Nucleolar Stress Induction by Oxaliplatin and Derivatives

Emily C. Sutton[#,†,‡], Christine E. McDevitt[#,§], Jack Y. Prochnau[§], Matthew V. Yglesias[‡§], Austin M. Mroz[§ǁ], Min Chieh Yang[§ǁ], Rachael M. Cunningham[§], Christopher H. Hendon[§ǁ], Victoria J. DeRose[*,‡,§,ǁ]

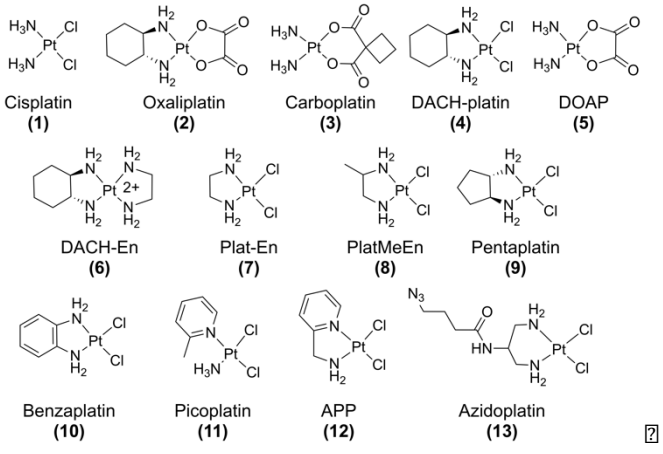
[†]Department of Biology, [‡]Institute of Molecular Biology, [§]Department of Chemistry and Biochemistry, [ǁ]Materials Science Institute — University of Oregon, Eugene, OR 97403

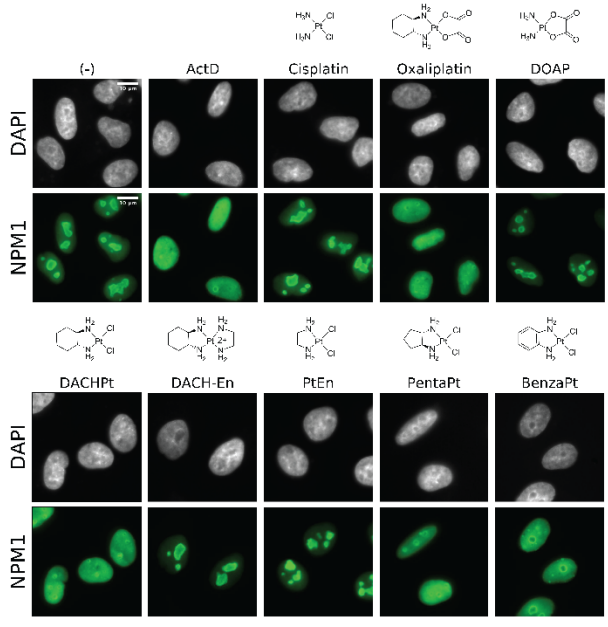
Supporting Information Placeholder

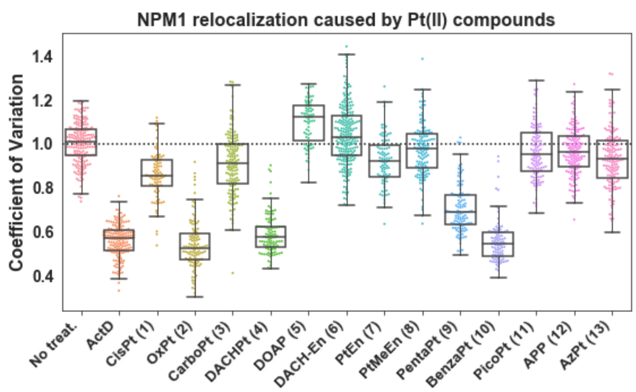
ABSTRACT: Platinum(II) compounds are a critical class of chemotherapeutic agents. Recent studies have highlighted the ability of a subset of Pt(II) compounds, including oxaliplatin but not cisplatin, to induce cytotoxicity via nucleolar stress rather than a canonical DNA damage response. In this study, influential properties of Pt(II) compounds were investigated using redistribution of nucleophosmin (NPM1) as a marker of nucleolar stress. NPM1 assays were coupled to calculated and measured properties such as compound size and hydrophobicity. The oxalate leaving group of oxaliplatin is not required for NPM1 redistribution. Interestingly, although changes in diaminocyclohexane (DACH) ligand ring size and aromaticity can be tolerated, ring orientation appears important for stress induction. The specificity of ligand requirements provides insight into the striking ability of only certain Pt(II) compounds to activate nucleolar processes.

