In Vitro Evaluation of Rhodium and Osmium RAPTA Analogues: The Case for Organometallic Anticancer Drugs Not Based on Ruthenium

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Reaction of the dimer $[(\eta^5\text{-}C_5Me_5)RhCl(\mu_2\text{-}Cl)]_2$ with 2 or 4 equiv of the water-soluble phosphine 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (pta) affords $[Rh(\eta^5\text{-}C_5Me_5)(pta)Cl_2]$ and $[Rh(\eta^5\text{-}C_5Me_5)(pta)_2Cl]Cl$, respectively. Both complexes have been characterized in solution by NMR spectroscopy and in the solid state by single-crystal X-ray diffraction, the latter as the chloride and BPh_4^- salts. In addition, the rhodium(I) complexes $[Rh(\eta^5\text{-}C_5Me_5)(CO)(pta)]$ and $[Rh(\eta^5\text{-}C_5H_5)(pta)_2]$ have been prepared from $[Rh(\eta^5\text{-}C_5Me_5)(CO)_2]$ and $[Rh(\eta^5\text{-}C_5H_5)(PPh_3)_2]$, respectively, by reaction with pta. An in vitro evaluation of these compounds, together with $[Os(\eta^6\text{-}C_{10}H_{14})(pta)Cl_2]$ and the well-characterized antimetastasis drug $[Ru(\eta^6\text{-}C_{10}H_{14})(pta)Cl_2]$, RAPTA-C, was undertaken using HT29 colon carcinoma, A549 lung carcinoma, and T47D breast carcinoma cells. In the HT29 cell line, the two nearest congeners to $[Ru(\eta^6\text{-}C_{10}H_{14})(pta)Cl_2]$, viz., $[Rh(\eta^5\text{-}C_5Me_5)(pta)Cl_2]$ and $[Os(\eta^6\text{-}C_{10}H_{14})(pta)Cl_2]$, demonstrated very similar cytotoxicity profiles. $[Rh(\eta^5\text{-}C_5Me_5)(pta)Cl_2]$ proved significantly more cytotoxic in A549 cells and $[Rh(\eta^5\text{-}C_5Me_5)(pta)_2Cl]Cl$ 3-fold more cytotoxic in T47D cells, both relative to RAPTA-C. These data suggest that the development of organometallic anticancer drugs based on the neighboring elements to ruthenium should not be overlooked.

Introduction

Following the success of cisplatin in the clinic since its discovery in 1965,[1] which remains the most widely used anticancer drug to this day, employed in the treatment of approximately 70% of all cancer patients, a large number of other platinum-based drugs have been prepared and evaluated.[2] Although cisplatin and platinum-based drugs, more generally, are arguably the most successful class of anticancer drugs in the world, they are not without problems; notably they exhibit a high general toxicity, leading to undesirable side-effects, although minimized by careful administration protocols, and they are also inactive against certain types of cancers.[3] Thus, other metal coordination compounds have been evaluated in cancer chemotherapy,[4] and organometallic compounds have also been evaluated extensively for their medicinal properties.[5] The earliest example of a highly successful organometallic anticancer compound is titanocene dichloride, pioneered by Köpf and Köpf-Maier, who investigated the antitumor activity of several early transition metal cyclopentadienyl (metallocene) complexes, in the 1970s.[6] Although titanocene dichloride successfully completed phase II clinical trials, it has not gained clinical approval.[7] We are not aware of any organometallic compound in clinical trials for cancer, although ferrocifen, a ferrocenyl derivative of tamoxifen,[7] looks set to enter clinical trials in the near future,[8] and related ferrocenyl derivatives are in clinical trials for malaria.[9] The only non-platinum transition metal compounds currently in clinical trials are two coordination compounds based on ruthenium, viz., [ImH][*trans*-$RuCl_4$(DMSO)Im] (NAMI-A)[10] and [ImH][*trans*-$RuCl_4Im_2$] (KP1019),[11] which has stimulated much interest in the medicinal properties of this metal. A gallium compound is also in clinical trials, and main group compounds have been extensively studied for their anticancer properties.[12] Both the ruthenium compounds demonstrate a low general

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toxicity, which contrasts to the pharmacological properties of platinum drugs, and this low toxicity has been ascribed to selective uptake of ruthenium compounds by cancer cells mediated via the transferrin cycle[13] and to mechanisms such as activation through reduction.[14] Interest in inorganic ruthenium drugs has extended to organometallic compounds, with a growing body of work on ruthenium(II)-arene derivatives.[15]

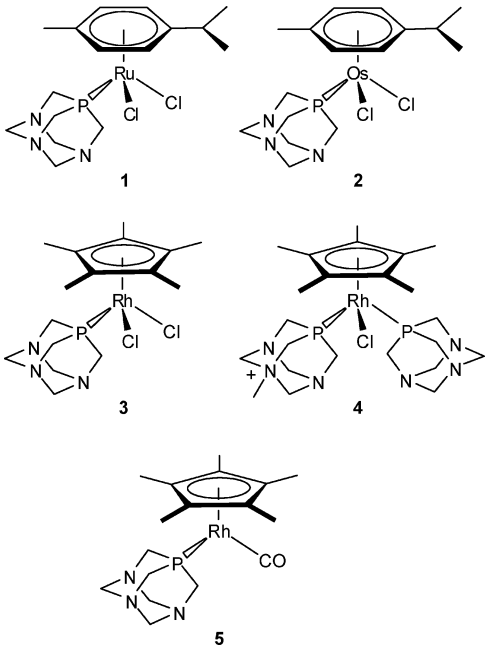
For example, ruthenium(II)-arene compounds with imidazole,[16] alanine- and guanine-derived co-ligands,[17] ethylenediamine,[18] disulfoxide,[19] and pta[20] co-ligands have been evaluated (pta = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane).[21] The pta-derived compounds appear to impart a pH-dependent activity, which is conducive to providing excellent pharmacological properties. In this paper we describe closely related congeners to the well-characterized (in vitro and in vivo) ruthenium(II)-arene-pta compounds based on osmium and rhodium and show that these metals are also worth considering within the context of organometallic anticancer drugs.

