Dalton Transactions

PERSPECTIVE

View Article Online
View Journal

Cite this: DOI: 10.1039/c8dt01858h

Anticancer activity of complexes of the third row transition metals, rhenium, osmium, and iridium

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The clinical success of the platinum-based chemotherapeutic agents has prompted the investigation of coordination and organometallic complexes of alternative metal centers for use as anticancer agents. Among these alternatives, the third row transition metal neighbors of platinum on the periodic table have only recently been explored for their potential to yield anticancer-active complexes. In this Perspective, we summarize developments within the last six years on the application of rhenium, osmium, and iridium complexes as anticancer drug candidates. This review focuses on studies that discuss the potential mechanisms of action of these complexes. As reflected in this Perspective, complexes of these metal ions induce cancer cell death *via* a diverse range of mechanisms. Notably, small structural changes can significantly alter the mode of cell death, hindering efforts to elucidate structure–activity relationships. This property may both benefit and hinder the clinical development of these compounds.

Received 8th May 2018,
Accepted 6th June 2018

DOI: 10.1039/c8dt01858h

rsc.li/dalton

1. Introduction

Despite significant advances over the last fifty years, cancer remains one of the leading causes of death worldwide.[1] In conjunction with surgical resection and radiotherapy, chemotherapy, the application of cytotoxic chemical compounds, is still the predominant strategy for cancer treatment. The toxic side effects associated with chemotherapeutic treatment regimens, however, have directed research efforts towards the development of targeted therapies.[2–4] These targeted approaches rely on the overexpression of specific receptors on cancer cells. In principle, targeted therapy provides a means to treat cancer without the off-target side effects associated with cytotoxic chemotherapeutic agents, but it is limited to cancers that exhibit specific targetable genotypes. For patients with cancers lacking these desired genotypes, chemotherapy remains the primary therapeutic option. As such, there is a need to develop more effective chemotherapeutic agents with superior toxicity profiles.

Among the most effective and well-studied class of chemotherapeutic agents are the platinum-based drugs, which comprise the FDA-approved compounds, cisplatin, carbopla-

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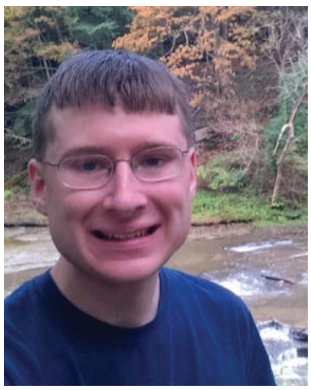
tin, and oxaliplatin.[5] These drugs, whose mechanisms of action rely on the formation of covalent Pt–DNA adducts,[6] suffer from many of the limitations of chemotherapy. Namely, they induce toxic side effects,[7] such as nephrotoxicity, ototoxicity, and peripheral neuropathy, which limit the doses that can be safely administered to patients.[8] In addition, cancer cells readily develop resistance to these drugs, rendering them useless for most relapsed forms of this disease.[9] In spite of these limitations, the platinum drugs are still the first line treatment for many cancer types as evidenced by their use in approximately 50% of all chemotherapeutic treatment regimens.[10] Although continued efforts to improve upon platinum anticancer agents have been made,[11,12] there have been no new drugs of this class brought to the market in the United States since the FDA approval of oxaliplatin in 2002.[13]

Given the clinical success of the platinum-based compounds, extensive research efforts have been directed towards investigating the anticancer activity of complexes of alternative metal ions. The aim of these studies is to capitalize on the novel therapeutic potential of inorganic complexes, while circumventing the limitations of the platinum drugs.[14] The majority of these efforts have focused on complexes of ruthenium, gold, and titanium.[15] Although several of these compounds have shown extremely promising activity that has warranted clinical trials, their chemistry is significantly different from that of platinum.[16] Specifically, their ligand substitution kinetics are several orders of magnitude larger than those of platinum. More closely aligned chemistry can be found for elements that directly neighbor platinum in the third row of the transition metals, namely rhenium, osmium, and iridium.[17–19] A potential advantage of complexes of these metal ions is their structural diversity compared to the typical square planar Pt(II) complexes. The unique molecular architectures of these complexes may enable novel modes of interaction with biomolecular targets.[20] As such, there has been an increasing number of investigations exploiting the attractive chemical properties of these elements for the development of anticancer agents.

In this Perspective, we summarize recent investigations of anticancer agents comprising the elements, rhenium, osmium, and iridium. The scope of this article is restricted to developments within the last six years, describing earlier studies only when necessary to give appropriate context for recent investigations. Additionally, an emphasis is placed on studies that investigated the mechanisms of action and *in vivo* properties of these complexes. Although a number of recent studies have demonstrated the use of rhenium,[21–26] osmium,[27,28] and iridium[29,30] complexes as photodynamic or photoactivated therapeutic agents, these light-activated compounds are beyond the scope of this Perspective. Collectively, the results summarized in this Perspective highlight the valuable anticancer activity of these complexes and demonstrate their growing potential as novel chemotherapeutic agents.



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Justin J. Wilson

Justin Wilson originates from Orange County, California, USA. He obtained his B.S. degree in Chemistry from the University of California, Berkeley (2008), which was awarded with Highest Honors and the Departmental Citation. Justin carried out his Ph.D. studies under the mentorship of Prof. Stephen J. Lippard at the Massachusetts Institute of Technology on the topic of platinum-based anticancer agents. He received the Davison Prize for his thesis, an annual prize awarded for the best inorganic thesis at MIT. Following completion of his graduate studies in 2013, Justin assumed a position as a Seaborg Institute Postdoctoral Fellow at Los Alamos National Laboratory (LANL), where he worked under the guidance of Dr. Eva Birnbaum in the Isotope Production Program. His work at LANL focused on the radiochemistry of therapeutic alpha-emitting radionuclides. Since assuming his independent position as an Assistant Professor of Chemistry and Chemical Biology at Cornell University in 2015, Justin's research efforts have been focused broadly on novel implementations of metals in medicine.

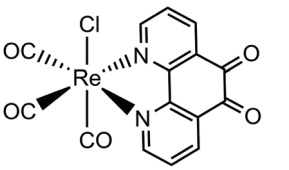
2. Rhenium complexes

Complexes of rhenium have long been neglected as potential anticancer agents. Only recently have a number of investigations arisen that demonstrate the potent anticancer activity of these complexes.[31] Rhenium attains a wide range of oxidation states and, as such, supports a diverse set of different ligand types and coordination geometries. Arguably, the most common structural motif applied in biological systems is the stable Re(I) tricarbonyl core.[32] By leveraging their rich spectroscopic properties, these compounds can be employed for *in vitro* fluorescence and vibrational microscopic imaging.[33,34] Additionally, their facile synthesis can be used to generate a wide range of compounds whose properties can be tuned to maximize biological activity. In addition to Re(I) tricarbonyl complexes, higher oxidation state rhenium compounds have also been explored for their anticancer activity. Efforts in this direction have mainly focused on rhenium compounds containing metal–ligand and metal–metal multiple bonds. Because the advent of rhenium in cancer therapy is still at its infancy, the mechanisms of action of these compounds remain largely unknown. Several review articles summarize the anticancer activity of these complexes but provide little discussion on their mechanisms of action.[31,32,35] In this section, we discuss recent studies that provide insight on the mechanistic aspects of their anticancer activity. We specifically focus our discussion on Re(I) tricarbonyl complexes and rhenium compounds of higher oxidation states that have been disclosed within the last six years.

2.1. Rhenium tricarbonyl complexes

Biological macromolecule-binding studies. The anticancer activity of Re(I) tricarbonyl complexes presumably results from their interactions with intracellular biomolecules. These interactions have been explored for several members of this class of complexes. Because DNA is often the presumed target for metal-based drugs, many of these studies have focused on nucleobase interactions. Early studies on *fac*-$[Re(CO)_3(OH_2)_3]^+$ compounds verified that they can bind covalently to guanine nucleobases *via* substitution of the labile aqua ligands.[36–38] A related diimine compound *fac*-$[Re(bpy)(CO)_3(CH_3CN)]^+$, where bpy = 2,2′-bipyridine, also reacts with guanine to form a covalent adduct *via* substitution of the axial acetonitrile ligand.[39] Although these results suggest that DNA might be an important target for these compounds *in vitro* and *in vivo*, more biologically relevant evidence is required to confirm this hypothesis.

Re(I) tricarbonyl complexes that do not contain labile ligands can non-covalently interact with DNA either through intercalation or groove binding. For example, a 1,10-phenanthroline-5,6-dione Re(I) tricarbonyl complex (**1**) interacts with DNA *via* binding to the minor groove.[40] The free ligand, in contrast, intercalates between DNA base pairs. The cytotoxic activities of this rhenium complex and its free ligand were assessed in glioblastoma, prostate, and breast cancer cell lines. In all cell lines, the free ligand was approximately 10 times more cytotoxic than the corresponding rhenium complex, suggesting that the mode of DNA binding might play an important role in the anticancer activity of these compounds. Similarly, the lipophilic rhenium complexes, **2a** and **2b**, which both bear axial pyridine ligands containing a long alkyl chain, interact with the minor groove of DNA.[41] Molecular docking studies confirm that the alkyl chain plays a key role in DNA binding because it allows for the complex to attain the proper orientation for fitting within the minor groove. The DNA-binding abilities of these complexes correlate with their cytotoxic activity in B and T cell lymphoma cancer cells. Complex **2b**, which is more hydrophobic and binds more strongly to DNA than **2a**, is also more cytotoxic. These studies illustrate the role that non-covalent DNA-binding interactions can have on the activity of Re(I) tricarbonyl complexes.



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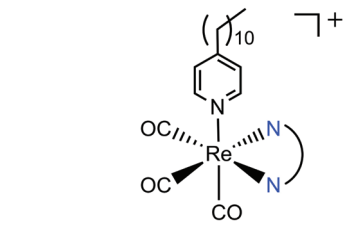
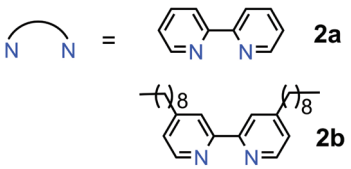
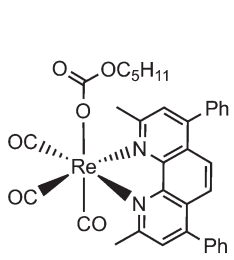




Re(I) tricarbonyl complexes bearing axial alkylcarbonate ligands, such as complex **3**, were reported to bind non-covalently to DNA *via* intercalation.[42] However, the hallmark features of intercalative DNA binding, namely hypochromism and high binding affinity,[43] are absent. For example, the binding constants for these rhenium alkylcarbonate complexes are on the order of 10^4 M^{-1}, whereas the binding constant of the well-established DNA intercalator, ethidium bromide, is on the order of 10^7 M^{-1}.[44] Thus, these complexes are most likely interacting with DNA *via* an alternative mechanism, such as major or minor groove binding. Covalent binding *via* loss of the axial alkycarbonate ligand is another possibility that was not explored in this study. A related series of rhenium diimine complexes bearing sulfonato and carboxylato axial ligands exhibit potent anticancer activity in a series of breast cancer cell lines.[45] The most effective complex, **4**, is active in the nanomolar range. Like the alkylcarbonate rhenium complexes, these compounds interact weakly with DNA, as reflected by

their low binding constants that range from 10^4–10^5 M^{-1}. Therefore, a groove-binding mechanism is most likely operative. Other related Re(I) tricarbonyl complexes also bind to DNA in a similar manner.[46–51] These results highlight the potential of DNA binding as a mechanism of action, but the importance of this target needs to be further verified *in vitro* and *in vivo*.



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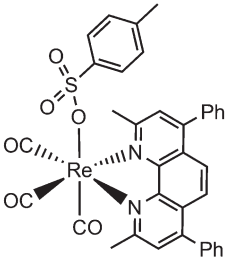

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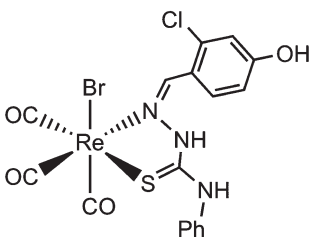
The possibility that these complexes target proteins has also been explored. This hypothesis is bolstered by the fact that histidine residues have been covalently modified with Re(I) tricarbonyl complexes as a well-established strategy to probe long-distance electron transfer pathways in proteins.[52,53] Furthermore, several proteins containing covalent Re(I) modifications have been crystallized. In almost all cases, the Re(I) center is covalently bound to a histidine residue,[54–61] reflecting the high affinity of these complexes for nitrogen donor ligands. More recently, co-crystallization experiments with hen egg white lysozyme (HEWL) and the *fac*-$[Re(CO)_3(H_2O)_3]^+$ complex revealed the presence of rhenium covalent modifications on the glutamate, aspartate, and C-terminal carboxylate groups, suggesting that these residues may also be suitable protein side chain targets.[62]

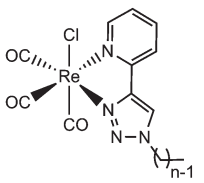
Modifications of the supporting ligands of Re(I) tricarbonyl complexes can be used to target specific proteins. For example, the Re(I) thiosemicarbazone complex (**5**) targets the estrogen receptor α (ERα).[63] Estrogen receptors are overexpressed in hormone-dependent breast cancer cells[64] and can be targeted by therapeutic agents to increase cancer cell selectivity.[65–67] Among a series of related Re(I) thiosemicarbazones, complex **5** binds to ERα with the highest affinity, illustrating the potential of rhenium thiosemicarbazones as protein-targeting anticancer agents. Further investigations on how ligand modifications drive selectivity for protein targets presents an interesting line of research for the development of rhenium-based anticancer agents.



5

Cellular localization and mechanisms of uptake. Although the application of Re(I) tricarbonyl complexes as anticancer agents is a relatively recent development, their use as molecular imaging agents has been explored in much greater detail.[34,68–72] Their long-lived triplet metal-to-ligand charge transfer (^{3}MLCT) luminescence can be exploited for fluorescence microscopy in living cells.[73–76] For some of these complexes, however, the luminescence quantum yield is too low to be useful for fluorescence imaging. An alternative detection method is vibrational microscopy, which probes the high-energy C≡O stretching modes of these complexes. The C≡O stretch occurs in a region that is transparent *in vivo*; no endogenous biomolecules absorb in the C≡O stretching region between 1900–2100 cm^{-1}. The localization of these compounds can, therefore, be detected directly *via* FTIR or Raman spectromicroscopy. These spectroscopic methods provide a useful means of probing compound uptake and intracellular localization. In combination with their therapeutic anticancer effects, the luminescence and vibrational imaging capabilities of these compounds make them interesting small-molecule theranostic agents,[77] complexes that possess therapeutic and diagnostic capabilities.

The power of vibrational microscopy techniques was demonstrated in a study on the anticancer active Re(I) tricarbonyl complexes bearing pyridyl–triazole ligands with extended alkyl chains (**6a–6c**).[78] Cytotoxicity measurements in breast cancer cells indicate that compound **6c** with the longest alkyl chain is the most potent compound, whereas compound **6a** with the shortest alkyl chain is the least active. Accordingly, FTIR spectromicroscopy revealed that complex **6a** was poorly taken up by the cells as no detectable vibrational signature of this compound was observed. In contrast, high concentrations of **6c** were found in these breast cancer cells. Therefore, the cytotoxicity of this class of compounds correlates with the cellular uptake. In turn, the cellular uptake appears to depend on the lipophilicity of the complex because the longer alkyl chain compounds were taken up more effectively. This result suggests that a passive diffusion mechanism of cell uptake, which is largely dependent on compound lipophilicity,[79,80] may be important for these compounds.

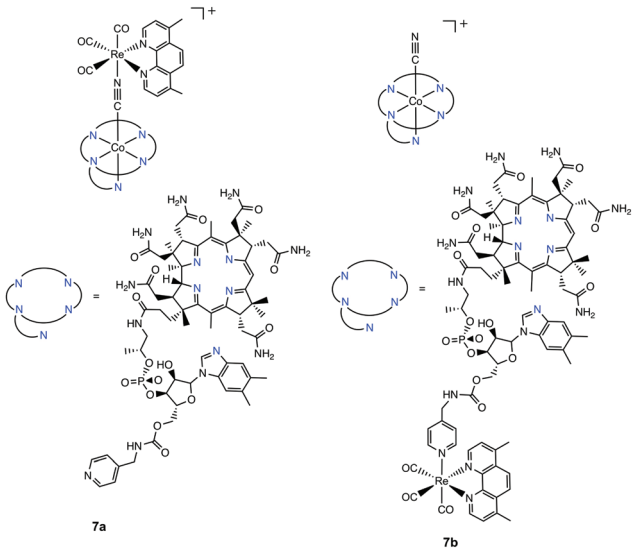


n = 4 (**6a**), 8 (**6b**), 12 (**6c**)

6a–6c

Vibrational microscopy has also been employed to determine intracellular distribution of Re(I) tricarbonyl complexes. For example, the vitamin B_{12}-conjugated Re(I) tricarbonyl complexes, **7a** and **7b**, were explored in this capacity.[81] These compounds, which exploit vitamin B_{12} to increase cel-

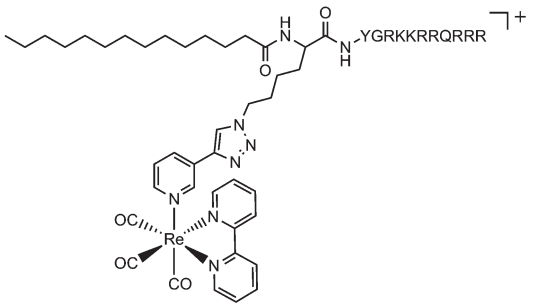
lular uptake *via* cobalamin transporters, exhibit cytotoxicity levels in the micromolar range in prostate cancer cells. Imaging their cellular uptake and intracellular distribution, however, is hindered by the fact that the vitamin B_{12} cofactor quenches the luminescence emission of the Re(I) tricarbonyl center. In this case, FTIR spectromicroscopy was successfully implemented to determine cell uptake and intracellular localization of these compounds. Complex **7b** was found to localize in the cytoplasm and perinuclear regions of the cell. Lipid CH_2 stretching modes were also measured because they are typical IR markers for organelles that are rich in membranes, such as the Golgi apparatus and the endoplasmic reticulum (ER).[82] Colocalization of the C≡O stretching mode with the lipid CH_2 modes was poor, indicating that this compound does not accumulate in the Golgi or ER.



