Medicinal Applications of Metal Complexes Binding to Biological Macromolecules

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Summary: Biological macromolecules present in living organisms, like proteins, DNA, have many metal-binding sites. As a consequence, coordination compounds can react with such cellular components, displaying possible toxic effects, or they also may have beneficial applications. So metal ions and compounds not only are essential for life in e.g. dioxygen transport, or biocatalysis, but they can also be used for controlled toxicity in medicinal applications. Well-known examples are the application of gold compounds in arthritis treatment, bismuth compounds for ulcer healing, and silver compounds for open skin protection. However, only in a few cases details of the mechanist are known, and if so, the biological macromolecules play a key role. Transition-metal coordination compounds that have metal-ligand exchange rates comparable to cell-division processes, i.e. in particular Pt and Ru, often appear to be highly active in killing cancers, as seen from the study of several cancer cell lines. Classical examples, like cisplatin, and new examples of Pt and Ru compounds will be discussed in some detail, with a focus on their binding to the DNA biomacromolecule. The classical compound *cis*-diamminedichloridoplatinum(II), often abbreviated as cisplatin, and its first-generation derivatives are known to bind to several biomacromolecules in a specific way, and eventually bind at DNA. Four important examples are shown in the figure. However, on its way to DNA other cellular components, like proteins and peptides might be intermediates before the final target is reached. Other metal-containing drugs, like Ru compounds, that show anti-cancer activity have a less well-known mode of action. Molecular-based mechanistic studies may result in improved clinical administration protocols.

Keywords: anticancer properties; biomacromolecules; DNA; Platinum; Ruthenium

Introduction

Metal-containing drugs, have for long been of interest, but the interest of the scientific community for medicinal aspects of metal compounds has rapidly grown in the last few decades.[1,2] The anticancer agents, cisplatin, carboplatin and oxaliplatin enjoy the status of the world's best selling anticancer drugs.[3,4] After the appearance of the first patents and papers on cisplatin (IUPAC name: *cis*-diamminedichloridoplatinum(II),) and its enormous success in the treatment of a variety of tumors, the research on Pt-antitumor chemistry has attracted much interest from chemists, pharmacologists, biochemists, biologists and medical researchers,[5] generating several studies on metal-macromolecule interactions, with special attention to DNA binding. As a positive result of these multidisciplinary studies, an already quite detailed knowledge of the mechanism of cisplatin and related drugs is available.[5,6] In addition, this knowledge has resulted in much improved clinical administration protocols, as well as research about and application of other, related drugs containing transition metals.

All chemotherapeutic drugs are known to have – often very serious – drawbacks,

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including intrinsic or acquired resistance and also disturbing toxicity. The platinum-based drugs are no exception, despite many efforts to reduce the side effects. Fortunately, improved understanding of the mechanism of action of cisplatin, originating from the efforts of many research groups during the last two decades has resulted in a variety of newly designed platinum drugs, and also drugs with other metals, like ruthenium.[7–11] Although many mechanistic questions have been answered, especially for the Pt compounds, several important questions remain, especially for the drugs containing non-Pt metals, in particular dealing with how these molecules interact with biomacromolecules, both kinetically and thermodynamically.[4–6]

Given the limited space, this paper will devote most attention to the interactions of the platinum species with macromolecules, in particular nucleic acids and proteins. I shall start with a brief introduction on the coordination chemistry of medicinal relevant metals, with a focus on Pt and other noble metals that have been shown to possess important biological properties. The main part will be dealing with a description of the state-of-the-art in metal-anticancer drugs and the current mechanistic insights for cisplatin and related platinum and ruthenium drugs. Interaction of the metal coordination compounds with the bio-macromolecules is a central them in the discussion. Finally, a brief discussion will be given to the design, synthesis, structure and biological activities of new bifunctional and multifunctional, platinum, ruthenium and mixed-metal compounds, their possible applications and binding to bio-macromolecules.

Metal Coordination Compounds and their Binding to Bio-Macromolecules

Metal-ion coordination bonds are of intermediate strength, i.e. much weaker than covalent bonds, and usually much stronger than the so-called non-covalent interactions (stacking, hydrogen bonding). For proper understanding of reactivity knowledge of electronic structures, thermodynamics and kinetics of M-L bond formation is therefore important.

In many biological, now often called supramolecular, systems these intermediate and weak interactions play an important role, not only in protein structures (secondary, tertiary and quaternary structures), and in DNA structures (stacks in the helix, double helices). Also in metal-binding reactions to bio-macromolecules such interactions play an important role, and many of these together may generate a high thermodynamic stability.

