ELSEVIER

Inorganica Chimica Acta 273 (1998) 62–71

Inorganica Chimica Acta

Rhodium(III) analogues of antitumour-active ruthenium(III) compounds: the crystal structure of [ImH][*trans*-$RhCl_4(Im)_2$] (Im = imidazole) [1]

Giovanni Mestroni [a,*], Enzo Alessio [a], Andrea Sessanta o Santi [a], Silvano Geremia [a], Alberta Bergamo [b], Gianni Sava [b], Angela Boccarelli [c], Angela Schettino [c], Mauro Coluccia [c]

[a] *Dipartimento di Scienze Chimiche, Università di Trieste, 34127 Trieste, Italy*

[b] *Dipartimento di Scienze Biomediche, Università di Trieste, 34127 Trieste, Italy*

[c] *Dipartimento di Scienze Biomediche e Oncologia Umana, Università di Bari–Policlinico, 70124 Bari, Italy*

Received 10 April 1997; revised 25 June 1997; accepted 10 July 1997

Abstract

A series of neutral and anionic Rh(III)-chloride compounds bearing ammonia or imidazole (Im) ligands, and isostructural to Ru(III) complexes endowed with antineoplastic activity, were synthesized and characterized spectroscopically. The X-ray crystal structure of [ImH][*trans*-$RhCl_4(Im)_2$] was determined. Crystal data: monoclinic, space group $C2/c$, $Z=4$, $a=13.133(3)$, $b=7.977(1)$, $c=16.683(4)$ Å, $\beta=113.84(2)°$. The solution behaviour and some biological parameters of the new rhodium compounds, including cytotoxicity, in vitro interactions with DNA and in vivo antitumour activity against a tumour model, were investigated and compared with those of the corresponding ruthenium analogues.

Keywords: Antitumour compounds; Rhodium complexes; Ruthenium complexes; Cytotoxicity; Crystal structures

1. Introduction

The square planar Pt(II) complex *cis*-$PtCl_2(NH_3)_2$ (cisplatin), whose antitumour activity was discovered in the late 1960s [1], has been widely investigated in different fields of science, ranging from classical synthetic inorganic chemistry to modern bioinorganic chemistry, from pharmacology to clinical practice. Cisplatin now has a well established leading position in the panel of chemotherapeutics against human malignancies; it is routinely administered for the treatment of testicular and ovarian cancer and is increasingly used against other malignancies, in particular cervical, bladder and head and neck tumours [2]; for a recent short but comprehensive review on cisplatin see Ref. [3]. Some second and third generation platinum drugs, with some improved characteristics compared with cisplatin, are currently being brought into clinical use. However, several tumours may have spontaneous (or acquired) resistance to platinum drugs.

It is widely accepted that the primary target of platinum drugs is DNA, to which they bind covalently, most frequently to neighbouring guanine-N7 sites. Chelation of platinum induces distortions of the double helix that affect both replication and transcription of DNA and ultimately lead to cell death [4].

These findings have long stimulated investigations into the field of non-platinum inorganic antitumour drugs [5]. Non-platinum active compounds are likely to have mechanisms of action, biodistribution and toxicity which are different from those of platinum drugs and might therefore be active against human malignancies that are resistant, or have acquired resistance, to them. This indeed proved to be the case for a series of ruthenium(III) compounds bearing heterocyclic N-donor ligands, such as [ImH][*trans*-$RuCl_4(Im)_2$] (**ICR**, Im = imidazole) and the analogous indazole (Ind) complex, which were shown to possess very good activity against a platinum-resistant colorectal tumour in rats [6]. On the other hand, a deceptively similar complex developed by us, [ImH][*trans*-$RuCl_4(Im)(Me_2SO)$], has, like its sodium salt [7], a specific activity against spontaneous lung metastases in animal models and is currently being developed into an antimetastatic

* Corresponding author. Tel.: +39 40 676 3955; fax: +39 40 676 3903; e-mail: mestroni@uts.univ.trieste.it

[1] Dedicated to Professor Ivano Bertini.

0020-1693/98/$19.00
PII S0020-1693(97)05915-X

drug [8]. The complex can be formally thought of as derived from [ImH][*trans*-$RuCl_4(Im)_2$] by replacement of one imidazole ligand with one S-bonded dimethyl sulfoxide molecule The mechanism of action of this ruthenium drug is still largely unknown, but the absence of relevant cytotoxicity suggests that DNA might not be one of its primary targets [9].

The establishment of the chemical behaviour of a new inorganic drug in aqueous solution, in particular under physiological conditions and in the presence of biologically relevant molecules, is the first step towards the understanding of its mechanism of action. In the case of ruthenium(III) drugs, these investigations have been complicated by their tendency to form polymeric oxo species at physiological pH and by the paramagnetism of the Ru(III) nucleus, which limits the use of ^{1}H NMR [10]. Moreover, ruthenium(III) compounds might undergo in vivo reduction to ruthenium(II) species [11].

In recent papers [12], we have shown that rhodium(III) forms a series of diamagnetic Me_2SO-chloride complexes which are isostructural with those formed by Ru(III) and behave quite similarly to them in aqueous solution as far as dissociation of the ligands is concerned. As a logical extension to that work, we report here the synthesis and spectroscopic characterization of a series of rhodium(III) complexes with ammonia or imidazole ligands which are isostructural to the antitumour-active ruthenium(III) compounds. Their cytotoxicity, in vitro interactions with DNA and in vivo antitumour activity against a tumour model have been investigated and are described here.

2. Experimental

Analytical grade solvents were used without further purification for synthetic purposes. Hydrated $RhCl_3$ was purchased from Johnson Mattey. ^{1}H NMR spectra were recorded in $CDCl_3$ (referenced to the solvent peak versus TMS at 7.26 ppm), DMSO-d_6 (referenced to the solvent peak versus TMS at 2.50 ppm) and D_2O (referenced to the solvent peak versus DSS at 4.80 ppm) on a Jeol EX400 spectrometer. Deuterated solvents were purchased from Aldrich and Cambridge Isotope Laboratories. Solid state IR spectra (Nujol) were recorded on a Perkin-Elmer 983G instrument between CsI windows. UV–Vis spectra were recorded on a Jasco V550 spectrophotometer. Elemental analyses were performed by Dr E. Cebulec (Dipartimento di Scienze Chimiche, Università di Trieste). The precursors *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ (**1**) and [$(Me_2SO)_2H$][*trans*-$RhCl_4(Me_2SO)_2$] (**2**) were synthesized according to the reported procedures [12].

