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Synthesis and anticancer activity of stable Pt(IV) monodentate phosphine complexes from their Pt(II) anticancer precursors.

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A) Full characterization and detailed synthesis of the complexes

B) Supplementary information of the reduction potential studies

C) Supplementary information for the protocols and cell lines

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E) Supplementary information of the pBR322 binding studies and conditions.

F) supplementary information of the reaction of complex 1 and GSH

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A) Full characterization and detailed synthesis of the complexes

Freshly synthesized Chlorine gas was bubble through a solution of *trans*-$PtCl_2(PPh_3)$(amine) in CH_2Cl_2 for 1 hour to give a crystalline intense yellow solid.

***t*-$PtCl_4(PPh_3)(NH(CH_3)_2)$** (**1**): Yellow solid. Yield: 64%. Elemental analysis CHN, found: C,36.71; H,3.78; N,2.37; calculated for $C_{20}H_{22}Cl_4NPPt$: C,37.28; H,3.44; N,2.17. NMR δ_H (300.13MHz; $CDCl_3$) 2.70(6H, sept CH_3-dma), 4.34(1H, br, NH), 7.41(6H, m, C_{meta}), 7.50(3H, m, C_{para}), 7.94(6H, m, C_{orto}). δ_C (75.47MHz; $CDCl_3$), 41.23(CH_3-dma), 127.6(CH-Ar), 132.1(CH-Ar), 138.5(CH-Ar). δ_P (121,5MHz; $CDCl_3$), 2.11($J_{P\text{-}Pt}$ 2121,15). δ_{Pt} (64.53MHz; $CDCl_3$) -1545 ($J_{Pt\text{-}P}$ 2122.32).

***t*-$PtCl_4(PPh_3)(NH_2CH(CH_3)_2)$** (**2**): Yellow solid. Yield: 52%. Elemental analysis CHN, found: C,38.26; H,4.02; N,2.07; calculated for $C_{21}H_{24}Cl_4NPPt$: C,38.32; H,3.67; N,2.13. NMR δ_H (300.13MHz; (Acetona D_6) 1.43(6H, d, $J_{H\text{-}H}$ 5.6, CH_3-dma), 3.72(1H, sept, CH-ipa), 4.73(1H, br, NH), 7.49(6H, m, C_{meta}), 7.56(3H, m, C_{para}), 7.61(6H, m, C_{orto}). δ_C (75.47 MHz; Acetona D_6), 23.4(CH_3-ipa), 127.5(CH-Ar), 131.9(CH-Ar), 135.7(CH-Ar). δ_P (121,5MHz; Acetona D_6), 0.79($J_{P\text{-}Pt}$ 2158,17). δ_{Pt} (64.53MHz; Acetona D_6) -1496 ($J_{Pt\text{-}P}$ 2159.43).

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B) Supplementary informacion of the reduction potential studies

