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The Platin-X Series: Activation, Targeting, and Delivery

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Abstract

Anticancer platinum (Pt) complexes have long been pronounced as one of the biggest success stories in the history of medicinal inorganic chemistry. Yet there still remains the hunt for the “magic bullet” which can satiate the requisites of an effective chemotherapeutic drug formulation. Pt(IV) complexes are kinetically more inert than the Pt(II) congeners and offer the opportunity to append additional functional groups/ligands for prodrug activation, tumor targeting or drug delivery. The ultimate aim for functionalization is to enhance the tumor selective action and attenuate systemic toxicity of the drugs. Moreover, increase in cellular accumulation to surmount the resistance of the tumor against the drugs is also of paramount importance in drug development and discovery. In this review, we will address some of the attempts in our lab to develop Pt(IV) prodrugs that can be activated and their targeted delivery using robust nanotechnology platforms.

Introduction

Platinum (Pt) compounds set their foot as chemotherapeutic agents following the accidental discovery of the inhibition of binary fission in *E. coli* bacteria by cisplatin when Barnett Rosenberg was studying the electrolysis of platinum electrodes in 1965.¹ Thus, began a surge in the synthesis and development of other potential platinum complexes that could negate the limitations posed by cisplatin treatment which includes systemic toxicities and drug resistance of various types of cancers.² With the approval of cisplatin, carboplatin, and oxaliplatin by the Food and Drug Administration (FDA) along with region specific approval of nedaplatin, lobaplatin, and heptaplatin (Figure 1); platinum complexes reflect one of the greatest success stories in the history of medicinal inorganic chemistry. Yet of late, there is a decline in the number of platinum compounds entering the clinical trials. With the profound knowledge of the mechanism of action of the drugs and the robust technologies available at hand, one can strive to formulate the next generation of platinum drugs to promote tumor targeted drug delivery, greater spatio-temporal control over the drug release at the tumor site, ameliorate the tumor selectivity, attenuate the systemic toxicity, and facilitate the enrichment

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of the active drug to specific cellular compartments to evade tumor resistance.^{3,4} In this review, we focus on the efforts of our group towards the development of platinum prodrugs that are suitably modified to achieve great efficacy with regard to their therapeutic potential.

Prodrugs are drug derivatives with abilities to undergo transformation to release the active drug for execution of the desired pharmacological effects.^{5,6} The drug release and activation of the prodrug may occur *via* different chemical pathways such as enzymatic action, redox reaction, photothermal activation, pH-dependent manner *etc.* In the present scenario of drug discovery, prodrug strategy has become the forerunner for ameliorating the physicochemical and pharmacological properties of potential drugs. Almost 10% of drugs approved worldwide can be classified as prodrugs.⁶

Recent trends in anticancer prodrug strategies show that the functionalized drug molecules have better tumor selectivity, aqueous solubility, lower toxicity, increased cellular uptake, and can overcome multidrug resistance.⁷ Pt(IV) complexes with kinetically inert d⁶ electronic configuration and six coordinate octahedral geometry are very attractive candidates for platinum based anticancer prodrugs.^{5,6} Not only can they avoid diversion to off-target biological nucleophiles before reaching the purine bases in nuclear DNA, the additional ligands also provide opportunities for tethering tumor targeting units, improve lipophilicity, enhance redox stability, and cellular uptake properties. The two additional ligands may also be exploited to append delivery vehicles such as nanoparticles (NPs) or other carrier systems. Pt(IV) complexes are known for DNA platination which takes weeks to occur.^{4,8} Unless prodrugs are reduced to the Pt(II) species, their therapeutic efficacy will be limited due to the systemic clearance from the body within a few hours to days.^{9,10} The hypoxic environment of the tumors is thought to facilitate the reduction of the Pt(IV) to the “classical active Pt(II)” species which is then expected to exhibit its therapeutic effect. The reduction can occur by cellular reducing agents such as glutathione or ascorbate *via* inner or outer sphere electron transfer. Furthermore, certain studies have revealed the role of high molecular weight cellular components specially proteins for the reduction.⁶

The other aspect of the prodrug strategy is to camouflage the active drug with a tumor targeting carrier molecule, preserving the targeting properties of the former and ensuring a controlled and systematic release of the latter at the requisite site.¹¹ This may be achieved by active or passive targeting. Active targeting of the drug relies on the interaction of the prodrug with a cell surface marker generally associated with the tumor such as an antigen or a receptor. Tumor associated receptors are well documented in the literature like transferrin, selectins, integrins, folate receptor, glucose transporter (GLUT), galectins, hyaluronic acid receptors, and the asialoglycoprotein receptor.¹² The prodrugs bearing the ligands bind to the receptors and are delivered inside the cancer cells *via* receptor mediated endocytosis. On the other hand, passive targeting aims to achieve greater drug accumulation in the tumors using large molecules or NPs as inert carriers that do not necessarily interact with tumor cells but have a strong influence on the bio-distribution of the drug. This is manifested in the enhanced permeability and retention (EPR) effect.¹³ The rapid proliferation of the vascular endothelium in tumors result in an increase in the number of fenestrations compared to the normal vessels, ranging from 200 nm to 1.2 μm .¹⁴ Consequently, macromolecules such as NPs can cross the tumor endothelial barrier passively through these fenestrations. The lack

of a proper lymphatic network does not allow the effective removal of excess fluid from the tumor tissue. All the factors combined together makes tumors susceptible to the circulating macromolecules, which can accumulate and remain there for substantial long periods of time.

In recent years, extensive research has led to the development of various approaches to minimize the systemic toxicity and drug resistance of Pt-based anticancer drugs. The ultimate goal yet to be achieved is to develop the “magic bullet” which will be specific for tumor tissues, can be administered at low dose, have minimal side effects and demonstrate an excellent overall therapeutic index. Since cancer cells show an altered metabolic pathway, glycosylated Pt-terpyridine complexes were developed and these complexes were found to be several hundred folds more cytotoxic than cisplatin.¹⁵ Monosaccharide conjugated to oxaliplatin like species were also developed and moderate *in vitro* cytotoxicity was observed for these complexes in various cancer cells.¹⁶ Adamantane conjugated carboplatin species were encapsulated in β -cyclodextrin (oligosaccharide comprising 6–8 glucopyranoside units) which showed better cytotoxicity to human neuroblastoma compared to carboplatin alone.¹⁷ On a similar line of thought, bisphosphonates have shown high affinity for bone tissues or other calcified tissues and were thus thought to have the potential to target the Pt drugs to the bones.¹⁸ Bisphosphonate tetraethyl ester analogues of picoplatin have shown some promising results in this regard.¹⁹ Another strategy is to use peptides and proteins as carriers for Pt(IV) prodrugs.²⁰ Integrins and aminopeptidase which can recognize specific peptides such as arginylglycylaspartic acid (RGD) and Asn-Gly-Arg (NGR) are upregulated in endothelial cells and hence these cell surface proteins hold promise as targets for chemotherapy.^{21,22} Thus, Pt(IV)-RGD conjugates were developed that were specifically cytotoxic to integrin overexpressing cell lines. Similar Pt(IV)-NGR conjugates were found to be less active than Pt(IV)-RGD complexes but are more cytotoxic than the nonspecific Pt-peptide controls.²³ Examples of Pt prodrug complexes or Pt-conjugated to various targeting motifs improving the efficacy and reducing side effects of platinum based chemotherapeutic agents are enlisted in Table 1. Different strategies of therapeutic activation, delivery, and targeting are pictorially represented in Figure 2.