2.1. Preparations

2.1.1. Na[*trans*-$RhCl_4(Me_2SO)_2$] (**2Na**)

A 0.56 g amount of [$(Me_2SO)_2H$][*trans*-$RhCl_4(Me_2SO)_2$] (1.0 mmol) was dissolved in a mixture of ethanol (16 ml) and water (0.2 ml). Filtration over fine paper yielded a clear orange solution, to which an 80 mg amount of NaCl (1.4 mmol) dissolved in 0.3 ml of water was added. Vigorous stirring or sonication induced the rapid precipitation of a light orange solid, which was recovered by filtration, washed with cold ethanol and diethyl ether and vacuum dried at room temperature (yield 0.34 g, 80%). *Anal.* Calc. for $C_4H_{12}Cl_4NaO_2RhS_2$ (M_r = 423.97): C, 11.3; H, 2.85. Found: C, 11.2; H, 2.76%. Selected IR absorption bands (Nujol, cm^{-1}): ν_{SO} 1114 (vs); $\nu_{Rh\text{-}S}$ 426 (m); $\nu_{Rh\text{-}Cl}$ 347 (s).

2.1.2. *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ (**3**)

A 0.5 g amount of *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ (1.12 mmol) was dissolved in a mixture of CH_2Cl_2 (12 ml) and DMSO (1 ml) in a flask closed with a stopcock. The flask was first connected to a vacuum line and then to a reservoir of gaseous ammonia. Within 30 min the solution turned from orange to yellow and a microcrystalline precipitate of the same colour began to form. After reacting overnight, the precipitate was recovered by filtration, washed with cold CH_2Cl_2 and diethyl ether, and vacuum dried at room temperature (yield 0.32 g, 75%). *Anal.* Calc. for $C_4H_{15}NCl_3O_2RhS_2$ (M_r = 382.55): C, 12.5; H, 3.95; N, 3.66; Cl, 27.8. Found: C, 12.4; H, 3.95; N, 3.76; Cl, 27.9%. ^{1}H NMR ($CDCl_3$, ppm): 3.53 (6H, s, Me_2SO); 3.47 (6H, s, Me_2SO). (D_2O): 3.52 (6H, s, Me_2SO); 3.50 (6H, s, Me_2SO). Selected IR absorption bands (Nujol, cm^{-1}): ν_{NH} 3317 (m), 3228 (m), 3180 (w, sh); ν_{SO} 1134 (s), 1123 (vs); $\nu_{Rh\text{-}S}$ 426 (m); $\nu_{Rh\text{-}Cl}$ 345 (s), 326 (m).

2.1.3. *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$ (**4**)

A 0.1 g amount of *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ (0.22 mmol) was partially dissolved in 2.5 ml of acetone and 0.068 g of imidazole (1 mmol) was added. The mixture was stirred at room temperature for 5 h, during which time the precipitate turned gradually from orange to light yellow. It was then recovered by filtration, washed with chloroform and diethyl ether and vacuum dried at room temperature (yield 57 mg, 60%). *Anal.* Calc. for $C_7H_{16}N_2Cl_3O_2RhS_2$ (M_r = 433.60): C, 19.4; H, 3.72; N, 6.46. Found: C, 19.3; H, 3.65; N, 6.54%. ^{1}H NMR (D_2O, ppm): 8.46 (1H, m, Im); 7.63 (1H, m, Im); 7.31 (1H, m, Im); 3.57 (6H, s, Me_2SO); 3.32 (6H, s, Me_2SO). ($CDCl_3$): 9.6 (br, 1H, NH); 8.29 (1H, m, Im); 7.67 (1H, m, Im); 6.95 (1H, m, Im); 3.61 (6H, s, Me_2SO); 3.49 (6H, s, Me_2SO). (DMSO-d_6, ppm): 12.8 (br, 1H, NH); 8.15 (1H, m, Im); 7.37 (1H, m, Im); 7.21 (1H, m, Im); 3.49 (6H, s, Me_2SO); 3.37 (6H, s, Me_2SO). Selected IR absorption bands (Nujol, cm^{-1}): ν_{SO} 1137, 1118 (vs); $\nu_{Rh\text{-}S}$ 428 (m); $\nu_{Rh\text{-}Cl}$ 354 (s), 326 (m).

2.1.4. *mer,cis*-$RhCl_3(Me_2SO)(Im)_2$ (**5**)

A 0.1 g amount of *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ (0.22 mmol) was partially dissolved in 10 ml of methanol and 0.030 g of imidazole (0.45 mmol) was added. The mixture was heated to reflux and a clear yellow solution was immediately obtained. After 30 min it was cooled, concentrated to ~3 ml and stored at room temperature. A yellow

precipitate formed within a few days. It was recovered by filtration, washed with chloroform and diethyl ether and vacuum dried at room temperature (yield 60 mg, 65%). *Anal.* Calc. for $C_8H_{14}N_4Cl_3ORhS$ (M_r=423.55): C, 22.7; H, 3.33; N, 13.22. Found: C, 22.3; H, 3.35; N, 13.3%. ^{1}H NMR (DMSO-d_6, ppm): (the two inequivalent imidazole ligands have been arbitrarily labelled A and B and their resonances assigned with a COSY spectrum) 12.7 (2H, br, NH); 8.04 (1H, m, H2 Im-B); 7.98 (1H, m, H2 Im-A); 7.20 (1H, m, H5 Im-A); 7.14 (2H, m, H4+H5 Im-B); 6.98 (1H, m, H4 Im-A); 3.42 (6H, s, Me_2SO). Selected IR absorption bands (Nujol, cm^{-1}): ν_{SO} 1126 (vs); $\nu_{Rh\text{-}S}$ 429 (m); $\nu_{Rh\text{-}Cl}$ 351 (s).

2.1.5. *Na[trans-RhCl$_4$(Me$_2$SO)(Im)]·2Me$_2$SO* (6)

A 0.2 g amount of Na[*trans*-$RhCl_4(Me_2SO)_2$] (0.47 mmol) was partially dissolved in a mixture of acetone (5 ml) and DMSO (0.65 ml). A 0.2 g amount of imidazole (3 mmol) was added and the mixture reacted at room temperature under magnetic stirring. A clear, deep orange solution was obtained within 1 h and was filtered over fine paper. The addition of a few drops of diethyl ether induced formation of orange crystals of the product. After standing overnight at room temperature, the product was recovered by filtration, washed with cold acetone and diethyl ether and vacuum dried at room temperature (yield 0.19 g, 70%). As was the case for the ruthenium analogue [7a], complex **6** crystallizes with two molecules of DMSO and is correctly formulated as Na[*trans*-$RhCl_4(Me_2SO)(Im)]\cdot 2Me_2SO$. *Anal.* Calc. for $C_9H_{22}N_2Cl_4NaO_3RhS_3$ (M_r=570.17): C, 18.9; H, 3.89; N, 4.91. Found: C, 18.9; H, 3.78; N, 5.05%. ^{1}H NMR (D_2O, ppm): 8.29 (1H, m, Im); 7.53 (1H, m, Im); 7.26 (1H, m, Im); 3.53 (6H, s, Me_2SO); 2.71 (12H, s, Me_2SO). Selected IR absorption bands (Nujol, cm^{-1}): ν_{SO} 1088 (vs, Me_2SO), 1028 (vs, Me_2SO); $\nu_{Rh\text{-}S}$ 438 (m); $\nu_{Rh\text{-}Cl}$ 361 (s), 346 (s). Visible spectrum in H_2O solution (λ_{max}, nm (ε, M^{-1} cm^{-1})): 419 (120); 495 (sh, br) (30).

