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Anticancer activity of new organo-ruthenium, rhodium and iridium complexes containing the 2-(pyridine-2-yl)thiazole *N,N*-chelating ligand

Michaël Gras [a], Bruno Therrien [a,*], Georg Süss-Fink [a], Angela Casini [b], Fabio Edafe [b], Paul J. Dyson [b]

[a] Institut de Chimie, Université de Neuchâtel, Case postale 158, CH-2009 Neuchâtel, Switzerland
[b] Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

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ABSTRACT

The dinuclear dichloro complexes $[(\eta^6\text{-arene})_2Ru_2(\mu\text{-Cl})_2Cl_2]$ and $[(\eta^5\text{-}C_5Me_5)_2M_2(\mu\text{-Cl})_2Cl_2]$ react with 2-(pyridine-2-yl)thiazole (pyTz) to afford the cationic complexes $[(\eta^6\text{-arene})Ru(pyTz)Cl]^+$ (arene = C_6H_6 **1**, *p*-^{i}PrC_6H_4Me **2** or C_6Me_6 **3**) and $[(\eta^5\text{-}C_5Me_5)M(pyTz)Cl]^+$ (M = Rh **4** or Ir **5**), isolated as the chloride salts. The reaction of **2** and **3** with $SnCl_2$ leads to the dinuclear heterometallic trichlorostannyl derivatives $[(\eta^6\text{-}p\text{-}^i\text{Pr}C_6H_4\text{Me})Ru(pyTz)(SnCl_3)]^+$ (**6**) and $[(\eta^6\text{-}C_6Me_6)Ru(pyTz)(SnCl_3)]^+$ (**7**), respectively, also isolated as the chloride salts. The molecular structures of **4**, **5** and **7** have been established by single-crystal X-ray structure analyses of the corresponding hexafluorophosphate salts. The *in vitro* anticancer activities of the metal complexes on human ovarian cancer cell lines A2780 and A2780cisR (cisplatin-resistant), as well as their interactions with plasmid DNA and the model protein ubiquitin, have been investigated.

1. Introduction

Organometallic transition metals complexes endowed with anticancer properties are currently attracting considerable attention due to their wide and diverse structural types and varied ligand bonding modes which offers considerable potential in fine-tuning their biological properties [1]. Titanocene dichloride was the first organo-transition metal compound to be extensively studied as an alternative to cisplatin and it underwent numerous clinical evaluations [2]. More recently, targeted ferrocene sandwich complexes and half-sandwich ruthenium complexes [3] are gaining increasing attention, with encouraging results observed in animal models [4].

Notably, half-sandwich ruthenium–arene complexes containing *N,N*-chelating ligands that are not only of interest in catalysis [5], are also of interest in bioorganometallic chemistry due to their anticancer properties [6]. The versatility of the pseudo-tetrahedral coordination geometry at the metal centre provides considerable flexibility in terms of rational functionalisation [7]; the aromatic ligand provides stability to the metal oxidation state and gives a lipophilic domain to the complex, while the halide is labile and usually undergoes hydrolysis in aqueous solution [8] or exchange with a substrate under catalytic conditions [9] and potentially physiological conditions. Aquation is believed to be a key aspect in the biological activity of the $[(\eta^6\text{-arene})Ru(en)Cl]^+$ (en = ethylenediamine) complexes, in which the *N,N*-chelating ligand endows the complexes with high cytotoxicity towards human ovarian cancer cells [10]. A structure–activity relationship (SAR) study showed that the most active complexes contain a coordinatively stable *N,N*-chelating ligand, a hydrophobic arene ligand and a labile halide group [11]. While osmium arene complexes have been investigated for their anticancer potential [12] and an osmium analogue of the $[(\eta^6\text{-arene})Ru(en)Cl]^+$-type complexes have been studied [10], very few studies of isoelectronic rhodium and iridium complexes have been undertaken. Nevertheless, rhodium half-sandwich compounds containing 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decanephosphine and chlorido co-ligands have been evaluated *in vitro* [6] and model reactions with a series of nucleosides were also studied [13]. Moreover, Sheldrick and co-workers have reported a series of related complexes based on rhodium and iridium containing *N,N*-chelating polypyridyl ligands that are cytotoxic and interact with DNA via coordination, intercalation or a combination of both [14]. In general, the results obtained indicate that antiproliferative effects are governed by the size of the polypyridyl ligands, with the complexes bearing the larger ligands being more cytotoxic.

In this paper, we describe the synthesis, structural characterisation and the biological evaluation of a new series of half-sandwich complexes based on ruthenium, rhodium and iridium incorporating the coordinatively stable *N,N*-chelating ligand 2-(pyridine-2-yl)thiazole (pyTz), a labile chlorido or, in the case of the ruthenium complexes, a trichlorostannyl group. It was found that all the compounds interact strongly with DNA, but somewhat surprisingly, are

* Corresponding author. Tel.: +41 (0) 32 718 24 99; fax: +41 (0) 32 718 25 11.
E-mail address: bruno.therrien@unine.ch (B. Therrien).

