Oxaliplatin and \([\text{Pt}(R,R'-\text{DACH})(\text{panobinostat}-2\text{H})]\) show nanomolar cytotoxicity towards diffuse intrinsic pontine glioma (DIPG)

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Electronic supplementary information

Materials and Methods

Chemistry:

HPLC-grade solvents and Millipore-filtered \(\text{H}_2\text{O}\) were used for the preparation of compounds and purification by HPLC. \(\text{K}_2[\text{PtCl}_4]\) was purchased from Precious Metals Online. Oxaliplatin was purchased from MedChemTronica and used as received. Panobinostat was purchased from Insight Biotechnology and suberohydroxamic acid (\(N,N'\)-dihydroxyoctane diamide, SubH) was purchased from Sigma-Aldrich. All other reagents were purchased from Sigma-Aldrich or Alfa Aesar and used as received. (IM) indicates use of a nylon syringe filter (pore size 0.2 \(\mu\text{M}\)). All manipulations were carried out under reduced lighting and solutions were prepared, stored, and handled with minimal exposure to light.

NMR spectroscopy. Spectra were acquired at 298 K unless otherwise stated, and processed using Topspin 3.2. All chemical shift (\(\delta\)) values are given in parts per million and are referenced to residual solvent unless otherwise stated. \(^1\text{H}\) NMR: were acquired on a Bruker AVIIIHD 500 MHz (500.13 MHz) equipped with a 5mm z-gradient broadband X-19F/1H BBFO SMART probe or a Bruker AVIIIHD 400 nanobay (400.17 MHz). \(^{13}\text{C}\) NMR: acquired on a Bruker AVII 500 MHz spectrometer equipped with a z-gradient triple resonance inverse \(^1\text{H}/^{19}\text{F}(^{13}\text{C})\) TXI probe. \(^{195}\text{Pt}\) NMR: \(^{195}\text{Pt}\) chemical shifts were externally referenced to \(\text{K}_2\text{PtCl}_6\) in 1.5 mM HCl in \(\text{D}_2\text{O}\) (\(\delta\) 0 ppm). Data were acquired at 298 K on a Bruker Avance III spectrometer with a 11.7 T magnet, inducing 500 MHz and 107 MHz Larmor frequencies for \(^1\text{H}\) and \(^{195}\text{Pt}\) respectively. Mass Spectrometry: low resolution ESI-MS were obtained with a Waters Micromass LCT Premier XE spectrometer. HRMS: obtained with a Thermofisher Exactive Plus with a Waters Acuity UPLC system. MS/MS experiments: were performed on an Acuity UPLC in flow injection analysis mode, equipped with a Waters Xevo G25 QTOF. HPLC: were performed with a Waters Autopurification system, equipped with a Waters X-Bridge OBD semi-prep column (5 \(\mu\text{m}\), 19 mm x 50 mm), with an injection loop of 1 ml, eluting with \(\text{H}_2\text{O}+0.1\%\ \text{NH}_2\text{OH}\) (pH 9)/MeCN +0.1% \(\text{NH}_3\text{OH}\). The crude samples (in \(\text{H}_2\text{O}/\text{MeCN}\) were filtered (nylon, 0.2 \(\mu\text{m}\)) and injected in 750 \(\mu\text{L}\) aliquots, with mass-directed purification with an ACQUITY QDa performance mass spectrometer. Analytical HPLC used the same solvents and system, with a Waters X-Bridge OBD column (5\(\mu\text{m}\), 4.6mm x 50mm) and an injection loop of 0.02 ml. HPLC data was processed using MassLynx 4.0. Retention times (\(t_R\)) are quoted for the solvent gradient: 0 min (95% A : 5% B); 1 min (95:5), 7.5 min (5:95) on the analytical column. UV-visible absorption spectra were obtained with the Waters HPLC.
Biological experiments:

HSJD-DIPG-007, SU-DIPG-IV, HSJD-DIPG-007 and BIOMEDE 194 patient-derived DIPG cell lines were generously provided by Professor Chris Jones (ICR, London). All DIPG cell lines were grown in a tumour stem cell media base consisting of a 1:1 solution of Dulbecco’s Modified Eagles Medium: Nutrient Mixture F12 (DMEM/F12) (11330-032) and Neurobasal-A Medium (10888-022), supplemented with 1 M HEPES solution (15630-080), 100 mM sodium pyruvate solution (11360-070), 10 mM non-essential amino acids solution (11140-050), 1x Glutamax-I Supplement (35050-061) and 1x Antibiotic-Antimycotic solution (15240-096). All media and supplements were obtained from ThermoFisher. Tumour stem cell base medium solution was supplemented with 1X B-27 Supplement, minus Vitamin A, (Thermo Fisher, 12587-010) and 20 ng.mL⁻¹ Human-EGF (AF-100-15), 20 ng.mL⁻¹ Human-FGF-basic (AF-100-18B), 20 ng.mL⁻¹ Human-PDGF-AA (AF-100-13A), 20 ng.mL⁻¹ Human-PDGF-BB (AF-100-14B) all obtained from Peprotech, and 2 µg.mL⁻¹ Heparin solution (0.2 %, Sigma, H3393). Cells were incubated at 37 °C in 5% CO₂.

384 well plates (Greiner, 781091) were pre-coated with 10 µL of 1µg.mL⁻¹ laminin (Sigma, L2020) and incubated overnight. Immediately prior to cell seeding, the laminin was removed and replaced with 20 µL of DIPG cells per well and incubated overnight. Cell seeding densities were: SU-DIPG-IV – 250 cpw; HSJD-DIPG-007 – 250 cpw; SU-DIPG-XXI - 625 cpw and BIOMEDE 194 – 625 cpw. Compounds were prepared in 100% DMF, excluding carboplatin which was prepared in dH₂O. A stock serial dilution ranging from 5 mM to 0.1 µM was prepared and 2 µL of this was diluted in 198 µL of complete media (1:100). 5 µL of diluted compound was added per well (1:5). All compounds were run in triplicate. After 72h cell viability was determined by CellTiter-Glo luminescence assay (Promega, G9683). IC₅₀ values were determined using GraphPad Prism, determined as the concentration at which cell viability was 50% of the DMSO-treated control wells.
Chemical Syntheses: Note: Care was taken to avoid exposure of both Ag and Pt solutions to ambient light, due to potential photosensitivity.

**Cis-[Pt(R,R-DACH)Cl₂]**¹

K₂PtCl₄ (1.82 g, 4.39 mmol, 1 equiv.) was dissolved in water (8.76 mL) with stirring, R,R-DACH (0.5 g, 4.39 mmol, 1 equiv.) was added and the solution stirred for 1 hr. The resultant crystalline solid was filtered, washed with ice-cold water and ethanol. To the filtrate, a second equivalent of R,R-DACH (0.25 g, 2.19 mmol) was added and the solution was stirred for 1 hr, obtaining a second crop of product (overall yield 1.006 g, 2.4641 mmol, 60%).

**¹H NMR (400 MHz, DMSO-d₆, ppm)** δ: 2.35 (m, 2H), 1.93 (dd, 2H), 1.50 (m, 2H), 1.27-1.38 (m, 2H), 1.02 (m, 2H).

**¹⁹⁵Pt NMR (107 MHz, DMSO-d₆, ppm)** δ: -2288.

**[Pt II(DACH)(Sub-H)] (1)**

A suspension of cis-[Pt(R,R-DACH)Cl₂] (0.248 g, 0.65 mmol) in water (7.5 mL) was treated with an aqueous solution of AgNO₃ (0.219 g, 1.29 mmol, 1.98 equiv.) and stirred in the dark overnight at room temperature. The AgCl precipitate was removed by filtration (IM) and SubH (0.204 g, 1.0 mmol) was added to the filtrate. The solution was stirred at 40 °C for 2 d. The solution was reduced to approximately 5 mL, purified by HPLC (selection mass M = 511.18) and the solvent removed by freeze-drying to collect 1 as a yellow solid (0.1632 g, 0.3188 mmol, 49%).