2.1.6. *[ImH][trans-RhCl$_4$(Im)$_2$] (7)*

A 0.5 g amount of hydrated $RhCl_3$ (1.9 mmol) was dissolved by gentle warming (1 h) in a mixture of ethanol (5 ml) and 2 M HCl (5 ml). A 1 g amount of imidazole (14.7 mmol) dissolved in 1 ml of 6 N HCl was then added to the warm brown–red solution; the warm mixture was allowed to react for 5 min. Red–brown crystals of the product slowly precipitated from the solution stored at room temperature. After 3 days they were recovered by filtration, washed with cold ethanol and diethyl ether and vacuum dried at room temperature (yield 0.42 g, 50%). *Anal.* Calc. for $C_9H_{13}N_6Cl_4Rh$ (M_r=449.96): C, 24.0; H, 2.91; N, 18.6. Found: C, 23.8; H, 2.99; N, 18.8%. ^{1}H NMR (D_2O, ppm): 8.68 (1H, s, ImH^+); 8.27 (2H, m, Im); 7.54 (2H, m, Im); 7.47 (2H, s, ImH^+); 7.23 (2H, m, Im). Selected IR absorption bands (Nujol, cm^{-1}): Im 1063 (s) and 614 (s), ImH^+ 1047 (s) and 623 (s); $\nu_{Rh\text{-}Cl}$ 353 (s). Visible spectrum in H_2O solution (λ_{max}, nm (ε, M^{-1} cm^{-1})): 443 (85); 505 (sh, br) (46).

2.2. X-ray crystallography

Crystals of [ImH][*trans*-$RhCl_4(Im)_2$] (**7**) suitable for X-ray crystallography were obtained with the synthetic procedure described above. Intensity data were collected on a CAD4 Enraf-Nonius single crystal diffractometer at room temperature by the ω scan technique using graphite-monochromated Mo Kα radiation (λ=0.7107 Å). Crystal data are reported in Table 1. The intensities were corrected for Lorentz and polarization factors. The structure was solved by conventional Patterson and Fourier methods and refined through full-matrix least-squares methods using unitary weight. The non-hydrogen atoms were treated anisotropically. The hydrogen atoms were added as fixed contributions at their calculated positions, with isotropic thermal parameters 1.3 times the value of B_{eq} of the atoms to which they are attached. The final $R(F_o)$ and $R_w(F_o)$ values are reported in Table 1. Complex neutral-atom scattering factors, including anomalous dispersion terms for all non-H atoms, were taken from the literature [13]. Calculations were carried out on a VAX 2000 using the Molen package [14]. Final non-H positional parameters and B_{eq} (Å^2) are given in Table 2; bond distances and angles for the complex are reported in Table 3.

Table 1
Crystallographic data for [ImH][*trans*-$RhCl_4(Im)_2$]

Formula	$C_9H_{13}Cl_4N_6Rh$	D_{calc} (g cm^{-3})	1.869
M	449.96	μ(Mo Kα) (cm^{-1})	17.2
a (Å)	13.133 (3)	F(000)	888
b (Å)	7.977(1)	Crystal size (mm)	0.05×0.3×0.2
c (Å)	16.683(4)	No. measured reflections	2553
β (°)	113.84(2)	No. independent reflections ($I \geq 3\sigma(I)$)	1644
V (Å^3)	1598.6	No. variables	116
Z	4	$R(F_o)$	0.039
Crystal system	monoclinic	$R_w(F_o)$	0.039
Space group	$C2/c$	Goodness of fit	1.21
2θ (Mo Kα) (°)	4–60	Residuals in F_{map} (e Å^{-3})	±0.3

Table 2
Positional parameters of non-hydrogen atoms for [ImH][*trans*-$RhCl_4(Im)_2$]

Atom	x	y	z
Rh	0.000	0.12784(7)	0.250
Cl1	0.000	−0.1637(2)	0.250
Cl2	0.000	0.4194(2)	0.250
Cl3	0.14978(9)	0.1273(2)	0.38708(7)
N1	0.1092(3)	0.1300(6)	0.1912(2)
N2	0.2491(3)	0.1988(8)	0.1598(3)
C1	0.1992(4)	0.2220(8)	0.2145(3)
C2	0.1887(4)	0.0841(9)	0.0999(3)
C3	0.1008(4)	0.0428(8)	0.1179(3)
N1b [a]	0.0036(7)	0.473(2)	0.4401(5)
N2b [a]	−0.0469(7)	0.563(1)	0.5383(7)
C1b [a]	−0.066(1)	0.576(2)	0.451(1)
C2b [a]	0.032(1)	0.453(2)	0.5695(7)
C3b [a]	0.065(1)	0.388(2)	0.5122(9)

[a] Occupancy factor 0.5.

Table 3
Bond distances (Å) and angles (°) for [ImH][*trans*-$RhCl_4(Im)_2$]

Atoms	Distance
Rh Cl1	2.326(2)
Rh Cl2	2.325(2)
Rh Cl3	2.337(1)
Rh N1	2.039(4)
N1 C1	1.310(7)
N1 C3	1.371(7)
N2 C1	1.333(8)
N2 C2	1.350(8)
C2 C3	1.345(9)
Atoms	Angles
Cl1 Rh Cl3	89.89(5)
Cl1 Rh N1	90.5(1)
Cl2 Rh Cl3	90.11(5)
Cl2 Rh N1	89.5(1)
Cl3 Rh N1	89.63(9)
N1 Rh N1′	179.0(2)
Rh N1 C1	126.0(4)
Rh N1 C3	127.3(3)
C1 N1 C3	106.6(5)
C1 N2 C2	107.2(5)
N1 C1 N2	110.7(5)
N2 C2 C3	107.5(5)
N1 C3 C2	107.9(5)

2.3. Human tumour cell lines

A2780 cell line, an ovarian carcinoma cell line derived from an untreated patient, and the cisplatin-resistant subline A2780/cp8 were kindly supplied by Dr R. Ozols (Fox Chase Cancer Center, Philadelphia, PA). LoVo and Calu are colon and lung carcinoma cell lines respectively, which were obtained from ATCC (Rockville, MD). The cell lines were maintained in the logarithmic phase at 37°C in a 5% CO_2 humified atmosphere in air using the following: (i) RPMI 1640 medium supplemented with 10% foetal calf serum, 2 μM glutamine, 10 mg ml^{-1} gentamycin and 10 μg ml^{-1} insulin for A2780 and A2780/cp8 cells; (ii) Ham's F12 medium supplemented with 20% foetal calf serum, 2 μM glutamine, 0.1% gentamycin for LoVo cells; (iii) Eagle's MEM medium with non-essential amino acids, sodium pyruvate 1 mM, 10% foetal calf serum for Calu cells.

