Synthesis, characterization and antiproliferative activity of amino- and DMSO complexes of platinum(II) containing *L*-carnitine.

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Keywords

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ABSTRACT

L-Carnitine, a biomolecule able to cross the blood-brain barrier exploiting specific transporters, behaves as mono or bidentate anionic ligand for Pt(II) in the new amino complexes *cis*-[Pt(*L*-carnitine-O)$_2$(NH$_3$)$_2$](BF$_4$)$_2$ (**1**), *cis*-[PtCl(*L*-carnitina-O)(NH$_3$)$_2$]BF$_4$ (**2**), [Pt(*L*-carnitine-O,O')(1,2-DACH)]BF$_4$ (**3**), [Pt(*L*-carnitine-O)$_2$(1,2-DACH)](BF$_4$)$_2$ (**4**), and [PtCl(*L*-carnitine-O)(1,2-DACH)](BF$_4$) (**5**). Four complexes with DMSO have been also prepared and characterized: the synthetic intermediate [Pt(CO$_3$)(DMSO)$_2$] (**6**), [Pt(*L*-carnitine-O,O')(DMSO)$_2$]BF$_4$ (**7**), *cis*-[Pt(*L*-carnitine-O)$_2$(DMSO)$_2$](BF$_4$)$_2$ (**8**) and *cis*-[PtCl(L-carnitine-O)(DMSO)$_2$]BF$_4$, (**9**).

The antiproliferative activity of three representative complexes **1**, **5** and **7** has been assayed against three human cancer cell lines A2780, K562 and SKOV3, and it was found comparable to that of the parent active compounds *cis*-[PtCl$_2$(1,2-DACH)] and cisplatin.

1. Introduction

L-Carnitine is an endogenous molecule, naturally occurring in animals, where is biosynthesized in the liver and kidneys from the amino acids *L*-lysine and *L*-methionine. It has a primary role in the transport of fatty acids from cytosol into the mitochondria, where their ß-oxidation to acetyl CoA is a step of the biochemical path which produces energy from the stored fat reserves [1].

For its role in fatty acids metabolism and for its antioxidant properties, *L*-carnitine is largely diffused as a nutritional supplement for wellness and as an adjuvant treatment for several diseases like myocardial infarction, angina pectoris, Alzheimer's disease, cancer [2]. It has also been introduced in drugs cocktails containing cisplatin because *L*-carnitine is considered able to mitigate some of cisplatin side effects like nephrotoxicity and intestine problems [3].

Moreover, because of its ability to cross the blood-brain barrier exploiting specific transporters, the conjugation of some poorly delivered drugs with *L*-carnitine has been recently proposed as a strategy for promoting their access to CNS. [4]

As we have underlined in a previous work [5], the chemical structure of *L*-carnitine allows its use as a ligand for metal ions without any chemical modification, and therefore it could be taken into account as a carrier for metal-based drugs to the CNS.

The aim of the present work is the preparation and characterization of *L*-carnitine complexes i) with Pt-amino ligands, namely NH_3 and 1,2-DACH, which have the role of carrier ligands in several Pt complexes with established antitumor activity, ii) with Pt-DMSO group, which has been recently reported as a component of active complexes. [6,7]
The introduction of *L*-carnitine in a Pt anticancer drug should be advantageous for many reasons: the positive charge of the quaternary ammonium group of *L*-carnitine is conserved in Pt complexes and is likely to favor the interaction with polyanionic DNA; *L*-carnitine Pt complexes could exploit its specific transporters and reach the CNS, where the cisplatin concentration is low; the antioxidant properties of *L*-carnitine could amplify the anticancer effect of Pt drugs and contribute to minimize their side effects.

2. Experimental section

2.1. Materials and instrument

All the manipulations were carried out in atmosphere unless otherwise noted. Elemental analyses were determined 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 (^{1}H at 300 MHz, ^{13}C at 75.43 MHz, ^{31}P at 121.50 MHz) or a Varian Mercury Plus (^{1}H at 400 MHz, ^{13}C at 100.58 MHz, ^{31}P at 161.92 MHz, ^{195}Pt at 85.64 MHz). The ^{13}C and ^{31}P spectra were run with proton decoupling, ^{13}C signals are reported in ppm relative to external tetramethylsilane (TMS) while ^{31}P signals are reported in ppm relative to an external 85% H_3PO_4 standard. The reference for ^{195}Pt NMR was Na_2PtCl_6 1M in D_2O. Commercial solvents and reagents were purchased and used without further purification. The parent metal complexes *cis*-$[PtI_2(NH_3)_2]$, *cis*-$[PtCl_2(NH_3)_2]$, [8] $[PtCl_2$(1,2-DACH)] [9], $[PtCO_3$(1,2-DACH)] [10] and *cis*-$[PtCl_2(DMSO)_2]$ [11] were prepared as described in the literature.

*2.2. Synthesis of amino complexes **1-5***

Complex cis-[Pt(L-carnitine-O)$_2$(NH$_3$)$_2$](BF$_4$)$_2$, **1**
cis-$[PtI_2(NH_3)_2]$ (0.400 g, MW 482.9 g mol^{-1}, 8.3· 10^{-4} mol) was suspended in 150 mL of water and kept under vigorous stirring at 50°C for 15 min; a solution of $AgBF_4$ (0.330 g, MW 194.7 g mol^{-1}, 1.7 · 10^{-3} mol, 2 eq) in 10 mL of H_2O was then added dropwise.
The mixture was kept under stirring in the dark at room temperature for 18 hours.

The yellow precipitate of AgI was then removed by filtration over a short column of celite, and the volume of the clear solution was reduced under vacuum. *L*-carnitine inner salt (0.274 g, $1.7 \cdot 10^{-3}$ mol, 2 eq), dissolved in one mL of water, was then added and the mixture was stirred for a further 4 hours, then taken to dryness under vacuum. The solid white residue was then dried over P_2O_5. (0.581 g, MW 725.1 g mol^{-1}, $8.0 \cdot 10^{-4}$ mol, yield 97%). Soluble in H_2O and DMSO.

Complex **1** found (% calculated for $C_{14}H_{36}B_2F_8N_4O_6Pt$): C 23.01 (23.19), H 5.09 (5.00) and N 7.67 (7.73).

^{1}H NMR (300 MHz *D_2O*, 25°C) δ = 2.28 (bm, 4H, CH_2COO), 3.05 (s, 18H, Me_3N^+), 3.27 (m, 4H, CH_2N), ca. 3.9 ppm (bm, 6H, $Pt(NH_3)_2$), 4.40 (m, 2H, CHOH) ppm. The signal at 3.9 ppm collapses and disappears completely in 6 hours; the other signals do not change over 30 hours.

^{1}H NMR (300 MHz DMSO-d_6, 25°C) δ = 2.00 (bm, 4H, CH_2COO), 3.10 (s, 18H, Me_3N^+), 3.25 (m, 4H, CH_2N), 4.00 (bm, 6H, NH_3), 4.40 (m, 2H, CHOH) ppm.

^{195}Pt NMR (85.64 MHz, DMSO, 25°C) δ = -3136 ppm.

MS-ESI: Major: observed m/z 275.53, calculated 551.28/2=275.62 for $C_{14}H_{36}N_4O_6Pt$ $(M-2BF_4)^{2+}$. Minor: observed 638.07, calculated 638.34 for $C_{14}H_{36}BF_4N_4O_6Pt$ $(M- BF_4)^+$.

Complex cis-[PtCl(L-carnitine-O)(NH_3)$_2$]BF_4, **2**

cis-[$PtCl_2(NH_3)_2$] (0.138 g, MW 300 g mol^{-1}, $4.6 \cdot 10^{-4}$ mol) suspended in 30 mL of H_2O was kept under vigorous stirring at 50°C until it turned into a pale yellow solution denoting the formation of aquo species. After 30 min a solution of $AgBF_4$ (0.09 g, MW 194.7 g mol^{-1}, $4.6 \cdot 10^{-4}$ mol, 1 eq) in 2 mL of H_2O was added and left under stirring at room temperature for 20 hours.

