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Metal Anticancer Compounds

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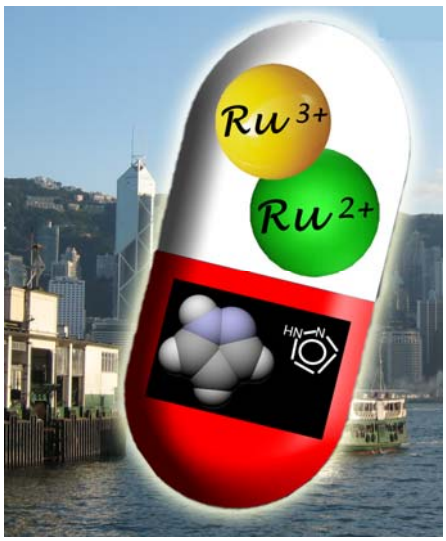

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Synthesis, molecular structure and evaluation of new organometallic ruthenium anticancer agents†

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A number of new ruthenium compounds have been synthesised, isolated and characterised, which exhibit excellent cytotoxicity against a number of different human tumour cell lines including a defined cisplatin resistant cell line and colon cancer cell lines. Addition of hydrophobic groups to the ruthenium molecules has a positive effect on the cytotoxicity values. Evidence is provided that, after incubation of a ruthenium compound with a 46 mer oligonucleotide duplex and subsequent nuclease treatment, ruthenium is bound to a guanine residue.

Introduction

Ruthenium compounds have shown promising anticancer activity and have been evaluated *in vitro* and *in vivo*[1-5] and two Ru^{III} complexes have entered clinical trials.[6-8] The anticancer potential of Ru^{II} "half-sandwich" arene complexes have also been evaluated and exhibit both *in vitro* and *in vivo* activity.[2-5,9] It has been shown that analogs containing the heavier congener osmium can be potently cytotoxic towards cancer cells with activity that is comparable to the clinical drugs carboplatin and cisplatin.[10-13] Modifications have been carried out to ruthenium arene complexes and the cytotoxic effects examined. Sadler *et al.* have synthesised Ru^{II} arene complexes of the type $[(\eta^6\text{-arene})Ru^{II}(en)X][PF_6]$ (en = ethylenediamine, X = halogen) which show *in vitro* and *in vivo* activity.[14-19] Dyson *et al.* have reported ruthenium arene systems with phosphine ligands and metallarectangles that have high anticancer activity.[20-22] Ruthenium arene complexes have also been prepared which incorporate bio-ligands in aqueous solution and comparative binding study investigations carried out.[23-28] Sheldrick and co-workers postulate that the binding of the Ru arene fragment to DNA will not only be affected by the shape of the intercalating ligand, but also the co-ligands.

As well as our interest in early transition metal anticancer complexes,[29-31] we have been interested in developing a range of ruthenium complexes incorporating ligands which have a high degree of flexibility, both sterically and electronically, in order to evaluate effects on cytotoxic behaviour. To this end we explored synthesising a range of novel ruthenium compounds, which also incorporated fragments for H-bonding and aryl groups to allow potential intercalation into DNA as well as affecting increased hydrophobic nature of the compounds. In addition to evaluating the ruthenium compounds *in vitro*, we were also interested in the mechanism of action and binding to duplex oligonucleotide strands and deriving the fate of the ruthenium species after incubation with nuclease enzymes.

Experimental

Preparation of ligands was carried out under air using wet solvents. Unless otherwise stated all manipulations were conducted using standard Schlenk line techniques, under an inert atmosphere of di-nitrogen or argon, using a modified dual vacuum/di-nitrogen line or in a Braun Labmaster 100 glove box under an atmosphere of di-nitrogen. Diethyl ether, petroleum ether (bp 40–60 °C), THF and toluene were pre-dried with Na metal and distilled over Na or Na/benzophenone under di-nitrogen. DCM, CH_3CN and methanol were pre-dried and distilled over CaH_2 under N_2. All solvents were subsequently stored in ampoules under N_2. The preparation of $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$ was achieved using literature methods.[32] Pyridine-2-carboxylic acid-(4-nitrophenyl)-amide was prepared as previously reported by Dutta *et al.*[33] ^1H and ^{13}C NMR spectra were recorded on Bruker 250 MHz, 300 MHz and 500 MHz spectrometers. High resolution mass spectrometry was performed by the University of Leeds mass spectrometry service. Elemental analyses were performed by the University of Leeds microanalytical services.

Crystallographic data collection and structure solution

X-ray crystallography. Data for compounds **1–7** were collected on a Nonius KappaCCD area-detector diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) using 1.0 ϕ rotation frames. Pertinent crystallographic details are given in Table 1. The crystal was cooled to 150 K by an Oxford Cryosystems low temperature device[34] before data collection using 1.0° φ rotation frames. The images were processed using the DENZO and SCALEPACK programs[35] followed by structure solution by direct methods *via* one of the SHELXS86[36] SIR92,[37] or SIR97[38] programs. All structures were refined by full-matrix least squares on F^2 using SHELXL97[39] Molecular graphics were plotted using POVray[40] *via* XSeed.[41] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were constrained with a

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Table 1 Crystallographic data for **1–6**

	1	2	3	4	5	6
Formula	$C_{16}H_{20}Cl_2N_2ORu$	$C_{23}H_{22}ClN_3O_3Ru$	$C_{22}H_{23}ClN_3O_3Ru$	$C_{26}H_{24}ClN_3O_3Ru$	$C_{29}H_{27}Cl_{10}N_3O_3Ru$	$C_{12}H_{10}ClNO_2Ru$
Mol. wt.	428.31	524.96	513.95	563.11	921.11	336.73
Temperature (K)	150(2)	150(2)	150(2)	150(2)	150(2)	150(2)
Wavelength [Mo-Kα] (Å)	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1/n$	$C2/c$	$C2/c$	$P2_1/n$	$P2_1/c$	$P\bar{1}$
a (Å)	9.8470(4)	29.8887(4)	31.444(6)	9.1994(2)	12.5540(2)	6.3800(2)
B (Å)	11.8300(6)	8.69930(10)	8.5608(17)	18.2026(4)	15.2770(3)	8.5720(3)
C (Å)	14.8190(7)	15.9600(3)	16.642(3)	14.1456(3)	19.4440(3)	10.8540(3)
α (°)	90	90	90	90	90	75.937(2)
β (°)	92.679(2)°	100.24(3)	113.19(3)	102.5280(9)	104.3070(10)	77.251(2)
γ (°)	90	90	90	90	90	79.785(2
Volume (Å^3)	1724.38(14)	4083.66(11)	4118.0(14)	2312.32(9)	3613.46(11)	556.79(3)
Z	4	8	8	4	4	2
Density (calcd) (Mg m^{-3})	1.65	1.281	1.2433	1.617	1.693	2.008
μ (mm^{-1})	1.221	0.698	0.691	0.828	1.21	1.633
Crystal size (mm)	0.13 × 0.03 × 0.01	0.26 × 0.13 × 0.07	0.2 × 0.2× 0.2	0.2 × 0.13 × 0.12	0.42 × 0.04 × 0.04	0.07 × 0.03 × 0.03
Reflections collected	15832	18405	109847	34376	54562	9665
Independent reflections	3934 [R(int) = 0.0868]	4009 [R(int) = 0.0856]	4002 [R(int) = 0.0534]	4544 [R(int) = 0.1118]	8300 [R(int) = 0.1409]	2499 [R(int) = 0.0815]
Data/restraints/ parameters	3934/0/205	4009/0/274	4002/0/274	4544/0/307	8300/0/419	2499/0/154
Goodness-of-fit on F^2	1.029	1.073	1.006	1.063	1.033	1.065
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0414, wR_2 = 0.0788	R_1 = 0.0356, wR_2 = 0.0895	R_1 = 0.0387, wR_2 = 0.0892	R_1 = 0.0418, wR_2 = 0.1062	R_1 = 0.0431, wR_2 = 0.0982	R_1 = 0.0323, wR_2 = 0.0815
R indices (all data)	R_1 = 0.0726, wR_2 = 0.0881	R_1 = 0.0474, wR_2 = 0.0956	R_1 = 0.0586, wR_2 = 0.0991	R_1 = 0.0571, wR_2 = 0.1154	R_1 = 0.0724, wR_2 = 0.1111	R_1 = 0.0348, wR_2 = 0.0833
Largest diff. peak and hole e Å^{-3}	0.727 and –1.181	0.842 and –1.894	0.628 and –0.902	1.12 and –1.357	0.701 and –1.123	0.625 and –1.12

riding model; U(H) was set at 1.2 × (1.5 × for methyl groups) U_{eq} for the parent atom.

