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Synthesis, Characterization and Cytotoxic Activity of New Platinum(II) Complexes with some Nitrogen Containing Ligands, Part 1: With β-Carboline Alkaloids

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New platinum(II) complexes of the general formula *cis*-[PtLL'X_2], where L = harmaline, harmine; L' = DMSO, 3,5-*dim*ethylpyrazole, cyclohexylamine and $X_2 = Cl_2$, $O_2(CO)_2C{-}CH_2CH_2CH_2CH_2$, C_2O_4 have been prepared as analogue to so called cisplatin, carboplatin (paraplatin) and oxaliplatin, respectively. These complexes have been characterized physico-chemically and spectroscopically. The cytotoxic activities of these complexes have been studied against Hep-2, HeLa, RD, L20B, BGM and Vero cell lines using the MTT-colorimetric assay. These activities were compared with cytotoxic activities of three reference standards; the cisplatin, carboplatin and oxaliplatin complexes. The significance of these results is discussed.

INTRODUCTION

An extensive research, on the synthesis and biological activity on platinum complexes, was done after the discovery of Rosenberg *et al.*[1] that cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, has a potent activity against tumour cells. There have been a large number of platinum complexes screened thereafter against certain types of tumour cell lines. Some of these complexes were already drugs, *e.g.*, carboplatin, $[Pt(NH_3)_2\{O_2(CO)_2C{-}CH_2CH_2CH_2CH_2\}]$[2]; others were under filling, *e.g.*, oxaliplatin[3] and still others were under various stages of pre-clinical and clinical trials[4].

As a continuation of our comprehensive investigation on the synthesis of metal complexes, *e.g.*, platinum complexes with various donating ligands[5–8] and their biological activity as anti-tumour agents[9–12], we are presenting here the synthesis and properties of new platinum(II) complexes of some β-carboline alkaloids (Scheme 1), and their cytotoxic activities against six tumour cell lines *in vitro*.

EXPERIMENTAL

The ^1H NMR spectra were recorded at Yarmook University, Irbid, Jordan, on

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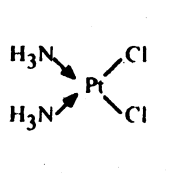
a Bruker-WH 80 DS spectrometer, using $CDCl_3$ or DMSO-d_6 as solvents with TMS as an internal standard. IR spectra were recorded on a Perkin-Elmer FT IR spectrometer using KBr discs in the range 4000–400 cm^{-1}. Analysis of the complexes was done by Atlantic Microlab., Inc., Norcross, Georgia-30091 (USA).

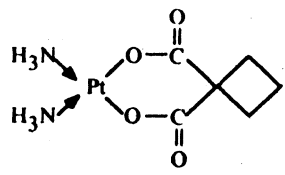
Preparation of starting materials

The compounds K_2PtCl_4, harmaline, harmine, cyclohexylamine, 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 in our previous work[6]. The complexes $[Pt(DMSO)_2C_2O_4]$, $[Pt(DMSO)_2\{O_2(CO)_2\overline{C—CH_2CH_2CH_2CH_2}\}]$ and *cis*-[Pt(DMSO)(harmaline)Cl_2] were prepared in our laboratories[13].

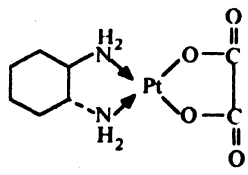
SCHEME I
THE NEW PLATINUM(II) COMPLEXES (1–5) PREPARED IN THIS STUDY AND SCREENED AGAINST SIX TUMOUR CELL LINES



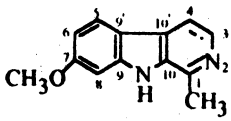
Cisplatin



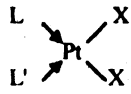
Carboplatine



Oxaliplatin



C3–C4, (Harmaline)
C3=C4, (Harmine)



(1) L = Harmaline, L′ = DMSO, $X_2 = O(CO)_2\overline{C—CH_2CH_2CH_2CH_2}$
(2) L = Harmine, L′ = DMSO, $X_2 = O(CO)_2\overline{C—CH_2CH_2CH_2CH_2}$
(3) L = Harmaline, L′ = DMSO, $X_2 = C_2O_4$
(4) L = Harmaline, L′ = 3,5-dimethylpyrazole, $X = Cl^-$
(5) L = Harmaline, L′= cyclohexylamine, $X = Cl^-$

