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Ru(η^6-arene) Metal-based drug the modern anticancer pharmacology

Degree's finals project

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Chemistry Degree

UNIVERSITAT ROVIRA I VIRGILI

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ABBREVIATIONS

CAP: 1,4,7-triaza-9-phosphatricyclo[5.3.2.1]tridecane
DNA: Deoxyribonucleic acid
EtOH: Ethanol
FDA: Food and Drug Administration
M: Molar
MeOH: Methanol
mg: Milligrams
mM: Millimolar
mL: Millilitres
NMR: Nuclear Magnetic Resonance
RACAP: $[(\eta^6\text{-arene})(CAP)RuX_2]$
RAPTA: $[(\eta^6\text{-arene})(PTA)RuX_2]$
pD: $-\log_{10}[a_{D+}]$
pH: $-\log_{10}[a_{H+}]$
PTA: 1,3,5-Triaza-7-phosphaadamantane

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A B S T R A C T

Research interests are based around the development of metal complexes as potential therapeutic agents and understanding their chemistry in biological systems.

One major focus currently is the development of new organometallic ruthenium complex as potential anticancer agents. [(η^6-arene)(Oxalate)(PTA)Ru] and [(η^6-arene)(PTA)$RuCl_2$][1,2] compounds have clearly demonstrated that the Ru(η^6-arene) can produce complex with high activity against primary and secondary tumours. It is intended to extend the structural diversity of Ru Compounds (η^6-arene) with the aim of improving selectivity and activity against metastasis.

The aim is to form a Ru mononuclear complex (η^6-arene) through the reaction of $RuCl_3.xH_2O$ with a diene as a precursor followed by reaction with a phosphine ligand or ligand chelate.

Various dienes are reacted with $RuCl_3.xH_2O$ in MeOH /EtOH or Acetone /H_2O to form an intermediate with formula [(η^6-arene$RuCl_2$]$_2$ and [(η^6-arene)(Oxalate)$RuCl_2$] followed by a reaction with PTA (1,3,5- Triaza-7-phosphateamantane).[3]

1. INTRODUCTION.

1.1. Faculty of science and Engineering, University of Hull.

At the University of Hull, the chemistry subject group-school of sciences carries out world-class research in many scientific areas, from imaging agents, miniaturised chemistry in lab on chip platforms to advanced functional materials and drug synthesis.

The medicinal and Imaging Chemistry theme use the multidisciplinary approach at the interface of chemistry and biology, the synthesis of inorganic and organic chemistry, bionanotechnology and materials science.

The therapeutic agents section research on the application of synthetic methods to health related problems. The members of the group study synthetic procedures to obtain molecules for use as drugs.

1.2. THE RESEARCH FOCUS: Metal-based drug the modern anticancer pharmacology

Metal-based drugs are playing an increasing role in the field of modern anticancer pharmacology. One of the most important chemotherapeutics in cancer treatments is Platinum-based. The prototype cisplatin was approved by the FDA in 1978, is currently used to treat testicular, bladder and ovarian cancers. The fact of using cisplatin in the clinic it is not without serious problems like severe side-effects such as nephrotoxicity, ototoxicity, neurotoxicity and problems associated with intrinsic or acquired tumour resistance.[4]

Several approved cisplatin analogues (carboplatin and oxaliplatin) were developed to mitigate the toxicity and resistance. More efficacious and less toxic anticancer drugs like ruthenium-based anticancer agents show more efficacious and less toxicity.

Since the synthesis of the first arene ruthenium complexes in 1967 by Winkhaus and Singer, the chemistry of this family of complex has investigated because their structural diversity. The ways the arene ligand can be functionalized, their versatile stereochemical controlling elements in areas such as catalysis and their potential as metalloligands.

The development of ruthenium(II)-arene compounds in the +2 oxidation state by the η^6–coordinated arene ligand, the RAPTA family ([Ru(η^6-arene)(PTA)RuX_2]), PTA= 1,3,5-triaza-7phosphaadamantane (**Fig.1.2.1**), have been studied. These compounds are cytotoxic to a cancer cell lines including cisplatin-resistant strains as shows A. Bergamo, A. Masi, A.F.A. Peacock, A. Habtemariam, P.J. Sadler, G. Sava, J. *Inorg. Biochem. 104 (2010) 79–86.* in a vivo study of [Ru(η^6-biphenyl)(en)Cl]$^+$ against mammary carcinoma, reducing the growth of the primary tumour and the development and growth of lung metastases. Moreover, wide variety of organometallic ruthenium (II) compounds with different structures have been prepared and examined their cytotoxicity to cancer cells with excellent reviews. [5]

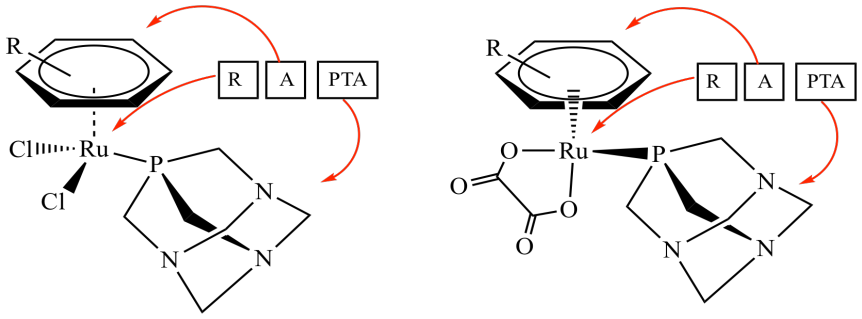

Fig. 1.2.1 Generic Structure of RAPTA compound.

The research focuses on the development of new RAPTA structures by the modulation of the arene structures, in this case, cymene and toluene. The piano-stool structure of the RAPTA compound is characteristic, three coordination sites of the Ru are occupied by a η^6-coordinated arene ligand which stabilize the Ru(II) oxidation state. The amphiphilic PTA ligand occupied another coordination site to leave available two coordination sites that are occupied by chloride or oxalate ligands in this case (see **Fig.1.2.1**).

2. SYNTHESIS AND CHARACTERISATION

2.1. Synthesis of [(η^6-P-cymene)(PTA)$RuCl_2$].

The intermediate dimer [(η^6-P-cymene) $RuCl_2$]$_2$, is synthesized by the addition of $RuCl_3xH_2O$ with 10 equivalents of α-phelladrene in 90% aqueous EtOH as shows **Fig.2.1.1**. The reaction is carried out in a reflux system for 5 hours. The brown solution is filtered to eliminate impurities and reagents that did not react. The complex beings to crystalize and is stored in the refrigerator overnight. The red precipitate is collected, washed with MeOH and dried (yield 85%).