$RuC_{16}H_{18}NO_2Cl$ 1

To a Schlenk tube charged with [Ru(η^6-*p*-cymene)Cl$_2$]$_2$ (0.25 g, 0.4 mmol) in THF (150 mL) was added $C_6H_4NO_2K$ (0.13 g, 0.8 mmol) with stirring. The mixture was allowed to stir overnight to yield an orange solution with a white precipitate of KCl. The solvent was removed *in vacuo* and the product extracted in dichloromethane (3 × 10 mL). Diethyl ether was added (10 mL) to afford a yellow precipitate which was washed in diethyl ether (3 × 5 mL) and dried *in vacuo* to afford a yellow powder. Crystals of the product suitable for X-ray crystallographic analysis were obtained *via* vapour diffusion from dichloromethane and petroleum ether (bp 40–60 °C). (0.2 g, 0.5 mmol, 62.5%). Found: C 48.35; H 4.61; N 3.55%. Calc. for $RuC_{16}H_{18}NO_2Cl$: C 48.92; H 4.62; 3.57%. ^{1}H NMR ($CDCl_3$, 500.13 MHz, 300 K) δ 8.87 [br. d, 1H, ^{3}J(^{1}H-^{1}H) = 7.7 Hz, C_5H_4N], 7.95 [d, 1H ^{3}J(^{1}H-^{1}H) = 7.7 Hz, C_5H_4N], 7.87 [t, 1H, ^{3}J(^{1}H-^{1}H) = 6.7 Hz, C_5H_4N], 7.50 [br. t, 1H, C_5H_4N], 5.55 [d, 1H, ^{3}J(^{1}H-^{1}H) = 5.8 Hz, CH of C_6H_4], 5.53 [d, 1H, ^{3}J(^{1}H-^{1}H) = 5.8 Hz, CH of C_6H_4], 5.39 [d, 1H, ^{3}J(^{1}H-^{1}H) = 5.8 Hz, CH of C_6H_4], 5.31 [d, 1H, ^{3}J(^{1}H-^{1}H) = 5.8 Hz, CH of C_6H_4], 2.81 [sept, 1H, CH(CH$_3$)$_2$], 2.23 [s, 3H, $C_6H_4CH_3$], 1.17 [m, 6H, CH(CH_3)$_2$]. ^{13}C{^{1}H} NMR ($CDCl_3$), 125.76 MHz, 300 K) δ 171.1 [CCO_2], 152.3 [CH of C_5H_4N], 151.3 [$C_5H_4NCCO_2$], 139.2 [CH of C_5H_4N], 127.9 [CH of C_5H_4N], 126.9 [CH of C_5H_4N], 102.6 [C of C_6H_4], 98.7 [C of C_6H_4], 82.8 [CH of C_6H_4], 82.5 [CH of C_6H_4], 81.5 [CH of C_6H_4], 80.9 [CH of C_6H_4], 31.1 [CH(CH$_3$)$_2$], 22.3 22.2 [2 × CH(CH$_3$)$_2$], 18.7 [$C_6H_4CH_3$].

$RuC_{22}H_{22}N_3O_3Cl$ 2

[Ru(η^6-*p*-cymene)Cl$_2$]$_2$ (0.1 g, 0.16 mmol) was dissolved in dry ethanol (50 mL). N-3-nitro-Ph-picolinamide (0.1 g) was added with stirring. The mixture was heated under reflux (1 h), cooled and filtered over NH_4PF_6 (0.1 g). The mixture was heated under reflux overnight to afford an orange solution. The volume of solvent was reduced and petroleum ether (bp 40–60 °C) added (50 mL) to afford an orange precipitate which was isolated *via* filtration, washed in petroleum ether (bp 40–60 °C) and dried *in vacuo* to obtain an orange powder (0.0908 g, 0.15 mmol, 92%). Crystals suitable for X-ray analysis were obtained from methanol. Quadrupolar ES MS (+): m/z 478.2. Found C 51.0; H 4.25; N 8.0% Calc. for $RuC_{22}H_{22}N_3O_3Cl$ C 51.51; H 4.32; 8.19% ^{1}H NMR ($CDCl_3$, 300.1 MHz, 300 K) δ 9.01 [d, 1H, ^{3}J(^{1}H-^{1}H) = 6 Hz, CH of C_5H_4N], 8.62 [d, 1H, ^{3}J(^{1}H-^{1}H) = 6 Hz, CH of $C_6H_4NO_2$], 8.11 [m, 2H, CH of C_5H_4N and CH of $C_6H_4NO_2$], 7.99 [m, 2H, C_5H_4N and CH of $C_6H_4NO_2$], 7.49 [m, 2H, C_5H_4N and CH of $C_6H_4NO_2$], 5.30 [d, 1H, ^{3}J(^{1}H-^{1}H) = 6 Hz, CH of C_6H_4], 5.22 [d, 1H, ^{3}J(^{1}H-^{1}H) = 6 Hz, CH of C_6H_4], 5.16 [d, 1H, ^{3}J(^{1}H-^{1}H) = 6 Hz], 4.79 [d, 1H, ^{3}J(^{1}H-^{1}H) = 6 Hz, CH of C_6H_4], 2.56 [m, 1H, CH of CH(CH$_3$)$_2$], 2.25 [s, 3H, CH$_3$ of $C_6H_4CH_3$], 1.08 [d, 6H, ^{3}J(^{1}H-^{1}H) = 6 Hz, 2 × CH$_3$ of CH(CH_3)$_2$]; ^{13}C{^{1}H} NMR ($CDCl_3$, 75.5 MHz, 300 K) δ 155.8 [C of CONR], 153.6 [C of C_5H_4N], 153.5 [CH of C_5H_4N], 151.8 [C of $C_6H_4NO_2$], 148.8 [C of $C_6H_4NO_2$], 139.2 [CH of C_5H_4N], 133.5 [CH of C_5H_4N], 129.3 [CH of C_5H_4N], 127.3 [CH of $C_6H_4NO_2$], 126.7 [CH of $C_6H_4NO_2$], 122.2 [CH of $C_6H_4NO_2$], 119.4 [CH of $C_6H_4NO_2$], 102.9 [C of C_6H_4], 101.5 [C of C_6H_4], 84.9 [CH of C_6H_4], 84.7 [CH of C_6H_4], 84.3 [CH of C_6H_4], 84.0 [CH of C_6H_4], 31.4 [CH of CH(CH$_3$)$_2$], 22.8 [CH$_3$ of CH(CH$_3$)$_2$], 22.3 [CH$_3$ of CH(CH$_3$)$_2$], 19.2 CH$_3$ of CH$_3$C$_6$H$_4$].

$RuC_{22}H_{22}N_3O_3Cl$ 3

$[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$ (0.1 g, 0.16 mol) was dissolved in dry ethanol (50 mL). N-4-nitro-Ph-picolinamide (0.1 g) was added with stirring. The mixture was heated under reflux (1 h), cooled and filtered over NH_4PF_6 (0.1 g). The mixture was allowed to stir (1 h) to afford a yellow precipitate. The precipitate was isolated *via* filtration, washed in petroleum ether (bp 40–60 °C) and dried *in vacuo* to afford a yellow powder (0.07 g, 0.14 mmol, 85%). Crystals suitable for X-ray analysis were obtained from the mother liquor. Found C 51.45; H 4.25; N 8.3% Calc. for $RuC_{22}H_{22}N_3O_3Cl$ C 51.51; H 4.32; 8.19% ^{1}H NMR ($CDCl_3$, 300.1 MHz, 300 K) δ 8.9 [d, 1H, $^3J(^1H\text{-}^1H) = 6$ Hz, CH of C_5H_4N], 8.2 [m, 2H, CH of $C_6H_4NO_2$], 8.1 [d, 1H, $^3J(^1H\text{-}^1H) = 6$ Hz, CH of C_5H_4N], 7.9 [m, 3H, CH of C_5H_4N, 2 × CH of $C_6H_4NO_2$], 7.6 [m, 1H, CH of C_5H_4N], 5.3 [d, CH of C_6H_4], 5.2 [d, CH of C_6H_4], 5.1 [d, CH of C_6H_4], 4.8 [d, CH of C_6H_4], 2.5 [sept., 1H, $^3J(^1H\text{-}^1H) = 6$ Hz, CH of $(CH_3)_2CH$], 2.3 [s, 3H, $C_6H_4CH_3$], 1.1 [m, 6H, $CH(CH_3)_2$]; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.5 MHz, 300 K) δ 19.3 [$CH(CH_3)_2$], 22.4 [$CH(CH_3)_2$], 22.7 [$CH(CH_3)_2$], 31.4 [$C_6H_4CH_3$], 83.8 [C_6H_4], 84.3 [C_6H_4], 84.6 [C_6H_4], 85.2 [C_6H_4], 101.9 [C of C_6H_4], 102.9 [C of C_6H_4], 119.6 [2 × CH of $C_6H_4NO_2$], 124.5 [2 × CH of $C_6H_4NO_2$], 125.6 [CH of C_5H_4N], 126.9 [CH of C_5H_4N], 127.5 [CH of C_5H_4N], 127.6 [CH of C_5H_4N], 138.4 [C of C_5H_4N], 139.3 [C of $C_6H_4NO_2$], 153.5 [C of $C_6H_4NO_2$], 159.3 [CONAr]. ES MS(+): *m*/*z* 478.0786 [M$^+$]–Cl.

