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Platinum Anticancer Coordination Compounds: Study of DNA Binding Inspires New Drug Design

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A brief overview is given of platinum anticancer drugs in routine clinical use and under clinical development worldwide. Details of the binding of these drugs with nucleic acids, the preferred binding site, etc. are discussed as well. Using the mechanistic knowledge at the molecular level, in particular DNA binding, possibilities for new drugs are explored. A major part of the review deals with design, synthesis and biochemical/biophysical studies of such new compounds and their possible applications as cytostatic drugs. Special attention is given to the application of bifunctionality in such new compounds.

Introduction

The medical application of the simple coordination compound, *cis*-$PtCl_2(NH_3)_2$ has reached freshmen chemistry textbooks worldwide. The clinical use of the compound which is also known as "cisplatin" and CDDP – first described 150 years ago[1] – is indeed a real success story, brought to a new life by the discoveries or Barnett Rosenberg and his team.[2,3] The structure is given in Figure 1, together with that of the other clinically applied Pt compounds. For the history of the discovery the reader is referred to a recent overview of Hambley et al.[4]

Hospitals not using cisplatin do perhaps not exist anymore, and in recent years, the interest in derivatives has grown tremendously. Cisplatin is routinely used for the treatment of testicular and ovarian cancer, and is increasingly used against other tumours, such as cervical, bladder and head/neck tumours;[5] typically applied doses administered to patients are 100–200 mg/day for up to 5 consecutive days by infusion.[5] A key reaction step in the mechanism of action appears to be the binding of the *cis*-$Pt(NH_3)_2$ unit

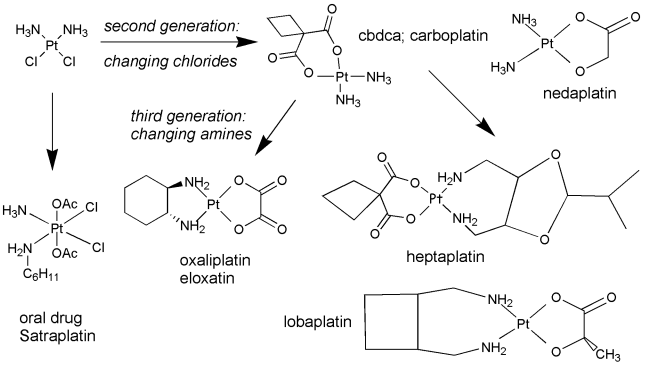

Figure 1. Schematic structures of 3 clinically applied anticancer drugs (worldwide), together with 3 regionally applied ones (China, South Korea, Japan), and the oral drug Satraplatin (the latter is not yet in routine use).

to cellular DNA at two neighbouring guanine bases, and more specifically at their N7 atoms.[6]

In addition to cisplatin in later years carboplatin [Pt(cbdca)$(NH_3)_2$] and oxaliplatin [Pt(1,2-dach) (ox)], with cbdca = 1,1-dicarboxylato-cyclobutane; dach = 1,2-diaminocyclohexane; ox = oxalato(2–) have been introduced, the latter specifically for the treatment of colorectal can-

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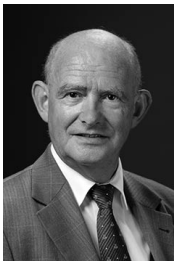

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cers.[5] Derivatives with the same *cis*-$PtX_2(amine)_2$ structural unit (X = leaving anionic group; amine = any primary or secondary amine), display similar or even improved biological activity in several cancer cell lines; however, up to now only about 10–15 Pt compounds have entered in advanced clinical trials; in China, Japan and South Korea three other derivatives (nedaplatin, lobaplatin and heptaplatin, resp.) have been in clinical use, and these have also been included in Figure 1. Studying the details of the binding (kinetics, structures) of such platinum compounds to nucleic acids may not only lead to a better understanding of the mechanism of action, but may also result in the development of new drugs, based on improved knowledge of their DNA binding.

Similar compounds from other group-10 elements (Ni, Pd) do rarely yield active compounds;[7] in fact a key factor that might explain why Pt is most useful, comes from the ligand-exchange kinetics, which for this type of ligands is of the order of a few hours, thereby preventing rapid equilibration reactions, where the metal and ligand would be separated.[6,8] A few compounds of other metals, like Ru, Ti, Ga and Sn have been reported to be active and some have entered clinical trials, like for Ru: NAMI-A and KP1019;[9,10] and for Ga: KP46;[11] these will not be discussed in this minireview. Earlier review reports in the field from the author[6,8,12–14] and several colleagues[5,15–20] are recommended for further reading. In a few cases, a few original papers will be given as well.

The present Microreview article will focus, after a brief introduction, on recent progress and emerging trends from us and others, not yet reviewed in detail. Only major chemistry facts that have led to a significant improvement of our insight into the mechanism of action will be discussed in detail. The inspiration in all cases originates from detailed and increased knowledge of the M-DNA binding.