Results and Discussion

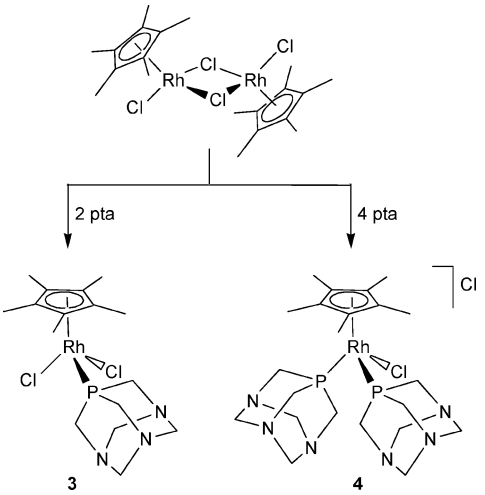
The compounds used in the comparative in vitro study (see below) are shown in Chart 1. $[Ru(\eta^6\text{-}C_{10}H_{14})(pta)Cl_2]$, RAPTA-C (**1**),[20a] and the osmium analogue **2**[22] have been reported previously, the former having been subjected to an in vivo study, proving it to be a highly selective agent for the treatment of secondary (metastasis) tumors with excellent pharmacokinetic properties.[23]

The rhodium compounds used in the study are new and were prepared from the dimer $[(\eta^5\text{-}C_5Me_5)RhCl(\mu_2\text{-}Cl)]_2$ according to the routes shown in Schemes 1 and 2. Direct reaction of the rhodium dimer with 2 or 4 equiv of pta affords the highly water soluble species $[Rh(\eta^5\text{-}C_5Me_5)(pta)Cl_2]$ (**3**) and $[Rh(\eta^5\text{-}C_5Me_5)(pta)_2Cl]Cl$ (**4**·Cl), respectively. The chloride counteranion in **4**·Cl is easily substituted by other anions, e.g., tetraphenylborate, by stirring with $NaBPh_4$ in MeOH to give $[Rh(\eta^5\text{-}C_5Me_5)(pta)_2Cl]BPh_4$ (**4g**·BPh_4). The electrospray ionization mass spectrum (ESI-MS) of **3** in MeOH is dominated by a peak at $m/z = 466$, which corresponds to the protonated parent ion $[Rh(\eta^5\text{-}C_5Me_5)\{pta(H)\}ptaCl_2]^+$, presumably in which the pta ligand is *N*-protonated. In fact, the *N*-sites of metal-pta complexes are readily protonated in protic solvents such as alcohols and

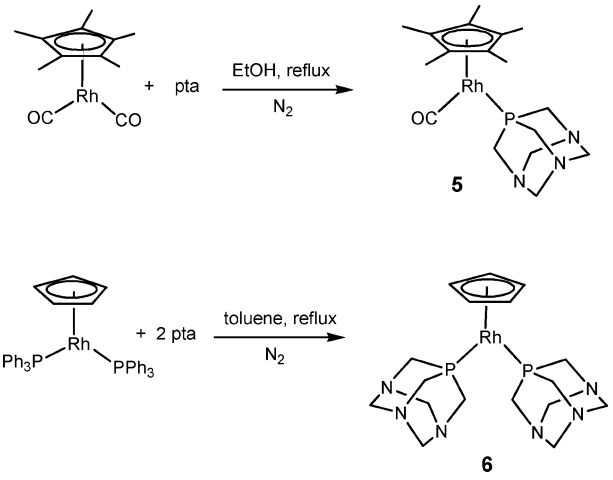
Chart 1



Scheme 1



Scheme 2



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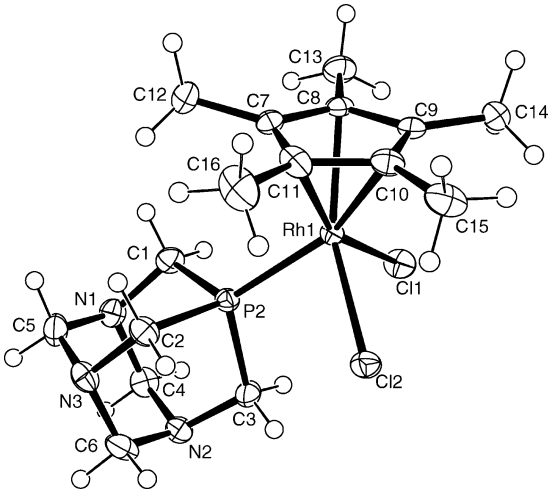

Figure 1. ORTEP representations of **3** drawn at 30% probability ellipsoids. Key bond lengths (Å) and angles (deg): Rh1−Cp* (centroid) 1.804, Rh1−P2 2.286(1), Rh1−Cl1 2.410(1), Rh1−Cl2 2.417(1), Cp*−Rh1−P2 29.75, Cp*−Rh1−Cl1 124.84, Cp*−Rh1−Cl2 124.53, P2−Rh−Cl1 83.95(4).

