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Bioorganic Chemistry 33 (2005) 1–15
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Synthesis of 17β-estradiol-linked platinum(II) complexes and their cytocidal activity on estrogen-dependent and -independent breast tumor cells

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Received 5 May 2004
Available online 6 August 2004

Abstract

The synthesis of two new highly potent 17β-estradiol-linked platinum(II) complexes is described. The new molecules are linked at position 16 of the steroid nucleus with an alkyl chain. They are made from estrone in nine chemical steps with an overall yield exceeding 10%. The biological activity of these compounds was evaluated in vitro on estrogen dependent and independent (ER^+ and ER^-) human breast tumor cell lines: MCF-7 and MDA-MB-231. The novel compounds prove to be highly cytotoxic against breast cancer cell lines. The most cytotoxic derivative shows high affinity for the estrogen receptor alpha.

Keywords: Antitumor agents; Breast cancer; Estradiol; Platinum(II) complexes; Hybrid estradiol-Pt(II) complexes

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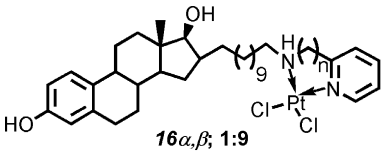
0045-2068/$ - see front matter
doi:10.1016/j.bioorg.2004.06.009

1. Introduction

Breast cancer is a major health problem among women in the world [1]. The successful treatment of this disease is limited by the fact that essentially all breast cancers become resistant to chemotherapy and endocrine therapy [2,3]. Moreover, it is also known that 40% of patients with estrogen receptor positive (ER^+) tumors do not respond to endocrine manipulation [4]. Recent evidence suggests that the progression to hormone resistance in some breast tumors is due to a variety of cellular changes mediated via the estrogen receptor-signalling pathway [5]. There is a need to develop new chemotherapeutic agents with increased efficacy and decreased systemic toxicity. Our goal is to design, based on our knowledge of triphenylethylene platinum(II) complexes (a non-steroidal cytotoxic estrogen) and on literature precedents, new highly potent 17β-estradiol-linked platinum(II) complexes. The estrogenic portion of the molecule would be used to direct the cytotoxic Pt(II) moiety toward the target cells thereby increasing specificity and reducing systemic toxicity.

The mechanism of growth regulation of breast cancer is a very complex process [6]. Nevertheless, it is well established that, in hormone-dependent breast cancer cell, there is an interaction of the native ligand 17β-estradiol (E_2) with the estrogen receptor (ER). This event leads to an array of cellular transformations, which culminate to cell growth. Agents that could interfere with this process could eventually give additional tools to treat breast cancer. More precisely, the synthesis of new antiestrogens bearing a cytotoxic moiety could be of prime interest because of their potential unique interaction with the ER and with DNA. This manuscript describes the synthesis of a new family of highly potent 17β-estradiol-linked Pt(II) complexes (see general structure **1**). It also reports the in vitro cytotoxic activity of these compounds on two neoplastic human breast cancer cell lines: MCF-7 and MDA-MB-231 (ER^+ and ER^-). For further evaluation, the novel Pt(II) complexes were tested on skin (B16-F10) and on intestine (HT-29) cancers.

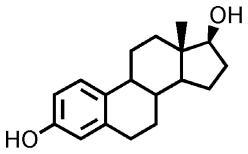


16α,β; 1:9

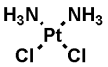
1, n = 1 (a), 2 (b)

cis-Diamminedichloroplatinum(II) (**2,** cisplatin) is one of the most successful chemotherapeutic agents used in the treatment of several human cancers. It is generally accepted that the biological effects of platinum(II) complexes are due to the formation of DNA adducts capable of blocking replication and/or transcription [7]. Unfortunately, these drugs present deleterious side effects, particularly nephrotoxicity [7]. Several new antiestrogen-linked Pt(II) complexes have been described recently in the literature [8]. Interestingly, depending on the nature of the antiestrogenic moiety the in vitro and in vivo biological activity do not necessarily coincide when tested on

