APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2005; **19**: 1–10
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.725

Development of organometallic (organo-transition metal) pharmaceuticals†

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Received 2 February 2004; Accepted 9 February 2004

This paper is aimed at introducing the organometallic chemist to the fascinating area of organometallic pharmaceuticals. It commences by identifying the properties of organometallic (transition metal) compounds that lend themselves to medical applications. Next, the specialized techniques and methods that are used to assess the medicinal properties of compounds are summarized, and although these techniques are not restricted to organometallic compounds, all examples are concerned with organometallic compounds. The design and evaluation of organometallic compounds for medicinal applications are described in context with the diseases they have been evaluated against, and areas are identified that may have most potential for organometallic pharmaceuticals. Some new results, including the first example of an organo-osmium compound that might exhibit effective anticancer properties, are also described. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: bioorganometallic; transition-metal-based drugs; anticancer; antiviral; antimicrobial

INTRODUCTION

The field of bioorganometallic chemistry (or more strictly bioorgano-transition metal chemistry) is now a recognized sub-discipline within organometallic chemistry, standing alongside more mature areas such as catalysis and organometallic materials chemistry. Two of the leading innovators in the field, Fish and Jaouen, recently summarized the core subjects that make up the field of bioorganometallic chemistry in a review published in *Organometallics*;[1] and a previous review on bioorganometallic chemistry that highlighted the potential of the subject is given by Jaouen *et al*.[2] Various bioorganometallic topics have been identified, including the synthesis of biologically relevant compounds via catalysis or organometallic intermediates, catalytic action on biomolecules, the synthesis of organometallic compounds with biologically relevant co-ligands, organometallic immunoassays, host–guest chemistry and molecular recognition, bioimaging, biosensors and bioprobes, and the use of organometallic compounds as pharmaceutical products in their own right. In this paper, originally presented at the XVth FECHEM Conference on Organometallic Chemistry, we describe the various properties of organometallic compounds that make them suitable for pharmaceuticals applications, together with the methods/strategies used to facilitate the development of organometallic pharmaceuticals.

CURRENT STATUS OF ORGANOMETALLIC PHARMACEUTICALS

The field of organometallic pharmaceuticals is not a new one and dates back to the pioneering work of Köpf and Köpf-Maier towards the end of the 1970s, who investigated the antitumour activity of early transition-metal cyclopentadienyl complexes.[3] Although organometallics have been evaluated most extensively as reagents to combat cancer, presumably since the coordination compound cisplatin remains the most widely used anticancer drug (still used to treat approximately 70% of all cancer patients since its discovery in 1965),[4] other diseases have also been investigated, including parasitic, viral, microbial and most recently cardioprotection using metal carbonyl complexes.[5] Table 1 lists the various organo-transition-metal compounds that have been evaluated for

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†Based on a lecture presented at the XVth FECHEM Conference on Organometallic Chemistry, held 10–15 August 2003, Zürich, Switzerland.
Contract/grant sponsor: Swiss National Science Foundation.
Contract/grant sponsor: COST (Switzerland).
Contract/grant sponsor: EPFL.

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Table 1. Organometallic compounds evaluated as pharmaceuticals for various diseases

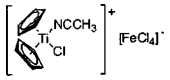
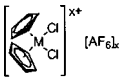
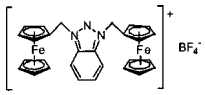
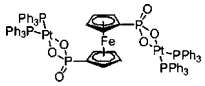
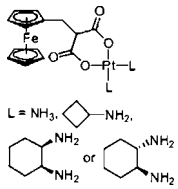
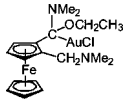
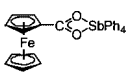
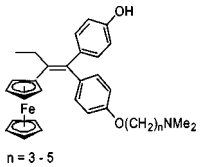
Compound	Disease	Comment	Ref.
 M= Ti, Nb or Mo	Cancer	Activity against various tumours (Ehrlich ascites tumour (EAT), sarcoma 180, B16 melanoma, colon 38 carcinoma and Lewis lung carcinoma). Titanocene dichloride is in Phase II clinical trials	5, 6, 9
	Cancer	Active against Ehrlich ascites tumour cells	7
 M = Nb, Mo or Re A = Sb [Nb, Mo] or As [Re] x = 1 [Nb, Re] or 2 [Mo]	Cancer	Active against Ehrlich ascites tumour cells	8, 9
 X = halide, acac, SCN, N_3 etc.	Cancer	Several complexes based on $V(Cp)_2$ have antitumour and antiproliferative properties. The most potent is $[(Cp)_2V(NCSe)_2]$	10
 X= I_3^-, picrate or $CCl_3CO_2^-$	Cancer	I_3^-: inhibit the development of experimentally reinoculated tumours. Active against Rauscher leukaemia virus. Picrate and $CCl_3CO_2^-$: 100% cure against EAT in CF1 mice	11, 12
	Cancer	Inhibits the development of experimentally reinoculated tumours	6
	Cancer	Moderate activity against P388 leukaemia cells (phosphonic acids inactive)	13
	Cancer	Good activity against both adriamycin-sensitive and -resistant P338 murine leukaemia cells (effect higher than cisplatin)	14
	Cancer	$IC_{50} = 1.5$ for HT1376 (bladder) $IC_{50} = 7.4$ for SW620 (colon)	15
	Cancer	Active against KB and HeLa human neoplastic cell lines	16
 n = 3 - 5	Oestrogen-dependent cancers	Antiproliferative activity on mammary tumours (MCF7, RT × 6, TD5, MDA-MB231). Ferrocifen is about to enter Phase I clinical trials	17,18

