In vitro Cytostatic Activity of Palladium(II) and Platinum(II) Halide Complexes with Thiocarbamic Esters

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Introduction

A wide interest in the heavy metal chemistry applied to pharmacological researches arose from Rosenberg's discovery of the antitumour activity of *cis*-$Pt(NH_3)_2Cl_2$ (DDP) [1]. Systematic investigations on platinum diamine analogues were carried out with the purpose of correlating chemical properties and biological activity, and some general criteria which are of importance in order to isolate active substances were reported [2–6]. At the same time new transition metal complexes were synthesized, with the aim to improve antitumour properties and therapeutic indices.

Chemotherapy with DDP alone or in combination with other drugs presents side effects such as nephrotoxicity. This toxicity seems to be due to interactions of the metal with thiol groups in tubular membrane-bound enzymes. Borch *et al.* [7] demonstrated that sodium diethyldithiocarbamate prevents nephrotoxicity without adverse effects on the tumour response to DDP. Moreover the DNA interstrand cross-link formation, in isolated DNA treated by DDP, may be prevented by thiourea and related compounds [8], although this effect *in vivo* depends on the thiourea concentration and on the interval between platinum and thiourea treatment [9].

Bearing in mind the cytotoxic action found for complexes of platinum with sulfur donor ligands [10], we have undertaken the synthesis of platinum and palladium complexes with thiocarbamate esters to test as possible antitumour agents.

This note reports the preparation and results of a preliminary test *in vitro* on KB cells by platinum(II) and palladium(II) halide complexes with N,N-dimethyl O-ethylthiocarbamate (DMTC), N-methyl O-ethylthiocarbamate (MTC) and O-ethylthiocarbamate (TC), which coordinate those metals through the sulfur atom.

Along with the square planar DMTC, MTC and TC 1:2 adducts, compounds of the type $Pt(MTC)_3X_2$ and $[M(MTC)_4]X_2$ (M = Pd, Pt; X = Cl, Br) have been isolated. In the solid state the 1:4 complexes have an ionic structure but in most solvents they release one or two ligand molecules to give the corresponding 1.3 and 1.2 derivatives [11–15].

Experimental

Preparation of the Complexes

trans-$Pt(DMTC)_2X_2$ (X = Br, I) and *trans*-$Pd(DMTC)_2X_2$ (X = Cl, Br, I) were as in ref. [11] A new method was used to synthesize *cis* and *trans*-$Pt(DMTC)_2Cl_2$: $PtCl_2$ (1.18 mmol) was added under agitation to an ethanol solution of DMTC (2.36 mmol in 12 ml). The reaction went on slowly to give an orange solution and a pink solid. After 5 h the solid was filtered, dried *in vacuo* and purified by benzene/n-hexane (M.p. = 119 °C). IR and ^1H NMR were in accordance with the data reported in ref. [11] for the *trans*-isomer. The orange solution was evaporated to dryness and the residue extracted with benzene. By addition of n-hexane an oily product separated, which when washed and kept under n-hexane, in three days gave orange crystals of the *cis*-isomer (M.p. = 102 °C). The structure of both the isomers was confirmed by X-ray analysis [12, 13].

The complexes *trans*-$Pd(MTC)_2X_2$ (X = Cl, Br, SCN) were as in ref. [14] and the platinum halide derivatives of MTC as in ref. [15].

cis-$Pt(TC)_2Cl_2$ was prepared by adding $PtCl_2$ (0.94 mmol) to a solution of TC (1.18 mmol) in acetone (20 ml). After 2 h a golden-yellow solution was formed, from which yellow needles of the compound precipitated by addition of n-hexane (M.p. = slow decomposition over 120 °C; found %: C = 15.4; H = 3.2; N = 5.8; Cl = 14.7; calcd. %: C = 15.1; H = 2.9; N = 5.9; Cl = 14.8). The compound is soluble in DMSO, acetone, H_2O, ethanol; slightly soluble in CH_2Cl_2; insoluble in ethyl ether, n-hexane benzene. The IR data are consistent with coordination of TC through the thiocarbonyl group; the intense bands at 321, 309 cm^{-1} were assigned to Pt–Cl stretching in a *cis*-arrangement.

cis-$Pt(NH_3)_2Cl_2$ was prepared as in ref. [16].

