Influence of ring modifications on nucleolar stress caused by oxaliplatin-like compounds

Christine E. McDevitt,[a][b] Andres S. Guerrero,[a][b] Haley M. Smith,[b] Victoria J. DeRose*[b]

[a] Co-first authors
[b] C.E. McDevitt, A.S. Guerrero, H.M. Smith, Prof. V.J. DeRose
Department of Chemistry and Biochemistry
Institute of Materials Science
University of Oregon
Eugene, OR, USA 97403
derose@uoregon.edu

Abstract: Oxaliplatin, a platinum compound in broad clinical use, can induce cell death through a nucleolar stress pathway rather than the canonical DNA damage response studied for other Pt(II) compounds. Previous work has found that the oxaliplatin 1,2-diaminocyclohexane (DACH) ring but not the oxalate leaving group is important to the ability to induce nucleolar stress. Here we study the influence of DACH ring substituents at the 4-position on the ability of DACH-Pt(II) compounds to cause nucleolar stress. We determine that DACH-Pt(II) compounds with 4-position methyl, ethyl, or propyl substituents induce nucleolar stress, but DACH-Pt(II) compounds with 4-isopropyl substituents do not induce nucleolar stress. This effect is independent of whether the substituent is in the axial or equatorial position relatively to the trans diamines of the ligand. These results suggest that spatially sensitive interactions could be involved in the ability of platinum compounds to cause nucleolar stress.

Introduction

Platinum chemotherapeutic drugs have broad clinical use.[1–3] Cisplatin, has been in use for over 40 years, while the newest U.S. FDA-approved derivative, oxaliplatin, has been in use for over 20 years.[4] Although they are both square-planar Pt(II) compounds, the 1,2-diaminocyclohexane (DACH) ligand and chelating oxalate leaving group of oxaliplatin differ significantly from the smaller diammine and dichloro ligands of cisplatin. Historically, oxaliplatin is used to treat colorectal cancers and lung cancers while cisplatin is used to treat bladder, testicular, cervical and ovarian cancer.[1] Despite their extensive clinical use, it was only recently discovered that oxaliplatin can cause cell death via induction of the nucleolar stress pathway, as opposed to the DNA damage response pathway induced by cisplatin.[4–7] The mononuclear Pt(II) compound phenanthriplatin also causes nucleolar stress.[5,6]

The differences in the mechanism of action between oxaliplatin and cisplatin led us to perform a limited structure-function study to determine characteristics that were necessary to cause nucleolar stress.[6] We found that exchange of the oxalate leaving group for dichloro ligands still supports nucleolar stress, indicating that the DACH carrier ligand is the relevant difference. Modification of the DACH ligand to an aromatic or diaminocyclopentane still supports nucleolar stress, although the latter smaller ring induces less of an effect.[6] Other ligands with similar bulk or hydrophobicity do not produce nucleolar stress, indicating the importance of ligand orientation. One notable example of the importance of ligand orientation was comparison of benzaplatin (*cis*-[1,2-phenylenediamine]dichloride platinum (II)) and APP (*cis*-[2-picolylamine]dichloride platinum (II)).[6] Both compounds include a six membered, aromatic ring situated similarly to the DACH ligand of oxaliplatin. In benzaplatin, which causes nucleolar stress, the benzene ring's steric bulk is located in the same orientation as the DACH ligand. For APP, however, the 2-picolylamine, shifts the orientation of the steric bulk of the aromatic ring with respect to the Pt-diamine plane, and this compound does not cause nucleolar stress. This strong dependence on size and orientation of the non-labile platinum ligand could point toward a specific interaction that is being disrupted by these platinum compounds in causing nucleolar stress. Here, we further examine derivatization of the ligand ring, specifically at the 4- and 5- positions on the DACH ligand, in order to determine a substitution tolerance for this class of Pt(II) compounds in causing nucleolar stress.

Derivatives of Pt(II)-DACH compounds have been studied previously by introducing various alkyl groups at the 4-position. Previously studied substituents include methyl, ethyl, propyl, tert-butyl, and phenyl.[8] Additionally, differences between alkyl group substitution in the axial and equatorial positions have also been investigated, with most investigation around the 4-methyl and 4-ethyl derivatives. Galanski and coworkers have found that generally the 4-methyl derivatives are more toxic than the 4-ethyl derivatives with the effect being more pronounced at longer time points and dependent on cell type.[8,9] A later study found that the addition of a bulky t-butyl group in the same position significantly lowered the toxicity of the platinum compound, indicating an important sensitivity to changes in structure despite the compound being more hydrophobic, which would enhance cellular uptake and potentially drive segregation to more hydrophobic regions of the cell.[8,10]

The influence of 4-methyl DACH ring substitutions as well as influence of *R,R* and *S,S* chirality have also been investigated by Abramkin and coworkers.[11] Interestingly, when measured in cell culture, the installation of a 4-methyl group in the axial orientation is more toxic than in the equatorial position, and this compound also has higher toxicity than oxaliplatin. However, in mouse tumor models, the effectiveness is flipped with the equatorial 4-methyl substituent being more effective. In mice, the equatorial 4-methyl Pt(II) compound is also more tolerated, leading to the possibility of higher survivability at more effective dose concentrations.[11] Additionally, Jungwirth and coworkers have found that an oxaliplatin derivative with a 4-methyl group in the axial position is more toxic than oxaliplatin or a derivative with an equatorial 4-methyl substituent when measured in HCT-116 cells exhibiting a p53 knock out as well as the corresponding oxaliplatin resistant cell lines.[12] This result is particularly interesting given that p53 is a known part of the nucleolar stress pathway.

Here we investigate how substitutions at the DACH 4,5 positions influence the ability of Pt(II) compounds to cause nucleolar stress (**Figure 1)**. We test 4-methylDACH-Pt **(2, 3)**, 4-ethylDACH-Pt **(4, 5),** and 4-propylDACH-Pt **(6, 7)** with the substituent in the axial or equatorial positions. Additionally, we investigate DACH-4-ene-Pt **(8)**, a compound with a double bond at the 4,5 position that has previously been shown to be more

effective than cisplatin, but less effective than oxaliplatin, particularly in colon cancer cell lines. Overall, it was able to overcome cisplatin and oxaliplatin-resistant cell lines, with the R,R enantiomer being more cytotoxic than the S,S enantiomer in DACH-4-ene-Pt(II) and S,S enantiomer being more cytotoxic than the R,R enantiomer in DACH-4-ene-Pt(IV). Treatment with DACH-4-ene-Pt(II/IV) compounds induced DNA fragmentation and condensation, which could be indicative of nucleolar stress.[13,14] Axial 4-acetamideDACH-Pt **(9)**, equatorial 4-isopropylDACH-Pt **(10)**, and axial 4-isopropylDACH-Pt **(11)** add increased bulk to the ring. We find that 4-methylDACH-Pt, 4-ethylDACH-Pt, and 4-propylDACH-Pt caused nucleolar stress whether substituents were in the axial or equatorial positions, and that the elimination of axial protons in DACH-4-ene-Pt **(8)** also does not affect induction of nucleolar stress. Interestingly, axial 4-acetamideDACH-Pt **(11)** and both axial and equatorial 4-isopropylDACH-Pt derivatives (**9, 10**) do not cause nucleolar stress, providing evidence for an interaction with a high specificity and selectivity that induces the nucleolar stress response by platinum compounds.

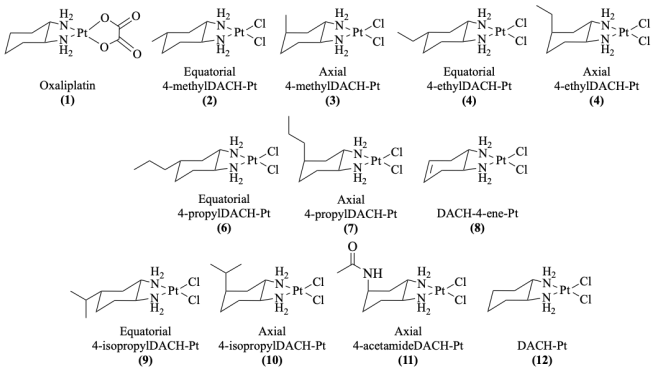


Figure 1. Pt(II) compounds tested for nucleolar stress induction.

