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Essay

Redox-Active Anticancer Complexes

Redox-Active Metal Complexes for Anticancer Therapy

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Abstract: The redox properties of both metals and ligands in transition metal complexes offer unusual routes for new mechanisms of anticancer therapy. Metal complexes can introduce artificial reductive and oxidative stress into cancer cells, including behavior as photoactivatable agents and catalysts. Relatively inert metal complexes ("prodrugs") can be activated by redox processes within cancer cells. Examples of pharmaceuticals activated by bioreduction include three Pt^{IV} and two Ru^{III} compounds that have already entered clinical trials. More recently, novel Co^{III}, Fe^{III}, Pt^{IV}, Ru(III/II), Os^{II}, and Ir^{III} complexes have been reported to exhibit redox-mediated anticancer activity. Redox activation strategies can introduce new methods to increase cancer cell selectivity and combat drug resistance. Using combination therapy together with redox modulators to increase potency is also possible. This essay focuses on metal complexes that are activated in the reducing environment of cancer cells.

Introduction

Hypoxia is a serious problem in cancer therapy. Tumors contain a more reducing environment compared with healthy tissues due to accelerated metabolic activity, high rates of cell growth, and proliferation.[1] Chemotherapy and radiotherapy are unsuccessful for tumor cells that up-regulate drug resistance genes in hypoxic environments.[2] However, studies show that hypoxia can be exploited for therapeutic selectivity, as it differentiates cancer cells from normal cells.[3] The redox properties of both metals and ligands in transition metal complexes offer unusual routes for redox activation. The reducing tumor microenvironment provides an opportunity for inert oxidized metal prodrugs to be selectively activated by cancer cells in hypoxic environments. Thus, there is much potential for the development of bioreducible metal prodrugs.

Metal complexes contain a variety of structural and electronic features that can be exploited in drug design.[3,4] The metal itself and its oxidation state can be varied, as well as coordination geometries and coordination numbers. These properties allow the fine-tuning of chemical reactivity, including the rates of ligand exchange, the strengths of metal–ligand bonds, metal- and ligand-based redox potentials, ligand conformations, and outer-sphere interactions.[3] As well as the metal, the ligands can also play important roles in biological activity. They can be involved in target recognition and, when released, interfere in biochemical pathways.[5]

In this essay, we discuss metal complexes activated by the redox balance in cancer cells. The redox activation mechanism provides a highly effective cancer therapy strategy, especially because it 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. Upon cellular reduction, platinum(IV) prodrugs can not only release an active Pt^{II} complex but also additional bioactive substances that function in a manner orthogonal to Pt^{II}, providing a "dual-threat" mode of action. We have studied a wide range of redox-active organometallic Ru^{II}/Os^{II}/Rh^{III}/Ir^{III} complexes as anticancer agents.[5,6] The anticancer activity of Os^{II}–arene complexes, for example, can achieve nanomolar potency toward cancer cells in combination with the redox modulator L-buthionine sulfoximine, an inhibitor of the synthesis of glutathione, which is an antioxidant in cells.[5] Here we discuss applications of metal-based drugs for anticancer therapy involving redox-activated prodrug strategies and redox modulation.

Redox Systems in Cells

The redox balance is tightly regulated in living organisms. The disturbance of this balance can cause, or arise from, many dis-

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eases, including cancer. The mitochondrial electron transport chain is the major radical oxygen species (ROS) generation site in cancer cells. Although the generation of ROS is involved in important cell signaling functions of living cells, excessive amounts of ROS are commonly found in neoplastic tissues.[7] The accumulated intracellular ROS attack proteins, lipids, carbohydrates, and nucleic acids inside cells.

Enzymes that catalyze ROS-generating chemical reactions include peroxidases, NADPH oxidase, NADPH oxidase isoforms (NOX), glucose oxidase, xanthine oxidase (XO), lipoxygenases (LOXs), myeloperoxidase (MPO), cyclooxygenases (COXs), and nitric oxide synthase.[8] Myeloperoxidase (MPO) is a heme enzyme localized in lysosomes of neutrophils, macrophages, and monocytes. This enzyme chlorinates H_2O_2 to give highly reactive HOCl (Table 1) and also catalyzes the oxidation of thiocyanate (SCN^-) to generate another ROS, hypothiocyanite ($OSCN^-$), by a similar reaction.[9]

Table 1. Major endogenous oxidative enzymatic reactions.

Enzyme	Reaction catalyzed
NADPH oxidase	$NADPH + 2O_2 \longrightarrow 2O_2^- + NADP^+ + H^+$
Xanthine oxidase	$Hypoxanthine + 2O_2 + NAD(P)H \longrightarrow Xanthine + 2O_2^- + NAD(P)^+ + H^+$ $Xanthine + 2O_2 + NAD(P)H \longrightarrow Uric\ acid + 2O_2^- + NAD(P)^+ + H^+$
Myeloperoxidase	$H_2O_2 + Cl^- + H^+ \longrightarrow HOCl + H_2O$ $HOCl \longrightarrow O_2 + H^+ + Cl^-$ (H_2O_2, H_2O)
Superoxide dismutase ($Z = Cu^{2+}/Cu^+$ or Mn^{3+}/Mn^{2+})	$EnZ_{ox} + O_2^- \longrightarrow EnZ_{red} + O_2$ $EnZ_{red} + 2H^+ + O_2^- \longrightarrow EnZ_{ox} + H_2O_2$
Glutathione peroxidase Glutathione reductase	$2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O$ or $2GSH + ROOH \longrightarrow GSSH + ROH + H_2O$ $GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$
Catalase	$2H_2O_2 \longrightarrow 2H_2O + O_2$

Reactive nitrogen species (RNS) include nitric oxide (•NO), nitrogen dioxide ($^{\bullet}NO_2$), peroxynitrite ($ONOO^-$), and dinitrogen trioxide (N_2O_3). RNS are often linked to ROS, for example, in the formation of peroxynitrite causing nitrosative stress. Oxidative and nitrosative stress have been etiologically implicated in a wide variety of disease processes and states: aging, hypertension, atherosclerosis, ischemia/reperfusion (I/R) injury, renal diseases, diabetic neuropathies, Alzheimer's disease and cancers.[10]

An antioxidant is most simply defined as a molecule capable of slowing down or preventing redox changes in cancer cells. Cancer cells have developed several endogenous antioxidant systems to deal with over-produced cellular ROS. The redox equilibrium is tuned by cellular antioxidants, which can be divided into enzymatic and non-enzymatic groups.

