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Article

Synthesis, Characterization and Cytotoxic Behavior against Hela of Iridium (Iii) Complexes, Half Sandwich Type

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Abstract: In this paper, we describe the synthesis and characterization of iridium (III) half sandwich complexes of stoichiometry $[\text{Cp}^+\text{IrCl}_2\text{L}]$ with $\text{Cp}^+ = \eta^5\text{-C}_5\text{EtMe}_4$; $\text{L} =$ alanine anhydride (I), *ortho*- (II) and *para*-aminophenol (III). For comparative purposes, the ionic complex $[\text{Cp}^+\text{IrCl}(\text{o-phen})](\text{PF}_6)$; $\text{Cp}^+ = (\eta^5\text{-C}_5\text{Me}_5)$ (IV) has been also synthesized and characterized. All the isolated complexes have been characterised by C, H and N elemental analysis; infrared and ^1H nuclear magnetic resonance spectroscopies; ^1H - ^1H COSY; mass spectrometry (ESI/TOF), thermogravimetry and, in the case of (IV), by conductivity measurements. The cytotoxic behaviour of the isolated complexes was studied against HeLa and complexes (III) and (IV) were found to have an IC_{50} close to 160-170 μM , quite close to the IC_{50} value of cisplatin which was found to be 134,7 μM , indicating that they are good cytotoxic agents. However, complexes (I) and (II) showed IC_{50} values above 250 μM indicating their low cytotoxicity.

Keywords: Iridium (III) complexes; Half sandwich; Synthesis and characterization; Anticancer activity Hela; MTT assay.

1. Introduction

The success achieved by certain platinum(II) complexes such as cisplatin, carboplatin and oxalipatin [1,2] has stimulated research into new metallodrugs that can inhibit side effects such as nausea and kidney toxicity [3]. Thus, numerous organometallic compounds have been described that also behave as metallodrugs such as Ir, Ru, Os and Rh compounds [4–14]. Moreover, it is worth noting a wide range of half sandwich organometallic compounds that exhibit an interesting and promising antitumor character. Among them we can highlight the following organometallic compounds of Ru(II) (15, 16), Rh (II) [16], Ir(III) [17] and Os (II) [14].

Thus, in summary, it can be stated that the medicinal properties of platinum and ruthenium compounds are well established [18–21], while those of iridium(III) compounds constitute a relatively new field of research [22,23]. However, iridium compounds have not only shown potent anticancer reactivity [24–27], but their mechanism of action (MoA) and spectrum of tumour sensitivity are different from those of conventional platinum-based drugs [28–29], and thus may find potential use in the treatment of Pt-resistant cancers.

The above named metal ions, together with their surrounding ligands, play a vital role in the reactivity towards biological target sites [30–33]. The main factors for the selection of good results are the ligand structure, its electron-giving properties and steric hindrances. In this respect, nitrogen-donor ligands remain very important. In this regard, nitrogen donor ligands remain very important because they significantly affect the activity of the metal complexes against tumours [34,35].

The pyridine ring itself is an important part of natural biomolecules such as nicotamide, adenine, dinucleotide phosphate NADP, or vitamin B6 and plays an important role in many biological

processes. Pyridine rings are also used in many synthetic drug preparations. For example, pyridine monodentate in cyclopentamethyl iridium complexes, behaves as antitumor against cell lines such as: A2780, A549, and MCF-7 [31].

Strategies have been improved in the design of complexes containing the pyridine ligand. Imino pyridyl type N^N Schiff base [30] ligands can be easily synthesized.

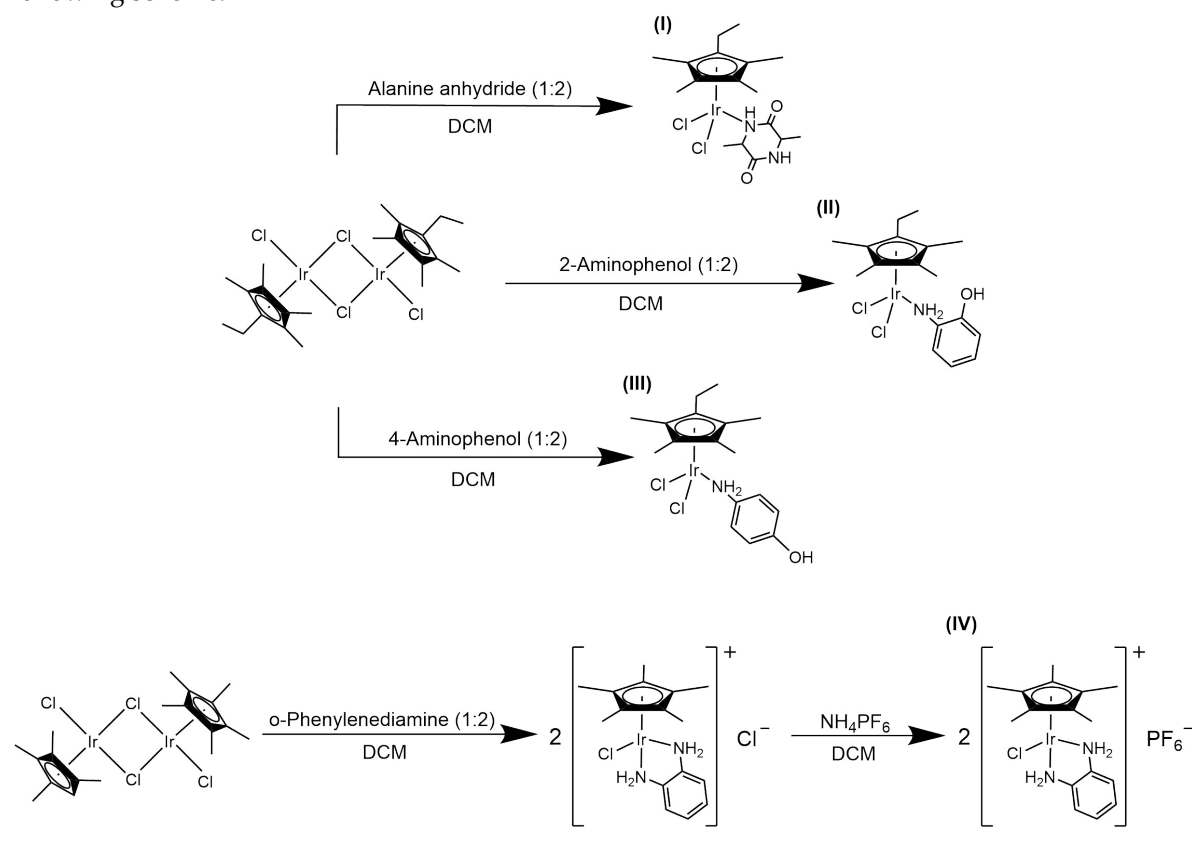
As a final comment, it is worth noting that the chemistry behind the synthesis, characterisation and cytotoxic behaviour of iridium(III) complexes with a "half sandwich" or "piano tool" structure is in constant development as can be seen in the references [36–43].

