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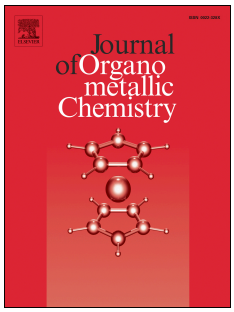

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Highly cytotoxic trithiolato-bridged dinuclear Rh(III) and Ir(III) complexes

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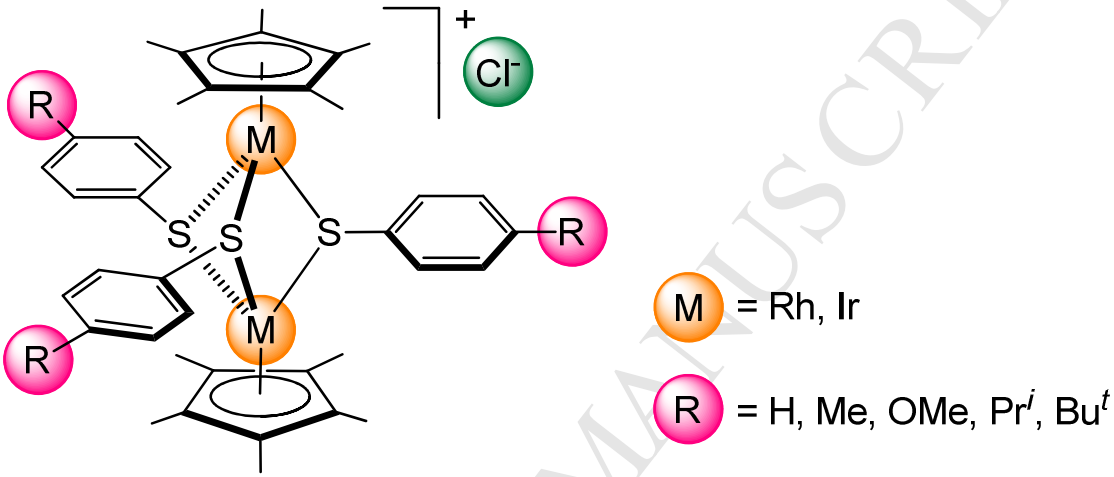
ABSTRACT

Water soluble trithiolato-bridged cationic complexes of the type $[(\eta^5\text{-}C_5Me_5)_2M_2(\mu\text{-}SC_6H_4\text{-}p\text{-}X)_3]^+$ (M = Rh, X = H, **1**; CH_3, **3**; OCH_3, **5**; Pr^i, **7**; Bu^t, **9**; M = Ir, X = H, **2**; CH_3, **4**; OCH_3, **6**; Pr^i, **8**; Bu^t, **10**) were synthesized and isolated as their chloride salts by reacting pentamethylcyclopentadienyl rhodium and iridium dimers $[(\eta^5\text{-}C_5Me_5)_2M_2(\mu\text{-}Cl)_2Cl_2]$ in ethanol with the corresponding thiophenol. All complexes were isolated in good yields and were fully characterized including single-crystal X-ray structure analysis on representative complexes. The complexes were found to have IC_{50} values in the nanomolar range in human ovarian A2780 cancer cells, but did not display selectivity with respect to noncancerous human HEK293 embryonic kidney cells.

Keywords: Half-sandwich complexes; Dinuclear complexes; Thiolato-bridging ligands; Lipophilicity; Anticancer activity.

GRAPHICAL ABSTRACT

Water-soluble trithiolato-bridged pentamethylcyclopentadienyl Rh(III) and Ir(III) complexes have been synthesized and characterized. The cytotoxicity of all complexes has been established using cancerous and noncancerous cell lines. The cationic complexes are highly cytotoxic, with IC_{50} values in the nanomolar range.



HIGHLIGHTS

· Synthesis of dinuclear half-sandwich complexes bridged by thiolato ligands.

· The single-crystal X-ray structure analysis of three derivatives is presented.

· The antiproliferative activity of all complexes has been evaluated in vitro.

· Highly cytotoxic complexes on cancerous and noncancerous cells.

1. Introduction

The medicinal properties of organometallic complexes is an area of growing interest and virtually all key types of ligands encountered in organometallic chemistry have been considered [1]. Of these, arene ruthenium complexes have received considerable attention [2], including dinuclear [3] and polynuclear [4] systems, while the half-sandwich rhodium and iridium analogues have been studied to a lesser extent [5].

Dinuclear complexes bridged by thiolato ligands are an important class of compounds. Such a structural motif is found at the active site of hydrogenases [6], which has inspired the development of synthetic catalysts based on this fragment [7]. Among thiolato-bridged dinuclear complexes those containing half-sandwich units have been known for many years [8]. However, only recently they were found to exhibit *in vitro* antiproliferative activity against various cancer cell lines in the nanomolar range [9].