$RuC_{26}H_{24}ClN_3O_3$ 4

To a Schlenk tube charged with $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$ (0.1 g, 0.16 mmol) in dry ethanol (20 mL) was added N-3-nitro-Ph-quinlinamide (0.096 g, 0.33 mmol). The mixture was then heated until dissolution occurred and filtered over NH_4PF_6 (0.1 g, excess). The volume of solvent was reduced *in vacuo* to afford an orange precipitate, which was isolated *via* filtration, washed in diethyl ether (3 × 10 mL) and dried *in vacuo* to afford an orange powder. Crystals suitable for X-ray crystallographic analysis were obtained from the mother liquor, (0.15 g, 0.27 mmol, 84%). Found C 55.30; H 4.45; N 7.55%. Calc. C 55.47; H 4.30; N 7.46% for $RuC_{26}H_{24}N_3O_3Cl$. ^{1}H NMR ($CDCl_3$, 300.13 MHz, 300 K) δ 8.95 [d, 1H, $^3J(^1H\text{-}^1H) = 8.34$ Hz, CH of C_9H_6N], 8.90 [t, 1H, $^3J(^1H\text{-}^1H) = 1.74$ Hz, CH of $C_6H_4NO_2$], 8.42 [d, 1H, $^3J(^1H\text{-}^1H) = 8.10$ Hz, CH of C_9H_6N & CH of $C_6H_4NO_2$], 8.30 [d, 1H, $^3J(^1H\text{-}^1H) = 8.40$ Hz, CH of C_9H_6N], 8.00 [m, 3H, 2 × CH of C_9H_6N & CH of $C_6H_4NO_2$], 7.79 [t, 1H, $^3J(^1H\text{-}^1H) = 6.0$ Hz, CH of C_9H_6N], 7.54 [t, 1H, $^3J(^1H\text{-}^1H) = 8.10$ Hz, CH of $C_6H_4NO_2$], 5.49 [d, 1H, $^3J(^1H\text{-}^1H) = 6.0$ Hz, CH of C_6H_4], 5.36 [d, 1H, $^3J(^1H\text{-}^1H) = 5.7$ Hz, CH of C_6H_4], 5.25 [d, 1H, $^3J(^1H\text{-}^1H) = 5.7$ Hz, CH of C_6H_4], 4.75 [d, 1H, $^3J(^1H\text{-}^1H) = 6.0$ Hz, CH of C_6H_4], 2.31 [m, 4H, $CH(CH_3)_2$ & CH_3], 1.06 [d, 3H, $^3J(^1H\text{-}^1H) = 6.9$ Hz, $CH(CH_3)_2$], 0.88 [d, 3H, $^3J(^1H\text{-}^1H) = 6.9$ Hz, $CH(CH_3)_2$]; $^{13}C\text{-}\{^1H\}$ NMR ($CDCl_3$, 75.47 MHz, 300 K) δ 167.45 [C=O], 157.70 [C of C_9H_6N], 153.42 [C of $C_6H_4NO_2$], 148.73 [C of $C_6H_4NO_2$], 148.67 [C of C_9H_6N], 139.88 [CH of C_9H_6N], 133.26 [CH of $C_6H_4NO_2$], 131.74 [CH of C_9H_6N], 130.70 [C of C_9H_6N], 129.37 [CH of $C_6H_4NO_2$], 129.34 [CH of $C_6H_4NO_2$], 129.29 [CH of C_9H_6N], 128.98 [CH of C_9H_6N], 122.69 [CH of C_9H_6N], 122.05 [CH of $C_6H_4NO_2$], 119.35 [CH of C_9H_6N], 103.28 [C of C_6H_4], 102.06 [C of C_6H_4], 85.50 [CH of C_6H_4], 84.88 [CH of C_6H_4], 84.58 [CH of C_6H_4], 84.42 [CH of C_6H_4], 31.26 [$CH(CH_3)_2$], 22.46 [$CH(CH_3)_2$], 22.40 [$CH(CH_3)_2$], 19.18 [CH_3].

$RuC_{26}H_{24}ClN_3O_3$ 5

To a Schlenk tube charged with a suspension of $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$, 0.08 mmol) in dry ethanol (20 mL) was added N-4-nitro-Ph-quinlinamide (0.047 g, 0.16 mmol). The mixture was then heated until dissolution occurred and filtered over NH_4PF_6 (0.05 g, excess). The volume of solvent was reduced *in vacuo* to afford an orange precipitate, which was isolated *via* filtration, washed in diethyl ether (30 mL) and dried *in vacuo* to afford an orange powder. Crystals suitable for X-ray crystallographic analysis were obtained from $CDCl_3$. (0.075 g, 0.1 mmol, 79%). Analysis for $RuC_{26}H_{24}N_3O_3Cl{\cdot}C_2H_5OH$. Found C 54.25; H 4.31; N 7.33%. Calc. C 54.50; H 4.74; N 7.06%. ^{1}H NMR ($CDCl_3$, 300.13 MHz, 300 K) δ 8.95 [d, 1H, $^3J(^1H\text{-}^1H) = 9$ Hz, CH of C_9H_6N], 8.43 [d, 1H, $^3J(^1H\text{-}^1H) = 6$ Hz, CH of C_9H_6N], 8.27 [m, 3H, 2 × CH of $C_6H_4NO_2$, CH of C_9H_6N], 8.23 [d, 2H, 2 × CH of $C_6H_4NO_2$], 8.01 [m, 1H, CH of C_9H_6N], 8.00 [m, 1H, C_9H_6N], 7.80 [t, 1H, $^3J(^1H\text{-}^1H) = 6$ Hz, C_9H_6N], 5.47 [d, 1H, $^3J(^1H\text{-}^1H) = 6$ Hz, CH of C_6H_4], 5.34 [d, 1H, $^3J(^1H\text{-}^1H) = 6$ Hz, CH of C_6H_4], 5.24 [d, 1H, $^3J(^1H\text{-}^1H) = 6$ Hz, CH of C_6H_4], 4.77 [d, 1H, $^3J(^1H\text{-}^1H) = 6$ Hz, CH of C_6H_4], 2.28 [m, 4H, CH_3 of $C_6H_4CH_3$, CH of $CH(CH_3)_2$], 1.07 [d, 3H, $^3J(^1H\text{-}^1H) = 9$ Hz, CH_3 of $CH(CH_3)_2$], 0.90 [d, 3H, $^3J(^1H\text{-}^1H) = 9$ Hz, CH_3 of $CH(CH_3)_2$];$^{13}C\text{-}\{^1H\}$ NMR ($CDCl_3$, 75.47 MHz, 300 K) δ 167.40 [C of CONR]; 159.08 [C of $C_6H_4NO_2$]; 157.59 [C of $C_6H_4NO_2$]; 148.69 [C of C_9H_6N]; 144.04 [C of C_9H_6N]; 139.99 [CH of C_9H_6N]; 131.82 [CH of C_9H_6N]; 130.75 [C of C_9H_6N]; 129.40 [CH of C_9H_6N]; 129.30 [CH of C_9H_6N]; 129.08 [CH of C_9H_6N]; 127.42 [2 × CH of $C_6H_4NO_2$]; 124.47 [2 × CH of $C_6H_4NO_2$]; 122.68 [CH of C_9H_6N]; 103.27 [C of C_6H_4]; 102.31 [C of C_6H_4]; 85.81 [CH of C_6H_4]; 84.69 [CH of C_6H_4]; 84.57 [CH of C_6H_4]; 84.52 [CH of C_6H_4]; 31.22 [CH_3]; 22.47 [CH_3 of $CH(CH_3)_2$]; 22.40 [CH_3 of $CH(CH_3)_2$]; 19.17 [CH of $CH(CH_3)_2$]; ES MS(+): *m*/*z* 528.0859 [M$^+$]–Cl$^-$.

$RuC_{12}H_{10}NO_2Cl$ 6

To a Schlenk tube charged with a suspension of $[Ru(\eta^6\text{-benzene})Cl_2]_2$ (0.05 g, 0.1 mmol) in THF (50 mL) was added potassium picolinate (0.03 g, 0.2 mmol) with stirring. The mixture was then heated under reflux (24 h) to afford a yellow suspension. The solvent was removed *in vacuo*, the product extracted in methanol (50 mL) and stored at –20 °C. Orange crystals suitable for X-ray crystallographic analysis were obtained from the mother liquor. (0.03 g, 0.1 mmol, 45%). Analysis for $RuC_{12}H_{10}NO_2Cl$. Found: C 42.60; H 3.11; N 4.25%. Calc.: C 42.80; H 2.99; N 4.16%. ^{1}H NMR (d^6-DMSO, 300.13 MHz, 300 K) δ 9.43 [d, 1H, $^3J(^1H\text{-}^1H) = 6.6$ Hz, CH of C_5H_4N], 8.11 [t, 1H, $^3J(^1H\text{-}^1H) = 6.6$ Hz, CH of C_5H_4N], 7.78 [m, 2H, 2 × CH of C_5H_4N], 5.95 [s, 6H, C_6H_6];$^{13}C\{^1H\}$ NMR (d^6-DMSO, 62.90 MHz, 300 K) δ 170.8 [C of CON], 154.5 [CH of C_5H_4N], 150.8 [C of C_5H_4N], 140.1 [CH of C_5H_4N], 128.3 [CH of C_5H_4N], 125.8 [CH of C_5H_4N], 83.7 [CH of C_6H_6].

$RuC_{18}H_{14}N_3O_3Cl$ 7

To a Schlenk tube charged with a suspension of $[Ru(\eta^6\text{-benzene})Cl_2]_2$ (0.1 g, 0.19 mmol) in methanol (50 mL) was added

N-4-nitro-Ph-picolinamide (0.1 g, 0.28 mmol) with stirring. The mixture was then heated under reflux (*ca.* 3 h.) and the cooled mixture filtered over NH_4PF_6 (0.1 g, excess) and heated under reflux overnight. On cooling the mixture an orange precipitate was formed. The precipitate was isolated *via* filtration, washed in petroleum ether (bp 40–60 °C) and dried *in vacuo* to afford an orange powder. Crystals suitable for X-ray crystallographic analysis were obtained *via* vapour diffusion from acetone and diethyl ether. (0.08 g, 0.175 mmol, 92%). Found C 47.13.; H 2.84.; N 8.97.% Calc. for $RuC_{18}H_{14}N_3O_3Cl$ C 47.32; H 3.09; N 9.20% ^{1}H NMR (d^6-DMSO, 300.13 MHz, 300 K) δ 9.41 [d, 1H, $^3J(^1H\text{-}^1H)$ = 6.2 Hz, CH of C_5H_4N], 8.23 [d, 2H, $^3J(^1H\text{-}^1H)$ = 9.0 Hz, 2 × CH of $C_6H_4NO_2$], 8.10 [1H, t, $^3J(^1H\text{-}^1H)$ = 3.3 Hz, CH of C_5H_4N], 7.89 [3H, m, 1 × CH of C_5H_4N, 2 × CH of $C_6H_4NO_2$], 7.70 [1H, t, $^3J(^1H\text{-}^1H)$ = 3.3 Hz, CH of C_5H_4N], 5.68 [6H, s, 6 × CH of C_6H_6];^{13}C-{^{1}H} (d^6-DMSO, 62.90 MHz, 300 K) δ 167.1 [C of *C*ON], 159.9 [C of C_5H_4N], 155.3 [CH of C_5H_4N], 154.6 [C of $C_6H_4NO_2$], 143.0 [C of $C_6H_4NO_2$], 139.7 [CH of C_5H_4N], 128.7 [CH of C_5H_4N], 127.7 [2 × CH of $C_6H_4NO_2$], 125.5 [CH of C_5H_4N], 124.0 [2 × CH of $C_6H_4NO_2$], 85.8 [6 × CH of C_6H_6]. ES MS(+): *m*/*z* 422.0161 [M$^+$]–Cl$^-$.

[RuC$_{26}$H$_{31}$N$_6$O$_7$][PF$_6$] 8

Guanosine hydrate (0.02 g, 0.08 mmol.) was added to a stirred solution of **1** (0.03 g, 0.08 mmol.) in distilled water. The mixture was allowed to stir at 65 °C (5 days) and filtered over NH_4PF_6 (0.1 g, excess). A green precipitate was immediately formed which was isolated *via* filtration, washed in diethyl ether and dried to afford a pale green powder. (0.025 g, 0.03 mmol, 40%) Analysis for $RuC_{26}H_{31}N_6O_7PF_6{\cdot}2.5H_2O$ Found: C 37.05; H 4.50; N 10.70%. Calc.: C 37.64; H 4.25; N 10.12%. ^{1}H NMR (D_2O, 300.13 MHz, 300 K) δ 9.49 [br. d, 1H, CH of C_5H_4N], 7.90 [t, 1H, $^3J(^1H\text{-}^1H)$ = 9.0 Hz, CH of C_5H_4N], 7.80 [d, 1H $^3J(^1H\text{-}^1H)$ = 9.0 Hz, N=C*H*-N], 7.63 [m, 2H, 2 × CH of C_5H_4N], 5.96 [m, 1H, CH of C_6H_4], 5.75 [m, 1H, CH of C_6H_4], 5.67 [m, 2H, 1 × CH of C_6H_4 1 × CH of $C_5H_9O_4$], 5.50 [d, 1H, $^3J(^1H\text{-}^1H)$ = 3.3 Hz, CH of C_6H_4], 4.42 [d of t, 1H, CH of $C_5H_9O_4$], 4.20 [d of t, 1H, CH of $C_5H_9O_4$], 4.11 [m, 1H, CH of $C_5H_9O_4$], 3.72 [m, 2H, CH_2OH], 2.50 [m, 1H, CH(CH$_3$)$_2$], 1.86 [s, 3H, CH_3], 1.05 [t, 3H, 1 × CH(CH_3)$_2$], [d, 3H, 1 × CH(CH_3)$_2$]; ^{13}C{^{1}H} NMR (D_2O, 75.47 MHz, 300 K) δ 174.5 [$C_5H_4NCO_2$], 156.5 [Guanine *C*=O], 155.6 [C_5H_4N], 154.8 [Guanine N=*C*-NH$_2$], 151.7 [Guanine N-*C*-C=O], 148.0 [Guanine N-*C*-N], 141.2 [C_5H_4N], 139.3 [N=*C*=N], 129.9 [C_5H_4N], 127.1 [C_5H_4N], 116.9 [C of $C_5H_4NCO_2$], 104.8 [C of C_6H_4], 102.4 [C of C_6H_4], 88.9 [Ribose N-*C*H], 86.0/85.7 [2 × CH of C_6H_4], 85.5 [Ribose HOCH$_2$-*C*H], 82.8/82.4 [2 × CH of C_6H_4], 74.5 [Ribose N-CH-*C*HOH], 70.4 [Ribose *C*HOH-CH-CH$_2$OH], 61.4 [Ribose *C*H$_2$OH], 31.0 [(CH$_3$)$_2$*C*H], 21.9 [(*C*H$_3$)$_2$CH], 21.18 [(*C*H$_3$)$_2$CH], 17.7 [*C*H$_3$].^{31}P NMR (D_2O, 101.26 MHz, 300 K) δ –60.76 [septet, $^1J(^{31}P\text{-}^{19}F)$ 708 Hz, *P*F$_6^-$]. ES-MS: *m*/*z* 641.1271 [M$^+$].

Typical SRB assay

The assay was carried out in 96 well plates with an average of 0.5 × 10^4 cells per mL with 100 µl in each well. The cells were then treated with varying concentrations of drug for 6 days and then incubated for 6 days in an atmosphere of 5% CO_2/95% air. After 6 days the cells were removed from the incubator and washed with cold trichloroacetic acid (TCA) to fix the cells to the bottom of the plates. These were then washed with water to ensure the removal of all TCA. The plates were then stained with a solution of 0.02% (w/v) SRB in 1.0% (v/v) acetic acid for 30 min. The plates were washed thoroughly with acetic acid and allowed to dry overnight. 100 µl of 10 mM unbuffered tris was added to each well to re-suspend the SRB dye. The plates were then read using a plate reader which records the mean absorbance at 570 nm of each concentration and using a linear interpolation the IC_{50} is calculated.

DNA nuclease treatment

10 µl DNA was incubated with 10 µl compound **3** or cisplatin for 45 min at room temperature. The reaction mixture was then treated with 8 µl (0.02 units) snake venom Phosphodiesterase I (purchased from Sigma-Aldrich Company Ltd.) for 45 min at room temperature to produce single nucleotides-5′-monophosphate. 2 µl (2 units) Shrimp Alkaline Phosphatase (purchased from Sigma-Aldrich Company Ltd.) was added to the nuclease reaction mixture, which was made up to 100 µl with nuclease buffer (32 mM Tris HCl, pH 7.4, 25 mM $MgCl_2$ in nuclease-free water) and incubated overnight at 37 °C in a DNA Engine PTC200 Peltier Thermal Cycler PCR following the method of Eadie *et al.*[42] The reaction mixture was purified by adding 10 µl NaCl (5 mM), together with 200 µl absolute ethanol at 0 °C, then chilled on dry ice for 30 min, and centrifuged for 30 min at 4 °C in a Biofuge fresco (top speed 13,000 r.p.m.). The supernatant was collected, and the total volume was made up to 1 ml with absolute ethanol. At that time the mixture was chilled for a further 30 min in dry ice, then centrifuged as before. The supernatant obtained from the second step was evaporated to dryness *via* lyophilisation in a speed vacuum lyophiliser to concentrate the samples which were dissolved in ultra pure nuclease free water and stored at –80 °C for mass spectrometry analyses.

Results and discussion

Compound **1** was synthesised by the reaction of potassium picolinate with [Ru(η^6-*p*-cymene)Cl$_2$]$_2$. Compound **1** was recrystallised from a mixture of ethanol/petroleum ether (bp 40–60 °C) and was characterised by NMR spectroscopy, microanalysis and X-ray crystallography. The molecular structure of compound **1** is shown in Fig. 1 and shows binding of the ligand through the pyridinyl nitrogen and the carbonyl oxygen (N/O). Selected bond lengths and angles are shown in Table 2. Compound **1** crystallised in a monoclinic cell and the structural solution was performed in the space group $P2_1/n$. The asymmetric unit comprises one molecule of **1**. Compound **1** (and all subsequent ruthenium arene complexes synthesised in this work) possess the characteristic pseudo-tetrahedral geometry exhibited by all η^6-ruthenium arene complexes. The η^6 π-bonded arene ring occupying one vertex of the tetrahedron, with the three other ligands occupying the remaining three sites. An alternative term for the molecular structure of ruthenium arene complexes is given by the "piano stool" analogy, whereby the η^6 π-bonded arene ligand forms the seat and the three remaining ligands form the legs of the stool.[43]

Compound **1** crystallised in the R_{Ru} configuration (*R* configuration at the ruthenium metal centre). The bond lengths observed

Table 2 Selected bond distances (Å) and bond angles (°) for compound **1**. Estimated standard deviations are given in parentheses

Ru(1)–O(1)	2.0809(17)	Ru(1)–N(1)	2.096(2)
Ru(1)–Cl(1)	2.4133(6)	Ru(1)–C(4)	2.190(3)
Ru(1)–C(5)	2.173(2)	Ru(1)–C(6)	2.175(3)
Ru(1)–C(9)	2.186(2)	Ru(1)–C(7)	2.216(3)
Ru(1)–C(10)	2.175(3)	O(1)–C(11)	1.290(3)
O(2)–C(11)	1.228(3)	C(4)–C(5)	1.436(4)
C(5)–C(6)	1.402(4)	C(6)–C(7)	1.438(4)
C(9)–C(7)	1.401(4)	C(4)–C(10)	1.410(4)
C(10)–C(9)	1.425(4)		
Ru(1)–C(Arene)	2.186(3)		
Average			
O(1)–Ru(1)–N(1)	77.95(7)	N(1)–Ru(1)–Cl(1)	84.04(6)
O(1)–Ru(1)–Cl(1)	85.56(5)	O(2)–C(11)–O(1)	124.4(2)
C(4)–C(10)–C(9)	120.9(2)	C(6)–C(5)–C(4)	121.5(2)
O(2)–C(11)–O(1)	124.4(2)		
C(4)–C(10)–C(9)	120.9(2)	C(6)–C(5)–C(4)	121.5(2)
C(5)–C(6)–C(7)	121.1(2)	C(7)–C(9)–C(10)	122.1(2)
C(9)–C(7)–C(6)	117.1(2)	C(10)–C(4)–C(5)	117.3(2)

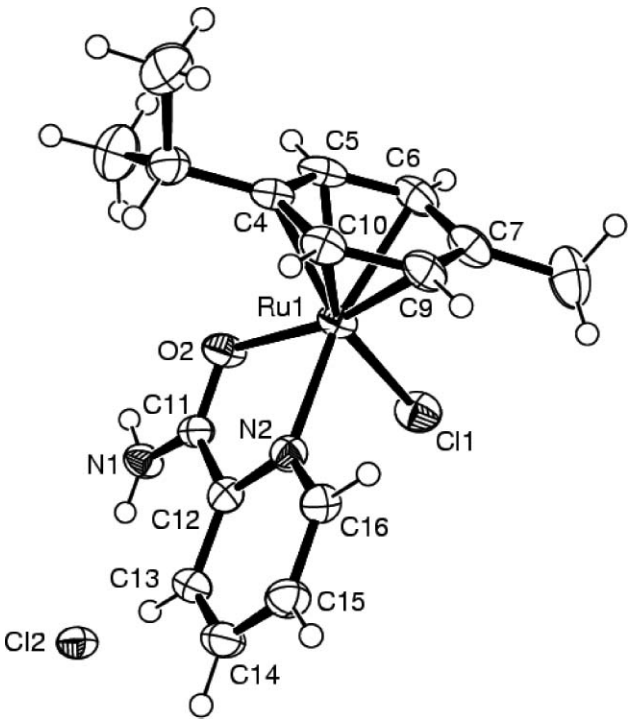


Fig. 1 The molecular structure of compound **1**, with 50% probability thermal ellipsoids.

in compound **1**: Ru(1)–O(1) of 2.0809(17) Å and Ru(1)–Cl(1) of 2.4133(6) Å are within the ranges observed for other ruthenium arene α-amino acidate complexes, of 2.079(11) Å–2.392(7) Å.[44] The Ru(1)–N(1) bond length of 2.096(2) Å is within the range of analogous ruthenium arene pyridyl complexes.[45]

The Ru–C(arene) average bond distance of 2.186(3) Å is within the range of ruthenium arene complexes of this type (as are the Ru–C(arene) bond lengths in all subsequent ruthenium arene complexes synthesised in this work).[46] As reported in previous works,[43] the bond length between the ruthenium centre and the substituted carbon atoms of the *p*-cymene ligand are longer by *ca.* 0.017–0.043 Å. This feature is observed in all subsequent ruthenium arene complexes reported in this work.

Table 3 Selected bond distances (Å) and bond angles (°) for compound **2**. Estimated standard deviations are given in parentheses

Ru(1)–N(1)	2.077(2)	Ru(1)–N(2)	2.107(2)
Ru(1)–C(9)	2.163(3)	Ru(1)–C(5)	2.183(3)
Ru(1)–C(4)	2.207(3)	Ru(1)–C(8)	2.210(3)
Ru(1)–C(6)	2.210(3)	Ru(1)–C(7)	2.239(3)
Ru(1)–Cl(1)	2.4067(8)	O(1)–C(17)	1.241(3)
N(1)–C(18)	1.343(3)	N(1)–C(22)	1.347(4)
N(2)–C(17)	1.345(4)	O(2)–N(3)	1.225(3)
O(3)–N(3)	1.225(4)	N(2)–C(11)	1.416(3)
C(11)–C(16)	1.399(4)	N(3)–C(15)	1.469(4)
C(13)–C(14)	1.383(4)	C(11)–C(12)	1.392(4)
C(15)–C(16)	1.388(4)	C(12)–C(13)	1.392(4)
C(18)–C(19)	1.377(4)	C(14)–C(15)	1.380(4)
C(20)–C(21)	1.385(4)	C(17)–C(18)	1.504(4)
C(19)–C(20)	1.381(4)	C(21)–C(22)	1.375(4)
N(1)–Ru(1)–N(2)	76.59(9)	N(1)–Ru(1)–Cl(1)	84.33(7)
N(2)–Ru(1)–Cl(1)	87.13(7)		

Table 4 Selected bond distances (Å) and bond angles (°) for compound **3**. Estimated standard deviations are given in parentheses

N(1)–Ru(1)	2.083(3)	N(2)–Ru(1)	2.093(3)
Cl(1)–Ru(1)	2.4173(10)	C(4)–Ru(1)	2.191(4)
C(5)–Ru(1)	2.161(4)	C(7)–Ru(1)	2.228(3)
C(9)–Ru(1)	2.187(3)	C(8)–Ru(1)	2.200(4)
C(6)–Ru(1)	2.197(4)	C(20)–N(3)	1.471(5)
O(2)–N(3)	1.211(5)	O(3)–N(3)	1.235(5)
C(4)–C(5)	1.402(5)	C(7)–C(6)	1.404(5)
C(7)–C(8)	1.425(5)	C(9)–C(8)	1.392(5)
C(5)–C(6)	1.420(5)	C(9)–C(4)	1.425(5)
C(16)–O(1)	1.242(4)		
Ru(1)–C(Arene)	2.194(4)		
Average			
Cg(2)–Ru(1)[a]	1.6801		
Cg(2)–Ru(1)–Cl(1)[a]	127.68	Cg(2)–Ru(1)–N(1)[a]	131.36
Cg(2)–Ru(1)–N(2)[a]	131.31	N(1)–Ru(1)–N(2)	76.45(11)
N(1)–Ru(1)–Cl(1)	85.12(8)	N(2)–Ru(1)–Cl(1)	87.15(9)
O(2)–N(3)–O(3)	124.3(4)		
C(5)–C(4)–C(9)	117.0(3)	C(6)–C(7)–C(8)	118.0(3)
C(4)–C(5)–C(6)	121.4(3)	C(7)–C(6)–C(5)	120.9(3)
C(9)–C(8)–C(7)	120.4(3)	C(8)–C(9)–C(4)	122.2(3)

[a] Cg(2) denotes centroid of the C_6 ring C(4)–C(9).

Compounds **2** and **3** were synthesised by the reaction of the N-3-nitro-Ph-picolinamide and N-4-nitro-Ph-picolinamide with $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$. Compounds **2** and **3** were recrystallised from ethanol and methanol respectively. Both compounds were characterised by NMR spectroscopy, microanalysis and X-ray crystallography. Selected bond lengths and angles are shown in Tables 3 and 4 respectively. The molecular structures are shown in Fig. 2 and 3. The ligands are bound through the pyridyl nitrogen and the amidinato nitrogen atoms (N/N) similar to their osmium analogues.[47] Thus, for compounds **2** and **3**, substituting a NO_2 group in the *p*- and *m*-position makes the ligand bind through the pyridyl N and amidinato N atoms, whilst the unsubstituted/alkyl substituted derivative tends to N/O binding. The increased acidity of the proton is caused by the electron withdrawing groups on the phenyl ring. Studies carried out for the *p*-substituted phenol, systems show that *p*-nitro has the largest σ^- (enhanced σ-value due to electron withdrawing resonance effects) value of 1.27.[48] This is reinforced by the fact that for the *p*-nitro derivative, the reaction proceeds smoothly at room temperature, whereas for the *m*-nitro derivative, heat is needed to ensure that the reaction proceeds to completion.

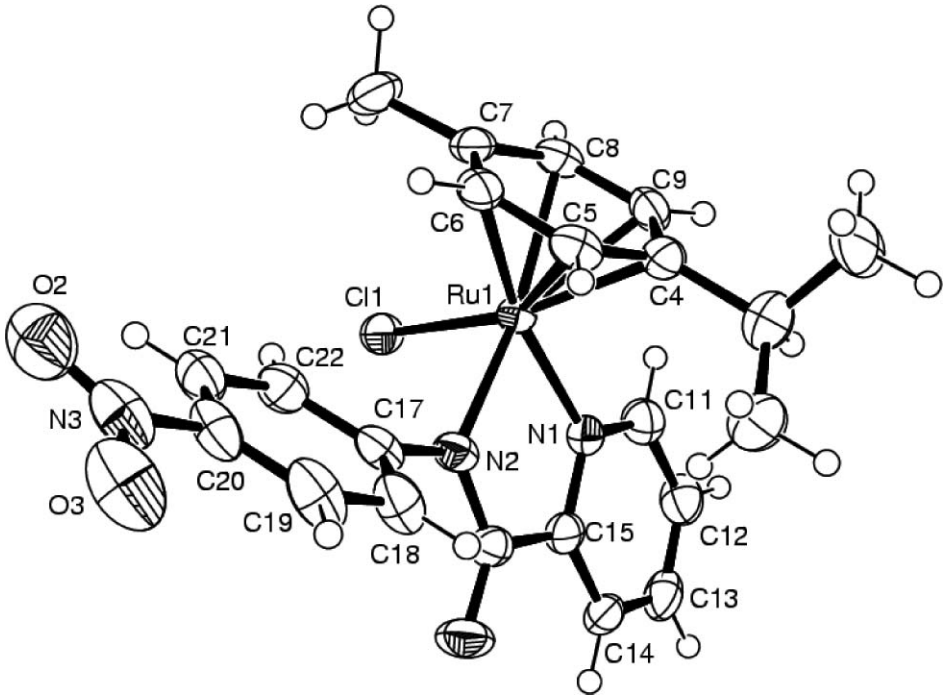


Fig. 2 The molecular structure of compound **2**, with 50% probability thermal ellipsoids.