We have previously synthesised and characterised half sandwich complexes of Ru(II) and Ir(III) and studied their cytotoxic behaviour [44–47].

In this work we describe the synthesis and characterization of four organometallic iridium(III) compounds of stoichiometry $[\text{Cp}^*\text{IrCl}_2\text{L}]$, where L = alanine anhydride, and *ortho*- and *para*-aminophenol.

2. Results and discussion

The new half sandwich iridium(III) complexes described in this work are presented in the following scheme.



Scheme 1. Synthesis of complexes I-IV.

The complexes $[\text{Cp}^*\text{Ir}(\mu\text{-Cl})_2]_2$; Cp^* being C_5Me_5 or C_5MeEt_4 reacts in dichloromethane with alanine anhydride and *ortho*- or *para*-aminophenol, in the proportion described in the experimental part to form the compounds described in the scheme 1, and are precipitated with hexane.

And present an adequate correct elemental analysis of C, H and N. Their yields are as shown in Table 1.

Table 1. Colour, yield, elemental analysis (a: calculated values in parenthesis); exact mass and melting point (b: M.P. decomposition temperatures obtained from thermogravimetric curves) for complexes I-IV.

COMPLEX	COLOUR	YIELD (%)	ANALYTICAL DATA ^A			MASS DATA Fragments	M.P. ^B
			C	H	N		
I	Dark Orange	63	35.39 (35.54)	4.59 (4.70)	8.17 (8.36)		332
II	Dark Orange	45	38.90 (39.16)	4.41 (4.58)	2.53 (2.69)		380
III	Dark Orange	41	38.94 (39.16)	4.38 (4.58)	2.67 (2.69)		434
IV	Yellow	92	31.06 (31.20)	3.59 (3.73)	4.53 (4.54)	435.1399 [M-Cl] ⁺ 421.1159 [M-PF ₆] ⁺	377

As far as the melting (decomposition) points are concerned, as can be seen from the thermograms attached in the supplementary material, they are quite high for organometallic compounds. In this table, the result of the mass spectra (ESI/TOF) of compound (IV) in dimethylsulphoxide (DMSO) is presented. Compounds I, II and III undergo a different decomposition pattern as follows. The results obtained can also be found in the accompanying supplementary material.

As far as the mass spectra of the compounds (I, II and III) are concerned, it appears that, in a DMSO medium which is a good ligand, equilibrium occurs:

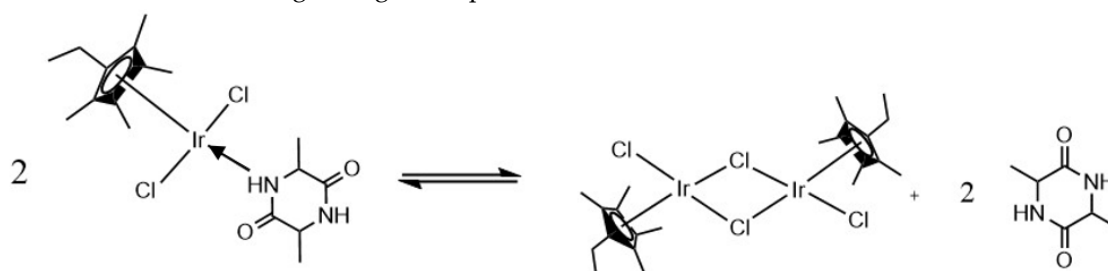


Figure 1. Reaction equilibrium between the starting complex and the final species.

On the other hand, as mentioned above, DMSO can act as a ligand that is coordinated by S (soft base), O (hard base) and even by the double bond, it is possible that 1, 2 or 3 DMSO molecules can enter the iridium(III) coordination sphere, in this case coordinated by oxygen as iridium(III) is a hard acid [48].

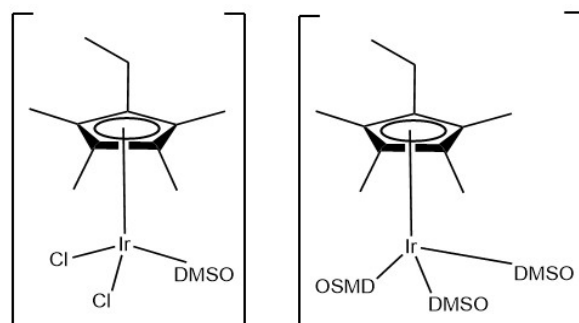


Figure 2. Possible species formed by equilibrium of the complex $[(\eta^5\text{-C}_5\text{EtMe}_4)\text{IrCl}_2]$ (alanine anhydride) with the solvent DMSO.

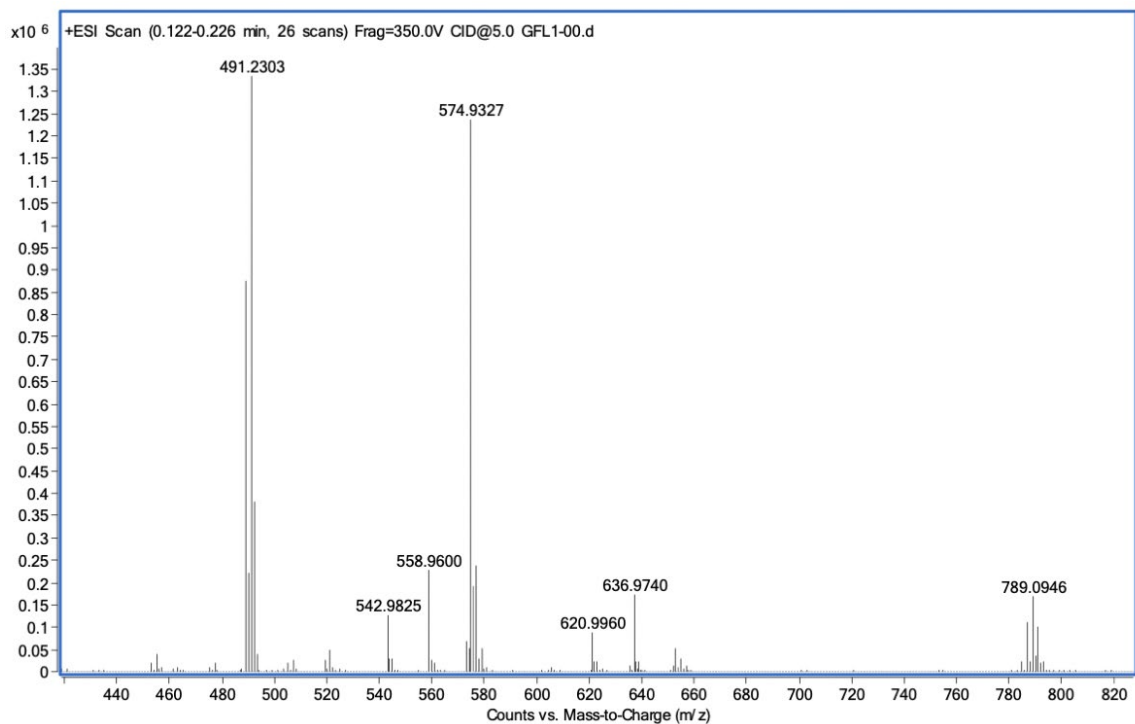


Figure 3. Mass spectrum of [(η⁵-C5EtMe4)IrCl₂ (alanine anhydride)].