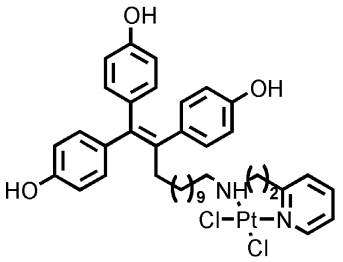
ER^+ cancers. Much work is needed to better understand the biological behaviour of this type of cytotoxic agent. We have been investigating for some time the synthesis of new triphenylethylene Pt(II) complexes designed for the treatment of breast cancer. We have shown that the presence of two or three hydroxyls groups on the triphenylethylene moiety of the molecule (see compound **3**) is essential for binding affinity to the ER [9]. Also, it was observed that the length of the side chain bearing the cytotoxic Pt(II) portion should be 11 or 12 carbon atoms long for optimal biological activity. More recently, we have incorporated several diamine ligands on a common triphenylethylene backbone in order to improve the cytotoxicity of this type of molecules. We have discovered that the Pt(II) complex **3** derived from 2-(2′-aminoethyl)pyridine possesses excellent in vitro cytotoxic activity [10]. Compound **3** (IC_{50} = 5 μM) is as active as cisplatin on MCF-7 breast cancer cells. Based on these results, we decided to incorporate the same pyridine derivatives on the 17β-estradiol nucleus (see compound **1**).



Estradiol

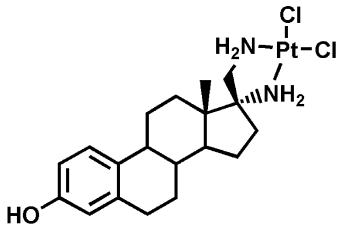


2, Cisplatin

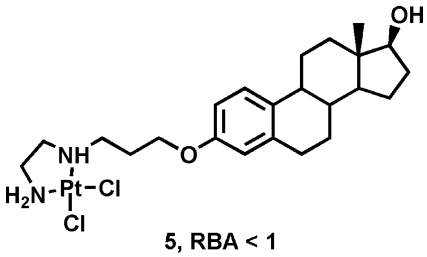


3, Triphenylethylene Pt(II) complex

It is known that in 17β-estradiol, the 3-hydroxy group is more important for binding than the 17β-OH. While 17β-estradiol possesses an ER relative binding affinity (RBA) of 100%, 17-deoxyestradiol has an RBA of 14%, whereas 3-deoxyestradiol has a RBA of 1.7% [11]. The relative importance of the 3- and 17-hydroxy functions can also be illustrated via a close examination of the RBA values of two known estradiol-linked Pt(II) complexes **4** and **5** (see structures below) [12]. The Pt(II) derivative **4** with a free phenol function possesses a RBA value of 6% while the Pt(II) derivative **5** with the free 17β-OH function possesses a RBA value of less than 1%. From these results, it is obvious that the presence of both hydroxyl functions on the estradiol nucleus is essential for high affinity to the ER. Therefore, the proposed Pt(II) complexes linked at C-16 would necessarily be better ligands for the ER.



4, RBA = 6



5, RBA < 1

2. Materials and methods

Anhydrous reactions were performed under an inert atmosphere, the setup assembled and cooled under dry nitrogen. Unless otherwise noted, starting material, reactant and solvents were obtained commercially and were used as such or purified, and dried by standard means [13]. Organic solutions were dried over magnesium sulfate ($MgSO_4$), evaporated on a rotatory evaporator and under reduced pressure. All reactions were monitored by UV fluorescence or staining with iodine. Commercial TLC plates were Sigma T 6145 (polyester silica gel 60 Å, 0.25 mm). Preparative TLC was performed on 1 mm silica gel 60 Å, 20×20 plates (Whatman, 4861 840). Flash column chromatography was performed according to the method of Still et al. [14] on Merck grade 60 silica gel, 230–400 mesh. All solvents used in chromatography had been distilled.

The infrared spectra were taken on a Nicolet Impact 420 FT-IR. Mass spectral assays were obtained using a VG Micromass 7070 HS instrument using ionization energy of 70 eV (University of Sherbrooke).

Nuclear magnetic resonance (NMR) spectra were recorded either on a Bruker AMX-II-500 equipped with a reversed or QNP probe (Pharmacor) or (when indicated) on a Varian 200 MHz NMR apparatus. Samples were dissolved in deuterochloroform ($CDCl_3$), deuteroacetone (acetone-d_6) or deuterodimethylsulfoxide (DMSO-d_6) for data acquisition using tetramethylsilane or chloroform as internal standard (TMS, δ 0.0 ppm for ^{1}H NMR and $CDCl_3$ δ 77.0 ppm for ^{13}C NMR). Chemical shifts (δ) are expressed in parts per million (ppm), the coupling constants (J) are expressed in hertz (Hz). Multiplicities are described by the following abbreviations: s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, m for multiplet, #m for several multiplets, and br s for broad singlet.