In addition to this ''thermodynamic'' stability of molecules and aggregates, the ''kinetic'' stability has to be considered, i.e. how fast are the bonds formed and broken. It is well known that ligand-exchange processes of ions like Mg(II), Ni(II), Ca(II), Na(I), are very fast indeed (up to 10^9/sec), whereas the ligand exchange processes of Pt(II), Ru(II), Os(II), Ir(III), Cr(III) and Pt(IV) are much slower; such processes may take hours (Pt, Ru) or even longer at ambient temperatures. A schematic presentation of ligand exchange rates for metal-aqua complexes is depicted in Figure 1; the figure is based on the early results published by Taube.[12] It is clearly seen that Pt(II) and Ru(II) have exchange reactions that are within the range of biological (cell division) processes.

Of course it should be realised that with other ligands, different rates may apply, and also that in chelates, like e.g. metal-porphyrins, the ligand exchange rates are much slowed down.[13]

Brief Overview Platinum Anticancer Drugs

The history of the discovery and development of cisplatin, starting with the serendipitous discovery of Rosenberg[14] and its reported anticancer activity,[15] is well documented, and the main work before 1999 is mentioned in the excellent monograph compiled by Lippert.[16] More recent

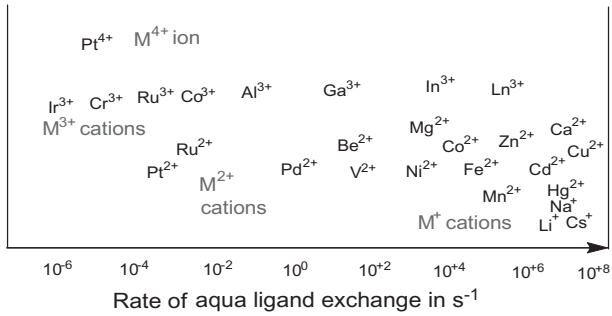

Figure 1.
Relative ranking of metal ions in water ligand exchange (after Taube,[12]).

updates are available from several sources, and are recommended for further reading.[5,17–21]

The number of compounds used routinely in the clinics, as per 2007, is still limited. Some examples are depicted in Figure 2. A recent overview of the used drugs is available.[22]

Cisplatin Mechanism

A brief summary can only be given here, and the reader is referred to recent reviews for details.[4,5,18–20,23] So, after the administration of cisplatin in the blood (usually by injection or infusion), the drug is well known to be transported over the body. The presence of a relatively high chloride concentration in the blood (about 100 mM), prevents ligand exchanges and hydrolysis. Nevertheless, the small fraction that does hydrolyse is known to be responsible for the acute toxicities, such as kidney damage. Carboplatin and oxaliplatin have much slower hydrolysis, and do hardly dissociate in blood, and therefore have smaller side effects. The Pt compounds do enter – almost all – types of cells, whereby use is made of passive or even active transport via specific receptors. It appears that the so-called CTR1 receptor (used by Nature for Cu transport), may assist or influence the Pt species to enter the cell, with or without changing the ligands around the metal.[5] During the process of entering the cells binding of the platinum species to one of the membrane components, i.e. phosphatidyl serine, has been proposed based on NMR analysis,[24]. A schematic structure of such a macromolecular adduct is presented in Figure 3.

Early studies of mechanistic research on cisplatin had made clear that DNA is a main target,[16] and therefore binding of Pt compounds to DNA and its oligomeric fragments have been the subject of many investigations.[25–30] From such studies it became soon clear that the guanine base binds much more rapidly to Pt than the other bases do, and also is held much longer at this position. This behaviour has been explained by a higher basic pK_a and by simultaneous H-bonding of the amine-NH

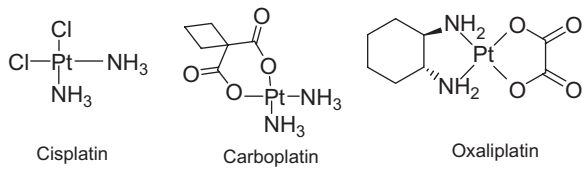

Figure 2.
Schematic structure of the 3 routinely-used platinum anticancer compounds.

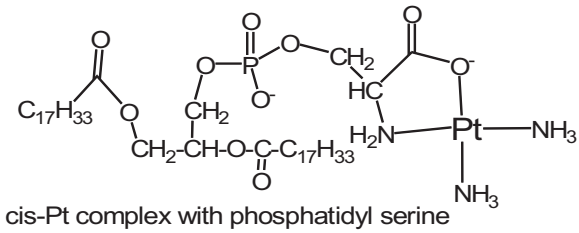

Figure 3.
Schematic structure of the reaction product of cisplatin with phosphatidylserine.

