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Highly cytotoxic diruthenium trithiolato complexes of the type $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR)_3]^+$: synthesis, characterization, molecular structure and *in vitro* anticancer activity†‡

Federico Giannini,[a] Lydia E. H. Paul,[a] Julien Furrer,*[a] Bruno Therrien[b] and Georg Süss-Fink*[b]

A new series of cationic dinuclear *p*-cymene ruthenium complexes bridged by three thiophenolato ligands containing various substituents mainly in *meta* and *ortho* positions, $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR)_3]^+$ (R = 3-C_6H_4Me: **1**; R = 3-C_6H_4OMe: **2**; R = 3-C_6H_4OEt: **3**; R = 3-$C_6H_4CF_3$: **4**; R = 3-$C_6H_4NH_2$: **5**; R = 3-C_6H_4Cl: **6**; R = 2-C_6H_4Me: **7**; R = 2-C_6H_4OMe: **8**; R = 2-$C_6H_4Pr^i$: **9**; R = 2-$C_6H_4CF_3$: **10**; R = npt: **11** (npt = 2-naphthyl); R = mco: **12** (mco = 4-methylcoumarinyl); R = 3,5-$C_6H_3Me_2$: **13**; R = 3,5-$C_6H_3(CF_3)_2$: **14**; R = 3,5-$C_6H_3Cl_2$: **15**; R = 3,4-$C_6H_3(OMe)_2$: **16**), have been prepared from the reaction of the neutral *p*-cymene diruthenium dichloride dimer, $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2Cl_4]$, with the corresponding thiophenol RSH. All cationic complexes have been isolated as their chloride salts and fully characterized by spectroscopic and analytical methods. The molecular structures of **10** and **15** have been solved by a single-crystal X-ray structure analysis of [**10**]Cl and [**15**]Cl, which show that the two ruthenium atoms adopt a pseudo-octahedral geometry without a metal–metal bond in accordance with the noble gas rule. All complexes are highly cytotoxic towards human ovarian cancer cells, the IC_{50} values being mostly in the nanomolar range. Complex **9** shows the highest cytotoxicity with an IC_{50} value of 0.03 μM towards the A2780 cell line and the cisplatin-resistant mutant A2780cisR. The cytotoxicity of these complexes, which belong to the most active ruthenium anticancer compounds reported so far, can be correlated with the lipophilicity of the corresponding thiols. In comparison with the previous series, the results demonstrate that the positions of the substituents in the thiopenolato bridges are not as important as the nature of the substituents, alkyl substituents being the best ones in line with their lipophilic character.

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1. Introduction

Water-soluble arene ruthenium complexes are easily taken up by living cells, because they seem to have the right balance between hydrophilic and lipophilic properties. Combined with the synthetic diversity and the fact that ruthenium is a non-toxic metal, water-soluble arene complexes are ideally suited for medicinal applications.[1]

In the search for anticancer agents containing metals other than platinum, ruthenium compounds turned out to be the most promising ones.[2] The ligand exchange kinetics of metal complexes in aqueous solution, which seem to be crucial for the anticancer activity, are very similar for platinum(II) and ruthenium(II) complexes.[3] Ruthenium complexes containing imidazole (imi) and indazole (ind) introduced by B. K. Keppler[4] and G. Sava[5] were found to be active against a number of tumors; after extensive preclinical tests, the compounds [indH][*trans*-$Ru(N\text{-ind})_2Cl_4$] (KP1019) and [imiH][*trans*-$Ru(N\text{-imi})(S\text{-dmso})Cl_4$] (NAMI-A) went into clinical trials.[2]

The field of arene ruthenium anticancer compounds was initiated in 1992 by D. Tocher, who had observed a cytotoxicity enhancement by coordinating the anticancer agent metronidazole to a benzene ruthenium dichloro fragment.[6] Later on, this field was pioneered by P. J. Dyson[1] and by P. J. Sadler,[7] who reported in 2001 the arene ruthenium complexes $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru(pta)Cl_2]$ (pta = 1,3,5-triaza-7-phospha-tricyclo-[3.3.1.1]decane),

[a] *Department für Chemie und Biochemie, Universität Bern, CH-3012 Bern, Switzerland. E-mail: julien.furrer@dcb.unibe.ch; Fax: +41 (0) 31 631 4887; Tel: +41 (0) 31 631 4383*
[b] *Institut de Chimie, Université de Neuchâtel, CH-2000 Neuchâtel, Switzerland. E-mail: georg.suess-fink@unine.ch; Fax: +41 (0) 32 718 2511; Tel: +41 (0) 32 718 2405*

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termed RAPTA-C[8] and $[(C_6H_5Ph)Ru(en)Cl]^+$ (en = 1,2-ethylenediamine)[9] to show antimetastatic or antitumoral properties; both groups studied arene ruthenium complexes of these types extensively.[10] Of particular interest are dinuclear arene ruthenium complexes, for which the cytotoxicity could be correlated with lipophilicity and water solubility: C. G. Hartinger reported a series of ruthenium complexes containing a pyridone-derived linker as an *O,O-O,O*-doubly bridging ligand, $(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru(O_2C_6H_5N\text{-}(CH_2)_nNC_6H_5O_2)Ru(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)$ (n = 2–12), which showed high activity against various cancer cell lines with a pronounced influence of the spacer length on the cytotoxicity.[11]

Recently, we found cationic dinuclear arene ruthenium complexes of general formula $[(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-}SR)_3]^+$ to be highly cytotoxic as chloride salts towards human ovarian cancer cells A2780 and their cisplatin-resistant mutant A2780cisR, the IC_{50} values being mostly in the submicromolar range.[12] Incubation with possible biological targets such as nucleotides, peptides and amino acids revealed interactions with only cysteine and glutathione, causing oxidation to cystine and oxidized glutathione (GSSG), respectively, as observed by NMR spectroscopy. The complexes can be recovered intact after oxidation, which prompted us to suggest a catalytic role of the ruthenium complex in its biological mode of action,[13,14] a mechanism that had been postulated for the first time by Sadler.[15]

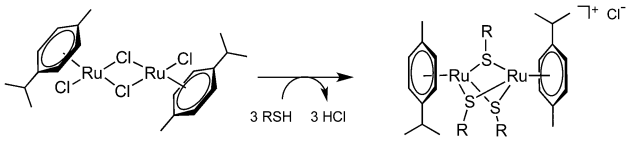
A systematic study of dinuclear trithiophenolato compounds of general formula $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-S-4-}C_6H_4X)_3]Cl$ (X being different functional groups in the *para* position of the thiophenolato substituents)[16] and $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR^1)_2(\mu_2\text{-}SR^2)]Cl$ (with different thiolato bridges)[17] suggested the cytotoxic activity to be influenced by the lipophilicity and the Hammett's constants of the corresponding thiols, although a direct correlation between cytotoxicity, catalytic oxidation activity and redox potentials could not be established, which is perhaps not surprising because the biological activity may be mainly determined by the different cellular uptake of the compounds.

