



Published in final edited form as:

Dalton Trans. 2009 December 28; (48): 10651–10659. doi:10.1039/b913896j.

Non-Traditional Platinum Compounds for Improved Accumulation, Oral Bioavailability, and Tumor Targeting

Katherine S. Lovejoy and Stephen J. Lippard

Department of Chemistry, Massachusetts Institute of Technology Cambridge, Massachusetts 02139, USA

Peter Sadler

Summary

The five platinum anticancer compounds currently in clinical use conform to structure-activity relationships formulated¹ shortly after the discovery² that *cis*-diamminedichloroplatinum(II), cisplatin, has antitumor activity in mice. These compounds are neutral, platinum(II) species with two am(m)ine ligands or one bidentate chelating diamine and two additional ligands that can be replaced by water through aquation reactions. The resulting cations ultimately form bifunctional adducts on DNA. Information about the chemistry of these platinum compounds and correlations of their structures with anticancer activity have provided guidance for the design of novel anticancer drug candidates based on proposed mechanisms of action. This article discusses advances in the synthesis and evaluation of such non-traditional platinum compounds, including cationic and tumor-targeting constructs.

Introduction

Cisplatin is a widely used platinum-based anticancer drug with antitumor activity that was first discovered in 1969.² It is currently used in bladder, non-small cell lung, head and neck, ovarian, cervical, and other cancers, being curative in nearly all cases of testicular cancer. A similar platinum complex with fewer toxic side effects, carboplatin (Figure 1), was introduced as a second generation modification of cisplatin and, more recently, oxaliplatin was introduced as a first-line treatment for colorectal cancer (Fig. 1).³

The three FDA-approved platinum compounds, cisplatin, carboplatin, and oxaliplatin, as well as nedaplatin, approved for use in Japan, and lobaplatin, approved for use in China, obey the originally devised structure-activity relationships for antitumor candidates in the platinum family. These rules specified that such compounds should be neutral, platinum(II) species with two am(m)ine ligands or one bidentate chelating diamine, and two ligands that can be replaced by aquation reactions.^{1, 4} Of platinum compounds currently or recently being evaluated in clinical trials, picoplatin (AMD473, Figure 2) and ProLindac⁵ obey the original rules.

Thirty-five years after formulation of the original structure-activity relationships, the search continues for platinum compounds having novel pre-clinical properties, such as activity in cisplatin-resistant cells or a pattern of cytotoxicity significantly different from that of cisplatin and carboplatin against a panel of cells lines of various origin.⁶ The findings include structures that significantly violate the original rules, including cationic complexes. In recent years, efforts by the platinum anticancer drug development community have significantly intensified, with substantial effort being expended to discover tactics for improving cellular accumulation, oral bioavailability, lifetime in blood, and tumor targeting.

Non-traditional compounds in clinical trials

The three-year interval between initial testing of cisplatin in mouse tumor models in 1968 to clinical trials with terminal cancer patients, which began in 1971, is unusually short by today's standards. Concerns about heavy metal toxicity were overcome in favor of rapid introduction of a promising new treatment.⁴ Platinum compounds currently in clinical trials that violate the canonical structure-activity relationships have had to overcome significant barriers on their way to the clinic, and the lag between bench top and clinical evaluation has been considerably longer than that of cisplatin.

Compounds having entered clinical trials include platinum(IV) complexes, such as satraplatin, *c,c,t*-[Pt(NH₃)₂(cyclohexylamine)Cl(COOCH₃)₂], the first orally available candidate. Polyplatinum compounds of the form [$\{\text{PtCl}_m(\text{NH}_3)_{3-m}\}_2(\text{H}_2\text{N-R-NH}_2)\]^{2(2-m)+}$ where $m = 0-3$ and R is a linear or substituted aliphatic linker, and picoplatin, *cis*-[Pt(NH₃)(2-picoline)Cl₂], (Figure 2) have also been evaluated.

The first patient received satraplatin in 1993.^{7, 8} A phase II trial for hormone-refractory prostate cancer showed a median overall survival of 14.9 months for the patients receiving satraplatin and prednisone and a survival of 11.9 months for patients receiving prednisone alone, and a phase III trial in patients with similar cancer profiles also revealed that patients on the combination therapy had an improved prognosis for disease progression.⁹

The dose-limiting side effect for satraplatin is myelosuppression, specifically, a reduction in the production of white blood cells and platelets. Biotransformation of satraplatin in the body produces several products, the major one arising results in reduction to platinum(II), *cis*-[PtCl₂(NH₃)(cyclohexylamine)].⁷ DNA adducts formed by this reduced and activated form of satraplatin are similar to those of cisplatin¹⁰ and can be repaired by the nucleotide excision repair pathway.¹¹ A comparison of excision repair *in vitro* for cisplatin, oxaliplatin, and *cis*-[PtCl₂(NH₃)(cyclohexylamine)], the reduced form of oxaliplatin, revealed only modest differences. The percent excision after 60 minutes was about 2.0% for site-specific 1,2-d(GpG) intrastrand cross-links of *cis*-[PtCl₂(NH₃)(cyclohexylamine)] compared to 1.5% and 1.0% for those of the analogous cisplatin and oxaliplatin adducts.¹¹

In addition to satraplatin, two other platinum(IV) complexes have advanced to clinical trials, namely, tetraplatin, tetrachloro-*trans-R,R*-cyclohexane-1,2-diamine-platinum(IV), and iproplatin (dichlorodihydroxobis(isopropylamine)platinum(IV)). Platinum(IV) anticancer drug candidates have been reviewed recently.^{12, 13}