2.4. In vitro growth inhibition assay

The growth inhibitory effect of rhodium complexes towards tumour cell lines was evaluated using the Cell Proliferation Kit I (MTT) of Boehringer Mannheim, following the manufacturer's protocol. Briefly, the cells grown in the culture flasks were trypsinized and 100 μl of medium containing 5×10^4 cells was inoculated into 96-well microplates. After 24 h from seeding, the cells were incubated with various concentrations of rhodium complexes freshly dissolved in the culture medium for 3 days at 37°C, followed by an additional 4 h incubation with 10 μl/well of tetrazolium salt solution (5 mg ml^{-1}). The cells were dissolved in 100 μl of 10% SDS solubilization solution, and the absorbance was measured at 570 nm. The drug concentration which inhibited cell growth by 50% (IC_{50}) was obtained from semi-logarithmic dose–response plots.

2.5. Primer extension footprinting assay

pGEM-4Z double stranded DNA (1.5×10^{-8} mol nucleotides) was reacted with rhodium complexes (drug/nucleotide molar ratio 0.01) in a total volume of 20 μl of 10 mM $NaClO_4$ for 16 h at 37°C. At the end of the reaction time, excess drug was removed by ethanol precipitation. After alkaline denaturation, the DNA was primed with 18-mer SP6(+) primer (New England Biolabs) and DNA synthesis was performed by Sequenase 2 enzyme (United States Biochemicals) in the presence of [α-^{32}P]dATP (370 KBq, 111 TBq $mmol^{-1}$, Amersham) and unlabelled dNTPs following the manufacturer's protocol. The products were separated by electrophoresis on a denaturing gel (6% polyacrylamide/7 M urea) in parallel to a sequence ladder performed on unreacted control DNA. Autoradiography was performed overnight with Kodak Ektamat G film.

2.6. DNA interstrand cross-link evaluation

The 2746-bp pGEM-4Z DNA linearized by Bam HI endonuclease (New England Biolabs) was mixed with rhodium complexes at drug/nucleotide formal ratios of 0.001 and 0.01 and then incubated in 10 mM $NaClO_4$ at 37°C. At different time intervals (0–72 h), the cross-linking reaction was stopped by adjusting the NaOH concentration to 30 mM and cooling the samples at −20°C. The samples were then analysed on a denaturing 1% agarose gel where DNA fragments containing interstrand cross-links migrate slower than fragments without interstrand cross-links. The percentage of

interstrand cross-linking was calculated from a densitometric scan of the resulting bands.

2.7. Ethidium bromide assay

Calf thymus DNA (Sigma) was reacted with rhodium complexes for 48 h at 37°C in 10 mM $NaClO_4$ at a drug/nucleotide ratio between 0 and 0.1. Fluorescence measurements of DNA modified by rhodium complexes in the presence of ethidium bromide (EtBr) were performed using a Perkin Elmer LS 5B spectrofluorimeter (excitation wavelength 546 nm, emission wavelength 590 nm). The fluorescence was measured in 0.4 M NaCl and the concentrations were 0.01 mg ml^{-1} for DNA and 0.04 mg ml^{-1} for EtBr.

2.8. Tumour line

The MCa mammary carcinoma of CBA mouse was obtained from the Rudjer Boskovic Institute, Zagreb, Croatia. The tumour line was maintained locally by bi-weekly passages of 10^6 viable tumour cells, injected into the calf of the left hind leg of CBA adult mice obtained from a locally established breeding colony. Tumour cell suspensions were prepared from primary tumours of donors similarly inoculated 2 weeks earlier. Tumour propagation for experimental purposes was similarly performed using male mice 6–8 weeks old.

2.9. Primary tumour and lung metastasis evaluation

Primary tumours were measured with caliper and their weight was estimated using the formula $(\pi/6)a^2b$, where a and b are perpendicular axes ($a < b$) and the tumour density is assumed equal to unity. Evaluation of the number and weight of lung metastases, formed spontaneously from the i.m. tumour implants, was performed after sacrifice of the animals by cervical dislocation. The number of lung metastases on the surface of the freshly removed lungs was evaluated using a low-power stereo microscope equipped with a calibrated grid. The weight of the metastatic tumour per mouse was calculated by determining the volume of each metastatic nodule by the formula reported above for primary tumours.

2.10. Animal treatment

Treatment was performed on days 1-5-9-13 after tumour implantation. The compounds were dissolved in 0.9% NaCl and administered i.p. to mice in volumes of 0.1 ml/10 g body weight. The dosage used was 50 mg/kg/day for Na[*trans*-$RhCl_4(Me_2SO)(Im)]\cdot 2Me_2SO$, Na[*trans*-$RhCl_4(Me_2SO)_2$], *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$, and 141 mg/kg/day for ImH[*trans*-$RhCl_4(Im)_2$]. Controls received only 0.9% NaCl.

3. Results

3.1. Synthesis and characterization of complexes

Sulfur bonded Me_2SO has a rather strong *trans*-labilizing effect that was exploited advantageously by us for the selective introduction of N-donor ligands in the rhodium-chloride-sulfoxide precursors *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ (**1**) and Na[*trans*-$RhCl_4(Me_2SO)_2$] (**2Na**). In **1** replacement of the Me_2SO *trans* the S-bonded sulfoxide with either ammonia or imidazole (Im) occurred easily at room temperature, yielding *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ (**3**) and *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$ (**4**) respectively. In the case of imidazole, if the reaction was not properly stopped at this stage by precipitation of the monosubstituted product, partial formation of the *cis*-disubstituted species *mer,cis*-$RhCl_3(Me_2SO)(Im)_2$ (**5**) occurred. Compound **5** was obtained selectively from **1** and an excess of imidazole under slightly more drastic reaction conditions (refluxing methanol). The reactivity of [Na][*trans*-$RhCl_4(Me_2SO)_2$] with imidazole was very straightforward and involved the selective replacement of only one of the two Me_2SO ligands *trans* to each other, yielding [Na][*trans*-$RhCl_4(Me_2SO)(Im)$] (**6**). The corresponding *trans* bis-imidazole complex was conveniently obtained as imidazolium salt, [ImH][*trans*-$RhCl_4(Im)_2$] (**7**), adopting a synthetic procedure very similar to that reported for the ruthenium analogue **ICR** [6c]. All the new compounds are very soluble in DMSO. The solubility in water is quite good for the ionic species [Na][*trans*-$RhCl_4(Me_2SO)(Im)$], while it becomes rather low (~mmolar) for [ImH][*trans*-$RhCl_4(Im)_2$] and the monosubstituted neutral complexes *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ and *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$ and lower yet (at the limit for ^1H NMR detection) for the bis-imidazole neutral species *mer,cis*-$RhCl_3(Me_2SO)(Im)_2$.

