CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.201000148

Advances in Platinum Chemotherapeutics

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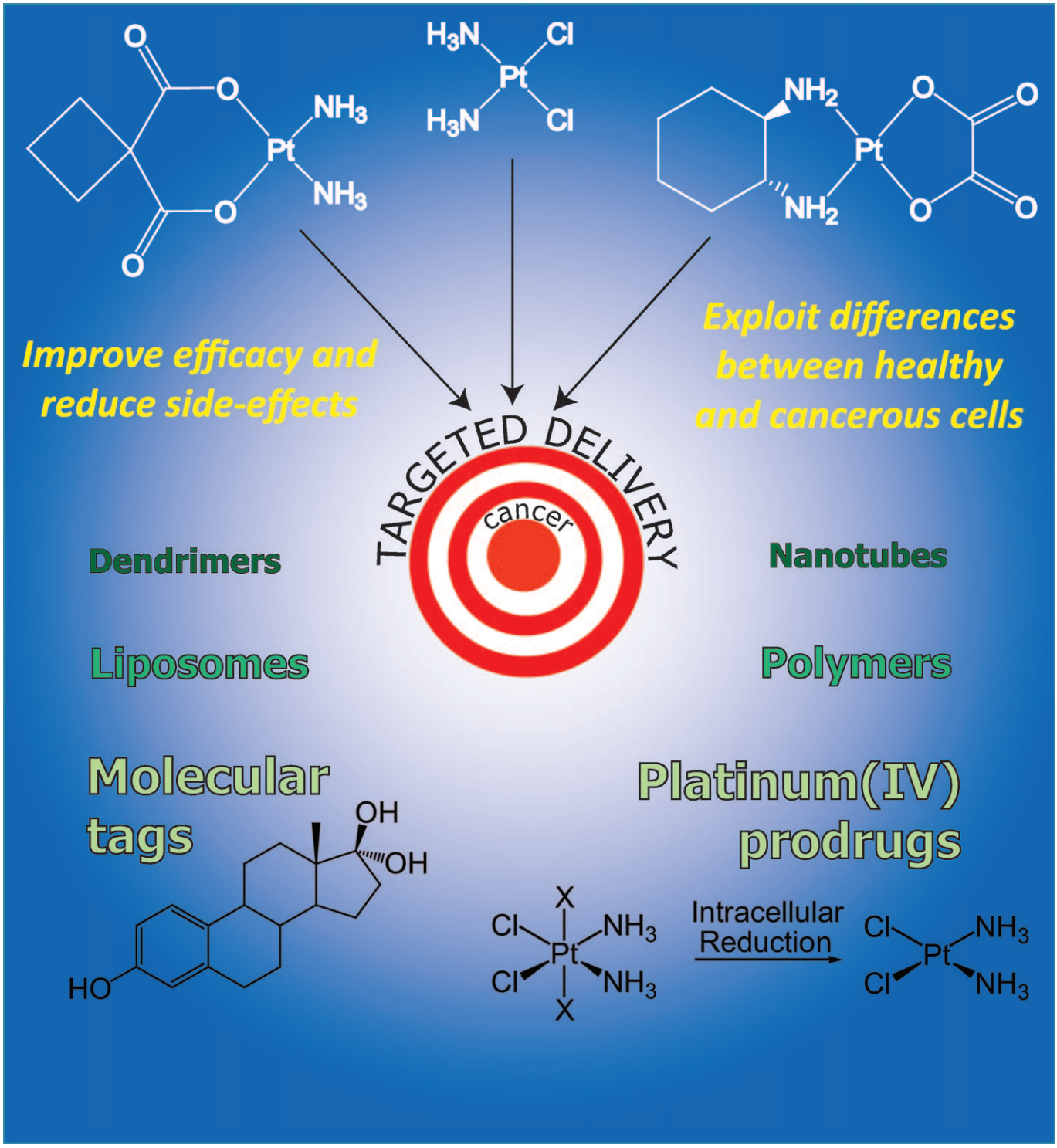

Abstract: The approved platinum(II)-based anticancer agents cisplatin, carboplatin and oxaliplatin are widely utilised in the clinic, although with numerous disadvantages. With the aim of circumventing unwanted side-effects, a great deal of research is being conducted in the areas of cancer-specific targeting, drug administration and drug delivery. The targeting of platinum complexes to cancerous tissues can be achieved by the attachment of small molecules with biological significance. In addition, the administration of platinum complexes in the form of platinum(IV) allows for intracellular reduction to release the active form of the drug, cisplatin. Drug delivery includes such technologies as liposomes, dendrimers, polymers and nanotubes, with all showing promise for the delivery of platinum compounds. In this paper we highlight some of the recent advances in the field of platinum chemotherapeutics, with a focus on the technologies that attempt to utilise the cytotoxic nature of cisplatin, whilst improving drug targeting to reduce side-effects.

Keywords: drug delivery · platinum · prodrugs · targeting

Introduction

In the 1960s it was discovered that *cis*-diamminedichloroplatinum(II) (cisplatin, Figure 1 a) inhibited the cell division of *Escherichia coli* (*E.coli*).[1] Cisplatin is now used both alone and in combination with other drugs for the treatment of numerous types of cancer, including: bladder, ovarian, head and neck, testicular and lung cancers. Once inside the cell, cisplatin is hydrolysed, forming the highly reactive species $[Pt(NH_3)_2Cl(OH_2)]^+$, which readily forms coordinate adducts with DNA.[2] These adducts induce changes to the secondary structure of DNA that inhibit transcription and replication,[3] consequently leading to cellular apoptosis.[4] Cisplatin administration is often limited by severe toxic side-effects,[5] as well as the intrinsic and acquired resistance possessed by various cancers. As such, a large number of platinum(II)-based compounds structurally similar to cisplatin have been developed and tested,[6] with only diammine(1,1-cyclobutadicarboxylato)platinum(II) (carboplatin, Figure 1 b) and (1*R*,2*R*)-diaminocyclohexane)oxalateplatinum(II) (oxaliplatin, Figure 1 c) receiving wide-spread approval. Carboplatin and oxaliplatin both form coordinate adducts with DNA, and whilst they possess some advantages over cisplatin, the issues of adverse side-effects and resistance/cross-resistance persist. Overlooking the drawbacks, platinum drugs are still the most common treatment for cancer patients, with an estimated 50–70 % being treated with platinum-based drugs.[7] To circumvent the drawbacks of current platinum(II)-based drugs there has been a search towards new platinum drugs that are structurally different to cisplatin or demonstrate different modes of binding. These compounds include multinuclear and intercalating complexes.[8,9]

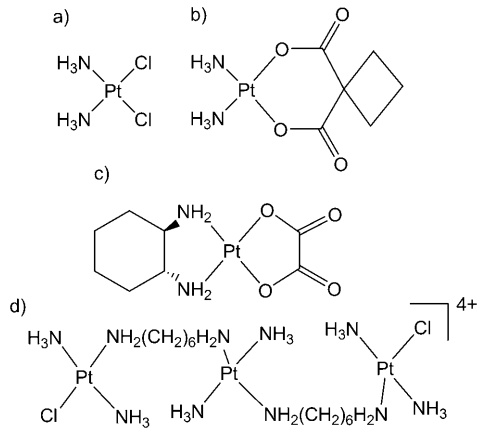

Figure 1. The chemical structures of a) cisplatin, b) carboplatin, c) oxaliplatin and d) BBR3464.

In recent years, a number of multinuclear platinum complexes have been developed with the aim of increasing activity and overcoming cisplatin side-effects and resistance. A number of these complexes have shown activity in both cisplatin-sensitive and cisplatin-resistant cell lines.[10] The trinuclear platinum complex BBR3464 (Figure 1 d) consists of two terminal *trans*-platinum(II) constituents capable of coordinate binding to DNA; each is bound to a 1,6-diaminocyclohexane ligand that is connected to the *trans*-platinum(II) centre, which is capable of minor groove binding. The relatively large positive charge (+4) of BBR3464, compared to cisplatin, increases both its binding affinity for DNA and cellular uptake.[11] While BBR3464 has shown promising in vitro and in vivo activity compared to cisplatin,[12,13] phase II clinical trials have shown only partial responses with cisplatin-resistant ovarian and non-small-cell lung cancers.[14] Minimal activity against gastric carcinomas was also observed, with significant toxicity and side-effects experienced by many patients, resulting in a poor maximum tolerated dose (MTD).[15] These results have prompted continued investiga-

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tions of platinum(II) complexes capable of different binding modes in an attempt to mitigate these issues.[15]

Another class of compounds that has been well-investigated over the past thirty years are the platinum intercalators. The early work of Jennette et al. focused on terpyridine complexes and demonstrated the intercalating ability of square-planar platinum complexes.[16] These compounds interact with DNA through the reversible insertion of the molecule between the base pairs; this causes unwinding and lengthening of the DNA, thereby preventing replication. McFayden et al. developed a series of complexes containing methylated derivatives of 1,10-phenanthroline, with a structure–activity relationship proposed based on the degree and position of methylation.[17] This work has been extended in our laboratory, in which a wide range of intercalating and ancillary ligand combinations have been examined to develop a structure–activity relationship.[8,9] The most active complex, [(5,6-dimethyl-1,10-phenanthroline)(1*S*,2*S*-diaminocyclohexane)platinum]Cl_2 (56MESS, Figure 2a) displayed cytotoxicity up to 1000 times greater than cisplatin in the L1210 cell line,[18] whilst also displaying activity in cisplatin resistant cell lines.[8] Preliminary in vivo studies on the related, but less active complex [(1,10-phenanthroline)(1*S*,2*S*-diaminocyclohexane)platinum]Cl_2 (PHENSS, Figure 2b) have shown low toxicity and reduced tumour growth,[19] demonstrating the potential of this class of compounds.

