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Water-soluble arene ruthenium complexes containing pyridinethiolato ligands: Synthesis, molecular structure, redox properties and anticancer activity of the cations $[(\eta^6\text{-arene})Ru(p\text{-}SC_5H_4NH)_3]^{2+}$

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ABSTRACT

The cationic complexes $[(\eta^6\text{-arene})Ru(SC_5H_4NH)_3]^{2+}$, arene being C_6H_6 (**1**), MeC_6H_5 (**2**), $p\text{-}^iPrC_6H_4Me$ (**3**) or C_6Me_6 (**4**), have been synthesised from the reaction of 4-pyridinethiol with the corresponding precursor $(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ and isolated as the chloride salts. The single-crystal X-ray structure of [**4**]$(PF_6)_2$ reveals three 4-pyridinethiol moieties coordinated to the ruthenium centre through the sulphur atom, with the hydrogen atom transferred from the sulphur to the nitrogen atom. The electrochemical study of **1**–**4** shows a clear correlation between the Ru(II)/Ru(III) redox potentials and the number of alkyl substituents at the arene ligand ($E^{o\prime}$ ($Ru^{II/III}$): **1** > **2** > **3** > **4**), whereas the cytotoxicity towards A2780 ovarian cancer cells follows the series **4** > **1** > **3** > **2**, the hexamethylbenzene derivative **4** being the most cytotoxic one. The corresponding reaction of the *ortho*-isomer, 2-pyridinethiol, with $(\eta^6\text{-}C_6Me_6)_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ does not lead to the expected 2-pyridinethiolato analogue, but yields the neutral complex $(\eta^6\text{-}C_6Me_6)Ru(\eta^2\text{-}SC_5H_4N)(\eta^1\text{-}SC_5H_4N)$ (**5**). The analogous complex $(\eta^6\text{-}C_6Me_6)Ru(\eta^2\text{-}SC_9H_6N)(\eta^1\text{-}SC_9H_6N)$ (**6**) is obtained from the similar reaction with 2-quinolinethiol.

1. Introduction

Cationic arene ruthenium complexes that are air-stable and water-soluble are finding increasing interest in many areas [1] including homogeneous catalysts [2], polymeric materials [3], nanocages [4] and nanoparticle precursors [5]. Notably, arene ruthenium compounds are also being explored for their medicinal properties as anticancer agents [6]. Arene ruthenium complexes of the type $(\eta^6\text{-arene})RuCl_2$(imidazole) [7] $(\eta^6\text{-arene})RuCl_2$(PTA) (PTA = 1,3,5-triaza-7-phosphaadamantane) [8], $[(\eta^6\text{-arene})RuCl(en)]^+$ (en = ethylenediamine) [9], $(\eta^6\text{-arene})RuCl_2$(DMSO) [10], have been studied for their antitumour activity *in vitro* and in some cases *in vivo*. The ethylenediamine series of complexes have been evaluated for activity both *in vitro* and *in vivo* against human ovarian cancer, which show high activity coupled to non cross-resistance in cisplatin resistant models [11]. The PTA series of compounds were found to effectively reduce the growth of lung metastases in CBA mice bearing the MCa mammary carcinoma [12]. Other anticancer compounds based on the arene ruthenium unit include those with modified paullone ligands [13] and ferrocene moieties [14] and bridging systems [15]. The arene ruthenium unit has even been attached via a pH cleavable linker to human serum albumin as a drug delivery vector [16].

Recently, we reported the synthesis of tris–thiolato-bridged complexes of the type $[(\eta^6\text{-arene})_2Ru_2(\mu\text{-}SR)_3]^+$ (arene = C_6H_6, $p\text{-}^iPrC_6H_4Me$, C_6Me_6; R = $p\text{-}C_6H_4Me$, CH_2CH_2OH, $p\text{-}C_6H_4OH$), accessible from the reaction of $(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ with the corresponding thiol [17] (Scheme 1). In contrast, the mono and dithiolato-bridged complexes $[(\eta^6\text{-arene})_2Ru_2(\mu\text{-}H)_2(\mu\text{-}SR)]^+$ and $[(\eta^6\text{-arene})_2Ru_2(\mu\text{-}H)(\mu\text{-}SR)_2]^+$ (arene = $p\text{-}C_6H_2Me_4$, C_6Me_6; R = $p\text{-}C_6H_4Me$, $p\text{-}C_6H_4Br$) may be obtained from $[(\eta^6\text{-arene})_2Ru_2(\mu\text{-}H)_3]^+$ by reaction with the corresponding thiophenol [18].

