Metal based drugs: from serendipity to design†

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The platinum anticancer drug cisplatin has made a major contribution to the treatment of testicular and ovarian cancer. This chance discovery has been the stimulus for research into other metal-based drugs. Inorganic chemistry offers many opportunities for medicinal chemistry, and the discovery of metal-based drugs has moved on from chance discovery to rational drug design. There are however, many challenges associated with the drug discovery and development process. The aim of this review is to provide case histories exemplifying the role of rational drug design in modern inorganic medicinal chemistry in the context of these challenges. The evolution of platinum drugs from cisplatin to third generation drugs is described. The molecular target for the platinum agents is DNA. Alternative molecular targets such as thiol-containing proteins and redox processes are proposed. The example of a simple, safe, efficacious metal-based drug, Fosrenoltm, is reviewed.

Introduction

Upon reviewing the literature on metal-based drugs from the perspective of the first decade of the 21st century it is fascinating to realize that the history of metals in medicine dates back to the earliest times of recorded history.† The author is indebted to many reviewers who have documented this, and whose writings have stimulated and inspired his own interest in this field. Several of these reviews are cited in the bibliography and the reader is encouraged to delve into these papers for his or herself. It is worth noting that metal-based medicines have been used since the early days of civilization. The medical use of gold can be traced back to 2500 BC in China. The ancient Egyptians used copper to sterilize water, and the Greek physician Hippocrates used mercury in 400 BC. The seventeenth century Swiss alchemist and physician Paracelsus pioneered the use of minerals in medicine using antimony, arsenic and mercury salts such as mercurous chloride as a diuretic. An English contemporary, the herbalist Nicholas Culpepper, advocated a gold elixir aurum potabile for ailments caused by a decrease in the vital spirits including melancholy, fainting, fevers and falling sickness.

The beginning of the 20th century saw metals making an impact on modern medicine with Erlich’s discovery of the arsenic organometallic drug Salvarsan for the treatment of syphilis. Other metal-based treatments for infectious diseases included antimony compounds for the treatment of the parasitic disease leishmaniasis, and gold cyanide for tuberculosis. The latter was championed by the German bacteriologist Robert Koch. Towards the end of the nineteenth century Robert Koch discovered the bacteriostatic properties of gold cyanide against the tubercle bacillus. The investigation of gold therapy for the treatment of rheumatoid arthritis resulted from the suggestion that the tubercle bacillus was the causative agent for this disease. Though ineffective against tuberculosis gold compounds were found to be efficacious against rheumatoid arthritis and as a result gold drugs have been in use since the early part of the twentieth century for the treatment of rheumatoid arthritis. The two most commonly used drugs were sodium aurothiomalate and aurothioglucose. Both are polymeric and have to be given by intramuscular injection. In 1985 an orally bioavailable, monomeric gold(t) phosphate drug was introduced for rheumatoid arthritis, Auranofin™.‡ These drugs, with their limited efficacy have since been superseded by more modern drugs.

A diversity of metal compounds is now available.¶ Minor gastrointestinal ailments can be addressed by over-the counter agents such as magnesium and aluminium oxides, calcium carbonate, and bismuth subsalicylate. Lithium carbonate is used to treat manic depression, and sodium nitroprusside is used in emergency surgical situations as an anti-hypertensive. One of the major medical breakthroughs for metal-based drugs was the
The multiple challenges of drug discovery and development

Before embarking on a voyage it is worthwhile looking ahead at some of the things you may need on the journey. Likewise with drug discovery it is helpful to be aware of the many challenges and hurdles that it is necessary to overcome on the way to new drug approval. These challenges are similar whether the drug is a small organic molecule, a biological macromolecule such as a protein or nucleic acid, or an inorganic molecule.

The first challenge is to identify a disease target. There are numerous unmet medical needs examples being intractable solid tumors such as glioblastoma, and colon cancers, autoimmune inflammatory diseases such as rheumatoid arthritis, and intensive care problems such as septic shock. However, identifying an unmet need is only the first stage, it is also necessary to identify a molecular target that is associated with the disease etiology and pathology. In the case of cancer DNA is a common molecular target though recent advances have identified other targets such as growth factor receptors. For an autoimmune disease, or an acute disease involving a multitude of inflammatory mediators such as septic shock the target is less clear. Though it is now possible to identify putative targets using genomics and proteomics, target validation is frequently a major challenge. This can be addressed by using antibodies to the target, gene knockout mouse models, or gene knockdown using siRNA. Once a molecular target has been identified it is necessary to establish systems to screen new chemical entities against the desired target. This challenge provides an ongoing debate within the drug discovery community. Over recent years the trend has been towards high throughput screening, usually in conjunction with large compound libraries. Screens utilizing a single molecular target such as an enzyme or receptor with a suitable readout of activity have been popular, however with the improvements in technology, particularly imaging technology, high content cell-based assays are becoming increasingly popular.

Turning a structural lead into a drug-like molecule is a significant challenge for the medicinal chemist. Many drug candidates fail either in late development or early stage clinical trials due to poor pharmacokinetic characteristics, i.e. the inability of the drug to reach its molecular target in vivo. This is defined by the ADME (absorption, distribution, metabolism and excretion) properties of the molecule. A set of rules for the organic medicinal chemist have been proposed by Lipinski, known as the Lipinski rule of 5, which can help to identify orally bioavailable drugs. The original four rules which make up the rule of 5 for a drug are that the molecule should: (1) have no more than 5 hydrogen bond donators, (2) no more than 10 hydrogen bond acceptors, (3) a molecular weight of less than 500, and (4) a partition coefficient (log P) of less than 5. Note: the rule of 5 refers to the multiples of 5 of the desired properties, not the number of rules. These rules have since been extended. Following the rules is not a guarantee that a molecule will be an ideal drug candidate, but empirical evidence indicates that it increases the likelihood of a molecule being “drug-like.” It should be borne in mind that these rules were based on the properties of approved drugs, all of which are organic molecules. These rules may not necessarily apply to inorganic molecules, but the principle of producing novel agents with “drug-like” properties is an important one.

The “drug-like” properties of a molecule are frequently investigated early in the drug discovery process using in vitro ADME tests. These can include an investigation of drug metabolism using liver microsomes, a sub cellular fraction containing the cytochrome P450 metabolizing enzymes. P450 inhibition can also be assessed in vitro, this is important to assist in predicting drug–drug interactions. Other tests can predict absorption either using artificial biological membranes or epithelial cell monolayers such as CaCo-2 cells. Toxicity can also be addressed at this early stage either using in vitro cytotoxicity or incorporating an in vivo maximum tolerated dose (MTD) study. Drug development candidates that pass all these tests can then proceed into more extensive toxicology studies and subsequently into Phase I clinical trials to assess toxicity and preliminary pharmacokinetics. In these later stages of drug development toxicity becomes increasingly important as the aim of any drug discovery program is to develop not only efficacious, but safe drugs.