**¹H NMR (500 MHz, D₂O)** δ: 5.87 (0.5H, br, DACH-NH₂), 5.19 (0.5H, br, DACH-NH₂), 2.38 (m, 4H, 2H₁ +H₆' + H₆''), 2.17 (m, 2H, 2H₅), 2.07 (m, 2H, 2H₄'), 1.60 (m, 6H, 2H₅ + 2H₆' +2H₆''), 1.31 (m, 6H, 2H₄' + 2H₅'), 1.17 (m, 2H, 2H₅'').

**¹³C NMR (126 MHz, DMF-d₇)** δ: 171.6 (CO-1), 170.7 (CO-2), 63.3 and 62.8 (C₆' and C₆''), 33.3 (C₅), 32.6 and 32.7 (two inequivalent C₄), 29.5, 29.19, 29.16 (C₄' and C₄''), 28.0 (C₃), 26.2, 26.12 and 26.06 (C₅' and C₅''), 25.24 and 25.22 (two inequivalent C₃).

**¹⁹⁵Pt NMR (107 MHz, D₂O, ppm)** δ: -1973. **HRMS (H₂O) m/z**: 512.1829 [M+H]+. UV-Vis (H₂O/MeCN): λmax 195 nm.

**[Pt⁴⁰(DACH)(panobinostat)] (2)**²

To a suspension of cis-[Pt(R,R-DACH)Cl₂] (0.08 g, 0.21 mmol) in water (2 mL) was added a solution of AgNO₃ (0.07 g, 0.415 mmol, 1.98 equiv.) in H₂O (5 mL) and stirred in the dark overnight at room temperature. The AgCl precipitate was removed by filtration (IM) and a DMF solution of panobinostat (0.088 g, 0.252 mmol in 5 mL) was added to the filtrate. The resultant solution was stirred at room temperature for 24 h. The solvent was removed and the residue redissolved in acetonitrile. The solution was purified by mass-directed prep-HPLC (selection mass M = 656.25) and the solvent removed by freeze-drying to give 2 as a yellow solid (0.0249 g, 0.0380 mmol, 18%).
$^1$H NMR (500 MHz, DMF-$d_7$, ppm) $\delta$: 13.23 (br s, 1H), 10.90 (br s, 1H), 9.35 (br, s, 1-2H), 7.72 (d, $J = 8.1$ Hz, 2H, Q and R), 7.66 (d, $J = 8.0$, 2H, P and O), 7.49 (d, $J = 8.0$, 1H, G), 7.45 (m, $J = 16.1$ Hz, 1H, T), 7.34 (d, $J = 8.1$, 1H, D), 7.05 (dd, $J = 7.6$, 1H, E), 6.98 (dd, $J = 7.4$, 1H, F), 6.91 (d, $J = 16.1$, 1H, U), 6.52 (d, br, 1.5H, DACH-NH$_2$), 5.74 (m, br, 1.5H, DACH-NH$_2$), 4.50 (s, 2H, M), 3.39 (br, 2H, K), 3.22 (t, $J = 8.5$ Hz, 2H, J), 2.44 (2H, m, H$_A$ and H$_A''$), 2.40 (s, 3H, A), 2.09 (obsced, 2H, 2H$_B$), 1.58 ((2H, m, 2H$_C$), 1.49 (2H, m, 2H$_B$), 1.19 (2H, m, 2H$_C$). $^{13}$C NMR (126 MHz, DMF-$d_7$, ppm) $\delta$: 166.4 (q, Pt-CO), 137.0 (q, N), 136.90 (T), 136.86 (q, C), 134.7 (q, S), 134.2 (q, B), 131.9 (O+P), 129.3 (q, H), 128.9 (R+Q), 121.4 (E), 119.6 (F), 118.2 (G), 113.4 (U), 111.7 (D), 106.3 (q, I), 63.6 and 63.2 (C$_A$ and C$_A''$), 51.7 (M), 49.0 (K), 32.9 and 32.7 (two inequivalent C$_B$), 25.42 and 25.39 (two inequivalent C$_C$), 22.1 (J), 11.6 (CH$_3$ A). $^{195}$Pt NMR (107 MHz, DMF-$d_7$, ppm) $\delta$: -1918. HRMS (DMSO-$d_6$ /MeOH) m/z : 657.2522 [M+H]$^+$. UV-vis (H$_2$O /MeCN) $\lambda$: 277 nm.
Commercial oxaliplatin: purity confirmation

