Preparation and anticancer activity of two tryptamine derived platinum complexes

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Abstract

In search for new antitumor drugs with target specificity [4-[10-(1*H*-indol-3-yl)-4,7-dioxo-3,8-diazadecyl]-1,2-benzenediolato(2–)-*O,O'*]bis(triphenylphosphine)platinum (**3**) and L-[4-(9-carboxy-10-(1*H*-indol-3-yl)-4,7-dioxo-3,8-diazadecyl]-1,2-benzenediolato(2–)-*O,O'*]bis(triphenylphosphine)platinum methyl ester (**4**) were prepared by condensation of [4-(6-carboxy-4-oxo-3-azahexyl)-1,2-benzenediolato(2–)-*O,O'*]-bis(triphenylphosphine)platinum (**2**) with tryptamine and tryptophane methyl ester, respectively. Compounds **3** and **4** were found to have cytotoxic activity against MDA-MB 231, a human breast cancer cell line, albeit in a somewhat lower effectiveness than that of the *cis*-diamminedichloroplatinum(II) drug.

Introduction

Previously we have shown that upon attachment of molecular carriers, such as steroidal hormones, to *cis*-diamminedichloroplatinum(II) complexes the antitumor properties are usually retained, and the effectiveness of the drug is increased [1, 2]. Some of the navigators were found, by labelling experiments, to direct the platinum metal almost exclusively towards a single organ [3]. Among the oldest biological carriers that have been bound to cytotoxic compounds are amino-acid derivatives [4]. However, many of the amino-acid conjugates are labile [5, 6] and in our experience, fail to facilitate transport of the drug to the required target.

In this paper we report the synthesis and the activity against MDA-MB 231 breast carcinoma cells of two highly stable platinum(II)-catecholato complexes [7] with attached tryptamine carriers. As these carriers are essential building blocks for the biosynthesis of serotonine [8, 9], they are expected to direct the platinum drug preferentially to the serotonine rich tumor cells in the gastrointestinal tract [10]. The complex is also expected to penetrate through the blood brain barrier, which highly limits the introduction of *cis*-DDP [11–14].

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Experimental

[4-(6-Carboxy-4-oxo-3-azahexyl)-1,2-benzenediolato(2–)-O,O']bis(triphenylphosphine)platinum (2)

A mixture of 100 mg (1 mmol) of succinic anhydride and 0.12 ml (0.87 mmol) of triethylamine was added to a solution of 600 mg (0.69 mmol) of [4-(2-aminoethyl)-1,2-benzenedioilato(2–)-*O,O'*]-bis(triphenylphosphine)platinum (**1**) [7] in 12 ml of CH_2Cl_2. The mixture was stirred at room temperature under argon atmosphere for 60 min. Dilution with 250 ml of CH_2Cl_2 followed by acidification with aqueous $KHSO_4$ to pH 3.5, washing with water and removal of the solvent under reduced pressure afforded **2** as a tan solid that was purified by addition of ether. Yield 600 mg (89%); m.p. (dec.) 195–196 °C. IR (KBr): 1720 (C=O), 1650, 1560 (HNC=O), 1480, 1265 (C–O) cm^{-1}. 300 MHz ^1H NMR ($CDCl_3$): δ 2.360 (m, 2H, CH_2CONH), 2.470 (m, 2H, CH_2COOH), 2.583 (t, 2H, $J=6.7$ Hz, CH_2CH_2NH), 3.368 (dt, 2H, $J_d=5.7$ Hz, $J_t=6.7$ Hz, CH_2CH_2NH), 5.900 (t, 1H, $J=5.7$ Hz, CH_2NH), 6.182 (dd, 1H, $J_{3,5}=1.8$ Hz, $J_{5,6}=7.9$ Hz, aromatic-H5), 6.261 (d, 1H, $J_{3,5}=1.8$ Hz, aromatic-H3), 6.367 (d, 1H, $J_{5,6}=7.9$ Hz, aromatic-H6), 7.12–7.55 (m, 30H, PC_6H_5). *Anal.* Calc. for $C_{48}H_{43}NO_5P_2Pt$: C, 59.38; H, 4.46; N, 1.44. Found: C, 59.10; H, 4.26; N, 1.28%.

