SUPPORTING INFORMATION


Tharushi P. Wijesinghe,1 Busra Kaya,1 Miguel A. Gonzálvez,2 Jeffrey R. Harmer,3 Mahan Gholam Azad,1 Paul V. Bernhardt,2 Mahendiran Dharmasivam,1* and Des R. Richardson,1,4*

1Centre for Cancer Cell Biology and Drug Discovery, Griffith Institute for Drug Discovery, Griffith University, Nathan, 4111, Australia; 2School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, 4072, Australia; 3Centre for Advanced Imaging, University of Queensland, Brisbane, 4072; 4Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan.

*Corresponding authors: Dr. Des R. Richardson and Dr. Mahendiran Dharmasivam, Centre for Cancer Cell Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan, Brisbane, 4111, Queensland, Australia. Email: d.richardson@griffith.edu.au; m.dharmasivam@griffith.edu.au
**Table of Contents**

*Supplementary Procedures*

Materials.............................................................................................................................................. S4

General methods.................................................................................................................................... S4

Crystallographic studies.......................................................................................................................... S4

Electron paramagnetic resonance spectroscopy..................................................................................... S5

Electrochemistry..................................................................................................................................... S5

Calculation of log \( P \) values (log \( P_{\text{calc}} \))..................................................................................... S5

Cell culture............................................................................................................................................... S6

Cellular proliferation assay..................................................................................................................... S6

Examination of ROS generation using H\(_2\)DCF..................................................................................... S7

Spectral analysis of the oxidation of oxy-Mb to met-Mb......................................................................... S7

Molecular docking studies...................................................................................................................... S8

Western blot analysis............................................................................................................................ S8

Statistics.................................................................................................................................................. S9

*Supplementary Tables*

Table S1: Crystal and refinement data table.......................................................................................... S10, S11

Table S2: Spin Hamiltonian parameters.................................................................................................. S12

Table S3: Physiochemical properties of complexes............................................................................... S13

*Supplementary Figures*

Figure S1: Unit cell packing diagrams................................................................................................. S13

Figure S2: Unit cell packing diagrams.................................................................................................. S14

Figure S3: Experimental and simulated EPR spectra............................................................................. S15
Figure S4: Correlation between the redox potentials and Hammett substituent parameter (σ_p) .................................................................S16
Figure S5: Cyclic voltammetry studies of 2:1 ligand:Cu(II) complexes ..................................................S17
Figure S6: Transmetallation studies ........................................................................................................S18
Figure S7: Cyclic voltammetry studies of the 2:1 ligand:Zn(II) complexes .................................................S19
Figures S8-S9: Relationship between the redox potentials and anti-proliferative activity ......S20, S21
Figures S10-S11: 1H and 13C NMR spectra of PPP4pT in d6-DMSO ..........................................................S22
Figures S12-S13: 1H and 13C NMR spectra of PPP4MepT in d6-DMSO ....................................................S23
Figures S14-S15: 1H and 13C NMR spectra of PPP4MeOpT in d6-DMSO ..................................................S24
Figures S16-S17: 1H and 13C NMR spectra of PPP4FpT in d6-DMSO ......................................................S25
Figures S18-S19: 1H and 13C NMR spectra of PPP4TFpT in d6-DMSO .....................................................S26
Figures S20-S21: 1H and 13C NMR spectra of PPP4ClpT in d7-DMF .......................................................S27
Figures S22-S23: 1H and 13C NMR spectra of PPP4NpT in d6-DMSO ......................................................S28
Figures S24-S25: 1H and 13C NMR spectrum of [Zn(PPP4pT)2] in d6-DMSO ............................................S29
Figures S26-S27: 1H and 13C NMR spectrum of [Zn(PPP4MepT)2] in d6-DMSO . .................................S30
Figures S28-S29: 1H and 13C NMR spectrum of [Zn(PPP4MeOpT)2] in d6-DMSO ..............................S31
Figures S30-S31: 1H and 13C NMR spectrum of [Zn(PPP4FpT)2] in d6-DMSO .................................S32
Figures S32-S33: 1H and 13C NMR spectrum of [Zn(PPP4TFpT)2] in d6-DMSO .................................S33
Figures S34-S35: 1H and 13C NMR spectrum of [Zn(PPP4ClpT)2] in d6-DMSO .................................S34
Figures S36-S37: 1H and 13C NMR spectrum of [Zn(PPP4NpT)2] in d6-DMSO .................................S35

