Five-coordinate platinum(II) complexes containing substituted olefins: synthesis and cytostatic activity

Simonetta Bartolucci
Dipartimento di Chimìca Organica e Biologica, Università di Napoli, via Mezzocannone 16, 80134 Naples (Italy)

Patrizia Carpinelli
Istituto di Biochimica delle Proteine ed Enzimologia del C.N.R., via Toiano 6, 80072 Arco Felice (Italy)

Vincenzo De Felice, Bruno Giovannitti
Facoltà di Agraria, Università del Molise, via Tiberio 21/A, 86100 Campobasso (Italy)

and Augusto De Renzi*
Dipartimento di Chimica, Università di Napoli, via Mezzocannone 4, 80134 Naples (Italy)

(Received April 13, 1992)

Abstract

The synthesis and characterization of five-coordinate platinum(II) complexes of general formula [PtClX(N-N)(uns)] (X = Cl, Me; N-N = bidentate nitrogen ligands; uns = substituted alkene containing carboxy or amino groups) are described. In some cases, the hindered rotation of the prochiral unsaturated ligand around the Pt–alkene bond, alone or in conjunction with a ligand environment affording a stereogenic metal centre, gives rise to a mixture of different stereoisomers. Some of the obtained complexes were tested on the neuroblastoma cell line SK-N-MC and a considerable inhibition of cellular proliferation was detected.

Introduction

Five-coordination is now becoming a common feature in the chemistry of platinum(II)–olefin complexes. Actually, since some relevant factors promoting the attainment of a coordinatively saturated configuration for d^8 ions have been elucidated [1], several synthetic procedures have been set up [1, 2] and the number of stable five-coordinate Pt(II)–olefin complexes is quickly increasing. However, the chemistry of these compounds is largely incomplete and even less is known about their biological properties.

On the other hand, several four-coordinate Pt(II) complexes show biological effectiveness against some types of tumours [3] two of them, namely *cis*-diamine-dichloroplatinum(II) (cisplatin) and *cis*-diamine-1,1-cyclobutanedicarboxylateplatinum(II) (carboplatin), are commonly used as anticancer drugs. Most of the four-coordinate Pt(II) complexes which show good antitumour activity [4] have the general formula *cis*-$[PtL_2X_2]$, where L_2 is a bidentate or a bis-monodentate nitrogen ligand bearing at least one hydrogen atom on the Pt(II)-coordinate nitrogens and X_2 is a bidentate or a bis-monodentate anionic ligand with fairly good leaving properties. These features are considered essential to establish a strong interaction with DNA [5]. Several experiments are in fact consistent with the formation of an intra-strand crosslinking in which the N-7 atoms of two adjacent guanines bind the platinum atom. In addition, a model by Lippard and co-workers [6] shows a strong hydrogen bond between the phosphate group and the Pt(II)-coordinate nitrogens of a $Pt(NH_3)_2$ moiety.

On these grounds, as the widest class of five-coordinate Pt(II) complexes has the general formula [PtXY(N-N)(olefin)] in which the two anionic ligands (X, Y = halogen and/or hydrocarbyl group) occupy the axial positions in a tbp geometry and N-N is a bidentate ligand with sp^2-hybridized nitrogens, the requirements reported above for antitumour activity do not appear to be fulfilled. However, a preliminary report [7] on the cytostatic activity of the five-coordinate complex [PtClMe(maleic acid)(6-Mepy-CH=NMe)] indicated a cell growth inhibition comparable to that of cisplatin towards the murine neuroblastoma cells 41A3.

Therefore, it is intended that an exploratory study on the cytostatic activity of five-coordinate Pt(II) com-

*Author to whom correspondence should be addressed.

0020-1693/92/$5.00

plexes be undertaken because of their potential interest. This work reports the synthesis and the cytostatic activity of five-coordinate compounds, of the type reported above, in which the olefin ligand bears carboxy or amino groups as polar substituents to ensure the solubility of the complexes in aqueous media in order to perform biological tests.

Experimental

^{1}H NMR spectra were recorded at 60 and 270 MHz on JEOL PMX and Bruker AC 270 spectrometers, respectively, in $CDCl_3$ or CD_3OD solution. IR spectra were made on a Perkin-Elmer 457 spectrometer. Solvents and reagents were of Analar grade and were used without purification. Ligand **6** is the only N-N ligand commercially available, the others being prepared by published methods [8]. $[PtClMe(Me_2S)_2]$ was synthesized by a known method [9].

*Synthesis of $[PtCl_2(N\text{-}N)(C_2H_4)]$ (**1a–6a**)*

0.386 g (1 mmol) of $K[PtCl_3(C_2H_4)]\cdot H_2O$ was dissolved in 10 ml of methanol and the stoichiometric amount of the appropriate N-N ligand (**2–6**) was added in the cold. After 10 min the formed precipitate was filtered off and recrystallized from a methylene chloride/methanol mixture. The complexes were obtained as yellow crystals in 80–90% yields.