Using a similar approach, $(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ was reacted with 4-pyridinethiol (4-mercaptopyridine), in order to synthesise tris–thiolato-bridged complexes analogous to those depicted in Scheme 1. Surprisingly, the reaction proceeds in a different way and gives rise to the formation of mononuclear cations, involving the rearrangement of three pyridinethiol molecules to pyridinium–thiolato ligands. In addition, the isomeric 2-pyridinethiol (2-mercaptopyridine) reacts with $(\eta^6\text{-}C_6Me_6)_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ in a different manner, leading to neutral mononuclear thiolato complexes. Furthermore, since pyridinium–thiolato ligands combined with platinum demonstrate excellent anticancer activity [19], we decided to explore the *in vitro* cytotoxicity of these compounds and to establish whether their activity correlates with their biologically accessible redox potential.

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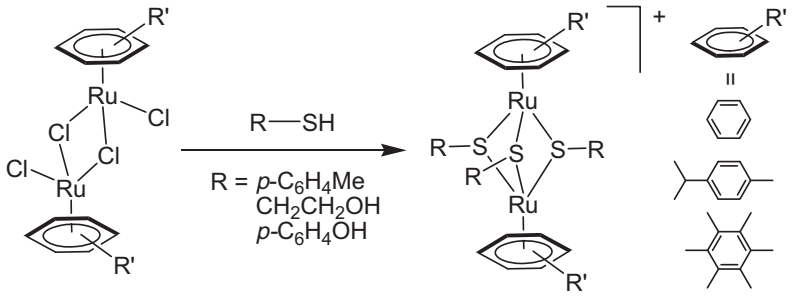


Scheme 1.

2. Results and discussion

2.1. Syntheses and characterisation of *1–4*

The dinuclear arene ruthenium complexes $(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ (arene = C_6H_6, C_6H_5Me, $p\text{-}^iPrC_6H_4Me$, C_6Me_6) react with 4-pyridinethiol (4-mercaptopyridine) in refluxing ethanol to give the mononuclear ruthenium complexes $[(\eta^6\text{-arene})\text{-}Ru(SC_5H_4NH)_3]^{2+}$ (**1**–**4**), the yields being higher if technical grade ethanol (5.6% water) is used. The reaction formally involves the transfer of the hydrogen atom in 4-pyridinethiol from sulphur to nitrogen to form a 4-pyridinium–thiolato ligand (Scheme 2).

Complexes **1**–**4** are isolated as the chloride salts which are yellow to red air-stable crystalline solids. They are soluble in water, methanol, ethanol, dimethylsulfoxide and acetonitrile, but not soluble in dichloromethane, chloroform, tetrahydrofuran or hydrocarbon solvents. The ^1H NMR spectra of **1**–**4** in DMSO-d_6 show a broad singlet for the pyridinium protons at $\delta = 14.20$ ppm for **1**, 14.05 ppm for **2**, 13.76 ppm for **3** and 13.99 ppm for **4**, which integrates to three N–H hydrogen atoms with respect to the other C–H signals.

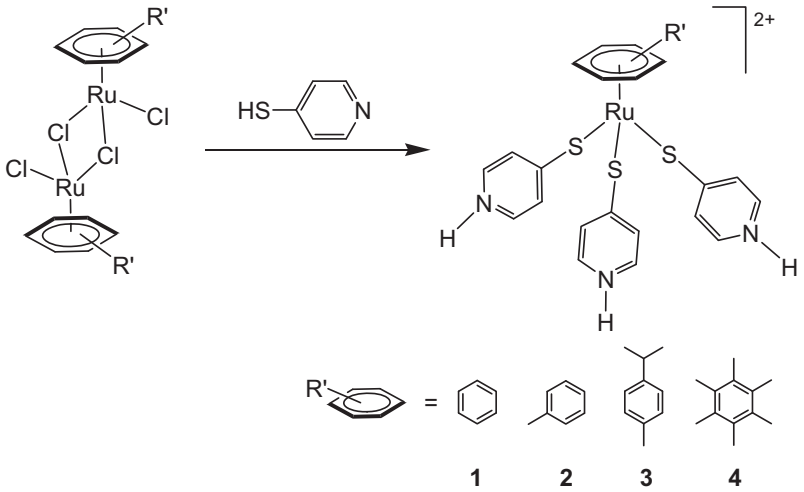
In order to obtain X-ray quality crystals, the hexafluorophosphate salt of **4** was prepared by anion exchange of [**4**]Cl_2 with KPF_6 in wet methanol. The compound [**4**]$(PF_6)_2$ crystallises from this water–methanol solution in the form of orange needles. The molecular structure of cation **4** shows the ruthenium atom to adopt a piano-stool geometry with the metal centre coordinated by the arene ligand and the three sulphur atoms of the three 4-pyridinium–thiolato ligands. An ORTEP drawing with the atom labelling scheme for complex **4** is shown in Fig. 1 together with selected bond lengths and angles.

