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Organoiridium Complexes: Anticancer Agents and Catalysts

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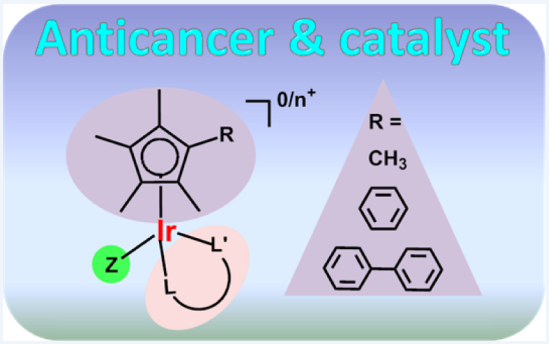
CONSPECTUS: Iridium is a relatively rare precious heavy metal, only slightly less dense than osmium. Researchers have long recognized the catalytic properties of square-planar Ir^{I} complexes, such as Crabtree's hydrogenation catalyst, an organometallic complex with cyclooctadiene, phosphane, and pyridine ligands. More recently, chemists have developed half-sandwich pseudo-octahedral pentamethylcyclopentadienyl Ir^{III} complexes containing diamine ligands that efficiently catalyze transfer hydrogenation reactions of ketones and aldehydes in water using H_2 or formate as the hydrogen source. Although sometimes assumed to be chemically inert, the reactivity of low-spin $5d^6$ Ir^{III} centers is highly dependent on the set of ligands. Cp* complexes with strong σ-donor C^C-chelating ligands can even stabilize Ir^{IV} and catalyze the oxidation of water. In comparison with well developed Ir catalysts, Ir-based pharmaceuticals are still in their infancy. In this Account, we review recent developments in organoiridium complexes as both catalysts and anticancer agents.



Initial studies of anticancer activity with organoiridium complexes focused on square-planar Ir^{I} complexes because of their structural and electronic similarity to Pt^{II} anticancer complexes such as cisplatin. Recently, researchers have studied half-sandwich Ir^{III} anticancer complexes. These complexes with the formula $[(Cp^{x})Ir(L\text{^}L')Z]^{0/n+}$ (with Cp* or extended Cp* and L^L′ = chelated C^N or N^N ligands) have a much greater potency (nanomolar) toward a range of cancer cells (especially leukemia, colon cancer, breast cancer, prostate cancer, and melanoma) than cisplatin. Their mechanism of action may involve both an attack on DNA and a perturbation of the redox status of cells. Some of these complexes can form Ir^{III}-hydride complexes using coenzyme NAD(P)H as a source of hydride to catalyze the generation of H_2 or the reduction of quinones to semiquinones. Intriguingly, relatively unreactive organoiridium complexes containing an imine as a monodentate ligand have prooxidant activity, which appears to involve catalytic hydride transfer to oxygen and the generation of hydrogen peroxide in cells. In addition, researchers have designed inert Ir^{III} complexes as potent kinase inhibitors. Octahedral cyclometalated Ir^{III} complexes not only serve as cell imaging agents, but can also inhibit tumor necrosis factor α, promote DNA oxidation, generate singlet oxygen when photoactivated, and exhibit good anticancer activity. Although relatively unexplored, organoiridium chemistry offers unique features that researchers can exploit to generate novel diagnostic agents and drugs with new mechanisms of action.

1. INTRODUCTION

Iridium (Ir) is a third-row transition metal, a congener of Co and Rh, a member of the platinum-group of "precious metals". It is a relatively rare element discovered in 1803 as an impurity in platinum. Iridium metal is inert and corrosion-resistant. The global demand for Ir in 2007 was 3700 kg, half being used for electrical and electrochemical applications and 20% for catalysis.[1]

The variety of oxidation states (especially Ir^{I}, Ir^{III}, Ir^{IV}), coordination numbers (mainly 4, 6) and coordination geometries of organoiridium complexes[2−5] are illustrated in Table 1. These have attracted much attention in a wide range of areas, especially catalysis.[6] For example, the dinuclear catalyst $[\{Ir(Cp^*)(Cl)\}_2(thbpym)]Cl_2$ (Table 2) provides a way to store hydrogen as an aqueous solution of formic acid.[7] Several groups have developed pincer-ligated Ir catalysts, e.g. for n-alkane metathesis, with potential for industrial applications.[8,9] The Cativa process is one of the largest-scale platinum-group metal-catalyzed carbonylation reactions (>4 million tons/yr); $[Ir(CO)_2I_2]^-$ is used as a catalyst for carbonylation of methanol to produce acetic acid.[2] Some examples of organoiridium catalysts[4,7,10−12] and their design features are summarized in Table 2.

In comparison with well developed Ir catalysts, Ir-based pharmaceuticals are still in their infancy. So far, there are three main applications for organoiridium compounds in biology: luminescent biological labels and probes,[13] protein inhibitors,[14,15] and anticancer agents.[16]

The clinical success and drawbacks of Pt anticancer drugs have stimulated the exploration of other metal-based anticancer compounds,[17] Figure 1, with different mechanisms of action (MoA), reduced side effects, and efficacy toward a wider range of cancers. Iridium complexes were first investigated for their anticancer activity shortly after the discovery of cisplatin. Over the period 1970−2000, attention was focused on $5d^8$ Ir^{I} compounds with square-planar geometry similar to cisplatin, such as $[Ir(acac)(cod)]$ (**1**)[18] and dinuclear $[IrCl(cod)]_2$ (**2**),[19] Figure 2. Encouragingly, **1** gave 100% cures in mice bearing Ehrlich ascites,

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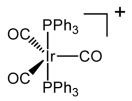
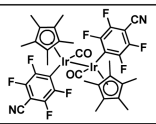
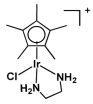
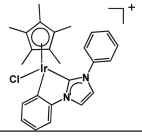
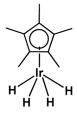
Table 1. Oxidation States and Geometries of Organoiridium Compounds

Oxidation state	Example	Geometry	Ref.
-1	$H[Ir(CO)_4]$	tetrahedral	
0	$Ir(CO)_4$	tetrahedral	
+1	$[Ir(CO)_2I_2]^-$	square-planar	2
		trigonal-bipyramidal	
+2		Pseudo-octahedral	3
+3		Pseudo-octahedral	
+4		Pseudo-octahedral	4
+5			5

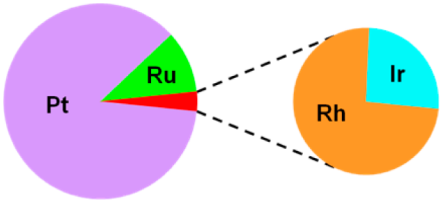


Figure 1. Relative number of publications on Ru, Rh, Ir, and Pt anticancer complexes (Scifinder 16.10.2013).

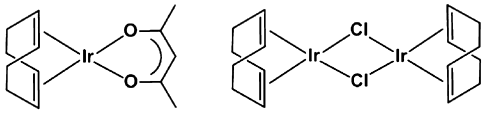
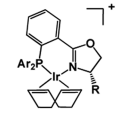
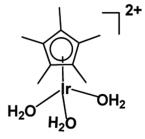
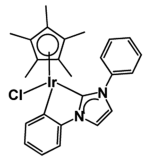
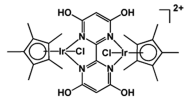


Figure 2. Early Ir^I anticancer compounds.

and inhibited growth of subcutaneous Lewis lung carcinoma in mice. Compound **2** showed antimetastatic activity in the Lewis lung model, but no inhibition of primary tumors. At that time very little work was done on their MoA.