Chemical Formula: C₈H₁₄N₂O₄Pt
Exact Mass: 397.06

**Oxaliplatin in D₂O**

**MB-F-F0**

1: Scan ES+  
401.04  
8.75e5

Figure S1. HPLC purity trace for commercial oxaliplatin (D₂O): detected as \( \text{d}_4 \)-deuterated compound following amine proton exchange \([M + H]^+ = 401.04 \text{ m/z (top)}\) integration of ESI-MS spectrum at 1.77 min.
Figure S2. $^1$H NMR spectrum (D$_2$O, 500 MHz) (commercial oxaliplatin) δ: 2.38 (2H, m, CH(NH$_2$), Pt satellites, H$\alpha$, H$\alpha'$), 2.08 (2H, d, $J = 13$ (geminal), 2H$\beta'$), 1.60 (2H, m, 2H$\gamma$), 1.33 (2H, m, 2H$\delta$), 1.18 (2H, m, 2H$\epsilon$).

$^1$H – $^1$H COSY: including weaker correlations (circled)

Figure S3. $^1$H – $^1$H COSY NMR spectrum (D$_2$O) (commercial oxaliplatin).
Figure S4. $^{13}$C NMR spectrum (D$_2$O, 126 MHz) (commercial oxaliplatin) $\delta$: 168.3 (oxalate CO), 62.2 ($C_A$), 31.7 ($C_B$), 23.9 ($C_C$).

Figure S5. $^{195}$Pt NMR spectrum (107 MHz, D$_2$O) (commercial oxaliplatin) $\delta$: -1996.
Figure S6. $^1$H-$^{13}$C HSQC NMR spectrum (D$_2$O) (commercial oxaliplatin).
**Figure S7.** $^1$H NMR spectrum (500 MHz, $d_6$-DMSO) (free SubH ligand) δ: 10.32 (br, 2OH), 8.66 (br, 2NH), 1.92 (4H, $J$ = 7.5, 4H$_1$), 1.46 (4H, m, 4H$_2$), 1.21 (4H, m, 4H$_3$).

**Figure S8.** $^{13}$C NMR spectrum (126 MHz, $d_6$-DMSO) (free SubH ligand) δ: 169.2 ((CO)NHR), 32.3 (CH$_2$, C$_1$), 28.4 (CH$_2$, C$_3$), 25.1(CH$_2$, C$_2$).
Figure S9. $^1$H – $^{13}$C HSQC of free SubH ligand (d$_6$-DMSO).
NMR spectral assignment of SubH complex 1

Figure S10. Putative structure of complex 1 and potential solution structure [1+H]^+; stabilisation could occur through either the terminal OH or OH or C=O group.
$^1$H NMR of complex 1 ($D_2O$)

Figure S11. $^1$H NMR of complex 1 (500 MHz, $D_2O$).
**Complex 1 (DMF-d$_7$)**

$^1$H NMR spectrum

* = DMF

**Figure S12.** $^1$H NMR of complex 1 (500 MHz, DMF-d$_7$).
Complex 1 (DMF-$d_7$) $^1$H - $^1$H COSY NMR spectra

Figure S13. $^1$H COSY NMR spectra of complex 1 (500 MHz, DMF-$d_7$).
Figure S14. $^1$H COSY and ROESY NMR spectra of complex 1 (500 MHz, DMF-$d_7$).
Figure S15. $^1$H-$^{13}$C HSQC NMR spectrum complex 1 (DMF-$d_7$).
$^{1}\text{H} - ^{13}\text{C}$ HMBC NMR spectrum of complex 1 (DMF-$d_7$)

**Figure S16.** $^{1}\text{H} - ^{13}\text{C}$ HMBC NMR spectrum (DMF-$d_7$) of complex 1 (region 1).
$^1$H-$^{13}$C HMBC NMR spectrum of complex 1 (DMF-$d_7$)

Figure S17. $^1$H-$^{13}$C HMBC NMR spectrum (DMF-$d_7$) of complex 1 (regions 2-5).
Figure S18. $^{13}$C NMR spectrum (126 MHz, DMF-$d_7$) of complex 1.
Panobinostat NMR spectroscopic assignment