With N-N ligand **1** the reaction was performed at −30 °C and the precipitate was simply washed with water omitting the recrystallization step owing to the extensive olefin release in solution. The complexes **2a**, **4a** [1a] and **6a** [1d] were identified by comparison with literature data.

Relevant ^{1}H NMR parameters: **1a** ($CDCl_3$, −30 °C) δ 8.93 (s, CH=N $J(^{195}PtH)$ 40 Hz), 4.32 (m, $NCHMe_2$), 3.52 (m, C_2H_4, $J(^{195}PtH)$ 72 Hz), 1.68 (d, Me_2); **3a** ($CDCl_3$) δ 8.88 (s, CH=N, $J(^{195}PtH)$ 45 Hz), 4.00 (s, NMe), 3.45 (m, C_2H_4, $J(^{195}PtH)$ 76 Hz), 3.15 (s, Me-py); **5a** ($CDCl_3$) δ 9.01 (s, CH=N, $J(^{195}PtH)$ 38 Hz), 3.51 (m, C_2H_4, $J(^{195}PtH)$ 71 Hz), 3.16 (s, Me-py), 1.56 (s, $NCMe_3$).

*Synthesis of $[PtClMe(N\text{-}N)(C_2H_4)]$ (**2b–6b**)*

0.370 g (1 mmol) of $[PtClMe(Me_2S)_2]$ was dissolved in 10 ml of methylene chloride and a 10% excess of the appropriate N-N ligand (**2–6**) was added. The reaction flask was pressurized with ethylene (3 atm) and allowed to stand at room temperature for 24 h. The mixture was concentrated *in vacuo* and crystallization of the complexes was obtained by adding diethyl ether. Yields of yellow crystalline compounds were in 65–75% range. With N-N ligand **1** only the corresponding [PtClMe(N-N)] complex was isolated.

The complexes **2b**, **3b**, **5b** and **6b** [10] were identified by comparison with literature data.

Relevant ^{1}H NMR parameters: **4b** ($CDCl_3$) δ 8.93 (s, CH=N, $J(^{195}PtH)$ 37 Hz), 4.22 (m, $NCHMe_2$), 3.08 (s, Me-py), 2.94 (d, $=CH_2$, $J(^{195}PtH)$ 82 Hz), 2.19 and 2.08 (two t, $=CH_2$, $J(^{195}PtH)$ 60 Hz), 1.67 and 1.52 (two d, Me_2), −0.05 (s, PtMe, $J(^{195}PtH)$ 71 Hz).

*Synthesis of $[PtCl_2(N\text{-}N)(uns)]$ (**5a$_1$–5a$_3$**)*

0.235 g (0.5 mmol) of **5a** containing ^{14}C–C_2H_4 was dissolved in the minimum amount of methylene chloride. A 10% excess of the appropriate unsaturated ligand dissolved in methanol was added. The solution was allowed to stand at room temperature until a negative test for radiochemical activity from a sample of the mixture was obtained (*c.* 24 h). The mixture was concentrated to a small volume and diethyl ether was added. A yellow microcrystalline solid was obtained in 60–70% yield.

When complexes **1a–4a** were used as starting materials, the above procedure afforded red crystals of the corresponding $[PtCl_2(N\text{-}N)]$ compounds.

*Synthesis of $[PtCl_2(N\text{-}N)(CH_2{=}CHCH_2NH_3]Cl$ (**6a$_4$**)*

0.5 g (0.5 mmol) of **6a** containing ^{14}C–C_2H_4 was dissolved in 10 ml of chloroform and a stoichiometric amount of allylammonium chloride was added. The mixture was allowed to stand at 50 °C overnight. The light yellow microcrystalline precipitate was filtered off (50% yield).

When the same procedure was applied with the unsaturated carboxylic acids, the highly insoluble $[PtCl_2$(2,9-Me_2-1,10-phenanthroline)] complex [1d] was formed.

*Synthesis of $[PtClMe(N\text{-}N)(uns)]$ (**1b$_1$–6b$_1$, 4b$_2$, 4b$_3$, 5b$_2$, 5b$_3$, 6b$_4$, 6b$_5$**)*

Method A. N-N = **2–5**. 0.5 mmol of the corresponding $[PtClMe(N\text{-}N)(C_2H_4)]$ complex was dissolved in a 1:2 mixture of acetone/methanol A 10% excess of the unsaturated ligand was added and the solution was allowed to stand at room temperature for 24 h. The formed yellow microcrystalline precipitate was filtered off and washed with methanol (65–85% yield).

Method B. N-N = **1–4**. 0.5 mmol of the corresponding [PtClMe(N-N)] complex was dissolved in 5 ml of methylene chloride. A 10% excess of the unsaturated ligand dissolved in methanol was added and the solution was allowed to stand at room temperature for 24 h. The products were crystallized by adding diethyl ether (65–75% yield).