The chemotherapeutic agent cisplatin has inspired the synthesis and investigation of thousands of Pt(II) analogs.[1] Of these, only two other compounds — carboplatin and oxaliplatin — have met FDA standards for medical use. Until recently, it was believed that the cytotoxicity of these compounds could be attributed solely to their DNA crosslinking abilities and subsequent induction of the DNA Damage Response (DDR), a known trigger of apoptotic pathways.[2] As the body of research on Pt(II) reagents has grown, a more complex picture has emerged of the mechanisms of action behind these ubiquitous drugs.[2] A striking recent discovery is that oxaliplatin, but not cisplatin or carboplatin, causes cytotoxicity via disruptions in ribosome biogenesis rather than DDR.[3] Ribosome biogenesis occurs in the nucleolus, a conserved and highly structured organelle in eukaryotes. Disruptions of the nucleolus or ribosome biogenesis trigger the nucleolar stress response, which leads to cell death or senescence via activation of the tumor suppressor p53. Because its molecular mechanisms are not yet fully understood, and due to its potential role as a chemotherapeutic target, this fascinating stress process is an area of intense interest in the fields of molecular biology and medicine.[4–7]

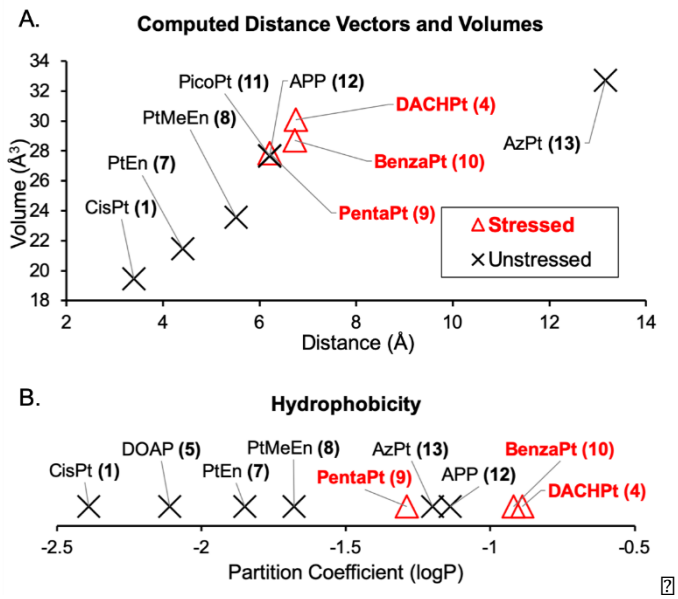
The specificity of oxaliplatin as a nucleolar stress inducer is intriguing when considered alongside other data indicating a relationship between Pt(II) compounds and the nucleolus.[8] Post-treatment fluorescent labeling of clickable Pt(II) drug analogs has shown localization of these compounds to the nucleolus,[8,9] and there is significant evidence that Pt(II) compounds associate with ribosomes and ribosomal RNA (rRNA).[10–16] The structural determinants and molecular mechanisms by which only specific Pt(II) compounds may cause a nucleolar stress response are not understood. Here, we explore properties of oxaliplatin and other Pt(II) compounds and find that a narrow window of derivatives is able to induce nucleolar stress. The results define a set of constraints for Pt(II) compounds to induce this unique cell death pathway.

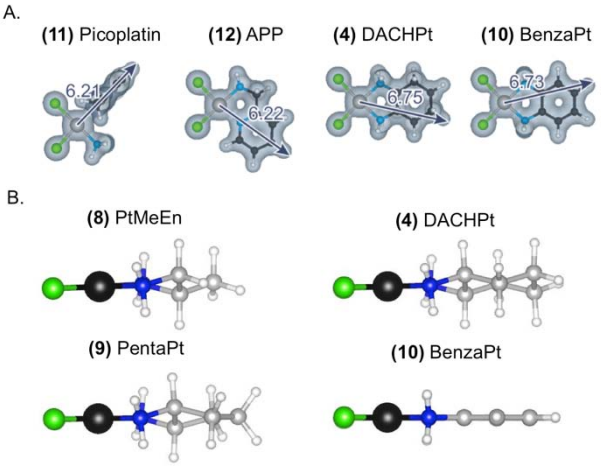

Cisplatin
(1)
Oxaliplatin
(2)
Carboplatin
(3)
DACH-platin
(4)
DOAP
(5)
DACH-En
(6)
Plat-En
(7)
PlatMeEn
(8)
Pentaplatin
(9)
Benzaplatin
(10)
Picoplatin
(11)
APP
(12)
Azidoplatin
(13)


