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Tuning the Cytotoxicity of Ruthenium(II) *para*-Cymene Complexes by Mono-Substitution at a Triphenylphosphine/Phenoxydiphenylphosphine Ligand

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Abstract

The new complexes [$RuCl_2(\eta^6$-*p*-cymene)(κ*P*-Ph_2PR)] [R = 4-$C_6H_4OSiMe_2{}^tBu$, **1**; R = 4-C_6H_4Br, **2**; R = $OC(=O)CHCl_2$, **3**; R = OPh, **4**; R = O(2-$C_6H_4SiMe_2{}^tBu$), **5**] and [$Ru(C_2O_4)(\eta^6$-*p*-cymene){κ*P*-$Ph_2PO(2\text{-}C_6H_4(SiMe_2{}^tBu))$}], **6**, were obtained in 83-98% yield from Ru(II) arene precursors by three different synthetic strategies. The unprecedented phosphine $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$ was synthesized in 86% yield from 2-$C_6H_4Br(OSiMe_2{}^tBu)$ and Ph_2PCl, via intramolecular *oxygen to carbon* 1,3 migration of the silyl group (retro-Brook rearrangement). All the complexes were fully characterized by analytical and spectroscopic methods, and by single crystal X-ray diffraction in the cases of **3**, **4**, **5** and **6**. Complexes **1-6** and the model compounds [$RuCl_2(\eta^6$-*p*-cymene)(κ*P*-PPh_3)] (**Ru-PPh$_3$**) and [$Ru(C_2O_4)(\eta^6$-*p*-cymene)(κ*P*-PPh_3)] (**Ru-PPh$_3$-O**) underwent slow degradation in chloroform solutions upon air contact; the mixed valence complex [(η^6-*p*-cymene)Ru(μ-Cl)$_3$$RuCl_2$(κ*P*-$PPh_3$)], **7**, was isolated from a solution of **Ru-PPh$_3$** in $CHCl_3$, and X-ray identified. The antiproliferative activity of **1-6** and **Ru-PPh$_3$**, **Ru-PPh$_3$-O** and [$RuCl_2(\eta^6$-*p*-cymene)(κ*P*-PTA)] (RAPTA-C) was assessed towards the triple-negative breast cancer cell line MDA-MB-231, the ovarian carcinoma cell line A2780 and human skin fibroblasts (HSF). Complexes **1**, **2**, **5** and **6** displayed IC_{50} values significantly lower than that of cisplatin, with **2** providing a more potent cytotoxic effect on MDA-MB-231 and A2780 cancer cells compared to the noncancerous cell line (HSF). The stability of all complexes in DMSO/water solution was elucidated by NMR and conductivity measurements, and in particular ^{35}Cl NMR spectroscopy was helpful to check the possible chloride dissociation. The stability studies suggest that the cytotoxic activity *in vitro* of the compounds is mainly ascribable to Ru(II) species still bound to the phosphorus ligand.

Keywords: metal-based drugs, ruthenium arene complexes, RAPTA complexes, triphenylphosphine ligands, breast cancer.

Introduction

Cancer represents the first cause of death in economically developed countries, and the second in developing countries.[1] A huge effort of scientific research is aimed to obtain new effective and selective metal drugs in view of overcoming the severe toxicity and acquired resistance issues associated with the use of platinum chemotherapics, which are currently used in clinical treatments.[2] Ruthenium compounds have been intensively investigated in this setting,[3] and especially Ru(II) arene complexes have aroused a great interest.[4] In particular, $[RuCl_2(\eta^6\text{-}p\text{-cymene})(\kappa P\text{-PTA})]$ (RAPTA complexes),[5] containing the amphiphilic phosphorus ligand 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (PTA), and $[RuCl(\eta^6\text{-arene})(\kappa^2 N\text{-}NH_2CH_2CH_2NH_2)][PF_6]$,[6] containing a bidentate ethylene-1,2-diamine ligand, have emerged as promising anticancer agents and are pointing to clinical trials (Figure 1).[7] With specific reference to RAPTA complexes, these display excellent antimetastatic and antiangiogenic behaviour *in vivo* and are able to reduce the growth of certain primary tumours.[8] These features have stimulated the search for the anticancer potential of many other similar compounds, and RAPTA-analogues bearing various alkyl/aryl-phosphine ligands in the place of PTA have been screened for their activity.[9] Noteworthy results have been achieved by Dyson and co-workers with perfluoro-substituted trialkylphosphines, supplying thermotropic behaviour to the respective complexes,[10] and carbohydrate-modified 3,5,6-bicyclophosphites, the resulting complexes showing a certain degree of selectivity against several cancer cells compared to non tumorigenic cell line.[11]

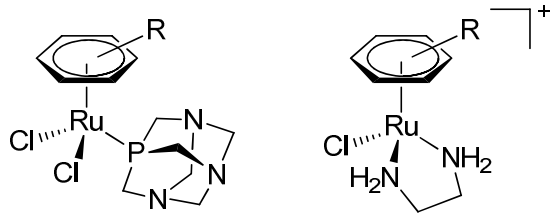


Figure 1. Ruthenium(II) arene anticancer complexes.

On the other hand, the introduction of triphenylphosphine as a ligand, being considerably more hydrophobic than PTA, usually leads to higher levels of cytotoxicity *in vitro*, although at the expense of selectivity in some cases.[12] For instance, $[RuCl(\eta^6\text{-}p\text{-cymene})(py^*)(PPh_3)]^+$, containing a substituted pyridine ligand (py*), exhibited activity against human leukemia tumour cell line comparable to that obtained with cisplatin.[13] Moreover, Hartinger and co-workers recently reported that the incorporation of PPh_3 (or PTA) in Ru(II) arene complexes containing a bidentate oxygen co-ligand (3-hydroxy-4-pyridone or 3-hydroxy-4-pyrone) determined a dramatic increase of the cytotoxic activity on different cell lines,[14] and a similar outcome was observed for $[Ru(\eta^6\text{-benzene})(\kappa N\text{-letrozole})(PPh_3)][BF_4]$ vs. $[Ru(\eta^6\text{-benzene})(\kappa N\text{-letrozole})_2][BF_4]$.[15] It has been demonstrated that the presence of a triphenylphosphine ligand is important also to facilitate the binding of the Ru complex to DNA and then distort its secondary and tertiary structure.[13]

The investigation on Ru(II) arene compounds bearing substituted triphenylphosphine ligands has been limitedly developed. More precisely, $Ph_2P(4\text{-}C_6H_4CO_2H)$ and $Ph_2P(4\text{-}C_6H_4OH)$ have been used to incorporate a variety of bioactive carboxylic acids within the Ru(II) *para*-cymene scaffold, through esterification reactions.[16] The resulting $[RuCl_2(\eta^6\text{-}p\text{-cymene})(\kappa P\text{-}Ph_2PAr^{BIO})]$ complexes display variable activity towards A2780 and A2780cisR cancer cell lines, the degree of activity being significantly influenced by the nature of the bioactive fragment.

Herein, we present the synthesis and the full characterization of six new Ru(II)-*p*-cymene complexes with differently mono-substituted triphenylphosphine or phenoxydiphenylphosphine ligands, including a dichloroacetic acid functionalized triphenylphosphine, a silyl ether substituted triphenylphosphine and a unprecedented silyl phenoxydiphenylphosphine. Dichloroacetic acid is a commercially available inhibitor of pyruvate dehydrogenase kinase, able to enhance cellular apoptosis.[17] The incorporation of

dichloroacetic acid in platinum based drugs was reported to provide several favourable effects,[18] and especially the Pt(IV) compound called "mitaplatin" displayed excellent characteristics of cytotoxicity and selectivity.[19] To the best of our knowledge, dichloroacetic acid has not been tethered to ruthenium complexes for medicinal applications hitherto. Silicon-containing phosphine ligands have been considered in the present work in view of the peculiar properties that organosilicon compounds may supply to pharmaceuticals.[20]

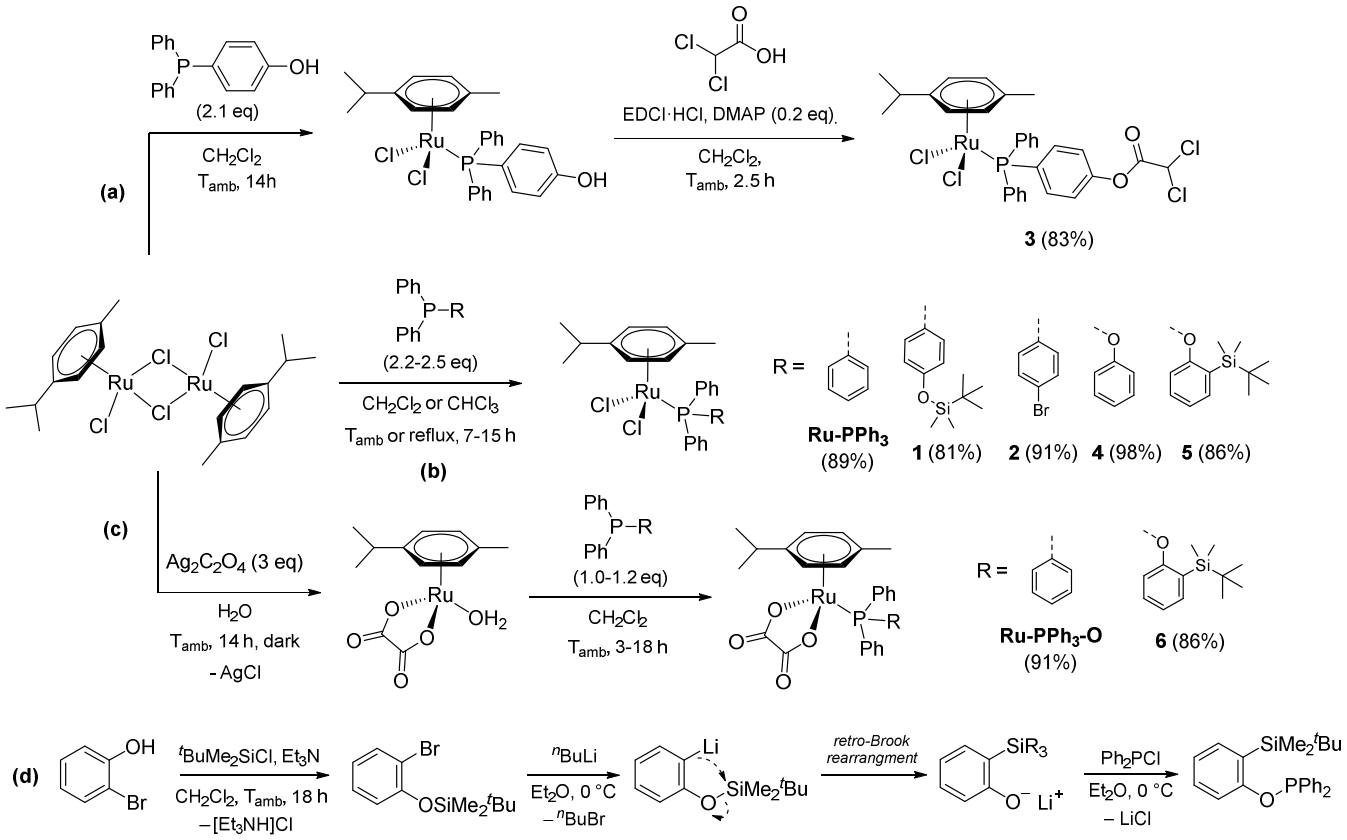
On account of the fact that platinum drugs are still the only viable options for the treatment of triple negative breast cancers and ovarian carcinomas, the new complexes have been assessed for their *in vitro* antiproliferative activity towards MDA-MB-231 (triple negative breast cancer) and A2780 (ovarian carcinoma) cancer cell lines, and human skin fibroblasts (HSF) as non-transformed primary cell line.

Results and discussion

1. Synthesis and characterization of compounds.

The reactions of the dinuclear compound $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ with $Ph_2P(4\text{-}C_6H_4OSiMe_2{}^tBu)$, $Ph_2P(4\text{-}C_6H_4Br)$, $Ph_2P(OPh)$ and $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$, respectively, were conducted in chlorinated solvents at ambient temperature or above, and afforded the novel complexes **1-2** and **4-5**, in good to excellent yields (Scheme 1b). The phosphine $Ph_2P(4\text{-}C_6H_4OSiMe_2{}^tBu)$ is an intermediate product along the convenient synthesis of $Ph_2P(4\text{-}C_6H_4OH)$, the *tert*-butyl dimethyl silyl moiety being a protecting group.[16a,21] $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$ is a unprecedented compound, and was synthesized in 86% yield from the aryl-silylether $2\text{-}C_6H_4Br(OSiMe_2{}^tBu)$ and *n*-BuLi/Ph_2PCl via [1,3] retro-Brook rearrangement (Scheme 1d).[22]

Complex **3** was obtained in 83% yield by direct esterification of [$RuCl_2(\eta^6$-*p*-cymene){κ*P*-$Ph_2P(4$-$C_6H_4OH)$}] with dichloroacetic acid (a bioactive molecule, *vide infra*) through EDCI/DMAP protocol (Scheme 1a). This procedure, negating the necessity of protecting strategies towards the labile Ru-Cl bonds, is convenient on account of the fact it does not require the manipulation of non coordinated, air sensitive $Ph_2P(4$-$C_6H_4OH)$.[16a] Complex **6**, differing from **5** in that two chloride ligands are replaced with an oxalate, was straightforwardly prepared from [$Ru(C_2O_4)(\eta^6$-*p*-cymene)(H_2O)] and $Ph_2P(O(2$-$C_6H_4SiMe_2{}^tBu))$ (Scheme 1c).



