Aminomalonato(1,2-diaminocyclohexane)platinum(II): A Competitive Antitumor Compound Within a New Class of Neutral, Chemically Stable, Water Soluble, Functionalized Platinum(II) Complexes

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Abstract

Antitumor, neutral, chemically stable, water soluble and functionalized aminomalonato–platinum(II) complexes have been prepared and their mode of coordination characterized by elemental analysis and infrared spectra. Among this new class of compounds, aminomalonato(1,2-diaminocyclohexane)platinum(II) has been selected for ^{13}C NMR measurements and for initial evaluation against L1210 and B 16 melanoma. The preliminary biological results reveal the high antineoplastic potential of this compound.

Introduction

It has been now largely recognized that there is an important need for an antitumor *cis*-dichlorodiamineplatinum(II) (*cis*-DDP) analog, which might be at least equally effective, but with less toxic side-effects, such as nausea or vomiting, ototoxicity, neurotoxicity and renal damage [1–3]. The complications which have been encountered so far among the second generation platinum complexes are still an excessive host toxicity, insufficient water solubility for intravenous (i.v.) administration and an undefined structure or lack of sufficient chemical stability for formulation development [4–7]. Even among the restricted number of selected compounds which have recently entered clinical trial, several appear to be affected by at least one of the above shortcomings [6]. Moreover, all the platinum complexes which have been reported in the literature do not appear to be designed for the possibility of becoming antitumor drugs with target specificity.

In recent years we have investigated the possibility of functionalization of organometallic derivatives, in order to utilize the chemical handle for covalently linking the complexes to carrier molecules [8–10]. In our opinion this approach could lead to the development of targeted platinum derivatives having an increased specificity toward malignant cells [10]. In spite of the encouraging biological results obtained *in vitro* with some of our prototypes (*i.e.*, platinum(II)–catecholamine complexes [11]), we had to overcome their very low aqueous solubility. Apparently the bulky lypophilic triphenylphosphine ligands, which appeared necessary to stabilize the platinum–*o*-catecholato coordination bond, were responsible for the lack of antitumor activity *in vivo* of the compounds [12]. In general, the combination of chemical stability and water solubility has also been a well-known problem for several among the second generation platinum complexes which contain neutral amine ligands [5, 13–15]. In this case an increase in water solubility has usually been achieved by replacing the chloride ions with less strongly bound acido-ligands, such as NO_3^-, $0.5SO_4^{2-}$, phthalates or substituted carboxylates [5, 16–19]. In most instances, such a replacement leads to kinetically reactive species which undergo partial or total hydrolysis [16, 19]. Consequently, either they produce an excess host toxicity or they present a significant problem for optimal chemical characterization [7, 20, 21]. On the other hand, the use of cyclic carboxylate ions, such as oxalate, malonate or substituted malonates, has provided antitumor compounds with increased chemical stability, but in most cases with insufficient water solubility [16, 19, 22].

In order to overcome the above-cited shortcomings for a potential antitumor platinum drug, we have developed a new class of chemically stable, water soluble platinum–malonato derivatives [23]. They are characterized by the presence of a free amine functional group, which confers to the complexes a high degree of water solubility and can be utilized for the covalent binding to carrier molecules.

In the present work we report on their synthesis and chemical characterization. Preliminary evaluation *in vivo* against L1210 leukemia and B 16 melanoma have been carried out with one of these compounds: the aminomalonato(1,2-diaminocyclohexane)platinum(II) complex, AM-DACHP. An additional screening of this compound against L1210 leukemia has

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been conducted in direct comparison with Tetraplatin, which is now undergoing clinical trial at the National Cancer Institute (NCI). The initial results show that AM-DACHP is emerging as a very competitive platinum antitumor agent, with a high possibility of exploitation.

Experimental

Physical Measurements and Microanalyses

Infrared spectra were recorded with a Perkin Elmer 457 Grating Infrared spectrophotometer, solid samples being run as KBr pellets. ^{13}C NMR spectra were taken on a Bruker WP-200 instrument in D_2O as solvent with both TMS and $CHCl_3$ as external standards. Carbon, hydrogen and nitrogen analyses were performed by the Microanalytical Service of the Chemistry Institute at the Hebrew University of Jerusalem.