(-)
ActD
Cisplatin
Oxaliplatin
DOAP
DAPI
NPM1
DACHPt
DACH-En
PtEn
PentaPt
BenzaPt
DAPI
NPM1



NPM1 relocalization caused by Pt(II) compounds
Coefficient of Variation
1.4
1.2
1.0
0.8
0.6
0.4
No treat.
ActD
CisPt (1)
OxPt (2)
CarboPt (3)
DACHPt (4)
DOAP (5)
DACH-En (6)
PtEn (7)
PtMeEn (8)
PentaPt (9)
BenzaPt (10)
PicoPt (11)
APP (12)
AzPt (13)


A.
Computed Distance Vectors and Volumes
Volume (Å³)
Distance (Å)
PicoPt (11)
APP (12)
DACHPt (4)
PtMeEn (8)
AzPt (13)
BenzaPt (10)
PtEn (7)
PentaPt (9)
CisPt (1)
Stressed
Unstressed
B.
Hydrophobicity
Partition Coefficient (logP)
CisPt (1)
DOAP (5)
PtEn (7)
PtMeEn (8)
PentaPt (9)
AzPt (13)
APP (12)
BenzaPt (10)
DACHPt (4)


A.
(11) Picoplatin
(12) APP
(4) DACHPt
(10) BenzaPt
6.21
6.22
6.75
6.73
B.
(8) PtMeEn
(4) DACHPt
(9) PentaPt
(10) BenzaPt

the biomolecular interactions that maintain nucleolar integrity. More work is needed to understand this fascinating biological stress process and to define the specific properties of Pt(II) compounds that cause it.

ASSOCIATED CONTENT

Supporting Information.
The Supporting Information is available free of charge on the ACS Publications website.

Supplementary figures, tables, materials and methods (PDF)

Supplementary NMR Spectra (PDF)

AUTHOR INFORMATION

Corresponding Author

*derose@uoregon.edu

Author Contributions

#Co-first authors

Funding Sources

No competing financial interests have been declared.

This work was supported by the National Science Foundation [CHE1710721 to VJD], the NIH [T32 GM007759-29 to ECS] and used the Extreme Science and Engineering Discovery Environment (XSEDE), which is supported by National Science Foundation [ACI-1548562]. Computations were also performed on the PICS Coeus high performance computer, which is supported by the National Science Foundation [1624776]. This work is also supported by the Department of Chemistry and Biochemistry, Department of Biology, Institute of Molecular Biology and the Material Science Institute at the University of Oregon.

REFERENCES

(1) Kelland, L. The Resurgence of Platinum-Based Cancer Chemotherapy. *Nat. Rev. Cancer* **2007**, *7* (8), 573–584.

(2) Wexselblatt, E.; Yavin, E.; Gibson, D. Cellular Interactions of Platinum Drugs. *Inorganica Chim. Acta* **2012**, *393*, 75–83.

(3) Bruno, P. M.; Liu, Y.; Park, G. Y.; Murai, J.; Koch, C. E.; Eisen, T. J.; Pritchard, J. R.; Pommier, Y.; Lippard, S. J.; Hemann, M. T. A Subset of Platinum-Containing Chemotherapeutic Agents Kills Cells by Inducing Ribosome Biogenesis Stress. *Nat. Med.* **2017**, *23* (4), 461–471.

(4) Tsai, R. Y. L.; Pederson, T. Connecting the Nucleolus to the Cell Cycle and Human Disease. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **2014**, *28* (8), 3290–3296.

(5) Woods, S. J.; Hannan, K. M.; Pearson, R. B.; Hannan, R. D. The Nucleolus as a Fundamental Regulator of the P53 Response and a New Target for Cancer Therapy. *Biochim. Biophys. Acta BBA - Gene Regul. Mech.* **2015**, *1849* (7), 821–829.

(6) Boulon, S.; Westman, B. J.; Hutten, S.; Boisvert, F.-M. M.; Lamond, A. I. The Nucleolus under Stress. *Mol. Cell* **2010**, *40* (2), 216–227.