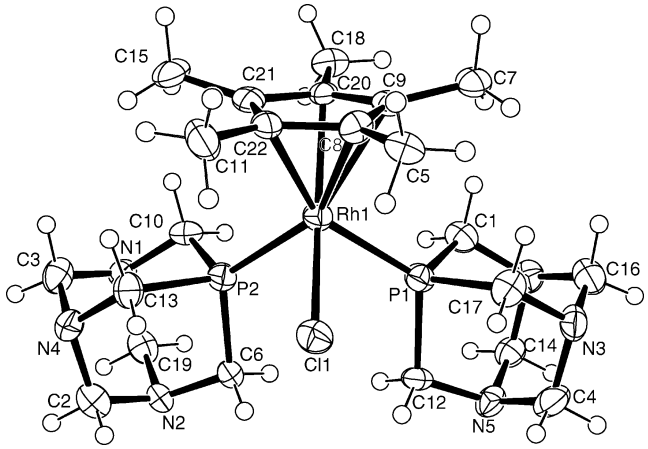

Figure 2. ORTEP representation of the complex cation **4** drawn at 30% probability ellipsoids with solvate and anion components removed. Key bond lengths (Å) and angles (deg) for **4**·Cl: Rh1−Cp* (centroid) 1.859, Rh1−P1 2.291(2), Rh1−P2 2.288(2), Rh1−Cl1 2.405(2), Rh1−Cl2 6.005(2), Cp*−Rh1−P1 125.13, Cp*−Rh1−P2 126.53, Cp*−Rh1−Cl1 125.13, P1−Rh−P2 96.93(7). Key bond lengths (Å) and angles (deg) for **4**·BPh_4: Rh1−Cp* (centroid) 1.860, Rh1−P1 2.2970(8), Rh1−P2 2.3059(8), Rh1−Cl1 2.4087(8), Cp*−Rh1−P1 125.42, Cp*−Rh1−P2 126.53, Cp*−Rh1−Cl1 126.88, P1−Rh−P2 97.44(3).

water,[24] which is, apparently, extremely facile in the case of rhodium-pta complexes. For example, both [RhCl{pta(H)}$(pta)_2$]Cl and [RhCl{pta(H)}$_3$(pta)]Cl_3 are prepared by refluxing either $RhCl_3$ or $RhCl(pta)_3$ with stoichiometric equivalents of pta in EtOH and H_2O, respectively.[25] Compound **4** is naturally charged, and in methanol, the only peak observed in the ESI-MS is that of the parent cation at *m*/*z* 587. Two Rh(I) complexes bearing η^5-C_5H_5 and one or two pta ligands, namely, [Rh(η^5-C_5Me_5)(CO)(pta)] (**5**) and [Rh(η^5-C_5H_5)$(pta)_2$] (**6**) were also synthesized by substituting one CO or two PPh_3 ligands from suitable Rh(I) precursors [Cp′RhL_2] (Cp′ = Cp, Cp*; L = CO, PPh_3) (Scheme 2).

The ^{1}H and ^{31}P{^{1}H} NMR spectra of **3** and **4** in $CDCl_3$ agree with the proposed formulas. The methyl protons of the η^5-C_5Me_5 group are observed at 1.69 ppm as a doublet (J_{HP} = 3.5 Hz) for **3** and at 1.84 ppm as a triplet (J_{HP} = 3.0 Hz) for **4**. Peaks of single resonances between 4.2 and 4.9 ppm correspond to the methylene protons within the pta ligand, and the simple chemical shift pattern indicates that the ligand is unprotonated. The solution ^{31}P{^{1}H} NMR spectrum of **3** and **4** exhibits a doublet resonance at −32.37 ppm (J_{PRh} = 141 Hz) and at −28.68 ppm (J_{PRh} = 131 Hz), respectively.

The two rhodium(I) complexes [Rh(η^5-C_5Me_5)(CO)(pta)] and [Rh(η^5-C_5H_5)$(pta)_2$] exhibit typical doublet resonances in their solution ^{31}P{^{1}H} NMR spectra at −36.8 ppm ($^1J_{RhP}$ = 187.2 Hz) and −25.32 (d, J_{PRh} = 205.0 Hz). The high magnitude of the $^1J_{RhP}$ is in line with the presence of a Rh(I) center.[26]

Single crystals of **3**, **4**·Cl, and **4**·BPh_4 were obtained (see Experimental Section) and their structures determined by X-ray crystallography. The molecular structure of **3** is depicted in Figure 1, with key bonding parameters listed in the caption.