The next crucial step is to be able to design a suitable proof-of-principle Phase II clinical trial. Ideally this should be a small, cost-effective trial with adequate end points, which can confirm clinical activity. One disease area where this is increasingly seen as a major challenge is in the development of cancer drugs, particularly those aimed at novel molecular targets. Many cancer drug candidates fail in Phase III trials because of inadequate proof-of-principle Phase II trials. This is not necessarily the fault of particular investigators, more it is the challenge of designing trials with suitable endpoints. Traditionally the readout for cancer drug trials has been shrinkage.

serendipitous discovery of the potent anti-tumor activity of the platinum drug cisplatin. Barnett Rosenberg was interested in the resemblance between the mitotic spindle of dividing cells and the orientation of iron filings around a magnetic field. In order to investigate this he took Eschericia coli, a prokaryote, grown in a culture apparatus containing platinum electrodes. Under these conditions the bacteria grew as large filaments. Subsequent investigations demonstrated that the filamentous growth was caused by the formation of platinum ammine salts. He then took the intuitive leap to test complexes of this type for anticancer activity hence leading to the discovery of cisplatin. The discovery of this drug, which is still widely used to treat testicular cancer, has been a major stimulus for inorganic medicinal chemistry drug discovery. These research efforts have not been limited solely to the search for improved platinum-based therapies, but also for alternative metals and for new therapeutic uses.

The discovery of the gold and platinum drugs was the result of chance discovery, serendipity. However rational design of metal-based drugs has become increasingly important, and must continue to be so, if we are to make new advances in metal-based drug discovery. These rules may not necessarily apply to inorganic molecules, but these rules which make up the rule of 5 for a drug are that the molecule should: (1) have no more than 5 hydrogen bond donators, (2) no more than 10 hydrogen bond acceptors, (3) a molecular weight of less than 500, and (4) a partition coefficient (log P) of less than 5. Note: the rule of 5 refers to the multiples of 5 of the desired properties, not the number of rules. These rules have since been extended. Following the rules is not a guarantee that a molecule will be an ideal drug candidate, but empirical evidence indicates that it increases the likelihood of a molecule being “drug-like.” It should be borne in mind that these rules were based on the properties of approved drugs, all of which are organic molecules. These rules may not necessarily apply to inorganic molecules, but the principle of producing novel agents with “drug-like” properties is an important one.

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of the primary tumor. However this endpoint may be unsuitable for drugs aimed at some of the newer targets where the aim is to reduce angiogenesis, cause tumor stasis, or inhibition of metastasis. Much thought is now going into designing new clinical trial protocols for agents targeting these processes.8

This leads us into the final challenge where we come full circle, developing a drug discovery and development strategy. To do this it can be argued that one should work backwards, initially asking the question, “Is it possible to design a cost-effective proof-of-principle clinical trial?” If this cannot be done, then however subtle the chemistry, and however brilliant the concept, the challenges of turning an idea into a drug may be insurmountable. Clinical trials can be very costly, particularly if large patient numbers are required. This is particularly an issue for small companies.

With the majority of drugs being organic molecules what does inorganic medicinal chemistry have to offer over traditional organic medicinal chemistry, and what are the distinctive properties of inorganic chemistry which allows metal-based drugs to successfully overcome the challenges described above? Metals can coordinate ligands in a precise three-dimensional configuration thus allowing the tailoring of the molecule to recognize and interact with a defined molecular target. This is further enhanced by the diversity of opportunity for chemical modification of ligands. Transition metals possess different oxidation states, which not only allows for modification of the three-dimensional space into which the molecule can fit, but significantly allows them to participate in biological redox chemistry. In addition the ability to undergo ligand exchange reactions offers opportunities for metals to interact and coordinate with biological molecules.

Tackling the challenges of drug discovery outlined above may be regarded as being more appropriate for a drug company, but having a view of a drug development pathway is also important from an academic perspective if one is truly serious about developing a realistically useful drug. The following case studies on metal-based drug discovery will be reviewed retrospectively with these challenges in mind.

Platinum cancer drugs

Cisplatin

Arguably the discovery of cisplatin was one of the most significant events for cancer chemotherapy in the 20th century. Cisplatin, a square planar Pt(II) complex (cis-dichlorodiamine platinum(II)) was approved for clinical use in 1978, and has become first line therapy for the treatment of testicular cancer. Platinum drugs are now widely used for the treatment of testicular and ovarian cancers.8

Cisplatin acts by binding to DNA.3,4 In aqueous biological media the chloro ligands are replaced by water to give a diaquo species which interacts with DNA to form inter- and intra-strand cross-links. The lesion causing cancer cell death is the intrastrand cross-link between adjacent guanine bases on the DNA strand. Interestingly the frequency of this lesion (> 60%) is too high to be accounted for by simple statistics and the cis-ammine ligands appear to interact with the DNA phosphate backbone to specifically direct this simple molecule in an orientation such as to promote this specific lesion. Cisplatin has several limitations, it is very toxic, particularly towards the kidneys and has to be given as a large volume intravenous infusion, it is not orally bioavailable, and there is a population of cancer cells which either are inherently resistant, or acquire resistance. These problems have led to extensive medicinal chemistry programs in many laboratories. Perhaps the most successful research program has been the collaboration between Johnson Matthey, and later AnorMED; and the Institute of Cancer Research and the Royal Marsden Hospital, UK (Fig. 1).

![Fig. 1 Structure of platinum complexes discovered and developed by the Johnson Matthey/AnorMED-Institute of Cancer Research collaboration.](Image)

Carboplatin: overcoming toxicity

Toxicity was associated with the rapid aquration of cisplatin allowing it to potentially interact with biological ligands en route to the target molecule, DNA. This process is slower with anionic chelating ligands such as O-donor malonates. Opening of the chelate ring allows formation of a monoqua species, however the reverse reaction can also occur readily. The chelate is subsequently more stable than the dichloro complex thus allowing more time for the drug to reach the target molecule. The monoqua species can react with N-donor ligands such as DNA. The subsequent adduct is sufficiently stable to allow displacement of the second carboxylate and formation of the DNA cross-link. Carboplatin (JM8), with the ligand cyclobutanedicarboxylic acid (CBDCA) was found to have reduced nephrotoxicity compared with the parent molecule cisplatin.89 Though its cytotoxicity towards cancer cells was an order of magnitude less then cisplatin this was compensated for by the reduced toxicity. Carboplatin has a similar clinical activity to cisplatin, significantly there is cross-resistance with cisplatin, not surprisingly as the mechanism of action is similar to cisplatin. Carboplatin was approved for clinical use in 1986.

Satraplatin: the first orally bioavailable platinum drug

Though the toxicity problem was overcome, the cross-resistance and lack of oral bioavailability led to the search for a third generation drug. Compounds with an ammine ligand and an...
organic amine ligand, the mixed amines, were investigated with the hypothesis that the mixed amine ligands would give a different interaction with DNA. Platinum(IV) complexes were investigated during the search for a second-generation platinum drug, as the Pt(IV) complexes were more inert to substitution than equivalent Pt(II) complexes. Iproplatin (JM9), with hydroxyl axial ligands, had reduced nephrotoxicity compared with cisplatin, but was less efficacious in tumor models than carboplatin. However, it was hypothesized that replacement of the hydroxyl ligands with carboxylate ligands would increase the compound lipophilicity and hence give an orally bioavailable drug. The combination of the mixed amine series with Pt(IV), led to the discovery of satraplatin, JM216, the first orally bioavailable platinum drug. Satraplatin, with an ammine and cyclohexylamine ligands, and axial acetato ligands, with an aqueous solubility of 0.3 mg ml$^{-1}$ in saline, 0.7 mg ml$^{-1}$ solubility in octanol, and with a partition coefficient of 0.1, was found to be orally bioavailable whilst retaining anti-tumor activity. Satraplatin was found to have nonlinear pharmacokinetics in patients, but this was overcome by a dose-splitting schedule, first identified in pre-clinical studies using human tumor xenografts, the dose being split between 5-daily doses. Satraplatin has a complex biotransformation pathway with both Pt(IV) and Pt(II) metabolites. In addition it is an inhibitor of several cytochrome P450 enzymes. This is reflected in the combination studies with etoposide, which is a substrate for CYP3A4. Lower doses of both are required in combination to avoid toxicity. This highlights the importance of performing early ADME (absorption, distribution, metabolism, excretion) studies as part of the drug discovery process. Satraplatin was found to have a similar activity profile to cisplatin and carboplatin in tumor models, but interestingly was also active against some acquired cisplatin-resistant tumor cell lines, which may be due to a different interaction with DNA, and/or susceptibility to different DNA repair mechanisms. Satraplatin is now (March 2007) in late stage Phase III clinical trials for hormone-refractory prostate cancer. GPC Biotech filed an NDA (New Drug Application) with the FDA in February 2007.