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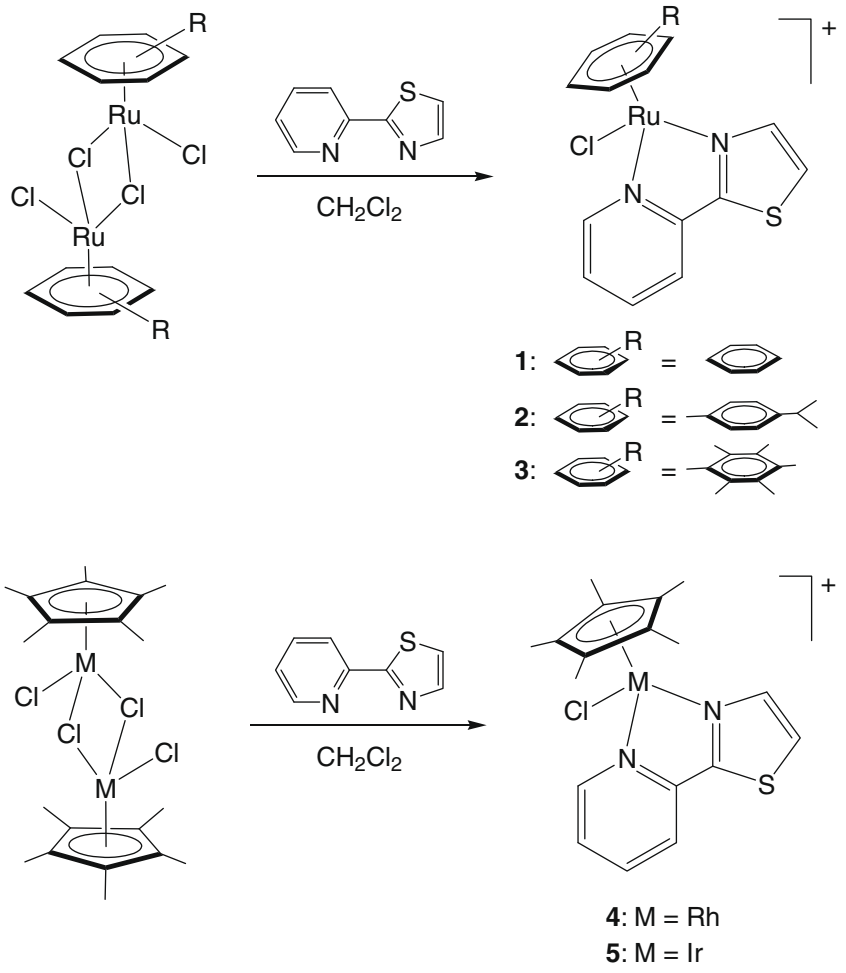


Scheme 1. Synthesis of cations **1**–**5**.

only slightly cytotoxic. The most cytotoxic compounds are those containing the trichlorostannyl group.

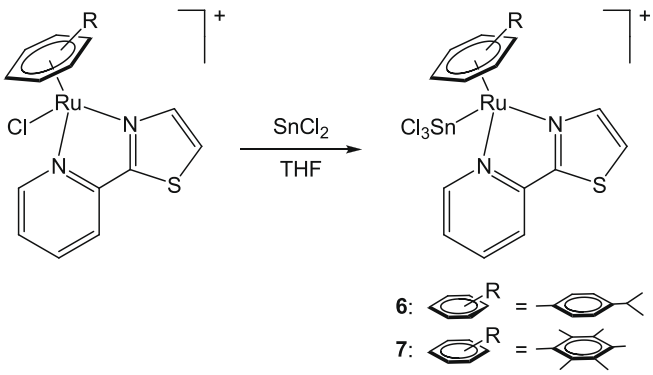
2. Results and discussion

2.1. Synthesis of the mononuclear complexes *1*–*7* as chloride salts

The arene or pentamethylcyclopentadienyl complexes $[(\eta^6\text{-arene})_2Ru_2(\mu\text{-}Cl)_2Cl_2]$ (arene = C_6H_6, *p*-^{i}PrC$_6$H$_4$Me, C_6Me_6) and $[(\eta^5\text{-}C_5Me_5)_2M_2(\mu\text{-}Cl)_2Cl_2]$ (M = Rh, Ir) react with 2 equiv of 2-(pyridine-2-yl)thiazole (pyTz) in dichloromethane to form the cationic arene ruthenium complexes $[(\eta^6\text{-}C_6H_6)Ru(pyTz)Cl]^+$ (**1**), $[(\eta^6\text{-}p\text{-}^iPrC_6H_4Me)Ru(pyTz)Cl]^+$ (**2**) and $[(\eta^6\text{-}C_6Me_6)Ru(pyTz)Cl]^+$ (**3**), and the pentamethylcyclopentadienyl complexes $[(\eta^5\text{-}C_5Me_5)Rh(pyTz)Cl]^+$ (**4**) and $[(\eta^5\text{-}C_5Me_5)Ir(pyTz)Cl]^+$ (**5**), see Scheme 1. All complexes are isolated as their chloride salts and were characterised by mass spectrometry, NMR spectroscopy and elemental analysis.

The electrospray ionisation mass spectra of these compounds show the expected molecular peaks for cations **1**–**5**. In the ^{1}H NMR spectra of **1**–**5**, the chelating pyTz ligand can be recognised by characteristic heteroaromatic signals, with the arene or pentamethylcyclopentadienyl ligand giving rise to a singlet for the methyl proton resonances in the case of **3**, **4** and **5**, to a singlet for the protons of the benzene ring in the case of **1** and the typical *para*-cymene pattern including the indicative septet in the case of **2** (for further details see Section 3).

Compounds [**2**]Cl and [**3**]Cl react with $SnCl_2$ in THF at room temperature to give $[(\eta^6\text{-}p\text{-}^iPrC_6H_4Me)Ru(pyTz)(SnCl_3)]Cl$ ([**6**]Cl) and $[(\eta^6\text{-}C_6Me_6)Ru(pyTz)(SnCl_3)]Cl$ ([**7**]Cl), respectively (Scheme 2). The analogous reaction with [**1**]Cl, [**4**]Cl and [**5**]Cl is hampered by the limited solubility of these compounds. In the mass spectra of **6** and **7** molecular ion peaks are not observed, but fragments that correspond to the loss of $SnCl_3$. However, the ^{119}Sn NMR spectra of **6** and **7** contain a peak at δ = -364.47 for **6** and δ = -265.26 ppm for **7**, indicative of the coordinated $SnCl_3$ unit [15]. Moreover, the ^{1}H NMR spectra show the expected signals of the pyTz ligand as well as those of the arene ligands.