Unfortunately, attempts to obtain crystals of the new compounds were unsuccessful. However, microcrystals were obtained, which guaranteed the purity of the species obtained for the rest of the tests.

The most significant data obtained from the infrared spectra are presented in Table 2

Table 2. Infrared data of compounds I-IV.

COMPLEX	V(N-H)	V(M-CL)	V(C=O)	V(O-H)	V(P-F)
I	3317 s, 3190 s	317 s, 278 s	1690 s	-	-
II	3365 s, 3196 s	314 s, 281 s	-	3601 s	-
III	3456 s, 3359 s	299 s, 278 s	-	3601 s	-
IV	3284 s, 3176 s	287 s	-	-	844 s, 559 s

All compounds show two ν(NH) bands which are attributed to the tension vibrations between nitrogen and hydrogen in the range 3176-3365 cm⁻¹. Compound I shows a signal at 1961 cm⁻¹ which is assigned to the ν(CO) from the carbonyl of the alanine anhydride ligand. Compounds II and III contain a band at 3601 cm⁻¹ assignable to the ν(OH) hydroxyl stress vibration of the ortho- and para-aminobenzene ligands. In all cases, two signals appear at approximately 280-310 cm⁻¹ due to the ν(IrCl) vibrations of the metal-chlorine bonds [44–48]. Finally, in the ionic complex IV, the PF₆⁻ anion, which is octahedral, presents the two ν(PF) bands, according to the literature [44–48].

Compound IV, which is ionic, has a conductivity value in 5x10⁻⁴ Molar acetone of 106 Ω⁻¹.cm².mol⁻¹ which is compatible with the existence of a 1:1 electrolyte in solution of this solvent. [49]

Table 3 presents the ¹H-NMR data for the four complexes assigned using, in addition, the ¹H-¹H COSY spectra. The results obtained are in agreement with what was expected [44–47].

Table 3. ¹H-NMR data of complexes I-IV in CDCl₃.

COMPLEX	¹ H Δ(CDCl ₃)	LIGAND STRUCTURE
I	5.81 (d, 2H, J = 13.5 Hz, Ala-H _{NH})	
	4.11 (qt, 1H, J = 7.5 Hz, Ala-H _F)	
	2.5 (qt, 2H, J = 7.5 Hz, Cp ^{et} -H _{B(-CH₂(CH₃))})	
	1.62 (s, 6H, Cp ^{et} -H _{D(-CH₃)})	
	1.59 (s, 6H, Cp ^{et} -H _{C(-CH₃)})	
	1.50 (d, 3H, J = 7.2 Hz, Ala-H _{E(-CH₃)})	
	1.08 (t, 3H, J = 7.5 Hz, Cp ^{et} -H _{A(-CH₂(CH₃))})	
II	10.24 (s, 2H, H-NH ₂)	
	7.8 (s, 1H, H-OH)	
	7.03 (m, 2H, -H _F)	
	6.89 (d, 1H, J = 7.2 Hz, -H _I)	
	6.8 (t, 1H, J = 7.2 Hz, -H _H)	
	2.08 (qt, 2H, J = 7.2 Hz, Cp ^{et} -H _{B(-CH₂(CH₃))})	
	1.82 (s, 6H, Cp ^{et} -H _{D(-CH₃)})	
III	1.66(s, 6H, Cp ^{et} -H _{C(-CH₃)})	
	1.06 (t, 3H, J = 8 Hz, Cp ^{et} -H _{A(-CH₂(CH₃))})	
	9.39 (s, 1H, H-OH)	
	8.44 (s, 2H, H-NH ₂)	
	7.03 (d, 2H, J = 8.8 Hz, -H _F)	
	6.71 (d, 2H, J = 8.4 Hz, -H _E)	
	2.35 (q, 2H, J = 7.2 Hz, H _{B(-CH₂(CH₃))})	
IV	1.81 (s, 6H, H _D)	
	1.79 (s, 6H, H _C)	
	1.03 (t, 3H, J = 3.6 Hz, H _{A(-CH₂(CH₃))})	
	7.31 (m, 2H, H _A)	
	7.20(m, 2H, H _B)	
	4.52 (s, 2H, -NH ₂)	
	1.83 (s, 15H, -H _{Cp*})	

Cytotoxicity of complexes I-III.

In vitro toxicity assays of complexes I-III were performed on cervical cancer cells of the HeLa tumour line. The tests were performed in triplicate. Cisplatin was used as the reference compound in the cytotoxic study.

At the time of seeding, the cells were dispersed in the medium and spherical in shape. After 24 hours in an atmosphere of 5% CO₂, 90% relative humidity, and 37.5°C, the cells acquired an elongated morphology, due to adhesion to the culture flask.

Subsequently, the corresponding complex synthesised in the same medium with DMSO (<1%) was introduced into the 96-well plate and serial dilutions were performed in a 1:2 ratio. After 48 hours, the plates were observed under the microscope to check the action of the drug on cell viability.

For comparison, the same was done with cisplatin. Thus, the possible cytotoxic action of the synthesised drug could be verified.

Next, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability colorimetric assay was performed, which shows a yellow colour. After addition of DMSO the metabolically active cells reduce MTT to Formazan which has a dark blue colour [50,51]. The dilution was measured at 570 nm, the fluorescence intensity of which is proportional to the amount of viable cells. As a control, HeLa cells untreated with complexes I-III were used. Eagles's (EMEM) and each of the added drugs were used as targets.

The data obtained are shown in the following table:

Table 4. Cytotoxicity data obtained for complexes I, II and III and for cisplatin.

Complex	IC ₅₀ (μM)
I	368.9587
II	166.9103
III	402.4583
cisplatin	134.7368

Two graphs have been constructed with these data:

Cell inhibition curves for the synthesised complexes show data above the reference complex, cisplatin. That is, the number of viable cells is higher than that of cisplatin.

