Published in Dalton Transactions 39, issue 11, 2816-2820, 2010
which should be used for any reference to this work

Anticancer activity of osmium metalla-rectangles

Nicolas P. E. Barry,[a] Fabio Edafe,[b] Paul J. Dyson[b] and Bruno Therrien*[a]

A series of cationic metalla-rectangles of the general formula $[(p\text{-cymene})_4Os_4(OO\cap OO)_2(N\cap N)_2]^{4+}$ have been obtained in methanol from the dinuclear arene osmium precursors $[(p\text{-cymene})_2Os_2(OO\cap OO)_2Cl_2]$ ($OO\cap OO$ = 2,5-dioxydo-1,4-benzoquinonato (dhbq), 2,5-dichloro-1,4-benzoquinonato (dcbq)) by reaction with bipyridine linkers ($N\cap N$ = 4,4′-bipyridine, 1,2-bis(4-pyridyl)ethylene) in the presence of $AgCF_3SO_3$. All complexes were isolated as triflate salts and characterised by NMR, IR and UV-visible spectroscopy. The cytotoxicities of the dinuclear and tetranuclear osmium complexes were established using ovarian A2780 cancer cell lines. The most active metalla-rectangle, $[(p\text{-cymene})_4Os_4(dhbq)_2(4,4'\text{-bipyridine})_2]^{4+}$, shows an IC_{50} value of 5.7 μM (comparable to cisplatin) against A2780 cancer cells and 7.5 μM against the cisplatin resistant A2780cisR cells.

Introduction

Almost 20 years have passed since Fujita's group synthesised the first metalla-squares, $[(en)_4M_4(4,4'\text{-bipyridine})_4]^{8+}$ (en = ethylenediamine; M = Pd, Pt) (Chart 1).[1] Only recently the biological potential of such compounds has been studied, despite the tremendous success of cisplatin,[2] one of the most widely used anticancer metal-based drugs.[3] These studies showed that the water soluble complex $[(en)_4Pt_4(4,4'\text{-bipyridine})_4]^{8+}$ possesses a good binding affinity for G-quadruplex DNA,[4] and a cytotoxicity comparable to cisplatin was observed against HL-60 tumour cells.[5]

The application of multinuclear Pt compounds to overcome serious side effects and resistance mechanisms associated with cisplatin is not new.[6] The trinuclear compound $[(NH_3)_2ClPtNH_2(CH_2)_6NH_2Pt(NH_3)_2NH_2(CH_2)_6NH_2PtCl(NH_3)_2]^{4+}$ (BBR3464) was found to be 2 to 3 orders of magnitude more active than cisplatin and even entered phase II clinical trials before being abandoned.[7] Arene ruthenium complexes have also been evaluated as putative anticancer agents, and appear to exert their cytotoxic effect *via* a different mode of action to that of cisplatin, and could potentially overcome the limitations of cisplatin.[8] However, the multinuclearity approach is a relatively under exploited concept in arene ruthenium systems.[9] The dinuclear complexes $[(p\text{-cymene})_2Ru_2(OO\cap OO)Cl_2]$ ($OO\cap OO$ = $MeC_5H_2O_2N(CH_2)_nNC_5H_2MeO_2$) show relevant cytotoxicities towards human cancer cell lines and unique DNA binding properties.[10] Similarly, the Ru_2Fe trinuclear complex $[(p\text{-cymene})_2Ru_2(N\cap N)Cl_2]$ ($N\cap N$ = $NC_5H_4OOC\text{-}C_5H_4FeC_5H_4\text{-}COOC_5H_4N$) was found to be equally cytotoxic against A2780 and A2780cisR (cisplatin resistant) cancer cells,[11] and the dinuclear complex $[(indane)_2Ru_2Cl_2(2,3\text{-bis}(2\text{-pyridyl})pyrazine)]$ has been studied as a potential photochemical agent in cancer cells.[12] Trinuclear arene ruthenium clusters have shown remarkable cytotoxicity[13] and a series of tetranuclear arene ruthenium complexes containing a porphyrin core demonstrated excellent photodynamic properties.[14] Tetra- and octanuclear arene ruthenium complexes attached to first and second generation polypyridyl dendritic cores were found to be cytotoxic with a good correlation between size and cytotoxicity.[15] We also synthesised rectangular tetranuclear arene ruthenium complexes incorporating 2,5-dioxydo-1,4-benzoquinonato (dhbq) or 2,5-dichloro-1,4-benzoquinonato (dcbq) and $N\cap N$ linkers ($N\cap N$ = 4,4′-bipyridine, 1,2-bis(4-pyridyl)ethylene) which proved to be

[a]*Institute of Chemistry, University of Neuchatel, 51 Ave de Bellevaux, CH-2000, Neuchatel, Switzerland. E-mail: bruno.therrien@unine.ch; Fax: +41 32 7182511*
[b]*Institut des Sciences et Ingénierie Chimique, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015, Lausanne, Switzerland*

