Electronic Supplementary Information (ESI)

Enhancement of the antiproliferative activity of $[RuCp(PPh_3)_2(dmoPTA\text{-}1\kappa P)]^+$ *via* its coordination to one $\{CoCl_2\}$ unit: synthesis, crystal structure and properties of $[RuCp(PPh_3)_2\text{-}\mu\text{-}dmoPTA\text{-}1\kappa P\text{:}2\kappa^2 N,N'\text{-}CoCl_2](OTf)\cdot 0.25H_2O$

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1.- Materials and instruments

All chemicals were reagent grade and, unless otherwise stated, were used and received by commercial suppliers. Likewise, all reactions were carried out in a pure argon atmosphere using standard Schlenk-tube techniques with freshly distilled and oxygen-free solvents. The complex $[RuCp(PPh_3)_2(HdmPTA)](OSO_2CF_3)_2$ (**1·2OTf**) was prepared as indicated recently.[1] Elemental analysis (C,H,N) were performed on a Fisons Instruments EA 1108 elemental analyzer. Infrared spectra (KBr, Aldrich) were measured with a ThermoNicolet Avatar 300FT-IR spectrometer. ^{1}H, COSY, $^{31}P\{^1H\}$ and $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker DRX300 spectrometer operating at 300.13 (^{1}H) and 75.47 MHz (^{13}C), respectively. Peak positions are relative to tetramethylsilane and were calibrated against the residual solvent resonance (^{1}H) or the deuterated solvent multiplet (^{13}C). $^{31}P\{^1H\}$ spectra were recorded on the same instrument operating at 121.49 and 282.40 MHz, respectively. Chemical shifts for $^{31}P\{^1H\}$ NMR were measured relative to external 85% H_3PO_4, it was measured with downfield values taken as positive. All NMR spectra were obtained at 25 °C.

2.- Experimental details

2.1. Synthesis of $[RuCp(PPh_3)_2\text{-}\mu\text{-}dmoPTA\text{-}1\kappa P\text{:}2\kappa^2 N,N'\text{-}CoCl_2]\cdot(OSO_2CF_3)\cdot 0.25H_2O$ (2·OTf·0.25H$_2$O)

Potassium *tert*-butoxide (0.010 g, 0.090 mmol) was added into a solution of **1·2OTf** (0.095 g, 0.083 mmol) in 10 mL of EtOH. After 15 min at room temperature finely grounded solid $CoCl_2\cdot 6H_2O$ (0.022 g, 0.094 mmol) was added. The resulting blue solution kept for 30 minutes at room temperature and then reduced to 5 mL under reduced pressure. The resulting blue solid was recrystallized in EtOH, providing green microcrystals that were filtered and air dried.

Yield: 0.043 g, 40.18%. $S_{25ºC,CHCl_3}$ > 97.3 mg/ml, $S_{25ºC,H_2O}$ < 0.5 mg/ml, $S_{25ºC,\ EtOH}$ = 12.3 mg/ml. $C_{49}H_{51}F_3Cl_2N_3O_{3.25}P_3RuCoS$ (1146,80 g mol^{-1}): Found C: 51.34; H 4.25; N 3.70; calcd. C 51.27; H 4.45; N 3.66. IR (KBr, cm^{-1}): $\nu_{(CarH)}$ 3071, 3057; $\nu_{(CH)}$ 2961,2915, 2861; $\delta as_{(CH)}$ 1434 (m); $\nu_{(OTF)}$ 1274, 1252, 1170, 1158; $\nu_{(C-N)}$ 1029 (m), 1071 (m); $\delta oop_{(Car-H)}$ 757 (m), 745 (m); $\delta oop_{(C=Car)}$ 69+ (s). ^{1}H NMR (300.13 MHz, 25ºC, $CDCl_3$): δ(ppm) 6.48 (s, Cp, 5H), 7.03-9.04 (bm, aromatic, 25H), 126.25 (bm, PPh_3, 5H).^{13}C{^{1}H} NMR (300.13 MHz, 25 °C, $CDCl_3$): δ(ppm) 91.0 (s, Cp), 141.3-127.7 (m, aromatic, PPh_3). ^{31}P{^{1}H} NMR (300.13 MHz, 25 °C, $CDCl_3$): δ(ppm) 40.72 (d, $^2J_{PP}$ = 29.87 Hz, PPh_3), 211.32 (bm, dmoPTA).

