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Synthesis, Characterization and Cytotoxic Activity of New Platinum(II) Complexes with Some Nitrogen Containing Ligands, Part (2): With 3,5-Dimethylpyrazole

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New platinum(II) complexes of the general formula *cis*-[PtLL'X_2], where L = L' = 3,5-dimethylpyrazole, X = 0.5 C_2O_4 or 0.5 $O_2(CO)_2C{-}CH_2CH_2CH_2CH_2$ or $O(CO)C_6H_{11}$ and L = 3,5-dimethylpyrazole, L' = DMSO, X = Cl, have been prepared as analogue to so called *cis*platin, carboplatin (paraplatin) and oxaliplatin; the known, anti-cancer drugs. The complexes obtained have been characterized physico-chemically and spectroscopically. The cytotoxic activities of these complexes have been studied against Hep-2, HeLa, RD, L_{20B}, BGM and Vero cell lines using the MTT-colorimetric assay. These activities were compared with cytotoxic activities of three reference standards: the *cis*platin, carboplatin and oxaliplatin complexes. The significance of the results obtained is discussed.

INTRODUCTION

We have presented in part (1) of this work[1] a brief historical view about the biological activities of platinum complexes with some nitrogen containing ligands. In this part, we are presenting the synthesis and properties of new platinum(II) complexes of 3,5-dimethylpyrazole (Scheme 1), and their cytotoxic activities against six cell lines *in vitro*.

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra were recorded at the University of Jordan, Amman, Jordan, on a Bruker-DPX 300 MHz spectrometer, using $CDCl_3$ as a solvent with TMS as an internal standard. IR spectra were recorded on a Perkin-Elmer FT IR spectrometer using KBr discs in the range 4000–400 cm^{-1}.

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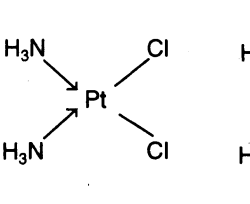
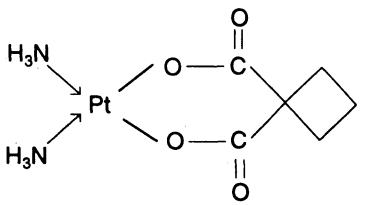
Preparation of Starting Materials

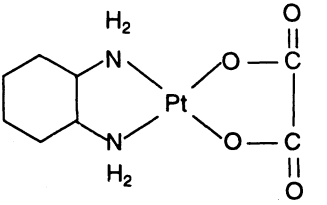
The compounds K_2PtCl_4, oxalic acid, cyclohexylcarboxylic acid and 1,1-cyclobutyldicarboxylic acid were commercial products (Fluka) and used without further purification. The compounds 3,5-dimethylpyrazole and *cis*-$[Pt(DMSO)_2Cl_2]$ were prepared as described previously[2]. The complexes $[Pt(DMSO)_2C_2O_4]$, *cis*-$[Pt(DMSO)_2(OCOC_6H_{11})_2]$ and $[Pt(DMSO)_2\{O_2(CO)_2C{-}CH_2CH_2CH_2CH_2\}]$ were prepared in our laboratories[3].



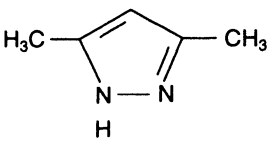
Cisplatin



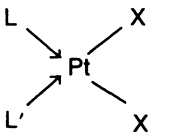
Carboplatin



Oxaliplatin



3,5-Dimethylpyrazole



(1) L=L'=pyrazole, X_2=C2O4
(2) L=L'=pyrazole, $X_2=O_2(CO)_2C{-}CH_2CH_2CH_2CH_2$
(3) L=pyrazole, L'=DMSO, X=Cl

(4) L=L'=pyrazole, X=O—CO-(cyclohexyl, 1, 2, 3, 4)

Scheme 1. The new platinum(II) complexes (1–4) prepared in this study and screened against six tumour cell lines.