The white precipitate of AgCl was then removed by filtration. *L*-carnitine inner salt (0.074 g, $4.6 \cdot 10^{-4}$ mol, 1 eq), dissolved in a few mL of water, was then added and the mixture was stirred for a further 20 hours, then taken to dryness under vacuum. The solid yellow residue was then dried under vacuum over P_2O_5. (0.173 g, MW 512.6 g mol^{-1}, $3.4 \cdot 10^{-4}$ mol, yield 73.4%). Soluble in DMSO and H_2O.

Complex **2** found (% calculated for $C_7H_{21}BClF_4N_3O_3Pt$): C 16.54 (16.40), H 4.23 (4.13) and N 8.12 (8.20).

^{1}H NMR (300 MHz *D_2O*, 25°C) δ = 2.36 (bm, 2H, CH_2COO), 3.05 (s, 9H, Me_3N^+), 3.28 (m 2H, CH_2N), 4.43 (m, 1H, CHOH) ppm.

^{1}H NMR (300 MHz, DMSO-d_6, 25°C) δ =2.26 (bm, 2H, CH_2COO), 3.10 (s, 9H, Me_3N^+), 3.32 (2s, 2H, CH_2N), 3.9-4.6 (bm, 4H, CHOH + NH_3) ppm.

MS-ESI: observed m/z 425.9 (M^+). (MW – BF_4), calculated 425.6 for $C_7H_{21}ClN_3O_3Pt$.

Complex [Pt(L-carnitine-O,O')(1,2-DACH)]BF_4, **3**

[$PtCO_3$(1,2-DACH)] (0.100 g, MW 369.3 g mol^{-1}, 2.7 · 10^{-4} mol) was dissolved in 20 mL of H_2O. A second solution containing *L*-carnitineBF_4 (0.038 g, 1.5 · 10^{-4} mol, 1 eq) in 3 mL of H_2O was then added dropwise to the previous. The mixture was kept under stirring for 20 hours and then taken to dryness giving a cream solid, soluble in H_2O and DMSO. (0.132 g, MW 556.3 g mol^{-1}, 2.4 · 10^{-4} mol, yield 87.8%).

Complex **3** found (% calculated for $C_{13}H_{29}BF_4N_3O_3Pt$): C 28.10 (28.02), H 5.22 (5.25) and N 7.52 (7.54).

^{1}H NMR (300 MHz *D_2O*, 25°C) δ = 1.0-1.1, 1.4, 1.85, 2.4 (bm, 10H, DACH), 2.27 e 2.29 (2 d, 2H, CH_2COO), 3.05 (s, 9H, Me_3N^+), 3.27 (m, 2H, CH_2N), 4.4 (m, 1H, CHO) ppm.

MS-ESI: observed m/z 469.13, calculated 469.26 for $C_{13}H_{28}N_3O_3Pt$ (M^+).

Complex [Pt(L-carnitine-O)$_2$(1,2-DACH)](BF_4)$_2$, **4**

A solution of $AgBF_4$ (0.103 g, 5.3 · 10^{-4} mol, 1 eq) in 3 mL of H_2O was added dropwise under stirring to a suspension of [$PtCl_2$(1,2-DACH)] (0.100 g, 2.6 · 10^{-4} mol) in 20 mL of H_2O. After ten minutes a solution of *L*-carnitine inner salt (0.085 g, 5.3 · 10^{-4} mol, 2 eq) in 3 mL of water was also added. The mixture was kept under stirring for 24 hours and then subject to centrifugation to remove AgCl. The remaining solution is then taken to dryness giving a cream solid (0.200 g, MW 805.3 g mol^{-1}, 2.5 · 10^{-4} mol, yield 94%), soluble in H_2O and DMSO.

Complex **4** found (% calculated for $C_{20}H_{44}B_2F_8N_4O_6Pt$): C 29.90 (29.83), H 5.58 (5.51) and N 7.01 (6.96).

^{1}H NMR (300 MHz *D_2O*, 25°C) δ =1.0-1.1 (4H), 1.4 (2H), 1.9 (2H), (bm, 8H, DACH), 2.2-2.25 (bm, 2H, DACH + 2d, 4H, CH_2COO), 3.05 (s, 18H, Me_3N^+), 3.25 (m, 4H, CH_2N), 4.4 (bm, 2H, CHOH).

^{1}H NMR (300 MHz DMSO-*d_6*, 25°C) δ = 1.03, 1.2, 1.5, 1.9, 2, 2.25 (bm, 10H, DACH), 1.95 (bm, 4H, CH_2COO), 3.1 (s, 18H, Me_3N^+), 3.2 (m, 4H, CH_2N), 4.2 (bm, 2H, CHOH), 7.7 (bs, 1H, OH) ppm.

MS-ESI: observed m/z 718.27, (718.11 calculated for $C_{20}H_{44}BF_4N_4O_6Pt$ (M^+)) and 315.6 (M^{2+})

Complex [PtCl(L-carnitine-O)(1,2-DACH)]BF_4, **5**

Complex **5** was prepared as above described for complex **4**, using 1 eq of $AgBF_4$ (0.051 g, 2.6 · 10^{-4} mol) and 1 eq of L-carnitine inner salt (0.042 g, 2.6 · 10^{-4} mol)

The product was obtained as a crystalline pale yellow solid (0.143 g, MW 592.7 g mol^{-1}, $2.4 \cdot 10^{-4}$ mol, yield 92%), soluble in water and DMSO.

Complex **5** found (% calculated for $C_{13}H_{29}BClF_4N_3O_3Pt$): C 26.33 (26.34), H 5.12 (4.93) and N 7.15 (7.09).

^{1}H NMR (300 MHz *D_2O*, 25°C) δ = 0.9-1.2 (4H), 1.44 (2H), 1.9 (2H), (bm, 8H, DACH), 2.3 (bm, 2H, DACH + 2d, 2H CH_2COO), 3.1 (s, 9H, Me_3N^+), 3.3 (m, 2H, CH_2N), 4.4 (bm, 1H, CHO) ppm. Unchanged over 30 hours.

^{1}H NMR (300 MHz DMSO-*d_6*, 25°C) δ = 1.0, 1.2, 1.4 (bm, 6H, DACH), 2.0 (bm, 4H DACH + 2H, CH_2COO), 3.1 (s, 9H, Me_3N^+), 3.2 (m, 2H, CH_2N), 4.2 (bm, 1H, CHO), 5-6 (bm, NH_2 DACH), 7.1 (bs, 1H, OH) ppm.

^{195}Pt NMR (85.64 MHz, DMSO, 25°C) δ = -3267 ppm.

MS-ESI: observed m/z 506.13, (506.15 calculated for $C_{13}H_{29}ClN_3O_3Pt$, M^+).

*2.3. Synthesis of DMSO complexes **6-9***

Complex $[PtCO_3(DMSO)_2]$, **6**

Finely grounded *cis*-$[PtCl_2(DMSO)_2]$ (0.254 g, PM 422.14 g mol^{-1}, $6.02 \cdot 10^{-4}$ mol, 1 eq) was suspended in 30 mL of H_2O, solid Ag_2CO_3 (0.166 g, $6.02 \cdot 10^{-4}$ mol, 1 eq) was added and the reaction was kept under stirring at room temperature in the dark for 20 hours to complete the precipitation of AgCl. The suspension was then filtered over a celite pad giving a clear colorless solution containing $[PtCO_3(DMSO)_2]$, which can be isolated as a pale yellow water soluble solid (0.220 g, MW 411.2 g mol^{-1}, $5.35 \cdot 10^{-4}$ mol, yield 89%).

^{1}H NMR (300 MHz *D_2O*, 25°C) δ = 3.31 ($^3J_{HPt}$ = 27.6 Hz, 12H, CH_3).

^{13}C NMR (400 MHz, *D_2O*, 25°C) δ = 165.65 (s, 1C, CO_3), 42.67 (s, $^2J_{CPt}$ = 38.4 Hz, 4C, CH_3,) ppm.

^{195}Pt NMR (85.64 MHz, *D_2O, 25°C*) δ = -3155.5 ppm

Complex $[Pt(L\text{-carnitine-}O,O')(DMSO)_2]BF_4$, ***7***

$[PtCO_3(DMSO)_2]$ was dissolved in 20 mL of H_2O and then *L*-carnitineBF_4 (0.09 g, $3.6 \cdot 10^{-4}$ mol, 1 eq) in 1 mL of H_2O was added (0.148 g, $3.6 \cdot 10^{-4}$ mol). The solution was kept under stirring for 3 hours and then taken to dryness leaving a sticky solid which was washed with acetone (0.215 g, MW 598.3 g mol^{-1}, $3.6 \cdot 10^{-4}$ mol, yield 100%). The product is soluble in H_2O e DMSO.