The Ru–S bond distances ranging from 2.3785(9) to 2.3923(9) Å in **4** are comparable to those observed in $[(\eta^5\text{-}C_5H_5)Ru(PPh_3)\text{-}(NO)(SC_5H_4NH)]BF_4$ [20] and $[(\eta^5\text{-}C_5H_5)Ru(PPh_3)(CO)(SC_5H_4N)]$ [20]. Interestingly, to minimise steric repulsion between the three 4-pyridinium–thiolato ligands, two S–Ru–S angles are quite obtuse [94.43(3)° and 97.14(3)°] while the remaining S–Ru–S angle is extremely acute [80.69(3)°]. Moreover, two pyridinium rings point away from each other, while the third pyridinium aromatic ring is directed under the $(\eta^6\text{-}C_6Me_6)Ru$ moiety. The S–C bond distances ranging from 1.712(4) to 1.719(4) Å in **4,** as well as the lengths of the C–C bonds ranging from 1.353(6) to 1.420(5) Å in the pyridinium ring are in accordance with the ligands being aromatic thiolato ligands rather than thione ligands.

The crystal of [**4**]$(PF_6)_2$ contains four water molecules around the dication and all the pyridinium moieties form a strong hydrogen bond with water molecules (Fig. 2): the N···O distances being N(1)···O(1) 2.798(5), N(2)···O(2) 2.738(5) and N(3)···O(1)^i 2.790(4) Å ($i = x, -1 + y, z$), respectively, with N–H···O angles of N(1)–H···O(1) 163(5)°, N(2)–H···O(2) 164(4)° and N(3)–H···O(1)^i 175(5)°. In addition, one PF_6 anion resides above the η^6-arene ligand with P–F···C distances ranging from 3.107(5) to 3.855(5) Å.

2.2. Electrochemistry of *1–4*

The redox behaviour of complexes **1**–**4** has been studied by cyclic voltammetry in the anodic region at a platinum disc electrode in ca. 0.5 mM aqueous solutions of the complexes containing 0.1 M



Scheme 2.

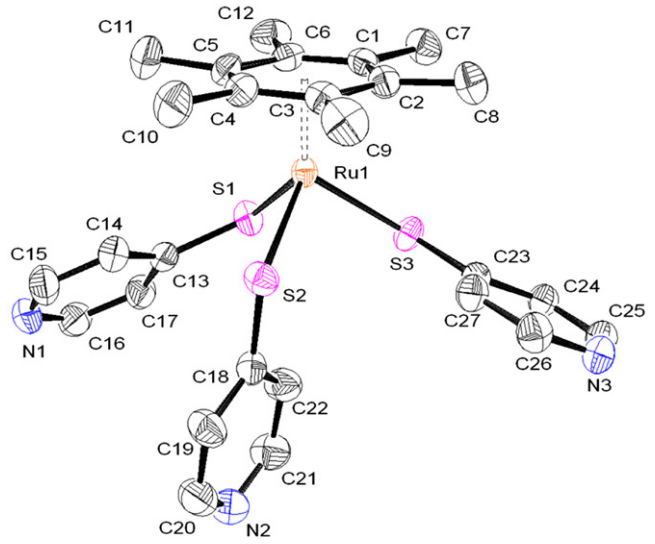


Fig. 1. ORTEP diagram of complex **4** with 50% probability thermal ellipsoids. Hydrogen atoms, water molecules and PF_6 anions are omitted for clarity. Selected bond lengths (Å) and angles (°): Ru(1)–S(1) 2.3923(9), Ru(1)–S(2) 2.3785(9), Ru(1)–S(3) 2.3843(9), S(1)–C(13) 1.712(4), S(2)–C(18) 1.719(4), S(3)–C(23) 1.714(3); S(1)–Ru(1)–S(2) 94.43(3), S(2)–Ru(1)–S(3) 97.14(3), S(1)–Ru(1)–S(3) 80.69(3).

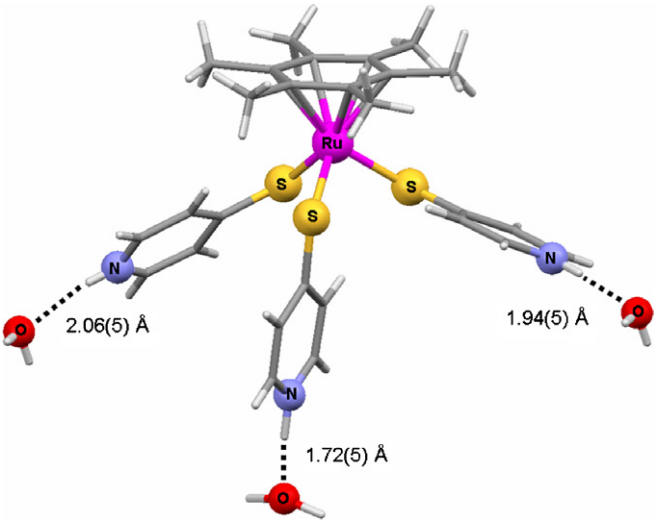


Fig. 2. Pyridinium···OH_2 hydrogen bonds observed in $[\mathbf{4}](PF_6)_2 \cdot 4H_2O$.

$NaClO_4$ as the supporting electrolyte. The pertinent data are summarised in Table 1. Generally, the complexes undergo two consecutive irreversible oxidations (the second wave is hardly detectable in the case of **1**). The redox response is markedly obscured by adsorption phenomena that result in extensive blocking of the electrode surface[1] and also make the determination of the redox potential somewhat less accurate. Nevertheless, an inspection of the data allows the first wave to be assigned to an irreversible $Ru^{II} \rightarrow Ru^{III}$ oxidation as observed in the case of $[(\eta^6\text{-arene})RuCl_2(NC_5H_4OOCC_5H_4FeC_5H_5)]$ complexes, where the alkyl substituents at the arene ligand have the same influence on the Ru^{II}/Ru^{III} redox potentials [14]. In the series, the associated redox potential decreases linearly (by 100 mV over the series) with increasing the number of alkyl the groups attached to the η^6-arene ring, thus reflecting a

[1] No response is observed after the first cycle, indicating full coverage of the electrode surface.

Table 1
Summary of the electrochemical data[a]

Compound	E_{pa} (V)
1	+0.67[b]
2	+0.65, ca. +0.81
3	+0.63, ca. +0.84
4	+0.58, +0.78

[a] Potentials are given relative to saturated calomel electrode. E_{pa} is the anodic peak potential.
[b] The wave due to second oxidation is not observed.

stronger electron-donating character that apparently makes the electron removal easier (Fig. 3).

With values between +0.58 and +0.67 V, the Ru^{II}/Ru^{III} redox potentials for **1**–**4** are significantly lower than those for the more cytotoxic complexes $[(\eta^6\text{-arene})RuCl_2(NC_5H_4OOCC_5H_4FeC_5H_5)]$, the E_{pa} values ranging from +0.91 to +1.00 V [14]. However, as for most arene ruthenium complexes with anti-cancer properties electrochemical data are missing, a clear correlation between E_{pa} values and IC_{50} values cannot be claimed so far.

2.3. Anticancer activity of *1*–*4*

The *in vitro* cytotoxicity of **1**–**4** was evaluated by the biological MTT assay (see Section 3) which measures mitochondrial dehydrogenase activity as an indication of cell viability using the A2780 ovarian cancer cell line. The compounds were incubated at various concentrations (in triplicate) in the A2780 cells and cell viability measured after an incubation period of 72 h. Each experiment was conducted in duplicate and the IC_{50} values listed in Table 2 were calculated as an average over the two experiments.

There is no clear correlation between the IC_{50} values and the E_{pa} data, since the benzene derivative **1** is more cytotoxic than the alkyl-substituted arene derivatives **2**–**4**, the latter ones correlating well with the electrochemical data. For ruthenium compounds the 'activation by reduction' mechanism, beside transferrin mediated uptake, has been proposed to account for the low general toxicity of Ru(III) compounds [21]. Nevertheless, there is convincing evidence to suggest that some metal-based anticancer drugs exert their cytotoxic effect via other redox processes [22]. It should be noted that the observed IC_{50} values are considerably higher than those characteristic of cisplatin and other platinum agents, nevertheless a number of ruthenium-based compounds have been found

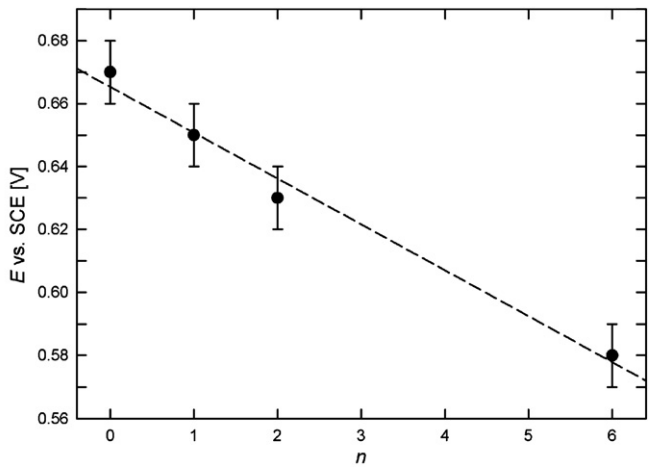


Fig. 3. Correlation between the redox potential of the first oxidation and the number of alkyl groups (n) at the arene ring (the least-squares linear fit is indicated with a dashed line).