Administration Protocols and Side Effects

Cisplatin is given by injection, and its toxic side effects (e.g. nausea, ear damage, vomiting, loss of sensation in hands, kidney toxicity) have been severe and in fact, this has stimulated research towards the design and synthesis of derivative compounds. These side effects have also resulted in the development of special drug-dosing protocols, making use of protective and rescue agents, like sodium dithiocarbamate and thiourea.[21] Figure 2 shows the most important ones of such protective and rescue agents. At an early stage we had already indicated that the interaction of such compounds with Pt^{II} would interfere with the mechanism of action and side effects[22,23] and perhaps could even be an intermediate on the way to DNA binding[24] (vide infra). Much chemistry studies of rescue agents needs to be done and are waiting for new research approaches.

The second-generation platinum drug carboplatin, $[Pt(C_6H_6O_4)(NH_3)_2]$, has also been in routine use and has less toxic side effects than cisplatin. Its much lower reactivity also allows that a higher dose can be administered (up to 2000 mg/day).[5]

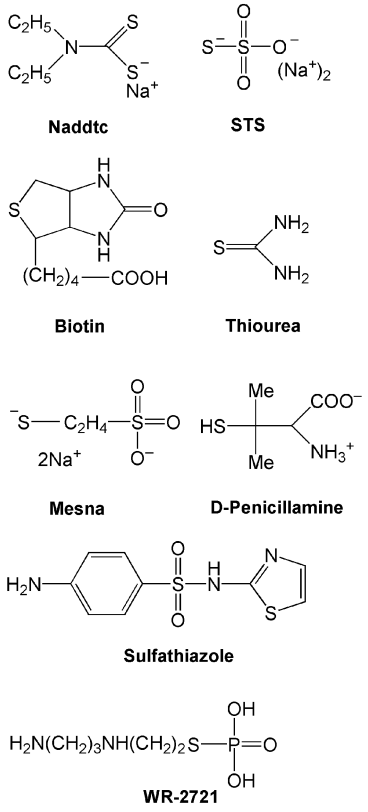

Figure 2. Selection of the most important protective and rescue agents for use in cisplatin treatment.

Later developments have shown that drug resistance may develop in certain tumours. This is in fact one of the main limitations when treating patients.[5] Such resistance is now easily detected using studies with tumour cell lines, so that potential, new drugs can be rapidly screened. As a result, a new group of compounds, with different amines, has evolved during the last decade. These compounds are often considered as the so-called third-generation drugs. They also include Pt^{IV} derivatives that may be administered orally, like the *cis*,*trans*,*cis*-$[PtCl_2(RCOO)_2(amine)_2]$ (R = alkyl). Figure 1 includes a selected number of clinically used, or tried Pt amine compounds of 2nd generation (changing the leaving groups) and 3rd generation (changing both the amine and the leaving groups).

Biologically Relevant Ligands and Summary of Mechanistic Investigations

Elementary coordination-chemistry knowledge would predict that in living systems S-donor ligands in proteins would rapidly bind and generate the most stable M–L bonds. Also binding of heavy-metal ions to lone-pairs of nitrogen atoms can be predicted to be strong. Other metal ions, like Mg^{II}, Mn^{II} would rather bind to the oxygen donor ligand, like phosphate groups of the DNA and carboxylates of protein side chains. Consequently, heavy-metal binding would involve amino-acid side chains from cysteine, methionine, histidine, and also the solvent-exposed N7 atoms of

adenine and guanine in double-stranded DNA. In single-stranded parts of DNA and RNA, the N3 of cytosine and N1 of adenine would also be accessible for metal binding. The N3 of the purine bases is sterically protected.

The influence of Pt–protein interactions in a variety of Pt compounds has been demonstrated repeatedly, and only a few recent papers are mentioned here.[25–27] So, in theory a large variety of biological molecules could be the ultimate target for platinum compounds. Early studies of the mechanism of action had made clear that cellular DNA is the most likely biological target, and the several potential DNA-binding sites for heavy metals are schematically depicted in Figure 3. This selectivity for DNA was initially seen as quite surprising, because such compounds on their way to reach DNA may meet and bind to other cellular components, like proteins and peptides. We now know that at least some of the platinum is temporarily bound to such S-donor ligands[12] (vide infra).