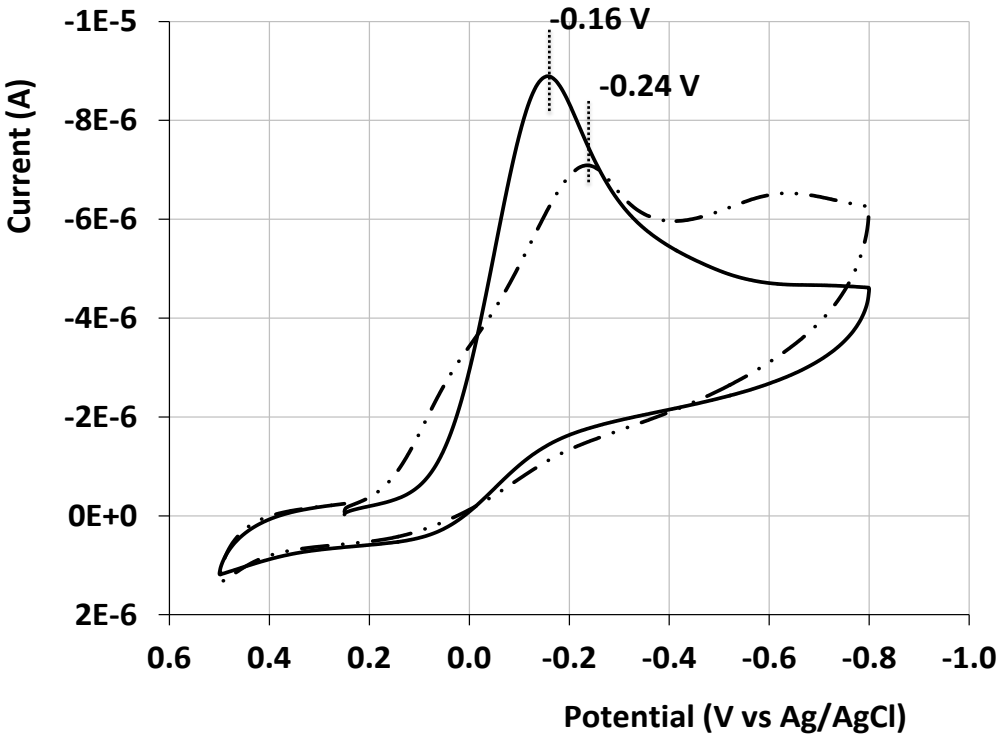

Figure SM1. Cyclic voltammograms of 3 mM complex **2** (solid line) and complex **1** (dash dotted line) in a 0.1 M of $NaClO_4$ water:DMSO (1:2) solution at a carbon disc electrode and scan rate of 100 $mV \cdot s^{-1}$.

Experimental part

The reduction potentials have been examined for *trans*-Pt^{IV} complexes **1** and **2**. The electrochemical study of Pt^{IV} analogues of cisplatin has been also included to control.

Generally, cyclic voltammetry (CV) is used for measuring the reduction potential (Ep) of Pt^{IV} complexes. The reduction of Pt^{IV} is an irreversible two electron process involving the loss of axial ligands and a return to square-planar geometry of Pt^{II}. The reported Ep values are cathodic half-wave potentials and are measured at the peak of the forward wave. The CV obtained for **1** and **2** complexes are shown in Figure SM1. The reduction potentials of all Pt^{IV} complexes are given in the manuscript.

C) Supplementary information for the protocols and cell lines

Cell lines. C A375 human metastatic melanoma and NCI-H460 lung cancer cell lines were purchased from ATCC. Colorectal carcinoma cell lines HCT116, and

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matched p53-deficient HCT116 (-/-) were a kind gift of Bert Vogelstein (John-Hopkins University, Baltimore, MD). These cell lines were cultured with DMEN (A375 and NCI-H460) or RPMI (HCT116) (Sigma) with 10% foetal bovine serum, 2mM L-glutamine, 100 U/mL penicilin, 100 μg/mL streptomicin in humidified air with 5% CO_2 at 37 ºC.

Assessment of Cytotoxicity. All tests were repeated at different times as controls..The compounds were tested in 96-well plates. Cells growing in a flask were harvested just before they became confluent, counted using a haemocytometer and diluted with media adjusting the concentration to the required number of cells per 0.2 ml (volume for each well). The cells were then seeded in the 96-well plates at a density depending of the cell size, between 1000 and 4000 cells/well. The cells were then left to plate down and grow for 24 hours before adding the drugs. The compounds were weighed out, dissolved in DMSO and diluted with PBS to reach 10 mM solutions. From here a "mother plate" with serial dilutions was prepared at 200X the final concentration in the culture. Each mother plate was stored at -20 ºC until further use. The appropriate volume of the compound solution (usually 2 μL) was added automatically (Beckman FX 96 tip) to media to make it up to the final concentration for each drug. The media was removed from the cells and replaced with 0.2ml of the media dosed with drug. Each concentration was assayed in triplicate. A set of control wells are left on each plate, containing media without drug. A second set of control wells were left on each plate, containing media with the same concentration of PBS. Another control set was obtained with the cells untreated just before adding the drugs (seeding control, number of cells starting the culture) The cells were exposed to the drugs for 96 hours and then washed twice with phosphate buffered saline before being fixed with 10% glutaraldehyde. Cells were washed twice and fixed with crystal violet 0.5% during 30 minutes, extensively washed and the absorbance was measured at 595 nm.

D) Supplementary information of the X-Ray characterization

Experimental data complex 1

A clear yellow prismatic-like specimen of $C_{20}H_{22}Cl_4NPPt$, approximate dimensions 0.20 mm x 0.24 mm x 0.32 mm, was used for the X-ray crystallographic analysis. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 37418 reflections to a maximum θ angle of 28.29° (0.75 Å resolution), of which 5342 were independent (average redundancy 7.004, completeness = 99.7%, R_{int} = 2.40%, R_{sig} = 1.35%) and 5247 (98.22%) were greater than $2\sigma(F^2)$.The final cell constants of a = 8.6761(7) Å, b = 9.8525(8) Å, c = 14.2672(11) Å, α = 73.172(3)°, β = 75.434(3)°, γ = 69.505(4)°, volume = 1078.02(15) $Å^3$, are based upon the refinement of the XYZ-centroids of 9996 reflections above 20 σ(I) with 5.978° < 2θ < 56.57°.Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.758. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.2102 and 0.3315. The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group P -1, with Z = 2 for the formula unit, $C_{20}H_{22}Cl_4NPPt$.The final