Enzymatic antioxidants include superoxide dismutases (SODs), catalase, peroxidases, and glutathione S-transferase (GST), several of which require trace metal cofactors.[11] For example, there are two types of SOD enzymes present in mammalian cells, Cu-Zn SOD (cytoplasmic/nuclear) and Mn SOD (mitochondrial). Hydrogen peroxide generated after SOD activity is further converted to water by catalase and peroxidases. Catalase is relatively limited in cellular distribution (e.g. peroxisomes and a few other locations). Glutathione peroxidase and peroxiredoxin systems, as classes, are of comparable, if not potentially greater, importance than catalase. Catalase catalyzes the decomposition of H_2O_2 to O_2 and H_2O. It is an important enzyme in protecting the cell from oxidative damage by ROS. But, under prolonged oxidative stress with oxidation of NADPH, catalase activity drops.[12,13]

Cellular redox systems also utilize non-enzymatic antioxidants such as the tripeptide glutathione (GSH, γ-L-Glu–L-Cys–Gly), vitamin C (ascorbic acid), and thioredoxin (Trx). Non-enzymatic antioxidants react directly with the oxidants. Such antioxidants are said to be "scavengers"; their roles are unavoidably suicidal. Ascorbic acid can directly scavenge hydroxyl radicals by forming the semidehydroascorbate free radical that is subsequently reduced by GSH.[14] GSH, present at concentrations of 0.5–10 mM, is the predominant non-protein thiol in cancer cells.

The glutathione system of reduced GSH, oxidized GSSG, and glutathione peroxidase (GPx) is important for maintaining the cellular redox balance.[15] It is a major thiol–disulfide redox buffer in the cell and acts as the central mechanism for reducing H_2O_2.[16] This complements catalase as a reducing system for H_2O_2 but exceeds catalase in its capacity to eliminate additional types of toxic peroxides. The key enzyme in the glutathione system responsible for the reduction of H_2O_2 is GPx.[17] The reducing capacity of GPx enzymes is based on high levels of GSH. GPx reduces hydrogen peroxide to water by oxidizing glutathione to its disulfide (GSSG) (Table 1). The GSSG is reduced back to GSH by the reaction of GSH reductase (GR) with NADPH.[18] This capacity to recycle GSH gives the glutathione system a key role in the antioxidant defense mechanism of a cell and prevents depletion of cellular thiols.[19] Curiously there are situations in which GSH appears to act as a pro-oxidant. For example, GSH can react non-enzymatically with superoxide ($O_2^{\bullet-}$), nitric oxide (NO), hydroxyl radical (•OH), and peroxynitrite ($ONOO^-$). GSH can also induce oxidation of metal thiolates (M-SR) to metal sulfenates [M-S(O)-R].[19,20]

Trx is an oxidoreductase enzyme containing a dithiol–disulfide active site (–Cys–Gly–Pro–Cys–).[21] Oxidized Trx contains a disulfide bridge (–S–S–) between two cysteines, whereas reduced Trx is a dithiol with two cysteines.[19] The thioredoxins are maintained in the reduced state by the flavoenzyme thioredoxin reductase, in a NADPH-dependent reaction. Trx is important in signal transduction, inflammatory responses, and other biological functions such as apoptosis, cell growth, and proliferation.[19–23]

Here we describe the potential role of redox modulation in the mechanism of action of metal anticancer prodrugs, particularly in cobalt, platinum, ruthenium, osmium, and iridium complexes. To what extent is modulation of cellular redox processes involved in their activity? High-oxidation-state metal complexes can undergo intracellular reduction and release anticancer drugs in the reductive environment in cancer cells, for example, Co^{III} is reduced to Co^{II}, and Pt^{IV} is reduced to Pt^{II}. Organometallic

complexes can act as biocatalysts for modulating the redox state of cancer cells.

Co^{III} Complexes

Cobalt complexes, in general, have two accessible oxidation states: Cobalt(III) is kinetically inert due to its low-spin $3d^6$ configuration, and Co^{II} is labile (high-spin $3d^7$). Thus, Co^{III} complexes can act as carriers for selective delivery of anticancer agents to the hypoxic regions of a tumor.[24–26] It has been demonstrated that coordination of anticancer agents to Co^{III} can inhibit their cytotoxic properties. When Co^{III} is reduced to Co^{II} in a hypoxic environment, the active molecule is released and restored to its active form to kill cells. Active Co^{III} complexes studied thus far include those with quinoline,[27] amine,[28] nitrogen mustard,[29,30] marimastat,[31] and curcumin ligands.[32]

Nitrogen mustards are highly toxic due to their DNA alkylation and cross-linking activity. In vivo they are not selective for tumor tissue; however, they can be deactivated by coordination to Co^{III} and released on reduction to Co^{II} in hypoxic tumor tissue, thereby reducing systemic toxicity.[33] The Co^{III} mustard complex $[Co(Meacac)_2(DCE)]^+$ [Figure 1a, Meacac = 3-methyl acetylacetonate, DCE = *N*,*N*-bis(2-chloroethyl)ethylenediamine] is 20 times more active against hypoxic cancer cells rather than normoxic cells.[34] For a series of Co Meacac complexes, the redox potential has been shown to be of importance for hypoxic selectivity. Recently, Hambley et al. reported a Co^{III} complex that releases a curcumin ligand upon reduction in a hypoxic environment (Figure 1b).[32] This curcumin-containing Co^{III} complex exhibits selective cytotoxicity to cancer cells over non-tumorigenic cells.

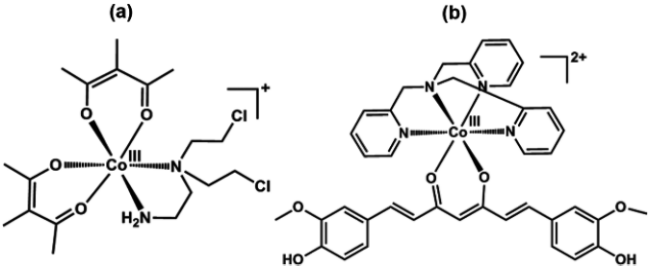

Figure 1. Co^{III} prodrugs with (a) nitrogen mustard and (b) curcumin ligands.