Method C. N-N = **5**, **6**. 0.37 g (1 mmol) of $[PtClMe(Me_2S)_2]$ was dissolved in 10 ml of methylene chloride. An equimolar amount of the N-N and a 10% excess of the unsaturated ligand, dissolved in methanol,

were added at once and the solution was allowed to stand at room temperature for 4 h. The products were crystallized by adding diethyl ether (65–85% yield).

Synthesis of ***6b***$_1$ *as the α-methylbenzylammonium salt*

0.116 g (1 mmol) of maleic acid was dissolved in 5 ml of THF and an equimolar amount of α-methylbenzylamine was added at 0 °C. The solution was concentrated *in vacuo* affording a white solid. 0.118 g (0.5 mmol) of this solid was dissolved in 5 ml of chloroform and added dropwise at 0 °C to a solution of 0.227 g (0.5 mmol) of [PtClMe(2,9-Me_2-1,10-phenanthroline)] in 15 ml of the same solvent. A white crystalline solid (0.303 g, 88% yield) was obtained after storing in the refrigerator. *Anal.* Found: C, 46.8; H, 4.3; N, 6.2. Calc. for $C_{27}H_{30}ClN_3O_4Pt$: C, 46.93; H, 4.38; N, 6.08%. ^{1}H NMR parameters (CD_3OD): 8.53 (d, 2 H), 7.98 (s, 2 H), 7.92 (d, 2 H), 7.4 (m, Ph), 4.45 (q, *CH*Me), 4.37 (s, CH=CH, $J(^{195}PtH)$ 84 Hz), 3.39 (s, Me_2), 1.65 (d, CH*Me*), 0.32 δ (s, PtMe, $J(^{195}PtH)$ 71 Hz).

Biological tests

Materials and methods

The human neuroblastoma cell line SK-N-MC was originally characterized by Biedler *et al.* [11]. Cells were grown in an incubator at 37 °C under a humidified atmosphere containing 5% CO_2 and 95% air, and maintained in minimal essential medium (MEM) with non-essential amino acids supplemented with 10% fetal calf serum, $NaHCO_3$ (2.2 g/l), penicillin (50 U/ml), streptomycin (50 μg/ml) and sodium pyruvate (110 mg/l).

Growth inhibition studies

Approximately 1×10^5 cells were seeded into 60 mm tissue culture dishes and treated 24 h later with the compounds indicated (final concentrations ranging between 0.5–5 μM). The drugs were dissolved in a 10 mM pH 7 K phosphate buffer, filtered with Millipore filters and stored at −20 °C at the stock concentration (1 mM). 48 h after treatment the cells were counted with a Burker hemocytometer.

Cytotoxicity assay

The cells (1×10^3) after a 24 h drug exposure were assayed for colony-forming ability by growth in a drug-free medium for 10 days. The colonies, fixed and stained with 50% methanol and methylene blue (5 g/l) for 15 min, were counted with an illuminating lamp.

Results and discussion

The synthesis of five-coordinate [PtClX(N-N)(uns)] compounds with X = Cl or Me was attempted for uns = acrylic (AA), maleic (MA) and fumaric acid (FA), allylammonium (Al) and trimethylallylammonium (MAl) chloride. The N-N ligands used are shown in Fig. 1 with the labelling scheme of the complexes.

Two procedures were adopted for the preparation of the compounds:

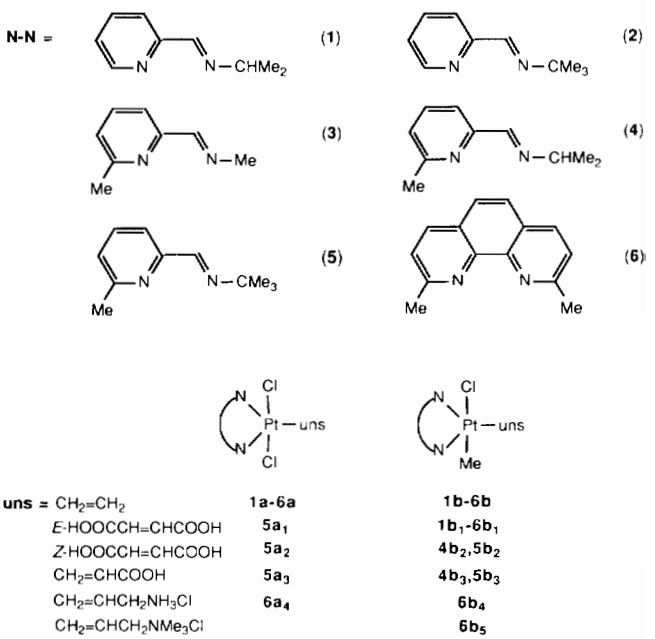
$$[PtClX(N\text{-}N)(C_2H_4)] + uns = [PtClX(N\text{-}N)(uns)] + C_2H_4 \quad (1)$$

$$[PtClX(N\text{-}N)] + uns = [PtClX(N\text{-}N)(uns)] \quad (2)$$

While method (1) is of almost general use, procedure (2) can be satisfactorily used when X = Me. The direct interaction of a simple olefin with $[PtCl_2(N\text{-}N)]$ species, followed by the isolation of a five-coordinate complex, was only observed [1d] when the whole process is favoured by steric factors concerning the N-N ligand (i.e. the presence of substituents on the carbons adjacent to the coordinated nitrogens and a skeletal rigidity, as in **6**). The failure of this procedure in the case of electron-deficient olefins could also be attributed to electronic reasons, as the metal centre in the dichloro species is harder than in [PtClMe(N-N)] complexes.

