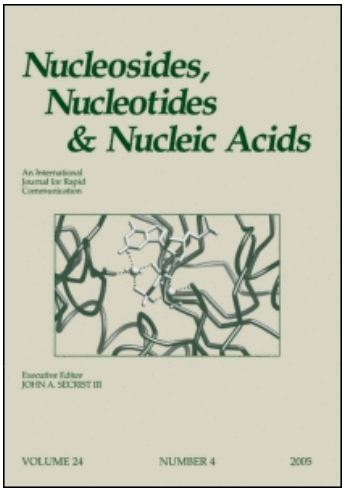

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Molecular Structure and Cytotoxicity of 3D-Transition Metal Complexes Capable to Form a Stable Metal-Nitrogen Bond

A. Marzotto[a]; D. A. Clemente[b]; A. Zampiron[c]; M. Carrara[c]
[a] Department of Inorganic, Metallorganic and Analytical Chemistry, University of Padova, Padova, Italy [b] Department of Materials Engineering and Applied Chemistry, University of Trieste, Trieste, Italy [c] Department of Pharmacology and Anesthesiology, University of Padova, Padova, Italy

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MOLECULAR STRUCTURE AND CYTOTOXICITY OF *3D*-TRANSITION METAL COMPLEXES CAPABLE TO FORM A STABLE METAL-NITROGEN BOND

A. Marzotto [*a], D.A. Clemente [b], A. Zampiron [c] and M. Carrara [c]

[a] *Department of Inorganic, Metallorganic and Analytical Chemistry, University of Padova, Via Loredan 4, 35100 Padova (Italy)*

[b] *Department of Materials Engineering and Applied Chemistry, University of Trieste, Via A. Valerio 2, 34127 Trieste (Italy)*

[c] *Department of Pharmacology and Anesthesiology, University of Padova, Largo E. Meneghetti 2, 35131 Padova (Italy)*

ABSTRACT The cytotoxicity of several Co(II), Ni(II), Cu(II) and Zn(II) complexes with various molecular structures and geometries, has been tested on LoVo and 2008 cells at 1-100 μM concentration for 24 h exposure. On the basis of 24 h results, the exposure time was prolonged to 48 and to 72 hours.The most potent complexes result $[Cu(tren)(H_2O)]^{2+}$ $2Cl^-$, **E**, $[CoCl_3(H_2Meppz)]$, **G**, and $[CoCl_3(HMe_2ppz)]$, **H**, (tren=tris(2-aminoethyl)amine, H_2Meppz=1-methylpiperazin-1-ium, HMe_2ppz=1,4-dimethylpiperazin-1-ium cations). Nevertheless, these complexes are able to induce cell growth reduction of about 50% at highest doses tested (1-100 μM) and after 72 h exposure.

INTRODUCTION

The discovery of the antitumour activity of cisplatin (*cis*-[diamminedichloro-platinum(II)], *cis*-DDP, *cis*-$[Pt(NH_3)_2Cl_2]$) by Rosenberg [1] triggered new developments in the synthesis of novel platinum complexes in an attempt to improve antitumor activity,

* To whom correspondence should be addressed (e-mail: marzotto@chim02.chin.unipd.it).

to reduce toxicity or to overcome resistance. Up to now, the only platinum analogue introduced to date in clinical practice is carboplatin, {diammine(cyclobutane-1,1-dicarboxylato(2-)-O,O')platinum(II), [$Pt(NH_3)_2$(CBDCA)], CBDCA=cyclobutane-1,1-dicarboxylato)}, which is a second choice drug for the treatment of ovarian cancer, because its activity is comparable to that of *cis*-DDP, but some of its toxicologic features, especially myelosuppression, are dose limiting [2].

Among a number of molecules currently in clinical development, of particular interest are: (ammine bis(acetato)dichlorocyclohexylamine)platinum(IV), JM216, the first orally bioavailable platinum complex [3] and the novel trinuclear platinum complex, BBR 3464, with *trans* configuration [4].

It is generally accepted that cytotoxic effects of *cis*-DDP are due to the formation of DNA adducts capable of blocking replication and/or transcription. *cis*-DDP forms several types of lesions in DNA [5,6], mainly including intrastrand and interstrand cross-links. The respective role of these lesions in the antitumour activity of the drug is not yet established [7]. Several studies have suggested that *cis*-DDP interstrand cross-links (ICL), which represent a small portion (5-10%) of the total lesions, could be responsible for the cytotoxicity of the drug. ICL are formed between *cis*-DDP and the **N(7)** atoms of two guanine residues.

