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Bis(dimethylsulfoxide)carbonateplatinum(II), a new synthon for a low-impact, versatile synthetic route to anticancer Pt carboxylates.

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The work describes a new low-impact synthetic route to Pt(II)-carboxylate complexes, a class of compounds provided with established anticancer activity. The process is based on the ligand substitution on [$PtCO_3(Me_2SO\text{-}S)_2$] (**1**), a new synthon that can be easily prepared in water with high yield, is stable as a solid, and is reactive in solution where all its ligands can be easily replaced. It reacts with acidic *O*-donors releasing CO_2 as the only side-product, whose development also supplies a driving force toward the products.

The substitution of the carbonate led to the new Pt-DMSO carboxylate complexes **2-4**, while the total substitution of the ligands of complex **1** gave the new Pt-phosphino carboxylates **5-9** in high yields.

The x-ray crystal structures of complex [Pt(*D*(-)-quinate-*O,O'*)($Me_2SO\text{-}S)_2$] (**3**), [Pt(salicylate)($Me_2SO\text{-}S)_2$] (**4**) and [Pt(salicylate)($PPh_3)_2$] (**6**) were determined.

The tests of the antiproliferative activity of complexes **1-9** on two human tumoral cell lines, A2780 (cisplatin-sensitive) and SKOV-3 (cisplatin-resistant), showed that the PTA complexes **7**-**9** were the most active on both cell lines.

Introduction

The coordination chemistry of Pt(II) was deeply explored, both with the aim of understanding fundamental reaction mechanisms and for its valuable practical applications in the fields of catalysis and medicine.[1-3] Most of the known Pt(II) complexes were prepared by ligand substitutions from a few versatile key intermediates. A huge number of cisplatin analogues reported in the literature have been synthesized starting from the ready accessible key compound *cis*-[$PtCl_2(Me_2SO\text{-}S)_2$], where coordinated Me_2SO can be replaced by a variety of N-donors, both mono-dentate and chelating, giving the corresponding dichlorides cis-[$PtCl_2$(NN')].[4-6]

Other Pt-containing starting materials undertake complete ligands substitution, e.g. [$PtMe_2$(1,5-cod)] (1,5-cod = 1,5 cyclooctadiene), characterized by chemical stability and by the presence of easily replaceable ligands. Emblematically, [$PtMe_2$(1,5-cod)] reacts in organic solvent with coordinating acids (e.g. carboxylic acids) via Pt-Me bonds protonolysis, and with neutral ligands (e.g. phosphines) by 1,5-cod substitution.[7-10] No similar synthon is available for reactions in water, which would be valuable above all for the preparation of Pt complexes with *O*-donor ligands for pharmaceutical applications.[11]

The presence of *O*-donors as anionic ligands is the common character of Pt-drugs provided with a better therapeutic index than cisplatin, namely carboplatin, oxaliplatin, nedaplatin and lobaplatin.[12-13] Among thousands of cisplatin analogues prepared since the late seventies, only this group has achieved international approval and has found wide clinical use.

Still today, research in the field of Pt-based anticancer drugs is oriented towards Pt-carboxylates with innovative properties, like the Pt(IV) complexes satraplatin which is orally active[14] and mitaplatin which contains the pro-apoptotic anion dichloroacetate.[15-16]

In new drug design, a great variety of carboxylate ligands were considered, while the neutral carrier ligands are mostly limited to NH_3 or primary amines like 1,2-diaminocyclohexane (1,2-DACH) and cyclohexylamine. The change of neutral ligands could open new perspectives in terms of therapeutic index, distribution and route of administration.

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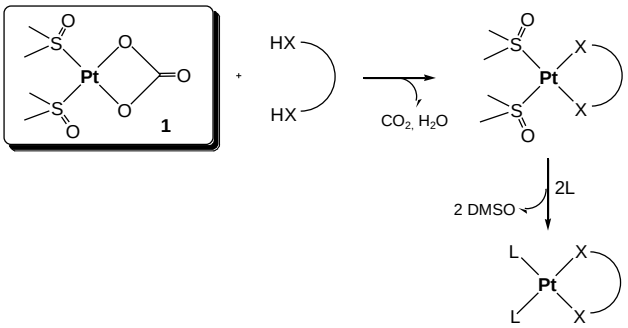
Electronic Supplementary Information (ESI) available:
Selected NMR and ESI-MS spectra, data of known products, crystallographic information is reported as Supporting Information.
Crystallographic data for the structural analysis of **3, 4** and **6** have been deposited with the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK, and are available on request, free of charge, from the Director on quoting deposit numbers CCDC 1438939, 1438940 and 1438941 for **3, 4** and **6**, respectively.

The main purpose of this work is to develop of a new, simple and eco-friendly synthetic path to Pt-carboxylate complexes, that may be suitable for complexes with non aminic carrier ligands, based on a new Pt-containing synthon.

The insertion of hard *O*-donors, e.g. the deprotonated form of dicarboxylic acids or hydroxy-carboxylic acids, is not straightforward on the soft acid Pt(II)[17] and needs to be assisted by one or more driving forces like: a) the presence of easily replaceable leaving groups (e.g. H_2O, NO_3^-) in the reagent complex, b) the formation of a 5 or 6-membered chelate ring in the product, c) the formation of volatile or precipitating side-products.

Pt(II) dicarboxylated bearing NH_3 or 1,2-DACH as neutral ligands are routinely obtained from the corresponding dihalo complexes by precipitation of the halides as water insoluble silver or barium salts.[18-19] These reactions occur in water where both the starting dihalo and the final dicarboxylate complexes are soluble. With poorly water-soluble reagents like several complexes of phosphines, DMSO, tertiary and aromatic amines, this route (being in practice an heterogeneous process) takes long time and gives low yields due to part of the product being co-precipitated with the silver salt.

We are proposing here the use of complex $[PtCO_3(Me_2SO\text{-}S)_2]$ (**1**) as a new synthon for Pt(II) coordination chemistry, whose ligands can all undergo substitution: the anionic carbonate via protonolysis with acids, DMSO by nucleophilic substitution at Pt(II). The side products are respectively CO_2 and DMSO, both of which are easy to remove (Scheme 1).



