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Ruthenium Polyaminocarboxylate Complexes

PROSPECTS FOR THEIR USE AS METALLOPHARMACEUTICALS

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Ruthenium (Ru) complexes containing polyaminocarboxylate (pac) ligands (Ru-pac) have features which indicate they may be suitable for biological applications. For instance, Ru-pac complexes can bind to biomolecules through a rapid and facile aquo-substitution reaction, and Ru-pac has a range of accessible oxidation states. Ru-pac also has some notable catalytic properties that mimic enzymatic hydrocarbon oxidation by cytochrome P-450 in homogeneous conditions. This is of immense significance towards developing Ru-pac based agents for oxidative cleavage of DNA and artificial nuclease in DNA foot-printing experiments. This review aims to highlight the scope of Ru-pac complexes as metallopharmaceuticals, and outlines their potential for certain biological applications.

The chemistry of ruthenium complexes containing polyaminocarboxylate (pac) ligands (Ru-pac) is of continuing interest. The donor character of the pac ligand is comparable with that of many biological enzymes which make use of the carboxylate and amine donors from amino acids to bind to a metal centre. The pac ligand can form very stable 1:1 (metal:ligand) complexes with ruthenium.

Early studies (1-3), later confirmed by crystallographic evidence (4-8), showed that the pac ligands in Ru-pac complexes function as pentadentate ligands, see Figure 1. The sixth coordination site of the ruthenium centre in Ru-pac complexes is occupied by a water molecule at low pH or by an hydroxide ion at high pH. Figure 1 shows structures and formulae of some [Ru(pac)(H_2O)] complexes, and Table I contains data on spectral, electrochemical and acid-dissociation constants for these [Ru^{III}(pac)(H_2O)] complexes.

The chemistry of [Ru^{III}(pac)(H_2O)] complexes is dominated by their lability towards the aquo-substitution reaction, which affords a facile and straightforward synthesis to mixed-ligand complexes (8). The reasons that ruthenium complexes containing 'pac' ligands demonstrate potential applications in metallodrugs are because of:

- the number of stable and accessible oxidation states they possess,
- their rapid rate of ligand exchange, and
- their ability to bind to certain biological molecules.

Furthermore, these Ru-pac complexes exhibit catalytic properties, in homogeneous conditions in the presence of oxygen atom donors, that mimic the biological enzymatic oxidation of hydrocarbons by cytochrome P-450 (8). Although the significance of Ru-pac complexes as chemotherapeutic agents has been reviewed (9), this article aims to examine the prospects of Ru-pac complexes for promoting studies towards the development of Ru-pac based pharmaceuticals, for a range of diseases. A glossary of terms used in the review is appended at the end of the paper.

The Ru-pac Complex as a Model for Enzymatic Oxidation

Dioxygen complexes of transition metals play an important role in a number of biological reactions. The formation of a Ru(IV)-peroxo complex species in the reaction of Ru(III)-edta and dioxygen was first reported by Ezerskaya and Solovykh (10,

