were acquired through a spectrophotometer of the type Perkin Elmer model FT-IR Frontier Single Range – MIR, in the region of 4000 to 220 cm-1. The measurements were performed with the aid of the accessory (ATR) with crystaldiamond, with samples in the solid state.

2.3 Instruments – Nuclear magnetic resonance spectroscopy

The ^{1}H nuclear magnetic resonance spectra were obtained in a Bruker spectrometer model Ascend 400 (400 MHz) from the Multiuser Laboratory from the Institute of Chemistry of the Federal University of Uberlândia (IQ-UFU). At samples were dissolved in deuterated solvents (Aldrich) and the peaks residual solvents were used as internal standards.

2.4 Instruments – Elementary Analysis

The carbon, hydrogen and nitrogen contents were analyzed in the Multiuser Laboratory of the Instituto de Química – UFU using the Perkin-Elmer equipment, model 2400 (series II) CHNS/O Elemental Analyzer.

3. Preparation of the complexes

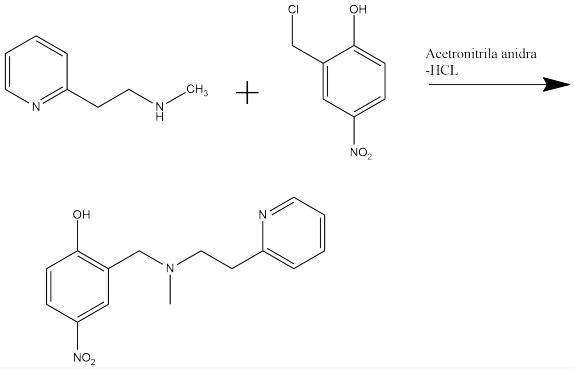
3.1 Preparation of the chelating agent

In this work, the ligand 2-(methyl(2-(pyridin-2-yl)ethyl)amino)-4-nitrophenol was prepared by addition in a round bottom flask containing; 1.35 g of anhydrous potassium carbonate and 15.0 ml of anhydrous acetonitrile were added. The flask was closed with a rubber septum, and the suspension was stirred in an inert atmosphere and ice bath for 5 minutes. Then, 0.90 ml of 2-(2 methylaminoethyl) pyridine was added to the suspension.

The flask was sealed with a rubber septum, and the suspension was kept under an inert atmosphere and an ice bath. By means of a syringe, a solution of 1.30 g of 2-chloromethyl-4-nitrophenol, previously dried under reduced pressure, in 10.0 ml of anhydrous acetonitrile, was added dropwise over a period of 30 minutes to the suspension. The suspension was stirred in an inert atmosphere at room temperature for 48 hours. The suspension was filtered, and the retained solid was washed with acetonitrile. The solvent from the filtrate was removed under reduced pressure, and the oil obtained was redissolved in 30.0 ml of ethyl acetate.

The solution was extracted twice with aqueous NaCl solution, and the organic phase was separated. The aqueous phase was washed once with 25.0 ml of ethyl acetate, and the organic phase was separated. The organic phases were mixed, and the resulting solution was dried over anhydrous sodium sulfate, and the solvent removed under reduced pressure. To the obtained oil, a 25.0 ml portion of dichloromethane was added and, after complete solubilization of the oil, the solvent was removed under reduced pressure. This step was performed three times. The final product is obtained as a very viscous oil with a reddish-orange color **(Scheme 1)**:

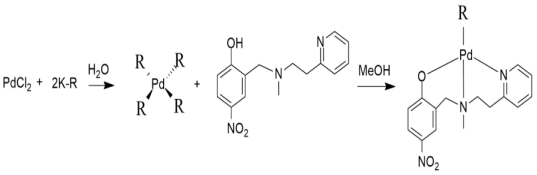
Scheme 1 - Synthetic route for ligand synthesis. 2-(methyl(2-(pyridin-2-yl)ethyl)amino)-4-nitrophenol.