**Picoplatin: a strategy to overcome resistance**

In parallel with the search for an orally bioavailable drug the Johnson Matthey–Institute of Cancer Research collaboration was focused on the search for a drug which could overcome cisplatin resistance. A twofold approach was adopted, the combination of novel chemistry with mechanism and disease-oriented biological testing. There are three main mechanisms of resistance to platinum drugs. (1) repair of the DNA–platinum lesion, (2) reduced intracellular accumulation or transport and (3) interaction with thiol-containing molecules such as glutathione leading to an inactive drug. The latter involves ligand substitution reaction with the platinum center. Attention of these substitution reactions should give a compound with reduced susceptibility to resistance via this mechanism. This was the rationale for the synthesis of picoplatin (JM473, ZD473, AMD473). Picoplatin is a Pt(II) mixed amine complex, cis-[amminedichloro(2-methylpyridine)platinum(II)]. The thiol substitution reaction with cisplatin occurs via an associative mechanism. The steric bulk provided by replacing one of the amines with a methyl substituted pyridine shifts the mechanism of substitution from an associative to a dissociative mechanism, thus lowering the rate of substitution and allowing the drug more time to reach its target, DNA.

The key feature of the biological testing was the use of a panel of ovarian cell lines, treatment of ovarian cancer being one of the main clinical applications of platinum drugs. This tumor cell panel contained three pairs of cisplatin sensitive and resistant cell lines, 41M/41McisR, CH1/CH1cisR, and A2780/A2780cisR. This cell line panel encompassed the main known mechanisms of cisplatin resistance. The mechanism of resistance in 41McisR was primarily due to decreased drug accumulation/drain transport, enhanced DNA repair/tolerance for CH1cisR, and a combination of decreased drug transport, enhanced DNA repair/tolerance, and elevated intracellular glutathione levels for A2780cisR. Picoplatin was found to be similarly active against the cisplatin sensitive and resistant cell lines with resistance factors (the ratio of the IC$_50$ of the resistant cell line to the sensitive cell line) of 1.3, 2.5 and 1.9 against the 41M, CH1, and A2780 cell line pairs, compared with cisplatin, which had resistance factors of 4.7, 6.4 and 16.2 respectively. This in vitro activity was reflected in the in vivo activity against solid tumor xenografts of the same cell lines. The dose limiting toxicity of picoplatin was myelosuppression with no nephrotoxicity. Furthermore mechanistic studies indicated that picoplatin bound to DNA in a different fashion to cisplatin and carboplatin, suggestive of a unique DNA lesion. Interestingly picoplatin is also orally bioavailable and was active against the CH1cisR xenograft when given by this route.

Picoplatin was initially licensed to AstraZeneca by AnorMED and tested in several clinical trials. Though demonstrating activity in cisplatin sensitive patients, it showed only minor improvement over cisplatin in cisplatin-resistant patients. This activity did not meet the activity requirements set by AstraZeneca who decided to halt development. However promising activity was seen in Phase II studies, responses were seen in platinum-resistant patients with ovarian cancer, non-small cell lung cancer, small cell lung cancer (SCLC) and mesothelioma. Picoplatin is now being developed by Poniard and is in clinical trials against small cell lung cancer, colorectal cancer, and prostate cancer.

The evolution of the platinum cancer drugs is a wonderful example of how inorganic chemistry has met a number of pharmacological and clinical challenges, by taking a fortuitous discovery and turning it into a drug. Though this initial discovery was a result of chance the subsequent success stories have been the result of combining chemical design with a focused development strategy. First to successfully tackle the challenge of toxicity by using a rational chemical approach. Then improving the properties to give an orally bioavailable molecule, and finally addressing the problem of drug resistance, one of the major challenges with cancer cytotoxic drugs.

**New molecular targets**

**Overturning the platinum paradigm**

Much of the early work on metal-based compounds focused on their interaction with DNA based on the platinum paradigm. The success of cisplatin and carboplatin stimulated the search for other transition metal-based cancer drugs. A wide range of metals were studied including palladium, iridium, rhodium, ruthenium, tin, copper, and gold. However it is now increasingly apparent that
many metal complexes with potential for antitumor activity do not behave like cisplatin. A series of ruthenium-arene complexes have been designed to interact with DNA in a novel fashion compared to the platinum drugs. Compounds such as RM175 are designed to interact with DNA in a bifunctional manner by both intercalation and direct metallation.\textsuperscript{15} Ruthenium complexes such as indazolium [bis-indazolotetrachlororuthenate] (KP1019), and imidazolium [trans-imidazolemethythiosulphidotetrachlororuthenate], NAMI-A have significantly different properties to cisplatin. The former, which has promising activity against models of colorectal cancer, is redox activated and is transported by the transferrin system.\textsuperscript{16} NAMI-A is only weakly cytotoxic, but interestingly has a profound and selective inhibitory effect on tumor metastasis (Fig. 2).\textsuperscript{17}

![Image](https://via.placeholder.com/150)

**Fig. 2** Overturning the “platinum paradigm.” Structures of metal complexes with biomolecular interactions significantly different to the DNA cross-linking of platinum anticancer drugs.

There are several examples where metal-based drugs target biological processes other than DNA replication. The superoxide anion, \(\text{O}_2^-\), is a mediator of both acute and chronic inflammation. Under normal circumstances \(\text{O}_2^-\) levels are controlled by the superoxide dismutase (SOD) metalloenzymes. The SOD enzymes are a class of oxidoreductase enzymes containing either copper or manganese at the active site. Small molecule SOD mimetics have been evaluated as therapeutic agents. A manganese(II) pentaazamacrocyclic complex, M40403, has been shown to have catalytic activity equivalent to the native enzyme, have activity in models of inflammatory disease, and has successfully completed a Phase I clinical trial.\textsuperscript{18}

