Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

An Aminophosphonate Ester Ligand-Containing Platinum(II) Complex Induces Potent Immunogenic Cell Death *In Vitro* and Elicits Effective Anti-Tumor Immune Responses *In Vivo*

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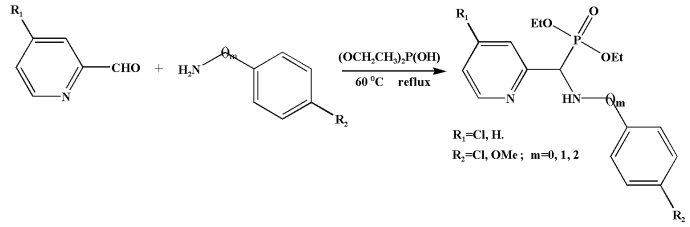
Materials and methods.

All chemicals, unless otherwise noted, were purchased from Sigma and Alfa Aesar. All materials were used as received without further purification unless noted specifically. Tris-HCl-NaCl (TBS) buffer solution (5 mM Tris, 50 mM NaCl, pH adjusted to 7.35 by titration with hydrochloric acid using a Sartorius PB-10 pH meter, Tris buffer was prepared using double distilled water. The TBE buffer (1×) were commercially available. ^{1}H NMR spectra were recorded on a Bruker AV-500 NMR spectrometer with $CDCl_3$ as solvent. Elemental analyses (C, H, and N) were carried out on a PerkinElmer Series II CHNS/O 2400 elemental analyzer. ESI-MS spectra for the characterization of complexes were performed on Thermo Fisher Scientific Exactive LC-MS Spectrometer. Details for the synthesis of ligands are shown in the Supporting Information.

1. Preparation of the ligands

General procedure for the synthesis of aminophosphonate esters ligands.

Equimolar amounts (0.2 mol) of pyridinealdehyde diethylacidphosphite and phenethylamine were refluxed for 1 h at 60°C, after the reacting mixture were cooled to room temperature, the oily residue was purified on a silica gel column (petroleum ether: ethyl acetate=1:1). Yellow oil-like product was obtained.



Scheme S1. synthesis of aminophosphonate ester ligands.

Data for $L^{Pt\text{-}1}$: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 5.3 Hz, 1H), 7.48 (s, 1H), 7.20 (d, *J* = 5.3 Hz, 1H), 7.07 (d, *J* = 8.8 Hz, 2H), 6.58 (d, *J* = 8.8 Hz, 2H), 4.89 (d, *J* = 22.5 Hz, 1H), 4.20 – 4.10 (m, 4H), 4.09 – 4.00 (m, 1H), 3.93 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H). ESI-MS: m/z 388.16 $[L^{Pt\text{-}1} + H]^+$.

Data for L^1: ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.60 (d, J = 3.7 Hz, 1H), 7.68 (m, 1H), 7.50 (d, J = 7.9 Hz, 1H), 7.24 (m, 1H), 7.04 (d, J = 8.6 Hz, 2H), 6.60 (d, J = 8.6 Hz, 2H), 4.97 (d, J = 22.3 Hz, 1H), 4.19 – 4.08 (m, 2H), 4.05 – 3.99 (m, 1H), 3.93 – 3.83 (m, 1H), 1.25 (t, J = 7.1 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H).^{13}CNMR (400 MHz, $CDCl_3$) δ 155.14 (s), 148.65 (s), 145.01 (s), 137.45 (s), 129.02 (s), 123.17 (s), 115.15 (s), 63.70 (s), 63.39 (s), 57.54 (s), 16.39 (s), 16.21 (s). ESI-MS: m/z 355.12 $[L^1 + H]^+$.

Data for L^2: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, J = 4.8 Hz, 1H), 7.64 – 7.57 (m, 1H), 7.49 – 7.40 (m, 1H), 7.19 – 7.10 (m, 1H), 6.68 (dd, J = 7.0, 5.1 Hz, 2H), 6.65 (dd, J = 7.0, 5.1 Hz, 2H), 4.90 (d, J = 22.4 Hz, 1H), 4.15 – 4.09 (m, 2H), 4.04 – 3.97 (m, 2H), 3.90 (m, 1H), 3.66 (s, 3H), 1.26 (t, J = 7.1 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H).^{13}C NMR (400 MHz, $CDCl_3$) δ 155.98 (s), 152.78 (s), 149.11 (s), 140.62 (s), 136.70 (s), 122.89 (s), 122.75 (s), 115.46 (s), 114.72 (s), 63.48 (s), 63.13 (s), 58.33 (s), 55.63 (s), 16.41 (s), 16.23 (s). ESI-MS: m/z 351.18 $[L^2+H]^+$.

Data for L^3:^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 5.3 Hz, 1H), 7.73 (s, 1H), 7.43 (d, *J* = 5.3 Hz, 1H), 6.73 (d, *J* = 9.0 Hz, 2H), 6.66 (d, *J* = 9.0 Hz, 2H), 5.17 – 4.91 (m, 1H), 4.09 (m, 2H), 4.03 – 3.77 (m, 3H), 3.63 (s, 3H), 1.25 (1.21, *J* = 7.0 Hz, 6H), 1.09 (t, *J* = 7.0 Hz, 3H). ESI-MS: m/z 384.05 $[L^3+H]^+$.

Data for L^4: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 5.0 Hz, 1H), 7.42 (s, 1H), 7.26 – 7.17 (m, 5H), 4.17 – 4.12 (m, 2H), 4.12 – 3.92 (m, 4H), 3.76 (d, *J* = 13.4 Hz, 1H), 3.54 (d, *J* = 13.4 Hz, 1H), 1.27 (t, *J* = 6.6 Hz, 3H), 1.18 (m, *J* = 6.6 Hz, 3H). ESI-MS: m/z 402.19 $[L^4 + H]^+$.

Data for L^5: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.59 (s, 1H), 7.34 (s, 1H), 7.14 (d, J = 2.7 Hz, 5H), 4.14 – 3.91 (m, 4H), 3.70 (s, 1H), 3.48 (s, 1H), 3.06 (s, 2H), 1.24 (m, 3H), 1.08 (m, 3H).^{13}C NMR (400 MHz, $CDCl_3$) δ 155.86 (s), 149.28 (s), 137.95 (s), 136.40 (s), 132.67 (s), 129.66 (s), 128.36 (s), 123.78 (s), 122.68 (s), 63.13 (s), 62.73 (s), 62.05 (s), 51.23 (s), 16.35 (s), 16.21 (s). ESI-MS: m/z 369.14 $[L^5 + H]^+$.

Data for L^6: ^{1}H NMR(400MHz, $CDCl_3$) δ 8.13 (d, J = 8.5 Hz, 1H), 7.83 – 7.79 (m, 1H), 7.59 (d, J = 8.3 Hz, 1H), 7.52 (dd, J = 7.8, 6.8 Hz, 1H), 7.19 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 4.41 (d, *J* = 21.7 Hz, 1H), 4.09 – 4.01 (m, 4H), 3.92 (m, 2H), 3.75 (s, 3H), 3.60 (d, J = 13.0 Hz, 1H), 1.26 (t, J = 7.0 Hz, 3H), 1.17 (t, J = 7.0 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 158.76 (s), 156.64 (s), 147.72 (s), 136.32 (s), 129.64 (s), 129.51 (s), 129.25 (s), 126.50 (s), 121.35 (d, J = 2.8 Hz), 113.74 (s), 63.33 (d, J = 6.8 Hz), 62.87 (d, J = 7.0 Hz), 61.21 (s), 55.25 (s), 51.76 (s), 16.42 (s), 16.30 (s). ESI-MS: m/z 365.18 $[L^6+H]^+$.

Data for L^7: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 5.2 Hz, 1H), 7.43 (s, 1H), 7.20 (d, *J* = 5.2 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 2H), 6.81 (d, *J* = 8.3 Hz, 2H), 4.12 (m, 1H), 4.12 – 3.90 (m, 4H), 3.80 – 3.70 (m, 5H), 3.51 (d, *J* = 13.0 Hz, 1H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H). ESI-MS: m/z 398.25 $[L^7+H]^+$.

Data for L^8: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 5.3 Hz, 1H), 7.36 (s, 1H), 7.21 (d, *J* = 5.3 Hz,1H), 7.19 (d, *J* = 8.2 Hz, 2H), 7.06 (d, *J* = 8.2 Hz, 2H), 4.16 (d, *J* = 21.5 Hz, 1H), 4.12 – 3.91 (m, 4H), 2.87 – 2.48 (m, 5H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.17 (t, *J* = 7.1 Hz, 3H). ESI-MS: m/z 412.16 $[L^8 + H]^+$.

Data for L^9: ^{1}H NMR (600 MHz,$CDCl_3$) δ 8.42 (d, J = 4.8 Hz, 1H), 7.58 – 7.49 (m, 1H), 7.27(m,1H), 7.06 (m, 3H), 6.95 (m, 2H), 4.01 – 3.99 (m, 2H), 3.97 – 3.92 (m, 2H), 3.87 (m, 1H), 3.07(dd, J = 6.7, 3.4 Hz, 2H), 2.64 (dd, J = 6.7, 3.4 Hz, 2H), 1.10 (t, J = 7.1 Hz, 3H), 1.05 – 0.99 (t, J = 7.1 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 155.87 (s), 149.06 (s), 138.19 (s), 136.42 (s), 131.66 (s), 130.01 (s), 128.24 (s), 123.43 (s), 122.66 (s), 63.10 (s), 62.75 (s), 61.69 (s), 49.60 (s), 35.35 (s), 16.24 (s), 16.14 (s). ESI-MS: m/z 383.15 $[L^9 + H]^+$.

Data for L^{10}: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 1.3Hz, 1H), 7.63 (m, 1H), 7.37 (dd, *J*=7.9, 1.1 Hz, 1H), 7.17 (dd, *J* = 9.5, 8.0, 1H), 7.06 – 7.04 (m, 1H), 7.04 – 7.02 (m, 2H), 6.79 – 6.77 (m, 2H), 4.21 (d, *J* = 21.3 Hz, 1H), 4.11 – 3.95 (m, 4H), 3.75 (s, 3H), 2.75 – 2.67 (m, 4H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.15 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 157.98

(s), 156.33 (s), 149.18 (s), 136.41 (s), 131.86 (s), 129.61 (s), 123.42 (s), 122.62 (s), 113.92 (s), 63.14 (s), 62.79 (s), 62.06 (s), 55.24 (s), 50.20 (s), 35.37 (s), 16.38 (s), 16.27 (s). ESI-MS: m/z 379.17 $[L^{10}+H]^+$.

Data for L^{11}: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 5.3 Hz, 1H), 7.39 (d, *J* = 5.3 Hz, 1H), 7.20 (dt, *J* = 15.9, 8.3 Hz, 1H), 7.08 – 7.00 (m, 2H), 6.87 – 6.65 (m, 2H), 4.21 (d, *J* = 21.4 Hz, 1H), 4.13 – 3.94 (m, 4H), 3.82 (s, 3H), 2.80 – 2.59 (m, 4H), 1.28 (m, *J* = 7.0 Hz, 3H), 1.19 (t, *J* = 7.0 Hz, 3H). ESI-MS: m/z 412.13 $[L^{11}+H]^+$.

2. Preparation of the platinum complexes

General procedure for the synthesis of the platinum complexes: to a solution of *cis*-$Pt(DMSO)_2Cl_2$ (84 mg, 0.2 mmol) was added with aminophosphonate ester ligand (0.2 mmol) dissolved in 30 mL mixtures of anhydrous dichloromethane and ethanol (1:1). The mixture was stirred in the dark at room temperature for 1 day. The resulting yellow solution was filtered, and crystals were obtained by slow evaporation of filtrate solution. ESI-MS was performed in the complex solutions in DMSO.

Data for Pt-1: ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.53 (d, *J* = 5.4 Hz, 1H), 7.69 (d, *J* = 5.4 Hz, 1H), 7.49 (s, 1H), 7.06 (d, *J* = 7.3 Hz, 2H), 6.79 (d, *J* = 7.3 Hz, 2H), 5.19 (d, *J* = 24.1 Hz, 1H), 4.38 – 4.20 (m, 1H), 4.14 – 4.00 (m, 4H), 1.17 (t, *J* = 7.1 Hz, 3H), 1.09 (t, *J* = 7.1 Hz, 3H). ESI-MS: m/z 697.42 $[M – Cl + DMSO]^+$. Elemental analysis calculated: C, 29.28; H, 2.69; N, 4.31; Found: C, 29.38; H, 2.77; N, 4.28.

Data for 1: ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.55 (d, *J* = 4.4 Hz, 1H), 7.81 (dd, *J* =14.5, 7.6 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.30 (dd, *J* = 22.6, 16.5 Hz, 1H), 7.06 (d, *J* = 8.8 Hz, 1H), 6.80 (d, *J* = 8.8 Hz, 1H), 5.11 (d, *J* = 23.6 Hz, 1H), 4.12 (m, 4H), 3.81(m, 1H), 1.19 (t, *J* = 7.0 Hz, 3H), 1.07 (t, *J* = 7.0 Hz, 3H).ESI-MS: m/z 660.54 $[M – Cl + DMSO]^+$. Elemental analysis calculated: C, 31.08; H, 3.14; N, 4.57; Found: C, 31.05; H, 3.09; N, 4.52.

Data for 2: ^{1}H NMR (400 MHz, d_6- DMSO) δ 8.59 (d, *J* = 4.6 Hz, 1H), 7.84 (dd, *J* = 15.9, 8.0 Hz, 2H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.35 (dd, *J* = 14.9, 8.7 Hz, 1H), 6.76 (d, *J* = 6.0 Hz, 2H), 6.71 (d, *J* = 6.0 Hz, 2H), 5.08 (d, *J* = 23.9 Hz, 1H), 4.36 (m, 2H), 4.24 (m, 1H), 4.13 (m, 2H), 3.64 (s, 3H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.11 (t, *J* = 7.0 Hz, 3H). ESI-MS: m/z 657.08 $[M – Cl + DMSO]^+$. Elemental analysis calculated: C, 33.21; H, 3.68; N, 4.54; Found: C, 33.18; H, 3.60; N, 4.57.

Data for 3: ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.70 (d, *J* = 5.3 Hz, 1H), 8.06 (d, *J* = 5.2 Hz, 1H), 7.80 (dd, *J* = 5.5, 3.5 Hz, 1H), 7.29 (d, *J* = 6.2 Hz, 2H), 7.05 (d, *J* =6.2 Hz, 2H), 4.26 (m, 2H), 4.08 (m, 4H), 3.77 (s, 3H), 1.22 – 1.17 (m, 6H). ESI-MS: m/z 690.57 $[M– Cl+ DMSO]^+$. Elemental analysis calculated: C, 31.51; H, 3.31; N, 4.28; Found: C, 31.42; H, 3.26; N, 4.31.

Data for 4: ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.86 (d, *J* = 6.4 Hz, 1H), 7.87 – 7.81 (m, 2H), 7.75 – 7.72 (m, 1H), 7.69 – 7.62 (m, 1H), 7.37 – 7.27 (m, 2H), 4.99 (d, *J* = 20.6 Hz, 1H), 4.59 – 4.47 (m, 1H), 4.40 – 4.28 (m, 2H), 4.29 – 4.12 (m, 4H), 1.31 (t, *J* = 7.1 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H). ESI-MS: m/z 708.34 $[M– Cl+ DMSO]^+$. Elemental analysis calculated: C, 31.08; H, 3.14; N, 4.57; Found: C, 30.56; H, 3.02; N, 4.19.

Data for 5: ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.84 (d, *J* = 5.7 Hz, 1H), 8.04 (d, *J* = 7.7 Hz, 1H), 7.65 (m, 1H), 7.63 (m, 1H), 7.47 (d, *J* = 7.9 Hz, 2H), 6.86 (d, *J* = 14.6 Hz, 2H), 4.87 (d, *J* = 20.4 Hz, 1H), 4.21 (m, 2H), 4.10 – 4.06 (m, 2H), 3.73 (m, 2H), 3.68 – 3.64 (m, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.16 (t, *J* = 7.0 Hz, 3H). ESI-MS: m/z 674.52 $[M - Cl + DMSO]^+$. Elemental analysis calculated: C, 32.34; H, 3.41; N, 4.38; Found: C, 32.22; H, 3.34; N, 4.42.

Data for 6: ^{1}H NMR (400 MHz, d_6-DMSO) ^{1}H NMR (400 MHz, d_6- DMSO) δ 8.89 (d, *J* = 5.7 Hz, 1H), 8.10 (dd, *J* = 7.7, 8.4 Hz, 1H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.45 (dd, *J* = 13.5, 7.0 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 2H), 4.92 (d, *J* = 20.4 Hz, 1H), 4.61 – 4.48 (m, 1H), 4.31 – 4.22 (m, 4H), 3.78 (s, 2H), 3.72 (s, 3H), 1.28 (t, *J* = 7.0 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 3H). ESI-MS: m/z 670.4 $[M- Cl+ DMSO]^+$. Elemental analysis calculated: C, 34.18; H, 3.92; N, 4.54; Found: C, 34.35; H, 3.84; N, 4.45.

Data for 7: ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.80 (d, *J* = 6.4 Hz, 1H), 7.67 – 7.62 (m,3H), 6.89 (d, *J* = 8.0 Hz, 2H), 6.77 (d, *J* = 8.0 Hz, 2H), 4.81 (d, *J* = 20.5 Hz, 1H), 4.54 – 4.41 (m, 1H), 4.14 (m, 4H), 3.70 (s, 3H), 3.33 (s, 2H), 1.27 (t, *J* = 7.0 Hz, 3H), 1.18 (t, *J* = 7.0 Hz, 3H). ESI-MS: m/z 704.48 $[M- Cl+ DMSO]^+$. Elemental analysis calculated: C, 32.68; H, 3.61; N, 4.18; Found: C, 32.57; H, 3.49; N, 4.22.

Data for 8: ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.08 (d, *J* = 6.3 Hz, 1H), 7.88 – 7.76 (m, 2H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 5.15 (d, *J* = 19.8 Hz, 1H), 4.69 – 4.54 (m, 1H), 4.47 – 4.15 (m, 4H), 3.23 – 3.01 (m, 4H), 1.35 (t, *J* = 7.0 Hz, 1H), 1.29 (t, *J* = 7.0 Hz, 1H). ESI-MS: m/z 722.48 $[M - Cl + DMSO]^+$. Elemental analysis calculated: C, 31.58; H, 3.24; N, 4.24; Found: C, 31.69; H, 3.25; N, 4.11.

Data for 9: ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.09 (d, J = 17.4, 1H), 8.23 (dd, J = 7.7, 21.2 Hz, 1H), 7.68 (m, 1H), 7.60 (d, J = 6.8 Hz, 1H), 7.15 (d, J = 8.3 Hz, 2H), 6.84 (d, J = 8.3 Hz,, 2H), 5.18 (d, J = 19.7 Hz, 1H), 4.60 – 4.52 (m, 1H), 4.33 – 4.13 (m, 4H), 3.07 – 2.98 (m, 4H), 1.29 (t, J = 7.0 Hz, 3H), 1.20 (t, J = 7.0 Hz, 3H). ESI-MS: m/z 688.10$[M - Cl + DMSO]^+$. Elemental analysis calculated: C, 33.44; H, 3.61; N, 4.27; Found: C, 33.37; H, 3.58; N, 4.32.

Data for 10: ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.16 (d, *J* = 7.9 Hz, 1H), 8.29 (t, *J* = 7.7 Hz, 1H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.65 (t, *J* = 6.8 Hz, 1H), 7.24 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 8.7 Hz ,2H), 5.94 (m, 1H), 5.23 (d, *J* = 19.7 Hz, 1H), 4.65 – 4.54 (m, 1H), 4.43 – 4.12 (m, 4H), 3.76 (s, 3H), 3.17 – 2.95 (m, 4H), 1.33 (t, *J* = 7.0 Hz, 3H), 1.25 (t, *J* = 7.0 Hz, 3H). ESI-MS: m/z 684.18 $[M- Cl+ DMSO]^+$. Elemental analysis calculated: C, 33.62; H, 3.99; N, 4.27; Found: C, 33.47; H, 4.07; N, 4.35.

Data for 11: ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.03 (m, 2H), 7.78 (d, *J* = 6.3 Hz,2H), 7.13 (d, *J* = 6.3 Hz, 2H), 6.86 – 6.83 (m, 2H), 5.10 (d, *J* = 19.8 Hz, 1H), 4.62 – 4.47 (m, 1H), 4.42 – 4.30 (m, 1H), 4.29 – 4.08 (m, 4H), 3.71 (s, 3H), 2.99 (m, 4H), 1.29 (t, *J* = 7.0 Hz, 1H), 1.23 (t, *J* = 7.0 Hz, 1H). ESI-MS: m/z 718.08 $[M - Cl + DMSO]^+$. Elemental analysis calculated: C, 33.68; H, 3.74; N, 4.19; Found: C, 33.67; H, 3.72; N, 4.13.

3. X-ray Crystallographic Analysis

X-ray diffraction data were collected on an Agilent Super Nova diffractometer with graphite-monochromatic Mo-Kα radiation (λ = 0.71073 Å) at room temperature. The crystal structures were solved by the direct method using the program SHELXS-97 and refined by the full-matrix least-squares method on F^2 for all non-hydrogen atoms using SHELXL-97 with anisotropic thermal parameters. Hydrogen atoms were located in calculated positions and refined isotropically. Hydrogen atoms of water molecules were fixed in a difference Fourier map and refined isotropically.

4. Cell lines, Culture Conditions, and Cytotoxicity Assay.

The cancer cell lines were obtained from the Shanghai Cell Bank in the Chinese Academy of Sciences. Tumor cell lines were grown in the RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 U/mL streptomycinin at 37 °C, in a highly humidified atmosphere of 95% air/5% CO_2. The cytotoxicity of the title compounds against cancer cell lines were examined by the microculture tetrozolium (MTT) assay. The growth inhibitory rate of treated cells was calculated using the data from three replicate tests as $(OD_{control} - OD_{test})/OD_{control} \times 100\%$. The compounds were incubated with various cell lines for 48 h at different concentrations of complex dissolved in fresh media; the range of concentrations used is dependent on the complex. The final IC_{50} values were calculated by the Bliss method (n = 5). For complexes with IC_{50}<20 μM, a maximum of 1% DMSO was used; while in case of IC_{50}>20 μM, a maximum of 5% DMSO (100 μM) was used, which may cause overestimation of cytotoxicity. But it does not influence the conclusion on the SAR study. All tests were independently repeated at least three times.

5. In-cell stability test

T-24 cells were seeded in Petri dishes and then were cultured for 24 h in the drug-free medium at 37 °C in a humidified atmosphere of 5% CO_2/ 95% air. The cells were followed by treatment with the **Pt-1** at 0.5 μM. After 24 h incubation, washing three times with the ice-cold phosphate-buffered saline (PBS) buffer, cells were harvested and lysed using the lysis buffer (150 mM NaCl, 100 mM Tris-HCl, pH 7.4, 10% glycerol, 1% Triton X-100, 10 mM NaF, 5 mM sodium pyrophosphate, 5 mM sodium orthovanadate, 0.1% SDS) with protease inhibitor. All proteins were precipitated by ethanol, then supernatant were collected by centrifugation and tested with ESI-MS.

6. Cellular uptake by ICP-MS

The T-24 cells were seeded in Petri dishes. The cells were cultured for 24 h in the drug-free medium at 37 °C in a humidified atmosphere of 5% CO_2/ 95% air, and then treated with the **Pt-1** at 2 μM. After 24 h incubation, washing three times with the ice-cold phosphate-buffered saline (PBS) buffer, the subcellular parts were separated by corresponding kit, and then which were further treated with concentrated HNO_3. The resulting solutions were diluted with double-distilled water to a final concentration of 5% HNO_3. The metal content in the diluted solutions was determined using inductively coupled plasma mass spectrometry (ICP-MS). The result is the mean of three experiments and reported as mean ± SD.

7. Subcellular Fractionation assay

To examine the amount of Pt complexes bound to the nucleus, mitochondria and ER in T-24 cells, the cells were seeded in 6-well tissue culture plates for 24 h and then treated with the complexes at 2 μM. After 24 h incubation, the cells were washed three times with the ice-cold phosphate-buffered saline (PBS) buffer. The cytoplasm, nucleus and mitochondria were extracted using Mitochondria Isolation Kit(ab110168) and Nuclear Extraction Kit (ab113474) and ER Extraction Kit (ER0100). Each sample was digested with concentrated HNO_3, and the resulting solutions were diluted with double-distilled water to a final concentration of 5% HNO_3. The metal content in the diluted solutions was determined by ICP-MS. Again, the results shown in Fig. S7 are the means of three experiments and reported as mean ± SD

8. Western blotting of proteins

Cancer cells (2×10^6) were cultured on 60 mm dish and incubated overnight before experiments, which were treated with complex **Pt-1** at labeled concentration for indicated time. After incubation, cells were harvested and lysed using the lysis buffer (150 mM NaCl, 100 mM Tris-HCl, pH 7.4, 10% glycerol, 1% Triton X-100, 10 mM NaF, 5 mM sodium pyrophosphate, 5 mM sodium orthovanadate, 0.1% SDS) with protease inhibitor. Total protein extracts (50 mg) were loaded onto suitable concentration SDS polyacrylamide gel, and were then transferred to polyvinylidene fluoride (PVDF) membranes. The membrane was blocked with 5% BSA in TBST buffer and incubated with corresponding primary antibodies at 4 ºC overnight. After washing, the membrane was incubated with secondary antibody conjugated with horseradish peroxidase (1:2500) for 120 min. The immunoreactive signals were detected using enhanced chemiluminescence kit (Pierce ECL Western Blotting Substrate) following the procedures given in the user manual.

9. Visualization of production of ROS in the ER by confocal microscopy

T-24 cells were plated at 2×10^6 cells/well on poly-L-lysine coated cover slips in 6-well plates in heat deactivated complete RPMI. Cells were treated with drugs for indicated time before they were washed thrice with pre-warmed PBS and incubated with 25 μM pre-warmed H_2DCFDA (488 nm/515 nm) for 20 min at 37 °C. 20 min later, H_2DCFDA was removed from the cells and 1 μM pre-warmed ER-Tracker Red (587 nm/ 615 nm) was added to the cells for 15 min. After the incubation time, cells were washed thrice with PBS before the cover slips were washed with ultrapure water, mounted onto slides and analysed with confocal microscopy (Carl Zeiss,USA) immediately. Images were taken using 20 × objective lens and processed using Zeiss FLUOVIEW Viewer.

10. Investigation of CRT exposure by confocal microscopy and flow cytometry

Cancer cells were seeded into 6-well plates at a density of 100,000 cells per well. After 2 h treatment with Pt-1, the cells were washed twice with PBS and fixed in 0.5% paraformaldehyde for 5 min. Fixed cells were stained by rabbit anti-calreticulin antibody overnight at 4 °C (1:400, Cell Signaling Technology, USA), without permeabilization. After 3 washes with cold PBS, the cells were stained with Alexa Fluor 488-conjugated secondary anti-rabbit antibody (1:1000, Life technologies, USA) for 60 min at room temperature. The images

were observed under a LSM 710 confocal microscope (Carl Zeiss,USA). In addition, the CRT positive cell was similarly analysed by flow cytometry.

11. HMGB1 and ATP release assays

Cancer cells were seeded into 6-well plates at a density of 2×10^6 cells per well. After 4, 12, 24h treatment with the complex Pt-1 (~IC_{50} concentration), the cell culture supernatant was collected. Release of HMGB1 in the cell culture supernatant was determined via western blot, with BSA used as the control protein. The HMGB1 in full cell lysate was also detected using western blot, and β-actin was used as the control protein. In addition, a chemiluminescence ATP Determination Kit (Life technologies, USA) was used to detect ATP concentration in the supernatant according to the manufacturer's instructions. Briefly, the samples after various treatments were added into reaction solution containing D-luciferin and firefly luciferase without ATP, followed by measurement of the luminescence. The amount of ATP was then calculated according to a standard curve generated by the ATP standard solution.

12. Immune responses of PBMCs and DCs

Briefly, PBMCs were isolated from healthy donors by Ficoll-Hypaque density gradient centrifugation and cultured in RPMI 1640 medium, containing 10% FBS for 5 days. On day 5, PBMCs were seeded into 6-well plates at a density of 2×10^6 cells per well, and the medium was replaced by conditioned medium of T-24 cells after treating with Pt-1 (IC_{50} concentration) for 48 h. The amount of the cytokines, TNF-α and IFN-γ, secreted from treated PBMCs after 24 h into the complete RPMI supernatant was measured using the commercially available human TNF-α and IFN-γ sandwich ELISA screening kits (Pierce) as per the manufacturer's protocol. Calibration standards provided by the kits were reconstituted and diluted in complete RPMI. Readings were adjusted to pg/10^6 cells for comparison. Human DCs were generated from the adherent fraction of PBMCs. In brief, the adherent cells in PBMCs were cultured for 6 days in RPMI 1640 medium, containing 10% FBS, 20 ng/mL human GM-CSF, and 10 ng/mL human IL-4. On day 6, DCs were seeded into 6-well plates at a density of 200,000 cells per well, and the medium was replaced by conditioned medium of T-24 cells treated with Pt-1 (IC_{50} concentration) for 48 h. After 24 h, the DCs were collected, and plasma proteins were obtained by the instructions of membrane extraction Kit (Sigma Aldrich), then the maturation markers for DCs, CD80 and CD83, were evaluated by Western blotting.

13. Cytotoxicity of ICD-activated human peripheral blood mononuclear cells against tumour cell-lines

In total, 2×10^6 PBMCs were incubated in 5 mL of complete RPMI for 24 h at 37 °C. Suspension cultures of PBMCs were activated by incubating 5.0×10^5 PBMCs with conditioned medium of T-24 cells treated with Pt-1 (~IC_{50} concentration) for 48 h. After activation, PBMCs were washed twice with complete DMEM to remove residual medium and resuspended in 1.2 mL of complete DMEM. Then 4.0×10^4 PBMCs were co-cultured with T-24 tumor cells (pre-seeded 24 h beforehand at a density of 4.0×10^3 cells/well in 100 µL aliquots into flat-bottomed 96-well tissue-culture plates) and incubated for 24, 48, 72 h. At the end of exposure, the medium was replaced by 100 µL/well MTT solution (0.5 mg/mL in PBS). After incubation for 4 h, MTT was aspirated and substituted with 100 µL/well DMSO. UV-vis absorbance was

measured at 570 nm using a microplate reader. Experiments were performed in triplicates for each activated PBMCs and carried out independently for three times. Cell viability was calculated from the absorbance value of the tumour cells cultured with activated PBMCs or non-activated PBMCs (A), the absorbance value of PBMCs only (B), the absorbance value of DMSO only (C), and the absorbance value of the tumour cells only (D) by the formula: $\frac{A-C-(B-C)}{D-C}\times 100\%$.

14. Anti-tumour vaccination

The vaccination experiment was conducted with C57BL/6 mice (6 weeks old, male, approximately 20 g), which were purchased from Peking Union Medical College. The mice were housed in the experimental animal facilities at the Guangxi Medical University in a temperature and humidity controlled environment with a 12 h light/12 h dark cycle. All mice were fed commercial chow and water ad libitum. All experiments were performed according to the guide for the care and use of laboratory animals of the Guangxi Medical University, and all the animal experiment procedures were approved by the Ethics Committee of Guangxi Medical University Cancer Institute. For vaccination experiment, a total of 4×10^6 MB-49 cells were treated with the different drug formulations (the concentrations of oxaliplatin and **Pt-1** were 1.3 and 0.5 μM, respectively) for 48 h *in vitro*, and then were inoculated subcutaneously into left flanks of 6-week old female C57BL/6 mice (n = 10 per group). After 7 days, the mice were re-challenged with live 1×10^6 MB-49 cells into the contralateral flanks. Tumour progress was monitored in the contralateral flank, with tumour-free defined as negligible tumour mass detected by palpation.

15. Inhibition of tumour growth in mouse models

For testing acute toxicity, six-week-old male and female C57BL/6 mice (weight 20-22 g) were randomly divided into 7 groups (n = 6) and used to study the *in vivo* safety of **Pt-1** and oxaliplatin by intraperitoneal injection at the indicated doses once a day for 12 days. The signs of toxicity were observed and body weight was recorded daily. Subcutaneous bladder cancer xenografts were formed in immunocompetent female C57BL/6 mice by injecting 2×10^6 MB-49 cells (5 mice/ group). When the tumour volume reached about 60 mm^3, oxaliplatin (6 mg/kg) and **Pt-1** (10 mg/kg) were injected through the intraperitoneal injection every 2 days. The tumour volumes were monitored every 3 days using digital calipers. The tumour volume (mm^3) was calculated using the following formula: tumour volume = (shortest diameter)^2 × (longest diameter) × 0.5. The tumour growth curves were plotted as the average tumour volume *vs* days after the first treatment. All the mice were sacrificed 18 days after treatment, and the tumours were collected, weighed and fixed in formalin for paraffin embedding. The inhibition rates of tumour growth (IRT) were calculated as follows: IRT = 100% × (mean tumour weight of the control group – mean tumour weight of the experimental group) / mean tumour weight of the control group. For determination of peripheral and infiltrating $CD3^+$ and $CD3^+CD8^+$ T lymphocytes, peripheral blood was collected from C57BL/6 mice. Spleen and tumour single-cell suspensions were prepared as previously[44]. Then the cells were co-stained with FITC-anti-CD3 and PE/Cy5-anti-CD8 antibodies (Biolegend, USA). Surface expression of CD3 and CD8 were analyzed by flow cytometry.

Fig. S1 ^{1}H NMR of **Pt-1** in d6-DMSO solution.

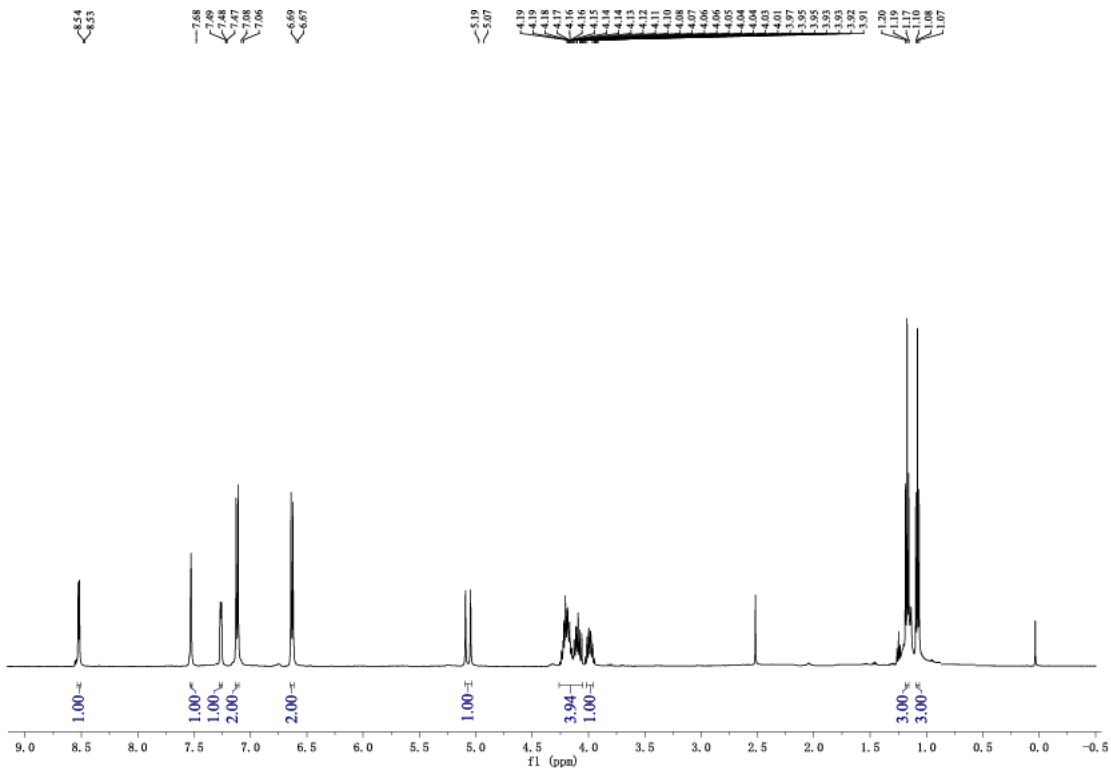


Fig. S2 ESI-MS spectrum of **Pt-1** DMSO solution.

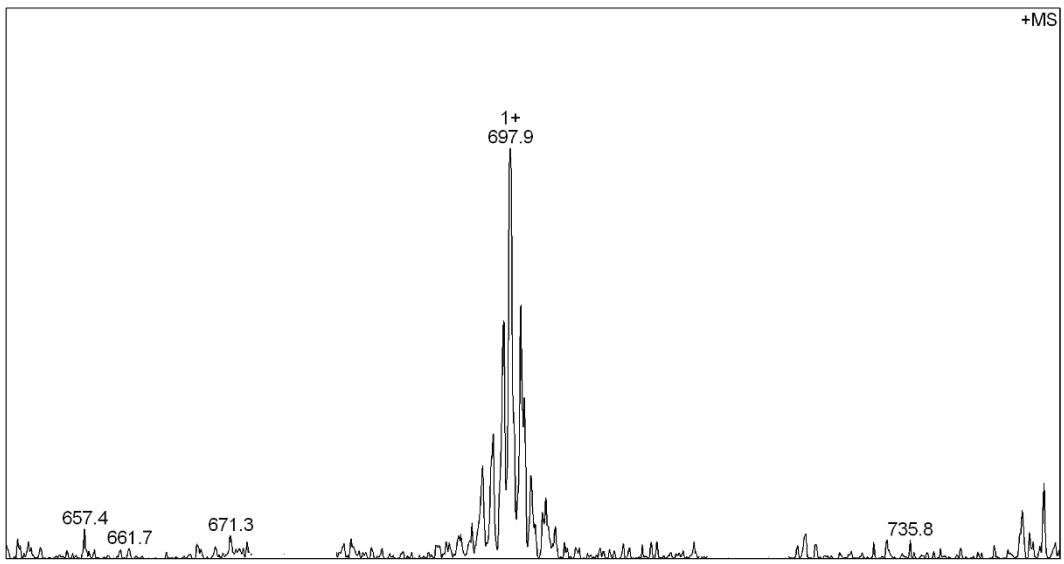
Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\kebin huang\txm\1104-lapt11.d		
Method	DEF_MS-Pos 20160701.M	Operator	hct
Sample Name	ESI Tuning Mix Pos	Instrument	HCT
Comment			

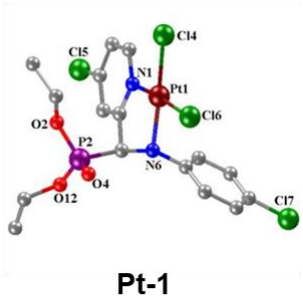
Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Std/Enhanced	Scan Begin	650 m/z	Scan End	750 m/z
Capillary Exit	140.0 Volt	Skimmer	40.0 Volt	Trap Drive	65.8
Accumulation Time	1495 µs	Averages	5 Spectra	Auto MS/MS	off

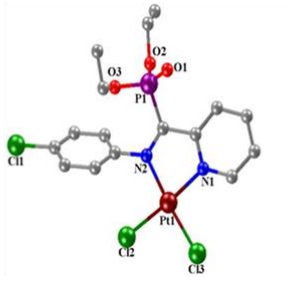


#	m/z	I
1	685.9	296364
2	692.2	307580
3	695.2	253971
4	696.2	269288
5	697.2	515447
6	697.9	888141
7	698.9	548446
8	699.2	349531
9	709.1	227882
10	711.1	226922

Fig. S3 ORTEP drawing of complexes **Pt-1**, **1**, **2**, **3**, **5**, **6**, **8**, **10**, and **11**.



Pt-1



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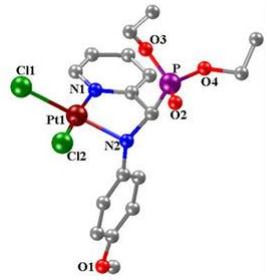

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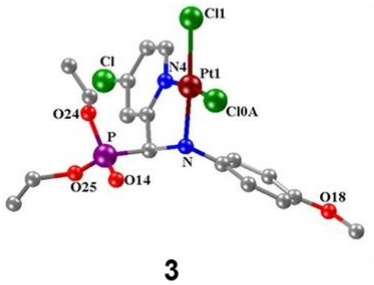

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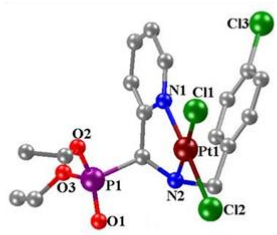

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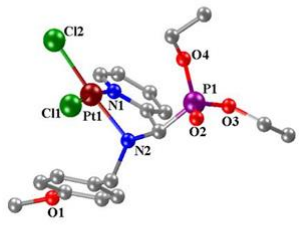

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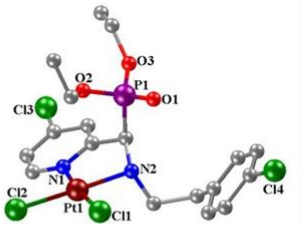

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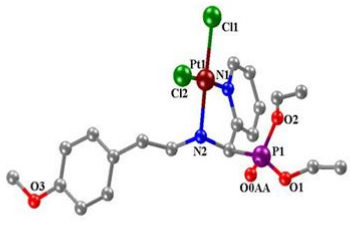

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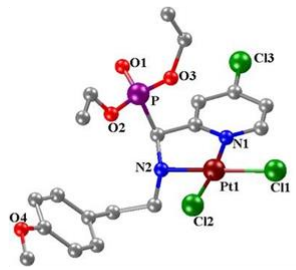


11

Table S1. Crystal data and structure refinement details for complexes **1**, **2**, **3, 4, 6, 8, 9, 10, Pt-1**

formula	$C_{16}H_{19}Cl_4N_2O_3PPt$ (**Pt-1**)	$C_{16}H_{18}Cl_3N_2O_3PPt$ (**1**)	$C_{17}H_{22}Cl_3N_2O_3PPt$(**2**)	$C_{17}H_{22}Cl_3N_2O_4PPt$(**3**)	$C_{17}H_{22}Cl_3N_2O_3PPt$ (**5**)	$C_{18}H_{25}Cl_2N_2O_4PPt$(**6**)	$C_{18}H_{23}Cl_4N_2O_3PPt$ (**8**)	$C_{19}H_{27}Cl_2N_2O_4PPt$ (**10**)	$C_{19}H_{26}Cl_3N_2O_4PPt$(**11**)
fw	655.19	618.73	616.33	650.78	634.78	630.33	683.23	644.39	676.13
T / K	293(2)	293(2)	293(2)	293(2)	293(2)	293(2)	293(2)	293(2)	293(2)
crystal system	monoclinic,	monoclinic	orthorhombic	orthorhombic	monoclinic,	monoclinic	triclinic	triclinic,	triclinic
space group	P21/c	P21/c	Pbca	Pbca	$P2_1/n$	P21/n	P -1	P^{-1}	P-1
a, Å	8.1410 (12)	9.6106(12)	10.0411(5)	12.064(2)	8.7652(3)	9.22386(14)	8.366(3)	9.3032(4)	8.5496(10)
b, Å	17.253 (3)	20.423(3)	14.3523(8)	16.098(3)	14.5838(6)	13.26158(18)	11.425(4)	11.5641(4)	11.5478(14)
c, Å	16.187 (3)	10.6731(13)	29.091(2)	22.941(4)	17.6187(11)	18.9212(3)	14.150(5)	12.6329(6)	12.5966(15)
α, º	90.00	90.00	90.00	90.00	90.00	90.00	95.168(6)	111.392(4)	83.444(2)
β, º	104.267(2)	96.686(2)	90.00	90.00	116.45(2)	102.5961(15)	106.359(6)	106.122(4)	85.190(2)
γ, º	90.00	90.00	90.00	90.00	90.00	90.00	106.779(6)	101.324(3)	71.014(2)
V, Å^3	2203.5 (6)	2080.7(4)	4192.4(4)	4455.1(13)	2252.21(19)	2258.79(6)	1220.7(8)	1146.41(8)	1166.9(2)
Z	4	4	8	8	4	4	2	2	2
D_c, g cm^{-3}	1.975	1.975	1.877	1.940	1.872	1. 593	1.859	1.867	1.932
μ, mm^{-1}	6.940	7.224	6.476	6.756	6.676	1.154	6.27	6.450	6.453
GOF on F^2	1.02	1.096	1.145	0.766	1.141	1.124	1.31	1.029	1.080
Reflns(collected/unique)	26711 /4497	13812/4220	19961/4088	38858/4573	19962/5088	12731/3973	9517/4172	9553/4682	13943/4816
R_{int}	0.044	0.0290	0.1216	0.0692	0.1416	0.0269	0.060	0.03661	0.0366
R_1[a] ($I > 2\sigma(I)$)	0.024	0.0274	0.0852	0.0272	0.0842	0.0823	0.1012	0.0352	0.0260
wR_2[b] (all data)	0.055	0.0877	0.2027	0.1073	0.2857	0.2613	0.3041	0.0844	0.0724
CCDC number	1542736	1542734	1542730	1542732	1542733	1542729	1542735	1542728	1542731

[a] $R_1 = \Sigma\, ||F_o| - |F_c||/\Sigma|F_o|$; [b] $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{½}$.

Fig. S4 HPLC spectra for **Pt-1** in aqueous solution (1 mg/mL) at time points of 0 h, 24 h, 48 h, respectively. Column: reversed-phase C18 column (YMC HPLC COLUMN, 150×4.6mm I. D.). Column temperature: 35ºC. Mobile phase: Methol/H_2O (80:20). Flow rate: 1.0 ml/min. Injection volume: 8 μL.

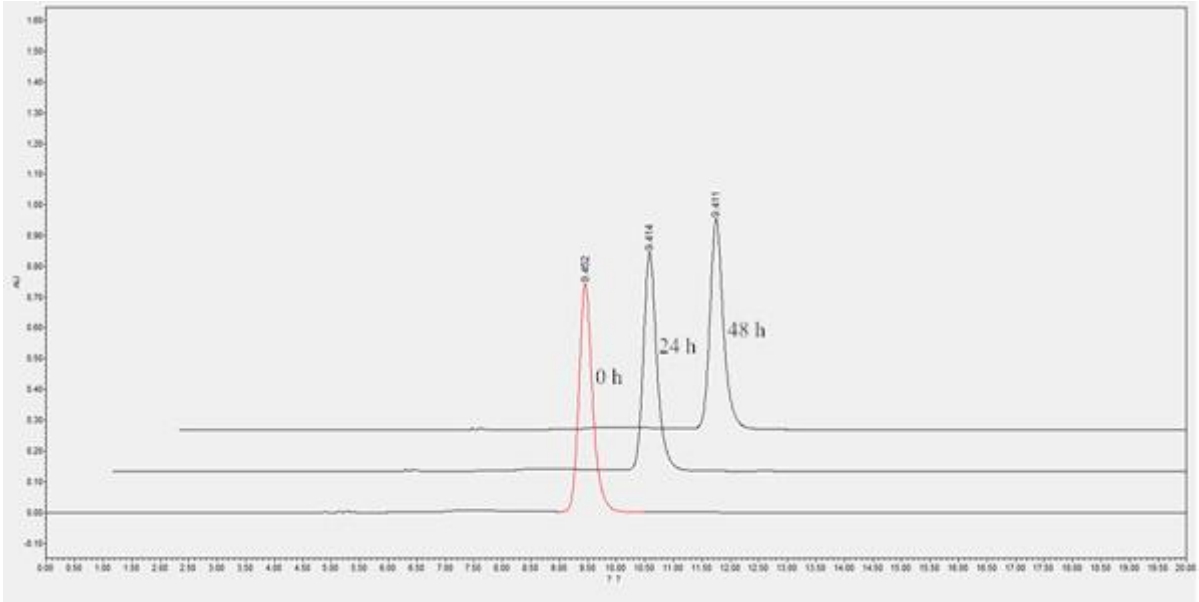


Table S2. IC_{50} (μM) values for the platinum(II) complexes towards tumor or normal cell lines.

Compound	T-24	MG-63	HepG-2	SK-OV-3	HL-7702
Pt-1	0.46 ± 0.31	0.25 ± 0.11	0.73 ± 0.13	1.2 ± 0.2	24.8 ± 2.4
1	6.3 ± 0.4	5.2 ± 0.3	7.9 ± 0.6	11.5 ± 0.2	64.6 ± 3.6
2	35.2 ± 2.4	68.5 ± 2.9	47.5 ± 3.4	88.7 ± 4.5	82.1± 6.2
3	22.6 ± 0.4	19.3 ± 0.9	18.3 ± 0.6	23.5 ± 2.2	>100
4	10.5 ± 0.6	11.3 ± 0.3	16.4 ± 0.5	14.8 ± 1.4	33.6 ± 2.8
5	14.6 ± 1.5	13.5 ± 0.4	17.5 ± 1.1	25.1 ± 1.5	54.6 ± 5.7
6	24.7 ± 1.5	22.7 ± 1.4	78.5 ± 5.5	74.6 ± 3.2	>100
7	18.3 ± 1.3	16.2 ± 0.2	18.8 ± 2.4	21.1 ± 2.1	>100
8	11.8 ± 2.1	16.6 ± 0.7	14.6 ± 1.4	11.4 ± 2.6	44.2 ± 3.7
9	23.5 ± 2.6	21.5 ± 0.8	28.2 ± 1.5	34.6 ± 3.1	76.4 ± 4.5
10	18.7 ± 1.3	28.2 ± 1.6	33.5 ± 2.2	62.2 ± 5.1	> 100
11	13.4 ±1.1	19.7 ± 0.4	18.7 ± 1.4	13.3 ± 0.7	88.6 ± 3.7
cisplatin	1.8 ± 0.2	7.5 ± 0.2	4.2 ± 0.3	8.1 ± 0.4	16.6 ± 1.8
oxaliplatin	0.87 ± 0.15	4.6 ± 0.1	1.2 ± 0.2	7.5 ± 0.6	13.6 ± 1.8

[a]IC_{50} is the drug effective concentration in inhibition rate of 50% for the cell growth analyzed by the MTT assay after 48 h of drug incubation. Each value is the mean of three independent measurements.

Fig. S5 Percentage of inhibition by L^{Pt-1} or **Pt-1** at 20 μM towards different cancer cells after 48 h incubation time.

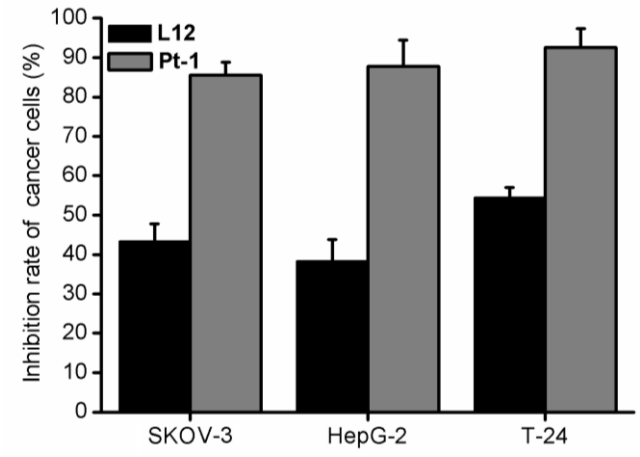


Fig. S6 ESI-MS analysis of the supernatant after ethanol precipitation of the cell lysates of T-24 cells incubated with **Pt-1** for 24 h.

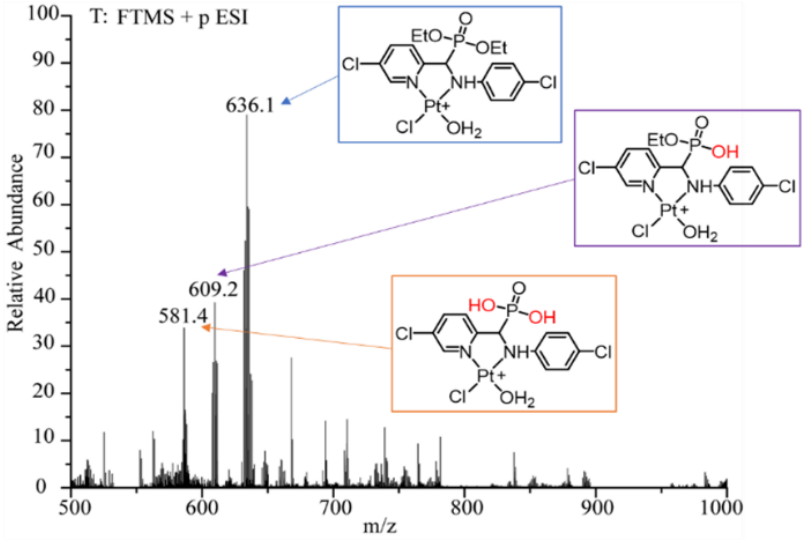


Fig. S7 Subcellular localization of **Pt-1** and **1** in nuclei, mitochondria and ER of cancer cells.

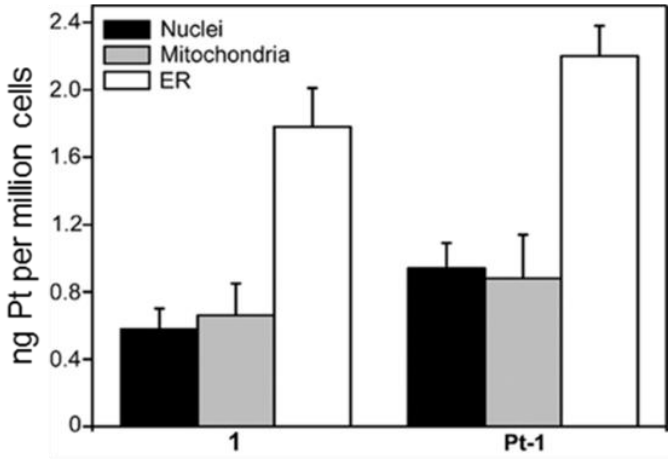


Fig. S8 Western blot analysis of ER-stress related proteins after treatment of T-24 cells with an IC_{50} concentration of **Pt-1** and **1** for 48 h.

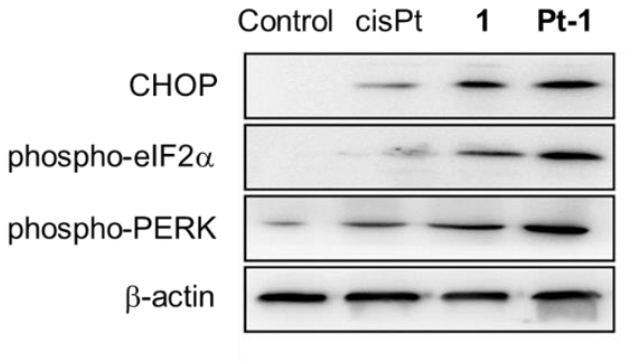


Fig. S9 Staining of cancer cells by DCFH and ER-Tracker after treating with **Pt-1** for different time.

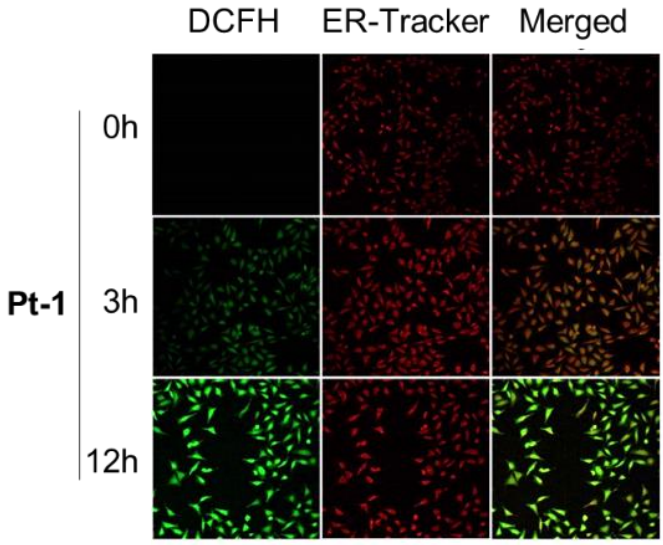


Fig. S10 Rapid surface exposure of CRT that was detected by confocal microscopy after drug incubation (2 h) of T-24 cells that were followed by surface immunofluorescence staining with anti-CRT mAb.

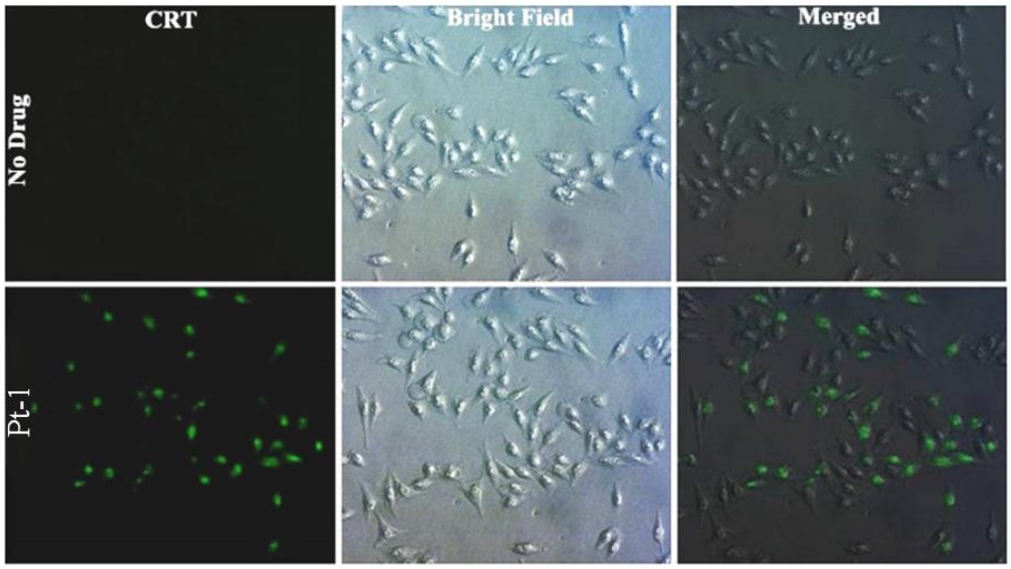
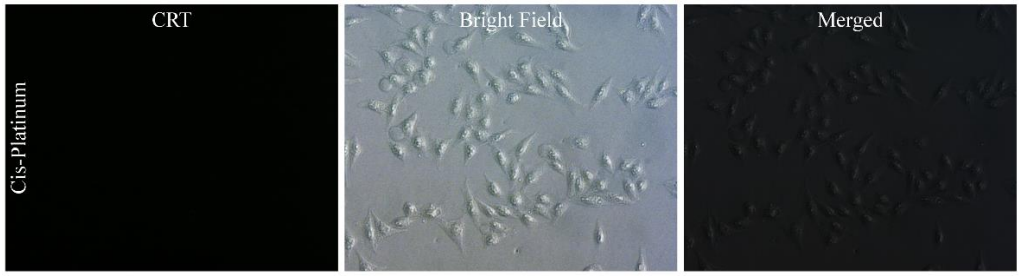


Fig. S11 Control experiment: detection of CRT exposure, ATP release and HMGB1 secretion after treating T-24 cells by cisplatin (2 μM, ~IC_{50} concentraion) for 2 h or for the time as indicated.

A)



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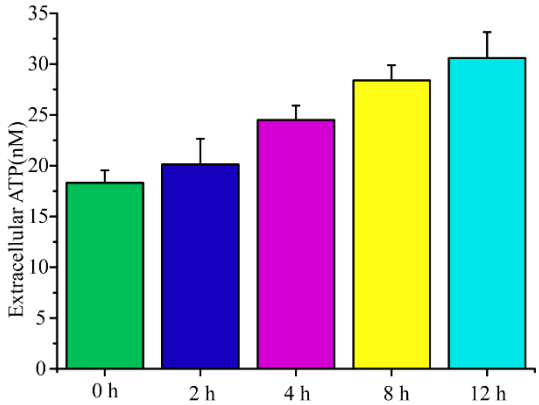

C)

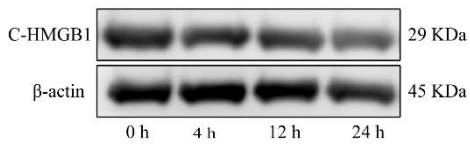

Table S3. Dosage used to test acute toxicities of oxliplatin and **Pt-1** (Alive/Total).

	10 μmol/kg	20 μmol/kg	30 μmol/kg
Oxliplatin	3/3	2/3	0/3
Pt-1	3/3	3/3	3/3

Fig. S12 Body weight change of C57BL/6 mice bearing MB-49 cells (n=5) after treatment with 9.8 mg/kg **Pt-1** or 6 mg/kg oxaliplatin.

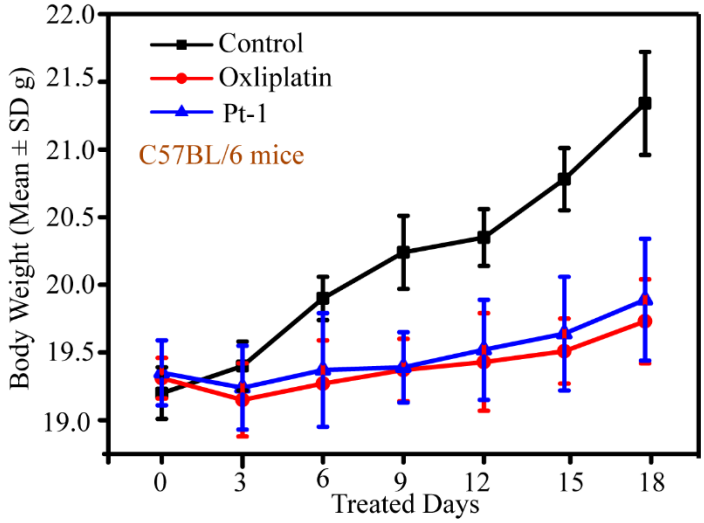

Fig. S13 Percentage of total $CD3^+$ T lymphocytes in the total cells isolated from peripheral blood, spleen, and tumours of C57BL/6 mice treated with **Pt-1** or oxaliplatin. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$