**Supplementary References**

References ........................................................................................................................................S36
Materials and Methods

Chemical Studies

Materials

Benzaldehyde, 2-acetylpyridine, phenyl isothiocyanate, 4-methylphenyl isothiocyanate, 4-methoxyphenyl isothiocyanate, 4-fluorophenyl isothiocyanate, 4-(trifluoromethyl)phenyl isothiocyanate, 4-chlorophenyl isothiocyanate, 4-nitrophenyl isothiocyanate, hydrazine monohydrate, NaOH, Fe(ClO₄)₃·6H₂O, CuCl₂·2H₂O, Cu(ClO₄)₂·6H₂O, Zn(ClO₄)₂·6H₂O, MTT, DFO, Triapine®, COTI-2, horse skeletal muscle myoglobin (95–100% purity), and all solvents were of the highest analytical grade possible and purchased from Sigma-Aldrich (St. Louis, MO).

General methods

¹H and ¹³C NMR spectra were performed using a Bruker Avance III 500 NMR spectrometer (Billerica, MA) using either DMSO-­d₆ or DMF-d₇. The chemical shift (δ) was referenced using the solvent peak as an internal standard. The elemental analysis (C, H, N, S) of the ligand and complexes was performed using a Thermo Scientific Flash 2000 CHNS/O analyzer (Waltham, MA). Liquid chromatography mass spectrometry data were collected using a Thermo Fisher ISQ™ EM Single Quadrupole Mass Spectrometer. Electronic absorption spectra were recorded between 200–800 nm using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan).

Crystallographic studies

X-ray crystallographic studies were performed at 190 K on an Oxford Diffraction Gemini diffractometer with Cu Kα radiation (1.54184 Å). Structures were solved with SHELXS¹ and refined with the SHELXL.² Thermal ellipsoid plots were generated with Mercury (CCDC). All crystallographic data in CIF format have been deposited with the CCDC (deposition numbers 2285443-2285449).
Electron paramagnetic resonance spectroscopy

Continuous-wave (CW) X-band (ca 9.42 GHz) electron paramagnetic resonance (EPR) spectra were recorded on a Bruker Elexsys E540 spectrometer equipped with an ElexSys Super High Sensitivity Probehead and LN2 cooling (Bruker ER4141VT temperature control unit). The magnetic field was calibrated with 2,2-diphenyl-1-picrylhydrazyl ($g = 2.0036$) and measurements were carried out at 105 K using a modulation frequency of 100 kHz modulation, and for Cu complexes a modulation amplitude of 0.15 mT and a microwave power of 1.26 mW (22 dB of 200 mW), and for Fe complexes a modulation amplitude of 0.1 mT and a microwave power of 5.02 mW (16 dB of 200 mW). All measurements were carried out under non-saturating conditions. Spectra were simulated with the program EasySpin.$^3$

Electrochemistry

Cyclic voltammograms of relevant Fe(III) and Cu(II) complexes were obtained using a Gamry Interface 1010B Potentiostat equipped with a non-aqueous Ag reference electrode ($\text{Bu}_4\text{NClO}_4$; 0.1 M in DMF), a glassy carbon working electrode, and a Pt wire auxiliary electrode. All complexes were dissolved at 1 mM in DMF (100%) and used $\text{Bu}_4\text{NClO}_4$ (0.1 M) as the supporting electrolyte. Before measurements, the solutions of complexes were subjected to comprehensive N$_2$ purging.

