Communications to the Editor

Cytostatic *trans*-Platinum(II) Complexes

Sir:

A principal feature of the structure–activity relationships of platinum antitumor complexes of general formula $[PtCl_2(amine)_2]$ is that the cis isomers (especially Cisplatin, *cis*-DDP, *cis*-$[PtCl_2(NH_3)_2]$) are active whereas the corresponding trans isomers are inactive.[1] The inactivity of trans compounds may be in part a consequence of their lower cytotoxicity. Trans complexes are reported to have ID_{50} values against cultured L1210 leukemia cells 1 or 2 orders of magnitude greater than cis compounds. Although trans compounds are often less toxic toward mice judging from their maximum tolerated dose (LD_0), sufficient drug cannot be administered to kill the tumor.[2] In contrast, an "antitumor" effect is observed if L1210 cells are treated ex vivo with *trans*-DDP and subsequently injected interperitoneally in mice.[3] At the molecular level, a possible explanation for this isomeric selectivity is that cis compounds form platinum–DNA adducts that inhibit DNA replication to a greater extent than those formed from trans compounds.[4] Alternatively, DNA adducts formed by trans compounds may be repaired more rapidly.[5]

In this paper we report that *trans*-Pt(II) complexes of pyridine and γ-picoline (4-methylpridine), $[PtCl_2L_2]$, are significantly more cytostatic than their corresponding cis isomers. These results have important implications for the role of geometrical isomerism in the structure–activity relations of platinum antitumor drugs. The complexes were prepared from $K_2[PtCl_4]$ by well-established methods.[6,7] All complexes gave satisfactory C, H, N analyses and isomeric purity was verified by IR and ^1H and ^{195}Pt NMR spectroscopy. The ^{195}Pt chemical shifts in d_7-DMF for the pyridine complexes were −1965 ppm (cis) and −1958 ppm (trans) relative to $PtCl_6^{2-}$. No isomerization was observed upon allowing the complexes to stand in DMF for several hours.

The L1210 leukemia cells, culture media, and growth conditions have been previously described. The bis(pyridine) and bis(γ-picoline) complexes were dissolved in DMF and diluted to a final DMF concentration of 0.25%. Growth inhibition was determined after 48 h in the presence of drug or 0.25% DMF.[2]

The ID_{50} values of Table I show that growth inhibition by *trans*-bis(pyridine) complexes is a factor of 5 larger than their corresponding cis isomers. In contrast, the ID_{50} of cis- and trans-DDP against L1210 leukemia cells in vitro are 2.3 and 67 μM, respectively.[2] Similarly, cis-DDP is 16 times more toxic toward CHO cells and 18 times more toxic toward HeLa cells than *trans*-DDP.[8,9] Independent NMR measurements showed no isomerization in DMF over a period of hours for either pyridine complex. These data and the results in Table I confirm that any explanation of the activity of the trans compound invoking a simple isomerization to an active cis isomer is invalid. Hence we show here that, contrary to previous results, the trans geometry does not impose molecular constraints that necessarily lead to lower cytostasis than the cis isomer.

Table I. Cytostatic and Toxic Effects of *cis*- and *trans*-$[PtCl_2L_2]$ (L = pyridine (py), 4-methylpyridine (γ-pic), and NH_3)

complex	L1210 leukemia: ID_{50},[a] μM	LD_0,[b] μmol/kg	P388 leukemia: %T/C[c]
cis-$[PtCl_2(py)_2]$	7.3 ± 0.7	236	109
trans-$[PtCl_2(py)_2]$	1.5 ± 0.2	236	113
cis-$[PtCl_2(\gamma\text{-pic})_2]$	13.5 ± 0.7	88	119
trans-$[PtCl_2(\gamma\text{-pic})_2]$	2.3 ± 0.8	332	105
cis-$[PtCl_2(NH_3)_2]$	2.3[d]	30[d]	205
trans-$[PtCl_2(NH_3)_2]$	67.0[d]	167[d]	116

[a] Mean ± range of two to three independent experiments. [b] Three to nine female BDF1 mice were treated intraperitoneally (ip) with increasing concentrations of platinum compounds dissolved in klucel. LD_0 is the maximum dose for which all mice survived after 30 days of treatment. [c] 10^6 cells were grafted ip in female BDF1 mice on day 0, six mice for each dose. On day 1, platinum compounds dissolved in klucel at three concentrations including the LD_0 were administered ip. Antitumor activity is expressed as the best %T/C, the median survival time of treated animals versus untreated controls. T/C >125% is considered significant antitumor activity. Experiments with L1210 leukemia gave the same results as P388 leukemia. [d] Reference 2.

Numerous derivatives of *cis*-$[PtCl_2(NH_3)_2]$ have been synthesized in attempts to produce less toxic and/or more active derivatives and preliminary screening of these compounds often involves cytostatic studies of the type used in this work. For example, Pt(II) chloroamines with ID_{50} values less than 10 μM and LD_0 greater than 10 μmol/kg show antitumor activity against murine tumors grafted ip after a single ip injection of the drug on day 1.[2] By these criteria both trans derivatives in Table I would be expected to be antitumoral. However, none of the pyridine complexes in this study showed reasonable antitumor activity (%T/C > 150) against P388 or L1210 leukemia. The reasons for these negative results are unknown. The reactivities of *trans*-$[PtCl_2(NH_3)_2]$ and *trans*-$[PtCl_2(py)_2]$ in substitution reactions such as chloride exchange are remarkably similar (with first-order rate constants of $k = 3.5 \times 10^{-5}$ s^{-1}).[10,11] Thus, hydrolysis rates are also not expected to vary dramatically in the two sets of compounds. The differences in cytotoxicity therefore are not likely to be due to a simple difference in hydrolysis.

(1) Cleare, M. J.; Hoeschele, J. D. *Bioinorg. Chem.* **1974**, *2*, 187.
(2) Macquet, J. P.; Butour, J. L. *J. Natl. Cancer Inst.* **1983**, *70*, 899.
(3) Johnson, N. P.; Lapetoule, P.; Razaka, H.; Butour, J. L. In *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*; Nicolini, M., Ed.; Martinus Nijhoff Publishing: Boston, 1988; p 3.
(4) Johnson, N. P.; Lapetoule, P.; Razaka, H.; Villani, G. In *Biochemical Mechanisms of Platinum Antitumor Drugs*; McBrien, D. C. H., Slater, T., Eds.; IRL Press: Oxford, 1986; p 1.
(5) Ciccarelli, R. B.; Solomon, M. J.; Varshavsky, A.; Lippard, S. J. *Biochemistry*, **1985**, *24*, 7533.
(6) Kauffman, G. B. *Inorg. Synth.* **1963**, *7*, 249.
(7) Thompson, L. K. *Inorg. Chim. Acta* **1980**, *38*, 117.
(8) Pascoe, J. M.; Roberts, J. J. *Biochem. Pharmacol.* **1974**, *23*, 1345.
(9) Johnson, N. P.; Hoeschele, J. D.; Rahn, R. O.; O'Neill, J. P.; Hsie, A. W. *Cancer Res.* **1980**, *40*, 1463.
(10) Pearson, R. G.; Gray, H. B.; Basolo, F. *J. Am. Chem. Soc.* **1960**, *82*, 787.
(11) Banerjea, D.; Basolo, F.; Pearson, R. G. *J. Am. Chem. Soc.* **1957**, *79*, 4055.

0022-2623/89/1832-2240$01.50/0

Perhaps complexes with pyridine and amine ligands have different biodistributions or the greater reactivity of the trans isomer may result in more rapid inactivation in the animal. Whatever the mechanism, our results raise questions concerning the general validity of in vitro criteria for antitumor activity of Pt(II) compounds based on the results of amine complexes.

In summary, we report *trans*-Pt(II) complexes with unusual biological activity that are significantly more cytostatic than their corresponding cis isomers. The metabolism and biodistribution of these compounds is of considerable interest. These molecules should be useful for investigating the detailed mechanism of isomeric selectivity of platinum antitumor compounds and the design of structurally novel platinum antitumor agents.

* Authors to whom correspondence should be addressed.
† University of Vermont.
‡ Laboratoire de Pharmacologie et de Toxicologie Fondamentales du CNRS.