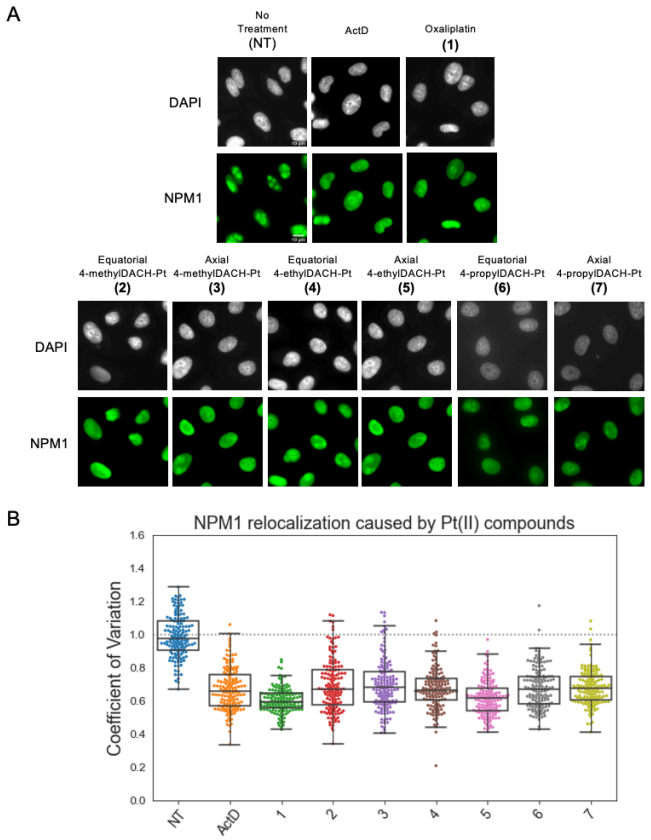


Figure 2. (A) Representative cell images of nucleolar stress induction at 24 hours observed by NPM1 immunofluorescence (green). Scale bar=10 um. Stock solution made with DMF (**2, 3, 4, 5, 6, 7**), water **(1)**, or DMSO **(ActD)**. Treatments are 10 uM for Pt(II) derivatives and 5 nM for Actinomycin D. (B) Quantification of NPM1 relocalization by Pt(II) compounds. For each treatment data set, boxes represent median, first and third quartiles, and vertical lines represent the range of data with outliers.

Results and Discussion

Pt(II)-DACH compounds containing 4-position methyl, ethyl, and propyl groups cause a nucleolar stress response

In order to investigate the effects of modifications to the DACH ring of oxaliplatin we created a small library of derivatives that increased steric bulk at the 4-position in a stepwise manner (**Figure 1**). We previously showed that substituting the oxalate leaving group with dichloro ligands did not influence nucleolar stress as observed at 24 hrs post-treatment,[6] and so the dichloro DACH-Pt(II) derivatives were used in this study. In order to identify whether compounds cause nucleolar stress, relocalization of NPM1 (nucleophosmin) was monitored and quantified by coefficient of variation (CV) analysis, as previously described.[6,7,13]

Treatment of A549 cells for 24 hrs with 4-methylDACH-Pt **(2, 3)**, 4-ethylDACH-Pt **(4, 5)**, and 4-propylDACH-Pt **(6,7)** causes nucleolar stress (**Figure 2)** as indicated by a median CV around 0.6 in all cases. This observation with methyl, ethyl, and propyl groups at the 4- position shows that small modifications of the DACH ligand still support nucleolar stress as the mechanism of action, and that this could be a viable position to utilize when designing platinum compounds that cause nucleolar stress.

It has been previously noted that the positioning of methyl groups on the DACH ring may influence activity. The isomers **2** and **3** have been reported to have different toxicity levels in cells and different dosage tolerances in mice.[11,12] The 4-methylDACH-Pt, 4-methylDACH-Pt, and 4-propylDACH-Pt compounds **(2-7)** cause nucleolar stress independent of whether the 4-substituent is in the axial or equatorial position (**Figure 2**). These results suggest that the mechanism of action on nucleolar processes remains the same for both axial and equatorial derivatives. The toxicity differences noted previously may be explained through other interactions that could occur between platinum and biomolecules, such as detoxification pathways.[12] We have previously noted that toxicity at 24 hrs does not correlate with nucleolar stress response.[6] For example, some Pt(II) compounds that cause robust nucleolar stress at 24 hrs do not induce significant cytotoxicity until later times.[6] Similarly, differences in the cytotoxicity of this small library of compounds as reported in prior studies[9,11,12,14] does not appear to influence whether these compounds cause nucleolar stress.

Axial protons at Pt(II)-DACH 4,5- positions are not necessary to cause nucleolar stress

In addition to methyl and ethyl derivatives, which increase the size and hydrophobicity of the DACH ligand of oxaliplatin, we also tested eliminating the protons at the 4- and 5-position by introducing a double bond with DACH-4-ene-Pt **(8)**.[10] When this compound was used to treat cells, NPM1 relocalized to the nucleoplasm indicating that the compound caused nucleolar stress **(Figure 3)**. The chair conformation found in the DACH ligand is preserved in DACH-4-ene-Pt **(8)**, while the axial protons

at the 4,5- positions are no longer present. This indicates that the DACH 4,5-position protons are likely not involved in a biomolecular interaction that is causing nucleolar stress.

Pt(II)-DACH compounds with bulky constituents at the 4-position do not cause nucleolar stress

In order to investigate the effect of adding bulk to the 4-position, axial 4-acetamideDACH-Pt **(11)**, equatorial 4-isopropylDACH-Pt **(9)**, and axial 4-isopropylDACH-Pt **(10)** were synthesized and tested for nucleolar stress induction. Neither axial 4-acetamideDACH-Pt **(11)** or axial or equatorial 4-isopropylDACH-Pt derivatives **(9, 10)** caused NPM1 to relocalize, showing CV values around 1 in all cases indicating that they did not cause nucleolar stress (**Figure 3**). This is in contrast to the 4-methylDACH-Pt, 4-ethylDACH-Pt, and 4-propylDACH-Pt derivatives **(2-7)** which all caused nucleolar stress (**Figure 2**)**.** It is striking that the addition of a second methyl to form the 4-isopropylDACH-Pt **(9, 10)** compounds creates enough increase in steric bulk to cause the platinum compounds to no longer cause nucleolar stress. Similarly, the larger acetamide present on 4-acetamideDACH-Pt **(11)** stops the compounds from causing nucleolar stress **(Figure 3)**. Previous literature has noted that a platinum compound containing a *t*-butyl at the 4- position has 10-fold lower cytotoxicity than the methyl- or ethyl-derivatized platinum compounds.[8] The significant differences in toxicity could be related to different mechanisms of action for platinum compounds with larger substituents on the DACH ring.

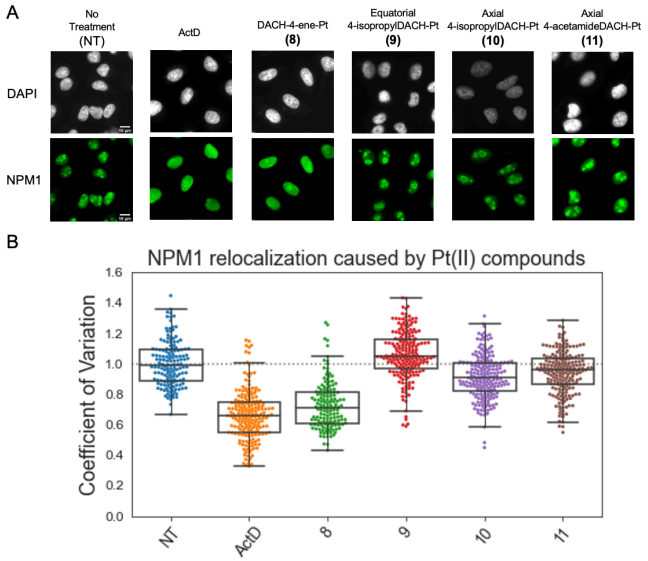


Figure 3. (A) Representative cell images of nucleolar stress induction at 24 hours observed by NPM1 immunofluorescence (green). Scale bar=10 um. Stock solution made with DMF (**9, 10, 11**) or DMSO **(ActD, 8)**. Treatments are 10 uM for Pt(II) derivatives and 5 nM for Actinomycin D. (B) Quantification of NPM1 relocalization by Pt(II) compounds. For each treatment data set, boxes represent median, first and third quartiles, and vertical lines represent the range of data with outliers.

