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Enhancement of the antiproliferative activity of $[RuCp(PPh_3)_2(dmoPTA\text{-}1\kappa P)]^+$ *via* its coordination to one $\{CoCl_2\}$ unit: synthesis, crystal structure and properties of $[RuCp(PPh_3)_2\text{-}\mu\text{-}dmoPTA\text{-}1\kappa P{:}2\kappa^2 N,N'\text{-}CoCl_2](OTf)\cdot 0.25H_2O$.

Zenida Mendoza,[a] Pablo Lorenzo-Luis,[a] Franco Scalambra,[b] José M. Padrón[c] and Antonio Romerosa*[b]

Synthesis, characterization and the antiproliferative activity of the new bimetallic complex $[RuCp(PPh_3)_2\text{-}\mu\text{-}dmoPTA\text{-}1\kappa P{:}2\kappa^2 N,N'\text{-}CoCl_2]OTf\cdot 0.25H_2O$ are described. The stability of the complex was studied under air and N_2 in $CDCl_3$, DMSO, water and the cell culture medium, at room temperature and 40 °C. The complex showed an enhanced antiproliferative activity (up to six-fold) when compared with its parent complex $[RuCp(PPh_3)_2(HdmoPTA)]^{2+}$ against human lung, cervix, breast, and colon solid tumor cell lines.

The biological activity of a metal complex depends on its components, metal and ligands, but also on its behaviour in a cellular medium, as showed by the intense research targeted since 1965 to understand how the first found antiproliferative active complex, cisplatin,[1] acts. Despite of the long time passed, this complex continues to be in medical use although it produces adverse effects on patients and its activity remains unknown.[2] Replacing platinum is mandatory to obtain less toxic and cheaper metal complexes but active against cancers.[3] Organometallic complexes have shown interesting antiproliferative activity.[4] In particular, {Ru-η^6-arene}-complexes incorporating aqua-soluble phosphines have attracted considerable interest due to their hydrophilic phosphane coligands and the possibility of the inclusion of labile groups prone to be substituted.[5] The study of the mechanism of action of these complexes is nowadays in progress as it has been proved that proceeds through a different path than that known for platinum complexes.[1-6]

Recently, we observed that the ruthenium complex $[RuClCp(PPh_3)(HdmoPTA)]^+$, containing the ligand $HdmoPTA^+$ (HdmoPTA = 3,7-H-3,7-dimethyl-1,3,7-triaza-5-phosphabicyclo[3.3.1]nonane),[7] (*the so-called 1st generation*, Fig. 1) showed one of the strongest ability to inhibit in vitro the proliferation of human cancer cell lines.[8] Notably, this complex exhibits an excellent antiproliferative profile against the colon cancer cell line WiDr (GI_{50} = 1.7 μM), which is known to exhibit a large resistance to cisplatin exposure. Our early results have demonstrated that the ligand $(HdmoPTA)^+$ is able to coordinate by both P and N_{CH_3} atoms,[7] giving rise to bis-heterometallic complexes with differentiate antiproliferative activity. They showed GI_{50} values in the range 0.8–6.5 μM, which are comparable to those obtained for the standard anticancer drug cisplatin.[8,9] A step ahead in the synthesis of more active antiproliferative complexes was obtained by exchanging the Cl by a PPh_3 in $[RuClCp(PPh_3)(HdmoPTA)]^+$, leading to the new complex $[RuCp(PPh_3)_2(HdmoPTA)]^{2+}$ (**1**) (*the so-called 2nd generation*, Fig. 1). The antiproliferative activity of **1·2OTf** against human lung, cervix, breast, and colon solid tumor cell lines (GI_{50} = 0.17–0.29 μM, Table 1) was much higher than that observed for $[RuClCp(PPh_3)(HdmoPTA)]^+$ but also for the known platinum drugs and the ruthenium complexes described until now.[1]

Altogether, the obtained results suggested that our next step should be targeted to the synthesis of bis-heterometallic-complexes by coordination of a metal unit to dmoPTA-N_{CH_3} atoms of **1**, expecting that the resulting bis-metallic complex displays an enlarged activity than starting complex and previous complexes $[RuClCp(PPh_3)(HdmoPTA)]^+$ and $[RuClCp(PPh_3)\text{-}\mu\text{-}dmoPTA\text{-}1\kappa P{:}2\kappa^2 N,N'\text{-}MQ]$ (M = Co, Ni, Zn; Q = acac, Cl_2).[8,9] Herein we report the synthesis, spectroscopic characterization and X-ray crystal structure of the Ru-Co complex $[RuCp(PPh_3)_2\text{-}\mu\text{-}dmoPTA\text{-}1\kappa P{:}2\kappa^2 N,N'\text{-}CoCl_2](OTf)\cdot 0.25H_2O$ (**2·OTf·0.25H$_2$O**), and its antiproliferative activity against a panel of human cell lines.