Despite the promise that many of the new types of platinum complexes offer in their high in vitro/in vivo activity and lack of cross-resistance with cisplatin, relatively few have entered clinical trials and none have received approval for use in cancer treatment. As such, a great deal of recent research has focused on improving current chemotherapeutics using approaches such as drug targeting, delivery and

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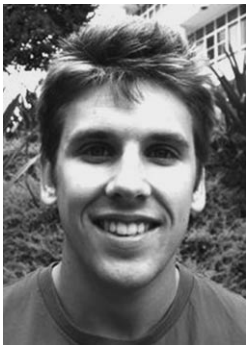

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Prof. Janice R. Aldrich-Wright received her Ph.D. from Macquarie University in 1993. She took up an academic position at UWS prior to finishing her Ph.D., where she is now a Professor in the School of Biomedical and Health Sciences. She has approximately 50 publications and is a co-inventor on four patents. Her research interests are firmly based in medicinal inorganic chemistry and coordination chemistry.

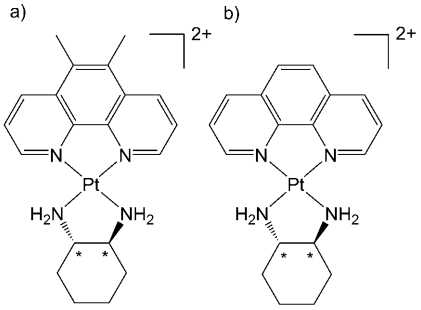

Figure 2. The chemical structures of a) 56MESS and b) PHENSS. Counterions have been omitted, but are usually chlorides.

prodrugs; here we present a review of the recent work in these areas.

Drug Targeting and Delivery

Targeting and delivery are two aspects of drug design that have been receiving much attention in recent years, because of the potential for improving efficacy and reducing unwanted side-effects. A variety of biological targets can be utilised to exploit the differences between "normal" and cancerous tissues. Current anticancer drugs are all of low molecular weight, which allows them to be rapidly passed through the membrane of most tissues, regardless of whether they are malignant or not. A method of selectively targeting cancer is to exploit the differences between cancerous and "normal" cells.

Cancerous tissue has poor lymphatic clearance and slow venous return, whilst extravasation into cancer tissue interstitium persists, and as such, tumours possess abnormal molecular-transport dynamics, with macromolecules accumulating in the tissue. This phenomenon was described by Maeda and Matsumura as the enhanced permeability and retention (EPR) effect.[20,21] The EPR effect is evident in a large number of tumour types and is used as a guiding strategy for the creation of novel cancer-specific drugs that are comprised of micelle, lipid particles and macromolecular motifs.[22]

Macromolecular delivery systems of the organic chemotherapeutics doxorubicin and paclitaxel (Figure 3) have been approved for cancer treatment. The liposomal formulation of doxorubicin and albumin-based delivery systems for paclitaxel (TAXOL™) utilise the EPR effect to allow better retention of the drug within cancerous tissue. Despite the advantages that these technologies have offered for organic chemotherapeutics, there have been no drug-delivery/targeting technologies approved for platinum drugs.

One of the most promising and highly developed drug carriers currently used are liposomes (phospholipid bilayer vesicles).[23] Liposomal delivery can improve the amount of drug available to a tumourous cell by increasing the vascular permeability and therefore the retention.[7] One of the oldest liposome models, as described by Bangham et al.,[24]

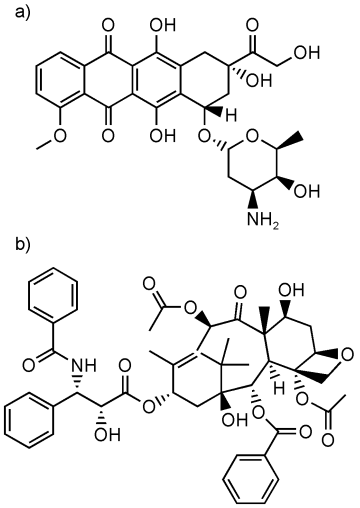

Figure 3. The chemical structures of a) doxorubicin and b) paclitaxel.

consisted of amphiphilic phospholipids and cholesterol placed in an aqueous environment, causing them to self-associate and form bilayers with an aqueous cavity.[23,24] Liposomes have many advantages such as biocompatibility, versatility (allowing enclosure of biologically active drugs either within the phospholipid bilayers or within the aqueous cavity), the potential to shield drugs from inactivation (before delivery to the target site) and the ability to deliver the drug directly into the cellular compartments (limiting adverse effects such as toxicity). The properties of the liposome can be varied by manipulation of size, charge and surface properties during formulation.[25] A liposomal formulation of doxorubicin is currently in use in the clinic.[26]

The anthracycline antibiotic doxorubicin (Figure 3a) is an organic intercalator that is used in the treatment of a variety of cancers. The commercially available liposomal formulation of doxorubicin HCl (Doxil®, Figure 4) has been approved for the treatment of ovarian cancer, AIDS-related Kaposi's sarcoma and multiple myeloma. Doxil® shows lower cardiotoxicity and greater efficacy than free doxorubicin. This is most likely due to the EPR of the larger molecules by the cancer vasculature.[27] Furthermore, there has

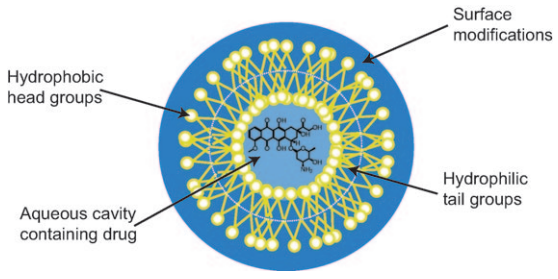

Figure 4. A schematic representation of the liposomal formulation of doxorubicin, Doxil®.

been some evidence that Doxil® is able to overcome doxorubicin resistance.[26] While liposomes have been shown to be effective in the delivery of doxorubicin, another area of current research is drug delivery by the protein human serum albumin (HSA).

HSA is a single chain protein of 585 amino acids (≈66 kDa), is the most abundant protein in plasma (≈40 mg mL^{-1}, 0.6 mM),[26] and is responsible for the transport of a variety of endogenous (fatty acids, hormones, etc.) and exogenous substances (drugs).[28] Drug binding by HSA can greatly influence the pharmacokinetics of the drug by affecting distribution and clearance.[29] A drug investigated for delivery by HSA is the miotic inhibitor paclitaxel. Paclitaxel (Figure 3b) is used to treat lung, ovarian, breast, head and neck cancer and advanced Kaposi's sarcoma. The drug has limited solubility and therefore is dissolved in Cremphor-EL® for intravenous administration, to which many patients experience hypersensitivity. Nanoparticle albumin-bound paclitaxel (known as nab™-paclitaxel, ABI-007 or Abraxane®) is approved by the FDA for the treatment of metastatic and recurrent breast cancer, and does not require the use of Cremphor-EL®. Furthermore, the albumin-bound paclitaxel has higher solubility, therefore reducing both the volume and infusion time.[30]

The positive advances in drug delivery and targeting of organic anticancer agents has led the focus of this research towards platinum drugs. Here we will detail some of the new research and technologies being investigated in an attempt to improve the efficacy and reduce the side-effects of the currently approved platinum(II) drugs. We will also summarise some of the new platinum complexes, particularly platinum(IV) drugs, which are being developed in the hope of improved targeting and reduced toxicity.

Platinum(II) targeting and delivery

Liposomes: There are several liposomal formulations of platinum drugs currently being explored. Lipoplatin™ is a cisplatin–liposome formulation with the liposome consisting of dipalmitoyl phosphatidyl glycerol, soy phosphatidyl choline, cholesterol and methoxy–polyethylene glycol–distearoyl phosphatidyl–ethanolamine.[31] The liposomes (diameter ≈110 nm) display preferential tumour uptake compared to surrounding non-cancerous tissues due to the EPR effect.[32] Lipoplatin™ also shows no nephrotoxicity and lacks the serious side-effects of cisplatin, while seeming to retain the efficacy of cisplatin.[5,31] A number of phase III clinical trials are currently in progress.[5]

Similarly, liposomal formulations of oxaliplatin analogues have been developed. Aroplatin™ (L-NDDP) is a liposomal formulation of oxaliplatin. A phase II clinical trial of Aroplatin™ in patients with advanced colorectal cancer resistant to 5-fluorouracil/leucovorin, capecitabine or irinotecan indicated that Aroplatin™ was effective to a degree.[33] The drawback of liposomal formulations like Aroplatin™ is that they are quickly removed from circulation by the macrophages of the reticuloendothelial system; however, this limitation has been overcome with the discovery of stearically stabilised liposomes.