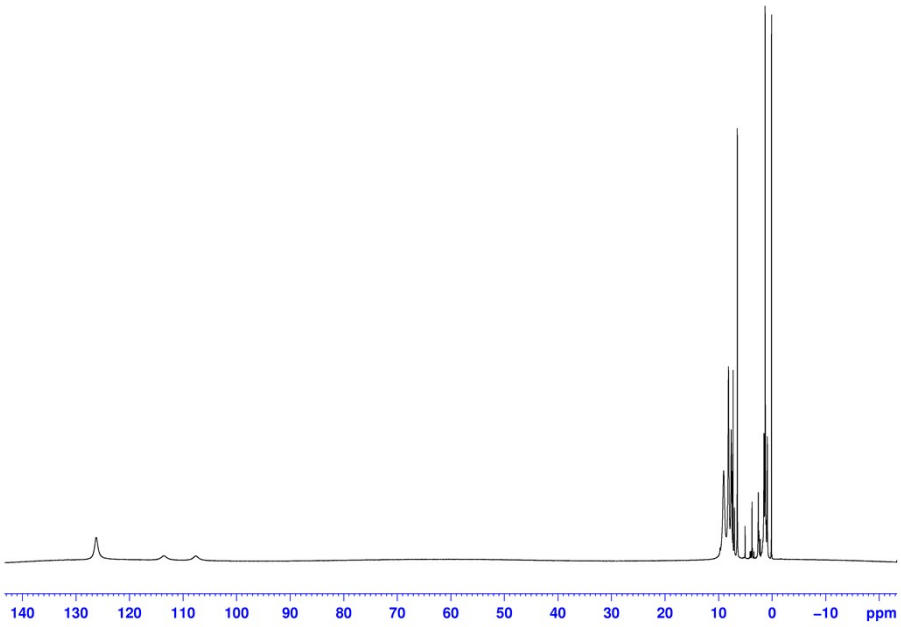

Figure S1. ^{1}H NMR for **2·OTf·0.25H_2O** in $CDCl_3$ at 25 °C.

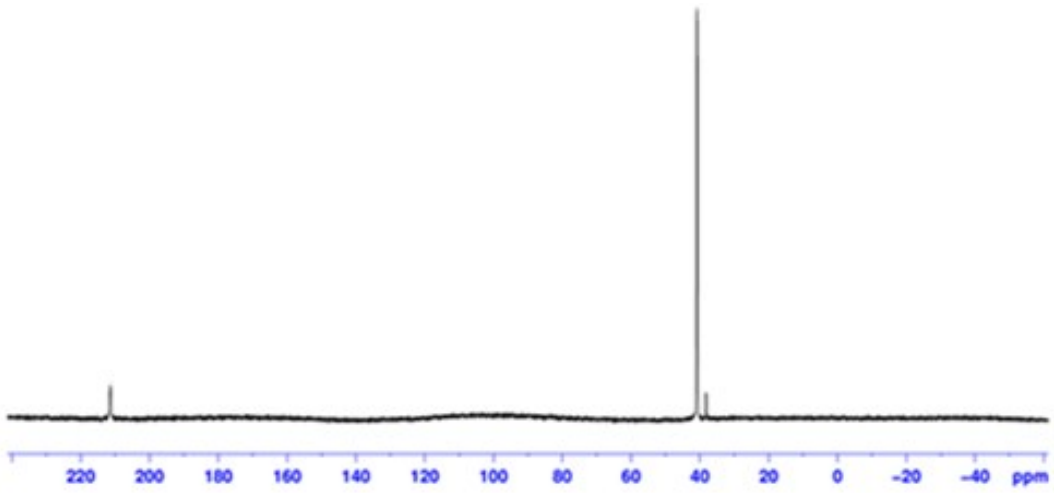

Figure S2. ^{31}P{^{1}H} NMR for **2·OTf·0.25H_2O** in $CDCl_3$ at 25 °C.