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†Electronic Supplementary Information (ESI) available: synthesis, $^{31}P\{^1H\}$ NMR characterisation and growth cell cycle (PDF). CCDC1545888. For crystallographic data in CIF or other electronic format see DOI: 10.1039/x0xx00000x

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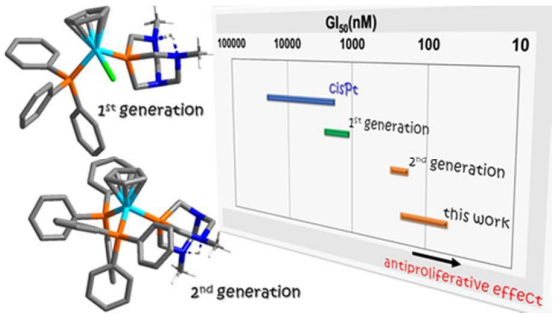
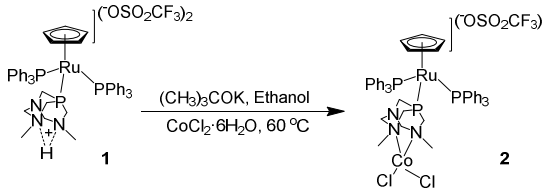


Fig. 1. Antiproliferative activity of cisplatin, 1st, 2nd generation HdmoPTA-Ru and the complex presented in this work against human solid tumour cell lines A549; HBL-100; HeLa; SW1573; T47D and WiDr, indicted as a range.

The synthesis of **2·OTf·0.25H$_2$O** (Scheme 1) was achieved by deprotonation of [RuCp(PPh$_3$)$_2$(HdmoPTA)](OTf)$_2$ (**1·2OTf**) with potassium *tert*-butoxyde and further reaction with $CoCl_2 \cdot 6H_2O$.



Scheme 1. Synthesis of **2·OTf 0.25H$_2$O**

Single crystals good enough for their analysis by single crystal X-ray diffraction were obtained by evaporating at room temperature under nitrogen from a EtOH solution of **2** (ESI). Complex **2·OTf·0.25H$_2$O** was found to be sparingly soluble in water ($S_{25ºC,H_2O}$ < 0.5 mg/mL) but soluble in a range of organic solvents such as chloroform ($S_{25ºC,CHCl_3}$ > 97.3 mg/mL). Its $^{31}P\{^1H\}$ NMR ($CDCl_3$) shows a multiplet at 211.35 ppm and a doublet at 40.77 ppm, far away from the chemical shift observed in **1·2OTf** ($\Delta\delta$ (**2**$_{dmoPTA}$ – **1**$_{HdmoPTA}$) = 172.75 ppm; $\Delta\delta$ (**2**$_{PPh_3}$ – **1**$_{PPh_3}$) = 54.86 ppm), as a consequence of the paramagnetism of the Co(II) coordinate to the dmoPTA-N_{CH_3}. A similar behavior was observed for parent bimetallic complexes [RuClCp(PPh$_3$)-μ-dmoPTA-1κ*P*:2κ^2*N*,*N*′-Co^{II}Q] (Q = acac, Cl$_2$).[9,10] The ^{1}H NMR signals were not possible to be undoubtedly assign but $^{13}C\{H\}$ NMR signals for aromatics (141.32-127.71 ppm) and Cp (91.03 ppm, which is shifted 5.54 ppm to that found in **1**).