7a **7b**

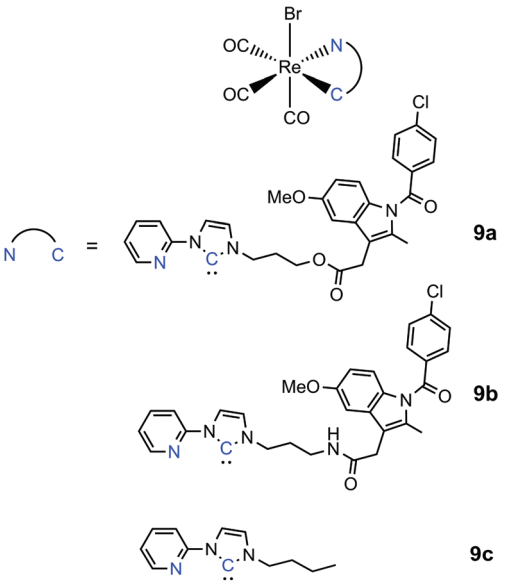
Additional cellular uptake studies have focused on Re(I) tricarbonyl complexes that bear receptor-targeting groups. For example, glucose- and fructose-conjugated compounds have been designed to exhibit increased cellular uptake in cancer cells *via* the overexpressed glucose transporter proteins (GLUTs).[83–85] The glucose-functionalized rhenium compounds do not have increased overall uptake compared to the non-functionalized molecules; however, their uptake is reduced in the presence of glucose,[86,87] which saturates the glucose transporters. This result suggests that their uptake is at least partly mediated by these transporters. Similarly, the fructose-conjugated compound is taken up through fructose transporters, and like the glucose analogues, it has lower uptake and cytotoxicity compared to the non-functionalized compound.

The implementation of peptides is an alternative method for improving cellular uptake.[88] For example, a Re(I) tricarbonyl complex (**8**) conjugated to the myristoylated HIV-1 Tat cell-penetrating peptide sequence (YGRKKRRQRRR) exhibits enhanced cellular uptake and cytotoxicity compared to the non-functionalized derivative.[89] HIV-1 Tat is a membrane translocation peptide sequence,[90] whereas myristic acid is a fatty acid.[91] Both of these compounds have been used to increase the cellular uptake of conjugated anticancer agents.[92,93] Therefore, the use of cell-penetrating peptides appears to be an effective strategy for increasing cellular uptake of rhenium-based anticancer agents.

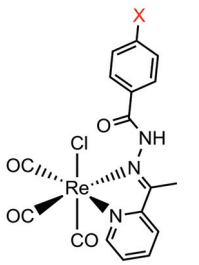


8

Cell death modes and insight into mechanisms of action. Once accumulated in the cell at sufficient concentrations, Re(I) tricarbonyl complexes can interact with biological targets to induce their cytotoxic activity. This process has been investigated for several members of this class of compounds. Within this class, we can broadly categorize them based on whether they bear a bidentate and monodentate ligand, species that are often referred to as [2 + 1] complexes,[94] or a single tridentate ligand. Whereas the [2 + 1] compounds are susceptible to ligand substitution reactions *via* displacement of the monodentate ligand,[95] compounds bearing tridentate ligands are generally inert.[96] Among the former complexes, the lability of the monodentate ligand is a contributing factor to their cytotoxic activity. For example, this correlation is observed for the Re(I) tricarbonyl complexes bearing bidentate indomethacin-based ligands (**9a–9c**).[97] Indomethacin is a non-steroidal anti-inflammatory drug (NSAID), which has been shown to potentiate the anticancer properties of metal-based drug candidates.[98–100] The cytotoxicity of these compounds is dependent on the nature of the axial ligand, which was varied to be either chloride, bromide, iodide, or thiocyanate. These studies revealed that complexes bearing more labile axial ligands, namely chloride and bromide, are more cytotoxic in pancreatic cancer cell lines. This result suggests that the formation of covalent bonds, which is gated by axial ligand substitution, is important for the biological activities exhibited by complexes of this type. In addition, complexes **9a–9c** arrest the G2/M phase of the cell cycle and inhibit the protein Aurora-A kinase, an enzyme that is involved in a number of oncogenic pathways.[101]



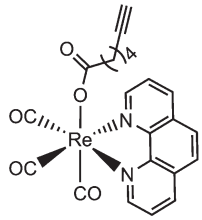
Another class of Re(I) tricarbonyl complexes, which bears bidentate hydrazine ligands (**10a** and **10b**), was investigated for anticancer activity.[102] These complexes were mildly cytotoxic as characterized by IC_{50} (50% growth inhibitory concentration) values ranging from 16 to 60 μM in H460 lung cancer cells. These agents induce cell death *via* an apoptotic mechanism and decrease intracellular levels of reactive oxygen species (ROS), suggesting that they also possess antioxidant properties.



X = Cl (**10a**), NO_2 (**10b**)

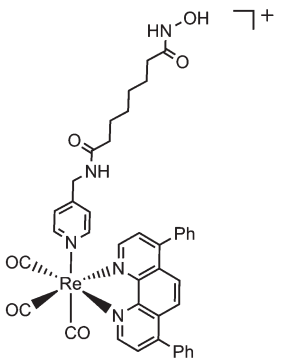
10a and 10b

In a more recent study, a Re(I) tricarbonyl phenanthroline compound bearing a labile carboxylate axial ligand (**11**) was tested for its reactivity with HEWL, which was chosen as a model representative protein.[103] Mass spectrometry analysis confirmed the formation of covalent rhenium-HEWL adducts *via* the loss of the axial carboxylate ligand. The luminescent properties of these rhenium complexes were also leveraged to image **11** in cervical cancer cells, revealing selective accumulation of this compound in the mitochondria. The production of ROS upon treatment with **11** was also observed. These results suggest that further interest should be placed on evaluating potential protein-based targets of Re(I) tricarbonyl anticancer agents.



11

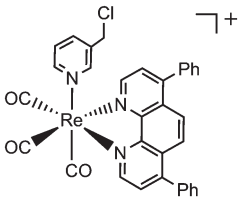
A significant amount of work has also been carried out on the investigation of Re(I) tricarbonyl complexes that have relatively inert axial pyridine ligands. For example, compound **12**, which bears a histone deacetylase (HDAC) inhibitor conjugated to the axial pyridine, was investigated as an anticancer agent.[104] HDAC inhibitors are already established as therapeutic drug candidates. The enzyme, HDAC, regulates gene expression by catalyzing the removal of the acetyl groups on histones, and its inhibition leads to events such as induction of cell death, reduction of angiogenesis, and immune responses.[105] When this HDAC inhibitor is coordinated to the axial position of the Re(I) tricarbonyl complex, mitochondrial localization is observed and cell death is induced *via* caspase-independent paraptosis, a pathway that gives rise to enlarged mitochondria, production of ROS, and cytoplasmic vacuolization.[106] Due to the large number of rhenium tricarbonyl complexes that induce apoptosis,[107–112] the paraptotic mechanism of complex **12** could be a valuable characteristic for potent *in vivo* activity and overcoming cisplatin-resistance.



12

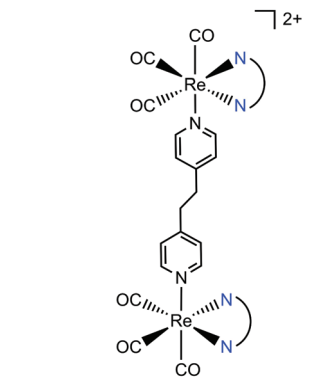
The axial pyridine ligands also provide a position for functional modification of these Re(I) anticancer agents. For example, the use of an electrophilic picolyl chloride ligand was employed to develop a Re(I) tricarbonyl complex (**13**) that is immobilized in the mitochondria upon reaction with nucleophilic thiols.[113] Complex **13** exhibits sub-micromolar toxicity in lung, cisplatin-resistant lung, and cervical cancer cell lines. Confocal fluorescence microscopy studies confirmed the localization of **13** in the mitochondria. Its immobilization in this organelle was verified by a series of attempted washes with phosphate-buffered saline (PBS). Unlike the pyridine analogue, complex **13** was retained in the mitochondria after these

thorough washes. Mechanistically, this compound inhibits mitochondrial respiration, increases intracellular ROS levels, and triggers caspase-dependent apoptosis to a greater extent than the non-immobilized complexes.



13

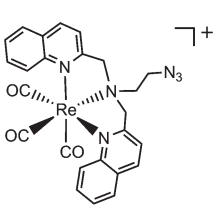
Dinuclear rhenium compounds, tethered *via* axial pyridine ligands, have also been explored.[114] The dinuclear compounds, **14a** and **14b**, are more cytotoxic than their mononuclear forms. When normalized per rhenium atom, however, compound **14b** is only 0.8 to 2.3 times as toxic as its mononuclear analogue, indicating that the additional rhenium center only contributes to the activity of these complexes in an additive, rather than synergistic, manner. In contrast, **14a** is several orders of magnitude more potent than its mononuclear analogue in a variety of cancer cell lines, demonstrating that the dinuclear structural motif gives rise to the activity of this compound. Additionally, compound **14b** is more active than **14a**, a property that can possibly be attributed to the higher lipophilicity of **14b**. Notably, the intracellular localization and mechanism of induced cell death of these compounds are different. Compound **14b** accumulates in the mitochondria and gives rise to paraptotic cell death, whereas **14a** localizes to the lysosome and induces caspase-independent apoptosis. These results may guide the development of the structure–activity relationships (SARs) for Re(I) tricarbonyl compounds. This study demonstrates that subtle structural and lipophilic changes can dramatically alter the mechanisms of action and potency of these complexes.



14a

14b

As mentioned above, inert Re(I) tricarbonyl complexes that bear tridentate ligands may also possess anticancer activity. For example, complex **15**, which bears a tridentate bisquinoline ligand, was evaluated for anticancer activity in human breast, osteosarcoma, and hepatocellular cancer cells lines, revealing IC_{50} values as low as 6 μM in breast cancer cells.[115] When **15** is administered at low concentrations, apoptosis induction is not observed. Rather, these concentrations induce ROS production and decrease cellular respiration while upregulating glycolytic pathways. In contrast, a related rhenium complex, which bears a tridentate bisphenanthridine ligand (**16**), activates both extrinsic receptor-mediated and intrinsic mitochondria-dependent apoptotic pathways.[116] The use of this compound on cells that overexpress the anti-apoptotic factor Bcl-2[117] had no effect on its efficacy and mechanism of cell death. Furthermore, this compound shows comparable activity in daunorubicin-resistant, vincristine-resistant, and wild-type cancer cell lines, demonstrating its potential value for the treatment of refractory forms of cancer.



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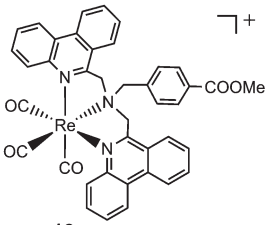


16

Inert complexes bearing organometallic cyclopentadienyl (Cp) ligands, rather than nitrogen donor ligands, also exhibit anticancer activity. The potent anticancer drug, doxorubicin, was conjugated to a Cp ligand on Re(I) tricarbonyl complexes (**17a** and **17b**) and tested against HeLa cervical cancer cells.[118] Although the established target of doxorubicin is nuclear DNA,[119] confocal fluorescence microscopy studies of HeLa cells treated with **17a** revealed that this compound localizes to the mitochondria (Fig. 1). However, intracellular rhenium quantification using inductively coupled plasma mass spectrometry (ICP-MS), showed substantial accumulation in the nucleus, comprising approximately 60% of the total internalized rhenium, whereas only about 30% was found in the mitochondria. The lack of luminescence observed in the nucleus compared to the mitochondria could be a consequence of quenching of the doxorubicin fluorescence by π-stacking between the nucleotide base pairs. Complex **17a** possesses potent anticancer activity, acting in a manner that depolarizes the mitochondrial membrane potential and induces apoptosis. These results demonstrate how the presence of the metal pharmacophore has the ability to alter the cellular distribution of established drugs.

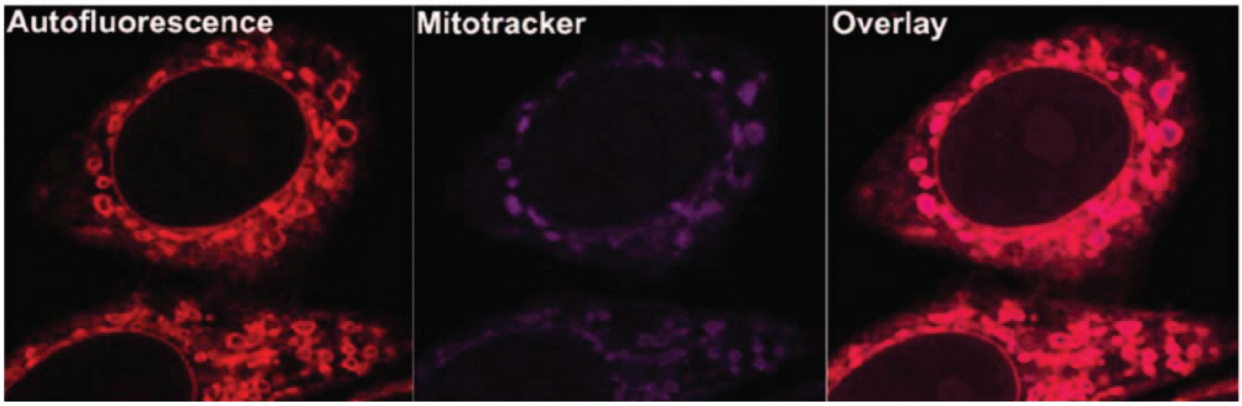
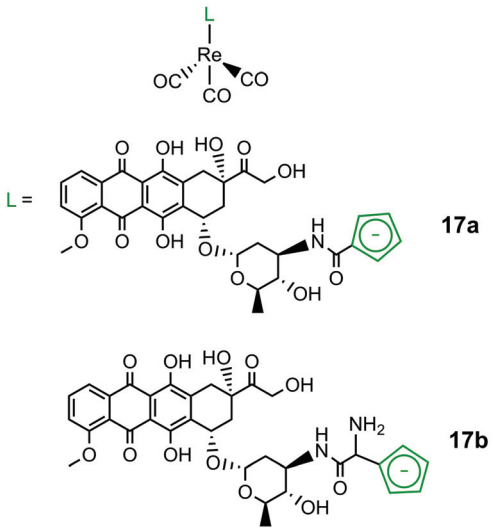
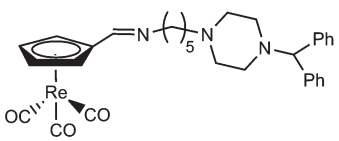


Fig. 1 Confocal fluorescence microscopy images of HeLa cells treated with compound **17a** (left), MitoTracker dye (middle), and overlay of **17a** and MitoTracker (right) shows localization to the mitochondrial membrane. The "autofluorescence" panel shows the emission of compound **17a**. Reprinted with permission from ref. 118. Copyright (2016), with permission from John Wiley and Sons.

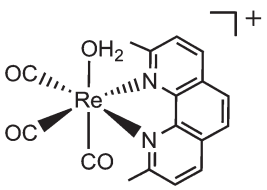


The utility of the inert Cp ligand as an attachment point for biologically active molecules was further investigated. Namely, an inhibitor of glycogen synthase kinase 3β (GSK-3β), a protein involved in glycogen metabolism,[120] was conjugated to the Cp ligand of a Re(I) tricarbonyl complex (**18**).[121] The activity of the enzyme, GSK-3β, is implicated in several forms of cancer and neurodegenerative diseases, thus rendering it an important drug target.[122] The cytotoxic activity of the rhenium–inhibitor conjugate was greater than the inhibitor alone, suggesting that the Re(I) tricarbonyl core potentiates the activities of this class of complexes. Computational docking studies at the binding site of GSK-3β confirm that the organic ligand and its rhenium conjugate fit into the active site with favorable binding energies.



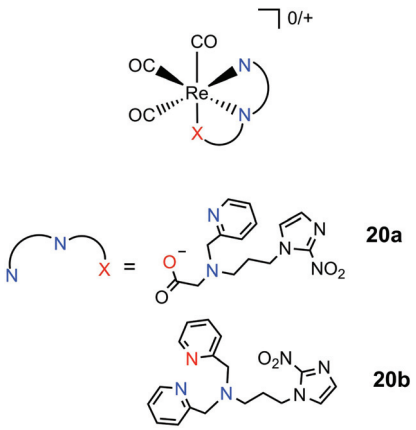
18

***In vivo* studies.** The majority of studies investigating the anticancer activities of Re(I) tricarbonyl complexes have focused solely on their *in vitro* properties. Fewer studies have explored the *in vivo* applicability of this class of compounds. There exists a significant theranostic potential for this class of compounds by virtue of the widespread availability of the isotope, technetium-99m (^{99m}Tc), which can be used for *in vivo* imaging *via* single-photon emission computed tomography (SPECT).[35] In principal, the similarity in the chemistry of rhenium and technetium may enable the use of ^{99m}Tc analogues as companion diagnostics for novel rhenium-based drug candidates. We have verified the feasibility of this application in our investigations of complex **19**.[123] Prior to *in vivo* studies, a series of these complexes, which each contain different diimine ligands, were explored for their anticancer activity in a range of cancer cell lines. The most potent complex, **19**, exhibited anticancer activity at concentrations lower than 5 μM. Furthermore, the ^{3}MLCT emission of this compound was used to probe the mechanisms of cellular uptake and intracellular distribution. These studies revealed that this compound is taken up *via* an energy-dependent mechanism, which is partially mediated by endocytosis, and that it localizes to the endosome and lysosomes. The mechanism of cell death induced by these compounds does not canonically fit into any of the established categories, such as apoptosis and necrosis, suggesting that a novel mode of action may be operative. Based on these promising *in vitro* data, the *in vivo* biodistribution of this compound and its ^{99m}Tc analogue were investigated in C57BL/6 mice. The biodistribution was evaluated at three time points, revealing similar organ uptake patterns between the two compounds. This similarity bodes well for the possibility of using ^{99m}Tc as a companion diagnostic agent with anticancer-active Re(I) tricarbonyl complexes.