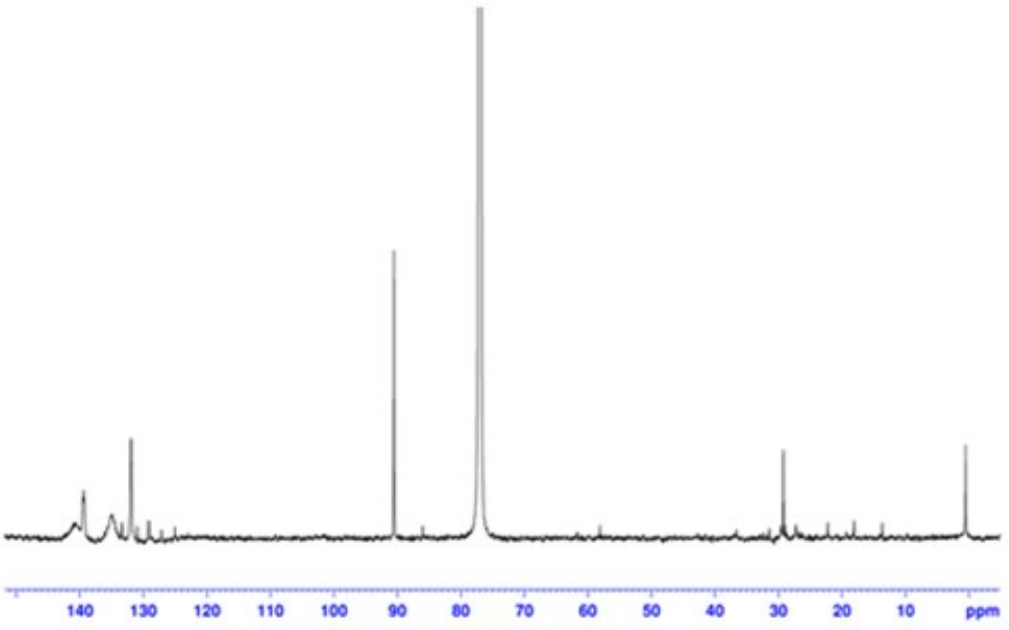

Figure S3. $^{13}C\{^{1}H\}$ NMR for **2·OTf·0.25H_2O** in $CDCl_3$ at 25 °C.

2.2. X-ray analysis of 2·OTf·0.25H_2O.

A single crystal with suitable dimensions (0.041 x 0.02 x 0.015) was mounted on a glass fibber with cyanoacrylate at room temperature. Data collection was performed on a Bruker APEX-II CCD diffractometer in the range $0.963 \leq 2\theta \leq 26.804$. Data were collected at 100(2)° K using graphite-monochromatized Mo-Kα ($\lambda = 0.71073$) in the range $-7 \leq h \leq 14$, $-25 \leq k \leq 26$, $-28 \leq l \leq 28$. The structure was determined by direct method and refined by least-squares procedures on F^2 (SHELX-XTL) by Olex2 package.[2,3] The function minimized during the refinement was $w = 1/[\sigma^2(F_0^2)+(0.0440P)^2 + 10.2700P]$. The final geometrical calculations, the graphical manipulations and the analysis of H-bond network and other crystallographic calculations were carried out with Olex2 package.[3] In the final least squares cycles all non-H atoms were allowed to vibrate anisotropically. The hydrogen atoms were located at the calculated positions and assigned a fixed displacement. The chloride ligand (Cl3) was found to be disordered (Uani = 0.27) and refined anisotropically. The 0.25 H_2O molecules are disordered between two positions with occupancy factors 0.405 (O0AA) and 0.095 (O1AA). Crystal data and data collection details are given in Table S1. **CCDC 1545888** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via the World Wide Web (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033 or emailing deposit@ccdc.cam.ac.uk)

Table S1. Crystal data and structure refinement information for **2·OTf·0.25H_2O**

