Supporting Information for

Construction and Application of a Rh-Pt DNA Metalloinsertor Conjugate

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Experimental Methods

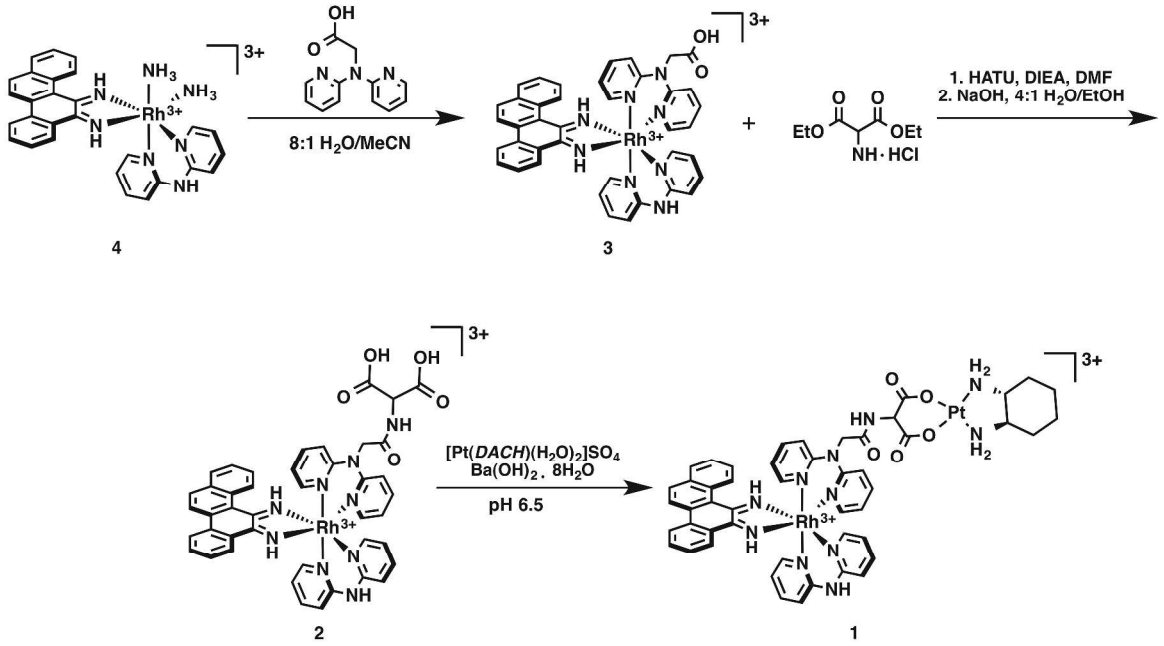
Materials

Cisplatin, oxaliplatin, and all organic reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. Commercially available chemicals were used as received without further purification. $RhCl_3$ and K_2PtCl_4 starting material were purchased from Pressure Chemical Co. Media and supplements were purchased from Invitrogen (Carlsbad, CA). BrdU, antibodies, and buffers were purchased in kit format from Roche Molecular Biochemical (Mannheim, Germany). Oligonucleotides were ordered from Integrated DNA Technologies and purified by HPLC using a C18 reverse-phase column (Varian, Inc; Corona, CA). All HPLC purifications were carried out on a Hewlett-Packard 1100 HPLC. DNA purity was confirmed by MALDI-TOF mass spectrometry and quantified by UV-vis using the extinction coefficients at 260 nm estimated for single-stranded DNA. UV-vis characterizations were performed on a Beckmann DU 7400 spectrophotometer. Radiolabeled [^{32}P]-ATP was purchased from MP Biomedicals (Santa Ana, CA).

The syntheses of chrysene-5,6-dione (chrysi), [Pt(*DACH*)$(H_2O)_2$]SO_4 (*DACH* = (1*R*,2*R*)-(-)-1,2-diaminocyclohexane), and di(pyridin-2-yl)glycine (dpa-AcOH*)* were carried out according to published procedures.[1-3] The synthesis of precursor [Rh(chrysi)(HDPA)$(NH_3)_2$]TFA_3 was carried out in a manner analogous to that of [Rh(chrysi)(phen)$(NH_3)_2$], as described by Mürner et al.[1]

RhPt Synthesis

The synthesis of conjugate RhPt is shown in Scheme 1, and was carried out as described below.