Fe^{III} Complexes

Iron(III) complexes with salen/salphen ligands and their derivatives have been extensively explored for anticancer activity.[35–37] Iron–salen/salphen complexes[35] having phenolato donors induce tumor-selective apoptosis and cytotoxicity toward cisplatin-resistant cancer cells due to Fe^{II}/Fe^{III} and salen/salphen-substituted ligands. Mandal and co-workers have described a water-soluble Fe^{III}–salen that cleaves DNA/RNA in vitro under a reducing environment and induces apoptosis in human cells via a mitochondrial pathway (Figure 2a).[35,36] Lange et al. and Lee et al. have explored the potential of Fe^{III}–salophene complexes for ovarian cancer therapy and leukemia, respectively.[37]

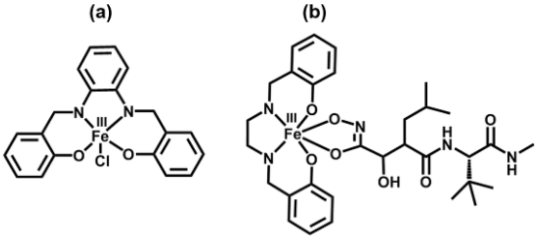

Figure 2. Fe^{III} complexes with (a) the salen ligand and (b) marimastat, a MMP inhibitor.

Marimastat exerts its anticancer activity by inhibiting matrix metalloproteinases (MMPs), which are overexpressed in cancer cells.[38] The high metal affinity of marimastat for MMPs has been exploited by ligand-releasing metal prodrugs, for example, Co^{III}–marimastat and Fe^{III}–marimastat prodrugs. An Fe^{III} marimastat–salen complex (Figure 2b) has been evaluated as a hypoxia-activated drug carrier. The complex provides a suitable framework for release of the MMP inhibitor at hypoxic tumor sites upon reduction to the more labile Fe^{II} oxidation state. Biological tests established that the complex is stable in non-reducing environments and serves to deliver intact MMP inhibitors to tumor sites.[39]

Pt^{IV} Complexes

Platinum anticancer drugs {e.g. cisplatin, *cis*-$[PtCl_2(NH_3)_2]$} are the most important antitumor agents currently available in the clinic, and they have proved to be highly effective towards a variety of solid tumors.[40] However, severe side-effects[41] as well as intrinsic or acquired drug resistance limit the applications of Pt^{II} complexes.[42] To address these drawbacks, a number of novel strategies are being explored, including Pt^{IV} prodrugs.[43] The administration of non-toxic Pt^{IV} prodrugs that can be activated selectively by reduction at tumor sites might reduce unwanted reactions with biomolecules and thus minimize the undesired side-effects. Potential agents for Pt^{IV} reduction in cancer cells include glutathione (Pt^{IV} + 2GSH → Pt^{II} + GSSG + $2H^+$),[44] ascorbate (vitamin C), NAD(P)H, and cysteine-containing proteins.[45] GSH is abundant inside cells (0.5–10 mM) as a reductant of Pt^{IV} complexes, but it can also coordinate to and deactivate the active Pt^{II} species.

So far, four octahedral Pt^{IV} prodrugs have entered clinical trials, namely, tetraplatin, iproplatin, satraplatin, and LA-12 (Figure 3a–d).[46] However, LA-12 failed in phase I trials, and tetraplatin could not be investigated further after phase I due to high neurotoxicity. Iproplatin had limited success in phase II trials. The first orally available Pt drug candidate, satraplatin, was abandoned recently in phase III trials.[46] The lower efficacy of these Pt^{IV} prodrugs with respect to that of cisplatin, together with variability in drug uptake and side-effects, has meant that these Pt^{IV} prodrugs have not yet been approved for clinical use. Thus, there is a need to explore other novel Pt^{IV} prodrugs with high anticancer efficacy, high cell uptake efficiency, and sensitivity to reduction.

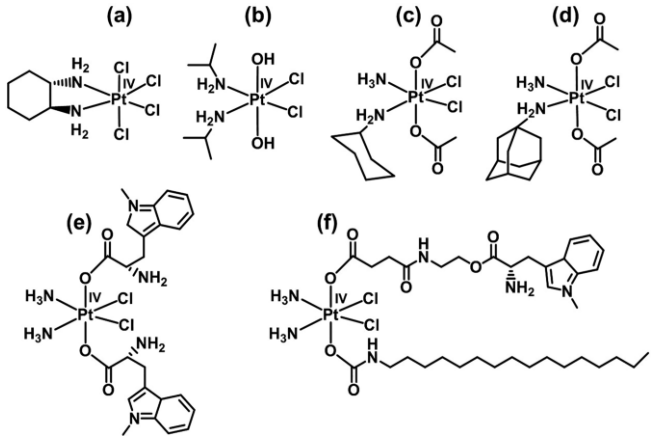

Figure 3. (a–d) Pt^{IV} anticancer complexes that have entered clinical trials: (a) tetraplatin, (b) iproplatin, (c) satraplatin, (d) LA-12, (e, f) Pt^{IV}-(D)-1-methyltryptophan conjugates for combined immunomodulation and DNA cross-link-triggered apoptosis for cancer "immuno-chemotherapy".

Lippard et al. have investigated a variety of Pt^{IV} prodrug approaches,[47] for example Pt^{IV}-(D)-1-methyltryptophan conjugates (Figure 3e, f), for combined immunomodulation and DNA cross-link-triggered apoptosis for cancer "immuno-chemotherapy".[48] These prodrugs kill hormone-dependent, cisplatin-resistant, human ovarian cancer cells effectively, inhibiting indoleamine-2,3-dioxygenase (IDO) by transcriptional deregulation of the autocrine-signaling loop IDO-AHR-IL6. IDO is an immunosuppressive enzyme found in human tumors, and it is involved in immune evasion and tumor tolerance. These compounds are the first Pt drug candidates with immune checkpoint blockade properties that induce kynurenine production and promote T-cell proliferation. They have low toxicity in mice and are stable in blood.

Photoactivatable Pt^{IV}-azide prodrugs, such as *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)(Py)]$ and $[Pt(N_3)_2(OH)_2(Py)_2]$ (Figure 4a, b),[49,50] upon irradiation with light, can be selectively activated to become potently cytotoxic toward a number of cancer cell lines. Perhaps surprisingly, in view of the role of amine NH groups in stabilizing DNA adducts of Pt^{II} ammine anticancer complexes, replacing one or two NH_3 ligands with pyridine (Py) in $[Pt(N_3)_2(OH)_2(NH_3)_2]$ leads to higher photocytotoxicity and visible-light activation. *Trans*-$[Pt(N_3)_2(OH)_2(NH_3)(Py)]$ forms *trans*-G adducts both with model G derivatives and with plasmid DNA. Moreover, DNA–protein cross-links also form readily, and DNA repair synthesis on plasmid DNA platinated by photoactivated $[Pt(N_3)_2(OH)_2(NH_3)(Py)]$ is markedly lower than that for transplatin.