Complex **7** found (% calculated for $C_{11}H_{26}BF_4NO_5PtS_2$): C 21.90 (22.08), H 4.45 (4.38) and N 2.30 (2.34).

^{1}H NMR (300 MHz D_2O, 25°C) δ = 2.2 (bm, 2H, CH_2COO), 3.1 (s, 9H, Me_3N^+), 3.2 (m, 2H, CH_2N), 3.5 (s, 12 H, CH_3 DMSO), 4.2 (m, 1H, CHO) ppm.
^{1}H NMR (300 MHz *d*$_6$-DMSO, 25°C) δ = 2.2 (bm, 2H, CH_2COO), 3.1 (s, 9H, Me_3N^+), 3.3 (m, 12 H, CH_3 of DMSO + 2H, CH_2N), 4.2 (m, 1H, CHO) ppm.
^{195}Pt NMR (85.64 MHz, DMSO, 25°C) δ = -3193.5 ppm.
MS-ESI: observed m/z 511, calculated 511.4 for $C_{11}H_{26}NO_5PtS_2$ (M^+).

Complex cis-[Pt(L-carnitine-O)$_2$(DMSO)$_2$](BF$_4$)$_2$, **8**

A solution of $AgBF_4$ (0.184 g, $9.4 \cdot 10^{-4}$ mol, 2 eq) in pochi mL di H_2O was added under stirring to a suspension of *cis*-[$PtCl_2(DMSO)_2$] (0.200 g, $4.7 \cdot 10^{-4}$ mol) in 30 mL di H_2O . After 3 hours AgCl was removed by filtration on a Celite pad, and then a solution of *L*-carnitine inner salt (0.152 g, $9.4 \cdot 10^{-4}$ mol, 2 eq) in water was added to the remaining clear solution. The mixture was kept under stirring in the dark for 15 hours and then taken to dryness, leaving a pale yellow sticky solid. (0.307 g, MW 847.3 g mol^{-1}, $3.6 \cdot 10^{-4}$ mol, yield 76%). Soluble in water and DMSO.
Complex **8** found (% calculated for $C_{18}H_{42}B_2F_8N_2O_8PtS_2$): C 25.55 (25.51), H 5.02 (5.00) and N 3.32 (3.31).
^{1}H NMR (300 MHz D_2O, 25°C δ = 2.28 (d, $^{3}J_{HH}$ 4.9 Hz, 2H, CH_2COO), 2.29 (d, $^{3}J_{HH}$ 6.24 Hz, 2H, CH_2COO), 3.06 (s, 18H, Me_3N^+), 3.27 and 3.29 (s, 4H, CH_2N), 3.34 (s, 12H, CH_3 DMSO), 4.4 (m, 2H, *CH*OH) ppm.
^{1}H NMR (300 MHz DMSO-d_6, 25°C) δ = 2.0 (2d, 4H, CH_2COO), 3.06 (s, 18H, Me_3N^+), 3.2 - 3.4 (m, 12H, CH_3 coordinated DMSO + m, 4H, CH_2N),4.2 (bm, 2H, CHOH) ppm, 7.2 ppm (OH).
MS-ESI: observed m/z 511, calculated 511.4 for $C_{11}H_{26}NO_5PtS_2$ (M^+- *L*-carnitine).

Complex cis-[PtCl(L-carnitine-O)(DMSO)$_2$]BF$_4$, **9**

Complex **9** was prepared as above described for **8**, except the reagents ratio, which was the following: *cis*-[$PtCl_2(DMSO)_2$] (0.200 g, $4.7 \cdot 10^{-4}$ mol), $AgBF_4$ (0.092 g, $4.74 \cdot 10^{-4}$ mol, 1 eq) and *L*-carnitine (0.076 g, $4.74 \cdot 10^{-4}$ mol, 1 eq). Complex **9** was obtained as a sticky pale yellow solid (0.143 g, MW 634.1 g mol^{-1}, $2.25 \cdot 10^{-4}$ mol, yield 95%). Soluble in H_2O and DMSO.
Complex **9** found (% calculated for $C_{11}H_{27}BClF_4NO_5PtS_2$): C 20.78 (20.81), H 4.31 (4.29) and N 2.20 (2.21).

^{1}H NMR (300 MHz D_2O, 25°C), δ = 2.26 (d, $^{3}J_{HH}$ 3.7 Hz, 2H, CH_2COO), 2.28 (d, $^{3}J_{HH}$ 4.3 Hz, 2H, CH_2COO), 3.08 (s, 9H, Me_3N^+), 3.2 - 3.5 (m, 2H, CH_2N + 12H, CH_3),4.43 (bm, 1H, CHOH) ppm.

^{1}H NMR (300 MHz, *d*$_6$-DMSO, 25°C), δ = 2.06 (m, $^{3}J_{HH}$ 3.7 Hz, 2H, *CH*$_2$COO), 3.11 (s, 9H, Me_3N^+), 3.08 - 3.34 (m, 2H, *CH*$_2$N + 12H, *CH*$_3$ DMSO),4.25 (bm, 1H, *CH*OH) ppm.

^{195}Pt NMR (85.64 MHz, DMSO, 25°C) δ = -3193.4 ppm

MS-ESI: observed m/z 546.9, calculated 547.3 per $C_{11}H_{27}ClNO_5PtS_2$ (M^+), m/z 511, calculated 511.4 for $C_{11}H_{26}NO_5PtS_2$ (M^+- Cl).