Preparation of the Complexes

The new platinum complexes (Scheme 1) were prepared as follows:

Cis-[Pt(Pyrazole)$_2$C$_2$O$_4$] (1)

The complex *cis*-$[Pt(DMSO)_2C_2O_4]$ (0.44 g, 1.0 mmol) was suspended in a mixture of 1 : 1 water/ethanol (20 mL) and 3,5-dimethylpyrazole (0.25 g, 2.6 mmol), was dissolved in ethanol and added to the suspension. The reaction mixture was heated with stirring until all solid had gone into solution; this step took about 30 min. The solution was taken to dryness and the solid thus obtained was washed several times with ether (to remove the excess pyrazole) and dried

under vacuum at 80°C for several hours. The product obtained is pure enough for further purposes. Yield is 0.24 (50%).

Cis-[Pt(Pyrazole)$_2${$O_2(CO)_2$C—$CH_2CH_2CH_2CH_2$}] (2)

The complex [Pt(DMSO)$_2${$O_2(CO)_2$C—$CH_2CH_2CH_2CH_2$}] (0.5 g, 1.0 mmol) was suspended in chloroform (20 mL) and 3,5-dimethylpyrazole (0.25 g, 2.6 mmol) was dissolved in chloroform (10 mL) and added to the suspension. The reaction mixture was gently heated for *ca.* 30 min with stirring until all solid had gone into solution. The mixture was filtered through celite and the clear filtrate was taken to dryness. The residual oil was washed several times with ether and dried under vacuum at 80°C for several hours to give an off-white solid. The product obtained is pure enough; nevertheless, it can be recrystallized from chloroform/ether. Yield is 0.38 g (72%).

Cis-[Pt(Pyrazole)(DMSO)Cl_2] (3)

The complex *cis*-[Pt(DMSO)$_2Cl_2$] (1.1 g, 2.6 mmol) was suspended in DMSO (7 mL) and 3,5-dimethylpyrazole (0.25 g, 2.6 mmol), was dissolved in DMSO (5 mL) and added to the suspension. The reaction mixture was gently heated for few minutes until all solid went into solution. After cooling to room temperature, 0.1N HCl was added until the solution became turbid. The product was extracted from the mixture with chloroform (*ca.* 100 mL) and the extract was dried over anhydrous Na_2SO_4. Evaporation of all chloroform left an oily material; it was washed several times with ether and dried under vacuum. The ^1H and ^{13}C NMR spectra of the product comprise the presence of two complexes with 70% and 30% proportions; identified the latter were to be the *cis-* and *trans-* isomers of the complex [Pt(DMSO)(Pyrazole)Cl_2]. The product was recrystallized several times from chloroform/n-hexane until removal of all the *trans-* isomer.

Cis-[Pt(Pyrazole)$_2${$O(CO)C_6H_{11}$}$_2$] (4)

The complex *cis*-[Pt(DMSO)$_2${$O(CO)C_6H_{11}$}$_2$] (0.6 g, 1.0 mmol) was dissolved in chloroform (15 mL) and 3,5-dimethylpyrazole (0.25 g, 2.6 mmol) was dissolved in chloroform (5 mL) and added to the above solution. The reaction mixture was allowed to stand at ambient temperature for *ca.* 30 min; then all chloroform was evaporated until dryness to leave an oily material, which was washed with small portions of ether and dried under vacuum. The ^1H and ^{13}C NMR spectra of the product comprise the presence of two complexes with 88% and 12% proportions. The latter was identified to be the *cis* and *trans-* isomers of the complex [Pt(pyrazole)$_2${$O(CO)C_6H_{11}$}$_2$]. The product was purified by extraction of the mixture several times with *n*-hexane (*ca.* 200 mL), in which all the *trans-* isomer had gone into solution, while the *cis-* isomer remained as insoluble solid. This was collected by filtration and dried under vacuum at 80°C for several hours. Yield is 0.3 g (50%).

