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Metals in Medicine

Zijian Guo and Peter J. Sadler*

Bioinorganic chemistry is a rapidly developing field and there is enormous potential for applications in medicine, not only for the 24 or so essential elements, but also for nonessential and even radioactive elements. Medicinal inorganic chemistry offers real possibilities to pharmaceutical industries, which have traditionally been dominated by organic chemistry alone, for the discovery of truly novel drugs with new mechanisms of action. The field has been stimulated by the success of cisplatin, the world's best selling anticancer drug, and platinum complexes with reduced toxicity, oral activity, and activity against resistant tumors are currently on clinical trial. The organometallic complex titanocene dichloride is also being injected into patients, and Ru^{III} complexes have promising antimetastatic activity. The toxicity of Gd^{III} complexes can be controlled so that they can be safely injected in gram quantities as magnetic resonance imaging (MRI) contrast agents, and ligand design allows paramagnetic ions to be targeted to specific organs. Designed ligands also enable the targeting of radiodiagnostic (e.g. ^{99m}Tc) and radiotherapeutic isotopes (e.g. ^{186}Re). There has been recent progress in understanding the coordination chemistry and biochemistry of older metallodrugs such as gold antiarthritic and bismuth antiulcer drugs, and further work might lead to their more effective use. Current areas with exciting clinical potential include manganese superoxide dismutase mimics, vanadium insulin mimics, ruthenium nitric oxide scavengers, lanthanide-based photosensitizers, and metal-targeted organic agents. Our increasing knowledge of metal biochemistry will provide scope for the design of new drugs (both inorganic and organic) in many other areas too, for example neuropharmaceutical and antiinfective agents. Progress in medicinal coordination chemistry is heavily dependent on understanding not only the thermodynamics (equilibria and structures) but also the kinetics (and mechanisms) of reactions of metal complexes, especially under biologically relevant conditions.

Keywords: bioinorganic chemistry · coordination chemistry · drug research · medicinal chemistry · metallodrugs

1. Introduction

Biomedical inorganic chemistry ("elemental medicine") is an important new area of chemistry. It offers the potential for the design of novel therapeutic and diagnostic agents and hence for the treatment and understanding of diseases which are currently intractable (Figure 1).[1–3] Inorganic elements play crucial roles in biological and biomedical processes, and it is evident that many organic compounds used in medicine do not have a purely organic mode of action; some are activated or biotransformed by metal ions including metalloenzymes,[4] others have a direct or indirect effect on metal ion metabolism. In this review we focus on metal complexes, and especially on recent developments relating to platinum anticancer, gold antiarthritic, and bismuth antiulcer agents. First, in Section 2, wider applications involving metal complexes which are either used, or close to being used, clinically are described briefly to illustrate the scope of the subject.

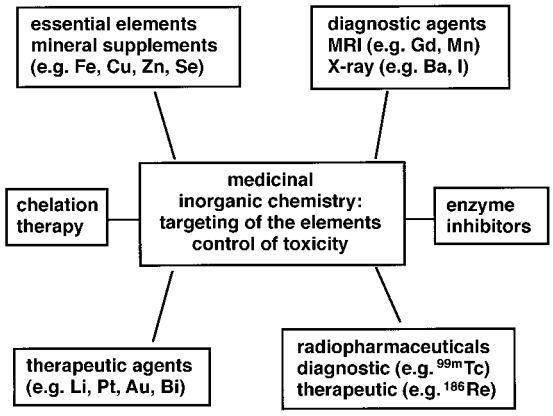

Figure 1. Some of the key areas of medicinal inorganic chemistry.

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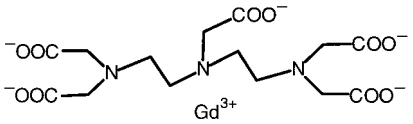
2. Design of Diagnostic and Therapeutic Agents

2.1. MRI Contrast Agents

Magnetic resonance imaging is now a powerful tool in clinical diagnosis.[5] Diseases can be detected from differences in ^{1}H NMR resonances (mainly of H_2O) between normal and abnormal tissues by administration of external paramagnetic agents, contrast agents. Most contrast agents contain Gd^{III}, Mn^{II}, or Fe^{III}, ions which have a large number of unpaired electrons (7, 5, and 5, respectively, high spin) and long electron spin relaxation times.[6, 7]

Four Gd^{III} complexes have been approved for clinical use, and are widely used, for example, for the detection of abnormalities of the blood–brain barrier.[15,199] Complexes containing DTPA (see **1**, Magnevist) and DOTA ligands (see **2**, Dotarem) are ionic, whereas those of BMA-DTPA (see **3**, Omniscan) and HP-DOTA (see **4**, Prohance) are neutral; their low osmolarity decreases the pain of the injections. All these agents are extracellular, and they diffuse rapidly into the interstitial space. The Gd^{III} center is nine-coordinate in each complex and contains one bound H_2O ligand. The crystal structure of $[Gd(dota)]^-$ (**2**) is shown in Figure 2. Water exchange on Gd^{III} is dissociative,[200] and steric crowding at the H_2O site enhances the exchange rate. Thus the H_2O exchange rate for [Gd(bma-dtpa)] (**3**), in which the distances between Gd and the amide oxygen atoms (2.44 Å) are longer than those between Gd and the carboxylate oxygen atoms in $[Gd(dtpa)]^{2-}$ (**1**, 2.40 Å), is seven times less than for **1** (Table 1). The Gd^{III} complexes of DTPA and DOTA are thermodynamically more stable (Table 1) than those of BMA-DTPA and HP-DOTA, respectively. Complexes **2** and **4** have a higher kinetic stability than **1** and **3**. The stabilities are highly pH dependent. For example, lg K for complex **1** decreases by a factor of about 4 from pH 7.4 to 5. This could



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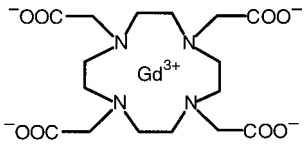

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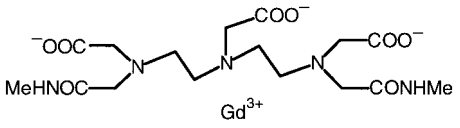

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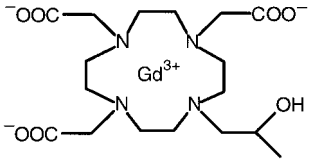

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Peter Sadler, born in 1946, received his DPhil in bioinorganic chemistry from the University of Oxford in 1972 under the supervision of Professors Allen Hill and Bob Williams. After spending two years as a Medical Research Council Fellow at the University of Cambridge and National Institute for Medical Research, Mill Hill, he was appointed to a lectureship in Chemistry at Birkbeck College, University of London, where he was subsequently appointed as Reader in Biological Inorganic Chemistry (1985), and Professor of Chemistry (1991). In 1996 he moved to the University of Edinburgh to take up the Crum Brown Chair of Chemistry. His research interests are concerned with the chemistry of metals in medicine, the chemical basis for the mechanism of action of metallodrugs, and the design of novel agents. He has published about 265 papers and was the recipient of the Royal Society of Chemistry Award for Inorganic Biochemistry in 1993. He was Chairman of the EC COST Action D8 "The Chemistry of Metals in Medicine", which coordinates research in this area in about 20 European countries, from 1996 to 1998.

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Zijian Guo, born in 1961, received his BSc from the University of Hebei, PR China, and was awarded his doctorate degree in Italy in 1994, carrying out research on transition metal chemistry with Professor G. Faraglia at the University of Padua. Since then he has been a postdoctoral research fellow in Professor Sadler's laboratory at Birkbeck College, University of London and the University of Edinburgh. His recent research has centered on the design and mechanism of action of platinum and ruthenium anticancer agents.

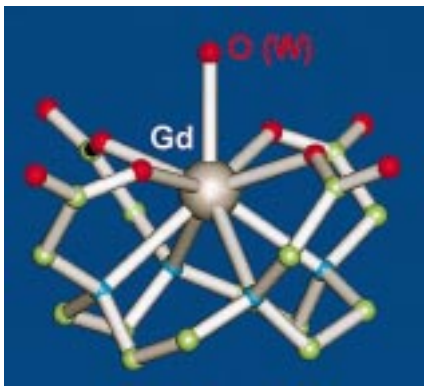


Figure 2. Crystal structure of $[Gd(dota)]^-$ in Na-**2**, an MRI contrast agent clinically used for detection of abnormalities in the blood–brain barrier. Adapted from ref. [197]. W indicates water.

Table 1. The relaxivity R_1 (20 MHz) and other properties of clinical contrast agents.[a]

Agent	k_{ex}^{298}[b] [$10^6\,s^{-1}$]	R_1 [$mM^{-1}\,s^{-1}$]	lg K[c]	Ref.
$[Gd(dtpa)]^{2-}$ (**1**)	3.3	4.5	22.5	[193]
$[Gd(dota)]^-$ (**2**)	4.8	3.4	25.8	[194]
$[Gd(bma\text{-}dtpa)]$ (**3**)	0.45	4.4	16.9	[194]
$[Gd(hp\text{-}dota)]$ (**4**)		3.6	23.8	[194]
$[Gd(bopta)]^{2-}$ (**5**)		4.4	22.5	[9]
$[Mn(dpdp)]^{4-}$ (**6**)		2.8	15.1	[10, 195]

[a] Relaxivity is the enhancement of proton relaxation rate in aqueous solution per unit of concentration in mM. Each agent has one bound water except **6**, which has no bound water. [b] k_{ex}^{298} = the water-exchange rate at 298 K. [c] K = stability constant at 298 K.

be significant in some biological compartments, for example lysosomes where the pH value can be as low as 5. The derivatization of soluble and insoluble polysaccharides allows the delivery of multiple paramagnetic ions.[8]

Complex **1** does not enter cells and is excreted almost exclusively by the kidney. Introduction of a benzyloxymethyl substituent on the α-C atom of a terminal acetate of DTPA as in BOPTA produces a Gd^{III} complex (**5**, Gadobenate, Figure 3) which enters hepatocytes and is excreted in bile.[9] The

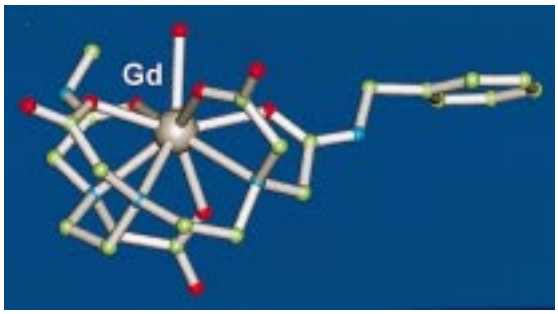


Figure 3. Crystal structure of $[Gd(bopta)]^{2-}$ in Na_2-**5**, an MRI contrast agent on clinical trial for liver imaging. Adapted from ref. [9].

coordination sphere of Gd^{III} in **5** is almost identical to that in **1** (nine-coordinate, distorted tricapped trigonal prism), and both complexes have similar stabilities and relaxivities (Table 1).[9]

The distorted octahedral complex **6** (Figure 4; Teslascan is the mangafodipir trisodium salt),[10] is in clinical use for enhancing contrast in the liver (detection of hepatocellular carcinomas).[11] The relaxivity of complex **6** (Table 1) is about

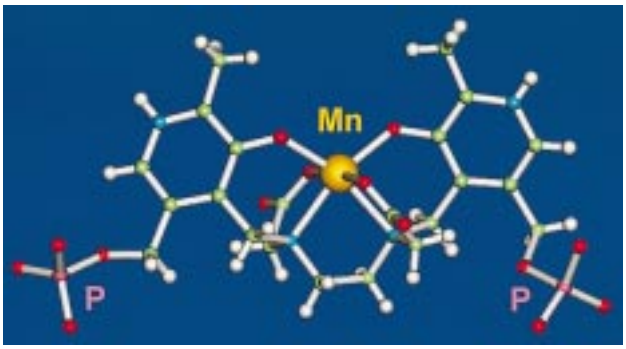
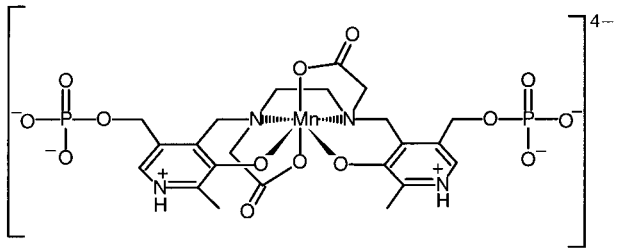


Figure 4. Crystal structure of $[Mn(dpdp)]^{4-}$ in $CaNa_2$-**6**, an MRI contrast agent for liver imaging. Adapted from ref. [10].



