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Modulation of the Solubility Properties of Arene Ruthenium Complexes Bearing Stannyl Ligands as Potential Anti-Cancer Agents

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Abstract

Cleavage of the known ruthenium dimer $[RuCl_2(\eta^6-C_6H_5OCH_2CH_2OH)]_2$ (1), bearing a hydrophilic substituent on the η^6 coordinated aromatic ring, with the phosphine ligands: triphenyl phosphine, triphenyl phosphite, trimethyl phosphite, and 1,3,5-triaza-7-phosphaadamantane (PTA) afforded the known complexes [RuCl₂(η^{6} - $[RuCl_2(\eta^6-C_6H_5OCH_2CH_2OH)\{P(OPh)_2\}]$ $C_{e}H_{2}OCH_{2}CH_{2}OH)(PPh_{2})$ (2a), (**2b**), $[RuCl_2(\eta^6 C_6H_5OCH_2CH_2OH)$ {P(OMe₃)}] (2c), and [RuCl₂ (η^6 - $C_6H_5OCH_2CH_2OH$)(PTA)] (4). The reaction of the known complex 2a with SnCl₂ afforded, by facile insertion of the SnCl₂ moiety into the Ru-Cl bond, the novel complex [RuCl(n⁶-C₆H₅OCH₂CH₂OH)(PPh₃)(SnCl₃)] (**3a**). Similarly, the reaction of complex **2b** with SnCl₂ afforded the novel complex $[RuCl(\eta^6-C_6H_5OCH_2CH_2OH){P(OPh)_3}(SnCl_3)]$ (3b). Complexes 3a and 3b were fully characterized by spectroscopy (Infrared (IR) -spectroscopy, ¹H, ³¹P and ¹¹⁹Sn Nuclear Magnetic Resonance (NMR) spectroscopy, UV-Vis spectroscopy and high resolution ESI-MS) and their thermal behaviour elucidated by Thermogravimetric Analysis (TGA). Density Functional Theory (DFT) calculations (Level of theory B3LYP, basis set for H, C, P, O, N and Cl is 6-31+G(d,p) and for Ru and Sn is DGDZVP) for complex 3a, 3b and 4 were also carried out, in particular to elucidate the bonding situation between Ru and Sn in complexes. The hitherto unprecedented anti-cancer activity of the complexes 2a - 2c as well as the novel stannyl complexes 3a and 3b were evaluated against MCF-7 (oestrogen receptor positive) human breast adenocarcinoma cell lines. All complexes show activity active against MCF-7 cell lines, indicating potential application as an anti-tumor agent.

Keywords: Ruthenium, Solubility, Stannyl ligands, Arene, Anti-cancer, MCF-7 Human Breast Adenocarcinoma Cells.

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1. Introduction

Cis-diamminedichloridoplatinum(II), commonly known as cisplatin, is administered intravenously to treat patients suffering from numerous types of cancers including testicular, cervical and certain lung cancers [1, 2]. In an attempt to increase the bioavailability and limit the side-effects of chemotherapeutic drugs, ruthenium complexes have been investigated as an alternative to platinum [3-8]. A detailed mechanistic study of the complex [Ru(p-cymene)(NHC)Cl₂] (NHC=1,3-bis(4-(tert-butyl)benzylimidazol-2-ylidene), has been reported and shows antiproliferate activity of EC109 cells is accompanied by up-regulation of p53 and p21 proteins and a down-regulation of cyclin D1 [9]. The aforementioned proteins are involved in the cell cycle, which can cause cell death if not properly regulated [9]. Ruthenium has potentially important pharmacological advantages, including its access to a range of oxidation states (II, III and IV) [6]. The energy barriers between each state are relatively low, allowing a change in oxidation state under relevant physiological conditions when inside the cell [6]. Despite the flexible oxidation states, ligand exchange rates in water are relatively slow and correlate with the time-scale of cellular reproduction [10]. If a ruthenium ion binds to a biomolecular target in the cell, it will most likely remain bound for the remainder of that cell's lifetime, which can be crucial for cytotoxic activity [10]. Research has been done on the cytotoxic activity of ruthenium arene complexes, giving promising results [3, 8]. One such example is RAPTA-C (Fig. 1) [11-13]. This piano-stool ruthenium arene complex bears a 1,3,5-triaza-7phosphaadamantane ligand and presents promise, similar to the drug NAMI-A, another active agent (Fig. 1) [14, 15]. Interesting chlorambucil derivatives of RAPTA-C have also been reported more recently in the literature [13].



Figure 1: The drug candidates RAPTA-C and NAMI-A are undergoing clinical trials for the treatment of secondary tumors [11, 14].