Equatorial 4-isopropylDACH-Pt **(9)**, and axial 4-isopropylDACH-Pt **(10)** are more hydrophobic and bulkier[10] than the 4-methylDACH-Pt **(1**, **2)**, and 4-ethylDACH-Pt **(3**, **4)** compounds yet they do not cause nucleolar stress. The hydrophobicity of 4-acetamideDACH-Pt **(11),** compared to compounds with only a DACH ligand, is less intuitive. In order to determine the hydrophobicity of the axial 4-acetamideDACH-Pt **(11)** we determined the logP value, allowing comparison with other nucleolar stress-inducing compounds.[6] The logP value of axial 4-acetamideDACH-Pt **(11)** was found to be -1.03±0.02. This value is less than that previously measured for DACH-Pt (logP = -0.89) but greater than that of the 5-membered ring compound penta-Pt (logP = -1.29), both of which cause nucleolar stress.[6] The hydrophobicities of compounds **9**, **10,** and **11** do not provide an explanation for why these compounds do not cause nucleolar stress. Instead, the increase in steric bulk at the 4- position appears to change the mechanism of action of these compounds, while derivatives **2-6** with smaller 4-position substituents cause nucleolar stress.

It is possible that the increased bulk of the isopropyl in equatorial 4-isopropylDACH-Pt **(9)**, axial 4-isopropylDACH-pt **(10)**, and the amide bond in axial 4-acetamideDACH-Pt **(11)** could help to explain why these compounds no longer cause nucleolar stress. DFT calculations were performed to obtain the volume taken up by the different compounds as well as to visually compare their properties. The main trends observed are that compounds with axial substituents tend to take up a larger total volume than that of the equatorial compounds **(Table S1, Figure 4)**, but interestingly a correlation was not able to be made that steric bulk alone was responsible for inducing nucleolar stress. For example, the axial 4-propylDACH-Pt **(7)** has a similar total volume to that of the axial 4-isopropylDACH-Pt **(10)** as well as axial 4-acetamideDACH-Pt **(11) (Figure 4).** The substituents in the isopropyl **(9, 10)** and the axial acetamide **(11)** compounds compared to that of the methyl, ethyl, and propyl **(2-7)** compounds mainly differ in disposition of steric bulk at the 4- position of the DACH ring, which may indicate a crucial structural difference influencing the nucleolar stress mechanism.

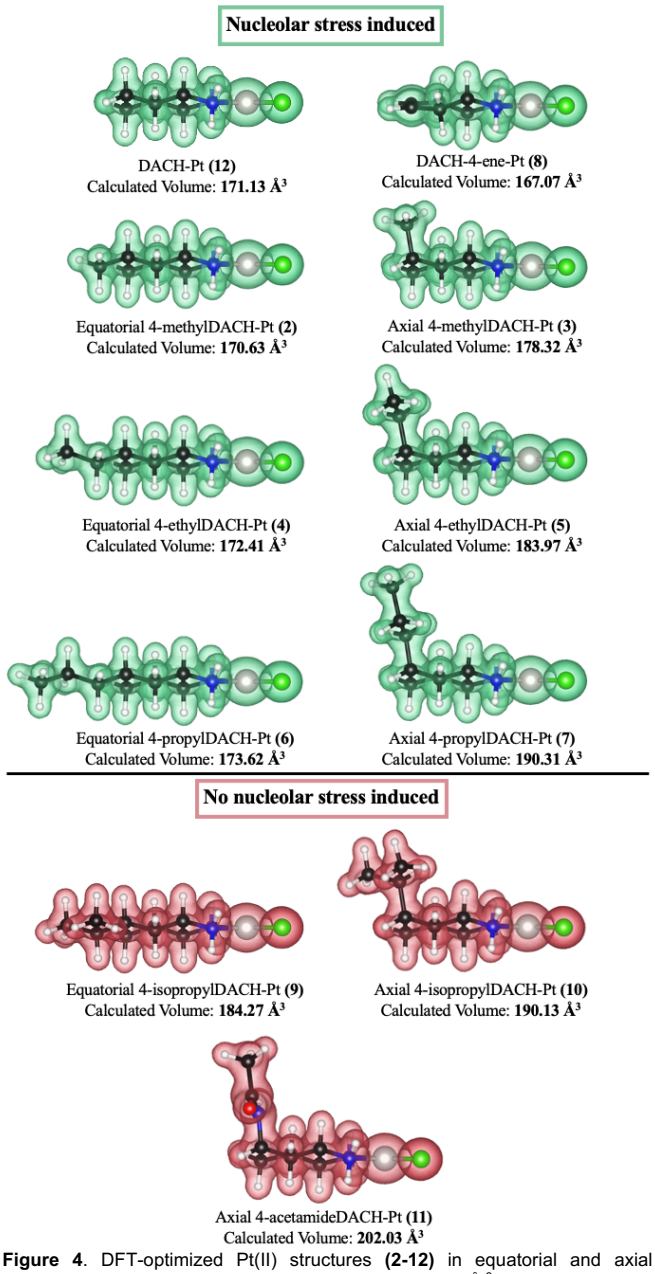


Figure 4. DFT-optimized Pt(II) structures **(2-12)** in equatorial and axial orientations are displayed at an isosurface level of 0.25 e/Å^{-3} for each compound, as implemented in VESTA. This illustrates the calculated volume.

DMSO coordination can inactivate the ability of Pt(II)-DACH compounds to induce nucleolar stress

Platinum can coordinate to a variety of ligands including nitrogen, oxygen, and sulfur, with sulfur being a preferred ligand. DMSO is known to coordinate to platinum compounds very quickly and is also known to limit side effects but also to deactivate effectiveness of platinum drugs in clinical trials.[15,16] We have previously shown that replacing the oxalate leaving group of oxaliplatin with dichloro ligands supports nucleolar stress induction by DACH-Pt(II) compounds, but that replacement with a chelating ethylenediamine (DACH-Pt(en)$^{2+}$) resulted in no nucleolar stress activity, indicating that cellular ligand exchange is important to the ability of Pt(II) compounds to cause nucleolar stress.[6] To further this result, we investigated the effects of DMSO coordination on NPM1 relocalization. We performed the NPM1 relocalization assay on 4-methylDACH-Pt **(2, 3)**, 4-ethylDACH-Pt **(3, 4)**, and 4-propylDACH-Pt **(6, 7)** as well as DACH-Pt **(12)** with stock solutions made in DMF or DMSO and stored for one day at room temperature. Compounds **2-6** dissolved for 24 hours in DMSO instead of DMF no longer caused nucleolar stress **(Figure 5)**. This indicates that for these compounds, DMSO coordination inhibits the relocalization of NPM1. These results reinforce the concept that care needs to be taken to ensure that results are not caused by inactivation of Pt(II) compounds through the coordination with DMSO.[15]

Of note, DACH-4-ene-Pt **(8)** was dissolved in DMSO for less than 20 minutes before cells were treated for the trials shown in **Figure 3**. Previous literature has found that coordination to DMSO requires >20 minutes in solution for some platinum compounds.[17,18] Varbanov and coworkers showed that the displacement of one chloride ligand of cisplatin with DMSO occurs in 50% of the sample at around 20 minutes after dissolving in PBS with 10% DMSO. The sample reached complete coordination with one DMSO and one water molecule around 80 minutes after dissolving as monitored by ESI-MS.[17] In our experiments, care was taken to minimize the time compounds were exposed to DMSO, and no compounds were stored for more than 20 minutes in DMSO before cell treatments where the effects of DMSO in the experiment were not being studied.