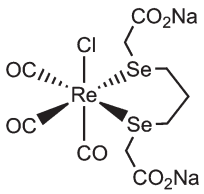


19

A number of other studies have also demonstrated the suitability of using ^{99m}Tc as an imaging analogue for rhenium.[124–127] In a recent example, Re(I) tricarbonyl complexes bearing hypoxia-targeting nitro-imidazole ligands (**20a** and **20b**) were explored in this context.[128] The nitro-imidazole group is well known to undergo selective reduction under hypoxic conditions to form electrophilic species that become trapped by biological nucleophiles.[129] Accordingly, compounds **20a** and **20b** were found to accumulate in much higher concentrations in hypoxic cells compared to normoxic cells. Although the mechanism of uptake was not explored for these compounds, this example shows how conventional drug-targeting strategies can be applied to these rhenium anticancer agents. The ^{99m}Tc analogue of the hypoxia-targeting rhenium complex, **20b**, was imaged in mice bearing renal cell carcinoma SK-RE-52 xenografts. This compound effectively localized in hypoxic tumor tissues, further illustrating that ^{99m}Tc can be swapped for rhenium without significantly altering its biological properties.

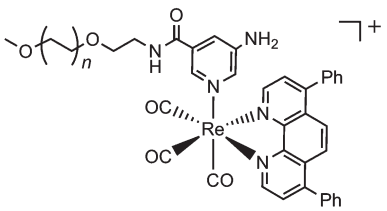


In contrast to the relatively common ^{99m}Tc imaging studies discussed above, there have been only several investigations on the *in vivo* antitumor activity of Re(I) tricarbonyl complexes.[130–134] For example, the diselenoether Re(I) complex, **21**, was one of the few compounds to be evaluated for *in vivo* activity.[131,133,134] This compound, which was designed to combine the cytotoxic effects of rhenium with the antiproliferative effects of selenium,[135] inhibits tumor growth in mice bearing MDA-MB-321 breast cancer xenografts. The tumor volume of mice treated with this compound was reduced by 43% relative to those administered with the saline control. Furthermore, no weight loss was observed in treated mice, reflecting the minimal *in vivo* toxic side effects of this compound.



21

In vivo studies involving zebrafish have also been explored for this class of compounds.[136] Complex **22**, which is tethered to a water-solubilizing and biocompatible poly(ethylene)glycol (PEG) chain,[137,138] exhibits micromolar toxicity in HeLa cells. In contrast, a structural analogue that does not contain the PEG group is more cytotoxic by a factor of 2. To see if these results translate *in vivo*, both **22** and its PEG-free analogue were evaluated for toxicity in zebrafish embryos. The PEG-free complex gave rise to a significant extent of developmental defects in these embryos in contrast to complex **22**, which was essentially non-toxic. These results indicate that the PEG group acts to decrease toxic effects of rhenium anticancer agents, thereby providing a rational strategy for improving their toxicological properties.[139] Furthermore, they validate the use of zebrafish as a model for evaluating metal-based anticancer agents.

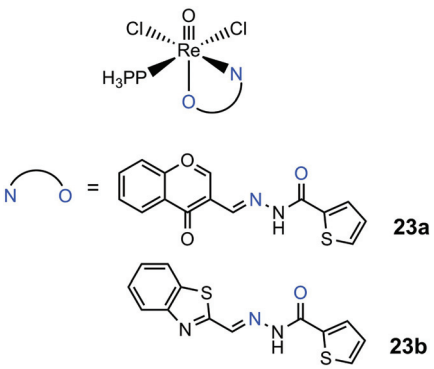


n = ca. 112

22

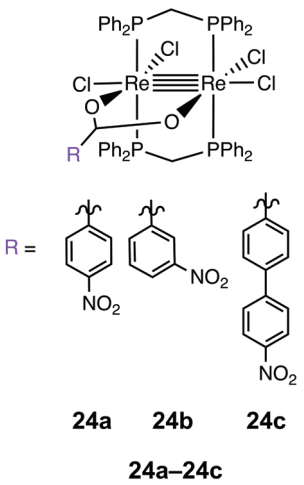
2.2. Higher oxidation state complexes

Rhenium can attain a wide range of oxidation states in addition to the +1 state that was discussed above. The investigation of complexes of rhenium in higher oxidation states as anticancer agents, however, has been far less explored. In this respect, bidentate Re(V) oxo complexes bearing Schiff base ligands **23a** and **23b** were investigated for their ability to interact with DNA.[140] These compounds interact with DNA weakly, as characterized by binding constants that fall near 10^4 M^{-1}. Additionally, they cleave DNA, indicating that they could be useful for further *in vitro* anticancer activity studies.



Dirhenium(III) complexes, which contain Re–Re quadruple bonds, are another class of high-valent rhenium compounds that exhibit anticancer activity.[141,142] In a recent study, a series of mixed-valent dirhenium(III,II) complexes with bridging nitro-

benzoate ligands (**24a–24c**) were investigated for anticancer activity.[143] When tested in MCF-7 breast cancer cells, these complexes exhibited IC_{50} values near 60 μM, indicating that they are moderately active. They induced overall shrinkage of the cancer cells and blebbing of the nuclear membrane. Additionally, the cell cycle was arrested in the G0/G1 phase upon treatment with these compounds. Notably, no differences in cytotoxicity or cell cycle arrest patterns were observed between **24a–24c**, indicating that the location of the nitro group on the bridging ligand does not significantly affect their potency. The precise molecular target and mechanism of action of these complexes, however, remains unknown.



24a **24b** **24c**

24a–24c

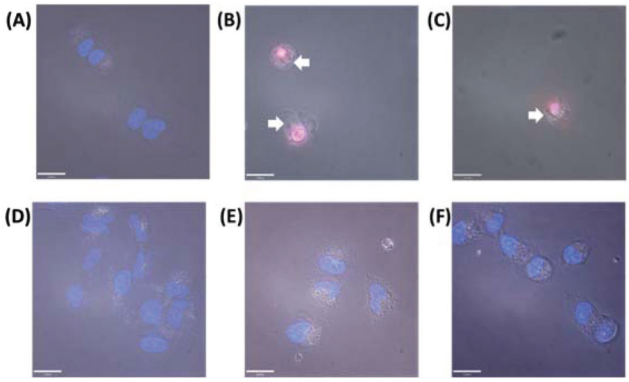
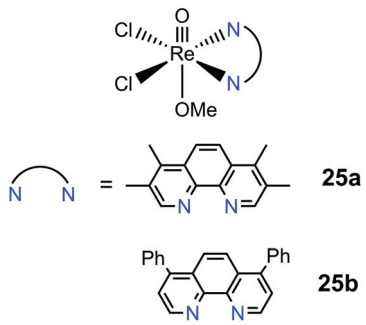


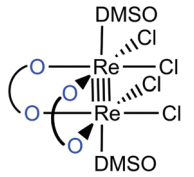
Fig. 2 Fluorescence microscopy images of A549 cells stained with Hoechst 33258 and propidium iodide, and treated with (A) growth media only, (B) 20 μM **25a** for 12 h, (C) 20 μM **25b** for 12 h, (D) necrostatin-1, (E) necrostatin-1 and **25a**, or (F) necrostatin-1 and **25b**. Arrows indicate signs of membrane disintegration. Scale bar = 21 μm. Reprinted with permission from ref. 144. Copyright (2015), with permission from American Chemical Society.

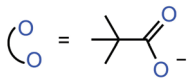


***In vivo* studies.** The Re(v) oxo compounds (**25a** and **25b**) were investigated in a panel of cancer cell lines, where they exhibit potent anticancer activities with IC_{50} values ranging from 45 to 695 nM.[144] These compounds induce a relatively unique form of cell death known as necroptosis. Necroptosis is a programmed form of cell death where the active degradation of mitochondrial, lysosomal, and plasma membranes occurs.[145] Evidence for necroptosis was supported by experiments that show necrostatin-1, a known inhibitor of necroptosis,[146,147] inhibits cell death induced by these compounds (Fig. 2). Further aspects of their cell death mechanism include the generation of intracellular ROS, the depolarization of the mitochondrial membrane potential, and the stalling of cells in the G1 phase of the cell cycle. Due to the potent *in vitro* activity and a unique form of cell death, these complexes were studied for their effects in healthy mice. The minimal *in vivo* toxicity of both **25a** and **25b** was evidenced by the observation that C57BL/6 mice treated with 36 mg kg^{-1} of the compound for 6 days showed no weight loss. Despite the promising features of these compounds and the lack of adverse side effects in healthy mice, *in vivo* anticancer studies were not carried out. Future studies of these compounds will hopefully provide further information about the mechanism of cell death *in vivo*.

As discussed above, dirhenium compounds are another class of high-valent rhenium anticancer agents. The dirhenium compound, **26**, was recently evaluated for *in vivo* anticancer activity.[148] This compound was found to inhibit tumor growth by 60% in rats implanted with Guerin's carcinoma T8 cell tumor xenografts. When administered in conjunction with cisplatin, **26** was more effective; the total tumor reduction was upwards of 85%, suggesting that this compound acts in a synergistic manner with cisplatin. Furthermore, the combination therapy of **26** and cisplatin did not cause nephrotoxicity, indicating that this treatment strategy is better tolerated than cisplatin alone. To evaluate the possibility of DNA as a target, the interactions of **26** with this biomolecule were investigated. These studies demonstrated that **26** binds DNA, most likely forming covalent interstrand crosslinks, and also cleaves plasmid DNA. To further improve the formulation of compound **26**, it was encapsulated in liposomes.[149] Liposomes, simple phospholipid vesicles, are commonly used as drug delivery vehicles to increase the cellular uptake and selectivity for cancer cells.[150] For example, liposomal formulations of cisplatin have successfully been implemented to reduce the toxic side effects of this drug.[151] To capitalize on their synergistic properties, compound **26** and cisplatin were encapsulated

together in liposomes. The liposomal formulation acts to suppress the hydrolysis of **26** and to decrease the toxic side effects of cisplatin. As anticipated, the implementation of these compounds co-encapsulated in the liposomes gave rise to more effective and less toxic *in vivo* anticancer activity. The enhanced activity might partially be attributed to the possible formation of a Re–Pt species in the liposome. This study highlights the value of exploring synergistic effects of rhenium anticancer agents with established drugs and also the potential use of novel drug delivery vehicles to improve their activity.





26

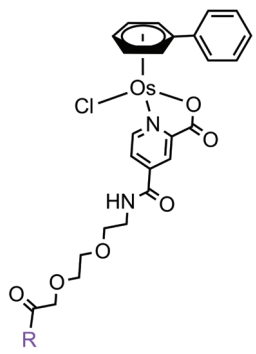
3. Osmium complexes

Complexes of osmium have received far less attention than those of ruthenium with respect to their utility in medicinal chemistry. The general aversion to osmium is partially motivated by the well-known toxicity of OsO_4.[152] In different chemical forms, however, osmium may possess properties that are useful for anticancer activity. In comparison to ruthenium, osmium prefers higher oxidation states and is generally more inert. Furthermore, the success of previously investigated ruthenium anticancer agents, such as NAMI-A[153,154] and KP1019,[155,156] which have progressed to clinical trials, provides a rational starting point for exploring the corresponding osmium analogues. The implementation of novel ligands and the diverse coordination geometries and oxidation states of osmium has led to the development of a wide range of osmium-based anticancer agents within the last six years. Despite the expansion in the design and application of these compounds as anticancer agents, their mechanisms of action remain largely elusive. Whereas some review articles have illustrated the development of osmium anticancer agents,[157,158] a comprehensive analysis on their modes of cell death is less discussed. In this section, we review efforts to understand the mechanisms of action of osmium anticancer agents, as categorized based on ligand type and formal oxidation state.

3.1. Osmium arene complexes

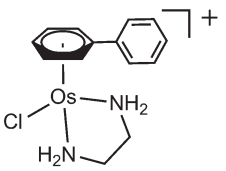
Biological macromolecule-binding studies. The most thoroughly investigated class of osmium anticancer agents comprise the Os(II) arene "piano-stool" complexes that have the general formula $[(arene)Os(NN)X]^+$, where NN is typically a bidentate nitrogen donor and X is a halogen or pseudo-halogen.[159,160] These osmium compounds were initially designed to match the corresponding ruthenium analogues, which exhibit potent anticancer activity and bind covalently to DNA.[158,161] Like the ruthenium compounds, the Os(II) arene compounds bind covalently to DNA as well.[162] However, attempts to correlate the activity of the osmium complexes to their DNA-binding properties have been somewhat tenuous.

Recent studies in this regard have been reported on complex **27**, a piano-stool complex bearing a bidentate picolinate ligand conjugated to octreotide, a somatostatin receptor-binding cyclic peptide.[163] The somatostatin receptor, which is overexpressed in a wide range of cancers, is a valuable target for cancer-selective therapeutic agents.[164] This compound forms covalent adducts with the DNA oligonucleotide, 5′-dCATGGCT, at the guanine sites. Despite its favorable DNA-binding properties, however, it was inactive against breast cancer cells. Conversely, a related ruthenium–octreotide conjugate, which did not interact with DNA, induced cytotoxic effects in breast cancer cells. This result suggests that the mechanism of action of these octreotide-conjugates does not stem from their abilities to bind to DNA. Although complex **27** was not tested further, the activity of the ruthenium complex was potentiated by overexpression of the somatostatin receptor and inhibited by saturation of the receptor with excess octreotide. This result confirms that the cellular uptake of these metal–octreotide conjugates is mediated by the somatostatin receptor and incorporation of the metal center does not unfavorably alter the uptake properties of octreotide.



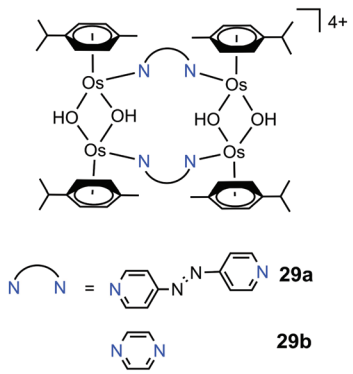
27

To further explore DNA as a potential target, the interaction of the Os(II) arene complex, **28**, with a short self-complementary 12-mer DNA oligonucleotide was investigated with ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS).[165] As expected, these studies revealed covalent binding of this complex to the sole guanine nucleobase. A more novel discovery, however, was that this complex also binds covalently to a cytosine nucleobase, an uncommon phenomenon for metal-based drugs. This result highlights that these osmium complexes may exhibit DNA-binding properties that are distinct from the platinum-based drugs.



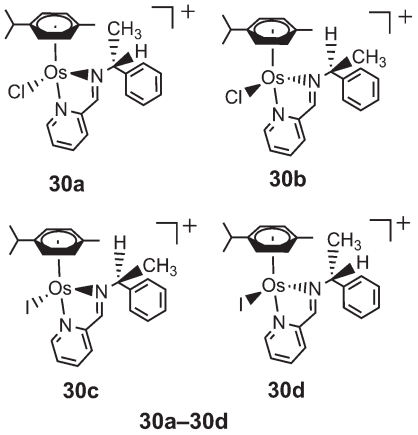
28

Anticancer active tetranuclear Os(II) arene complexes were also investigated for their DNA-binding properties.[166] The osmium centers in these complexes were linked either by a 4,4′-azopyridine (**29a**) or a pyrazine moiety (**29b**). Complex **29a** exhibited 2-fold greater activity in ovarian and lung cancer cells in comparison to **29b**. Because supramolecular metal clusters are known to bind to DNA,[167,168] the interactions of **29a** and **29b** with calf thymus DNA (ct-DNA) were evaluated. Both of these highly charged clusters bind ct-DNA, inducing its condensation. The interactions of these complexes with pBR322 plasmid DNA were further studied with atomic force microscopy (AFM). Both complex **29a** and **29b** form adducts with the plasmid DNA. Complex **29a** is more toxic as compared to **29b**, a property that may be attributed to the larger induction of DNA condensation caused by **29a**.



Computational studies on Os(II) picolinate compounds, [(η^6-arene)Os(pic)Cl] (η^6-arene = benzene, biphenyl, or *p*-cymene and pic = 2-picolinic acid) have been carried out to assess the stability of these species with respect to aquation and nucleobase ligand substitution.[169] Consistent with earlier experimental results,[170] the osmium compounds are predicted to be more stable and resistant to aquation than their ruthenium analogues and to also kinetically prefer binding to 9-ethylguanine (9-EtG) over 9-ethyladenine (9-EtA). These calculations indicate that the arene ligand plays a minor role in the nucleobase binding properties of these complexes. Evidently, the differing arenes significantly alter the steric interactions associated with ligand substitution of these complexes. For example, the compound containing the most hindered arene, *p*-cymene, also had the slowest nucleobase substitution rates. Although these results effectively demonstrate how computational studies can predict reactivity patterns, the extrapolation of their conclusions to highly complex biological systems should be taken with caution.

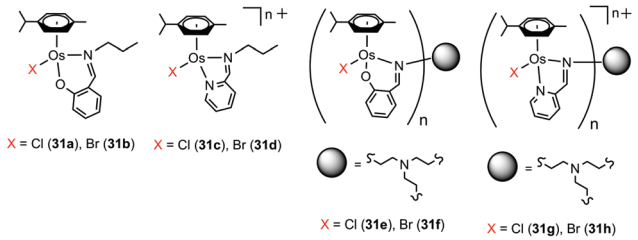
A set of osmium *p*-cymene diastereomers bearing iminopyridine ligands (**30a–30d**) were screened for activity in ovarian cancer cells.[171] The activity of these complexes is primarily dictated by the halide ligand. For example, the iodido complexes, **30c** and **30d**, are over 20-fold more active than the chlorido complexes, **30a** and **30b**, but the diastereomeric pairs are equally active. Based on their potent activity, the iodido complexes were screened against the NCI-60 (National Cancer Institute) cell line panel.[172,173] In this panel, the relative efficacy of the complexes in 60 types of cancer cells was determined. Using the NCI COMPARE algorithm,[174] the varying activities of these complexes in the NCI-60 panel were correlated to other known anticancer drugs in the NCI database. The algorithm results showed a strong correlation between the complexes and the anti-microtubular drug, vinblastine sulfate. Whereas vinblastine operates by inhibiting polymerization of tubulin,[175] complexes **30c** and **30d** did not. This result suggests that these complexes operate *via* an alternative mechanism, despite displaying a similar spectrum of activity as vinblastine.