Because the central mechanism of cytotoxicity activity is the formation of a Pt(II)-N(aromatic) bond and because other metal ions are able to form a similar metal-nitrogen bond we have examined, as antitumor agents, complexes containing metal ions essential for life as Co(II), Co(III), Ni(II), Cu(II) and Zn(II). However one must not forget that the Pt(II)-N bond is stronger than the other M(II)-nitrogen bonds, in fact Mortimer *et al.* [8] found that the bond dissociation energy $\overline{D}$(Pt-N) between Pt(II) and the **N(7)** atom of 9-methyladenine has an upper limit of 217 kJ mol^{-1} while Dunstan [9] found that the bond dissociation energy $\overline{D}$(Ni-N), for the bond between Ni(II) acetylacetonate and the nitrogen atom of pyridine or various methyl-pyridines, ranges from 94 to 108.6 kJ mol^{-1}. In addition, Dunstan [10] showed that Ni-N bonds are slightly stronger ($\Delta\overline{D}$= 1-6 kJ mol^{-1}) than the corresponding Co-N bonds in the analogous adducts of Co(II) acetylacetonate formed with the same heterocyclic pyridines bases.

Furthermore, taking into account not only the thermodynamic aspect but also the kinetic side, it must be remembered that, during a nucleophylic substitution reaction of

associative mechanism, the square-planar Pt(II) complexes possess a relative reactivity that is much more lower (near 10^{-5}-10^{-6} times) than that of analogous metal(II) complexes, as for example Ni(II) or Pd(II) [11]. This means that cisplatin, when is bonded to DNA-bases, is much more inert than the analogous metal complexes. In line with this, every time the metal-nitrogen bond is weakened, complexes with lower antitumoral activity are obtained, for example the square-planar Pt(II) zwitterionic complexes *cis*-[Pt(HN^+~N)(L)Cl_2] (HN^+~ N=protonated diamines, L=SCN^-, NO_2^-, Cl^-, Br^-, F^-) show a reduction in the antitumor activity (against murine leukemic cells L1210) when the ligand (L) possesses a higher *trans*-effect (more labilizing: SCN^-, NO_2^-, Br^-) [12].

These considerations prompted us to synthesize and to characterize by physico-chemical methods, including X-ray diffraction, several novel transition metal complexes having different structural and geometrical features in order to ascertain: **(i)** the mechanism governing the molecular recognition of DNA nitrogen binding sites by metal complexes, **(ii)** the electronic and steric factors favouring the formation of a strong metal-nitrogen bond with DNA nucleobases. The aim is to identify metal complexes able to form selective and stable metal-nitrogen bonds such to inhibit the tumor cells growth.

First of all, the cytotoxicity of the new complexes has been evaluated on human colon carcinoma cell lines LoVo and on human ovarian carcinoma cell lines 2008 after 24 hours treatment. On the basis of these results, we decided to prolong the exposure time (48 h and 72 h) to the six complexes found to possess on LoVo cells an interesting activity: [Co(hp)$(H_2O)_2$], **A**, [Ni($C_{22}H_{22}N_4$)], **B**, [Cu(tren)$(H_2O)]^{2+}$ $2Cl^-$, **E**, [ZnCl(tren)]$^+$ $Cl^-.3H_2O$, **F**, [$CoCl_3$(H_2Meppz)], **G**, [$CoCl_3$(HMe_2ppz)], **H**, (tren=tris(2-aminoethyl)-amine, H_2Meppz=1-methylpiperazin-1-ium, HMe_2ppz=1,4-dimethypiperazin-1-ium cations). The obtained results indicate as the most interesting tested complexes [Cu(tren)$(H_2O)]^{2+}2Cl^-$, **E**, [$CoCl_3$(H_2Meppz)], **G**, and [$CoCl_3$(HMe_2ppz)], **H**.

RESULTS AND DISCUSSION

The complexes synthesized and tested by us can be classified into four distinct groups with regard to their molecular structure and geometry:

1) Square-planar Co(II), Ni(II) and Pt(II) complexes: $[Co(hp)(H_2O)_2]$ (H_2hp=hemiporphyrazine) [13,14] $[Ni(C_{22}H_{22}N_4)]$ [15] ($C_{22}H_{24}N_4$=6,8,15,17-tetramethyldibenzo-5,9,14,18-tetraazacyclotetradecene), *trans*-$[Pt(HMeppz)_2]^{2+}$ $2Cl^-.4H_2O$[16], *trans*-$[PtCl_2(HMeppz)_2]$[17], $[PtCl_3(H_2Meppz)]$[18], *trans*-$[PtCl_2(H_2Meppz)_2]^{2+}$ $2Cl^-.2H_2O$ [18], *cis*-$[PtCl_2(Me_2ppz)]$ [18] (that resembles cisplatin) and *cis*-$[PtI_2(HMeppz)]$[19], HMeppz=1-methylpiperazine, Me_2ppz= 1,4-dimethylpiperazine.

