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Review

Using coordination chemistry to design new medicines☆

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Contents

Abstract

The rich diversity of coordination chemistry provides exciting prospects for the design of novel therapeutic agents with unique mechanisms of action. Central to such discovery is the understanding of both the kinetics and thermodynamics of reactions of metal complexes under conditions of biological relevance, and consideration of the roles of both the metal and its ligands in recognition processes. Examples from our recent work are reported here and discussed.

Xylylbicyclam is a potent anti-HIV agent and is in clinical use as a stem-cell-mobilizing drug (AMD3100, "Mozobil"). Its target is the 7-helix membrane receptor CXCR4. Specific metallomacrocycle configurations can be recognized by proteins *via* metal coordination to specific amino acid side-chains, H-bonding and hydrophobic interactions, allowing optimisation of drug design.

Photoactivation of octahedral *cis* and *trans* diam(m)ino diazido Pt(IV) complexes can lead to unusual redox and substitution reactions. Such activated complexes can kill cancer cells by novel mechanisms of action, providing a basis for a novel form of photochemotherapy.

Substitution and redox reactions and the anticancer activity of Ru(II) arene complexes of the type [(η^6-arene)Ru(X)(YZ)] are highly dependent on the nature of the arene, and monodentate (X) and chelated (YZ) ligands. Understanding of the factors which control such reactions has led to the rational design of analogous osmium anticancer complexes.

Keywords: Cyclams; Proteins; Platinum; Photochemistry; Ruthenium; Osmium; Arene

Abbreviations: acac, acetylacetonate; 5′-AMP, 5′-adenosinemonophosphate; bip, biphenyl; cGMP, 3′,5′-cyclic-guanosinemonophosphate; 5′-CMP, 5′-cytidinemonophosphate; Cp, cyclopentadienyl; cyclam, 1,4,8,11-tetraazacyclotetradecane; cym, *p*-cymene; d(GpG), 2′-deoxyguanylyl-(3′ → 5′)-2′-deoxyguanosine; DHA, dihydroanthracene; dppz, dipyrido[3,2-*a*:2′,3′-*c*]phenazine; DTPA, diethylenetriaminepentaacetate; en, ethylenediamine; 5′-GMP, 5′-guanosinemonophosphate; GPCR, G-protein-coupled receptor; GSH, glutathione; HSQC, heteronuclear single quantum coherence; Im, imidazole; In, indazole; L-MetH, L-methionine; MRI, magnetic resonance imaging; PBS, phosphate buffered saline (0.01 M phosphate pH 7.4, 0.138 M NaCl, 0.0027 M KCl); py, pyridine; THA, tetrahydroanthracene; 5′-TMP, 5′-thymidinemonophosphate

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1. Medicinal inorganic chemistry

Medicinal inorganic chemistry is a discipline of growing significance in both therapeutic and diagnostic medicine. Inorganic compounds have been used in medicine for many centuries, but often only in an empirical way with little attempt to design the compounds to be used, and with little or no understanding of the molecular basis of their mechanism of action. In the late 1960s, the discovery and development of the antitumour compound cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) played a profound role in establishing the field of medicinal inorganic chemistry [1]. Cisplatin, and the second generation alternatives carboplatin and oxaliplatin, are still the most widely used chemotherapeutic agents for cancer, greatly improving the survival rates of patients worldwide. The success of cisplatin has aroused great interest in the development of new metal complexes to diagnose and/or treat diseases including diabetes, Alzheimer's, and cancer. The history and basic concepts of medicinal inorganic chemistry have been widely reviewed [2].

As indicated in Fig. 1, key areas in the design of active compounds are the control of toxicity (side-effects) and targeting of the metal to specific tissues, organs, or cells where activity is needed. Metal ions can be introduced into a biological system either for therapeutic effect or as diagnostic aids. Alternatively, metal ions can be removed from a biological system by judicious use of metal binding molecules (ligands) [3]. Thus, biomedical inorganic chemistry offers the potential for the design of novel therapeutic and diagnostic agents and for the treatment and understanding of diseases which are currently intractable [4].

It is seldom useful to describe elements as "toxic" or "non-toxic". Even so-called "toxic compounds" can usually be tolerated in low doses, and may exhibit therapeutic effects within narrow concentration ranges, and biologically-essential elements can become toxic at high doses (Fig. 2, the Bertrand diagram). Moreover, the same element may be beneficial or noxious depending on speciation (the nature of the molecule or ion that contains the element). About 24 elements are currently thought to be essential for mammalian life: H, C, N, O, F, Na, Mg, Si, P, S, Cl, K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, Sn, and I. However, this list may not be complete. For example, boron [5] and chromium may turn out to be essential, and it has been suggested that silicon is essential only to prevent aluminium from being toxic [6]. The biological roles of Cr are surrounded by controversy [7]. Currently, Cr(VI) compounds are among 88 recognized (class I) human carcinogens. By contrast, most nutritionists regard Cr(III) as an essential micronutrient, acting as an insulin activator, although this opinion has been disputed. No Cr(III)-dependent biomolecules, such as enzymes or cofactors, have yet been unambiguously described.

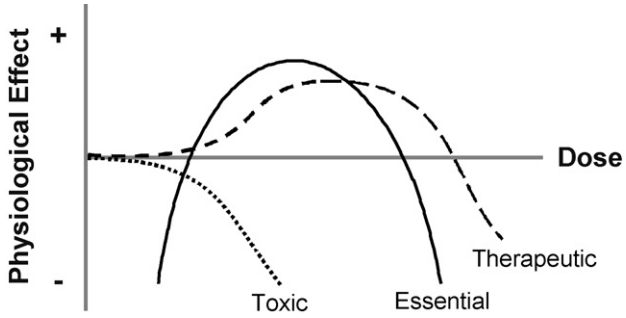

Fig. 2. A Bertrand diagram, showing that physiological and toxic effects are a continuum (adapted from G. Bertrand, 8th Int. Congr. Appl. Chem. 28 (1912) 30).

Inorganic elements play crucial roles in biological and biomedical processes [2b], and it is evident that many organic compounds used in medicine do not have a purely organic mode of action; some are activated or biotransformed by metal ions including metalloenzymes [8], others have a direct or indirect effect on metal ion metabolism. Indeed, many proteins need to bind one or more metal ions to perform their functions (metalloproteins), either because the metal ion is involved in the catalytic mechanism or because it stabilizes/determines the protein tertiary or quaternary structure. Metal ions are also very important for the structure and function (in the case of RNA) of nucleic acids. The intracellular concentration of several metals as well as their distribution among the various cell compartments and their incorporation into metalloproteins is tightly controlled

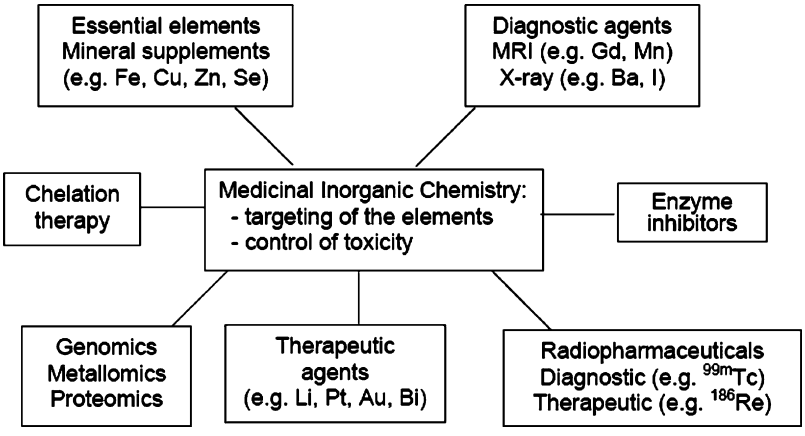

Fig. 1. Some of the areas of medicinal inorganic chemistry.

[9]; a proper balance of the equilibria involved in these control processes is necessary for a healthy phenotype.

Genome sequencing projects have provided us with the sequences of all proteins that the various organisms can produce (proteome) [10]. The investigation of the linkages between inorganic elements (essential and noxious) and the information obtainable from genome sequences, as well as of the mechanisms that warrant homeostasis in cell compartments, represent a further challenge for bioinorganic chemists in the so-called "post-genomic era" [11]. But this is a difficult task. In fact, even though elaborate analyses and annotations have been enabled by a rich and ever-increasing portfolio of bioinformatics tools, metal binding properties remain difficult to predict as well as to investigate experimentally. How do we recognize the codes for essential elements in the human genome? How do genomes code for the Periodic Table? Codes for essential elements are programs which control uptake, transport, delivery, and excretion of metals; they are mainly based on proteins which recognize specific complexes utilizing both thermodynamic (oxidation state, number and types of ligands, coordination geometry) and kinetic (ligand exchange) aspects of metal properties. With knowledge of the genomes of microorganisms, we could use differences in codes to design selective drugs. But this is a challenging task, as genomes code for ligands (proteins) which bind both in the first and second coordination spheres of metal ions.

The mammalian biochemistry of several essential elements is poorly understood. For example, chromium, nickel, and tin seem to be essential for life, but we do not know whether there are codes for them, and we do not know how to use them effectively in medicine, yet. For some elements the genetic code involves proteins which recognize specific metal complexes. For example, only one specific cobalt compound, the coenzyme vitamin B_{12}, appears to be essential [12], and in *Escherichia coli* the molybdate-dependent transcriptional regulator ModE acts as a sensor of intracellular molybdate (MoO_4^{2-}) concentration and as a regulator for the transcription of several operons that control the uptake and utilization of molybdenum [13]. Zinc is essential for life, and is the second most abundant transition metal ion in living organisms after iron. It has essentially two possible roles: catalytic or structural. Zinc may also modulate signalling events, as it occurs in processes maintaining zinc homeostasis. An attempt to compile a catalogue of proteins encoded by the human genome, which may require zinc for their physiological functions, has been recently reported [14]. Some 10% of the proteins coded by the human genome (the proteome) potentially have zinc-binding sequences. Selenium provides a good example of the importance of speciation: it is an essential element and yet some of its compounds are highly toxic (*e.g.* H_2Se). Genes which code for selenium incorporation into amino acids have been characterised [15].