Previous work has shown that tin groups possess cytotoxic properties [16], and the effectiveness of a tin dichloride (SnCl₂) insertion on ruthenium arene complexes facilitates an improvement in cytotoxicity [17]. In this vein, we have recently reported neutral complexes of the type [RuCl(η^6 -C₆H₆)(PR₃)(SnCl₃)] (R = OPh, Ph, OMe), the ionic complexes [Ru(η^6 -C₆H₆)(PPh₃)(SnCl₃)(DMAP)]⁺BF₄⁻ and [(RuCl(η^6 -C₆H₆)(PPh₃)(DMAP)]⁺BF₄⁻ and [(RuCl(η^6 -C₆H₆)(PPh₃)(DMAP)]⁺BF₄⁻; the latter exhibit promising cytotoxic activity towards the cell lines A2780 (human ovarian cancer) and A2780cisR (human ovarian cisplatin-resistant cancer) (Fig. 2) [18]. In particular, [Ru(η^6 -C₆H₆)(PPh₃)(SnCl₃)(DMAP)]⁺BF₄⁻ exhibits some cancer cell selectivity. The neutral complexes [RuCl(η^6 -C₆H₆)(PR₃)(SnCl₃)] were shown to be less cytotoxic and exhibited in some cases low cytotoxicity. In a further study, complexes of the type [RuCl(η^6 -Arene)(PR₃)(GeCl₃)] were shown to exhibit very low cytotoxicity towards A2780 which we proposed was linked to the rapid aquation of the complexes [19]. Possibly, the lower than expected cytotoxicity in the complexes [RuCl(η^6 -C₆H₆)(PR₃)(SnCl₃)] could be linked to the their extreme insolubility in aqueous or non-polar media. Hence, solubility in aqueous media seemed to play a distinctive role in the cytotoxic activity: very insoluble

complexes exhibited low activity, despite having $SnCl_3$ groups which could be expected to enhance cytotoxicity [18]. Inspired by these findings, and as part of our ongoing investigation into elucidating the effect of incorporating a stannyl group into the ligand sphere of a Ru (II) arene complex and its effect on cytotoxicity and cancer cell selectivity, we synthesized a series of both known and novel complexes of the type [RuClX(η^6 -C₆H₅OCH₂CH₂OH)(PR₃)] (X = Cl or SnCl₃, R = OMe, Ph, OPh). These complexes bear a hydrophilic tail on the arene moiety, which might enhance solubility in aqueous media. The cytotoxicity towards MCF-7 (oestrogen receptor positive) human breast adenocarcinoma cells is reported here. Compounds are benchmarked against the cisplatin clinical drug (CDDP).



Figure 2: Ruthenium arene complexes exhibiting promising cytotoxic activity towards the cell lines A2780 (human ovarian cancer) and A2780cisR (human ovarian cisplatin-resistant cancer) [18].

2. Results and Discussion

2.1. Synthesis of the complexes

The precursor selected for this synthesis is the easily accessible ruthenium dimer $[RuCl_2(\eta^6-C_6H_5OCH_2CH_2OH)]_2$ (1) [20], which enables facile entry to the known piano-stool complexes **2a**, **2b** and **2c** through simple ligand cleavage reactions with the appropriate phosphine (Scheme 1) [20, 21]. For the synthesis of the known complexes **2a** and **2b** a modified procedure from Lastra-Barreira *et al.* was used [20]. Amendments were made by stirring the solution for an extended time period and eliminating the necessity of column chromatography to afford a simplified procedure with a higher overall yield (77.9 % and 91.0 % respectively). Subsequent reaction of complexes **2a** and **2b** with SnCl₂ affords the novel complexes [RuCl(η^6 -C₆H₅OCH₂CH₂OH)(PPh₃)(SnCl₃)] (**3a**) and [RuCl(η^6 -C₆H₅OCH₂CH₂OH){P(OPh)₃}(SnCl₃)] (**3b**) respectively by insertion of SnCl₂ into the Ru-Cl bond. An analogous complex to RAPTA-C was synthesized utilizing a PTA ligand, affording the known complex **4**.



Scheme 1: Overview of complexes synthesized in this study. (i) 2 equiv. $PPh_3/P(OPh)_3/P(OMe)_3$ in DCM, r.t., 17 h; (ii) 1 equiv. $SnCl_2$ in DCM, reflux, 4.5 h; (iii) 2 equiv. PTA in DCM : methanol (1:1), 50°C, 30 min.