Preparation of Compounds 1–10

In a typical preparation, a 1:1 aqueous solution of redistilled 1,2-diaminocyclohexane (DACH) was added dropwise to a freshly prepared stirred solution of K_2PtI_4 [24]. After 4 h at room temperature, the reaction mixture was stored at 0 °C overnight. The bright yellow precipitate of (DACH)PtI_2 was filtered, washed with H_2O, EtOH and dried *in vacuo*. A suspension of 5 g of (DACH)PtI_2 in 160 ml of H_2O, containing 2.63 g of silver sulfate, was stirred in the dark for 3.5 h at 38 °C. The precipitate of silver iodide was separated with the aid of a sintered glass filter and the filtrate pipetted into 2.94 g of barium aminomalonate. The reaction mixture was stirred overnight at room temperature, cooled to 0 °C and filtered, and the residue washed with icy water. The clear filtrate was concentrated under reduced pressure below 45 °C to a volume of about 3 ml. Addition of EtOH afforded a white precipitate, which was filtered out, washed with EtOH, acetone and dried *in vacuo*. In this experiment (DACH)$Pt(OCO)_2CH-NH_2$, Am-DACHP, was obtained as dihydrate with a yield of 89%. The other complexes listed in Table I were prepared by a similar method [23].

In Vivo Assays

The i.v. evaluation against L1210 leukemia was performed at the Department of Pharmacology, College of Medicine of the University of Vermont, Burlington, Vt., under the supervision of Prof. J. J. McCormack. The intraperitoneal (i.p.) evaluations against L1210, as a single dose, and against B 16 melanoma, were carried out in the laboratories of Bristol Myers, Syracuse, N.Y. The comparison against L1210, as multiple dose, was conducted at the Southern Research Institute, Birmingham, Ala., under the supervision of Dr. Daniel Griswold (NCI-Exp. A8-00139).

Results and Discussion

As a modification of the classic method, reported by Cleare and Hoeschele [25] for the preparation of platinum malonato complexes, sulphate amine–platinum intermediates reacted with barium aminomalonate yielding the corresponding dicarboxylato complexes. The overall procedure is outlined by the following scheme:

$$(amine)PtI_2 + Ag_2SO_4 \longrightarrow (amine)PtSO_4 + 2AgI\downarrow$$

$$(amine)PtSO_4 + Ba(OCO)_2CH-NH_2 \longrightarrow (amine)Pt(OCO)_2CH\text{-}NH_2 + BaSO_4\downarrow$$

The use of barium as the counter ion for the formation of the substituted-malonato salt, has proven useful in our case in order to stabilize the aminomalonato intermediate and to maintain an almost

TABLE I. Analytical Data for the Platinum(II)–Aminomalonato Complexes (am = $(OCO)_2CH-NH_2$)

Compound	Yield (%)[a]	Decomposition temperature (°C)	Found(calc.) (%)		
			C	H	N
$(H_3N)_2Pt$ am·H_2O (1)	75	250	9.90(9.89)	2.91(3.05)	11.47(11.53)
(en)Pt am·H_2O (2)	89	254	15.54(15.38)	3.24(3.36)	10.34(10.76)
(DACH)Pt am·$2H_2O$ (3)	88	250	23.69(23.36)	4.55(4.58)	8.80(9.09)
(aziridine)$_2$Pt am·H_2O (4)	53	200	20.07(20.18)	3.48(3.64)	9.81(10.09)
(c-propylamine)$_2$Pt am (5)	67	217	25.13(25.34)	3.93(4.03)	9.98(9.85)
(c-butylamine)$_2$Pt am·$2H_2O$ (6)	65	132	27.15(26.92)	5.00(5.14)	8.12(8.56)
(c-pentylamine)$_2$Pt am (7)	82	190	32.13(32.34)	5.47(5.23)	8.65(8.71)
(c-hexylamine)$_2$Pt am·H_2O (8)	42	164	34.13(34.10)	5.90(5.92)	7.79(7.95)
(i-propylamine)$_2$Pt am·H_2O (9)	81	174	23.86(24.10)	5.28(5.18)	9.21(9.37)
(n-propylamine)$_2$Pt am (10)	91	175	25.15(25.10)	5.23(4.93)	9.29(9.76)

[a]Calculated from the parent compound (amine)PtI_2.

TABLE II. Selected IR Bands[a] for the Platinum(II)–Aminomalonato Complexes

Compound	ν(C=O) (cm^{-1}, br, vs)	ν(C–O) (cm^{-1}, m)	ν(PtN–H) (cm^{-1}, br, s)	ν(C–N–H) (bending, cm^{-1})
1	1680	1390	3260	1590(m)
2	1650	1400	3200	1600(sh)
3	1640	1400	3180	1570(sh)
4	1670	1390	3200	1560(m)
5	1660	1380	3180	1530(sh)
6	1650	1370	3080	1570(sh)
7	1640	1370	3080	1520(m)
8	1640	1380	3090	1520(m)
9	1650	1380	3080	1540(sh)
10	1640	1380	3080	1530(sh)