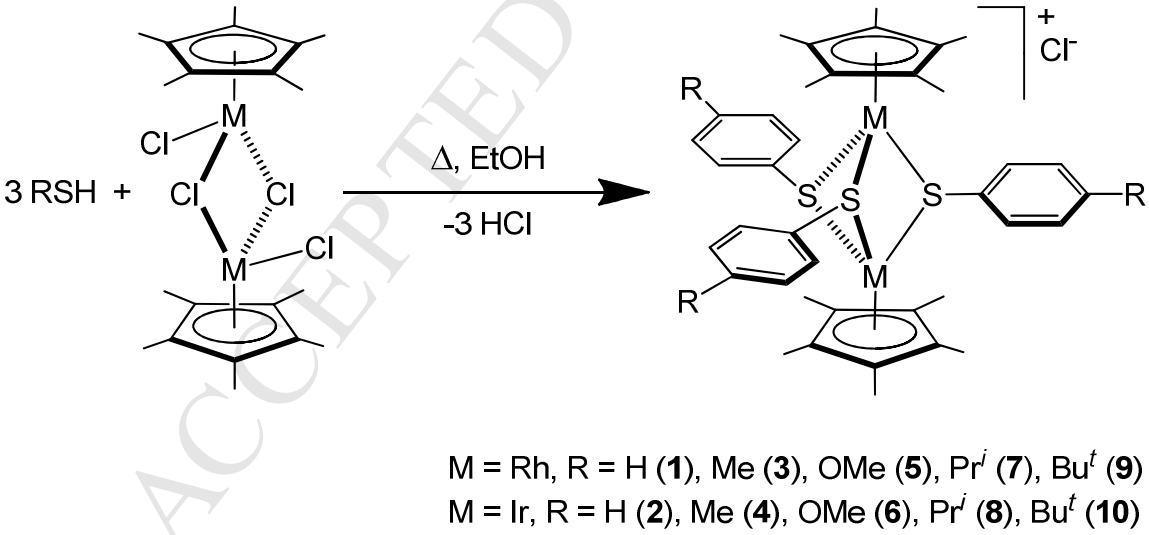
Trithiolato-bridged dinuclear arene ruthenium complexes have yielded IC_{50} values as low as 30 nM against human ovarian cancer cells with the most active compound being the *tert*-butyl thiolato-bridged *p*-cymene ruthenium derivative $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Bu^t)_3]^+$ [10]. These ruthenium derivatives also catalyze the oxidation of glutathione, which in cells could lead to an increase of reactive oxygen species (ROS) since glutathione is a natural ROS scavenger [11]. Other mechanisms/targets for the potent cytotoxicity of these complexes cannot be excluded.

Recently, we showed that the thiolato-bridged pentamethylcyclopentadienyl (C_5Me_5) rhodium and iridium analogues exhibit similar cytotoxicity to the arene ruthenium compounds with IC_{50} values also in the submicromolar range [12]. In contrast to the dinuclear arene ruthenium complexes, the rhodium and iridium thiolato-bridged species do not catalyze the

oxidation of glutathione, indicating another mode of action [12a]. In an extension of these nascent studies, herein, we describe a series of new trithiolato-bridged Rh(III) and Ir(III) half-sandwich complexes and their cytotoxicity on ovarian cancer (A2780) cancer cells and noncancerous human embryonic kidney (HEK293) cells.

2. Results and discussion

Reaction of the dinuclear dichloro-bridged complexes $[(\eta^5\text{-}C_5Me_5)_2M_2(\mu\text{-}Cl)_2Cl_2]$ (M = Rh and Ir) with three equivalents of *para*-substituted thiols in ethanol under reflux led to the formation of the cationic trithiolato-bridged complexes of the general formula $[(\eta^5\text{-}C_5Me_5)_2M_2(\mu\text{-}SC_6H_4\text{-}p\text{-}X)_3]^+$ (M = Rh; R = H, **1**; R = Me, **3**; R = OMe; **5**, R = Pr^i, **7**; R = Bu^t, **9**; M = Ir; R = H, **2**; R = Me, **4**; R = OMe; **6**, R = Pr^i, **8**; R = Bu^t, **10**). All complexes were isolated in good yield as the chloride salt (Scheme 1). The salts [**1**]Cl and [**3**]PF_6 have been previously reported [13].



Scheme 1. Synthesis of the trithiolato-bridged complexes [**1**]Cl–[**10**]Cl.

These compounds are not hygroscopic and are stable in air and in solution. They are sparingly soluble in polar solvents including dichloromethane, chloroform, acetone, and

acetonitrile and insoluble in non-polar solvents. In addition, [**1**]Cl–[**10**]Cl are moderately soluble in water. All complexes were isolated in good yields and fully characterized by elemental analysis, ^{1}H, ^{13}C NMR and UV-visible spectroscopy, and electro-spray ionization mass spectrometry (see experimental section). The analytical data of the compounds are consistent with the expected structures. The resonances of the thiolato ligands are observed at lower frequencies than those of the uncoordinated thiols in the ^{1}H NMR spectra of the complexes. Electrospray ionization mass spectra for [**1**]Cl–[**10**]Cl display the expected molecular ion peak corresponding to the intact cation. In the electronic absorption spectra of dichloromethane solutions of the complexes, intense transitions in the visible region are observed and assigned to MLCT (metal-ligand charge transfer) bands, while intra-ligand transitions are observed at longer wavelengths.