Both essential and non-essential metals can be used in therapy and diagnosis, and examples of compounds in current clinical use are listed in Table 1. It is important to ask which parts of the active compound are essential for activity: the metal itself, the ligands, or the intact complex of metal plus, at least, some of the ligands? Many metallodrugs are "pro-drugs", and they undergo ligand substitution and/or redox reactions before they reach the target site. Thus, a rational design of potential therapeutic/diagnostic agents is needed to achieve specific targeting features and to control toxicity (side-effects), by controlling thermodynamic and kinetic processes of metal complexes. Tailored, multifunctional ligands for metal-based medicinal drugs offer many exciting possibilities, including targeting specific tissues, membrane receptors, or endogenous molecules, and can play an integral role in modulating the potential toxicity of a metallodrug to achieve success in areas of diagnosis and therapy.

Examples from our recent work are here reported and discussed. Topics covered include the design and mechanism of action of anti-HIV and stem-cell-mobilizing metallomacrocycles, photoactivatable platinum anticancer agents, and organometallic ruthenium and osmium arene anticancer complexes.

Table 1
Some metal compounds in clinical use

Compound Example (brand name)	Function	Comment
Active complexes		
cis-$[Pt^{II}Cl_2(NH_3)_2]$ (Cisplatin)	Anticancer	*trans*-isomer is inactive
$[Gd^{III}(DTPA)(H_2O)]^{2-}$ (Magnevist)	Extracellular contrast agent for MRI	Low toxicity
$[^{99m}Tc^{I}(CNCH_2C(CH_3)_2OCH_3)_6]^{+}$ (Cardiolite)	Myocardial imaging	Positively-charged complex taken up by the heart
Vitamin B_{12}	Coenzyme	Deficiency causes pernicious anaemia
Active metals		
Li_2CO_3	Prophylaxis for bipolar disorders	Li forms weak complexes, labile
$[Au^{I}$(thiomalate)] (Myocrisin)	Antirheumatoid arthritic	Facile thiol exchange on Au^{I}
Ammonium potassium Bi^{III} citrate (De-Nol)	Antibacterial, antiulcer	Strong binding of Bi to thiols, facile exchange
$Na_2[Fe^{II}(CN)_5(NO)]\cdot 2H_2O$ (Nipride)	Hypotensive	Releases NO, relaxes vascular muscles
(Bleomycin)	Anticancer	Requires Fe for DNA attack
p-xylyl-bicyclam-8HCl (AMD3100)	Anti-HIV, stem cell mobilization	May bind metals *in vivo*
CaCOs, $Mg(OH)_2$	Antacid	Slow release of alkali
$La_2^{III}(CO_3)_3$ (Fosnol)	Chronic renal failure	Reduces phosphate absorption

2. Metallomacrocycles

Cyclams are of interest in fields as diverse as catalysis, selective metal recovery and recycling, sensors, and therapy and diagnosis [16]. There is current medical interest in the drug AMD3100, a bicyclam containing two cyclam units connected by a *p*-phenylenebis(methylene) (*p*-xylyl) linker (AMD3100, *p*-xylylbicyclam, "Mozobil", Chart 1). AMD3100 is in phase II clinical trials for stem cell transplantation used in the treatment of patients who have cancers involving the blood and immune system (mobilizes stem cells from bone marrow) [17]. It is also one of the most potent anti-HIV agents known [18] and has recently been on clinical trial for the treatment of AIDS; nevertheless, severe side-effects resulted in trials being discontinued. AMD3100 blocks entry of T-lymphotropic HIV-1 and HIV-2 strains by specific binding to the CXCR4 co-receptor which assists the entry of HIV into cells and anchors stem cells in the bone marrow [19]. CXCR4 (receptor number 4 for natural chemotactic cytokine proteins containing a conserved Cys-X-Cys disulfide sequence, mediators of white blood cell trafficking and activation) is a member of the classical G-protein-coupled receptor (GPCR) family of membrane proteins, which contain seven transmembrane helices. G-protein-coupled receptors are involved in the regulation of nearly all physiological processes and *ca*. 40% of all therapeutic interventions [20]. On binding to diffusible extracellular ligands, they switch to active conformations capable of interacting with hundreds of G-proteins.

Cyclams are strong metal-chelating agents. Complexation of AMD3100 to Zn(II) enhances co-receptor binding strength and anti-HIV activity, whereas Pd(II) binding inactivates the drug, the activity order being [21]:

$$\mathrm{Zn(II)_2} > \mathrm{AMD3100} > \mathrm{Ni(II)_2} > \mathrm{Cu(II)_2} \gg \mathrm{Co(III)_2} \gg \mathrm{Pd(II)_2}.$$

Cyclam has a high affinity for Zn(II) (log $K = 15.5$) [22] and at physiologically-attainable concentrations of AMD3100 in blood (micromolar) [23] where the Zn(II) concentration is *ca*. 19 μM, it can be calculated that nearly all the drug would exist as a Zn(II) complex at physiological pH of 7.4 at 310 K [24]. Metal cyclam complexes commonly adopt one of the configurations shown in Chart 2, which differ in the chirality of the bound N atoms. Based on our recent NMR studies of complexes, such as $[\mathrm{Zn(cyclam)(H_2O)_2}]^{2+}$, which has a *trans*-III configuration in the crystalline state, including Kaplus-type analyses of vicinal couplings [25], it is possible to conclude that Zn(II) is taken up rapidly by cyclam and this gives rise to the unusual *trans*-I/*cis*-V

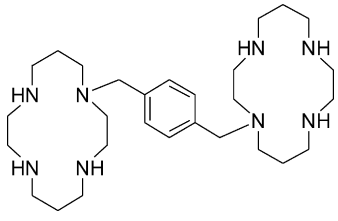

Chart 1. The antiviral macrocyclic bicyclam AMD3100. Metal ions, such as Ni(II), Cu(II) and Zn(II) can bind strongly to each ring.

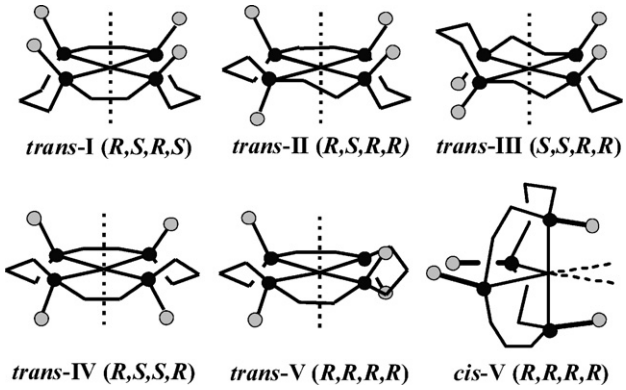

Chart 2. Structures of the major configurations of metal cyclam complexes. *Trans* configurations show the chiral N atoms with NH bonds pointing up or down, together with *cis*-V, the folded form of *trans*-V.

configurations of Zn(II) cyclam which slowly equilibrate with the *trans*-III configuration, usually the most stable configuration in the solid state.

For metal-bicyclam complexes, there is a close correlation [21] between antiviral activity and binding to the co-receptor CXCR4 as monitored by inhibition of 12G5 mAb binding and the intracellular Ca^{2+} signal induced by SDF-1α chemokine. The affinity of AMD3100 for the CXCR4 receptor is enhanced by factors of 7, 36, and 50 by incorporation of Cu^{2+}, Zn^{2+}, or Ni^{2+}, respectively, into the cyclam rings [26], and a similar metal-induced enhancement in CXCR4 affinity is observed for cyclam itself, although the affinity for the receptor is much lower. Our results also suggest that, surprisingly, Zn(II) binds to cyclam more rapidly than Cu(II) at physiological pH (7.4), thus slowing the uptake of the thermodynamically-favoured metal ion [24]. The ability of Zn(II) to exert kinetic control over the uptake of Cu(II) into specific binding sites is a principle which may be of wider significance in biology, *e.g.* in the assembly of the active sites of metalloproteins. Thus, we conclude that it is likely to be both thermodynamically and kinetically favourable for cyclam anti-HIV drugs to form Zn(II) complexes in blood plasma. Moreover, the specificity of recognition of metal cyclams by membrane co-receptor proteins may be determined by the configuration of the macrocycle. Our studies suggest that metal complexation *in vivo* is a factor which should be considered in attempts to understand the mechanism of action of (apo) cyclam drugs, both in terms of their antiviral activity and their side-effects.

The orientation of the N-H bonds is important for CXCR4 co-receptor recognition, and direct binding of metal ions in metallobicyclam complexes to CXCR4 carboxylate groups is likely to be a major factor in determining the strength of the interaction. We have shown that acetate binding to Zn_2-(*p*-xylylbicyclam) (acetate as a model for carboxylate side-chains of aspartate and glutamate residues) induces the rapid formation of the unusual *cis*-V configuration *via* chelation, and second coordination sphere double H-bonding to diagonal cyclam NH protons [27]. Molecular modelling suggests that the carboxylate groups of Asp-262 and Glu-288 can stabilize a similar *cis*-V configuration for one Zn(II)-cyclam unit of Zn_2-(*p*-xylylbicyclam)

