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New PTA (1,3,5-triaza 7-phosphaadamantane) derivatives associating zwitterionic structure and coordinative ability

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ABSTRACT

The reactions of PTA (1,3,5-triaza 7-phosphaadamantane) and HMTA (1,3,5,7-tetraazaadamantane) with 1,3-propanesultone or 1,4-butanesultone gave the water soluble zwitterionic derivatives $PTA^+C_3H_6SO_3^-$ (**1**), $PTA^+C_4H_8SO_3^-$ (**2**), $HMTA^+C_3H_6SO_3^-$ (**3**) and $HMTA^+C_4H_8SO_3^-$ (**4**). The crystal structure of $HMTA^+C_3H_6SO_3$ is reported. The coordinative ability of **1**–**4** towards Pt(II) and Ru(II) has been investigated and the antiproliferative activity of ligands and complexes has been tested in two human ovarian cancer cell lines, A2780 and SKOV3.

1. Introduction

Hydrophilicity combined with the lack of net charge makes zwitterionic compounds suitable for many applications: e.g. they have been exploited as non-denaturating detergents for proteins [1], metal salt extractants [2], ionic liquids [3], quantum dots components [4] and also as drugs with low affinity for blood serum albumin and high oral biodisponibility, e.g. the antibiotics ciprofloxacin and amoxicillin [5].

Zwitterionic species, both naturally occurring like phosphocholine and synthetically prepared or modified like sulfabetaines, have also attracted a great deal of attention as functional groups for polymeric structures with valuable properties [6].

The presence of zwitterionic moieties produces innovative biomaterials of great potential in biochemistry, and in drugs and diagnostics development [7]: conjugation to zwitterionic biomaterials represents a new strategy for stabilising peptide-based drugs and for protecting implantable electrochemical glucose biosensors from biofouling [8]. The zwitterions used for these purposes are not pH sensitive, bearing quaternary ammonium groups coupled with anionic groups which are weak bases (e.g. RSO_3^-, RPO_4^{2-}) and therefore they are not de-protonable and they are protonable only at very low pH values.

Because of the above described advantageous interactions with bio-systems, we reasoned that it could be of interest to produce species where the zwitterionic character is coupled with the presence of a coordinative site for metal ions, particularly for those with well-known pharmaceutical activity, like Pt(II) and Ru(II). Being these soft acidic ions, the introduction of a soft donor like phosphorus is likely to favour the metal coordination [9].

We designed the new ligands **1** and **2**, which are water-soluble zwitterionic phosphines, easily obtainable through PTA (1,3,5-triaza 7-phosphaadamantane) N-alkylation.

We have recently prepared some cationic PTA derivatives exploiting the alkylation process which occurs invariably to a single nitrogen atom, with complete regioselectivity, leaving vacant the P-donor, the favourite site for soft metals coordination [10].

In this paper we describe the synthesis, properties and coordination to platinum and ruthenium of **1** and **2**, two zwitterionic phosphines where short polymethylene chains connect the PTA cage to a sulfonated group, joining in the same molecule the two groups traditionally exploited to make a phosphine water soluble (PTA and SO_3^-) [11].

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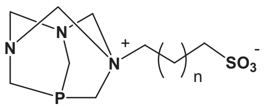
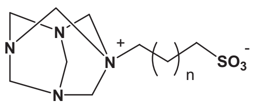


1, n = 1
2, n = 2

The aminic analogues **3** and **4**, obtained from HMTA (1,3,5,7-tetraazaadamantane), have been also prepared and characterised.



3, n = 1
4, n = 2

2. Experimental

2.1. General procedures

All manipulations were carried out under argon atmosphere with the use of standard Schlenk techniques unless otherwise noted. Elemental analyses were carried out using a Carlo Erba instrument model EA1110. The ESI mass spectra were acquired with a Micromass LCQDuo Finningan. NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer (^1H at 300.23 MHz, ^{13}C at 75.43 MHz, ^{31}P at 121.50 MHz) or a Varian Mercury Plus (^1H at 399.97 MHz, ^{13}C at 100.58 MHz, ^{31}P at 161.92 MHz). The ^{13}C and ^{31}P spectra were run with proton decoupling and ^{31}P spectra are reported in ppm relative to an external 85% H_3PO_4 standard, with positive shifts downfield. ^{13}C NMR spectra are reported in ppm relative to external tetramethylsilane (TMS), with positive shifts downfield. The solubility in water for **1**–**4**, was determined by progressively adding 50 μL volumes of water to 0.1 g of solid product until it appears completely dissolved. For Pt and Ru complexes the concentration of saturated solutions have been measured by a Perkin-Elmer atomic absorption spectrometer (Analyst 800) against the appropriate standards.

Solvents were distilled and dried prior to use. Commercial sultones were purchased and used without further purification. PTA [12] and the metal complexes precursors [$PtCl_2$(1,5-COD)] (1,5-COD = 1,5-cyclooctadiene) [13] and [CpRuCl(PPh_3)(PTA)] [14] were prepared as described in the literature.

2.2. Synthesis of $PTA^+C_3H_6SO_3$ (**1**)

0.100 g of PTA (0.64 mmol) were dissolved in AcOEt (12 mL) and 2.2 eq (0.173 g, 1.42 mmol) of 1,3-propanesultone were added. The mixture was stirred at room temperature for 18 h. The product precipitated as a white solid and was separated by filtration and washed with ether (0.110 g, 0.40 mmol, 62.5%).

Solubility in water (25 °C): 50 mg/mL.

Data for **1** were as follows. ^1H NMR (300 MHz, D_2O, 25 °C): δ 2.10 (m, 2H, $CH_2SO_3^-$), δ 2.85 (pst, $_{HH}$ = 7.22 Hz, 2H, $CH_2\mathit{CH_2}CH_2$), δ 3.00 (m, 2H, $CH_2\mathit{CH_2}N^+$), δ 3.80 (m, 4H, $P\mathit{CH_2}N$), δ 4.24 (s, 1H, PCH_2N^+), δ 4.25 (s, 1H, PCH_2N^+), δ 4.36 (d, $^2J_{HH}$ = 13.6 Hz, 1H, $N\mathit{CH_2}N$), δ 4.50 (d, $^2J_{HH}$ = 13.6 Hz, 1H, $N\mathit{CH_2}N$), δ 4.72 (d, $^2J_{HH}$ = 11.4 Hz, 2H, $N\mathit{CH_2}N^+$), δ 4.93 (d, $^2J_{HH}$ = 11.4 Hz, 2H, $N\mathit{CH_2}N^+$).