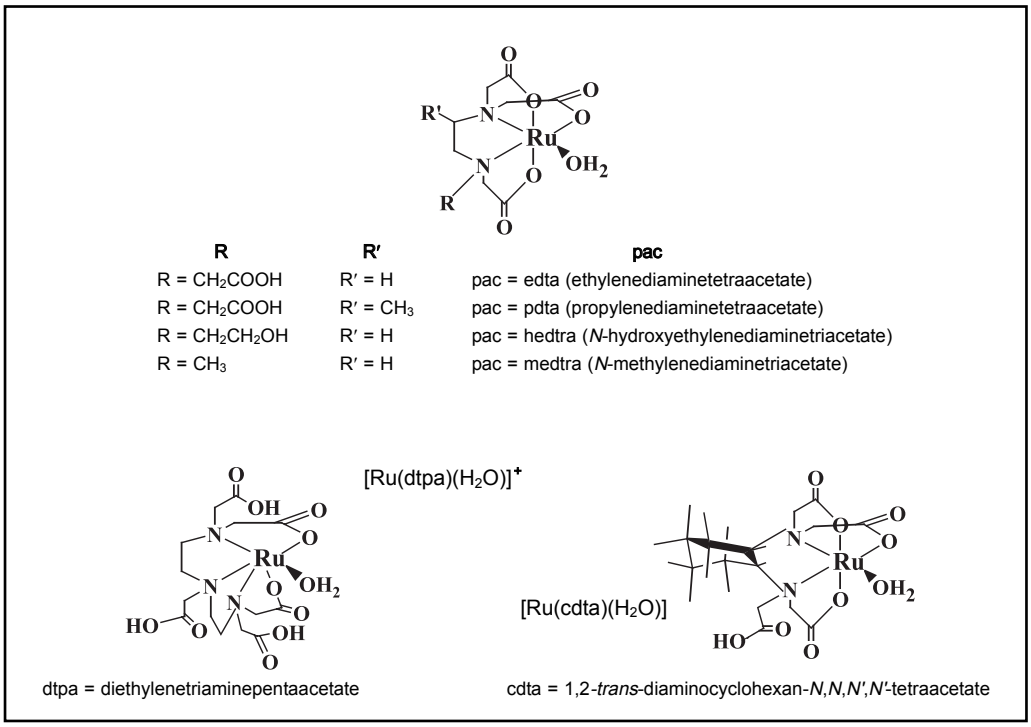

Fig. 1 Schematic representation of ruthenium complexes [Ru^{III}(pac)(H_2O)] bearing a pac ligand. The complexes are depicted as in a low pH solvent, when the sixth coordination site is occupied by OH_2. The coordinated H_2O molecule is labile towards substitution reactions around this site

Table I

Spectral, Electrochemical and Acid-Dissociation Constants for Common [Ru^{III}(pac)(H_2O)] Complexes

Complex	Spectral data, λ_{max}, nm (ε_{max}, M^{-1} cm^{-1}) in water	Electrochemical data, $E_{1/2}$ (V vs. NHE)	Acid dissociation constant, pK_1, at 25°C	Acid dissociation constant, pK_2, at 25°C	Ref.
[Ru^{III}(Hedta)(H_2O)]	280 (2800 ± 50) 350 (680 ± 30)	−0.04	2.4	7.6	8
[Ru^{III}(Hpdta)(H_2O)]	282 (2890 ± 50) 370 (940 ± 50)	−0.05	2.3	8.1	8
[Ru^{III}(hedtra)(H_2O)]	285 (1950 ± 20) 350 (850 ± 20)	−0.07	-	4.9	8
[Ru^{III}(medtra)(H_2O)]	290 (2400 ± 30)	−0.10	-	6.3	8

Hedta and Hpdta represent the protonated pendant COOH group in [Ru^{III}(Hedta)(H_2O)] and [Ru^{III}(Hpdta)(H_2O)], respectively. M is mol dm^{-3}

11) in the 1960s, while spectral, electrochemical, and kinetic evidence in favour of the formation of the $[\{Ru^{IV}(edta)\}_2(O_2)]^{2-}$ peroxo species during catalytic hydrocarbon oxidation was reported by Taqui Khan and coworkers (12, 13). Ru-pac complexes (pac = edta, hedtra) in the presence of the single

oxygen atom donors: NaOCl, PhIO, *t*-BuOOH and $KHSO_5$, were found to be active in catalysing the epoxidation of olefins and the hydroxylation of the C-H bond (14, 15); both of these reactions resemble enzymatic oxidation by cytochrome P-450 monooxygenase (an enzyme catalysing oxo-transfer reactions). Further, a system comprising $[Ru^{III}(edta)(H_2O)]^-$ /ascorbic acid/H_2O_2 (or O_2) that was able to perform the hydroxylation of cyclohexane to cyclohexanol, as an analogue of the Udenfriend system (16), was also reported (17). Ferryl intermediates (Fe^V=O) are the active DNA cleavage agents in O_2-activated DNA cleavage by bleomycin. Bleomycin epoxidises stilbenes *via* its ferryl form. Similarly, $[Ru^V(pac)O]$ was reported to be the active species in the olefin epoxidation and hydroxylation of saturated hydrocarbons (14, 15a). Reports that the oxo-functionalisation of the C=C/C-H bond in hydrocarbon oxidation is catalysed by Ru-pac complexes seem to be highly significant for developing Ru-pac based agents for oxidative cleavage of DNA and artificial nuclease in DNA foot-printing experiments (15b).

Prospects for Ru-pac Complexes as Antitumour Agents

Although in cell culture studies a correlation has been observed between the cytotoxicity of some ruthenium complexes and their DNA binding ability (18), the mechanism of the drug action of these ruthenium complexes is still largely unknown. Octahedral Ru(III) and Ru(II) complexes containing ligands, such as ammines, N-heterocycles and dimethylsulfoxides, exhibited various degrees of biological activity, including antitumour action *in vivo* (19). Considering that the above Ru(III) complexes are more inert than the corresponding Ru(II) analogues, an 'activation by reduction' mechanism was proposed to explain the antitumour activity of such complexes (19).