Figure 4 shows the percentage inhibition of cell activity in which it can be seen that at a complex II concentration of 261 μM, 90% of the Hela cell activity is inhibited; this is comparable to what happens at a cisplatin concentration of 242 μM (98.90% of activity is inhibited).

Figure 5 shows the absorbance versus the logarithm of the concentration for each of the complexes studied. From this graph it can be deduced that the higher the concentration of the cytotoxic compound, the lower the absorbance. This is due to the fact that the cells are no longer viable and cannot reduce the MTT so that Formazan is not produced [51]

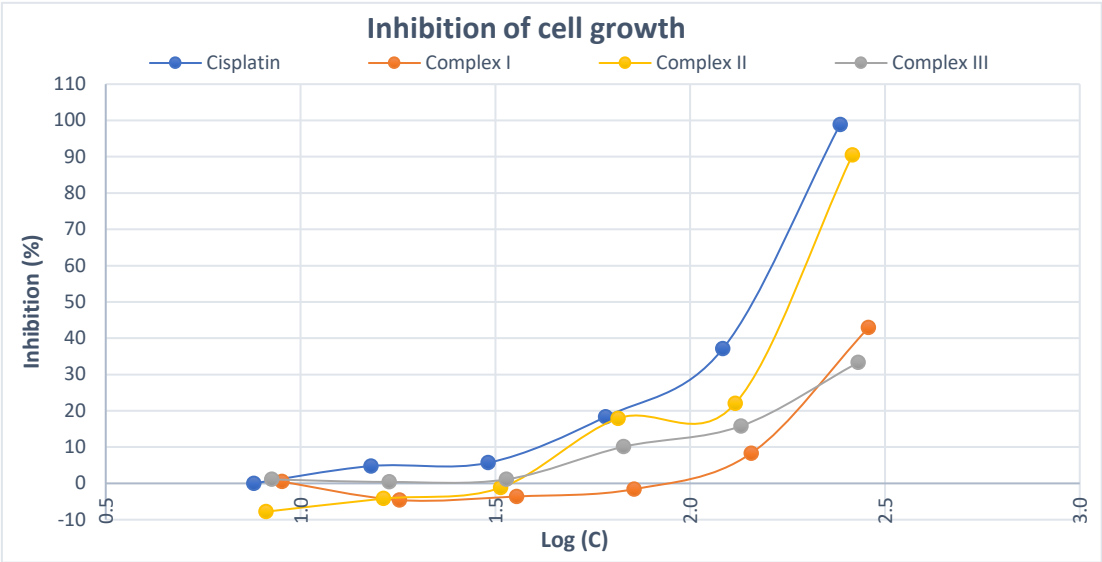


Figure 4. Percentage of inhibition of cellular activity after the addition of the synthesized complexes and cisplatin.

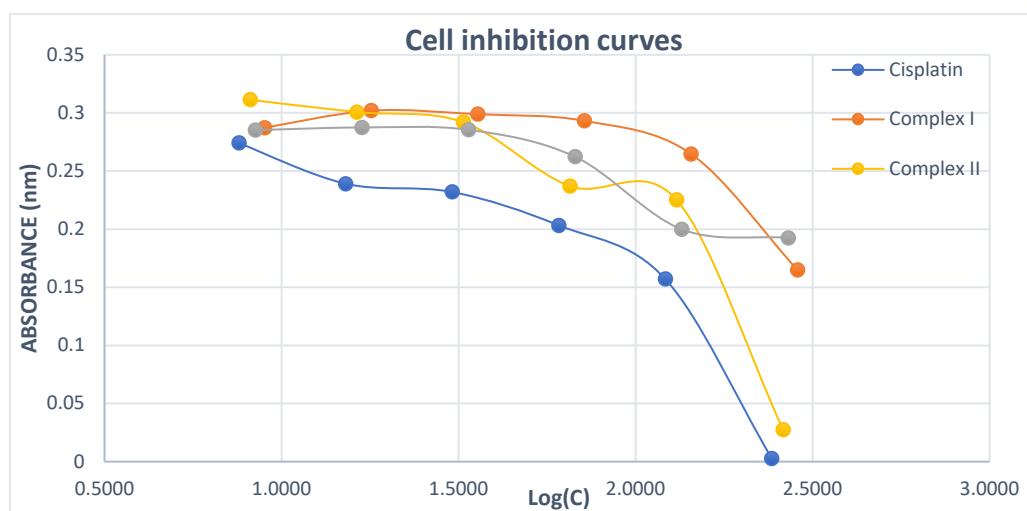


Figure 5. Inhibition curves of HeLa cells against the synthesized complexes, using cisplatin as a reference.

From table 4 the IC₅₀ can be obtained by extrapolation of the data obtained [44,52]. For the calculation of the IC₅₀ of each complex, a graph (Figure 4) has been made in which the absorbance is plotted against the concentration of each complex in μM . Using the equation of the line obtained for each compound, the IC₅₀ can be determined from the following equation [53]:

$$IC_{50} = \frac{50 - b}{a}$$

Equation 1. Linear calculation of IC₅₀

Where a is the slope of the line and b the ordinate at the origin.

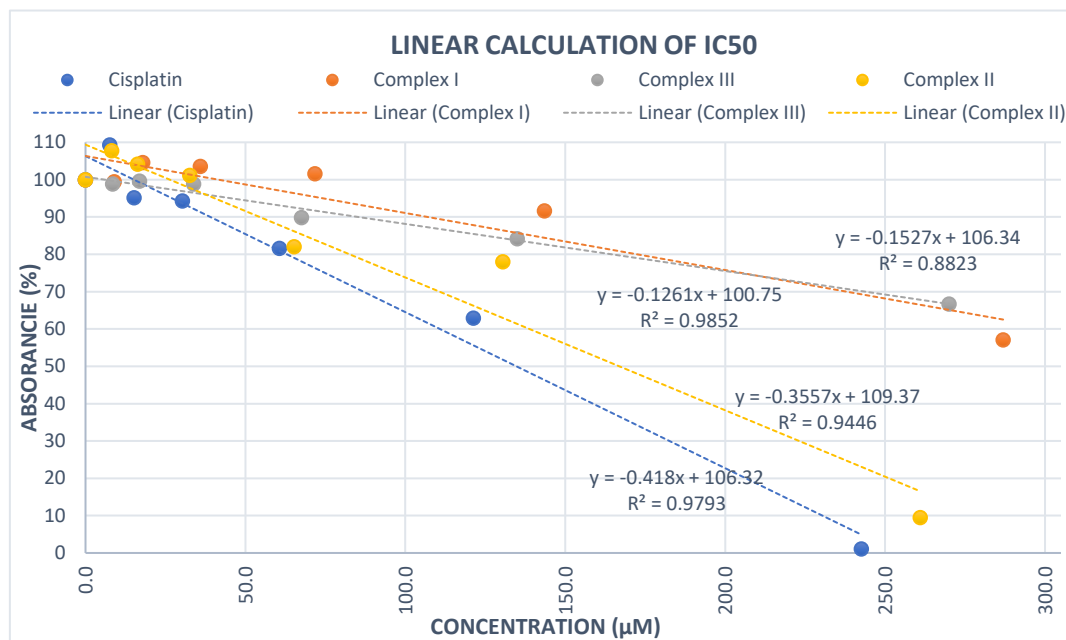


Figure 6. Linear calculation of IC₅₀ of cisplatin and complexes I, II and III.