Nicholas Farrell,*,† Tam T. B. Ha‡
Jean-Pierre Souchard,‡ Franz L. Wimmer‡
Suzy Cros,‡ Neil P. Johnson*,‡

Department of Chemistry
University of Vermont
Burlington, Vermont 05405
and Laboratoire de Pharmacologie et de Toxicologie
Fondamentales du CNRS
205 Route de Narbonne
Toulouse 31400, France

Received February 16, 1989

Articles

5-Piperazinylalkyl-2(3*H*)-oxazolones with Neuroleptic Activity

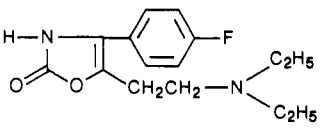
Giuseppe Cascio,* Elso Manghisi, and Giancarlo Fregnan

Lusofarmaco,† Research Division, Milan, Italy. *Received December 14, 1988*

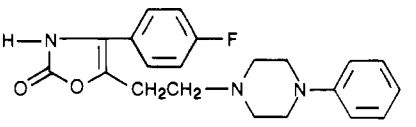
A series of new 4-aryl-5-[ω-(4-aryl-1-piperazinyl)alkyl]-2(3*H*)-oxazolones was synthesized and tested for neuroleptic activity in mice and rats. Several compounds exhibited interesting neuroleptic activity with very low liability to the extrapyramidal side effects. In particular the activity of 4-(4-fluorophenyl)-5-[2-[4-(3,5-dichlorophenyl)-1-piperazinyl]ethyl]-2(3*H*)-oxazolone (**14**) was greater than that of butropipazone and fluanisone, while of the same order of that of chlorpromazine; however, the product showed a longer lasting activity and minor ability to produce catalepsy as compared with the reference drugs.

2(3*H*)-Oxazolones have received little attention as molecules of potential pharmacological interest; however, sedative and muscle relaxant activity were found to be associated with some 4-aryl-2(3*H*)-oxazolones.[1] As part of a program aimed at discovering new series of therapeutically active compounds, a number of 4-aryl-2(3*H*)-oxazolones bearing an aminoethyl group in the 5-position have been synthesized in our laboratories; pharmacological evaluation of such compounds revealed the pronounced antiarrhythmic activity of 4-(4-fluorophenyl)-5-[2-(diethylamino)ethyl]-2(3*H*)-oxazolone (**1**).[2,3] Substitution of the diethylamino group of **1** with 4-phenylpiperazine was subsequently shown to result in compound **2** (Table I) exhibiting central nervous system (CNS) depressant activity in mice and rats. This result prompted us to prepare a number of 4-arylpiperazine derivatives **3–41** (listed in Table I), which being mostly prepared from the corresponding 2-hydroxy-4-aminobutyrophenones (**2a–29a**), might be considered structurally related to well-known 4-aminobutyrophenones endowed with neuroleptic activity. Therefore, it was of some interest to ascertain the biological activity of analogues in which the butanone side chain was modified and partially incorporated into a heterocyclic structure.

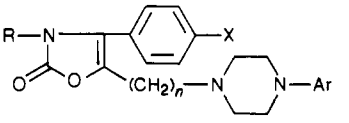
The structure–activity relationships of the above oxazolones are discussed herein. Also a detailed pharmacological profile of two selected compounds, **5** and **14**, is reported (Table II).



1



2



3-41

Chemistry

Oxazolones **2–29** were obtained by cyclization of the corresponding ketols **2a–29a** with phosgene and NH_3 (method A). Similarly, cyclization of ketol **2a** with phosgene and the proper substituted amine provided the oxazolones **31–35** (method A). Alternatively, compounds

† A company linked to "A. Menarini–Industrie Farmaceutiche Riunite s.r.l.".

(1) Bottari, F.; Nannipieri, E.; Saettone, M. F.; Serafini, M. F.; Tellini, N. *J. Med. Chem.* **1972**, *15*, 39.
(2) Cascio, G.; Fregnan, G. B.; Manghisi, E. In *La ricerca scientifica nell'industria farmaceutica in Italia*; Ferro, Ed.; Milan, 1977; p 635.
(3) Fregnan, G. B.; Prada, G.; Subissi, A. In *La ricerca scientifica nella industria farmaceutica in Italia*; Ferro, Ed.; Milan, 1977; p 645.

0022-2623/89/1832-2241$01.50/0