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anisotropic full-matrix least-squares refinement on F^2 with 246 variables converged at R1 = 1.22%, for the observed data and wR2 = 7.77% for all data. The goodness-of-fit was 1.196. The largest peak in the final difference electron density synthesis was 1.165 $e^-/Å^3$ and the largest hole was -1.644 $e^-/Å^3$ with an RMS deviation of 0.482 $e^-/Å^3$. On the basis of the final model, the calculated density was 1.985 g/cm^3 and F(000), 620 e^-.

Table SM4. Sample and crystal data for complex 1.

Chemical formula	$C_{20}H_{22}Cl_4NPPt$	
Formula weight	644.25	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal size	0.20 x 0.24 x 0.32 mm	
Crystal habit	clear yellow prismatic	
Crystal system	triclinic	
Space group	P -1	
Unit cell dimensions	a = 8.6761(7) Å	α = 73.172(3)°
	b = 9.8525(8) Å	β = 75.434(3)°
	c = 14.2672(11) Å	γ = 69.505(4)°
Volume	1078.02(15) $Å^3$	
Z	2	
Density (calculated)	1.985 Mg/cm^3	
Absorption coefficient	7.084 mm^{-1}	
F(000)	620	

Table SM5. Data collection and structure refinement for 1.

Theta range for data collection	1.51 to 28.29°	
Index ranges	-11<=h<=11, -13<=k<=13, -19<=l<=19	
Reflections collected	37418	
Independent reflections	5342 [R(int) = 0.0240]	
Coverage of independent reflections	99.7%	
Absorption correction	multi-scan	
Max. and min. transmission	0.3315 and 0.2102	
Structure solution technique	direct methods	
Structure solution program	SHELXS-97 (Sheldrick, 2008)	
Refinement method	Full-matrix least-squares on F^2	
Refinement program	SHELXL-97 (Sheldrick, 2008)	
Function minimized	$\Sigma\, w(F_o^2 - F_c^2)^2$	
Data / restraints / parameters	5342 / 0 / 246	
Goodness-of-fit on F^2	1.196	
Δ/σ_{max}	0.001	
Final R indices	5247 data; I>2σ(I)	R1 = 0.0122, wR2 = 0.0454
	all data	R1 = 0.0158, wR2 = 0.0777
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.0558P)^2+0.7680P]$ where $P=(F_o^2+2F_c^2)/3$	
Largest diff. peak and hole	1.165 and -1.644 $eÅ^{-3}$	

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R.M.S. deviation from mean	0.482 eÅ^{-3}

Table SM6. Selected bond lengths (Å) for 1.

Pt1-Cl1	2.3274(8)	**Pt1-Cl2**	2.3267(9)
Pt1-Cl3	2.3098(8)	**Pt1-Cl4**	2.3138(8)
Pt1-N1	2.197(3)	**Pt1-P1**	2.3429(9)

Experimental data complex 2

A clear yellow prismatic-like specimen of $C_{21}H_{24}Cl_4NPPt$, approximate dimensions 0.10 mm x 0.19 mm x 0.22 mm, was used for the X-ray crystallographic analysis. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 30087 reflections to a maximum θ angle of 26.83° (0.79 Å resolution), of which 4662 were independent (average redundancy 6.454, completeness = 96.0%, R_{int} = 3.40%, R_{sig} = 2.38%) and 4337 (93.03%) were greater than $2\sigma(F^2)$.The final cell constants of a = 9.0349(8) Å, b = 9.8658(9) Å, c = 14.1981(13) Å, α = 72.771(5)°, β = 75.680(4)°, γ = 71.733(4)°, volume = 1131.09(18) Å^3, are based upon the refinement of the XYZ-centroids of 9880 reflections above 20 σ(I) with 5.244° < 2θ < 53.04°.Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.732. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.3181 and 0.5516. The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group P -1, with Z = 2 for the formula unit, $C_{21}H_{22}Cl_4NPPt$.The final anisotropic full-matrix least-squares refinement on F^2 with 255 variables converged at R1 = 2.20%, for the observed data and wR2 = 12.64% for all data. The goodness-of-fit was 1.097. The largest peak in the final difference electron density synthesis was 1.366 e$^-$/Å^3 and the largest hole was -3.017 e$^-$/Å^3 with an RMS deviation of 0.472 e$^-$/Å^3. On the basis of the final model, the calculated density was 1.927 g/cm^3 and F(000), 632 e$^-$.

