

Review

Targeting platinum anti-tumour drugs: Overview of strategies employed to reduce systemic toxicity

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Abstract

Selective drug delivery is an important approach with great potential for overcoming problems associated with the systemic toxicity of chemotherapy, in particular, platinum-based chemotherapy. Finding successful strategies for the targeting of platinum anticancer drugs has therefore been a subject of extensive research. This review paper gives an overview of some of the different approaches that have recently been used in the development of targeted platinum anticancer drugs.

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1. Introduction

Cisplatin is one of the leading drugs currently used in the treatment of a number of solid malignancies (1; Fig. 1) [1]. For reasons of toxicity and drug resistance, however, there has been a widespread search for related complexes with similar or improved activity. Although cisplatin can induce apoptosis selectively in cancer cells through binding to DNA, the drug undergoes many non-selective reactions with a variety of biomolecules, such as proteins and phospholipids [2]. Furthermore, the drug is rapidly distributed throughout the whole body upon administration, interacting

with both healthy and cancerous tissue [3]. This interaction gives rise to the dose-limiting nephro- and hepatotoxicities, as well as to drug resistance [4]. Targeting of the drug to the DNA of tumour cells is therefore highly desirable. This targeting can be achieved via different strategies, e.g. improving plasma stability, regulating tumour-selective uptake and increasing the affinity of the drug for its ultimate target, nuclear DNA.

Most targeted platinum compounds consist of a vector ligand tethered to a platinum drug moiety. Cisplatin and the other platinum drugs that have entered the clinic, all have two leaving groups in *cis*-position facing two amine groups [5]. The cisplatin pharmacophore can be conjugated to the vector either via the leaving groups, or via the amine groups. In the first case, the drug can dissociate from the carrier ligand,

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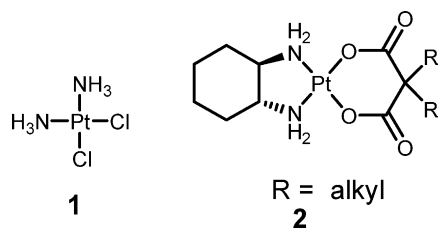


Fig. 1. Cisplatin (1) and a lipophilic malonate derivative of *trans*-R,R-1,2-diaminocyclohexaneplatinum(II) (2).

resulting in the release of an active, aquated drug species nearby the target. The precisely correct rate of dissociation of the platinum from the carrier is crucial for the cytotoxic properties of the conjugate. Alternatively, the vector ligand may be coordinated to the amine functionalities. In this case, the vector will remain coordinated to the drug while entering the cell and even upon DNA binding, modifying significantly the physical and chemical properties of the drug and its DNA adducts. Although the vector may achieve the desired targeting effect, it may also impair with the cytotoxic properties of the compound. Therefore, one can also envisage a so-called prodrug approach, where a known platinum drug is released from the vector at the target. Several Pt(IV) drugs are known to be reduced to Pt(II) species inside the cell [6]. Targeting functionalities introduced to the axial ligands of Pt(IV) species will therefore dissociate from the platinum inside the cell. Prodrugs containing an enzymatically cleavable linker between the platinum drug moiety and the vector will generally release a drug moiety containing some kind of trace from the linker, which will inevitably influence the cytotoxicity.

In addition to reduction of the problems associated with systemic toxicity, resistance may be overcome by carefully targeted platinum drugs. Resistance to platinum drugs can be dependent on many different factors, however, most resistant tumours show an impaired accumulation of the drug [7]. Recent studies have shown that the high-affinity copper transporters are an important factor in the regulation of cisplatin uptake [8–10]. Targeting other uptake mechanisms may therefore be a strategy that can lead to the development of new drugs overcoming cisplatin resistance. Increasing the affinity of a platinum complex for DNA may overcome resistance based on an increased concentration of deactivating thiols. The targeted drug will be less exposed to these thiols as the rate of DNA binding would be increased [11]. Finally, the different nature of the DNA adduct formed by some targeted platinum complexes may overcome resistance based on improved nucleotide excision repair of cisplatin DNA adducts [11].

This paper presents an overview of recent strategies that have been employed for the targeting of platinum drugs. Four different types of targeting are discussed: passive targeting, receptor mediated targeting, enzymatically activated prodrugs and DNA targeting. Accordingly, the article is divided in four different sections.