Scheme 1

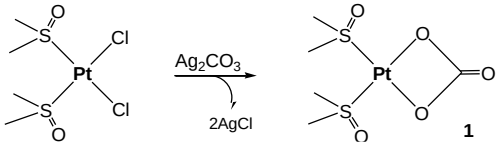
The reactivity of complex **1** parallels that of the above described $[PtMe_2(1,5\text{-cod})]$, enhanced by environment-friendly characters: in fact **1** is prepared in a reactor open to the laboratory atmosphere, with high yields, and is soluble and reactive in water. The Pt coordinated carbonate can deprotonate the incoming acidic ligands giving CO_2 as a harmless side product, which also leaves two vacant coordinative sites on Pt and supplies a driving force toward the products.

A few carbonate Pt complexes have been described before, e.g. $[PtCO_3(PPh_3)_2]$[20-21] and $[PtCO_3(1,2\text{-DACH})]$[22] and used as convenient starting materials in organic solvents.

Results and discussion

Synthesis and characterization of $[PtCO_3(Me_2SO\text{-}S)_2]$ (1)

By treating the dichloride *cis*-$[PtCl_2(Me_2SO\text{-}S)_2]$ with Ag_2CO_3 in water (Scheme 2), we have prepared and isolated the new carbonate complex $[PtCO_3(Me_2SO\text{-}S)_2]$ (**1**). Complex **1** has been characterized by NMR in D_2O.



Scheme 2

In ^1H NMR, the coordinated DMSO is found at 3.27 ppm as a singlet with broad satellites ($^3J_{PtH}$ of 20.5 Hz), due to the superposition of the spectra of two isotopomers, one containing non-active Pt which produces the central signal and the other containing 195-Pt (33.7%) which gives a doublet (Pt-satellites).

The difference in line broadening between the central signal (sharp) and its satellites (broad) can be ascribed to the relaxation of ^{195}Pt via the chemical shift anisotropy mechanism.[23] This signal is clearly distinguishable from the corresponding signal in its precursor *cis*-$[PtCl_2(Me_2SO\text{-}S)_2]$, which falls at 3.42 ppm in D_2O and $^3J_{HPt}$ = 25.3 Hz.[24]

The ^{13}C signal of the coordinated DMSO is at 42.69 ppm with $^2J_{CPt}$ = 38.4 Hz (in D_2O). It is possible to observe also the signal of coordinated carbonate at 165.65 ppm, which is too weak to detect the Pt satellites. The ^{195}Pt NMR in D_2O shows a single signal at -3155.3 ppm.

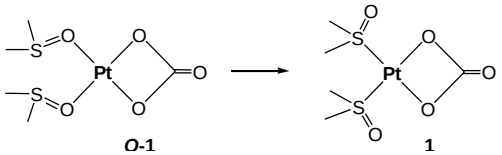
Complex **1** is soluble in water, slightly soluble in DMSO, CH_3OH and acetone.

Equilibria of complex 1 in aqueous solution

While in the solid state complex **1** can be stored for a long time without modification (unchanged ^1H NMR signal after 5 months), in solution it gives rise to two consecutive transformations: O/S isomerization and DMSO/H_2O exchange.

O/S isomerization[25-26]**:** in the ^1H-NMR spectrum of a freshly prepared solution in D_2O ($1.5 \cdot 10^{-2}$ M), the main signal for DMSO is a singlet at 3.10 ppm with no satellites and the minor is at 3.27 ppm with $^3J_{PtH}$ 20.5 Hz (2.7:1 ratio); we attribute the first signal to the *O*-coordinated isomer ***O*-1**, which turns almost completely to the *S*-isomer **1** (1:20 ratio) (Scheme 3) in 2 hours (Fig. 1). The signals of a mixed S/O-coordinated species have not been observed.

The presence of small residual amounts of the *O*-isomer does not influence the subsequent substitution reactions.



Scheme 3

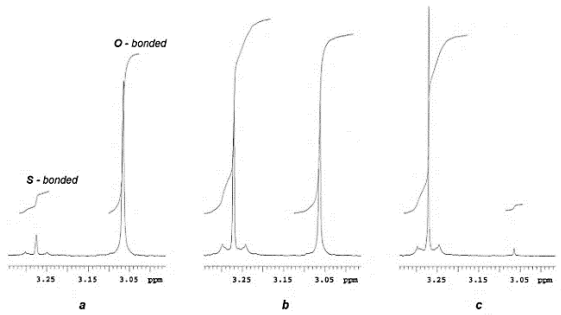
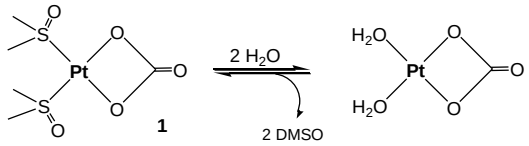


Fig. 1 **–** *O*-bonded/*S*-bonded isomerization observed by ^{1}H-NMR signals of coordinated DMSO (at 400 MHz): ***a***, after 2 min; ***b***, 18 min; ***c***, 2 hours.

The *O*-bonded/*S*-bonded isomerization of Pt-coordinated DMSO could be due to the conversion of the kinetically favoured O-bonded isomer into the thermodynamically favoured S-isomer occurring at a rate compatible with the NMR-scale. [27-29]

DMSO/H_2O exchange: [$PtCO_3(Me_2SO\text{-}S)_2$] (**1**) is soluble in water, but it slowly undertakes a partial replacement of DMSO by water (Scheme 4).



Scheme 4

In a $1.5\cdot10^{-2}$ M solution, the ratio of free to coordinated DMSO was estimated from the integrals ratio of the ^{1}H NMR signals at 2.58 ppm and 3.27 ($^{3}J_{PtH}$ 20.5 Hz ppm), respectively. It increases from 1:22 (ratio 0.045) in a fresh solution to an equilibrium state of 1:10.5 (ratio 0.095) in 6 days, as reported in Fig 2.