Empirical formula	$C_{49}H_{51}F_3Cl_2N_3O_{3.25}P_3RuCoS$
Formula weight	1148.81
Crystal system	Triclinic (N°. 2)
Space group	P-1
a (Å)	11.1351(8)
b (Å)	20.8269(15)
c (Å)	22.1074(16)
α *(º)*	106.3010(10)
β *(º)*	93.3240(10)
γ *(º)*	94.3000(10)
V(Å^3)	4890.2(6)
Z	4
Calculated density (g·cm^{-3})	1.560
Absorption coefficient (mm^{-1})	0.956
F(000)	2340
Data/restraints/parameters	19829/1243/1212
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0430, wR_2 = 0.1004
wR_2 R indices (all data)	R_1 = 0.0517, wR_2 = 0.1085
Goodness of fit (GOF) on F^2	1.054
Largest difference in peak and hole (e·Å^{-3})	0.845 and -0.879

Table S2. Selected bond lenghts for **2·OTf·0.25H_2O**

Atom 1	Atom 2	Bond length (Å)
Ru1	P1	2.370(9)
Ru1	P2	2.378(4)
Ru1	P3	2.319(7)
Ru1	Cp_{centr}	1.893
N2B	Co1	2.058(3)
N1B	Co1	2.056(3)
Co1	Cl1	2.210(8)
Co1	Cl2	2.224(2)
Ru1A	P1A	2.390(9)
Ru1A	P2A	2.343(6)
Ru1A	P3A	2.305(3)
Ru1A	$Cp1_{centr}$	1.881
N1A	Co1A	2.056(3)
N2A	Co1A	2.051(3)
Co1A	Cl1A	2.212(8)
Co1A	Cl2A	2.222(6)

Table S3. Selected bond lenghts for **2·OTf·0.25H_2O**

Atom 1	Atom 2	Atom 3	Angle (º)
P1	Ru1	P2	103.62(3)
P2	Ru1	P3	99.73(3)
P3	Ru1	P1	92.77(3)
P1	Ru1	Cp_{centr}	119.15
P2	Ru1	Cp_{centr}	114.58
P3	Ru1	Cp_{centr}	122.95
Cl1	Co1	Cl2	115.26(17)
N1B	Co1	N2B	91.26(11)
P1A	Ru1A	P2A	102.48(3)
P2A	Ru1A	P3A	97.10(3)
P3A	Ru1A	P1A	96.97(3)
P1A	Ru1A	$Cp1_{centr}$	118.60
P2A	Ru1A	$Cp1_{centr}$	116.23
P3A	Ru1A	$Cp1_{centr}$	121.37
Cl1A	Co1A	Cl2A	121.58(4)
N1A	Co1A	N1A	91.92(11)

2.3. Stability of 2·OTf·0.25H_2O in DMSO-d_6.

Crystals of **2·OTf·0.25H_2O** (5.4 mg; 0.0047 mmol) were added into a 5 mm NMR containing 0.5 mL of DMSO-d_6 under N_2. The recorded $^{31}P\{^1H\}$ NMR after 10 minutes showed that the {$CoCl_2$} unit was realized from complex **2·OTf·0.25H_2O** to give rise to the deprotonated complex [RuCp(PPh_3)$_2$(dmoPTA-1κ*P*)]$^+$. $^{31}P\{^1H\}$ NMR (300.13 MHz, 25 °C, DMSO-d_6): δ(ppm) -6.94 (t, dmoPTA), 43.44 (d, $^2J_{pp}$ = 93.88 Hz, PPh_3)