2. Passive tumour targeting based on the EPR effect

Tumours are often hyperpermeable towards macromolecules as a result of compromised vasculature [12]. Combined with a lack of effective lymphatic drainage, also common in tumours, accumulation of such macromolecules can take place. This ‘enhanced permeability and retention effect’ (so-called EPR effect) can lead to increased drug concentration within the tumour tissue if the therapeutic agent is coupled to a macromolecular carrier, or packed inside a nano-sized particle. Subsequent endocytosis will ensure drug uptake into the tumour cell. This passive tumour targeting has proved to be successful in the development of several non-platinum chemotherapeutic agents such as Doxil[®], a liposomal formulation of doxorubicin [13]. It is important to note that in vitro studies are not always able to predict the in vivo behaviour of macromolecular drug delivery systems. This has been explained by the slow kinetics of endocytosis compared to the rapid uptake of low-molecular weight reference compounds [14]. Some examples of the EPR effect exploited in the development of novel platinum anti-cancer therapies are outlined below in some detail.

The poor water solubility and low lipophilicity of cisplatin makes it difficult to efficiently encapsulate the drug in a liposome. Nevertheless, many different liposomal formulations of platinum drugs have been prepared. In general, such formulations show much longer half-lives in vivo. As a result, high drug accumulation in the tumour, and mild toxicity profiles can be observed. Lipoplatin, for example, shows a 2–50-fold increased drug concentration in the tumours during Phase-I clinical trials [15]. A stealth liposome-encapsulated cisplatin (SPI-77) has undergone several Phase-I and Phase-II clinical trials [16–23]. It shows very favourable pharmacokinetics and reduced urine excretion. As a result, the maximum tolerated dose is increased 5-fold with respect to free cisplatin. Unfortunately, this benefit has not yet translated into a curative advantage over cisplatin [24]. Using novel encapsulation methods, it is possible to form nanocapsules of cisplatin in a lipid bilayer with very high drug-to-lipid ratio [25]. These products show promising in vitro activity [26]. It is also possible to form liposomes of cisplatin derivatives in order to overcome the problems with solubility and lipophilicity. Lipophilic malonate derivatives of *trans*-R,R-1,2-diaminocyclohexaneplatinum(II) complexes (2; Fig. 1) can be trapped in multilamellar liposomal vesicles [27]. Due to their stability and low absorption into systemic circulation they present a promising class of compounds, currently under clinical investigation [28].

In polymer drug delivery systems, the active drug is linked to a polymeric carrier while circulating in the blood to be released at the target [29]. The polymer bound drug must possess high plasma stability and be non-toxic. Through coordination of cisplatin to carboxymethyl-dextran polymers via substitution of the chloride atoms it is possible to load up to 15 or 60 mole of platinum to 10,000 or 40,000 Da polymers, respectively. These polymers display favourable