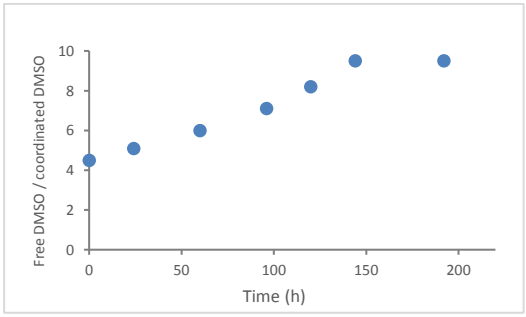
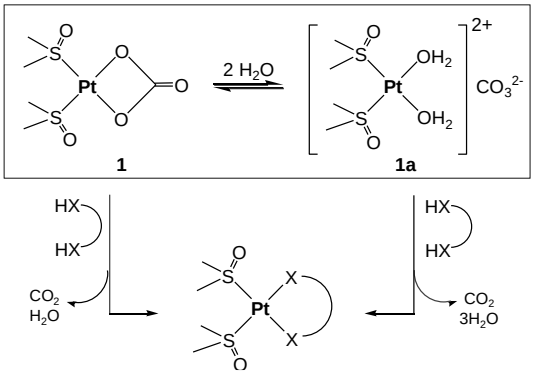


Fig 2. Kinetic plot for DMSO release from complex **1**.

Due to this reactivity, the use of freshly prepared solution is recommended when **1** is used for reactions in water.

CO_3^{2-}/H_2O exchange. Complex [$PtCO_3(Me_2SO\text{-}S)_2$] is very soluble in water (unlike the parent dichloride complex). This unexpected high water solubility suggested the hypothesis that the real nature of complex **1** in water is an equilibrium state between carbonate and the aquo species **1a**, which does not preclude the possibility for complex **1** to react with acids (Scheme 5).



Scheme 5

In the MS-ESI spectrum, the major peak M^+ at 734.87 is consistent with an *O*-bridged dinuclear species $[(Me_2SO\text{-}S)_4Pt_2\mu(O)\mu(OH)]^+$, which is probably originated from **1** via the aquo species **1a**, followed by dimerization. Although it cannot be excluded that this species is produced under specific electrospray conditions, the MS-ESI supports the hypothesis described above.

Reactivity of [$PtCO_3(Me_2SO\text{-}S)_2$] (1)

Protonolysis and substitution of CO_3^{2-}: a series of [Pt(Me_2SO-*S*)$_2$(dicarboxylate)] have been obtained by Pierpont *et al*[30] by exploiting the reaction of *cis*-[$PtCl_2(Me_2SO\text{-}S)_2$] with the silver salt of the dicarboxylic acid in water. We obtained some of the products reported in ref. 30 from the reaction of **1** with the appropriate acids, which produced H_2CO_3 (CO_2) as the only side-product. [Pt(CBDC)($Me_2SO\text{-}S)_2$] (**A**) and [Pt(malonate)($Me_2SO\text{-}S)_2$] (**B**) were identified by comparison with the NMR data of ref. 30, while [Pt(oxalate)($Me_2SO\text{-}S)_2$] (**C**) was indirectly identified after the exchange of DMSO with PPh_3 in $CDCl_3$ which gave [Pt(oxalate)($PPh_3)_2$] (^{31}P NMR: 7.73 ppm, 3780 Hz), as showed by comparison with the reported ^{31}P NMR data (7.7 ppm, 3770 Hz).[31]

The reaction of [$PtCO_3(Me_2SO\text{-}S)_2$] with one equivalent of *L*-carnitineBF_4 gave the chelate complex (**D**) [Pt(*L*-carnitina-*O,O'*)($Me_2SO\text{-}S)_2$]BF_4, as we already reported.[32]

These reactions showed that **1** can react with bicarboxylic acids (CBDA, malonic, oxalic) giving Pt/DMSO/carboxylate complexes. We then focussed on α and β hydroxyacids whose coordination to Pt is relevant because a large number of such molecules are natural, biocompatible or even provided with various therapeutic activities which could improve the performance of Pt-based drugs.

The coordination of alcoholic-*O* to Pt is quite rare, due to the low affinity of soft Pt to hard *O*-donors, and occurs exclusively when favoured by a side driving force like the formation of a chelate ring and/or the development of a volatile side product.[33]

The reaction of **1** with α-hydroxycarboxylic acids produces DMSO-Pt complexes containing a five-membered chelate ring where the donors are a COO^- and an RO^- group. The same pattern is present in the platinum-based drug nedaplatin.[34]

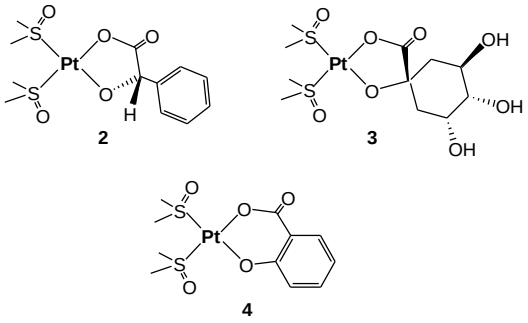
The α-hydroxycarboxylic *L*(+)-mandelic acid has been used in medicine as an antibacterial, particularly in the treatment of urinary tract infections. It is a useful precursor to various drugs and Pt complexes.[35]

The reaction of complex **1** with 1 eq of *L*(+)-mandelic acid in water produced the new complex [Pt(*L*(+)-mandelate)(Me_2SO-*S*)$_2$], **2**, in 2 hours in high yield, with CO_2 and water as the only side product.

In the ^{1}H NMR in D_2O, the CH signal of coordinate mandelate was found at 5.13 ($^4J_{PtH}$ 41 Hz). It is also possible to observe four signals for the methyl groups of DMSO (3.31, 3.34, 3.39, 3.40 ppm with satellites, coupling constants not computable), while the ^{13}C shows three singlets (probably due to a fortuitous coincidence of two signals) at 42.70, 42.97, 43.47 ppm. The inequivalence of the four CH_3 groups of coordinated DMSO is due to two different *O* donors in *trans* to coordinated DMSO and to the presence of a chiral center which makes the methyl groups of each coordinated DMSO diastereotopic.

Another example of α-hydroxycarboxylic acid is the *D*(-)-quinic acid, which is exploited as a versatile chiral starting material for the synthesis of new pharmaceuticals[36] and is also known as an effective carrier for some metal ions, due to the formation of coordination compounds.[37]

The reaction of complex **1** with *D*(-)-quinic acid in water gave complex **3**, [Pt(*D*(-)-quinate-*O,O'*)(Me_2SO-*S*)$_2$], whose characterization is reported in the Experimental section.



The slow evaporation of a solution of **3** in MeOH gave crystals suitable for the acquisition of the x-ray crystal structure (Fig. 3).

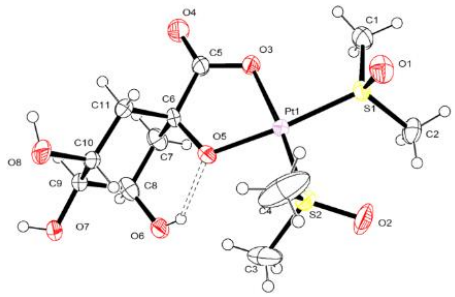


Fig. 3 - ORTEPIII view[38] and atom numbering scheme for complex **3**, [Pt(*D*(-)-quinate-*O,O'*)(Me_2SO-*S*)$_2$]. Thermal ellipsoids are drawn at the 40% probability level. The intramolecular hydrogen bond is drawn as a dashed line.

The reaction of **1** with ß-hydroxycarboxylic acids produces DMSO-Pt complexes containing a six- membered chelate ring with a COO^- group and an RO^- as donor groups; for example, complex **4**, [Pt(salicylate)(Me_2SO-*S*)$_2$] was obtained by treating **1** in water with salicylic acid, a well-known anti-inflammatory drug.[39]

The ^{1}H NMR of complex **4** in acetone shows two signals of coordinated DMSO at 3.46 (19.0 Hz) and 3.54 (20.5 Hz) due to two DMSO in *trans* to different *O*-donors. Crystals of **4** suitable for x-ray diffractometric determination were obtained from an acetone solution (Fig. 4).

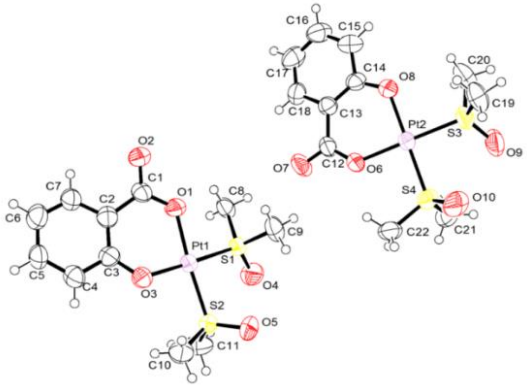


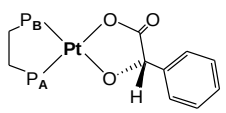
Fig. 4 ORTEPIII view[38] and atom numbering scheme for complex **4**, [Pt(salicylate)(Me_2SO-*S*)$_2$]. Thermal ellipsoids are drawn at the 40% probability level.

Total substitution of the ligands in [$PtCO_3$(Me_2SO-*S*)$_2$] (1): the total substitution of the ligands in complex **1** can be obtained via two possible sequences: *i)* substitution of DMSO with neutral ligands followed by the protonolysis of CO_3^{2-} by the incoming acids or *ii)* protonolysis of **1** with the desired acid followed by the substitution of DMSO. Several experiments showed that the more convenient sequence is *ii)*, which avoids the formation of side products probably due to the DMSO *trans* effect, which we observed using sequence *i)*.

In most cases, these syntheses can be carried out in one pot, saving time and chemicals. For example, the new complex **5**, bearing the chelating diphosphine dppe, was prepared starting from [$PtCO_3$(Me_2SO-*S*)$_2$] treated with one equivalent of *L*(+)-mandelic acid in water. One equivalent of dppe dissolved in acetone was then added to the previous solution, triggering the immediate precipitation of **5** as a white solid.

The ^{31}P NMR of **5** in acetone shows two doublets coupled with ^{195}Pt at 28.4 (d, $^1J_{PPt}$ 3671 Hz, *trans* to COO, P_A) ppm and 33.3 ppm (d, $^1J_{PPt}$ 3113 Hz, *trans* to OR, P_B), due to two inequivalent P, coupled each other with a $^2J_{PP}$ 10.7 Hz.[40]

On the contrary, the preparation of the known complex **6** requires a two-steps procedure: after the formation of [Pt(Me_2SO-*S*)$_2$(salicylate)] in water, it is necessary to change the solvent (to acetone) because PPh_3 is insoluble in water.



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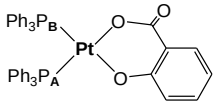

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Complex **6** has been reported before[41], but has not been characterized by a crystal structure, which we determined on crystals of **6** grown in acetone (Fig. 5).

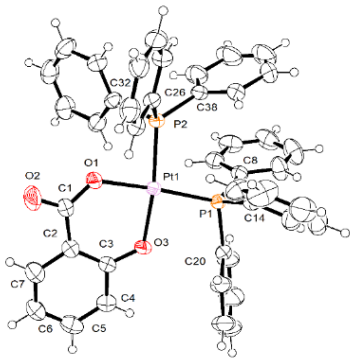


Fig. 5 ORTEPIII view[38] and atom numbering scheme for complex **6,** [Pt(Me_2SO-*S*)$_2$(salicylate)]. Thermal ellipsoids are drawn at the 40% probability level.

One of the problems in using phosphines as ligands for Pt and other metal ion complexes aimed to pharmaceutical applications is the lack of solubility in water, which is particularly relevant with aromatic phosphines like PPh_3. The water soluble phosphine PTA has been proposed by us[42] and others[43-47] as a convenient alternative.

Therefore, we prepared the PTA analogue of **6**, the new complex **7**, by a one-pot reaction from complex **1** by addition of salicylic acid (1 eq) followed by PTA (2 eq) in acetone.

The ^{31}P NMR of the product in DMSO shows two doublets coupled with ^{195}Pt at -64.55 ($^1J_{PPt}$ 3156 Hz, *trans* to OR, P_B) and -63.12 ($^1J_{PPt}$ 3416 Hz, *trans* to COO, P_A) ppm, due to two inequivalent P atoms, coupled each other with a $^2J_{PP}$ 25.5Hz.

The new complex **8,** [Pt(*D*(-)quinate-*O,O'*)$_2$(PTA)$_2$], was also obtained by a one-pot synthesis in CH_3OH, starting from **1** which was converted into the DMSO intermediate complex **3**. In the same solvent, two equivalents of PTA were added and complex **8** was isolated after one hour. The characterization of **8** is based on ^{31}P NMR in DMSO, showing two doublets coupled with ^{195}Pt at -63.33 ($^1J_{PPt}$ 2995 Hz, *trans* to OR, P_B) and -62.04 ($^1J_{PPt}$ 3340 Hz, *trans* to COO, P_A) ppm, due to two inequivalent P atoms, coupled each other with a $^2J_{PP}$ 24.0 Hz.

The new complex [Pt(*L*-carnitine)(PTA)$_2$]BF_4, **9**, was prepared from the DMSO intermediate [Pt(*L*-carnitine)(Me_2SO-*S*)$_2$]BF_4, **D**[32], which was treated with two equivalents of PTA in acetone.

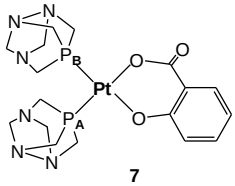
The ^{31}P NMR of **9** in CH_3OH shows two doublets coupled with ^{195}Pt at -61.88 ($^1J_{PPt}$ 3018 Hz, *trans* to OR, P_B) and -58.63 ($^1J_{PPt}$ 3692 Hz, *trans* to COO, P_A) ppm, due to two inequivalent P, coupled each other with a $^2J_{PP}$ 24.3 Hz.

Test of inhibition of cellular proliferation on human tumoral cell lines. Activity of complexes 1-9.

The Pt complexes **1**-**9** together with their precursor *cis*-[$PtCl_2$(Me_2SO-*S*)$_2$] were tested in vitro for antiproliferative activity on two human tumoral cell lines, A2780 (cisplatin-sensitive) and SKOV-3 (cisplatin-resistant) at 0.01 µM, 0.1 µM, 1 µM, 10 µM, 100 µM and 400 µM.

The cell growth %, for each complex at each dose, was determined in three independent experiments. The results, obtained by using a Coulter counter after 72 h treatment, and expressed as IC_{50}, are reported in Table 1.

The results in Table 1 indicate a low activity of DMSO complexes **1-4** on both cell lines. The dppe complex **5** shows a remarkable activity on the A2780 cell line and an even better activity on the cisplatin-resistant SKOV-3 cell line, where it seems to be more active than cisplatin. The PPh_3 complex **6** shows a low antiproliferative effect on A2780 cell line, but its activity interestingly increases on SKOV-3 cells.



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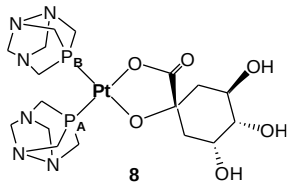

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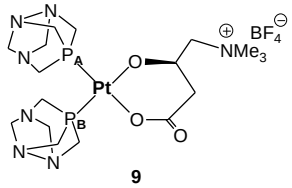

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The PTA complexes **7** and **8** are quite active on A2780 and very active on SKOV-3, the best being complex **7**. A comparison between complex **4**, [Pt(salicylate-*O,O'*)(Me_2SO-*S*)$_2$], (not active on SKOV-3) and complex **7**, [Pt(salicylate-*O,O'*)(PTA)$_2$], the most active on the same cell line, underlines how much the activity is affected by the neutral ligands on Pt.

The remarkable activity of the here described phosphinic complexes confirms that this group of Pt complexes deserves renewed attention, especially when the presence of hydrophilic phosphines like PTA improves their solubility in water.

Table 1. IC_{50} of complexes **1-10** on A2780 and SKOV-3 cell lines**.**

	Complexes	A2780		SKOV-3	
		IC_{50} µM	sd	IC_{50} µM	sd
DMSO complexes	$[PtCl_2(Me_2SO\text{-}S)_2]$	96.03	1.78	186.05	7.14
	1	111.97	10.90	42.45	1.34
	2	42.07	1.63	327.50	0.71
	3	101.30	15.27	176.35	14.92
	4	49.03	7.98	205.25	9.55
dppe	**5**	6.60	1.73	4.70	1.84
PPh_3	**6**	55.07	9.27	7.55	0.35
PTA complexes	**7**	6.37	2.54	0.77	0.19
	8	13.97	2.42	0.89	0.05
	9	3.07	1.10	3.90	0.14
cisplatin		2.93	0.15	5.94	0.08

Experimental

All the manipulations were carried out in atmosphere unless otherwise noted. Commercial solvents and reagents were purchased and used without further purification. *cis*-$[PtCl_2(Me_2SO\text{-}S)_2]$ was prepared as described in the literature,[48] as well as PTA.[49] Elemental analyses were determined using a Carlo Erba instrument model EA1110. The ESI mass spectra were acquired with a Micromass LCQ Duo 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 , ^{31}P and ^{195}Pt spectra were run with proton decoupling, ^{13}C signals are reported in ppm relative to external tetramethylsilane (TMS), ^{31}P signals are reported in ppm relative to an external 85% H_3PO_4 standard and the reference for ^{195}Pt NMR was Na_2PtCl_6 1M in D_2O.

Synthesis of $[PtCO_3(Me_2SO\text{-}S)_2]$ (1).

A suspension of complex *cis*-$[PtCl_2(Me_2SO\text{-}S)_2]$ (422 mg, $1{\cdot}10^{-3}$ mol, MW 422.1 g/mol) in 40 mL of H_2O was kept under vigorous stirring for 15 minutes and then solid Ag_2CO_3 (268 mg, $0.97{\cdot}10^{-3}$ mol, MW 275.7 g/mol, 0.97 eq) was added. In order to avoid silver carbonate aggregates, a very accurate mixing is essential. The reaction mixture was kept in an oil bath at 40 °C in the dark for two hours, and then it was filtered on Celite for eliminating the precipitate of AgCl. The clear solution, taken to dryness, left **1** as a pale yellow solid (373 mg, $0.91{\cdot}10^{-3}$ mol, MW 411.2 g/mol, yield 93%).

1 · $2H_2O$, $C_5H_{16}O_7S_2Pt$ (447): % found (% calc. for) C 13.01 (13.42), H: 3.50 (3.57), S 14.55 (14.32). ^{1}H NMR (D_2O): δ = 3.27 (s,$^3J_{PtH}$ 20 Hz, 12H, DMSO) ppm. ^{13}C NMR (D_2O): δ = 42.69 (s, C, $^2J_{PtC}$ 38.42 Hz, DMSO), 165.65 ppm (s, CO_3). ^{195}Pt NMR (D_2O): - 3155.3 ppm. MS-ESI: m/z = 734.87 $(C_8H_{25}O_6S_4Pt_2)^+$, m/z = 656.80 $(C_6H_{19}O_5S_3Pt_2)^+$. $S_{25°C}$ (H_2O): 470 g L^{-1}.

New Pt complexes obtained from 1

Synthesis of [Pt(*L*(+)mandelate-*O,O'*)(Me_2SO-*S*)$_2$] (2).

Complex $[PtCO_3(Me_2SO\text{-}S)_2]$, **1,** (100 mg, $2.43{\cdot}10^{-4}$ mol, MW 411.2 g/mol, 1 eq), solubilized in 10 mL of H_2O, was kept under vigorous stirring. *L*-mandelic acid (37 mg, $2.43{\cdot}10^{-4}$ mol, MW 152.1 g/mol, 1eq), solubilized in 2 mL of H_2O, was added to the first solution. No change of color or precipitate was noted. The solution was left for two hours under stirring, then taken to dryness leaving **2** as a yellow-white solid (112 mg, $2.23{\cdot}10^{-4}$ mol, MW 501.5 g/mol, yield 91%).

$C_{12}H_{18}O_5S_2Pt$ (501.5): % found (% calc. for) C 28.73 (28.74), H: 3.52 (3.62), S 12.83 (12.79). ^{1}H NMR (D_2O): δ = 3.31, 3.34, 3.39, 3.40 (4s, $^3J_{HPt}$ unresolved, 12H, DMSO), 5.13 (s, $^3J_{HPt}$ = 41 Hz, 1H, CH), 7.2-7.4 (m, 5H, Ph) ppm. ^{13}C NMR (D_2O): δ = 42.70, 42.97, 43.47 (s, CH_3, DMSO), 81.28 (s, CH), 128.95, 128.63 and 126.50 (3s, Ph), 191.54 (s, COO) ppm. ^{195}Pt NMR (D_2O): δ = - 3202 ppm. $S_{25°C}$ (H_2O): 6.7 g L^{-1}.

Synthesis of [Pt(*D*(-)quinate-*O,O'*)(Me_2SO-*S*)$_2$] (3).

Complex **3** was prepared in the same way as complex **2**, using the following quantities: $[PtCO_3(Me_2SO\text{-}S)_2]$ 100 mg ($2.43{\cdot}10^{-4}$ mol, MW 411.2 g/mol) in 10 mL of H_2O and *D*(-)quinic acid, 47 mg ($2.43{\cdot}10^{-4}$ mol, MW 192.2 g/mol, 1 eq) in 2 mL of H_2O. A pale yellow solid (107 mg, $1.98{\cdot}10^{-4}$ mol, MW 541.5 g/mol, yield 81%) was obtained.

$C_{11}H_{22}O_8S_2Pt$ (541.5): % found (% calc. for) C 24.23 (24.40), H: 4.15 (4.09), S 12.03 (11.84).

^{1}H NMR (D_2O): δ = 1.42-1.78 (m, 2H, CH_2), 2.0-2.32 (m, 2H, CH_2), 3.2-3.5 (m, 13H, CHOH + DMSO), 3.85 (m, 1H, CHOH), 3.95 (m, 1H, CHOH) ppm. ^{1}H NMR (CD_3OD): δ = 1.57-1.87 (m, 2H, CH_2), 2.0-2.33

(m, 2H, CH_2), 3.47 (m, 13H, CHOH + DMSO), 4.00 (m, 2H, 2CHOH) ppm. ^{13}C NMR (CD_3OD): δ = 42.24, 44.10, 44.90, 45.94 (s, 2 signals CH_3, DMSO and 2 CH_2), 68.95, 73.23, 78.20, 84.73, 193.13 (s, COO) ppm. ^{195}Pt NMR (CD_3OD): δ = - 3265 ppm. $S_{25°C}$ (H_2O): 48 g L^{-1}. The crystallographic structure of **3** was determined on crystals grown in MeOH (see Fig. 3).

Synthesis of [Pt(salicylate-*O,O'*)(Me_2SO-*S*)$_2$] (4).

Complex **4** was prepared in the same way as complex **3**, using the following quantities:

[$PtCO_3(Me_2SO$-*S*$)_2$], 100 mg ($2.43\cdot10^{-4}$ mol, MW 411.2 g/mol) in 10 mL of H_2O and 34 mg of salicylic acid ($2.43\cdot10^{-4}$ mol, MW 138.1 g/mol, 1eq) in 20 mL of H_2O. A pale yellow solid (94 mg, $1.92\cdot10^{-4}$ mol, MW 487.4 g/mol, 79%) was obtained in 2 hours.

$C_{11}H_{16}O_5S_2Pt$ (487.4): % found (% calc. for) C 27.03 (27.10), H: 3.45 (3.31), S 13.07 (13.16).^{1}H NMR (D_2O): δ = 3.22-3.37 (m, 12H, DMSO), 6.70 (2H), 7.20 (1H), 7.63 (1H) (m, Ph) ppm. ^{1}H NMR (acetone-d_6): δ = 3.46 (s, $^3J_{PtH}$ 19 Hz, 6H, DMSO), 3.54 (s, $^3J_{PtH}$ 20.5 Hz, 3H, DMSO), 3.54 (s, $^3J_{PtH}$ 20.5 Hz, 3H, DMSO), 6.68 (m), 6.81 (d), 7.14 (m) (4H, Ph) ppm. ^{13}C NMR (acetone-d_6): δ = 41.23, 41.30 (s, 2 CH_3, DMSO); 116.70, 120.20, 131.73 and 132.53 (Ph); 164.51(C-O), 165.37 (s, COO) ppm. ^{195}Pt NMR (acetone): δ = - 3118 ppm. $S_{25°C}$ (H_2O): 5.0 g L^{-1}. The crystallographic structure of **4** was determined on crystals grown in acetone for two weeks (see Fig. 4).

One-pot synthesis of [Pt(*L*(+)mandelate-*O,O'*)(dppe)] (5).

L(+)mandelic acid (37 mg, $2.43\cdot10^{-4}$ mol, MW 152.1 g/mol, 1 eq) dissolved in 2 mL of water was added to a solution of [$PtCO_3(Me_2SO$-*S*$)_2$] (100 mg, $2.43\cdot10^{-4}$ mol, MW 411.2 g/mol) in 10 mL of water. The formation of the intermediate [Pt(*L*-mandelate-*O,O'*)(Me_2SO-*S*$)_2$], **2**, gave $2.43\cdot10^{-4}$ mol, assuming complete conversion.

After one hour, bis(diphenylphosphino)ethane (dppe, 96.7 mg, $2.43\cdot10^{-4}$ mol, MW 398.4 g/mol, 1 eq), dissolved in 5 mL of acetone, was added to the previous solution. Complex **5** precipitated immediately as a white solid (163 mg, $2.19\cdot10^{-4}$ mol, MW 743.5 g/mol, yield 90.1%).

$C_{34}H_{30}O_3P_2Pt$ (743.6): % found (% calc. for) C 54.93 (54.92), H: 4.14 (4.07). ^{1}H NMR (acetone-d6): δ = 2.65 (bs, PCH_2CH_2P, 4H), 5.35 (bs, CHCOO), 7.1-8.2 (m, 5H, Ph), ppm. ^{31}P NMR (acetone): δ_A = 28.42 (d, $^1J_{PtPA}$ 3671 Hz, P_A), δ_B = 33.30 (d, $^1J_{PtPB}$ 3113 Hz, P_B), ppm, $^2J_{PAPB}$ 10.7 Hz. $S_{25°C}$ (H_2O) < 1 g L^{-1}.

Exchange of DMSO for PPh_3 in [Pt(salicylate-*O,O'*)(Me_2SO-*S*$)_2$] giving [Pt(salicylate-*O,O'*)(PPh_3)$_2$] (6).

Two eq of PPh_3 were added to a solution of **4** in acetone. The observation of ^{31}P NMR after a few minutes showed the formation of complex **6** (by comparison with reported data).[35]

^{31}P NMR (acetone): δ_B = 13.4 (d, $^1J_{PtPB}$ 3560 Hz, Ph_3P_B) ppm, δ_A = 14.18 (d, $^1J_{PtPA}$ 3862 Hz, Ph_3P_A), $^2J_{PAPB}$ 27 Hz. $S_{25°C}$ (H_2O) < 1 g L^{-1}. The crystallographic structure was determined on crystals of **6** grown from an acetone solution (see Fig. 5).

One-pot synthesis of [Pt(salicylate-*O,O'*)(PTA)$_2$] (7).

Salicylic acid (34 mg, $2.43\cdot10^{-4}$ mol, MW 138.1 g/mol, 1 eq) dissolved in 5 mL of acetone was added to a suspension of [$PtCO_3(Me_2SO$-*S*$)_2$] (100 mg, $2.43\cdot10^{-4}$ mol, MW 411.2 g/mol) in 5 mL of acetone. In the course of one hour, the mixture became completely clear and the reaction presumably gave $2.43\cdot10^{-4}$ mol of the intermediate [Pt(salicylate-*O,O'*)(Me_2SO-*S*$)_2$], **4**, assuming complete conversion.

A solution of PTA (76 mg, $4.86\cdot10^{-4}$ mol, MW 157.0 g/mol, 2 eq) in acetone (5 mL) was then added under nitrogen to the previous solution. Complex **7** precipitated as a white solid. After 30 min, it was filtered and washed with acetone (131 mg, $2.03\cdot10^{-4}$ mol, MW 645.5 g/mol, yield 84%).

$C_{19}H_{28}O_3N_6P_2Pt$ (645.5): % found (% calc. for) C 34.99 (35.35), H: 4.34 (4.37), N: 12.95 (13.02). ^{1}H NMR (D_2O): δ = 4.20 (d, NCH_2P, $^2J_{HP}$ 14 Hz), 4.40 and 4.42 (2s, NCH_2N), 6.65 (m, 2H, Ph), 7.20 (m, 1H, Ph) and 7.60 (m, 1H, Ph) ppm. ^{1}H NMR (DMSO-d_6): δ = 4.25 (d, NCH_2P, $^2J_{HP}$ 14 Hz), 4.50 (m, NCH_2N), 6.5 (m, 1H, Ph), 6.6 (m, 1H, Ph), 7.12 (m, 1H, Ph) and 7.65 (m, 1H, Ph) ppm. ^{31}P NMR (D_2O): δ_B = - 64.98 (d, $^1J_{PtPB}$ 3182 Hz, PTA_B), δ_A = - 63.55 (d, $^1J_{PtPA}$ 3393 Hz, PTA_A) ppm, $^2J_{PAPB}$ 25.7 Hz. ^{31}P NMR (DMSO): δ_B = - 64.55 (d, $^1J_{PtPB}$ 3156 Hz, PTA_B), δ_A = - 63.12 (d, $^1J_{PtPA}$ 3416 Hz, PTA_A) ppm, $^2J_{PAPB}$ 25.5 Hz. $S_{25°C}$ (H_2O) = 42 gL^{-1}.

One-pot synthesis of [Pt(*D*(-)quinate-*O,O'*)(PTA)$_2$] (8).

The above described intermediate **3** was formed by adding *D*(-)-quinic acid (47 mg, $2.43\cdot10^{-4}$ mol, MW 192.2 g/mol, 1 eq) in 5 mL of CH_3OH to a suspension of [$PtCO_3(Me_2SO$-*S*$)_2$] (100 mg, $2.43\cdot10^{-4}$ mol, MW 411.2 g/mol) in 20 mL of CH_3OH. The mixture was left under vigorous stirring for one night. The intermediate complex [Pt(*D*(-)quinate-*O,O'*)(Me_2SO-*S*$)_2$], **3** was obtained ($2.43\cdot10^{-4}$ mol, assuming total conversion) and left in solution. PTA (76 mg, $4.86\cdot10^{-4}$ mol, MW 157.1 g/mol, 2 eq), solubilized in 10 mL of CH_3OH, was then added under nitrogen. Complex **8** precipitated as a white solid and then was filtered and washed with methanol (142 mg, $2.03\cdot10^{-4}$ mol, MW 699.4 g/mol, yield 84%).

