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Synthesis and anticancer activity evaluation of η^5-$C_5(CH_3)_4R$ ruthenium complexes bearing chelating diphosphine ligands†

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The complexes [RuCp*(PP)Cl] (Cp* = C_5Me_5; [1], PP = dppm; [4], PP = Xantphos), [RuCp$^{\#}$(PP)Cl] (Cp$^{\#}$ = $C_5Me_4(CH_2)_5OH$; [2], PP = dppm; [5], PP = Xantphos) and [RuCp*(dppm)(CH_3CN)][SbF_6] [3] were synthesized and evaluated *in vitro* as anticancer agents. Compounds 1-3 gave nanomolar IC_{50} values against normoxic A2780 and HT-29 cell lines, and were also tested against hypoxic HT-29 cells, maintaining their high activity. Complex 3 yielded an IC_{50} value of 0.55 ± 0.03 µM under a 0.1% O_2 concentration.

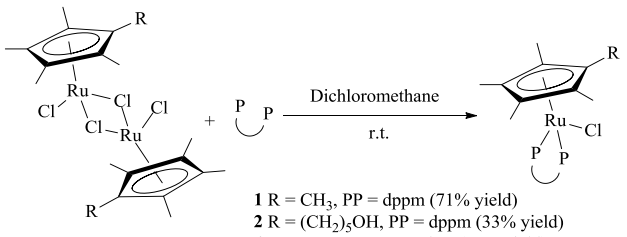
Numerous organometallic (η^6-arene)-ruthenium complexes have been screened as anticancer agents with promising results, for instance, compounds of the types [(η^6-arene)Ru(NN)Cl]$^+$ (NN = chelating nitrogen ligands, especially ethylenediamine (en)),[1-3] [(η^6-arene)Ru(NO)Cl] (NO = 3′-fluorophenyl-3-(phenylamino)-2-buten-1-one),[4] [(η^6-arene)Ru(OO)X] (OO = 3-hydroxyflavone derivatives, X = Cl, Br or I)[5, 6] or [(η^6-arene)Ru(pta)Cl_2] (RAPTA) (pta = 1,3,5-triaza-7-phosphatricyclo [3.3.1.1] decane).[7, 8] Samuelson and co-workers have published the use of η^6-*p*-cymene ruthenium complexes with different diphosphines acting as either monodentate or chelating ligands, which showed good growth inhibitions against several cancer cell lines.[9] In contrast, fewer examples of η^5-cyclopentadienyl (Cp) or pentamethylcyclopentadienyl (Cp*) compounds have been biologically evaluated. Sava reported the synthesis and activity against TS/A adenocarcinoma of the compounds [(η^5-C_5H_5)Ru(pta)$_2$Cl] and [(η^5-C_5Me_5)Ru(pta)$_2$Cl], as equivalents to the RAPTA complexes.[10] Compounds of the type [(η^5-C_5H_5)Ru(PP)L][X] (PP = 2 × PPh_3 or 1,2-bis(diphenylphosphino)ethane, L = planar nitrogen σ-bonded ligand and X = CF_3SO_3 or PF_6) have been synthesised by Moreno *et al*. and some of them show better cytotoxicities than cisplatin.[11-13] However, none of these Cp/Cp* ruthenium complexes has been tested under hypoxic conditions. Some diphosphines have demonstrated cytotoxicity against various cell lines,[14] but it has been observed that, upon coordination to metals, diphosphines produce complexes with improved anticancer activity compared to the free ligands; a general hypothesis considers that the metal protects the ligands from oxidation before they interact with the corresponding biological target.[15]

Here we present the results obtained from cell line assays carried out under normoxic and hypoxic conditions with ruthenium complexes containing chelating diphosphine ligands such as 1,1-bis(diphenylphosphino)methane (dppm) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos). The complexes have general structures [RuCp*(PP)Cl] (**1**, PP = dppm; **4**, PP = Xantphos), [RuCp$^{\#}$(PP)Cl] (Cp$^{\#}$ = $C_5Me_4(CH_2)_5OH$; **2**, PP = dppm; **5**, PP = Xantphos) and [RuCp*(PP)(CH_3CN)][SbF_6] (**3**, PP = dppm). We investigated the biological activity of both ligands and the effect of complexation. We were interested in assessing the impact of hydrophilic functionalisation of Cp* with an –OH group and the different cytotoxicities shown by analogous neutral and charged complexes. The anticancer activities were assessed against A2780 and HT-29 cell lines, for HT-29 both at 21% and 0.1% O_2 (hypoxic conditions) concentrations.