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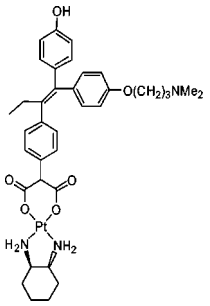
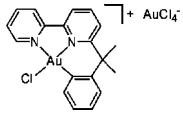
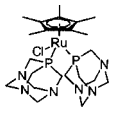
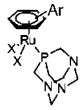
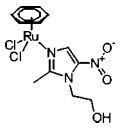
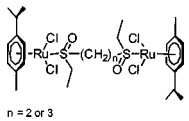
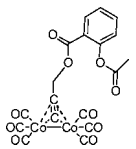
Table 1. (*Continued*)

Compound	Disease	Comment	Ref.
	Oestrogen-dependent cancers	Antiproliferative activity on mammary tumour MCF7 and abtioestrogenic effect on MVLN cells	19
 X= Cl, OAc	Cancer	Active against *Staphylococcus aureus* and *Enterococcus faecalis*. More cytotoxic to CHO cells than cisplatin; equally against HT1376 bladder tumour and SK-OV-3 ovarian tumour cells	20
	Cancer	Active against cisplatin-resistant A2780S, A2780R and SKOV3 tumour cell lines	21
	Cancer	Tested on TS/A murine adenocarcinoma tumour cells	22
 X = Cl, Br, I or SCN	Cancer and antimicrobial activity	Active on hypoxic tumour cells and microbes	23,24
	Cancer	Active on hypoxic cells	25
 L= DMSO, 3-aminopyridine	Cancer	Topoisomerase II poisoning. Active against *in vitro* breast and colon carcinoma cells	26,27
	Cancer	Inhibition of the growth of the human ovarian cancer cell line A2780	28
 n = 2 or 3	Cancer	Dimethylsulfoxide (DMSO)(MTT) assays show *in vitro* anticancer activity against a human mammary cancer line with IC_{50} values of 360 ($n = 2$) and 55 μM ($n = 3$)	29
	Cancer	More active than cisplatin on MCF-7 and MDA-MB-231 mammary tumour cells lines	30,31
	Cancer	Activite on Ehrlich ascites tumors	32

(*continued overleaf*)

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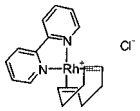
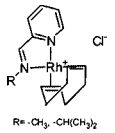
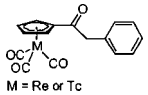
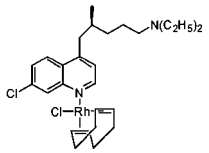
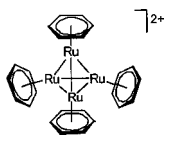
Table 1. (*Continued*)

