Published in final edited form as:

Mol Pharm. 2016 August 1; 13(8): 2677–2682. doi:10.1021/acs.molpharmaceut.6b00240.

Systemic Delivery and Biodistribution of Cisplatin *in Vivo*

Yu-Hsuan Chu[†], Martha Sibrian-Vazquez[†], Jorge O. Escobedo[†], Amanda R. Phillips[‡], D. Thomas Dickey[‡], Qi Wang[‡], Martina Ralle[§], Peter S. Steyger[‡], and Robert M. Strongin^{*,†}

[†]Department of Chemistry, Portland State University, Portland, Oregon 97201, United States

[‡]Oregon Hearing Research Center, Oregon Health & Science University, Portland, Oregon 97239, United States

[§]Department of Molecular & Medical Genetics, Oregon Health & Science University, Portland, Oregon 97239, United States

Abstract

Cisplatin is widely used to treat a variety of cancers. However, ototoxicity and nephrotoxicity remain serious side effects of cisplatin-based chemotherapy. In order to inform the study of cisplatin's off-target effects, a new drug-fluorophore conjugate was synthesized that exhibited utility as a tracer to determine the cellular uptake and *in vivo* distribution of cisplatin. This probe will serve as a useful tool to facilitate investigations into the kinetics and biodistribution of cisplatin and its associated side effects in preclinical models after systemic administration.

Graphical abstract

Keywords

cisplatin; Texas Red; conjugate; ototoxicity; tracer

*Corresponding Author: strongin@pdx.edu.

Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.6b00240.

Detailed spectral and characterization data and HPLC purity analysis of conjugates (PDF)

INTRODUCTION

Cisplatin (*cis*-diamminedichloroplatinum[II]) is a platinum-containing anti-cancer drug, the bioactivity of which was discovered by Rosenberg et al. in 1965.¹ It has become a cornerstone of antineoplastic chemotherapy to treat many types of cancer, including ovarian, cervical, stomach, bladder, head, and neck.^{2–6} Cisplatin is especially efficacious for testicular cancer, which has an overall cure rate greater than 90% and nearly 100% at stage I.⁵ There are more than 1 million patients receiving cisplatin or its derivatives in North America and Europe every year. Unfortunately, the clinical use of cisplatin is limited by acquired drug resistance and severe dose-dependent side effects, such as ototoxicity and nephrotoxicity. Clinical studies have shown that, in >60% of the patients who have taken multiple doses of cisplatin, permanent hearing loss^{7,8} and acute renal dysfunction were significant side effects.⁹ Drug-induced hearing loss can severely affect an individual's quality of life. Consequences can include social isolation, depression, and loss of income. Children can exhibit delayed development of communication and learning skills, and have a relatively challenging time developing social interactions.^{7,8} Therefore, an understanding of the events that lead to cisplatin-induced ototoxicity and nephrotoxicity can have a profound impact on human health, and can enable the design of potentially less harmful therapeutic alternatives.

The mechanism of cisplatin uptake is poorly understood. There have been several prior studies to identify the biodistribution of cisplatin in cells and tissues. These include the use of positron emission tomography, radiolabeled cisplatin and high-performance liquid chromatography (HPLC).^{10–12} Morphologically, these techniques have relatively low anatomic resolution and cannot provide the cellular distribution of cisplatin in heterogeneous cellular tissues, such as the cochlea in the inner ear. In order to investigate the cellular uptake and intracellular distribution of cisplatin, the use of fluorogenic probes is a promising alternative to these techniques.

Prior investigations of fluorophore–cisplatin conjugates and their cellular uptake have been reported. For example, a fluorescein–cisplatin conjugate (**1**, Figure 1) entered cells via endocytosis.¹³ A tetramethylrhodamine–cisplatin conjugate entered the sensory hair cells of the zebrafish lateral line via their apical membranes, potentially via the mechanoelectrical transduction channel-dependent mechanism or calmodulin-dependent endocytosis.¹⁴ In addition, other conjugates, including cyanine, coumarin, and dinitrophenyl fluorophores, have been studied.¹⁵ Results of these prior studies have suggested that a cationic charge on the fluorophore can enhance electrostatic interactions of the cisplatin conjugate toward a guanine base. Therefore, we hypothesized that conjugation of cisplatin to a cationic dye will better preserve its desired bioactive properties, thus enabling *in vivo* studies of its cellular trafficking mechanisms involving ion channels. This hypothesis is also based on related investigations of other cationic pharmaceuticals, such as the aminoglycosides, which are also known to cause ototoxicity and nephrotoxicity.¹⁶ Specifically, it was envisioned that tagging cisplatin with a rhodamine-type fluorophore, akin to gentamicin–Texas Red,^{17,18} would better preserve cisplatin's properties related to its cationic charge as compared to conjugation to a negatively charged fluorophore.¹⁶