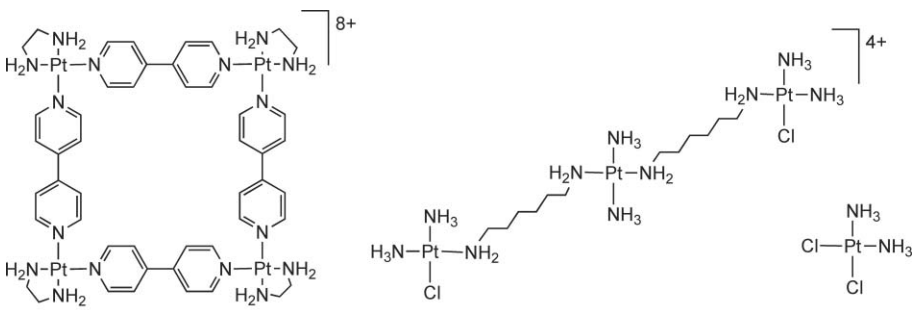


Chart 1

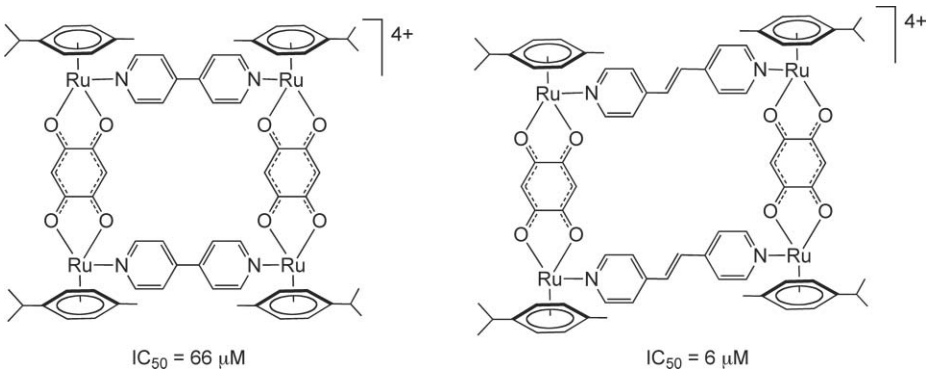


Chart 2

active against A2780 cancer cells.[16] The smaller metalla-rectangle $[(p\text{-cymene})_4Ru_4(dhbq)_2(N\cap N)_2]^{4+}$ containing 4,4′-bipyridine ligands was less cytotoxic [$IC_{50} = 66$ μM] than the larger metalla-rectangle containing 1,2-bis(4-pyridyl)ethylene ligands [$IC_{50} = 6$ μM], which suggested a pronounced size effect (Chart 2).

Therefore, in order to compare the cytotoxicities and to better understand the mechanisms of action in human ovarian cancer cell lines, we have prepared a new series of tetranuclear metalla-rectangles containing the same building blocks, but with osmium centres, $[(p\text{-cymene})_4Os_4(OO\cap OO)_2(N\cap N)_2]^{4+}$ ($N\cap N$ = 4,4′-bipyridine, 1,2-bis(4-pyridyl)ethylene). Indeed, in recent years, arene osmium complexes have started to be evaluated for anticancer activity and some exhibit promising cytotoxicities.[17]

Results and discussion

The tetranuclear arene osmium complexes $[(p\text{-cymene})_4Os_4(OO\cap OO)_2(N\cap N)_2]^{4+}$ ($OO\cap OO$ = 2,5-dioxydo-1,4-benzoquinonato (dhbq), 2,5-dichloro-1,4-benzoquinonato (dcbq); $N\cap N$ = 4,4′-bipyridine, 1,2-bis(4-pyridyl)ethylene) were prepared from the dinuclear complexes $[Os_2(p\text{-cymene})_2(OO\cap OO)Cl_2]$ (**1**, $OO\cap OO$ = dhbq; **2**, $OO\cap OO$ = dcbq). The coordinatively unsaturated intermediate formed upon addition of silver triflate reacts at room temperature in the presence of the $N\cap N$ donor ligands to give the corresponding tetranuclear cations **3–6**, which are stabilised as triflate salts, $[(p\text{-cymene})_4Os_4(dhbq)_2(4,4'\text{-bipyridine})_2][CF_3SO_3]_4$ ([**3**][CF_3SO_3]$_4$), $[(p\text{-cymene})_4Os_4(dcbq)_2(4,4'\text{-bipyridine})_2][CF_3SO_3]_4$ ([**4**][CF_3SO_3]$_4$), $[(p\text{-cymene})_4Os_4(dhbq)_2(1,2\text{-bis(4-pyridyl)ethylene})_2][CF_3SO_3]_4$ ([**5**][CF_3SO_3]$_4$) and $[(p\text{-cymene})_4Os_4(dcbq)_2(1,2\text{-bis(4-pyridyl)ethylene})_2][CF_3SO_3]_4$ ([**6**][CF_3SO_3]$_4$), see Scheme 1. Compounds [**3–6**][CF_3SO_3]$_4$ are soluble in polar organic solvents such as acetonitrile, acetone, methanol and also dimethylsulfoxide. It is worth noting that the precursor compounds **1** and **2** are only sparingly soluble in these solvents.