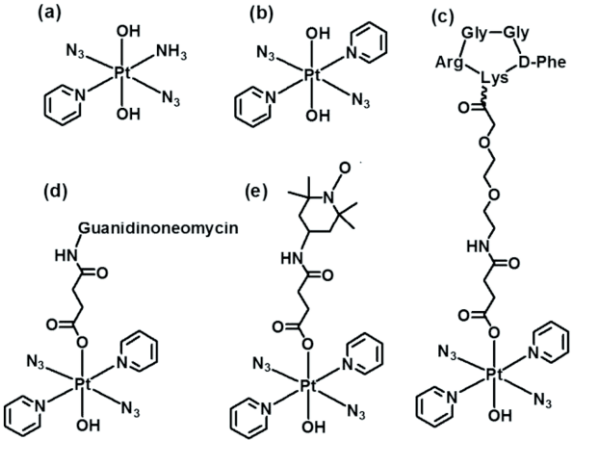

Figure 4. Photoactivatable Pt^{IV} prodrugs. (a) $[Pt(N_3)_2(OH)_2(NH_3)(Py)]$, (b) $[Pt(N_3)_2(OH)_2(Py)_2]$, (c) $[Pt(N_3)_2(py)_2(OH)]$ (RGD sequence), (d) $\{Pt(N_3)_2(py)_2(OH)\}$ (guanidinoneomycin conjugate), (e) $[Pt(N_3)_2(py)_2(OH)]$ (TEMPO conjugate).

The complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(py)_2]$, conjugated to a cyclic peptide containing the RGD sequence (–Arg–Gly–Asp–) (Figure 4c), is selectively recognized by $\alpha V\beta 3$ and $\alpha V\beta 5$ integrins.[51] Upon visible-light irradiation, phototoxicity is induced preferentially in SK-MEL-28 melanoma cancer cells overexpressing $\alpha V\beta 3$ integrin compared to that in control DU-145 human prostate carcinoma cells. Photoactivation of the platinum–guanidinoneomycin conjugate (Figure 4d) in the presence of 5′-guanosine monophosphate (5′-GMP) leads to the formation of *trans*-$[Pt(N_3)(py)_2(5'\text{-GMP})]^+$, as does the photoactivation of the parent platinum(IV) complex. Binding of the Pt^{II} photoproduct $\{PtN_3(py)_2\}^+$ to guanine nucleobases in a short, single-stranded oligonucleotide is also observed.[52] This provides a novel approach to visible-light-driven dual control of cancer selectivity and drug release. Moreover, the released active *trans*-Pt^{II} complexes have a different anticancer spectrum from that of cisplatin. Recently, the nitroxide spin-labelled photoactivatable Pt^{IV} prodrug *trans,trans,trans*-$[Pt(N_3)_2(OH)(OCOCH_2CH_2CONH\text{-TEMPO})(Py)_2]$(Pt-TEMPO, TEMPO = 2,2,6,6-tetramethylpiperidine 1-oxyl) (Figure 4e) has been reported, which is activated by photoreduction.[53] Irradiation with blue visible light gives rise to Pt^{II} and azidyl as well as nitroxyl radicals. Pt-TEMPO exhibited low toxicity in the dark, and on photoactivation, it was as active as the clinical photosensitizer chlorpromazine and more active than cisplatin toward human ovarian cancer cells under the same conditions. The anticancer activity of Pt-TEMPO may be the result of attack on DNA as well as the activity of the reactive azidyl and TEMPO radicals. The complex might be suitable for the treatment of surface cancers such as bladder and oesophageal cancers.

Ru(III/II) Complexes

Three Ru^{III} coordination compounds have entered clinical trials: [ImH][transRuCl$_4$(DMSO)Im] (NAMI-A, Im = imidazole), [InH][trans-RuCl$_4$In$_2$] (KP1019, In = indazole), and NKP-1339 (the sodium salt of KP1019) (Figure 5a–c).[54] The first Ru-based anticancer drug candidate in clinical trials was NAMI-A, followed by KP1019 in 2003. Both successfully completed phase I, but NAMI-A has recently been withdrawn from the clinic after phase I/II because of unconvincing efficacy; the likelihood of further clinical studies of NAMI-A is uncertain.[55,56] These Ru^{III} complexes may be activated in vivo by reduction to Ru^{II}. The Ru^{III}/Ru^{II} redox potentials of KP1019 and NAMI-A in 0.20 M phosphate buffer at pH 7.0 are 0.03 and 0.25 V vs. NHE, respectively,[57] almost unaffected by the buffer system used, and physiologically accessible by intra- and extracellular reducing agents (e.g. glutathione, $E^{0\prime} = -0.25$ V or ascorbic acid, $E^{0\prime} = +0.06$ V vs. NHE

at pH 7.0), as well as some proteins.[57] Thus the complexes can readily undergo reduction in biological systems.[58]

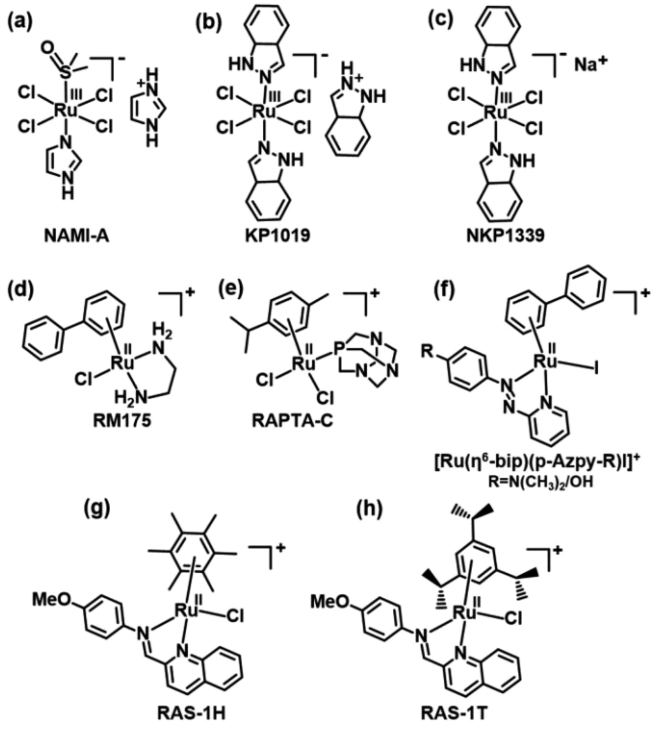

Figure 5. Structures of ruthenium anticancer complexes. (a–c) Ru^{III} anticancer complexes that have undergone clinical trials; (d) RM175, (e) RAPTA-C, (f) $[Ru(\eta^6\text{-bip})(p\text{-Azpy-R})I]^+$ {R = $N(CH_3)_2$/OH}; (g, h) Ru^{II} Schiff base (RAS) complexes: (g) RSA-1H and (h) RSA-1T.

