

Ruthenium metallopharmaceuticals

Michael J. Clarke *

Merkert Chemistry Center, Boston College, 2609 Beacon Street, Chestnut Hill, MA 02467-3860, USA

Received 11 October 2001; accepted 18 January 2002

Contents

Abstract	209
1. Introduction	210
2. Anticancer compounds	211
2.1 Activation by reduction	211
2.2 Active Ru(II) complexes	211
2.3 Transferrin transport	212
2.4 Covalent DNA binding	213
2.5 DNA damage generated by covalently bound Ru	215
2.6 Modulation of DNA binding by glutathione	216
2.7 Cytotoxicities of Ru complexes	217
2.8 Complexes with EDTA-type ligands	218
2.9 Complexes with R ₂ SO	218
2.10 Photodynamic therapy	219
3. Ruthenium red and Ru360: inhibitors of Ca ²⁺ utilization	222
4. Nitric oxide complexes	223
5. Immunosuppressants	227
6. Radiopharmaceuticals	227
7. Conclusion	228
Acknowledgements	229
References	229

Abstract

The well-developed synthetic chemistry of ruthenium, particularly with ammine, amine and imine ligands, provides for many approaches to innovative new metallopharmaceuticals. Advantages of utilizing ruthenium am(m)ine complexes in drug development include, (1) reliable preparations of stable complexes with predictable structures; (2) the ability to tune ligand affinities, electron transfer and substitution rates, and reduction potentials; and (3) an increasing knowledge of the biological effects of ruthenium complexes. Many Ru(II) and Ru(III) am(m)ine complexes selectively bind to imine sites in biomolecules. Collectively, these lend ruthenium complexes to redox-activation and photodynamic approaches to therapy as well as the development of radio-

Abbreviations: Ade, A, adenine; 5'AMP, adenosine-5'-monophosphate; bpy, 2,2'-bipyridyl; bzac, 1-phenylbutane-1,3-diketone; CD, circular dichroism; cdt, 1,2-cyclo-hexanediaminetetraacetate; chrysi, 5,6-chrysenequinone diimine; CT-DNA, calf thymus DNA; Cyt, C, cytosine; 5'-CMP, cytidine-5'-monophosphate; Cp, cyclo-pentadienide; cyclam, 1,4,8,11-tetraazocyclo-tetradecane; dppz, dipyrrodo[3,2-a:2',3'-c] phenazine; en, ethylenediamine; 9EtGua, 9-ethylguanine; GSH, glutathione (γ -glutamate–cysteine–glycine); 5'-dGMP, deoxyguanosine-5'-monophosphate; Gua, G, guanine; dGuo, guanosine; dGuo, dG, 2'-deoxyguanosine; Hyp, hypoxanthine; Icyt, isocytidine; ICR, imidazolium *trans*-tetrachlorobisimidazolruthenium(III); Imm, imidazole; Ind, indazole; Isn, isonicotinamide; Me, methyl; Et, ethyl; mcp, Methylguanidinium phenanthroline; 7MeGua, 7-methylguanine; 1MeGuo, 7-methylguanosine; 7MeHyp, 7-methylhypoxanthine; 6MeICyt, 6-methylisocytosine; 1,3Me₂Xan[−], deprotonated 1,3-dimethylxanthine; MM2 molecular mechanics 2; NAMI, Na[*trans*-(Me₂SO)(Im)Cl₄Ru]; NAMI-A, ImH[*trans*-(Me₂SO)(Im)Cl₄Ru]; NHE, normal hydrogen electrode; 8-OG, 8-oxo-guanine; 8-Oguo, 8-oxo-guanosine; PIH, pyridoxal isonicotinoyl hydrazone; quin, 8-hydroxyquinoline; Ox, oxalate; pdta, 1,2-propylenediaminetetraacetate; phen, 1,10-phenanthroline; py, pyridine; Pyr, pyridine derivative; SSCE, standard saturated calomel electrode; 5'-TMP, thymidine-5'-monophosphate; 5'dTMP, deoxythymidine-5'-monophosphate; tpy, 2,2':6'',2''-terpyridine; Tf, transferrin; TfR, transferrin receptor; trien, triethylenetetraammine.

* Tel.: +1-617-552-3624; fax: +1-617-552-2705

E-mail address: clarke@bc.edu (M.J. Clarke).

pharmaceuticals containing one of several radionuclides of ruthenium. Ruthenium red and the related Ru360 strongly inhibit calcium ion uptake in the mitochondria. A number of ruthenium compounds with anticancer activity appear to penetrate tumors through a transferrin-mediated process and bind to cellular DNA following intracellular activation by reduction. Ruthenium complexes exhibit both nitric oxide release and scavenging functions that can affect vasodilation and synapse firing. Simple ruthenium complexes are unusually effective in suppressing the immune response by inhibiting T cell proliferation.

© 2002 Elsevier Science B.V. All rights reserved.

Keywords: ruthenium; metallopharmaceutical; immunosuppressant; anticancer; transferring; DNA; photodynamic; nitric oxide; radiopharmaceutical

1. Introduction

Along with an increased understanding of metalloprotein function and some excellent models of metal ion active sites [1], recent advances in understanding how naturally-occurring metal ions are delivered to these active sites and how metal ions are involved in some diseases indicate new roles for metal ions in therapeutic strategies [2]. The current array of successful metallopharmaceuticals, which include the platinum anticancer drugs [3–9], radiodiagnostic agents that contain $^{99\text{m}}\text{Tc}$ and other radionuclides [10,11], Gd(III) MRI agents [12,13], β -emitting radiotherapeutic compounds [14], complexes involving vanadium [15–17] as an insulin mimic and Cr(III) as an intermediary in activating insulin receptors [18,19], all indicate the utility of complexes as both therapeutic and diagnostic agents.

Most of these metal-containing pharmaceuticals have been developed in academic laboratories or by relatively small or ‘start-up’ pharmaceutical enterprises [15,20–22]. The very success of pharmaceutical chemists in synthesizing broad ranges of carbon-based compounds tends to eliminate less common elements from their synthetic programs. The lack of experience of traditional medicinal chemists and pharmacologists in dealing with biologically active metal complexes poses a substantial activation energy barrier to their identifying active metal complexes and shepherding them to the clinic. These factors coupled with a tendency of pharmaceutical houses and government screening programs to view transition metal ions as toxic ‘heavy metals’ (despite the body’s utilization of gram quantities of Fe, Cu and Zn) retards the development of metallopharmaceuticals. On the other hand, this also provides enterprising transition metal chemists with opportunities to pioneer the development of exciting new drugs.

While the statistical success of metal-based compounds in reaching the clinic through the NIH anticancer screening program is about the same as for carbon-based compounds (1 in 6000 tested), the movement of new transition metal chemotherapeutic agents

toward the clinic has been slow. Keppler has pointed out the inherent bias in testing antitumor metal compounds in cell and animal systems, which are sensitive to cisplatin, and the difficulty in formulating metal complexes, particularly those with low solubility [23].

The synthetic chemistry of the transition metal, ruthenium is well developed, particularly with ammine, amine and imine ligands, and provides for many approaches to innovative new metallopharmaceuticals. Due to strong ligand-field stabilization, the more common oxidation states (Ru(II), Ru(III), and Ru(IV)) in aqueous solution are usually octahedral and are often fairly inert to ligand substitution. The drug-like effects of ruthenium red, which has been used as a cytological stain for over a century, have long been known. Advantages of utilizing ruthenium am(m)ine complexes in drug development include, (1) reliable methods of synthesizing stable complexes with predictable structures; (2) the ability to tune ligand affinities, electron transfer and substitution rates, and reduction potentials; and (3) an increasing knowledge of the biological effects of ruthenium complexes.

Collectively these lend ruthenium complexes to redox-activation and photodynamic approaches to therapy as well as the development of radiopharmaceuticals containing one of several radionuclides of ruthenium [24–32]. Finally, many am(m)ine complexes of Ru(II) and Ru(III) complexes of ruthenium tend to selectively bind to imine sites in biomolecules, which (as opposed to amine sites) do not protonate at neutral pH, thereby leaving their nitrogen lone pairs available for metal ion coordination. Consequently, ruthenium complexes often selectively coordinate histidyl imidazole nitrogens on proteins [33–35] and the N7 site on the imidazole ring of purine nucleotides, and so can take advantage of the properties of proteins, oligonucleotides and nucleic acids to target specific tissues [36]. Thiolato complexes are also possible, but these are often kinetically unstable [37], particularly in air [38]. Complexes with flavins and pterins are also known [39–41], but tend to be photochemically unstable.

2. Anticancer compounds

2.1. Activation by reduction

Due to the octahedral structure of Ru(II) and Ru(III) complexes as opposed to the square-planar geometry of Pt(II), ruthenium antitumor complexes probably function in a manner differently than cisplatin, which appears to bend DNA by crosslinking adjacent Gua thereby causing a class of DNA binding proteins to adhere to the site [4,5,8]. In what has become known as the ‘activation by reduction’ hypothesis, we suggested that Ru(III) complexes may serve as prodrugs that are activated by reduction in vivo to coordinate more rapidly to biomolecules [42–44]. Since tumors rapidly utilize oxygen and other nutrients and the development of new blood vessels (known as neovascularization or angiogenesis) often fails to keep pace with tumor growth, there is usually a lower O₂ content (hypoxia) in tumor cells [45–49]. Consequently, cancer cells depend more on glycolysis for energy and generate an excess of lactic acid, which lowers the pH in tumor cells [50]. Due to these metabolic differences, the relative electrochemical potential inside tumors is generally lower than in the surrounding normal tissue, particularly at the center of the tumor [51]. These differences in tumor relative to normal cell metabolism should favor the production of Ru(II) relative to Ru(III) in tumors, compared with normal tissue.

In the absence of π -bonding effects, the lower charge on Ru(II) would cause it to be more actively substituting than Ru(III) in a similar coordination environment. As reduction of Ru(III) to Ru(II) fills the d_{π} (t_{2g}) orbitals,

π -donor ligands that coordinate firmly to Ru(III) are no longer able to do so with Ru(II) and bind less strongly. In the case of Ru(II) am(m)ine complexes, acido ligands are lost fairly rapidly ($k = 1-10 \text{ s}^{-1}$) [52,53]. This coupled with the expected higher [Ru(II)]/[Ru(III)] ratio in tumor cells should lead to increased intracellular binding and hence somewhat selective tumor toxicity. Glutathione (GSH) and a number of redox proteins are capable of reducing Ru(III) complexes in vivo [54]. Single-electron-transfer proteins, which exist in both the mitochondrial electron-transfer chain and in microsomal electron-transfer systems, can also reduce Ru(III) with the microsomal proteins being the more efficient for $[\text{Cl}(\text{NH}_3)_5\text{Ru}]^{2+}$ [54]. In addition, ammineruthenium complexes can also be reduced by transmembrane electron transport systems, so that it is not necessary for the complexes to enter cells in order to be reduced [55]. Oxidation of Ru(II) back to Ru(III) can occur by molecular oxygen [56], cytochrome oxidase [56–58], and other oxidants, but this is relatively less likely to occur in hypoxic tumor cells. The effect of hypoxia in increasing DNA binding and thereby enhancing the toxicity of the anticancer agents *cis*- $[\text{Cl}_2(\text{NH}_3)_4\text{Ru}]\text{Cl}$ (CCR) and (ImH) *trans*- $[(\text{Im})_2\text{Cl}_4\text{Ru}]$ (ICR) against HeLa cells has been clearly demonstrated (see Fig. 1) [59]. There is also evidence that the first ruthenium compound to enter clinical trials, $\text{Na}\{trans-[\text{Cl}_4(\text{DMSO})(\text{Im})\text{Ru}]\}$ (NAMI), is activated by reduction [60,61].

2.2. Active Ru(II) complexes

While the modest antitumor activity of Ru(II) complexes such as *cis*- $\text{Cl}_2(\text{DMSO})_4\text{Ru}$ were studied relatively early [62,63], significant activity has been seen only recently with Ru(II) complexes stabilized with heteroaromatic ligands [64–66]. The isomer of *cis*- $[\text{Cl}_2(\text{azpy})_2\text{Ru}]$ (azpy = 2-(phenylazo)pyridine) with C_2 symmetry exhibits substantial activity against several cell lines [67,68]. Possible reasons for this are, (1) the decrease in the rate of chloride aquation due the π -acceptor effect of the imine ligands increasing the effective charge on the metal ion so that the hydrolysis rates are in the range of cisplatin; (2) increased hydrophobic or intercalative interactions with DNA, which may facilitate covalent binding; (3) geometric effects exerted by the ligands, which may facilitate (or inhibit) protein binding to the nucleic acid.

Arene ligands stabilize Ru(II) and provide a hydrophobic face that may enhance recognition and transport through cell membranes and at least one such complex inhibits topoisomerase II, which is essential in cell division. Topoisomerase II (DNA gyrase) activity is inhibited by what is reported to be $[\text{Cl}_2(\text{Me}_2\text{SO})(\text{C}_6\text{H}_6)\text{Ru}(\text{II})]$, but not by $[(\text{saldox})_2\text{Ru}(\text{II})]$ (*sic*), where sal = salicylaldoximate [69]. Topoisomerase II

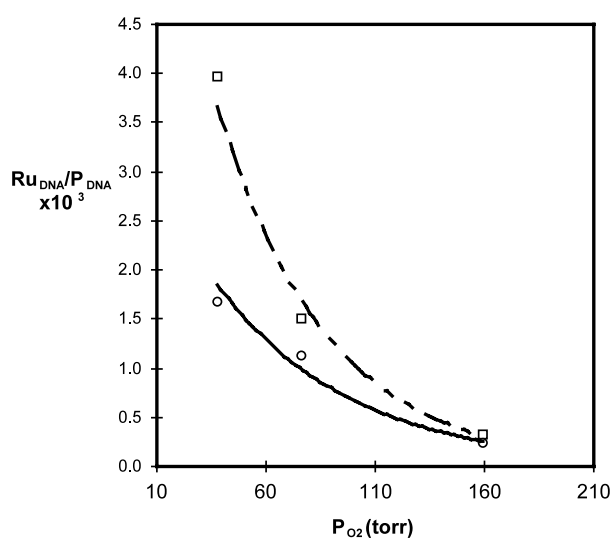


Fig. 1. DNA binding for ruthenium anticancer complexes as a function of hypoxia. Correlation between the amount of Ru bound to DNA at $[\text{Ru}] = 10 \text{ }\mu\text{M}$ and $\log[\text{PO}_2]$. Circle points, *cis*- $[\text{Cl}_2(\text{NH}_3)_4\text{Ru}]\text{Cl}$ (CCR); square points, $[\text{ImH}]\text{trans}-[(\text{Im})_2\text{Cl}_4\text{Ru}]$ (ICR). Curves are fitted to exponential lines [59].

alters the topological properties of DNA, thereby helping to maintain the structural organization of the mitotic chromosomal scaffold in the replication, transcription, recombination, and segregation of chromosomal pairs during cell division [69,70]. As these roles are particularly important in proliferating cancers, selectively targeting topoisomerase II could inhibit neoplastic cells division and possibly induce apoptosis by fragmenting DNA. At the low halide concentration inside the cell, $[\text{Cl}_2(\text{Me}_2\text{SO})(\text{C}_6\text{H}_6)\text{Ru}(\text{II})]$ likely exists as *fac*- $[(\text{H}_2\text{O})_3(\text{C}_6\text{H}_6)\text{Ru}(\text{II})]$ and so might function analogously to *fac*- $\text{Cl}_3(\text{NH}_3)_3\text{Ru}$ (see below).

Piano-stool organometallic complexes of the type $[\text{X}(\eta^6\text{-arene})(\text{en})\text{Ru}(\text{II})]^+$, where arene is benzene or substituted benzene, en is ethylenediamine or *N*-ethylethylenediamine, and X is a halide, inhibit the growth of the human ovarian cancer cell line A2780, but do not inhibit topoisomerase I or II [71]. Several complexes of this type are as active as carboplatin, but are not as active as cisplatin. Hydrolysis of the reactive Ru–X bond is essential for activity, and Ru–Cl hydrolysis can be suppressed by chloride ions. In solution, $[\text{Cl}(\eta^6\text{-p-cymene})(\text{en})\text{Ru}(\text{II})]^+$ binds selectively to Gua bases on DNA oligonucleotides to form monofunctional adducts and slightly lowers the DNA melting temperature [69]. Since these complexes are monofunctional, their biological mechanisms are probably different from either the cisplatin or NAMI types of drugs.

The complex $[\text{Cl}(\text{terpy})(\text{tmphen})\text{Ru}]^+$ (tmphen = tetramethylphenanthroline) binds predominantly at DNA purine sites and inhibits cell growth, which also appears dependent on the lability of the chloride [72]. Complexes of the type $[\text{L}(\text{bpy})_2\text{Ru}(\text{II})]^+$, where L represents the antimitotics colchicine (Col) and trimethylcolchicinic acid (Tmca), in which coordination appears to involve an anionic tropolone ring on the ligands, exhibit activity approximately the same as the free ligands. Since the complexes do not appear to dissociate in solution, the activity against human chronic myelogenous leukemia (K562) and human colon adenocarcinoma (COLO 205) was ascribed to the intact complexes [73].

2.3. Transferrin transport

Ruthenium ammine and imine complexes, such as $[(\text{H}_2\text{O})(\text{NH}_3)_5\text{Ru}(\text{II})]^{2+}$, $[\text{Cl}(\text{NH}_3)_5\text{Ru}]^{2+}$ *trans*- $[(\text{Im})_2\text{-Cl}_4\text{Ru}]^-$ and *trans*- $[(\text{Im})(\text{DMSO})\text{Cl}_4\text{Ru}]^-$, bind readily to surface accessible histidyl imidazole nitrogens on a number of proteins. In the blood, albumin and transferrin are responsible for most binding [34,35] and transferrin appears to facilitate its entry into cells [74]. However, both the kinetics and equilibria of ruthenium uptake and release are highly dependent on the complex involved. As expected on the basis of the relative pK_a of

the ligands, ruthenium binding to imidazoles on protein surfaces is greater than to Gua on DNA [34,37,75,76].

The elevated requirements of tumor cells for nutrients coupled with their higher membrane permeability and angiogenesis with associated increased blood flow result in both specific and nonspecific uptake of metallopharmaceuticals. Specific intake for several metal ions appears to be mediated by transferrin [77–79]. Transferrin is a glycoprotein of ca. 80 kDa [80] and consists of a single polypeptide chain, arranged in two similar lobes. Each lobe binds one Fe^{3+} through two tyrosines, a histidine, an aspartate and a bidentate carbonate [81]. Release of Fe^{3+} from transferrin is induced at low pH, which may involve protonation of the histidine ligand and/or a pH-induced change in hydrogen-bonding between lysines in the N-lobe [82].

Tracer studies with $^{103}\text{RuCl}_3$ were an early indication of substantial transferrin (Tf) binding [74,83,84], and injection of $^{103}\text{Ru}(\text{III})\text{-Tf}$ demonstrated high tumor uptake [74,84,85]. The diaqua intermediates of $\text{Na}\{trans\text{-}[\text{Cl}_4(\text{DMSO})(\text{Im})\text{Ru}]\}$ and its indazole analogue bind to the iron binding site of apotransferrin with a 2:1 stoichiometry with the heterocycles remaining attached. The Im-Ru-DMSO core can be reversibly removed with citrate [86–89].

In contrast, citrate alone does not remove the metal ion from $[(\text{NH}_3)_5\text{Ru}(\text{III})]_2\text{Tf}$. However, following reduction in the presence of GSH or citric acid about half of the Ru is displaced. Unlike $\text{Tf-Fe}(\text{III/II})$ [90], the $\text{Tf-Ru}(\text{III/II})$ reduction potential should be biologically accessible. Such reduction should facilitate release of Ru from histidine sites on Tf particularly in the lower pH of tumor tissue or the Tf endosome (pH 5.6) [91].

In addition to the histidines in each of the two iron binding sites, human Tf contains 17 other accessible histidines [92,93], which also appear to serve as potential binding, lower-affinity, sites for Ru(II) and Ru(III). The histidines in the Fe binding sites appear to have a high affinity for Ru(III), while the solvent exposed, surface histidines exhibit a lower affinity [89]. Approximately 6–8 of the accessible histidines bind $[(\text{H}_2\text{O})(\text{NH}_3)_5\text{-Ru}(\text{II})]^{2+}$ [92].

Binding of the antitumor agent, *trans*- $[\text{Cl}_4(\text{Im})_2\text{Ru}]^-$, to apotransferrin takes several hours, while *trans*- $[\text{Cl}_4(\text{Ind})_2\text{Ru}]^-$ takes only a few minutes [94]. Both also bind to surface histidyl imidazoles on albumin in the blood [95] as does NAMI [35]. Hydrolytic intermediates are formed in buffer solution and the carbonate complex may be taken into the protein [95]. Crystallographic data shows that *trans*- $[\text{Cl}_4(\text{Im})_2\text{Ru}]^-$ binds to a histidine at both iron binding sites [87–89]. While carbonate assists binding [95], the refined crystal structure of *trans*- $[(\text{Ind})_2\text{Cl}_4\text{Ru}]^-$ exhibits no evidence of carbonate binding [89]. Holotransferrin loaded with 2, 4 or 8 Ru/Tf of *trans*- $[(\text{Ind})_2\text{Cl}_4\text{Ru}]^-$ was ca. 80-fold more effective at inhibiting the growth of SW707 human

colon cancer cells in culture on a molar basis than the Ru complex alone [96]. Efficacy was independent of ruthenium loading and decreased when Ru-apotransferrin was used [96]. While the binding of ruthenium to both iron-binding and surface accessible histidyl imidazoles was taken to be quantitative in cytotoxic studies with *trans*-[L₂Cl₄Ru][−] [96], the estimated net affinities of pentammineruthenium ions for imidazole sites on Tf are only 10²–10⁵ M^{−1} [97].