2.2. Spectroscopic characterization of the novel complexes 2.2.1. $[RuCl(\eta^6-C_6H_5OCH_2CH_2OH)(PPh_3)(SnCl_3)]$ (3a)

A downfield shift of 3 ppm can be observed in the ${}^{31}P{}^{1}H$ NMR spectrum of complex 3a in comparison to the precursor 2a. This is in close analogy to our earlier observations, however, in contrast to our earlier findings [18], no ¹¹⁹Sn or ¹¹⁷Sn satellites were observed. Due to the insertion of SnCl₂ into the Ru-Cl bond of the precursor, the Ru center is rendered chiral and this results in loss of symmetry of the arene ring: in the ¹H NMR spectrum several resonance signals are observed corresponding to the asymmetric nature of the arene ring. This is also in close analogy to our earlier reported findings [18, 19]. Indeed, starting from the achiral precursor, 2a, the insertion of SnCl₂ induces a chiral center at Ru which should afford a racemic mixture of both enantiomers (rac-3a). This would be expected to afford one set of resonance signals in all NMR spectra (we represent, for simplicity in all our schemes, one of these stereoisomers for 3a and 3b). The IR spectrum of 3a shows a very weak and broad stretching vibration at 3078 cm⁻¹, which confirms the presence of the OH group. TGA shows an onset of 3.84% (29.26 g/mol) at 75 °C which may be due to trace impurities. The greatest mass loss of 14.22% (108.35 g/mol) occurred at the thermal event at temperatures in the range 321.01-342.26 °C. The weight of the leftover sample at the temperature of 493.45 °C corresponded to 44.40% (339.30 g/mol) which could be attributed to the Ru metal center and Sn. The observed decomposition point at 205 °C is in accordance with the thermal event in the range 128 -237 °C. The high resolution mass spectrum (ESI) of the complex shows a complex fragmentation pattern, with the base peak corresponding to the loss of the trichlorostannyl ligand from the parent ion i.e. [M-SnCl₃]⁺. This suggests that the stannyl ligand is somewhat weakly bound to the metal centre, which is supported by our DFT calculations showing a Wiberg Bond Index of 0.723 (see below). This fragmentation is in analogy to our previously reported analogous germyl complexes [19].

2.2.2. $[RuCl(\eta^6-C_6H_5OCH_2CH_2OH)(\{P(OPh)_3\}(SnCl_3)](3b)$

The chirality at the ruthenium center results in different environments for each proton on the arene ring

visible in the ¹H NMR spectrum in analogy to **3a**. The OH group can be assigned to a distinct doublet of doublets at $\delta = 4.51$ ppm. This is a result of the adjacent diastereotopic CH₂ hydrogens which appear to be in distinct environments. The OCH₂ attached to the arene ring appears as an unresolved broad peak ($\delta = 3.93-3.83$ ppm) as a result of complex ³*J* coupling to each of the diastereotopic H atoms on the adjacent CH₂. When comparing the resonance signal in the ³¹P{¹H} NMR to the precursor **2b** a downfield shift (by 7.3 ppm) upon the tin insertion is found. This is comparable to what is observed for complex **3a** and its precursor **2a** and our earlier findings [18]. In the ¹¹⁹Sn {¹H} NMR spectrum of **3b** a doublet resonance signal is observed at $\delta = -169.3$ ppm confirming the attachment of the SnCl₃ moiety to the Ru (II) center, as the doublet arises from a ²*J*_{Sn,P} coupling. This confirms additionally, that also in solution, no fluxional process is occurring, and the SnCl₃ is statically bound to the Ru center, as any decomplexation equilibrium would quench the coupling to P (Fig. 3). In close analogy to **3a**, complex **3b** exhibits a high resolution mass spectrum (ESI) which has a complex fragmentation pattern, with one of the high intensity signals corresponding to the loss of SnCl₃ from the parent ion.



Figure 3: ¹¹⁹Sn NMR spectrum of complex **3b**.

In the IR spectrum recorded for this sample a very weak and broad peak at 3000 cm⁻¹ denotes the presence of the OH group as in complex **3a**. The analysis using UV-Vis spectroscopy shows the highest absorbance at the $\lambda_{max} = 454$ nm. When compared to the precursor **2b**, a blue shift of 30 nm has been observed, which is in agreement with the visible colour change. The TGA analysis of complex **3b** reveals the initial mass loss of 3.27 % (26.50 g/mol) at a temperature of 135 °C. The biggest mass decrease of 18.40 % (149.00 g/mol) was recorded at the thermal event at temperatures in the range 225.24–339.11 °C.

2.2.3 Water solubility

By the addition of a hydrophilic substituent on the arene ligand an improved water solubility of the complexes was intended. Simple tests were conducted to assess the relative water solubilities of the complexes. Complexes **3a** and **4** were found to be somewhat water soluble at 20 °C (0.03 mg/mL and 10 mg/mL respectively). However, **2b** and **3b** were found to be insoluble.