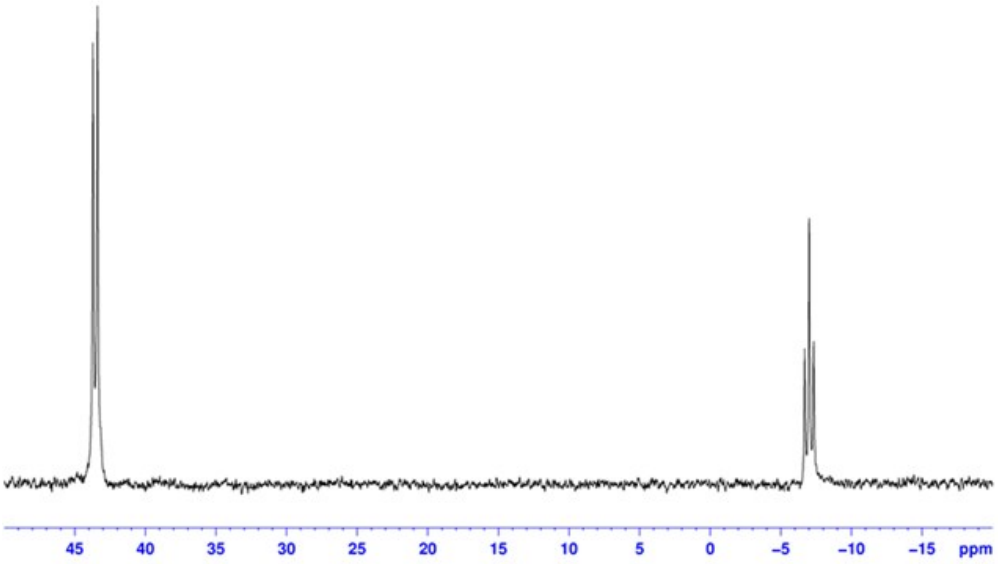

Figure S4. $^{31}P\{^1H\}$ NR for **2·OTf·0.25H_2O** in DMSO-d_6 at 25 °C.

2.4. Stability of 2·OTf·0.25H_2O first with DMSO-d_6 and immediately with cell culture medium.

Two 5 mm NMR tubes were prepared containing **2·OTf·0.25H_2O** (5.4 mg; 0.0047 mmol). Into one of them was introduced 0.5 mL of DMSO-d_6. In other, 0,5 mL of DMSO-d6 was added and 0.6 mL of the used cell culture medium. The resulting dissolutions were kept at room temperature and recorded by $^{31}P\{^1H\}$ NMR along the time. In both tubes the NMR the obtained results were similar. The complex initially releases slowly the {$CoCl_2$} unit and further also slowly the release of one PPh_3 starts. Similar results were obtained when air was bubbled into the solutions. A faster but similar reaction was observed when both solutions were kept at 40 °C.

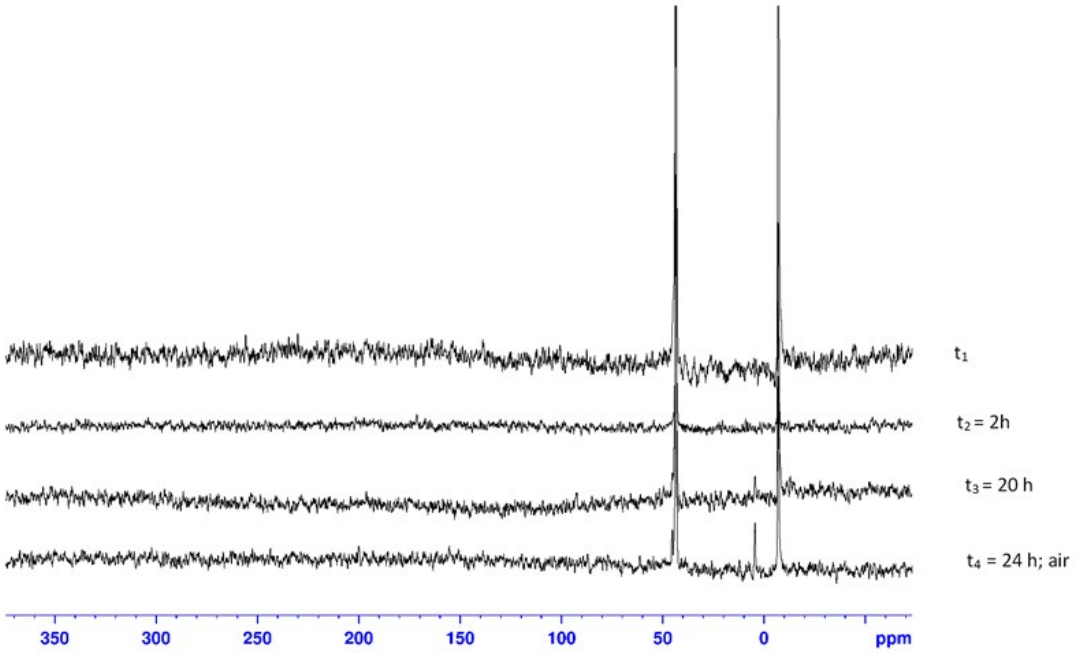

Figure S5. $^{31}P\{^1H\}$ NMR for **2·OTf·0.25H_2O** first with DMSO-d_6 (0.5 mL) at 25°C