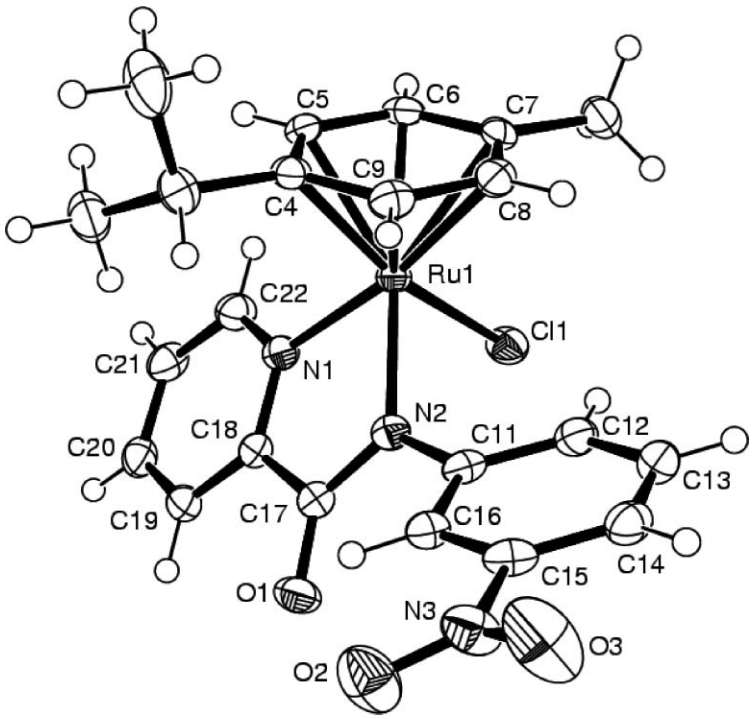


Fig. 3 The molecular structure of compound **3**, with 50% probability thermal ellipsoids.

Studies have revealed that increasing the hydrophobic character of a complex resulted in a concomitant increase in the activity of that complex against a range of tumour types.[16] Quinoline-2-carboxylic acid (quinaldic acid) is structurally similar to picolinic acid, and the quinoline residue is abundant in naturally occurring molecules. Quinaldic acid possesses an extra four-membered ring relative to picolinic acid and as a result may possess more hydrophobic character than the less substituted pyridine analogue. There is also the possibility for π–π stacking. In order to investigate these effects on the cytotoxic activity of ruthenium arene complexes, a series of quinaldic acid derivatives (analogues of the picolinic acid complexes) (*vide supra*) were synthesised.

Synthetic routes to a range of quinaldamide derivatives were originally developed by Davis *et al.* for studies of the potential

application of quinaldic acid for the identification and isolation of amino acids, peptides and proteins.[49] Initially quinaldic acid was converted to the acid chloride *via* treatment with excess thionyl chloride. Condensation of quinaldic acid with the appropriate aniline in the presence of base afforded quinaldamide ligands in moderate to good yields (they are also commercially available). Few examples of quinaldic acid and quinaldamide species with metal ions have been reported in the literature. Amongst these, there have been studies on the coordination modes of ruthenium complexes containing the phosphine ligand, bis(diphenylphosphino)methane (DPPM).[50] The interaction and coordination modes of the ligands, *N*-(2-aminophenyl)quinoline-2′-carboxamide and *N*-(3-aminophenyl)quinoline-2′-carboxamide with cobalt, copper, zinc and nickel metal ions has been investigated.[51]

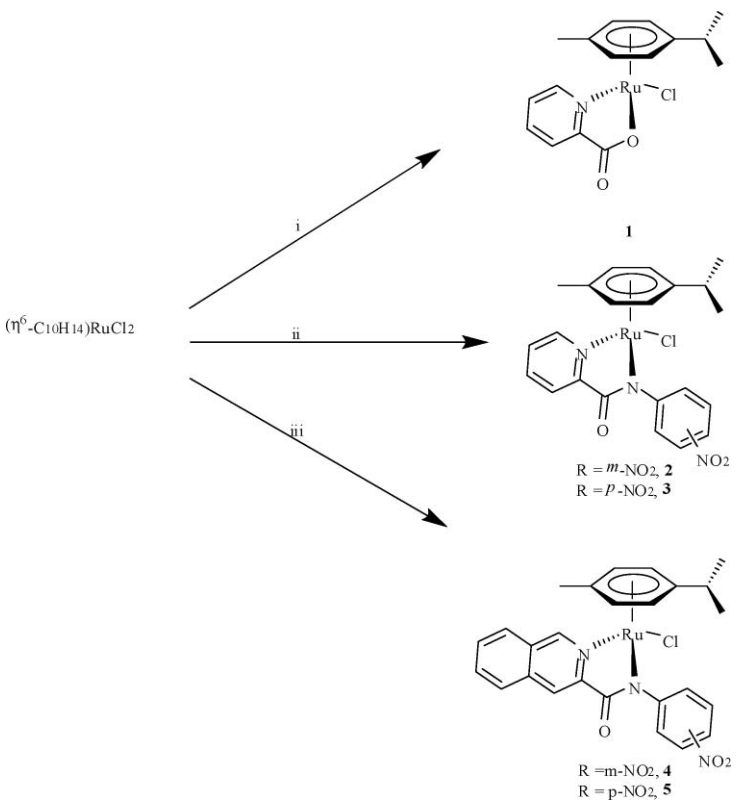
Compounds **4** and **5** were synthesised according to Scheme 1. $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$ was heated with a slight excess of N-3-nitro-Ph-quinlinamide or N-4-nitro-Ph-quinlinamide in dry ethanol. The mixture was then filtered over an excess of NH_4PF_6 to afford $[RuC_{26}H_{24}N_3O_3Cl]$ **4** and $[RuC_{26}H_{24}N_3O_3Cl]$ **5**. As observed in the ruthenium arene picolinic acid complexes (**2** & **3**) the quinaldamide derivatives incorporating a nitro-functional group exhibit preference for the formation of a neutral species in which the ligand is bound through the pyridyl nitrogen and the amidato nitrogen (*vide supra*). Compounds **4** and **5** were obtained as analytically pure orange powders in 84% and 79% yields respectively as air stable racemic mixtures ($[\alpha]_D^{25} = 0°$). Both complexes were characterised by NMR spectroscopy, mass spectrometry, elemental analyses and X-ray crystallography.

Table 5 Selected bond distances (Å) and bond angles (°) for compound **4**. Estimated standard deviations are given in parentheses

Ru(1)–N(13)	2.088(3)	Ru(1)–N(2)	2.112(3)
Ru(1)–Cl(1)	2.4091(9)	Ru(1)–C(20)	2.220(4)
Ru(1)–C(21)	2.181(4)	Ru(1)–C(22)	2.249(4)
Ru(1)–C(23)	2.261(3)	Ru(1)–C(24)	2.184(3)
Ru(1)–C(25)	2.165(3)	O(12)–C(12)	1.232(4)
O(16A)–N(16)	1.215(4)	N(16)–O(16B)	1.217(5)
C(20)–C(25)	1.407(5)	C(20)–C(21)	1.419(5)
C(25)–C(24)	1.408(5)	C(24)–C(23)	1.424(5)
C(23)–C(22)	1.399(5)	C(21)–C(22)	1.416(5)
Ru(1)–C(Arene) Average	2.188(2)	Cg(4)–Ru(1)[a]	1.7003(15)
Cg(4)–Ru(1)–Cl(1)[a]	129.70(6)	Cg(4)–Ru(1)–N(2)[a]	132.18(9)
Cg(4)–Ru(1)–N(13)[a]	128.66(9)	N(13)–Ru(1)–N(2)	77.21(10)
N(13)–Ru(1)–Cl(1)	86.43(8)	N(2)–Ru(1)–Cl(1)	84.57(7)
O(16A)–N(16)–O(16B)	123.1(3)	O(12)–C(12)–N(13)	128.3(3)
C(25)–C(20)–C(21)	116.4(3)	C(20)–C(25)–C(24)	122.4(3)
C(25)–C(24)–C(23)	120.2(3)	C(22)–C(23)–C(24)	118.2(3)
C(22)–C(21)–C(20)	121.8(3)	C(23)–C(22)–C(21)	120.7(3)

[a] Cg(4) denotes centroid of the C_6 ring C(20)–C(25).

Orange single crystals of **4** suitable for X-ray crystallographic analysis were obtained from $CDCl_3$ in an NMR tube. The molecular structure is given in Fig. 4. Selected bond lengths and angles are given in Table 5. Compound **4** crystallised in a monoclinic cell and the structural solution was performed in the space group $P2_1/n$. The asymmetric unit comprises one molecule of **4**. Compound **4** crystallised in the S_{Ru} configuration (*S* configuration at the ruthenium metal centre).



Scheme 1 (i) potassium picolinate, THF; (ii) N-3-nitro-Ph-picolinamide and N-4-nitro-Ph-picolinamide, THF; NH_4PF_6 (iii) N-3-nitro-Ph-quinlinamide and N-4-nitro-Ph-quinlinamide, THF; NH_4PF_6.