As an extension of this work, we herein report the synthesis and molecular structure, the *in vitro* anticancer activity and the catalytic glutathione oxidation activity of a new series of sixteen trithiophenolato-bridged dinuclear *p*-cymene ruthenium complexes of general formula $[(\eta^6\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR)_3]^+$, R being aromatic substituents with various functional groups mainly in the *meta* and *ortho* positions.

2. Results and discussion

2.1. Synthesis and characterization

The *p*-cymene ruthenium complex $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2Cl_4]$ reacts in refluxing ethanol with an excess of the corresponding thiol to give the trithiolato complexes $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR)_3]^+$ (R = 3-C_6H_4Me: **1**; R = 3-C_6H_4OMe: **2**; R = 3-C_6H_4OEt: **3**; R = 3-$C_6H_4CF_3$: **4**; R = 3-$C_6H_4NH_2$: **5**; R = 3-C_6H_4Cl: **6**; R = 2-C_6H_4Me: **7**; R = 2-C_6H_4OMe: **8**; R = 2-$C_6H_4Pr^i$: **9**; R = 2-$C_6H_4CF_3$: **10**; R = npt: **11** (npt = 2-naphthyl); R = mco: **12** (mco = 4-methylcoumarinyl); R = 3,5-$C_6H_3Me_2$: **13**; R = 3,5-$C_6H_3(CF_3)_2$: **14**; R = 3,5-$C_6H_3Cl_2$: **15**; R = 3,4-$C_6H_3(OMe)_2$: **16**), which are isolated by chromatographic methods as orange to red air stable chloride salts in good to excellent yields (Scheme 1). The analytical data are given in the Experimental section.



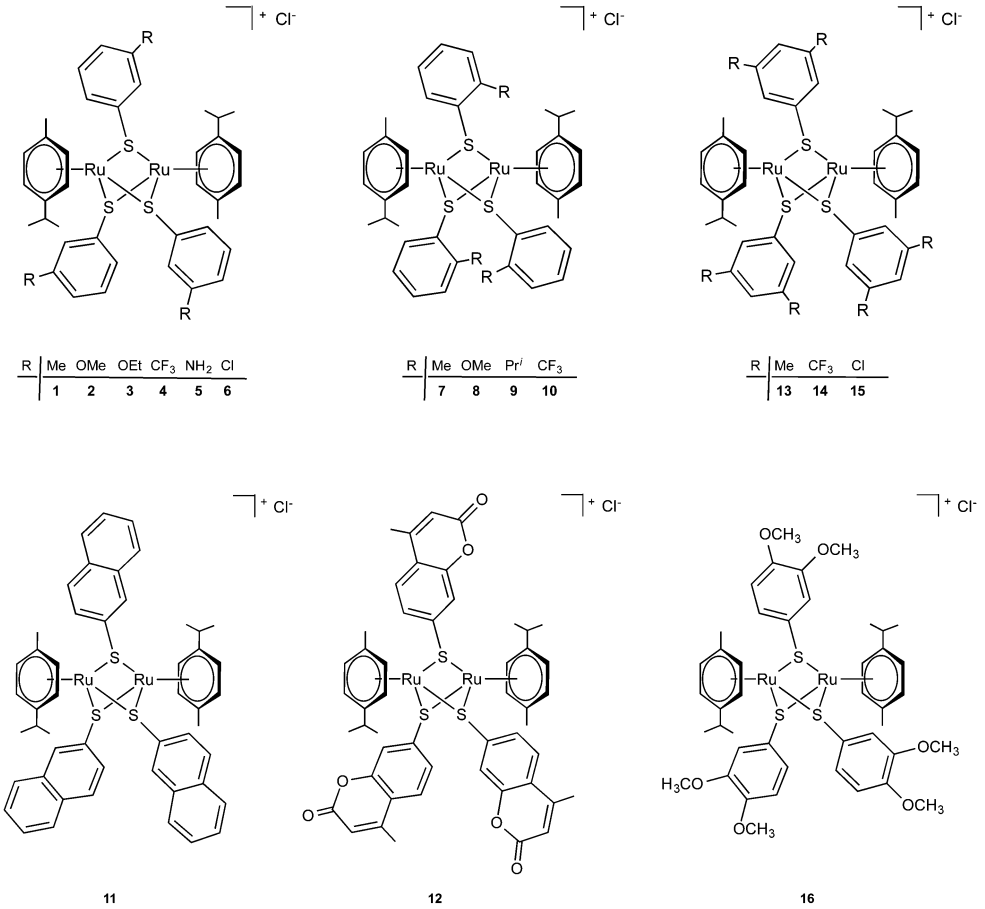
Scheme 1 Synthesis of complexes **1–16** as the chloride salts.

All compounds have been fully characterized by spectroscopic and analytical methods, the ^1H and ^{13}C NMR data being particularly meaningful. For instance, the ^1H NMR spectrum of complex **1** presents four resonances in the aromatic region between 7.8 and 7.1 ppm: a singlet at 7.69 ppm being assigned to the *ortho* proton in position 2 of the aromatic ring of the thiophenolato ligands, the two doublets at 7.67 and 7.17 ppm being attributed to the *ortho* and *para* protons, and a doublet of doublets at 7.27 ppm related to the *meta* proton. The *meta* methyl groups of the thiopenolato ligands give rise to a singlet at 2.45 ppm. Characteristic for *p*-cymene ruthenium complexes is the low field shift for the resonances of the aromatic *p*-cymene protons, for which the coordination to the ruthenium center causes a chemical shift of the four doublets to lower frequencies between 5.5 and 5.0 ppm. The resonances in the low frequency region are caused by the aliphatic protons of the *p*-cymene ligands: a septuplet at 1.94 ppm and the two doublets at 0.91 and 0.81 ppm caused by the two isopropyl groups and the singlet at 1.63 ppm caused by the two methyl substituents (Scheme 2).

As an example of the complexes with an *ortho* substituent on the thiophenolato ligands, the ^1H NMR spectrum of complex **8** is discussed, while the ^1H NMR spectrum of complex **15** serves as an example of complexes with two substituents on the thiophenolato ligands; the spectrum of **8** reveals three characteristic resonances in the aromatic region: a doublet at 7.85 ppm due to the *ortho* proton, a triplet of doublets at 7.36 ppm being assigned to the *para* proton and a multiplet at 7.00 ppm being attributed to the two *meta* protons. The region between 5.6 and 4.90 ppm presents four doublets caused by the aromatic protons of the two *p*-cymene rings. The methoxy group gives rise to a strong singlet at 4.10 ppm, a septuplet at 1.99 ppm and two doublets at 0.86 and 0.67 ppm are assigned to the isopropyl protons, and a singlet at 1.64 ppm is assigned to the methyl group of the *p*-cymene rings. The ^1H NMR spectrum of complex **15** shows nearly the same signals in the low frequency region, but in the aromatic region between 8 and 7 ppm only two singlets at 7.79 and 7.41 ppm show up, assignable to the two *ortho* protons and the *para* proton of the thiophenolato ligands. Interestingly, only one doublet at 5.56 ppm for the aromatic protons of the two *p*-cymene ligands is observed in this case, whereas the remaining protons collapse in a tight multiplet.