$^{13}C\{^1H\}$ NMR (50.320 MHz, D_2O, 25 °C): δ 16.7 (s, $CH_2SO_3^-$), δ 46.9 (d, $^1J_{PC}$ = 20.6 Hz, PCH_2N), δ 49.0 (s, $CH_2\mathit{CH_2}CH_2$), δ 54.0 (d, $^1J_{PC}$ = 33 Hz, PCH_2N^+), δ 62.2 (s, $CH_2\mathit{CH_2}N^+$), δ 70.5 (s, NCH_2N), δ 80.2 (s, NCH_2N^+).

$^{31}P\{^1H\}$ NMR (121.5 MHz, D_2O, 25 °C): δ −83.67 ppm (s). (121.5 MHz, DMSO, 25 °C): δ −84.76.

Anal. Calc. for $C_9H_{18}N_3O_3PS$ (279): C, 38.70; H, 6.50; N, 15.05; S, 11.46. Found: C, 38.68; H, 6.45; N, 14.90; S, 11.36%.

Electrospray MS (in H_2O): observed *m/z* 280 (M+1), 302 (M+23), calcd 280 for $C_9H_{19}N_3O_3PS$ (MH+}.

2.3. Synthesis of $PTA^+C_4H_8SO_3^-$ (**2**)

0.300 g (1.9 mmol) of PTA were mixed with 780 μL (7.6 mmol, 4 eq) of 1,4-butanesultone for 20 h without any solvent. 5 mL of ether were then added and the white solid product was separated by filtration and washed with ether (0.483 g, 1.65 mmol, 86.8%).

Solubility in water (25 °C): 504 mg/mL.

Data for **2** were as follows: ^1H NMR (300 MHz, D_2O, 25 °C): δ 1.63 (m, 2H, $CH_2\mathit{CH_2}SO_3^-$), δ 1.80 (m, 2H, $\mathit{CH_2}CH_2SO_3^-$), δ 2.82 (m, 4H, $CH_2\mathit{CH_2CH_2}N^+$), δ 3.80 (m, 4H, $P\mathit{CH_2}N$), δ 4.22 (s, 1H, $P\mathit{CH_2}N^+$), δ 4.23 (s, 1H, $P\mathit{CH_2}N^+$), δ 4.38 (d, $^2J_{HH}$ = 14 Hz, 1H, $N\mathit{CH_2}N$), δ 4.52 (d, $^2J_{HH}$ = 14 Hz, 1H, $N\mathit{CH_2}N$), δ 4.72 (d, $^2J_{HH}$ = 11 Hz, 2H, $N\mathit{CH_2}N^+$), δ 4.90 (d, $^2J_{HH}$ = 11 Hz, 2H, $N\mathit{CH_2}N^+$).

$^{13}C\{^1H\}$NMR (75.43 MHz, D_2O, 25 °C): δ 18.1 (s $CH_2SO_3^-$), δ 21.2 (s, $CH_2\mathit{CH_2}CH_2SO_3^-$), δ 30.1 (s, $CH_2\mathit{CH_2}CH_2N^+$), δ 45.2 (d, $^1J_{PC}$ = 21.4 Hz, PCH_2N), δ 50.4 (s, $CH_2CH_2\mathit{CH_2}N^+$), δ 52.9 (d, $^1J_{PC}$ = 34 Hz, PCH_2N^+), δ 62.2 (s, $CH_2\mathit{CH_2}N^+$), δ 69.4 (s, NCH_2N), δ 78.8 (s, NCH_2N^+).

$^{31}P\{^1H\}$ NMR (121.5 MHz, D_2O, 25 °C): δ −82.86 ppm (s).

Anal. Calc. for $C_{10}H_{20}N_3O_3PS$ (293): C, 40.94; H, 6.88; N, 14.33; S, 10.91. Found: C, 41.03; H, 6.97; N, 14.25; S, 10.82%.

Electrospray MS (in H_2O): observed *m/z* ($M+H^+$) 294, ($M+Na^+$) 316, calcd 294 for $C_{10}H_{21}N_3O_3PS$ (MH^+).

2.4. Synthesis of $HMTA^+C_3H_6SO_3^-$ (**3**)

0.500 g of HMTA (3.27 mmol) were dissolved in refluxing toluene and 0.522 g of 1,3-propanesultone (4.28 mmol, 1.2 eq) were added. The mixture was refluxed for 18 h. The product was separated by filtration as a white solid and washed with ether (0.840 g, 3.21 mmol, 98.2% yield).

Solubility in water (25 °C): 180 mg/mL.

Data for **3** were as follows. ^1H NMR (300 MHz, D_2O, 25 °C): δ 2.10 (m, 2H, $CH_2SO_3^-$), δ 2.80 (pst, $^2J_{HH}$ = 7.00 Hz, 2H, $CH_2\mathit{CH_2}CH_2$), δ 2.95 (m, 2H, $CH_2\mathit{CH_2}N^+$), δ 4.44 (d, 3H, $^2J_{HH}$ = 14 Hz, $N\mathit{CH_2}N$), δ 4.62 (d, 3H, $^2J_{HH}$ = 14 Hz, $N\mathit{CH_2}N$), δ 5.07 (s, 6H, $N\mathit{CH_2}N^+$).

$^{13}C\{^1H\}$ NMR (75.43 MHz, D_2O, 25 °C): δ 15.6 (s, $\mathit{CH_2}SO_3$), δ 47.4 (s, $CH_2\mathit{CH_2}CH_2$), 55.3 (s, $CH_2\mathit{CH_2}N^+$), δ 70.0 (s, NCH_2N), δ 78.2 (s, NCH_2N^+).

Anal. Calc. for $C_9H_{18}SO_3N_4$ (262): C, 41.20; H, 6.92; N, 21.37; S, 12.20. Found: C, 41.20; H, 6.92; N, 20.80; S, 12.97%.

Electrospray MS (in H_2O): observed *m/z* 263 ($M+H^+$), 285 ($M+Na^+$), 417, 525 ($2M+H^+$), 547 ($2M+Na^+$) 686, calcd 263 for $C_9H_{19}SO_3N_4$ = (MH^+).

2.5. Synthesis of zwitterions $HMTA^+C_4H_8SO_3^-$ (**4**)

0.230 g of HMTA (2.44 mmol) and 1 mL of 1,4-butanesultone (1.330 g, 9.76 mmol, 6 eq) were mixed and kept under stirring at room temperature for 3 days. Diethylether was then added to the white slurry and the white solid was filtered and washed with ether (0.440 g, 1.59 mmol, 65.2%). Data for **4** were as follows.

Solubility in water (25 °C): 385 mg/mL.