2.2.4 Single Crystal X-Ray Investigation of **3b**

Crystals, suitable for single crystal X-ray diffraction analysis, of complex **3b** were obtained by slow evaporation of a dilute sample of **3b** in dichloromethane at 7 °C (Fig. 4), providing additional evidence of SnCl₂ insertion into the Ru-Cl bond of its precursor **2b**. Complex **3b** has features typical of piano-stool Ru(II) arene complexes of this type (see [18] and references therein). Complex **3b** features a Ru-Sn bond length of 2.5627(19) Å, which is comparable to the complex $[(\eta^6-C_6H_6)RuCl(SnCl_3)(P(OPh)_3)]$ (2.5686 Å) reported by us earlier [18]. The complex exhibits a slightly distorted tetrahedral geometry about the Sn center evidenced by the slight deviations from a perfect tetrahedral geometry: Cl(1)-Sn(1)-Ru(1) 120.30(16), Cl(3)-Sn(1)-Ru(1) 118.9(4), Cl(2)-Sn(1)-Ru(1) 115.34(17). Similarly, the geometry around the Ru centre can be described as distorted tetrahedral.



Figure 4: ORTEP representation of the X-ray structure of complex *rac*-**3b** at the 30 % probability level. Disorder at Cl3 not represented. Selected bond lengths [Å]: Sn(1)-Ru(1) 2.5627(19), Cl(3)-Sn(1) 2.409(9), Sn(1)-Cl(2) 2.351(5), Sn(1)-Cl(1) 2.377(7), Ru(1)-P(1) 2.246(5). Selected bond angles [°]: P(1)-Ru(1)-Sn(1) 86.56(13), Cl(4)-Ru(1)-Sn(1) 85.46(14), P(1)-Ru(1)-Cl(4) 85.80(17), Cl(1)-Sn(1)-Ru(1) 120.30(16), Cl(3)-Sn(1)-Ru(1) 118.9(4), Cl(2)-Sn(1)-Ru(1) 115.34(17). (Other metric parameters found in the supporting information).

2.3. Cytotoxic testing

The anti-cancer activity of Ru(II) arene complexes (5 & 10 μ M prepared in DMSO) on the MCF-7 (oestrogen-receptor positive) human breast adenocarcinoma, was evaluated using the MTT colorimetric assay (Fig. 5) [22]. In all assays, DMSO diluted in free medium was included as a negative control and the clinical drug cisplatin (CDDP) was included as a positive control with an IC₅₀ value of 5.47 μ M (see figure S9), comparable to that reported in literature for the same cell-line (5.75 μ M) [23]. The % cell viability against the Ru(II) arene complexes is herein reported in figure 5. Complexes **2a** – **2c**, **3a** and **4**

show activity against the MCF-7 cells, at both concentrations tested with complex **3b** showing low activity. Complex **2b** is the most active (<50% cell survival) at 10 μ M which may be attributed to the presence of the triphenyl phosphate ligand. This possibly enhances the solubility of the drug molecule upon direct contact or interaction with the human albumin serum (HSA) protein enabling its transportation to the biologically active target at enhanced rates [24]. Compound **4** is suspected to undergo an aquation reaction in solution to yield its complex cation and the respective counter ion which may account for the observed trend specifically for this compound. The tin compounds **3a** and **3b** exhibit some cytotoxicity as compared with the complexes reported earlier of the type [RuCl(η^6 -C₆H₆)(PR₃)(SnCl₃)] which were largely inactive. This provides some evidence that attachment of the hydrophilic tail increases the water solubility and hence the cytotoxic activity.







Figure 5: MTT cell viability assays for MCF-7 cells treated for 24 hours with Ru arene organometallic complexes **2a-c**, **3a**, **3b** & **4** prepared at (a) 5 & 10 μ M and (b) 0 - 10 μ M or vehicle (DMSO) or Cisplatin (CDDP). Graphs show mean cell viability as a percentage of vehicle control \pm SEM for each concentration of complexes determined from three independent experiments performed in quadruplicate [*p<0.5; **p<0.01; ***p<0.001].

Although compound **2b** was insoluble in water, it was soluble in DMSO and is the most active against the MCF-7 cells in this series. Compound **3b** was also insoluble in water and only partially soluble in DMSO and it showed only some activity against the MCF-7 cells. The hydrophobicity of **3b** may be attributed to the presence of the trichlorostannyl ligand coordinated to the ruthenium metal center, impeding the solubility of the compound in DMSO. This phenomenon was also observed and reported for the half-sandwich ruthenium complex, $[(\eta^6-C_6H_6)Ru(SnCl_3)_2(P(OMe)_3)]$, bearing two trichloro stannyl

ligand moieties [18], for which the antiproliferative activity was measured against ovarian cancer cells after 72 hours of continuous drug action.