2.5. Reactivity of NaCl in a solution of 2·OTf·0.25H2O first dissolved in DMSO and immediately with D_2O.

NaCl (12.8 mg, 0.219 mmol) was added into a solution obtained by dissolution of **2·OTf·0.25H_2O** (50 mg, 0.043 mmol) first in 10 mL of DMSO and immediately in 50 mL of water. The resulting solution was stirred at room temperature over nigh and the solvent evaporated under vacuum. The obtained powder showed the same $^{31}P\{^1H\}$ NMR that complex $[RuCpCl(PPh_3)(HdmoPTA\text{-}\kappa P)]^+$ previously published by us.[4] We must note that, single crystals good enough to be used for X-ray structure determination were obtained from ethanol by slow evaporation at room temperature. The resolution of the crystal structure showed that the obtained complex is $[RuCpCl(PPh_3)(HdmoPTA\text{-}\kappa P)]^+$ as suggested by $^{31}P\{^1H\}$ NMR.

2.6. Stability of 2·OTf·0.25H_2O in $CDCl_3$.

Into a 5 mm NMR tube was dissolved **2·OTf·0.25H_2O** (8.3 mg, 0.007 mmol) in 0.5 mL of $CDCl_3$. The $^{31}P\{^1H\}$ NMR showed that the complex slowly releases a PPh_3 initially and further the elimination of the $\{CoCl_2\}$ unit was started. Reaction was accelerated at 40 °C. Bubbling air into the solution does not produce any modify of the reaction tendency. The $^{31}P\{^1H\}$ NMR shows that after 3 days **2·OTf·0.25H_2O** still is present in the reaction along with $[RuCp(PPh_3)_2(dmoPTA\text{-}\kappa P)]$, $[RuClCp(PPh_3)(dmoPTA\text{-}\kappa P)]$, $[RuClCp(PPh_3)\text{-}\mu\text{-}dmoPTA\text{-}1\kappa P\text{:}2\kappa^2 N,N'\text{-}CoCl_2]$.