Scheme 2. Synthesis of the cationic trichlorostannyl complexes **6** and **7**.

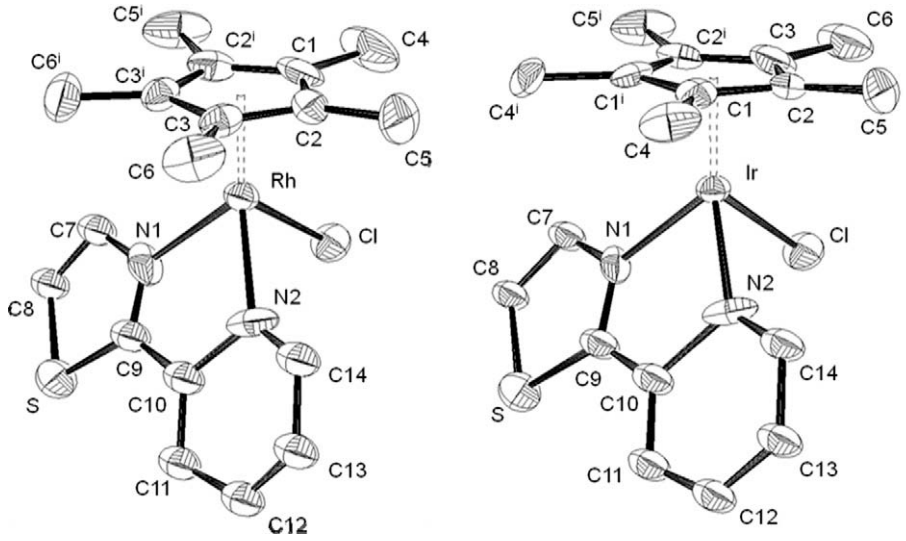


Fig. 1. ORTEP diagrams of cations **4** (left) and **5** (right) with 50% probability thermal ellipsoids. Hydrogen atoms and counter anions are omitted for clarity. Symmetry code: (i) $1 - x, y, z$.

2.2. Molecular structures of [4]PF_6, [5]PF_6 and [7]PF_6 in the solid state

The molecular structures of cations **4**, **5** and **7** have been established by single-crystal X-ray structure analysis as their hexafluorophosphate salts, which were obtained by diffusion of a dichloromethane solution of the corresponding chloride salts [**4**]Cl, [**5**]Cl and [**7**]Cl in the presence of KPF_6 into a diethylether layer. Salts [**4**]PF_6 and [**5**]PF_6 crystallise in the orthorhombic space group $Pmn2_1$ and [**7**]PF_6 crystallises in the orthorhombic space group *Pnma*. The complexes show a typical piano-stool geometry with the metal centre being coordinated by an arene or pentamethylcyclopentadienyl ring, a chlorido ligand and a chelating 2-(pyridine-2-yl)thiazole ligand. The nitrogen atoms of the 2-(pyridine-2-yl)thiazole ligand are bound to the metal centre and not the sulfur atom. ORTEP drawings with the atom labelling scheme for complexes **4** and **5** are shown in Fig. 1, while complex **7** is presented in Fig. 2. Selected bond lengths and angles for **4**, **5** and **7** are listed in Table 1.

Table 1
Selected bond lengths and angles for [**4**]PF_6, [**5**]PF_6 and [**7**]$PF_6{\cdot}CH_2Cl_2$.

	[**4**]PF_6	[**5**]PF_6	[**7**]PF_6
Interatomic distances			
M–N1	2.125(5)	2.070(10)	2.108(4)
M–N2	2.125(5)	2.070(10)	2.108(4)
M–Cl	2.382(2)	2.385(4)	
Ru–Sn			2.5952(9)
M-centroid	1.773	1.784	1.730
Angles			
N1–M–N2	77.1(3)	78.1(7)	
N1–M–Cl	87.49(15)	85.5(3)	
N2–M–Cl	87.49(15)	85.5(3)	
N1–Ru–N2			75.8(2)
N1–Ru–Sn			84.98(11)
N2–Ru–Sn			84.98(11)

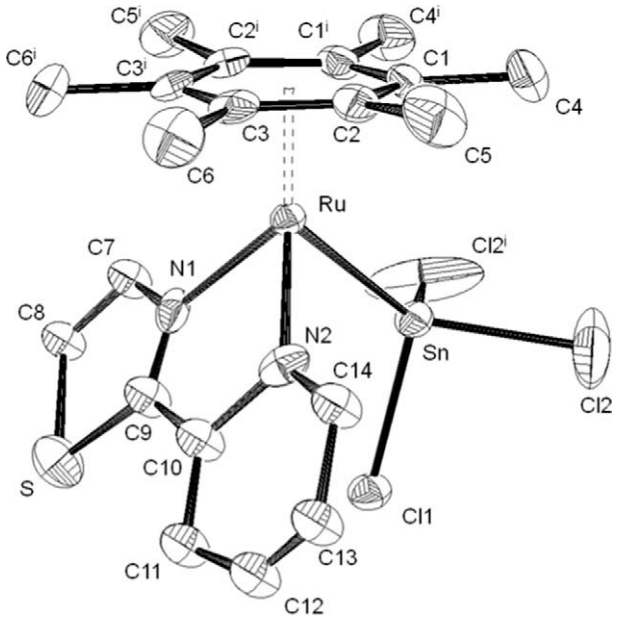


Fig. 2. ORTEP diagram of cation **7** with 50% probability thermal ellipsoids. Hydrogen atoms, dichloromethane solvate and the hexafluorophosphate anion are omitted for clarity. Symmetry code: (i) $x, 1/2 - y, z$.