30a–30d

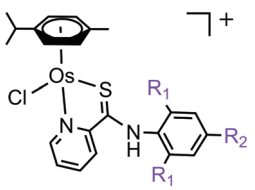
Although now a well-recognized phenomenon,[176] the ability of metal-based anticancer agents to target proteins remains a relatively understudied topic, especially for osmium complexes. In this context, a series of mononuclear (**31a–31d**) and trinuclear (**31e–31h**) Os(II) arene complexes bearing either pyridyl imine or phenoxy imine ligands exhibit potent anticancer activity in osteosarcoma and cisplatin-resistant ovarian cancer cell lines in a manner that is mediated by protein-based targets.[177] These complexes are more cytotoxic in the osteosarcoma cancer cells. Among them, compound **31b** is the most active complex with an IC_{50} value of 2.0 μM, which is comparable to the IC_{50} value of cisplatin (1.5 μM). The phenoxy imine complexes (**31a**, **31b**, **31e**, and **31f**) are more cytotoxic than the pyridyl imine complexes (**31c**, **31d**, **31g**, and **31h**). Additionally, for both the trinuclear and mononuclear species, the bromido complexes (**31b**, **31d**, **31f**, and **31h**) are more active than the chlorido complexes, indicating that substitution kinetics of this position are important for mediating the activities of these compounds. Further studies were performed to compare these complexes with similar ruthenium-

based compounds. For instance, many Ru(II) drugs inhibit topoisomerase I through DNA intercalation.[178] Thus, the structurally analogous Os(II) complexes (**31b**, **31e**, and **31f**) were tested for topoisomerase I inhibition. This enzyme is a viable drug target for several established anticancer drugs like topotecan.[179] All of the osmium complexes tested inhibit topoisomerase I, indicating that this enzyme could be a potential target for future mononuclear and trinuclear osmium pyridyl imine complexes.



31a–31h

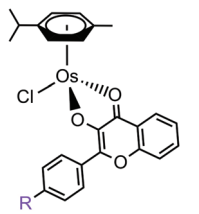
Other possible drug targets that involve both DNA and proteins are the nucleosomes, the unit of chromatin that comprises DNA coiled about a histone protein octamer.[180,181] The Os(II) arene complexes bearing *N*-substituted 2-pyridinecarbothioamides (PCA) ligands (**32a** and **32b**), which exhibit potent anticancer activity in lung and ovarian cancer cells, were found to form covalent adducts with the histone proteins.[182] Crystals of the nucleosome core particle were soaked in solutions containing complexes **32a** and **32b**. Single-crystal X-ray diffraction revealed that these complexes bind covalently to two histidine residues at the histone H2B site. The apparent preference for this complex to bind the protein histidine side chains, rather than nucleobase sites on the DNA, is unexpected given the known DNA-binding activities of related analogues. This result also suggests that these compounds could hinder chromatin mobility as a mechanism of action.



$R_1 = R_2 = H$ (**32a**)
$R_1 = H$, $R_2 = F$ (**32b**)

32a and 32b

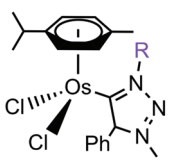
The ability of the Os(II) arene complexes, **33a–33d**, to interact covalently with a diverse set of proteins was studied in comparison to their ruthenium and rhodium analogues.[183] These complexes contain flavonoid-based ligands, which are naturally occurring plant constituents[184] that have been shown to inhibit topoisomerase IIα.[185,186] The ruthenium, rhodium, and osmium compounds exhibit extremely potent *in vitro* anticancer activities with IC_{50} values in the nanomolar range. Among these metal complexes, the most cytotoxic species were those bearing the flavonoid ligand with a *p*-Cl substituent. The interactions of these complexes with ubiquitin and cytochrome *c* were investigated by mass spectrometry. These studies verified that the complexes form covalent adducts with ubiquitin, binding selectively at histidine side chains. However, no such adducts were detected on cytochrome *c*, indicating that there may be sequence specificity associated with the ability of these complexes to bind proteins. These complexes also bind covalently to the nucleotide triphosphates, 5′-dGTP and 5′-dATP. Notably, the ruthenium and osmium complexes bind preferentially to 5′-dGTP, whereas the rhodium complex selectively interacts with 5′-dATP. This selectivity reflects a subtle difference in the coordination preferences of these metal ions. However, when incubated in the presence of a mixture of amino acids and nucleotides, all metal complexes preferentially bind histidine, providing further evidence of the importance of protein targets.



R = H (**33a**), *p*-CH_3 (**33b**), *p*-F (**33c**), *p*-Cl (**33d**)

33a–33d

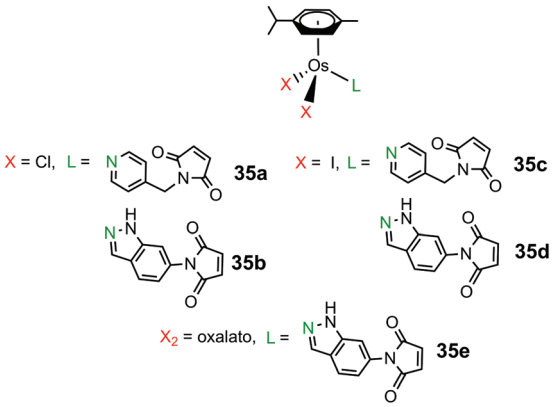
The exceedingly slow ligand substitution kinetics of osmium complexes, especially in comparison to its second row congener ruthenium, give rise to new facets in its medicinally relevant biological chemistry. For example, Os(II) arene complexes bearing N-heterocyclic carbene (NHC) ligands, such as **34**, are kinetically inert, yet highly cytotoxic in ovarian cancer cells.[187] The osmium complexes, unlike their ruthenium analogues, do not interact covalently with ubiquitin. The kinetic inertness of these osmium complexes may be advantageous because many metal complexes are deactivated by intracellular nucleophiles like glutathione (GSH). Among this series of related Os(II) NHC complexes, compound **34**, which bears the lipophilic dodecyl alkyl chain, is the most active in comparison to the less lipophilic alkyl substituents. This result suggests that the lipophilicity of the complex is the primary determinant of activity for this class of compounds.



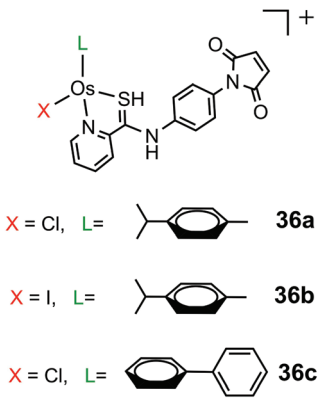
R = *n*-dodecyl

34

The slow ligand substitution kinetics of these Os(II) arene complexes can also be utilized to exploit outer-sphere ligand-based reactivity. For example, the thiol-reactive maleimide groups were conjugated to the osmium complexes, **35a–35e**[188] and **36a–36c**.[189] These compounds react with thiols, like cysteine, at the maleimide site without any direct ligand substitution reaction at the osmium center. The most active complexes, **35a** and **35b**, exhibit almost identical IC_{50} values of 9.0 and 8.0 μM, respectively. In contrast, complexes **36a–36c** showed no *in vitro* anticancer activity at all. The differing activities of these osmium maleimide compounds highlight the effect that ligand modifications can have on the function of metal complexes.

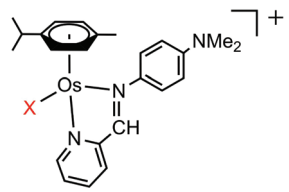


35a–35e



36a–36c

Mechanism of cell death. The mechanisms of cell death induced by structurally similar Os(II) arene complexes can be remarkably dependent on the nature of the supporting ligands. As a representative example, the differing activity and mechanism of action of a set of osmium complexes bearing iminopyridine ligands (**37a** and **37b**) were explored and compared to their largely investigated osmium azopyridine derivatives.[190] Complexes **37a** and **37b**, which differ only by the nature of the halide ligand, exhibit potent anticancer activity against ovarian and lung cancer cell lines. Their activity is correlated to their ability to produce ROS and oxidize NADH. Unlike the ruthenium azopyridine analogues,[191] these complexes cannot readily oxidize GSH but are competent oxidants for NADH, converting it to NAD^+ *via* a hydride transfer to the Os(II) center. Between **37a** and **37b**, the chlorido complex, **37a**, is substantially less cytotoxic than the iodido complex. Furthermore, the iodido complex, **37b**, is equally potent in wild-type and cisplatin- and oxaliplatin-resistant cell lines. In contrast, the activity of the chlorido complex, **37a**, is diminished by a factor of 4 in the drug-resistant cell lines.[192] This result suggests that **37b** operates under a distinct mechanism of action from both the platinum drugs and its chlorido analogue **37a**. Additional mechanistic studies on **37b** revealed that its activity is not dependent on the expression levels or mutation status of p53 and that it also induces late-stage apoptosis with increased activity in the presence of L-buthionine sulfoximine (BSO), a glutathione S-transferase (GST) inhibitor.[193] These studies have provided insight on the effect that small changes in ligand substituents can have on the mechanism of action of structurally analogous metal complexes.



37a and 37b

Modifications of the chelating ligand also play a large role in affecting the mechanism of action of these complexes. For example, the osmium complex, **38**, which contains an azopyridine ligand, induces cell death *via* a different mechanism than its iminopyridine analogue.[194] Complex **38** exhibits greater activity than cisplatin in lung cancer cells. Similar to **37a** and **37b**, the halide ligand plays an important role, as evidenced by the substantially greater cytotoxicity of **38** compared to its chlorido analogue. Compound **38** induces cell death *via* disruption of mitochondrial function as indicated by depolarization of the mitochondrial membrane potential and release of cytochrome *c* into the cytosol. Similar to the iminopyridine complexes, **37a** and **37b**, **38** is selective for cancer cells over non-cancerous fibroblasts, and its activity is inversely related to intracellular GSH concentration.[195] Complex **38** also operates through a ROS-dependent pathway and modulates metabolic processes.[196] Because the activity of **38** is dependent on GSH concentration, the role of this tripeptide in mediating the activity of **38** was further explored.[197] HPLC studies showed that the Os–I bond of this complex hydrolyzes to form the presumed active species, the hydroxido complex [(η^6-*p*-cymene)Os(*p*-NMe_2-azopyridine)(OH)], in the presence of GSH. This hydroxido species can then interact with excess GSH to form the osmium–thiolato (GS^-) or osmium–sulfenato (GSO^-) adducts. To probe the intracellu-

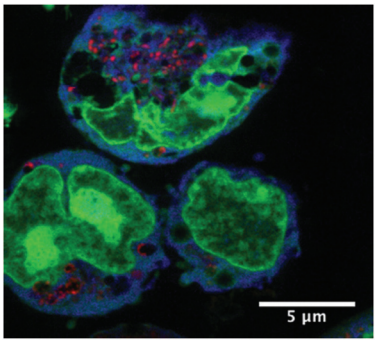
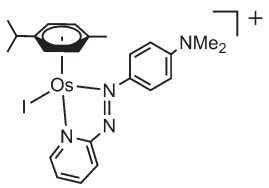


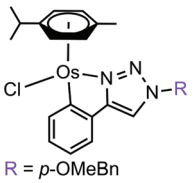
Fig. 3 X-ray fluorescence map of osmium (red), zinc (green), and calcium (blue) in A2780 ovarian cancer cells treated with **38**. Reprinted with permission from ref. 198. Copyright (2017), with permission from John Wiley and Sons.

lar localization of **38**, synchrotron-based X-ray fluorescence imaging was employed.[198] This technique allows for simultaneous mapping of specific elements based on their distinct X-ray emission energies.[199] Ovarian cancer cells treated with **38** were imaged to track the intracellular localization of osmium. The osmium of complex **38** localized primarily in the mitochondria (Fig. 3). Imaging of calcium revealed that these ions were depleted from the ER and enriched in the cytosol, suggesting that **38** alters intracellular calcium trafficking. Because the export of calcium from the ER is a hallmark feature of apoptosis,[200] this result suggests that **38** can trigger this mode of cell death. Collectively, the detailed studies of **38** indicate that this compound mediates cell death *via* a mechanism of action that is distinct from that of cisplatin. The primary feature of this mechanism include its ability to increase intracellular ROS levels, which leads to apoptosis. Additionally, related iridium azopyridine complexes operate *via* similar mechanisms of action, which will be discussed in Part 4 of this review.[201]



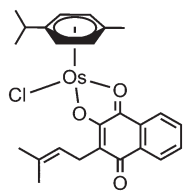
38

Based on their structural similarity to the osmium iminopyridine and azopyridine complexes described above, cyclometalated Os(II) arene complexes bearing 1-substituted 4-phenyl-1,2,3-triazole ligands were explored for their anticancer activity.[202] The most potent compound in this series, **39**, stalls cells in the S phase of the cell cycle. These compounds were also evaluated as inhibitors of topoisomerase IIα. However, they failed to inhibit this enzyme at biologically relevant concentrations, suggesting that topoisomerase IIα is not a likely target and that a different mechanism of action is operative.



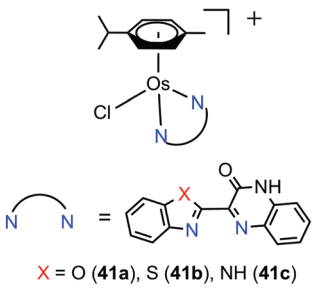
39

An alternative approach to control osmium-mediated cell death mechanisms is the use of bioactive ligands, which have known mechanisms of action, on Os(II) arene complexes. Lapachol, a natural product with anticancer activity that stems from its ability to generate ROS,[203] was employed as a ligand for Os(II) arene complex **40**.[204] The Os(II) complex triggered apoptosis in cancer cells *via* the production of ROS, resulting in an IC_{50} value that is similar to free lapachol. Because no significant enhancement of activity was observed for the complex, the cytotoxicity of **40** is most likely a consequence of the lapachol moiety.

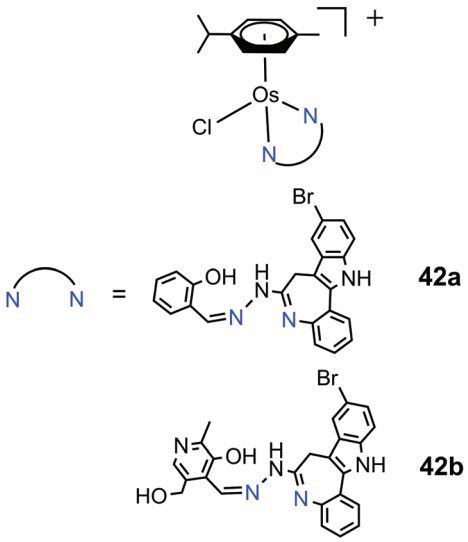


40

Protein kinase inhibitors, a well-established class of anticancer drugs,[205,206] have also been implemented as ligands for Os(II) arene complexes. Complexes **41a–41c**, which bear quinoxalinone-based protein kinase inhibitor-like ligands, gave rise to significant cytotoxic effects in a panel of cancer cell lines.[207] They killed cells *via* an apoptotic pathway but did not substantially alter the cell cycle. Paullones, another type of cyclic-dependent kinase (Cdk-2) inhibitors,[208] were used as ligands on the Os(II) arene complexes, **42a** and **42b**.[209] Like **41a–41c**, these complexes showed potent anticancer activity but did not affect the cell cycle. The Cdk-2 inhibitory activities of these complexes were substantially reduced relative to the free ligands, suggesting that alternative causes of cell death are operational for this class of compounds.

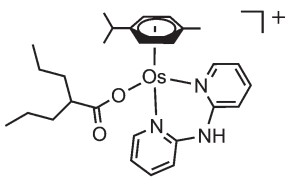


41a–41c



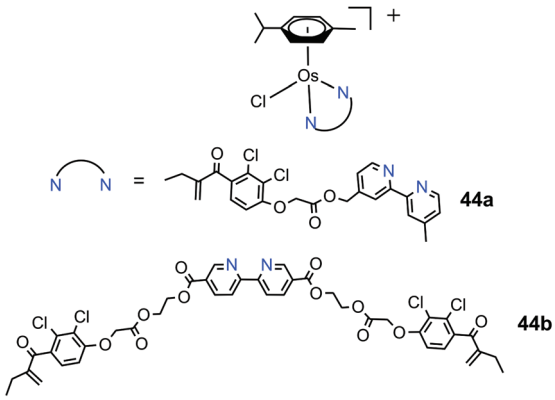
42a **42b**

An Os(II) arene complex bearing valproic acid (**43**), a drug used to treat bipolar disorder and seizures,[210] was investigated for use as an anticancer agent.[211] Valproic acid inhibits HDAC, a property that gives rise to its antiproliferative effects.[212] Compared to an analogous complex without valproic acid, **43** exhibits a 3-fold higher cytotoxic activity in ovarian cancer cells. Complex **43** was also shown to induce apoptosis, produce ROS, and disrupt the mitochondrial membrane potential. Lastly, **43** inhibits HDAC with similar potency as free valproic acid, suggesting that this enzyme is a critical target for this complex.

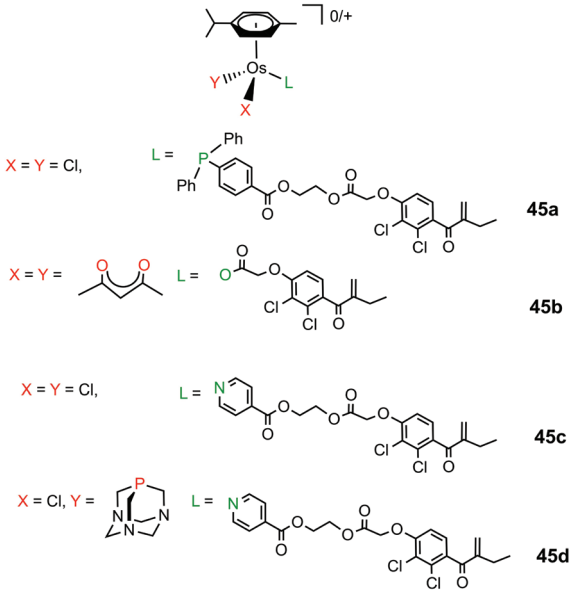


43

The enzyme GST, which catalyzes the conjugation of glutathione to metal-based anticancer agents to deactivate them, is upregulated in multidrug-resistant cancers.[213] An inhibitor of GST, ethacrynic acid (EA),[214] could therefore help overcome drug-resistance in cancer cells. To explore this hypothesis, EA was conjugated to osmium to yield complexes **44a**, **44b**,[215] and **45a–45d**.[216] These complexes were designed to deliver EA upon intracellular cleavage of the ester bonds. Accordingly, these compounds show GST inhibitory activity and are also effective in cisplatin-resistant cancer cell lines.