Calculation of log P values ($\log P_{\text{calc}}$)

Logarithmic $P$ values were calculated using the ChemDraw Professional program, version 12.0.2. The average log $P$ values were calculated by taking the average values estimated using Crippen’s fragmentation method,$^4$ Viswanadhan’s fragmentation method,$^5$ and Broto’s method.$^6$ The following physicochemical properties that are necessary to evaluate Lipinski’s rules, namely topological polar surface area (TPSA) and the number of hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), and rotatable bonds, were calculated using the Cheminformatics tool (http://www.molinspiration.com/).

S5
Biological Studies

Cell culture
All cell-types were purchased from the American Type Culture Collection (ATCC; Manassas, VA). The SH-SY5Y NB cells were grown in Roswell Park Memorial Institute 1640 media (RPMI 1640; Sigma-Aldrich). The BE(2)-C cells were grown in a 1:1 ratio of MEM and Ham’s F12 nutrient mixture (Thermo Fisher Scientific, MA, USA). All media were supplemented with 10% fetal bovine serum, non-essential amino acids (1 mM), sodium pyruvate (1 mM), L-glutamine (2 mM), penicillin (100 U/mL), streptomycin (100 U/mL), and Fungizone (0.5 µg/mL). Cells were grown at 37°C under a 5% CO₂-humidified atmosphere in an incubator using well established methodology.7

Cellular proliferation assay
Cellular proliferation was assessed using the well-established 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) proliferation assay validated by viable cell counts using standard methods.8 The ligands and complexes were dissolved in DMSO to prepare a stock solution of 10 mM and then diluted in culture media. For cell culture experiments, the maximum concentration of DMSO did not exceed 0.5% (v/v), which had no influence of proliferation.8 Cells were seeded in 96-well plates (6000-15,000 cells/well) and were incubated with serial dilutions of the agents for 24- or 72-h/37°C. After these incubations, MTT (5 mg/mL in PBS) was added to the cells and incubated for 2 h/37°C and then the overlying media carefully aspirated from the wells. The cells were subsequently solubilized by adding DMSO (100 µL) and after shaking the plates for 5 min, the absorbance read using a CLARIOstar microplate reader (BMG LabTech, Germany) at a wavelength of 570 nm. MTT color formation was demonstrated to be directly correlated with cell number.8 Analysis of the data was performed using MARS Data Analysis Software (BMG LabTech; version 3.30). The concentration of agents required to inhibit cellular proliferation by 50% (IC₅₀) was then calculated.


**Examination of ROS generation using H<sub>2</sub>DCF**

The production of ROS was evaluated by well characterized procedures examining H<sub>2</sub>DCF oxidation. Solutions of CuCl<sub>2</sub>, tetrathiomolybdate (TM), the ligands (DpC, PPP44mT, and the PPP4pT series analogues) and their 1:1 and 2:1 ligand:Cu(II) complexes were prepared at 5 μM in either HBSS (pH 7.4) or 150 mM acetate buffer (pH 5) to mimic the cytosolic and lysosomal environment, respectively. To this solution, L-cysteine (100 μM) was added as a reducing agent, followed by H<sub>2</sub>DCF (5 μM). Then, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>; 100 μM) was added to initiate hydroxyl radical generation. The HBSS or acetate buffer with L-cysteine, H<sub>2</sub>DCF and H<sub>2</sub>O<sub>2</sub>, but without the addition of ligands or metal complexes, was implemented as a control. Fluorescence was analyzed implementing a CLARIOstar Plus microplate reader (BMG LABTECH, Australia) at λ<sub>excitation</sub> = 485 nm and λ<sub>emission</sub> = 530 nm.

