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Importance of the π-Ligand: Remarkable Effect of the Cyclopentadienyl Ring on the Cytotoxicity of Ruthenium PTA Compounds

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Summary: The water-soluble complexes [(Cp′OR)RuCl(PTA)$_2$] (Cp′OR = η^5-1-alkoxy-2,4-di-tert-butyl-3-neopentylcyclopentadienyl; R = Me, Et; PTA = 1,3,5-triaza-7-phosphaadamantane) are considerably more cytotoxic (ca. 2 orders of magnitude) than the cyclopentadienyl analogue [CpRuCl(PTA)$_2$] (i.e., IC_{50} = 4−10 vs >1000 μM, depending on the cell line). The structure of [(Cp′OMe)RuCl(PTA)$_2$] is reported, together with that of the precursor [(Cp′OEt)Ru(μ-Cl)]$_2$.

Platinum-based drugs are among the most effective agents for the treatment of cancer, with cisplatin, carboplatin, and oxaliplatin in widespread clinical use.[1] However, a plethora of non-platinum-metal complexes have also been prepared and tested for anticancer activity.[2] Notably, ruthenium complexes have been found to be effective against cancers that cannot be treated with platinum drugs and they also tend to exhibit lower general toxicities compared to platinum compounds.[3] Two ruthenium compounds (the indazole complex KP1019 and the imidazole complex NAMI-A) that have a spectrum of activity different from that of platinum drugs[4] are currently undergoing clinical evaluation.[5]

More recently, increasing interest has focused on organometallic compounds,[6] especially those based on group 8 metals.[7] Specifically, certain ruthenium(II)−arene compounds show excellent antiproliferative properties in vitro and/or in vivo.[4,8] Our research has focused on compounds of the general formula [(η^6-arene)RuCl$_2$(PTA)] (PTA = 1,3,5-triaza-7-phosphaadamantane), the prototype being [(η^6-*p*-cymene)RuCl$_2$(PTA)], termed RAPTA-C. Several structurally diverse RAPTA derivatives have been studied, and their in vitro cytotoxicity has been

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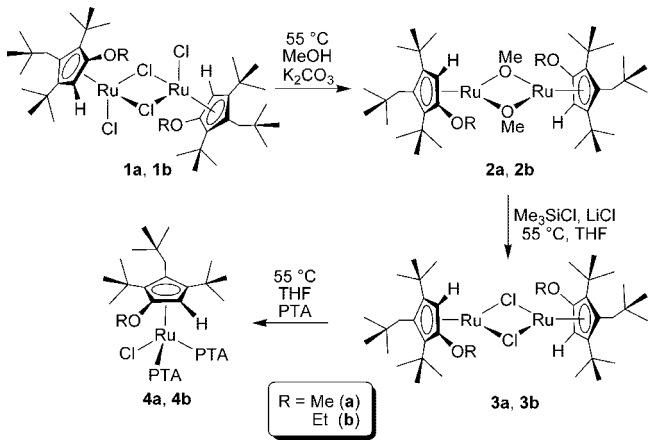
Scheme 1



evaluated.[9] In addition, some related ruthenium cyclopentadienyl complexes containing the PTA ligand, viz. $[CpRuCl(PTA)_2]$ and $[Cp^*RuCl(PTA)_2]$, have also been reported.[10] All these compounds are similar in that they display only weak in vitro activity, and while RAPTA-C shows excellent in vivo characteristics comparable to those of NAMI-A,[11] this behavior is only achieved at higher doses. Thus, one of the aims in this area is to find compounds that maintain the structural features of these compounds, i.e. a π-ligand and PTA and halide ligands, but are more cytotoxic, such that lower doses may reduce tumor mass in vivo. Below we describe two compounds which meet the desired criteria and are not only 2 orders of magnitude more active than a close model structure but also show excellent activity in a cisplatin-resistant cancer cell line.