44a **44b**

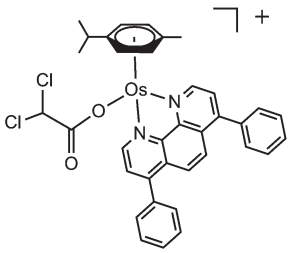


45a **45b** **45c** **45d**

45a–45d

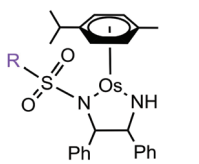
Similar to the approach described above of using osmium complexes conjugated to EA, the osmium complex, **46**, and its ruthenium analogue were designed to contain dichloroacetate (DCA) as a bioactive ligand.[217] Sodium dichloroacetate is used for the treatment of lactic acidosis.[218] Its activity is attributed to its ability to inhibit pyruvate dehydrogenase kinase (PDK), an enzyme that is important to cancer cells for maintaining their unique metabolic profile of aerobic glycolysis.[219,220] Complex **46** and its ruthenium analogue exhibit similar micromolar toxicities in ovarian cancer cells. Although the cytotoxic activities of the two complexes were comparable, only the ruthenium complex gave rise to a substantial population of cells in the sub-G1 phase. Additionally, the ruthenium analogue gave rise to higher levels of mitochondrial cytochrome *c* in the cytosol. This study illustrates

the potential of bioactive molecule-releasing osmium complexes as potent anticancer agents and demonstrates that ruthenium analogues may have distinct mechanisms of action.



46

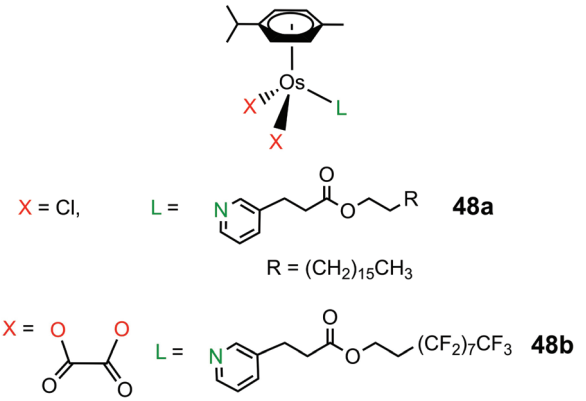
In a new direction, the exploitation of the catalytic properties of Os(II) arene complexes to mediate anticancer activity has been investigated for complex **47** and related derivatives.[221] This compound catalyzes the enantio-selective transfer hydrogenation reduction of pyruvate to lactate using formic acid as a hydride source. Furthermore, the catalytic activity of **47** is retained in live cells. The compounds are only cytotoxic against cancer cells in the presence of excess formate, suggesting that toxicity is dependent on the ability of these complexes to catalyze this reaction. Additionally, a more biologically relevant hydride source, formylmethionine, also enables this reactivity in cells. The development of catalytic metallodrugs is an important field that may usher in the next generation of metal-based anticancer agents.[190,191,222–224]



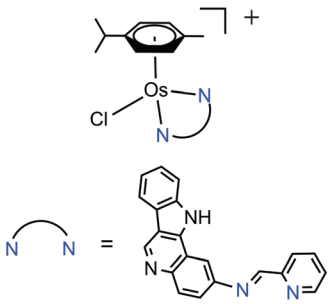
R = 4-methylphenyl

47

***In vivo* studies.** Only a handful of Os(II) arene complexes have been investigated with *in vivo* models of cancer.[225–229] Among several of the more recent examples, Os(II) arene complexes bearing alkyl and perfluoroalkyl groups (**48a** and **48b**) were investigated in chicken embryos to probe their anti-angiogenic effects.[226] Using the chicken embryo chorioallantoic membrane (CAM) model,[230] the complexes were shown to disrupt vascular activity through induction of vaso-occlusion of the vasculature, signifying their potential use for preventing tumor angiogenesis.

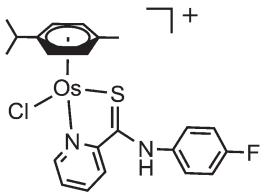


The *in vivo* anticancer activity of the indolo[3,2-*c*]quinoline osmium complex (**49**) was evaluated in mice bearing xenografts of CT-26 murine colon cancer cells.[227] The osmium complex administered over the span of five days at low doses, both intraperitoneally and orally, was found to reduce tumor growth to a greater extent than its ruthenium analogues. The greater *in vivo* activity of the osmium complex is surprising because the ruthenium analogue was actually more potent in the *in vitro* cancer models. This result highlights the challenges in attempting to use *in vitro* models to predict the *in vivo* activity of novel drug candidates.



49

Given the growing interest in osmium anticancer agents, a validated protocol for analyzing the biodistribution of osmium in mice *via* ICP-MS was recently reported.[225] For standard *in vivo* biodistribution studies, animal organs are digested with strongly oxidizing acids.[231] Under these conditions, however, osmium is lost in the form of the highly toxic and volatile OsO_4. In this study, mild digestion conditions were established to prevent oxidation and loss of osmium. To demonstrate the efficacy of this procedure, the biodistribution patterns of the Os(II) arene complex, **50**, and its Ru(II) congener were evaluated in mice. Both compounds displayed similar uptake profiles. In general, higher concentrations of osmium were detected compared to ruthenium, indicating that the osmium complexes clear less effectively *in vivo*. The compounds were taken up preferentially in the liver with only moderate uptake in the tumor. This study validates a new osmium quantification method that could be useful for exploring the *in vivo* anticancer activities of complexes containing this element.

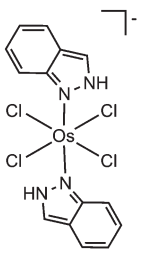


50

Following the method described above for the ICP-MS analysis of osmium in biological samples, the biodistribution of complex **32b** was investigated and compared to that of its ruthenium analogue.[232] Complex **32b**[182] exhibits potent *in vitro* cytotoxic activity and can be administered orally *in vivo* as an anticancer agent. When administered orally over the course of 14 days, both the ruthenium and osmium complexes inhibited tumor growth in mice bearing CT-26 colon cancer tumor xenografts. After this initial 14-day treatment period, the mice were further observed for an additional 7 days without additional compound administration. After cessation of treatment, tumors on mice that were treated with the ruthenium complex returned to volumes that matched those found on the untreated controls. In contrast, the mice treated with osmium complex **32b** did not display observable regrowth of the tumors. This result indicates the long-lasting effects of **32b** on tumor growth and may illustrate its potential for preventing cancer relapse in patients. The biodistribution of these complexes was evaluated by ICP-MS, and the metal localization was imaged with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), a highly sensitive element-specific imaging technique.[233] The liver and kidney had the highest concentrations of the metal complexes. LA-ICP-MS showed that the ruthenium and osmium concentrations were higher in the cortex rather than the medulla of the kidney. This result is consistent with kidney distribution patterns of cisplatin.[234] With respect to the tumor localization, the ruthenium analogue was found to penetrate deeper into the interior of the tumor compared to the osmium complex, which primarily localized to the outer edge of the tumor as shown in Fig. 4. These results highlight the application of LA-ICP-MS for determining the spatial distribution of osmium in tissues.

3.2. Osmium indazole complexes

The Ru(III) complex, *trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III)], also known as KP1019, is one of the few non-platinum metal-based compounds to progress to clinical trials.[155,156] This complex is activated both by reduction and ligand substitution reactions.[235] Given the substantially different redox chemistry and slower ligand exchange kinetics of third row transition metals compared to their second row analogues, it was expected that osmium derivatives may exhibit different and possibly complementary anticancer activities to KP1019. The osmium analogue of KP1019 has only recently been investigated.[236] An interesting feature of this osmium complex is the accessibility of different isomers that vary based on the tautomeric state of the coordinating indazole ligand. For example, the *trans* isomer (**51**) contains the 2*H*-indazole tautomer, but the *cis* isomer (**52a**) bears the 1*H*-indazole tautomer. The ability of 1*H*-indazole to tautomerize into 2*H*-indazole could be important for the physiological and biological properties of these complexes. To investigate this hypothesis, a series of osmium complexes with different tautomeric forms of indazole were investigated for their anticancer activity and compared to the ruthenium complex, KP1019.[237] Unlike KP1019, these compounds were essentially inert to ligand substitution reactions with amino acids and nucleobases. Despite their kinetic inertness, they still exhibit moderate anticancer activity, albeit less than that of KP1019. Related anticancer active Os(III) and Os(IV) 1*H*- and 2*H*-indazole complexes (**52a–52c**) were subjected to mechanistic studies to understand how they induce cell death.[238] Complexes **52a** and **52b**, which are substantially less cytotoxic than cisplatin, induce late-stage apoptosis, characterized by both the flipping of phosphatidylserine to the outside of the cell and plasma membrane permeabilization. Among these osmium indazole complexes, the most active compound is the *trans* 2*H*-indazole complex, **51**. The other compounds are substantially less potent, indicating that coordination isomers and ligand tautomeric forms can have large effects on the cytotoxic activity of this class of compounds.



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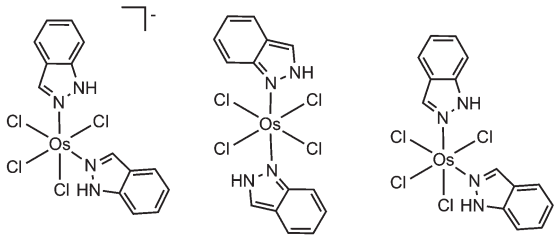


52a **52b** **52c**

52a–52c

***In vivo* studies.** Within this class of compounds, only complexes **53a** and **53b**, which contain a single indazole ligand in either the 1*H*- (**53a**) or 2*H*- (**53b**) tautomeric forms, were tested for *in vivo* anticancer activity in hematoma Hep3B SCID mouse xenotransplantation models.[239] Like compounds **51** and **52a–52c**, which were only tested *in vitro*, the different tautomeric forms of the indazole ligand gave rise

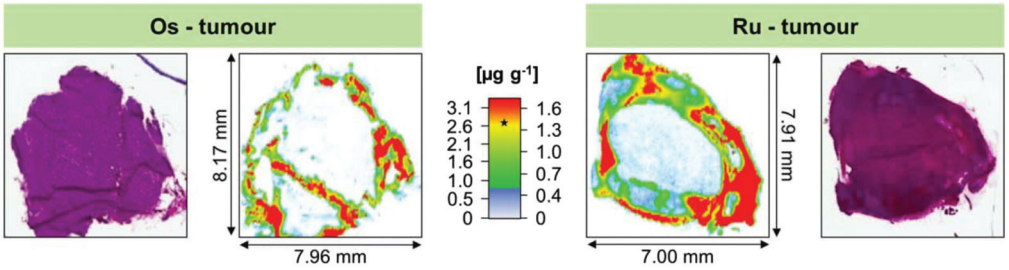
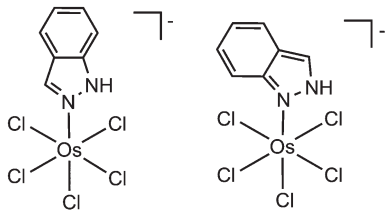


Fig. 4 Osmium (left) and ruthenium (right) spatial distribution in CT-26 tumor xenografts after oral administration of 15 mg kg^{-1} of either compound **32b** or its ruthenium analogue. The images in purple illustrate the tumors with haematoxylin–eosin (H&E) staining and the other images show the metal distribution using LA-ICP-MS. The average metal concentration is illustrated on the scale with an asterisk. Reprinted with permission from ref. 232. Copyright (2018), with permission from Royal Society of Chemistry.

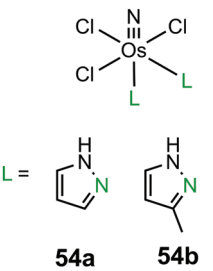
to substantially different *in vivo* activities. In contrast to compound **53a**, which partially reduces tumor growth, **53b** has no effect on tumor size. Despite its inability to inhibit tumor growth, **53b** enhances the long-term survival of mice. These studies highlight the subtle effects of ligand conformations on modulating the biological activity of coordination complexes.



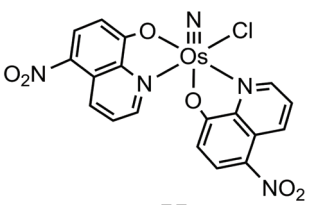
53a **53b**

3.3. Osmium nitrido complexes

The stability of higher oxidation states of osmium gives access to novel metal–ligand multiple bond architectures. For example, the osmium nitrido unit, in which osmium is formally in the +6 oxidation state, is a stable structural motif that supports an Os–N triple bond. This class of compounds has previously been investigated for N-atom transfer reactions and in various catalytic roles.[240,241] Members of this class of compounds were also recently discovered to possess anticancer activity. The Os(VI) nitrido complexes bearing pyrazole (**54a** and **54b**)[242] and quinolinolato (**55**)[243] ligands, for example, both give rise to cytotoxic effects in a panel of cancer cell lines. These compounds induce apoptosis and arrest the cell cycle in the S phase. Compound **54b** also led to phosphorylation of the histone protein H2AX, a known marker for DNA damage.[244,245] Additionally, **54a** and **54b** cleave supercoiled plasmid DNA. Taken together, these results implicate DNA as a major target for this class of compounds.

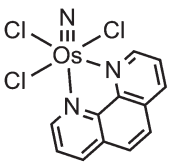


54a **54b**



55

A related class of Os(VI) nitrido compounds, which bear chelating diimine ligands, give rise to potent, sub-micromolar cytotoxicity in cancer cells. One of the most potent compounds, **56**, was shown to induce cell death *via* DNA damage, as reflected by the phosphorylation of H2AX and apoptosis induction.[246] Another potent compound, an analogue of **56** with a 4,7-diphenyl-1,10-phenanthroline ligand, causes ER stress instead of DNA damage, signifying the important role of the supporting ligands in mediating the anticancer activity of these complexes. Remarkably, complex **56** possesses enhanced toxicity in breast cancer stem cells (CSCs) as well.[247] Because cancer stem cells are largely responsible for cancer relapse and patient mortality,[248–251] the ability of this class of compounds to target CSCs provides interesting therapeutic possibilities for the use of Os(VI) nitrido complexes.



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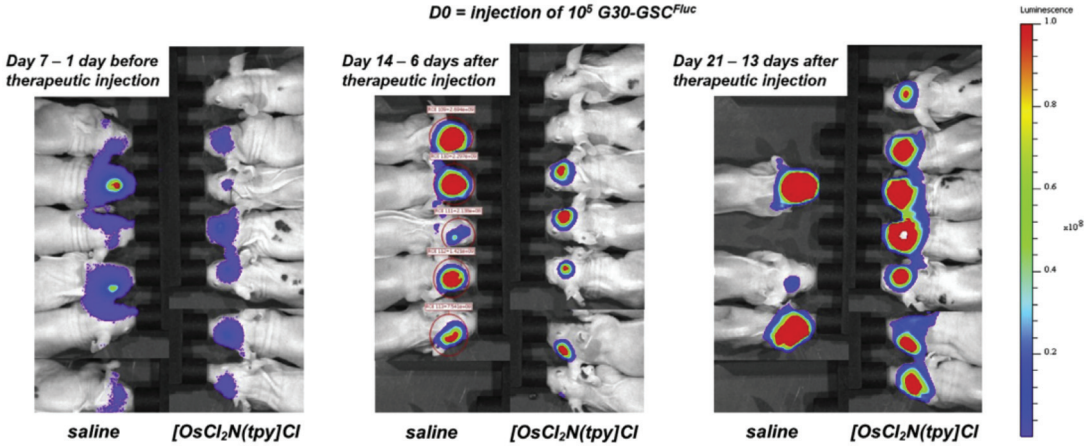
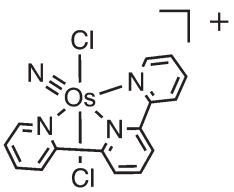


Fig. 5 Efficacy of **57** in mice bearing patient-derived glioblastoma xenografts. The mice shown on the left side of each panel are the untreated control group (injected with saline) and the mice shown on the right side of each panel were treated with **57**. The luminescence, arising from the luciferase expression of the cancer cells, illustrates tumor growth after 13 days post injection. Reprinted with permission from ref. 252. Copyright (2018), with permission from Elsevier.

***In vivo* studies.** *In vivo* studies of Os(VI) nitrido complexes remain fairly limited at this stage. Most recently, the cancer stem cell-selective complex, **56**, and a related terpyridine-bearing Os(VI) nitrido complex **57** were evaluated *in vivo* for antitumor activity.[252] These *in vivo* studies were prompted by the potent *in vitro* cytotoxic activity of these complexes in glioblastoma cells. These compounds were injected intracranially into mice bearing patient-derived glioblastoma xenografts. Tumor growth was monitored by luminescence detection, capitalizing on the luciferase expression of the tumor model used. Fig. 5 shows the luminescence intensity of the tumor in both untreated and treated mice at 6 and 13 days after compound administration. Although both complex **56** and **57** increase the survival times of mice compared to the untreated control, compound **57** was substantially more effective than **56** *in vivo*. This result contradicts the *in vitro* studies, which demonstrated **56** to be the more potent of the two compounds. This result highlights the challenges of relying on *in vitro* studies to predict *in vivo* compound efficacy, a phenomenon that was discussed earlier in the context of compound **53b**. These early results are promising for establishing this class of osmium complexes as anticancer agents. Further studies, however, should help determine their *in vivo* mechanisms of action more precisely.



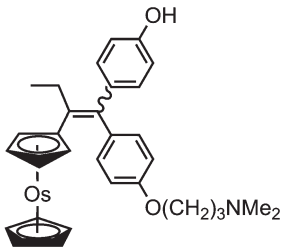
57

3.4. Osmocifen complexes

Tamoxifen is an organic nonsteroidal antiestrogenic drug used for the treatment of breast and ovarian cancer.[253] A second-generation analogue of tamoxifen is the organometallic drug candidate, ferrocifen, in which one of the phenyl groups in tamoxifen is replaced by a highly stable bis (cyclopentadienyl) Fe(II) (ferrocene) core.[254–256] Ferrocifen has shown several advantages over tamoxifen. In addition to maintaining the antiestrogenic properties of tamoxifen, it also catalyzes the generation of ROS through redox cycling of the Fe(II) center.[255] The promising preclinical studies with ferrocifen have prompted an investigation of the corresponding ruthenium and osmium analogues, which are often referred to as "ruthenocifen" and "osmocifen" complexes, respectively.