A survey of the CCDC for compounds where a monocoordinate phosphine is attached to the Cp*$RhCl_2$ moiety reveals in total 45 structures. Surprisingly, the search demonstrated very few systems containing a simple homoleptic phosphine such as PPh_3 or PMe_3; however, both [Cp*$RhCl_2${$P(OEt)_3$}] and [Cp*$RhCl_2${$P(OPh)_3$}] are known.[27] A comparison of the former with **3** reveals a slightly shorter Rh−P bond ([Cp*$RhCl_2$-{$P(OEt)_3$}], 2.268(3) Å; **3**, 2.286(1) Å). The only previously reported Rh−P(pta) distance within the complex Rh(pta)$(pta\text{-}me)_3$Cl, where the metal center has a +1 oxidation state, is significantly shorter (2.206(1) Å) than ones for the Cp*Rh(III) species reported in this paper.[25] Conversely, the Rh−Cl bond distances in [Cp*$RhCl_2${$P(OEt)_3$}] differ from one another (2.381(3), 2.411 (4) Å), whereas in **3**, they are equivalent (2.410-(1) and 2.417(1) Å). This is perhaps due to intermolecular interactions within the lattice system. The molecular packing of **3** is characterized by a small number of contacts, the shortest being between a nitrogen center of pta and a methyl hydrogen of the η^5-C_5Me_5 ligand, 2.547 Å, and a longer contact between one chlorine substituent and a CH_2 group of pta, 2.898 Å. Interestingly, the other Cl group has no other interactions aside from its bonding to the metal, which is also the case for [Cp*$RhCl_2${$P(OEt)_3$}].

The structure of the cation **4** is depicted in Figure 2, with key bonding parameters listed in the caption. Two different crystal structures have been obtained, which differ by the type of anion and solvate contained within the lattice.

The Rh−Cl and Rh−P bond distances in **3** and the two salts of **4** are essentially equivalent. The major difference between the mono- and bis-pta species lies in the coordination of the η^5-C_5Me_5 ring to the Rh center. In **3**, the two methyl substituents that eclipse the pta ligand are lifted out of the C_5 ring plane, by 5.49°. In contrast, the presence of two pta units in **4** results in a lengthening of the Rh−Cp(centroid) distance as compared to **3** [1.861 versus 1.804 Å] and in all of the methyl groups being bent above the C_5 ring plane, ranging from 9.86° to 3.07°. Similarly, a CCDC search on $[RhCp^*Cl(PR_3)_2]^+$ revealed only four systems with nonchelating phosphines, where PR_3 = $P(Ph_2)CH_2CH_2NEt_2$, $P(PPh)_2CCPh$, $P(Ph)_2CHCH_2$, and $P(Ph)_2$-CH_2CHCH_2. A comparison of **4** with the isoelectronic complex [Ru(η^5-C_5Me_5)$(pta)_2$Cl][24] reveals the primary difference between

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the two molecules is a 5° rotation of the cyclopentadienyl ring with respect to the Ru−Cl bond. Interestingly, for the bis-pta species, the Ru−P and Rh−P bond lengths are equal [Ru(η^5-C_5Me_5)(pta)$_2$Cl: av 2.285(4) Å; **4**BPh_4^-: 2.297(1), 2.288(1) Å; **4**Cl^-: 2.291(2), 2.288(2) Å], but the Ru−Cl bond is longer than that of Rh−Cl [Ru(η^5-C_5Me_5)(pta)$_2$Cl: av 2.467(2) Å, **4**BPh_4^-: 2.4055(8) Å, **4**Cl^-: 2.405(2) Å]. Similarly, the Ru−Cp*(centroid) distance (1.860, 1.862 Å) is equivalent to the Rh−Cp*(centroid) separation [1.865 Å], and a similar amount of bending of the methyl groups above the C_5 plane is also observed. In **3** the P−Rh−P bond angle (**4**BPh_4^-: 96.61(1)°, **4**Cl^-: 96.93(7)°) is slighter greater than the P−Ru−P bond angle (av 93.34(9)°), but whereas the P−Rh−Cl angles (84.30(1)°, 85.61(1)°) are nearly equal in **4**, for [Ru(η^5-C_5Me_5)(pta)$_2$-Cl, the Cl is pushed to one side, resulting in unequal P−Ru−Cl bond angles (av 84.33(10)° versus 90.82(11)°).

In Vitro Evaluation. Compounds **1**−**5** (see Figure 1) were evaluated in vitro by testing for inhibition of cell proliferation activity against the HT29 colon carcinoma, A549 lung carcinoma, and T47D breast carcinoma cell lines. Due to air sensitivity, it was not possible to test complex **6** along with **1**−**5**. The effects of **1**−**5** on the growth of these cell lines were evaluated after 72 h treatment, and the results from these experiments are displayed in Figure 3. The experiments were repeated twice for all the compounds, and the corresponding IC_{50} values resulting from an average of the two experiments are listed in Table 1 for the three cell types.

From Figure 3 and Table 1 it can be seen that for the HT29 cell line RAPTA-C, the model compound in this study, and the osmium [Os(η^6-$C_{10}H_{14}$)(pta)Cl_2] and rhodium [Rh(η^5-C_5Me_5)-(pta)Cl_2] analogues exhibit essentially the same order of cytotoxicites [IC_{50} at 72 h 380−456 μM]. The cationic rhodium complex [Rh(η^5-C_5Me_5)(pta)$_2$Cl]Cl (**4**) is considerably less cytotoxic, and the rhodium(I) complex [Rh(η^5-C_5Me_5)(CO)(pta)] (**5**) showed no discernible cytotoxicity up to 200 μM in the HT29 cells or the other cells used; it was not possible to evaluate **5** at greater concentrations due to its limited solubility in the culture medium. Of all the compounds tested against the A549 cells, only **3** was cytotoxic to any significant extent. This result clearly demonstrates the superior activity of the rhodium analogue of RAPTA-C in this cell line. Previously, it has been suggested that the greater lipophobicity of the cyclopentadienyl ligand related to *p*-cymene and other C_6-arene ligands reduces uptake in cells and accordingly lowers their cytotoxicity.[22] It would appear that the pentamethylcyclopentadienyl ring does not impede activity in this way, which is not unreasonable given the presence of the methyl groups. In the T47D cells it is only the rhodium complexes **3** and **4** that are cytotoxic, this time the cationic bis-pta species being the most active.