[a] As KBr pellets.

neutral pH during the course of the reaction [23]. Analytical data and IR-measurements of some representative aminomalonato complexes are listed in Tables I and II, respectively. The coordination of the dicarboxylic acid to the platinum nucleus was confirmed by the disappearance of the three strong bands at 1630, 1590 and 1570 cm^{-1}, characteristic of the $Ba(OCO)_2CH–NH_2$ salt, and by the appearance of a new asymmetric coordinated $–CO_2$ absorption at about 1670 cm^{-1} [18, 26]. Broad absorptions between 3260–3080 cm^{-1} have been attributed to the coordinated neutral amine ligands. These bands appear in the same region as the intermediate (amine)PtI_2 complexes. A new absorption of medium intensity in the 1590–1530 cm^{-1} region (appearing in some cases as a shoulder to the strong vicinal carboxylate band) was found consistent with the C–N–H bending frequency of the non-coordinated amine group [11, 26].

As mentioned, the structural design of this new class of complexes is in accordance with the chemical stability of platinum(II)–malonato analogs [22]. The stability of platinum containing cyclic oxalato and malonato ligands in solution has been rationalized by the chelating effect of the dicarboxylato group, which has been shown to be maximum for 5 and 6 membered rings [19]. In addition to this, the free amine group attached to the malonate was found to confer a high aqueous solubility to the complexes. The solubility was generally dependent upon the degree of alkylation of the amino ligands and proved to be above 40 mg/ml for all the compounds.

It is well known that the antitumor activity of platinum(II) complexes is very sensitive to the type of coordinating amines [17, 19, 22]. We have noticed in our case that the amino group in position 2 of the malonate can have an influence on the physical properties of the amine ligands coordinated to the platinum. An example of this influence is given by the following ^{13}C NMR data, obtained for compound **3** (AM-DACHP). It should be noted that the 1,2-diaminocyclohexane used in our synthesis consisted of a mixture of *trans*- and *cis*-isomers in the ratio 5:4. Consequently, the resulting platinum complex was also a mixture of stereoisomers. Although no attempts were made to have them separated, they could be identified by their distinctive ^{13}C NMR spectra. Table III indicates three ^{13}C resonance peaks characteristic for the three pairs of α, β and γ carbons in each of the isomers of DACH. Upon complexation of the diamine ligand with platinum, the corresponding ^{13}C chemical shifts were changed by -4 to $+7$ ppm. These values are in excellent agreement with those observed by Ericson *et al.* [27], for some other platinum derivatives of *trans*-DACH. However, owing to the diastereotopic nature of the central carbon atom of the amino-malonato ligand, the number of ^{13}C NMR signals for the cyclohexane carbon atoms doubled. The effect of the position of the NH_2 group in the malonate moiety (above or below the plane of the ligand) on the resonance of the α-cyclohexane carbon atoms was found to be

TABLE III. ^{13}C NMR Data for Free DACH and for Isomeric AM-DACHP[a]

Chemical shifts, δ (ppm)				Assignment
Free DACH		AM-DACHP		
trans	*cis*	Product of *trans*	Product of *cis*	
23.70	20.34	22.79, 22.90	19.18, 19.24	γ-carbon atom
32.40	28.57	30.81, 30.90	24.68[b]	β-carbon atom
55.12	50.06	59.60, 61.48	55.19, 57.40	α-carbon atom

[a]The spectra were taken at 50 MHz in D_2O with both TMS and $CHCl_3$ as external standards. In measuring the spectrum of the complex the numbers of scans was 54 000, the pulse width 2.5 μs and the relaxation delay 0.0 s. Under these conditions the carbon atoms of the coordinated amine-malonate do not show up in the NMR spectra. [b]Two overlapping signals.

considerably larger than on the remote β- and γ-peaks. The various ^{13}C NMR signals of the *cis*- and *trans*-DACH isomers as well as the corresponding platinum complexes are summarized in Table III.

Antitumor Activity

Several of the most promising second generation platinum complexes are characterized by the presence of DACH as the stable, neutral amine ligand [5, 7, 17, 20]. This ligand was found to confer to the complexes excellent antitumor properties such as reduced nephrotoxicity and lack of cross-resistance with *cis*-DDP. For this reason AM-DACHP has been chosen among this new class of water soluble aminomalonato–platinum(II) derivatives for initial biological testings.