The geometry of the neutral compounds **3–5** was established unambiguously by combined ^1H NMR and IR spectroscopy. The ^1H NMR spectra of *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ and *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$ have two singlet resonances in the region of S-bonded Me_2SO, each accounting for six protons, which were attributed to the equivalent methyl groups of the two inequivalent Me_2SO ligands. A *fac* geometry, where the two equivalent Me_2SO ligands have inequivalent methyl groups, would also give a similar NMR pattern, but the strong Rh–Cl stretching bands in the IR spectra are consistent with the presence of a *mer*, rather than *fac*, disposition of the chlorides. Moreover, all the structurally characterized monosubstituted derivatives of *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ have a *mer,cis* geometry [15–17]. The neutral compounds *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ and *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$ are linkage isomers compared with the corresponding ruthenium(III) analogues *mer,cis*-$RuCl_3(Me_2SO)(Me_2SO)(NH_3)$ and *mer,cis*-$RuCl_3(Me_2SO)(Me_2SO)(Im)$, where the sulfoxide *cis* to the N-ligand is bound through oxygen [7a].

The ^{1}H NMR spectrum of the disubstituted species $RhCl_3(Me_2SO)(Im)_2$ (**5**), which shows two inequivalent imidazole ligands and a single Me_2SO resonance, is in agreement only with a *mer,cis* geometry. Complex **5** is isostructural to the bis-1-methylimidazole ruthenium species *mer,cis*-$RuCl_3(Me_2SO)(1Me\text{-}Im)_2$, whose X-ray structure has been recently reported by us [18]. The crystal structure of *mer,cis*-$RhCl_3(Me_2SO)(py)_2$ has also been described [19].

The monoanionic species [Na][*trans*-$RhCl_4(Me_2SO)(Im)$] (**6**) and [ImH][*trans*-$RhCl_4(Im)_2$] (**7**) have very simple ^{1}H NMR spectra, which might be in agreement also with a *cis* geometry of the two neutral ligands. A *cis* geometry for the anionic species would not be unprecedented, since we found that [*cis*-$RhCl_4(Me_2SO)_2]^-$ is thermodynamically more stable than the corresponding *trans* isomer [12b]. The *trans* geometry of **6** and **7** was established unambiguously by the analysis of their IR and electronic absorption spectra. The IR spectra of **6** and **7** are indeed very similar to those of the crystallographically known ruthenium analogues Na[*trans*-$RuCl_4(Me_2SO)(Im)$] [7a] and [ImH][*trans*-$RuCl_4(Im)_2$] [6c] respectively. Moreover, the visible spectra of both Na[*trans*-$RhCl_4(Me_2SO)(Im)]\cdot 2Me_2SO$ and [ImH][*trans*-$RhCl_4(Im)_2$] have a characteristic absorption at about 500 nm, typical of a *trans* geometry. In fact, this band is present in the structurally well characterized *trans*-$[RhCl_4(Me_2SO)_2]^-$ species [20], but it is absent in the corresponding *cis* isomer [12b]. In the case of [ImH][*trans*-$RhCl_4(Im)_2$] the X-ray crystal structure was also determined.

3.2. X-ray crystallography

An Ortep drawing of the structure of [ImH][*trans*-$RhCl_4(Im)_2$] with the atom-numbering scheme is shown in Fig. 1. The crystal consists of $[RhCl_4Im_2]^-$ anions and $[ImH]^+$ cations. The Rh, Cl(1) and Cl(2) atoms lie on a twofold rotation axis; consequently, the asymmetric unit consists of only half a complex anion. The cation is located around a crystallographic inversion centre so that it is disordered in two superpositions with occupancy factor 0.5. The crystals of the Rh complex are isomorphous to those of the analogous Ru derivative [6c]. A comparison between the

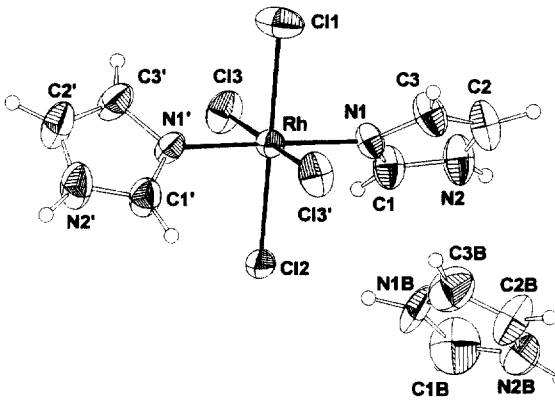

Fig. 1. ORTEP drawing of [ImH][*trans*-$RhCl_4(Im)_2$] (**7**) with 50% probability for the thermal ellipsoids.

two isomorphous structures shows that the Rh–Cl bond lengths (mean value 2.329 Å) are shorter than the corresponding Ru–Cl lengths (mean value 2.349 Å), in agreement with the increase in the atomic number of the metal. This difference between the coordination distances in the two metals is more marked for the binding distance of the Im ligand, which is 2.039(4) Å in the Rh complex and 2.079(3) Å in the Ru analogue [6c].

The planes of the two *trans* N-coordinated ligands are nearly perpendicular to one another (84°). The disordered cation is involved in a short contact (3.18(1) Å) between the chlorine atom Cl2 and protonated nitrogen N1b. Furthermore it forms a stacking interaction with the coordinated Im.

3.3. Chemical behaviour in aqueous solution

The chemical behaviour of a light-protected aqueous solution of the soluble Rh compounds was investigated at room temperature by ^{1}H NMR spectroscopy. Millimolar solutions of both *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ and *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$ are rather inert and their spectrum remained basically unchanged over a 72 h period. Very limited changes, attributable to a minor dissociation of Me_2SO, were found over a 7 day period. No loss of the nitrogen ligand was observed. The anionic compounds were found to be relatively more labile compared with the neutral compounds. In a D_2O solution of [ImH][*trans*-$RhCl_4(Im)_2$] a new set of resonances for coordinated imidazole (7.33, 7.53 and 8.30 ppm) appeared within 24 h and slowly increased over time. The new species amounted to ~25% of the original complex after 7 days; since the free/bound imidazole ratio did not change over time, the new resonances can be safely attributed to the *mer,trans*-$RhCl_3(Im)_2(H_2O)$ species, derived from the parent compound **7** upon chloride dissociation. As in the case of ruthenium, replacement of one imidazole ligand with Me_2SO resulted in a more rapid and extensive chloride dissociation process [10,21]. In fact, in a D_2O solution of Na[*trans*-$RhCl_4(Me_2SO)(Im)]\cdot 2Me_2SO$, several minor species were detectable after 24 h and after 7 days ~50% of the original complex had been transformed into new species, one of which was largely predominant. Since the free/bound Me_2SO ratio was constant over time and no free imidazole could be detected, all the new species derived from the parent compound **6** upon chloride loss. The resonances of the main new species (bound imidazole at 8.32, 7.52 and 7.35 ppm and Me_2SO signal overlapped by that of **6**) can be attributed to a *mer,trans*-$RhCl_3(Im)(Me_2SO)(H_2O)$ complex, by comparison with those of *mer,trans*-$RhCl_3(Im)_2(H_2O)$ reported above.