Ru-pac complexes, due to their lability towards aquo-substitution, bind DNA constituents in a facile and straightforward manner (20-22) and thus have oncological significance. Antitumour activity has been reported for labile Ru(IV)-cdta (cdta = 1,2-*trans*-diaminocyclohexan-*N*,*N*,*N*′,*N*′-tetraacetate) (23, 24), while *cis*-$[Ru^{III}(pdta)Cl_2]$ (pdta = propylenediaminetetraacetate) is known to damage nuclear DNA and inhibit DNA recognition by enzyme restriction (25). A crosslinking with a guanine base unit of DNA has been proposed as an explanation for the observed activity. However, the generation of a superoxide radical in NADPH oxidase, triggered by the presence of the Ru-pdta complex, may be another reason for the observed cytotoxity (9).

Mixed-ligand complexes of Ru(II)-pac with a series of DNA bases have been reported by Shepherd's group (15c) and the binding sites of the DNA constituents have been discussed with regard to their significance in chemotherapy. They reported a novel η^2-coordination mode for Ru-pac (pac = hedtra, ttha; ttha = triethylenetetraaminehexaacetate) at the C5=C6 olefinic double bonds of uridine- and cytidine-related bases, along with coordination at the normal binding sites (N3 and N1). Although the 'pac' environment favours π-donation by the ruthenium centre, no experimental evidence for η^2-attachment was observed in the case of thymidine. This assumes the pyrimidine structure is important for η^2-coordination. The affinity of Ru(II)-pac complexes to the η^2-pyrimidine site was shown to be linked to a balance between electronic and steric factors, and thus Ru-pac could be significant as a DNA crosslinking agent (15c).

Our Ru-pac Work

In our laboratory, kinetic and mechanistic aspects of the interaction of $[Ru(pac)(H_2O)]$ complexes with DNA have been explored in attempts to find mechanisms of possible drug activity. Our previous studies on the kinetics and mechanism of binding of $[Ru^{III}(edta)(H_2O)]^-$ with DNA bases, nucleosides and nucleotides, led us to conclude that $[Ru^{III}(edta)(H_2O)]^-$ binds an adenine base unit of single strand calf-thymus DNA in a kinetically preferred pathway (20-22).

Other kinetic studies (26) have suggested that there is rapid coordination through the N7 of the adenine moiety of adenosine monophosphate (AMP) followed by a ring closure step in which the exocyclic NH_2 group (at C6 in the adenine base) of AMP binds to the ruthenium centre by dislodging

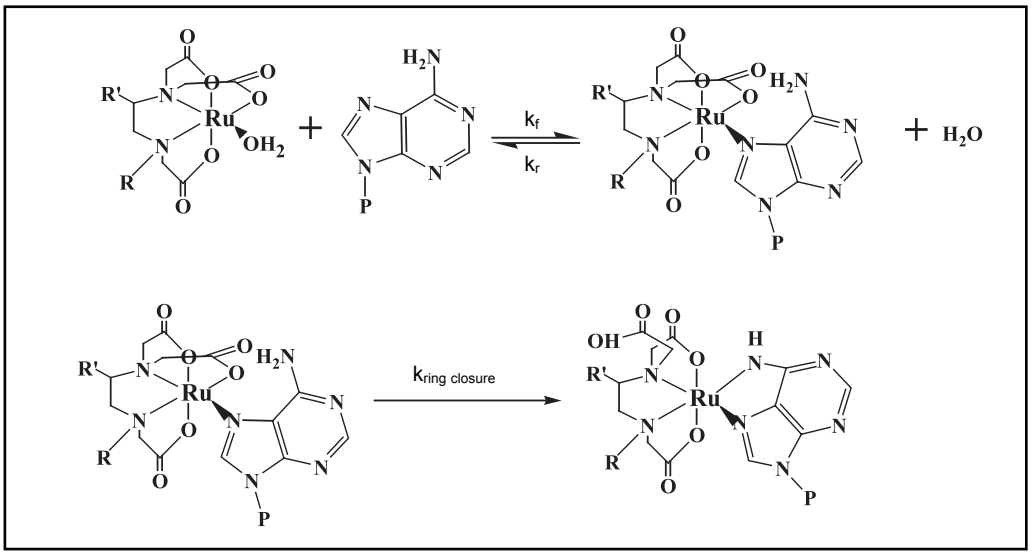

Fig. 2 Mechanism showing how $[Ru^{III}(edta)(H_2O)]^-$ binds an adenine base unit. The N7 of the adenine moiety of AMP binds to the Ru-edta complex at the position where a COO^- unit, coordinated to Ru, has been dislodged. k_f is the forward rate constant of the reaction; k_r is the backward rate constant of the reaction P is phosphosugar

the adjacent carboxylate group of the coordinated pac ligand, see Figure 2.