The synthetic route used to prepare the target compounds $[(Cp'OR)RuCl(PTA)_2]$ (**4a**, R = Me; **4b**, R = Et) is shown in Scheme 1. Complexes **4a**,**b** were generated in good yield by reaction of the chloro-bridged complexes **3a**,**b** with PTA.[12] The synthesis and structure of the precursor **3a** has been described recently.[13] It can be obtained in two steps from the easily accessible **1a**.[14] The ethoxy complex **3b** was prepared in an analogous fashion by reaction of **1b** with excess K_2CO_3 in MeOH, followed by exchange of the μ-OMe ligands with μ-Cl ligands by treatment with Me_3SiCl in the presence of LiCl.

The spectroscopic characterization of **4a**,**b** corroborates the expected structure; of note, the $^{31}P\{^1H\}$ NMR spectra in $C_6D_5CD_3$ contain two doublets of equal relative intensity at −38.14 (d, $^2J_{PP}$ = 35 Hz) and −48.22 (d, $^2J_{PP}$ = 35 Hz) for **4a** and −37.84 (d, $^2J_{PP}$ = 36 Hz) and −47.64 (d, $^2J_{PP}$ = 36 Hz) for **4b**, indicating that the two P atoms of the PTA ligands are chemically different by virtue of the planar chirality of the ring.

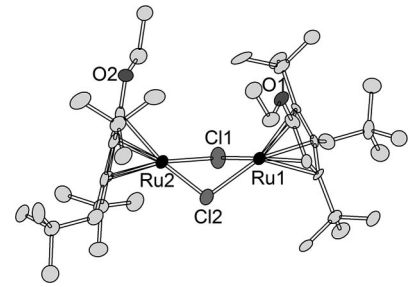


Figure 1. Graphic representation of the molecular structure of complex **3b** in the crystal state. Thermal ellipsoids are at the 50% probability level. The hydrogen atoms are not shown for clarity. Selected bond lengths (Å) and angles (deg): Ru1−Cl1 = 2.382(4), Ru1−Cl2 = 2.428(4), Ru2−Cl1 = 2.409(4), Ru2−Cl2 = 2.416(4); Ru1−Cl1−Ru2=89.10(13),Ru1−Cl2−Ru2=87.89(13),Cl1−Ru1−Cl2 = 83.68(13), Cl1−Ru2−Cl2 = 83.36(13).

The dimeric structure of the previously reported intermediate **3a** is unusual, in view of the fact that the frequently used Cp* analogue $[Cp^*Ru(\mu\text{-}Cl)]_4$ displays a distorted heterocubane structure in the solid state.[15] This motivated us to perform a crystallographic analysis of the ethoxy complex **3b**. As is observed for **3a**, an electronically unsaturated dimer is obtained and not a tetramer (Figure 1).[16]

The solid-state structure of **3b** shows a Ru···Ru separation of 3.361(2) Å, which is significantly shorter than the Ru···Ru distance of 3.6023(5) Å observed for **3a**[13] but still too long for any significant intermetallic interaction. In line with the shorter Ru···Ru distance is the observation that the $Ru_2(\mu\text{-}Cl)_2$ core of **3b** is more bent (fold angle 138.62°) than that of **3a** (fold angle 160.50°). Since the π-ligands of **3a**,**b** are very similar, we assume that the differences in structure are mainly due to packing effects. This is in line with calculations, which have