Table SM1. Sample and crystal data for complex 2.

Chemical formula	$C_{21}H_{24}Cl_4NPPt$	
Formula weight	656.26	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal size	0.10 x 0.19 x 0.22 mm	
Crystal habit	clear yellow prismatic	
Crystal system	triclinic	
Space group	P -1	
Unit cell dimensions	a = 9.0349(8) Å	α = 72.771(5)°
	b = 9.8658(9) Å	β = 75.680(4)°
	c = 14.1981(13) Å	γ = 71.733(4)°
Volume	1131.09(18) Å^3	

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Z	2
Density (calculated)	1.927 Mg/cm^3
Absorption coefficient	6.754 mm^{-1}
F(000)	632

Table SM2. Data collection and structure refinement for complex 2.

Theta range for data collection	1.52 to 26.83°	
Index ranges	-11<=h<=11, -12<=k<=12, -17<=l<=17	
Reflections collected	30087	
Independent reflections	4662 [R(int) = 0.0340]	
Coverage of independent reflections	96.0%	
Absorption correction	multi-scan	
Max. and min. transmission	0.5516 and 0.3181	
Structure solution technique	direct methods	
Structure solution program	SHELXS-97 (Sheldrick, 2008)	
Refinement method	Full-matrix least-squares on F^2	
Refinement program	SHELXL-97 (Sheldrick, 2008)	
Function minimized	$\Sigma\, w(F_o^2 - F_c^2)^2$	
Data / restraints / parameters	4662 / 0 / 255	
Goodness-of-fit on F^2	1.097	
Final R indices	4337 data; I>2σ(I)	R1 = 0.0220, wR2 = 0.0706
	all data	R1 = 0.0305, wR2 = 0.1264
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.1000P)^2+0.8847P]$ where $P=(F_o^2+2F_c^2)/3$	
Largest diff. peak and hole	1.366 and -3.017 $eÅ^{-3}$	
R.M.S. deviation from mean	0.472 $eÅ^{-3}$	

Table SM3. Selected bond lengths (Å) for complex 2.

Pt1-Cl1	2.332(2)	**Pt1-Cl3**	2.315(2)
Pt1-Cl2	2.328(2)	**Pt1-Cl4**	2.319(2)
Pt1-N1	2.148(5)	**Pt1-P1**	2.350(2)

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E) Supplementary information of the pBR322 binding studies and conditions.

The DNA binding studies were done in a total volumen of 20µl. The DNA alicuots containing 8mL of DNA-pBR322 (10ng/mL stock) in 10mM Tris-HCl (pH 7.6) and 1mM EDTA were incubated with the platinum compounds at several ri values (0.001 a 0.5) using the corresponding amount of platinum from either stock solution 5µM or 50 µM. The samples were incubated at 37º for 24 h, after which time 2µL of a loading dye containing 50% glycerol, 0.25% bromophenol blue and 0.25% Xylenecyanol was added. The total of the sample (20µL) was loaded in the wells of a 0.8% agarose gel. Electrophoresis was carried out for a period of 2.5h approximately at 50V. After electrophoresis the gel was immersed in 800mL of Millipore water containing 64µL from a 10mg/mL stock solution of ethidium bromide for 30 m. to stain the DNA.

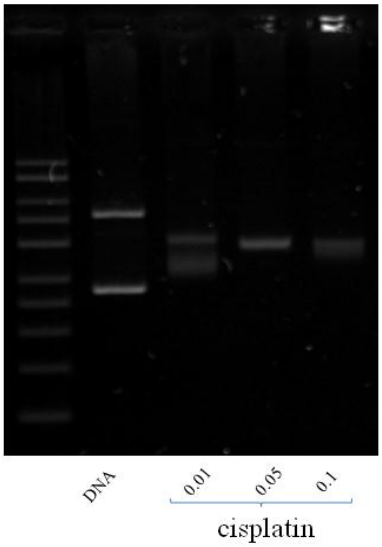

Figure SM2. Agarose gel electrophoresis of pBR322 plasmid treated with the cisplatin. Lane 1: Marker, lane 2: DNA of control plasmid. Plasmid DNA incubated at r_i = 0.01 to 0,2, with cisplatin (lanes 3 to 6). oc = open circular DNA form; ccc =covalently closed circular DNA form