to the O6 of guanine.[4,8] Detailed analysis had shown earlier a much larger amount of GG adducts than statistically expected (about 2/3 of all Pt binds at GG).[31] This binding process has been studied in detail, starting with the mononuclear level,[32,33] followed by dinucleotide and trinucleotide species,[34,35] including crystal structure determinations,[25,30]. More recently also double-stranded DNA coordinated as a macromolecular ligand to the cisplatin unit, has been studied. In all cases a clear kinked chelated structure is formed, as shown by several NMR and XRD structure determinations.[26,36–38] In the next stages, particularly through the work of Lippard,[39,40] it was found that proteins, known to recognize the kinked DNA do bind to the kinked, platinated DNA. Even a 3D crystal structure of such a protein, bound to platinated DNA has been determined recently,[41] showing that the overall kinked structure is not changed and that the protein more or less embraces the platinated DNA; a phenylalanine side chain, located between the two coordinated guanine bases, possibly stabilises the kink. The structure of this impressive ternary bio-macromolecular complex is redrawn in Figure 4. It might be that as a direct, or indirect, consequence of this this protein binding the tumor cell might be killed by apoptosis .[5]

From the beginning of mechanistic studies it had been puzzling why all platinum species would not bind at the numerous sulfur-donor ligands (which are

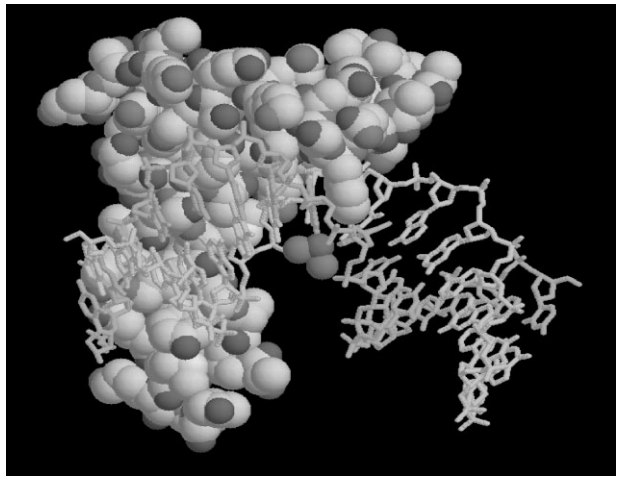

Figure 4.
Structure of the HMG protein fragment (space filling), stabilizing the cisplatin-bound kinked DNA (2-colour stick model), after Lippard.[41] The phenylalanine stacking in between the 2 Guanines is clearly visible.

known platinophilic) in the cells, and never reach the DNA. In fact the possibility of rapid S-donor ligand binding to the Pt species, was considered some time ago already, in particular to act as an intermediate[42–44] on its way to the DNA in the nucleus. Despite the fact that several S donor ligands do react differently from one another, such a temporary binding to molecules like glutathione and methionine is highly likely,[20,45], but also retardation of DNA binding has been proven.[46]

The questions how Pt species can pass the cytoplasm and find its way to the nucleus have been studied,[47] using a cis-diamine-Pt compound carrying a fluorescent label; this will allow the processes in cells to be followed in real time, i.e. from entering the cell, entering the nucleus, and leaving the cell via the Golgi apparatus. We have shown this also to be possible with other Pt compounds.[48,49]

Mechanistic Studies on Related Pt Compounds

Rather early in the history of cisplatin numerous derivatives were prepared and studied, in a search for structure-activity relationships.[21] Initially variations on cisplatin were dealing with the changes of the amines, and in the anionic ligands. These studies have resulted in carboplatin (see Figure 2); later on compounds with other amines, were synthesized, like oxaliplatin,[50,51] which is now frequently used in the treatment of colon cancer, Oxaliplatin, also sometimes called Eloxatin, was discovered over two decades ago by Kidani[52] and subsequently developed;[51] however, it took a long time before it was fully accepted and only recently is has been routinely used in the clinic. This compound is especially interesting as it is sensitive to tumors to which cisplatin does not well respond. In fact almost the same Pt-DNA adducts have been reported as for cisplatin, including a 3D structure of a synthetic adduct with a double-stranded piece of DNA.[53]

Initially trans-Pt compounds based on primary amines were all found to be inactive; more recently, it has been shown that sterically hindered amines and imines, even when in trans positions, generate active species for aromatic imines [54,55] and for aliphatic amines and mixed imine-amine complexes.[56–59] .These and other possible developments are schematically depicted in Figure 5.

Often ideas of new compounds arise from mechanistic findings with previous generations of drugs. Some important new developments that may lead to, or just have

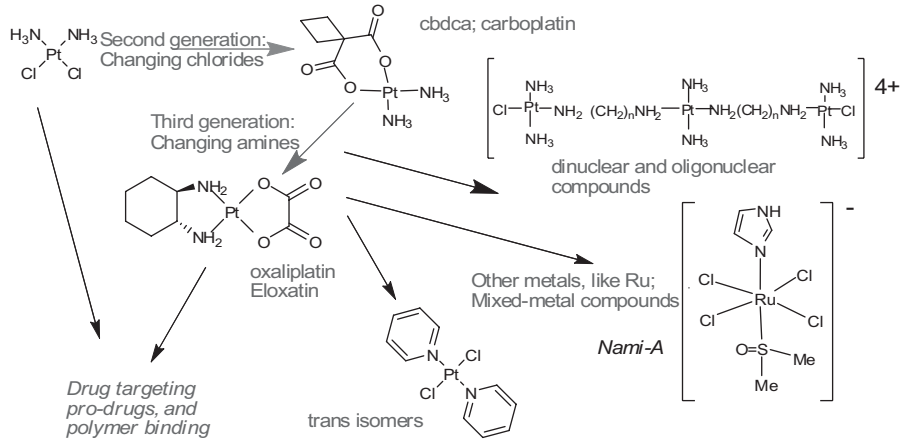

Figure 5.
Platinum drug development in a schematic way, starting from cisplatin, up to oxaliplatin, and NAMI-A.