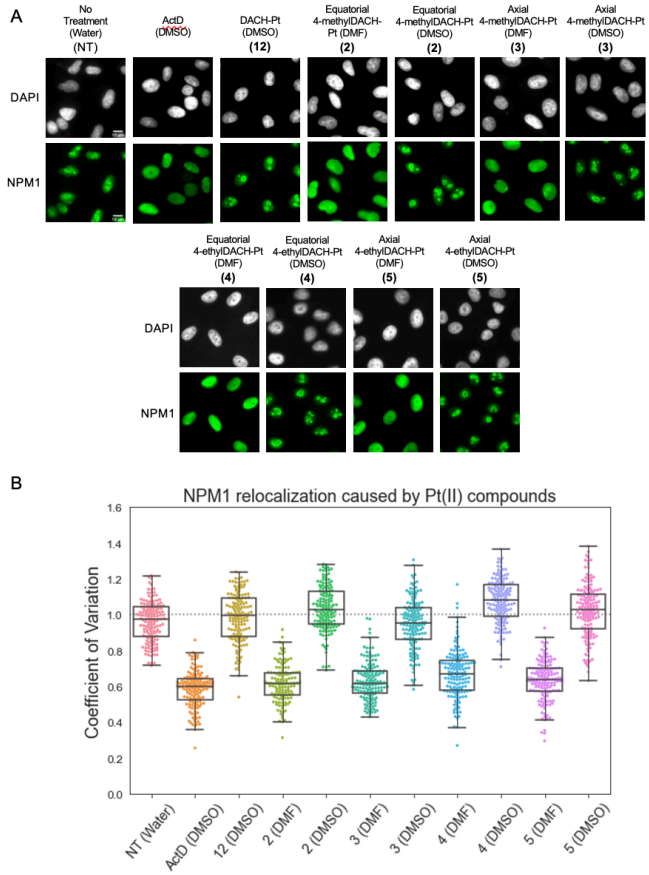


Figure 5. (A) Representative cell images of nucleolar stress induction at 24 hours illustrating the effects of solvents DMF or DMSO. Scale bar=10 um. Stock solutions made with DMF or DMSO as noted. Treatments are 10 uM for Pt(II) derivatives and 5 nM for Actinomycin D. (B) Quantification of NPM1 relocalization by Pt(II) compounds. For each treatment data set, boxes represent median, first and third quartiles, and vertical lines represent the range of data with outliers.

The inhibition of some platinum compounds when coordinated to DMSO could be an indicator of the strength of the coordination at the target biomolecule that is occurring to cause nucleolar stress. The Pt(II)-biomolecular coordination that causes nucleolar stress could involve oxygen or nitrogen ligands, both of

which are weaker coordination partners for platinum than is DMSO.

Discussion

The mechanism by which oxaliplatin and related Pt(II) compounds induce a distinctive nucleolar stress response is of interest for the field of nucleolar biology as well as platinum therapeutics. In this work we sought to explore the structural threshold for modifications to the oxaliplatin DACH ligand that would still support a nucleolar stress response. We used an NPM1 redistribution assay[6,14] to directly observe nucleolar stress in A549 cells following 24-hr treatment with each compound. We find that the addition of a methyl, ethyl, or propyl substituent at the 4-position of the DACH ligand in either axial or equatorial orientations (compounds **2-7**) still supports nucleolar stress induction. Additionally, we probed the elimination of protons through incorporation of a 4-5 double bond (**8**), and this compound also still supports a nucleolar stress response. These results indicate that the process by which Pt(II) compounds cause nucleolar stress tolerates substitutions at least through the size of a propyl group at the 4-position of the DACH ring.

To further compare the influences of steric bulk, rigidity, and hydrophobicity on 4-position substituents, we also tested an axial acetamide and an isopropyl substitution in both the axial and equatorial position (compounds **9**, **10**, and **11)**. While the addition of a 4-methyl, ethyl or propyl substituent **(2- 7)** was tolerated with respect to nucleolar stress induction by DACH-Pt(II) compounds, compounds with a 4-acetamide **(11)** or isopropyl **(9, 10)** substituent no longer caused nucleolar stress. The acetamide and isopropyl substituents both increase steric bulk, but differ substantially in hydrophobicity, indicating that hydrophobicity is not the major factor determining the ability of Pt compounds to cause nucleolar stress.[6] Further, while both acetamide and isopropyl substituents increase steric bulk, the overall volumes of the 4-isopropyl derivatives do not exceed those of stress-inducing 4-propyl derivatives (**Figure 4**). However, in both the 4-isopropyl and 4-acetamide DACH compounds, the extra steric bulk is constrained to certain orientations in comparison with a 4-propyl substituent, which has more degrees of freedom.

The structure-function relationships observed here suggest that the binding mode or process induced by oxaliplatin-like Pt(II) compounds that causes nucleolar stress can be evaded if the DACH ring substituents are sufficiently rigid or held in a certain orientation with respect to the Pt(II)-amine ligand plane, as is the case with 4-isopropryl and acetamide substituents from this study, as well as cis-(2-picolylamine)dichloride Pt(II) previously shown to not cause nucleolar stress.[6] Overall, these studies highlight the unique structural requirements that oxaliplatin satisfies in order to cause a different mechanism of action than cisplatin.

The chair conformation of the DACH ligand of oxaliplatin provides interesting positioning of functional groups that remain rigid after coordination to the platinum atom. The observation that the ability to cause nucleolar stress appears to be insensitive to changes in both the axial and equatorial positions points towards similar flexibility at both locations. However, the finding that the axial 4-acetamideDACH-Pt **(11)** and both axial and equatorial 4-isopropylDACH-Pt derivatives **(9, 10)** do not cause nucleolar stress indicates that there is a limited tolerance for bulk around the DACH ring 4-position in Pt(II) compounds that cause nucleolar stress. The mechanism by which these compounds might cause nucleolar stress is not yet known, but it is interesting to consider models that include a specific biomolecular interaction that is affected by certain platinum compounds. One possible mechanism could be similar to a fairly stringent lock and key type interaction in which the DACH ligand of oxaliplatin is uniquely situated as the "best fit." In this scenario, Pt compounds with smaller ligands, such as ethylene diamine which shows no nucleolar stress as well as 1,2-diaminocyclopentane found in pentaplatin which shows diminished NPM1 relocalization,[6] and larger ligands, such as axial 4-acetamideDACH-Pt **(11),** equatorial 4-isopropylDACH-Pt **(9)**, and axial 4-isopropylDACH-Pt **(10)** discussed here, are no longer able to function similarly and their binding results in no primary nucleolar stress response.

Although the current state of structure-function relationships seem to indicate a specific interaction between Pt(II) compounds and a nucleolar target leading to nucleolar stress, less specific interactions may also be important to the process of nucleolar stress caused by platinum compounds. For example, it is possible that initial inhibition of rRNA transcription, demonstrated with oxaliplatin but not cisplatin,[7] could lead to changes in the nucleolar structure allowing additional non-specific interactions between the DACH ring of oxaliplatin and previously inaccessible compartments of the nucleolus to influence more widespread changes to the nucleolus. One observation that could indicate a more widespread effect on the nucleolus after a potential initial, specific, disruption is the change in nucleolar morphology from eccentrically shaped nucleoli to round structures when NPM1 is relocalized. The change in morphology has been noted in previous studies, yet the interaction with platinum that causes the change is currently unknown.[5–7]

Conclusion

Here we test limits on the structural requirements for oxaliplatin derivatives to cause nucleolar stress. We find that increasing sizes of DACH ring 4-position substituents through a propyl group support nucleolar stress, but Pt(II)-DACH compounds with 4-isopropyl and acetamide substituents do not cause nucleolar stress. It is also found that DMSO coordination inhibits the abilities of these compounds to cause nucleolar stress. More research is needed to determine the exact mechanism and biomolecule target by which ring-containing Pt(II) compounds induce nucleolar stress, including both initial effects of platinum binding as well as the results of such disruption. This understanding can help to better hone a new generation of platinum compounds that exploit the nucleolar stress pathway.