The determination of the crystal structure (see ESI for the data) of this complex provided the final support to its Ru-Co bimetallic character. The crystal structure is shown in Fig. 2, selected crystallographic parameters, bonds and angles are respectively display in Table S1, S2 and S3. The asymmetric unit of **2·OTf·0.25H$_2$O** is constituted by two bi-metallic cationic complex [RuCp(PPh$_3$)$_2$-μ-dmoPTA-1κP:2k^{2}N,N′-CoCl$_2$]$^+$, two OTf anion and two disordered 0.25 water molecules. The distribution of ligands around rutheniums (Fig. 2a) in both complex molecules are basically the same to those found for previous structures containing HdmoPTA-Ru.[1,7] The coordination P_{dmoPTA}-Ru distance is found to be 2.370(9) Å, which is slightly shorter than in **1** (2.3208(1) Å)[1] but greater than that found for [RuClCp(PPh$_3$)HdmoPTA] (2.2767(3) Å).[7] The P_{PPh_3}-Ru distances are similar in one of the molecule (2.378(4) Å, 2.370(9) Å), which are similar to those in **1** (average value: 2.3777 Å), but significantly different in the other one (2.2911(9) Å, 2.3438(8) Å). Interestingly, the P1-Ru1-P3 angle in **2** is shorter to that in **1** (92.77(3)° versus 96.58(4)°) but **2** displays the largest angle (P2-Ru1-P3 = 99.73(3)°). This angle is also larger than those in bis-metallic complexes [RuClCp(PPh$_3$)dmoPTA-MCl$_2$] (M = Ni, Zn) (av. 96.8°)[9] and {[RuClCp(PPh$_3$)dmoPTA-M(acac-κ^2*O,O*′)$_2$] (M = Co, Ni, Zn) (av. 99.0°).[10] Additionally, the angle Cl3-Co1-Cl2 in **2** is found to be 126.7(10)°, which is in the range of those found in complexes [RuClCp(PPh$_3$)dmoPTA-MCl$_2$] [M = Ni (129.4(3)°), Zn (121.8(6)°)], unfortunately the respective complex with M= Co(II) was not structurally characterized yet.[9] No significant differences were found in the rest of the bond distances and angles with respect to parent dmoPTA-cyclopentadienyl Ru(II) complexes. The crystal packing diagram (Fig. 2b) shows weak intermolecular interactions among the molecules (C18A-H18A···F3 = 3.458(4), C23A-H23A···F2 = 3.259(4) Å) and C-H/π interactions between the aromatic centroid and the adjacent phenyl-C-H groups (centroid-to-C-H distances from 3.311(3) Å to 3.463(4) Å), which probably provide an additional stabilization of the structure of the complex.[1]

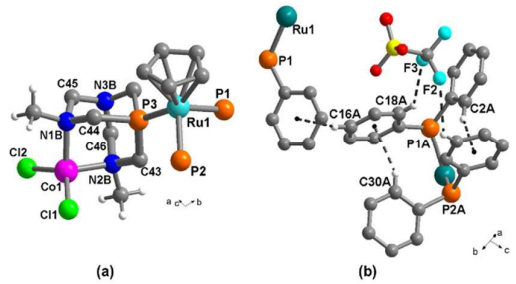


Fig. 2. A ball and sticks perspective drawing of **2** (a). Dashed lines represent the selected intermolecular interactions (b). For the sake of clarity most of the hydrogen and the dashed line C23A-H23A···F2 were omitted.

The antiproliferative activity tests of **2·OTf·0.25H$_2$O** using the standard protocol (see ESI) on six humans solid tumour cells lines together with those for **1·2OTf** and cisplatin, which were also tested at the same time for the sake of comparison, are shown in Table 1.

Table 1. GI_{50} values (μM) of complexes **1·2OTf**[a], **2·OTf·0.25H$_2$O**[b] and *cis*platin against several human solid tumor cells lines.

	Cell line (*origin*)					
	A549 (lung)	HBL-100 (breast)	HeLa (*cervix*)	SW1573 (*lung*)	T-47D (*breast*)	WiDr (*colon*)
1	0.29 (0.09)	0.21 (0.04)	0.17 (0.04)	0.20 (0.02)	0.25 (0.04)	0.20 (0.03)
2	0.062 (0.019)	0.088 (0.008)	0.084 (0.022)	0.054 (0.013)	0.21 (0.05)	0.065 (0.010)
cisPt	4.9 (0.2)	1.9 (0.2)	1.8 (0.5)	2.7 (0.4)	17 (3.3)	23 (4.3)

(**a**) From ref. 1. (**b**) Means of at least three experiments.