The cytotoxic activity was evaluated for two complexes of this group: $[Co(hp)(H_2O)_2]$, **A**, and $[Ni(C_{22}H_{22}N_4)]$, **B**, whereas some Pt(II) complexes have already been tested (see ref.18 and references therein). Compounds **A** and **B** exhibit an interesting activity after 24 hours exposure on both cell lines after 24 hours (**TABLE 1**). In particular on 2008 cells, complex **A** is able to induce a significant cell growth inhibition (about 20%) also at the lowest concentration tested, 1 μM (**TABLE 1**). On LoVo cells, complex **A** exhibits after 24 hours an interesting cytotoxiticity only at 10 and 100 μM. Prolonging the exposure time, this complex loses activity after 48 and 72 hours. Analogously, the compound **B** shows a cytotoxic effect similar to that of **A** (**FIG. 1**).

Since the octahedral $[Co(hp)(H_2O)_2]$ (**FIG. 2**) can easily lose the two axial water molecules [14] becoming a near planar molecule and $[Ni(C_{22}H_{22}N_4)]$ (**FIG. 3**) complex is already planar, one could hypothesize a mechanism of action in which the Co(II) and Ni(II) complexes form with DNA nucleobases an axial metal-nitrogen bond similar to that found for $[Co(III)hp(2\text{-})bis(1\text{-methylimidazole})]^+ I_3^-$ [20]. However, this mechanism must be ruled out by the severe steric hindrance between the adenine amino group or the guanine ketonic oxygen and the extended molecular plane of the complexes. The most probable mechanism of action is the formation of an intercalation compound favoured when the planar part of the molecule possesses a surface area larger than 28 $Å^2$ [21]. In fact, the surface area of [Co(hp)], calculated considering the circumstance that this complex is isomorphous with Ni(hp) [22,23], is near 175 $Å^2$ while that of $[Ni(C_{22}H_{22}N_4)]$ is near 100 $Å^2$. Moreover, hemiphorphyrazine metal complexes resemble the porphyrin

TABLE 1. Cytotoxic effect on LoVo and 2008 cells after 24 h exposure
*p<0.05; ** p<0.01.

COMPOUNDS	DOSES μM	LoVo cells	2008 cells
A $[Co(hp)(H_2O)_2]$	1	80.9	78.3*
	10	70.4*	73.9*
	100	59.8**	69.4**
B $[Ni(C_{22}H_{22}N_4)]$	1	88.8	82.1
	10	70.9**	71.3**
	100	63.1**	69.8**
C $[NiCl(tren)(H_2O)]^+Cl^-\cdot H_2O$	1	95.4	100
	10	89.1	97.0
	100	79.9	85.5
D $[CoCl(tren)]^+Cl^-\cdot H_2O$	1	92.8	99.0
	10	82.3	89.8
	100	76.6*	74.1*
E $[Cu(tren)(H_2O)]^{2+}2Cl^-$	1	93.7	96.8
	10	87.2	83.5
	100	73.7**	57.6**
F $[ZnCl(tren)]^+Cl^-\cdot 3\ H_2O$	1	97.1	99.2
	10	90.0	93.9
	100	82.6	80.7*
G $[CoCl_3(H_2Meppz)]$	1	85.6	90.2
	10	73.2**	82.2*
	100	68.5**	60.7**
H $[CoCl_3(HMe_2ppz)]$	1	92.7	94.6
	10	88.4	87.1
	100	82.8	79.8*

ones which are known for their ability to bind DNA by intercalation when they are free from axial ligands [24,25].

2) Octahedral Ni(II) complex, $[NiCl(tren)(H_2O)]^+ Cl^-.H_2O$ [26] (compound **C**), is almost inactive on both cell lines at tested doses (**TABLE 1**) although it is able to form a stable metal-nitrogen bond being characterized by two *cis*-positions free for bonding (as occurs in cisplatin). In fact, we have demostrated through X-ray analysis that the Ni(II) complex reacts with neutral imidazole or adenine base forming the ternary complexes $[Ni(tren)(ImH)(H_2O)]^{2+}\ 2Cl^-.H_2O$ [27] and $[NiCl(tren)(HAde)]^+Cl^-$ [27], respectively (**FIG. 4**)