Cytotoxicity of Complex IV

As in the three previous cases, the tests were performed in triplicate using the complex $[\text{Cp}^*\text{IrCl}(o\text{-fen})]\text{PF}_6$, $\text{Cp}^* = \text{C}_5\text{Me}_5$; ($o\text{-fen}$ = o -phenylenediamine) against human cervical cancer cells (HeLa). The complex starts to show a slight cytotoxic character at concentrations above 62 μM . The table below shows the absorbances associated with each of the compound concentrations.

Table 5. Cell viability table for Complex IV versus HeLa.

Concentration (μM)	Absorbance (%)
250	2,7
234	3,3
218	15,4
203	23,8
187	43,1
172	45,6
156	64,4
140	70,0
125	82,5
109	88,7
94	91,9
78	94,0
47	99,5
16	100
0	100

Two complementary graphs have been constructed with the above data, using the percentages of cell inhibition in one graph and the observed observations in the other. They provide relevant information, since the IC₅₀ and IC₉₀ can be obtained by extrapolation of the data [51]

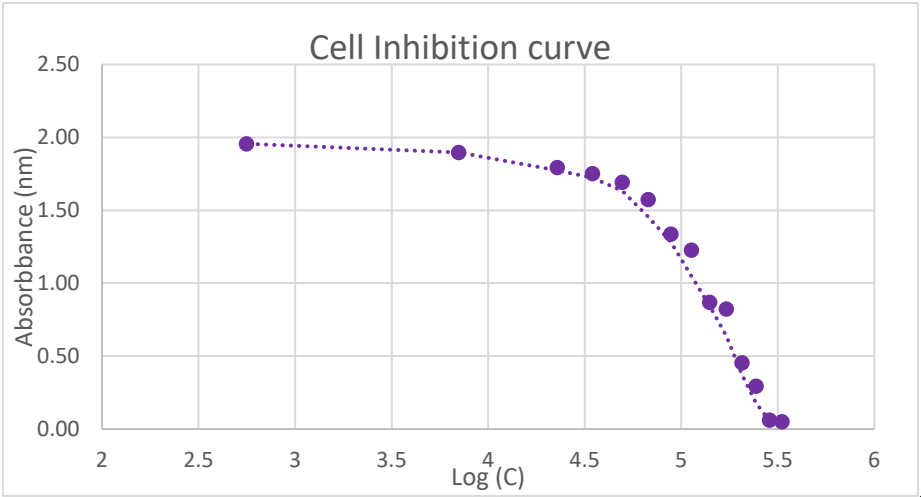


Figure 7. Inhibition curves of HeLa cells against complex IV.

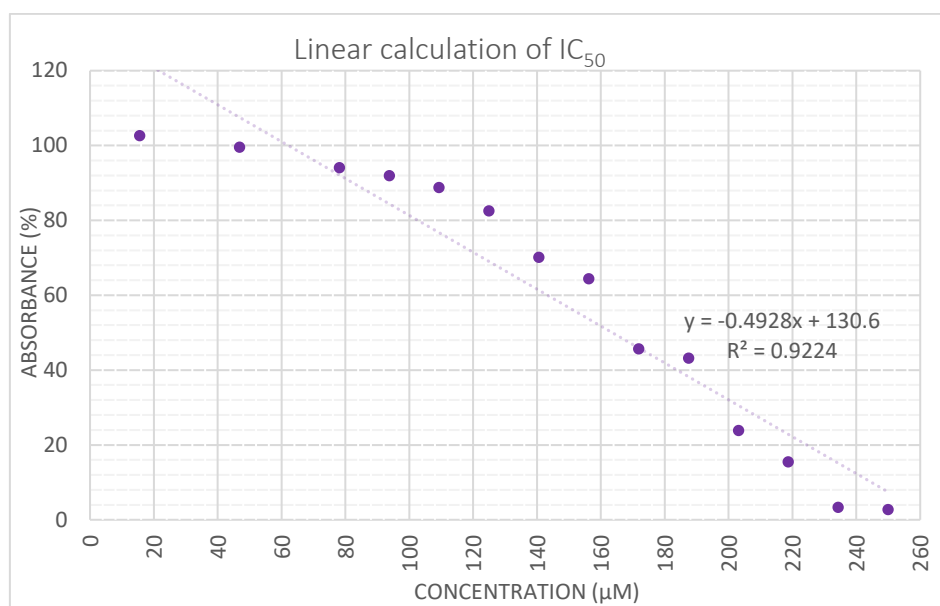


Figure 8. Linear calculation of IC_{50} of complex IV.

The compound concentration at which IC_{50} is reached is close to 160 μM (163.5552 μM) [53]

3. Materials and Methods.

Solvents: methanol (99.5%), diethylether (99.5%), hexane (95%), dichloromethane and chloroform (99.5%) were purchased from Panreac.

The deuterated solvents: chloroform and dimethyl sulfoxide are from Euriso-top.

1-ethyl-2,3,4,5-tetramethylcyclopentadiene (Cp^*), alanine anhydride and ortho- and para-aminophenol were from Sigma Aldrich; $IrCl_3 \cdot 3H_2O$ from Johnson Matthey.

Instrumentation:

Infrared spectra were performed on a spectrophotometer 100 FT-IR in the range 4000-200 cm^{-1} . Nuclear magnetic resonance spectra were obtained from Bruker Avance 400 MHz and 600 MHz spectrometers. The solvents used were $CdCl_2$ and $DMSO-d_6$. Elemental analyses of C, H and N were obtained on a Leco CNHS-932 microanalyzer. Mass spectra were obtained on a Fluostar Omega spectrometer (ESI). Ions are detected by "time of flight" (TOF). Conductivity measurements were made with a Crison MicroCM2200 conductivitymeter. Finally, thermogravimetric analyses were recorded on a TA Instruments TGA-DTA Simultaneous Analyzer.

The in vitro cytotoxicity studies were carried out by Central Services for Experimental Sciences of the University of Murcia (SACE), with a Biosafety Level 2.