3. Synthesis of 17β-estradiol-linked Pt(II) complexes

3.1. Synthesis of 3-benzyloxy-1,3,5(10)-estratrien-17-one (7)

To a solution of estrone **6** (1.00 g, 3.70 mmol) in dichloromethane (10 mL), was added benzylbromide (0.53 mL, 4.44 mmol), tetrabutylammonium hydrogen sulfate (100 mg), and a solution of sodium hydroxide (10% w/v, 5 mL). The reaction mixture was stirred vigorously at reflux for 24 h. Then, the mixture was diluted with diethyl ether (30 mL) and water (30 mL) and washed with water (4×75 mL). The organic phase was dried with magnesium sulfate, filtered, and evaporated to yield a solid compound. The residue was triturated with hexanes to give a white solid in 99% yield which was homogeneous by TLC. It was used without further purification at the next step. M.P.: 128–129 °C. IR (KBr, ν_{max}, cm^{-1}): 1731 (C═O), 1614 (C═C), 1230, and 1008 (C═O). ^{1}H NMR ($CDCl_3$, δ ppm): 7.41 (2H, d, J = 7.6 Hz, a-CH), 7.36 (2H, t, J = 7.5 Hz, b-CH), 7.29 (1H, t, J = 7.2 Hz, c-CH), 7.18 (1H, d, J = 8.6 Hz, 1-CH), 6.78 (1H, dd, J = 2.5 Hz and J = 8.5 Hz, 2-CH), 6.71 (1H, d, J = 2.1 Hz, 4-CH), 5.01 (2H, s, C**H$_2$**Ph), 2.88 (2H, m, 6-CH_2), 2.50–1.39 (13H, #m, 3× CH and 5× CH_2), 0.89 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 220.5 (17-C), 156.8 (3-C), 137.7 (**C**CH_2O), 137.2 (5-C), 132.2 (10-C), 128.4 (b-C), 127.7 (c-C), 127.3 (a-C), 126.2 (1-C), 114.8 (4-C), 112.3 (2-C), 69.8 (**C**H_2Ph), 50.3, 47.9, 43.9, 38.3, 35.8, 31.5, 29.6, 26.5, 25.8, 21.5, 13.8 (C-18). MS (*m/e*): 360 (M^+), 269 (M^+—C_7H_7). Exact mass. Calculated for $C_{25}H_{28}O_2$ = 360.2089; found = 360.2095.

NB: a-CH, b-CH, and c-CH are *ortho*, *meta*, and *para* protons on the benzyl protecting group. Similarly, a-C, b-C, and c-C are the corresponding carbons on the benzyl group.

3.2. Synthesis of 3-benzyloxy-16α,β-(methoxycarbonyl)-1,3,5(10)-estratrien-17-one (8)

A solution of 3-benzyloxy-1,3,5(10)-estratrien-17-one (**7**) (4.00 g, 11.1 mmol) in dry THF (5 mL) was added over a period of 30 min to a solution of dimethylcarbonate (2.34 mL, 27.8 mmol) and potassium hydride (1.42 g, 34.7 mmol) in dry THF (40 mL). Then, the mixture was heated to reflux for a period of 3 h. Most of the solvent was then evaporated and the residue was diluted with ethyl acetate (100 mL) and treated with a saturated ammonium chloride solution (50 mL). The organic phase was washed with water (6 × 40 mL), dried and evaporated to give a yellowish solid. Trituration of the residue with a mixture of acetone: hexanes (1:1) yielded the title compound in 90% yield as a white solid which was homogeneous by TLC. It was used without further purification at the next step. M.P.: 152–154 °C. IR (NaCl, ν_{max}, cm^{-1}): 1747 (C═O, ester), 1721 (C═O, ketone), 1609 (C═C), 1226 (C—O). ^{1}H NMR ($CDCl_3$, δ ppm): 7.46 (2H, d, J = 7.4 Hz, a-CH), 7.41 (2H, t, J = 7.5 Hz, b-CH), 7.35 (1H, t, J = 7.2 Hz, c-CH), 7.22 (1H, d, J = 8.7 Hz, 1-CH), 6.83 (1H, dd, J = 2.5 Hz and J = 8.5 Hz, 2-CH), 6.77 (1H, s, 4-CH), 5.06 (2H, s, C**H$_2$**Ph), 3.79 (3H, s, COOC**H$_3$**), 3.24 (1H, t, J = 9.2 Hz, 16α-CH), 2.92 (2H, m, 6-CH_2), 1.31–2.46 (11H, #m, 3× CH, 4× CH_2), 1.02, and 0.99 (3H, 2s, 18-CH_3, 16α,β (1:4)). ^{13}C NMR ($CDCl_3$, δ ppm),