We have also shown that the solution behaviour of the ruthenium(III)-Me_2SO analogues was strongly influenced by the presence of small amounts of monoelectronic reducing agents [10]; in particular, the addition of a stoichiometric amount of either ascorbic acid or cysteine to an aqueous solution of Na[*trans*-$RuCl_4(Me_2SO)(Im)$] induced the immediate and complete reduction to ruthenium(II) species.

Table 4
Cytotoxicity (IC_{50} expressed as μmol concentrations) of rhodium compounds against human tumour cell lines compared with cisplatin

Compound	A2780	A2780/cp8	LoVo	Calu
cisplatin	3.2 ± 1	8.5 ± 1.6	2.5 ± 1	12 ± 2
mer,cis-$RhCl_3(Me_2SO)_2(Me_2SO)$	> 200	> 200	> 100	n.d.
mer,cis-$RhCl_3(Me_2SO)_2(NH_3)$	1.5 ± 0.4	2.5 ± 1	0.4 ± 0.2	9
mer,cis-$RhCl_3(Me_2SO)_2(Im)$	15.6 ± 2	14 ± 0.9	6.7 ± 3	70
mer,cis-$RhCl_3(Me_2SO)(Im)_2$	> 200	> 200	40 ± 15	> 200
Na[*trans*-$RhCl_4(Me_2SO)_2$]	> 200	> 200	> 200	n.d.
Na[*trans*-$RhCl_4(Me_2SO)(Im)$] · $2Me_2SO$	> 100	n.d.	> 100	n.d.
[ImH][*trans*-$RhCl_4(Im)_2$]	> 1000	> 1000	> 200	n.d.

n.d. not done.

We found instead that the visible absorption spectrum of aqueous solutions of Na[*trans*-$RhCl_4(Me_2SO)(Im)$] is unaffected by the presence of such reductants in stoichiometric ratio.

3.4. Cytotoxicity

The cytotoxicity of the rhodium compounds was tested against four human tumour cell lines, one of which (A2780/cp8) is cisplatin resistant (Table 4). While the neutral bisimidazole complex *mer,cis*-$RhCl_3(Me_2SO)(Im)_2$ (**5**) and the anionic compounds Na[*trans*-$RhCl_4(Me_2SO)_2$] (**2Na**), Na[*trans*-$RhCl_4(Me_2SO)(Im)$] · $2Me_2SO$ (**6**) and [ImH][*trans*-$RhCl_4(Im)_2$] (**7**) had a negligible cytotoxicity, *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$ (**4**) and in particular *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ (**3**) showed a remarkable and selective cytotoxic activity, similar to that of cisplatin (Table 4).[2] Moreover, the cytotoxicity of the two mono-substituted rhodium compounds on the cisplatin-resistant cell line was comparable with that on the parent line (cisplatin is at least two to three times less cytotoxic on the resistant line compared with the parent line). Instead, the precursor of **3** and **4**, *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$, was considerably less cytotoxic against all cell lines.

3.5. In vitro metal–DNA interactions

The interactions of the rhodium compounds with DNA in vitro were investigated by different techniques. The primer extension footprinting assay [22] allowed us to evaluate the sequence selectivity of DNA modification by rhodium complexes (Fig. 2). All rhodium complexes, with the exception of *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$, reacted extensively with DNA, as shown by the stop sites on the sequencing gel. Numerous faint bands, resulting from premature termination of DNA synthesis, corresponded to nearly every nucleotide of the template strand. Unlike cisplatin and other metal complexes such as *trans*-$RuCl_2(Me_2SO)_4$ [23], the rhodium compounds did not show a preferential binding for adjacent guanine residues; *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$, *mer,cis*-$RhCl_3(Me_2SO)(Im)_2$, Na[*trans*-$RhCl_4(Me_2SO)(Im)$] and [ImH][*trans*-$RhCl_4(Im)_2$] showed indeed similar, and quite uncommon, behaviour, with preferential stop sites corresponding to pyrimidin residues.

The DNA interstrand cross-links are among those critical molecular lesions known to inactivate the DNA as a template for replication. We evaluated the ability of rhodium complexes to induce such lesions in vitro by gel electrophoresis under denaturing conditions of a linearized fragment of plasmidic DNA, as described by Lemaire et al. [24]. At a drug/nucleotide ratio of 0.001, only *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ showed some activity, inducing 4% of interstrand cross-linked DNA after 72 h of incubation at 37°C. Assuming the presence of one interstrand cross-link per DNA molecule, the amount of rhodium involved in this bifunctional interaction can be estimated as being less than 1% of the total. For the sake of comparison, under the same conditions the antimetastatic active Ru(III) complex Na[*trans*-$RuCl_4(Me_2SO)(Im)$] was found by us to induce 30% of interstrand cross-linked DNA, accounting for 5.3% of the total ruthenium. At the high drug/nucleotide ratio of 0.01, *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ also showed some activity, inducing 15% of cross-linked DNA after 72 h of incubation at 37°C.

The DNA binding mode of rhodium complexes was further investigated using EtBr as fluorescent probe to distinguish between perturbations induced by monofunctional and bifunctional DNA adducts [25]. The modification of double helical DNA induced by the rhodium compounds at drug/nucleotide ratios between 0 and 0.1 did not decrease the EtBr fluorescence, a result similar to that obtained with monodentate platinum complexes such as [Pt(dien)Cl]Cl (not shown).

Taken together, the rather low interstrand cross-linking ability of rhodium complexes and the EtBr fluorescence measurements suggest that in the interaction with DNA prevailingly monofunctional adducts are formed.

3.6. Antitumour activity

The antitumour activity of selected rhodium compounds was tested in vivo against the MCa mammary carcinoma, an i.m. implanted solid tumour of CBA mice which gives spon-

[2] Compounds **3** and **4** are being sent to NCI for a screening of their cytotoxicity on a wider panel of cell lines.