Scheme 1. Synthesis of RhPt **(1).**

[Rh(HDPA)(chrysi)(dpa-AcOH](TFA)$_3$ (3)

[Rh(HDPA)(chrysi)(NH3)$_2$](TFA)$_3$ (**4**) (620 mg, 0.69 mmol) and di(pyridin-2-yl)glycine (dpa-AcOH) (240 mg, 1.05 mmol) were dissolved in 8:1 H_2O:MeCN (90 ml) and refluxed for 24 h. The solvent was removed *in vacuo*, and the crude product was purified by HPLC using a C_{18} reverse-phase column (Varian, Inc.) on a Hewlett Packard 1100 HPLC (85:15 to 40:60 H_2O (0.1 % TFA):MeCN). Complex **3** was isolated as a dark red, hygroscopic solid. Yield: 0.55 g (73%). ^{1}H NMR (300 MHz, DMSO-d6) δ 11.47 (broad s, 1H), 9.64 (s, 1H), 9.04 (d, J = 6.2 Hz, 1H), 8.77 (dd, J = 15.0, 6.8 Hz, 1H), 8.52 (d, J = 6.1 Hz, 1H), 8.24 (dd, J = 5.0, 1.8 Hz, 4H), 8.08 (dt, J = 16.7, 9.5 Hz, 2H), 8.01 – 7.84 (m, 4H), 7.80 – 7.74 (m, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.67 (s, 1H), 7.56 – 7.40 (m, 3H), 7.38 – 7.29 (m, 1H), 7.19 (t, J = 8.4 Hz, 4H), 6.99 – 6.86 (m, 3H), 6.86 – 6.74 (m, 1H), 4.63 (s, 2H). ESI-MS (cation): *m/z* calc 757.17 (M – 2H$^+$), 379.59 (M – H^{2+}), obs. 756.9, 379.1.

[Rh(HDPA)(chrysi)(diethyl-2-(2-(di(pyridin-2-yl)amino)acetamido)malonate] $(TFA)_3$

[Rh(HDPA)(chrysi)(dpa-AcOH](TFA)$_3$ (**3**) (100 mg, 0.09 mmol), diethyl aminomalonate hydrochloride (38 mg, 0.18 mmol), and (dimethylamino)-*N*,*N*-dimethyl(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yloxy)methaniminium hexafluorophosphate (HATU, 83 mg, 0.22 mmol) were combined in a vial and dried under vacuum to remove all water. The solids were dissolved in anhydrous DMF (1.3 ml) under argon and stirred at room temperature for 10 min. Ethyldiisopropylamine (DIPEA, 95 µl, 0.54 mmol) was added, and the solution was allowed to stir at room temperature for 12 h. The solvent was removed *in vacuo*, and the intermediate was purified by HPLC as described above. Yield: 41 mg (36% by HPLC). ESI-MS (cation): *m/z* calc 914.24 ($M - 2H^+$), 457.12 ($M - H^{2+}$), obs. 913.9, 457.8

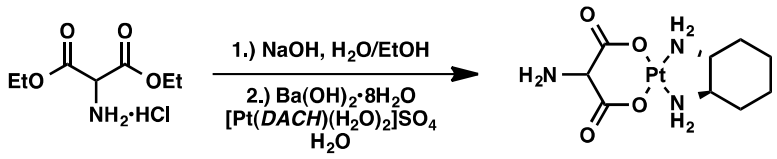
[Rh(HDPA)(chrysi)(2-(2-(di(pyridin-2-yl)amino)acetamido)-3-ethoxy-3-oxopropanoic acid)] $(TFA)_3$ ("Rh(Amal)") (2)

