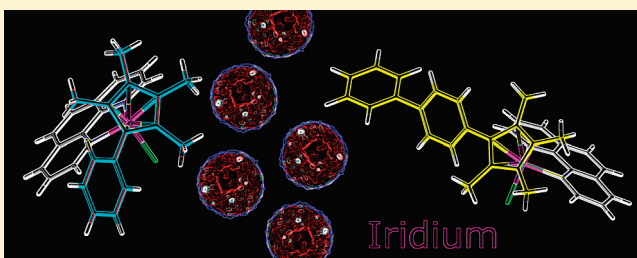


Organometallic Half-Sandwich Iridium Anticancer Complexes

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S Supporting Information

ABSTRACT: The low-spin $5d^6$ Ir^{III} organometallic half-sandwich complexes $[(\eta^5\text{-Cp}^x)\text{Ir}(\text{XY})\text{Cl}]^{0/+}$, $\text{Cp}^x = \text{Cp}^*$, tetramethyl(phenyl)cyclopentadienyl (Cp^{xph}), or tetramethyl(biphenyl)cyclopentadienyl (Cp^{xbiph}), $\text{XY} = 1,10\text{-phenanthroline}$ (4–6), 2,2'-bipyridine (7–9), ethylenediamine (10 and 11), or picolinate (12–14), hydrolyze rapidly. Complexes with N,N-chelating ligands readily form adducts with 9-ethylguanine but not 9-ethyladenine; picolinate complexes bind to both purines. Cytotoxic potency toward A2780 human ovarian cancer cells increases with phenyl substitution on Cp^* : $\text{Cp}^{\text{xbiph}} > \text{Cp}^{\text{xph}} > \text{Cp}^*$; Cp^{xbiph} complexes 6 and 9 have submicromolar activity. Guanine residues are preferential binding sites for 4–6 on plasmid DNA. Hydrophobicity ($\log P$), cell and nucleus accumulation of Ir correlate with cytotoxicity, $6 > 5 > 4$; they distribute similarly within cells. The ability to displace DNA intercalator ethidium bromide from DNA correlates with cytotoxicity and viscosity of Ir–DNA adducts. The hydrophobicity and intercalative ability of Cp^{xph} and Cp^{xbiph} make a major contribution to the anticancer potency of their Ir^{III} complexes.



INTRODUCTION

The clinical success of cisplatin, carboplatin, and oxaliplatin¹ has stimulated the search for other transition metal complexes that possess anticancer activity. New metal-based anticancer drugs may be able to widen the spectrum of treatable cancers, reduce toxic side effects, and overcome platinum resistance. Interest in bio-organometallic chemistry and the design of organometallic complexes as anticancer agents is currently increasing.² Carbon-bound π -bonded arenes and cyclopentadienyl ligands can provide control of the hydrophilicity and hydrophobicity of the faces of the coordination complex (which influences cell uptake and targeting).³ Most metallodrugs are prodrugs, and control over ligand substitution is vital if the complex is to reach and react with its target site. In this respect, octahedral low-spin d^6 complexes are attractive for drug design since they are often kinetically inert. Inertness increases from the first to second to third row of transition metals.⁴ The lifetime for exchange of an aqua ligand on $[\text{Ir}(\text{H}_2\text{O})_6]^{3+}$, for example, is about 300 years!⁵ There are only a limited number of reported studies on the biological activity of iridium complexes. Early studies were concerned with nonorganometallic Ir^I and Ir^{III} complexes,⁶ and more recently, a few studies of organometallic Ir^{III} complexes have been reported.⁷ Iridium(III) complexes are generally thought to be too inert to possess high reactivity. Indeed, the inertness of Ir^{III} has allowed the design of complexes that function as rigid scaffolds and inhibit kinase enzymes, for example.⁸ The biological inactivity of *trans*- $[\text{IrCl}_4(\text{DMSO})(\text{Im})][\text{ImH}]^9$ and *trans*- $[\text{IrCl}_4(\text{Im})_2][\text{ImH}]$ (ImH = imidazole),¹⁰ Ir^{III} analogues of the Ru^{III} anticancer drugs NAMI-A and the imidazole analogue of

the indazole complex KP1019, respectively, has been attributed to the kinetic inertness of Ir^{III}.

Organometallic Ru^{II} and Os^{II} arene anticancer complexes of the type $[(\eta^6\text{-arene})(\text{Ru}/\text{Os})(\text{NN})\text{Cl}]^+$, where NN is a chelating diamine ligand, can be activated by hydrolysis of the Ru/Os–Cl bond, prior to binding to DNA.¹¹ The arene is important in determining the anticancer activity and nature of the DNA distortions. In particular, when the arene has an extended ring system (e.g., biphenyl or tetrahydroanthracene), direct binding to DNA bases (largely guanine) can be accompanied by arene intercalation between the bases.¹²

Neutral arene ligands do not stabilize Ir^{III}. In contrast, negatively charged pentamethylcyclopentadienyl (Cp^*) is an excellent stabilizing ligand for Ir^{III}. In the work reported here, we apply the design concepts discovered for Ru^{II} and Os^{II} arene complexes to Ir^{III} Cp^* and functionalized Cp^* complexes $[(\eta^5\text{-Cp}^x)\text{Ir}(\text{XY})\text{Cl}]^{0/+}$ containing N,N-bound 1,10-phenanthroline (phen), 2,2'-bipyridine (bpy), ethylenediamine (en), and N,O-bound picolinate (pico) as chelating ligands. Iridium(III) Cp^* complexes have attracted recent attention as catalysts, for example, in hydrogen transfer reactions.¹³ Only a few iridium complexes containing functionalized Cp^* ligands have been reported previously.¹⁴ We have studied the effect of Cp^* functionalization on the rate of hydrolysis, acidity of the aqua adducts, interactions with nucleobases, hydrophobicity (octanol/water partition), cell accumulation (the net effect of uptake and efflux)

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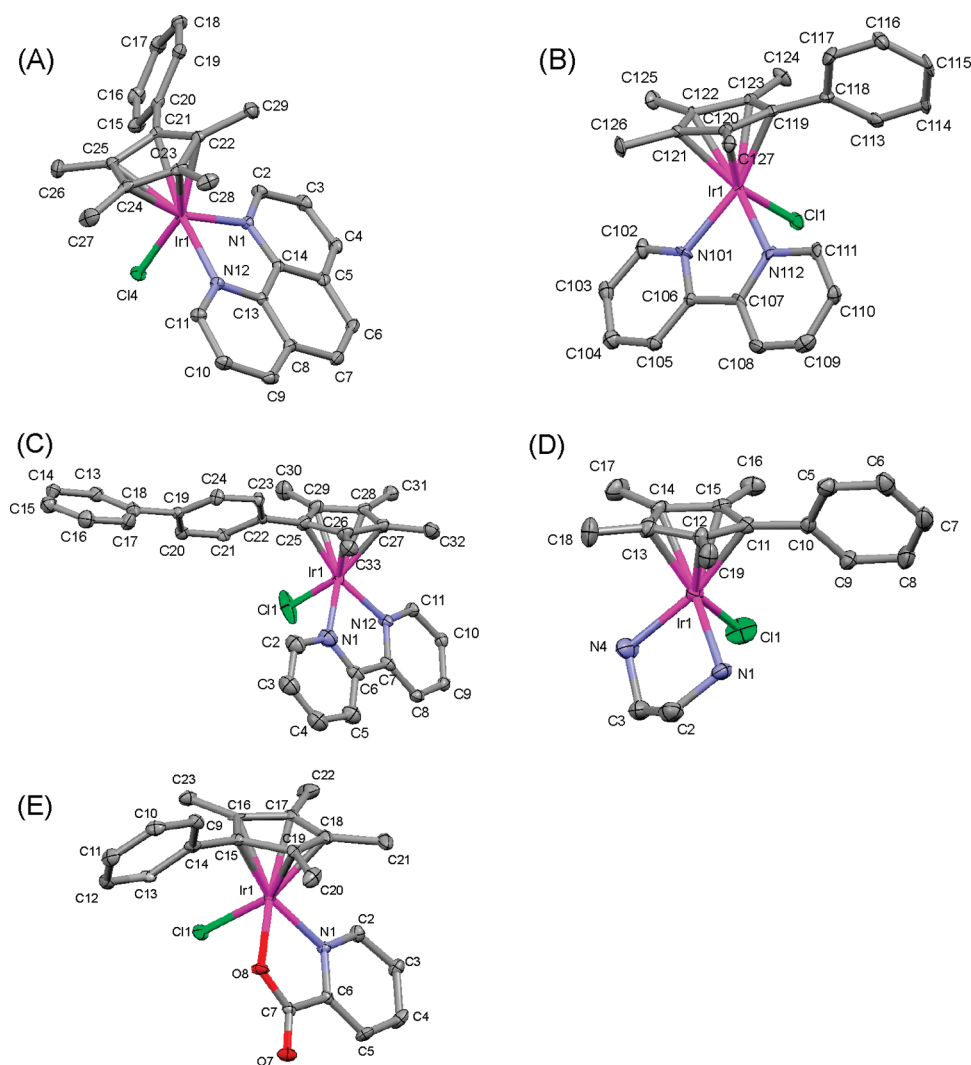


Figure 1. X-ray crystal structures with atom numbering schemes for (A) $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})\text{Cl}]\text{PF}_6$ ($5 \cdot \text{PF}_6$), (B) $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ ($8 \cdot \text{PF}_6$), (C) $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ ($9 \cdot \text{PF}_6$), (D) $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{en})\text{Cl}]\text{BPh}_4$ ($11 \cdot \text{BPh}_4$), and (E) $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})\text{Cl}]$ (13), with thermal ellipsoids drawn at 50% probability. The hydrogen atoms and counterions have been omitted for clarity.

experimental X-ray structures in the case of $5 \cdot \text{PF}_6$, $8 \cdot \text{PF}_6$, $9 \cdot \text{PF}_6$, and $11 \cdot \text{BPh}_4$. DFT calculations show that Ir–Cl and Ir–cyclopentadienyl ring bond distances remain similar on changing Cp* to substituted Cp* groups.