3.2 Standard synthesis of complexes

For the preparation of palladium complexes, the reaction was initially carried out to synthesize a palladium complex containing 4 ligand groups of interest (Cl, I, SCN), the whole process being carried out in distilled water. The precursor was generated in situ. After 10 minutes of reaction, the ligand 2-(methyl(2-(pyridin-2-yl)ethyl)amino)-4 nitrophenol dissolved in 3 ml of methanol was added to the metallic precursor. All reactions were refluxed at 65°C for three hours (**Scheme 2**):

Scheme 2 - Standard synthesis for complexes of the [PdR(L1)] type where R = Cl-, I- and SCN-.

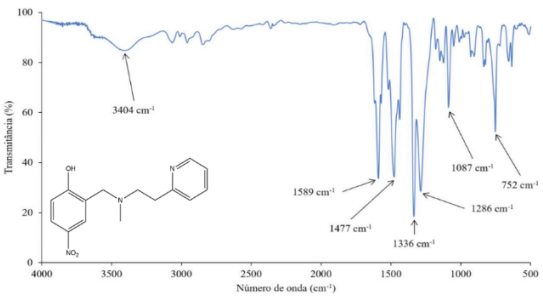


4 Result and Discussion

4.1 Absorption spectrum in the infrared region of the ligand(L1)

Through the spectra, it was possible to verify that the synthesis of the ligand was effective, as it is possible to identify the absorption bands characteristic of the functional groups present in the structure, as shown in Figure 4:

Fig. 4 - Absorption spectrum in the infrared region in cm-1 for the L1 ligand.



As analyzed, in the IR spectrum it is possible to observe several bands that are characteristic of groups present in the ligand, mainly with the stretching bands ν(O-H), ν(C=N), two bands of ν(N-O) characteristic of the nitro group. **Table 1** below presents some stretches that characterize the manufacture of the ligand:

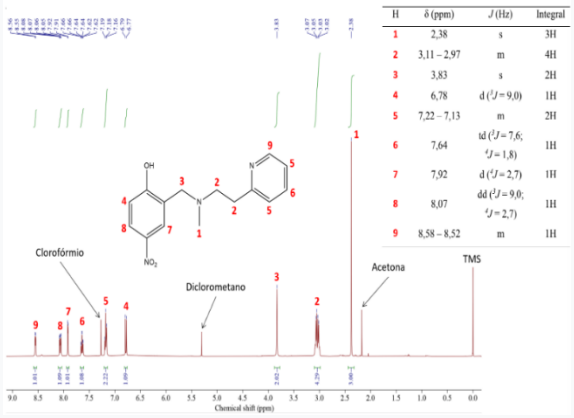
Tab. 1 - Main bands presented by the HL1 ligand.

Banda (cm^{-1})	Atribuição
3404	ν(O–H)
1589	ν(C=N) + ν(C=C)
1477	ν_{as}(N–O)
1336	ν_{s}(N–O)
1286	ν(C–N)
1087	ν(C–O)
752	δ(N–O)

4.2 Hydrogen Nuclear Magnetic Resonance of the ligand

The ligand was also characterized in solution by hydrogen nuclear magnetic resonance. From the hydrogen count, which is equivalent to the number of hydrogens present in the structure, it is possible to state that the compound was successfully obtained. The chemical shifts are consistent as shown in Figure 5:

Fig. 5 - 1H NMR spectrum of the ligand 2-(methyl(2-(pyridin-2-yl)ethyl)amino)-4-nitrophenol in DMSO-d6 solution (δ = ppm).



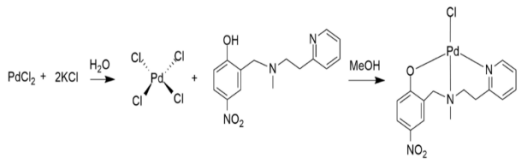
4.3 Complex [PdCl(L1)]

The reaction to obtain [PdCl(L1)] was carried out starting from 0.564mmol of $PdCl^2$ and 1.13mmol of KCl in 3 ml of water. The reaction was refluxed for ten minutes. After this time, 0.564mmol of ligand dissolved in 3 ml of methanol was added to the round bottom flask. Subsequently, another 3 ml of methanol were added at reflux at 65°C.