2.4. Growth inhibition assays

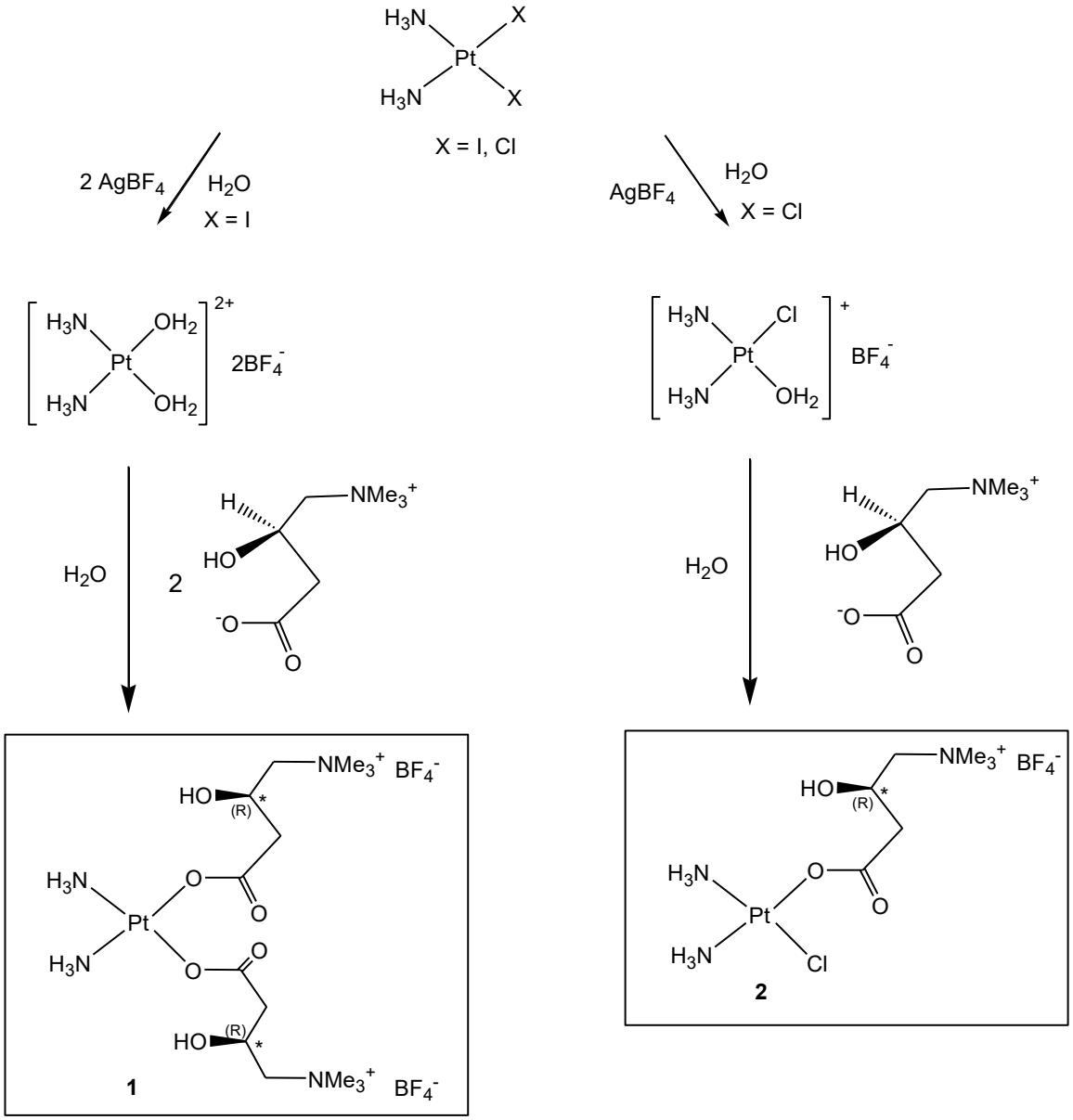
Cell growth inhibition assays were carried out using the leukemia cell line K562 and two human ovarian cancer cell lines, A2780 and SKOV3; K562 and A2780 cells are cisplatin-sensitive and SKOV3 cells are cisplatin-resistant. Cell lines were obtained from ATCC (Manassas, VA) and maintained in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), penicillin (100 Units mL-1), streptomycin (100 μg mL-1) and glutamine (2mM) (complete medium); the pH of the medium was 7.2 and the incubation was performed at 37 °C in a 5% CO_2 atmosphere. Adherent cells were routinely used at 70% of confluence and passaged every 3 days by treatment with 0.05% trypsin-EDTA (Lonza). K562 cells were routinely fed every 3 days. The antiproliferative activity of the compounds was tested with 3-(4,5-dimethylthiozol-2-yl)2,5-diphenyltetrazolium bromide solution (MTT) assay [12]. The cells were seeded in triplicate in 96-well trays at the density of $5 \cdot 10^3$ in 50 μL of complete medium. Stock solutions (20 mM) of compounds **1, 5, 7,** cisplatin and *L*-carnitineBF_4 were made in water, while stock solutions (20 mM) of [$PtCl_2$(1,2-DACH)] and *cis*-[$PtCl_2(DMSO)_2$] were made in DMSO. All solutions were diluted in complete medium to give final concentrations of 10, 1 and 0.1 μM. Cisplatin was employed as a control for the cisplatin-sensitive A2780 and K562 cell lines, 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 hours, and then 25 μL of a 12 mM solution of MTT were added. After two hours of incubation, 100 μL of lysing buffer (50% DMF + 20% sodium dodecyl sulfate (SDS), pH 4.7) were added to convert the MTT solution into a violet colored formazane. After additional 18 hours the solution absorbance, proportional to the number of live cells, was measured by spectrophotometer at 570 nm and converted into % of growth inhibition.

3. Results and discussion

*3.1. Synthesis and characterization of L-carnitine Pt-amino complexes **1**-**5**.*

All the Pt complexes which have been approved as drugs and have been successfully employed in clinics since many years and still now, have a common chemical character: they contain amino ligands, namely NH_3 or 1,2-DACH [13,14]. For this reason we have focused our interest on NH_3 and 1,2-DACH Pt complexes bearing *L*-carnitine as anionic ligand, both as mono and bidentate chelating ligand.

The bicarboxylate complex *cis*-[Pt(*L*-carnitine-O)$_2$(NH_3)$_2$](BF_4)$_2$ **1,** has been prepared from *cis*-[PtI_2(NH_3)$_2$] in water as described in the Experimental section and in Scheme 1.



Scheme 1

Complex **1** has been characterized by ^{1}H-NMR in D_2O, which shows the peaks of coordinated *L*-carnitine (2.28 CH_2COO, 3.05 NMe_3^+, 3.27 CH_2N, 4.40 CHOH) and a very broad signal around 3.9 ppm due to $Pt(NH_3)_2$ [15], which slowly exchange with D_2O disappearing over 6 hours. All the other signals do not undergo variations in 30 hours, proving the stability of complex **1** in an aqueous medium. In DMSO-d_6, all the signals are found at very similar shifts, at 4.0-4.4 ppm a broad signal is observed due to NH_3 overlapped to C<u>H</u>OH. In both solvents, the CH_2 signals of CH_2COO and CH_2N are found as unresolved multiplets because the protons of each pair are diastereotopic and therefore inequivalent: two close signals are expected coupled each other and coupled to vicinal protons with different unresolved coupling constants. The presence of a single species is confirmed by ^{195}Pt NMR, showing one signal at -3136 ppm.

The identity of **1** has been confirmed by its MS-ESI spectrum where the doubly charged peak M^{2+} (MW- $2BF_4$) is observed at 275.5 and a minor one at 638.2 corresponding to mono-charged M^+, due to the loss of a single BF_4^- (MW- BF_4).

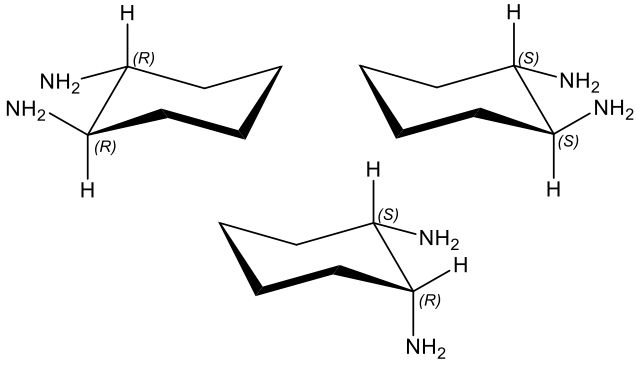
A further confirmation of the identity of **1** is the exchange of NH_3 for PPh_3 in DMSO solution. After a few minutes, the ^{31}P NMR spectrum of the known phosphinic bicarboxylate complex *cis*-[Pt(*L*-carnitine-O)$_2$(PPh_3)$_2$](BF_4)$_2$ is observed as a singlet with satellites at 6.24 ppm ($^{1}J_{PtP}$ 3723 Hz), coincident with the data reported in our previous paper on PPh_3-Pt complexes with carnitine [5]. Although isomerization processes cannot be excluded, the formation of the *cis* isomer of the phosphinic product (as proved by the value $^{1}J_{PtP}$) supports the hypothesis of a *cis* geometry also for complex **1**.

Complex **2**, *cis*-[PtCl(*L*-carnitine-O)(NH_3)$_2$]BF_4, containing a single *L*-carnitine as a monodentate carboxylate ligand, has been prepared from *cis*-[$PtCl_2$(NH_3)$_2$] as reported in the Experimental section and in Scheme 1.

Complex **2** has been characterized by ^{1}H-NMR in D_2O, showing the peaks of coordinated *L*-carnitine, whose data are similar to those of complex **1** in the same solvent; in DMSO-d_6 the signals of **2** are found at 2.26 ppm CH_2COO, 3.10 ppm Me_3N^+, 3.32 ppm CH_2N and the signal of NH_3 is observed as a broad peak between 3.9-4.6 pm, overlapped with the signal of C<u>H</u>OH.

The MS-ESI spectrum of **2** shows a signal at 425.9, corresponding to the monocharged cation M^+ [MW - BF_4]$^+$.

1,2-DACH is a chelating diamine largely employed in platinum anticancer drugs [14]. It presents three isomeric forms: two optically active *trans* forms (1*R*, 2*R* and 1*S*, 2*S*) and one *cis* meso form. We have used the *trans* form (1*R*, 2*R*)-(-)-1,2-diaminocyclohexane.