Biological Methods

(1) *Complexes*: The four complexes, 1–4 (Scheme 1) were dissolved in 10% DMSO. Three serial dilutions of 0.1, 1.0 and 10.0 μg/mL were used and millipore (0.2 nm) filtered under laminar flow conditions.

Reference standards (*cis*platin and carboplatin) were purchased from Bristol Myers (USA) and oxaliplatin was perpared, characterized and purified (HPLC) in our laboratories[3].

(2) *Cell lines*: Hep-2 (human carcinoma of larynx), HeLa (human cervical carcinoma), RD (human embryonal rhabdomyosarcoma), L_{20B} (mouse L-cells containing human polio-virus receptors[4]), BGM (African green monkey kidney cells) and Vero (African green monkey kidney cells) were kindly supplied by Dr. M. Abdul-Majeed, Al-Basheer Hospital, Amman, Jordan. All cells except L_{20B} and Vero cells were maintained in Minimum Essential Medium (MEM) and supplementecd with 5% fetal calf serum (ICN-Flow Laboratories, UK), L-glutamine and antibiotics (100 units of penicillin and 100 μg mL^{-1} of streptomycin). L_{20B} cells were maintained in Dulbecco's MEM (DMEM) (Sigma Chemical Co., USA) and supplemented with 10% fetal calf serum and antibiotics. Whereas Vero cells were maintained in Medium-199 (Sigma Chemicals Co., USA) and supplemented with 5% fetal calf serum and antibiotics.

(3) *Cytotoxicity tests*: MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay was performed in a 96-well plate[5, 6]. The above cell lines (1×10^6 cells mL^{-1}) were seeded in each well with 100 μL of growth medium and 10% fetal calf serum and antibiotics. After overnight incubation (37°C, 5% CO_2), 10 μL of the sample solution was added to each well and incubated for 72 h. Then 10 μL of MTT (5 mg mL^{-1}) was added to each well and the plates were incubated for a further 4 h. Later, 25 μL of 10% SDS-0.01 M HCl solution was added to each well. The optical density was recorded using a microplate reader at 540 nm. Three separate sets of controls containing the solvents (10% DMSO) were used in each plate. The IC_{50} (μg mL^{-1}) was calculated using the probit test.

RESULTS AND DISCUSSION

The physical properties of the pyrazole ligand and its complexes $[PtLL'X_2]$ (Scheme 1) are listed in Table-1 and their ^1H and ^{13}C NMR data are listed in Tables 2 and 3, respectively.

Reaction of *cis*-$[Pt(DMSO)_2X_2]$, X=0.5 C_2O_4, 0.5 $O_2(CO)_2C{-}CH_2CH_2CH_2CH_2$, $O(CO){-}C_6H_{11}$ with two moles of 3,5-dimethylpyrazole (L) leads to a complete displacement of DMSO and affords set of complexes of the general formula $[PtL_2X_2]$. In the case where X = Cl, the reaction leads to the formation of a mixture of several complexes and attempts to identify them were unsuccessful, while the reaction of *cis*-$[Pt(DMSO)_2Cl_2]$ with one mole of the pyrazole ligand leads to the formation of a mixture of both, the *cis*- and *trans*- isomers with 70% and 30% proportions, respectively. The remaining DMSO molecule in $[Pt(DMSO)LCl_2]$ was identified by IR and ^1H and ^{13}C NMR spectroscopy (Tables 1–3).

In the case where X = O(CO)—C_6H_{11}, the reaction again leads to the formation

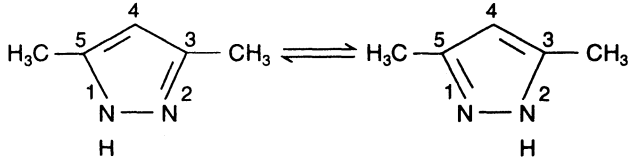
of both, the *cis-* and *trans-* isomers with 88% and 12% proportions, respectively. Removal of the *trans*-isomer out of the products 3 and 4 (Scheme 1) was carried out by the successive crystallization of the product as mentioned in the experimental part.

