Antitumor Complexes of Platinum with Carrier Molecules. 2 [1]. Mixed Complexes of Amino Acids and tert-Butylamine

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Received July 20, 1984

Abstract

In the search for a fine modulation of cisplatin analogues we have synthesized complexes with two different inert ligands bound to platinum in the *cis*- position. This paper reports on compounds of formula *cis*-$[PtCl_2(aaH)(tba)]$ (aaH, amino acid; tba, tert-butylamine). These complexes have been synthesized with the aim of obtaining liposoluble cisplatin analogues bound to natural carrier groups. The derivatives of glycine, *D*-alanine, *L*-threonine, and *L*-serine were found to be moderately active against murine P388 and L1210 leukemia models. The compound $K[PtCl_3(tba)]$ was also found to be active against the same tumor models. Their activity and potency was, however, much lower than that of cisplatin.

Introduction

The toxic side effects of cisplatin** pose severe limitations on its clinical utility [2]. Much effort has therefore been devoted to finding cisplatin analogues with lower toxicity and improved effectiveness [3, 4]. Since most of the toxic effects of the antitumor drugs arise from their lack of selectivity, it has been proposed that antitumor agents should be bound to carrier molecules [5], with the hope of obtaining compounds which display their cytotoxic effects only in the tumor tissues.

In our laboratories we have applied this concept to cisplatin analogues, preparing and testing for antitumor activity platinum complexes in which the chemically active site of the drug, *i.e.* the *cis*-PtX_2 moiety (X = a leaving group), is bound to ligands which could act as carrier groups. One example has been a platinum complex of sulfadiazine (sulfanilamidopyrimidine) which has been reported to accumulate in cancer tissues [6]. This complex has been found to display an antitumor activity towards murine P388 leukemia higher than that expected from the *in vitro* cytotoxicity data [1].

The high requirement and uptake of important nutrients, such as amino acids, by tumor cells [7, 8], has been exploited to produce selective cytotoxic effects towards specific tumor tissues, by depriving cells of an adequate supply of the metabolic precursor (*i.e.* depleting enzyme therapy [9]). These nutrients have already been used as carriers of alkylating agents [10]. In the field of cisplatin analogues it has been reported that solutions of tetrachloroplatinate(II) and amino acids (*viz.* glutamine, asparagine, but also glycine and serine) show some activity against P388 and L1210 leukemias in mice [11, 12]. Moreover $K[PtCl_2(gly)]$ (in which glycine is chelated), although it does not inhibit sarcoma 180 growth [13], is active against murine L1210 leukemia [14]. Beck [15, 16] has reported that *cis*-dichlorobis(ethylglycylglycinate)platinum(II) (where the peptide ester molecules are *N*-coordinated) is active against some experimental tumors, and, more important, that analysis of the Pt content of the tissues of the treated rats suggests that these ligands induce some selectivity in the platinum complex towards the tumor cells [16, 17]. Finally, compounds of the type *cis-O*-[Pt(amino acidato)$_2$] (in which the amino acid moieties are chelated) were found to be inactive [18].

The results of a recent thorough study [19] have suggested that toxicity of cisplatin analogues correlates, at least in the first approximation, with the lability of the leaving groups, while cytotoxicity can

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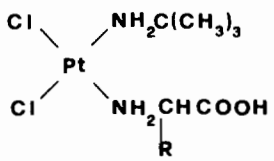
**Abbreviations. Cisplatin, *cis*-dichlorodiamminoplatinum-(II); aaH, amino acid; glyH, glycine; alaH, alanine; pheH, phenylalanine; serH, serine; threH, threonine; lysH, lysine; tba, tert-butylamine, DMF, dimethylformamide. aa is an amino acid anion.

0020-1693/84/$3.00

TABLE I. Analytical Data.

Compound	Found (Calcd.) %		
	C	H	N
cis-[$PtCl_2$(glyH)(tba)] (**1**)	17.4	4.2	6.8
	(17.4)	(3.9)	(6.8)
cis-[$PtCl_2$(*L*-alaH)(tba)] (*L*-**2**)	19.5	4.3	6.5
	(19.6)	(4.2)	(6.5)
cis-[$PtCl_2$(*D*-alaH)(tba)] (*D*-**2**)	19.4	4.2	6.4
	(19.6)	(4.2)	(6.5)
cis-[$PtCl_2$(*L*-pheH)(tba)]·H_2O (**3**)	29.9	4.7	5.4
	(29.9)	(4.7)	(5.5)
cis-[$PtCl_2$(*L*-serH)(tba)] (**4**)	18.8	4.3	6.0
	(18.9)	(4.1)	(6.3)
cis-[$PtCl_2$(*L*-threH)(tba)] (**5**)	20.9	4.0	6.1
	(21.0)	(4.3)	(6.1)
cis-K[$PtCl_2$(*L*-lys)(tba)]·$2H_2O$ (**6**)	21.8	4.6	7.0
	(21.5)	(4.6)	(7.5)
K[$PtCl_3$(tba)] (**7**)	11.5	2.8	3.4
	(11.6)	(2.8)	(3.4)
K[$PtCl_2$(gly)]	6.5	1.1	3.6
	(6.3)	(1.1)	(3.7)

be modulated by the nature of the inert ligands. Up to now the majority of the reported cisplatin analogues are of the type *cis*-[PtX_2L_2] (X = leaving group, L = inert ligand], but we believe that a finer tuning of the antitumor activity can be achieved by preparing compounds of the type *cis*-[PtX_2AB], *i.e.* by binding two different inert ligands, A and B, to the platinum ion, in order to confer some specific properties to the drugs. In this paper we wish to report the properties of compounds **1**–**6** in which platinum is bound to *N*-coordinated amino acids, as potential carrier groups, and to tert-butylamine, which should increase the liposolubility of the compounds.



1 R = H
2 R = CH_3 (*L* and *D*)
3 R = $CH_2C_6H_5$ (*L*)
4 R = CH_2OH (*L*)
5 R = $CHOHCH_3$ (*L*, threo)
6 R = $(CH_2)_4NH_2$ (*L*)

Experimental

Analyses, see Table I, were from the microanalytical laboratory, the University, Milan. Infrared spectra were obtained on a Nicolet MX-1 FTIR instrument. Circular dichroism spectra were recorded on a Jobin Yvon Mark III. All chemicals were reagent grade.

Preparation of the Complexes

Potassium Dichloroglycinatoplatinate(II)

It was prepared according to ref. [20].

Potassium tert-Butylaminetrichloroplatinate(II) (7)

This compound was prepared following a suggestion of Kong and Rochon [21], by heating at 70 °C for 15 hours a DMF solution of potassium tetrachloroplatinate(II) and freshly distilled tert-butylamine in the ratio 1:1. (Typically two mmol of reactants in 40 ml). The reaction mixture was then evaporated to 6–7 ml under reduced pressure and stored at 5 °C for 24 h. The filtered, clear orange solution was then treated with a large excess of ether. The crude product was dissolved in cold water, and the filtered solution was evaporated to dryness under reduced pressure at a temperature below 45 °C. Yields 70%. The product must be stored in the dark.

cis-Amino acidtert-butylaminedichloroplatinum-(II)

General procedure for compounds **1**–**5**. 1 mmol of the amino acid (glycine, alanine, phenylananine, serine, and threonine) was treated, in 30 ml of water, with 1 mmol of 7, and heated at 50–60 °C for 10 h. During this time the pH of the slurry decreases from

TABLE II. Cytotoxic Activity Against HeLa Cells *in vitro*.[a]

Compound	ID_{50} (μg/ml)
cisplatin	0.14
5	1.1
7	1.6
1	2.5
4	3.0
6	11.0

[a]For details see experimental.

5 to approximately 2.5. The filtered solution was evaporated to dryness under reduced pressure, and the residue was treated with methanol. After removal of KCl by filtration, addition of ether gave a yellow product, the analysis of which showed it to be a mixture of $[PtCl_2(aaH)(tba)]$ and $K[PtCl_2(aa)(tba)]$. The desired 'acidic' complex could be easily obtained by addition of 0.1 N HCl to the water solution of the mixture, to pH 1.5. Evaporation to dryness afforded $[PtCl_2(aaH)(tba)]$ which could be freed from KCl by dissolving the product in the minimum amount of $CHCl_3$/methanol (8/2 v/v) and precipitating the complex by addition of a large excess of ether to the filtrate. Yields 50–70% based on **7**.

For the preparation of **6**, the amino acid was liberated *in situ* from lysine hydrochloride by treatment with KOH. The solution was heated at 60 °C for 10 h, the final pH being about 9. From this solution the potassium salt $K[PtCl_2(lys)(tba)]$ obtained was analytically pure (see Table I) and was used as such.

Biological Expeirments

Cell Survival Determination

HeLa cells were exposed to the drugs for 24 h. Survival was determined by the ability of the treated cells to form colonies. After treatment the medium was removed and cells were washed and suspended in Eagle's minimal essential medium containing 10% fetal calf serum, and plated colonies were counted after 8 days of incubation. ID_{50} values were determined by dose-response curves and are reported in Table II.

Tumor Models and Antitumor Testing

BDA/2 and BDF1 mice of both sexes were obtained from Charles River Laboratories (Calco, Como, Italy). After arrival, the animals were acclimated for a week and randomized into experimental groups. Tumor lines were maintained as previously described [22, 23]. The experiments with L1210 and P388 leukemias were carried out in BDF1 female mice inoculated i.p. with 1×10^5 and 1×10^6 cells per mouse respectively. The drugs were administered i.p. as a single dose on day 1 after tumor cell transplantation. The dose range was deduced approximately by cytotoxic activity. Animals were observed daily. Comparative antitumor effects of various doses were determined from the median survival time (MST), in days, for treated (T) *versus* control (C) groups and expressed as T/C (%). A drug was considered active if it produced a T/C % higher than 125. The results are collected in Table III.

TABLE III. Antitumor Activity against P388 and L1210 Leukemia Models.[a]

Compound	Dose range tested (mg/Kg)	OD (mg/Kg)[b]	T/C (%)
P388:			
cisplatin		7–10	246 (170–322)
7	5–200	50	165
1	10–80	80	155
$K[PtCl_2gly]$	50–60	50	150 (144–156)
5[c]	12.5–100	>100	150
***D*-2**	25–200	100	155
4	25–200	25	133
3[c]	25–200	25	110
***L*-2**	25–200	25	85
6	100–200	100	20
L1210:			
cisplatin		7	178 (168–187)
7	10–28.5	>28.5	137
5[c]	10–250	200	137
***L*-2**	10–25	15	115

[a]For details see experimental. [b]Optimal dose, corresponding approximately to LD_{10}. [c]These compounds were suspended in saline with hydroxymethylcellulose.

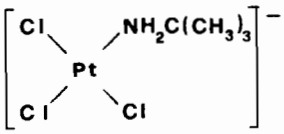
Results and Discussion

Preparation and Characterization of the Compounds

Potassium tert-Butylaminetrichloroplatinate(II) (7)

If the equimolar amounts of an amine and $K_2[PtCl_4]$ are reacted in water the insoluble *cis*-$[PtCl_2(amine)_2]$ precipitates eventually. However, following a suggestion of Kong and Rochon [21], compounds of the type $[PtCl_3(amine)]^-$ are easily prepared in DMF solution presumably because of the solubility of the *cis*-dichlorodiamine complex in this solvent. The other methods of preparation of $[PtCl_3(amine)]^-$ are time and reagent consuming [21, 24, 25]. Although we could prepare by the DMF method a number of derivatives of other amines in reasonably good yields (70–80%) we failed to prepare the ammonia derivative in a yield higher than 10%, in contrast to what was reported in the original paper [21].

The infrared spectrum of **7** (nujol mull on CsI disks) shows three bands attributable to the Pt–Cl stretching vibrations (312, 325, and 330 cm^{-1}) as expected for a $[PtCl_3L]$ species [25, 26] (*cf.*, for instance, $[PtCl_3(NMe_3)]^-$, 314, 329, and 337 cm^{-1} [25]). The molar conductivity of a 10^{-3} mol dm^{-3} water solution of **7** (110 ohm^{-1} cm^2 mol^{-1}) is consistent with a 1:1 electrolyte. The structure of this compound is therefore as in **7**.