^1H NMR (300 MHz, D_2O, 25 °C): δ 1.68 (m, 2H, $\mathit{CH_2}SO_3^-$), δ 1.76 (m, 2H, $\mathit{CH_2}CH_2SO_3^-$), δ 2.82 (pst, $^2J_{HH}$ = 7.00 Hz, 4H, $N^+\mathit{CH_2CH_2}CH_2$), δ 4.42 (d, 3H, $^2J_{HH}$ = 14 Hz, $N\mathit{CH_2}N$), δ 4.61 (d, 3H, $^2J_{HH}$ = 14 Hz, $N\mathit{CH_2}N$), δ 5.00 (s, 6H, $N\mathit{CH_2}N^+$).

$^{13}C\{^1H\}$ NMR (75.43 MHz, D_2O, 25 °C): δ 18.4 (s, $\mathit{CH_2}SO_3^-$), δ 21.6 (s, $\mathit{CH_2}CH_2SO_3$), δ 50.0 (s, $CH_2\mathit{CH_2}CH_2$), δ 56.7 (s, $CH_2\mathit{CH_2}N^+$), δ 70.3 (s, NCH_2N), δ 78.5 (s, NCH_2N^+).

Anal. Calc. for $C_{10}H_{20}N_4O_3S$ (276): C, 43.46; H, 7.30; N, 20.29; S, 11.58. Found: C, 43.56; H, 7.67; N, 20.03; S, 11.75%.

Electrospray MS (in H_2O): observed *m/z* 277.1 ($M+H^+$), 553.2 ($2M+H^+$), calcd 277 for $C_{10}H_{21}N_4O_3S = (MH^+)$.

2.6. X-ray crystal structure of **3**

The crystal data for $HMTA^+C_3H_6SO_3^-$ (**3**) were collected at room temperature (T = 295 K) using a Nonius Kappa CCD diffractometer with graphite monochromated Mo Kα radiation and corrected for Lorentz and polarisation effects. The structure was solved by direct methods (SIR97 [15]) and refined using full-matrix least-squares. All non-hydrogen atoms were refined anisotropically and hydrogens isotropically. All the calculations were performed using SHELXL-97 [16] and PARST [17] implemented in WINGX [18] system of programs. The crystal data and refinement parameters are summarized in Table 1. Selected structural parameters are given in Table 2.

Table 1
Crystal data and details of data collection for **3**.

Compound	$HMTA^+C_3H_6SO_3^-$
Formula	$C_9H_{18}N_4O_3S \cdot 3H_2O$
M	316.38
System	monoclinic
Space group	$P2_1/c$
a (Å)	10.1098(3)
b (Å)	6.3722(2)
c (Å)	22.8048(6)
β (°)	96.237(1)
V (Å^3)	1460.43(4)
Z	4
D_{calc} (g cm^{-3})	1.439
T (K)	295
μ (cm^{-1})	2.53
θ_{min}–θ_{max}/°	3.67–27.88
Collected reflections	8254
Unique reflections	3420
R_{int}	0.025
Observed reflections [$I > 2\sigma(I)$]	2716
R (Observed reflections)	0.0400
wR (All reflections)	0.1129
S	1.023
$\Delta\rho_{max}$; $\Delta\rho_{min}$ (e Å^{-3})	0.26; −0.38
CCDC Deposit No.	CCDC 894267

Table 2
Selected structural parameters (Å, °) for compound **3**.

Bond distances			
N1–C1	1.534(2)	N4–C4	1.440(2)
N1–C2	1.536(2)	N4–C5	1.471(2)
N1–C4	1.533(2)	N4–C6	1.474(2)
N1–C7	1.497(2)	C7–C8	1.515(2)
N2–C1	1.439(2)	C8–C9	1.519(2)
N2–C3	1.474(2)	C9–S1	1.779(2)
N2–C5	1.475(2)	S1–O1	1.441(1)
N3–C2	1.435(2)	S1–O2	1.450(1)
N3–C3	1.471(2)	S1–O3	1.457(1)
N3–C6	1.469(2)		
Bond angles			
C1–N1–C2	107.2(1)	C8–C9–S1	113.3(1)
C1–N1–C4	107.9(1)	C9–S1–O1	107.5(1)
C1–N1–C7	112.3(1)	C9–S1–O2	106.7(1)
C2–N1–C4	107.5(1)	C9–S1–O3	105.0(1)
C2–N1–C7	108.0(1)	O1–S1–O2	111.9(1)
C4–N1–C7	113.6(1)	O1–S1–O3	112.4(1)
N1–C7–C8	115.9(1)	O2–S1–O3	112.9(1)
C7–C8–C9	107.9(1)		
Torsion angles			
N1–C7–C8–C9	176.9(1)	C7–C8–C9–S1	−172.4(1)

2.7. Coordination reactions of $PTA^+C_3H_6SO_3^-$ (**1**) and $PTA^+C_4H_8SO_3^-$ (**2**)

2.7.1. Synthesis of *cis*-[$PtCl_2(PTA^+C_3H_6SO_3^-)_2$], (**5**)

A solution of K_2PtCl_4 (0.083 g, 2×10^{-4} mol) in water (5 mL) was added to a suspension of $PTA^+C_3H_6SO_3^-$ (0.111 g, 4×10^{-4} mol) in 10 mL of ethanol.

The sudden precipitation of a pink-white solid and the decoloration of the solution are observed. The solid was filtered, washed with water and dried (0.140 g, 1.7×10^{-4} mol, 85.0% yield).

The product **5** has a very low solubility in water (178 mg/L at 25 °C, measured by atomic absorption), but its solubility in a NaCl solution or in 5 M HCl, although scarce, allowed spectroscopic characterization.

^{1}H NMR (300 MHz, D_2O sat NaCl, 25 °C): δ 2.38 (m, 2H, CH_2CH_2*CH_2*SO_3^-), δ 3.15 (m, 3H) + 3.52 (m, 1H) ($^+$N*CH_2CH_2*CH_2) 4.9 (bm, 6H, P*CH_2*N^+ e P*CH_2*N), δ 5.20 (bs, 2H, N*CH_2*N), δ 5.42 (dd, 4H, $^2J_{HH}$ = 11 Hz, N*CH_2*N^+).

$^{31}P\{^1H\}$ NMR (121.5 MHz, D_2O sat NaCl, 25 °C): δ −37.98 ppm ($^1J_{PtP}$ 3534 Hz).

$^{31}P\{^1H\}$ NMR (121.5 MHz, HCl 5 M, 25 °C): δ −38.27 ppm ($^1J_{PtP}$ 3534 Hz).

Anal. Calc. for $C_{18}H_{36}Cl_2N_6O_6S_2P_2Pt$ (824): C, 26.21; H, 4.40; N, 10.19; S, 7.76. Found: C, 25.95; H, 4.42; N, 9.85; S, 7.75%.