The distance between the metal ion and the centroid of the η^5-C_5Me_5 ring is shorter in **4** (1.773 Å) than in **5** (1.784 Å), but are comparable to those in related η^5-C_5Me_5 rhodium and iridium complexes [5d]. The M–Cl bond lengths are 2.382(2) Å (in **4**) and 2.385(4) Å (in **5**), which are almost identical to the reported cationic polypyridyl rhodium complex [(η^5-C_5Me_5)RhCl(4′-phenyl-2,2′:6′,2″-terpyridine)]$^+$ [2.3984(1) Å] [16]. In **7** the Ru–Sn bond length is comparable to those found in other mononuclear arene ruthenium trichlorostannyl complexes [15].

In the presence of dichloromethane, compound [**7**]PF_6 crystallises with one molecule of dichloromethane per asymmetric unit giving rise to multiple intermolecular interactions between the different components of the cell. These intermolecular interactions are dominated by C–H···F and C–H···Cl contacts. The C···F distances range from 2.970 to 3.154 Å with angles ranging from 139.3° to 178.8°, and the C···Cl distances range from 3.568 to 3.868 Å with angles ranging from 131.7° to 175.9°.

2.3. Biological activity of compounds [1–7]Cl

The antiproliferative properties of **1**–**7** were established by monitoring their ability to inhibit cell growth using the MTT assay

Table 2
IC_{50}[a] values of [**1**–**7**]Cl in the A2780 and A2780cisR cell lines.

Compound	IC_{50} (μM) A2780	IC_{50} (μM) A2780cisR
Cisplatin	1.6	8.6
[**1**]Cl	>300	>300
[**2**]Cl	258.3 ± 2.0	214.4 ± 3.3
[**3**]Cl	182.4 ± 2.6	178.2 ± 2.3
[**4**]Cl	>300	>300
[**5**]Cl	>300	>300
[**6**]Cl	46.1 ± 0.6	117.8 ± 1.7
[**7**]Cl	161.9 ± 2.3	124.9 ± 4.3

[a] IC_{50} is the drug concentration necessary for 50% inhibition of cell viability.

(see Section 3). Cytotoxic activity was determined on the human ovarian cancer (A2780) cell line, and its cisplatin-resistant variant (A2780cisR), after 72 h exposure to the compounds (see Table 2). All the compounds are less cytotoxic than cisplatin (used as a control) in both cell lines.

The cytotoxicity of the compounds is low, in the same order of magnitude as bifunctional ruthenium compounds, that despite being only weakly active *in vitro* display good antimetastatic activity on *in vivo* models [4a–c]. Indeed, NAMI-A, a ruthenium(III) coordination complex inactive *in vitro*, but exhibiting selective antimetastatic activity *in vivo*, is now in phase II clinical trials [17]. However, compared to other organometallic compounds with *N,N*-chelating ligands [10,11], **1**–**7** are significantly less cytotoxic. Indeed, the most active compounds are those containing the trichlorostannyl fragment which is not unexpected since tin compounds are highly cytotoxic [18].

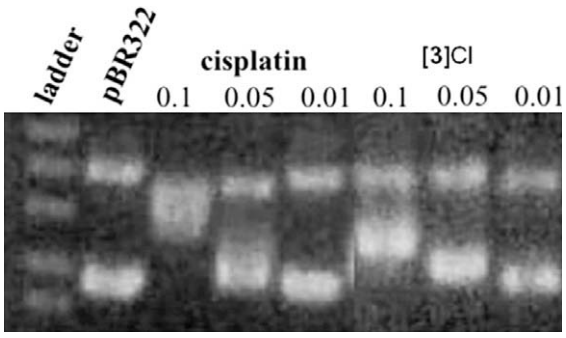


Fig. 3. Gel showing pBR322 plasmid DNA unmodified or treated with different concentrations of cisplatin or [**3**]Cl (metal complex: DNA base pairs ratio = 0.1, 0.05, 0.01).

It is tempting to attribute their low cytotoxicity to reduced DNA interactions due to the absence of either an intercalating group or good hydrogen bond donors on the *N,N*-chelating ligand in **1**–**7**. To test this hypothesis **1**–**7** were incubated with plasmid DNA pBR322 for 12 h at 37 °C at different metal: base pair ratios (r = 0.1, 0.05 and 0.01) and the resulting mixtures separated by gel electrophoresis. A representative gel of pBR322 plasmid treated with **3** in comparison to cisplatin is shown in Fig. 3. Notably, the migration and the quantitative distribution of the two most abundant forms of plasmid DNA (super-coiled and nicked) are affected by the metal complex similarly to cisplatin, the effects being evident at the highest metal: base pairs ratios. Similar effects have also been observed for other related compounds [19] and presumably interact with the DNA via coordination. Since **1**–**7** damage DNA under these conditions to a similar degree as cisplatin it seems more likely that *in vitro* these compounds do not enter the cell nucleus or mitochondria, or are easily detached from the DNA, and therefore do not interfere with DNA function.