In the in vitro cell proliferation assays the chloride concentration in the medium is ca. 120 mM, and at this concentration hydrolysis of RAPTA-C is almost completely suppressed, as is clear from Figure 4, which shows the ^{31}P NMR spectra of RAPTA-C in water with varying concentrations of chloride. At high chloride concentration, i.e., that characteristic of the blood, only RAPTA-C is present. In the absence of chloride the hydrolysis product Ru(η^6-$C_{10}H_{14}$)(pta)Cl(H_2O)]$^+$ dominates, although it is in equilibrium with RAPTA-C. With the osmium complex **2** at the chloride concentration in the medium hydrolysis is almost completely suppressed, as for RAPTA-C. In contrast, with the rhodium complex **3** somewhat more than 50% of the complex has been hydrolyzed. Despite the difference in the extent of hydrolysis between **3** and the other two complexes, and thus the charged state of the complexes, little difference is observed with respect to their cytotoxicities. This similarity is probably due to the fact that within the cell, where the chloride

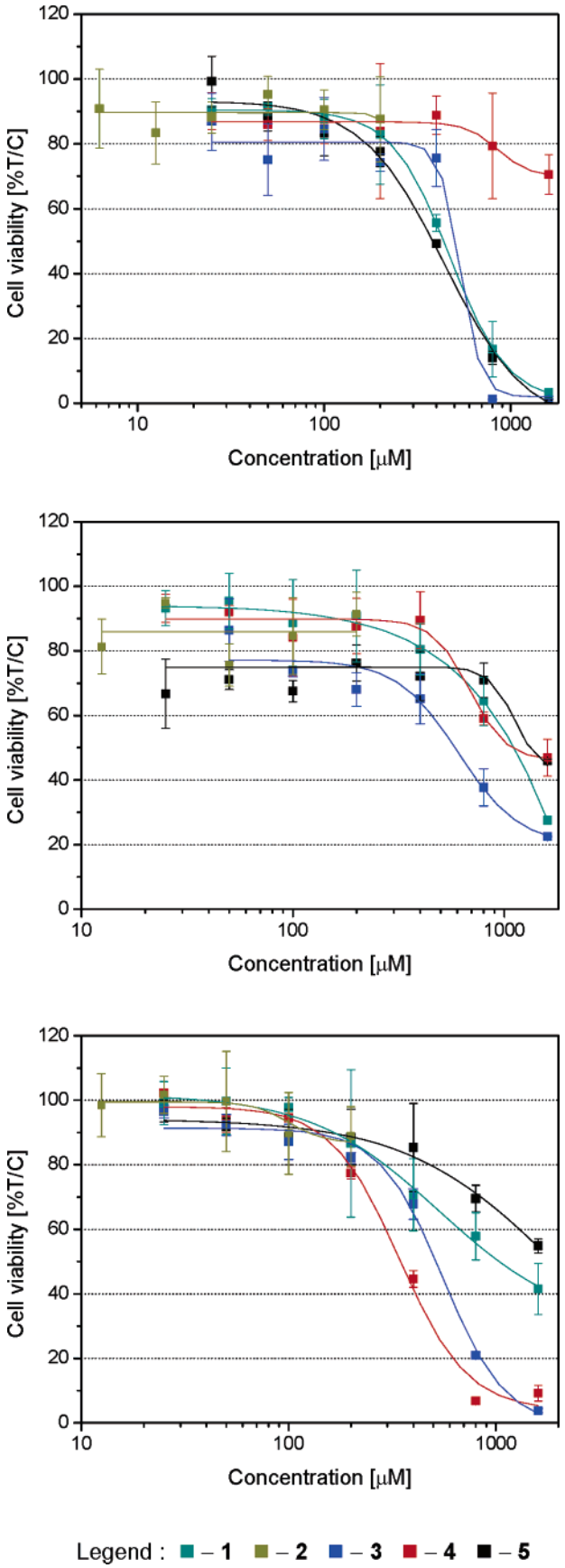

Figure 3. Inhibition of cell growth proliferation in (top) HT29 cells, (middle) A549 cells, and (bottom) T47D cells, after 72 h of exposure to the complexes.

Table 1. Inhibition of Cell Proliferation as Determined by the MTT Assay

	IC_{50} (μM)		
complex	HT29	A549	T47D
1	441	1105	1034
2	456	1430	>1600
3	380	584	512
4	>1600	956	346
5[a]	>200	>200	>200

[a] The compound was tested at a maximum concentration of 200 μM, which is its limit of solubility in the culture medium.

