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

Paola Bergamini [a,*], Lorenza Marvelli [a], Giulia Spirandelli [a], Eleonora Gallerani [b]

[a] Dipartimento di Scienze Chimiche e Farmaceutiche, Università degli Studi di Ferrara, Via Fossato di Mortara 17, 44121 Ferrara, Italy
[b] Dipartimento di Scienze della Vita e Biotecnologie, Sezione di Biochimica e Biologia Molecolare, Università degli Studi di Ferrara, Via Fossato di Mortara 74, 44121 Ferrara, Italy

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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

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

* Corresponding author. Tel.: +39 0532455129; fax: +39 0532455167.
E-mail address: bgp@unife.it (P. Bergamini).

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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 1 M 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**

2.2.1. 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}, $8.3 \cdot 10^{-4}$ 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}, $1.7 \cdot 10^{-3}$ 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 h.

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 h, 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 h; the other signals do not change over 30 h.

^{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 $C_{14}H_{36}BF_4N_4O_6Pt$ $(M-BF_4)^+$.

2.2.2. 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 h.

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 h, 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 $C_7H_{21}ClN_3O_3Pt$.

2.2.3. 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}, $2.7 \cdot 10^{-4}$ mol) was dissolved in 20 mL of H_2O. A second solution containing L-carnitineBF_4 (0.038 g, $1.5 \cdot 10^{-4}$ mol, 1 eq) in 3 mL of H_2O was then added dropwise to the previous. The mixture was kept under stirring for 20 h and then taken to dryness giving a cream solid, soluble in H_2O and DMSO. (0.132 g, MW 556.3 g mol^{-1}, $2.4 \cdot 10^{-4}$ 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^+).

2.2.4. Complex [Pt(L-carnitine-O)$_2$(1,2-DACH)](BF$_4$)$_2$, **4**

A solution of $AgBF_4$ (0.103 g, $5.3 \cdot 10^{-4}$ 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, $2.6 \cdot 10^{-4}$ mol) in 20 mL of H_2O. After ten minutes a solution of L-carnitine inner salt (0.085 g, $5.3 \cdot 10^{-4}$ mol, 2 eq) in 3 mL of water was also added. The mixture was kept under stirring for 24 h 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}, $2.5 \cdot 10^{-4}$ 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+}).

2.2.5. 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, $2.6 \cdot 10^{-4}$ mol) and 1 eq of L-carnitine inner salt (0.042 g, $2.6 \cdot 10^{-4}$ 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 h.

^{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^+).