Table 2
IC_{50} values of compounds **1**–**4** in the A2780 ovarian cancer cell line

Compound	IC_{50} (μM)
1	148
2	294
3	195
4	74

to show very good *in vivo* activity while displaying only a low *in vitro* cytotoxicity [12,23].

2.4. Syntheses and characterisation of **5** and **6**

For comparison, $(\eta^6\text{-}C_6Me_6)_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ was reacted with 2-pyridinethiol, the structural isomer of 4-pyridinethiol. The reaction does not lead to a cationic pyridinium–thiolato complex, but instead to the neutral complex, $(\eta^6\text{-}C_6Me_6)Ru(\eta^2\text{-}SC_5H_4N)(\eta^1\text{-}SC_5H_4N)$ (**5**) (Scheme 3). Complex **5** is a yellow air-stable powder soluble in dichloromethane, chloroform, acetone, DMSO and acetonitrile and is insoluble in methanol, ethanol and hydrocarbons. The ^{1}H NMR spectrum of **5** in DMSO-d_6 shows a singlet at δ = 2.03 ppm for hexamethylbenzene hydrogen atoms and the expected signals for the two heteroaromatic rings [δ = 6.47 ppm (d), 6.60 ppm (d), 6.61 ppm (d), 7.04 ppm (m), 7.22 ppm (dd), 8.06 ppm (d) and 8.13 ppm (d)]. The ESI mass spectrum of **5** displays a peak at *m*/*z* 374.20 corresponding to the fragment ion $[(\eta^6\text{-}C_6Me_6)Ru(\eta^2\text{-}SC_5H_4N)H]^+$, but does not show the molecular peak. However, the molecular constitution of **5** is confirmed by the correct elemental analysis.

In a similar way, $(\eta^6\text{-}C_6Me_6)_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ reacts with the analogous 2-quinolinethiol in refluxing ethanol to give $(\eta^6\text{-}C_6Me_6)Ru(\eta^2\text{-}SC_9H_6N)(\eta^1\text{-}SC_9H_6N)$ (**6**) in 66% yield (Scheme 3). Complex **6** is a red air-stable solid with solubility properties similar to **5**. The ^{1}H NMR spectrum of **6** in DMSO-d_6 exhibits a singlet at δ = 2.13 ppm for hexamethylbenzene hydrogen atoms together with signals for the two heteroaromatic rings [δ = 6.63 ppm (d), 7.19 ppm (m), 7.30 ppm (m), 7.55 ppm (m), 7.78 ppm (d) and 8.03 ppm (d)]. The ESI mass spectrum of **6** contains a peak at *m*/*z* 424.30 corresponding to the fragment $[(\eta^6\text{-}C_6Me_6)Ru(\eta^2\text{-}SC_9H_6N)H]^+$, in keeping with the spectrum of **5**, with elemental analysis confirming the assignment. Since **5** and **6** are insoluble in water, their cytotoxicities were not assessed.

3. Experimental

3.1. General remarks

All reagents were purchased either from Aldrich or Fluka and used as received. The complexes $(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ were prepared according to the literature methods [24]. NMR spectra were recorded on a Bruker AMX 400 spectrometer using the residual proton resonance of the deuterated solvent as internal standard. Elemental analyses were performed by the Laboratory of Pharmaceutical Chemistry, University of Geneva (Switzerland) or by the Mikroelementaranalytisches Laboratorium, ETH Zürich (Switzerland). Electrospray mass spectra were performed by the Department of Chemistry of the University of Fribourg (Switzerland). Electrochemical measurements were performed with a computer-controlled multipurpose polarograph AUTOLAB III (Eco Chemie, Netherlands) at room temperature using a standard Metrohm three-electrode cell with platinum disc electrode (AUTOLAB RDE; 3 mm diameter) as the working electrode, platinum sheet auxiliary electrode, and saturated calomel electrode as the reference. The analysed compounds were dissolved in water (deionised and subsequently distilled under argon) to give a solution containing 5×10^{-4} M of the analyte and 0.1 M $NaClO_4$ (Merck, p.a.). The solutions were degassed with argon prior to the measurement and then kept under an argon blanket. The redox potentials were reproducible within ca ±10 mV.