Organelle specific targeting of anticancer agents adds a new dimension to drug discovery and development research. A clear understanding of the role of different organelles in the disease is of prime importance if one endeavors to deliver the drug in its active form to its target site of action inside the cell. The nucleus forms the spearhead of the eukaryotic cell; thus regulation of gene expression and other cellular processes are virtually controlled by the nucleus alone. Nuclear DNA (nDNA) is a potent target for chemotherapeutic agents and the mechanism of action of cisplatin elucidates how the nuclear DNA is platinated, resulting in inhibition of DNA replication and mitosis. Though thought to be a promising strategy, recent research established that base/excision repair mechanisms operative inside the nucleus render these molecules ineffective with repeated doses.^{24–26} Mitochondria targeting is an attractive alternate strategy for the development of chemotherapeutic agents. Mitochondria are the hub of various cellular processes including oxidative phosphorylation (OXPHOS), regulation of apoptosis, and modulation of the intracellular calcium concentration. Mitochondrial dysfunction due to the glycolytic pathway was first implicated by Otto Warburg and came to be known as the Warburg Effect.^{27,28} The most well studied method of

mitochondria targeting exploits the mitochondrial membrane potential (Ψ_m) which prevails across the membranes and permits positively charged lipophilic moieties to cross the barriers. Targeting the lysosomes, endosomes, Golgi apparatus and endoplasmic reticulum also holds promise and are new targets for upcoming cancer drugs.²⁹

Another challenge for improving drug efficacy is to ensure selective delivery of the molecule at the tumor site. There are multiple options available for developing a robust drug delivery platform and extensive research is still being conducted for the ideal formulation. Nanotechnology holds promise to resolve many of the current drawbacks and can provide many therapeutic advantages, such as, better aqueous solubility of drug, protection of the drug under physiological pH, increased blood circulation time, controlled release, greater spatio-temporal control over drug release, and co-delivery of multiple drugs for combination therapy.³⁰

Polymers such as poly(amino acids), poly(amidoamine) dendrimers, polyesters poly lactide (PLA), poly glycolide (PGA), poly(lactide-*co*-glycolide) (PLGA), and *N*-(2-hydroxypropyl)methacrylamide (PHPMA) are used successfully as carriers for Pt-based compounds. Polymeric NPs are thought to improve accumulation of platinum drugs in tumors by the EPR effect. Pt-based compounds that are encapsulated into the inner core of polymeric micelles by chemical or physical means provide relatively high stability to the drug molecules. Liposomal formulations of platinum drugs like LipoplatinTM and AroplatinTM which are derivatives of cisplatin and oxaliplatin, respectively have made their way to the clinical trials.¹⁴ Recent studies evidenced the potentials of carbon nanotubes as carriers for drug delivery because of their unique physicochemical and physiological characteristics. Single walled carbon nanotubes (SWCNT) and single walled carbon nanohorns are mostly used for delivery of Pt-based anticancer agents.³¹ The low toxicity, easy surface functionalization and high loading capacity make these attractive drug delivery agents.^{32,33} There has been a surge in the number of research articles demonstrating the excellent drug delivery properties of poly(lactic-*co*-glycolic acid) (PLGA)-PEG polymers because of their biocompatibility and effective clearance from the body.³⁴ Among the other drug delivery platforms, mesoporous silica nanoparticles (MSNPs) and metal organic frameworks (MOF) have shown interesting results which may translate to an effective formulation for drug delivery.^{35,36} MSNPs facilitate the diffusion of loading of drugs into the microchannels, and prevent prerelease of the loaded drugs. The pore structure of the MSNPs can be easily modulated by varying the surfactants and thus can be used to load different types of chemotherapeutic agents, proteins, siRNAs or DNA.³⁵ On the other hand MOFs, have emerged as a suitable platform for drug delivery, due to the high drug loading capacity, biodegradability, and versatile functionality.³⁶ These are a new class of hybrid materials comprising metal ions and organic bridging ligands with high surface areas and large pore sizes for drug encapsulation. Pt-based nanoscale coordinating polymers were developed as composites to impart anticancer effects.^{37,38} Significant progress was achieved to activate, target, and deliver potential drug candidates to targeted sites. In the following sections, we discuss our endeavor to develop novel Pt-based systems, the Platin-*X* series (Figure 3, the concept of this drawing was adapted based on a recent review³⁹), that were synthesized by new strategies, in search for new targets to overcome the many hurdles of Pt-chemotherapy.

Activation

Among the various modes of activation of Pt prodrugs, reduction of Pt(IV) prodrug to the active Pt(II) drug under the influence of reducing cellular environment is the one of the most widely utilized approach.⁶⁵ Along this direction, we and others have worked towards the development of novel Pt(IV) prodrugs that not only evade cisplatin resistance characteristic to cancer cells but also perform multiple therapeutic roles.⁶⁶ Here, as we discuss these examples, one would see how these prodrugs release multiple active entities upon intracellular activation and affect various pathways to bring a combined effect (Figure 4).

1. Platin-A

Prostate cancer in the castrate setting is often associated with an array of complex immune-mediated processes and inflammation which in turn contributes to metastasis.⁶⁷ Nonsteroidal anti-inflammatory drugs (NSAIDs) are capable of tackling chemotherapeutics associated inflammation during cancer treatment.⁶⁸ Cyclooxygenase isoforms are the primary targets of NSAIDs and acetylsalicylic acid or aspirin is one of the most widely used NSAIDs that are known to inhibit COX-1 and COX-2 irreversibly, thereby inhibiting prostaglandins biosynthesis.^{69,70} Also, aspirin along with its metabolite, salicylate, is capable of inducing the secretion of anti-inflammatory cytokines.⁷¹ So, we decided to use aspirin as a means of chemoprevention during cisplatin treatment. Since free drug administration suffers obstacles like definitive exposure to the target sites and different biodistribution and pharmacokinetics at the target organs, we decided to design a Pt(IV) prodrug that releases cisplatin and aspirin simultaneously upon reduction.

Platin-A is the first ever example of a cisplatin analogue that has aspirin as one of the axial ligands (Figure 4A and 4B).^{42,72} Standard biochemical experiments showed that it is capable of forming Pt(II) adducts with deoxyguanosine 5'-monophosphate (5'-dGMP) which is indicative of the fact that Platin-A is capable of releasing cisplatin which can bind to nuclear DNA and stall replication in cells. It showed remarkable cytotoxic potential against both androgen-responsive LNCaP cells and androgen-unresponsive PC3 and DU145 cells with IC₅₀ values comparable to that of cisplatin. A high level of apoptotic cell deaths induced by Platin-A in cancer cells was found to be similar to that of cisplatin while aspirin alone didn't show any significant cytotoxic potential.

High performance liquid chromatography (HPLC) studies confirmed the release of aspirin from Platin-A upon reduction, which further gets converted to salicylic acid. Both aspirin and salicylate are potent inhibitors of COX-1 and COX-2 and so Platin-A could potentially reduce tumor-associated inflammation. COX inhibitory property of Platin-A was assessed using biochemical assays. It is also capable of reducing tumor necrosis factor- α (TNF- α) induced COX-2 secretion in PC-3 cells. Pro-inflammatory cytokines, IL-6 and TNF- α , activate NF- κ B which in turn modulates inflammation-induced carcinogenesis. Platin-A not only suppressed LPS-induced secretion of IL-6 and TNF- α it was also able to enhance production of anti-inflammatory cytokine, IL-10.