After the addition of the binder, precipitate formation and color change from yellow to orange was observed, and the solution became cloudy. After the compound went through recrystallization and

drying in the oven, 182 mg of final compound were obtained, with 241.9 mg expected, thus a reaction with 75% yield. This compound has a slightly greenish color and is soluble in the following solvents: DMSO, acetonitrile, methanol, dichloromethane and ethanol. Below, in **Scheme 3**, The synthesis of this complex is shown below.

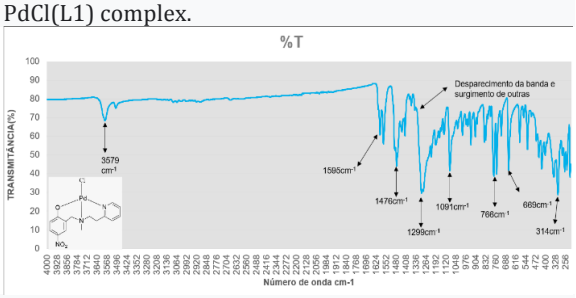
Scheme 3 - Synthetic route of the PdCL(L1) complex.



4.4 Analysis by infrared absorption spectroscopy

Figure 6 demonstrates:

Fig. 6 - Infrared absorption spectrum in cm-1 for the PdCl(L1) complex.

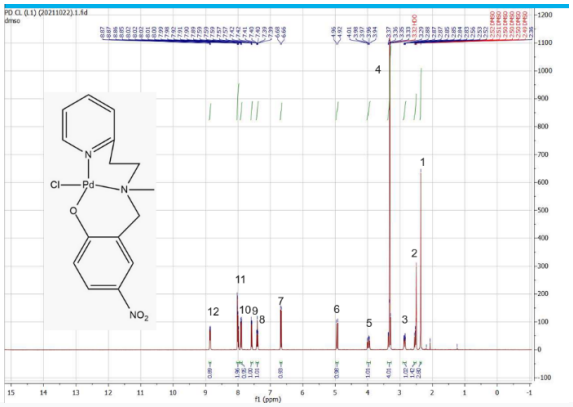


In this spectrum shown in Figure 8, it is possible to observe some important characteristics about this compound, when compared to the ligand. It can be seen that the bands of the nitro group remain in a very similar range, but with a displacement at 1595cm^{-1}, such as a decrease in the ν(O-H) band and a large displacement of it, probably indicating that the compound was still wet. A new band around 314cm-1 is observed, characteristics of the link between palladium and chlorine that suggest the complexation of palladium to the ligand and, finally, the appearance of a band at 669cm^{-1} characteristic of a link between palladium and nitrogen.

4.5 Hydrogen Magnetic Resonance Analysis

Shows **Figure 6**:

Fig. 6 - ^{1}H NMR spectrum of the PdCl(L1) complex in DMSO-d^{6}(δ = ppm) solution.



In this spectrum presented in **Figure 6**, it is possible to count the 16 hydrogens contained in the expected formula of the complex, in addition to a change in ppm in the hydrogens, indicating a complexation with palladium, mainly in carbons 1, 2 and 3, indicating a approximation due to this complexation. **Table 2** below presents the values of chemical shifts, integral and coupling constant obtained by the spectrum:

Tab. 2 - 1H NMR data of the PdCl(L1) complex.

H	δ(ppm)	*J*(Hz)	Integral
12	8.88-8.83	A(d)	1H
11	8,01	m	2H
10	7,9	dd	1H
9	7,58	d	1H
8	7,41	m	1H
7	6,67	d	1H
6	4,94	d	1H
5	3,97	dd	1H
4	3,36	d	1H
3	2,85	m	1H
2	2,54	d	1H
1	2,36	s	3H

4.6 Elementary Analysis

The expected mass percentage calculations for this compound would be: Carbon = 42.08%; Hydrogen = 3.77%; and Nitrogen = 9.81% as shown in **Table 3** Finally, it is possible to compare what was obtained experimentally with what was expected. It is noticed that there is a low variation – error below 3% –, making the analysis of the analyzed complex satisfactory.

Tab. 3 - Elemental analysis results obtained for the complex [PdCl(L1)].

