Supplementary Material

Osmium (II) polypyridyl probe with a therapeutic twist: disrupting the mitochondrial membrane potential for targeted therapy

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$^1$H NMR spectra of tpybenzCOOH and Os(II) complexes

![NMR spectra](image)

Figure S1: $^1$H-NMR spectra of tpybenzCOOH in DMSO-$d_6$ (top) and TFA-$d$, (bottom) 600 MHz
Figure S2: $^1$H NMR of purified [Os(tpybenzCOOH)$_2$]$^{2+}$ in DMSO-$d_6$, 400 MHz
Figure S3: COSY spectrum of [Os(tpybenzCOOH)₂]²⁺ in MeCN-d₃, 600 MHz
Figure S4: $^1$H NMR spectrum of Os$^{5+}$MPP in MeOH-d$_4$, 400 MHz with key regions in the aromatic and aliphatic region highlighted.
Mass Spectrometry Analysis

Figure S5: HRMS (TOF ES+) of [Os(tpybenzCOOH)_2]^{2+}
Figure S6: HPLC chromatogram (above) and Mass Spectrum (below) of Os\textsuperscript{II} MPP complex following LC-MS analysis (Q-Exactive). Conditions; gradient extended from 10- 90% (Formic Acid 0.1%, 80% MeCN).
Figure S7: LC-MS analysis of Os\(^{18}\)MPP Zoomed m/z 456-470 (RT 21-23 min)

Figure S8: LC-MS analysis of Os\(^{18}\)MPP Zoomed m/z 747-754 (RT 21-23 min)
Figure S9: LC-MS analysis of Os\textsuperscript{II}MPP Zoomed m/z 458-468 (RT 23-25 min)

Figure S10: LC-MS analysis of Os\textsuperscript{II}MPP Zoomed m/z 560-566 (RT 23-25 min)
HPLC Analysis of parent and conjugate Os(II) complex
Figure S11: Top: HPLC Chromatogram of Os\textsuperscript{II} parent complex and Os\textsuperscript{II} MPP conjugate obtained RP-C18 HPLC with MeCN mobile phase. Bottom three traces show traces using gradient mobile phase detected at 280 nm. (Gradient MeCN/ H\textsubscript{2}O 0.1% TFA gradient, 1ml min\textsuperscript{-1}). Elution of Os\textsuperscript{II}MPP conjugate at 8.3 min (Channel 1: 280 nm and Channel 2: 490nm). Diode array detection was used and the HPLC UV-vis spectrum of each peak was obtained during Os\textsuperscript{II} MPP analysis and is shown below.
Figure S12: HPLC UV-vis spectra obtained for peaks observed during Os\(^{\text{III}}\)MPP HPLC analysis illustrating the absence of a component with absorbance only in the UV-vis region as the MLCT band at a longer wavelength confirms Osmium-coordinated complex for each peak. The distribution of peaks in the conjugate product is suspected to originate from different ionization states of the conjugate.

Spectroscopic Measurements of Os(II) parent complex and Os(II) conjugate

Figure S13: Absorbance spectrum of parent complex [Os(tpybenzCOOH)]\(^{2+}\) 1 in acetonitrile solution (25\(\mu\)M).
Figure S14: Emission spectra of parent complex [Os(tpybenzCOOH)$_2$]$^{3+}$ in acetonitrile (50μM) under aerated and deaerated conditions (slit widths 10 nm / 10 nm). Solution was deaerated using nitrogen gas and oxygen concentration was measured using PreSence O$_2$ sensor.
Figure S15: Absorbance spectra of parent complex [Os(tpybenzCOOH)₂]²⁺ (50μM/ PBS pH 7.4) following continuous photo-irradiation with ARC Lamp 150W for 3 h at Room Temperature.
Emission Decays of Os (II) parent and Os\textsuperscript{II} MPP complex – Time- correlated single photon counting (TCSPC) measurements using NanoHarp 2.1, FluoTime100 (PicoQuant) and mono-exponentially fitted using PicoQuant Fluofit software.

\[ \int_{-\infty}^{t} IRF(t') \sum_{i=1}^{x} A_i e^{-\frac{t-t'}{\tau_i}} \, dt' \]

Figure S16: Emission Decays of parent complex [Os(tpybenzCOOH)\textsubscript{2}]\textsuperscript{2+} in aerated and deaerated acetonitrile (50\textmu M); Residual plots for the exponential fitting of both curves are shown below each plot.
\[
\int_{-\infty}^{t} IRF(t') \sum_{i=1}^{X} A_i e^{-\frac{t-t'}{\tau_i}} dt'
\]