In the present study, the compound was used after centrifugation of the stock solution to help aid solubility before dilution with the supplemented RPMI medium. Complexes **2a**, **3a** and **3b** at 10 μ M indicate an increase in the cell viability, possibly due to the uptake limit of the complexes. A multi-dose cell viability of compounds **3a** and **3b** is shown in figure 5b indicating an approximate cell death of 30 % at 8 μ M, beyond which an increase in the cell viability is observed.

2.4 Density Functional Calculations

The optimized structures of complexes 3a, 3b and 4 are shown in figure 6.



Figure 6: Density Functional Theory optimized structures for complexes 3a (left), 3b (middle) and 4 (right). Level of theory B3LYP, basis set for H, C, P, O, N and Cl is 6-31+G(d,p) and for Ru and Sn is DGDZVP.

In all the optimized structures, the Ru atoms show pseudo-tetrahedral geometry. For complexes 3a and 3b, the Sn atoms also show pseudo-tetrahedral geometry; in addition, the optimized structures of 3a and 3b are very similar in the arene and SnCl₃ moieties. A visual representation of the highest occupied energy orbitals (HOMO) for complexes 3a, 3b and 4 can be found in figure 7; while a visual representation of the lowest unoccupied energy orbitals (LUMO) is depicted in figure 8.



Figure 7: Graphical representation of the highest occupied energy orbitals (HOMO) for complexes **3a** (left), **3b** (middle) and **4** (right). Level of theory B3LYP, basis set for H, C, P, O, N and Cl is 6-31+G(d,p) and for Ru and Sn is DGDZVP.

For complex **3b**, the DFT-optimized structure matches the crystallographic structure obtained by X-ray analysis within 8 % difference according to selected values of bond length and angles reported in

	X-Ray		DFT optimized		
	Bond	Angles	Bond	Angles	Difference
	Lengths	Angles	Lengths	Angles	
	[Å]	[^o]	[Å]	[⁰]	(%)
Sn1-Cl1	2.377		2.432		2
Sn1-Cl2	2.351		2.434		3
Sn1-Cl3	2.409		2.384		-1
Sn1-Ru1	2.563		2.638		3
Ru1-Cl4	2.379		2.447		3
Ru1-P1	2.246		2.276		1
Ru1-Sn1-Cl4		85.46		85.47	0
Ru1-Sn1-P1		86.56		88.77	2
Cl1-Sn1-Cl2		96.30		99.34	3
Cl1-Sn1-Cl3		108.00		101.86	-6
Cl2-Sn1-Cl3		92.50		100.77	8

Table 1: Comparison of the DFT-optimized structure and the X-ray crystallographic data

For complexes **3a** and **3b**, the HOMO is mainly located on the Ru-Sn bond and the Ru and Cl atoms. This indicates that the oxygen atoms connected to the phosphorous do not significantly influence the HOMO of complex **3b** with respect to complex **3a**. For complex **4**, the HOMO is mainly located on the Cl atoms and along the Ru-arene coordination.



Figure 8: Graphical representation of the lowest unoccupied energy orbitals (LUMO) for complexes 3a (left), 3b (middle) and 4 (right). Level of theory B3LYP, basis set for H, C, P, O, N and Cl is 6-31+G(d,p) and for Ru and Sn is DGDZVP.

For complexes **3a** and **3b** the LUMO is mainly located on the Sn-Cl bonds as well as on the Ru, Ru-Cl bond and delocalized over the arene. Likewise, the analysis of the HOMOs, the LUMOs of the optimized structures for **3a** and **3b** are very similar, denoting a negligible effect of the oxygens bound to the phosphorous on the LUMO. Similar results are obtained for the LUMO of complex **4** where the LUMO is located on the Ru-Cl bonds and delocalized on the arene. Natural bond analysis (NBO) was performed to establish the nature of bonds with particular focus on the ruthenium-tin bonds and atoms of complexes **3a** and **3b**. Table 2 summarizes the DFT results of the natural bond order analysis for complexes **3a** and **3b**.

Atom	Bond Polariz. (%)	s-character (%)	p-character (%)	d-character (%)	Mulliken Charge	Wiberg Bond Index				
Complex 3a										
Ru	50.47	23.52	27.73	48.75	-0.709	0.723				
Sn	49.53	82.12	17.51	0.37	0.602					
Complex 3b										
Ru	52.62	22.87	25.93	51.20	-0.895	0.711				
Sn	47.38	80.27	19.32	0.41	0.648					

Table 2: Natural bond analysis calculation for complexes 3a and 3b.