Compound	Disease	Comment	Ref.
	Cancer	Effective on L1210 leukaemia	30,31
	Cancer	Activity against MCa mammary carcinoma, Lewis lung carcinoma and lung metastatic tumours	33
	Cancer	Activity comparable to cisplatin on Ehrlich ascites carcinoma	34
$[RhI(cod)L_2]^+X^-$ cod = 1,3-cyclooctadiene, L = classical antiparasitic drug (*benznidazole*, nifurtimox, niridazole) and X = Cl^-, ClO_4^-, NO_3^-, $[BPh]^-$ and the anion of ethylfumaric acid	Cancer	Studied on mice with Ehrlich ascitic, Landschutz ascitic, S-180 and P-388 leukaemic tumours	35,36
$[Rh(CO)_2L]$ L = sulfamethoxydiazine, dithiocarbamate, diphenyl-dithiocarbamate	Cancer	Active in different biological systems	37,38
	Cancer	Radiopharmaceutical applications in diagnosis (^{99m}Tc) and therapy (^{188}Re and ^{186}Re)	39,40
	Cancer	Radiopharmaceutical applications in diagnosis (^{99m}Tc) and therapy (^{188}Re and ^{186}Re)	41
	Antimalaria	Equipotent to chloroquine	42
	Cardio-protective action	Cardiac cells pretreated with CORM-3 (10 to 50 μM) become more resistant to the damage caused by hypoxia–reoxygenation and oxidative stress	43
	Polio	Active in BSC-1 cells (African Green Monkey kidney cells ATCC CCL 26) infected with Polio virus type 1 (Pfizer vaccine strain)	24

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their therapeutic properties. One compound, titanocene dichloride, is already in Phase II clinical trials,[44] and a second compound, ferrocifen, which is a ferrocene derivative of tamoxifen,[19,45] looks set to enter clinical trials in the near future (Jaouen G, personal communication). Main-group organometallic complexes are not listed in Table 1, although some compounds such as diorganotin(IV) derivatives have been known to have antiproliferative activity on specific cancers for many years.[46,47]

It is evident from Table 1 that the majority of organometallic compounds have been evaluated as anticancer compounds, resulting from the success of cisplatin in the treatment of many different types of tumour.[48,49] However, a number of generalizations can be made regarding the utility of organometallics in pharmaceutical applications and, accordingly, compounds can be categorized as follows.

(1) Those where the ligands are labile and are displaced prior to reaching the diseased cell. For example titanocene dichloride, while being a stable compound by organometallic standards, readily loses the cyclopentadienyl rings under physiological conditions. In fact, such ligand loss could be important in its therapeutic action, as it has been found that $Ti(\eta\text{-}C_5H_5)_2Cl_2$ binds to the iron-transport protein transferrin with displacement of both cyclopentadienyl rings and it induces a similar conformational change to iron(III) binding.[50] It has been hypothesized that transferrin might serve as a potential drug transport and delivery system,[51] since many diseased cells have a high iron(III) requirement to facilitate rapid cell growth, which may be satisfied by increasing the number of transferrin receptors on the cell surface,[52] thereby sequestering a greater amount of the circulating metal-loaded transferrin.
(2) Attaching organometallic groups to coordination complexes that have proven therapeutic activity. For example, ferrocene has been linked to both platinum and gold centres, which have well-characterized anticancer effects. In a slight modification to this strategy, organometallics with ligands related to those in effective anticancer coordination complexes are also used in combination with organic ligands. For example, the diamine ligand in $[Ru(\eta\text{-arene})(en)Cl]^+$ has some similarity to the ammonia ligands of cisplatin.[28]
(3) Attaching organometallic groups to compounds of known biological function. Perhaps the best example here is the substitution of the phenyl ring in tamoxifen with a ferrocene unit. The resulting compound, ferrocifen, is expected to enter clinical trials very soon.[17,18] The most widely used antimalarial drug, chloroquine, is no longer active on many parasites, which have developed resistance to its widespread use over many years. However, attaching organometallic fragments, either via coordination bonds or covalent interactions, restores activity to the compound.[42]
(4) Harnessing functional organometallic ligands that have well-defined properties in organometallic chemistry which could be exploited in biological systems. Such a concept may be illustrated using $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2(pta)]$ (pta = 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane), which does not affect the growth of healthy cells, but is toxic to hypoxic cells (those with slightly lower pH than normal cells, such as cancer cells).[22] It would appear that the pta ligand is of critical importance in this mechanism, in that it could facilitate the ability of the compound to cross a cell membrane and exhibit the pH-dependent DNA damage.
(5) Water-soluble organometallics based on radioactive elements such as ^{99m}Tc, ^{188}Re and ^{186}Re can be used to diagnose and potentially treat cancer.[53,54] In these compounds it is the radioactive metal centre that provides the activity, although the ligands attached to the metal centre will ultimately determine the selectivity and efficiency of these compounds to target diseased cells.