Picoplatin was designed with additional steric bulk protecting the axial positions at the platinum center to reduce potential inactivation by intracellular thiols such as glutathione.⁷ In support of this approach is the fact that cisplatin binding to DNA is significantly less in the presence of 5 mM glutathione. Moreover, cells that are resistant to cisplatin are not cross-resistant to picoplatin.¹⁴ Picoplatin exhibits synergistic behavior when administered in combination therapy with paclitaxel, as does cisplatin. Phase II clinical trials of picoplatin demonstrated a survival benefit for patients with small cell lung cancer who were treated with the drug after relapsing within six months of initial therapy with other agents. A phase III trial of picoplatin, SPEAR (Study of Picoplatin Efficacy After Relapse), is currently running in Europe and India, with a focus on patients with small cell lung cancer. A phase I trial in colorectal cancer in combination therapy with 5-fluorouracil and a phase II trial in prostate cancer (in combination therapy with docetaxel and prednisone) are also in progress.

Cationic non-traditional compounds

A Cationic Triplatinum Complex in Clinical Trials, $[\{trans\text{-PtCl}(\text{NH}_3)_2\}_2\{\mu\text{-trans-Pt}(\text{NH}_3)_2(\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2)_2\}]^{4+}$

The trinuclear cation $[\{trans\text{-PtCl}(\text{NH}_3)_2\}_2\{\mu\text{-trans-Pt}(\text{NH}_3)_2(\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2)_2\}]^{4+}$ is composed of two *trans*- $\{\text{PtCl}(\text{NH}_3)_2\}^+$ units linked by a bridging tetra-amine *trans*- $\{\text{Pt}(\text{NH}_3)_2\{\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2\}_2\}^{2+}$ unit (Figure 2) and is undergoing phase II clinical trials. The platinum atoms at either end of the linear chain react in a monofunctional manner with non-adjacent DNA bases to form a variety of adducts. It is uncertain clear which adduct, if any, predominates *in vivo*.¹⁵ The charge on the internal platinum atom is important to the anticancer activity of the complex and is associated with increased cellular accumulation proposed to be involve polyamine transporters.¹⁶ The dose-limiting suppression of blood cells produced in the bone marrow and gastrointestinal side effects, which reduce the maximum-tolerated dose below that of cisplatin, limit the potency of the drug and may prevent it from advancing further clinical trials. For patients with advanced gastric or gastroesophageal adenocarcinomas receiving 1.1 mg/m² every four weeks, 5 of 7 experienced side effects requiring a reduction in dose. Of 17 patients on a reduced dose of 0.9 mm m⁻², only 1 showed a significant response.¹⁷ A second generation compound, BBR3610, contains only two coordinated platinum units¹⁵, displays p53-independent cytotoxic activity in colon cancer cells,¹⁸ has an IC₉₀ 250 times less than that of cisplatin in glioma cells,¹⁹ and is currently being developed by Cell Therapeutics.²⁰

Cationic Drug Candidates

Cationic platinum compounds are not commonly thought of as active species, sometimes attributed to an inability to diffuse through the neutral, hydrophobic lipid bilayer. Once oxaliplatin was identified as a potential substrate for cation transporters in cell membranes, 21 investigations of other cationic platinum complexes were undertaken. Once inside the cell, the cationic nature of these compounds renders them analogs of the aquated, cationic forms of cisplatin, which has an affinity for polyanionic DNA. As a practical matter, cations are significantly more water soluble than their neutral counterparts, which aids drug formulation. Also, unlike some organic drugs, they are unlikely to partition into or adhere to hydrophobic plastics used in common clinical and cell culture practice.

Active cationic complexes fall into several categories. One involves a Pt(II) framework analogous to cisplatin having with three non-labile nitrogen donor ligands and one chloride leaving group (Figure 3, A).²² A variation on this structure involves linking two such monofunctional complexes to form binuclear, bifunctional,²³ or trinuclear, bifunctional (Figure 3, F)²⁴ complexes. Besides $[\{trans\text{-PtCl}(\text{NH}_3)_2\}_2\{\mu\text{-trans-Pt}(\text{NH}_3)_2(\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2)_2\}]^{4+}$, discussed above, another cationic, multinuclear platinum(II) drug candidate is $[(cis\text{-}\{\text{Pt}(\text{NH}_3)_2\})_2(\mu\text{-OH})(\mu\text{-pL})]^{2+}$ (pL = pyrazolate), which contains two platinum centers that are each capable of binding DNA monofunctionally after loss of the bridging hydroxide (Scheme 1). DNA adducts formed by this complex include the intrastrand 1,2-d(GpG) cross-link, which is the most prevalent cisplatin–DNA adduct. Following global platination of plasmid DNA by $[(cis\text{-}\{\text{Pt}(\text{NH}_3)_2\})_2(\mu\text{-OH})(\mu\text{-pL})]^{2+}$, the duplex is unwound by ~ 15°. Unlike cisplatin, $[(cis\text{-}\{\text{Pt}(\text{NH}_3)_2\})_2(\mu\text{-OH})(\mu\text{-pL})]^{2+}$ does not induce a significant bend in the helix axis nor does it alter the melting temperature.²⁶ The compound is more toxic than cisplatin in most tested cell lines, displaying 37-fold-greater potency than the parent drug in a melanoma cell line M19 and 39-fold greater cytotoxicity in a breast cancer cell line MCF-7.²⁷ Advances in the design of multinuclear platinum are reviewed more extensively elsewhere.²⁴ Highly charged, cationic, multinuclear platinum(II) complexes that do not appear to interact covalently with DNA have also recently been described.²⁸