2.2. Molecular structures of 10 and 15

Suitable crystals for X-ray structure analysis were obtained for complexes [**10**]Cl (R = 2-$C_6H_4CF_3$) and [**15**]Cl (R = 3,5-$C_6H_3Cl_2$). Both salts co-crystallize with solvent molecules and the



Scheme 2 Schematic representation of compounds [**1**]Cl–[**16**]Cl.

stoichiometry in the crystals is [**10**]Cl·CH_2Cl_2 and [**15**]Cl·$2CHCl_3$, respectively. These solvent molecules not only fill the voids in the crystal packing, they also form a series of weak C–H···Cl interactions with their neighboring cations ([**10**]$^+$ or [**15**]$^+$) and anions (Cl^-): the C···Cl distances ranging from 3.3 to 3.5 Å in [**10**]Cl·CH_2Cl_2 and from 3.3 to 3.7 in [**15**]Cl·$2CHCl_3$. The Ortep drawings including the atom labeling scheme for **10** and **15** are shown in Fig. 1 and 2, together with selected bond lengths and angles.

In both structures, the ruthenium atoms adopt a pseudo-octahedral geometry with three sulfur atoms and the *p*-cymene ligand that formally occupies three coordination sites. The Ru_2S_3 unit forms a trigonal-bipyramidal framework with no metal–metal bond; the Ru···Ru distances being 3.3472(8) in **10** and 3.3518(5) Å in **15**. The Ru–S bond distances in both cations range from 2.3821(17) to 2.4239(15) Å and the Ru–S–Ru angles range from 87.85(4) to 88.82(5)°; these values are similar to those found in the *p*-cymene derivatives $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}S\text{-}4\text{-}C_6H_4Br)_3]^+$ and $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}S\text{-}4\text{-}C_6H_4Me)_3]^+$.[18,19] In both structures, the R groups of the thiolato bridges, R = 2-$C_6H_4CF_3$ in **10** and 3,5-$C_6H_3Cl_2$ in **15**, are not in the plane of the three sulfur atoms, thus generating a propeller type chirality. The tilt of the R groups remains in solution, for which diastereotopic protons for the *p*-cymene ligands are observed by NMR spectroscopy (see the Experimental part).

2.3. Cytotoxicity studies

Complexes **1–16** were evaluated as the chloride salts for their *in vitro* anticancer activity towards the human ovarian cancer cell line A2780 and its cisplatin-resistant mutant A2780cisR, using the cell counting kit 8 assay (Dojindo), which measures mitochondrial dehydrogenase activity as an indication of cell viability. The IC_{50} values, which represent the concentration of the complex that is required for 50% *in vitro* inhibition, are reported in Table 1.

All complexes except **12** are highly cytotoxic towards human ovarian cancer cells, with IC_{50} values in the nanomolar range. All complexes except **16** exhibit similar activities against both cell lines, A2780 and A2780cisR. Complex **9** (where R = 2-$C_6H_4Pr^i$) shows the highest cytotoxic effect for both ovarian cancer cell lines, with an IC_{50} value of 0.03 μM towards A2780 and A2780cisR cell lines. Following the tendency we found for the other classes of dinuclear trithiolato *p*-cymene ruthenium complexes,[13,14,16,17] complexes **1–16** are amongst the most active anticancer arene ruthenium compounds ever reported. For comparison, we also included cisplatin as a benchmark

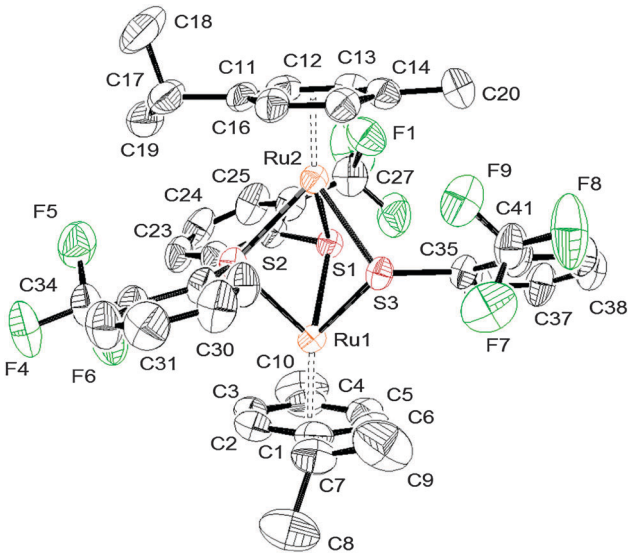


Fig. 1 Ortep diagram of **10** with 35% probability level thermal ellipsoids, hydrogen atoms, chloride and dichloromethane being omitted for clarity. Selected bond lengths (Å) and angles (°): Ru1–Ru2 3.3472(8), Ru1–S1 2.4033(15), Ru1–S2 2.4028(15), Ru1–S3 2.3821(17), Ru2–S1 2.4093(15), Ru2–S2 2.3857(16), Ru2–S3 2.4239(15); Ru1–S1–Ru2 88.26(5), Ru1–S2–Ru2 88.82(5), Ru1–S3–Ru2 88.41(5).

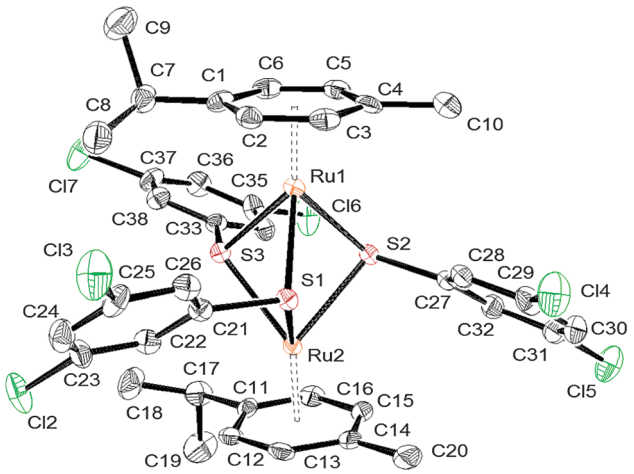


Fig. 2 Ortep diagram of **15** with 35% probability level thermal ellipsoids, hydrogen atoms, chloride and chloroform molecules being omitted for clarity. Selected bond lengths (Å) and angles (°): Ru1–Ru2 3.3518(5), Ru1–S1 2.4193(12), Ru1–S2 2.3952(12), Ru1–S3 2.4080(12), Ru2–S1 2.4126(11), Ru2–S2 2.4106(12), Ru2–S3 2.3974(12); Ru1–S1–Ru2 87.85(4), Ru1–S2–Ru2 88.45(4), Ru1–S3–Ru2 88.46(4).

(IC_{50} 2.94 ± 0.17 μM for A2780 and 21.90 ± 0.80 μM for A2780cisR).