Synthesis of the Complexes

The starting compounds $[Cp^*IrCl(\mu-Cl)]_2$, $Cp^* = C_5Me_5$ (compounds I and III) and C_5EtMe_4 (compound IV) have been prepared according to previously described methods [54,55].

Compound I. $[Cp^*IrCl(\mu-Cl)]_2$ (1.0040 g, 0.121 mmol) was reacted in a round-bottomed flask with 0.0351 g (0.247 mmol) of alanine anhydride in 45 mL of dichloromethane. The mixture, initially yellow in colour, was kept under gentle heating and stirring for one hour. Subsequently, hexane was added until the solid precipitated. It was then filtered over a porous non-vacuum plate to obtain a green solid. Finally, the solid obtained was recrystallized with dichloromethane-hexane to obtain a solid of the same colour with a yield of 63%.

Compound II. 0.1025 g (0.124 mmol) of the starting product, $[Cp^*IrCl(\mu-Cl)]_2$ was weighed and dissolved in a round-bottomed flask in 40 mL dichloromethane. Then 0.0272 g (0.250 mmol) of o-aminophenol was added. The initially dark green solution was kept under stirring and gentle heating

for one hour. After this time, it was kept in the refrigerator for 24 hours. Then, a dark green solution with dark green particles was obtained and separated by filtration. The mother liquor was concentrated by heating and stirring. The volume was reduced by gentle heating to 1/3 of the initial volume. Hexane was added until a green solid precipitated which was separated by filtration. In the end 0.0642 g of compound was obtained and recrystallised with dichloromethane/hexane in 45% yield.

Compound III.- 0.0767 g (0.0930 mmol) of starting compound were weighed $[\text{Cp}^*\text{IrCl}(\mu\text{-Cl})_2]$ and 0.0245 (0.225 mmol) *p*-aminophenol. Both compounds were dissolved in 15 ml chloroform in a round-bottomed flask. The orange-coloured solution was kept under stirring and moderate heating for one hour. It was then refrigerated for 24 hours. A mixture of the orange solution and a brown solid was obtained. The product was filtered and washed with chloroform. The solution was concentrated under vacuum to one third volume, hexane was added as precipitant and a brown solid was obtained which was recrystallised from chloroform/hexane. The final solid (0.0359 g) was obtained in 41% yield.

Preparation of $[\text{Ir}(\text{C}_5\text{Me}_5)\text{Cl}(o\text{-phen})]\text{Cl}$.- 0.2002 g of the starting dimer was weighed and poured into a 50 ml round bottom flask together with 20 mL of dichloromethane. Then 0.0596 g of *o*-phenylenediamine was added to 15 mL of dichloromethane. The flask was stoppered and the resulting yellow solution was left to stir at room temperature for one hour. After one hour, the stirring was stopped and a yellow precipitate was observed and filtered, washed three times with a 1:2 dichloromethane/ethyl ether mixture and a yellow solid was obtained, which was recrystallised from dichloromethane/ethyl ether, for 24 hours in the refrigerator. At the end 0.2293 g of product was obtained with a yield of 90%.

Compound IV.- 0.1026 g of $[\text{Ir}(\text{C}_5\text{Me}_5)\text{Cl}(o\text{-phen})]\text{Cl}$ was weighed and poured into a round bottom flask together with 20 ml dichloromethane. To the resulting solution 0.0339 g of ammonium hexafluorophosphate in 20 mL of additional dichloromethane was added while stirring for one hour and a yellow solid was observed which was separated by filtration, washed three times with dichloromethane/ethyl ether. The beige solid obtained was recrystallised from a dichloromethane/ether mixture. The desired compound was obtained with a yield of 92%.

4. CONCLUSIONS

1) In this work, we have described the synthesis of four "half sandwich" complexes of iridium (III), three of them neutral containing $(\text{Cp}^*=\text{C}_5\text{EtMe}_4)$ of stoichiometry $[\text{Cp}^*\text{IrCl}_2\text{L}]$, with L = alanine anhydride and ortho and para aminophenol. And an anionic one of stoichiometry $[\text{Cp}^*\text{IrCl}(o\text{-phen})]\text{PF}_6$.

2) The compounds, with the exception of IV, are not very soluble in usual solvents and have exceptional decomposition points indicating the existence of strong metal-ligand bonds.

3) The compounds have been obtained as air-stable solids and characterized by elemental analysis, IR and ^1H -NMR spectroscopies with the help of ^1H - ^1H COSY, mass spectrometry (ESI/TOF) and thermogravimetry.

4) The cytotoxicity studies show that while the neutral compound III, which contains *p*-aminophen as a neutral ligand, and the ionic compound IV present IC_{50} values of 166.9103, respectively, values quite similar to that of cisplatin, 134, 7368. The other two compounds have IC_{50} values above 250 mM, which is why they are considered inactive for the proposed purpose.

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References.

1. L. Kelland, The resurgence of platinum-based cancer chemotherapy *Nat. Rev. Cancer*, **2007**, 7, 573-584.
2. D. Wang and S. J. Lippard, Cellular processing of platinum anticancer drugs. *Nat. Rev. Drug Discovery*, **2005**, 4, 307-320.