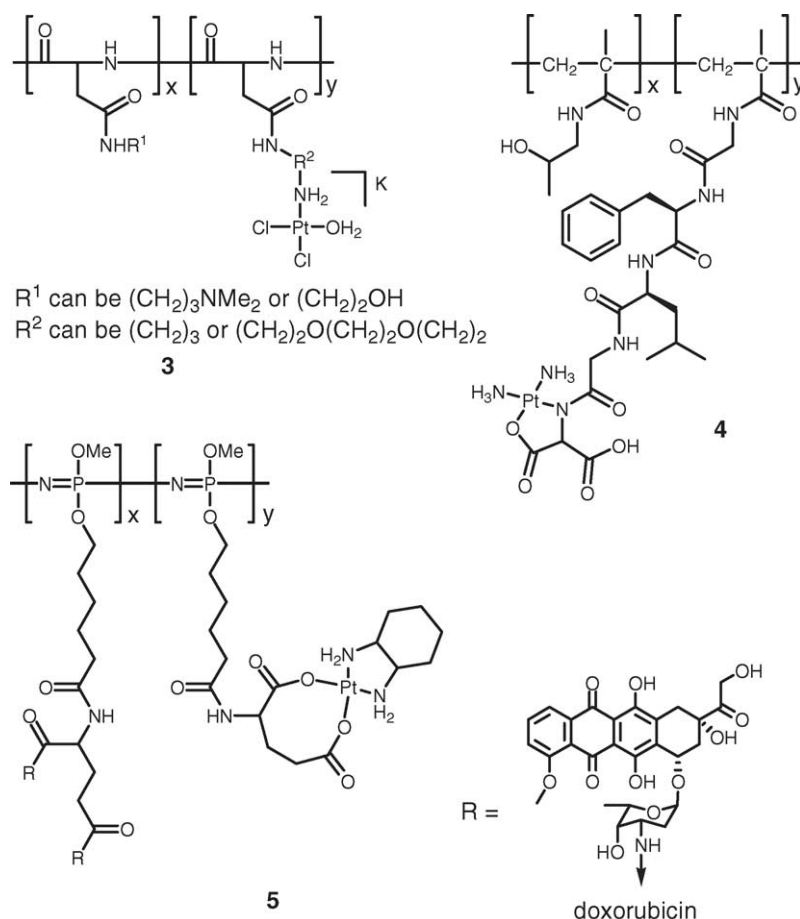


Fig. 2. Polymer platinum conjugates: carboxymethyl-dextran polymers containing platinum(II) monoamine species (3) [34], HPMA copolymer-bound platinum therapeutic agent AP5280 (4) [38] and a poly(organophosphazene) carrier loaded with platinum(dach) and doxorubicin (5) [42].

antitumour properties compared to cisplatin [30–33]. Similarly water-soluble poly-aspartamide can be loaded with the tetrachloroplatinate dianion in aqueous medium to yield an unusual polymer-bound platinum(II) monoamine species (3; Fig. 2) [34–36]. This binding mode of platinum would normally lead to charged species that might have difficulty penetrating biological barriers. When anchored to a polymeric carrier, however, these compounds can be endocytosed across cellular membranes and display high in vitro cytotoxicity.

The *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-bound platinum therapeutic agent AP5280 has a platinum(II) diamine linked to the polymer via an N,O-chelating unit (4; Fig. 2). Inside the cell, this chelate is thought to be released, either due to proteolytic cleavage of the short peptide bridge linking the drug to the polymer, or through release of the platinum from the chelating ligand via hydrolysis [37,38]. The in vitro cytotoxicity of this polymer is 2.0–2.7-fold higher than carboplatin. In mouse models, however, toxicity was found to be decreased 6-fold without significant change in tumour growth inhibition compared to carboplatin. The drug can be formulated for clinical use [39,40] and has entered Phase-I clinical trials [41].

Different anti-cancer drugs can be loaded onto the same polymeric carrier. By conjugating a platinum(dach) moiety, as well as doxorubicin, to a poly(organophosphazene) carrier in differing relative ratio's, a single agent that effectively attains combination therapy is produced (5; Fig. 2). The polymer is selectively taken up into the tumour cells due to the EPR effect, after which the two drugs can be released. Indeed high in vivo activities were observed for these complex conjugates [42].

Small cisplatin-containing particles can be formed through the reaction between cisplatin and [PEG-P(Asp)] [43], or [PEG-P(Glu)] [44] in aqueous medium, leading to the spontaneous formation of cisplatin loaded micelles (6; Fig. 3). These micelles have a narrow size distribution of around 20 nm and accumulate selectively in solid tumours. Although the particles are much less cytotoxic than free cisplatin, almost complete recovery of the cytotoxic activity can be attained though pre-incubation in saline solution. The in vivo evaluations of these micelles show that the combination of tumour targeting and time-modulated drug release can lead to drugs with improved therapeutic indices compared to cisplatin [45].

