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Synthesis and Biological Studies of Steroidal *cis*-Platinum(II) Complexes*

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Abstract

Several new steroidal *cis*-platinum(II) complexes (**4a**, **4b**, **4c** and **4d**) were synthesized by treatment of K_2PtCl_4 with appropriately substituted steroids. The receptor binding affinities (*RBA*) of the prepared complexes, which may be indicative of their selectivity, were evaluated along with the antitumor testing of one of these steroidal *cis*-platinum(II) complexes. The latter was found to possess activity comparable to cisplatin, with respect to both activity and therapeutic index.

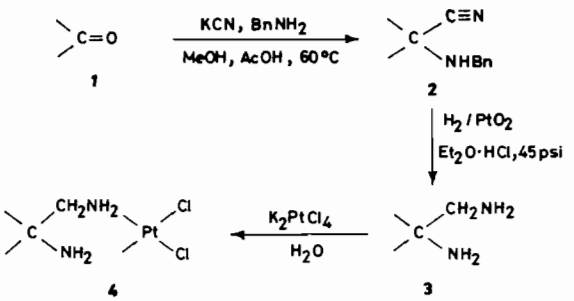
Introduction

The clinical superiority of platinum complexes over other neoplastic agents has been demonstrated in the treatment of several types of tumors [1]. However, the lack of selectivity and high toxicity of these compounds limit their effective use in cancer chemotherapy.

Taking into consideration the resistance of hormone-dependent mammary carcinoma against cisplatin, we have investigated the incorporation of dicholoroplatinum with diamino analogs of estrogenic and androgenic hormones, hoping to retain the antitumor activity of these complexes and to decrease their systematic toxicity (kidney, intestial, hemopoetic organs). This approach is based on the assumption that these *cis*-platinum(II) complexes are translocated into the nucleus of the mammary tumor cell by the steroidal hormone receptor systems, thereby causing a specific activity against the corresponding hormone-dependent cancers [2]. Efforts in this direction have also been made by other authors by linking cytotoxic agents to estrogens or antiestrogens [3]. In this paper we describe the synthesis and characterization of new steroidal platinum(II) complexes and their biological properties.

Results and Discussion

The diamino ligands of the complexes have been synthesized as dihydrochlorides via a modified Strecker reaction and subsequent hydrogenation of the received α-benzylaminonitriles formed (Scheme 1). More specifically, $\Delta^{1,3,5(10)}$-estratrien-3-ol-17-one



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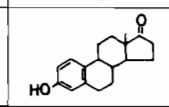
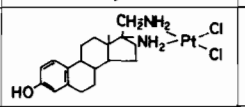
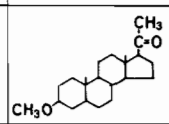
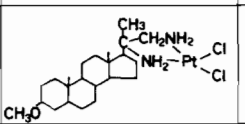
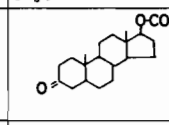
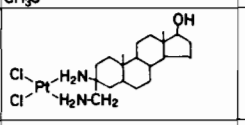
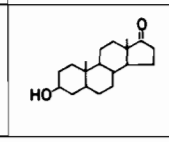
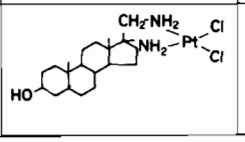
a			48 %
b			46 %
c			52 %
d			54 %

Scheme 1. 3-Methoxy-pregnan-20-one (1b) was prepared from 4-pregnene-3,20-dione by protection (MeOH, H+/DMF) of the 3-position enolate and subsequent hydrogenation (H_2, Pd/C, EtOAc). 5α-Androstan-17β-3-one propionate (1c) was prepared from Δ^4-5α-androstan-17β-3-one propionate by hydrogenation of the double bond (H_2, Pd/C, EtOAc). The ester was removed by hydrogenolysis at the diamine formation stage 2c → 3c.

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0020-1693/87/$3.50

(**1a**), 3-methoxypregnan-20-one (**1b**), 5α-androstan-17β-3-one propionate (**1c**) and 5α-androstan-3β-ol-17-one (**1d**) have been used as the carbonyl-containing precursors for the synthesis of the diamino analogs of steroidal hormones. The complexation of the above diamines (**3a**, **3b**, **3c** and **3d**) was carried out in water using potassium tetrachloroplatinate as complexation agent. The pH of the reaction was adjusted to 6.5 and monitored (with a 0.1 N NaOH solution) until the end of the reaction [4]. The *cis*-platinum(II) complexes (**4a**, **4b**, **4c** and **4d**) formed were precipitated out and purified by recrystallization from DMF/H_2O. The formation of the complexes and their configurations were verified by analysis and their compatible ^1H NMR and IR spectra. Absorptions in the IR region (Table I) reveal that the N–H stretching vibration has considerably changed upon the formation of a metal–nitrogen bond. The weak absorptions in the region of 530 cm^{-1} are characteristic of the metal–nitrogen stretching vibration. Furthermore, the two weak absorptions in the far-infrared close to 320 cm^{-1} indicate a *cis*-metal–chlorine structure [5].

TABLE I. IR Data of the Diamines and their *cis*-Pt(II) Complexes

Compound	ν(N–H) (cm^{-1})	ν(Pt–N) (cm^{-1})	ν(Pt–Cl) (cm^{-1})
3a	3280		
4a	3220	530	318, 323
3b	3300		
4b	3210	532	318, 323
3c	3380		
4c	3130	530	318, 327
3d	3310		
4d	3205	537	317, 325

Receptor Binding Affinities

The receptor binding affinities, expressed as *RBA* values (*i.e.* relation to the standard compounds = 100) are summarized in Table II.

The prepared *cis*-platinum(II) complexes may be classified in two groups on the basis of their *RBA* values. Those with low binding affinity (**4b**, **4d**) and those with moderate *RBA* values (**4a**, **4c**). Since the latter *RBA* values are from the best values reported so far in the literature for steroidal *cis*-platinum(II) complexes [2, 6], our findings may be valuable for the design and synthesis of new, more selective and potent, steroidal *cis*-platinum(II) complexes.

Anticancer Activity

Experiments for evaluation of the antitumor activity were performed only with one of the synthesized complexes. ADJ/PCG solid tumors in female Balb/C mice were used as the testing system and the steroidal *cis*-platinum(II) complex **4b** was found to possess activity comparable to cisplatin, with respect to both activity and therapeutic index. The results are depicted in Table III.

TABLE II. Receptor Binding Affinities (*RBA*) of the Synthesized Compounds

Compound	Receptor[a]	*RBA* (%)
3a	ER	~0
4a	ER	6.03
3b	PgR	0.15
4b	PgR	0.12
2c	AR	19.7
3c	AR	6.4
4c	AR	1.4
4d	AR	~0

[a]ER = estrogen receptor, PgR = progesterone receptor, AR = androgen receptor.

TABLE III. Anticancer Activity[a]

Compound	LD_{50} (mg/kg)	ID_{90}[b] (mg/kg)	Therapeutic index
4b	35	~1	15–20
cisplatin	15–20	2.9	12

[a]Solvent = oil; route = i.p.; schedule = single dose; first injection on day 20 after implant. [b]Dose for 90% tumor weight inhibition relative to controls.

Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian EM 360 (60 MHz) spectrometer using TMS as internal standard. IR spectra were obtained on a Perkin-Elmer Model 283 B (4000–200 cm^{-1}) spectrometer.

The starting materials were donated by Schering and Remek pharmaceutical companies as analytical grade reagents and were used without any further purification.

3-Hydroxy-$\Delta^{1,3,5(10)}$-estratrienyl-17β-benzylamino-17α-carbonitrile (2a)

To a solution of $\Delta^{1,3,5(10)}$-estratrien-3-ol-17-one (**1a**) (7 mM), potassium cyanide (7.1 mM) in 100 ml absolute methanol, benzylamine (8 mM) and glacial acetic acid (8.5 mM) were added. The reaction mixture was heated with stirring at 60 °C and the reaction was run for 20 h. The solvent was removed under reduced pressure and the residue was dissolved in 150 ml water–chloroform (1:1). The organic layer was separated, dried over $MgSO_4$ and the solvent was evaporated to yield a slurry. Addition of anhydrous etherial HCl gave α-benzylaminonitrile as the hydro-

chloride (white solid). Yield 70%, melting point (m.p.) 208 °C decomposition (dec.). *Anal.* Calc. for $C_{26}H_{31}ON_2Cl$: C, 73.88; H, 7.41; N, 6.54. Found: C, 73.65; H, 7.83; N, 6.74%.

20β-Amino-20α-carbonitrile of methoxypregnan-20-one (2b)

This compound was prepared as for compound **2a**. Yield 65%, m.p. 172–174 °C. *Anal.* Calc. for $C_{23}H_{38}ON_2$: C, 77.05; H, 10.68; N, 7.81. Found: C, 76.80; H, 10.82; N, 7.55%.

O-Propionatotestosteron-3-yl-17β-ol-3β-benzylamino-3α-carbonitrile (2c)

This compound was prepared as for compound **2a**. Yield 95%, m.p. 223 °C. *Anal.* Calc. for $C_{30}H_{42}O_2N_2$: C, 77.87; H, 9.15; N, 6.06. Found: C, 77.51; H, 9.35; N, 6.26%.

5α-Androstan-3β-ol-17β-benzylamino-17α-carbonitrile (2d)

This compound was prepared as for compound **2a**. Yield 88%, m.p. 189 °C (dec.) *Anal.* Calc. for $C_{27}H_{39}ON_2Cl$: C, 73.19; H, 8.88; N, 6.32. Found: C, 72.93; H, 9.05; N, 6.15%.