To hydrolyze the ethyl esters, [Rh(HDPA)(chrysi)(diethyl-2-(2-(di(pyridin-2-yl)amino)acetamido)malonate] $(TFA)_3$ (41 mg, 0.033 mmol) was dissolved in a 5:1 H_2O:EtOH mixture (12 ml). 1N NaOH was added to pH 10, and the reaction was stirred at room temperature for 24 h. The solvent was removed by rotary evaporation, and Rh(Amal) was purified by HPLC as described above. The chloride salt was obtained from a Sephadex QAE anion-exchange column equilibrated with 0.1 M $MgCl_2$. Yield: 12 mg (30% by HPLC). ^{1}H NMR (500 MHz, D_2O): δ 8.91 (dd, J = 6.2, 1.6 Hz, 1H), 8.23 – 8.15 (m, 1H), 8.14 – 8.11 (m, 2H), 8.08 (dd, J = 13.5, 7.6 Hz, 1H), 8.00 (ttd, J = 8.3, 6.9, 1.8 Hz, 3H), 7.94 – 7.90 (m, 1H), 7.90 – 7.85 (m, 1H), 7.83 (d, J = 7.6 Hz, 1H), 7.79 (d, J = 1.7 Hz, 1H), 7.78 – 7.76 (m, 1H), 7.59 (ddd, J = 7.9, 6.5, 1.3 Hz, 1H), 7.51 – 7.45 (m, 3H), 7.36 (td, J = 6.9, 6.3, 1.4 Hz, 2H), 7.34 – 7.30 (m, 1H), 7.29 (d, J = 1.2 Hz, 1H), 7.27 (ddd, J = 4.6, 2.4, 1.0 Hz, 1H), 7.26 (q, J = 2.2 Hz, 1H), 7.25 – 7.22 (m, 1H), 7.21 – 7.17 (m, 1H), 7.11 – 7.06 (m, 3H), 6.91 (ddd, J = 7.7, 6.5, 1.4 Hz, 1H), 4.87 (s, 2H), 3.67 (s, 1H). ESI-MS (cation): *m/z* calc 858.18 ($M - 2H^+$), 429.09 ($M - H^{2+}$), obs. 857.7, 429.5. UV-vis (H_2O, pH 7): 259 nm (53,500 M^{-1} cm^{-1}), 287 nm (39,300 M^{-1} cm^{-1}), 402 nm (6,400 M^{-1} cm^{-1}).

"RhPt"

To a solution of Rh(Amal) (12 mg, 0.01 mmol) in H_2O (10 ml) was added aqueous $Ba(OH)_2 \bullet 8H_2O$ (54 mg, 0.17 mmol in 5 ml H_2O) to pH 11. The yellow suspension was sonicated and added dropwise to a stirred solution of $[Pt(DACH)(H_2O)_2]SO_4$ (76 mg, 0.17 mmol) in H_2O (10 ml) at ambient temperature. The solution turned orange upon addition of the Ba/Rh mixture, and $BaSO_4$ crashed out as a white precipitate. The remaining $Ba(OH)_2 \bullet H_2O$ stock was added to the mixture until a pH 7 was reached, and the reaction was allowed to stir at room temperature for 24 h. The $BaSO_4$ byproduct was filtered, and the filtrate was concentrated *in vacuo* and purified by HPLC. The chloride salt was obtained from a Sephadex QAE anion-exchange column equilibrated with 0.1 M $MgCl_2$. Yield: 7.1 mg (57% by HPLC). ^{1}H NMR (300 MHz, D_2O): δ 10.06 (s, 1H, chrysi NH), 8.19 (d, J = 6.9 Hz, 1H, chrysi CH), 8.12 (d, J = 13.2 Hz, 1H, chrysi CH), 8.05 (d, J = 9.2 Hz, 1H, chrysi CH), 8.00 – 7.92 (m, 1H, chrysi CH), 7.89 (d, J = 10.0 Hz, 1H, chrysi CH), 7.83 (s, 1H, chrysi CH), 7.80 (s, 1H, chrysi CH), 7.74 (s, 1H, CONH), 7.64 (d, J = 8.1 Hz, 2H, py), 7.55 (d, 1H, chrysi CH), 7.54 – 7.47 (m, 2H, py), 7.47 – 7.33 (m, 1H, chrysi CH), 7.23 (d, J = 6.1 Hz, 1H, chrysi CH), 7.13 (dt, J = 13.3, 6.5 Hz, 2H, py), 6.97 (dd, J = 14.5, 7.1 Hz, 2H, py), 6.42 – 5.93 (m, 2H, Pt-NH_2), 5.57 – 5.03 (m, 2H, Pt-NH_2), 2.43 (d, J = 1.3 Hz, 2H, dach CH), 1.92 (d, J = 10.1 Hz, 1H, dach CH), 1.43 (s, 2H, dach CH), 1.16 (s, 1H, dach CH), 0.99 (t, J = 10.2 Hz, 2H, dach CH). ESI-MS (cation): *m/z* calc 1165.24 (M – $2H^+$), 583.12 (M – H^{2+}), obs. 1165.9, 582.9. UV-vis (H_2O, pH 7): 315 nm (27,000 M^{-1} cm^{-1}), 389 nm (5,420 M^{-1} cm^{-1}).

Synthesis of Pt(Amal) (Scheme 2)



Scheme 2. Synthesis of Pt(Amal).