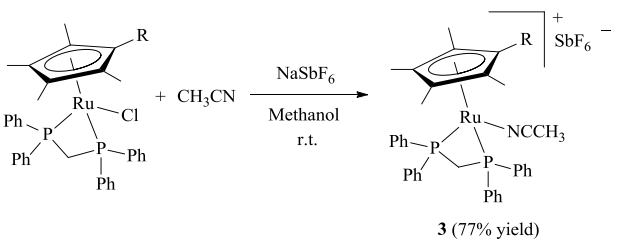
Complexes **1** and **4** were synthesised from [RuCp*Cl_2]$_2$, which was obtained following literature methods.[16, 17] A similar method was employed for compounds **2** and **5**, starting from the novel [RuCp$^{\#}Cl_2$]$_2$ complex (Scheme 1). This in turn was prepared by reaction of (5-hydroxypentyl)-tetramethylcyclopentadiene[18] with $RuCl_3$ in ethanol at reflux. Compounds **1**[19, 20] and **4**[21] had been previously reported, but not biologically tested. Complex **3** was obtained from complex **1**, acetonitrile and $NaSbF_6$ in methanol at room temperature (Scheme 2). This method was adapted from the published synthesis of [RuCp*(PP)(CH_3CN)][PF_6] complexes, where PP = chiral diphosphines.[22] The structure of complex **3** was determined by single crystal X-ray diffraction. Compound **3** crystallised in a triclinic cell from pentane/chloroform, and the structural solution was performed in the space group $P\bar{1}$. The asymmetric unit comprises one molecule of compound **3**, including the counterion SbF_6. The molecular structure of **3** is shown in Figure 1 and selected bond lengths and angles are given in Table 1. Compound **3** presents the characteristic piano-stool geometry typical of η^5- and η^6-organometallic ruthenium species. The N(1)-C(11) triple bond length is 1.153(2) Å.

The cytotoxic activities of compounds **1**-**5**, along with cisplatin, dppm and Xantphos were tested on the A2780 and HT-29 cell lines after five-day exposures at 37 ºC and 21% O_2. The IC_{50} results are shown in Table 2. The most active complexes were those formed from dppm, **1**, **2** and **3**, all with better cytotoxicities, in the nanomolar range, than cisplatin for both HT-29 and A2780 cell lines.

Dppm was active by itself, with IC_{50} values below 1.5 μM. However, ^{1}H and ^{31}P NMR spectroscopy experiments in deuterated DMSO showed no de-coordination of dppm from complexes **1** and **3** after five days. The observation that diphosphines do not dissociate is further reinforced by the fact that complexes **4** and **5** gave moderate to good activities, which are not due to a possible release of the ligand, because Xantphos did not show anticancer behaviour on its own. This contradicts the previous hypothesis that the activities of these types of diphosphine complexes depend on possible de-coordinations of the ligands.[15] The extremely different behavior of dppm and Xantphos provides interesting material for further future studies. Complexes **4** and **5** were more active against A2780 cells, with IC_{50} values close to cisplatin. The presence of the $(CH_2)_5OH$ chain in the $Cp^{\#}$ compounds **2** and **5** produced no great effect on their anticancer activities, compared to those of the Cp* complexes **1** and **4**. The best cytotoxicity was observed for the positively charged complex **3**.



Scheme 1. General synthesis of complexes **1**, **2**, **4** and **5**.



Scheme 2. Synthesis of complex **3**.

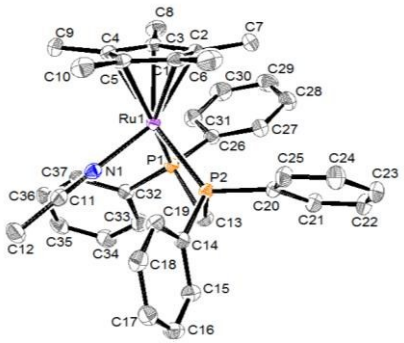


Figure 1. ORTEP structure of complex **3** (cation) with thermal ellipsoids set at 50% probability. Hydrogen atoms omitted for clarity.

To assess the extent of hydrolysis[23] in complexes **1** and **3**, 10 mM samples of both complexes in 0.6 ml of deuterated solvent (90% deuterated DMSO + 10% deuterium oxide) were prepared in NMR tubes and analysed by ^{1}H NMR spectroscopy every 24 hours during five days at room temperature. A new set of peaks at 5.14 and 1.61 ppm appeared gradually in both samples (see Fig. S1 in the ESI). The new species formed, after five days, in 48% yield from complex **1** and in 67% yield from complex **3**. Mass spectrometry of this new species showed the same peaks observed for the chloride complex **1**, where the chloride ligand was lost. By inference, the new species is believed to be the aqua species, which entails that monocationic complex **3** hydrolyses to a higher extent under the same conditions, and this coincides with its higher anticancer activity.