Complex **2·OTf·0.25H$_2$O** showed an enhanced antiproliferative activity in five of the six cell lines tested with respect to **1·2OTf** and much better activity than cisplatin. In addition, the salt $CoCl_2 \cdot 6H_2O$ was tested, confirming that it is inactive against cell lines checked. Next, we studied the effect of complex **2·OTf·0.25H$_2$O** on the cell cycle of the same panel of tumour cell lines (Fig. S8). Similarly, to that observed for complex **1·2OTf**,[1] the exposure of studied cell lines to **2·OTf·0.25H$_2$O** produces the accumulation of cells in the G_1

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compartment of the cell cycle. From this result we anticipate that both complexes display a similar mechanism of action against the studied cell lines.

Complex **2·OTf·0.25H$_2$O** in DMSO-d$_6$ at room temperature releases in 10 min. the {CoCl$_2$} unit, which is transformed in the [Co(DMSO)$_4$Cl$_2$] complex,[11] to provide the neutral deprotonated complex [RuCp(PPh$_3$)$_2$(dmoPTA)]$^+$. In contrast, addition of DMSO and quick further addition of water or the cell culture medium avoid the fast elimination of the {CoCl$_2$} unit. Therefore, the protocol to study the antiproliferative activity of **2·OTf·0.25H$_2$O** does not produce a fast decomposition of the compound. The evolution of a similar solution at room temperature and 40 °C showed that the complete release of the {CoCl$_2$} unit was achieved after 2 hours. Further an additional reaction was observed: the elimination of one PPh$_3$. This reaction is so slow that after 24 h at room temperature and 16 h at 40 °C only a 5 % of the phosphine was eliminated. When a similar reaction was preformed but containing 5 eq. of NaCl one of the PPh$_3$ in **2** was exchanged by Cl$^-$, giving rise to the stoichiometric production of complex [RuClCp(PPh$_3$)(HdmoPTA)]$^+$.[7] However, in a lipophilic environment the behavior of **2·OTf·0.25H$_2$O** was showed to be different. In a CDCl$_3$ solution the complex first releases a PPh$_3$ molecule and further the {CoCl$_2$} unit, indicating that its stability is quite dependent on the environment.

In summary, the obtained results indicate that the coordination of a {CoCl$_2$} unit to **1**-HdmoPTA-N$_{CH3}$ atoms leads to a new bis-metallic complex with antiproliferative activity enhanced than starting complex **1·2OTf**, which was one of the most active anticancer agent known until now. The NMR studies targeted to know how stable is the complex **2·OTf·0.25H$_2$O** in cell culture medium, pointing out that the Co-unit is released slowly but at the moment there are no evidences to know if this reaction is significantly produced before **2** goes into the cancer cell. In any case the CoCl$_2$ salt was found inactive against the studied cancer cells.[12] This result support that this salt outside the cell could not be the responsible of the enhanced activity of **2·OTf·0.25H$_2$O** but inside the cell could be and antiproliferative agent and/or synergize the activity of other compounds. If this is correct, complex **2·OTf·0.25H$_2$O** acts as a "Trojan horse" to introduce into the cell the {CoCl$_2$} unit. It is important to stress that cobalt is an essential microelement in the human body, playing an important physiological role in the metabolism of iron, synthesis of hemoglobin, methionine metabolism and as a component of Vitamin B$_{12}$.[13] The exchange of one of the PPh$_3$ bonded to the metal by a ligand in the solution was also observed, being this reaction slower than the release of {CoCl$_2$} unit. It is reasonable to suppose that this reaction should occur into the cell. This reaction was observed to be the fastest one when the complex was dissolved in a lipophilic solvent as CDCl$_3$. Therefore, not only **2·OTf·0.25H$_2$O** but also products formed by its decomposition could be the real responsible of its so high antiproliferative activity. However, it is evident that the combination in **2** of the metal units containing Ru and Co leads to a significant enhance of the antiproliferative activity of both metal units separated.

More detailed kinetic studies and biological experiments would be necessary to establish the exact role of all possible products derived from **2·OTf·0.25H$_2$O** and to understand how this compound displays a so large antiproliferative activity, better than most of the known metal complexes.[14] Additionally, new bis-metallic Ru-M complexes are in synthesis to know if different to Co metals produce similar active antiproliferative compounds. Our findings open the possibility to develop new organometallic drugs that can rely on selecting metals and ligands to modulate the pharmacological properties of the final drug.