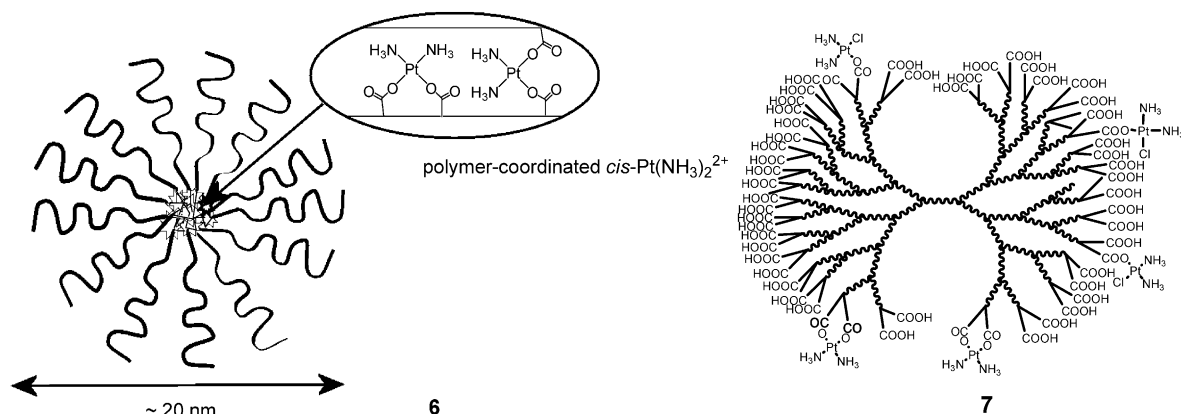


Fig. 3. A schematic representation of cisplatin-containing micelles (6) [44] and platinated PAMAM[®] generation 3.5 dendrimer (7) [46].

Dendrimers offer an attractive alternative to the use of polymers as drug-delivery vehicles. Unlike polymers, dendrimers are mono-dispersed and their surface chemistries are well defined and can be tailored to suit specific needs. Interestingly, platinum dendrimers prepared with polyamidoamine (PAMAM[®]) dendrimer generation 3.5 and cisplatin

showed poor in vitro cytotoxic properties (7; Fig. 3). In a carefully chosen mouse-model, however, where preferential tumour accumulation by the EPR effect is allowed, the platinum dendrimer showed both highly increased accumulation and increased therapeutic efficacy compared to free cisplatin [46].