major isomer 16β-CO_2CH_3: 211.9 (17-C), 169.8 (**C**OOCH$_3$), 156.9 (3-C), 137.6 (**C**CH$_2$O), 137.1 (5-C), 131.9 (10-C), 128.4 (b-C), 127.8 (c-C), 127.3 (a-C), 126.2 (1-C), 114.8 (4-C), 112.4 (2-C), 69.8 (**C**H$_2$Ph), 54.0 (COO**C**H$_3$), 52.4, 48.8, 47.8, 43.9, 37.8, 31.8, 29.5, 26.4, 26.3, 25.7, 13.2 (18-C). MS (*m/e*): 418 (M^+), 386 (M^+—CH_3O). Exact mass: Calculated for $C_{27}H_{30}O_4$ = 418.2144; found = 418.2136.

*3.3. Synthesis of 3-benzyloxy-16α,β-(methoxycarbonyl)-16α,β-(11′-tetrahydropyranyloxyundecanyl)-1,3,5(10)-estratrien-17-one (**9**)*

A stirred solution of derivative **8** (0.98 g, 2.33 mmol), 1-tetrahydropyranyloxy-11-bromoundecane (3.12 g, 9.32 mmol), benzyltriethylammonium chloride (150 mg) and sodium hydroxide 10% w/v (8 mL) in dichloromethane (12 mL) was heated to reflux for 20 h. Then, the mixture was diluted with diethyl ether (40 mL) and extracted with a saturated ammonium chloride solution (2 × 20 mL) and with water (4 × 50 mL). The organic phase was dried, filtered, and concentrated to an oil. Purification by flash chromatography with a mixture of hexanes and acetone (9:1) gave 1.12 g (70%) of a viscous oil. IR (NaCl, ν_{max}, cm^{-1}): 1747 (C=O, ester), 1722 (C=O, ketone), 1604 (C=C), 1231, and 1031 (C—O). ^{1}H NMR ($CDCl_3$, δ ppm): 7.43 (2H, d, J = 7.6 Hz, a-CH), 7.38 (2H, t, J = 7.4 Hz, b-CH), 7.32 (1H, t, J = 7.3 Hz, c-CH), 7.19 (1H, d, J = 8.5 Hz, 1-CH), 6.79 (1H, dd, J = 2.0 Hz and J = 8.8 Hz, 2-CH), 6.74 (1H, s, 4-CH), 5.04 (2H, s, C**H$_2$**Ph), 4.58 (1H, t, J = 3.6 Hz, OCHO), 3.90–3.36 (4H, 4m, C**H$_2$**OCHOC**H$_2$**) 3.73 (3H, s, COOCH$_3$), 2.91 (2H, m, 6-CH$_2$), 2.41–1.20 (37H, #m, 3× CH, 17× CH$_2$), 0.93 and 0.91 (3H, 2s, 18-CH$_3$, 16α,β-CO_2CH_3 (1:6)). ^{13}C NMR ($CDCl_3$, δ ppm), major isomer 16β-CO_2CH_3: 214.0 (17-C), 171.8 (**C**OOCH$_3$), 156.9 (3-C), 137.7 (**C**CH$_2$O), 137.2 (5-C), 132.1 (10-C), 128.5 (b-C), 127.8 (c-C), 127.4 (a-C), 126.2 (1-C), 114.8 (4-C), 112.4 (2-C), 98.8 (OCHO), 69.9 (**C**H$_2$Ph), 67.6 (**C**H$_2$OCH on THP ring), 62.3 (**C**H$_2$OCH on aliphatic chain), 60.1, 52.5 (COO**C**H$_3$), 50.4, 49.4, 45.9, 44.0, 37.9, 35.5, 32.0, 31.5, 30.7, 30.5, 29.74, 29.70, 29.5, 29.4, 29.3, 26.5, 26.2, 25.7, 25.5, 25.4, 19.7, 14.0 (18-C). MS (*m/e*): 672 (M^+), 587 (M^+—C_5H_9O), 497 (M^+=C_5H_8O and C_7H_7). Exact mass: Calculated for $C_{43}H_{60}O_6$ = 672.4390; found = 672.4398.