led to clinical applications of platinum drugs are the recent dinuclear and trinuclear compounds of the third generation Pt anticancer compounds; in fact all such compounds have at least a H-donor function available on one of the amines. The role of this N-H group has been explained kinetically in its approach of the guanine, and in the additional stabilisation of the formed GG chelates by hydrogen bonding to a DNA backbone phosphate,[8,60] making them less prone to reversion by binding to the S-donor ligands in the cell.

Surprisingly also the inert Pt(IV) can yield compounds that are anti-cancer active; they were initially assumed to be reduced to Pt(II) *in vivo*, before binding to the DNA. Later studies have shown that also certain Pt(IV) compounds may react with DNA and with DNA fragments,[61] and that traces of Pt(II) catalyse this reaction.[62–65] The mechanism of reduction also involves the phosphate groups, as elegantly proven for 5′-GMP.[66]

A most surprising new class of dinuclear and trinuclear compounds, with diamines as bridging ligands has been studied in great detail by Brabec and Farrell.[67–76] The flexible linker between the Pt ions does allow multiple binding on the DNA chain, and this has resulted in interesting geometrical differences between isomers.[77]

A quite different approach deals with rigid ligand bridges between the Pt ions. After the first experiments by Kozelka[78], Komeda[79–81] found that the rigidly bridged dinuclear Pt compounds, containing either pyrazole or triazole bridge, may generate very active species, that do bind to DNA. Earlier attempts with imidazoles yielded mononuclear compounds upon binding to transition metals,[82] followed by platinum.[83] These compounds initially turned out to be rather inactive, but their use in trans compounds[84] and with the azolato anion as a bridging ligand, showed very high cytostatic activities.[79,80,85] In Figure 6 a structure determined by high-resolution NMR studies on double-stranded DNA with such a (pyrazolato)Pt_2 unit bound[86] is redrawn; this structure was also confirmed by DFT-based theoretical calculations.[87]

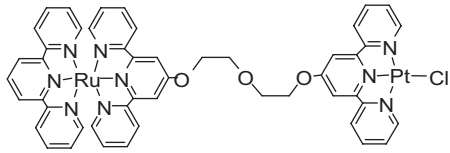

Figure 6.
Dinuclear cationic species containing Ru(II) and Pt(II), with a variable spacer, and a chloride as a leaving group.

Ru Compounds and Mixed-Metal Compounds

The similarity in ligand exchange behaviour for Pt(II) and Ru(II) has led to a variety of new Ru(II) and Ru(III) compounds that show promising activity in cell lines and animal studies. After the early work of Clarke,[88,89] important work from the Trieste groups on the NAMI-class compounds,[90] and related work by Keppler,[91] the field of Ru anticancer research has now rapidly evolved.[10] Here only a few complexes can be discussed, as the binding to DNA and other macromolecules has not yet been that far developed, compared to cisplatin. To be mentioned in particular are the three rather recently reported types of Ruthenium compounds:

a) NAMI-type compounds (see Figure 5 for a structural drawing of the anionic Ru species in NAMI-A).
b) The aza-pyridine compounds, in which different isomers show significantly different cytostatic activity.[92–94]
c) The organometallic half-sandwich compounds of formula [Ru(sandwich)(diamine)Cl], in which the amine ligand is an important factor for the activity;[95–97] also here hydrogen bonding plays an importance role.

The mechanism of action of the Ru compounds is hardly known, and even the fact that DNA is an important target is not

sure as yet; a recent review is recommended for further studies.[98]

Finally, a few new mixed-metal Pt compounds should be mentioned here. If two different metals can be linked, in a kinetically inert way, i.e. by using a spacer of variable length, fine-tuning and a wealth on new compounds become accessible. Using both Pt and Ru, a few cases have been reported already[99,100] including a 3D structure determination; the corresponding chemical structure is redrawn in Figure 6.