More recently, organo-Ir^{III} anticancer agents have shown promising antiproliferative activity toward cancer cells. In this Account, we focus on recent advances in (1) the rational design of organometallic Ir^{III} half-sandwich cyclopentadienyl anticancer complexes; (2) the discovery of Ir biocatalysts, and attempts to understand their MoA. It is interesting to compare the design features which have been incorporated into organo-Ir catalysts with those in anticancer complexes.

Table 2. Some Design Features in Organoiridium Catalysts

Catalyst/ Application (Ref.)	Design Features
 Hydrogenation (10)	Ligand cod functions as a leaving group. P^N-chelating ligand causes more effective regiocontrol than symmetric ligands.
 Water oxidation (11,12)	Water soluble. Cp* provides an electron-rich environment to stabilize Ir(IV) intermediates. Precursor, easily forms robust catalytic iridium oxides. H_2O ligands readily substituted by substrate.
 Water oxidation (4)	Strong σ-donor C^C-chelating ligand assists stabilization of Ir(IV) intermediates. Leaving group Cl may hydrolyze in aqueous solution, forming more active aqua complex, readily substituted by substrate.
 $[\{Ir(Cp^*)(Cl)\}_2(thbpym)]Cl_2$, stores and releases H_2 (7)	Functional groups in chelating ligand modulate catalytic activity by protonation and deprotonation.

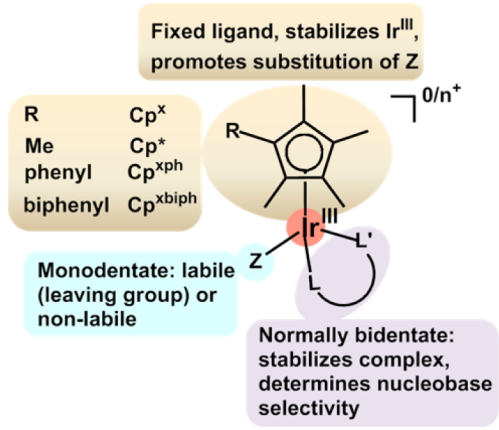


Figure 3. General structure of half-sandwich Ir^{III} cyclopentadienyl complexes. The ligands tune the chemical and biological activity.

2. ORGANOMETALLIC IRIDIUM ANTICANCER AGENTS

2.1. General Features

Until recently much of the research on the design of metal-based anticancer complexes in our laboratory was focused on Ru^{II}, Os^{II}, and Pt^{IV}.[20] Ir^{III} is often considered to be one of the most inert low-spin d^6 metal ions. However, inertness and stability might also be desirable properties for drug design, allowing the complex to reach its target site without modification. Indeed, the ligands may play important roles in target-site recognition. In addition, Ir^{III} is a more stable oxidation state than Ir^I with a higher

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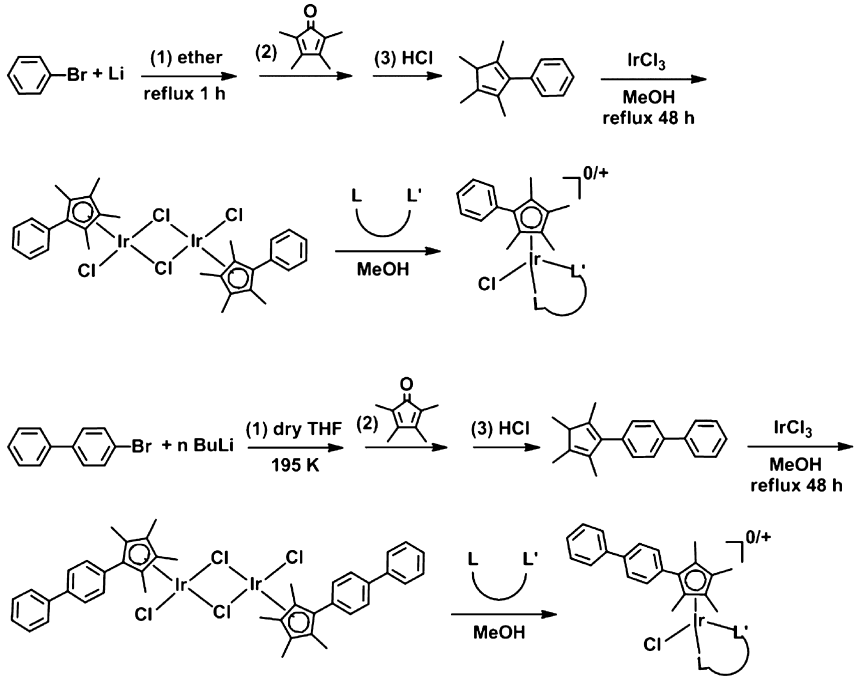
Scheme 1. Synthesis of Ligands $Cp^{xph}H$, $Cp^{xbiph}H$, and Respective Half-Sandwich Ir^{III} Complexes



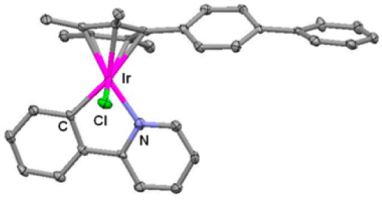


Figure 4. X-ray crystal structure of $[(\eta^5\text{-}Cp^{xbiph})Ir(phpy)Cl]$ (**3**). Based on data from ref 24.

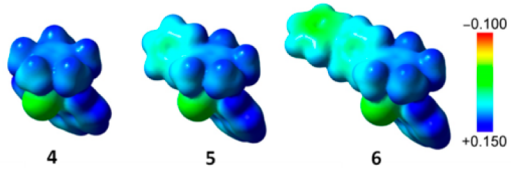


Figure 5. Electrostatic potential surfaces of complexes $[(\eta^5\text{-}Cp^*)Ir(phen)Cl]^+$ (**4**), $[(\eta^5\text{-}Cp^{xph})Ir(phen)Cl]^+$ (**5**), and $[(\eta^5\text{-}Cp^{xbiph})Ir(phen)Cl]^+$ (**6**). Electrostatic potential: red, negative; blue, positive. Reprinted with permission from ref 22. Copyright 2011 American Chemical Society.

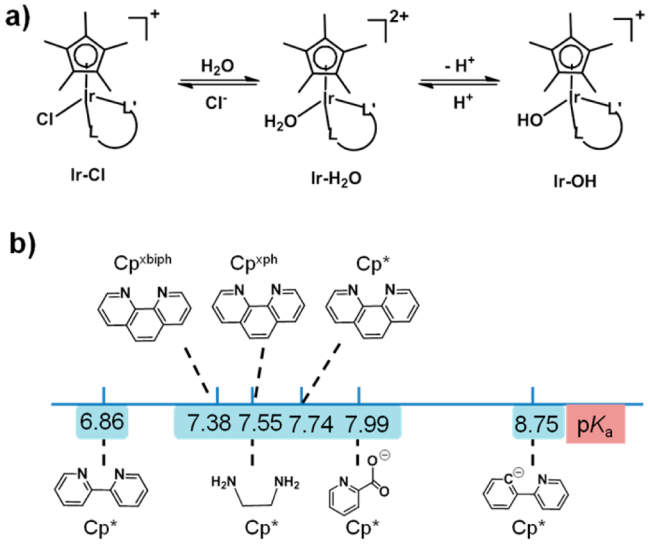


Figure 6. (a) Cp* Ir aqua species. (b) Effects of ligands on pK_a values of aqua complexes $Ir-H_2O$.

coordination number (6 versus 4), which can provide extensive structural diversity from a wide range of ligands. Unlike Ru^{II} or Os^{II} complexes, Ir^{III} complexes are relatively unstable with arenes (benzene derivatives) as ligands;[21] instead cyclopentadienyl ligands, especially electron-rich pentamethylcyclopentadienyls (Cp*), are suitable.