Although the classifications given above are quite useful, not all the compounds fit well into them. The cluster $[H_4Ru_4(\eta^6\text{-}C_6H_6)]^{2+}$ is difficult to classify and was essentially discovered following a large screening of water-soluble organometallic compounds.[24] Other organometallic cluster compounds, including $Ru_3(CO)_9(pta)_3$, $[Pt_3(\mu_3\text{-CO})(\mu\text{-dppm})_3]^{2+}$, $HOs_3(CO)_9(\mu\text{-L}-H)L'$ (L = 3-amino quinoline, $L' = Na_3[PC_6H_4SO_3)_3]$ or $[P(OCH_2CH_2NMe_3)_3]I_3$; L = 3-(2-phenyl acetimido) quinoline, $L' = [P(OCH_2CH_2NMe_3)_3]I_3$ or L = phenanthridine, $L' = [P(OCH_2CH_2NMe_3)_3]I_3$) and $Rh(\mu_3\text{-}S)_2(\eta\text{-}C_5Me_5)_3]^{2+}$, have also been tentatively postulated as exhibiting pharmacological properties based on their ability to damage DNA.[55,56] Interest in clusters originates from the possibility of specifically targeting them to the tumour site, exploiting differences between the healthy and tumour tissues. The 'enhanced permeability and retention' effect is a common difference that results in a dramatic increase in blood vessel permeability within diseased tissues compared with normal tissues.[57] The normal endothelial layer surrounding the blood vessels feeding healthy tissues is intact, restricting the size of molecules that can diffuse from the blood. In contrast, the endothelial layer of blood vessels in diseased tissues is more porous to large molecules, providing access to the surrounding tissue. Further, diseased tissue does not generally have a lymphatic drainage system; hence, once macromolecules have entered the tissue they are retained. With size-dictated selectivity in mind, a water-soluble dendrimer-platinate compound that slowly releases platinum *in vitro* is currently being evaluated.[58]

Preliminary biological characterization of potential organometallic pharmaceuticals

Usually, the first screen for activity of any potential pharmaceutical compound, which may provide an estimate of how the drugs will behave *in vivo*, is to study the effect of the compound on cell culture. Normal cells do not grow in

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tissue culture, but the genetic modifications that take place in cancer cells allow them to be grown *in vitro*. Caution should always be applied to the results obtained from cell studies, as the actual environment differs from that of real systems. At the start of the experiment, growth conditions are optimized; thus, the environment is more like that of healthy cells in the body. However, as the cells grow and waste products accumulate, the environment is more representative of hypoxic tumour cells. At this point the cells will begin to die, so most experiments use cancer cells growing under optimum conditions, which are incubated with the compound of interest to observe whether it causes the cells to die. One of the simplest ways to determine the 'health' of cells (or cell death) in culture is colorimetrically using the dye MTT. In healthy cells, MTT is reduced in the mitochondria to form formazan crystals, the concentration of which can be determined spectrophotometrically following dissolution in dimethylsulfoxide (DMSO). If a compound damages a cell or inhibits cell growth, then the mitochondrial activity is reduced and this reduction can be quantified spectrophotometrically, which in turn can be correlated to drug activity.

The cytotoxicity of a compound is helpful in determining the usefulness of the compound for a specific application, but it does not give an insight into the molecular mechanism of drug activity. It is very difficult to identify all of the biomolecules with which the drug interacts, due to the complexity of mammalian cells; each cell contains at least 10 000 different proteins plus other small molecules, nucleotides and lipids, not to mention the molecules that the compound may interact with or be modified by under physiological conditions.

An important biological target for metal-based anticancer drugs is DNA, since DNA replication is integral to the progression of these diseases; typically, the modifications to the control system of the cell that occur in diseased cells make them more likely to die as a result of DNA damage than healthy cells. The differences in recovery level of DNA-damaged healthy and cancer cells are thought to be one mechanism by which cisplatin exhibits its effect. The gel shift assay represents a simple and rapid method to probe drug–DNA modifications. Typically, compounds are incubated with DNA and then separated by electrophoresis and stained with a dye enabling the visualization of the DNA and, consequently, the effect the compound has had on the DNA. Figure 1 shows a stained gel after electrophoresis, which has been used to monitor the effect of some ruthenium compounds on the structure of plasmid DNA. The first lane on the left (lane 1 in Fig. 1) contains unmodified DNA, which consists of about 5% open circular (OC) DNA and 95% supercoiled (SC) DNA. When the gel in incubated with $[H_4Ru_4(C_6H_6)_4]^{2+}$ or $Ru_3(CO)_9(pta)_3$, lanes 2 and 3 respectively, DNA damage alters the pattern of migration, whereas incubation with $H_4Ru_4(CO)_{12}$, lane 4, does not affect migration of the DNA species, suggesting that there is no interaction under these conditions (possibly due to poor aqueous solubility).