Mass spectrometric approaches have proven potential for the analysis of metal complex: protein binding [20]. The compounds were therefore investigated for their ability to bind a model protein, ubiquitin (Ub), by electrospray ionisation mass spectrometry (ESI-MS) using established procedures [21]. A comparative analysis of the intensity of the peaks of the free protein with those of the adducts provides a qualitative indication of the degree of metallation. In addition, analysis of the peaks allows identification of the stoichiometry of the adducts and of the nature of protein bound metallic fragments. Each metal complex was reacted with Ub at a 3:1 metal:protein molar ratio over 24 h at 37 °C. The only compounds that were observed to form adducts with Ub under the given conditions are **1**, **3** and **4**. A representative mass spectrum for **3** (focusing on the +11 and +10 ions) is shown in Fig. 4. The spectrum contains peaks at 779 (+11 ions) and 857 m/z (+10 ions) characteristic of Ub and additional peaks that are attributed to protein adducts derived from **3**. Notably, the main adduct formed by **3** [peaks at 795 (+11 ions) and 875 m/z (+10 ions)] corresponds to protein species containing a $[(\eta^6\text{-}C_6Me_6)Ru]^{2+}$ fragment. Addi-

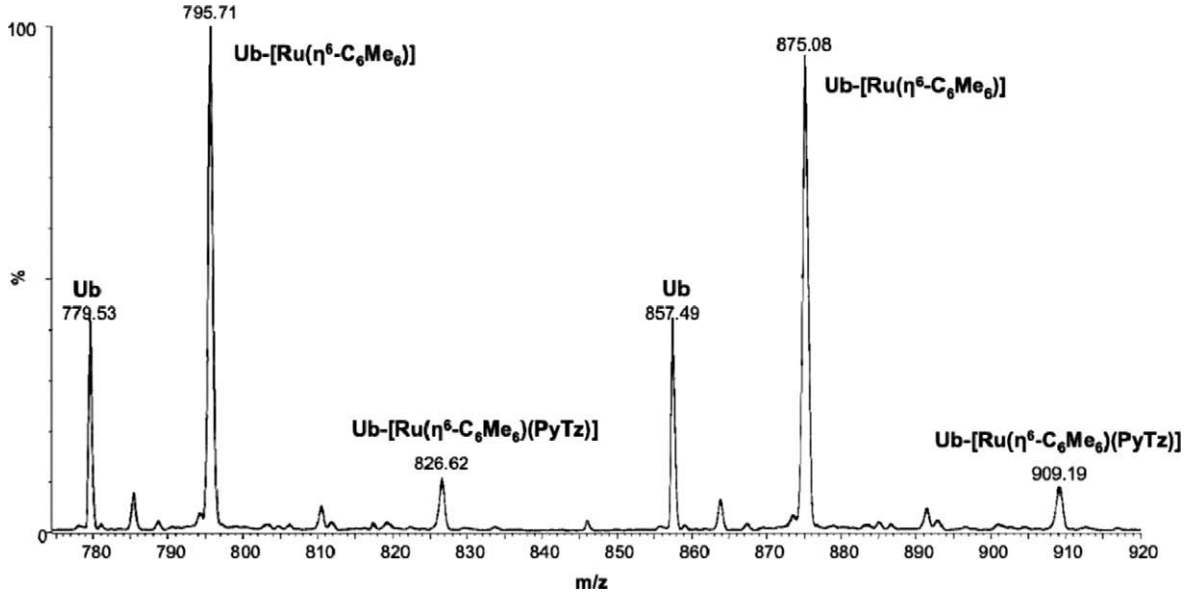


Fig. 4. ESI mass spectrum (+11 and +10 ions) of Ub treated with [**3**]Cl (3:1, metal:protein ratio) in water after 24 h incubation at 37 °C.

tional less intense signals [peaks at 826 (+11 ions) and 909 *m*/*z* (+10 ions)] demonstrate the presence of [(η^6-C_6Me_6)Ru(pyTz)]$^+$-Ub bound metallo-fragments, which presumably form first prior to loss of the *N,N*-chelating ligand.

In conclusion, a series of organometallic ruthenium, rhodium and iridium complexes containing 2-(pyridine-2-yl)thiazole as a co-ligand were prepared and characterised. It was possible to prepare trichlorostannyl adducts of the ruthenium species. All the compounds were either not cytotoxic or, in the case of the trichlorostannyl derivatives, displayed modest cytotoxicity towards human ovarian cancer cells. Since all the compounds interact extensively with DNA, presumably via coordination, and yet display only modest cytotoxicity, it seems reasonable to conclude that they do not reach the DNA target *in vitro* or, if they do reach the DNA, they must be easily removed by various DNA repair mechanisms [22]. Protein targets have also been implicated in the biological role of ruthenium–arene compounds [23] and it would appear that at least some of the compounds reported herein can bind efficiently to proteins. Such protein interactions presumably play a role in the mechanism of action of these compounds, in terms of both cytotoxicity and detoxification pathways.

3. Experimental

3.1. General

All reagents were purchased from either Aldrich or Fluka and used as received. [(η^6-arene)$_2$Ru$_2$(μ-Cl)$_2$Cl$_2$] [24] and [(η^5-C_5Me_5)$_2$M$_2$(μ-Cl)$_2$Cl$_2$] (M = Rh, Ir) [25] were prepared according to the literature methods. All manipulations were carried out in air. The NMR spectra were recorded on a Bruker AvanceII 400 spectrometer using the residual protonated solvent as internal standard. Elemental analyses were performed by the Laboratory of Pharmaceutical Chemistry, University of Geneva (Switzerland) or by Mikroelementaranalytisches Laboratorium, ETH Zürich (Switzerland). Electrospray mass spectra were obtained in positive-ion mode with an LCQ Finnigan mass spectrometer or recorded at the Department of Chemistry of the University of Fribourg (Switzerland) by Prof. Titus Jenny.