Electrostatic potential surfaces (EPSs) for phen chlorido complexes 4–6 and aqua adduct 6A and the pico chlorido complexes 12–14 and aqua adduct 14A were calculated. N,N-chelating phen complexes 4–6 show more positive electrostatic potentials than the N,O-chelating pico complexes 12–14 (Figure 2). Moreover, a higher electron density is present on the second phenyl ring of the Cp^xbiph ligand in complexes 6 and 14. The same trend is observed in the EPSs of the aqua derivatives $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})(\text{H}_2\text{O})]^{2+}$ (6A) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})(\text{H}_2\text{O})]^+$ (14A), which as expected show more positive surfaces as compared to their chlorido analogues, 6 and 14.

Hydrolysis Studies. Hydrolysis of M–Cl bonds can represent an activation step for transition metal anticancer complexes.¹⁷ M–OH₂ aqua complexes are often more reactive than the relevant chlorido complexes.¹⁸ The hydrolysis of compounds 4–10 and 12–14 in 5% MeOD-*d*₄/95% D₂O (v/v) was

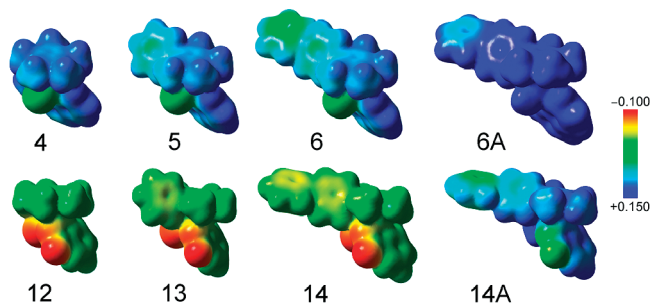


Figure 2. EPSs of the phen chlorido complexes 4–6 and aqua adduct 6A and the pico chlorido complexes 12–14 and aqua adduct 14A. EPS surfaces are shown in both space (with positive and negative regions in blue and red, respectively) and mapped on electron density (isovalue 0.004) of the molecules. The electrostatic potential is represented with a color scale going from red (–0.100 au) to blue (0.150 au).

monitored by ¹H NMR at different temperatures from 278 to 293 K. The presence of methanol ensured the solubility of the complexes.

Table 1. Hydrolysis Data for Complexes 4–10 and 12–14 at Various Temperatures

complex ^a	<i>k</i> (min ⁻¹)					<i>K</i> _{aq} (mM) ^b
	<i>t</i> _{1/2} (min)					
	278 K	283 K	288 K	293 K	310 K ^c	
4	<i>c</i>					0.04
5	0.027	0.047	0.083	<i>d</i>	0.65	0.06
	25.4	14.6	8.3	<i>d</i>	1.1	
6	0.022	<i>d</i>	0.044	0.065	0.18	0.29
	31.8	<i>d</i>	15.9	10.7	3.8	
7	<i>c</i>					0.05
8	0.031	0.062	0.099	<i>d</i>	0.89	0.08
	22.1	11.1	7.0	<i>d</i>	0.8	
9	0.026	0.041	<i>d</i>	0.078	0.23	1.45
	26.7	16.9	<i>d</i>	8.8	3.0	
10	<i>c</i>					0.44
12	<i>c</i>					0.78
13	<i>c</i>					1.11
14	<i>c</i>					2.33

^aThe course of the hydrolysis of complex **11** was difficult to interpret from NMR spectra. ^b278 K. ^cToo fast to be measured. ^dNot determined. ^eObtained from the Arrhenius equation.

All of these Ir^{III} complexes undergo relatively rapid hydrolysis. Complexes $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phen})\text{Cl}]^+$ (**4**), $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{bpy})\text{Cl}]^+$ (**7**), and $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{en})\text{Cl}]^+$ (**10**) containing Cp* and $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{pico})\text{Cl}]^+$ (**12**), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})\text{Cl}]^+$ (**13**), and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})\text{Cl}]^+$ (**14**) containing picolinate hydrolyzed too rapidly for the rates to be determined by ¹H NMR spectroscopy even at 278 K: there was little change in the spectra between 5 min and 24 h, Figure S5 in the Supporting Information. Attempts to observe hydrolysis of these complexes by UV–vis at 288 K were also unsuccessful: Equilibrium was reached before the first UV–vis spectrum was acquired (<1 min), Figure S6 in the Supporting Information.

To confirm the hydrolysis of these complexes, NaCl (1–4 mol equiv) was added to equilibrium solutions. With an increase in the NaCl concentration, ¹H NMR peaks for the chlorido adducts increased while peaks for the aqua form decreased in intensity (Figures S7–S9 in the Supporting Information). Similarly, the addition of NaCl to an aqueous solution of the aqua complex **4A** gave rise to peaks for the chlorido complex **4** (Figure S10 in the Supporting Information).

The hydrolysis of complexes $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})\text{Cl}]^+$ (**5**), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})\text{Cl}]^+$ (**6**), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]^+$ (**8**), and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]^+$ (**9**) was slow enough to be studied by ¹H NMR at low temperature. Hydrolysis was monitored at temperatures ranging from 278 to 293 K by observing the appearance of new ¹H NMR peaks over time. The time dependence for formation of the aqua adducts of **5**, **6**, **8**, and **9** was fitted to pseudo first-order kinetics (Figure S11 in the Supporting Information), and their hydrolysis rate constants, half-lives, and extent of hydrolysis were determined (Table 1). At 278 K, the half-life for hydrolysis of the biphenyl-substituted Cp^{xbiph} complex **6** was 32 min, about 1.3 times slower than that of the phenyl-Cp* complex **5** (25 min; Table 1). The half-lives and extent of hydrolysis of complexes **4–6** at 278 K increase with the size of the ring system in the order Cp^{xbiph} > Cp^{xph} > Cp*. This trend is also observed for complexes **7–9**.

Table 2. p*K*_a* and p*K*_a Values^a for the Deprotonation of the Coordinated D₂O in Complexes 4A–10A and 12A–14A

aqua complex	p <i>K</i> _a *	p <i>K</i> _a
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phen})(\text{D}_2\text{O})]^{2+}$ (4A)	7.88	7.74
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})(\text{D}_2\text{O})]^{2+}$ (5A)	7.68	7.55
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})(\text{D}_2\text{O})]^{2+}$ (6A)	7.50	7.38
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{bpy})(\text{D}_2\text{O})]^{2+}$ (7A)	6.94	6.86
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})(\text{D}_2\text{O})]^{2+}$ (8A)	6.31	6.28
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})(\text{D}_2\text{O})]^{2+}$ (9A)	6.68	6.63
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{en})(\text{D}_2\text{O})]^{2+}$ (10A)	7.66	7.54
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{pico})(\text{D}_2\text{O})]^+$ (12A)	8.15	7.99
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})(\text{D}_2\text{O})]^+$ (13A)	7.75	7.62
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})(\text{D}_2\text{O})]^+$ (14A)	7.65	7.52

^ap*K*_a values were calculated from p*K*_a* according to Krezel and Bal.²⁰

The hydrolysis rate constants and half-lives of **5**, **6**, **8**, and **9** at 310 K (body temperature) were calculated using the Arrhenius equation and are listed in Table 1. These range from 1 min for complexes **5** and **8** to 4 min for complex **6**. In each series of complexes containing different chelating N,N- or N,O-ligands, the equilibrium constants (*K*_{aq}) for hydrolysis at 278 K increase with increasing phenyl substitution on the Cp^x ligand (Table 1).

The effect of added chloride on the aqueous chemistry of **7** was investigated. ¹H NMR spectra of 1 mM **7** in 104, 23, or 4 mM NaCl in D₂O (mimicking the chloride concentrations in blood plasma, cell cytoplasm, and cell nucleus, respectively)^{18b,19} were recorded within 10 min of sample preparation and after incubation at 310 K for 24 h. On the basis of ¹H NMR peak integrals, almost no hydrolyzed complex **7** (**7A**) was found to be present in 104 mM [Cl] or in 23 mM [Cl], and only 5% of aqua complex **7A** was observed at 4 mM [Cl] after 10 min with no further change after 24 h.

p*K*_a* Determination. The p*K*_a of coordinated water can have a significant influence on its reactivity since M–OH bonds are often much less labile than M–OH₂ bonds;^{11b} moreover, hydroxide is a good bridging ligand and can give rise to oligomeric species. Changes in the ¹H NMR chemical shifts for coordinated chelating ligands in aqua complexes **4A–9A**, **12A–14A**, and methyl groups of Cp* in aqua complex **10A** were followed with a change in pH* over a range of 2–11 (Figure S12 in the Supporting Information). 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 p*K*_a* values of the coordinated water were determined. This gave rise to p*K*_a values between 6.28 and 7.99 (Table 2), with the bpy complexes being the most acidic (p*K*_a values 6.28–6.86) and the pico complexes the least acidic (p*K*_a values 7.52–7.99).

Interactions with Nucleobases. Because DNA is a potential target site for transition metal anticancer complexes,²¹ the binding of 9-ethylguanine (9-EtG) and 9-ethyladenine (9-EtA) to complexes **4–14**, and aqua complex **5A** was studied. The extent of nucleobase adduct formation by these complexes based on ¹H NMR peak integrals is shown in Table 3.

