Electronic Supplementary Material (ESI) for Dalton Transactions.

Platinum(IV) N-heterocyclic carbene complexes: synthesis, characterisation and cytotoxic activity.

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A) X-ray data for 1_{Cl}

B) Kinetic studies in DMSO

C) Apoptosis analysis by Annexin V-Propidium Iodide labelling

D) Supplementary information for ROS quantification and mitochondrial respiratory activity

A) X-ray data for $\mathbf{1_{Cl}}$

X-ray diffraction data collection was carried out on a Nonius Kappa-CCD diffractometer equipped with an Oxford Cryosystem liquid N_2 device, using Mo-Kα radiation (λ = 0.71073 Å). The crystal-detector distance was 36mm. The cell parameters were determined (Denzo software)[1] from reflections taken from one set of 10 frames (1.0° steps in phi angle), each at 20s exposure. The structures were solved by direct methods using the program SHELXS-2013.[2] The refinement and all further calculations were carried out using SHELXL-2013.[3] The H-atoms were included in calculated positions and treated as riding atoms using SHELXL default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F2. A semi-empirical absorption correction was applied using the MULscanABS routine in PLATON.[4]

[1] "Kappa CCD Operation Manual", Nonius B. V., Ed.; Delft: The Netherlands 1997.
[2] G. M. Sheldrick, *Acta Cryst.* 1990, **A46**, 467.
[3] G. M. Sheldrick, *Acta Cryst.* 2008, **A64**, 112.
[4] Spek, A.L. *J.Appl.Cryst.* 2003, **36**, 7.

CCDC number 1452899

Complexes	**1_{Cl}**
Empirical formula	C16 H17 Cl4 N3 Pt
Formula weight	588.21
Temperature	173(2) K
Wavelength	0.71073 A
Crystal system	*Monoclinic*
space group	*C 2/c*
Unit cell dimensions	
a (Å)	a = 28.198(2) A
b (Å)	b = 8.8580(6) A
c (Å)	c = 16.9570(14) A
α (°)	alpha = 90 deg
β (°)	beta = 117.956(3) deg
γ (°)	gamma = 90 deg
Volume ($Å^3$)	3741.2(5) A^3
Z	8
Calculated density (Mg/m^3)	2.089 Mg/m^3
Absorption coefficient (mm^{-1})	8.075 mm^-1
F(000)	2240
Crystal size (mm)	0.10 x 0.09 x 0.04 mm
Theta range (°)	1.635 to 27.493 deg
Limiting indices	-36<=h<=36, -11<=k<=10, -21<=l<=15
Reflections collected / unique / R_{int}	14528 / 4274 [R(int) = 0.1174]

Completeness to theta	100.0 %
Absorption Correction	Semi-empirical from equivalents
Max. and min. transmission	0.64917 and 0.44581
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4274 / 0 / 176
Goodness-of-fit on F_2	1.042
Final R indices *R1, wR2 (I>2σ(I))*	R1 = 0.0619, wR2 = 0.1278
R1, wR2 (all data)	R1 = 0.1503, wR2 = 0.1802
Largest diff. peak and hole (e.Å^{-3})	2.415 and -3.796 e.A^-3
Extinction coefficient	n/a

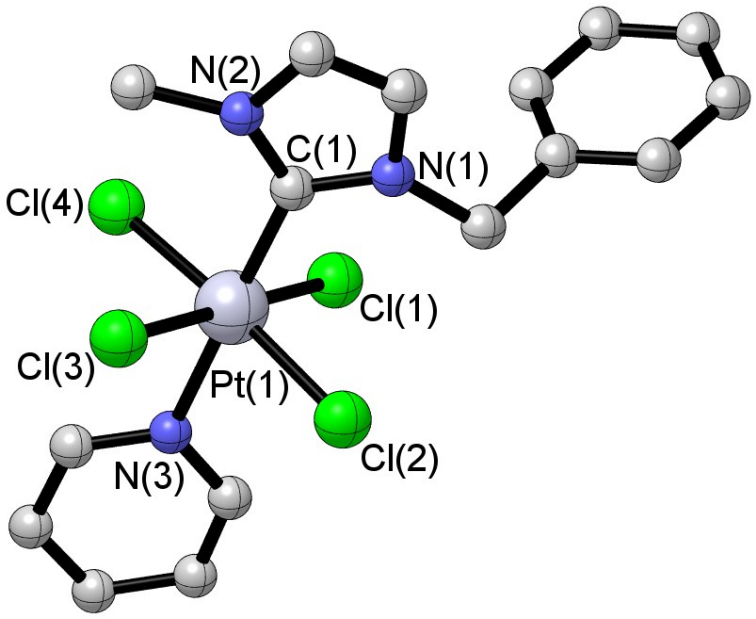

Figure S1. Molecular structure of the Pt(IV) NHC complex $\mathbf{1_{Cl}}$. Selected bond distances (Å) and angles (deg): C(1)-Pt(1), 2.034(11); Cl(1)-Pt(1), 2.329(3); Cl(2)-Pt(1), 2.327(3); Cl(3)-Pt(1),

2.336(3); Cl(4)-Pt, 2.330(3); N(3)-Pt(1), 2.127(9); C(1)-Pt(1)-N(3), 178.4(4); C(1)-Pt(1)-Cl(2), 91.6(4); N(3)-Pt(1)-Cl(2), 89.2(3); Cl(2)-Pt(1)-Cl(4), 177.34(12); Cl(1)-Pt(1)-Cl(4), 91.85(12).

B) Kinetic studies in DMSO

General procedure for reduction of (NHC)$PtCl_4$(amine) complexes in DMSO

The stability of the complexes was monitored in d_6-DMSO by ^{1}H NMR at 25 °C and at a concentration of 10^{-3}M of platinum. The formation of *cis* (NHC)PtX_2(DMSO) species was confirmed by comparison with synthetic samples.[5] The kinetic of the reaction was monitored via integration of the ^{1}H NMR signals attributed to the ortho-positions in the pyridine moiety in free pyridine (δ = 8.8 ppm) versus its platinum-coordinated derivative (δ = 9.1 ppm) (in d_6-DMSO, see example Figure S2).

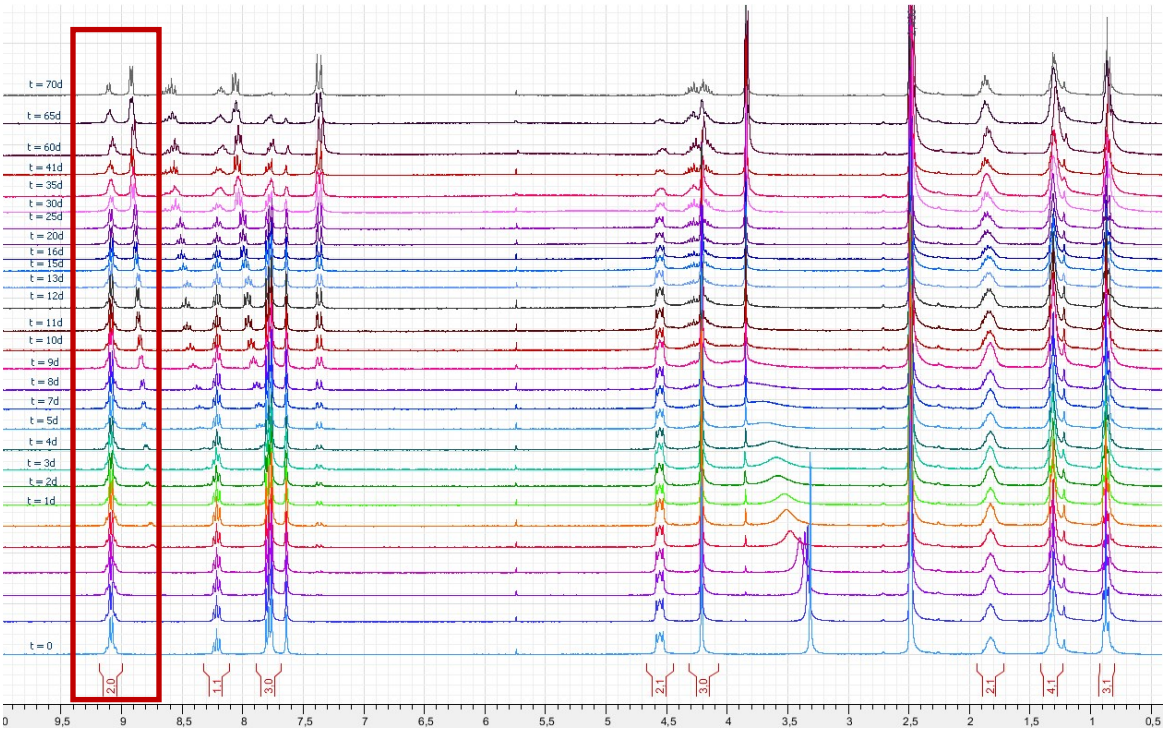

Figure S2. Reaction progress with platinum complex $\mathbf{4_{Cl}}$ (representative example) at 25 °C in d_6-DMSO.

[5] a) J. K. Muenzner, T. Rehm, B. Biersack, A. Casini, I. A. M. de Graaf, P. Worawutputtapong, A. Noor, R. Kempe, V. Brabec, J. Kasparkova and R. Schobert, *J. Med. Chem.* 2015, **58**, 6283; b) J. J. Hu, F. Li and T. S. A. Hor, *Organometallics* 2009, **28**, 1212.

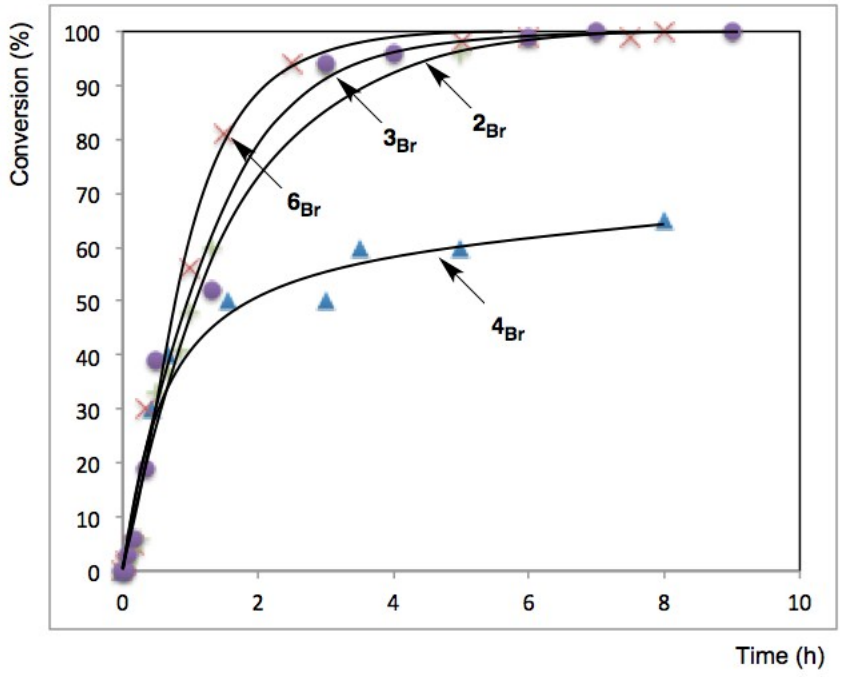

Figure S3. Conversion of (NHC)$PtBr_4$(amine) complexes into the corresponding (NHC)$PtBr_2$(amine) compound as a function of time (25 °C, DMSO, 10^{-3}M).

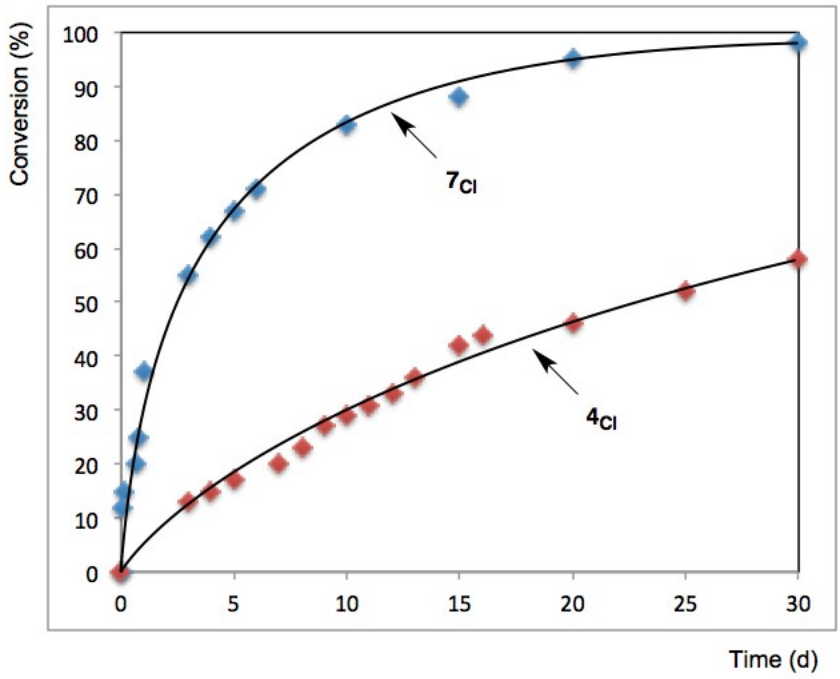

Figure S4. Conversion of (NHC)$PtCl_4$(amine) complexes into the corresponding (NHC)$PtCl_2$(amine) compound as a function of time (25 °C, DMSO, 10^{-3}M).

General procedure for reduction of (NHC)$PtCl_4$(pyridine) complex 4_{Cl} by glutathione

Selected complex $\mathbf{4_{Cl}}$ (0.4 mg) was solubilised in d_6-DMSO (final concentration, C = 10^{-3}M) with 1 equiv. glutathione (0.2 mg) and evolution was followed by ^{1}H NMR at 25 °C until full conversion (Figure S5). The kinetic of the reaction was monitored via integration of the ^{1}H NMR signals attributed to the ortho-positions in the pyridine moiety in free pyridine (δ = 8.8 ppm) versus its platinum-coordinated derivative (δ = 9.1 ppm) (in d_6-DMSO).

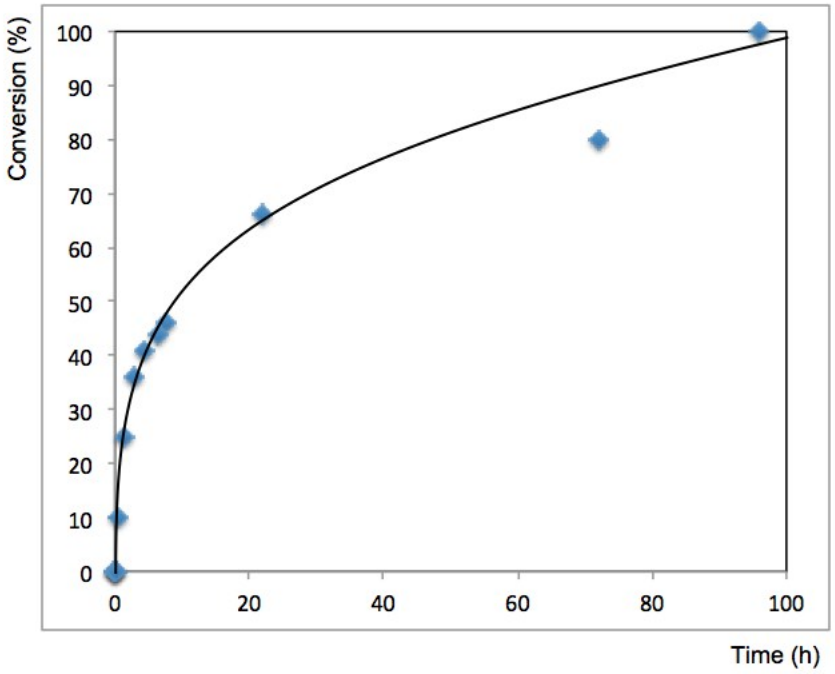

Figure S5. Conversion of (NHC)$PtCl_4$(pyridine) complex $\mathbf{4_{Cl}}$ in the presence of one equiv. of glutathione as a function of time (25 °C, d_6-DMSO, 10^{-3}M) monitored by ^{1}H NMR.

C) <u>Apoptosis analysis by Annexin V-Propidium Iodide labelling (4_{Cl} and 4_{Br})</u>

The human colon cancer cells HCT116 p53 KO were plated ($5*10^4$ cells/well) in 96-well plates. The next day, they were treated either with $\mathbf{4}_{Cl}$ (0.1, 1, 10 and 100 µM) or with $\mathbf{4}_{Br}$ (0.1, 1, 10 and 100 µM) or with oxaliplatin (0.1, 1, 10 and 100 µM), a platinum derivative used as anticancer agent. After 24 hours at 37 °C and 5% CO_2, the cells were dissociated using trypsin-EDTA (Sigma-Aldrich) and incubated with Annexin-V-APC (Becton Dickinson Pharmingen) at 25°C in the dark for 15 minutes. Cells were then stained with Propidium Iodide (PI) (Sigma Aldrich, 0.01 mg/mL) and analysed by a FacsCalibur (Becton Dickinson) flow cytometer and the CellQuest Pro Software. Between 2000 and 5000 cells were acquired for each experimental condition and the results were processed with FlowJo Data Analysis Software. Experiments were performed in triplicate.