Shortly after our report on Platin-A, Liu *et al.* reported similar findings on the same aspirin-cisplatin complex which was termed as Asplatin (Figure 4A).⁴³ Asplatin demonstrated

significant inhibitory effect on the growth of cancer cells. In a follow up publication, this group also demonstrated *in vivo* properties of Asplatin using a self-assembled nanocarrier.⁷³ In this regard, Hey-Hawkins and coworkers also reported covalently linked conjugates of cisplatin and cyclooxygenase COX inhibitors which demonstrated similar mechanism of action after intracellular cleavage of the components (Figure 4A).⁷⁴ They used indomethacin or ibuprofen as the axial ligands. Preliminary studies could not decipher the precise reasons for improved cytotoxicity and increased lipophilicity of the overall molecules leading to greater cellular uptake was thought to be the predominating factor. Although the indomethacin conjugates were potent COX inhibitors, they were found to operate by COX independent mechanisms and showed similar cytotoxicities in different cell lines irrespective of COX-2 expression.

Platin-*A* proved to be a unique example of chemo-anti-inflammatory molecule that releases active Pt(II) drug, cisplatin, and anti-inflammatory aspirin concurrently upon activation inside cells. Cisplatin, by virtue of its anti-proliferative properties, kills the cancer cells apoptotically whereas aspirin participates in tumor-related inflammation at the site of treatment. To achieve a control over the relative stoichiometry of the chemotherapeutic drug and the anti-inflammatory drug, we designed a cocktail NP which is highlighted in the targeting and delivery section of this review.

2. Platin-*B*

Cancer cells are known to develop cisplatin resistance and among the various ways of cisplatin detoxification, one is the reaction of aqueous cisplatin with glutathione either spontaneously or catalyzed by GSH-S-transferase- π enzyme.⁷⁵ This reaction results in the formation of GS-Pt adducts that get excreted out of the cells actively by glutathione S-conjugate export (GS-X) pump.^{76,77} Another way in which GSH gives rise to cisplatin resistance is by enhancing Pt-DNA adduct repair mechanism.⁷⁸ GSH is also capable of reducing reactive oxygen species and thereby suppressing apoptosis in cancer cells. Other thiol containing proteins, metallothioneins, are also known to detoxify cisplatin.⁷⁹

Designing of Pt(IV) drugs could act as an alibi to avoid deactivation of the drug by the high levels of GSH in resistant cancer cells. A combination of such Pt(IV) prodrugs with thiol reactive alkylating agents such as pipobroman would act as a protecting shield for the active drug from intracellular thiols.⁸⁰ Keeping this approach in mind, Platin-*B* was developed which is a combination of alkylating agent, and classic DNA cross-linker, cisplatin (Figure 4).⁸¹ *In vitro* reduction of Platin-*B* with ascorbic acid verified our hypothesis that it is capable of releasing Pt(II) species. Under such simulated reducing environment, Platin-*B* leads to the formation of adducts with 5'-dGMP confirming the DNA cross-linking ability of the released Pt(II) species. On the other hand, interaction of Platin-*B* with the most abundant intracellular reductant, glutathione, formed a new Pt(IV) species. This observation supported the initial hypothesis that Platin-*B* forms a Platin-*B*-GSH adduct which in turn protects the Pt(II) center from thiol-induced deactivation. This also reinforces the statement that the prodrug is capable of interacting with cellular thiols.

Platin-*B*, when tested for its cytotoxic properties in a number of cell lines showed that is active in all those cells irrespective of whether it is cisplatin sensitive or resistant. Moreover,

it is many folds more cytotoxic when compared to its analogous molecules or the parent drug, cisplatin. Addition of classical γ -GCS inhibitor, L-buthione sulfoximine (BSO)⁸², or GSH did not have any significant effect on the cytotoxic potential of Platin-*B* which further indicates the GSH-resistant property of this Pt(IV) prodrug.

Several other qualities of the Platin-*B* make it more efficient prodrug than its analogous compounds or the parent drug cisplatin. This prodrug, by virtue of its high lipophilicity accumulates in greater quantities inside the cells. Pt-mt-DNA adducts were detected in addition to nuclear DNA-Pt adducts in cells treated with Platin-*B* which indicated mitochondrial localization of the compound. Hexokinase 2 protein is overexpressed on outer mitochondrial membrane and the ability of Platin-*B* to associate itself by using the pendent – Br groups to this protein was proposed to be the mechanism of mitochondrial association of the molecule.⁸³ Analyses of oxygen consumption rates (OCRs), a measure of mitochondrial health, of cells treated with Platin-*B* in presence of additives which affect mitochondrial electron transport showed significant inhibition of mitochondrial respiration. Inhibited citrate synthase activity of treated cells signified loss of mitochondrial mass further confirming that mitochondria are also targets for this Pt(IV) prodrug. So, the design of Platin-*B* not only acts as a protective shield for the active drug, it also helps it in getting taken up by the cells because of high lipophilicity and possibly HK 2 mediated mitochondrial uptake. These aspects of the compound help it to evade GSH mediated detoxification and enhanced nuclear DNA repair rates in resistant cancer cells.

3. Platin-Az and Platin-CLK

Kinetically inert Pt(IV) complexes with two available axial ligand sites for the incorporation of suitable ligands serve as attractive candidates for the development of novel Pt(IV) prodrugs. Acid anhydrides are widely used for introduction of multiple functionalities on the Pt(IV) metal center.^{42,81} However, acid anhydrides, due to their low stability may end up giving impure products and low yields. Also, a large number of molecules of interest may lack an acid functionality making them unsuitable for transformation to their corresponding anhydrides. Although click chemistry may appear to be an easy alternative strategy for the installation of a molecule of interest onto a Pt(IV) complex, one must be skeptical to use copper(I) in such reactions in order to avoid the possibility of Pt(IV) reduction by the Cu(I).^{84,85} Recently, click like approaches were used to introduce functional groups on to the Pt(IV) scaffolds using triazole, maleimide, and hydrazone as connecting moieties.^{83,86,87} Strain-promoted alkyne-azide cycloaddition (SPAAC) approach^{88–90} enabled us to overcome this hurdle and provide an alternative reaction pathway for easy installation of azadibenzocyclooctyne (ADIBO) conjugated functionalities on Pt(IV) prodrugs.

As a proof of concept, we have synthesized a new Pt(IV) complex, Platin-Az, with terminal azides appended to the axial ligands which can be used as a precursor for a wide range of SPAAC reactions with ADIBO derivative of a molecules of interest (Figure 5A). A one-step reaction of Platin-Az with ADIBO-COOH resulted in its conversion to Platin-CLK (Figure 5B).⁹¹ The ease of performing this reaction successfully indicates that SPAAC approach can be used to suitably functionalize a Pt(IV) compound with multiple therapeutics, targeting moieties, imaging probes, delivery vehicles and many more. Attaching the ADIBO

functionality did not change the redox potential of the Pt(IV) center significantly. Moreover, the relatively non-toxic nature of Platin-CLK when compared to Platin-Az makes it a safer option to use it for theranostic applications as well.