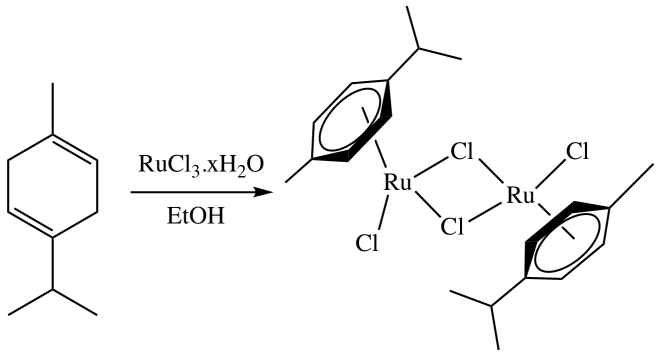

Fig 2.1.1. Synthesis of [(η^6-P-cymene)$RuCl_2$]$_2$

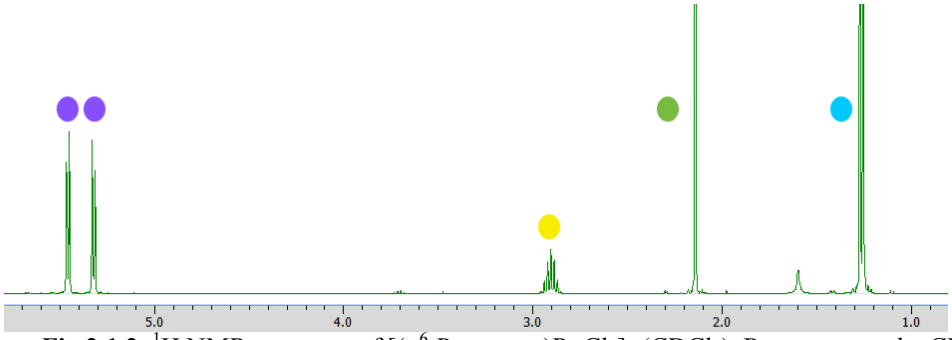

Fig 2.1.2. ^{1}H NMR spectrum of [(η^6-P-cymene)$RuCl_2$]$_2$ ($CDCl_3$). Ru arene-purple, CH-yellow, CH_3 (isopropyl)-blue, CH_3 (methyl)-green. (Not dry at all)

Once the dimer is obtained and verified by ^{1}H NMR spectra (**Fig.2.1.2**) it is reacted with 2 equivalents of PTA (1,3,5-Triaza-7-phosphate.amantane) in dichloromethane for 3 hours (reaction scheme **Fig.2.1.3)**. The solvent is evaporated using the rotavapor and dried in vacuum line overnight. Checked by ^{1}H NMR spectra (**Fig.2.1.4**) the evidence that the complex [(η^{6}-P-cymene)(PTA)$RuCl_2$] has been obtained (yield 61%).

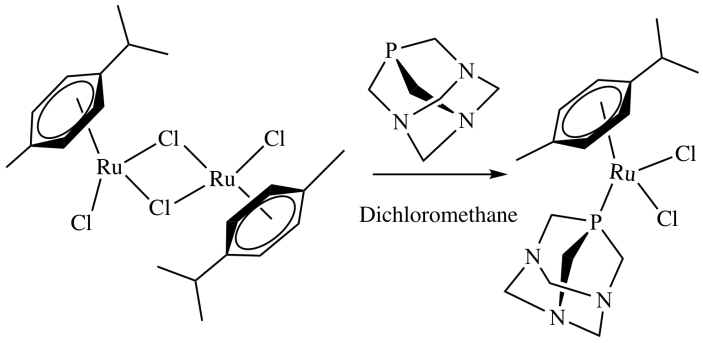

Fig 2.1.3. Synthesis of [(η^{6}-P-cymene)(PTA)$RuCl_2$].

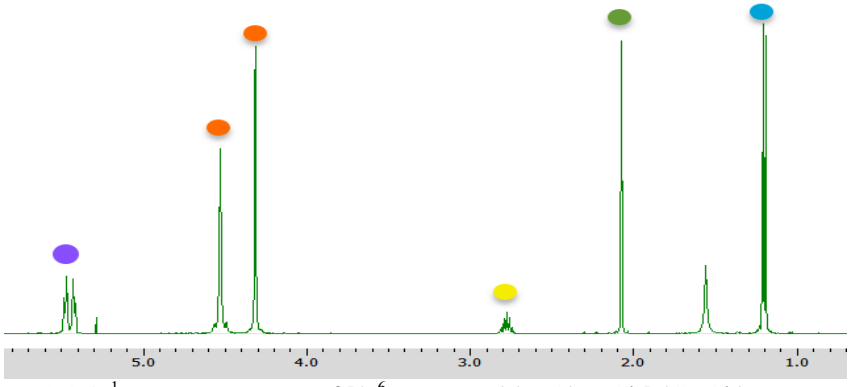

Fig 2.1.4. ^{1}H NMR spectrum of [(η^{6}-P-cymene)(PTA)$RuCl_2$] ($CDCl_3$). Ru arene-Purple, PTA- orange, CH- yellow, CH_3 (isopropyl)-blue, CH_3 (methyl)-green. (Not dry at all)

2.2. Synthesis of [(η^6-P-cymene)(oxalate)(PTA)Ru].

From the dimer [(η^6-P-cymene)$RuCl_2$]$_2$ obtained in section *2.1. Synthesis of [(η^6-P-cymene)(PTA)$RuCl_2$]*, is reacted with 2,5 equivalents of silver oxalate in water for 5 hours (**Fig 2.2.1**).The round bottom flask where the reaction is carried out must be covered with foil. The yellow solution is vacuum filtered using silica gel to prevent the AgCl (white precipitate) passes to the solution. The solvent is evaporated to obtain [(η^6-P-cymene)(oxalate)(H_2O)Ru]. The yellow precipitate is dried in vacuum and checked by ^{1}H NMR (**Fig 2.2.2)** that the desired complex has been obtained with 75% of yield.

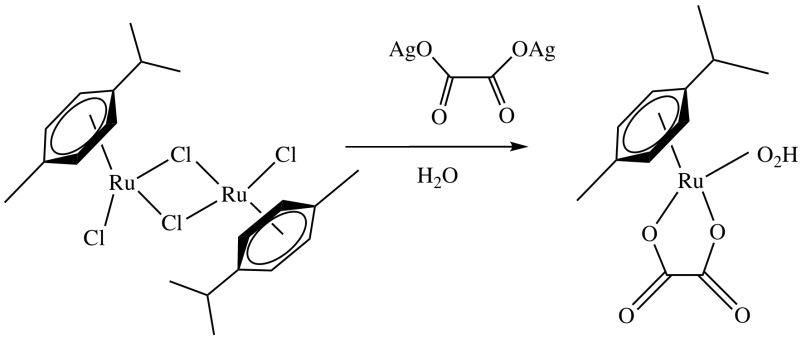

Fig 2.2.1. Synthesis of [(η^6-P-cymene)(oxalate)(H_2O)Ru]