Binding the ammineruthenium complexes to Tf and Fe₂Tf had relatively little effect on the amount of nuclear cellular DNA binding at the ruthenium IC₅₀ concentrations [97]. On the other hand, an order of magnitude higher DNA binding was required to achieve 50% inhibition of growth compared with those of *cis*-[Cl₂(NH₃)₄Ru]Cl and (ImH)*trans*-[(Im)₂Cl₄Ru] against HeLa cells ([Ru]_{DNA}/[P]_{DNA} = 0.3 × 10^{−3} and 0.4 × 10^{−3}, respectively, 24 h incubation) [59]. This difference in the amount of [Ru]_{DNA}/[P]_{DNA} required for IC₅₀ cell toxicity may be related to the increase in the IC₅₀ values for *cis*-[Ru(Cl₂(NH₃)₄)Cl] between the HeLa (3.5 μM) [59] and Jurkat T_{ag} (190 μM) cell lines [97]. On the other hand, the IC₅₀ toxicity of (ImH)*trans*-[(Im)₂Cl₄Ru] remained essentially the same between the two cell lines (2.0 μM, HeLa and 1.3 μM, Jurkat); but also required an order of magnitude higher [Ru]_{DNA}/[P]_{DNA} (0.20, HeLa and 2.2–4.9, Jurkat) [59,97]. Since the amount of Ru bound to nuclear Jurkat cell DNA required for 50% inhibition of growth was in the same range for all complexes studied, but was achieved at much lower media concentrations of (ImH)*trans*-[(Im)₂Cl₄Ru], the difference in toxicity between cell lines may have to do with a cellular uptake and release mechanism in Jurkat cells that better utilizes the higher affinity and reversible release of the imidazole complex by Tf.

In contrast to the quantitative removal of the metal from Cl₃(Im)₂Ru–Tf by citrate at low pH [87], neither Ru(III) ammine complex dissociated from Tf under these conditions. When a 100-fold excess of citric acid (pH 3.5, under argon over Zn–Hg amalgam, 24 h, 23 °C) was used to remove Ru from apotransferrin complexes under reducing conditions, ca. 50% of the original amount of [(NH₃)₅Ru[−]] and 36% of the original amount of *cis*-[(NH₃)₄Ru[−]] were removed.

An 80-fold increase (per mole of Ru) in toxicity is also seen for the apotransferrin form of [(NH₃)₅Ru(III)]₂Tf against Jurkat T_{ag} cells; however, no increase in activity was observed for *cis*-[(NH₃)₄Ru(III)] coordinated to either apo- or holotransferrin [98]. Inhibition of HeLa cell growth in tissue culture increased with added Tf for both *cis*-[Cl₂(NH₃)₄Ru]Cl and (ImH)*trans*-[(Im)₂Cl₄Ru] [59]. Since they are preferentially taken into tumor cells, Ru–Tf complexes may provide a new family of less toxic and more effective antitumor agents. The enhanced toxicity of *trans*-[(Im)₂Cl₄Ru][−] relative to *cis*-[Cl₂(NH₃)₄Ru(III)] against some cell lines may be due

to the former's greater uptake by Tf in the blood and more efficient release in the low-pH Tf-receptor vesicle inside the cell, rather than greater DNA binding for the same concentration within the cell or generating more effective DNA lesions. While efficient uptake of Ru by Tf and intracellular dissociation from it appears to be necessary, it is also possible that the differences in cytotoxicity and DNA binding between the pentaammine and *trans*-imidazole complexes among cell lines results from metabolic differences other than differences in the Ru uptake through the Tf receptor [97].

2.4. Covalent DNA binding

Relatively 'soft' transition metal ions such as Ru(II) and Ru(III) tend to bind to nitrogen sites on DNA bases. In the case of [L(NH₃)₅Ru^{III}], where L is a purine, various N-bound types of linkage isomers have been isolated and spectroscopically [36,99–103] and structurally characterized (see Fig. 2). Linkage isomers are restricted by the sugar in nucleosides, so that N⁷ coordination is most frequently observed in purine nucleosides. When this restriction is lifted nearly all linkage isomers are obtained. The rate of the pH-dependent linkage isomerization between the N3 and N9 sites of Hyp (see Fig. 2) follows the rate law $-d[N3]/dt = (k_1[H^+] + k_o K_a)/([H^+] + K_a)[N3]$, where [N3] is the concentration of [Hypκ^{N3}(NH₃)₅Ru(III)], $k_1 = 1.25 \times 10^{-4} \text{ s}^{-1}$, $k_o = 2.2 \times 10^{-6} \text{ s}^{-1}$, and pK_a 4.82 [99,101]. Stable *exocyclic* linkage isomers have been obtained with cytosine, adenine (Ade, A) and related derivatives [100,102,104,105]. The single exception appears to be coordination to the *exocyclic* N² of Gua.

Since cationic metal complexes have an electrostatic attraction to polyanionic nucleic acids, the rate of nucleic acid binding of Gκ^{N7} sites by [(H₂O)(NH₃)₅Ru(II)]²⁺ proceeds fairly rapidly and is strongly dependent on ionic-strength [76]. In DNA, a second reactive phase probably has to do with coordination of interior sites exposed upon separation of the nucleic acid strands [75]. The affinity binding constants of [(H₂O)(NH₃)₅Ru(II)]²⁺ are 5100 and 7800 M^{−1} for helical and single-stranded CT-DNA, respectively [75]. Binding to RNA is somewhat lower, probably because the additional sugar oxygen has a modest effect on the basicity of the purine N⁷ [76].

Unlike [(H₂O)(NH₃)₅Ru(II)]²⁺ [75], covalent binding of *trans*-[(H₂O)(py)(NH₃)₄Ru(II)]²⁺ to DNA is Gκ⁷-specific [106], with K_G = 1 × 10⁴ M^{−1}. Pyridine ligands (Pyr) slow DNA binding by *trans*-[(H₂O)(Pyr)(NH₃)₄Ru(II)]²⁺ relative to [(H₂O)(NH₃)₅Ru(II)]²⁺ and favor the Ru(III/II) reduction by about 150 mV

¹ The indicator κ, as in Gκ⁷, denotes the coordinating atom when linkage isomers are possible.

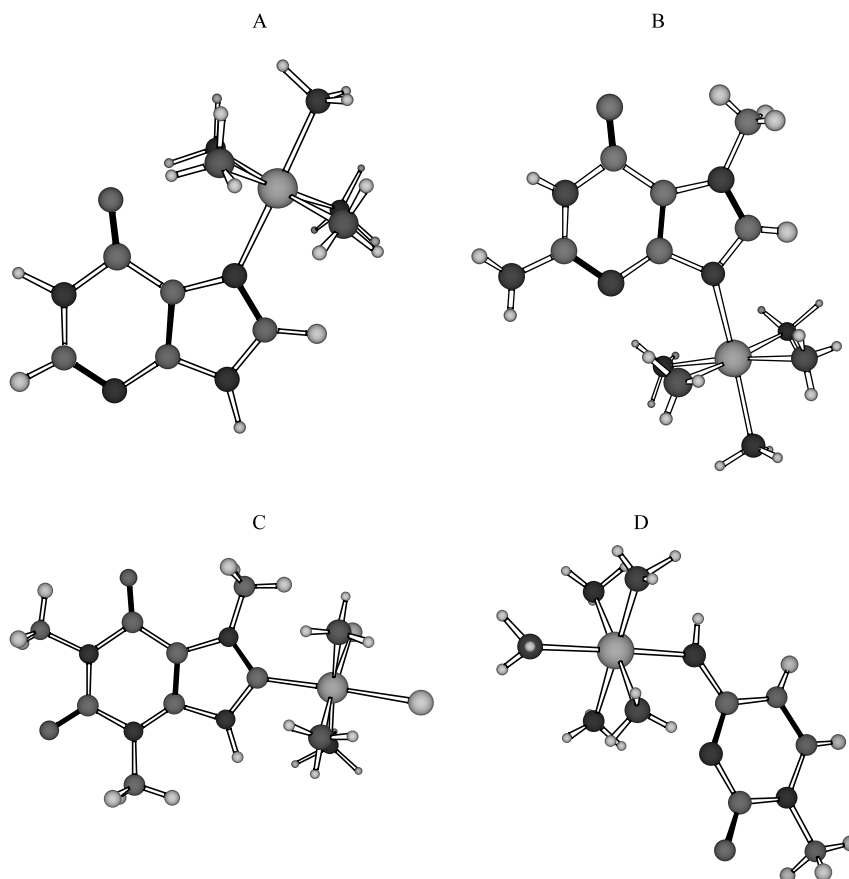


Fig. 2. Structures of a) $[(\text{Hyp}\kappa^7)(\text{NH}_3)_5\text{Ru}]^{3+}$ showing the typical N7 coordination of many transition metal ions at the N7 of a purine [101]; b) $[(7\text{MeGuak}^7)(\text{NH}_3)_5\text{Ru}]^{3+}$, depicting one of the linkage isomers present when N9 is also available for bonding [36]; c) *cis*- $[\text{Cl}_2(1,3,7\text{Me}_3\text{Xan}\kappa^8)-(\text{NH}_3)_3\text{Ru}]^{3+}$ illustrating an unusual carbon-bound form of caffeine bound to ruthenium; and d) $[(1\text{MeCyt}\kappa^{\text{N}4})\text{Cl}(\text{NH}_3)\text{Ru}]^{2+}$ portraying binding to an ionized exocyclic amine.

[106]. The air oxidation of $[(\text{py})(\text{NH}_3)_4\text{Ru(II)}]_n\text{-DNA}$ to $[(\text{py})(\text{NH}_3)_4\text{Ru(III)}]_n\text{-DNA}$ at pH 6 occurs with a pseudo first-order rate constant of $5.6 \times 10^{-4} \text{ s}^{-1}$ at $\mu = 0.1$ and 25°C [106]. Stabilization of Ru(II) by pyridine ligands also promotes the disproportionation of Ru(III) to the corresponding complexes of Ru(II) and, presumably, Ru(IV), which facilitates both hydrolysis and autoxidation for both monomers and DNA [103]. Surprisingly, the rate limiting step in the hydroxide-dependent disproportionation pathway is not electron transfer between Ru(III)'s, but deprotonation of an ammine.

In the anticancer complex, *trans*- $[\text{Cl}_4(\text{Im})_2\text{Ru}]^-$ (ICR), aquation occurs stepwise by sequential loss of two chlorides at an initial rate of $9.6 \times 10^{-6} \text{ s}^{-1}$ at 25°C and $5.26 \times 10^{-5} \text{ s}^{-1}$ at 37°C [107]. The formation of blue-green ($\lambda_{\text{max}} = 585 \text{ nm}$) precipitates in serum and physiological buffer suggest that hydrolyzed forms anate to form carbonato or carboxylato species [95]. After hydrolysis, ICR may cross link $\text{G}\kappa^7$ sites on DNA with histidyl imidazoles on proteins [108]. The surprisingly high reduction potential of *trans*- $[\text{Cl}_4(\text{Im})_2\text{Ru}]^-$ (-0.24 V) may allow in vivo reduction [107], which

would cause the chlorides to dissociate more rapidly, and an activation by reduction mechanism has been postulated [108,109]. It may be that a hydrido intermediate is responsible for the high E° , which may account for the rapid reaction between *trans*- $[\text{Cl}_4(\text{Im})_2\text{Ru}]^-$ and GSH [108].

In complexes such as $[(\text{Cyt}\kappa^{\text{N}4})^-(\text{NH}_3)_5\text{Ru(III)}]^{2+}$, the Ru(III) coordinates to the exocyclic amine and rotates around the N4–C4 axis. Since such coordination forces the unusual imine tautomeric form, the relative stability of the two rotational isomers is a function of pH, with the rotamer shown in Fig. 3 being stabilized above the pK_a (~ 3.2) by hydrogen bonding to N3. Figs. 3 and 4 illustrate the types of redox-dependent linkage isomerization and pH-dependent rotamerization that can occur in $[\text{L}(\text{NH}_3)_5\text{Ru(III)}]$, where L is a ligand with a ring imine nitrogen adjacent to an exocyclic ammine, such as 2-aminopyrimidineadenine (as illustrated in Fig. 3), Ade, Cyt, or isocytosine (as illustrated in Fig. 4). Due to the difference in distance between the metal ion and the ionization site, the rotamers in cytidine and adenosine complexes exhibit different pK_a . The interconversion may be facilitated by proton dissociation and very

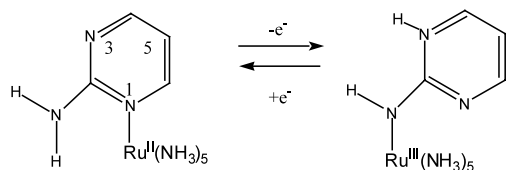


Fig. 3. Redox-dependent linkage isomerization of $[(2\text{AmPym}\kappa^{\text{N}1})(\text{NH}_3)_5\text{Ru(II)}]^{2+}$ and $[(2\text{AmPym}\kappa^{\text{N}2})(\text{NH}_3)_5\text{Ru(III)}]^{3+}$ and pH dependent rotamerization. The linkage isomers exhibit distinct reduction potentials and the rotational isomers display distinct $^1\text{H-NMR}$ signals [104].

rapid rotation rates ($3000\text{--}6000\text{ s}^{-1}$, $T = 25\text{ }^\circ\text{C}$) are evident with small activation values ($\Delta H^\ddagger \sim 1.6\text{ kcal}$) [104]. The $\kappa^{\text{N}2}$ to $\kappa^{\text{N}1}$ linkage isomerization rate for the 2-aminopyrimidine complex following reduction to Ru(II) is 3.0 s^{-1} at $23\text{ }^\circ\text{C}$ [104].

Full structural characterization of *trans*- $[\text{Cl}_3(\text{dmp})_2(9\text{-MeAde})\text{Ru}]$ (*dmp* = 5,7-dimethyl[1,2,4]-triazolo[1,5-*a*]pyrimidine; 9-MeAde = 9-methyladenine) also reveals the Ru(III), coordinated through the exocyclic N6 nitrogen. Two intramolecular hydrogen bonds stabilize the coordination of the neutral-ligand Ade with the proton at N1. This complex exhibits a surprisingly short C6–N6 distance ($1.293\text{ \AA}$) [105].

Imidazolyldine (C^8 -coordinated) complexes form spontaneously with xanthine ligands [36,110] and have been induced by chelation through a pendant amine in $[\text{Cl}_2\text{L}(\text{DMSO})_2\text{Ru}]$ *L* = 9-[2-(2-aminoethylamino)ethyl]-Ade).

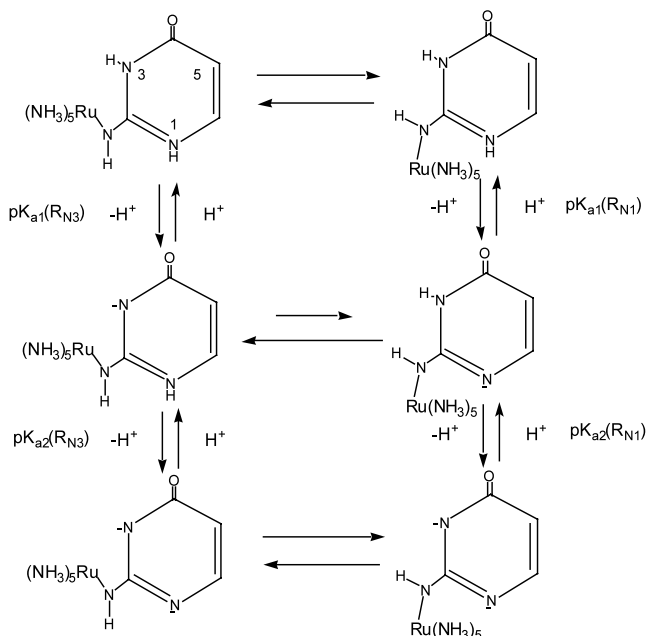


Fig. 4. Rotameric, tautomeric and ionization equilibria of $[\text{ICyt}\kappa^{\text{N}2}(\text{NH}_3)_5\text{Ru(III)}]^{3+}$ [104].

2.5. DNA damage generated by covalently bound Ru

Ru(III) functions as a general acid in promoting the hydrolysis of the *N*-glycosidic bond in $[(\text{dG}\kappa^{\text{N}7})-(\text{NH}_3)_5\text{Ru(III)}]$ ($t_{1/2} = 1.5\text{ days}$, $56\text{ }^\circ\text{C}$, pH 7) [111]; however, this has not been observed on DNA [75]. Ru(III) N7-coordinated to nucleosides also facilitates their base-catalyzed air oxidation to 8-oxo-nucleosides [36,112]. This reaction probably proceeds by hydroxide attack at C8 induced by Ru(III) followed by sequential single-electron transfers via the Ru to oxygen. Following autoxidation, the glycosidic bond undergoes base-catalyzed cleavage; however, this also has not been observed to cleave DNA [75]. Autoxidation is hindered by the electron-donating amine at C2 and proton ionization of N1 [30,36].

Ruthenium(IV) at $\text{G}\kappa^{\text{N}7}$ on DNA is a stronger general acid catalyst, which also better facilitates Gua autoxidation. A convenient route to Ru(IV) on DNA is through the disproportionation of $[\text{py}(\text{NH}_3)_5\text{Ru(III)}]$ to Ru(II) and Ru(IV) [103,113]. Following the disproportionation of *trans*- $[\text{Guo}\kappa^{\text{N}7}(\text{py})(\text{NH}_3)_4\text{Ru(III)}]$, the appearance of *trans*- $[\text{Guak}^{\text{N}7}(\text{py})(\text{NH}_3)_4\text{Ru(III)}]$ and free ribose is consistent with general acid hydrolysis of the glycosidic bond induced by Ru(IV), which is subsequently reduced. In the presence of oxygen, *trans*- $[\text{8-OGuo}\kappa^{\text{N}7}(\text{py})(\text{NH}_3)_4\text{Ru(III)}]$ is also detected [103]. In contrast to the monomer, the Ru-induced autoxidation of Gua on DNA is favored over *N*-glycolysis by a factor of 1.7 at pH 11 and $25\text{ }^\circ\text{C}$. Under these conditions, a dynamic steady-state arises in which reduction of Ru(IV) produces additional Ru(II)– G_{DNA} , which is air-oxidized to Ru(III)– G_{DNA} followed by disproportionation back to Ru(II)– G_{DNA} and Ru(IV)– G_{DNA} . The Ru(IV)– G_{DNA} can hydrolyze to give Ru–G or undergo autoxidation to yield Ru-8OG products. This dynamic system slowly, but catalytically, damages DNA [106].

Strand cleavage of plasmid DNA can also occur by Fenton, Haber–Weiss or oxo-metal ion chemistry for a number of ruthenium(III) amines. However, the covalently bound metal in $[(\text{NH}_3)_5\text{Ru(III)}]_n\text{--DNA}$ is fairly inefficient at generating oxygen radicals [75]. Surprisingly, base-catalyzed cleavage by covalently bound $[\text{Cl}(\text{py})(\text{NH}_3)_4\text{Ru(III)}]$ is more efficient than O_2 activation, even at neutral pH [106].

The *A*-enantiomer of *cis*- $[\text{Cl}_2(\text{phen})_2\text{Ru(II)}]$ selectively associates with B-DNA through electrostatic and hydrophobic interactions before coordinating, presumably at $\text{G}\kappa^{\text{N}7}$ and a number of mono- and diaqua polypyridyl complexes of Ru(II) covalently bind to DNA, but at a relatively low level [114,115]. Interstrand crosslinking has also been suggested for *cis*-diaqua polypyridyl complexes [116]. Covalent binding of *mer*- $[\text{Cl}_3(\text{tpy})\text{Ru}]$ to DNA occurs with about 2% interstrand cross-linking, presumably through *trans*-($\text{G}\kappa^{\text{N}7}$)₂ coor-

dination, which may be responsible for its antitumor activity [117]. A series of complexes with thiazole and phosphine ligands has been prepared for testing and the structure of *mer*-[Cl₃(thz)₃Ru], (thz = 1,3-thiazole) has been determined [118,119].

The complexes *trans*-[Cl(SO₂)(NH₃)₄Ru]⁺ [120], *mer*-[Cl₃(Me₂SO)₃Ru], *trans*-[Cl₄(Me₂SO)₂Ru][−], and *mer*,-*cis*-[Cl₃(H₂O)₂(Me₂SO)Ru] all produce DNA inter-strand crosslinks [121]. Adjacent intrastrand Gκ^{N7}–Gκ^{N7} crosslinks with *cis*-ruthenium ions may be possible, but are sterically more crowded by the octahedral geometry [65,122–124]. For example, *trans*-[Cl₄(Me₂SO)₂Ru][−] reacts with d(GpG) to yield a macrocyclic chelate with the likely formulation, *cis*-[d(Gκ^{N7}pGκ^{N7})Cl(H₂O)(Me₂SO)₂Ru(II)], in which the sugars are in *anti* configurations and the Gua are destacked in a head-to-head arrangement [125].