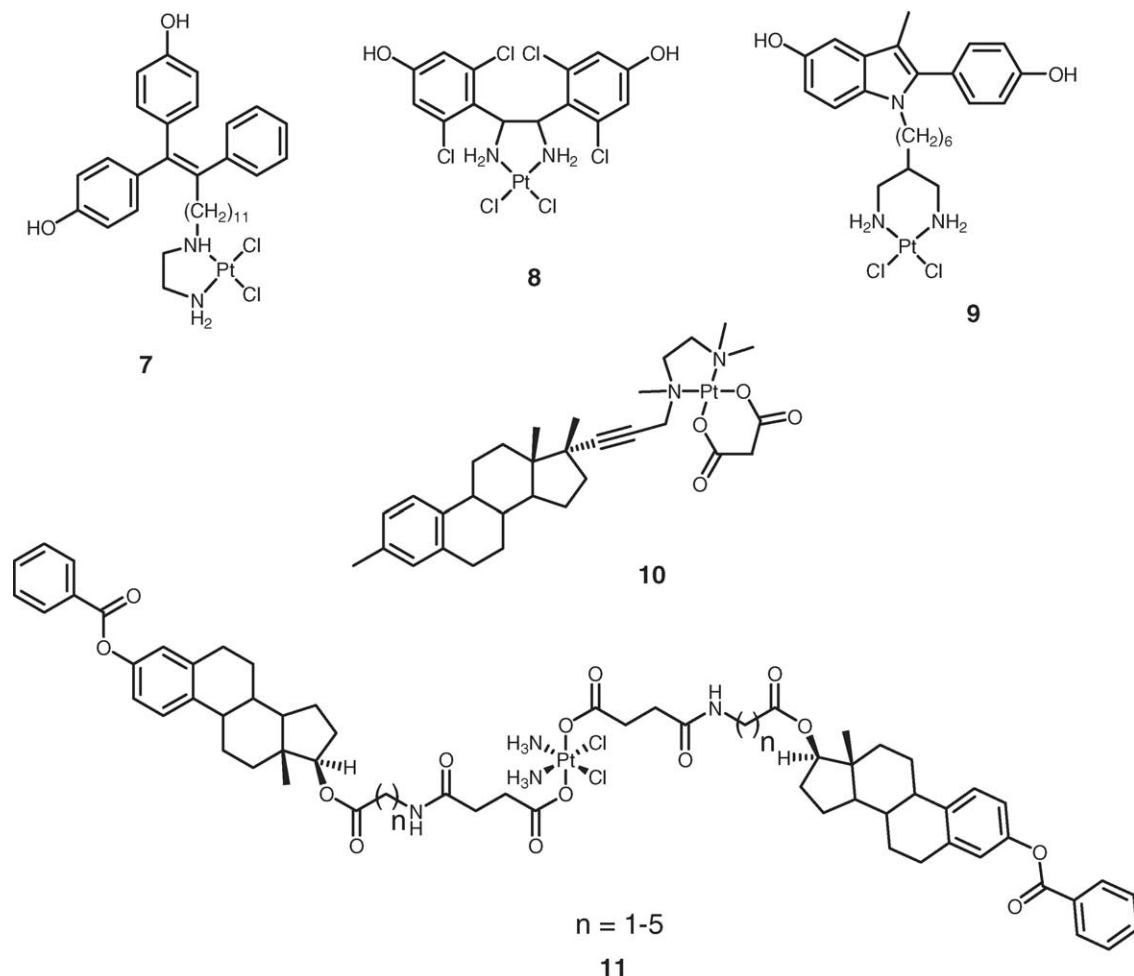


Fig. 4. Platinum compounds targeted towards the estrogen receptor, containing polyaromatic ligands (7–9) [49–51] or estradiol derivatives (10 and 11) [54,55].

3. Receptor mediated targeting

Tumour-associated membrane-bound antigens provide cellular targets for targeted delivery to a certain tumour. By tailoring a drug to have high-affinity for such a receptor, the drug can be endocytosed specifically into cells displaying the receptor. Receptors associated with cell growth and division are often overexpressed on a tumour, thus providing obvious targets for cancer therapy. Many platinum compounds with receptor-specific appended ligands have been prepared [47]. These ligands can be based on the natural substrate or an antibody against the appropriate receptor. It is important that the conjugated drug does not impair with the affinity of the vector for its receptor, and vice versa, the vector must of course not reduce the potency of the cytotoxic agent. Platinum-based drugs targeted to estrogen receptors, folic acid receptors and oncofetal protein receptors have been reported, some examples of which are discussed in more detail.

The estrogen receptor is overexpressed in some breast and prostate tumours. Platinum compounds with polyaromatic ligands (**7–9**; Fig. 4) [48–51], or appended estradiol derivatives (**10**; Fig. 4) [52–54], can be internalised via the estrogen receptor. However, these complexes show low relative binding affinities (RBA) to the estrogen receptor ranging from 0.05 to 5.2% relative to an RBA of 100% for estradiol. Therefore, little or no increased cytotoxicity of the complexes in estrogen receptor-positive cells (ER⁺) compared to estrogen receptor-negative cells (ER[−]) is observed. Since treatment with estrogen can sensitize ER⁺ cells towards cisplatin, a double prodrug is formed by tethering estrogen to platinum(IV) (**11**; Fig. 4) [55]. The estrogen–platinum conjugate is internalised in the target cell. Inside the cell reduction of Pt(IV) to Pt(II) releases both the active platinum species and the estrogen. In this case, a factor 2 increase in activity was observed between the ER⁺ and ER[−] cells.

The folate receptor is another confirmed target for cancer chemotherapy. Surface receptors for folic acid are generally overexpressed in human cancer cells, and many examples are known of folate–drug conjugates internalised successfully via the folate receptor [56]. A carboplatin moiety conjugated to a folic acid functionalised PEG carrier was shown to be taken up into tumour cells via the folate receptor [57]. Surprisingly, the PEG–Pt conjugate lacking the folic acid, used as a reference structure, displayed higher cellular accumulation, as well as higher cytotoxicity. These results suggest that even highly targeted compounds can have several different cellular uptake mechanisms. This work was further extended by introducing a nuclear localisation (NLS) peptide to the PEG–Pt conjugate, targeting the complex towards the nucleus [58]. In this system, the NLS peptide increased internalisation allowing increased accumulation in the nucleus. The increased accumulation did not, however, lead to increased Pt–DNA adduct formation, or to increased cytotoxicity compared to the PEG–Pt conjugate lacking the NLS peptide. The authors suggest this behaviour may be due to the fact that carboplatin requires cytosolic activation prior to

DNA binding, and therefore carboplatin-based drugs do not necessarily benefit from a rapid transport from the cytosol to the nucleus.