[Pt(DACH)(aminomalonate)] ("Pt(Amal)")

Diethyl aminomalonate hydrochloride (110 mg, 0.52 mmol) was hydrolyzed in a solution of 4:1 H_2O:EtOH (10 ml) basified with 1N NaOH (pH 13). The reaction was stirred at room temperature overnight, neutralized with 1N HCl, and dried *in vacuo* to afford the diacid as a white solid. The resulting aminomalonic acid hydrochloride (78 mg, 0.366 mmol) was added to a suspension of $Ba(OH)_2 \bullet 8H_2O$ (58 mg, 0.183 mmol) in 10 ml H_2O. The mixture was added dropwise to a solution of $[Pt(DACH)(H_2O)_2]SO_4$ (81 mg, 0.183 mmol) in H_2O (20 ml) and stirred at room temperature, pH 7, for 3h. The $BaSO_4$ precipitate was removed by vacuum filtration, and the filtrate was left to stand at 4 °C. A yellow precipitate was filtered and dried under vacuum. The residue was dissolved in a minimum volume of water, filtered through Celite, and dried under vacuum again to give Pt(Amal) as a pale yellow solid. Yield: 10 mg (13%). ^{1}H NMR (300 MHz, D_2O): 3.96 (s, 2H), 3.74 (s, 1H), 2.26 (m, 2H), 1.98 (m, 2H), 1.40 (m, 2H), 1.31 (m, 2H), 0.99 (m, 2H). ESI-MS (cation): *m/z* calc 426.09, obs. 449.0 ($M + Na^+$).

Photocleavage Competition Titrations

A single-stranded DNA oligomer with the sequence 5′-TTAGGATCATCCATATA-3′ (underline denotes the mismatch) was labeled at the 5′-end with [^{32}P]-ATP using polynucleotide kinase (PNK) at 37 °C for 1 h. The radiolabeled DNA was purified by gel electrophoresis and annealed to its mismatched complement (containing a CC mismatch) by heating to 90 °C in buffer (100 mM NaCl, 20 mM NaP_i, pH 7.1), followed by slow cooling to ambient temperature over 3 h, to give a final concentration of 2 μM duplex DNA. Racemic solutions of the RhPt conjugate were prepared in Milli-Q water over a range of concentrations (100 nM – 50 μM). For each sample, 4 μM *rac*-$[Rh(bpy)_2chrysi]Cl_3$ (5 μl), which photocleaves DNA at mismatched sites, 2 μM annealed mismatched duplex DNA (10 μl), and the non-photocleaving RhPt at various concentrations (5 μl) were combined to give 1 μM *rac*-$[Rh(bpy)_2chrysi]Cl_3$ and 1 μM duplex DNA as the final concentrations. A "light" control, (ØRh, ØPt) consisting of 2 μM DNA mixed with 10 μl Milli-Q water, and a "dark" control (Ø *hυ*), containing the DNA mixed with the highest concentration of RhPt without irradiation, were also prepared. The samples were vortexed and, except for the dark control, irradiated on an Oriel (Darmstadt, Germany) 1000-W Hg/Xe solar simulator (340-440 nm) for 15 min.

The samples were then incubated at 37 °C for 10 minutes to degrade any metastable products and dried under vacuum. The irradiated samples were electrophoresed on a 20% denaturing polyacrylamide gel and exposed to a phosphor screen. The amounts of DNA in each band were analyzed by autoradiography and quantitated by phosphorimagery (ImageQuant).

Binding Constant Determination

As the RhPt complex does not photocleave DNA upon irradiation, the binding affinity for a CC mismatch was determined *via* a competition titration against *rac*-$[Rh(bpy)_2chrysi]^{3+}$, which does photocleave DNA at mismatched sites. To assess the binding of the rhodium subunit of RhPt at the CC mismatch, the fraction of cleaved DNA was quantified and expressed as a percentage of the total DNA in each lane and plotted against the log of the concentration of RhPt. The data from three independent titration experiments were each fit to a sigmoidal curve using OriginPro 8.5. The concentration of rhodium at the inflection point at the curve ($[Rh_{50\%}]$) was then used to solve simultaneous equilibria involving DNA, $[Rh(bpy)_2chrysi]Cl_3$, and RhPt in Mathematica 8.0 to obtain the binding constant (K_B). A representative photocleavage titration curve can be seen in Figure S1.

DNA platination was analyzed in a similar manner, wherein the fraction of platinated DNA was quantified and expressed as a percentage of the total DNA in each lane and plotted against the log of the concentration of RhPt. The data from three independent titrations were each plotted in OriginPro 8.5. A representative DNA platination titration curve can be seen in Figure S2.