Cationic complexes with ligand sets comprising a bidentate (Figure 3, B) or tridentate (Figure 3, C) nitrogen donor ligand plus a halogen, thiourea,^{29, 30} or sulfoxide fourth ligand have also been evaluated as anticancer drug candidates. Cationic complexes with relatively non-labile thiourea ligands, instead of the more labile sulfoxides, bind one equivalent of 5'-GMP or γ (GpG), even in the presence of excess nucleotide.³⁰ Neither of two thiourea-containing mononuclear complexes tested, [PtCl(en)(tutu)]NO₃ and [PtCl(dach)(tutu)]NO₃ (tutu = 1,1,3,3-tetramethylthiourea), were cytotoxic in murine L1210 leukemia cells. The Pt–S distance of 2.281(2) Å in [PtCl(dach)(tutu)]⁺ and the ¹⁹⁵Pt NMR chemical shift of –2895 both suggest weak acceptor properties of tutu, especially compared with the sulfoxide complexes, in which the ¹⁹⁵Pt chemical shifts are ~ –3300 for each.³¹ The thiourea S-atom shields the platinum nucleus less than the sulfoxide S-atom, but more than the chloride ion in cisplatin (δ ~ –2149 ppm).³²

A thiourea-based monofunctional platinum-acridine complex (Pt-ACRAMTU, Figure 4) binds to N3 of adenine in the minor groove as a consequence of an intercalator-mediated minor groove association of the pendant acridine ring at adenine-containing base pair steps.³³ The complex is more active than cisplatin in a non-small-cell cancer cell line.³⁴

Another framework involves replacement of one ammine on cisplatin or *trans*-[Pt(NH₃)₂Cl₂] with a positively charged, non-labile nitrogen-donor ligand, such as piperazine (Figure 3, D and E).³⁵ The cisplatin derivative affords a compound with reduced cytotoxicity, whereas the corresponding replacement on *trans*-[Pt(NH₃)₂Cl₂] enhances cytotoxicity. A related series of cationic compounds that display significant activity have the general formula *trans*-[PtCl₂(Am)(pip-pip)]HCl (pip-pip = 4-piperidinopiperidine) (Figure 4).³⁶ Of the seven compounds in this series and cisplatin, the derivative with Am = NH₃ was most cytotoxic against the cisplatin-resistant ovarian cancer cell lines A2780cisR, CH1cisR, and 41McisR. In a murine carcinoma line C-26, cellular accumulation of *trans*-[PtCl₂(NH₃)(pip-pip)]HCl was 10- to 25-fold greater than cisplatin, although the level of platinum bound to DNA was approximately the same. The charged complexes also bind DNA up to 10-fold more rapidly than neutral complexes such as cisplatin and *trans*-[Pt(NH₃)₂Cl₂].³⁷

Ligand Choice

Within the aforementioned basic structural frameworks, a variety of ligand choices are possible. Variation of N-donor strength is one way to tune the biological properties of the compounds. The initial series of compounds of general formula *cis*-[Pt(NH₃)₂(N-donor)Cl]⁺ revealed that, although the ¹⁹⁵Pt NMR chemical shift could be related to the relative strength of the N-donor ligand, cytotoxicity did not correlate with either of these parameters.²²

Bulky ligands protect the platinum center in biological milieu from deactivation by non-nucleoside nucleophiles such as glutathione or cysteine. The added lipophilicity of such ligands can also aid in passage through the lipid bilayer that comprises cell membranes. Evaluating the lipophilicity of platinum complexes is complicated, and different methods lead to conflicting results. Available methodologies have been recently reviewed.³⁸

Choice of a bidentate or tridentate N-donor ligand adds stability to cationic platinum complexes and decreases the possibility of undesired substitution reactions at the chelate. For complexes with the *trans*-[Pt(cationic N-donor)(NH₃)₂Cl₂] ligand set and stereochemistry, choice of a planar cationic N-donor can activate the *trans* geometry.³⁹ The choice of leaving group also plays a role in the biological fate and antitumor properties of this class of compounds. The lability of the Pt–X bond is crucial for delivery of platinum to DNA without prior deactivation by other biological nucleophiles. An extremely labile bond will most likely lead either to deactivation before arrival at the tumor site or toxicity owing to accumulation of platinum in healthy tissue. An inert leaving group will reduce the level of

DNA-bound platinum, with a high proportion of the compound passing through the body intact. Chloride, defined as the preferred leaving group in the original formulation of platinum anticancer drug structure-activity relationships,¹ is also useful as a leaving group in the cationic class of compounds. Use of iodide as the leaving group, often available as a result of synthetic convenience, is expected to depart slowly from the platinum coordination sphere and lead to more sluggish DNA modification, as illustrated by the decrease in rate constant⁴⁴ for chloride vs. iodide for the reaction: $[\text{Pt}(\text{dien})\text{X}]^+ + \text{pyridine} \rightarrow [\text{Pt}(\text{dien})\text{pyridine}]^{2+} + \text{X}^-$.