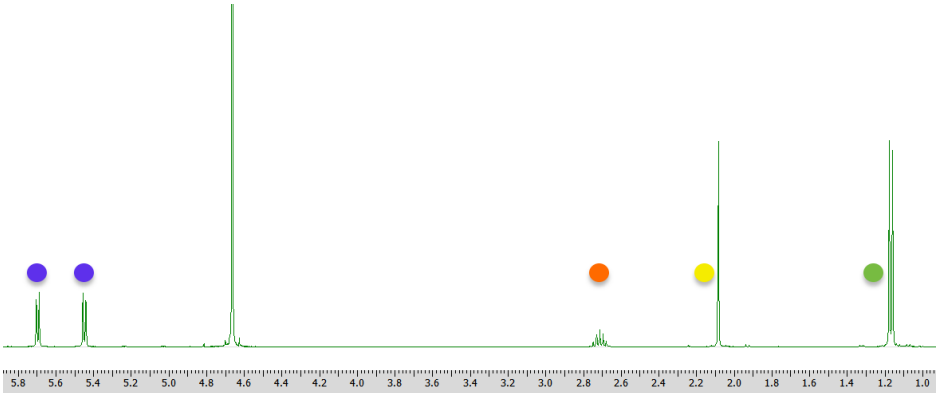

Fig 2.2.2. ^{1}H NMR spectrum of [(η^6-P-cymene)(oxalate)(H_2O)Ru] (D_2O). Ru arene-purple, CH- orange, CH_3 (isopropyl)-green, CH_3 (methyl)-yellow.

It is then reacted with 1 equivalent of PTA in EtOH for 3 hours (**Fig 2.2.3**). All the solvent is evaporated with the rotavapor to obtain the complex [(η^6-P-cymene)(oxalate)(PTA)Ru]. For the crystallization, it is dissolved in the minimum quantity of water to make the solution as concentrated as possible. The saturated solution is added in a vial, which is introduced into a flask containing acetone, whose vapours are slowly introduced into the vial (crystallization by vapour diffusion), causing the crystallization of the [(η^6-P-cymene)(oxalate)(PTA)Ru]. Yellow crystals are checked by ^{1}H NMR spectra (**Fig.2.2.4**) the evidence that the complex [(η^6-P-cymene)(PTA)RuCl$_2$] has been obtained and is totally dried with 65% of yield.

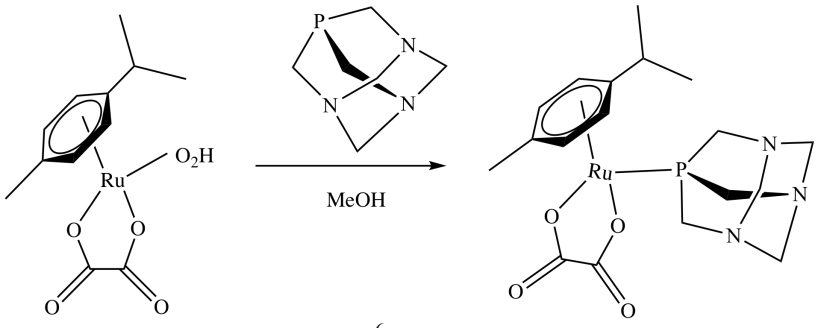

Fig 2.2.3. Synthesis of [(η^6-P-cymene)(oxalate)(PTA)Ru].

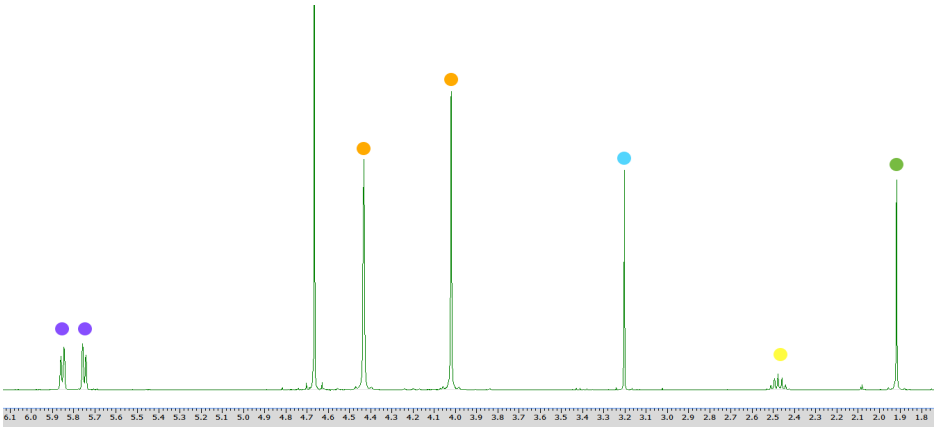

Fig 2.2.4. ^{1}H NMR spectrum of [(η^6-P-cymene)(oxalate)(PTA)Ru] (D_2O). Ru arene-purple, PTA- orange, CH_3 (isopropyl)-green, CH-yellow, CH_3 (methyl)- blue.

2.3. Synthesis of [(η^6-Toluen)(oxalate)(PTA)Ru].

The intermediate dimer [(η^6-Toluen)$RuCl_2$]$_2$ is prepared by the reaction of $RuCl_3xH_2O$ with 5 equivalents of 1-Methyl-1,4- Cyclhexadiene in MeOH with reflux system for 12 hours as shows **Fig 2.3.1**. The red precipitate is collected and washed with MeOH (yield 84%).

When the dimer [(η^6-Toluen)$RuCl_2$]$_2$ is synthesized, it is reacted with 2,5 equivalents of silver oxalate in water for 3 hours (**Fig 2.3.1).** The round bottom flask where is carried out the reaction must be covered with foil. The yellow solution is filtered with vacuum using silica gel in order to prevent the AgCl (white precipitate) passes to the solution. The solvent is evaporated with the rotavapor and verify with ^{1}H NMR the evidence of [(η^6-Toluen)(oxalate)(H_2O)Ru] is obtained (**Fig.2.3.2**) with 73% of yield.

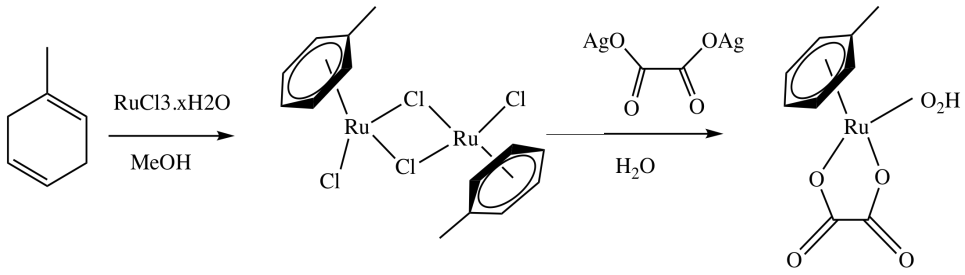

Fig.2.3.1. Synthesis of [(η^6-Toluene)$RuCl_2$]$_2$ and [(η^6-Toluene)(oxalate)(H_2O)Ru]