The Ru–Pt dinuclear complex, [*cis*,*fac*-(RuCl₂(Me₂SO)₃)}μ-NH₂(CH₂)₄NH₂-{*cis*-(Pt(NH₃)Cl₂)}], rapidly loses Me₂SO and chloride from the ruthenium center and crosslinks DNA repair proteins to DNA [126,127]. The DNA lesion responsible for efficient DNA-protein cross-linking is most probably a DNA–DNA inter-strand crosslink by the platinum end of the molecule [127]. Analogous complexes of the type [(bpy)₂M-(dpb)PtCl₂]Cl₂ (where M = Ru(II) or Os(II), and dpb = 2,3-bis(2-pyridyl)benzoquinoxaline) also form both intrastrand DNA crosslinks, due to the *cis*-Cl₂Pt(II) moiety, and interstrand crosslinks, which are probably made through the second metal center [128,129]. Complexes of the type, [(bpy)₂Ru-(dpb)PtCl₂]Cl₂ covalently bind to DNA through the Pt but may provide for photochemical effects through the Ru [129].

2.6. Modulation of DNA binding by glutathione

Glutathione (γ-glutamate–cysteine–glycine = GSH) [130] is present in cells at concentrations of 0.1–10 mM, but is readily oxidized to the disulfide (GSSG, *E*° = −0.246 V vs. NHE) [131]. GSH helps protect cells from: reactive oxygen intermediates, UV radiation, and heavy metal toxicity [132]. Decreased GSH levels have been implicated in cytochrome-*c* escape from the mitochondria, which is a triggering event in apoptosis [133]. GSH reduces some metal ions, such as CrO₄^{2−} and Pt(IV) anticancer drugs [134,135], to species that coordinate or otherwise react with DNA [136–140]. On the other hand, GSH binding to Pt(II) inhibits DNA binding and appears to contribute to cisplatin resistance in tumor cells [141–143]. GSH (0.1 M, pH 6, apparently in air) rapidly reduces the anticancer complex *trans*-[Cl₄(Im)₂Ru][−] (*E*° = −0.24 V), which then dissociates its imidazole ligands within 1 h GSH coordination of *trans*-[Cl₄(Im)₂Ru][−] followed by electron transfer has been assumed [108].

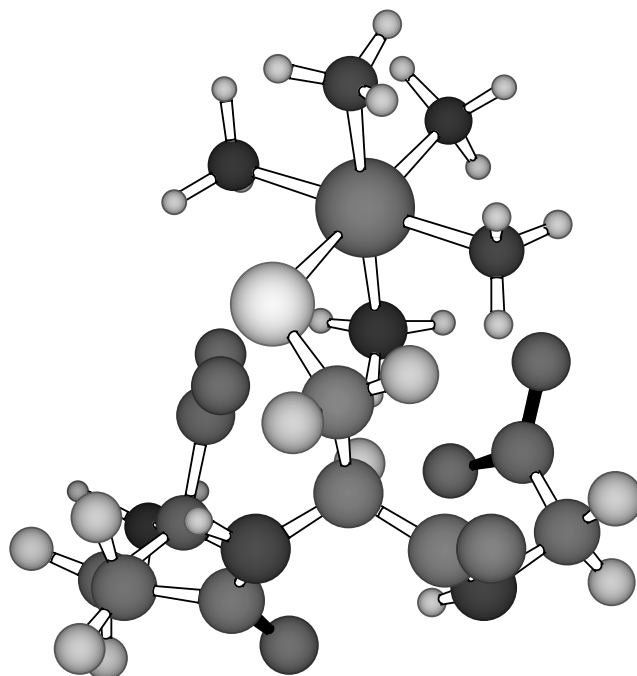


Fig. 5. MM2 energy-minimized structure for the peptide portion of [(GS)(NH₃)₅Ru(III)]⁺ illustrating the probable wrapping of the carboxylates around the metal cation [38,353,354].

The aerobic reaction of GSH with [Cl(NH₃)₅-Ru(III)]²⁺ is first order in [GSH] and yields only [OH(NH₃)₅Ru(III)]²⁺ and GSSG. Since GSH only slowly reduces [Cl(NH₃)₅Ru]²⁺ under physiological conditions (*t*_{1/2} = ~10 min) and the Ru(II) product is readily oxidized by air, this mode of activating Ru to bind to biopolymers by reduction may not be important in tissues under normal oxygen tensions, but may be in the hypoxic environment of tumors [54,59]. Since oxygen also effectively prevents GSH coordination, this could circumvent some thiol-based resistance to rutheniumammine anticancer agents.

The anaerobic reaction of GSH with [Cl(NH₃)₅Ru]²⁺ proceeds through reduction of Ru(III) by GSH to give [H₂O(NH₃)₅Ru(II)]²⁺, followed by coordination to produce [GSH(NH₃)₅Ru(II)]²⁺ and then oxidation by [OH(NH₃)₅Ru(III)]²⁺ or GSSG to yield [GS(NH₃)₅-Ru(III)]⁺ (Fig. 5) [38]. The reduction potential of [(GS)(NH₃)₅Ru(III)] is pH dependent with: *E* = *E*° − 0.59 log{*K*_a/([H⁺] + *K*_a)}, where *E*° = −0.44 V and p*K*_a = 7.1.

Depending on its relative concentration, GSH both facilitates and inhibits ruthenium binding to DNA. At [GSH]/[Ru(III)] ≤ 1, the coordination of [Cl(NH₃)₅-Ru(III)]²⁺ to DNA is facilitated by GSH reduction to the more substitution-labile [H₂O(NH₃)₅Ru(II)]²⁺. However, at [GSH]/[Ru(III)] ≥ 1, DNA binding is inhibited by GSH, which coordinates the Ru(II) and facilitates oxidation back to Ru(III) because of the low *E*° of [GS(NH₃)₅Ru(III)]⁺. Consistent with this is the

increased toxicity of $[\text{Cl}(\text{NH}_3)_5\text{Ru}]^{2+}$ to Jurkat T-cells, when GSH levels are suppressed [38].

Inhibition of DNA binding by GSH is most evident at $\text{G}\kappa^{\text{N}7}$ and GSH removes most of the metal ion from $\text{G}\kappa^{\text{N}7}$ sites on DNA. It is less effective in preventing binding or removing the metal from $\text{A}\kappa^{\text{N}6}$ and $\text{C}\kappa^{\text{N}4}$ sites owing to the lower $\text{Ru}(\text{III},\text{II})$ reduction potential [100], when the metal ion is attached to the exocyclic ammine of these ligands [102]. The ability of Ade to provide strong π -binding sites for both $\text{Ru}(\text{II})$ (N1) and $\text{Ru}(\text{III})$ (ionized N6) may account for its maintaining ruthenium binding even at high [GSH]. Such altering of DNA binding at physiological concentrations of GSH may have a significant effect on the mechanism of ruthenium antitumor compounds by favoring A and C binding over G [38], but speciation of nuclear DNA with respect to Ru-binding has not been determined.

Since GSH partially removes ammineruthenium(III) ions from Tf, NH_3 from $[(\text{NH}_3)_6\text{Ru}]^{3+}$ and pyridine (py) from $[\text{py}(\text{NH}_3)_5\text{Ru}]^{3+}$ to form $[\text{GS}(\text{NH}_3)_5\text{Ru}(\text{III})]^+$ [38], it may be involved in the metabolism of many types of ruthenium pharmaceuticals.

2.7. Cytotoxicities of Ru complexes

There appears to be a correlation between cytotoxicity and DNA binding for the representative ruthenium am(m)ine anticancer compounds, $\text{cis}-[\text{Cl}_2(\text{NH}_3)_4\text{Ru}(\text{III})]\text{Cl}_2$ and $(\text{HIm})[\text{trans}-(\text{Im})_2\text{Cl}_4\text{Ru}(\text{III})]$ in cell cultures [59]. Also consistent with DNA binding in vivo, a number of ammine, amine and heterocyclic complexes of ruthenium exhibit: inhibition of DNA replication [43], mutagenic activity and induction of the SOS repair mechanism [144], binding to nuclear DNA [59,145], and reduction of RNA synthesis [146]. EDTA-type complexes of $\text{Ru}(\text{III})$ and even $\text{Ru}(\text{IV})$ have shown anticancer activity, apparently through DNA binding [147,148]. The compound $\text{mer}-[\text{Ru}(\text{terpy})\text{Cl}_3]$ ($\text{tpy} = 2,2':6'',2''\text{-terpyridine}$) exhibits antitumor activity midway between those of cisplatin and carboplatin in the L1210 cell line, which possibly involves interstrand G–G crosslinks [117].

More recently, $\text{Ru}(\text{II})$ complexes have shown good activity. In particular, the two isomers of $\text{cis}-[\text{Cl}_2(\text{azpy})_2\text{Ru}]$ ($\text{azpy} = 2\text{-(phenylazo)pyridine}$) are remarkably

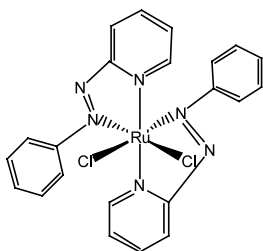


Fig. 6. The α -isomer of $\text{cis}-[\text{Cl}_2(\text{azpy})_2\text{Ru}]$ ($\text{azpy} = 2\text{-(phenylazo)pyridine}$) [67,68].

different in their cytotoxicities with the α -atropisomer (with C_2 symmetry, see Fig. 6) being far more active than the β -isomer or the (*trans*-head-to-head) γ -isomer [67,68]. As with $\text{cis}-[\text{Cl}_2(\text{bpy})_2\text{Ru}]$, steric constraints hinder coordination and only monoguanine adducts have been characterized to date [67]. However in contrast to the inactive $\text{cis}-[\text{Cl}_2(\text{bpy})_2\text{Ru}]$ [149], the more flexible azapy analogue allows for more orientations for a coordinated Gua, which may allow for binding of more than one DNA base. Indeed, the inactivity of $\beta-[\text{Cl}_2(\text{azpy})_2\text{Ru}]$ may also be due to greater steric constraints relative to the α -isomer. It is possible that the details of rotation between the phenyl rings on the azpy ligands and the rotational flexibility of the Gua or other DNA base ligands is important to understanding the differences in toxicity among the otherwise structurally similar $\text{Ru}(\text{II})$ complexes with polypyridine ligands [67,68].

While some monoacido complexes, such as $[\text{CH}_3\text{CH}_2\text{CO}_2(\text{NH}_3)_5\text{Ru}](\text{ClO}_4)_2$ are active, multichloro compounds such as $\text{cis}-[\text{Cl}_2(\text{NH}_3)_4\text{Ru}]\text{Cl}$, *fac*- $[\text{Cl}_3(\text{NH}_3)_3\text{Ru}]$ [44,150–152] and $(\text{HIm})\text{trans}-[\text{Cl}_4(\text{Im})_2\text{Ru}]$ [153,154] exhibit the best activity against primary tumors.

Solubility can be enhanced by increasing the number of chlorides, and *trans*-complexes of the type $(\text{LH})[\text{Cl}_4\text{L}_2\text{Ru}]$ (where L = imidazole or indazole), which show activity against a number of cancer cell lines [155–157] are particularly effective against colorectal tumors [107,109,153,158–161]. These and other multiacido ruthenium(III) complexes, particularly di-through tetrachloro complexes, appear to be transported in the blood by Tf and albumin (HSA), with the major portion (80%) binding to the latter [74,83,84,87,88]. Albumin can bind up to five (hydrolyzed) units of $[\text{Cl}_4\text{L}_2\text{Ru}]^-$ [94], which disrupts the protein α -helical structure. Quenching of the HSA Trp 214 fluorescence is consistent with Ru binding to the nearby His 242 so as to alter the local structure and expose Trp 214 to water [162]. Similarly, a substantial reduction of heme and bilirubin binding is attributed to Ru–histidine coordination at or near the HSA–heme binding site. Solubility can be increased by utilizing dialkylsulfoxide (R_2SO) analogs, as in complexes such as *trans*- $[\text{Cl}_2(\text{Me}_2\text{SO})_4\text{Ru}]$, $[\text{Cl}_3(\text{Me}_2\text{SO})_2\text{BRu}]$ (B = imidazole or indazole) and $\text{Na}\text{trans}-[\text{Cl}_4(\text{R}_2\text{SO})_2\text{Ru}]$, where R = methyl and tetramethylene [60,121,163].

Mixed-valent, μ -carboxylato complexes of the type, $\mu-[(\text{RCO}_2)_4\text{ClRu}_2]$ (R = CH_3 , CH_3CH_2) [160], are active against P388 lymphocytic leukemia [160], possibly by binding to DNA along the lines of the structurally similar rhodium complexes. Complexes with μ -*N,N'*-diphenylformamidinate and μ -(fluoroanilino)pyridinates have also been prepared [164–166]. The compound $\mu-[(\text{F}_3\text{CCO}_2)_4(\text{F}_3\text{CCO}_2)\text{Ru}_2]$ forms $\text{cis}-[\mu-(\text{F}_3\text{CCO}_2)_4\mu-(9\text{EtGua})\text{Ru}(\text{II})_2(\text{CH}_3\text{OH})_2]^{2+}$, where $9\text{EtGua} = 9\text{-}$

ethylguanine, in which the Gua bridge between the two Ru(II) atoms in a N7–O6 head-to-tail fashion [167].

Preparations of ‘ruthenium red’ (see below) bind to polyanions such as plant pectins and the protective mucopolysaccharide coat on some tumor cells [168]. Consequently, Ru-red concentrates in tumors [169] and inhibits tumor growth, perhaps by inhibiting Ca^{2+} transport [170,171]. The complex $\mu\text{-O}[(\text{H}_2\text{O})_2(\text{bpy})_2\text{Ru(III)}]_2^{4+}$ coordinates to DNA at relatively low levels ($[\text{Ru}]_{\text{DNA}}/[\text{P}]_{\text{DNA}} = 0.02$) with low stereoselectivity, which may favor the *AA* isomer. Coordination at this level also stabilizes the thermal melting of DNA by about 8 °C and may involve interstrand crosslinks [116].

Some nitrosylruthenium(II) species may be active by releasing toxic nitric oxide upon reduction in vivo [172–174]. The binuclear complex $\{[\text{Ru}(\text{NO})\text{Cl}_3]_2(\text{tpda})\}$ (*tpda* = *N,N,N',N'*-tetrakis(2-pyridylmethyl)adipamide) contains two DNA-coordinating, photo-labile Ru(II) centers, and a groove-spanning tether moiety [175].

2.8. Complexes with EDTA-type ligands

Ruthenium complexes with polyaminopolycarboxylic chelating ligands constitute a relatively new group of anticancer compounds [176,177]. The complex $[(\text{H}_2\text{O})(\text{edta})\text{Ru(II)}]^{2-}$ coordinates to both the N7 (30%) and N3 (70%) sites on 5'-GMP, but the Ru(III) form binds only at N7. $[(5'\text{-GMP})(\text{edta})\text{Ru(III)}]^{n-}$ has a reduction potential of 0.01 V (22 °C), but ionizes a proton from N1 at a $\text{p}K_{\text{a}}$ of 7.2, which should cause its reduction potential to decrease at higher pH [178].

In $[\text{Cl}_2(\text{cdta})\text{Ru(IV)}]$, where *cdta* = 1,2-*cyclo*-hexanediaminetetraacetate, the chlorides are *cis* to one another and the carboxylates appear to be labile. The Ru(IV,III) reduction potential occurs at 0.78 V, while that for the Ru(III,II) couple is at –0.01 V [177], so that these complexes may belong to the class of multi-acido ruthenium(III) complexes, whose activity involves transport by Tf [34]. The compound, *cis*- $[\text{Cl}_2(\text{pdta})\text{Ru(III)}]$, where *pdta* = 1,2-propylenediaminetetraacetate, also shows good antitumor activity, possibly by cross-linking Guas on DNA; and a model complex, $[(\text{Gua})_2(\text{pdta})\text{Ru(III)}]$, has been isolated. The solid state structure of *cis*- $[\text{Cl}_2(\text{pdta})\text{Ru(III)}]$ reveals coordination by two nitrogens and two monodentate carboxylates [148]. In solution, the chlorides dissociate to produce a number of reactive Ru(III) species; however, the metal ion maintains its oxidation state as well as the *pdta* ligand [179]. The complex rapidly binds to albumin, apotransferrin or diferric Tf to produce relatively stable adducts in which $(\text{pdta})\text{Ru(III)}$ is probably bound to histidines on the protein surface [34]. *Cis*- $[\text{Cl}_2(\text{pdta})\text{Ru(III)}]$ damages nuclear DNA, inhibits DNA recognition and DNA lysis by restriction enzymes [180], alters the conformation of

pHV14 DNA [180], stimulates NADPH oxidase and a respiratory burst in phagocytic neutrophils, and elicits phosphorylation of tyrosine residues [147,148].

The complex $[(\text{hedta})\text{Ru(II)}]^{-}$ binds to the usual N3 position of pyrimidines, but can also bind in a η^2 -fashion to C5–C6 [181,182]. A distribution between η^1 binding at both N1 and N3 of pyrimidine, which can be either stereochemically rigid or fluxional, as well as η^2 -binding is observed [183]. The complex $[\text{Ru}_2(\text{II})-(\text{ttha})(\text{DMU})_2]^{2-}$ (*ttha* = triethylenetetraminehexaacetate; *DMU* = 1,3-dimethyluracil) suggests an interstrand cross link between uracils [181]. As η^2 -coordination across C5–C6 raises the Ru(III/II) reduction potential to ~0.5 V, the η^2 -Ru(II)–DNA adducts may be stable in vivo. The complex $\{[\text{Ru(II)}(\text{hedta})]_2(\text{tpda})\}^{2-}$ (*tpda* = *N,N,N',N'*-tetrakis(2-pyridyl)adipamide) is being investigated as a DNA crosslinking agent as an extended bridge between DNA and proteins [184].

2.9. Complexes with R_2SO

The compound *cis*- $[\text{Cl}_2(\text{Me}_2\text{SO})_4\text{Ru}]$ exhibits only marginal antitumor activity, but was the origin of efforts in the investigation of other dialkylsulfoxide ruthenium complexes as anticancer agents. Covalent binding of this compound to DNA does not significantly affect the conformation of B-DNA but does increase its thermal stability. Comparisons with the inactive *trans* complex may also be instructive. *Trans*- $[\text{Cl}_2(\text{Me}_2\text{SO})_4\text{Ru}]$ binds much more rapidly to DNA with greater changes in its CD spectra, which are attributed to disruption of the DNA structure due to crosslinking [185]. While individual guanosines bind reversibly to *trans*- $[\text{Cl}_2(\text{Me}_2\text{SO})_4\text{Ru}]$ [186] and 5'-dGMP forms an N7- PO_4 chelate rather than a bis-5'-dGMP complex [65], NMR evidence suggests the formulation of *cis*- $[\text{d}(\text{G}\kappa^7\text{pG}\kappa^7)\text{Cl}(\text{H}_2\text{O})-(\text{Me}_2\text{SO})_2\text{Ru(II)}]$ [125], in which the sugars are in *anti* configurations and the Gua are destacked in a head-to-head arrangement similar to that of cisplatin [125]. Both the *cis* and *trans* isomers induce the B to Z transition in poly(dGdC), with the *trans* complex being much more effective. DNA extracted from cells, which were separately treated with each isomer, showed a five-fold higher content of Ru in the *trans* case [64,187,188].

Since metastatic cancer is particularly difficult to treat, the antimetastatic activity of the ruthenium dimethylsulfoxide complexes, particularly *Natrans*- $[\text{Cl}_4(\text{Me}_2\text{SO})(\text{Im})\text{Ru}]$ (NAMI) represents an important development. Such complexes could be particularly useful in minimizing the growth of undetected micro-metastases following surgery or radiotherapy [189,190]. While structurally similar to $(\text{ImH})\text{trans}-[\text{Cl}_4(\text{Im})_2\text{Ru}]$ (ICR, $E^\circ = -0.24$ V), NAMI has a significantly higher Ru(III/II) reduction potential (0.235 V) [107,153,191] owing to the π -acceptor effect of the S-bound DMSO, which also exerts a kinetic *trans*-effect. Relatively high

concentrations ($>100\text{ }\mu\text{M}$) are needed to produce a cytotoxic effect, which depends upon the lipophilicity of the complex and the presence of serum and plasma proteins [192]. Of those tested, the most lipophilic complex, *Natrans*-[RuCl₄(TMSO)Iq] (TMSO = tetramethylsulfoxide; Iq = isoquinoline), causes DNA fragmentation similar to cisplatin [193]. While preferentially binding to G–C rich regions, NAMI is much less effective than cisplatin at altering DNA conformation, affecting DNA electrophoretic mobility, and inhibiting DNA recognition and cleavage by restriction enzymes [180,194]. Comparison with cisplatin and other similar cytotoxic ruthenium complexes indicate that the antimetastatic action of NAMI does not result from direct cytotoxic effects [193]. NAMI can be administered orally [163,192] and is active against a broad range of tumors including Lewis lung carcinoma, B16 melanoma, and MCA mammary carcinoma [195].

NAMI appears to increase resistance to the formation of metastases [192,196,197], but not through an enhanced antigenicity or an immunological response [189,198]. While NAMI binds to DNA and forms DNA–protein crosslinks, it does not significantly form DNA interstrand crosslinks (less than 1%) [189,199]. While the antimetastatic action of NAMI does not appear to involve DNA binding, 80–90% of the complex in solution binds to CT-DNA within 24 h at 37 °C. Such binding stabilizes DNA in low salt media (0.01 M NaClO₄), but alterations in the CD spectrum suggest unwinding of the DNA. Binding also inhibits the B to Z transition in poly(Dg–dC) and significantly inhibits DNA and RNA polymerases, with termination occurring preferentially at Gua residues [189].