The order of reactivity of $[Ru^{III}(pac)(H_2O)]$ complexes towards binding nucleotides is:

$$[Ru^{III}(pdta)(H_2O)]^{3-} \geq [Ru^{III}(edta)(H_2O)]^{-} >> [Ru^{III}(hedtra)(H_2O)]^{-} > [Ru^{III}(medtra)(H_2O)]^{-}$$

All the Ru-pac complexes exhibit a similar order of reactivity towards nucleotides: AMP >> inosine monophosphate (IMP) > guanosine monophosphate (GMP).

The results of cell proliferation studies with Ru-pac complexes (26) using cell lines for MCF-7 (breast cancer), NCI-H460 (lung cancer) and SF-268 (central nervous system) revealed that $[Ru(edta)(H_2O)]^-$ and $[Ru(pdta)(H_2O)]^-$, are much more efficient inhibitors of these cell lines than complexes where pac = $hedtra^{3-}$ and $medtra^{3-}$. $[Ru(hedtra)(H_2O)]$ and $[Ru(medtra)(H_2O)]$ have insignificant activity which, presumably, is associated with a much lower rate of binding to purine-based nucleotides than in the case of the '$edta^{4-}$' and '$pdta^{4-}$' complexes.

The order of growth inhibition for these three cancer cell lines, due to $[Ru(edta)(H_2O)]^-$ is: SF-268 > NCI-H460 > MCF-7, and the estimated GI_{50} values (in mM) of $[Ru(edta)(H_2O)]^-$ are: 0.57 for SF-268, 0.65 for NCI-H460 and 0.78 for MCF-7, respectively.

Furthermore, in cancer cells, binding of an active agent with sulfur-containing biomolecules available in the cells is considered to be one reason for 'drug resistance' and 'toxicity' of cisplatin-like drugs in the postulated mechanism for activity (27). Binding with thio-macromolecules in the cell decreases the intracellular accumulation of metallodrugs, so they cannot reach sufficient numbers to bind with the DNA in the cell to cause cell death. Thus it appears that understanding the kinetic interactions of these metal complexes with DNA fragments, with regard to sulfur-containing biomolecules, is important for understanding their antitumour activity as well as their toxicity.

We have recently reported that the binding rate of $[Ru^{III}(pac)(H_2O)]$ with such thio-ligands is much lower than the binding rate with AMP (28). This indicates that Ru-pac complexes could have a lower toxic effect, due to their lower reactivity with sulfur-containing biomolecules, and thus be of pharmacological significance. Such possible metallodrugs could perhaps be tolerated at higher dosage with fewer side effects.

Ru-pac Complexes as NO Scavengers

The enzyme, nitric oxide synthase (NOS) catalyses the conversion of L-arginine to L-citrulline, during which reaction NO is produced (29). There are several isoforms of NOS and these are divided into the Ca^{2+}-dependent constitutive NOS (cNOS) and the Ca^{2+}-independent inducible NOS (iNOS) groups.

Effects of a Decrease in NO

NO seems to play a role in many disease states (30a); for instance, a decrease in NO production (from cNOS) can lead to severe hypertension. This is a disease state that is treated by vasodilators (NO donors), such as nitroprusside (30b). The route of NO production from nitroprusside is:

$$[Fe^{II}(CN)_5(NO^+)]^{2-} \rightarrow (\text{RSH; ascorbate}) \rightarrow [Fe^{II}(CN)_5(NO)]^{3-} \quad \text{(i)}$$

$$[Fe^{II}(CN)_5(NO)]^{3-} \rightarrow CN^- + [Fe(CN)_4(NO)]^{2-} \rightarrow NO + [Fe(CN)_4(H_2O)_2]^{2-} \quad \text{(ii)}$$

The NO produced acts as:

$$NO \rightarrow \text{guanylate cyclase sites} \rightarrow \text{GMP production} \rightarrow \text{blood pressure control} \quad \text{(iii)}$$

Effects of Excess NO

On the other hand, sepsis and toxic shock are caused in patients by the overproduction of NO – in response to a pathogenic invasion of the bloodstream (31-42). The result is a precipitous drop in blood pressure that could lead to multi-organ failure, including renal failure. The excess NO in the bloodstream is present due to action by macrophages (mononuclear phagocytic cells that reside in tissues). This situation with the pathogens may be treated by other antibiotics, while the excess NO can be absorbed by a metal complex acting as a scavenger. Ru-pac complexes meet the basic requirements for effective NO scavenging as they undergo a rapid substitution reaction and form stable nitrosyl complexes (43). In addition, the Ru-NO bond is reasonably stable, and persists through a variety of substitution and redox reactions. This allows the properties of the Ru-pac complex to be finely tuned so it can become an effective NO scavenger:

$$\text{macrophage CAM cell} \rightarrow (\text{pathogen/cytokine}) \rightarrow \text{NO production} \quad \text{(iv)}$$

$$[Ru^{III}(\text{edta})(H_2O)]^- + NO \rightarrow [Ru^{II}(NO^+)(\text{edta})]^- + H_2O \quad \text{(v)}$$

In vitro studies have shown that Ru-edta complexes are successful in scavenging NO in biological systems and suggest that they could play a role in novel therapeutic strategies aimed at alleviating NO-mediated disease states (44). For instance, the addition of Ru-edta complexes (100 μM) to gamma-activated RAW 264 cells (a murine macrophage cell line) was found to reduce NO levels. The effect of Ru-edta complexes on NO-mediated tumour cell killing by gamma-activated macrophages (RAW 264) was studied in a co-culture system. A non-adherent murine mastocytoma (P815) line was the 'target' cell. Ru-edta complexes (100 μM of JM1226 and JM6245) when added to the culture medium, gave some protection from macrophage-mediated cell killing. The 'target' cell viability increased from 54.5 ± 3.3% to 93.2 ± 7.1% and 80.0 ± 4.6%, respectively, (n = 6).

The vasodilator response of isolated, perfused, precontracted rat tail arteries caused by a one-off injection (10 μl) of S-nitroso-*N*-acetyl-penicillamine (SNAP) was attenuated by adding Ru-edta complexes (100 μM) to the perfusate. The ED_{50} increased from 6.0 μM (Krebs only) to 1.8 mM (Krebs + JM6245) and from 7 μM (Krebs only) to 132 μM (Krebs + JM1226). Male Wistar rats were injected with bacterial LPS (4 mg kg^{-1} intraperitoneal of lipopolysaccharide) to induce endotoxaemia. When the JM1226 Ru-edta complex (100 μM) was administered 20 hours afterwards, the LPS fully reversed the hypotension associated with the endotoxaemia.

A brief study of the kinetics of interaction of Ru-edta complexes with NO, aimed at understanding the mechanisms of drug action (45), showed that the rate of the aquo-substitution of $[Ru^{III}(\text{edta})(H_2O/OH)]^{-/2-}$ with NO is very fast ($1.95–3.29 \times 10^7$ M^{-1} s^{-1} at 7.3°C) in pH range 6.5-8.0. However, at pH 8.0, the higher value for the rate of aquo-substitution (3.29×10^7 M^{-1} s^{-1} at 7.3°C), than at pH 6.5 (2.18×10^7 M^{-1} s^{-1} at 7.5°C), does not agree with the pH dependence of the rate constants for the aquo-substitution reaction of the

Ru-edta complex with other entering nucleophiles (8). This apparently anomalous observation (45) could probably be explained by assuming the pK_2 value for the acid-dissociation:

$$[Ru^{III}(edta)(H_2O)]^- \leftrightarrow [Ru^{III}(edta)(OH)]^{2-} + H^+$$

pK_2 would, at 7.3°C, be higher than the value (7.6) reported at 25°C. As a result, the concentration of the generally labile $[Ru^{III}(edta)(H_2O)]^-$ species would be higher at low temperature (7.3°C) even at pH > 7.6. Therefore, the decrease in the rate constant at pH > 7.6 reportedly observed for the substitution of Ru-edta complex at 25°C was not seen at 7.3°C by Slade *et al.* (45).

The thermodynamics and kinetics of Ru-edta complexes as efficient scavengers of NO was recently reported by Eldik's group (46). The results of FTIR and ^{15}N-NMR spectroscopy studies clearly afforded evidence of the NO^+ character of NO coordinated to the Ru-edta complex. The value of the overall equilibrium constant (K_{NO}) determined from UV-Vis spectroscopic and electrochemical methods, is 9.1×10^7 M^{-1} at 25°C and pH = 5.0. The effect of buffer components (acetate buffer) became clear, while the value of the rate constant (1×10^5 M^{-1} s^{-1} at 8°C and pH 5.0) was two-orders of magnitude less than that reported by Slade *et al.* (45). An attempt to make direct measurements of the rate of NO binding, using laser flash photolysis was unsuccessful, though the formation of a disubstituted $[Ru^{II}(edta)(NO^+)(NO_2^-)]^{2-}$ was detected by ^{15}N-NMR spectroscopy. Laser flash photolysis of this complex was complicated by the number of chemical reaction steps.

In other recent reports, the preparation, characterisation, kinetics and biochemical activity of various species of Ru-pac complexes (pac = edta; dtpa) were reported (47, 48). The report reaffirmed the NO scavenging ability of Ru-pac complexes and reported similar rate constant data as Slade *et al.* for the Ru-edta complex (45). However, the binding of NO with Ru-dtpa was slower (3×10^5 M^{-1} s^{-1} at 20°C and pH = 7.4) (50 mM phosphate buffer) than that observed for the Ru-edta complex (45).

The reaction of $[Ru^{III}(edta)(H_2O)]^-$ with H_2O_2 in the presence of arginine produces NO, in the form of $[Ru^{II}(edta)(NO^+)]^-$, and citrulline (49). This affords a simple model of NOS. A working mechanism has been proposed for this reaction involving the hydroxylation of arginine by $[Ru^{V}(edta)(O)]^-$ species (formed by reaction of $[Ru^{III}(edta)(H_2O)]^-$ with H_2O_2) to resemble the first monooxygenase step of the NOS reaction (49). In a subsequent step, the oxidation of *N*-hydroxyarginine to citrulline and NO is proposed to take place *via* a 'peroxide shunt' mechanism (49).

Organ Rejection Studies

In 2002 it was reported that NO derived from the regulation of inducible NO synthase (iNOS) might play an important role in organ rejection (50). In experimental models of acute cardiac transplant rejection (without immunosuppression), treatment using NOS inhibitors to prevent acute rejection yielded conflicting results. This is suggested to be most likely due to potential inhibition of constitutive NOS (cNOS). Accordingly, agents that trap NO directly may have some advantage. The efficacy of the Ru-edta complex alone and in combination with low-dose cyclosporine (CsA, which is an immunosuppressive drug that delays graft rejection - a model of delayed graft rejection) for inhibiting the nitrosylation of myocardial protein, and for prolonging cardiac allograft survival in a model of acute cardiac transplant rejection (without immunosuppression) has been evaluated (50). Treatment with the Ru-edta complex either prolonged the graft survival and/or caused a marked decrease in myocardial nitrosylprotein formation, as determined by EPR spectroscopy. *In vivo* scavenging of NO by the ruthenium complex was verified by high-performance liquid chromatography analysis of the nitrosylated drug in plasma samples. Low-dose CsA given alone or in combination with the Ru-edta complex completely blocked the formation of myocardial nitrosylprotein complexes. While low-dose CsA alone prolonged graft survival, the combined therapy of CsA and the Ru-edta complex produced a synergistic effect on graft survival. The studies explored the possibility of using the

Ru-edta complex alone and in combination with CsA to protect myocardial proteins from post-transcriptional modification and to prolong cardiac graft survival.

NO in Angiogenesis and Tumour Progression

NO also plays a role in angiogenesis and tumour progression (51-62). These studies suggest that increased levels of NO correlate with tumour growth and spread in human cancers. Drugs that interfere in the nitric oxide synthase (NOS) pathway could thus be useful against angiogenesis-dependent tumours, and the Ru^{III}-edta complex was found to be effective in this regard (62). The key steps of angiogenesis, endothelial cell proliferation and migration stimulated by vascular endothelial growth factor (VEGF) or NO donor drugs, were reportedly blocked by the Ru^{III}-edta complex (62).

Ru-pac Complexes as Protease Inhibitors

Cysteine proteases (thiol protease in older literature) have recently been discovered in viruses of poliomyelitis and hepatitis A (63, 64), and their pathological role (65-69) in brain trauma, muscular dystrophy, arthritis, cardiac ischaemia and Alzheimer's disease is reasoned to occur *via* degradation of concerned proteins by the enzyme. Cysteine proteases have a control aspect on HIV-1, myocardial repair, periodontal disease and cytomeglavirus (herpes).

Recently, evidence from molecular, immunological and pharmacological studies has indicated that cysteine cathepsins (peptidases belonging to the papain family) play a role in the malignant progression of human tumours (70). It has been suggested that cysteine cathepsins, most likely with serine proteases, degrade the extracellular matrix, thereby facilitating tumour growth into surrounding tissues and vasculature (70). Clinically, the levels, activities and localisation of cysteine cathepsins and their endogenous inhibitors have been shown to have diagnostic and prognostic value (70).