The introduction of polyethylene glycol (PEG) units on to the phospholipid bilayers stearically stabilises the liposomes, thereby extending the circulation time.[34] The attachment of PEG units on to drug/carrier molecules can improve drug solubility, uptake and retention. The increased size of the PEGylated molecule allows it to selectively accumulate in tumour tissue, as described by the EPR effect.[35] Lipids incorporating PEG units are known as STEALTH® liposomes.[34] Unlike classical liposomes, in which typically 80–90% of the liposomes are deposited in the liver, STEALTH® liposomes remain mainly in the blood with only 10–15% reaching the liver, so the circulation half-life can be greater than 40 h.[36] STEALTH® liposomes containing cisplatin, known as SPI-77, are of particular interest for drug delivery. A phase II study of SPI-77 on non-small-cell lung cancer showed that the stearically stabilised liposomal cisplatin formulation has limited toxicity, even at large doses.[34]

Contradictory to the above findings, more recent research suggests that liposomal formulations like SPI-77 are not a great improvement on the free drug, due to their slow release kinetics making the concentration of the bioavailable drug so low that the therapeutic effect is negligible. To overcome this limitation, pH-sensitive liposome–cisplatin (spHL-CDDP) formulations have been designed utilising the fusogenic properties of the membrane to release cisplatin into the cytosol. The cytotoxicity of spHL-CDDP on human lung cancer cell line has proven this formulation to be active.[37] Ultrasound-triggered release of cisplatin from the STEALTH® liposomes is another area that has been explored to overcome this issue, with some success.[38]

Dendrimers: Dendrimers are one type of macromolecule that have been developed as anticancer drug carriers as their structure allows for functionalisation, encapsulation or conjugation of numerous molecules on the surface or at the core of the dendrimer. These carrier molecules are therefore an area of interest for drug delivery and targeting of platinum(II)-based drugs. Dendrimers are extensively divided dendritic molecules that have distinct features such as a bulky molecular size, narrow size distribution, spherical structure and derivatisation of the end functional groups.[39] There are numerous forms of dendrimers that are made from polyamidoamines, polyamines, polypeptides, poly(aryl ethers), polyesters, carbohydrates or DNA. The most frequently reported dendrimers are the polyamidoamines (PAMAM) type (Figure 5), which are available with an extensive range of generations and end functional groups.[40] The generations refer to the number of diverging points contained by the dendrimers from the interior to the exterior. Half-generation PAMAM dendrimers are terminated with a carboxylate surface group, whilst full generations hold amino-terminal groups.

The multivalent nature of dendrimers enables the covalent attachment of drug molecules to the dendrimer periph-

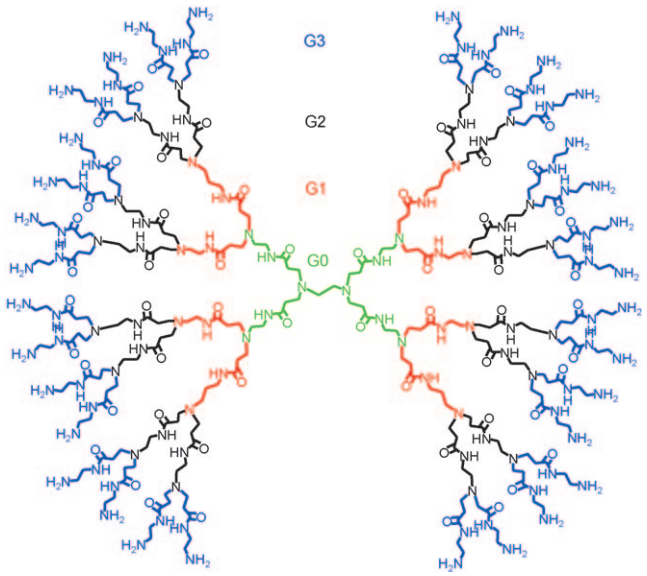

Figure 5. The chemical structure of a G3 PAMAM dendrimer.

ery. Drug loading can be altered by varying the generation number of the dendrimer and release of the drug can also be controlled by the use of degradable linkages binding the drug and dendrimer together.[41] Duncan and co-workers conjugated PAMAM dendrimers (G3.5) to cisplatin through the functionalised sodium carboxylate surface.[42] As a conjugate, the potent anticancer drug demonstrated increased solubility, decreased systemic toxicity, selective accumulation in solid tumours and the ability to release cisplatin slowly in vitro. Also, in the treatment of subcutaneous B16F10 melanoma, the cisplatin-PAMAM dendrimer complex has shown increased efficiency, compared to cisplatin alone.[41,43]

Dendrimers can also be targeted towards cancerous cells in order to prevent the cytotoxic drug from being indiscriminately delivered to healthy cells also. Cancerous cells have a higher cellular uptake of folic acid compared to "normal" cells. In addition, a number of tumour types (such as ovarian, endometrial, breast, lung, renal and colon) display over-expression of the folic acid receptors.[43–45] This over-expression on the surface of cancer cells has identified folic acid receptors as targets for the delivery of therapeutic cancer agents.[46] Folic acid/dendrimer systems are covalently conjugated to methotrexate to specifically destroy cells over-expressing folic acid receptors, after delivering the drug by means of endocytosis.[47] Quintana and co-workers synthesised an ethylenediamine core PAMAM dendrimer (G5) that was covalently attached to folic acid, fluorescein and methotrexate.[48] This complex provided targeting, intracellular drug delivery and imaging with 100 times decreased toxicity in comparison to free methotrexate.

Polymers: In 1975, Ringsdorf was the first to attach a physiologically active compounds to a water-soluble polymer.[49] Polymer therapeutics exhibit a number of advantageous properties including; high stability, allowing for extended time in the circulatory system; a high drug-loading capacity and a long drug retention time. These factors make polymers suitable carriers for anticancer drugs.[50] The high-molecular-weight polymer–drug conjugates allow for longer retention in the cell as they can only gain access by endocytosis.[51] These properties overcome many of the short comings that are inherent with platinum drugs by reducing side-effects and enhancing the therapeutic efficacy.

Polymers are typically comprised of two or three block copolymers with alternate repeating units. The design of polymers for drug delivery offers a considerable degree of variety; with a typical triblock polymer containing solubilising and targeting units, as well as loading units with a drug bound, which is subsequently released at the target site (Figure 6).[52] The targeting and solubilising units allow the

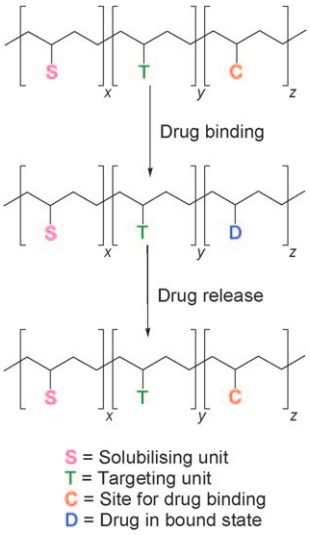

Figure 6. A schematic representation of the binding of a drug to a polymer and its release at the intended target. Adapted from Neuse et al.[52]

polymers to become more biologically available.[53] Targeting units can include antibodies, proteins, peptides or other small molecules.[54] The drug is bound to the polymer by a spacer that can be cleaved when the therapeutic target is reached. Often the spacer is composed of a sequence of self-immolative amino acids that contain a predetermined site at which breakage occurs, releasing the drug into the cell after the polymer has reached its target. Spacers can be synthesised to be cleaved by enzymatic or hydrolytic reactions. Non-degradable spacers can also be synthesised to allow attachment of moieties that need to remain adhered to the polymer such as targeting or solubilising units.

The polymer that has most often been conjugated to anticancer compounds is *N*-(2-hydroxy)propylmethacrylamide (HPMA), because its non-toxic profile is known from its earlier use as a plasma expander.[55] The first macromolecular prodrug to utilise the HPMA copolymer was PK1, which combined HPMA with doxorubicin (Figure 7a).[56] In the instance of PK1 the tetrapeptide spacer was synthesised so ly-

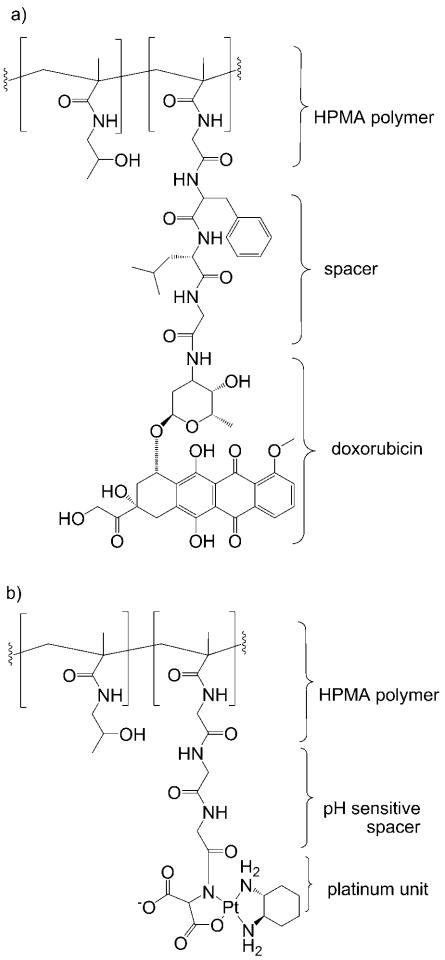

Figure 7. The chemical structures of diblock polymer drugs a) *N*-(2-hydroxypropyl)methacrylamide polymer bound doxorubicin (PK1) and b) AP5346.

sosomal enzymes within the tumourous tissue would break it down, releasing doxorubicin.[56] Early work with HPMA copolymers produced the first generation of polymers that contained platinum moieties, but was discontinued due to inconsistent formulation.[57] The third-generation HPMA copolymer AP5346 (Figure 7b) was synthesised with a pH-sensitive spacer incorporated, to enable the release of [diaminocyclohexaneplatinate]$^{2+}$ at the low pH characteristic of hypoxic tumour tissue.[58] AP5346 has undergone phase I and II clinical trials for patients suffering from reoccurring ovarian cancer. The trials demonstrated that AP5346 has equal to, if not higher efficacy than oxaliplatin and displayed exceptional tolerability.[57] The greater tolerability of AP5346 is attributed to its ability to deliver the oxaliplatin directly to the tumour.[58]

Cyclodextrin-based polymers, poly(ethyleneglycol)–camptothecin, carboxymethyldextran–exatecan and poly(ethyleneglycol)–poly(aspartic acid)–doxorubicin micelles are a few other polymer-based drugs that have entered the clinical trial pipeline since their inception as a viable therapeutic strategy.[59] Future developments in this field need to include advances in modulating the degradation rates of the polymers, allowing for a more versatile approach to attach targeting moieties, more highly specific and accurate polymerisation techniques to produce consistent products with exact molecular weights and molecular weight distributions and the addition of imaging tags so drug delivery can be easily monitored.[59] Another direction of polymer research is the addition of thermosensitive polymers to platinum compounds.