The antitumor activity of this prototype complex is limited, but as a result new Pt-Ru and Pt-M compounds have been investigated. When combining the slow metal with a faster metal, like Cu(II), a well-known DNA cleaving agent, completely new systems become possible.[101]

In fact combining the principle of Figure 6, to the DNA cutting capabilities of the Cu systems, a powerful bifunctional system becomes accessible, where the Pt species on the DNA does the selective binding, where the Cu part can do the cutting, at fixed distance, determined by the space lengths. The prototype of this system has just been proven and is schematically shown in Figure 7.[102]

Final Remarks

The work summarized above has been aimed shown that the ''heavy metals'' platinum and ruthenium, when coordinated to the proper ligands, may act on macromolecules, while acting as powerful anticancer drugs. The important property of these metals appears to be they are ''slow'' in ligand-exchange reactions, in fact the exchange of some of their ligands falls within the same time scale as many of the cellular division processes takes place. This may explain why these compounds are not defragmented before any of their biological targets is reached. It is realized that the present short overview can only be far from complete; however, I have tried to focus on some issues that Pt and Ru have in common (ligand exchange kinetics), and on their binding to biologically relevant macromolecules, in particular DNA. The list of references given below, including some specialist reviews, should help the interested reader to find more details.

Figure 7.
Schematic structure of a bifunctional compound with a Cu-containing DNA-cleaving moiety and a Pt ion as the anchor, capable of recognizing and chelating to a GG site in the double-stranded DNA, after de Hoog et al.[102]

Acknowledgements: The author wishes to thank Johnson & Matthey (Reading, UK) for their generous loan of K_2PtCl_4 and $RuCl_3 \bullet 3H_2O$. Continuous support from the Netherlands Chemical Research Council (CW) is gratefully acknowledged. Many students and postdocs contributed to the research highlighted above; their names are listed in the references as my co-authors. Support and sponsorship obtained by several COST Actions (D1, D8, D20 and D39), allowing several student exchanges, are kindly acknowledged.

[1] K. H. Thompson, C. Orvig, *Science* **2003**, *300*, 936–939.
[2] S. J. Lippard, *Nat. Chem. Biol.* **2006**, *2*, 504–507.
[3] J. Reedijk, in *Macromolecular Nanostructured Materials*, N., Ueyama, A. Harada, Eds., Springer, Edition 2004, vol. *78*, pp. 244–257.
[4] J. Reedijk, *Proc. Natl. Acad. Sc. (USA)* **2003**, *100*, 3611–3616.
[5] D. Wang, S. J. Lippard, *Nat. Rev. Drug Discov.* **2005**, *4*, 307–320.
[6] J. Reedijk, in *Symposium Series No. 903 Medicinal Inorganic Chemistry*, J. L., Sessler, S. R., Doctrow, T. J. McMurry, S. J. Lippard, American Chemical Society, Washington DC Edition 2005, pp. 80–109.
[7] O. Novakova, J. Kasparkova, O. Vrana, P. M. van Vliet, J. Reedijk, V. Brabec, *Biochem.* **1995**, *34*, 12369–12378.
[8] J. Reedijk, *Chem. Commun.* **1996**, 801–806.
[9] E. Alessio, E. Iengo, B. Serli, G. Mestroni, G. Sava, *J. Inorg. Biochem.*, **2001**, *86*, 21–21.
[10] P. J. Dyson, G. Sava, *Dalton Trans.*, **2006**, 1929–1933.