Scheme 1. Synthetic routes to ruthenium triphenylphosphine complexes (**a-c**) and $Ph_2P(O(2$-$C_6H_4SiMe_2{}^tBu))$ ligand (**d**). Yields are given in parentheses.

All the complexes **1-6** and the ligand $Ph_2P(O(2$-$C_6H_4SiMe_2{}^tBu))$ were characterized by analytical methods, IR and NMR spectroscopy (see Table 1S provided as Supporting Information). In the IR spectrum of **3** (solid state), the ester group manifests itself with a broad band around 1770 cm^{-1}, while

the carbonyl functions of **6** have been detected at 1666-1697 cm^{-1}. In **4-5** and $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$, a strong absorption in the range 870-892 cm^{-1} has been attributed to the stretching vibration of the P-O moiety. The dichloroacetate group in **3** is featured also by the ^{13}C NMR resonances occurring at 162.5 (*C*=O) and 64.2 (*C*HCl_2) ppm. The $^{31}P\{^1H\}$ NMR spectra of **1-3** display a typical singlet at ca. 24 ppm; the ^{31}P NMR resonance related to the aryloxy-diphenylphosphine compounds **4**, **5** and $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$, in $CDCl_3$ solution, falls at significantly lower fields. The phosphorus nucleus of $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$ resonates at 108.3 ppm in the uncoordinated molecule, and at 120.3 ppm in **5**. The ^{29}Si NMR spectra of **5** and $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$, comprising a carbon-bound silicon atom, consist of a singlet at approximately 3 ppm; on the other hand, the resonance related to the oxygen-bound silicon nucleus falls at 21.7 ppm in **1**.

Crystals suitable to X-ray analysis were collected for **3**, **4**, **5** and **6**; views of the ORTEP molecular structures are shown in Figures 2-5, while relevant bonding parameters are provided as SI (Tables 12S-15S). Compounds **3-6** comprise the expected three-leg piano-stool geometry typical of other Ru(II)-arene compounds,[23] and the bonding parameters around the Ru(II) centres are similar to those reported for related [$RuCl_2$(*p*-cymene)(phosphine)] and [$Ru(C_2O_4)$(*p*-cymene)(phosphine)] structures.[24]

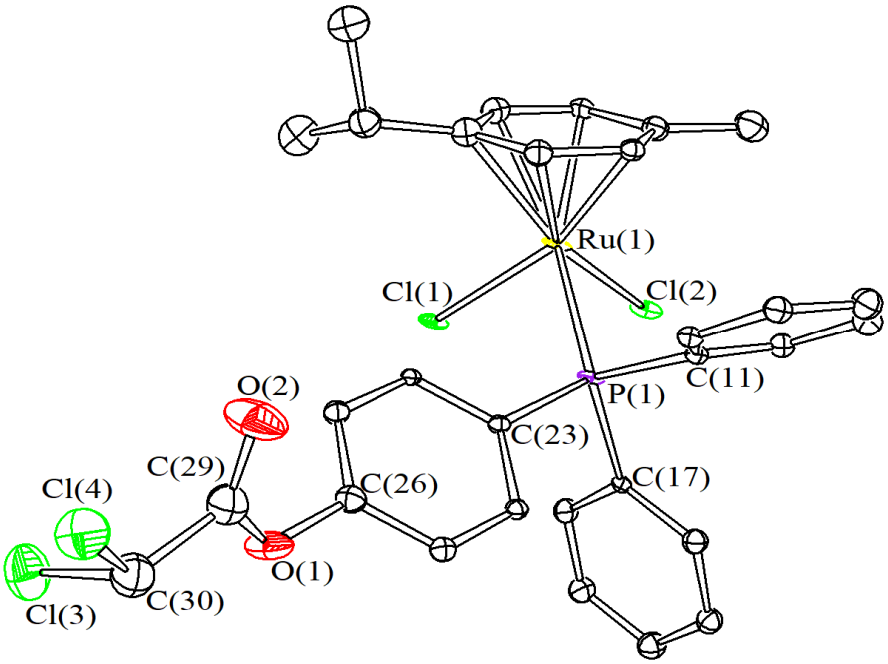


Figure 2. Molecular structure of [$RuCl_2(\eta^6$-*p*-cymene){κ*P*-$Ph_2P(4$-$C_6H_4OCOCHCl_2)$}], **3**. Displacement ellipsoids are at the 30% probability level. H-atoms have been omitted for clarity.

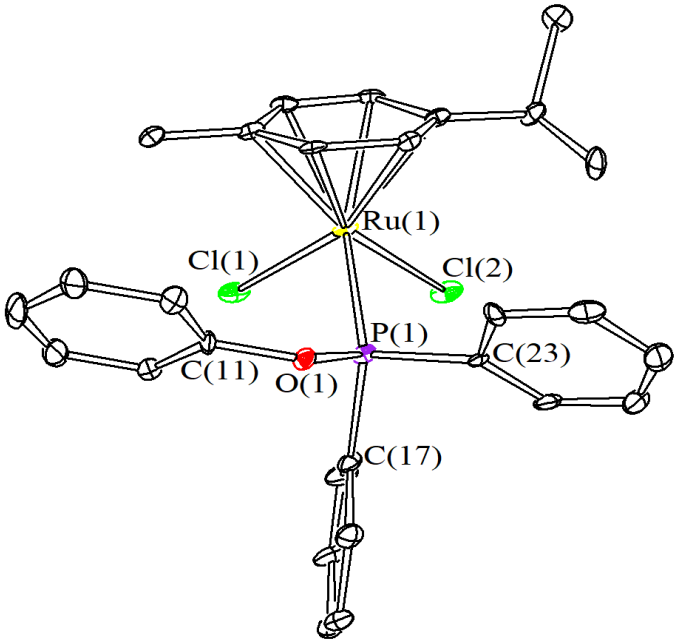


Figure 3. Molecular structure of [$RuCl_2(\eta^6$-*p*-cymene){κ*P*-$Ph_2P(OPh)$}], **4**. Displacement ellipsoids are at the 30% probability level. H-atoms have been omitted for clarity.

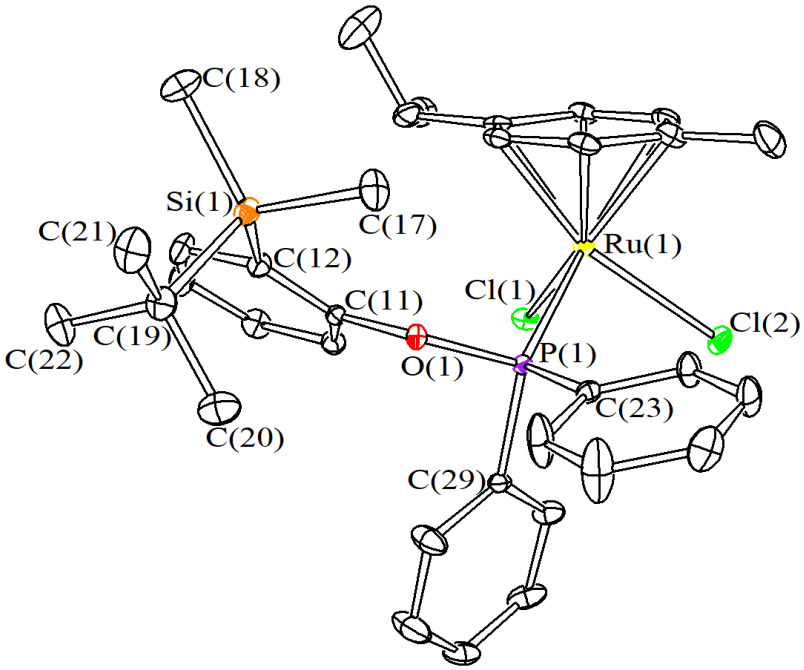


Figure 4. Molecular structure of [$RuCl_2(\eta^6$-*p*-cymene){κ*P*-$Ph_2PO(2\text{-}C_6H_4(SiMe_2{}^tBu))$}], **5**. Displacement ellipsoids are at the 30% probability level. H-atoms have been omitted for clarity.

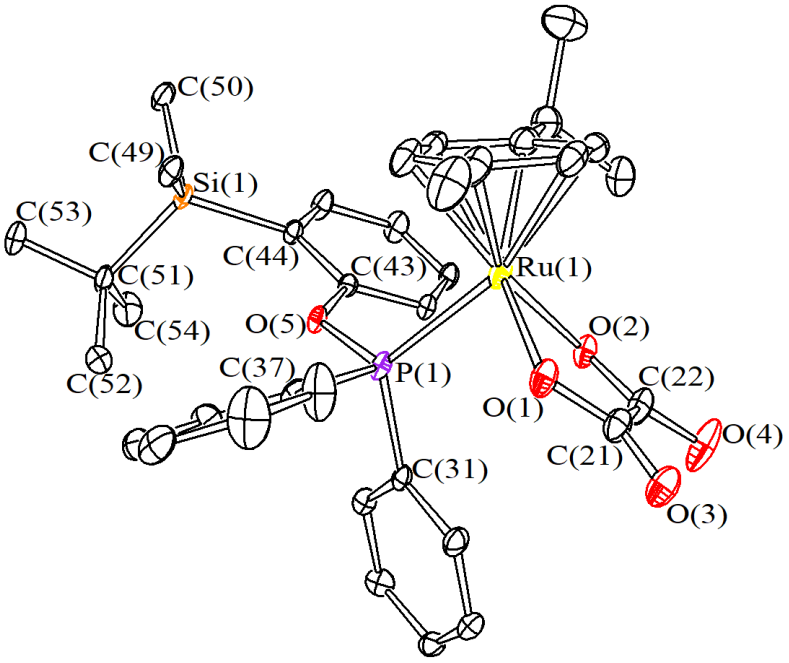
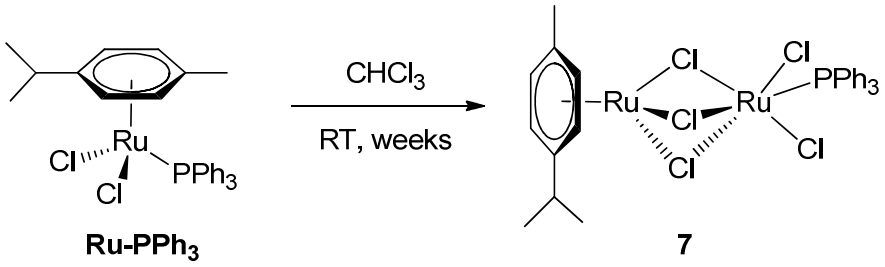


Figure 5. Molecular structure of [Ru(C_2O_4)(η^6-*p*-cymene){κ*P*-$Ph_2PO(2\text{-}C_6H_4(SiMe_2{}^tBu))$}], **6**. Displacement ellipsoids are at the 50% probability level. H-atoms have been omitted for clarity.

Complexes **1-6** are air stable and well soluble in chlorinated solvents, but not indefinitely stable in chloroform upon air contact. According to IR/NMR experiments (see Supporting Information for details), **1** and **6** in chloroform completely degraded after two weeks, the red/yellow solutions progressively turning to green and affording a complicated mixture of species including *p*-cymene. A similar behaviour was found for [$RuCl_2$(η^6-*p*-cymene)(κ*P*-PPh_3)] (**Ru-PPh$_3$**); in this case, some green crystals of the dinuclear, mixed valence Ru^{II}-Ru^{III} compound [Ru(μ-Cl)$_3$(η^6-*p*-cymene)$RuCl_2$(κ*P*-PPh_3)], **7**,[25] were isolated from the reaction solution, and then identified by X-ray diffraction (Scheme 2). Complexes belonging to the family [Ru(μ-Cl)$_3$(η^6-*p*-cymene)$RuCl_2$(κ*P*-PR_3)] (R = Ph, Cy, n-Bu) were previously reported as prepared from [$RuCl_2$(η^6-*p*-cymene)]$_2$ and the appropriate phosphine, upon ethylene addition.[26,]

Instability of Ru(η^6-arene) derivatives in chlorinated solvents is documented in the literature[27] and possibly associated to the decomposition of the solvent into HCl and carbon radicals.[28] Interestingly,

Ru(II) complexes have been used as catalysts for the photodegradation of chloroform and other chlorinated compounds.[28b,29]



Scheme 2. Degradation route of Ru(II)-arene triphenylphosphine complex in chloroform solution upon air contact.