Dimethyl Sulfate Footprinting of Platinated DNA

DNA footprinting of guanine by dimethyl sulfate (DMS) was carried out according to literature procedures.[4] Briefly, single stranded DNA with the sequence 5′-TTAGGATCATCCATATA-3′ (underline denotes the mismatch) was labeled at the 5′-end with [^{32}P]-ATP and annealed with its CC mismatched complement as described above. A solution of 1 μM annealed DNA was platinated with either RhPt or oxaliplatin at the concentrations indicated by incubation at 37 °C for 90 min. After cooling to 25 °C,

the samples were dried *in vacuo* and taken up in 5 µl Milli-Q water. The samples were diluted with DMS buffer (50 mM sodium cacodylate, 1 mM EDTA, pH 7.5), and 2 mM calf-thymus DNA (4 µl) was added as a carrier. Samples were cooled to 0 °C and treated with 5 µl DMS (10% v/v in EtOH) for 5 min at 25 °C. The reaction was quenched *via* addition of the DMS stop solution (1.5 M NaOAc, 1 M β-mercaptoethanol, 250 µg/ml yeast tRNA) at 0 °C. Following ethanol precipitation of the DNA, samples were treated with 10% aqueous piperidine and heated to 90 °C for 30 min. The piperidine was removed *in vacuo*, and samples were electrophoresed on a 20% denaturing polyacrylamide gel and exposed to a phosphor screen. The amounts of DNA in each band were analyzed by autoradiography and quantitated by phosphorimagery (ImageQuant).

Cell Culture

HCT116O. HCT116O cells were grown in RPMI medium 1640 supplemented with 10% fetal bovine serum, 400 µg/ml Geneticin (G418), 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were grown in tissue culture flasks (Corning Costar, Acton, MA) at 37 °C under a humidified atmosphere (5% CO_2).

Cellular Proliferation ELISA

The antiproliferative effects of conjugate RhPt, oxaliplatin, cisplatin, Rh(Amal) and Pt(Amal) were studied *via* enzyme-linked immunosorbent assay (ELISA). HCT116N and HCT116O cells were plated in 96-well plates at 2000 cells/well and given 24 h to adhere. The cells were incubated with varying concentrations of metal complex (0 – 2 µM) and grown for an additional 24 h. In the case of Rh and Pt combination treatment, both Rh(Amal) and cisplatin were administered from 0 – 2 µM. The media was then replaced with fresh media free of Rh or Pt for the remainder of the 72 h experiment. Cells were labeled with BrdU 24 h before analysis, and BrdU incorporation was quantified by antibody assay. Cellular proliferation was expressed as the amount of BrdU incorporated into treated cells compared to that of the untreated controls. Errors were calculated from 5 replicates. The results are shown in Figures S3-S4.

ICP-MS Assay for Whole-Cell Rh and Pt Levels

HCT116O cells (1.0×10^6) were seeded in 6-well plates containing 3 ml media and allowed 24 h to adhere. The cells were treated with 2 μM of RhPt, Rh(Amal), Pt(Amal), cisplatin, or oxaliplatin and incubated for periods of 1, 3, 6, 12, or 24 h. After the incubation period, the media was decanted and the wells were washed with 4 x 5 ml PBS. The cells were lysed with 1 ml of a 1% sodium dodecyl sulfate (SDS) solution and sonicated using a Qsonica Ultrasonic processor for 20 s at 20% amplitude. A 750 μl aliquot was diluted with 750 μl of a 2% HNO_3 (v/v) solution and analyzed for rhodium and platinum content on a Thermo X Series II ICP-MS unit. ICP-MS measurements for platinum content were measured only for the three most abundant naturally occurring isotopes, ^{194}Pt (33%), ^{195}Pt (34%), and ^{196}Pt (25%). The remainder of the cell lysate was analyzed for protein content *via* a bicinchoninic assay (BCA).[5] Rhodium and platinum counts were normalized to protein content to obtain ng [Rh/Pt]/mg [protein], and standard errors were calculated from three replicates.