$C_{19}H_{34}O_6N_6P_2Pt$ (699.5): % found (% calc. for) C 32.52 (32.62), H: 4.94 (4.90), N: 12.15 (12.01). ^{1}H NMR (D_2O): δ = 1.6 – 2.0 (m, 4H), 3.38 (m, 1H), 3.85 (m, 2H) (cyclohexane), 4.2 (d, 12H, PTA), 4.45 (s, 12H, PTA). ^{31}P NMR (D_2O): δ_B = - 60.81 (s, $^1J_{PtPB}$ 3083 Hz, PTA_B), δ_A = - 60.81 (s, $^1J_{PtPA}$ 3479 Hz, PTA_A) ppm. ^{31}P NMR (DMSO): δ_B = - 63.33 (d, $^1J_{PtPB}$ 2995 Hz, PTA_B), δ_A = - 62.04 (d, $^1J_{PtPA}$ 3340 Hz, PTA_A) ppm, $^2J_{PAPB}$ 24 Hz. ^{13}C NMR (DMSO): ca 40 ppm, under solvent signal (CH_2 quinic acid); 49.51 and 49.91 (d, NCH_2P, $^1J_{CP}$ = 24.5 and 24.2 Hz), 67.08 (s, 2 CHOH quinic acid), 71.61 and 71.68 (s, NCH_2N), 77.03 (s, 1 CHOH quinic acid), 80.50 (s, COHCOOH), 173.06 (s, COO). $S_{25°C}$ (H_2O) = 64 g L^{-1}.

Two-step synthesis of [Pt(*L*-carnitine-*O,O'*)(PTA)$_2$]BF_4 (9).

Complex **9** was obtained by a two-step synthesis, the first occurring in water and the second in acetone.

Step 1: formation of **D**, as reported in ref 18.

Step 2: [Pt(*L*-carnitine-*O,O'*)(Me_2SO-*S*$)_2$]BF_4, **D**, (263 mg, $4.40\cdot10^{-4}$ mol, MW 598.2 g/mol) was suspended in 20 mL of acetone, and PTA (138 mg, $8.79\cdot10^{-4}$ mol, MW 157.1 g/mol, 2 eq in 20 mL of acetone) was added under nitrogen. The mixture was kept under vigorous stirring for 12 hours. The pale yellow precipitate, **9**, was then filtered and dried under vacuum over P_2O_5. (200 mg, $2.64\cdot10^{-4}$ mol, MW 756.4 g/mol, yield 60%).

$C_{19}H_{38}BF_4O_3N_7P_2Pt$ (756.4): % found (% calc. for) C 30.12 (30.17), H: 5.23 (5.06), N: 12.85 (12.96). ^{31}P NMR (CH_3OH): δ_B = - 61.88 (d, $^1J_{PtPB}$

3018 Hz, PTA_B), δ_A = - 58.63 (d, $^1J_{PtPA}$ 3692 Hz, PTA_A) ppm, $^2J_{PAPB}$ 24.3 Hz. ^{1}H NMR (CD_3OD): δ = 2.45 (m, 2H, CH_2COO), 3.20 (s, 9H, NMe_3), 3.42 (m, 2H, CH_2N), 4.32 (d, 13H, PTA + CH), 4.60 (s, 12H, PTA). $S_{25°C}$ (H_2O) = 8.5 g L^{-1}.

X-Ray Crystallography

Single-crystal diffraction data for complexes **3**, **4** and **6** were collected on a Nonius Kappa diffractometer equipped with a CCD detector with graphite-monochromatized MoKα radiation (λ = 0.71069 Å). Intensities were corrected for Lorentz, polarization and absorption effects.[50] The structures were solved by direct methods with the SIR97 suite of programs[51] and refinement were performed on F^2 by full-matrix least-squares methods with all non-hydrogen atoms anisotropic. The absolute configuration of the quinic ligand in complex **3** was confirmed by the value of the Flack parameter (0.007(6)). In complex **6** the electron density difference map showed some rather large, broad peaks located among the complexes. Attempts to identify the solvent molecules failed. Instead, a new set of F^2 (hkl) values with the contribution from solvent molecules withdrawn was obtained by the *SQUEEZE* procedure implemented in *PLATON*.[52] The potential solvent volume in the crystals was 176.4 $Å^3$ per unit cell volume. In **3**, hydrogen atoms of the hydroxyl groups were found in the difference Fourier map and refined isotropically; all other hydrogens were included on calculated positions, riding on their carrier atoms. All calculations were performed using SHELXL-97[53], implemented in the system of programs WINGX.[54] Crystal data are given in Supporting Material.

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. 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 (2 mM) (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).

Pure derivatives were added at serial dilutions and incubated for 3 days. After this time, cells were washed with PBS 1X and detached with trypsin. Cells were suspended in physiological solution and counted with a ZBI Coulter Counter (Coulter Electronics, Hialeah, FL, USA). The cell number/ml was determined as IC_{50} after 3days of culture, when untreated cells are in log phase of cell growth.[55] Stock solutions (10 mM) of compounds were made in water, while stock solution (10 mM) of complexes **5** and **6** was made in H_2O/DMSO 5%. All solutions were diluted in complete medium to give final concentrations. Cisplatin was employed as a control for the cisplatin-sensitive A2780, 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 1000 μL total volume, for 72 hours.

Conclusions

As a general final consideration about the quite extended group of reactions of complex [$PtCO_3(Me_2SO\text{-}S)_2$], **1**, here reported, it is possible to affirm that the substitution of carbonate with carboxylic acids or hydroxycarboxylic acids through protonolysis was successful in every case we tested, included the monocarboxylic acid DCA (dichloroacetic acid), which does not benefit from the stabilizing effect of a metal-chelate formation, and which will be object of another paper.

This reactivity can be exploited for preparing a variety of DMSO-Pt-carboxylate complexes. The subsequent substitution of DMSO with a phosphine, e.g. PPh_3, dppe or PTA, occurs smoothly, opening an access to a new group of potential Pt anticancer drugs.

Conflict of interest

The authors declare no competing financial interest.

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