In the design of compounds for activity in cisplatin-resistant tumors, replacement of a chloride ligand with a sulfoxide has provided compounds such as $[\text{PtCl}(\text{R}'\text{R}''\text{SO})\text{-(diamine ligand)}]\text{NO}_3$ that are active against cisplatin-resistant cell lines. The biological activity of these complexes in an L1210 leukemia mouse model was, in some instances, as good as that of cisplatin.³¹ The sulfoxide ligand has strong pi-acceptor properties, is labile, and is easily substituted. Consequently, it is possible that replacement by chloride ion occurs prior to cell entry. Moreover, reaction to form diaqua species intracellularly would facilitate in the formation of Pt-DNA adducts like those of cisplatin.⁴⁵

A summary of structural parameters for eight known cationic, monofunctional platinum(II) complexes is provided in Table 1. Weakening of the Pt–X₁ bond is reflected by variations in bond length shown in the table. Although this parameter probably plays a role by influencing the ability of compounds of this class to bind to DNA, a clear correlation between cytotoxicity and Pt–X₁ bond length is not apparent because of the variety of tumor models and cell types used to evaluate cytotoxicity. For one pair of compounds that were studied in the same model, the Pt–X₁ distance of 2.309(2) Å for *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{N}3\text{-cytosine})\text{Cl}]\text{Cl}$ is marginally shorter than the corresponding value of 2.312(4) Å for *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{N}1\text{-pyridine})\text{Cl}]\text{Cl}$. For this pair of compounds and in the sarcoma 180 ascites mouse model, the longer Pt–X₁ bond corresponded to slightly superior antitumor activity. *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{N}1\text{-pyridine})\text{Cl}]\text{Cl}$ showed a 103% increase in mean survival time, whereas *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{N}3\text{-cytosine})\text{Cl}]\text{Cl}$ resulted in a 92% increase in mean survival time.²²

Stability of Cationic Compounds

The stability of platinum(II) compounds with three am(m)ines and one leaving group is not always guaranteed. The possible loss or displacement of an am(m)ine by a biological nucleophile to form a bifunctional, potentially neutral species must be considered. For compounds with general formula *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{N-donor})\text{Cl}]^+$, ammonia remains bound even after extended incubation in the presence of excess dG,⁴⁶ sodium diethyldithiocarbamate, thiourea, cysteine, or methionine,⁴⁷ and neither pyridine nor ammonia is lost upon binding to DNA. The monofunctional adducts formed by this class of compounds pose a processing challenge to the cell that is distinctly different from that of bifunctional adducts of the kind formed by cisplatin and carboplatin. Examples of complexes that might be expected to be monofunctional and cationic, but which lose a ligand to form bifunctional adducts with DNA, include compounds of the form $[\text{PtCl}(\text{R}'\text{R}''\text{SO})\text{diamine}]\text{NO}_3$ ⁴⁵ and those with bulky ligands such *N*-methyl-2,7-diazapyrenium.^{48, 49}

Mechanisms of action

The activity of platinum(II) anticancer compounds involves in their chemistry with DNA in the nucleus. Aquation yields cationic, highly electrophilic species that react with various nucleophiles in the cell, including amino acid sulfhydryl groups and nitrogen donor atoms on nucleic acids.⁵⁰ Reactions with sulfhydryl groups have been invoked to explain platinum resistance.⁵¹ Reactions with DNA, the most nucleophilic sites of which are the N7 atoms of

guanosine bases in the major groove, are the critical anticancer interactions and induce major distortions in the DNA.⁵²

DNA distortions induced by platinum lesions^{53, 54} disrupt vital cellular processes, such as replication and transcription, and elicit cellular defense mechanisms. The cell has several programs for responding to platinum-induced DNA damage. Four types of pathways that assist the cell in managing DNA damage have been identified.⁵⁵ DNA can be repaired by one of several mechanisms that restore the undamaged state. Secondly, the cell cycle can be arrested by activation of a DNA damage checkpoint, a process that allows the cell time to repair the damage. Additionally, changes in the transcription of genes in response to DNA damage can aid cell survival. Finally, cells that have sustained significant damage undergo apoptosis, or programmed cell death.

Damage that involves covalent modifications of DNA is processed by DNA repair and recombination pathways that can be divided into five categories: direct repair, base excision repair, nucleotide excision repair, double-stranded break repair, and repair of interstrand cross-links.⁵⁵

The downstream events that evolve following identification of DNA damage, for which RNA polymerase II is especially important,^{56, 57} and lead to cell cycle arrest depend on the type of damage. Cisplatin modifications of DNA the cell cycle in S phase, eventually progressing to a block in G2 phase.^{58, 59} While the cell cycle is paused, changes in gene transcription occur that allow the cell to respond to the damage. Many enzymes involved in gene repair are not transcribed at significant levels until DNA adducts are detected, after which they are synthesized in sufficient quantity to endow the cell with the capability to repair the damage.⁶⁰ If the cell fails to respond sufficiently, it succumbs to the damage and apoptosis is triggered. Designing drugs that cause damage to DNA but do not induce cancer cells to mount a checkpoint response or begin DNA repair is crucial to the improvement of anticancer therapy.⁵⁵

Influx transporters and platinum compounds

The mechanisms by which platinum anticancer compounds accumulate in cells have been of interest since the early work on cisplatin, where it was already conjectured that cisplatin is taken up by passive diffusion through cell membranes.⁴ Although the small, neutral cisplatin may not need to take advantage of an active transport mechanism, new drug candidates that are either sterically more hindered, charged, or both, have been shown to interact with influx and efflux transporters. The role of cellular accumulation in determining sensitivity to platinum-based chemotherapy has recently been reviewed.⁶¹ It is of particular interest to determine how cationic complexes accumulate in the cell and to understand whether compounds can be designed to take advantage of the various modes of accumulation.