The calculation results show no significant differences between the bond polarizations and the contributions of the different atomic orbitals of Ru and Sn of complexes **3a** and **3b**. Complex **3b** shows a slightly more negative Mulliken charge on the Ru atom most likely due to an increased electron density generated by the presence of the oxygen atoms directly connected to the P atoms coordinated to the Ru centre. The Wiberg bond index for the Ru-Sn bond is 0.723 for Complex **3a** and 0.711 for complex **3b**, which is in close agreement with our earlier reported findings for a series of analogous Germyl complexes [19].

3. Experimental Section

3.1 Materials and methods

All reactions were carried out under nitrogen overpressure. Analysis of all complexes proceeded in air as the complexes were air and moisture stable. All commercially available chemicals used were obtained from Sigma-Aldrich and used without further purification. Dimer **1** was prepared according to Soleimannejad *et al.* [25]. Complex **2c** was prepared following a published procedure from Lastra-Barreira *et al.* [20]. For complexes **2a** and **2b** the same procedure was used with only minor amendments. Complex **4** was prepared using a procedure reported by Matsina *et al.* [21]. NMR spectra were recorded on a Bruker Ultrashield 300 or in the case of ¹¹⁹Sn NMR (111.8 MH_z): a Varian Unity INOVA **300** spectrometer. IR spectra were recorded on a Shimadzu MIRacle IR (ATR), UV-Vis on a Shimadzu UV 3600, and TGA spectra were recorded on a TGA Q-500 at the University of Maastricht Brightlands Chemelot Campus, Netherlands. The NMR signals are reported with reference to their solvent residual signals. The peaks in the IR spectra were reported according to their relative intensities: s: strong; w: weak; m: medium; vs: very strong; vw: very weak. The high resolution ESI-MS of **3a** and **3b** was recorded at the University of Stellenbosch Central Anlytical Facilities on a Waters Synapt G2 spectrometer with a cone voltage of 15 V.

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X-ray structure determination: For X-ray structure analyses the crystals were mounted onto the tip of glass fibers, and data collection was performed with a BRUKER-AXS SMART APEX CCD diffractometer using graphite-monochromated Mo K α radiation (0.71073 Å). The data were reduced to F_0^2 and corrected for absorption effects with SAINT [26] and SADABS [27, 28], respectively. Structures were solved by direct methods and refined by full-matrix least-squares method (SHELXL97 and SHELX2013) [29, 30]. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed in calculated positions to correspond to standard bond lengths and angles. All diagrams were drawn with 30 % probability thermal ellipsoids and all hydrogen atoms were omitted for clarity.

Crystallographic data for the structure of compounds **3b** reported in this paper has been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 1585436 (**3b**). Copies of data can be obtained free of charge at: http://www.ccdc.cam.ac.uk/products/csd/request/. Figures of solid state molecular structures were generated using Ortep-3 as implemented in WINGX [31] and rendered using POV-Ray 3.6 [32].

3.2 Cell culture

RPMI 1640 medium (Highveld Biological, Lyndhurst, UK) was used to maintain the human breast adenocarcinoma MCF-7 (oestrogen receptor positive) cell line. The medium was supplemented with 10% Foetal Bovine Serum (FBS), 100 U/ml penicillin and 100 μ g/ml streptomycin. Cells were maintained at 37 °C in a 5 % CO₂-95 % air-humidified incubator.

3.3 In vitro cytotoxicity evaluation-MTT assay protocol

The complex solutions were prepared by dissolving compounds in DMSO to give 10 mM stock solutions which were used on the same day of treatment. MCF-7 cells were seeded in 96 well plates at a density of 4.5 x 10^3 cells per well. After 48 hrs, cells were treated for 24 hours with medium (200 µL) containing the test compounds (0, 5 and 10 µM) or vehicle (10 µM DMSO). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyyltetrazolium bromide (MTT) assay (11465007001, Sigma-Aldrich) [22] was used to determine the cell viability according to the manufacturer's instructions. Briefly, MTT (10 µL) solution was added to each well and the plates were incubated for 4 h at 37 °C. After this period, the solubilizing buffer (10% SDS in 0.01 M HCl) was added to each well and the plates were determined for each well. Mean cell viability was calculated as a percentage of the mean vehicle control. At least three independent experiments in quadruplicate were performed.

3.4 Statistical analysis

Statistical analysis was conducted with the acquisition of the data represented as mean values and SEM (standard error of the means) of three independent experiments. The t-test (a student's statistical hypothesis test) was used to compare the two experimental frames and a value of p < 0.5 was accepted as statistically significant.

3.5 Density Functional Theory calculations

DFT calculations were performed to model the complexes **3a**, **3b** and **4**. The Guassian09 software package was used. The level of theory used for all calculations is B3LYP with the basis set 6-31+G(d,p) for H, C, O, P, Cl and N atoms; while for Ru and Sn atoms the DGDZVP basis set was used. Geometry



optimizations were calculated without any constraints. All the optimized geometries show non imaginary frequency. Energies and Natural Bond Order analyses were conducted on the optimized structures [33-36].