Herein, cisplatin was conjugated to the commercially available fluorophore Texas Red to afford **2** (Figure 1). Following systemic injection, its distribution in fixed rodent tissues, including the cochlea, was determined using confocal microscopy. This is the first example of the successful use of a fluorescently tagged cisplatin probe in mammals, to the best of our knowledge. Compound **2** exhibited an unprecedented degree of functionality. For example, the compound crossed the blood–labyrinth barrier and entered the blood–brain barrier at the choroid plexus; therefore, it has substantial potential utility as a tracer.

RESULTS AND DISCUSSION

Platinum complex **3** (Figure 1) was selected as a target as it contains a short linker for conjugation and retains the chemical properties of cisplatin, as reported by Karasawa et al.¹⁹ The target cisplatin–Texas Red conjugate (**2**) was synthesized from the reaction of Texas Red *N*-succinimidyl ester (TR-SE, mixture of regioisomers) (**4**) with platinum complex **3** under basic conditions in 88% yield with 97% purity (Supporting Information).

In order to determine that **2** had spectral properties similar to those of Texas Red-derived **4**, absorbance and fluorescence spectra were evaluated. As shown in Figure 2, conjugate **2** exhibited similar maximum absorption intensities as compared to **4**, with the fluorescence excitation spectra slightly red-shifted by 5 nm in **2**. Relatively negligible changes in the emission spectra were observed, and, as expected, there was no significant improvement in the quantum yield of **4** compared to **2**.

To verify that **2** retained the cytotoxic properties of cisplatin, the relative toxicity values of cisplatin and **2** were compared using zebrafish neuromast hair cells. Larvae were treated with a dose range of **2** or **4** for 4 h and allowed to recover for 3 h.²⁰ Alexa Fluor 488-conjugated phalloidin labeling of the hair bundle and cuticular plate was used to determine the number of surviving hair cells. Hair cells can cleave their hair bundles following drug exposure, yet the loss of the hair bundle or cuticular plate is an ototoxic event. After treatment with **2** (10–200 $\mu\text{g/mL}$) for 4 h, plus 3 h of recovery, the number of surviving hair cells decreased in a dose-dependent manner (Figures 3A–C,E). Treatment with **4** revealed negligible fluorescence in the hair cells, suggesting that the forced uptake of Texas Red, via conjugation to cisplatin, was responsible for the enhanced toxicity of **2** (Figure 3D). To quantify and normalize this decrease, untreated controls provided a baseline to determine the ratio of surviving hair cells in zebrafish neuromasts. Both cisplatin and **2** decreased hair cell numbers in a dose-dependent manner (Figure 3E).

To determine the *in vivo* distribution of **2**, rats were injected intravenously with 2 mg/kg of **2**. In the renal cortex, proximal, but not distal cells avidly took up diffuse and punctate fluorescence within 1 h (Figure 4A). By 3 h, the intensity of cytoplasmic and punctate fluorescence had diminished, indicating clearance, which was further evident at 24 h (Figure 4B,C). Similar uptake and clearance kinetics were also seen in liver hepatocytes (Figure 4D,E). In the choroid plexus, a double endo- and epithelial barrier in the blood–brain barrier, **2** was diffusely dispersed in the cytoplasm of ependymal cells within 1 h (Figure 4G). By 3 h, fluorescent puncta occurred within the laden cytoplasm (Figure 4H). After 24 h, more

intense puncta were present within the laden cytoplasm (Figure 4I), indicating an apparent lack of cellular clearance.