3. DL Ma, M Wang, Z Mao, C Yang, CT Ng, CH Leung. Rhodium complexes as therapeutic agents. *Dalton Trans.*, 2016, 45, 2762-2771.
4. G. S. Yellol, A. Donaire, V. Vasylyeva, C. Janiak, and J. Ruiz, On the antitumor properties of novel cyclometalated benzimidazole Ru (II), Ir (III) and Rh (III) complexes
Chem. Commun., 2013, 49, 11533-11535.
6. C. H. Leung, H. J. Zhong, D. S. H. Chan and D. L. Ma, Bioactive iridium and rhodium complexes as therapeutic agents *Coord. Chem. Rev.*, 2013, 257, 1764-1776.
7. J. P. C. Coverdale, I. Romero-Canelon, C. Sanchez-Cano, G. J. Clarkson, A. Habtemariam, M. Wills and P. J. Sandler. Asymmetric transfer hydrogenation by synthetic catalysts in cancer cells , *Nat. Chem.*, 2018, 10, 347-354.
8. J. M. Hearn, I. Romero-Canelon, B. Qamar, Z. Liu, I. Hands-Portman and P. J. Sandler, The Potent Oxidant Anticancer Activity of Organoiridium, Catalysts, *ACS Chem. Biol.*, 213, 8, 1335-1343.
9. W. Liu and R. Gust, Metal N-heterocyclic carbene complexes as potential antitumor metallodrugs, *Chem. Soc. Rev.*, 2013, 42, 755-773.
10. G. Gasser, I. Ott and N. Metzler-Nolte, Organometallic Anticancer Compounds, *J. Med. Chem.*, 2013, 56, 1291-1300.
11. C. G. Hartinger, N. Metzler-Nolte and P. J. Dyson, Ruthenium(II)-Arene RAPTA Type Complexes Containing Curcumin and Bisdemethoxycurcumin Display Potent and Selective Anticancer Activity *Organometallics*, 2012, 31, 5677-5685.
12. I. Romero-Canelon, L. Salassa and P. J. Sadler, The Contrasting Activity of Irido versus Chlorido Ruthenium and Osmium Arene Azo- and Imino-pyridine Anticancer Complexes: Control of Cell Selectivity, Cross-Resistance, p53 Dependence, and Apoptosis Pathway *J. Med. Chem.*, 2013, 56, 1291-1300.
13. F. J. Ballester, E. Ortega, V. Porto, H. Kostyrhunova, N. Davila-Ferreira, D. Bautista, V. Brabec, F. Dominguez, M. D. Santana and J. Ruiz, New half-sandwich ruthenium(II) complexes as proteosynthesis inhibitors in cancer cells, *Chem. Commun.*, 2019, 55, 1140-1143.
14. W. X. Ni, W. L. Man, S. M. Yiu, M. Ho, M. T. W. Cheung, C. C. Ko, C. M. Che, Y. W. Lam and T. C. Lau, Halide Control of N, N-Coordination versus N, C-Cyclometalation and Stereospecific Phenyl Ring Deuteration of Osmium(II) p-Cymene Phenylazobenzothiazole Complexes, *Chem. Sci.*, 2012, 3, 1582-1588.
15. E. Ortega, F. Ballester, A. Hernandez-Garcia, S. Hernandez-Garcia, M. A. Guerrero-Rubio, D. Bautista, M. D. Santana and J. Ruiz, *Inorg. Chem. Front.*, 2021, 8, 141-155.
16. J. C. Dabrowiak, "Metals in Medicine", John, Wiley and Sons Ltd, Chichester, 2009, p. 149.
17. O. Domotorr, V. F. S Pape, N. V. May, G. Szakac and E. A. Enyedy, Comparative solution equilibrium studies of antitumor ruthenium (η^6 -p-cymene) and rhodium (η^5 -C₅Me₅) complexes of 8-hydroxyquinolines, *Dalton Trans.*, 2017, 46, 4382-4396.
18. Z. Liu, L. Salassa, A. Habtemariam, A. M. Pizarro, G. J. Clarkson and P. J. Sadler, Potent Half-Sandwich Iridium(III) Anticancer Complexes Containing C_N-Chelated and Pyridine Ligands, *Inorg. Chem.*, 2011, 50, 5777-5783.
19. Z. Zhu, Z. Wang, Y. Hao, C. Zhu, J. Jiao, H. Chen, Y. -M. Wang, J. Yan, Z. Guo and X. Wang, Glutathione boosting the cytotoxicity of a magnetic platinum(IV) nano-prodrug in tumor cells,
Chem. Sci., 2016, 7, 2864-2869.
21. S. Dilruba and G. V. Kalayda, Platinum-based drugs: past, present and future, *Cancer Chemother. Pharmacol.*, 2016, 77, 1103-1124.
22. C. Johnstone T, K. Suntharalingam and J. S. Lippard, The next generation of platinum drugs: targeted Pt (II) agents, nanoparticle delivery, and Pt (IV) prodrugs, *Chem. Rev.*, 2016, 116, 3436-3486.
23. C. G. Hartinger, N. Metzler-Nolte and P. J. Dyson, Challenges and opportunities in the development of organometallic anticancer drugs, *Organometallics*, 2012, 31, 5677-5685
24. Z. Liu, I. Romero-Canelon, B. Qamar, J. M. Hearn, A. Habtemariam, N. P. Barry, A. M. Pizarro, G. K. Clarkson and P. J. Sadler, The Potent Oxidant Anticancer Activity of Organoiridium Catalysts, *Angew. Chem., Int. Ed.*, 2014, 53, 3941-3946.
25. Y. Li, C. P. Tan, W. Zhang, L. He, L. N. Ji and Z. W. Mao, Phosphorescent iridium(III)-bis-N-heterocyclic carbene complexes as mitochondria-targeted theranostic and photodynamic anticancer agents, *Biomaterials*, 2015, 39, 95-104.
26. Z. Liu, A. Habtemariam, A. M. Pizarro, S. a. Fletcher, A. Kisova, O. Vrana, L. Salassa, P. C. A. Buijnninx, G. J. Clarkson, V. Brabec, and P. J. Sadler, Organometallic Half-Sandwich Iridium Anticancer Complexes, *J. Med. Chem.*, 2011, 54, 3011-3026.
27. C. Wang, J. Liu, Z. Tian, M. Tian, L. Tian, W. Zhao and Z. Liu, Half-sandwich iridium N-heterocyclic carbene anticancer complexes, *Dalton Trans.*, 2017, 46, 6870-6883.
28. L. He, C. P. Tan, R. R. Ye, Y. Z. Yao, Y. H. Liu, Q. Zhao, L. N. Ji and Z. W. Mao, L. He, C. P. Tan, R. R. Ye, Y. Z. Yao, Y. H. Liu, Q. Zhao, L. N. Ji and Z. W. Mao, Half-sandwich iridium N-heterocyclic carbene anticancer complexes, *Angew. Chem., Int. Ed.*, 2014, 53, 12137-12141.