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(12) Synthesis of complex **4a**: PTA (68 mg, 0.43 mmol) was added to a solution of complex **3a** (100 mg, 0.12 mmol) in dry THF (3 mL), and the mixture was stirred at 55 °C. The solution gradually changed from orange-red to bright yellow, along with the appearance of a yellow precipitate. After 3 h, the reaction mixture was filtered and the solid was washed with THF and dried under high vacuum. Yield: 152.5 mg (86%). ^1H NMR ($C_6D_5CD_3$, 25 °C): δ (ppm) 4.75 (s, 1 H, Cp H), 4.35−4.55 (m, 12 H, NCH_2N), 4.05−4.30 (m, 12 H, PCH_2N), 3.71 (s, 3 H, OCH_3), 2.87 (d, $^2J_{HH}$ = 16 Hz, 1 H, CH_2), 2.79 (d, $^2J_{HH}$ = 16 Hz, 1 H, CH_2), 1.38 (s, 9 H, *t*-Bu), 1.21 (s, 9 H, *t*-Bu), 1.16 (s, 9 H, *t*-Bu). $^{31}P\{^1H\}$ NMR ($C_6D_5CD_3$, 25 °C): δ (ppm) −38.14 (d, $^2J_{PP}$ = 35 Hz, 1 P, PTA), −48.22 (d, $^2J_{PP}$ = 35 Hz, 1 P, PTA). Synthesis of complex **4b**: the synthesis was performed analogously to that of complex **4a**, using 66 mg of PTA (0.42 mmol) and 100 mg of complex **3b** (0.117 mmol) in dry THF (3 mL). Yield: 152.6 mg (88%). ^1H NMR ($C_6D_5CD_3$, 25 °C): δ (ppm) 4.83 (s, 1 H, Cp H), 4.45−4.65 (m, 12 H, NCH_2N), 4.25−4.45 (m, 12 H, PCH_2N), 3.67 (dq, $^2J_{HH}$ = 16 Hz, $^3J_{HH}$ = 7 Hz, 1 H, Cp−OCH_2CH$_3$), 3.39 (dq, $^2J_{HH}$ = 16 Hz, $^3J_{HH}$ = 7 Hz, 1 H, Cp−OCH_2CH$_3$), 3.20 (d, $^2J_{HH}$ = 16 Hz, 1 H, CH_2), 3.07 (d, $^2J_{HH}$ = 16 Hz, 1 H, CH_2), 1.83 (s, 9 H, *t*-Bu), 1.45 (s, 9 H, *t*-Bu), 1.34 (s, 9 H, *t*-Bu), 1.16 (t, $^3J_{HH}$ = 7 Hz, 3 H, Cp−OCH$_2$CH_3). $^{31}P\{^1H\}$ NMR ($C_6D_5CD_3$, 25 °C): δ (ppm) −37.84 (d, $^2J_{PP}$ = 36 Hz, 1 P, PTA), −47.64 (d, $^2J_{PP}$ = 36 Hz, 1 P, PTA). ^{13}C NMR data are given in the Supporting Information.

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(16) Crystal data for complex **3b**: $C_{40}H_{70}Cl_2O_2Ru_2$, M_r = 856.00, triclinic, a = 9.6933(19) Å, b = 12.9082(19) Å, c = 17.281(3) Å, α = 101.758(17)°, β = 99.882(18)°, γ = 92.274(13)°, V = 2079.2(7) Å^3, T = 100(2) K, space group $P\bar{1}$, Z = 2, μ(Mo Kα) = 0.710 73 Å, 25 709 reflections collected, 5868 independent reflections, R_{int} = 0.0924, R1 ($I > 2\sigma(I)$) = 0.0904, wR2 (all data) = 0.2361.

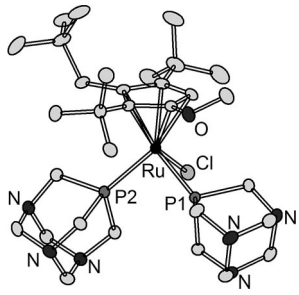

Figure 2. Graphic representation of the molecular structure of **4a** in the crystal state. Thermal ellipsoids are at the 50% probability level. The hydrogen atoms and the solvent molecule (0.5 toluene) are not shown for clarity.

Table 1. Selected Bond Lengths (Å) and Angles (deg) for the Complexes [CpRuCl(PTA)$_2$], [Cp*RuCl(PTA)$_2$], and 4a

	[CpRuCl(PTA)$_2$][a]	[Cp*RuCl(PTA)$_2$][b]	**4a**
Ru−Cl	2.445(2)	2.465(2); 2.468(2)	2.4716(4)
Ru−P(1)	2.258(3)	2.284(1); 2.285(2)	2.2873(4)
Ru−P(2)	2.247(3)	2.285(2); 2.287(2)	2.3195(4)
av Ru−C	2.197(7)	2.211(6); 2.208(8)	2.231
P(1)−Ru−P(2)	96.85(5)	93.30(5); 93.37(7)	93.378(15)
P(1)−Ru−Cl	91.61(7)	90.69(6); 90.94(9)	81.224(14)
P(2)−Ru−Cl	86.46(7)	84.38(6); 84.27(8)	85.260(14)

[a] Data from ref 19. [b] Data from ref 10; there are two independent molecules in the asymmetric unit.