The character of the substituents in the thiolato ligands plays an important role in the differences in terms of cytotoxicity observed for this series of complexes, whereas the position of the substituents in the thiolato ligands seems to have little influence on the *in vitro* anticancer activity. Whatever the substituent position is, complexes with no heteroatom exhibit increasing IC_{50} values that parallel the aliphatic character and lipophilicity of the corresponding thiol, going from **1** (R = 3-C_6H_4Me) to **13** (R = 3,5-$C_6H_3Me_2$) and **9** (R = 2-$C_6H_4Pr^i$), see Table 1. Ligands containing alkoxyl substituents such as complex **3** (R = 3-C_6H_4OEt), **2** (R = 3-C_6H_4OMe) and **8** (R = 2-C_6H_4OMe) have approximately the same cytotoxic values between 0.12 and 0.16 μM. For these complexes, as well as for those containing only aliphatic substituents on the thiolato ligands, the disubstitution has a beneficial influence on the cytotoxicity: indeed, complex **16** [R = 3,4-$C_6H_3(OMe)_2$] has a lower IC_{50} value as complex **2** (R = 3-C_6H_4OMe). The heteroatom-containing complexes show almost the same cytotoxicity without correlation to the position in the thiophenolato ligand, the IC_{50} values being around 0.15 μM, with the exception of **4** (R = 2-$C_6H_4CF_3$) with a lower and **5** (R = 3-$C_6H_4NH_2$) with a higher IC_{50} value.

Table 1 Comparison of cytotoxicities of **1–16** with the calculated log *P* values for the corresponding thiols

Complex	Log *P* (RSH)	IC_{50} [μM] A2780	IC_{50} [μM] A2780cisR
1	2.98 ± 0.28	0.235 ± 0.033	0.230 ± 0.019
2	2.54 ± 0.30	0.153 ± 0.014	0.201 ± 0.004
3	3.07 ± 0.30	0.126 ± 0.024	0.177 ± 0.013
4	3.49 ± 0.36	0.085 ± 0.004	0.075 ± 0.005
5	1.27 ± 0.29	0.869 ± 0.059	2.249 ± 0.271
6	3.10 ± 0.29	0.177 ± 0.012	0.216 ± 0.016
7	2.98 ± 0.28	0.332 ± 0.015	0.354 ± 0.011
8	2.24 ± 0.30	0.155 ± 0.011	0.210 ± 0.012
9	3.86 ± 0.28	0.030 ± 0.001	0.031 ± 0.001
10	3.55 ± 0.36	0.122 ± 0.019	0.119 ± 0.006
11	3.75 ± 0.28	0.070 ± 0.012	0.049 ± 0.004
12	2.83 ± 0.42	7.73 ± 1.491	≥10
13	3.44 ± 0.28	0.083 ± 0.005	0.044 ± 0.002
14	4.74 ± 0.47	0.075 ± 0.004	0.059 ± 0.005
15	3.69 ± 0.32	0.066 ± 0.005	0.063 ± 0.003
16	2.38 ± 0.32	0.070 ± 0.012	0.638 ± 0.057

2.4. Correlation between cytotoxicity and lipophilicity

The *in vitro* activity of anticancer drugs can often be related in part to their lipophilic character, the resulting hydrophobicity may contribute to an increased uptake of the compound by the cells, thereby enhancing the antiproliferative activity.[20] In the case of the complexes **1–16**, the Ru_2S_3 core with two *p*-cymene ligands remains the same for all complexes, the lipophilicity should vary only as a function of the RSH log *P* parameters, where the partition coefficient log *P*, calculated using the ACD/ChemSketch software,[21,22] reflects the lipophilicity of the substituents.

The partition coefficients of the thiols given in Table 1 are plotted in Fig. S1 (ESI‡) against the IC_{50} values of the related complexes **1–16**. From these plots a correlation between lipophilicity and cytotoxic activity is evidenced by the black regression line; complexes derived from thiols with increasing log *P* parameters up to the value 4 show a steady decrease of their IC_{50} values, which seem to increase again after log *P* = 4.5, the tendency being the same for both cell lines. From these plots it can be seen that complexes with ligands derived from thiols with log *P* values in the range between 3.5 and 4.0 show the highest cytotoxicities, whereas complexes with ligands derived from thiols with log *P* values less than 3.5 belong to the less active one of the series, presumably due to an insufficient

cellular uptake, since the less lipophilic compounds have more difficulties to cross cell membranes.

2.5. Catalytic glutathione oxidation

The tripeptide glutathione, a thiol present in living cells with concentrations ranging from 0.1 to 10 mM, is the major intracellular reducing agent; it deactivates reactive oxygen species and serves as a cofactor for redox modulating enzymes.[23] Glutathione is found in living cells either free or bound to proteins. Free glutathione is present mainly in its reduced form (GSH), which can be converted to the oxidized disulfide form (GSSG) during oxidative stress and reverted to the reduced form by glutathione reductase.[24] Maintaining an optimal GSH : GSSG ratio in the cells is critical for survival, and a deficiency of GSH can result in oxidative damage. This ratio is superior to 100 in normal resting cells, whereas in various situations of oxidative stress this ratio is reported to decrease to values between 10 and 1.[25] An imbalance of GSH levels is observed in a wide range of pathologies, including cancer;[26] elevated GSH levels increase antioxidant capacity and resistance to oxidative stress, and this is observed in many types of cancer.[26–28] A depletion of GSH could therefore affect the ability of cancer cells to cope with oxidation damage.[29]

Recently, we found the series of highly cytotoxic complexes of the general formula $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR^1)_2(\mu_2\text{-}SR^2)]^+$ (where R^1 are aliphatic groups and R^2 are *p*-substituted phenyl groups) to catalyze efficiently the oxidation of GSH to GSSG. However, it was not possible to establish a direct correlation between their *in vitro* anticancer activity (IC_{50}) and their catalytic activity (TOF_{50}).[17]

For the present series of complexes $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR)_3]^+$ containing thiophenolato ligands with *ortho* and *meta* substituents, the catalytic activity for the oxidation of GSH to GSSG was also analyzed by NMR spectroscopy. Since the glutathione autoxidation in the presence of O_2 is less than 5% in 24 h, we incubated the two most cytotoxic complexes **9** and **11** (lowest IC_{50}) and the two least cytotoxic complexes **5** and **12** (highest IC_{50}) with GSH in a 1 : 100 ratio, in a solution of D_2O/DMSO-d_6 (99 : 1), at pD 7 and 37 °C and in an aerobic atmosphere (see Table 2).

The ^{1}H-NMR spectra confirm the complete oxidation of GSH to GSSG within 24 h in the four experiments, as evidenced by the complete disappearance of the β-CH_2 resonances of GSH at δ 3.01 ppm and the simultaneous appearance of two new signals at δ 3.06 ppm and δ 3.34 ppm, and by the replacement of the α-CH resonance of GSH at δ 4.63 ppm with a new signal at δ 4.81 ppm. The TOF_{50} values, which correspond to the turnover frequencies for each complex as a catalyst at about 50% conversion of GSH to GSSG, are reported in Table 2, in comparison with the corresponding IC_{50} values. All the complexes studied are completely intact after a catalytic run and active for further runs. Since the $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR)_3]^+$ complexes are inert to substitution and stable towards oxygen and water, we expect them to enter the cancer cells intact.