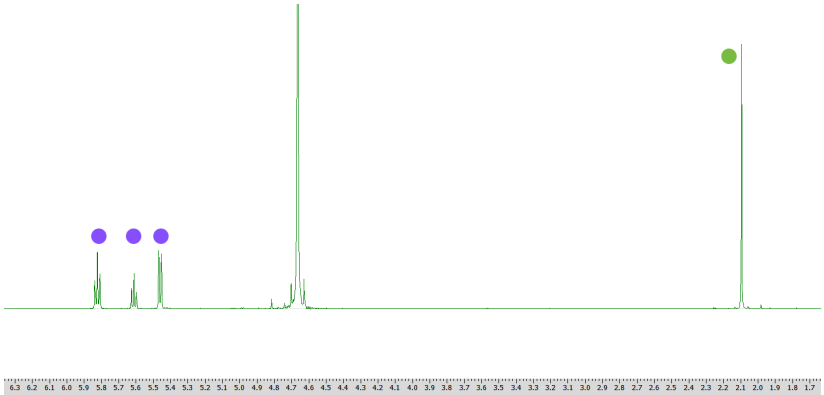

Fig 2.3.2. ^{1}H NMR spectrum of [(η^6-Toluene)(oxalate)(H_2O)Ru] (D_2O). Ru arene-purple, CH_3 (methyl)-green.

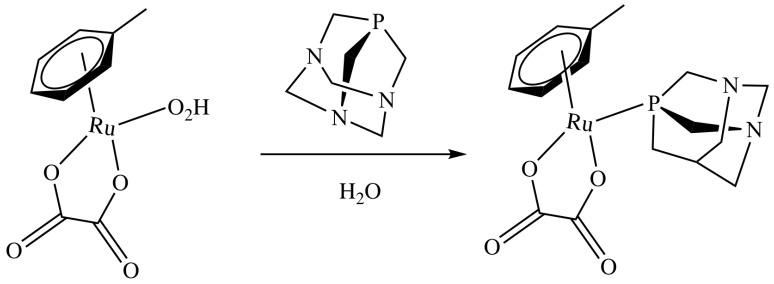

Fig 2.3.3. Synthesis of [(η^6-Toluene)(oxalate)(PTA)Ru]

As **Fig 2.3.3** shows, the complex [(η^6-Toluen)(oxalate)(H_2O)Ru] is reacted with 1 equivalent of PTA in water for 2 hours. The solvent is evaporated because a chromatographic column will have to be made with MeOH/Water (1:1) as solvent. The fractions with the complex, [(η^6-Toluen)(oxalate)(PTA)Ru], is collected and the solvent is removed with the rotavapor. For the crystallization, it is dissolved in the minimum quantity of MeOH to make the solution as concentrated as possible. The saturated solution is added in a vial, which is introduced into a flask containing diethyl eter, whose vapours are slowly introduced into the vial (crystallization by vapour diffusion), causing the crystallization of the [(η^6-Toluen)(oxalate)(PTA)Ru]. Yellow crystals are checked by ^{1}H NMR (**Fig.2.3.4**), ^{13}C NMR (**Fig.2.3.5**) and ^{31}P NMR (**Fig.2.3.6**) spectra the evidence that the complex [(η^6-Toluen)(oxalate)(PTA)Ru] is formed because this is the first time is synthesised with 56% of yield.

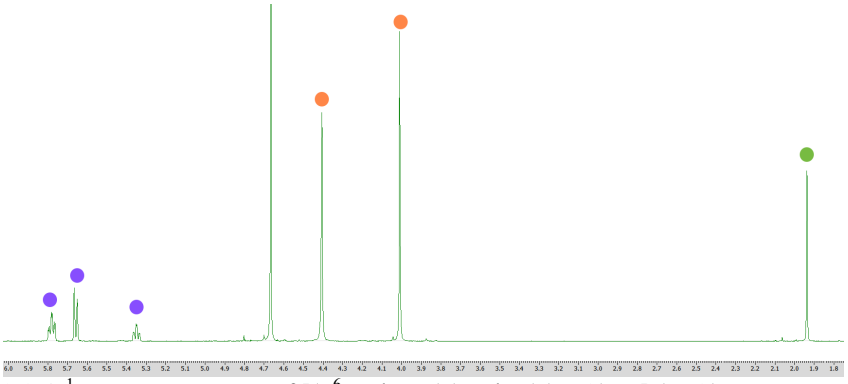

Fig 2.3.4. ^{1}H NMR spectrum of [(η^6-Toluene)(oxalate)(PTA)Ru] (D_2O). Ru arene-purple, PTA- orange, CH_3 (methyl)-green.

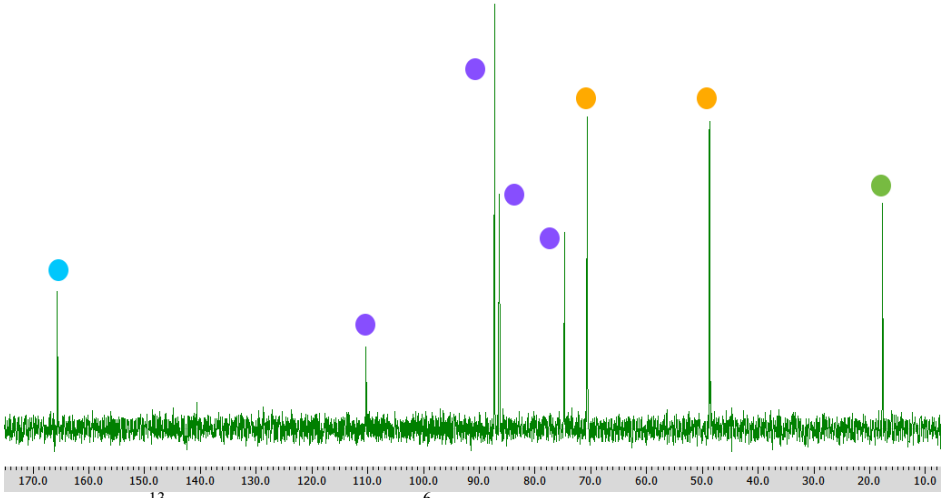

Fig 2.3.5. ^{13}C NMR spectrum of [(η^6-Toluene)(oxalate)(PTA)Ru] (D_2O). Ru arene-purple, PTA- orange, CH_3 (methyl)-green, C(oxalate)- blue.

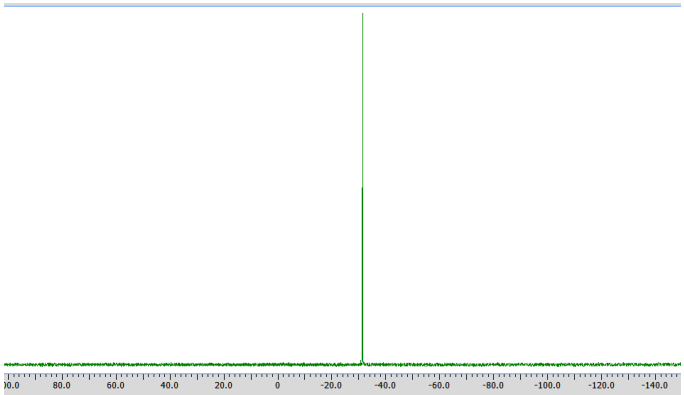

Fig 2.3.6. ^{31}P NMR spectrum of [(η^6-Toluene)(oxalate)(PTA)Ru] (D_2O). Phosphorus PTA signal.