Osmocifen complexes were compared to iron and ruthenium analogues with respect to *in vitro* anticancer activity.[257] The most potent osmocifen complex, **58**, is significantly cytotoxic in both hormone-dependent and hormone-independent breast cancer cells.[258] Hormone-dependent breast cancer cells generally rely on the presence of external hormones, such as estrogen and progesterone, for their continued proliferation. In contrast, hormone-independent breast cancer grows and metastasizes aggressively in the absence of these hormones. Tamoxifen, an antiestrogenic drug, is used exclusively for hormone-dependent breast cancer.[259] As such, the ability of the osmocifen complexes to induce cell death in hormone-independent breast cancer cells indicates that the osmium center alters the mechanism of action of tamoxifen, expanding its therapeutic utility to this type of aggressive cancer. Because ferrocifen induces cellular senescence, an irreversible cell cycle arrest pathway,[260,261] **58** and its ruthenium analogue were investigated in this capacity. These studies indicate that the extent of senescence induction was less for these compounds compared to ferrocifen. Although less potent than ferrocifen, the ruthenium and osmium derivatives exhibit low micromolar toxicity. To further probe the SARs for this class of compounds, complex **59**, which lacks the $O(CH_2)_3NMe_2$ tail of **58**, was investigated for anti-

cancer activity. This $O(CH_2)_3NMe_2$ structural motif is apparently important for mediating the biological activity of this class of compounds, as evidenced by the diminished activity of **59** compared to **58**. For example, complex **58** increases production of ROS, decreases the mitochondrial membrane potential, and oxidizes cytosolic and mitochondrial thioredoxins, proteins that are critical for maintaining the cellular redox status. These effects are attributed to the inhibition of thioredoxin reductase, an enzyme that controls the redox status of thioredoxin.[262] Similar results were observed for complex **59** but to a lesser extent. These studies highlight the potential of osmocifen complexes as potent thioredoxin reductase inhibitors.



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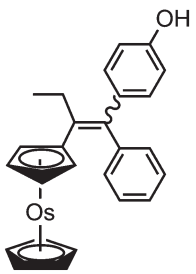
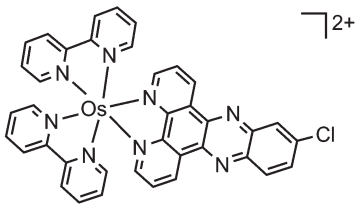


59

3.5. Osmium polypyridyl complexes

The biological activity of Os(II) polypyridyl complexes was first investigated in 1952.[263] Since then, few studies have explored this class of compounds as anticancer agents. The lack of labile ligands in these compounds suggests that they bind with intracellular targets *via* non-covalent interactions. Although these complexes may potentially bind to DNA *via* intercalation, an interesting application lies in their abilities to interact with protein-based targets. Complex **60**, for example, was shown to inhibit STAT5B,[264] a transcription factor that is known to upregulate oncogenic pathways in cancer cells.[265–267] The inhibitory activity of **60** was demonstrated in living cells based on decreased transcription activity of STAT5B. The complex directly binds with STAT5B, preventing its dimerization to the functionally active form. The inhibition of STAT5B manifests in the cytotoxic effects of **60** against cancer cells. Another class of Os(II) polypyridyl complexes were investigated as inhibitors of the hypoxia-inducible factor (HIF) pathway.[268] HIF regulates the hypoxic response in mammals.[269] Under hypoxic conditions the HIF-1α and HIF-1β transcription factors dimerize to form the active HIF heterodimer, triggering the transcription of genes involved in cell proliferation and tumorigenesis.[270–272] However, under normoxic conditions HIF-1α is degraded and the HIF heterodimer does not form. Among the compounds investigated in this study, **61** most effectively inhibits the HIF pathway by disrupting the HIF-1α-p300 protein complex, which is directly linked to the activation of tumorigenic gene transcription.[271–273] These recent studies on Os(II) polypyridyl complexes revealed novel opportunities to target cancer-related proteins, such as STAT5B and HIF-1α. Although not considered in these studies, the luminescence properties of these complexes may be leveraged for simultaneous imaging as well,[274] rendering these complexes an interesting new class of anticancer agents.



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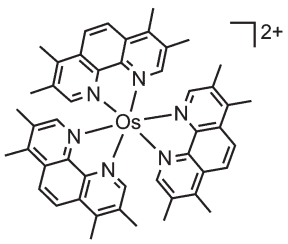
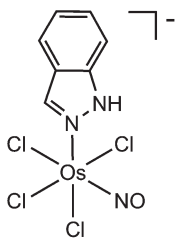


61

3.6. Osmium nitrosyl and carbonyl complexes

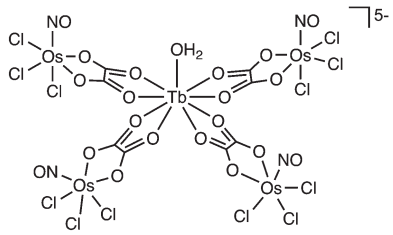
Carbon monoxide (CO) and nitric oxide (NO) mediate a wide range of biological processes,[275–279] and recent studies have shown that they may possess favorable antiproliferative properties for use in cancer therapy.[280,281] The application of coordination complexes as selective delivery agents for these gaseous molecules for cancer treatment is an expanding field of interest.[282–285] The use of osmium complexes in this context, however, is much less explored. Among recent investigations, a library of twenty osmium and ruthenium complexes bearing azole heterocycle and nitrosyl ligands of the general formula, $[MCl_4(NO)(Hazole)]^-$, Hazole = azole heterocycle, M = Ru or Os, were compared for their antiproliferative properties.[286] The osmium complexes were inert in the presence of ubiquitin, myoglobin, and the reducing agent, ascorbic acid. In contrast, the ruthenium analogues underwent rapid reduction upon treatment with ascorbic acid even though they were unreactive to ubiquitin and myoglobin. The ruthenium complexes are more cytotoxic than the osmium complexes in a panel of cancer cell lines, which could possibly be due to their more rapid reduction. The orientation of

ligands about the osmium center, however, plays a large role in mediating the activity of the complexes. For example, the most cytotoxic osmium complex, **62**, was about 3 to 10 times more active than its *trans* isomer depending on the cell line. Furthermore, consistent with its slow ligand exchange kinetics, complex **62** did not bind covalently to human serum albumin (HSA).[287] It did, however, interact non-covalently with two hydrophobic binding sites within this protein. This result suggests that non-covalent adducts could play a role in the mechanism of action of this compound. Complex **62** and the *cis* and *trans* ruthenium derivatives were further investigated for their mechanism of action.[288] The *cis* and *trans* ruthenium analogues effectively depolarize the mitochondrial membrane potential, induce apoptosis, and increase ROS levels. In contrast, these effects were much less pronounced for the osmium analogue, **62**. Although NO release was not directly investigated, intracellular levels of cyclic guanosine monophosphate (cGMP), which is produced intracellularly upon NO exposure,[289] were determined. The cGMP levels were substantially higher for cells treated with the ruthenium complex in comparison to the osmium complex, **62**. The poorer activity of the osmium complexes may be attributed to the stronger Os–NO bond, which prevents NO release and the formation of covalent adducts with the osmium center.



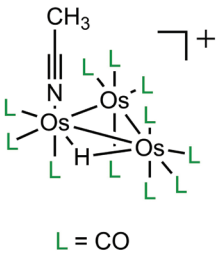
62

Multinuclear osmium and ruthenium nitrosyl complexes linked to a central lanthanide ion *via* bridging oxalate ligands were developed as potential theranostic agents, simultaneously capitalizing on the therapeutic properties of the ruthenium and osmium centers and diagnostic imaging capabilities of the luminescent lanthanide core.[290] Consistent with several of the compounds discussed above, the osmium complexes were substantially less active than their ruthenium analogues. The cellular uptake of the most active compound, **63**, was investigated by ICP-MS. Consistent with the relative *in vitro* anticancer activities of these complexes, the ruthenium analogue was taken up to a much greater extent compared to the osmium complex, **63**. Unfortunately, the luminescence or magnetic contrast properties of the lanthanides were not explored. However, the concept of capitalizing on the novel imaging properties of the lanthanides ions in combination with osmium-based anticancer agents is a promising strategy for the development of new theranostic drug candidates.



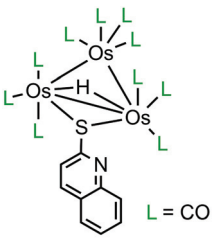
63

The activity of osmium carbonyl complexes was explored in a series of trisosmium carbonyl clusters.[291] These complexes were found to be more active against breast cancer cell lines lacking the estrogen receptor, indicating that they may operate through a receptor-independent pathway. Additionally, the most potent complex, **64**, induces apoptosis. The cytotoxicity of these complexes may arise from the labile acetonitrile ligand that could enable this cluster to form covalent biological adducts. Release of CO may also give rise to cytotoxic activity. For this class of compounds, however, more studies are necessary to understand their mechanism of action.



64

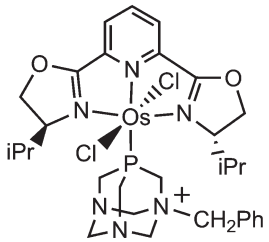
***In vivo* studies.** A related trisosmium carbonyl complex (**65**) was found to be cytotoxic in colorectal cancer cells and induce apoptosis through mitochondrial stress and ROS production.[292] It was also evaluated in combination with cisplatin and doxorubicin. The combined therapy gave rise to synergistic effects that facilitated the induction of tumor cell apoptosis. Following these *in vitro* results, this compound was tested *in vivo*. The administration of **65** to mice bearing HCT116 colorectal cancer xenografts results in decreased tumor growth rate and an overall 50% reduction in tumor volume. This *in vivo* study demonstrates the therapeutic potential of trisosmium carbonyl clusters.



65

3.7. Osmium pybox complexes

Ruthenium pybox complexes bearing the water-soluble phosphine ligand, 1,3,5-triaza-7-phosphaadamantane (PTA), have previously been shown to possess potent anticancer activity.[293] Motivated by these results for ruthenium, a series of Os(II) complexes bearing PTA and enantiopure (*S*,*S*) pybox ligands were investigated for both anticancer and antimicrobial activity.[294] The compounds partially decrease the electrophoretic mobility of plasmid DNA, indicating that they unwind the DNA double helix to a small extent. The most active complex, **66**, exhibits cytotoxicity in the micromolar range against cervical cancer cells and induces apoptosis like the ruthenium analogues. This class of osmium complexes highlights how novel ligands can be applied to generate cytotoxic compounds.



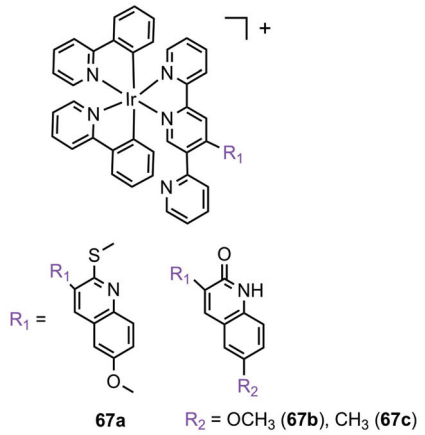
66

4. Iridium complexes

Like rhenium and osmium, the anticancer properties of iridium complexes remain relatively less explored. In recent years, however, there has been a surge in the application of these complexes as anticancer agents and imaging probes.[295–298] Although many of these compounds exhibit cytotoxicity in cancer cells, as highlighted in recent review articles,[295,299] their mechanisms of action have only been scarcely investigated. In this section, we summarize recent studies on the mechanism of action of anticancer iridium complexes, as categorized based on their compound structural types.

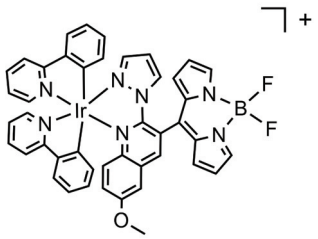
4.1. Cyclometalated iridium complexes

Octahedral cyclometalated iridium complexes represent a common structural motif. This class of compounds has predominantly been investigated for their promising photophysical properties, which have enabled their use in photoredox catalysis.[300] These compounds have also recently been shown to possess useful imaging and anticancer properties.[301–307] Additionally, some complexes target DNA or inhibit specific proteins.[303,308–313] For example, complexes **67a–67c**,[314] and **68**[315] kill cancer cells *via* an apoptotic mechanism. Molecular docking studies suggest that their activity stems from their ability to bind the minor groove of DNA.



67a R_2 = OCH_3 (**67b**), CH_3 (**67c**)

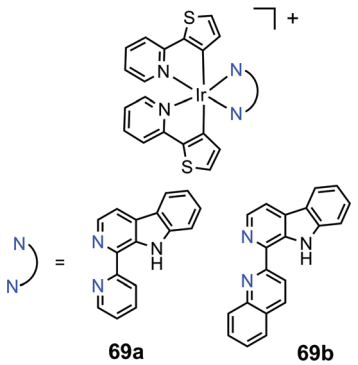
67a–67c

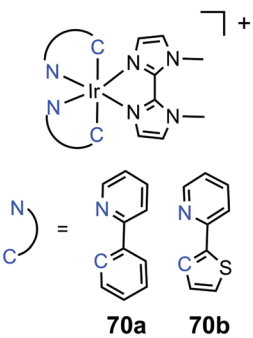


68

Modifications of the peripheral ligands can substantially alter the activity of these complexes. For example, β-carboline alkaloid moieties, which are kinase inhibitors,[316] were coordinated to cyclometalated Ir(III) complexes **69a** and **69b**.[317] Complex **69b** inhibited the mammalian target of rapamycin (mTOR) signaling pathway, a kinase that regulates cell growth and autophagy,[318] ultimately leading to cell death *via* autophagy. The ability of this complex to inhibit mTOR confirms how the implementation of the β-carboline alkaloid moiety can rationally lead to a desired mechanism of action. In another example, phosphorescent Ir(III) complexes containing 1,1′-dimethyl-2,2′-biimidazole ancillary ligands and two 2-phenylpyridine (**70a**) or two 2-thienylpyridine (**70b**) bidentate moieties were explored for anticancer activity.[319] These complexes were designed to help understand the cellular processes during mitophagy, a type of autophagy that selectively degrades the mitochondria (Fig. 6b).[320] Compounds **70a** and **70b** were found to accumulate in the mitochondria of A549 lung cancer cells and induce mitophagy, resulting in increased protein expression of PINK1, a protein that manages the autophagic clearance of damaged mitochondria after mitophagy. The results also showed increased expression of the protein Parkin, a regulator of cancer cell migration.[321] As shown in Fig. 6a, complex **70a** (green) was imaged using time-lapse confocal fluorescence microscopy in A549 cells stained with eGFP-LC3 (an autophagosome marker, red) and LysoTracker Deep Red (a lysosome marker, LTDR, pink). These images show the process of mitophagy over the course of 12 min. After 3 min, the iridium complex colocalizes with the eGFP-LC3 marker, signifying the for-

mation of mitochondria-containing autophagosomes. Over longer time periods, the emission arising from the autophagosome diminished, a consequence of the hydrolytic digestion of these structures. The time-lapse microscopy experiment confirms that this class of compounds induces autophagy through mitochondrial damage. Overall, these studies demonstrate how ligand design and modification can impact the mechanism of action of these complexes to give rise to substantial anticancer activity.





A related cyclometalated complex bearing a 2-(4-cyanophenyl)imidazole[4,5-*f*][1,10]phenanthroline ligand (**71**) was evaluated for anticancer activity in MDA-MB-231 breast cancer cells.[322] Confocal fluorescence microscopy revealed that these compounds accumulate in the mitochondria, and flow cytometry studies showed that complex **71** induces apoptosis. These results suggest that this complex's mode of cell death proceeds *via* an intrinsic mitochondria-mediated apoptotic pathway. A similar iridium complex that contains a novel ligand BTCP (2-bicyclo[2.2.1]hept-en-yl-1*H*-1,3,7,8-tetraazacyclopenta[*l*]phenanthrene) (**72**) was studied for activity in a variety of cancer cell lines.[323] Complex **72** induces ROS production and depolarization of the mitochondrial membrane potential. Complex **72** also stalls cells in the G0/G1 phase of the cell cycle. Fluorescence microscopy, in conjunction with fluorescent autophagosome marker monodansylcadaverine (MDC), was used to study cell invasion and induction of autophagy.[324] These results showed increases in MDC fluorescence intensity with increasing concentrations of **72**, indicating that this compound induces autophagy. Furthermore, the complex upregulates the pro-apoptotic proteins caspase-3,

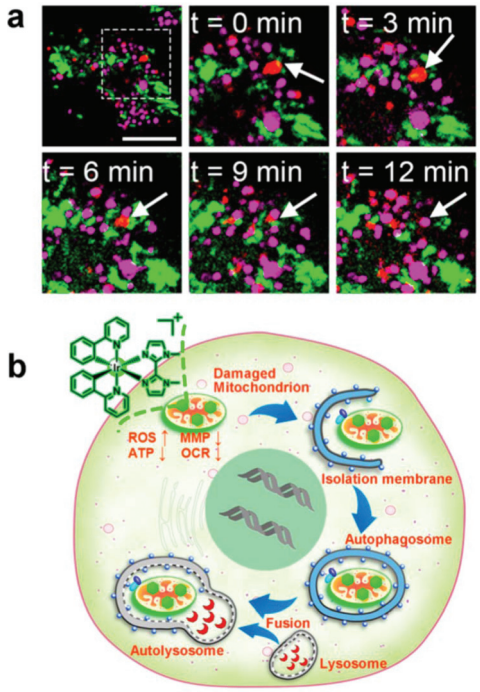
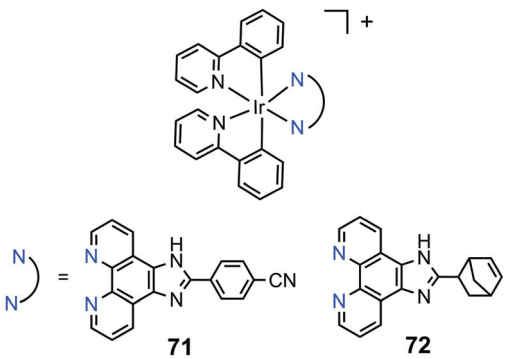


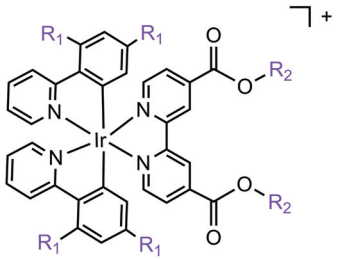
Fig. 6 (A) Time-lapse confocal fluorescence imaging in A549 cells expressing eGFP-LC3 (red) treated with **70a** (15 μM, green) and LTDR (50 nM, pink) for 6 h. Scale bar: 10 μm. (B) Schematic illustration of mitophagy. Reprinted with permission from ref. 319. Copyright (2017), with permission from American Chemical Society.