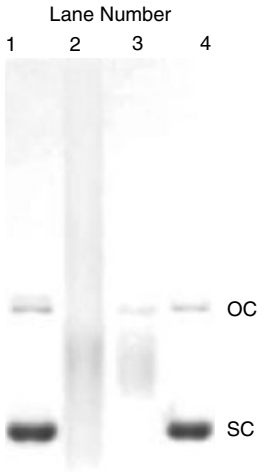

Figure 1. An agarose gel showing the effect of organometallic clusters on the structure of DNA.

Despite the focus on DNA as a target for cancer therapy, it is highly likely that this is not the only target in the molecular mechanism of drug activity, and alternative assays are used and new ones are being developed. Increasingly, compounds are designed with a specific biological target in mind. The advantage of rational drug design is that there are a number of direct assays that can be performed looking at both specific interactions and drug activity in whole cells, which are not possible if the target is unknown. For example, topoisomerase II is a common target for a range of drugs, including anticancer, antimalarial and antiviral compounds. Topoisomerase II is an enzyme that plays an important role in replication, transcription, recombination and segregation of chromosomal pairs during cell division. The gene expressing the enzyme has been cloned from a number of sources and topoisomerase II can be made recombinantly, providing an easily accessible, pure and homogeneous source for characterization. Availability of the enzyme in pure form allows the effect of the drug on enzyme activity to be probed directly, and by using gel electrophoresis ruthenium compounds have been shown to inhibit topoisomerase II.[26] The interaction of metallodrugs with plasma proteins is also relevant, as platinum and ruthenium compounds may bind to proteins such as serum transferrin and albumin (see below), which are normally used to deliver iron to the cells. Figure 2 shows the results of IEF gel electrophoresis used to investigate the interaction of metal complexes with transferrin. In this experiment the gel has a pH gradient and the protein migrates until it reaches the pH that neutralizes its charge so that it is no longer affected by the potential difference applied across the gel. The majority of the apo-transferrin (lane 1) sample is focused just below pH 6.5, with a fraction that contains some residual iron focusing below pH 6.0. When the sample is incubated with iron this distribution reverses (lane 2), showing that iron interacts. Similarly, cisplatin (lane 3) and $Ru(\eta^6 - p\text{-cymene})Cl_2(pta)$ (lane 4) interact with the protein

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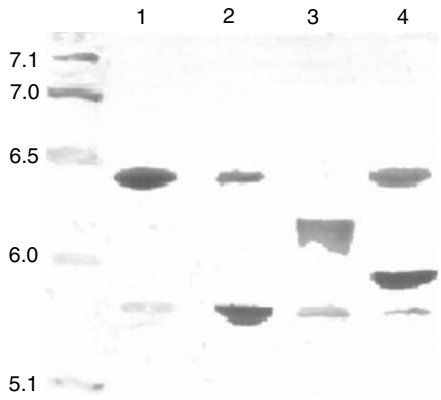

Figure 2. Polyacrylamide isoelectric focusing (IEF) gel electrophoresis of: apo-transferrin (lane 1); transferrin incubated with an iron(III) compound (lane 2); transferrin incubated with a platinum(II) compound (lane 3), transferrin incubated with a ruthenium(II) compound (lane 4).

and alter its charge (Allardyce CS, Dyson PJ, unpublished results). Although IEF gel electrophoresis shows that there is an interaction between the protein and the metal complexes, it does not provide any information regarding the binding of the platinum(II) and ruthenium(II) species. Mass spectrometry has been used to provide information on the actual drug binding site in the protein.[59]

Investigating drug interactions with proteins from whole-cell extracts represents a major challenge. In addition to the interactions at the specific binding site of a protein, non-specific interactions may occur with various proteins in the cell, which could lead to undesirable side effects. Therefore, it is useful to test compounds with complex mixtures of protein or whole-cell systems to gauge the general toxicity and identify non-specific interactions. Protein gel electrophoresis methods can be used to resolve species from whole-cell extracts into individual bands or spots, and these methods, including two-dimensional gel electrophoresis, play an integral role in proteomics methods. Despite the many advances that have made proteomics a powerful tool for probing metabolic disease, these techniques alone are of limited use when trying to identify drug targets. Recently, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been used to identify regions of gels that contain metals such as platinum and ruthenium from protein samples extracted from cells treated with the drug.[60] Subsequent extraction of the protein band where the metal drug has been identified by the ICP-MS experiment and identification of the protein using proteomics methods rapidly identifies the protein targets of the drug. Although application of LA-ICP-MS to identify drug targets is still in its infancy, the power of the technique to pick out drug–protein adducts from hugely complicated systems could lead to accelerated drug discovery.