Although Ru(II) arene complexes with phosphine ligands may undergo, in chloroform in contact with air, degradation routes including Ru(II) to Ru(III) oxidation, the same compounds are not expected to be engaged in redox processes in the course of the *in vitro* cytotoxicity analyses (vide infra). In fact, former electrochemical studies on a variety of Ru(II) arene compounds did not evidence redox activity within a biologically relevant range of potentials.[16e,30]

2. In vitro cytotoxicity studies.

The ability of the newly prepared compounds **1-6** to inhibit cell growth was evaluated against the triple-negative breast cancer cell line MDA-MB-231 (Figure 6A and Table 1), and the human ovarian carcinoma cell line A2780 (Figure 6B and Table 1). The analyses were extended to [$RuCl_2(\eta^6$-*p*-cymene)(κP-PPh_3)] (**Ru-PPh$_3$**), [$Ru(C_2O_4)(\eta^6$-*p*-cymene)(κP-PPh_3)] (**Ru-PPh$_3$-O**) and [$RuCl_2(\eta^6$-*p*-cymene)(κP-PTA)] (RAPTA-C), as reference compounds. Cells were incubated with increasing concentration of compounds dissolved in DMSO.

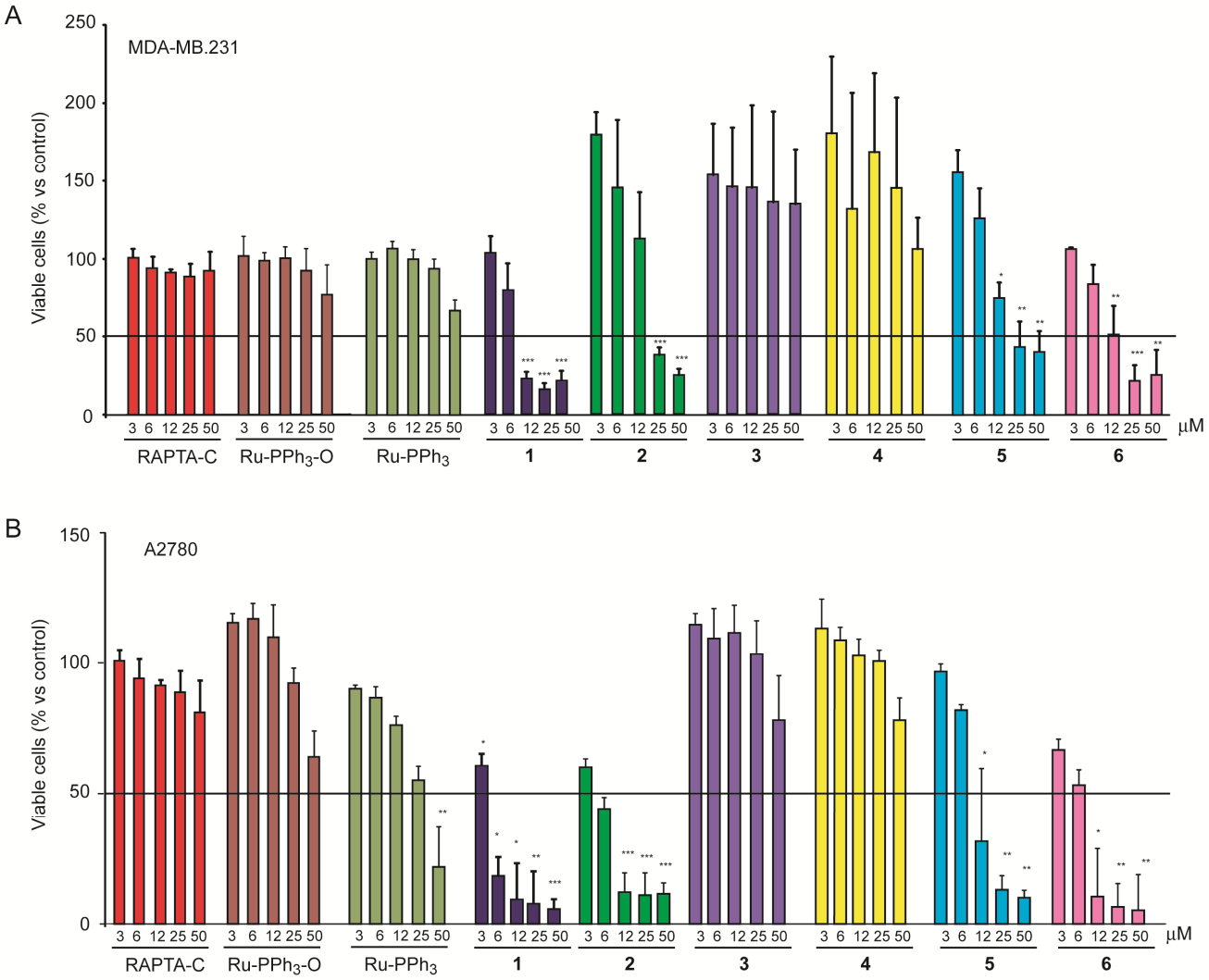


Figure 6. Cytotoxic activity of **1-6, Ru-PPh$_3$, Ru-PPh$_3$-O** and RAPTA-C towards: A) MDA-MB-231 cell line; B) A2780 cell line. Each bar represents the mean ± SD of three independent experiments. Inhibitors versus control: *P <0.05; **P <0.01; ***P <0.001.

Comparable cytotoxic effects were ascertained for the respective compounds on the two tumoral cell lines. In particular, **1**, **2**, **5** and **6** showed a considerable cytotoxic activity, although a tendency to stimulate the growth of MDA-MB-231 cells was observed for **2** and **5** at low concentrations (see Figure 6). The IC_{50} values are substantially lower than the values obtained with cisplatin on the same cell lines under the same experimental conditions. Otherwise, **3**, **4**, **Ru-PPh$_3$**, **Ru-PPh$_3$-O** and RAPTA-C did not

reduce the cell viability by more than 50% at the maximal tested concentration (50 μM). It should be noted that RAPTA-C was previously found to be non cytotoxic against a panel of cell lines.[5]

These results clearly indicate that the mono-substitution of one phenyl ring, in PPh_3 or $PPh_2(OPh)$, can lead to a significant increase in the cytotoxicity of the resulting complexes (compare **Ru-PPh$_3$** with **1**-**2**, and **4** with **5**-**6**). The relatively high activity exhibited by **1**, **5** and **6** could be somehow related to the presence of the *tert*-butyl dimethyl silyl substituent. As a matter of fact, *tert*-butyl dimethyl silyl, tethered to tetrahydropyran rings, was previously indicated as an enhancer of cytotoxicity against HL60 human leukemia cells and MCF7 breast cancer cells *in vitro*, due to its lipophilicity favouring the cellular uptake of the drug.[31]

It is possible that the drop of activity observed on moving from **1**-**2** to **3** is related to some unexpected effect associated with dichloroacetic acid,[19] which may be released from **3** inside the cells by means of intracellular esterases.[32] Compounds homologous to **3**, $[RuCl_2(\eta^6\text{-}p\text{-cymene})(\kappa P\text{-}Ph_2PAr^{BIO})]$, derivatized with bioactive carboxylic acids different from $CHCl_2CO_2H$, displayed variable cytotoxic activity towards A2780 and A2780cisR cancer cell lines (see Introduction) and, in one case (derivatization with Indomethacin), not appreciable activity.[16a]

We further explored the possible effect of **3** on cell viability by analysing the cell cycle progression after 24h incubation of A2780 cells (Table 2). We detected a lowering in the percentage of cells in S and G2/M phase compared to untreated cells, suggesting a possible interference of **3** with the progression from G1 to S phase. No sub G0 cells were observed, in agreement with the relatively low cytotoxicity exhibited by the complex and the absence of induction of apoptosis, under the employed experimental conditions.

In order to assess the possible selectivity of the more active compounds (**1**, **2**, **5** and **6**) towards cancer cells rather than non transformed cells, we extended the analysis of the antiproliferative activity to human skin fibroblasts (HSF). Complexes **1**, **5** and **6** are not endowed with cancer cell selectivity, i.e.

they are cytotoxic also to the HSF. Conversely, a moderate selectivity has been observed in the case of the Br-functionalized complex **2** [selectivity indexes are 2.3 (HSF/MDA-MB-231) and 4.6 (HSF/A2780)]: according to this result, the inclusion of $Ph_2P(4\text{-}C_6H_4Br)$ in ruthenium arene compounds might represent a privileged choice with respect to the use of PPh_3 (see Introduction).

Compound	MDA-MB-231	A2780	HSF
1	9.2 ± 1.1	3.6 ± 0.6	7.2 ± 1.0
2	13.9 ± 1.2	7.0 ± 1.6	31.9 ± 1.1
3	>50	>50	N/T
4	>50	>50	N/T
5	29.1 ± 1.4	9.4 ± 2.6	7.8 ± 1.1
6	14.4 ± 1.2	7.6 ± 1.2	12.5 ± 1.0
Ru-PPh_3	>50	>50	N/T
Ru-PPh_3-O	>50	>50	N/T
RAPTA-C	>50	>50	N/T
Cisplatin	59.4 [33]	31.5	N/T

Table 1. IC_{50} values (μM) determined for **1-6**, **Ru-PPh_3**, **Ru-PPh_3-O**, RAPTA-C and cisplatin, on human breast (MDA-MB-231) cancer cells and human skin fibroblasts (HSF) cells at 48 h. Values are given as the mean ± SD. N/T not tested.

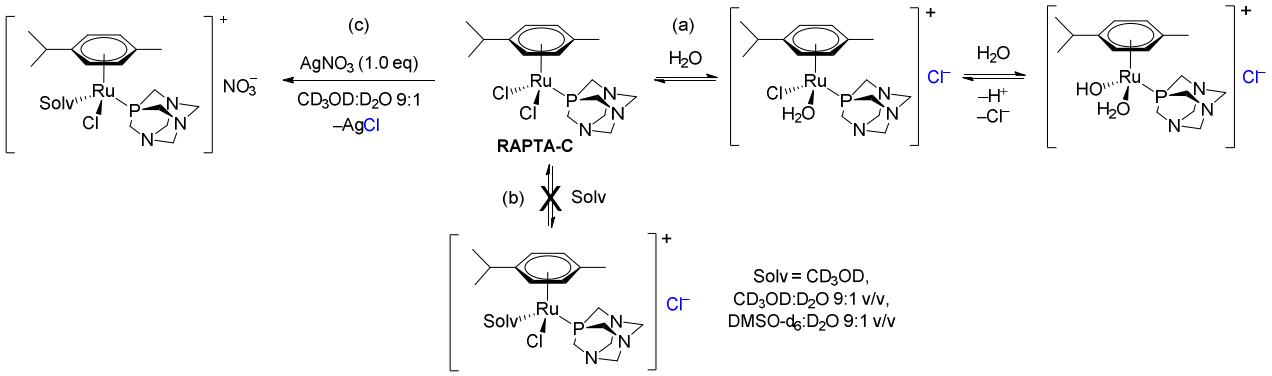
Compound	G0/G1 phase (%)	S phase (%)	G2/M phase (%)
Control	60.5	20.1	19.4
3	78.9	4.5	16.7
T-test	p<0.001	p<0.001	P=0.04

Table 2. Effect of complex **3** on cell cycle progression of A2780 cell line. Cells were incubated for 24h either in the presence or in the absence of **3** (50μM). At the end of this incubation period, the cell cycle analysis was performed.

3. Chloride/solvent exchange on model compounds and stability of 1-6 in aqueous solution.

We performed NMR experiments (^1H, ^{31}P, ^{35}Cl) and conductivity measurements to assess the stability of **1-6** and model compounds under pseudo-physiological conditions (see also Experimental Section and Supporting Information). In particular, ^{35}Cl NMR spectroscopy revealed to be a helpful tool to

detect the solvolysis of the Ru-Cl bonds. ^{35}Cl is a quadrupolar nucleus, thus covalently bound chlorines give raise to broad resonances in the ^{35}Cl NMR spectrum and are difficult to observe in a reasonable time. Otherwise, the chloride anion can be readily recognized: for instance, NaCl (D_2O solution) and $[Et_3NH]Cl$ (CD_3OD solution) display sharp ^{35}Cl resonances at 0.0 ppm (reference) and –22.7 ppm, respectively (see Experimental). In agreement with literature reports,[34] we clearly detected fast chloride/water exchange on RAPTA-C in D_2O solution by ^{35}Cl NMR (Scheme 3a). On the other hand, ^{35}Cl NMR indicated no Cl^-/solvent exchange when RAPTA-C was dissolved in CD_3OD, and even in CD_3OD/D_2O 9:1 and DMSO-d_6/D_2O 9:1 mixtures (Scheme 3b). Under these conditions, a single set of ^{1}H and ^{31}P NMR resonances was observed, in accordance with the RAPTA-C structure. Conversely, when one equivalent of $AgNO_3$ was added to the CD_3OD/D_2O 9:1 solution (Cl^- abstraction, Scheme 3c), the ^{1}H NMR pattern was in agreement with the structure $[RuCl(\eta^6\text{-}p\text{-cymene})(Solv)(PTA)]^+$ (stereogenic Ru-centre), and a marked increase in molar conductivity was measured too (from 35 to 81 $S\cdot cm^2\cdot mol^{-1}$).