The ^{1}H NMR spectra of **1**, **3** and **5** display a singlet due to the dhbq protons (H_{dhbq}) at $\delta \approx 5.9$ ppm. Complexes **5** and **6** display a singlet due to the ethylene protons ($H_{C=C}$) at 7.4 ppm. Upon formation of the cationic tetranuclear metalla-rectangles, the methyl and isopropyl signals of the *p*-cymene ligands in **3–6** remain almost unchanged as compared to complexes **1** and **2**. In contrast, the aromatic protons of the *p*-cymene ligands are shifted downfield in the dcbq metalla-rectangles **4** and **6** relative to their dhbq analogues **3** and **5**, see Fig. 1. Similarly, a downfield shift of the pyridyl protons of the 4,4′-bipyridine linkers in **3** and **4** is observed compared to their 1,2-bis(4-pyridyl)ethylene analogues **5** and **6**. The infrared spectra of **3–6** are dominated by absorptions of the coordinated $N\cap N$ and $OO\cap OO$ ligands, which are only slightly shifted as compared to the free ligands. In addition to the $N\cap N$ and $OO\cap OO$ signals, strong absorptions due to the triflate anions [1260(s), 1030(s), 638(m) cm^{-1}] are also observed in the infrared spectra of the salts [**3–6**][CF_3SO_3]$_4$.

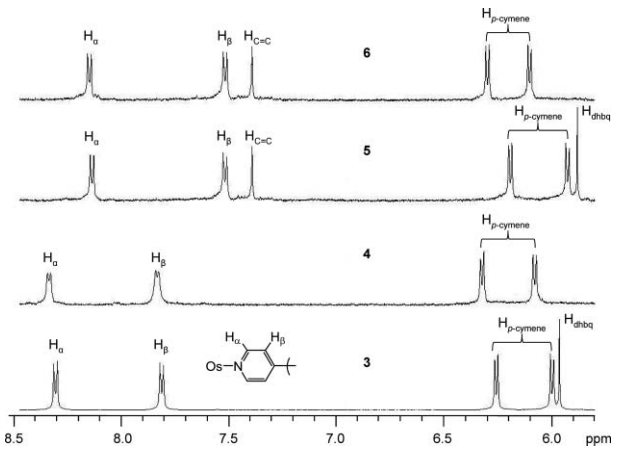
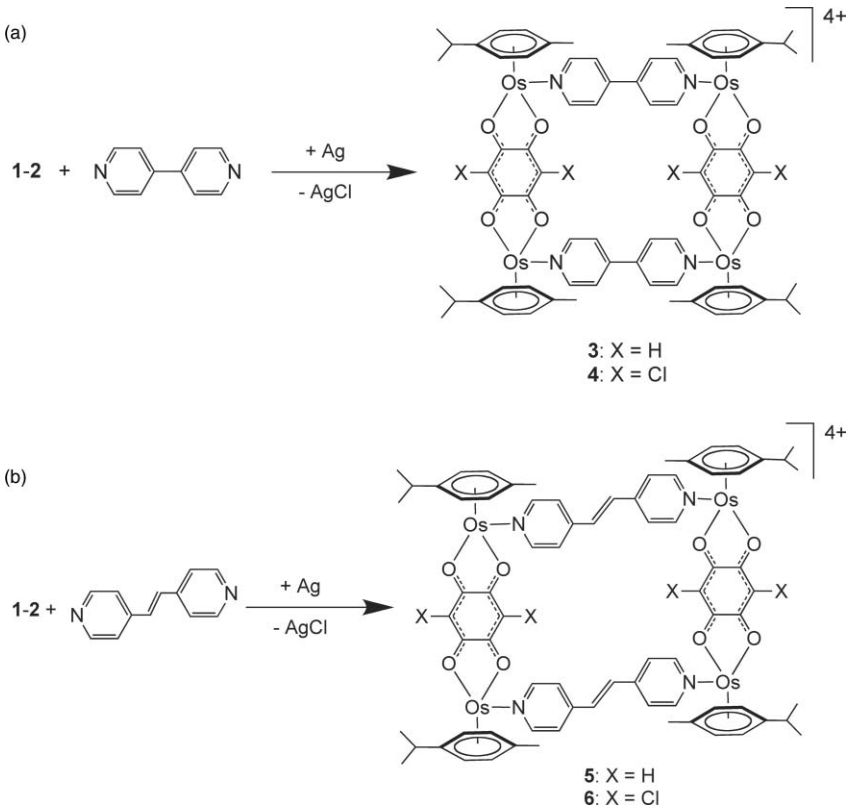


Fig. 1 ^{1}H NMR spectra in CD_3CN of metalla-rectangles **3–6**, showing the pyridyl and ethylene region of the $N\cap N$ linkers and the aromatic region of the *p*-cymene and $OO\cap OO$ ligands.