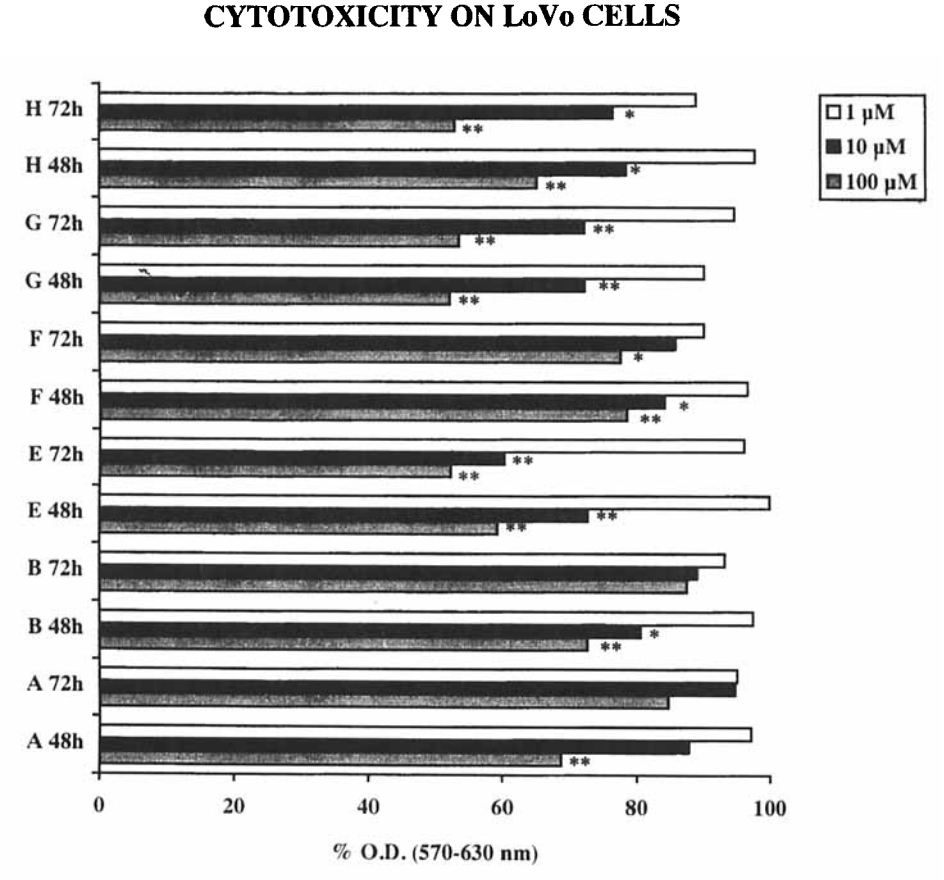

FIG. 1. Effects on MTT reduction induced by 48 and 72 h treatment with: **A**=[Co(hp)$(H_2O)_2$], **B**=[Ni$(C_{22}H_{22}N_4)$], **E**=[Cu(tren)$(H_2O)]^{2+}2Cl^-$, **F**= [ZnCl(tren)]$^+$ $Cl^-.3H_2O$, **G**= [$CoCl_3(H_2Meppz)$], **H**=[$CoCl_3(HMe_2ppz)$] *p<0.05; **p<0.01.

(tren=tris(2-aminoethyl)amine, ImH=neutral imidazole, HAde=neutral adenine). In the latter compound the adenine base is bound to the Ni(II) ion in an unusual fashion i.e. through its pyrimidine **N(3)** nitrogen atom (a binding site unknown before) which, on the other hand, is difficult to be reached inside the DNA strands [28]. However through theoretical molecular models, we have evidenced that the **N(9)** site (or alternatively the **N(7)** site when **N(9)** is blocked) becomes available for bonding a metal ion when the **N(3)**

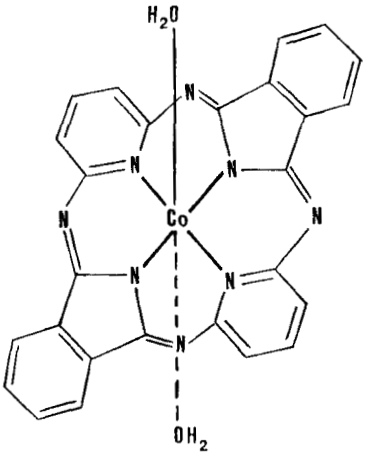

FIG. 2 Structure scheme of the octahedral $[Co^{II}(hp)(H_2O)_2]$ complex, **A**.

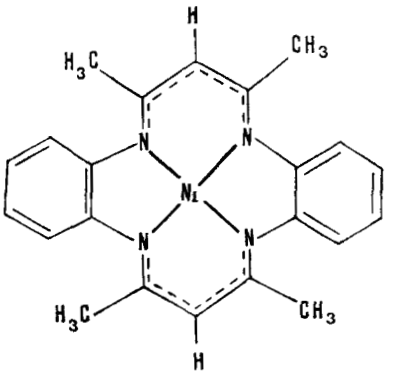

FIG. 3 Structure scheme of the square-planar $[Ni^{II}(C_{22}H_{22}N_4)]$ complex, **B**.

site is sterically hindered [27]. Then, the low activity of complex **C** is very likely due to the difficulty of DNA bases to reach the Ni atom inside the crowded octahedral complex.

3) D_{3h} trigonal-bipyramidal Co(II), Cu(II) and Zn(II) complexes: $[CoCl(tren)]^+ Cl^- .H_2O$ [29] (compound **D**), $[Cu(tren)(H_2O)]^{2+} 2Cl^-$ [30] (compound **E**) and $[ZnCl(tren)]^+ Cl^- .3H_2O$ [31] (compound **F**), having only one binding site along the tree-dimensional axis of the trigonal-bipyramidal structure. Also these complexes are able to form a metal-

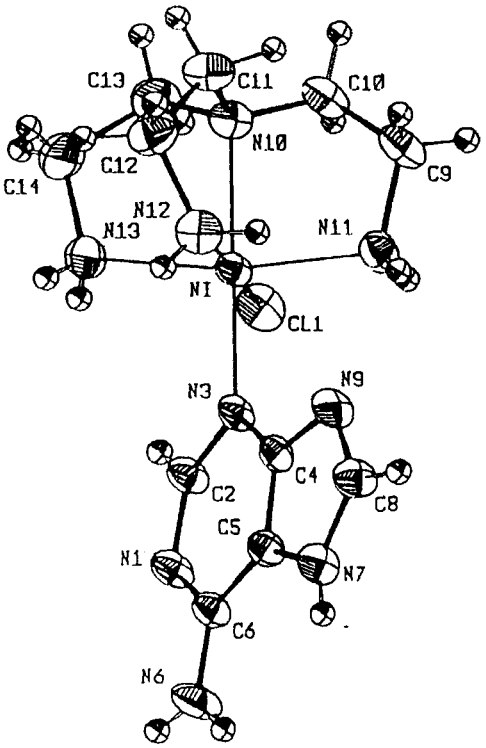

FIG. 4 Molecular structure of the octahedral $[Ni^{II}(tren)(HAde)]^{+}$ cation.

nitrogen bond, for example compound **E** gives with the adenine nucleobase the $[Cu(tren)(Ade)]^{+}\,Cl^{-}.2H_2O$ [30] ternary complex **(FIG. 5)**, in which the adenine molecule binds the Cu(II) ion as adeninato monoanion (a negative uncommon state of adenine) using its imidazole **N(9)** nitrogen atom.

Thus, we have experimentally observed that the octahedral $[NiCl(tren)(H_2O)]^{+}\,Cl^{-}.H_2O$ is able to recognize selectively the pyrimidine **N(3)** nitrogen of adenine whereas the trigonal-bipyramidal $[Cu(tren)(H_2O)]^{2+}\,2Cl^{-}$ binds the adeninato monoanion through its imidazole **N(9)** nitrogen atom. These results demonstrate that it is possible to address an appropriate metal complex to a pre-determined nitrogen atom of adenine nucleobase taking into account the following factors: **(i)** the coordination geometry and the steric hindrance of the metal complex; **(ii)** the oxidation state of metal and adenine molecule;

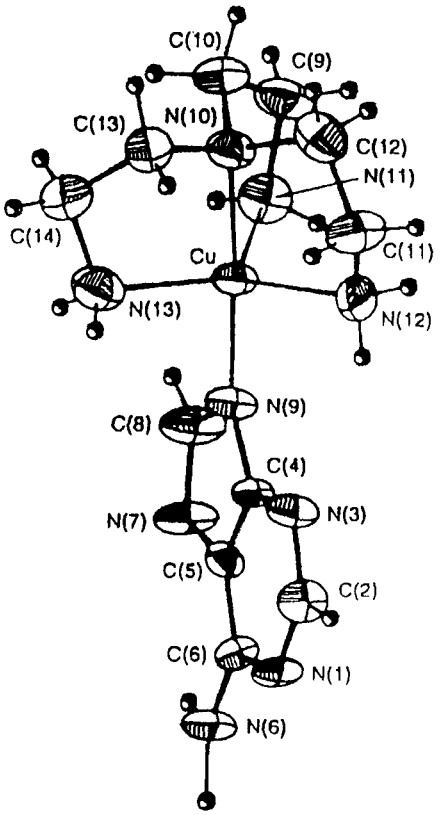

FIG. 5 Molecular structure of the trigonal-bipyramidal $[Cu^{II}(tren)(Ade)]^{+}$ cation.

(iii) the steric hindrance of the metal complex and adenine molecule; **(iv)** the formation of intramolecular hydrogen bonds.