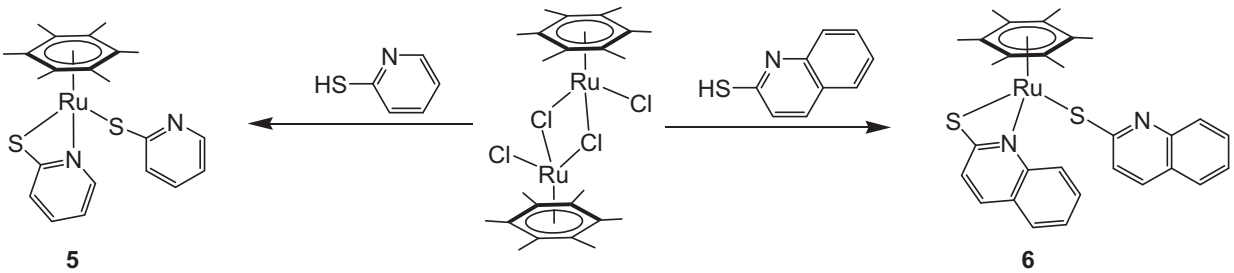
3.2. Synthesis

3.2.1. Synthesis of complexes **1**–**4**

The complex $(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-}Cl)_2Cl_2$ (0.20 mmol, 100 mg for **1**, 105 mg for **2**, 128 mg for **3**, 133 mg for **4**) was refluxed in 50 mL of N_2-saturated technical grade ethanol. When the complex has completely dissolved, a solution of 4-mercaptopyridine (133 mg, 1.20 mmol) in technical ethanol (5 mL) was added drop-wise to the hot solution. The resulting mixture was refluxed for 16 h. After cooling to room temperature, the red solution was filtered through celite in order to remove degradation products, and then the solvent was removed under reduced pressure. The residue was dissolved in methanol (5 mL), and the product was precipitated by addition of diethyl ether (50 mL), isolated by filtration and dried *in vacuo*.

*3.2.1.1. Spectroscopic data for $[(\eta^6\text{-}C_6H_6)Ru(SC_5H_4NH)_3]Cl_2$ (cation **1**).* Dark-yellow solid, yield 200 mg (86%). ^{1}H NMR (400 MHz, DMSO-d_6): 6.13 (s, 6H, C_6H_6), 7.58 (d, ^3J = 7.2 Hz, 6H, *H*–Ar), 7.96 (d, ^3J = 7.2 Hz, 6H, *H*–Ar), 14.20 (s, 3H, N*H*). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6): 89.77 (C_6H_6), 126.57 (*C*Ar), 135.65 (*C*Ar), 179.07 (S–*C*). MS (ESI, *m*/*z*): 401 $[M-SC_5H_4NH]^+$, 290 $[M-2(SC_5H_4NH)]^+$. Anal. Calc. for $C_{21}H_{21}Cl_2N_3RuS_3 \cdot H_2O$: C, 41.93; H, 3.85; N, 6.98. Found: C, 42.26; H, 3.92; N, 6.77%.

*3.2.1.2. Spectroscopic data for $[(\eta^6\text{-}MeC_6H_5)Ru(SC_5H_4NH)_3]Cl_2$ (cation **2**).* Dark-yellow solid, yield 197 mg (81%). ^{1}H NMR (400 MHz, DMSO-d_6): 2.18 (s, 3H, CH_3), 5.97 (d, ^3J = 6 Hz, 2H, *H*–Ar), 6.08 (m, 3H, *H*–Ar), 7.63 (d, ^3J = 6.8 Hz, 6H, *H*–Ar), 7.98 (d, ^3J = 6.8 Hz, 6H, *H*–Ar), 14.05 (s, 3H, N*H*). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6): 18.34 (CH_3), 85.47 (*C*Ar), 87.07 (*C*Ar), 91.77 (*C*Ar), 107.81 (*C*–CH_3), 126.69 (*C*Ar), 135.45 (*C*Ar), 178.91 (S–*C*). MS (ESI, *m*/*z*): 415 $[M-SC_5H_4NH]^+$, 304 $[M-2(SC_5H_4NH)]^+$. Anal. Calc. for



5 **6**

Scheme 3.

$C_{21}H_{21}Cl_2N_3RuS_3 \cdot H_2O$: C, 42.92; H, 4.09; N, 6.83. Found: C, 42.77; H, 4.08; N, 6.71%.