2.4. Synthesis of [(η^6-Toluene)(PTA)$RuCl_2$].

With the dimer [(η^6-Toluen)$RuCl_2$]$_2$ obtained in section *2.3. Synthesis of [(η^6-Toluene)(oxalate)(PTA)Ru]*, is reacted with 1 equivalent of PTA in MeOH for 3 hours as **Fig 2.4.1** shows. If at the end of the reaction there is precipitate, it would mean this is filtered. With the red solution, helping with the rotavapor the necessary volume is evaporated until the solution is saturated, then it is left in the refrigerator to obtain the complex [(η^6-Toluene)(PTA)$RuCl_2$]. In addition, the red solid is filtered and checked by ^{1}H NMR spectra (see **Fig 2.4.2**).the evidence that the complex has been obtained with a 78% of yield.

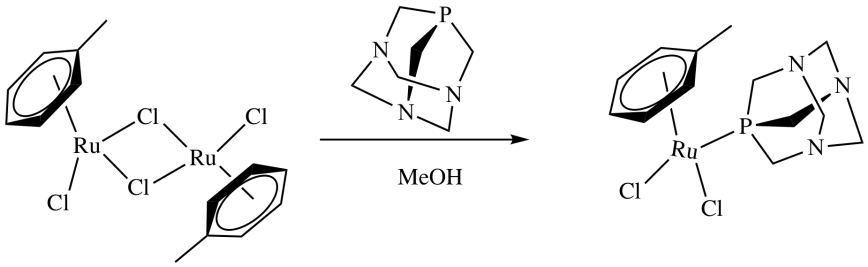

Fig 2.4.1. Synthesis of [(η^6-Toluene)(PTA)$RuCl_2$]

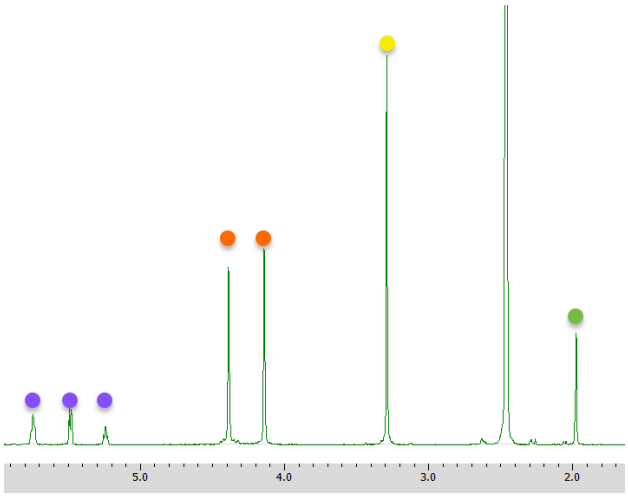

Fig 2.4.2. ^{1}H NMR spectrum of [(η^6-Toluene)(PTA)$RuCl_2$] (DMF-d_7). Ru arene-purple, CH_3 (methyl)-green, PTA-orange, MeOH- yellow.

3. RESULTS AND DISCUSSION.

3.1. Synthesis of [(η^6-P-cymene)(PTA)$RuCl_2$].

The final research with dichloromethane as solvent in the last reaction in section *2.1.Synthesis of [(η^6-P-cymene)(PTA)$RuCl_2$]* makes the final compound pure enough as shown in the elemental analysis in **Table 3.1.1.** The reaction of *of* [(η^6-P-cymene)$RuCl_2$]$_2$ with PTA had first been tried with MeOH as solvent but the impurities persisted. With Acetone/ dichloromethane (1:1) as solvent, the acetone reacted with the compound releasing the aromatic ring from the metal complex. Finally, with only having dichloromethane as a solvent in the reaction of [(η^6-P-cymene)$RuCl_2$]$_2$ with PTA allows to obtain the complex pure and in a simple way.

Table 3.1.1. Results of elemental analysis of [(η^6-P-cymeno)(PTA)$RuCl_2$]

	Analysis % expected	**Results % found**
C	41,45	41,11
H	5,66	5,55
N	9,07	8,81

3.2. Synthesis of [(η^6-P-cymene)(oxalate)(PTA)Ru].

With a typical crystallization by slow cooling of a saturated solution, crystals with sufficient purity are not obtained. A simple crystallization method is vapour diffusion crystallization technique carried out and commented in section *2.2 Synthesis of* [(η^6-P-cymene)(oxalate)(PTA)Ru] which allows to obtain small crystals. Correspondingly elemental analysis is carried out to verify if they are pure enough to be able to realize the studies in cells.

As presented in **Table 3.2.1** the complex *of* [(η^6-P-cymene)(oxalate)(PTA)Ru] is pure enough to be able to execute the studies in cells.

Table 3.2.1. Results of elemental analysis of [(η^6-P-cymeno)(Oxalate)(PTA)Ru]

	Analysis % expected	Results % found
C	44,98	44,73
H	5,46	5,64
N	8,75	8,63

3.3. Synthesis of [(η^6-Toluen)(oxalate)(PTA)Ru].

Vapor diffusion crystallization technique is carried out and mentioned in section *2.3 Synthesis of [(η^6-Toluen)(oxalate)(PTA)Ru]* which allows to obtain small crystals, which an elemental analysis is carried out to verify if they are pure enough to be able to realize the studies in cells. With a crystallization by slow cooling of a saturated solution, crystals with sufficient purity are not obtained.

As the illustration of **Table 3.3.1** shows the complex [(η^6-Toluen)(oxalate)(PTA)Ru] is pure enough to be able to execute the studies in cells.

Tabla 3.3.1. Resultados análisis elemental of [(η^6-Toluen)(Oxalate)(PTA)Ru]

	Analysis % expected	Results % found
C	38,71	38,87
H	4,98	4,81
N	9,03	9,11

3.4. Synthesis of [(η^6-Toluen)(PTA)RuCl$_2$].

The compound is obtained quickly, simply and with enough pure to be able to comprehend studies in cells with metastases as can be verified in the results of elemental analysis in **Table 3.4.1.**

Tabla 3.4.1. Resultados análisis elemental of [(η^6-Toluene)(PTA)$RuCl_2$]

	Analysis % expected	Results % found
C	37,04	36,96
H	4,79	4,60
N	9,98	9,68

4. AQUATION AND STABILITY STUDIES

It was found that a pH > 7 the DNA is not damage but below pH 7 DNA damage is predominant. Healthy cells grow at pHs typically 7,4 and cancer cells have lower pH, typically pH 6,8.It has been studied that ruthenium(II)-arene complex with PTA as ligand show DNA damaged in a pH typical of hypoxic tumour cells and at pH of healthy cells, the DNA is little or no damage[6,7]. The process, which it is generally believed the key activation step inside the cell before the drug achieves the intracellular DNA target, is the hydrolysis. The neutral complex in water releases chloride in equilibrium, leaving an hydrolysis product (see **Fig 4.1**) that is believed it is the real active antitumor agent[8]. It has been proposed that the hydrolysis of the Ru-Cl bond may activate the complex for DNA binding[9]. Moreover, when the hydrolysis is done, the PTA ligand when is protonated at low pH (**Fig 4.2**) and this protonated form is considered to be the active agent and this form can cause damage to the DNA of cancerigen cells.