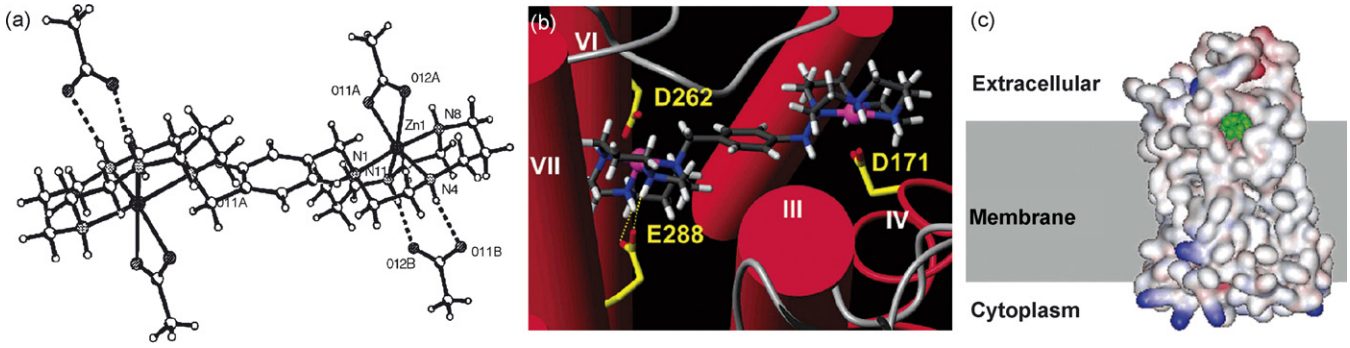

Fig. 3. First and second coordination sphere interactions of Zn_2-(*p*-xylylbicyclam) with carboxylate groups. (A) X-ray crystal structure of [Zn_2-(*p*-xylylbicyclam)$(OAc)_2$]$(OAc)_2 \cdot 2CH_3OH$ (CH_3OH not shown). Both cyclam units adopt the *cis*-V configuration with chelation by acetate on one cyclam face and double H-bonding on the other. (B) Model of Zn_2-(*p*-xylylbicyclam) bound to the CXCR4 co-receptor. Transmembrane helices are red cylinders, except helix IV, which is depicted as a ribbon for clarity. One of the Zn(II)-cyclams has axial coordination to the oxygens of Asp-262 and double H-bonds between two of its NH groups and the oxygens of Glu-288 on the opposite cyclam face and is in the *cis*-V configuration, resembling that in part (a) above. The second Zn(II)-cyclam is *trans*-I with axial coordination to Asp-171. (C) View of the model of CXCR4 showing the location of the proposed Zn_2-(*p*-xylylbicyclam) binding site. The *cis*-V cyclam is visible through a channel on one side of the protein. The likely position of the membrane is indicated in grey, Zn_2-(*p*-xylylbicyclam) is green, and electrostatic potentials are coloured blue-positive, red-negative, and white-neutral. The unstructured N-terminus (residues 1–38) and C-terminus (residues 320–352) have been omitted (reproduced from Ref. [27] with permission of the copyright holders).

bound to CXCR4. The second Zn(II)-cyclam unit can bind to Asp-171 with the bicyclam connecting across the main co-receptor binding site. Such a network of interactions allows a strong induced-fit recognition and is consistent with the high antagonistic potency of Zn_2-(*p*-xylylbicyclam) (Fig. 3).

Our studies of lysozyme have revealed structural details of protein-metallocyclam interactions that may be important for receptor recognition [28]. Solution NMR studies show that Cu-cyclam interacts with specific tryptophan residues of lysozyme (Trp-62, Trp-63, and Trp-123). Two major binding sites for both Cu-cyclam and Cu_2-(*p*-xylylbicyclam) were detected by X-ray crystallography (Fig. 4). In the first site, Cu^{2+} in one cyclam ring of Cu_2-(*p*-xylylbicyclam) adopts a *trans* configuration and is coordinated to a carboxylate oxygen of Asp-101, whereas for Cu-cyclam two ring NH groups form H-bonds to the carboxylate oxygens of Asp-101, stabilizing an unusual *cis* (folded) cyclam configuration. For both complexes in this site, a cyclam ring is sandwiched between the indole side-chains of two tryptophan residues (Trp-62 and Trp-63). In the second site, a *trans* cyclam ring is stacked on Trp-123 and H-bonded to the backbone car-

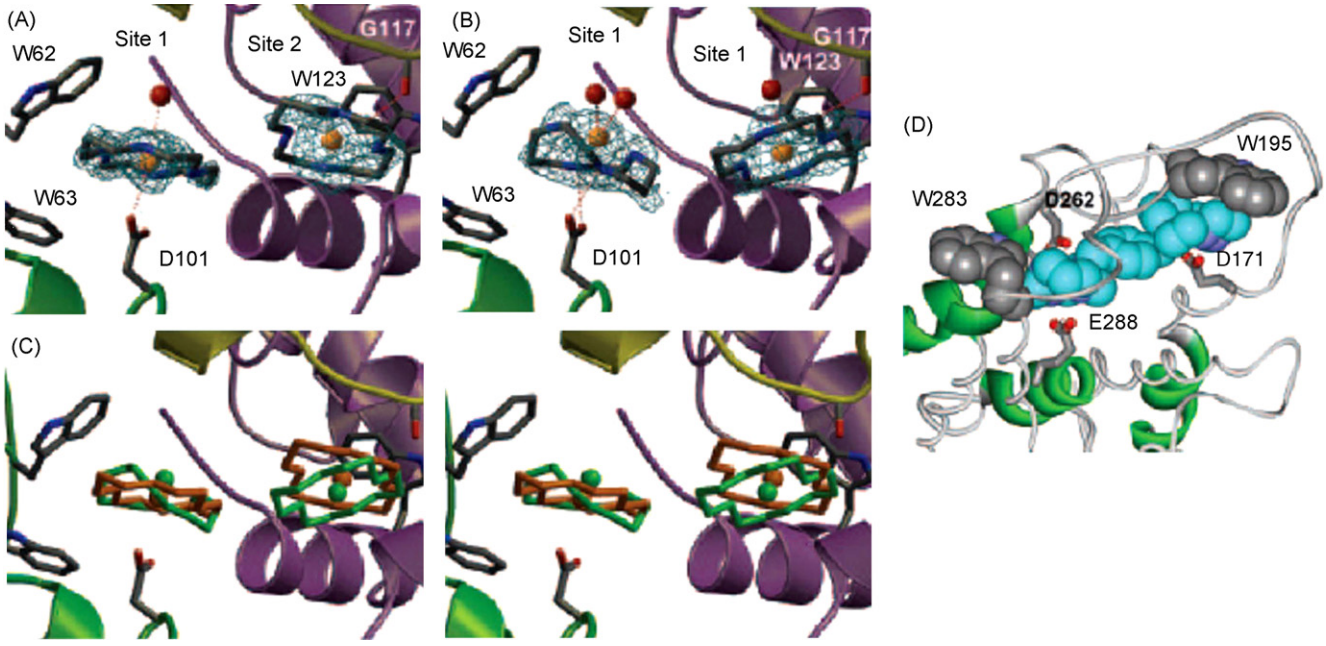

Fig. 4. The two Cu-cyclam and Cu_2-(*p*-xylylbicyclam) binding sites in crystals of HEWL. (A) Cu_2-(*p*-xylylbicyclam)-HEWL. (B) Cu-cyclam-HEWL. (C) Stereoview of an overlay of the cyclam positions in lysozyme. Colour code: orange, Cu_2-(*p*-xylylbicyclam); green, Cu-cyclam. The sites are at the intersection of three lysozyme molecules in the crystal. Molecule 1 is green and contains Asp-101, Trp-62, and Trp-63; molecule 2 is purple and contains Trp-123; and molecule 3 is yellow and contains Gly-117. (D) A model showing [Zn_2-(*p*-xylylbicyclam)]$^{4+}$ bound to the human co-receptor CXCR4 [27]. One of the cyclam rings in a *trans* configuration is stacked on Trp-195 and its Zn is bound to Asp-171. The other cyclam ring has a folded configuration (*cis*) and is close to Trp-283; its Zn is bound to Asp-262, and two ring NH groups are H-bonded to the oxygens of Glu-288 (reproduced from Ref. [28] with permission of the copyright holders).

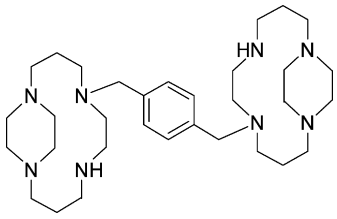

Chart 3. Constrained analogue of AMD3100.

bonyl of Gly-117. We have shown that there is a pocket in a model of the human CXCR4 co-receptor in which *trans* and *cis* configurations of metallobicyclam can bind by direct metal coordination to carboxylate side-chains, cyclam-NH···carboxylate H-bonding, together with hydrophobic interactions with tryptophan residues. These studies provide a structural basis for the design of macrocycles that bind stereospecifically to G-protein-coupled and other protein receptors.

On the basis of the reported results, we have also suggested that configurationally fixed AMD3100 analogues would have the advantage of presenting only one configuration in solution for coordinate bond formation on binding to the protein. Thus, our study aimed to produce a series of configurationally fixed complexes and showed the key importance of the coordination interaction for drug binding. In an attempt to rationalize the effects of cyclam configuration and to produce new specific antagonists for CXCR4, Archibald et al. have successfully synthesized a configurationally fixed bis-macrocyclic compound (Chart 3) and its zinc(II) complex [29]. The X-ray structure (Fig. 5) reveals an asymmetric unit containing one macrocyclic ring complexed to a six-coordinate zinc ion. The two zinc centres in one molecule are identical and related by crystallographic symmetry. Analysis of the macrocyclic configuration reveals the equivalent of a *trans*-II-type arrangement. The coordination of the bound acetate corresponds to the proposed interaction of each of the macrocyclic units with CXCR4 aspartate residues, Asp-171 and Asp-262. To prove the ability of both the macrocyclic ligand and its zinc complex to bind to the CXCR4 chemokine receptor and inhibit infection by HIV, the compounds were tested in an assay monitoring viral infection. They both bind to the CXCR4 receptor as detected by flow cell cytometry displacement studies with an anti-CXCR4 antibody. But whereas the constrained bis-macrocycle is considerably less-active than the analogue AMD3100, an enhancement of three orders of magnitude in the anti-HIV activity is observed for its Zn complex, which is 3× more potent than $[Zn_2(AMD3100)]^{4+}$. This demonstrates the importance of coordination to the chemokine receptor and validates the strategy of configurational restriction [29].

3. Photoactivated platinum complexes

The platinum diammino complexes cisplatin and carboplatin are highly effective anticancer drugs, but their use is limited by dose-limiting side-effects, by their restricted spectrum of anticancer activity, and by the development of resistance after repeated use in treatment [30]. As an approach to avoid toxic side-effects, we are exploring the use of non-toxic platinum prodrugs which can be activated by light leading to the release of active antitumour agents directly into the cancer cells.

Light in combination with photosensitive substances has been used therapeutically for several years to treat various diseases, in particular of the skin [31]. Indeed, four thousand years ago, the ancient Egyptians attempted to use sunlight plus topically-applied extracts (containing psoralens) of the fruit of Bishop's weed or Bullwort to treat vitiligo, a pigmentation disorder of the skin. Recent advances in laser and fibre optic technologies have spurred the development of novel medical applications based on the use of light [32]. These methods allow physicians to irradiate not only the skin but also internal organs with light of highly defined intensity and wavelength. This is a major advance in the phototherapy of cancer because many solid tumours (*e.g.* oesophagus, head and neck, lung, bladder, cervix and colon tumours) grow in body cavities accessible to such devices. A site-specific activation of photochemotherapeutic agents is therefore possible, which minimizes the severe side-effects of chemotherapy.