3.6 $[RuCl(\eta^6-C_6H_5OCH_2CH_2OH)(PPh_3)(SnCl_3)]$ (3a)

To a solution of **2a** (0.300 g, 0.524 mmol) in DCM (35 mL), anhydrous tin dichloride (0.109 g, 0.576 mmol) was added. The mixture was refluxed for 4.5 h. The solution was filtered, and the solvent removed *in vacuo* affording a dark red solid. Yield: 0.319 g (0.418 mmol, 79.8 %). ¹H NMR (300.1 MHz, DMSO-*d*₆, 296.2 K, ppm) δ 7.66-7.41 (m, 15H, PP*h*₃), 6.40 (t, ³*J*_{H,H} = 5.9 Hz, 1H, C6*H*₅), 6.09 (d, ³*J*_{H,H} = 5.9 Hz, 1H, C₆*H*₅), 5.94 (d, ³*J*_{H,H} = 6.3 Hz, 1H, C₆*H*₅), 5.59-5.54 (ps q, 1H, C₆*H*₅), 5.02 (broad s, 1H, OH), 4.24-4.17 (m, 1H, C₆*H*₅), 4.12-4.05 (m, 2H, C*H*₂O), 3.71 (broad s, 2H, C*H*₂OH). ¹³C{¹H} NMR (75.5 MHz, DMSO-*d*₆, 296.7 K, ppm) δ 143.4 (d, C_{ipso}, PP*h*₃), 133.9 (d, C_{meta}, PP*h*₃), 131.4 (d, C_{para}, PP*h*₃), 129.3 (d, C_{ortho}, PP*h*₃), 77.9 (d, ²*J*_{C,P} = 9.5 Hz C_{meta}, *C*₆*H*₅), 72.2 (s, C_{para}, *C*₆*H*₅), 62.0 (s, *CH*₂O), 59.4 (s, C_{ortho}, *C*₆*H*₅) 55.4 (s, *CH*₂OH) (C¹ of C₆*H*₅ ring not visible). ³¹P {¹H} NMR (121.5 MHz, DMSO-*d*₆, 298.2 K, ppm) δ 33.2 (s). FTIR 3078 (w, broad), 2959 (vvw), 1528 (m), 1464 (w), 1435 (m), 1265 (m, broad), 1092 (m), 1072-999 (w, broad), 910 (w, broad), 845 (w), 800 (w, broad), 746 (m), 692 (s), 667 (m) cm⁻¹. UV-Vis (nm) $\lambda_{max} = 376$. TGA: (Weight % decrease) 80 °C – 128.17 °C (3.847 %), 128.17 °C – 237.71 (6.69 %), 237.71 °C – 283.11 °C (6.52 %), 283.11 °C – 321.01 °C (11.13 %), 321.01 °C – 342.26 °C (14.22 %), 342.26 °C – 375.16 °C (7.55 %), 375.16 °C – 493.45 °C (5.70 %). Melting point: 205 °C (dec.). S20°C (H2O) soluble, 0.03 mg/mL. ESI-MS 537.0331 (found), 537.0342 (calcd.) ([M-SnCl₃]⁺, 100 %).

3.7 $[RuCl(\eta^6 - C_6H_5OCH_2CH_2OH)\{P(OPh)_3\}(SnCl_3)]$ (3b)