[11] A. H. Velders, A. C. G. Hotze, G. A. van Albada, J. G. Haasnoot, J. Reedijk, *Inorg. Chem.* **2000**, *39*, 4073–4080.
[12] H. Taube, *Chem. Rev.* **1952**, *50*, 69–78.
[13] C. E. Housecroft, A. G. Sharpe, *Inorganic Chemistry*, Prentice Hall, Harlow, **2001**.
[14] B. Rosenberg, L. Van Camp, T. Krigas, *Nature* **1965**, *205*, 698–699.
[15] B. Rosenberg, L. Van Camp, J. E. Trosko, V. H. Mansour, *Nature* **1969**, *222*, 385–387.
[16] B. Lippert, *Cisplatin, Chemistry and Biochemistry of a Leading Anticancer Drug*, Wiley-VCH, Weinheim **1999**.
[17] G. Natile, L. G. Marzilli, *Coord. Chem. Rev.* **2006**, *250*, 1315–1331.
[18] M. Galanski, M. A. Jakupec, B. K. Keppler, *Curr. Med. Chem.* **2005**, *12*, 2075–2094.
[19] M. J. Piccart, H. Lamb, J. B. Vermorken, *Ann. Oncol.* **2001**, *12*, 1195–1203.
[20] J. Reedijk, *Chem. Rev.* **1999**, *99*, 2499–2510.
[21] E. Wong, C. M. Giandomenico, *Chem. Rev.* **1999**, *99*, 2451–2466.
[22] G. Momekov, D. Momekova, *Expert Opin. Ther. Patents* **2006**, *16*, 1383–1403.
[23] M. Ober, D. Wang, M. A. Wallace, S. J. Lippard, *J. Biomol. Struct. Dyn.* **2007**, *24*, 675–675.
[24] G. Speelmans, W. H. H. M. Sips, R. J. H. Grisel, R. W. H. M. Staffhorst, A. M. J. Fichtinger-Schepman, J. Reedijk, B. de Kruijff, *Biochim. Biophys. Acta* **1996**, *1283*, 60–66.
[25] G. Admiraal, J. L. van der Veer, R. A. G. de Graaff, J. H. J. den Hartog, J. Reedijk, *J. Am. Chem. Soc.* **1987**, *109*, 592–594.
[26] J. H. J. den Hartog, C. Altona, J. H. van Boom, G. A. van der Marel, C. A. G. Haasnoot, J. Reedijk, *J. Am. Chem. Soc.* **1984**, *106*, 1528–1530.
[27] J. Kozelka, M. A. Elizondo-Riojas, *J. Inorg. Biochem.* **2001**, *86*, 64–64.
[28] L. G. Marzilli, T. P. Kline, D. Live, G. Zon, in *"Metal-DNA Chemistry", ACS Symposium Series (Tullius, T.D., ed.),* **1989**, *402*, 119.
[29] L. G. Marzilli, J. S. Saad, Z. Kuklenyik, K. A. Keating, Y. H. Xu, *J. Am. Chem. Soc.* **2001**, *123*, 2764–2770.
[30] S. E. Sherman, S. J. Lippard, *Chem. Rev.* **1987**, *87*, 1153–1181.
[31] A. M. J. Fichtinger-Schepman, J. L. van der Veer, J. H. J. den Hartog, P. H. M. Lohman, J. Reedijk, *Biochem.* **1985**, *24*, 707–713.
[32] A. T. M. Marcelis, C. Erkelens, J. Reedijk, *Inorg. Chim. Acta* **1984**, *91*, 129–135.
[33] A. T. M. Marcelis, C. G. van Kralingen, J. Reedijk, *J. Inorg. Biochem.* **1980**, *13*, 213–221.
[34] J. H. J. den Hartog C Altona, J. H. van Boom, A. T. M. Marcelis, G. A. v. d. Marel, L. J. Rinkel, G. Wille-Hazeleger, J. Reedijk, *Eur. J. Biochem.* **1983**, *134–141*, 485.
[35] A. T. M. Marcelis, J. Reedijk, *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 212–213.
[36] S. T. Sullivan, A. Ciccarese, F. P. Fanizzi, L. G. Marzilli, *J. Am. Chem. Soc.* **2001**, *123*, 9345–9355.