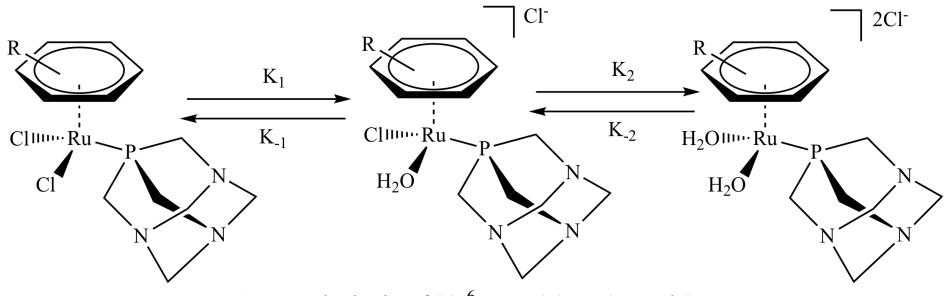

Fig 4.1. Hydrolysis of [(η^6-arene)(PTA)$RuCl_2$]

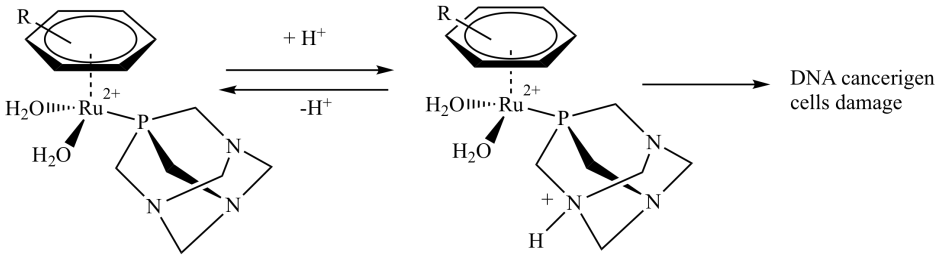

Fig 4.2 Protonation of the PTA ligand in a [(η^6-arene)(PTA)$RuCl_2$] complex.

To test that hypothesis, the RAPTA compounds [(η^6-P-cymene)(PTA)$RuCl_2$] and [(η^6-toluene)(PTA)$RuCl_2$] are studied paying attention to their aqueous chemistry with a buffer solution and chloride concentration using ^{1}H NMR to measure the rate of Hydrolysis.

A Phosphate buffer solution of 0,1997M is made with a pH 7,0. From this solution another buffer solution is made with pH 7,4 and concentration of 0,3464M. To be able to use them in the studio using ^{1}H NMR an exchange of protons is made by deuterium. In addition, 10 ml of each buffer solutions being added into different round bottom flasks, the solvent is then evaporated with the rotavapor and the solid is dried in the vacuum line. Furthermore, 2 mL of D_2O is added to dissolve the solid, the solvent is removed with the rotavapor and the solid is dried in vacuum (this step is repeated 3 times for each solutions). After the protons exchange by deuterium is done, 10mL of D_2O are added in each round bottom flask having a concentration of $1{,}997 \cdot 10^{-3}$M from the 0,1997M solution, $3{,}464 \cdot 10^{-3}$M from the 0,3464M solution with pD 7,43 and 7,84 respectively (pD = pH + 0,4).

To be representative of blood plasma it is necessary to work with the pD 7,43 solution with 150mM NaCl and 5mMNaCl, extra and intracellular NaCl concentration respectively. Then, for the 100mM NaCl solution, 43,8 mg were added into 5mL of the pD 7,43 solution. For the 5mM NaCl concentration, a extra solution with 73mg of NaCl in 1mL D_2O is done, then 20µL of this solutions were added into 5 mL of the pD 7,43 solution.

4.1. Hydrolysis of [(η^6-P-cymene)(PTA)$RuCl_2$] in aqueous, buffered and salt solutions.

Hydrolysis of [(η^6-P-cymene)(PTA)$RuCl_2$] was studied using ^{1}H NMR spectrometry. First of all, 23mg of the complex was dissolved in 0,5ml pD 7,43 buffered solution and extracellular NaCl concentration (150mM) making a concentration of 0,01M [(η^6-P-cymene)(PTA)$RuCl_2$] . Paying attention in the aromatic part of the spectrum (**Fig 4.1.1**) it shows that we already have a starting material and the complex definitely reacts with the solvent, the phosphate buffered solution or with the NaCl forming new complex (the exact nature of the new complex is not characterized). Nevertheless, the signal tiny take out really comparing with the original complex (see **Fig 4.1.1)**, so does not place great importance concluding that the complex it is not hydrolysed and is stable in these conditions. The complex is characterized 24h later and the ^{1}H NMR spectrum does not change.

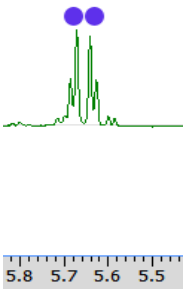

Fig 4.1.1. ^{1}H NMR aromatic part spectrum of the [(η^6-P-cymene)(PTA)$RuCl_2$] hydrolysis of complex with a buffer solution pD 7,43 and 150mM NaCl. Ru-arene-purple.

The behaviour is remarkable different dissolving the same quantity of [(η^6-P-cymene)(PTA)$RuCl_2$] with the buffer solution pD 7,43 and 5mM NaCl concentration. As **Fig 4.1.2** shows, a new complex is formed because there are new aromatic signals next to the originals, which become smaller. The complex is characterized each 2h and the ^{1}H NMR spectrum but it does not change.

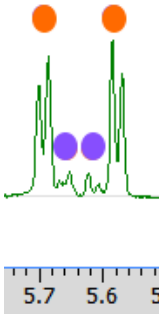

Fig 4.1.2. ^{1}H NMR aromatic part spectrum of the [(η^{6}-P-cymene)(PTA)RuCl$_2$] hydrolysis of complex with a buffer solution pD 7,43 and 5mM NaCl. Original complex Ru-arene- purple, different complex – orange.