ICP-MS Assay for Nuclear Rh and Pt Levels

HCT116O cells were plated at 1.0×10^7 cells in 10 ml media and incubated for 24 h. The cells were treated with 2 μM of RhPt, Rh(Amal), Pt(Amal), cisplatin, or oxaliplatin and incubated for an additional 24 h. The cells were harvested by trypsinization, washed with cold PBS, and the nuclear fractions were isolated according to established procedures.[6] The nuclear pellets were suspended in 800 μl of Milli-Q water and sonicated on a Qsonica Ultrasonic processor for 20 s at 40% amplitude. A 750 μl aliquot was diluted with 750 μl of a 2% HNO_3 (v/v) solution and analyzed for rhodium and platinum content on a Thermo X Series II ICP-MS unit as previously described. The remainder of each sample was used for quantification of nuclear protein content by BCA analysis. The protein content was then converted to number of nuclei by the conversion factor 3.28×10^{-8} mg [nuclear protein]/nuclei.[6] Rhodium and platinum counts were then normalized to the number of nuclei, and standard errors were calculated from three replicates.

ICP-MS Assay for Mitochondrial Rh and Pt Levels

HCT116O cells were plated at 1.5 x 10^7 cells/plate and allowed 24h to adhere. The cells were treated with 2 μM of RhPt, Rh(Amal), Pt(Amal), cisplatin, or oxaliplatin and incubated for an additional 24 h. The cells were harvested by trypsinization, washed with cold PBS, and the mitochondrial fractions were isolated according to established procedures.[6] The mitochondrial pellets were suspended in 800 μl of Milli-Q water and sonicated on a Qsonica Ultrasonic processor for 20 s at 40% amplitude. A 750 μl aliquot was diluted with 750 μl of a 2% HNO_3 (v/v) solution and analyzed for rhodium and platinum content on a Thermo X Series II ICP-MS unit as previously described. The remainder of each sample was used for quantification of mitochondrial protein content by BCA analysis. Rhodium and platinum counts were normalized to protein content to obtain ng [Rh/Pt]/mg [mitochondrial protein], and standard errors were calculated from three replicates.

MTT Cytotoxicity Assay

The cytotoxic effects of conjugate RhPt, Rh(Amal), Pt(Amal), oxaliplatin, and cisplatin were studied *via* MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay in the HCT116O cell line.[7] Cells were plated in 96-well plates at 50,000 cells/well and incubated with varying concentrations of metal complex (100 nM – 100 μM). For caspase-inhibition assays, Z-VAD-FMK was added to a final concentration of 20 μM. For poly-ADP ribose polymerase (PARP) assays, the inhibitor 3,4-dihydro-5[4-(1-piperindinyl)butoxy]-1(2*H*)-isoquinoline (DPQ) was added to a final concentration of either 25 or 50 μM. Cells were incubated under humidified atmosphere for 72 h and labeled with MTT for an additional 4 h at 37 °C, 5% CO_2. The ensuing formazan crystals were dissolved with a lysis buffer (10% SDS in 10 mM HCl) according to the manufacturer's instructions. MTT reduction to formazan was quantified by electronic absorption at 570 nm (background: 690 nm), and percent viability was expressed as the amount of formazan in treated cells compared to that of the untreated controls. The data were plotted in OriginPro 8.5 and fit to a sigmoidal curve. Errors were calculated from 5 replicates. The dose-response curves for HCT116O cells are shown in Figure S5

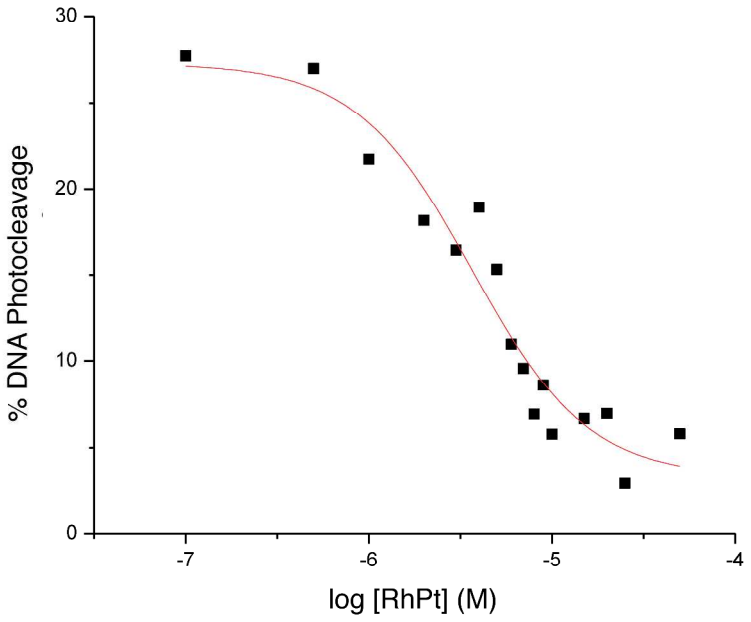


Figure S1. Representative sigmoidal curve fit of photocleavage competition titrations between 1 μM $[Rh(bpy)_2chrysi]^{3+}$ 0-50 μM of RhPt for binding constant determination at the CC mismatch.