The stability of the cations [**1**]$^{+}$–[**10**]$^{+}$ was studied by ^{1}H NMR spectroscopy in D_2O/DMSO-d_6 (90%/10%, v/v) solutions: Solvents used to prepare the stock solutions of the compounds for biological tests. Over a period of 24 h, no changes were observed in the spectra, suggesting that the complexes are suitable for antiproliferative activity studies.

Molecular structures

Crystals suitable for single-crystal X-ray structure analysis were obtained for [**3**]Cl, [**4**]Cl and [**5**]Cl. The molecular structure of the cations [**3**]$^{+}$, [**4**]$^{+}$ and [**5**]$^{+}$ are shown in Figures 1-3, respectively. The molecular structures of the cations comprise a closed trigonal bipyramid M_2S_3 framework, with each metal being coordinated to an η^5-C_5Me_5 ligand and three sulfur atoms. The M-S bond distances [ranging from 2.3846(7) to 2.4215(5) Å] and M-S-M angles [ranging from 76.11(3) to 80.16(2)°] are similar to those found in analogous trithiolato-bridged half-sandwich complexes [8]. The Rh···Rh distance is 3.2250(3) Å in [**3**]$^{+}$ and 3.2374(3) Å in [**5**]$^{+}$, and in [**4**]$^{+}$ the Ir···Ir distance is 3.3172(2) Å. Overall, all geometrical

parameters are consistent with those found in similar trithiolato-bridged half-sandwich complexes [8].

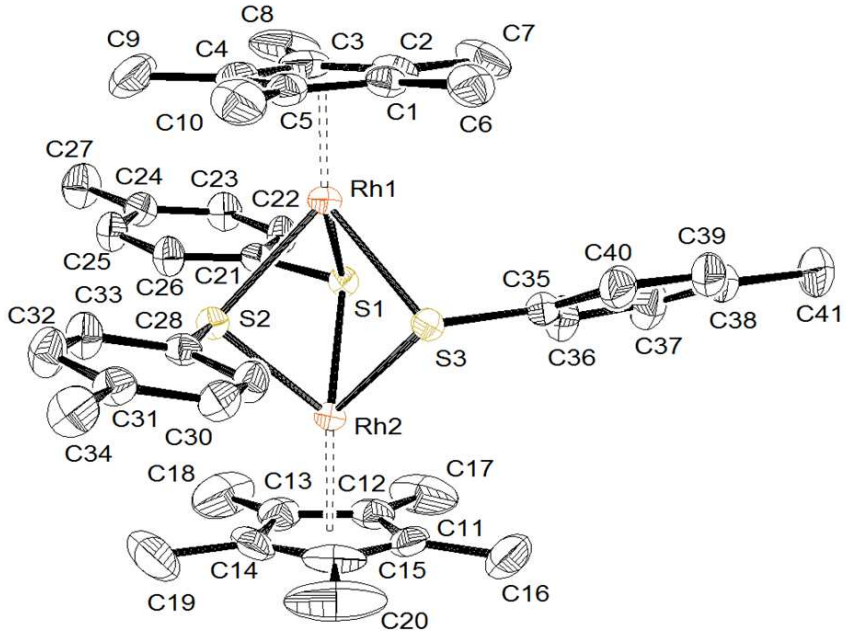


Figure 1. Molecular structure of [**3**]$^+$ with ellipsoids at the 50% probability level; H atoms, Cl^- and $CHCl_3$ molecules omitted for clarity.

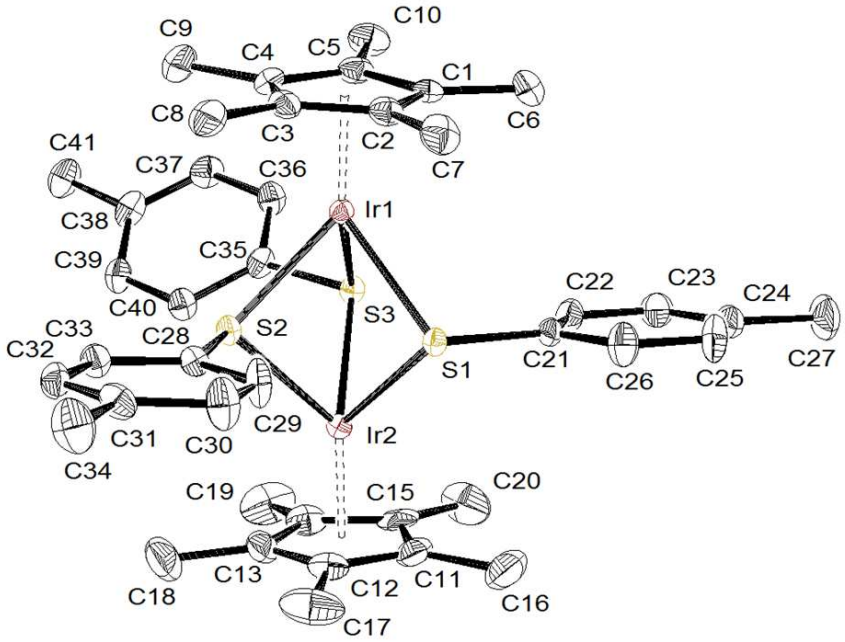


Figure 2. Molecular structure of [**4**]$^+$ with ellipsoids at the 50% probability level; H atoms, Cl^- and CH_2Cl_2 molecule omitted for clarity.

