Communications to the Editor

A *trans*-Platinum Complex Showing Higher Antitumor Activity than the Cis Congeners

Mauro Coluccia,*,† Anna Nassi,† Francesco Loseto,† Angela Boccarelli,† Maria A. Mariggio,† Domenico Giordano,† Francesco P. Intini,‡ Paola Caputo,‡ and Giovanni Natile*,‡

Dipartimento di Scienze Biomediche e Oncologia Umana, Sezione di Patologia Generale e Oncologia Sperimentale, Università di Bari, Policlinico, Piazza Giulio Cesare 11, I-70124 Bari, Italy and Dipartimento Farmaco-Chimico, Università di Bari, via E. Orabona, 4, I-70125 Bari, Italy

Received November 20, 1992

Structure–activity relationships that define the class of antitumor-active platinum compounds were summarized in 1973.[1] *cis*-Diamminedichloroplatinum(II) (cis-DDP) and related analogues produce bifunctional lesions on DNA; the most common adduct is an intrastrand cross-link between adjacent guanines that has been shown to inhibit DNA replication and/or transcription.[2,3] More recently, platinum cations of the form $[PtCl(NH_3)_2(Am)]^+$ (Am = pyridine, pyrimidine, purine, piperidine, or aniline ligand) have been proposed as a new class of platinum antitumor complexes[4] characterized by a monofunctional interaction with DNA, a binding mode previously unexpected to result in antitumor activity.[5] Moreover, activation of the trans geometry has been reported to occur in platinum(II) complexes with planar ligands, $[PtCl_2L_2]$ (L = pyridine, *N*-methylimidazole, thiazole, or quinoline).[6-8] These observations suggest the possibility of identifying new classes of platinum complexes structurally different from the classical analogues.

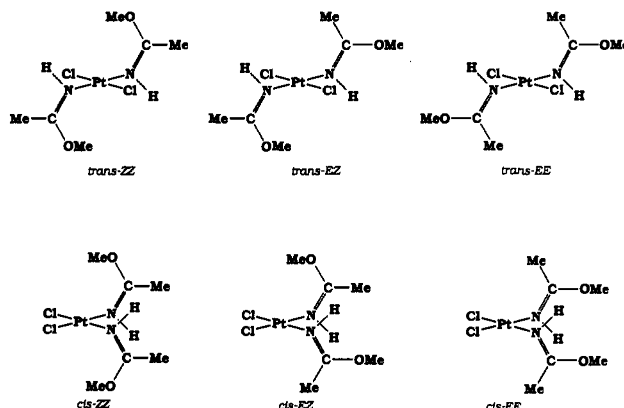
We have substituted imino ether for ammine in *cis*- and *trans*-DDP and found that in the new compounds the biological activity of cis and trans isomers is reversed. The complex with trans geometry shows the greatest in vitro cytotoxicity against P388 leukemia cells and displays a relevant antitumor activity on P388- and P388/DDP-bearing mice.

Imino ethers, like amines, are potential N-donor ligands and have a N-bound hydrogen suitable for hydrogen bond formation. They can have geometrical isomerism (*E* or *Z*) about the CN double bond so that, in addition to the possible cis and trans configurations of the platinum complex, there is additional isomerisn at the coordinated ligands.[9]

Platinum complexes with imino ethers are readily prepared by addition of alcohol to platinum nitriles.[9,10] The geometry of the metal center does not change during the reaction so that the *cis*-imino ether species are formed by starting with the *cis*-nitrile and similarly for the trans isomer. The *E* or *Z* configuration of the imino ether allows the formation of three different isomers for the cis and three for the trans isomer of $[PtCl_2(\text{imino ether})_2]$. A scheme of different isomers is given in Chart I. The *ZZ* isomer is favored on a kinetic ground (trans addition of the alcohol to the nitrile triple bond) while the *EE* isomer is favored on a thermodynamic basis. Therefore the initially formed *ZZ* isomer isomerizes subsequently to the *EZ* and *EE* isomers in the presence of a catalytic amount of base; in the absence of base this transformation is not observed and the *ZZ* isomer can be regarded as a stable species. *cis-EE*, *cis-ZZ*, and *trans-EE* (the yield of *EZ* isomers was always small; *trans-ZZ*, having a low solubility in water, was not investigated) were tested for in vitro cytotoxicity and in vivo antitumor activity against P388 leukemia. The in vitro inhibitory effects of increasing doses of platinum–imino ether complexes on the growth of P388 cells are shown in Figure 1. The drug sensitivity is also expressed as 50% growth inhibitory concentration, ID_{50}. The ID_{50} values of *cis-EE* and *cis-ZZ* are greater than that of *cis*-DDP (ID_{50} = 2.05 μM) by a factor of 4 and 9, respectively; on the contrary, the ID_{50} of *trans-EE* proved to be smaller than that of *trans*-DDP (ID_{50} = 85 μM) by a factor of 40, and very close to that of *cis*-DDP. These results indicate that the substitution of imino ethers for ammines brings only slight changes in the activity of the cis complexes, while it has a dramatic effect on the behavior of the trans species. The reasons for the enhanced cytotoxicity of the *trans-EE* compound are under investigation; preliminary results indicate that *trans-EE* inhibits the DNA synthesis in P388 cells in vitro (unpublished), suggesting a role for DNA binding in its mechanism of action. Moreover, other mechanisms such as enhanced uptake or different reactivity with intracellular thiols might be involved.[11]

As far as the in vivo effects are concerned, the results obtained with P388 leukemia-bearing mice (Table I) indicate that *cis-EE* and *trans-EE* are endowed with significant antitumor activity, while *cis-ZZ* is inactive. The antitumor activity of *cis-EE* does not depend upon the treatment schedule, and a cross-resistance of the same compound with *cis*-DDP was observed on the P388/DDP subline. In contrast *trans-EE* is more active when administered daily for 7 consecutive days (%T/C = 170) and has significant effect also on the *cis*-DDP-resistant subline (%T/C = 133). This is the first unambigous evidence for an antitumor activity of a *trans*-platinum complex.

Chart I. Schematic Drawing of the Structural Formulas of the Imino Ether Complexes



* Authors to whom correspondence should be addressed.
† Dipartimento di Scienze Biomediche e Oncologia Umana.
‡ Dipartimento Farmaco-Chimico.

0022-2623/93/1836-0510$04.00/0 © 1993 American Chemical Society

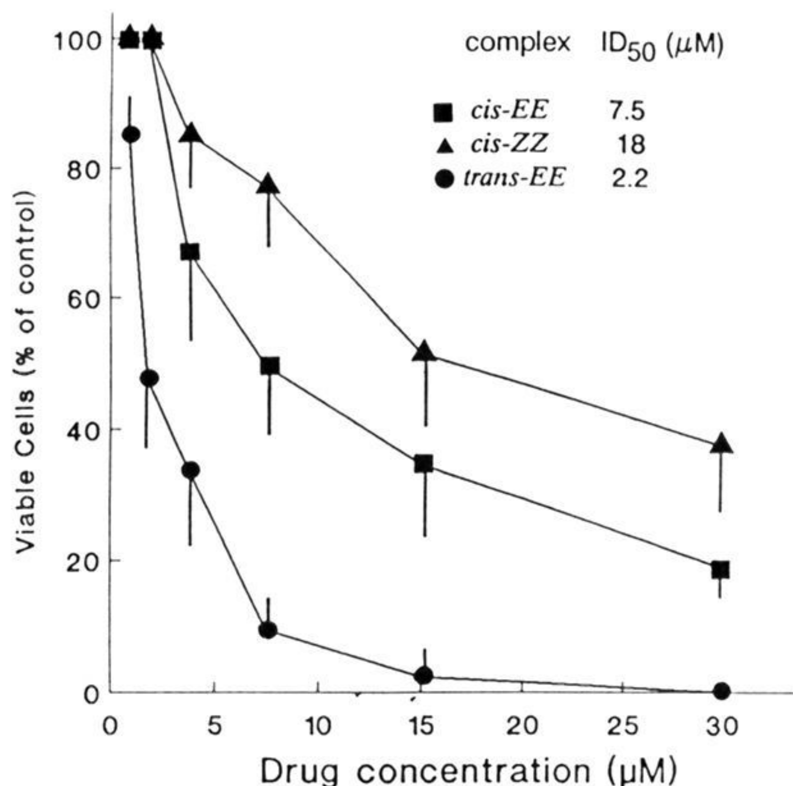


Figure 1. Survival curves of P388 cells (10^5 cells/mL in exponential growth phase) exposed to increasing concentrations of *cis-EE* (■), *cis-ZZ* (▲), and *trans-EE* (●) for 1 h at 37 °C. After drug removal, the cells were incubated for an additional 48 h, and then their number and viability (trypan blue exclusion test) were assessed. Each point represents the mean of five experiments performed in triplicate ± standard deviations.

Table I. Effects of Platinum–Imino Ether Complexes and *cis*- and *trans*-DDP on Mice Bearing P388 or P388/DDP Leukemia[a]

complex	dose (μmol/Kg)	treatment (days)	Tumor system, % T/C[b] P388	P388/DDP
cis-EE	19.6	1	117	
	39.2	1	145[d]	
	78.4	1	84	
	9.8[c]	1–7	144[e]	113
cis-ZZ	19.6	1	109	
	39.2	1	101	
	78.4	1	114	
cis-DDP	26	1	202	95
	2[c]	1–7	203	90
trans-EE	19.6	1	110	
	39.2	1	133[d]	
	78.4	1	121	
	19.6[c]	1–7	170[e]	133
trans-DDP	193	1	105	107

[a] P388 and P388/DDP leukemia cells (10^6/mouse) were implanted ip into B6D2F1 mice on day 0 (6 animals/group, 8 controls); the treatment was performed ip with the complexes dissolved in water just before use. The P388/DDP subline was established in vivo in our laboratory by ip treatment with a single dose of *cis*-DDP (20 μmol/kg) given 2 days after the passage of 10^6 leukemic cells over successive generations in B6D2F1 mice. [b] % T/C = median survival time (×100) of treated animals versus untreated controls; T/C > 125% is considered significant antitumor activity. [c] Equitoxic doses corresponding to $LD_{0.05}$ were calculated separately using the method of Litchfield and Wilcoxon (tumor-bearing mice, 1–7 day schedule) (Litchfield, J.; Wilcoxon, F. A. A Simplified Method of Evaluating Dose-Effects Experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113). [d] The difference between the two indicated groups is not statistically significant. [e] The difference between the two indicated groups is statistically significant ($p < 0.05$, Student–Newman–Keuls test).

The sequence specificity of DNA modification by Pt–imino ether complexes in comparison with *cis*- and *trans*-DDP was investigated by the primer extension footprinting assay (Figure 2). Pt–imino ether complexes (lanes 2–4) appear to block the DNA polymerase to a minor extent in comparison to *cis*- and *trans*-DDP (lanes 1 and 5, respectively) as indicated by the different amount of radioactivity in the corresponding lanes at the top of the gel (i.e. at the positions of the completed molecules). This

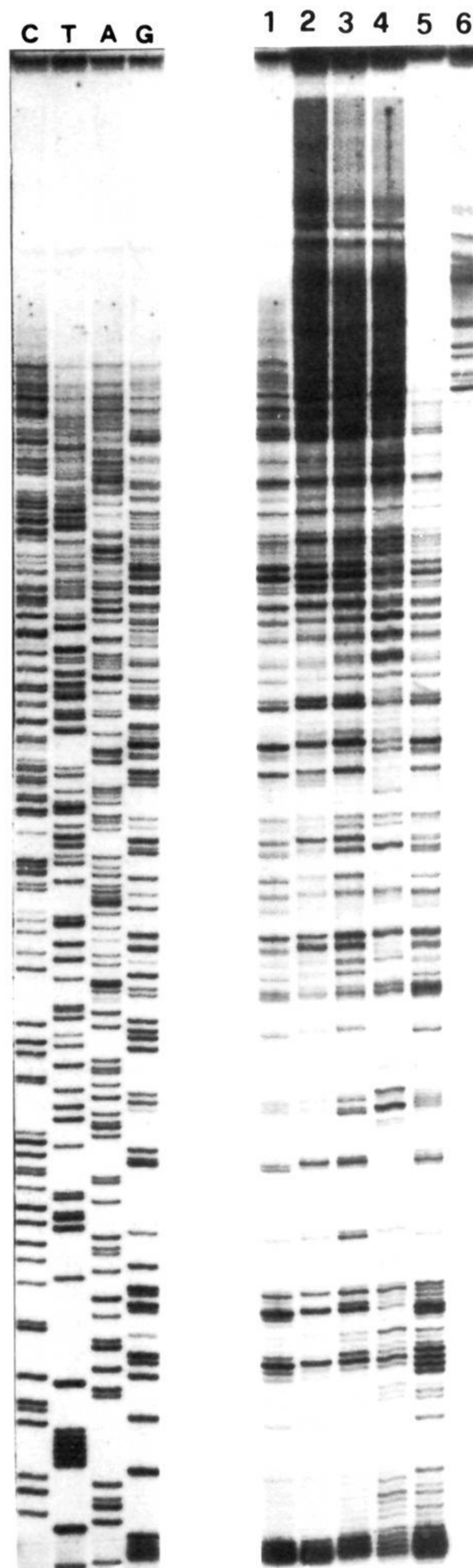


Figure 2. Autoradiogram of a 6% polyacrylamide/7 M urea sequencing gel showing the blocks to Sequenase 2 T7 DNA polymerase (United States Biochemicals) on drug-treated pBR322 DNA. The DNA (1.5 × 10^{-8} mol nucleotides) was reacted with freshly dissolved platinum complexes at drug/nucleotide molar concentration ratio of 0.02 for 1 h at 37 °C. At the end of incubation, excess drug was removed by centrifugation through Sephadex G-50 columns and an overnight post-treatment incubation period at room temperature was performed. After alkaline denaturation, the DNA was primed with 16-mer pst(+) primer, and the synthesis performed by Sequenase 2 in the presence of [α-^{32}P]dATP and unlabeled dNTPs. The products of synthesis were electrophoresed in parallel to a sequence ladder performed on unreacted DNA. Lanes designed C, T, A, and G refer to the base positions on the template strand: lane 1, *cis*-DDP-modified DNA; lane 2, *cis-EE*-modified DNA; lane 3, *cis-ZZ*-modified DNA; lane 4, *trans-EE*-modified DNA; lane 5, *trans*-DDP-modified DNA; lane 6, no reagent.

behavior might be related to a lower capability of interaction with double stranded DNA of Pt–imino ether complexes as compared with *cis*- and *trans*-DDP; however, direct measurement of platinum bound to DNA is necessary before this hypothesis can be conclusively demonstrated. All tested compounds show a great affinity for neighboring guanines (e.g. the d(pG_5) region at the bottom of the gel), even though the minor intensity of *trans-EE* stop bands (lane 4) at the d(pG_5) site indicates a lower preference of this complex for multiple guanines as compared to the cis congeners and to *cis*- and *trans*-DDP. In agreement with analogous experiments performed with Klenow polymerase[12] and Sequenase[5] on M13mp18 single-stranded DNA, the sites of inhibition of DNA synthesis correspond mainly to d(pGG) and, to a minor extent, to d(pAG) sites on *cis*-DDP-treated pBR322 template; in most cases the synthesized DNA chains extending up to the first platinated nucleotide. *cis-EE* exhibits the greatest binding specificity for d(pGG) sites while *cis-ZZ* is able to react with the same efficacy with d(pGG) and d(pAG) sites. The most intense stop bands on the *trans-EE*-treated template correspond to guanines within 3'AGN sites (where N = G or C) with a general pattern more similar, in the number and regioselectivity of blocking lesions, to that of *cis*-imino ether complexes and *cis*-DDP than to that of *trans*-DDP, which forms a wide variety of adducts in the G-rich regions.

The results of the present study demonstrate, for the first time, that a *trans*-[$PtCl_2$(imino ether)$_2$] complex is not only more cytotoxic than the cis congeners but also endowed with significant antitumor activity. The reasons for the activity of *trans-EE* are presently unknown although, in the context of the established relevance of DNA binding mode in the mechanism of action of *cis*-DDP, it may be hypothesized that the different selectivity in the interaction with DNA of *trans-EE* as compared with *trans*-DDP could play a role in determining its biological activity.

Detailed investigations on the interaction between *trans-EE* and DNA (i.e., bifunctional or monofunctional type of binding) and on the activity of this compound against solid murine metastasizing tumors will allow the establishment of the relevance of this type of compounds as a new class of platinum anticancer agents.

Acknowledgment. This work was supported by the contributions of M.U.R.S.T. (40%) and C.N.R. (A.C.R.O. Project).

