Supporting Information

Spin-labelled photo-cytotoxic diazido platinum(IV) anticancer complex

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Materials and methods:

Chloroform-d 99.8 atom % D, sodium azide (99%) and N-hydroxysuccinamide (97%) were purchased from Aldrich. $K_2[PtCl_4]$ was obtained from Precious Metals Online. Pyridine was purchased from Fischer Scientific UK, and other chemicals from Sigma Aldrich.

Caution! Heavy metal azides can be shock-sensitive detonators. We did not encounter any problems during the work reported here but due care and attention with appropriate precautions should be taken in their synthesis and handling.

^{1}H NMR spectra were recorded on a 400 MHz Bruker Avance III HD spectrometer. ESI-MS spectra were recorded on an Agilent 6130B single quadrupole detector instrument and ESI-HR-MS data was collected on a Bruker microTOF instrument.

X-band EPR spectra were recorded using a Bruker EMX spectrometer under ambient conditions. For photoactivation experiments, aqueous samples were contained in a flat-cell (WG812) positioned in a TM_{110} cavity (ER4103 TM), and were not degassed. For kinetic measurements, the EPR parameters were: sweep width 8 mT, 1024 points, time constant 10.24 ms and conversion time 20.48 ms, giving a sweep time of 21 s. Field modulation was applied at 100 kHz and 0.05 mT, and the microwave attenuation was 18 dB (~3.2 mW). Sample illumination was provided by a 100 mW laser diode operating at 405 nm (RS Pro DL-405-0.10), mounted in a temperature controlled mount (Thorlabs TCLDM9). The diode was focussed into a 0.50 NA, 1000 µm diameter multimode optical fibre (Thorlabs FP1000URT), with power output at the fibre end measured at 42 mW under the operating conditions used. The fibre tip was inserted into the EPR cavity via the front optical grill such that the diverging beam illuminated the full width of the EPR flat-cell.

EPR spectral simulations were performed in Matlab using the EasySpin package.[1] In order to determine the concentration of the spin-adduct formed,

experimental data were fitted with a linear combination of the individual simulated spectra of Pt-TEMPO and DMPO-N_3. As the solution spectra are in the fast motional regime, the routine *garlic* was used for all simulations.

To allow spectral simulation of the azidyl adduct, an effective g-value was determined using EasySpin's *esfit* routine as g = 2.00595, with hyperfine coupling constants taken from the literature.[2] The phenomenological lineshape used contained Lorentzian and Gaussian components having an equal full-width at half-maximum of 0.1 mT, and dynamic effects were neglected. The full EasySpin system structure is given below (linewidths are in units of mT and hyperfine couplings in units of MHz):

```
SysDMPO =
      S:  0.5
      g:  [2.00595]
   Nucs:  '14N,H,14N'
      A:  [40.6362 41.7572 8.8559]
     lw:  [0.1000 0.1000]
```

Simulations of the Pt-TEMPO complex were performed using parameters derived from fitting to the spectrum without spin-adduct recorded prior to illumination. Hyperfine couplings arising from both the nitroxide nitrogen and statistical abundance of nearest neighbour ^{13}C nuclei were included, the former being constrained to allow axial distortion only ($a_{\parallel}^{N} = 3.4287$ mT, $a_{\perp}^{N} = 0.8626$ mT) and the latter to be fully isotropic ($a_{\beta}^{C} = 0.7523$ mT). A rhombic g-tensor was used and dynamics considered in the fast motional regime, $\tau_c \approx 120$ ps. The full EasySpin system structure used was:

```
SysPtTEMPO =
      S:  0.5
      g:  [2.0080 2.0077 2.0025]
   Nucs:  'N,(12,13)C'
     A_:  48.1465   23.9718   0
          21.0829   0        0
  Abund:  {[1]  [0.9786 0.0214]}
     lw:  [0.1695 0.0522]
logtcorr: -9.9276
```

The EPR spectrum shown in Fig 1c was recorded using 0.5 mM Pt-TEMPO in dichloromethane within a 2.2 mm inner diameter quartz tube (Wilmad 705-SQ) degassed by repeated freeze-pump-thaw cycling. Spectrometer settings were: sweep width 6 mT, 8192 points, time constant and conversion time 10.24 ms, with field modulation applied at 100 kHz and 7 μT, and the average of 8 scans displayed. Under these conditions, in the absence of oxygen-induced exchange broadenings, small proton hyperfine couplings are partially resolved on all but the high-field line.

Cell Culture: A2780 human ovarian carcinoma cells were obtained from the European Collection of Cell Cultures (ECACC) and were grown in Roswell Park Memorial Institute medium (RPMI-1640) with or without phenol red according to the protocol below. All media were supplemented with 10% v/v of foetal calf serum, 1% v/v of 2 mM glutamine and 1% v/v penicillin/streptomycin. All cells were grown as adherent monolayers at 310 K in a 5% CO_2 humidified atmosphere and passaged regularly at approx. 80% confluence.

In vitro **Growth Inhibition Assay**: Briefly, 5000 cells were seeded per well in 96-well black glass-bottom plates using independent duplicate plates (one for dark conditions and one for irradiation). The cells were pre-incubated in drug-free media with phenol red containing medium at 310 K for 72 h before adding different concentrations of the compounds to be tested. In order to prepare the stock solution of the drugs, complexes were dissolved first in DMSO and then diluted in a 50:50 v/v mixture of phenol red-free RPMI-1640:saline. These stock solutions were further diluted using phenol-red free cell culture medium until working concentrations were achieved, in these solutions the maximum DMSO concentration was in all cases < 0.5% v/v. Cells were exposed to the different concentrations of the drugs for 1h. After this, one plate was irradiated for 1h using blue light (50 mW at 465 nm) while the dark plate was kept in the incubator. After irradiation, supernatants of both plates were removed by suction and each well was washed with PBS buffer. A further 24 h was allowed for the cells to recover in drug-free phenol-containing medium at 310 K. Percentages of cell survival are determined by comparison to untreated controls which were only exposed to the maximum concentration of vehicle (0.5% v/v of DMSO). Untreated controls were also compared between the irradiated and the

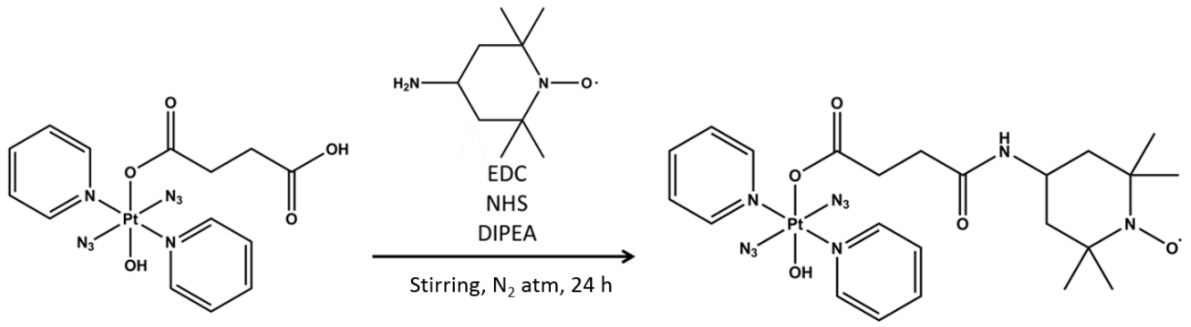
non-irradiated plates to ensure that the differences in cell survival were not statistically relevant, hence guaranteeing that the light source by itself was not causing cell death. The SRB assay was used to determine cell viability. Absorbance measurements of the solubilised dye (on a BioRad iMark microplate reader using a 500 nm filter) allowed the determination of viable treated cells compared to untreated controls. IC_{50} values (concentrations which caused 50% of cell death), were determined as the average of triplicates and their standard deviations were calculated. Stock concentrations for all metal complexes used in these biological assays were adjusted/verified after ICP-OES metal quantification.

Synthesis of *trans,trans,trans*-[$Pt(N_3)_2(OH)(succinate)(Py)_2$]:

Trans,trans,trans-[$Pt(N_3)_2(OH)(succinate)(Py)_2$] was prepared by following the synthetic procedure reported previously.[3]

Synthesis of *trans,trans,trans*-[$Pt(N_3)_2(OH)(OCOCH_2CH_2CONH$-TEMPO)$(Py)_2$]:

Trans,trans,trans-[$Pt(N_3)_2(OH)(succinate)(Py)_2$] (0.035 g, 0.0612 mmol) was treated with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC; 0.019 g, 0.122 mmol) and N-hydroxysuccinimide (NHS; 0.007 g, 0.060 mmol) in DMF (1.5 mL) as solvent. After 2 h stirring at room temperature under N_2 atmosphere in the dark, 4-amino-TEMPO containing DIPEA (10.77 µL, 0.0619 mmol) was added to the reaction mixture dropwise and the reaction was allowed to stir for 24 h in the dark. Then the solvent was removed under high vacuum and the product was separated on a silica column eluting with ($MeOH/CH_2Cl_2$). The yellow coloured oily product obtained was solubilised in few drops of acetone and precipitated by the addition of diethyl ether. The yield obtained was 33% (0.015 g, 0.020 mmol). Anal. Calculated for $C_{25}H_{39}N_{10}O_6Pt^{\cdot}$ (**1**): C, 38.96; H, 5.10; N, 18.17. Found: C, 39.06 H, 4.86; N, 17.35. HRMS (H_2O): $[M+Na]^+$ calculated: 747.2177, found: 747.2184.



Scheme S1 Synthesis of *trans,trans,trans*-[$Pt(N_3)_2(OH)(OCOCH_2CH_2CONH$-TEMPO)$(Py)_2$].

Characterization of *trans,trans,trans*-[$Pt(N_3)_2(OH)(OCOCH_2CH_2CONH$-TEMPO)$(Py)_2$]:

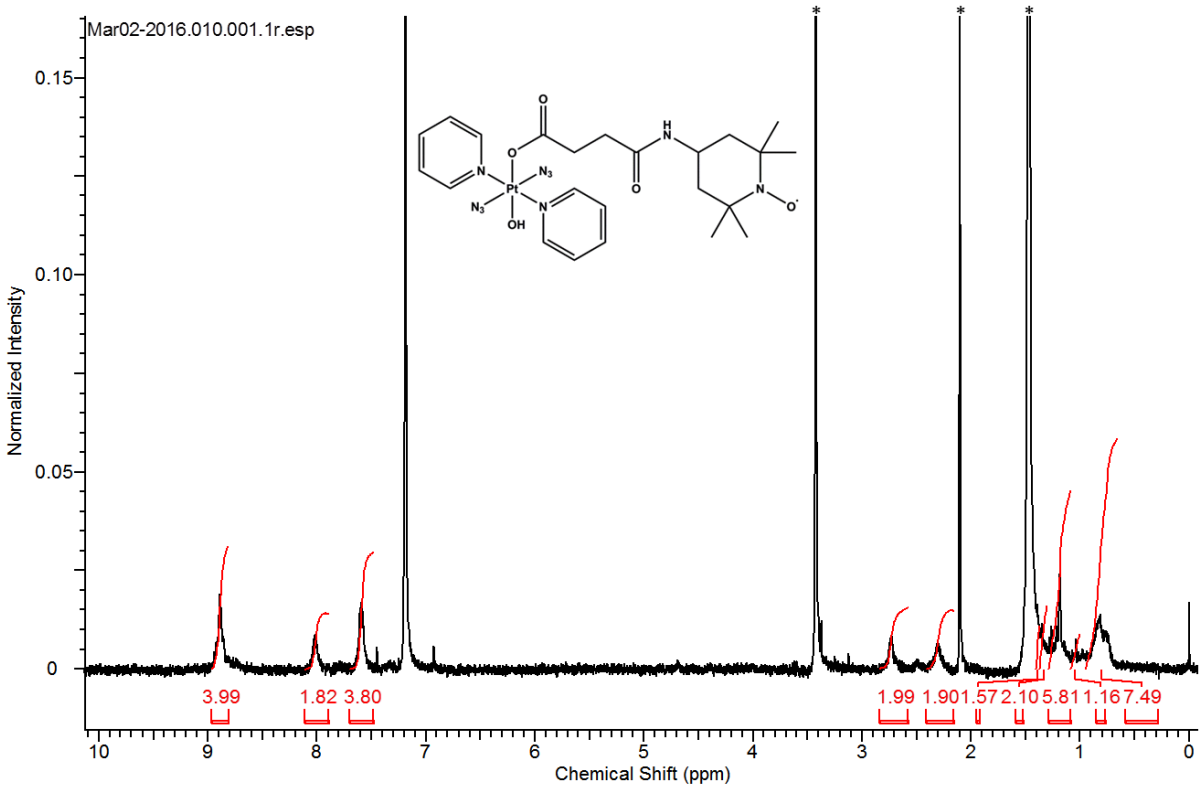


Fig. S1 400 MHz ^{1}H NMR spectrum of Pt-TEMPO in $CDCl_3$ (* corresponds to residual solvents peaks of diethyl ether and acetone).

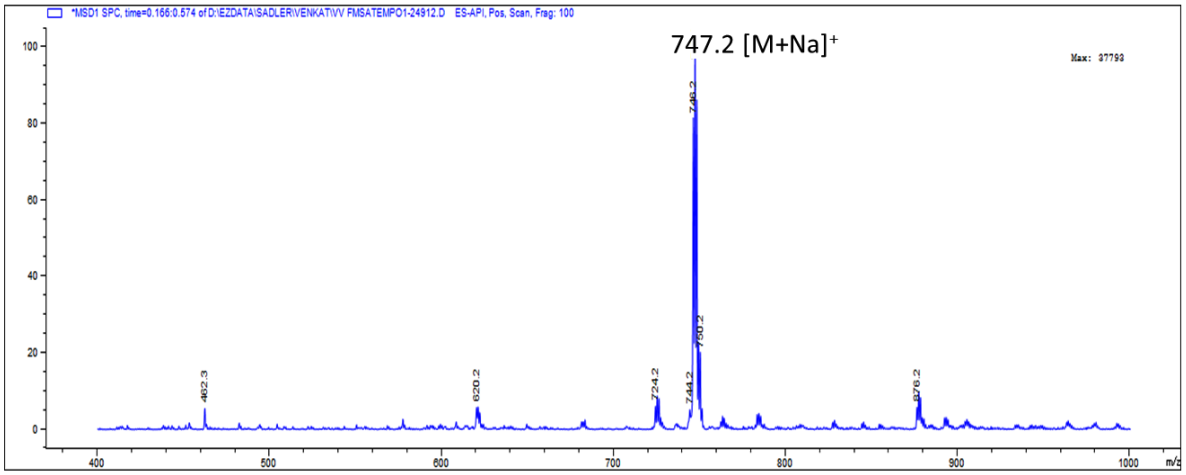


Fig. S2 ESI-MS spectrum of Pt-TEMPO in H_2O.

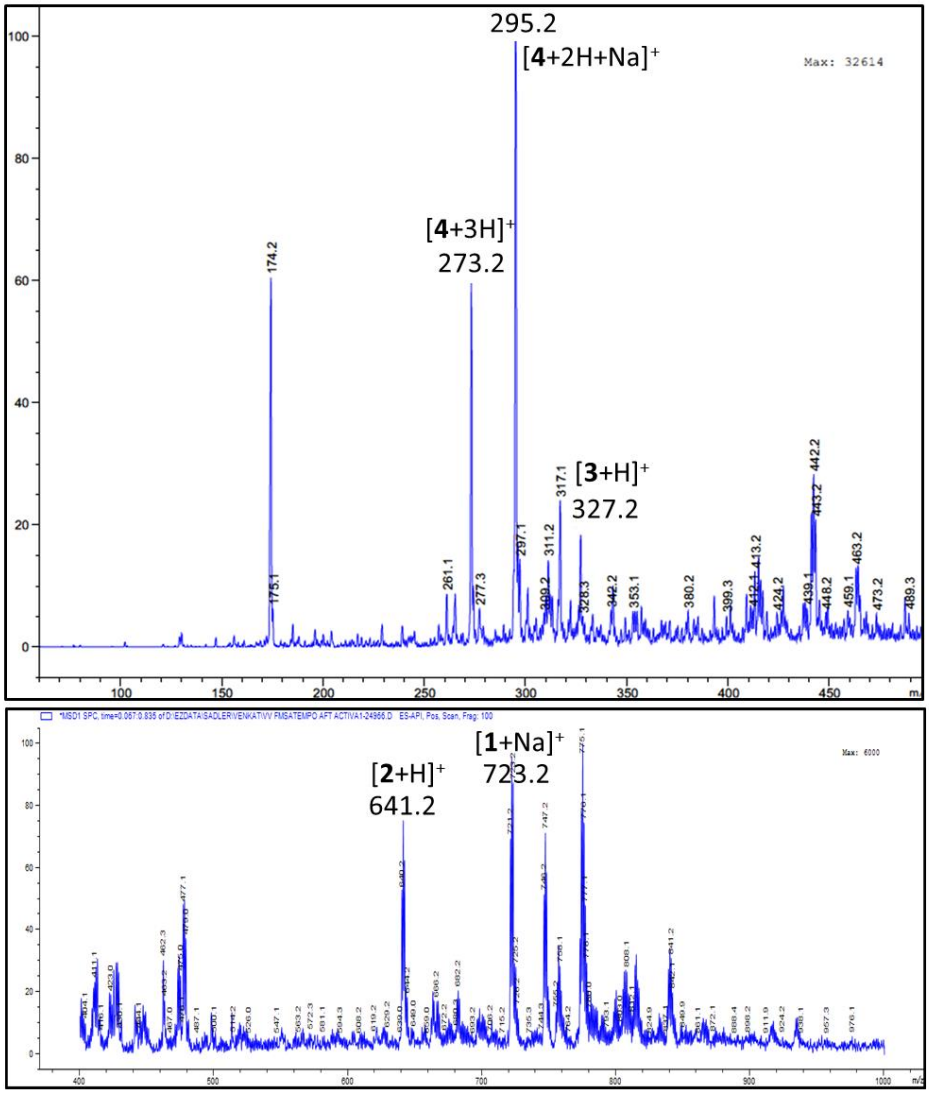


Fig. S3 ESI-MS spectrum of Pt-TEMPO after photoactivation for 2 h in H_2O, showing the presence of different species in solution from **1-4**.

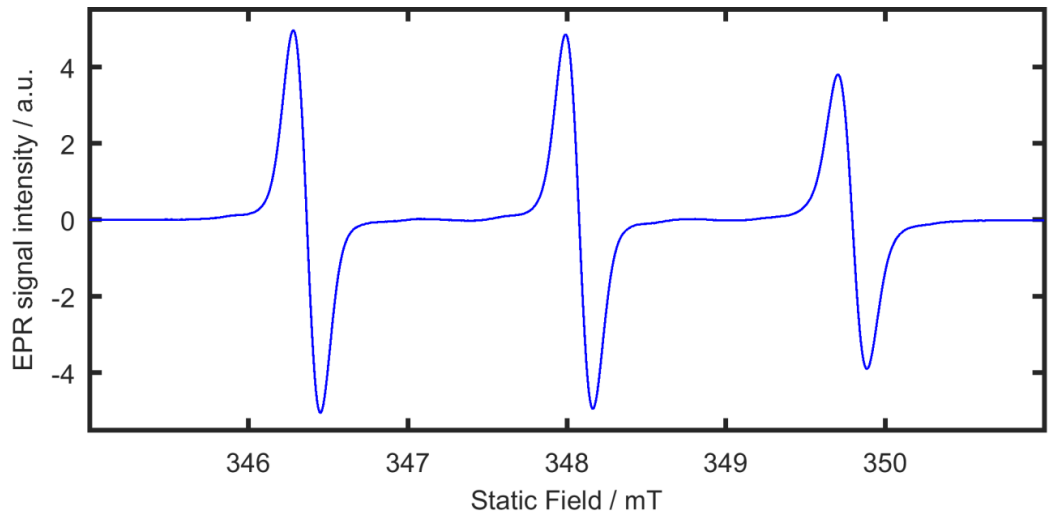


Fig. S4 EPR spectrum of Pt-TEMPO (1 mM in H_2O) with DMPO (25 mM) before illumination and without sample deoxygenation.

Photoactivation:

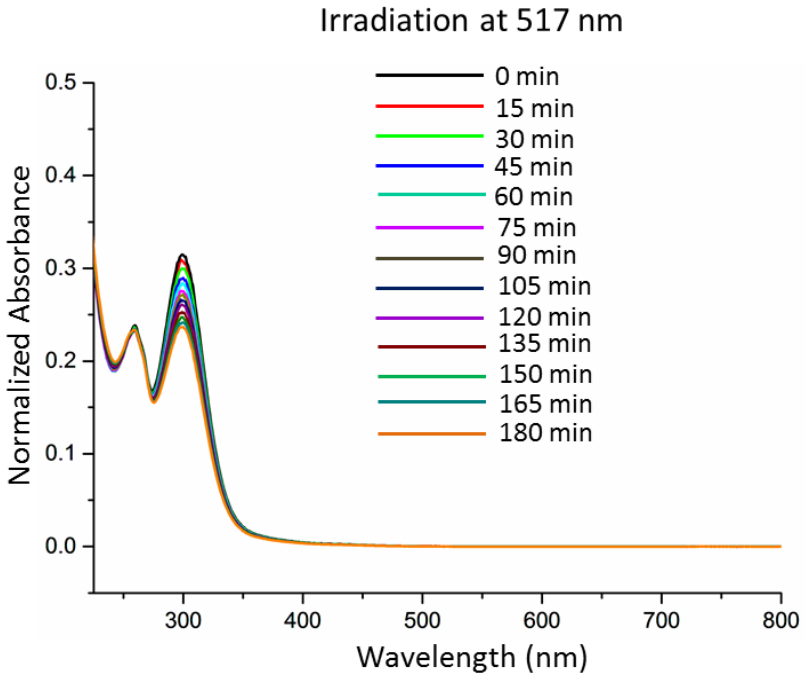


Fig.S5 UV-Vis spectrum of Pt-TEMPO, before and after irradiation with 517 nm (green) light for 3 h in H_2O.

Growth curves for A2780 human ovarian carcinoma cells:

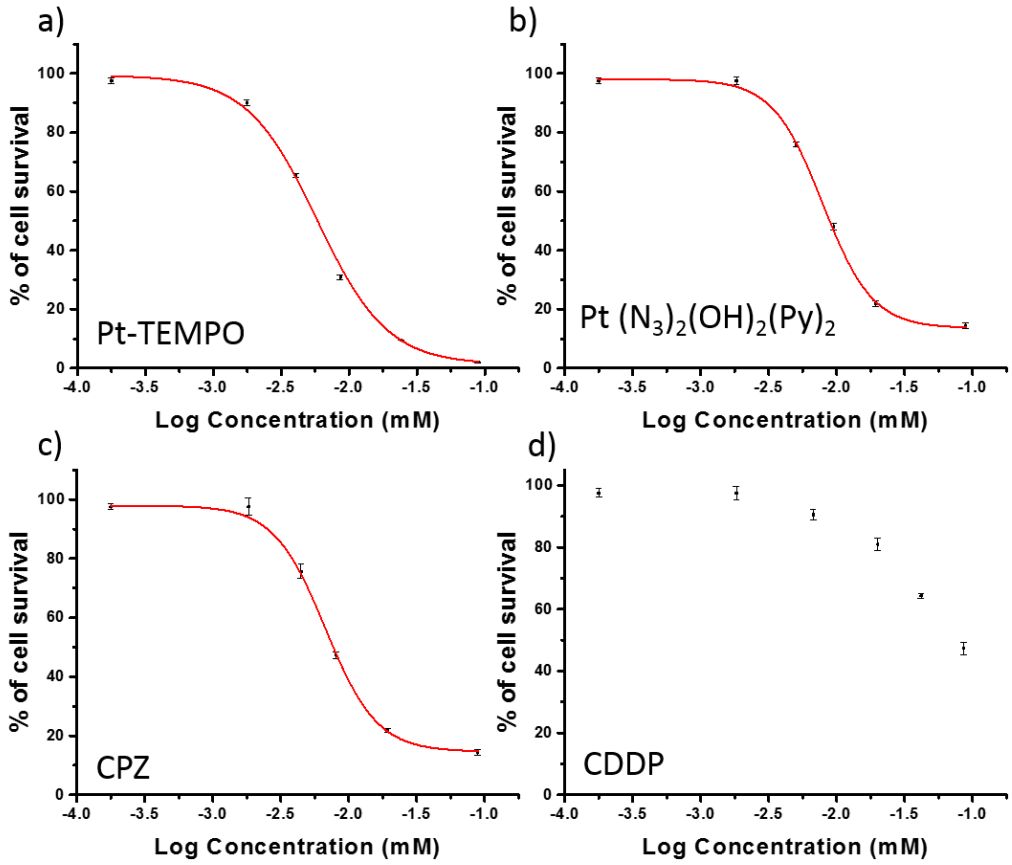


Fig.S6 Cell growth curve of A2780 human ovarian carcinoma cells in the presence of (a) Pt-TEMPO, (b) $[Pt(N_3)_2(OH)_2(Py)_2]$, (c) CPZ, and (d) cisplatin (CDDP).

References

1) S. Stoll, A. Schweiger, *J. Magn. Reson*., 2006, **178**, 42.
2) J. S. Butler, J. A. Woods, N. J. Farrer, M. E. Newton, and P. J. Sadler, *J. Am. Chem. Soc.*, 2012, **134**, 16508.
3) E. Shaili, PhD thesis, University of Warwick, 2013. http://wrap.warwick.ac.uk/59800/