3.2. Preparation of [**1**]Cl–[**3**]Cl

To a solution of the dinuclear dichloro complex [(η^6-arene)$_2$Ru$_2$(μ-Cl)$_2$Cl$_2$] (0.20 mmol, 100 mg for **1**, 122 mg for **2**, 133 mg for **3**) in CH_2Cl_2 (30 mL), 65 mg (0.40 mmol) of solid 2-(pyridin-2-yl)thiazole were added. The resulting mixture was stirred at room temperature for 16 h. Then the solution was filtered through Celite in order to remove solid particles, and the solvent was removed under reduced pressure. The residue was dissolved in methanol (5 mL), and the product was precipitated by addition of diethylether (50 mL), isolated by filtration and dried *in vacuo*.

3.2.1. Spectroscopic data for [(η^6-C_6H_6)Ru(pyTz)Cl]Cl ([**1**]Cl)

Dark-orange solid, yield 75 mg (91%). ^{1}H NMR (400 MHz, CD_3OD-d_4): 6.18 (s, 6H, C_6H_6), 7.74 (t, $^3J_{H-H}$ = 8 Hz, 1H, C_5H_4N), 8.23 (m, 2H, C_5H_4N), 8.30 (d, 1H, $^3J_{H-H}$ = 8 Hz, Tz-*H*), 8.76 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 9.56 (d, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD-d_4): 87.66, 125.47, 126.66, 128.64, 129.35, 141.68, 147.24, 152.15, 157.17. Mass (ESI, *m*/*z*): 377.1 [M−Cl]$^+$. Anal. Calc. for $C_{14}H_{12}Cl_2N_2RuS$: C, 40.78; H, 2.93; N, 6.79. Found: C, 40.98; H, 3.02; N, 6.77 (%).

3.2.2. Spectroscopic data for [(η^6-*p*-^{i}PrC_6H_4Me)Ru(pyTz)Cl]Cl ([**2**]Cl)

Orange solid, yield 83 mg (88%). ^{1}H NMR (400 MHz, CD_3OD-d_4): 1.09 (d, $^3J_{H-H}$ = 8 Hz, 6H, $(CH_3)_2CH$), 2.25 (s, 3H, CH_3), 2.69 (sept, $^3J_{H-H}$ = 8 Hz, 1H, $(CH_3)_2CH$), 5.94 (d, $^3J_{H-H}$ = 8 Hz, 2H, *H*-Ar), 6.18 (d, $^3J_{H-H}$ = 8 Hz, 2H, *H*-Ar), 7.76 (t, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N), 8.26 (m, 2H, C_5H_4N), 8.34 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 8.74 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 9.51 (d, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD-d_4): 19.06, 22.32, 22.47, 32.47, 84.35, 85.15, 86.77, 86.90, 125.53, 127.19, 128.92, 141.64, 147.33, 157.16. Mass (ESI, *m*/*z*): 433.1 [M−Cl]$^+$. Anal. Calc. for $C_{18}H_{20}Cl_2N_2RuS$: C, 46.15; H, 4.30; N, 5.98. Found: C, 46.17; H, 4.18; N, 6.83 (%).

3.2.3. Spectroscopic data for [(η^6-C_6Me_6)Ru(pyTz)Cl]Cl ([**3**]Cl)

Red solid, yield 85 mg (89%). ^{1}H NMR (400 MHz, CD_3OD-d_4): 2.20 (s, 18H, CH_3), 7.78 (t, $^3J_{H-H}$ = 8 Hz, 1H, C_5H_4N), 8.21 (m, 3H, C_5H_4N, Tz-*H*), 8.32 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 8.90 (d, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD-d_4): 16.06, 96.70, 125.08, 127.05, 128.81, 141.24, 144.87, 152.11, 154.77, 166.55. Mass (ESI, *m*/*z*): 461.1 [M−Cl]$^+$. Anal. Calc. for $C_{20}H_{24}Cl_2N_2RuS$: C, 48.39; H, 4.87; N, 5.64. Found: C, 48.50; H, 4.99; N, 5.75 (%).

3.3. Preparation of [**4**]Cl and [**5**]Cl

To a solution of dinuclear dichloro complex [(η^5-C_5Me_5)$_2$M$_2$(μ-Cl)$_2$Cl$_2$] (0.12 mmol, 74 mg for **4,** 100 mg for **5**) in CH_2Cl_2 (30 mL), 41 mg (0.25 mmol) of solid 2-(pyridin-2-yl)thiazole were added. The resulting mixture was stirred at room temperature for 16 h. Then, the solution was filtered through Celite in order to remove solids particles, and the solvent was removed under reduced pressure. The residue was dissolved in methanol (5 mL), and the product was precipitated by addition of diethylether (50 mL), isolated by filtration and dried *in vacuo*.

3.3.1. Spectroscopic data for [(η^5-C_5Me_5)Rh(pyTz)Cl]Cl ([**4**]Cl)

Orange solid, yield 49 mg (85%). ^{1}H NMR (400 MHz, CD_3OD-d_4): 1.79 (s, 15H, CH_3), 7.86 (t, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N), 8.29 (m, 3H, C_5H_4N, Tz-*H*), 8.39 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 9.01 (d, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD-d_4): 9.16, 98.68, 125.35, 127.51, 129.55, 141.99, 143.63, 151.42, 153.38, 167.61. Mass (ESI, *m*/*z*): 435.1 [M−Cl]$^+$. Anal. Calc. for $C_{18}H_{22}Cl_2N_2RhS$: C, 45.78; H, 4.70; N, 5.93. Found: C, 45.74; H, 4.75; N, 6.12 (%).