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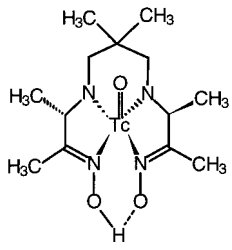
35% greater than that of Mn complexes of DTPA and DOTA, which also do not contain directly coordinated water.[12]

Superparamagnetic nanoparticles consisting of iron oxide coated with dextran are also being used as MRI contrast agents. The distribution of injected particles in the body is dependent on particle size: those of diameter 30 nm (Sinerem)[13] are useful for blood pool imaging, of diameter 150 nm (Endorem)[14] for specific liver imaging, and larger particles of diameter 300 nm (Lumirem)[15] can be administered orally for gastrointestinal tract imaging, as can Gadolite,[16] a zeolite containing trapped Gd^{III}.

2.2. Radiopharmaceuticals

Clinical interest in radionuclides centers not only on high-intensity γ-ray emitters, especially ^{99m}Tc and ^{201}Tl, ^{111}In, ^{67}Ga, ^{51}Co, ^{51}Cr, and ^{169}Yb for diagnostic imaging, but also on β emitters, for example ^{89}Sr, ^{153}Sm, and ^{186}Re, for therapy.[1,201] Many ^{99m}Tc-based radiopharmaceuticals and several other radionuclides are currently used in clinical diagnosis. Complex **7** ($[^{99m}Tc^{V}(dl\text{-hm-pao})]$, Ceretec) is an approved cerebral perfusion imaging agent for evaluation of stroke. It is taken up by the brain and is transformed into a more hydrophilic species which is retained in the brain.



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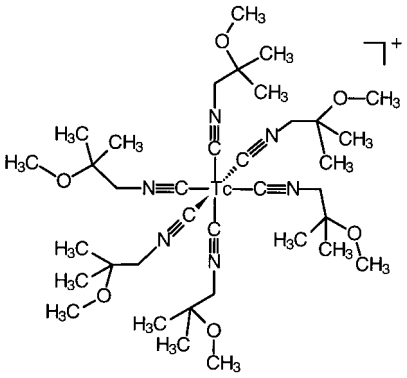
Intriguingly, the isomer containing *dl*-HM-PAO is retained in brain significantly longer than that containing the *meso* ligand.[17]

Complex **8** ([^{99m}Tc^{I}(sestamibi)]$^{+}$, Cardiolite) is used for myocardial perfusion imaging. It was designed on the basis



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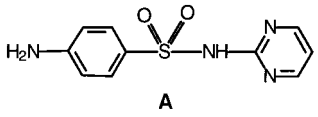
that lipophilic cationic complexes behave as potassium mimics and are taken up by the myocardium.[18] The sequential metabolism of the six identical methoxy groups of **8** to hydroxyl groups in the liver leads to formation of ^{99m}Tc complexes with greater hydrophilicity which are not retained in myocardial tissues.[19]

Monoclonal antibodies (mAbs) conjugated with radionuclides such as ^{111}In satumomab pendetide (which contains the murine mAb B72.3, which is directed to TAG-72, an antigen expressed by many adenocarcinomas) are used clinically for diagnosis of colorectal and ovarian cancer.[20] Several other murine mAbs linked to ^{99m}Tc and ^{111}In are in clinical trials.[21] Substantial progress has been made recently in the development of ^{99m}Tc-based receptor-specific radiopharmaceuticals.[22] Encapsulation in fullerenes may also provide a novel method for the delivery of radionuclides to target sites.[23, 24]

2.3. Antiinfective Agents

Silver and its compounds have long been used as antimicrobial agents in medicine. Silver is active at low concentrations and has a low toxicity. The practice of instilling the eyes of infants with 1% $AgNO_3$ solution immediately after birth is still common in some countries for prevention of ophthalmia neonatorum.[25] Silver sulfadiazine **9**, made from Ag^{I} and sulfadiazine **A**, is used clinically as an antimicrobial and antifungal agent. It is an insoluble polymeric compound which releases Ag^{I} ions slowly, and is applied topically as a cream to prevent bacterial infections in cases of severe burns. Of industrial importance is the slow release of antimicrobial Ag^{I} ions from inorganic or organic polymer matrices.[26]



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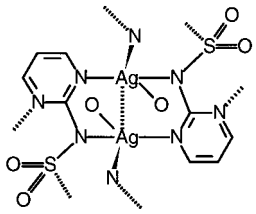

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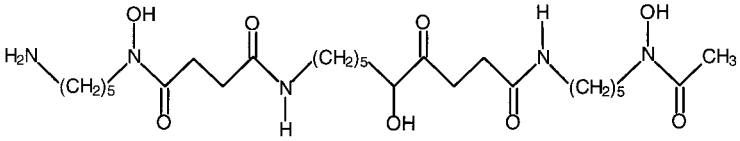
The mechanism of Ag^{I} cytotoxicity is unknown. Cell-wall damage may be important, and it has been shown that Cys150 in phosphomannose isomerase, an essential enzyme for the biosynthesis of cell walls of *Candida albicans*, is a Ag^{I} target in this organism.[27] Silver-resistant bacteria are known,[25] and progress in understanding the mechanism of resistance is now being made.[202]

Antimony has been used for medicinal purposes for many centuries. Complexes of Sb^{III} are generally more toxic than those of Sb^{V}. Two Sb^{V} drugs, *N*-methylglucamine antimonate (Glucantime) and sodium stibogluconate (Pentostam), are used clinically for the treatment of leishmaniasis, a disease caused by intracellular parasites.[28] The carbohydrates in the drugs may serve to deliver Sb^{V} to macrophages. The Sb^{V} complexes may be prodrugs for the more toxic Sb^{III} formed at or near the site of action. There is current interest in improving the stability, solubility, and efficacy of Sb drugs, and Sb^{III} and Sb^{V} complexes with yeast mannan are reported to be promising.[29, 30]

The iron chelator desferrioxamine (**10**) is clinically approved for the treatment of malaria. Its activity may arise from disruption of Fe^{III} metabolism within the digestive vacuole of malaria parasites.[31]



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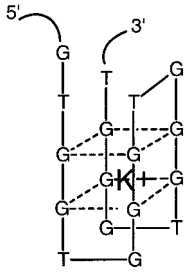
Several antisense oligonucleotides are potent inhibitors of HIV-1 integrase,[32] and are currently on clinical trial. These have key sequences such as 5′-d(GTGGTGGGTGGGTGG-GT) (**11**, T30175), and are composed entirely of deoxyguanosine and thymidine. They fold up in the presence of K^{+} ions to give four-stranded structures dominated by two stacked guanine-quartet motifs (Figure 5).[33]

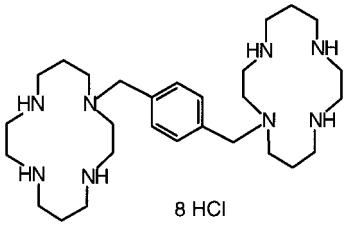


K-11

Figure 5. The folding of anti-HIV oligonucleotide **11** in the presence of potassium ions.

This structure is very stable under physiological conditions (e.g., K-**11** is resistant to serum nucleases with a half-life of 5 h) and is probably critical for biological activity.

Macrocyclic bicyclam ligands such as **12** (JM3100) are amongst the most potent inhibitors of HIV ever described, being active at nanomolar levels.[34] Since they are nontoxic at



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micromolar levels, they have a high selectivity index (ca. 10^5). The zinc complex of **12** is also active.[35] The bicyclams appear to block HIV-1 entry and membrane fusion through interaction with the CXCR4 coreceptor during the early stages of the retrovirus replicative cycle.[36]

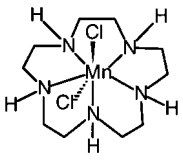
2.4. Superoxide Dismutase Mimics

The free radical superoxide, O_2^-, is a product of activated leukocytes and endothelial cells, and has been postulated to be a mediator of ischemia-reperfusion injury as well as inflammatory and vascular diseases. It can react with NO to form damaging peroxynitrite, ONO_2^-. The metalloenzyme superoxide dismutase (SOD) can destroy O_2^-: Cu,Zn-SOD in the cytoplasm of eukaryotic cells, Mn-SOD in mitochondria [Eqs. (1) and (2)]. However, the use of SOD in therapy is

$$M^{n+1} + O_2^- \rightarrow M^{n+} + O_2 \quad (1)$$

$$M^{n+} + O_2^- + 2H^+ \rightarrow M^{n+1} + H_2O_2 \quad (2)$$

limited by its short plasma half-life (clearance by the kidney) and inability to penetrate cell membranes (i.e., extracellular activity only). Low molecular mass mimics of SOD are therefore of much potential pharmaceutical interest.[37] For example, a variety of Mn- and Fe-based porphyrins and macrocyclic complexes exhibit SOD mimic activity.[38–41] Mn^{II} and Mn^{III} macrocycles appear to be particularly promising.[42, 43] For example, complex **13** (SC-52608) is able to scavenge superoxide and therefore effectively protect the regionally ischemic and reperfused myocardium from injury.[44] Another example is manganese(III) 5,10,15,20-tetrakis(4-benzoic acid)-porphyrin (MnTBAP), which can protect against neurodegeneration and is therefore of potential interest for the treatment of brain diseases such as Parkinson's and Alzheimer's diseases.[45]



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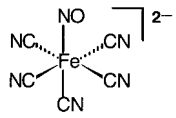
2.5. Cardiovascular System

The low-spin Fe^{II} complex sodium nitroprusside (**14**) is the only clinically used metal–nitrosyl complex.[46] It is often used to lower blood pressure in humans. Its hypotensive effect is evident within seconds after infusion, and the desired blood pressure is usually obtained within one to two minutes. It is also useful in cases of emergency hypertension, heart attacks, and surgery.[47] The therapeutic effects of **14** depend on release of nitric oxide, which relaxes vascular smooth muscle. Activation in vivo may involve reduction to $[Fe(CN)_5(NO)]^{3-}$, which then releases cyanide to give $[Fe(CN)_4(NO)]^{2-}$ and then nitric oxide.[48, 49]



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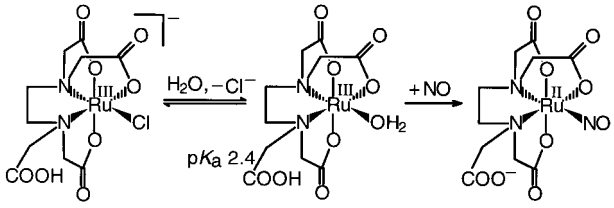
Ruthenium complexes such as K[Ru(Hedta)Cl] K-**15** (JM1226) have been proposed as nitric oxide scavengers for the control of NO levels under conditions of medical interest.