Organoruthenium(II) complexes, such as $[Ru(\eta^6\text{-bip})(en)Cl]^+$ (RM175)[59] and RAPTA-C,[60] have promising anticancer activity (Figure 5d, e). Interestingly, although RM175 reacts with the thiol in GSH to form $[Ru(\eta^6\text{-bip})(en)(SG)]^+$, this is not the end product. Oxygen addition to the bound thiolate sulfur easily affords the sulfenate complex $[Ru(\eta^6\text{-bip})(en)(S(O)G)]^+$. Further oxidation can take place to give the sulfinate adduct $[Ru(\eta^6\text{-bip})(en)(S(O)_2G)]^+$.[61] Unlike the behavior of Pt^{II} drugs, such binding to GSH, when followed by oxidation, promotes binding to guanine in DNA. Displacement of the sulfenate ligand by guanine N7 provides a redox-mediated pathway to DNA binding for these arene–Ru^{II}–diamine complexes.[62]

$[Ru(\eta^6\text{-bip})(p\text{-Azpy-}NMe_2)I]^+$ and $[Ru(\eta^6\text{-bip})(p\text{-Azpy-OH})I]^+$ (Figure 5f), in which NMe_2Ph-Azpy- and HO-Ph-Azpy are *para*-substituted phenylazopyridine ligands, contain N=N azo bonds in the ligand, which give rise to reduction potentials that are biologically accessible (ca. –0.3 V). These complexes can oxidize GSH to GSSG under physiological conditions and generate elevated levels of ROS in A549 lung cancer cells, which can be scavenged by *N*-acetyl-L-cysteine (NAC).[63] The mechanism of formation of these ROS is not clear but may involve ligand-based reduction and appears to be catalytic.

Gaiddon and co-workers[64] investigated two organoruthenium Schiff base complexes, RAS-1H (Figure 5g) and RAS-1T (Figure 5h), and demonstrated that, although they both induce non-apoptotic programmed cell death (PCD) through endoplasmic reticulum (ER) stress pathways, their modes of action are drastically different despite modest structural variations. RAS-1T acts through ROS-mediated ER stress, while RAS-1H is ROS-independent. They further showed that the complexes are more efficacious towards apoptosis-resistant cells than clinical drugs, including oxaliplatin. This work provides the basis for underpinning ER stress modulation using metal complexes to bypass apoptosis resistance.

Ir^{III} Complexes

Iridium complexes have attracted much recent attention in a wide range of areas, especially catalysis. Organoiridium(III) complexes have interesting biological (e.g. as luminescent probes),[65] and anticancer applications.[66]

Unlike Ru^{II} and Os^{II}, it is not possible to stabilize Ir^{III} with an arene ligand, and instead cyclopentadienyl and preferably pentamethylcyclopentadienyl ligands are used. A range of organometallic Ir^{III} cyclopentadienyl complexes of the type $[(\eta^5\text{-}Cp^x)Ir(L\,L)Z]^{0/n+}$ {where Cp^x = Cp*, Cp^{xph} (phenyltetramethylcyclopentadienyl) or Cp^{xbiph} (biphenyltetramethylcyclopentadienyl), L L = bidentate ligand with nitrogen and/or carbon donor atoms, Z = Cl or py} have been synthesized and characterized as potential anticancer agents (Figure 6).[67–69] There are effective strategies for switching on and/or controlling the anticancer activity, involving modifications to the three ligands. In the phen/Cl series (Figure 6a), addition of phenyl substituents to the Cp* ring markedly increases the potency. In the bpy series, replacement of a chelated N by isoelectronic C^- causes a dramatic increase in activity (Figure 6b), and further addition of a biphenyl substituent and replacement of Cl^- by pyridine achieves nanomolar activity (IC_{50} = 100 nM; Figure 6c).[70]

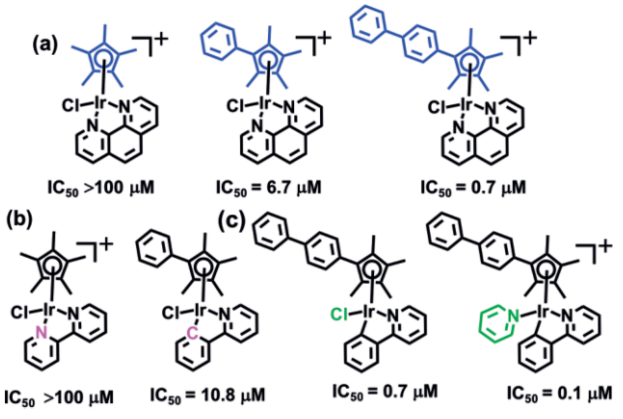

Figure 6. Half-sandwich organometallic Ir^{III} cyclopentadienyl complexes $[(\eta^5\text{-}Cp^x)Ir(L\,L)Z]^{0/+}$ and their IC_{50} values.

Facile conversion of coenzyme NADH to NAD^+ can be achieved through hydride transfer using Ir^{III} Cp* complexes.[71] Hydride transfer from NADH results in the formation of Ir–H species (^{1}H NMR Ir–H peak at ca. 15 ppm). The hydride can further be transferred to oxygen to generate H_2O_2.[72] Thus it is possible to perturb the intracellular ratio of NADH/NAD^+ as well

as carry out reductions which might normally be achieved by enzymes, such as the conversion of pyruvate to lactate (lactate dehydrogenase). These organoiridium complexes can have potent antiproliferative activity towards a wide range of cancer cells and will provide a means of probing NADH-mediated cell signaling pathways and coupling hydrogenations to biological processes.

Os^{II} Complexes

In general, the redox activity of Os^{II} complexes is associated with the formation of ROS in cells and, as in the case of Ru complexes, might lead to activation in the reductive environment of tumors.[73,74] Changing the arene from *p*-cymene to biphenyl and the monodentate ligand from chloride to iodide in the library of Os^{II} complexes of the formula $[Os(\eta^6\text{-arene})(L)X]^+$ {L = azopyridine derivatives (Azpy-R) or iminopyridine *N*,*N*-chelators, X = Cl or I, arene = *p*-cymene or biphenyl} results in a significant increase in anticancer activity (Figure 7).[75–79] Azopyridine Os^{II} complexes with electron-donating substituents on the phenyl ring (e.g. OH or NMe_2) or electron-withdrawing groups on the pyridine ring (e.g. F, Cl, Br or I) are an order of magnitude more active than their unsubstituted analogs. This might be related to the involvement of redox processes associated with the azo group[74,75] (e.g. reductive attack by glutathione[80]). Notably, $[Os(\eta^6\text{-biphenyl})(Azpy\text{-}NMe_2)I]PF_6$ (Figure 7a, R = NMe_2, X = I) has more than ten times higher anticancer potency than cisplatin (CDDP) against the kinds of tested cancer cell lines.