**Chemdraw prediction:**

**Figure S19.** $^1$H NMR of free panobinostat, assigned through 2D experiments, comparison with Chemdraw and literature. $^3$H NMR (500 MHz, DMF-$d_7$) $\delta$: 10.68 (br s, 1H, OH), 7.55 (m, $J = 15.8$ Hz, 1H, T) plus (d, $J = 8.0$ Hz, 2H, Q and R), 7.46 (d, $J = 8.0$, 1H, G), 7.42 (d, $J = 8.0$, 2H, P and O), 7.29 (d, $J = 8.0$, 1H, D), 7.00 (m, $J = 7.4$, 1H, E), 6.94 (m, $J = 7.4$, 1H F), 6.62 (d, $J = 15.8$, 1H, U), 3.86 (s, 2H, M), 2.93 (t, $J = 7.5$ Hz, 2H, K plus *DMF - residual solvent)), 2.84 (t, $J = 7.5$ Hz, 2H, J), 2.39 (s, 3H A).
Figure S20. $^1$H COSY NMR of free panobinostat (500 MHz, DMF-$d_7$).
Figure S21. $^{13}$C NMR of free panobinostat, assigned through 2D experiments, comparison with Chemdraw and literature$^{3}$ (126 MHz, DMF-d$_7$) δ: 164.2 (q, CO), 143.8 (T), 139.5 (q, N), 136.9 (q, C), 134.8 (q, S), 133.2 (q, B), 129.9 (q, H), 129.6 (O+P), 128.5 (R+Q), 121.0 (E), 119.6 (U), 119.2 (F), 118.5 (G), 111.4 (D), 109.6 (q, l), 52.8 (M), 51.1 (K), 25.7 (J), 11.9 (CH$_3$ A). * = residual DMF.
Figure 22. $^1\text{H} - ^{13}\text{C}$ HSQC NMR of free panobinostat (DMF-$d_7$).
NMR spectral assignment of panobinostat complex 2

Figure S23. Putative structure of complex 2 and potential solution structure [2+H]⁺. Alternative binding modes 2b and 2c are less consistent with ESI-MS and HRMS data.
Figure S24. $^1$H NMR (DMF-$d_7$, ppm) of oxaliplatin (400 MHz, top); panobinostat (500 MHz, middle) and complex 2 (500MHz, bottom).
Figure S25. $^1$H-$^{13}$C HSQC NMR spectra of complex 2 (DMF-$d_7$) (aliphatic region).
Figure S26. $^1$H-$^{13}$C HSQC NMR spectra of complex 2 (DMF-$d_7$) (aliphatic region).
Figure S27. $^1$H-$^{13}$C HMBC NMR of complex 2 (DMF-$d_7$) (aromatic and CO regions).
Figure S28. $^1$H-$^{13}$C HMBC NMR spectra of complex 2 (DMF-$d_7$) (aliphatic regions).
**Figure S29.** $^{13}$C NMR spectrum (126 MHz, DMF-$d_7$, ppm) of complex 2. δ: 166.4 (q, Pt-CO), 137.0 (q, N), 136.90 (T), 136.86 (q, C), 134.7 (q, S), 134.2 (q, B), 131.9 (O+P), 129.3 (q, H), 128.9 (R+Q), 121.4 (E), 119.6 (F), 118.2 (G), 113.4 (U), 111.7 (D), 106.3 (q, I), 63.6 and 63.2 (C$_{\alpha'}$ and C$_{\alpha''}$), 51.7 (M), 49.0 (K), 32.9 and 32.7 (two inequivalent C$_b$), 25.42 and 25.39 (two inequivalent C$_c$), 22.1 (J), 11.6 (CH$_3$ A).
Figure S30. $^{195}$Pt NMR spectra of complex 1 (D$_2$O: -1973 ppm, $d_7$-DMF: -1947 ppm) and complex 2 (DMF-$d_7$, -1918 ppm).
Figure S31. $^1$H NMR spectra (500 MHz) in DMF-$d_7$ of top: panobinostat and bottom: precipitate formed following mixing of panobinostat and oxaliplatin. * = DMF.
Figure S32. HRMS of \([1 + H]^+ \ (C_{14}H_{28}N_4O_4PtH)\) \(m/z\): 512.1829 found (left); 512.18211 calculated (right) (1.5 ppm error).