The IR spectra were registered by a Perkin-Elmer 580 B Spectrophotometer; the ^1H NMR

Abbreviations:
BES, (N,N-bis[2-Hydroxyethyl]-2-aminoethane sulfonic acid); HEPES, (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid); TES, (N-tris[Hydroxymethyl] methyl-2-aminoethanesulfonic acid); DMSO = dimethylsulfoxide.

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spectra by a Varian FT 80A NMR Spectrometer.

In vitro *Cytostatic Activity*

The substances were tested on KB cells (Flow Laboratories) cultivated in Lieghton tubes, according to Geran *et al.* [17]. Minimal Eagle's medium (MEM) [18] supplemented with 10% non essential amino acids and 10% calf serum inactivated at 56 °C for 30 min was used. The medium was buffered with TES (3 m*M*), HEPES (3 m*M*), BES (3 m*M*) and Tricine (3 m*M*) [19]. The cells were seeded at rate of 10^5 cells per tube and incubated at 37 °C. After 24 h, the cells were attached to the glass and the compounds to be tested dissolved immediately before use in sterile DMSO or acetone. The final concentration of DMSO or acetone in MEM (0.5%) was tested for non-toxicity. Incubation was carried out at 37 °C for 72 h. As a positive control 6-mercaptopurine was always included ($ED_{50} \cong 0.1$ μg/ml) We have also tested the three ligands by using the same *in vitro* procedure also at the doses present in 1 μg/ml of each respective complex in order to examine if the activity of the complexes can be ascribed to the ligand itself.

Cell growth was estimated by counting the viable cells detached from the glass wall with trypsin [20]. The statistical evaluation of results was done by the Student *t* test.

The cytostatic activity was calculated as CA% = 100 − T − B/C − B·100, where B is the Baseline (initial number of viable cells), and T and C are the viable cells in the treated and control tubes respectively after 72 h incubation [17]. The CA% values were plotted against log D, D being the drug concentration in μg/ml. From the sigmoidal curve log ED_{50} (where ED_{50} is the drug concentration which inhibits by 50% the cell growth in respect to controls) was determined.

Results and Discussion

The ED_{50} values for all the compounds tested are reported in Table I; an $ED_{50} \leqslant 10$ μg/ml was taken as being indicative of significant cytostatic activity.

The three ligands did not show any activity up to 10 μg/ml.

All the 1:2 DMTC adducts have low activity in both solvents (Table I). The ^1H NMR spectra of those complexes is d_6-DMSO are identical to the spectrum of DMTC, indicating that DMSO, which coordinates through the sulfur atom, is a stronger donor than DMTC. The drug is then a mixture of free DMTC and of a complex of type $M(DMSO)_2X_2$ (M = Pd, Pt; X = halide). *Cis*-$Pt(DMSO)_2Cl_2$, whose crystal structure has been reported [21], is not an antitumour compound [22]. In acetone the 1:2 complexes are generally monomeric, but their *trans* configuration is not in favour of an active behaviour [5]. The only complex with a *cis* structure, *cis*-$Pt(DMTC)_2Cl_2$, has an appreciable tendency to isomerize, as observed in benzene [11].

TABLE I. *In vitro* Cytostatic Activity against KB Cells.

Compound	ED_{50} (micrograms/ml)	
	Acetone	DMSO
cis-$Pt(DMTC)_2Cl_2$	>10	>10
trans-$Pt(DMTC)_2Cl_2$	>10	>10
trans-$Pt(DMTC)_2Br_2$	>10	>10
trans-$Pt(DMTC)_2I_2$	>10	>10
trans-$Pd(DMTC)_2Cl_2$	>10	>10
trans-$Pd(DMTC)_2Br_2$	>10	>10
trans-$Pd(DMTC)_2I_2$	>10	>10
cis-$Pt(MTC)_2Cl_2$	0.7	5.3
trans-$Pt(MDTC)_2Cl_2$	0.8	>10
$Pt(MTC)_3Cl_2$	0.3	0.7
$[Pt(MTC)_4]Cl_2$	5 9	>10
trans-$Pt(MTC)_2Br_2$	0.4	7.6
$Pt(MTC)_3Br_2$	0.5	1.8
$[Pt(MTC)_4]Br_2$	1 5	1.6
trans-$Pd(MTC)_2Cl_2$	>10	>10
trans-$Pd(MTC)_2Br_2$	[a]	>10
trans-$Pd(MTC)_2(SCN)_2$	[a]	>10
$[Pd(MTC)_4]Cl_2$	>10	>10
cis-$Pt(TC)_2Cl_2$	>10	>10
cis-$Pt(NH_3)_2Cl_2$	[a]	0.1

[a] Insufficiently soluble.

When the ligand is MTC, the palladium complexes are inactive, whereas all the platinum derivatives present a more or less evident activity in both solvents. We thought then to investigate the behaviour of MTC and its platinum complexes in d_6-acetone and d_6-DMSO by ^1H NMR spectroscopy. From Table II, MTC Presents a broad signal (around 8–9 ppm) due to the NH proton and two NCH_3 doublets of different intensity, assigned to the *syn* and *anti* isomers of the planar MTC molecule [14, 15, 23 and ref. therein].

The ethyl proton resonances also differ in the two isomers, but are too close to be integrated. *Cis*- and *trans*-$Pt(MTC)_2Cl_2$ have in d_6-DMSO identical spectra (Fig. 1), with two NH signals of equal intensity due respectively to free (9 ppm) and coordinated (10.2 ppm) MTC. For the other groups of protons the lower field resonances (marked with solid circles in the figure) belong to the complexed ligand. The spectra of $Pt(MTC)_3Cl_2$ and $[Pt(MTC)_4]Cl_2$ are similar, except for the amount of coordinated MTC

TABLE II. ^{1}H NMR Spectra of MTC and Complexes (t° ≅ 29 °C; the chemical shifts are in ppm).[a]

Compound	Solvent	NH	NCH_3	O-CH_2-CH_3	O-CH_2-CH_3
MTC	d_6-DMSO	8.9	2.82 (75%) 2.66	1.21 s 1.24	4.36 s 4.41
cis-Pt(MTC)$_2$Cl$_2$	d_6-DMSO	9.0(50%) 10.2(50%)	2.84 s 2.70 2.93	1 22 s 1.25 1.37	4.41 s 4.45 4.59
trans-Pt(MTC)$_2$Cl$_2$	d_6-DMSO	8.9(50%) 10.2(50%)	2.84 s 2.65 2.89	1.21 s 1.26 1.44	4.36 s 4.40 4.55
Pt(MTC)$_3$Cl$_2$	d_6-DMSO	9.0(68%) 10.2(32%)	2.80 s 2 66 2.87	1.24 s 1.25 1.37	4.40 s 4.42 4.55
MTC	d_6-acetone	7.9	2.98(57%) 2.83	1.24 s 1.31	4.42 s 4.44
cis-Pt(MTC)$_2$Cl$_2$[b]	d_6-acetone	9.5	3.04 s 3.00	1.41	4.57 s 4.60
trans-Pt(MTC)$_2$Cl$_2$[b]	d_6-acetone	9.6	3.06 3.02 s	1.44	4.57 4.60 s
Pt(MTC)$_3$Cl$_2$	d_6-acetone	11.2[c](65%)	2.9[d]	1.42[e]	4.59[e]
[Pt(MTC)$_4$]Cl$_2$	d_6-acetone	8.1 vbr	3.0[f] 2.8	1.22 s (25%) 1.25	4.38 s 4.42
		11.2(60%)	3.02[f] 2.94 s	1.40	4.54

[a]The stronger between two very close signals is indicated by s. [b]Within 10′ from solubilization. After 2 h both the compounds give the spectrum of Fig. 2; after one day a strong singlet, increasing with time with a parallel decrease of the NH signal is observed. [c]Two hardly appreciable NH signals are at about 9.6 and 8.0 ppm. [d]Stronger signal which overlaps weaker resonances probably due to MTC and Pt(MTC)$_2$Cl$_2$. [e]Very weak free ligand signals are at 1.22 and 4.44 ppm. [f]The assignment is doubtful; the two signals have apparently a comparable intensity.

which is about 32% and 25% of the total respectively. It can be concluded that in DMSO the three compounds release partially MTC to form a complex of the type Pt(MTC)(DMSO)Cl$_2$. Several platinum halide complexes, containing one sulfur bonded DMSO and another ligand (generally N-donor), with either *cis* or *trans* structure, have been isolated [24–29].

When dissolved in acetone, the platinum MTC complexes have a cytostatic activity higher than in DMSO, and comparable with that of *cis*-Pt(NH$_3$)$_2$Cl$_2$. In fact for this compound ED$_{50}$ is 3 × 10^{-7} *M*, while it is ≅1.5 × 10^{-6} *M* for *cis*- and *trans*-Pt(MTC)$_2$Cl$_2$, 5 × 10^{-7} *M* for Pt(MTC)$_3$Cl$_2$, 7 × 10^{-7} *M* for Pt(MTC)$_2$Br$_2$ and Pt(MTC)$_3$Br$_2$ and 8 × 10^{-6} *and 2* × 10^{-6} *M* for the 1:4 chloro- and bromo-derivative respectively.

It is of interest to note that the cells which survived the treatment with our complexes did not show the morphological change observed by us [30] and other authors [31] after DDP treatment.

The very close ED$_{50}$ values for the *cis*- and *trans*-chloro-complexes can be due to the isomerization equilibrium in acetone. The ^{1}H NMR spectrum of *cis*-Pt(MTC)$_2$Cl$_2$, taken within 10′ from solubilization presents a strong doublet at 3.04 ppm, with a hardly visible doublet at 3.00 ppm; the more intense CH_2 quadruplet is at 4.57 ppm (the weak one at 4.60 ppm). In one hour the spectrum is that of Fig. 2 (where the weaker signals are of the *trans* form) and remains unchanged after 5 h. *Trans*-Pt(MTC)$_2$Cl$_2$ is almost insoluble in acetone, by heating up to 50 °C it slowly dissolves giving a spectrum similar to that of the *cis* isomer, but where the intensity of the signals is reversed, indicating a predominance of the *trans* form. In two hours the spectrum resembles that of Fig. 2; in acetone, as in benzene [11], an identical equilibrium situation is reached whatever the starting isomer. In acetone the 1:4 and 1:3 MTC complexes release MTC; the species in solution are free ligand and the 1:3 and 1:2 adducts [14, 15]. The spectrum of [Pt(MTC)$_4$]Cl$_2$ in acetone resembles that in benzene [15] but the N-CH_3 resonances are very close and consist of two doublets of comparable intensity (one of MTC, the other is the weaker signal of the

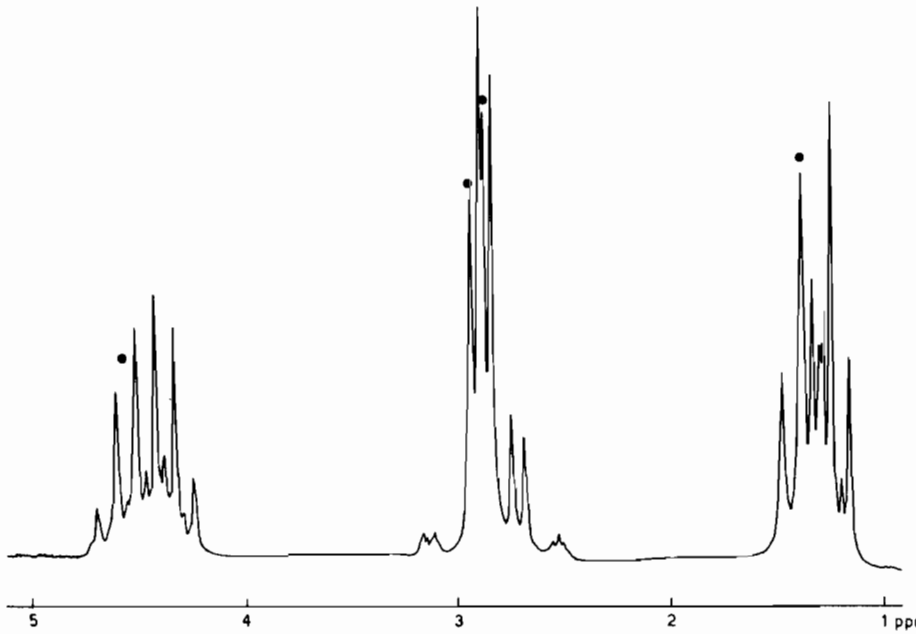

Fig. 1. ^{1}H NMR spectrum of *trans*-Pt(MTC)$_2$Cl$_2$ in d_6-DMSO (20 mg in 0.5 ml). The solid circles indicate the signals due to the complexed MTC.

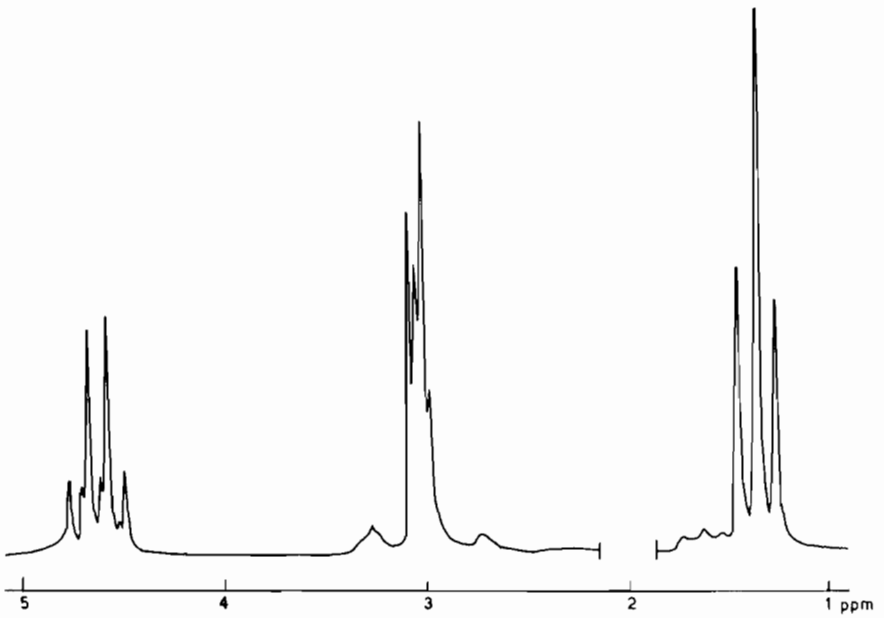

Fig. 2. ^{1}H NMR spectrum of *cis*-Pt(MTC)$_2$Cl$_2$ in d_6-acetone (15 mg in 0.5 ml) after one hour from solubilization.

1:3 complex) and one more intense doublet which is the stronger signal of the 1:3 complex. The aspect of the Pt(MTC)$_3$Cl$_2$ spectrum changes with concentration At higher concentrations the 1:3 species predominates (NH at 11.2 ppm); on diluting the amount of 1.2 (NH at 9.5 ppm) and MTC (NH at 8.0 ppm) increases, making it difficult to assign the almost superimposed NCH_3 resonances.

The possibility to have more species in acetone probably plays an important role in the cytostatic activity of the MTC complexes; in fact the 1:4 complexes have ED_{50} molar values appreciably higher

with respect to that of the 1:2 and 1:3 analogues, suggesting that free MTC affects the cell–complex interactions. In DMSO, where all the complexes release MTC, the cytostatic activity is generally lower than in acetone.

MTC complexes being the more promising from the preliminary data reported in this note, we think it worthwhile to extend the study in this field, with particular attention to the effects of water and chloride ions on the complexes, since the cytostatic activity is evaluated in an aqueous medium where the chloride ions are predominant [32].

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