Water soluble, cyclometalated Pt(II)-Ln(III) conjugates towards novel bimodal imaging agents

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Electronic Supplementary Information

Synthesis

Scheme S1. Step-wise synthesis of the heterometallic Pt-Ln targets.

All reactions were performed with the use of vacuum line and Schlenk techniques. Reagents were commercial grade and were used without further purification. 1H NMR spectra were recorded on a Bruker Avance dpx 400 or 250 MHz spectrometer and Bruker
AvanceIII-400 spectrometer, $^{13}$C{$^1$H} NMR spectra on a Joel Eclipse 300 MHz or Bruker Avance dpx 500 MHz spectrometer and $^{195}$Pt NMR spectra on Bruker Avance dpx 500 MHz spectrometer, and were recorded in CDCl$_3$, D$_2$O, CD$_3$CN or d$_6$-DMSO solutions. $^1$H, $^{13}$C{$^1$H} and $^{195}$Pt NMR chemical shifts ($\delta$) were determined relative to internal tetramethylsilane, Si(CH$_3$)$_4$ and are given in ppm. Low-resolution mass spectra were obtained by the staff at Cardiff University. High-resolution mass spectra were carried out by the staff at Cardiff University and the EPSRC National Mass Spectrometry Service at Swansea University, UK. All photophysical data was obtained on a JobinYvon-Horiba Fluorolog-3 spectrometer fitted with a JY TBX picosecond photodetection module and a Hamamatsu R5509-73 detector (cooled to $-80$ °C using a C9940 housing) was used for near-IR luminescence measurements. For the near-IR lifetimes the pulsed laser source was a Continuum Minilite Nd:YAG configured for 355 nm output. Emission spectra were uncorrected and excitation spectra were instrument corrected. The pulsed source was a Nano-LED configured for 295, 372 or 459 nm output operating at 500 kHz or 1 MHz. Luminescence lifetime profiles were obtained using the JobinYvon–Horiba FluoroHub single photon counting module and the data fits yielded the lifetime values using the provided DAS6 deconvolution software. IR spectra were recorded on an ATR equipped Shimadzu IRAffinity-1 spectrophotometer. UV-vis data were recorded as solutions on a Perkin Elmer Lambda20 spectrophotometer.

**DFT studies**
Non-relativistic calculations were performed on the Gaussian 09 program.$^1$ Geometry optimisations were carried out without constraints using the B3LYP functional.$^2$ The Stuttgart-Dresden basis set was used for the Pt centres,$^3$ and was invoked with pseudopotentials for the core electrons, with a 6-31G(d) basis set$^4$ for all remaining atoms. TD-DFT studies were performed using the same functional, but with 6-31+G(d,p) on all non-metal atoms, and also included a simulated MeCN environment using the polarized continuum model (PCM) approach.$^5$ For prediction of absorption spectra, the geometry used to calculate orbital and other properties was used without modification. For the prediction of emission energies, however, the triplet state was allowed to relax to its optimal geometry using unrestricted B3LYP in the gas phase, prior to solvated TD-DFT.

**Confocal microscopy**
Cells tested were MCF-7 human breast adenocarcinoma cell line and *Schizosaccharomyces pombe* (fission yeast).
MCF-7: Cells were detached from culture flask using trypsin-EDTA, re-suspended in DMEM medium and concentrated by centrifugation. Cells were re-suspended 7 ml fresh DMEM medium, aliquoted into 1 ml volumes in Eppendorfs and incubated at 37°C (under 5% CO₂ and 95% air) with 20µl of each compound (final concentration was 100 µg/ml for test compounds) for 30 min. Cells were washed in PBS (pH 7.2) and re-suspended in 100 µl HMEM for imaging.

*S. pombe*: 1 ml overnight cell suspension incubated initially with 20 µl of each compound separately (final concentration was 100 µg/ml). These were incubated at room temperature for 30 min. Cells were then washed 2x in PBS (pH 7.2) to remove medium and background fluorescence signal, and re-suspended in 1 ml PBS for imaging.

Cell preparations were viewed by epifluorescence and transmitted light (Nomarski differential interference contrast optics) using a Leica TCS SP2 AOBS confocal laser microscope (Leica, Germany) using ×63 or ×100 objectives. Imaging parameters were λ_ex 405 nm diode laser and λ_em 580-640 nm which corresponds to the 3MLCT emission energy of the Pt(II) lumophore.

**Synthesis of pq-1:** Following a general procedure but using 2-phenyl-4-quinolinecarboxylic acid (0.521 g, 2.095 mmol) and 2-(2-aminoethoxy)ethanol (0.200 g, 1.905 mmol). Yield = 0.416 g, (65%). ¹H NMR (400 MHz, CDCl₃): δ_H 8.10 (1H, d, ³J_HH = 8.8 Hz, Ar), 8.06 – 8.03 (3H, m, Ar), 7.76 (1H, s, Ar), 7.69 – 7.66 (1H, m, Ar), 7.50 – 7.43 (4H, m, Ar), 7.14 (1H, br. t, ³J_HH = 4.4 Hz, NH), 3.71 – 3.64 (6H, m, CH₂), 3.56 – 3.50 (2H, m, CH₂), 2.77 (1H, br. S, OH) ppm. ¹³C(¹H) NMR (75.6 MHz, CDCl₃): δ_C 167.7 (CO), 156.6 (Ar), 148.2 (Ar), 143.0 (Ar), 138.4 (Ar), 130.9 (Ar), 130.3 (Ar), 129.9 (Ar), 129.6 (Ar), 128.9 (Ar), 127.5 (Ar), 127.3 (Ar), 125.1 (Ar), 123.4 (Ar), 72.4 (CH₂), 69.6 (CH₂), 61.7 (CH₂), 39.9 (CH₂) ppm. MS(ES) found m/z = 359.14 for [M + H]+. UV-vis (CHCl₃): λ_max (ε / dm³ mol⁻¹ cm⁻¹) 261 (30500), 329 (69900) nm. IR (thin film): ν_max 3404, 3273, 1643, 1546 cm⁻¹.

**Synthesis of pq-2:** Following a general procedure but using 2-phenyl-4-quinolinecarboxylic acid chloride (0.200 g, 0.748 mmol) and diethylene glycol monomethyl ether (0.135 g, 1.121 mmol). Yield = 0.252 g, (96%). ¹H NMR (400 MHz, CDCl₃): δ_H 8.75 (1H, dd, ³J_HH = 8.4 Hz, ⁴J_HH = 1.2 Hz, Ar), 8.42 (1H, s, Ar), 8.24 – 8.19 (3H, m, Ar), 7.80 – 7.75 (1H, m, Ar), 7.65 – 7.61 (1H, m, Ar), 7.57 – 7.48 (3H, m, Ar), 4.68 – 4.60 (2H, m, CH₂), 3.98 – 3.93 (2H, m, CH₂), 3.76 – 3.71 (2H, m, CH₂), 3.62 – 3.57 (2H, m, CH₂), 3.39 (3H, s, Me) ppm. ¹³C(¹H) NMR (125.8 MHz, CDCl₃): δ_C 166.5 (CO), 156.8 (Ar), 149.3 (Ar), 138.8 (Ar), 135.9 (Ar), 130.3 (Ar), 129.9 (Ar), 129.7 (Ar), 128.9 (Ar), 128.8 (Ar),
127.6 (Ar), 127.5 (Ar), 125.5 (Ar), 124.0 (Ar), 120.4 (Ar), 72.0 (CH₂), 70.7 (CH₂), 69.1 (CH₂), 64.8 (CH₂), 59.1 (CH₃) ppm. HR-MS: calcd. for [C₂₅H₄ₒN₂O₇]⁺ 538.2984, found m/z = 538.2974; and calcd. 560.2803 for [NaC₂₄H₃₉N₂O₇]⁺, found m/z = 560.2792. UV-vis (CHCl₃): λ_max (ε / dm³ mol⁻¹ cm⁻¹) 265 (29900), 339 (7680) nm. IR (thin film): ν_max 3061, 2876, 1723, 1591, 1549, 1495, 1447, 1343, 1246, 1231, 1194, 1107, 1020, 770, 692 cm⁻¹.

Synthesis of [Pt(pq-2)Cl]₂: Based on a modified literature methodology⁷ using potassium tetrachloroplatinate(II) (0.100 g, 0.241 mmol) and pq-2 (0.064 g, 0.182 mmol). A solution of potassium tetrachloroplatinate(II) (1.0 eq.) in water (2 mL) was added to a stirring solution of pq-2 (1.0 eq.) in 2-ethoxyethanol (6 mL) under dinitrogen and heated to 80 °C for 16 h in a foil-wrapped flask. Brine (10 mL) was added to the cooled solution and the resultant precipitate was collected on a sinter and washed with water (2 x 10 mL) and dried. The isolated solid was used without purification. Yield = 0.062 g, (59%).

Synthesis of [Pt(pq-2)(DMSO)Cl]: Adapted from a literature methodology⁸ using [Pt(pq-2)Cl]₂ (0.062 g, 0.053 mmol). Crude [Pt(pq-2)(µ-Cl)]₂ was dissolved in a minimum volume of DMSO before being precipitated with brine (10 mL), filtered on a sinter and washed with water (2 x 20 mL) and dried. The dark brown compound was purified by dissolving in a minimum volume of dichloromethane and precipitating dark brown impurities by slow addition of diethyl ether. The filtrate was then dried and triturated with diethyl ether until the sticky oil formed a bright orange precipitate. Yield = 0.049 g, (70%). ¹H NMR (400 MHz, CDCl₃): δ_H 9.06 (1H, d, 3_J_HH = 8.8 Hz, Ar), 8.58 (1H, dd, 3_J_HH = 8.8 Hz, 4_J_HH = 1.2 Hz, Ar), 8.37 – 8.34 (1H, m, Ar), 8.32 (1H, s, Ar), 7.78 – 7.73 (1H, m, Ar), 7.71 – 7.69 (1H, m, Ar), 7.65 – 7.61 (1H, m, Ar), 7.29 – 7.26 (2H, m, Ar), 4.70 – 4.67 (2H, m, CH₂), 3.95 – 3.92 (2H, m, CH₂), 3.76 – 3.74 (2H, m, CH₂), 3.67 (6H, s, with satellites 3_J_HPt = 21.2 Hz, 2 x Me from coordinated DMSO), 3.62 – 3.60 (2H, m, CH₂), 3.41 (3H, s, Me) ppm. ¹³C(^1H) NMR (125.8 MHz, CDCl₃): δ_C 166.5 (CO), 165.2 (Ar), 147.6 (Ar), 145.6 (Ar), 143.0 (Ar), 139.7 (Ar), 133.8 (Ar), 130.8 (Ar), 129.7 (Ar), 128.5 (Ar), 128.3 (Ar), 126.0 (Ar), 125.4 (Ar), 125.2 (Ar), 124.9 (Ar), 117.7 (Ar), 72.0 (CH₂), 70.7 (CH₂), 68.9 (CH₂), 65.3 (CH₂), 59.1 (CH₃), 46.2 ((CH₃)₂SO) ppm. ¹⁹⁵Pt (107.5 MHz, CDCl₃): δ_Pt -3665 ppm. MS(ES) found m/z = 586.08 for [M – SO(CH₃)₂ – Cl + CH₃CN]⁺ and 627.10 for [M – SO(CH₃)₂ – Cl + 2CH₃CN]⁺. HR-MS: calcd. 626.1550 for [C₂₅H₂₆N₃O₄]₁⁹⁴Pt⁺, found m/z = 626.1567. UV-vis (CHCl₃): λ_max (ε / dm³ mol⁻¹ cm⁻¹) 253, (12500), 291 (8790), 362 (4130), 424 (1410) nm. IR (thin film): ν_max 2876, 1728, 1597, 1580, 1545, 1454, 1379, 1294, 1275, 1250, 1198, 1138, 1022, 766, 731 cm⁻¹.
Synthesis of $\text{P}2$: 4-pyridinecarboxaldehyde (0.083 g, 0.779 mmol) was added to a stirring solution of $\text{P}1$ (0.476 g, 0.779 mmol) in 1,2-dichloroethane (8 mL) and was stirred under dinitrogen. After 16 h, Na(OAc)$_2$BH (0.500 g, excess) was added and the reaction was left stirring for 16 h. NaHCO$_3$ (sat. soln., 10 mL) was added and the product was extracted with dichloromethane (20 mL). The organic phase was washed with water (2 x 20 mL) and brine (20 mL) before being dried over MgSO$_4$, filtered and dried in vacuo. Yield = 0.505 g (92%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 8.99 (1H, br, NH), 8.47 (2H, d, $^3$J$_{HH}$ = 6.0 Hz, Ar), 7.46 (2H, d, $^3$J$_{HH}$ = 6.0 Hz, Ar), 4.69 (2H, br. s, CH$_2$), 3.92 (2H, br. s, CH$_2$), 3.67 – 1.87 (28H, br. m, CH$_2$), 1.39 – 1.36 (27H, m, tert-Bu) ppm. $^{13}$C($^1$H) NMR (125.8 MHz, CDCl$_3$): $\delta$C 171.3 (CO), 171.1 (CO), 148.7 (Ar), 145.6 (Ar), 123.1 (Ar), 81.1 (C(CH$_3$)), 81.0 (CO(CH$_3$)), 55.1 (CH$_2$), 54.8 (CH$_2$), 54.7 (CH$_2$), 50.3 (CH$_2$), 46.8 (CH$_2$), 37.1 (CH$_2$), 27.2 (C(CH$_3$)), 27.0 (C(CH$_3$)) ppm. MS(ES) found m/z = 728.45 for [M + Na]$^+$. Trifluoroacetic acid (3 mL, excess) was then added to a stirring solution of the tert-butyl protected precursor (0.505 g, 0.717 mmol) in dichloromethane (3 mL). The reaction was stirred for 48 h before the solvent was removed in vacuo. The residue was dissolved in a minimal volume of acetonitrile and added, dropwise, to a vigorously stirred vessel of ice-cold diethyl ether (20 mL). The resultant precipitate was filtered under dinitrogen to yield $\text{P}2$ as a white solid. Yield = 0.350 g, (91%). $^1$H NMR (500 MHz, D$_2$O): $\delta$H 8.87 (2H, d, $^3$J$_{HH}$ = 7.0 Hz, Ar), 8.21 (2H, br. m, Ar), 4.70 (2H, s, CH$_2$), 3.97 – 2.88 (28H, br. m, CH$_2$) ppm. $^{13}$C($^1$H) NMR (125.8 MHz, D$_2$O): $\delta$C 162.2 (CO), 161.9 (CO), 150.8 (Ar), 141.1 (Ar), 126.7 (Ar), 65.1 (CH$_2$), 54.2 (CH$_2$), 50.2 (br, CH$_2$), 48.4 (br CH$_2$), 47.3 (br CH$_2$), 34.6 (CH$_2$) ppm. HR-MS: calcd. 501.2900 for [C$_{35}$H$_{37}$N$_2$O]$^+$, found m/z = 501.2889.

Synthesis of [Gd-py]: Gadolinium(III) triflate (0.112 g, 0.186 mmol) and $\text{P}2$ (0.100 g, 0.186 mmol) were dissolved in methanol (10 mL) and heated to 50 °C for 24 h under dinitrogen. The solution was cooled and diethyl ether (20 mL) was added. The precipitate was filtered under dinitrogen to yield an off-white solid. Yield = 0.112 g, (87%). HR-MS: calcd. 687.1947 for [C$_{24}$H$_{37}$Gd$_7$O$_{77}$]$,^+$, found m/z = 687.1941. UV-vis (CHCl$_3$): $\lambda_{max}$ (ε / dm$^3$ mol$^{-1}$ cm$^{-1}$) 201 (7240), 256 (1840) nm. IR (thin film): $\nu_{max}$ 3397, 1582, 1402, 1244, 1225, 1165, 1082, 1026, 635 cm$^{-1}$.

Synthesis of [Yb-py]: Ytterbium(III) triflate (0.115 g, 0.186 mmol) and $\text{P}2$ (0.100 g, 0.186 mmol) were dissolved in methanol (10 mL) and heated to 50 °C for 24 h under dinitrogen. The solution was cooled and diethyl ether (20 mL) was added. The precipitate was filtered
under dinitrogen to yield an off-white solid. Yield = 0.121 g, (92%). $^{1}$H NMR (400 MHz, D$_2$O): $\delta_{H}$ +133.44, +125.59, +120.46, +119.57, +33.43, +26.95, +23.45, +22.16, +21.00, +18.62, +16.42, +13.35, +8.97, +3.18, -1.77, -2.38, -24.53, -27.53, -31.67, -42.19, -45.47, -57.61, -58.91, -74.55, -76.88. HR-MS: calcd. 705.2102 for [C$_{24}$H$_{37}$N$_{7}$O$_{7}$Yb]$^{+}$, found m/z = 705.2110. UV-vis (CHCl$_{3}$): $\lambda_{\text{max}}$ ($\epsilon$ / dm$^3$ mol$^{-1}$ cm$^{-1}$) 201 (7800), 256 (1430) nm. IR (thin film): $\nu_{\text{max}}$ 3408, 1601, 1410, 1258, 1167, 1083, 1028, 800, 640 cm$^{-1}$.

**Synthesis of [Pt-Gd]:** [Pt(pq-2)(DMSO)Cl] (0.052 g, 0.079 mmol) and Gd-py (0.050 g, 0.072 mmol) were dissolved in acetone and heated to 40 °C for 48 h under dinitrogen. The solvent was removed in vacuo and the solid dissolved in minimal acetonitrile and precipitated with diethyl ether. The solid was filtered and washed with dichloromethane to yield a bright orange solid. Yield = 0.062 g, (68%). HR-MS: calcd. 1233.2922 for [C$_{45}$H$_{56}$N$_{8}$O$_{11}$Gd$^{154}$Pt]$^{+}$, found m/z = 1233.2953. UV-vis (CHCl$_{3}$): $\lambda_{\text{max}}$ ($\epsilon$ / dm$^3$ mol$^{-1}$ cm$^{-1}$) 203 (25000), 246 (13300), 285 (9320), 364 (4450) nm. IR (thin film): $\nu_{\text{max}}$ 3433, 1732, 1591, 1456, 1404, 1246, 1167, 1084, 1028, 941, 903, 841, 762, 723, 637 cm$^{-1}$.

**Synthesis of [Pt-Yb]:** [Pt(pq-2)(DMSO)Cl] (0.051 g, 0.078 mmol) and Yb-py (0.050 g, 0.071 mmol) were dissolved in acetone and heated to 40 °C for 48 h under dinitrogen. The solvent was removed in vacuo and the solid dissolved in minimal acetonitrile and precipitated with diethyl ether. The solid was filtered and washed with dichloromethane to yield a bright orange solid. Yield = 0.072 g, (79%). HR-MS: calcd. 1249.3059 for [C$_{45}$H$_{56}$N$_{8}$O$_{11}$Yb$^{171}$Pt]$^{+}$, found m/z = 1249.3076. UV-vis (CHCl$_{3}$): $\lambda_{\text{max}}$ ($\epsilon$ / dm$^3$ mol$^{-1}$ cm$^{-1}$) 203 (26300), 248 (14100), 284 (9920), 364 (4640) nm. IR (thin film): $\nu_{\text{max}}$ 3408, 1730, 1595, 1458, 1400, 1389, 1248, 1165, 1084, 1028, 943, 909, 843, 762, 727, 638 cm$^{-1}$.
Figure S1. $^1$H NMR spectra of Yb-py recorded in D$_2$O (400 MHz).
Figure S2. Comparison of the observed (bottom) and theoretical (top) isotopic distribution for the [M-Cl]$^+$ ion of Pt-Gd.

Figure S3. Calculated orbital representations for [PtCl(epqc)(DMSO)] (top, l-to-r: HOMO, LUMO) and [PtCl(epqc)(py)] (bottom, l-to-r: HOMO-1, HOMO, LUMO).
X-ray diffraction

A suitable crystal was selected and measured following a standard method on a Rigaku AFC12 goniometer equipped with an enhanced sensitivity (HG) Saturn724+ detector mounted at the window of a FR-E+ SuperBright molybdenum rotating anode generator with HF Varimax optics (100µm focus) at 100K. Cell determination, data collection, reduction, cell refinement and absorption correction carried out using CrystalClear-SM Expert 3.1b27. Structures solved using SUPERFLIP and refined using SHELXL-2013.

Crystal data for [PtCl(pq-2)(DMSO)] : C_{23}H_{26}ClNO_5PtS, M = 659.05, dark orange blade, a = 9.5830(7), b = 9.8654(7), c = 14.2517(10) Å, α = 104.643(5), β = 95.718(3), γ = 114.866(4), V = 1149.74(15) Å³, Triclinic, space group P-1, Z = 2, μ = 6.345 mm⁻¹, reflections collected = 15635, independent reflections = 5262, R_{int} = 0.0403, R1 = 0.0237, wR2 = 0.0.619 [F² > 2σ(F²)] , R1 = 0.0245, wR2 = 0.0623 (all data).

Table S1. Selected bond lengths (Å) and angles (deg).

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Table S2. Calculated d-orbital contributions to the frontier orbitals.

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<td>[PtCl(epqc)(DMSO)]</td>
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**Figure S4.** $^1$H NMRD for 1mM Gd-py and Pt-Gd recorded in water at 25 °C and 37 °C (pH 6.5-6.7).

**Figure S5.** Confocal fluorescence microscopy images of *S. pombe* using Pt-Gd ($\lambda_{ex}$ 405 nm and $\lambda_{em}$ 580-640 nm) showing fluorescence, transmitted and superimposed images.
Figure S6. Confocal fluorescence microscopy images of *S. pombe* using Pt-Gd (λ<sub>ex</sub> 405 nm and λ<sub>em</sub> 580-640 nm) showing fluorescence, transmitted and superimposed images.

References


10) CrystalClear-SM Expert 3.1 b27, 2013, Rigaku