*3.4. Synthesis of 3-benzyloxy-16α,β-(11′-hydroxyundecanyl)-1,3,5(10)-estratrien-17-one (**10**)*

A solution of 3-benzyloxy-16α,β-(methoxycarbonyl)-16α,β-(11′-tetrahydropyranyloxyundecanyl)-1,3,5(10)-estratrien-17-one (**9**) (0.41 g, 0.61 mmol), lithium chloride (0.57 g, 13.37 mmol), and water (0.24 mL, 13.37 mmol) in *N*,*N*-dimethylformamide (8 mL) was stirred and heated to reflux for 20 h. Afterwards, the solvent was partly evaporated and the residue transferred into an extraction funnel using ethyl acetate (40 mL) and water (30 mL). The organic phase was washed twice with hydrochloric acid (20 mL, 10% v/v) and with water 4 × 50 mL. The organic phase was dried, filtered, and concentrated to an oil. Purification by flash chromatography with a mixture of hexanes and acetone (9:1) gave 80% of the final product as an oil. IR (NaCl, ν_{max}, cm^{-1}): 3200–3600 (O—H), 1731 (C=O), 1604 (C=C), 1231, and 1021 (C—O). ^{1}H NMR ($CDCl_3$, δ

ppm): 7.45 (2H, d, $J = 7.3$ Hz, a-CH), 7.39 (2H, t, $J = 7.4$ Hz, b-CH), 7.33 (1H, t, $J = 7.3$ Hz, c-CH), 7.21 (1H, d, $J = 8.6$ Hz, 1-CH), 6.80 (1H, dd, $J = 2.0$ Hz and $J = 8.8$ Hz, 2-CH), 6.75 (1H, s, 4-CH), 5.05 (2H, s, C**H$_2$**Ph), 3.65 (2H, t, $J = 6.6$ Hz, C**H$_2$**OH), 2,91 (2H, m, 6-CH_2), 2.47–1.25 (33H, #m, 4× CH, 14× CH_2, OH), 0.96 and 0.88 (3H, 2s, 18-CH_3, 16α, β-11′-hydroxyundecanyl (1:1.7)). MS (*m/e*): 530 ($M^+ + H^+$), 439 ($M^+ - C_7H_6$). Exact mass: Calculated for $C_{36}H_{50}O_3$ ($M + H^+$) = 530.3768; found = 530.3760.

*3.5. Synthesis of 3-benzyloxy-16α,β-(11′-hydroxyundecanyl)-1,3,5(10)-estratrien-17β-ol (intermediate precursor of **11**)*