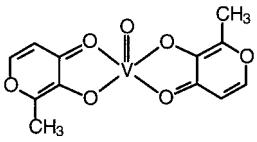
15 **16** **17**

Complex **15** in water exists in equilibrium with the aqua species **16** (JM6245; the pK_a of the dangling arm carboxyl group is 2.4).[50] Both complexes bind to NO very rapidly (rate constant $> 10^8\,M^{-1}s^{-1}$ at body temperature 310 K) and tightly ($K > 10^8\,M^{-1}$), forming the linear nitrosonium $Ru^{II}-NO^+$ adduct **17**.[50]

Complex **16** has been shown to reverse the poor response of the artery to vasoconstrictor drugs,[51] which is a major clinical problem in the treatment of patients with septic shock (caused by very high levels of circulating bacteria in the body). The excessive production of NO not only appears to be a major contributory factor in septic shock, but also in diabetes, arthritis, inflammation, and epilepsy.

2.6. Insulin Mimetics

It was discovered nearly 20 years ago that V^V as vanadate and V^{IV} as vanadyl can mimic some of the effects of insulin (stimulate glucose uptake and oxidation as well as glycogen synthesis).[52, 53] Vanadium complexes with organic ligands are often less toxic and can have improved aqueous solubility and lipophilicity.



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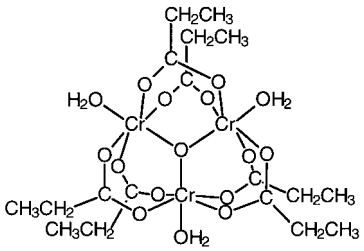
The orally active complex bis-(maltolato) oxovanadium(IV) (**18**, BMOV)[54] is three times more effective in vivo as an insulin-mimetic agent than $VOSO_4$.[55] In the solid-state, complex **18** has a five-coordinate square-

pyramidal geometry with the oxo ligand in the axial position and *trans* maltolato ligands.[56] In aerobic aqueous solutions, the complex is rapidly oxidized to the dioxovanadium(v) species.[57]

Low molecular weight chromium-binding substance (LMWCr), a naturally occuring oligopeptide (ca. 1500 Da, consisting of Cr^{III}, Asp, Glu, Gly, and Cys in a 4:2:4:2:2 ratio), has been found to activate the insulin-dependent tyrosine kinase activity of the insulin receptor protein, with the activity being proportional to the Cr content of the oligopeptide (maximium activity with four Cr^{III} per oligopeptide).[58] The trinuclear cation **19** can activate tyrosine kinase activity of the



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insulin receptor protein in a fashion almost identical to that of LMWCr.[59] Intriguingly when acetate replaces propionate in **19**, the complex does not activate but rather inhibits both the membrane phosphatase and kinase activity. Its potential for diabetes treatment has yet to be established.

2.7. Organic Drugs Targeted at Metals

Metal ions can play an important role in the mechanism of action of organic drugs, as is briefly illustrated by the following examples.

Organic drugs Galardin (glycomed), Ro319790, batimastat (BB-94), and BB-2516 are being evaluated in clinical trials for the treatment of diseases such as arthritis, cardiovascular disorders, and cancer. They are inhibitors of matrix metalloproteinases (MMPs), a family of zinc-dependent enzymes that degrade the major component of the extracellular matrix. Over-expression and activation of these enzymes have been linked with several diseases including cancer, arthritis, and multiple sclerosis. Inhibitors of these enzymes usually possess a group which can bind (chelate) to the active-site zinc(II) ion, and a peptide backbone which matches the requirements of the peptide cleavage site (Figure 6).[60]

Batimastat, 4-(*N*-hydroxyamino)-(2*R*)-isobutyl-(3*S*)-[2-(thienylthiomethyl)succinyl]-L-phenylalanine-*N*-methylamide, is a broad spectrum inhibitor with nanomolar activity against MMPs, and is in phase II clinical trial for treatment of breast and ovarian cancer.[61] The crystal structure of the catalytic domain of human neutrophil collagenase with bound batimastat (Figure 7) shows that batimastat coordinates to the catalytic Zn^{II} center in a bidentate manner through the hydroxyl and carbonyl oxygen atoms of the hydroxamate group.[62]

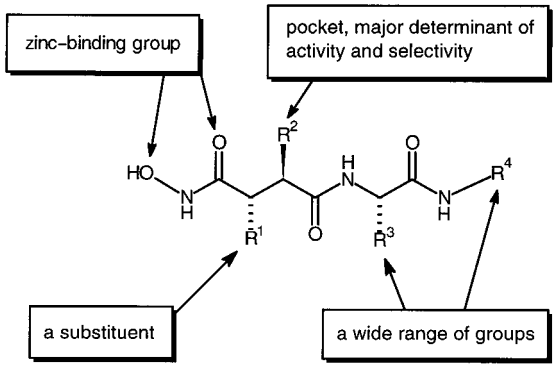


Figure 6. Backbone structure of matrix metalloproteinase inhibitors.

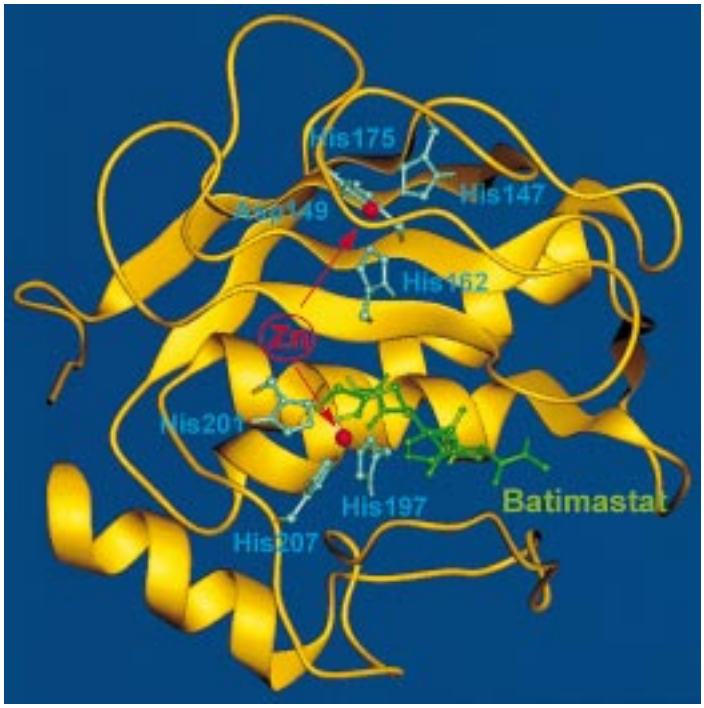
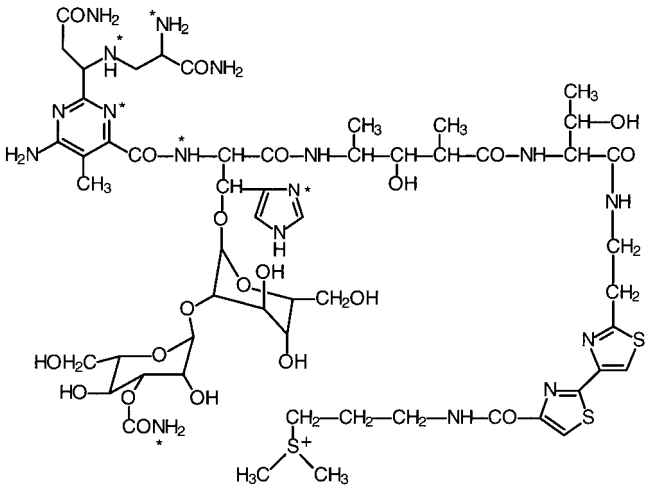


Figure 7. Crystal structure of the batimastat–human neutrophil collagenase complex, showing the inhibitor (green) coordinated to the catalytic Zn^{II} center. Adapted from ref. [62] (structure reference: PDBID, 1JAQ).

The bleomycins (BLMs; e.g. A2 (**20**); the asterisks represent metal-binding sites) are a group of glycopeptide antibiotics which possess antitumor activity against several types of tumors. Bleomycin sulfate is used clinically in combination chemotherapy for the treatment of head and neck cancer, certain lymphomas, and testicular cancer.[63]

The cytotoxicity of BLM is believed to result from its ability to bind iron (**20**-Fe),[64] activate oxygen, and cleave DNA[63] and possibly RNA.[65] The ability of the Fe^{II}–BLM complex to bind to oxygen and produce oxygenated BLM species such as O_2^{-}-Fe^{III}-BLM or O_2-Fe^{II}-BLM may be due to the presence of delocalized π electrons around iron and the strong iron–pyrimidine π backbonding.[66, 67] Oxygenated BLM accepts an additional electron to form activated low-spin ferric-peroxide BLM (O_2^{2-}-Fe^{III}-BLM).[66, 67] The structural features of Fe–BLM responsible for DNA (or RNA) degradation remain unclear.



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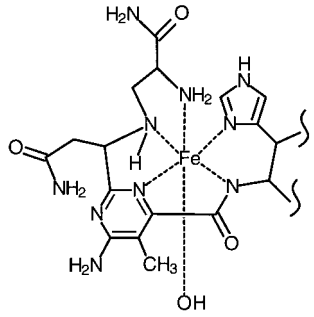
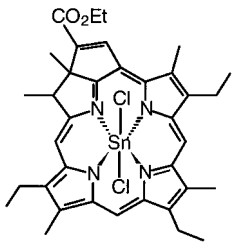


20-Fe

2.8. Photodynamic Therapy

Photodynamic therapy involves the treatment of diseased tissues and cells with a photosensitizer and visible light.[203,204] Most of the clinical interest is focused on cancer, porphyrias, and hematological diseases, and various forms of jaundice. Photosensitisers are required which show some selectivity for photodamage to tumor tissue.