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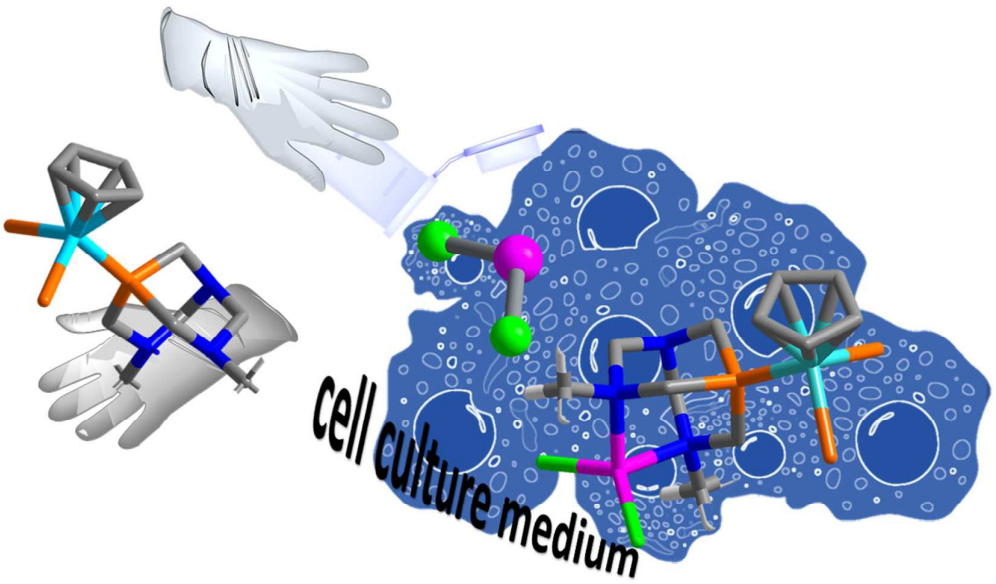

The complex $[RuCp(PPh_3)_2\text{-}\mu\text{-}dmoPTA\text{-}1\kappa P{:}2k^2 N,N'\text{-}CoCl_2]\cdot OTf\cdot 0.25H_2O$ shows an antiproliferative activity significantly better (up to 354-fold for WiDr-colon cells) than cisplatin and with enhanced activity up to six-fold than its starting complex $[RuCp(PPh_3)_2(HdmoPTA)]^{2+}$.

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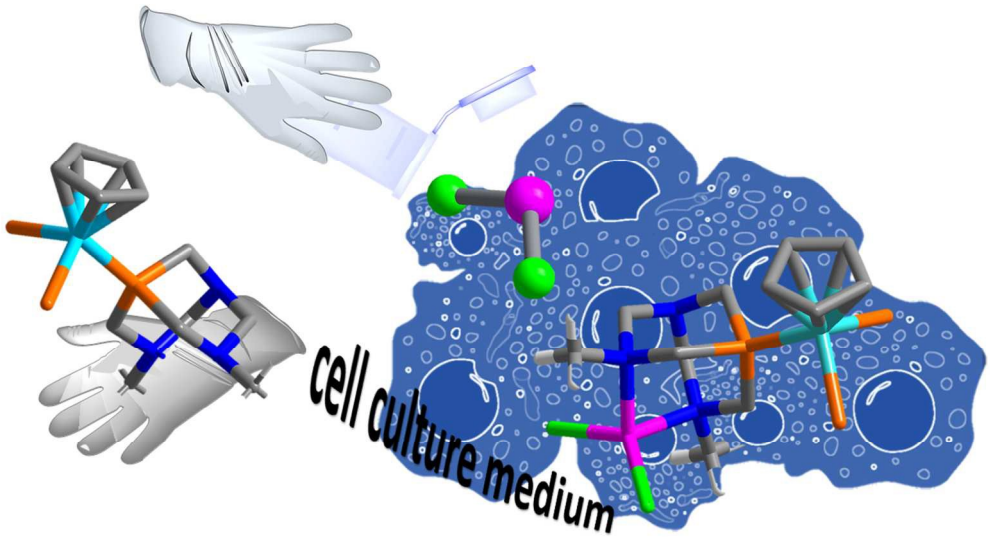

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