Table 1. Selected bond lengths [Å] and angles [°] in the structure of compound **3** with s.u.s. in parenthesis.

Ru(1)-N(1)	2.0775(16)
Ru(1)-P(1)	2.3201(6)
Ru(1)-P(2)	2.3503(6)
N(1)-C(11)	1.153(2)
C(11)-C(12)	1.481(3)
Ru(1)-Ring Centroid	1.884
Ru(1)-C(Cp*)	2.25226
P(1)-Ru(1)-P(2)	71.626(19)
N(1)-C(11)-C(12)	178.5(2)
C(11)-N(1)-Ru(1)	178.90(16)
P(2)-C(13)-P(1)	93.53(8)

Table 2 gives the IC_{50} results obtained for the most active compounds **1-3** against hypoxic HT-29 cells at an oxygen concentration of 0.1%. Cancerous cells are known to proliferate within hypoxic environments, with oxygen content below 2%,[24] therefore hypoxic experiments tend to reproduce the conditions found in human solid tumours. Apart from cisplatin, whose activity remains practically unmodified, tirapazamine, a drug known to be hypoxia sensitive,[25] was also employed as reference. Interestingly, the IC_{50} of dppm under hypoxic conditions increased considerably from 1.47 μM to 17.19 μM. A possible explanation for this is that the active species might be an oxidized form of dppm. However, Samuelson *et al*. have reported that, while dppm is moderately active against H460 lung cells (IC_{50} = 18.2 μM), mono-oxidised dppm shows no cytotoxic activity (IC_{50} > 250 μM),[9] and similar conclusions had been drawn by Sadler *et al*.[14] The activities of complexes **1-3** improved slightly at a low O_2 concentration. Complex **3** showed again the best performance, with an IC_{50} of 0.55 ± 0.03 μM, and is of particular significance and interest.

Table 2. IC_{50} values for complexes **1-5** along with cisplatin, tirapazamin, dppm and Xantphos. The drugs were incubated for 5 days at 37 ºC.

	IC_{50} (μM) at 21% O_2				**IC_{50} (μM) at 0.1% O_2**	
Compound	**A2780**	**±**	**HT-29**	**±**	**HT-29**	**±**
Cisplatin	1.4	0.3	2.52	0.09	2.4	0.4
Tirapazamine	-	-	31	3	2.8	0.4
dppm	1	1	1.47	0.02	17.19	0.08
1	1.1	0.2	0.73	0.05	0.66	0.03
2	0.9	0.1	0.791	0.007	0.76	0.03
3	0.70	0.02	0.61	0.01	0.55	0.03
Xantphos	>250	-	>250	-	-	-
4	3.6	0.4	10.1	0.5	-	-
5	4.0	0.3	11.9	0.7	-	-

Conclusions

In summary, a series of Cp*-based diphosphine ruthenium complexes (**1**-**5**) was prepared and biologically tested against A2780 and HT-29 cancerous cell lines. Both normoxic and hypoxic studies showed activities in the nanomolar range. The best anticancer activity was obtained with complex **3**, which maintained a low IC_{50} value even under hypoxic conditions with 0.1% O_2 concentration, and showed a higher degree of hydrolysis than its neutral analogue **1** under the same conditions. Future studies could include cationic versions of **2**, **4**, **5** and similar complexes to check whether they are generally more effective. Testing other free and coordinated phosphines and phosphine oxides could shed some light on the effect that the oxidation state and structure of the ligand have on cytotoxic activity.

Notes and references

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Electronic Supplementary Information (ESI) available: experimental procedures for compounds, cell line experimental and crystal structure determination details (CCDC 957987). See DOI: 10.1039/c000000x/

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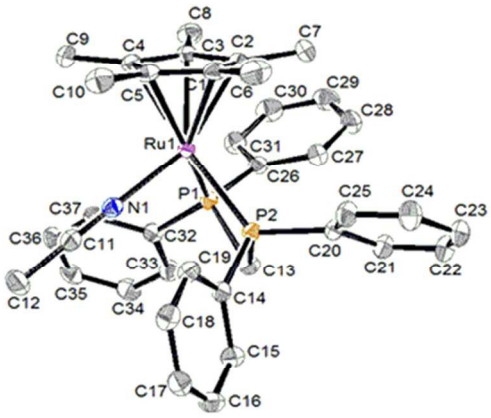
A series of ruthenium complexes with η^5-$C_5(CH_3)_4R$ and diphosphine ligands have been synthesised and evaluated *in vitro* as anticancer agents. IC_{50} values in the nanomolar range have been obtained against two types of cell lines.



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