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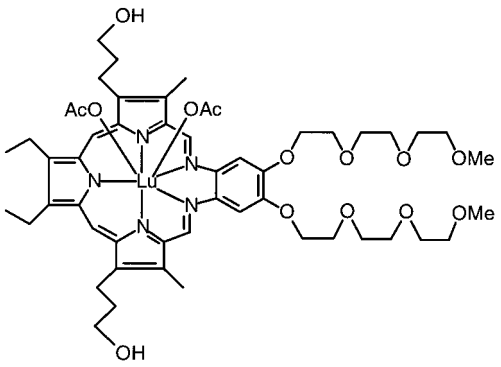
Tin(IV) ethyl etiopurpurin (**21**, SnET2)[68] is a second-generation photosensitiser currently under clinical evaluation. It is preferentially bound to high-density lipoproteins in blood plasma.[69] Complexes of texaphyrins (expanded porphyrins) with Cd^{II}, La^{III}, and Lu^{III} (**22**) are effective photosensitizers.[70, 71] They absorb strongly in the physiologically important far-red spectral region (700–800 nm), give rise to long-lived triplet states, and are highly efficient in the production of singlet oxygen.

The lutetium complex **22** is currently on clinical trial as a photosensitiser for the treatment of cancer. This complex



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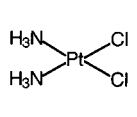
possesses a strong broad absorption band centered at 732 nm.[72] Upon absorption of light, it becomes activated to a long-lived triplet state complex that reacts with O_2 to generate cytotoxic singlet oxygen. Complex **22** is also in clinical trials as a photosensitiser for the treatment of atherosclerosis, the vascular disease caused by deposition of cholesterol and other fatty materials in the walls of blood vessels.

3. Anticancer Agents—Platinum

3.1. Clinical Platinum Complexes

Platinum complexes are now amongst the most widely used drugs for the treatment of cancer.[205,206] Four injectable Pt^{II} compounds have been approved for clinical use and several other *cis*-diam(m)ine complexes are on clinical trials, including an oral Pt^{IV} complex. Today cisplatin (**23**) is one of the



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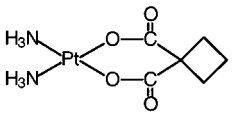

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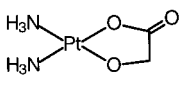

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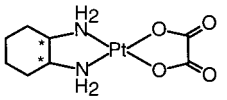

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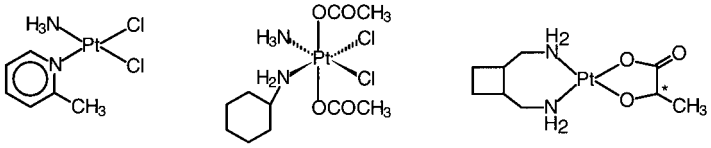
most widely used anticancer drugs, together with the second-generation drug carboplatin [$Pt(NH_3)_2$(CBDCA-*O*,*O*′)] (**24**). The glycolato complex **25** (nedaplatin, 254-S) and oxalato complex **26** (oxaliplatin, *l*-OHP, which contains *R*,*R*-1,2-diaminocyclohexane, DACH) have been approved for clinical use in Japan and France, respectively. These drugs are particularly effective in combination chemotherapy for treatment of advanced lung, colorectal, and ovarian cancers.[73, 74]

The sterically-hindered complex **27** (ZD0473) is active (by injection and oral administration) against an acquired cisplatin-resistant subline of a human ovarian carcinoma xenograph,[75] and entered clinical trials in 1997. It is less reactive



27 28 29

than cisplatin, for example inducing DNA interstrand cross-links in cells and binding to plasma proteins more slowly. The steric hindrance provided by the 2-methyl group, which lies over the top of the Pt-centered square plane, is evident in Figure 8.[76] The hydrolysis rates of **27** are two to three times slower than those of cisplatin (Table 2).[76]

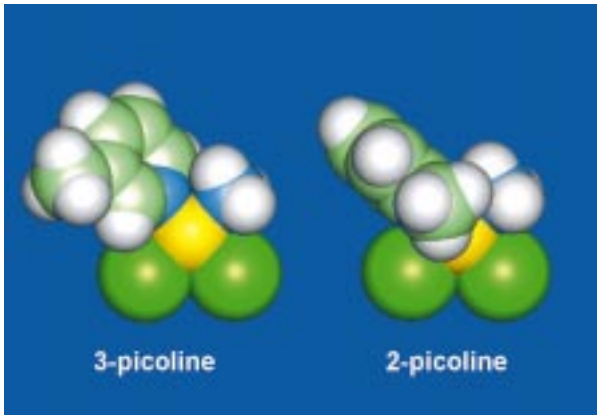
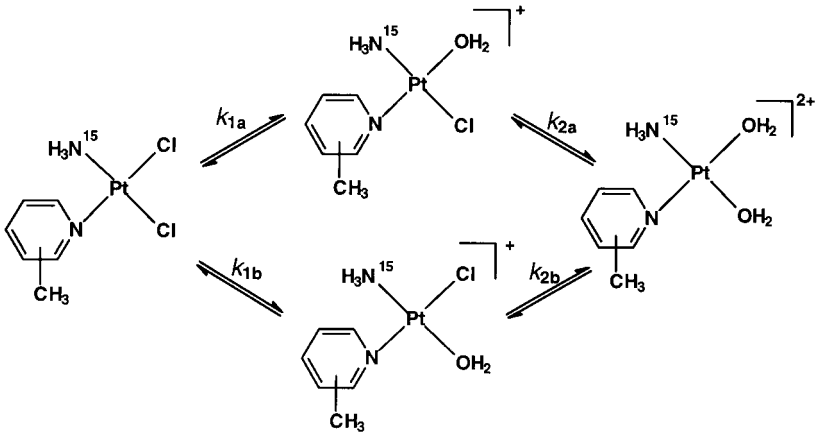


Figure 8. Crystal structure of the sterically hindered 2-picoline anticancer complex **27** in comparison with its 3-picoline analogue. The 2-methyl group in **27** lies directly over the Pt-centered square plane ($H_3C\cdots Pt$ 3.22 Å). Adapted from ref. [76].

Table 2. Rate constants k (Scheme 1) for hydrolysis of the platinum–picoline anticancer complex **27** and the 3-picoline analogue at 310 K (0.1 M $NaClO_4$),[76] in comparison with cisplatin (308 K, 0.32 M KNO_3).[196]

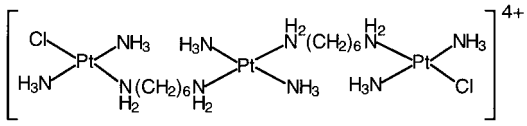
Compound	k [$\times 10^{-6}$ s^{-1}]	Compound	k [$\times 10^{-6}$ s^{-1}]
2-picoline–Pt complex **27**	k_{1a}: 31.9 ± 1.5 k_{1b}: 22.1 ± 1.4 k_{2a}: 73 ± 14 k_{2b}: 3.5 ± 2.5	3-picoline–Pt complex	k_{1a}: 44.7 ± 1.9 k_{1b}: 103 ± 4 k_{2a}: 35.0 ± 1.7 k_{2b}: 78 ± 60
cisplatin (**23**)	k_1: 75.9		



Scheme 1. Reaction steps in the hydrolysis of am(m)ineplatinum complexes.

The orally active complex **28** (JM216) is currently in phase II clinical trials. It is reported to have superior in vitro and in vivo activity compared to cisplatin against human cervical, small cell lung, and ovarian carcinoma cell lines.[77] When **28** is incubated with human plasma it is converted into at least six biotransformation products, which include mono- and dihydroxo Pt^{IV} complexes and the dichloroplatinum(II) complex *cis*-[$PtCl_2(NH_3)$(cyclohexylamine)] as the major metabolite.[78, 79] Complex **29** (Lobaplatin, D-19466) was introduced into clinical trials in 1992.[80] It is currently in phase II trials for treatment of cisplatin-resistant ovarian cancer,[81] advanced head and neck cancers,[82] and small-cell lung cancer.[83]

The trinuclear complex **30** (BBR3464, counteranion = NO_3^-), in which the central Pt unit is capable only of hydrogen-bonding interactions with DNA is now on clinical



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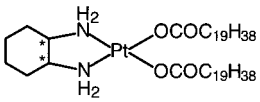
trial.[84–86] The overall charge of +4 greatly enhances DNA affinity, characterized by long-range interstrand cross-linking up to six base pairs apart with significant unwinding and efficient, irreversible conversion of B- to Z-DNA. The adducts terminate DNA synthesis in vitro. The cytotoxicity of the complex is insensitive to the p53 status of cisplatin-resistant cells (effective against tumors carrying a mutant p53). It is reported to be up to 100-fold more potent than cisplatin against human tumor cell lines resistant to cisplatin.

cis-Bis(nonadecanoato)(*trans*-*R*,*R*-1,2-diaminocyclohexane)-platinum(II) **31** (N-DDP) is a liposome-incorporated lipophilic cisplatin analogue that has shown promising in vivo activity against tumors resistant to cisplatin and liver metastases,[87] and is currently on clinical trial.



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3.2. Platination of DNA

Guanine N7 is the most electron-rich site on DNA (most easily oxidized), and the major adducts of platinum drugs with DNA are 1,2-GpG and 1,2-ApG intrastrand cross-links. The properties of these adducts have been extensively characterized and reviewed.[88] The NMR solution structure of *cis*-[{$Pt(NH_3)_2$}$^{2+}${d(CCTG*G*TCC)·d(GGACCAGG)}] (where * denotes Pt-bound G) indicates that the B-DNA backbone conformation is significantly altered to accommodate the platinated lesion (Figure 9).[89] A recent X-ray crystal structure of the cisplatin adduct of the duplex

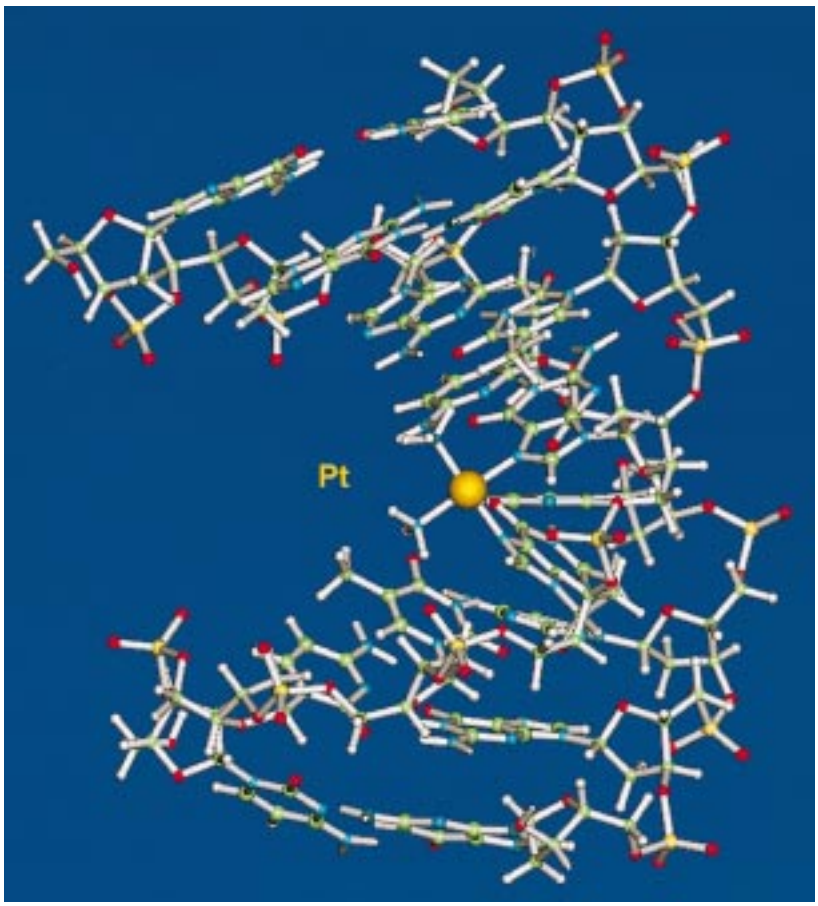


Figure 9. The refined structure based on NMR data of *cis*-[{Pt$(NH_3)_2$}$^{2+}${d(CCTG*G*TCC)·d(GGACCAGG)}], showing that the DNA is significantly altered from the B form to accommodate the platination lesion[89] (structure reference: PDBID, 1AU5).