3.3.2. Spectroscopic data for [(η^5-C_5Me_5)Ir(pyTz)Cl]Cl ([**5**]Cl)

Yellow solid, yield 59 mg (88%). ^{1}H NMR (400 MHz, CD_3OD-d_4): 1.78 (s, 15H, CH_3), 7.83 (t, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N), 8.28 (m, 2H, C_5H_4N), 8.31 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 8.46 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 9.00 (d, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD-d_4): 8.85, 90.73, 125.48, 128.21, 129.98, 141.98, 143.24, 152.36, 153.31, 169.41. Mass (ESI, *m*/*z*): 525.1 [M−Cl]$^+$. Anal. Calc. for $C_{18}H_{22}Cl_2IrN_2S$: C, 38.50; H, 3.95; N, 4.99. Found: C, 38.34; H, 4.02; N, 4.76 (%).

3.4. Preparation of [**6**]Cl and [**7**]Cl

To a solution of the mononuclear complexes [(η^6-arene)$_2$Ru$_2$(-pyTz)Cl]Cl (0.20 mmol, 94 mg of [**2**]Cl**,** 99 mg of [**3**]Cl) in THF (30 mL), 53 mg (0.28 mmol) of solid $SnCl_2$ were added. The resulting mixture was stirred at room temperature for 16 h. Then, the solution was filtered through Celite in order to remove solids particles, and the solvent was removed under reduced pressure. The residue was dissolved in methanol (5 mL), and the product was precipitated by addition of diethylether (50 mL), before being isolated by filtration and dried *in vacuo*.

3.3.3. Spectroscopic data for [(η^6-*p*-^{i}PrC_6H_4Me)Ru(pyTz)($SnCl_3$)]Cl ([**6**]Cl)

Orange solid, yield 94 mg (72%). ^{1}H NMR (400 MHz, DMSO-d_6): 0.98 (d,$^3J_{H-H}$ = 8 Hz, 6H, $(CH_3)_2CH$), 2.16 (s, 3H, CH_3), 2.63 (sept, $^3J_{H-H}$ = 8 Hz, 1H, $(CH_3)_2CH$), 6.03 (d, $^3J_{H-H}$ = 8 Hz, 2H, *H*-Ar), 6.26 (d, $^3J_{H-H}$

= 8 Hz, 2H, *H*-Ar), 7.78 (t, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N), 8.25 (t, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N), 8.43 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 8.83 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 954 (d, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6): 18.26, 21.60, 30.48, 82.75, 83.54, 85.16, 124.37, 126.66, 127.43, 140.43, 146.10, 149.94, 155.87, 164.81. ^{119}Sn NMR (149 MHz, DMSO-d_6): −364.47. Mass (ESI, *m*/*z*): 433.4 $[M-SnCl_3]^+$. Anal. Calc. for $C_{18}H_{20}Cl_4N_2RuSSn$: C, 32.85; H, 3.06; N, 4.26. Found: C, 32.91; H, 3.10; N, 4.37 (%).

3.3.4. Spectroscopic data for [(η^6-C_6Me_6)Ru(pyTz)($SnCl_3$)]Cl ([**7**]Cl)

Orange solid, yield 99 mg (71%). ^1H NMR (400 MHz, DMSO-d_6): 2.11 (s, 18H, CH_3), 7.79 (t, $^3J_{H-H}$ = 8 Hz, 1H, C_5H_4N), 8.22 (t, $^3J_{H-H}$ = 8 Hz, 1H, C_5H_4N), 8.24 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 8.42 (d, 1H, $^3J_{H-H}$ = 4 Hz, Tz-*H*), 8.96 (d, $^3J_{H-H}$ = 4 Hz, 1H, C_5H_4N). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6): 15.75, 95.09, 124.27, 126.99, 127.91, 140.18, 143.86, 150.37, 153.72, 164.82. ^{119}Sn NMR (149 MHz, DMSO-d_6): −265.26. Mass (ESI, *m*/*z*): 461.4 $[M-SnCl_3]^+$. Anal. Calc. for $C_{20}H_{24}Cl_4N_2RuSSn$: C, 35.01; H, 3.53; N, 4.08. Found: C, 35.15; H, 3.46; N, 4.22 (%).

3.5. Biological studies

3.5.1. Cell culture and inhibition of cell growth

Human A2780 and A2780cisR ovarian carcinoma cell lines were obtained from the European Centre of Cell Cultures (ECACC, Salisbury, UK) and maintained in culture as described by the provider. The cells were routinely grown in RPMI 1640 medium containing 10% foetal calf serum (FCS) and antibiotics at 37 °C and 6% CO_2. For evaluation of growth inhibition tests, the cells were seeded in 96-well plates (Costar, Integra Biosciences, Cambridge, MA) and grown for 24 h in complete medium. The stock solutions of metal complexes were prepared by dissolving the compounds in 1 mL of DMSO to reach a concentration of 10^{-2} M. They were then diluted in RPMI medium and added to the wells (100 μL) to obtain a final concentration ranging between 0 and 80 μM. DMSO at comparable concentrations did not show any effects on cell cytotoxicity. Complexes stock solutions were diluted directly in culture medium to the required concentration and added to the cell culture. After 72 h incubation at 37 °C, 20 μL of a solution of MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) in PBS (2 mg mL^{-1}) was added to each well, and the plates were then incubated for 2 h at 37 °C. The medium was then aspirated and DMSO (100 μL) was added to dissolve the precipitate. The absorbance of each well was measured at 580 nm using a 96-well multiwell-plate reader (iEMS Reader MF, Labsystems, Bioconcept, Switzerland) and compared to the values of control cells incubated without complexes. The IC_{50} values for the inhibition of cell growth were determined by fitting the plot of the percentage of surviving cells against the drug concentration using a sigmoidal function (Origin v7.5).