Table 2 Comparison of cytotoxicity (IC_{50}) with catalytic activity (TOF_{50}) of the most and the least cytotoxic complexes

Complex	R	TOF_{50} (h^{-1})	IC_{50} [μM] A2780	IC_{50} [μM] A2780cisR
9	2-$C_6H_4Pr^i$	5.39	0.030 ± 0.001	0.031 ± 0.001
11	npt	13.30	0.070 ± 0.012	0.049 ± 0.004
5	3-$C_6H_4NH_2$	6.09	0.869 ± 0.059	2.249 ± 0.271
12	mco	5.59	7.73 ± 1.491	≥10

As expected, a direct correlation between cytotoxicity and catalytic glutathione oxidation activity could not be established. One of the most cytotoxic complexes of the series, **11** (with IC_{50} values of 0.07 μM for A2780 and 0.05 μM for A2780cisR), has a high catalytic activity (TOF_{50} = 13.30 h^{-1}) in line with its high cytotoxicity (low IC_{50}). Complex **12** (IC_{50} > 7 μM for both cell lines) shows a lower TOF_{50} value of 5.59 h^{-1}, as expected for lower cytotoxicity (high IC_{50}). However, the most cytotoxic complex **9** (IC_{50} = 0.03 μM for both cell lines) has only a TOF_{50} of 5.39 h^{-1}, in the same order of magnitude as the two least cytotoxic complexes.

The lack of a direct correlation between the catalytic oxidation activity and the cytotoxicity of these complexes is not really surprising, since the biological activity of compounds depends also on the cellular uptake and on the ability to penetrate cancer cell membranes. This ability is mainly a function of their lipophilicity and correlates therefore with the partition coefficients of the compounds. Nevertheless, the catalytic glutathione oxidation may play a role in the general mode of action of these complexes.

3. Conclusion

To the best of our knowledge, the new diruthenium trithiolato complexes, all obtained in good to excellent yields, are among the most cytotoxic arene ruthenium compounds ever reported. Interestingly, all complexes show comparable effects on both, cisplatin-sensitive and cisplatin-resistant human ovarian cancer cells. Based on our results for the compounds $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}S\text{-}4\text{-}C_6H_4X)_3]Cl$ with different functional groups X in the *para* position of the thiophenolato substituents,[16] it can be assumed that, also for the new complexes with substituents in *meta* and *ortho* positions, the catalytic oxidation of GSH to GSSG plays a role in the biological activity of these complexes, but other modes of action probably involving non-covalent interactions with enzymes and/or DNA, may also be involved.

From the results obtained, we can confirm that the lipophilicity plays an important role for these complexes. A comparison with the *para*-substituted analogues $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}S\text{-}4\text{-}C_6H_4X)_3]Cl$[16] shows that the position of the substituent in the aromatic rings of the thiolato bridges is not so important as the nature of the substituent. In the series of the mixed thiolato derivatives $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR^1)_2(\mu_2\text{-}SR^2)]Cl$,[17] it turned out that with different thiolato bridges it is possible to compensate the effect of one thiolato bridge by the other one. Overall, it can be generalized that the cytotoxicity of this type of complexes without heteroatoms in the thiolato bridges parallels the aliphatic character and the lipophilicity of the corresponding thiols.

4. Experimental

4.1. Materials and methods

The starting material $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2Cl_4]$ was prepared according to published methods.[30] All other reagents were commercially available and were used as received. NMR spectra were recorded with Bruker 400 MHz and 500 MHz spectrometers. Electrospray mass spectra were obtained in positive- or negative-ion mode with an LCQ Finningan mass spectrometer. Microanalyses were performed by the Mikroelementaranalytisches Laboratorium, ETH, Zürich (Switzerland).

4.2. Synthesis of complexes $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR)_3]^+$ (1–16)

A solution of $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2Cl_4]$ (122.4 mg, 0.2 mmol) in technical grade EtOH (40 ml) was heated under reflux. As soon as the starting material was completely dissolved, a solution of 6 equivalents of the corresponding thiol in 5 ml EtOH was added dropwise (1,3-MeC_6H_4SH: 143 μl; 1,3-$MeOC_6H_4SH$: 148 μl; 1,3-$EtOC_6H_4SH$: 169 μl; 1,3-$CF_3C_6H_4SH$: 163 μl; 1,3-$NH_2C_6H_4SH$: 128 μl; 1,3-ClC_6H_4SH: 140 μl; 1,2-MeC_6H_4SH: 142 μl; 1,2-$MeOC_6H_4SH$: 146 μl; 1,2-$Pr^iC_6H_4SH$: 182 μl; 1,2-$CF_3C_6H_4SH$: 159 μl; npt-SH: 192 mg; mco-SH: 230 mg; 1,3,5-$Me_2C_6H_3SH$: 164 μl; 1,3,5-$(CF_3)_2C_6H_3SH$: 203 μl; 1,3,5-$Cl_2C_6H_3SH$: 215 mg; 3,4-$(MeO)_2C_6H_3$-1-SH: 174 μl). The resulting solution was refluxed for 18 h. Then, the solvent was evaporated, and the residue was purified by column chromatography on silica gel using dichloromethane–ethanol (5 : 1) as an eluent. The yellow to reddish products were isolated as chloride salts and dried under vacuum.

4.2.1. Data for [1]Cl. Yield: 145.0 mg (83%). $C_{41}H_{49}ClRu_2S_3\cdot\frac{1}{4}CH_2Cl_2$ (896.86): calcd C 55.24, H 5.56%; found C 55.07, H 6.06%. ESI MS (MeOH): m/z = 841.11 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.69 (s, 3 H, SC_6H_4-*m*-CH_3), 7.67 (d, ^3J = 7.42 Hz, 3 H, SC_6H_4-*m*-CH_3), 7.27 (m, 3 H, SC_6H_4-*m*-CH_3), 7.17 (d, ^3J = 7.42 Hz, 3 H, SC_6H_4-*m*-CH_3), 5.35 (d, ^3J = 5.39 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.21 (d, ^3J = 5.39 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.11 (d, ^3J = 5.39 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.08 (d, ^3J = 5.39 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 2.45 (s, 9 H, SC_6H_4-*m*-CH_3), 1.94 [sept., ^3J = 6.95 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.63 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.91 [d, ^3J = 6.83 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.81 [d, ^3J = 6.83 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 138.9, 137.6, 133.2, 129.5, 129.3, 129.1, 107.4, 99.8, 85.5, 84.8, 83.55, 82.3, 30.6, 22.0, 21.6, 21.4, 17.7 ppm.