Experimental Section

Cell Culture and Treatment

A549 human lung carcinoma cells (#CCL-185, American Type Culture Collection) were cultured in 5% CO_2 at 37 °C in Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS) and 1% antibiotic-antimycotic. Treatments were performed on cells that had been grown for 11-26 passages to 70% confluency. All treatments were performed for 24 hours at 10 uM concentrations of the platinum compounds. Compounds were made in 5mM stocks. For treatments with DMSO (excluding DACH-4-ene-Pt), the solutions were stored for 24 hours at room temperature to ensure coordination of platinum complexes with DMSO. DACH-4-ene-Pt DMSO solutions were made directly before treatment with cells (<10min in solution). The compound stock solutions were made with DMF (**2, 3, 4, 5, 6, 7, 9, 10, 11**), water (**1**), or DMSO (ActD, **8**, DMSO trials with **2, 3, 4, 5, 12**). Stock solutions were diluted into media immediately prior to drug treatment. Treatments were performed in triplicate and additional replicates are available from the corresponding author upon reasonable request.

Immunofluorescence

Cells were grown on coverslips (Ted Pella product no. 260368, round glass coverslips, 10mm diam. 0.16-0.19mm thick) as described above. After treatment was complete, cells were washed with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde (PFA) in PBS for 20 minutes at room temperature. PFA was removed using aspiration and cells were permeabilized with 0.5% Triton-X in PBS for 20 minutes at room temperature. Two ten-minute blocking steps were then performed with 1% bovine serum albumin (BSA) in PBST (PBS with 0.1% Tween-20). Cells were incubated for one hour in primary antibody (NPM1 monoclonal antibody, FC-61991, from ThermoFisher, 1:200 dilution in PBST with 1%BSA) and 1 hour in secondary antibody (Goat Anti-Mouse IGG H&L Alexa Fluor® 488, ab150113, Abcam, 1:1000 dilution in PBST with 1% BSA), with three five-minute wash steps using PBST between antibody incubations. They were washed again in the same manner before mounting the slides. Coverslips were then mounted on slides with ProLong™ Diamond Antifade Mountant with DAPI (Thermo Fisher) according to manufacturer's instructions.

Image Processing and Quantification

Images were taken using a HC PL Fluotar 63x/1.3 oil objective mounted on a Leica DMi8 fluorescence microscope with Leica Application Suite X software. The quantification of NPM1 relocalization was performed in an automated fashion using a Python 3 script. Images were preprocessed in ImageJ,[27,28] to convert the DAPI and NPM1 channels into separate 16-bit grayscale images. Between 50-250 cells were analyzed for each treatment group. Nuclei were segmented using the DAPI images using Li thresholding function in the Scikit-Image Python package.[29] The coefficient of variation (CV) for individual nuclei, which is defined as the standard deviation in pixel intensity divided by the mean pixel intensity, was calculated from the NPM1 images using the SciPy Python package. All the data was normalized to the no-treatment in each experiment. NPM1 imaging results for each compound were observed in triplicate. Data are represented as boxplots generated using Seaborn within Python.

Measurement of partition coefficients

Water was mixed with octanol for 24 hours and left to stand for a minimum of 24 hours to obtain water-saturated octanol and octanol-saturated water that were used for determining partition coefficients. A saturated solution of platinum compound in water was created and the solution was filtered through a 0.1 um disposable filter (Whatman). Measurements of the partition coefficients were performed using the classical shake-flask method according to OECD guidelines. The water stock solution of platinum was mixed with octanol in a 1:1 ratio and vortexed for 30 minutes. The mixtures were then centrifuged for 30 minutes at 10,000 rpm. Samples of the water layer were then used to determine the concentration of platinum using ICP-MS. ICP-MS solutions were made by diluting 1:1000 the water solutions with 2% nitric acid. The saturated stock solution of water was used to determine the total concentration of platinum. The concentration of platinum that partitioned into octanol was assumed to be the difference between the saturated stock solution and the concentration of platinum in the water layer after 30 minutes of vortexing. The partition coefficient was found by dividing the calculated concentration of platinum in octanol by the concentration in water. The measurements were performed in triplicate and a standard deviation was determined.

Computations

Computations were performed as previously reported.[6,13] Briefly, compounds were optimized using density functional theory (DFT) in Gaussian09.[19] Optimizations to geometry were performed using a RMS force convergence criterion of 10^{-5} hartree. The electronic wavefunction was minimized using GGA functional PBE[20,21] with the LANL2DZ basis set. We did not explicitly include relativistic effects as these were not expected to impact the geometries of the compounds significantly.[22] In order to quantitatively assess the size of the molecules we used the presence of an electric field, as derived from the electrostatic potential, to signify the location of the chemical system. DFT yields the electrostatic potential of the optimized, non-hydrolyzed compound structures and tools previously developed and reported were used to analyze the electrostatic potential of a chemical system.[23]

Synthesis

Materials

Oxaliplatin was purchased from TCI. Unless otherwise noted, starting materials were purchased from Millipore Sigma Aldrich and TCI. DACH-Pt **(12)** was synthesized according to previously published methods.[6]

Cis-[4-methyl-trans-(+/-)-1,2-cyclohexanediamine]dichloride platinum (II) (Equatorial 4-methylDACH-Pt) **(2)**

Equatorial 4-methyl-1,2-cyclohexadiamine was synthesized according to literature procedure[9] and without the use of L-tartaric acid[11] (see Supporting Information (SI)).

Crude 4-methyl-1,2-cyclohexadiamine (108 mg, 0.84 mmol) was dissolved in 1 mL water with DBU (35 mg, 0.23 mmol). Potassium tetrachloroplatinate(II) (348 mg, 0.84 mmol) was added to the reaction and allowed to react for 6 hours. The yellow solid was pelleted, washed with water (3x) and ether (1x), and dried to produce 6.8 mg of product (2% yield). ^{1}H NMR (500 MHz, DMF-d_7) δ 5.59 (s, 2H), 5.02 (d, J = 10.9 Hz, 2H), 2.65 – 2.57 (m, 1H), 2.52 (dt, J = 11.2, 4.3 Hz, 1H), 2.05 (ddd, J = 11.2, 8.7, 4.4 Hz, 2H), 1.58 – 1.48 (m, 2H), 1.46 – 1.36 (m, 1H), 1.21 (q, J = 12.0 Hz, 1H), 0.98 – 0.83 (m, 4H). ^{13}C NMR (151 MHz, DMF- d_7) δ 64.62, 64.47, 41.43, 34.26, 32.75, 32.21, 21.90. ^{195}Pt NMR (129 MHz, DMF- d_7) δ -2263.87.

Cis-[4-methyl-trans-(+/-)-1,2-cyclohexanediamine]dichloride platinum (II) (Axial 4-methylDACH-Pt) (**3**)

Axial 4-methyl-1,2-cyclohexadiamine was synthesized according to literature procedure,[9] with the use of 4-methyl-1-cyclohexene as starting material and without L-tartaric acid[11] (see SI).

Crude 4-methyl-cyclohexane-1,2-diamine (67 mg, 0.52 mmol) was dissolved in 1.0 mL water with DBU (35 mg, 0.23 mmol). Potassium tetrachloroplatinate (109 mg, 0.26 mmol) is added to the reaction and allowed to react for 6 hours. The yellow solid was pelleted, washed with water (3x) and ether (1x), and dried to produce 3.5 mg of product (2% yield). ^{1}H NMR (500 MHz, DMF-d_7) δ 5.52 (d, J = 64.8 Hz, 2H), 4.96 (s, 2H), 2.43 (dq, J = 8.8, 6.0, 4.5 Hz, 1H), 1.91 (dq, J = 9.4, 4.6 Hz, 2H), 1.82 – 1.49 (m, 4H), 1.48 – 1.31 (m, 2H), 0.94 (dd, J = 11.5, 6.8 Hz, 3H). ^{13}C NMR (151 MHz, DMF-d_7) δ 64.81, 59.56, 38.38, 32.38, 28.49, 27.55, 17.99. ^{195}Pt NMR (129 MHz, DMF-d_7) δ -2275.44.