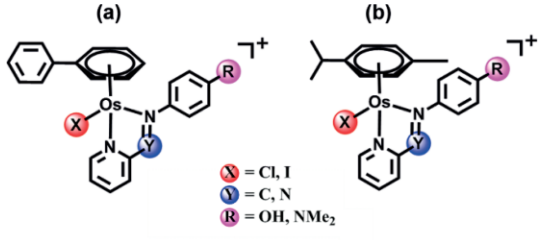

Figure 7. Organometallic Os^{II} anticancer complexes $[Os(\eta^6\text{-arene})(L)X]^+$ {L = azopyridine derivatives (Azpy-R) or iminopyridine *N*,*N*-chelators; X = Cl or I; (a) arene = biphenyl, (b) arene = *p*-cymene}.

FY26 (Figure 7b, R = NMe_2, X = I, Y = N) is highly active towards cancer cell lines;[76,77] in particular, it exhibits submicromolar activity in A2780 ovarian, MCF7 breast, A549 lung, and HCT116 colon cancer cell lines. FY26 is more potent than cisplatin in the NCI-60 cell line screen (the average GI_{50} value is 0.28 μM for FY26 but 10.3 μM for cisplatin) as well as in the 809-cell line screen of the Sanger Institute (the average GI_{50} value is 0.75 μM for FY26 but 36.7 μM for cisplatin). The potency of FY26 can be increased by coadministration with non-toxic doses of the redox modulator L-buthionine sulfoximine (L-BSO), which reduces GSH levels in cells by inhibiting the enzyme γ-glutamylcysteine synthetase. The potency of FY26 in A2780 cancer cells increases 2.3-fold when FY26 is coadministered with 5 μM of L-BSO, the IC_{50} value decreasing from 160 ± 10 to 69 ± 5 nM. FY26 rapidly induces the formation of ROS in cells, especially superoxide. Recently, XRF mapping of FY26 osmium in cancer cells has provided evidence for targeting of mitochondria.[81] Cancer cells have malfunctioning mitochondria, and attack on their redox balance can provide some selectivity over normal cells.

Active iminopyridine complexes (Y = C, Figure 7) induce a remarkable increase in the ROS level in A549 lung cancer cells. They can oxidize NADH to NAD^+. The oxidation of NADH might occur through the formation of an Os–H intermediate, which causes interference in the redox signaling pathways in cancer cells.[78] Moreover, these complexes are selective for cancer cells over healthy cells and have high accumulation in cell membranes. Their mode of action is related to cell growth arrest in the G1 phase and caspase 3 activation, and their activities are independent of p53 status.[79]

Perspectives

The development of resistance is a major clinical problem with current anticancer drugs, including platinum compounds. Multitargeting by metallodrugs, or by metallodrugs in combination with clinical drugs, might provide a strategy to address this problem. In particular, the redox balance in cancer cells and the difference in the ability of cancer cells to cope with changes in the levels of redox-active species such as ROS, provides a means for selective attack on cancer cells. The unique ability of metal complexes to undergo redox activation processes involving both metal and ligand redox centers that can be tuned to specific potentials should provide them with the novel mechanisms of action required to overcome resistance. Further research in this field is now required to investigate these new possibilities for drug design.

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[1] J. M. Brown, *Cancer Biol. Ther.* **2002**, *1*, 453–458.
[2] A. M. Shannon, D. J. Bouchier-Hayes, C. M. Condron, D. Toomey, *Cancer Treat. Rev.* **2003**, *29*, 297–307.
[3] I. Romero-Canelón, M. Mos, P. J. Sadler, *J. Med. Chem.* **2015**, *58*, 7874–7880.
[4] M. Galanski, M. A. Jakupec, B. K. Keppler, *Curr. Med. Chem.* **2005**, *12*, 2075–2094.
[5] I. Romero-Canelón, P. J. Sadler, *Inorg. Chem.* **2013**, *52*, 12276–12291.
[6] Z. Liu, P. J. Sadler, *Acc. Chem. Res.* **2014**, *47*, 1174–1185.
[7] M. Diehn, R. W. Cho, N. Lobo, T. Kalisky, M. J. Dorie, A. N. Kulp, D. Qian, J. S. Lam, L. E. Ailles, M. Wong, B. Joshua, M. J. Kaplan, I. Wapnir, F. M. Dirbas, G. Somlo, C. Garberoglio, B. Paz, J. Shen, S. K. Lau, S. R. Quake, J. M. Brown, I. L. Weissman, M. F. Clarke, *Nature* **2009**, *458*, 780–783.