The addition of 1 mol equiv of 9-EtG to an equilibrium solution of complex **5**, $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})\text{Cl}]^+$ (1.0 mM), in 5% MeOD-*d*₄/95% D₂O (v/v, pH* 7.2) at 310 K resulted in 15% of **5** reacting after 10 min and a new 9-EtG H8 peak appearing at 7.68 ppm (species **5G**, Figure S13 in the Supporting Information),

Table 3. Extent of 9-EtG and 9-EtA Adduct Formation for Complexes 4–14 and 5A (ca. 1 mM) at 310 K after 24 h

	Cp ^x	XY	G adduct (%)	A adduct (%)
4	Cp*	phen(N,N–)	83	0
5	Cp ^{xph}	phen(N,N–)	42	0
5A	Cp ^{xph}	phen(N,N–)	74	0
6	Cp ^{xbiph}	phen(N,N–)	90	0
7	Cp*	bpy(N,N–)	61	0
8	Cp ^{xph}	bpy(N,N–)	47	0
9	Cp ^{xbiph}	bpy(N,N–)	65	0
10	Cp*	en(N,N–)	100	0
11	Cp ^{xph}	en(N,N–)	100	0
12	Cp*	pico(N,O–)	100	81
13	Cp ^{xph}	pico(N,O–)	100	76
14	Cp ^{xbiph}	pico(N,O–)	100	71

shifted by 0.15 ppm to high field relative to that of free 9-EtG. After 24 h, 42% of **5** had reacted. The ESI-MS of an equilibrium solution (Figure S14 in the Supporting Information) contained a major peak at *m/z* 374.6, confirming the formation of the 9-EtG adduct **5G**, $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})(9\text{-EtG})]^{2+}$ (calcd *m/z* 374.5). However, 65% of aqua complex **5A** (prepared by treating a solution of **5** with 1 mol equiv of AgNO₃) reacted with 9-EtG to form **5G** after 10 min and 73% after 24 h. More than 80% of complexes **4** and **6** reacted with 9-EtG under the same conditions.

Complexes $[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{en})\text{Cl}]^+$ (**10**) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{en})\text{Cl}]^+$ (**11**) interestingly showed an exceptionally high affinity for 9-EtG with 100% nucleobase adduct formation within 10 min. On addition of a solution of 1 mol equiv of 9-EtG gradually to an equilibrium solution of **10** (0.9 mM) in 5% MeOD-*d*₄/95% D₂O (v/v) at 310 K, Figure S15 in the Supporting Information, the methyl peak for **10** and **10A** decreased in intensity and eventually disappeared. A new Cp* methyl peak (for **10G**) appeared at 1.65 ppm. When 9-EtG was in excess, a set of new peaks assignable to free 9-EtG was clearly visible (Figure S15 in the Supporting Information).

The geometry of the adduct (**7G**) between complex **7** and 9-EtG was optimized by DFT calculations. These gave an Ir–N7(9-EtG) distance of 2.14 Å. The Ir–N and Ir–cyclopentadienyl ring bond distances increase by 0.1–0.2 Å as compared to complex **7**. The highest occupied molecular orbital (HOMO) of the adduct is 9-EtG-centered and the lowest unoccupied molecular orbital (LUMO) has bpy character (see Table S3 in the Supporting Information).

The addition of 1 mol equiv of 9-EtA to an equilibrium solution of **5** (1.0 mM) in 5% MeOD-*d*₄/95% D₂O (v/v) at 310 K resulted in no additional ¹H NMR peaks over a period of 24 h (Figure S16 in the Supporting Information). Similarly, no reaction with 9-EtA was observed for other complexes containing N,N-chelating ligands (Table 3).

In contrast, compounds **12**–**14** containing pico as chelating ligand formed both 9-EtG and 9-EtA adducts to the extent of 100% and more than 70% completion, respectively, after 24 h. Two adenine nucleobase adducts are formed in the reaction of complexes **12**–**14** with 9-EtA, most likely through iridium binding to N1 or N7 of adenine forming 9-EtA adducts in 1:3.1, 1:3.2, and 1:3.0 ratios, respectively (Figure S17 in the Supporting Information).

Cancer Cell and DNA Studies. *Cytotoxicity.* The cytotoxicity of complexes **4**–**14** toward A2780 human ovarian cancer cells was investigated; see Table 4. The IC₅₀ values (concentration at

Table 4. Inhibition of Growth of A2780 Human Ovarian Cancer Cells by Complexes 4–14 and Comparison with Cisplatin

complex	IC ₅₀ ^a (μM)
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{phen})\text{Cl}]\text{Cl}$ (4 ·Cl)	>100
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})\text{Cl}]\text{PF}_6$ (5 ·PF ₆)	6.70 ± 0.62
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})\text{Cl}]\text{PF}_6$ (6 ·PF ₆)	0.72 ± 0.01
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{Cl}$ (7 ·Cl)	>100
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ (8 ·PF ₆)	15.86 ± 1.49
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ (9 ·PF ₆)	0.57 ± 0.09
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{en})\text{Cl}]\text{PF}_6$ (10 ·PF ₆)	>100
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{en})\text{Cl}]\text{BPh}_4$ (11 ·BPh ₄)	16.97 ± 0.05
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Ir}(\text{pico})\text{Cl}]$ (12)	>100
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})\text{Cl}]$ (13)	>100
$[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})\text{Cl}]$ (14)	16.30 ± 0.32
cisplatin	1.22 ± 0.12

^aThe drug treatment period was 24 h.

Table 5. log *P* Values for Complexes 4–6^a

complex	log <i>P</i>	
	mean	SD
4	−0.82	0.01
5	0.48	0.03
6	1.11	0.17

^aResults are the means of three independent experiments and are expressed as means ± SDs.

which 50% of the cell growth is inhibited) for Cp* complexes **4**, **7**, **10**, and **12** and the Cp^{xph} pico complex **13** were all >100 μM and are thus deemed as inactive. However, compounds **5**, **6**, **8**, **9**, **11**, and **14** were all active. Complexes **5**, **8**, and **11** containing Cp^{xph} and **14** containing Cp^{xbiph} showed good activity, displaying IC₅₀ values of 6–17 μM.

Complexes **6** and **9** containing Cp^{xbiph} exhibited potent cytotoxicity with IC₅₀ values of 0.7 and 0.6 μM, respectively, ca. twice as active as cisplatin in the A2780 cell line (IC₅₀ = 1.2 μM). For all four series of complexes containing different chelating N,N- or N,O-ligands, the trend of increasing of activity with increasing phenyl substitution was the same: Cp^{xbiph} > Cp^{xph} > Cp*.

Hydrophobicity (log P). The octanol/water partition coefficients (log *P*) for the phen complexes **4**–**6** were determined since lipophilicity correlates with cytotoxic potency for some reported series of metallodrugs.²² The determined values are listed in Table 5. The addition of NaCl (200 mM) was used to suppress hydrolysis of the compounds, ensuring that log *P* values for the chlorido and not aqua complexes were determined. The log *P* values increase in the order 4 < 5 < 6. Only complex **4**, containing the unsubstituted Cp* ligand, has a negative log *P* value (partitions preferentially into water, Table 5).

Cell Accumulation and DNA Binding. Because increased lipophilicity has often been linked to increased cell uptake and cytotoxicity,²³ the accumulation and DNA binding of complexes **4**–**6** by A2780 ovarian cancer cells was determined after 24 h of exposure to 5 μM concentrations of the complexes. DNA from A2780 cells was isolated, and the Ir content was determined. The Cp^{xbiph} complex **6** gave rise to the highest level of iridium on

Table 6. Iridium Accumulation and Binding to DNA in A2780 Human Ovarian Cancer Cells^a

complex	cell accumulation (ng Ir/10 ⁶ cells)		DNA binding (ng Ir/10 ⁶ cells)	
	mean	SD	mean	SD
4	3.9	0.2	0.3	0.04
5	23.5	3.7	1.3	0.3
6	88.8	20.0	5.3	1.6

^aThe drug treatment period was 24 h with 5 μ M Ir^{III} complexes. Each value represents the mean \pm SD for two independent experiments done in triplicate.

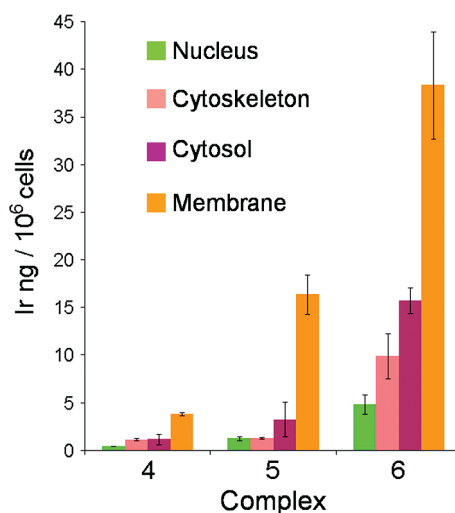


Figure 3. Iridium content of the nucleus, cytosol, membrane, and cytoskeleton fractions (ng Ir/10⁶ cells) of A2780 cells after 24 h of exposure to 5 μ M 4–6. Results are the means of two independent experiments in triplicate and are expressed as means \pm SDs.

DNA, ca. 4 \times that of complex 5 and 20 \times that of complex 4 (Table 6). Of the total Ir taken up by the cells, 7.7% for 4, 5.5% for 5, and 6.0% for 6 was bound to DNA.

Distribution of Iridium in Cell Fractions. The iridium content of the nucleus, cytosol, membrane, and cytoskeleton fractions isolated from A2780 cells after 24 h of exposure to the phen complexes 4–6 was determined, and the results are shown in Table S5 in the Supporting Information and Figure 3. The extent of accumulation of the three complexes into the different cell fractions was similar to that observed for whole cell accumulation: 6 > 5 > 4. The highest concentration of iridium was in the cell membrane/particulate fraction, accounting for 54 (6), 74 (5), and 59% (4) of the total Ir in the cell. For all complexes, the next highest concentration of Ir was in the cytosol, accounting for 24 (6), 15 (5), and 17% (4).

For the two remaining fractions, the Ir concentration dropped significantly. For complexes 4 and 6, the cytoskeleton was the next major compartment for Ir accumulation. The amount of Ir in the nucleus was significant and similar for all three Ir^{III} complexes, 7.2% of the total Ir for 6, 5.8% for 5, and 6.4% for 4, and similar in proportion to that bound to DNA.

Replication Mapping of Iridium-DNA Adducts. This procedure involved the extension by VentR(exo-) DNA polymerase of the 3'-end of the primer up to the metal adduct on the template

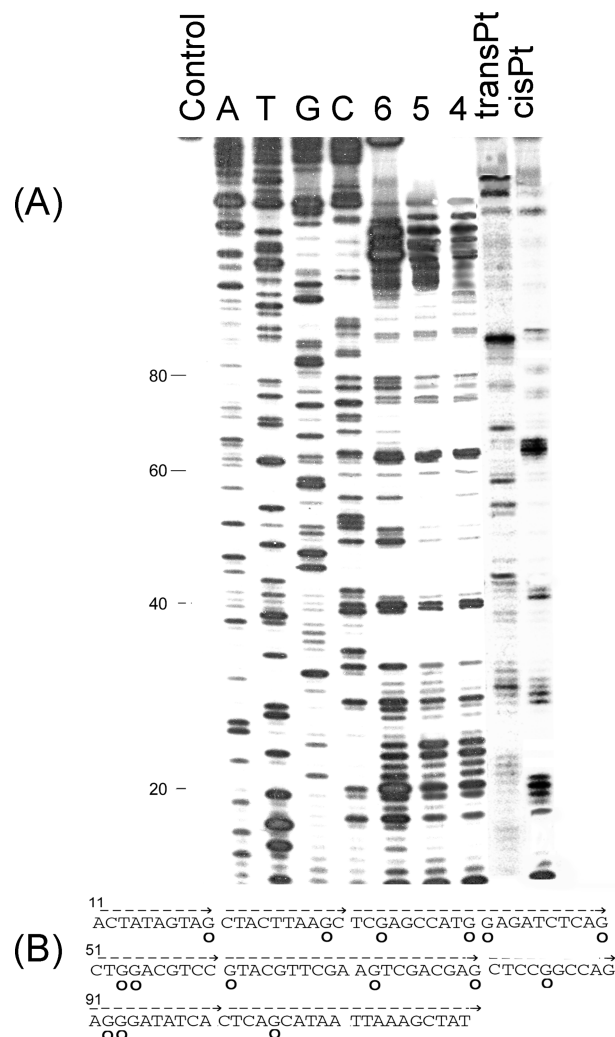


Figure 4. Replication mapping of Ir–DNA adducts. (A) Autoradiogram of 6% polyacrylamide/8 M urea sequencing gel showing inhibition of DNA synthesis by VentR DNA polymerase on the pSP73KB plasmid DNA linearized by *Hpa*I restriction enzyme and subsequently modified by Ir^{III} complexes, cisplatin, or transplatin. Lanes: control, unmodified template; A, T, G, and C, chain-terminated marker DNAs (note that these dideoxy sequencing lanes give the sequence complementary to the template strand); 4–6, DNA modified by complexes 4–6 at $r_b = 0.01$, respectively; cisPt, DNA modified by cisplatin at $r_b = 0.01$; and transPt, DNA modified by transplatin at $r_b = 0.01$. The numbers correspond to the nucleotide sequence in panel B. (B) Schematic diagram showing a portion of the sequence used to monitor inhibition of DNA synthesis on the template containing adducts of Ir^{III} complexes. The arrow indicates the direction of the synthesis. O, major stop signals from panel A, lanes 4–6. The numbering of the nucleotides in this scheme corresponds to the numbering of the nucleotides in the pSP73KB nucleotide sequence map.

strand of pSP73KB DNA linearized by *Hpa*I restriction endonuclease. The products of the synthesis were then examined on DNA sequencing gels, and the sequence specificity of iridium adduct formation was determined to the exact base pair. In vitro DNA synthesis on DNA templates containing the adducts of the phen complexes 4–6 generated a population of DNA fragments, indicating that the adducts of these complexes effectively terminated DNA synthesis (Figure 4A, lanes 4–6). Complexes 4–6 exhibit a sequence dependence of the inhibition clearly different from that of cisplatin. The Ir compounds form more blocks on

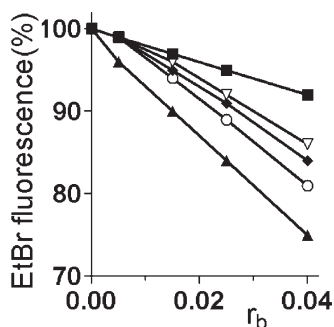


Figure 5. Plots showing the dependence of EtBr fluorescence on r_b for calf thymus DNA modified by Ir^{III} complexes 4–6, cisplatin, and [Pt(dien)Cl]Cl in 10 mM NaClO₄ at 310 K for 24 h: complex 4, ▽; complex 5, ◆; complex 6, ○; cisplatin, ▲; and [Pt(dien)Cl]Cl, ■. Data points measured in triplicate varied on average ±3% from their mean.

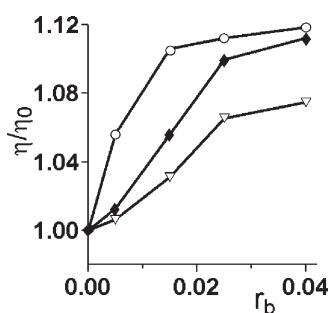


Figure 6. Plots showing the dependence of relative viscosity on r_b for calf thymus DNA modified by Ir^{III} complexes 4–6. The viscosity was measured in 10 mM NaClO₄, pH 6, at 310 K. Complex 4, ▽; complex 5, ◆; and complex 6, ○.

DNA for DNA polymerase than cisplatin, and some of them occur at different sequences. These results are consistent with a less regular sequence specificity of complexes 4–6 in comparison with cisplatin. Identical patterns of blocks on DNA were observed if the template DNA was incubated with the metal complex for 8, 24, or 72 h (data not shown).

Ethidium Bromide (EtBr) Displacement. The ability of the complexes to displace the DNA intercalator EtBr from CT DNA was probed by monitoring the relative fluorescence of EtBr bound to DNA after treating the DNA with varying concentrations of 4–6. Figure 5 shows a plot of relative fluorescence vs r_b for complexes 4–6, cisplatin, and monofunctional chloridobis(2-aminoethyl)amineplatinum(II) chloride ([Pt(dien)Cl]Cl).

The adducts of monofunctional Ir^{III} complexes competitively replaced intercalated EtBr more effectively than the adducts of monofunctional [Pt(dien)Cl]Cl but slightly less than the adducts of bifunctional cisplatin. Notably, the trend in ability to displace DNA intercalator EtBr from CT DNA was 6 > 5 > 4, which correlates with their cytotoxicity (Table 4).

Viscometry. The effects of complexes 4–6 on the viscosity of rodlike CT DNA (0.15 mg/mL or 0.47 mM in phosphorus content) are shown in Figure 6. On increasing the amounts of 4–6 bound to DNA (in the range of r_b values of 0.005–0.04), the relative viscosity of CT DNA increased steadily; the effect follows the order 6 > 5 > 4, which correlates with their cytotoxicity (Table 4) as well as with their ability to displace DNA intercalator EtBr from DNA (Figure 5).

DISCUSSION

The clinical success of cisplatin and related platinum anti-cancer drugs¹ has led to the search for active complexes of other transition metals, especially to combat the limited spectrum of activity of platinum, drug resistance, and side effects. Can the design features found in platinum drugs be introduced into other transition metal complexes so as to rationalize the design process? From studies of organometallic half-sandwich arene complexes of the type $[(\eta^6\text{-arene})M(\text{diamine})\text{Cl}]^+$ ($M = \text{Ru}^{\text{II}}$ or Os^{II}), there is evidence that they can.^{2c,24} These organometallic complexes contain an M–Cl bond, which can be activated by hydrolysis, and a neighboring NH group can stabilize binding to the nucleobase guanine via NH–C6O H-bonding. Unlike cisplatin, which contains two labile Pt–Cl bonds and can cross-link bases on DNA,^{1b} these complexes are monofunctional but nevertheless can cause replication stop-sites when bound to DNA, unlike monofunctional Pt^{II} adducts, such as [Pt(dien)Cl]Cl.²⁵ Their strong selectivity for G versus A can be reversed by changing the NH group on the chelated ligand (e.g., ethylenediamine) to an H-bond acceptor oxygen such as in acetylacetonate.²⁶ The interaction of Ru^{II} and Os^{II} arene complexes with DNA and their potency toward cancer cells can be increased by incorporating an extended arene, which can intercalate between base pairs neighboring the metal coordination site.^{25,27}

In the present work, we sought to apply these design concepts to Ir^{III} complexes since the biological activity of iridium complexes has received relatively little attention.^{6,7} The low-spin d⁶ metal ion Ir^{III} is often described in the literature as totally inert, perhaps because water exchange on [Ir(H₂O)₆]³⁺ ions has long been known to take hundreds of years.^{5a} On the other hand, it is also known that water exchange in $[(\eta^5\text{-Cp}^*)\text{Ir}(\text{H}_2\text{O})_3]^{2+}$ is ca. 10¹⁴ times faster.²⁸ In general, the dependence of ligand exchange reactions on the nature of the ligands in Ir^{III} complexes has been little studied. We have investigated Ir^{III} complexes containing Cp* ligands since they form highly stable Ir–C bonds, unlike arenes. The catalytic properties of Ir^{III} Cp* complexes have recently been explored.^{13,29} We have prepared the Cp* 1,10-phenanthroline complex $[(\eta^5\text{-Cp}^*)\text{Ir}(\text{phen})\text{Cl}]^+$ (4, a complex recently reported to be inactive toward MCF-7 breast and HT-29 colon cancer cells)³⁰ and compared its aqueous chemistry (hydrolysis and acidity of aqua adduct), nucleobase binding, transcription mapping, cytotoxicity toward A2780 human ovarian cancer cells, hydrophobicity (octanol/water partition), cellular distribution, and DNA intercalation with that of related novel phen complexes containing phenyl- and biphenyl-substituted Cp* ligands (5 and 6) and with analogous complexes containing bipyridine (bpy, 7–9) or ethylenediamine (en, 10 and 11) as the N, N-chelating ligand or picolinate (pico, 12–14) as an N,O-chelating ligand.

Structures of the Complexes. A search of the Cambridge Database revealed that no structure of metal complexes containing the ligand Cp^{xiph} has been reported. Complex $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ (9·PF₆) therefore appears to be the first such structure. The crystal structures of $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{IrCl}_2]_2$ (2), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})\text{Cl}]\text{PF}_6$ (5·PF₆), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ (8·PF₆), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{en})\text{Cl}]\text{BPh}_4$ (11·BPh₄), and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})\text{Cl}]$ (13) are the first reported with $(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)$ (Cp^{xiph}) coordinated to iridium.

The bond distances and angles in the dimer $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{IrCl}_2]_2$ (2) compare well to those found for the corresponding Cp* analogue $[(\eta^5\text{-C}_5\text{Me}_5)\text{IrCl}_2]_2$ (1).³¹ The Ir–Cl(bridging)–Ir and Cl(bridging)–Ir–Cl(bridging) angles in 2 are 1.2° more acute and obtuse, respectively, than those of the Cp* analogue. The Ir–Cl

bond lengths in complexes **8**·PF₆ and **9**·PF₆ [2.3859(19) and 2.3840(14) Å, respectively, Table S2 in the Supporting Information] are almost the same; however, the Ir–Cl bond length in complex **7** is slightly longer [2.404(2) Å].³² The twist angles in complex **9**·PF₆ are similar to those angles in Ru^{II} terphenyl arene complexes.^{27c} The Ir–Cl bond length in complex **13** [2.3860(10) Å] is similar to that in complex **12** [(η⁵-C₅Me₅)Ir(pico)Cl] [2.3997(15) Å].³³

The distance between the Ir^{III} and the centroid of Cp ring in [(η⁵-C₅Me₄C₆H₅)Ir(phen)Cl]PF₆ (**5**·PF₆) (1.783 Å) is similar to that in [(η⁵-C₅Me₅)Ir(phen)Cl]CF₃SO₃ (1.780 Å),³⁴ and the Ir–Cl bond length in **5**·PF₆ (2.3891(5) Å) is similar to that in [(η⁵-C₅Me₅)Ir(phen)Cl]CF₃SO₃ (2.395 Å).³⁴ On changing Cp* to substituted Cp* ligands, no significant change is observed by DFT calculations for Ir–Cl and Ir–cyclopentadienyl ring bond distances. These results suggest that the introduction of phenyl substituent on the Cp* ring does not give rise to significant change in structure.

Hydrolysis and pK_a of Aqua Adducts. There are only a few previous studies of the aquation of organometallic Ir^{III} complexes.^{5a,35} In general, all of the complexes studied in this work hydrolyze rapidly. Complexes **4**, **7**, and **10** containing Cp* and **12**–**14** containing picolinate hydrolyzed too rapidly to be observed by conventional UV–vis at 288 K (*t*_{1/2} < 1 min). Even complex [(η⁵-C₅Me₄C₆H₄C₆H₅)Ir(phen)Cl]⁺ (**6**), which hydrolyzed the slowest of these complexes, has a calculated half-life at 310 K of <4 min (Table 1). These results illustrate that Ir^{III} complexes are not always inert and can be quite labile. The hydrolysis of Ir–Cl bonds in iridium complexes is strongly dependent on the coordinated ligands. These Cp^x Ir^{III} complexes undergo even faster hydrolysis than low-spin d⁶ arene Ru^{II} and Os^{II} phen complexes,^{11a,36} more than 2 orders of magnitude faster than Os^{II} for example. The electron donor methyl groups on the Cp ring may contribute to the fast hydrolysis. These increase the effective charge on Ir and facilitate chloride loss. This behavior is consistent with that of hexamethylbenzene Ru^{II} complexes.³⁷

Previous studies on the hydrolysis rates of Os^{II} arene compounds of the type [(η⁶-arene)Os(XY)Cl]ⁿ⁺ have shown that the aqueous reactivity of these complexes is highly dependent on the nature of the chelating ligand.³⁸ In particular, the negatively charged electron-donating picolinate ligand increases the rate of hydrolysis as compared to complexes with diamine ligands, as seen here for complexes **12**–**14**.

The presence of bpy as a π-acceptor in complex [(η⁶-bip)Ru(bpy)Cl]PF₆, where bip = biphenyl, decreases the rate of hydrolysis by a factor of 2 as compared to the en analogue.^{36,39} The π-acceptor ligands bpy and phen can withdraw electron density from a metal center, increasing the positive charge on the metal, making it less favorable for Cl[−] to leave, slowing down the hydrolysis. As a result, complexes **5**, **6**, **8**, and **9** containing bpy or phen as the chelating ligand hydrolyzed much more slowly than the en complex **10** and pico analogues, complexes **13** and **14**. However, despite the electron-withdrawing ability of bpy and phen, the hydrolysis rates of complexes **4** and **7** are still relatively fast and appear to be controlled by the powerful electron donor Cp*.

Our previous work has shown that the interaction of [(η⁶-bip)Ru(en)Cl]⁺ with amino acids,⁴⁰ proteins,⁴⁰ peptides,⁴¹ and DNA bases^{11b} involves aquation (substitution of Cl by H₂O) as the first step. The anticancer drug cisplatin also undergoes aquation prior to platination of the target site, DNA.^{18b,42} The equilibrium constants at 278 K for hydrolysis of complexes **4**–**6**, **7**–**9**, and **12**–**14**, decrease in the order **6** > **5** > **4**, **9** > **8** > **7**, and **14** > **12**, **13** (Table 1), which parallels their cytotoxicity

(Table 4), perhaps indicating that activation by aquation is important for the mechanism of their cytotoxic action.

At chloride concentrations typical of blood plasma (104 mM) and cell cytoplasm (23 mM), the inactive complex [(η⁵-C₅Me₅)Ir(bpy)Cl]⁺ (**7**) (IC₅₀ > 100 μM, Table 4) was found to be almost all present in solution as the intact chlorido species, which is relatively unreactive as compared to the aqua complex **7A**. At a chloride concentration of 4 mM, close to that of the cell nucleus, only about 5% of **7** was present as the reactive aqua species.

When the pK_a values of the aqua complexes (Table 2) are compared, it is evident that the presence of phenyl or biphenyl substituent lowers the pK_a value by ca. 0.4 units consistent with withdrawal of electron density from the Ir center. Replacement of the π-acceptor ligand bpy in aqua complex **7A** by the chelating diamine donor en to give **10A** leads to a significant increase in pK_a by ca. 0.7 units, consistent with an increased electron density on the metal center. Similarly, the replacement of the neutral chelated bpy ligand by the anionic pico ligand raises the pK_a by 1.1 units. There appears to be no correlation between the pK_a values of the aqua adducts and the cytotoxicity of these complexes. The pK_a values of the pico, en, and phen aqua complexes **4A**, **6A**, **10A**, **12A**–**14A** suggest that they will be present largely as the reactive aqua adducts as opposed to the less reactive hydroxo adducts at physiological pH (7.4), whereas the pK_a values of the bpy complexes **7A**–**9A** are significantly lower, especially **8A**, and therefore most of the hydrolyzed bpy complexes would be present as the hydroxido form (at pH 7.4). Despite this, complexes **8** and **9** exhibit good activity. Their reactivity would be aided by the small lowering of pH, which is thought to occur in tumors.⁴³

For Ru^{II} complexes that contain biphenyl as the arene and bpy as chelating ligand, loss of the arene is observed in aqueous solution.³⁶ In contrast, no loss of any Cp^x ligands was observed for any of the Ir^{III} complexes studied in this work.

Interactions with Nucleobases. DNA is often a target for cytotoxic transition metal anticancer complexes.²¹ There is little reported work on interactions of iridium complexes with nucleobases.⁴⁴ In the present study, the reactions of complexes **4**–**14** and aqua complex **5A** with 9-EtG and 9-EtA were investigated. Complexes **4**–**11** and **5A** containing a neutral N, N-chelating ligand all bind selectively to 9-EtG as compared to 9-EtA, with which no reaction was observed after 24 h. This result is consistent with the replication mapping experiments (Figure 4), which show that G residues are the preferential binding sites on polymeric DNA modified with complexes **4**–**6**. The less regular sequence specificity of complexes **4**–**6** in comparison with cisplatin might arise from the faster DNA binding of **4**–**6**. The selectivity in nucleobase binding can be rationalized in terms of H-bonding, nonbonding repulsive interactions between the chelating ligand and the nucleobase substituents, and the electronic properties of the various nucleobase coordination sites.^{38a} Our previous studies of Ru–N7 guanine adducts have revealed a strong H-bonding interaction between one en NH and G C6O.^{11b} This may explain the strong affinity of 9-EtG for the Ir^{III} en complexes **10** and **11**. A phenanthroline ligand cannot provide a donor NH group, but instead, the interaction with G C6O may be stabilized by a C–H H-bond similar to that observed in a bipyridine complex of Ru^{II}.³⁶ This possibility was indicated by the DFT optimized structure of the 9-EtG adduct of **6** (Figure S18 and Table S5 in the Supporting Information).

Complexes 4–11 containing an N,N-chelating ligand did not react with 9-EtA, most likely due to the steric hindrance of the NH₂ group on the 6-position of the adenine ring. As compared to complexes containing an N,N-chelating ligand, compounds 12–14 that contain the N,O-chelating ligand pico bind significantly (70–100%) to both nucleobases; see Table 3. These picolate Ir^{III} adducts of complexes 12–14 with 9-EtA (12Ad, 13Ad, and 14Ad, respectively) can be stabilized by hydrogen bonding between the NH₂ group of adenine and a carboxylate oxygen of the picolate ligand.⁴⁵ As expected, the aqua complex 5A reacted to a greater extent with 9-EtG as compared to the chlorido complex 5, see Table 3, consistent with the increased reactivity of aqua adducts as compared to their chlorido forms,^{18a} but the selectivity for G versus A was the same.

Hydrophobicity (log *P*) and Cell Accumulation. log *P* values for octanol/water partition provide a measure of hydrophobicity that is often a factor relevant for cell uptake and anticancer activity. For several classes of metallo-anticancer complexes, a correlation between increased hydrophobicity and increased cytotoxic activity has been reported.^{22,23}

In this study, as expected, the log *P* values (Table 5) and hydrophobicity increase with increasing size of the substituted Cp* ligand. Additionally, the hydrophobicity, cancer cell activity, and cell accumulation correlate significantly, following the order 6 > 5 > 4. Complex [(η⁵-C₅Me₅)Ir(phen)Cl]⁺ (4) is the least hydrophobic, the least cytotoxic, and the least taken up by the cells, whereas complex [(η⁵-C₅Me₄C₆H₄C₆H₅)Ir(phen)Cl]⁺ (6) displays the highest hydrophobicity, is the most cytotoxic, and the most taken up by the cells. These data suggest that in the ovarian A2780 cancer cell line, the log *P* value is a useful parameter for predicting the cytotoxicity of this class of iridium complexes. These data also show that using more extended coordinated Cp* ligands such as Cp^{xbiph} gives rise to an increased hydrophobicity, leading to higher cellular uptake and higher cytotoxicity. For Os^{II} arene complexes, this range of log *P* values (−0.51 and 0.86) is also accompanied by promising anticancer activity.⁴⁶ Such complexes are hydrophobic enough to partition efficiently into cells and yet hydrophilic enough to exhibit reasonable aqueous solubility.

Distribution of Iridium in Cells. The accumulation of the three phen complexes 4–6 into the different cell fractions was studied. A significant proportion of the total iridium (54–74%) was in the cell membrane fraction; see Figure 3. This may be related not only to Ir being transported into the cytoplasm but also to Ir being exported by cells. For all complexes, the next highest concentration of iridium was found in the cytosol showing that passage through the outer membrane readily occurs.

Although the lowest proportion of iridium was found in the nucleus, especially for complexes 4 and 6, it is notable that there is a correlation between nucleus accumulation and cytotoxicity of the complexes, both of which follow the order 6 > 5 > 4, suggesting that penetrating the nucleus and binding to nuclear DNA may provide an important contribution to the mechanism of cytotoxicity. A similar relationship between nucleus accumulation and cytotoxicity was observed for Os^{II} arene complexes.⁴⁶

DNA Binding in A2780 Carcinoma Cells. The amount of iridium found on the DNA of A2780 cells (Table 6) incubated with the complexes for 24 h follows the order 6 > 5 > 4, which correlates with their cytotoxicity, hydrophobicity (log *P*), and cellular accumulation (Table 6) and is similar to the total accumulation by cell nuclei. The extent of iridium binding of

4–6 to DNA, 5.5–7.7% of the total iridium taken up by the cells, is higher than that reported for cisplatin (~1%)⁴⁷ and Os^{II} arene complexes.⁴⁶ DNA may therefore be a potential target for these cytotoxic iridium complexes, although we cannot rule out the possibility that nuclear DNA may not be the only target.⁴⁸

EtBr Displacement and Viscometry. The fluorescent probe EtBr can be used to distinguish between intercalating and nonintercalating ligands.^{25,49} Viscosity measurements are also useful for probing the nature of DNA interactions since viscosity is sensitive to alterations in DNA length. For instance, complexes or ligands that intercalate cause an increase in overall DNA contour length due to the increase in separation of base pairs at the intercalation sites, which leads to an increase in viscosity of DNA solutions. On the other hand, drug molecules that bind in DNA grooves cause less pronounced changes in the viscosity of DNA solutions.⁵⁰

Modification of CT DNA by complexes 4–6 resulted in a decrease of EtBr fluorescence intensity (Figure 5) and an increase in the relative viscosity of CT DNA (Figure 6) in the same order 6 > 5 > 4, which correlates with their cytotoxicity (IC₅₀ values). These results indicate that addition of phenyl substituents to the Cp* ring in these iridium complexes enhances the intercalative ability into DNA. Dual-mode intercalation/G N7 coordination DNA binding may therefore play an important role in the cytotoxicity of these Ir^{III} complexes. This observation parallels that of Ru^{II} and Os^{II} arene complexes for which extended arenes can also intercalate and increase the potency of the complexes.^{12a,24c} For example, the Ru^{II} anticancer complex [(η⁶-*p*-terp)Ru(en)Cl]⁺ (where *p*-terp = *para*-terphenyl, a similar arene ligand to Cp^{xbiph}) also exhibits combined intercalative and monofunctional (coordination) binding to DNA.^{27c}

The DFT-computed EPSs of the phen complexes 4–6 show more positive values than those of pico complexes 12–14 (same trend for the aqua derivatives 6A and 14A). This may favor interaction with negatively charged DNA in the case of phen complexes. The higher electron density on the second phenyl ring of the Cp^{xbiph} ligands (Figure 2), together with the lower steric hindrance of the terminal phenyl ring, can explain the better intercalation properties of 6. Complex 6 is likely to be present in the nucleus largely as the aqua complex 6A, which has an even higher positive charge distributed over its surface (Figure 2).

Cytotoxicity. Complexes [(η⁵-C₅Me₅)Ir(phen)Cl]Cl (4·Cl), [(η⁵-C₅Me₅)Ir(bpy)Cl]Cl (7·Cl), [(η⁵-C₅Me₅)Ir(en)Cl]PF₆ (10·PF₆), and [(η⁵-C₅Me₅)Ir(pico)] (12) containing Cp* were nontoxic (IC₅₀ > 100 μM) toward the human ovarian A2780 cancer cell line (Table 4). The cytotoxicity of complexes [(η⁵-C₅Me₄-C₆H₅)Ir(phen)Cl]PF₆ (5·PF₆) and [(η⁵-C₅Me₄-C₆H₄-C₆H₅)Ir(phen)Cl]PF₆ (6·PF₆) containing phenyl and biphenyl substituents, respectively, on the tetramethylcyclopentadienyl ring increases dramatically as compared to the parent Cp* complex 4. Complex 6 containing Cp^{xbiph} is ca. twice as potent toward A2780 human ovarian cancer cells as the anticancer drug cisplatin (Table 4). The introduction of phenyl and biphenyl substituents also resulted in significant increases in activity for complexes 7–9, 10–11, and 12–14, suggesting that these phenyl groups play a crucial role in the mechanism of action. This is consistent with our previous observations that the cytotoxicity of η⁶-arene Ru^{II} compounds increases with the size of the arene ring system in the order benzene < *p*-cymene < biphenyl < dihydroanthracene < tetrahydroanthracene.^{3c} The increase in potency on addition of phenyl substituents to the Cp ring, by about an order of magnitude for each of the additions (from 4 to 5 to 6), is more dramatic than in the case of ruthenium arene

ethylenediamine complexes.^{27,51} For these iridium complexes, the phenyl substituents not only enhance lipophilicity and cell accumulation but also introduce an additional mode of DNA interaction (intercalation).

Both complexes $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]^+$ (**8**) and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})\text{Cl}]$ (**13**) contain Cp^{xph} , however, **8** exhibits activity toward A2780 cancer cells whereas **13** is inactive, which suggests that the chelating ligand also plays a role. Replacement of neutral bpy by anionic pico as the chelating ligand increases the rate and extent of hydrolysis, the pK_a of the aqua complex (from 6.31 to 7.75 for ring = Cp^{xph} , Table 2), and changes the nucleobase specificity. For complexes **7–9** containing neutral bpy, there is exclusive binding to 9-EtG. In contrast, complexes **12–14**, containing anionic pico, bind strongly to both 9-EtG and 9-EtA. These results are in agreement with previous work on Ru^{II} and Os^{II} complexes in which neutral en is replaced by negatively charged acetylacetonate (acac).^{26,38a} The rapid hydrolysis, low acidity of aqua adducts, and strong binding to biomolecules may account for the low cytotoxicity of the picolinate complexes. The highly reactive aqua species may interact with other cellular biomolecules before they reach target sites, thus effectively being deactivated. For example, the amino acids (methionine or cysteine) or tripeptides can form S-bound adducts with Ir^{III} Cp^* complexes.^{44a}

Interestingly, some ruthenium^{27a} and osmium^{11a} arene complexes containing phen or bpy derivatives show poor or no activity against A2780 cells. The inactivity of phen complex **4** and bpy complex **7** may be correlated with their low extent of hydrolysis and poor cellular accumulation. Although complex **7** may reach the cell nucleus, its hydrolysis is largely suppressed (only 5% hydrolyzed in the presence of 4 mM NaCl), and the complex therefore remains predominately as the less-reactive intact chlorido species. These factors may explain the inactivity of **4** and **7** and also the inactivity of **10**.

CONCLUSIONS

The goal of the present study was to explore the rational design of monofunctional organometallic half-sandwich Ir^{III} anticancer complexes based on knowledge of the features that contribute to the activity of half-sandwich Ru^{II} and Os^{II} arene complexes.^{2,24a,52} The biological and medicinal chemistry of iridium complexes has been little explored previously,^{6,7} perhaps because it is often assumed that low-spin $5d^6$ Ir^{III} complexes are highly kinetically inert.^{5,9,10} Our data show that this is not always the case. Cyclopentadienyl ligands, while stabilizing Ir^{III} , can confer kinetic lability on trans monodentate ligands such as chloride. Moreover, phenyl substituents on the Cp^* ring as in Cp^{xph} and Cp^{xbiph} can have a major effect on the chemical and biological behavior of $[(\eta^5\text{-Cp}^{\text{x}})\text{Ir}(\text{XY})\text{Cl}]^{0/+}$ complexes.

This appears to be the first time that Cp^{xph} and Cp^{xbiph} ligands have been used in iridium complexes. The crystal structure of complex $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ (**9**· PF_6) appears to be the first reported structure containing the ligand Cp^{xbiph} . The crystal structures of $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{IrCl}_2]_2$ (**2**), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{phen})\text{Cl}]\text{PF}_6$ (**5**· PF_6), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{bpy})\text{Cl}]\text{PF}_6$ (**8**· PF_6), $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{en})\text{Cl}]\text{BPh}_4$ (**11**· BPh_4), and $[(\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5)\text{Ir}(\text{pico})\text{Cl}]$ (**13**) are the first to be reported with $\eta^5\text{-C}_5\text{Me}_4\text{C}_6\text{H}_5$ coordinated to iridium.

Figure 7 provides an overview of the relationships between cancer cell cytotoxicity, intercalative ability, cellular accumulation, hydrophobicity, and rates and extents of hydrolysis for the phen

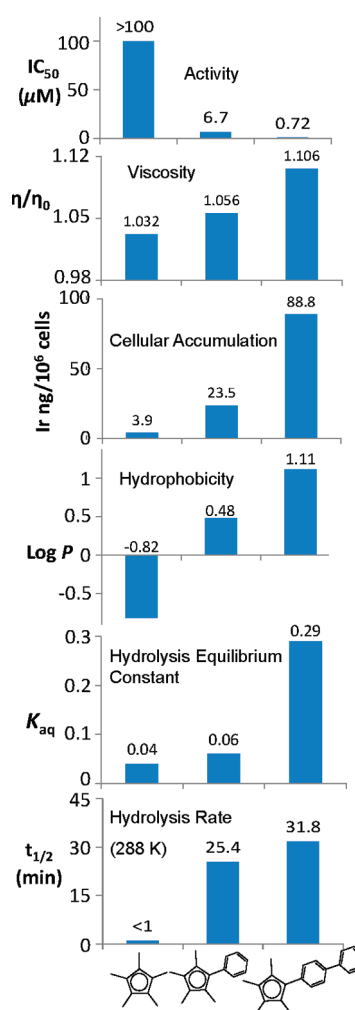


Figure 7. Bar charts illustrating the relationship between cytotoxicity toward human cancer cells, intercalative ability, cellular accumulation, hydrophobicity, and rates (288 K) and equilibrium constants (278 K) of hydrolysis for iridium complexes $[(\eta^5\text{-Cp}^{\text{x}})\text{Ir}(\text{phen})\text{Cl}]^+$ containing $\text{Cp}^{\text{x}} = \text{Cp}^*$ (**4**), Cp^{xph} (**5**), and Cp^{xbiph} (**6**).

complexes **4–6**. Figure 8 shows how the chelating and Cp^{x} ligands affect the anticancer activity, nucleobase binding, and aqueous chemistry of the bpy complexes **7–9** and pico complexes **12–14**. These $[(\eta^5\text{-Cp}^{\text{x}})\text{Ir}(\text{XY})\text{Cl}]^{0/+}$ complexes hydrolyze rapidly (the slowest half-life <4 min at 310 K), and the nature of the cyclopentadienyl and chelating ligands significantly influence their aqueous chemistry. In general, the introduction of phenyl and biphenyl Cp^* ring substituents slows down the hydrolysis rate, increases the extent of hydrolysis, and increases the acidity of the respective aqua species. The chelating ligand appears to determine the selectivity of nucleobase binding. The complexes containing N,N-chelating ligands discriminate strongly between the purine nucleobases guanine and adenine, showing little binding to the latter for either for Cp^* , Cp^{xph} , or Cp^{xbiph} complexes. In contrast, complexes **12–14** containing the N,O-chelating ligand picolinate bind to both 9-EtG and 9-EtA.

The introduction of a phenyl substituent into the Cp^* ring switches on cancer cell cytotoxicity. For example, the tetramethyl(phenyl)-cyclopentadienyl complex **5** is more than 1 order of magnitude more potent than the Cp^* complex **4**, and the biphenyl complex **6** is more than 2 orders of magnitude more potent than complex **4** and twice as potent as cisplatin in the same cell line. This increase in activity

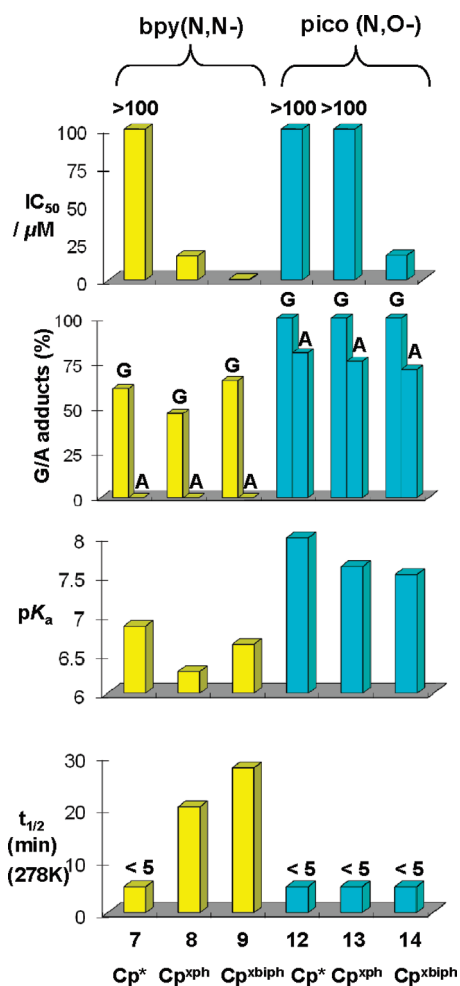


Figure 8. Bar charts illustrating the influence of N,N- and N,O-chelating ligands on the cytotoxicity, nucleobase binding, hydrolysis, and pK_a of the aqua adducts of the bpy complexes 7–9 and pico complexes 12–14.

parallels the increase in hydrophobicity, increase in cell accumulation, and DNA binding. Complexes 5 and 6 can exhibit dual mode binding to DNA: iridium binding to G N7 is accompanied by intercalation of the phenyl substituents on the Cp* ring. Significant increases in activity for complexes 7–9, 10–11, and 12–14 are also observed. On the other hand, the chelating ligand can also play an important role in the anticancer activity. For example, the pico complex 13 shows no activity toward the A2780 cell line, while the bpy analogue complex 8 shows good activity, and the bpy complex 9 is more than 1 order of magnitude more potent than the pico analogue, complex 14.

The work reported here demonstrates that rational chemical design can be applied to Ir^{III} complexes to achieve potent cancer cell cytotoxicity. It is notable that Cp ring substituents can also play a major role in controlling the chemical and biological properties of ferrocenyl and titanocenyl anticancer complexes.⁵³ In general, organometallic complexes offer much promise for the design of novel therapeutic agents.^{2,24a,52a}

EXPERIMENTAL SECTION

Details of the materials used and the synthesis and characterization of complexes are in the Supporting Information. The general synthetic route to the iridium complexes involved reaction of 1,10-phenanthroline, 2,2'-bipyridine, ethylenediamine, or 2-picolinate, with the appropriate dimer

[(η^5 -Cp^x)IrCl₂]₂ in methanol. CHN elemental analyses were carried out for all complexes and synthesized ligands, by which their purities were confirmed to be in excess of 97.0%.

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 α radiation. Absorption corrections were applied using ABSPACK.⁵⁴ 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)⁵⁵ with additional light atoms found by Fourier methods. Complexes were refined against F² using SHELXL,⁵⁶ and hydrogen atoms were added at calculated positions and refined riding on their parent atoms.

X-ray crystallographic data for complexes 2, 5·PF₆, 8·PF₆, 9·PF₆, 11·BPh₄, and 13 are available as Supporting Information and have been deposited in the Cambridge Crystallographic Data Centre under the accession numbers CCDC 802289, 802288, 802287, 802291, 802290, and 802286, respectively. X-ray crystallographic data in CIF format are available from the Cambridge Crystallographic Data Centre (<http://www.ccdc.cam.ac.uk/>).

NMR Spectroscopy. ¹H NMR spectra were acquired in 5 mm NMR tubes at 298 K (unless stated otherwise) on either Bruker DPX 400 (¹H = 400.03 MHz) or AVA 600 (¹H = 600.13 MHz) spectrometers. ¹H NMR chemical shifts were internally referenced to (CHD₂)(CD₃)SO (2.50 ppm) for DMSO-*d*₆, CHCl₃ (7.26 ppm) for chloroform-*d*₁, or to 1,4-dioxane (3.75 ppm) for aqueous solutions. All data processing was carried out using XWIN-NMR version 3.6 (Bruker UK Ltd.).

