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Next-Generation Metal Anticancer Complexes: Multitargeting via Redox Modulation

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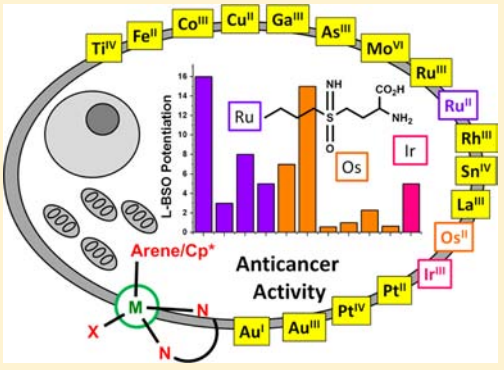
Supporting Information

ABSTRACT: Platinum complexes are widely used anticancer drugs. New generations of metal chemotherapeutics offer the prospect of combating platinum resistance and expanding the range of treatable cancers. Such new complexes might be effective if they form distinctly different lesions on DNA. In this Forum Article, we discuss the possibility that targeting the redox balance in cancer cells may also be a highly effective strategy, especially because it is a multiple-site approach and offers selectivity over normal cells. Metal complexes can interfere in cellular redox chemistry in several ways: directly through metal or ligand redox centers or indirectly by binding to biomolecules involved in cellular redox pathways. We illustrate that a surprisingly large number of active metal anticancer agents have a potential redox arm to their mechanism of action. For such complexes, the possibility arises of using combination therapy together with redox modulators to increase the anticancer potency: attractive for lowering the doses of metal complexes that need to be administered. We illustrate that organometallic ruthenium(II) and osmium(II) arene complexes and iridium(III) cyclopentadienyl complexes of the type $[(\text{arene/Cp}^{\text{xPh}})\text{M}(N,N)\text{Cl/I}]^{n+}$ can achieve nanomolar potency toward cancer cells in combination with the redox modulator L-buthionine sulfoximine. Our discussion highlights the importance of determining not only the distribution of metal anticancer complexes in cells but also their speciation, the chemical form of the metal complex, including the oxidation state of the metal, the fate of the ligands, and dynamic processes such as efflux. This will be aided in the future by proteomic and genomic analyses but needs to be supplemented by new analytical methods that have the sensitivity and spatial and temporal resolution to reveal such information. To achieve this, major new funding programs are needed that support global research on the design of novel metal-based drugs with new mechanisms of action, tailored to patient needs.



INTRODUCTION

The most well-known metal-based anticancer drug cisplatin (CDDP) arose from serendipity.[1,2] Although the methods of modern molecular biology have given us a more detailed understanding of its target site and mechanism of action, there is still much to be explained.[3]

Metal complexes provide a highly versatile platform for drug design. Besides variations in the metal and its oxidation state, metal ions have a range of geometries and coordination numbers that allow the fine-tuning of their chemical reactivity in terms of both kinetics (rates of ligand exchange) and thermodynamics (strengths of metal−ligand bonds, redox potentials, etc.). Not only the metal but also the ligands can play important roles in biological activity, ranging from outer-sphere recognition of the target site to the activity of any released ligands and ligand-centered redox processes.

Here we explore the potential role of redox modulation in the mechanism of action of metal anticancer complexes, in particular for platinum, gold, gallium, arsenic, ruthenium, osmium, and iridium complexes. To what extent is modulation of cellular redox processes involved in their activity? Our discussion will highlight an important area for further work in this field: the need for advances in elucidating the spatial and temporal speciation of metallodrugs in organs, tissues, and cells and the need to determine the fate of both the metal and ligands. If there are changes in the redox state of the cell, do they involve redox processes centered on the metal and/or on the ligands? Or do they arise indirectly from interference in normal redox processes in cells? Table 1 gives examples of the direct, indirect, or potential involvement of redox reactions in the mechanism of action of metal-based anticancer complexes.

In general, metal complexes are "pro-drugs" that become transformed by ligand substitution and redox processes before they reach the target site. It is challenging to define the "pharmacophores" for metal complexes: the steric and electronic features necessary for target recognition and for triggering the biological response.[4,5] This is straightforward only for truly inert metal complexes, such as some third-row low-spin d^6 transition-metal complexes.[6] However, even such low-spin d^6 complexes can be

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Table 1. Examples of the Involvement of Redox Reactions in the Activity of Metal Anticancer Complexes

metal	redox reaction	example	ref
Fe	formation of phenoxy radicals	hydroxyferrocifen	213
	ferrocene-mediated proton-coupled electron transfer	ferrocifen derivatives	214, 215
Co	reduction of Co^{III} to Co^{II}		216, 217
			218, 219
	lipid peroxidation	$[Co(acac)_2(en)(nic)_2]Br$	220
		$[Co(acac)_2(en)(isonic)_2]Br$	
	generation of $O_2^{\bullet-}$	$[Co(acac)_2(en)(nic)_2]Br$	221
Cu	altered GSH levels and changes in the mitochondrial membrane potential	$[Cu(4,7\text{-}Me_2\text{-}phen)(gly)]NO_3$	222
Ga	interference in Fe^{III} metabolism		109
	increased ROS levels and depleted GSH	$Ga(NO_3)_3$	109
	changes in the mitochondria membrane potential	$GaCl_3$, $Ga(maltolate)_3$	110
	inhibition of ribonucleotide reductase	KP46	112
	disturbed endoplasmic reticulum function	KP2235	114
As	increased ROS and depleted GSH	As_2O_3	118, 119
	increased H_2O_2 levels	As_2O_3	120
Mo	increased ROS	tetrathiomolybdate	208
Ru	Ru^{III} reduced to Ru^{II}	NAMI-A	156, 169
		KP1019	169
	reaction with GSH	NAMI-A	169
		KP1019	
		RM175	175, 176
	nitric oxide ($NO^{\bullet}$) scavengers	NAMI-A, KP1019	171
	reduction of the N═N bond	$[Ru(\eta^6\text{-cym})(p\text{-Azpy-NMe}_2)I]^+$	180
	inhibition of TrxR	RAPTA-C	148
La	inhibits ribonucleotide reductase	$[La^{III}(1,10\text{-phenan})_3](CN)_3$ (KP772)	223, 224
Os	generates ROS	$[Os(\eta^6\text{- }p\text{-cym})(p\text{-impy-NMe}_2)I]^+$	189
		$[Os(\eta^6\text{-bip})(p\text{-Azpy-NMe}_2)I]^+$	
	oxidizes NADH to NAD^+	$[Os(\eta^6\text{-}p\text{-cym})(p\text{-impy-NMe}_2)Cl]^+$	189
Ir	generates ROS	*fac*-$[IrCl_3(DMSO)(phen)]$	149
		$[Ir(\eta^5\text{-}C_5Me_4C_6H_5)(phpy)(py)]PF_6$,	192
	oxidizes NADH to NAD^+	$[Ir(\eta^5\text{-}C_5Me_4C_6H_5)(phen)Cl]PF_6$	193
	oxidizes quinones to semiquinones	$[Ir(C_5Me_4)(phen)H_2O]PF_6$	194
Pt	reaction with intracellular GSH	CDDP	69
		$[Pt(1,2\text{-DACH})Cl_2]$	82
	inhibition of TrxR	CDDP	22, 70
		nedaplatin	81
	depletion of NADPH causing increased $OH^{\bullet}$ and O_2^- levels	CDDP	22, 72
	increased levels of H_2O_2	OXA	38, 78
	decomposition after reaction with sulfur-containing proteins	BBR3464	86, 87
	photoactivation from nontoxic Pt^{IV} to potent Pt^{II}	FM190	161, 162
	generation of azidyl radicals	FM190	164
Au	inhibition of TrxR	auranofin	22, 91
		AUOXO1	104
		$[(\hat{C}\hat{N}\hat{C})_2Au^{III}{}_2(m\text{-dppp})](CF_3SO_3)_2$	102
		$[Au^{I}X(PPhen_3)]$	93, 94
		AUL12	90, 98
		$[Au^{I}(H_2Ac4Me)Cl]$	92
	generation of ROS, particularly H_2O_2	AUL12	90, 99
	changes in the mitochondrial potential	$[Au(TPP)]^+$	100

relatively labile, depending on the choice of ligands. For many complexes, the challenge is to control the activation by substitution (ligand exchange) and/or redox processes in order to trigger the biological response in the desired region of tissues and at the optimum time. If this can be achieved, then such drug design offers unique features not available to purely organic drugs.

CANCER AND REDOX CHEMISTRY

Cancer, defined by the World Health Organization as "the uncontrolled growth and spread of cells", is responsible for at least 13% of deaths worldwide. Current statistics indicate that 1 in every 3 people will develop some form of cancer during their lifetime. It is estimated that by 2030 there will be 21.4 million new cases diagnosed every year.[7]

During the first half of the 20th century, surgery and radiotherapy dominated cancer treatment. Chemotherapy, as a viable alternative, was first considered in the 1940s when nitrogen mustards were used against lymphomas. Since then, extensive research into drugs that combat the proliferation of

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malignant tissue has produced significant cures and/or improvement of life expectancy for cancer patients.[8]

The lack of selectivity has often been presented as the first downside of chemotherapy, mostly because of the severity of undesired side effects, which range from nausea and vomiting to acute renal failure. Theoretically, a reduction of side effects could be achieved by increasing the dosage of the drug that reaches the diseased tissue while reducing the dosage that reaches and affects normal surrounding tissue/organs. However, in practice, selectivity is a much more complicated issue. Most anticancer agents currently in clinical use rely on the high proliferation rate of neoplastic tissue as a way of achieving selectivity.[9] This results in side toxicity to tissues and organs that also exhibit frequent cellular replacement such as the bone marrow and gastrointestinal tract.[10]

The second problem encountered with chemotherapy is the high incidence of resistance, which can be classified as inherent or acquired. Inherent resistance usually has pleiotropic origins and determines the selectivity of a neoplastic lesion to chemotherapy. This resistance is the basis of the Goldie–Coldman hypothesis,[11] which states that resistance can arise from spontaneous mutations that inevitably occur in cell proliferation as part of intrinsic genetic instability.[12] In comparison, acquired resistance develops after an initial exposure to chemotherapeutics. Multidrug resistance (MDR) can emerge as a cellular response to chemotherapeutic agents.[13] The family of proteins known as MDR-associated proteins (MRPs) are often organic anion transporters, although MRP1, MRP2, and MRP3 can also transport neutral molecules, which function as efflux pumps in order to reduce intracellular drug concentrations. The most widely known protein of this family is P-glycoprotein discovered in 1976.[14,15] Mutations in drug targets as a mechanism of acquired resistance have been extensively investigated. Such is the case for chronic myeloid leukemia. This type of cancer is often treated with imatinib. However, up to 40% of cases develop some resistance because of a mutation in the kinase domain of breakpoint cluster region protein (BCR).[16−18]

Besides mutations in drug targets, other prominent molecular mechanisms of acquired resistance include increased drug efflux, activation of downstream or parallel signaling pathways, and altered drug metabolism.[19] The development of drug resistance is not limited to cancer treatment and can be a critical factor in the management of diseases such as malaria, tuberculosis, and human immunodeficiency virus (HIV).[20,21]

The redox balance is tightly regulated in living organisms. The disturbance of this balance is at the center of many diseases, including cancer[22,23] and neurological disorders.[24] Although the generation of reactive oxygen species (ROS) is a normal process occurring in all living cells with important signaling functions,[25] increased levels of $OH^{\bullet}$, $O_2^{\bullet-}$, and H_2O_2 are common in neoplastic tissue because cancer cells usually exhibit disturbed mitochondrial function.[22,26,27] It is also important to note that the redox environment in a cell is very varied and differs within different cellular compartments.[28]

This tight redox balance inside cells is maintained by enzymatic and nonenzymatic reactions. The latter involve, for example, glutathione (GSH) and thioredoxin (Trx), while the former involve reductases, oxidases, and peroxidases. GSH is mainly involved in the deactivation of hydroxyl radicals $OH^{\bullet}$ to produce water, and superoxide $O_2^{\bullet-}$ to generate H_2O_2, which can subsequently be involved in Fenton-like reactions that transform H_2O_2 to H_2O.[29] Table 2 shows the most common biologically relevant reactions involving ROS.[22]

Table 2. Some Redox Potentials for ROS (from Reference 22)

ROS	reaction	E° (V)
$OH^{\bullet}$	$OH^{\bullet} + e^- + H^+ \rightleftharpoons H_2O$	+2.31
$O_2^{\bullet-}$	$O_2^{\bullet-} + e^- + 2H^+ \rightleftharpoons H_2O_2$	+0.94
	$O_2 + e^- \rightleftharpoons O_2^{\bullet-}$	−0.16
H_2O_2	$H_2O_2 + e^- + H^+ \rightleftharpoons H_2O + OH^{\bullet}$	+0.32

Manipulation of the ROS levels may provide a highly effective strategy for treating cancer, as highlighted recently by Watson,[30] Jungwirth et al.,[22] and Trachootham et al.[31] Redox vulnerability in cells arises from excessive energy requirements and can be exploited by the use of antioxidant-lowering or ROS-producing drugs.[30] The latter strategy is being used to target K-ras mutant cancer cells selectively. In this case, the ROS produced by lanperisone are inefficiently scavenged in mutant cells, which, in turn, results in nonapoptotic cell death.[32] Although the molecular mechanism by which elevated ROS levels result in cell death is still poorly understood, there is much current effort to target the stress response to ROS using small organic molecules.[33−37]

The generation of free radicals by anticancer drugs, metal-based or not, and subsequent elevated levels of oxidative stress have been related to oncogenic stimulation.[23,38] However, the activation of antioxidant cascades that modify the tumor microenvironment has been associated with a positive patient prognosis, particularly in the case of breast cancer.[39]

INDIRECT INVOLVEMENT OF REDOX CHEMISTRY IN THE ACTIVITY OF METAL-BASED ANTICANCER DRUGS

Platinum(II). Since the serendipitous discovery that CDDP (Figure 1a) can arrest cell division of *Escherichia coli*, platinum coordination complexes have been widely investigated as anticancer agents.[3] CDDP was first synthesized in 1845 by Perone[40] and its structure proposed in 1893 by Werner, but it was not until 1965 when the observations of Rosenberg et al.[1,2] started a new field for platinum chemistry and its medical applications.[41] CDDP was the first platinum-based drug with antineoplastic activity approved by the U.S. Food and Drug Administration (FDA; 1978). Currently, it is used alone or in combination with other chemotherapeutic drugs against bladder and advanced cervical cancer that cannot be treated with surgery or radiotherapy, as well as in nonsmall cell lung or ovarian cancer that are locally advanced or have metastasized. It is also used to treat malignant mesothelioma, squamous cell carcinoma of the head and neck, and testicular cancer.[42]

It is widely accepted that the antineoplastic properties of CDDP rely on its interaction with DNA, which, in turn, activates apoptosis.[3] However, this is a reductionist view of a process in which several important events are involved from drug administration to cellular death. CDDP is administered directly into the bloodstream. The chloride concentration in blood plasma is ca. 100 mM,[43] and this allows CDDP to remain intact. It is thought that protein binding can deactivate the platinum drug at this stage, especially binding to sulfur-containing amino acid side chains (Met and Cys) in metallothionein or albumin.[44] Cellular accumulation of CDDP occurs by means of diffusion and active transport via the copper transporter CTR1.[45−47] Once in the cytoplasm of the cell, the concentration of chloride is reduced to ca. 20 mM and partial aquation of CDDP can occur, and even more so in the nucleus, where the chloride concentration is ca. 4 mM. Aquated forms of CDDP are more

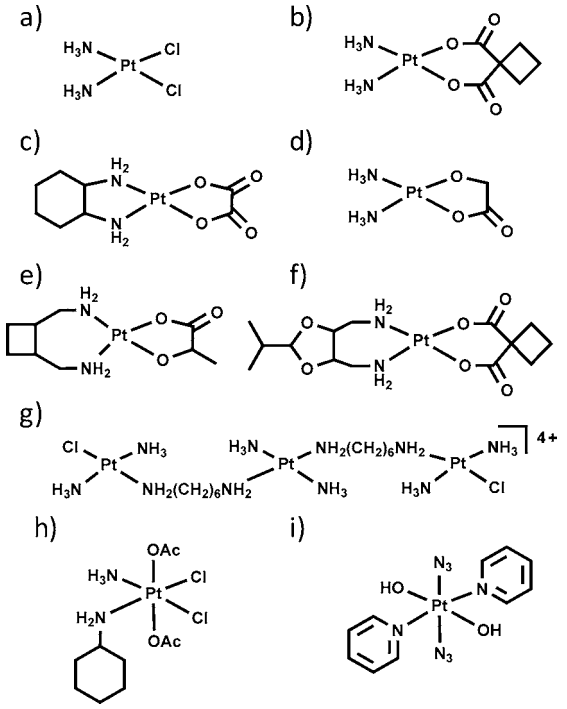

Figure 1. Platinum anticancer agents: (a) CDDP; (b) carboplatin; (c) OXA; (d) nedaplatin; (e) lobaplatin; (f) heptaplatin; (g) BBR3464; (h) JM216; (i) FM190.

reactive and bind to DNA and can form monofunctional adducts that further evolve into cross-links, especially intrastrand (GG) cross-links.[43,48−51]

CDDP−DNA lesions can be repaired by three distinct mechanisms: (1) nucleotide excision repair, NER; (2) mismatch repair, MMR; (3) DNA-dependent protein kinase repair, DNA-PK. NER is the most common mechanism of the three. An ATP-dependent protein recognizes the DNA lesion, especially 1,2-intrastrand cross-links, and excises the damaged DNA strand section for subsequent filling of the gap by DNA polymerase.[52] Although the DNA lesions caused by CDDP and their repair processes have been extensively investigated, the detailed mechanism by which they lead to apoptosis remains poorly understood.[43,53]

One of the major challenges in the use of CDDP as a chemotherapeutic agent for cancer treatment is to combat the high incidence of resistance.[54] Such resistance can result from one of the following mechanisms: (a) impaired cellular accumulation as a consequence of reduced cellular uptake or increased cellular efflux; (b) deactivation by binding to sulfur-containing proteins; (c) increased repair of DNA lesions.[55−61] CDDP administration can also cause severe side effects, including nephrotoxicity,[62,63] neurotoxicity,[64,65] ototoxicity,[66,67] nausea, and vomiting. These side effects are mostly caused by the lack of drug selectivity.

Although DNA interactions play a significant role in the mechanism of action of CDDP, only 1% of intravenously administered CDDP reaches the cell nucleus. Therefore, it is of interest to consider the fate of the remainder of the drug. CDDP is known to react with GSH. Although there are reports that at least 60% of CDDP reacts with GSH to form the product $[Pt(GS)_2]$,[68] it has been debated that the formation of 1:1 or 1:2 GSH complexes is not responsible for the inactivation of the drug.[69] CDDP, as well as the GSH−CDDP adducts, are capable of inhibiting the selenoenzyme thioredoxin reductase (TrxR).[22,70] TrxR contains FAD and NADPH binding domains and a redox-active disulfide (Cys−Cys) bond in its active site. It transfers electrons to Trx, which, in turn, reduces disulfide bonds and other substrates. Mammalian TrxRs contain a second redox-active site, a C-terminal −Cys−SeCys− (SeCys = selenocysteine).[71] Also, CDDP treatment alters mitochondrial function by depleting NADPH, which may increase $OH^{\bullet}$[22] and O_2^{-}.[72] This involvement of CDDP with redox homeostasis has been linked to side effects such as nephrotoxicity[73,74] and hepatic malfunction.[75] Hence, indirectly, some of the biological effects of CDDP appear to be linked to redox perturbations in the cell. It has also been suggested that, in head and neck cancer, the interaction of CDDP with mitochondria is crucial for the clinical activity of the drug.[76,77]

Two other *cis*-diam(m)ineplatinum(II) complexes have gained FDA approval, carboplatin (Figure 1b) in 1989 and oxaliplatin (OXA; Figure 1c) in 2002, the latter gaining European approval in 1996. Carboplatin is approved for treatment of nonsmall cell lung cancer and ovarian cancer with locally advanced lesions or derived from tumor recurrence. Advanced or recurrent colorectal cancers or stage III colon cancers may be treated with OXA.[42] The failure of combination therapy involving OXA and cetuximab for the treatment of colorectal cancer with wild-type K-Ras has its origins in redox-related mechanisms. The efficiency of the platinum drug is highly linked to the production of H_2O_2,[38] while the monoclonal antibody inhibits H_2O_2 production via inhibition of EGFR/Ras/Nox1; these opposing effects result in limited drug efficacy.[78]