Protein phosphorylation by kinase enzymes, and subsequent dephosphorylation by phosphatase enzymes, is a major regulatory mechanism for protein activity and transmission of intracellular signals. Staurosporine, an indolocarbazole alkaloid, is a relatively non-selective protein kinase inhibitor. Cyclopentadienyl halftsandwich ruthenium complexes are being used as a structural scaffold for staurosporine mimetics (Fig. 2). By combining features of the indolocarbazole within the half-sandwich complex highly potent and specific protein kinase inhibitors have been identified for the protein kinases Pim1, MSK1, and glycogen synthase kinase (GSK3\textalpha.).\textsuperscript{19} Insulin binds to the insulin receptor activating the Insulin Receptor Tyrosine Kinase (IRTK) triggering a sequence of downstream events. Down-regulation of insulin signaling is mediated by IRTK-associated phosphatases. Oxovanadium compounds, both V(IV) vanadyl, and V(V) vanadate, have been investigated as small molecule insulin mimetics.\textsuperscript{20} One of the most extensively studied compounds is bis(maltolato)oxovanadium(IV), also known as BMOV (Fig. 2). BMOV is orally absorbed, and lowers plasma glucose levels in streptozotocin-induced diabetic rats. An analogue of BMOV, bis(ethylmaltolato)oxovanadium(IV) (BEOV) has completed a Phase I clinical trial. Oxovanadium compounds have been shown to exert their insulin-mimetic effect by inhibiting IRTK-associated phosphatases, thus activating the insulin receptor. The situation is more complex with vanadyl compounds. It has been suggested that vanadyl may be oxidized to vanadate, alternatively vanadyl may directly stimulate cytosolic protein kinases thus bypassing the insulin receptor. Protein phosphorylation therefore presents itself as a unique target for metal-based drugs.

There has been an ongoing interest in gold compounds for the treatment of cancer. The mechanism of gold anti-arthritis drugs is probably multi-factorial, resulting in modulation of the immune response.\textsuperscript{21} The early promising indication of activity in animal cancer models for gold(I) phosphine compounds, ([Au(dppe)]\textsubscript{2}Cl was a lead candidate in preclinical development until it was found to be cardiotoxic) prompted an interest in gold compounds. These initial studies pointed towards DNA as the target for these compounds. Recent work with gold(I) carbene complexes with potential antitumor activity indicate, however, that they target mitochondrial membranes causing mitochondrial membrane permeability, possibly by an interaction with the mitochondrial permeability transition pore.\textsuperscript{22}

Our own work with Au(III) cyclometalated complexes has also shown that DNA is not the target for these molecules. In collaboration with UMIST, UK, we adopted an integrated approach in our search for new metal-based cancer drugs. This incorporated inorganic medicinal chemistry, \textit{in vitro} screening using panels of human tumor-derived cell lines, \textit{in vivo} testing using human tumor xenograft models, and mechanistic studies.\textsuperscript{23} Au(III) was chosen as its complexes are square planar, with Au(III) being isoelectronic with Pt(II). Au(III) complexes are generally very reactive with the gold being readily reduced to Au(II). However the Au(III) structure is stabilized by using a single mononegative bidentate chelating ligand, \(2-[(\text{dimethylamino})\text{methyl}]\text{phenyl}\), (damp). This ligand forms part of a 5-membered chelate ring with Au(III) in which the nitrogen of the amine and the carbon of the phenyl ring bond to the metal. The remaining two coordination positions are taken by either two monodentate ligands (chloride, acetato) or one bidentate ligand (oxalato, malonato, thiosalicyalato (Fig. 3A)). These ligands are readily hydrolyzed allowing substitution reactions with biological molecules.\textsuperscript{24}
Initial testing was undertaken using two different human tumor cell line panels. The primary panel consisted of cells from tumors representative of different tissue types, and different sensitivities to cisplatin. Differential cytotoxicity, as opposed to non-selective toxicity, was used as a selection criterion. Most of these cell lines could be grown as solid tumor xenografts. The hydrolysable ligands significantly affected the solubility of the compounds with the chloro and oxalato complexes being less soluble than the acetato and malonato complexes. This was reflected in the better \textit{in vivo} activity of the acetato complex against the tumor xenografts.

Mechanistic studies focused on the acetato complex which had modest \textit{in vivo} activity against human tumor xenografts (Fig. 3B). The ability of this compound to interact with DNA was investigated using a number of techniques. The initial studies examined the binding of the compound to a closed circular DNA plasmid, Col E1, by monitoring its mobility on an agarose gel.

Fig. 3  Gold(III) 2-[(dimethylamino)methyl]phenyl (damp) complexes. (A) Structure. (B) Antitumor activity against the CH1 ovarian tumor xenograft. (C) Comparison of cisplatin and [Au(damp)(acetato)$_2$] on plasmid mobility.
The plasmid exists in two forms, a relaxed form, and a supercoiled form. A DNA binding agent such as cisplatin significantly changes the mobility of the plasmid by unwinding the supercoiled form, making it more bulky and impeding its mobility on the gel. The acetato-analogue [Au(acetato)(damp)] was only able to do this at concentrations up to 1500 times the concentration required to kill tumor cells in vitro (Fig. 3C). Alkaline elution was used to assess DNA cross-linking. This is a filtration technique in which cross-linking impedes the flow of DNA through a filter. DNA cross-linking by cisplatin can be easily demonstrated using this technique. Unlike the platinum drug [Au(acetato)(damp)] was unable to cross link DNA. Cisplatin causes a block in the cell cycle at the point where DNA repair occurs, the S/G2 interface. This can be assessed using flow cytometry. Cells are stained with propidium iodide, a DNA fluorochrome that binds quantitatively to DNA. The degree of fluorescence is therefore directly proportional to the DNA content of the cell, which in turn is an indicator of the point in the cell cycle that the cells are in at a given time. Using this technique it was apparent that [Au(acetato)(damp)] was not exerting a cell cycle specific effect. Further supporting evidence came from pharmacological studies. The acetato complex was similarly active against the CH1 and CH1cisR cell line whose mechanism of resistance is repair of cisplatin/DNA lesions. Also [Au(acetato)(damp)] had little activity against the mouse ADJ/PC6 tumor, which is known to be particularly sensitive to compounds which cross-link DNA.

Collectively these data point to a different mechanism of action of the Au(III) compounds indicating that the analogy of d⁸, square planar Au(III) and Pt(II) complexes does not extend to their interaction with cells. ¹³C-NMR studies of model reactions with biological ligands showed that the damp complexes have a preference for S-donor ligands such as glutathione and cysteine, with only limited reactivity against nucleosides and their bases. Though the activity of these compounds was modest compared with the platinum complexes described above, the example of the Au(III) damp complexes demonstrates that the combination of an appropriate testing strategy, coupled with novel chemistry, can point to new structural classes with novel mechanisms of action.

It is therefore apparent that there are other potential molecular targets for metal-based drugs. As discussed earlier the properties of the transition metals lend themselves to drug discovery, particularly those pertinent to interactions with biological molecules. Two features in particular are the ability to undergo ligand exchange reactions, and the ability to participate in redox reactions. Interaction with DNA is an example of the first, and the activation by reduction of ruthenium complexes is an example of the second. With the plethora of available molecular and biochemical targets to choose from which of these are amenable to attack by the inorganic chemist? Two examples are given below, cysteine proteases, and the free radical nitric oxide.

**Biological thiol-containing proteins**

Cysteine proteases such as the lysosomal protease cathepsin B contain a cysteine at their active site. This cysteine acts in concert with a histidine, the two together forming a stable thiolate-imidazolium ion pair. Nucleophilic attack by the thiolate on the carbonyl carbon of the peptide bond results in the formation of an acyl intermediate. The collapse of the acyl intermediate results in release of the cleaved peptide and regeneration of the active site.