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F) supplementary information of the reaction of complex 1 and GSH

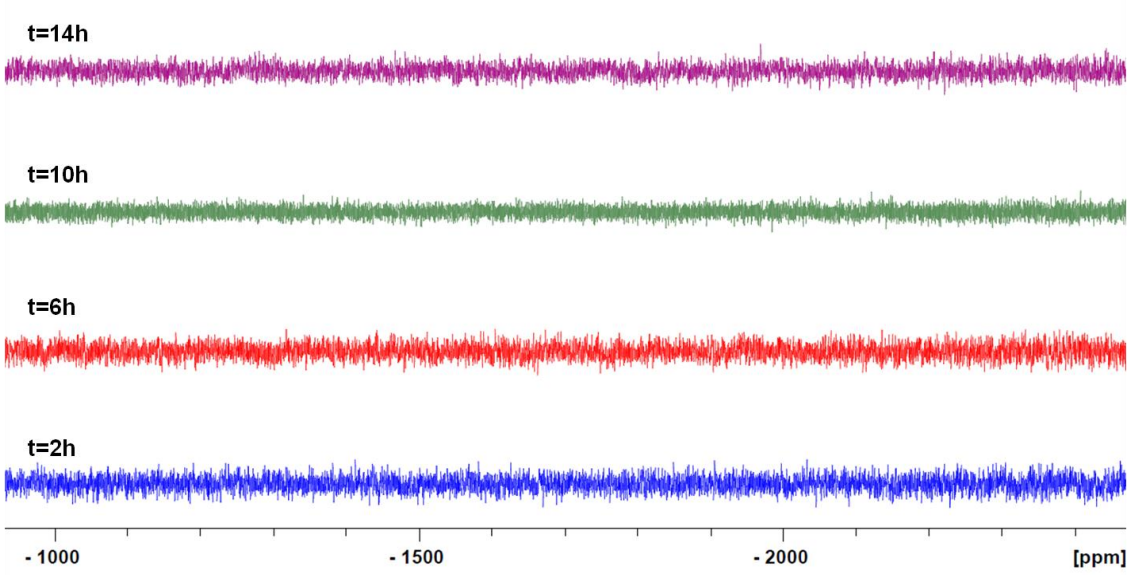

Figure SM3. ^{195}Pt NMR spectra in the area of Pt(IV) for complex **1** with GSH (ratio 1:2) in DMSO recorded from 2h to 14h.

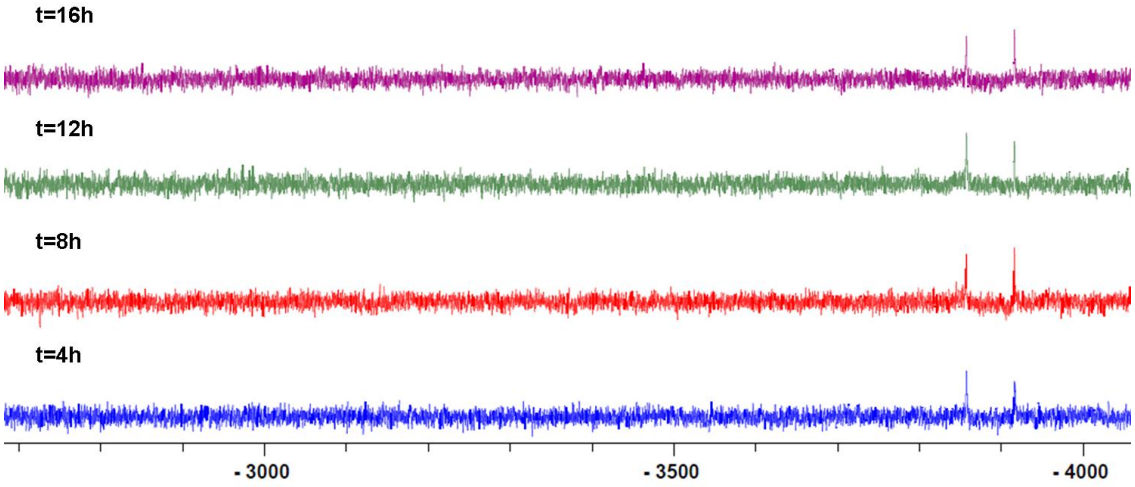

Figure SM4. ^{195}Pt NMR spectra in the area of Pt(II) for complex **1** with GSH (ratio 1:2) in DMSO recorded from 2h to 16h.