At levels that cause a dramatic reduction in lung metastases, NAMI greatly alters the ratio between the mRNAs of MMP-2 (a metalloproteinase capable of degrading the extracellular matrix) and TIMP-2, the specific tissue inhibitor of this enzyme [200,201]. This corresponds with a pronounced increase of extracellular matrix components in the tumor parenchyma and around tumor blood vessels, which probably hinders metastasis formation and blood flow to the tumor [202]. Overall, NAMI appears to down regulate type-IV collagenolytic activity and the metastatic potential of MCA mammary carcinoma [198]. Combining NAMI with 5-fluoruracil achieved better results in mice against the solid metastasizing MCA mammary carcinoma and lymphocytic leukemia P388 [203].

NAMI can undergo a number of hydrolysis and electron transfer reactions under physiological conditions. Loss of DMSO and imidazole following chloride dissociation results in polyoxo complexes of ruthenium. At 25 °C, hydrolysis of the first chloride occurs within an hour, while the second takes more than twice as long. At physiological pH, *trans*-[Cl₄(Im)(Me₂SO)Ru][−] is more labile to substitution than *trans*-[Cl₄(Im)₂Ru][−]

($t_{1/2} = 19.7\text{ h}$ at 25 °C) [107]. Chloride loss for the former is catalyzed by reduction to Ru(II), which is expected to occur under physiological conditions and is enhanced in vitro by traces of biological reductants, such as ascorbic acid or cysteine [60,61]. Consequently, a redox-catalytic (activation by reduction) mechanism is suspected.

NAMI-A, (ImH)*trans*-[Cl₄(Me₂SO)(Im)Ru], is a more stable and reproducible solid than NAMI, while exhibiting similar pharmacological properties [204,205]. It selectively interferes with the growth of Lewis lung, MCA mammary carcinoma and TS/A adenocarcinoma metastases already settled in rodent lungs in a manner that is independent of the stage of the metastatic growth [206] and not simply related to a larger concentration in the lungs than in other tissues [207]. When administered intraperitoneally, NAMI-A is rapidly distributed to the body and rapidly cleared from the blood by the kidneys [207], with only 10% of the original dose remaining in the blood after 5 min [189]. It significantly increases the percentage of CD8⁺ cells at three dose levels, while CD4⁺ cells increased only at the lowest dose and remain unchanged at medium and high doses. NAMI-A significantly increases the thickness of the connective tissue of the tumor capsule and around tumor blood vessels, and impairs MMP-2, possibly at the level of its gene and/or its inhibitor TIMP-2 [189]. NAMI-A appears to be less toxic than cisplatin, does not modify cell growth, and causes a transient cell cycle arrest of tumor cells in the premitotic G2/M phase; whereas, cisplatin causes a dose-dependent disruption of cell cycle phases and reduces cell proliferation [206].

2.10. Photodynamic therapy

In photodynamic therapy (PDT) light is used to kill undesired cells in the body. The activity of PDT agents depends on their ability to associate with biopolymers or aggregates such as cell membranes or DNA. Cell membranes can be disrupted by the conversion of light energy to heat by nonradiative conversion, whereby photothermal sensitizer molecules produce localized hyperthermal damage. Damage to DNA can also occur by photoinduced electron transfer between the excited state of the PDT molecule and DNA, thereby damaging the cell's ability to function. Alternatively, light absorption by a photosensitizing molecule can lead to energy transfer to activate another molecule, such as the conversion of O₂ to the excited singlet state [32,208–210]. Photoinduced ligand substitution is also possible whereby a metal complex would either release a biologically active molecule or bind to nucleic acids or protein active sites [210,211].

To be useful in PDT, the ground state complex should be stable and generally nontoxic. The complex should also have well described photophysical properties with

an efficient photochemical conversion to the biologically active form. Since mammalian tissue is highly absorbant in the higher energy part of the visible spectrum, PDT complexes must absorb strongly at the relatively long wavelengths (640–850 nm) that penetrate tissues fairly well [210].

Metal complexes of phthalocyanines, naphthalocyanines, porphyrins and texaphyrins absorb at long wavelengths and often show tumor localization. Since complexes of these molecules with paramagnetic ions often have short triplet lifetimes and decay by non-radiative pathways releasing their energy as heat, they can act as photothermal sensitizers [210].

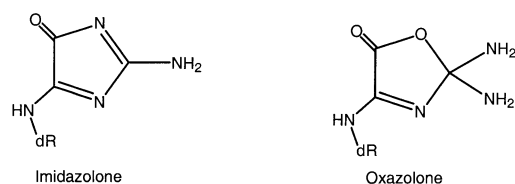
Diamagnetic Ru(II) phthalocyanines are thought to react by direct electron transfer to the photoexcited state (type I mechanism) [212,213]. A series of complexes of the type *trans*-[Ru(II)(nc)X₂], where nc = 2,3-naphthalocyanine, prepared from *trans*-[Ru(II)(nc)(PhCN)₂] (PhCN = benzonitrile) that have fairly strongly absorbing Q-bands (710–760 nm, $\epsilon \sim 10^5 \text{ M}^{-1} \text{ cm}^{-1}$) exhibit activity against HeLa cells at micromolar concentrations [214,215]. The greatest phototoxicity and relatively low chemotoxicity was observed when aromatic nitrogen heterocycles (nicotinic acid, pyrazinic acid, 3-pyridine-sulfonic acid) were coordinated in the axial positions to enhance solubility [215].

In the case of anticancer photodynamic therapy, DNA is usually the target. Metal complexes can interact with DNA by, (1) electrostatic binding between a cationic metal complex and the DNA polyanion [216,217]; (2) noncovalent hydrophobic surface binding; (3) intercalating between DNA bases, and 4) covalent binding [218]. Any of these types of binding may also be incorporated into shape-specific binding to particular nucleic acid substructures [219]. Due to their intense metal-to-ligand charge transfer transitions and long-lived excited states, ruthenium(II) complexes with *N*-heterocyclic ligands are particularly attractive as potential PDT agents [32,220–222], not only for DNA, but also for specific DNA or RNA substructures [219,223,224]. Polypyridyl and related ligand complexes of Ru(II) are usually activated by a metal to ligand charge transfer excitation, which leads to a triplet MLCT state (³MLCT) [211]. The ³MLCT can convert by the following methods with the corresponding rate constants, (1) radiative emission of light (k_r) around 600 nm; (2) radiationless deactivation as heat (k_{nr}); and (3) thermal conversion to a higher energy, metal-centered triplet (³MC). The lifetime (τ) of the ³MLCT is thus controlled by the rates of these three processes [211].

$$1/\tau = k_r + k_{nr} + k_{MC}e^{-\Delta E/RT}$$

The ³MLCT state, which is simultaneously a better oxidant and reductant than the ground state, often leads to an electron transfer reaction. In contrast, the ³MC state often involves populating a higher energy d-orbital

Type I products.



Type II products.

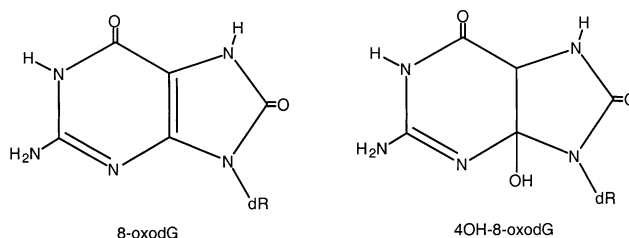


Fig. 7. Type I oxidation products of deoxyguanosine following photoinduced single-electron oxidation, and Type II oxidation products initially following oxidation by singlet oxygen.

with antibonding character thereby weakening one or more of the Ru–N bonds resulting in ligand substitution [211,221]. The ‘flash-quench’ approach involves oxidizing the (usually ³MLCT) excited state by an added oxidant, such as [(NH₃)₆Ru]³⁺, to prepare a more powerful ground state oxidant such as [(bpy)₃Ru]³⁺.

Gua, which is the most easily oxidized DNA base, is most commonly affected [225,226]. However, oxidation of the other bases as well as the sugar is also possible [227]. Different products are observed depending on whether deoxyguanosine (dG) is oxidized by electron transfer to the photoexcited complex (Type I) or by oxidation by ¹O₂ (Type II) [228,229]. Single-electron oxidation tends to give 2-aminoimidazolone and oxazolone products, which are piperidine labile and lead to DNA cleavage [230]. Singlet oxygen attack initially forms 8-oxoguanine and 4-hydroxy-8-oxoguanine products but further reaction with ¹O₂ yields the oxalone and additional 4-hydroxy-8-oxoguanine (see Fig. 7). Photoinduced single-electron oxidation of 8OG can also yield the imidazolone [229]. Stacked G’s are predicted to be thermodynamically more easily oxidized than single G’s and single-electron oxidation takes place preferentially at the 5′-G of 5′GG-3′ sequences [231,232].

Table 1 summarizes the affinities of a number of ruthenium complexes for noncovalent association with DNA and indicates which appear to bind through intercalation. Single strand cleavage resulting from the photoexcitation of [(bpy)₃Ru]²⁺ or [(phen)₃Ru]²⁺, which bind to the surface of DNA, in the presence of air results primarily from the formation of ¹O₂ [233].

Table 1
Binding constants for trisbidentate complexes of Ru(II)

Complex	<i>K</i>	Site size	Enantio-selectivity	Likely intercalator	Reference
[Ru(bpy) ₃] ²⁺	7×10^2	6–12	no	no	[344]
[Ru(bpy)(tpy)(H ₂ O)] ²⁺	6.6×10^2			no	[345]
[Ru(bpy) ₂ phen] ²⁺	7×10^2	10–14	Δ	no	[344]
[Ru(bpy) ₂ dip] ²⁺	1.7×10^3	12–18	Δ	yes	[344]
[Ru(bpy) ₂ ppz] ²⁺	5.5×10^3	3–4	yes	partial	[346,347]
[Ru(bpy) ₂ qpy] ²⁺	1.3×10^4	2–3	no	yes	[346]
[Ru(bpy) ₂ Me ₂ qpy] ⁴⁺	2.8×10^4	3	yes	yes	[346]
[Ru(phen) ₃] ²⁺	3×10^4	3–4	Δ	no(?)	[348]
[Ru(NH ₃) ₄ dppz] ²⁺	1.24×10^5			yes	[349]
[Ru(bpy) ₂ phi] ²⁺	1.6×10^5	4	yes	yes	[344]
[Ru(tpy) ₂ dppz(H ₂ O)] ²⁺	7.3×10^5			yes	[345]
{(dpp)(NH ₃) ₄ Ru(II)} ₂ ⁴⁺	8.3×10^5	6		yes	[350]
[Ru(phen) ₂ dppz] ²⁺	$\sim 10^8$	2	yes	yes	[351,352]

Enantioselectivity is indicated as to whether binding by the Δ or Λ is preferred. If the reference indicates enantioselective binding, but does not indicate which isomer a 'yes' is indicated.

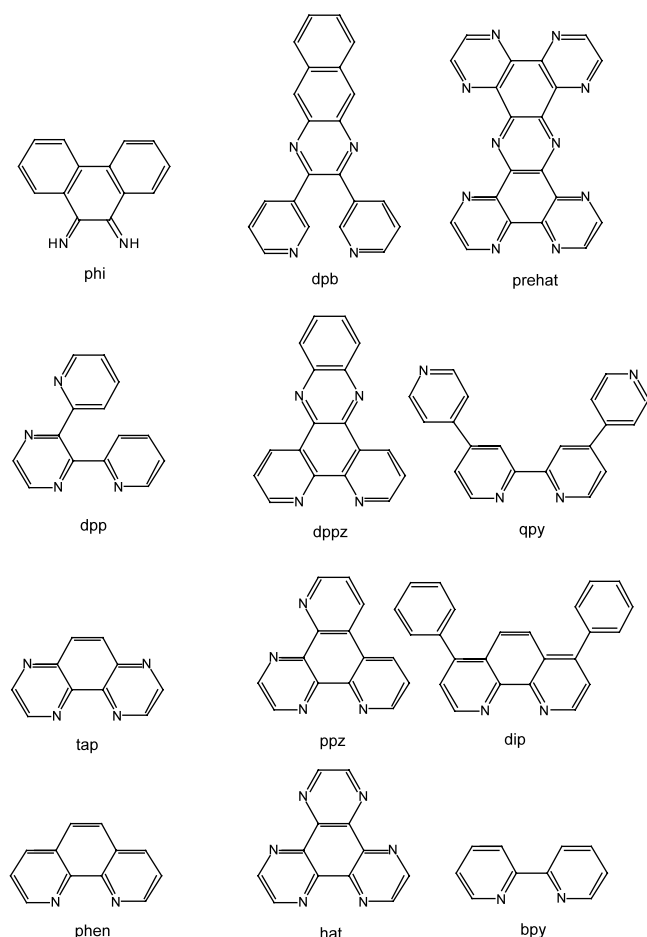


Fig. 8. Structures of N-heteroaromatic ligands.

For [(bpy)₃Ru]²⁺ the quantum yield increases from 1.2×10^{-6} under anaerobic conditions to 6.6×10^{-6} in air [32]. On the other hand, the excited states of a number of complexes of the type [L(bpy)₂Ru]²⁺, where L = hat, tap and bpz (see Fig. 8) and similar ligands

react primarily by electron-transfer rather than by generating singlet oxygen [234]. Covalently bound DNA adducts involving the exocyclic amine of Gua also occurs for some complexes whose excited states oxidize guanine. This probably occurs by radical reactions involving the reduced (radical ligand) ruthenium complex and oxidized guanine radicals [32].

When [dip(tap)₂Ru]²⁺ was tethered to oligodeoxyribonucleotides at the 5' position of a thymidine, photoinduced electron transfer from the guanines on the complementary strand occurred. Photooxidation of Gua is probably involved in the formation of photo-product(s) that irreversibly crosslink the two strands [235]. Flash-quenched generated [dppz(phen)₂Ru]³⁺ bound to DNA produces Gua radicals that are capable of crosslinking to tyrosine [236] and tryptophan [237] radicals that may also be generated by long range electron transfer in DNA, which implies that DNA–protein crosslinked proteins may be introduced by PDT [238].

The dinuclear complex, μ-hat[(phen)₂Ru]₂⁴⁺, exhibits the same photoreactivity as the mononuclear hat complex, but forms strong ion pairs with nucleotides and interacts more strongly with single- than double stranded DNA. Analogous complexes with dppz are significantly better intercalators, but have shorter excited life times than [(bpy)₃Ru]²⁺ and so generate less ¹O₂ [219]. A complex in which two [(bpy)₃Ru]²⁺ ions are tethered exhibited an affinity for DNA ca. 100-fold greater than that of the monomer and also yielded more efficient DNA strand cleavage [239].

While [(phen)₂(prehat)Ru]²⁺, which does not emit in water, luminesces upon intercalation, the luminescence of [(tap)₂(prehat)Ru]²⁺ is quenched by binding to DNA or in the presence of GMP. This strongly suggests photoinduced electron transfer from the Gua residues of

GMP or DNA to the excited triplet complex indicating it as a good DNA photoreagent [240]. Bis intercalation of dimeric complexes that must thread through the DNA helix or in which the linker has to sling itself around opening base pairs in order to thread has been suggested as a way of increasing the affinity of PDT complexes for DNA and extending their residence lifetime on this biopolymer [241,242]. While threading does not always occur in tethered dimers [242], the complex μ -(cpdppz)₂[(phen)₂Ru]₂⁴⁺, in which two chiral mono-intercalator complexes of [dppz(phen)₂Ru]²⁺ are tethered to each other via the dppz moieties and an aliphatic diamide linker, does thread through DNA [241]. Binding titrations indicate that the size of the binding site is about four base pairs, consistent with a nearest-neighbor-exclusion binding model, in which the two intercalated dppz subunits of the complex are separated by two base pairs. The linear dichroism spectrum of the *meso* (Δ,Δ) stereoisomer bound to DNA was an average of those of the two opposite enantiomers (Δ,Δ) and (Δ,Δ) suggesting that the binding geometry of each subunit depends solely upon its absolute configuration and not upon any diastereomeric intramolecular interactions between the Ru(phen)₂ moieties.

A supramolecular tetraruthenated tetrapyrrolylporphyrin, μ -[*meso*-5,10,15,20-(py)₄porphyrin][Cl(bpy)₂Ru(II)]₄ (TRP), introduces single-strand breaks into DNA in the presence of light. This occurs by both Type I and Type II mechanisms (see Fig. 7) but with 8-oxoguanosine predominating as the product through the formation of singlet oxygen [243]. In model studies using dG alone, the Type II/Type I product ratio was 2.3 [209].

Ogawa has attempted to utilize a synthetic 'leucine zipper' (bZIP) DNA-binding peptide to carry a ruthenium photosensitizer to DNA [244]. A photoactive ruthenium site, [Ru(bpy)₂(phen-IA)], where IA = iodoacetate, was attached to a surface-exposed cysteine residue of the bZIP peptide. While the free metal complex causes Gua-specific damage and the synthetic Ru-bZIP peptide recognizes and binds the consensus AP-1 DNA element, it does not damage DNA. It may be that DNA photodamage does not occur because the Ru binds at a point on the peptide that places the metal within the coiled-coil region of the protein [244].

The complex [(bpz)₃Ru(II)]²⁺ (bpz = trisbipyrazyl) photolytically damages both single- and double-stranded oligonucleotides by oxidative mechanisms involving both excited state electron transfer and production of singlet oxygen, both of which are enhanced by Cu/Zn superoxide dismutase [245].

A unique 'photodynamic' approach is to use the Mössbauer absorption of γ -rays by ruthenium complexes bound to DNA to induce Auger electrons to damage the nucleic acid [246].

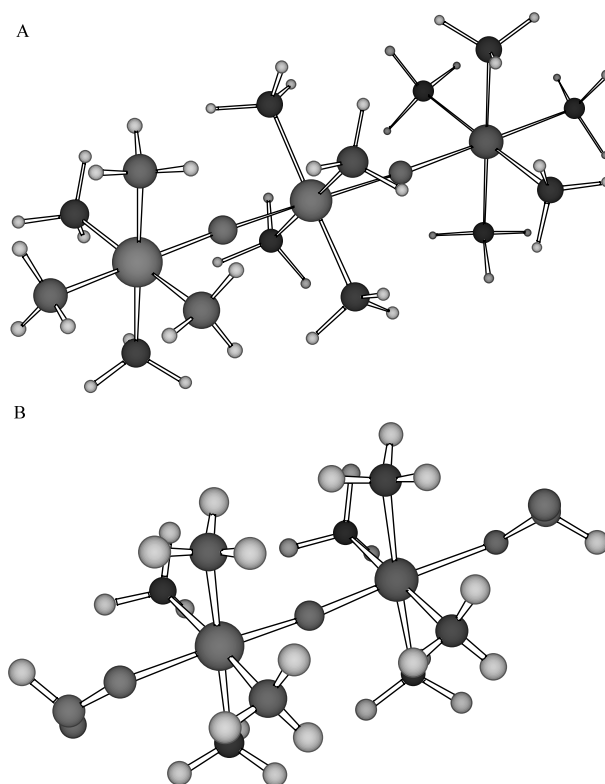


Fig. 9. Structure of (A) ruthenium red [247,355] and (B) Ru360 [266].

3. Ruthenium red and Ru360: inhibitors of Ca²⁺ utilization

Since preparations of the mixed-valent complex ruthenium red (Ru-red, see Fig. 9A), [(NH₃)₅Ru(III)ORu(IV)(NH₃)₄Ru(III)(NH₃)₅]⁶⁺ [247], have been used as a cytological stain for over a century, its biological properties are well known [149,248,249]. The affinity of Ru-red for mucopolysaccharides provides for the radiosciintigraphic visualization of tumors, which often generate a protective coating consisting largely of hyaluronic acid [168]. Ruthenium red binds to a number of calcium-binding proteins and interfere's with metabolism involving Ca²⁺ [250–255] and is also an antagonist for capsaicin [256,257]. Ruthenium red injected into rat brains concentrates in neuronal somas located near the injected areas. In the case of the CA1 region of the hippocampus, there is remarkable damage (vacuolization) of the pyramidal neurons followed by cell loss and disruption of the CA1 cell layer. The damage caused by selective penetration of ruthenium red into neuronal bodies results in hyperactivity of glutamatergic neurotransmission that leads to pronounced changes in motor function [258].

Nanomolar levels of Ru-red strongly inhibit respiration-driven Ca²⁺ uptake in mitochondria [259], which has led to increasing interest in Ru-red as a drug [170,260–262]. Ru-red's ability to inhibit calcium ion uptake by the mitochondria has also facilitated the

characterization of Ca^{2+} utilization in this organelle [263,264].

Commercial preparations of ruthenium red often contain substantial impurities. The dimeric impurity, $[\text{X}(\text{NH}_3)_4\text{Ru(III)ORu(IV)(NH}_3)_4\text{X}]^{3+}$ (Ru360, $\text{X} = \text{Cl}^-$ or OH^- see Fig. 9B) [265], is often responsible for most of the inhibition of Ca^{2+} uptake in mitochondria [259,266]. Ru360 specifically blocks uptake of Ca^{2+} into the mitochondria of cardiac myocytes; but it does not exhibit antitumor activity in cell culture screens [266]. Ru360's tripositive charge, hydrogen bonding capability, and ability to readily deform probably allow it to bind to a variety of anionic carboxylate sites on proteins including Ca^{2+} receptors. Decomposition of the hydroxo capped complex at physiological pH also yields a substance that irreversibly binds to polyanionic materials.

The bridging Ru–O bond in Ru360 is quite short (1.8240(6) Å), while the Ru–O_{formate} bond length (2.033(3) Å) is typical for a Ru–O single bond. The crystallographic equivalence of the two Ru atoms, together with magnetic ($\mu_{\text{eff}} = 1.15 \text{ B}$) and electrochemical evidence ($E_1^\circ = 1.17 \text{ V}$, $E_2^\circ = 0.05 \text{ V}$; $\Delta E^\circ = 1.12 \text{ V}$) indicate strong electronic coupling between the mixed-valent Ru's. The apical formates are easily replaced by chlorides in HCl. Aquation rates of both the formate and chloro complexes are pH dependent [266].

Since Ru360 is at least an order of magnitude more potent than ruthenium red and specifically inhibits the uptake of Ca^{2+} by the mitochondria (Ru360 $\text{IC}_{50} = 0.184 \text{ nM}$; ruthenium red $\text{IC}_{50} = 6.85 \text{ nM}$), it should be preferred for probing the effects of Ca^{2+} in this organelle [252,253]. ^{103}Ru 360 binds to isolated mitochondria with a high affinity ($K_d = 0.34 \text{ nM}$) [267]. The inhibition of the calcium ion uniporter by Ru360 is noncompetitive with Ca^{2+} ($K_i = 9.89 \text{ nM}$) [263,265]. The binding of Ru360 to the uniporter protein has been reported as $\sim 5 \text{ pmol mg}^{-1}$ protein in one study [263] and $0.08 \text{ pmol mg}^{-1}$ in another [267]. Ruthenium red and Ru360 are mutually exclusive inhibitors of the mitochondrial uniporter and appear to involve the same, low-affinity Ca^{2+} binding sites [263,268]. Since La^{3+} bound to the high affinity Ca^{2+} site was not displaced by Ru360, it has been speculated that the binding site in the uniporter for both Ru-red and Ru360 involves a negatively charged phospholipid membrane region, which may be closely associated with the uniporter inhibitor-binding site [263]. Ru360 has also been used as an affinity ligand in an effort to isolate the calcium ion uniporter protein [268].

The ability of Ru360 to bind to similar, if not the same sites, as ruthenium red is probably due the two ions being somewhat similar in their cationic charge density, ability to hydrogen bond through their ammine ligands, and capability to readily deform along the Ru–O–Ru axis. These properties should allow both type of

ions to bind to a variety of anionic carboxylate sites on proteins including low-affinity Ca^{2+} receptors as well as anionic phospholipids in the membrane. The smaller size of Ru360 and its ability to covalently bind to amino acid carboxylate groups through its apical sites may account for its greater biochemical activity relative to ruthenium red. Ru360 is probably also responsible for at least a portion of ruthenium red's ability to stain for electron microscopy.

4. Nitric oxide complexes

Nitric oxide (NO) is involved in a wide range of physiological processes [172,269–271]. When produced by macrophages to kill invasive tumor cells, bacteria and fungi [272], activation is caused by interferon- γ , endotoxins (bacterial cell wall lipopolysaccharides that elicit inflammatory responses), or T cells [272,273]. The toxic effect appears to derive from inhibition of enzyme activity through NO binding to nonheme protein iron-centers and release of intracellular iron from targeted cells [274–276]. In toxic-shock syndrome, excessive macrophage NO-synthase (NOS) activity leads to arterial expansion and cardiovascular collapse [277].

Nitric oxide synthase (NOS) catalyzes a net $5e^-$ oxidation of a terminal nitrogen of arginine to yield citrulline and nitric oxide. There are three major classes of NOS, endothelial, neuronal and immunological [269]. To some degree all have inducible and constitutive forms and all are NADPH-dependent cytosolic oxygenases that incorporate oxygen from O_2 into both products [271,278,279]. NOS contains both a P450-type domain and a P450-reductase domain. As a P450 type enzyme, it is activated by a $2e^-$ -reduction in which an oxygen from O_2 is added to arginine to produce *N*-hydroxy-arginine as an intermediate. The second step involves a tetrahydrobiopterin cofactor [280], which appears to facilitate the odd-electron oxidation to yield $\text{NO}^\bullet$ [281,282].

Sodium nitroprusside, $\text{Na}_2[\text{NO}(\text{CN})_5\text{Fe}] \cdot 2\text{H}_2\text{O}$, which is clinically used to rapidly lower blood pressure, is activated by reduction in vivo to $[\text{Fe}(\text{CN})_5\text{NO}]^{3-}$ in which an antibonding orbital becomes populated to facilitate the loss of NO [283]. Consequently, the biological reduction of metallonitrosyls is already a proven method of delivering NO to a desired tissue in a clinical setting. Since varying levels of NO at specific sites through the controlled binding/release of NO to/from metallopharmaceuticals would be beneficial in a number of biomedical applications, it is important to be able to modulate the rate of NO association/dissociation while minimizing the occurrence of side reactions involving the metal.

NO, functioning as a neuromodulator in the hippocampus of the brain, plays a fundamental role in the

basic neuronal processes involved in short-term memory and neuronal development and thereby in learning and experience [284,285]. As these abilities are among the very essences defining humanity, understanding them at the molecular level is of fundamental importance. Memory begins with an event that potentiates a network of synapses to fire again upon the same or similar stimuli. Since this potentiation must be relatively long-lasting, even for short-term memory, this process is called long-term potentiation (LTP). LTP involves the growth of new synapses between neurons and usually involves either a strong signal or simultaneous activation by many inputs [286]. Since learning tends to occur in situations that are, (a) new or striking; (b) reinforced through many senses; or (c) repetitive, there are probably parallel mechanisms for potentiating neural networks to fire (i.e. thoughts to occur) under the appropriate stimuli. The precise role of NO has been controversial [287], as LTP and memory occur in animals not possessing the hippocampal NOS gene [288]. Curiously, these animals also exhibit extremely aggressive behavior. Consequently, NO seems to be involved as a diffusive agent coordinating nearby neurons as well as playing a role in the post/presynaptic LTP feedback [289]. As LTP requires erasure to form new memories, it may be that NO is involved in the inverse process, long-term-depression (LDP) [290].

Communication across hippocampal synapses occurs by vesicular release of neurotransmitters, particularly glutamate. The glutamate travels from the presynaptic neuron into the synapse and then arrives at (among others) both an NMDA receptor and a non-NMDA receptor on the postsynaptic membrane. On binding glutamate, the non-NMDA receptor opens ion channels that allow Na^+ to rush in, thereby depolarizing the membrane. This depolarization opens a Ca^{2+} -channel associated with the NMDA receptor. Calmodulin binds the incoming Ca^{2+} and then activates NOS directly and indirectly through stimulating several Ca^{2+} -calmodulin dependent kinases (α -CaMKII, protein kinase C and tyrosine kinase) [291–293], which can also be involved in LTP independent of NOS. NO released from NOS [294,295] diffuses out to neighboring synapses and back to the presynaptic neuron, where it activates guanylate cyclase, initiating biochemical changes that stimulate additional glutamate synthesis and release [275,285]. NO thereby closes a feedback loop, which is initiated by exceeding an activity-dependent threshold and whose result is potentiating the neuron to fire again through added synaptical junctions [296].

As the noninnocence of the nitrosyl group, it can be coordinated as NO^+ , $\text{NO}^\bullet$ or NO^- , in which the M–N–O bond angle can vary from linear to markedly bent. At least in Fe and Ru nitrosyl complexes, the π^* -nitrosyl orbital generally lies between the metal d_π (t_{2g}) and σ^* (e_g) orbitals [297–299]. In the case of nitropursside,

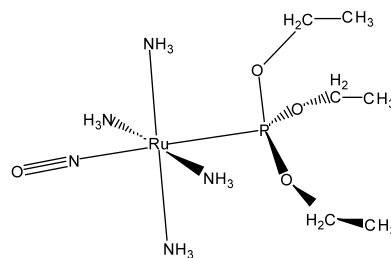


Fig. 10. Structure of the NO-releasing complex, *trans*-[(NO)(P(OCH₂CH₃)(NH₃)₄Ru(II)] [321].

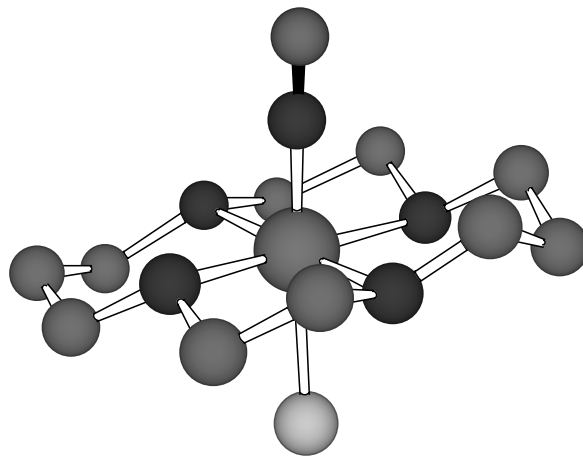


Fig. 11. Structure of the ruthenium nitrosyl vasodilator complex *trans*-[Cl(NO)cyclamRu]²⁺ with hydrogens omitted for clarity [321].

ZINDO (INDO/1) calculations suggest the HOMO's are primarily cyano rather than metal in character [300]. Reduction of these complexes populates a π molecular orbital with both π^* -nitrosyl and metal d_π character [301]. This probably decreases the π -bonding interaction with the metal, but leaves the σ -bond intact. In the case of nitropursside the *trans* cyano is preferentially lost. This, in turn, lowers the energy of the metal d_{z^2} -orbital, so that it lies below that of the π^* -NO. Consequently, this σ^* orbital now becomes partially populated and all the σ -bonds become labilized, leading to loss of both NO and CN^- .

While a number of workers have pointed out that the relative stability of ruthenium(II) nitrosyl complexes provides a good model for the systematic study of various nitrosyl association and dissociation pathways [172], this approach has been most actively pursued by Franco, Tfouni and coworkers in Brazil. The *trans*-[(NO)L(NH₃)₄Ru]³⁺ and related *trans*-[(NO)L(cyclam)Ru]ⁿ⁺ systems (Figs. 10 and 11) are generally soluble in water with the equatorial nitrogen ligands being inert to substitution [302,303]. The related complexes *trans*-[(NO)L(cyclam)Ru] (L = Cl[−] and OH[−], dioxocyclam = 1,4,8,11-tetraaza-*cyclo*-tetradecane-5,7-dione) have been recently prepared and characterized by NMR, IR and ESI-MS techniques [304].

In ruthenium chemistry, tertiary phosphorus ligands are very effective in stabilizing Ru(II) and in labilizing firmly bound *trans* ligands, such as nitric oxide [305–307]. Owing to the phosphite's *trans*-effect, *trans*- $[(\text{NO})(\text{P}(\text{OEt})_3)(\text{NH}_3)_4\text{Ru}]^{3+}$ releases nitric oxide following reduction. Unfortunately, the strong π -acceptor nature of this ligand also facilitates hydroxide attack on the coordinated NO to yield NO_2^- , which dissociates fairly readily. The relevant reactions are summarized below.

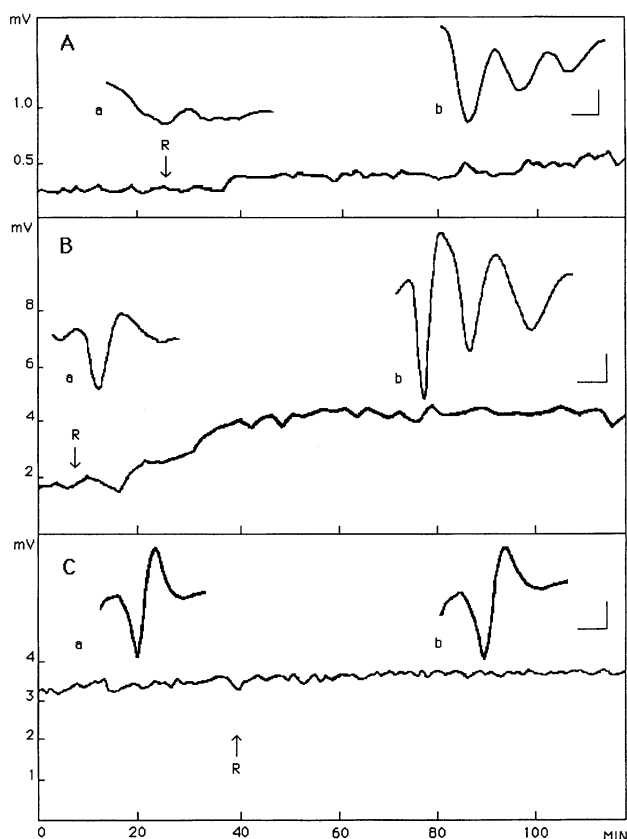
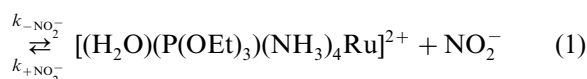
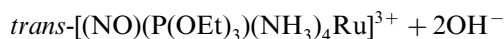
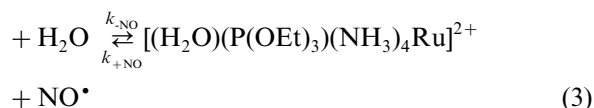
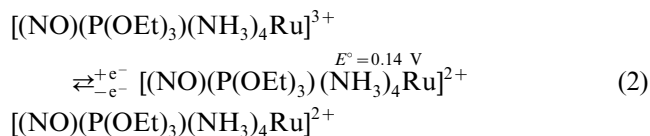


Fig. 12. The influence of $[(\text{NO})(\text{P}(\text{OEt})_3)(\text{NH}_3)_4\text{Ru}](\text{PF}_6)_3$ on hippocampal, evoked potentials recorded from the CA1 region. The upper part of each figure shows the magnitude of 10 averaged potentials recorded 10 min before (potentials a) and 60 min after (potentials b) application of the compound. The lower parts of each figure depict the changes in the magnitude of the potentials recorded during the whole experiment. (A) Amplification of the magnitude of EPSP depicting the slow but persistent increase in the magnitude of the potential after addition (marked by an arrow). Calibration, 5 ms, 0.3 mV. (B) Facilitation of the population spike following addition of the compound. Calibration, 5 ms, 1 mV. The multiple potential waves in (A) and (B) following application of indicate firing of nearby neurons. (C) Ineffectiveness of molecule R added to the slices, which were preincubated with oxyhemoglobin to scavenge released NO. Calibration, 5 ms, 1 mV.



The facilitated release of NO from *trans*- $[(\text{NO})(\text{P}(\text{OEt})_3)(\text{NH}_3)_4\text{Ru}](\text{PF}_6)_3$ (Fig. 10) is responsible for the biological activity of this complex in inducing neuronal firing in hippocampal slices upon an electrical stimulus [308,309]. Fig. 12 illustrates a typical experiment with this compound and Table 2 contains the average results of pertinent experiments. The increase in the amplitude of the wave form shown in Fig. 8 indicates an increase in the number of neurons firing and appears to be due to the in situ production of NO. Related complexes without NO or without a *trans*-labilizing ligand are inactive (Table 2). Similarly, the addition of an NO-scavenger, oxyhemoglobin, also eliminates the effect Fig. 13.

The phosphito complex also exhibits a vasodilator capability as do a number of complexes of the type *trans*- $[(\text{NO})\text{L}(\text{NH}_3)_4\text{Ru}]^{3+}$, where L = an aromatic nitrogen heterocycle, and *trans*- $[(\text{NO})\text{Cl}(\text{cyclam})\text{Ru}]^{3+}$ [173,310]. The vasodilator function appears to be open to complexes with weaker *trans*-effects, which release NO more slowly, than the phosphito complex.

Due to the thermal instability of nitrosyl complexes with iron porphyrins, the possibilities of photodynamic therapy involving ruthenium(II) nitrosyl complexes is being explored. Complexes of the type *trans*- $[(\text{NO})(\text{N}-\text{O}_2)(\text{TPP})\text{Ru}]$ release both NO and NO_2^- upon irradiation at 355 nm, but regenerate the original complex in the presence of NO [311,312].

Effective scavengers of NO, such as $\text{K}[\text{Cl}(\text{EDTA})\text{Ru}(\text{III})]$, may be useful in treating toxic shock syndrome by lowering the dangerously high level of NO in the bloodstream [313–315]. In aqueous solution, this aquates rapidly to give $[\text{H}_2\text{O}(\text{EDTA})\text{Ru}(\text{III})]^-$, in which the EDTA is pentadentate with a pendant carboxylate ($\text{pK}_a(\text{COOH}) = 2.4$, $\text{pK}_a(\text{H}_2\text{O}) = 7.6$), which varies between the four possible sites, thereby labilizing the molecule [316–318]. At pH 7.4 and 7 °C, the complex coordinates NO in a second-order reaction to form $[\text{NO}(\text{EDTA})\text{Ru}(\text{II})]^-$, ($k = 2 \times 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$, $K \approx 10^8 \text{ M}^{-1}$) [319]. The EDTA complex exhibited a protective effect against nitric oxide toxicity in several cell lines and inhibits the vasodilator response to NO-releasing agents [313]. In a rat model for toxic shock [314] animals treated with $\text{K}[\text{Cl}(\text{EDTA})\text{Ru}(\text{III})]$ returned to normal blood pressure after 9 h as opposed to 24–28 h for untreated animals [314]. Similar effects were seen in a porcine model [320]. Overall, polyaminocarboxylate complexes of Ru(III) appear to be effective scavengers

Table 2

Influence of *trans*-[(NO)(P(OEt)₃)(NH₃)₄Ru](PF₆)₃ and related control complexes on the hippocampal population spike [309]

Compound	Concentration (mM)	E° ^a	<i>N</i>	% Increase in P.S.
<i>trans</i> -[(NO)(P(OEt) ₃)(NH ₃) ₄ Ru](PF ₆) ₃	1–2.5	0.14	14	178 ± 46
<i>trans</i> -[(H ₂ O)(P(OEt) ₃)(NH ₃) ₄ Ru](PF ₆) ₂	0.25–0.75 ^b	0.69	8	2.0 ± 9.3
<i>trans</i> -[(P(OEt) ₃) ₂ (NH ₃) ₄ Ru](PF ₆) ₂	1–2.5	0.89	7	21.5 ± 21.4
[(NO)(NH ₃) ₅ Ru]Cl ₃	1–2.5	−0.012	9	30 ± 18
<i>trans</i> -[(NO)(P(OEt) ₃)(NH ₃) ₄ Ru](PF ₆) ₃ + oxyhemoglobin	1–2.5	0.14	5	4.2 ± 2.3
NaNO ₂	0.2–0.3		9	inhibition
<i>trans</i> -[(H ₂ O)(P(OEt) ₃)(NH ₃) ₄ Ru](PF ₆) ₂	1.0	0.69	16	74 ± 11
+ NaNO ₂	1–2.8			

^a Relative to SHE.^b Concentration limited by solubility. E° , reduction potential; P.S., population spike.

of NO in a variety of biological models and may provide an alternative therapeutic strategy to NOS inhibitors for the treatment of NO-mediated diseases [313].

The administration of *trans*-[(NO)Cl(cyclam)-Ru(II)]²⁺ reduces blood pressure in Wistar rats, especially in hypertensive rats. Notably, the slower rate of release of NO• from this complex following reduction produces a hypotensive effect 20 times longer than that

generated by sodium nitroprusside in either normotensive or hypertensive rats. The rate of NO• release is (k_{-NO}) is $2.2 \times 10^{-3} \text{ s}^{-1}$ at 35 °C following chemical reduction ($E^\circ = 0.10 \text{ V}$ vs. NHE) of *trans*-[(NO)Cl(cyclam)Ru(II)]²⁺ [321]. The continuous infusion of *trans*-[Cl(CF₃SO₃)(cyclam)Ru(III)]⁺ blocks the hypotensive effect of the *trans*-[(NO)Cl(cyclam)-Ru(II)]²⁺ probably by scavenging the NO• released

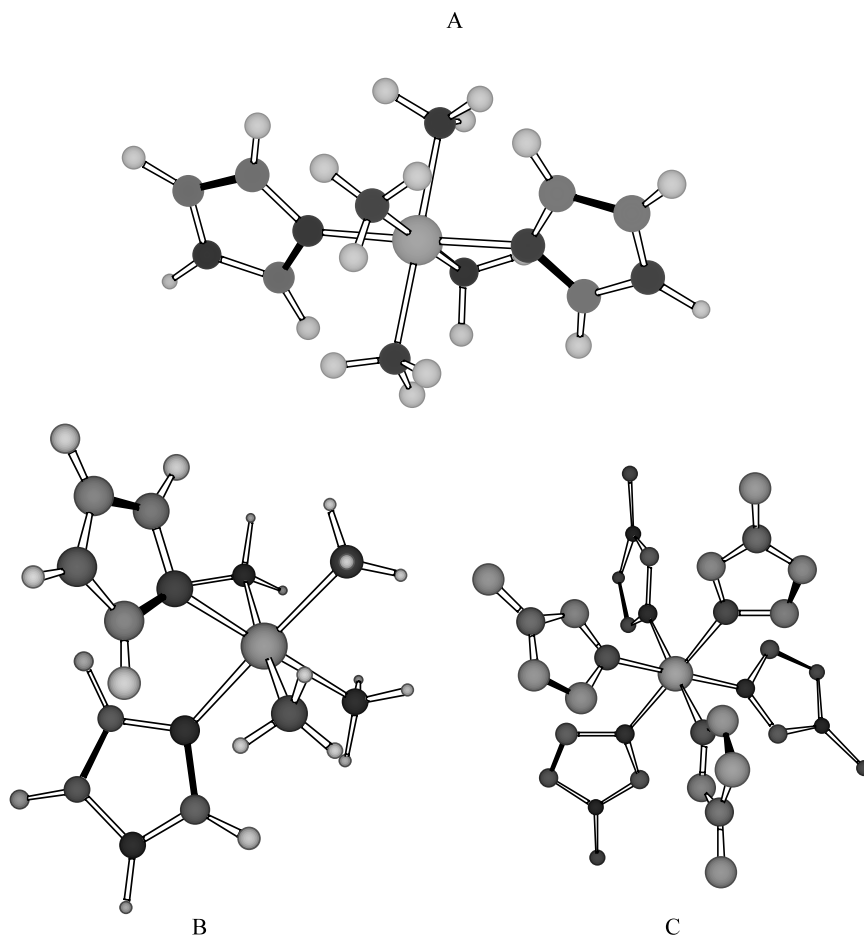


Fig. 13. Structures of ruthenium immunosuppressant complexes: (A) *trans*-[(Im)₂(NH₃)₄Ru]³⁺ [356], (B) *cis*-[(Im)₂(NH₃)₄Ru]²⁺, (C) [(1MeIm)₆-Ru]²⁺ [324]. Hydrogen atoms omitted from C for clarity.

after reduction. Taken together, these results suggests that ruthenium cyclam complexes could be used as a ‘NO-buffer’ system to control physiological levels of available NO [322].