Cysteine proteases have been implicated in the pathophysiology of several diseases including inflammatory airway diseases, bone and joint disorders, parasitic diseases, and cancer. Cathepsin K is a validated drug target for bone diseases such as osteoporosis. It is found in osteoclasts and plays a role in bone resorption. Cathepsin L plays a role in antigen presentation and has been implicated in tissue degenerative diseases such as rheumatoid arthritis, and also atherosclerosis, and cancer. The most extensively studied lysosomal cysteine protease is cathepsin B. Cathepsin B is unique amongst the cathepsin cysteine proteases in that it has both endopeptidase and carboxyexopeptidase activity, whereas other members of this class of proteases are endopeptidases. This is due to a unique structural feature of cathepsin B, the occluding loop that extends over the active site. It is believed that a specific histidine, His111, in the occluding loop is responsible for positioning peptide substrates so that cleavage can occur from the C-terminal. This feature potentially provides a means to build in specificity into a molecule for cathepsin B over other cathepsin cysteine proteases.²⁵

Cathepsin B is capable of degrading components of the extracellular matrix in such diseases as muscular dystrophy and rheumatoid arthritis. Cathepsin B expression is located at the outer edge of solid tumors suggesting it is involved in degrading the tumor extracellular matrix thus playing a role in tumor metastasis. Increased expression and secretion of cathepsin B have been shown to be associated with numerous human and experimental tumors, and has been proposed to be a prognostic marker for several types of cancer. The exact role for cathepsin B in solid tumors has yet to be defined, but it has been proposed to be involved in metastasis, angiogenesis, and tumor progression. Carcinoma cell invasion and metastasis can be inhibited by the nonspecific, irreversible, cysteine protease inhibitor E-64. Cathepsin B therefore presents itself as a possible therapeutic target for the control of tumor progression.²⁶

Our hypothesis was that a metal complex could inhibit a cysteine protease by ligand exchange with the thiol of the active site cysteine. We investigated several classes of metal complexes including cyclometalated organo Au(III) and Pd(II) complexes. The most promising compounds came from a series of rhenium(V) ‘3 + 1’ mixed ligand oxorhenium complexes, where there is one tridentate ligand, and one monodentate ligand. The tridentate ligands were either of the type S,S,S or S-pyridyl-S. Rhenium was chosen as rhenium and technetium complexes have been used for tumor imaging and there is therefore a precedent for the clinical use of similar compounds. The ‘3 + 1’ ligand set has been used to deliver rhenium and technetium to tissues, this ligand set in addition provides many opportunities for chemical modification. This is particularly relevant from the viewpoint of obtaining a cathepsin B specific inhibitor, our aim being to use this property to target the occluding loop. The monodentate ligand can undergo substitution reactions with thiolis, and its reactivity can be modified. Furthermore thiolate ligands can stabilize the ReO₄⁻ core.²⁷

These compounds were tested for their ability to inhibit cathepsin cysteine proteases (Table 1). These compounds were potent inhibitors of cathepsin B, showing selectivity for cathepsin B over cathepsin K. Inhibitory potency for cathepsin B was in part determined by the leaving group with the monochloride...
The tridentate ligand also had an effect on activity. For example, comparing complexes with the methoxyphenolate leaving group, the S-pyridyl-S complex was more active than the S,S,S complex, presumably due to stabilization of the leaving group by the trans sulfur. The S,S,S chloro complex was however very active. It was hypothesized that this complex adopts a trigonal bipyramidal geometry, readily losing the chloride, to form the preferred square pyramidal geometry upon substitution with the cysteine thiolate at the enzyme active site.

The mechanism of inhibition was also studied for two of the compounds \([\text{ReO(SpyS)}(\text{SPhOMe-})] \text{ and } [\text{ReO(SSS-2,2′)}] \text{Cl}\). With standard determinations of enzyme kinetic parameters, active-site titration with inhibitors, and active-site protection with the reversible inhibitor chymostatin, it was demonstrated that both compounds were selective active-site directed inhibitors of cathepsin B. Active site titration indicated a 1 : 1 stoichiometry of compound : enzyme active site. Inhibition could be protected by addition of the active site directed inhibitor, chymostatin. Both these results indicated that the compounds were directed to the active site. Mass spectrometry studies showed accumulation of a cathepsin B-rhenium complex intermediate for both compounds, providing further conclusive evidence of the active-site-directed nature of the inhibition. Mechanistically, however, there were subtle differences with the S-pyridyl-S compound being a tight-binding reversible inhibitor, with its activity being restored in the presence of excess exogenous cysteine, whereas the S,S,S compound was a time-dependent inhibitor, only slowly reversed in the presence of exogenous cysteine. Based upon this data a hypothetical model of compound binding was constructed with the rhenium inhibitor being coordinated to the active site cysteine after ligand substitution of the monodentate ligand (Fig. 4). These promising results suggest that these complexes can target cysteine protease-mediated pathologies.

**Nitric oxide: a free radical target**

One of the most significant discoveries to impact inorganic biochemistry in recent years was the biological role for nitric oxide as both a cell signaling and regulatory molecule in the cardiovascular system, and peripheral and central nervous system, and as a component of the immune system. Unlike most cell signaling molecules, such as hormones, neurotransmitters and cytokines, which are either organic molecules or large peptides or proteins, nitric oxide is a simple diatomic inorganic molecule. Not surprisingly for such an important and ubiquitous molecule, disruption of the NO signaling pathway has been implicated in the pathophysiology of many disease states, including septic shock, inflammatory disease, diabetes, allograft rejection, the immunopathology of graft-vs-host disease, and neurological...
disorders such as epilepsy, cerebral ischemia and chronic neurodegenerative disorders such as multiple sclerosis. Nitric oxide has also been implicated as a mediator in reperfusion injury after surgery contributing to the oxidative damage by reactive oxygen species (ROS). Reperfusion injury is a potential contributory factor to the impairment in cardiac, pulmonary and neurological function observed immediately after cardiac bypass surgery. NO-associated reperfusion injury has also been shown to contribute to myocardial dysfunction following myocardial infarction. This simple molecule with its unique chemistry therefore presents itself as an attractive therapeutic target to the inorganic medicinal chemist.

The major approach to the treatment of diseases where there is an overproduction of nitric oxide has focused primarily on the development of inhibitors of the nitric oxide synthase enzyme (NOS). However, there are three isoforms of NOS, two constitutive forms (NOS I and NOS III) and one inducible (NOS II). The primary challenge however is to find an inhibitor which is specific for the desired isoform implicated in the particular disease pathology. NO exerts many of its biological functions by interaction with iron-containing proteins such as hemoglobin, guanylate cyclase, cytochrome oxidase and aconitase. An alternative approach to NOS inhibition is to scavenge or remove the excess NO. Ruthenium complexes were investigated as potential NO scavengers. Ruthenium is the next transition metal below iron in group 8 of the periodic table, and the formation of nitrosyl complexes is a marked feature of ruthenium chemistry. Both compounds exhibited little or no toxicity. Both are water soluble and are not orally bioavailable, and therefore had to be administered by parenteral routes such as by intravenous or intraperitoneal injection. Rodent models of septic shock in particular have been used extensively to study NOS inhibitors. The majority of these models have used surgically operated, anaesthetised animals. Anaesthesia causes hemodynamic changes so we used a model of endotoxemia using conscious rats in which blood pressure was monitored using a tail cuff apparatus after a single bolus injection of LPS. Endotoxic rats were treated with AMD1226 at the nadir of the hypotension, 20 h after LPS injection. In contrast to the control animals, there was a rapid recovery following administration of AMD1226 manifested by an accelerated rise in blood pressure, which returned to normal 9 h after drug treatment. Significantly isolated rat tail artery preparations showed that arteries from these LPS-treated rats had a decreased responsiveness to the vasoconstrictor phenylephrine compared with those from control animals. The responsiveness to phenylephrine could be restored by addition of either AMD1226, or AMD6245, or the arginine analogue L-Nω-monomethyl-L-arginine (L-NMMA).