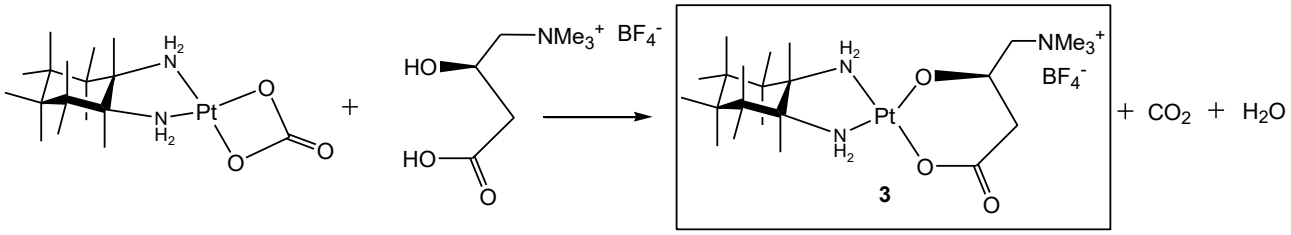
The NH_2 groups on vicinal carbons act as chelating sites including the metal into a stable five-membered ring.

The here presented complexes have been obtained from [$PtCl_2$(1,2-DACH)], whose synthesis was firstly reported in 1985 [9].

For preparing the bis-chelate complex **3** (Scheme 2), [$PtCl_2$(1,2-DACH)] needs to be converted into the carbonato-complex [$PtCO_3$(1,2-DACH)], also described before [10]. When *L*-carnitine is added to a solution of [$PtCO_3$(1,2-DACH)] in water, the carbonate acts both as a diprotic base and as a leaving group. *L*-carnitine, doubly deprotonated at the carboxylate and at γ-CHOH, replaces CO_3^{2-}, which leaves as CO_2 and H_2O, giving complex **3** [Pt(*L*-carnitine-O,O')(1,2-DACH)]BF_4. The deprotonation and coordination of γ-CHOH occurs because is driven by the formation of a 6-membered chelated ring and of a volatile side product (CO_2).

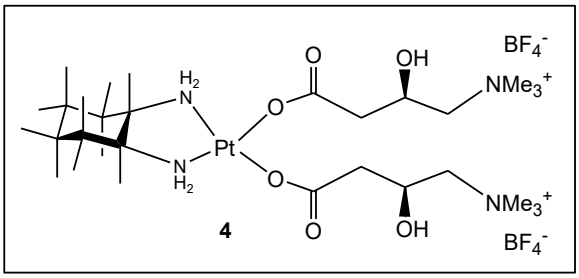
The characterization of **3** is based on ^{1}H NMR, which shows 4 multiplets of chelating 1,2-DACH between 1.0 and 2.4 ppm (total 10H) and the signals of *L*-carnitine at 2.27 and 2.29 ppm (2 doublets of diastereotopic CH_2COO), a singlet at 3.05 ppm integrating for 9H, unresolved multiplets at 3.27 (CH_2N) and 4.4 (CHO) ppm.

The MS-ESI shows a peak at 469.13 corresponding to M^+ (MW - BF_4) $^+$.



Scheme 2

The bicarboxylate complex [Pt(*L*-carnitina-O)$_2$(1,2-DACH)](BF$_4$)$_2$, **4,** has been prepared by treating [PtCl$_2$(1,2-DACH)] with two equivalents of AgBF$_4$ and two of *L*-carnitine inner salt. The ^{1}H-NMR in D$_2$O shows the signals of coordinated 1,2-DACH and *L*-carnitine as above, but in a 1:2 ratio, while the MS-ESI is characterized by M$^+$ (MW - BF$_4$)$^+$ at 718.



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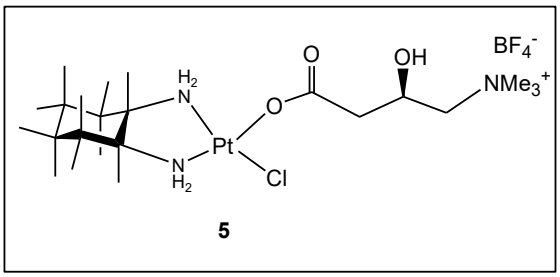
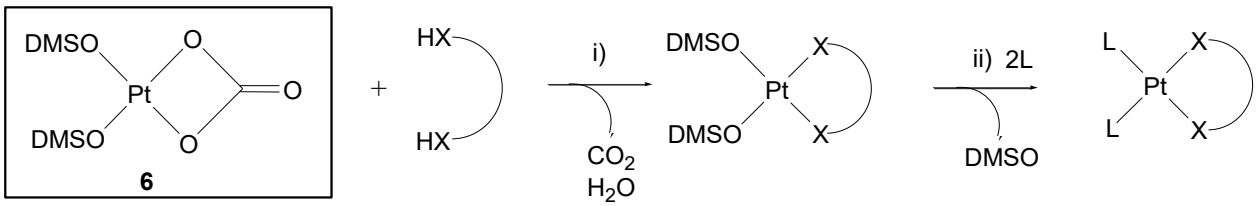


5

The chlorocomplex **5,** [PtCl(*L*-carnitina-O)(1,2-DACH)](BF$_4$), has been obtained in the same way using a single equivalent of AgBF$_4$ followed by one equivalent of *L*-carnitine inner salt. The ^{1}H NMR of **5** in D$_2$O is similar to the previous, except that the integration shows a 1:1 ratio between 1,2-DACH and *L*-carnitine. No variations in the ^{1}H NMR in D$_2$O have been noticed in 30 hours observation at room temperature, supporting the stability of complex **5** in such conditions. The MS-ESI shows the M$^+$ (MW - BF$_4$)$^+$ peak at 506.13, with a few other minor peaks.

*3.2. L-carnitine Pt-DMSO complexes **6**-**9**.*

Pt complexes bearing *S*-coordinated DMSO as neutral ligand can be regarded as versatile synthetic tools for the preparation of other complexes by DMSO replacement or by the substitution of the ligand in *trans* position to DMSO favored by its high *trans* effect.
Moreover, recent investigations have revealed interesting results for Pt(II) complexes with a DMSO moiety, especially with respect to their nucleoside binding capacities [16]
With the aim of finding a synthetic way to the chelate complex **7**, we have isolated for the first time the carbonato complex [PtCO$_3$(DMSO)$_2$], **6**, a versatile synthon which allows the coordination of chelating bi-acid ligands by protonolysis of Pt-O bonds, releasing only CO$_2$ (Scheme 3, step i) as side product. Moreover the neutral ligands DMSO can also be replaced by other neutral ligands (Scheme 3, step ii).

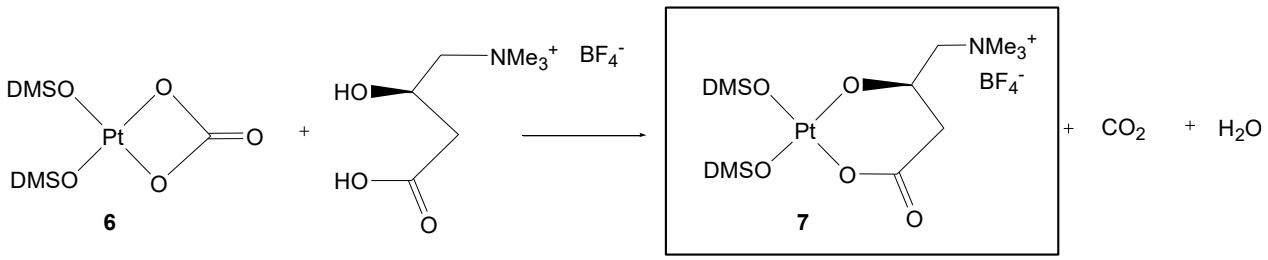


Scheme 3

Complex $[PtCO_3(DMSO)_2]$, obtained treating the dichloride with Ag_2CO_3, has been characterized by NMR in D_2O. The ^1H and ^{13}C signals of the CH_3 of coordinated DMSO are found at 3.31 ppm with $^3J_{PtH}$ di 27.6 Hz (^1H) and 42.67 ppm $^2J_{CPt}$ di 38.4 Hz (^{13}C) [17-19]. The presence of Pt-Me coupling confirms that DMSO is S-coordinated. In the ^{13}C NMR it is possible to observe also the signal of coordinated CO_3^{2-} at 165.65 ppm, too weak to detect the Pt satellites. For comparison, the corresponding $PtCO_3$ signal in *cis*-$[PtCO_3(PPh_3)_2]$ was found at 166.9 ppm with $^2J_{PtC}$ 66 Hz [20-22].

