Preparation, characterization and antileukemic properties of diaminemalonatoplatinum(II) complexes tethered to ferrocene

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Abstract

In search for new antitumor agents with target specificity we have prepared four new complexes in which diaminemalonatoplatinum(II) moieties are covalently tethered to ferrocene — and organ specific biological carrier. The four $PtAm_2$[(ferrocenemethyl)propanedioic acid] complexes (where $Am_2 = (NH_3)_2$, bis(aminocyclobutane), *cis*- and *trans*-1,2-diaminocyclohexane) were characterized by ^{195}Pt NMR spectroscopy and by elemental analysis. Their activity was assessed *in vitro* against P388 leukemia cells. They showed considerable activity (ED_{50} in the 5–45 μM range) though to a smaller extent than *cis*-diamminedichloroplatinum(II). They are, however, more active than the previously reported complexes in which a bis(phosphinecatecholato)platinum(II) moiety was tethered to ferrocene or to ruthenocene.

Introduction

cis-Diamminedichloroplatinum(II) (*cis*-DDP) is a highly effective antitumor agent which is in broad clinical use against testicular, ovarian, head and neck cancers [1–3]. Despite its international application, it still suffers from some major drawbacks with include: a narrow spectrum of activity, nephrotoxicity, and from the resistance acquired by the tumor cells [4]. Nephrotoxicity can be significantly reduced by replacing the two *cis*-oriented chloride ligands with a dicarboxylate moiety such as malonate (as in Malonatoplatin) or cyclobutyldicarboxylate (as in Carboplatin) [5]. Attempts to increase the spectrum of activity by further modification of the compounds, and by attaching the platinum complexes to conventional molecular carriers which can direct the platinum complex to a specific organ, have so far met with limited success [6].

Ferrocene and some of its derivatives are highly selective molecular carriers (see, for example, ref. 7) that possess antineoplastic properties [8]. Therefore, conjugates of ferrocene and antitumor platinum drugs should be of particular interest. So far, however, only a few examples of such bimetallic complexes have been reported [9].

Recently [10] we prepared a triphenylphosphine-stabilized ferrocene bound platinum–catecholato complex and studied its biodistribution. The metallocene proved to navigate the drug almost entirely to the liver and the spleen. Unfortunately, this bimetallic complex had only negligible antitumor activity.

Thus, we decided to combine the proven targeting of the metallocene with a second generation platinum drug in order to obtain a new antitumor agent with target specificity. In this paper we report the synthesis, characterization and anticancer properties against P388 leukemia cells of four novel complexes in which a diaminedicarboxylateplatinum(II) is tethered to ferrocene.

Experimental

The ^1H NMR spectra of the various compounds were taken on a Bruker WP-200 instrument. ^{195}Pt NMR spectra were measured at 64.374 MHz on a Varian VXR-300s spectrometer equipped with a 5 mm computer switchable probehead. Typical acquisition parameters include a 100 000 Hz sweep width, 7 μs pulse

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width and 200 Hz line broadening. The ^{195}Pt chemical shifts were referenced externally to K_2PtCl_4 in D_2O at −1624 ppm. For further details see ref. 11.

General procedure for the preparation of cis-[(ferrocenemethyl)propanedioate]aminoplatinum(II) complexes (5a–d)

A suspension of 0.8 mmol of the appropriate platinum complex $PtAm_2I_2$ [12], 272 mg (1.6 mmol) of $AgNO_3$ and 36 ml of triply distilled water (MeOH for the preparation of **5c**) was stirred in the dark at 20 °C for 24 h. The reaction mixture was filtered through a sintered glass funnel, the AgI precipitate was washed with 15 ml of warm H_2O (or MeOH) and the combined filtrates of $[PtAm_2(H_2O)_2]^{2+}[NO_3]_2^{2-}$ (**2**) (or $PtAm_2(MeOH)_2]^{2+}[NO_3]_2^{2-}$) [13] were added to 243 mg (0.8 mmol) of (ferrocenemethyl)propanedioic acid (**4**) (prepared according to Hauser and Lindsay from ferrocene (**1**) via trimethyl(ferrocenemethyl)ammonium iodide (**3**) [14]). The mixture was stirred at room temperature for 24 h during which aqueous KOH was added to keep the pH at 4. The products were worked up as follows. Compound **5a** (from $Pt(NH_3)_2I_2$ [15]) that precipitated as a yellow powder was washed successively with H_2O and MeOH; yield 68%. Compound **5b** (from Pt(aminocyclobutane)$_2I_2$ [16]) was dissolved in MeOH, freed from insoluble impurities by filtration, and reprecipitated with water; yield 31%. Compound **5c** (from Pt(*cis*-1,2-diaminocyclohexane)Cl_2 [17]) was obtained in 63% yield after concentration of the reaction mixture to a volume of 1 ml. Water was added and the precipitate was washed successively with H_2O and dry Et_2O. Compound **5d** (from Pt(*trans*-1,2-diaminocyclohexane)I_2 [17]) that precipitated as yellow crystals was washed successively with H_2O, MeOH and Et_2O; yield 63%. ^{195}Pt NMR data, m.p.s and elementary analyses of compounds **5a–d** are given in Table 1.

In vitro cytoxicity studies

Adriamycin sensitive P388 murine leukemia cells (S/ADR) and a subline resistant to adriamycin (R/ADR), were propagated continuously in suspension culture. Cells were grown in Rosewell Park Memorial Institute 1640 medium (Grand Island Biological Co, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 10 μM 2-mercaptoethanol (Sigma Chemical Co, St. Louis, MO), 50 units/ml of penicillin base and 50 μg/ml of streptomycin base (both from GIBCO). Cell growth was assessed by measuring cell density in a Coulter counter (Coulter Electronics Ltd, Harpenden, Hartfordshire, UK). An inoculum of cells was transferred to fresh medium once every 4 days, in order to maintain growth in an exponential phase. Initial cell density was 10^5 cells/ml and after 4 days in culture, it reached $1–2\times10^6$ cells/ml. Cell growth rates were calculated from the culture densities measured once a day for 4 days.

The sensitivity of a cell line to compounds **5a–d** was assessed as follows. Cells were cultured in the presence of various concentrations of the drug for 4 days and the slope of the log cell density versus time plot was calculated by linear regression analysis. The growth rate at each drug concentration was expressed as a percentage of the control growth rate. In this manner, dose–effect curves were produced and used to determine the compound's drug concentration effective at inhibiting the growth rate by 50%. The adriamycin ED_{50} for the drug sensitive and the drug resistant cell lines was $2–6\times10^{-8}$ and $1–2\times10^{-6}$ M, respectively. No change in the drug sensitivity of either cell line was observed during 8 years of continuous *in vitro* culture. The ED_{50} values obtained have standard errors of less than 10% of the mean. These results are summarized in Table 2.

Results and discussion

Preparation of the ferrocene-containing moiety

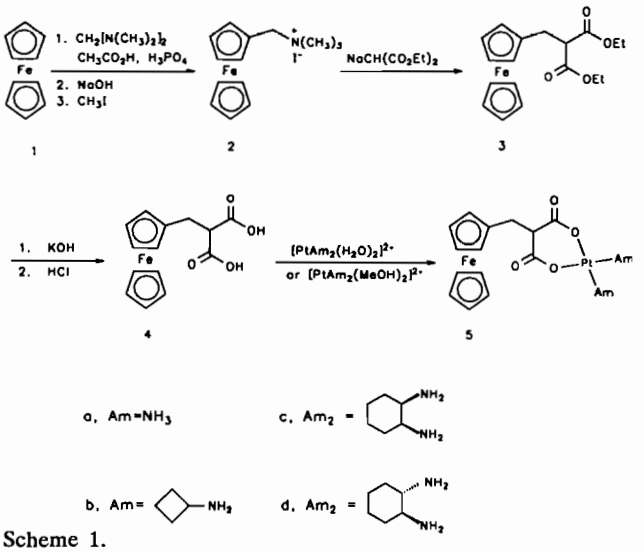
The preparation of (ferrocenemethyl)propionic acid was accomplished by modification of the method of Hauser and Lindsay [14] and is outlined in Scheme 1. This scheme is favorable since (i) it is easier to prepare the quaternary ammonium salt **2** than ferrocenemethyl chloride (from ferrocenemethanol and PCl_3 [18]), and (ii) the alkylation of sodium diethyl malonate proceeds often more smoothly with ammonium salts than with halides [19]. The only drawback of the synthesis of **4**