Electronic absorption spectra of the tetranuclear metalla-rectangles **3–6** as well as the dinuclear complexes **1** and **2** were acquired in acetone at 10^{-5} M concentration in the range 250–800 nm, see Fig. 2. The UV-visible spectra of these complexes are characterised by an intense high-energy band centred at 310 nm, which is assigned to a ligand-localised or intra-ligand $\pi \rightarrow \pi^*$ transition, as well as broad low-energy bands associated with metal-to-ligand charge transfer (MLCT) transitions. In **1** and **2**, only one MLCT band is found ($\approx$ 600 nm), while in metalla-rectangles **3–6** an additional band centred at 400 nm is observed as well. The UV-visible spectra of **3–6** remain the same after several weeks in solution (acetone and acetonitrile), thus indicating a great stability of the metalla-rectangles.

The antiproliferative activity of all the complexes was evaluated against the A2780 (cisplatin sensitive) and A2780cisR (cisplatin resistant) human ovarian cancer cell lines. The cytotoxicities of the



Scheme 1 Synthesis of the metalla-rectangles **3–6**.

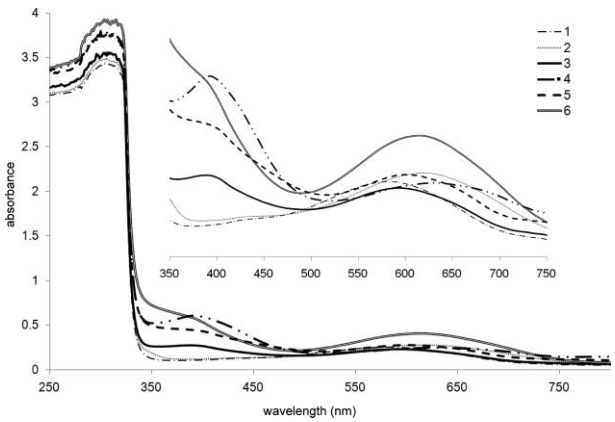


Fig. 2 UV-visible spectra of **1–6** in acetone (10^{-5} M).

dinuclear complexes **1** and **2** and the tetranuclear arene osmium complexes **3–6** and comparisons with appropriate ruthenium analogues are presented in Table 1. The majority of the compounds display either limited or no cytotoxicity towards both cell lines with the exception of **5** that is slightly cytotoxic in the A2780 cell line (IC_{50} = 59 μM) and **3** that is very cytotoxic towards both the sensitive and resistant cell lines (5.7 and 7.5 μM, respectively). Note that both **3** and **5** contain the linker that is devoid of chlorine. What is particularly striking from Table 1 is the poor correlation of the cytotoxicities between the osmium and ruthenium analogues. The ruthenium analogue of **3** is the least cytotoxic of the series and the ruthenium analogue of **5** is the most cytotoxic of the ruthenium compounds available for comparison. Nevertheless,

Table 1 IC_{50} values of complexes **1–6** in A2780 and A2780cisR cell lines

Compound	A2780 (IC_{50}, μM)[a]		A2780cisR (IC_{50}, μM)
1	135.4 ± 4.5		>300
2	223.3 ± 18.2		176.0 ± 21.9
3	5.4 ± 0.6	[66 μM]	7.4 ± 0.4
4	>300	[43 μM]	>300
5	56.8 ± 0.9	[6 μM]	132.5 ± 13.5
6	168.4 ± 20.4	[29 μM]	201.1 ± 32.3
cisplatin	1.6		8.6

[a] In brackets the IC_{50} values of the ruthenium analogues are given.[16]

the ruthenium analogue of **5** and the osmium compound **3** are of essentially equivalent activity and are both significantly more active than cisplatin in the A2780cisR cell line, although it should be noted that there are four times more metal ions present.

Conclusion

A series of cationic metalla-rectangles based on osmium have been prepared and characterised. The compounds are stable, and based on promising results obtained for analogous ruthenium compounds, were evaluated for *in vitro* anticancer activity. One of the tetraosmium complexes was found to be as active as one of the ruthenium-based analogues (although not the direct analogue). In general osmium compounds are thought to be less cytotoxic than ruthenium compounds and therefore this study may lead to increased interest in the anticancer (and other biological) properties of osmium compounds.

Experimental

General

2,5-Dihydroxy-1,4-benzoquinone, 2,5-dichloro-1,4-benzoquinone, silver trifluoromethanesulfonate, 4,4′-bipyridine and 1,2-bis(4-pyridyl)ethylene were purchased from Sigma-Aldrich and used as received. $[(p\text{-cymene})_2Os_2(dhbq)Cl_2]$ and $[(p\text{-cymene})_2Os_2(dcbq)Cl_2]$ were prepared according to published methods[18] from $[(p\text{-cymene})OsCl_2]_2$,[19] instead of $[(p\text{-cymene})RuCl_2]_2$. All other reagents were commercially available (Sigma-Aldrich) and used as received. The ^1H, $^{13}C\ \{^1H\}$, and ^1H ROESY NMR spectra were recorded on a Bruker AvanceII 400 spectrometer using the residual protonated solvent as internal standard. Infrared spectra were recorded as KBr pellets on a Perkin-Elmer FTIR 1720 X spectrometer. UV-visible absorption spectra were recorded on an Uvikon 930 spectrophotometer using precision cells made of quartz (1 cm). Elemental analyses were performed by the Laboratory of Pharmaceutical Chemistry, University of Geneva (Switzerland).