The addition of a thermosensitive cyclotriphosphazene polymer to platinum(II) is a novel strategy developed by Song et al.[60] This thermosensitive polymer possesses a low critical solution temperature (LCST) at which a phase transition takes place, precipitating the polymer from the solution. Development of the various polymers has led to a complex exhibiting a LCST of 15 °C, which importantly is lower than body temperature, and therefore may be administered directly to the tumour site with minimum side-effects. A LCST below body temperature allows for administration of the drug as a liquid to the tumour site, at which it can precipitate out at body temperature and be retained due to the EPR effect. The LCST depends on factors such as hydrogen-bonding capacity and hydrophobicity between the polymer and water in a delicate balance that is highly sensitive to the surrounding temperatures. Out of the ten cyclotriphosphazene–platinum(II) complexes published, only two exhibited LCSTs below body temperature, meaning that they are suitable for therapeutic use for direct local delivery to a tumour site.[60] The antitumour activity of these complexes has shown them to be very effective both in vitro and in vivo against murine leukaemia L1210 with the effective dose (ED_{50}, dose to produce a therapeutic response) comparable to cisplatin. In addition, dosages of up to twenty times more than cisplatin could be tolerated, while still retaining a much higher mean survival time.[60]

Nanotubes: Carbon nanotubes (CNT, Figure 8) are stable and allow easy incorporation of drugs within the inner void, with the exterior surface being able to undergo chemical modifications. The toxicity of CNTs appears to be low,[61] and a type of CNT, the single-walled carbon nanohorns (SWNH) have shown no toxicity in vitro.[62] SWNHs with individual diameters of 2–5 nm, form large spherical aggre-

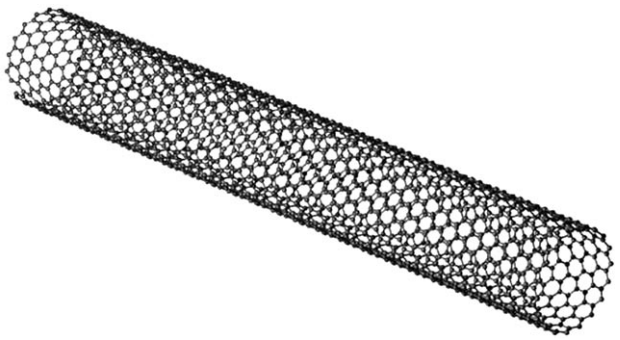

Figure 8. A single-walled carbon nanotube.

gates (80–100 nm)[63] that allows them to take advantage of the EPR effect. Cisplatin that is released from inside oxidised SWNHs still displays antitumour effects.[64] This work has been continued with cisplatin and SWNHs containing holes of 0.5–1.5 nm in diameter along the tubes. These nanohorns have exhibited greater activity than cisplatin both in vitro and in vivo, with greater tumour retention.[21,65]

There have been recent developments in targeted single-wall nanotubes (SWNT), whereby cisplatin and epithermal growth factor were attached to the SWNT in order to selectively target head and neck squamous cancer. In vivo studies have shown greater tumour regression than untargeted controls and highlight the potential of this form of selective tumour targeting.[66]

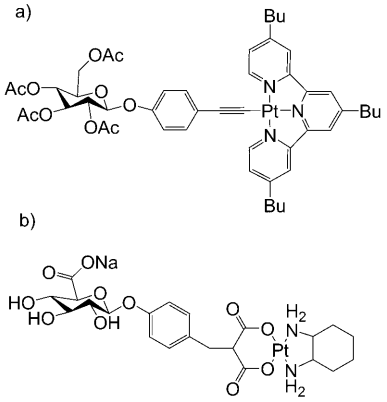

Figure 9. The chemical structures of a) glycosylated acetylide terpyridine-platinum[70] and b) the β-glucuronyl–platinum[71] complexes.

Platinum(II) Molecular Tags

As further research is conducted into increasing the understanding of cellular mechanisms involved with the growth, proliferation and actions of cancerous cells the discovery of new and exciting therapeutic targets also increases. Promising research into drug targeting involves the identification of the over-expression of certain genes, proteins and receptors. Drug targeting that exploits tumour cell characteristics, such as increased angiogenesis and mitosis, or the tumour microenvironment, such as the hypoxic and acidic nature, is an ideal focus.[67] Therapeutic targeting is a key strategy for improving the efficacy of treatment and greatly reducing toxic side-effects of current platinum-based drugs.

An alternative to targeting the cellular and environmental aspects of tumours involves the differences in biochemical metabolism between "normal" and cancerous cells. One well-known difference, as mentioned previously, is an increased cellular uptake of folic acid and over-expression of the folic acid receptors on a number of tumour types. Due to the increased uptake of folic acid by cancerous cells, folic acid receptors are an apt target for anticancer chemotherapeutic agents. A second difference between the metabolism of "normal" and cancerous cells is an increase in aerobic glycolysis for ATP generation known as the Warburg effect.[68] Glycolysis has been shown to be the predominant form of energy production in tumour cells and is therefore another therapeutic target.[69] One study where sugar moieties have been utilised is with terpyridineplatinum(II) complexes with various glycosylated acetylide and arylacetylide ligands (Figure 9a). The results of cell-line testing against five human cell carcinoma lines showed a potency of up to 100 times greater than that of cisplatin.[70] The addition of a glucal unit to oxaliplatin derivatives has also been achieved with the production of a cytotoxic drug utilising antibody-directed enzyme therapy (Figure 9b).[71]

While folic acid and glycolysis intermediates are viable options for therapeutic targets, it is worth noting that there are many other biological targets. Sex hormones such as estrogens and testosterone are of interest due to their importance in cancers of the reproductive system.[72] Estrogenic hormone tags have been attached to cisplatin derivatives due to an over-expression of estrogen receptor in several types of cancers including breast, uterine and ovarian.[73]

There are two main benefits from attaching estrogen to anticancer compounds; drug targeting due to increased cellular uptake, and also the potential for the cell to be sensitised to the compound. Estrogenic steroids are an attractive approach for cellular targeting and delivery as they are transported via the blood (when bound to steroid transport proteins), cross the cell membrane,[74] bind to the estrogen receptor in the cytoplasm and then are transferred to the nucleus.[75] Tissues (such as breast cancer) that are rich in estrogen receptors (ER) accumulate molecules such as steroids that have high binding affinities for these receptors.[72] Due to the high binding affinity of steroids localising in specific tissue (breast), some level of targeted drug delivery can be attained.[74] For this reason there has been much interest in using estrogenic steroids to deliver platinum-based compounds into cells. As well as introducing some degree of cellular targeting, estrogen also acts in sensitising cells to cisplatin. Estrogen induces over-expression of HMGB1, a protein that shields cisplatin–DNA adducts from nucleotide excision repair.[77,78]

Previous work focused on cisplatin derivatives linked to estrogen receptors through one of the two steroid hydroxy groups;[79] however, these two groups are integral for receptor recognition,[80] hence little was gained from this linkage. Studies showed that estrogen-*cis*-platinum(II) complexes bound at either the 3- or 17-hydroxyl position showed receptor binding affinity and biological activity similar to that of cisplatin.[81] Jaouen has demonstrated that organometallics could be attached to the 17α-position of estradiol preserving some estrogen receptor binding ability.[77,82] Jaouen revealed that a short linker between the estrogen 17α-position and the organometallic molecule was optimal for estrogen receptor binding. An ethynyl group was a successful linker as it provided adequate separation between the steroid and the molecule; however, it limited conformational flexibility.[77] It was also reported that directing the substituent away from the β-face (containing the hydroxyl group) of estrogen de-

creased steric interference coinciding with the receptor pocket.

Hannon and co-workers have designed and synthesised an estrogen–terpyridine platinum(II) complex in which an alkynl group is bonded to the 17α-position on the estrogen and the 4′-position on the terpyridine (Figure 10). This molecule was synthesised in order to produce a biomolecule with increased cellular uptake, whilst retaining estrogen receptor binding affinity.[74]

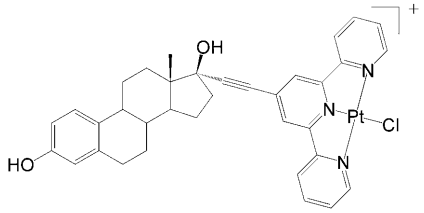

Figure 10. The structure of 17α-[4′-ethynyl-2,2′:6′,2″-terpyridine]-17β-estradiol platinum(II) chloride.

It is now more widely known that attaching estrogen to the *cis*-platinum(II) unit, while leaving the hydroxyl groups unaltered to associate with the estrogen receptor (Figure 11), gives a complex that has optimal receptor binding affinity as determined by molecular modelling analysis.[73] Berube[73,83] coupled estrogen to cisplatin-like agents at the 16-position producing highly active compounds, which demonstrates the effectiveness of this approach. These complexes were up seven times more cytotoxic than cisplatin, indicating the synthesis of compounds with high receptor binding affinity is a very promising therapeutic strategy.[73]

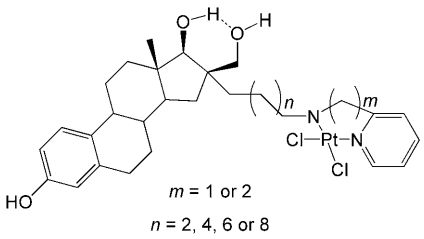

Figure 11. The chemical structure of estrogen–platinum(II) molecules with high receptor binding affinity.[73]

Platinum(IV) Prodrugs

Whilst research groups have added molecular tags to platinum(II) centres with the aim of increasing the targeting ability or decreasing reactivity, other groups have focused on molecules containing platinum(IV) centres. Platinum(IV) complexes have many advantages over platinum(II), including a lower toxicity profile, an increased kinetic inertness and reduced activity. Platinum(IV) drugs are perfect examples of prodrugs, whereby a sufficiently stable and inert complex can be transported around the body until it reaches the desired target, where it can be converted to its active cytotoxic form, which in this case is platinum(II) (Figure 12).[84,85] Conversion to the cytotoxic cisplatin may

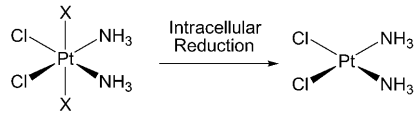

Figure 12. Structure of a platinum(IV) prodrug and its reduction to cisplatin.

be achieved by a number of mechanisms, depending on the ligands coordinated. These mechanisms can involve enzymatic breakdown, specific antibodies or even factors specific to certain areas such as pH.[84] Due to their increased stability, the complexes can be transported around the body within the bloodstream or administered orally.[86] The relative inertness of platinum(IV) complexes is also advantageous due to prevention of side-reactions with in vivo components, such as intracellular proteins, that typically deactivate platinum(II) complexes.