Cis-[4-ethyl-trans-(+/-)-1,2-cyclohexanediamine]dichloride platinum (II) (Equatorial 4-ethylDACH-Pt) (**4**)

Equatorial 4-ethyl-1,2-cyclohexadiamine was synthesized with a modified literature procedure utilizing 4-ethylcylohexanol as the starting material[8,9,11,24] (see SI).

Crude 4-ethyl-cyclohexane-1,2-diamine (103 mg, 0.72 mmol) is dissolved in 1.5 mL water with DBU (35 mg, 0.23 mmol). Potassium tetrachloroplatinate(II) (113 mg, 0.27 mmol) is added to the reaction and allowed to react for 6 hours. The yellow solid was pelleted, washed with water (3x) and ether (1x), and dried to produce 9.6 mg of product (3% yield). ^{1}H NMR (500 MHz, DMF-d_7) δ 5.58 (s, 2H), 5.01 (s, 2H), 2.66 – 2.51 (m, 1H), 2.13 – 2.04 (m, 1H), 1.79 – 1.68 (m, 1H), 1.66 – 1.58 (m, 1H), 1.50 (dtt, J = 22.2, 8.4, 4.1 Hz, 2H), 1.36 – 1.22 (m, 3H), 1.19 (dt, J = 10.5, 6.0 Hz, 1H), 0.87 (dd, J = 9.4, 5.2 Hz, 4H). ^{13}C NMR (126 MHz, DMF-d_7) δ 64.90, 64.59, 39.41, 39.12, 32.17, 31.84, 29.62, 12.47. ^{195}Pt NMR (129 MHz, DMF-d_7) δ -2259.16.

Cis-[4-ethyl-trans-(+/-)-1,2-cyclohexanediamine]dichloride platinum (II) (Axial 4-ethylDACH-Pt) **(5)**

Axial 4-ethyl-1,2-cyclohexadiamine was synthesized with a modified literature procedure utilizing 4-ethylcylohexanol as the starting material[9,11,24,24] (see SI).

Crude 4-ethyl-cyclohexane-1,2-diamine (107 mg, 0.75 mmol) is dissolved in 1.5 mL water with DBU (35 mg, 0.23 mmol). Potassium tetrachloroplatinate(II) (108 mg, 0.26 mmol) is added to the reaction and allowed to react for 6 hours. The yellow solid was pelleted, washed water (3x) and ether (1x), and dried to produce 5.4 mg of product (2% yield). ^{1}H NMR (500 MHz, DMF-d_7) δ 5.53 (d, J = 43.9 Hz, 2H), 4.95 (s, 2H), 2.68 – 2.41 (m, 2H), 2.07 (d, J = 12.4 Hz, 1H), 2.00 – 1.80 (m, 1H), 1.79 – 1.59 (m, 2H), 1.52 (dd, J = 28.5, 17.8 Hz, 2H), 1.42 – 1.27 (m, 2H), 1.26 – 1.14 (m, 1H), 0.97 – 0.74 (m, 3H). ^{13}C NMR (151 MHz, DMF-d_7) δ 64.91, 59.92, 29.41, 29.12, 28.07, 24.61, 12.81, 12.26. ^{195}Pt NMR (129 MHz, DMF-d_7) δ -2271.33.

Cis-[4-propyl-1,2-dyclohexanediamine]dichloride platinum (II) (Equatorial 4-propylDACH-Pt) **(6)**

Equatorial 4-propyl-1,2-cyclohexadiamine was synthesized with a modified literature procedure utilizing 4-propylcylohexanone as the starting material[8,9] (see SI).

Crude 4-propyl-cyclohexane-1,2-diamine (83 mg, 0.53 mmol) is dissolved in 1.5 mL water with DBU (35 mg, 0.23 mmol). Potassium tetrachloroplatinate (123 mg, 0.30 mmol) is added to the reaction and allowed to react for 6 hours. The yellow solid was pelleted, washed water (3x) and ether (1x), and dried to produce 32.9 mg of product (15% yield). ^{1}H NMR (500 MHz, DMF-d_7) δ 5.60 (d, J = 13.1 Hz, 2H), 5.05 (s, 2H), 2.72 – 2.46 (m, 2H), 2.09 (ddq, J = 16.2, 12.2, 3.3 Hz, 1H), 1.71 (s, 1H), 1.64 – 1.58 (m, 1H), 1.53 (qd, J = 12.9, 3.8 Hz, 1H), 1.28 (tt, J = 23.0, 13.9 Hz, 4H), 1.23 – 1.14 (m, 2H), 0.87 (q, J = 7.5 Hz, 4H). ^{13}C NMR (151 MHz, DMF-d_7) δ 64.45, 64.14, 39.04, 38.87, 36.98, 31.82, 31.79, 20.91, 14.65. ^{195}Pt NMR (129 MHz, DMF-d_7) δ -2260.24. HRMS (ESI+): m/z calcd for $C_9H_{21}Cl_2N_2Pt$: 422.0730 [M+H]+; found: 422.0727.

Cis-[4-isopropyl-1,2-cyclohexanediamine]dichloride platinum (II) (Axial 4-propylDACH-Pt) **(7)**

Axial 4-propyl-1,2-cyclohexadiamine was synthesized with a modified literature procedure utilizing 4-propylcylohexanone as the starting material[8,9] (see SI).

Crude 4-propyl-cyclohexane-1,2-diamine (173 mg, 1.11 mmol) is dissolved in 1.5 mL water with DBU (35 mg, 0.23 mmol). Potassium tetrachloroplatinate(II) (169 mg, 0.41 mmol) is added to the reaction and allowed to react for 6 hours. The yellow solid was pelleted, washed water (3x) and ether (1x), and dried to produce 22.9 mg of product (5% yield). ^{1}H NMR (500 MHz, DMF-d_7) δ 5.54 (d, J = 57.0 Hz, 2H), 4.97 (dd, J = 27.9, 15.3 Hz, 2H), 2.69 – 2.42 (m, 2H), 2.15 – 2.00 (m, 1H), 1.90 (dd, J = 12.8, 3.8 Hz, 1H), 1.69 (ddd, J = 26.7, 13.9, 4.6 Hz, 1H), 1.60 (td, J = 12.4, 4.7 Hz, 1H), 1.47 (d, J = 14.4 Hz, 1H), 1.42 – 1.34 (m, 1H), 1.33 – 1.26 (m, 3H), 1.26 – 1.16 (m, 1H), 0.88 (dt, J = 11.8, 7.0 Hz, 4H). ^{13}C NMR (151 MHz, DMF-d_7) δ 65.09, 60.15, 36.57, 34.31, 33.98, 29.64, 28.30, 21.78, 14.98. ^{195}Pt NMR (129 MHz, DMF-d_7) δ -2271.08. HRMS (ESI+): m/z calcd for $C_9H_{21}Cl_2N_2Pt$: 422.0730 [M+H]+; found: 422.0720.

Cis-[trans-4-cyclohexane-1,2-diamine]dichloride platinum (II) (DACH-4-ene-Pt) **(8)**

A similar synthesis procedure of DACH-4-ene-Pt was shown in literature.[25]

Trans-4-cyclohexene-1,2-diamine dichloride (49 mg, 0.26 mmol) is dissolved in 1ml water with DBU (83 mg, 0.54 mmol). Potassium tetrachloroplatinate(II) (112 mg, 0.26 mmol) is added to the reaction and allowed to react for 4 hours. The yellow solid is pelleted, collected, and air dried to produce 20 mg of product (20% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.74 (d, J = 10 Hz, 2H), 5.40 – 5.31 (m, 2H), 5.16 (s, 2H), 2.41 – 2.35 (m, 2H), 2.31 (d, J = 16.6 Hz, 2H), 2.13 (dd, J = 16.2, 8.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 125.20, 59.76, 32.34. ^{195}Pt NMR (129 MHz, DMSO-d_6) δ -3271.83. HRMS (ESI+): m/z calcd for $C_6H_{13}Cl_2N_2Pt$: 378.0104 [M+H]+; found: 378.0101.