Scheme 3. Behaviour of RAPTA-C in aqueous solutions; presence/absence of Cl^- ascertained by ^{35}Cl NMR spectroscopy.

Due to limited solubility in water, stability studies on $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$, **Ru-PPh$_3$**, **Ru-PPh$_3$-O** and **1-6** were carried out in DMSO/water 9:1 solutions. An overview of the different NMR identified species after 72 h is shown in Scheme 4, while the fraction of the starting material detected after 0, 24

and 48 h is given in Table 3. Analogously to what found for RAPTA-C in DMSO-d_6/D_2O 9:1, ^{35}Cl NMR experiments conducted on freshly prepared solutions suggested to rule out fast chloride dissociation from **1-3**, **Ru-PPh$_3$** and [$RuCl_2(\eta^6$-*p*-cymene)]$_2$.[35] Coherently, the respective ^{1}H and ^{31}P spectra, displaying a single set of resonances for the *p*-cymene ligand (C_s symmetry), did not modify upon addition of 0.15 M NaCl. Moreover, molar conductivity of the DMSO:water 9:1 solutions was significantly lower (≈ 20-25 S·cm^2·mol^{-1}) than expected for a 1:1 electrolyte in this solvent (≈ 50 S·cm^2·mol^{-1}, see SI). The set of signals (^{1}H, ^{31}P) belonging to the starting Ru-dichlorido complex persisted throughout the stability experiments (37°C, 72 h), and the only other set of observed Ru-arene ^{1}H signals was due to the formation of [$RuCl_2(\eta^6$-*p*-cymene)(DMSO)] (see Scheme 4).

The dimeric compound [$RuCl_2(\eta^6$-*p*-cymene)]$_2$ was cleanly cleaved into [$RuCl_2(\eta^6$-*p*-cymene)(DMSO)], **S1**, in DMSO/water 9:1 as well as in pure DMSO.[36] It should be mentioned that a sharp ^{35}Cl signal was previously reported for [$RuCl_2(\eta^6$-$C_6Et_6)]_2$ in methanol solution at −50 °C, but not at ambient temperature. Such phenomenon was attributed to the existence of the equilibrium [$RuCl_2(\eta^6$-$C_6Et_6)]_2$ ⇆ [$Ru_2Cl_3(\eta^6$-$C_6Et_6)$]Cl, shifting in favour of the ionic species at low temperature, rather than to a possible fast chloride/solvent exchange on the NMR timescale.[27b]

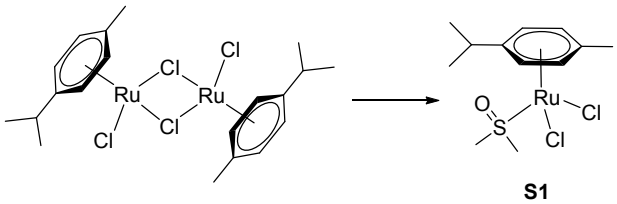
Progressive phosphine/DMSO exchange was detected for **1-5** and **Ru-PPh$_3$** during 72 h. In **6** and **Ru-PPh$_3$-O**, lacking of chloride ligands but containing a bis-carboxylate group, the phosphine release was significantly inhibited; the solvato-species [$Ru(C_2O_4)(\eta^6$-*p*-cymene)(DMSO)], **S2**, was formed from **6** in low amount only after 72 h.

It seems reasonable that, in accordance with the behaviour of RAPTA-C (Scheme 3) and some water-soluble [$RuCl_2(\eta^6$-*p*-cymene)(PAr_3)] complexes,[37] **1-6** may undergo Cl^-/solvent exchange more easily in diluted (micromolar) solutions with a higher relative content of water, as is the case of cell culture. This process is expected to afford cationic species, basically [$RuCl(\eta^6$-*p*-cymene)(H_2O)(PPh_2R)]$^+$, whereby the solvolysis of the phosphine ligand should be inhibited with respect to what observed on

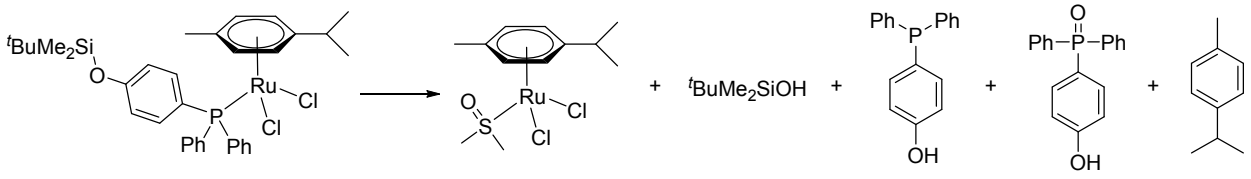
the parent neutral derivatives, [$RuCl_2(\eta^6$-*p*-cymene)(PPh_2R)], in DMSO:D_2O 9:1 (Scheme 4). In other terms, the degree of phosphine dissociation recognized for the complexes in DMSO/water is probably overestimated respect to the real situation of the *in vitro* trials.

The disruptive effect of DMSO is likely to be responsible also for the observed, partial release of the *p*-cymene ligand from **1-6**, **Ru-PPh_3** and **Ru-PPh_3-O** (Scheme 4).[38] Indeed, arene dissociation from Ru(II) complexes in water is generally not observed[39] unless under UV irradiation.[40]

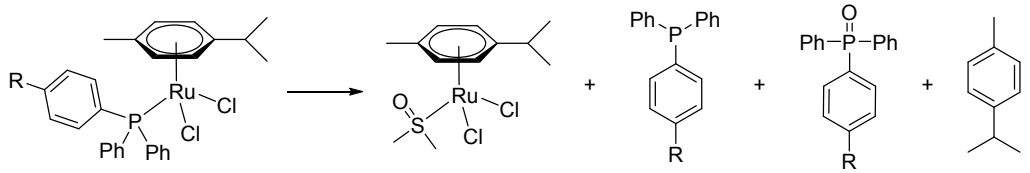
Accounting all of these considerations, the cytotoxic activity of **1-6** is plausibly related for the most part to non dissociated ruthenium-phosphine species.



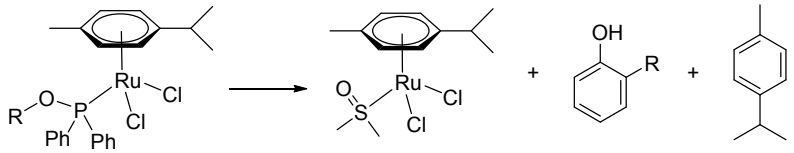
S1



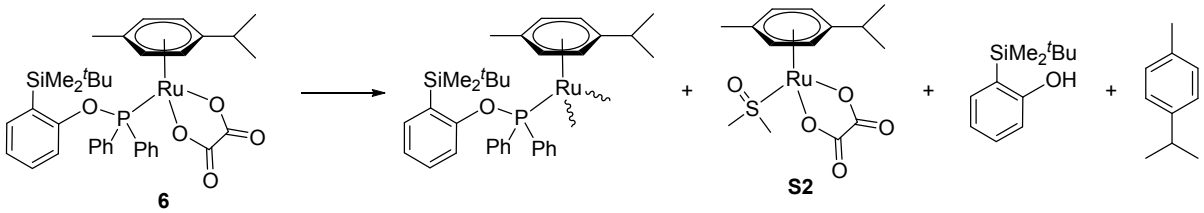
1 **S1**



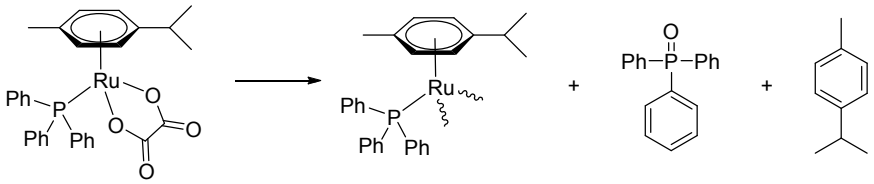
R = Br (**2**), $OCOCHCl_2$ (**3**), H (**Ru-PPh$_3$**) **S1**



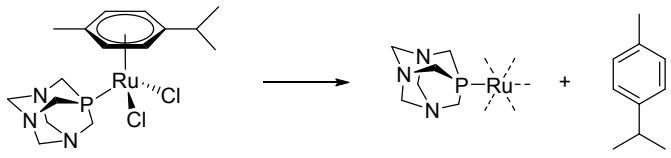
R = H (**4**), 2-$C_6H_4(OSiMe_2{}^tBu)$ (**5**) **S1**



6 **S2**



Ru-PPh$_3$-O



RAPTA-C

Scheme 4. Compounds detected in DMSO-d_6/D_2O solutions of **1**-**6**, $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$, **Ru-PPh$_3$**, **Ru-PPh$_3$-O** and RAPTA-C maintained at 37 °C for 72 h.

Compound	% (^{1}H NMR *vs.* internal standard)		
	t = 0	t = 23.5 h	t = 48 h
1	97	70	24
2	97	67	45
3	95	56	37
4	94	25	14
5	96	33	6
6	100	75	56
Ru-PPh$_3$	98	77	56
Ru-PPh$_3$-O	99	77	74
RAPTA-C	100	90 [a]	63 [b]

Table 3. Fraction of complexes **1**-**6**, **Ru-PPh$_3$**, **Ru-PPh$_3$-O** and RAPTA-C in DMSO-d_6/D_2O at 37 °C after 24 h and 48 h; % values are based on ^{1}H NMR spectroscopy (dimethyl sulfone as internal standard). [a] t = 16.5 h. [b] t = 40.5 h.

Conclusions

Since a variety of ruthenium(II) arene complexes containing a phosphine ligand have aroused interest for their possible anticancer activity, we have prepared and characterized a series of new Ru(II) arene complexes containing a triphenylphosphine or phenoxydiphenylphosphine ligand, variably mono-substituted at one phenyl ring. In general, the substitution leads to an increase of the cytotoxic activity of the complexes (MDA-MB-231 and A2780 cancer cell lines), some of them displaying IC_{50} values much lower than those related to cisplatin. A moderate level of selectivity towards cancer cells respect to non tumorous cells has been observed with (4-bromophenyl)diphenylphosphine, whose use might become convenient when the synthetic design of antitumoral ruthenium arene compounds includes the

incorporation of a triphenylphosphine moiety. According to stability studies carried out in aqueous solutions, we presume that the *in vitro* cytotoxic activity of the compounds is largely ascribable to phosphine-bound Ru(II) species. Although the cross combination with other techniques seems needed for conclusive information, ^{35}Cl NMR spectroscopy is helpful to study the possible solvolysis of Ru-Cl bonds, which represents a largely accepted mechanism for drug activation.

Experimental Section.