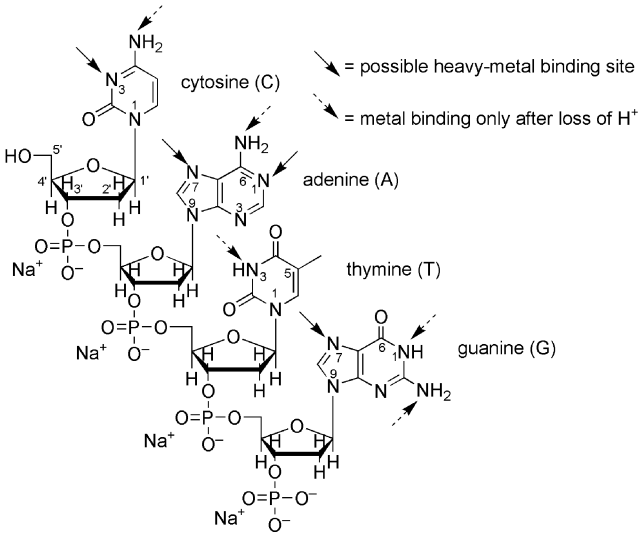

Figure 3. Possible binding sites for heavy metals in a single-stranded tetranucleotide; N3 sites in purines are sterically protected for M binding.

Outside the cell the chloride concentration is sufficiently high to prevent or hamper hydrolysis.[4,14] After entering the cell, through passive, or as more recently found, active transport at so-called CTR1 sites,[28,29] inside the cell the drug will react by hydrolysis, and/or reaction with other ligands, like S-donor ligands, such as methionine and glutathione.[8,30,31]

The early mechanistic studies by several groups soon pointed towards a specific cisplatin binding to cellular DNA, eventually shown to be the guanine-N7 atoms, by a variation of biochemical techniques[32,33] and has been reviewed earlier.[13] Inside the nucleus (and likewise inside mitochondria) a subsequent reaction with DNA will occur, and as known for several years now,[33] about $^2/_3$ of all platinum is chelated to two neighbouring guanines at their N7 sites, resulting in a kink of the DNA structure. This kink and unwinding is recognized by certain cellular proteins, which may result in either repair of the DNA, by cutting out Pt and resynthesizing at the open sites, or in no repair followed by apoptosis and cell killing.[34,35] The remaining platinum appears to leave the cell via – amongst – others the Golgi apparatus[36] and where normally the copper transporters ATP7A and ATP7B are located.[37–39] These proteins are believed to mediate efflux of the platinum drugs or to sequester them away from their target, nuclear DNA[29,40] A simplified picture of this process is depicted in Figure 4.

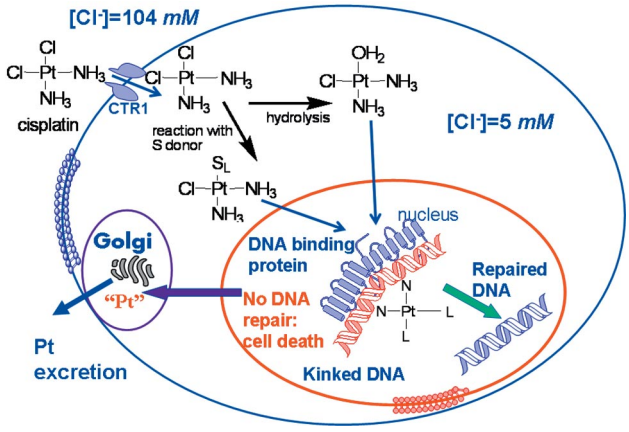

Figure 4. Simplified overview of the pathway of cisplatin in the cell, towards the DNA and its consequences afterwards.

The process leading towards the current view of the kinked platinated DNA recognized by a HMG-type protein, in fact started with the NMR structure of platinated ds DNA, followed by the proof of Den Hartog[41,42] that DNA was kinked at the Pt-binding site, and confirmed by a variety of studies, such as gel electrophoresis, to study the bending of the DNA.[43] Moreover both XRD and NMR, on several double-stranded sequences,[43–47] have confirmed the kink and the unwinding. For details and structures, the reader is referred to earlier reviews. Also other binding sites on the DNA, occurring in much smaller quantities, resulted in kinks, like at the AG sites, the interstrand GG crosslink and even monofunctional binding to double-stranded DNA was found to result in some distortion.[48] This monofunc-

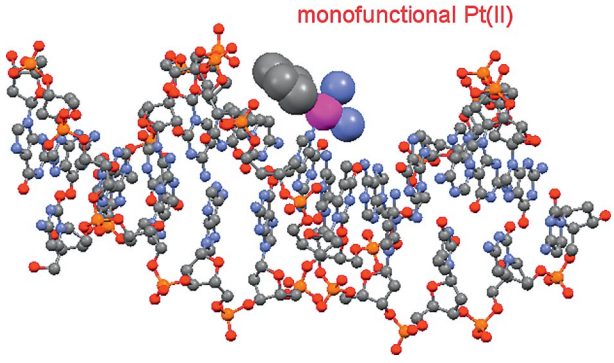

Figure 5. Projection of the structure of a ds-DNA platinated monofunctionally at G-N7 of a synthetic dodecamer; the pyridine group is located in the major groove.[49]

tional GN7 binding has received a new boost by the recent finding of Lippard et al.[49] that the classical compound *cis*-[PtCl(NH_3)$_2$(pyridine)]Cl containing the cationic species cis-diammine(pyridine)chloridoplatinum(II) is very active. The compound is transported into the cells by the cationic transport sites OCT1 and OCT2, known to be the preferred entrance gates for oxaliplatin.[50] As expected the DNA is hardly kinked after binding of the monofunctional compound at a Guanine-N7 site of a synthetic double-stranded oligonucleotide. In Figure 5 a projection of the structure is redrawn, based on the coordinates of Lovejoy.[49]