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[4-[10-(1H-Indol-3-yl)-4,7-dioxo-3,8-diazadecyl]-1,2-benzenediolato(2–)-O,O']-bis(triphenylphosphine)-platinum (3)

To a solution of 210 mg (0.216 mmol) of **2**, 44 mg (0.216 mmol) of dicyclohexylcarbodiimide (DCC) in 2 ml of CH_2Cl_2 was added a solution of 42.5 mg (0.216 mmol) of tryptamine hydrochloride and 43 mg (0.280 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in 0.9 ml of the same solvent. The mixture was stirred under argon for 4 h at 0 °C and then for 10 h at room temperature. Treatment with 70 ml of CH_2Cl_2 and 25 ml of H_2O was followed by acidification with aqueous $KHSO_4$ to pH 3.5. The organic layer was washed with water, dried and concentrated. PLC chromatography on neutral alumina using mixtures of CH_2Cl_2 and MeOH (from 3 to 50% MeOH) as eluent gave crude **3**. Further purification was accomplished by dissolving the complex in anhydrous acetone, removal of traces of dicyclohexylurea by filtration and recrystallization from ether. Yield 33 mg (13%); orange crystals of m.p. 140–142 °C. IR (KBr): 1640, 1520 (HNC=O), 1480, 1275 (C–O) cm^{-1}. 200 MHz ^1H NMR ($CDCl_3$): δ 2.223 (m, 4H, CH_2CO), 2.532 (t, 2H, $J=6.3$ Hz, $CH_2C_6H_3$), 2.872 (t, 2H, $J=6.6$ Hz, $CH_2C_8H_6N$), 3.267 (dt, 2H, $J_d=5.1$, $J_t=6.3$ Hz, $CH_2CH_2C_6H_3$), 3.479 (dt, $J_d=5.3$, $J_t=6.6$ Hz, $CH_2CH_2C_8H_6N$), 5.511 (t, 1H, $J=5.1$ Hz, $NHCH_2CH_2C_6H_3$), 6.133 (dd, 1H, $J_{3,5}=1.9$, $J_{5,6}=8.0$ Hz, phenyl-H5), 6.159 (t, 1H, $J=5.3$ Hz, $NHCH_2CH_2C_8H_6N$), 6.269 (d, 1H, $J_{3,5}=1.9$ Hz, phenyl-H3), 6.392 (d, 1H, $J_{5,6}=8.0$ Hz, phenyl-H6), 6.692 (d, 1H, $J=2.1$ Hz, indanyl-H2), 7.00–7.55 (m, 34H, indanyl-H4, -H5, -H6, -H7 and PC_6H_5), 8.722 (br s, 1H, indanyl-NH). 64 MHz $^{195}Pt\{^1H\}$ NMR ($CDCl_3$, K_2PtCl_4 as external standard): δ −4096 (t, $J_{Pt,P}=3566$ Hz). *Anal.* Calc. for $C_{58}H_{53}N_3O_4P_2Pt$: C, 62.58; H, 4.80; N, 3.77. Found: C, 62.26; H, 5.03; N, 3.43%.

L-[4-[9-Carboxy-10-(1H-indol-3-yl)-4,7-dioxo-3,8-diazadecyl]-1,2-benzenediolato-(2–)-O,O']-bis(triphenylphosphine)platinum methyl ester (4)