Platinum(IV) drugs that have entered clinical trials include iproplatin (Figure 13a), although with limited success in phase II trials,[87] along with the more promising drugs oxoplatin (Figure 13b) and satraplatin (Figure 13c). Oxoplatin is relatively inert under mild conditions and therefore can be administered orally. Oxoplatin is reduced to cisplatin by intracellular reducing agents, as well as ascorbic acid and hydrochloric acid; exposure to 0.1 M HCl resulted in a twofold increase in activity.[86] The reduction/activation under acidic conditions is promising as it can be utilised in the acidic microenvironment of solid tumours. Satraplatin was the first orally active platinum analogue and has recently completed phase III trials with very promising outcomes, including an increase in progression-free survival, a decrease in pain re-

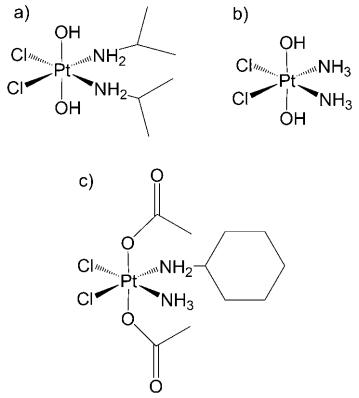

Figure 13. The chemical structure of platinum(IV) complexes a) iproplatin, b) oxoplatin and c) satraplatin.

sponse and encouraging results for prostate-specific antigen response.[88] Satraplatin has shown similar anticancer activity to carboplatin, but lacks the nephrotoxicity, neurotoxicity and ototoxicity, with patient responses indicating that it is much better tolerated than cisplatin.[88]

One method for active drug release under current research is that of photoactivatable platinum complexes. This method employs platinum(IV) as a prodrug with light-sensitive ligand(s) that are photoactivated at the site of the tumour to release active antitumour agents. Development of this strategy has been advanced with the development of fibre optic and laser technologies, that is, methods that can focus irradiation with a defined wavelength and intensity to internal organs.[89] The platinum photoactivatable classes developed thus far are typically based on diiodoplatinum(IV) (Figure 14a), and diazidoplatinum(IV) diammine complexes

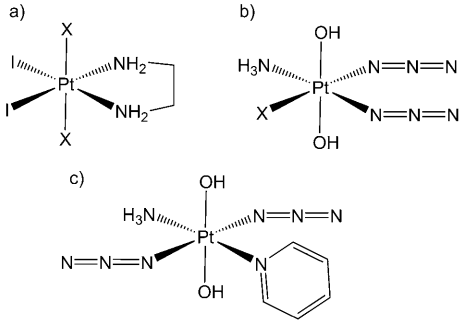

Figure 14. The general structure of photoactivatable complexes based on a) diiodoplatinum(IV) and b) diazidoplatinum(IV) complexes.

(Figure 14b), which appear to have a unique photoactivation ability based on the oxidation state of the platinum centre and metal-to-ligand charge transitions.[89,90] Unlike platinum(II) complexes, the *trans* stereoisomers of diazido–platinum(IV) diammine complexes have been shown to be more cytotoxic in comparison to the *cis* isomers.[91] After UVA irradiation the IC_{50} of the complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)(py)]$ (Figure 14c) decreased from 244.3 to 1.9 μM, while the IC_{50} of cisplatin remained almost unchanged at approximately 151 μM.[92]

While there appears to be successes in the field of platinum(IV) prodrugs with drugs like satraplatin, the search continues for new drugs based on this prodrug model. Exploration into the addition of tags capable of therapeutic targeting (as mentioned previously) is continuously being investigated. Recently, platinacyclobutanes bearing biological components have been synthesised by Stocker et al., in which platinum(IV) complexes containing biological components such as thymidine (Figure 15a), cholesterol (Figure 15b), glucose (Figure 15c) and proline (Figure 15d) were coupled to a cyclopropylmethanol. These complexes then undergo reduction to produce platinum(II) complexes.[93] Recent advances in prodrug design offers immense potential for future chemotherapeutic treatment. Further work combines the prodrug approach of platinum(IV) with

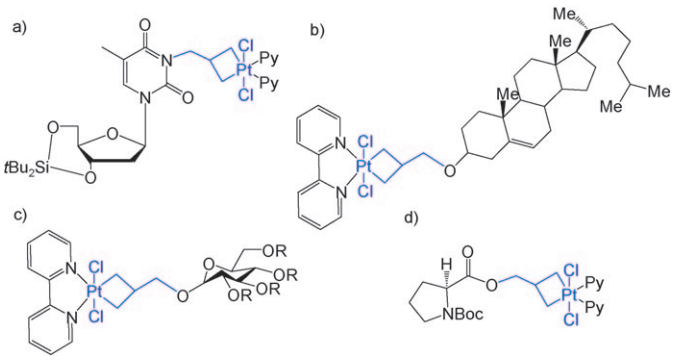

Figure 15. The chemical structure of platincyclobutanes bearing a) thymidine, b) cholesterol, c) glucose and d) proline substituents.

targeting cancer-specific antigens and biological components in drug delivery.

Platinum(IV) prodrug targeting and delivery

While prodrug development is one promising area of drug design, the encapsulation for targeted delivery of these drugs is of much interest, because although platinum(IV) prodrugs are stable, they are not impervious to degradation. Controlled release polymeric nanoparticles (NPs) with a functionalised surface can include the attachment of peptides, antibodies or aptamers that can increase the specificity for particular cancerous cells.[85] Drug encapsulation by a NP protects the drug from degradation before reaching cancerous cells and also protects non-cancerous cells from the effects of the drug.

One strategy for treating cancer, particularly prostate cancer, entails targeting antigens specific for the cancerous tissue.[85] The most appealing antigen is the prostate-specific membrane antigen (PSMA) as it is over-expressed on prostate cancer cells, offering a suitable target for cancer therapy.[94] Dhar et al.[85] used functionalised NPs developed from poly(D,L-lactic-*co*-glycolic acid) (PLGA)[95] and PEG to encapsulate a hydrophobic platinum(IV) prodrug, with PSMA-specific aptamers attached to the surface for specific delivery to $PSMA^+$ LNCaP prostate cancer cells. The platinum(IV) PLGA-PEG polymers are particularly useful because of their safety in clinical use and systemic clearance times.[96] The platinum(IV) prodrug contained two linear hexyl chains (Figure 16), which optimised encapsulation and controlled release from the NP into the cancerous cell.[85] Once inside the cell, the platinum(IV) drug is reduced to deliver cisplatin directly to the cancerous cell.

Another strategy to circumvent the disadvantages of cisplatin is by attaching cell-sensitising estradiol units to platinum(IV) compounds.[97] As previously discussed, the use of estrogen in drug targeting and delivery has two main advantages; estrogen receptors are over-expressed on the surface of many cancer cells, and, as estrogen induces over-expression of the human gene high-mobility group box 1 (HMGB1), it can prevent repair of cisplatin–DNA ad-

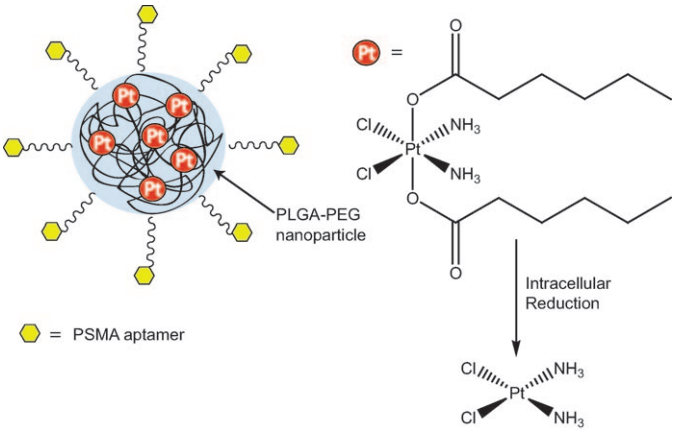

Figure 16. The chemical structure of the encapsulated platinum(IV) prodrug within the PLGA-PEG nanoparticle. The nanoparticle allows targeting to prostate cancer cells using PSMA aptamers. Once inside the cell, the drug is reduced to cisplatin.

ducts.[98,99] As such, the use of estrogen attached to platinum(IV) prodrugs is of great interest. In these compounds, the steroid is released from the platinum upon reduction and before DNA binding,[99] releasing both cisplatin and estrogen. Lippard and co-workers[100] revealed that estrogen-receptor-positive (ER(+)) cells treated with estrogen are sensitised to cisplatin.[99] As such, the release of both cisplatin and estrogen sensitises the cancerous cells to the platinum(II) delivered at the same time.[100]

The proposed mechanism of action of these compounds, as proposed by Lippard,[100] suggests that when the drug-steroid complex enters the cell, the complex is reduced releasing cisplatin and two equivalents of modified estrogen. DNA binding to cisplatin and hydrolysis of the ester may lead to HMGB1 upregulation and cell sensitisation, resulting in shielding repair of the cisplatin-DNA adducts (Figure 17).