Preparation of the complexes

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

***Cis*-[Pt(DMSO)(harmaline) $\{O_2(CO)_2\overline{C—CH_2CH_2CH_2CH_2}\}$] (1)**

The complex *cis*-$[Pt(DMSO)_2\{O_2(CO)_2\overline{C—CH_2CH_2CH_2CH_2}\}]$ (1.15 g, 2.3 mmol) was suspended in chloroform (50 mL) and harmeline (0.50 g, 2.3 mmol)

was added at once and the reaction mixture was heated under reflux for *ca.* 2 h, during which time the mixture became a clear yellow solution, then turned turbid. This was filtered and the small amount of solid remains on the filter paper was washed with chloroform (25 mL). Chloroform was evaporated until the volume became *ca.* 20 mL, then *n*-hexane was added to the point of turbidity. The yellow solid thus formed was filtered off, washed with *n*-hexane and dried under vacuum for several hours. The product is pure enough for further purposes; nevertheless, it can be recrystallized from chloroform/*n*-hexane. The yield is not less than 70%.

Cis-[Pt(DMSO)(harmine) $\{O_2(CO)_2\overline{C—CH_2CH_2CH_2CH_2}\}$] (2)

The complex *cis*-[Pt(DMSO)$_2$$\{O_2(CO)_2\overline{C—CH_2CH_2CH_2CH_2}\}$] (1.15 g, 2.33 mmol) was added in portions to a hot solution of harmine (0.50 g, 2.35 mmol) in ethanol (100 mL) and the reaction mixture was heated under reflux for *ca.* 2 h, during which time, all the solid had gone into solution, then turned turbid with some solid. On cooling to room temperature, the off-white solid was filtered off, washed with small portions of ethanol, then with *n*-hexane and dried under vacuum for several hours. The product can be recrystallized from large amount of ethanol. The yield is not less than 70%.

Cis-[Pt(DMSO)(harmaline)C_2O_4] (3)

This was prepared by a similar method to that of complex (1) above, by treating the complex *cis*-[Pt(DMSO)$_2$$C_2O_4$] (1.10 g, 2.5 mmol) with harmaline (0.53 g, 2.5 mmol) in chloroform (100 mL). The yellow product thus precipitated was filtered off, washed with *n*-hexane and dried under vacuum for several hours. The yield is above 70%.

Cis-[Pt(Pyrazole)(harmaline)Cl_2] (4)

A solution of 3,5-dimethylpyrazole (0.10 g, 1.0 mmol) in chloroform (10 mL) was added to a suspension of the complex *cis*-[Pt(DMSO)(harmaline)Cl_2] (0.56 g, 1.0 mmol) in chloroform (20 mL). The reaction mixture was gently heated until no solid was left. The mixture was filtered through celite and the clear yellow solution was evaporated until the volume became *ca.* 5 mL and ether was added to the point of turbidity and the mixture was left in the refrigerator for overnight. The solid thus obtained was filtered off, washed several times with ether and dried under vacuum at 80°C for several hours. The yield is 0.5 g (87%).

Cis-[Pt($C_6H_{11}NH_2$)(harmaline)Cl_2] (5)

This was prepared by a similar method to that of complex (4) above, by treating the complex *cis*-[Pt(DMSO)(harmaline)Cl_2] (0.56 g, 1.0 mmol) with cyclohexylamine (0.15 g, 1.5 mmol) in chloroform (30 mL). After heating of the mixture for few minutes, it was taken to dryness and the yellow oil thus obtained was treated with ether under vigorous stirring until complete solidification. The solid formed was separated by decantation, washed several times with ether and dried under vacuum for several hours. The product is pure enough for further purposes, nevertheless, it can be recrystallized from chloroform/ether. The yield is *ca.* 50%.

Biological Methods

(1) *Complexes*: The five complexes, 1-5 (Scheme 1) were dissolved in 10% DMSO. 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 (cisplatin and carboplatin) were purchased from Bristol Myers (USA) and oxaliplatin was prepared, characterized and purified (HPLC) in our laboratories[13].