Sample (Id)	***mass (mg)***	***%C***	***% H***	***%N***

ACETANILIDE (STANDARD)	1,725	71,16	6,91	10,35
PdCl(L1)	2,143	41,74	3,86	9,69

4.7 Complex [PdI(L1)]

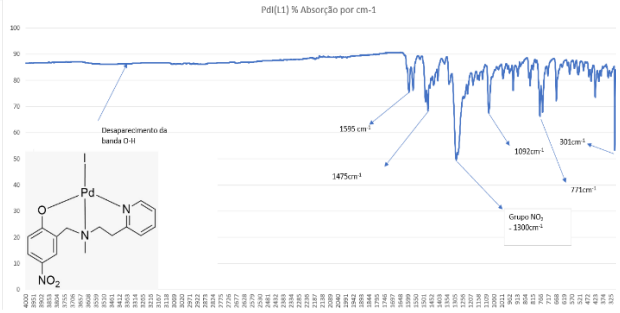
For the synthesis of [PdI(L1)], 0.564mmol of $PdCl^2$ was first added to 3 ml of distilled water; after that, 2.25mmol of KCl was added to this mixture; the reaction was refluxed for ten minutes. After this reaction, 0.564mmol of ligand, dissolved in 6 ml of methanol, was slowly added with a pipette to a flask containing the previously produced solution. This reaction was visible to the naked eye, turning the solution black. The reaction was refluxed at 65ºC for 3 hours and then filtered and washed with 2 ml of methanol, ethyl alcohol and hexane were also used, all in the amount of 2 ml. 295 mg were obtained, with 299 mg expected.

Such amount suggested a high yield, but suggested that there were impurities; therefore, the compound is subsequently filtered again, as described above, and the final amount obtained has not yet been obtained in a timely manner, since the recrystallization and filtration process is still being carried out. Below are the characterizations made for the [PdI(L1)] complex so far.

4.8 Analysis by infrared absorption spectroscopy

Figure 7 demonstrates:

Fig. 7 - Infrared absorption spectrum in cm-1 for the PdI(L1) complex.



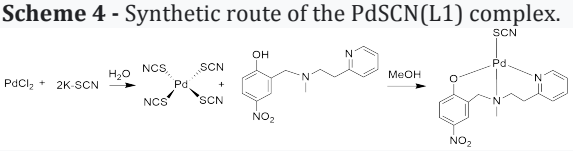
In this spectrum, shown in **Figure 7**, one can again observe some bands appearing and others disappearing, indicating the formation of the complex. The main band that disappeared was the O-H band, which is located around 3400cm-1, and a band around 301cm-1 appeared again.The other characterizations were not performed in a timely manner.

4.9 Complex[PdSCN(L1)]

Initially, 1.12mmol of potassium thiocyanate was dissolved in 2 ml of water, later being added 0.564mmol of PdCl2, the reaction took place at reflux for ten minutes. After that, 0.564mmol of ligand was dissolved in 5 ml of methanol, being taken to ultrasound to aid in dissolution. Subsequently, with the aid of a pipette, the dissolved ligand was slowly added to the reaction. There was visible formation of precipitate. 71 mg were obtained, whereas the expected figure was 262.12 mg. Subsequently, 22 ml of acetonitrile were added and the compound was filtered hot. Your solution looks red.

The synthetic route is shown in **Scheme 4**. The complex is soluble in acetonitrile, DMSO and in acetone. It showed poor solubility in methanol and dichloromethane. Below are the characterizations made for the [PdSCN(L1)] complex so far:

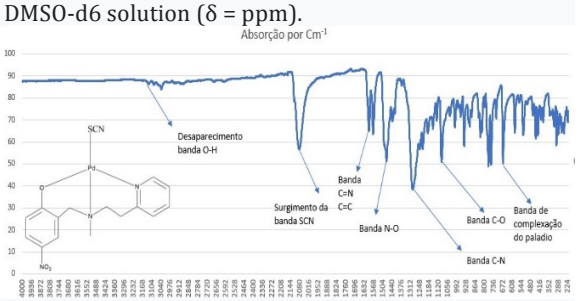
Scheme 4 - Synthetic route of the PdSCN(L1) complex.