To a solution of derivative **10** (0.23 g, 0.43 mmol) in dry THF (8 mL) at −78 °C under an inert nitrogen atmosphere, was slowly added a solution of lithium aluminum hydride 1 M/THF (4.3 mL, 4.3 mmol). The resulting mixture was stirred for 1 h. Then, ethyl acetate (1 mL) was added to destroy the excess $LiAlH_4$. The reaction mixture was diluted with ethyl acetate (40 mL), extracted with a solution of hydrochloric acid (10% v/v, 3×20 mL) and with water (5×50 mL). The organic phase was dried, filtered, and evaporated to an oil. Purification by flash chromatography with a mixture of hexanes and acetone (4:1) gave a total of 0.18 g (78%) of a viscous oil. In this specific experiment, the two isomers were isolated 60% (0.14 g) of the 16α isomer and 18% (0.04 g) of the 16β isomer. However, on larger scale, the mixture of isomers was used for the synthesis of the final platinum(II) complexe. IR (NaCl, ν_{max}, cm^{-1}): 3650–3100 (O—H), 1609 (C=C), 1221, and 1022 (C—O). ^{1}H NMR ($CDCl_3$, δ ppm): 7.44 (2H, d, $J = 7.5$ Hz, a-CH), 7.38 (2H, t, $J = 7.4$ Hz, b-CH), 7.32 (1H, t, $J = 7.2$ Hz, c-CH), 7.19 (1H, d, $J = 8.6$ Hz, 1-CH), 6.78 (1H, dd, $J = 1.9$ Hz, and 8.1 Hz, 2-CH), 6.72 (1H, d, $J = 0.8$ Hz, 4-CH), 5.03 (2H, s, C**H$_2$**Ph), 3.79 (1H, d, $J = 11.2$ Hz, CHO**H**), 3.62 (2H, t, $J = 6.7$ Hz, C**H$_2$**OH), 3,50 (1H, d, $J = 10.9$ Hz, C**H**OH, 16α), 3,45 (1H, s, CH_2O**H**), 2.82–2,80 (2H, m, 6-CH_2), 2.30–1.07 (32H, #m, 4× CH, 14× CH_2), 0.88 (3H, s, 18-CH_3, 16α). ^{13}C NMR ($CDCl_3$, δ ppm), 16α pure isomer: 156.7 (3-C), 137.9 (**C**CH_2O), 137.2 (5-C), 132.9 (10-C), 128.5 (b-C), 127.8 (c-C), 127.4 (a-C), 126.2 (1-C), 114.8 (4-C), 112.2 (2-C), 90.5 (CHOH), 69.9 (**C**H_2Ph), 62.9 (CH_2OH), 47.6, 46.7, 44.8, 43.8, 39.3, 37.92, 37.89, 33.1, 32.7, 30.5, 29.7, 29.59, 29.57, 29.5, 29.3, 27.4, 26.2, 25.7, 24.7, 11.9 (18-C). MS (*m/e*): 532 (M^+), 423 (M^+-H_2O and C_7H_7). Exact mass: Calculated for $C_{36}H_{52}O_3$ = 532.3905; found = 532.3916.

*3.6. Synthesis of 3-benzyloxy-16α,β-(11′-bromoundecanyl)-1,3,5(10)-estratrien-17β-ol (**11**)*

The diol intermediate (0.16 g, 0.30 mmol) was solubilized in dichloromethane (10 mL), then carbon tetrabromide (0.40 g, 1.20 mmol) and triphenylphosphine (0.31 g, 1.20 mmol) are added to the diol. The reaction mixture under an inert atmosphere of nitrogen was stirred at room temperature for 1–2 h. The organic phase was diluted with diethyl ether (50 mL) and washed with a saturated ammonium chloride solution (25 mL) and with water (5×60 mL). The ethereal phase was dried, filtered,

and evaporated to an oil. The residue was purified by flash chromatography with a mixture of hexanes and acetone (9:1) to give 0.11 g (64%) of the title derivative as an oil. IR (NaCl, ν_{max}, cm^{-1}) : 3650–3150 (O—H), 1604 (C=C), 1232, and 1022 (C—O). ^1H NMR ($CDCl_3$, δ ppm): 7.47 (2H, d, J = 7.4 Hz, a-CH), 7.42 (2H, t, J = 7.5 Hz, b-CH), 7.35 (1H, t, J = 7.1 Hz, c-CH), 7.24 (1H, d, J = 8.5 Hz, 1-CH), 6.83 (1H, dd, J = 1.7 and 8.1 Hz, 2-CH), 6.76 (1H, s, 4-CH), 5.07 (2H, s, C**H$_2$**Ph), 3.77 (0,7H, d, J = 10.1 Hz, C**H**OH, 16β), 3.44 (2H, t, J = 6.9 Hz, CH_2Br), 3.30 (0,3H, d, J = 7.6 Hz, C**H**OH, 16α), 2.89 (2H, m, 6-CH_2), 2.35–1.02 (33H, #m, 4× CH, 14× CH_2, -OH), 0.80, and 0.78 (3H, 2s, 18-CH_3, 16α,β (1:2.4)). MS (*m/e*) : 594 (M^+), 504 (M^+—C_7H_6). Exact mass: Calculated for $C_{36}H_{51}O_2Br$ = 594.3045; found = 594.3072.