(2) *Cell lines*: Hep-2 (human carcinoma of larynx), HeLa (human cervical carcinoma), RD (human embryonal rhabdomyosarcoma), L20B (mouse L-cells containing human polio-virus receptors[14]), BGM (African green monkey kidney cells) were kindly supplied by Dr. M. Abdul-Majeed, Al-Basheer Hospital, Amman, Jordan. All cells except L20B and Vero cells were maintained in minimum essential medium (MEM) and supplemented with 5% fetal calf serum (ICN-Flow Laboratories, UK), L-glutamine and antibiotics (100 units of penicillin and 100 μg mL^{-1} of streptomycin). L20B 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-diphenyltetrazolium bromide) colorimetric assay was performed in a 96-well plate[15, 16]. 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 complexes *cis*-[PtLL'X_2] (Scheme 1) are listed in Table 1 and their ^1H NMR data are listed in Table-2. The reaction of *cis*-[Pt(DMSO)$_2X_2$], $X_2 = Cl_2$, C_2O_4, $O_2(CO)_2\overline{C—CH_2CH_2CH_2CH_2}$ with one mole of the β-carboline alkaloid (harmaline or harmine) affords a good type of complex intermediates, in which the alkaloid displaces one DMSO molecule and coordinates with platinum in a monodentate fashion *via* the most reactive donating site, *i.e.*, N^2 (Scheme 1). The remaining DMSO molecule in the complex intermediates (1–3) was identified by its ν(S=O) IR absorption band, which appeared clearly at 1140 cm^{-1}, assigning S-bonding with platinum[10a]. Further support for this argument, is the proton signal appearing in the ^1H NMR spectra at $\delta = 3.3$ ppm with $3J(^{195}Pt—S—C—^1H) = 24$ Hz, assigning the presence of DMSO in the complex with S-bonding[5]. The most interesting feature with the remaining DMSO in the complex is that the proton chemical shift of NH group in the alkaloid moiety showed significant downfield shift when measured in

TABLE-1
PHYSICAL PROPERTIES OF PLATINUM(II) COMPLEXES

Complex	Colour	m.p. (°C) (Dec.)	Analysis % Found (Calcd.)			Selected IR bands (cm^{-1})†				
			C	H	N	ν(N—H)	ν(C=O)	νC=C)	ν(C=N)	ν(S=O)
1	bright yellow	196–198	40.14 (40.06)	4.22 (4.13)	4.53 (4.45)	3467 b	1688 s	1629 s	1544 s	1143 s
2	off-white	240–250	41.07 (40.19)	3.92 (3.83)	4.46 (4.47)	3450 b	1670 s	1635 s	1550 m	1140 m
3	bright yellow	190–200	35.75 (35.48)	3.65 (3.48)	4.88 (4.87)	3445 b	1705 s	1635 s	1544 m	1143 m
4	deep yellow	150–160	*	*	*	3400 vb	—	1626 s	1544 s 1565 s	—
5	yellow	140–146	*	*	*	3420 b	—	1615 s	1550 s, sh	—

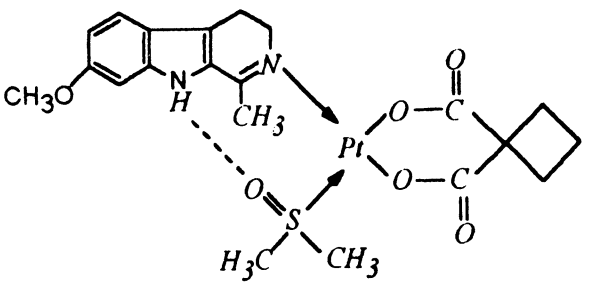
*Data were not recorded. †for IR data: b, broad; vb very broad; m, medium; s, strong and sh, shoulder bands.