shown that the $M_2(\mu\text{-Cl})_2$ core of dinuclear, chloro-bridged complexes can be quite flexible.[17] The average Ru−Cl distance in **3b**, 2.409 Å, is smaller than that in [Cp*Ru(μ-Cl)]$_4$ (2.524 Å).[15]

The target complex **4a** was also analyzed by X-ray crystallography.[18] It displays the expected three-legged piano-stool geometry with two PTA and one chloro ligand coordinated opposite to the π-ligand (Figure 2). Overall, the bond lengths and angles are similar to those reported for [CpRuCl(PTA)$_2$][19] and [Cp*RuCl(PTA)$_2$][10] (Table 1). Of note, a slight increase of the Ru−P, Ru−C, and Ru−Cl bond distances on going from Cp via Cp* to Cp′OMe is observed; this is presumably a reflection of the increasing electron-donating and steric requirements of the respective π-ligands.

The in vitro anticancer activities of water-soluble **4a**,**b**, the reference compound [CpRuCl(PTA)$_2$],[10] and cisplatin as a benchmark were determined using the MTT assay on the human ovarian cancer cell line A2780 and its cisplatin-resistant analogue A2780cisR. The effects of the compounds on the growth of these cells were evaluated after 72 h treatment, and the results from these studies are displayed in Table 2. The experiments were repeated twice (each in triplicate) for all the compounds, and the corresponding IC_{50} values result from an average of the two experiments for both cell types. Compounds **4a**,**b** are remarkably cytotoxic toward both cell lines, displaying activity similar to that of cisplatin. In contrast, the reference compound [CpRuCl(PTA)$_2$] remains inactive at 1000 μM. This is in line with the observations of Peruzzini et al.,[10] who found that [CpRuCl(PTA)$_2$] did not show any antiproliferative effects when tested with TS/A murine adenocarcinoma tumor cells. It should be noted that, in this assay, the Cp* analogue [Cp*RuCl(PTA)$_2$] showed only modest cytotoxicity.[10] It is also noteworthy that an extensive range of different arene ligands have been studied for the related complexes [(η^6-arene)RuCl$_2$(PTA)] and only small differences in (generally low) cytotoxicity are observed.[9a,c]

Table 2. IC_{50} Values of 4a,b on the Human Ovarian Cancer Cell Lines after 72 h Incubation Together with Other Compounds for Comparison Purposes

	IC_{50} (μM)	
compd	A2780	A2780cisR
4a	5	6
4b	4	10
[CpRuCl(PTA)$_2$]	>1000	>1000
cisplatin	2	9

The precise reason for the vast differences in activity between **4a**,**b** and [CpRuCl(PTA)$_2$] is likely to be multifactoral. Of importance is probably the increased lipophilicity of **4a**,**b**,[20] but facilitated exchange reactions due to the bulky π-ligand can contribute as well. It is well-known that hydrophobic compounds can cross cell membranes more readily than hydrophilic compounds and, therefore, uptake into cells tends to be higher. Overall, our results show that, in contrast to what has been suggested in the literature,[21] the cyclopentadienyl ligand of Ru(PTA) half-sandwich complexes does have a very pronounced effect on the biological activity.

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Supporting Information Available: Text giving experimental details and analytical data for the complexes **2b**, **3b**, and **4a**,**b** and a CIF file giving crystallographic data for the complexes **3b** and **4a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Crystal data for complex **4a**·0.5(toluene): $C_{34.5}H_{61}ClN_6OP_2Ru$, M_r = 774.35, triclinic, a = 10.1217(2) Å, b = 11.7586(4) Å, c = 15.8825(5) Å, α = 94.871(3)°, β = 90.261(2)°, γ = 99.551(2)°, V = 1857.02(9) Å^3, T = 140(2) K, space group $P\bar{1}$, Z = 2, μ(Mo Kα) = 0.710 73 Å, 15 365 reflections collected, 6520 independent reflections, R_{int} = 0.0119, R1 ($I > 2\sigma(I)$) = 0.0187, wR2 (all data) = 0.0488.

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(20) The complexes **4a**,**b** can be dissolved in toluene and diethyl ether, whereas [CpRuCl(PTA)$_2$] is not soluble in these solvents.

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