TABLE 1. Analytical data, decomposition temperatures and ^{195}Pt NMR for complexes **5a–d**

Compound	Formula	Found (calc.) (%)			Decomposition temperature (°C)	^{195}Pt NMR (ppm)
		C	H	N		
5a	$C_{14}H_{18}FeN_2O_4Pt$	31.21 (31.77)	3.43 (3.43)	4.81 (5.29)	109	−1790
5b	$C_{22}H_{30}FeN_2O_4Pt$	41.75 (41.45)	4.59 (4.74)	3.91 (4.39)	125	−1947
5c	$C_{20}H_{26}FeN_2O_4Pt$	39.75 (39.42)	4.32 (4.30)	4.67 (4.60)	164	−1914
5d	$C_{20}H_{26}FeN_2O_4Pt$	39.95 (39.42)	4.31 (4.30)	4.09 (4.60)	177	−1943

TABLE 2. Antileukemic properties of *cis*-DDP and of complexes **5a–d**

Compound	ED_{50} (P388) (μM)	
	S/ADR	R/ADR
cis-DDP	0.19	1.4
5a	5	45
5b	12	20
5c	10	>10
5d	10	>10



Scheme 1.

is the sluggish KOH-promoted hydrolysis of **3** that furnishes the dicarboxylic acid only in moderate yield.

Preparation of the bimetallic complexes

The diaminediiodoplatinum(II) complexes, $PtAm_2I_2$, where Am_2 is $(NH_3)_2$, bis(aminocyclobutane) and *trans*-1,2-diaminocyclohexane, were prepared by the method of Dhara [15] and subsequently converted with the aid of 1.98 equiv. of $AgNO_3$ into the appropriate reactive diaqua species (mostly of formula $[PtAm_2(H_2O)_2]^{2+}[NO_3]_2^{2-}$). Interaction of the latter compounds with **4** resulted in the formation of *cis*-[(ferrocenemethyl)propanedioate]diamineplatinum(II) complexes **5a, 5b** and **5d** that separated from the reaction mixtures. As the reaction progressed, the pH dropped as the result of the deprotonation of the dicarboxylic acid. Thus, it was necessary to add continuously small amounts of a 0.1 M KOH solution to maintain the pH at 4.

The bimetallic complex **5c** could not be obtained by interaction of **4** and the corresponding diaqua platinum species in water. The synthesis was, however, successful when Pt(*cis*-diaminocyclohexane)$_2$Cl$_2$ was reacted with $AgNO_3$ in MeOH, and the resulting methanol complex treated with **4** in the same solvent. Complex **5c** was then precipitated by addition of water. Complexes **5a–d** were characterized by ^{195}Pt NMR spectroscopy. The displayed resonances given in Table 1 are in good agreement with the literature values for these types of compounds [11, 20].

Biological studies

The ED_{50} values for compounds **5a–d** listed in Table 2 indicate that the complexes have considerable activity against both adriamycin sensitive and adriamycin resistant P338 murine leukemia cells. The values are, however, higher than those for *cis*-DDP. Complex **5a** was shown to be approximately twice as potent as **5b, 5c** and **5d** against adriamycin sensitive cells, but substantially less active against the adriamycin resistant cell culture. The isomeric complexes **5c** and **5d** are equally active against both kinds of the leukemia cells.

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