**Spectral analysis of the oxidation of oxy-Mb to met-Mb**

Mb solutions were prepared using standard procedures. Briefly, reduced Mb in PBS (pH 7.2) were prepared by adding 1.2 mL of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> (12 mM) to 38 mL of metMb solution (40 μM). The Mb was then passed through a PBS equilibrated PD-10 column (Pharmacia; prepacked 8.3 mL of Sephadex G-25). OxyMb were then generated by gently bubbling 150 mL of O<sub>2</sub> (from a syringe filled with air) through the reduced Mb solution, which removes residual Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>. The effect of the Fe(III), Cu(II), and Zn(II) complexes of PPP4pT series analogues were compared to [Fe(DFO)], [Fe(Dp44mT)<sub>2</sub>]<sup>+</sup>, [Fe(DpC)<sub>2</sub>]<sup>+</sup>, [Fe(COTI-2)<sub>2</sub>]<sup>+</sup>, and [Fe(Triapine®)<sub>2</sub>]<sup>+</sup>. The [Fe(DFO)], [Fe(COTI-2)<sub>2</sub>]<sup>+</sup>, and [Fe(Triapine®)<sub>2</sub>]<sup>+</sup> were prepared as complexes in-situ. All ligands were dissolved in 1,2-propanediol and diluted to 10 μM in PBS (pH 7.2) and the experiment performed over 0–3 h. Spectra (300–700 nm) were obtained at 20°C using a Shimadzu UV–Vis spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan). Concentrations of met/reduced/oxy Mb were determined at the
following wavelengths, namely metMb at 409 nm; reduced-Mb at 435 nm; and oxy-Mb at 544 and 582 nm, respectively.12

**Molecular docking studies**

Molecular docking studies were carried out using AutoDock 4 and AutoDock Vina.13, 14 The structures of [Fe(Dp44mT)$_2$]$^+$, [Fe(DpC)$_2$]$^+$, [Fe(PPP44mT)$_2$]$^+$, and the Fe(III) complexes of all 7 PPP4pT analogues were converted into PDB format from mol format by OPENBABEL.15 The oxyMb crystal structure was downloaded from the protein data bank (http://www.rcsb.org/pdb); (PDB ID: 1MBO). Visualization of the docked position was done using UCSF Chimera16 and Discovery Studio molecular graphics programs.17

**Western blot analysis**

DFO (Sigma-Aldrich Chemical Co., St. Louis) was dissolved in medium to a final concentration of 100 µM, while Bp2mT, Dp44mT, DpC, PPP44mT, and the PPP4pT analogues were examined at 5 µM and incubated with the cells for 24 h/37º C. Total protein was extracted from cells and western analysis performed using established methods.18 The primary antibodies used were against: human transferrin receptor 1 (TfR1; 1:1,000; Cat. # 13-6890; Invitrogen, MA, USA); ferritin (1:1000; Cat. # 4393; Cell Signaling Technology); NDRG1 (1:2,000; Cat. # ab37897; Abcam Inc., Cambridge, MA); cyclin D1 (1:1,000; Cat. # 2978; Cell Signaling Technology); and N-myc (1:1,000; Cat. # 51705; Cell Signaling Technology). The secondary antibodies were used at 1:5,000 and included anti-goat (Cat. # A5420), anti-rabbit (Cat. # A4416), and anti-mouse (Cat. # A6154) from Sigma-Aldrich. The Sapphire Biomolecular Imaging System (Azure Biosystems, Dublin, CA) was used for protein visualization. Densitometric analysis of blots was performed using ChemiDoc Image Lab software (Bio-Rad, Hercules, CA). All proteins were normalized to the β-actin loading control using an anti-β-actin antibody (1:5,000; Cat. # A5316; Sigma-Aldrich)
Statistics

Experimental data were analyzed using Student’s t-test. Results were expressed as mean ± standard deviation (SD) and mean ± standard error of the mean (SEM) and considered to be statistically significant when $p < 0.05$. 
Table S1 Crystal and refinement data for the PPP4pT series of ligands evaluated.