Figure S33. HRMS of \([2 + H]^+ \ (C_{27}H_{35}N_5O_2PtH)\) \(m/z\): 657.2522 found (left); 657.2511 calculated (right) (1.7 ppm error).
Detection of carboxylic acid species derived from hydroxamic acids during HPLC

\[
\text{[SubH +H]^+} \xrightarrow{\text{"-NH"}} \text{F}
\]

Formula: C₈H₁₇N₂O₄

detected 205.15 m/z; predicted 205.12 m/z

\[
\text{[panobinostat +H]^+} \xrightarrow{\text{"-NH"}} \text{G}\quad \text{and}\quad \text{H}
\]

Formula: C₂₁H₂₃N₃O₂

(positive ionisation mode) detected 335.16 m/z; predicted: 335.18 m/z

(negative ionisation mode) detected 333.12 m/z; predicted: 333.16 m/z

**Figure S34.** Species detected by LCMS corresponding to conversion of hydroxamic acid group to carboxylic acid group for SubH and panobinostat.
Attempted oxidation of 1 and 2 to platinum (IV) complexes

Synthesis of [Pt\textsuperscript{IV}(R,R-DACH)(Sub\textsubscript{H})(OH)\textsubscript{2}] (3). Complex 1 (20 mg, 0.039 mmol) was suspended in a solution of H\textsubscript{2}O (5 ml) and H\textsubscript{2}O\textsubscript{2} (11 µL, 3.98 mg, 3 eq. 30% H\textsubscript{2}O\textsubscript{2}), before stirring at ambient temperature for 24 h. The solvent was removed by lyophilisation and the oxidised product 3 purified by mass-directed HPLC (mass selection M = 545.18). Solvent from the collected fractions was removed by lyophilisation to produce 3 as a yellow solid; 3 was analysed by HRMS (C\textsubscript{14}H\textsubscript{30}N\textsubscript{4}O\textsubscript{6}PtH; 546.1887 m/z found; 546.1886 m/z calculated, 0.18 ppm error).

Figure S35. HRMS of [3 + H]\textsuperscript{+} (C\textsubscript{14}H\textsubscript{30}N\textsubscript{4}O\textsubscript{6}PtH\textsuperscript{+}; 546.1887 m/z found; 546.1886 m/z calculated (0.18 ppm error).
Figure S36. A) Extracted ESI-MS of region of HPLC trace corresponding to the product [3+H]+ (546.19 m/z) B) Assignments: Free [Sub+H]+ ligand is observed at 205.10 m/z; the platinum species at 510.15 m/z is suggested to be formed through dehydration of [3+H]+, which could be stabilised (for example) by delocalisation of the charge or interaction of the hydroxamic acid ligand with the platinum centre. The species [(3)2 + H]+ is seen at 1091.45 m/z (model 1091.37 m/z); and [(3)2 − H2O]+ is detected at 1073.20 m/z (model 1073.36 m/z).

**Attempted oxidation of complex 2**

Complex 2 (5 mg, MW 657.702, 0.0075 mmol) was dissolved in minimum DMF. 35% H2O2 (22 μL, 0.00765 g, 30 eq., 0.225 mmol) was added and the solution stirred at 50°C for 2 h, with monitoring at regular intervals by HPLC which showed consumption of 2, but no evidence of the dihydroxido Pt(IV) product.
Cytotoxicity data

**Figure S37.** Cytotoxicity curves for compounds in three DIPG cell lines after 72h exposure. Cytotoxicity of carboplatin, oxaliplatin, SubH and panobinostat in A: BIOMEDE 194 B: SU-DIPG-XXI and C: SU-DIPG-IV. Cytotoxicity of 1:1 oxaliplatin and subH, oxaliplatin + panobinostat and complex 1 and 2 in D: BIOMEDE 194, E: SU-DIPG-XXI and F: SU-DIPG-IV. All experiments were conducted in minimum of triplicate, with three biological repeats.

**References**