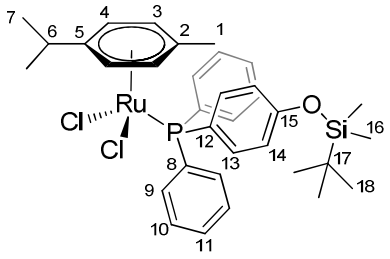
General experimental details. $RuCl_3 \cdot 3H_2O$ (99.9%) was purchased from Strem, while all the other reactants were obtained from Alfa Aesar, Sigma Aldrich or TCI Europe, and were of the highest purity available. The following reagents were stored under nitrogen or argon as received: 2-bromophenol (under protection from the light), triethylamine, *tert*-butyldimethylsilyl chloride (TBDMSCl), butyl lithium (2.5 M solution in hexanes, 4 °C), chlorodiphenylphosphine, phenoxydiphenylphosphine (4 °C), PCl_5, oxalyl chloride (4 °C), ethyl(diisopropylamino)carboxydiimide hydrochloride (EDCI·HCl, –20 °C). Dichloroacetic acid was distilled under reduced pressure, dried under vacuum over P_2O_5 and stored under nitrogen. $Ph_2P(4\text{-}C_6H_4Br)$,[41] $[Ru(C_2O_4)(\eta^6\text{-}p\text{-cymene})(H_2O)]$,[24c] $[RuCl_2(\eta^6\text{-}p\text{-cymene})(\kappa P\text{-}PPh_3)]$,[42] and $[Ru(C_2O_4)(\eta^6\text{-}p\text{-cymene})(\kappa P\text{-}PPh_3)]$[43] were prepared according to modified literature procedures (see Supporting Information). $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$,[44] $[RuCl_2(\eta^6\text{-}p\text{-cymene})(PTA)]$ (RAPTA-C),[45] $Ph_2P(4\text{-}C_6H_4OSiMe_2{}^tBu)$[16a] and $[RuCl_2(\eta^6\text{-}p\text{-cymene})(Ph_2P(4\text{-}C_6H_4OH))]$[16a] were prepared according to the literature. All reactions, except the preparation of $[Ru(C_2O_4)(\eta^6\text{-}p\text{-cymene})(H_2O)]$, were carried out under a nitrogen atmosphere using standard Schlenk techniques and solvents distilled from appropriate drying agents. Once isolated, $o\text{-}C_6H_4Br(OSiMe_2{}^tBu)$, $Ph_2PO(2\text{-}C_6H_4(SiMe_2{}^tBu))$, $o\text{-}C_6H_4(OH)(SiMe_2{}^tBu)$ (under protection from the light), $Ph_2P(4\text{-}C_6H_4OSiMe_2{}^tBu)$ and $Ph_2P(4\text{-}C_6H_4Br)$ were stored under nitrogen, all the other products being air stable. Compound $[Ru(C_2O_4)(\eta^6\text{-}p\text{-cymene})(H_2O)]$ was either used some days after its preparation or stored under

nitrogen for longer periods. Silica gel (Merck, 70-230 mesh) was dried at 150 °C overnight and stored under nitrogen. NMR spectra were recorded at 298 K on a Bruker Avance II DRX400 instrument equipped with a BBFO broadband probe. Chemical shifts (expressed in parts per million) are referenced to the residual solvent peaks[46] (^{1}H, ^{13}C) or to external standard (^{31}P to 85% H_3PO_4; ^{29}Si to TMS, ^{35}Cl to 1 M NaCl in D_2O). Spectra were assigned with the assistance of ^{1}H{^{31}P}, DEPT-135 spectra and ^{1}H-^{1}H (COSY), ^{1}H-^{13}C (*gs*-HSQC and *gs*-HMBC) correlation experiments.[47] Infrared spectra of solid samples were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer, equipped with a UATR sampling accessory. Infrared spectra of CH_2Cl_2 solutions were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer with a CaF_2 liquid transmission cell. UV-Vis spectra were recorded on a Ultraspec 2100 Pro spectrophotometer with 0.1 cm quartz cuvettes. IR and UV-Vis spectra were processed with Spectragryph software.[48] Carbon, hydrogen and nitrogen analysis was performed on a Carlo Erba mod. 1106 instrument. Melting/decomposition temperatures were determined on a STMP3 Stuart scientific instrument with a capillary apparatus. Conductivity measurements[49] were carried out at 21°C using an XS COND 8 instrument (cell constant = 1.0 cm^{-1}).

Synthesis of new compounds.

A. Synthesis of [RuCl$_2$(η^6-*p*-cymene){κ*P*-Ph$_2$P(4-C$_6$H$_4$OSiMe$_2$^{t}Bu)}], 1.

Chart 1. Structure of **1** (numbering refers to carbon atoms).

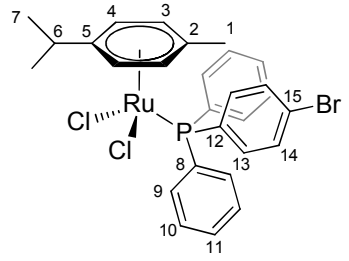


In a 25 mL Schlenk tube, [RuCl$_2$(η^6-*p*-cymene)]$_2$ (254 mg, 0.415 mmol) and Ph$_2$P(4-C$_6$H$_4$OSiMe$_2$^{t}Bu)

(389 mg, 0.991 mmol) were dissolved in CH_2Cl_2 (10 mL). The resulting deep red solution was stirred at ambient temperature for 7.5 hours and the formation of a ruthenium-coordinated phosphine compound was assessed by TLC, ^{29}Si and ^{31}P NMR. Therefore volatiles were removed under vacuum and the residue was suspended in pentane (20 mL). The suspension was cooled to 0 °C and filtered; the resulting orange-brown solid was washed with a small volume of cold pentane and dried under vacuum (45 °C). Yield: 469 mg, 81%. The title compound is soluble in DMSO, EtOH, CH_2Cl_2 and Et_2O, less soluble in pentane/petroleum ether and insoluble in H_2O. Anal. calcd. for $C_{34}H_{43}Cl_2OPRuSi$: C, 58.44; H, 6.20; Cl, 10.15. Found: C, 58.16; H, 6.25; Cl, 10.02. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3055w, 2957w, 2929w, 2896w, 2885w, 2857w, 1591m, 1497s, 1482m-sh, 1471m, 1435m, 1401w, 1388w, 1361w, 1257s-br, 1176s ($\nu_{O\text{-}Ar}$), 1093s, 1057w, 1029w, 1005w, 907s, 838s, 824s-sh, 806s, 781s, 744m, 719w, 693s, 673m-sh. ^1H NMR ($CDCl_3$): δ/ppm = 7.83–7.76 (m, 4H, C9-H), 7.70 (pseudo-t, $^3J_{HH} = {}^3J_{HP}$ = 9 Hz, 2H, C13-H), 7.40–7.32 (m, 6H, C10-H + C11-H), 6.82 (d, $^3J_{HH}$ = 7.3 Hz, 2H, C14-H), 5.19 (d, $^3J_{HH}$ = 5.8 Hz, 2H, C4-H), 4.98 (d, $^3J_{HH}$ = 5.5 Hz, 2H, C3-H), 2.87 (hept, $^3J_{HH}$ = 6.1 Hz, 1H, C6-H), 1.86 (s, 3H, C1-H), 1.10 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C7-H), 0.97 (s, 9H, C18-H), 0.20 (s, 6H, C16-H). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 157.7 (C15), 136.4 (d, $^2J_{CP}$ = 11 Hz, C13), 134.5 (d, $^1J_{CP}$ = 46 Hz, C8), 134.3 (d, $^2J_{CP}$ = 9 Hz, C9), 130.2 (d, $^4J_{CP}$ = 2 Hz, C11), 128.0 (d, $^3J_{CP}$ = 10 Hz, C10), 125.0 (d, $^1J_{CP}$ = 50 Hz, C12), 119.7 (d, $^3J_{CP}$ = 11 Hz, C14), 111.1 (d, $^2J_{CP}$ = 3 Hz, C5), 96.0 (C2), 89.1 (d, $^2J_{CP}$ = 3 Hz, C3), 87.3 (d, $^2J_{CP}$ = 6 Hz, C4), 30.4 (C6), 25.7 (C17), 22.0 (C7), 18.3 (C18), 17.9 (C1), -4.5 (C16). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 23.2. $^{29}Si\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 21.7.

B. Synthesis of [$RuCl_2(\eta^6$-*p*-cymene){κ*P*-$Ph_2P(4\text{-}C_6H_4Br)$}], 2.

Chart 2. Structure of **2** (numbering refers to carbon atoms).

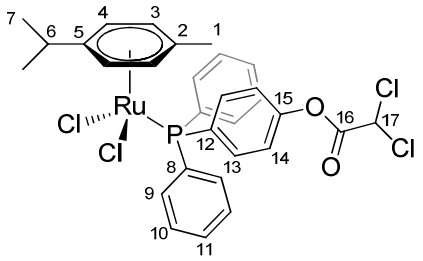


In a 25 mL Schlenk tube, $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ (65 mg, 0.106 mmol) and $Ph_2P(4\text{-}C_6H_4Br)$ (79 mg, 0.232 mmol) were dissolved in $CHCl_3$ (12 mL). The resulting red solution was heated at reflux for 15 hours and the progress of reaction was checked by TLC. The reaction mixture was cooled to ambient temperature, volatiles were removed under vacuum and the residue was dissolved in a small volume of CH_2Cl_2. Addition of hexane under vigorous stirring caused the precipitation of the title compound as a red solid. The suspension was filtered; the solid was washed with hexane and then with a small volume of hexane/Et_2O mixture (1:1 *v*/*v* ratio), and finally dried under vacuum (40 °C). Yield: 125 mg, 91%. **2** was obtained in admixture with minor products when the reaction was performed at ambient temperature. The title compound is soluble in DMSO, acetone and chlorinated solvents, less soluble in MeOH, poorly soluble in Et_2O and insoluble in H_2O and hexane. Anal. calcd. for $C_{28}H_{28}BrCl_2PRu$: C, 51.95; H, 4.36; Cl, 10.95. Found: C, 52.06; H, 4.23; Cl, 10.84. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3049w, 2960w, 2925w, 2870w, 1572w, 1556w, 1480m, 1468m-sh, 1434m, 1384m, 1325w, 1277w, 1189m, 1161w, 1117w, 1094m, 1070m, 1058m-sh, 1028w, 1009m, 951w, 926w, 896w, 869w, 811m, 799m-sh, 752m, 725s, 698s. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.83–7.76 (m, 4H, C9-H), 7.70 (pseudo-t, $^3J_{HH} = {}^3J_{HP}$ = 8.9 Hz, 2H, C13-H), 7.47–7.34 (m, 8H, C10-H + C11-H + C14-H), 5.21 (d, $^3J_{HH}$ = 4.9 Hz, 2H, C3-H/C4-H), 4.99 (d, $^3J_{HH}$ = 4.4 Hz, 2H, C3-H/C4-H), 2.84 (hept, $^3J_{HH}$ = 6.5 Hz, 1H, C6-H), 1.85 (s, 3H, C1-H), 1.10 (d, $^3J_{HH}$ = 6.7 Hz, 6H, C7-H). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 136.3 (d, $^2J_{CP}$ = 10 Hz, C13), 134.2 (d, $^2J_{CP}$ = 10 Hz, C9), 133.8 (d, $^1J_{CP}$ = 45 Hz, C8), 132.3 (d, $^1J_{CP}$ = 46 Hz, C12), 131.0 (d, $^3J_{CP}$ = 10 Hz, C14), 130.6 (d, $^4J_{CP}$ = 2 Hz, C11), 128.3 (d, $^3J_{CP}$ = 10 Hz, C10), 125.3 (d, $^4J_{CP}$ = 3 Hz, C15),

111.5 (d, $^2J_{CP}$ = 3 Hz, C5), 96.3 (C2), 89.1 (d, $^2J_{CP}$ = 3 Hz, C3/C4), 87.4 (d, $^2J_{CP}$ = 5 Hz, C3/C4), 30.4 (C6), 22.0 (C7), 17.9 (C1). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 24.4.

C. Synthesis of [$RuCl_2(\eta^6$-*p*-cymene){κ*P*-$Ph_2P(4\text{-}C_6H_4OCOCHCl_2)$}], 3.

Chart 3. Structure of **3** (numbering refers to carbon atoms).