4.2.2. Data for [2]Cl. Yield: 162.0 mg (88%). $C_{41}H_{49}O_3ClRu_2S_3$ (923.61): calcd C 53.32, H 5.35%; found C 53.42, H 5.95%. ESI MS (MeOH): m/z = 889.09 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (m, 3 H, SC_6H_4-*m*-OCH_3), 7.43 (m, 3 H, SC_6H_4-*m*-OCH_3), 7.32 (t, ^3J = 8.06 Hz, 3 H, SC_6H_4-*m*-OCH_3), 6.90 (m, 3 H, SC_6H_4-*m*-OCH_3), 5.42 (d, ^3J = 5.80 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.26 (d, ^3J = 5.80 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.17 (d, ^3J = 5.80 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.12 (d, ^3J = 5.80 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 3.93 (s, 9 H, SC_6H_4-*m*-OCH_3), 1.98 [sept., ^3J = 6.95 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.66 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.93 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.82 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 159.6, 139.2, 130.1, 125.1, 118.5, 113.4, 107.6, 99.9, 85.5, 85.1, 84.9, 83.8, 55.9, 30.6, 22.6, 22.0, 17.7 ppm.

4.2.3. Data for [3]Cl. Yield: 173.4 mg (90%). $C_{44}H_{55}ClO_3Ru_2S_3\cdot\frac{1}{2}CH_2Cl_2$ (1008.17): calcd C 53.02, H 5.60%; found C 52.97, H 5.70%. ESI MS (MeOH): m/z = 930.7 $[M]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49 (d, ^3J = 7.6 Hz, 3 H, SC_6H_4-*m*-OCH_2CH_3), 7.44 (m, 3 H, SC_6H_4-*m*-OCH_2CH_3), 7.34 (t, ^3J = 8.4 Hz, 3 H, SC_6H_4-*m*-OCH_2CH_3), 6.94 (d, ^3J = 7.6 Hz, 3 H, SC_6H_4-*m*-OCH_2CH_3), 5.37 (d, ^3J = 4 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.23 (d, ^3J = 4 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.13 (d, ^3J = 4 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.10 (d, ^3J = 4 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 4.13 (q, ^3J = 6.8 Hz, 6 H, SC_6H_4-*m*-OCH_2CH_3), 1.97 [sept., ^3J = 6.80 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.65 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 1.50 (t, ^3J = 6.8 Hz, 9 H, SC_6H_4-*m*-OCH_2CH_3), 0.92 [d, ^3J = 4 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.80 [d, ^3J = 8 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 158.9, 138.9, 130.1, 124.8, 119.0, 114.3, 107.5, 99.9, 85.5, 84.9, 84.8, 83.7, 63.9, 30.6, 22.7, 21.9, 17.7, 14.9 ppm.

4.2.4. Data for [4]Cl. Yield: 149.40 mg (72%). $C_{41}H_{40}F_9ClRu_2S_3\cdot\frac{1}{2}EtOH$ (1060.57): calcd C 48.69, H 4.37%; found C 48.85, H 4.58%. ESI MS (MeOH): m/z = 1003.02 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.50 (d, ^3J = 7.4 Hz, 3 H, SC_6H_4-*m*-CF_3), 8.05 (s, 3 H, SC_6H_4-*m*-CF_3), 7.72 (t, ^3J = 6.82 Hz, 3 H, SC_6H_4-*m*-CF_3), 7.64 (d, ^3J = 6.82 Hz, 3 H, SC_6H_4-*m*-CF_3), 5.68 (m, 2 H, *p*-$MeC_6H_4Pr^i$), 5.34 (m, 4 H, *p*-$MeC_6H_4Pr^i$), 5.26 (m, 2 H, *p*-$MeC_6H_4Pr^i$), 1.89 [sept., ^3J = 6.90 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.62 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.88 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.79 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 138.9, 137.3, 133.1, 130.7, 128.8, 125.1, 125.0, 108.1, 100.5, 85.9, 85.6, 85.3, 84.1, 30.7, 22.4, 21.8, 17.6 ppm.

4.2.5. Data for [5]Cl. Yield: 141.09 mg (80%). $C_{38}H_{46}N_3ClRu_2S_3\cdot\frac{1}{2}CH_2Cl_2$ (921.05): calcd C 50.21, H 5.14%; found C 50.13, H 5.60%. ESI MS (MeOH): m/z = 844.09 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.55 (s, 3 H, SC_6H_4-*m*-NH_2), 7.11 (d, ^3J = 7.90 Hz, 3 H, SC_6H_4-*m*-NH_2), 7.03 (t, ^3J = 7.90 Hz, 3 H, SC_6H_4-*m*-NH_2), 6.61 (d, ^3J = 7.90 Hz, 3 H, SC_6H_4-*m*-NH_2), 5.57 (d, ^3J = 5.75 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.32 (d, ^3J = 5.75 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.25 (d, ^3J = 5.75 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.19 (d, ^3J = 5.75 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 2.06 [sept., ^3J = 6.95 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.66 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.94 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.83 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 147.4, 138.9, 129.1, 121.7, 119.5, 114.8, 107.1, 99.7, 85.5, 85.3, 84.6, 84.3, 30.6, 22.8, 22.0, 17.6 ppm.

4.2.6. Data for [6]Cl. Yield: 110.5 mg (59%). $C_{38}H_{40}Cl_4Ru_2S_3\cdot\frac{4}{5}EtOH$ (973.73): calcd C 49.33, H 4.76%; found C 49.20, H 4.94%. ESI MS (MeOH): m/z = 902.94 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (d, ^3J = 7.80 Hz, 3 H, SC_6H_4-*m*-Cl), $\frac{1}{3}\frac{2}{3}$7.80 (m, 3 H, SC_6H_4-*m*-Cl), 7.46 (t, ^3J = 7.85 Hz, 3 H, SC_6H_4-*m*-Cl), 7.36 (d, ^3J = 7.85 Hz, 3 H, SC_6H_4-*m*-Cl), 5.57 (d, ^3J = 5.95 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.33 (d, ^3J = 5.95 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.28 (d, ^3J = 5.95 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.25 (d, ^3J = 5.95 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 1.99 [sept., ^3J = 6.85 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.67 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.95 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.85 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 139.6,

134.2, 131.9, 131.6, 131.1, 128.7, 107.9, 100.4, 85.8, 85.3, 85.2, 83.9, 30.8, 22.5, 22.0, 17.8 ppm.

4.2.7. Data for [7]Cl. Yield: 112.95 mg (65%). $C_{41}H_{49}ClRu_2S_3\cdot\frac{1}{4}CH_2Cl_2$ (896.86): calcd C 55.24, H 5.56%; found C 55.13, H 6.04%. ESI MS (MeOH): m/z = 841.11 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.96 (d, ^3J = 7.85 Hz, 3 H, SC_6H_4-*o*-CH_3), 7.29 (m, 3 H, SC_6H_4-*o*-CH_3), 7.27 (m, 3 H, SC_6H_4-*o*-CH_3), 7.23 (m, 3 H, SC_6H_4-*o*-CH_3), 5.34 (d, ^3J = 5.75 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.19 (d, ^3J = 5.75 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.13 (d, ^3J = 5.75 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.09 (d, ^3J = 5.75 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 2.86 (s, 9 H, SC_6H_4-*o*-CH_3), 1.95 [sept., ^3J = 6.95 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.58 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.84 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.70 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 140.7, 137.0, 133.0, 130.7, 128.7, 127.2, 107.2, 99.9, 84.7, 83.8, 83.4, 83.0, 30.7, 22.6, 22.0, 21.3, 17.7 ppm.