Syntheses

Metalla-Clips 1 and 2. A mixture of $[(p\text{-cymene})OsCl_2]_2$ (500 mg, 0.71 mmol) and the appropriate quinone (**1**, 2,5-dihydroxy-1,4-benzoquinone, 99.5 mg, 0.71 mmol; **2**, 2,5-dichloro-1,4-benzoquinone, 125.7 mg, 0.71 mmol) in methanol (100 mL) was stirred at room temperature for 2 h, then filtered. The black precipitate was washed with diethyl ether, and dried under vacuum.

1. Yield: 486 mg (80%). IR: ν/cm^{-1}: 3056 (w, aromatic, C=C), 1515 (s, dhbq, C=O). ^1H NMR (400 MHz, $CDCl_3$, 298 K): δ (ppm) = 6.25 (d, ^3J = 6.2 Hz, 4H, H_{ar}), 5.94 (d, 4H, H_{ar}), 5.90 (s, 2H, H_q), 2.70 (sept, 2H, C**H**$(CH_3)_2$), 2.26 (s, 6H, CH_3), 1.32 (d, ^3J = 2.3 Hz, 12H, CH(C**H**$_3)_2$). $^{13}C\ \{^1H\}$ NMR (100 MHz, $CDCl_3$, 298 K): δ (ppm) = 185.9 (**C**O), 101.1 (**C**H_q), 92.3 (**C**$_{ar}$), 89.2 (**C**$_{ar}$), 74.3 (**C**H_{ar}), 70.9 (**C**H_{ar}), 32.9 (**C**H$(CH_3)_2$), 22.8 (CH(**C**$H_3)_2$), 19.3 (**C**H_3). UV-vis (1.0×10^{-5} M, $(CH_3)_2CO$): λ_{max} 583 nm ($\varepsilon = 2.52 \times 10^4$ M^{-1} cm^{-1}). Elemental Analysis (%): Calc. for $C_{26}H_{30}Cl_2O_4Os_2$ (857.9): C, 36.40; H, 3.52; Found: C, 36.56; H, 3.62.

2. Yield: 490 mg (74%). IR: ν/cm^{-1}: 3056 (w, aromatic, C=C), 1515 (s, dcbq, C=O). ^1H NMR (400 MHz, $CDCl_3$, 298 K): δ (ppm) = 6.32 (d, ^3J = 6.3 Hz, 4H, H_{ar}), 6.16 (d, 4H, H_{ar}), 2.81 (sept, 2H, C**H**$(CH_3)_2$), 2.40 (s, 6H, CH_3), 1.35 (d, ^3J = 2.3 Hz, 12H, CH(C**H**$_3)_2$). $^{13}C\ \{^1H\}$ NMR (100 MHz, $CDCl_3$, 298 K): δ (ppm) = 186.1 (**C**O), 110.5 (**C**$_q$), 94.7 (**C**$_{ar}$), 90.5 (**C**$_{ar}$), 74.6 (**C**H_{ar}), 71.5 (**C**H_{ar}), 32.8 (**C**H$(CH_3)_2$), 22.8 (CH(**C**$H_3)_2$), 19.2 (**C**H_3). UV-vis (1.0×10^{-5} M, $(CH_3)_2CO$): λ_{max} 618 nm ($\varepsilon = 2.81 \times 10^4$ M^{-1} cm^{-1}). Elemental Analysis (%): Calc. for $C_{26}H_{28}Cl_4O_4Os_2$ (926.8): C, 33.69; H, 3.02; Found: C, 33.36; H, 3.08.

Metalla-Rectangles [3–6][CF_3SO_3]$_4$. $AgCF_3SO_3$ (149.0 mg, 0.58 mmol) was added to a suspension of $[(p\text{-cymene})_2Os_2(dhbq)Cl_2]$ (**3** and **5**, 248.5 mg, 0.29 mmol) or $[(p\text{-cymene})_2Os_2(dcbq)Cl_2]$ (**4** and **6**, 268.1 mg, 0.29 mmol) in methanol (50 mL) at room temperature and stirred for 3 h, followed by filtration to remove AgCl. Then, 4,4′-bipyridine (**3** and **4**, 45.3 mg, 0.29 mmol) or 1,2-bis(4-pyridyl)ethylene (**5** and **6**, 52.8 mg, 0.29 mmol) was added to the filtrate. The solution was stirred at reflux for 12 h. The solvent was removed and the residue extracted with dichloromethane. The filtrate was concentrated to about 2 mL and diethyl ether was added to give the corresponding products as black powders.