4.10 Analysis by infrared absorption spectroscopy

Figure 8 demonstrates:

Fig. 8 - ^1H NMR spectrum of the PdSCN(L1) complex in DMSO-d6 solution (δ = ppm).



In the spectrum of [PdSCN(L1)], shown in **Figure 8**, it is observed the disappearance of the O-H band and the appearance of the SCN band, in addition to the remaining bands referring to the ligand. Furthermore, there is the appearance of bands around 600cm-1 to 300cm-1, thus suggesting complexation with sulfur and nitrogen.The other characterizations were not done in a timely manner.

5. Conclusion

The initially proposed project presented some challenges and, therefore, a different ligand was used in order to create complexes that were successfully synthesized and with good yields. The spectroscopic techniques performed to date indicate that the complexes were successfully synthesized.

For this, it is essential to observe the disappearance of certain bands and the appearance of others, as observed in the [PdI(L1)] and [PdSCN(L1)] complexes, where the ν(O-H) bands and the emergence of several bands around 600cm -1 to 300cm-1indicating complexation with palladium. It is also worth mentioning that in the complex [PdSCN(L1)] there is the appearance of the characteristic band of thiocyanate.

Still from the perspective of absorption spectroscopy in the infrared region, the complex [PdCl(L1)] presented the characteristic bands of complexation between palladium and chlorine, as it maintained all the binding bands of the ligand, showing a small displacement, indicating the complexation of the ligand with the metallic core. It is also valid to indicate that the Hydrogen Nuclear Magnetic Resonance showed varied chemical shifts, as well as accounted for the amount of expected hydrogens in the complex.

Finally, the elemental analysis estimated the mass percentages of carbon, hydrogen and nitrogen of the [PdCl(L1)] complex, being approximated with an error smaller than 3% of the expected.

6. Work Projection

The perspectives for this work include the completion of the spectroscopic characterizations, among them collecting the spectra of Magnetic Resonances of Hydrogen of the compounds with iodine and thiocyanate radicals, as well as performing their elemental analyses. We will also perform absorption spectroscopy in the visible ultraviolet region of all compounds and also perform Carbon Magnetic Resonance on all of them. If we obtain quality crystals, we will also perform single crystal X-ray diffraction. Finally, we will evaluate the biological activity of the compounds.

7. References

[1] Cheng H., et al. Synthesis, characterisation, activities, cell uptake and DNA binding of a trinuclear complex: [{trans-PtCl(NH3)}2#-{transPd(NH3)(2-hydroxypyridine)-(H2N(CH2)6NH2)2]Cl4. *Eur J Med Chem*. 2006; 41: 896-903.

[2] Kartalou, M., Essigmann, J. M. *Mechanisms of resistance to cisplatin. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 2001. doi: https://www.sciencedirect. com/science/article/abs/pii/S00 27510701001415. 26 feb. 2022.

[3] Knupp V. F. et al. *Síntese e caracterização estrutural de complexos mono e bimetálicos de paládio e platina com ligantes contendo nitrogênio e enxofre*. Departamento de Química – ICEx – Universidade Federal de Minas Gerais, Belo Horizonte-MG; 1997; 42 p.

[4] Silva L. E. da. *Heterociclos Aromáticos Nitrogenados: síntese e potencial Quimioterápico*. Tese (doutorado) – Universidade Federal de Santa Catarina, Centro de Ciências Físicas e Matemáticas. Programa de Pós-Graduação em Química; 2006; 186 p.

[5] Souza G. de et al. Silver: Brief history, properties and applications. *ScienceDirect;* 2013; 24 [1]: 14-16. doi: https://www.sciencedirect.com/science/article/ pii/S0187893X13731896.

[6] Thompson K. H., Orvig C. *Science 300*. Washington, DC, USA; 2003 May 9;300(5621):936-9. doi: 10.1126/science.1083004.

[7] Tušek-Božić L. et al. *Synthesis, characterization and antitumor activity of palladium(II) complexes of monoethyl 8-quinolylmethylphosphonate*. Zagreb, Croatia; 2008; 27[4]:1317-1328.