*3.7. Synthesis of 16α,β-(11′-bromoundecanyl)-1,3,5(10)-estratrien-3,17β-diol (precursor of derivatives **12a** and **12b**)*

A stirred suspension of 3-benzyloxy-16α, β-(11′-bromoundecanyl)-1,3,5(10)-estratrien-17β-ol (**11**, 300 mg, 0,50 mmol) and 10% Pd/C (150 mg) in dry THF (4 mL) was stirred under hydrogen atmospheric pressure for 3–6 h. The reaction was followed by TLC until completion. The insoluble material was filtered off with diethyl ether (70 mL) and the filtrate was concentrated to give the crude product. It was purified by flash chromatography (hexanes: acetone (4:1)) to give 225 mg (90%) of a white solid. M.P.: 128–130 °C. IR (NaCl, ν_{max}, cm^{-1}): 3600–3100 (O—H), 1604 (C=C), 1247, and 1068 (C—O). ^1H NMR ($CDCl_3$, δ ppm): 7.15 (1H, d, J = 8.5 Hz, 1-CH), 6.62 (1H, d, J = 8.7 Hz, 2-CH), 6.56 (1H, s, 4-CH), 4.75–4.35 (1H, br s, 3-OH), 3.73 (1H, d, J = 10.1 Hz, C**H**OH, 16β), 3.41 (2H, t, J = 6.8 Hz, CH_2Br), 3.27 (1H, d, J = 7.6 Hz, C**H**OH, 16α), 2.82 (2H, m, 6-CH_2), 2.30–0.97 (33H, #m, 4× CH, 14× CH_2, 17-OH), 0.80 and 0.77 (3H, 2s, 18-CH_3, 16α,β (1:3.9)).^{13}C NMR ($CDCl_3$, δ ppm), major 16β isomer: 153.5 (3-C), 138.1 (5-C), 132.5 (10-C), 126.4 (1-C), 115.2 (4-C), 112.7 (2-C), 82.4 (CHOH, 16β), 48.5, 44.1, 44.0, 39.9, 38.3, 37.6, 34.0, 32.8, 32.3, 31.4, 29.8, 29.61, 29.56, 29.5, 29.4, 28.7, 28.6, 28.1, 27.4, 26.2, 12.3 (18-C). MS (*m/e*): 504 (M^+), 426 (M^+—^{78}Br). Exact mass: Calculated for $C_{29}H_{45}O_2Br$ = 504.2592; found = 504.2603.

*3.8. Synthesis of 16α,β-[11′-(2″-picolylamino)undecanyl]-1,3,5(10)-estratrien-3,17β-diol (**12a**)*

The title compound was made as described for the synthesis of the amine **12b** below. In this case the following quantities were used: 16α, β-(11′-bromoundecanyl)-1,3,5(10)-estratrien-3,17β-diol (70 mg, 0.14 mmol), 2-(aminomethyl)pyridine (80 μL, 0.84 mmol), methanol (2 mL). The reaction mixture was heated to reflux for 21 h under an inert atmosphere of nitrogen. The extraction was done using a mixture of diethyl ether and dichloromethane (4:1, 30 mL). The crude amine was obtained quantitatively as a yellowish oil. The title amine was used without further purification in the next step. IR (NaCl, ν_{max}, cm^{-1}): 3650–3100 (O—H and N—H), 1588 (C=C), 1246, and 1072 (C—O). ^1H NMR ($CDCl_3$, δ ppm): 8.56 (1H, d,

J = 4.9 Hz, a′-CH), 7.64 (1H, t, J = 8.2 Hz, c′-CH), 7.30 (1H, d, J = 7.8 Hz, d′-CH), 7.20–7.14 (2H, m, b′-CH and 1-CH), 6.61 (1H, dd, J = 2.2 and J = 8.7 Hz, 2-CH), 6.56 (1H, s, 4-CH), 3.93 (2H, s, C**H$_2$**pyridyl), 3.73 (1H, d, J = 10.0 Hz, C**H**OH, 16β), 3.26 (1H, d, J = 7.3 Hz, C**H**OH, 16α), 2.82 (2H, m, 6-CH_2), 2.67 (1H, t, J = 5.5 Hz, NH), 2.60–0.86 (36H, #m, 4× CH, 15× CH_2, 2× OH), 0.80 and 0.77 (3H, 2s, 18-CH_3, 16α,β (1:4)). MS (*m/e*): 532 (M^+), 426 (M^+—$C_6H_6N_2$). Exact mass: Calculated for $C_{35}H_{52}O_2N_2$ = 532.4022; found = 532.4029.