5. Immunosuppressants

Since the most widely used immunosuppressive agent, cyclosporin A, interferes with a Ca-dependent step in the immune response [323] and also prevents the Ca^{2+} -dependent opening of a megachannel permeability transition pore in the inner mitochondrial membrane, it is conceivable that other Ca^{2+} blocking agents, such as ruthenium red and its related complexes, might interfere with the immune response. Procept Inc. found that ruthenium red inhibited the antigen-induced proliferation of T cells with an IC_{50} of 35 nM, which is comparable to that of cyclosporin A [249]. Subsequent studies revealed that simple, substitution-inert Ru(II/III) complexes with nitrogen ligands (NH_3 , imidazole, py) can be potent immunosuppressants [324–326]. For example, nanomolar concentrations of [4Mepy(NH_3)₅-Ru(III)] (PRO-2844) and *cis*-[(Im)₂(NH_3)₄Ru(III)] (PRO-2846) strongly inhibit antigen-stimulated T lymphocyte proliferation with few or no acute toxic side effects. While showing nM activity against antigen-stimulated T cells, the compounds are notably less toxic ($\text{IC}_{50} > 10 \mu\text{M}$) against a Jurkat cell line [326]. Since these Ru drugs are mechanistically distinct from the clinically used agents, cyclosporin A and FK506, and may function through an electron-transfer pathway [324], they may point the way toward an exciting new class of immunosuppressive drugs [326,327].

The reported active Ru-immunosuppressant agents, so far have involved six, small N-coordinated ligands (N_6 -coordinate) [324,326]. Among these compounds, there is a window of activity between ~ 50 and ~ 400 mV, which not only indicates a redox process in their mechanism, but also suggests why only Ru complexes have thus far been reported to be active [327]. If electron transfer is involved, this should be facile and take place within a biologically accessible range of reduction potentials. As has been well documented in studies of Ru-anticancer agents, simple Ru complexes with nitrogen ligands fulfill this requirement [149]. It seems likely that the small size of the active complexes (among the largest are [(1MeIm)₆Ru]³⁺) allows them to approach electron-transfer sites inside proteins. The higher activity of those complexes with aromatic ligands, is in keeping with Gray’s studies indicating that aromatic ligands penetrate deeper into electron-transfer sites in proteins [328]. Also consistent with this is the reduction of a highly active immunosuppressant agent, [py(NH_3)₅Ru]³⁺ by HeLa cells [98].

Given the ‘redox window’ and the high activity of these complexes, it is apparent that they must function catalytically, i.e. by continually short-circuiting one or more redox processes by accepting an electron from one molecule and passing it to another. It is possible that oxygen serves as the oxidant, which would bypass much of the mitochondrial respiratory redox chain and produce toxic superoxide. While active complexes such as [py(NH_3)₅Ru]²⁺ are so slowly oxidized by O_2 [56], that it would seem that another biological oxidant might be more likely, autoxidation is accelerated if a ligand is lost thereby allowing O_2 to interact in an inner-sphere fashion, or if a catalyst, such as Fe(II/III) is present. It may be that a reductant at least as reducing as ubiquinone in the mitochondrial respiratory redox chain and/or an oxidant around cyt-oxidase or O_2 are involved in redox cycling of the Ru(II/III) to short-circuit the production of ATP.

Given hydrolysis rates for a number of N_6 Ru(II) complexes, ligand loss is a very real possibility over the time spans (1 day–1 week) of tissue culture experiments and would certainly be expected to occur over the long periods required for drug administration needed to suppress rejection of organ transplants [329]. The reaction of GSH with [(4-picoline)(NH_3)₅Ru]³⁺ and [(NH_3)₆Ru]³⁺ also produces some [GS(NH_3)₅-Ru(III)]²⁺, which is indicative of a reduced intermediate that eliminates nitrogen ligands, and suggests that GSH is conceivably involved in the ruthenium immunosuppressant activity. Ligand loss would give rise to complexes with substitutable sites of the type discussed under anticancer agents that would be expected to bind to DNA, interfere with nucleic acid metabolism, and would probably be mutagenic.

6. Radiopharmaceuticals

Metal containing radiopharmaceuticals can be classified into two broad types, (1) Those that find their target by virtue of the properties of aqua metal ion or the metal ion complex itself; and (2) those that localize in the desired tissue by virtue of being attached to a targeting protein or polypeptide. Localizing α - or β -emitting radionuclides in a tumor can be used to kill the tumor cells. Utilization of a γ -emitting radionuclides, such as the widely used ^{99m}Tc, allow for diagnostic organ imaging. Radioscintigraphy refers to the visualization of organs that have concentrated a γ -emitting radionuclide [10]. The γ -rays are detected by a photodetector and converted into a visible image by computer methods in real time. This provides not only for visualizing the organ, but observing its function as well. For example, the clearance of a radioactive compound from the blood by the liver can be seen both as a decrease in activity in the blood and an increase in activity in the liver,

followed by the progression of activity into the gall bladder and finally the intestine.

The radiophysical properties of ^{97}Ru are nearly ideal for some types of radiodiagnostic imaging. It decays by electron capture with a 2.88 days half-life and emits a 216 keV γ -ray that can be used in common radio-scintigraphic instruments. Unfortunately, ^{97}Ru is not commercially available. An alternative isotope, ^{103}Ru is commercially available, but emits a 497 keV γ -ray, which is of only fair utility in radioscintigraphy, and has a longer (39.35 days) half-life. On the other hand, its longer half-life and lower specific activity can be an advantage in developing synthetic methods [330].

A corollary of the activation-by-reduction hypothesis for chemotherapy involving Ru(III) ammine complexes is that they should concentrate in tumors as a result of reduction and intracellular binding. Indeed, a number of ruthenium ammine complexes exhibit good tumor uptake [330,331]. Unfortunately, they also remain in the blood through binding to albumin and Tf. RuCl_3 exhibits some nonspecific binding to erythrocytes [332]. The coordination of ruthenium to Tf can be used to enhance tumor uptake, since neoplastic cells, especially those in rapidly growing tumors, have a high iron requirement and display a large number of Tf receptors on their membrane surfaces. The approach of using a protein to localize rutheruthenium in a tumor was demonstrated by Srivastava, Larson, et al. who showed that the uptake of ^{103}Ru -labelled Tf by EMT-6 sarcoma in mice was almost twice as high as that of a widely used tumor-imaging agent, ^{67}Ga -citrate [74,330,333].

Effective radio-imaging agents for the liver have been developed using rutheruthenium(III) with iminodiacetate ligands containing lipophilic groups that facilitate uptake by the liver and biliary tract [334–336]. Several lipophilic ruthenocene derivatives are excreted in both urine and bile following hydroxylation in the liver and formation of a glucuronide conjugate [337] and some ruthenocene derivatives have been designed to localize in the adrenals and other organs [338,339]. Ruthenocene-haloperidol exhibited a high affinity for the lung, but not the brain, in rats [340]. The complex $[(\text{pyal})(\text{NH}_3)_5\text{Ru(III)}]^{3+}$ (pyal = β -(4-pyridyl)- α -alanine) displayed good uptake by the pancreas [341]. Ruthenium complexes with phosphate and phosphonates tend to localize in bone [84]. Ruthenium red [247] binds in vitro and in vivo to cell surfaces high in acidic glycoproteins and has been used to image tumors [169,171,342]. The complex $[(\text{BLM})(\text{NH}_3)_5^{103}\text{Ru}]$, where BLM = bleomycin, a clinically used antitumor antibiotic that tends to localize in tumors, yielded a tissue distribution in normal and tumor-bearing mice essentially the same as BLM itself [330,343].

Aside from uptake owing to nonspecific causes such as increased blood perfusion and higher cell permeability in tumors, overall it is likely that tumor accumulation

of simple ammineruthenium complexes proceeds by two pathways. First, rapid tumor uptake proceeds through activation of the Ru(III)-prodrug toward binding by reduction in the tumor. Since small ions are excreted fairly readily by the kidneys, this mode of binding should decrease rapidly with time. Second, Tf mediated tumor binding, which may occur for many days following injection.

7. Conclusion

The versatile synthetic chemistry of ruthenium opens up a wide range of complexes with a variety of ligands that could provide an arsenal of compounds for clinical uses. Ruthenium compounds appear to penetrate tumors well and bind to cellular DNA. While the initial DNA binding site of many ruthenium complexes is the same as that of cisplatin, i.e. the N7 of Gua, their antitumor mechanisms are different. In the case of the ruthenium compound now in clinical trials, NAMI, its antimetastatic activity is independent of DNA coordination, and may depend on the regulation of a matrix proteinase. Better understanding Tf mediated Ru transport into tumors could lead to more effective and probably less toxic agents. Use of the radionuclide, ^{97}Ru , could have considerable advantages over $^{99\text{m}}\text{Tc}$ in that its longer half life allows for more complicated synthetic methods, purification and quality control while still providing excellent radioscintigraphic images. The substantial body of work on the photochemistry of Ru(II) complexes with aromatic nitrogen ligands, particularly those that intercalate into DNA, offer the potential for novel photodynamic approaches to cancer therapy.

Particularly exciting is the unusual ability of simple ruthenium complexes to suppress the immune response by inhibiting the proliferation of T cells. While the initial set of complexes tested are likely to give rise to others that would bind to DNA and possibly be mutagenic, the idea of short-circuiting electron transfer pathways synergetically coupled with other subcellular interactions is one with rich possibilities for controlling the division and multiplication of selected cell types.

The ability to tune both the uptake and release of the ubiquitous intercellular messenger molecule, NO, by manipulating other ligands on Ru lays out a definite way in which coordination chemists can contribute not only to new classes of drugs to affect a variety of biological functions, but also possibly to the development of a molecular tool chest that can be used to probe NO function in the same way that ruthenium red has been used to probe that of Ca^{2+} .

Acknowledgements

Funding for this work was provided by NIH Grant GM26390.

References

- [1] R.H. Holm, P. Kennepohl, E.I. Solomon, *Chem. Rev.* 96 (1996) 2239.
- [2] S.J. Lippard, J.M. Berg, *Curr. Opin. Chem. Biol.* 4 (2000) 137.
- [3] Q. He, C.H. Liang, S.J. Lippard, *Proc. Natl. Acad. Sci. USA* 97 (2000) 5768.
- [4] A. Gelasco, S.J. Lippard, *Top. Biol. Inorg. Chem.* 1 (1999) 1.
- [5] E.R. Jamieson, S.J. Lippard, *Chem. Rev.* 99 (1999) 2467.
- [6] A.T. Yarnell, S. Oh, D. Reinberg, S.J. Lippard, *J. Biol. Chem.* 276 (2001) 25736.
- [7] C.J. Ziegler, A.P. Silverman, S.J. Lippard, *J. Biol. Inorg. Chem.* 5 (2000) 774.
- [8] N. Farrell, Y. Qu, J.D. Roberts, *Top. Biol. Inorg. Chem.* 1 (1999) 99.
- [9] J. Zehnulova, J. Kasparkova, N. Farrell, V. Brabec, *J. Biol. Chem.* 276 (2001) 22191.
- [10] M.J. Clarke, L. Podbielski, *Coord. Chem. Rev.* 78 (1987) 253.
- [11] A.B. Packard, J.F. Kronauge, M.W. Brechbiel, *Top. Biol. Inorg. Chem.* 2 (1999) 45.
- [12] P. Caravan, J.J. Ellison, T.J. McMurphy, R.B. Lauffer, *Chem. Rev. (Washington, DC)* 99 (1999) 2293.
- [13] M.F. Tweedle, K. Kumar, *Top. Biol. Inorg. Chem.* 2 (1999) 1.
- [14] M.J. Heeg, S.S. Jurisson, *Acc. Chem. Res.* 32 (1999) 1053.
- [15] C. Orvig, M.J. Abrams, in: J. Michl, J.A. Gladysz, K.L. Rowlen (Eds.), *Chemical Reviews*, American Chemical Society, Washington, DC, 1999.
- [16] K.H. Thompson, C. Orvig, *Dalton* (2000) 2885.
- [17] M. Melchior, S.J. Rettig, B.D. Liboiron, K.H. Thompson, V.G. Yuen, J.H. McNeill, C. Orvig, *Inorg. Chem.* 40 (2001) 4686.
- [18] R.A. Anderson, *J. Am. Coll. Nutr.* 17 (1998) 548.
- [19] J.B. Vincent, *Acc. Chem. Res.* 33 (2000) 503.
- [20] M.J. Clarke, P.J. Sadler, *Top. Biol. Inorg. Chem.* 1 (1999) 200.
- [21] M.J. Clarke, P.J. Sadler, *Top. Biol. Inorg. Chem.* 2 (1999) 216.
- [22] Z.J. Guo, P.J. Sadler, *Angewandte Chemie-Int. Ed.* 38 (1999) 1513.
- [23] T. Pieper, K. Borsky, B.K. Keppler, *Top. Bioinorg. Chem.* 1 (1999) 171.
- [24] M. Hartmann, B.K. Keppler, *Comm. Inorg. Chem.* 16 (1995) 339.
- [25] N. Farrell, *Transition Metal Complexes as Drugs and Chemotherapeutic Agents*, Kluwer Academic, Boston, 1989.
- [26] B.K. Keppler, *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim, Germany, 1993.
- [27] M.J. Clarke, *Ruthenium and Other Non-Platinum Metal Complexes in Cancer Chemotherapy*, Springer-Verlag, Heidelberg, Germany, 1989.
- [28] M.J. Clarke, S. Limauro, in: G. Berthon (Ed.), *Handbook on Metal–Ligand Interactions in Biological Fluids*, Marcel Dekker, New York, 1995, pp. 1010–1018.
- [29] M.J. Clarke, M. Stubbs, *Met. Ions Biol. Syst.* 32 (1996) 727.
- [30] M.J. Clarke, in: S.J. Isied (Ed.), *Electron Transfer Reactions*, American Chemical Society Symposium Series, Washington, D.C., 1997, pp. 349–365.
- [31] H. Ali, J.E. van Lier, *Chem. Rev.* 99 (1999) 2379.
- [32] J.M. Kelly, C.-M. Moucheron, A. Kirsch-De Mesmaeker, *Struct. Bond.* 92 (1999) 163.
- [33] H.B. Gray, J.R. Winkler, *Ann. Rev. Biochem.* 65 (1996) 537.
- [34] L. Messori, F.G. Vilchez, R. Vilaplana, F. Piccoli, E. Alessio, B. Keppler, *Met. Based Drugs* 7 (2000) 335.
- [35] L. Messori, P. Orioli, D. Vullo, E. Alessio, E. Iengo, *Eur. J. Biochem.* 267 (2000) 1206.
- [36] V.M. Bailey, K.J. LaChance-Galang, P.E. Doan, M.J. Clarke, *Inorg. Chem.* 36 (1997) 1873.
- [37] C.G. Kuehn, H. Taube, *J. Am. Chem. Soc.* 98 (1976) 689.
- [38] D. Frasca, M.J. Clarke, *J. Am. Chem. Soc.* 121 (1999) 8523.
- [39] M.J. Clarke, M.G. Dowling, *Inorg. Chem.* 20 (1981) 3506.
- [40] M.J. Clarke, M.G. Dowling, in: V. Massey, C. Williams (Eds.), *Proceedings of the Seventh International Flavin Symposium*, Elsevier, New York, 1982, pp. 579–583.
- [41] A. Abelleira, R.D. Galang, M.J. Clarke, *Inorg. Chem.* 29 (1990) 633.
- [42] M.J. Clarke, F. Zhu, D. Frasca, *Chem. Rev.* 99 (1999) 2511.
- [43] A.D. Kelman, M.J. Clarke, S.D. Edmonds, H.J. Peresie, *J. Clin. Hematol. Oncol.* 7 (1977) 274.
- [44] M.J. Clarke, in: B.K. Keppler (Ed.), *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim, 1993, pp. 129–157.
- [45] B.D. Palmer, W.R. Wilson, S.M. Pullen, *J. Med. Chem.* 33 (1990) 112.
- [46] G.K. Snyder, *J. Appl. Physiol.* 65 (1988) 2332.
- [47] R.G. Steen, *Am. J. Roentgenol.* 157 (1991) 243.
- [48] P. Okunieff, E.P. Dunphy, P. Vaupel, *Adv. Exp. Med. Biol.* 345 (1994) 485.
- [49] J.E. Biskupiak, K.A. Krohn, *J. Nucl. Med.* (1993) 411.
- [50] J.L. Wike-Hooley, J. Haveman, H.S. Reinhold, *Radiother. Oncol.* 2 (1984) 343.
- [51] D. Miklavcic, G. Sersa, S. Novakovic, *J. Bioelect.* 9 (1990) 133.
- [52] J.A. Marchant, T. Matsubara, P.C. Ford, *Inorg. Chem.* 16 (1977) 2160.
- [53] G.M. Coleman, J.W. Gesler, E.A. Shirley, J.R. Kuempel, *Inorg. Chem.* 12 (1973) 1036.
- [54] M.J. Clarke, S. Bitler, D. Rennert, M. Buchbinder, A.D. Kelman, *J. Inorg. Biochem.* 12 (1980) 79.
- [55] J.F. Laliberté, I.L. Sun, F.L. Crane, M.J. Clarke, *J. Bioenerg. Biomembr.* 19 (1987) 69.
- [56] D.M. Stanbury, O. Haas, H. Taube, *Inorg. Chem.* 19 (1980) 518.
- [57] D.M. Stanbury, D. Gaswick, G.M. Brown, H. Taube, *Inorg. Chem.* 22 (1983) 1975.
- [58] D.M. Stanbury, W.A. Mulac, J.C. Sullivan, H. Taube, *Inorg. Chem.* 19 (1980) 3735.
- [59] D. Frasca, J. Ciampa, J. Emerson, R.S. Umans, M.J. Clarke, *Metal-Based Drugs* 3 (1996) 197.
- [60] G. Mestroni, E. Alessio, G. Sava, S. Pacor, M. Coluccia, A. Boccarelli, *Metal-Based Drugs* 1 (1994) 41.
- [61] G. Mestroni, E. Alessio, G. Sava, S. Pacor, M. Coluccia, in: B.K. Keppler (Ed.), *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim, 1993, pp. 157–185.
- [62] C. Monti-Bragadin, L. Ramani, L. Samer, G. Mestroni, G. Zassinovich, *Antimicrob. Agents Chemother.* 7 (1975) 825.
- [63] G. Sava, S. Zorzet, T. Giraldo, G. Mestroni, G. Zassinovich, *Eur. J. Cancer Clin. Oncol.* 20 (1984) 841.
- [64] G. Sava, S. Pacor, S. Zorzet, E. Alessio, G. Mestroni, *Pharmacol. Res.* 21 (1989) 617.
- [65] E. Alessio, Y. Xu, S. Cauci, G. Mestroni, F. Quadrioglio, P. Viglino, L.G. Marzilli, *J. Am. Chem. Soc.* 111 (1989) 7068.
- [66] G. Mestroni, E. Alessio, M. Calligaris, W.M. Attia, F. Quadrioglio, S. Cauci, G. Sava, S. Zorzet, S. Pacor, C. Monti-Bragadin, M. Tamaro, L. Dolzani, *Progress in Clinical Biochemistry and Medicine*, Springer-Verlag, Berlin, 1989.
- [67] A.C.G. Hotze, A.H. Velders, F. Ugozzoli, M. Biagini-Cingi, A.M. Manotti-Lanfredi, J.G. Haasnoot, J. Reedijk, *Inorg. Chem.* 39 (2000) 3838.
- [68] A.H. Velders, H. Kooijman, A.L. Spek, J.G. Haasnoot, D. De Vos, J. Reedijk, *Inorg. Chem.* 39 (2000) 2966.
- [69] Y.N.V. Gopal, D. Jayaraju, A.K. Kondapi, *Biochemistry* 38 (1999) 4382.
- [70] L. Stryer, *Biochemistry*, Freeman, New York, 1995.