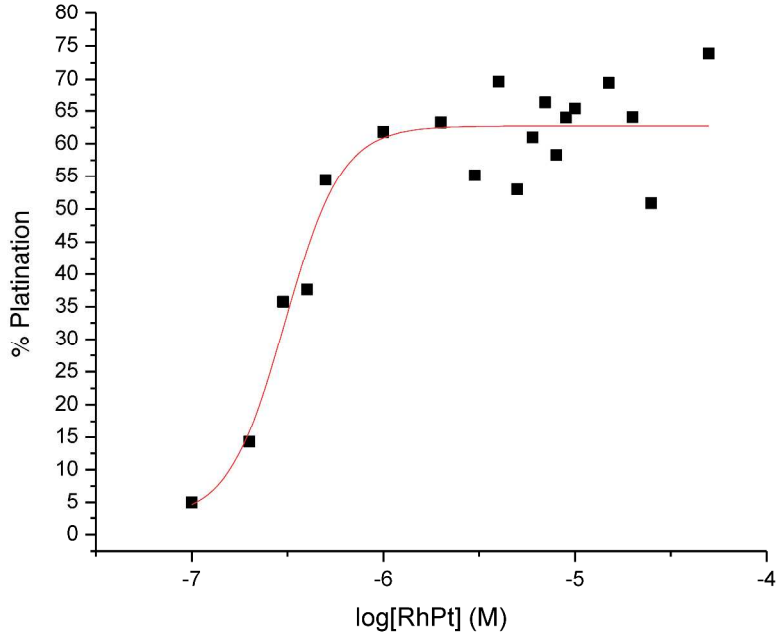


Figure S2. Representative sigmoidal curve fit of DNA platination by the platinum subunit of RhPt, from 0-50 μM.

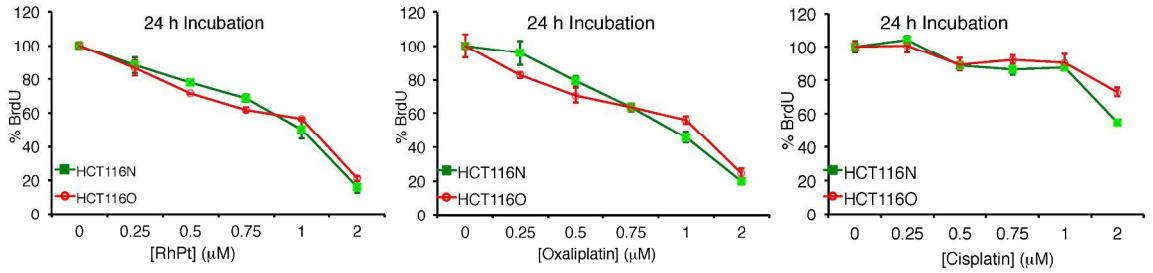


Figure S3. Inhibitory effects of RhPt (left), oxaliplatin (center) and cisplatin (right) on cellular proliferation. DNA synthesis is shown as a function of percent BrdU incorporation normalized to that of untreated cells. MMR-proficient HCT116N (green) and MMR-deficient HCT116O (red) cells were plated in 96-well plates and allowed 24 h to adhere. Cells were then treated with 0-2 μM of the indicated metal complex for 24 h, after which the medium was removed and replaced with fresh, drug-free medium for the remainder of the 72 h period. BrdU was added to the medium 24 h prior to ELISA analysis. Standard error bars were calculated from 5 replicates.

