Supporting Information

Organometallic cyclopentadienyl iridium(III) anticancer complexes with new mechanisms of action: NCI-60 screening, mitochondrial targeting and apoptosis in human cancer cells

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MATERIALS

For complex synthesis, $IrCl_3 \cdot nH_2O$ was purchased from Precious Metals Online (PMO pty Ltd). All solvents were obtained from commercial sources such as Fisher Scientific and Sigma-Aldrich and were used without further purification; ethanol was obtained from the same suppliers but dried over Mg/I_2 before use. 1,10-Phenanthroline monohydrate, 2,2′-bipyridine, 2-phenylpyridine, 4-(2-pyridylazo)-N,N-dimethylaniline ligands were purchased from Sigma. Deuterated solvents were purchased from Cambridge Isotopes Limited. ICP-MS standards (Ir) were obtained from Inorganic Ventures.

For the biological experiments, RPMI-1640, medium, as well as foetal bovine serum, L-glutamine, penicillin/streptomycin mixture, trypsin, trypsin/EDTA, phosphate buffered saline (PBS) were purchased from PAA Laboratories GmbH. CDDP (≥ 99.9%), trichloroacetic acid (≥ 99%), sulforhodamine B (75%), sodium phosphate monobasic monohydrate (≥ 99%), sodium phosphate dibasic heptahydrate (≥ 99%), acetic acid (≥ 99%), L-BSO (>97%), staurosporine, PI (>94%) and RNAse A were obtained from Sigma Aldrich together with the Annexin V-FITC Apoptosis Detection Kit and Abcam JC-10 Mitochondrial Membrane Potential Assay kit for flow cytometry.

For TEM, glutaraldehyde (2%), cacodylate buffer at pH 7.6, 2% uranyl acetate in maleate buffer, 100% propylene oxide and Embed 812 resin were all purchased from Agar Scientific.

NMR data (^{1}H, ^{13}C) were acquired using 5 mm NMR tubes in the NMR Spectroscopy Facility of Warwick University on either a 500-MHz spectrometer Bruker DRX-500 or a 400-MHz Bruker spectrometer, experiments were carried out at 298 K unless otherwise stated. ^{1}H-NMR

chemical shifts were internally referenced. Typically, the spectral width was 20 ppm. Spectra were processed using Bruker Topspin 2.1. Elemental analysis (percentages of C, H and N) was carried out on a CE-440 Exeter Elemental Analyzer by the Warwick Analytical Service.

METHODS

Compound synthesis and characterization. [(η^5-$C_5Me_4C_6H_5$)Ir(azpy-NMe_2)Cl]PF_6 (4). [(η^5-$C_5Me_4C_6H_5$)$IrCl_2$]$_2$ (45 mg, 0.05 mmol), and ligand 4-(2-pyridylazo)-N,N-dimethylaniline (23 mg, 0.10 mmol) in MeOH (20 mL) were stirred for 12 h at ambient temperature. The volume was slowly reduced to half on a rotary evaporator and NH_4PF_6 (45 mg, 0.28 mmol) was added. After standing at 277 K, a microcrystalline product formed. This was collected by filtration, washed with diethyl ether, and recrystallised from methanol/diethyl ether. Yield: 56 mg (70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.34 (d, 1H, *J* = 8.0 Hz), 8.25 (d, 1H, *J* = 5.5 Hz), 8.11–8.07 (m, 2H), 8.03 (t, 1H, *J* = 8.0 Hz), 7.49–7.41 (m, 5H), 7.13 (t, 1H, *J* = 7.0 Hz), 6.68–6.64 (m, 2H), 3.31 (s, 6H), 1.75 (s, 3H), 1.69 (s, 3H), 1.65 (s, 3H), 1.49 (s, 3H). Anal. Calcd for $C_{28}H_{31}ClF_6IrN_4P$ (796.15): C, 42.24; H, 3.92; N, 7.04. Found: C, 42.32; H, 3.91; N, 7.16.