RUTHENIUM-BASED ANTICANCER DRUGS

Despite the success of platinum-based anticancer compounds, there is still a need for other drugs due to the inability of platinum compounds to tackle some types of cancer of high social incidence and the side effects induced by the platinum compounds in clinical use. Apart from platinum, the anticancer properties of ruthenium compounds (both coordination and organometallic) are now being studied intensively. The first ruthenium anticancer drug [ImH][*trans*-$RuCl_4$(DMSO)Im], NAMI-A, is progressing through clinical trials,[61] and another compound [ImH][*trans*-$RuCl_4Im_2$], KP1019, is poised to enter clinical trials.[62] The nature of the cation is also important, as the sodium salt of [*trans*-$RuCl_4$(DMSO)Im] was too unstable to enter clinical trials. Recently, some anionic transition-metal complexes have been combined with the 1-butyl-3-methylimidazolium cation, which gives rise to a low melting-point salt that is liquid at room temperature, and stable.[63,64] Using this cation with anticancer compounds might facilitate drug delivery, and we intend to explore this possibility in the near future. Based on a number of different ruthenium compounds, there appear to be three main properties that make ruthenium compounds well suited to medicinal application:

(1) The ligand exchange kinetics of ruthenium(II) and ruthenium(III) complexes are similar to that of platinum(II) complexes. Ligand exchange is an important determinant of biological activity, as very few metal drugs reach the biological target without being modified, and although some exchange reactions are essential for inducing the appropriate therapeutic properties, others can lead to drug deactivation and detoxification.
(2) Ruthenium is unique amongst the platinum group metals, in that the oxidation states II, III and IV are all accessible under physiological conditions. Ruthenium(III) complexes tend to be more biologically inert than related ruthenium(II) and ruthenium(IV) complexes. In biological systems, glutathione, ascorbate and single-electron-transfer proteins are able to reduce ruthenium(III) and ruthenium(IV), and molecular oxygen and cytochrome oxidase readily oxidize ruthenium(II). The redox potential of ruthenium compounds can be exploited to improve the effectiveness of drugs. For example, a ruthenium(III) prodrug can be administrated, which is activated by reduction in diseased tissues. In many cases the altered metabolism associated with cancer results in a lower oxygen concentration in these tissues (compared with healthy tissues) and elevated levels of glutathione, and this promotes a reducing environment. If the active ruthenium(II) complex leaves the low oxygen environment, it may be converted back to ruthenium(III) by a variety of biological oxidants.
(3) It is thought that ruthenium may mimic iron in binding to biological molecules such as serum transferrin and albumin, reducing the general toxicity of ruthenium

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drugs. These two proteins are used by mammals to solubilize and transport iron, and since rapidly dividing cells, e.g. cancer cells, have a greater requirement for iron, they increase the number of transferrin receptors located on their cell surface in order to sequester more of the circulating metal-loaded transferrin.

A number of ruthenium compounds have been shown to bind to DNA *in vitro*, and there appears to be a correlation between DNA binding and the cytotoxicity of ruthenium(III) amine complexes in tissue culture. The mode of DNA binding by certain ruthenium complexes involves cross-links between DNA strands, presumably favoured by the steric restrictions imposed by the octahedral geometry of the complexes. This binding mechanism differs from the intrastrand cross-links favoured by cisplatin; consequently, the cancer cell lines that have developed resistance to cisplatin by accelerating the rate of repair of intrastrand cross-links are often susceptible to ruthenium anticancer drugs.

RUTHENIUM(II)–ARENE COMPLEXES

One of the main problems associated with ruthenium coordination complexes, in terms of progress into clinical trials, is their instability and complicated ligand exchange chemistry. Accordingly, it has been postulated that the increased stability of organoruthenium complexes might provide better drug candidates. Several years before much of the current interest in ruthenium anticancer compounds began, the organometallic compound $Ru(\eta^6\text{-}C_6H_6)Cl_2$(metronidazole) (metronidazole = 1 − β-hydroxyethyl-2-methyl-5-nitroimidazole; see Table 1) was evaluated as an anticancer agent by Dale *et al*.[25] Although it was found that the complex had a greater selective cytotoxicity than metronidazole itself under hypoxis reducing conditions, as far as we are aware further studies were not forthcoming. In the last few years, however, several groups have commenced investigating related ruthenium(II)–arene compounds, but each with a slightly different strategy.