A solution of 60 mg (0.212 mmol) of L-tryptophane methyl ester hydrochloride and 30 mg (0.30 mmol) of triethylamine in 2.6 ml of CH_2Cl_2 was mixed with a solution of 206 mg (0.212 mmol) of **2** and 50 mg (0.237 mmol) of DCC in 2.5 ml of CH_2Cl_2. The mixture was stirred under argon for 15 h, diluted with 100 ml of CH_2Cl_2, washed with water, dried and concentrated under reduced pressure. The residue was digested with a mixture of 0.5 ml of CH_2Cl_2 and 4 ml of anhydrous acetone. Solid dicyclohexylurea was filtered off and the filtrate concentrated and chromatographed twice on alumina that had been deactivated with 18% of water (activity I), using CH_2Cl_2–MeOH mixtures (from 0 to 50% MeOH) as eluent. Yield 50 mg (20%) of **4**; m.p. 144–146 °C (from ether); $[\alpha]_{546}^{20}+3.4\pm0.1°$ (c, 0.47, CH_2Cl_2). IR (KBr): 1735 (C=O), 1655, 1530 (HNC=O), 1485, 1275 (C–O) cm^{-1}. 200 MHz ^1H NMR ($CDCl_3$): δ 2.008 (m, 2H, CH_2CO), 2.335 (m, 2H, CH_2CO), 2.546 (t, 2H, $J=5.8$ Hz, CH_2CH_2NH), 3.256 (ABX pattern, 2H $J_{AB}=15$, $J_{AX,BX}=6.9$ Hz, CH_2CH), 3.279 (dt, 2H, $J_d=5.0$, $J_t=5.8$ Hz, CH_2NH), 3.676 (s, 3H, CH_3), 4.828 (dt, 1H, $J_d=5.1$, $J_t=6.9$ Hz, $CHCH_2$), 5.382 (t, 1H, $J=5.0$ Hz, CH_2NH), 6.158 (dd, 1H, $J_{3,5}=1.8$ Hz, $J_{5,6}=$ phenyl-H5), 6.286 (d, 1H, $J_{3,5}=1.8$ Hz, phenyl-H3), 6.422 (d, 1H, $J_{5,6}=8.2$ Hz, phenyl-H6), 6.424 (d, 1H, $J=5.1$ Hz, CHNH), 6.626 (d, 1H, $J=2.1$ Hz, indanyl-H2), 6.98–7.54 (m, 34H, indanyl-H4, H5, H6, H7 and PC_6H_5), 8.926 (br s, 1H, indanyl-NH). 64 MHz $^{195}Pt\{^1H\}$ NMR ($CDCl_3$, K_2PtCl_4 as external standard): δ −4096 (t, $J_{Pt,P}=3566$ Hz). *Anal.* Calc. for $C_{60}H_{55}N_3O_6P_2Pt$: C, 61.53; H, 4.73; N, 3.59. Found: C, 61.24; H, 4.70; N, 3.53%.

Cell cultures

A human breast carcinoma cell line MDA-MB 231 [15] was cultivated in Earle's based minimal essential medium supplemented with 10% fetal bovine serum (Grand Island Biological Co. NY), non-essential amino acids, 2 mM glutamine, 10 U/ml of penicillin and 10 mg/ml of streptomycine. Cells (in monolayers) were subcultured following suspension in a 0.1% EDTA solution in phosphate buffered saline (PBS) without calcium and magnesium and incubation in a humidified 5% CO_2 incubator at 37 °C. For growth inhibition studies, exponentially growing cells were suspended, diluted in the above medium and plated into 1.6 cm, 24 wells, tissue culture plates; approximately $2-3\times10^4$ cells in each well. After incubation of the cells for about 24 h, to reach an exponential growth phase, the appropriate platinum complex was added. Compounds **3** and **4** were dissolved in a DMSO-PBS solution and further diluted in the medium. The final concentration of DMSO in the medium was <0.6% which *per se* did not inhibit growth. The control drug *cis*-DDP was dissolved in PBS. The cells were then incubated with the drugs for 16 h, washed twice with PBS and reincubated in a drug-free medium for 48–70 h. The cells were quantitated by counting in a hemocytometer after suspension of the monolayers in the above PBS-EDTA solution.

Thymidine incorporation experiments

The drugs were added into the exponentially growing cells (at almost confluency) in 24 well plates and after incubation for 3 h the drug treatment was terminated as described above. [^3H]Thymidine (60

pmol/0.4 μCi) was added in 1 ml of serum-depleted medium, and the cells were incubated at 37 °C for 1 h. Thymidine incorporation was terminated by three washings of the cell layers with ice-cold PBS, and the radioactivity determined in the cold 5% trichloroacetic acid precipitate dissolved in 1 N NaOH. The solutions were neutralized with 1 N HCl and 0.35 ml samples were added to 3.5 ml of a Triton–toluene scintillation fluid (prepared from 1 l of toluene, 0.5 l of Triton X-100, 8.25 g PPO and 0.15 g POPOP) and counted in a Packard liquid scintillation spectrometer.