[8] A. Bhattacharyya, R. Chattopadhyay, S. Mitra, S. E. Crowe, *Physiol. Rev.* **2014**, *94*, 329–354.
[9] I. Aldib, J. Soubhye, K. Z. Boudjeltia, M. Vanhaeverbeek, A. Rousseau, P. G. Furtmüller, C. Obinger, F. Dufrasne, J. Nève, P. Van Antwerpen, M. Prévost, *J. Med. Chem.* **2012**, *55*, 7208–7218.
[10] V. Sánchez-Valle, N. C. Chavez-Tapia, M. Uribe, N. Méndez-Sánchez, *Curr. Med. Chem.* **2012**, *19*, 4850–4860.
[11] B. Halliwell, J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, New York, **2015**.
[12] S. Sabuncuoglu, A. Eken, A. Aydin, H. Ozgunes, H. Orhan, *Drug Chem. Toxicol.* **2015**, *38*, 375–382.
[13] C. C. Winterbourn, A. J. Kettle, *Antioxid. Redox Signaling* **2013**, *18*, 642–660.
[14] U. Jungwirth, C. R. Kowol, B. K. Keppler, C. G. Hartinger, W. Berger, P. Heffeter, *Antioxid. Redox Signaling* **2011**, *15*, 1085–1127.
[15] F. Q. Schafer, G. R. Buettner, *Free Radical Biol. Med.* **2001**, *30*, 1191–1212.
[16] M. L. Fishel, M. P. Gamcsik, S. M. Delaney, E. G. Zuhowski, V. M. Maher, T. Karrison, R. C. Moschel, M. J. Egorin, M. E. Dolan, *Cancer Chemother. Pharmacol.* **2005**, *55*, 333–342.
[17] E. E. Ramsay, P. J. Dilda, *Front. Pharmacol.* **2014**, *5*, 1–16.
[18] J. E. Heffner, J. E. Repine, *Am. Rev. Respir. Dis.* **1989**, *140*, 531–554.
[19] S. A. A. Comhair, S. C. Erzurum, *Antioxid. Redox Signaling* **2010**, *12*, 93–124.
[20] H. Petzold, P. J. Sadler, *Chem. Commun.* **2008**, 4413–4415.
[21] T. Hoshino, M. Okamoto, S. Takei, Y. Sakazaki, T. Iwanaga, H. Aizawa, *Antioxid. Redox Signaling* **2008**, *10*, 769–783.
[22] J. Nordberg, E. S. Arner, *Free Radical Biol. Med.* **2001**, *31*, 1287–1312.
[23] M. Genestra, *Cell Signal* **2007**, *19*, 1807–1819.
[24] I. C. A. de Souza, L. V. Faro, C. B. Pinheiro, D. T. G. Gonzaga, F. D. C. da Silva, V. F. Ferreira, F. da Silva Miranda, M. Scarpellini, M. Lanznaster, *Dalton Trans.* **2016**, *45*, 13671–13674.
[25] N. Yamamoto, A. K. Renfrew, B. J. Kim, N. S. Bryce, T. W. Hambley, *J. Med. Chem.* **2012**, *55*, 11013–11021.
[26] A. K. Renfrew, N. S. Bryce, T. W. Hambley, *Chem. Sci.* **2013**, *4*, 3731–3739.
[27] F. L. S. Bustamante, J. M. Metello, F. A. V. Castro, C. B. Pinheiro, M. D. Pereira, M. Lanznaster, *Inorg. Chem.* **2013**, *52*, 1167–1169.
[28] D. C. Ware, P. J. Brothers, G. R. Clark, W. A. Denny, B. D. Palmer, W. R. Wilson, *J. Chem. Soc., Dalton Trans.* **2000**, 925–932.
[29] L. L. Parker, S. M. Lacy, L. J. Farrugia, C. Evans, D. J. Robins, C. C. O'Hare, J. A. Hartley, M. Jaffar, I. J. Stratford, *J. Med. Chem.* **2004**, *47*, 5683–5689.
[30] P. R. Craig, P. J. Brothers, G. R. Clark, W. R. Wilson, W. A. Denny, D. C. Ware, *Dalton Trans.* **2004**, 611–618.
[31] T. W. Failes, T. W. Hambley, *Dalton Trans.* **2006**, 1895–1901.
[32] A. K. Renfrew, N. S. Bryce, T. Hambley, *Chem. Eur. J.* **2015**, *21*, 15224–15234.
[33] C. R. Munteanu, K. Suntharalingam, *Dalton Trans.* **2015**, *44*, 13796–13808.
[34] D. C. Ware, B. D. Palmer, W. R. Wilson, W. A. Denny, *J. Med. Chem.* **1993**, *36*, 1839–1846.
[35] K. I. Ansari, S. Kasiri, J. D. Grant, S. S. Mandal, *J. Biomol. Screening* **2011**, *16*, 26–35.
[36] T. S. Lange, C. McCourt, R. K. Singh, K. K. Kim, A. P. Singh, B. S. Luisi, O. Alpturk, R. M. Strongin, L. Brard, *Drug Des. Dev. Ther.* **2009**, *3*, 17–26.
[37] S. Y. Lee, A. Hille, I. Kitanovic, P. Jesse, G. Henze, S. Wölfl, R. Gust, A. Prokop, *Leuk. Res.* **2011**, *35*, 571–572.
[38] T. W. Failes, C. Cullinane, C. I. Diakos, N. Yamamoto, J. G. Lyons, T. W. Hambley, *Chem. Eur. J.* **2007**, *13*, 2974–2982.
[39] T. W. Failes, T. W. Hambley, *J. Inorg. Biochem.* **2007**, *101*, 396–403.
[40] B. W. Harper, A. M. Krause-Heuer, M. P. Grant, M. Manohar, K. B. Garbutcheon-Singh, J. R. Aldrich-Wright, *Chem. Eur. J.* **2010**, *16*, 7064–7077.
[41] X. Yao, K. Panichpisal, N. Kurtzman, K. Nugent, *Am. J. Med. Sci.* **2007**, *334*, 115–124.
[42] L. Kelland, *Nat. Rev. Cancer* **2007**, *7*, 573–584.
[43] M. D. Hall, T. W. Hambley, *Coord. Chem. Rev.* **2002**, *232*, 49–67.
[44] G. R. Gibbons, S. Wyrick, S. G. Chaney, *Cancer Res.* **1989**, *49*, 1402–1407.
[45] J. L. van der Veer, A. R. Peters, J. Reedijk, *J. Inorg. Biochem.* **1986**, *26*, 137–142.
[46] N. Graf, S. J. Lippard, *Adv. Drug Delivery Rev.* **2012**, *64*, 993–1004.
[47] T. C. Johnstone, K. Suntharalingam, S. J. Lippard, *Chem. Rev.* **2016**, *116*, 3436–3486.
[48] S. G. Awuah, Y. R. Zheng, P. M. Bruno, M. T. Hemann, S. J. Lippard, *J. Am. Chem. Soc.* **2015**, *137*, 14854–14857.
[49] F. S. Mackay, J. A. Woods, P. Heringova, J. Kasparkova, A. M. Pizarro, S. A. Moggach, S. Parsons, V. Brabec, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20743–20748.
[50] N. J. Farrer, J. A. Woods, L. Salassa, Y. Zhao, K. S. Robinson, G. Clarkson, F. S. Mackay, P. J. Sadler, *Angew. Chem. Int. Ed.* **2010**, *49*, 8905–8908; *Angew. Chem.* **2010**, *122*, 9089–9092.
[51] A. Gandioso, E. Shaili, A. Massaguer, G. Artigas, A. González-Cantó, J. A. Woods, P. J. Sadler, V. Marchán, *Chem. Commun.* **2015**, *51*, 9169–9172.
[52] E. Shaili, M. Fernández-Giménez, S. Rodríguez-Astor, A. Gandioso, L. Sandín, C. García-Vélez, A. Massaguer, G. J. Clarkson, J. A. Woods, P. J. Sadler, V. Marchín, *Chem. Eur. J.* **2015**, *21*, 18474–18486.
[53] V. Venkatesh, C. J. Wedge, I. Romero-Canelón, A. Habtemariama, P. J. Sadler, *Dalton Trans.* **2016**, *45*, 13034–13037.
[54] C. G. Hartinger, M. A. Jakupec, S. Zorbas-Seifried, M. Groessl, A. Egger, W. Berger, H. Zorbas, P. J. Dyson, B. K. Keppler, *Chem. Biodiversity* **2008**, *5*, 2140–2155.
[55] S. Leijen, S. A. Burgers, P. Baas, D. Pluim, M. Tibben, E. van Werkhoven, E. Alessio, G. Sava, J. H. Beijnen, J. H. M. Schellens, *Invest. New Drugs* **2015**, *33*, 201–214.
[56] C. S. Allardyce, P. J. Dyson, *Dalton Trans.* **2016**, *45*, 3201–3209.
[57] E. Reisner, V. B. Arion, B. K. Keppler, A. J. L. Pombeiro, *Inorg. Chim. Acta* **2008**, *361*, 1569–1583.
[58] A. M. Pizarro, A. Habtemariam, P. J. Sadler, *Top. Organomet. Chem.* **2010**, *32*, 21–56.
[59] R. E. Morris, R. E. Aird, P. D. S. Murdoch, H. Chen, J. Cummings, N. D. Hughes, S. Parsons, A. Parkin, G. Boyd, D. I. Jodrell, P. J. Sadler, *J. Med. Chem.* **2001**, *44*, 3616–3621.
[60] C. Scolaro, A. Bergamo, L. Brescacin, R. Delfino, M. Cocchietto, G. Laurenczy, T. J. Geldbach, G. Sava, P. J. Dyson, *J. Med. Chem.* **2005**, *48*, 4161–4171.
[61] F. Wang, J. Xu, A. Habtemariam, J. Bella, P. J. Sadler, *J. Am. Chem. Soc.* **2005**, *127*, 17734–17743.
[62] K. Wu, W. Hu, Q. Luo, X. Li, S. Xiong, P. J. Sadler, F. Wang, *J. Am. Soc. Mass Spectrom.* **2013**, *24*, 410–420.
[63] S. J. Dougan, A. Habtemariam, S. E. McHale, S. Parsons, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11628–11633.
[64] M. J. Chow, C. Licona, D. Y. Q. Wong, G. Pastorin, C. Gaiddon, W. H. Ang, *J. Med. Chem.* **2014**, *57*, 6043–6059; M. J. Chow, C. Licona, D. Y. Q. Wong, G. Pastorin, C. Gaiddon, W. H. Ang, *Chem. Sci.* **2016**, *7*, 4117–4124.
[65] K. K.-W. Lo, S. P.-Y. Li, K. Y. Zhang, *New J. Chem.* **2011**, *35*, 265–287.
[66] Y. Geldmacher, M. Oleszak, W. S. Sheldrick, *Inorg. Chim. Acta* **2012**, *393*, 84–102.
[67] Z. Liu, A. Habtemariam, A. M. Pizarro, S. A. Fletcher, A. Kisova, O. Vrana, L. Salassa, P. C. A. Bruijnincx, G. J. Clarkson, V. Brabec, P. J. Sadler, *J. Med. Chem.* **2011**, *54*, 3011–3026.
[68] Z. Liu, L. Salassa, A. Habtemariam, A. M. Pizarro, G. J. Clarkson, P. J. Sadler, *Inorg. Chem.* **2011**, *50*, 5777–5783.
[69] Z. Liu, A. Habtemariam, A. M. Pizarro, G. J. Clarkson, P. J. Sadler, *Organometallics* **2011**, *30*, 4702–4710.
[70] Z. Liu, I. Romero-Canelón, A. Habtemariam, G. J. Clarkson, P. J. Sadler, *Organometallics* **2014**, *33*, 5324–5333.
[71] S. Betanzos-Lara, Z. Liu, A. Habtemariam, A. M. Pizarro, B. Qamar, P. J. Sadler, *Angew. Chem. Int. Ed.* **2012**, *51*, 3897–3900; *Angew. Chem.* **2012**, *124*, 3963.
[72] Z. Liu, I. Romero-Canelón, B. Qamar, J. M. Hearn, A. Habtemariam, N. P. E. Barry, A. M. Pizarro, G. J. Clarkson, P. J. Sadler, *Angew. Chem. Int. Ed.* **2014**, *53*, 3941–3946; *Angew. Chem.* **2014**, *126*, 4022.
[73] J. Lu, A. Holmgren, *Antioxid. Redox Signaling* **2012**, *7*, 1738–1747.
[74] M. Hanif, M. V. Babak, C. G. Hartinger, *Drug Discovery Today* **2014**, *19*, 1640–1648.
[75] Y. Fu, A. Habtemariam, A. M. Pizarro, S. H. van Rijt, D. J. Healey, P. A. Cooper, S. D. Shnyder, G. J. Clarkson, P. J. Sadler, *J. Med. Chem.* **2010**, *53*, 8192–8196.
[76] S. D. Shnyder, Y. Fu, A. Habtemariam, S. H. van Rijt, P. A. Cooper, P. M. Loadman, P. J. Sadler, *MedChemComm* **2011**, *2*, 666–668.
[77] Y. Fu, A. Habtemariam, A. M. Basri, D. Braddick, G. J. Clarkson, P. J. Sadler, *Dalton Trans.* **2011**, *40*, 10553–10562.