Photodynamic therapy is already well known in clinics, and involves the selective damage of target tissue by using a photosensitizing drug and light. The mechanism of action of photosensitizers (such as porphyrins) involves oxygen. The photosensitizer absorbs energy from a light source and becomes excited to an energetically higher electronic state. This energy is then transferred to oxygen which is converted from a triplet ground state ($^3O_2$) to a singlet excited state ($^1O_2$). The photosensitizer is then regenerated and so works catalytically. Singlet oxygen ($^1O_2$) is a short-lived but highly reactive molecule which reacts rapidly with cellular components. These reactions cause damage to the cell that ultimately leads to cell death [33]. However, the requirement for oxygen is a major drawback as many malignant and most aggressive cancer cells are hypoxic [33]. Therefore, photoactivatable compounds, which do not require the presence of oxygen, might be more effective in killing tumour cells *via* a different mechanism.

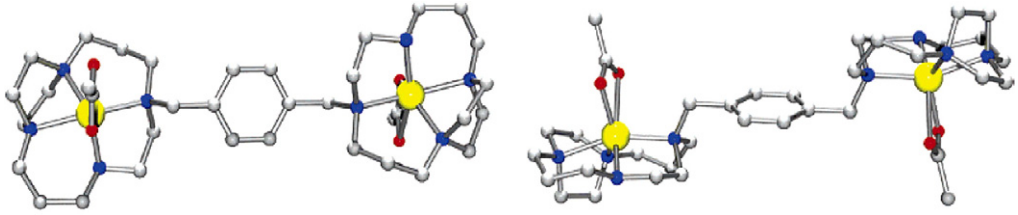

Fig. 5. Ball and stick representations of the single crystal X-ray structure of $[Zn_2(OAc)_2(L)]^{2+}$. Hydrogens have been omitted for clarity (reproduced from Ref. [29] with permission of the copyright holders).

Octahedral Pt(IV) complexes are considerably more inert kinetically than their square-planar Pt(II) analogues [34]; in fact, substitution reactions of the ligands take place very slowly or not at all under physiological conditions. Because of their chemical inertness, efforts have been made to develop orally-active Pt(IV) antitumour agents, such as satraplatin [35]. It is widely accepted that reduction to the Pt(II) oxidation state by biological reducing agents is necessary for Pt(IV) complexes to exert their anticancer activity, so their pharmacology depends on how readily they are reduced, and this in turn depends on the nature of the axial ligands. Reduction occurs most readily when the axial ligands are chlorides, least readily when they are hydroxides, and is intermediate when they are carboxylates (with different chain lengths) [36]. There is a clear correlation between reduction potential and the pharmacology of Pt(IV) complexes, confirming that it is possible to tune these properties.

Another interesting mechanism of reduction from Pt(IV) to Pt(II) is photochemical. Pt(IV) complexes can be made photolabile by choosing appropriate ligands. Such platinum complexes can then undergo a variety of photochemical reactions, including photoreduction. A novel application of these phenomena for therapeutic purposes is the development of light-sensitive Pt(IV) pro-drugs that can be photoactivated to antitumour Pt(II) agents directly at the site of the tumour with laser light. This selective activation of a light-sensitive pro-drug would be expected to lead to an increase in the therapeutic index and, hence, the clinical usefulness of platinum antitumour agents might be expanded. The development of such drugs has been recently reviewed [37].

Examples of such agents, Pt(IV)-diaminodiiodo compounds, have been reported by Bednarski and co-workers [38,39], but, although these can platinate DNA on photoactivation with visible light, they are not stable towards reducing agents, such as glutathione and albumin [40]. We have therefore sought alternative Pt(IV) compounds that are better suited to this purpose, especially those more stable towards reducing agents [41].

Pt(IV)-azido complexes are well known to be photoactive. Photoreactions, using UV light ($\lambda < 400$ nm), have been reported for Pt(IV)-hexa- and -diazido compounds in water, leading to a reductive elimination of the azido ligands and the production of N_2 [42,43]. To photoreduce Pt(IV) to Pt(II), two azido ligands are required. One possible mechanism is that upon irradiation the azido ligands form $N_3^{\bullet}$ radicals. Azidyl radicals are very unstable and rapidly decompose in water into molecular nitrogen (N_2), thus preventing reoxidation of the platinum centre (Chart 4). This fast decomposition is believed to be the reason for the efficiency of photoredox reactions involving complexes containing azido ligands.

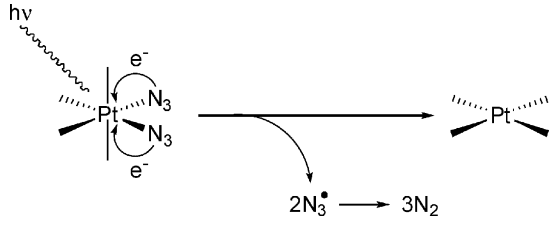

Chart 4. Possible mechanism for the photoreduction of a Pt(IV)-diazido complexes.

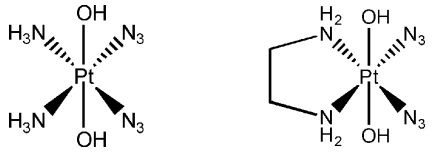

Chart 5. Chemical drawings of *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ and *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$.

On the basis of these considerations, we have reported the first crystal structures of the Pt(IV)-diazidodiam(m)ino complexes *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ and *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$ (Chart 5), and shown that these complexes can be activated by light to give highly reactive Pt(II) species which bind rapidly and stereospecifically to nucleotides, thereby forming known *cis*-platinum-nucleotide cross-links [44]. The hydroxo ligands enhance aqueous solubility and also stabilize the Pt(IV) oxidation state [36,45]. Changing the am(m)ino ligands does not significantly alter the reduction potential of Pt(IV) compounds so these ligands can be varied to improve properties, such as lipophilicity, without affecting the stability [36].

The stability of photoactivatable complexes under physiological conditions is an important factor to consider for potential photochemotherapeutic drugs. Pt(II) compounds are generally more reactive than their Pt(IV) counter-parts [34]. The tripeptide glutathione (γ-L-Glu-L-Cys-Gly) is the most abundant reducing agent found in cells with concentrations up to 10 mM [46], therefore it is preferable that photoactive Pt(IV) compounds are not readily reduced by it. Also of concern is whether the diazido complexes are stable in solution and towards potential targets, such as the guanine bases of RNA and DNA in the dark. NMR studies of *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ and *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$ have shown that both are stable towards hydrolysis for more than 90 days, inert to reaction with nucleobases, such as 5′-GMP and d(GpG) in the dark, and, most significantly, only react very slowly with glutathione over a period of several weeks [44].

The interaction of both Pt(IV)-diazido complexes with nucleobases upon irradiation has been also studied. Irradiation of *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ with blue light (457.9 nm, 10 mW cm^{-2}, 7.7 h) and UV light (365.0 nm, 10 mW cm^{-2}, 30 min), in the presence of two molar equivalents of 5′-GMP resulted in the same five major Pt(II) species. One was assigned as *cis*-$[Pt(NH_3)_2(5'\text{-GMP-}N^7)_2]^{2+}$, but there was evidence for photoisomerisation processes, since *trans*-$[Pt(NH_3)_2(5'\text{-GMP-}N^7)_2]^{2+}$ was also detected [47]. In the presence of one molar equivalent of d(GpG), reduction and binding of both complexes was seen after irradiation with blue or UVA light. The reaction of *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$ yielded only a few minor Pt(IV) side-products with UVA light (Fig. 6), and none with blue light. The major product in both cases was *cis*-$[Pt(en)(d(GpG)\text{-}N^7,N^7)]^{2+}$ [48].

Further studies have been carried out to determine whether *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$ can bind preferentially to guanine bases to form GG cross-links on strands of DNA [49]. The profile of DNA platination of a 212-bp fragment of plasmid DNA was determined by transcription mapping of the DNA adducts. Platination produces premature termination of duplex transcrip-

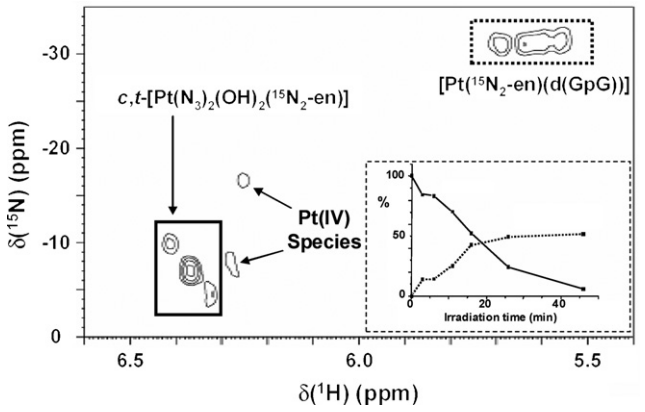

Fig. 6. 2D [^{1}H,^{15}N] HSQC NMR spectroscopy of a solution containing *cis*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(^{15}N$_2$-en)] (0.75 mM) and d(GpG) (0.5 mM) after irradiation for 26 min (λ_{irr} = 365.0 nm). The inset graph (– *cis*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(^{15}N$_2$-en)]; - - - [Pt(^{15}N$_2$-en)(d(GpG))]) shows that formation of the cross-linked Pt(II) adduct is complete within 30 min. Some photoinduced isomerisation/substitution of the Pt(IV) starting complex is also evident (adapted from Ref. [48]).

tion. Platinum determination by FAAS showed that no platinum binding to DNA occurred in the dark, but platination levels of r_b = 0.01 (r_b is defined as the number of moles of platinum bound per nucleotide) were attained on irradiation of the sample. The major stop sites on the irradiated fragment were similar to those produced by cisplatin and appeared at guanine residues mainly contained in GG sequences (Fig. 7). HPLC analysis of enzymatic digests of a synthetic 40-bp oligonucleotide duplex photoirradiated in the presence of *cis*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(en)] also suggested that the major binding sites involve bifunctional GG adducts, in agreement with the transcription mapping results.