In order to achieve selective inhibition of cysteine proteases, cysteine protease inhibitor (CPI) should have an active site which could, selectively, be highly reactive with the cysteine residue of the enzyme to produce an inert covalent enzyme-inhibitor complex. In this context, the use of metal complexes is conspicuously absent from the literature. However, we have recently discovered that Ru-pac complexes possess cysteine protease inhibition activity (26, 71).

The protease inhibition activity of Ru-pac complexes was studied using three cysteine protease enzymes: bromalien, papain and ficin with azoalbumin as the substrate. In order to understand cysteine protease inhibition by Ru-pac complexes, the interaction of Ru-pac complexes with cysteine (a thio-amino acid and cysteine protease contain this unit) and other thio-amino acids was studied, leading to the formation of S-coordinated species. The ability of Ru-pac complexes to inhibit cysteine protease activity was attributed to the high affinity of the ruthenium complexes towards binding the –SH group in the cysteine residue of the enzymes *via* a rapid aquo-substitution reaction. The protease activity of the enzyme was thus inhibited by the formation of a stable Ru(edta)-enzyme complex, see Figure 3.

These studies demonstrate that the $[Ru^{III}(edta)(H_2O)]^-$ complex effectively inhibits the protease activity of the three enzymes, whereas, the $[Ru^{III}(hedtra)(H_2O)]$ complex, although able to reduce the hydrolysis of azoalbumin by bromalein at a certain level, failed to do so with papain. The lower efficacy of the Ru-hedtra complex, than the Ru-edta complex, towards inhibiting protease activity of bromalein may be linked with the lower affinity of Ru-hedtra towards binding the –SH group in cysteine.

However, the absence of inhibition activity by the Ru-hedtra complex for papain, and the significantly lower efficacy for ficin suggest that the protease inhibition activity of the Ru^{III}-pac complexes is enzyme specific.

Very recently it has been reported (72) that the S-atom of cysteine reacts to bind the N-atom of the nitrosyl complex of Ru-edta to form a 1:1 intermediate species, which subsequently converts into another intermediate by reacting with another molecule of cysteine and ultimately produces

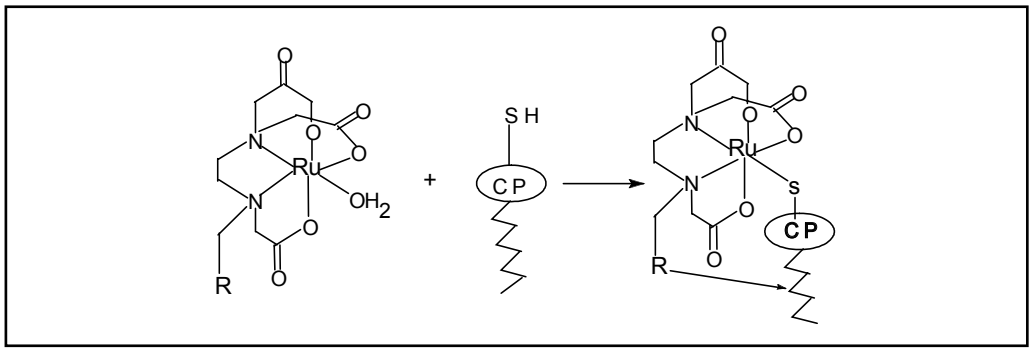

Fig. 3 The protease activity of the enzyme, cysteine protease (CP) bearing an SH group, is inhibited by Ru-pac to form a stable Ru(edta)-enzyme complex. R is a peptidal unit that can recognise enzymes selectively. If R were known, then a reactive drug could be developed

$[Ru^{III}(edta)(H_2O)]^-$. Cysteine is returned to the reacting system together with the release of N_2O. This observation is of significance, implying the catalytic reduction of NO to N_2O in a biological system.

Concluding Remarks

In this article, we have primarily reviewed the kinetics and mechanism of the interaction of Ru-pac complexes with biomolecules. The ability of Ru-pac complexes to perform hydrocarbon oxidation in a manner that resembles the enzymatic system, cytochrome P-450, appears to be useful for developing Ru-pac based agents for oxidative cleavage of DNA and artificial nuclease in DNA footprinting experiments. The ability of Ru-pac complexes to bind to DNA constituents at a faster rate than to sulfur-containing ligands, points towards exploring the possibility of a new family of ruthenium-based anticancer drugs of low toxicity. The [Ru(pac)(NO)] complexes offer a number of features as NO carriers or scavengers.