d(CCTCTG*G*TCTCC)·d(GGAGACCAGAGG) (Figure 10) shows that cisplatin bends DNA by 35–40° in the direction of the major groove with a dihedral angle of 26° between the two guanine rings.[90, 91] The duplex adopts a juxtaposition of A-like and B-like helical DNA segments. It is notable that the conformation surrounding the GG platination site in the solid-state X-ray crystal structure[91] is remarkably similar to that in solution.[89, 92] The Pt ion is displaced from the planes of the coordinated G bases by about 0.8 Å, suggesting the presence of significant strain in this lesion. The nature and properties of minor cisplatin–DNA lesions which account for about 10% of the adducts, including 1,3-d(GpNpG) intrastrand and interstrand cross-links and monofunctional adducts, are less well understood.

Interstrand cross-linking of DNA occurs predominantly between two guanine residues on opposite strands, and requires a distance of approximately 3 Å between two N7 atoms. The most common interstrand cross-linking sequences are 5′-CG and 3′-CG.[93] In these sequences the two guanine residues are separated by at least 7–9 Å; therefore a large distortion of double-helical B-DNA is necessary to achieve the cross-linking. The solution NMR structure (Figure 11) of *cis*-[{Pt$(NH_3)_2$}$^{2+}${d(CATAGCTATG)$_2$}] shows that the duplex undergoes a significant rearrangement at the lesion site so that the platinum atom is located in the minor groove.[94] The deoxyribose of the platinated G residue is inverted so that O4′ is pointing in the opposite direction compared to the remaining nucleotides. Moreover, the C residue which was originally base-paired to the platinated G is extruded and becomes extra-helical (Figure 11). In the solution NMR structure of the interstrand cross-linked [{Pt$(NH_3)_2$}$^{2+}$}-{d(CCTCG*CTCTC)·d(GAGAG*CGAGG)}], the *cis*-{Pt-$(NH_3)_2$}$^{2+}$ moiety is also located in the minor groove.[95] The stacking of the two cross-linked guanine residues with the surrounding bases induces a bend of 40° towards the minor groove. A similar bend occurs in the X-ray crystal structure of this interstrand cross-link. The two cytosin residues are extruded from the double helix, and the platinum residue is embedded in a cage of nine water molecules.[207]

The general mechanism of formation of GG cross-links on DNA by cisplatin is shown in Scheme 2. Cisplatin is hydrolyzed to give monoaqua (and diaqua) species with a half-life of 1.7 h at 310 K. The aqua complexes are believed to be the active species towards DNA.

Unexpected was the finding that the 5′-G monofunctional adduct formed during the reaction of cisplatin with the 14-mer DNA duplex d(ATACATGGTACATA)·d(TATGTACCATGTAT) is very long lived with a half-life of 80 h at 298 K.[96, 97] The lifetimes of the two monofunctional G adducts of the GG single strand are similar, suggesting that the three-dimensional structure of DNA plays a role in stabilizing the 5′-G adduct either by shielding the Cl ligand from hydrolysis, or by constraining the position of the incoming 3′-G N7 ligand. Molecular modeling studies demonstrate that hydrogen-bonding between the NH_3 ligands and carbonyl groups on DNA plays a major role in determining the orientation of the Pt–Cl bonds and their accessibility. Molecular mechanics calculations show that although the

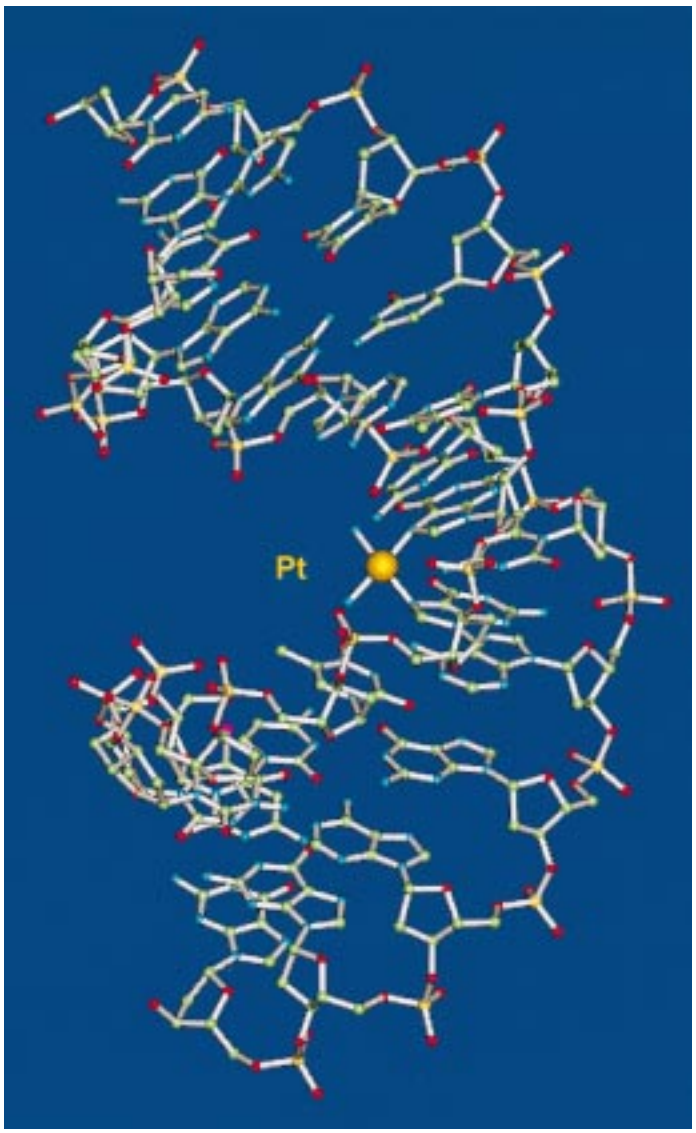


Figure 10. Crystal structure of *cis*-[{Pt(NH$_3$)$_2$}$^{2+}${d(CCTCTG*G*TCTCC)·d(GGAGACCAGAGG)}], showing that the DNA duplex is kinked by about 40° towards the major groove and has a juxtaposition of A-like and B-like helical DNA segments[90, 91] (structure reference: PDBID, 1AIO).

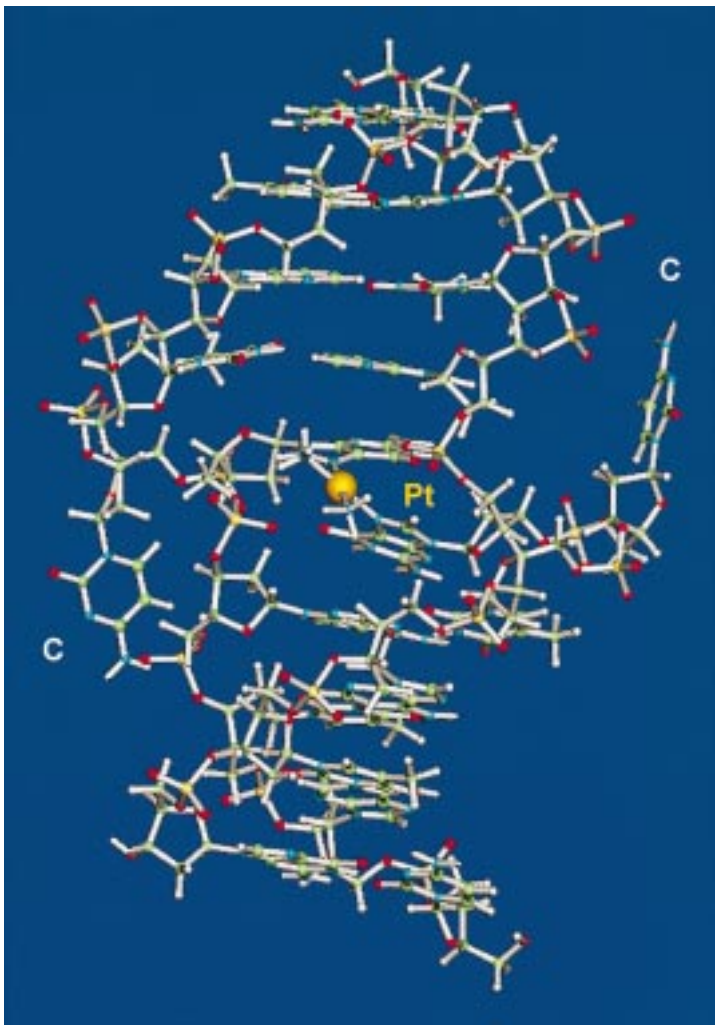
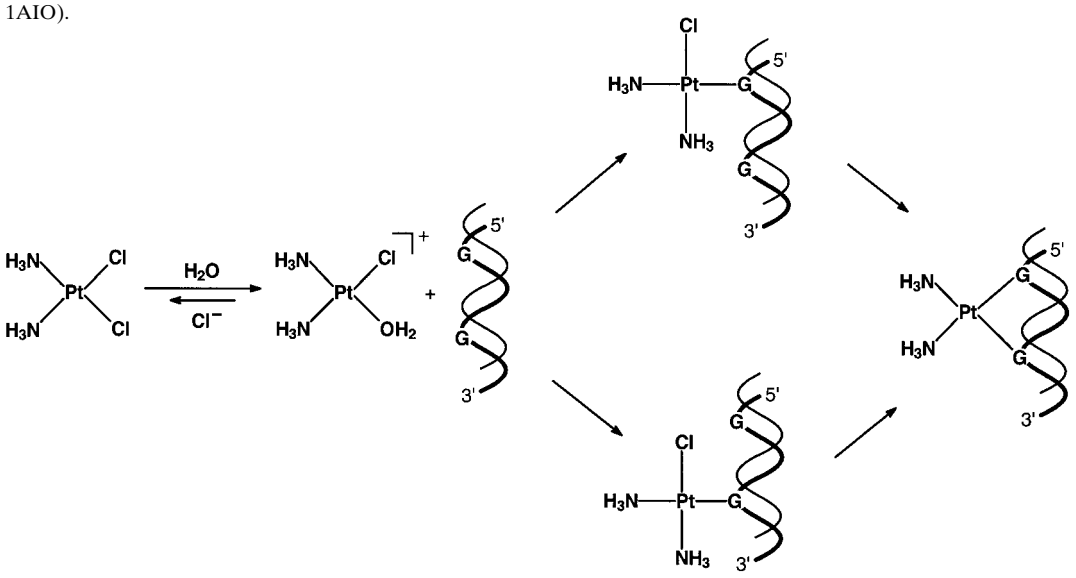


Figure 11. The refined structure based on NMR data of interstrand cross-linked *cis*-[{Pt(NH$_3$)$_2$}$^{2+}${d(CATAGCTATG)$_2$}]. The deoxycytosine residues opposite to the platinated deoxyguanines are excluded and become extra-helical[94] (structure reference: PDBID, 1DDP).