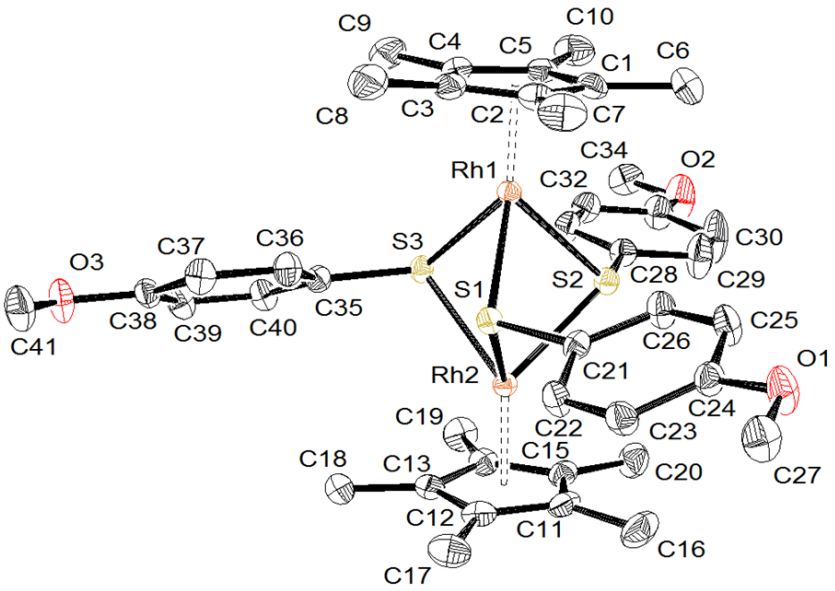


Figure 3. Molecular structure of [**5**]$^+$ with ellipsoids at the 50% probability level; H atoms, Cl^- and HCl molecule omitted for clarity.

HCl is present in the crystal packing of $[(\eta^5\text{-}C_5Me_5)_2Rh_2(\mu\text{-}SC_6H_4\text{-}p\text{-}OMe)_3]Cl$ ([**5**]Cl), which is also apparent from the elemental analysis of this salt (the HCl is generated during the synthesis, see Scheme 1). However, we were unable to distinguish between the HCl and the Cl^- in the crystal, the hydrogen being fixed arbitrary on one position. A HCl molecule was previously observed in the crystal packing of the analogous complex $[(\eta^5\text{-}C_5Me_5)_2Ir_2(\mu\text{-}SCH_2CH_2C_6H_5)_3]Cl \cdot HCl$ [12a].

Antiproliferative activity

The antiproliferative activity of [**1**]Cl–[**10**]Cl was evaluated against human A2780 ovarian cancer cells and on human HEK293 embryonic kidney cells, as a model for healthy cells. The IC_{50} values (IC_{50} is the drug concentration necessary for 50% inhibition of cell viability) after 72 h of cell exposure are listed in Table 1, together with their resistance factors (RF). For

comparison, the IC_{50} values of the corresponding trithiolato-bridged dinuclear *p*-cymene ruthenium complexes (Ru-analogues) are also listed in Table 1.

Table 1. IC_{50} values of compounds [**1**]Cl–[**10**]Cl toward human cancer cells A2780 and healthy cells HEK293 after 72 h exposure (RF = resistance factor).

compound	A2780 (μM)	HEK293 (μM)	RF
cisplatin	0.86±0.06	4.41±0.37	5.1
$[(\eta^5\text{-}C_5Me_5)_2Rh_2(\mu\text{-}SC_6H_5)_3]Cl$ ([**1**]Cl)	0.07±0.01	0.03±0.01	0.4
$[(\eta^5\text{-}C_5Me_5)_2Ir_2(\mu\text{-}SC_6H_5)_3]Cl$ ([**2**]Cl)	0.07±0.01	0.03±0.01	0.4
(*p*-cymene)Ru-analogue	0.24±0.01	0.80±0.03	3.3
$[(\eta^5\text{-}C_5Me_5)_2Rh_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Me)_3]Cl$ ([**3**]Cl)	0.05±0.01	0.03±0.01	0.6
$[(\eta^5\text{-}C_5Me_5)_2Ir_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Me)_3]Cl$ ([**4**]Cl)	0.04±0.01	0.03±0.01	0.8
(*p*-cymene)Ru-analogue	0.13±0.01	NA	
$[(\eta^5\text{-}C_5Me_5)_2Rh_2(\mu\text{-}SC_6H_4\text{-}p\text{-}OMe)_3]Cl$ ([**5**]Cl)	0.04±0.01	0.04±0.01	1.0
$[(\eta^5\text{-}C_5Me_5)_2Ir_2(\mu\text{-}SC_6H_4\text{-}p\text{-}OMe)_3]Cl$ ([**6**]Cl)	0.06±0.01	0.03±0.01	0.5
(*p*-cymene)Ru-analogue	0.18±0.03	0.29±0.02	1.6
$[(\eta^5\text{-}C_5Me_5)_2Rh_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Pr^i)_3]Cl$ ([**7**]Cl)	0.04±0.01	0.06±0.01	1.5
$[(\eta^5\text{-}C_5Me_5)_2Ir_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Pr^i)_3]Cl$ ([**8**]Cl)	0.05±0.01	0.08±0.01	1.6
(*p*-cymene)Ru-analogue	0.08±0.01	0.16±0.02	2.0
$[(\eta^5\text{-}C_5Me_5)_2Rh_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Bu^t)_3]Cl$ ([**9**]Cl)	0.06±0.01	0.16±0.02	2.7
$[(\eta^5\text{-}C_5Me_5)_2Ir_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Bu^t)_3]Cl$ ([**10**]Cl)	0.03±0.01	0.07±0.01	2.3
(*p*-cymene)Ru-analogue	0.03±0.01	0.03±0.01	1.0