[3][CF_3SO_3]$_4$. Yield: 43 mg (12%). IR: ν/cm^{-1}: 3071 (m, aromatic, C=C), 1630 (s, dhbq, C=O), 1261 (s, triflate, C–F). ^1H NMR (400 MHz, CD_3CN, 298 K): δ (ppm) = 8.31 (d, ^3J = 8.3 Hz, 8H, H_α), 7.82 (d, 8H, H_β), 6.26 (d, ^3J = 6.3 Hz, 8H, H_{ar}), 6.00 (d, 8H, H_{ar}), 5.97 (s, 4H, H_q), 2.75 (sept, 4H, C**H**$(CH_3)_2$), 2.18 (s, 12H, CH_3), 1.29 (d, ^3J = 2.3 Hz, 24H, CH(C**H**$_3)_2$). $^{13}C\ \{^1H\}$ NMR (100 MHz, CD_3CN, 298 K): δ (ppm) = 186.6 (**C**O), 154.8 (**C**H_α), 145.8 (**C**$_{pyr}$), 124.4 (**C**H_β), 101.8 (**C**H_q), 95.7 (**C**$_{ar}$), 91.7 (**C**$_{ar}$), 76.8 (**C**H_{ar}), 73.9 (**C**H_{ar}), 32.5 (**C**H$(CH_3)_2$), 22.8 (CH(**C**$H_3)_2$), 18.4 (**C**H_3). UV-vis (1.0×10^{-5} M, $(CH_3)_2CO$): λ_{max} 400 nm ($\varepsilon = 2.66 \times 10^4$ M^{-1} cm^{-1}), λ_{max} 593 nm ($\varepsilon = 2.31 \times 10^4$ M^{-1} cm^{-1}). Elemental Analysis (%): Calc. for $C_{76}H_{76}F_{12}N_4O_{20}Os_4S_4 \cdot CH_2Cl_2$ (2567.5): C, 36.02; H, 3.06; N, 2.18; Found: C, 36.09; H, 2.94; N, 2.04.

[4][CF_3SO_3]$_4$. Yield: 33 mg (9%). IR: ν/cm^{-1}: 3070 (m, aromatic, C=C), 1628 (s, dcbq, C=O), 1260 (s, triflate, C–F). ^1H NMR (400 MHz, CD_3CN, 298 K): δ (ppm) = 8.33 (d, ^3J = 8.3 Hz, 8H, H_α), 7.83 (d, 8H, H_β), 6.32 (d, ^3J = 6.3 Hz, 8H, H_{ar}), 6.09 (d, 8H, H_{ar}), 2.75 (sept, 4H, C**H**$(CH_3)_2$), 2.17 (s, 12H, CH_3), 1.35 (d, ^3J = 2.9 Hz, 24H, CH(C**H**$_3)_2$). $^{13}C\ \{^1H\}$ NMR (100 MHz, CD_3CN, 298 K): δ (ppm) = 180.7 (**C**O), 154.9 (**C**H_α), 145.7 (**C**$_{pyr}$), 124.5 (**C**H_β), 110.2 (**C**$_q$), 89.2 (**C**$_{ar}$), 88.8 (**C**$_{ar}$), 77.0 (**C**H_{ar}), 74.9 (**C**H_{ar}), 32.6 (**C**H$(CH_3)_2$), 22.7 (CH(**C**$H_3)_2$), 18.6 (**C**H_3). UV-vis (1.0×10^{-5} M, $(CH_3)_2CO$): λ_{max} 404 nm ($\varepsilon = 4.22 \times 10^4$ M^{-1} cm^{-1}), λ_{max} 607 nm ($\varepsilon = 2.75 \times 10^4$ M^{-1} cm^{-1}). Elemental Analysis (%): Calc. for $C_{76}H_{72}Cl_4F_{12}N_4O_{20}Os_4S_4$ (2620.4): C, 34.82; H, 2.75; N, 2.14; Found: C, 35.12; H, 2.98; N, 2.35.

[5][CF_3SO_3]$_4$. Yield: 38 mg (10%). IR: ν/cm^{-1}: 3070 (m, aromatic, C=C), 1630 (s, dhbq, C=O), 1262 (s, triflate, C–F). ^1H NMR (400 MHz, CD_3CN, 298 K): δ (ppm) = 8.15 (d, ^3J = 6.9 Hz, 8H, H_α), 7.53 (d, 8H, H_β), 7.39 (s, 4H, $H_{C=C}$), 6.19 (d, ^3J = 6.5 Hz, 8H, H_{ar}), 5.92 (d, 8H, H_{ar}), 5.89 (s, 4H, H_q), 2.73 (sept, 4H, C**H**$(CH_3)_2$), 2.17 (s, 12H, CH_3), 1.30 (d, ^3J = 5.8 Hz, 24H, CH(C**H**$_3)_2$). $^{13}C\ \{^1H\}$ NMR (100 MHz, CD_3CN, 298 K): δ (ppm) = 186.6 (**C**O), 154.0 (**C**H_α), 153.9 (**C**$_{pyr}$), 134.8 (**C**H=**C**H), 124.7 (**C**H_β), 101.6 (**C**H_q), 91.4 (**C**$_{ar}$), 89.2 (**C**$_{ar}$), 76.6 (**C**H_{ar}), 73.9 (**C**H_{ar}), 32.5 (**C**H$(CH_3)_2$), 22.8 (CH(**C**$H_3)_2$), 18.4 (**C**H_3). UV-vis (1.0×10^{-5} M, $(CH_3)_2CO$): λ_{max} 393 nm ($\varepsilon = 5.75 \times 10^4$ M^{-1} cm^{-1}), λ_{max} 617 nm ($\varepsilon = 2.45 \times 10^4$ M^{-1} cm^{-1}). Elemental Analysis (%): Calc. for $C_{80}H_{80}F_{12}N_4O_{20}Os_4S_4$ (2534.7): C, 37.90; H, 3.16; N, 2.21; Found: C, 37.36; H, 3.02; N, 2.18.