Dichloro derivatives

An attempt was made to react all the six five-coordinate ethylene complexes **1a–6a**, prepared by treat-



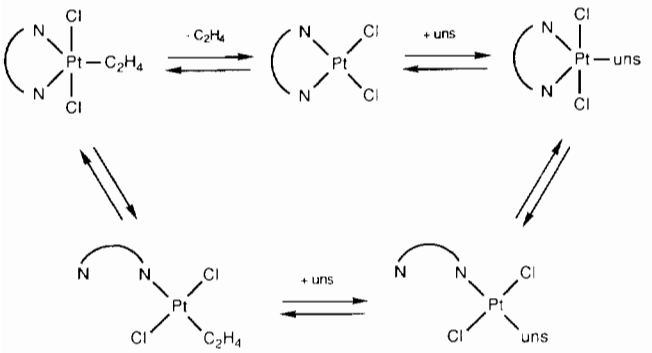
uns =	1a-6a	1b-6b
CH_2=CH_2		
E-HOOCCH=CHCOOH	$5a_1$	$1b_1$-$6b_1$
Z-HOOCCH=CHCOOH	$5a_2$	$4b_2$,$5b_2$
CH_2=CHCOOH	$5a_3$	$4b_3$,$5b_3$
CH_2=$CHCH_2NH_3Cl$	$6a_4$	$6b_4$
CH_2=$CHCH_2NMe_3Cl$		$6b_5$

Fig. 1. [PtClX(N-N)(uns)] complexes and N-N ligands with the notations used in the text.

ing $K[PtCl_3(C_2H_4)]$-^{14}C–C_2H_4 with the appropriate N-N ligand according to a known general procedure [12], with the unsaturated ligands. Only in the case of complex **5a** did the exchange occur, affording the expected compounds. Precipitation of the square-planar species $[PtCl_2(N\text{-}N)]$ was otherwise observed.

Two different mechanisms could, in principle, be responsible for the olefin exchange, both requiring the formation of a four-coordinate intermediate (Scheme 1). However, as described above, the first process is ineffective in the case of the dichloro complexes. Therefore, the achievement of an olefin exchange is related to the 'stability' of the starting five-coordinate compound towards the ethylene release and to the tendency of the N-N ligand to act as a monodentate. This intermediate has already been proposed for olefin exchange in the case of five-coordinate Pt(II)–olefin complexes containing α-diimines [13]. Complexes **1a–4a** show a moderate stability in solution and an irreversible ethylene dissociation occurs. The skeletal rigidity of the N-N ligand **6** prevents the olefin exchange with stable **6a**: a very slow ethylene release is generally observed in boiling chloroform. However, the exchange with allylammonium chloride gave rise to a moderate yield of the expected $\mathbf{6a}_4$ compound. Only complex **5a** offers the suitable reactivity window to allow the exchange to be successful in all cases.

The characterization data of the obtained compounds are listed in Table 1. With reference to the coordination geometry, it has been widely reported [1] that ^1H NMR spectroscopy is the diagnostic tool for its assignement in Pt(II)–olefin complexes, since an upfield shift of 1.5–2 ppm is observed for the resonances of the olefinic protons in a trigonal-bipyramidal arrangement with respect to the more usual square-planar geometry. In this case such a comparison is not feasible, as scarce examples of four-coordinate Pt(II) complexes containing an electron-deficient olefin are known [14]. On the other hand, the general characterization data (mol. wt. determinations, IR and ^1H NMR spectra) strongly support a tpb geometry for complexes $\mathbf{5a}_1$–$\mathbf{5a}_3$ and $\mathbf{6a}_4$, as confirmed by X-ray structural determinations on a similar compound [15].



Scheme 1.

The ^1H NMR spectrum of $\mathbf{5a}_3$ (uns = acrylic acid) shows the presence of two stereoisomers (actually two enantiomeric pairs) in an approximate 3:2 ratio, as a consequence of a hindered rotation around the Pt–alkene bond (Fig. 2). In the case of $\mathbf{5a}_1$ (uns = maleic acid) the two rotamers are in an enantiomeric relationship, but the hindered rotation is evidenced by the resonance pattern of the olefinic protons that give rise to an AB quartet. A single enantiomeric pair is expected for complex $\mathbf{5a}_2$, containing the prochiral FA. A broad =CH proton resonance agrees with the hindered rotation of the unsaturated ligand.