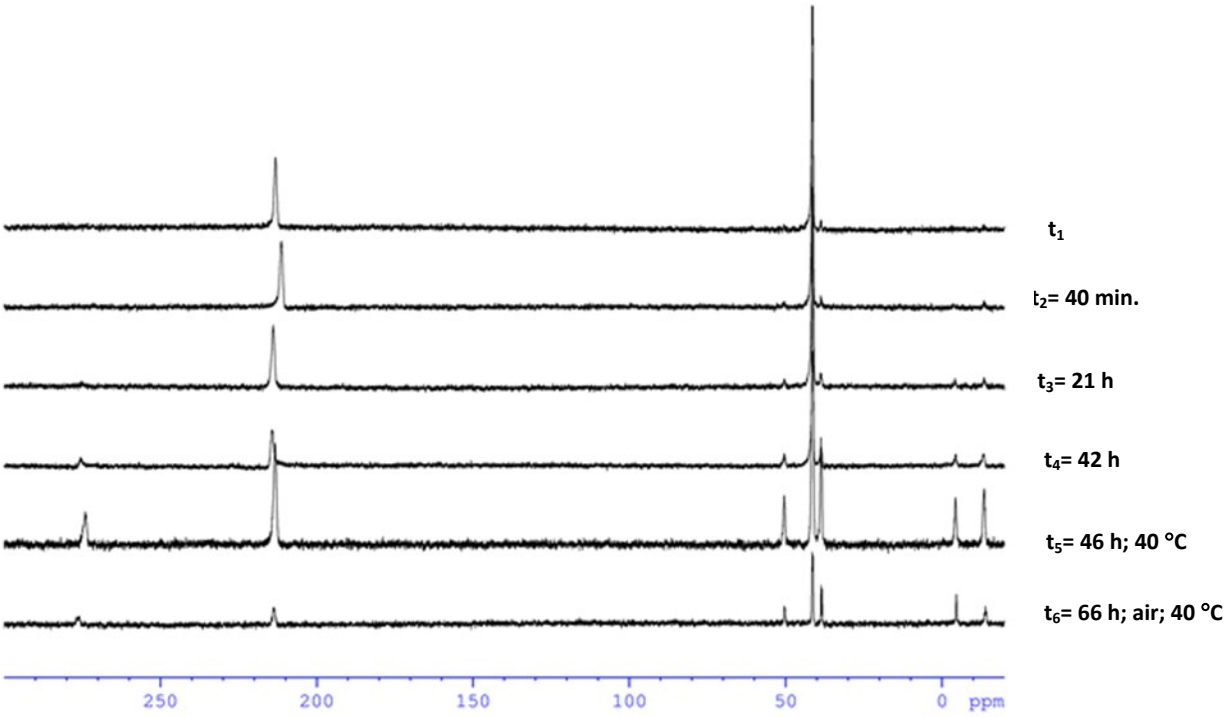

Figure S7 $^{31}P\{^1H\}$ NMR for **2·OTf·0.25H_2O in $CDCl_3$** versus the time

2.7. Growth inhibition assays

The human solid tumor cell lines A549, HBL-100, HeLa, SW1573, T-47D and WiDr were used in this study. These cell lines were a kind gift from Prof. G. J. Peters (VU Medical Center, Amsterdam, Netherlands). Cells were maintained in 25 cm^2 culture flasks in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum and 2 mM L-glutamine in a 37 °C, 5% CO_2, 95% humidified air incubator. Exponentially growing cells were trypsinized and re-suspended in antibiotic containing medium (100 units of penicillin G and 0.1 mg of streptomycin per mL). Single cell suspensions displaying >97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto 96-well microtiter plates. Cells were inoculated in a volume of 100 µL per well at densities of 2 500 (A549, HBL-100 and HeLa) and 5 000 (SW1573, T-47D and WiDr) cells per well, based on their doubling times. Compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, negative control). Each agent was tested in triplicate at different dilutions in the range of 1–100 µM.

The drug treatment was started on day 1 after plating. Drug incubation times were 48 h, after which time cells were precipitated with 25 µL ice-cold TCA (50% w/v) and fixed for 60 min at 4 °C. Then the SRB assay was performed. The optical density (OD) of each well was measured at 530 nm, using BioTek's PowerWave XS Absorbance Microplate Reader. Values were corrected for background OD from wells only containing medium.

2.8. Cell cycle assays

Cells were seeded in six well plates at a density of 2.5–5 × 10^5 cells/well. After 24 h the products were added to the respective well and incubated for an additional period of 24 h. Cells were trypsinized, harvested, transferred to test tubes (12 × 75 mm) and centrifuged at 1500 rpm for 10 min. The supernatant was discarded, and the cell pellets were resuspended in 200 mL of cold PBS and fixed by the addition of 1 mL ice-cold 70% EtOH. Fixed cells were incubated overnight at 20 °C, after which time they were centrifuged at 1500 rpm for 10 min. The cell pellets were resuspended in 500 mL of phosphate-buffered saline (PBS), and 5 mL of DNase-free RNase solution (10 mg/mL) was added. The mixture was incubated at 37 °C for 30 min. Finally, 5 mL of propidium iodide (PI; 0.5 mg/mL) was added. Flow cytometric determination of DNA content (20000 cells/sample) was analyzed on an Accuri C6 Flow Cytometer (Becton Dickinson, San Jose, CA, USA). The fractions of´the cells in G_0/G_1, S, and G_2/M phases were analyzed with BD Accuri C6 software.