[6][CF_3SO_3]$_4$. Yield: 47 mg (12%). IR: ν/cm^{-1}: 3072 (m, aromatic, C=C), 1632 (s, dcbq, C=O), 1258 (s, triflate, C–F). ^1H NMR (400 MHz, CD_3CN, 298 K): δ (ppm) = 8.13 (d, ^3J = 7.1 Hz, 8H, H_α), 7.51 (d, 8H, H_β), 7.40 (s, 4H, $H_{C=C}$), 6.30 (d, ^3J = 6.5 Hz, 8H, H_{ar}), 6.09 (d, 8H, H_{ar}), 2.74 (sept, 4H, C**H**$(CH_3)_2$), 2.14 (s, 12H, CH_3), 1.32 (d, ^3J = 5.2 Hz, 24H, CH(C**H**$_3)_2$). $^{13}C\ \{^1H\}$ NMR (100 MHz, CD_3CN, 298 K): δ (ppm) = 183.2 (**C**O), 153.8 (**C**H_α), 153.6 (**C**$_{pyr}$), 134.7 (**C**H=**C**H), 123.2 (**C**H_β), 111.3 (**C**$_q$), 92.6 (**C**$_{ar}$), 90.2 (**C**$_{ar}$), 77.2 (**C**H_{ar}), 74.8 (**C**H_{ar}), 32.5 (**C**H$(CH_3)_2$), 22.7 (CH(**C**$H_3)_2$), 18.5 (**C**H_3). UV-vis (1.0×10^{-5} M, $(CH_3)_2CO$): λ_{max} 387 nm ($\varepsilon = 5.91 \times 10^4$ M^{-1} cm^{-1}), λ_{max} 622 nm ($\varepsilon = 4.01 \times 10^4$ M^{-1} cm^{-1}). Elemental Analysis (%): Calc. for $C_{80}H_{76}Cl_4F_{12}N_4O_{20}Os_4S_4$ (2672.5): C, 35.94; H, 2.84; N, 2.09; Found: C, 35.16; H, 2.61; N, 2.03.

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 RPMI 1640 medium with GlutaMAX(tm) containing 5% foetal calf serum (FCS) and antibiotics (penicillin and ciproxin) at 37 °C and 6% CO_2. For the evaluation of growth inhibition tests, the cells were seeded in 96-well plates and grown for 24 h in complete medium. Complexes were diluted to the required concentration and added to the cell culture for 72 h incubation. Solutions of the compounds were applied by diluting a freshly prepared stock solution of the corresponding compound in aqueous RPMI medium with GlutaMAX(tm) (20 mM). The MTT test was performed in the last 2 h without changing the culture medium. Following drug exposure, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to the cells at a final concentration of 0.25 mg ml^{-1} and incubated for 2 h, then the culture medium was aspirated and the violet formazan (artificial chromogenic precipitate of the reduction of tetrazolium salts by dehydrogenases and reductases) dissolved in DMSO. The optical density of each well (96-well plates) was quantified three times in tetraplicates at 540 nm using a multiwell plate reader (iEMS Reader MF, Labsystems, US), and the percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. The IC_{50} values for the inhibition of cell growth were determined by fitting the plot of the logarithmic percentage of surviving cells against the logarithm of the drug concentration using a linear regression function. An MS-Excel add-in for calculating the standard deviation of the median absolute deviation (SMAD) is available on the website of the Royal Society of Chemistry (http://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/Software/RobustStatistics.asp). The median value and the median absolute deviation were obtained from this add-in and those values are reported in Table 1.