Peptide libraries provide a powerful tool to explore protein interactions, therefore peptides are highly suited for the development of receptor-specific vector molecules. Appending a targeting peptide to a drug can be a successful strategy for obtaining highly targeted delivery of the therapeutic agent [59]. Platinum–peptide conjugates for targeted drug delivery can be prepared using solid-phase methodologies [60,61]. Via this approach both mononuclear and dinuclear platinum–peptide conjugates were prepared in our laboratory, using a number of peptides previously described as vector molecules for the transport of biomolecules [62,63]. Initial cytotoxic assays indicate an increase in activity of the targeted compounds compared to untargeted analogues [64].

Oncofetal proteins, such as the α -fetoprotein, are endocytosed by tumour cells specifically due to the 10 times higher abundance of the receptor on T-lymphoma cells compared to normal proliferating T-lymphocytes. Cisplatin can be conjugated to the α -fetoprotein in different drug-to-protein ratios and purified via dialysis. With a protein/drug ratio of 1/3 up to 1/5, respectively, the conjugates were found to be significantly more toxic than the free drug. For higher drug ratios, the toxicity became similar to that of the free drug, suggesting at this point that the conjugated cisplatin disrupts the recognition of the protein by the receptor. The specificity of the conjugates was successfully illustrated *in vitro*, showing a factor 2–3 increase in activity in tumour cells compared to normal cells [65].

4. Enzymatically activated prodrugs

Non-toxic prodrugs that can be selectively activated by enzymes at a tumour provide an exciting opportunity for the development of targeted therapies. In antibody-directed enzyme prodrug therapy (ADEPT), selectivity for the target is achieved by administration of an enzyme fused to an antibody followed by a prodrug that can be activated by the enzyme [66]. A number of enzymes are found in highly increased concentration around tumour tissue, allowing the use of prodrug monotherapy (PMT). This involves a single administration of the prodrug, which is activated locally to yield the active drug [67,68]. These concepts were explored for a cephalosporin–platinum complex, which can be activated by β -lactamases (**12**; Fig. 5) [69] and a β -glucuronyl–platinum conjugate, which can be activated by β -glucuronidase (**13**; Fig. 5) [70]. Although both results show that the enzyme is indeed capable of activating the prodrug, in either case a modified oxaliplatin-type species is released whose cytotoxic activity was not investigated separately [69,70]. Esterases are abundant in all cells, but some stereoselectivity can be observed between normal and cancer cells. Cell-specific activation of a platinum compound by stereoselective ester hydrolysis is therefore a promising possibility. Platinum compounds with

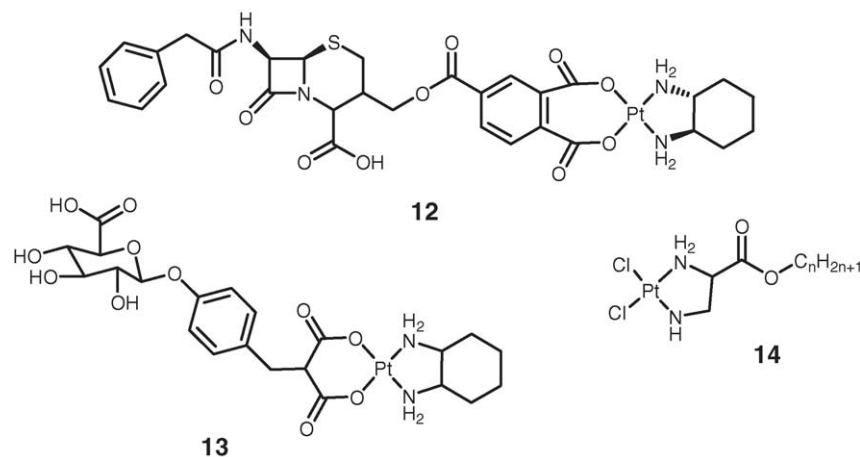


Fig. 5. Enzymatically activated platinum prodrugs **12** [69], **13** [70] and **14** [71].

hydrophobic side-chains linked via an ester bond were prepared (**14**; Fig. 5) [71]. These compounds show improved cell-permeability with respect to cisplatin. In a cell line which shows high-stereoselectivity for ester hydrolysis, a 10-fold difference in activity between the (R)-enantiomer and the (S)-enantiomer was observed, indicating that ester hydrolysis indeed activates these compounds [71].