2.7.2. Synthesis of *cis*-[$PtCl_2(PTA^+C_4H_8SO_3^-)_2$] (**6**)

$PTA^+C_4H_8SO_3^-$ (0.117 g, 4×10^{-4} mol) was dissolved in 5 mL of water and added at 0 °C to a solution of K_2PtCl_4 (0.083 g, 2×10^{-4} mol) in water (2.5 mL).

An off-white solid precipitated while the solution shaded. The solid was separated by centrifugation, washed with water and dried (0.135 g, 1.6×10^{-4} mol, 80.0% yield).

The product **6** has a very low solubility in water (36.5 mg/L at 25 °C, measured by atomic absorption), but its solubility in a NaCl solution or in 5 M HCl allowed spectroscopic characterization.

^{1}H NMR (300 MHz, D_2O sat NaCl, 25 °C): δ 2.10 (m, 2H, CH_2*CH_2* SO_3^-), δ 2.25 (m, 2H, *CH_2*$CH_2SO_3^-$), δ 3.35 (m, 3H) + 3.60 (m, 1H) (CH_2*CH_2CH_2*N^+) 4.9 (bm, 6H, P*CH_2*N^+ and P*CH_2*N), δ 5.20 (bs, 2H, N*CH_2*N), δ 5.42 (dd, 4H, $^2J_{HH}$ = 11 Hz, N*CH_2*N^+).

$^{31}P\{^1H\}$ NMR (121.5 MHz, D_2O sat NaCl, 25 °C): δ −37.99 ppm ($^1J_{PtP}$ 3546 Hz).

Electrospray MS (in H_2O): observed *m/z* 875 ($M+Na^+$), calcd 875 for $C_{20}H_{40}Cl_2N_6P_2Pt\ O_6S_2Na$.

Anal. Calc. for $C_{20}H_{40}Cl_2N_6P_2PtO_6S_2$ (852): C, 28.16; H, 4.73; N, 9.86; S, 7.50. Found: C, 28.43; H, 4.84; N, 9.73; S, 7.55%.

2.7.3. Synthesis of [$Cp(PPh_3)(PTA^+C_3H_6SO_3^-)RuCl$], (**7**)

Method A: 102 mg of [$Cp(PPh_3)_2RuCl$] (0.14 mmol) was mixed with $PTA^+C_3H_6SO_3^-$ (39 mg, 0.14 mmol) in 20 mL of ethanol and 2 mL of distilled water. The mixture was refluxed for 1 h. Concentration of the solution to *ca.* half volume and the addition of diethylether (5 mL) gave [$Cp(PPh_3)(PTA^+C_3H_6SO_3^-)RuCl$], **7**, as a dark yellow solid, which was filtered off and dried with diethyl ether (3 × 2 mL). (50.0% yield).

Method B: A solution of [$Cp(PPh_3)(PTA)RuCl$] (0.200 g, 3.2×10^{-4} mol) in 15 mL of CH_2Cl_2 was treated with an excess of 1,3-propanesultone (0.314 g, 2.57×10^{-3} mol) and the mixture was refluxed for 3 h under nitrogen. The colour slowly turned from yellow to dark orange; a dark yellow solid precipitated, which was filtered out and washed with dichloromethane (2 × 2 mL) and diethyl ether (3 × 2 mL) (0.150 g, 2×10^{-4} mol, 62.5% yield).

Complex **7** has a very low solubility in water (41 mg/L at 25 °C, measured by atomic absorption), but its solubility in DMSO allowed spectroscopic characterization.

^{1}H NMR (300 MHz, *DMSO* d_6, 25 °C): δ 1.85 (m, 2H, CH_2*CH_2*SO_3^-), δ 2.10 (m, 2H, *CH_2*$CH_2SO_3^-$), δ 2.98 (m, 2H, CH_2CH_2*CH_2*N^+), δ 3.65 (bm, 1H, P*CH_2*N), δ 3.80 (bm, 1H, P*CH_2*N), δ 3.97 (bm, 1H, P*CH_2*N),

δ 4.09 (bm, 1H, PCH_2N), δ 4.22 (bm, 2H, PCH_2N^+), δ 4.47 (m, 5H, Cp), δ 4.72 (m, 4H, NCH_2N^+), δ 4.98 (m, 2H, NCH_2N).

$^{31}P\{^1H\}$ NMR (121.5 MHz, *DMSO* d6, 25 °C): δ 46.56 (d, $^2J_{PP}$ 43.90 Hz), δ −15.35 ppm (d, $^2J_{PP}$ 43.90 Hz).

Anal. Calc. for $C_{32}H_{38}ClN_3P_2RuO_3S$ (743): C, 51.70; H, 5.16; N, 5.66; S, 4.30. Found: C, 52.12; H, 5.35; N, 5.59; S, 4.25%.

2.7.4. Synthesis of [Cp(PPh3)(PTA+C4H8SO3−)RuCl] (8)

Method A: Complex [Cp(PPh_3)($PTA^+C_4H_8SO_3^-$)RuCl], **8**, was obtained by treating [Cp(PPh_3)$_2$RuCl] (0.15 mmol) with 1 eq of $PTA^{+-}C_4H_8SO_3^-$ as above described for [Cp(PPh_3)($PTA^+C_3H_6SO_3^-$)RuCl], (0.093 mmol, yield 62.0%).

Method B: A Schlenk tube was charged with [Cp(PPh_3)(PTA)RuCl] (0.200 g, 3.2×10^{-4} mol) and 2 mL of liquid 1,4-butanesultone; a dark yellow solid was formed from the reaction mixture stirred at 50 °C for 2 h. Slow addition of diethyl ether (3 mL) completed the precipitation of the product, which was filtered and washed with diethyl ether (3 × 2 mL). (0.180 g, 2.36×10^{-4} mol, 73.8% yield).

Complex **8** has a very low solubility in water (35.0 mg/L at 25 °C, measured by atomic absorption), but its solubility in DMSO allowed spectroscopic characterization.

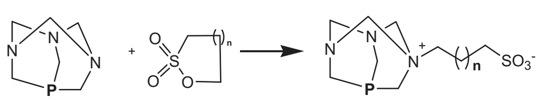
^1H NMR (300 MHz, *DMSO* d6, 25 °C): δ 1.70 (m, 4H, $CH_2CH_2SO_3^-$), δ 2.05 (m, 2H, $CH_2CH_2CH_2SO_3^-$), δ 2.86 (m, 2H, $CH_2CH_2N^+$), δ 3.6–4.3 (6H, PCH_2N), δ 4.48 (m, 5H, Cp), δ 4.78 (m, 4H, NCH_2N^+), δ 4.90 (m, 2H, NCH_2N).