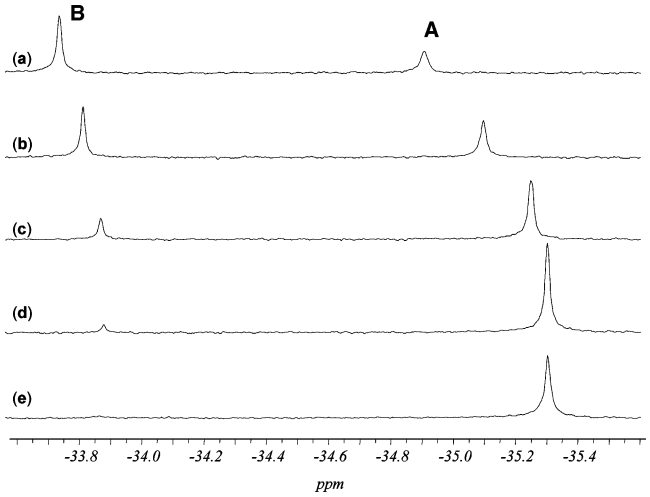

Figure 4. Influence of chloride concentration on the hydrolysis of RAPTA-C determined by ^{31}P NMR spectroscopy. [RAPTA-C] = 5 mM, [$NaNO_3$] = 1 M: (a) [Cl^-] = 0 mM, (b) [Cl^-] = 5 mM, (c) [Cl^-] = 22.7 mM, (d) [Cl^-] = 104 mM, (e) [Cl^-] = 200 mM. The chloride in solution resulting from hydrolysis of RAPTA-C is not included. A corresponds to unmodified RAPTA-C, B to Ru(η^6-$C_{10}H_{14}$)(pta)Cl(H_2O)]$^+$.

concentration is much lower, complexes **1**, **2**, and **3** are nearly completely hydrolyzed and therefore equally reactive toward the potential target, i.e., DNA or RNA.

RAPTA-C is the prototype compound that shows considerable potential as an antimetastasis agent in vivo.[23] Attempts to improve its efficacy by modification of the arene ligand for functionalized arenes[28] and substitution of the arene by chelating six-electron donor ligands[29] have already been attempted. The results emanating from this study, however, indicate that in addition to ligand modification it may prove interesting to study compounds based on different metals; certainly the osmium and rhodium compounds described herein are worth studying further. As mentioned in the Introduction, other types of ruthenium(II)-arene compounds are under investigation for their antitumor properties. Some [Ru(η^6-arene)(en)Cl] (en = ethylenediamine) complexes exhibit IC_{50} values as low as those as cisplatin in certain types of cancer cells, whereas the osmium analogues are not cytotoxic, apparently due to the formation of the inactive hydroxyl species [Os(η^6-arene)(en)(OH)]. Such a limitation is not deemed likely with the bis-chloride osmium compound **2** described herein. Perhaps it should be no surprise that the rhodium compounds described herein are active since Fish has shown that rhodium(III)-pentamethylcyclopentadienyl aqua complexes readily react with DNA model compounds,[30] and although ruthenium-chloride pta complexes have been reported to interact with DNA,[31] the actual target of the compounds described herein may not be DNA since we have shown that strong and specific interactions with proteins also occur.[32]

As far as we are aware, the only other class of metal-based anticancer drugs to be systematically studied with respect to identifying the optimum metal are the organometallic selective estrogen receptor modulator compounds developed by Jaouen. His group has studied the role of various metals including titanium,[33] rhenium,[34] ruthenium,[35] osmium,[36] platinum,[37] and rhodium,[38] with an optimum effect provided by iron in the ferrocene derivative of tamoxifen.[39] A series of different metals have also been studied for metal-carbonyl-releasing compounds that have pharmacological properties in suppressing organ graft rejection, and while the most effective compound discovered thus far is based on ruthenium, much of the evaluation has been directed by synthetic limitations.[40] This research, like that described herein, demonstrates the need for a more thorough and methodical approach with respect to metal selection in medicinal organometallic chemistry, which is less well investigated than the related area of medicinal coordination complexes.[4]

Experimental Section

General Procedures. All synthetic procedures were carried out using standard Schlenk glassware under an inert atmosphere of dry nitrogen. The ligand pta[41] and the complexes [Rh(η^5-C_5Me_5)$(CO)_2$],[42] [(η^5-C_5Me_5)RhCl(μ_2-Cl)]$_2$,[43] [Rh(η^5-C_5H_5)$(PPh_3)_2$],[44] [Ru(η^6-$C_{10}H_{14}$)(pta)Cl_2],[20] and [Os(η^6-$C_{10}H_{14}$)(pta)Cl_2][22] were prepared as described in the literature. Other reagents were obtained from commercial suppliers and used without further purification. Solvents were distilled and degassed according to standard procedures.[45]

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Table 2. Crystal Data and Details of the Structure Determination for 3, 4·Cl, and 4·BPh_4

	3	**4**·Cl	**4**·BPh_4
chemical formula	$C_{16}H_{27}Cl_2N_3PRh$	$C_{22}H_{40}Cl_2N_6O_2P_2Rh$	$C_{46.70}H_{61.81}BClN_6O_{0.705}P_2Rh$
fw	466.19	656.35	929.54
cryst syst	orthorhombic	triclinic	monoclinic
space group	$P2_12_12_1$	$P\bar{1}$	$P2_1/c$
a (Å)	8.500(3)	9.1729(18)	11.9850(10)
b (Å)	3.382(6)	12.325(3)	30.388(2)
c (Å)	16.361(9)	13.028(3)	13.0020(9)
β (deg)	90	83.11(3)	107.800(7)
volume (Å^3)	1861.0(15)	1362.5(6)	4508.6(6)
Z	4	2	4
D_{calc} (g cm^{-3})	1.664	1.607	1.369
$F(000)$	952	684	1946
μ (mm^{-1})	1.293	0.973	0.551
temp (K)	200	150	200
wavelength (Å)	0.71073	0.71073	0.71073
no. of measd reflns	2310	14 259	101 216
no. of unique reflns	2255	4815	8037
no. of unique reflns [$I > 2\sigma(I)$]	2180	2640	7960
no. of data/params	2255/213	4815/323	8037/530
R^a [$I > 2\sigma(I)$]	0.0190	0.0635	0.0454
$wR_2{}^a$ (all data)	0.0480	0.1218	0.0971
GoF^b	1.00	0.982	1.164

