

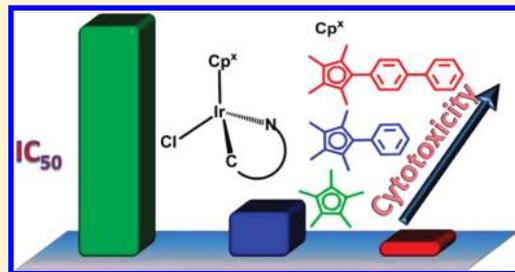
Organometallic Iridium(III) Cyclopentadienyl Anticancer Complexes Containing C,N-Chelating Ligands

Zhe Liu, Abraha Habtemariam, Ana M. Pizarro, Guy J. Clarkson, and Peter J. Sadler*

Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, U.K.

S Supporting Information

ABSTRACT: Organometallic Ir^{III} cyclopentadienyl complexes $[(\eta^5\text{-Cp}^x)\text{Ir}(\text{C}^{\wedge}\text{N})\text{Cl}]$ $\{\text{Cp}^x = \text{Cp}^*, \text{C}^{\wedge}\text{N} = 2\text{-}(p\text{-tolyl})\text{pyridine (1), 2-phenylquinoline (2), 2-(2,4-difluorophenyl)pyridine (3), Cp}^x = \text{tetramethyl(phenyl)cyclopentadienyl (Cp}^{\text{xph}}, \text{C}^{\wedge}\text{N} = 2\text{-phenylpyridine (4), and Cp}^x = \text{tetramethyl(biphenyl)cyclopentadienyl (Cp}^{\text{xbiph)}, \text{C}^{\wedge}\text{N} = 2\text{-phenylpyridine (5)}\}$ have been synthesized and characterized. The X-ray crystal structures of **2** and **5** have been determined and show typical “piano-stool” geometry. All the complexes hydrolyzed rapidly in aqueous solution (<5 min) even at 278 K. The pK_a values of the aqua adducts **1A–5A** are in the range 8.31–8.87 and follow the order **1A** > **2A** > **4A** > **5A** \approx **3A**. Hydroxo-bridged dimers $\{[(\eta^5\text{-Cp}^x)\text{Ir}]_2(\mu\text{-OD})_3\}^+$ ($\text{Cp}^x = \text{Cp}^*, \mathbf{6}; \text{Cp}^{\text{xph}}, \mathbf{7}; \text{Cp}^{\text{xbiph}}, \mathbf{8}$) are readily formed during pH titrations at ca. pH 8.7. Complexes **1** and **3–5** bind strongly to 9-ethylguanine (9-EtG), moderately strongly to 9-methyladenine (9-MeA), and hence preferentially to 9-EtG when in competition with 9-MeA. The extent of guanine and adenine binding to complex **2** was significantly lower for both purines due to steric hindrance from the chelating ligand. All complexes showed potent cytotoxicity, with IC_{50} values ranging from 6.5 to 0.7 μM toward A2780 human ovarian cancer cells. Potency toward these cancer cells increased with additional phenyl substitution on Cp^* : $\text{Cp}^{\text{xbiph}} > \text{Cp}^{\text{xph}} > \text{Cp}^*$. Cp^{xbiph} with complex **5** exhibited submicromolar activity ($2\times$ as active as cisplatin). These data demonstrate how the aqueous chemistry, nucleobase binding, and anticancer activity of C,N-bound Ir^{III} cyclopentadienyl complexes can be controlled and fine-tuned by the modification of the chelating and cyclopentadienyl ligands.



INTRODUCTION

Organometallic complexes offer enormous scope for the design of anticancer candidates due to their versatile structures, potential redox features, and wide range of ligand substitution rates.¹ For examples, titanocenyl, ferrocenyl, and Ru^{II} arene anticancer complexes are attracting current attention.² However, there are only a limited number of reported studies on the anticancer activity of organometallic iridium complexes.³ In general iridium(III) complexes are usually thought to be relatively inert.⁴

The negatively charged pentamethylcyclopentadienyl ligand (Cp^*) is often an excellent stabilizing ligand for organometallic iridium(III) complexes. However, a number of $\text{Cp}^* \text{Ir}^{\text{III}}$ complexes of the type $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{XY})\text{Cl}]^{0/+}$, where XY = N,N-bound 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (PTA),³ⁱ ethylenediamine (en), 2,2'-bipyridine (bpy), 1,10-phenanthroline (phen), or N,O-bound picolinate (pico),^{3a} have been reported to be inactive toward A2780 human ovarian cancer cells. However, the electronic and steric properties of the ligands can have a major effect on the chemical and biological activities of transition metal complexes.⁵ Recently we have demonstrated that introduction of phenyl or biphenyl substituents on Cp^* can improve cancer cell cytotoxicity of N,N-chelated Ir^{III} complexes significantly.^{3a} In addition, we have found that replacement of the neutral N,N-bound chelating ligand bpy by the negatively

charged C,N-bound 2-phenylpyridine (phpy) ligand in a $\text{Cp}^* \text{Ir}^{\text{III}}$ chlorido complex can switch on biological activity.^{3b} This finding has led us to make detailed investigations of $\text{Cp}^* \text{Ir}^{\text{III}}$ complexes with C,N-bound ligands and to study the influence of phenyl- or biphenyl-substituted Cp^* on their chemical and biological properties.

We report studies of the aqueous chemistry (hydrolysis, acidity of the resultant aqua adducts), nucleobase binding, and cancer cell toxicity of Ir^{III} complexes containing C,N-chelating ligands based on 2-phenylpyridine with electron-donating or electron-withdrawing substituents and with Cp^* and substituted Cp^* ligands, Chart 1. The results suggest that this new class of organometallic Ir(III) complexes is well suited for development as anticancer agents.

