Synthesis and Antitumor Activity of a Novel Diplatinum Complex of the Binucleating Naphthazarinato Ligand

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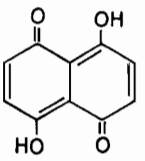
Abstract

In trying to combine the pharmacological properties of cisplatin and adriamycine, the representative prototypes of the two very active and most used groups of cytotoxic drugs, namely the *cis*-platinum-(II) series and the anthracycline cytostatic antibiotics, we came on the idea to use as ligand for cisplatin molecules the binucleating naphthazarinato moiety, knowing that the naphthoquinone part of the anthracycline molecule is essential for the antineoplastic activity. Synthesis was carried out by the reaction of naphthazarine with K_2PtCl_4 in the presence of ammonia. This reaction gives a binuclear complex with the stoichiometry $Pt_2(C_{10}H_4O_4)(NH_3)_2Cl_2$ and a planar structure confirmed by IR and electronic spectra. The complex has a very potent antineoplastic activity comparable with that of cisplatin, but much lower nephrotoxicity. The activity spectrum on transplantable tumors is similar to that of cisplatin.

Introduction

It is well known that *cis*-dichlorodiamineplatinum (*cis*-DDP), being the prototype of a new class of cytotoxic agents, plays a significant role in the chemotherapy of human cancer [1–3]. Moreover, many other platinum(II) compounds have been synthesized and investigated in an effort to identify compounds with increased antitumor effectiveness and or decreased toxicity, as well as to shed light on the mechanism accounting for their biological activity [4–5]. Also of considerable importance were the synergistic therapeutic effects observed, both at experimental and clinical level, by using DDP in combination with other cytostatics. Accordingly, the most effective and widely used is the DDP–adriamycin combination, which, in fact, demonstrated an interference of the antitumor activities of its constituents.

Adriamycin, which is an anthracyclin antibiotic with very potent antineoplastic activity [6, 7], can be considered as a derivative of the naphthazarin molecule **1**. This very interesting quinonic molecule



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provides the basic skeleton of a vast number of naturally occurring compounds which exhibit important biological and pharmacological properties [8–9]. Among these compounds alkannin and its antipode shikonin, as well as some of their esters have shown remarkable anticancer activity [10–11]. Furthermore, the wound healing properties of alkannin and its esters have been thoroughly studied and the regeneration of necrotic tissues verified [11].

As a result of our interest in the interference of the biological activity, we thought it would be advisable to synthesize model compounds involving in the same molecule both the biologically active naphthazarinato moiety and the platinum(II) metal centers. The synthesis of such a molecule seems to be possible, since the naphthazarinato moiety, being a potential binucleating chelate ligand, could be coordinated to two platinum(II) metal centers in an end-to-end fashion. In the present paper we report on the results concerning the synthesis, characterization and screening work of a diplatinum com-

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plex, namely (diamine)(dichloro)(μ-naphthazarinato)diplatinum, $Pt_2(\mu\text{-}C_{10}H_4O_4)(NH_3)_2Cl_2$, **2**. The role of the chelating ability of the binucleating naphthazarinato ligand on the antitumor activity of the platinum(II) centers is also discussed.

Experimental

All chemicals were reagent grade and used as received.

Synthesis

The diplatinum complex was obtained by the following method. To a solution of 5,8-dihydroxy-1,4-naphthalene-dione(naphthazarin) (0.19 g, 1 mmol) in 50 ml of ethanol, an aqueous solution of K_2PtCl_4 (0.83 g, 2 mmol) in 50 ml of water was added slowly under continuous magnetic stirring, followed by addition of an excess (5 ml) of 25% aqueous ammonia solution. The resulting mixture was left aside for 1 day at room temperature under continuous magnetic stirring. During this period a microcrystalline black solid was precipitated, which was removed by filtration, washed several times with small portions of water, followed by ethanol and diethylether and dried under vacuum. The isolated compound is soluble in dimethylformamide (DMF) and dimethylsulfoxide (DMSO) and slightly soluble in water and most of the common organic solvents. The analytical data were as follows: Calc. for ($C_{10}H_{10}O_4N_2Cl_2Pt_2$): C, 17.54; H, 1.47; N, 4.09; Pt, 57.10. Found: C, 17.52; H, 1.48; N, 4.11; Pt, 56.98%.

Physical Measurements

Infrared spectra were recorded in the region of 4000–250 cm^{-1} on a Perkin-Elmer 467 spectrophotometer using KBr discs and/or Nujol mulls. Electronic spectra were obtained on a Perkin-Elmer Hitachi 200 spectrophotometer and on a Cary 17D spectrophotometer, using freshly prepared solutions in DMF. Carbon, hydrogen and nitrogen were determined using a Perkin-Elmer 240 Elemental Analyzer.