The results from the rodent model demonstrated the ability of the ruthenium(III) edta complex to reverse NO mediated hypotension. Rats, however, are known to produce far more nitric oxide after challenge with endotoxin than that seen in human disease. In order to investigate the potential efficacy of the ruthenium nitric oxide scavengers in a more clinically relevant model a porcine model of endotoxia was used. This model mimics several of the manifestations of septic shock and acute lung injury in humans. Anaesthetised male, random-bred Yorkshire swine were given LPS by infusion over a period of 1 h. At this point animals were treated with a single bolus injection of AMD6245 (5 mg kg⁻¹). Treatment with AMD6245 restored mean arterial pressure to control levels, but had no effect on pulmonary arterial pressure. The ruthenium compound however improved two aspects of pulmonary function, pulmonary compliance and pulmonary shunt. This improvement in lung function is indicative of protection against endotoxin-associated acute lung injury.

Although hypotension is an obvious symptom of septic shock, restoration of blood pressure is no guarantee of survival. Pharmacological NOS inhibitors have been shown to reverse sepsis or LPS-induced hypotension but worsen organ injury. It is noteworthy, therefore, that AMD6245 conferred significant protection against LPS-induced acute lung injury in the porcine model. Release of NO may be a regulatory mechanism that counters the development of pulmonary hypertension during sepsis or endotoxemia. Seen in this context, the ability of the ruthenium-based NO scavenger to ameliorate the LPS induced lung injury is potentially significant.
Fig. 5  NO binding by ruthenium(III) polyaminocarboxylates. (A) Scheme of NO binding by AMD6245 and AMD6221. (B) ORTEP diagrams of AMD6245 (Ru(III)) and the linear Ru–N–O nitrosyl adduct AMD6263 (Ru(II)). (C) Identification of the ruthenium nitrosyl adduct (AMD3689) of AMD6221 in the cell culture supernatant of LPS/Interferon-γ-stimulated RAW264 macrophages. HPLC chromatogram of the cell culture supernatant from unstimulated cells incubated with 100 μM AMD6221 for 18 h (left) compared with HPLC chromatogram of the cell culture supernatant from LPS/IFN-stimulated cells incubated with 100 μM AMD6221 for 18 h containing the nitrosyl adduct (right).
release of inflammatory mediators including cytokines, and free radical species such as superoxide and NO. An example of surgical intervention resulting in initiation of the inflammatory cascade is cardiopulmonary bypass (CPB). The clinical significance of the pro-inflammatory cytokine release during cardiopulmonary bypass include at least a temporary heart and pulmonary dysfunction and possibly longer term neurocognitive defects. The pro-inflammatory cytokines activate downstream signaling leading to the generation and release of other inflammatory mediators including NO and matrix metalloproteinases.

Using a canine model we tested if nitric oxide scavenging using AMD6221 would influence specific clinical outcomes, and secondarily would alter markers of inflammation following cardiopulmonary bypass. Animals treated with AMD6221 had reduced phenylephrine requirements which correlates with the reversal of the hypo-responsiveness seen in arteries from LPS-treated rats, reduction of fluid administration, reduced expression of CD18 on neutrophils indicative of an overall reduction of the inflammatory response, and lower creatinine kinase levels which is a marker for acute impairment of both cardiac and cerebrovascular function. Interestingly the increase in matrix metalloproteinase activity seen upon surgery was reduced in AMD6221 treated dogs. These results provide a preliminary indication that NO scavenging may improve clinical outcome following surgical procedures such as CPB.

Another surgical procedure in which NO has been implicated is organ rejection following graft surgery. There is a growing body of evidence that NO produced by iNOS plays a significant role in the mechanism of organ rejection. Elevated levels of iNOS mRNA have been observed in cardiac allografts in experimental animals and in rejecting cardiac transplants in humans. However therapeutic intervention with NOS inhibitors has yielded conflicting effects on graft survival. It has been postulated that a scavenger, which removes excess NO, whilst maintaining functional iNOS may retain the beneficial NO-mediated antimicrobial function.

AMD6221 was evaluated in a rat cardiac allograft model in which heterotopic abdominal cardiac transplantation was performed using rat strains (Wistar Furth WF:RT1 and Lewis LEW:RT1) with disparities at both major and minor histocompatibility loci. AMD6221 administered with a dose of 75 mg kg−1 twice daily prolonged graft survival, furthermore a synergistic improvement on graft survival was seen with a combination of AMD6221 and low dose cyclosporin compared with either compound used alone. Evidence for a reduction in NO was shown by a reduction in heme nitrosylprotein formation in treated animals compared with untreated controls at post-operative day 6 as measured by EPR, and a reduction in plasma nitrite/nitrate levels after a single pulse injection of AMD6221. In addition the formation of the ruthenium nitrosyl adduct in plasma was demonstrated using HPLC. This study therefore not only demonstrated the therapeutic potential for NO scavenging by AMD6221 in graft rejection, but also provided evidence for NO scavenging by a ruthenium(III) polyaminocarboxylate complex in vivo.

The kinetics of NO binding was studied using stopped-flow techniques. AMD6245 bound NO with a 1 : 1 stoichiometry and a second order rate constant for the substitution of the coordinated water molecule with NO of 2.24 × 107 M−1 s−1 at 7.3 °C (pH = 7.4; 50 mM phosphate buffer). AMD6221 also binds NO with a 1 : 1 stoichiometry, but with a second order rate constant of k = 3 × 103 M−1 s−1 (pH = 7.4 50 mM PBS, 20 °C), and a binding constant, K_a = 2 × 107 M−1. This binding constant is considerably lower than that of AMD6245 (or AMD1226) and NO (K_a > 109 M−1) indicating that the affinity of AMD6221 for NO is lower than that of AMD6245. Interestingly the compounds also exhibited different pharmacokinetic profiles when administered i.v. with AMD6221 being cleared more rapidly from plasma than AMD6245. This suggests that the pharmacological properties of the ruthenium(III) polyaminocarboxylate NO scavengers can be modulated by chemical modification of the ligand set around the metal center. Intravenous administration is a suitable dosing route for the intensive care unit setting. It allows controlled delivery of the drug, and under the intensive patient monitoring in the intensive care unit setting, allows titration of the dose to a physiological effect such as blood pressure. However there are other disease settings where oral administration is preferable. With this in mind, and in an attempt to make a more “drug-like” molecule, modifications were made to the chelate backbone in order to enhance lipophilicity of the molecules and improve oral absorption. Modifications to both the edta and dtpa framework, which included incorporating a pyridyl moiety into the edta framework so that one N atom of the edta ligand was replaced by a pyridyl N atom, and incorporating pendant functional groups to the central N atom of dtpa, resulted in either retention or improvement of the NO scavenging activity compared with the parent compounds.