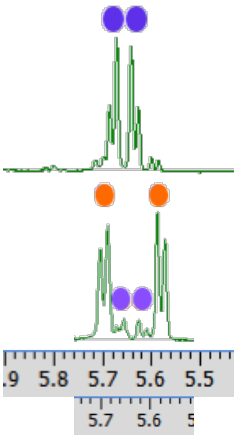

Fig. 4.1.3. ^{1}H NMR aromatic part spectrum of the [(η^{6}-P-cymene)(PTA)RuCl$_2$] hydrolysis of complex with a buffer solution pD 7,43 and 150mM NaCl (up) and 5mM NaCl (down). Original complex Ru-arene- purple, new complex – orange.

It is not known whether the compound is hydrolysed because the exact nature of the new complex is not characterized, but definitively at low concentration of NaCl the compound [(η^{6}-P-cymene)(PTA)RuCl$_2$] reacts as **Fig 4.1.3** shows in a comparative pictures. This would mean the compound begins to react with NaCl, H_2O or the phosphate buffered solution in intracellular conditions but does not react in extracellular conditions.

4.2. Hydrolysis of [(η^6-toluene)(PTA)$RuCl_2$] in aqueous, buffered and salt solutions.

23mg of [(η^6-toluene)(PTA)$RuCl_2$] is dissolved in a pD 7,43 buffered solution with a 150mM of NaCl (extracellular NaCl concentration) making 0,01M [(η^6-toluene)(PTA)$RuCl_2$] concentration for the hydrolysis study. Aromatic signals of the spectrum (**Fig 4.2.1**) show something reacts but the starting material is still in the highest quantity. The complex is characterized 24h later and the ^{1}H NMR spectrum does not change. The different signals as the original compound are tiny as **Fig 4.2.1** shows, so it does not place great importance again, concluding that the complex it is not hydrolysed and is table in these conditions.

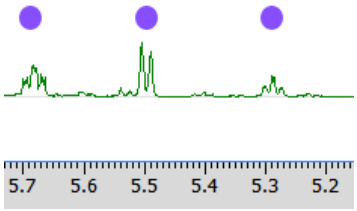

Fig 4.2.1. ^{1}H NMR spectrum of the [(η^6-toluene)(PTA)$RuCl_2$] hydrolysis of complex with a buffer solution pD 7,43 and 150mM NaCl. Original complex: aromatic- purple.

Using the intracellular NaCl concentration (5mM NaCl) in the pD 7,43 buffered solution the complex, as can be seen in **Fig 4.2.2,** the complex reacts forming a new compound at first time. With the step from the hours (see **Fig 4.2.3, Fig 4.2.4** and **Fig 4.2.5**), the starting material reacts completely. Finally, only remains the new complex whose nature is not characterized but concluding that at low intracellular concentration of NaCl the compound [(η^6-toluene)(PTA)$RuCl_2$] reacts, while with the extracellular concentration of NaCl does not reacts.

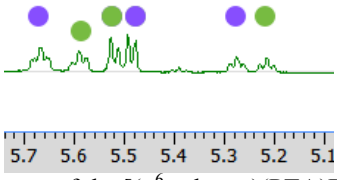

Fig 4.2.2. ^{1}H NMR spectrum of the [(η^{6}-toluene)(PTA)$RuCl_2$] hydrolysis of complex with a buffer solution pD 7,43 and 5mM NaCl. Original complex: aromatic- purple. New complex: aromatic-green.

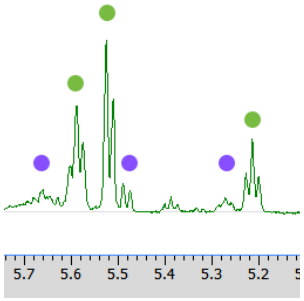

Fig 4.2.3. ^{1}H NMR spectrum of the [(η^{6}-toluene)(PTA)$RuCl_2$] hydrolysis of complex with a buffer solution pD 7,43 and 5mM NaCl 2 hours later. Original complex: aromatic-purple. New complex: aromatic-green.

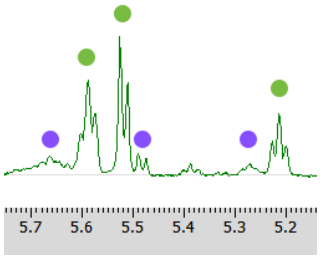

Fig 4.2.4. ^{1}H NMR spectrum of the [(η^{6}-toluene)(PTA)$RuCl_2$] hydrolysis of complex with a buffer solution pD 7,43 and 5mM NaCl 4 hours later. Original complex: aromatic-purple. New complex: aromatic-green.

5. COMPARATION WITH THE CAPTA-Ru COMPOUNDS.

RAPTA-type compounds with their solubility in water give by the PTA ligand have been identified as promising anticancer agents with low toxicity and tolerance to low pH. The modification of the PTA ligand sometimes increases cytotoxicity, but the selectivity to the cancer cells is lost. The new ligand 1,4,7-triaza-9-phosphatricyclo[5.3.2.1]tridecane, CAP, was discovered[10]. CAP has a cage similar but with a higher cage flexibility because the presence of two CH_2 between the N atoms, bringing different reactivities (see **Fig 5.1**). The protonation at N atoms of CAP ligand due to the more electron donicity that P and N showed of CAP.

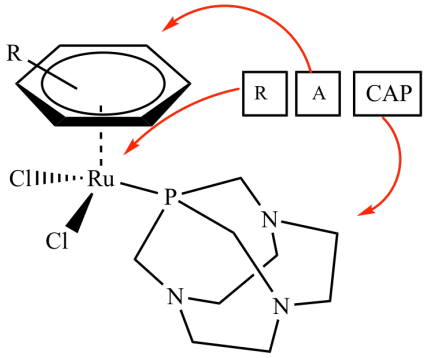

Fig. 5.1. Generic Structure of RACAP compounds.

The stability of $[(\eta^6\text{-P-cymene})(CAP)RuCl_2]$ under pseudopharmacological conditions were determinate in aqueous $NaCl/D_2O$ (100mM) by ^1H NMR. Complex $[(\eta^6\text{-P-cymene})(CAP)RuCl_2]$ is stable in these conditions[11]. Moreover, the molecular structure was studied and compared with the analogue RAPTA compound showing the Ru-P bond length is significantly longer that PTA structure. The same applies to the Ru-C bond lengths.

The cytotoxicity to human ovarian carcinoma cells of $[(\eta^6\text{-P-cymene})(CAP)RuCl_2]$ was studied in Guerriero, A.; Oberhauser, W.; Riedel, T.; Peruzzini, M.; Dyson, P. J.; Gonsalvi, L. *Inorg. Chem.* **2017**, *56* (10), 5514–5518. Compared to $[(\eta^6\text{-P-cymene})(PTA)RuCl_2]$, the CAP analogue is more cytotoxic toward cell lines and the cancer cell selectivity is maintained.

In conclusion, this new ruthenium(II) arene half-sandwich complex bearing the CAP ligand is stable in extracellular conditions as the PTA analogue. Furthermore, the CAP compound is more cytotoxic to cancer cells with a reasonable selectivity to cancer cells.