Carboplatin and OXA are thought to possess mechanisms of action similar to those of CDDP, related to attack on DNA.[79] Although carboplatin shows reduced side effects and OXA shows improved performance toward colorectal cancers, CDDP is still the chemotherapeutic agent of choice and is more widely used. These second-generation platinum compounds suffer from the same high incidence of acquired resistance and lack of selectivity, for which the development of novel platinum complexes has been a major goal during the last two decades.[43]

Compounds similar in structure to CDDP, *cis*-$[PtX_2(amine)_2]$, where X = hydrolyzable anionic ligand, have been widely studied, including both primary and secondary amines. As a result, nedaplatin (Figure 1d) is in clinical use in China, while lobaplatin (Figure 1e) and heptaplatin (Figure 1f) are used in Japan and South Korea, respectively. However, their clinical use has not spread worldwide.[80] The mechanism of action of nedaplatin, in particular, has been associated with the inhibition of TrxR.[81]

Mononuclear complexes such as $[Pt(1,2\text{-DACH})Cl_2]$, where DACH = diaminocyclohexane, undergo in vitro reactions similar to those of CDDP with biologically relevant thiols. In this case, $[Pt(1,2\text{-DACH})Cl_2]$ gives rise to 1:1 Pt/GS complexes like $[Pt(1,2\text{-DACH})(\mu_2\text{-SG})]$, as well as 2:2 and 2:1 analogues. These GSH derivatives have been studied under physiologically relevant conditions, at pH 7 and 310 K, and may well aid the transport of the complex or hinder its activity.[82]

Di- and trinuclear platinum complexes with metal centers linked by an alkanediamine chain can also have potent anticancer activity.[83] Complex $[\{trans\text{-}PtCl(NH_3)_2\}_2\text{-}\mu\text{-}\{trans\text{-}Pt(NH_3)_2(H_2N(CH_2)_6NH_2)_2\}]^{4+}$ (BBR3464; Figure 1g) was the first of this series to enter clinical trials.[84] BBR3464 forms inter- and intra-strand cross-links with DNA and shows increased potency, compared to CDDP, in a broad range of tumors.[85] In particular, the complex can undergo decomposition upon reaction with sulfur-containing proteins: substitutions

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of the chlorine in Pt−Cl bonds by sulfur can result in bridge cleavage.[86,87]

There are examples of direct redox reactions of platinum(II) anticancer complexes that may be of biological importance, although their significance has yet to be demonstrated in a biological system. The first involves the reduction of disulfide bonds. For example, $[Pt(en)Cl_2]$ reacts with GSSG to give the dinuclear thiolate-sulfur-bridged dimer $[\{Pt(en)(\mu^2\text{-SG})\}_2]$.[88] Such reactions might also occur with disulfide bonds in proteins. The second involves the spontaneous (air) oxidation of Pt^{II} to Pt^{IV}, especially *trans*-diamine anticancer complexes. For example, *trans*-$[PtCl_2(NH_3)$(2-Me-butylamine)$]$, a complex with activity comparable to that of CDDP, undergoes facile oxidation in an aqueous solution.[89] Could oxidation of Pt^{II} to Pt^{IV} occur in cells bearing in mind that not only O_2 but other oxidants are present? If this occurred on DNA, for example, then the platinum would become very firmly bound.

Gold(I). The activity of gold compounds is often associated with inhibition of the enzyme TrxR, although validation of this target has not usually been investigated. Gold(I) has a high affinity for "soft" ligands such as sulfur and selenium, and both cysteine and selenocysteine residues play important roles in the function of this enzyme. Gold(I) can bind strongly to thiolates and selenates by ligand-exchange reactions, which are normally quite rapid for linear two-coordinate gold(I).[90] TrxR inhibition by gold(I) complexes, in general, can disturb mitochondrial function and generate elevated ROS levels, which, in turn, may decrease the mitochondrial membrane potential. Tetraacetyl-β-D-thioglucosegold(I) triethylphosphine (auranofin; Figure 2a), however, does not cause lipid peroxidation by the generation of H_2O_2 or enhanced nitric oxide production, nor does it alter the GSH levels, regardless of the potency of TrxR inhibition.[22] Auranofin does not inhibit glutathione peroxidase or glutathione reductase, in contrast to some gold(III) complexes.[91] It is not yet clear whether oxidative stress contributes to auranofin's anticancer activity. Thiosemicarbazone derivatives of gold(I), like $[Au(H_2Ac4Me)Cl]$ where H_2Ac4Me = *N*-(4)-methyl-2-acetylpyridine thiosemicarbazone (Figure 2b), inhibit TrxR at micromolar concentrations and are active in acute myeloid leukemia HL60.[92] The selenoenzyme TrxR is also inhibited by gold(I) phosphines such as $[AuX(PEt_3)]$, where X = Cl, Br, CN, or SCN, which exhibit submicromolar activities against lung (A549) and breast (MCF7) cancers, as well as leukemia (HL60). Interestingly, similar to the mechanism observed for auranofin, these gold(I) complexes selectively inhibit TrxR over glutathione reductase or glutathione peroxidase.[93] The same selectivity has been observed in (alkynylphosphine)gold(I) derivatives such as [2-(4-methoxyphenyl)ethyn-1-yl](triphenylphosphine)gold(I) or [3-(phenylmethoxy)-prop-1-yn-1-yl](triphenylphosphine)-gold(I), for which the ratio between the inhibitory activity for glutathione reductase and TrxR is 228 and 406, respectively.[94]

Disruption of other mitochondrial functions, besides TrxR inhibition, is also possible with cationic N-heterocyclic carbene (NHC) complexes. These are capable of interfering with mitochondrial membrane potentials and subsequent induction of apoptosis. Newly investigated targets for NHC complexes also include protein tyrosine phosphatases and the activation of cellular proteins such as Chk1, Chk2, tumor suppressor protein 53 (p53), and p21.[95−97]

Gold(III). Gold(III) complexes are isoelectronic ($5d^8$) and isostructural (square-planar) with platinum(II) and so have attracted interest as potential anticancer agents. However, rather rigid chelation is required to achieve stability, and the active

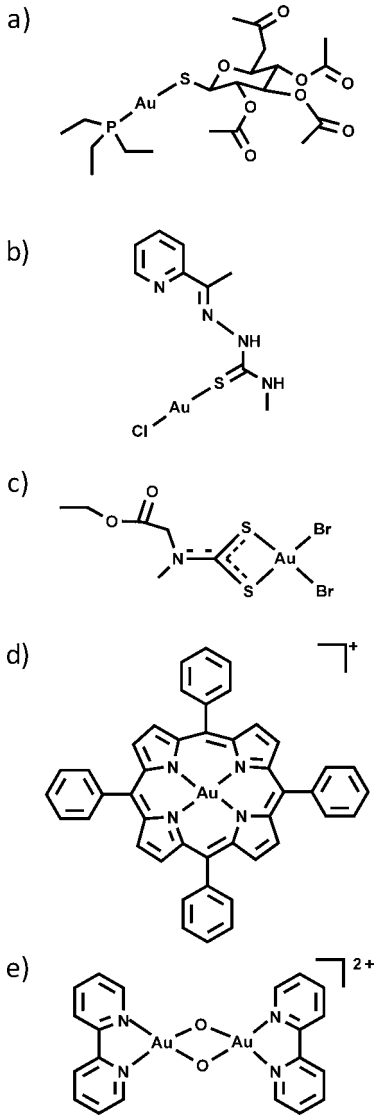

Figure 2. Gold anticancer complexes: (a) Auranofin; (b) [Au(*N*-4-methyl-2-acetylpyridine)Cl]; (c) AUL12; (d) [Au(TPP)]Cl; (e) AUOXO1.

gold(III) complexes that have emerged appear to act via a mechanism different from that of CDDP.

The gold(III) dithiocarbamato complexes developed by the Fregona group[98] trigger cell death after generating ROS, in particular H_2O_2, and promoting modifications of the mitochondrial function.[99] These dithiocarbamato complexes do not alter mitochondrial respiration, so it is proposed that their activity is linked to a disturbed TrxR system rather than to an increased activity in the electron-transport chain.[90] In vivo studies of $[Au^{III}Br_2(ESDT)]$ (AUL12; Figure 2c) toward murine tumor models show up to 80% inhibition of tumor growth with reduced nephrotoxicity and minimum weight loss. Other promising gold(III) candidates are the porphyrinato derivatives developed by Che and Sun, which include the complex $[Au(TPP)]^+$ (Figure 2d).[100] This has shown good activity in nasopharyngeal and hepatocellular carcinoma, colon cancer, neuroblastoma, melanoma, and promyelocytic leukemia. Moreover, it has shown satisfactory in vivo activity in an orthotropic rat HCC model with McA-RH7777 inducing necrosis as well as apoptosis in the tumor tissue. This gold porphyrin complex is able to inhibit tumor pro-

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liferation without causing considerable weight loss.[101] $[Au(TPP)]^+$ appears to cause changes in the mitochondrial potential and, subsequently, suppresses the Bcl-2 protein, inducing cell death by apoptosis.[100] Also, this complex binds noncovalently to HSA, which might play a role in its transport. Che et al. have also investigated phosphine complexes that target TrxR, such as $[(C\hat{}N\hat{}C)_2Au_2(\mu\text{-dppp})](CF_3SO_3)_2$, where C^N^C = 2,6-diphenylpyridine and dppp = bis(diphenylphosphino)-propane.[102]

The dinuclear oxo-bridged gold(III) complexes $[Au^{III}_2(2,2'\text{-bipy})_2(\mu\text{-O})]^{2+}$ (AUOXO1; Figure 2e) and AUXO5 developed in the Messori group are active toward A2780 ovarian cells, and, most importantly, they retain potency in the CDDP-resistant A2780cis cell line.[103] In these binuclear complexes, the gold centers are linked through a dioxo bridge and each Au^{III} ion is bound to an N,N-chelating ligand, 2,2′-bipyridine.[104] In this case, the mechanism of action seems to be primarily related to protein interaction because their direct inhibition of TrxR activates the release of cytochrome *c*. However, COMPARE analysis of AUOXO1 has been linked to the inhibition of protein kinase C.[105,106]