TABLE-2
PROTON CHEMICAL SHIFTS (δ ppm)* AND PLATINUM-PROTON COUPLING CONSTANTS (J Hz) FOR PLATINUM(II) COMPLEXES

Complex	Alkaloid (L)					Ligand (L′)				Carboxylate δ(CH_2)
	δ(CH_3)	δ(CH_3O)	δ(CH_2)	δ(NH)	δ(aromatic)	δ(CH_3/DMSO) (3J(^{195}Pt—CH)	δ(CH_3)	δ(CH), δ(NH)	δ(C_6H_{11})	
							3,5-dimethylpyrazole			
1	3.29 s	3.9 s	3.0 m	11.4 s	6.7–7.3 m	3.33 (24.3)	—	—	—	2.1 q (2H) 3.0 t (4H)
2	3.35 s	3.9 s	—	12.1 s	6.9–8.7 m	exchanged with DMSO-d_6	—	—	—	1.8 q (2H) 2.9 t (4H)
3	2.40 s	3.8 s	3.0 m	11.1 s	6.7–7.4 m	3.30 (23.0)	—	—	—	—
4	2.60 s	3.8 s	3.0 m	10.8 s	6.6–7.4 m	—	2.33 s, 2.64 s	5.85 s, 9.6 s	—	—
5	2.60 s	3.8 s	3.1 m	10.9 s	6.6–7.4 m	—	—	—	1.2–2.5 m	—

*Downfield from internal TMS, using $CDCl_3$ as a solvent, except complex 2 in which DMSO-d_6 was used. Abbreviations s, t, q, m are for singlet, triplet, quintet and multiplet signals, respectively.

SCHEME 2
THE SUGGESTED STRUCTURE FOR COMPLEXES 1, 2 AND 3 (BUT WITH OXALATO GROUP), SHOWING THE HYDROGEN BONDING BETWEEN O OF DMSO AND H OF NH GROUP



$CDCl_3$ (*ca.* 8.0 ppm in the free alkaloid and *ca.* 11.5 ppm in its platinum complex). This means that DMSO molecule had been intramolecularly interacted with H of NH group *via.* its oxygen atom by hydrogen bonding (Scheme 2), just like the intermolecular interaction occurring between the free alkaloid and DMSO when the ^1H NMR of the latter was measured in DMSO-d_6 (δ(NH) = 12 ppm)[9].

On the contrary, this phenomenon was not observed in the complex *cis*-[Pt(alkaloid)(DMSO)Cl_2] in which δ(NH) = 8.5 ppm (in $CDCl_3$)[13]. This may be due to the fact that carboxylato group in complexes 1–3 influences the complex to have the *cis*-isomer with smaller N—Pt—S bond angle compared to that when Cl_2 is used instead of carboxylato group, *i.e.*, more freedom for the Cl_2-complex to have larger N—Pt—S bond angle and in turn makes the distance between O atom of DMSO and H of NH larger, and hence weakening the hydrogen bond thereafter.

However, the remaining DMSO in the complexes 1, 2 and 3 could well be displaced by a stronger ligand, *i.e.*, pyrazole or cyclohexylamine to give the final complexes *cis*-[PtLL'Cl_2], such as complexes 4 and 5 (Scheme 1), which can be prepared from *cis*-[Pt(harmaline)(DMSO)Cl_2] and pyrazole or cyclohexylamine, respectively. The total displacement of DMSO was confirmed by the complete disappearance of both the S=O absorption band in the IR spectra and the CH_3 signals of DMSO in the ^1H NMR spectra of the resulting complexes 4 and 5.

The δ(NH) value of the alkaloid in complexes 4 and 5 is fairly smaller (*ca.* 10.8 ppm) than that of complexes 1, 2 and 3 (*ca.* 11.5 ppm) and larger than that of the free alkaloid (*ca.* 8.5 ppm), and this may be due to some intramolecular interaction between the NH group of the alkaloids and the other ligand in the complex.

The purity of some selected complexes, *i.e.*, 1 and 2, was checked by HPLC, using Spheresorb, 5 ODS, 5 microns, 25 × 4.6 cm at a wavelength of 254 nm. Both the solvent and the mobile phase used were methanol, at a flow rate of 1 mL min^{-1}. Both complexes (concentration *ca.* 10 mg%) gave a single line with very close retention times of *ca.* 2.4 min.