Consequences of Structural Changes in DNA After Pt Binding

A very important question deals with the steps that occur after the Pt chelation to the 2 neighbouring guanines. The biological consequences of the kinking, unwinding and subsequent protein binding, which has been elegantly and convincingly shown in the study of Lippard et al.,[35] is still a matter of some controversy.[51] It could be that the protein (at least in some tumour cells) prevents repair of the damage, so that the cell is killed eventually; it could also be that the protein binding induces repair, and prevents the cell from killing.[52,53] Our studies with a fluorescently labelled Pt compound, which was followed in time and space using a microscope, have shown that the Golgi apparatus may be accumulating the remaining Pt before excretion.[36]

In all cases studied so far, the base pairs remain basically present, even after protein binding[35,45,54] and the DNA bends or kinks by an angle of 30–55° depending on the base sequence. Over the years, this picture has hardly changed and has been confirmed by a number of other sequences from several laboratories. It had been realised that a weak point in the use of 2D NMR for the determination of such structures is that no direct measurement of the bending angle of the DNA can be obtained; however, the use XRD in the solid state has confirmed the details of the kink and unwinding in a few cases in recent years.[35,45,54]

Therefore, in some cases cell death by apoptosis occurs, whereas in other cases repair of the platinated DNA has been found. What is known for a long time is that that the progression of DNA polymerase along the DNA chain is blocked at GpG-Pt sites.[55,56] Also the progression of *E. coli* RNA polymerase is known to be blocked,[56] indicating that platination has an effect both at replication and transcription.

Most recent work[34,57] has shown that the effect of repair is fundamental for the mechanism of action. In fact, by repairing the damage on DNA caused by cisplatin treatment, the cell might increase the probability of its survival. The detailed description of the biological consequences goes beyond the scope of this review, and the reader is referred to recent reviews of Lippard and Kelland[5,52] The transport in and out the cell has been elegantly reviewed by Hall et al.[58]

Structure–Activity Relationships and Design of New Derivatives

Derivatives of cisplatin were of course made soon after the discovery, some of which showed activity and others not. A large variety of studies had been undertaken with attempts to formulate structure-activity relationships for such Pt compounds. Initially in all cases reported, the cis geometry of two amines (symmetric, asymmetric, chelating or not), was seen as a first requirement.[59] Also leaving groups with a weaker *trans* effect than the amine, were found to be required. Later on the presence of only one N–H group on the amine was found to be sufficient.[14] The highly promising compound picoplatin [*cis*-(ammine)-dichlorido(2-picoline)platinum(II)] is a nice illustration of this requirement.[60] The steric bulk of the methyl group in the picoline ligand slows down the ligand exchange kinetics at the platinum. Water solubility should be good and toxic side effects should be minimized. In addition, possible reactions in the blood with ligands containing S-donor atoms should be suppressed.[5,14]

For all third-generation drugs (vide supra) binding to a guanine-N7 site appears to occur and to be essential. Different compounds, however, appear to have different binding kinetics, and also the structural details of the resulting DNA adducts appeared to differ to some degree.[61] This might have important biological consequences, as will be become evident below.

Design and development of new structures to overcome the above-mentioned problems of resistance against cisplatin, which patients may develop, no doubt will result in better treatment of tumours that are not sensitive enough to cisplatin. Therefore, new drugs are sought for two major reasons, i.e. to further reduce toxicity, and to circumvent resistance. In addition, controlled release and drug targeting have been motivating research to new Pt compounds.

So all new platinum compounds should lack cross-resistance to cisplatin and carboplatin. It is well known by now that this requirement can be reached e.g. by using non-ammonia ligands, by dinuclear and by oligonuclear compounds[62,63] and by compounds with the *trans* geometry.[64–68] Even imines in *trans* geometry can give highly active compounds.[69]

It is now generally accepted that metal coordination compounds having metal–ligand exchange rates comparable to cell-division processes, i.e. mainly Pt and Ru compounds, often appear to be highly active as anticancer agents;[8,70] consequently, much research towards new compounds is focused on these two metals.

From the above it will be clear that a continuous need for new platinum compounds exists and research towards the design of new classes will no doubt be a lively area of research in the next years. So in principle new drugs can be designed and synthesized by applying careful variations in: a) The eventually **selected amine ligands** at platinum: chelating or not; H-bond donor; steric effects; presence of side arms for secondary DNA interactions; attached carrier groups; activating or directing groups.

b) The **selected leaving groups** at platinum: non-toxic; optimal ligand-exchange kinetics; possibility to act as a prodrug.

In recent years several of such and additional variations have been introduced, and the most important ones will be presented below in some detail. They all deal with one or more of the following aspects:

i. Keeping the administered drug inert for some time after administration, and release them at a desired time or at a specific location (target). This may involve compounds containing (tissue-specific) carrier molecules as ligands to reach higher drug concentrations, or several variations with slower or gradual release in or at certain tumour tissues. The activation can occur in several ways, like chemical dissociation (from a polymer, from a nanoparticle, or from a vesicle), by photochemical or redox activation, or by simply changing the pH. Also compounds bearing protecting groups that are released (by e.g. antibody-linked enzymes) only at the surface of the (specific) tumour cells can be considered.

ii. Compounds have also been designed that are chemically attached to other chemotherapeutic agents, e.g. intercalators with anticancer activity, or with biologically active co-ligands, to generate synergistic effects; even compounds with radio-sensitizers as co-ligands for use in radiation therapy have been used.

iii. Compounds containing more than one platinum atom connected by a bridge; such a bridge can be either flexible, or rigid. The appended metal can also be a different metal and/or another DNA-binding group.

In the discussion below, a selection of examples from our own work, and work of others will be addressed, with a focus on the DNA binding and its (expected) induced structural changes

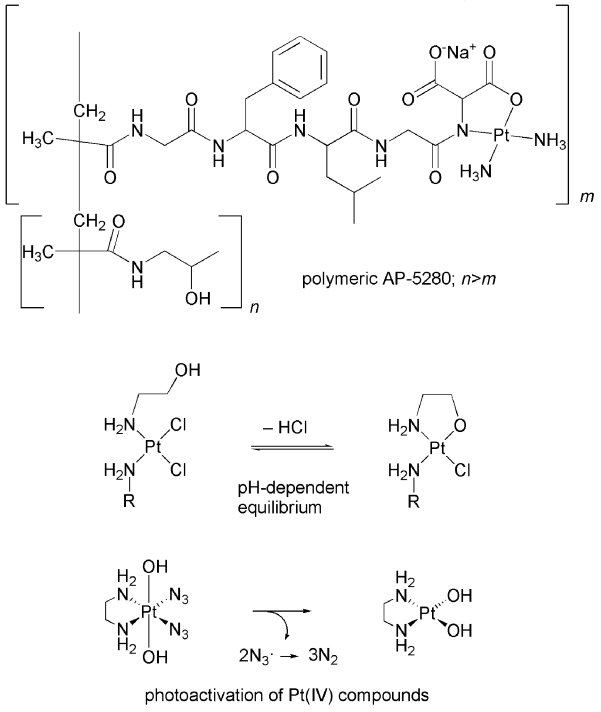

Figure 6. Examples of drugs to be released upon changing pH or by photochemical activation.

1. Slow and Controlled Release of the Pt Drugs

In Figure 6, a selection of the three important approaches is given.

The approach of binding the Pt species for a short time to a polymer (chemically) has been known for some years and some applications are close to clinical use. As tumours may hyperpermeable towards macromolecules as a result of compromised vasculature and therefore this "enhanced permeability and retention effect" (the so-called EPR effect) may result in an increased drug concentration within the tumour tissue.[71] This is possible when the therapeutic agent is coupled to a macromolecular carrier, or may be packed inside a nanosized particle, which after endocytosis will result in drug uptake into the tumour cells.

The well-known acrylamide-based drug AP5280 with a peptide spacer is given for illustration.[72,73] Also by simply changing the pH to more acidic, a Pt–O bond may dissociate making the drug more free to DNA binding.[74]

A most spectacular activation was first described by Bednarski and Sadler,[75] where azido ligands coordinated to Pt^{IV}, can be photochemically, reductively released, generating Pt^{II} species, to react with DNA. Very recently Lippard et al. have extended this approach by attachment to single-walled carbon nanotubes; such species carry some 60–70 Pt-drug molecules, which can enter the cell by endocytosis, after which reduction takes place.[76] Also very recently Wheat has reported an improved delivery of the drugs by attaching the Pt to cucurbiturils.[77] An as yet chemically not well understood approach is based on the inclusion of cisplatin (and related compounds) in vesicles. This both increases the solubility and results in the slow release and high activity.[78,79]

2. Target Towards Specific Sites

In Figure 7, a selection of three important approaches towards drug targeting is presented. Here the co-ligand can have a certain specific affinity to some biological sites, such as bones, where calcium activation can occur, or the specific surface of certain tumour cells, where e.g. special agents, like enzymes may be present.[71]

In a search for osteotropic Pt complexes we used compounds that have leaving groups containing phosphates, which may be released by adding calcium near the bones.[80] Indeed dissociation by adding Ca^{II} dissociation of the phosphates was found by ^{31}P NMR analysis.