Bak, and Bid, while downregulating anti-apoptotic proteins Bcl-1, Bcl-x, and procaspase 7. Based on these results, it is apparent that complex **72** induces both autophagy and apoptotic cell death.



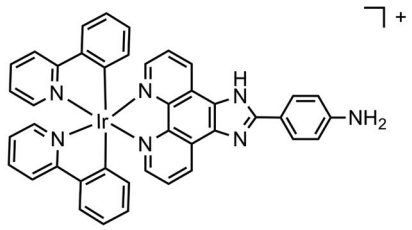
Recently, iridium complexes have been shown to localize in the mitochondria. Many of these complexes give rise to an increase in ROS, depolarization of the mitochondrial membrane potential, and activation of caspases, signifying the mitochondria to be an important target for this class of compounds.[325–329] For example, the ester functional groups on complexes **73a–73h** undergo intracellular hydrolysis, trapping the resulting anionic complex in the cell where it induces autophagy and apoptosis.[330] Colocalization studies with mitochondrial dyes

confirm that these complexes accumulate in the mitochondria, as shown for related structural analogues. Following this trend, complex **74** accumulates in the mitochondria and has an IC_{50} value in the micromolar range.[331] Upon inducing cell death, it downregulates the expression of the anti-apoptotic proteins, Bcl-1 and Bcl-x, while upregulating expression of the pro-apoptotic protein, Bak, as shown by Western blotting. It also stalls the cell cycle in the G0/G1 phase in PC-12 rat adrenal pheochromocytoma tumor cells. These studies suggest that the mitochondria are an important target for this class of compounds.



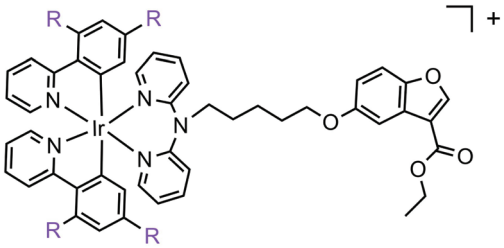
R_1 = H, R_2 = CH_3 (**73a**)
R_1 = H, R_2 = CH_2CH_3 (**73b**)
R_1 = H, R_2 = $(CH_2)_3CH_3$ (**73c**)
R_1 = H, R_2 = $CH_2CH(CH_3)_2$ (**73d**)
R_1 = F, R_2 = CH_3 (**73e**)
R_1 = F, R_2 = CH_2CH_3 (**73f**)
R_1 = F, R_2 = $(CH_2)_3CH_3$ (**73g**)
R_1 = F, R_2 = $CH_2CH(CH_3)_2$ (**73h**)

73a–73h



74

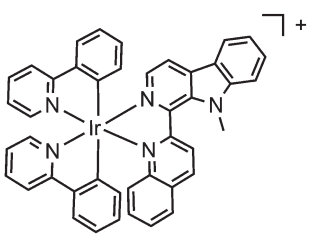
***In vivo* studies.** Two Ir(III) (**75a** and **75b**) and two Rh(III) benzofuran-conjugated complexes were synthesized and screened against DU-145 prostate cancer cells.[332] Benzofuran was chosen based on its known ability to act as an inhibitor of the autophagy-regulating kinase, mTOR,[333] and phosphoinositide 3-kinase (PI3), an enzyme involved in cell growth and proliferation.[334] Complex **75a** has an IC_{50} value of 3 μM and induces antiproliferative effects by directly inhibiting translocation and the activities of the transcription factors, STAT3[335] and NF-κB,[336] which are involved in tumor growth and cell proliferation, respectively. *In vivo* studies in RM-1 murine prostate cancer xenograft mouse models demonstrate dose-dependent tumor growth suppression without adverse side effects for both complexes. These studies suggest that iridium complexes of this type may provide a new basis for the logical design of dual inhibitor anticancer drugs.



R = H (**75a**), F (**75b**)

75a and 75b

Another cyclometalated iridium complex (**76**) was studied for *in vivo* activity in a mouse model.[337] This complex has potent anticancer activity against A549 lung cancer cells as characterized by an IC_{50} value of 0.93 μM. Confocal laser scanning microscopy studies revealed that **76** colocalizes with MitoTracker Green in A549 cells but does not localize with LysoTracker Green DND-26. This result indicates that complex **76** may selectively target the mitochondria. In order to investigate apoptosis as a possible mode of cell death, cells treated with this complex were stained with Hoechst dye and subjected to fluorescence microscopy studies. These microscopy studies showed the formation of cytoplasmic vacuoles with the plasma membrane and nuclei intact. In contrast, cells treated with cisplatin exhibited significant membrane blebbing and nuclear fragmentation. Cytoplasmic vacuoles can potentially signify autophagic or paraptotic cell death.[338] In order to distinguish between the two pathways, cells were co-treated with **76** and well-known autophagy inhibitors 3-methyladenine, chloroquine, wortmannin, and bafilomycin A1.[339,340] Because the activity of **76** remains unchanged after co-treatment with these inhibitors, autophagy was ruled out as a possible mechanism. The possibility of paraptosis, which is characterized by mitochondrial dilation, was investigated with transmission electron microscopy (TEM).[341] These TEM studies confirmed that the vacuoles originated from enlarged mitochondria, suggesting that paraptosis is the operative mechanism of cell death. The *in vivo* activity of this complex was evaluated in mice bearing A549 xenografts. Complex **76** was able to significantly inhibit tumor growth in a manner that was superior to an equivalent treatment regimen with cisplatin. In addition, mice treated with **76** did not show any significant change in body weight during the experiment, whereas the body weight of cisplatin-treated mice dropped by 10% on average. These results highlight the ability of this complex to kill lung cancer cells through a paraptotic cell death mechanism with limited side effects in mice.

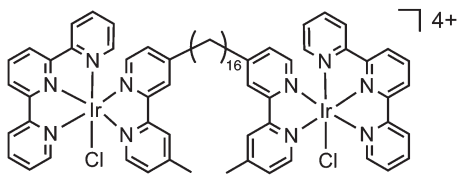


76

4.2. Multinuclear complexes

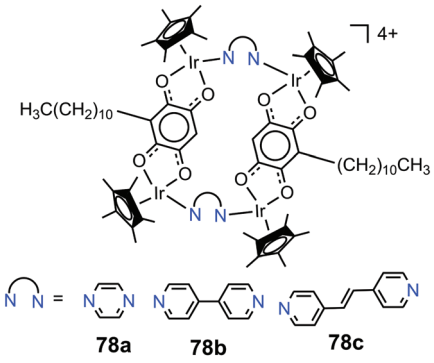
Multimetallic molecular frameworks offer interesting possibilities for the design of new anticancer agents to exploit the activity of several metal ions simultaneously. This approach has been very successful in the development of multinuclear analogues of cisplatin, as some of these compounds have progressed towards clinical trials.[342] This strategy is also relatively successful for alternative metal ion complexes. In this context, several homo- and heteronuclear iridium complexes have been evaluated as anticancer agents.

A set of highly charged cationic dinuclear Ir(III) complexes were investigated for their anticancer activity.[343] The alkane linker between the two Ir(III) centers was varied, ranging from 7 up to 16 carbons, to study its effect on the biological activities of the compounds. The most potent compound, **77**, contained a 16-carbon chain linker. By virtue of their cationic charge, the complexes bind to ct-DNA with high affinity as shown by circular dichroism (CD) spectroscopy. Complex **77** induces significant changes in the CD spectral intensity of DNA samples, indicating that **77** binds to this biomolecule with high affinity and causes significant structural distortion. Further mechanistic studies may help in understanding the mode of cell death such as the induction of an apoptotic response. Additional studies comparing wild-type and cisplatin-resistant cell lines may provide more insight on the potency of this complex.



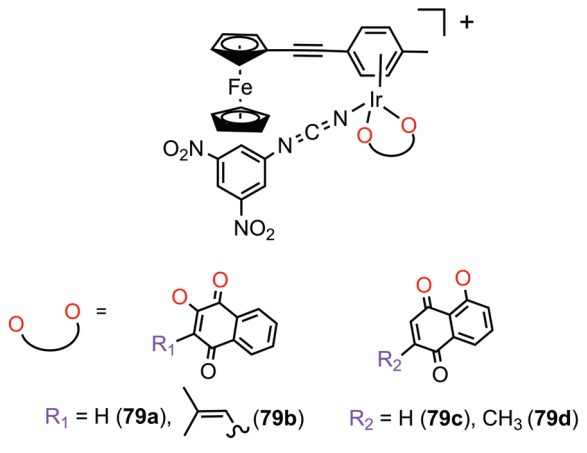
77

Tetranuclear iridium complexes **78a–78c** were tested for anticancer activity against prostate, lung, and cervical cancer cell lines.[344] The Ir(III) fragments were linked *via* the hydrophobic benzoquinone embelin, strategically chosen based on its cell permeability and ability to inhibit X-linked inhibitors of apoptosis protein (XIAP), an anti-apoptotic protein.[345] Two additional bridging ligands, comprising either a pyrazine, 4,4′-bipyridine, or 1,2-bis(4-pyridyl)ethylene, were employed to complete the metallo-rectangular structures. The most cytotoxic iridium complex, **78c**, gave rise to IC_{50} values that were less than 1 μM in all three cell lines. Cell cycle analysis showed cell population accumulation in the sub-G1 phase, suggesting that treatment with these complexes leads to DNA fragmentation. An annexin V/propidium iodide (PI) assay, which measures populations of cells undergoing apoptosis *versus* necrosis,[346] demonstrated the ability of the complexes to induce apoptosis in both the early and late stages. These results confirmed that embelin successfully inhibits the anti-apoptotic protein, XIAP, resulting in apoptotic cell death.



78a–78c

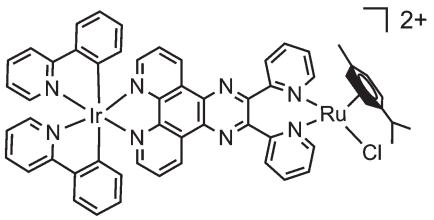
The SARs for a series of iridium complexes bearing a ferrocenyl moiety (**79a–79d**) were explored.[347] As described above for the example of ferrocifen, the presence of ferrocene as a functional group may potentiate the biological activity of anticancer agents.[348] These four complexes exhibit high cytotoxicity against HT-29 colon, HepG-2 liver, MCF-7 breast, HCT-8 colon, and A2780 ovarian cancer cell lines. The SARs revealed that the lipophilicity of the compounds is the major factor that dictates their cytotoxicity. Accordingly, the most lipophilic compound, **79b**, gave rise to the most potent anticancer activity as characterized by IC_{50} values ranging from 4 to 8 μM in a panel of different cell lines. An annexin V/PI assay indicated that complexes **79a–79d** induce early- and late-stage apoptosis in breast cancer cells (Fig. 7). As evidenced by the DCFH-DA (2′,7′-dichlorodihydrofluorescin diacetate) assay, these compounds also induce the production of intracellular ROS. The origin of this ROS production was explored in the context of the enzyme thioredoxin reductase, which plays a key role in maintaining cellular redox status. These compounds were shown to effectively inhibit thioredoxin reductase, further strengthening the potential for ROS production to contribute to cytotoxicity.



79a–79d

The dinuclear iridium–ruthenium complex with the metal centers bridged by a pyrazino[2,3-*f*][1,10]phenanthroline

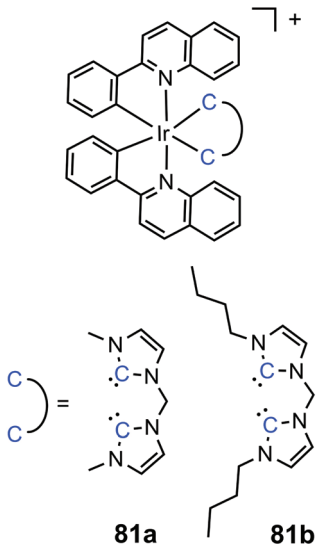
ligand (**80**) was investigated for anticancer activity.[349] This complex gives rise to more potent cytotoxic effects compared to the homonuclear diruthenium analogue. The mode of cell death induced by **80** was further explored *via* flow cytometric analysis of the cell cycle, which revealed stalling of the cells in the G1 phase. Western blots were carried out to check for changes in expression levels of proteins responsible for apoptosis in cells upon treatment with **80**. Specifically, the apoptotic regulators Bcl-2 and Bax and the cleavage of poly(ADP-ribose) polymerase (PARP), which is involved in DNA repair and apoptosis, were investigated in response to treatment with compound **80**.[350] No significant increase in the expression levels of any of these three proteins was detected, indicating that **80** induces a non-apoptotic form of cell death. Studies on the morphology of cells treated with **80** revealed extensive cytoplasmic vacuolization, a feature of autophagy.[351] Western blot analysis of the LC3II protein, a diagnostic marker for autophagic cell death,[352] confirmed that **80** induces autophagy in these cells.



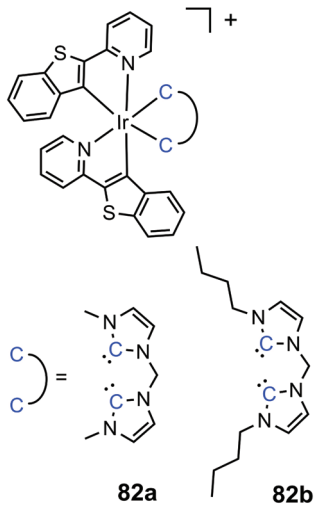
80

4.3. Iridium N-heterocyclic carbene complexes

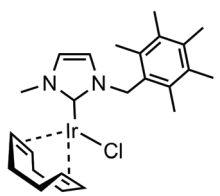
N-Heterocyclic carbene ligands form remarkably stable complexes with late transition metal ions.[353] As such, they have an increasingly important role in the development of new metal-based drug candidates.[354] For example, the Ir(III) complexes **81a**, **81b**, **82a**, and **82b** bearing bidentate NHC ligands were shown to be more effective than cisplatin in eradicating A549 lung cancer cells.[355] These compounds triggered an increase in intracellular ROS levels and depolarization of the mitochondrial membrane potential, ultimately resulting in caspase-dependent apoptosis. A structurally distinct Ir(I) NHC complex (**83**) was also found to possess anticancer activity against MCF-7 breast and HT-29 colon cancer cells.[356] Reactivity of **83** with a representative protein, horse heart cytochrome *c*,[357] was investigated using high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). The resulting HR-ESI-MS spectra revealed the formation of an **83**–cytochrome *c* adduct, confirming that this compound binds covalently. Although these studies illustrate *in vitro* anticancer activity, further *in vivo* investigations are needed to establish this class of complexes as potential drug candidates.



81a **81b**



82a **82b**



83

4.4. Peptide-conjugated iridium complexes

Increasing selectivity for cancer cells is a strategy that should minimize toxic side effects of chemotherapeutic agents. An attractive method for drug design is to utilize peptide sequences that can act as targeting moieties for different cellular receptors.[358,359] For example, complex **84** bearing the peptide sequence, KKGG, was tested for its cytotoxicity against Jurkat lymphocytic leukemia, Molt-4 lymphoblastic leukemia, HeLa cervical, and A549 lung cancer cells, revealing potent activity as characterized by IC_{50} values in the micromolar range.[360] Time-lapse microscopy studies at 37 °C showed significant morpho-

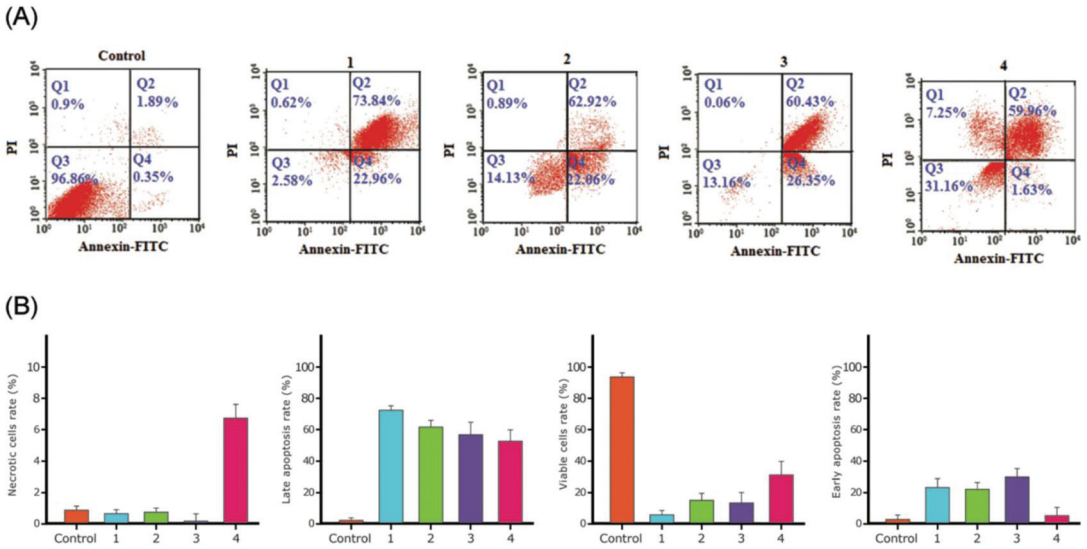
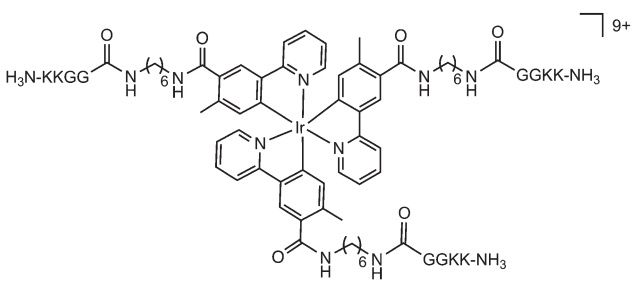


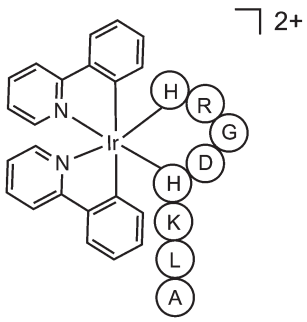
Fig. 7 (A) Flow cytometry analysis of MCF-7 breast cancer cells after an annexin V/PI assay. (B) Flow cytometry analysis data transformed into density plots for untreated cells and treated cells with 10 μM (24 h) of **79a–79d** (labeled 1–4, respectively) are shown. Reprinted with permission from ref. 347. Copyright (2017), with permission from Royal Society of Chemistry.

logical changes of Jurkat cells as they underwent swelling and membrane blebbing. Analogous studies at 4 °C indicated that the complex does not alter cell morphology at low temperatures, which is possibly a consequence of its diminished cellular uptake under these conditions. When cells were treated with Z-VAD-fmk, a pan-caspase inhibitor, and necrostatin-1, a necroptosis inhibitor, in conjunction with **84**, no cytoprotective effects were observed. These results suggest that **84** kills cancer cells through a necrotic pathway, which is independent of the caspases and necroptosis-inducing protein machinery. These complexes were further studied to identify their biological targets. The KKGG peptide sequence was replaced with a KKKGG peptide that was conjugated to the 3-trifluoromethyl-3-phenyldiazirine (TFPD) photoaffinity label. Diazirine photoaffinity label-conjugated compounds can be effectively used to isolate protein targets in complex biological media.[361,362] For this Ir(III) complex, the proteins that were targeted upon photoirradiation were purified by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and complexation of **84** to the protein target was confirmed by liquid chromatography-mass spectrometry (LC-MS/MS). The Ca^{2+} sensor protein, calmodulin,[363–365] was found to be an important target for **84**, indicating that this complex may alter intracellular calcium trafficking as part of its mechanism of action.