Mass Spectrometry. Electrospray ionization mass spectra (ESI-MS) were obtained by preparing the samples in 50% CH₃CN and 50% H₂O (v/v) and infusing into the mass spectrometer (Bruker Esquire 2000). 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 effects of deuterium on glass electrode) of NMR samples in D₂O were measured at ca. 298 K directly in the NMR tube, before and after recording NMR spectra, using a Corning 240 pH meter equipped with a microcombination electrode calibrated with Aldrich buffer solutions of pH 4, 7, and 10.

UV-vis Spectroscopy. A Cary 300 UV-vis recording spectrophotometer was used with 1 cm path-length quartz cuvettes (0.5 mL) and a PTP1 Peltier temperature controller. Spectra were processed using UVWinlab software. Experiments were carried out at 288 K unless otherwise stated.

Kinetics of Hydrolysis. Solutions of complexes 4–10 and 12–14 with final concentrations of 0.2–0.7 mM in 5% MeOD-*d*₄/95% D₂O (v/v) were prepared by dissolution of the complexes in MeOD-*d*₄ followed by rapid dilution with D₂O. ¹H NMR spectra were recorded after various time intervals. The rates of hydrolysis were determined by fitting plots of concentrations (determined from ¹H NMR peak integrals) versus time to a first-order rate equation using ORIGIN version 8.1. The hydrolysis of complexes 4, 7, 10, 12–14 was monitored by ¹H NMR at 278 K (to slow down the rate and avoid freezing the samples) and for complexes 5, 6, 8, and 9 at 278, 283, 288, and 293 K. The Arrhenius equation ln(*k*) = ln(*A*) – *E*_a/*RT* was used to calculate the hydrolysis rate constants and half-lives of 5, 6, 8, and 9 at 310 K.

Determination of pK_a Values. To generate the aqua complexes, chlorido complexes were dissolved in D₂O, and 0.98 mol equiv of AgNO₃ were 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 D₂O), the pH* values of solutions of the aqua complexes in this study in D₂O were varied from ca. pH* 2 to 11 by the addition of dilute NaOD and DClO₄, and ¹H NMR spectra were recorded. The chemical shifts of the chelating ligand protons and/or methyl protons of

Cp^x 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 Bal²⁰ for comparison with related values in the literature.

Computational Details. The Gaussian 03 package⁵⁷ was employed for all calculations. Geometry optimization calculations for complexes 4–14, aqua complexes 6A and 14A, and 9-EtG adduct 7G were performed in the gas phase with the gradient-corrected correlation functional PBE0.⁵⁸ The LanL2DZ basis set and effective core potential⁵⁹ were used for the Ir atom, and the 6-31G** basis set was used for all other atoms.⁶⁰ The nature of all stationary points was confirmed by performing a normal-mode analysis. EPSs for complexes 4–6, 12–14, 6A, and 14A were calculated and mapped on electron density (isovalue 0.004) of the molecules. The electrostatic potential is represented with a color scale ranging from red (−0.100 au) to blue (0.150 au).

Interactions with Nucleobases. The reaction of complexes 4–14 and 5A (ca. 1 mM) with nucleobases typically involved addition of a solution containing 1 mol equiv of nucleobase in D_2O to an equilibrium solution of complexes 4–14 in 5% MeOD- d_4 /95% D_2O (v/v) or to a solution of the aqua complex 5A (prepared by the addition of 1 mol equiv of AgNO_3 to a solution of 5 and removal of AgCl by filtration). ^1H NMR spectra of these solutions were recorded at 310 K after various time intervals.

Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) Instrumentation and Calibration. All ICP-MS analyses were carried out on an Agilent Technologies 7500 series ICP-MS instrument. The water used for ICP-MS analysis was doubly deionized (DDW) using a Millipore Milli-Q water purification system and a USF Elga UHQ water deionizer. The iridium Specpure plasma standard (Alfa Aesar, 1000 ppm in 10% HCl) was diluted with 3% HNO_3 DDW to freshly prepare calibrants at concentrations 1000, 800, 400, 200, 100, 50, 10, 1, and 0.1 ppb. The ICP-MS instrument was set to detect ^{193}Ir with typical detection limits of ca. 8 ppt using no-gas mode.

Cell Culture and Cytotoxicity. The A2780 ovarian cell line was obtained from the ECACC (European Collection of Animal Cell Culture, Salisbury, United Kingdom). The cells were maintained in RPMI 1640 media, which was 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 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 100 μM , depending on the preliminary activity data obtained in screening assays. 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), and then the protein content (proportional to cell survival) measured using the sulforhodamine B (SRB) assay.⁶¹ The standard errors are based on two independent experiments of three replicates each.

log P Determination. Octanol-saturated water (OSW) and water-saturated octanol (WSO) were prepared using analytical grade octanol and 0.2 M aqueous NaCl solution (to suppress hydrolysis of the chlorido complexes). Aliquots of stock solutions of iridium complexes in OSW were added to equal volumes of WSO and shaken in an IKA Vibrax VXC basic shaker for 4 h at 500 g/min to allow partition at ambient temperature (~ 298 K). The aqueous layer was carefully separated from the octanol layer for iridium analysis. ^{193}Ir was quantified from aliquots taken from the octanol-saturated aqueous samples before and after partition. Partition coefficients of Ir^{III} complexes were calculated using the equation $\log P = \log ([\text{Ir}]_{\text{WSO}}/[\text{Ir}]_{\text{OSW}})$, where $[\text{Ir}]_{\text{WSO}}$ was

obtained by subtraction of the Ir content of the aqueous layer after partition from the Ir content of the aqueous layer before partition.

Cell Accumulation, Cellular Distribution, and DNA Binding in A2780 Human Carcinoma Cells. A2780 cells were plated at a density of 5×10^6 cells/100 mm Petri dish in 9 mL of culture medium on day 1 (three dishes were prepared per compound tested, and three untreated control dishes, in two independent experiments). On day 2, cells were exposed to the Ir^{III} complexes 4–6. Stock solutions of the iridium compounds were prepared fresh in DMSO and diluted in 0.9% saline and medium (1:1; 0.5% v/v DMSO final concentration) to a final concentration of Ir on the plates of 5 μM . After 24 h of drug exposure at 310 K on a 5% CO_2 incubator, the drug-containing medium was removed, and the cells were washed, trypsinized, and counted using a hemocytometer. One-third of the cells were centrifuged, quickly washed with PBS, and stored at 253 K for determination of total cell accumulation (the net effect of uptake and efflux) of iridium. Another third of the samples was used for cytosol, nucleus, membrane/particulate, and cytoskeleton fractionation, using a FractionPREP cell fractionation kit from BioVision (Mountain View, CA). The last third of the samples was used for quantification of Ir bound to DNA using the Nucleon genomic DNA extraction kit (GE healthcare, Amersham, United Kingdom; BACC-1 protocol). All of the cell pellets and solid cell fractions were digested in freshly distilled 72% HNO_3 in Wheaton V-Vials with a PTFE-faced rubber-lined cap (Sigma-Aldrich) for 16 h at 373 K. After they were cooled, the samples were diluted with DDW to a maximum final concentration of 7.2% HNO_3 (suitable for ICP-MS analysis) prior to quantification of iridium.

Sequence Preference of DNA Adducts. The primer extension footprinting assay was used to evaluate the sequence selectivity of DNA modification by complexes 4–6. A fragment of pSP73KB DNA linearized by *HpaI* (2464 bp) was incubated with Ir^{III} complexes in 10 mM NaClO_4 for 24 h at 310 K to obtain $r_b = 0.01$ (bound Ir/base). The excess of drug was removed by ethanol precipitation. Circum Vent™ Thermal Cycle Sequencing Kit with Vent(exo[−]) DNA polymerase was used along with the protocol for thermal cycle DNA sequencing with 5'-end-labeled 20-mer SP6 primer recommended by the manufacturer with small modifications.⁶² The synthesis products were separated by electrophoresis on a denaturing polyacrylamide (PAA) gel [6% polyacrylamide (PAA)/8 M urea]; sequence ladders were obtained in parallel using untreated control DNA fragments.

Fluorescence Measurements. These measurements were performed on a Shimadzu RF 40 spectrofluorophotometer using a 1 cm quartz cell. Fluorescence measurements of CT DNA modified by Ir^{III} complexes, cisplatin, or $[\text{Pt}(\text{dien})\text{Cl}]\text{Cl}$ (dien = diethylenetriamine), in the presence of EtBr, were performed at an excitation wavelength of 546 nm, and the emitted fluorescence was analyzed at 590 nm. The fluorescence intensity was measured at 298 K in 0.4 M NaCl to avoid secondary binding of EtBr to DNA.⁶³ The concentrations were 0.01 mg/mL for DNA and 0.04 mg/mL for EtBr, which corresponded to the saturation of all intercalation sites for EtBr on DNA.^{63a}

Viscometry. The relative viscosity of the solutions of CT DNA nonmodified or modified by complexes 4–6 at the concentration of 150 $\mu\text{g}/\text{mL}$ was measured by microviscometry (AMVn Automated Micro Viscometer, Anton Paar GmbH, Austria) using a 1.6 mm capillary tube at 310 K. The densities of the solutions were measured using a Density Meter DMA 4500 instrument (Anton Paar GmbH, Austria).

■ ASSOCIATED CONTENT

Supporting Information. Details of the materials used, synthesis and characterization of ligands and complexes, crystallographic data (Tables S1 and S2 and Figures S1–S4), calculations (Tables S3, S4, S6, and S18), aqueous chemistry (Figures S5–S12), nucleobase studies (Figures S13–S17), and

distribution in cell fractions (Table S5). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ABBREVIATIONS USED

Cp*, pentamethylcyclopentadienyl; Cp^{xph}, tetramethyl(phenyl)cyclopentadienyl; Cp^{xbiph}, tetramethyl(biphenyl)cyclopentadienyl; 9-EtG, 9-ethylguanine; 9-EtA, 9-ethyladenine; IC₅₀, half-maximum inhibitory concentration

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