Gallium(III). Gallium-based compounds have been explored in the clinic as antineoplastic agents.[107,108] Oral administration of gallium salts such as $Ga(NO_3)_3$ results in low toxicity, which allows chronic treatment. Gallium nitrate has reached phase II clinical trials with promising results in the treatment of bladder carcinoma and lymphomas. Although it is known that this complex increases intracellular ROS and lowers the levels of GSH within 1 h of exposure, it is possible that redox imbalance may not be the primary mechanism of action involved in its cytotoxicity because coadministration experiments of gallium nitrate and NAC do not modulate the induction of apoptosis.[109]

Gallium chloride and maltolate (Figure 3a) have also been investigated;[108] the latter underwent phase I clinical trials on

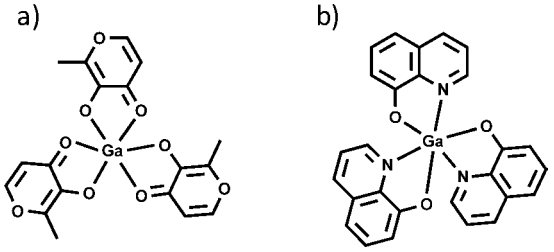

Figure 3. Gallium complexes: (a) gallium(III) tris(maltolate); (b) KP46.

patients with prostate cancer, refractory bladder cancer, and malignant lymphoma. It has also been studied in combination with the proteasome inhibitor bortezomib, showing a loss in the mitochondrial membrane potential and activation of caspase 3.[110] In general, gallium(III) compounds appear to induce calcium efflux from mitochondria, an important step that triggers apoptosis.[108] More specifically, cells exposed to gallium(III) complexes undergo intrinsic apoptosis after Bax activation and cytochrome *c* release.[109]

The complex tris(8-quinolinolato)gallium(III) (KP46; Figure 3b) is capable of inhibiting tumor growth, and there is clinical evidence of its activity in renal cell carcinoma.[111−113] In this case, the lipophilic ligands allow oral administration of the drug.[98,111] It is generally believed that the antiproliferative activity of gallium maltolate and KP46 is directly related to inhibition of ribonucleotide reductase, the iron-dependent enzyme essential for DNA synthesis.[112] More recently, another gallium-based compound, KP2235, which targets the functionality of the endoplasmic reticulum, has completed preclinical trials.[114]

Gallium(III) can often substitute for iron(III) in proteins, which can result in disrupted-iron homeostasis.[109] However, unlike iron, gallium(III) is not redox-active under physiological conditions,[115,116] One strategy to improve the activity of gallium(III) complexes is the preadministration of iron chelators. This strategy allows higher binding to transferrin and subsequently increases cellular uptake via transferrin-receptor-mediated endocytosis. The ability to inhibit ribonucleotide reductase is probably a consequence of gallium−iron substitution.[115]

Arsenic(III). Arsenic trioxide, commercialized as Trisenox, has been in clinical use for acute promyelocytic leukemia (APL) since its FDA approval in 2000, although its antileukemic properties were first reported in 1878 in Boston when Fowler's solution was shown to affect white blood cell counts.[117] Arsenic exposure disturbs the natural redox balance in cells because it is capable of reducing GSH levels. Increments in ROS levels have been related to both the anticancer[118] and mutagenic properties of arsenic derivatives.[119]

The mechanism of action of arsenic trioxide toward APL seems to be linked to the down-regulation of Bcl-2, leading to apoptosis. In mitochondria, As_2O_3 [$As(OH)_3$ in solution] inhibits glutathione peroxidase, causing higher levels of H_2O_2 and subsequent changes in the membrane potential. These events result in cytochrome *c* release into the cytosol and the activation of programmed cell death via caspase-dependent pathways.[120]

More recently, arsenic trioxide has been investigated in solid tumors, such as cervical cancer. In this case, the cytotoxic activity is initiated by changes in the mitochondrial membrane potential, which allows translocation of the apoptosis-inducing factor to the nucleus and subsequent cell death via a caspase-independent pathway. This mechanism also requires PARP-1 to be activated by ROS-mediated mechanisms.[118]

Ruthenium(II). Ruthenium(II/III) complexes have been widely developed as alternatives to platinum chemotherapeutics. They offer the promise of novel modes of action, independent of DNA binding, that could overcome inherent and acquired resistance to platinum drugs.[121,122] Promising series of ruthenium(II) complexes include organometallic diamine/arene complexes such as $[Ru(\eta^6\text{-bip})(en)Cl]^+$ (RM175; Figure 4a),[123] RAPTA-C (Figure 4b),[124] and polypyridyl complexes.[125] The arene substituents and choice of mono- and bidentate ligands in organometallic ruthenium(II) "piano-stool" complexes of the type $[Ru(arene)(XY)Z]^{n+}$ allow fine-tuning of the physical and chemical properties.[126−129] These complexes include three basic building blocks: an arene ligand, which stabilizes the oxidation state of the metal and provides an hydrophobic face, a monodentate ligand, Z, initially included as an activation site, and a bidentate ligand, XY.[130,131]

Recent evidence suggests that ruthenium(II) arene complexes are most likely to be multitargeted. Although complexes with a labile Ru−Z (e.g., Cl) bond can attack DNA, this mechanism may be only partly responsible for the observed antineoplastic activity, and other targets may be involved.[132] Their biological activity may also be linked to interaction with protein kinases, carbonic anhydrases,[133] and topoisomerases.[134−136]

Organometallic ruthenium(II) piano-stool complexes can undergo activation by the loss/replacement of the monodentate

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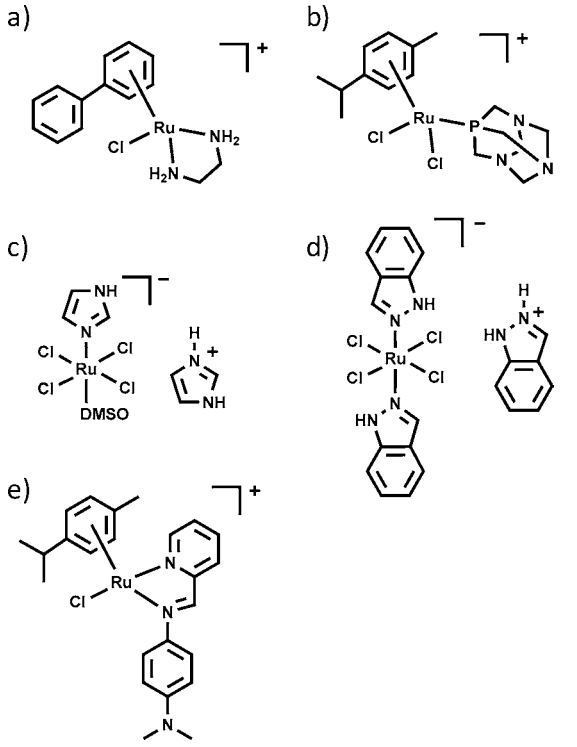

Figure 4. Ruthenium(II/III)-based antineoplastic agents: (a) RM175; (b) RAPTA-C; (c) NAMI-A; (d) KP1019; (e) $[Ru(\eta^6\text{-}p\text{-cym})(p\text{-Impy-NMe}_2)Cl]^+$.

ligand Z. This gives rise to a free coordination position that can bind to DNA or other biological molecules.[130] Some cell-free studies show that aquation of the complexes can occur with subsequent binding to nucleobases, especially G.[137,138]

Pyridocarbazole half-sandwich ruthenium(II) complexes with phosphorus-donor ligands and some other octahedral ruthenium(II) complexes can inhibit protein kinases GSK3α (glycogen synthase kinase 3) and PAK1 (serine/threonine-protein kinase 1).[139,140] Ruthenium polypyridyl complexes such as Δ-$[Ru(bpy)_2(UIP)]^{2+}$ [where UIP = 2-(5-uracil)-1*H*-imidazo-[4,5-*f*][1,10]phenanthroline] or $[Ru(bpy)_2(dpq)]^{2+}$ can interact with DNA by intercalation,[134,141,142] but they can also induce mitochondria-mediated[143] and caspase-dependent[144] apoptosis. This mechanism of cell death activation is also observed for organometallic "piano-stool" ruthenium(II) complexes.[145−147] Ruthenium(II) complexes have been reported to inhibit TrxR, in particular RAPTA-C derivatives.[148]

Iridium(III). Regardless their small therapeutic window, which could hinder future clinical development, polypyridyl-iridium(III) complexes with the general formula *fac*-$[IrCl_3(DMSO)(pp)]$, where pp = bpy, phen, dpq, dppa, or dppn (Figure 5a), have been reported to have low micromolar activity against MCF7 breast cancer and HT-29 colon carcinoma cells.[149] A related complex containing the N,N-bidentate 5,6-dimethyl-phenanthroline ligand has been shown to cause concentration-dependent apoptosis in Jurkat leukemia cells accompanied by rising levels of ROS.[149] Inert iridium(III) complexes, such as [Ir(DBCOT)(octasporine)(SeCN)(Me)] where DBCOT = dibenzo[*a*,*e*]cyclooctatetraene (Figure 5b), selectively inhibit the protein kinase FLT4, as a result of its rigid scaffold.[150] The metal center in this complex fulfils two functions: first it holds in place the structural scaffold for molecular recognition of the ATP binding site by the kinase, and second it allows light-induced ligand-exchange reactions, which, in turn, cause apoptosis in cancer cells.[150,151]

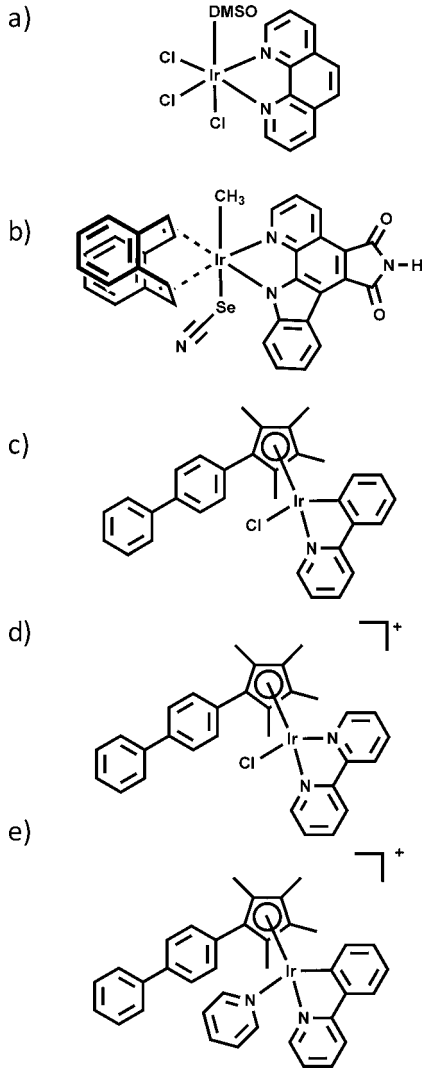

Figure 5. Iridium anticancer agents: (a) *fac*-$[IrCl_3(DMSO)(bpy)]$; (b) [Ir(DBCOT) (octasporine)(SeCN)(Me)]; (c) $[Ir(\eta^5\text{-}C_5Me_4C_6H_4C_6H_5)(phpy)Cl]^+$; (d) $[Ir(\eta^5\text{-}C_5Me_4C_6H_4C_6H_5)(bpy)\text{-}Cl]^+$; (e) $[Ir(\eta^5\text{-}C_5Me_4C_6H_4C_6H_5)(phpy)py]^+$.