We then tested whether **2** crossed the cochlear blood–labyrinth barrier (akin to the blood–brain barrier) into the stria vascularis (SV) and entered hair cells. In cryostat sections of the cochlea, at 1 and 3 h (Figure 5) after injection, fluorescence was brightest in the SV in the lateral wall of the cochlea, compared to other cochlear locations, as for fluorescently tagged gentamicin.¹⁸ Renal and cochlear tissues did not exhibit any fluorescence from hydrolyzed forms of **4** (data not shown) as previously described.^{18,21} In whole mounted tissues of the cochlear lateral wall, 1 h after injection, fluorescence was visible in strial marginal cells, intrastrial tissues (particularly capillary endothelial cells), and basal cells, with negligible fluorescence in spiral ligament (SL) fibrocytes (Figure 6A–D). After 3 h, the intensity of **2** in strial cells was increased, but less so in fibrocytes (Figure 6E–H). After 24 h, clearance was evident in strial tissues (Figure 6I–K), while fibrocytes continued to take up **2** (Figure 6L). The clearance of **2** was statistically significant at later times compared to earlier time points (Figure 7), as for fluorescently tagged gentamicin.¹⁸

We then examined the uptake of **2** by cochlear hair cells in the organ of Corti. One hour after injection, outer hair cells (OHCs) displayed weak fluorescence, particularly on their stereocilia (Figure 8A). Inner hair cells (IHCs) did not exhibit fluorescence at 1 h after injection (Figure 8B), although punctate fluorescence was visible (in supporting cells surrounding IHCs, Figure 8C), and identified as nonspecific autofluorescence (likely lipofuscin) as previously described.²¹ Three hours after injection, OHC and IHC stereocilia and cell bodies displayed diffuse fluorescence from **2** (Figure 8D, E), and this fluorescence intensity was maintained 24 h later (Figure 8F, G). These data demonstrated that **2** crossed the blood–labyrinth barrier and entered cochlear hair cells of healthy rats, reminiscent of previous studies using Texas Red-conjugated gentamicin.^{18,22}

Using induction-coupled plasma mass spectrometry, we found that the serum kinetics of cisplatin and **2** were similar following i.v. injection. The serum half-life ($t_{1/2}$) of total **2** was calculated to be 6.8 h from 60 min to 24 and 26.1 h from the 24-h to 5-day time points. The $t_{1/2}$ for native cisplatin in rats was initially 6.3 h,²³ and then 14.7 h between 12 and 48 h.²⁴ Thus, the serum kinetics of **2** was similar to the biphasic kinetics of cisplatin, although with a longer secondary $t_{1/2}$ than published studies, perhaps due to the removal of the hydration step²⁵ in our protocol, as well as the larger molecular mass of the conjugate (~1000) compared to native cisplatin (~300).

CONCLUSION

Cisplatin–Texas Red conjugate **2** was synthesized using platinum complex **3** and commercially available TR-SE **4** in 97% purity. Spectral evaluation showed that the cisplatin conjugate **2** essentially retained the spectral characteristics of the parent probe **4**.

Cisplatin is a relatively small molecule; thus, conjugation could alter the kinetics of its uptake as well as its cytotoxicity. Cellular studies revealed that Texas Red conjugate **2** successfully undergoes uptake and exhibits cytotoxicity to hair cells similar to that of native

cisplatin. Moreover, conjugate **2** enters and is cleared from a variety of mammalian organs, in addition to crossing the blood–labyrinth barrier to enter cochlear hair cells in rats. The cellular uptake and *in vivo* distribution of **2** largely replicate our previous studies using Texas Red-conjugated gentamicin.¹⁸ Despite a larger molecular mass (~1000), **2** crosses an intact blood–labyrinth barrier and enters cochlear hair cells within 1 h. Systemically administered **2** was preferentially associated with the SV compared to the spiral ligament and organ of Corti. Previous studies have shown cisplatin toxicity in the SV, with concomitant loss of cochlear function, implying stria uptake of cisplatin.²⁶ This conjugate will therefore facilitate investigations into the kinetics and biodistribution of cisplatin, and its associated side effects, in preclinical models after systemic administration, as for fluorescently tagged gentamicin.²⁷ One way to reduce the volume of distribution of cisplatin *in vivo* is to attach cisplatin to polymers, nanoparticles, or monoclonal antibodies that can more specifically target tumors compared to systemic delivery of native cisplatin.^{28,29} This can be particularly effective if the binding ligand is labile and cleaved in the vicinity of the tumor or within cancer cells.³⁰ However, polymers can often be entrapped within the fibrous sheath surrounding the tumor, or otherwise heterogeneously distributed throughout the tumor.^{31,32} These approaches, however, may ameliorate cisplatin-induced ototoxicity if they retain their tumoritoxic potential. The fluorescence tagging of cisplatin was designed to determine the cellular distribution of cisplatin within tissues with heterogeneous cell types, like the cochlea, to determine which cells had a preferential uptake of the conjugate, and thereby of the native drug, akin to similar studies for gentamicin and tagged gentamicin.^{18,27}