Cis-[4-isopropyl-1,2-cyclohexanediamine]dichloride platinum (II) (Equatorial 4-isopropylDACH-Pt) **(9)**

Equatorial 4-isopropyl-1,2-cyclohexadiamine was synthesized with a modified literature procedure using 4-isopropylcyclohexanol as the starting material[9] (see SI).

Crude 4-isopropyl-cyclohexane-1,2-diamine (114 mg, 0.73 mmol) is dissolved in 1.5 mL water with DBU (35 mg, 0.23 mmol). Potassium tetrachloroplatinate(II) (109 mg, 0.26 mmol) is added to the reaction and allowed to react for 6 hours. The yellow solid was pelleted, washed water (3x) and ether (1x), and dried to produce 28.5 mg of product (9% yield). ^{1}H NMR (500 MHz, DMF-d_7) δ 5.57 (s, 2H), 5.00 (s, 2H), 2.61 – 2.46 (m, 2H), 2.14 – 2.04 (m, 2H), 1.75 (s, 1H), 1.58 (d, J = 13.6 Hz, 1H), 1.50 (dd, J = 11.1, 4.3 Hz, 1H), 1.27 (q, J = 11.8 Hz, 1H), 1.16 – 1.08 (m, 1H), 1.00 – 0.93 (m, 1H), 0.86 (dd, J = 6.8, 2.1 Hz, 6H). ^{13}C NMR (126 MHz, DMF-d_7) δ 64.47, 64.42, 43.49, 32.47, 31.71, 28.36, 20.30, 20.22. ^{195}Pt NMR (129 MHz, DMF-d_7) δ -2257.17. HRMS (ESI+): m/z calcd for $C_9H_{21}Cl_2N_2Pt$: 422.0730 [M+H]+; found: 422.0715.

Cis-[4-isopropyl-1,2-cyclohexanediamine]dichloride platinum (II) (Axial 4-isopropylDACH-Pt) **(10)**

Axial 4-isopropyl-1,2-cyclohexadiamine was synthesized with a modified literature procedure using 4-isopropylcyclohexanol as the starting material[9] (see SI).

Crude 4-isopropyl-cyclohexane-1,2-diamine (127 mg, 0.81 mmol) is dissolved in 1.5 mL water with DBU (35 mg, 0.23 mmol). Potassium tetrachloroplatinate(II) (133 mg, 0.32 mmol) is added to the reaction and allowed to react for 6 hours. The yellow solid was pelleted, washed water (3x), ether (1x), DMF (5x), and dried to produce 5.2 mg of product (2% yield). ^{1}H NMR (500 MHz, DMF-d_7) δ 5.57 (s, 2H), 4.96 – 4.86 (m, 2H), 2.62 – 2.55 (m, 1H), 2.47

– 2.40 (m, 1H), 2.27 (d, J = 13.1 Hz, 1H), 1.91 (dd, J = 13.0, 3.8 Hz, 1H), 1.72 (d, J = 16.4 Hz, 1H), 1.65 (d, J = 12.4 Hz, 1H), 1.51 (td, J = 12.6, 4.3 Hz, 1H), 1.32 – 1.23 (m, 2H), 1.16 (s, 1H), 0.99 – 0.81 (m, 6H). ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.56 – 5.44 (m, 2H), 4.94 (s, 2H), 2.23 (d, J = 11.9 Hz, 1H), 2.07 (d, J = 11.3 Hz, 1H), 1.99 (d, J = 13.1 Hz, 1H), 1.65 (d, J = 12.5 Hz, 1H), 1.56 (d, J = 13.7 Hz, 1H), 1.48 (d, J = 12.5 Hz, 1H), 1.39 (d, J = 12.5 Hz, 1H), 1.25 (d, J = 12.6 Hz, 1H), 1.09 (d, J = 13.3 Hz, 1H), 1.05 (s, 1H), 0.84 (dd, J = 25.0, 6.5 Hz, 6H). ^{13}C NMR (126 MHz, DMF-d_7) δ 64.77, 59.83, 41.54, 27.90, 27.41, 26.63, 21.86, 21.29. ^{195}Pt NMR (129 MHz, DMF-d_7) δ -2270.85. HRMS (ESI+): m/z calcd for $C_9H_{21}Cl_2N_2Pt$: 422.0730 [M+H]+; found: 422.0742.

Cis-[4-acetamido-1,2-cyclohexanediamine]dichloride platinum (II) (Axial 4-acetamideDACH-Pt) **(11)**

1,2-(ditert-butyl carbamate) 4-acetamide cyclohexane was synthesized according to literature procedure.[26]

1,2-(ditert-butyl carbamate) 4-acetamide cyclohexane (130 mg, 0.34 mmol) is reacted for 1 hour in 4M HCl in dioxane. The solution is dried until a solid is present and is no longer acidic. The resulting salt is dissolved in 1ml of water and DBU (69 mg, 0.45 mmol). Potassium tetrachloroplatinate(II) (180 mg, 0.43 mmol) is then added and allowed to react overnight. A brown solid precipitates and is pelleted and air dried to produce 105 mg of product (71% yield). ^{1}H NMR (500 MHz, DMF-d_7) δ 7.67 (t, J = 6.4 Hz, 1H), 5.77 (s, 1H), 5.60 (s, 1H), 5.12 (s, 2H), 3.92 (d, J = 32.6 Hz, 1H), 3.24 (s, 1H), 2.24 – 2.15 (m, 1H), 1.94 (q, J = 6.9, 3.9 Hz, 1H), 1.89 (d, J = 6.7 Hz, 3H), 1.84 (dd, J = 7.3, 3.8 Hz, 1H), 1.74 (qd, J = 8.1, 2.8 Hz, 1H), 1.68 – 1.51 (m, 2H), 1.50 – 1.39 (m, 1H). ^{13}C NMR (151 MHz, DMF-d_7) δ 170.15, 64.53, 60.59, 45.77, 37.19, 27.54, 24.97, 23.61. ^{195}Pt NMR (129 MHz, DMF-d_7) δ -2283.40.

Acknowledgements

We would like to acknowledge the contributions of University of Oregon undergraduate researchers Haley Rice and Nathan Nguyen. This work was supported by the National Science Foundation [CHE-2109255 to VJD]. Haley Rice was supported by the UO Knight Campus Undergraduate Scholars program. Computations were performed with access to the University of Oregon HPC facility, Talapas. 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.

Keywords: oxaliplatin • nucleolar stress • ribosome biogenesis stress • platinum • structure-activity relationship

[1] T. C. Johnstone, K. Suntharalingam, S. J. Lippard, "The Next Generation of Platinum Drugs: Targeted Pt (II) Agents, Nanoparticle Delivery, and Pt (IV) Prodrugs" *Chem. Rev.* **2016**, *116*, 3436–3486.

[2] R. A. Alderden, M. D. Hall, T. W. Hambley, "The Discovery and Development of Cisplatin" *J. Chem. Educ.* **2006**, *83*, 728.

[3] "Discovery – Cisplatin and The Treatment of Testicular and Other Cancers - National Cancer Institute," https://www.cancer.gov/research/progress/discovery/cisplatin, **2014**.

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

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

[6] E. C. Sutton, C. E. McDevitt, J. Y. Prochnau, M. V. Yglesias, A. M. Mroz, M. C. Yang, R. M. Cunningham, C. H. Hendon, V. J. DeRose, "Nucleolar Stress Induction by Oxaliplatin and Derivatives" *J. Am. Chem. Soc.* **2019**, *141*, 18411–18415.

[7] E. C. Sutton, V. J. DeRose, "Early Nucleolar Responses Differentiate Mechanisms of Cell Death Induced by Oxaliplatin and Cisplatin" *J. Biol. Chem.* **2021**, *296*, 100633.