Another method of drug delivery of platinum(IV) is the use of carbon nanotubes. Functionalised soluble SWNTs utilise the reducing environment of endosomes to selectively release the drug into the cancerous cell,[101] releasing cisplatin.[100] Feazell et al.[97] synthesised a SWNT-tethered platinum(IV) ($[Pt(NH_3)_2Cl_2(OEt)(O_2CCH_2CH_2CO_2H)]$) compound. This platinum(IV) compound is tethered through one of its axial ligands to an amine functional group on the surface of the SWNT. The SWNT transports the inert platinum(IV) drug to the cancerous target, where it is reduced to cisplatin (Figure 18). On average, a SWNT is able to carry 65 platinum(IV) centres per nanotube.

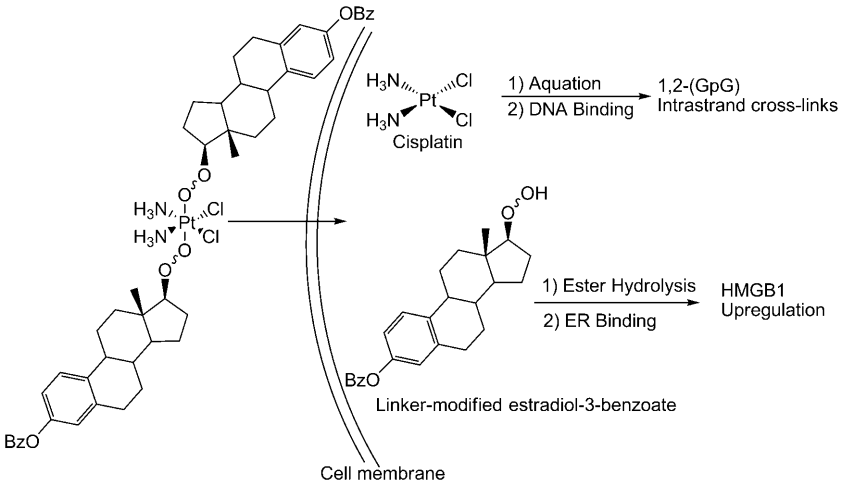

Figure 17. Proposed mechanism of action for estrogen-tethered platinum(IV) complexes. Within the cell the platinum(IV) complex is reduced to platinum(II), releasing cisplatin and two equivalents of modified estrogen. Upregulation of HMGB1 prevents cisplatin-DNA crosslinks from repair and increases cell death.[100]

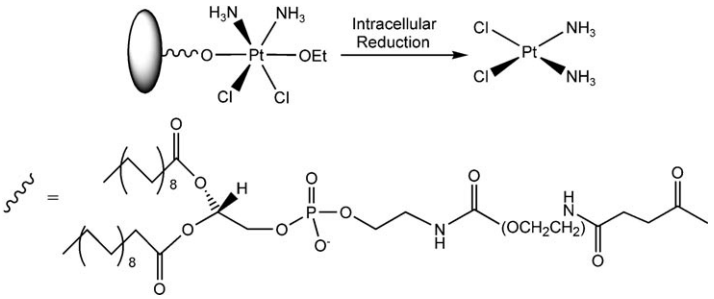

Figure 18. The platinum(IV)-tethered SWNT prodrug that is converted to cisplatin after intracellular reduction.

As already mentioned, a variety of receptors have been identified as a way of targeting cancerous cells, such as folic acid.[45] Dhar et al.[102] synthesised a platinum(IV)–SWNT complex, with the main objective of targeting, protecting and delivering cisplatin (upon intracellular reduction) to the cancerous cell. The platinum(IV) complex designed for this purpose, *cis,cis,trans*-$[Pt(NH_3)_2])_2Cl_2(O_2CCH_2CH_2CO_2H)$-$(O_2CCH_2CH_2CONH$-PEG-folic acid)] (Figure 19), contains succinate as one of its axial ligands for attachment to the amine functionalised SWNT[97] and a folic acid derivative as the other axial ligand, separated from the platinum centre by a PEG spacer, making the compound more water soluble and biocompatible.[103]

Another type of nanoparticle delivery vehicle studied by Dhar et al. involves platinum(IV) polyvalent oligonucleotide gold nanoparticle conjugates (platinum(IV)/DNA/gold-NPs).[89,102,104] As a drug delivery vehicle they have appealing characteristics, including high levels of cellular uptake in a number of cell types, no observed toxicity and resistance to enzymatic degradation.[104] The gold nanoparticle is functionalised with thiolated 28 mer oligonucleotides containing a terminal dodecyl amine for conjugation. The platinum(IV)

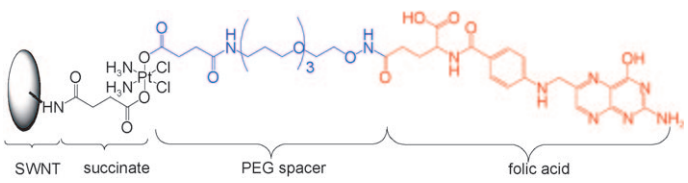

Figure 19. Folic acid receptor mediated targeting and SWNT mediated delivery of a platinum(IV) complex, which releases cisplatin upon intracellular reduction.

complex used, *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OH)(O_2CCH_2CH_2CO_2H)]$, was tethered to an amine-functionalised DNA/gold-NP surface through amide linkages. Treatment of the platinum(IV) compound with EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide) and NHS (*N*-succinimidyl ester) activated the compound, which readily formed amide linkages with the amines on the DNA/gold-NP surface, resulting in a platinum(IV)/DNA/gold-NP (Figure 20). This compound was designed to allow the platinum(IV) drug to travel safely in the blood stream to the tumour site, before the release of cisplatin upon intracellular reduction. Electrochemical studies of this conjugated oligonucleotide revealed irreversible reduction due to loss of the axial ligands. Fluorescence spectroscopy showed that after 6 h the conjugate had localised in the cell vesicles and after 12 h had localised in the cytosol.

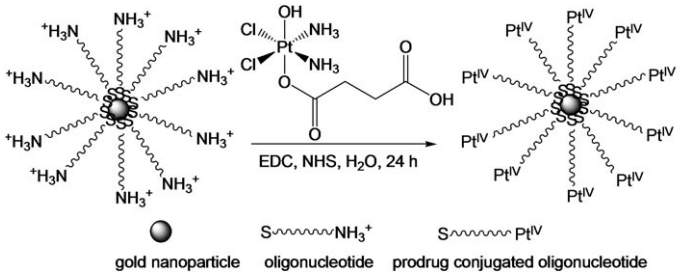

Figure 20. An overview of the synthesis of platinum(IV)–DNA–gold nanoparticle through peptide bond formation.[105]

Summary and Outlook

There have been numerous recent advances in drug delivery and the targeting of platinum anticancer compounds that are already in trial in the clinic. Using a variety of approaches, drug encapsulation, targeting and delivery have been used to conserve the tumour cytotoxicity, whilst reducing toxicity to "normal" tissues, results in a higher therapeutic index and increased efficacy. These developments show great promise for the improvement in the delivery of the platinum anticancer compounds that have already been used to treat many cancer patients over the past thirty years. The more recent developments in both drug delivery and molecular tagging have dramatically increased the interest in new techniques and methods for future developments of anticancer drugs.

Acknowledgements

B.W.H., A.M.K.-H., M.P.G. and K.B.G.-S. were supported by University of Western Sydney Australian Postgraduate Awards. This work was supported by UWS internal grants and an International Science Linkage grant.