DIRECT INVOLVEMENT OF REDOX CHEMISTRY IN THE ACTIVITY OF METAL-BASED ANTICANCER DRUGS

Platinum(IV). Classically-inert low-spin $5d^6$ platinum(IV) anticancer complexes are usually thought to be involved in redox reactions in vivo, which lead to the reductive activation of these "pro-drugs". These platinum(IV) pro-drugs include tetraplatin and satraplatin (JM216; Figure 1h). The latter was the first example of a platinum antineoplastic agent that could be administered orally.[152] However, the complex was abandoned after phase III trials.[153]

Platinum(IV) compounds can undergo reductive elimination reactions during intracellular reactions with GSH[154] (Pt^{IV} + 2GSH → Pt^{II} + GSSG + $2H^+$) or ascorbate and be converted into their platinum(II) analogues while maintaining their cytotoxic profiles.[154] X-ray absorption near-edge structure studies of platinum(IV) complexes 24 h after administration show a cellular distribution similar to that of CDDP, suggesting that reduction to

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platinum(II) occurs during this period.[155] Possible reducing agents include ascorbate, GSH, and cysteine-containing proteins.[156] Glutathione, in particular, is found in cells at concentrations around 1−10 mM.[157] The reduction potentials of platinum(IV) complexes are strongly influenced by the nature of the axial ligands.[158] Table 3 shows the reduction potentials of selected platinum(IV) complexes. Although reaction with GSH is a possibility, complexes such as JM216 (Figure 1h) do not undergo such a reduction, nor is it reduced by cytochrome *c* alone. However, reaction with hemoglobin in the presence of NADH occurs rapidly to generate the corresponding platinum(II) complex.[159] Other complexes not readily reduced by GSH include *cis,trans,cis*-$[PtCl_2(OCOCH_3)_2(NH_3)(amine)]$, where amine = cyclohexylamine or adamantylamine.[160]

Table 3. Selected Redox and Half-wave Potentials for Species Directly Involved in the Redox Chemistry and Activity of Metal-Based Anticancer Drugs

metal	compound	mV	ref
	GSH	$E^{o\prime} = -160^a$	225
		$E^{o\prime} = -250^b$	168
	Ascorbic acid	$E^{o\prime} = +60^b$	168
	$Ru^{III} \rightarrow Ru^{II}$		
Ru	NAMI-A	$E^{o\prime} = +250^b$	168
	KP1019	$E^{o\prime} = -430^b$	
		$E^{o\prime} = +30^b$	
	$Pt^{IV} \rightarrow Pt^{II}$		
	$[Pt(NH_3)_2Cl_4]$	$E_p = -260^d$	226
	$[Pt(en)Cl_4]$	$E_p = -260^d$	
	$[Pt(OAc)_2(NH_3)_2Cl_2]$	$E_p = -635^d$	
Pt	$[Pt(OH)_2(NH_3)_2Cl_2]$	$E_p = -880^d$	
	$[Pt(OH)_2(en)Cl_2]$	$E_p = -884^e$	
	$[Pt(ipa)(OH)_2Cl_2]$	$E_p = -730^e$	227
	$[Pt(OAc)_2(en)Cl_2]$	$E_p = -546^e$	
	$[Pt(DACH)Cl_4]$	$E_p = -90^e$	

[a]pH 7.4, 298 K, 0.1 M phosphate buffer, SHE. [b]pH 7.0, 0.20M phosphate buffer, NHE. [c]$[n\text{-}Bu_4N][BF_4]$/DMF, NHE. [d]0.1 M KCl, Ag/AgCl. [e]pH 7.0, 0.1M KCl, Ag/AgCl.

Another strategy to activate platinum(IV) is the use of spatially directed light: photoactivated chemotherapy. This allows the administration of nontoxic platinum(IV) complexes that are reduced to to active platinum(II) active platinum(II) complexes only upon light irradiation of the neoplastic lesion, sparing the surrounding tissue from side toxicity. The diazidodipyridine complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(py)_2]$ (FM190; Figure 1i), for example, is stable in the dark and unreactive toward reduction by GSH. However, upon irradiation at 365 nm (UVA), the complex is potently phototoxic toward A2780 ovarian carcinoma and its CDDP-resistant derived cell line A2780cis, as well as esophageal adenocarcinoma OE19.[161] The complex can also be activated by blue and green light. Structurally-related complexes, such as *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(py)(NH_3)]$, are known to cause cell death by mechanisms that are apoptosis-independent when activated by UVA.[162] These diazidoplatinum(IV) complexes can form unusual (Pt^{II})DNA lesions[163] and also generate azidyl radicals.[164] The role played by such radicals in the mechanism of photocytotoxicity is not yet understood.

Ruthenium(III). Two ruthenium(III) antineoplastic complexes, NAMI-A (Figure 4c) and KP1019/NKP-1339 (Figure 4d), have reached human clinical trials.[165] NAMI-A has shown antimetastatic potential,[166,167] while the latter induces apoptosis in primary tumors. These may be activated in vivo by reduction to ruthenium(II). The Ru^{III}/Ru^{II} redox potential for KP1019 is −0.43 V and that for NAMI-A +0.25 V, both within the biologically relevant window[168] and independent of the pH.[169] The complexes can be activated not only by direct reduction but also by aquation.[170]

These octahedral ruthenium(III) complexes have a high affinity for serum proteins, and such interactions may be responsible for the adverse effects observed in the clinical trials of KP1019. Reduction to Ru^{II} can promote substitution of the two chlorido ligands by water molecules, facilitating interaction with biomolecules. Chloride/water ligand exchange has been achieved in cell-free experiments by reaction of the ruthenium(III) complex with ascorbic acid or GSH. After reaction with GSH, the ruthenium complex KP1019 binds to GMP.[169] NKP1339 (the sodium salt of KP1019) and NAMI-A are reported to be nitric oxide ($NO^{\bullet}$) scavengers. They form $Ru^{II}NO$ adducts, which inhibit endothelial cell migration, and could be responsible for the angiogenic properties of NAMI-A.[171]

ORGANOMETALLIC RUTHENIUM(II), OSMIUM(II), AND IRIDIUM(III) COMPLEXES

We and others have attempted to design new generations of transition-metal anticancer complexes that might overcome clinical problems with platinum drugs, notably resistance,[54] the limited spectrum of activity,[42] and side effects.[62,64,66]

Initially, to avoid cross-resistance, we designed complexes that could form distinctly different structural lesions on DNA. Thus, N,N-chelated ruthenium(II) arene complexes show activity in vitro and in vivo with little cross-resistance, and if the arene is extended with substituents such as phenyl rings, it can also intercalate between DNA bases adjacent to the ruthenation site. Like CDDP, these chlorido complexes can also undergo activation by aquation,[130,170] and the rate of aquation as well as pK_a of the resulting aqua complex can be controlled by the choice of the chelating ligand (e.g., NN vs NO vs OO). Cell-free studies have shown that piano-stool ruthenium(II) complexes such as $[Ru(\eta^6\text{-}p\text{-terp})(en)Cl]^+$ or $[Ru(\eta^6{:}\eta^1\text{-}C_6H_5(CH_2)_3NH_2)(NO_3)_2]$ can interact with calf-thymus DNA, as well as cellular DNA[172,173] and activate NER mechanisms after the formation of Ru-DNA adducts.[174]

However, it has recently become apparent that complexes in this class do not all act by a classical DNA-attack mechanism, and moreover even those that can attack DNA may be involved in redox reactions in cells. For example, the active complex RM175 (Figure 4a) reacts with GSH to form $[Ru(\eta^6\text{-bip})(en)(SG)]^+$, which, surprisingly, undergoes facile oxidation to the sulfenato complex $[Ru(\eta^6\text{-bip})(en)(S(O)G)]^+$ and even the sulfinate $[Ru(\eta^6\text{-bip})(en)(S(O)_2G)]^+$.[175,176] Such oxidation appears to promote rather than inhibit binding to guanine and DNA, a different behavior from that of platinum thiolate complexes. The sulfenate ligand can be displaced by guanine N7, and so this provides a potential redox-mediated pathway to DNA binding.[177]

An important property of these ruthenium(II) piano-stool complexes is their ability to circumvent platinum resistance. RM175 is active toward CDDP-resistant A2780cis ovarian carcinoma cells.[178] This complex causes G1/G2 arrest in HCT116 cells in a p53- and p21/cyclin-dependent kinase inhibitor 1 (WAF1)-dependent manner after short drug-exposure periods.[179]

Although aquation of ruthenium(II) arene complexes can be a first step toward anticancer activity, the antiproliferative activity

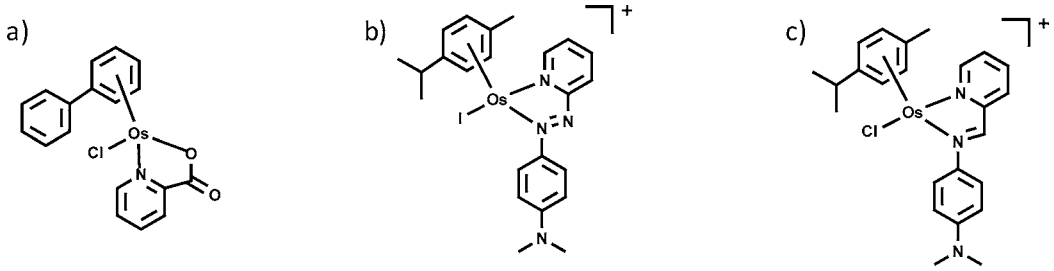

Figure 6. Osmium complexes: (a) [Os(η^6-bip)(picolinate)Cl]; (b) FY26; (c) [Os(η^6-*p*-cym)(*p*-Impy-NMe_2)Cl]PF_6.

of some related complexes is independent of hydrolysis. Piano-stool complexes with azopyridine ligands (strong π acceptors) exhibit potent antiproliferative activity in ovarian (A2780) and lung (A549) cancer despite being inert toward hydrolysis.[138] In the case of complexes [Ru(η^6-bip)(*p*-Azpy-NMe_2)I]$^+$ and [Ru(η^6- bip)(*p*-Azpy-OH)I]$^+$ where *p*-Azpy-NMe_2 and *p*-Azpy-OH are para-substituted phenylazopyridine ligands, the inclusion of the azo N═N bond in the ligand achieves reduction potentials that are biologically accessible (−0.36 and −0.26 V, respectively). These complexes oxidize GSH to GSSG under physiological conditions and generate elevated levels of ROS in A549 lung cancer cells that can be scavenged by *N*-acetyl-L-cysteine (NAC).[180] The mechanism of formation of these ROS is not clear but may involve ligand-based reduction and may even be catalytic.