Human genes that encode transporters make up 3.0% of all open reading frames and code for a predicted 841 transporter proteins.⁶² Solute transporters are divided into four major classes, according to a transporter classification system.⁶³ Channels move water, ions, or hydrophilic small molecules down a gradient. Primary active transporters utilize ATP hydrolysis to drive the process. Secondary transporters operate by an ion or solute electrochemical gradient. Finally, group transporters are characterized by their requirement for modification of the substrate, such as by phosphorylation, during transport.⁶² In humans, the percentages of channels, primary transporters, secondary transporters, and phosphotransferases are 43.3, 14.9, 38.9, and 0, respectively.⁶²

As a class, secondary transporters accept a wide variety of substrates, including sugars, lipophilic molecules, cations and anions, and nucleosides. Organic cation transporters (OCTs) are members of the solute carrier family of secondary transporters (SLC22A) with a broad range of substrate specificity. Of interest for drug metabolism, they are found, among other organs, in the intestine, liver, and kidney⁶⁴⁻⁶⁶ and facilitate the movement of endogenous and xenobiotic substrates across cell membranes. The presence of these transporters in the intestine facilitates drug uptake from the gastrointestinal tract, whereas presence in the liver and kidney affects excretion of drugs and can cause renal and nephrotoxicity. Examples of drugs that are organic cations can be found among antihistamines, β -adrenergic antagonists, calcium channel blockers, and skeletal muscle relaxants⁶⁷ Examples of endogenous substrates of the organic cation transporters include guanidine, a small molecule formed during protein metabolism, and thiamine, which is needed for the metabolism of carbohydrates and lipids.⁶⁸

The presence of OCTs in the intestinal lining has both desirable and undesirable biomedical consequences. Organic cations that would otherwise not be orally bioavailable are removed from the intestine by organic cation transporters and passed into the bloodstream. Certain foods such as caffeine may interfere with the uptake of organic cations, as demonstrated for the model substrate MPP⁺ (1-methyl-4-phenylpyridinium).⁶⁹ Changes in pH of the contents of the small intestine may also affect the uptake of some drugs. Additionally, mutations in the transporters can lead to variability in drug accumulation among different patient populations, and the identification of such polymorphisms in human OCTs can be valuable for identifying patients at risk for adverse drug reactions.⁷⁰

Studies of the colorectal adenocarcinoma cell line Caco-2 under conditions that simulate the *in vivo* environment and allow differentiation between apical and basolateral membranes have clarified the location of OCTs.⁶⁶ The transporter hOCT1 is localized at the basolateral membrane and plays a role in uptake and efflux at the interstitium (Figure 5). Uptake of cations occurs under a normal membrane potential of -60 mV. Alternately, efflux of a particular cation can occur if the intracellular concentration becomes much higher than the extracellular concentration.

Other connections between transport and platinum sensitivity

Besides organic cation transporters, other transporters that have proved to be influential determining in the efficacy of platinum anticancer drug candidates. Folate receptor- α , which is overexpressed on the cell membrane of a variety of human tumors, has been targeted using platinum-folate constructs. An early example, carboplatin was modified with a folic acid-PEG construct to produce a complex that efficiently entered folate receptor-positive cells, but seemed then to be sequestered in a way that prevented reaction of the platinum with nuclear DNA.⁷¹ In a more successful example, a platinum(IV) complex was modified with a folic acid derivative at one axial position and a single-walled carbon nanotube at the other axial site.⁷² The Pt(IV) construct underwent reductive release of cisplatin inside the cell, producing increased platinum-DNA adducts in, and selective destruction of, folate receptor-positive cells.

The homeostasis of copper ions in the human body is mediated largely by the copper transporter CTR1 (SLC31A1), and this transporter has been linked to the accumulation of platinum compounds in cancer cells.⁷³ The presence of copper increases the accumulation but decreases the potency of cisplatin. Copper increases both accumulation and potency of $[\{trans\text{-PtCl}(\text{NH}_3)_2\}_2\{\mu\text{-trans-Pt}(\text{NH}_3)_2(\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2)_2\}]^{4+}$.⁷⁴

Other transporters that have been associated with the efficacy of platinum anticancer drugs and drug candidates include steroid receptors. Targeting cancer cells using platinum

complexes tethered to estrogen derivatives has been successful in pre-clinical experiments.⁷⁵⁻⁷⁷ The combination of estrogen and platinum complexes is of interest because estrogen potentiates the upregulation of HMGB1,⁷⁸ a protein that binds with high affinity to platinum-DNA adducts.

Influence of HMGB1 on cellular processes

The high-mobility group box-1 protein is an abundant, highly conserved protein that is a critical component of a wide variety of cellular processes. It binds to cisplatin-modified DNA with a specificity between 10- and 100-fold over unmodified DNA with K_d values ranging from 0.3-370 nM.⁷⁹ At a concentration of 8 μ M, HMGB1 inhibits the overall repair of cisplatin-DNA adducts > 70%, where the DNA substrate concentration is 20 pM.⁸⁰ This finding suggested that cells with high levels of HGMB1 may have increased susceptibility to cisplatin owing to decreased excision repair of the DNA adducts. In support of this hypothesis, the cytotoxicity of cisplatin was potentiated by pre-treatment of breast and cervical cancer tumor cells with estrogen and progesterone,⁷⁸ a protocol that increases the levels of HMGB1 in the nucleus.^{81, 82}

Paradoxically, increased expression of HMGB1 is also linked to enhanced resistance to cisplatin. Activity in the HMGB1 promoter region was 3- to 10-fold higher in cisplatin-resistant KB-CP20 cells than in the parental KB cell line.⁸³ These data and other correlations between HMGB1 and the cancer evolution have led to an alternate hypothesis. Cisplatin-modified DNA may sequester HMGB1, inhibiting its roles in the progression of cancer. In this model, cisplatin acts as an anti-HMGB1 agent in a way that may potentiate its anticancer drug activity.⁸⁴

The two hypotheses arise from the dual nature of HMGB1. It acts both as a transcription factor in the nucleus but also as a cytokine in the cytosol.