(7) Farley-Barnes, K. I.; McCann, K. L.; Ogawa, L. M.; Merkel, J.; Surovtseva, Y. V.; Baserga, S. J. Diverse Regulators of Human Ribosome Biogenesis Discovered by Changes in Nucleolar Number. *Cell Rep.* **2018**, *22* (7), 1923–1934.

(8) Pickard, A. J.; Bierbach, U. The Cell's Nucleolus: An Emerging Target for Chemotherapeutic Intervention. *ChemMedChem* **2013**, *8* (9), 1441–1449.

(9) Wirth, R.; White, J. D.; Moghaddam, A. D.; Ginzburg, A. L.; Zakharov, L. N.; Haley, M. M.; DeRose, V. J. Azide vs. Alkyne Functionalization in Pt(II) Complexes for Post-Treatment Click Modification: Solid State Structure, Fluorescent Labeling, and Cellular Fate. *J. Am. Chem. Soc.* **2015**, *137* (48), 15169-15175.

(10) Plakos, K.; DeRose, V. J. Mapping Platinum Adducts on Yeast Ribosomal RNA Using High-Throughput Sequencing. *Chem. Commun.* **2017**, *53* (95), 12746–12749.

(11) Hostetter, A. A.; Osborn, M. F.; DeRose, V. J. Characterization of RNA-Pt Adducts Formed from Cisplatin Treatment of *Saccharomyces cerevisiae*. *ACS Chem Biol* **2012**, *7* (1), 218–225.

(12) Osborn, M. F.; White, J. D.; Haley, M. M.; DeRose, V. J. Platinum-RNA Modifications Following Drug Treatment in *S. cerevisiae* Identified by Click Chemistry and Enzymatic Mapping. *ACS Chem. Biol.* **2014**, *9* (10), 2404-2411.

(13) Melnikov, S. V.; Soll, D.; Steitz, T. A.; Polikanov, Y. S. Insights into RNA Binding by the Anticancer Drug Cisplatin from the Crystal Structure of Cisplatin-Modified Ribosome. *Nucleic Acids Res.* **2016**, *44* (10), 4978–4987.

(14) Rijal, K.; Chow, C. S. A New Role for Cisplatin: Probing Ribosomal RNA Structure. *Chem. Commun.* **2007**, *0* (1), 107-109.

(15) Saunders, A. M.; DeRose, V. J. Beyond Mg2+: Functional Interactions between RNA and Transition Metals. *Curr. Opin. Chem. Biol.* **2016**, *31*, 153–159.

(16) Sutton, E. C.; McDevitt, C. E.; Yglesias, M. V.; Cunningham, R. M.; DeRose, V. J. Tracking the Cellular Targets of Platinum Anticancer Drugs: Current Tools and Emergent Methods. *Inorganica Chim. Acta* **2019**, 118984.

(17) Rubbi, C. P.; Milner, J. Disruption of the Nucleolus Mediates Stabilization of p53 in Response to DNA Damage and Other Stresses. *EMBO J.* **2003**, *22* (22), 6068–6077.

(18) Yang, K.; Wang, M.; Zhao, Y.; Sun, X.; Yang, Y.; Li, X.; Zhou, A.; Chu, H.; Zhou, H.; Xu, J.; Wu, M.; Yang, J; Yi, J. A Redox Mechanism Underlying Nucleolar Stress Sensing by Nucleophosmin. *Nat. Commun.* **2016**, *7*, 13599.

(19) Bursac, S.; Brdovcak, M. C.; Donati, G.; Volarevic, S. Activation of the Tumor Suppressor P53 upon Impairment of Ribosome Biogenesis. *Biochim. Biophys. Acta* **2014**, *1842* (6), 817–830.

(20) Nicolas, E.; Parisot, P.; Pinto-Monteiro, C.; de Walque, R.; De Vleeschouwer, C.; Lafontaine, D. L. J. Involvement of Human Ribosomal Proteins in Nucleolar Structure and P53-Dependent Nucleolar Stress. *Nat. Commun.* **2016**, *7*, 11390.

(21) Bruno, P. M.; Liu, Y.; Park, G. Y.; Murai, J.; Koch, C. E.; Eisen, T. J.; Pritchard, J. R.; Pommier, Y.; Lippard, S. J.; Hemann, M. T. A Subset of Platinum-Containing Chemotherapeutic Agents Kills Cells by Inducing Ribosome Biogenesis Stress. *Nat. Med.* **2017**, *23* (4), 461–471.