<table>
<thead>
<tr>
<th></th>
<th>PPP4pT</th>
<th>PPP4MepT</th>
<th>PPP4FpT</th>
<th>PPP4TFpT</th>
<th>PPP4ClpT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C$<em>{21}$H$</em>{18}$N$_4$S</td>
<td>C$<em>{22}$H$</em>{20}$N$_4$S</td>
<td>C$<em>{21}$H$</em>{17}$FN$_4$S</td>
<td>C$<em>{23.5}$H$</em>{20}$F$_3$N$<em>4$O$</em>{0.5}$S</td>
<td>C$<em>{21}$H$</em>{17}$ClN$_4$S</td>
</tr>
<tr>
<td>M.W</td>
<td>358.45</td>
<td>372.48</td>
<td>376.45</td>
<td>455.49</td>
<td>392.9</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
<td>Monoclinic</td>
<td>Monoclinic</td>
<td>Triclinic</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>$P2_1/c$</td>
<td>$P2_1/c$</td>
<td>$P2_1/c$</td>
<td>$P$ 1</td>
<td>$P2_1/c$</td>
</tr>
<tr>
<td>$a$ (Å)</td>
<td>16.308(1)</td>
<td>17.6111(6)</td>
<td>16.4047(8)</td>
<td>7.0417(7)</td>
<td>17.6945(4)</td>
</tr>
<tr>
<td>$b$ (Å)</td>
<td>6.9157(4)</td>
<td>7.0332(2)</td>
<td>6.9924(4)</td>
<td>17.846(1)</td>
<td>7.0224(1)</td>
</tr>
<tr>
<td>$c$ (Å)</td>
<td>18.030(2)</td>
<td>17.9064(7)</td>
<td>18.007(9)</td>
<td>21.259(2)</td>
<td>17.8444(5)</td>
</tr>
<tr>
<td>$\alpha$ (°)</td>
<td></td>
<td></td>
<td></td>
<td>66.832(8)</td>
<td></td>
</tr>
<tr>
<td>$\beta$ (°)</td>
<td>116.64(1)</td>
<td>117.654(5)</td>
<td>115.670(6)</td>
<td>82.773(8)</td>
<td>118.169(3)</td>
</tr>
<tr>
<td>$\gamma$ (°)</td>
<td></td>
<td></td>
<td></td>
<td>88.974(6)</td>
<td></td>
</tr>
<tr>
<td>$V$ (Å$^3$)</td>
<td>1817.6(3)</td>
<td>1964.6(1)</td>
<td>1861.7(2)</td>
<td>2435.1(4)</td>
<td>1954.69(9)</td>
</tr>
<tr>
<td>$T$ (K)</td>
<td>190</td>
<td>190</td>
<td>190</td>
<td>190</td>
<td>190</td>
</tr>
<tr>
<td>$Z$</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$R_1$ (obs. data)</td>
<td>0.0363</td>
<td>0.0362</td>
<td>0.0407</td>
<td>0.0746</td>
<td>0.0332</td>
</tr>
<tr>
<td>$wR_2$ (all data)</td>
<td>0.0885</td>
<td>0.0939</td>
<td>0.1084</td>
<td>0.2162</td>
<td>0.0899</td>
</tr>
<tr>
<td>GOF</td>
<td>1.043</td>
<td>1.054</td>
<td>1.034</td>
<td>0.939</td>
<td>1.051</td>
</tr>
<tr>
<td>CCDC No.</td>
<td>2285443</td>
<td>2285444</td>
<td>2285445</td>
<td>2285446</td>
<td>2285447</td>
</tr>
</tbody>
</table>
Table S1 (continued) Crystal and refinement data for the Fe(III) and Zn(II) complex of PPP4pT.

<table>
<thead>
<tr>
<th></th>
<th><a href="ClO%E2%82%84">Fe(PPP4pT)₂</a>·2EtOH</th>
<th>[Zn(PPP4pT)₂]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C₄₀H₄₆ClFeN₈O₆S₂</td>
<td>C₄₂H₃₄N₈S₂Zn</td>
</tr>
<tr>
<td>M.W</td>
<td>962.33</td>
<td>780.26</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
<td>Orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>P 2₁/c</td>
<td>P b c n</td>
</tr>
<tr>
<td>a (Å)</td>
<td>17.9639(4)</td>
<td>23.8477(15)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>14.2593(3)</td>
<td>9.8152(8)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>19.0206(4)</td>
<td>15.2362(10)</td>
</tr>
<tr>
<td>α (°)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>β (°)</td>
<td>113.671(3)</td>
<td>90</td>
</tr>
<tr>
<td>γ (°)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>V (Å³)</td>
<td>4462.26(19)</td>
<td>3566.3(4)</td>
</tr>
<tr>
<td>T (K)</td>
<td>190(2)</td>
<td>190(2)</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>R₁ (obs. data)</td>
<td>0.0575</td>
<td>0.0556</td>
</tr>
<tr>
<td>wR₂ (all data)</td>
<td>0.1407</td>
<td>0.1199</td>
</tr>
<tr>
<td>GOF</td>
<td>1.034</td>
<td>1.039</td>
</tr>
<tr>
<td>CCDC No.</td>
<td>2285448</td>
<td>2285449</td>
</tr>
</tbody>
</table>
Table S2 Spin Hamiltonian parameters of [Fe(PPP4pT)₂]^+, [Fe(PPP4MepT)₂]^+, [Fe(PPP4MeOpT)₂]^+, [Fe(PPP4FpT)₂]^+, [Cu(PPP4pT)Cl], [Cu(PPP4MepT)Cl], [Cu(PPP4MeOpT)Cl], and [Cu(PPP4FpT)Cl].

<table>
<thead>
<tr>
<th>Complex</th>
<th>( g_1 ) (MHz)</th>
<th>( g_2 ) (MHz)</th>
<th>( g_3 ) (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Fe(PPP4pT)₂]^+</td>
<td>2.1868</td>
<td>2.1360</td>
<td>1.9983</td>
</tr>
<tr>
<td>[Fe(PPP4MepT)₂]^+</td>
<td>2.1873</td>
<td>2.1359</td>
<td>1.9980</td>
</tr>
<tr>
<td>[Fe(PPP4MeOpT)₂]^+</td>
<td>2.1873</td>
<td>2.1373</td>
<td>1.9982</td>
</tr>
<tr>
<td>[Fe(PPP4FpT)₂]^+</td>
<td>2.1883</td>
<td>2.1368</td>
<td>1.9976</td>
</tr>
<tr>
<td>[Cu(PPP4pT)Cl]</td>
<td>2.0416 (47)</td>
<td>2.0416 (39)</td>
<td>2.1745 (521)</td>
</tr>
<tr>
<td>[Cu(PPP4MepT)Cl]</td>
<td>2.0448 (60)</td>
<td>2.0414 (66)</td>
<td>2.1767 (520)</td>
</tr>
<tr>
<td>[Cu(PPP4MeOpT)Cl]</td>
<td>2.0418 (30)</td>
<td>2.0427 (90)</td>
<td>2.1764 (519)</td>
</tr>
<tr>
<td>[Cu(PPP4FpT)Cl]</td>
<td>2.0485 (18)</td>
<td>2.0388 (84)</td>
<td>2.1744 (517)</td>
</tr>
</tbody>
</table>
Table S3: Physiochemical properties of [Fe(PPP4pT)₂]⁺, [Cu(PPP4pT)Cl], [Cu(PPP4pT)₂] and [Zn(PPP4pT)₂] compared to the parent ligand, PPP4pT, calculated using cheminformatics (http://www.molinspiration.com/).³

<table>
<thead>
<tr>
<th>Compounds</th>
<th>M.W.</th>
<th>log P (calc.)</th>
<th>HBA (N + O)</th>
<th>HBD (NH + OH)</th>
<th>Rot. bonds</th>
<th>TPSA (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPP4pT</td>
<td>358.46</td>
<td>4.77</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>48.78</td>
</tr>
<tr>
<td>[Fe(PPP4pT)₂]⁺</td>
<td>770.77</td>
<td>8.87</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>62.56</td>
</tr>
<tr>
<td>[Cu(PPP4pT)Cl]</td>
<td>456.46</td>
<td>4.94</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>31.28</td>
</tr>
<tr>
<td>[Cu(PPP4pT)₂]</td>
<td>778.47</td>
<td>8.98</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>62.56</td>
</tr>
<tr>
<td>[Zn(PPP4pT)₂]</td>
<td>780.31</td>
<td>9.22</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>62.56</td>
</tr>
<tr>
<td>Required</td>
<td>&lt; 500</td>
<td>&lt; 5</td>
<td>&lt; 10</td>
<td>&lt; 5</td>
<td>&lt; 10</td>
<td>&lt; 140</td>
</tr>
</tbody>
</table>
Figure S1. Unit cell packing diagrams (H-atoms omitted) for the ligands, namely: (A) PPP4pT, (B) PPP4MepT, (C) PPP4FpT, (D) PPP4TFpT-\text{C}_3\text{H}_6\text{O}, and (E) PPP4ClpT. Note that the packing is identical in panels (A), (B), (C), and (E).
Figure S2. Unit cell packing diagrams (H-atoms and disordered atoms not shown) for the complexes, namely: (A) \([\text{Fe(PPP4pT)2}](\text{ClO}_4)\cdot 2\text{EtOH}\) and (B) \([\text{Zn(PPP4pT)2}]\).
Figure S3. (A) Experimental and simulated EPR spectra of [Cu(PPP4MeOpT)Cl] with $^{14}$N hyperfine coupling included in the model. Spin Hamiltonian parameters = $g_1 = 2.0418$ ($A_{1,Cu}$ 30 MHz), $g_2 = 2.0427$ ($A_{2,Cu} = 90$ MHz, $A_{N1} = A_{N2} = 39$ MHz) $g_3 = 2.1764$ ($A_{3,Cu} = 520$ MHz). (B) Experimental (solid lines) and simulated (dotted line) X-band EPR spectra (105 K) of [Cu(PPP4pT)Cl], [Cu(PPP4MeT)Cl], [Cu(PPP4MeOpT)Cl], and [Cu(PPP4FpT)Cl] in DMF with $^{14}$N hyperfine coupling excluded from the model. Spin Hamiltonian parameters are listed in Table S2.
Figure S4. Correlation between the redox potential and Hammett substituent parameter ($\sigma_p$)\cite{19} of: (A) Fe(III) and (B) Cu(II) complexes of the PPP4pT analogues.
Figure S5A-F. Cyclic voltammograms of 2:1 Cu(II) complexes of the PPP4pT analogues in the presence or absence of a 6-fold equivalent of the respective ligand. All cyclic voltammetry was performed using 1 mM solutions of the complexes dissolved in DMF/0.1 M Bu$_4$NClO$_4$. The sweep rate was 100 mV s$^{-1}$ and all sweeps were initiated in the direction of the arrow.
Figure S6. (A) Incremental addition of Fe(III) (FeCl₃; 0–2.3 equiv.) to [Cu(PPP₄pT)₂] (50 µM) (red line) in DMSO results in a spectrum that is a mixture of [Cu(PPP₄pT)₂] and [Fe(PPP₄pT)₂]⁺ (blue line). (B) Stoichiometric analysis of the data in (A) derived by plotting the absorption maximum at 523 nm versus [Fe³⁺]/[Cu(PPP₄pT)₂]. (C) Incremental addition of Fe(III) (as FeCl₃; 0–2.3 equiv.) to [Cu(PPP₄pT)Cl] (50 µM) in DMSO results in a spectrum that is consistent with [Cu(PPP₄pT)Cl]. (D) Incremental addition of Fe(III) (FeCl₃; 0–2.3 equiv.) to [Zn(PPP₄pT)₂] (50 µM; red line) in DMSO followed by a 24 h incubation at 20 ºC results in the formation of a spectrum that is consistent with a mixture of [Fe(PPP₄pT)₂]⁺ (blue line) and [Zn(PPP₄pT)₂]. (E) Stoichiometric analysis of the data in (D). Plotting the absorption maximum at 523 nm versus [Fe³⁺]/[Zn(PPP₄pT)₂]. Upon adding Fe(III) to [Zn(PPP₄pT)₂], an increase in absorbance at 523 nm occurred that is characteristic of [Fe(PPP₄pT)₂]⁺.
Figure S7. Cyclic voltammograms of 2:1 Zn(II) complexes of the PPP4pT analogues. All cyclic voltammetry was performed using 1 mM solutions of the complexes dissolved in DMF, 0.1 M Bu4NClO4. The sweep rate was 100 mV s⁻¹ and all sweeps were initiated in the direction of the arrow.
Figure S8. The relationship between the redox potentials of Fe$^{III/II}$ and Cu$^{II/1}$ and the anti-proliferative activity (IC$_{50}$) in both SH-SY5Y (A, B) and BE(2)-C cells (C, D) after 24 h of incubation with Fe(III) and Cu(II) complexes of PPP4pT analogues.
Figure S9. The relationship between the redox potentials of Fe$^{III/II}$ and Cu$^{II/I}$ and the anti-proliferative activity (IC$_{50}$) in both SH-SY5Y (A, B) and BE(2)-C cells (C, D) after 72 h of incubation with Fe(III) and Cu(II) complexes of PPP4pT analogues.
Figure S10. $^1$H NMR spectrum of PPP4pT in $d_6$-DMSO.

Figure S11. $^{13}$C NMR spectrum of PPP4pT in $d_6$-DMSO.
Figure S12. $^1$H NMR spectrum of PPP4MepT in $d_6$-DMSO.

Figure S13. $^{13}$C NMR spectrum of PPP4MepT in $d_6$-DMSO.
Figure S14. $^1$H NMR spectrum of PPP4MeOpT in $d_6$-DMSO.

Figure S15. $^1$H and $^{13}$C NMR spectra of PPP4MeOpT in $d_6$-DMSO.
Figure S16. $^1$H NMR spectrum of PPP4FpT in $d_6$-DMSO.

Figure S17. $^{13}$C NMR spectrum of PPP4FpT in $d_6$-DMSO.
**Figure S18.** $^1$H NMR spectrum of PPP4TFpT in $d_6$-DMSO.

**Figure S19.** $^{13}$C NMR spectrum of PPP4TFpT in $d_6$-DMSO.
Figure S20. $^1$H NMR spectrum of PPP4ClpT in d$_7$-DMF.

Figure S21. $^{13}$C NMR spectrum of PPP4ClpT in d$_7$-DMF.
Figure S22. $^1$H NMR spectrum of PPP4NpT in $d_6$-DMSO.

Figure S23. $^{13}$C NMR spectrum of PPP4NpT in $d_6$-DMSO.
Figure S24. $^1$H NMR spectrum of $[\text{Zn(PPP4pT)}_2]$ in $d_6$-DMSO.

Figure S25. $^{13}$C NMR spectrum of $[\text{Zn(PPP4pT)}_2]$ in $d_6$-DMSO.
Figure S26. $^1$H NMR spectrum of [Zn(PPP4MepT)$_2$] in $d_6$-DMSO.

Figure S27. $^{13}$C NMR spectrum of [Zn(PPP4MepT)$_2$] in $d_6$-DMSO.
Figure S28. $^1$H NMR spectrum of [Zn(PPP4MeOpT)$_2$] in $d_6$-DMSO.

Figure S29. $^{13}$C NMR spectrum of [Zn(PPP4MeOpT)$_2$] in $d_6$-DMSO.
Figure S30. $^1$H NMR spectrum of [Zn(PPP4FpT)$_2$] in $d_6$-DMSO.

Figure S31. $^{13}$C NMR spectrum of [Zn(PPP4FpT)$_2$] in $d_6$-DMSO.
Figure S32. $^1$H NMR spectrum of [Zn(PPP4TFpT)$_2$] in $d_6$-DMSO.

Figure S33. $^{13}$C NMR spectrum of [Zn(PPP4TFpT)$_2$] in $d_6$-DMSO.
Figure S34. $^1$H NMR spectrum of [Zn(PPP4ClpT)$_2$] in $d_6$-DMSO.

Figure S35. $^{13}$C NMR spectrum of [Zn(PPP4ClpT)$_2$] in $d_6$-DMSO.
Figure S36. $^1$H NMR spectrum of $[\text{Zn(PPP4NpT)}_2]$ in $d_6$-DMSO.

Figure S37. $^{13}$C NMR spectrum of $[\text{Zn(PPP4NpT)}_2]$ in $d_6$-DMSO.
References:


