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Antitumor Steroidal–*cis*-Platinum(II)–*o*-Catecholato Conjugates: Preliminary Evaluation on Breast Cancer MCF-7 Cells

OTTAVIO GANDOLFI*, JOCHANAN BLUM

Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

and FREDERIKA MANDELBAUM-SHAVIT

Department of Bacteriology, Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel

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*Six new steroidal–*cis*-platinum(II)–*o*-catecholato complexes (5–9 and 15) were prepared by treatment of either [4-(2-aminoethyl)1,2-benzenediolato(2–)-O,O']–bis(triphenylphosphine)platinum(II) or [3,4-dihydroxybenzenepropionic acid (2–)-O^3, O^4]–bis-(triphenylphosphine)platinum(II) with appropriate functionalized steroids. The biological effect of the air-stable conjugates on a human breast tumor cell line, MCF-7, was compared with that of* cis*-dichlorodiaminoplatinum(II) (*cis*-DDP). The activity of the new compounds proved to be of the same order of magnitude as* cis*-DDP.*

Introduction

The discovery of the powerful antitumor properties of *cis*-dichlorodiamino-platinum(II)(*cis*-DDP) [1], has led to the synthesis of a large number of biologically active transition metal complexes [2]. The clinical superiority of the platinum complexes over other antineoplastic agents has been demonstrated in the treatment of several types of tumors [3]. However, the high toxicity of this compound limits its effective use in cancer chemotherapy.

It has been shown previously that receptor-proteins specific for estrogen, glucocorticoids and androgens in breast cancer cells [4], and for glucocorticoids in renal cancer tissue [5], are present in higher concentration in the latter than in the normal tissue. Human meningiomas as well as several forms of lymphatic leukemia also retain an endocrine response mechanism [6, 7]. It therefore appears to us that binding the antitumor platinum complex to a hormonally active steroid may increase the drug specificity for hormone-dependent tumors and consequently will allow its effective use at lower concentrations. Improved therapeutic indexes have already been found for alkylating agents bound to steroids [8]. A similar approach, based on the use of estrogen-bridged purines, against P388 murine leukemia and adriamycin-resistant subline P388/ADR has recently been reported [9].

The synthesis of such hormone-anchored platinum compounds would require the provision of the two units (*i.e.* the transition metal complex and the lipophilic carrier) with a connecting handle, so that the resulting modified bioorganic molecule may still retain a high binding affinity towards the specific receptor-protein.

In our previous papers we reported the synthesis of a number of platinum group metals coordinated to functionalized *o*-catechols [10–12]. We have shown that the presence of a free amine or carboxyl function in the *o*-catecholato ligand is a convenient tool for covalently coupling either simple organic molecules [11], or more elaborate structures, such as derivatives of estrone, estradiol and testosterone [13]. The *cis*-platinum(II)–*o*-catecholato complexes were found already to have marked cytotoxicity on the growth of L 1210 leukemia cells [14].

In the present paper we report the synthesis of new steroidal–*cis*-platinum(II) complexes and a preliminary evaluation of the activity of some of these compounds against a human breast tumor cell line, MCF-7.

Experimental

Infrared spectra were recorded with a Perkin-Elmer 457 spectrophotometer, solid samples were run as KBr pellets. ^{1}H NMR spectra were obtained using a WH 300 Bruker spectrometer, $CDCl_3$ was used as solvent and TMS as internal standard. Microanalyses were performed by the microanalytical laboratories of our university.

All the reactions were performed under an argon atmosphere. The subsequent work-up of the reaction mixture was carried out in air. Reagent-grade

*Author to whom correspondence should be addressed.

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materials were used throughout the experiments. Solvents, unless analytical grade, were purified as described in the literature [15].

Cis-dichlorodiamino platinum(II) (*cis*-DDP) [16], [3,4-dihydroxybenzene-propionic acid (2−)-O^3, O^4]bis(triphenylphosphine)platinum(II) *1* [14], [4-(2-aminoethyl)1, 2-benzenediolato(2−)-O,O']bis-(triphenylphosphine)platinum(II) *2* [14], 17β-amino-estra-1,3,5(10)-trien-3-o1 *3* [17], and [estra-1,3,5-(10)-trien-3,17β-diol-3-O-[N-[2-(3,4-dihydroxyphenyl) ethyl]acetamidato(2−)]]bis(triphenylphosphine)platinum(II) *4* [13] were prepared as previously reported.

General Synthesis of [3,7,12-trioxo-5β-cholan-24-[N-[2-(3,4-Dihydroxyphenyl)ethyl]carboxamidato(2−)]] bis(triphenylphosphine)platinum(II) 5, *[3α-hydroxy-5β-cholan-24-[N-[2-(3,4-dihydroxyphenyl)ethyl]carboxamidato(2−)]]bis(triphenylphosphine)platinum-(II)* 6, *[3α,12α-dihydroxy-5β-cholan-24-[N-[2-(3,4-dihydroxyphenyl)ethyl]carboxamidato(2−)]]bis(triphenylphosphine)platinum(II)* 7, *[preg-4-en-3-one-20β-[N-[2-(3,4-dihydroxyphenyl)ethyl]carboxamidato(2−)]]bis(triphenylphosphine)platinum(II)* 8, *and [3-oxoandrost-4-en-17-[N-[2-(3,4-dihydroxyphenyl)-ethyl]carboxamidato(2−)]]bis(triphenylphosphine)-platinum(II)* 9

In a typical preparation, 0.4 mmol of dicyclohexylcarbodiimide (DCC), dissolved in 1 mL of THF, was added at 0 °C to a 3 mL THF solution, containing a stoichiometric amount of N-hydroxysuccinimide (NHS) and of the appropriate steroid (3,7,12-trioxo-5β-cholan-24-oic acid *10*, 3α-hydroxy-5β-cholan-24-oic acid *11*, 3α,12α-dihydroxy-5β-cholan-24-oic acid *12*, preg-4-en-3-one-20β-carboxylic acid *13*, 3-oxo-androst-4-en-17β-carboxylic acid *14*). The solution was stirred for 2 h at 0 °C and overnight at room temperature. The white precipitate of N,N'-dicyclohexylurea was filtered off. The filtrate was concentrated under reduced pressure and the residue dissolved in CH_2Cl_2 and filtered from remaining traces of N,N'-dicyclohexylurea. The filtrate was evaporated to dryness and the white solid dried *in vacuo*. The resulting active ester was used in the next step without further purification. A 2 mL THF solution, containing a stoichiometric amount of the appropriate active ester, was added to a 3 mL THF solution, containing 0.172 mmol of *2* and 3.0 mmol of triethylamine at 0 °C. The mixture was stirred for 2 h at 0 °C and for 16 h at room temperature. The solid was filtered off and the solution evaporated to dryness. The product was washed with water on a synthered filter, dried *in vacuo* and chromatographed on deactivated alumina (deactivation being 15% for *5*, 20% for *6*, 24% for *7* and 18% for *8* and *9*) eluting with increasing amounts of CH_2Cl_2 in C_6H_6 (compounds *6–9*), C_6H_6/CH_2Cl_2 (1:1) followed by 100% CH_2Cl_2 and 100% EtOAc (compound 5). The eluted solution was concentrated under reduced pressure and rechromatographed on a short silica gel column, with CH_2Cl_2/MeOH (33:1). The eluate was evaporated and the orange microcrystalline solid washed with ether.

[Estra-1,3,5(10)-trien-3-o1-17β-amino-17β-N-[3,4-dihydroxybenzenepropanamidato(2−)-O^3,O^4]]bis-(triphenylphosphine)platinum(II) 15

To a solution of 0.44 mmol of *3* in 3 mL of THF were added successively at 0 °C 0.2 mL of triethylamine and a solution of 0.23 mmol of the active ester derivative of *1* [11] in 3 mL of THF. The reaction mixture was stirred first at 0 °C for 1 h and then at room temperature overnight. After removal of the solvent under reduced pressure the solid was washed on sintered filter successively with water, 0.5 N aqueous HOAc and water. The product was chromatographed on 18% deactivated alumina eluting with C_6H_6/CH_2Cl_2 (1:1). The eluted solution containing the product was concentrated under reduced pressure and filtered through a silica gel column with CH_2Cl_2 containing 1% MeOH. The orange solid was washed with ether.

Biological Assay

MCF-7 cells, obtained from Professor George Klein (Karolinska Institute, Stockholm, Sweden) were cultivated as monolayers in Earle's based minimal essential medium (MEM), supplemented with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.), 2 m*M* glutamine, 1 m*M* sodium pyruvate, non essential amino acids (Grand Island Biological Co.), 0.2 U/mL of insulin (Nordisk Insulin Laboratorium Copenhagen, Denmark), 100 U/mL of penicillin and 100 μg/mL of streptomycin. Cells were subcultured every week following resuspensions in a trypsin solution (0.25% trypsin and 0.02% EDTA in saline) and incubated in a humidified incubator with 5% CO_2 at 37 °C. The cultures were repeatedly tested and shown to be free of mycoplasma contamination.

Compounds *2*, *4* and *9* were added to the culture medium as diluted DMSO solutions. *Cis*-DDP was dissolved in Dulbecco's phosphate buffered saline (PBS). Stock solutions were prepared at concentrations 100 times higher than those in the medium. The final concentration of DMSO did not exceed 0.5%, an amount which did not affect the growth or viability of the MCF-7 cells.

Drug Susceptibility Studies

Cells, growing in log phase, were suspended in the trypsin solution, diluted in medium and 2 mL of the cell suspension, containing about 10^5 cells, and plated into 35 × 10 mm tissue culture dishes. After incubation for 16 h to allow the cells to attach, the drug was added at a volume of 20 μL and the cultures were incubated for 3 h. The drug treatment was terminated by aspiration of the medium, washing of the cell

layers with 2 mL PBS and adding 2 mL of drug-free medium. The cells were cultivated for 4 days, then resuspended in trypsin solution and counted in a hemocytometer. The viability of the cells counted was assessed using the trypan blue exclusion method.

Results and Discussion

The carboxyl-functionalized steroids *10–14* (Fig. 1) were converted into their respective active ester

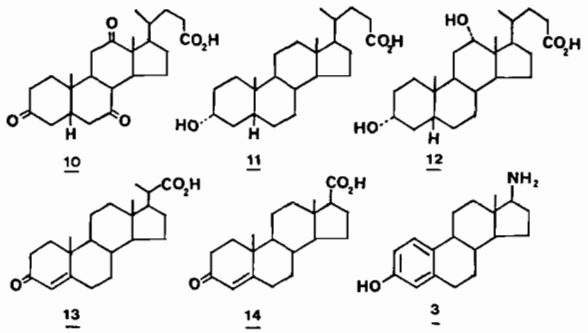
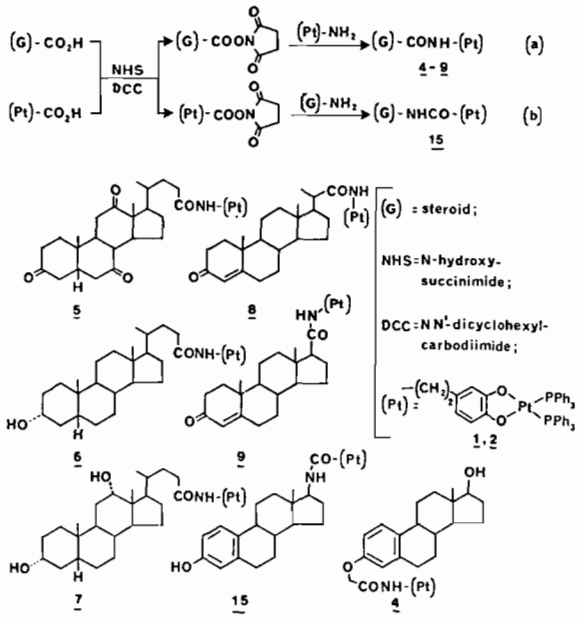


Fig. 1

derivatives by treatment with equimolar amounts of N-hydroxysuccinimide (NHS) and N,N′-dicyclohexylcarbodiimide (DCC) in THF. The active esters so formed [characterized by the $COCH_2CH_2CO$ singlets at ~2.8 ppm and by the ester carbonyl absorptions at ~1740(vs) and ~1780(m) were reacted with the amine-functionalized-platinum catecholato complex *2* in the presence of excess triethylamine. The resulting amides *5–9* showed distinct amide-carbonyl bands at ~1560 and ~1645 cm^{-1} [11–13] and a catechol-phenyl ring absorption at ~1265 and ~1480 cm^{-1} [10]. Analytical and detailed spectroscopic data for the new complexes are listed in Tables I and II respectively. The two step synthesis of *5–9* is shown by route (a) of Scheme I.

Similarly, route (b) describes the reaction of the active ester of the platinum-carboxyl functionalized complex *1* with the amine-steroid *3* (Fig. 1) which



Scheme 1

affords the corresponding adduct *15* (for analytical and spectroscopic data see Tables I and II). The analytical results for all the steroid-platinum conjugates were within ±0.4% of the theoretical values.

The steroidal–*cis*-platinum(II) complexes *5–9* and *15* proved to be chemically stable and did not deteriorate even when left for several days in solutions of CH_2Cl_2, EtOH, THF and DMSO under ambient atmosphere.

For a preliminary biological evaluation we used a human breast carcinoma MCF-7 cell line, derived from a patient with methastatic breast cancer [18]. This cell line is known to possess estrogen and progesterone receptors [19] and is estrogen-responsive by increased growth and net DNA synthesis [20, 21]. We have chosen compounds *9* and *4* [13], which are examples of antitumor *cis*-platinum catecholato complexes [14] bridged to a progesterone- and an estrogen-derivative respectively, and the results were compared with those of the unconjugated platinum-

TABLE I. Analytical Data for the New Steroidal–*cis*-Platinum(II) Complexes *5–9*, *15*; L = PPh_3, G_1 = 3,7,12-trione-5β cholanic acid; G_2 = 3α-ol-5β cholanic acid; G_3 = 3α,12α diol-5β-cholanic acid; G_4 = 4-pregnen-3-one, 20β-carboxyl acid; G_5 = 4-androsten-3-one, 17β-carboxyl acid; G_6 = estra-1,3,5(10)-trien-3-ol-17β-yl-amine.