In summary, conjugate **2** facilitates the study of the origin of cisplatin's side effects in mammals. Prior studies with fluorescently tagged cisplatin have been limited to *in vitro* investigations. In heterogeneous structures such as the cochlea and other biological tissues, conjugate **2** enables the microscopic analysis of cisplatin's biodistribution within tissues, complementing related techniques, such as ICP-MS, for native cisplatin.

EXPERIMENTAL SECTION

General

All chemicals were used without further purification. ¹H NMR, ¹³C NMR, and ¹⁹⁵Pt NMR were acquired on an ARX-400 Avance Bruker spectrometer. Chemical shifts (δ) are given in ppm relative to TMS and DMF-*d*₇ was used as solvent. For ¹⁹⁵Pt-NMR, K₂PtCl₄ was used as external standard. Mass spectra was conducted at the PSU Bioanalytical Mass Spectrometry Facility on a ThermoElectron LTQ-Orbitrap Discovery high resolution mass spectrometer coupled to an Accela HPLC system. Another HPLC system was used for the spectrophotometric detection consisting on a 1525 binary delivery module (Waters), and a 2996 photodiode array detector (Waters). A Supelco Discovery C-18 (25 cm × 2.1 mm, 5 μ m) column was used for the separation in both HPLC systems. Fluorescence measurements were carried out on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies), and absorption measurements were performed on a Cary Eclipse 50 Bio UV–vis spectrophotometer.

Synthesis of Cisplatin–Texas Red Conjugate, **2**

Platinum complex **5** (10.55 mg, 26.93 μmol) was dissolved in 1.2 mL of anhydrous DMF and sonicated for 10 min. Et_3N (10.24 μL , 73.44 μmol) was added in one portion and the mixture sonicated for another 5 min, resulting in a clear pale yellow solution; if necessary, DI H_2O was added in increments of 20 μL and sonicated until completely dissolved. Compound **4** (TR-SE, 20 mg, 24.48 μmol ; Invitrogen, CA) was added, and the reaction mixture stirred at rt for 1 h prior to evaporation under vacuum to afford a purple precipitate. The residue was centrifuged and washed with cold H_2O ($2 \times 10 \text{ mL}$) and Et_2O ($3 \times 10 \text{ mL}$) until the supernatant became almost clear, and dried under vacuum to afford a purple solid (yield: 88%, 22.8 mg). HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{40}\text{H}_{51}\text{Cl}_2\text{N}_6\text{O}_7\text{PtS}_2$: 1056.2282; observed 1056.2354.

Zebrafish

Wild-type zebrafish larvae, 5 days after fertilization, were treated with a dose range of **2** or native cisplatin (10–400 $\mu\text{g}/\text{mL}$ in standard E3 medium) for 4 h and recovered for 3 h prior to fixation in 4% formaldehyde containing 0.5% Triton X-100.²⁰ Larvae were then labeled with Alexa Fluor 488-conjugated phalloidin to determine the number of surviving hair cell bundles, as described previously.¹⁸