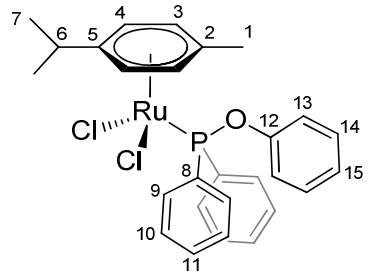
In a 25 mL Schlenk tube, CH_2Cl_2 (6 mL), Cl_2CHCO_2H (30 µL, 0.36 mmol), [$RuCl_2(\eta^6$-*p*-cymene){κ*P*-$Ph_2P(4\text{-}C_6H_4OH)$}] (75 mg, 0.13 mmol), EDCI·HCl (97 mg, 0.51 mmol) and DMAP (6 mg, 0.05 mmol) were introduced in the order given. The resulting red solution was stirred at ambient temperature and aliquots of the solution were taken for ^1H and ^{31}P NMR analysis. After 2.5 hours, volatiles were removed under vacuum. Shortly afterwards, the residue was dissolved in a small volume of CH_2Cl_2, the solution was diluted with EtOAc (10 mL) and then extracted with water (3x20mL). Volatiles were removed under vacuum from the organic phase and the residue was dissolved in a small volume of DCM/Et_2O (1:1 *v*/*v*). Hexane addition under intense stirring caused the precipitation of the title compound as a red-brown powder. The suspension was filtered and the solid was washed with hexane and dried under vacuum (40 °C). Yield: 75 mg, 83%. Note: it is important for the workup to be performed immediately after the end of the reaction. The title compound is soluble in chlorinated solvents and DMSO, less soluble in Et_2O, insoluble in hexane and water. X-ray quality crystals of **3**·acetone were obtained from an acetone solution of **3** layered with heptane and settled aside at -20 °C. Anal. calcd. for $C_{30}H_{29}Cl_4O_2PRu$: C, 51.81; H, 4.20; Cl, 20.39. Found: C, 51.67; H, 4.28; Cl, 20.26. IR

(solid state): ῡ/cm^{-1} = 3055w, 2962w, 2929w-sh, 2870w, 1785m-sh and 1768m ($\nu_{C=O}$), 1587w, 1493m, 1483m, 1471w, 1435m, 1396w, 1386w, 1289m, 1234m, 1205m, 1168s ($\nu_{O\text{-}Ar}$), 1139m, 1093m, 1057w, 1028w, 1017m, 999w, 938w, 858m, 822m, 798m, 747m, 695s. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.90 (pseudo-t, $^{3}J_{HH}$ = $^{3}J_{HP}$ = 8.2 Hz, 2H, C13-H), 7.81 (pseudo-t, $^{3}J_{HH}$ = $^{3}J_{HP}$ = 8.2 Hz, 4H, C9-H), 7.46–7.36 (m, 6H, C10-H + C11-H), 7.12 (d, $^{3}J_{HH}$ = 7.5 Hz, 2H, C14-H), 6.13 (s, 1H, C17-H), 5.20 (d, $^{3}J_{HH}$ = 4.2 Hz, 2H, C3-H/C4-H), 5.01 (d, $^{3}J_{HH}$ = 3.7 Hz, 2H, C3-H/C4-H), 2.81 (hept, $^{3}J_{HH}$ = 6.1 Hz, 1H, C6-H), 1.85 (s, 3H, C1-H), 1.09 (d, $^{3}J_{HH}$ = 6.1 Hz, 6H, C7-H). ^{13}C{^{1}H} NMR ($CDCl_3$): δ/ppm = 162.5 (C16), 151.4 (d, $^{4}J_{CP}$ = 2 Hz, C15), 136.3 (d, $^{2}J_{CP}$ = 10 Hz, C13), 134.2 (d, $^{2}J_{CP}$ = 10 Hz, C9), 133.8 (d, $^{1}J_{CP}$ = 45 Hz, C8), 132.0 (d, $^{1}J_{CP}$ = 47 Hz, C12), 130.6 (d, $^{4}J_{CP}$ = 1 Hz, C11), 128.3 (d, $^{3}J_{CP}$ = 10 Hz, C10), 120.1 (d, $^{3}J_{CP}$ = 11 Hz, C14), 111.3 (C5), 96.2 (C2), 89.2 (d, $^{2}J_{CP}$ = 2 Hz, C3/C4), 87.3 (d, $^{2}J_{CP}$ = 5 Hz, C3/C4), 64.2 (C17), 30.3 (C6), 21.9 (C7), 17.8 (C1). ^{31}P{^{1}H} NMR ($CDCl_3$): δ/ppm = 24.0. Freshly-prepared solutions of **3** in $CDCl_3$ and DMSO-d_6 show broadening of NMR resonances, probably due to association phenomena. A regular ^{1}H spectrum can be obtained after some hours at ambient temperature. ^{1}H NMR (CD_3OD): δ/ppm = 7.91 (pseudo-t, $^{3}J_{HH}$ = $^{3}J_{HP}$ = 9.1 Hz, 2H, C13-H), 7.88–7.80 (m, 4H, C9-H), 7.52–7.36 (m, 6H, C10-H + C11-H), 7.19 (d, $^{3}J_{HH}$ = 7.4 Hz, 2H, C14-H), 6.71 (s, 1H, C17-H), 5.32 (d, $^{3}J_{HH}$ = 5.5 Hz, 2H, C3-H/C4-H), 5.22 (d, $^{3}J_{HH}$ = 4.9 Hz, 2H, C3-H/C4-H), 2.63 (hept, $^{3}J_{HH}$ = 7.1 Hz, 1H, C6-H), 1.88 (s, 3H, C1-H), 1.07 (d, $^{3}J_{HH}$ = 6.7 Hz, 6H, C7-H). ^{31}P{^{1}H} NMR (CD_3OD): δ/ppm = 24.0.

Solutions containing $Cl_2CHCOCl$, freshly prepared from dichloroacetic acid (see SI), reacted with [$RuCl_2$(η^6-*p*-cymene)($Ph_2P(4\text{-}C_6H_4OH)$)] and Et_3N affording mixtures of products including **3**.

D. Synthesis of [$RuCl_2$(η^6-*p*-cymene)(κ*P*-Ph_2POPh)], 4.

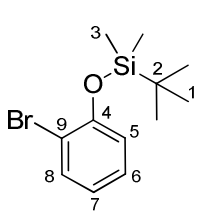
Chart 4. Structure of **4** (numbering refers to carbon atoms).

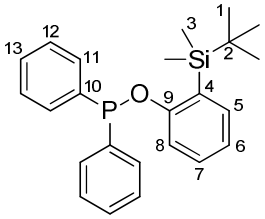


In a 25 mL Schlenk tube, $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ (133 mg, 0.218 mmol) and $Ph_2P(OPh)$ (145 mg, 0.521 mmol) were dissolved in $CHCl_3$ (7 mL). The resulting red solution was stirred at ambient temperature for 15 hours and the progress of reaction was checked by TLC. Volatiles were removed under vacuum and the residue was suspended in petroleum ether (20 mL). The suspension was filtered and the resulting orange-red solid was washed with petroleum ether and dried under vacuum (40 °C). Yield: 251 mg, 98%. The title compound is soluble in DMSO and chlorinated solvents, less soluble in acetone and EtOH and insoluble in H_2O and hydrocarbons. Crystals suitable for X-ray analysis were obtained from CH_2Cl_2 solutions of **4** layered with either Et_2O or hexane, and settled aside at -20 °C. Anal. calcd. for $C_{28}H_{29}Cl_2OPRu$: C, 57.54; H, 5.00; Cl, 12.13. Found: C, 57.46; H, 4.92; Cl, 12.21. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3055w, 2965w, 2874w, 1589m, 1491m, 1481m, 1435m, 1389w, 1374w, 1288w, 1238w, 1208s ($\nu_{O\text{-}Ar}$), 1185m, 1174m, 1158w, 1104m-sh, 1093m, 1075w, 1056w, 1028w, 998w, 956w, 926w, 889s ($\nu_{P\text{-}O}$), 859m, 826w, 798w, 764s, 750s, 727s, 708s, 697s, 689s. ^1H NMR ($CDCl_3$): δ/ppm = 8.04–8.00 (m, 4H, C9-H), 7.38–7.31 (m, 6H, C10-H and C11-H), 7.31–7.22 (m, 4H, C13-H + C14-H), 7.04 (t, $^3J_{HH}$ = 7.0 Hz, 1H, C15-H), 5.26 (pseudo-q, $^3J_{HH}$ = 5.8 Hz, 4H, C3-H + C4-H), 2.54 (hept, $^3J_{HH}$ = 6.6 Hz, 1H, C6-H), 1.50 (s, 3H, C1-H), 0.85 (d, $^3J_{HH}$ = 6.8 Hz, 6H, C7-H). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 152.9 (d, $^2J_{CP}$ = 5 Hz, C12), 136.2 (d, $^1J_{CP}$ = 48 Hz, C8), 132.1 (d, $^2J_{CP}$ = 11 Hz, C9), 130.9 (d, $^4J_{CP}$ = 1 Hz, C11), 129.8 (C14), 128.0 (d, $^3J_{CP}$ = 10 Hz, C10), 123.7 (C15), 120.8 (d, $^3J_{CP}$ = 6 Hz, C13), 110.5 (C5), 97.3 (C2), 91.4 (d, $^2J_{CP}$ = 4 Hz, C3/C4), 87.6 (d, $^2J_{CP}$ = 5.8 Hz, C3/C4), 30.1 (C6), 21.5 (C7), 17.3 (C1). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 113.7.

E. Synthesis and characterization of $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$.

Chart 5. Structures of $2\text{-}C_6H_4(Br)(OSiMe_2{}^tBu)$ (left) and $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$ (right) (numbering refers to carbon atoms).





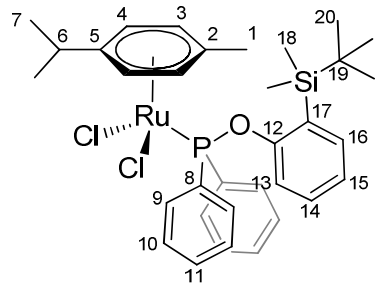
Step 1.[50] In a 100-mL Schlenk tube, Et_3N (1.8 mL, 13 mmol) and TBDMSCl (1.88 g, 12.5 mmol) were added to a solution of 2-bromophenol (1.0 mL, 9.3 mmol) in CH_2Cl_2 (15 mL). The pale yellow solution was stirred at ambient temperature for 4.5 hours under protection from the light and the progress of reaction was monitored by TLC. The resulting pale yellow suspension was extracted with H_2O (3x20 mL), then the volatiles were removed from the organic phase under vacuum (50 °C). The product $2\text{-}C_6H_4(Br)(OSiMe_2{}^tBu)$ was obtained as a pale yellow liquid. Yield: quantitative. ^1H NMR ($CDCl_3$): δ/ppm = 7.63 (dd, $^3J_{HH}$ = 7.9, $^4J_{HH}$ = 1.6 Hz, 1H, C8-H), 7.28 (td, $^3J_{HH}$ = 7.7, $^4J_{HH}$ = 1.6 Hz, 1H, C6-H), 6.99 (dd, $^3J_{HH}$ = 8.1, $^4J_{HH}$ = 1.5 Hz, 1H, C5-H), 6.92 (td, $^3J_{HH}$ = 7.4, $^4J_{HH}$ = 1.4 Hz, 1H, C7-H), 1.17 (s, 9H, C1-H), 0.37 (s, 6H, C3-H). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 152.6 (C4), 133.5 (C8), 128.3 (C6), 122.4 (C7), 120.4 (C5), 115.5 (C9), 26.0 (C1), 18.6 (C2), -4.0 (C3).

Step 2. In a 100 mL Schlenk tube, *n*-BuLi (3.8 mL of a 2.5 M solution in hexanes, 9.5 mmol) was slowly added (15') to a colourless solution of $2\text{-}C_6H_4(Br)(OSiMe_2{}^tBu)$ (2.70 g, 9.4 mmol) in Et_2O (20 mL), at 0 °C under vigorous stirring. The resulting pale yellow solution was allowed to reach ambient temperature and stirred for additional 1.5 hours. Therefore Ph_2PCl (1.75 mL, 9.5 mmol) was added dropwise to the solution along five minutes time at 0 °C, and precipitation of a colourless solid (LiCl)

occurred. The resulting suspension was allowed to reach ambient temperature and then stirred for additional 20 hours. The reaction mixture was concentrated under reduced pressure, loaded on top of a silica column (h = 4 cm, d = 3 cm) and eluted with hexane. The title compound was obtained as a colourless solid after solvent removal under vacuum (50 °C). Yield: 3.12 g, 86%. Anal. Calcd. for $C_{24}H_{29}OPSi$: C, 73.43; H, 7.45. Found: C, 73.22; H, 7.56. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3073w, 3058w, 3005w, 2950w, 2924m, 2880w, 2852m, 1584m, 1564w, 1468s, 1432s, 1421m-sh, 1385w, 1360w, 1306w, 1268m, 1258m, 1242w, 1200s ($\nu_{O\text{-}Ar}$), 1183m-sh, 1161w-sh, 1125m, 1094m, 1075m, 1041m, 1026m, 1009w, 938w, 914w, 870s ($\nu_{P\text{-}O}$), 848m, 833s, 821s, 806s, 772s, 757s, 744s, 731s, 693s, 683s-sh. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.64–7.58 (m, 4H, C11-H), 7.46–7.42 (m, 7H, C5-H + C12-H + C13-H), 7.31 (dt, $^{3}J_{HH}$ = 15.4 Hz, $^{4}J_{HH}$ = 1.6 Hz, 1H, C7-H), 7.10 (dd, $^{3}J_{HH}$ = 8.1 Hz, $^{4}J_{HP}$ = 2.6 Hz, 1H, C8-H), 7.06 (t, $^{3}J_{HH}$ = 7.3 Hz, 1H, C6-H), 0.81 (s, 9H, C1-H), 0.14 (s, 1H, C3-H). ^{13}C{^{1}H} NMR ($CDCl_3$): δ/ppm = 162.3 (d, $^{2}J_{CP}$ = 9 Hz, C9), 140.0 (d, $^{1}J_{CP}$ = 17 Hz, C10), 136.8 (C5), 131.7 (d, $^{2}J_{CP}$ = 23 Hz, C11), 130.8 (d, $^{4}J_{CP}$ = 2 Hz, C7), 130.0 (C13), 128.5 (d, $^{3}J_{CP}$ = 7 Hz, C12), 127.2 (d, $^{3}J_{CP}$ = 3 Hz, C4), 121.7 (C6), 116.0 (d, $^{3}J_{CP}$ = 23 Hz, C8), 27.1 (C1), 17.7 (C2), -4.4 (C3). ^{31}P{^{1}H} NMR ($CDCl_3$): δ/ppm = 108.3. ^{29}Si{^{1}H} NMR ($CDCl_3$): δ/ppm = 3.3.