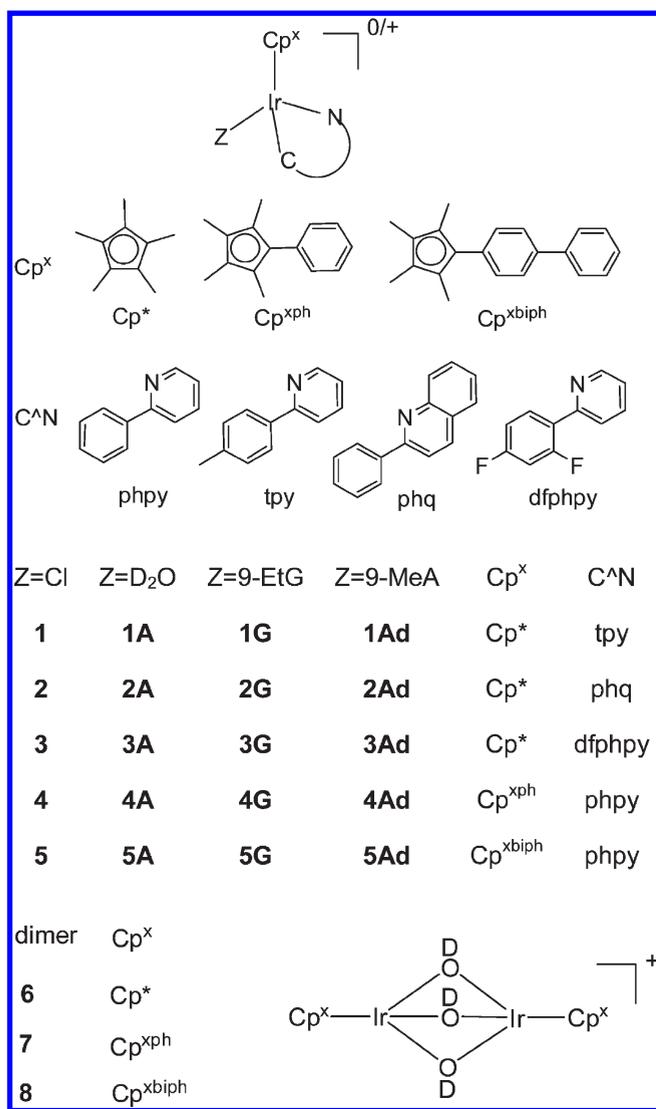
EXPERIMENTAL SECTION

Materials. 2-Phenylpyridine, 2-(2,4-difluorophenyl)pyridine, 2-(*p*-tolyl)pyridine, 2-phenylquinoline, 9-ethylguanine, and 9-methyladenine were purchased from Sigma-Aldrich. Methanol was distilled over magnesium/iodine prior to use. Dimers $[(\eta^5\text{-C}_5\text{Me}_5)\text{IrCl}_2]_2$,⁶ $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{IrCl}_2]_2$,^{3a} and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{IrCl}_2]_2$ ^{3a} were prepared according to reported methods.

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Chart 1. Iridium Cyclopentadienyl Complexes Studied in This Work



Syntheses. $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})\text{Cl}]\text{Cl}$ (**1**). A solution of $[(\eta^5\text{-C}_5\text{Me}_5)\text{IrCl}_2]_2$ (48 mg, 0.06 mmol), 2-(*p*-tolyl)pyridine (20 mg, 0.12 mmol), and sodium acetate (20 mg, 0.24 mmol) in CH_2Cl_2 (15 mL) was stirred for 2 h at ambient temperature. The solution was filtered through Celite. The filtrate was evaporated to dryness on a rotary evaporator and washed with diethyl ether. The product was recrystallized from CHCl_3 /hexane. Yield: 45 mg (70%). $^1\text{H NMR}$ (CDCl_3): δ 8.65 (d, 1H, $J = 5.7$ Hz), 7.75 (d, 1H, $J = 8.3$ Hz), 7.62 (m, 2H), 7.57 (d, 1H, $J = 8.0$ Hz), 7.03 (t, 1H, $J = 6.3$ Hz), 6.86 (d, 1H, $J = 7.8$ Hz), 1.68 (s, 15H). $^{13}\text{C NMR}$ (CDCl_3): δ 151.29, 140.79, 136.74, 128.35, 123.27, 121.65, 118.43, 88.41, 77.36, 8.83. Anal. Calcd for $\text{C}_{22}\text{H}_{25}\text{ClIr}$ (531.14): C, 49.75; H, 4.74; N, 2.64. Found: C, 49.66; H, 4.65; N, 2.68. MS: m/z 496 $[\text{M} - \text{Cl}]^+$.

$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]\text{Cl}$ (**2**). The synthesis was performed as for **1** using $[(\eta^5\text{-C}_5\text{Me}_5)\text{IrCl}_2]_2$ (48 mg, 0.06 mmol), 2-phenylquinoline (25 mg, 0.12 mmol), and sodium acetate (20 mg, 0.24 mmol). Yield: 43 mg (75%). $^1\text{H NMR}$ (CDCl_3): δ 8.71 (d, 1H, $J = 8.8$ Hz), 8.02 (d, 1H, $J = 8.7$ Hz), 7.93 (d, 2H, $J = 8.8$ Hz), 7.77 (m, 2H), 7.69 (t, 1H, $J = 8.1$ Hz), 7.53 (t, 1H, $J = 6.7$ Hz), 7.24 (t, 1H, $J = 7.8$ Hz), 7.07 (t, 1H, $J = 7.7$ Hz), 1.57 (s, 15H). $^{13}\text{C NMR}$ (CDCl_3): δ 156.22, 137.76, 136.49, 131.36,

130.87, 130.33, 127.97, 126.34, 125.30, 122.11, 116.56, 89.11, 77.35, 76.71, 9.26. Anal. Calcd for $\text{C}_{25}\text{H}_{25}\text{ClIr}$ (567.13): C, 52.94; H, 4.44; N, 2.47. Found: C, 53.06; H, 4.41; N, 2.42. MS: m/z 531 $[\text{M} - \text{Cl}]^+$. Crystals suitable for X-ray diffraction were obtained by slow evaporation of a methanol/diethyl ether solution at ambient temperature.

$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfphpy})\text{Cl}]\text{Cl}$ (**3**). The synthesis was performed as for **1** using $[(\eta^5\text{-C}_5\text{Me}_5)\text{IrCl}_2]_2$ (48 mg, 0.06 mmol), 2-(2,4-difluorophenyl)pyridine (23 mg, 0.12 mmol), and sodium acetate (20 mg, 0.24 mmol). Yield: 46 mg (70%). $^1\text{H NMR}$ (CDCl_3): δ 8.71 (d, 1H, $J = 6.0$ Hz), 8.19 (d, 1H, $J = 8.8$ Hz), 7.69 (t, 1H, $J = 8.0$ Hz), 7.31 (d, 1H, $J = 8.8$ Hz), 7.10 (t, 1H, $J = 6.3$ Hz), 6.49 (t, 1H, $J = 9.5$ Hz), 1.67 (s, 15H). $^{13}\text{C NMR}$ (CDCl_3): δ 151.54, 143.53, 137.52, 122.7, 117.28, 98.20, 89.02, 77.35, 8.78. Anal. Calcd for $\text{C}_{21}\text{H}_{21}\text{ClF}_2\text{NIr}$ (553.10): C, 45.60; H, 3.83; N, 2.53. Found: C, 45.76; H, 3.71; N, 2.46. MS: m/z 517 $[\text{M} - \text{Cl}]^+$.

$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]\text{Cl}$ (**4**). A solution of $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{IrCl}_2]_2$ (46 mg, 0.05 mmol), 2-phenylpyridine (15 mg, 0.10 mmol), and sodium acetate (16 mg, 0.20 mmol) in CH_2Cl_2 (15 mL) was heated under reflux in an N_2 atmosphere for 24 h. The solution was filtered through Celite. The filtrate was evaporated to dryness on a rotary evaporator and washed with diethyl ether. The product was recrystallized from CHCl_3 /hexane. Yield: 37 mg (57%). $^1\text{H NMR}$ ($\text{MeOD-}d_4$): δ 8.60 (d, 1H, $J = 5.3$ Hz), 8.04 (d, 1H, $J = 8.3$ Hz), 7.84 (m, 2H), 7.65 (d, 1H, $J = 7.8$ Hz), 7.38 (m, 3H), 7.33 (m, 2H), 7.16 (t, 1H, $J = 6.1$ Hz), 7.13 (t, 1H, $J = 7.2$ Hz), 7.09 (t, 1H, $J = 7.3$ Hz), 1.85 (s, 3H), 1.74 (s, 3H), 1.72 (s, 3H), 1.56 (s, 3H). $^{13}\text{C NMR}$ (CDCl_3): δ 151.53, 137.13, 135.65, 131.14, 128.77, 127.33, 123.90, 122.20, 118.96, 77.35, 9.66. Anal. Calcd for $\text{C}_{26}\text{H}_{25}\text{ClIr}$ (579.16): C, 53.92; H, 4.35; N, 2.42. Found: C, 53.77; H, 4.31; N, 2.41. MS: m/z 543 $[\text{M} - \text{Cl}]^+$.