Rats

Male Long–Evans rats (~200 g) received one i.v. injection of 2 mg/kg of compound **2** (in a 60/40% mixture of EtOH/PBS, pH 7.4), and at various time points (0.5, 3, 6, 24, and 96 h) were anesthetized, and serum was collected prior to cardiac perfusion with phosphate-buffered saline (PBS), followed by 4% formaldehyde containing 0.5% Triton X-100. Choroid plexi, cochleae, kidneys, and liver tissues were excised and fixed overnight. Alternatively, rats received an equivalent dose of **4** prior to the above procedures. All tissues were counter-labeled with Alexa Fluor 488-conjugated phalloidin to label filamentous actin. All specimens were whole-mounted in VectaShield (Vector Labs, CA) and observed using a Bio-Rad MRC 1024 ES laser scanning confocal system attached to a Nikon Eclipse TE300 inverted microscope.¹⁸ For cryostat sections, cochleae were decalcified in 10% EDTA, infiltrated with OCT, cryostat sectioned at 8 μm , and mounted on gelatin-coated glass slides.¹⁸ Alexa Fluor 488 and Texas Red images were collected sequentially. For each dosing regimen, all specimens were imaged at the same laser intensity and gain settings, including control tissues. Representative images from each dose regimen were identically prepared for publication using Adobe Photoshop.¹⁸ Platinum in cisplatin and **2** was detected using isocratic HPLC and ICP-MS (running in time-resolved mode).³³

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institute on Deafness and Other Communication Disorders (R21 DC010231; R01 DC012588) and the National Center for Research Resources (RR025512) at the National Institutes

of Health for elemental analysis and by the National Science Foundation (Grant 0741993) for the purchase of the LTQ-Orbitrap Discovery.

ABBREVIATIONS

TR-SE	Texas Red <i>N</i> -succinimidyl ester
HPLC	high-performance liquid chromatography
DMF	<i>N,N</i> -dimethylformamide
i.v	intravenous
SV	stria vascularis
SL	spiral ligament
OHCs	outer hair cells
IHCs	inner hair cells
PBS	phosphate-buffered saline

References

1. Rosenberg B, Van Camp L, Krigas T. Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. *Nature*. 1965; 205:698–699. [PubMed: 14287410]
2. Roberts, D.; O'Dwyer, P.; Johnson, S. Platinum Complexes for the Treatment of Cancer. In: Minev, BR., editor. *Cancer Management in Man: Chemotherapy, Biological Therapy, Hyperthermia and Supporting Measures*. Vol. 13. Springer; The Netherlands: 2011. p. 145-164.
3. Wong E, Giandomenico CM. Current Status of Platinum-Based Antitumor Drugs. *Chem Rev*. 1999; 99:2451–2466. [PubMed: 11749486]
4. Weiss RB, Christian MC. New cisplatin analogues in development. A review. *Drugs*. 1993; 46:360–377. [PubMed: 7693428]
5. Einhorn LH. Curing metastatic testicular cancer. *Proc Natl Acad Sci U S A*. 2002; 99:4592–4595. [PubMed: 11904381]
6. Kelland L. The resurgence of platinum-based cancer chemotherapy. *Nat Rev Cancer*. 2007; 7:573–584. [PubMed: 17625587]
7. Knight KR, Kraemer DF, Neuwelt EA. Ototoxicity in children receiving platinum chemotherapy: underestimating a commonly occurring toxicity that may influence academic and social development. *J Clin Oncol*. 2005; 23:8588–8596. [PubMed: 16314621]
8. Knight KR, Kraemer DF, Winter C, Neuwelt EA. Early changes in auditory function as a result of platinum chemotherapy: use of extended high-frequency audiometry and evoked distortion product otoacoustic emissions. *J Clin Oncol*. 2007; 25:1190–1195. [PubMed: 17401008]
9. Skinner R, Pearson AD, English MW, Price L, Wyllie RA, Coulthard MG, Craft AW. Cisplatin dose rate as a risk factor for nephrotoxicity in children. *Br J Cancer*. 1998; 77:1677–1682. [PubMed: 9635848]
10. Ginos JZ, Cooper AJ, Dhawan V, Lai JC, Strother SC, Alcock N, Rottenberg DA. [¹³N]cisplatin PET to assess pharmacokinetics of intra-arterial versus intravenous chemotherapy for malignant brain tumors. *J Nucl Med*. 1987; 28:1844–1852. [PubMed: 3500286]
11. Ning S, Yu N, Brown DM, Kanekal S, Knox SJ. Radiosensitization by intratumoral administration of cisplatin in a sustained-release drug delivery system. *Radiother Oncol*. 1999; 50:215–223. [PubMed: 10368046]