[a] $R = \sum||F_o| - |F_c||/\sum|F_o|$, $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$. [b] GoF $= \{\sum[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$ where n is the number of data and p is the number of parameters refined.

Infrared spectra were recorded on a Perkin-Elmer Spectrum BX series FT-IR spectrometer in KBr disks. The ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on a Bruker AC200 spectrometer operating at 200.13 and 50.32 MHz, respectively. Peak positions are relative to tetramethylsilane and were calibrated against the residual solvent resonance (^{1}H) or the deuterated solvent multiplet (^{13}C). ^{31}P{^{1}H} NMR spectra were recorded on the same instrument operating at 81.01 mHz. Chemical shifts were measured relative to external 85% H_3PO_4, with downfield shifts considered positive. All the NMR spectra were recorded at room temperature (20 °C) unless otherwise stated. Elemental analyses (C, H) were performed using a Carlo Erba model 1106 elemental analyzer by the Microanalytical Service of the Department of Chemistry at the University of Florence. ESI-MS of the complexes were obtained on a Thermofinigan LCQ Deca XP Plus quadrupole ion trap instrument set in positive mode (solvent: methanol; flow rate: 5 μl/mn; spray voltage: 5 kV; capillary temperature: 100 °C; capillary voltage: 20 V), as described previously.[46] The percentage given in brackets belongs to the relative intensity of the peaks.

Synthesis of [Rh(η^5-C_5Me_5)(pta)Cl_2], 3. A solution of [(η^5-C_5Me_5)Rh(Cl)(μ_2-Cl)]$_2$ (100 mg, 0.162 mmol) and pta (51 mg, 0.324 mmol) in $CHCl_3$ (25 mL) was left standing for 12 h. After evaporation, the residue was washed with ether (3 × 10 mL) and $CHCl_3$ (3 × 5 mL) to give a fine red microcrystalline powder (110 mg, 0.236 mmol, 73%). Recrystallization from methanol gave red crystals of **3** suitable for X-ray diffarction analysis.

^{1}H NMR ($CDCl_3$): δ 1.69, (d, Cp-CH_3, J_{HP} = 3.5 Hz), δ 4.33 (s, PCH_2N), δ 4.51 (s, NCH_2N). ^{31}P{^{1}H} NMR ($CDCl_3$): δ −32.37 (d, J_{PRh} = 141 Hz). ESI-MS: m/z = 430.0 [Rh(η^5-C_5Me_5)(pta)(Cl)]$^+$ (10%), m/z = 465.9 [Rh(η^5-C_5Me_5){pta(H)}ptaCl_2]$^+$ (100%), and m/z = 932.6 [Rh_2(η^5-C_5Me_5)$_2${pta(H)}pta(pta)Cl_4]$^+$ (38%). Anal. Calcd for $C_{16}H_{27}N_3Cl_2PRh$: C, 41.2; H, 5.8; N, 9.0. Found: C, 41.0; H, 5.9; N, 9.1.

Synthesis of [Rh(η^5-C_5Me_5)(pta)$_2$Cl]Cl, 4·Cl. A mixture of [(η^5-C_5Me_5)Rh(Cl)(μ_2-Cl)]$_2$ (100 mg, 0.162 mmol) and pta (110 mg, 0.700 mmol) in $CHCl_3$ (25 mL) was left standing for 24 h at room temperature to give dark orange crystals, which were separated from the mother liquor by decantation, collected by filtration in the air, and washed with $CHCl_3$ (2 × 3 mL) and n-pentane (2 × 5 mL) before being dried at the pump (155 mg, 0.249 mmol, 77%).

^{1}H NMR ($CDCl_3$): δ 1.84, (t, Cp-CH_3, J_{HP} = 3.0 Hz), δ 4.4−4.9 (PCH_2N, NCH_2N, 7 peaks, 24 H). ^{31}P{^{1}H} NMR ($CDCl_3$): δ −28.68 (d, J_{PRh} = 131 Hz). ESI-MS m/z = 587.00 [Rh(η^5-C_5Me_5)(pta)$_2$Cl]$^+$ (100%). Anal. Calcd for $C_{22}H_{39}N_6Cl_2P_2Rh$: C, 42.4; H, 6.3; N, 13.5. Found: C, 42.2; H, 6.3; N, 13.3.