Work on these ruthenium arene complexes has also revealed the subtle effects of the ligands on mechanisms of cellular influx and efflux. The uptake of chlorido(iminopyridine)arene-ruthenium(II) complex [Ru(η^6-*p*-cym)(*p*-Impy-NMe_2)Cl]$^+$ (Figure 4e) into A2780 human ovarian cancer cells occurs mainly via active transport, whereas the iodido analogue [Ru(η^6-*p*-cym)(*p*-Impy-NMe_2)I]$^+$ is taken up via passive diffusion.[181] Subtle differences in the electrostatic charge distribution in these derivatives appear to lead to significant changes in their subcellular distribution and, in turn, to the activation of different apoptotic pathways. The iodido complexes are more selective for cancer cells compared to normal cells (fibroblasts) and, importantly, are not dependent on p53 for activity, in contrast to CDDP, which loses potency in p53 mutant cancer cells.[182]

These findings for ruthenium were mirrored in our work on the heavier congener osmium. Ruthenium(II) complex RM175 (Figure 4a) is active against MCa mammary carcinoma in vivo and causes metastasis reduction, whereas the osmium(II) analogue [Os(η^6-bip)(en)Cl]$^+$ is not active in vivo.[183] Aquation of osmium(II) arene half-sandwich complexes is typically 50−100 slower than that of analogous ruthenium(II) arenes, and the aqua adducts of osmium(II) are ca. 1.5 pK_a units more acidic.[184,185] The slowness of the substitution reactions can be countered by choosing N,O- instead of N,N-chelating ligands, and indeed picolinate complexes, such as [Os(η^6-bip)(picolinate)Cl] (Figure 6a), exhibit good anticancer activity in vitro and bind to DNA at rates similar to those of CDDP.[186] Interestingly, the cytotoxicity can be switched off by introducing a sterically demanding ortho substituent onto the pyridine ring.[187] Cell accumulation of osmium from such complexes is enhanced by attachment to cell-penetrating peptides such as Arg_8, but only if albumin is depleted in the cell culture medium.[184]

Again although DNA is a viable target for osmium(II) arene complexes, switching the chelated ligand to an azopyridine, and specifically to a phenylazopyridine with OH or NMe_2 substituents on the phenyl ring, confers inertness on the complex toward substitution and yet produces highly potent anticancer complexes. Azopyridineosmium(II) complexes such as [Os(η^6-bip)(*p*-Azpy-OH)I]PF_6, [Os(η^6-*p*-cym)(*p*-Azpy-OH)I]PF_6, [Os(η^6-bip)(*p*-Azpy-NMe_2)I]PF_6, and [Os(η^6-*p*-cym)(*p*-Azpy-NMe_2)I]PF_6 are an order of magnitude more potent than CDDP in the ovarian cancer cell line A2780.[188] In particular, [Os(η^6-*p*-cym)-(*p*-Azpy-NMe_2)I]PF_6 (FY26; Figure 6b) exhibits submicromolar IC_{50} values in lung A549, colon HCT116, breast MCF7, and prostate cancer PC3 cell lines. This complex has also shown good in vivo tumor reduction for HCT116 xenografts and exhibits low toxicity and negligible deleterious effects.[186,188] As with the ruthenium(II) analogues, the presence of electron-donating substituents on the phenyl ring (e.g., OH or NMe_2) is important for the high activity.

Analogous iminopyridine complexes, such as [Os(η^6-bip)(*p*-Impy-OH)I]PF_6, [Os(η^6-*p*-cym)(*p*-Impy-OH)I]PF_6, [Os(η^6-bip)(*p*-Impy-NMe_2)Cl]PF_6, and [Os(η^6-*p*-cym)(*p*-Impy-NMe_2)Cl]PF_6, have also been investigated. In contrast to the azopyridine complexes, [Os(η^6-*p*-cym)(*p*-Impy-NMe_2)Cl]PF_6 (Figure 6c) undergoes total hydrolysis after 24 h and, importantly, oxidizes NADH to NAD^+ in a phosphate buffer, forming an Os−H hydride, although it remains unreactive toward GSH. This reaction, which may occur by a hydride transfer, probably accounts for the ca. 2 times increase of the NAD^+/NADH ratio (from 2.5 ± 0.4 to 5.4 ± 0.3) in A2780 ovarian cancer cells after 6 h of exposure to 1.5 μM complex. Its antiproliferative activity may be linked to this redox behavior because it increases ROS levels in A549 lung cancer cells.[189]

In a similar manner, we have investigated related low-spin $5d^6$ iridium(III) complexes. Negatively-charged cyclopentadienyl ligands, and in particular pentamethylcyclopenadienyl (Cp*), form stable carbon-bound iridium(III) complexes, whereas arenes do not. Despite possessing a classically-inert electronic configuration, the rates of ligand substitution on iridium(III) depend critically on the ligands. For example, the highly active complexes [Ir(η^5-$C_5Me_4C_6H_4C_6H_5$)(phpy)Cl]$^+$ (Figure 5c) and [Ir(η^5-$C_5Me_4C_6H_4C_6H_5$)(bpy)Cl]$^+$ (Figure 5d) containing biphenyl substituents on the Cp* ring undergo facile hydrolysis and form stable complexes with 9-ethylguanine, but not 9-ethyladenine. Their IC_{50} values for A2780 ovarian cancer cells are in the submicromolar range (0.72 ± 0.01 and 0.57 ± 0.07 μM, respectively).[190] COMPARE analysis of the IC_{50} values of iridium(III) complexes in the NCI panel of 60 cancer cell lines indicates that their mechanism of action is novel and unrelated to that of CDDP or OXA.[191]

The structurally related complex [Ir(η^5-$C_5Me_4C_6H_5$)(phpy)-(py)]PF_6 (Figure 5e) has recently been prepared in our laboratory. The presence of the pyridine ligand blocks hydrolysis. The complex has an IC_{50} value of 0.12 ± 0.02 μM toward A2780 ovarian cancer cells, and after 1 h of drug exposure, there is a dramatic increase in ROS production (>1000-fold).[192]

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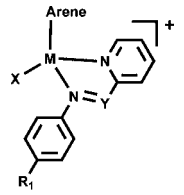
In some cases, the redox activity in A2780 cancer cells exhibited by these ruthenium(II), osmium(II), and iridium(III) organometallic complexes may be related to hydride formation and changes in the $NAD^+/NADH$ ratio. Iridium(III) complexes $[Ir(\eta^5\text{-}C_5Me_4C_6H_5)(phpy)Cl]PF_6$ and $[Ir(C_5Me_4)(phpy)Cl]\text{-}PF_6$ can catalytically oxidize NADH to NAD^+ with a turnover number (TON) of 75 and a turnover frequency (TOF) of 4.3 h^{-1}. Moreover, the tetramethyl(biphenyl)cyclopentadienyl complex can increase the $NAD^+/NADH$ ratio in A2780 ovarian cancer cells, almost doubling from 7.95 ± 0.10 to 14.84 ± 0.77 after 6 h of exposure to the complex, perhaps involving transfer of hydride from NADH to biologically available substrates, modulating the redox balance in cells.[193] The possibility also arises that such complexes can catalyze the production of H_2 in cells. The aqua derivatives of these iridium(III) complexes $[Ir(\eta^5\text{-}C_5Me_4C_6H_5)\text{-}(phpy)H_2O]PF_6$ and $[Ir(C_5Me_4)(phpy)H_2O]PF_6$ can also catalyze the reduction of quinones (e.g., menadione, vitamin K_3) by NADH hydride transfer. Intriguingly, overall this reduction appears to be a one-electron process, giving the semiquinone (detectable by electron paramagnetic resonance) as the product rather than a two-electron reaction to give the quinol.[194]

The realization that the mechanism of action of these organometallic transition-metal complexes may involve alteration in the redox status of cancer cells has led us to investigate synergy with the redox modulator L-buthionine sulfoximine (L-BSO). We have previously demonstrated that L-BSO, at nontoxic concentrations, greatly potentiates the anticancer activity of organometallic azopyridine osmium(II) arene complexes.[195] For example, 50 μM L-BSO coadministered with 1 μM FY26 (Figure 6b) increased the antiproliferative activity of the osmium complex by ca. 2 times in A2780 ovarian cancer cells (from ca. 20% to 10% survival) and ca. 3 times in the case of A549 lung cancer (60% versus 20% cell survival in the combination treatment). Additionally, complex $[Ir(\eta^5\text{-}C_5Me_4C_6H_5)(phpy)\text{-}(py)]PF_6$ (Figure 5e) increases its potency by ca. 2 times when coadministered with 5 μM L-BSO.[192]

Inhibition of γ-glutamylcysteine synthetase, an enzyme involved in the rate-limiting step of GSH synthesis, by L-BSO decreases intracellular GSH levels. When used at low concentrations, L-BSO has been reported to increase ROS levels at the same time as augmenting the sensitivity to anticancer drugs that depend on GSH-mediated detoxification.[56,58] Such is the case when GSH depletion restores CDDP sensitivity in resistant cell lines and improves the activity of some platinum−urea complexes.[196,197] Other metal-based drugs that benefit from this approach include the ruthenium(II) complex KP1019.[22]

When used as a single agent at high concentrations, L-BSO is capable of increasing ROS levels to the point of causing apoptosis.[198,199] However, the use of these high concentrations of the inhibitor can also cause deactivation of anticancer drugs such as paclitaxel because it interferes with the cell cycle changes produced by this taxane drug.[200] This demonstrates the importance of optimizing the concentration of L-BSO to achieve the desired effect.