12. Ekborn A, Lindberg A, Laurell G, Wallin I, Eksborg S, Ehrsson H. Ototoxicity, nephrotoxicity and pharmacokinetics of cisplatin and its monohydrated complex in the guinea pig. *Cancer Chemother Pharmacol.* 2003; 51:36–42. [PubMed: 12497204]
13. Safaei R, Katano K, Larson BJ, Samimi G, Holzer AK, Naerdemann W, Tomioka M, Goodman M, Howell SB. Intracellular localization and trafficking of fluorescein-labeled cisplatin in human ovarian carcinoma cells. *Clin Cancer Res.* 2005; 11:756–767. [PubMed: 15701866]
14. Thomas AJ, Hailey DW, Stawicki TM, Wu P, Coffin AB, Rubel EW, Raible DW, Simon JA, Ou HC. Functional mechanotransduction is required for cisplatin-induced hair cell death in the zebrafish lateral line. *J Neurosci.* 2013; 33:4405–4414. [PubMed: 23467357]
15. Heetebrij RJ, Talman EG, Von Velzen MA, Von Gijlswijk RPM, Snoeijers SS, Schalk M, Wiegant J, Von der Rijke F, Kerkhoven RM, Raap AK, Tanke HJ, Reedijk J, Houthoff HJ. Platinum(II)-based coordination compounds as nucleic acid labeling reagents: Synthesis, reactivity, and applications in hybridization assays. *ChemBioChem.* 2003; 4:573–583. [PubMed: 12851925]
16. Dulon D, Zajic G, Aran JM, Schacht J. Aminoglycoside antibiotics impair calcium entry but not viability and motility in isolated cochlear outer hair cells. *J Neurosci Res.* 1989; 24:338–346. [PubMed: 2585554]
17. Dai CF, Steyger PS. A systemic gentamicin pathway across the stria vascularis. *Hear Res.* 2008; 235:114–124. [PubMed: 18082985]
18. Wang Q, Steyger PS. Trafficking of systemic fluorescent gentamicin into the cochlea and hair cells. *J Assoc Res Otolaringol.* 2009; 10:205–219.
19. Karasawa T, Sibrian-Vazquez M, Strongin RM, Steyger PS. Identification of Cisplatin-Binding Proteins Using Agarose Conjugates of Platinum Compounds. *PLoS One.* 2013; 8:e66220. [PubMed: 23755301]
20. Owens KN, Santos F, Roberts B, Linbo T, Coffin AB, Knisely AJ, Simon JA, Rubel EW, Raible DW. Identification of genetic and chemical modulators of zebrafish mechanosensory hair cell death. *PLoS Genet.* 2008; 4:e1000020. [PubMed: 18454195]
21. Dai CF, Mangiardi D, Cotanche DA, Steyger PS. Uptake of fluorescent gentamicin by vertebrate sensory cells in vivo. *Hear Res.* 2006; 213:64–78. [PubMed: 16466873]
22. Li H, Steyger PS. Systemic aminoglycosides are trafficked via endolymph into cochlear hair cells. *Sci Rep.* 2011; 1:159. [PubMed: 22355674]
23. Takada K, Kawamura T, Inai M, Masuda S, Oka T, Yoshikawa Y, Shibata N, Yoshikawa H, Ike O, Wada H, Hitomi S. Pharmacokinetics of cisplatin in albuminemic rats. *Biopharm Drug Dispos.* 1999; 20:421–428. [PubMed: 10951431]
24. Hall KS, Lien B, Endresen L, Bergan A, Rugstad HE. Changes in pharmacokinetics of cisplatin after hepatic resection in rats. *Drug Metab Dispos.* 1991; 19:725–728. [PubMed: 1680646]
25. Ciarimboli G, Deuster D, Knief A, Sperling M, Holtkamp M, Edemir B, Pavenstadt H, Lanvers-Kaminsky C, Zehnhoff-Dinnesen A, Schinkel AH, Koepsell H, Jurgens H, Schlatter E. Organic cation transporter 2 mediates cisplatin-induced oto- and nephrotoxicity and is a target for protective interventions. *Am J Pathol.* 2010; 176:1169–1180. [PubMed: 20110413]
26. Laurell G, Ekborn A, Viberg A, Canlon B. Effects of a Single High Dose of Cisplatin on the Melanocytes of the Stria Vascularis in the Guinea Pig. *Audiol Neuro-Otol.* 2007; 12:170–178.
27. Koo JW, Quintanilla-Dieck L, Jiang M, Liu J, Urdang ZD, Allensworth JJ, Cross CP, Li H, Steyger PS. Endotoxemia-mediated inflammation potentiates aminoglycoside-induced ototoxicity. *Sci Transl Med.* 2015; 7:298ra118.
28. Paraskar AS, Soni S, Chin KT, Chaudhuri P, Muto KW, Berkowitz J, Handlogten MW, Alves NJ, Bilgicer B, Dinulescu DM, Mashelkar RA, Sengupta S. Harnessing structure-activity relationship to engineer a cisplatin nanoparticle for enhanced antitumor efficacy. *Proc Natl Acad Sci U S A.* 2010; 107:12435–12440. [PubMed: 20616005]
29. Barth RF, Wu G, Meisen WH, Nakkula RJ, Yang W, Huo T, Kellough DA, Kaumaya P, Turro C, Agius LM. Design, synthesis, and evaluation of cisplatin-containing EGFR targeting bioconjugates as potential therapeutic agents for brain tumors. *OncoTargets Ther.* 2016; 9:2769–2781.
30. Callari M, Aldrich-Wright JR, de Souza PL, Stenzel MH. Polymers with platinum drugs and other macromolecular metal complexes for cancer treatment. *Prog Polym Sci.* 2014; 39:1614–1643.