In an attempt to explore the potential of Platin-CLK to be used successfully as a near-IR emitting fluorescent reporter, ADIBO-Cy5.5 was clicked onto Platin-CLK to synthesize Platin-Cy5.5 (Figure 5B). Confocal microscopy studies reveal that cells can easily take up Platin-Cy5.5 and the intracellular localization behavior of the molecule can easily be monitored as a bright near-IR fluorescent signal. The heavy metal fluorescence quenching effect of Pt(IV) center is also minimal because of the long chain linker separating the metal center and the fluorophore. Small molecule based technologies, owing to their poor biodistribution and pharmacokinetic properties, face hurdles in getting translated clinically. NPs, on the other hand, are promising candidates as drug delivery vehicles because of their longer circulation lifetimes, lesser off-target effects and controlled drug release.^{92–94} Platin-CLK, when encapsulated in biodegradable PLGA-*b*-PEG nanoparticles, showed much higher loading efficiencies compared to the parent molecule Platin-Az. This further shows the potential of this technology to be translated to a nanoformulation for clinical usage.

Platin-CLK is a proof of principle that presents SPAAC as an alternative strategy for its one-pot conversion to a multifaceted Pt(IV) prodrug performing multiple functions at the same time. Another example of this strategy is the design and synthesis of Platin-M where a mitochondria targeting triphenylphosphonium moiety was clicked onto Platin-Az. This has been discussed in the targeting and delivery section of this review.

As an alternate approach for the synthesis of Pt(IV) prodrugs, Pichler *et al.* reported maleimide-functionalized complexes as a novel synthetic platform.⁸⁶ They reported the first class of Pt(IV) complexes with maleimide ligands at the axial positions which could be further used to tether thiol containing targeting groups with an ease. Human serum albumin (HSA) which contains a single free thiol group was used to prepare drug–HSA conjugates using maleimide as a coupling moiety as it accumulates in malignant tissues due to EPR effect. The efficacy of the conjugates was tested in murine CT-26 colon cancer models *in vivo* which showed their potent anticancer properties and reduced tumor growth. Zheng *et al.* reported a series of Pt(IV) prodrugs with the axial ligands mimicking fatty acids. The rationale was to exploit HSA as the delivery vehicle for the platinum prodrugs.⁹⁵

Ang and coworkers attempted to expand the application of Pt(IV) scaffold as cisplatin prodrugs using novel chemo-selective ligation strategies.⁹⁶ In this regard, their efforts towards the development of hydrazone or oxime bond by chemo-selective reaction between amine and carbonyl group is noteworthy. Such reactions are among the fastest and mildest bio-conjugation reactions which occur in aqueous medium at physiological pH and with low stoichiometric concentrations of the reactants. As a proof of concept, the authors reported a six amino acid peptide as a mimic of the anti-inflammatory protein ANXA1 which demonstrated anti-proliferative activities in various cell lines.

Targeting and Delivery of Platin-X

Incorporating targeting moieties in the core structure and developing suitable delivery vehicles with additional targeting ligands can markedly improve therapeutic index of Pt-based compounds. This strategy can effectively shield the active drug from other biological species to prevent off-target attack. Though the mechanism of cisplatin uptake by cells is still a controversial debate that the therapeutic action relies on the formation of platinum adducts with the purine bases.^{2,97,98}

1. Platin-M

The major concern for cisplatin therapy is however the chemoresistance acquired by the cancer cells with prolonged or repeated dosage. This is related to the extensive repair of Pt-nDNA adducts in the nucleus using nucleotide excision repair (NER). Thus, in our group we developed a Pt(IV) prodrug, Platin-M to target mtDNA with the aim of overcoming chemoresistance, since mitochondrial base excision repair machinery does not have ability to repair Pt-mtDNA adducts (Figure 6).⁹⁹ Recently, a collaborative work from Kelley and Lippard groups documented anticancer properties of a mitochondria-acting Pt(II) compound.¹⁰⁰

There are several studies that were focused on the possibility whether cisplatin can act on mitochondria, the hub of various cellular processes.^{101–104} Given that mitochondrial base excision repair machinery is inactive in repairing Pt-DNA adducts, targeting Pt-based prodrugs to the mitochondria can provide an alternative to conventional chemotherapy. Furthermore, mitochondrial dysfunction is associated with the process of carcinogenesis and the metabolic reprogramming in cancerous cells makes the mitochondria a potent target for cancer specific chemotherapy. Thus, we developed Platin-M, a hydrophobic, mitochondria targeting Pt(IV) prodrug which can accumulate in the hyperpolarized mitochondria of tumor cells where upon *in situ* reduction it can generate the active Pt(II) species (Figure 6).^{8,99} Platin-M was synthesized by appending two delocalized and lipophilic triphenylphosphonium (TPP) cations at the axial positions of the cisplatin core using a SPAAC reaction between Platin-Az and azadibenzocyclooctyne coupled to a TPP unit which is mentioned in the aforementioned section. For potential *in vivo* translation with improved biodistribution and pharmacokinetic properties of this Pt(IV) prodrug and to adopt a dual targeting approach, Platin-M was encapsulated in a PLGA-PEG polymer functionalized with a TPP unit (PLGA-*b*-PEG-TPP)^{105,106} with demonstrated mitochondrial association properties and long blood circulation half live, and optimum distribution parameters (Figure 6). The NPs formed from this polymer uses a hydrophobic, delocalized –TPP cation covered surface for their mitochondrial association by utilizing the substantial negative mitochondrial membrane potential which exists across the membranes. The targeted Platin-M bearing NPs (T-Platin-M-NPs) exhibited pronounced brain accumulation properties compared to the non-targeted analogue (NT-Platin-M-NP) in a rat model.⁹⁹ Analysis of intracellular components mitochondria, cytosol, and nucleus isolated from PC3 cells treated with cisplatin, Platin-M, NT-Platin-M-NPs, and T-Platin-M-NPs demonstrated higher Pt concentration in the mitochondrial proteins and greater Pt-mtDNA adduct formation when Platin-M was delivered with T-NPs. Platin-M alone or encapsulated in the T-NP was also

more cytotoxic compared to cisplatin in human neuroblastoma SH-SY5Y, human prostate cancer PC3, and cisplatin resistant ovarian cancer A2780/CP70 cells. Platin-M displayed higher activity than cisplatin in the resistant cell line and the efficacy escalated ~5 times with the T-Platin-M-NPs. Further evidence of mitochondrial activity of Platin-M or T-Platin-M-NPs was manifested in the significantly low basal oxygen consumption rate (OCR) levels of cells treated with Platin-M or T-Platin-M-NPs. JC-1 red/green fluorescence measurements in SH-SY5Y cells verified the loss of membrane potential when treated with T-Platin-M-NPs. Thus, Platin-M represents a unique dual targeting strategy for delivering a cisplatin prodrug to the mitochondria that lacks the NER machinery which may be beneficial for cisplatin resistant cancers.

These results motivated us to conduct *in vivo* studies in a canine model which has higher anatomical and physiological similarity to human. Safety profile of T-Platin-M-NPs was determined for a period of 14-day.¹⁰⁷ The T-Platin-M-NPs showed greatly improved cytotoxicity and mitochondria toxicity in canine brain tumor cells *in vitro*.¹⁰⁷ The *in vivo* studies in beagle demonstrated safety profile of T-Platin-M-NPs with no obvious nephrotoxic side effects, no adverse effects in brain, and no significant alterations in the major organ samples upon 2.2 mg/kg dosing of T-Platin-M-NPs up to 14 days. Further preclinical safety and therapeutic studies are needed before realization of clinical applications of T-Platin-M-NPs.