References

1 M. Fujita, J. Yazaki and K. Ogura, *J. Am. Chem. Soc.*, 1990, **112**, 5645.
2 B. Rosenberg, L. Van Camp, J. E. Trosko and V. H. Mansour, *Nature*, 1969, **222**, 385–386.
3 (*a*) J. Reedijk, *Chem. Commun.*, 1996, 801; (*b*) E. Wong and C. M. Giandomenico, *Chem. Rev.*, 1999, **99**, 2451; (*c*) *Cisplatin*, ed B. Lippert, Verlag Helvetica Chimica Acta, Zürich, 1999; (*d*) T. Boulikas and M. Vougiouka, *Oncol. Rep.*, 2003, **10**, 1663; (*e*) Y. Jung and S. J. Lippard, *Chem. Rev.*, 2007, **107**, 1387; (*f*) D. Gibson, *Dalton Trans.*, 2009, 10681.
4 R. Kieltyka, P. Englebienne, J. Fakhoury, C. Autexier, N. Moitessier and H. F. Sleiman, *J. Am. Chem. Soc.*, 2008, **130**, 10040.
5 M. Mounir, J. Lorenzo, M. Ferrer, M. J. Prieto, O. Rossell, F. X. Avilès and V. Moreno, *J. Inorg. Biochem.*, 2007, **101**, 660.
6 C. Manzotti, G. Pratesi, E. Menta, R. Di Domenico, E. Cavalletti, H. H. Fiebig, L. R. Kelland, N. Farrell, D. Polizzi, R. Supino, G. Pezzoni and F. Zunino, *Clin. Cancer Res.*, 2000, **6**, 2626.
7 T. A. Hensing, N. H. Hanna, H. H. Gillenwater, M. Gabriella Camboni, C. Allievi and M. A. Socinski, *Anti-Cancer Drugs*, 2006, **17**, 697.
8 (*a*) M. J. Clarke, *Coord. Chem. Rev.*, 2003, **236**, 209; (*b*) Y. K. Yan, M. Melchart, A. Habtemariam and P. J. Sadler, *Chem. Commun.*, 2005, 4764; (*c*) W. H. Ang and P. J. Dyson, *Eur. J. Inorg. Chem.*, 2006, 4003; (*d*) P. J. Dyson and G. Sava, *Dalton Trans.*, 2006, 1929.
9 G. Süss-Fink, *Dalton Trans.*, 2010, **39**, 1673.
10 (*a*) M.-G. Mendoza-Ferri, C. G. Hartinger, R. E. Eichinger, N. Stolyarova, K. Severin, M. A. Jakupec, A. A. Nazarov and B. K. Keppler, *Organometallics*, 2008, **27**, 2405; (*b*) M. G. Mendoza-Ferri, C. G. Hartinger, M. A. Mendoza, M. Groessl, A. E. Egger, R. E. Eichinger, J. B. Mangrum, N. P. Farrell, M. Maruszak, P. J. Bednarski, F. Klein, M. A. Jakupec, A. A. Nazarov, K. Severin and B. K. Keppler, *J. Med. Chem.*, 2009, **52**, 916; (*c*) M. G. Mendoza-Ferri, C. G. Hartinger, A. A. Nazarov, R. E. Eichinger, M. A. Jakupec, K. Severin and B. K. Keppler, *Organometallics*, 2009, **28**, 6260; (*d*) O. Novakova, A. A. Nazarov, C. G. Hartinger, B. K. Keppler and V. Brabec, *Biochem. Pharmacol.*, 2009, **77**, 364.
11 M. Auzias, B. Therrien, G. Süss-Fink, P. Štěpnička, W. H. Ang and P. J. Dyson, *Inorg. Chem.*, 2008, **47**, 578.
12 S. W. Magennis, A. Habtemariam, O. Novakova, J. B. Henry, S. Meier, S. Parsons, I. D. H. Oswald, V. Brabec and P. J. Sadler, *Inorg. Chem.*, 2007, **46**, 5059.
13 B. Therrien, W. H. Ang, F. Chérioux, L. Vieille-Petit, L. Juillerat-Jeanneret, G. Süss-Fink and P. J. Dyson, *J. Cluster Sci.*, 2007, **18**, 741.
14 (*a*) F. Schmitt, P. Govindaswamy, G. Süss-Fink, W. H. Ang, P. J. Dyson, L. Juillerat-Jeanneret and B. Therrien, *J. Med. Chem.*, 2008, **51**, 1811; (*b*) F. Schmitt, P. Govindaswamy, O. Zava, G. Süss-Fink, L. Juillerat-Jeanneret and B. Therrien, *JBIC, J. Biol. Inorg. Chem.*, 2009, **14**, 101.
15 P. Govender, N. C. Antonels, J. Mattsson, A. K. Renfrew, P. J. Dyson, J. R. Moss, B. Therrien and G. S. Smith, *J. Organomet. Chem.*, 2009, **694**, 3470.
16 J. Mattsson, P. Govindaswamy, A. K. Renfrew, P. J. Dyson, P. Štěpnička, G. Süss-Fink and B. Therrien, *Organometallics*, 2009, **28**, 4350.
17 (*a*) A. Dorcier, P. J. Dyson, C. Gossens, U. Rothlisberger, R. Scopelliti and I. Tavernelli, *Organometallics*, 2005, **24**, 2114; (*b*) A. Dorcier, C. G. Hartinger, R. Scopelliti, R. H. Fish, B. K. Keppler and P. J. Dyson, *J. Inorg. Biochem.*, 2008, **102**, 1066; (*c*) P. C. A. Bruijnincx and P. J. Sadler, *Adv. Inorg. Chem.*, 2009, **61**, 1and references therein; (*d*) S. H. van Rijt, A. J. Hebden, T. Amaresekera, R. J. Deeth, G. J. Clarkson, S. Parsons, P. C. McGowan and P. J. Sadler, *J. Med. Chem.*, 2009, **52**, 7753.
18 (*a*) B. Therrien, G. Süss-Fink, P. Govindaswamy, A. K. Renfrew and P. J. Dyson, *Angew. Chem., Int. Ed.*, 2008, **47**, 3773; (*b*) J. Mattsson, P. Govindaswamy, J. Furrer, Y. Sei, K. Yamaguchi, G. Süss-Fink and B. Therrien, *Organometallics*, 2008, **27**, 4346.
19 R. Castarlenas, M. A. Esteruelas and E. Oñate, *Organometallics*, 2005, **24**, 4343.