All compounds exhibit high cytotoxicity towards both the A2780 and HEK293 cell lines (IC_{50} values of 30-160 nM), with IC_{50} values ca. 10 – 30 fold lower than cisplatin. Within this series there is little difference in the cytotoxicity values for analogous Rh and Ir complexes, an exception being compounds [**9**]Cl and [**10**]Cl, where the rhodium complex is less cytotoxic than the iridium analogue in both cell lines. Complexes bearing unsubstituted or *p*-Me-substituted thiophenol bridges, i.e. [**1**]Cl–[**4**]Cl, lower IC_{50} values are observed in the HEK293 cell line than the cancerous A2780 cell line. With the more bulky and lipophilic, Pr^i and Bu^t-substituted thiophenol this cytotoxicity profile is reversed, with the compounds

[**7**]Cl–[**10**]Cl being more active toward the A2780 cell line than the HEK293 cell line. This selectivity was most pronounced for [**9**]Cl with a RF of 2.7, however, this selectivity profile is still lower than that of cisplatin (5.1). The related ruthenium structures are all less cytotoxic than their iridium or rhodium analogues with the exception of the compound containing Bu^t-substituted thiophenol bridges, which is as cytotoxic as [(η^5-C_5Me_5)$_2$$Ir_2$($\mu$-$SC_6H_4$-*p*-$Bu^t$)$_3$]Cl ([**10**]Cl) in the A2780 cell line and more cytotoxic than [**10**]Cl in the HEK293 cell line.

The iridium and rhodium complexes with the *para*-substituents on the thiophenol bridges do not significantly contribute to the observed cytotoxicity in the A2780 cell line, illustrated by the IC_{50} values remaining relative constant across the series. In the HEK293 cell line the compounds with the most lipophilic Pr^i- and Bu^t-thiophenol substituents are slightly less cytotoxicity relative to complexes with H-, Me- and MeO-substituents. In contrast, for the ruthenium complexes the thiophenol substituents are strongly linked to the overall cytotoxicity with increasing substituent lipophilicity translating into increased cytotoxicity against both cell lines. The cytotoxicity of the related ruthenium dinuclear complexes may be readily modulated by changing the nature of the bridging ligand whereas, in contrast, the activity of the rhodium and iridium systems is less influenced by the bridging ligand with the cytotoxicity remaining high regardless of the substituent on the bridging thiophenol ligand.

CONCLUSION

A series of dinuclear pentamethylcyclopentadienyl rhodium and iridium complexes, in which the two metals are connected via bridging thiolato ligands to afford compounds with a M_2S_3 core, are highly cytotoxic, but display limited discrimination between cancerous and noncancerous cancer cells. The influence of the substituents on the bridging thiolato ligands is relatively innocuous and therefore precludes a clear path to the systematic development of more selective derivatives.

EXPERIMENTAL

Materials and methods

The starting materials $[(\eta^5\text{-}C_5Me_5)_2Rh_2(\mu\text{-}Cl)_2Cl_2]$ and $[(\eta^5\text{-}C_5Me_5)_2Ir_2(\mu\text{-}Cl)_2Cl_2]$ were prepared according to published methods [14]. All other reagents were commercially available and were used without further purification. ^1H and $^{13}C\{^1H\}$ NMR spectra were recorded on Bruker Avance II 400 MHz spectrometers using the residual protonated solvent as internal standard. Electrospray mass spectra were obtained in positive ion mode with an LCQ Finnigan mass spectrometer. UV-visible absorption spectra were recorded using an Uvikon 930 spectrophotometer (10^{-5} M in CH_2Cl_2). Microanalysis was carried out by the Mikroelementaranalytisches Laboratorium, ETH Zürich (Switzerland).

Synthesis of [1]Cl–[10]Cl.