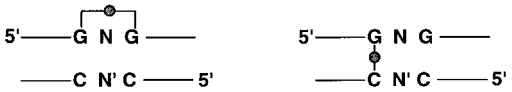


Scheme 2. Mechanism of reaction of cisplatin (**23**) with DNA.

chloride ligand in the monofunctional adduct faces outward, away from the helix, the aqua ligand which replaces it after hydrolysis faces inwards on account of its strong hydrogen-bonding properties.[97] Modeling of transition states is now required.

Kinetic analyses based on HPLC results allow the accurate determination of the rates of both platination and chelation steps for reactions of cisplatin diaqua with oligonucleotides.[98, 99] For the double-stranded oligonucleotide $d(TTGGCCAA)_2$ the formation of the 5′-G monoaqua adduct is faster than that of the 3′-G monoadduct, and macrochelate ring closure of the 5′-G monoaqua to give the bifunctional adduct (half-life of 3.2 h at 293 K) is much slower than that of the 3′-G monoadduct. The biological significance of long-lived monofunctional adducts on DNA remains to be determined, but these alone may be sufficient to kill cells if they are not repaired, which seems to be the case for the active *trans* iminoether complex **33** (see Section 3.4).[100] Long-lived monofunctional adducts may also promote the formation of DNA–protein cross-links.

The stability of the Pt–N7G bond is known to be very high, and it can be broken only by very strong nucleophiles such as cyanide or thiourea. However, recent studies have shown that this bond can be labile in certain DNA adducts. The adduct *trans*-[{$Pt(NH_3)_2$}$^{2+}${d(TCTACG*CG*TTCT)}] (1,3-GG cross-link) is unstable at neutral pH and rearranges to form the linkage isomer *trans*-[{$Pt(NH_3)_2$}$^{2+}${d(TCTAC*GCG*TTCT)}] (1,4-CG cross-link).[101] It was found subsequently that intra- and interstrand transplatin–DNA adducts undergo isomerization in both single- and double-stranded DNA.[102–104] For example interstrand cross-links between a platinated 5′-G and the complementary C residue can be formed (Scheme 3).[102]



Scheme 3. Rearrangement of a *trans*-[{$Pt(NH_3)_2$}-DNA] intrastrand cross-link (left) to an interstrand cross-link (right).

The isomerization of the intrastrand d(CCTG*G*TCC)·d(GGACCAGG) cross-link to a d(CCTG*GTCC)·d(GGACCAGG*) interstrand cross-link shows that even Pt–N7 bonds in Pt–1,2-GpG cross-links can be destabilized.[89] The process appears to be assisted by Cl^-.

3.3. Protein Recognition

In tumor cells the excision repair of platinated DNA lesions may be protected by a class of proteins known as high mobility group (HMG) proteins.[106, 107]

The protein HMG1 has three structural domains, two of them, domains A and B, are positively charged, and the third domain comprises a 30-amino-acid stretch of acidic residues at the C terminus. Several NMR structures of HMG domains have been determined.[108, 109] Domain B of HMG1 consists of three α-helical regions joined by small loops, and folded into an L-shaped structure. The positively charged domain A and the central domain B bind to DNA, while the acidic C-terminal domain interacts with histones.

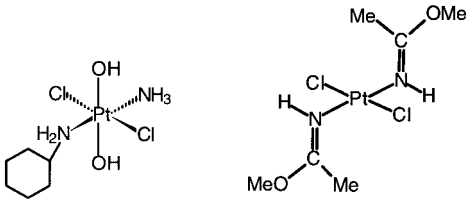
The binding affinity of HMG1 towards a series of cisplatin-modified 15-mer DNA duplexes d(CCTCTCN1G*G*N2TCTTC)·d(GAAGAN3CCN4GAGAGG) is highly dependent on the bases adjacent to the Pt lesion.[110] The affinity for HMG1 domain A decreases by over two orders of magnitude in the order N2 = dA > T > dC. When N1 = N2 = dA, Pt–DNA binds 100-fold stronger to HMG1 domain A ($K_d = 1.6$ nM) than to HMG1 domain B ($K_d = 134$ nM). The HMG binding increases the bending of platinated DNA to as much as 90°.[111] NMR studies of a GG 14-mer platinated with cisplatin suggest that the kinked duplex binds in the elbow region of HMG1 domain A,[112] and recent X-ray studies[105] of HMG1 domain A bound to a 16-base-pair DNA fragment containing a cisplatin–DNA 1,2-d(GpG) intrastrand cross-link shows that the protein inserts a Phe side chain into the platinum site.

Nuclear protein, the linker histone H1, also binds much more strongly to cisplatin-modified DNA than to transplatin or unmodified DNA.[113] Competitive binding of a cisplatin-modified 123-base-pair DNA fragment shows that histone H1 binding is stronger than that of HMG1. Also the promoter recognition factor TATA box-binding protein (TBP)/TFIID binds selectively to and is sequestered by cisplatin-damaged DNA. This may lure TBP/TFIID away from its normal promoter sequence explaining the inhibition of RNA synthesis.[114] These proteins may therefore also play important roles in the mechanism of action of platinum drugs.

3.4. Active *trans* Complexes

Most of the work on the design of platinum anticancer complexes has concentrated on *cis*-diam(m)ine complexes, since it was discovered early on that *trans*-$[PtCl_2(NH_3)_2]$ is inactive. However, the report that *trans*-$[PtCl_2(py)_2]$ (py = pyridine) is potently cytotoxic has stimulated renewed interest in *trans* complexes.[115]

The *trans* Pt^{IV} complex **32** is active against both murine and human subcutaneous tumor models,[116] and efficiently promotes DNA interstrand cross-links and single-strand



32 **33**

breaks.[117] These properties may account for its unusual cytotoxicity profile against cisplatin-resistant tumors.[118] Intriguingly the corresponding *trans* Pt^{II} complexes (without the axial hydroxo groups) are inactive. Direct reactions of the Pt^{IV} complexes with DNA may be important, but in vivo reduction could produce reactive intermediates.

trans-Imino ether platinum complexes such as **33** have much higher antitumor activity than the *cis* analogues (OMe and Pt are *cis* with respect to C=N in the *Z* isomer, and *trans* in the *E* isomer).[119, 120] The mechanism of action of these agents appears to be different from that of cisplatin and may be related to the properties of the imino ether ligands.[121] Although these *trans* complexes react with DNA more slowly than cisplatin, they achieve the same level of DNA binding after 24 h. The *trans E*,*E* complex is the most effective in inhibiting DNA synthesis and cell proliferation, but does not induce large local DNA conformational changes.[122] It preferentially forms monofunctional adducts at guanine residues in double-helical DNA even after long incubation times (48 h at 310 K).[123a] The reactivity of the second chloride ligand in the monofunctional adduct is markedly reduced. The *trans E*,*E* monofunctional adducts are not recognized by HMG proteins.[123b,c]

3.5. Biotransformation

L-Methionine (L-MetH) may play an important role in the metabolism of platinum anticancer drugs. The Pt^{II}–L-Met complexes form rapidly in plasma after injection of cisplatin into rats.[124] The complex [Pt(Met-*S*,*N*)$_2$] has been detected in the urine of patients[125] and exists predominantly as the *cis* isomer (*cis*:*trans* = 87:13) in solution (Figure 12).[126]

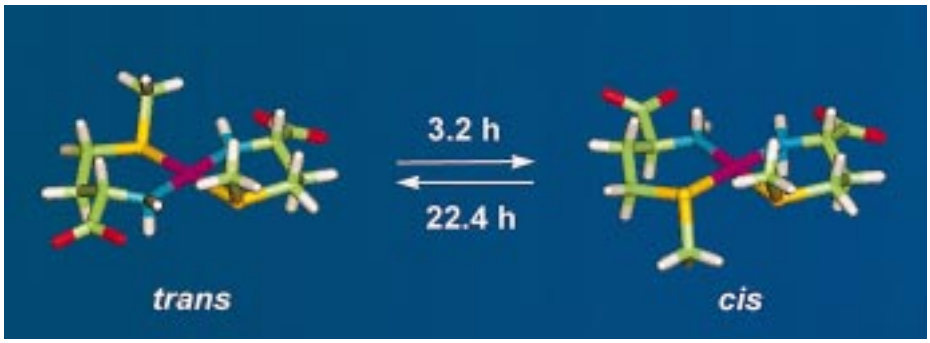


Figure 12. Isomerization of *R*,*R*-*trans*- and *S*,*R*-*cis*-[Pt(Met)$_2$], a metabolite of cisplatin. Adapted from ref. [126].

Human serum albumin (HSA) is a single-chain 66-kDa protein which contains 17 disulfide bridges and one free thiol group at Cys34, as well as six Met residues: M87, M123, M298, M329, M446, and M548. Reaction between cisplatin and HSA is thought to be the main route for platinum binding in human blood plasma.[127] Recent data suggest that cisplatin reacts mainly with methionine residues of albumin, forming a Met-*S*,*N* macrochelate together with minor adducts with Cys34 and monodentate Met-*S* residues.[128]

The reaction of carboplatin **24** with L-Met leads to a surprisingly stable ring-opened intermediate with a half-life of 28 h at 310 K.[129] Similar ring-opened complexes appear to be present in urine after carboplatin administration.[130] Therefore it is possible that the reaction of carboplatin with Met or its derivatives could provide an activation pathway for the drug. Binding of the methionine sulfur atom is reversible, and it can be displaced by guanine N7.[208]

Intracellular thiols such as glutathione (the tripeptide γ-Glu-Cys-Gly, GSH), often present at concentrations of 3–10 mM, may inactivate platinum drugs. The major product from reaction between cisplatin and GSH is a high molecular mass polymer with a Pt:GSH ratio of 1:2.[131] The formation of such polymers may be responsible for depletion of platinum from the circulation. The active export of cisplatin from cells is one of the major mechanisms of resistance, and a GS-X pump, an ATP-dependent export pump for GS-conjugates, is able to pump out GS–Pt complexes from tumor cells.[132] Reaction between the cysteine-rich protein metallothionein (MT) and cisplatin leads to displacement of the ammine ligands and gives rise to Pt_{7-10}MT containing PtS_4 clusters.[133] This provides another pathway for the inactivation of Pt drugs.