2. Platin-4 Cocktail NP

The development of Platin-*A* has added a new dimension to the idea of combination chemotherapeutics. This strategy modulates various pathways inside the cancer cells and overcomes resistance mechanism thereby offering maximum therapeutic effect. As described in one of the earlier sections, Platin-*A* simultaneously delivers cisplatin and the well-known anti-inflammatory drug aspirin. Platin-*A* demonstrated that the use of combination of cisplatin with anti-inflammatory agents can provide better therapeutic outcome over the use of standard therapies under *in vitro* settings.⁴² Platin-*A* was capable of inhibiting COX-1 and 2 comparable to aspirin treatment and showed cytotoxic effects similar to cisplatin alone. These results warrant exploration of *in vivo* therapeutic properties of Platin-*A* and thus we are actively involved in combining anti-inflammatory drugs to chemotherapeutics such as cisplatin. However, when drug combinations are administered in their free forms, the major challenges faced are: correct choice of dosages of individual drug to achieve the correct ratio of the combinations which can show synergistic effects, exposure of individual drugs to their targets of interest, control over individual biodistribution patterns and pharmacokinetic parameters. The use of prodrug as we described in case of Platin-*A*, we tend to face challenges when low molecular weight compounds are used under *in vivo* settings. Polymeric NPs of biodegradable polymers offer advantages of controlled drug release, enhanced stability of individual drugs, and the ability to carry thousands of multiple drug molecules per NP. Thus, one way to combine cisplatin with aspirin will be to use a backbone functionalized polymers such as PLA or PLGA. However, a key shortcoming of these polymers is the lack of functional groups on the aliphatic backbones restricting the number of sites for chemical conjugation of drug combinations. Moreover, backbone functionalized poly(lactide) (PLA) or PLGA derivatives disrupts the biodegradable FDA structure of these

polymers¹⁰⁸ and raises the questions of potential immunogenicity, toxicity, and biodegradability. Often construction of such multi drug containing polymers requires rigorous functionalization of drug molecules, use of metal-based catalysts which might compromise the safety profiles of such combination therapeutic containing polymers or their NPs.¹⁰⁹ The idea behind the design of Platin-4 cocktail NP is to take this Platin-*A* concept to the next level wherein; one can achieve control over the stoichiometry of the drugs to be added in combination. Also, clever design of the molecule allows temporal control over the release of the active drugs inside the cells. To circumvent these limitations and establish therapeutic efficacy, we recently adopted a unique approach to synthesize PLA with multiple terminal functionalities keeping the polymer backbone close to the FDA-approved skeleton.^{72,110} We achieved this by end functionalization with biodegradable dendrons with varied number of functionalities for loading of Pt(IV) prodrug and aspirin with precise and predictable loadings. The strategy utilized a combination of click chemistry and dendrons to functionalize the two ends of a PLA polymer with a Pt(IV) prodrug and aspirin with a ratio of 4:3 (Figure 7). The Pt(IV) compound, Platin-4, used in this polymer is shown in Figure 7. Quantitative analyses indicated ~71 wt% conjugation of Platin-4 and ~75 wt% aspirin conjugation in (Asp)₄-PLA-(Platin-4)₃. The Janus polymer, (Asp)₄-PLA-(Platin-4)₃, resulted controlled release NPs upon blending with a co-block polymer such as PLGA-*b*-PEG-OH (Figure 7) with abilities to release the Pt-compound and aspirin following temporal release patterns. The choice of linkages to attach the drug molecules to the PLA polymer allowed controlled and temporal release of both aspirin and Pt(IV). The Pt(IV) prodrugs were linked to the PLA ends via one of the two axial ligands while aspirin was tethered to PLA using ester bonds. As a result, Pt(IV) gets released in response to a stimulus that is reducing in nature whereas aspirin gets released under the action of esterases. This polymeric platform opens up several possibilities such as the surface charge of the NPs resulting from the Janus polymer can easily be varied by blending with a suitable block copolymer (Figure 7), intracellular or extracellular targeting properties can be achieved by using an amphiphilic polymer with an appropriate terminal ligand (Figure 7). The wide plethora of controlled release polymeric NPs that exist, are unable to deliver combination of two or more drugs at a desired therapeutic ratio. The technology developed herein, used functionalized biodegradable polymers to deliver a combination of therapeutics at a specific stoichiometric proportion. This technology also enabled stimuli responsive control over the release of the drugs. This can serve as a template for the synthesis and development of NP mediated delivery of combination therapeutics for diseases requiring multi-drug treatment.

Conclusions

In the quest for ideal Pt-based anticancer formulations, we are actively addressing various facets of drug discovery and development. Our strategies are based on the activation of Pt(IV) prodrugs with suitable functionalities to target specific tumors and/or certain organelles and ensure effective release/delivery of the active drug at the requisite time. Platin-*A* which was formulated using anticancer drug, cisplatin and anti-inflammatory agent, aspirin exhibited excellent cytotoxicity profile and distinct ability to suppress intracellular COX-2. Platin-*B*, a combination of cisplatin and a potent alkylating agent mimicking pipobroman could induce changes in mitochondrial functions forming adducts with mt-

DNA, leading to the loss of mitochondrial mass in resistant cells. Platin-Az was synthesized as a tool to help us incorporate additional functionalities with ease. As examples, we demonstrated synthesis of Platin-CLK and Platin-Cy5.5 using Platin-Az precursor and a novel SPAAC approach. Platin-M reflects one of the many uses of the Platin-Az formulation where a mitochondria targeting moiety was successfully appended on the Pt-prodrug to detour cisplatin to avail the mitochondria pool to exert its therapeutic effect. The NP cocktail comprising an anti-inflammatory agent and a chemotherapeutic unit mounted on dendrons and attached with a linker is yet another manifestation of our unique strategies to rationally design drugs for combination therapy. It is now widely accepted that no “wonder drug” alone can tackle the multiple factors associated with cancers. In this regard, we can only strive to develop robust formulations for multiple drug cocktail therapy to make a major impact on the therapeutic efficacy.

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References

1. Rosenberg B, Van Camp L, Krigas T. *Nature*. 1965; 205:698. [PubMed: 14287410]
2. Wang D, Lippard SJ. *Nat Rev Drug Discov*. 2005; 4:307. [PubMed: 15789122]
3. Wang X, Wang X, Guo Z. *Acc Chem Res*. 2015; 48:2622. [PubMed: 26247558]
4. Johnstone TC, Suntharalingam K, Lippard SJ. *Chemical Reviews*. 2016; 116:3436. [PubMed: 26865551]
5. Rautio J, Kumpulainen H, Heimbach T, Oliyai R, Oh D, Jarvinen T, Savolainen J. *Nat Rev Drug Discov*. 2008; 7:255. [PubMed: 18219308]
6. Huttunen KM, Raunio H, Rautio J. *Pharmacol Rev*. 2011; 63:750. [PubMed: 21737530]
7. Singh Y, Palombo M, Sinko PJ. *Curr Med Chem*. 2008; 15:1802. [PubMed: 18691040]
8. Dolman RC, Deacon GB, Hambley TW. *J Inorg Biochem*. 2002; 88:260. [PubMed: 11897339]
9. Akshintala S, Marcus L, Warren KE, Murphy RF, Sissung TM, Srivastava A, Goodspeed WJ, Goodwin A, Brewer CC, Zalewski C. *Pediatr Blood Cancer*. 2015; 62:603. [PubMed: 25556988]
10. Ricart AD, Sarantopoulos J, Calvo E, Chu QS, Greene D, Nathan FE, Petrone ME, Tolcher AW, Papadopoulos KP. *Clin Cancer Res*. 2009; 15:3866. [PubMed: 19458055]
11. Bae YH, Park K. *J Control Release*. 2011; 153:198. [PubMed: 21663778]
12. Kratz F, Müller IA, Ryppa C, Warnecke A. *ChemMedChem*. 2008; 3:20. [PubMed: 17963208]
13. Fang J, Nakamura H, Maeda H. *Adv Drug Deliv Rev*. 2011; 63:136. [PubMed: 20441782]
14. Wang X, Guo Z. *Chem Soc Rev*. 2013; 42:202. [PubMed: 23042411]
15. Wai-Yin Sun R, Ma D-L, Wong EL-M, Che C-M. *Dalton Trans*. 2007:4884. [PubMed: 17992273]
16. Berger I, Nazarov AA, Hartinger CG, Groessl M, Valiahdi SM, Jakupc MA, Keppler BK. *ChemMedChem*. 2007; 2:505. [PubMed: 17340670]
17. Prashar D, Shi Y, Bandyopadhyay D, Dabrowiak JC, Luk YY. *Bioorg Med Chem Lett*. 2011; 21:7421. [PubMed: 22050886]
18. Papapoulos SE. *Bone*. 38:613. [PubMed: 16504613]
19. Xue Z, Lin M, Zhu J, Zhang J, Li Y, Guo Z. *Chem Commun*. 2010; 46:1212.