Sadler and co-workers[28,50] have studied ruthenium (II)–arene complexes with ethylenediamine ligands, viz. $[Ru(\eta^6\text{-arene})Cl(dien)]^+$ (dien = ethylenediamine). They are stable, quite soluble in water and exhibit anticancer activity both *in vitro* and *in vivo*, including activity against cisplatin-resistant cancer cells. The use of the ethylenediamine ligand derives from an analogy with the ammonia ligands in cisplatin, which are thought to participate in the cytotoxicity by forming a hydrogen bond with the DNA in addition to the covalent interactions. Since DNA represents a potential target for ruthenium(II) complexes, studies were carried out which indicate strong and selective binding to N7 of guanine bases on DNA oligomers. The reactivity of the various binding sites of nucleobases toward $[Ru(\eta^6\text{-biphenyl})Cl(dien)]^+$ decreases in the order G(N7) > I(N7) > I(N1), T(N3) > C(N3) > A(N7), A(N1), and such site selectivity appears to be controlled by the ethylenediamine NH_2 groups, which hydrogen bond with exocyclic oxygenations, but are non-bonding and repulsive toward the exocyclic amino groups of the nucleobases. It also appears that hydrophobic interactions between the arene ligand and DNA could facilitate DNA binding, and a direct correlation between cytotoxicity and the size of the arene was observed. Combined *in vitro* and *in vivo* data suggest that the most active complexes contain the most hydrophobic η^6-arene ligands. It also appears that arene–purine-basestacking plays a significant role in stabilizing the transition state in the reaction of the complex with DNA; the rate of reaction of cGMP with $[Ru(\eta^6\text{-arene})(en)Cl]^+$ decreased in the order: tetrahydroanthracene > biphenyl > dihydroanthracene >> *p*-cymene > benzene, suggesting that N7 binding is promoted by favourable arene–purine hydrophobic interactions in an associative transition state. Reactions of complexes containing tetrahydroanthracene, biphenyl and dihydroanthracene, which can take part in $\pi-\pi$ stacking, are up to an order of magnitude faster than those containing arenes, which cannot.

The anticancer properties of ruthenium coordination complexes with sulfur-based ligands have been widely explored and show considerable promise, presumably leading to their evaluation in organometallic systems by James and co-workers.[29] They prepared a series of ruthenium(II)–arene compounds with disulfoxide ligands and tested them *in vitro* for anticancer activity. It was found that the most active were naturally charged compounds, which is in keeping with other studies, including our own. Sheldrick and co-workers have also explored the interactions of the ruthenium(II)–arene derivatives $[Ru(\eta^6\text{-arene})(amino\ acid)(dppz)]^{n+}$ ($n = 1–3$), where dppz is the large intercalator co-ligand dipyrido[3,2-*a*:2′, 3′-*c*]phenazine.[65] It was found that the preferred groove for DNA binding via intercalation of the dppz ligand depends on the steric bulk of the ruthenium–arene unit and other ancillary ligand. Bulky arene ligands disfavour front-on intercalation and deeper dppz penetration, although specific intercalation interactions were still observed.

We have focused our attention on the properties of pta in the complexes $Ru(\eta^6\text{-arene})Cl_2$(pta). This ligand has been widely used in aqueous-organic biphasic catalysis (e.g. see Refs 66–70), and one property that we thought might lend itself to the physiological environment is that protonation of the pta ligand influences solubility, with the protonated species having a higher solubility in water and the deprotonated species having a higher solubility in hydrophobic solvents. Such a property could be useful in facilitating the migration of pta complexes across cell membranes and in increasing the likelihood of DNA interactions. Although the mechanism by which $Ru(\eta^6\text{-arene})Cl_2$(pta) binds to DNA remains uncertain, binding is pH dependent, being greater at lower pH. Furthermore, the compounds are inactive in P388 tumour