To a solution of 2b (0.300g, 0.484 mmol) in DCM (35 mL), anhydrous tin dichloride (0.100 g, 0.532 mmol) was added. The mixture was refluxed for 4.5 h. The orange solution was filtered, and the solvent removed in vacuo affording a viscous red oil. The crude product was left to crystallize from a mixture of diethyl ether and DCM at -25°C obtaining bright red crystals. Yield: 0.212 g (0.261 mmol, 54.0%). ¹H NMR (300.1 MHz, CDCl₃, 296.2 K, ppm) δ 7.38-7.25 (m, 15H, P(OPh)₃), 6.33 (t, ^xJ_{HH} = 5.4 Hz, 1H, C_6H_5), 5.76-5.74 (m, 1H, C_6H_5), 5.60-5.55 (m, 1H, C_6H_5), 4.52 (dd, ${}^3J_{H,H} = 2.03$ Hz, ${}^3J_{H,H} = 3.35$ Hz, 1H, OH), 4.16-4.10 (m, 1H, C_6H_5), 4.04-3.99 (m, 1H, C_6H_5), 3.93-3.86 (m, 2H, CH_2O), 3.71 (ps t, ${}^3J_{H,H} = 6.0$ Hz, 1H, CH₂^AOH), 2.45 (t, 1H, CH₂^BOH). ¹³C{¹H} NMR (75.5 MHz, DMSO-*d*₆, 295.2 K, ppm) δ 150.9 (d, ${}^{2}J_{C,P}$ = 10.0 Hz, C_{ipso}, P(OPh)₃), 130.0 (s, C_{meta}, P(OPh)₃), 125.9 (s, C_{para}, P(OPh)₃), 121.7 (d, ${}^{3}J_{C,P}$ = 4.4 Hz Cortho, P(OPh)₃), 94.6 (s, C_{meta}, C₆H₅), 92.2 (s, C_{para}, C₆H₅), 72.7 (s, CH₂O), 72.3 (s, C_{ortho}, C₆H₅), 60.4 (s, CH₂OH) (C¹ of C₆H₅ ring not visible). ³¹P{¹H} NMR (121.5 MHz, CDCl₃, 296.2 K, ppm) δ 123.7 (s, $^{2}J_{\text{Sn}, P}^{119} = 896 \text{ Hz}, ^{2}J_{\text{Sn}, P}^{117} = 849 \text{ Hz}). ^{119}\text{Sn} \{^{1}\text{H}\} \text{ NMR} (111.8 \text{ MHz}, \text{CDCl}_{3}, 298.2 \text{ K}, \text{ppm}) \delta - 169.4 \text{ Hz}$ (d, ${}^{2}J^{119}_{\text{Sn}, P} = 974$ Hz). FTIR 3000 (vw, broad), 1540 (vw), 1485 (vw), 1270 (vw, broad), 1190 (vw), 1150 (vw), 1080 (vw), 1020 (vw), 940 (vw), 900 (w, broad), 760 (w), 690 (w) cm⁻¹. UV-Vis (nm) λ_{max} = 452. TGA (Weight % decrease) 150 $^{\circ}$ C – 177.74 $^{\circ}$ C (3.27 %), 177.74 $^{\circ}$ C – 225.24 (8.59 %), 225.24 $^{\circ}$ C - 339.11 °C (18.40 %), 399.11 °C - 404.81 °C (12.89 %), 404.81 °C - 485.32 °C (14.81 %). Melting point: 169 °C (dec.). S20°C (H₂O) insoluble. ESI-MS 584.9194 (found), 585.0172 (calcd.) ([M-SnCl₃]⁺, 27 %).

4. Conclusion

Two novel half-sandwich Ru(II) compounds bearing trichlorostannyl ligands were successfully synthesized as potential anti-cancer agents: $[RuCl(\eta^6-C_6H_5OCH_2CH_2OH)(PPh_3)(SnCl_3)]$ (3a) and $[RuCl(\eta^6-C_6H_5OCH_2CH_2OH){P(OPh)_3}(SnCl_3)]$ (3b) and their cytotoxicity compared with the related known complexes: [RuCl₂(η^6 -C₆H₅OCH₂CH₂OH)(PPh₃)] (**2a**), [RuCl₂(η^6 -C₆H₅OCH₂CH₂OH){P(OPh)₃}] (2b), $[\operatorname{RuCl}_2(\eta^6 - C_6H_5OCH_2CH_2OH) \{P(OMe_3)\}]$ (2c), and $[\operatorname{RuCl}_2(\eta^6 - C_6H_5OCH_2CH_2OH)(PTA)]$ (4). Both new complexes were characterized using IR-spectroscopy, multinuclear NMR spectroscopy, UV-Vis spectroscopy and TGA as well as high resolution ESI-MS. Complex 3b was also characterized by single crystal X-ray diffraction analysis. The aim to increase the bioavailability via increasing hydrophilicity was gauged using a simple water solubility test finding the complexes 3a and 4 to be water soluble, while **3b** was insoluble. Compounds **2(a-c)**, **3(a)** and **4** is somewhat cytotoxic to MCF-7 breast cancer cells at the concentrations tested. The low cytotoxicity of compound **3b** is likely due to the only partial solubility of the compound in DMSO, thus offsetting the compound's capability to induce cellular apoptosis. Hence in this study the complexes 2a and 2b are more cytotoxic than their trichlorotin analogues **3a** and **3b**, suggesting that despite attachment of a hydrophilic substituent to the η^6 -arene ring, insolubility caused by the trichlorostannyl ligand still might impede cytotoxicity of the latter. An investigation into the solubility of these compounds in a synthetic buffer like that of the human body may provide more accurate results.

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Highlights

- Modulating solubility of potential anti-cancer agents by attachment of a hydrophilic tail trichlorostannyl complexes.
- In vitro testing against MCF-7 (oestrogen receptor positive) human breast adenocarcinoma cell lines
- DFT (density functional theory) results.