$[CoCl(tren)]^{+}Cl^{-}.H_2O$, **D,** exhibits a low activity also at the highest dose of 100 μM after 24 h exposure and $[ZnCl(tren)]^{+}Cl^{-}.3H_2O$, **F**, is almost inactive even if the exposure time is prolonged to 72 h. On the contrary, $[Cu(tren)(H_2O)]^{2+}2Cl^{-}$, **E**, possesses on LoVo cells a significant cytotoxic, that is dose- and time-dependent, with a nearly 50% cell growth inhibition after 72 h exposure at 100 μM. (**TABLE 1** and **FIG. 1**). It must be remarked that this complex induces an interesting dose-dependent cytotoxiticity also on ovarian cells 2008 (**TABLE 1**).

4) Tetrahedral Co(II) and Zn(II) complexes, $[H_2Meppz)]^+$ $[CoCl_3(HMeppz)]^-$ [35], $[CoCl_3(H_2Meppz)]$ [36], $[CoCl_3(HMe_2ppz)]$ [36], $[ZnCl_3(H_2Meppz)]$ [37], $[ZnCl_3(HMe_2ppz)]$ [37] (**FIGURES 6,7**). At present, the cytotoxitic activity has been studied only for $[CoCl_3(H_2Meppz)]$ and $[CoCl_3(HMe_2ppz)]$ (compounds **G** and **H**, respectively) and the results obtained, in particular on LoVo cells, indicate these two complexes, together complex **E**, as the most interesting tested compounds being able to reduce cell growth to about 50% after 72 h exposure at 100 μM.

CONCLUSIONS

The cytotoxic tests on the present transition metal complexes which possess different steric hindrance and geometry, ranging from square-planar, tetrahedral, trigonal-bypiramidal to octahedral, indicate that these complexes are generally less active than *cis*-DDP when tested under the same experimental conditions [39,40]. In fact, while this Pt-drug shows on LoVo cells an IC_{50}=12,3 (10.6-14.2) μM and on 2008 cells an IC_{50}=13.8 (11.5-16.5) μM after 24 h exposure, the cytoxiticity of the new complexes is significant only after treatment at high doses or long time exposure. Nevertheless, it can be interesting to better study the cytotoxic effect of complexes **E**, **G** and **H** above all at long time exposure also in consideration that cobalt and copper are naturally present and essential for life.

EXPERIMENTAL SECTION

Materials and Methods

Cell lines - The human colon adenocarcinoma cells LoVo were maintained in Ham's F 12 medium plus 10% heat inactivated FCS, 1% antibiotics (all products Biochrom KG Seromed) and 2 mM L-glutamine (Merck).

The human ovarian adenocarcinoma cells 2008 were maintained in RPMI 1640 supplemented with 10% heat-inactivated FCS, 1% antibiotics (all products Biochrom KG Seromed) and 2 mM L-glutamine (Merck).

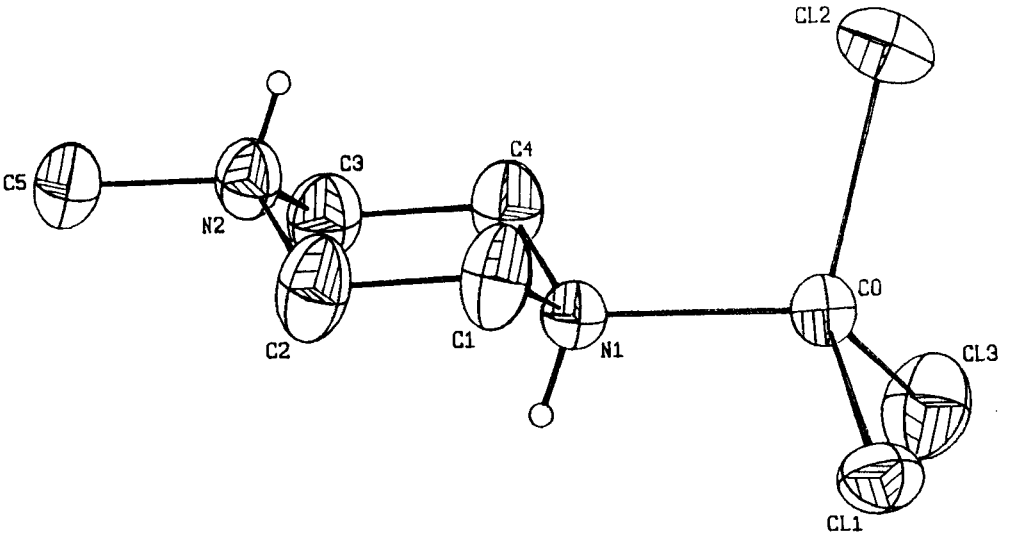

FIG. 6 Molecular structure of pseudotetrahedral zwitterionic $[Co^{II}Cl_3(H_2Meppz)]$, **G**.