IR Spectra

The routine IR spectral measurements were carried out using KBr discs. The spectral data of the free ligand were recorded for comparison with those of the complexes prepared. Some characteristic bands due to N—H, C=C, C=N stretching frequencies of the free ligand were shifted to either higher or lower values, *i.e.*, no systematic variations, assigning a coordination of the ligand with platinum (Table-1). Other band appearing at *ca.* 1710 cm^{-1} for complexes 1, 2 and 4 is clearly attributed to ν(C=O) of the carboxylato groups. Additional bands due to other functional group's stretching frequencies were also observed.

NMR Spectra

The ^1H NMR spectral data of the free pyrazole showed one single signal for the two methyl groups, *i.e.*, they have the same environments, due to the fact that this moiety exists in the following two tautomeric forms:



(1) (2)

Similarly, the ^{13}C NMR spectral data of this ligand showed single signal for the carbon atom of both methyl groups, as well as single signal for carbon-3 and-5, again because both methyl groups and both carbon-3 and-5 have the same environments.

Upon coordination with platinum, most possibly, *via* the quaternary nitrogen of the ligand (nitrogen atom bearing no hydrogen), the two tautomeric forms become one and hence the two methyl groups will then have two different environments. This is clear from the ^1H NMR spectral data of all the complexes prepared (Table-2), in which two characteristic signals for both methyl groups were shown and the broad signal of the NH groups of the free ligand was shifted downfield by 1.5–4.5 ppm, upon coordination. This significant shift of the NH group was occured due to an inductive effect caused by the coordination of the adjacent nitrogen with platinum.

Similar results were observed with the ^{13}C NMR spectral data, in which the methyl groups showed two well defined signals and C-3 and C-5, also, showed two differentiated signals upon coordination (Table-3). The higher chemical shift value obtained is assigned to the methyl group attached to the nitrogen atom coordinated to platinum. Other carbon atoms of the pyrazole moiety and of the carboxylato groups are also recorded and assigned[7] (Table-3).

TABLE-1

PHYSICAL PROPERTIES OR PLATINUM(II) COMPLEXES[a]

Compound	Color	m.p. (°C) (Dec)	Selected IR bands ($(cm^{-1})^b$)				
			ν(N—H)	ν(C=O)	ν(C=C)	ν(C=N)	ν(others)
Free ligand	Off-white	100–102	3200 m, b	—	1593.5 s, sh	1486 s	1424, 1304, 1154, 1030, 853
1	Off-white	>300	3204, 3137 m	1704 s	1580 s	1528 s	1408.5, 1304, 1195, 1072, 810
2	Off-white	148–158	3204, 3130 m	1725 m	1598 s	1537 s	1410, 1305, 1219, 1150, 1025, 800
3 (*cis*-)	Off-white	164–174	3185 m, b	—	1582 s	1486 s	1416, 1293, 1200, 1130[c], 1111, 1026, 810
4 (*cis*-)	White	206–210	3140 m	1717 m	1594 s	1536 s	1420, 1310, 1201, 1076, 960, 741

a. Satisfactory elemental analyses were obtained.
b. For IR data: b, broad; m, medium; s, strong; sh, shoulder bands.
c. Strong band assigned to ν(S=O) of coordinated DMSO.