3.5.2. DNA electrophoresis

Samples with pBR322 plasmid DNA were prepared by adding the required volume of a freshly prepared solution of metal complexes in MilliQ water. The concentration of plasmid in the reaction mixture was 75 ng/L and the concentration of the complexes was varied to give different metal-to-base pair ratios (0.1, 0.05 and 0.01). The mobility of the metal complex-treated pBR322 samples was analysed by gel electrophoresis on a 0.8% (w/v) agarose gel (Boehringer-Mannheim, Mannheim, Germany) at 90 V/cm at 25 °C in Tris–acetate/EDTA buffer. The gel was stained for 20 min in 0.5 g/mL (w/v) ethidium bromide and the bands were analysed with a UVP gel scanner.

3.5.3. ESI-MS with ubiquitin

Samples were prepared by mixing ubiquitin 100 μM (Sigma, U6253) with an excess of the appropriate metal complex (3:1, metal:protein ratio) in MilliQ water (pH 6) and incubated over 24 h at 37 °C. Prior to analysis samples were extensively ultrafiltered using a Centricon YM-3 filter (Amicon Bioseparations, Millipore Corporation) in order to remove the unbound complex. ESI-MS data were acquired on a Q-Tof Ultima mass spectrometer (Waters) fitted with a standard Z-spray ion source and operated in the positive ionisation mode. Experimental parameters were set as follows: capillary voltage 3.5 kV, source temperature 80 °C, desolvation temperature 120 °C, sample cone voltage 100 V, desolvation gas flow 400 L/h, acquisition window 300–2000 *m*/*z* in 1 s. The samples were diluted 1:10 in water and 5 μL was introduced into the mass spectrometer by infusion at a flow rate of 20 μL/min with a solution of ACN/H_2O/HCOOH 50:49.8:0.2

Table 3
Crystallographic and structure refinement parameters for complexes [**4**]PF_6, [**5**]PF_6 and [**7**]$PF_6{\cdot}CH_2Cl_2$.

	[**4**]PF_6	[**5**]PF_6	[**7**]$PF_6{\cdot}CH_2Cl_2$
Chemical formula	$C_{18}H_{21}ClF_6N_2PRhS$	$C_{18}H_{21}ClF_6N_2PIrS$	$C_{21}H_{26}Cl_5F_6N_2PRuSSn$
Formula weight	580.76	670.05	880.48
Crystal system	Orthorhombic	Orthorhombic	Orthorhombic
Space group	$Pmn2_1$ (no. 31)	$Pmn2_1$ (no. 31)	*Pnma* (no. 62)
Crystal colour and shape	Orange block	Red block	Orange rod
Crystal size	0.24 × 0.18 × 0.16	0.19 × 0.18 × 0.14	0.23 × 0.16 × 0.14
a (Å)	12.763(3)	12.739(3)	23.176(5)
b (Å)	8.123(2)	8.179(2)	13.078(3)
c (Å)	10.531(2)	10.486(2)	10.099(2)
V (Å^3)	1091.8(4)	1092.6(4)	3061.0(11)
Z	2	2	4
T (K)	173(2)	173(2)	173(2)
D_{calc} (g cm^{-3})	1.767	2.037	1.911
μ (mm^{-1})	1.132	6.460	1.919
Scan range (°)	2.51 < θ < 26.16	2.52 < θ < 26.14	2.35 < θ < 25.61
Unique reflections	2237	2129	2828
Reflections used [$I > 2\sigma(I)$]	1944	1981	1894
Flack parameter	−0.02(7)	0.02(3)	
R_{int}	0.0430	0.0486	0.0417
Final *R* indices [$I > 2\sigma(I)$]*	0.0406, wR_2 0.1052	0.0506, wR_2 0.1334	0.0350, wR_2 0.0696
R indices (all data)	0.0477, wR_2 0.1092	0.0542, wR_2 0.1408	0.0614, wR_2 0.0734
Goodness-of-fit	1.059	1.128	0.860
Max, min $\Delta\rho$ (e Å^{-3})	0.768, −0.590	1.439, −4.903	0.923, −0.697

* Structures were refined on F_o^2: $wR_2 = [\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma w(F_o^2)^2]^{1/2}$, where $w^{-1} = [\Sigma(F_o^2) + (aP)^2 + bP]$ and $P = [\max(F_o^2, 0) + 2F_c^2]/3$.

(v:v:v). External calibration was carried out with a solution of phosphoric acid at 0.01%. Data were processed using the MassLynx 4.1 software.

3.6. Single-crystal X-ray structure analyses

Crystals of compounds [**4**]PF_6, [**5**]PF_6 and [**7**]$PF_6{\cdot}CH_2Cl_2$, prepared by diffusion of a dichloromethane solution of [**4**]Cl, [**5**]Cl and [**7**]Cl in the presence of KPF_6 into a diethylether layer, were mounted on a Stoe Image Plate Diffraction system equipped with a ϕ circle goniometer, using Mo Kα graphite monochromated radiation ($\lambda = 0.71073$ Å) with ϕ range 0–200°. The structures were solved by direct methods using the program SHELXS-97, while the refinement and all further calculations were carried out using SHELXL-97 [26]. The H-atoms were found on Fourier difference map or included in calculated positions and treated as riding atoms using the SHELXL default parameters. The non-H-atoms were refined anisotropically, using weighted full-matrix least-square on F^2. Crystallographic details are summarised in Table 3. Figs. 1 and 2 were drawn with ORTEP [27].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2010.01.020.

CCDC 755206 [**4**]PF_6, 755207 [**5**]PF_6 and 755208 [**7**]$PF_6{\cdot}CH_2Cl_2$ contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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