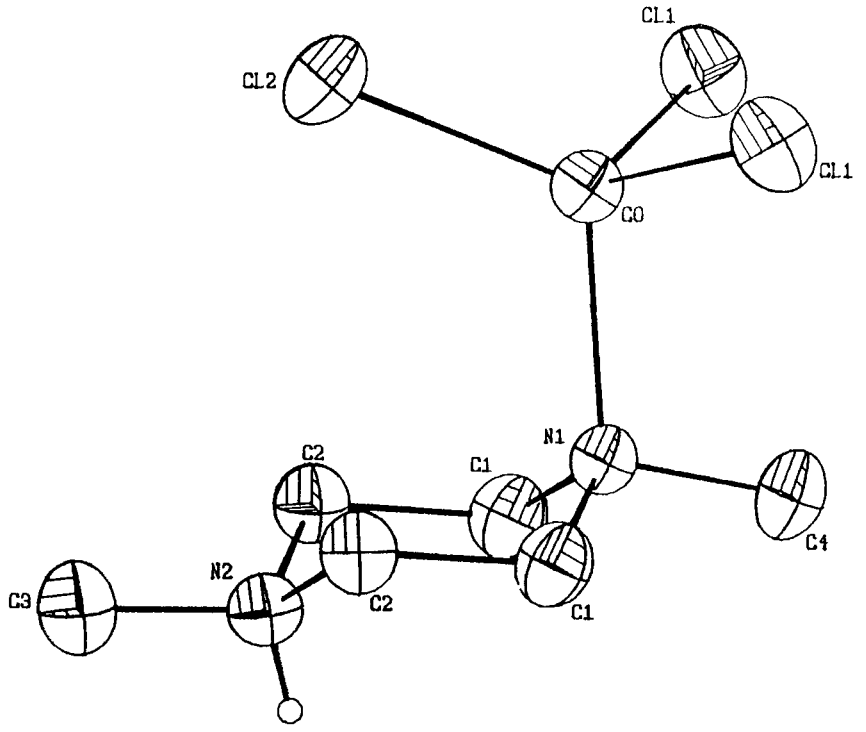

FIG. 7 Molecular structure of pseudotetrahedral zwitterionic $[Co^{II}C_3l(HMe_2ppz)]$, **H**.

Tetrazolium salts assay (MTT) – The cytotoxicity assay was performed according to the method reported by Mosmann [38]. The cells were seeded at $5x10^4$/ml in 96-well tissue plates (Falcon) and treated 24 h later with each agent at different concentrations. Viable cell growth was determined by MTT reduction assay after 24 h of incubation for all complexes and after 24, 48 and 72 h for selected compounds. 20 µl of MTT solution (5 mg/ml in PBS) were added to each well and plates were incubated for 4 h at 37°C. DMSO (200 µl) was then added to all wells and mixed thoroughly to dissolve the dark blue crystals. The absorbance was measured on a micro culture plate reader (Titertek Multiscan) using a test wavelength of 570 nm and a reference wavelength of 630 nm.

Statistical analysis – For each assay three different experiments were performed in triplicate. The results were reported as a percentage of controls and statistically evaluated by Student's *t*-test.

Synthesis of the metal complexes

The Co(II) and Co(III), Ni(II), Cu(II), Zn(II) and Pt(II) complexes have been synthesized according to the methods described respectively in the references: *trans*-$[Pt(HMeppz)_2]^{2+}$ $2Cl^-.4H_2O$ [16], *trans*-$[PtCl_2(HMeppz)_2]$ [17], $[PtCl_3(H_2Meppz)]$ [18], *trans*-$[PtCl_2(H_2Meppz)_2]^{2+}2Cl^-.2H_2O$ [18] and *cis*-$[PtCl_2(Me_2ppz)]$ [18], *cis*-$[PtI_2(HMeppz)]$ [19], $[Co^{III}hp(2\text{-})bis(1\text{-methylimidazole})]^{3+}I_3^-$ [20], $[NiCl(tren)(H_2O)]^+Cl^-$ [26], $[Ni(tren)(ImH)(H_2O)]^{2+}$ $2Cl^-.H_2O$ [27] and $[NiCl(tren)(HAde)]^+$ Cl^- [27], $[Cu(tren)(Ade)]^+$ $Cl^-.2H_2O$ [30], $[ZnCl(tren)]^+Cl^-.3H_2O$ [31], $[H_2Meppz]^+$ $[CoCl_3(HMeppz)]^-$ [35], $[ZnCl_3(H_2Meppz)]$ [37] and $[ZnCl_3(HMe_2ppz)]$ [37].

$[Co(hp)(H_2O)_2]$ (refs.13,14) has been prepared by refluxing for 4 hours in a dimethylformamide (DMF) solution (60 ml) hemiporhyrazine (4.03g, 9.1 mmol) with a slight excess of $Co(CH_3COO)_2.4H_2O$ (2.75g, 11.0 mmol). After the mixture cooled to room temperature, the black precipitate was filtered, washed with dimethylformamide (DMF) and methanol and recrystallized from 1/1 DMF/MeOH solution. The crude product was dried at 353 K for 2 h. *Analytical data.* Found: C, 58.8; H, 3.2; N, 21.0%.