IC_{50} determinations. A2780 cells were seeded in 96 well places with ca. 5000 cells per well. The plates were incubated at 310 K for 48 h. A 2 mM stock solution of each compound was prepared in 5% DMSO, 95% saline, from which final dilutions ranging from 0.1 to 100 μM were made with RPMI-1640. Drug exposure time was 24 h at 310 K, after which supernatants were removed cells were allowed to recover for 72 h in drug-free medium at 310 K.

Sulforhodamine B assay was used to determine cell survival. IC_{50} values (as the half maximal inhibitory concentration) were determined from duplicates of triplicates in independent experiments and standard deviations were calculated.

IC_{50} modulation by co-incubation with L-BSO. Cell viability studies were carried out as described in the above section with the following experimental modification. Cells were co-exposed to L-BSO and each of the Ir complexes, using solutions which was prepared independently and added within 5 min of each other.

TEM sample preparation. A2780 cells were seeded at a density of 5 x 10^6 cells/100mm Petri dish using 10 mL RPMI-1640 medium. On day 1 the cells were exposed to 5 μM of compound **1**. After 24 h the medium was removed and the cells were washed with PBS, fixed with 2% glutaraldehyde (Agar Scientific) in cadodylate buffer at pH 7.6 (Agar Scientific) and left for 1 h agitating on a shaker. Plates were rinsed with cadodylate buffer and the cells were transferred to falcon tubes, and centrifuged. Finally, cells were dehydrated with graded levels of ethanol (20-100% ethanol). Samples were then infiltrated with 100% propylene oxide for 1 h followed by 1:1 mixture of propylene oxide and embed 812 resin for 6 h. This was then replaced with several changes of 100% resin over an 18 h period before curing for 24 h at 333 K. Blocks were trimmed and sectioned on a Leica Ultracut E ultramicrotome (Leica Microsystems). Sections of 70-80 nm thick were collected on 400-mech Cu grids and stained with 2% uranyl acetate. Sections were imaged on a JOEL 1200EXII TEM with a Gatan 1 k × 1 k CCD camera.

Mitochondrial polarization assay. These experiments were carried out using the JC-10 Mitochondrial Membrane Potential Assay kit from Abcam, according to the manufacturer's instructions. Briefly, Cells were seeded at 1 x 10^6 cells per well in 6-well plates and left to incubate for 24 h. Drug solutions were added at the IC_{50} and 1/3 of the IC_{50} (Table 1) and the cells left to incubate for a further 4 or 24 h. Cells were washed twice with PBS and transferred to 12 x 75 mm flow cytometry test tubes in 500 µL solution of dye (125 µL JC-10 in 25 mL binding buffer) and the tubes protected from light for an incubation period of 30 min at ambient temperature. CCCP was used as positive control, with a drug exposure time of 30 min. Samples were immediately run on Beckton Dickinson FACScan with fluorescence detection, where FL2 fluorescence decreases as membrane polarization occurs.

Table S1A. NCI/NIH database correlations for complex **1**

Correlations to Standard Agents and Synthetics database	Pairwise correlation coefficient (*r*)
64414	0.839
634791	0.835
754708	0.881
754721	0.902
754722	0.849
755637	0.755
755639	0.881
755640	0.828
761277	0.916
Actinomycin D	0.698
Asiaticoside	0.79
Aurantomycin B	0.819
Bisantrene HCl	0.739
Bouvardin	0.806
Bouvardin ternafolia	0.712
Bruceantin	0.557
C.I Basic Red 2	0.773
Calendulaglycoside D2	0.766
Chromomycin A3	0.829
Daunorubicin	0.514
Deoxybouvardin	0.856
Ellipticine deriv	0.714
Gamitrinib-TPP	0.908
Harringtonine	0.707
Macbecin II	0.602
Malform A	0.761
Mito-tempol	0.765
Mitramycin	0.614
Nogalomycin C	0.71
Olivomycin	0.821
Phermerol	0.762
Phyllanthoside	0.929
Quassinoid	0.783
Rhodium dimer	0.802
Romidepson	0.713
Sempervirine, nitrate, dihydrate	0.729
Stendomycin salicylate	0.769
Taxol	0.756
Undulatone	0.76
Vinblastine sulfate	0.804
Vinorelbine tartrate	0.83

Table S1B. NCI/NIH database correlations for complex **2**

Correlations to Standard Agents and Synthetics database	Pairwise correlation coefficient (*r*)
634791	0.86
644614	0.821
754708	0.874
754722	0.865
755637	0.745
755639	0.858
755640	0.775
756057	0.927
761277	0.832
Actinomycin D	0.58
Anthrapyrazole	0.501
Asiaticoside	0.757
Aurantimycin B	0.76
Bisanrene HCl	0.727
Bouvardin	0.791
Chromomycin A3	0.768
CI.Basic red 2	0.739
Daunorubicin	0.514
Deoxybouvardin	0.805
Doxorubicin	0.501
Echinomycin	0.517
Ellipticine deriv	0.722
Macbecin II	0.696
Malformin A	0.772
Mito-tempol	0.766
Mitramycin	0.535
Phemerol	0.738
Phyllanthoside	0.769
Quassinoid	0.71
Rhodium dimer	0.743
Sempervirine, nitrate, dihydrate	0.703
Stendomycin salicylate	0.75
Taxol	0.693
Vinblastine sulfate	0.767
Vinorelbine tartrate	0.692

Table S1C. NCI/NIH database correlations for complex **3**

Correlations to Standard Agents and Synthetics database	Pairwise correlation coefficient (*r*)
634791	0.627
644614	0.686
754708	0.627
754722	0.6
755639	0.606
755640	0.647
756057	0.643
761277	0.604
761278	0.67
5,6-ethylenediquino[3,2-B]	0.676
A-7	0.552
Berberine idodide	0.554
C.I basic red 2	0.661
Diethylcyanine	0.591
Juncusol deriv	0.6
Laurodin	0.575
Leucinostatin A	0.582
Mito-tempol	0.574
Phemerol	0.653
Rhodium dimer	0.573
Stendomycin salicylate	0.606
Trichloromethyldihydroberbrine, 8-	0.583
Vancide 26	0.619
Vinblastine sulfate	0.507

Table S1D. NCI/NIH database correlations for complex **4**

Correlations to Standard Agents and Synthetics database	Pairwise correlation coefficient (*r*)
754709	0.738
755638	0.711
2-Hydroxy discorhabdin D	0.759
9-Alpha-hydroxyparthenolide	0.705
Aquaymycin	0.688
Bis(helenalinyl)glutarate	0.741
Bishelenalinyl malonate	0.678
Carquinostatin B	0.693
Cycloalkannin	0.698
Diccorhabdin B	0.708
Discorhabdin C, TFA	0.778
Discorhabdin G	0.71
Discorhabdin I	0.707
Enhydrin A	0.698
Eupacurvin	0.739
Farinosin, dehydro	0.68
Geldamycin	0.72
Herbimycin	0.729
Julimycin B2	0.714
L-cysteine analogue	0.544
Landomycin A	0.694
Melampodinin	0.704
Nanomycin	0.677
Pibenzimol HCl	0.585
Rifamycin	0.759
Rifamycin SV	0.677
Rifamycin SV	0.759
Streptonigrin	0.701
Tolypomycin Y	0.677
Tris(4-methyltropolonato)bismuth(III)	0.689
Weisiensin A	0.703
Withacnistin	0.7
Withanolide, 4-B-hydroxy	0.677

Table S1E. NCI/NIH database correlations for cisplatin

Correlations to Standard Agents and Synthetics database	Pairwise correlation coefficient (*r*)
2,2'-bioxirane	0.724
8,9- dimethoxycamptothecin	0.649
Amsacrine	0.619
Aphidicolin glycinate	0.637
Aziridinyl benzoquinone	0.651
AZQ	0.718
Bizelesin	0.659
Bleomycin A5, sulfate	0.625
Camptothecin, acetate	0.602
Carboplatin	0.783
Chlorambucil	0.717
Chlorambucil	0.671
Chlorozotocin	0.616
Dianhydrogalatitol	0.713
Didemnin B	0.515
Dijodbenzotef	0.677
Epoxypiperazine	0.667
Flavoneacetic acid	0.688
Hepsulfam	0.652
Imidazoquinoline	0.691
Isophosphoramide mustard	0.623
K31	0.682
K33	0.68
Kedarcidin	0.624
Malonatodiammine platinum (II)	0.815
Melphalan	0.673
Mitomycin C	0.652
Piperazine alkylator	0.671
Pipobroman	0.63
Porfiromycin	0.64
Thio-tepa	0.708
Tomaymycin	0.601
Triethlenemelamine	0.667
Uracil nitrogen mustard	0.706

Table S1F. NCI/NIH database correlations for oxaliplatin

Correlations to Standard Agents and Synthetics database	Pairwise correlation coefficient (*r*)
1,2-Diamino-cyclohexane-Pt-SEO3	0.747
1,6- Dimethoxyphenanzine DI-N-oxide	0.577
3-Deazauridine	0.548
4-Chloro-3-nitrobenzanilide	0.601
5-Fluorouracil	0.534
Acivicin	0.508
Anguidine	0.538
Bispyridocarbazollum DMS	0.553
Brequinar	0.506
Carboplatin	0.715
Cyclopentenylcytosine	0.565
Dichloroallyl lawsone	0.529
Largomycin	0.555
Methotrexate	0.557
Miliisane B	0.584
Miliusate	0.608
N,N- dibenzyldaunomycin	0.668
N,N- dibenzyldaunomycin	0.668
Niveusin A	0.579
Tetraplatin	0.644
Tetraplatin	0.644
Uridine, 5-fluoro	0.605

Table S2. Summary of NCI/NIH correlations for compounds **1**−**4**, cisplatin (cis) and oxaliplatin (ox).

Class	Agent	1	2	3	4	cis	ox
DNA inter	actinomycin D*						
	bisantrene HCl	+	+				
	chromomycin A3		+				
	daunorubicin*						
	echinomycin						
	mitramycin						
	rifamycin SV				+		
	olivomycin	++					
	rhodium dimer	++	+				
DNA antimet[§]	macbecin II						
	landomycin A						
Alkyl	L-cysteine analogue						
Topo	doxorubicin*						
	anthrapyrazole						
	berberine iodide						
Prot syn[§]	phyllanthoside	+	+				
	bruceantin						
	didemnin B						
	anguidine						
	aurantimycin B	++					
	ellipticine deriv	+	+				
	bouvardin	++	+				
	harringtonine*	+					
	undulatone	+					
Mitosis	taxol*	+					
	vinblastine sulfate*	++	+				
	malformin A	+	+				
	vinorelbine tartrate*	++					
Oxidat[§]	asiaticoside	+	+				
	cycloalkannin						
	nogalomycin C	+					

(*****) Indicates a compound in clinical trials for the treatment of cancer.
(+) indicates a compound with a correlation where $r > 0.7$ and (++) where $r > 0.8$.
(§) DNA inter: DNA intercalators; DNA antimet: DNA antimetabolite; Alkyl: alkylating agents; Topo: topoisomerase inhibitors; Prot syn: protein synthesis inhibitors; Mitosis: mitosis inhibitors; Oxidat: inhibitors of oxidative processes.

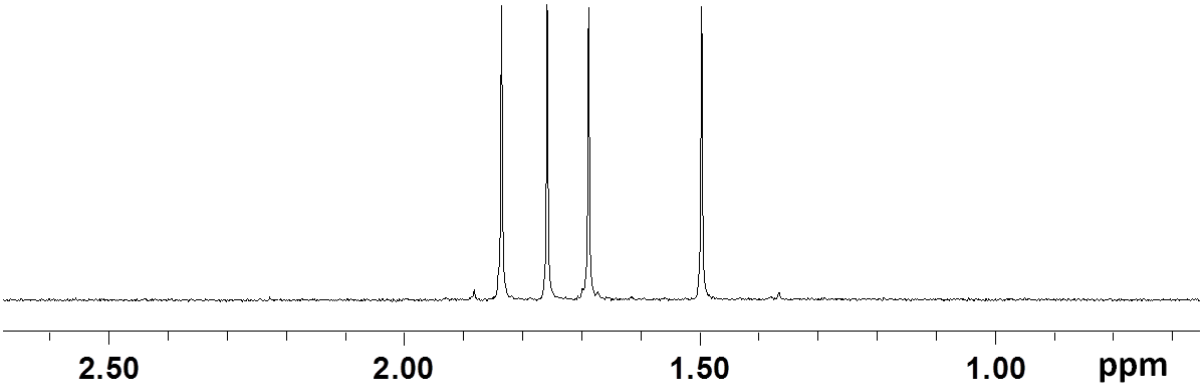

Figure S1. ^{1}H NMR spectrum showing the methyl region of **4** (0.7 mM) in 10% MeOD-d_4/90% D_2O (v/v). By comparison with the spectrum of the aqua complex (prepared using $AgNO_3$ to remove the chloride ligand), it can be concluded that the above spectrum is that of intact complex 4 and that no hydrolysis was observed after 24 h. Also the addition of NaCl did not cause further changes to the spectrum.

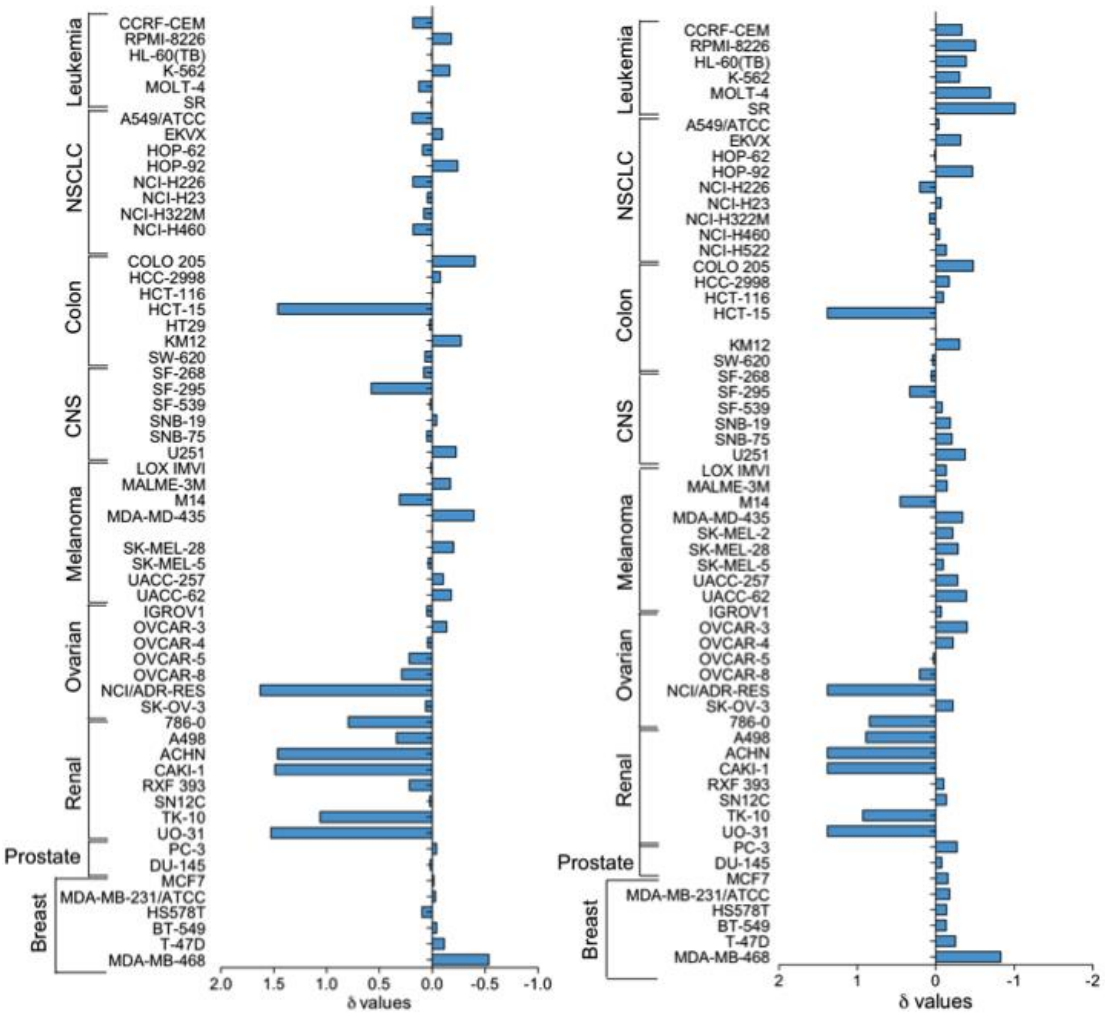

Figure S2. NCI60 GI_{50} mean graphs for **1** (left) and **2** (right).

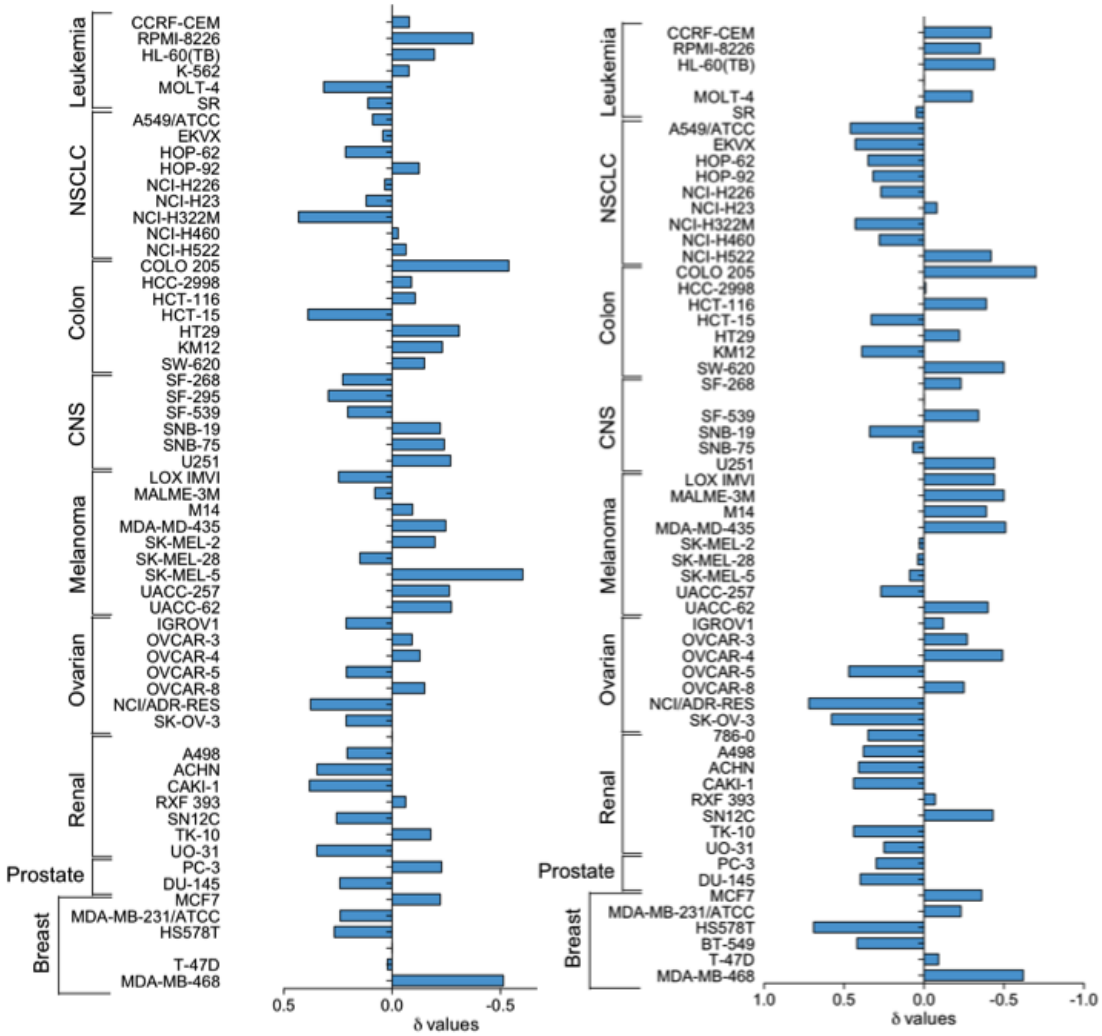

Figure S3. NCI60 GI_{50} mean graphs for **3** (left) and **4** (right).