$[PtCO_3(DMSO)_2]$ is soluble in water, although after long time in solution it decomposes giving hydrolysis products as reported for other Pt-DMSO complexes [23].

The reaction of $[PtCO_3(DMSO)_2]$ with one equivalent of *L*-carnitineBF_4 gives the chelate complex **7,** [Pt(*L*-carnitine-O,O')$(DMSO)_2]BF_4$. (Scheme 4).



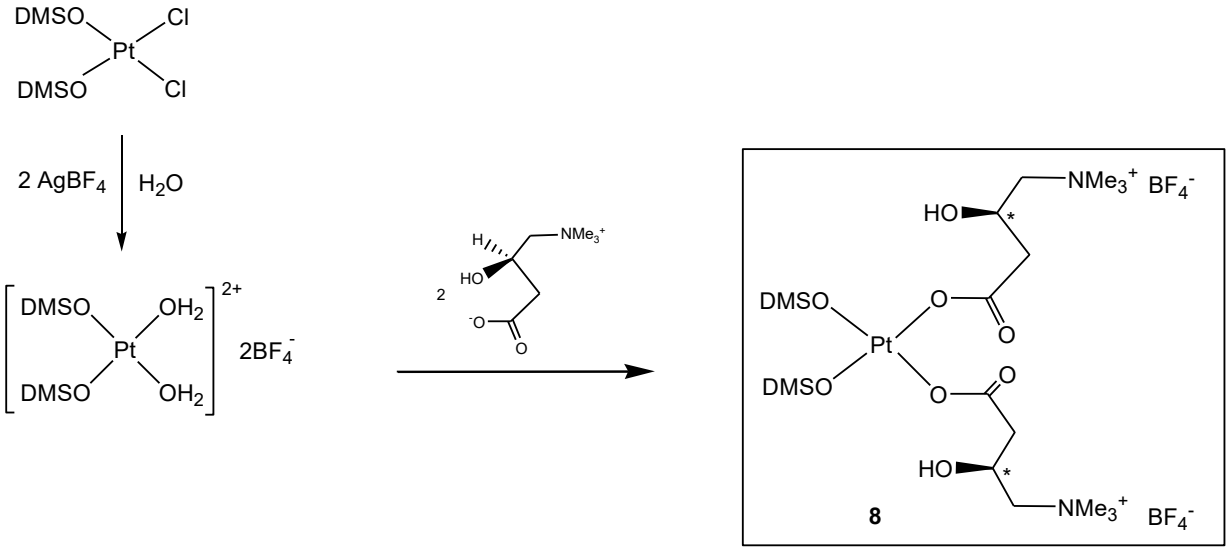
Scheme 4

Complex **7** has been characterized by ^1H NMR and MS-ESI. ^1H-NMR in DMSO shows the signals of coordinated *L*-carnitine close to those of the free molecule and a multiplet at 3.3 ppm integrating for 14 protons (12 of DMSO and 2 of CH_2N), while the MS-ESI shows M^+ at 511.0.

In order to confirm the identity of the chelate **7,** we exchanged both its DMSO ligands for PPh_3, obtaining the known complex [Pt(*L*-carnitine-O,O')$(PPh_3)_2]BF_4$, as observed by its ^{31}P NMR (10.5 ppm, $^1J_{PtP}$ 3943 Hz, *trans* to COO e 10.1 ppm, $^1J_{PtP}$ 3447 Hz, *trans* to O, $^2J_{PP}$ = 22 Hz) [5]. The formation of [Pt(*L*-carnitine-O,O')$(PPh_3)_2]BF_4$ is an example of total ligands substitution on complex **6**, completed in two steps**.**

The bicarboxylate analogue *cis*-[Pt(*L*-carnitine-O)$_2$(DMSO)$_2$](BF$_4$)$_2$, **8** has been prepared from *cis*-[PtCl$_2$(DMSO)$_2$] as described in Section 2.2 and in Scheme 5.

In the ^{1}H NMR in DMSO-d$_6$ and in D$_2$O the signal of coordinated DMSO is found at 3.34 ppm, partially overlapped with the peaks of the diastereotopic protons of CH$_2$N$^+$ at 3.29 and 3.27 ppm. In the MS-ESI a species containing a single *L*-carnitine (M$^+$ - *L*-carnitine) is observed at 511.

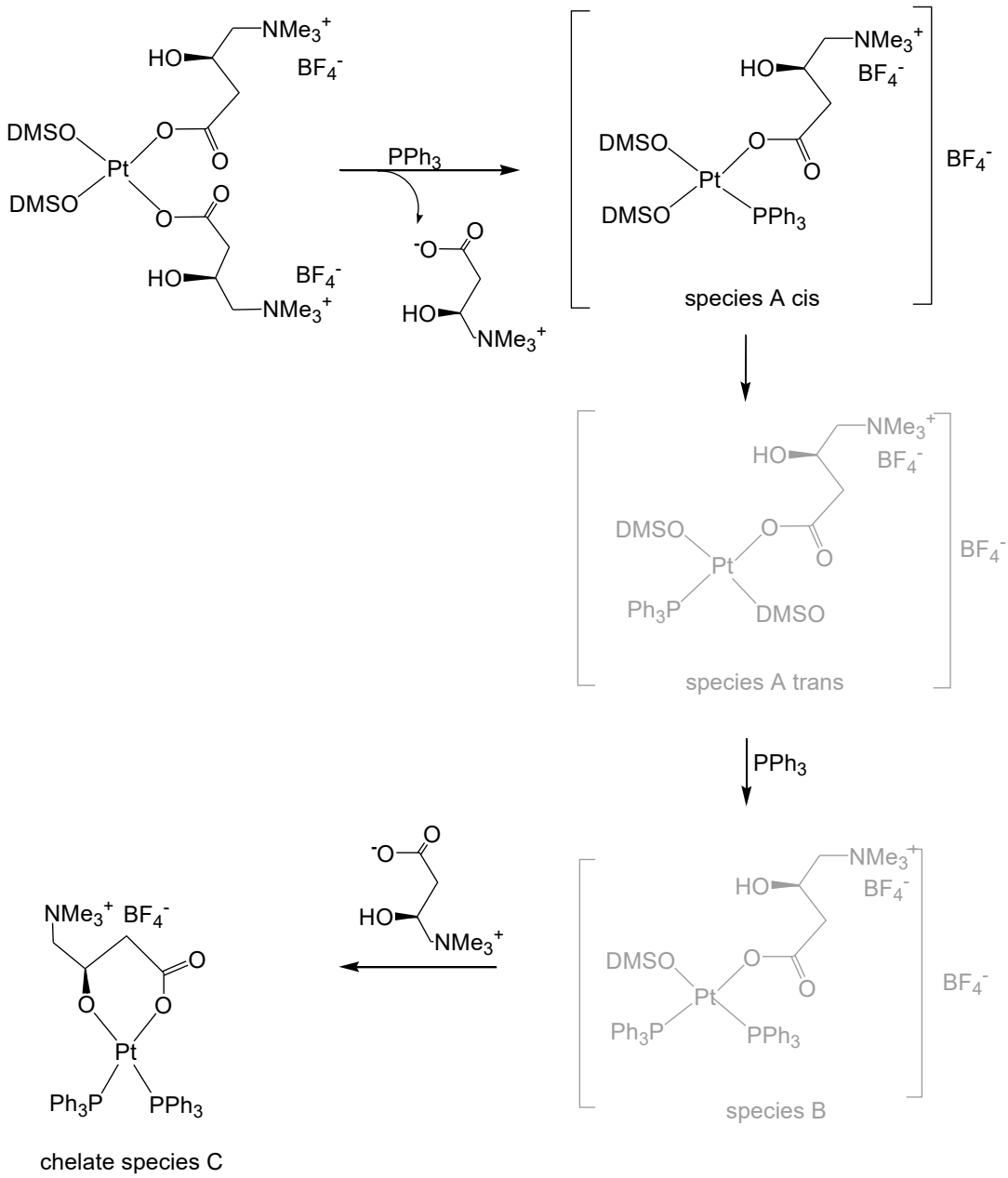


Scheme 5

The exchange of coordinated DMSO for PPh$_3$ in a DMSO solution of complex **8**, gave an unexpected result.