- [71] R.E. Morris, R.E. Aird, P.d.S. Murdoch, H.M. Chen, J. Cummings, N.D. Hughes, S. Parsons, A. Parkin, G. Boyd, D.I. Jodrell, P.J. Sadler, *J. Med. Chem.* 44 (2001) 3616, J. Cummings, R.E. Aird, R.E. Morris, H.M. Chen, P.d.S. Murdoch, P.J. Sadler, J.F. Smyth, D.I. Jodrell, *Clin. Cancer Res.* 6 (2000) 142.
- [72] C.-C. Cheng, W.-L. Lee, J.-G. Su, C.-L. Liu, *J. Chin. Chem. Soc. (Taipei)* 47 (2000) 213.
- [73] J. Helms, B.L. Huang, R. Price, B.P. Sullivan, B.A. Sullivan, *Inorg. Chem.* 34 (1995) 5335.
- [74] P. Som, Z.H. Oster, K. Matsui, G. Gugliemi, B. Persson, M.L. Pellettieri, S.C. Srivastava, P. Richards, H.L. Atkins, A.B. Brill, *Eur. J. Nucl. Med.* 8 (1983) 491.
- [75] M.J. Clarke, B. Jansen, K.A. Marx, R. Kruger, *Inorg. Chim. Acta* 124 (1986) 13.
- [76] M. McNamara, M.J. Clarke, *Inorg. Chim. Acta* 195 (1992) 175.
- [77] W.R. Harris, *Struct. Bond.* 92 (1998) 121.
- [78] F. Kratz, M.T. Schutte, *Cancer J.* 11 (1998) 60.
- [79] H. Sun, H. Li, P.J. Sadler, *Chem. Rev.* 99 (1999) 2817.
- [80] H.S. Li, P.J. Sun, *Eur. J. Biochem.* 242 (1996) 387.
- [81] S.E. Bailey, R.W. Garratt, G. Richard, *Biochemistry* 27 (1988) 5804.
- [82] R.T.A. MacGillivray, M.C. Bewley, C.A. Smith, Q.-Y. He, A.B. Mason, R.C. Woodworth, E.N. Baker, *Biochemistry* 39 (2000) 1211.
- [83] S.C. Srivastava, P. Richards, G.E. Meinken, S.M. Larson, Z. Grunbaum, in: R.P. Spencer (Ed.), *Radiopharmaceuticals*, Grune & Stratton, Inc, New York, 1981, p. 207.
- [84] S.C. Srivastava, L.F. Mausner, M.J. Clarke, in: M.J. Clarke (Ed.), *Ruthenium and other Non-Platinum Metal Complexes in Cancer Chemotherapy*, Springer-Verlag, Heidelberg, 1989, pp. 111–150.
- [85] A. Anto, I. Anto, T. Hiraki, K. Hisada, *Int. J. Rad. Appl.* 15 (1988) 133.
- [86] L. Messori, F. Kratz, E. Alessio, *Met.-Based Drugs* 3 (1996) 1.
- [87] F. Kratz, M. Hartmann, B. Keppler, L. Messori, *J. Biol. Chem.* 269 (1994) 2581.
- [88] F. Kratz, B.K. Keppler, L. Messori, C. Smith, E.N. Baker, *Metal-Based Drugs* 1 (1994) 169.
- [89] C.A. Smith, A.J. Sutherland-Smith, B.K. Keppler, F. Kratz, E.N. Baker, *J. Biol. Inorg. Chem.* 1 (1996) 424.
- [90] D.C. Kraitier, O. Zak, P. Aisen, A.L. Crumbliss, *Inorg. Chem.* 37 (1998) 964.
- [91] F. Kratz, L. Messori, *J. Inorg. Biochem.* 49 (1993) 79.
- [92] D.M. Martin, N.D. Chasteen, J.K. Grady, *Biochim. Biophys. Acta* 1076 (1991) 252.
- [93] P. Aisen, in: G.L. Eichhorn (Ed.), *Inorganic Biochemistry*, Elsevier, Amsterdam, 1973, pp. 280–305.
- [94] F. Kratz, N. Mulinacci, L. Messori, I. Bertini, B.K. Keppler, in: J. Anastassopoulou, P. Collery, J.-C. Etienne, T. Theophanides (Eds.), *Metal Ions in Biology and Medicine*, John Libbey Limited Eurotext, Paris, 1992, pp. 69–74.
- [95] B.K. Keppler, in: B.K. Keppler (Ed.), *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim, Germany, 1993, pp. 187–220.
- [96] F. Kratz, B. Keppler, M. Hartmann, L. Messori, R. Berger, *Met.-Based Drugs* 3 (1996) 15.
- [97] D.R. Frasca, L.E. Gehrig, M.J. Clarke, *J. Inorg. Biochem.* 83 (2001) 139.
- [98] D.R. Frasca, Ph.D. thesis, Boston College, 2000.
- [99] M.J. Clarke, *Inorg. Chem.* 16 (1977) 738.
- [100] M.J. Clarke, *J. Am. Chem. Soc.* 100 (1978) 5068.
- [101] M.E. Kastner, K.F. Coffey, M.J. Clarke, S.E. Edmonds, K. Eriks, *J. Am. Chem. Soc.* 103 (1981) 5747.
- [102] V.M. Bailey, M.J. Clarke, *Inorg. Chem.* 36 (1997) 1611.
- [103] K. LaChance-Galang, M. Zhao, M.J. Clarke, *Inorg. Chem.* 35 (1996) 6021.
- [104] K.J. LaChance-Galang, I. Maldonado, M.L. Gallagher, W. Jian, A. Prock, J. Chaklos, R.D. Galang, M.J. Clarke, *Inorg. Chem.* 40 (2001) 485.
- [105] A.H. Velders, B. Van der Geest, H. Kooijman, A.L. Spek, J.G. Haasnoot, J. Reedijk, *Eur. J. Inorg. Chem.* (2001) 369.
- [106] M. Zhao, M.J. Clarke, *J. Biol. Inorg. Chem.* 4 (1999) 325.
- [107] O.M. Dhubhghaill, W. Hagen, B.K. Keppler, K.-G. Lipponer, J. Sadler, *J. Chem. Soc. Dalton Trans.* (1994) 3305.
- [108] M. Hartmann, K.-G. Lipponer, B.K. Keppler, *Inorg. Chim. Acta* 267 (1998) 137.
- [109] B.K. Keppler, M. Hartmann, *Metal-Based Drugs* 1 (1994) 145.
- [110] M.J. Clarke, H. Taube, *J. Am. Chem. Soc.* 97 (1975) 1397–1403, C. Price, M.R.J. Elsegood, W. Clegg, N.H. Reese, A. Houlton, *Angew. Chem., Intl. Ed.* 36 (1997) 172–1764.
- [111] M.J. Clarke, P.E. Morrissey, *Inorg. Chim. Acta* 80 (1984) L69.
- [112] K.C. Gariepy, M.A. Curtin, M.J. Clarke, *J. Am. Chem. Soc.* 111 (1989) 4947.
- [113] D.P. Rudd, H. Taube, *Inorg. Chem.* 10 (1971) 1543.
- [114] J.K. Barton, E. Lolis, *J. Am. Chem. Soc.* 107 (1985) 708.
- [115] N. Grover, N. Gupta, H.H. Thorp, *J. Am. Chem. Soc.* 114 (1992) 3390.
- [116] N. Grover, T.W. Welch, T.A. Fairley, M. Cory, H.H. Thorp, *Inorg. Chem.* 33 (1994) 3544.
- [117] P.M. van Vliet, M.S. Sarinten, S.M.S. Toekimin, J.G. Haasnoot, J. Reedijk, O. Nováková, O. Vrána, V. Brabec, *Inorg. Chim. Acta* 231 (1995) 57.
- [118] C. Pifferi, R. Cini, *J. Chem. Soc. Dalton Trans.* (1998) 2679.
- [119] C. Pifferi, R. Cini, *Acta Crystallogr. Sect. C-Cryst. Struct. Commun.* 56 (2000) E439.
- [120] S. Isied, H. Taube, *Inorg. Chem.* 15 (1976) 3070.
- [121] S. Pacor, G. Sava, V. Ceschia, F. Bregant, G. Mestroni, E. Alessio, *Chem. Biol. Interact.* 78 (1991) 223.
- [122] L.G. Marzilli, M. Iwamoto, E. Alessio, L. Hansen, M. Calligaris, *J. Am. Chem. Soc.* 116 (1994) 815.
- [123] E. Alessio, E. Zangrando, R. Roppa, L. Marzilli, *Inorg. Chem.* 37 (1998) 2458.
- [124] M. Iwamoto, E. Alessio, L.G. Marzilli, *Inorg. Chem.* 35 (1996) 2384.
- [125] G. Esposito, S. Cauci, F. Fogolari, E. Alessio, M. Scocchi, F. Quadrifoglio, P. Viglino, *Biochemistry* 31 (1992) 7094.
- [126] N. Farrell, Y. Qu, *Inorg. Chem.* 34 (1995) 3573.
- [127] B. Van Houten, S. Illenye, Y. Qu, N. Farrell, *Biochemistry* 32 (1993) 11794.
- [128] M. Milkevitch, B.W. Shirley, K.J. Brewer, *Inorg. Chim. Acta* 264 (1997) 249.
- [129] M. Milkevitch, H. Storrie, E. Brauns, K.J. Brewer, B.W. Shirley, *Inorg. Chem.* 36 (1997) 4534.
- [130] A. Corazza, I. Harvey, P. Sadler, *Eur. J. Biochem.* 236 (1996) 697.
- [131] D.L. Rabenstein, K.K. Millis, K.H. Weaver, *J. Org. Chem.* 58 (1993) 4144.
- [132] T.M. Buttke, P.A. Sandstrom, *Immunol. Today* 15 (1994) 7.
- [133] S. Coppola, L. Ghibelli, *Biochem. Soc. Trans.* 28 (2000) 56.
- [134] L. Chen, P.F. Lee, S.Y. Wong, J.D. Ranford, J.J. Vittal, *J. Chem. Soc. Dalton Trans.* (1999) 1209.
- [135] K. Lemma, J. Berglund, N. Farrell, L.I. Elding, *J. Biol. Inorg. Chem.* 5 (2000) 300.
- [136] H.J. Berkovits, R.A. Floyd, K.E. Wetterhahn, J. Aiyar, *Environ. Health Perspect.* E10 (1991) 53.
- [137] K.E. Wetterhahn, S.L. Brauer, *J. Am. Chem. Soc.* 113 (1991) 3001.
- [138] A. Eastman, *Biochem. Pharmacol.* 36 (1987) 4177.
- [139] N.A. Kratochwil, Z. Guo, P.D.S. Murdoch, J.A. Parkinson, P. Bednarski, P. Sadler, *J. Am. Chem. Soc.* 120 (1998) 8253.
- [140] J. Reedijk, *Chem. Rev.* 99 (1999) 2499.
- [141] T. Ishikawa, F. Ali-Osman, *J. Biol. Chem.* 268 (1993) 20116.

- [142] Y. Chen, Z. Guo, J.A. Parkinson, P.J. Sadler, *J. Chem. Soc. Dalton Trans.* (1998) 3577.
- [143] J.-M. Teuben, J. Reedijk, *J. Biol. Inorg. Chem.* 5 (2000) 463.
- [144] R.E. Yasbin, C.R. Matthews, M.J. Clarke, *Chemico-Biol. Interact.* 30 (1980) 355.
- [145] K.A. Marx, R. Kruger, M.J. Clarke, *Molec. Cell. Biochem.* 86 (1989) 155.
- [146] K.A. Marx, C. Seery, P. Malloy, *Mol. Cell. Biochem.* 90 (1989) 37.
- [147] M. Carballo, R. Vilaplana, G. Marquez, M. Conde, F.J. Bedoya, F. Gonzalez-Vilchez, F. Sobrino, *Biochem. J.* 328 (1997) 559.
- [148] R. Vilaplana, M.A. Romero, M. Quiros, J.M. Salas, F. Gonzalez-Vilchez, *Metal-Based Drugs* 2 (1995) 211.
- [149] M.J. Clarke, *Met. Ions Biol. Syst.* 11 (1980) 231.
- [150] F. Bottomley, *Can. J. Chem.* 55 (1977) 2788.
- [151] J.R. Durig, J. Danneman, W.D. Behnke, E.E. Mercer, *Chem.-Biol. Interact.* 13 (1976) 287.
- [152] M.J. Clarke, *Prog. Clin. Biochem. Med.* 10 (1989) 25.
- [153] B.K. Keppler, W. Rupp, U.W. Juhl, H. Endres, R. Niebl, W. Balzer, *Inorg. Chem.* 26 (1987) 4366.
- [154] T. Pieper, B.K. Keppler, *Analisis* 26 (1998) M84.
- [155] A. Galeano, M.R. Berger, B.K. Keppler, *Arzneimittel-Forschung/Drug Research* 42-1 (1992) 821.
- [156] E.D. Kreuser, B.K. Keppler, W.E. Berdel, A. Piest, E. Thiel, *Semin. Oncol.* 19 (1992) 73.
- [157] H. Depenbrock, S. Schmelcher, R. Peter, B.K. Keppler, G. Weirich, T. Block, J. Rastetter, A.R. Hanauske, *Eur. J. Cancer* 33 (1997) 2404.
- [158] M.H. Seelig, M.R. Berger, B.K. Keppler, *J. Cancer Res. Clin. Oncol.* 118 (1992) 195.
- [159] M.R. Berger, F.T. Garzon, D. Schmal, B.K. Keppler, *Anticancer Res.* 9 (1989) 761.
- [160] B.K. Keppler, M. Henn, U.M. Juhl, M.R. Berger, R. Niebel, F.E. Wagner, in: M.J. Clarke (Ed.), *Ruthenium and Other Non-Platinum Metal Complexes in Cancer Chemotherapy*, Springer-Verlag, Heidelberg, 1989, pp. 41–70.
- [161] B.K. Keppler, W. Balzer, V. Seifried, *Arzneim.-Forsch.* 37 (1987) 770.
- [162] L. Trynda-Lemiesz, B.K. Keppler, H.K. Koslowski, *J. Inorg. Biochem.* 73 (1999) 123.
- [163] G. Sava, S. Pacor, G. Mestroni, E. Alessio, *Clin. Exp. Metastasis* 10 (1992) 273.
- [164] J.L. Bear, B. Han, K.M. Kadish, *Inorg. Chem.* 35 (1996) 3012.
- [165] J.L. Bear, Y. Li, K.M. Kadish, *Inorg. Chem.* 35 (1996) 3053.
- [166] J.L. Bear, Y. Li, K.M. Kadish, *Inorg. Chem.* 36 (1997) 5449.
- [167] K.R. Dunbar, J.H. Matonic, V.P. Saharan, C.A. Crawford, G. Christou, *J. Am. Chem. Soc.* 116 (1994) 2201.
- [168] M.A. Oberer-Greenwood, L.M. Muul, M.K. Gately, P.L. Kornblith, B.W. Smith, *J. Neurooncol.* 3 (1986) 387.
- [169] L.H. Anghileri, *Strahlentherapie* 149 (1975) 173.
- [170] T.W. Hurley, *Am. J. Physiol.* 23 (1988) 621.
- [171] L.J. Anghileri, C. Marchal, M. Matrat, M.C. Crone-Escanya, *Neoplasma* 33 (1986) 603.
- [172] M.J. Clarke, J.B. Gaul, *Struct. Bonding* 81 (1993) 147.
- [173] S.S.S. Borges, C.U. Davanzo, E.E. Castellano, J. Zukerman-Schpector, S.C. Silva, D.W. Franco, *Inorg. Chem.* 37 (1998) 2670.
- [174] M.G. Gomes, C.U. Davanzo, S.C. Silva, L.G.F. Lopes, P.S. Santos, D.W. Franco, *J. Chem. Soc. Dalton Trans.* (1998) 601.
- [175] J.M. Slocik, R.A. Kortess, R.E. Shepherd, *Met.-Based Drugs* 7 (2000) 67.
- [176] S.R. Vilaplana, M.G. Basallote, C. Ruiz-Valero, E. Gutierrez, F. González-Vilchez, *J. Chem. Soc. Chem. Commun.* (1991) 100.
- [177] R.A. Vilaplana, F. Gonzalez-Vilchez, C. Ruiz-Valero, *Inorg. Chim. Acta* 224 (1994) 15.
- [178] D. Chatterjee, M.S. Ward, R.E. Shepherd, *Inorg. Chim. Acta* 285 (1999) 170.
- [179] F. Gonzalez-Vilchez, R. Vilaplana, G. Blasco, L. Messori, *J. Inorg. Biochem.* 71 (1998) 45.
- [180] E. Gallori, C. Vettori, E. Alessio, F. Gonzalez-Vilchez, R. Vilaplana, P. Orioli, A. Casini, L. Messori, *Arch. Biochem. Biophys.* 376 (2000) 156.
- [181] R.E. Shepherd, in: S. Isied (Eds.) *Electron Transfer Reactions*, American Chemical Society Symposium Series Washington, DC, 1997, pp. 367–399.
- [182] R.E. Shepherd, Y. Chen, F.-T. Lin, *Inorg. Chem.* 36 (1997) 818.
- [183] Y. Chen, F.-T. Lin, R.E. Shepherd, *Inorg. Chim. Acta* 268 (1998) 287.
- [184] R.E. Shepherd, Y. Chen, R.A. Kortess, M.S. Ward, *Inorg. Chim. Acta* 303 (2000) 30.
- [185] G. Mestroni, E. Alessio, M. Calligaris, W.M. Attia, F. Quadri-foglio, S. Cauci, G. Sava, S. Zorzet, S. Pacor, C. Monti-Bragadin, M. Tamaro, L. Dolzani, in: M.J. Clarke (Ed.), *Ruthenium and Other Non-Platinum Metal Complexes in Cancer Chemotherapy*, Springer-Verlag, Heidelberg, 1989, pp. 74–88.
- [186] S. Cauci, P. Viglino, G. Esposito, *J. Inorg. Biochem.* 43 (1991) 739.
- [187] E. Alessio, G. Mestroni, G.A. Nardin, M. Wahib, M. Calligaris, G. Sava, S. Zorzet, *Inorg. Chem.* 27 (1988) 4099.
- [188] E. Alessio, W. Attia, M. Calligaris, S. Cauci, L. Dolzani, G. Mestroni, C. Monti-Bragadin, G. Nardin, F. Quadri-foglio, G. Sava, *Dev. Oncol.* 54 (1988) 617.
- [189] G. Sava, E. Alessio, E. Bergamo, G. Mestroni, *Top. Biol. Inorg. Chem.* 1 (1999) 143.
- [190] G. Sava, S. Pacor, M. Coluccia, M. Mariggio, M. Cocchietto, E. Alessio, G. Mestroni, *Drug Invest.* 8 (1994) 150.
- [191] E. Alessio, G. Balducci, A. Lutman, G. Mestroni, M. Calligaris, W.M. Attia, *Inorg. Chim. Acta* 203 (1992) 205.
- [192] G. Sava, S. Pacor, A. Bergamo, M. Cocchietto, G. Mestroni, E. Alessio, *Chem. Biol. Interact.* 95 (1995) 109.
- [193] I. Capozzi, K. Clerici, M. Cocchietto, G. Salerno, A. Bergamo, G. Sava, *Chem. Biol. Interact.* 113 (1998) 51.
- [194] L. Messori, A. Casini, D. Vullo, S.G. Haroutianian, E.B. Dalian, P. Orioli, *Inorg. Chim. Acta* 303 (2000) 283.
- [195] G. Sava, S. Pacor, G. Mestroni, E. Alessio, *Anti-Cancer Drugs* 3 (1992) 25.
- [196] R. Gagliardi, G. Sava, S. Pacor, G. Mestroni, E. Alessio, *Clin. Exp. Metastasis (DFC)* 12 (1994) 93.
- [197] A. Bergamo, M. Cocchietto, I. Capozzi, G. Mestroni, E. Alessio, G. Sava, *Anticancer Drugs* 7 (1996) 697.
- [198] G. Sava, G. Salerno, A. Bergamo, M. Cocchietto, R. Gagliardi, E. Alessio, G. Mestroni, *Metal Based Drugs* 3 (1996) 67.
- [199] A. Barca, B. Pani, M. Tamaro, E. Russo, *Mut. Res. Fundam. Mole. Mechan. Mutagenesis* 423 (1999) 171.
- [200] E. Morgunova, A. Tuuttila, U. Bergmann, M. Isupov, Y. Lindqvist, G. Schneider, K. Tryggvason, *Science* 284 (1999) 1667.
- [201] M. Whittaker, C.D. Floyd, P.D. Brown, J.H. Gearing, *Chem. Rev.* 99 (1999) 2735.
- [202] G. Mestroni, E. Alessio, G. Sava, *PCT Int. Appl.* (1996) WO 9800431 A1 980108, WO 97-EP3401, 970630, IT 96-MI1359 960702.
- [203] M. Coluccia, G. Sava, G. Salerno, A. Bergamo, S. Pacor, G. Mestroni, E. Alessio, *Metal-Based Drugs* 2 (1995) 195.
- [204] G. Sava, R. Gagliardi, M. Cocchietto, K. Clerici, I. Capozzi, M. Marrella, E. Alessio, G. Mestroni, R. Milanino, *Pathol. Oncol. Res.* 4 (1998) 30.
- [205] S. Geremia, E. Alessio, F. Todone, *Inorg. Chim. Acta* 253 (1996) 87.
- [206] A. Bergamo, R. Gagliardi, V. Scarcia, A. Furlani, E. Alessio, G. Mestroni, G. Sava, *J. Pharmacol. Exp. Ther.* 289 (1999) 559.
- [207] G. Sava, K. Clerici, I. Capozzi, M. Cocchietto, R. Gagliardi, E. Alessio, G. Mestroni, A. Perbellini, *Anticancer Drugs* 10 (1999) 129.