3-Hydroxy-$\Delta^{1,3,5(10)}$-estratrienyl-17β-amino-17α-methylamine (3a)

To a solution of 1 g 3-hydroxy-$\Delta^{1,3,5(10)}$-estratrienyl-17β-benzylamino-17α-carbonitrile hydrochlorate in 200 ml EtOH–MeOH 9:1, 5 ml of EtOH·HCl 2 N was added and the mixture was hydrogenated over platinum oxide (0.15 g) under 3 atm pressure for 4.5 h. The catalyst was removed by filtration and the solvent evaporated under reduced pressure, yielding the diamine dihydrochloride. The product was recrystallized from MeOH–Et_2O. Yield 85%, m.p. 192 °C (dec.). *Anal.* Calc. for $C_{19}H_{30}ON_2Cl_2$: C, 61.12; H, 8.09; N, 7.51. Found: C, 61.35; H, 8.26; N, 7.13%.

3-Methoxy-20β-amino-20α-methylaminopregnane (3b)

This was prepared as for compound **3a**. Yield 86%, m.p. 161–162 °C. *Anal.* Calc. for $C_{23}H_{44}ON_2Cl_2 \cdot 2H_2O$: C, 58.58; H, 10.26; N, 5.94. Found: C, 58.82; H, 10.49; N, 5.78%.

Androstan-3-yl-17β-ol-3β-amino-3α-methylamine (3c)

This was prepared as for compound **3a**. Yield 75%, m.p. 228 °C. *Anal.* Calc. for $C_{20}H_{38}ON_2Cl_2$: C, 61.05; H, 9.74; N, 7.12. Found: C, 60.90; H, 9.83; N, 6.77%.

5α-Androstan-3β-ol-17β-amino-17α-methylamine (3d)

This was prepared as for compound **3a**. Yield 88%, m.p. 219 °C (dec.). *Anal.* Calc. for $C_{20}H_{38}ON_2Cl_2$: C, 61.05; H, 9.74; N, 7.12; Cl, 18.03. Found: C, 61.25; H, 9.99; N, 6.80; Cl, 17.80%.

cis-Dichloro-[3-hydroxy-$\Delta^{1,3,5(10)}$-estratrienyl-17β-amino-17α-methylamino]platinum(II) (4a)

To a solution of 3-hydroxy-$\Delta^{1,3,5(10)}$-estratrienyl-17β-amine-17α-methyl-amine hydrochlorate in 5 ml water, a solution of potassium tetrachloroplatinate in 5 ml water was added with stirring. The pH of the reaction was monitored (and maintained) to 6.5. After 4 h the resulting white precipitate was filtered, dried and recrystallized from DMF/H_2O. Yield 79%, m.p. 243 °C (dec.). *Anal.* Calc. for $C_{19}H_{28}ON_2PtCl_2$: C, 40.29; H, 4.98; N, 4.95; Pt, 34.44. Found: C, 40.35; H, 5.03; N, 4.78; Pt, 34.70%.

cis-Dichloro-[3-methoxy-20β-amino-20α-methylaminopregnan]platinum(II) (4b)

This was prepared as for compound **4a**. Yield 82%, m.p. 308 °C (dec.). *Anal.* Calc. for $C_{23}H_{42}ON_2PtCl_2$: C, 43.94; H, 6.74; N, 4.45; Pt, 31.05. Found: C, 43.74; H, 6.72; N, 4.51; Pt, 31.23%.

cis-Dichloro[5α-androstan-3-yl-17β-ol-3β-amino-3α-methylamino]platinum(II) (4c)

This was prepared as for compound **4a**. Yield 72.30%, m.p. 283 °C (dec.). *Anal.* Calc. for $C_{20}H_{36}ON_2PtCl_2$: C, 40.94; H, 6.19; N, 4.77; Pt, 33.28. Found: C, 40.71; H, 6.08; N, 4.51; Pt, 33.49%.

cis-Dichloro-[5α-androstan-3β-ol-17β-amino-17α-methylamino]platinum(II) (4d)

This was prepared as for compound **4a**. Yield 69.5%, m.p. 166 °C (dec.). *Anal.* Calc. for $C_{20}H_{36}ON_2PtCl_2$: C, 40.94; H, 6.19; N, 4.77; Pt, 33.28. Found: C, 40.78; H, 6.33; N, 4.55; Pt, 33.52%.

Binding Affinities

The receptor binding affinities of the synthesized compounds were determined in competitive radiometric binding assays, using:

(i) For progesterone receptor: [^{3}H]R 5020 (promegestone) as a tracer, progesterone as a standard and estrogen-induced rat uterus as a source of receptor.

(ii) For estrogen receptor: [^{3}H]estradiol as tracer and standard [6] and lamb uterine cytosol as a source of receptor.

(iii) For androgen receptor: [^{3}H]R 1881 as a tracer, dihydrotestosterone as standard and ventral postal from 24 h castrate rats as a source of receptor.

Anticancer Testing

The synthesized complexes were tested against the ADS/PC6 plasma cell tumor in female Balb/C mice by the European Organization for Research and Treatment of Cancer (EORTC), Amsterdam, according to their screening protocol.

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