5. Compounds targeted towards cellular DNA

Cellular DNA is the single most studied target for platinum anti-tumour drugs. It is believed that direct coordination of platinum to the nucleophilic nitrogens of the nucleobases is responsible for the induction of apoptosis in tumour cells [72]. As a result many platinum complexes have been designed to optimise the platinum–DNA interaction. Such targeting can improve the drugs in several ways. Increased affinity for DNA leads to reduced exposure of the platinum to other cellular nucleophiles, such as deactivating thiols. This may lead to both a reduction in side-effects and the overcoming of resistance based on increased glutathione concentrations. Furthermore, the damage inflicted by the compounds may be different and perhaps more severe, compared to the damage inflicted by a platinum compound alone. This difference could change the spectrum of activity and overcome

resistance mechanisms based on increased or improved DNA repair [11].

Introducing an additional positive charge in platinum complexes will induce charge–charge interactions with DNA. Positively charged polynuclear platinum compounds with aliphatic diamine linkers have increased affinity for the negatively charged phosphate backbone of DNA [73,74]. This may be one of the possible reasons that these drugs show high-activities, which has spurred Phase-I clinical trials [75,76]. Conversely, platinum–peptide conjugates with increased affinity for DNA did not show increased cytotoxic activity [77]. One may conclude therefore that cytotoxicity is the result of a number of factors including cellular trafficking and chemical reactivity, as well as target affinity.

More elaborate targeting of DNA involves the tethering of different types of DNA targeting ligands to platinum complexes. The type of ligands used include oligonucleotides, intercalators and DNA-groove binders. A single stranded oligonucleotide has been tethered to platinum(II) and to platinum(IV) species (**15**; Fig. 6) [78,79]. Alternatively, PNA can be used to improve the stability of the complex [80]. These compounds have been shown to overcome multidrug resistance associated with altered DNA topoisomerase II. Furthermore, some sequence specific inhibition of oncogenes can be observed, determined by the sequence of the tethered oligonucleotide [81].

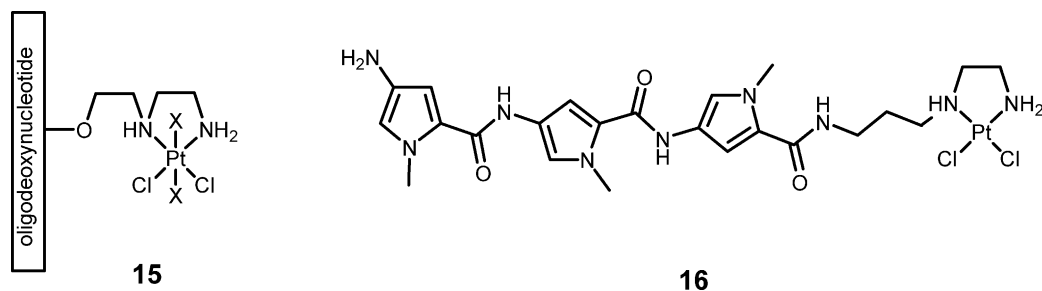


Fig. 6. Generic form of oligonucleotide platinum(VI) complexes **15** [78] and a groove-binder platinum complex **16** [82].