[78] Y. Fu, M. J. Romero, A. Habtemariam, M. E. Snowden, L. Song, G. J. Clarkson, B. Qamar, A. M. Pizarro, P. R. Unwin, P. J. Sadler, *Chem. Sci.* **2012**, *3*, 2485–2494.
[79] I. Romero-Canelón, L. Salassa, P. J. Sadler, *J. Med. Chem.* **2013**, *56*, 1291–1300.
[80] R. J. Needham, C. Sanchez-Cano, X. Zhang, I. Romero-Canelón, A. Habtemariam, M. S. Cooper, L. Meszaros, G. J. Clarkson, P. J. Blower, P. J. Sadler, *Angew. Chem. Int. Ed.* **2017**, *56*, 1017–1020; *Angew. Chem.* **2017**, *129*, 1037–1040.
[81] C. Sanchez-Cano, I. Romero-Canelón, Y. Yang, I. J. Hands-Portman, S. Bohic, P. Cloetens, P. J. Sadler, *Chem. Eur. J.* **2017**, DOI: 10.1002/chem.201605911.

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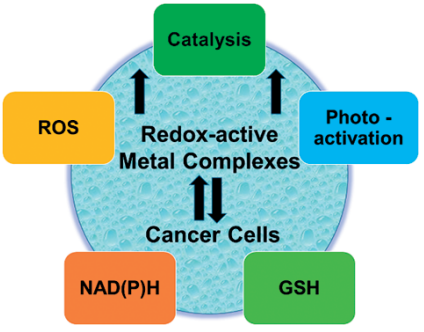

Redox reactions in the reducing environment of cancer cells can activate metal complexes so as to deliver bioactive ligands or modulate the redox state of cancer cells. Such redox activation strategies can provide novel mechanisms of action that increase drug selectivity and combat resistance.

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