3.6. Photoactivation

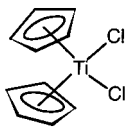
Both Pt^{II} and Pt^{IV} complexes have long been known to be photoreactive, and there is much scope for the application of photodynamic reactions in biochemistry and pharmacology.

A novel approach to DNA platination involves the use of iodoplatinum(IV) complexes which are activated by visible light.[134] The toxicity of *trans*,*cis*-[Pt(en)(OAc)$_2$I$_2$] towards human bladder cancer cells is enhanced (by 35%) when the treated cells are irradiated with light of $\lambda > 375$ nm. It has been shown that visible light can induce the aquation of this complex followed by photoreductive platination of guanosine monophosphate, in contrast to the dihydroxo analogue.[135] Reactions of iodoplatinum(IV) ethylenediamine complexes with glutathione in the absence of light involve the initial attack of thiol on an iodide ligand of Pt^{IV} to generate reactive chelate ring-opened intermediates.[136] Electron transfer driven labilization of *trans* ligands provides a new concept in drug design.

UV light induced cleavage of both intra- and interstrand cross-links involving Pt–G bonds has been observed. Irradiation of DNA modified by cisplatin with UV light ($\lambda > 300$ nm) can induce specific cross-links to the protein HMG1, thought to involve Lys6 in domain B with labilization of a Pt–purine bond.[137, 138]

3.7. Other Metal Anticancer Agents

The antitumor activity of titanocene dichloride (**34**) was first recognised in 1979,[139] and, since then, the activities of some other metallocenes (V, Nb, Mo, Fe, Ge, and Sn) have been reported.[140] Complex **34** is active against a diverse range of human carcinomas, including gastrointestinal and breast carcinomas, but not against head and neck cancers, and is now in phase II clinical trials. There appears to be a lack of cross-resistance between **34** and cisplatin.[141]



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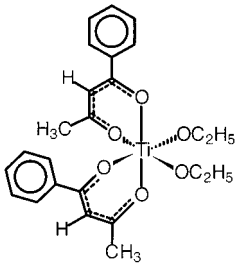

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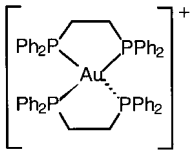
The bis(β-diketonato) Ti^{IV} complex **35** (budotitane, shown as the predominant *cis,cis,cis* isomer) entered phase I clinical trials in Germany in 1986 for the treatment of colon cancer.[142] The complex is very susceptible to hydrolysis, and to minimize this, the coprecipitate of Cremophor EL, 1,2-propylene glycol in ethanol, and the drug in a ratio of 9:1:1 is dissolved in water prior to administration.[142] The dose-limiting toxicity appears to be cardiac arrhythmia.[143] Ti^{IV} binds strongly to human serum transferrin,[144] and this protein could serve to deliver Ti^{IV} to cancer cells.

Tetrahedral bis(diphosphanyl)gold(I) complexes such as **36** are much less reactive, for example towards thiols, than linear Au^{I} antiarthritic complexes. Complex **36** (lactate salt) exhibits activity in a range of cancer models. It has a different mechanism of action to cisplatin, and is targeted to mitochondria, where it destroys membrane potentials. Complex **36** proved to be too cardiotoxic for clinical use, but the introduction of pyridylaryl substituents on P increases the hydrophilicity of bischelated Au^{I} diphosphane complexes, and gives rise to potent and selective activity towards cisplatin-sensitive and resistant ovarian tumor cells.[145] It remains to be seen whether the cardiotoxicity is reduced.



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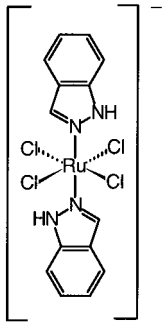
Gallium salts are known to exhibit anticancer activity, and Ga^{III} is probably delivered to tumor cells through the serum protein transferrin. Recent interest in gallium salts arises from their synergistic effect with cisplatin in the treatment of lung cancer[146] and carcinoma of the urothelium.[147] Gallium(III) maltolate has recently entered clinical trials for the treatment of bone disease and related conditions.[148]

Ruthenium(III) complexes are more active against metastases than against primary tumors, and Ru^{III} is also thought to be delivered to tumor cells through transferrin.[149] The indazole complex **37**, which is especially active against colorectal tumors,[150] binds reversibly to His253 of apolactoferrin, one of the Fe^{III} ligands in the iron-binding cleft of the N lobe, with displacement of a chloride ligand.[151] Another promising candidate for clinical trial as an anti-metastatic agent is complex **38** (as the imidazolinium salt, NAMI-A).[152] Ruthenium(III) complexes may be activated by reduction to Ru^{II} in vivo.

Both Ru^{II} and Ru^{III} complexes are known to bind preferentially to N7 of G on DNA, but can also bind to A and C residues.[153, 154] Although most ruthenium antitumor agents have two reactive coordination sites, GG intrastrand cross-links on DNA appear to be sterically unfavorable. The only example appears to be that of *trans*-$[Ru^{II}Cl_2(dmso)_4]$ with GpG, which has been structurally characterized by NMR spectroscopy.[155] In this complex, the two N7-coordinated G residues adopt a head-to-head conformation and are strongly destacked.



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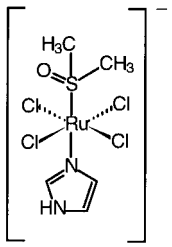

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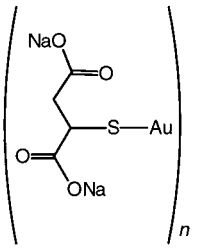
4. Gold Antiarthritic Drugs

Several injectable 1:1 Au^{I} thiolato complexes are used clinically for the treatment of difficult cases of rheumatoid arthritis, including sodium aurothiomalate (**39**, Myocrisin), aurothioglucose (**40**, Solganol), and sodium aurothiopropanol sulfonate (**41**, Allochrysine).[209]



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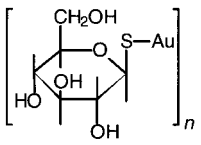

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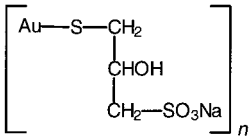

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Most of the gold thiolates have a gold-to-ligand ratio close to 1:1, and are polymeric complexes (e.g. chain or ring forms) with thiolate S-bridging and linear Au^{I} ions, as shown by EXAFS[156] and WAXS data.[157] Crystal structures of the gold thiolate drugs themselves have been elusive, the only example resulting from the recent elegant crystallization of **39** by Bau[158] using techniques for crystallization of macromolecules. The linear S-Au-S units are arranged into polymeric double-helical chains (Figure 13). Hexameric Au_6S_6 rings of the type predicted previously[159] have also been shown to exist in crystals with 2,4,6-tri(isopropyl)thiophenol as the thiolato ligand (Figure 14).[160] The formulated gold thiolate drugs often contain a small molar excess (e.g. 10%) of thiol over Au^{I}, and readily undergo thiolate addition and thiolate exchange reactions.[161]

The only oral gold antiarthritic compound in clinical use is the phosphane complex **42** (auranofin, Ridaura) shown in Figure 15. Complex **42** is also highly cytotoxic to tumor cells in culture, and is also reported to be active topically against psoriasis.[162]

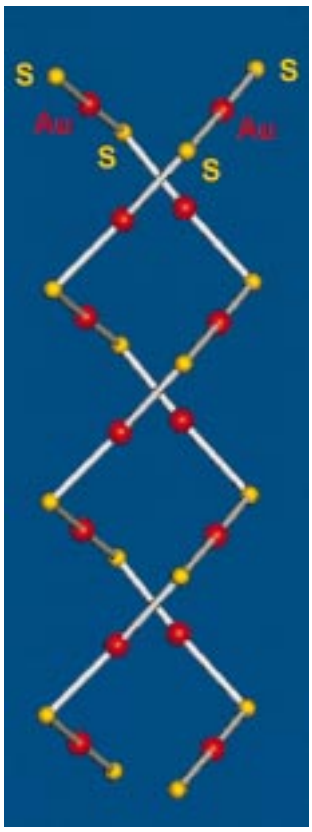

Figure 13. Crystal structure of **39** showing the double-helical chains. The right-handed helix shown contains thiomalate ligands with the *R* absolute configuration. Au–S bond lengths : 2.283, 2.286 Å; S-Au-S angles: 178.88°, 169.41°. Adapted from ref. [158].

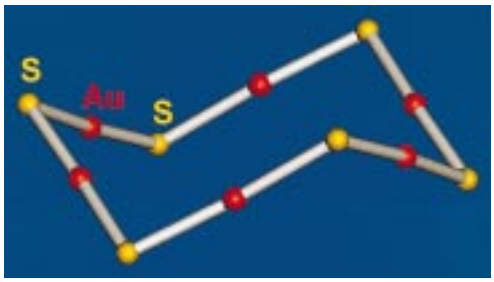

Figure 14. The hexameric ring structure in gold(I) 2,4,6-tri(isopropyl)thiophenolate. Substituents on S have been omitted for clarity. Au–S bond lengths: 2.28 – 2.29 Å. S-Au-S bond angles: 175 – 177°. Adapted from ref. [160].

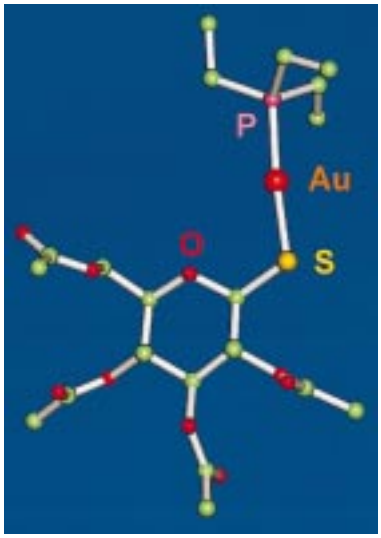

Figure 15. Crystal structure of the oral antiarthritic drug auranofin (**42**). Adapted from ref. [198].

A common metabolite found in the urine and plasma of patients treated with gold drugs is $[Au(CN)_2]^-$,[163] an ion which readily enters cells and can inhibit the oxidative burst of white blood cells. It may therefore be an active metabolite of gold drugs. It also exhibits anticancer and anti-HIV activity.[209] The high Au content of the red blood cells of smokers receiving gold therapy has been attributed to the inhalation of HCN in smoke.[164]

Under conditions mimicking red blood cell concentrations, Elder et al.[165] have observed that $[Au(CN)_2]^-$ reacts with GSH to form $[Au(SG)_2]^-$, which is very stable. $[Au(SG)_2]^-$ is likely to be formed inside cells both from gold thiolate drugs and from auranofin **42**, and may be responsible for the inhibition of enzymes such as the Se-enzyme glutathione peroxidase.[166]

Compound **39** is a potent inhibitor of neutrophil collagenase, a zinc enzyme which contains Cys ligands in the metal-binding site.[167, 168] Such interactions may be important in joint tissue.