*3.2.1.3. Spectroscopic data for [(η^6-p-^{i}PrC$_6$H$_4$Me)Ru(SC$_5$H$_4$NH)$_3$]Cl$_2$ (cation **3**).* Orange solid, yield 236 mg (92%). ^{1}H NMR (400 MHz, DMSO-d_6): 1.21 (d, ^3J = 6.8 Hz, 6H, (CH_3)$_2$CH), 2.19 (s, 3H, CH_3), 2.77 (sept, ^3J = 7.2 Hz, 1H, (CH$_3$)$_2$C*H*), 5.79 (m, 4H, *H*–Ar), 7.7 (d, ^3J = 7 Hz, 6H, *H*–Ar), 7.88 (d, ^3J = 7 Hz, 6H, *H*–Ar), 13.76 (s, 3H, N*H*). ^{13}C {^{1}H} NMR (100 MHz, DMSO-d_6): 17.38 ((*C*H$_3$)$_2$CH), 22.01 (*C*H$_3$), 30.02 ((CH$_3$)$_2$*C*H), 86.88 (*C*Ar), 89.54 (*C*Ar), 106.21 (*C*Ar), 108.24 (*C*Ar), 126.76 (*C*Ar), 135.22 (*C*Ar), 178.46 (S-*C*). MS (ESI, *m*/*z*): 457 [M−SC$_5$H$_4$NH]$^+$, 346.1 [M−2SC$_5$H$_4$NH]$^+$. Anal. Calc. for $C_{25}H_{29}Cl_2N_3RuS_3 \cdot 2H_2O$: C, 44.44; H, 4.92; N, 6.22. Found: C, 44.63; H, 4.66; N, 6.21%.

*3.2.1.4. Spectroscopic data for [(η^6-C$_6$Me$_6$)Ru(SC$_5$H$_4$NH)$_3$]Cl$_2$ (cation **4**).* Red solid, yield 250 mg (94%). ^{1}H NMR (400 MHz, DMSO-d_6): 1.98 (s, 18H, CH_3), 7.57 (d, ^3J = 8 Hz, 6H, *H*–Ar), 7.85 (d, ^3J = 8 Hz, 6H, *H*–Ar), 13.99 (s, 3H, N*H*). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): 14.81 (*C*H$_3$), 98.28 (*C*Ar), 126.49 (*C*Ar), 134.83 (*C*Ar), 178.1 (S–*C*). MS (ESI, *m*/*z*): 485.1 [M−SC$_5$H$_4$NH]$^+$, 374.1 [M−2SC$_5$H$_4$NH]$^+$. Anal. Calc. for $C_{27}H_{33}Cl_2N_3RuS_3 \cdot H_2O$: C, 47.29; H, 5.14; N, 6.13. Found: C, 47.39; H, 5.34; N, 5.75%.

*3.2.2. Synthesis of complexes **5** and **6***

The complex (η^6-C$_6$Me$_6$)$_2$Ru$_2$(μ_2-Cl)$_2$Cl$_2$ (100 mg, 0.15 mmol) was refluxed in N$_2$-saturated technical grade ethanol (50 mL). When the complex had completely dissolved, a solution of 2-mercaptopyridine (100 mg, 0.9 mmol) or 2-quinolinethiol (145 mg, 0.9 mmol) in technical ethanol (5 mL) was added drop-wise to the hot solution. Then the resulting mixture was refluxed for another 16 h. After cooling to room temperature, the solution was filtered through celite in order to remove degradation products, and then the solvent was removed under reduced pressure. In the case of **5**, the residue was dissolved in methanol (5 mL), and the product was precipitated by addition of diethyl ether (50 mL), isolated by filtration and dried *in vacuo*. Compound **6** was purified by column chromatography (aluminium oxide, CH$_2$Cl$_2$/acetone 1:1, $R_f \approx 0.8$) and was isolated by evaporation of the solvent and dried *in vacuo*.

*3.2.2.1. Spectroscopic data for (η^6-C$_6$Me$_6$)Ru(η^2-SC$_5$H$_4$N)(η^1-SC$_5$H$_4$N) (**5**).* Orange solid, yield 88 mg (61%). ^{1}H NMR (400 MHz, DMSO-d_6): 2.03 (s, 18H, CH_3), 6.47 (d, ^3J = 8 Hz, 1H, C*H*), 6.61 (dd, 2H, C*H*), 7.04 (m, 2H, C*H*), 7.22 (dd, ^3J = 8 Hz, 1H, C*H*), 8.06 (d, 1H, C*H*), 8.13 (d, ^3J = 8 Hz, 1H, C*H*). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): 15.62 (*C*H$_3$), 93.14 (*C*Me), 115.18, 115.37 (*C*H), 128.19, 133 (*C*H), 128.89 (*C*H), 134.25 (*C*H), 147.57 (*C*H), 148.99 (*C*H), 134.83 (*C*H), 171.19, 177.64 (S–*C*). MS (ESI, *m*/*z*): 506.9 [M+Na]$^+$, 484.9 [M+H]$^+$, 374.2 [M−(SC$_5$H$_4$N)+H]$^+$. Anal. Calc. for $C_{22}H_{26}N_2RuS_2$: C, 54.63; H, 5.42; N, 5.79. Found: C, 54.60; H, 5.44; N, 5.70%.