20. Massaguer A, González-Cantó A, Escribano E, Barrabés S, Artigas G, Moreno V, Marchán V. *Dalton Trans.* 2015; 44:202. [PubMed: 25369773]
21. Arosio D, Manzoni L, Araldi EMV, Caprini A, Monferini E, Scolastico C. *Bioconjug Chem.* 2009; 20:1611. [PubMed: 21141806]
22. Regberg J, Srimanee A, Langel U. *Pharmaceuticals (Basel, Switzerland).* 2012; 5:991.
23. Mukhopadhyay S, Barnés CM, Haskel A, Short SM, Barnes KR, Lippard SJ. *Bioconjug Chem.* 2008; 19:39. [PubMed: 17845003]
24. Kothandapani A, Dangeti VSMN, Brown AR, Banze LA, Wang X-H, Sobol RW, Patrick SM. *J Biol Chem.* 2011; 286:14564. [PubMed: 21357694]
25. Kothandapani A, Sawant A, Dangeti VSMN, Sobol RW, Patrick SM. *Nucleic Acids Res.* 2013:gkt479.
26. Martin LP, Hamilton TC, Schilder RJ. *Clin Cancer Res.* 2008; 14:1291. [PubMed: 18316546]
27. Koppenol WH, Bounds PL, Dang CV. *Nat Rev Cancer.* 2011; 11:325. [PubMed: 21508971]
28. Warburg O. *Science.* 1956; 123:309. [PubMed: 13298683]
29. Sakhrani NM, Padh H. *Drug Des Devel Ther.* 2013; 7:585.
30. Sun T, Zhang YS, Pang B, Hyun DC, Yang M, Xia Y. *Angew Chem Int Ed.* 2014; 53:12320.
31. Dhar S, Liu Z, Thomale J, Dai H, Lippard SJ. *J Am Chem Soc.* 2008; 130:11467. [PubMed: 18661990]
32. Fabbro C, Ali-Boucetta H, Ros TD, Kostarelos K, Bianco A, Prato M. *Chem Commun.* 2012; 48:3911.
33. Miyawaki J, Yudasaka M, Azami T, Kubo Y, Iijima S. *ACS Nano.* 2008; 2:213. [PubMed: 19206621]
34. Acharya S, Sahoo SK. *Adv Drug Deliv Rev.* 2011; 63:170. [PubMed: 20965219]
35. Rosenholm JM, Sahlgren C, Linden M. *Nanoscale.* 2010; 2:1870. [PubMed: 20730166]
36. Kuppler RJ, Timmons DJ, Fang Q-R, Li J-R, Makal TA, Young MD, Yuan D, Zhao D, Zhuang W, Zhou H-C. *Coord Chem Rev.* 2009; 253:3042.
37. Liu D, Poon C, Lu K, He C, Lin W. *Nat Commun.* 2014; 5:4182. [PubMed: 24964370]
38. Rieter WJ, Pott KM, Taylor KM, Lin W. *J Am Chem Soc.* 2008; 130:11584. [PubMed: 18686947]
39. Wilson JJ, Lippard SJ. *Chem Rev.* 2014; 114:4470. [PubMed: 24283498]
40. Ang WH, Khalaila I, Allardyce CS, Juillerat-Jeanneret L, Dyson PJ. *J Am Chem Soc.* 2005; 127:1382. [PubMed: 15686364]
41. Yang J, Sun X, Mao W, Sui M, Tang J, Shen Y. *Mol Pharm.* 2012; 9:2793. [PubMed: 22953987]
42. Pathak RK, Marrache S, Choi JH, Berding TB, Dhar S. *Angew Chem Int Ed.* 2014; 53:1963.
43. Cheng Q, Shi H, Wang H, Min Y, Wang J, Liu Y. *Chem Commun.* 2014; 50:7427.
44. Dhar S, Lippard SJ. *Proc Natl Acad Sci.* 2009; 106:22199. [PubMed: 20007777]
45. Suntharalingam K, Song Y, Lippard SJ. *Chem Commun.* 2014; 50:2465.
46. Farrer NJ, Woods JA, Salassa L, Zhao Y, Robinson KS, Clarkson G, Mackay FS, Sadler PJ. *Angew Chem Int Ed.* 2010; 49:8905.
47. Jeong YI, Kim ST, Jin SG, Ryu HH, Jin YH, Jung TY, Kim IY, Jung S. *J Pharm Sci.* 2008; 97:1268. [PubMed: 17674407]
48. Götte M, Yip GW. *Cancer Res.* 2006; 66:10233. [PubMed: 17079438]
49. Sasanelli R, Boccarelli A, Giordano D, Laforgia M, Arnesano F, Natile G, Cardellicchio C, Capozzi MAM, Coluccia M. *J Med Chem.* 2007; 50:3434. [PubMed: 17583333]
50. Graf N, Mokhtari TE, Papayannopoulos IA, Lippard SJ. *J Inorg Biochem.* 2012; 110:58. [PubMed: 22465700]
51. Abramkin S, Valiahdi SM, Jakupec MA, Galanski M, Metzler-Nolte N, Keppler BK. *Dalton Trans.* 2012; 41:3001. [PubMed: 22281694]
52. Xing R, Wang X, Zhang C, Zhang Y, Wang Q, Yang Z, Guo Z. *J Inorg Biochem.* 2009; 103:1039. [PubMed: 19501911]
53. Margiotto N, Ostuni R, Rinaldo R, Denora N, Laquintana V, Trapani G, Liso G, Natile G. *J Med Chem.* 2007; 50:1019. [PubMed: 17286392]