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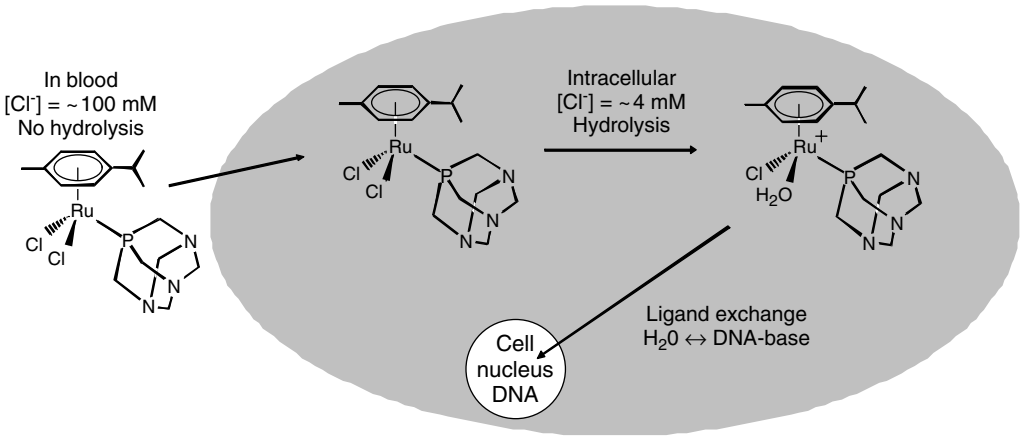

Figure 3. Exchange of the chloride ligands with water is prevented outside of the cell by the high free chloride concentrations. Inside the cell, ligand exchange occurs, activating the compounds for covalent modification of biological targets.

cells grown at the pH typical of healthy cells, whereas at the slightly lower pH characteristic of a real cancer cell *in vivo* the Ru(η^6-arene)Cl_2(pta) complexes inhibit their growth (many diseased cells have a reduced pH, due to metabolic changes, in part associated with the accelerated cell division). Some progress has been made in delineating the events that lead to activity of the Ru(η^6-arene)Cl_2(pta) compounds, and these are summarized in Fig. 3. It is worth noting that naturally charged Me–pta$^+$ analogues Ru(η^6-arene)Cl_2(pta–Me)$^+$ are toxic to cells grown at both normal and low pH.

Further studies are currently in progress to establish the way in which Ru(η^6-arene)Cl_2(pta) complexes interact with DNA. It is interesting to note that the binding of cisplatin to DNA also increases at low pH, according to a recent mass spectroscopic study. The cyclopentadienyl complexes Ru(η^5-arene)Cl(pta)$_2$ (arene = C_5H_5 or C_5Me_5) have been screened against TS/A murine adenocarcinoma tumor cells and only the latter was found to inhibit cell proliferation.[22] The effect of pH was not examined.

Although the interactions of the ruthenium(II)–arene compounds described above have been most extensively studied with DNA, Ru(η^6-C_6H_6)Cl_2(Me_2SO) has been shown to inhibit topoisomersae II.[26,27] In light of these studies, the ethylenediamine derivatives were screened for activity as topoisomersae II inhibitors, but they were found to be inactive. However, the pta derivatives have been shown to have very specific interactions with proteins, at least compared with cisplatin, using the LA-ICP-MS method described above, and we intend to report on the principal protein interactions of the Ru(η^6-arene)Cl_2(pta) compounds in due course.

OTHER DIRECTIONS

Ruthenium(II)–arene complexes are also finding applications in other areas with potential medical applications. For example, Severin and co-workers[71,72] have recently shown that trinuclear metallamacrocycles based on the ruthenium(II)–arene unit, as well as other related systems, display an extremely high affinity and selectivity for lithium and sodium ions. Since lithium salts such as Li_2CO_3 are among the most frequently used drugs for the treatment of manic depression, and owing to its narrow therapeutic range, the Li^+ concentration in the blood of the patients needs to be carefully controlled, and compounds like [($C_6H_5CO_2Et$)Ru(L)]$_3$ (L = 3-oxo-pyridonate ligand) selectively extract LiCl from an aqueous solution containing a large excess of alkali and alkaline earth metal salts that could prove to be of therapeutic use.

Several metal–arene or metal–cyclopentadienyl compounds related to ruthenium(II)–arene compounds are also being studied as potential anticancer drugs. For example, it should be feasible to prepare osmium analogues of any ruthenium(II)–arene complex, and in this context we have prepared Os(η^6-*p*-cymene)Cl_2(pta) and commenced evaluating its anticancer properties. In fact, osmium analogues of all the ruthenium(II)–arene complexes might be interesting to study, as the rate of ligand exchange is slower and this might be better suited to physiological conditions. Other related compounds prepared in our laboratory include Rh(η^5-C_5Me_5)Cl_2(pta) and [Rh(η^5-C_5Me_5)Cl(pta)$_2$]$^+$, and we will report on their biological activity in due course.

Acknowledgements

We thank the Swiss National Science Foundation, COST (Switzerland) and the EPFL for financial support.

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