[37] S. U. Dunham, C. J. Turner, S. J. Lippard, *J. Am. Chem. Soc.* **1998**, *120*, 5395–5406.
[38] F. Reeder, Z. J. Guo, P. D. Murdoch, A. Corazza, T. W. Hambley, S. J. BernersPrice, J. C. Chottard, P. J. Sadler, *Eur. J. Biochem.* **1997**, *249*, 370–382.
[39] E. R. Jamieson, M. P. Jacobson, C. M. Barnes, C. S. Chow, S. J. Lippard, *J. Biol. Chem.* **1999**, *274*, 12346–12354.
[40] E. R. Jamieson, S. J. Lippard, *Chem. Rev.* **1999**, *99*, 2467–2498.
[41] U. M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo, S. J. Lippard, *Nature* **1999**, *399*, 708–712.
[42] E. L. M. Lempers, K. Inagaki, J. Reedijk, *Inorg. Chim. Acta*, **1988**, *152*, 201–207.
[43] E. L. M. Lempers, J. Reedijk, *Inorg. Chem.* **1990**, *29*, 217–222.
[44] S. S. G. E. van Boom, J. Reedijk, *J. Chem. Soc. Chem. Commun.* **1993**, 1397–1398.
[45] Z. J. Guo, P. J. Sadler, in, *Adv. Inorg. Chem., Edition* **2000**, vol. *49*, pp. 183–306.
[46] O. Vrana, V. Brabec, *Biochem.* **2002**, *41*, 10994–10999.
[47] C. Molenaar, J. M. Teuben, R. J. Heetebrij, H. J. Tanke, J. Reedijk, *J. Biol. Inorg. Chem.* **2000**, *5*, 655–665.
[48] G. V. Kalayda, G. F. Zhang, T. Abraham, H. J. Tanke, J. Reedijk, *J. Med. Chem.* **2005**, *48*, 5191–5202.
[49] G. V. Kalayda, B. A. J. Jansen, P. Wielaard, H. J. Tanke, J. Reedijk, *J. Biol. Inorg. Chem.* **2005**, *10*, 305–315.
[50] G. Mathe, Y. Kidani, M. Segiguchi, M. Eriguchi, G. Fredj, G. Peytavin, J. L. Misset, S. Brienza, F. Devassals, E. Chenu, C. Bourut, *Biomed. Pharmacother.* **1989**, *43*, 237–250.
[51] M. Noji, R. Kizu, Y. Takeda, N. Akiyama, I. Yoshizaki, M. Eriguchi, Y. Kidani, *Biomed. Pharmacother.* **2005**, *59*, 224–229.
[52] G. Mathe, Y. Kidani, K. Triana, S. Brienza, P. Ribaud, E. Goldschmidt, E. Ecstein, R. Despax, M. Musset, J. L. Misset, *Biomed. Pharmacother.* **1986**, *40*, 372–376.
[53] B. Spingler, D. A. Whittington, S. J. Lippard, *J. Inorg. Biochem.* **2001**, *86*, 440–440.
[54] N. Farrell, T. T. B. Ha, J. P. Souchard, F. L. Wimmer, S. Cros, N. P. Johnson, *J. Med. Chem.* **1989**, *32*, 2240–2241.
[55] A. Zakovska, O. Novakova, Z. Balcarova, U. Bierbach, N. Farrell, V. Brabec, *Eur. J. Biochem.* **1998**, *254*, 547–557.
[56] A. G. Quiroga, J. M. Perez, C. Alonso, C. Navarro-Ranninger, N. Farrell, *J. Med. Chem.* **2006**, *49*, 224–231.
[57] E. Pantoja, A. Gallipoli, S. van Zutphen, D. M. Tooke, A. L. Spek, C. Navarro-Ranninger, J. Reedijk, *Inorg. Chim. Acta* **2006**, *359*, 4335–4342.
[58] D. Gibson, Y. Najajreh, J. Kasparkova, V. Brabec, J. M. Perez, C. Navarro-Ranniger, *J. Inorg. Biochem.* **2003**, *96*, 42–42.
[59] J. A. R. Navarro, E. Freisinger, B. Lippert, *Inorg. Chem.* **2000**, *39*, 1059–1065.