A range of half-sandwich organometallic Ir^{III} cyclopentadienyl complexes of the type $[(\eta^5\text{-}Cp^x)Ir(L\text{^}L')Z]^{0/n+}$ (where Cp^x = Cp*, Cp^{xph} (phenyltetramethylcyclopentadienyl) or Cp^{xbiph} (biphenyltetramethylcyclopentadienyl), L^L′ = bidentate ligand with nitrogen, oxygen and/or carbon donor atoms, for example, N^N-, N^O-, O^O-, or C^N-chelating ligand, Z = Cl or py) has been synthesized and characterized as potential anticancer agents.[22−24] The general structure of these pseudo-octahedral complexes is shown in Figure 3. The π-bound negatively charged Cp^x ligand, occupies one face of the octahedron (3 coordination sites) and affects the stability of the remaining of ligands through modification of their electronic behavior. The extended arene in the functionalized Cp* ligand may play a role in interactions with a target, for example, by intercalation into DNA base pairs, and the hydrophobicity of the arene enhances cellular uptake of the complex. The chelating ligand L^L′ provides additional stability for the complex and contributes to tuning the electronic properties of the iridium center. The monodentate ligand Z, such as chloride, can provide a labile site for substitution reactions with target sites.

The synthetic route to functionalized cyclopentadienyl ligands and half-sandwich Ir^{III} compounds, is summarized in Scheme 1. Our work appeared to provide the first synthesis of $\{Ir(Cp^{xph})\}$ and $\{Ir(Cp^{xbiph})\}$ compounds; these normally are air-stable and yellow in color. The X-ray crystal structures of $\{Ir(Cp^{xbiph})\}$

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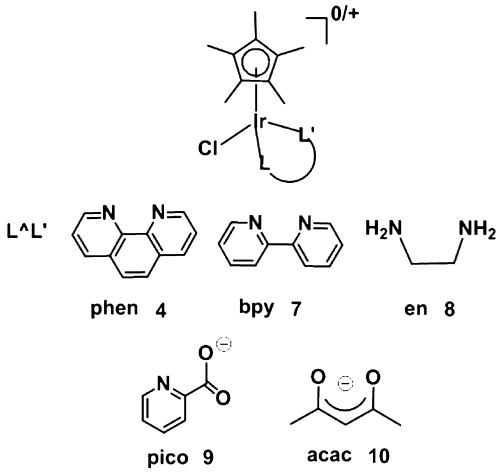
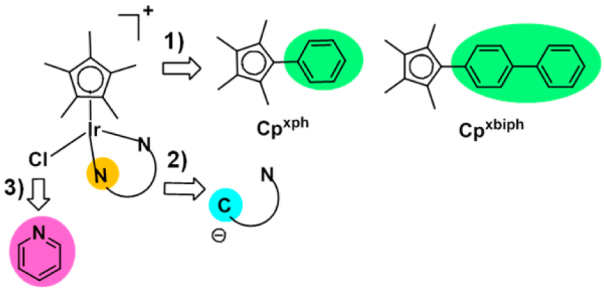


Figure 7. Inactive Cp* Ir^{III} complexes.

Scheme 2. Strategies Used to Improve the Anticancer Activity of Cyclopentadienyl Ir^{III} Complexes



compounds, such as $[(\eta^5\text{-Cp}^{xbiph})Ir(phpy)Cl]$ (**3**, phpy = 2-phenylpyridine, Figure 4), show a twist between the central phenyl ring and the adjacent two rings (48.9° and 21.5°), while the Cp ring and the terminal phenyl ring are almost parallel.[24] Electrostatic potential surfaces for 1,10-phenanthroline (phen) complexes **4**–**6** show that higher electron density is present on the terminal phenyl ring of the Cp^{xbiph} ligand in **6**, Figure 5.[22]

Hydrolysis is often considered as an activation step in the MoA of transition metal anticancer agents,[25,26] but Ir^{III} complexes are often thought to be too inert to possess high

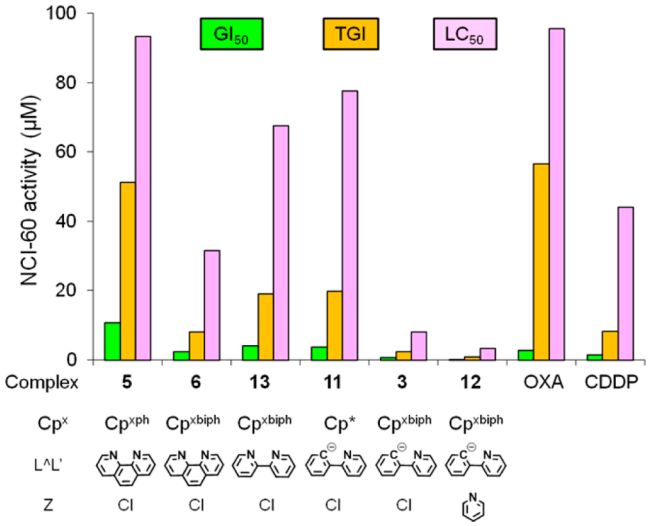


Figure 9. Anticancer activity of Ir compounds, oxaliplatin (OXA), and cisplatin (CDDP) in the NCI-60 screen. GI_{50} and TGI, concentration causing 50% and 100% cell growth inhibition, respectively. LC_{50}, concentration decreasing the original cell number by 50%. Based on data from refs 33 and 34.

activity. For example, the lifetime for a water molecule in the first shell of $[Ir(H_2O)_6]^{3+}$ is >300 years.[27] The compound *trans*-$[IrCl_4(DMSO)(Im)][ImH]$ (DMSO = dimethyl sulfoxide, ImH = imidazole), the Ir analogue of NAMI-A[28] (Ru antitumor metastasis inhibitor on clinical trials), is inert toward hydrolysis and lacks biological activity.[29] Surprisingly, many of the half-sandwich cyclopentadienyl Ir–Cl complexes we have studied hydrolyze rapidly (minutes) in aqueous solution and form aqua complexes Ir–H_2O (Figure 6a), illustrating that Ir^{III} complexes can be quite labile. The electron-rich methyl groups on the Cp ring may contribute to the fast hydrolysis by increasing the negative charge on the metal, making it favorable for Cl^- to leave. These results are consistent with a previous report of a 14-orders-of-magnitude increase in the rate of water exchange in $[Ir(H_2O)_6]^{3+}$ by introduction of Cp*.[30] Introduction of phenyl or biphenyl Cp* substituents slows down hydrolysis rate, but increases the extent of hydrolysis, Figure 11e.

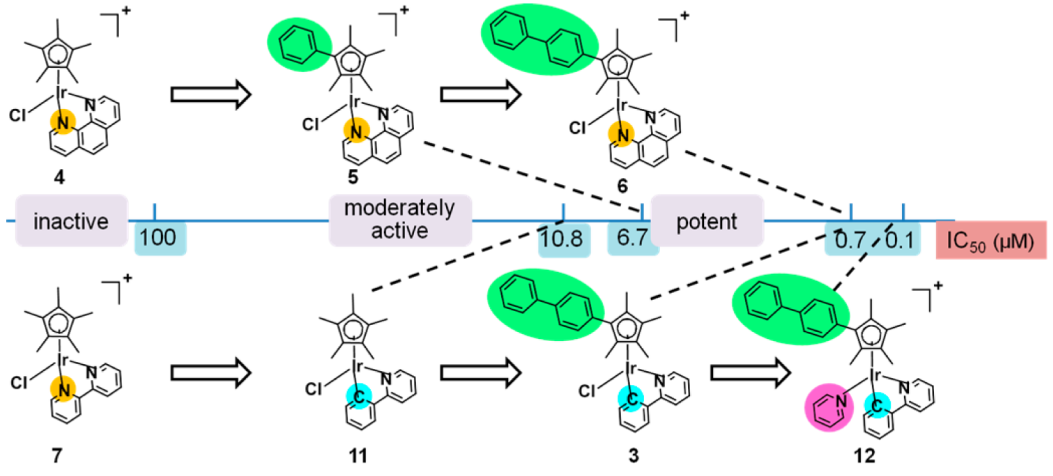


Figure 8. Comparison of cytotoxicity of $[(\eta^5\text{-Cp}^x)Ir(L^{\wedge}L')Z]^{0/+}$ complexes against A2780 cancer cells. Inactive: <50% growth inhibition at 100 μM. Moderate: 80% to 50% growth inhibition at 50 μM. Potent: >50% growth inhibition at 5 μM.

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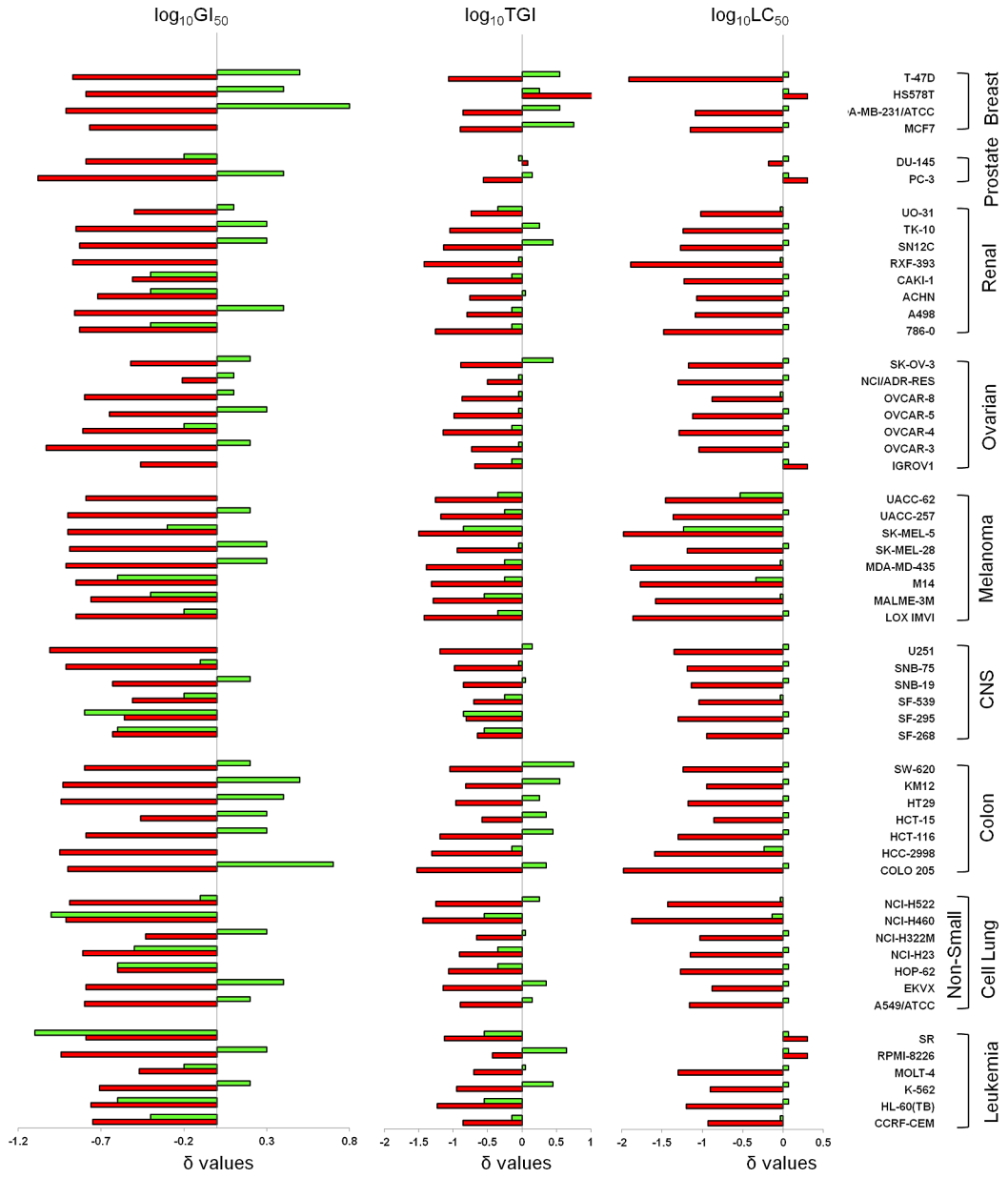

Figure 10. GI_{50}, TGI, and LC_{50} mean graphs for CDDP in the NCI-60 panel of cancer cell lines; the green bars show how the values deviate from the mean ($\delta = 0$ corresponds to $\log_{10}$ $GI_{50} = -5.83$, $\log_{10}$ TGI $= -5.08$, and $\log_{10}$ $LC_{50} = -4.36$,). GI_{50}, TGI, and LC_{50} values for **12** are plotted as red bars relative to the mean values for CDDP, and show that **12** is more active (lower GI_{50}, TGI, and LC_{50} values) than CDDP in almost all the cell lines. Based on data from ref 34.

The activated aqua adduct is acidic in aqueous solution, Figure 6a; the deprotonated form Ir−OH is usually less reactive. X-ray crystal structures of organometallic Ir aqua complexes are rare.[30] We found that complexes containing negatively charged chelating ligands have higher Ir−H_2O pK_a values than those bearing neutral chelating ligands, Figure 6b, similar to Ru^{II} and Os^{II} arene complexes.[31] For example, C^N-chelated Ir complexes have pK_a values ca. 1.9 units higher than N^N-chelated analogues, thus ensuring that most of the hydrolyzed complexes would be in the active Ir−H_2O form at physiological pH.[24]

Ir−H_2O species formed after hydrolysis react more readily with nucleobases than Ir−Cl complexes.[22]

2.2. Anticancer Studies: Structure−Activity Relationships

In our early work, we discovered that Cp* complexes $[(\eta^5\text{-Cp}^*)\text{Ir(L^L')Cl}]^{0/+}$ containing N^N- (phen, bpy = 2,2′-bipyridine, en = ethylenediamine), N^O- (pico = picolinate), or O^O-chelating ligand (acac = acetylacetonate), Figure 7, were all inactive with IC_{50} values (dose which inhibits cell growth by 50%) of >100 μM toward A2780 human ovarian cancer cells. Poor cellular accumulation may account for their inactivity, Figure 11c.[22]

We subsequently discovered effective strategies for switching on anticancer activity involving modifications to the three ligands: (1) extending the Cp* ring by introduction of a phenyl or biphenyl substitutent; (2) replacement of the neutral N^N-bound chelating ligand with a negatively charged isoelectronic C^N-bound analogue; and (3) changing the leaving group Cl^- to an amine such as pyridine (py), Scheme 2.

These modifications produced significant increases in activity as illustrated in Figure 8 for A2780 cells. In the phen/Cl series, addition of phenyl substituents to the Cp* ring increased potency markedly. In the bpy series, replacement of a chelated N by

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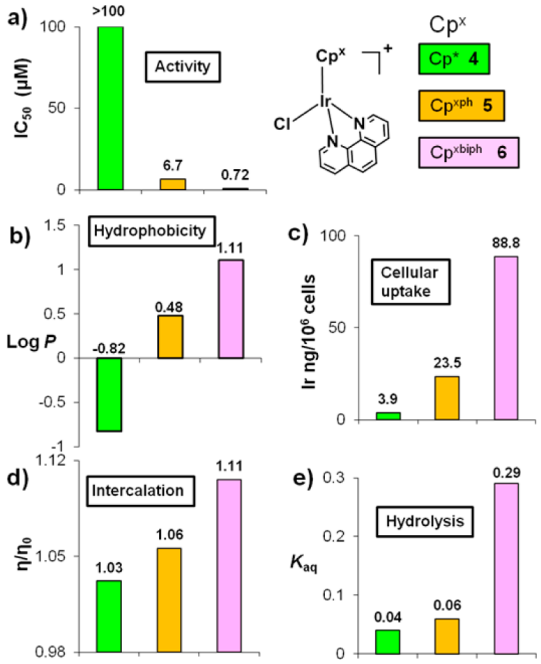

Figure 11. Influence of substitutents on the cyclopentadienyl ligands on the behavior of complexes **4**–**6**. Based on data from ref 22.

isoelectronic C^- caused a dramatic increase in activity, and further addition of a biphenyl substituent and replacement of Cl^- by pyridine achieved nanomolar activity.

Screening against the NCI panel of 60 cancer cell lines (Figures 9 and 10) confirmed that this class of Ir^{III} complexes can have comparable or even higher activity than the clinical drugs oxaliplatin (OXA) and cisplatin (CDDP). This screen identified high activity towards several other types of cancer cells, notably leukemia, colon, breast, prostate, and melanoma.[32] COMPARE analysis of NCI data suggests a distinct difference between Ir compounds and Pt anticancer drugs, and a different MoA.[33,34] Detailed structure–activity relationships are discussed below.

2.2.1. From Cp* to Cp^{xph} and Cp^{xbiph}. Interestingly, cytotoxic potency toward cancer cells increases markedly with the number of phenyl rings on Cp*: Cp^{xbiph} > Cp^{xph} ≫ Cp*; see Figures 8, 9, and 11a. The increased hydrophobicity of Cp^{xph} and Cp^{xbiph}, assists passage across cell membranes, Figure 11b and c, and they can also intercalate into DNA. Indeed, modification of calf thymus DNA (CT DNA) by **4**–**6** results in a decrease of EtBr fluorescence intensity and increase in the relative viscosity of CT DNA in the same order **6** > **5** > **4**, Figure 11d.

Cp^{xph} and Cp^{xbiph} complexes, for example, **5** and **6**, are dual-function DNA binding agents. On the one hand, they can modify DNA by intercalation, and on the other they can coordinate directly to DNA bases, especially to guanine (G), and can block DNA replication.[22] Our data show that complexes **4**–**6** can indeed gain access to the nucleus in A2780 cancer cells in the order **4** < **5** < **6**, and subsequently bind to DNA, indicating that DNA is an important biological target.[22]

2.2.2. Modification of Chelated Ligand. The anticancer activity of Cp* complexes can also be switched on by changing a neutral N^N-chelating ligand to a negatively charged C^N-bound analogue, Figures 8 and 12a. The Cp* complex [(η^5-Cp*)Ir(phpy)Cl] (**11**) is as potent as the Cp^{xbiph} complex [(η^5-Cp^{xbiph})Ir(bpy)Cl]$^+$ (**13**) toward NCI-60 cell lines, Figure 9.

The change in chelated ligand affects the selectively of binding to nucleobases: complex **7** containing bpy (N^N) forms 9-ethylguanine

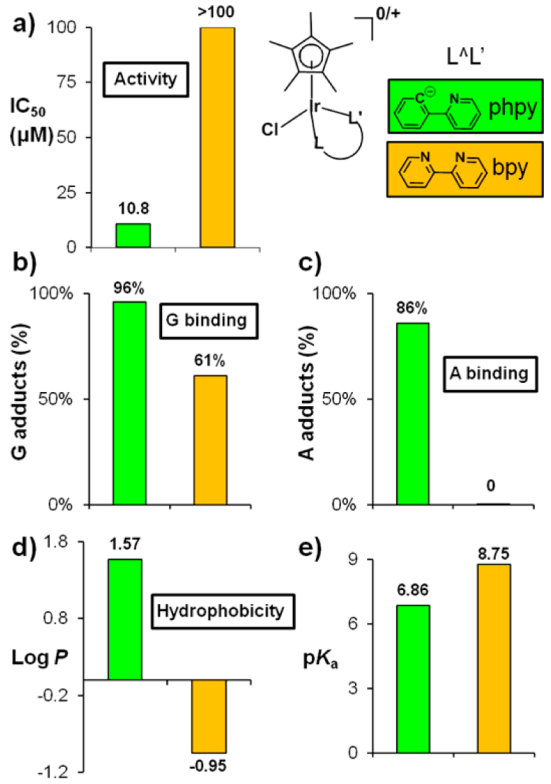

Figure 12. Influence of chelated ligands on the behavior of complexes [(η^5-Cp*)Ir(bpy)Cl]$^+$ (**7**) and [(η^5-Cp*)Ir(phpy)Cl] (**11**). Based on data from ref 23.

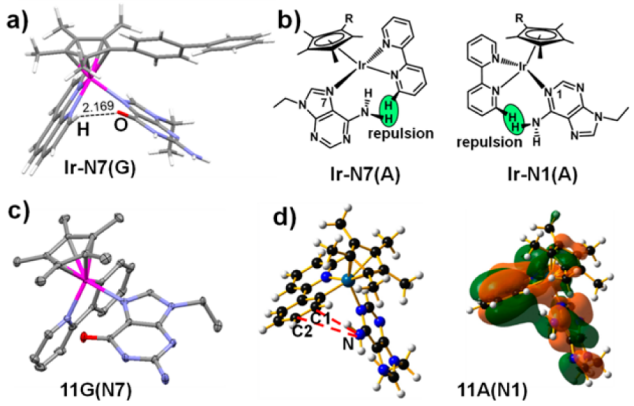

Figure 13. Guanine (G) and adenine (A) adducts of Ir^{III} anticancer complexes. Reprinted with permission from refs 22 and 23. Copyright 2011 American Chemical Society.

(9-EtG) but not 9-ethyladenine (9-EtA) adducts; however, C^N-complex **11** readily binds to both purines and to a much higher extent, Figure 12b and c. Moreover, replacing N^N- with C^N- increases hydrophobicity significantly, leading to higher cellular uptake and higher anticancer activity, Figure 12d.

The selectivity toward nucleobase binding appears to result from both electronic and steric effects, including H-bonding (e.g., C6O of G, Figure 13a) and repulsive interactions between protons of the bpy ligand and exocyclic amine groups (C6NH_2 of A, Figure 13b). The X-ray structure of the guanine adduct [(η^5-Cp*)Ir(phpy)(9-EtG-N7)]$^+$ (**11G**) has been determined, Figure 13c.[23]

Interestingly the C^N- complex **11** forms two Ir–A adducts through binding to N1 and N7 of adenine. Density functional

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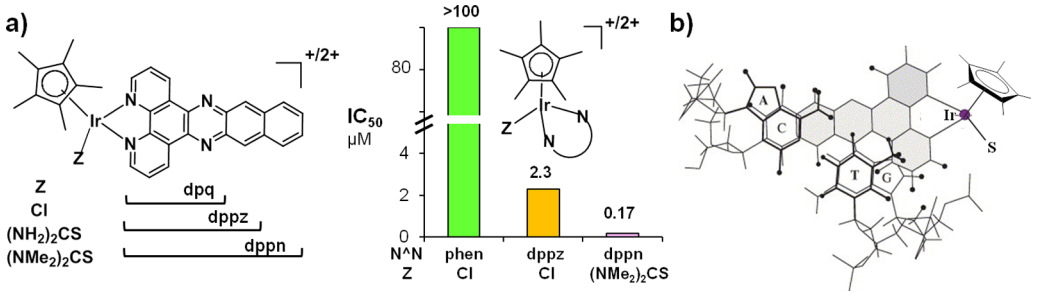
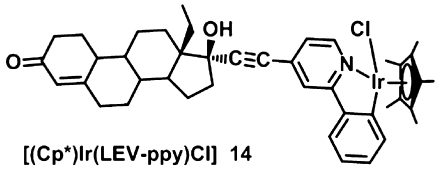


Figure 14. (a) Influence of the size of polypyridyl ligands on cytotoxicity toward MCF-7 breast cancer cells. (b) Illustration of side-on intercalation. Adapted from ref 36.

theory calculations indicate that N1 binding is more stable than N7 by 18.36 kJ/mol,[23] and that the A adduct **11A** may be stabilized by a π-orbital interaction between N (NH_2 of adenine) and C1 and C2 (phpy), Figure 13d.

Increasing the size of the planar chelated ligand is also an effective strategy to improve anticancer potency. For example, the phen complex $[(\eta^5\text{-Cp}^{xbiph})\text{Ir(phen)Cl}]^+$ (**6**) has higher cytotoxicity than its bpy counterpart $[(\eta^5\text{-Cp}^{xbiph})\text{Ir(bpy)Cl}]^+$ (**13**), Figure 9. The size of the polypyridyl ligand in the complexes $[(\eta^5\text{-Cp*})\text{Ir(N^N)(Z)}]^{n+}$, where N^N = dpq (dipyrido[3,2-f:2′,3′-h]-quinoxaline), dppz (dipyrido[3,2-a:2′,3′-c]phenazine), dppn (benzo[*i*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine), correlates with anticancer activity: dpq < dppz < dppn, Figure 14a.[35] Increasing the surface area of the polypyridyl ligand generally results in a significant increase in the intercalative binding strength and cellular uptake. A side-on intercalation mode was established by 2D NOESY NMR for the interaction of a dppz complex with the hexanucleotide $d(GTCGAC)_2$, Figure 14b.[36]

The steroid hormone conjugate $[(\eta^5\text{-Cp*})\text{Ir(LEV-phpy)Cl}]$ (**14**, LEV-phpy =17-*α*-[2-phenylpyridyl-4-ethynyl]-19-nortestosterone), targeted to steroidal receptors,[37] displays 6-fold and 2-fold greater potency than CDDP and unfunctionalized **11**, respectively.



[(Cp*)Ir(LEV-ppy)Cl] 14

Cp* complexes **15**–**20** containing other N^O-, O^O-, or N^N-chelating ligands differ in their anticancer activity, Figure 15.[38−41] The N^O-bound complexes **15**–**17** are all active, especially **15** which is more potent than CDDP toward HeLa and HL60 cancer cells. Complexes **18** and **19** show moderate activity, and **20** is inactive. Further work is needed to understand the MoA of these complexes.

2.2.3. Changing Monodentate Ligand Chloride to Pyridine. Changing the monodentate ligand from chloride to pyridine (py) in Ru arene complexes blocks hydrolysis,[42] and can greatly reduce activity toward cancer cells. Surprisingly, $[(\eta^5\text{-Cp}^{xbiph})\text{Ir(phpy)(py)}]^+$ (**12**) exhibits high anticancer activity, and is ca. 3× more active than the chlorido ananogue $[(\eta^5\text{-Cp}^{xbiph})\text{Ir(phpy)Cl}]$ (**3**), and 6−13× more active than CDDP against NCI-60 cancer cell lines, Figure 9.[34] Complex **12** is a strong inducer of reactive oxygen species (ROS) in A2780 cancer cells, Figure 16a. Excitingly, complex **12** can generate higher levels of ROS in cancer cells than in normal cells, resulting in 13× higher potency toward cancer cells than normal cells. The generation of ROS is an effective way of killing cancer cells.[43]

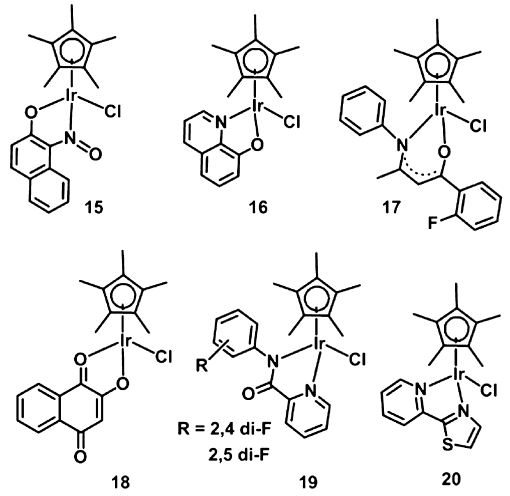


Figure 15. Cp* Ir^{III} anticancer complexes containing N^O-, O^O-, or N^N-chelating ligands.

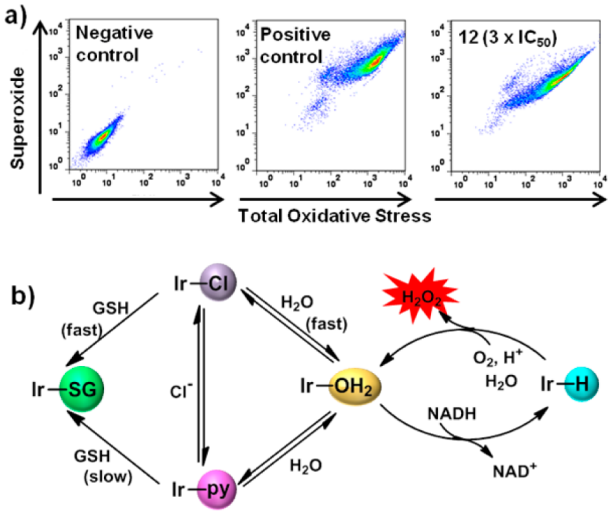


Figure 16. (a) ROS levels in A2780 cells induced by **12** after 1 h exposure at 310 K. Adapted from ref 34. (b) Reactions of Ir^{III} pyridine complex **12** and its Cl analogue and possible pathways for generation of H_2O_2. More details of hydride transfer from NADH to Ir are in Figure 19.

Replacement of the chlorido ligand by py appears to be an effective strategy to avoid deactivation. Compared to py complex **12**, complex **3** is more reactive, hydrolyzes rapidly, and reacts readily with the abundant intracellular thiol glutathione (GSH), Figure 16b. Therefore, less reactive complex **12** is more likely to reach intracellular target sites.

