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Targeting and delivery of platinum-based anticancer drugs

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Platinum-based anticancer drugs occupy a crucial role in the treatment of various malignant tumours. However, the efficacy and applicability of platinum drugs are heavily restricted by severe systemic toxicities and drug resistance. Different drug targeting and delivery (DTD) strategies have been developed to prevent the shortcomings of platinum-based chemotherapy. These approaches can be roughly categorized into two groups; namely, active and passive tactics. Active DTD is realized through specific molecular interactions between the drugs and cell or tissue elements, while passive DTD is achieved by exploiting the enhanced permeability and retention effect in tumour tissues. The principal methods for active DTD include conjugation of platinum drugs with selective targeting moieties or encapsulation of platinum drugs in host molecules. Bioactive substances such as hormones, carbohydrates, bisphosphonates, peptides and proteins are commonly used in active DTD. Passive DTD generally involves the fabrication of functionalized polymers or nanoparticles and the subsequent conjugation of platinum drugs with such entities. Polymeric micelles, liposomes, nanotubes and nanoparticles are frequently used in passive DTD. In some cases, both active and passive mechanisms are involved in one DTD system. This review concentrates on various targeting and delivery techniques for improving the efficacy and reducing the side effects of platinum-based anticancer drugs. The content covers most of the related literatures published since 2006. These innovative tactics represent current state-of-the-art developments in platinum-based anticancer drugs.

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1 Introduction

Platinum-based anticancer agents are a mainstay of clinical drugs for the treatment of various solid tumors such as genitourinary, colorectal, and non-small cell lung cancers.[1–3] The leading anticancer drug, cisplatin, has been used for more than three decades in standard chemotherapy regimens either as a single therapeutic modality or in combination with other cytotoxic agents or radiotherapy.[4,5] However, platinum-based anticancer chemotherapy is associated with severe side effects because of poor specificity.[6] In the case of cisplatin, systemic toxicities like nephrotoxicity, neurotoxicity, ototoxicity, and emetogenesis inflict serious disorders or injuries on the patients during the treatment, which badly restrict its efficacy.[7–9]

In addition to systemic toxicities, the efficacy of cisplatin is often limited by the intrinsic and acquired resistance possessed by various cancers.[10] Multiple mechanisms have been proposed to elucidate the cellular resistance to cisplatin and its analogues in preclinical models.[11,12] The four representatives include: (i) decreased drug accumulation or increased drug efflux;[13] (ii) increased detoxification of the drug by sulfur-containing molecules within the cells;[14,15] (iii) enhanced repair and increased tolerance to DNA damage;[16] and (iv) changes in molecular pathways involved in the regulation of cell survival or cell death.[17] Since these mechanisms have been expounded in a series of reviews,[18–22] we shall skip the details here for brevity.

The disadvantages of cisplatin have created a sustained momentum for the improvement of platinum-based anticancer drugs.[23] Over the last 40 years, thousands of platinum complexes have been prepared in the hope of finding new drugs with a more tolerable toxicological profile and higher efficacy.[24,25] These efforts have brought five more drugs into clinical use, *i.e.* carboplatin, oxaliplatin, nedaplatin, lobaplatin, and heptaplatin, and about 10 other complexes are currently under clinical trials.[26–28] Each of the latecomers shows some qualities that are not revealed by cisplatin. For example, nedaplatin displays less nephrotoxicity and neurotoxicity than cisplatin and carboplatin,[29,30] and oxaliplatin demonstrates less toxicity and little or no cross resistance to cisplatin or carboplatin.[31] However, since most of

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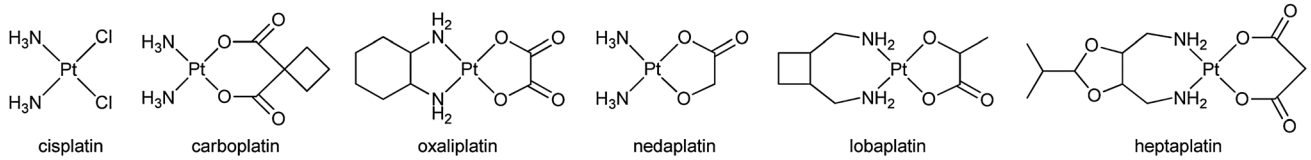
these drugs operate *via* a similar non-specific mechanism of action, some defects of cisplatin are consequently retained, albeit to a lesser extent. Thus, simple modification of the ligands seems unlikely to bring about a quality leap from an indiscriminative drug to a "magic bullet".



Systemic toxicity and drug resistance are the main concerns in current development of platinum anticancer agents. Ideally, future platinum drugs should attack exclusively cancerous cells without affecting normal ones, and enter the former more readily than the latter. However, this goal is virtually unattainable for such a complicated disease as cancer. Nonetheless, it is possible to approach the ideal situation by developing platinum-based prodrugs that are safe in the administered form but are cytotoxic within the cancer cells after being activated under certain conditions. Obviously, the realization of this ideal is determined by the tumour selectivity of platinum complexes.

Generally speaking, at least three options are viable in the design of new platinum drugs: (i) constructing complexes that display different DNA-binding modes; (ii) exploiting prodrugs that can be activated only in the tumour tissues; and (iii) improving drug accumulation at the tumour site by means of an accurate targeting and delivery strategy.[32] The first category comprises polynuclear platinum complexes, *trans*-platinum complexes, and monofunctional platinum complexes.[33] The second category includes complexes that exploit the unique features of solid tumours, such as acidic pH,[34] and hypoxic or reducing conditions. For instance, inert platinum(IV) complexes can be reduced to cytotoxic platinum(II) complexes with the loss of the two axial ligands under hypoxic conditions in the tumour tissue, and thus, act as prodrugs.[35,36] This review will focus on the third category, that is, ameliorating the selective accumulation of platinum drugs in tumour tissues through targeting and delivery techniques. Since many valuable reviews and books on this topic have appeared over the years,[37–46] the materials of this review are exclusively sourced from literature published since 2006.

2 Drug targeting and delivery

Drug targeting and delivery (DTD) represents a highly active field of research for drugs that can go straight to their biological targets as "magic bullets".[47] In comparison with traditional chemotherapy, targeted therapy for cancers has two major advantages: avoidance of damage to normal tissues, and restraint of drug resistance. In recent years, various DTD approaches have been developed in an attempt to minimize the systemic toxicity and drug resistance of platinum-based anticancer drugs.[48,49] The ultimate goal of these endeavors is to obtain platinum drugs that are highly selective for tumour tissues and can be administered at lower doses with fewer side effects and an improved therapeutic index.

DTD can be active or passive. Active DTD is based on the specific biomolecular interactions between drugs and cell or tissue elements. This approach can be applied to tumours containing biochemical entities whose quantity or functionality differs from those of normal tissues. In a typical active DTD system, the pharmacophore is bound to the targeting moiety

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via a spacer and a linker; the specific functionality of the transporter-, antigen- or receptor-based conjugate drives the drug towards the tumour tissue by virtue of its specific binding affinity.[50] Bioactive substances, such as hormones, sugars, amino acids, proteins, and bis-phosphonates, are commonly employed to fulfil the targeting function. Additionally, biodegradable molecules, such as polysaccharides, polyamino acids, proteins, and water soluble poly(ethylene glycol) (PEG), are adopted to perform the delivery function. The addition of targeting functionality to the drug makes it possible to distinguish cancerous cells or tissues form healthy ones, and thereby ensures the high efficacy and low side effects of the drug.[51]

Passive DTD is achieved by taking advantage of the enhanced permeability and retention (EPR) effect in tumour tissues.[52] Tumour vasculature is conspicuously disorganized and twisted as compared with the vasculature in normal tissues. The vascular endothelium in tumours proliferates rapidly and discontinuously, resulting in a higher number of fenestrations and open junctions than normal vessels, ranging from 200 nm to 1.2 μm.[53] Consequently, particles that are small enough, typically measuring on the order of a few hundred nanometers, can passively cross the tumour endothelial barrier through fenestrations. Moreover, the lack of a functional lymphatic network prevents the efficient removal of excess fluid from the solid tumour tissue. The combination of these two effects makes tumours hyperpermeable to the circulating macromolecules, which extravasate and accumulate in the solid tumour tissue because of inefficient drainage by the lymphatic system, and remain there for substantial periods of time (Fig. 1).[54] For platinum-based anticancer drugs, carriers used in passive DTD usually contain donor groups capable of coordinating with the platinum moiety.

The EPR effect is evident in a large number of tumour types; it is applicable for any biocompatible macromolecular compounds above 40 kDa. The drug concentration in tumour tissue can be 10–30 times higher than that in the blood. It is noteworthy that the EPR effect not only brings on the momentary passive DTD for tumour tissues, but also prolongs the drug retention for more than several weeks or longer.[55] In fact, to exert a substantial EPR effect, drugs need to circulate in the bloodstream for at least 6 h. With the help of the EPR effect, novel cancer-specific platinum drugs can be created by exploiting the properties of nanoparticles and macromolecules such as micelles and liposomes.

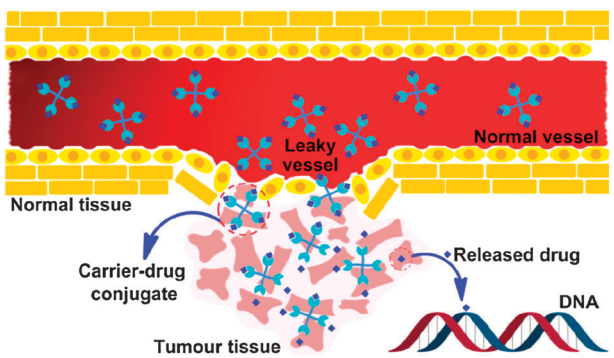


Fig. 1 Schematic representation of the EPR effect: normal vessels have a tight endothelium, while tumour vessels are disorganized and leaky, allowing preferential extravasation of circulating macromolecules. In tumour tissues, the carrier-drug conjugate is cleaved to generate the active platinum species, leading to the formation of cell-lethal DNA adducts.

The microenvironmental difference between normal and tumour cells plays a key role in some DTD systems to generate the active platinum species. Normal cells typically use mitochondrial oxidative phosphorylation to metabolize glucose and switch over to glycolysis only when there is little or no oxygen, producing lactate as a byproduct. By contrast, the metabolism of cancer cells is characterized by the increase in aerobic glycolysis and the dependency on the glycolytic pathway for ATP generation, known as the Warburg effect. Glycolysis is a series of metabolic processes by which one molecule of glucose is catabolized into two molecules of pyruvate, a net gain of two ATP, and two protons:[56]

$$\text{Glucose} + 2P_i + 2ADP + 2NAD^+ \rightarrow 2\text{Pyruvate} + 2ATP + 2NADH + 2H^+ + 2H_2O$$

The glycolytic reaction under hypoxic conditions results in a pH decrease in tumour tissues, and even leads to acidosis in the peritumoural region, so that the pH value in tumour tissues is often 0.5–1.0 units lower than that in normal tissues. Therefore, platinum conjugates showing reduced activity under normal physiological conditions (pH 7.4) could rearrange the ligands under the acidic conditions in the tumour tissue, leading to the generation of an active species. However, the prerequisite for this rearrangement is the presence of a pH-sensitive hydrolysable group in the spacer, and the pH-dependent stability of the carrier–drug bond should fit into the narrow window of 2–3 pH units.

3 Active targeting and delivery

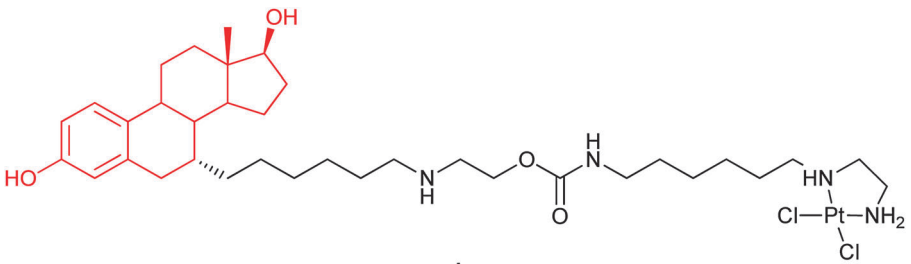
3.1 Estrogens as carriers

Estrogens can easily cross the cellular membrane by passive diffusion because of high lypophilicity, bind to the estrogen receptor (ER) in the cytoplasm and then be transferred to the nucleus, hence they are attractive carriers for the active DTD. Tissues and the corresponding cell lines that express ERs are defined as estrogen responsive ER(+). In addition to the classical estrogen receptor α (ERα), a new estrogen receptor β (ERβ) is also involved in the growth modulation of normal and malignant breasts and other tissues. ERα mediates the proliferative effect of estrogen in breast cancer cells, whereas ERβ seems to be antiproliferative. Since the distribution of the two ERs is not uniform in the target cells, the hormone derivatives could lead to either agonistic or antagonistic biological effects.[57]

ERβ is an interesting therapeutic target in breast cancer cells and ERβ-selective agonists are potential drugs for the therapy of ERα(+)/ERβ(+) breast cancer due to their antiproliferative and antiangiogenic properties.[58] Tissues rich in ERs, such as breast and ovarian cancers, tend to accumulate molecules that have high binding affinity for these receptors. Therefore, molecules that bind to the ER and have favorable cellular transport properties can be incorporated into platinum complexes to target these cancers.