Gold(I) has a very high affinity for thiolate S, but binds only weakly to O and N ligands. Hence proteins containing cysteine thiolate groups (especially those with low pK_a values) are targets for Au^I, but binding of Au^I to DNA is weak. Moreover, thiolate exchange reactions are usually rapid. During therapy, Au levels in the blood typically reach 20 μM, and Au is transported by albumin bound to Cys34. The rate of gold binding is determined by the rate of opening of the cleft containing Cys34,[169, 170] and Au binding appears to induce a "flip-out" of this residue.[171] Gold(I) drugs can bind strongly to thiol groups in DNA-binding proteins such as the transcription factors Jun – Jun and Jun – Fos, giving rise to the possibility that gold can regulate transcription factor activity.[172]

The oxidation of Au^I to Au^{III} in vivo may be responsible for some of the toxic side effects of gold drugs.[173, 174] In inflammatory situations, strong oxidants such as ClO^- and H_2O_2 are potentially available in vivo and can oxidize the metal centers in **39**, **40** and **42** to Au^{III}.[175] Gold(III) has the unusual ability to deprotonate peptide amide groups even at low pH values (e.g. pH 2)[176] and may modify peptide recognition by T cells. Gold(III) can oxidize disulfide bridges in albumin and insulin,[177] and methionine residues of ribonuclease.[178]

5. Bismuth Antiulcer Drugs

Bismuth compounds have been used for treating gastrointestinal disorders for more than two centuries.[179] These include nitrate, salicylate salts, and colloidal bismuth subcitrate, all Bi^{III} compounds. Bismuth(V) is usually a strong oxidant. The structures of Bi drugs are largely unknown. The coordination number of Bi^{III} is highly variable (3 – 10), and the coordination geometry often irregular.[180] Bi^{III} is highly acidic in water (first pK_a ca. 1.5) and has a strong tendency to form stable hydroxo- and oxo-bridged clusters. Figure 16 shows the core structure of $[Bi_6O_4(OH)_4](NO_3)_6 \cdot 4H_2O$, known since the 17th century as "magisterium bismuti" and used as a beauty care product, which crystallizes at pH values below 1.2.[181]

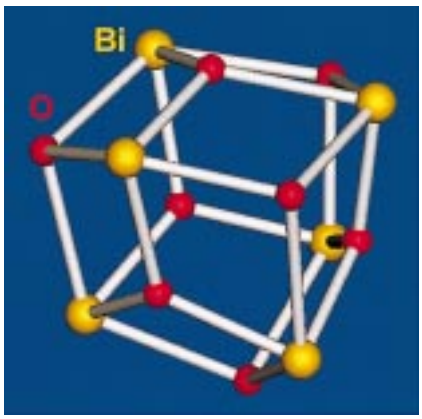

Figure 16. Crystal structure of $[Bi_6O_4(OH)_4](NO_3)_6 \cdot 4H_2O$, showing hydroxo- and oxo-bridged Bi^{III} centers. Adapted from ref. [181].

The best understood in structural terms are the citrate complexes, for which several X-ray structures have been determined,[182] although none has exactly the same composition as the drugs themselves. The dominant feature is the dimeric $[(cit)BiBi(cit)]^{2-}$ unit (H_4cit = citric acid), which contains bridging citrate anions. The Bi–O(alkoxide) bond is very short (2.2 Å) and strong, and a stereochemical role for the $6s^2$ lone pair is apparent in the structure. These dimers aggregate into chains and sheets in the crystal through a network of hydrogen bonds involving citrate, counter ions, and water to give polymers such as that shown in Figure 17. Such polymers may be deposited on the surface of ulcers. Bismuth(III) citrate complexes appear to be stable in solution over a pH range of about 3.5 to 7.5. $[Bi^{III}(Hcit)]$ itself can be solubilized by a variety of amines,[183] and the adduct with the organic histamine antagonist ranitidine, ranitidine bismuth citrate,[184, 185] has recently been marketed as a new drug.

The antimicrobial activity of Bi^{III} against the bacterium *Helicobacter pylori* appears to be important for its antiulcer activity.[186] This organism may also be involved in other conditions such as cancer. The biological effects of Bi^{III} are probably largely due to binding to proteins and enzymes. The binding of Bi^{III} to DNA is, as yet, unknown. There is a strong correlation between the strength of binding of Bi^{III} to a variety of ligands and that of Fe^{III} (Figure 18). Although Bi^{III} (ionic radius 1.03 Å) is larger than Fe^{III} (ionic radius 0.64 Å, high spin) it also binds strongly to the serum Fe^{III} transport protein transferrin.[187] The strength of binding of transferrin to Bi^{III} can be correlated with the high acidity of Bi^{III} (Figure 19). Related proteins (periplasmic iron-binding proteins) are involved in Fe uptake by some virulent bacteria. Bismuth(III)

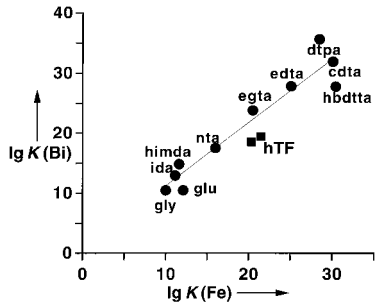

Figure 18. Linear free energy relationship for complexation of Fe^{III} and Bi^{III} with oxygen and nitrogen donor ligands. Adapted from ref. [187a]. hbdtta = *N*,*N*′-bis(2-hydroxybenzyl)diethylenetriamine-*N*,*N*′,*N*″-triacetate, cdta = *trans*-1,2-diaminocyclohexane, dtpa = diethylenetriamine pentaacetate, gly = glycine, glu = glutamate, ida = iminodiethanoate, himda = *N*-(2-hydroxyethyl)iminodiethanoate, nta = nitrilotriacetate, hTF = human transferrin, egta = ethylenebis(oxyethylenenitrilo)tetraacetate, edta = ethylenediaminetetraacetate.

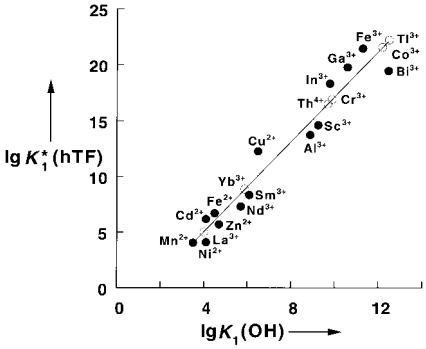

Figure 19. Correlation of the strength of metal binding to human serum transferrin hTF (bicarbonate independent binding constant) with metal ion acidity. Open circles are predicted values. Adapted from ref. [187b].

can readily displace Zn^{II} from the Cys-rich protein metallothionein, and bismuth metallothionein is stable even under strongly acidic conditions (pH 2).[188]

Cysteine and glutathione may play a role in the transport of Bi^{III} in cells and biofluids. These thiols can prevent the precipitation of colloidal bismuth subcitrate (CBS) at pH 2.0, and animal studies have shown that simultaneous oral administration of bismuth salts and thiols produces a significant rise in the bismuth concentration in blood plasma.[189, 190] The complex $[Bi(SG)_3]$ is very stable ($\lg K = 29.6$) over a wide pH range (2–10) with binding through the S atom only.[191] Exchange of GSH between free and bound forms is relatively rapid at biological pH (ca. 1500 s^{-1}).

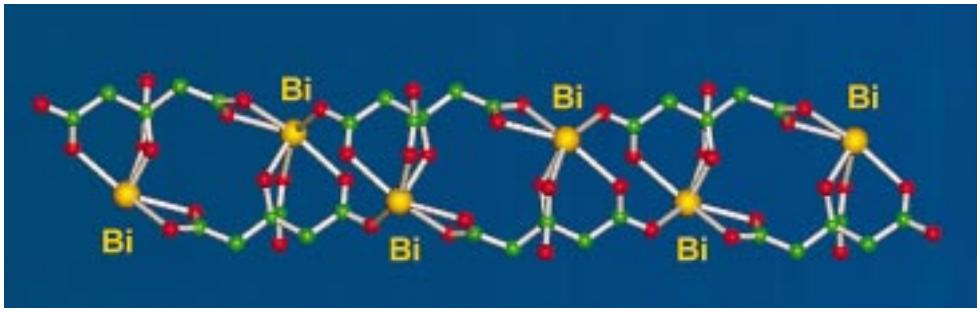

Figure 17. Crystal structure of the polymeric Bi–citrate complex $Na_2[Bi_2(cit)_2]$. Adapted from ref. [182d].

6. Summary and Outlook

It is clear that inorganic chemistry will have an important role to play in medicine in the future. The range of elements traditionally available to the pharmacologist (largely H, C, N, O, F, S, P) is being widened to include all 24 elements thought to be essential to mammalian life together with many other elements in the periodic table, both stable and radioactive. Metal ions in particular play crucial roles in physiological processes and offer much scope for innovative drug design.

The success of cisplatin (the world's best selling anticancer drug) for the treatment of cancer is clear. The mechanism of action of platinum-based agents is much better understood than those of other widely used metallodrugs such as gold antiarthritic, bismuth antiulcer, lithium antidepressive (used by about 1 in 1000 of the population of the UK), or antimony antiparasitic agents. These elements all present significant challenges, as do elements new to the clinic such as Ti, V, Mn, Ru, and Gd.

The improved design of metallodrugs depends on a better knowledge of coordination chemistry under biologically relevant conditions, knowledge not only of the thermodynamics (equilibrium constants and structures of products) but also of the kinetics (mechanisms, pathways, ligand-exchange dynamics) of substitution and redox reactions. Biological systems often operate far from thermodynamic equilibrium. Understanding the nature of the interactions of metallodrugs with cell membranes, proteins, enzymes, and DNA is particularly important. Our increasing knowledge of the role of metals in genetic regulation and the nature of metal sensor, transport, and chaperone proteins will eventually have a major impact on drug design. For example, iron is a corepressor for the diphtheria toxin gene in virulent pathogenic bacteria,[192] and inherited human diseases of copper transport can now be linked directly to genes encoding copper-transporting ATPases.[210] There is much further scope for using metals to deliver biologically active ligands, for example NO, and the recent clinical introduction of titanocene dichloride illustrates that organometallic complexes too can have uses in medicine, provided they are formulated appropriately.

As a final illustration of how medicinal inorganic chemistry is likely to have a major impact on pharmacology, we highlight neuropharmacoclogy. The roles of Na^+, K^+, and Ca^{2+} in neurochemistry are well-known, but it is also apparent that Fe and Cu enzymes can control neurotransmitter biosynthetic pathways, and there are millimolar levels of Zn^{2+} in the hippocampus during neurotransmission. Moreover, Mn is abundant in the brain in enzymes such as glutamine synthase and superoxide dismutase. We can speculate that the control of metal neurochemistry is vital for the prevention of neuronal degradation, and for an understanding, and perhaps effective treatment, of conditions such as Parkinson's disease, senile dementia, Alzheimer's disease, and even Creutzfeldt–Jakob disease (CJD). Prion protein, believed to be the cause of CJD, is thought to be a copper protein in vivo.[211] We can expect medicinal inorganic chemistry to rise to such challenges.

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