4.2.8. Data for [8]Cl. Yield: 109.9 mg (60%). $C_{41}H_{49}O_3ClRu_2S_3\cdot\frac{3}{4}CH_2Cl_2$ (987.33): calcd C 51.09, H 5.20%; found C 50.95, H 5.75%. ESI MS (MeOH): m/z = 888.1 $[M]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (d, ^3J = 7.80 Hz, 3 H, SC_6H_4-*o*-OCH_3), 7.36 (t, ^3J = 7.80 Hz, 3 H, SC_6H_4-*o*-OCH_3), 7.00 (m, 6 H, SC_6H_4-*o*-OCH_3), 5.43 (d, ^3J = 5.60 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.33 (d, ^3J = 5.60 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.18 (d, ^3J = 5.60 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.02 (d, ^3J = 5.60 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 4.10 (s, 9 H, SC_6H_4-*o*-OCH_3), 1.99 [sept., ^3J = 6.80 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.64 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.86 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.67 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 160.0, 134.5, 129.7, 125.7, 121.6, 111.5, 107.3, 100.4, 84.5, 83.9, 83.8, 83.6, 56.4, 30.6, 22.8, 21.3, 17.8 ppm.

4.2.9. Data for [9]Cl. Yield: 111.9 mg (58%). $C_{47}H_{61}ClRu_2S_3\cdot\frac{1}{2}CH_2Cl_2$ (1002.25): calcd C 56.92, H 6.24%; found C 56.66, H 6.63%. ESI MS (MeOH): m/z = 925.2 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.95 (d, ^3J = 7.90 Hz, 3 H, SC_6H_4-*o*-Pr^i), 7.36 (m, 6 H, SC_6H_4-*o*-Pr^i), 7.20 (m, 3 H, SC_6H_4-*o*-Pr^i), 5.28 (d, ^3J = 5.70 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.18 (d, ^3J = 5.70 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.05 (d, ^3J = 5.70 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 4.99 (d, ^3J = 5.70 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 4.19 [sept., ^3J = 6.80 Hz, 3 H, SC_6H_4-*o*-$CH(CH_3)_2$], 1.97 [sept., ^3J = 6.80 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.60 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 1.50 [d, ^3J = 6.90 Hz, 18 H, SC_6H_4-*o*-$CH(CH_3)_2$], 0.80 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.68 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 151.3, 135.3, 133.3, 129.3, 126.8, 125.7, 106.8, 100.0, 84.6, 83.7, 83.6, 83.5, 31.0, 30.5, 23.9, 22.5, 21.2, 17.8 ppm.

4.2.10. Data for [10]Cl. Yield: 151.2 mg (73%). $C_{41}H_{40}F_9ClRu_2S_3$ (1037.54): calcd C 47.46, H 3.89%; found C 47.17, H 4.07%. ESI MS (MeOH): m/z = 1003.0 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.31 (d, ^3J = 7.2 Hz, 3 H, SC_6H_4-*o*-CF_3), 7.78 (d, ^3J = 7.2 Hz, 3 H, SC_6H_4-*o*-CF_3), 7.61 (m, 6 H, SC_6H_4-*o*-CF_3), 5.18 (m, 8 H, *p*-$MeC_6H_4Pr^i$), 2.01 [sept., ^3J = 6.80 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.57 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.83 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.71 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 137.2, 135.5, 133.4, 133.0, 129.3, 127.1, 122.7, 108.0, 100.7, 84.9, 84.5, 84.4, 83.6, 30.5, 22.7, 20.9, 17.6 ppm.

4.2.11. Data for [11]Cl. Yield: 137.7 mg (70%). $C_{50}H_{49}ClRu_2S_3\cdot EtOH$ (1029.78): calcd C 60.65, H 5.38%; found C 60.35, H 5.50%. ESI MS (MeOH): m/z = 949.3 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.52 (s, 3 H, $SC_{10}H_7$), 8.04 (m, 6 H, $SC_{10}H_7$), 7.92 (m, 6 H, $SC_{10}H_7$), 7.57 (m, 6 H, $SC_{10}H_7$), 5.51 (d, ^3J = 5.5 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.31 (d, ^3J = 5.5 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.28 (d, ^3J = 5.5 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.24 (d, ^3J = 5.5 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 1.96 [sept., ^3J = 6.40 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.58 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.81 [d, ^3J = 6.80 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.71 [d, ^3J = 6.80 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 135.4, 133.4, 133.0, 132.2, 129.8, 129.1, 128.0, 127.9, 127.3, 127.0, 108.0, 99.7, 85.9, 85.3, 84.6, 83.7, 30.8, 22.7, 21.9, 17.8 ppm.

4.2.12. Data for [12]Cl. Yield: 183.5 mg (85%). $C_{50}H_{49}O_6ClRu_2S_3\cdot EtOH\cdot CH_2Cl_2$ (1210.71): calcd C 52.58, H 4.75%; found C 52.84, H 5.00%. ESI MS (MeOH): m/z = 1045.3 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.33 (d, ^3J = 8.4 Hz, 3 H, $SC_6H_3C_3HO_2$-7-CH_3), 7.85 (d, ^3J = 8.4 Hz, 3 H, $SC_6H_3C_3HO_2$-7-CH_3), 7.73 (s, 3 H, $SC_6H_3C_3HO_2$-7-CH_3), 6.36 (s, 3 H, $SC_6H_3C_3HO_2$-7-CH_3), 5.79 (d, ^3J = 6.0 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.42 (d, ^3J = 6.0 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.38 (d, ^3J = 6.0 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.31 (d, ^3J = 6.0 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 2.53 (s, 9 H, $SC_6H_3C_3HO_2$-7-CH_3), 1.98 [sept., ^3J = 6.90 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.67 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.91 [d, ^3J = 6.80 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.76 [d, ^3J = 6.80 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 160.6, 153.2, 152.7, 143.0, 130.1, 126.2, 120.1, 119.9, 115.3, 108.3, 100.8, 85.7, 85.6, 85.4, 84.3, 30.5, 22.9, 21.9, 19.0, 18.1 ppm.

4.2.13. Data for [13]Cl. Yield: 119.3 mg (65%). $C_{44}H_{55}ClRu_2S_3\cdot EtOH$ (963.78): calcd C 57.32, H 6.38%; found C 56.99, H 6.64%. ESI MS (MeOH): m/z = 883.16 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45 (s, 6 H, SC_6H_3-3,5-CH_3), 6.98 (s, 3 H, SC_6H_3-3,5-CH_3), 5.27 (d, ^3J = 5.60 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.17 (d, ^3J = 5.60 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.07 (d, ^3J = 5.60 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.03 (d, ^3J = 5.60 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 2.41 [s, 18 H, SC_6H_3-3,5-CH_3], 1.96 [sept., ^3J = 6.80 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.65 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.93 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.85 [d, ^3J = 6.90 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 138.6, 137.4, 130.3, 130.2, 107.5, 99.6, 85.5, 84.7, 84.6, 83.4, 30.5, 22.5, 22.0, 21.5, 17.7 ppm.