In order to evaluate the *in vivo* efficacy of AM-DACHP, the complex was administered intravenously and intraperitoneally as a single dose to mice inoculated the previous day with 1×10^6 L1210 cells. It is noteworthy to recall that when platinum compounds are administered intravenously, their antitumor effect is greatly reduced, as has also been observed for *cis*-DDP [28]. The results in Table IV show that, even when injected intravenously and at single low doses, AM-DACHP revealed a significant antitumor potency which is far beyond the criterion level of activity outlined by the NCI protocols for this tumor model in a less advanced stage ($T/C \geqslant 150$).

In addition to its antileukemic activity, AM-DACHP was found to be also very effective against B 16 melanoma. Table V summarizes the results of AM-DACHP when administered i.p. to mice inoculated the previous day with 0.5 ml of a 10% brei suspension. Even at very low doses the compound produced an activity comparable to *cis*-DDP [4] and far above the criterion level of the NCI protocols ($T/C \geqslant 150$ for the B 16 melanoma system).

As a further biological test, the compound was also evaluated on a multiple dose treatment against L1210 *in vivo*. This experiment was conducted in direct comparison with its DACH analog, tetrachloro(1,2-diaminocyclohexane)Pt(IV), Tetraplatin (NSC-363812). The latter has been recently recommended for development to clinical trial by the Platinum Analog Working Group, Decision Network Committee (NCI), after a thorough selection among a great number of DACH-containing platinum derivatives. The results, summarized in Table VI, indicate that the efficacy of AM-DACHP against L1210 is equivalent to Tetraplatin even at doses which are still not considered the optimum ones. Moreover, the

TABLE IV. Antitumor Activity of AM-DACHP Against Advanced L1210 Leukemia: Dose-response Comparisons of i.v. and i.p. Treatments at Single Doses[a]

Compound	Host: DBF_1 M		Host: CDF_1 F	
	i.v. Dose (mg/kg)	Median T/C (%)	i.p. Dose (mg/kg)	Median T/C (%)
AM-DACHP	100	toxic	128	toxic
	50	153	64	214
	25	169	32	186
	12.5	159	16	136

[a]L1210 cells (1×10^6 suspended in 0.1 ml of physiological saline solution) were inoculated i.p. into groups of DBF_1 M mice and into groups of CDF_1 F mice. The compound, dissolved in 5% dextrose solution, was administered i.v. or i.p. as a single dose the following day. Animals that received no drug treatment died between 7 and 9 days after tumor inoculation.

TABLE V. Antitumor Activity of AM-DACHP Against B 16 Melanoma[a]

Compound	Dose (mg/kg)	T/C (%)
AM-DACHP	24	toxic
	16	175
	12	167
	6	192
	3	170

[a]Groups of BDF_1 M mice were implanted i.p. with 0.5 ml of a 10% brei suspension. AM-DACHP, dissolved in 5% dextrose solution, was administered as a daily dose for 9 days. Animals that received no drug treatment died between 20 and 21 days.

TABLE VI. Dose-response Comparisons of Tetraplatin and AM-DACHP Against i.p.-Implanted L1210 Leukemia[a]

Compound	i.p. Dose (mg/kg)	Median T/C (%)	30-Day survivors/ total
Tetraplatin	16	–	2/6
	8	315	3/6
	4	218	1/6
	2	221	0/6
	1	123	0/6
AM-DACHP	64	315	5/6
	32	263	2/6
	16	242	1/6
	8	166	0/6
	4	134	0/6
Diluent control			0/30

[a]L1210 cells (1×10^5) were implanted i.p. into CDF_1 F mice on day 0. Drug treatments were given i.p. as single injections at the doses indicated on days 1, 5 and 9 after tumor implantation. Tetraplatin was freshly prepared daily and administered in solution in saline, while AM-DACHP was administered in aqueous solution. Animals that received no drug treatment died between 8 and 11 days.

compound appeared highly schedule-dependent. Its activity can be greatly enhanced when administered i.p. on a multiple dose treatment as observed also for other DACH analogs [7].

The preliminary biological results obtained so far are indicative of the high antitumor potency of AM-DACHP which warrants further pharmacological investigation.

Finally, we would like to stress that, in addition to the chemical stability and high water solubility of AM-DACHP and its platinum analogs, the amine group in position 2 of the malonato ligand can be utilized in order to anchor the compounds to carrier molecules, either synthetic or natural, or even to macromolecules such as polypeptides or proteins. The covalent attachment of these complexes to several steroid-hormones has already been accomplished in our laboratories [29]. Since the success of this approach is highly dependent upon the appropriate choice of the carrier molecule, the possibility of developing platinum drugs specific for a particular tumor system might necessitate the investigation of an extensive number of different conjugates. Mutual collaboration between research groups is therefore highly recommended. We do believe that AM-DACHP and its aminomalonato platinum analogs can be highly exploited for the development of selective targeted platinum drugs.

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