HMGB1 in the Nucleus

In resting, healthy cells, HMGB1 is found in the nucleus and acts as a transcription factor and chromatin remodeler. Two nuclear localization signal sequences, at amino acids 28-44 and 180-185, occur in the sequence of human HMGB1.⁸⁵ In its role as a transcription factor, HMGB1 is involved in the regulation of a limited number of genes. Steroid receptors, for example, including the progesterone and estrogen receptors, are activated upon steroid binding and bind to DNA at sites that contain hormone response elements of target genes, thereby stimulating their transcription. Because of its ability to induce substantial DNA bending, HMGB1 can assist in the formation protein-protein interactions involving these steroid receptors. The structural distortion also stabilizes the steroid receptor-DNA interaction. HMGB1 therefore enhances the interaction of the steroid receptor and its hormone response element, which leads to increased transcription of the associated genes.⁸²

HMGB1 in the Cytosol

In addition to its activity in the nucleus, HMGB1 is also released into the cytosol and into the extracellular environment by both active and passive mechanisms. Movement out of the nucleus occurs in cells dying by a deregulated, necrotic death and not in cells that succumb to apoptosis.⁸⁶

HMGB4

Besides HMGB1, there are several other members of the HMG-box family of proteins exist, which differ mainly in the organs where they are expressed. A recently identified member of the family, HMGB4 (NCBI Reference Sequence: NP_660206.2), has been characterized.⁸⁷

It is expressed in testis and sperm cells, which is of interest because of the particular efficacy of cisplatin in testicular cancer. Sequence alignment analysis (Figure 6) indicates that a disulfide bond formed in HMGB1 between Cys23 and Cys4588 cannot be formed in HMGB4 because of the absence of cysteine at position 23. Additionally, the acidic C-terminal tail in HMGB1, which reduces the affinity of HMGB1 for DNA,⁸⁹ is absent in HMGB4. The binding affinity of HMGB4 for cisplatin-modified DNA is unknown but, given the lack of disulfide bond formation and inhibitory acidic tail in HMGB4, it is likely to be strong. We hypothesize that this protein may be important in sensitizing testicular cancer cells to cisplatin through inhibition of nucleotide excision repair by the repair shielding mechanism discussed above.

Conclusions

Recent insights into the cellular processing of platinum compounds and the downstream effects of platinum damage to the cell have led to a diverse class of new compounds and have aroused interest in candidates long ago neglected. The major challenges that confront the synthetic chemist designer of platinum anticancer drug candidates include the imperative that new compounds be significantly different in their spectrum of activity from those of cisplatin, oxaliplatin, and carboplatin. Guidelines developed by the NCI and researchers in the field regarding potency, mechanisms of action, and similarity to drugs currently on the market and available in generic form will guide the development of the next generation of platinum-based anticancer drugs.

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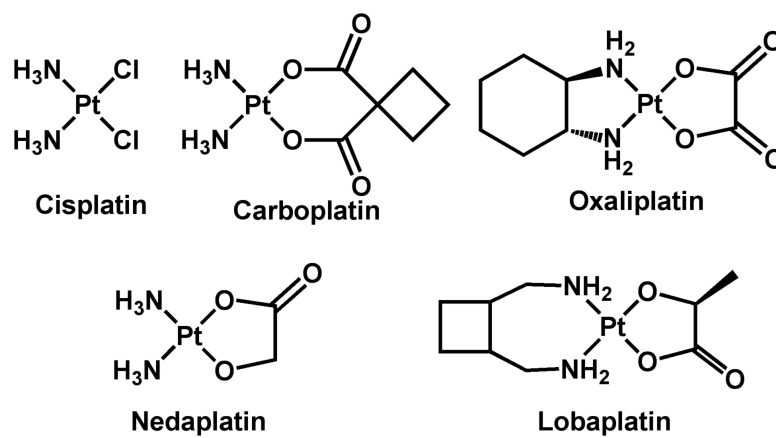


Figure 1.
Structures of the platinum compounds currently in clinical use.

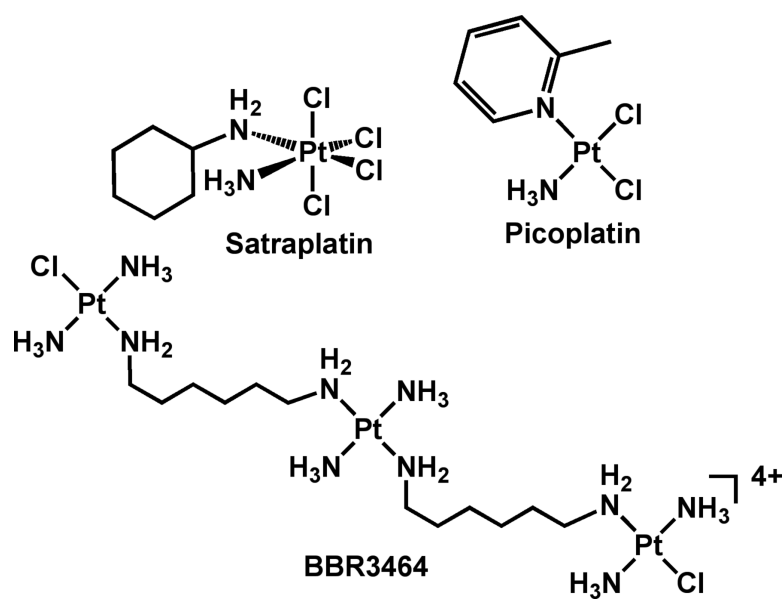


Figure 2.
New and non-traditional compounds in clinical trials.