Calculated for $C_{26}H_{18}CoN_8O_2$: C, 58.54; H, 3.40; N, 21.00%. Thermogravimetric (TG and DTG) and differential scanning calorimetry (DSC) measurements showed that the two water molecules are both coordinated to Co(II) being lost at the same time between 493 and 558 K.

$[CoCl(tren)]^+ Cl^-.H_2O$ (ref.29) was sinthesized by reacting in 100 ml of EtOH the tren ligand (1.15 ml, 7.5 mmol) with $CoCl_2 \cdot 6H_2O$ (1.785g, 7.5 mmol) under stirring at room temperature for 2 hours. By concentration of the violet solution after a week a blue-violet compound precipitated which was filtered, washed and dried under vacuum. *Analytical data.* Found: C, 24.81; H, 6.65, N, 19.17; Cl, 23.92 %. Calculated for $C_6H_{20}CoCl_2N_4O$: C, 24.50; H, 6.85; N, 19.05; Cl, 24.11 %.

$[CoCl_3(H_2Meppz)]$, *Trichloro(1-methylpiperazin-1-ium-N^4)cobalt(II)* (ref.36), has been synthesized by adding dropwise under stirring a blue-violet solution, previously prepared at 313 K, of anhydrous $CoCl_2$ (0.129 g, 1 mmol) in 10 ml EtOH, to a solution of 1-methylpiperazine (HMeppz) (0.113 ml, 1 mmol) in 5 ml EtOH. After mixing, a blue powder compound precipitates and the solution was stirred for 2 hours and then cooled to room temperature. The solid product was filtered, washed with EtOH-Et_2O (1:2 v/v) and dried under vacuum for 4 hours. Then, the complex was nearly quantitatively extracted by refluxing the precipitate in CH_3CN at 353 K for 8 h (solubility in CH_3CN, 0.5 mg/ml). After filtration, the solution was allowed to evaporate slowly for 4 days. The sky-blue crystals were filtered off, washed with CH_3CN-EtOH (1:1 v/v) and dried under vacuum. *Analytical data.* Found: C, 22.48; H, 5.04; N, 10.47; Cl, 39.87 %. Calculated for $C_5H_{13}N_2Cl_3Co$: C, 22.54; H, 4.92; N, 10.52; Cl, 39.91 %.

$[CoCl_3(HMe_2ppz)]$, *Trichloro(1,4-dimethylpiperazin-1-ium-N^4)cobalt(II)* (ref.36), was prepared and recrystallized following the same procedures used for the precedent complex except for 1,4-dimethylpiperazine (Me_2ppz) (0.138 ml, 1 mmole) instead of 1-methylpiperazine. *Analytical data.* Found: C, 25.62; H, 5.46; N, 10.15; Cl, 37.85 %. Calculated for $C_6H_{15}N_2Cl_3Co$: C, 25.69; H, 5.39; N, 9.99; Cl, 37.92 %

$[Ni(C_{22}H_{22}N_4)]$ (ref.14) was prepared by refluxing at 338 K for 48 hours in anhydrous methanol (MeOH, 50 ml), $Ni(CH_3COO)_2 \cdot 4H_2O$ (5g, 20.0 mmol), o-phenylen-

diamine (4.3 g, 40 mmol) and 2,4 penthadione (4.1 ml, 40 mmol) under nitrogen atmosphere. After cooling at room temperature, a blue-pink product precipitates which was filtered, washed with MeOH and dried at 353 K. *Analytical data.* Found: C, 64.96; H, 5.62; N, 13.58 %. Calc. for $C_{22}H_{22}NiN_4$: C, 65.87; H, 5.53; N, 13.97%.

$[Cu(tren)(H_2O)]^{2+}$ $2Cl^-$ (ref.30) was sinthesized by reaction between $CuCl_2 \cdot 2H_2O$ (1.278g, 7.5 mmol), dissolved in ethanol (40 ml), and tren (1.15 ml, 7.5 mmol). The resulting blue solution was stirred at room temperature for 2 hours. The addition of diethyl ether precipitated a blue compound which was filtered off, washed with ETOH-Et_2O (1:2 v/v) and dried under vacuum. *Analytical data.* Found: C, 24.10; H, 6.70; Cl, 23.65; N, 18.60%. Calc. for $C_6H_{20}Cl_2CuN_4O$: C, 24,12; H, 6.75; Cl, 23.75; N, 18.75%. The assignment of the molecular structure was supported by UV/VIS and IR spectra and, in particular, by thermogravimetric measurements indicating that the water molecule is coordinated to Cu(II).

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