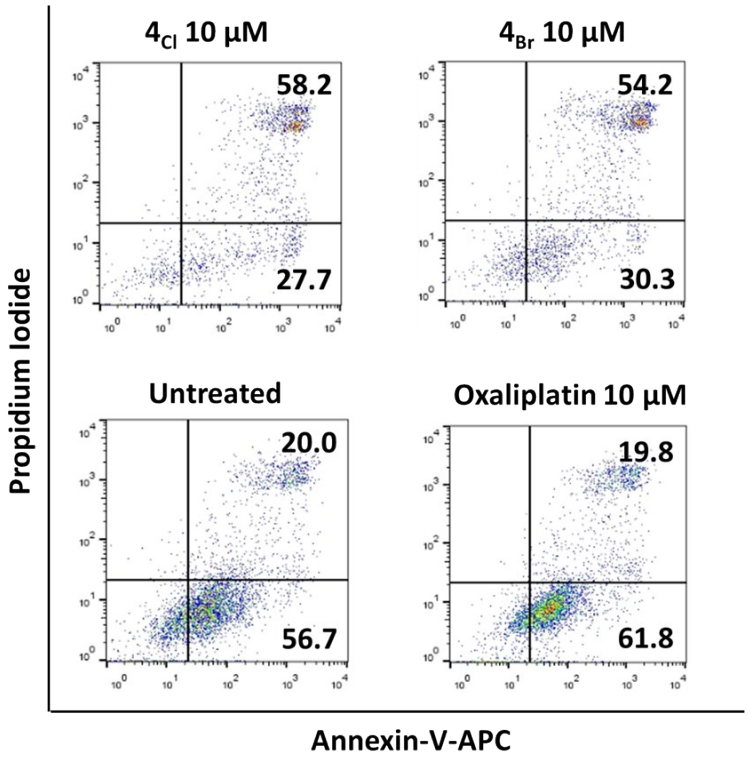

Figure S6. Evaluation of apoptosis on HCT116 p53 KO cell lines after treatment with $\mathbf{4}_{Cl}$, $\mathbf{4}_{Br}$ or oxaliplatin at indicated concentrations (24 h of incubation). After treatment, cells were labelled Annexin V-APC and Propidium Iodide and analysed by flow cytometry. Significant flow cytometry dot plots showing apoptosis induction are represented. The percentage of early apoptotic cells (Annexin V^+/PI^-) and late apoptotic cells (Annexin V^+/PI^+) are indicated.

D) <u>Supplementary information for ROS quantification and mitochondrial respiratory activity (MitoSox and MitoTracker staining)</u>

The human colon cancer cells HCT116 were plated ($5*10^4$ cells/well) in 96-well plates and after they had adhered, they were treated either with $\mathbf{4}_{Cl}$ (0.1, 1, 10 and 100 µM) or $\mathbf{4}_{Br}$ (0.1, 1.0, 10 and 100 µM) or with oxaliplatin (0.1, 1, 10 and 100 µM, Sigma-Aldrich), a platinum derivative used as anticancer agent, or with staurosporine (0.1, 1, 10 and 100 µM, Sigma-Aldrich), a compound known to induce ROS production. After 12 hours at 37°C and 5% CO_2, the cells were dissociated using trypsin-EDTA (Sigma-Aldrich) and incubated with either MitoSox Red mitochondrial superoxide indicator (1.0 µM, Invitrogen) or with MitoTracker Green FM and MitoTracker Deep Red 633 (0,2 µM, Invitrogen) followed by an incubation at 37°C in the dark for 30 minutes. Between 2000 and 5000 cells were acquired for each experimental condition using a FacsCalibur (Becton Dickinson) flow cytometer and the CellQuest Pro Software. The results were processed with FlowJo Data Analyis Software. Experiments were performed in triplicate.