For example, the biological affinity between 17β-estradiol and its cognate receptor can theoretically be used to direct a platinum agent to the target cells. Conjugates of platinum complexes with natural or synthetic estrogens and antiestrogens targeting the nuclear ERs have been reviewed recently.[59] These conjugates are targeted cytotoxic agents for ER-rich tissues such as hormone-dependent breast cancer.[60] At least two benefits are possible from attaching estrogen to platinum complexes: increasing cellular uptake of the drug, and sensitizing cells to the drug. The following examples are some new platinum complexes designed on such principles.

In complex **1**, a DNA damaging warhead, [Pt(ethylenediamine)Cl_2], is tethered through a carbamate-containing linker to the steroid residue. The ligand has 28% relative binding affinity for the ER as compared to 17β-estradiol. After covalent binding to a synthetic DNA duplex 16-mer, the affinity of **1** for the ER is still retained. Complex **1** shows higher cytotoxicity against the ER(+) ovarian cancer cell line CAOV3 than the control compound. It is also more toxic to the ER(+) MCF-7 than to the ER(−) MDA-MB-231 breast cancer cell lines. These results indicate that both the presence of the estradiol moiety in **1** and the expression of the ER in target cells contribute to the enhanced activity.[61]



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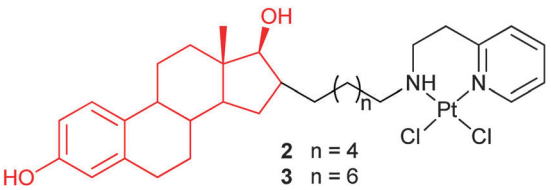
Complexes **2–5** are made of 17β-estradiol and a cisplatin analogue linked together by a 6–14 carbon atom chain. These complexes are 4–9 times more cytotoxic than cisplatin on the ER(+) MCF-7 and ER(−) MDA-MB-231 breast cancer cell lines. Among them, **4** exhibits a promising cytotoxicity towards all the tested cancer cells, while **2** shows the least cytotoxicity. This may be due to the short linkage which causes additional steric hindrance around the 17-hydroxyl group of the steroid, which is necessary for binding to the ER. As expected, these complexes display similar binding affinities for the ERα and ERβ, close to that of 17β-estradiol, and cisplatin shows no affinity for the ERs. Their strong cytotoxic activity may be partly attributed to their interactions with the ERα and ERβ. The length of the alkyl chain separating the estradiol from the platinum moiety does not seem to play a crucial role for the ER binding affinity and cytotoxicity. Unexpectedly, these complexes show no clear specific action on estrogen-dependent cells as compared to estrogen-independent cells *in vitro*. However, several *in vivo* assays with **4** on nude mouse xenografted model show strong and selective anticancer activity on hormone-dependent breast and ovarian cancers. Consequently, complexes **2–5** have the potential to target the ER *in vivo* and reduce the systemic toxicities.[62]

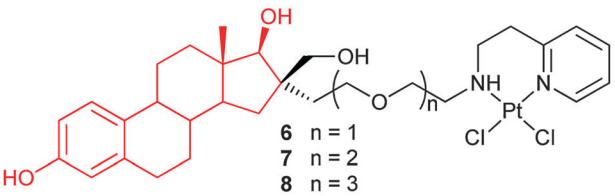
In analogues **6–10**, the alkyl linking chain is replaced with a PEG linking chain of various lengths. The length of the PEG chain does not influence the solubility of the complexes. The most active complex **10** contains 5 ethylene glycol units and is equipotent to cisplatin against breast cancer cell lines MCF-7 and MDA-MB-231. However, they also present no specific toxicity towards ER(+) MCF-7 cells *in vitro*.[63]

Estradiol-linked carboplatin and oxaliplatin analogues have also been prepared. Their antiproliferative activities on MCF-7 and MDA-MB-231 cell lines are at the micromolar range and more active than carboplatin and oxaliplatin alone but less active than their cisplatin counterparts. Oxaliplatin derivatives show a high affinity for ERα whereas carboplatin derivatives show a very low affinity for ERα, suggesting that the nature of the platinum(II) unit is important for the antiproliferative activity and estrogen-selectivity of the vector complexes.[64]

The cellular accumulation and inhibitory effect of several dichloro(6-aminomethylnicotinate)platinum(II) steroid conjugates have been investigated on ER(+)/ER(−) MCF-7 and MDA-MB-231 breast cancer cells. Complex **11**, with 3-*O*-linked estrogen, distinctly inhibits the growth of ER(+) MCF-7 cells, but has little if any effect on ER(−) MCF-7 cells. The same behavior of complex **12**

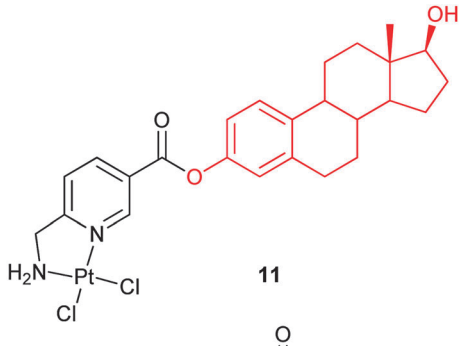


2 n = 4
3 n = 6
4 n = 9
5 n = 12



6 n = 1
7 n = 2
8 n = 3
9 n = 4
10 n = 5

with 17-*O*-linked androgen against ER(+) and ER(−) breast cancer cell lines is less effective. These complexes bind strongly to sex hormone-binding globulin, and **11** also binds strongly and agonistically to the nuclear ERα. The results suggest that the accumulation of **11** and **12** in ER(+) breast cancer cells is a receptor-mediated process. Interestingly, the most cytotoxic



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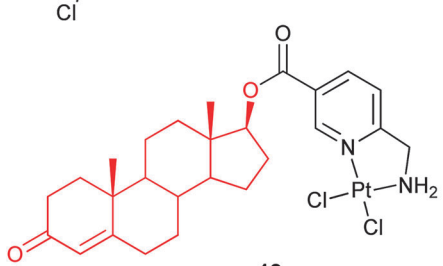

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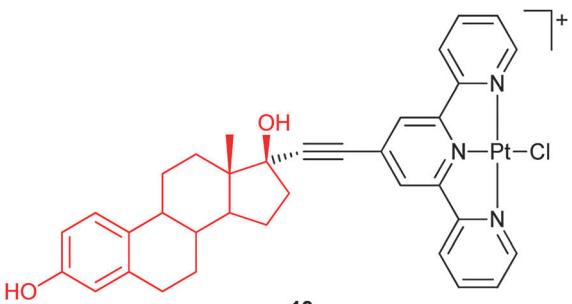
complex **11** exerts no DNA distorting effect, suggesting that its mode of action is different from that of cisplatin.[65]

Steroids are effective delivery vehicles, even for charged platinum complexes. In complex **13**, the ligand terpyridine is attached to the 17α-position on the estrogen *via* an ethynyl link. The aim of this design is to add the targeting quality of estrogen to the platinum moiety and thereby to increase the drug accumulation in tumour cells. A whole cell ER assay on ER(+) MCF-7 breast cancer cells confirms the binding of **13** to the ER. Complex **13** also binds to both human and bovine serum albumin (SA) and to DNA, in each case the biomolecule is linked to the platinum(II) center through coordination with the displacement of the labile chloride ligand. Circular dichroism indicates that a termolecular entity involving **13**, SA and DNA is formed.[66]



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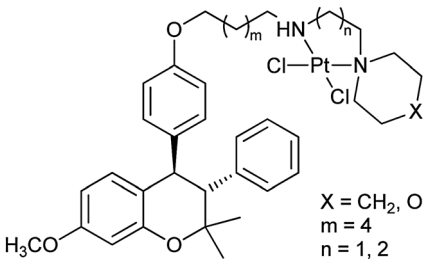
Nonsteroidal estrogens have also been used to selectively target mammary tumour cells.[67] For example, benzopyran-based platinum complexes such as **14** and **15** are synthesized as hybrids of selective ER modulators and cytotoxic anticancer agents. The diamminodichloroplatinum(II) group functions as the cytostatic moiety, whereas 3,4-diarylbenzopyran nucleus, well known for its affinity for ERs with selectivity for ERα, acts as the carrier moiety. The platinum(II) moiety is linked with the carrier through a linker of various lengths at a position corresponding to the 7α- or 11β-position of the 17β-estradiol. These complexes are expected to reach the target site efficiently and the possible free amine metabolite could act as a selective ER modulator. Actually, they show significant *in vitro* cytotoxic activity against different ER-dependent and -independent breast cancer cell lines. Furthermore, complexes from aromatic amines are more potent than their aliphatic amine analogues. However, the length of the linker chain has little effect on the biological activity. These complexes may potentially be used as cytotoxic agents and selective ER modulators in cancer treatment.[68] The selective ER modulator tamoxifen, a leading agent in the adjuvant treatment of breast cancer, has also been linked *via* a spacer to the platinum anticancer group, but no particular benefit is obtained.[69]



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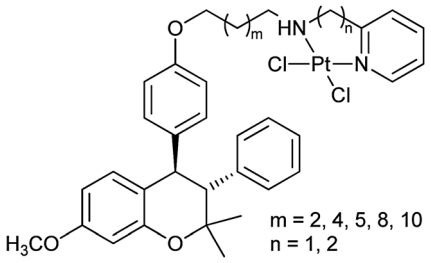

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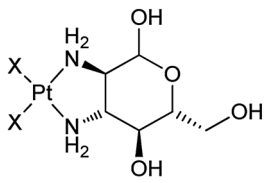
3.2 Carbohydrates as carriers

Carbohydrates such as mono- and polysaccharides are fundamental components of glycolipids and glycoproteins, and are building blocks of nucleotides. Cancer cells commonly display altered sugar metabolism, for instance, increased glucose consumption for energy production *via* glycolysis to survive in the profoundly hypoxic environment of malignant lesions. Therefore, carbohydrates can be exploited as carriers for platinum-based anticancer drugs. Natural carbohydrates and synthetic derivatives possess manifold donors endowing them with the ability to coordinate with metal centers and provide some advantages over other ligands, for example, biocompatibility, membrane permeability, non-toxicity, enantiomeric purity, and water solubility. More importantly, the existence of specific saccharide receptors exclusively expressed by some cancer cells could enhance the specificity and recognition of platinum drugs. In recent years, a number of platinum complexes with carbohydrates suitably functionalized with mono- and bidentate amine ligands, phosphines or other P-donor groups, S- or Se-donor ligands or alcohols have been synthesized, and some of them are promising anticancer drugs.[70]

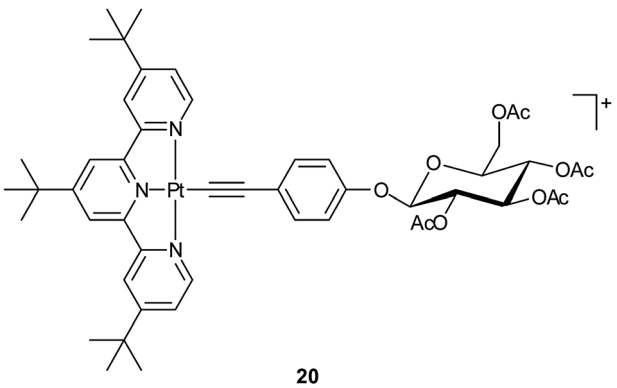
3.2.1 Monosaccharide. Aminosugar 2,3-diamino-2,3-dideoxy-D-glucopyranose can be used to form oxaliplatin-like platinum complexes with different leaving groups. This is exemplified by complexes **16–19**, in which the carbohydrate moiety is bound to

the platinum center *via* amino groups. The *in vitro* cytotoxicity of these complexes has been determined in human cervix carcinoma HeLa, ovary carcinoma CH1, colon carcinoma SW480, and osteosarcoma U2OS cell lines. In general, these complexes are moderately cytotoxic, with IC_{50} values being in the micromolar range. The most active chlorido complex **16** is one to two orders of magnitude less cytotoxic than oxaliplatin, but comparable to carboplatin in two of the four cell lines. Complex **16** binds to dGMP in a similar way to cisplatin, whereas the iodido complex **17** reacts at a much lower rate, probably due to slower hydrolysis. The reactivity of complexes **18** and **19** to dGMP is lower than that of **16** and **17** due to the stability of the dicarboxylato ligands, which partially corresponds to their cytotoxicity. These complexes may share a similar mechanism of action, probably forming the same carbohydrate–Pt–DNA adducts, whereas the nature of the leaving groups only affect the reaction kinetics. However, a correlation between cytotoxicity and DNA binding ability is only observed in SW480 and U2OS cells. The results indicate that the binding of DNA is not the only determinant of the cytotoxicity. As the glycolytic energy production of cells can only be exploited properly in living organisms, *in vivo* tests are needed to reveal the real potential of these complexes.[71]



16 X = Cl
17 X = I
18 X = oxalato
19 X = malonato

A water-soluble [Pt(terpy)(glycosylated arylacetylide)]$^+$ complex **20** shows up to ~100 times higher cytotoxicity against human cancer cell lines than cisplatin, and is at least 8-fold more cytotoxic than [Pt(terpy)Cl]$^+$. It is approximately 10-times more active than the non-glycosylated analogous complex. Judged from the morphology and cell membrane integrity, complex **20** induces 52% apoptosis with only 5% necrosis in the large cell lung cancer cells (NCI-H460). Besides, treatment of NCI-H460 cells with **20** results in significant alteration in 111 types of gene expressions. The Pt(II)-glycosylated arylacetylide moiety remains intact in aqueous solution for 72 h at room temperature. This organometallic compound represents a rare example of platinum complexes linked to sugar molecules exhibiting lower IC_{50} values than cisplatin in cell culture studies.[72]



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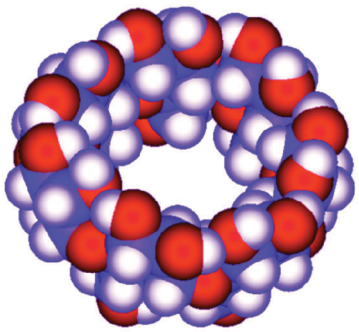
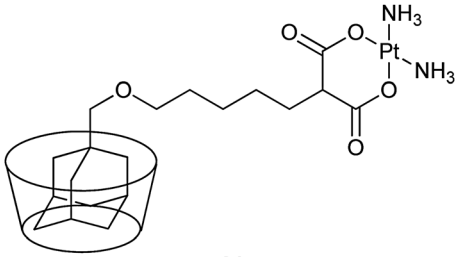


Fig. 2 Molecular model of β-cyclodextrin.