F. Synthesis of [$RuCl_2$(η^6-*p*-cymene){κ*P*-$Ph_2PO(2\text{-}C_6H_4(SiMe_2{}^tBu))$}], 5.

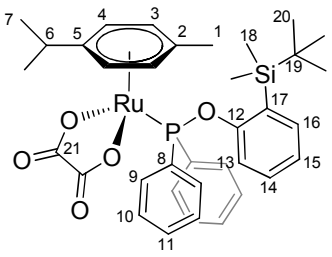
Chart 6. Structure of **5** (numbering refers to carbon atoms).



In a 25 mL Schlenk tube, $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ (66 mg, 0.11 mmol) and $Ph_2P(O(2\text{-}C_6H_4SiMe_2{}^tBu))$ (111 mg, 0.283 mmol) were dissolved in CH_2Cl_2 (6 mL). The resulting red solution was stirred at ambient temperature for 14 hours and the progress of reaction was checked by TLC. Volatiles were removed under vacuum and the resulting red oily residue was triturated and suspended in hexane (20 mL). The suspension was filtered and the red solid was washed with hexane and dried under vacuum (40 °C). Yield: 129 mg, 86%. The title compound is soluble in DMSO and chlorinated solvents, poorly soluble in EtOH and Et_2O and insoluble in hexane and H_2O. Crystals suitable for X-ray analysis were obtained from a $CHCl_3$ solution of **5** layered with either hexane or heptane, and settled aside at -20 °C. Anal. calcd. for $C_{34}H_{43}Cl_2OPRuSi$: C, 58.44; H, 6.20; Cl, 10.15. Found: C, 58.32; H, 6.12; Cl, 9.97. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3063w, 3041w, 2955m, 2925m, 2882w, 2853w, 1590w, 1568w, 1542w, 1469m, 1435m, 1427m, 1378w, 1359w, 1322w, 1292w, 1273w, 1262w, 1255w, 1185s ($\nu_{O\text{-}Ar}$), 1130m, 1096s, 1078m, 1058w, 1037w, 1006w, 975w, 936w, 909w, 885s ($\nu_{P\text{-}O}$), 859w-sh, 835m, 821s, 807s, 776m, 758s, 737s, 722w, 705m-sh, 696s, 687s-sh. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.92 (pseudo-t, $^3J_{HH} = {}^3J_{HP}$ = 8 Hz, 4H, C9-H), 7.62 (d, $^3J_{HH}$ = 8.3 Hz, 1H, C13-H), 7.52 (d, $^3J_{HH}$ = 7.6 Hz, 1H, C16-H), 7.39–7.28 (m, 6H, C10-H + C11-H), 7.19 (dt, $^3J_{HH}$ = 7.7 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, C14-H), 7.04 (t, $^3J_{HH}$ = 7.2 Hz, 1H, C15-H), 5.39 (d, $^3J_{HH}$ = 6.2 Hz, 2H, C4-H), 5.33 (d, $^3J_{HH}$ = 6.0 Hz, 2H, C3-H), 2.56 (hept, $^3J_{HH}$ = 6.9 Hz, 1H, C6-H), 1.67 (s, 3H, C1-H), 1.08 (s, 9H, C20-H), 0.91 (d, $^3J_{HH}$ = 7.0 Hz, 6H, C7-H), 0.40 (s, 6H, C18-H). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 158.2 (d, $^2J_{CP}$ = 4 Hz, C12), 137.5 (C16), 135.5 (d, $^1J_{CP}$ = 31 Hz, C8), 132.6 (d, $^2J_{CP}$ = 11 Hz, C9), 131.0 (C11), 130.9 (d, $^4J_{CP}$ = 2 Hz, C14), 127.7 (d, $^3J_{CP}$ = 11 Hz, C10), 126.0 (d, $^3J_{CP}$ = 7 Hz, C17), 122.6 (C15), 122.0 (d, $^3J_{CP}$ = 10 Hz, C13), 110.6 (C5), 97.2 (C2), 91.1 (C3), 87.4 (C4), 30.3 (C6), 27.5 (C20), 21.7 (C7), 18.1 (C19), 18.0 (C1), -3.5 (C18). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 120.3. $^{29}Si\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 3.0

G. Synthesis of $[Ru(C_2O_4)(\eta^6\text{-}p\text{-cymene})\{\kappa P\text{-}Ph_2PO(2\text{-}C_6H_4(SiMe_2{}^tBu))\}]$, 6.

Chart 7. Structure of **6** (numbering refers to carbon atoms).



In a 25 mL Schlenk tube, freshly prepared [Ru(C_2O_4)(η^6-*p*-cymene)(H_2O)] (114 mg, 0.334 mmol) and Ph_2P(O(2-$C_6H_4SiMe_2{}^tBu$)) (170 mg, 0.433 mmol) were dissolved in CH_2Cl_2 (5 mL). The reaction mixture was stirred at ambient temperature, affording a yellow-orange solution in 30'. After 18 hours, the progress of reaction was checked by TLC and volatiles were removed under vacuum. The residue was suspended in Et_2O (20 mL) and the suspension was filtered. The resulting golden yellow solid was washed with Et_2O and dried under vacuum (40 °C). Yield: 206 mg, 86%. The title compound is soluble in DMSO, EtOH and chlorinated solvents, insoluble in Et_2O, hexane and H_2O. X-ray quality crystals of **6**·hexane were obtained from a CH_2Cl_2 solution of **2** layered with hexane and settled aside at -20 °C. Anal. calcd. for $C_{36}H_{43}O_5PRuSi$: C, 60.40; H, 6.05. Found: C, 60.31; H, 5.96. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3058w, 2956w, 2928w, 2856w, 1697s, 1674s and 1666s-sh ($\nu_{C=O}$), 1586w, 1564w, 1471m, 1434m-sh, 1427m, 1360s, 1257w, 1180s ($\nu_{O\text{-}Ar}$), 1128m, 1100m, 1074m, 1036w, 1008w, 937w, 892s ($\nu_{P\text{-}O}$), 835m, 822m, 807m, 778m, 764m, 739s, 710m-sh, 697s. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.63-7.53 (m, 5H, C9-H + C13-H), 7.45 (dt, $^3J_{HH}$ = 7.7 Hz, $^4J_{HH}$ = 1.4 Hz, 1H, C14-H), 7.41–7.31 (m, 7H, C10-H + C11-H + C16-H), 7.22 (t, $^3J_{HH}$ = 7.3 Hz, 1H, C15-H), 5.31 (d, $^3J_{HH}$ = 5.8 Hz, 2H, C3-H/C4-H), 5.11 (d, $^3J_{HH}$ = 5.9 Hz, 2H, C3-H/C4-H), 2.26 (hept, $^3J_{HH}$ = 6.6, 1H, C6-H), 1.79 (s, 3H, C1-H), 1.16 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C7-H), 1.06 (s, 9H, C20-H), 0.40 (s, 6H, C18-H). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 165.4 (C21), 159.2 (C12), 137.8 (C16), 133.9 (d, $^1J_{CP}$ = 47 Hz, C8), 131.7 (C11), 131.1 (C14), 131.0 (d, $^2J_{CP}$ = 12 Hz, C9), 128.9 (d, $^3J_{CP}$ = 11 Hz, C10), 123.5 (C15), 121.6 (d, $^3J_{CP}$ = 10 Hz, C13), 111.6 (C5), 99.5

(C2), 87.6 (C3/C4), 87.3 (d, $^{2}J_{CP}$ = 3.7 Hz, C3/C4), 46.0 (C20), 27.3 (C6), 22.4 (C7), 18.3 (C1/C19), 18.0 (C1/C19), 8.8 (C18). $^{31}P\{^{1}H\}$ NMR ($CDCl_3$): δ/ppm = 124.3. $^{29}Si\{^{1}H\}$ NMR ($CDCl_3$): δ/ppm = 3.4.

Stability studies in DMSO/water solutions.

General features (see Supporting Information for details). Ruthenium complexes were dissolved in DMSO-d_6/D_2O 9:1 *v*/*v* (0.8 mL; [Ru] = $1.5\cdot10^{-2}$ mol·L^{-1}). An aliquot of the resulting solution (0.40 mL) was transferred into a NMR tube, maintained at 37°C for 72 hours and analyzed by NMR as a function of time. The remaining solution was diluted up to 4.0 mL with DMSO/H_2O 9:1 *v*/*v* (final [Ru] = $1.5\cdot10^{-3}$ mol·L^{-1}), maintained at 37 °C for 72 hours and its conductivity was measured as a function of time. Both NMR and conductivity measurements were performed upon brief cooling to ambient temperature, then Ru-containing solutions were heated again at 37°C. Dimethyl sulfone ($5.5\cdot10^{-3}$ mol·L^{-1}) was used as a reference for ^{1}H NMR spectra (δ/ppm = 2.97 (s, 6H) in DMSO-d_6/D_2O 9:1 *v*/*v*). Molar conductivity (Λ_m) was calculated with reference to the starting material. Percent values of compounds in solution are based on ^{1}H NMR spectroscopy and refer to identified compounds only. NMR signals in braces {} indicate superimpositions with other species.

Chloride/solvent exchange experiments.

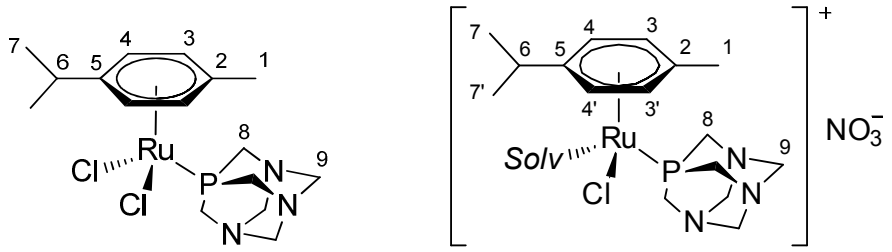
General procedures. Solutions of RAPTA-C and [$RuCl_2(\eta^6$-*p*-cymene)(κ*P*-PPh_3)] (**Ru-PPh$_3$**) in D_2O, CD_3OD or CD_3OD:D_2O 9:1 *v*/*v* were analyzed by $^{1}H/^{35}Cl/^{31}P$ NMR spectroscopy and conductivity measurements. The subsequent addition of 1.0 eq. of $AgNO_3$ was performed from a 0.2 M solution of $AgNO_3$ in CD_3OD:D_2O 9:1 *v*/*v*. Freshly-prepared solutions of RAPTA-C, **Ru-PPh$_3$** and **1-3** in DMSO-d_6:D_2O 9:1 *v*/*v* (c_{Ru} = $1.5\cdot10^{-2}$ mol·L^{-1}) were analysed by $^{1}H/^{35}Cl/^{31}P$ NMR spectroscopy. ^{1}H and ^{31}P NMR spectra were then repeated after the addition of NaCl (c_{NaCl} = 0.15 mol·L^{-1}).

A. ^{35}Cl NMR reference data.

NaCl. ^{35}Cl NMR (D_2O, acq. time 1 min): δ/ppm = 0 ($\Delta\nu_{1/2}$ = 40 Hz). ^{35}Cl NMR (CD_3OD, acq. time 1 min): δ/ppm = -28.3 ($\Delta\nu_{1/2} = 1.7\cdot10^2$ Hz). ^{35}Cl NMR (DMSO-d_6:D_2O 9:1, c = 0.11 mol·L^{-1}, acq. time 5 min): δ/ppm = 46.4 ($\Delta\nu_{1/2} = 5.8\cdot10^2$ Hz). ^{35}Cl NMR (DMSO-d_6:D_2O 9:1, c = $1.5\cdot10^{-2}$ mol·L^{-1}, acq. time 30 min): δ/ppm = 48.5 ($\Delta\nu_{1/2} = 5.2\cdot10^2$ Hz). *[Et$_3$NH]Cl*. ^{35}Cl NMR (CD_3OD, acq. time 1 min): δ/ppm = -22.7 ($\Delta\nu_{1/2} = 2.3\cdot10^2$ Hz). ^{35}Cl NMR ($CDCl_3$, acq. time 5 min): δ/ppm = 8.4 ($\Delta\nu_{1/2} = 1.3\cdot10^3$ Hz).

B. [($RuCl_2$(η^6-*p*-cymene)(PTA)] (RAPTA-C).

Chart 8. Structures of **RAPTA-C** (left) and solvato-complex formed by Cl-abstraction with $AgNO_3$ (right) (numbering refers to carbon atoms).