Cytotoxicity evaluations

All the new complexes prepared were already purified before testing for cytotoxicity by recrystallization from chloroform/*n*-hexane. Their cytotoxic activities against different cell lines are shown in Table-3. It appears that all complexes showed no cytotoxic activities against all the cell lines used at concentration ≤ 10 μg mL^{-1} with the exception of complex 4. Furthermore, all the complexes including the reference standards showed no activity against L20B cell line. Complex 4 exhibited a moderate cytotoxic activity against Hep-2, HeLa and Vero cells (IC_{50} values were 1.7, 5.5 and 8.5 μg mL^{-1}, respectively). This activity is almost certainly approaching to that of cisplatin against the same cell lines (IC_{50} values were 1.8, 5.5 and 8.0 μg mL^{-1}, respectively). On the other hand, the IC_{50} values of carboplatin against all the cell lines used were > 10 μg mL^{-1}, whereas oxaliplatin showed an IC_{50} values of 8.0 and 9.0 μg mL^{-1} against Hep-2 and HeLa cells respectively and > 10 μg mL^{-1} against the remaining cell lines.

TABLE-3
CYTOTOXIC ACTIVITIES OF PLATINUM(II) COMPLEXES WITH STANDARD REFERENCES AGAINST DIFFERENT TUMOUR CELL LINES

Complex	IC_{50} (μg mL^{-1})					
	Hep-2	HeLa	RD	L20B	BGM	Vero
1	>10	>10	>10	>10	>10	>10
2	>10	>10	>10	>10	>10	>10
3	>10	>10	>10	>10	>10	>10
4	1.7	5.5	8.0	>10	>10	8.5
5	>10	>10	>10	>10	>10	>10
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

Preliminary, it is notable that the complex 4, *cis*-[Pt(harmaline)(pyrazole)Cl_2], which is analogous to cisplatin, *cis*-[Pt(NH_3)Cl_2], is the most promising complex among the rest of complexes (1–3 and 5), which showed almost no activity at the concentration used. The activity of complex 4 may be attributed to the nature of the organic ligand (pyrazole). Yet, further *in vivo* studies are necessary to confirm these activities in the animal models.

REFERENCES

1. B. Rosenberg, L. Van Camp, J.E. Trosko and V.H. Mansour, *Nature (London)*, **222**, 385 (1969).
2. K.R. Harrap, *Cancer Treat. Rev.*, **12A**, 21 (1985).
3. T. Yamashita, J. Hirose, M. Noji, R. Saito, H. Tomida and Y. Kidani, *Biol. Pharm. Bull.*, **16**, 1014 (1993).
4. (a) R.B. Weiss and M.C. Christien, *Drugs*, **46**, 360 (1993).
   (b) L.R. Kelland, *Crit. Rev. Oncol. Hematol.*, **15**, 191 (1993).

5. T.A.K. Al-Allaf and A.Z. M. Sheet, *Polyhedron*, **14**, 239 (1995).
6. T.A.K. Al-Allaf and R.I.H. Al-Bayati, *Asian J. Chem.*, **7**, 465 (1995).
7. S.A. Al-Jibori, Z.M. Kalay and T.A.K. Al-Allaf, *Transition Met. Chem.*, **19**, 293 (1994).
8. T.A.K. Al-Allaf, I.A. Mustafa and S.E. Al-Mukhtar, *Transition Met. Chem.*, **18**, 1 (1993).
9. T.A.K. Al-Allaf, M.T. Ayoub and L.J. Rashan, *J. Inorg. Biochem.*, **38**, 47 (1990).
10. (a) T.A.K. Al-Allaf, P. Castan, R. Turpin, S. Wimmer and G. Bernadinelli, *Transition Met. Chem.*, **17**, 579 (1992).
    (b) T.A.K. Al-Allaf, P. Castan, R. Turpin and S. Wimmer, *C.R. Acad. Sci. Paris*, **314**, 1029 (1992).
11. T.A.K. Al-Allaf, L.J. Rashan and K.D. Sulayman, *Arch. Biotech. (Jordan)*, **2**, 13 (1993).
12. T.A.K. Al-Allaf, M. Ayoub and L.J. Rashan, *Dirasat (Jordan)*, **21B**, 128 (1994).
13. T.A.K. Al-Allaf and L.J. Rashan, Unpublished Observations (1995).
14. P.A. Pipkin, D.J. Wood, V.R. Racaniello and P.D. Minor, *J. Virol. Methods*, **41**, 333 (1993).
15. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).
16. C.M. Tsai, A.F. Gazdar, C. Allegra, R.P. Perng and B.S. Kramer, *Int. J. Cancer*, **46**, 101 (1990).

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