The complex $[(\eta^5\text{-}C_5Me_5)_2M_2(\mu\text{-}Cl)_2Cl_2]$ (M = Rh, 100 mg, 0.16 mmol; M = Ir, 100 mg, 0.13 mmol) was heated to reflux in ethanol (25 mL). Following complete dissolution of the complex, ca. 1 h, an ethanolic solution (5 mL) of the corresponding thiol (M = Rh, 0.48 mmol; HSC_6H_4-*p*-X, X = H, 50 µL (**1**); X = Me, 61 µL (**3**); X = OMe, 60 µL (**5**); X = Pr^i, 76 µL (**7**); X = Bu^t, 84 µL (**9**): M = Ir, 0.39 mmol; HSC_6H_4-*p*-X, X = H, 39 µL (**2**); X = Me, 47 µL (**4**); X = OMe, 47 µL (**6**); X = Pr^i, 59 µL (**8**); X = Bu^t, 65 µL (**10**)) was added dropwise to the hot solution. The resulting solution was heated to reflux for 3 h, during which the color of the solution changed to red for the rhodium derivatives and to yellow for the iridium derivatives. The mixture was cooled to RT and the solvent removed under reduced pressure. The resulting oil was washed with diethyl ether and hexane to obtain a powder. The powder was dissolved in dichloromethane (ca. 5 mL), precipitated with diethyl ether (ca. 100 mL), filtered and the solid dried under vacuum.

Compound [(η^5-C_5Me_5)$_2$Rh$_2$(μ-SC_6H_5)$_3$]Cl ([**1**]Cl).

Yield: 105 mg (77%). Elemental analysis (%): calcd. for [$C_{38}H_{45}Rh_2S_3$]Cl · HCl: C, 52.12; H, 5.29; found C, 51.87; H, 5.69. ESI-MS (MeOH, CH_2Cl_2): m/z = 804.0 $[M\text{-}Cl]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.81 (m, 6H, $SC_6\mathit{H}_5$), 7.35 (m, 9H, $SC_6\mathit{H}_5$), 1.34 (s, 30H, C_5Me_5) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 8.78, 97.91, 128.83, 128.92, 132.49, 133.24 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 249 (50500), 286 (52300), 340 (23300), 396 (19400).

[(η^5-C_5Me_5)$_2$Ir$_2$(μ-SC_6H_5)$_3$]Cl ([**2**]Cl).

Yield: 100 mg (78%). Elemental analysis (%): calcd. for [$C_{38}H_{45}Ir_2S_3$]Cl · HCl: C, 43.29; H, 4.40; found C, 43.31; H, 4.58. ESI-MS (MeOH, CH_2Cl_2): m/z = 982.2 $[M\text{-}Cl]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (m, 6H, $SC_6\mathit{H}_5$), 7.37 (m, 9H, $SC_6\mathit{H}_5$), 1.38 (s, 30H, C_5Me_5) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 8.50, 91.52, 128.75, 129.35, 130.56, 132.99 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 243 (78500), 331 (20700).

[(η^5-C_5Me_5)$_2$Rh$_2$(μ-SC_6H_4-*p*-Me)$_3$]Cl ([**3**]Cl).

Yield: 107 mg (75%). Elemental analysis (%): calcd. for [$C_{41}H_{51}Rh_2S_3$]Cl · HCl: C, 53.66; H, 5.71; found C, 53.31; H, 5.94. ESI-MS (MeOH, CH_2Cl_2): m/z = 846.0 $[M\text{-}Cl]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.66 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6\mathit{H}_4CH_3$), 7.13 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6\mathit{H}_4CH_3$), 2.36 (s, 9H, $SC_6H_4C\mathit{H}_3$), 1.32 (s, 30H, C_5Me_5) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 8.76, 21.24, 97.66, 128.86, 129.54, 133.14, 138.97 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 252 (84700), 281 (82000), 310 (63800), 396 (30600).

[(η^5-C_5Me_5)$_2$Ir$_2$(μ-SC_6H_4-*p*-Me)$_3$]Cl ([**4**]Cl).

Yield: 115 mg (86%). Elemental analysis (%): calcd. for [$C_{41}H_{51}Ir_2S_3$]Cl: C, 46.46; H, 4.58; found C, 46.28; H, 5.04. ESI-MS (MeOH, CH_2Cl_2): m/z = 1024.4 $[M\text{-}Cl]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6\mathit{H}_4CH_3$), 7.13 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6\mathit{H}_4CH_3$), 2.38 (s, 9H, $SC_6H_4C\mathit{H}_3$), 1.36 (s, 30H, C_5Me_5) ppm. $^{13}C\{^1H\}$ NMR (100 MHz,

$CDCl_3$): δ = 8.50, 21.18, 91.30, 127.08, 129.41, 132.86, 139.42 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 244 (63400), 331 (17500).

[(η^5-C_5Me_5)$_2$$Rh_2$(μ-$SC_6H_4$-*p*-OMe)$_3$]Cl ([**5**]Cl).

Yield: 125 mg (83%). Elemental analysis (%): calcd. for [$C_{41}H_{51}O_3Rh_2S_3$]Cl · HCl: C, 50.99; H, 5.43; found C, 50.83; H, 5.52. ESI-MS (MeOH, CH_2Cl_2): m/z = 894.0 $[M\text{-}Cl]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.72 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4OCH_3$), 6.88 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4OCH_3$), 3.87 (s, 9H, OCH_3), 1.34 (s, 30H, C_5Me_5) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 8.83, 55.55, 97.63, 114.39, 122.75, 134.47, 160.38 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 251 (64600), 281 (65200), 333 (45100), 400 (24400).