NMR methods have proved useful in the investigation of platinum drugs from the time that cisplatin was first introduced into the clinic more than 30 years ago. In particular, the introduction of 2D [^{1}H,^{15}N] NMR techniques in the early 1990s made it possible to follow the reactions of cisplatin and related platinum anticancer complexes under physiologically-relevant conditions [50]. Provided that am(m)ino ligands are ^{15}N-labeled, photoreactions of the Pt(IV) complexes *cis*,*trans*,*cis*-[Pt(N$_3$)$_2$(OH)$_2$(^{15}NH$_3$)$_2$] and *cis*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(^{15}N$_2$-en)] can be followed by 1D ^{1}H and 2D [^{1}H,^{15}N] HSQC NMR spectroscopy. Irradiation of *cis*,*trans*,*cis*-[Pt(N$_3$)$_2$(OH)$_2$(^{15}NH$_3$)$_2$] (488.0 nm, 10 mW cm^{-2}) for 20 h did not lead to photoreduction [51]. Several new Pt(IV) species were observed which could be either photoisomers, or photosubstitution products. Photoreduction did take place upon UVA irradiation, and three major Pt(II) species appeared after 26 min. A precipitate is also formed during the reaction which may be polynuclear hydroxo-bridged platinum species [48]. Photoreactions of *cis*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(^{15}N$_2$-en)] with blue and UVA light appeared to generate different products. Irradiation for 4 h with blue light (457.9 nm, 20 mW cm^{-2}) resulted in many new species, especially Pt(IV) species. Irradiation at 365.0 nm for only 11 min produced one new Pt(II) and one new Pt(IV) species. Again an insoluble precipitate was formed [48]. The development of 1D ^{14}N NMR methods has provided us with new insights into the mechanism of photoactivation pathways. In particular, this technique has allowed us to follow the photoreactions involving the azido ligands. Preliminary results (Fig. 8), have confirmed the production of N$_2$ upon irradiation, but also new pathways (*e.g.* involving release of N_3^- and/or am(m)ino ligands) can occur, and the photoproducts seem to be highly dependent on the solution conditions (sol-

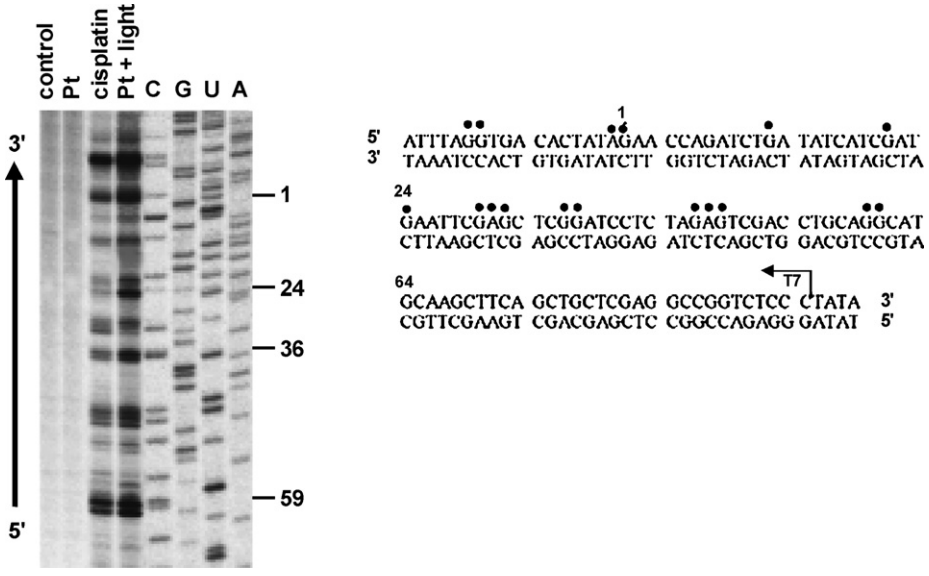

Fig. 7. Inhibition of RNA synthesis by T7 RNA polymerase on the *Nde*I/*Hpa*I fragment of pSP73KB plasmid modified by *cis*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(en)] and cisplatin. (A) Autoradiogram of 6% polyacrylamide/8 M urea sequencing gel. Lanes: control (non-modified template); Pt (the template modified by *cis*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(en)] and not irradiated); cisplatin (the template modified by cisplatin); Pt + light (the template modified by *cis*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(en)] and irradiated). (B) Schematic diagram showing the portion of the nucleotide sequence of the template of the *Nde*I/*Hpa*I fragment used to monitor inhibition of RNA synthesis by *cis*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(en)] and cisplatin. The arrow indicates the start of the T7 RNA polymerase. (●): Major stop signals. The numbers correspond to the nucleotide numbering in the sequence map of pSP73KB plasmid (adapted from Ref. [49]).

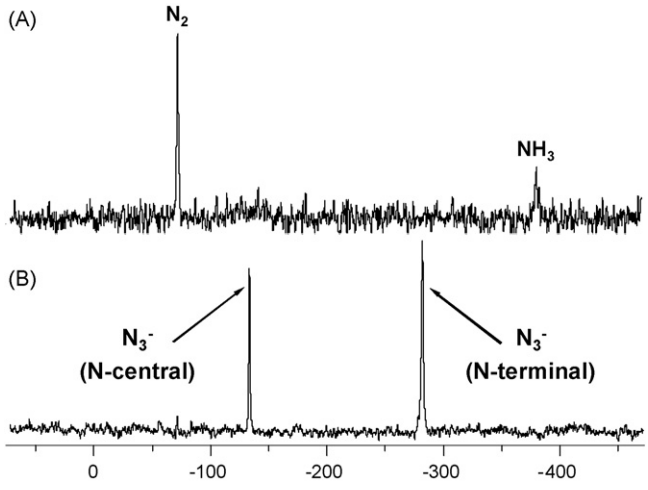

Fig. 8. $^{14}N\{^{1}H\}$ NMR spectra (referenced to external neat CH_3NO_2 at 0.00 ppm) of: (A) *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(^{15}NH_3)_2]$ 12.89 mM in 90% H_2O/10% D_2O after 2 h of irradiation (365.0 nm, 6.83 mW cm^{-2}) at 310 K. Before irradiation, the pH was adjusted to 5.15, and Ar was bubbled into the sample for 30 min. The final pH was 10.73, and a precipitate formed. (B) *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(^{15}NH_3)_2]$ 14.80 mM in 90% PBS/10% D_2O after 2 h of irradiation (365.0 nm, 6.87 mW cm^{-2}) at 310 K. Before irradiation, the starting pH was 7.36 (not adjusted), and Ar was bubbled into the sample for 30 min. The final pH was 9.87, and a precipitate formed.

vent, pH, concentration), including the presence of biomolecules [52].

The effect of *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ and *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$ on the growth of 5637 human bladder cancer cells has been studied with and without light [48]. These Pt(IV)-diazido complexes are non-toxic to 5637 human bladder cancer cells in the dark, but are toxic to the cells upon irradiation, with IC_{50} values (50% growth inhibitory concentration) decreased significantly from >300 μM to 49 and 63 μM for *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ and *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$, respectively. Photoactivation causes dramatic effects on the morphology of bladder cancer cells, including disintegration of their nuclei, whereas in the absence of irradiation, no changes in the cells were detected. The mechanism by which they kill cancer cells, therefore, appears to be different from that of cisplatin. Analogous experiments have been carried out with cisplatin-resistant 5637-Pt cells; intriguingly, both Pt(IV)-diazido complexes were found to be equally cytotoxic to both cisplatin-sensitive and -resistant cell lines. Cell uptake studies showed that light had no significant effect on platinum uptake up to 8 h. About five times as much platinum was found in the cells treated with cisplatin as those treated with *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ or *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$ which could explain the higher activity of cisplatin [48].

The two Pt(IV)-azide complexes described so far both contain azide ligands in the *cis* position; incorporation of azides in a *trans* configuration could potentially lead to a different mechanism of photodecomposition. Although the *trans* isomer of cisplatin, *trans*-$[PtCl_2(NH_3)_2]$ (transplatin) is relatively non-toxic to cancer cells itself [53], it can be activated by light, whereupon it becomes as active as cisplatin. Recent studies show that light activates both chloride ligands of transplatin, and experiments on plasmid DNA and a 23 base-pair DNA duplex show that irradiation can greatly enhance formation of interstrand cross-links and of DNA-protein cross-links (which are not formed in the dark) [54]. Moreover, replacement of one or both of the NH_3 groups with an aliphatic amine, a heterocyclic amine or an iminoether can result in cytotoxic trans compounds [55–57]. A major advantage of some *trans* compounds over their *cis* counter-parts is their increased activity in cisplatin-resistant cell lines [58,59]. The aqueous solubility of *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ is greater than its *cis* isomer, and solutions of nearly 20 mM can be prepared. The intense azide-to-Pt(IV) charge-transfer band is also shifted to longer wavelength [47]. This *trans* complex is stable to 5′-GMP in the dark, but after irradiation for only one minute 7% of the starting material underwent photoreduction and photosubstitution to produce *trans*-$[Pt(NH_3)_2(5'\text{-GMP-}N^7)]^{2+}$. This is remarkable as only a very small amount of reduction was seen after 60 min irradiation of *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ in the absence of nucleotides. After 1 h, more than 75% was bound to 5′-GMP as the *trans* bis-GMP adduct. This reaction is much more efficient than that of *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ or *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$. The phototoxicity of the compounds *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ and its *trans*,*trans*,*trans*-isomer towards human HaCaT keratinocytes has been investigated [47]. In the absence of light both were non-toxic, but upon irradiation they were as effective as cisplatin. On the basis of these results, it is apparent that *trans*-Pt(IV)-diazidodiam(m)ino complexes are worthy of further exploration as photochemotherapeutic agents. Preliminary phototoxicity tests performed on some *trans* analogues (*e.g. trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(NH_3)(py)]$ [60]) have shown that they can exhibit an higher cytotoxicity than cisplatin towards several cell lines.

This new class of photoactivatable platinum complexes could, therefore, be useful in the treatment of cancers that are accessible to light, including bladder, lung, oesophagus, and skin cancers. Since activation can be localized to the area of irradiation, this procedure has potential for avoiding the side-effects that often accompany the use of cisplatin.