3.2.2 Oligosaccharide. Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides typically constituted by 6–8 glucopyranoside units, taking the shape of toroids with the larger and the smaller openings exposing to the solvent. Although the interior of the toroids is not hydrophobic, it is considerably less hydrophilic than the aqueous environment and thus able to host other hydrophobic molecules. In contrast, the exterior is sufficiently hydrophilic. CDs have attracted much interest in pharmaceutical applications because their inclusion compounds can greatly modify the physical and chemical properties of the guest molecule, particularly water solubility. In most cases, the release of the bioactive species from the inclusion compounds is mediated by pH changes or enzymes, leading to the cleavage of hydrogen or ionic bonds between the host and the guest molecules, or the cleavage of α-1,4 linkages between glucose monomers. βCD is made up of seven sugar ring molecules (Fig. 2). Similar to other hydrophilic and membrane-impermeant molecules, βCD can be internalized by mammalian cells *via* pinocytosis and delivered to late endosomes/lysosomes. Mammalian cells lack the enzymes for degradation of CDs, so CDs should remain intact.[73] This suggests the possibility of enhancing the cellular uptake and potency of platinum-based drugs by noncovalent modification with βCD.

In complex **21**, an adamantane-tethered carboplatin analogue has been encapsulated in the cavity of βCD. This inclusion complex exhibits higher cytotoxicity towards human neuroblastoma SK-*N*-SH cells and a higher binding to plasmid pBR322 DNA than carboplatin. However, the cellular uptake rate of carboplatin is about 4 times higher than the inclusion complex. The result suggests that the higher cytotoxicity of **21** is not related to the amount of platinum that enters the cell, but perhaps to the more effective transport of **21** to the nucleus *via* the appended βCD moiety and thereby more efficient binding to nuclear DNA. Factors such as intracellular transport, receptor binding, and heterogeneous distributions inside cells seem to play more important roles than just the cellular uptake for the cytotoxic effect of the drug.[74] Water-insoluble *trans*-dichloro(dipyridine)platinum(II) is difficult to be used in biological settings. The encapsulation of this complex with βCD increases its solubility to 1.6 mg mL^{-1}. Moreover, the cytotoxicity of the inclusion complex **22** *in vitro* is much higher than that of *trans*-dichloro(dipyridine)platinum(II) and cisplatin against CT26 colon carcinoma and B16F10 melanoma cell

lines.[75] These examples demonstrate that βCD is an effective carrier for improving the DNA binding activity and other pharmaco-properties of platinum-based anticancer drugs.



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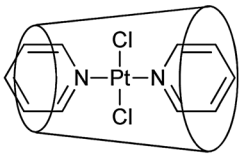

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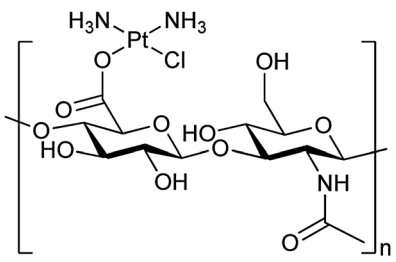
3.2.3 Polysaccharide. Polysaccharides are among the most common carriers in the synthesis of drug-conjugates. Polysaccharides such as hyaluronic acid (HA) have been employed to form microspheres with high loading of platinum drugs. HA is a polymer of disaccharides composed of D-glucuronic acid and D-*N*-acetylglucosamine units, linked *via* alternating β-1,4 and β-1,3 glycosidic bonds, with molecular weight ranging from 5000 to 20 000 000 Da *in vivo*. Increased presence and uptake of HA have been correlated with the progression and metastasis of prostate and breast cancers.[76,77] The carboxyl groups on HA are appropriate for the binding of platinum units. For example, HA could form stable conjugate with cisplatin.[78] Since breast cancer cells are known to have greater uptake of HA than normal tissues, and invasive breast cancer cells overexpress CD44, the primary receptor for HA, and are dependent on high concentrations of CD44-internalized HA for proliferation,[79] cisplatin–HA conjugates may be efficacious against lymphatic metastases.

The tissue distribution and anticancer activity of a cisplatin–HA conjugate **23** have been tested to determine whether the targeted cisplatin can increase the localized dose in lymphatic metastases without systemic toxicities. Conjugate **23** releases drug with a half-life of 10 h in saline and shows high anticancer activity *in vitro* similar to cisplatin in highly metatstatic MCF-7 and MDA-MB-231 human breast cancer cells. In addition, the conjugate is well tolerated in rodents with no signs of injection site morbidity or major organ toxicity after 96 h. Fluorescence imaging confirms the accumulation of **23** in the lymph nodes.[80]



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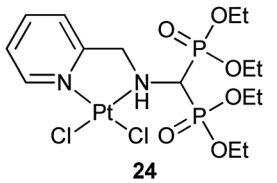
This is the first *in vivo* study of cisplatin–HA conjugate and the first examination of HA-drug conjugates designed for lymphatic deposition and retention.

Recently, oxaliplatin has been conjugated to HA-coupled chitosan nanoparticles for targeted delivery to colorectal tumours. In murine models, the drug delivery system results in relatively high local drug concentration in colonic tumours with prolonged exposure time, reflecting its targeting potential with enhanced antitumor efficacy and low systematic toxicity.[81]

3.3 Bisphosphonates as carriers

Bisphosphonates (BPs) have shown a high affinity for bone and other calcified tissues, and have widely been used as therapeutic agents for several bone-related diseases.[82] The ability to chelate calcium ions is the basis for the bone-targeting property of BPs. Since BPs can be absorbed onto bone surfaces and show significant inhibition to osteoclastic resorption or antitumor effects in preclinical models,[83] they are potential bone-targeting carriers for platinum drugs.

In our attempt to seek more specific platinum-based anticancer drugs, analogues of picoplatin (ZD0473), a developing drug candidate at Phase III stage, are linked to a bisphosphonate tetraethyl ester targeting carrier. The resultant complexes **24–27** exhibit excellent solubility in both organic and aqueous solutions. In cytotoxicity assay against human osteosarcoma MG-63 and ovarian cancer COC1 cell lines, complexes **25** and **27** demonstrate much higher activity than **24** and **26**, which can be correlated to the length of the linkers between the platinum moiety and the targeting bisphosphonate ester. The apoptotic assay with the most cytotoxic complex **27** reveals a different mode of cell death compared to cisplatin. In accordance with this, complexes **24–27** hardly bind to DNA, which is again very different from cisplatin. Thus, these complexes represent a new class of non-classical platinum anticancer agents with promising bone-targeting property.[84]



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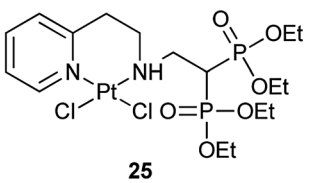

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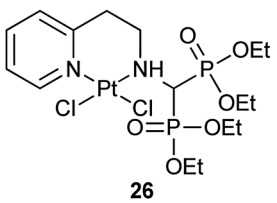

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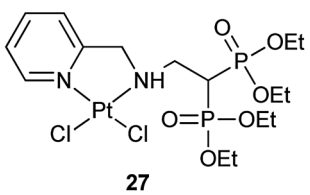
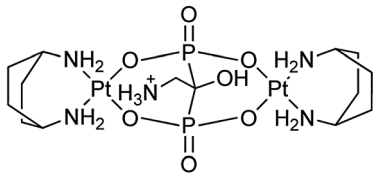


27

A series of dinuclear platinum(II) complexes with bridging geminal BPs have been reported as prodrugs with potential activity at the bone surface after embedment in inorganic matrices and implantation at the tumour site.[85,86] For example, complexes **28** and **29** show a cytotoxic activity with mean IC_{50} values of 22.46 and 13.57 μM, respectively, towards a panel of 13 human tumor cell lines. More impressively, they are able to

overcome the cisplatin-resistance with mean resistance factors of 0.92 for A431 and A431/Pt cervical carcinoma cells and of 0.93 for 2008 and cisplatin-resistant C13* ovarian adenocarcinoma cells, which are roughly 3 and 15 times lower than those calculated for cisplatin in cervical and ovarian carcinoma cells, respectively. Complexes **28** and **29** can also circumvent the multi-drug resistance in the colorectal cell line pair LoVo/LoVo-MDR, with a resistance factor of 1.25.[87] Nevertheless, BPs attached to a platinum center as leaving groups are likely to be detached during the delivery in physiological conditions, leading to the loss of bone-targeting property.



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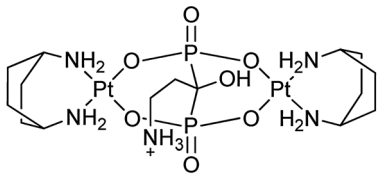

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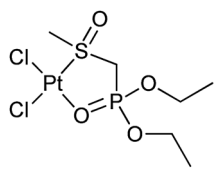
In addition to the bone-targeting ability, some BP-tethered platinum complexes also show an inhibition effect against matrix metalloproteinases (MMPs), which are zinc-dependent endopeptidases mediating the homeostasis of the extracellular matrix and are upregulated in almost every type of human cancers. MMPs are expressed mainly by cancer cells and their overexpression has been correlated with tumour progression; therefore, they are attractive therapeutic targets.[88] Platinum complexes **30** and **31** with diethyl[(methylsulfinyl)methyl]phosphonate as the carrier ligand have been proved to selectively inhibit MMP-9, -3, and -12 through a noncompetitive mechanism. In contrast, cisplatin, carboplatin, and the ligand are inactive. The growth inhibitory effects of **30** and **31** are markedly lower than those of cisplatin and carboplatin toward cisplatin-sensitive A2780 ovarian cancer cells, and maintain their activity toward cisplatin-resistant A2780cisR cells. These results demonstrate that BP-modifications to platinum complexes can be exploited to target biological substrates distinct from DNA.[89] Such a non-DNA-binding mode is also observed in platinum-pyrophosphato complexes.[90]



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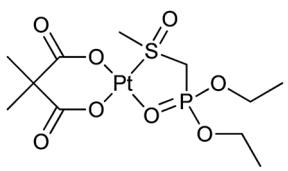

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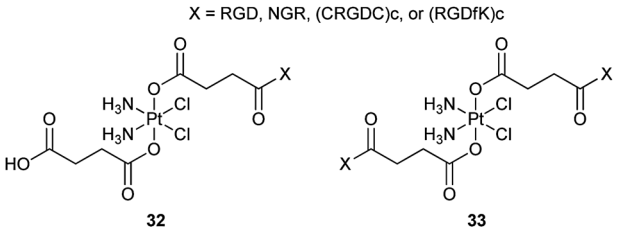
3.4 Peptides and proteins as carriers

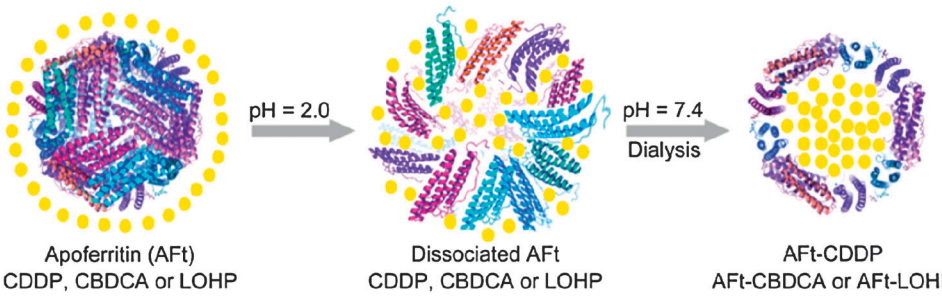
Tumour growth and metastasis are driven by angiogenesis; meanwhile, the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins and aminopeptidase N (APN) are upregulated in endothelial cells, thus, these cell surface proteins may serve as targets for chemotherapy. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ and APN can recognize the peptide motifs RGD (Arg-Gly-Asp) and NGR (Asn-Gly-Arg), respectively. Therefore, peptide motifs containing RGD, NGR, cyclic pentapeptide (CRGDC)c or (RGDfK)c have been appended to a series of mono- and difunctionalized platinum(IV) complexes **32** and **33** to specifically target the tumour vasculature. Cyclic peptides are chosen because they target angiogenic endothelial cells more efficiently compared to their linear counterparts. Cell inhibition assays show that the Pt(IV)–RGD conjugates are highly and specifically cytotoxic to cell lines containing the integrins, approaching the activity of cisplatin; the Pt(IV)–NGR conjugates are less active than Pt(IV)–RGD complexes but are more active than the nonspecific Pt-peptide controls.[91] These results suggest that some surface-protein-recognizable peptide motifs could be exploited as targeting devices for selective delivery of platinum-based anticancer drugs.



32 **33**

Chlorotoxin (CTX) is a 36-amino-acid peptide with four disulfide bridges. This peptide binds preferentially to glioma cells and many non-glioma tumour cell lines derived from lung, prostate, and melanoma cancers, but not to normal non-transformed cells. Thus, CTX could be integrated into platinum complexes as a targeting agent for the tumour-specific chemotherapy. In order to deliver cisplatin selectively to cancer cells, a conjugate structurally similar to **32** (X = CTX) was prepared recently. Like most platinum(IV) derivatives, the cytotoxicity of the conjugate is lower in cell cultures than that of cisplatin, but greater than those of its Pt(IV) precursor and CTX in several cancer cell lines.[92]

The native iron-storage protein ferritin (Ft) could be a promising vehicle for the active DTD since the binding sites and endocytosis of Ft have been identified in some tumour cells, and the internalization is associated with membrane-specific receptors. Ft receptors have shown potential value in the delivery of anticancer drugs to the brain. Ft can be easily demineralized into apoferritin (AFt), a hollow protein cage with internal and external diameters of 8 and 12 nm, respectively. This protein cage can be employed to deliver platinum drugs, which may enhance the drug selectivity for cell surfaces that express Ft receptors. On these grounds, we developed an active, as well as a passive, DTD system using AFt to improve the specificity of platinum drugs. Cisplatin, carboplatin, and oxaliplatin have been successfully encapsulated in the cavity of AFt. The encapsulation was achieved through manipulating the pH-dependent unfolding–refolding process of AFt at pH 2.0 and 7.4, respectively, in saturated drug solution (Scheme 1).[93] The structural

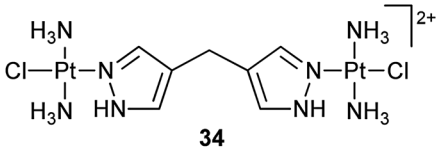


Scheme 1 Schematic illustration of the pH-mediated encapsulation of cisplatin (CDDP), carboplatin (CBDCA), or oxaliplatin (LOHP) by apoferritin (AFt) *via* an unfolding–refolding process.

integrity of the protein shell remains intact after encapsulation and hence the potential recognition nature should not be affected. *In vitro* assays on the rat pheochromocytoma PC12 cell line show that AFt–cisplatin inhibits the cells in a slow but sustaining mode and the cellular uptake of platinum is enhanced by AFt.[94] These protein-coated drugs are expected to improve the toxicity profiles of the naked ones and finally to overcome the detrimental effects of platinum-based drugs.

3.5 Cucurbiturils as carriers

Cucurbit[*n*]urils (*n* = 6, 7, 8) have become the most attractive macrocycles used for encapsulating platinum anticancer drugs in recent years.[95] Such systems have shown particular potential as protective carriers in drug delivery. Cucurbit[*n*]urils contain two symmetrical hydrophilic carbonyl lined portals, capping a central hydrophobic cavity, thus imparting an amphipathic nature to the macrocycles (Fig. 3). The hydrophobic inner cavity provides a favorable binding site for non-polar molecules, while the carbonyl units of the macrocycles are sites for hydrogen bonding and electrostatic interactions with cationic moieties.[96] These barrel-shaped molecules could be used as molecular hosts for neutral and charged mono- and multinuclear platinum anticancer agents.[97–99]



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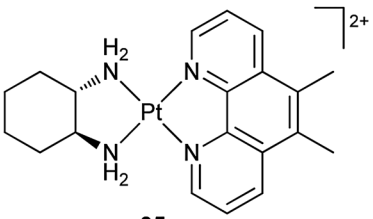


35

Partial or full encapsulation within cucurbit[*n*]urils creates steric hindrance to drug degradation by peptides and proteins, and allows for the tuning of drug release rates, cytotoxicity and toxicity.[100] For example, dinuclear platinum complex **34** has been included inside a cucurbit[7]uril macrocycle. Its cytotoxicity against the L1210 cell line and the corresponding cisplatin-resistant L1210/DDP sub-line is not affected heavily by the encapsulation, but the reactivity of the platinum center is reduced at least 3-fold.[101] Complex **35** is a DNA intercalator that displays cytotoxicity against a panel of human cancer cell lines, with some activity significantly higher, *e.g.* up to 100-fold greater in the L1210 and A-427 cell lines, than that of cisplatin. Partial encapsulation of **35** by cucurbit[6]uril barely changes its cytotoxicity;[102–105] however, such encapsulation by cucurbit[*n*]-urils (*n* = 6, 7, 8) can drastically reduce the deactivation by glutathione.[106] The size of the cavity and the binding affinity are closely relevant to the cytotoxicity, in that small changes of the size could either decrease or increase the activity (up to 2.5 fold) of the platinum complexes. The decrease in activity may result from the protective effects of the macrocycles on the encapsulated complexes. Nevertheless, *in vitro* results may not be sufficient to determine the fate of the encapsulated complexes. Recently, it is demonstrated that although the encapsulation of cisplatin in cucurbit[7]uril exhibits no effect on the *in vitro* cytotoxicity of cisplatin in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant sub-lines A2780/cp70 and MCP1, a significant effect on the *in vivo* cytotoxicity is observed using human tumour xenografts, in that both A2780 and A2780/cp70 tumours are sensitive to the host–guest complex. The total concentration of the circulating complex over a period of 24 h is significantly higher than that of free cisplatin when administered at the equivalent dose, implying that the improved pharmacokinetics plays a key role in overcoming the drug resistance.[107]

In some cases, encapsulation by cucurbit[*n*]urils can significantly affect the cytotoxicity and limit the water solubility of platinum complexes.[108] For these reasons, a number of other macrocycles such as β-cyclodextrin (*vide ante*) and calix[4]arene are investigated as potential alternatives. For instance, encapsulation of **35** in these macrocycles increases its stability to glutathione threefold, but shows no significant enhancement of the cytotoxicity against the LoVo human colorectal cancer cell line.[109] Similar result is also observed for **34** after such encapsulation.[110]

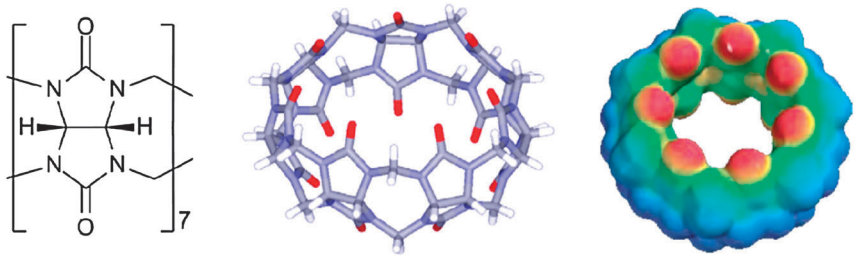
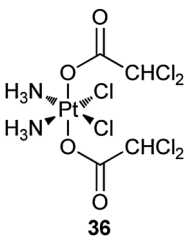


Fig. 3 Chemical structure (left), X-ray crystal structure (middle) and electrostatic potential map (right) of cucurbit[7]uril.

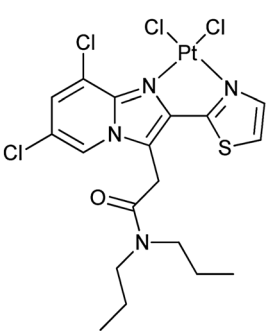
3.6 Other alternative carriers

Dichloroacetate (DCA) can reverse the Warburg effect (*vide ante*) by inhibiting a key enzyme, pyruvate dehydrogenase kinase (PDK), for the process, thereby causing cancer cells to commit suicide by apoptosis.[111] In complex **36** (Mitaplatin), two DCA units are appended to the axial positions of a platinum(IV) center. After crossing the plasma membrane, **36** is reduced to release the active drugs cisplatin and DCA. DCA inhibits mitochondrial PDK while cisplatin enters the nucleus to form 1,2-intrastrand d(GpG) DNA cross-links. By this unique mechanism, the complex attacks both nuclear DNA and mitochondria. Complex **36** is equally or more cytotoxic than all known platinum(IV) complexes and is comparable to cisplatin in a variety of cancer cell lines, but is nontoxic in normal cells. These properties demonstrate that a DCA-modified platinum(IV) complex is only effective in cancer cells and therefore could be an alternative avenue for active DTD of platinum anticancer agents.[112] A recent study indicates that **36** induces more apoptosis in cisplatin-resistant human epidermoid adenocarcinoma KB-CP 20 and hepatoma BEL 7404-CP 20 cancer cells compared with cisplatin on an equal molar basis, accumulates more than cisplatin in these cells due to enhanced transmembrane permeability, and shows special targeting to mitochondria. As a result, **36** is able to circumvent cisplatin resistance *via* the dual mechanism.[113]



36

Peripheral benzodiazepine receptor (PBR) is overexpressed in many tumour types, such as brain, liver, breast, and ovary cancers, with its overexpression grade correlating with the malignancy of the tumour. Thus, PBR-binding ligands have been widely explored as carriers for receptor-mediated drug delivery. For example, complex **37** contains a ligand with specific affinity for PBR; as a result, it possesses high affinity and selectivity for the PBR, which makes this compound a potential selective drug for tumours.[114] Complexes **38** and **39** have a similar carrier ligand with a nanomolar affinity for PBR. *In vitro* studies on human and rat glioma cells show that **38** and **39** keep high affinity and selectivity for PBR (nanomolar concentration) and are as cytotoxic as cisplatin. Moreover, they appear to be equally active against cisplatin-sensitive and -resistant A2780 cells. Similar to cisplatin, these complexes induce apoptosis but show a favorable 10- to 100-fold enhanced accumulation in the glioma cells.[115]



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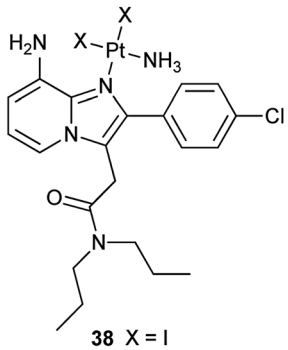
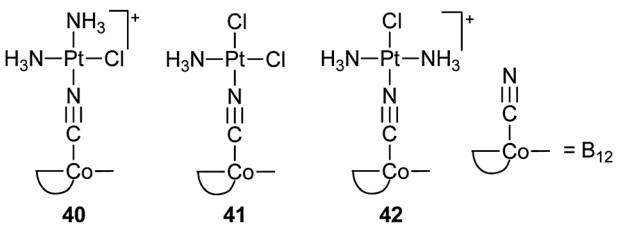


38 X = I
39 X = Cl

Fast proliferating cells require a higher amount of vitamin B_{12} than normal cells; therefore, vitamin B_{12} is an attractive DTD carrier for platinum complexes to enhance the tumour accumulation *via* the receptor-mediated uptake system. For example, the cyanide group in vitamin B_{12} can coordinate to various square-planar Pt^{II} complexes, forming the central $\{B_{12}–CN–Pt^{II}\}$ motif. These $\{B_{12}–CN–Pt^{II}\}$ conjugates are still recognizable by the intracellular adenosylation enzyme. Release of the platinum complexes from the conjugates is driven by the intracellular reduction of Co^{III} to Co^{II} to Co^{I} and subsequent adenosylation catalyzed by the adenosyltransferase. Thus, $\{B_{12}–CN–Pt^{II}\}$ conjugates can be considered as prodrugs suitable for targeted delivery of platinum complexes.[116] However, preliminary *in vitro* cytotoxicity studies using the $\{B_{12}–CN–Pt^{II}\}$ conjugates **40–42** indicate that they are less active than cisplatin against the human breast carcinoma cell line MCF-7 and human ovarian cancer cell line A2780, probably owing to a low uptake of the conjugates.[117]



40 **41** **42**

Cisplatin is usually administered intravenously as a short-term infusion. This yields a high drug concentration in the injection area and quick distribution in the body, leading to high local and systemic toxicity. Carbonated hydroxyapatite crystals (HACs) are similar to the porous structure in bones and hence, can be used to deliver cisplatin. This method has resulted in tumour inhibition and lower systemic toxicity. Cisplatin is adsorbed in the crystals instead of being included as solids. The adsorption depends on the physical and chemical properties of HACs such as the composition, the morphology, the surface area or the size; while the release of the drug depends on temperature, chloride concentration in the medium and crystallinity of HACs. Lower crystallinity leads to higher adsorption and slower release, and temperature slightly increases the drug release rate. The shape of HACs is important for the adsorption and desorption of cisplatin. Although both plate- and needle-shaped HACs have similar Ca/P bulk ratios, the surface areas and Ca/P surface ratios are different. The lower amount of calcium in the surface of needle-shaped HACs allows easier loading of the positively charged aquated platinum species. However, cisplatin release is the same for both shapes.[118]

4 Passive targeting and delivery

4.1 General concerns

Polymer-drug conjugates are emerging as an important class of anticancer nanomedicines,[119] particularly as potential passive DTD systems for platinum-based anticancer drugs.[120] Advantages of polymer-drug conjugates include: (i) enhanced cellular uptake because of the EPR effect and dynamic endocytosis characteristic of tumour cells;[121] (ii) prolonged circulation time in blood vessels and drug retention time in tumours; and (iii) high drug loading capacity and water solubility. Besides, polymer-drug conjugates can be modified with targeting moieties to actively target the tumour cells or vasculature.[122] These properties are helpful in overcoming the multidrug resistance and systemic toxicities that are inherent in current platinum anticancer drugs.

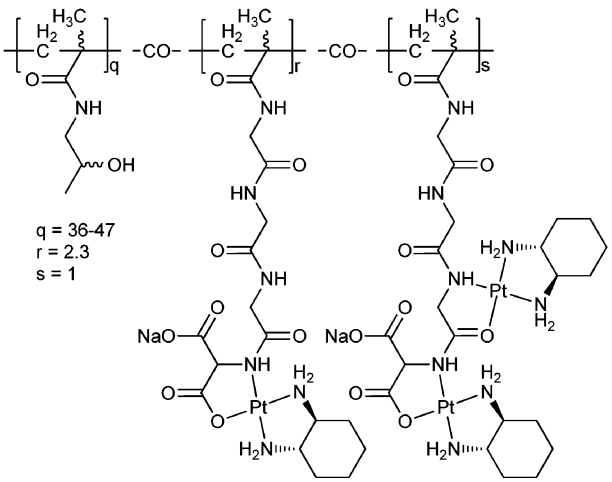
Both natural and synthetic polymers could be used as polymeric carriers for the delivery of platinum drugs.[123] Polymeric carriers used for DTD should be biodegradable and nontoxic,[124] commonly contain binding groups, and may also contain solubilising and targeting units. The binding groups can form covalent links with platinum moieties, and the solubilising and targeting units make the polymers more bioavailable and specific for cancers.[125] Targeting units could be antibodies, proteins, peptides or other small molecules.[126] In a typical polymer-platinum conjugate, the platinum moiety is linked to the polymeric backbone by a cleavable spacer, and a solubilising or hydrophilic group and a cellular targeting moiety may be attached at different points on the polymer backbone. The polymer-platinum linker must be stable during transport, but capable of releasing the platinum moiety at the therapeutic target. In general, the cleavage of the spacer is accomplished by some enzymes up-regulated in the tumour environment or by pH-sensitive hydrolytic reactions.

In addition to polymer-drug conjugates, passive DTD systems for platinum anticancer drugs also involve nanoparticulate drug delivery systems such as polymeric micelles, liposomes, lipids, dendrimers, nanospheres, and nanoparticles.[127–129] Compared to polymer-drug conjugates with size generally around 10 nm or less, the size of these systems is typically in a range of 20–300 nm.[130] Ideally, the size of an engineered particle should be in the range of 100–200 nm in diameter. Particles over 300 nm are rapidly recognized and sequestered by the reticulo-endothelial system, resulting in either poor drug accumulation at the target site or a short circulation half-life.[131] Examples of approved polymers include poly(ethylene glycol) (PEG), poly-(vinylpyrolidone) (PVP), poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA), and poly(ethylene oxide) (PEO).[132]

4.2 Polymer-drug conjugates

Polymer-platinum conjugates are formed between a polymer with suitable donor groups and a platinum moiety through coordination bonds. Polymers such as poly(amino acids), poly(amidoamine) dendrimers, and PHPMA are generally used as carriers because they contain inherent, pendant or terminal ligating groups.

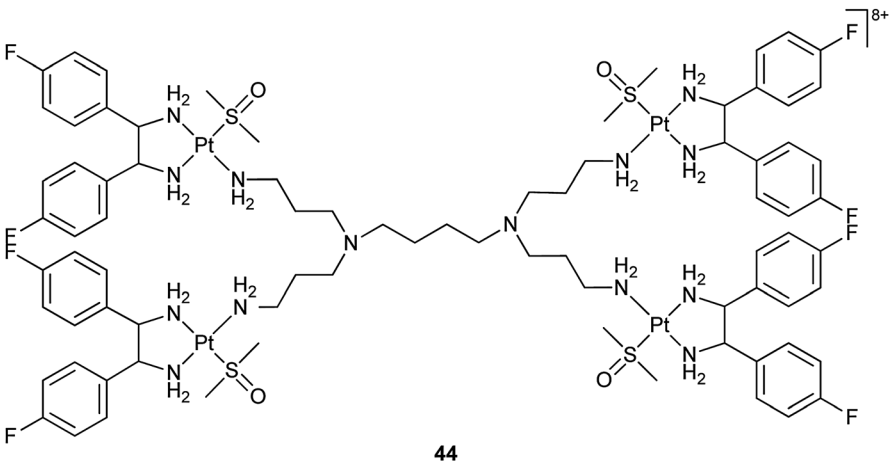
So far, PHPMA is one of the most successful polymers that have been used to construct polymer-platinum conjugates. One of such conjugates, **43** (AP5346), have entered clinical trials. In **43**, 1,2-diaminocyclohexaneplatinum(II) (DACHPt) moiety, a fragment of oxaliplatin, is bound to a hydrophilic biocompatible polymer with pH-sensitive triglycine side chains and an aminomalonic acid terminal group.[133] The conjugate is much more effective than oxaliplatin with exceptional tolerability in a large number of murine tumour models. The greater tolerability is attributed to the improved drug delivery toward the tumour, in that the platinum release rate of **43** is only 3.5% in 24 h in buffer at pH 7.4, but it is 7-fold higher in a slightly acidic environment (pH 5.4).[134] This means that **43** is not active until it reaches the tumour tissues where the environment is more acidic than that of normal tissues. In fact, **43** is capable of delivering 16-fold more platinum to the tumour than oxaliplatin, and 14-fold more platinum-DNA adducts are formed in the nucleus of tumour cells when it is administered at a dose of toxicity equal to that of



43

oxaliplatin. Moreover, at least five times more diaminocyclohexane-platinum units could be administered to patients with **43** compared to oxaliplatin.[135] Phase II results indicate that a clinically effective stabilization of disease has been achieved with **43**, and there is no indication of the acute neurotoxicity associated with oxaliplatin. Sustained levels of activity are also observed in patients with known resistance to platinum drugs.[136] Compound **43** has progressed through phase I trials and a phase II study in patients with recurrent ovarian cancer has been completed under the commercial name of Prolindac™.[137]

Dendrimers are highly branched tree-like polymers with multiple end groups. The functional groups on the surface of a dendrimer can be used to link platinum drugs *via* a cleavable chemical bond. Besides, attachment of various moieties to the backbone can provide targeting and other properties to the dendrimer. Poly(amidoamine) dendrimers are commercially available and have been investigated as potential drug delivery systems for several drugs including cisplatin.[138] A interesting example is the relatively simple polyimine dendrimer complex **44** in which 4 platinum(II) moieties are bonded to the linker *N*,*N*,*N*′,*N*′-tetrakis(3-aminopropyl)butane-1,4-diamine. This complex strongly binds to human serum albumin by hydrophobic and electrostatic interactions. It shows a 20-fold higher cellular uptake and a *ca.* 700-fold higher DNA binding than cisplatin in MCF-7 breast cancer cells without serum medium. The complex crosses the cell membrane through a passive transport and the polyimine dendrimer seems to serve as a carrier for the shuttling of platinum into the cell nuclei. As a result, **44** exhibits a relatively high cytotoxicity in MCF-7 cells.[139] Mechanistic studies on the cellular uptake with a dinuclear analogue of **44** suggest that the drug accumulation in the MCF-7 breast cancer cells is caused by macropinocytosis, which is only expressed shortly in normal cells, but is responsible for increased motility and metastasis in cancer cells. Thus, such polynuclear platinum complexes may target cancer cells more selectively than conventional platinum drugs.[140]



44

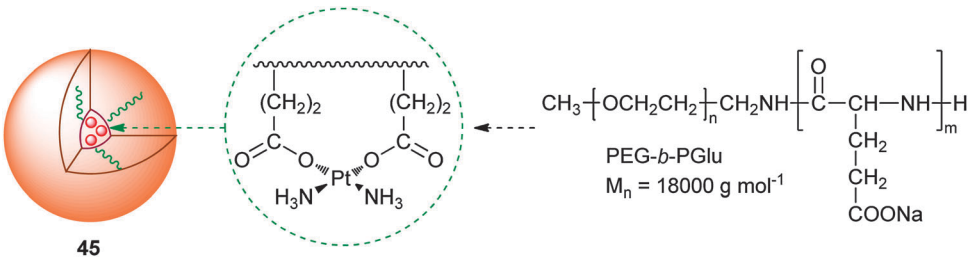
Hyperbranched polyglycerols (PG2) and aliphatic polyesters (H40) based on 2,2-bishydroxymethylpropionic acid are commercially available and have many hydroxyl terminal groups. Both PG2 and H40 have been modified with carboxylic acid groups capable of acting as ligands for platinum complexes derived from cisplatin. An advantage of these polyethers and polyesters over dendrimers is their easy availability. Modified PG2 form strongly bound platinum complexes and give controlled release of the drug over 7 days; while modified H40 forms a higher proportion of weakly bound platinum complexes and give similar release profiles in both water and saline. Modified PG2 appears to be more suitable as a drug delivery carrier for cisplatin.[141]

4.3 Polymeric micelles

Polymeric micelles are expected to enhance the accumulation of platinum drugs in tumour tissues by the EPR effect. Typically, platinum drugs are incorporated into the inner core of polymeric micelles by chemical conjugation or physical entrapment with relatively high stability. To prevent the micelles passing through normal vessels, their size can be controlled within the range of 20–100 nm, which is helpful in reducing the side effects of platinum anticancer drugs. Polymeric micelles could protect platinum complexes from degradation and achieve controlled release in the delivery, substantially improve the efficacy of platinum-based chemotherapy.

A representative example of polymeric micelle carrier systems for platinum drugs is NC-6004 (**45**), which is a block copolymer of PEG and poly(glutamic acid) (PGlu) coordinated with *cis*-diammineplatinum moieties. The hydrophilic PEG chain constitutes the outer shell of the micelles, and the PGlu-Pt complex chain comprises the inner core of the micelles. The molecular weight of PEG-*b*-PGlu as a sodium salt is approximately 18 000. The platinum release rates of **45** are 19.6 and 47.8% at 24 and 96 h, respectively.[142] A phase I clinical trial of **45** is under way in the UK on patients with solid tumors. The starting dose is 10 mg m^{-2}, and administered once every 3 weeks with only 1000 mL water loading. In general, **45** is well tolerated with minimal nephrotoxicity and no significant myelosuppression, emesis or neurotoxicity but a high rate of hypersensitivity reactions. Disease stabilization has been seen in heavily pre-treated patients.[143] Similarly, DACHPt has also been incorporated into

the PEG-*b*-PGlu block copolymer with different lengths of the PGlu block (20, 40, and 70 U). The resulting polymeric micelles are *ca.* 40 nm in diameter and have a narrow size distribution. *In vivo* distribution and antitumor activity experiments on CDF1 mice bearing the murine colon adenocarcinoma C-26 show that DACHPt-micelles accumulate at the tumour site 20-fold greater than oxaliplatin and achieve substantially higher antitumor efficacy. DACHPt-micelles also show a very strong antitumor activity against the multiple metastases generated from injected bioluminescent HeLa (HeLa-Luc) cells. These results suggest that DACHPt-micelles could be an outstanding DTD system for oxaliplatin in the treatment of solid tumours, especially the PEG-*b*-PGlu micelles prepared with 20 U of PGlu.[144] Such DACHPt-micelles, with 30 nm diameter, efficiently penetrate and accumulate in an orthotopic scirrhous gastric cancer model, leading to the inhibition of the tumour growth. Moreover, the elevated localization of systemically injected DACHPt-micelles in metastatic lymph nodes can inhibit the growth of metastatic tumours.[145]



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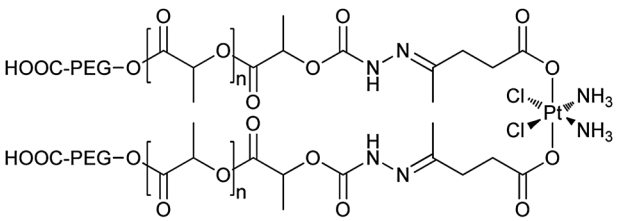
DACHPt and magnetic resonance imaging contrast agent gadolinium–diethylenetriaminepentaacetic acid (Gd-DTPA) have been incorporated into PEG-*b*-PGlu through reversible complexation of platinum, forming core-shell polymeric micelles. Both DACHPt and Gd-DTPA are released from the micelles in a sustained manner under physiologic conditions and colocalize in the tumour interior. Simultaneous therapy and imaging are achieved in an orthotopic animal model of intractable human pancreatic tumour by this nontoxic formulation. This study provides an effective design of theranostic micelles with high contrast enhancement and site-specific clinical potential.[146]

Another type of micelles are synthesized using block copolymer ionomers of PEO, poly(methacrylic acid) (PMA) and divalent metal cations as templates. The core of the micelles comprises a network of the cross-linked polyanions, which is surrounded by the shell of hydrophilic PEO chains. Cisplatin has been incorporated into the ionic core of the micelles with remarkably high efficiency (22%, w/w). The drug-loaded micelles are stable in aqueous dispersions without aggregation or precipitation for a prolonged period of time. Platinum complexes release in a slow and sustained manner from the micelles in physiological saline. *In vitro* studies using human A2780 ovarian carcinoma cells demonstrate that the cross-linked micelles can be rapidly internalized into cells.[147] These results indicate that polymeric micelles with cross-linked ionic cores are promising DTD carriers for platinum anticancer drugs.

The acid-responsive polymer–platinum conjugate **46** is a newly reported drug delivery vehicle for cisplatin. This nanoparticulate system is constructed by a covalent conjugation of the platinum(IV) prodrug to the hydrophobic segment of two biocompatible diblock copolymer chains through a pH-sensitive hydrazone bond. The conjugate can readily precipitate to form sub-100 nm nanoparticles in aqueous solution due to their low critical micelle concentration. The uniqueness of **46** lies in its highly differential drug release profile at different environmental acidity. During circulation in the blood, the nearly neutral pH (7.4) prevents any release of the drug from **46**; upon entering the cancer cells by endocytosis, the acidic intracellular pH (~5.6) stimulates a rapid release of the drug from **46**. The rapid release of drugs in high doses inside cancer cells could suppress the chemoresistance of cancer cells and thereby improve the therapeutic efficacy of the drug. The conjugate shows well-controlled platinum loading yield, excellent drug release kinetics, and an enhanced *in vitro* cytotoxicity against A2780 ovarian cancer cells as compared to free cisplatin.[148]



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4.4 Liposomal formulations

Formulation is an attractive option for improving the efficacy and reducing side effects of platinum-based anticancer drugs. This approach releases the therapeutic moiety at a tumour target in a controllable manner whilst masking the drugs from deactivation by plasma proteins or macrophages. Lipoplatin™ and Aroplatin™ (L-NDDP) are two successful liposomal formulations of platinum anticancer drugs. Lipoplatin™ is a formulation of cisplatin composed primarily of dipalmitoyl phosphatidyl glycerol (DPPG), soy phosphatidylcholine, and methoxypolyethylene glycol-distearoylphosphatidylethanolamine (cisplatin 8.9%, lipids 91.1%, w/w), with an average diameter of 110 nm (Fig. 4). The preparation of Lipoplatin™ begins with the formation of reverse micelles of DPPG with cisplatin and subsequent

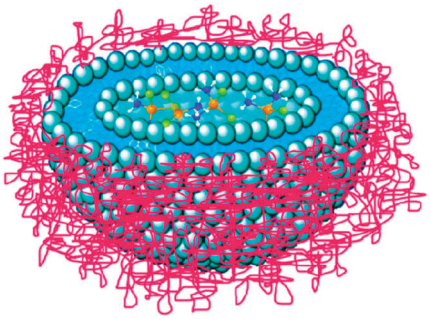
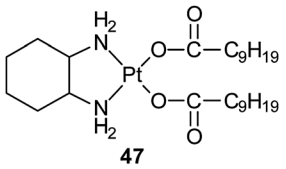


Fig. 4 An illustration of Lipoplatin™ shows the encapsulation of cisplatin in the lipid bilayer with the surface of the liposome being coated by hydrophilic PEG molecules.

conversion to liposomes upon interactions with neutral lipids. PEG units are introduced onto the phospholipid bilayers to sterically stabilise the liposomes, thereby extending the circulation time and improving the uptake, accumulation and retention in tumour tissues due to the EPR effect.[149,150] The solubility of cisplatin is enhanced from 1 to 3 mg mL^{-1} in saline, and the circulation time is prolonged from 6 to 117 h with the aid of liposomes, which is necessary for targeted extravasation into the permeable blood vessels of tumour tissues. In human studies, Lipoplatin™ preferentially concentrates in the primary tumours and their metastases because of the EPR effect and the subsequent avid uptake by the tumour cells either *via* phagocytosis or by direct fusion with the cell membrane, which lead to a 200-fold higher damage to cancer tissues compared to normal tissues and contribute to the low side effects of the drug. In animal studies, Lipoplatin™ kills not only tumour cells but also endothelial cells of the tumour vasculature after systemic delivery; therefore, it may act both as a chemotherapy drug and an antiangiogenesis agent.[151] In preclinical studies, Lipoplatin™ shows a superior cytotoxicity against non-small cell lung cancer (NSCLC) and renal cell carcinoma cell lines and a much lower toxicity in normal cells compared with cisplatin.[152] In phase I–III clinical studies, Lipoplatin™ alone or in combination with other anticancer drugs such as gemcitabine, 5-fluorouracil, and paclitaxel has demonstrated substantially reduced systemic toxicities like nephrotoxicity, neurotoxicity and ototoxicity, with an efficacy higher than or similar to that of cisplatin. Lipoplatin™ has finished successfully phase III clinical trials as a first line treatment against NSCLC, and has been granted phase II/III studies on pancreatic cancer as an orphan drug by the European Medicines Agency.[153,154]

Aroplatin™ is a liposomal formulation of *cis*-bis(neodecanoato)-(*trans-R,R*-1,2-diaminocyclohexane)platinum(II) (**47**), a structural analogue of oxaliplatin. Preclinical data show that Aroplatin™ has different biodistribution and toxicity profiles than cisplatin or carboplatin, and does not share cross-resistance with cisplatin. In a phase I trial, its maximum tolerated dose is 312.5 mg m^{-2} with myelosuppression as the dose limiting toxicity. Aroplatin™ has reached phase II trials in refractory metastatic colorectal cancer.[155]



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Transferrin (TF) receptors are overexpressed on tumour cells and TF receptor-mediated endocytosis is a normal physiological process through which TF delivers iron ions into cells. Therefore, TF receptors could be a viable target for cancer therapy. Liposomes modified with TF-conjugated PEG (TF–PEG-liposomes) have been used as both an active and a passive DTD carrier to realize the tumour-selective delivery of oxaliplatin. This approach has significantly reduced oxaliplatin partitioning to erythrocytes and improved the circulation time of oxaliplatin in Colon 26 tumour-bearing mice, resulting in enhanced extravasation of liposomes into tumours. Intravenously administered oxaliplatin encapsulated within TF–PEG-liposomes has been shown to maintain a high concentration in tumours for over 72 h and suppress tumour growth more effectively than free oxaliplatin.[156] Since selectivity and membrane permeability are inherent features of TF–PEG-liposomes and additional affinity for tumour cells is unnecessary for the delivered species, this DTD system may be applicable to other platinum anticancer drugs targeting at various types of tumours that overexpress TF receptors.

Hybrid molecules derived from nucleotides and lipids are ideal candidates for the encapsulation of platinum drugs. Their distinctive supramolecular capabilities and nontoxic properties have been exploited to develop a novel approach to the delivery of cisplatin. The method involves two steps: (i) encapsulation of the cisplatin nanoprecipitate *via* an anionic nucleotide-lipid; and (ii) stabilization of the resulting anionic nanoparticles using a cationic nucleoside-lipid. The nucleoside polar heads guide the self-assembly of the aggregates into highly loaded and stable nanoparticles (Fig. 5). The cytotoxic activity of the nanoparticles is significantly higher than that of free cisplatin. More impressively, the nanoparticles are much more effective than the free drug against cisplatin-resistant cell lines.[157]

4.5 Carbon nanotubes

Carbon nanotubes are attractive carriers for DTD because of their unique physical, chemical and physiological properties. The structural stability of carbon nanotubes could prolong the circulation time and bioavailability of the loaded drugs.[158] The functionalized soluble single-walled carbon nanotubes (SWNTs) and single-walled carbon nanohorns (SWNHs) are two kinds of the most used nanotubes for the delivery of platinum-based anticancer drugs. SWNTs and SWNHs have plenty of inner spaces that render the incorporation of the drugs possible; moreover, the tube walls can physically adsorb the drugs and various functional molecules. The exterior surface or the edges of the tube holes have oxidized functional groups where further chemical modifications are feasible. More importantly, carbon nanotubes show low toxicity *in vitro* and

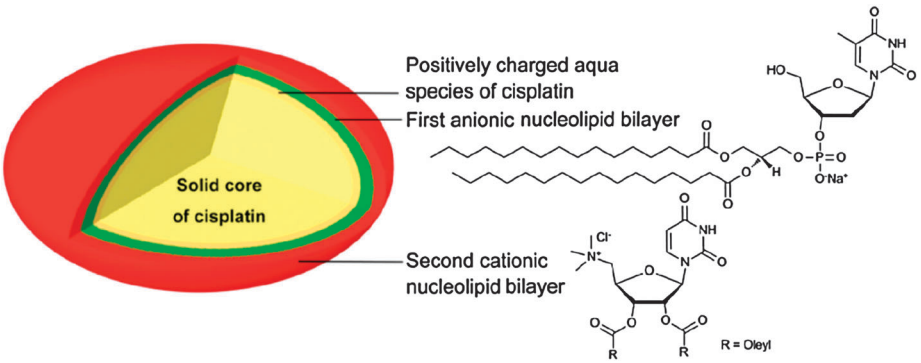
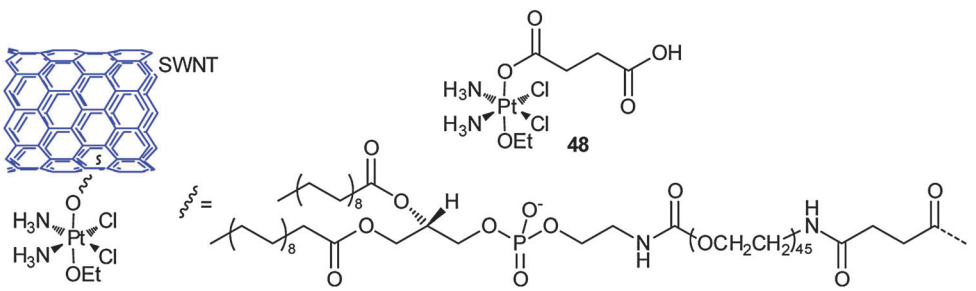


Fig. 5 Schematic diagram of a nucleotide-lipid-based nanoparticulate DTD system for cisplatin.

have negligible impact on a living body.[159] In view of the shuttling capacity of SWNTs, a platinum(IV) complex **48** has been tethered to SWNTs through covalent bonding. Upon intracellular reduction of the conjugate, a lethal dose of cisplatin is released. On average, 65 platinum(IV) centers are attached to each SWNT and they enter the cell through endocytosis, leading to higher levels of platinum in the cell than the untethered complex or cisplatin. The SWNT-Pt^{IV} conjugate shows a substantial increase in cytotoxicity against the testicular carcinoma cell line NTera-2 as compared with that of the free complex and cisplatin.[160]

FR-mediated endocytosis. An example of such a conjugate is shown in Fig. 6, where the platinum(IV) complex bearing succinates as its axial ligands is tethered to the amine functionalised SWNT and a folic acid derivative. The folate moiety serves as the targeting agent and SWNT as the delivery molecule. The PEG spacer between the platinum centre and folate group makes the conjugate more water soluble and biocompatible. The conjugate indeed delivers the platinum(IV) pharmacophore selectively into the FR(+) cancer cells that overexpress the FR on their surface and releases cisplatin upon intracellular reduction of Pt^{IV} to Pt^{II}, forming a high level of 1,2-d(GpG) intrastrand cross-link with



Increased cellular uptake of folic acid and overexpression of folate receptor (FR) are well-known biochemical characters of many tumour types. Therefore, FR, a glycoprotein on the cellular membrane with high affinity for folic acid, is a proper target for anticancer chemotherapeutic agents. Folate-like molecules represent an intriguing class of carriers for DTD specific to FR(+) tumour cells. Folic acid, linked *via* one of its carboxyl groups to a molecule, can enter cancer cells through nuclear DNA. The IC_{50} values of the conjugate towards FR(+) human choriocarcinoma JAR and human nasopharyngeal carcinoma KB cell lines are significantly lower than those of cisplatin or the free complex.[161] This is a telling example for an ideal DTD structure mode, that is, to incorporate the targeting and delivery moieties into a single molecule.

Epidermal growth factor receptor (EGFR) is overexpressed in a number of cancers such as ovary and lung cancers.[162,163]

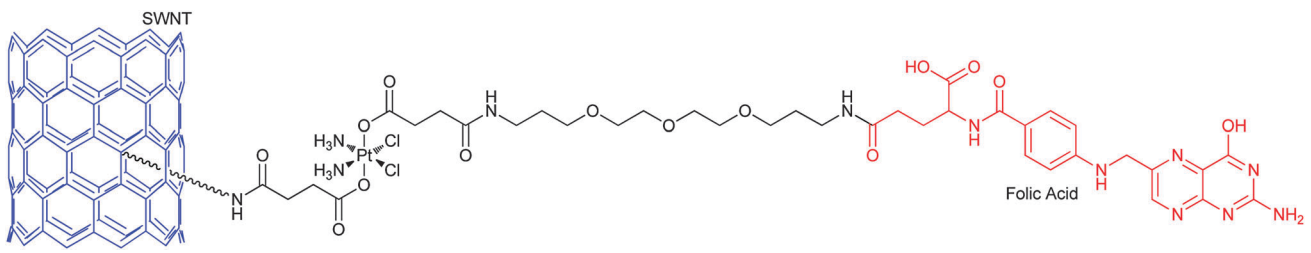


Fig. 6 SWNT-tethered platinum(IV) prodrug with targeting property for folate receptor, which releases cisplatin upon intracellular reduction in tumour cells.

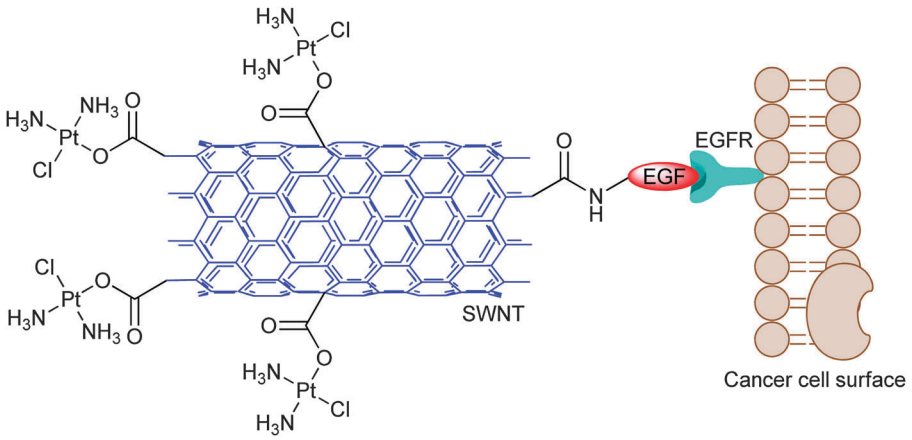
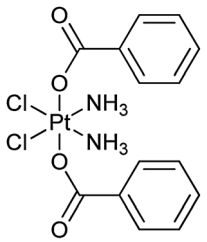


Fig. 7 Schematic diagram of the cisplatin-SWNT-EGF conjugate targeting the cell surface EGFR on a single HNSCC cell.

Accordingly, SWNT-tethered cisplatin is attached to epidermal growth factor (EGF) to specifically target head and neck squamous carcinoma cells (HNSCC) that overexpress EGFR (Fig. 7). The uptake of platinum in both *in vitro* and *in vivo* systems is higher for the targeted conjugate than for the untargeted controls. The conjugate enters into the cell through EGFR-directed endocytosis, as demonstrated by the lack of uptake in the absence of EGFR or EGF. In short-term studies on mice, the conjugate mainly distributes in tumours, with only small amounts distributing in various vital organs. The conjugate kills cancer cells more efficiently and inhibit tumour growth in mice more rapidly than cisplatin and the untargeted SWNT-cisplatin. This is the first SWNT-tethered platinum DTD system showing selective anticancer activity *in vivo*.[164]

SWNHs have a spherical structure between 80 and 100 nm assembled by several hundreds of SWNTs, and the size is adequate for drug delivery through vascular EPR effect. Cisplatin has been loaded into the SWNHs through a selective precipitation process in water. The amount of incorporated cisplatin is 46%, and the total released quantity of cisplatin is 100% over 48 h. As a result, *in vitro* anticancer efficiency of the drug-loaded SWNHs is 4–6 times greater than that of free cisplatin, and *in vivo* anticancer activity against the growth of transplanted tumours in mice is also better than cisplatin and remains for a long time (25 days). Since cisplatin-SWNHs adhere to the cell surfaces *in vitro* and stay within the tumour tissues *in vivo*, the released cisplatin can reach high concentrations locally in cells and in tissues, leading to an efficient attack against the tumour cells.[165]

Platinum(II) complexes tethered to the surface of SWNTs may risk being prematurely released from the carrier and binding non-specifically to endogenous nucleophiles. To address this problem, multiwalled carbon nanotubes are adopted as a protective shell to entrap stable hydrophobic platinum(IV) prodrug **49** within its inner cavity *via* hydrophobic–hydrophobic interactions. Upon chemical reduction by cellular reductants, **49** is converted to its hydrophilic and cytotoxic Pt^{II} form and released from the carrier due to the drastic reversal in hydrophobicity. In this way, controlled release of Pt^{II} species can be achieved intracellularly to exert its cytotoxic activity.[166]



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4.6 Nanoparticles

Nanoparticles could reach certain solid tumours *via* the EPR effect and thereby change the tissue distribution and pharmacokinetics of the loaded drugs, which would significantly improve their specificity for tumour tissues.[167,168] In comparison with other delivery strategies, nanoparticle-based DTD possesses many advantages, such as enhanced drug accumulation in tumour tissues, reduced systemic toxicity, and sustained drug release in an environmentally responsive manner.[169] Besides, nanoparticles could protect the loaded drug from degradation before reaching cancerous cells, and thereby prolong the blood circulation time of the drug and shield the body from undesired side effects. Surface-functionalized nanoparticles by peptides, antibodies or aptamers can further increase the specificity for particular cancerous cells.[170] Nanoparticles used for platinum DTD *in vivo* should be biocompatible, biodegradable, in appropriate size, and have high affinity for the platinum drug to avoid premature release before entering tumour cells. Several nanoparticle-based anticancer drugs have been approved by the FDA, and an interest in the development of nanoparticle formulations for effective delivery of platinum anticancer drugs has increased persistently.[171] For example, phospholipid nanocapsules have been exploited to deliver both cisplatin and carboplatin.[172–174] Polymeric nanoparticles as sequential release carriers are becoming more and more valuable in the drug delivery for cancer therapy.[175] Nanoparticles made of chitosan or *N*-trimethyl chitosan,[176] glycol chitosan,[177] poly(lactide-*co*-glycolide)-methoxy-poly(ethylene glycol),[178] poly[2-(*N*,*N*-diethylamino)ethyl

methacrylate]-*block*-poly(ethylene glycol),[179] and glucosamine-functionalized polyisobutylene-maleic acid[180] have been examined as carriers for the delivery of cisplatin in various cancer cells or tumour-bearing mice.

4.6.1 Targeted nanoparticles. As mentioned above, EGFR could be an ideal target for the platinum DTD system. The EGFR-specific binding and internalization of single-chain variable fragment anti-EGFR antibody (ScFvEGFR) have been demonstrated.[181] ScFvEGFR has been successfully conjugated to nanoparticles, resulting in compact ScFvEGFR-nanoparticles that can specifically be bound and internalized by EGFR-expressing cancer cells.[182] A DTD system of cisplatin to lung cancer has been developed using EGFR-targeted heparin-cisplatin nanoparticles. The nanoparticles are formed by assembly of cisplatin and heparin through coordination between Pt^{II} and carboxyl groups. ScFvEGFR is conjugated to the nanoparticles as an EGFR-targeting ligand. These nanoparticles significantly increase the intracellular concentrations of cisplatin and Pt-DNA adducts in EGFR-expressing non-small cell lung cancer H292 cells *via* an EGFR-mediated pathway. The blood circulation time, pharmacokinetics, biodistribution of Pt and antitumor activity of cisplatin are significantly improved by the nanoparticles; and the toxicity to the kidney and spleen in nude mice bearing H292 cell tumours is markedly reduced as compared with free cisplatin.[183]

Poly(D,L-lactic-*co*-glycolic acid) (PLGA)-PEG polymers are particularly useful in encapsulating platinum drugs for targeted delivery because of their safety in clinical use and systemic clearance times.[184] Prostate-specific membrane antigen (PSMA) is abundantly expressed in prostate cancer, its metastatic form, the hormone-refractory form and the neovasculature of many non-prostate solid tumours, offering a suitable target for cancer chemotherapy. To construct the PSMA-targeted nanoparticles, a hydrophobic platinum(IV) prodrug **50** is encapsulated in PLGA-PEG polymers by nanoprecipitation and subsequent conjugation of PSMA aptamers (Apt) (Fig. 8). The PSMA targeting aptamers on the surface of the nanoparticles direct **50** specifically to the human PSMA-overexpressing LNCaP prostate cancer cells. Upon internalization through endocytosis and intracellular reduction, a lethal dose of cisplatin is released from the polymeric nanoparticles. Controlled release of **50** from the nanoparticles extends over 60 h. The nanoparticles are highly cytotoxic to the LNCaP cells (IC_{50} = 0.03 μM); under the same conditions, they are 80 times more effective than cisplatin.[185] Recently, *in vivo* studies in different normal and PSMA-expressing animal models reveal that the pharmacokinetics, biodistribution, tolerability, and efficacy of Pt-Apt-NPs are enhanced when compared to cisplatin. Prolonged drug persistence in blood circulation and decreased accumulation of Pt in the kidneys are observed. Pt-Apt-NPs display a significant dose-sparing character of the drug, with equivalent antitumor efficacy in LNCaP xenografts at 1/3 the dose of cisplatin. This system provides a remarkable improvement in the therapeutic index of cisplatin for prostate cancer chemotherapy.[186]

Integrins are heterodimeric transmembrane proteins involved in cell adhesion and cell signaling, and their expression is commonly upregulated in cancers. The $\alpha_v\beta_3$ integrin is differentially upregulated on angiogenic endothelial cells as well as on many cancer cells. RGD motif (Arg-Gly-Asp) is a tumour vasculature-homing peptide existing in many extracellular matrix components and capable of binding integrins on the cell surface (*vide ante*). Recently, a PLGA-PEG nanoparticulate system comprising encapsulated **50** and cyclic pentapeptide c(RGDfK) as $\alpha_v\beta_3$-targeting moieties has been developed for anticancer therapy. The RGD-modified Pt(IV)-PLGA-PEG nanoparticles display enhanced cytotoxicity as compared to cisplatin in prostate and breast cancer epithelial cells *in vitro*; moreover, they are more efficacious and better tolerated in comparison to cisplatin in an orthotopic human breast cancer xenograft model *in vivo*.[187] This system combines both active and passive targeting approaches, resulting in an enhanced antitumor efficacy and reduced toxicity for platinum drugs.

On the same concept, **51** is encapsulated into nanoscale coordination polymers (NCPs) for targeted delivery to cancer cells. NCPs are constructed from Tb^{III} cations and **51** simply by precipitating from their aqueous solution *via* the addition of a poor solvent, where Tb^{III} ions act as connectors to form the metal–ligand polymers. The Pt-loaded NCPs are stabilized with shells of amorphous silica to prevent rapid dissolution and to effectively control the release of the platinum species. The silica shells extend the half-release time to 5.5 or 9 h, depending on the size of the coating (2 or 7 nm). These release rates would allow sufficient time for the Pt-loaded NCPs to circulate throughout the body and accumulate in tumour tissue. In order to enhance the cellular uptake of Pt-NCPs *in vitro*, silyl-derived c(RGDfK) is grafted onto the surface, which could enhance the binding affinity for the $\alpha_v\beta_3$ integrin. The targeted Pt-NCPs show IC_{50} values lower than that of cisplatin for angiogenic

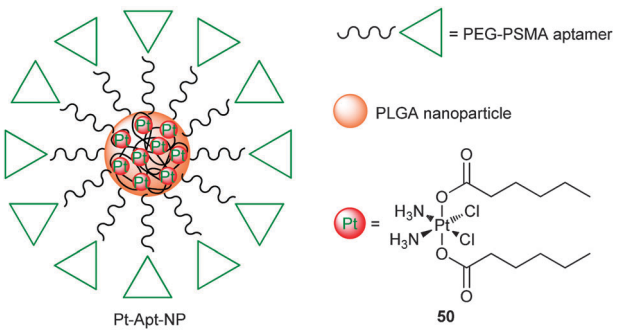
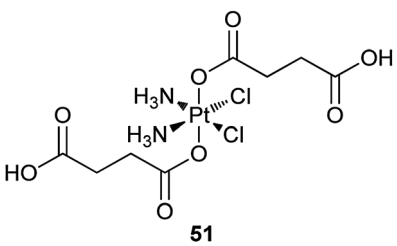


Fig. 8 The chemical structure of the encapsulated platinum(IV) prodrug within the PLGA-PEG nanoparticle.



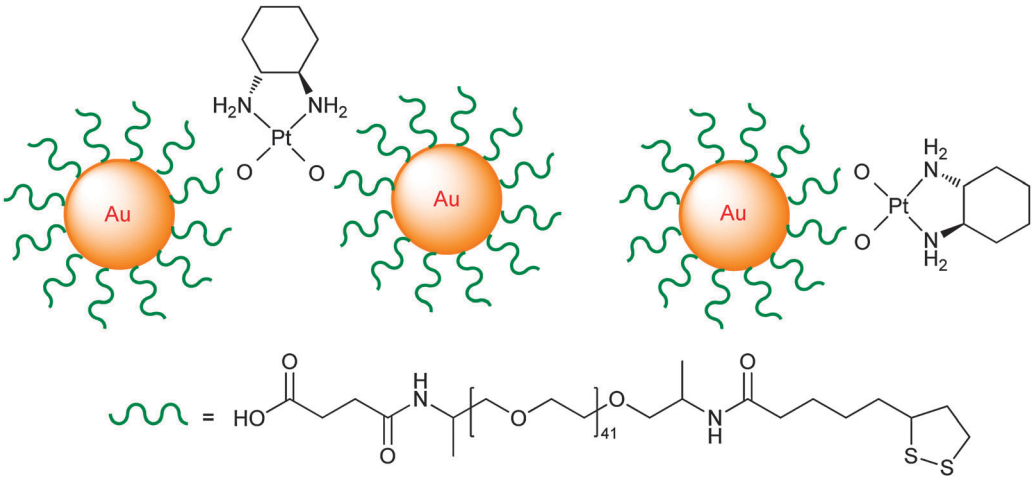
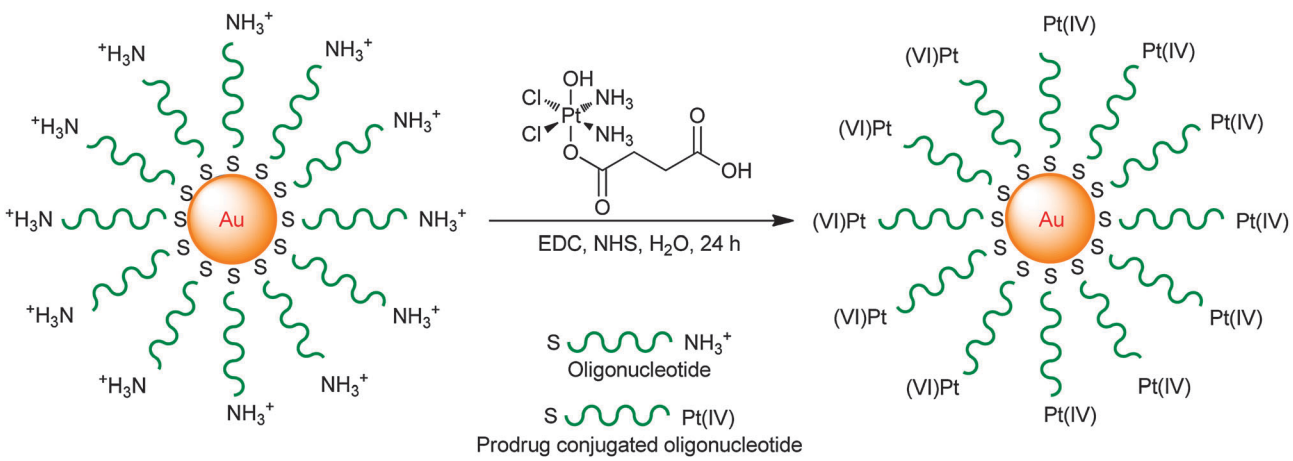


Fig. 9 The linking mode of thiolate-PEG-Au nanoparticles with the oxaliplatin fragment.

human colon carcinoma cell line HT29. The cytotoxicity against the MCF-7 cell line that does not overexpress the $\alpha_v\beta_3$ integrin is similar to that of cisplatin.[188]

4.6.2 Gold nanoparticles. Inorganic nanoparticles have emerged as highly effective DTD systems for platinum-based anticancer drugs in recent years. Among them, gold nanoparticles are particularly captivating because they are inert, nontoxic, biocompatible, and easy to be prepared and functionalized.[189–192] Most gold nanoparticles enter cells through endocytosis.[193] Cisplatin has been loaded onto gold nanoparticles of the form Au–Au_2S for potential cancer therapy.[194] Carboplatin has also been conjugated onto gold nanoparticles containing an FDA approved anti-EGFR antibody cetuximab for the treatment of EGFR overexpressing cancers. The nanoconjugate shows an enhanced therapeutic efficacy towards both EGFR(+) A549 lung and OVCAR5 ovarian cancer cell lines as compared to its non-targeted counterpart.[195] More recently, the active component of oxaliplatin has been tethered to gold nanoparticles for improved drug delivery, where the nanoparticles are functionalized with a thiolated PEG monolayer capped with a carboxylate group (Fig. 9). The platinum-tethered nanoparticles demonstrate as good as or significantly better inhibition activity than oxaliplatin against the A549 lung epithelial cancer and several colon cancer cell lines. Particularly, they show an unusual ability to penetrate the nucleus in the lung cancer cells.[196]

A novel DTD vehicle involving platinum(IV) polyvalent oligonucleotide gold nanoparticle conjugates has been developed. The gold nanoparticles are functionalized with thiolated 28-mer oligonucleotides containing a terminal dodecyl amine for platinum conjugation. A platinum(IV) complex, *cis*,*cis*,*trans*-$[Pt(NH_3)_2Cl_2(OH)(O_2CCH_2CH_2CO_2H)]$, is tethered to the amine-functionalized DNA–Au nanoparticle surface through amide linkages, resulting in Pt(IV)–DNA–Au nanoparticles (Scheme 2). The conjugates allow the platinum(IV) prodrug to travel safely in the bloodstream before reaching the tumour site. They are internalized by tumour cells and reduced to release cisplatin, which enters the nucleus and forms 1,2-d(GpG) intrastrand cross-links with DNA. This nanoparticulate conjugate shows a killing ability superior to cisplatin against human lung carcinoma A549, human prostate cancer PC3, cervical cancer HeLa, and human osteosarcoma U2OS cell lines. In contrast, the parent prodrug displays no significant killing under the same conditions.[197]



Scheme 2 The synthesis of Pt(IV)–DNA–Au nanoparticles through peptide bond formation.

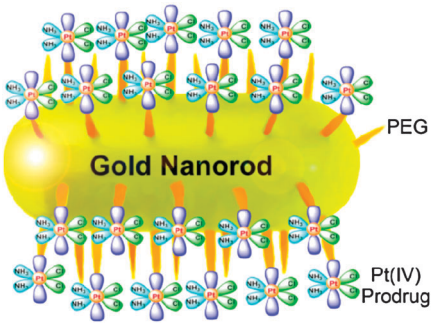
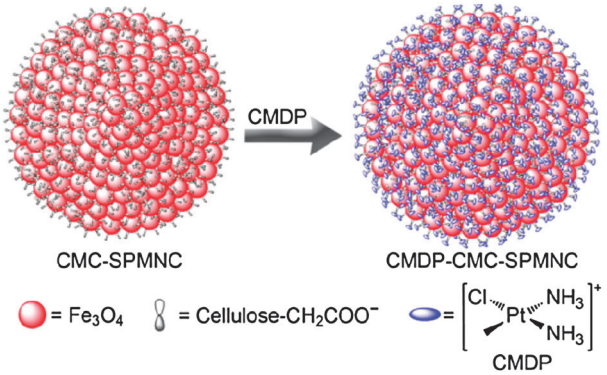


Fig. 10 Illustration of the PEGylated gold nanorod as a DTD carrier for a platinum(IV) prodrug.



Scheme 3 Fabrication route of the CMDP–CMC–SPMNC conjugate.

The appealing characteristics of such nanoparticles include high levels of cellular uptake in a number of cell types, nontoxicity and resistance to enzymatic degradation.[198,199]

The cellular uptake of gold nanoparticles is not only dependent on their size, but also on their shape.[200,201] A peculiar class of gold nanoparticles is the nonspherical gold nanorods (GNRs). In comparison with spherical gold nanoparticles, GNRs have longer circulation time *in vivo*, which enhances the efficiency of nanoparticle accumulation in tumours.[202] Recently, platinum(IV) prodrug *cis*,*cis*,*trans*-$[Pt(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)_2]$ has been tethered to the amine-functionalized PEG-GNRs (Fig. 10). This conjugate is stable under physiological conditions, but is liable to be reduced by cellular reductants, releasing the active Pt^{II} species. Compared with cisplatin, the conjugate shows an enhanced cellular platinum accumulation and superior cytotoxicity against cervical cancer HeLa, human lung carcinoma A549 and human breast adenocarcinoma MCF-7 cells lines.[203] More importantly, the conjugate can overcome the drug resistance in cisplatin-resistant A549R cells because it gets into cells through endocytosis and low expression of copper transport protein (Ctr1) in A549R cells does not affect its uptake. In addition, the platinum(IV) prodrug attached to PEG-GNRs is more inert than cisplatin, which would reduce the deactivation induced by glutathione and metallothionein.[204] These results suggest that PEG-GNRs are effective carriers for the delivery of platinum drugs.

4.6.3 Magnetic iron oxide nanoparticles. Magnetic nanoparticles are promising DTD carriers because they could guide drugs preferentially to the biological target through external magnet and hence abate the lesions in normal tissues.[205,206] Besides, a variety of tumour-targeting ligands such as monoclonal antibodies, peptides, or small molecules could be linked to magnetic nanoparticles to facilitate their entering into tumour cells.[207] Superparamagnetic iron oxide nanoparticles are attractive drug carriers in virtue of their biocompatibility, biodegradability, aqueous dispersibility and magnetisability;[208–210] after coating with biocompatible materials, they may coordinate with therapeutic or diagnostic moieties to form targeted formulations.[211–213] For example, Au–Fe_3O_4 nanoparticles can act as target-specific nanocarriers to deliver platinum drug into Her2(+) breast cancer cells. The platinum moiety is simply anchored to the Au side by reacting Au–S–$CH_2CH_2N(CH_2COOH)_2$ with cisplatin, and the Her2-specific monoclonal antibody Herceptin is linked to Fe_3O_4 through PEG_{3000}–CONH–Herceptin as a targeting agent. The core structure contains magnetic Fe_3O_4 and optically active gold nanoparticles, which can serve as both a magnetic and optical probe for tracking the platinum complex in cells and biological systems. The release of the therapeutic platinum species under low-pH conditions renders the nanoparticle conjugate more toxic to the Her2(+) Sk-Br3 breast cancer cells than free cisplatin.[214]

Recently, we fabricated the carboxymethylcellulose-modified superparamagnetic magnetite nanocrystal clusters (CMC–SPMNCs) as nanocarriers for the delivery of platinum drugs. Dechlorinated cisplatin, namely *cis*-monochlorodiammineplatinum(II) (CMDP), was loaded onto the clusters through the abundant carboxylate groups on the surface of the nanoparticles, forming CMDP–CMC–SPMNC conjugates with a mean diameter of 290.6 nm (Scheme 3). The conjugates display excellent dimensional uniformity, good aqueous dispersibility and strong magnetisability. In comparison with cisplatin, the conjugates can more readily enter cancer cells and exert higher cytotoxicity towards the human cervical cancer HeLa cells and the human hepatocarcinoma HepG2 cells. These nanoparticles are likely to be used as targeted carriers to deliver platinum anticancer drugs.[215]

5 Conclusions

The lack of tumour specificity is one of the major problems for platinum-based anticancer drugs. The nonselective distribution of platinum drugs in normal and cancer cells not only induces excessive systemic toxicity, but also reduces drug accumulation in tumour cells, resulting in tumour resistance to platinum drugs. In addition, unrestrained interactions of the drugs with plasma and tissue proteins may lead to rapid inactivation of platinum drugs and thereby suboptimal treatment for the tumour. Therefore, targeting platinum anticancer drugs to specific tumour tissues is an important issue in platinum-based chemotherapy.[216,217] The use of special delivery carriers to selectively transport platinum agents to tumours is very attractive to address these problems. A variety of DTD approaches have been developed to improve the efficacy of platinum anticancer drugs.[218] These approaches are classified

as active or passive strategies. Active DTD contributes to the selectivity of a platinum drug towards a specific kind of tumour according to its biochemical properties, while passive DTD leads to the beneficial accumulation of a platinum drug in the tumour mass because of the EPR effect. The rational design of DTD systems for platinum complexes has resulted in a number of ''magic bullets'' with therapeutic indexes better than those of cisplatin and its derivatives. This review presents the major achievements in this area in the past few years. These creative designs may inspire even more ingenious inventions come into being in the future development of platinum-based anticancer drugs.

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