(22) McDevitt, C. E.; Yglesias, M. V.; Mroz, A. M.; Sutton, E. C.; Yang, M. C.; Hendon, C. H.; DeRose, V. J. Monofunctional Platinum(II) Compounds and Nucleolar Stress: Is Phenanthriplatin Unique? *J. Biol. Inorg. Chem.* **2019**. *24* (6), 899-908.

(23) Chaney, S. G.; Campbell, S. L.; Bassett, E.; Wu, Y. Recognition and Processing of Cisplatin- and Oxaliplatin-DNA Adducts. *Crit. Rev. Oncol. Hematol.* **2005**, *53* (1), 3–11.

(24) Faivre, S.; Chan, D.; Salinas, R.; Woynarowska, B.; Woynarowski, J. M. DNA Strand Breaks and Apoptosis In-

duced by Oxaliplatin in Cancer Cells. *Biochem. Pharmacol.* **2003**, *66* (2), 225–237.
(25) Woynarowski, J. M.; Faivre, S.; Herzig, M. C.; Arnett, B.; Chapman, W. G.; Trevino, A. V.; Raymond, E.; Chaney, S. G.; Vaisman, A.; Varchenko, M.; Juniewicz, P.E. Oxaliplatin-Induced Damage of Cellular DNA. *Mol. Pharmacol.* **2000**, *58* (5), 920–927.
(26) Jerremalm, E.; Videhult, P.; Alvelius, G.; Griffiths, W. J.; Bergman, T.; Eksborg, S.; Ehrsson, H. Alkaline Hydrolysis of Oxaliplatin—Isolation and Identification of the Oxalato Monodentate Intermediate. *J. Pharm. Sci.* **2002**, *91* (10), 2116–2121.
(27) Zacharioudakis, E.; Agarwal, P.; Bartoli, A.; Abell, N.; Kunalingam, L.; Bergoglio, V.; Xhemalce, B.; Miller, K. M.; Rodriguez, R. Chromatin Regulates Genome Targeting with Cisplatin. *Angew. Chem. Int. Ed Engl.* **2017**, *56* (23), 6483–6487.
(28) Brunner, H.; Schellerer, K.-M. New Porphyrin Platinum Conjugates for the Cytostatic and Photodynamic Tumor Therapy. *Inorganica Chim. Acta* **2003**, *350*, 39–48.
(29) Siew, Y.-Y.; Neo, S.-Y.; Yew, H.-C.; Lim, S.-W.; Ng, Y.-C.; Lew, S.-M.; Seetoh, W.-G.; Seow, S.-V.; Koh, H.-L. Oxaliplatin Regulates Expression of Stress Ligands in Ovarian Cancer Cells and Modulates Their Susceptibility to Natural Killer Cell-Mediated Cytotoxicity. *Int. Immunol.* **2015**, *27* (12), 621–632.
(30) Englinger, B.; Pirker, C.; Heffeter, P.; Terenzi, A.; Kowol, C. R.; Keppler, B. K.; Berger, W. Metal Drugs and the Anticancer Immune Response. *Chem. Rev.* **2019**, *119* (2), 1519–1624.
(31) Terenzi, A.; Pirker, C.; Keppler, B. K.; Berger, W. Anticancer Metal Drugs and Immunogenic Cell Death. *J. Inorg. Biochem.* **2016**, *165*, 71–79.
(32) McKeage, M. J.; Hsu, T.; Screnci, D.; Haddad, G.; Baguley, B. C. Nucleolar Damage Correlates with Neurotoxicity Induced by Different Platinum Drugs. *Br. J. Cancer* **2001**, *85* (8), 1219–1225.
(33) Florent, T; Parmentier, B; El Fajoui, Z; Estornes, Y; Chayvialle, J; Saurin, J; Abello, J. P53 Dependent and Independent Sensitivity to Oxaliplatin of Colon Cancer Cells. *Biochem. Pharmacol.* **2007**, *74* (3), 392–406.
(34) Keck, M. V.; Lippard, S. J. Unwinding of Supercoiled DNA by Platinum-Ethidium and Related Complexes. *J. Am. Chem. Soc.* **1992**, *114* (9), 3386–3390.
(35) Malina, J.; Novakova, O.; Vojtiskova, M.; Natile, G.; Brabec, V. Conformation of DNA GG Intrastrand Cross-Link of Antitumor Oxaliplatin and Its Enantiomeric Analog. *Biophys. J.* **2007**, *93* (11), 3950–3962.

TOC figure: