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Synthesis of 17β-Estradiol Platinum(II) Complexes: Biological Evaluation on Breast Cancer Cell Lines

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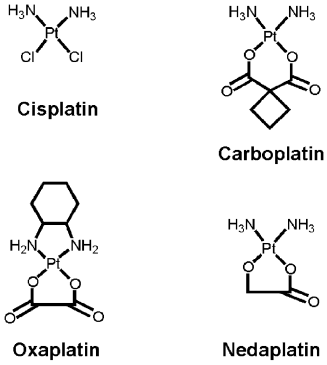
Abstract—The synthesis of a novel series of 17β-estradiol-linked platinum(II) complexes is described. The new molecules are linked with an alkyl chain at position 16α of the steroid nucleus and bear a 16β-hydroxymethyl side chain. They are made from estrone in five chemical steps with an overall yield exceeding 28%. The biological activity of these compounds was evaluated in vitro on estrogen dependent and independent (ER^+ and ER^-) human breast cancers. The derivatives incorporating a 2-(2′-amino-ethyl)pyridine ligand displayed good activity against the cell lines particularly when the connecting arm is 10 carbon atoms long.

Several platinum coordination complexes such as *cis*-diamminedichloroplatinum(II) (cisplatin) and diamine[1,1-cyclobutanedicarboxylato]-*O,O′*-platinum(II) (carboplatin) are currently used in chemotherapy of neoplastic diseases (see Scheme 1).[1] These complexes of a non-essential heavy metal, exhibit a remarkable antitumor effectiveness and a broad spectrum of activity. Recent literature reviews present a broad overview of the actual knowledge of platinum-based antitumor agents as well as their mechanisms of action.[1,2] It is generally accepted that the antitumor activity of platinum drugs is a consequence of their interaction with DNA. Cisplatin binds readily to guanine residues of DNA molecules thereby blocking replication and/or transcription.[2,3] Cisplatin has proved very successful in the treatment of a variety of human solid tumors such as genitourinary and gynecologic tumors as well as head, neck and lung tumors. Unfortunately, the development of cellular resistance to cisplatin in mammalian cells is common and is believed to occur via three main mechanisms: (a) increased efficiency of repair of platinum–DNA lesions, (b) increased detoxification by thiol containing scavenger molecules such as glutathione (GSH) and metallothionein (MT), and (c) decreased cellular uptake of the drug.[2] Its toxic effects, particularly kidney toxicity and neurotoxicity, also limit the clinical utility of the drug.[3] It is noteworthy that carboplatin is less toxic than cisplatin and can be given at a much higher dose (up to 2000 mg/dose for carboplatin as compare to a typical dose of 100 mg/day for cisplatin).[1] However carboplatin is less effective than cisplatin.[1,2]

More recently, two other platinum(II) derivatives were approved for use in some countries (see Scheme 1). (*trans*-L-diaminocyclohexane)oxalatoplatinum(II) (oxaliplatin) has been approved for the secondary treatment of metastatic colorectal cancer in France and other European countries. *cis*-Diammine-glycoloato-*O,O′*-platinum(II) (nedaplatin) has received approval for use



Scheme 1. Structure of the known platinum(II) complexes.

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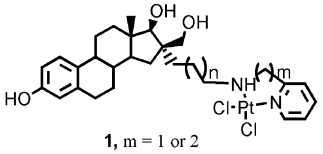
in Japan.[1] Unfortunately, oxaplatin and nedaplatin have not shown any distinct advantages over cisplatin and carboplatin. Thus, the design of novel platinum(II) complexes with a broader spectrum of activity, less toxicity and improved selectivity towards cancerous cells is still of great importance.

This paper describes the straightforward synthesis of a new family of 17β-estradiol-linked Pt(II) complexes (see general structure **1**). The new molecules bear a 16β-hydroxymethyl side chain and a platinum(II) complex locked in position 16α of the steroid nucleus. It also reports the in vitro cytotoxic activity of these compounds on estrogen dependent and independent (ER^+ and ER^-) human breast cancer cell lines.

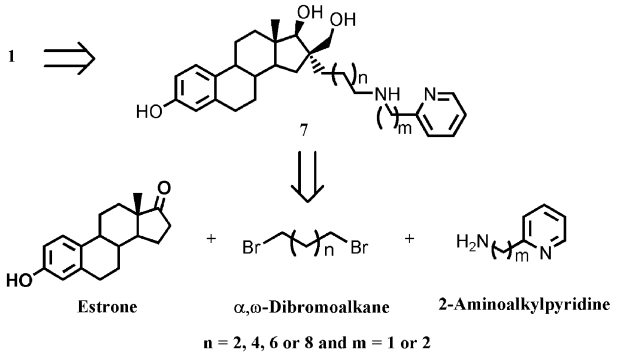


1, m = 1 or 2
n = 2, 4, 6 or 8

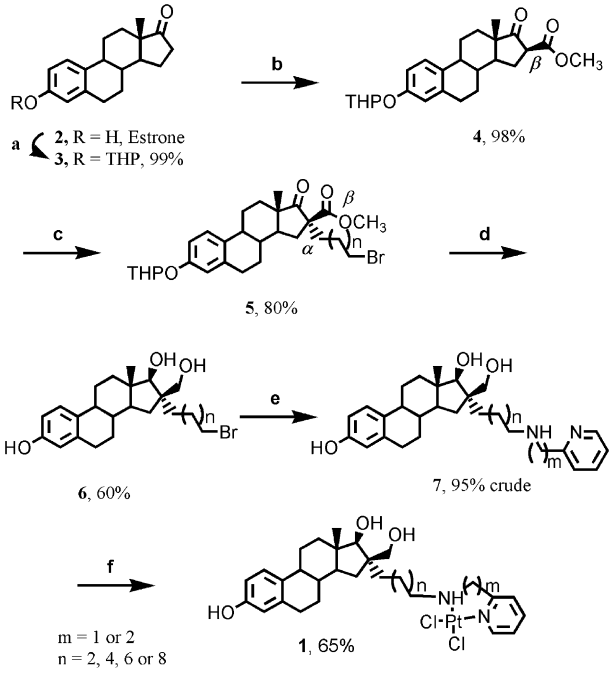
A retrosynthetic analysis of the target molecules is presented in Scheme 2. As one can observe, the Pt(II) complex **1** are derived from the 17β-estradiol aminopyridine derivative **7** upon a complexation reaction with potassium tetrachloroplatinate(II). The latter is obtained by the stepwise combination of three key components. Hence, the aminopyridine **7** can easily be prepared from estrone, a α,ω-dibromoalkane and a suitable 2-aminoaklylpyridine.

As shown in Scheme 3, the synthesis involves only five chemical steps starting from estrone (**2**) as the steroid template. The 17β-estradiol Pt(II) complexes **1** were obtained efficiently in high yield (28% overall) using a very simple reaction sequence.

Initially, estrone (**2**) was protected as a tetrahydropyranyl ether (R = THP) under standard reaction conditions. Accordingly, estrone was treated with dihydropyran in dichloromethane in the presence of pyridinium *p*-toluenesulfonate.[4] The yield of the protection reaction is 99%. The derivative **3** was transformed into the β-cetoester **4** upon treatment with dimethyl carbonate in the presence of a mixture of NaH/KH in dry tetrahydrofuran.[5,6] Derivative **4** was obtained with 98% yield. Treatment of derivative **4** with a suitable α,ω-dibromoalkane under phase transfer catalyst (PTC) reaction conditions gave compound **5** in 80% yield. The bromoalkane side chain was added to the less hindered α face of the molecule as shown by the presence of a single peak for the 18-CH_3 at δ 0.93 in the ^1H NMR spectrum and at δ 14.9 ppm in the ^{13}C NMR spectrum. Reduction of the β-ketoester moiety with lithium borohydride in dry ether at 0 °C followed by the cleavage of the tetrahydropyranyl ether of derivative **5** gave the triol **6**.[4] It was obtained in 60% overall yield as a single 17β-hydroxy isomer as shown by a sole signal for the 18-CH_3 at δ 0.89 in the ^1H NMR spectrum and at δ 12.5 ppm in the ^{13}C NMR spectrum. The stereochemistry of the 17β-hydroxy function was confirmed by comparison with ^{13}C NMR spectral data of known 17β- and 17α-estradiol derivatives.[7]

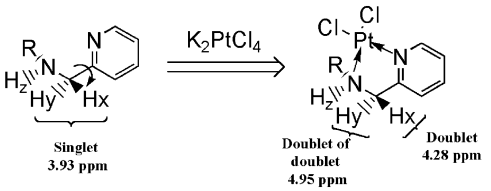


Scheme 2. Retrosynthetic analysis for the estradiol-linked Pt(II) complexes.

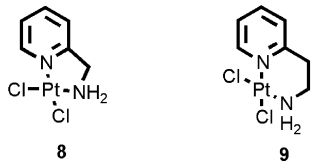


Scheme 3. Synthesis of 17β-estradiol-linked platinum(II) complexes. Reagents: (a) DHP, PPTs, CH_2Cl_2, 22 °C, 24 h, 99%; (b) KH, dimethyl carbonate, THF, reflux, 3 h, 98%; (c) $Br(CH_2)_nBr$, $Et_3N + BnCl^-$, NaOH 10% p/v, CH_2Cl_2, reflux, 20 h, 80%; (d) (1) $LiBH_4$, Et_2O, 0 °C, 3 h and 22 °C, 24 h; (2) PPTs, EtOH, 22 °C, 17 h, 60% (e) 2-aminomethyl pyridine ($m = 1$) or 2-(2′-aminoethyl)pyridine ($m = 2$), CH_3OH, reflux, 3 days, 95% crude; (f) K_2PtCl_4, DMF/H_2O (2:1), 22 °C, 2/3 days, 65%.

The final 17β-estradiol-linked Pt(II) complexes **1** were obtained in a two-step chemical sequence.[8] Firstly, the triol **6** was treated with an excess 2-aminoalkylpyridine to give derivative **7** for a yield of 80–100%. Secondly, the triol-aminopyridine intermediates were treated with potassium tetrachloroplatinate in a mixture of dimethylformamide and water to give the corresponding 17β-estradiol-linked Pt(II) complexes **1** with $m = 1$ or 2 and $n = 2$, 4, 6, 8. Consequently, the new cytotoxic molecules possess an alkyl side chain varying from 4 to 10 carbon atoms long. Complexation of the aminomethylpyridine intermediates was readily confirmed by the presence of two signals at δ 4.28 ppm and δ 4.95



Scheme 4. Complexation of the aminomethylpyridine.



Scheme 5. Structures of reference derivatives **8** and **9**.

ppm representing the methylene group locked in an heteronuclear ring system of the final products instead of the initial singlet at δ 3.93 ppm in the ^{1}H NMR spectrum of the starting material (Scheme 4).

Scheme 5 shows the structure of two known Pt(II) complexes (**8** and **9**) that were made as reference products for the biological evaluation study.[9] All new compounds synthesized were characterized by IR and NMR spectroscopy.[10]

The toxicity of the 17β-estradiol-linked Pt(II) complexes was evaluated on four human breast tumor cell lines using the MTT colorimetric assay.[11,12] The cytotoxicity of the compounds was tested along with controls (cisplatin, **8** and **9**) on both estrogen-receptor positive (ER^+, MCF-7 and ZR-75-1) and estrogen-receptor negative (ER^-, MDA-MB-231 and HS578T) human mammary carcinomas.[13] The MTT assay was performed over an incubation period of 72 h.

As shown by the MTT assays on the human breast cancer cell lines, the new Pt(II) complexes do not present any apparent specific toxicity towards ER^+ breast cancer cells (Tables 1 and 2). The reference derivatives **8** and **9** did not show any toxicity at the maximum (40 μM) concentration tested (data not shown in the tables). Hence, the linkage of this kind of cytotoxic moiety to a steroid nucleus improves the biological activity. One can speculate that a large organic portion enhance the cellular penetration of the membranes to the nucleus. The Pt(II) complexes **1**, $m=1$ are, in general, less toxic than those where $m=2$. This was previously observed with a series of triphenylethylene Pt(II) complexes.[8a] The length of the side chain seems to be optimal at $n=6$ or 8 for both types of aminopyridine analogues ($m=1$ or 2). The derivatives with a short side chain ($n=2$) are essentially inactive when compared with cisplatin. The Pt(II) complex with $m=2$ and $n=8$ is the most interesting derivative of the series. It presents an activity three to four times greater than cisplatin on all types of breast cancer cells (ER^+ and ER^-). These data confirm that the Pt(II) complex bearing the 2-(2′-aminoethyl)pyridine ligand presents higher activity than those bearing the 2-aminomethylpyridine ligand.

Molecular mechanics (MM2) and semi-empirical quantum mechanical calculations (AM1)[14] were used to study derivatives **1** ($m=1$, 2), **8** and **9**. The conformation studies were calculated in vacuo. It is observed that the amino group of the reference compounds **8** and **9** possess an electron density of −0.418 and −0.404, respectively. For the corresponding E_2–Pt(II) complexes, the amino group have an electron density of −0.357 and −0.348. This is in agreement with the theory as a secondary amino group is more basic than a primary amino group. Hence, there is a better coordination of the secondary amino groups with the platinum atom. However, the predicted bond length, resulting

Table 1. Inhibitory concentration[a] of 1 ($m=1$) and of cisplatin on both ER^+ and ER^- breast cancer cell lines

Breast cancer cell lines	ER	Cisplatin	1 $n=2$	1 $n=4$	1 $n=6$	1 $n=8$
MDA-MB-231	−	12.7±0.6	NR	18.5±0.4	9.2±0.2	12.7±4.7
HS578T	−	9.1±1.2	NR	28.0±5.3	12.4±1.5	17.3±3.1
MCF-7	+	16.7±5.7	NR	31.5±3.7	13.4±3.2	13.4±3.8
ZR-75-1	+	9.3±1.5	31.5±4.1	16.4±1.3	9.3±2.3	14.0±3.7

[a]Inhibitory concentration (IC_{50}, μM) as obtained by the MTT assay. Experiments were performed in duplicates and the results represent the mean±SEM of three independent experiments. NR = IC_{50} > 40 μM.

Table 2. Inhibitory concentration[a] of 1 ($m=2$) and of cisplatin on both ER^+ and ER- breast cancer cell lines

Breast cancer cell lines	ER	Cisplatin	1 $n=2$	1 $n=4$	1 $n=6$	1 $n=8$
MDA-MB-231	−	12.7±0.6	NR	4.8±0.1	8.6±0.1	4.7±2.6
HS578T	−	9.1±1.2	22.9±4.8	4.1±0.1	2.7±0.7	3.5±1.1
MCF-7	+	16.7±5.7	20.0±4.6	4.3±0.3	5.8±3.0	4.0±0.6
ZR-75-1	+	9.3±1.5	15.8±5.4	5.0±1.6	4.2±1.6	2.5±0.5

[a]Inhibitory concentration (IC_{50}, μM) as obtained by the MTT assay. Experiments were performed in duplicates and the results represent the mean±SEM of three independent experiments. NR = IC_{50} > 40 μM.

Table 3. Bond angles of selected platinum(II) complexes and of cisplatin

Bond angles (°)	**8**	**1**	**9**	**1**	Cisplatin
		$m=1$, $n=8$		$m=2$, $n=8$	
LNPtN	78.23	78.86	82.1	90.36	84.86
LClPtCl	88.15	88.15	89.5	87.65	90.26
LNPyPtCl	99.04	98.79	95.2	92.53	92.46
LNPtCl	94.58	94.19	93.3	89.35	92.42

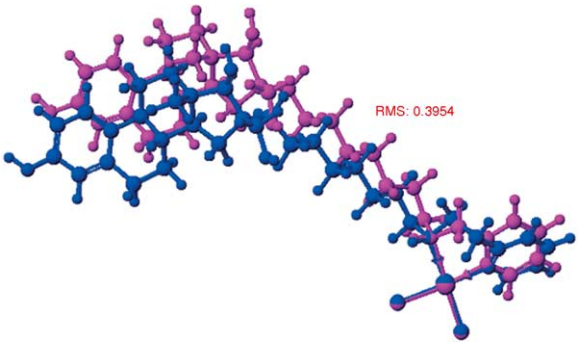


Figure 1. Superimposition of the most stable structures of E_2–Pt(II) $m=1$, $n=8$ (pink) and E_2–Pt(II) $m=2$, $n=8$ (blue) showing structural error (RMS = 0.3954).

from the combination of both factors basicity and steric hindrance, are similar on all the models studied. The difference in activity might be simply due to the conformation of the E_2–Pt(II) complexes as compared to the base derivatives. For instance, the shape of the PtN_2Cl_2 core of derivative **9** and **1** ($m=2$) is considerably different (see Table 3). Whereas, the shape of the PtN_2Cl_2 core of derivative **8** and **1** ($m=1$) is rather similar (Table 3). However, when compared to one another, the shape of the PtN_2Cl_2 core of **1** ($m=1$) and **1** ($m=2$) is significantly different (Table 3). Therefore, superimposition (Fig. 1) of the most stable conformation of derivatives **1**, $m=1$ and $m=2$, showed that they were structurally different as indicated by the RMS value (structural error) of 0.3954. The conformation of the PtN_2Cl_2 core of molecule linked to E_2 when $m=2$ could be available for binding to the DNA more readily without requiring additional energy as compared to other molecules studied and thereby present better biological activity than the references derivatives themselves.

In summary, this manuscript presents a facile synthesis of cytotoxic 17β-estradiol Pt(II) complexes. They are made form estrone in only five chemical transformations with an overall yield exceeding 28%. Using this strategy a large variety of Pt(II) complexes could easily be synthesized either with an alkyl side chain or a polyethylene glycol side chain. Furthermore, other diamine ligands could, without difficulty, be coupled to the bromide intermediate **6**. Molecular mechanics (MM2) and semi-empirical quantum mechanical calculations (AM1) showed that the combination of a side chain and the six-member ring [$N(CH_2)_2N$ Pt] to derivatives **9** induce a much greater structural change of the PtN_2Cl_2 core as compared to derivative **8**, same chain length but form a five-member ring [$N(CH_2)N$ Pt]. This could account for the discrepancies in cytotoxic activities observed for derivatives **1**, $m=1$ and **1**, $m=2$. This kind of estrogen-linked Pt(II) complexes could present several advantages over the known cisplatin analogues. Theoretically, the estrogenic portion of the molecule may direct the cytotoxic Pt(II) moiety towards the target cells in vivo, increasing specificity and reducing systemic toxicity. Further research will be necessary to evaluate the complete biological potential of this novel class of 17β-estradiol-linked Pt(II) complexes.

Acknowledgements

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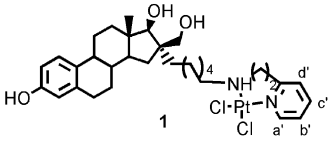
10. Spectral data for 16β-hydroxymethyl-16α-(6′-bromohexyl)-1,3,5(10)-estratrien-3,17β-diol (**6**, $n=4$): **IR** (NaCl, ν_{max}, cm^{-1}) 3355 (OH). ^1H NMR (200 MHz, acetone-d_6) δ 7.98 (1H, br s, OH), 7.08 (1H, d, $J=8.6$ Hz, 1-CH), 6.58 (1H, dd, $J=2.7$ Hz and $J=8.6$ Hz, 2-CH), 6.51 (1H, d, $J=2.7$ Hz, 4-CH), 4.33 (1H, br d, $J=2.3$ Hz, CHOH), 3.80–3.30 (4H, m, OH, CH_2OH), 3.50 (2H, t, $J=7.0$ Hz, CH_2Br), 2.76 (2H, m, 6-CH_2), 2.40–1.10 (21H, m, 3×CH, 9×CH_2), 0.89 (3H, s, 18-CH_3). ^{13}C NMR (200 MHz, acetone-d_6): δ 155.9, 138.4, 132.1, 126.9, 116.0, 113.6, 90.6, 67.1, 48.5, 47.6, 45.8, 44.8, 40.2, 39.2,

38.9, 34.8, 34.6, 33.7, 30.5, 28.9, 28.3, 27.2, 25.1, 12.5 (18-C).

Spectral data for 16β-hydroxymethyl-16α-[6-(2-pyridin-2-yl-ethylamino)-hexyl]-1,3,5(10)-estratrien-3,17β-diol dichloro-platinum (II) (**1**, $n=4$, $m=2$): **IR** (NaCl, ν_{max}, cm^{-1}): 3600–3050 (O–H and N–H), 1609 (C=C), 1241 and 1062 (C–O). ^1H NMR (500 MHz, acetone-d_6) δ 9.13 (1H, d, $J=5.5$ Hz, a′-CH), 8.03 (1H, t, $J=7.5$ Hz, c′-CH), 7.98 (1H, s, OH), 7.53 (1H, d, $J=7.5$ Hz, d′-CH), 7.43 (1H, t, $J=6.5$ Hz, b′-CH), 7.08 (1H, d, $J=8.4$ Hz, 1-CH), 6.59 (1H, dd, $J=1.3$ Hz and $J=8.3$ Hz, 2-CH), 6.53 (1H, s, 4-CH), 6.08 (1H, br s, NH), 4.31 (1H, t, $J=3.3$ Hz, CH_2**OH**), 3.72, 3.61, 3.45, 3.20 and 2.90 (10H, 5m, RC**H$_2$**NHC**H$_2$CH$_2$**-pyridyl, C**H**O**H** and C**H$_2$**OH), 2.75 (2H, m, 6-CH_2), 2.40–1.00 (21H, m, 3×CH, 9×CH_2), 0.87 (3H, s, 18-CH_3). ^{13}C NMR (500 MHz, acetone-d_6) δ 160.5 (pyridyl-C), 155.9 (a′-C), 154.4 (3-C), 140.1 (c′-C), 138.4 (5-C), 132.1 (10-C), 127.0 (1-C), 125.6 (d′-C), 124.6 (b′-C), 116.0 (4-C), 113.6 (2-C), 90.5 (CHOH), 67.2, 57.2, 48.4, 47.5, 46.6, 45.8, 44.8, 40.5, 40.2, 39.2, 38.9, 34.7, 30.9, 30.9, 30.7, 28.5, 28.4, 27.2, 25.1, 12.6 (18-C)



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