[1] B. Rosenberg, L. van Camp, T. Krigas, *Nature* **1965**, *205*, 698–699.
[2] E. R. Jamieson, S. J. Lippard, *Chem. Rev.* **1999**, *99*, 2467–2498.
[3] S. L. Bruhn, J. H. Toney, S. J. Lippard, *Prog. Inorg. Chem.* **1990**, *38*, 477–516.
[4] A. Eastman, *Cancer Cells* **1990**, *2*, 275–280.
[5] T. Boulikas, *Expert Opin. Invest. Drugs* **2009**, *18*, 1197–1218.
[6] R. B. Weiss, M. C. Christian, *Drugs* **1993**, *46*, 360–377; D. Lebwohl, R. Canetta, *Eur. J. Cancer* **1998**, *34*, 1522–1534.
[7] M. J. Hannon, *Pure Appl. Chem.* **2007**, *79*, 2243–2261.
[8] A. M. Krause-Heuer, R. Grünert, S. Kühne, M. Buczkowska, N. J. Wheate, D. D. L. Pevelen, L. R. Boag, D. M. Fisher, J. Kasparkova, J. Malina, P. J. Bednarski, V. Brabec, J. R. Aldrich-Wright, *J. Med. Chem.* **2009**, *52*, 5474–5484.
[9] C. R. Brodie, J. G. Collins, J. R. Aldrich-Wright, *Dalton Trans.* **2004**, 1145–1152.
[10] M. A. Fuertes, J. Castilla, C. Alonso, J. M. Perez, *Curr. Med. Chem.* **2002**, *9*, 539–551.
[11] A. L. Harris, J. J. Ryan, N. Farell, *Mol. Pharmacol.* **2005**, *69*, 666–672; A. L. Harris, M. Yang, A. Hegmans, L. Povirk, J. J. Ryan, L. Kelland, N. Farell, *Inorg. Chem.* **2005**, *44*, 9598–9600.
[12] T. Boulikas, M. Vougiouka, *Oncol. Rep.* **2003**, *10*, 1663–1682; M. A. Jakupec, M. Galanski, B. K. Keppler, *Rev. Physiol. Biochem. Pharmacol.* **2003**, *146*, 1–53.
[13] J. D. Roberts, J. Peroutka, G. Beggiolin, C. Manzotti, L. Piazzoni, N. Farrell, *J. Inorg. Biochem.* **1999**, *77*, 47–50; 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*, 528–534; P. Perego, L. Gatti, C. Caserini, R. Supino, D. Colangelo, R. Leone, S. Spinelli, N. Farrell, F. Zunino, *J. Inorg. Biochem.* **1999**, *77*, 59–64; G. Pratesi, P. Perego, D. Polizzi, S. C. Righetti, R. Supino, C. Caserini, C. Manzotti, F. C. Giuliani, G. Pezzoni, S. Tognella, S. Spinelli, N. Farrell, F. Zunino, *Br. J. Cancer* **1999**, *80*, 1912–1919; C. Manzotti, G. Pratesi, E. Menta, R. D. Domenico, E. Cavalletti, H. H. Fiebig, L. R. Kelland, N. Farrell, D. Polizzi, R. Supino, G. Pezzoni, F. Zunino, *Clin. Cancer Res.* **2000**, *6*, 2626–2634.
[14] N. Farrell in *Metal Ions in Biological Systems Vol. 42 Metal Complexes in Tumour Diagnosis and as Anticancer Agents*, (Eds.: A. Sigel, H. Sigel), FontisMedia S.A. & Marcel Dekker Inc., New York, **2004**, Chapter 8, pp. 251–296.
[15] D. I. Jodrell, T. R. J. Evans, W. Steward, D. Cameron, J. Prendiville, C. Aschele, C. Noberasco, M. Lind, J. Carmichael, N. Dobbs, G. Camboni, B. Gatti, F. De Braud, *Eur. J. Cancer* **2004**, *40*, 1872–1877.
[16] K. W. Jennette, J. T. Gill, J. A. Sadownick, S. J. Lippard, *J. Am. Chem. Soc.* **1976**, *98*, 6159–6168; K. W. Jennette, S. J. Lippard, G. A. Vassiliades, W. R. Bauer, *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 3839–3843.
[17] W. D. McFadyen, L. P. G. Wakelin, I. A. G. Roos, V. A. Leopold, *J. Med. Chem.* **1985**, *28*, 1113–1116.
[18] N. J. Wheate, R. I. Taleb, A. M. Krause-Heuer, R. L. Cook, S. Wang, V. J. Higgins, J. R. Aldrich-Wright, *Dalton Trans.* **2007**, 5055–5064.
[19] D. M. Fisher, R. R. Fenton, J. R. Aldrich-Wright, *Chem. Commun.* **2008**, 5613–5615.
[20] Y. Matsumura, H. Maeda, *Cancer Res.* **1986**, *46*, 6387–6392.

[21] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, *J. Controlled Release* **2000**, *65*, 271–284.
[22] H. Maeda, G. Y. Bharate, J. Daruwalla, *Eur. J. Pharm. Biopharm.* **2009**, *71*, 409–419.
[23] P. Sapra, T. M. Allen, *Prog. Lipid Res.* **2003**, *42*, 439–462.
[24] A. D. Bangham, M. M. Standish, J. C. Watkins, *J. Mol. Biol.* **1965**, *13*, 238–252.
[25] V. P. Torchilin, *Nat. Rev. Drug Discovery* **2005**, *4*, 145–160.
[26] K. Ogawara, K. Un, K. Tanaka, K. Higaki, T. Kimura, *J. Controlled Release* **2009**, *133*, 4–10.
[27] Y. Malam, M. Loizidou, A. M. Seifalian, *Trends Pharmacol. Sci.* **2009**, *30*, 592–599.
[28] U. Kragh-Hansen, *Pharmacol. Rev.* **1981**, *33*, 17–53.
[29] I. Petitpas, T. Grüne, A. A. Bhattacharya, S. Curry, *J. Biol. Chem.* **2001**, *276*, 22804.
[30] T. E. Stinchcombe, *Nanomedicine* **2007**, *2*, 415–423.
[31] G. P. Stathopoulos, T. Boulikas, M. Vougiouka, G. Deliconstantinos, S. Rigatos, E. Darli, V. Viliotoy, J. G. Stathopoulos, *Oncol. Rep.* **2005**, *13*, 589–595.
[32] T. Boulikas, G. P. Stathopoulos, N. Volakakis, M. Vougiouka, *Anticancer Res.* **2005**, *25*, 3031–3039.
[33] T. Dragovich, D. Mendelson, S. Kurtin, K. Richardson, D. V. HoV, A. Hoos, *Cancer Chemother. Pharmacol.* **2006**, *58*, 759–764.
[34] S. White, P. Lorigan, G. Margison, J. Margison, F. Martin, N. Thatcher, H. Anderson, M. Ranson, *Br. J. Cancer* **2006**, *95*, 822–828.
[35] D. J. Bharali, M. Khalil, M. Gurbuz, T. M. Simone, S. A. Mousa, *Int. J. Nanomed.* **2009**, *4*, 1–7.
[36] W. C. Zamboni, *Clin. Cancer Res.* **2005**, *11*, 8230–8234.
[37] Á. D. C. Júnior, L. G. Mota, E. A. Nunan, A. J. A. Wainstein, A. P. D. L. Wainstein, A. S. Leal, V. N. Cardoso, M. C. D. Oliveira, *Life Sci.* **2007**, *80*, 659–664.
[38] A. Schroeder, R. Honen, K. Turjeman, A. Gabizon, J. Kost, Y. Barenholz, *J. Controlled Release* **2009**, *137*, 63–68.
[39] M. K. Calabretta, A. Kumar, A. M. McDermott, C. Cai, *Biomacromolecules* **2007**, *8*, 1807–1811.
[40] C. C. Lee, J. A. Mackay, J. M. J. Frechet, F. C. Szoka, *Nat. Biotechnol.* **2005**, *23*, 1517–1525.
[41] E. R. Gillies, J. M. J. Fréchet, *Drug Discovery Today* **2005**, *10*, 35–43.
[42] N. Malik, E. G. Evagorou, R. Duncan, *Anti-Cancer Drugs* **1999**, *10*, 767–776; R. Duncan, N. Malik, *Proc. Int. Symp. Control. Release Bioact. Mater.* **1996**, *23*, 105–106.
[43] G. Toffoli, C. Cernigoi, A. Russo, A. Gallo, M. Bagnoli, M. Boiocchi, *Int. J. Cancer* **1997**, *74*, 193–198.
[44] S. D. Weitman, R. H. Lark, L. R. Coney, D. W. Fort, V. Frasca, V. R. Zurawski, B. A. Kamen, *Cancer Res.* **1992**, *52*, 3396–3401; J. F. Ross, P. K. Chaudhuri and M. Ratnam, *Cancer* **1994**, *73*, 2432–2443; A. Krause-Heuer, M. P. Grant, N. Orkey, J. R. Aldrich-Wright, *Aust. J. Chem.* **2008**, *61*, 675–681; L. T. Mantovani, S. Miotti, S. Ménard, S. Canevari, F. Raspagliesi, C. Bottini, F. Bottero, M. I. Colnaghi, *Eur. J. Cancer* **1994**, *30 A*, 363–369.
[45] A. C. Antony, *Blood* **1992**, *79*, 2807–2820.
[46] Y. Bae, W. D. Jang, N. Nishiyama, S. Fukushima, K. Kataoka, *Mol. BioSyst.* **2005**, *1*, 242–250; C. Müller, P. A. Schubiger, R. Schibli, *Eur. J. Nucl. Med. Mol. Imaging* **2006**, *33*, 1162–1170.
[47] A. K. Patri, J. F. Kukowska-Latallo, J. R. Baker, *Adv. Drug Delivery Rev.* **2005**, *57*, 2203–2214.
[48] A. Quintana, E. Raczka, L. Piehler, *Pharm. Res.* **2002**, *19*, 1310–1316.
[49] H. Ringsdorf, *J. Polym. Sci. Polym. Symp.* **1975**, *51*, 135–153.
[50] M. L. Adams, A. Lavasanifar, G. S. Kwon, *J. Pharm. Sci.* **2003**, *92*, 1343–1355; M. E. Gindy, R. K. Prud′homme, *Expert Opin. Drug Delivery* **2009**, *6*, 865–878.
[51] R. Satchi-Fainaro, R. Duncan, C. M. Barnes, *Adv. Polym. Sci.* **2006**, *193*, 1–65.
[52] E. W. Neuse, *Met.-Based Drugs* **2007**, 1–19.
[53] F. Kratz, I. A. Muller, C. Ryppa, A. Warnecke, *ChemMedChem* **2008**, *3*, 20–53.
[54] M. E. Davis, Z. Chen, D. M. Shin, *Nat. Rev. Drug Discovery* **2008**, *7*, 771–782.
[55] J. Kopecek, H. Bazilova, *Eur. Polym. J.* **1973**, *9*, 7–14.
[56] P. A. Vasey, S. B. Kaye, R. Morrison, C. Twelves, P. Wilson, R. Duncan, A. H. Thomson, L. S. Murray, T. E. Hilditch, T. Murray, S. Burtles, D. Fraier, F. E. , J. Cassidy, *Clin. Cancer Res.* **1999**, *5*, 83–94.
[57] D. P. Nowotnik, E. Cvitkovic, *Adv. Drug Delivery Rev.* **2009**, *61*, 1214–1219.
[58] J. R. Rice, J. L. Gerberich, D. P. Nowotnik, S. B. Howell, *Clin. Cancer Res.* **2006**, *12*, 2248–2254.
[59] C. Li, S. Wallace, *Adv. Drug Delivery Rev.* **2008**, *60*, 886–898.
[60] S.-C. Song, S. B. Lee, B. H. Lee, H.-W. Ha, K.-T. Lee, Y. S. Sohn, *J. Controlled Release* **2003**, *90*, 303–311.
[61] M. L. Becker, J. F. Fagan, N. D. Gallant, B. J. Bauer, V. Bajpai, E. K. Hobbie, S. H. Lacerda, K. B. Migler, J. P. Jakupciak, *Adv. Mater.* **2007**, *19*, 939.
[62] J. Miyawaki, M. Yudasaka, T. Azami, Y. Kubo, S. Iijima, *ACS Nano* **2008**, *2*, 213–226.
[63] S. Iijima, M. Yudasaka, R. Yamada, S. Bandow, K. Suenaga, F. Kokai, K. Takahashi, *Chem. Phys. Lett.* **1999**, *309*, 165–170.
[64] K. Ajima, M. Yudasaka, T. Murakami, A. Maigne, K. Shiba, S. Iijima, *Mol. Pharm.* **2005**, *2*, 475–480.
[65] K. Ajima, T. Murakami, Y. Mizoguchi, K. Tsuchida, T. Ichihashi, S. Iijima, M. Yudasaka, *ACS Nano* **2008**, *2*, 2057–2064; K. Ajima, M. Yudasaka, K. Suenaga, D. Kasuya, T. Azami, S. Iijima, *Adv. Mater.* **2004**, *16*, 397–401.
[66] A. A. Bhirde, V. Patel, J. Gavard, G. Zhang, A. A. Sousa, A. Masedunskas, R. D. Leapman, R. Weigert, J. S. Gutkind, J. F. Rusling, *ACS Nano* **2009**, *3*, 307–316.
[67] H. J. Broxterman, N. H. Georgopapadakou, *Drug Resist. Updates* **2005**, *8*, 183–197; A. K. Lyer, G. Khaled, J. Fang, H. Maeda, *Drug Discovery Today* **2006**, *11*, 812–818.
[68] H. Pelicano, D. S. Martin, R.-H. Xu, P. Huang, *Oncogene* **2006**, *25*, 4633–4646; O. Warburg, K. Posener, E. Negelein, *Biochem. Z.* **1924**, *152*, 319–344.
[69] M. E. Beckner, L. A. Liotta, E. Schiffmann, *J. Natl. Cancer Inst.* **1990**, *82*, 1836–1840.
[70] D.-L. Ma, T. Y.-T. Shum, F. Zhang, C.-M. Che, M. Yang, *Chem. Commun.* **2005**, 4675–4677.
[71] R. A. Tromp, S. S. G. E. van Boom, C. M. Timmers, S. van Zutphen, G. van der Marel, H. S. Overkleeft, J. H. van Boom, J. Reedijk, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4273–4276.
[72] G. G. Chen, Q. Zeng, G. M. K. Tse, *Med. Res. Rev.* **2004**, *24*, 954; W. Somboonporn, S. R. Davies, *Endocr. Rev.* **2004**, *25*, 374.
[73] V. Gagnon, M.-E. St-Germain, C. Descôteaux, J. Provencher-Mandeville, S. Parent, S. K. Mandal, E. Asselin, G. Bérubé, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5919–5924.
[74] M. J. Hannon, P. S. Green, D. M. Fisher, P. J. Derrick, J. L. Beck, S. J. Watt, S. F. Ralph, M. M. Sheil, P. R. Barker, N. W. Alcock, R. J. Price, K. J. Sanders, R. Pither, J. Davis, A. Rodger, *Chem. Eur. J.* **2006**, *12*, 8000–8013.
[75] C. Sanchez-Cano, M. J. Hannon, *Dalton Trans.* **2009**, 10702–10711.
[76] A. Jackson, J. Davis, R. J. Pither, A. Rodger, M. J. Hannon, *Inorg. Chem.* **2001**, *40*, 3964.
[77] S. Top, H. E. Hafa, A. Vessieres, J. Quivy, J. Vaissermann, D. W. Hughes, M. J. McGlinchy, J. P. Moron, E. Thoreau, G. Jaouen, *J. Am. Chem. Soc.* **1995**, *117*, 8372.
[78] N. G. Knebel, E. V. Angerer, *J. Med. Chem.* **1991**, *34*, 2145–2152; J. P. DiZio, K. E. Carlson, C. J. Bannochie, M. J. Welsh, E. V. Angerer, J. A. Katzenellenbogen, *J. Steroid Biochem. Mol. Biol.* **1992**, *42*, 363–373.
[79] J. Altman, T. Castrillo, W. Beck, G. Bernhardt, H. Schonenberger, *Inorg. Chem.* **1991**, *30*, 4085–4088; C. Chesne, G. Leclereq, P. Pointeau, H. Patin, *Eur. J. Med. Chem.* **1986**, *21*, 321–387.
[80] A. M. Brzozowski, A. C. W. Pike, Z. Dauter, R. E. Hubbard, T. Bonn, O. Engestrom, L. Ohmann, G. L. Greene, J. A. Gustafsson, M. Carlquist, *Nature* **1997**, *389*, 753–758.