- [208] M.J. Abrams, *Plat. Met. Rev.* 39 (1995) 14.
- [209] J.L. Ravanat, J. Cadet, K. Araki, H.E. Toma, M.H.G. Medeiros, P. Di Mascio, *Photochem. Photobiol.* 68 (1998) 698.
- [210] G. Stochel, A. Wanat, E. Kulis, Z. Stasicka, *Coord. Chem. Rev.* 171 (1998) 203.
- [211] C. Moucheron, A. Kirsch-De Mesmaeker, J.M. Kelly, *J. Photochem. Photobiol. B* 40 (1997) 91.
- [212] P. Charlesworth, T.G. Truscott, R.C. Brooks, B.C. Wilson, *J. Photochem. Photobiol. B* 26 (1994) 277.
- [213] G.E. Bossard, M.J. Abrams, M.C. Darkes, J.F. Vollano, R.C. Brooks, *Inorg. Chem.* 34 (1995) 1524.
- [214] M.J. Abrams, *Plat. Met. Rev.* 39 (1995) 14.
- [215] J.F. Vollano, G.E. Bossard, S.A. Martellucci, M.C. Darkes, M.J. Abrams, R.C. Brooks, *J. Photochem. Photobiol. B* 37 (1997) 230.
- [216] G.S. Manning, *Q. Rev. Biophys.* 11 (1978) 179.
- [217] G.S. Manning, J. Ray, *J. Biomol. Struct. Dyn.* 16 (1998) 461.
- [218] G. Yang, J.Z. Wu, L. Wang, L.N. Ji, X. Tian, *J. Inorg. Biochem.* 66 (1997) 141.
- [219] K.E. Erkkila, D.T. Odom, J.K. Barton, *Chem. Rev.* 99 (1999) 2777.
- [220] R.A. Krause, *Struct. Bonding (Berlin)* 67 (1987) 1.
- [221] E. Tfouni, *Coord. Chem. Rev.* 196 (2000) 281.
- [222] J.S. Bridgewater, L.M. Vogler, S.M. Molnar, K.J. Brewer, *Inorg. Chim. Acta* 208 (1993) 179.
- [223] A. Kirsch-De Mesmaeker, J.-P. Lecomte, J.M. Kelly, *Top. Curr. Chem.* 177 (1996) 25.
- [224] C.S. Chow, J.K. Barton, *J. Am. Chem. Soc.* 112 (1990) 2839.
- [225] S.K. Tyagi, G. Dryhurst, *J. Electroanal. Chem.* 216 (1987) 137.
- [226] I. Saito, M. Takayama, H. Sugiyama, K. Nakatani, *J. Am. Chem. Soc.* 117 (1995) 6406.
- [227] B. Armitage, *Chem. Rev.* 98 (1998) 1171.
- [228] K. Kino, I. Saito, H. Sugiyama, *J. Am. Chem. Soc.* 120 (1998) 7373.
- [229] H. Ikeda, I. Saito, *J. Am. Chem. Soc.* 121 (1999) 10836.
- [230] J. Cadet, M. Berger, G.W. Buchko, P.C. Joshi, S. Raoul, J.-L. Ravanat, *J. Am. Chem. Soc.* 116 (1994) 7403.
- [231] H. Sugiyama, I. Saito, *J. Am. Chem. Soc.* 118 (1996) 7063.
- [232] S. Kawanishi, S. Oikawa, M. Murata, H. Tsukitome, I. Saito, *Biochemistry* 38 (1999) 16733.
- [233] C.V. Kumar, J.K. Barton, N.J. Turro, *J. Am. Chem. Soc.* 107 (1985) 5518.
- [234] J.-P. Lecomte, A. Kirsch-De Mesmaeker, J.M. Kelly, *Inorg. Chem.* 34 (1995) 6481.
- [235] I. Ortmans, S. Content, N. Boutonnet, A. Kirsch-De Mesmaeker, W. Bannwarth, J.F. Constant, E. Defrancq, J. Lhomme, *Chem. Eur. J.* 5 (1999) 2712.
- [236] H.-A. Wagenknecht, E.D.A. Stemp, J.K. Barton, *Biochemistry* 39 (2000) 5483.
- [237] H.-A. Wagenknecht, E.D.A. Stemp, J.K. Barton, *J. Am. Chem. Soc.* 122 (2000) 1.
- [238] K.L. Nguyen, M. Steryo, K. Kurbanyan, K.M. Nowitzki, S.M. Butterfield, S.R. Ward, E.D.A. Stemp, *J. Am. Chem. Soc.* 122 (2000) 3585.
- [239] F. O'Reilly, J. Kelly, A. Kirsch-De Mesmaeker, *Chem. Commun.* 9 (1996) 1013.
- [240] C. Moucheron, A. Kirsch-De Mesmaeker, *J. Phys. Org. Chem.* 11 (1998) 577.
- [241] B. Oenfelt, P. Lincoln, B. Norden, *J. Am. Chem. Soc.* 121 (1999) 10846.
- [242] D.W. Dixon, N.B. Thornton, T. Netzel, *Inorg. Chem.* 38 (1999) 5526.
- [243] J. Onuki, A.V. Ribas, M.H.G. Medeiros, K. Araki, H.E. Toma, *Photochem. Photobiol.* 63 (1996) 272.
- [244] R.C. Lasey, S.S. Banerji, M.Y. Ogawa, *Inorg. Chim. Acta* 300–302 (2000) 822.
- [245] E. Gicquel, N. Paillous, P. Vicendo, *Photochem. Photobiol.* 72 (2000) 583.
- [246] F.M. Macdonnell, *Biochemistry* 34 (1995) 12871.
- [247] M.A.A.F. d. C.T. Carrondo, W.P. Griffith, J.P. Hall, A.C. Skapski, *Biochem. Biophys. Acta* 627 (1980) 332–334.
- [248] Y. Hirabayashi, T. Sakagami, K. Yamada, *Acta Histochem. Cytochem.* 23 (1990) 165.
- [249] D.S. Dwyer, K. Esenther, US Patent 5,238,689 (1996).
- [250] J.H. Charuk, C.A. Pirraglia, R.A. Reithmeier, *Anal. Biochem.* 188 (1990) 123.
- [251] S.S. Smaili, J.T. Russell, *Cell Calcium* 26 (1999) 121.
- [252] R.A. Bassani, T.R. Shannon, D.M. Bers, *Cell Calcium* 23 (1998) 433.
- [253] Z. Zhou, M.A. Matlib, D.M. Bers, *J. Physiol.* 507 (1998) 379.
- [254] I. Velasco, R. Tapia, *J. Neurosci. Res.* 60 (2000) 543.
- [255] M.T. Taylor, D.W. Bonhaus, *Neuropharmacology* 39 (2000) 1267.
- [256] R. Amann, C.A. Maggi, *Life Sci.* 49 (1991) 849.
- [257] A. Dray, *Life Sci.* 51 (1992) 1759.
- [258] E. Belmar, G. Garcia-Ugalde, R. Tapia, *Mol. Chem. Neuro-pathol.* 26 (1995) 285.
- [259] K.C. Reed, F.L. Bygrave, *Biochem. J.* 140 (1974) 143.
- [260] B. Antoniu, D.H. Kim, M. Morii, N. Ikemoto, *Biochim. Biophys. Acta* 816 (1985) 9.
- [261] M. Chiesi, R. Schwaller, G. Galviello, *Biochem. Biophys. Res. Commun.* 154 (1988) 1.
- [262] P.M. Vassilev, M.P. Kanazirska, L.J. Charamella, N.V. Dimitrov, H.T. Tien, *Cancer Res.* 47 (1987) 519.
- [263] C. Zazueta, M.E. Sosa-Torres, F. Correa, A. Garza-Ortiz, *J. Bioenerg. Biomembr.* 31 (1999) 551.
- [264] T. Pozzan, R. Rizzuto, P. Volpe, J. Meldolesi, *Physiol. Rev.* 74 (1994) 595.
- [265] W.-L. Ying, J. Emerson, M.J. Clarke, D.R. Sanadi, *Biochemistry* 30 (1991) 4949.
- [266] J. Emerson, M.J. Clarke, W.-L. Ying, D.R. Sanadi, *J. Am. Chem. Soc.* 115 (1993) 11799.
- [267] M.A. Matlib, Z. Zhou, S. Knight, S. Ahmed, K.M. Choi, J. Krause-Bauer, R. Phillips, R. Altschuld, Y. Kaatsube, N. Sperelakis, D.M. Bers, *J. Biol. Chem.* 273 (1998) 10223.
- [268] C. Zazueta, G. Zafra, G. Vera, C. Sanchez, E. Chavez, *J. Bioenerg. Biomembr.* 30 (1998) 489.
- [269] J.M. Fukuto, D.A. Wink, *Met. Ions Biol. Syst.* 36 (1999) 547.
- [270] D.A. Wink, M. Feelisch, Y. Vodovotz, J. Fukuto, M.B. Grisham, *React. Oxygen Species Biol. Syst.* (1999) 245.
- [271] J.W. Denninger, M.A. Marletta, *Biochim. Biophys. Acta* 1411 (1999) 334.
- [272] M.A. Marletta, P.S. Yoon, R. Iyengar, C.D. Leaf, J.S. Wishnok, *Biochemistry* 27 (1988) 8706.
- [273] S. Moncada, R.M.J. Palmer, E.A. Higgs, *Pharmacol. Rev.* 43 (1991) 109.
- [274] J.B. Hibbs, R.R. Taintor, Z. Varvin, *Biochem. Biophys. Res. Commun.* 157 (1988) 87.
- [275] J. Garthwaite, *TINS* 14 (1991) 60.
- [276] S. Moncada, E.A. Higgs, Nitric Oxide from L-Arginine: a Bioregulatory System: Proceedings of a Symposium Held at the Royal Society, London, 14–15 September, 1989, Elsevier, Amsterdam, 1990.
- [277] A. Zembowicz, J.R. Vane, *Proc. Natl. Acad. Sci. USA* 89 (1992) 2051.
- [278] A.M. Leone, R.M.J. Palmer, R.G. Knowles, P.L. Francis, D.S. Ashton, S. Moncada, *J. Biol. Chem.* 266 (1991) 23790.
- [279] M.A. Marletta, *J. Biol. Chem.* 268 (1993) 12231.
- [280] T.L. Poulos, H. Li, C.S. Raman, *Curr. Opin. Chem. Biol.* 3 (1999) 131.
- [281] A.R. Hurshman, C. Krebs, D.E. Edmondson, B.H. Huynh, M.A. Marletta, *Biochemistry* 38 (1999) 15689.
- [282] C.S. Raman, H. Li, P. Martasek, V. Kral, B.S.S. Masters, T.L. Poulos, *Cell (Cambridge, MA)* 95 (1998) 939.
- [283] C. Glidewell, I.L. Johnson, *Inorg. Chim. Acta* 132 (1987) 145.
- [284] D.S. Bredt, S.H. Snyder, *Neuron* 8 (1992) 3.
- [285] S.H. Snyder, D.S. Bredt, *Scientific American* (1992) 68.

- [286] R. Nowak, J. NIH Res. 4 (1992) 49.
- [287] C.L.M. Bon, J. Garthwaite, *Neuropharmacology* 40 (2001) 501–507. R.D. Hawkins, S. Hyeon, O. Arancio, *Prog. Brain Res.* 118 (1998) 153–172.
- [288] T.J. O'Dell, P.L. Huang, T.M. Dawson, J.L. Dinerman, S.H. Snyder, E.R. Kandel, M.C. Fishman, *Science* 265 (1994) 542.
- [289] K. Mori, H. Togashi, M. Matsumoto, M. Yoshioka, *Acta Physiol. Scand.* 169 (2000) 79.
- [290] I.I. Abramets, Y.V. Kuznetsov, I.M. Samoi'lovich, *Neurophysiology* 33 (2001) 86–93.
- [291] A.J. Silva, C.F. Stevens, S. Tonegawa, Y. Wang, *Science* 257 (1992) 201.
- [292] J.C. Hirsch, F. Crepel, *Synapse* 10 (1992) 173.
- [293] D.S. Bredt, C.D. Ferris, S.H. Snyder, *J. Biol. Chem.* 267 (1992) 10976.
- [294] D.S. Bredt, P.M. Hwang, S.H. Snyder, *Nature* 347 (1990) 768.
- [295] D.S. Bredt, P.M. Hwang, C.E. Glatt, C. Lowenstein, R.R. Reed, S.H. Snyder, *Nature* 351 (1991) 714.
- [296] N. Toni, P.A. Buchs, I. Nikonenko, C.R. Bron, D. Muller, *Nature* 402 (1999) 421.
- [297] B.F.G. Johnson, J.A. McCleverty, *Prog. Inorg. Chem.* 7 (1966) 277.
- [298] P.T. Manoharan, H.B. Gray, *J. Am. Chem. Soc.* 87 (1965) 3340.
- [299] R. Eisenberg, C.D. Meyer, *Acc. Chem. Res.* 8 (1975) 26.
- [300] F. Bottomley, F. Grein, *J. Chem. Soc., Dalton Trans.* (1980) 1359.
- [301] S.C. Da Silva, D.W. Franco, *Spectrochim. Acta Part A* 55A (1999) 1515.
- [302] P.C. Ford, R.D. Foust, Jr., R.E. Clarke, *Inorg. Chem.* 9 (1970) 1933.
- [303] H. Taube, *Comments Inorg. Chem.* 1 (1981).
- [304] J.M. Slocik, M.S. Ward, R.E. Shepherd, *Inorg. Chim. Acta* 317 (2001) 290.
- [305] D.W. Franco, H. Taube, *Inorg. Chem.* 17 (1978) 571.
- [306] D.W. Franco, *Inorg. Chim. Acta* 48 (1981) 1.
- [307] J.C. Nascimento-Filho, B.S. Lima-Neto, J.B. Lima, D.W. Franco, *J. Mol. Catalysis* 90 (1994) 257.
- [308] L.G.F. Lopes, E.E. Castellano, J. Zukerman-Schpector, A.G. Ferreira, C.U. Davanzo, M.J. Clarke, A. Wieraszko, D.W. Franco, unpublished results (2000).
- [309] A. Wieraszko, M.J. Clarke, D.R. Lang, L.G.F. Lopes, D.W. Franco, *Life Sci.* 68 (2001) 1535.
- [310] M.H. Krieger, A. Souza-Torsoni, F.G. Marcondes, D.W. Franco, J.C. Toledo, E. Tfouni, A.A. Ferro, M. Haun, M. Sumitami, *Hypertension* 33 (1999) 159.
- [311] K.M. Miranda, X. Bu, I. Lorkovic, P.C. Ford, *Inorg. Chem.* 36 (1997) 4838.
- [312] I.M. Lorkovic, K.M. Miranda, P.C. Ford, *J. Am. Chem. Soc.* 120 (1998) 11674.
- [313] S.P. Fricker, *Met. Ions Biol. Syst.* 36 (1999) 665.
- [314] S.P. Fricker, E. Slade, N.A. Powell, O.J. Vaughan, G.R. Henderson, B.A. Murrer, I.L. Megson, S.K. Bisland, F.W. Flitney, *Br. J. Pharmacol.* 122 (1997) 1441.
- [315] Y. Chen, F.-T. Lin, R.E. Shepherd, *Inorg. Chem.* 38 (1999) 973.
- [316] T. Matsubara, C. Creutz, *J. Am. Chem. Soc.* 100 (1978) 6255.
- [317] T. Matsubara, C. Creutz, *Inorg. Chem.* 18 (1979) 1956.
- [318] H.C. Bajaj, R. Van Eldik, *Inorg. Chem.* 29 (1990) 2855.
- [319] N.A. Davies, M.T. Wilson, E. Slade, S.P. Fricker, B.A. Murrer, N.A. Powell, G.R. Henderson, *Chem. Commun.* (1997) 47.
- [320] A.G. Baggs, S. Fricker, M. Abrams, C. Lee, M.P. Fink, *Surg. Forum* 48 (1997) 84.
- [321] D.R. Lang, J.A. Davis, L.G.F. Lopes, A.A. Ferro, L.C.G. Vasconcellos, D.W. Franco, E. Tfouni, A. Wieraszko, M.J. Clarke, *Inorg. Chem.* 39 (2000) 2294.
- [322] F.G. Marcondes, A.A. Ferro, A. Souza-Torsoni, M. Sumitami, M.J. Clarke, D.W. Franco, E. Tfouni, M.H. Krieger, *Life Sciences* (2002) accepted for publication.
- [323] P.J. Belshaw, S.L. Schreiber, *J. Am. Chem. Soc.* 119 (1997) 1805.
- [324] M. Clarke, V.M. Bailey, P. Doan, C. Hiller, K.J. LaChance-Galang, H. Daghljan, S. Mandal, C. Bastos, D. Lang, *Inorg. Chem.* 35 (1996) 4896.
- [325] T.D. Ocain, C.M. Bastos, K.A. Gordon, R.D. Granstein, J.C. Jenson, B. Jones, D.J. McAuliffe, J.R. Newcomb, *Transplant Proc.* 28 (1996) 3032.
- [326] C.M. Bastos, K.A. Gordon, T.D. Ocain, *Bioorg. Med. Chem. Lett.* 8 (1998) 147.
- [327] J.D. Franolic, C.M. Bastos, T.D. Ocain, K.A. Gordon, in: *Abstracts of Papers. 212th National Meeting of the American Chemical Society, Orlando, FL, Fall, American Chemical Society, Washington, D.C., 1996*, pp. INOR 327.
- [328] D. Cummins, H.B. Gray, *J. Am. Chem. Soc.* 99 (1977) 5158.
- [329] R.E. Shepherd, H. Taube, *Inorg. Chem.* 12 (1973) 1392.
- [330] S.C. Srivastava, L.F. Mausner, M.J. Clarke, *Prog. Clin. Biochem. Med.* 10 (1989) 111.
- [331] S.C. Srivastava, P. Richards, G.E. Meinken, P. Som, H.L. Atkins, S.M. Larson, A. Grunbaum, J.S. Rasey, M.G. Dowling, M.J. Clarke, in: J.A. Sorensen (Ed.), *Radiopharm. 2, Proc. Int. Symp., Second ed., Society of Nuclear Medicine, New York, 1979*, pp. 265–274.
- [332] T. Marik, B. Bibr, M. Kselikova, J. Petz, M. Becicova, *Radio-biol. Radiother.* 27 (1986) 359.
- [333] S.C. Srivastava, P. Richards, G.E. Meinken, S.M. Larson, Z. Grunbaum, *Radiopharm. Struct.-Act. Relat., [Proc. Symp.]* (1981) 207.
- [334] E.R. Schachner, M.C. Gil, H.L. Atkins, P. Som, S.C. Srivastava, J. Badia, D.F. Sacker, R.G. Fairchild, P. Richards, *J. Nucl. Med.* 22 (1981) 352.
- [335] E.R. Schachner, M.C. Gil, P. Som, Z.H. Oster, H.L. Atkins, G. Subramanian, J. Badia, S.C. Srivastava, P. Richards, S. Treves, *Nucl. Med. Commun.* 4 (1983) 94.
- [336] I. Zanzi, S.C. Srivastava, G.E. Meinken, W. Robeson, L.F. Mausner, R.G. Fairchild, D. Margouleff, *Nucl. Med. Biol.* 16 (1989) 397.
- [337] A.J. Taylor, M. Wenzel, *Biochem. J.* 172 (1978) 77.
- [338] R. Herken, M. Wenzel, *Eur. J. Nucl. Med.* 10 (1985) 56.
- [339] M. Wenzel, I.H. Park, *Int. J. Appl. Radiat. Isot.* 37 (1986) 491.
- [340] M. Wenzel, Y. Wu, *Appl. Radiat. Isot.* 39 (1988) 1237.
- [341] C.D. Meyer, M.A. Davis, C.J. Periana, *J. Med. Chem.* 26 (1983) 737.
- [342] R.V. Iozzo, *J. Biol. Chem.* 262 (1987) 1888.
- [343] R. Margalit, H.B. Gray, L. Podbielski, M.J. Clarke, *Chem-Biol. Interact.* 59 (1986) 231.
- [344] A.M. Pyle, J.P. Rehmann, R. Meshoyrer, *J. Am. Chem. Soc.* 111 (1989) 3051.
- [345] W. Kalsbeck, H.H. Thorp, *J. Am. Chem. Soc.* 115 (1993) 7146.
- [346] A.D. Baker, R.J. Morgan, T.C. Strekas, *J. Am. Chem. Soc.* 113 (1991) 1411.
- [347] S.A. Tysoe, R.J. Morgan, A.D. Baker, T.C. Strekas, *J. Chem. Phys.* 97 (1993) 1707.
- [348] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires, *Biochemistry* 31 (1992) 9319.
- [349] R.B. Nair, E.S. Teng, C.J. Murphy, *Inorg. Chem.* 37 (1998) 139.
- [350] D.L. Carlson, D.H. Huchital, E.J. Mantilla, R.D. Sheardy, W.R. Murphy, *J. Am. Chem. Soc.* 115 (1993) 6424.
- [351] C. Hiort, P. Lincoln, B. Norden, *J. Am. Chem. Soc.* 115 (1993) 3448.
- [352] R.B. Nair, C.J. Murphy, *J. Inorg. Biochem.* 69 (1998) 129.
- [353] M. Rubenstein, S. Rubenstein, *Chem3D Pro*, Cambridge Scientific Computing, Inc, Cambridge, MA, 1997.
- [354] N.L. Allinger, *J. Am. Chem. Soc.* 99 (1977) 8127.
- [355] J.E. Earley, P.P. Smith, T. Fealey, J.V. Silverton, *Inorg. Chem.* 10 (1971) 1943.
- [356] K.J. LaChance-Galang, P.E. Doan, M.J. Clarke, U. Rao, A. Yamano, B. Hoffman, *J. Am. Chem. Soc.* 117 (1995) 3529.