6. SUBSTANCES TO BE USED

Starting materials, reagents and solvents, products and by-products.	***Hazards***	***Exposure limit (WEL) – if applicable***	***Source of information (requires appropriate detail so it can be traced)***
α-phellandrene	R10: Flammable R22: Harmful if swallowed R36/37/38: Irritating to eyes, respiratory system and skin R43: May cause sensitization by skin contact.		Sigma Aldrich product page
1-Methyl-1,4-cyclohexadiene	R11: Highly Flammable. R36: Irritating to the eyes.		Sigma Aldrich product page
$RuCl_3.xH_2O$	R43: May cause sensitization by skin contact.		Sigma Aldrich product page
Ethanol	R11: Highly Flammable	1000 ppm/1920 $mg.m^{-3}$ (8 hr time weighted average reference period).	Sigma Aldrich product page and http://www.hse.gov.uk/pubns/priced/eh40.pdf
Acetone	R11: Highly Flammable R36: Irritating to the eyes R66: Repeated exposure may cause skin dryness or cracking R67: Vapors may cause drowsiness and dizziness	500 ppm/1210 $mg.m^{-3}$ (8 hr time weighted average reference period).	Sigma Aldrich product page http://www.hse.gov.uk/pubns/priced/eh40.pdf

$[\eta^6$-areneRuCl$_2]_2$	These compounds have not previously been evaluated for risks. It is reasonable to exercise the usual caution when handling a novel compound and assume the hazards are those emanating from the constituent starting materials. Assume:- R34: Causes burns R36/37/38: Irritating to eyes, respiratory system and skin R43: May cause sensitization by skin contact.		Sigma Aldrich product page
Silver oxalate	Not commercially available – treat as potentially harmful and exercise caution when handling.		
PTA	None given – handle with care.		
Hexane	R11: Highly flammable R38: Irritating to the skin R48/20: Harmful: danger of serious damage to health by prolonged exposure through inhalation R51/53: Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment R62: Possible risk of impaired fertility R65: Harmful: May cause lung damage if swallowed. R67: Vapors may cause drowsiness and dizziness	20 ppm/72 mg.m^{-3} (8 hr time weighted average reference period).	http://www.hse.gov.uk/pubns/priced/eh40.pdf Sigma Aldrich product page
Dichloromethane	R36/37/38: Irritating to eyes, respiratory system and skin R40: limited evidence of a carcinogenic effect R67: vapours may cause drowsiness and dizziness	100 ppm/350 mg.m^{-3} (8 hr time weighted average reference period).	http://www.hse.gov.uk/pubns/priced/eh40.pdf Sigma Aldrich product page

Methanol	R11: highly flammable R23/24/25: Toxic by inhalation, in contact with skin and if swallowed. R39/23/24/25: Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed.	200 ppm/266 $mg.m^{-3}$ (8 hr time weighted average reference period).	http://www.hse.gov.uk/pubns/priced/eh40.pdf Sigma Aldrich product page
Diethyl ether	R12: Extremely flammable R19: May form explosive peroxides R22: Harmful if swallowed R66: Repeated exposure may cause skin dryness or cracking R67: Vapors may cause drowsiness and dizziness	100 ppm/310 $mg.m^{-3}$ (8 hr time weighted average reference period).	http://www.hse.gov.uk/pubns/priced/eh40.pdf Sigma Aldrich product page
Silica gel	Eye: May cause eye irritation. Skin: May cause skin irritation. May be harmful if absorbed through the skin. Ingestion: May cause irritation of the digestive tract. May be harmful if swallowed. Inhalation: May cause respiratory tract irritation. May be harmful if inhaled. Chronic: Chronic inhalation of dust may lead to silicosis. Chronic inhalation can cause pneumoconiosis.	0.08 $mg.m^{-3}$ (8 hr time weighted average reference period).	http://www.hse.gov.uk/pubns/priced/eh40.pdf http://www.fishersci.com/ecomm/servlet/msdsproxy?productName=S70425&productDescription=SILICA+GEL+60+200M+GR62+25KG&catNo=S704-25&vendorId=VN00033897&storeId=10652
Mononuclear ruthenium product.	These complexes are novel and will be treated as potentially toxic/harmful due to their ability to coordinate with endogenous ligands.		
D_2O	N/A	N/A	N/A
$CDCl_3$	H30: Harmful if swallowed. H315: Causes skin irritation. H319: Causes serious eye irritation. H331: Toxic if inhaled. H351: Suspected of causing cancer. H361d: Suspected of damaging	2 ppm/9.9 $mg.m^{-3}$ (8 hr time weighted average reference period).	http://www.hse.gov.uk/pubns/priced/eh40.pdf Sigma Aldrich product page

	the unborn child. H372 Causes damage to organs through prolonged or repeated exposure.		
DMF-d_7	H226: Flammable liquid and vapour. H312 + H332: Harmful in contact with skin or if inhaled. H319: Causes serious eye irritation. H360: May damage fertility or the unborn child.	5 ppm/15 $mg.m^{-3}$ (8 hr time weighted average reference period).	http://www.hse.gov.uk/pubns/priced/eh40.pdf Sigma Aldrich product page

7. FUTUR RESEARCHS

Currently, the development of new organometallic ruthenium complex as potential anticancer agents is booming. The great diversity of ligands, which can be used in theses compounds and the study of this variety of compounds in cancerigen cells, is in the research focus in the main research laboratories.

The perfect antidote against cancer is in process.

8. CONCLUDING REMARKS

Extensive studies on the anticancer properties of ruthenium(II) compounds have been reported, two classes comprising [(η^6-arene)(Oxalate)(PTA)Ru] and [(η^6-arene)(PTA)$RuCl_2$. Four different compounds have been synthetized, with enough purity for studies in cancerigen cells, quick and easily, [(η^6-P-cymente)(PTA)$RuCl_2$]. [(η^6-toluene)(PTA)$RuCl_2$], [(η^6-P-cymente)(oxalate)(PTA)Ru] and [(η^6-toluene)(oxalate)(PTA)Ru].

In comparison with others reports, the hydrolysis of [(η^6-P-cymente)(PTA)$RuCl_2$], the results obtained coincided with those expected, the non-hydrolysis with extracellular liquid and the hydrolysis with intracellular liquid. For the [(η^6-toluene)(PTA)$RuCl_2$] the results expected are obtained as well as [(η^6-P-cymente)(PTA)$RuCl_2$][12] .

The hydrolysis studies for the [(η^6-arene)(Oxalate)(PTA)Ru] were not studied because the limit time of the training. It is supposed the oxalate group plays as a protecting group role avoiding the hydrolysis with high and low concentration of NaCl[13].

Great variety of different RAPTA compounds can be used to study their behaviour in cancerigen cells. About 40% of men and women will receive a diagnosis of cancer at any point in their life, finding the cure is a very important fact.

9. REFERENCES

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