4. Metal arene complexes

There is increasing interest in the application of organometallic chemistry to biology and medicine. Organometallic chemistry evolved rapidly during the second half of the 20th century [61] and bioorganometallic chemistry is now establishing itself as an important branch of the subject [62]. In particular, organometallic complexes, *i.e.* complexes with at least one direct metal-carbon bond, offer much potential for exploration as anticancer agents due to the large diversity of structure and bonding modes that are unique to them [63]. Natural biomolecules with metal-carbon bonds have been shown to exist, a well-established example being methylcobalamin (methyl-B_{12}), which contains a distinct Co–CH_3 bond. Methylcobalamin functions as a methylating agent in many important biochemical reactions, such as the synthesis of methionine from homocysteine [64].

Titanocene dichloride, $[TiCl_2Cp_2]$, is an example of organometallic anticancer complex. It was originally investigated because it was believed that the *cis*-($TiCl_2$) motif would react with DNA in a similar manner to cisplatin, and lead to the formation of bifunctional cross-links which might in turn induce apoptosis and cancer cell death. However, the complex binds only weakly to DNA bases, and more strongly to the phosphate backbone [65]. Titanocene dichloride is difficult to formulate for administration because of its ease of hydrolysis and ready formation of hydroxo- and oxo-bridged species. The Cp ligand is also readily displaced and readily protonated, *e.g.* on reaction with the protein transferrin in the blood [66]. Responses to titanocene dichloride in the clinic were not encouraging and the trials have now been abandoned.

Early interest in the anticancer activity of ruthenium complexes stemmed from the observations of Clarke that Ru(III) ammines, *e.g.* $[RuCl_3(NH_3)_3]$, are active anticancer agents [67]. However, these were too insoluble for clinical use. Two other Ru(III) complexes (Chart 6), *trans*-$[RuCl_4(Im)(DMSO)]ImH$ (NAMI-A) [68] and *trans*-$[RuCl_4(Ind)_2]IndH$ (KP1019) [69] are undergoing clinical trials. Whilst KP1019 is cytotoxic to cancer cells, NAMI-A is relatively non-toxic but has antimetastatic activity (prevents the spread of cancer). Clarke has proposed that the activity of Ru(III) complexes, which are usually relatively inert towards ligand substitution, is dependent on *in vivo* reduction to more labile Ru(II) complexes [67,70]. With this in mind, we have explored the activity of Ru(II) complexes. Since arenes are known to stabilize ruthenium in its +2 oxidation state, we have investigated the potential of Ru(II) arene complexes as anticancer agents and their associated aqueous chemistry [71]. We have found that "half-sandwich" Ru(II) monoarene complexes often possess good aqueous solubility (an advantage for clinical use) and that the arene ligand is relatively inert towards displacement under physiological conditions.

A typical structure of a half-sandwich "piano-stool" [(η^6-arene)Ru(X)(Y)(Z)] complex is shown in Chart 7, where the arene forms the seat of the piano-stool and the ligands resemble the legs. Linking the ligands Y and Z to form a bidentate chelating ligand (L) seems to be advantageous for anticancer activity. The structure of Ru(II) half-sandwich complexes allows for variations of the three main building blocks, the monodentate ligand X, the bidentate ligand L and the arene, to fine-tune the pharmacological properties of these complexes [72].

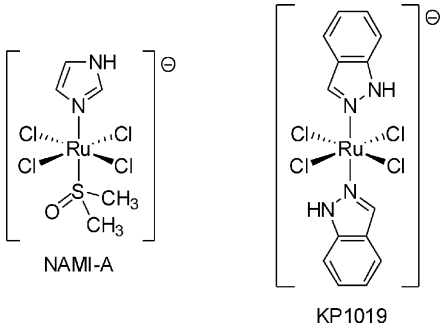

Chart 6. Ruthenium anticancer complexes *trans*-$[RuCl_4(Im)(DMSO)]ImH$ (NAMI-A) and *trans*-$[RuCl_4(Ind)_2]IndH$ (KP1019).

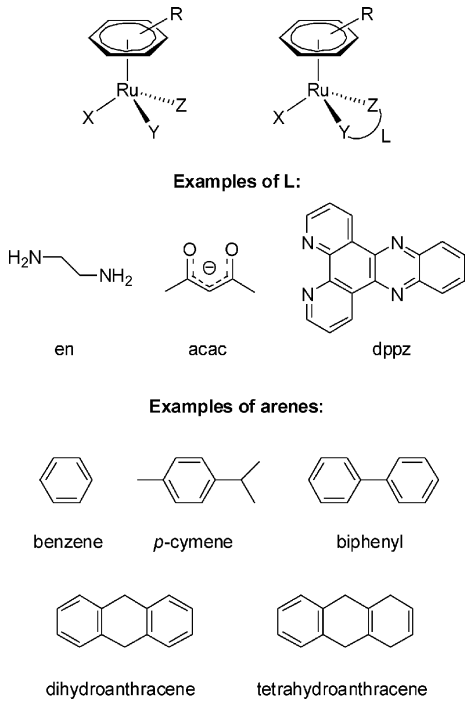

Chart 7. Typical structures of Ru(II) half-sandwich complexes and selected examples of chelating ligands (L) and arenes (Ph-R).

Reproducible cytotoxicities against A2780 human ovarian cancer cells are exhibited by the complexes [(η^6-arene)Ru(en)Cl]$^+$ [73]. Activity appears to increase with the size of the coordinated arene:

$$\text{benzene} < p\text{-cymene (cym)} < \text{biphenyl (bip)} < \text{dihydroanthracene (DHA)} \ll \text{tetrahydroanthracene (THA)}$$

such that, in this cell line, the bip complex has similar cytotoxicity to the anticancer drug carboplatin ($IC_{50} = 6\ \mu M$) and the THA complex is as active as cisplatin ($IC_{50} = 0.6\ \mu M$) [74]. The complexes [(η^6-cym)Ru(X)(Y)(Z)] (X, Y or Z = halide, acetonitrile or isonicotinamide), with three monodentate ligands, are however inactive towards A2780 human ovarian cancer cells *in vitro* [73]. These complexes may be too reactive with components of the cell culture medium and/or the cells, and are deactivated by biomolecules before they reach their target sites. Substitution of chloride by other halides, such as iodide has only a small effect on the cytotoxicity of en complexes [74]. From the above results, it appears that a more hydrophobic arene ligand and a single ligand exchange site (the other two coordination sites being occupied by a stable bidentate chelating ligand) are associated with high cytotoxicity.

Recent cytotoxicity tests on a more extensive range of Ru(II) arene complexes have indicated, however, that the structure-activity relationship is more complex [75]. For example, when en is replaced by acetylacetonate, the cym and bip complexes are much more cytotoxic than the DHA complex. Replacing en by *N*,*N*,*N*′,*N*′-tetramethylethylenediamine or 2,2′-bipyridine results in complexes with insignificant cytotoxicity, whilst

complexes with 1,2-diaminobenzene as the chelating ligand show comparable or enhanced cytotoxicity compared to the en analogues. Complexes which lack NH groups on the coordinated diamine are often inactive (*e.g.* $Me_2NCH_2CH_2NMe_2$, bipyridine, phenanthroline), but intriguingly bipyridine diol derivatives which can deprotonate and rigidify the bipyridine ring system *via* –C–O···H–O–C hydrogen-bonding, can exhibit high cytotoxicity levels [76].

In biological systems, $[(\eta^6\text{-arene})Ru(L)(X)]^{n+}$ complexes will encounter an array of biomolecules with which they could potentially react. Hence, it is important to gain a detailed understanding of such interactions with ligands ranging from water and chloride to nucleobases, oligonucleotides, DNA, amino acids and proteins. In aqueous media, the chloride ligand of $[(\eta^6\text{-arene})Ru(L)Cl]^+$ complexes can exchange with water to form aqua complexes $[(\eta^6\text{-arene})Ru(L)(H_2O)]^{2+}$. For L = en, the chloride-containing complexes generally undergo substitution reactions very much more slowly than the corresponding aqua compounds [77], hence it is important to understand the thermodynamics and kinetics of formation of the aqua complexes (*i.e.* activated form of the ruthenium arene complexes). The rates of aquation of $[(\eta^6\text{-arene})Ru(en)Cl]^+$ (η^6-arene = bip, DHA and THA) at 310 K and ionic strength (I, $NaClO_4$) of 0.1 M are an order of magnitude faster than that of cisplatin [78]. The reverse anation reactions in the presence of 100 mM NaCl (similar concentration to that in blood plasma) are also very rapid. The aquation and anation reactions are *ca.* two times faster for the DHA and THA complexes compared to the bip complex, suggesting that variations in the steric and electronic effects of the arene ligands modulate the ligand exchange reactions [78]. Hence, at physiologically-relevant concentrations of the ruthenium(II) arene complexes (0.5–5 μM), the complexes should be present in blood plasma largely as the less-reactive chloro complexes (>89%), whereas in the cell nucleus ($[Cl^-]$ *ca.* 4 mM) significant amounts (45–65%) of the more reactive aqua species would be formed readily. The coordinated aqua ligand of $[(\eta^6\text{-arene})Ru(en)(H_2O)]^{2+}$ undergoes acid dissociation to give the hydroxo complex $[(\eta^6\text{-arene})Ru(en)(OH)]^+$ which is less susceptible to substitution reactions than the aqua complex [77]. The pK_a values range from 7.71 to 7.89 and 8.01 for the bip, DHA and THA aqua complexes, respectively [78]. Since the pK_a values are high, only small amounts of the hydroxo species (<10% of the total Ru arene complex) would be present at physiological pH (7.2–7.4). Density functional calculations suggest that aquation occurs by means of a concerted ligand interchange mechanism in which bond-making is of greater importance than bond-breaking [79]. The calculated reaction barriers and overall reaction energies for the aquation of the halide and azide $\{(\eta^6\text{-arene})Ru(en)\}^{2+}$ complexes follow the order Br < Cl < I < N_3, in agreement with the experimental hydrolysis rates, Cl ≈ Br < I < N_3, confirming that the higher activation energies are responsible for the slower hydrolysis of the I and N_3 complexes. In general, for the arene complexes studied here, those that hydrolyze exhibit high cytotoxicity, and those that do not hydrolyze are inactive or weakly active. An intriguing exception is the thiophenolate complex, $[(\eta^6\text{-hexamethylbenzene})Ru(en)(SPh)]^+$, which undergoes little hydrolysis and appears to be activated by a different mechanism. It seems likely that it is activated by means of oxidation of the bound SPh by oxygen to the sulfenate or sulfinate, which is readily displaced from ruthenium by other biomolecules, such as guanine derivatives [79].