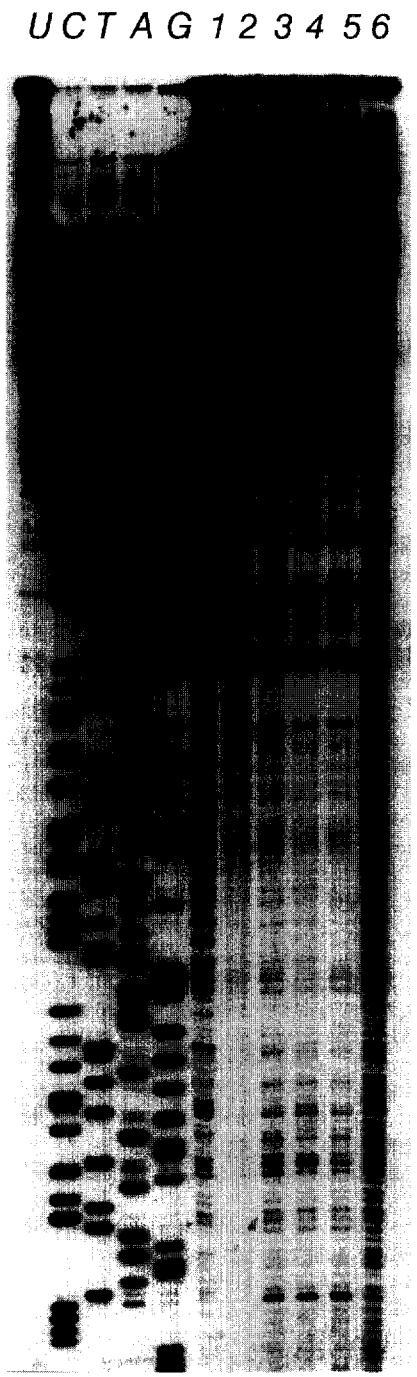

Fig. 2. Autodiagram of a 6% polyacrylamide/7 M urea sequencing gel showing the blocks to Sequenase 2 T7 DNA polymerase on drug-treated (drug/nucleotide molar ratio 0.01) pGEM 4z DNA. Lanes designated C, T, A, and G refer to the base positions on the template strand: lane 1 *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$; lane 2 *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$; lane 3 *mer,cis*-$RhCl_3(Me_2SO)(Im)_2$; lane 4 $Na[trans\text{-}RhCl_4(Me_2SO)(Im)]\cdot 2Me_2SO$; lane 5 $[ImH][trans\text{-}RhCl_4(Im)_2]$; lane 6 *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$; lane U no reagent.

taneous lung metastases. The effect of the treatment on both the growth of the primary tumour and on the number and weight of lung metastases was evaluated (Table 5). The aim of this experiment was to acquire a direct comparison with the most active ruthenium analogues and therefore the anionic rhodium complexes were privileged. Preliminary investigations showed that, in general, the rhodium-Me_2SO compounds are more toxic than the corresponding ruthenium compounds; the dosages used here were relatively non-toxic, as determined by the loss of body weight gain versus untreated controls (e.g. −5.7% and −3.8% for $Na[trans\text{-}RhCl_4(Me_2SO)(Im)]\cdot 2Me_2SO$ and *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ respectively). $[ImH][trans\text{-}RhCl_4(Im)_2]$ was found to be relatively less toxic, and therefore used at a higher dosage.

Under these experimental conditions only $Na[trans\text{-}RhCl_4(Me_2SO)(Im)]\cdot 2Me_2SO$ and *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ modified slightly the growth of the primary tumour (25% reduction versus untreated controls). The most interesting result is the remarkable and selective activity of *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ against the growth of spontaneous metastases, comparable with that of $Na[trans\text{-}RuCl_4(Me_2SO)(Im)]$ under similar experimental conditions. In fact, *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ significantly reduced the number of metastatic nodules (70% versus untreated controls, $p < 0.05$ t-Test for grouped data). The same compound caused an 80% reduction, versus untreated controls, of the weight of metastases. Despite the strong decrease, this effect is not statistically significant, probably because of the great variability inside some groups. As in the case of active ruthenium compounds, the antimetastatic activity of *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ seems to be unrelated to the in vitro cytotoxicity of the complex.

4. Discussion

The reactivity of the Rh(III)-chloride-Me_2SO precursors with N-donor ligands closely reflects the behaviour already observed by us with the corresponding Ru(III) compounds, and was exploited successfully for the synthesis of neutral and anionic derivatives which are structurally very similar to ruthenium(III) complexes endowed with antineoplastic activity. Despite this structural analogy, the rhodium(III) compounds in general did not show any relevant antitumour activity, with the remarkable exception of *mer,cis*-$RhCl_3(Me_2SO)_2(Me_2SO)$ which possessed a selective antimetastatic activity. The reason for the general inactivity might be found in the greater inertness of the rhodium complexes compared with the corresponding ruthenium analogues, evidenced by ^1H NMR solution studies, and by their lower capability to be activated by redox mechanisms. The inertness of the rhodium complexes might also account for their minor interactions with double stranded DNA in vitro, leading mainly to monofunctional adducts, and for their general lack of in vitro cytotoxicity. Surprisingly instead, the two inert complexes *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ and *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$ have a rather high cytotoxicity, comparable with that of cisplatin. This activity is apparently unrelated to DNA interaction properties similar to those of cisplatin and might be attributed to interactions with other

Table 5
Effects of in vivo treatment of MCa mammary carcinoma with rhodium compounds

Compound	Primary tumour (g)	Metastasis	
		Number	Weight (mg)
Control	3.89 ± 0.65	23 ± 4	17.66 ± 5.10
Na[*trans*-$RhCl_4(Me_2SO)_2$]	3.84 ± 0.45	42 ± 10	42.22 ± 4.30
Na[*trans*-$RhCl_4(Me_2SO)(Im)$] · $2Me_2SO$	2.84 ± 0.24	27 ± 6	16.66 ± 5.88
ImH[*trans*-$RhCl_4(Im)_2$]	3.47 ± 0.14	32 ± 10	28.88 ± 9.51
mer,cis-$RhCl_3(Me_2SO)_2(Me_2SO)$	2.82 ± 0.22	7 ± 3	3.48 ± 2.06

Five CBA males per group were injected with 10^6 tumour cells on day 0 and treated i.p. on days 1-5-9-13 with the compounds at the indicated doses. Primary tumour and metastasis evaluation was performed on day 23.

cellular components. Experiments aimed to evaluate the antitumour and antimetastatic activity of these two cytotoxic compounds are in progress.

5. Conclusions

A series of neutral and anionic Rh(III)-chloride compounds bearing ammonia or imidazole (Im) ligands, and isostructural to Ru(III) compounds endowed with antineoplastic activity, have been synthesized and characterized spectroscopically. The solution behaviour and some biological parameters of the new rhodium compounds, including cytotoxicity, in vitro interactions with DNA and antitumour activity against a tumour model, have been investigated and compared with those of the corresponding ruthenium analogues. Two neutral complexes, *mer,cis*-$RhCl_3(Me_2SO)_2(NH_3)$ and *mer,cis*-$RhCl_3(Me_2SO)_2(Im)$, were found to possess a cytotoxicity comparable with that of cisplatin against human tumour cell lines; their antineoplastic activity against tumour models is currently being investigated further.