Chloromethyl derivatives

The synthetic procedure based on the olefin exchange starting from the corresponding ethylene derivatives (eqn. (1)) can be satisfactorily applied in the case of complexes **2b–5b**. The direct interaction of the unsaturated ligand with the [PtClMe(N-N)] compounds (eqn. (2)) also works well for the N-N ligands **1–4**. In the case of N-N ligands **5** and **6** the best procedure involves a one-step addition of N-N and of the alkene to the $[PtClMe(Me_2S)_2]$ complex.

The characterization data of the obtained compounds are listed in Table 1. The tpb geometry is attributed to these complexes following the same general observations reported above. By comparing the ^1H NMR data, an upfield shift of the olefinic proton resonances was observed with respect to the dichloro derivatives that can be attributed to a higher metal-to-olefin π-backdonation. In addition, referring to the free olefin, it was noted that the upfield shift is markedly higher for the protons on the unsubstituted unsaturated carbon. A possible explanation is related to the polarization of the π-electronic density on the double bond [16] thus favouring an increased π-backdonation towards the unsubstituted carbon.

The MA containing complexes ($\mathbf{1b}_1$–$\mathbf{5b}_1$) are generally obtained as a mixture of two stereoisomers (actually two enantiomeric pairs), due to the hindered rotation of the unsaturated ligand and the stereogenic properties of the metal center (Fig. 3). The ratio goes from 3:2 to 3:1 and no variations are observed with time. On the contrary, a single (or a largely predominant) rotamer is obtained with symmetric ligand **6**. It is worth noting that the same effect was found in Pd(II) five-coordinate complexes containing olefins with electron-withdrawing substituents such as maleic anhydride or acroleyn [17]. Two diastereoisomers (an enantiomeric pair each) are obtained with the prochiral symmetrically *E*-disubstituted FA (complexes $\mathbf{4b}_2$ and $\mathbf{5b}_2$), while four enantiomeric pairs are possible with the monosubstituted prochiral AA (complexes $\mathbf{4b}_3$ and $\mathbf{5b}_3$). In these latter

TABLE 1. Relevant ^{1}H NMR data [δ (ppm), J (Hz)] for five-coordinate [PtClX(N-N)(uns)] complexes[a]

Complex	Pt–Me	N=CH	NCH_xMe_{3-x}	Me(H)–C(Het.)	uns
1b$_1$ [b,c]	0.37(68, s)	9.33(52, s)	1.50(d)	[d]	4.30(80, ABq, 2H)
2b$_1$	0.37(69, s) 0.50(66, s)	9.28(54, s) 9.23(54, s)	1.60(s) 1.60(s)	9.12(d) [d]	4.31(78, ABq, 2H) 3.45(75, ABq, 2H)
3b$_1$ [e]	0.36(68, s) 0.24(68, s)	9.36(56, s) 9.40(56, s)	4.05(s) 4.02(s)	3.02(s) 3.07(s)	3.45(80, d, 1H), 3.22(70, d, 1H) 4.28(80, ABq, 2H)
4b$_1$ [c]	0.44(72, s)	9.37(60, s)	1.56(d), 1.44(d)	3.03(s)	4.25(84, ABq, 2H)
5b$_1$	0.48(70, s) 0.49(70, s)	9.32(54, s) 9.30(54, s)	1.58(s) 1.60(s)	3.04(s) 3.00(s)	4.23(81, ABq, 2H) 3.38(81, ABq, 2H)
6b$_1$ [c,f]	0.22(70, s)			3.25(s)	4.08(89, s, 2H)
5a$_1$		9.31(49, s)	1.68(s)	3.19(s)	4.68(78, ABq, 2H)
4b$_2$ [c]	0.41(70, s)	9.32(50, s)	1.5(m)	3.09(s)	4.46(82, d, 1H), 3.94(71, d, 1H)
5b$_2$	0.41(68, s) 0.57(68, s)	9.28(49, s) 9.28(49, s)	1.62(s) 1.62(s)	3.15(s) 3.04(s)	4.51(87, d, 1H), 3.83(70, d, 1H) 4.49(87, d, 1H), 3.69(70, d, 1H)
5a$_2$		9.24(54, s)	1.68(s)	[d]	4.38(76, bs, 2H)
4b$_3$ [g]	0.19(72, s), 0.23(72, s)	9.23(42, s), 9.33(42, s)	1.66(d), 1.56(d), 1.47(d), 1.45(d)	3.11(s), 3.04(s)	4.15(m, two superimp. H), 2.91(dd, 1H), 2.78(dd, 1H), 2.69(dd, 1H), 2.58(dd, 1H)
5b$_3$ [g]	0.20(70, s), 0.27(70, s)	9.20(41, s), 9.31(50, s)	1.63(s)	3.12(s), 3.00(s)	4.1(m, two superimp. H), 2.8(m, two superimp. H), 2.65(m, two superimp. H)
5a$_3$		9.14(40, s) 9.27(48, s)	1.65(s)	3.10(s)	4.6(m, 1H), 3.8(m, 1H), 2.7(m, 1H) [d]
6b$_4$	0.19(70, s)			3.32(s), 3.30(s)	3.01(70, d, 1H), 2.65(m, 1H), 2.44(68, d, 1H)
6b$_5$ [c]	0.30(72, s)			3.33(s), 3.30(s)	4.05(m, 1H), 3.85(m, 1H), 2.55(m, 1H)
6a$_4$				3.46(s), 3.41	4.1(m, 1H), 3.65(d, 1H), 3.50(d, 1H)

[a]Spectra recorded in CD_3OD (99.95% isotopic purity, CD_2H 3.31 δ) solutions. Abbreviations: s=singlet; d=doublet; q=quartet; m=multiplet; bs=broad singlet. The chemical shifts of the heteroaromatic protons of the N-N ligands are in the range 8.7–7.5 ppm. [b]The complex appreciably dissociates in solution affording the corresponding four-coordinate [PtClMe(N-N)] species. [c]A second stereoisomer is present in less than 20% abundance. [d]Obscured by other signals. [e]Spectrum recorded in CD_3COCD_3 solution, see ref. 7. [f]Spectrum recorded in DMSO-d_6 solution. [g]The reported chemical shifts refer to the two stereoisomers in 1:1 ratio and a precise assignement is not feasible. The other two stereoisomers are in less than 10% abundance. The ^{195}Pt coupling of the unsaturated protons is not valuable.

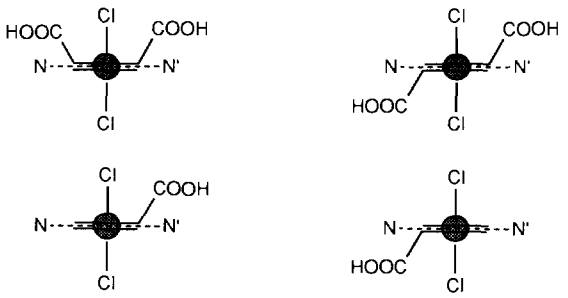


Fig. 2. Schematic view of [$PtCl_2$(N-N')(uns)] complexes along the equatorial plane (N-N' = chelating ligand with chemically non-equivalent nitrogens).

cases, however, two stereoisomers largely predominate, the other two being less than 10% of the product.

On attempting a configurational assignment, it must be noted that the more relevant difference in the ^{1}H NMR parameters of the two stereoisomers of the MA containing complexes (Table 1: **2b$_1$**, **3b$_1$**, **5b$_1$**) concerns the chemical shift of the olefin protons. A *c.* 0.8 ppm difference is actually observed (4.2 versus 3.4 δ). As the two stereoisomers are rotamers around the Pt–MA bond, this feature could be related to the facing of the =CH protons to a different axial ligand (Cl or Me). The observation that the two olefin protons of the coordinated FA (Table 1: **4b$_2$** and **5b$_2$**) show two distinct resonances at *c.* 4.5 and *c.* 3.8 δ agrees with this hypothesis. NOE experiments performed on similar compounds [18] indicate that the signal at higher δ must be attributed to the olefin proton facing the axial halogen. On this basis, the two more abundant stereoisomers in complexes **4b$_3$** and **5b$_3$** (=CH– resonance at 4.1 δ) should contain the carboxy group of the coordinated AA turned away from the chlorine. No other speculations can be made at present, concerning the side orientation of this substituent with respect to the unsymmetric N-N ligand.

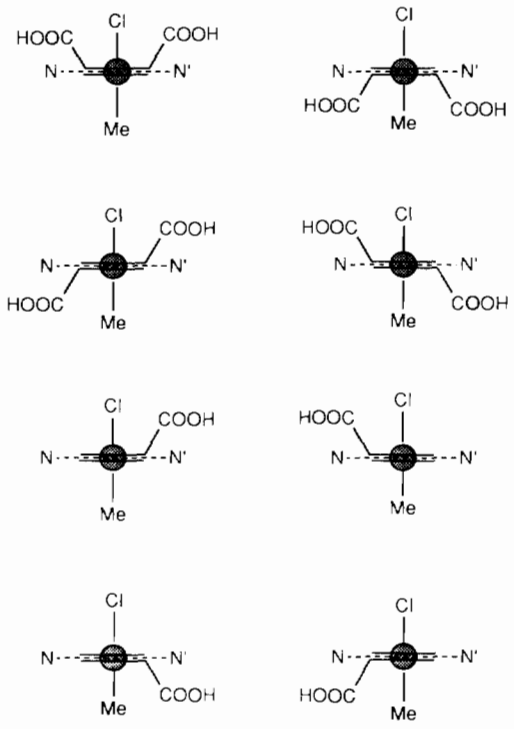


Fig. 3. Schematic view of [PtClMe(N-N')(uns)] complexes along the equatorial plane (N-N' = chelating ligand with chemically non equivalent nitrogens).