29. J. M. Hearn, I. Romero-Canelon, B. Qamar, Z. Liu, I. Hands-Portman and P. J. Sadler, Organometallic Iridium(III) Anticancer Complexes with New Mechanisms of Action: NCI-60 Screening, Mitochondrial Targeting, and Apoptosis, *ACS Chem. Biol.*, 2013, 8, 1335-1343.
30. V. Novohradsky, L. Zerzankova, J. Stepankova, A. Kisova, H. Kostrhunova, Z. Liu, P. J. Sadler, J. Kasparkova and V. Brabec, A dual-targeting, apoptosis-inducing organometallic half-sandwich iridium anticancer complex, *Metallomics*, 2014, 6, 1491-1501.
31. Z. Liu, A. Habtemariam, A. Pizarro, G. J. Clarkson and P. J. Sadler, Organometallic iridium (III) cyclopentadienyl anticancer complexes containing C, N-chelating ligands, *Organometallics*, 2011, 30, 4702-4710.
32. Z. Liu, V. Lebrun, T. Kitanosono, H. Mallin, V. Köler, D. Häussinger, D. Hilvert, S. Kobayashi and T. R. Ward, Upregulation of an Artificial Zymogen by Proteolysis, *Angew. Chem., Int. Ed.*, 2016, 11587-11590.
33. Z. Liu, L. Salassa, A. Habtemariam, A. M. Pizarro, G. J. Clarkson and P. J. Sadler, Contrasting Reactivity and Cancer Cell Cytotoxicity of Isoelectronic Organometallic Iridium(III) Complexes, *Inorg. Chem.*, 2011, 50, 5777-5783.
34. L. He, Y. Li, C.-P. Tan, R.-R. Ye, M.-Chen, J.-J. Cao, L.-N. Ji and Z.-W. Mao, Cyclometalated iridium(III) complexes as lysosome-targeted photodynamic anticancer and real-time tracking agents, *Chem. Sci*, 2015, 6, 5401-5418.
35. Z. Liu and P. J. Sadler, Formation of glutathione sulfenate and sulfinate complexes by an organoiridium (III) anticancer complex, *Inorg. Chem. Front.*, 2014, 1, 668-672.
36. Z. Liu, Z. Romero-Canelon, A. Habtemariam, G. J. Clarkson and P. J. Sadler, Potent Half-Sandwich Iridium(III) Anticancer Complexes Containing C $\wedge$ N-Chelated and Pyridine Ligands, *Organometallics*, 2014, 33, 5324-5333.
37. G. Giambastiani, L. Tuconi, R. L. Kuhlman and P. D. Hustad, "Imino-and Amido-Pyridinate d-Block Metal Complexes in Polymerization/Oligomerization Catalysis, in *Olefin Upgrading Catalysis by Nitrogen-based Metal Complexes I*, Springer, 2011, vol. 34, pp. 197-281.
38. X. Xiong, L.-Y. Liu, Z.-W. Mao, T. Zou, Approaches Towards Understanding the Mechanism-of-action of Metallodrugs, *Coord. Chem. Rev.*, 2022, 453, 214311.
39. K. Peng, Y. Zheng, W. Xia and Z.-W. Mao, Organometallic anti-tumor agents: targeting from biomolecules to dynamic bioprocesses, *Chem. Soc. Rev.*, 2023, 52, 2790-2832.
40. A. Kastner, T. Mendrina, F. Bachmann, W. Berger, B. K. Keppler, P. Heffeter and C. R. Kowol, Tumor-Targeted Dual-Action NSAID-Platinum(IV) Anticancer prodrugs, *Inorg. Chem. Front.*, 2023, 10, 4126-4138.
41. G. H. Ribeiro, A. R. Costa, A. R. de Souza, F. V. da Silva, F. T. Martins, A. M. Plutin and A. A. Batista, An Overview on the Anticancer Activity of Ru(II)/Acylthiourea Complexes, *Coord. Chem. Rev.*, 2023, 488, 215161.
42. J. Li and T. Chen, Transition Metal Complexes as Photosensitizers for Integrated Cancer Theranostic Applications, *Coord. Chem. Rev.*, 2020, 418, 213355.
43. X. He, L. Wei, J. Chen, S. Ge, M. Kandawa-Shultz, G. Shao and Y. Wang, Folate-targeted Iridium Complexes Amplify Photodynamic Therapy Efficacy Through Ferroptosis, *Inorg. Chem. Front.*, 2023, 10, 4780-4788.
44. H. Yuan, Z. Han, Y. Chen, F. Qi, H. Fang, Z. Guo, S. Zhang and W. He, Ferroptosis Photoinduced by New Cyclometalated Iridium(III) Complexes and Its Synergism with Apoptosis in Tumor Cell Inhibition, *Angew. Chem., Int. Ed.*, 2021, 60, 8174-8181.
45. X. Liu, A. Lv, P. Zhang, J. Chang, R. Dong, M. Liu, J. Liu, X. Huang, X.-A. Yuan, Z. Liu, *Dalton Trans.*, 2024, 53, 552-563.
46. María Angeles Pujante-Galián, Sergio A. Pérez, Mercedes G. Montalbán, Guzmán Carissimi, Marta G. Fuster, Gloria Villora, Gabriel García. P-Cymene Complexes of Ruthenium (II) as Antitumor agents. *Molecules*, 2020, 25, 5063-5077.
47. Marta G. Fuster, Imane Moulefera, Mercedes G. Montalbán, José Pérez, Gloria Villora and Gabriel García. Synthesis and Characterization of New Ruthenium (II) Complexes of Stoichiometry [Ru(p-Cymene) Cl₂L] and Their Cytotoxicity against HeLa-Type Cancer Cells. *Molecules*, 2022, 27, 7264-7275.
48. Natalia Sáez, Alfonso Canales-Martínez, Marta G. Fuster, Imane Moulefera, Delia Bautista, José Pérez, Gloria Villora and Gabriel García, Synthesis and characterization of new iridium(III) complexes containing the fragment [Cp*IrCl₂] and the ligands 2- and 4-aminobenzonitril and 2- and 4-aminopyridine. *J. Coord. Chem.*, 2024, 516-524. doi.org/10.1080/00958972.2024.2343787
49. Alfonso Canales-Martínez, Rosa María Pérez Pastor, Gloria Villora and Gabriel García. *Pure Appl Chem.*, 2024 (in the press).
50. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds (Part B)*, Sixth Edition, 2009, John Wiley & Sons, INC., Hoboken, New Jersey, USA.
51. W. G. Geary, The Use of Conductivity Measurements in Organic Solvents for the Characterisation Compounds, *Coord. Chem. Rev.*, 1971, 7, 81-122.

52. Stockert, J. C.; Blázquez-Castro, A.; Cañete, M.; Horobin, R. W.; Villanueva, A.; MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. *Acta Histochemica*, 2012, 114, 785-796.
53. G Eisenbrand, B Pool-Zobel, V Baker, M Balls, B J Blaauboer, A Boobis, A Carere, S Kevekordes, J-C Lhuguenot, R Pieters, J Kleiner.; *Methods of in vitro toxicology, Food Chem Toxicol*, 2002, 40, 193-236.
54. B. Zoehler, A. Melo de Aguiar, and G. Ferreira Silveira; SAEDC: Development of a technological solution for exploratory data analysis and statistics in cytotoxicity. *Pharmaceut. Statist.* 2003, 2, 167-174.
55. AAT Bioquest, Inc. (2022, June 26). Quest GraftM IC50 Calculator. AAT Bioquest. Accessed 25 June 2022.
56. Dooley, T.; Fairhurst, G.; Tiza, C. T.; Tabataian, K.; Blanco, C.; Ethyltetramethylcyclopentadienyl complexes of cobalt, rhodium, iridium and ruthenium. *Trans. Metal Chem.* 1978, 3, 299-302.
57. Bennett, M. A.; Huang, T. N.; Matheson, T. W.; Smith, K.; *Inorganic Synthesis*; John Wiley & Sons, Hoboken, NJ, USA, 1982, Volume 21, pp. 74-77.

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