D_2O. Orange solution. ^{1}H NMR: δ/ppm = 6.05 (d, *J* = 5.8 Hz), 5.96 (d, *J* = 5.9 Hz), 5.89 (d, *J* = 5.5 Hz), 5.84 (m-br), 5.79 (d, *J* = 5.5 Hz), 4.60 (s), 4.57 (s), 4.33 (s), 4.31 (s), 4.28 (s), 4.22 (s), 2.59 (m), 2.10 (s), 2.03 (s), 1.99 (m), 1.24–1.17 (m). ^{31}P{^{1}H} NMR: δ/ppm = -23.0, -32.6, -34.1, -35.1 (major). ^{35}Cl NMR (acq. time 1 min): δ/ppm = 0.49 ($\Delta\nu_{1/2}$ = 35 Hz).

CD_3OD. Orange-red solution. ^{1}H NMR: δ/ppm = 5.72 (d, $^3J_{HH}$ = 5.5 Hz, 2H, C3-H/C4-H), 5.68 (d, $^3J_{HH}$ = 5.7 Hz, 2H, C3-H/C4-H), 4.57 (s, 6H, C9-H), 4.31 (s, 6H, C8-H), 2.66 (hept, $^3J_{HH}$ = 6.6 Hz, 1H, C6-H), 2.00 (s, 3H, C1-H), 1.21 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C7-H). ^{31}P{^{1}H} NMR (CD_3OD): δ/ppm = -36.4.

CD_3OD:D_2O 9:1 v/v. Orange-red solution. Λ_m (c = $1.5\cdot10^{-3}$ mol·L^{-1}) = 35 S·cm^2·mol^{-1}. ^{1}H NMR: a single set of signals was observed, with negligible chemical shift variation with respect to that in CD_3OD. ^{31}P{^{1}H} NMR: δ/ppm = -36.1. ^{35}Cl NMR (acq. time 10 min): no signal.

$CD_3OD:D_2O$ 9:1 v/v + $AgNO_3$ (1 eq.). Yellow-orange solution + AgCl precipitate. Λ_m (c = $1.5\cdot10^{-3}$ mol·L^{-1}) = 81 S·cm^2·mol^{-1}. NMR data indicate quantitative formation of [(η^6-*p*-cymene)RuCl(Solv)(PTA)]$^+$. ^{1}H NMR: δ/ppm = 6.05 (d, $^3J_{HH}$ = 5.9 Hz, 1H), 5.96 (d, $^3J_{HH}$ = 6.1 Hz, 1H), 5.80 (d, $^3J_{HH}$ = 5.5 Hz, 1H), 5.76 (d, $^3J_{HH}$ = 5.5 Hz, 1H, C3-H + C3-H' + C4-H + C4'-H); 4.63 (s, 6H, C9-H), 4.41–4.29 (m, 6H, C8-H), 2.65 (hept, $^3J_{HH}$ = 6.5 Hz, 1H, C6-H), 2.07 (s, 3H, C1-H), 1.27 (d, $^3J_{HH}$ = 6.9 Hz, 3H, C7-H), 1.24 (d, $^3J_{HH}$ = 6.4 Hz, 3H, C7'-H). ^{31}P{^{1}H} NMR: δ/ppm = -34.9.

DMSO-d_6:D_2O 9:1 v/v. Orange solution. Λ_m (c = $1.5\cdot10^{-3}$ mol·L^{-1}) = 24 S·cm^2·mol^{-1}. ^{1}H NMR: δ/ppm = 5.72 (d, $^3J_{HH}$ = 5.7 Hz, 2H, C3-H/C4-H), 5.69 (d, $^3J_{HH}$ = 5.7 Hz, 2H, C3-H/C4-H), 4.41 (s, 6H, C9-H), 4.14 (s, 6H, C8-H), 1.86 (s, 3H, C1-H), 1.09 (d, $^3J_{HH}$ = 6.8 Hz, 6H, C7-H). ^{31}P{^{1}H} NMR: δ/ppm = -34.1. ^{35}Cl NMR (acq. time 30 min): no signal.

DMSO-d_6:D_2O 9:1 v/v + NaCl. ^{1}H NMR and ^{31}P{^{1}H} NMR: a single set of signals was observed, identical to that without NaCl.

C. [$RuCl_2$(η^6-*p*-cymene)(κ*P*-PPh_3)] (Ru-PPh_3).

$CD_3OD:D_2O$ 9:1 v/v. Orange-brown solution. Λ_m (c = $1.5\cdot10^{-3}$ mol·L^{-1}) = 68 S·cm^2·mol^{-1}. ^{1}H NMR: δ/ppm = 7.83–7.76 (m, 4H), 7.53–7.38 (m, 6H), 5.33 (d, $^3J_{HH}$ = 6.1 Hz, 2H), 5.21 (d, $^3J_{HH}$ = 5.9 Hz, 2H), 2.72–2.62 (m, 1H), 1.89 (s, 3H), 1.09 (d, $^3J_{HH}$ = 6.8 Hz, 6H). ^{31}P{^{1}H} NMR: δ/ppm = 24.5. ^{35}Cl NMR (acq. time 10 min): no signal.

$CD_3OD:D_2O$ 9:1 v/v + $AgNO_3$ (1 eq.). Yellow solution + precipitate. Λ_m (c = $1.5\cdot10^{-3}$ mol·L^{-1}) = 103 S·cm^2·mol^{-1}. Two major sets of signals, in *ca.* 1:1 ratio, were identified, along with other minor products. First set (identical to that of **Ru-PPh_3**). ^{1}H NMR: δ/ppm = 7.82–7.76 (m, 4H), 7.54–7.38 (m, 6H), 5.36 (d, $^3J_{HH}$ = 6.2 Hz, 2H), 5.21 (d, $^3J_{HH}$ = 5.7 Hz, 2H), 2.69 (m, $^3J_{HH}$ = 7.1 Hz, 1H), 1.89 (s, 3H), 1.11 (d, $^3J_{HH}$ = 6.9 Hz, 6H). ^{31}P{^{1}H} NMR: δ/ppm = 24.9. Second set. ^{1}H NMR: δ/ppm = 7.70-7.63 (m), {7.54–7.38 (m)}, 5.70 (s-br, 2H), 5.31 (s-br, 1H), 5.15 (s-br, 1H), 2.79 (hept, $^3J_{HH}$ = 6.4 Hz, 1H), 1.82 (s-br, 3H), 1.29 (s-br, 6H). ^{31}P{^{1}H} NMR: δ/ppm = 31.2 (m-br).

DMSO-d_6:D_2O 9:1 v/v. ^{1}H NMR: δ/ppm = 7.77–7.66 (m, 6H), 7.46–7.35 (m, 9H), 5.27 (d, *J* = 5.9 Hz, 2H), 5.21 (d, *J* = 5.6 Hz, 2H), 1.74 (s, 3H), 0.93 (d, *J* = 6.8 Hz, 6H). ^{31}P{^{1}H} NMR: δ/ppm = 24.2. ^{35}Cl NMR (acq. time 30 min): no signal.

DMSO-d_6:D_2O 9:1 v/v + NaCl. ^{1}H NMR and ^{31}P{^{1}H} NMR: a single set of signals was observed, identical to that without NaCl.

D. [$RuCl_2$(η^6-*p*-cymene){κ*P*-Ph_2P(4-C_6H_4O-R)}], R = $OSiMe_2{}^tBu$ (1), Br (2), $OCOCHCl_2$ (3).

*R = $OSiMe_2{}^tBu$, **1**. DMSO-d_6:D_2O 9:1 v/v*. ^{1}H NMR: δ/ppm = 7.73–7.66 (m, 4H), 7.60 (t, *J* = 9.2 Hz, 2H), 7.44–7.33 (m, 6H), 6.85 (d, *J* = 7.9 Hz, 2H), 5.27 (d, *J* = 6.0 Hz, 2H), 5.20 (d, *J* = 5.7 Hz, 2H), 1.74 (s, 3H), 1.00–0.85 (m, 15H), 0.18 (s, 6H). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 23.2. ^{35}Cl NMR (acq. time 30 min): no signal.

DMSO-d_6:D_2O 9:1 v/v + NaCl. ^{1}H NMR and ^{31}P{^{1}H} NMR: a single set of signals was observed, identical to that without NaCl.

*R = Br, **2**. DMSO-d_6:D_2O 9:1 v/v*. ^{1}H NMR: δ/ppm = 7.74–7.67 (m, 4H), 7.65 (t, *J* = 9.0 Hz, 2H), 7.54 (d, *J* = 7.6 Hz, 2H), 7.50–7.40 (m, 6H), 5.30 (d, *J* = 6.0 Hz, 2H), 5.23 (d, *J* = 5.8 Hz, 2H), 1.74 (s, 3H), 0.93 (d, *J* = 6.9 Hz, 6H). ^{31}P{^{1}H} NMR: δ/ppm = 24.5. ^{35}Cl NMR (acq. time 30 min): no signal.

DMSO-d_6:D_2O 9:1 v/v + NaCl. ^{1}H NMR and ^{31}P{^{1}H} NMR: a single set of signals was observed, identical to that without NaCl.

*R = $OCOCHCl_2$, **3**. DMSO-d_6:D_2O 9:1 v/v*. ^{1}H NMR: δ/ppm = 7.72–7.65 (m, 4H), 7.54 (t, *J* = 8.8 Hz, 2H), 7.46–7.32 (m, 6H), 6.77 (d, *J* = 7.6 Hz, 2H), 6.37 (s, 1H), 5.26 (d, *J* = 5.3 Hz, 2H), 5.17 (d, *J* = 4.7 Hz, 2H), 1.73 (s, 3H), 0.94 (d, *J* = 6.5 Hz, 6H). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 23.1.

DMSO-d_6:D_2O 9:1 v/v + NaCl. ^{1}H NMR and ^{31}P{^{1}H} NMR: a single set of signals was observed, identical to that without NaCl.

X-ray crystallography.

Crystal data and collection details for **3·CH_3COCH_3**, **4**, **5** and **6·C_6H_{14}** are reported in Table 16S. Data were recorded on a Bruker APEX II diffractometer equipped with a CCD detector using Mo–Kα radiation. Data were corrected for Lorentz polarization and absorption effects (empirical absorption correction SADABS).[51] The structures were solved by direct methods and refined by full-matrix least-squares based on all data using F^2.[52] Hydrogen atoms were fixed at calculated positions and refined by a riding model. All non-hydrogen atoms were refined with anisotropic displacement parameters.

In vitro cytotoxicity studies.

Reagents. Eagle's minimum essential medium (MEM) was purchased from Sigma Aldrich, while trypsin-EDTA, penicillin, streptomycin, sodium pyruvate, non-essential amino acid solution, fetal calf serum (FCS), plates and Petri dishes were purchased from EuroClone. The compounds were dissolved in dimethyl sulfoxide (DMSO) before performing each experiment. The maximal concentration utilized was 50 μM, due to limited water solubility; cisplatin was tested up to 100 μM.**Errore. Il segnalibro non è definito.** The same volume of solvent was added to control conditions and did not exceed 0.25% *v/v*.

Cell culture. Human triple negative cancer cell line MDA-MB-231, human ovarian carcinoma cell line A2780 and Human Skin Fibroblasts (HSF) were cultured in MEM supplemented with 10% FCS, non-essential amino acids, and penicillin/streptomycin at 37°C in a humidified atmosphere (5% CO_2 and 95% air).

Cell viability assay. Sulphorhodamine B (SRB) assay was performed to assess the cell viability after treatments. $5x10^3$ cells/well were seeded in a 96-well tray in triplicate. After 24h of incubation, the cells were treated with different concentrations of compounds. SRB assay were performed after 48h according to the method of Skehan et al.[53]

Cell cycle analysis. Cell cycle analysis was performed as previously described.[54] In brief, cells were seeded (250,000/35 mm petri dish) and incubated with DMEM supplemented with 10% FCS; 24 h later the medium was replaced with one containing 10% FCS in the presence or absence (control) of 50 μM of complex **3**, and the incubation was continued for a further 24 h. At the end of this incubation period cells were re-suspended with permeabilizing buffer (NaCl 100 mM, TRIS pH 7.4 150 mM; $CaCl_2$ 1 mM; $MgCl_2$ 0.5 mM; NP-40 0.1% containing 5 mM propidium iodide and 40 mg/ml of RNAse A) and the DNA nuclear content was analyzed with FACScan™ flow cytometer and BD CellQuest™ (both from BD Biosciences, San Jose, CA, USA).

Statistical analysis. Experimental data are expressed as mean ± S.D. The effects of the complexes versus control were analyzed by two-tailed Student's t test for unpaired data. The concentration of compounds required to reduce cell viability by 50% (IC_{50}) was calculated by nonlinear regression curve (GraphPad Prism, Version 5.01).

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Supporting Information Available

CCDC reference numbers 1571529 (**3**), 1571530 (**4**), 1571531 (**5**) and 1571532 (**6**) contain the supplementary crystallographic data for the X-ray studies reported in this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge

Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (internat.) +44-1223/336-033; e-mail: deposit@ccdc.cam.ac.uk).

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