4.2.14. Data for [14]Cl. Yield: 134.1 mg (54%). $C_{44}H_{37}F_{18}ClRu_2S_3\cdot 3$ EtOH (1287.61): calcd C 43.53, H 4.02%; found C 43.75, H 3.77%. ESI MS (MeOH): m/z = 1206.99 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.35 (s, 6 H, SC_6H_3-3,5-CF_3), 7.94 (s, 3 H, SC_6H_3-3,5-CF_3), 5.62 (m, 2 H, *p*-$MeC_6H_4Pr^i$), 5.35 (m, 6 H, *p*-$MeC_6H_4Pr^i$), 1.89 [sept., ^3J = 6.90 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.71 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.90 [m, 12 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 140.5, 132.5, 124.0, 122.5, 121.3, 109.2, 101.0, 86.8, 86.0, 84.9, 84.0, 31.0, 22.3, 21.6, 17.9 ppm.

4.2.15. Data for [15]Cl. Yield: 141.4 mg (68%). $C_{38}H_{37}Cl_7Ru_2S_3$ (1040.22): calcd C 43.88, H 3.59%; found C 43.87, H 3.73%. ESI MS (MeOH): m/z = 1004.9 $[M]^+$. ^{1}H NMR

(400 MHz, $CDCl_3$): δ = 7.79 (s, 6 H, SC_6H_3-3,5-Cl), 7.41 (s, 3 H, SC_6H_3-3,5-Cl), 5.56 (d, ^3J = 5.6 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.32 (m, 6 H, *p*-$MeC_6H_4Pr^i$), 2.06 [sept., ^3J = 6.80 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.64 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 1.02 [d, ^3J = 6.8 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.95 [d, ^3J = 6.8 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 140.6, 135.5, 130.6, 129.1, 108.7, 100.8, 86.1, 85.4, 85.1, 83.9, 31.0, 22.5, 22.0, 18.1 ppm.

4.2.16. Data for [16]Cl. Yield: 166 mg (82%). $C_{44}H_{55}ClO_6Ru_2S_3\cdot\frac{1}{8}CH_2Cl_2$ (1024.32): calcd C 51.35, H 5.41%; found C 51.65, H 5.60%. ESI MS (MeOH): m/z = 979.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.55 (d, ^3J = 8.4 Hz, 3 H, SC_6H_3-3,4-OCH_3), 7.50 (s, 3 H, SC_6H_3-3,4-OCH_3), 6.95 (d, ^3J = 8.4 Hz, 3 H, SC_6H_3-3,4-OCH_3), 5.47 (d, ^3J = 5.6 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.30 (d, ^3J = 5.6 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.23 (d, ^3J = 5.6 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 5.17 (d, ^3J = 5.6 Hz, 2 H, *p*-$MeC_6H_4Pr^i$), 4.07 (s, 9 H, SC_6H_3-3,4-OCH_3), 3.94 (s, 9 H, SC_6H_3-3,4-OCH_3), 1.98 [sept., ^3J = 6.80 Hz, 2 H, *p*-$MeC_6H_4CH(CH_3)_2$], 1.63 (s, 6 H, *p*-$CH_3C_6H_4Pr^i$), 0.95 [d, ^3J = 7.2 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$], 0.87 [d, ^3J = 6.8 Hz, 6 H, *p*-$MeC_6H_4CH(CH_3)_2$] ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 149.6, 148.7, 128.9, 125.7, 115.7, 111.9, 107.3, 99.3, 85.8, 85.2, 84.6, 83.6, 56.9, 56.2, 30.7, 22.8, 22.1, 17.7 ppm.

4.3. Single-crystal X-ray structure analyses

Crystals of compounds [**10**]Cl·CH_2Cl_2 and [**15**]Cl·2$CHCl_3$, obtained by the slow evaporation of a dichloromethane solution of [**10**]Cl and [**15**]Cl, respectively, were 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 structures were solved by direct methods using the program SHELXS-97, while the refinement and all further calculations were carried out using SHELXL-97.[31] The H-atoms were 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 (Table 3). Fig. 1 and 2 were drawn with ORTEP.[32]

The files CCDC 927646 [**10**]Cl·CH_2Cl_2 and 927647 [**15**]Cl·2$CHCl_3$ contain the supplementary crystallographic data for this paper.

Table 3 Crystallographic and structure refinement parameters for [**10**]Cl·CH_2Cl_2 and [**15**]Cl·2$CHCl_3$

	[**10**]Cl·CH_2Cl_2	[**15**]Cl·2$CHCl_3$
Chemical formula	$C_{42}H_{42}Cl_3F_9Ru_2S_3$	$C_{40}H_{39}Cl_{13}Ru_2S_3$
Formula weight	1122.43	1278.88
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$ (no. 14)	$P2_1/n$ (no. 14)
Crystal colour and shape	Red block	Orange block
Crystal size	0.19 × 0.15 × 0.15	0.20 × 0.19 × 0.15
a (Å)	13.2365(13)	13.2082(5)
b (Å)	19.4764(19)	13.1769(4)
c (Å)	17.2337(15)	28.5766(9)
β (°)	94.607(11)	92.029(3)
V (Å^3)	4428.5(7)	4970.4(3)
Z	4	4
T (K)	173(2)	173(2)
D_c (g cm^{-3})	1.683	1.709
μ (mm^{-1})	1.073	1.463
Scan range (°)	2.28 < θ < 26.09	1.68 < θ < 29.26
Unique reflections	8248	13 452
Observed refls [$I > 2\sigma(I)$]	4556	9346
R_{int}	0.0699	0.1486
Final R indices [$I > 2\sigma(I)$][a]	0.0472, wR_2 0.1129	0.0699, wR_2 0.0898
R indices (all data)	0.0911, wR_2 0.1255	0.1153, wR_2 0.0991
Goodness-of-fit	0.844	1.096
Max, min $\Delta\rho/e$ (Å^{-3})	0.740, −0.855	1.374, −1.214

[a] 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 $P = [\max(F_o^2, 0) + 2F_c^2]/3$.

4.4. Cell culture and inhibition of cell growth

Human A2780 and A2780cisR ovarian carcinoma cells 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 RPM1 1640 medium which contained fetal calf serum (FCS) (10%), 2 mM Gln and 1% antibiotics (penicillin/streptomycin) at 37 °C and CO_2 (5%). Cytotoxicity was determined using the cell counting kit 8 (Dojindo). Therefore, the cells were seeded in 96-well plates as monolayers with 100 μL of cell solution (approximately 10 000 cells) per well. Compounds were dissolved in DMSO, then dissolved in the culture medium and serially diluted to the appropriate concentration, to give a final DMSO concentration of 1%. 100 μL of drug solution was added to each well and the plates were incubated for 96 h. After incubation the culture medium was removed completely and subsequently, 10 μL kit solution and 100 μL fresh medium were added to the cells. The plates were incubated for a further 90 minutes. The optical density, directly proportional to the number of surviving cells, was quantified at 450 nm using a multiwall plate reader and the fraction of surviving cells was calculated from the absorbance of untreated control cells. Evaluation is based on means from four independent experiments, each comprising four microcultures per concentration level.

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