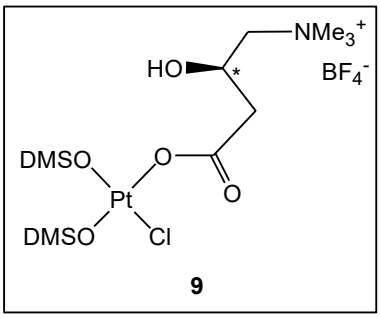
After a few minutes two species in a 10:1 ratio are shown in ^{31}P NMR: the main species is a singlet at 12.84 ppm with $^1J_{PtP}$ 4148 Hz and the other is the known spectrum of the above mentioned chelate [Pt(*L*-carnitine-O,O')(PPh$_3$)$_2$]BF$_4$. After 24 hours, a further observation of the ^{31}P-NMR of the solution, showed the same species but in a 1:6 ratio, which remained unchanged after a further 24 hours.

The singlet with satellites at 12.84 ppm is consistent with the species **A cis**, deriving from the substitution of a coordinated carnitine with PPh$_3$ (probably due to the high *trans* effect of DMSO). A second molecule of PPh$_3$ replaces then a DMSO (which could also be in *trans* to DMSO, after a *cis-trans* isomerization producing the species **A trans**) to give the intermediate **B,** which rapidly undergoes ring closure to produce the chelate species **C** [Pt(*L*-carnitine-O,O')(PPh$_3$)$_2$]BF$_4$.



Schema 6

The monocarboxylate *cis*-[PtCl(*L*-carnitine-O)(DMSO)$_2$](BF$_4$)$_2$, **9**, was similarly obtained from *cis*-[PtCl$_2$(DMSO)$_2$] (see Experimental). The ^{1}H NMR of **9** in D$_2$O shows the same peaks as **8**, and in the MS-ESI the M$^+$ peak (MW - BF$_4$) at 547 is observed together with a minor species at 511, corresponding to the monocharged fragment (M$^+$ - Cl) .



9

*3.3. Test of inhibition of cellular proliferation on human cell lines. Activity of complex **1**, **5, 7** and their corresponding dichlorides.*

A representative complex for each neutral ligand (NH_3, 1,2-DACH and DMSO) was chosen for biological tests, on the basis of their higher solubility in water and purity, The *L*-carnitine-Pt complexes **1**, **5** and **7** together with their precursors cisplatin, [$PtCl_2$(1,2-DACH)], *cis*-[$PtCl_2(DMSO)_2$] and *L*-carnitineBF_4, have been tested in vitro for antiproliferative activity on three human tumoral cell lines, A2780, K562 (cisplatin sensitive) and SKOV 3 (cisplatin-resistant) at 10, 1 e 0.1 μM. The results, after 72 h, obtained by MTT test, [12] are reported in Table 1 and Diagrams 1, 2 and 3

Compounds	μM	A2780	est. IC50	K562	est. IC50	SKOV 3	est. IC50
1	10	68 ± 0.034	1 μM	55 ± 0.074	10 μM	52 ± 0.12	10 μM
	1	49 ± 0.13		25 ± 0.047		32 ± 0.10	
	0.1	24 ± 0.28		5 ± 0.058		1 ± 0.094	
cisplatin	10	52 ± 0.061	10 μM	52 ± 0.15	10 μM	40 ± 0.083	10 μM
	1	30 ± 0.030		20 ± 0.021		15 ± 0.17	
	0.1	5 ± 0.083		1 ± 0.075		1 ± 0.18	
5	10	79 ± 0.41	0.1 μM	78 ± 0.10	1 μM	54 ± 0.18	10 μM
	1	66 ± 0.33		55 ± 0.029		8 ± 0.16	
	0.1	55 ± 0.31		15 ± 0.10		1 ± 0.16	
[$PtCl_2$(1,2-DACH)]	10	75 ± 0.11	1 μM	82 ± 0.11	1 μM	54 ± 0.30	10 μM
	1	65 ± 0.26		52 ± 0.084		20 ± 0.22	
	0.1	44 ± 0.39		20 ± 0.074		1 ± 0.19	
7	10	12 ± 0.019	>10 μM	3 ± 0.11	>10 μM	1 ± 0.096	10 μM
	1	14 ± 0.010		2 ± 0.091		1 ± 0.16	
	0.1	18 ± 0.13		1 ± 0.023		4 ± 0.24	
***cis*-[$PtCl_2$($DMSO)_2$]**	10	2 ± 0.55	>10 μM	10 ± 0.011	>10 μM	1 ± 0.27	>10 μM
	1	3 ± 0.46		10 ± 0.12		1 ± 0.24	
	0.1	4 ± 0.46		6 ± 0.23		1 ± 0.29	
L-carnitine	10	6 ± 0.12	>10 μM	7 ± 0.55	>10 μM	5 ± 0.1	>10 μM
	1	5 ± 0.22		5 ± 0.46		5 ± 0.16	
	0.1	2 ± 0.20		4 ± 0.12		5 ± 0.2	

Table 1 – Antiproliferative activity of complexes **1, 5, 7** and their precursors on A2780, K562 and SKOV3 cell lines at 10, 1 and 0.1 μM, and estimated IC50.

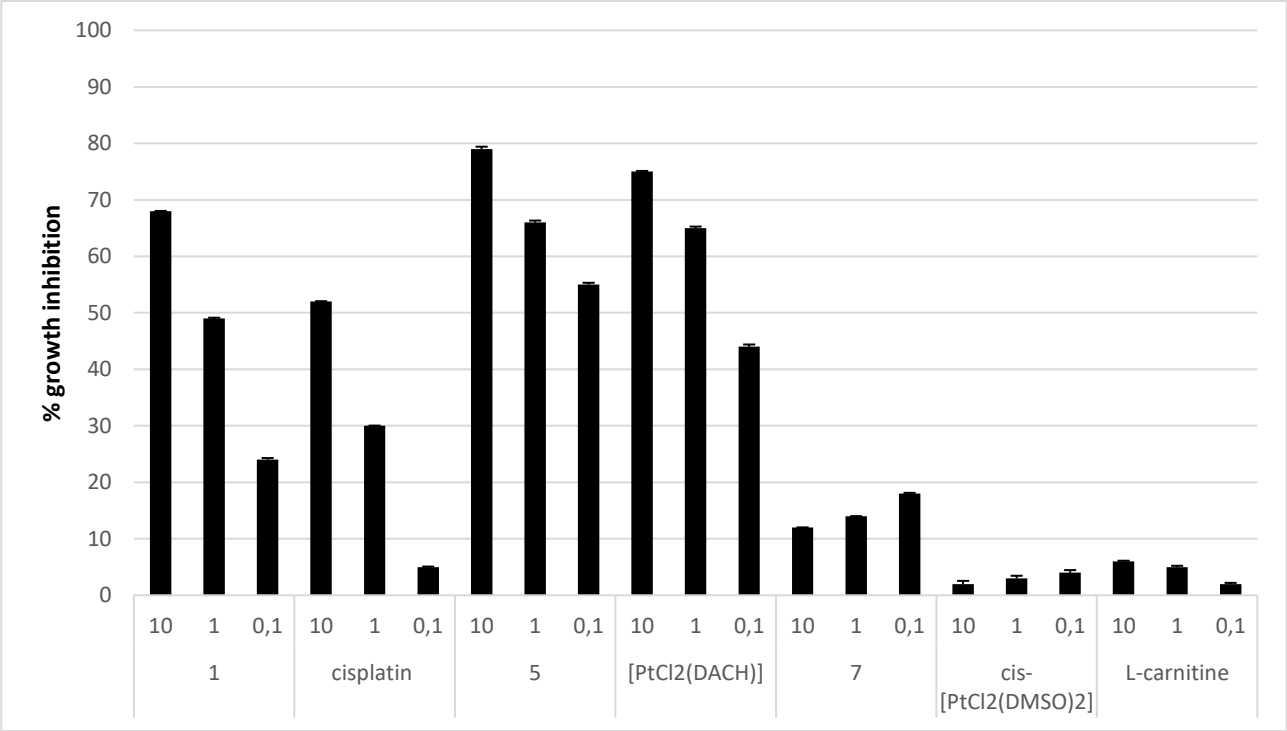


Diagram 1 – Antiproliferative activity at 10, 1 and 0.1 μM of complexes **1, 5, 7** and their precursors on A2780 cell line.