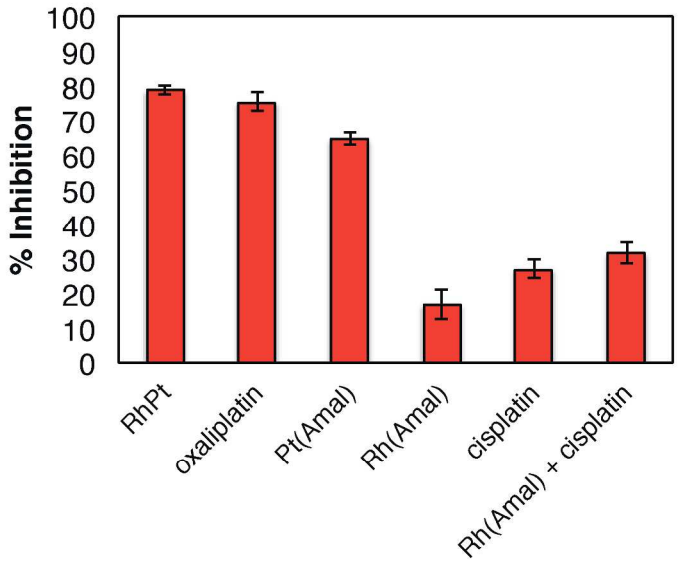


Figure S4. Inhibitory effects of all complexes on cellular proliferation in HCT116O cells after 24 h treatment with 2 μM of each complex. For combination treatment, cells were treated with 2 μM each of cisplatin and Rh(Amal). Treatment of cells with the unconjugated Rh(Amal) in combination with the relatively non-potent cisplatin compound has no significant effect on overall inhibition of growth. Percent inhibition is expressed as the normalized percent BrdU incorporation subtracted from 100% (i.e., untreated cells, normalized). Standard errors were calculated from 5 replicates.

Table S1. Cellular Distribution and Cytotoxicity of Complexes in HCT116O Cells[8,9]

Complex	Nuclear	Mitochondrial[a]	LC_{50}[b]
RhPt	18 ± 2 μM Pt 37 ± 2 μM Rh	52 ± 13 Pt 10 ± 0.4 Rh	9.0 μM
Rh(Amal)	4 ± 1 μM	9.8 ± 0.9	43.3 μM
Pt(Amal)	13 ± 1 μM	54 ± 5	57.2 μM
Oxaliplatin	15 ± 2 μM	68 ± 2	27.5 μM
Cisplatin	14 ± 2 μM	73 ± 17	29.5 μM

[a]Mitochondrial metal content is normalized to mitochondrial protein using by BCA analysis, and is expressed as (ng [metal]/mg [mito protein]). [b]LC_{50} refers to the concentration at which 50% of HCT116O cells are viable after 72 h treatment, as determined by MTT assay.

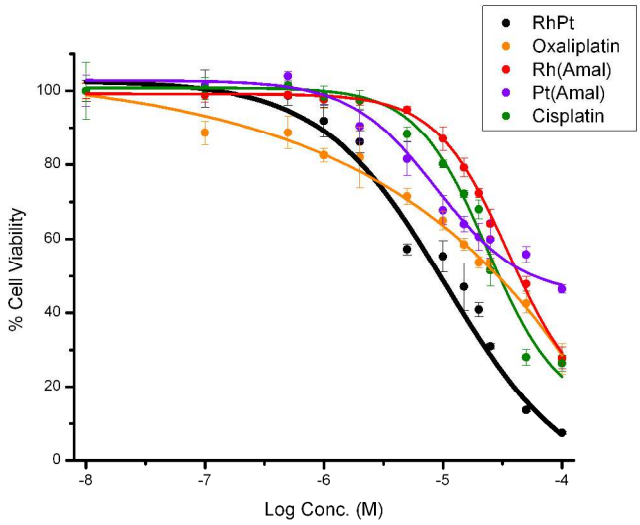


Figure S5. Dose-response cytotoxicity curves of HCT116O cells treated with RhPt (black), oxaliplatin (orange), Rh(Amal) (red), Pt(Amal) (purple), and cisplatin (green). Cells were treated with 0-100 μM of each metal complex and incubated for 72 h. After the incubation period, cells were treated with the MTT reagent for 4 h, and the resulting formazan crystals were solubilized with acidified SDS. Percent cell viability is defined as the percentage of formazan normalized to that of untreated cells. Data were fit to a sigmoidal curve, and LC_{50} values were obtained. Standard errors were calculated from 5 replicates.

References

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(8) Nuclear versus mitochondrial metal uptake are normalized differently, mitigating their comparison.

(9) Nuclear concentrations were obtained by dividing metal content by the volume of the nucleus, estimated as a sphere with radius 4 μm. Errors were calculated from three replicates. See Fujioka, A.; Terai, K.; Itoh, R. E.; Aoki, N.; Nakamura, T.; Kuroda, S.; Nishida, E.; Matsuda, M. *J. Biol. Chem.* **2006**, *281*, 8917-8926.