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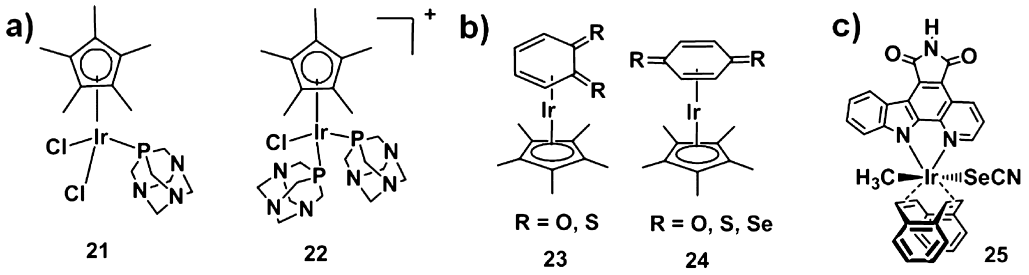

Figure 17. (a) Inactive half-sandwich Ir^{III} complexes with three monodentate ligands. (b) Stable thioquinones and diselenobenzoquinone sandwich Ir^{III} complexes with anticancer activity. (c) Photoactive octahedral Ir^{III} anticancer complex.

Some Ir^{III} aqua complexes can oxidize coenzyme NADH to NAD^+ (Figure 19) by accepting hydride to form an Ir hydride adduct (vide infra).[44,45] Our recent work demonstrates that potent Ir anticancer complexes such as **3** and **12** can accept hydride from NADH in aqueous solution (^{1}H NMR Ir−H − 14.7 ppm). The hydride can further be transferred to oxygen to generate H_2O_2, Figure 16b.[34] This is a new strategy for generating ROS in cancer cells using organometallic complexes and intracellular antioxidants. These complexes may provide highly effective oxidant-based therapy as a new approach to curing cancer.

2.3. Other Types of Organometallic Ir^{III} Anticancer Agents

The anticancer activity of half-sandwich phosphane complexes $[(Cp^*)Ir(PTA)Cl_2]$ (**21**) and $[(Cp^*)Ir(PTA)_2Cl]$ (**22**), where PTA = 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane, containing three monodentate ligands has also been explored, Figure 17a. In contrast to Ru *p*-cymene analogues, all of these Ir^{III} complexes were noncytotoxic (IC_{50} > 300 μM) toward A2780 cells.[46]

Labile thioquinones and diselenobenzoquinones have been stabilized by {Cp*Ir} fragments, Figure 17b.[47] The selenoquinone complex *p*-[Cp*Ir(η^4-$C_6H_4Se_2$)] is the most cytotoxic toward A2780 cells, comparable to cisplatin.

Organoiridium complexes containing pyridocarbazole as chelating ligand have been explored as kinase inhibitors.[15] Interestingly, complex **25** (Figure 17c) is not only a kinase inhibitor, but also photocytotoxic with an IC_{50} of 0.23 μM toward HeLa cells after irradiation with visible light for 1 h, 34× more potent than in the dark.[48] Photoinduced ligand substitution of the selenocyante group (−SeCN) with chloride occurs after 30 min of irradiation with 20-fold excess of chloride. The labilization of the Ir−SeCN bond results in apoptotic cell death. Multinuclear Cp* Ir complexes are also promising anticancer agents, especially thiolato-bridged dinuclear complexes.[49−52]

Cyclometalated Ir^{III} complexes can have long-lived emission in the visible region, making them attractive as biological probes. The triplet metal-to-ligand charge-transfer (^{3}MLCT) transition is the most common emissive state. Cyclometalated Ir^{III} complexes such as 26 (Figure 18a),[53] containing C^N- and N^N-chelating ligands, have attractive photophysical properties.[13] Recently, the rich luminescent properties of cyclometalated Ir^{III} probes have been utilized in oligonucleotide-based sensing.[54−56] In addition, these complexes are kinetically inert, and useful as structurally diverse molecular scaffolds for the design of selective enzyme inhibitors and anticancer agents, such as **27**.[57] In general, the cytotoxicity of cyclometalated Ir compounds is related to their cellular uptake efficiency.[58]

Cyclometalated Ir^{III} complexes are also effective photoinduced singlet oxygen producers. The complexes $[Ir(C^N)_2(XY)]$ (**28**), XY = two monodentate ligands or N^N-, C^N-, N^O-, or O^O-chelating ligands, function as photosensitizers for generating $^1O_2$, and lead to cell death, Figure 18b.[59−61]

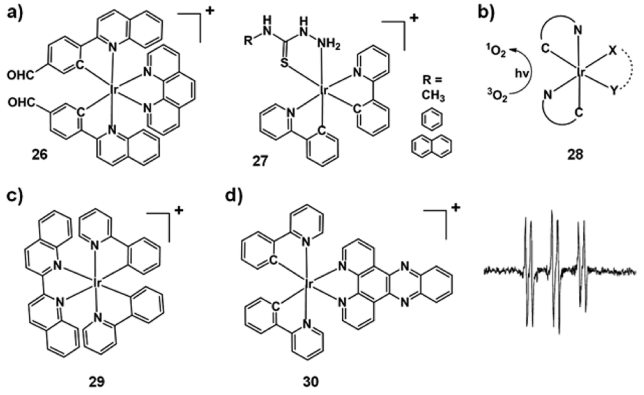

Figure 18. Examples of cyclometalated octahedral Ir^{III} complexes. (a) Bioimaging (**26**)[53] and anticancer (**27**)[57] agents. (b) Singlet oxygen photoinducer.[59−61] (c) Tumor necrosis factor-α inhibitor.[62] (d) EPR spectrum of guanine radicals generated by photoirradiation of complex **30** and DNA.[63] (Adapted from ref 61.)

Complex **29** (Figure 18c) is an inhibitor of tumor necrosis factor-α (TNF-α), a cytokine involved in systemic inflammation and other biological processes.[62] Both enantiomers Δ-1 and Λ-1 are potent inhibitors of TNF-α in human hepatocellular carcinoma cells with IC_{50} values of 2.5 and 4 μM, respectively.

Complex **30** (Figure 18d) has interesting excited-state redox properties. Irradiation produces redox damage on DNA by oxidizing purines and reducing pyrimidine nucleobases.[63] Guanine radicals can be observed by EPR due to photoinduced electron-transfer after only 90 s irradiation of complex **30** and $[poly(dG\text{-}dC)]_2$ at λ > 350 nm.