[(η^5-C_5Me_5)$_2$$Ir_2$(μ-$SC_6H_4$-*p*-OMe)$_3$]Cl ([**6**]Cl).

Yield: 90 mg (65%). Elemental analysis (%): calcd. for [$C_{41}H_{51}Ir_2O_3S_3$]Cl · 2 HCl: C, 41.70; H, 4.52; found C, 41.72; H, 4.44. ESI-MS (MeOH, CH_2Cl_2): m/z = 1072.3 $[M\text{-}Cl]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.69 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4OCH_3$), 6.88 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4OCH_3$), 3.86 (s, 9H, OCH_3), 1.38 (s, 30H, C_5Me_5) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 8.60, 55.61, 91.32, 114.22, 121.11, 134.21, 160.53 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 249 (73200), 280 (52100), 329 (24800), 361 (17500).

[(η^5-C_5Me_5)$_2$$Rh_2$(μ-$SC_6H_4$-*p*-$Pr^i$)$_3$]Cl ([**7**]Cl).

Yield: 90 mg (58%). Elemental analysis (%): calcd. for [$C_{47}H_{63}Rh_2S_3$]Cl · HCl: C, 56.34; H, 6.44; found C, 56.66; H, 6.37. ESI-MS (MeOH, CH_2Cl_2): m/z = 930.1 $[M\text{-}Cl]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4CH(CH_3)_2$), 7.18 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4CH(CH_3)_2$), 2.92 (sept, 3H, $SC_6H_4CH(CH_3)_2$), 1.31 (s, 30H, C_5Me_5), 1.27 (s, 18H, $SC_6H_4CH(CH_3)_2$) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 8.69, 23.87, 33.77, 97.71, 126.83, 129.22, 133.15, 150.04 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 251 (55400), 281 (51000), 310 (40000), 398 (18700).

$[(\eta^5\text{-}C_5Me_5)_2Ir_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Pr^i)_3]Cl$ ([**8**]Cl).

Yield: 91 mg (63%). Elemental analysis (%): calcd. for $[C_{47}H_{63}Ir_2S_3]Cl \cdot HCl$: C, 47.82; H, 5.46; found C, 47.80; H 5.34. ESI-MS (MeOH, CH_2Cl_2): m/z = 1108.4 $[M\text{-}Cl]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.64 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4CH(CH_3)_2$), 7.19 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4CH(CH_3)_2$), 2.93 (sept, 3H, $SC_6H_4CH(CH_3)_2$), 1.36 (s, 30H, C_5Me_5), 1.27 (s, 18H, $SC_6H_4CH(CH_3)_2$) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 8.45, 23.91, 33.72, 91.34, 126.71, 127.43, 132.87, 150.46 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 245 (78500), 333 (20800).

$[(\eta^5\text{-}C_5Me_5)_2Rh_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Bu^t)_3]Cl$ ([**9**]Cl).

Yield: 123 mg (76%). Elemental analysis (%): calcd. for $[C_{50}H_{69}Rh_2S_3]Cl \cdot H_2O$: C, 58.56; H, 6.98; found C 58.88; H 6.96. ESI-MS (MeOH, CH_2Cl_2): m/z = 972.0 $[M\text{-}Cl]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.69 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4C(CH_3)_3$), 7.33 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4C(CH_3)_3$), 1.33 (s, 30H, C_5Me_5), 1.32 (s, 27H, $SC_6H_4C(CH_3)_3$) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 8.73, 31.31, 34.95, 98.04, 125.98, 129.50, 133.27, 152.60 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 289 (68400), 389 (18000).

$[(\eta^5\text{-}C_5Me_5)_2Ir_2(\mu\text{-}SC_6H_4\text{-}p\text{-}Bu^t)_3]Cl$ ([**10**]Cl).

Yield: 125 mg (84%). Elemental analysis (%): calcd. for $[C_{50}H_{69}Ir_2S_3]Cl \cdot 3\ H_2O$: C, 48.42; H, 6.10; found C, 48.53; H 5.65. ESI-MS (MeOH, CH_2Cl_2): m/z = 1150.3 $[M\text{-}Cl]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.64 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4C(CH_3)_3$), 7.33 (d, $^3J_{HH}$ = 8 Hz, 6H, $SC_6H_4C(CH_3)_3$), 1.35 (s, 30H, C_5Me_5), 1.32 (s, 27H, $SC_6H_4C(CH_3)_3$) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 8.48, 31.33, 34.93, 91.67, 125.87, 127.71, 132.99, 153.03 ppm. UV-vis (1.0 x 10^{-5} M, CH_2Cl_2, 298 K): λ_{max} nm (ε cm^{-1}) = 241 (64500), 320 (15800).