*3.2.2.2. Spectroscopic data for (η^6-C$_6$Me$_6$)Ru(η^2-SC$_9$H$_6$N)(η^1-SC$_9$H$_6$N) (**6**).* Red solid, yield 115 mg (66%). ^{1}H NMR (400 MHz, DMSO-d_6): 2.13 (s, 18H, CH_3), 6.63 (d, 1H, C*H*), 7.19 (m, 2H, C*H*), 7.30 (m, 2H, C*H*), 7.55 (m, 5H, C*H*), 7.78 (d, 1H, C*H*), 8.03 (d, 1H, C*H*). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): 16.34 (*C*H$_3$), 93.72 (*C*Me), 121.84 (*C*H), 125.53 (*C*H), 127.84 (*C*H), 128.61 (*C*H), 129.88 (*C*H), 131.42 (*C*H) 132.11 (*C*H), 132.53 (*C*H), 133.64 (*C*H), 176.32 (S–*C*). MS (ESI, *m*/*z*): 584.9 [M+H]$^+$, 424.3 [M−(SC$_5$H$_4$N)+H]$^+$. Anal. Calc. for $C_{30}H_{30}N_2RuS_2$: C, 61.72; H, 5.18; N, 4.80. Found: C, 61.68; H, 5.25; N, 4.74%.

3.3. Single crystal X-ray structure analysis

A crystal of [**4**](PF$_6$)$_2 \cdot 4H_2O$ was mounted on a Stoe Image Plate Diffraction system equipped with a ϕ circle goniometer, using Mo Kα graphite monochromated radiation (λ = 0.71073 Å) with ϕ range 0–200°. The structure was solved by direct methods using the program SHELXS-97 [25]. 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. Fig. 1 was drawn with ORTEP [27] and Fig. 2 with the software MERCURY [28].

3.4. Cytotoxicity study

The human A2780 ovarian cancer cell line was obtained from the European Collection of Cell Cultures (Salisbury, UK). Cells were grown routinely in RPMI medium containing glucose, 5% foetal calf serum (FCS) and antibiotics at 37 °C and 5% CO_2. Cytotoxicity was determined using the MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). Cells were seeded in 96-well plates as monolayers with 100 μL of cell solution (approximately 20 000 cells) per well and preincubated for 24 h in medium supplemented with 10% FCS. Compounds were dissolved directly in the culture medium and serially diluted to the appropriate concentration. 100 μL of drug solution was added to each well and the plates were incubated for another 72 h. Subsequently, MTT (5 mg/mL solution) was added to the cells and the plates were incubated for a further 2 h. The culture medium was aspirated, and the purple formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 540 nm using a multiwell plate reader and the fraction of surviving cells was calculated from the absorbance of untreated control cells. Evaluation is based on means from two independent experiments, each comprising three microcultures per concentration level.

Table 3
Crystallographic and structure refinement parameters for complex **4**

	[**4**](PF$_6$)$_2 \cdot 4H_2O$
Chemical formula	$C_{27}H_{41}F_{12}N_3O_4P_2RuS_3$
Formula weight	958.82
Crystal system	Triclinic
Space group	$P\bar{1}$ (no. 2)
Crystal colour and shape	Orange block
Crystal size (mm)	0.12 × 0.09 × 0.08
a (Å)	8.1214(6)
b (Å)	15.2254(12)
c (Å)	17.0575(13)
α (°)	67.194(6)
β (°)	79.343(6)
γ (°)	88.307(6)
V (Å^3)	1908.7(3)
Z	2
T (K)	203(2)
D_c (g cm^{-3})	1.668
μ (mm^{-1})	0.754
Scan range (°)	1.32 < θ < 25.66
Unique reflections	7201
Observed reflections [$I > 2\sigma(I)$]	5463
R_{int}	0.0521
Final R indices [$I > 2\sigma(I)$][a]	R_1 0.0387, wR_2 0.0928
R indices (all data)	R_1 0.0544, wR_2 0.0964
Goodness-of-fit	0.938
Max, Min $\Delta\rho$ (eÅ^{-3})	1.025, −0.665

[a] Structures were refined on F_0^2: $wR_2 = [\sum[w(F_0^2 - F_c^2)^2]/\sum w(F_0^2)^2]^{1/2}$, *where* $w^{-1} = [\sum(F_0^2) + (aP)^2 + bP]$ and $P = [\max(F_0^2, 0) + 2F_c^2]/3$.

Supplementary material

CCDC 687524 contains the supplementary crystallographic data for this complex [**4**]$(PF_6)_2 \cdot 4H_2O$. 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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