[81] O. Gandolfi, J. Blum, *Inorg. Chim. Acta* **1984**, *91*, 257–261; M. P. Georgiadis, S. A. Haroutounian, *Inorg. Chim. Acta* **1987**, *138*, 249–252.
[82] H. I. El Amouri, A. Vessieres, D. Vichard, S. Top, M. Gruselle, G. Jaouen, *J. Med. Chem.* **1992**, *37*, 3130–3135.
[83] V. Perron, D. Rabouin, E. Asselin, S. Parent, R. C. C. Gaudreault, G. Berube, *Bioorg. Chem.* **2005**, *34*, 1.
[84] M. J. Ferguson, F. Y. Ahmed, J. Cassidy, *Drug Resist. Updates* **2001**, *4*, 225–232.
[85] S. Dhar, F. X. Gu, R. Langer, O. C. Farokhzad, S. J. Lippard, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17356–17361.
[86] U. Olszewski, F. Ach, E. Ulsperger, G. Baumgartner, R. Zeillinger, P. Bednarski, G. Hamilton, *Met.-Based Drugs* **2009**, 1–11.
[87] L. Pendyala, J. W. Cowens, G. B. Chheda, S. P. Dutta, P. J. Creaven, *Cancer Res.* **1988**, *48*, 3533–3536; J. B. Vermorken, S. Gundersen, M. Clavel, J. F. Smyth, P. Dodion, J. Renard, S. B. Kaye, *Ann. Oncol.* **1993**, *4*, 303–306.
[88] H. Choy, C. Park, M. Yao, *Clin. Cancer Res.* **2008**, *14*, 1633–1638.
[89] P. J. Bednarski, F. S. Mackay, P. J. Sadler, *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 75–93.
[90] P. Bednarski, R. Grünert, M. Zielzki, A. Wellner, F. S. Mackay, P. J. Sadler, *Chem. Biol.* **2006**, *13*, 61–67.
[91] F. S. Mackay, J. A. Woods, P. Heringova, J. Kasparkova, A. M. Pizarro, S. A. Moggach, S. Parsons, V. Brabec, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20743–20748; N. J. Farrer, J. A. Woods, V. P. Munk, F. S. Mackay, P. J. Sadler, *Chem. Res. Toxicol.* **2010**, *23*, 413–421.
[92] F. S. Mackay, J. A. Woods, H. Moseley, J. Ferguson, A. Dawson, S. Parsons, P. J. Sadler, *Chem. Eur. J.* **2006**, *12*, 3155–3161.
[93] B. L. Stocker, J. O. Hoberg, *Organometallics* **2009**, *28*, 4537–4541.
[94] R. S. Israeli, C. T. Powell, W. R. Fair, W. D. W. Heston, *Cancer Res.* **1993**, *53*, 277; G. P. Murphy, A. A. Elgamal, S. L. Su, D. G. Bostwick, E. H. Holmes, *Cancer* **1998**, *83*, 2259–2269.
[95] R. Langer, *Nature* **1998**, *392*, 5–10.
[96] F. Alexis, E. Pridgen, L. K. Molnar, O. C. Farokhzad, *Mol. Pharm.* **2008**, *5*, 505–515; R. Gref, Y. Minamitake, M. T. Peracchia, V. Trubetskoy, V. Torchilin, R. Langer, *Science* **1994**, *263*, 1600–1603.
[97] R. P. Feazell, N. Nakayama-Ratchford, H. Dai, S. J. Lippard, *J. Am. Chem. Soc.* **2007**, *129*, 8438–8439.
[98] J. C. Huang, D. B. Zamble, J. T. Reardon, S. J. Lippard, A. Sancar, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 10394–10398; K. Y. Chau, H. Y. P. Lam, K. L. D. Lee, *Exp. Cell Res.* **1998**, *241*, 269–272.
[99] Q. He, C. H. Liang, S. J. Lippard, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5768–5772.
[100] K. R. Barnes, A. Kutikov, S. J. Lippard, *J. Chem. Biol.* **2004**, *11*, 557.
[101] B. Arunachalam, U. T. Phan, H. J. Geuze, P. Cresswell, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 745–750.
[102] S. Dhar, Z. Liu, L. Thomale, H. Dai, S. J. Lippard, *J. Am. Chem. Soc.* **2008**, *130*, 11467–11476.
[103] S. Zalipsky, *Adv. Drug Delivery Rev.* **1995**, *16*, 157–182.
[104] D. A. Giljohann, D. S. Seferos, P. C. Patel, J. E. Millstone, N. L. Rosi, C. A. Mirkin, *Nano. Lett.* **2007**, *7*, 3818–3821; N. L. Rosi, D. A. Giljohann, C. S. Thaxton, A. K. R. Lytton-Jean, M. S. Han, C. A. Mirkin, *Science* **2006**, *312*, 1027–1030.
[105] S. Dhar, W. L. Daniel, D. A. Giljohann, C. A. Mirkin, S. J. Lippard, *J. Am. Chem. Soc.* **2009**, *131*, 14652–14653.