In a more recent study we have used a β-glucuronyl-based prodrug strategy.[81] High levels of β-glucuronidase are known to be present in necrotic areas of tumours; this is expected to lead to a selective activation of relatively non-toxic prodrugs, such as shown in Figure 6. In fact, the enzymatic cleavage of the platinum conjugate (due to a change in lipophilicity in the tumour tissue) could be proven by

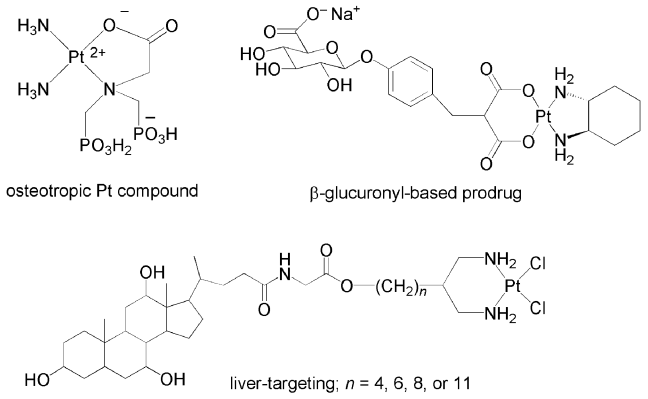

Figure 7. Selected examples of Pt compounds, likely to bind to DNA as cisplatin and oxaliplatin, but requiring activation near the tumour cell.

NMR analysis. Immediately after addition of the enzyme cleavage of the conjugate could be demonstrated in the ^{1}H NMR spectrum.[81] It has been shown also possible to apply this principle to target drugs to reach the liver.[82]

Combining this approach with the other methods mentioned above, could lead to highly efficient drug delivery approaches.

3. Platinum-Based Drugs Containing Another Potential Drug

In Figure 8, a selection of the most important results and approaches is given. The idea of generating synergistic effects by combining, if possible in the same molecule, both a cisplatin-like compound and another drug, has been in the literature for quite some time.[83,84] Combination of the Pt-amine units with heterocyclic ligands and polar alcohol groups, with and without H bonds, resulted in interesting and active compounds.[85] In all such cases the Platinum is expected to bind to guanines in a kinetically inert mode. Even monofunctional DNA binding, for molecules with extended spacers, potentially bearing a drug at the end, was demonstrated, after aquation of one labile chloride ligand.[86]

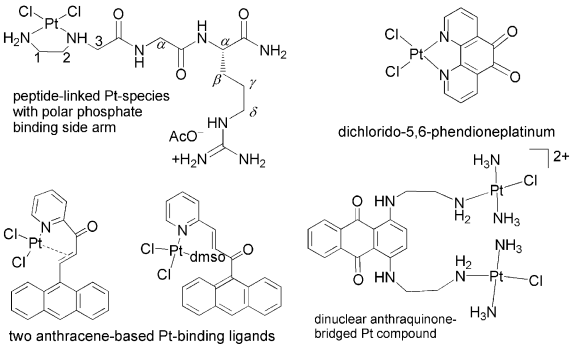

Figure 8. Pt drugs with rigid and flexible spacers that may display synergism in DNA binding.

More recently the attachment of flat aromatic molecules to cisplatin-based fragments has been extended by several groups, including ours.[87–89] Peptide-based drugs can be easily attached to Pt-amine units, and we were able to even synthesize these peptide-linked compounds on a resin, using solid-state peptide synthesis[90] (see Figure 8). Dyson et al.[91] cleverly used the etachrynic acid, a diuretic in clinical use, attached to a satraplatin-like Pt^{IV} compound and succeeded to overcome glutathione-S-transferase mediated drug resistance.[91]

Very recently the simple attachment of platinum to rigid intercalators, like 1,10-phenanthroline-5,6-dione, was shown to yield both anticancer active compounds, that simultaneously showed antimicrobial activity.[92] However, when the intercalating part is more flexible, the anticancer activity depends on the geometry of the molecule, although in both cases, DNA binding does occur, as shown by a recent study of Marqués with two isomeric anthracene-based ligands.[93] An additional advantage of these compounds is their fluorescent behaviour, allowing to follow the pathway in cell using digital fluorescent microscopy. Such processes have also been shown possible by using dinuclear Pt amine compound (each binding monofunctionally), as shown in Figure 8.[94] Also the very recent finding Aldrich-Wright needs to be mentioned here, where specificity of the platinated intercalator [Pt(*S*,*S*-dach)(phen)]$^{2+}$ was found in leukaemia cells and also in vivo studies showed a high promise.[95]

Other groups had focused on different approaches where a radiosensitizer or radioactive drugs had been attached to the platinum unit, but the reader is referred to earlier papers and reviews in this field.[13,84,96]

4. Dinuclear and Oligonuclear Platinum-Based Compounds

In Figure 9, a selection of the most important approaches with flexible linkers between the Pt^{II} ions is given. The first ideas to add a second metal in an anticancer drug were developed primarily by Farrell.[97] In fact the observa-