7

Reaction of 7 with Amino Acids

When equimolar amounts of **7** and an amino acid are reacted in water solution, a decrease of pH, from about 5 to about 2.5 is observed, since HCl is liberated in the reaction

$$K[PtCl_3(tba)] + aaH \longrightarrow K[PtCl_2(aa)(tba)] + HCl$$

The slightly acidic pH should favour *N*-coordination, rather than chelation of the amino acid. The pH decrease should also favour protonation of the *N*-coordinated amino acid carboxylic group (or rather disfavour its deprotonation). Since however the *N*-coordinated amino acids are completely protonated at pHs of about 1.5 (see below), at pH 2.5 the crude product is a mixture of the acidic complex and its potassium salt $[PtCl_2(aaH)(tba)]$ and $K[PtCl_2(aa)(tba)]$, as inferred also from spectroscopic evidence. In fact the infrared spectra of this mixture (KBr discs or nujol mulls) show a band at about 1730 cm^{-1} (C=O of the unionized carboxylic group) and two bands at about 1620 and 1400 cm^{-1} attributable to the free, uncomplexed ionic carboxylate [27–29]. Incidentally these data also confirm the unidentate (*N*-coordinate) nature of the amino acid [29].

By treatment with dilute aqueous HCl to pH 1.5 the pure acidic form $[PtCl_2(aaH)(tba)]$ can be isolated, whereas from basic solutions we were unable to obtain the anionic complex: at pHs higher than 8 the compounds are not stable and materials with irreproducible analyses were obtained. At physiological pH the compounds are stable enough and could be recovered after 48 h. In the preparation of the lys derivative the final pH is about 9, and the compound was obtained as its potassium salt (see Table I) and used as such.

All the mixed aaH–tba complexes are soluble both in water and in polar organic solvents.

Substitution of a chloride ligand in **7** should give rise to complexes with the *cis*-configuration because an amino group has a poorer *trans* effect than Cl. The *cis*-configuration of compounds **1–6** was confirmed by the Kurnachev test and by the appearance of two Pt–Cl stretching vibrations (330 and 340 cm^{-1}) in the infrared spectrum [26, 29]. The structure of the complexes is therefore as in **1–6**.

An interesting feature of these complexes is their conformation in solution, as inferred from circular dichroism spectra. In fact the *L*-phe derivative displays a spectrum opposite to those of complexes of the other amino acids of the same absolute configuration. This situation is not altered either at different pHs or in DMF solution. Similar behaviour has been reported for another series of *N*-coordinated amino acid complexes of platinum(II) [30]. Presumably this difference in the circular dichroism spectra arises from a peculiar conformation of the phe moiety dictated by the bulky phenyl group.

Biological Studies

The complexes were tested for *in vitro* cytotoxic activity on HeLa cells and for antitumor activity against i.p. transplanted leukemia models. The results are collected in Tables II and III respectively, together with the data obtained with cisplatin as a standard compound of this class of agents.

The *in vitro* cytotoxicity of these compounds, as well as the potency in the *in vivo* experiments, is one to two orders of magnitude lower than that of cisplatin. The activity of **7**, which possesses only one inert ligand, is not surprising, since $[PtCl_3(NH_3)]^-$ has already been reported to be active [13, 19] and suggested to be the prototype of a new class of antitumor compound [19]. It is likely, however, that this type of complex transforms, *in vivo*, into *cis*-dichloro derivatives by reaction with some nucleophile in the body fluid.

The activity of $K[PtCl_2(gly)]$ against these leukemia models has been confirmed [14]. The only active mixed tba aaH complexes are the derivatives of glycine (**1**), *D*-alanine (*D*-**2**), serine (**4**), and threonine (**5**). The lysine and the *L*-alanine complexes are inactive and toxic, the former being particularly so. With these exceptions the other compounds are less toxic than cisplatin.

In the single injection treatment, the antitumor effects were not always dose-dependent. For instance a dose of 25 mg/kg of **4** produced an increase of life span similar to that obtained with 100 mg/kg. The therapeutic potential of these compounds should therefore be further evaluated following a multiple injection treatment.

The data of Tables II and III suggest that the structure of the amino acid plays some role in the potency, toxicity, and therapeutic potential which could arise from some transport discrimination, although this should be demonstrated by a pharmacokinetic study, which we hope to carry out in the future. We believe that such a mechanism could be the origin of the differences observed between *D*-**2** and *L*-**2**, since chiral discrimination between chiral cisplatin analogues and DNA (which is the likely [31], although not widely accepted [32], target molecule) is very unlikely [33–36]. Preliminary results from our laboratories indicate the absence of such discrimination also for these compounds, since the circular dichroism spectra of the adducts between calf thymus DNA and *D*-**2** and *L*-**2** are indistinguishable. Relevant to this discussion is the fact that, while the two enantiomers of cyclophosphamide have similar antitumor activities [37] (the small differences probably arising from some difference in the metabolism [38]), the derivatives of phosphoramide with *D*- and *L*-amino acids display different activities and toxicities [10].

It also tempting to correlate the inactivity of **3** with its anomalous conformation in solution, but it is rather difficult to prove this hypothesis.

Finally the lower activity of these complexes in comparison with that of cisplatin, could arise, *inter alia,* from the fact that at physiological pHs the carboxylic group of the amino acid is ionized, giving rise to negatively charged species, less reactive towards the nucleophilic sites of the target molecule inside the cells. As a matter of fact all the anionic Pt complexes which have been found to display some antitumor activity (including **7** of the present study) are less active and potent than cisplatin [1, 13, 19].

Conclusions

Amino acid derivatives of cytotoxic agents were synthesized on the grounds of the concept that attachment of an antitumor agent to a naturally occurring carrier might increase the drug effectiveness because of the increased affinity for certain tumor cells, or the possibility of involvement in various metabolic pathways of tumor cells [5, 10, 39]. However, in the case of the complexes reported in this paper, only compounds with low activity have been obtained, and there seems to be little hope that further modulation of these compounds will produce more cytotoxic drugs. As a matter of fact one can even be doubtful of the role of the amino acid, since the activity of the precursor **7** is similar to that of the active amino acid derivatives **1**, **2**, and **5**. In conclusion the limited activity of these compounds has discouraged further tests on different schedules or different tumor models.

Up to now the results reported on the binding of carrier molecules to antitumor drugs are rather contradictory [1, 5, 6, 10]. In fact one of the major drawbacks of this approach is the fact that the chemical and biological properties of both the carrier and the drug molecules become unpredictable when bound together to form a new molecule. For instance, a sulfadiazine-mustard derivative has been reported to lack the selective uptake by tumor tissues of sulfadiazine itself [6], whereas the peptide platinum complex reported by Beck [15–17] has shown some degree of specificity.

Although the results reported in this paper are not encouraging, we believe that the study of antitumor drugs linked to carrier molecules must be developed, together with studies aimed at understanding the mechanism of drug transport and the difference in the mechanisms of drug uptake probably existing between normal and tumor cells [39, 40].

Finally the results here reported show that the binding of two different inert ligands to the *cis*-PtX_2 moiety increases the possibility of modulating the antitumor properties of a cisplatin analogue.

Acknowledgement

This work was supported in part by the Italian CNR, Rome (Progetto Finalizzato Controllo della Crescita Neoplastica).

References

1 A. Pasini, E. Bersanetti, F. Zunino and G. Savi, *Inorg. Chim. Acta, 80,* 99 (1983).
2 J. J. Roberts, in H. M. Pinedo, (Ed.), 'Cancer Chemotherapy 1982', Excerpta Medica, Amsterdam, 1982, p. 95.
3 W. C. Rose, J. E. Schurig, J. B. Huftalen and W. T. Bradner, *Cancer Treat. Rep., 66,* 135 (1982).

4 P. C. Hydes, in M. P. Hacker, E. B. Douple and I. H. Krakoff, (Eds.),'Platinum Coordination Complexes in Cancer Chemotherapy', Martinus Nijoff, Boston, 1984, p. 216.
5 D. S. Zaharko, M. Przybylski and V. T. Oliverio, *Meth. Cancer Res., 16,* 347 (1979).
6 C. Abel, T. A. Connors, W. C. Ross, N. H. Nam, H. Hoellinger and L. Pichot, *Eur. J. Cancer, 9,* 49 (1973).
7 D. F. H. Wallach, *J. Mol. Med., 1,* 97 (1976).
8 D. R. Williams, *Chem. Rev., 72,* 203 (1972).
9 L. J. Hanka, *Cancer Treat. Rep., 63,* 1009 (1979).
10 M. Szekerke, *Cancer Treat. Rep., 60,* 347 (1976).
11 R. J. Banner, A. J. Charlson, R. P. Gale, N. T. McArdle, K. E. Trainor and E. C. Watton, *Cancer Treat. Rep., 61,* 469 (1977).
12 A. J. Charlson, R. J. Trainor and E. C. Watton, *J. Clin. Emat. Oncol., 7,* 293 (1977).
13 M. J. Cleare and J. D. Hoeschele, *Bioinorg. Chem., 2,* 187 (1973).
14 A. J. Charlson, personal communication.
15 W. Beck, B. Purucker, M. Girnth, H. Scoenenberger, H. Seidenberger and G. Ruckdeschel, *Z. Naturforsch., Teil B:, 31,* 832 (1976).
16 W. A. Beck, in A. Mueller and E. Dueman (eds.) 'Transition Metal Chemistry, Current Problems and the Biological as well as the Catalytical Relevance', Verlag Chemie, Weinheim, 1981, p. 3.
17 R. Zeisler F. Lux, H. Seidenberger, H. Schoenenberger and W. Beck, in 'Nuclear Activation Techniques in the Life Science', Int. Atomic Energy Agency, Vienna, 1979, p. 467.
18 R. J. Speer, H. Ridgway, L. M. Hall, D. P. Stewart, K. E. Howe, D. Z. Lieberman, A. D. Neuman and J. M. Hill, *Cancer Chemother. Rep., 59,* 629 (1975).
19 J. P. Macquet and J. L. Butour, *J. Nat. Cancer Inst., 70,* 899 (1983).
20 L. E. Erickson, J. W. McDonald, J. K. Howie and R. C. Clow, *J. Am. Chem. Soc., 90,* 6371 (1968).
21 P. C. Kong and F. D. Rochon, *J. Chem. Soc., Chem. Commun.,* 599 (1975).
22 A. M. Casazza, G. Pratesi, F. Giuliani and A. Di Marco, *Tumori, 66,* 549 (1980).
23 F. Zunino, F. Giuliani, G. Savi, T. Dasdia and R. Gambetta, *Int. J. Cancer, 30,* 465 (1982).
24 T. S. Elleman, J. W. Reishus and D. S. Martin, Jr., *J. Am. Chem. Soc., 80,* 536 (1958).
25 P. L. Goggin, R. J. Goodfellow and F. J. S. Reed, *J. Chem. Soc., Dalton Trans.,* 1298 (1972).
26 J. R. Ferraro in 'Low Frequency Vibrations of Inorganic and Coordination Compounds', Plenum, New York, 1971.
27 J. Fujita, K. Konya and K. Nakamoto, *Inorg. Chem., 9,* 2794 (1970).
28 B. Purucker and W. Beck, *Z. Naturforsch., Teil B:, 27,* 1140 (1972).
29 J. A. Kieft and K. Nakamoto, *J. Inorg. Nucl. Chem., 29,* 2561 (1967).
30 O. P. Slyudkin and B. Nordén, *Inorg. Chem., 22,* 2637 (1983).
31 J. J. Roberts and M. F. Pera, Jr., in S. J. Lippard (ed.) 'Platinum, Gold and Other Metal Chemotherapeutic Agents', ACS Symposium Series 209, Washington, 1983, p. 3.
32 J. P. Macquet, J. L. Butour, N. Johnson, B. Salles and M. Wright, in M. P. Hacker, E. B. Douple and I. H. Krakoff, (eds.) 'Platinum Coordination Complexes in Cancer Chemotherapy', Martinus Nijoff, Boston, 1984. p. 27.
33 A. Pasini, A. Velcich and A. Mariani, *Chem.-Biol. Interact, 42,* 311 (1982).
34 M. Gullotti, A. Pasini, R. Ugo, S. Filippeschi, L. Marmonti and F. Spreafico, *Inorg. Chim. Acta, 91,* 223 (1984).
35 B. Wappes, M. Jennerwein, E. V. Angerer, J. Engel, H. Schoenenberger, H. Brunner, M. Schmidt, M. Berger, D. Schmahl and S. Seeber, *J. Cancer Res. Clin. Oncol., 107,* 15 (1984).
36 W. R. Leopold, R. P. Batzinger, E. C. Miller, J. A. Miller and R. H. Earhart, *Cancer Res., 41,* 4368 (1981).
37 A. E. Wroblewski, S. M. Socol, A. Okruszek and J. G. Verkade, *Inorg. Chem., 19,* 3713 (1980) and references therein.
38 P. J. Cox, P. B. Farmer, M. Jarman, R. W. Kinas and W. J. Stec, *Drug Metab. Dispos., 6,* 617 (1978).
39 D. T. Vistika, *Blood, 56,* 427 (1980).
40 F. M. Sirotnak, P. L. Chello and R. W. Brockman, *Meth. Cancer Res., 16,* 381 (1979).