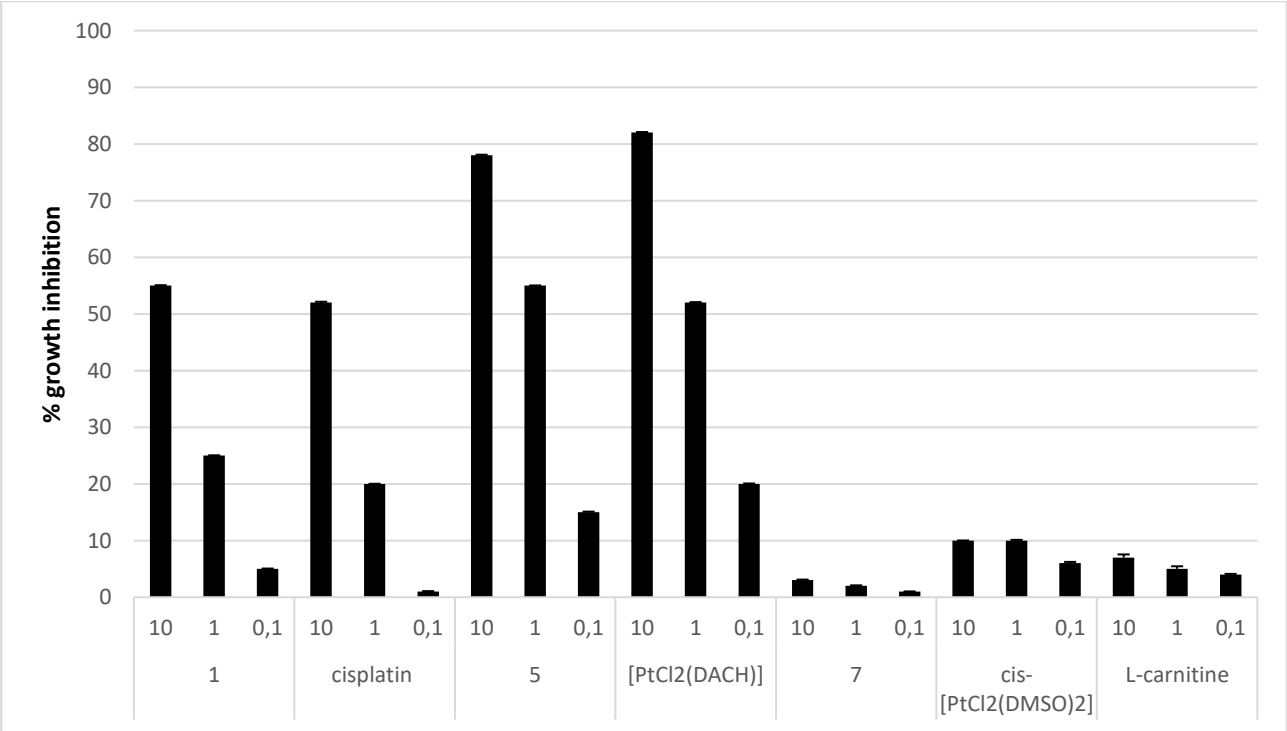


Diagram 2 – Antiproliferative activity at 10, 1 and 0.1 μM of complexes **1, 5, 7** and their precursors on K562 cell line.

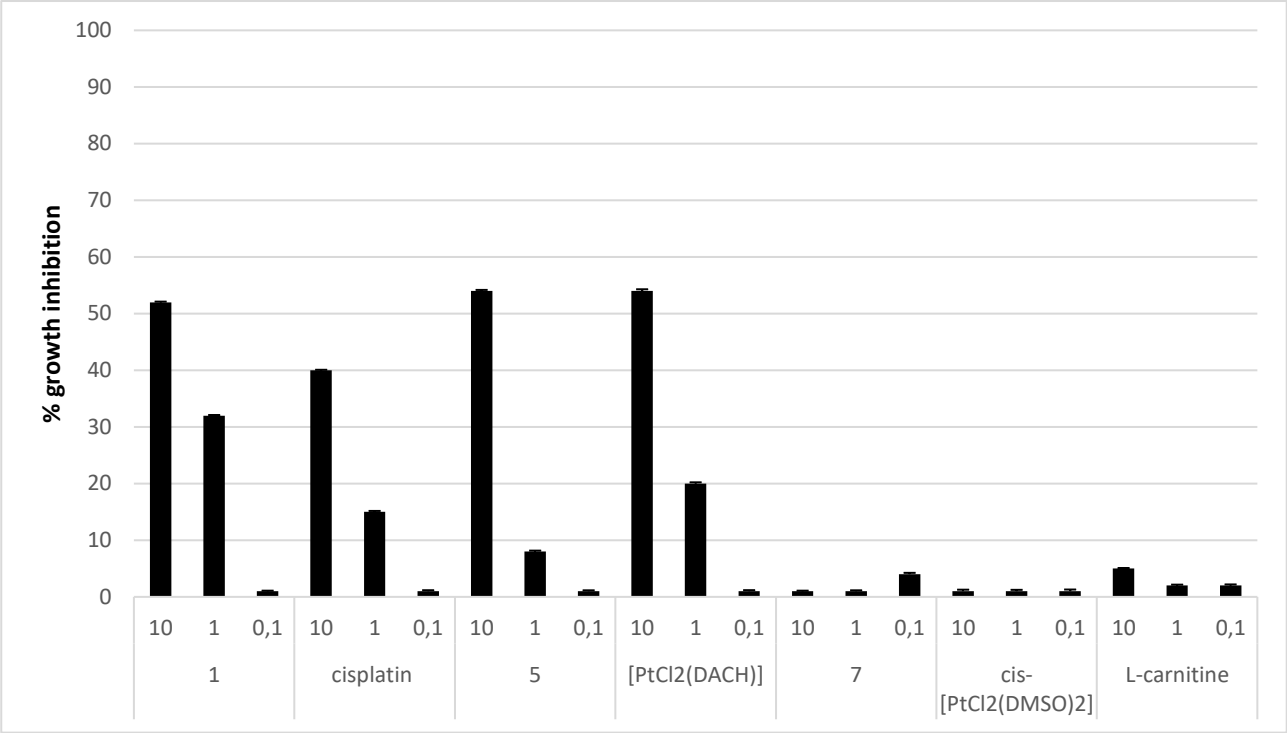


Diagram 3 – Antiproliferative activity at 10, 1 and 0.1 μM of complexes **1, 5, 7** and their precursors on SKOV 3 cell line.

The trend of the antiproliferative activity of each compound is the same for the three examined cell lines: the bicarboxylate complex **1**, *cis*-[Pt(*L*-carnitine-O)$_2$(NH$_3$)$_2$](BF$_4$)$_2$, has an activity comparable with cisplatin, while complex **5** [PtCl(*L*-carnitine-O)(1,2-DACH)] and its precursor [PtCl$_2$(1,2-DACH)] are more active (although on SKOV 3 only at the highest dose). The DMSO complex **7**, [Pt(*L*-carnitine-O,O')(DMSO)$_2$]BF$_4$ and its precursor *cis*-[PtCl$_2$(DMSO)$_2$] are poorly active on the three cell lines.

The antiproliferative activity of well-known anticancer complexes cisplatin and [PtCl$_2$(1,2-DACH)] is not modified when they are conjugated to *L*-carnitine and this observation is positive in view of testing their ability to cross the BBB exploiting the *L*-carnitine transporters.

Conclusion

Some examples of Pt complexes containing *L*-carnitine as monodentate or chelating bidentate anionic ligand, together with neutral ligands appropriate for antitumor activity (NH_3, 1,2-DACH, DMSO) have been prepared and characterized.

The antiproliferative activity of three representative complexes (**1**, **5** and **7)**, in comparison with their precursors cisplatin, [$PtCl_2$(1,2-DACH)] and *cis*-[$PtCl_2(DMSO)_2$], have been tested against three human cancer cell lines. It has been found that the *L*-carnitine conjugated **1** and **5,** containing NH_3 and DACH, maintain the remarkable activity of their precursors and therefore deserve further investigation.

The DMSO complex **7** and its precursor *cis*-[$PtCl_2(DMSO)_2$] showed no activity and therefore their value is related only to synthetic purposes, as precursors of active complexes by DMSO substitution.

Acknowledgments

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