$^{31}P\{^1H\}$ NMR (121.5 MHz, *DMSO* d6, 25 °C): δ 46.52 (d, $^2J_{PP}$ 43.90 Hz), δ −15.03 ppm (d, $^2J_{PP}$ 43.90 Hz).

Anal. Calc. for $C_{33}H_{40}ClN_3P_2RuO_3S$ (757): C, 52.33; H, 5.33; N, 5.55; S, 4.22. Found: C, 52.02; H, 5.39; N, 5.51; S, 4.18%.

2.8. Growth inhibition assays

Cell growth inhibition assays were carried out using two human ovarian cancer cell lines, A2780 and SKOV3; A2780 cells are cisplatin-sensitive and SKOV3 cells are cisplatin-resistant. Cells were maintained in RPMI 1640, supplemented with 10% newborn bovine serum, penicillin (100 U/mL), streptomycin (100 U/mL) and glutamine (2 mM); the pH of the medium was 7.2 and the incubation was at 37 °C in a 5% CO_2 atmosphere. Cells were routinely passed every three days. MTT test was used to study the compounds antiproliferative activity. The cells were seeded in triplicate in 96-well trays at a density of 25×10^3 in 50 μl of AIM-V medium for A2780 and SKOV3. Stock solutions (10 mM) of each compound were made in DMSO and diluted in AIM-V medium to give final concentrations of 10, 50 and 100 μM. Cisplatin was employed as a control for the cisplatin-sensitive A2780 cell line and for the cisplatin-resistant SKOV3. Untreated cells were placed in every plate as a negative control. The cells were exposed to the compounds, in 100 μl total volume, for 72 h, and then 25 μl of a 3-(4,5-dimethylthiozol-2-yl)2,5-diphenyltetrazolium bromide solution (MTT) (12 mM) were added. After 2 h of incubation, 100 μl of lysing buffer (50% DMF + 20% SDS, pH 4.7) were added to convert the MTT solution into a violet coloured formazane. After additional 18 h the solution absorbance, proportional to the number of live cells, was measured by spectrophotometer at 570 nm and converted into% of growth inhibition [19].



Scheme 1. Synthesis of $PTA^+C_3H_6SO_3$ (**1**) and $PTA^+C_4H_8SO_3^-$ (**2**).

3. Results and discussion

3.1. Zwitterionic phosphines PTA+C3H6SO3 (1) and PTA+C4H8SO3− (2)

From the reaction of PTA with 1,3-propane- and 1,4-butanesultone, the zwitterionic phosphines **1** and **2** were obtained in good yield (Scheme 1).

The reaction of PTA with solid 1,3-propanesultone to give **1** was carried out in AcOEt, while **2** was prepared mixing PTA with liquid 1,4-butanesultone without solvent.

Polialkylation, described with HMTA [20], was never observed for PTA in these experiments.

Compounds **1** and **2** were characterised by spectroscopic techniques. In both cases, the ^{31}P NMR in D_2O showed a single peak, at −83.67 and −82.86, respectively. ^1H and ^{13}C data are reported in the Experimental, the signals have been assigned through COSY (Fig. 1) and HETCOR (Fig. 2): the multiplet integrating 2H at δ 2.10 has been assigned to $CH_2SO_3^-$, the pseudotriplet (2H) at δ 2.85 ($^2J_{HH}$ = 7.22 Hz) is due to the central chain CH_2, while the CH_2 protons close to $PTAN^+$ give a signal at 3.00 ppm; the cage CH_2 have been found at 3.80 ppm (m, 4H, PCH_2N), 4.25 (d, $^2J_{HH}$ = 6.04 Hz, 2H, PCH_2N^+), at δ 4.36 and 4.50 (two doublets due to reciprocally coupled protons of NCH_2N $^2J_{HH}$ = 13.6 Hz), at δ 4.72 and 4.93 also two doublets due to reciprocally coupled protons NCH_2N^+, ($^2J_{HH}$ = 11.4 Hz).

The ^{13}C NMR shows the chain CH_2 as singlets at δ 16.7 ($CH_2SO_3^-$), δ 49.0 ($CH_2CH_2CH_2$) and δ 62.2 ($CH_2CH_2N^+$), while the cage CH_2 are found at δ 46.9 as a doublet due to PCH_2N ($^1J_{PC}$ = 20.6 Hz) and at δ 54.0 for PCH_2N^+, also a doublet with $^1J_{PC}$ = 33 Hz. At 70.5 and 80.2 ppm two singlets due to NCH_2N e NCH_2N^+ are observed.

The ES-MS shows a peak at 280, due to molecular weight plus an H^+, and one at 302, due to the addition of a Na^+ ion.

Similarly, it has been carried out the characterization of $PTA^+C_4H_8SO_3^-$, which presents analogue NMR feature (see Section 2) and, in ESI-MS, peaks at 294 (M+H^+), and 316 (M+Na^+).

3.2. Zwitterionic amines HMTA+C3H6SO3− (3) and HMTA+C4H8SO3− (4)

The aminic analogues of the above described zwitterionic phosphines, were obtained by alkylation of HMTA (Scheme 2).

$HMTA^+C_3H_6SO_3^-$ (**3**) was prepared refluxing HMTA with a small excess of 1,3-propanesultone in toluene for 18 h, while $HMTA^+C_4H_8SO_3^-$ (**4**) was obtained by mixing HMTA with pure 1,4-butanesultone at room temperature for 3 days.

Compounds **3** and **4** have been characterised by NMR and mass.

The ^1H NMR spectrum of $HMTA^+C_3H_8SO_3^-$ (**3**) is similar to that of $PTA^+C_3H_6SO_3^-$ for the alkylic chain pattern, while the aminic cage, more simmetrical than PTA, is characterised by two signals at 4.55 ppm (dd, 6H, NCH_2N^+) and 5.07 ppm (s, 6H, NCH_2N) in ^1H and at 70.0 (s, NCH_2N) and 78.2 ppm (s, NCH_2N^+) in ^{13}C NMR. In ESI mass the peak M+H^+ is observed at 263 and M+Na^+ at 285, together with dimeric species at 525 (2M+H^+) and 547 (2M+Na^+).

The NMR characterization of $HMTA^+C_4H_8SO_3^-$ (**4**), Fig. 3, is similar that of **3:** the peaks M+H^+ at *m/z* 277.1 and 2 M + H^+ at *m/z* 553.2 have been identified in the mass spectrum.

Suitable crystals of **3** were obtained and the X-ray crystal structure was determined.

The ORTEP [21] view of the trihydrated zwitterionic compound **3** is shown in Fig. 4. The crystal packing projected down the *b* axis is displayed in Fig. 5. The positive charge localised on the quaternary N1 nitrogen gives rise to significant lengthening of N1–C bonds (1.52[2] Å on average) with respect to the other N–C bond distances (1.46[2] Å on average) involving neutral N2, N3 and N4 nitrogens. The three-methylene linker adopts a full extended conformation (Table 2) as observed in other similar zwitterionic

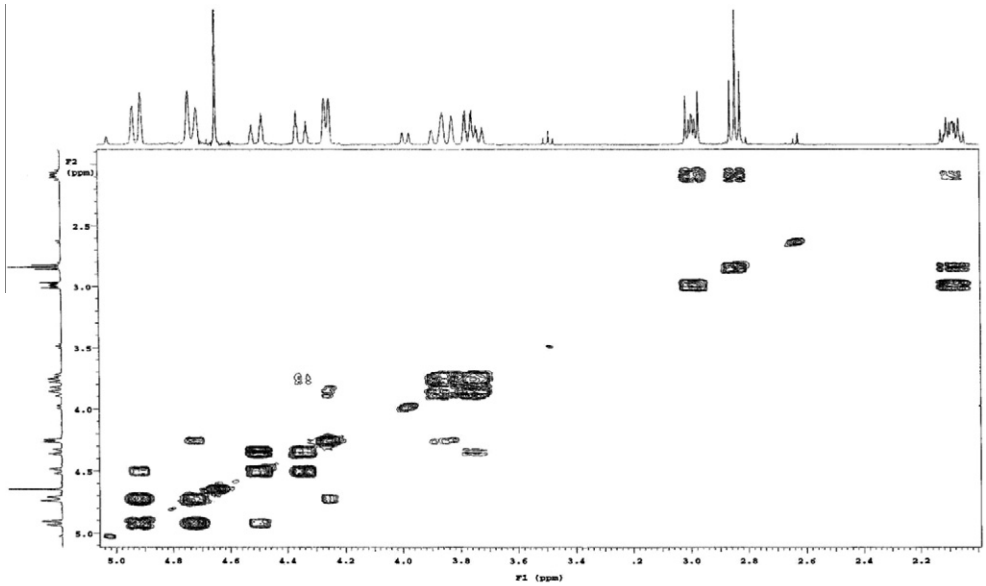


Fig. 1. COSY of $PTA^{+}C_3H_6SO_3^{-}$ (**1**).

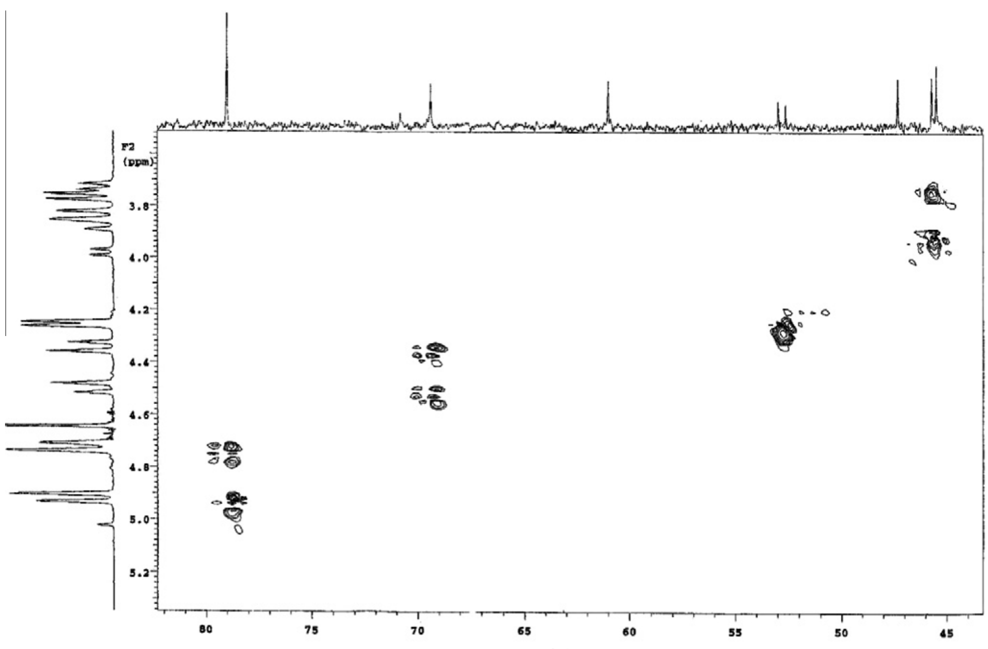
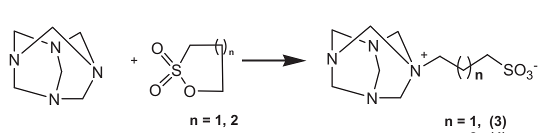


Fig. 2. HETCOR of $PTA^{+}C_3H_6SO_3^{-}$ (PTA cage detail).

compounds [22]. The molecules are packed in the crystal by means of C–H···O(sulfonate) and C–H···N($HMTA^{+}$) hydrogen bonds forming channels, parallel to *b* axis, which include all the molecules of water linked in infinite chains by means of strong hydrogen bonds and fixed to the channel walls through Ow–H···O(sulfonate) hydrogen bonds (Figs. 5, 6 and Table 3 inserted as Supplementary material).



Scheme 2. Synthesis of $HMTA^{+}C_3H_6SO_3^{-}$ (**3**) and $HMTA^{+}C_4H_8SO_3^{-}$(**4**).

3.3. Coordination of $PTA^{+}C_3H_6SO_3^{-}$ (1) and $PTA^{+}C_4H_8SO_3^{-}$ (2) to platinum

The coordination of the ligands $PTA^{+}C_3H_6SO_3^{-}$, $PTA^{+}C_4H_8SO_3^{-}$, $HMTA^{+}C_4H_8SO_3^{-}$ and $HMTA^{+}C_3H_8SO_3^{-}$ to platinum (II) has been attempted in various conditions with different precursors. The most successful reactions were performed dissolving the phosphine $PTA^{+}C_3H_6SO_3^{-}$ or $PTA^{+}C_4H_8SO_3^{-}$ in water and then adding a water solution of K_2PtCl_4 in a 2:1 ligand to metal ratio (Scheme 3).

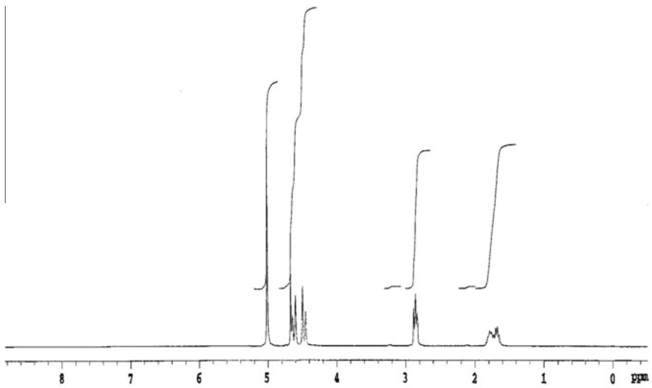


Fig. 3. ^{1}H NMR of $HMTA^{+}C_4H_8SO_3^{-}$, **4**, in D_2O.

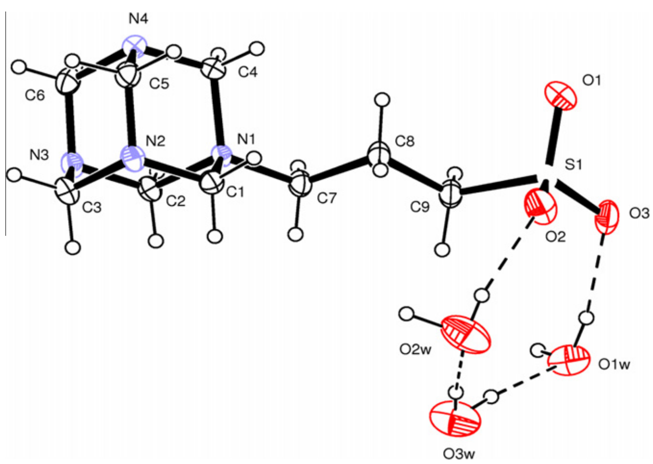


Fig. 4. ORTEP view of compound $HMTA^{+}C_3H_6SO_3^{-}$ (**3**). Thermal ellipsoids are drawn at 30% probability level.

In both cases the off-white products precipitated in a colloidal form from the solution, and after 10 min stirring at room temperature they were isolated by centrifugation and dried under vacuum.

The complexes **5** and **6** are poorly soluble in DMSO, H_2O, CH_3CN, CH_2Cl_2. In order to reach the concentration required by a reasonably fast acquisition of NMR spectra, we dissolved them in a 5 M HCl solution or in an aqueous NaCl saturated solution, where their solubility is higher then in pure water. In these media, interactions between Na^{+} (or H^{+}) and SO_3^{-} and between Cl^{-} and N^{+} are likely to be established. The observed increase of solubility could be due to the electrostatic interactions between the salt ions and the zwitterion surface charges, which prevent the formation of solute solid aggregates and stabilise the soluble forms [23].

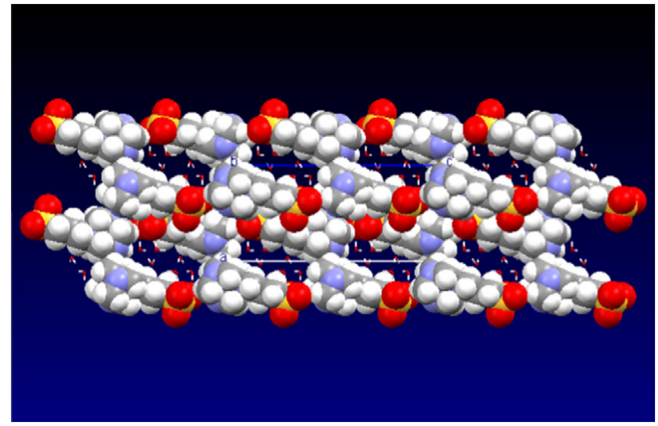


Fig. 6. A space filling representation of crystal packing of host–guest compound $HMTA^{+}C_3H_6SO_3^{-}\cdot 3H_2O$ (**3**) as viewed down the crystallographic *b* axis. The guest water molecules, included in the channels built up by the host $HMTA^{+}C_3H_6SO_3^{-}$ zwitterionic molecules, are shown using the stick representation.

Table 3
Estimated IC50 (μM) of **1**–**4** on A2780 and SKOV3 cell lines. Results are presented as a mean ± SD of three independent experiments performed in triplicates.

IC50	A2780	SKOV3
$PTA^{+}C_3H_6SO_3^{-}$, **1**	83.87 ± 0.78	>100
$PTA^{+}C_4H_8SO_3^{-}$, **2**	75.01 ± 1.67	>100
$HMTA^{+}C_3H_8SO_3^{-}$, **3**	39.58 ± 1.56	>100
$HMTA^{+}C_4H_8SO_3^{-}$, 4	38.22 ± 3.18	>100

The ^{31}P NMR of $[PtCl_2(PTA^{+}C_3H_6SO_3^{-})_2]$, **5**, has been acquired in HCl 5 M showing the presence of a signal with satellites at −38.27 ppm ($^{1}J_{PtP}$ = 3534 Hz) typical of a Pt(II) chloride PTA complex with *cis* geometry [24]. The ^{31}P NMR data in water saturated with NaCl are −37.98 ppm ($^{1}J_{PtP}$ 3534 Hz), indicating the presence of the same compound.

Similarly the ^{31}P NMR of complex $[PtCl_2(PTA^{+}C_4H_8SO_3^{-})_2]$, **6**, in D_2O saturated with NaCl, is a singlet with satellites at −37.99 ppm ($^{1}J_{PtP}$ = 3546 Hz). The ESI-MS analysis shows the $M+Na^{+}$ species at 875.

It is worth noticing that **5** and **6** are a rare type of zwitterionic complex where both the positive and negative charge belong to the ligand. In fact in the frequently reported so-called *platinum group metal zwitterions* the negative charge is due to the ligand and the positive to the metal [25].