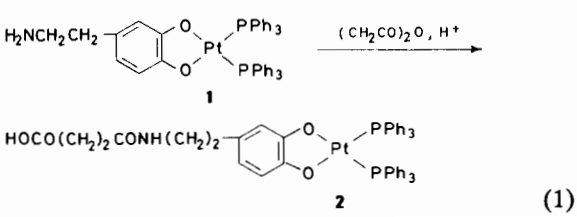
Results and discussion

Preparation of the complexes

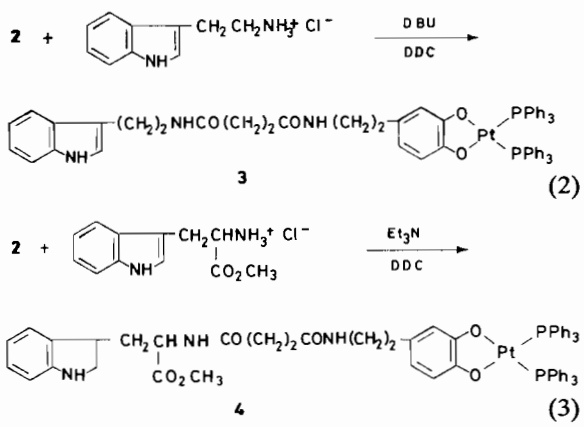
In analogy to our previous studies on the preparation of steroidal–*cis*-platinum(II) compounds [1] we attached the tryptamine derivatives to a chemically stabilized platinum–catecholamine complex. The side chain of the antitumor drug [4-(2-aminoethyl)-1,2-benzenediolato(2–)-*O*,*O'*]bis(triphenylphosphine)-platinum (**1**) (prepared as described [7] from *cis*-dichlorobis(triphenylphosphine)platinum(II) and L-dopamine) was extended by reaction with succinic anhydride in the presence of Et_3N (eqn. (1)). The amidic acid complex **2**, so formed, was then treated with either tryptamine–HCl or with tryptophane methyl ester–HCl in the presence of a base and DCC to give **3** and **4**, respectively (eqns. (2) and (3)).

It is remarkable that while tryptophane methyl ester is smoothly liberated from its hydrochloride by reaction with excess Et_3N, tryptamine hydrochloride gives the free base only in the presence of a stronger base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

The central succinyl moiety in **3** as well as in **4** serves as a spacer that provides complete separation between the anticancer platinum component and the biological navigator. The spacing is also likely to prevent undesired complexation of the tryptamine moiety to the metal by which the navigating properties of the carrier might have been negatively affected (cf. ref. 16).



(1)



The structures of **3** and **4** were established by elemental analyses, and by their IR and ^{1}H NMR spectra (see 'Experimental'). The close similarity of the ^{195}Pt NMR spectrum of **3** and that of **4** ($\delta = -4096$ with a ^{195}Pt–^{31}P coupling constant of 3566 Hz for both compounds [17]) suggests a similar chemical environment of the platinum nucleus in both complexes.

Biological studies

Experiments were performed to compare the growth inhibitory effect of the newly synthesized complexes with that of *cis*-DDP. It was found that the cytotoxicity of **3** and **4** for MDA-MB 231 cells is at a similar concentration range which is, however, lower than that of *cis*-DDP (Table 1). Our results for *cis*-DDP are in good agreement with those previously reported [18]. The lower effectiveness of **3** and **4** as compared to that of *cis*-DDP can be rationalized by the limited water-solubility of the former compounds.

Results obtained in studies on DNA synthesis, assessed by incorporation of [^{3}H]thymidine into the trichloroacetic acid-insoluble fraction have shown that exposure of the cells for 3 h either to **3** or to

TABLE 1. Inhibition of growth of MDA-MB 231 cells by *cis*-DDP and the analogs **3** and **4**[a, b]

Compound	Concentration (μM)	
	ID_{25}	ID_{50}
cis-DDP	1.0	2.2
3	1.5	3.6
4	1.2	3.5

[a]The cells were drug treated for 16 h, then washed twice with PBS and reincubated in a drug-free medium. Further experimental details as described in 'Experimental'. [b]The figures given are mean values of three experiments in triplicate.

4 at the concentration of 10 μM does not cause any significant inhibition in incorporation of the nucleoside. This observation is in contrast to a 50% inhibition obtained upon incubation with 5 μM of *cis*-DDP.

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