Synthesis of [Rh(η^5-C_5Me_5)(pta)$_2$Cl]BPh_4, 4·BPh_4. Metathesis reaction of **4**·Cl (0.5 mmol) with $NaBPh_4$ (0.65 mmol) was carried out dissolving the rhodium precursor in warm EtOH and adding the salt in an acetone solution at room temperature. By slow evaporation, the product was obtained as a microcrystalline powder, which was recrystallized from EtOH/acetone to yield single dark orange crystals suitable for X-ray structure determination. Yield: 90%. Anal. Calcd for $C_{46}H_{59}N_6BClP_2Rh$: C, 60.9; H, 6.6; N, 9.3. Found: C, 61.0; H, 6.5; N, 9.1.

Synthesis of [Rh(η^5-C_5Me_5)(CO)(pta)], 5. Solid [Rh(η^5-C_5Me_5)(CO)$_2$] (147 mg, 0.5 mmol) was added to an EtOH solution (20 mL) of pta (78.5 mg, 0.5 mmol) under nitrogen. The resulting orange solution was stirred and refluxed for 24 h, cooled to RT, and filtered under N_2 through a cotton plug. Solvent evaporation under a nitrogen stream gave orange crystals. These were then filtered off, washed with petroleum ether, and dried under vacuum (127 mg, 0.432 mmol, 60%).

IR (cm^{-1}): ν (CO) 1907 (s). ^{1}H NMR (CD_2Cl_2): δ 2.01 (dd, Cp-CH_3, J_{HP} = 1.9 Hz, J_{HRh} = 0.5 Hz, 15H), 3.98 (m, NCH_2N, 6H), 4.46 (m, PCH_2N, 6H). ^{31}P{^{1}H} NMR (CD_2Cl_2): δ −36.75 (d, J_{PRh} = 187.2 Hz). ^{13}C{^{1}H} NMR (CD_2Cl_2): δ 11.9 (s, Cp-CH_3), 56.37 (d, NCH_2P, $^3J_{CP}$ = 16.4 Hz), 74.0 (d, NCH_2N, J_{CP} = 6.7 Hz), 99.2 ppm (dd, Cp, $^2J_{CP}$ = 3.7 Hz, J_{CRh} = 1.8 Hz), 197.9 ppm (dd, CO, $^2J_{CP}$ = 85.8 Hz, J_{CRh} = 25.5 Hz). Anal. Calcd for $C_{17}H_{27}N_3OPRh$: C, 48.2; H. 6.4; N, 9.9. Found: C, 48.2; H, 6.4; N, 9.8.

Synthesis of [Rh(η^5-C_5H_5)(pta)$_2$], 6. [Rh(η^5-C_5H_5)(PPh_3)$_2$] (180 mg, 0.26 mmol) was added to a degassed EtOH solution (150 mL) of pta (81.7 mg, 0.52 mmol). The resulting orange solution was stirred and refluxed under nitrogen for 2 h. The solution was then concentrated to 8 mL, and reddish-orange crystals were slowly formed over 12 h. These crystals were filtered off under N_2, washed with EtOH/n-hexane (1:3), and dried under vacuum.

Yield: 43.1 mg (30%). ^{1}H NMR (CD_2Cl_2): δ 3.83 (br s, NCH_2N, 12H), 4.45 (br s, NCH_2P, 12H), 5.13 (s, Cp, 5H). ^{31}P{^{1}H} NMR (CD_2Cl_2): δ −25.32 (d, J_{PRh} = 205.0 Hz). Anal. Calcd for $C_{17}H_{29}N_6P_2Rh$: C, 42.3; H, 6.1; N, 17.4. Found: C, 42.8; H, 6.7; N, 17.0.

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Crystallography. The data were solved using direct methods with SHELXS and refined using SHELXL97.[47] The graphics interface package used was PLATON,[48] and the figures were generated using the ORTEP 3.07[49] generation package. For both compounds all non-hydrogen atoms have been refined anisotropically, and all the hydrogen atoms were placed using a riding model. For compound **4**·Cl there is a disordered, partially occupied dichloromethane solvent molecule.

Cell Culture. Human T47D breast carcinoma, A549 lung carcinoma, and HT-29 colon carcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). All other cell culture reagents were obtained from Gibco-BRL, Basel, Switzerland. The cells were routinely grown in DMEM medium containing 4.5 g/L glucose, 10% fetal calf serum (FCS), and antibiotics at 37 °C and 6% CO_2. For the MTT tests, the cells were seeded in 48-well plates (Costar, Integra Biosciences, Cambridge, MA) as monolayers for 24−48 h in complete medium to reach confluence, then fresh complete medium with 5% FCS was added together with the organometallic drugs, and culture was continued for 72 h. The test (see below) was performed for the last 2 h without changing the culture medium.

Determination of Cell Viability. The compounds were dissolved directly in culture medium, supplemented with 5% FCS. For **5**, the maximum concentration test was 200 μM due to the limited solubility of the compound in culture medium. Cell viability was determined using the MTT assay, which allows the quantification of the mitochondrial activity in metabolically active cells. Following drug exposure, MTT (final concentration 0.2 mg/mL) was added to the cells for 2 h, then the culture medium was aspirated and the violet formazan precipitate dissolved in 0.1 N HCl in 2-propanol. The optical density, which is directly proportional to number of surviving cells, was quantified at 540 nm using a multiwell plate reader (iEMS Reader MF, Labsystems), and the fraction of surviving cells, expressed as an average of three independent tests, was calculated from the absorbance of untreated control cells.

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Supporting Information Available: Crystallographic information files (CIF) for **3**, **4**·Cl, and **4**·BPh_4. This material is available free of charge via the Internet at http://pubs.acs.org.

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