AMD6221 has exhibited pharmacological activity in models of tumor growth, ocular inflammatory disease, and cardiovascular complications of diabetes. Yet, despite this apparent pharmacological activity of the NO scavengers, and their low toxicity, they have not progressed into clinical development. In many respects these are molecules in search of a disease and represent the problems and necessity of having a clear development strategy. Septic shock remains a major unmet clinical need, which was addressed by many companies. However the clinical trial failure rate in septic shock led to this disease being regarded as the graveyard of the biotechnology industry. Several agents were tested which attempted to intervene at one stage of the inflammatory cascade, frequently a target that appeared to be validated in animal models, but failed due to the pharmacological redundancy inherent in a multifactorial disease process.

Nitric oxide presents its own challenges as a disease target. On the one hand NO is essential to normal cardiovascular and neuronal functioning, yet on the other hand it is a mediator of disease pathology. This dual face of NO makes effective intervention in the NO pathway very challenging. Non-specific inhibition of all NOS isoforms is more likely to be detrimental than beneficial, and certainly in the case of septic shock this was found to be the case when a non-specific NOS inhibitor trial led to higher mortality in the treated arm than the control arm. Arguably this may have been the result of poor clinical trial design, but was equally likely due to the non-specific NOS inhibition.

In addition, what can appear to be a sound scientific idea may be difficult to translate into clinical practice. This is the case with CPB. The ideal clinical scenario for drug development is to conduct a small proof-of-principle Phase II clinical trial that should demonstrate efficacy prior to embarking on the randomized
Phase III trials required by the regulatory bodies for market approval. The clinical endpoints, transient pulmonary and cardiovascular function, neurocognitive impairment, quality of life require large numbers of patients in order to demonstrate a statistically significant difference between treated and untreated patients. For a small biopharmaceutical company this type of trial can be cost-prohibitive. Targeting NO, with its contradictory beneficial and detrimental effects, is a good example of the challenge of identifying a simple, effective, proof-of-principle Phase II clinical trial.

**Fosrenol™: a simple, effective, and safe metal-based drug**

In contrast Fosrenol™ represents a success for metal-based drug development. Fosrenol™ is a simple lanthanide salt, lanthanum carbonate, and is a safe and efficacious drug. It was approved for the treatment of hyperphosphatemia in patients with end stage renal disease (ESRD) by the FDA in October 2004 and by Sweden, the Reference Member State in the European Union Mutual Recognition Procedure for Fosrenol™, in March 2004. It was launched on the US market in January 2005 and had 8% of the US phosphate binding market by December 2005. Fosrenol™ is now available in Australia, Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Iceland, Ireland, Korea, The Netherlands, Sweden, Taiwan, the USA, and was recently launched in the UK in March 2007.

ESRD is associated with progressive secondary hyperparathyroidism, soft tissue calcification, cardiac complications, and increased morbidity. Hyperphosphatemia, increased serum phosphate levels, is one of the clinical consequences that accompany end stage renal disease. Normal adult serum phosphate levels range from 2.17–4.34 mg dl⁻¹ compared with the elevated levels of 6.2–9.3 mg dl⁻¹ seen in ESRD patients on hemodialysis. Phosphate metabolism is intimately linked with calcium metabolism, and is regulated by parathyroid hormone (PTH) and vitamin D. The average phosphate intake is around 1000–1500 mg day⁻¹. Phosphate is absorbed from the diet in the intestine and excreted via the kidney resulting in a net phosphate balance of zero under normal, healthy conditions. The production of PTH, which controls phosphate balance in the body, increases in response to the high phosphate levels in an attempt to correct the hyperphosphatemia. However vitamin D metabolism in the kidney is impaired in ESRD resulting in reduced calcium absorption and hypocalcemia. This decrease in calcium can in turn stimulate PTH secretion. This physiological response to hyperphosphatemia therefore occurs at the expense of increased parathyroid activity, a state described as secondary hyperparathyroidism (Fig. 6).

The pathological consequences of hyperphosphatemia are severe. These interacting events lead to several bone malformations in the joint, a disease known as renal osteodystrophy. Nearly half the deaths of dialysis patients are due to cardiovascular complications prevalent in patients with ESRD. Cardiovascular disease causing arrhythmias, left ventricular dysfunction, damage to heart valves, and ultimately complete heart block. In addition hyperphosphatemia and hypocalcemia are associated with atherosclerosis and calciphylaxis (a complication of extraskeletal calcification). Calcification of soft tissues is found in organs such as the lung, kidney, gastric mucosa, cornea and conjunctiva, and cutaneous and subcutaneous tissues.

Though the associated hypocalcemia and secondary parathyroidism can be treated with calcium supplements and calcitriol (a vitamin D derivative) hyperphosphatemia interferes with calcitriol therapy. Unfortunately hyperphosphatemia cannot be controlled by normal dialysis. It is difficult to control dietary intake of phosphate as phosphate is associated with protein intake thus decreasing dietary phosphate is difficult without significant reduction in protein intake. This puts patients with impaired renal function at risk of malnutrition. This means that alternative treatment options are needed for hyperphosphatemia. The binding of dietary phosphate in the gut has been the favored option. The ideal phosphate binder should have a high affinity for phosphate and should be able to bind phosphate rapidly. It should have low solubility and little or no systemic absorption. It should be non-toxic, available as a palatable oral dosage form, with a low pill burden. Aluminium-based binders such as aluminium hydroxide were used in the 1970s and early 1980s because aluminium readily forms insoluble and nonabsorbable aluminium phosphate precipitates. However, aluminium hydroxide, which is readily soluble, is absorbed from the gut and was found to be toxic. Aluminium causes CNS toxicity and encephalopathy. There are associated increases in hypercalcemia and cardiovascular calcification, and osteomalacia and adynamic (low turnover) bone disease, and associated bone and muscle pain. As a result calcium phosphate binders replaced aluminium-based phosphate binders. There are several available calcium-based phosphate binders including calcium carbonate, acetate, alginite and ketoglutarate with calcium carbonate and acetate being the most commonly used. Both are effective phosphate binders in dialysis patients and until recently have been the agents of choice. However, the problem with calcium-based agents is that the calcium can be absorbed...
provides a high density of positive charges, which can interact with phosphate anions resulting in strong phosphate binding. Renagel™ was shown in clinical trials to be at least as effective as calcium acetate at lowering phosphate levels. Renagel™ was approved by the FDA in 1998 and gained approval in Europe in 2000. An alternative solution is now available to physicians in the form of Fosrenol™.

The concept for Fosrenol™ was initially proposed and investigated by the Biomedical Technology Department of Johnson Matthey. It came out of an exploratory project investigating the biological interactions of lanthanides with a view to identifying potential therapeutic applications. Work sponsored by Johnson Matthey at the University of Surrey, UK, highlighted the ease with which the lanthanides formed precipitates with phosphate, frequently a challenge for the investigator. In collaboration with Shire Pharmaceuticals a project was initiated to examine lanthanides as phosphate binders for hyperphosphatemia. In vitro phosphate binding studies comparing a number of lanthanide salts identified lanthanum carbonate as having good phosphate binding properties. Further studies demonstrated that the tetrahydrate $\text{La}_2(\text{CO}_3)_3 \cdot 4\text{H}_2\text{O}$ possessed the best phosphate binding properties, with optimal binding at pH 3–5 whilst retaining binding activity across the full pH range of 1–7. This means that lanthanum carbonate can bind phosphate at the low pH of the stomach, as well as at the higher pH values found in the small intestine, duodenum and jejunum, unlike calcium carbonate. In addition comparative in vivo distribution studies showed that lanthanum carbonate had the best distribution profile with little to no oral absorption and tissue accumulation, and effectively complete elimination in the feces. A chemical process was developed for manufacture of the tetrahydrate, and with these proof-of-principle studies, Shire Pharmaceutical took on the clinical development of lanthanum carbonate. Patent rights to lanthanum carbonate were assigned to AnorMED. Fosrenol™ is produced and marketed by Shire Pharmaceuticals.