3. ORGANOMETALLIC IR^{III} COMPLEXES AS BIOCATALYSTS

The ability of iridium complexes to act as efficient catalysts[6] makes them attractive as potential biocatalysts. For example, Cp^*Ir^{III} complexes have been used as hydrogen transfer catalysts for the amination of 1,3-propanediol, produced by *C. butyricum* grown on glycerol.[64]

Ir^{III} and Rh^{III} Cp* complexes can catalyze the reduction of NAD^+ to NADH with formate as the hydride source.[65] We demonstrated the facile conversion of NADH to NAD^+ through hydride transfer using Ir^{III} Cp* (Figures 16 and 19b) and Ru^{II} arene complexes.[34,44] Hydride-transfer from NADH resulted in formation of Ir−H species. Interestingly, the bound hydride can be protonated under nitrogen, with catalytic generation of H_2, Figure 19c.[44] A TON (turnover number) of 75 after 24 h and TOF (turnover of frequency) up to 4.3 h^{-1} for catalytic oxidation of NADH by complex **32** were achieved under physiologically relevant conditions (pH 7.4, 310 K, water). The reduction of

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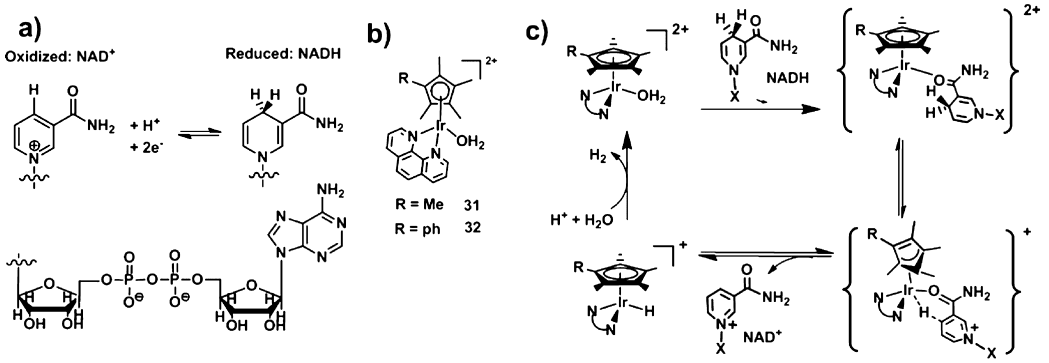

Figure 19. (a) Chemical structure of NADH and NAD^+. (b) Ir catalysts utilized for NAD^+/NADH conversion. (c) Proposed mechanism for catalytic hydride transfer from NADH to Ir−H_2O complexes. Adapted from ref 44.

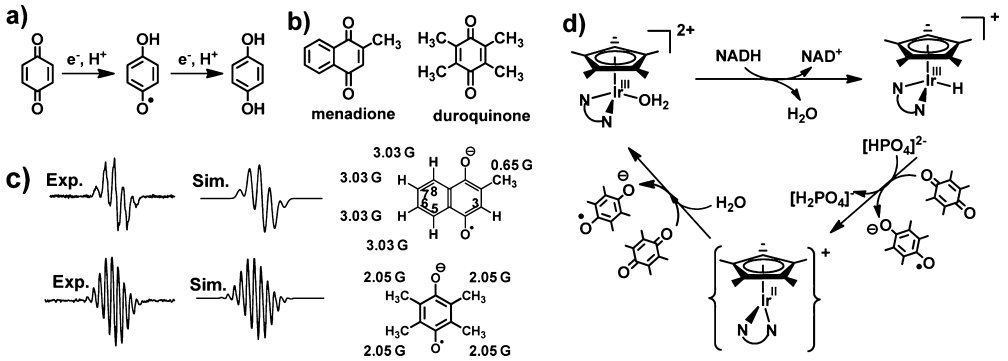

Figure 20. (a) One- and two-electron reduction of a quinone leading to semiquinone and hydroquinone, respectively. (b) Chemical structures of menadione and duroquinone. (c) EPR spectra of menadione (top) and duroquinone (down) radical anions. (d) Proposed mechanism for the catalytic reduction of duroquinone by NADH/Ir system. Adapted from ref 45.

pyruvate to lactate, catalyzed by lactate dehydrogenase, can be achieved by the NADH/Ir system in the absence of enzyme. Moreover, in A2780 cells, **32** (35 μM) can cause a doubling of the NAD^+/NADH ratio from 7.95 ± 0.10 to 14.84 ± 0.77 after 6 h treatment.[44] These findings open up possibilities for control of the redox status of cells, interference in NADH-mediated cell signal pathways, and coupling hydrogenations to biological processes.

We have investigated the potential for application of hydride Ir−H complexes as effective reductants in aqueous reactions. Quinones were studied since they can function as electron carriers in electron-transport chains, Figure 20a and b. Ir−H species are highly active in the catalytic reduction of duroquinone and menadione, generating semiquinone radicals (detectable by EPR, Figure 20c), with the TON 56.6 and TOF 12.4 h^{-1} in buffer at pH 7.2. Interestingly, the mechanism appears to involve two one-electron transfers to the quinone and a transient Ir^{II} state, Figure 20d, which then transfers the second electron to a second quinone, generating the second quinone radical and regenerating Ir^{III}. The Ir^{II} oxidation state is not common, but has been characterized by EPR in other systems such as PNP pincer complexes at 86 K.[66] Ir^{II} can also be stabilized in a dinuclear compound, often supported by an Ir−Ir bond. Only a few X-ray structures of Ir^{II} compounds have been determined.[3,67] We also considered the possibility that the semiquinone might arise from reaction of a quinol product with the quinone, but as yet have been unable to demonstrate this experimentally.

The catalytic transfer of hydride from NADH by Ir C^N complexes and generation of H_2O_2 is discussed in section 2.2.3 above. Recently, biotinylated iridium complexes have displayed promising catalytic activity as artificial metalloenzymes based on biotin/(strept)avidin technology.[68] The Ir-biotin artificial transfer hydrogenase can even combine with a variety of natural enzymes to perform concurrent tandem catalysis.[69]

4. CONCLUSIONS

Ir^{III} complexes with their low-spin $5d^6$ configurations are often thought to be kinetically inert, but this is not always the case. Their reactivity is strongly dependent on the ligand set. Ligand exchange reactions of some organoiridium Cp* complexes, for example, can take place in seconds. Recently, a new class of highly potent half-sandwich organometallic Ir^{III} anticancer agents has emerged. Some bind to DNA, and others can perturb the redox balance in cells. Attack on coenzyme NAD(P)H can lead to formation of iridium hydride adducts in water and catalytic reactions involving, for example, hydrogen production, quinone reduction, and hydrogen peroxide formation. If such reactions can occur in cells, then these complexes might function as catalytic drugs, albeit with the possibility of rapid poisoning of the catalyst. Pro-oxidant drugs can be very effective as anticancer agents and, being multitargeted, can combat resistance, a clinical problem. Promising bioactivities including enzyme inhibition, production of singlet oxygen, purine oxidation and photoactivation have been observed for cyclometalated Ir^{III} complexes.

The design concepts discussed in this Account can be applied to structure−activity relationships for other organometallic anticancer complexes and may introduce novel mechanisms of action.

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Notes

Z.L. and P.J.S. and are named inventors on a patent application relating to the iridium complexes used in their own work filed by the University of Warwick.
The authors declare no competing financial interest.

Biographies

Zhe Liu received his B.Sc. and M.Sc. at Shandong Normal University, P. R. China. Then he was appointed as a lecturer in Chemistry at Qingdao University in 2006. Between 2008 and 2011, he undertook a Ph.D. in Chemistry at the University of Warwick under the supervision of Professor Peter J. Sadler. Since then he has been a research fellow in the Sadler group. His research interests are centered on the design and mechanism of action of iridium anticancer agents and biocatalysts.

Peter Sadler obtained his BA, MA, and DPhil at the University of Oxford. Subsequently he was a Medical Research Council Research Fellow at the University of Cambridge and National Institute for Medical Research. From 1973 to 1996 he was at Birkbeck College, University of London, and from 1996 to 2007 Crum Brown Chair of Chemistry at the University of Edinburgh. In June 2007, he took up a Chair in Chemistry at the University of Warwick. He is a Fellow of the Royal Society of Edinburgh (FRSE) and the Royal Society of London (FRS), a European Research Council Advanced Investigator, and Mok Hing Yiu Distinguished Visiting Professor in Chemistry at the University of Hong Kong. His research interests are centered on the chemistry of metals in medicine.

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