Potentiation of Anticancer Activity Using L-BSO. Here we report the dose-dependent effects of L-BSO on the activity of organometallic ruthenium(II) and osmium(II) arene and iridium(III) cyclopentadienyl complexes. The principal aim of the coadministration of nontoxic doses of L-BSO is to achieve an increase in the potency that might allow a significant reduction in the dose of the metal-based drug because this reduction of the dose is likely to impact favorably on the reduction of side effects. We determined the IC_{50} values for imino- and azopyridine-ruthenium(II) and -osmium(II) arene complexes **1**−**6** (Figure 7) in A2780 human ovarian cancer cells in combination with L-BSO



	Compound	M	X	Y	R_1	Arene
1	$[Ru(\eta^6\text{-}p\text{-cym})(p\text{-Impy-NMe}_2)Cl]PF_6$	Ru	Cl	CH	NMe_2	*p*-cym
2	$[Ru(\eta^6\text{-}p\text{-cym})(p\text{-Impy-NMe}_2)I]PF_6$	Ru	I	CH	NMe_2	*p*-cym
3	$[Ru(\eta^6\text{-}p\text{-cym})(p\text{-Azpy-NMe}_2)Cl]PF_6$	Ru	Cl	N	NMe_2	*p*-cym
4	$[Ru(\eta^6\text{-}p\text{-cym})(p\text{-Azpy-NMe}_2)I]PF_6$	Ru	I	N	NMe_2	*p*-cym
5	$[Os(\eta^6\text{-}p\text{-cym})(p\text{-Impy-NMe}_2)Cl]PF_6$	Os	Cl	CH	NMe_2	*p*-cym
6	$[Os(\eta^6\text{-}p\text{-cym})(p\text{-Impy-NMe}_2)I]PF_6$	Os	I	CH	NMe_2	*p*-cym
7	$[Os(\eta^6\text{-}p\text{-bip})(p\text{-Azpy-NMe}_2)Cl]PF_6$	Os	Cl	N	NMe_2	bip
8	$[Os(\eta^6\text{-}p\text{-bip})(p\text{-Azpy-NMe}_2)I]PF_6$	Os	I	N	NMe_2	bip
9	$[Os(\eta^6\text{-}p\text{-cym})(p\text{-Azpy-NMe}_2)I]PF_6$	Os	I	N	NMe_2	*p*-cym
10	$[Os(\eta^6\text{-}p\text{-bip})(p\text{-Azpy-OH})I]PF_6$	Os	I	N	OH	bip
11	$[Ir((\eta^5\text{-}C_5Me_4C_6H_5)(p\text{-Azpy-NMe}_2)Cl]PF_6$	Ir	Cl	N	NMe_2	Cp^{*xph}

Figure 7. Organometallic half-sandwich "piano-stool" ruthenium(II), osmium(II), and iridium(III) complexes used in the redox modulation experiments.

at three different concentrations (1, 5, and 50 μM). The chemical structures of ruthenium(II) and osmium(II) complexes **1**−**6** are closely related. They all contain the same arene (*p*-cymene) and differ in the monodentate ligand (Cl vs I) or the chelating ligand [(*N*,*N*-dimethylphenyl)iminopyridine vs (*N*,*N*-dimethylphenyl)azopyridine]. The potency of these complexes greatly increases when coincubated with L-BSO (Figure 8a and Table S1 in the Supporting Information). The antiproliferative activity of ruthenium complex $[Ru(\eta^6\text{-}p\text{-cym})(p\text{-Impy-NMe}_2)Cl]PF_6$ (**1**) increased for all three doses of L-BSO (1, 5, and 50 μM). The greatest increase, by ca. 16-fold, was observed when **1** was coincubated with 5 μM L-BSO (IC_{50} decrease from 16.2 ± 0.9 to 1.0 ± 0.3 μM). The activity of the iodido analogue **2** was not affected by the presence of 1 μM L-BSO, but its potency increased by ca. 3-fold with 5 or 50 μM L-BSO, with its IC_{50} decreasing from 3.0 ± 0.2 to 1.05 ± 0.02 μM.

The activity of the ruthenium *p*-Azpy-NMe_2 complex **3** increased ca. 8-fold (IC_{50} decreased from 13.1 ± 0.5 to 1.63 ± 0.02 with 5 μM L-BSO), while only a ca. 2-fold decrease in IC_{50} was achieved when coadministered with 50 μM of the redox modulator. Strikingly, the IC_{50} value of the iodido analogue $[Ru(\eta^6\text{-}p\text{-cym})(p\text{-Azpy-NMe}_2)I]PF_6$ (**4**) and the corresponding osmium chlorido- and iodidoiminopyridine complexes **5** and **6** decreased to the submicromolar range. In particular, the complex $[Os(\eta^6\text{-}p\text{-cym})(p\text{-Impy-NMe}_2)I]PF_6$ (**6**) exhibits a 15-fold improvement in its potency to nanomolar values when coincubated with 5 μM L-BSO (IC_{50} = 80 ± 2 nM). In these experiments, the decrease in the GSH levels caused by L-BSO, together with the redox perturbation within the cell caused by the metal complex, appear to generate a ROS imbalance that results in higher cell death.

The greatest increases in the L-BSO-induced potency for complexes **1**−**6** are seen for the iminopyridine complexes. However, there is no clear structure−activity relationship that relates the improvements in potency to the structural changes between these six complexes. The aqueous behavior of the complexes does not relate to the observed potentiation. Complex **4** is inert to aquation while **6** is fully converted to the aqua species within 24 h and also complexes **1**, **2**, or **5** undergo only partial

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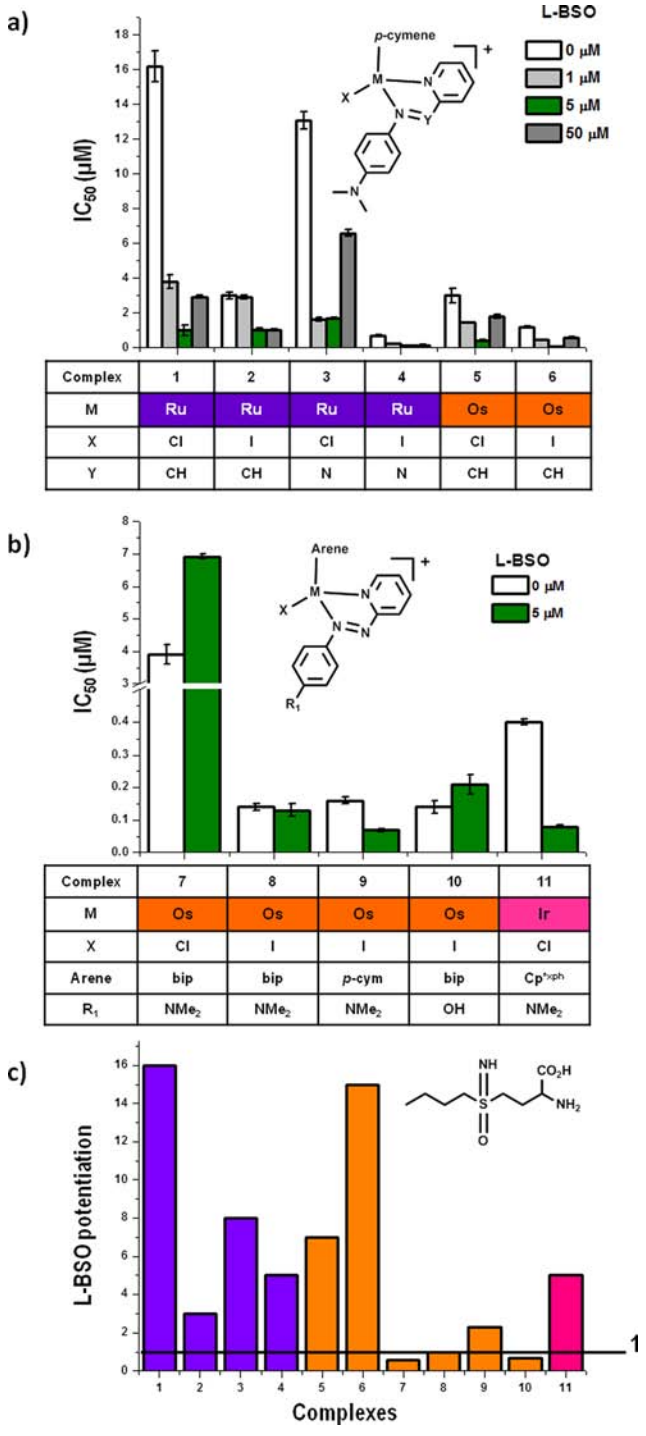

Figure 8. IC_{50} values in A2780 human ovarian cancer cells: (a) for complexes **1**−**6** after coincubation with 0, 1, 5, or 50 μM L-BSO; (b) for complexes **7**−**11** after coincubation with 5 μM L-BSO. The experiments included 48 h of preincubation time in a drug-free medium, 24 h of drug exposure, and 72 h of cell recovery time at 37 °C in a 5% CO_2 humidified atmosphere. (c) Degree of potentiation of activity toward A2780 cancer cells by L-BSO (dose 5 μM) defined as $IC_{50}(-\text{L-BSO})/IC_{50}(+\text{L-BSO})$.

aquation.[182] Interestingly, the optimum concentration of L-BSO to achieve the maximum potency with all of these complexes is as low as 5 μM. This dependence on the concentration of L-BSO is not linear because the highest concentration (50 μM) does not achieve further improvement. Perhaps at high concentrations of the redox modulator, complexes **1**, **3**, **5**, and **6** are deactivated in a manner similar to that of taxol. In the case of the taxane drug, it is thought that L-BSO prevents condensation of the microtubules involved in its antiproliferative activity.[200] The tubulin-related activity of these osmium(II) complexes is currently being explored. This might also be related to the behavior observed with ruthenium(II) complexes **1** and **3** when using high concentrations of the redox modulator (50 μM).