Binding studies of ruthenium(II) arene complexes with nucleobases are of special interest since DNA is the primary target of the archetypal metal-based anticancer drug, cisplatin [80]. Therefore, we have investigated reactions of complexes $[(\eta^6\text{-arene})Ru(en)(X)]^{n+}$, where arene = bip, THA, DHA, cym and Ph, X = Cl or H_2O, with nucleic acid derivatives as models of DNA [77]. For mononucleosides, $\{(\eta^6\text{-arene})Ru(en)\}^{2+}$ binds only to N7 of guanosine (G) and to N3 of thymidine (T). Binding to N3 of cytidine (C) is weak, and almost no binding to adenosine (A) is observed. The reactivity of the various binding sites of nucleobases towards Ru(II) at neutral pH decreases in the order G(N7) > T(N3) > C(N3) > A(N7), A(N1). Although this parallels the preference of cisplatin for binding with guanine over adenine [81], the diamino Ru(II) arene complexes are more highly discriminatory between G and A bases than Pt(II) complexes. This site selectivity appears to be enhanced by the en NH_2 groups, which H-bond with exocyclic oxygens (*e.g.* C6O of G) but are non-bonding and repulsive towards exocyclic amino groups of the nucleobases (*e.g.* C6NH_2 of A). For mononucleotides [77], the same pattern of site selectivity is observed; in competitive reactions of $[(\eta^6\text{-arene})Ru(en)Cl]^+$ with 5′-GMP, 5′-AMP, 5′-CMP and 5′-TMP, the only final adduct is $[(\eta^6\text{-arene})Ru(en)(5'\text{-GMP-}N^7)]^{2+}$. Significant amounts of the 5′-phosphate-bound species (40–60%) are also present at equilibrium for 5′-TMP, 5′-CMP and 5′-AMP. Reactions with nucleotides proceed *via* aquation of $[(\eta^6\text{-arene})Ru(en)Cl]^+$ followed by rapid binding to the 5′-phosphate group, and then rearrangement to give N7-, N1- or N3-bound products.

Comparative studies have shown that $[(\eta^6\text{-arene})Ru(en)Cl]^+$ complexes (arene = bip, DHA, THA or cym) bind relatively rapidly to calf thymus DNA at 310 K, with 50% binding in 3 h for the cym complex and 10–15 min for the others [82]. Circular dichroism and differential pulse polarography data suggest that the bip and anthracene complexes cause nondenaturational changes in DNA conformation (like cisplatin); in contrast, the cym complex distorts the DNA more severely (like transplatin). The circular dichroism data also suggest that intercalation and/or minor groove binding to calf thymus DNA are involved in the binding of the bip and anthracene derivatives, but not the cym complex [82]. These data have been also confirmed by NMR studies [83]. More recent studies [84] have revealed unique modes of binding of the anticancer complex $[(\eta^6\text{-bip})Ru(en)Cl]^+$ to the 14-mer DNA duplex III:

$$A_1\ T_2\ A_3\ C_4\ A_5\ T_6\ G_7\ G_8\ T_9\ A_{10}C_{11}A_{12}T_{13}A_{14}$$

$$T_{28}A_{27}T_{26}G_{25}T_{24}A_{23}C_{22}C_{21}A_{20}T_{19}G_{18}T_{17}A_{16}T_{15}$$

The monofunctional fragment $\{(\eta^6\text{-bip})Ru(en)\}^{2+}$ is highly specific for N7 of G, but mobile at elevated temperature where migration between guanines is facile. In contrast, such migration of Pt(II)-am(m)ino complexes is rare. This behaviour suggests that organometallic Ru(II) arene complexes can be readily removed from DNA, which may be beneficial for reversing DNA

damage in cells. The sites of ruthenation and intercalation on duplex III are G7, G8, G18 and G25. The specificity of $\{(\eta^6\text{-bip})Ru(en)\}^{2+}$ for G is aided by strong H-bonding between an NH of en and C6 carbonyl of G, and by π-π stacking involving the noncoordinated phenyl ring of the biphenyl ligand and DNA bases. Such stacking can occur *via* intercalation between DNA bases (G and T bases in the adducts III-Ru-G7 and III-Ru-G18i, where i = intercalated), or with a partially extruded T base, as in adduct III-Ru-G18*n* (*n* = non-intercalated). Arene-base stacking may play a role in determining the rates of reactions of Ru(II) arene en complexes with DNA, as appears to be the case for mononucleotides [77]. Other examples of metal complexes containing DNA-intercalating ligands include Ru(II), Rh(III) and di-Rh(II) complexes with phenanthroline derivatives [85] and Pt(II) complexes with directly-bound or pendent acridine arms [86]. It is well known that DNA structures are relatively flexible. Some intercalators can rotate within intercalation sites [87]. Our data suggest that ruthenium arene intercalation is dynamic in nature: equilibria can exist between intercalated (duplex III-Ru-G18i) and non-intercalated (duplex III-Ru-G18n) conformers. Our studies provide a structural basis for understanding how that the nature of the arene in $[(\eta^6\text{-bip})Ru(en)Cl]^+$ complexes can exert a significant effect on cytotoxicity [71], on excision-repair of DNA lesions [88], and on DNA destabilization [82].

Reactions between the sulfur-containing amino acids cysteine and methionine and ruthenium(II) arene anticancer complexes are of much interest in view of the strong influence of sulfur amino acids on the intracellular chemistry of platinum drugs, in particular their involvement in detoxification and resistance mechanisms [89]. Protein targets may also play a role in the mechanism of action of Ru(II) arene complexes, including the possibility that ruthenium can substitute for iron in proteins. We found that $[(\eta^6\text{-bip})Ru(en)Cl]^+$ reacts slowly, and only to about 50% completion, with the thiol amino acid L-cysteine in aqueous solution at 310 K, pH 2–5, and a 1:2 molar ratio [90]. Reactions appeared to involve aquation as the first step followed by initial formation of 1:1 adducts *via* substitution of water by S-bound or O-bound cysteine. Two dinuclear complexes were also detected as products from the reaction. These arise from the loss of chelated ethylenediamine, and contain one or two bridging cysteines. The unusual cluster species $\{(\eta^6\text{-bip})Ru\}_8$ was also formed, especially at higher cysteine concentrations. Reactions with cysteine are suppressed in 50 mM triethylammonium acetate solution at pH *ca*. 5 or in 100 mM NaCl, suggesting that thiols may not readily inactivate Ru(II)-en arene complexes in blood plasma or in cells. Similarly, interaction with the thioether sulfur of methionine appears to be relatively weak. Only 27% of $[(\eta^6\text{-bip})Ru(en)Cl]^+$ reacted with L-methionine at an initial pH of 5.7 after 48 h at 310 K, and gave rise to only one adduct, $[(\eta^6\text{-bip})Ru(en)(L\text{-MetH-}S)]^{2+}$.

In recent work [91], competition between the tripeptide glutathione and cGMP for the complex $[(\eta^6\text{-bip})Ru(en)Cl]^+$ was investigated using HPLC, LC–MS and [^{1}H,^{15}N] NMR spectroscopy. In unbuffered solution (pH *ca*. 3), the reaction with GSH gave rise to three intermediates: an S-bound thiolato adduct $[(\eta^6\text{-bip})Ru(en)(GS\text{-}S)]$ and two carboxylate-bound glutathione products $[(\eta^6\text{-bip})Ru(en)(GSH\text{-}O)]^+$ during the early stages (<6 h), followed by en displacement and formation of a tri-GS-bridged dinuclear Ru(II) complex $[((\eta^6\text{-bip})Ru)_2(GS\text{-}\mu\text{-}S)_3]^{2-}$. Under physiologically-relevant conditions (micromolar ruthenium concentrations, pH 7, 22 mM NaCl, 310 K), the thiolato complex $[(\eta^6\text{-bip})Ru(en)(GS\text{-}S)]$ was unexpectedly readily oxidized by dioxygen to the sulfenato complex $[(\eta^6\text{-bip})Ru(en)(GS(O)\text{-}S)]$ instead of forming the dinuclear complex $[((\eta^6\text{-bip})Ru)_2(GS\text{-}\mu\text{-}S)_3]^{2-}$. Under these conditions, competitive reaction of $[(\eta^6\text{-bip})Ru(en)Cl]^+$ with GSH and cGMP gave rise to the cGMP adduct $[(\eta^6\text{-bip})Ru(en)(cGMP\text{-}N^7)]^+$ as the major product, accounting for *ca*. 62% of total ruthenium after 72 h, even in the presence of a 250-fold molar excess of GSH. The oxidation of coordinated glutathione in the thiolato complex $[(\eta^6\text{-bip})Ru(en)(GS\text{-}S)]$ to the sulfenate derivative $[(\eta^6\text{-bip})Ru(en)(GS(O)\text{-}S)]$ appears to provide a facile route for displacement of S-bound glutathione by G(N7). Redox reactions of cysteinyl adducts of these Ru(II) arene anticancer complexes could therefore play a significant role in their biological activity.

The patterns of activity established *in vitro* for $[(\eta^6\text{-bip})Ru(en)Cl]^+$ are mirrored to a large degree *in vivo*, with the compound effecting significant growth delays against both cisplatin-sensitive and -resistant human ovarian A2780 tumours grafted on mice (xenografts) whilst being inactive against the Adriamycin-resistant xenograft [74]. Recently [92], $[(\eta^6\text{-bip})Ru(en)Cl]^+$ has been evaluated in a 13-cell line panel. Particular sensitivity (*ca*. 10-fold lower than mean IC_{50}) was noted in breast cancer and non-small cell lung cancer cell lines. In addition, it caused a significant growth delay in a xenograft model.