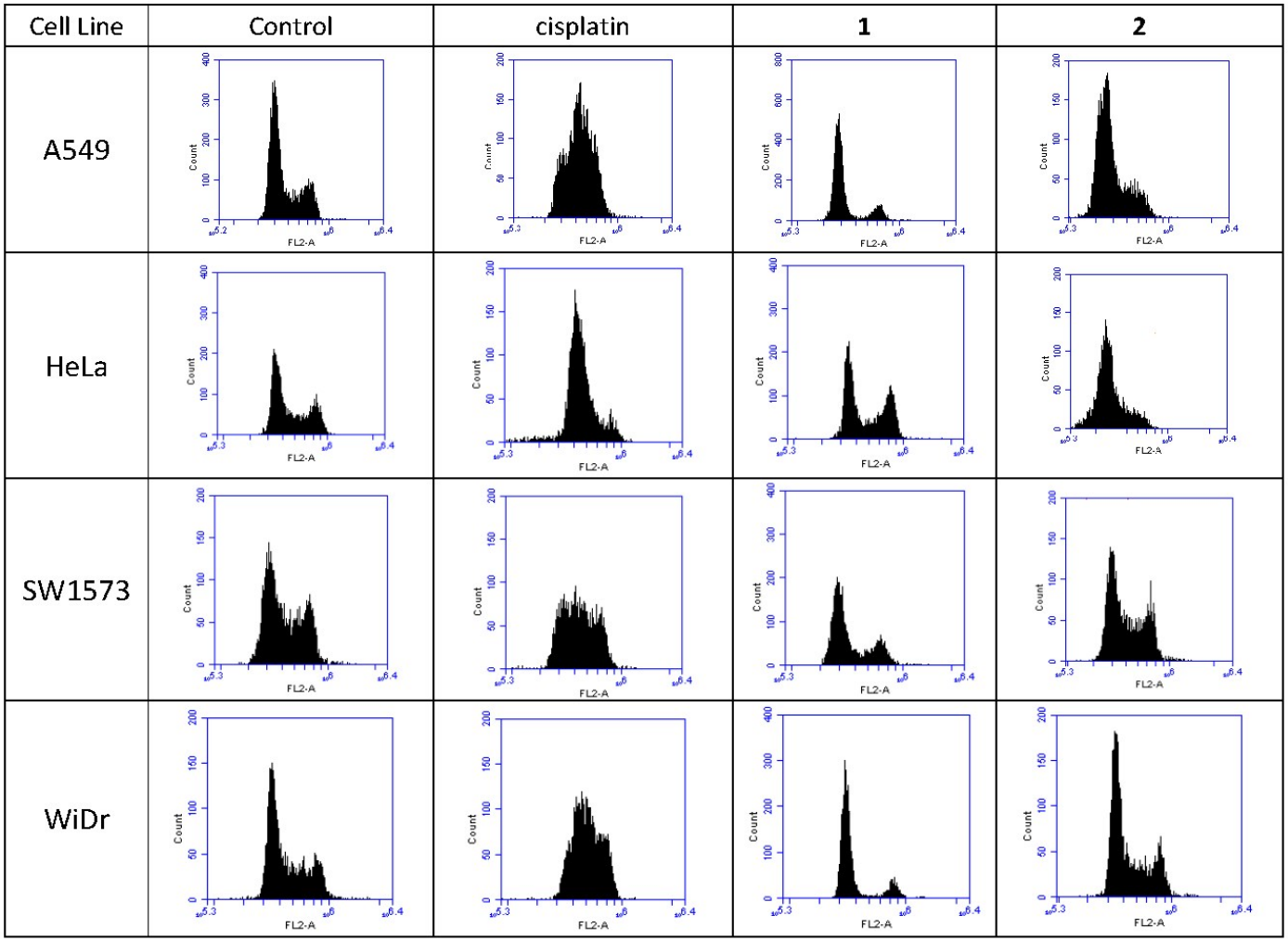

Figure S8. Representative histograms of cells treated with complex **1·2Otf** (0.5 µM) or complex **2·OTf·0.25H_2O** (0.1 µM) for 24 h. Cisplatin was used as reference drug.

References

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4. A. Mena-Cruz, P. Lorenzo-Luis, A. Romerosa, M. Saoud and M. Serrano-Ruiz, Inorg. Chem., 2007, **46**, 6120.