Results and Discussion

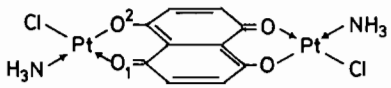
The reaction of naphthazarin (5,8-dihydroxy-1,4-naphthoquinone), **1**, with K_2PtCl_4 in the co-presence of an excess of ammonia affords a binuclear complex, formulated as $Pt_2(C_{10}H_4O_4)(NH_3)_2Cl_2$. This diplatinum complex is a microcrystalline black solid stable in air, soluble in DMF and DMSO giving dark violet solutions, slightly soluble in water and insoluble in most of the common organic solvents.

Insights concerning the structure and bonding of **2** were gained through the examination of its IR and electronic spectral data. The IR spectrum of **2** exhibits two strong absorption bands in the region of 1200–1700 cm^{-1}, which are due to the ν(C···O) stretching vibrations. Both these two bands are characteristic bands of the binucleating naphthazarinato ligand. The first band occurring at 1318 cm^{-1} is assigned to the stretching vibration of the phenolic C–O bonds [12, 13], whereas the second one at 1610 cm^{-1} is attributed to the stretching vibration of the carbonyl, C=O bond [12, 13]. The ν(C–O) band is shifted to higher frequencies (*ca.* 86 cm^{-1}) relative to the corresponding band of the free naphthazarin molecule, a fact which is consistent with the coordination of the phenolic oxygen donor atom in the complex. On the other hand, the ν(C=O) band is shifted to lower frequencies (*ca.* 11 cm^{-1}) relative to the corresponding band of the free naphthazarin molecule, thus providing evidence for the coordination of the quinonic oxygen donor atom as well. It is worth noting that in analogous complexes of the naphthazarinato ligand with Cu(II), Ni(II) and Zn(II), the shift of the ν(C=O) band to lower frequencies is much higher amounting to *ca.* 60–80 cm^{-1} [14]. The smaller shift of the ν(C=O) band in the diplatinum complex strongly suggests that the interactions between the quinonic oxygen donor atoms and the Pt(II) centers are much weaker than those of the complexes with the other transition metal centers. This is not surprising considering the soft Pearson acidity of the Pt(II) ions and the rather hard Pearson basicity of the quinonic oxygen donor atoms. In this respect, it is expected that the diplatinum complex would easily undergo nucleophilic substitution reactions of the naphthazarinato ligand by other nucleophiles possibly by an S_N1 type mechanism. Such behaviour of the diplatinum complex would be very important in determining its biological activity.

According to the IR spectral data discussed previously, it is clear that the naphthazarinato moiety acts as a binucleating ligand in the complex, bridging the two fragments bearing the Pt(II) centers in an end-to-end fashion. This is further supported by the presence in the IR spectrum (at *ca.* 455 cm^{-1}) of the weak absorption bands due to the stretching vibrations of the Pt–O bonds [15]. In the same region of the spectrum (320 cm^{-1}) also occurs the weak absorption band due to the ν(Pt–Cl) stretching vibrations [16], thus confirming the presence of Pt–Cl bonds in the complex. Finally, the IR spectrum of **2** exhibits all the characteristic bands due to the coordinated NH_3 ligands [17]. It is important to note that the observed narrowing of the ν(C···O) bands in the complex, as well as the absence of any splitting of the bands discussed above, strongly suggests the presence of the same environment around the two Pt(II) centers. Accordingly, the binucleating naphthazarinato ligand adopts in **2** its more stable

structure possessing the C_{2h} symmetry [13, 14]. This structure is further supported by the electronic spectral data of **2**. Thus, all the observed intraligand transitions at 264, 364 and 530 nm are in accordance with those predicted theoretically on the grounds of quantum chemical calculations performed on the C_{2h} structure of the free naphthazarin molecule [14]. The two shoulders observed at 565 and 612 nm may be due to charge transfer transitions either of the LMCT* or MLCT* type. However, due to the high intensity of both the intraligand and CT* transitions it was not possible to detect any crystal field band in the electronic spectrum of **2**. The same was also true for the analogous complexes of the naphthazarinato ligand with the Ni(II), Cu(II) and Zn(II) metal ions [14].

On the basis of the infrared and electronic spectral data, as well as the diamagnetic nature of **2** the following structure **3** can be proposed.