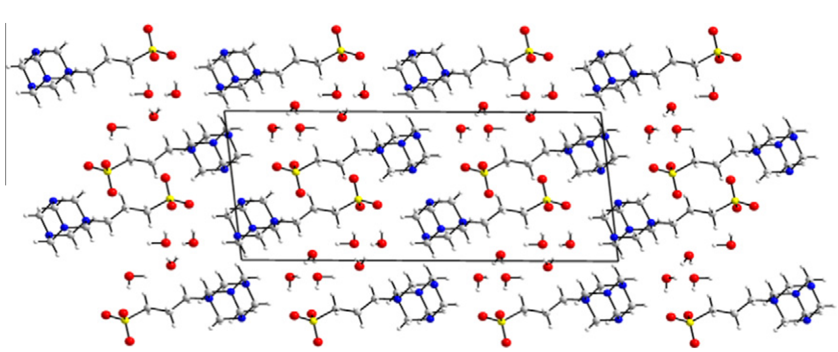
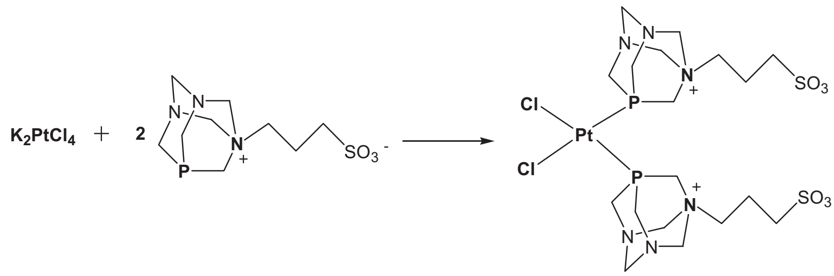
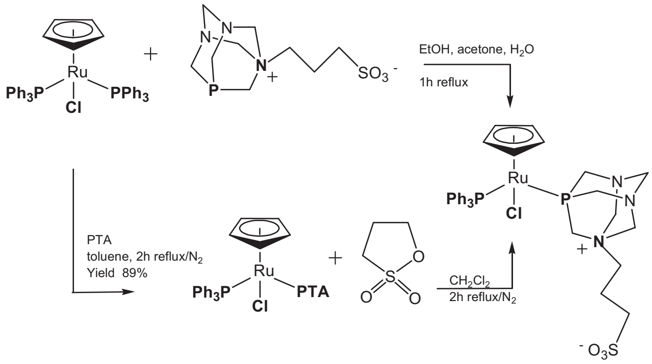


Fig. 5. Compound $HMTA^{+}C_3H_6SO_3^{-}$ (**3**), crystal packing projected down the *b* axis.



Scheme 3. Synthesis of the Pt complex cis-[$PtCl_2(PTA^+C_3H_6SO_3^-)_2$], **5**.



Scheme 4. Synthesis of the Ru complex **7**.

Table 4
Estimated IC50 (μM) of **1**, **5**, **7**, **2**, **6** and cisplatin on A2780 and SKOV3 cell lines. Results are presented as a mean ± SD of three independent experiments performed in triplicates.

IC50	A2780	SKOV3
$PTA^+C_3H_6SO_3^-$, **1**	83.87 ± 0.78	>100
[$PtCl_2(PTA^+C_3H_6SO_3^-)_2$], **5**	37.97 ± 1.52	>100
[$Cp(PPh_3)(PTA^+C_3H_6SO_3^-)RuCl$], **7**	>100	>100
$PTA^+C_4H_8SO_3^-$, **2**	75.01 ± 1.67	>100
[$PtCl_2(PTA^+C_4H_8SO_3^-)_2$], **6**	37.33 ± 3.94	>100
Cisplatin	10. 85 ± 1.10	>100

*3.4. Synthesis of Ru complexes of $PTA^+C_3H_6SO_3^-$ (**1**) and $PTA^+C_4H_8SO_3^-$ (**2**)*

Ru complexes NAMI-A and KP1019 are two anticancer ruthenium drugs which have already entered clinical trials. [26] The RAPTA-type complexes, ruthenium-arene-PTA organometallic species, have shown potential for the further development of anticancer remedies [27] and represent the reference for Ru complexes of new PTA derivatives.

Complexes **7** and **8** have been prepared in two ways (Scheme 4): (i) by replacing Ru coordinated PPh_3 in [$CpRuCl(PPh_3)_2$] with **1** or **2** or (ii) by treating the known parent complex [$CpRuCl(PPh_3)(PTA)$] with the commercial 1,3-propanesultone or 1,4-butanesultone in dichloromethane.

The proposed structures for **7** and **8** is consistent with the spectroscopic data. In the ^{31}P NMR spectra the products are characterised by an AM spin system of two doublets at *ca.* δ 46.56 ppm and at *ca.* δ −15.35 ppm, respectively, according to the presence of Ru-coordinated PPh_3 ligand and alkyl-PTA, reciprocally coupled with $^2J_{PP}$ = 43.0 Hz.

In the ^1H NMR spectra, the characteristic singlet of the cyclopentadienyl ligand is observed at 4.47 ppm; the signals of the aliphatic chains do not undergo a significant shift with respect to the free ligand, while for the cage protons, a broadening and a partial signals overlap is observed.

The coordination of ligands **3** and **4** to platinum and ruthenium was attempted unsuccessfully in a variety of conditions[1].

*3.5. Antiproliferative activity of $PTA^+C_3H_6SO_3^-$ (**1**), $PTA^+C_4H_8SO_3^-$(**2**), $HMTA^+C_4H_8SO_3^-$ (**3**), and $HMTA^+C_3H_8SO_3^-$(**4**)*

The antiproliferative activity of $PTA^+C_3H_6SO_3^-$, $PTA^+C_4H_8SO_3^-$, $HMTA^+C_3H_8SO_3^-$ and $HMTA^+C_4H_8SO_3^-$ have been tested on two human ovarian cancer cell lines, A2780 and SKOV3 and the results are reported in Table 3.

These figures indicated that the organic free ligands show some activity on A2780, higher for the amino HMTA derivatives.

In order to verify if the coordination of **1**–**4** to Pt or Ru would have improved the antiproliferative activity, we tried to obtain their complexes with these metal ions.

We found that only the zwitterionic phosphines $PTA^+C_3H_6SO_3^-$ and $PTA^+C_4H_8SO_3^-$ give isolable pure complexes. All the attempts (see Section 2) to obtain Pt complexes of the zwitterionic amines $HMTA^+C_3H_8SO_3^-$ and $HMTA^+C_4H_8SO_3^-$, both by direct coordination and by alkylation of [$PtCl_2(HMTA)_2$] failed.

In Table 4 we report the IC50 of Pt complexes of $PTA^+C_3H_6SO_3^-$ and $PTA^+C_4H_8SO_3^-$ compared with the corresponding ligands and cisplatin. As expected, the introduction of Pt does not improve the activity on cisplatin-resistant SKOV3, while on cisplatin-sensitive A2780, the activity of Pt complexes is higher than that of the corresponding phosphine.

The coordination to Ru decreases the antiproliferative activity of ligand **1** on both cell lines.

4. Conclusion

Phosphines **1** and **2** are the first reported example of PTA zwitterionic derivatives. Their antiproliferative activity, found significant on two human cancer cell lines, is increased upon coordination to Pt(II). The amino HMTA derivatives **3** and **4** present a higher activity than the corresponding PTA derivatives **1** and **2**.

These observations indicate that compounds **1**–**4** deserve further investigation aimed to exploit their zwitterionic properties and stereospecific coordinative ability for pharmaceutical purposes.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2012.12.006.

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