The discovery of the protease inhibition activity of Ru-pac complexes may be of significance in developing antiviral agents in which Ru-pac complexes could act as metallo-inhibitor agents for disease progression.

Although the above features make Ru-pac complexes promising for clinical application, a better mechanistic understanding of the different modes of drug action of Ru-pac complexes would probably yield more effective chemotherapeutic agents.

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Glossary

arginine is a nonessential amino acid in adults and supplies the amidine group for the synthesis of creatine. Arginine is also formed by the transfer of an N atom from aspartate to citrulline in the urea cycle. Arginine is important for NO production by the enzyme nitric oxide synthase. NO is important for maintaining cardiovascular health. However, most arginine is utilised in the liver and kidneys, and only a fraction is available for this purpose.

citrulline, **L-citrulline** is a nonessential amino acid that supports the body in optimising blood flow through its conversion to L-arginine and then, *via* nitric oxide synthase, to nitric oxide. Citrulline is synthesised in the intestinal tract from glutamine, and converts to arginine in the endothelial cells. Citrulline allows for increased and sustained NO production in the endothelium to support circulatory function.

cytidine is a purine nucleoside: cytosine linked by its N9 nitrogen to the C1 carbon of ribose. It is a component of ribonucleic acid (RNA) and its nucleotides are important in the synthesis of a variety of lipid derivatives.

effective dose 50, ED_{50}, is the amount of drug required to produce 50 percent of the maximum response in a pharmacological test. It is usually calculated from a plot of log(Dose) *vs.* response.

growth inhibitory concentration 50, GI_{50}, is the concentration required to inhibit growth of tumour cells in an *in vitro* test by 50 percent relative to a control.

JM1226, is now known as **AMD1226**, K[Ru(Hedta)Cl], potassium chloro[hydrogen(ethylenedinitrilo)tetraacetato]ruthenate.
A nitric oxide scavenger first developed at Johnson Matthey and now being further developed by AnorMED.

JM6245, is now known as **AMD6245**, [Ru(Hedta)(H_2O)], aqua[hydrogen(ethylenedinitrilo)tetraacetato]ruthenium.
A nitric oxide scavenger first developed at Johnson Matthey and now being further developed by AnorMED.

lipopolysaccharide, LPS also known as endotoxin, is composed of lipid and polysaccharide moieties. LPS is a component of the cell wall of gram-negative bacteria that is released from dying bacteria and stimulates many of the innate immune responses, including synthesis of nitric oxide. Lipopolysaccharide from *Escherichia coli* is a commonly used immune cell activator in laboratory immunology.

macrophage is a mononuclear phagocyte found in tissues, and plays an important role in the innate and adaptive immune response. Macrophages are produced from stem cells in bone marrow which develop into monocytes, enter the blood, and later into tissue where they develop into macrophages. Macrophages kill ingested microorganisms. They can be activated by endotoxin and cytokines such as γ-interferon.

NADPH is nicotinamide adenine dinucleotide phosphate (reduced form).

nucleoside is a heterocyclic nitrogenous base, a purine or pyrimidine, in a N-glycosidic linkage with a pentose sugar. It is often used to denote a compound obtained by hydrolysis of nucleic acids, a purine or pyrimidine linked to ribose (in RNA) or deoxyribose (in DNA).

nucleotide is a phosphate ester of a nucleoside, particularly the 5´-phosphate of a pyrimidine or purine in N-glycosidic linkage with ribose or deoxyribose, as occurs in the nucleic acids, RNA and DNA, respectively.

pyrimidine is a metadiazine, $C_4H_4N_2$, which is the fundamental form of the pyrimidine bases. There are mostly oxy or amino derivatives, for example, 2,4-dioxypyrimidine is uracil, 2-oxy-4-aminopyrimidine is cytosine, and 2,4-dioxy-5-methylpyrimidine is thymine. Uracil, cytosine and thymine are constituents of nucleic acid.

RAW 264 cells are a murine macrophage cell line. RAW 264 can be activated with LPS to produce cytokines and nitric oxide.

SNAP is S-nitroso-N-acetyl-D,L-penicillamine, an organic nitric oxide donor molecule.

thymidine is a pyrimidine nucleoside, thymine linked by its N1 nitrogen to the C1 carbon of deoxyribose. It is one of the four nucleotides that make up DNA.

uridine is a pyrimidine nucleoside, uracil linked by its N1 nitrogen to the C1 carbon of ribose. It is a component of ribonucleic acid (RNA), and its nucleotides participate in the biosynthesis of polysaccharides and some polysaccharide-containing compounds.