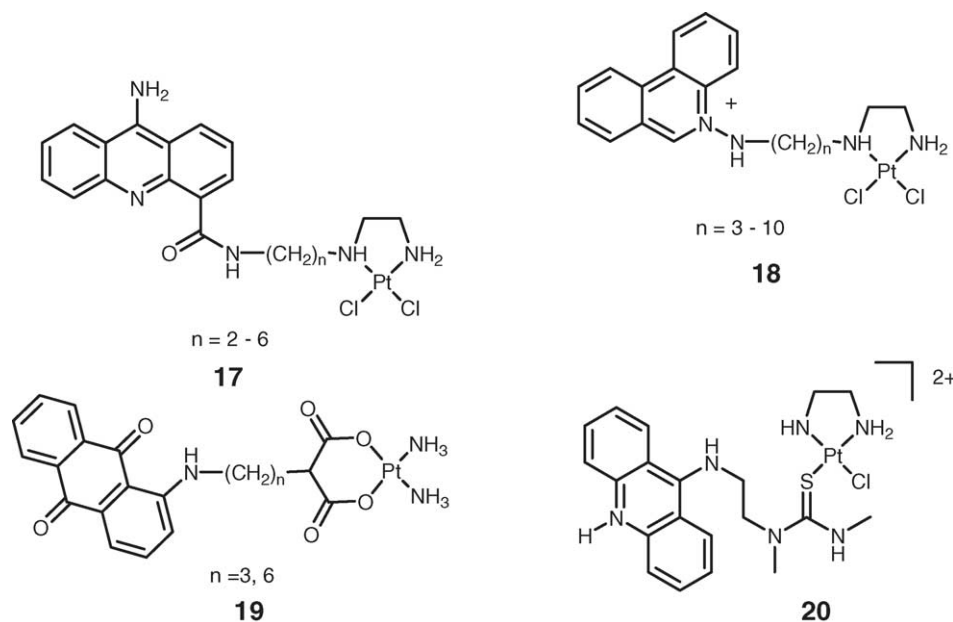


Fig. 7. Various intercalators tethered to platinum drug moieties: an acridine derivative (**17**) [108] phenanthridinium cation (**18**) [99], anthraquinone (**19**) [103] and a 9-aminoacridine derivative (**20**) [98].

Groove binders such as netropsin and distamycin owe their antineoplastic properties to their ability to bind DNA [83]. Platinum-groove binder conjugates may interact with DNA differently to the platinum complex or the groove binder alone, and therefore a different spectrum of activity can be expected from this class of compounds. A bis(amine)dichloride-platinum(II) compound can be linked to either the C or the N terminus of the oligopeptides to yield targeted platinum compounds (**16**; Fig. 6) [82,84,85]. Much study for this type of complex is focussed on understanding the (altered) DNA interactions with respect to the parent drug cisplatin. Interestingly, it was found that in certain cases the groove-binder is targeted away from the minor groove by the platinum towards the major groove [84].

Intercalators are ligands with very high-DNA affinity. Not surprisingly, they have received considerable attention as targeting ligands for platinum complexes. By joining a platinum drug moiety to an intercalator via a flexible linker of variable length one can envisage the two interacting with DNA simultaneously and even synergistically. Many different DNA intercalators have been explored to this end, including acridine derivatives (**17**; Fig. 7) [86–98], phenantroline (**18**; Fig. 7) [99,100], phenazine [101,102] and anthraquinone (**19**; Fig. 7) [103]. Based on the crystal structure of acridine intercalated into DNA [104] one may envisage the platinum in acridine conjugates to either bind in the major groove (**17**; Fig. 7), or the minor groove (**20**; Fig. 7) of DNA. Some of these compounds show cytotoxic activities surpassing cisplatin. In particular, the 9-aminoacridine-4-carboxamides homologues show both good in vitro and in vivo activities (**20**; Fig. 7) [105–107]. The interaction of these compounds with chromosomal DNA in an intact cellular environment was studied and indeed a changed DNA-binding specificity with respect

to cisplatin was observed [108]. This suggests that the intercalator positions the platinum in such a way that otherwise kinetically unfavourable DNA adducts can be formed as the major product. Furthermore, the rate of reaction between the platinum and DNA is greatly increased through an appended intercalator ligand. These observations help to explain why these compounds show good activities in cisplatin-resistant cell-lines [109].

6. Concluding remarks and outlook

Of the vast amount of different targeted platinum drugs, which have been synthesised and tested, only a small number are currently under clinical development. These are mostly formulations of existing drugs rather than radically new drugs. Though this may be the result of conservative choices made at the pharmaceutical industries capable of developing drugs beyond the initial in vitro and in vivo biological evaluations, it also leads to the conclusion that cisplatin is in fact a very good and hard-to-beat drug. Nearly four decades after its serendipitous discovery by Rosenberg the drug is still among the best platinum anti-tumour compound known to man. The platinum drugs presented in this overview with specific targeting functionalities, show promising properties and encouraging biological results. To make the development of these compounds from the lab to the patient economically viable, however, they need to be many times better than cisplatin with respect to tumour specificity, reducing dose-limiting side-effects or making a larger range of malignancies curable, in particular those resistant to cisplatin. As the biochemical understanding of cancer increases, new cancer-specific targets are identified. The challenge which chemists

face is to combine this new knowledge with the ability to synthesise different types of targeted platinum complexes, in order to develop the much-needed improved therapies.

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