6. Supplementary material

Hydrogen atom coordinates, anisotropic thermal parameters, tables of all bond lengths and angles, and a list of final calculated and observed structure factors for [ImH][*trans*-$RhCl_4(Im)_2$] are available from the authors.

Acknowledgements

Financial support for this work was provided by Italian MURST (40% grant), Italian CNR, ACRO Project and European COST Project D1/0001/95. Angela Schettino is a recipient of a CARSO fellowship.

References

[1] B. Rosenberg, L. Van Camp and T. Krigas, Nature, 205 (1965) 698.
[2] C.F.J. Barnard, Platinum Met. Rev., 33 (1989) 162.
[3] J. Reedijk, Chem. Commun., (1996) 801 and Refs. therein.
[4] P.M. Takahara, A.C. Rosenzweig, C.A. Frederick and S.J. Lippard, Nature, 377 (1995) 649.
[5] (a) M.J. Cleare and P.C. Hydes, in H. Siegel (ed.), Metal Ions in Biological Systems, Marcel Dekker, New York, 1980, pp. 1–62; (b) N. Farrell (ed.), in Transition Metal Complexes as Drugs and Chemotherapeutic Agents, Kluwer, Dordrecht, 1989; (c) I. Haiduc and C. Silvestru, Coord. Chem. Rev., 99 (1990) 253; (d) B.K. Keppler (ed.), Metal Complexes in Cancer Chemotherapy, VCH, Weinheim, 1993.
[6] (a) B.K. Keppler and W. Rupp, J. Cancer Res. Clin. Oncol., 111 (1986) 166; (b) F.T. Garzon, M.R. Berger, B.K. Keppler and D. Schmähl, Cancer Chemother. Pharmacol., 19 (1987) 347; (c) B.K. Keppler, W. Rupp, U.M. Juhl, H. Endres, R. Niebl and W. Balzer, Inorg. Chem., 26 (1987) 4366; (d) B.K. Keppler, K.-G. Lipponer, B. Stenzel and F. Kratz, in B.K. Keppler (ed.), Metal Complexes in Cancer Chemotherapy, VCH, Weinheim, 1993, p. 187 ff.
[7] (a) E. Alessio, G. Balducci, A. Lutman, G. Mestroni, M. Calligaris and W.M. Attia, Inorg. Chim. Acta, 203 (1993) 205; (b) G. Sava, S. Pacor, E. Alessio, G. Mestroni, R. Gagliardi, M. Cocchietto and M. Coluccia, Drugs Future, 18 (1993) 894; (c) R. Gagliardi, G. Sava, S. Pacor, G. Mestroni and E. Alessio, Clin. Exp. Metastasis, 12 (1994) 93; (d) M. Coluccia, G. Sava, G. Salerno, A. Bergamo, S. Pacor, G. Mestroni and E. Alessio, Metal-Based Drugs, 2 (1995) 195; (e) G. Sava, I. Capozzi, A. Bergamo, R. Gagliardi, M. Cocchietto, L. Masiero, M. Onisto, E. Alessio, G. Mestroni and S. Garbisa, Int. J. Cancer, 68 (1996) 60.
[8] G. Mestroni, E. Alessio and G. Sava, Italian Patent MI96A001359, 1996.
[9] G. Sava, S. Pacor, A. Bergamo, M. Cocchietto, G. Mestroni and E. Alessio, Chem.-Biol. Interact., 95 (1995) 109.
[10] G. Mestroni, E. Alessio, G. Sava, S. Pacor, M. Coluccia and A. Boccarelli, Metal-Based Drugs, 1 (1994) 41.
[11] M.J. Clarke, in H. Sigel (ed.), Metal Ions in Biological Systems, Vol. 11, Marcel Dekker, New York, 1980, p. 231.
[12] (a) E. Alessio, P. Faleschini, A. Sessanta o Santi, G. Mestroni and M. Calligaris, Inorg. Chem., 32 (1993) 5756; (b) E. Alessio, A. Sessanta o Santi, P. Faleschini, M. Calligaris and G. Mestroni, J. Chem. Soc., Dalton Trans., (1994) 1849.
[13] International Tables for X-ray Crystallography, Vol. IV, Kynoch, Birmingham, 1974.
[14] B.A. Frenz, The Enraf-Nonius CAD 4 SDP — a real-time system for concurrent X-ray data collection and crystal structure determination, in H. Schenk, R. Olthof-Hazekamp, H. van Koningsveld and G.C. Bassi (eds.), Computing in Crystallography, Delft University Press, Netherlands, 1978.
[15] F.D. Rochon, P.C. Kong and R. Melanson, Can. J. Chem., 61 (1983) 1823.
[16] W.J.J. Smeets, A.L. Spek, F.G.M. Niele, C.F. Mertens and R.J.M. Nolte, Acta Crystallogr. Sect. C, 43 (1987) 2330.

[17] S.N. Gamage, R.H. Morris, S.J. Rettig and B.R. James, J. Organomet. Chem., 309 (1986) C59.
[18] S. Geremia, E. Alessio and F. Todone, Inorg. Chim. Acta, 253 (1996) 87.
[19] P. Colamarino and P. Orioli, J. Chem. Soc., Dalton Trans., (1976) 845.
[20] (a) V.I. Sokol, N.D. Rubstova and A.Y. Gribernyuk, J. Struct. Chem. (Engl. Transl.), 15 (1974) 296; (b) B.R. James, R.H. Morris, F.W.B. Einstein and A. Willis, J. Chem. Soc., Chem. Commun., (1980) 31; (c) S.N. Gamage, R.H. Morris, S.J. Rettig, D.C. Thackray, I.S. Thorburn and B.R. James, J. Chem. Soc., Chem. Commun., (1987) 894; (d) S.N. Gamage, B.R. James, S.J. Rettig and J. Trotter, Can. J. Chem., 66 (1988) 1123.
[21] (a) O.M. Ni Dhubhghaill, W.R. Hagen, B.K. Keppler, K.-G. Lipponer and P.J. Sadler, J. Chem. Soc., Dalton Trans., (1994) 3305; (b) J. Chatlas, R. van Eldik and B.K. Keppler, Inorg. Chim. Acta, 233 (1995) 59.
[22] M. Coluccia, A. Nassi, F. Loseto, A. Boccarelli, M.A. Mariggiò, D. Giordano, F.P. Intini, P. Caputo and G. Natile, J. Med. Chem., 36 (1993) 510.
[23] M. Coluccia, G. Sava, F. Loseto, A. Nassi, A. Boccarelli, D. Giordano, E. Alessio and G. Mestroni, Eur. J. Cancer, 29A (1993) 1873.
[24] M.A. Lemaire, A. Schwartz, A.R. Rahmouni and M. Leng, Proc. Natl. Acad. Sci. USA, 88 (1991) 1982.
[25] J.L. Butour and J.P. Macquet, Eur. J. Biochem., 78 (1977) 455.