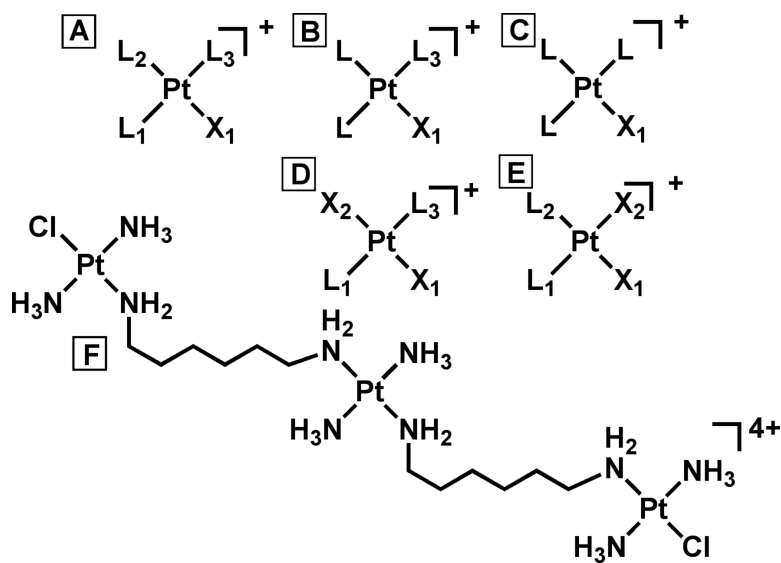
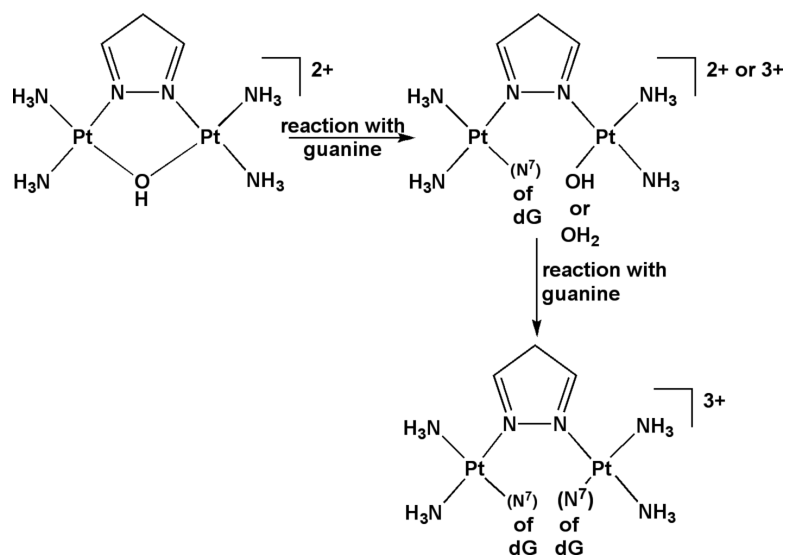


Figure 3. Structural frameworks for cationic platinum(II) complexes with antitumor activity.

**Scheme 1.**

Proposed reaction of $[(\text{cis-}[Pt(NH_3)_2])_2(\mu\text{-OH})(\mu\text{-pL})]^{2+}$ with adjacent dG residues.²⁵

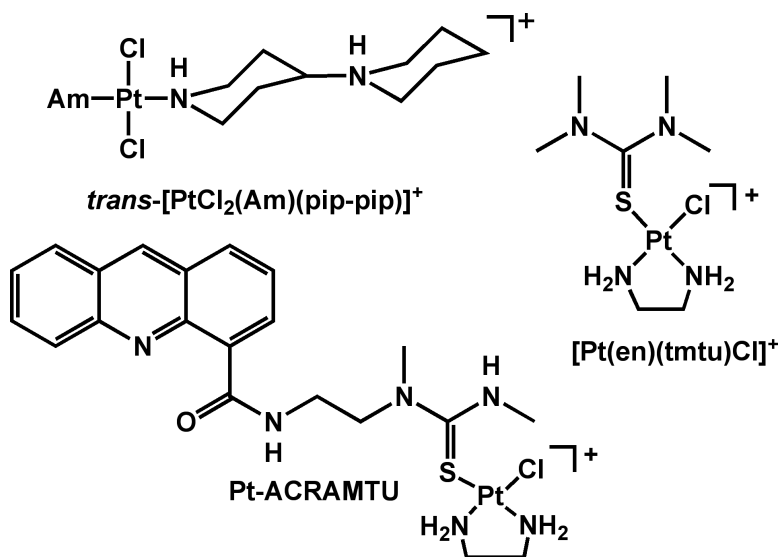


Figure 4.
Three cationic platinum(II) anticancer drug candidates.