[60] J. Reedijk, *Inorg. Chim. Acta* **1992**, *198–200*, 873–881.
[61] O. Novakova, O. Vrana, V. Kiseleva, V. Brabec, *Eur. J. Biochem.* **1995**, *228*, 616–622.
[62] S. Choi, S. Delaney, L. Orbai, E. J. Padgett, A. S. Hakemian, *Inorg. Chem.* **2001**, *40*, 5481–5486.
[63] R. M. Roat, M. J. Jerardi, C. B. Kopay, D. C. Heath, J. A. Clark, J. A. DeMars, J. M. Weaver, E. Bezemer, J. Reedijk, *J. Chem. Soc. Dalton Trans.* **1997**, 3615–3621.
[64] R. M. Roat, J. Reedijk, *J. Inorg. Biochem.* **1993**, *52*, 263–274.
[65] E. G. Talman, W. Brüning, J. Reedijk, A. L. Spek, N. Veldman, *Inorg. Chem.* **1997**, *36*, 854–861.
[66] S. Choi, R. B. Cooley, A. S. Hakemian, Y. C. Larrabee, R. C. Bunt, S. D. Maupas, J. G. Muller, C. J. Burrows, *J. Am. Chem. Soc.* **2004**, *126*, 591–598.
[67] R. Zaludova, A. Zakovska, J. Kasparkova, Z. Balcarova, V. Kleinwachter, O. Vrana, N. Farrell, V. Brabec, *Eur. J. Biochem.* **1997**, *246*, 508–517.
[68] J. Kasparkova, O. Novakova, O. Vrana, N. Farrell, V. Brabec, *Biochem.* **1999**, *38*, 10997–11005.
[69] M. Kloster, H. Kostrhunova, R. Zaludova, J. Malina, J. Kasparkova, V. Brabec, N. Farrell, *Biochem.* **2004**, *43*, 7776–7786.
[70] B. Moriarity, O. Novakova, N. Farrell, V. Brabec, J. Kasparkova, *Arch. Biochem. Biophys.* **2007**, *459*, 264–272.
[71] N. Farrell, *Chem. World* **2006**, *3*, 32–32.
[72] M. E. Oehlsen, A. Hegmans, Y. Qu, N. Farrell, *Inorg. Chem.* **2005**, *44*, 3004–3006.
[73] N. Farrell, in *Metal Ions In Biolgical Systems, Vol 42: Metal Complexes In Tumor Diagnosis And As Anticancer Agents*, Edition **2004**, vol. *42*, pp. 251–296.
[74] J. Kasparkova, J. Zehnulova, N. Farrell, V. Brabec, *J. Biol. Chem.* **2002**, *277*, 48076–48086.
[75] P. Perego, C. Caserini, L. Gatti, N. Carenini, S. Romanelli, R. Supino, D. Colangelo, I. Viano, R. Leone, S. Spinelli, G. Pezzoni, C. Manzotti, N. Farrell, F. Zunino, *Mol. Pharmacol.* **1999**, *55*, 1108–1108.
[76] M. J. Bloemink, J. Reedijk, N. Farrell, Y. Qu, A. I. Stetsenko, *J. Chem. Soc. Chem. Commun.* **1992**, 1002–1003.
[77] J. Zhang, D. S. Thomas, M. S. Davies, S. J. Berners-Price, N. Farrell, *J. Biol. Inorg. Chem.* **2005**, *10*, 652–666.
[78] J. Kozelka, E. Segal, C. Bois, *J. Inorg. Biochem.* **1992**, *47*, 67–80.
[79] S. Komeda, S. Bombard, S. Perrier, J. Reedijk, J. F. Kozelka, *J. Inorg. Biochem.* **2003**, *96*, 357–366.
[80] S. Komeda, M. Lutz, A. L. Spek, M. Chikuma, J. Reedijk, *Inorg. Chem.* **2000**, *39*, 4230–4236.
[81] S. Komeda, M. Lutz, A. L. Spek, Y. Yamanaka, T. Sato, M. Chikuma, J. Reedijk, *J. Am. Chem. Soc.* **2002**, *124*, 4738–4746.
[82] J. Reedijk, *Recl. Trav. Chim. Pays-Bas* **1970**, *89*, 605–613.
[83] B. J. Graves, D. J. Hodgson, C. G. van Kralingen, J. Reedijk, *Inorg. Chem.* **1978**, *17*, 3007–3011.
[84] E. Pantoja, A. Gallipoli, S. van Zutphen, S. Komeda, D. Reddy, D. Jaganyi, M. Lutz, D. M. Tooke, A. L. Spek, C. Navarro-Ranninger, J. Reedijk, *J. Inorg. Biochem.* **2006**, *100*, 1955–1964.
[85] S. Komeda, H. Ohishi, H. Yamane, M. Harikawa, K. Sakaguchi, M. Chikuma, *J. Chem. Soc., Dalton Trans* **1999**, 2959–2962.
[86] S. Teletchea, S. Komeda, J. M. Teuben, M. A. Elizondo-Riojas, J. Reedijk, J. Kozelka, *Chem. Eur. J.* **2006**, *12*, 3741–3753.
[87] A. Magistrato, P. Ruggerone, K. Spiegel, P. Carloni, J. Reedijk, *J. Phys. Chem. B* **2006**, *110*, 3604–3613.
[88] M. J. Clarke, *Coord. Chem. Rev.* **2003**, *236*, 207–233.
[89] M. J. Clarke, F. Zhu, D. R. Frasca, *Chem. Rev.* **1999**, *99*, 2511–2533.
[90] A. Bergamo, S. Zorzet, B. Gava, A. Sorc, E. Alessio, E. Iengo, G. Sava, *Anti-Cancer Drugs* **2000**, *11*, 665–672.
[91] H. Depenbrock, S. Schmelcher, R. Peter, B. K. Keppler, G. Weirich, T. Block, J. Rastetter, A. R. Hanauske, *Eur. J. Cancer* **1997**, *33*, 2404–2410.
[92] A. C. G. Hotze, M. Bacac, A. H. Velders, B. A. J. Jansen, H. Kooijman, A. L. Spek, J. G. Haasnoot, J. Reedijk, *J. Med. Chem.* **2003**, *46*, 1743–1750.
[93] A. C. G. Hotze, A. H. Velders, B. A. J. Jansen, J. G. Haasnoot, J. Reedijk, *J. Inorg. Biochem.* **2001**, *86*, 269.
[94] A. C. G. Hotze, A. H. Velders, F. Ugozzoli, M. Biagini-Cingi, A. M. Manotti-Lanfredi, J. G. Haasnoot, J. Reedijk, *Inorg. Chem.* **2000**, *39*, 3838–3844.
[95] A. F. A. Peacock, A. Habtemariam, R. Fernandez, V. Walland, F. P. A. Fabbiani, S. Parsons, R. E. Aird, D. I. Jodrell, P. J. Sadler, *J. Am. Chem. Soc.* **2006**, *128*, 1739–1748.
[96] H. K. Liu, F. Y. Wang, J. A. Parkinson, J. Bella, P. J. Sadler, *Chem. Eur. J.* **2006**, *12*, 6151–6165.
[97] Y. K. Yan, M. Melchart, A. Habtemariam, P. J. Sadler, *Chem. Commun.* **2005**, 4764–4776.
[98] W. H. Ang, P. J. Dyson, *Eur. J. Inorg. Chem.* **2006**, 4003–4018.
[99] B. Van Houten, S. Illenye, Y. Qu, N. Farrell, *Biochem.* **1993**, *32*, 11794–11801.
[100] K. van der Schilden, F. Garcia, H. Kooijman, A. L. Spek, J. G. Haasnoot, J. Reedijk, *Angew. Chem. Int. Edit.* **2004**, *43*, 5668–5670.
[101] M. Pitié, C. Boldron, H. Gornitzka, C. Hemmert, B. Donnadieu, B. Meunier, *Eur. J. Inorg. Chem.* **2003**, 528–540.
[102] P. de Hoog, C. Boldron, P. Gamez, K. Sliedregt-Bol, I. Roland, M. Pitié, R. Kiss, B. Meunier, J. Reedijk, *J. Med. Chem.* **2007**, *50*, 3148–3155.