The mechanism of action of L-BSO involves the prevention of cellular detoxification by GSH through a decrease in the GSH levels, as well as an increase in the redox activity. This results in an imbalance of the ROS levels in the cell because the low levels of GSH affect the equilibrium between GSH and its oxidized form GSSG.[199]

Having determined that, of the three doses studied, 5 μM L-BSO is an optimum dose for complexes **1**−**6**, we explored modulation of the antiproliferative activity of the *p*-cymene/biphenylosmium(II) azopyridine complexes **7**−**10** and pentamethylcyclopentadienyliridium(III) azopyridine complex **11** (Figures 7 and 8b and Table S2 in the Supporting Information). Osmium(II) complexes **7**−**9** share $-NMe_2$ as a substituent on the N,N-chelating ligand but differ in the arene (*p*-cymene vs biphenyl) and also the monodentate ligand (Cl vs I). Complex **10** is the only one in the series that includes an hydroxyl group instead of a dimethylamino substituent on the phenyl ring of the bidentate ligand. To our surprise, in in this series(**7** - **10**), the complex $[Os(\eta^6\text{-}p\text{-cym})(p\text{-Azopy-NMe}_2)I]PF_6$(**9**) was the only osmium(II) compound to exhibit an improvement in activity. Its potency doubled with a decrease in the IC_{50} value from 0.16 ± 0.01 to 69 ± 5 nM. Curiously, complexes **7** and **10**, which are structurally related, show a decrease in their potency of ca. 1.5-fold. Complexes **7**, **8**, and **10** all contain a biphenylarene ligand. Finally, the iridium(III) phenyltetramethylcyclopentadienyl complex **11** shows a 5-fold improvement to nanomolar potency, an IC_{50} of 80 ± 5 nM for A2780 human ovarian cancer cells.[191] The potentiation of activity induced by L-BSO across this series of complexes (Figure 8c) follows the order

$$\mathbf{1 > 6 > 3 > 4 = 5 = 11 > 2 > 9}$$

Ruthenium(II) complexes appear to respond most to co-administration with L-BSO compared to osmium(II) complexes. At the same time, complexes that include an iminopyridine group as the N,N-chelating ligand are also more responsive than azopyridine derivatives. Finally, chlorido and *p*-cymene derivatives seem to exhibit higher improvements than their corresponding iodido and biphenyl analogues. The best example of this is ruthenium(II) complex **1** containing a chloride as the monodentate ligand, an iminopyridine as the chelating ligand, and *p*-cymene as the arene, with the IC_{50} value decreasing from 16.2 ± 0.9 to 1.0 ± 0.3 μM.

Osmium(II) complexes have been investigated in relation to their reactivity toward GSH and NADH because the latter is also closely related to maintenance of the redox balance in cells. In general, complexes with the formula $[(\eta^6\text{-arene})Os(N,N)Cl/I]^+$, where N,N is an iminopyridine or an azopyridine ligand, do not react directly with GSH. However, when N,N is an azopyridine, the complexes are capable of oxidizing NADH, while the iminopyridine analogues do not undergo such a reaction.[189]

Such an observation, that the complexes do not react with GSH, makes a stronger case for the potentiation to be a cooperative redox effect between the intrinsic antiproliferative activity of the metal complexes and the effect of L-BSO. It is clear that subtle structural modifications, such as a change from chloride to iodide as the ligand, can have major implications for the mechanism of

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action.[181] However, it is not understood how these changes affect the redox behavior of the complexes inside the cells.

The osmium(II) complexes, in particular, are capable of increasing ROS and changing the mitochondrial potential of A2780 cells before causing cytochrome *c* release into cytosol.[201] Further work is needed to establish the involvement of the redox-active N═N bond in the activity of azopyridine derivatives. Do ligand-centered redox reactions modify the mechanism of action of these complexes? Can redox reactions react to the behavior observed when L-BSO is coadministered?

We have previously reported that the mean graph of the COMPARE analysis for the iridium(III) complex **11** does not correlate its antiproliferative activity to the mode of action of either CDDP or OXA.[191] Instead, it shows a positive correlation only to drugs with known mechanisms of action in three categories: DNA interactors, topoisomerase inhibitors, and, more interestingly, cytotoxic redox mediators.[191] Is the mode of action of complex **11** dictated by its inability to undergo hydrolysis, and how does this relate to the increment of potency induced by coadministration of L-BSO?

OUTLOOK AND CONCLUDING REMARKS

We have explored the involvement of redox reactions in the antiproliferative activity of metal-based anticancer complexes. Variations in the redox state of cells induced by metal complexes either directly or indirectly may contribute to the activity of metal-based drugs but can also be responsible for undesirable side effects, as appears to be the case for CDDP. Disruption of mitochondrial function is an attractive target for anticancer drug design because coadministration of a redox modulator can achieve considerable dose reductions. Our data for several organometallic ruthenium(II), osmium(II), and iridium(III) complexes illustrate how dose reductions of up to 15 times and nanomolar activity can be achieved using the redox modulator L-BSO (Figure 8c).

Although much effort has been devoted to the investigation of the cellular redox behavior of ruthenium(III)[202] and platinum(IV)[203,204] drugs, there is much still to be done. For example, there appear to be few reports of investigations of the interaction of metal anticancer drugs with ferredoxins and, in particular, the various components of the mitochondrial electron-transport chain, which play a major role in the redox chemistry of all cells. Ferredoxin reductase, for example, is involved in increased ROS production in lung cancer cells, interacting with the Fhit protein in mitochondria.[205] Interestingly, ferredoxin reductase also mediates p53-dependent apoptosis in colorectal cancer,[206] particularly in patients under 5-fluorouracil treatment.[207] Other components of the mitochondrial electron-transport chain also need to be investigated as possible targets for metal-based anticancer complexes.

The involvement of direct or indirect redox mediation in anticancer activity may be a general finding for metal-based complexes. We have illustrated this for several metals of current interest, and the discussion can probably be extended to other promising families of metal complexes such as $[MoS_4]^{2-}$ [208] and organometallic tin(IV),[209] titanium(IV),[210] and rhodium(III) complexes,[211] but more work is needed to explore such links. Most interestingly, $[Mo^{VI}S_4]^{2-}$ is itself a redox modulator that can sensitize cancer cells to organic anticancer agents in an ROS-dependent manner.[206]

An understanding of the molecular mechanisms of metallodrug activity would benefit greatly from advances in being able to monitor the temporal and spatial speciation of metallodrugs in cells at physiologically relevant concentrations. This is a major challenge that merits increased multidisciplinary effort and appropriate investment of research funding, perhaps on the scale of a global challenge. Metallodrugs offer the prospect of agents with novel mechanisms of action but will be accepted widely only when their target sites are better understood at the molecular level and side effects are minimized. They are likely to offer unique therapy in the coming era of personalized medicine guided by proteomic and genomic screening.

EXPERIMENTAL SECTION

The synthesis and characterization of the ruthenium(II) complexes **1**−**4**, osmium(II) complexes **5**−**10**, and iridium(III) complex **11** have been previously reported.[182,180,188,189] Osmium(II) and iridium(III) complexes were the kind gifts of Dr. Ying Fu and Dr. Zhe Liu.

For the biological experiments, the RPMI-1640 medium, as well as fetal bovine serum, L-glutamine, a penicillin/streptomycin mixture, trypsin, and phosphate-buffered saline (PBS) were purchased from PAA Laboratories GmbH. Trichloroacetic acid (≥99%), sulforhodamine B (75%), sodium phosphate monobasic monohydrate (≥99%), sodium phosphate dibasic heptahydrate (≥99%), acetic acid (≥99%), and L-BSO (≥97%) were obtained from Sigma Aldrich.

Cell Culture. A2780 human ovarian carcinoma cells were obtained from the European Collection of Cell Cultures and used between passages 5 and 18. Cells were grown in a Roswell Park Memorial Institute medium (RPMI-1640) supplemented with 10% fetal calf serum, 1% 2 mM glutamine, and 1% penicillin/streptomycin. All cells were grown as adherent monolayers at 310 K in a 5% CO_2 humidified atmosphere.

In Vitro Growth Inhibition Assay. Briefly, 5000 cells were seeded per well in 96-well plates. The cells were preincubated in drug-free media at 310 K for 48 h before adding different concentrations of the compounds to be tested. The drug exposure period was 24 h. After this, supernatants were removed by suction, and each well was washed with PBS. A further 72 h was allowed for the cells to recover in a drug-free medium at 310 K. The SRB assay[212] was used to determine the cell viability. Absorbance measurements of the solubilized dye (on a BioRad iMark microplate reader using a 470 nm filter) allowed determination of viable treated cells compared to untreated controls. IC_{50} values (concentrations that caused 50% of cell death) were determined as duplicates of triplicates in two independent sets of experiments, and their standard deviations were calculated.

Redox Modulation Assays. These experiments were performed using the protocol previously described for IC_{50} determination with the following modifications. Briefly, a 96-well plate was seeded with 5000 A2780 ovarian cells per well. Cells were preincubated in a drug-free medium for 48 h at 310 K, before the metal complexes were added together with L-BSO. In order to prepare the stock solution of the drug, the solid complex was dissolved first in dimethyl sulfoxide (DMSO) and then diluted in a 50:50 mixture of PBS/saline. Separately, a stock solution of L-BSO was prepared in saline. Both solutions were added to each well independently, but within 5 min of each other. After 24 h of exposure, drugs were removed by suction, cells were washed with PBS (100 μL per well), and a fresh medium was added to the plate (200 μL per well). Cells were allowed to recover in a drug-free medium for 72 h at 310 K. At the end of this period, the SRB assay was used to determine the cell viability.

ASSOCIATED CONTENT

Supporting Information

Experimental section: in vitro determination of the antiproliferative activity of complexes **1**−**11** and modulation of IC_{50} values for complexes **1**−**6** coincubated with 1, 5, and 50 μM L-BSO and for complexes **7**−**11** with 5 μM L-BSO. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

I.R.-C. carried out the activity modulation experiments. Both authors wrote the manuscript.

Notes

The authors declare the following competing financial interest(s): P.J.S. is named inventor on a patent application relating to the osmium and iridium complexes used in this work filed by the University of Warwick.

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