Supplementary Material Available: The experimental section describing the preparation of the complexes, determination of drug sensitivity in vitro, and primer extension footprinting assay and a table of proton chemical shifts of platinum compounds with imino ethers (4 pages). Ordering information is given on any current masthead page.

References

(1) Cleare, M. J.; Hoeschele, J. D. Antitumor Activity of Group VIII Transition Metal Complexes. I. Platinum(II) Complexes. *Bioinorg. Chem.* **1973**, *2*, 187–210.

(2) Sherman, S. E.; Lippard, S. J. Structural Aspects of Platinum Anticancer Drug Interaction with DNA. *Chem. Rev.* **1987**, *87*, 1153–1181.

(3) Heiger-Bernays, W. J.; Essigmann, J. M.; Lippard, S. J. Effect of the Antitumor Drug *cis*-diamminedichloroplatinum(II) and Related Platinum Complexes on Eukaryotic DNA Replication. *Biochemistry* **1990**, *29*, 8461–8466.

(4) Hollis, L. S.; Amundsen, A. R.; Stern, E. W. Chemical and Biological Properties of a new series of *cis*-diammineplatinum(II) Antitumor Agents Containing Three Nitrogen Donors: *cis*-[Pt$(NH_3)_2$(N-donor)Cl]$^+$. *J. Med. Chem.* **1989**, *32*, 128–136.

(5) Hollis, L. S.; Sundquist, W. I.; Burstyn, J. N.; Heiger-Bernays, W. J.; Bellon, S. F.; Ahmed, K. J.; Amundsen, A. R.; Stern, E. W.; Lippard, S. J. Mechanistic Studies of a Novel Class of Trisubstituted Platinum(II) Antitumor Agents. *Cancer Res.* **1991**, *51*, 1866–1875.

(6) Farrell, N.; Ha, T. T. B.; Souchard, J. P.; Wimmer, F. L.; Cros, S.; Johnson, N. P. Cytostatic *trans*-Platinum(II) Complexes. *J. Med. Chem.* **1989**, *32*, 2240–2241.

(7) Van Beusichem, M.; Farrell, N. Activation of the Trans Geometry in Platinum Antitumor Complexes. Synthesis, Characterization and Biological Activity of Complexes with the Planar Ligands Pyridine, *N*-Methylimidazole, Thiazole, and Quinoline. Crystal and Molecular Structure of *trans*-Dichlorobis(thiazole)platinum(II). *Inorg. Chem.* **1992**, *31*, 634–639.

(8) Farrell, N.; Kelland, L. R.; Roberts, J. D.; Van Beusichem, M. Activation of the *trans* Geometry in Platinum Antitumor Complexes: A survey of the Cytotoxicity of *trans* Complexes Containing Planar Ligands in Murine L1210 and Human Tumor Panels and Studies on Their Mechanism of Action. *Cancer Res.* **1992**, *52*, 5065–5072.

(9) Fanizzi, F. P.; Intini, F. P.; Natile, G. Nucleophilic Attack of Methanol on Bis(benzonitrile)dichloroplatinum: Formation of Mono- and Bis-imido Ester Derivatives. *J. Chem. Soc. Dalton Trans.* **1989**, 947–951.

(10) Casas, J. M.; Chisholm, M. H.; Sicilia, M. V.; Streib, W. E. Imino-ether Complexes of Platinum: cis-[$PtCl_2$(HN=C(OR)Me)$_2$] and [$PtCl_4$(HN=C(OR)Me)$_2$], where R = Me, Et and Pr^i. Preparation, Characterization and X-Ray Structure for [$PtCl_2$(NH=C(OPr^i)-Me)$_2$]$_2$. *Polyhedron* **1991**, *10*, 1573–1578.

(11) Kelland, L. R.; Mistry, P.; Abel, G.; Loh, S. W.; O'Neill, C. F.; Murrer, B. A.; Harrap, R. Mechanism-related Circumvention of Acquired *cis*-Diamminedichloroplatinum(II) Resistance Using Two Pairs of Human Ovarian Carcinoma Cell Lines by Ammine/Amine Platinum(IV) Dicarboxylates. *Cancer Res.* **1992**, *52*, 3857–3864.

(12) Pinto, A. L.; Lippard, S. J. Sequence-dependent Termination of in vitro DNA Synthesis by *cis*- and *trans*-diamminedichloroplatinum(II). *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4616–4619.