Single-Crystal X-Ray Structure Analysis

Orange crystals were grown by slow diffusion of diethylether into a chloroform and acetone solution for [**3**]Cl · 2 $CHCl_3$ and [**5**]Cl · HCl, while yellow crystals of [**4**]Cl · CH_2Cl_2 were obtained by vapour deposition of pentane into a dichloromethane solution of [**4**]Cl. Data were collected on a Stoe Image Plate Diffraction system equipped with a ϕ circle goniometer, using Mo-Kα graphite monochromatic radiation (λ = 0.71073 Å) with ϕ range 0-200º. 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 [15]. 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. Crystallographic details for [**3**]Cl · 2 $CHCl_3$, [**4**]Cl · CH_2Cl_2 and [**5**]Cl · HCl are summarized in Table 2. Figures 1, 2 and 3 were drawn with Ortep [16].

Table 2. Crystallographic and structure refinement parameters for [**3**]Cl · 2 $CHCl_3$, [**4**]Cl · CH_2Cl_2 and [**5**]Cl · HCl.

	[**3**]Cl · 2 $CHCl_3$	[**4**]Cl · CH_2Cl_2	[**5**]Cl · HCl
Chemical formula	$C_{43}H_{53}Cl_7Rh_2S_3$	$C_{42}H_{53}Cl_3Ir_2S_3$	$C_{41}H_{52}Cl_2O_3Rh_2S_3$
Formula weight	1120.00	1144.77	965.73
Crystal system	Monoclinic	Triclinic	Triclinic
Space group	*P* 2_1 (no. 4)	*P* -1 (no. 2)	*P* -1 (no. 2)
Crystal colour and shape	Orange block	Yellow block	Orange block
Crystal size	0.24 x 0.22 x 0.19	0.17 x 0.15 x 0.14	0.26 x 0.23 x 0.21
a (Å)	10.5286(6)	10.5985(5)	10.5899(5)
b (Å)	21.6699(12)	10.8746(5)	13.4561(6)
c (Å)	10.8306(6)	19.3788(8)	15.5188(8)
α (°)	90	76.066(3)	86.528(4)
β (°)	95.918(4)	78.199(3)	71.991(4)
γ (°)	90	82.418(3)	79.192(4)
V (Å^3)	2457.9(2)	2113.9(2)	2065.7(2)
Z	2	2	2
T (K)	173(2)	173(2)	173(2)
D_c (g·cm^{-3})	1.513	1.799	1.553
μ (mm^{-1})	1.208	6.656	1.117
Scan range (°)	1.88 < θ < 29.28	1.94 < θ < 29.19	2.05 < θ < 29.21
Unique reflections	13302	11406	11164
Observed refls [I>2σ(I)]	12595	9256	8435
R_{int}	0.0397	0.0551	0.0630
Final *R* indices [I>2σ(I)]*	0.0225, wR_2 0.0517	0.0296, wR_2 0.0470	0.0400, wR_2 0.0605
R indices (all data)	0.0249, wR_2 0.0528	0.0456, wR_2 0.0497	0.0665, wR_2 0.0652
Goodness-of-fit	1.011	1.038	1.031
Max, Min Δρ/e (Å^{-3})	0.579, - 0.549	0.911, - 1.823	0.584, - 0.876

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

CCDC 989349 [**3**]Cl · 2 $CHCl_3$, 989350 [**4**]Cl · CH_2Cl_2 and 989351 [**5**]Cl · HCl 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.

Cell Culture and Inhibition of Cell Growth

Human A2780 ovarian carcinoma cells and HEK293 cells were obtained from the European Collection of Cell Cultures (ECACC) (Salisbury, UK). Cells were cultured in either RPMI-1640 with GlutaMAX (A2780) or DMEM (Dulbecco's Modified Eagle Medium) high glucose with GlutaMAX (HEK 293) medium containing 10% fetal bovine serum (FBS) and penicillin at 37°C and 5% CO_2. Cytotoxicity was determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) assay (see below). Cells were seeded in 96 well plates by the addition of cells as a suspension in their respective media containing 10% FBS (100 μL per well, approximately 4300 cells) and pre-incubated for 24 h.

Fresh stock solutions of the compounds were prepared in DMSO just before injections, then the stock solution were diluted by addition to the culture medium [RPMI (Roswell Park Memorial Institute medium) or DMEM for A2780 and HEK 293, respectively]. The stock solutions were serially diluted to give compound solutions of the desired concentrations. Complex solutions (100 μL) were then added to plate wells (yielding final compound solutions in the range 0 to 5 or 0 to 1 μM) and the plates incubated for a further 72 h.

Subsequently, MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) solution (20 μL, 5 mg/mL in H_2O) was added to each well and the plates incubated for a further 2 h. The culture medium was then aspirated and the violet formazan precipitate produced by mitochondrial dehydrogenases of living cells was dissolved by the addition of DMSO (100 μL) to each well. The absorbance of the resultant solutions at 590 nm, which is directly proportional to the number of surviving cells, was recorded using a multiwell plate reader. The percentage of surviving cells was determined by measurement of the absorbance of wells corresponding to untreated control cells. The reported IC_{50} values are based on the mean values from two independent experiments; each concentration level per experiment was evaluated in triplicate, and those values are reported in Table 1.

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