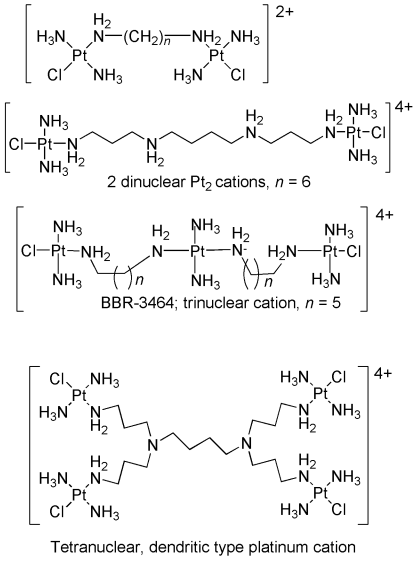

Figure 9. Dinuclear, trinuclear and tetranuclear Pt^{II} cations, with one DNA-binding site per platinum.

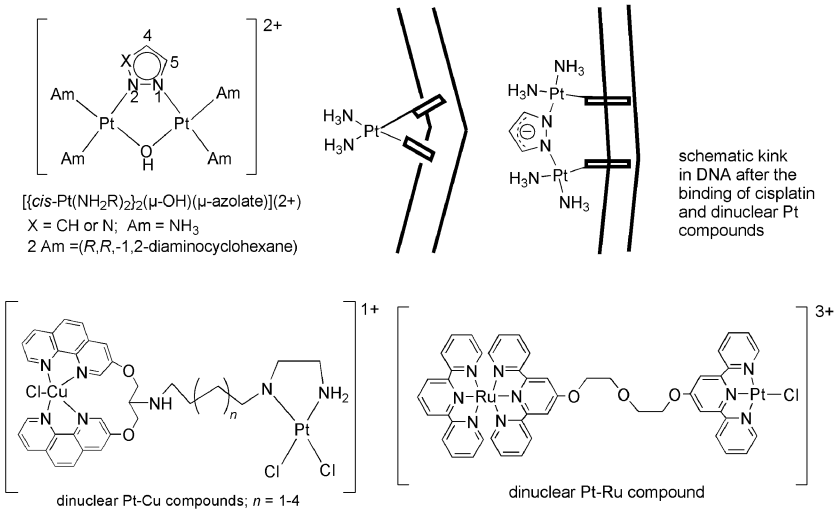

Figure 10. Dinuclear rigid Pt^{II} cationic species and schematic kink in DNA after binding (top) and heterodinuclear complexes with flexible linkers (bottom).

tion that nucleobases and nucleotides have more than one binding site, already led to the early suggestion of taking an excess Pt in such binding reactions.[86] The work of Lippert's group has subsequently shown that such multiple binding may occur on a relatively large scale,[98] although it seems unlikely that such binding would occur under physiological conditions. Therefore, a search to chemically linked metal ions, by bridging ligands with kinetically stable M–L bonds is required to study such compounds.

The first dinuclear Pt compounds with flexible linkers from the Farrell group, showed a promising activity,[99,100] and quite interesting binding with DNA has been observed, including hairpin folding after binding.[63,101]

The success of the dinuclear compounds was soon followed by the trinuclear species, with bifunctional DNA binding.[102–104] The DNA binding of such compounds is quite interesting and can span long distances, as shown by advanced NMR studies.[105–107]

However, a tetranuclear-based dendritic Pt^{II} compound, despite its ability to bind to DNA, was found inactive against several cancer cell lines.[108] Also dinuclear Pt^{II} compounds with peptides linking the two units were found to be only marginally active.[109]

In two other recent approaches, we have been using two **different** metal ions with flexible linkers, and also a group of dinuclear Pt^{II} compounds with rigid linkers, as shown in Figure 10. Realizing that the kink in the DNA caused by Pt^{II} amine compound when chelating to two neighbouring guanines was relative large (perhaps up to 40–55°, as shown above), Komeda has developed a group of azole-bridged Pt compounds, where the co-bridging OH group, is the leaving group.[110–114] Simple geometric considerations already led to the prediction that, upon loss of the OH group, each Pt would be able to bind at a Guanine-N7, which would result in a very small kink of double-stranded DNA when the two G bases would be adjacent.

The DNA binding of these dinuclear rigid compounds was as expected, resulting in a very small distortion of the helix only,[115] and the activity of the compounds in several cancer cell lines was found to be an order of magnitude better than that of cisplatin.[116] The NMR and MD refined structure of the DNA oligomer after platination is redrawn in Figure 11, and the cytotoxicity data in cell lines are given in Table 1. It is clearly seen that for almost all the used cell lines the IC_{50} values are about 10 times lower than that of the reference compound, cisplatin. So further and advanced studies on these compounds, using animal models, should make clear whether they will be suitable for presentation in clinical trials.