When allylammonium and trimethylallylammonium chloride are used as unsaturated ligands, the two corresponding **6b**$_4$ and **6b**$_5$ complexes are isolated. Two rotamers are obtained in the latter case in an approximate 3:1 ratio, while a single one is observed for **6b**$_4$. A possible explanation could be related to the formation of an intramolecular hydrogen bond N–H....Cl [19] that stabilizes the corresponding stereoisomer.

All the obtained complexes, except the **5** and **6** N-N ligands derivatives, slowly release the olefin in chloroform solution. Although no quantitative rate determinations have been made, the stability order is related to the steric features of the N-N ligand, as observed [1c] for analogous ethylene complexes.

Biological tests

The objective of this study was to determine whether these newly synthesized compounds could induce loss of proliferative potential. The different compounds were tested in an *in vitro* system consisting of human neuroblastoma cells. Neuroblastoma is a common childhood solid tumour of the autonomic nervous system with a poor prognosis in the presence of disseminated disease or loss of cellular differentiation. The inhibitory effect on the cellular proliferation was compared with that caused by cisplatin, a known cancer chemotherapy agent, as previously reported [7] for a murine line with the compound **3b**$_1$.

The tested complexes are N-N ligand **6** derivatives (**6b**$_1$, **6a**$_4$ and **6b**$_4$), chosen in order to face with a single (or largely predominant) stereoisomer and to minimize the effect of the olefin release with loss of the chemical identity of the compound. Complex **6b**$_1$ was used as the monoammonium salt to increase its solubility. Complex **3b**$_1$, for which data are reported towards murine neuroblastoma cells, and its dissociation product [PtClMe(6-Me-py-2-CH=NMe)] were also tested.

Figure 4 shows the effect of the newly synthesized compounds on cellular proliferation compared with that of cisplatin. The cells were treated for 24 h with concentrations ranging between 0.5 and 5 μM. The maximal effects on the inhibition of cellular proliferation were observed in the presence of compounds **6a**$_4$ and **6b**$_4$ with an *ID*50 of about 2.2 and 1.9 μM, respectively. The inhibitory effect for these compounds was dose-dependent in this range unlike the other three compounds tested. Cisplatin also produced inhibition at a lower concentration. The decrease in cell proliferation rate was more evident than that observed in the murine line.

A further comparison of the cytotoxic effect of the five cisplatin analogs was achieved by determining the clonogenic ability of the cells treated with the drugs. The human neuroblastoma cell line was exposed to the same concentrations of these compounds for a longer culture time and the cell survival determined. It does not require continuous exposure to the drugs as found

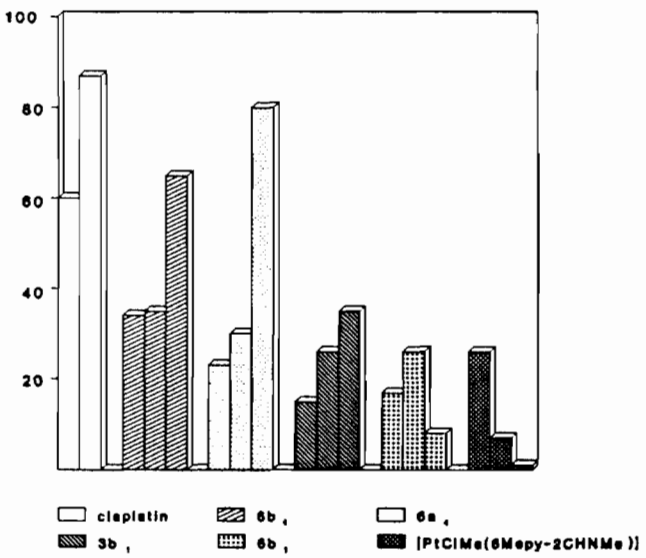


Fig. 4. Effect of cisplatin and analogs on cellular growth of SK-N-MC human neuroblastoma cells. Cellular inhibition is reported as percent of control. For cisplatin the effect is shown after treatment for 48 h at concentrations of 0.1 and 0.5 μM. For the other compounds the effect is shown at concentrations of 0.1, 1 and 5 μM.

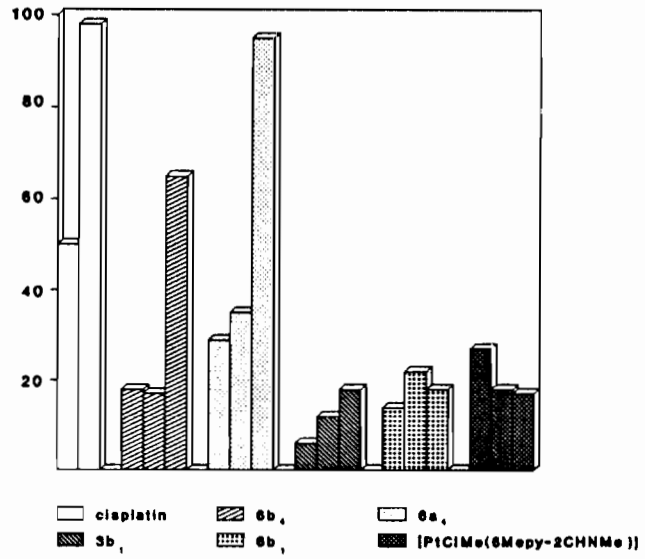


Fig. 5. Effect of cisplatin and analogs on colony formation by SK-N-MC human neuroblastoma cells. Cell kill is reported as percent of control. Concentrations used for the different compounds are the same as in Fig. 4.