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In this structure each Pt(II) center is four-coordinated by two oxygen, one nitrogen and one chloride donor atoms. Since all four-coordinated Pt(II) complexes are square-planar, the diplatinum complex under investigation is planar belonging to the C_{2h} point group. The inequivalence of the two Pt–O bonds in each Pt(II) center could be attributed to the different *trans*-effect of the chloride and ammonia ligands. The stronger *trans*-effect of the chloride ligand is responsible for the weaker Pt–O bond in the *trans*-position. The weakening of the Pt–O bonds has as a result the easy substitution of the naphthazarinato ligand by other nucleophiles (e.g. donor solvent molecules, or nitrogen donor atoms of the DNA and RNA). Such nucleophilic substitution reactions seem to be responsible for the antitumor activity of the diplatinum complex. Taking into consideration that the antitumor activity of the Pt(II) compounds is correlated to their coordinating ability towards DNA and RNA [18] it would be expected that the coordinatively unsaturated $Pt(NH_3)Cl$ species exert analogous antitumor activity to that of the *cis*-$Pt(NH_3)_2Cl_2$. In fact this is the case for the diplatinum complex under investigation.

Antitumor Activity

The diplatinum complex was tested against lymphoid leukemia L1210 Ehrlich ascites tumor (EAT), adenocarcinoma AC-755 and leukemia P388. Comparative work was carried out for the complex, cisplatin and naphthazarine. The toxicological study was closed before the screening work. The LD curves (10 days observation) are shown in Fig. 1. For the antitumor assay we used doses at about the LD_{10} level. Death of the animals (mice) after toxic doses of the complex occurred between the 4th and the 7th day after intraperitoneal (ip) injection. At doses calculated to be around LD_{10}, no organ lesions were observed (both macroscopically and on tissue histology). In comparison, in equivalent cisplatin doses, cylinders on the renal tubuli (after 48 h) and some degree of atrophy of the tubular epithelium (after the 8th day) are constant. For the antitumor activity we used the standard treatment schedules and evaluation methods [19, 20] The results are summarized in Table I. Naphthazarine was practically inactive in the tested tumor systems and is not included in Table I. Other details on biological activity are now in further investigation.

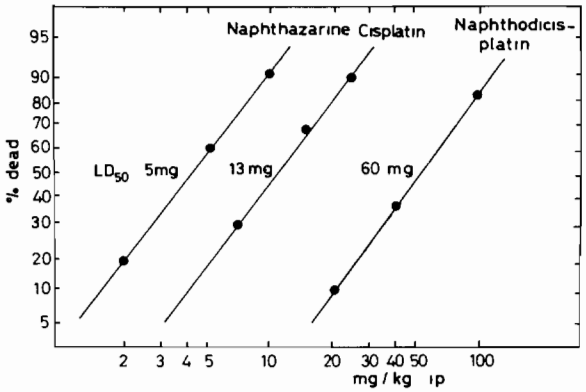

Fig. 1. LD curves of naphthazarine, cisplatin and naphthodicisplatin. Each curve has been estimated on a group of 20 mice. The observation time for calculating the LD was 10 days. The doses noticed in the middle of the curves are the corresponding LD_{50} doses

It is obvious that this preliminary biological screen work indicates the activity of the diplatinum complex to be comparable to that of cisplatin and there will be much interest in its low nephrotoxicity.

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*LMCT, ligand to metal charge transfer; MLCT, metal to ligand charge transfer, CT, charge transfer.

TABLE I. Results of the Screening Work on the Diplatinum Complex

Compound	Vehicle	Schedule (days)	Dose (mg) (ip)	T/C % (cures not included)	Cures	Comment
Leukemia L1210						
Controls				100	0/10	
Diplatinum	corn	1	28	111	0/10	
complex	oil	1.6	20	133	0/10	active
Cisplatin	NaCl	1	6	105	0/10	
		1.6	4	133	0/10	active
Leukemia P388						
Controls				100	0/10	
Diplatinum	corn	1	28	189	0/10	active
complex	oil	1.6	20	217	2/10	highly active
Cisplatin	NaCl	1	6	179	0/10	active
		1.6	4	207	0/10	active
Ehrlich ascites tumor						
Controls				100	0/10	
Diplatinum	corn	1	28	120	7/9	highly active
complex	oil	1.6	20	120	7/10	highly active
Cisplatin	NaCl	1	6	103	0/10	
		1.6	4	113	3/10	active
Adenocarcinoma AC-755						
Controls				100	0/10	
Diplatinum	corn	1	28	137	0/10	active
complex	oil	1.6	20	148	0/10	active
Cisplatin	NaCl	1	6	183	0/10	active
		1.6	4	226	0/10	active

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