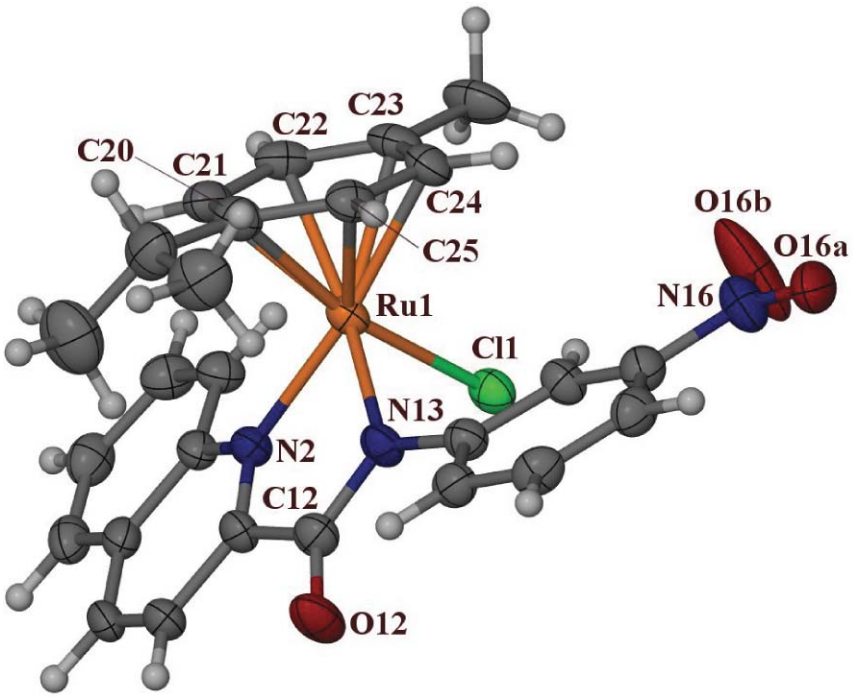


Fig. 4 The molecular structure of compound **4**, with 50% probability thermal ellipsoids.

Table 6 Selected bond distances (Å) and bond angles (°) for compound **5.** Estimated standard deviations are given in parentheses

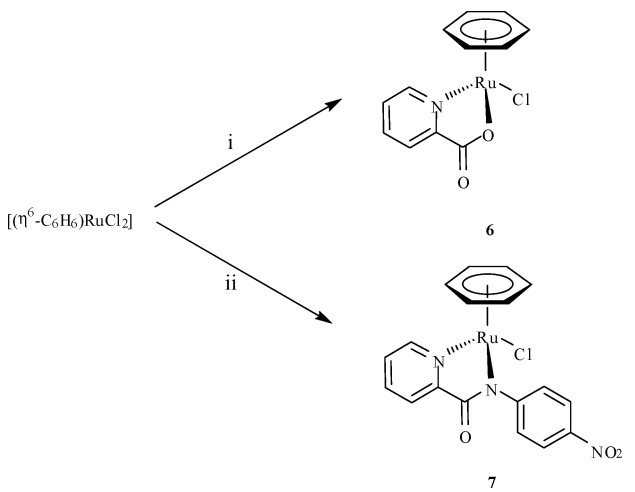
Ru(1)–N(2)	2.092(2)	Ru(1)–N(1)	2.119(2)
Ru(1)–Cl(1)	2.4081(8)	Ru(1)–C(20)	2.206(3)
Ru(1)–C(21)	2.182(3)	Ru(1)–C(22)	2.215(3)
Ru(1)–C(23)	2.230(3)	Ru(1)–C(25)	2.200(3)
Ru(1)–C(26)	2.169(3)	O(1)–C(10)	1.237(4)
O(2)–N(3)	1.241(4)	O(3)–N(3)	1.231(4)
C(20)–C(21)	1.425(4)	C(21)–C(22)	1.396(5)
C(22)–C(23)	1.426(5)	C(23)–C(25)	1.408(5)
C(23)–C(24)	1.504(4)	C(25)–C(26)	1.425(4)
Ru(1)–C(Arene) Average	2.200(3)		
Cg(4)–Ru(1)[a]	1.6837		
Cg(4)–Ru(1)–Cl(1)[a]	128.95	Cg(4)–Ru(1)–N(1)[a]	132.46
Cg(4)–Ru(1)–N(2)[a]	128.72	N(2)–Ru(1)–N(1)	77.26(9)
N(2)–Ru(1)–Cl(1)	87.54(7)	N(1)–Ru(1)–Cl(1)	84.16(7)
O(3)–N(3)–O(2)	122.7(3)	O(1)–C(10)–N(2)	127.5(3)
C(21)–C(22)–C(23)	120.5(3)	C(25)–C(23)–C(22)	118.1(3)
C(23)–C(25)–C(26)	120.9(3)	C(20)–C(26)–C(25)	121.3(3)
C(22)–C(21)–C(20)	122.4(3)	C(21)–C(22)–C(23)	120.5(3)

[a] Cg(4) denotes centroid of the C_6 ring C(20)–C(26).

Orange single crystals of **5** suitable for X-ray crystallographic analysis were obtained from $CDCl_3$ in a NMR tube. The molecular structure is given in Fig. 5. Selected bond lengths and angles are given in Table 6. Compound **5** crystallised in a monoclinic cell and the structural solution was performed in the space group $P2_1/c$. The asymmetric unit comprises one molecule of **5**. Compound **5** crystallised in the S_{Ru} configuration (S configuration at the ruthenium metal centre).

In order to determine the effect of substituents on the arene ring on the anticancer activity of the ruthenium arene complexes studied in this work, a selection of analogous half-sandwich η^6-benzene ruthenium complexes were synthesised. From the synthesis of these complexes, the effect of the *p*-cymene ligands on the anticancer activity of the complexes synthesised in this work may be elucidated. The half-sandwich η^6-benzene ruthenium complexes were synthesised using analogous methods to those employed for the synthesis of ruthenium arene complexes **1–5**.

Compound **6** was synthesised according to Scheme 2. Reaction of $[Ru(\eta^6\text{-benzene})Cl_2]_2$ with two equivalents of potassium picolinate in THF solution afforded $[RuC_{12}H_{10}NO_2Cl]$ **6**. Compound **6** was characterised by NMR spectroscopy, microanalysis, mass spectrometry and X-ray crystallography. In contrast to all other complexes synthesised in this work, only one signal, with an integration corresponding to six protons is observed in the ^1H and $^{13}C\{^1H\}$ NMR spectra for the aromatic protons of the



Scheme 2 (i) potassium picolinate, THF; (ii) N-4-nitro-Ph-picolinamide, THF.

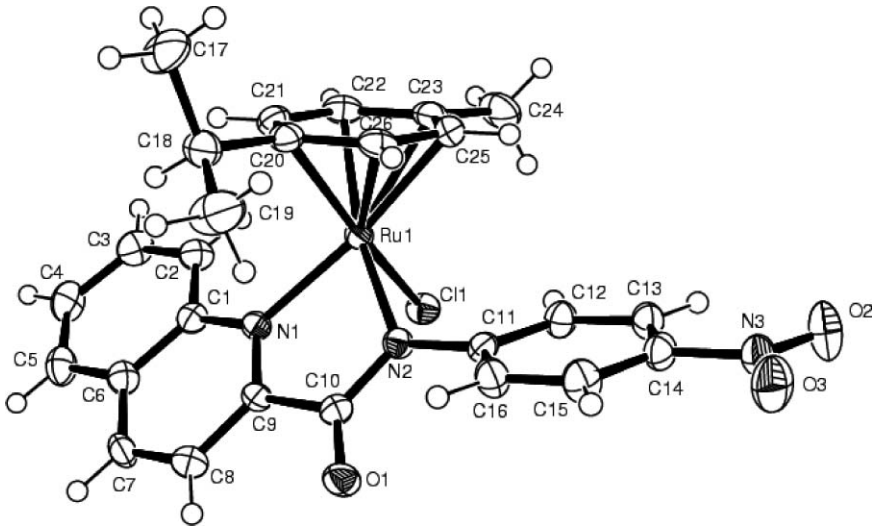


Fig. 5 The molecular structure of compound **5**, with 50% probability thermal ellipsoids.

η^6-bound arene ring; with chemical shifts of δ 5.95 ppm and 83.7 ppm respectively.

Orange single crystals of **6** suitable for X-ray crystallographic analysis were obtained from a saturated methanol solution of **6** at 4 °C, over a prolonged period of time in an environment free from disturbance and vibration. The molecular structure is shown in Fig. 6. Selected bond lengths and angles are given in Table 7. Compound **6** crystallised in a triclinic cell and the structural solution was performed in the space group $P\bar{1}$. The bond lengths and angles observed for **6** are comparable to those observed for compound **1**. The average Ru–C(arene) bond lengths for **6** are within the range observed for similar ruthenium benzene complexes.[52]

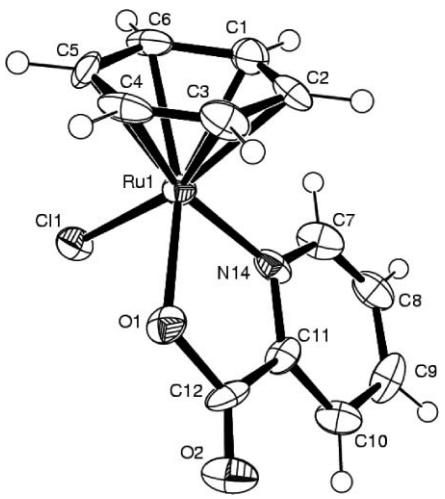


Fig. 6 (a) The molecular structure of compound **6**, with 50% probability thermal ellipsoids.

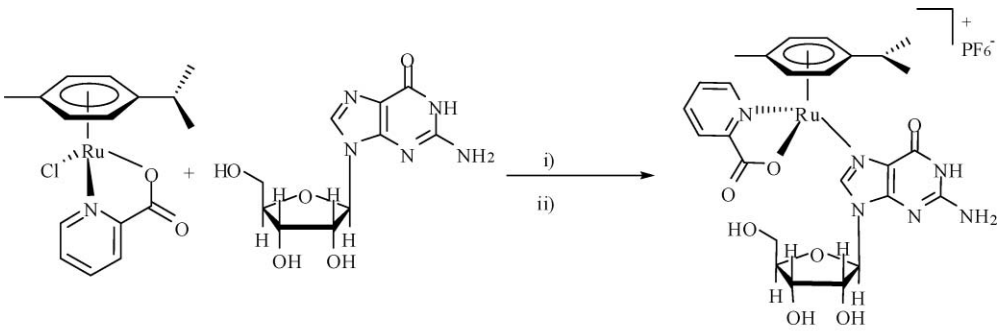
Compound **7** was synthesised (Scheme 2) when [Ru(η^6-benzene)Cl_2]$_2$was heated with two equivalents of N-4-nitro-Ph-picolinamide in dry methanol until dissolution occurred. The mixture was then filtered over an excess of NH_4PF_6 and heated to afford [$RuC_{18}H_{14}N_3O_3Cl$] (**7**). As observed previously in this work, the nitro functionalized ligand binds with the pyridyl and amidato nitrogen atoms. Compound **7** was obtained as an analytically pure orange powder in 92% yield as a racemic mixture ($[\alpha]_D^{25}$ = 0°). Compound **7** was characterised by NMR spectroscopy, mass spectrometry, elemental analysis and X-ray crystallography. Orange single crystals of **7** suitable for X-ray crystallographic analysis were obtained *via* vapour diffusion of diethyl ether into a saturated acetone solution of **7**. (The molecular structure is given in Fig. S1. Selected bond lengths and angles are given in Table S1—ESI.†)

Table 7 Selected bond distances (Å) and bond angles (°) for compound **6**. Estimated standard deviations are given in parentheses

Ru(1)–N(14)	2.046(10)	Ru(2)–N(15)	2.117(7)
Ru(1)–O(1)	2.068(9)	Ru(2)–O(3)	2.085(8)
Ru(1)–Cl(1)	2.413(3)	Ru(2)–Cl(2)	2.413(3)
Arene C–C Average	1.414(10)	Arene C–C Average	1.414(10)
Ru(1)–C(Arene) Average	2.178(11)	Ru(2)–C(Arene) Average	2.179(13)
Cg(3)–Ru(1)[a]	1.660(5)	Cg(4)–Ru(2)[a]	1.665(6)
Cg(3)–Ru(1)–Cl(1)[a]	130.3(2)	Cg(4)–Ru(2)–Cl(2)[a]	127.5(2)
Cg(3)–Ru(1)–N(14)[a]	131.4(3)	Cg(4)–Ru(2)–O(3)[a]	129.6(4)
Cg(3)–Ru(1)–O(1)[a]	127.6(3)	Cg(4)–Ru(2)–N(15)[a]	133.8(3)
N(14)–Ru(1)–O(1)	78.2(4)	O(3)–Ru(2)–N(15)	77.1(4)
N(14)–Ru(1)–Cl(1)	84.3(3)	N(15)–Ru(2)–Cl(2)	84.2(3)
O(1)–Ru(1)–Cl(1)	87.5(3)	O(3)–Ru(2)–Cl(2)	86.8(3)

[a] Cg(3) & Cg(4) (denote centroids of the C_6 rings C(1)–C(6) & C(13)–C(18) respectively).

The reaction of potential anticancer agents with guanine residues has led to the synthesis of model complexes which provide an insight into the mechanisms of activity of the agents *in vitro* and *in vivo*.[15,44] In double stranded nucleic acids, some of the potential binding sites of nucleobases are occupied in hydrogen bonding to the complementary strand.[53] The N7 sites of both adenine and guanine residues are free, and are thus found to be the major sites for binding to metal ions in DNA. Furthermore, N7 of guanine is more basic than N7 of adenine and thus coordination of metal ions to the guanine residues is favoured over the other available binding sites.[54]



8

Scheme 3 (i) H_2O, Δ (ii) NH_4PF_6.

The binding capability of a η^6-arene ruthenium complex synthesised in this work was investigated by reaction with guanosine hydrate (Guo). The guanine adducts developed in previous investigations were synthesised by heating the complex with the guanine residue for a period of time, ranging from one hour[44] to four days.[15] The reaction conditions employed by Sadler *et al.*[15] were modified and applied here. Compound **8** was synthesised according to Scheme 3. Compound **1** was reacted with Guo at 338 K in distilled water for 5 days. The resulting green solution was concentrated and filtered over an excess of ammonium hexafluorophosphate (NH_4PF_6) to afford $[RuC_{26}H_{31}N_6O_5][PF_6]$ (**8**) Scheme 3. Compound **8** was obtained as a green powder in 40% yield as a racemic mixture ($[\alpha]_D^{25} = 0°$) and was characterised by mass spectrometry, NMR spectroscopy and microanalysis.

Study of the NMR spectra of **8** reveal that one guanosine residue is bound to the ruthenium metal centre to afford the cationic species, **8**. ^{31}P NMR spectroscopy revealed the presence of the PF_6 counter anion as a septet, with a coupling constant of $^1J(^{31}P\text{-}^{19}F)$ 708 Hz at a chemical shift of δ −60.76 ppm. The ^{1}H NMR signals observed are similar to those found for other guanine residues[44] and the ^{1}H NMR signals assigned to the ribose unit of **8** are comparable to those observed for other literature examples.[15] The NMR spectroscopic evidence for **8** suggests that one guanosine residue is bound to the ruthenium centre *via* the N7 site.

Biological results

Cancer cell cytotoxicity

The cytotoxicity of a selection of the complexes was tested towards SRB assays of ovarian A2780; ovarian cisplatin resistant A2780cis; colon adenocarcinoma LS174T; breast adenocarcinoma MCF7 and colon adenocarcinoma LoVo. Each compound was incubated over a period of 6 days. Complexes **6** and **7** (Table 8) were found to be non-toxic in all three cell lines up to the highest test concentration of 50 μM. Their IC_{50} values (concentration at which 50% of the cell growth is inhibited) are therefore likely to be > 100 μM and the compounds can therefore be described as inactive. Compound **1** shows moderate activity, however, extremely promising activity was observed for the neutral *N*,*N*-coordinated compounds **2**, **3**, and **5** as shown in Table 8.

Compounds **2** and **3** exhibited IC_{50} values in the same order of magnitude as carboplatin. It is interesting to note that compound **3** shows a promising cell cytotoxicity profile and has favourable comparisons with respect to the breast and ovarian cancer cell line testing as the osmium congener [(η^6-*p*-cym)Os(*N*-4-nitro-Ph-picolinamide)Cl].[47] Compound **5** exhibited excellent cytotoxic activity against all the human tumour cell lines investigated, with values in the same order of magnitude as either cisplatin or carboplatin. Significantly, compounds **2**, **3** and **5** exhibited activity against the cisplatin resistant cell line A2780cis; which eventually develops resistance to treatment with cisplatin. The activity of compound **5** against the colon adenocarcinomas, LS174T and LoVo is of significance owing to the fact that colorectal tumours are at present intractable.[55]

Table 8 *In vitro* cytotoxicity of complexes **1**, **2**, **3**, **5**, **6** and **7** for a range of human tumour cell lines (6 day exposure) IC_{50} (μmol L^{-1})

Compound	A2780	A2780cis	LS174T	MCF7	LoVo
Cisplatin	0.8	3.3	4.6	2	0.7
1	42.5	36.1	N/A	49.9	78.3
2	7.6	10.7	22.5	11.5	16.4
3	4.6	5.6	18.4	14.4	9.1
5	6.1	6.4	7.7	3.4	8.1
6	>50	>50	>50	>50	>50
7	>50	>50	>50	>50	>50

Comparison of the anticancer activity of compound **1** with the analogous compound **6** reveal that, upon removal of the lipophilic substituents on the arene functionality (*p*-cymene to benzene) the activity of the complex is reduced. Furthermore, the most promising compound in the series, compound **5**, possesses the most hydrophobic ligand system (quinaldic acid as opposed to picolinic acid). These observations suggest that an increase in the hydrophobic character and possible π–π stacking character of a complex may result in a concomitant increase in the anticancer activity of that complex.

Nuclease treatment assay

We were interested in the DNA binding ability and mechanistic implications of the most cytotoxic molecule, compound **5**. In order to investigate the DNA binding nature and subsequent breakdown of the ruthenated DNA fragments, nuclease cleavage experiments were performed. Two polynucleotides (Fig. 7) were annealed together in the DNA Engine PTC200 Peltier Thermal Cycler PCR together to yield the double stranded section of DNA. Compound **4** was incubated with the double stranded DNA fragment for 45 min. The incubated material was treated with

5′-ATGTGACGACAGTACCAGACATGCAGTCTGGCAACTACACGTCAGT-3′

5′-ACTGACGTGTAGTTGCCAGACTGCATGTCTGGTACTGTCGTCACAT-3′

Fig. 7 Two 46-mer oligonucleotides used for the double stranded DNA fragment incubation experiment.

two enzymes, phosphodiesterase I, which cleaves DNA at 3′ ends, and alkaline phosphatase, which releases the phosphate from the 5′-end. Mass spectrometry analyses were carried out at the end of the experiment, which showed that, compound **4** formed an adduct with guanine nucleotides with a peak at 795.97.

Conclusion

A series of η^6-arene ruthenium complexes containing ligands with variable hydrophobic characteristics were synthesised and characterised. Some of the ruthenium compounds have exhibited very promising cytotoxic activity against a series of human tumour cell lines with IC_{50} values within the same order of magnitude as cisplatin and the cisplatin derivative carboplatin. The most active complexes tested were found to be those in which the ligand system incorporates a nitro group, either in the *meta* or *para* positions. The excellent activity of a functionalised quinaldimide against the colon adenocarcinomas, LS174T and LoVo is of significance owing to the fact that colorectal tumours are at present intractable.[55] It has been shown that addition of hydrophobic groups to the arene moiety of the ruthenium molecules has a positive effect on the cytotoxicity values. From a mechanistic aspect, we have proven that after incubation of a functionalised quinaldimide ruthenium compound with a 46 mer oligonucleotide duplex and subsequent nuclease treatment, ruthenium is bound to a guanine residue.

Acknowledgements

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