Figure S17: Emission Decays of parent complex \([\text{Os(tpybenzCOOH)}_2]^2+\) in aerated and deaerated PBS pH 7.4 (50\(\mu\)M); The residual plots for the exponential fitting of both curves are shown below.
\[ \int_{-\infty}^{t} IRF(t') \sum_{i=1}^{x} A_i e^{-\frac{t-t'}{\tau_i}} \, dt' \]

Figure S18: Emission decay of conjugate Os\textsuperscript{II} MPP under aerated and deaerated conditions (25μM/ PBS pH 7.4); Residual Plots of the exponential fitting for both curves are shown below.
Cytotoxicity Studies and determination of IC$_{50}$ of Os$^{II}$ MPP

Figure S19: Cell Viability of HeLa and MCF 7 cells after 24hr exposure to Os$^{II}$ MPP probe. Live cells were treated with the probe followed by addition of Resazurin for 6 h. Absorbance was read at 570 nm with background at 600 nm subtracted (n=3).

Figure S20: Determination of EC$_{50}$. HeLa cells were incubated in the presence of Os$^{II}$ MPP (0.1μM to 150μM) for 24 h. Cell proliferation was assayed with Resazurin (n=3). The IC$_{50}$ value, the minimal amount of Os$^{II}$ MPP required to inhibit 50 % viability of HeLa cells was found to be 30.61μM.
Confocal Imaging

Figure S21: Confocal Imaging studies of [Os(tpybenzCOOH)$_2$]$^{2+}$. Cells were treated with [Os(tpybenzCOOH)$_2$]$^{2+}$ at 30µM for 2 h. (A) Osmium Channel: uptake of the parent complex was not evident; (B) Background channels showing HeLa cells. Cells were excited with 490 nm WLL and emission was collected between 650nm and 850 nm.

Figure S22: Confocal microscopy of HeLa cells incubated with Os$^{II}$ MPP at 30µM for (A) 4 h, (B) 6 h and (C) 24 h in cell media at 37 °C in the absence of light. (a-c) Overlay images with the background channel. Os$^{II}$MPP was excited using a 490 nm white light laser and emission was collected between 650nm and 850 nm.
Figure S23: Confocal imaging of OsII MPP in HeLa and MCF 7 cells at increased concentrations; Uptake of OsII MPP probe is evident but changes are observed in the morphological features of cells indicative of cell death.

Figure S24: Co-staining with DRAQ7 following OsII MPP 30uM/2h; (A) Absence of nuclear staining confirms cells are viable; observed in blue is the emission of MitoTracker Deep Red due to co-excitation of MitoTracker Deep Red at 633 nm. (B) Overlay of all channels
Polycaspase FAM-FLICA and MitoPT TMRE Assay

Table 1: MitoPT and FLICA Assay: Cell Population Experimental Conditions

<table>
<thead>
<tr>
<th>Assay</th>
<th>Negative Control Populations</th>
<th>Positive Control Population</th>
<th>Experimental Populations</th>
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<tbody>
<tr>
<td>Mito PT</td>
<td>(1) Non-exposed population</td>
<td>CCCP (20μM/ 2 h)</td>
<td>Os&quot;MPP 30μM/ 2 h</td>
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<tr>
<td></td>
<td>(2) DMSO (100μM/ 1 h)</td>
<td></td>
<td>Os&quot;MPP 100μM/ 1 h</td>
</tr>
<tr>
<td>FLICA</td>
<td>(1) Non-exposed population</td>
<td>Staurosporine (1μM/ 3 h)</td>
<td>Os&quot;MPP 30μM/ 2 h</td>
</tr>
<tr>
<td></td>
<td>(2) DMSO (100μM/ 1 h)</td>
<td></td>
<td>Os&quot;MPP 100μM/ 1 h</td>
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Figure S25: Control studies for the FLICA assay (n=3) in HeLa and MCF 7 cell line. Cells were cultivated at 3 x 10^5 cells/well and were exposed to negative control 1 (non-treated) (B) negative control 2 DMSO (10% v/v) (C) positive control (staurosporine 1μM/ 3 h). Cells were spiked with 30X FAM-FLICA reagent (v/v ratio of 1:30) and incubated for 45 minutes at 37°C. Samples were analyzed in triplicate (3 x 100 μl) in a black bottomed 96-well plate using Tecan Plate fluorescence plate reader set at 488 nm excitation and 520 nm emission.