31. Seymour LW, Ulbrich K, Steyger PS, Brereton M, Subr V, Strohalm J, Duncan R. Tumour tropism and anti-cancer efficacy of polymer-based doxorubicin prodrugs in the treatment of subcutaneous murine B16F10 melanoma. *Br J Cancer*. 1994; 70:636–641. [PubMed: 7917909]
32. Steyger PS, Baban DF, Brereton M, Ulbrich K, Seymour LW. Intratumoural distribution as a determinant of tumour responsiveness to therapy using polymer-based macromolecular prodrugs. *J Controlled Release*. 1996; 39:35–46.
33. Hann S, Stefánka Z, Lenz K, Stingeder G. Novel separation method for highly sensitive speciation of cancerostatic platinum compounds by HPLC–ICP–MS. *Anal Bioanal Chem*. 2005; 381:405–412. [PubMed: 15455190]

Figure 1.
Top: Structures of cisplatin conjugates **1** and **2**. Bottom: Synthesis of **2**.

Figure 2. Top: Absorption spectra. Bottom: Excitation/emission fluorescence spectra of 1.25 μM solutions in MeOH of compounds **2** and **4**.

Figure 3. Cytotoxicity in zebrafish neuromast hair cells with a range of dose after 3 h recovery. (A–C) Neuromast hair cells treated with various concentrations of conjugate **2** (50–200 µg/mL) for 4 h. Phalloidin labeling (green) revealed the actin cytoskeleton of individual cells. (D) Treatment with **4**. (E) Quantification of surviving zebrafish neuromast hair cells after treatment with cisplatin or conjugate **2** (0–100 µg/mL). $N = 15$ neuromasts per dose, error bars = 95% confidence intervals.

Figure 4.

Uptake and clearance of **2** (red) in kidney (A–C), liver (D,E), and choroid plexus cells (G–I) over time. (F) Liver tissues of rats treated in the absence of **2**, showing only weak punctate autofluorescence. Alexa Fluor 488-conjugated phalloidin (green) revealed the actin cytoskeleton of individual cells. p, proximal tubule cells; d, distal tubule cells.

Figure 5.

Three hours after injection of **2**, fluorescence is most prominent in the stria vascularis (SV). SL, spiral ligament; OoC, organ of Corti; SpL, spiral limbus; M, modiolus.

Figure 6. Uptake and clearance of **2** in marginal cells (A,E,I), intrastratial tissues (B,F,J), basal cells (C,G,K), and spiral ligament fibrocytes (D,H,L) over time.

Figure 7. Uptake and clearance of **2** in marginal cells, intermediate cells, basal cells, and spiral ligament fibrocytes over time. *, significance difference ($P < 0.05$) versus all other time points (except with marginal cells at 24 h); error bars = standard error of the mean, $N = 3$.

Figure 8. Uptake and clearance of **2** in OHCs (A,D,F) and IHCs (B,E,G) over time. (C) Note the punctate autofluorescence (at 568 nm) in supporting cells adjacent to IHCs (not visible) of rats treated without **2** or **4**.