54. Ruiz-Sanchez P, Konig C, Ferrari S, Alberto R. *J Biol Inorg Chem*. 2011; 16:33. [PubMed: 20803225]
55. Kim E, Rye PT, Essigmann JM, Croy RG. *J Inorg Biochem*. 2009; 103:256. [PubMed: 19054566]
56. Raveendran R, Braude JP, Wexselblatt E, Novohradsky V, Stuchlikova O, Brabec V, Gandin V, Gibson D. *Chem Sci*. 2016; 7:2381.
57. Yang X-D, Xiang H-J, An L, Yang S-P, Liu J-G. *New J Chem*. 2015; 39:800.
58. Chin CF, Yap SQ, Li J, Pastorin G, Ang WH. *Chem Sci*. 2014; 5:2265.
59. Min Y, Mao C, Xu D, Wang J, Liu Y. *Chem Commun*. 2010; 46:8424.
60. Dai Y, Kang X, Yang D, Li X, Zhang X, Li C, Hou Z, Cheng Z, Ma Pa, Lin J. *Adv Healthc Mater*. 2013; 2:514.
61. Cheng Z, Dai Y, Kang X, Li C, Huang S, Lian H, Hou Z, Ma P, Lin J. *Biomaterials*. 2014; 35:6359. [PubMed: 24816364]
62. Rieter WJ, Pott KM, Taylor KML, Lin W. *J Am Chem Soc*. 2008; 130:11584. [PubMed: 18686947]
63. Taylor-Pashow KML, Rocca JD, Xie Z, Tran S, Lin W. *J Am Chem Soc*. 2009; 131:14261. [PubMed: 19807179]
64. Reiter RE, Gu Z, Watabe T, Thomas G, Szigeti K, Davis E, Wahl M, Nisitani S, Yamashiro J, Le Beau MM, Loda M, Witte ON. *Proc Natl Acad Sci*. 1998; 95:1735. [PubMed: 9465086]
65. Hall MD, Mellor HR, Callaghan R, Hambley TW. *J Med Chem*. 2007; 50:3403. [PubMed: 17602547]
66. Gibson D. *Dalton Trans*. 2016
67. Sturge J, Caley MP, Waxman J. *Nat Rev Clin Oncol*. 2011; 8:357. [PubMed: 21556025]
68. Thun MJ, Henley SJ, Patrono C. *J Natl Cancer Inst*. 2002; 94:252. [PubMed: 11854387]
69. Ricciotti E, FitzGerald GA. *Arterioscler Thromb Vasc Biol*. 2011; 31:986. [PubMed: 21508345]
70. Tosco P, Lazzarato L. *ChemMedChem*. 2009; 4:939. [PubMed: 19301318]
71. Grilli M, Pizzi M, Memo M, Spano P. *Science*. 1996; 274:1383. [PubMed: 8910280]
72. Pathak RK, Dhar S. *Synlett*.
73. Cheng Q, Shi H, Huang H, Cao Z, Wang J, Liu Y. *Chem Commun (Camb)*. 2015; 51:17536. [PubMed: 26482339]
74. Neumann W, Crews BC, Sárosi MB, Daniel CM, Ghebreselasie K, Scholz MS, Marnett LJ, Hey Hawkins E. *ChemMedChem*. 2015; 10:183. [PubMed: 25318459]
75. Goto S, Iida T, Cho S, Oka M, Kohno S, Kondo T. *Free Radic Res*. 1999; 31:549. [PubMed: 10630679]
76. Kasherman Y, Sturup S, Gibson D. *J Med Chem*. 2009; 52:4319. [PubMed: 19537717]
77. Ishikawa T, Wright CD, Ishizuka H. *J Biol Chem*. 1994; 269:29085. [PubMed: 7961875]
78. Brozovic A, Ambriovi -Ristov A, Osmak M. *Crit Rev Toxicol*. 2010; 40:347. [PubMed: 20163198]
79. Kelley S, Basu A, Teicher B, Hacker M, Hamer D, Lazo J. *Science*. 1988; 241:1813. [PubMed: 3175622]
80. Brusamolino E, Salvaneschi L, Canevari A, Bernasconi C. *J Clin Oncol*. 1984; 2:558. [PubMed: 6374054]
81. Pathak RK, Dhar S. *Chem Eur J*. 2016; 22:3029. [PubMed: 26807548]
82. Russo A, DeGraff W, Friedman N, Mitchell JB. *Cancer Res*. 1986; 46:2845. [PubMed: 2421885]
83. Patra Krushna C, Wang Q, Bhaskar Prashanth T, Miller L, Wang Z, Wheaton W, Chandel N, Laakso M, Muller William J, Allen Eric L, Jha Abhishek K, Smolen Gromoslaw A, Clasquin Michelle F, Robey RB, Hay N. *Cancer Cell*. 2013; 24:213. [PubMed: 23911236]
84. Kolb HC, Finn MG, Sharpless KB. *Angew Chem Int Ed*. 2001; 40:2004.
85. Zhang JZ, Bonnitcha P, Wexselblatt E, Klein AV, Najajreh Y, Gibson D, Hambley TW. *Chem Eur J*. 2013; 19:1672. [PubMed: 23255183]
86. Pichler V, Mayr J, Heffeter P, Dömötör O, Enyedy ÉA, Hermann G, Groza D, Köllensperger G, Galanksi M, Berger W. *Chem Commun*. 2013; 49:2249.

87. Gao M, Chen C, Fan A, Zhang J, Kong D, Wang Z, Zhao Y. *Nanotechnology*. 2015; 26:275101. [PubMed: 26066389]
88. Sletten EM, Bertozzi CR. *Angew Chem Int Ed*. 2009; 48:6974.
89. Sletten EM, Bertozzi CR. *Acc Chem Res*. 2011; 44:666. [PubMed: 21838330]
90. Ledin PA, Kolishetti N, Boons G-J. *Macromolecules*. 2013; 46:7759. [PubMed: 24511157]
91. Pathak RK, McNitt CD, Popik VV, Dhar S. *Chem Eur J*. 2014; 20:6861. [PubMed: 24756923]
92. Farokhzad OC. *Expert Opin Drug Deliv*. 2008; 5:927. [PubMed: 18754745]
93. Kamaly N, Xiao Z, Valencia PM, Radovic-Moreno AF, Farokhzad OC. *Chem Soc Rev*. 2012; 41:2971. [PubMed: 22388185]
94. Wheate NJ. *Nanomedicine*. 2012; 7:1285. [PubMed: 22994954]
95. Zheng Y-R, Suntharalingam K, Johnstone TC, Yoo H, Lin W, Brooks JG, Lippard SJ. *J Am Chem Soc*. 2014; 136:8790. [PubMed: 24902769]
96. Wong DYQ, Lau JY, Ang WH. *Dalton Trans*. 2012; 41:6104. [PubMed: 22426542]
97. Ishida S, Lee J, Thiele DJ, Herskowitz I. *Proc Natl Acad Sci*. 2002; 99:14298. [PubMed: 12370430]
98. Blair BG, Larson CA, Safaei R, Howell SB. *Clin Cancer Res*. 2009; 15:4312. [PubMed: 19509135]
99. Marrache S, Pathak RK, Dhar S. *Proc Natl Acad Sci*. 2014; 111:10444. [PubMed: 25002500]
100. Wisnovsky SP, Wilson JJ, Radford RJ, Pereira MP, Chan MR, Laposa RR, Lippard SJ, Kelley SO. *Chem Biol*. 2013; 20:1323. [PubMed: 24183971]
101. Cullen KJ, Yang Z, Schumaker L, Guo Z. *J Bioenerg Biomembr*. 2007; 39:43. [PubMed: 17318397]
102. Yang Z, Schumaker LM, Egorin MJ, Zuhowski EG, Guo Z, Cullen KJ. *Clin Cancer Res*. 2006; 12:5817. [PubMed: 17020989]
103. Murata T, Hibasami H, Maekawa S, Tagawa T, Nakashima K. *Biochem Int*. 1990; 20:949. [PubMed: 2112385]
104. Olivero OA, Semino C, Kassim A, Lopez-Larrazza DM, Poirier MC. *Mutat Res*. 1995; 346:221. [PubMed: 7753115]
105. Marrache S, Dhar S. *Proc Natl Acad Sci*. 2012; 109:16288. [PubMed: 22991470]
106. Marrache S, Tundup S, Harn DA, Dhar S. *ACS nano*. 2013; 7:7392. [PubMed: 23899410]
107. Feldhaeuser B, Platt SR, Marrache S, Kolishetti N, Pathak RK, Montgomery DJ, Reno LR, Howerth E, Dhar S. *Nanoscale*. 2015; 7:13822. [PubMed: 26234400]
108. Kolishetti N, Dhar S, Valencia PM, Lin LQ, Karnik R, Lippard SJ, Langer R, Farokhzad OC. *Proc Natl Acad Sci*. 2010; 107:17939. [PubMed: 20921363]
109. Liao L, Liu J, Dreaden EC, Morton SW, Shopsowitz KE, Hammond PT, Johnson JA. *J Am Chem Soc*. 2014; 136:5896. [PubMed: 24724706]
110. Pathak RK, Dhar S. *J Am Chem Soc*. 2015; 137:8324. [PubMed: 26086212]

Figure 1.