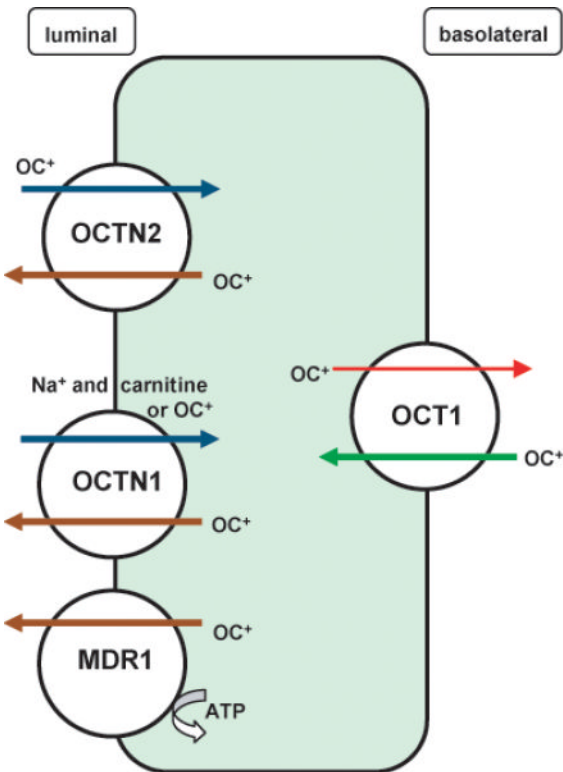


Figure 5.

Transporters of organic cations in enterocytes of the human small intestine. OCT1 transports cations in either direction depending on the electrochemical potential. In the presence of a normal membrane potential (-60 mV), cation uptake (thick arrows) is preferred. Efflux can occur if the intracellular concentration of the cation is 10 times higher inside the cell than outside the cell.


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HMGB4 : MGKEIQLKPKANVSSYVHFLNLRNKFKEQQPNTYVGFKEFSRKCSEKWR
HMGB1 : MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWK

HMGB4 : SISKHEKAKYEALAKLDKARYQEEMNYV---GKRKKRRKRDPQEP RPP
HMGB1 : TMSAKEKGFEDMAKADKARYEREMKTYIPPKGETKKKFK-DPNAK RPP

HMGB4 : SSFLLFCQDHYAQLKRENPNWSVVOVAKATGKMWSTATDLEKHPYEQRVA
HMGB1 : SAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTAADDKQPYEKKAA

HMGB4 : LIRAKYFELELYRKQCNARKKYRMSARNRCRGKRVRQS-----
HMGB1 : KIKKYEKDIAAVRAKGPDA-AKKGVVKAEKSKKKKEEEDEEEDDEE

HMGB4 : -----
HMGB1 : EEEDEEDEDDEEDDDDE

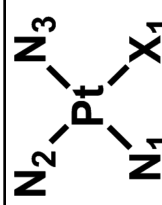
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Figure 6.

Sequence alignment of human HMGB4 and human HMGB1. Identical residues are highlighted in black, similar residues are highlighted in grey, and the Phe38, Cys23, and Cys45 residues are underlined.

Table 1

Structural parameters for some cationic complexes designed as antitumor drugs.



Compound	L ₁	L ₂	L ₃	X ₁	Pt-L ₁	Pt-L ₂	Pt-L ₃	Pt-X ₁	N ₂ -Pt-N ₁	N ₂ -Pt-N ₃	N ₁ -Pt-X ₁
<i>cis</i> -[Pt(NH ₃) ₂ (N7-cytosine)Cl]Cl 22	NH ₃	NH ₃	cytosine	Cl	2.045(7)	2.059(7)	2.033(7)	2.309(2)	90.1(3)°	90.1(3)°	91.5(2)°
<i>cis</i> -[Pt(NH ₃) ₂ (N7-pyridine)Cl]Cl 21	NH ₃	NH ₃	pyridine	Cl	2.054(13)	2.028(13)	2.002(13)	2.312(4)	89.9(6)°	89.2(5)°	89.7(4)°
[Pt(Me ₂ phac)(AmPIC)]I 40	2,9-dimethyl-1,10-phenanthroline		6-amino-2-picoline	I	2.044(6)	2.079(6)	2.036(6)	2.592(1)	80.9(2)°	96.7(2)°	98.8(2)°
[Pt ₃ (HPTA)PtCl ₃](ClO ₄) ₂ 41 (trinuclear compound with 3 monofunctional Pt centers)		2,2'-bis(pyridylmethyl)amine		Cl	1.986(8)	2.027(8)	2.001(8)	2.283(2)	81.8(3)°	85.1(3)°	96.6(2)°
[PtCl(en)(C ₁₀ H ₁₃ N ₄)](NO ₃) ₂ 34	en		N-[2-(acridin-9-ylamino)ethyl]-N'-methylpropionamide	Cl	2.013(7)	2.025(7)	2.023(3)	2.313(2)	83.3(3)°	90.9(2)°	90.5(2)°
[PtCl(en)(PIC-C-N)] 42 (neutral form, only closed form is cationic)	en		6-(methylpyridin-2-yl)acetate	Cl	2.033(3)	2.047(3)	2.048(3)	2.3093(9)	83.78(13)°	93.82(12)°	91.96(10)°
[Pt(bamtu)Cl] ⁺ 43			C-(6-aminomethylpyridin-2-yl)methylamine	Cl	2.033(6)	1.936(5)	2.040(6)	2.308(2)	83.2(2)°	81.4(2)°	97.4(2)°
[PtCl(dach)Pt(NO ₃) ₂]	dach		1,1,3,3-tetramethylthiourea	Cl	2.080(6)	2.042(6)	2.281(2)	2.298(2)	83.2(2)°	92.3(2)°	92.7(2)°