It is intriguing to consider the possible design of Os(II) arene anticancer complexes. In Group 10 of the Periodic Table, the stark contrast between the rapid reaction kinetics of the $4d^8$ ion Pd(II) compared to the $5d^8$ ion Pt(II) has led to difficulties in designing active palladium(II) complexes. If there is a parallel in Group 8, then strategies for the kinetic activation of Os(II) will be required. In general, as a third-row transition metal ion, Os(II) might be expected to be relatively inert compared to the second row ion Ru(II). However, the presence of an arene ligand can greatly increase the kinetic lability [93], and, importantly, our data show that the chelating ligand also plays a major role.

With en as the chelating ligand, hydrolysis of the Os(II) complex $[(\eta^6\text{-bip})Os(en)Cl]^+$ is *ca*. 40 times slower than that of the Ru(II) analogue, and the pK_a value (6.34) of the resulting aqua adduct is 1.4 units lower (Fig. 9) [94]. This implies that the less-reactive hydroxo adduct would predominate at physiological pH (7.4). Indeed, reactions with 9-ethylguanine were slower and occurred to a lower extent than those of the Ru(II) complexes.

In contrast, the acac complexes $[(\eta^6\text{-arene})Os(acac)Cl]$ (Fig. 9) hydrolyze rapidly (<10 min, 298 K) to give a mixture of a monoaqua complex, $[(\eta^6\text{-arene})Os(acac)(H_2O)]^+$ and an hydroxo-bridged dimer, $[(\eta^6\text{-arene})Os(\mu^2\text{-}OH)_3Os(\eta^6\text{-arene})]^+$ with loss of the acac ligand. Surprisingly this dimer forms readily even at micromolar concentrations and even in the presence of 0.1 M chloride (biological test conditions). The pK_a values of the coordinated water molecule in the monoaqua acac arene complexes is *ca*. 1.8 units lower than that of the Ru(II) analogues, for which the hydroxo-bridged dimer is not observed as an hydrolysis product (except in very basic solu-

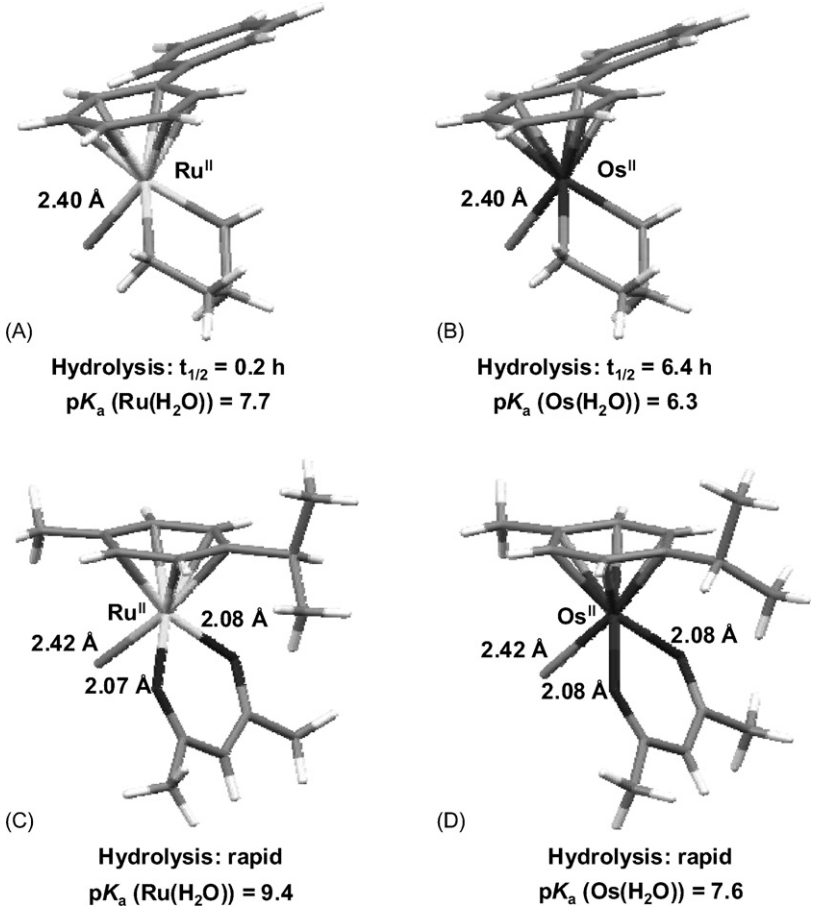

Fig. 9. X-ray crystal structures and comparison of the solution properties of Ru(II) arene complexes and their Os(II) analogues: (A) $[(\eta^6\text{-bip})Ru(en)Cl]^+$; (B) $[(\eta^6\text{-bip})Os(en)Cl]^+$; (C) $[(\eta^6\text{-cym})Ru(acac)Cl]$; (D) $[(\eta^6\text{-cym})Os(acac)Cl]$.

tions, and then only as a very minor product). The monoaqua acac complex $[(\eta^6\text{-arene})Os(acac)(H_2O)]^+$ reacts with purine bases but not with pyrimidines; this is the same base selectivity that was observed for the Ru(II) analogues [75]. However, the hydroxo-bridged dimer, $[(\eta^6\text{-cym})Os(\mu^2\text{-OH})_3Os(\eta^6\text{-cym})]^+$ is unreactive toward both purine and pyrimidine bases. This may be the key to the inactivity of the Os(II) arene acac complexes, since the hydroxo-bridged dimer was the only species present in micromolar aqueous solutions similar to those used in the biological tests [94].

These results demonstrate how the kinetics of ligand substitution reactions of Os(II) arene complexes in aqueous solution can be controlled by variation of the chelating ligand. Further tuning of substitution rates may be achievable by variations of the chelating ligand, monodentate leaving group and the arene. Such a systematic approach should contribute to the rational design of Os(II) arene complexes as potential anticancer agents. Preliminary data on complexes of the general type $[(\eta^6\text{-arene})Os(N,O\text{-chelate})Cl]$ [95], have allowed us to design complexes with high *in vitro* cytotoxicity.

5. Conclusions

It is exciting to explore the potential use of the wide range of elements in the Periodic Table as therapeutic (and diagnostic) agents. Not least because over 20 elements are thought to be essential for mammalian biochemistry. However, there is debate about the essentiality of several of them, *e.g.* chromium, nickel, silicon, tin. They may be essential, but we currently know very little about their biochemistry. As genome sequences became available, we should ask how codes (if they exist) for the elements are incorporated into genomes. Already we can read the sequences in terms of their protein products to recognize specific binding patterns for metal ions, such as zinc (over 600 gene products, especially zinc finger proteins containing $(His)_2(Cys)_2$ binding motifs, *ca.* 10% of genome), and for copper and for iron [14]. But metal binding proteins are not totally specific for individual metal ions, and it is a matter of the relative binding constants (Fig. 10) and rates (ligand exchange kinetics). It will be a challenge to deliver metal ions correctly to their specific pathways to correct abnormalities, or indeed to remove metal ions which end up in the wrong place.

In this short review, we have focused on recent work from our own laboratory involving both essential (Zn) and non-essential (Pt, Ru) metals. The work on antiviral and stem-cell-mobilizing zinc macrocycles illustrates how protein recognition can depend in subtle ways on direct bonding of the metal to amino acid side-chains, on the configuration of the macrocycle, on H-bonding interactions between cyclam NH groups and axial ligands, and hydrophobic interactions between the periphery of the ligand

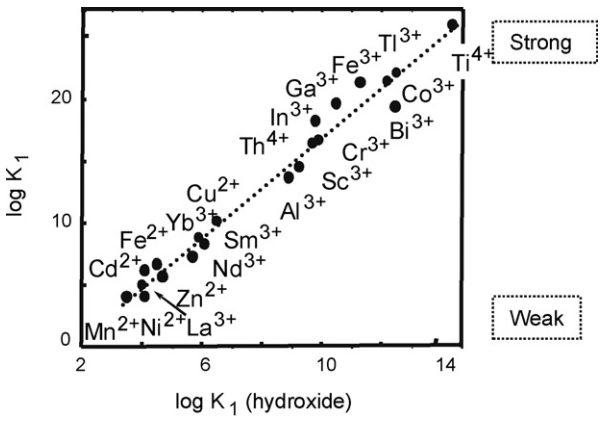

Fig. 10. Strength of metal binding to human transferrin (adapted from H. Li, P.J. Sadler, H. Sun, Eur. J. Biochem. 242 (1996) 387, with additional data for Ti(IV) from A.D. Tinoco, A.M. Valentine, J. Am. Chem. Soc. 127 (2005) 11218).

and hydrophobic protein side-chains (*e.g.* indole rings of Trp residues). Constraint of the macrocycle could therefore lead to even more potent agents, and this has been demonstrated [29].

Platinum(IV) diazido complexes offer the prospect of non-toxic pro-drugs which can be activated *in vivo* selectively by light at the sites where tumour cell destruction is required. Light activation allows the use of electronically excited states in biochemical reactions and gives the prospect of achieving novel reactions which cannot be readily achieved with ground-state complexes. For example, bis-guanine adducts can readily be produced upon photoexcitation of *trans* diamine platinum complexes, whereas monoguanine adducts are readily formed in the ground state. Such reactions can lead to DNA cross-links which may not be readily recognized by repair enzyme systems and lead to novel cytotoxicity pathways.

Organometallic "piano-stool" ruthenium and osmium arene complexes contain a range of features useful in drug design: variation of the arene, and the "legs" of the stool, either monodentate or chelating ligands. These ligands can exert fine control over both the thermodynamics and kinetics of reactions of importance to their pharmacology. These include hydrolysis reactions of Ru-halide bonds, the acidity of Ru-OH_2 adducts, and steric and electronic effects (including intercalation into DNA) of the arene ligand. Studies of the aqueous solution chemistry of these complexes under biological test conditions (micromolar concentrations) sometimes reveal reactions which cannot readily be predicted, leading, in the case of Os(II) arene acac complexes, to inert hydroxo-bridged dimers. This knowledge allows the re-design of complexes to achieve activity.

Despite the long period which has elapsed since the introduction of cisplatin (over 30 years), and the demonstration that the activity of platinum complexes depends intimately on the oxidation state, nature and geometry of the ligands, it is not yet widely accepted that similar considerations may well apply to many other metals. Part of the excitement of medicinal coordination chemistry stems from the possibility of demonstrating that this is the case. The exploration of such coordination chemistry offers the real prospect of providing new understanding of intractable diseases and of devising novel therapeutic and diagnostic agents.

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