A list of approved Pt-based drugs and their structures.

Figure 2.
Schematic representation of modes of activation, targeting, and delivery of therapeutic agents such as Pt-based compounds.

Figure 3.

A cartoon of Platin-*X* series with an intact cisplatin skeleton containing the leaving ligand and carrier groups.

Figure 4.
 (A) Structures of Pt(IV) prodrugs which contain anti-inflammatory components. (B)
 Combination therapeutic approaches used in Platin-A and Platin-B by activation of the Pt-center.

Figure 5.
 (A) Use of Platin-Az, ADIBO functionalized molecules, and SPAAC to generate Pt(IV) compounds along with possible functionalities which can be included using this approach.
 (B) Two such examples where this chemistry was used to generate new Pt(IV) compounds.

Figure 6. Mechanism of action of a mitochondria-targeted Pt(IV) prodrug, Platin-M, when it is delivered to cells using a targeted NP formulation.

Figure 7. Structures of Platin-4, prodrug of aspirin, and Janus polymer, $(\text{Asp})_4\text{-PLA}-(\text{Platin-4})_3$. Schematic representations of different types NP formation by using blending the Janus polymer with block copolymers and opportunities for further development to include other types of surface functionalities.

Table 1

Platinum complexes, conjugates used for activation, targeting, and delivery

Pt-based complex	Action	Ref.
Examples of Pt(IV) Activation		
Ormaplatin, Iproplatin, Satraplatin	Activated by reduction to produce cisplatin analogue	2
Ethacraplatin	Activation by reduction to produce cisplatin and GST inhibitor, ethacrynic acid	40
Platinum(IV) divalproate	Activation by reduction to produce cisplatin and valproic acid, a potent histone deacetylase (HDAC) inhibitor	41
Platin- <i>A</i> /Asplatin	Activation by reduction to produce cisplatin and aspirin, an anti-inflammatory agent	42,43
Mitaplatin	Activation by reduction to produce cisplatin and dichloroacetate	44
Cisplatin- α -TOS	Activation by reduction to produce cisplatin and α -TOS to inhibit antiapoptotic proteins Bcl-2 and Bcl-xL	45
<i>t,t,t</i> -[Pt(N ₃) ₂ (OH) ₂ (py) ₂]	Activation by photolysis with loss of azide ligands	46
Examples of Targeting by Pt Compounds		
PtCl ₂ (2,3-diamino-2,3-dideoxy-D-glucose), [Pt(tpy)-glycosylated arylacetylde] ⁺	Thought to target glucose transporters	15
Adamantane-carboplatin- β -cyclodextrin	Efficient transport to the nucleus and effective binding to nuclear DNA	17
Cisplatin-hyaluronic acid (CP-HA)	Target CD44, the primary receptor for HA	47
Oxaliplatin-HA-chitosan NP	Targeted delivery to colorectal tumors	48
Picoplatin-biphosphonate tetraethylester, Pt-diethyl[(methylsulfinyl)methyl]-phosphonate	Thought to target bone and other calcified tissues	19,49
Platinum(IV)-chlorotoxin	Thought to target bone and other calcified tissues	50
Oxaliplatin-TAT (Trans-acting Activator of Transcription)	Target proteins like matrix metalloproteinase 2 (MMP2), annexin A2, and chloride ion channels	51
Cisplatin-apoferritin	Target membrane specific receptors for ferritin	52
Platinum-peripheral benzodiazepine	Target membrane specific receptors for ferritin	53
Pt ^{II} complexes, with B ₁₂ -CN-Pt ^{II}	Release of the platinum complex from the conjugates is driven by the intracellular reduction of Co ^{III} to Co ^{II} to Co ^I and target vitamin B ₁₂ receptors	54
[Pt(en-17 β -estradiol)Cl ₂], [Pt(tpy-17 α -estradiol)Cl]	Target estrogen receptor	55

Pt-based complex	Action	Ref.
<i>ctc</i> -[Pt(NH ₃) ₂ (PhB) ₂ Cl ₂]	Target mitochondria apoptotic pathway	56
Examples of Delivery of Pt Compounds		
SWCNT- <i>c,c,t</i> -[Pt(NH ₃) ₂ Cl ₂ (disuccinate)]-folate, CNT- <i>c,t,c</i> -[Pt(N ₃) ₂ (OH) ₂ (NH ₃)(3-NH ₂ py)]-folate	Target folate receptor and deliver the platinum anticancer agent using carbon nanotube	31,57
MWCNT- <i>c,c,t</i> -[Pt-(NH ₃) ₂ Cl ₂ (O ₂ CC ₆ H ₅) (O ₂ CCH ₂ CH ₂ CO ₂ H)]-doxorubicin	Deliver the platinum anticancer agent using carbon nanotube	58
GNP- <i>c,c,t</i> -[Pt(NH ₃) ₂ Cl ₂ (O ₂ CCH ₂ CH ₂ CO ₂ H) ₂]-CRGDK	Target neuropilin-1 receptor and deliver to tumor site using AuNP	59
cisplatin-poly(ethylenimine)-NaYF ₄ -FA	Release of cisplatin by reduction, target folate receptor and NP used as a delivery vehicle	60
<i>c,c,t</i> -[Pt(NH ₃) ₂ Cl ₂ (O ₂ CCH ₂ CH ₂ CO ₂ H) ₂]-Fe ₂ O ₃ NP	Activated by the action of pancreatic enzyme	61
SWCNT-FA-Tb ₂ (H ₂ O) ₁₂ -(<i>c,c,t</i> -[Pt(NH ₃) ₂ Cl ₂ (O ₂ CCH ₂ CH ₂ CO ₂ H) ₂]) ₃	Platinum prodrug activated by reduction, target folate receptor and carbon nanotube is used for drug delivery	62
<i>c,c,t</i> -[Pt(NH ₃) ₂ Cl ₂ (OEt)-(O ₂ CCH ₂ CH ₂ CO ₂ H)]-FeNP MOF	MOF used as drug delivery vehicle	63
<i>c,t,c</i> -[Pt(NH ₃) ₂ (O ₂ CCH ₂ CH ₂ CH ₂ CH ₂ CH ₃) ₂ Cl ₂ - PLGA- <i>b</i> -PEG NP-PSMA targeting aptamers	Platinum prodrug activated by reduction, target prostate specific membrane antigen (PSMA) and PLGA- <i>b</i> -PEG NP used as a delivery platform	64