[8] M. Galanski, A. Yasemi, M. A. Jakupec, N. Graf v. Keyserlingk, B. K. Keppler, "Synthesis and Structure-Activity Relationships of mono- and dialkyl- substituted Oxaliplatin Derivatives" *Monatshefte Für Chem. Chem. Mon.* **2005**, *136*, 693–700.

[9] L. Habala, C. Dworak, A. A. Nazarov, C. G. Hartinger, S. A. Abramkin, V. B. Arion, W. Lindner, M. Galanski, B. K. Keppler, "Methyl-substituted Trans-1,2-cyclohexanediamines as New Ligands for Oxaliplatin-type Complexes" *Tetrahedron* **2008**, *64*, 137–146.

[10] I. Buß, G. V. Kalayda, A. Lindauer, M. R. Reithofer, M. Galanski, B. K. Keppler, U. Jaehde, "Enhancing Lipophilicity as a Strategy to Overcome Resistance Against Platinum Complexes?" *J. Biol. Inorg. Chem.* **2012**, *17*, 699–708.

[11] S. A. Abramkin, U. Jungwirth, S. M. Valiahdi, C. Dworak, L. Habala, K. Meelich, W. Berger, M. A. Jakupec, C. G. Hartinger, A. A. Nazarov, M. Galanski, B. K. Keppler, "{[1R, 2R, 4R)-4-Methyl-1,2-cyclohexanediamine}oxalatoplatinum(II): A Novel Enatiomerically Pure Oxaliplatin Derivative Showing Impreoved Anticancer Activity in Vivo" *J. Med. Chem.* **2010**, *53*, 7356–7364.

[12] U. Jungwirth, D. N. Xanthos, J. Gojo, A. K. Bytzek, W. Körner, P. Heffeter, S. A. Abramkin, M. A. Jakupec, C. G. Hartinger, U. Windberger, M. Galanski, B. K. Keppler, W. Berger, "Anticancer Activity of Methyl-substituted Oxaliplatin Analog" *Mol. Pharmacol.* **2012**, *81*, 719–728.

[13] C. E. McDevitt, M. V. Yglesias, A. M. Mroz, E. C. Sutton, M. C. Yang, C. H. Hendon, V. J. DeRose, "Monofunctional Platinum(II) compounds and nucleolar stress: Is phenanthriplatin unique?" *J. Biol. Inorg. Chem.* **2019**, *24*, 899–908.

[14] M. Galanski, A. Yasemi, S. Slaby, M. A. Jakupec, V. B. Arion, M. Rausch, A. A. Nazarov, B. K. Keppler, "Synthesis, Crystal Structure and Cytotoxicity of New Oxaliplatin Analogues Indicating that Improvements of Anticancer Activity is Still Possible" *Eur. J. Med. Chem.* **2004**, *39*, 707–714.

[15] M. D. Hall, K. A. Telma, K.-E. Chang, T. D. Lee, J. P. Madigan, J. R. Lloyd, I. S. Goldlust, J. D. Hoeschele, M. M. Gottesman, "Say No to DMSO: Dimethylsulfoxide Inactivates Cisplatin, Carboplatin and Other Platinum Complexes" *Cancer Res.* **2014**, *74*, 3913–3922.

[16] E. Wexselblatt, E. Yavin, D. Gibson, "Cellular Interactions of Platinum Drugs" *Inorganica Chim. Acta* **2012**, *393*, 75–83.

[17] H. P. Varbanov, D. Ortiz, D. Höfer, L. Menin, M. Galanski, B. K. Keppler, P. J. Dyson, "Oxaliplatin Reacts with DMSO Only in the Presence of Water" *Dalton Trans.* **2017**, *46*, 8929–8932.

[18] E. Fischer-Fodor, N. Moldovan, P. Virag, O. Soritau, I. Brie, P. Lönnecke, E. Hey-Hawkins, L. Silaghi-Dumitrescu, "The CellScan Technology for in vitro Studies on Novel Platinum Complexes with Organoarsenic Ligands" *Dalton Trans.* **2008**, *0*, 6393–6400.

[19] M. Frisch, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, G. Scalmani, V. Barone, G. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. Janesko, R. Gomperts, B. Mennucci, H. Hratchian, J. Ortiz, A. Izmaylov, J. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. Montgomery, Jr, J. Peralta, F. Ogliaro, M. Bearpark, J. Heyd, E. Brothers, K. Kudin, V. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. Burant, S. Iyengar, J. Tomasi, M. Cossi, J. Millam, M. Klene, C. Adamo, R. Cammi, J. Ochterski, R. Martin, K. Morokuma, O. Farkas, J. Foresman, D. Fox, *Gaussian09*, Gaussian Inc. Wallingford CT, **2016**.

[20] J. P. Perdew, K. Burke, M. Ernzerhof, "Generalized Gradient Approximation Made Simple" *Phys. Rev. Lett.* **1996**, *77*, 3865–3868.

[21] J. Perdew, K. Burke, M. Ernzerhof, "Errata: Generalized Gradient Approximation Made Simple" *Phys. Rev. Lett.* **1997**, *78*, 1396.

[22] F. Pansini, A. Neto, M. de Campos, de Aquino R, "Effects of All-electron Basis Sets and the Scalar Relativistic Corrections in the Structure and Electronic Properties of Niobium Clusters" *J. Phys. Chem. A* **2017**, *121*, 5728–5734.

[23] K. Butler, C. Hendon, A. Walsh, "Electronic Chemical Potentials of Porous Metal-Organic Frameworks" *J. Am. Chem. Soc.* **2014**, *136*, 2703–2706.

[24] L. Habala, M. Galanski, A. Yasemi, A. A. Nazarov, N. G. von Keyserlingk, B. K. Keppler, "Synthesis and Structure-Activity Relationships of Mono- and Dialkyll-substituted Oxaliplatin Derivatives" *Eur. J. Med. Chem.* **2005**, *40*, 1149–1155.

[25] P. Papadia, A. Barbanente, N. Ditaranto, J. D. Hoeschele, G. Natile, C. Marzano, V. Gandin, N. Margiotta, "Effect of Chirality in the Anticancer Activity of Pt(II) and Pt(IV) Complexes Containing the 1R,2R and 1S,2S Enantiomers of Trans-1,2-diamino-4-cyclohexene ligand (DACHEX), and Analogue of the Diaminocyclohexane Used in Oxaliplatin" *Dalton Trans.* **2021**, 15655-15668.

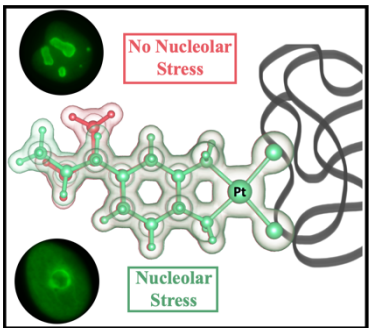
[26] G. V. Kalayda, M. Kullmann, M. Galanski, S. Gollos, "A Fluorescent Oxaliplatin Derivative for Investigation of Oxaliplatin Resistance Using Imaging Techniques" *J. Biol. Inorg. Chem.* **2017**, *22*, 1295–1304.

[27] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Tinevez, D.J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, A. Cardona, ‘‘Fiji: An Open-Source Platform for Biological-Image Analysis.“ *Nat. Methods* **2012**, *9*, 676–682.

[28] C.T. Rueden, J. Schindelin, M.C. Hiner, B.E. DeZonia, A.E. Walter, E.T. Arena, K.W. Eliceiri, ‚ImageJ2: ImageJ for the next Generation of Scientific Image Data.“ *BMC Bioinformatics* **2017**, *18*, 529.

[29] S. van der Walt, J.L. Schönberger, J. Nunez-Iglesias, F. Boulogne, J.D. Warner, N. Yager, E. Gouillart, T. Yu. ‘‘Scikit-Image: Image Processing in Python.“ *PeerJ* **2014**, *2*, e453.

Table of Contents



Substituents at the 4-position of the DACH ring of Pt(II)-based compounds influence nucleolar stress induction