$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]\text{Cl}$ (**5**). The synthesis was performed as for **4** using $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{IrCl}_2]_2$ (53 mg, 0.05 mmol), 2-phenylpyridine (15 mg, 0.10 mmol), and sodium acetate (16 mg, 0.20 mmol). Yield: 37 mg (57%). $^1\text{H NMR}$ (CDCl_3): δ 8.51 (d, 1H, $J = 5.3$ Hz), 7.81 (d, 1H, $J = 7.3$ Hz), 7.72 (m, 2H), 7.64 (m, 5H), 7.51 (m, 4H), 7.37 (d, 1H, $J = 7.6$ Hz), 7.16 (t, 1H, $J = 7.3$ Hz), 7.05 (t, 1H, $J = 6.0$ Hz), 6.94 (t, 1H, $J = 7.3$ Hz), 1.92 (s, 3H), 1.82 (s, 3H), 1.79 (s, 3H), 1.67 (s, 3H). $^{13}\text{C NMR}$ (CDCl_3): δ 151.45, 139.90, 136.98, 135.57, 131.05, 129.01, 127.22, 123.93, 122.51, 118.94, 77.34, 9.88. Anal. Calcd for $\text{C}_{32}\text{H}_{29}\text{ClIr}$ (655.25): C, 58.66; H, 4.46; N, 2.14. Found: C, 58.46; H, 4.35; N, 2.18. MS: m/z 619 $[\text{M} - \text{Cl}]^+$. Crystals suitable for X-ray diffraction were obtained by slow evaporation of a methanol/diethyl ether solution at ambient temperature.

METHODS AND INSTRUMENTATION

X-ray Crystallography. All diffraction data were obtained on an Oxford Diffraction Gemini four-circle system with a Ruby CCD area detector using Mo $K\alpha$ radiation. Absorption corrections were applied using ABSPACK.⁷ The crystals were mounted in oil and held at 100(2) K with the Oxford Cryosystem Cobra. The structures were solved by direct methods using SHELXS (TREF)⁸ with additional light atoms found by Fourier methods. Complexes **2** and **5** were refined against F^2 using SHELXL,⁹ and hydrogen atoms were added at calculated positions and refined riding on their parent atoms.

X-ray crystallographic data for complexes **2** and **5** have been deposited in the Cambridge Crystallographic Data Centre under the accession numbers CCDC 829525 and 829524, respectively.

NMR Spectroscopy. $^1\text{H NMR}$ spectra were acquired in 5 mm NMR tubes at 298 K (unless stated otherwise) on either Bruker DPX 400 ($^1\text{H} = 400.03$ MHz) or AVA 600 ($^1\text{H} = 600.13$ MHz) spectrometers. $^1\text{H NMR}$ chemical shifts were internally referenced to CHCl_3 (7.26 ppm) for chloroform- d_1 , CHD_2OD (3.33 ppm) for methanol- d_4 , or 1,4-dioxane (3.75 ppm) for aqueous solutions. All data processing was carried out using XWIN-NMR version 3.6 (Bruker UK Ltd.).

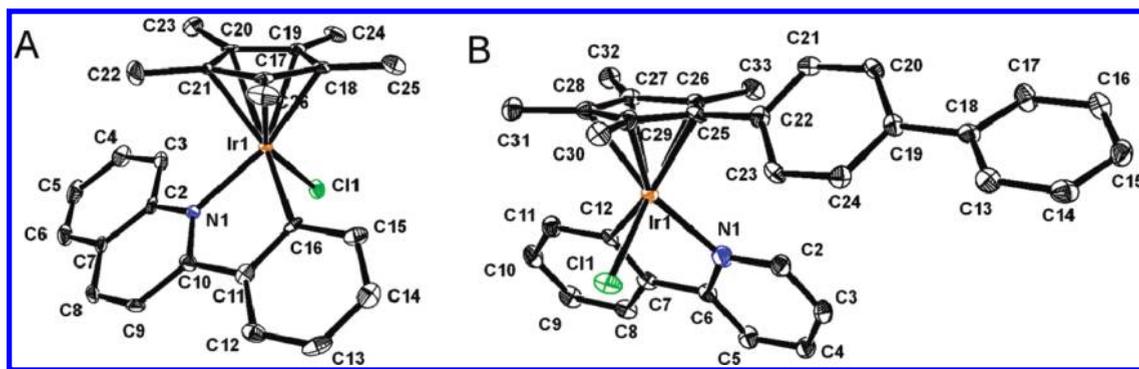


Figure 1. X-ray crystal structures and atom-numbering schemes for complexes $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ (**2**) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**5**).

Mass Spectrometry. Electrospray ionization mass spectra (ESI-MS) were obtained on a Bruker Esquire 2000 ion trap spectrometer. Samples were prepared in 50% CH_3CN and 50% H_2O (v/v). The mass spectra were recorded with a scan range of m/z 50–1000 for positive ions.

Elemental Analysis. CHN elemental analyses were carried out on a CE-440 elemental analyzer by Exeter Analytical (UK) Ltd.

pH Measurement. pH^* values (pH meter reading without correction for the effect of deuterium on the glass electrode) of NMR samples in D_2O were measured at ca. 298 K directly in the NMR tube, before and after recording the NMR spectra, using a Corning 240 pH meter equipped with a micro combination electrode calibrated with Aldrich buffer solutions of pH 4, 7, and 10.

Determination of pK_a Values. To generate the aqua complexes, chlorido complexes were dissolved in D_2O and 0.98 molar equiv of AgNO_3 was added. The solution was stirred for 24 h at 298 K, and AgCl was removed by filtration. For determinations of pK_a^* values (pK_a values for solutions in D_2O), the pH^* values of solutions of the aqua complexes in this study were varied from ca. pH^* 2 to 10 by the addition of dilute NaOD and DClO_4 , and ^1H NMR spectra were recorded. The chemical shifts of the chelating ligand protons and/or of the methyl protons of Cp^* were plotted against pH^* . The pH^* titration curves were fitted to the Henderson–Hasselbalch equation, with the assumption that the observed chemical shifts are weighted averages according to the populations of the protonated and deprotonated species. These pK_a^* values can be converted to pK_a values by use of the equation $\text{pK}_a = 0.929\text{pK}_a^* + 0.42$, as suggested by Krezel and Bal¹⁰ for comparison with related values in the literature.

Interactions with Nucleobases. The reaction of complexes **1–5** (ca. 1 mM) with nucleobases typically involved addition of a solution containing 1 molar equiv of nucleobase in D_2O to an equilibrium solution of complexes **1–5** in 20% $\text{MeOD-}d_4$ /80% D_2O (v/v). ^1H NMR spectra of these solutions were recorded at 310 K after various time intervals.

Cytotoxicity. The A2780 human ovarian cancer cell line was obtained from the ECACC (European Collection of Animal Cell Cultures, Salisbury, UK). The cells were maintained in RPMI 1640 media (supplemented with 10% fetal calf serum, 1% L-glutamine, and 1% penicillin/streptomycin). All cells were grown at 310 K in a humidified atmosphere containing 5% CO_2 . Stock solutions of the Ir^{III} complexes were first prepared in DMSO to assist dissolution (maximum final DMSO concentration 2%) and then diluted into 0.9% saline and medium (1:1). After plating 5000 A2780 cells per well on day 1, Ir^{III} complexes were added to the cancer cells on day 3 at concentrations ranging from 0.05 to 50 μM . Cells were exposed to the complexes for 24 h, washed with PBS, supplied with fresh medium, and allowed to grow for three doubling times (72 h). Protein content (proportional to cell survival) was then measured using the sulforhodamine B assay.¹¹ The

standard errors are based on two independent experiments carried out in triplicate.

RESULTS

Five Ir^{III} half-sandwich complexes of the type $[(\eta^5\text{-Cp}^x)\text{Ir}(\text{C}^{\wedge}\text{N})\text{Cl}]$, where Cp^x is pentamethylcyclopentadienyl Cp^* , or its phenyl (Cp^{xph}) or biphenyl ($\text{Cp}^{\text{x biph}}$) derivatives, and the C, N-chelating ligands are 2-(*p*-tolyl)pyridine (tpy, **1**), 2-phenylquinoline (phq, **2**), 2-(2,4-difluorophenyl)pyridine (dfphpy, **3**), or 2-phenylpyridine (phpy, **4** and **5**), were synthesized in good yields by reaction of the chelating ligand with the appropriate dimer $[(\eta^5\text{-Cp}^x)\text{IrCl}_2]_2$ in CH_2Cl_2 . All the synthesized complexes were fully characterized by ^1H NMR and ^{13}C NMR spectroscopy, ESI-MS, and CHN elemental analysis. All the complexes in this study are chiral, but no attempt was made to resolve them, and racemates are used in the following studies.

X-ray Crystal Structures. The X-ray crystal structures of $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ (**2**) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**5**) were determined. The structures and atom-numbering schemes are shown in Figure 1. Crystallographic data are shown in Table 1, and selected bond lengths and angles are listed in Table 2. Complexes **2** and **5** adopt the expected half-sandwich pseudo-octahedral “three-legged piano-stool” geometry with the iridium bound to a η^5 -cyclopentadienyl ligand (Ir to ring centroid distances of 1.829 and 1.825 Å, respectively). The Ir–Cl bond distances are 2.3989(16) and 2.3886(8) Å for **2** and **5**, respectively. The Ir–C(chelating ligand) bond lengths in **2** and **5** [2.045(6) and 2.057(3) Å, respectively] are significantly shorter than the Ir–N bond lengths [2.128(5) and 2.080(3) Å, respectively]. For complex **5**, the twist angle between the bound cyclopentadienyl ring and the central phenyl ring is 48.93°, while that between the bound ring and the terminal phenyl ring is 28.50°. The phenyl rings are twisted by 21.55°. No intermolecular π -ring stacking in the unit cell is observed in the two crystal structures.

Hydrolysis Studies. The hydrolysis of complexes **1–5** in 20% $\text{MeOD-}d_4$ /80% D_2O (v/v) was monitored by ^1H NMR spectroscopy at different temperatures. The presence of methanol ensured the solubility of the complexes. All these Ir^{III} complexes undergo rapid hydrolysis. Equilibrium was reached by the time the first ^1H NMR spectrum was acquired (~ 5 min) even at 278 K. At equilibrium 20–50% of complexes **1–5** was in the hydrolyzed form, based on ^1H NMR peak integrals.

To confirm the hydrolysis of the complexes, NaCl was added to equilibrium solutions containing the chlorido complexes and

Table 1. Crystallographic Data for $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ (2) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{2-phpy})\text{Cl}]$ (5)

	2	5
formula	C ₂₅ H ₂₅ ClIrN	C ₃₂ H ₂₉ ClIrN
MW	567.11	655.21
cryst color	orange block	orange block
cryst size (mm)	0.40 × 0.40 × 0.10	0.22 × 0.18 × 0.12
λ (Å)	0.71073	0.71073
temp (K)	100	100
cryst syst	orthorhombic	monoclinic
space group	P2(1)2(1)2(1)	P2(1)/n
a (Å)	8.05090(13)	10.0094(4)
b (Å)	15.9169(3)	22.9497(6)
c (Å)	16.0586(3)	11.2128(4)
α (deg)	90	90
β (deg)	90	103.473(3)
γ (deg)	90	90
vol (Å ³)	2057.84(6)	2504.84(14)
Z	4	4
density(calc) (Mg·m ⁻³)	1.830	1.737
abs coeff (mm ⁻¹)	6.628	5.459
F(000)	1104	1288
θ range (deg)	3.11 to 30.45	3.10 to 29.33
index ranges	−11 ≤ h ≤ 6, −20 ≤ k ≤ 22, −22 ≤ l ≤ 11	−13 ≤ h ≤ 12, −31 ≤ k ≤ 31, −14 ≤ l ≤ 15
reflms collected	10 123	23 877
indep reflms	5496 [R(int) = 0.0266]	6211 [R(int) = 0.0542]
data/restraints/params	5496/0/253	6211/0/320
final R indices [I > 2σ(I)]	R1 = 0.0368, wR2 = 0.0856	R1 = 0.0293, wR2 = 0.0680
R indices (all data)	R1 = 0.0407, wR2 = 0.0886	R1 = 0.0377, wR2 = 0.0699
GOF	1.053	0.999
largest diff peak and hole (e Å ⁻³)	6.037 and −1.728	1.754 and −2.149

their aqua adducts to final concentrations of 4, 23, and 104 mM NaCl, mimicking the chloride concentrations in cell nucleus, cell cytoplasm, and blood plasma, respectively.¹² ¹H NMR spectra were then recorded within 10 min of the Cl[−] additions at 298 K. Upon addition of NaCl, the ¹H NMR peaks corresponding to the chlorido complexes increased in intensity, while peaks for the aqua adducts decreased, Figures 2 and S1. These data confirm the formation of the aqua adducts and the reversibility of the process. On the basis of ¹H NMR data, reaction of aqua complexes **1A–5A** was almost complete in 104 mM [Cl[−]] or in 23 mM [Cl[−]], and ca. 5% of aqua complex was observed at 4 mM [Cl[−]] after 10 min with no further change after 24 h.

pK_a* Determination. Changes in the ¹H NMR chemical shifts of the methyl protons of Cp* or protons of the coordinated chelating ligands in aqua complexes **1A–5A** and $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$ (for comparison) were followed with change in pH* over a range of 2–10 (Figure S2). ¹H NMR peaks assigned to aqua complexes gradually shifted to high field with increase in pH*. The resulting pH titration curves were fitted to the Henderson–Hasselbalch equation, from which the pK_a* values

Table 2. Selected Bond Lengths (Å) and Angles (deg) for $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ (2) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{2-phpy})\text{Cl}]$ (5)

bond(s)	2	5
Ir–C(Cp ^x)	2.139(6)	2.151(3)
	2.157(6)	2.163(3)
	2.167(5)	2.183(3)
	2.243(6)	2.240(3)
	2.304(6)	2.243(3)
Ir–C(centroid)	1.829	1.825
Ir–C	2.045(6)	2.057(3)
Ir–N	2.128(5)	2.080(3)
Ir–Cl	2.3989(16)	2.3886(8)
C–Ir–N	77.4(2)	78.27(13)
C–Ir–Cl	89.66(17)	88.20(9)
N–Ir–Cl	87.23(13)	86.34(8)

of the coordinated water were determined. This gave pK_a values between 8.31 and 8.87 (Table 3), with the Cp^{xbiph} aqua complex **5A** and fluoro-substituted phenylpyridine Cp* complex **3A** having the lowest pK_a values (8.31 and 8.32, respectively) and the methyl-substituted phenylpyridine complex **1A** having the highest (8.87).

During the pH titrations for aqua complexes **1A–5A**, the appearance of a new set of peaks was detected with increasing pH* (>8.7). The new peaks are attributable to the free C₅N-chelating ligands and to the hydroxo-bridged dimers $\{[(\eta^5\text{-Cp}^x)\text{Ir}]_2(\mu\text{-OD})_3\}^+$ (Cp^x = Cp*, **6**; Cp^{xph}, **7**; Cp^{xbiph}, **8**; see Chart 1). The ¹H NMR peaks for hydroxo-bridged dimers **6–8** increased in intensity with increase in pH*. For complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfphpy})(\text{D}_2\text{O})]^+$ (**3A**), the amount of dimer **6** increased from 23% at pH* 9.0 to 50% at pH* 9.6, Figure 3. ESI-MS studies on the diluted sample (0.2 mM) gave a major peak at *m/z* 709.2, consistent with the presence of $\{[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}]_2(\mu\text{-OD})_3\}^+$ (calcd *m/z* 710.2).

Interactions with Nucleobases. Since DNA is a potential target for transition metal anticancer drugs,¹³ nucleobase binding reactions of complexes $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})\text{Cl}]$ (**1**), $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ (**2**), $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfphpy})\text{Cl}]$ (**3**), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**4**), and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**5**) with 9-ethylguanine (9-EtG) and 9-methyladenine (9-MeA) were investigated. Solutions of **1–5** (ca. 1 mM, containing an equilibrium mixture of **1–5** and their respective aqua adducts **1A–5A**) and 1 molar equiv of 9-EtG or 9-MeA in 20% MeOD-*d*₄/80% D₂O (v/v) were prepared, and ¹H NMR spectra were recorded at different time intervals at 310 K. The percentages of nucleobase adducts formed by the complexes after 24 h reaction, based on ¹H NMR peak integrals, are shown in Table S1 and Figure 4.

In the ¹H NMR spectrum of a solution containing **4** (0.8 mM) and 1 molar equiv of 9-ethylguanine (20% MeOD-*d*₄/80% D₂O, pH* 7.4, 310 K), one set of new peaks assignable to the 9-EtG adduct **4G** appeared, showing that 100% of **4** had reacted after 10 min (Figure S3). A significant change in chemical shift from 8.62 ppm for the chlorido complex **4** to 9.28 ppm for 9-EtG adduct **4G** for the CH=N (phpy ligand) proton was observed. A new 9-EtG H8 peak appeared at 7.44 ppm (singlet), shifted by 0.34 ppm to high field relative to that of free 9-EtG. After 24 h, no further change was observed. The ESI-MS of an equilibrium solution

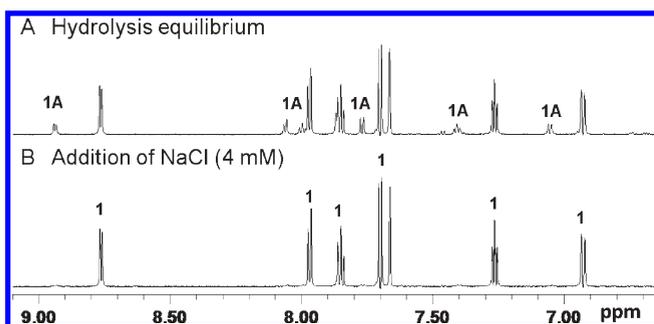


Figure 2. Confirmation of hydrolysis of Ir^{III} complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})\text{Cl}]$ (**1**). (A) ^1H NMR spectrum of an equilibrium solution of **1** (1 mM) in 20% MeOD- d_4 /80% D₂O (v/v) at 298 K. (B) ^1H NMR spectrum recorded 10 min after addition of NaCl (final concentration, 4 mM) to the equilibrium solution of **1**. Complex **1A** corresponds to the aqua complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})(\text{D}_2\text{O})]^+$. The peaks for the chlorido complex **1** increased in intensity, while peaks for the aqua complex **1A** decreased (ca. 5% **1A** remaining) upon addition of NaCl.

Table 3. pK_a^* and pK_a Values^a for the Deprotonation of the Coordinated D₂O in Complexes **1A**–**5A** and $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$

aqua complex	pK_a^*	pK_a
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})(\text{D}_2\text{O})]^+$ (1A)	9.10	8.87
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})(\text{D}_2\text{O})]^+$ (2A)	8.86	8.65
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfphpy})(\text{D}_2\text{O})]^+$ (3A)	8.51	8.32
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$ (4A)	8.64	8.45
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$ (5A)	8.50	8.31
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$	8.97	8.75

^a pK_a values calculated from pK_a^* according to Krezel and Bal.¹⁰

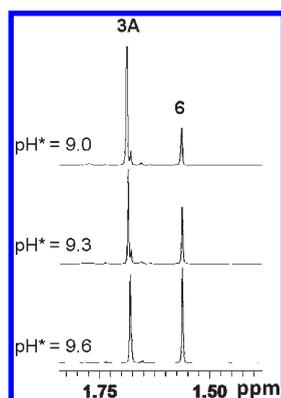


Figure 3. Methyl region of ^1H NMR spectra from the pH titration of the aqua complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfphpy})(\text{D}_2\text{O})]^+$ (**3A**), showing an increase in intensity of the peak for the hydroxo-bridged dimer $\{[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}]_2(\mu\text{-OD})_3\}^+$ (**6**) with increase in pH^* .

contained a major peak at m/z 723.2, confirming the formation of the 9-EtG adduct **4G**, $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})(9\text{-EtG})]^+$ (calcd m/z 722.9). Similarly, complexes **1**, **3**, and **5** also formed 9-EtG adducts to the extent of 100% after 24 h. Only complex **2**, containing 2-phenylquinoline, showed less strong binding to 9-EtG (45%, Figure 4 and Table S1).

Complexes **3**–**5** formed moderately strong 9-MeA adducts (35–63% at equilibrium after 24 h, Table S1). Only complex **1**

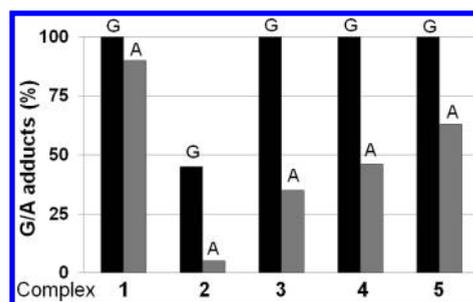


Figure 4. Bar chart showing the extent of binding of complexes **1**–**5** (ca. 1 mM in 20% MeOD- d_4 /80% D₂O) to the nucleobases 9-EtG and 9-MeA at equilibrium (24 h), based on ^1H NMR peak integrals.

$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})\text{Cl}]$ showed an exceptionally high affinity for 9-MeA, with 90% adduct formation after 24 h. Complex **2**, containing 2-phenylquinoline, formed almost no 9-MeA adduct (<5%). Except for **2**, two adenine nucleobase adducts were formed in the reactions between complexes **1** and **3**–**5** with 9-MeA, most likely through iridium binding to N1 or N7 of adenine in a ratio typically of 1:5.

Competition reactions between equimolar amounts of 9-EtG and 9-MeA and complexes **1** and **3**–**5** (ca. 1 mM) in 20% MeOD- d_4 /80% D₂O (v/v, pH^* ca. 7.4) at 310 K were investigated. In the competitive experiment, 9-EtG adduct **3G**, **4G**, or **5G** was observed as the only product for complexes **3**–**5** and as 80% 9-EtG adduct for complex **1** (20% 9-MeA adduct), Table S1. The ^1H NMR spectra for the competition reaction for **3** are shown in Figure 5.

Reactions of complexes $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**4**) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**5**) with 9-EtG were also investigated in the presence of 4 mM NaCl, Figure 6. A solution of **4** (ca. 0.8 mM) in 20% MeOD- d_4 /80% D₂O (v/v) containing an equilibrium mixture of **4** and aqua adduct **4A** was prepared, and its ^1H NMR spectrum was recorded, Figure 6A. Then NaCl was added to give a 4 mM $[\text{Cl}^-]$ solution. The aqua adduct **4A** was suppressed to ca. 5%, Figure 6B. Addition of ca. 1 molar equiv of 9-EtG resulted in complete formation of 9-EtG adduct after 24 h at 310 K, Figure 6C. Complex **5** also formed a guanine adduct quantitatively in the presence of 4 mM $[\text{Cl}^-]$.

Cytotoxicity. The cytotoxicity of complexes **1**–**5** toward A2780 human ovarian cancer cells was investigated, Table 4. The IC_{50} value (concentration at which 50% of the cell growth is inhibited) for Cp^* Ir^{III} complexes **1**–**3** is comparable with that of cisplatin. Complexes **4** and **5**, containing Cp^{xph} or Cp^{xbiph} , were even more potent, especially complex **5**, with an IC_{50} value of 0.7 μM (ca. twice as active as cisplatin). Overall, the cytotoxic potency increases with phenyl substitution on Cp^* : $\text{Cp}^{\text{xbiph}} > \text{Cp}^{\text{xph}} > \text{Cp}^*$, Table 4 and Figure S4.

DISCUSSION

X-ray Crystal Structures. A search of the Cambridge Crystallographic Database showed that the crystal structure of complex **5** is only the second example to be reported of a metal complex containing the Cp^{xbiph} ligand. The only other example is the complex $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ (where bpy = bipyridine).^{3a} The two complexes are structurally very similar. In complex **5**, the chelating ligand is closer to the Ir^{III} center than in $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ since the Ir–C bond length [2.057(3) Å] in

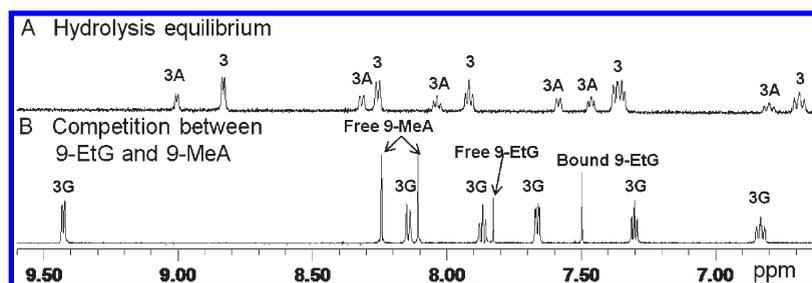


Figure 5. Low-field region of the ^1H NMR spectra for the competitive reaction between 9-EtG and 9-MeA and complex **3** $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfp}(\text{hpy}))\text{Cl}]$. (A) Equilibrium solution of **3** (1.0 mM) in 20% MeOD- d_4 /80% D $_2$ O (v/v, pH* 7.4) at 310 K, containing both the chlorido complex **3** and its aqua adduct **3A**. (B) 10 min after addition of equimolar amounts of 9-EtG and 9-MeA, showing the complete formation of 9-EtG adduct **3G**.

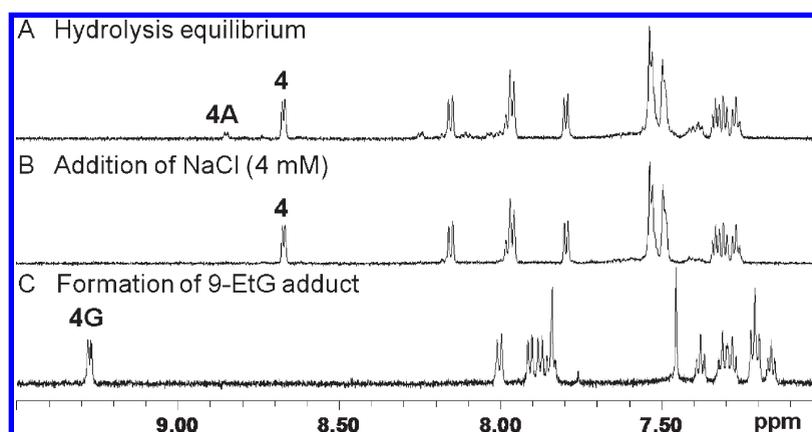


Figure 6. Low-field region of the ^1H NMR spectra for the reaction between 9-EtG and complex **4** $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{p}(\text{hpy}))\text{Cl}]$ at 310 K. (A) Equilibrium solution of **4** (0.8 mM) in 20% MeOD- d_4 /80% D $_2$ O (v/v, pH* 7.4), containing both the chlorido complex **4** and its aqua adduct **4A**. (B) 10 min after addition of NaCl (final concentration, 4 mM) to the equilibrium solution. (C) The complete formation of 9-EtG adduct **4G** after addition of 1 molar equiv of 9-EtG.

Table 4. Inhibition of Growth of A2780 Human Ovarian Cancer Cells by Complexes 1–5 and Comparison with Cisplatin

complex	IC $_{50}^a$ (μM)
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})\text{Cl}]$ (1)	3.28 ± 0.14
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ (2)	2.55 ± 0.03
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfp}(\text{hpy}))\text{Cl}]$ (3)	6.53 ± 0.50
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{p}(\text{hpy}))\text{Cl}]$ (4)	2.14 ± 0.50
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{p}(\text{hpy}))\text{Cl}]$ (5)	0.70 ± 0.04
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{p}(\text{hpy}))\text{Cl}]^b$	10.78 ± 1.72
cisplatin	1.19 ± 0.12

^a Drug-treatment period was 24 h. ^b Ref 3b.

complex **5** is significantly shorter than the Ir–N bond length [2.091(5) Å]^{3a} in the latter complex. The short Ir–C(phenylpyridine) distance causes a slight elongation of the Ir–cyclopentadienyl (centroid) bond with a distance of 1.825 Å in complex **5** (Table 2), compared to 1.787 Å in complex $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$. The Ir–Cl bond lengths are similar in the two complexes, with distances of 2.3886(8) Å (Table 2) and 2.3840(14) Å,^{3a} respectively. This behavior is similar to that observed for the C,N-chelated complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{p}(\text{hpy}))\text{Cl}]$ ¹⁴ when compared to the N,N-chelated complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{Cl}$.^{3b}

The bond lengths and bond angles in complexes $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ (**2**) and $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{p}(\text{hpy}))\text{Cl}]$ ¹⁴ are similar, except that the Ir–N bond for **2** [2.128(5) Å] is longer than that of $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{p}(\text{hpy}))\text{Cl}]$ [2.080(2) Å], implying weaker σ donation from N to the iridium center due to the electron-withdrawing phenyl ring in the quinoline moiety.

Hydrolysis and pK $_a$ of Aqua Adducts. Since M–OH $_2$ (M = metal) aqua complexes are often more reactive than the equivalent chlorido complexes,^{3a,12,15} hydrolysis of the M–Cl bonds can represent an activation step for transition metal anticancer complexes.¹⁶ There are only a few previous studies of the aquation (substitution of Cl by H $_2$ O) of organometallic Ir^{III} complexes.^{3a,4a,17} In the work reported here all the $[(\eta^5\text{-Cp}^x)\text{Ir}(\text{C}^{\wedge}\text{N})\text{Cl}]$ complexes **1–5** hydrolyzed too rapidly for determination of their hydrolysis rates by NMR, even for the biphenyl-substituted Cp^xbiph Ir^{III} complex **5** $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(2\text{-p}(\text{hpy}))\text{Cl}]$. We have previously reported half-lives for the hydrolysis of some Cp^xph or Cp^xbiph Ir^{III} complexes containing N,N-bound 2,2'-bipyridine (bpy) or 1,10-phenanthroline (phen) chelating ligands of ca. 4 min at 310 K.^{3a} The electron-donor methyl groups on the Cp ring and the negatively charged C, N-chelating ligands may together contribute to the fast hydrolysis observed for the complexes reported here since the increased effective charge on the Ir center may facilitate chloride loss. Fast hydrolysis rates also have been reported for some hexamethylbenzene Ru^{II} complexes,¹⁸ acetylacetonate Ru^{II} and Os^{II} complexes,¹⁹ and picolinate Ir^{III} complexes.^{3a} These results illustrate that Ir^{III}

complexes are not always inert and that Ir–Cl bonds can be labile.

At chloride concentrations typical of blood plasma (104 mM) and cell cytoplasm (23 mM), complexes **1–5** were found to be almost all present in solution as the relatively unreactive chlorido species, and only about 5% of complexes **1–5** were present as the reactive aqua species at a chloride concentration of 4 mM close to that of the cell nucleus. These data suggest that aquation is suppressed almost totally at the saline concentration in blood. However, complexes **1–5** might be activated by aquation in the cell nucleus. Another possibility is that the complexes might react by direct substitution of chloride by nucleobases (DNA).

The aqua complexes $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})(\text{D}_2\text{O})]^+$ (**1A**), $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})(\text{D}_2\text{O})]^+$ (**2A**), $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfphpy})(\text{D}_2\text{O})]^+$ (**3A**), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$ (**4A**), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$ (**5A**), and $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$ have similar pK_a values, ranging from 8.31 to 8.87 (Table 3). Although the substituents on the Cp* ring and the 2-phenylpyridine chelating ligand do not significantly affect the acidity of the bound water, the observed trend caused by these substituents is clear, following the order **1A** > **2A** > **4A** > **5A** \approx **3A**. The presence of phenyl and biphenyl substituents on the Cp* ring lower the pK_a value by ca. 0.3 to 0.4 units, consistent with the electron-withdrawing properties of these groups. Substitution of cyclometalated 2-phenylpyridine by the fluorinated chelating ligand 2-(2,4-difluorophenyl)pyridine leads to a decrease in pK_a by 0.4 unit. However, replacing cyclometalated 2-phenylpyridine by 2-(*p*-tolyl)pyridine or by the more π -delocalized 2-phenylquinoline has little effect on the pK_a value (ca. 0.1 unit).

However, the aqua complexes $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$, $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$ (**4A**), and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})(\text{D}_2\text{O})]^+$ (**5A**), containing the C,N-chelated 2-phenylpyridine ligand, have significantly higher pK_a values (average 1.9 units higher) than those for the structurally similar Ir^{III} Cp^x analogues bearing the N,N-bound 2,2'-bipyridine (bpy) ligand.^{3a} Therefore, the replacement of the neutral bpy ligand by the anionic phpy ligand plays a significant role in decreasing the acidity of the aqua complexes, consistent with previous reports.^{3a,19a} The high pK_a values of **1A–5A** thus ensure that most of the hydrolyzed complexes would be present in the active aqua form at physiological pH.

The pH titration also showed that additional species were formed for all complexes studied here above pH 8.7 (Figure 3), indicating the formation of the hydroxo-bridged dimers $\{[(\eta^5\text{-Cp}^x)\text{Ir}]_2(\mu\text{-OD})_3\}^+$ (**6–8**, Chart 1). However, this does not occur when the chelating ligand is an N,N-chelated bipyridine ligand.^{3a} Clearly the Ir–C bond shows less stability with respect to the formation of dimers **6–8** than the Ir–N bond. It seems likely that the mechanism of formation of **6–8** involves initial cleavage of the Ir–C bond, followed by Ir–N bond breakage. Some Os^{II} and Ru^{II} complexes containing N,O-bound or O,O-bound ligands have been reported to form hydroxo-bridged dimers readily during their hydrolysis, resulting in their inactivity toward cancer cell lines.²⁰ In this work, no hydroxo-bridged dimers of **6–8** were observed during hydrolysis, and all the complexes are active toward A2780 human ovarian cancer cells. This indicates that the C,N-chelated Ir^{III} complexes are stable in aqueous solution and the dimers **6–8** formed only appear at high pH and have no negative effect on their cytotoxicity at physiological pH.

Interactions with Nucleobases. Interaction with DNA is often associated with the cytotoxicity of metal anticancer drugs.¹³

In this study, the interactions of model nucleobases, 9-EtG and 9-MeA, with complexes **1–5** were investigated (Figure 4 and Table S1). All the C,N-chelated Ir^{III} complexes, except **2**, showed an exceptionally high nucleobase affinity, with 100% guanine adduct formation for 9-EtG, which may contribute to their high cytotoxicity. Complexes $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**4**) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**5**) still bind strongly to guanine in the presence of 4 mM [Cl[−]] (typically the chloride concentration in the cell nucleus) (Figure 6), which suggests that this class of iridium complexes might interact with DNA in the cell nucleus. Compared with N,N-chelated Ir^{III} complexes,^{3a} complexes containing a C,N-bound chelating ligand bind more significantly to 9-EtG, which may be due to their inherent advantage of having higher pK_a values. Under similar pH conditions, hydrolyzed C,N-complexes are more likely to be present as the reactive aqua form compared to the N,N-chelated complexes.

Complexes **1–5** bind more weakly to adenine compared to guanine. The competition between 9-EtG and 9-MeA for the C,N-bound Ir^{III} complexes give rise to 9-EtG adducts as the only product for **3–5** and as the major product for **1** (Figure 4 and Table S1), confirming that binding to guanine is stronger than to adenine. This may be due to the steric hindrance caused by the NH₂ group at the 6-position of the adenine ring. In addition, guanine is usually considered to be a stronger electron donor than adenine.²¹ The widely used anticancer drug cisplatin also prefers guanine over adenine.²² Organometallic Ru^{II}, Os^{II}, and Ir^{III} complexes containing N,O-chelating ligands or O,O-chelating ligands such as picolinate and acetylacetonate, which possess oxygen as an H-bond acceptor for adenine C6NH₂, also bind to both guanine and adenine residues.^{3a,19a,20b,23}

Ir^{III} cyclopentadienyl complexes containing a neutral N,N-chelating ligand (phen, bpy, or ethylenediamine) bind selectively to 9-EtG, but not to adenine.^{3a} The formation of adenine adducts in this work may be due to the interaction between NH₂ of 9-MeA and negatively charged carbons on the C,N-chelating ligand.^{3b} Thus complexes **1–3** containing different substituents on the phenylpyridine bind to 9-MeA differently. The electron-donating methyl group on the phenyl ring in complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})\text{Cl}]$ (**1**) increases electron density and may facilitate the interaction with 9-MeA (>90%). In contrast, only 35% of complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfphpy})\text{Cl}]$ (**3**) formed 9-MeA adducts, which may be due to the presence of the electron-withdrawing fluoro group. Complex **2** $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ has the lowest affinity for both model nucleobases among the five complexes, with 45% and <5% binding to 9-EtG and 9-MeA, respectively. The weaker binding is most likely due to the steric hindrance caused by the quinoline ligand.

Cytotoxicity. We have reported that some Cp* Ir^{III} complexes containing N,N- or N,O-chelating ligands are inactive toward A2780 human ovarian cancer cells.^{3a} However, Cp* complexes $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{tpy})\text{Cl}]$ (**1**), $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ (**2**), and $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{dfphpy})\text{Cl}]$ (**3**) studied here showed promising activity toward the human ovarian A2780 cancer cell line with IC₅₀ values ranging from 2.5 to 6.5 μM (Table 4), close to the value we reported recently for $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phpy})\text{Cl}]$.^{3b} Thus the introduction of C,N-chelating ligands is an effective method for switching on the cancer cell cytotoxicity of Cp* Ir^{III} complexes. The strong binding of Ir to nucleobases, especially to guanine bases, may provide an important contribution toward the cytotoxicity. Also the neutral C,N-complexes display more hydrophobic character than

the positively charged N,N-complexes^{3b} and therefore possess enhanced cellular uptake, which may also contribute to the cytotoxicity. The introduction of substituents on the phenylpyridine ring enhanced the cytotoxicity of $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phpy})\text{Cl}]$, Table 4. Particularly active is the complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phq})\text{Cl}]$ (**2**), which is 4 times more potent than $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phpy})\text{Cl}]$. The ability of the phq ligand to intercalate into DNA²⁴ may contribute to this enhanced potency.

The introduction of phenyl or biphenyl substituents onto the tetramethylcyclopentadienyl ring to give complexes $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**4**) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phpy})\text{Cl}]$ (**5**) results in a dramatic increase in cytotoxicity compared to the parent Cp* complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phpy})\text{Cl}]$, Table 4 and Figure S4. The activity of Ru^{II} arene complexes also increases with the size of the coordinated arene.²⁵ This suggests that the phenyl groups may play a crucial role in the mechanism of action of these phenylpyridine complexes. First, the phenyl or biphenyl ring increases the hydrophobicity of the molecule, which may assist with passage across cell membranes. In addition, the extended phenyl rings can intercalate into DNA, thus causing distortion of DNA structure. We have reported that the intercalative ability of 1,10-phenanthroline Ir^{III} chlorido complexes increases in the order of $\text{Cp}^{\text{xbiph}} > \text{Cp}^{\text{xph}} > \text{Cp}^*$.^{3a} Complexes **4** and **5**, containing phenyl or biphenyl substitutions, may interact with DNA by a dual mode: nucleobase binding to iridium accompanied by intercalation of the phenyl groups, which is a different mechanism of action from that of cisplatin.

CONCLUSIONS

Iridium-based anticancer agents, including organometallic iridium complexes, are currently attracting attention as potential anticancer agents with novel mechanisms of action. We have studied here the effects of changing the Cp^x ligand and negatively charged C,N-chelating ligand of Ir^{III} cyclopentadienyl complexes of the type $[(\eta^5\text{-Cp}^x)\text{Ir}(\text{C}^{\wedge}\text{N})\text{Cl}]$ on the hydrolysis of the chlorido complexes, acidity of the aqua adducts, nucleobase binding, and cancer cell cytotoxicity.

All the complexes undergo rapid hydrolysis (<5 min at 278 K) due to the strongly electron-donating methyl groups on the Cp ring and negatively charged C,N-chelating ligand. However, the complexes are likely to be present in their unhydrolyzed forms in the extracellular fluid and cell cytoplasm (typically 104 and 23 mM $[\text{Cl}^-]$), whereas they are likely to be activated by hydrolysis in the cell nucleus ($[\text{Cl}^-]$ ca. 4 mM). Generally, the aqua adducts of the C,N-complexes studied here possess low acidity, with pK_a values 1.9 units higher than N,N-analogues, which ensures that the active aqua adduct is the major form after hydrolysis at physiological pH and may contribute to the strong binding to guanine. The substituents on both the Cp* ring and on 2-phenylpyridine can fine-tune the acidity of aqua adducts according to their electronic effects. Hydroxo-bridged dimers $\{[(\eta^5\text{-Cp}^x)\text{Ir}]_2(\mu\text{-OD})_3\}^+$ (**6–8**) are observed at high pH (>8.7), which suggests the stability of C,N-complexes is not as high as N,N-analogues under strongly basic conditions.

Complexes **1** and **3–5** show strong binding to the nucleobase guanine, even in the presence of 4 mM $[\text{Cl}^-]$. Unlike the Ir^{III} complexes containing N,N-chelating ligands,^{3a} the C,N-complexes can also bind to adenine. However, they show a strong preference for binding to guanine over adenine. Complex **2** displayed the lowest extent of nucleobase binding among the

complexes studied due to steric hindrance from the chelating ligand 2-phenylquinoline.

All the C,N-complexes showed very promising anticancer activity toward A2780 human ovarian cancer cells. The Cp* complexes possess activity comparable to cisplatin. The introduction of a phenyl or biphenyl substituent significantly improved their cytotoxicity, especially for the Cp^{xbiph} complex **5**, which has submicromolar activity against A2780 cancer cells. The strong binding to guanine bases and hydrophobicity may contribute to their high activity. This study shows that desirable features can be introduced into this class of complexes to optimize their design as anticancer agents.

ASSOCIATED CONTENT

S Supporting Information. Details of the extent of nucleobase binding (Table S1), aqueous chemistry (Figures S1 and S2), nucleobase studies (Figure S3), and IC₅₀ comparison (Figure S4). This material is available free of charge via the Internet at <http://pubs.acs.org>. X-ray crystallographic data in CIF format are available from the Cambridge Crystallographic Data Centre (<http://www.ccdc.cam.ac.uk>).

AUTHOR INFORMATION

Corresponding Author

*E-mail: P.J.Sadler@warwick.ac.uk.

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