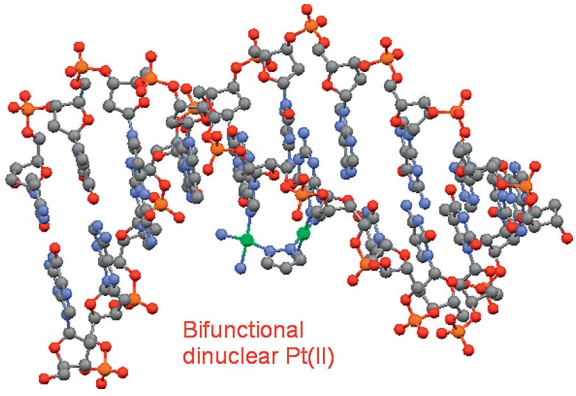

Figure 11. The structure of a ds DNA oligomer, coordinated to the dinuclear species $[Pt_2(pyrazolato)(NH_3)_4]^+$ at two Guanine-N7 sites.

Table 1. IC_{50} values of Pt compounds against 7 different cell lines.[a]

	MCF7	EVSA-T	WIDR	IGROV	M19	A498	H226
Cisplatin	2.33	1.41	3.22	0.56	1.86	7.51	10.9
Pt-pz	0.06	0.15	0.12	0.59	0.05	0.53	0.68
Pt-trz	0.09	0.32	0.40	0.13	0.19	1.24	2.72

[a] MCF7: breast cancer, EVSA-T: breast cancer, WIDR: colon cancer, IGROV: ovarian cancer; M19: melanoma, A498: renal cancer, H226: non-small cell lung cancer.

Realizing that Cu-phenanthroline compounds can cut DNA in an oxidative way, de Hoog has combined Cu-phenanthroline with several Pt-amines using a variety of spacers, primarily aiming for bifunctionality.[117–119] In several cases a high activity was found, and many of the newly prepared compounds were shown to be effective DNA cutting agents. Yet no structure-activity relationships for this class of compounds could be established.[117–119]

Replacing the Cu part by a fluorescent group in such compounds with relatively flexible spacers had provided strong evidence for the pathway in the cells of Pt compounds.[36] However, changing the Cu to Ru, as studied by Van der Schilden,[120] (see Figure 10) did not result in any significant anticancer activity, despite DNA binding taking place.

Perspectives

As summarized and illustrated above, the design and development of DNA-binding platinum-containing drugs, requires detailed coordination-chemistry knowledge of the M-DNA binding processes, regarding structure, thermodynamics and kinetics. During the last decade, others and we have been giving attention to new approaches, like bifunctionality. This approach had started with attention to hydrogen bonding as a secondary interaction, and has developed towards attached intercalators, attached co-drugs and a second (or third) metal, all with a major aim to avoid resistance development or synthesis of tumour-specific drugs.

The phenomenon of resistance development upon treatment with cisplatin and other compounds, mentioned above, remains still far from being understood on the molecular level. The resistance may have many origins, like a decreased Pt uptake by tumour cells, increased levels of S-donor ligands (such as known for metallothioneins and glutathione) in the tumour cells, migration of Pt from one site to another on the DNA and increased repair of Pt-caused DNA damage. Recent papers of Vasak et al. have eluded on the interaction of cisplatin and other Pt compounds with metallothioneins.[121,122] Interestingly, it was shown that while all ligands in *cis*-Pt^{II}-based compounds were replaced by cysteine thiolates, *trans*-Pt^{II} compounds retain their N-donor ligands, thereby remaining in a potentially active form.

Also underdeveloped, but less directly related to DNA binding, is the control of the toxic side effects; development of the coordination chemistry of Pt compounds with rescue and protective agents (usually S-donor ligands) and especially the reactions of these compounds with other cellular components and their cell-wall transport needs serious attention. More directly related to DNA binding of metal compounds is the migration of Pt units along the DNA chain. In an early study we found that under certain conditions (such as the presence of halide ions in solution) the "classical" bis-GN7 cisplatin adduct in a sequence may be converted to a different interstrand adduct.[123]

Fascinating new possibilities for research on Pt-based coordination compounds will appear in the coming decades, and the multifunctionality will no doubt increase. In addition to selective DNA binding, the other functions of the drug may serve for targeting, suppressing side effects, cell wall transport, nuclear membrane transport, and circumventing DNA repair.[5,16]

Finally it should be mentioned again that some of the new compounds possessing chemical and biological properties related to those of cisplatin, might be very active, but show weak binding or no binding at all to DNA. Lots of other metal compounds may be shown to be active in cancer treatment, and may primarily interact with other biological targets.[16,34] To understand the overall pharmacological and toxicological profile of Pt drugs and to improve the design of new drugs, alternative intracellular pathways and interactions for platinum compounds must also be considered in the planning of new active molecules.

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