84

Similarly, a set of iridium complexes bearing phenylpyridine ligands was covalently attached to short peptides *via* histidine side chains.[366] The most potent complex (**85**), which contains the peptide HRGDHKLA, exhibits an IC_{50} value of 4.5 μM in A549 lung cancer cells. Furthermore, it induces membrane blebbing and nuclear condensation, hallmark features of apoptosis. Unfortunately, the specific biomolecular target was not determined in this study. Further mechanistic studies are required to conclusively identify the macromolecular target for this complex and to verify the mode of cell death that it induces.

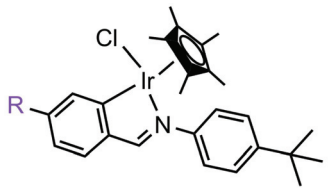


85

4.5. Iridium arene complexes

Among the most common class of iridium anticancer agents studied are those bearing Cp ligands. These so-called "piano-stool" and "half-sandwich" complexes exhibit a diverse range of anticancer activity and varying mechanisms of action.[367–374] For example, compounds **86a–86c** exhibit anticancer activity against lung cancer cells and arrest the cell cycle in the G2/M phase.[368] Results from an annexin V/PI assay indicate that these compounds give rise to apoptotic cell death when administered at a concentration of 5 μM. When dosed at a higher concentration of 10 μM, however, necrotic cell death was oper-

ational. Further mechanistic studies will elucidate how these complexes induce cell death, guiding the rational design of improved analogues.



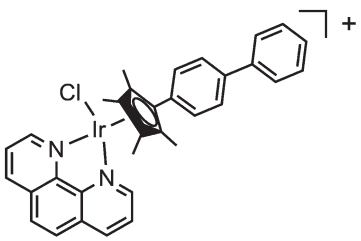
R = COOMe (**86a**), NO_2 (**86b**), CN (**86c**)

86a–86c

This class of compounds can also be used with established anticancer drugs to potentiate their activity. For example, half-sandwich iridium complexes bearing bpy ligands are non-toxic to cancer cells alone. However, when used in conjunction with the platinum-based drugs, cisplatin and carboplatin, a significant synergistic effect was observed. This study indicates that this class of compounds might have broader applications as chemosensitizing agents.[375]

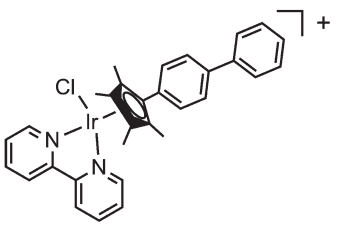
Although usually not considered in most studies, there is recent evidence to suggest that the counterion of cationic half-sandwich Ir(III) complexes may affect biological activity. The previously reported iridium complex,[376] **87**, was prepared with the different counterions, Cl^-, PF_6^-, BF_4^-, SbF_6^-, $CF_3SO_3^-$, BPh_4^-, and $BArF^-$, where $BArF^-$ = $[3,5\text{-}(CF_3)Ph]_4B^-$.[377] Compounds with the Cl^-, PF_6^-, BF_4^-, SbF_6^-, and $CF_3SO_3^-$ counterions were equally effective anticancer agents that gave rise to micromolar IC_{50} values in several cancer cell lines. The compound with the $CF_3SO_3^-$ counteranion induces both early- and late-stage apoptotic cell death and depolarizes the mitochondrial membrane potential. In contrast, the compounds containing the BPh_4^-, and $BArF^-$ counteranions were effectively inactive in lung cancer cells, as indicated by IC_{50} values that exceeded 100 μM. As expected, the inactive compound with the BPh_4^- anion did not perturb cellular function in a manner that was noticeably different from the untreated control. Although these results suggest that the counterion may play an important role in modulating the anticancer activity of cationic complexes, studies to investigate their impact on solubility, which may indirectly alter their biological properties, should be undertaken.

A half-sandwich iridium complex (**88**) induces apoptosis in ovarian and leukemia cancer cells and also fragments nuclear DNA.[378] Cell cycle analysis indicated that **88** stalls cells in the G0/G1 phase. Additionally, apoptosis was investigated as a potential mode of cell death for this complex using enzyme-linked immunosorbent assay (ELISA), a technique used to detect biological targets. This method can be exploited to probe cytoplasmic nucleosomes that are released during the early apoptotic stages caused by DNA fragmentation.[379] The increase in DNA fragmentation, as detected by ELISA, is consistent with an apoptotic mode of cell death. Confocal fluorescence microscopy was employed to visualize the mitochondrial membrane potential, using the probe tetramethylrhodamine ethyl ester (TMRE). This imaging study showed a decrease in mitochondrial membrane potential, as indicated by a decrease in the fluorescence signal of TMRE. Mitochondrial dysfunction was further supported by the ability of complex **88** to induce ROS production. Overall, complex **88** may operate through a dual mechanism of action by causing both nuclear DNA damage and mitochondrial dysfunction.

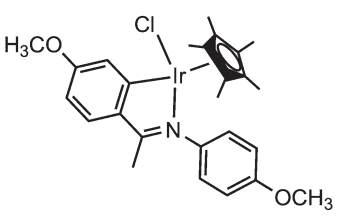


88

Related half-sandwich iridium complexes also induce apoptosis through a caspase-mediated pathway.[380,381] One such complex, **89**, was tested in K562 leukemia cell lines, revealing a remarkably low IC_{50} value of 0.26 μM.[382] Similar to the other iridium piano-stool complexes, **89** increases ROS levels in cells and decreases the mitochondrial membrane potential. Western blot studies showed the upregulation of pro-apoptotic proteins Bax and caspase-9, the downregulation of anti-apoptotic Bcl-2, and the release of mitochondrial cytochrome *c* into the cytosol. These results suggest that **89** induces cell death through a caspase-dependent apoptotic pathway.

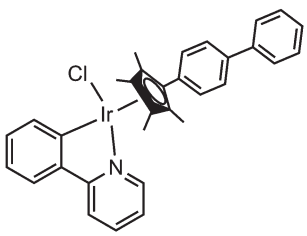


87

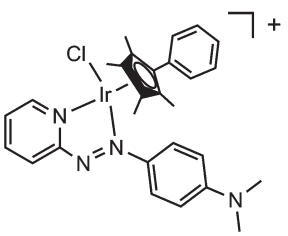


89

Ir(III) complexes (**90a** and **90b**) containing phenylpyridine and *N*,*N*-dimethylphenylazopyridine ligands were screened against 916 cell lines in the Sanger's Genomics of Drug Sensitivity in Cancer panel.[201] The resulting spectra of activity of these complexes in cancer cell lines can further be compared to those of 253 drugs in the Sanger Cancer Genome Database to identify compounds with similar mechanisms of action.[201,383] The results of this study revealed **90b** to have a novel spectrum of activity compared to the majority of the drugs in the database. Compound **90b** was found to be highly active in cell lines containing mutations in the *KIT* gene, which codes for the cytokine receptor, C-KIT, that is over-expressed in blood and bone cancers.[384] A good correlation between the spectra of activity of **90b** and the related Os(II) arene complex, **38**, which bears the same azopyridine ligand was observed, suggesting that this ligand plays a critical role in mediating the mechanism of cell death. Cells treated with **90a** and **90b** were stained with the nuclear-localizing dye, DAPI, and the NucView caspase activity indicator and imaged using confocal fluorescence microscopy (Fig. 8). These results revealed that complex **90b** reduces cell count and induces apoptosis to a greater extent than **90a**. Cell cycle analysis shows arrest in the S/G2 phase, which suggests that the compounds disrupt DNA replication or processes after the cloning of DNA. In contrast, the osmium analogue, **38**, arrested the cell cycle in the G1 phase. Despite this difference, both complex **90b** and **38** induce ROS production and apoptosis. As discussed above, the striking similarities in mechanisms of cell death are most likely a consequence of the azopyridine ligand. These results highlight the important role of the ligand in mediating the activities of metal-based anticancer agents.

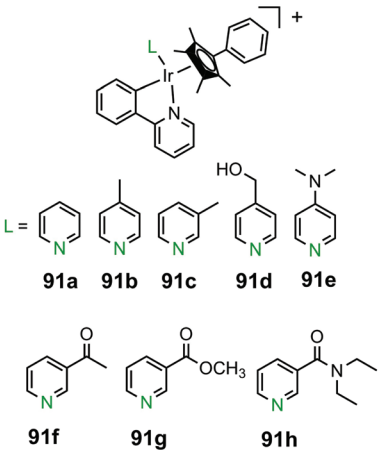


90a



90b

Another series of Ir(III) arene complexes (**91a–91h**) exhibit antiproliferative effects in A2780 ovarian, A549 lung, and MCF-7 breast cancer cell lines.[385] Studies to determine the mode of cell death, however, were inconclusive because hallmark signs of apoptosis or cell cycle arrest were not obvious. Additional studies could shed light on the mechanism of cell death for these complexes and aid in the rational design of future half-sandwich compounds.

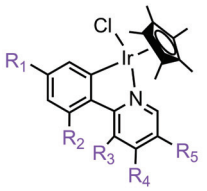


91a **91b** **91c** **91d** **91e**

91f **91g** **91h**

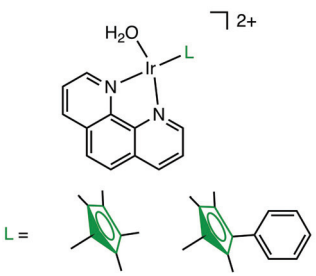
91a–91h

A key component of the mechanism of action of Ir(III) half-sandwich complexes is their ability to cause redox imbalances in cancer cells. This property has been capitalized upon to generate the series of complexes (**92**), which exhibit potent anticancer activities and are competent catalysts for NADH oxi-

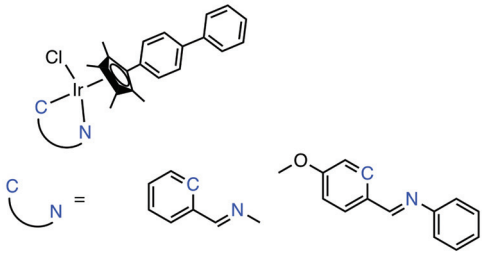


R_1 = H, F, CHO, CH_2OH, OH, NO_2
R_2 = H, F, CH_3
R_3 = H, F
R_4 = H, NO_2, CH_2OH, CH_3
R_5 = H, F, CHO, OH, CH_3

92



93a **93b**



94a **94b**

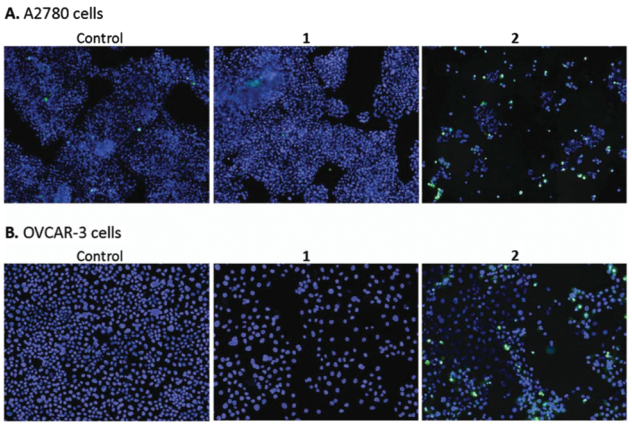


Fig. 8 Fluorescence microscopy images of (A) A2780 and (B) OVCAR-3 ovarian cancer cells after treatment with growth media (control), **90a** (1) at 2.5 μM, and **90b** (2) at 2.5 μM. DAPI stain (blue) was used to locate nuclei and NucView reagent was used as a stain for caspase activity in apoptotic cells (green). Reprinted with permission from ref. 201. Copyright (2017), with permission from Royal Society of Chemistry.

dation.[386] These complexes covalently bind 9-EtG through displacement of the labile halide ligand, indicating that DNA may be a potential biological target as well. Another recent study has implicated a mechanism of action that involves the disruption of cellular NADH levels.[387] This interference results from the ability of two Ir(III) complexes (**93a** and **93b**) to use NADH as a hydride source to catalyze transfer hydrogenation reactions. This process leads to the catalytic consumption of NADH, irreparably altering the cellular redox status. Similar to the complexes above, **93a** and **93b** exhibit potent anticancer activity in a panel of cancer cell lines. These compounds undergo rapid aquation and, like **92**, covalently bind 9-EtG.[386] Upon treatment with these complexes, the intracellular redox balance was disrupted in a manner that was attributed to the catalytic oxidation of NADH to NAD^+. Complexes of a similar type, **94a** and **94b**, were synthesized and tested against cervical cancer cells.[388] No binding to 9-EtG or 9-methyladenine was observed by ^1H NMR spectroscopy. However, catalytic hydride transfer from NADH to NAD^+ and increased ROS levels were detected. Cell cycle analysis indicated that both **94a** and **94b** stall the G1 phase. These results illustrate that the compounds operate *via* a cell cycle-dependent pathway and induce apoptosis through ROS generation.

5. Conclusion

In this Perspective, we summarized recent developments on the use of complexes of rhenium, osmium, and iridium as anticancer agents. Collectively, these studies highlight how the diverse structural, redox, and ligand substitution properties of these complexes can result in varying anticancer activities. Several characteristics emerge with respect to the mechanism of action of these complexes. Namely, many of these compounds trigger the formation of ROS and irreparably impair mitochondrial function. However, small alterations of ligand scaffolds in structurally related compounds can drastically change the mechanism of action, suggesting that a change in target is occurring as well. Although these features are interesting from a fundamental science perspective, they present a challenge for future development and optimization of new drug candidates. The large sensitivity of the mechanism of action for these complexes suggests that a variety of non-specific drug targets is possible. If properly vetted and optimized, however, this feature can lead to the development of improved chemotherapeutic agents that are substantially less susceptible to drug-resistant pathways. One clear advantage for using rhenium, osmium, and iridium complexes is their ability to be exploited for simultaneous imaging applications due to their rich spectroscopic properties. As highlighted in this Perspective, the structural diversity and unique features of these compounds will drive continued research on their development as new drug candidates for cancer treatment.

Abbreviations

AFM	Atomic force microscopy
$BArF^-$	$[3,4\text{-}(CF)_3Ph]_4B^-$
bpy	2,2′-Bipyridine
BSO	L-Buthionine sulfoximine
BTCP	2-Bicyclo[2.2.1]hept-en-yl-1*H*-1,3,7,8-tetraazacyclopenta[*l*]phenanthrene
CAM	Chorioallantoic membrane
CD	Circular dichroism
Cdk-2	Cyclic-dependent kinase
cGMP	Cyclic guanosine monophosphate
CO	Carbon monoxide
Cp	Cyclopentadienyl
CSCs	Cancer stem cells
ct-DNA	Calf thymus DNA
DCA	Dichloroacetate
DCFH-DA	2′,7′-Dichlorodihydrofluorescin diacetate
EA	Ethacrynic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ERα	Estrogen receptor α
9-EtA	9-Ethyladenine
9-EtG	9-Ethylguanine
FT-ICR MS	Fourier transform ion cyclotron resonance mass spectrometry
GLUTs	Glucose transporter proteins
GSH	Glutathione
GSK-3β	Glycogen synthase kinase 3β
GST	Glutathione S-transferase
HDAC	Histone deacetylase
H&E	Haematoxylin–eosin
HEWL	Hen egg white lysozyme
HIF	Hypoxia-inducible factor
HR-ESI-MS	High-resolution electrospray ionization mass spectrometry

HSA	Human serum albumin
IC_{50}	50% growth inhibitory concentration
ICP-MS	Inductively coupled plasma mass spectrometry
LA-ICP-MS	Laser ablation inductively coupled plasma mass spectrometry
LC-MS/MS	Liquid chromatography-mass spectrometry
LTDR	LysoTracker deep red
MDC	Monodansylcadaverine
^{3}MLCT	Triplet metal-to-ligand charge transfer
mTOR	Mammalian target of rapamycin
NCI	National Cancer Institute
NHC	N-Heterocyclic carbene
NO	Nitric oxide
NSAID	Non-steroidal anti-inflammatory drug
PARP	Poly(ADP-ribose) polymerase
PBS	Phosphate-buffered saline
PCA	2-Pyridinecarbothioamide
PDK	Pyruvate dehydrogenase kinase
PEG	Poly(ethylene)glycol
PI	Propidium iodide
PI3	Phosphoinositide 3-kinase
PTA	1,3,5-Triaza-7-phosphaadamantane
ROS	Reactive oxygen species
SARs	Structure–activity relationships
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
SPECT	Single-photon emission computed tomography
TEM	Transmission electron microscopy
TFPD	Trifluoromethyl-3-phenyldiazirine
TMRE	Tetramethylrhodamine ethyl ester
XIAP	X-Linked inhibitors of apoptosis protein

Conflicts of interest

The authors declare no competing financial interests.

Acknowledgements

We thank Samantha Davalos for assistance in preparing the artwork associated with this manuscript. Work in our laboratory is supported by Cornell University and by the Office of the Assistant Secretary of Defense for Health Affairs through the Ovarian Cancer Research Program under award no. W81XWH-17-1-0097.

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