for other antiproliferative agents. Figure 5 shows qualitatively similar results when clonogenic assays were used in the assessment of the inhibitory effect, indicating that the drugs could induce a neuronal differentiation as demonstrated by Parodi *et al.* [20] with cisplatin. Although the mechanism of cytotoxicity for these compounds is unknown, the different response of the cells to the drugs seems to depend on their structure; in fact in this assay it was also worth noting that the cationic five-coordinate $\mathbf{6a}_4$ and $\mathbf{6b}_4$ are the most active compounds. Furthermore, the dose-dependent inhibition for these compounds suggested an interference with some metabolic pathways, unlike the unspecific cytotoxicity caused by other compounds.

Thus these results might suggest a chemotherapeutic potential for these drugs which produce a considerable inhibition of cellular proliferation at concentrations comparable to those used for cisplatin.

Acknowledgements

The authors thank the Consiglio Nazionale delle Ricerche and the Ministero dell'Università e della Ricerca Scientifica e Tecnologica for financial support, and the Centro Interdipartimentale di Metodologie Chimico-Fisiche for the use of the Bruker AC-270 NMR spectrometer.

References

1 (a) H. van der Poel and G. van Koten, *Inorg. Chem., 20* (1981) 2950; (b) V. G. Albano, F. Demartin, A. De Renzi, G. Morelli and A. Saporito, *Inorg. Chem., 24* (1985) 2032; (c) M. E. Cucciolito, V. De Felice, A. Panunzi and A. Vitagliano, *Organometallics, 8* (1989) 1180; (d) F. P. Fanizzi, F. P. Intini, L. Maresca, G. Natile, M. Lanfranchi and A. Tiripicchio, *J. Chem. Soc., Dalton Trans.,* (1991) 1007, and refs. therein.
2 (a) V. De Felice, M. Funicello, A. Panunzi and F. Ruffo, *J. Organomet. Chem., 403* (1991) 243; (b) V. G. Albano, C. Castellari, V. De Felice, A. Panunzi and F. Ruffo, *J. Organomet. Chem., 425* (1992) 177.
3 M. P. Hacker, E. B. Douple and I. H. Krakoff (eds.), *Platinum Coordination Compounds in Cancer Chemotherapy,* Martinus Nijhoff, Boston, MA, 1984.
4 A. Pasini and F. Zunino, *Angew. Chem., Int. Ed. Engl., 26* (1987) 615.
5 J. Reedijk, *Pure Appl. Chem., 59* (1987) 181.
6 J. Kozelka, G. A. Petsko, G. J. Quigley and S. J. Lippard, *Inorg. Chem., 25* (1986) 1075.
7 S. Bartolucci, M. Rossi, M. Estenoz, A. Panunzi and A. Vitagliano, *Inorg. Chim. Acta, 137* (1987) 53.
8 H. van der Poel, G. van Koten, M. Kokkes and C. H. Stam, *Inorg. Chem., 20* (1981) 2941.
9 J. D. Scott and R. J. Puddephatt, *Organometallics, 2* (1983) 1643.
10 V. G. Albano, D. Braga, V. De Felice, A. Panunzi and A. Vitagliano, *Organometallics, 6* (1987) 517.
11 J. L. Biedler, L. Helson and B. A. Spengler, *Cancer Res., 33* (1973) 2643.
12 L. Maresca, G. Natile and L. Cattalini, *Inorg. Chim. Acta, 14* (1975) 79.
13 H. van der Poel, G. van Koten and G. C. van Stein, *J. Chem. Soc., Dalton Trans.,* (1981) 2164.
14 M. A. M. Meester, D. J. Stufkens and K. Vrieze, *Inorg. Chim. Acta, 21* (1977) 251.
15 V. G. Albano, F. Demartin, A. De Renzi and G. Morelli, *Inorg. Chim. Acta, 149* (1988) 253.
16 D. J. Cooper and J. Powell, *Inorg. Chem., 15* (1976) 1959.
17 V. G. Albano, C. Castellari, M. E. Cucciolito, A. Panunzi and A. Vitagliano, *Organometallics, 9* (1990) 1269.
18 P. S. Prcgosin and A. Vitagliano, personal communication.
19 (a) C. Pedone and E. Benedetti, *J. Organomet. Chem., 31* (1971) 403; (b) A. de Renzi, B. Di Blasio, A. Panunzi, C. Pedone and A. Vitagliano, *J. Chem. Soc., Dalton Trans.,* (1978) 1392.
20 M. T. Parodi, L. Varesio and G. P. Tonini, *Cancer Chemother. Pharmacol., 25* (1989) 114.