No.	Compound	Yield %	m.p. °C	% Found (Calcd.)		
				C	H	N
5	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NH\text{-}G_1)L_2, 2H_2O$	12	177	62.95(63.23)	6.11(5.86)	1.36(1.08)
6	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NH\text{-}G_2)L_2, H_2O$	15	166	65.33(65.41)	6.30(6.39)	1.37(1.12)
7	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NH\text{-}G_3)L_2, H_2O$	28	168	64.44(64.64)	6.52(6.31)	1.03(1.11)
8	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NH\text{-}G_4)L_2, 2H_2O$	13	172	64.25(64.21)	5.87(6.05)	1.37(1.13)
9	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NH\text{-}G_5)L_2, 2H_2O$	34	168	63.66(63.72)	5.54(5.86)	1.27(1.16)
15	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2CO\text{-}G_6)L_2, 2H_2O$	17	184	63.85(63.62)	5.50(5.52)	1.32(1.18)

TABLE II. IR Bands[a] and NMR Data[b] for Steroidal–*cis*-Platinum Complexes *5–9, 15*.

Compound	Amide-carbonyl bands cm^{-1}	Catecholato bands cm^{-1}	Other frequencies cm^{-1}	NMR (δ)[c]					
				CH_2–N	CH_2–4-[*o*-catecholato]	NH	18-CH_3	19-CH_3	21-CH_3	Other resonances
5	1650s, 1560w	1480vs, 1270vs	1710vs (ketone-C=O)	2.58(t)	3.35(t)	1.67(s)	1.4(s)	1.1(s)	0.81(d)	
6	1650s, 1560w	1485vs, 1265vs		2.58(t)	3.35(t)	1.66(s)	0.63(s)	0.91(s)	0.87(d)	
7	1645s, 1565w	1485vs, 1270vs		2.58(t)	3.40(m)	1.78(s)	0.67(s)	0.91(s)	0.93(d)	
8	1640s, 1570w	1485vs, 1265vs	1670s (Ketone-C=O)	2.57(t)	3.40(m)	1.76(s)	0.68(s)	0.18(s)	1.1(d)	5.73(s, OCCH=CH)
9	1655s, 1570w	1480vs, 1265vs	1670s (Ketone-C=O)	2.57(m)	3.40(m)	1.67(s)	0.70(s)	1.17(s)		5.73(s, OCCH=CH)
15	1640s, 1550m	1480vs, 1270vs			3.80(m)	1.80(s)	0.74(s)			

[a]KBr pellets. [b]In $CDCl_3$. [c]Proton resonances for $P(C_6H_5)_3$ and 1,2-$O_2C_6H_3$ occurring as multiplets at δ = 7.5–7.32 and δ = 6.43–6.28 respectively for all the compounds.

(II)–catecholamine precursor *2*, in a first effort to evaluate the possibility of recognition by the progesterone and estradiol receptors within these cells. Since to the best of our knowledge the effect of *cis*-DDP on the MCF-7 cell line has not been determined, we considered it imperative to compare the biological activity of our compounds with that of *cis*-DDP itself.

TABLE III. Growth Inhibition Obtained with MCF-7 Cells.

Compound	(%) Inhibition at 5 μ*M*
cis-DDP	68.46 ± 0.50
2	63.72 ± 0.46
4	55.05 ± 0.40
9	37.70 ± 0.28

The values are means ± S.D. of four experiments performed in triplicate.

The results of the antitumor activity of the *cis*-platinum(II)–*o*-catecholato complexes *2*, *4* and *9* against the MCF-7 line are listed in Table III and provide an additional indication of the cytotoxicity of the bisphosphine-platinum(II) catecholamine complexes. It may be recalled that in the evaluation against L 1210 leukemia cells, the antitumor activity was suggested to be a consequence of the intracellular release of active *cis*-DDP species and/or the catalytic conversion of the coordinated *o*-catechol into the corresponding active *o*-quinonoid form [14]. In the case of the MCF-7 tumor system the percentage inhibition appears to be of the same order of magnitude as that of *cis*-DDP.

Nevertheless, on the basis of our present data, it is not possible to attribute the lower inhibitory effects of the steroidal–*cis*-platinum(II) complexes *4* and *9*, with respect to the uncoupled *2* and *cis*-DDP, to a low steroid-receptor binding affinity. Apparently the presence of an anchored steroid further decreases the solubility of the bis(triphenylphosphine)platinum (II)–*o*-catecholates in the aqueous medium, thus rendering the available data *in vitro* difficult to interpret.

Further studies with other tumor cell systems on the role of solubility vs receptor affinity using this new class of antitumor Pt–steroid complexes are presently underway.

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