TABLE-2

PROTON CHEMICAL SHIFTS (δ ppm)* AND PLATINUM-PROTON COUPLING CONSTANTS (J Hz) FOR PLATINUM(II) COMPLEXES

Compound	Ligand (L) 3,5-dimethylpyrazole			Ligand (L') DMSO		Carboxylate
	δ(CH_3)	δ(CH)	δ(NH)	δ(CH_3)	3J(^{195}Pt-S-CH)	
Free ligand	2.27 s (6H)	5.80 s (1H)	10.30 s (1H)			
1	2.24 s (3H), 2.35 s (3H)	5.75 s (1H)	13.80 s (1H)			
2	2.28 s (3H), 2.40 s (3H)	5.80 s (1H)	13.20 s (1H)			2.20 q (2H) 2.70 t (4H), J = 8.0 Hz
3 (*cis*-)	2.10 s (3H), 2.53 s (3H)	5.90 s (1H)	11.70 s (1H)	3.50 s (6H)	24.5	
3 (*trans*-)	2.20 s (3H), 2.49 s (3H)	5.90 s (1H)	11.07 s (1H)	3.55 s (6H)	24.0	
4 (*cis*-)	2.28 s (3H), 2.43 s (3H)	5.70 s (1H)	14.90 s (1H)	—	—	1.30–2.10 m (11H)
4 (*trans*-)	3.00 s (3H), 3.48 s (3H)	5.75 s (1H)	13.10 s (1H)			1.28–2.07 m (11H)

*Downfield from internal TMS, using $CDCl_3$ as a solvent. Abbreviation s, t, q, m are for singlet, triplet, queintet and multiplet signals, respectively.

TABLE-3
CARBON-13 NMR DATA, δ(ppm)[a] AND J(HZ) FOR PLATINUN(II) COMPLEXES

Compund	Ligand (L) 3,5-dimethylpyrazole			Ligand (L') DMSO		Carboxylate	
	$\delta(CH_3)$	δ(CH)	$\delta(C—CH_3)$	$\delta(CH_3)$	$2J(^{195}Pt\text{-}S\text{-}^{13}C)$	δ(CO)	δ (others)
Free ligand	12.1	104.0	144.3				
1	11.0, 13.7	106.0	144.0, 150.5			170.0	
2	11.0, 13.7	105.4	143.6, 150.1			179.0	16.9 (CH_2), 30.1 (CH_2)$_2$, 40.9 (C)
3 (*cis*-)	11.0, 14.7	106.1	143.0, 150.2	44.8	48.0		
3 (*trans*-)	11.0, 14.2	106.6	143.0, 151.0	43.7	46.5		
4 (*cis*-)[b]	11.1, 13.7	105.1	143.5, 149.5	—	—	183.2	46.7 (C-1), 30.5 (C-2), 26.2 (C-3), 26.4 (C-4)

a. Downfield from internal TMS using $CDCl_3$ as a solvent.
b. Signal to noise ratio is not sufficient to assign the *trans* isomer.

Cytotoxicity evaluations:

The cytotoxic activities of these complexes against the different cell lines used in the present study, compared with the reference standards are illustrated in Table-4. It is evident that all of the complexes showed no cytotoxic activities against all cell lines at the concentrations used, except complex 2. This complex showed moderate activity against one cell line only, *i.e.*, Hep-2; its IC_{50} value was 2.1 μg mL^{-1}. However, this activity is somewhat significant when compared

TABLE-4
CYTOTOXIC ACTIVITIES OF PLATINUM(II) COMPLEXES WITH STANDARD REFERENCES AGAINST DIFFERENT TUMOUR CELL LINES

Complex	IC_{50} (μg mL^{-1})					
	Hep-2	HeLa	RD	L_{20B}	BGM	Vero
1	>10	>10	>10	>10	>10	>10
2	2.1	>10	>10	>10	>10	>10
3 (*cis*-)	>10	>10	>10	>10	>10	>10
4 (*cis*-)	>10	>10	>10	>10	>10	8.5
Cisplatin	1.8	5.5	>10	>10	>10	8.0
Carboplatin	>10	>10	>10	>10	>10	>10
Oxaliplatin	8	9.0	>10	>10	>10	>10

with the IC_{50} value of reference standards (*cis*platin, carboplatin and oxaliplatin; their IC_{50} values were 1.8, > 10 and 8.0 μg mL^{-1}, respectively) against the same cell line.

It is obvious that the moderate activity exhibited by complex 2 only against Hep-2 in comparison with the remaining complexes could be attributed to the nature of the complex itself. Yet, further *in vivo* tests in animal models are necessary to confirm this activity.

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