Detailed pre-clinical studies confirmed the phosphate-binding ability of lanthanum carbonate. The relative efficacy of lanthanum carbonate was compared with other phosphate binders in a rat model of renal failure. Lanthanum carbonate was equally effective as aluminium hydroxide, and more effective than either sevelamer or calcium carbonate, at reducing urine phosphate levels in this model. Lanthanum carbonate is very insoluble and the $\text{La}^{3+}$ cation does not cross biological membranes. Pharmacokinetic studies in animals confirmed that lanthanum carbonate is poorly absorbed when given by the oral route with >90% excreted in the feces, and <0.001% absorbed. No toxicity was observed in animal studies, in particular there were no cardiovascular or CNS effects, or any direct effects on calcium, vitamin D, or PTH metabolism. No effect on bone has been observed in normal animals. However, at high doses (1000–2000 mg kg$^{-1}$) an impairment of bone mineralization was seen in rats with chronic renal failure. No such effects have been seen in patients in the clinic, on the contrary improvements are seen in those patients with low turnover bone disease when treated with lanthanum carbonate. This favorably compares with calcium carbonate where there is a tendency to an increase in adynamic bone disease. Further studies in rats have shown that the observed impairment in bone mineralization was due to phosphate depletion at the high doses given, rather than to a direct effect on bone.

This favorable pre-clinical profile was repeated in clinical trials. When compared with placebo lanthanum carbonate reduced and maintained phosphate levels. Lanthanum carbonate was able to maintain phosphate levels in long-term trials for up to 2 years at levels of <5.9 or <5.6 mg dl$^{-1}$ depending upon the endpoint set by the trial. Dose levels of lanthanum carbonate range from 225–3000 mg La day$^{-1}$ in the trials. To achieve the best effect the tablets have to be taken with or immediately after food. One of the difficulties for patients is the high pill burden associated with phosphate binders. Significantly the pill burden with Fosrenol™ appears to be lower than that with other phosphate binders.

Fosrenol™ has an excellent safety profile. It is safe and well tolerated over the long term with some patients now having taken the drug for over 4 years. Most reported adverse events are mild–moderate, with gastrointestinal events being the most common, occurring with a frequency similar to that seen with calcium carbonate. The frequency of other adverse events is similar to that seen in patients on other phosphate binders and is primarily associated with the problems of treating a very sick patient population rather than drug related. No changes in serum calcium levels have been reported in patients treated with lanthanum carbonate, and though PTH levels are difficult to assess generally PTH levels remained stable or showed an increase in lanthanum carbonate treated patients.

Fosrenol™ therefore represents a significant improvement in treatment options for patients with end-stage renal disease. None of the available calcium- or aluminium-based phosphate binders match the requirements for an ideal agent, each having its own limitations. Knowing the challenges and difficulties in taking a drug from concept, through pre-clinical research and development, the costly hurdles of clinical trials, and navigating the regulatory submission process, what are the features of Fosrenol™ which have led it to successful market approval? Firstly there was a clinical need with a well defined and validated molecular target. Secondly the simplicity of the concept, a metal which will bind phosphate forming a non-absorbable product with resultant low toxicity, coupled with the expertise of Shire Pharmaceuticals (they were already marketing Calichew™, a calcium-based phosphate binder) provided a clear development plan. Fosrenol™ binds to the target and effectively neutralizes it; in the acidic environment of the stomach lanthanum carbonate dissociates sufficiently to allow formation of a highly insoluble phosphate. It has the required pharmacokinetic properties, it is poorly absorbed, with both the parent molecule and the phosphate product being eliminated in the feces. Because of the lack of absorption it has no systemic toxicity, it has no detrimental effect on calcium, vitamin D or PTH metabolism, and is safe and well tolerated. In addition its effectiveness as a phosphate binder appears in clinical studies to result in a lower pill burden for patients giving it an advantage over competing medications.
Conclusion

The medical application of metals has attracted humankind throughout history, yet many of those attractions have been based on subjective criteria rather than objective scientific evidence. Without doubt there are useful over-the-counter medicines such as the antacids, gold drugs have had some success for the treatment of rheumatoid arthritis, and metals have found a niche in diagnostic imaging, but the majority of modern approved drugs are either small organic molecules, or biologicals. The notable exception is the platinum-based anti-cancer drugs, which have been one of the great success stories of cancer therapy. The history of platinum has advanced from a serendipitous discovery to rational design of drugs to meet the challenges of toxicity, bioavailability, and resistance. What can we learn from these successes and limitations of metal-based drugs?

The platinum drugs indicate that the given right clinical need, and there is hardly any greater need than that for new cancer treatment, metal drugs can find a niche. Once that need has been identified, together with an amenable molecular target, and a pre-clinical and clinical development plan, transition metal chemistry is well suited to solve many of the challenges turning a structural lead into a “drug-like” molecule. One suggested way forward is to identify molecular targets that are amenable to inorganic chemistry. Certainly the examples of the investigative ruthenium anticancer drugs indicate that there are novel ways in which metal-based drugs can attack cancer other than by cytotoxic DNA lesions as in the example of platinum drugs. Novel targets for cancer could include thiol-based targets such as cysteine proteases including cathepsins and caspasps, the thioedoxin reducates system, transcription factors, and redox and free radical chemistry such as superoxide dismutase mimics and NO scavengers.

Assuming all these criteria can be met one of the remaining major challenges is the prejudice against metal-based drugs based on their perceived toxicity. It is true that heavy metals are toxic, most people are aware of mercury and lead poisoning. However it is a simplistic generalization to extrapolate and say that all metal compounds are toxic. The inorganic chemist knows that the biological properties of a metal are determined both by speciation and the ligand set around the metal center. Two good examples come from platinum and ruthenium chemistry. The simple platinum chloro salts are known sensitizers eliciting a potential fatal allergic reaction, whereas the neutral complex cis-dichlorodiamine platinum(tt) complex is one of the most successful cancer drugs of recent years.42 Ruthenium red is a potent neurotoxin and inhibitor of mitochondrial calcium transport,43 whereas NAMI-A is not cytotoxic, and the ruthenium(III) polyaminocarboxylates appear to have little or no in vivo toxicity. Ruthenium red is a mixed-valent, three metal center, positively charged (6+) complex whereas both NAMI-A and the NO scavenger molecules are monomeric neutral or anionic complexes. It is the chemistry of the molecule that determines its biological properties. Fosrenol™, lanthanum carbonate, represents the latest metal-based drug to gain regulatory approval. This drug meets an important clinical need, has a simple molecular target, and it has the desired pharmacokinetic properties. Furthermore, it has an excellent safety profile.

For the field of metals in medicine to continue to move forward it is important that the inorganic medicinal chemist recognize the challenges of drug discovery inherent in producing “drug-like” molecules, together with a clear development strategy. Additionally a commitment is required to the continual education of their colleagues in drug discovery; medicinal chemists, biological scientists involved in pre-clinical research and development, clinicians, and regulatory bodies, in order to overcome the prejudices against metal-based drugs.

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