*3.9. Synthesis of 16α,β-[11′-(2″-pyridylethylamino)undecanyl]-1,3,5(10)-estratrien-3,17β-diol (**12b**)*

A stirred solution of 16α, β-(11′-bromoundecanyl)-1,3,5(10)-estratrien-3,17β-diol (0.15 g, 0.30 mmol) and 2-(2-aminoethyl)pyridine (0.36 mL, 3 mmol), in methanol (5 mL) was heated to reflux for 3 days under an inert atmosphere of nitrogen. Then, the solvent was evaporated and the residue dissolved in diethyl ether (30 mL) was washed with water (5 × 50 mL). The aqueous phases are extracted with diethyl ether (2 × 15 mL). The combined organic phases were dried, filtered, and evaporated to an oil. The crude amine was obtained in 80% yield and was used without further purification at the next step. IR (NaCl, v_{max}, cm^{-1}): 3550–3050 (O—H and N—H), 1588 (C=C), 1241, and 1072 (C—O). ^{1}H NMR ($CDCl_3$, δ ppm): 8.48 (1H, d, J = 4.2 Hz, a′-CH), 7.63 (1H, t, J = 7.7 Hz, c′-CH), 7.18 (2H, m, d′-CH and b′-CH), 7.12 (1H, d, J = 8.2 Hz, 1-CH), 6.63 (1H, d, J = 8.3 Hz, 2-CH), 6.57 (1H, s, 4-CH), 3.73 (1H, d, J = 10.0 Hz, C**H**OH, isomer 16β), 3.26 (1H, d, J = 7.2 Hz, C**H**OH, isomer 16α), 3.16 (4H, m, NHC**H$_2$**C**H$_2$**pyridyl), 2.79 (4H, m, $(CH_2)_{10}$C**H$_2$**NH and 6-CH_2), 2.29–1.00 (35H, m, 4× CH, 14× CH_2, 2× OH and NH), 0.80 and 0.76 (3H, 2s, 18-CH_3, 16α,β (1:4)). ^{13}C NMR ($CDCl_3$, δ ppm), major isomer 16β: 159.4 (pyridyl-C), 154.0 (3-C), 148.8 (a′-C), 138.1 (5-C), 137.0 (c′-C), 132.2 (10-C), 126.4 (1-C), 123.6 (d′-C), 121.9 (b′-C), 115.4 (4-C), 112.9 (2-C), 82.5 (CHOH, 16β), 48.8, 48.6, 48.2, 44.1, 44.0, 40.0, 38.4, 37.7, 32.4, 31.4, 29.8, 29.7, 29.6, 29.5, 29.2, 28.6, 28.3, 27.5, 27.0, 26.3, 12.4 (18-C). MS (*m/e*): 546 (M^+), 454 (M^+—C_6H_4N). Exact mass: Calculated for $C_{36}H_{54}O_2N_2$ = 546.4198; found = 546.4185.

*3.10. Synthesis of 16α,β-[11′-(2″-picolylamino)undecanyl]-1,3,5(10)-estratrien-3, 17β-diol dichloroplatinate (II) (**1a**)*

This platinum(II) complex was made as described for the synthesis of the complex **1b** below. In this case the following quantities were used : amine **12a** (63 mg, 0.12 mmol), potassium tetrachloroplatinate (II) (60 mg, 0.14 mmol), DMF: H_2O (2:1, 3 mL). Purification by flash chromatography with hexanes: acetone (1:1) gave the title compound in 61% yield in the form of yellow crystals. M.P.: > 124 °C decomposed. IR (NaCl, v_{max}, cm^{-1}): 3600–3050 (O—H and N—H), 1609 (C=C), 1241 and 1062 (C—O). ^{1}H NMR ($CDCl_3$ + Acetone-d_6 (9 :1), δ ppm): 9.20 (1H, d, J = 5.6 Hz, a′-CH), 8.13 (1H, t, J = 7.8 Hz, c′-CH), 7.84 (1H, s, 3-OH), 7.69 (1H, d, J = 7.8 Hz, d′-CH), 7.45 (1H, t, J = 6.7 Hz, b′-CH), 7.15 (1H, d, J = 8.4 Hz, 1-CH), 6.67 (1H, d, J = 8.2 Hz, 2-CH), 6.62 (1H, s, 4-CH), 6.27 (1H, br s, NH), 4.95 (1H, dd, J = 6.2 Hz, and

J = 16.5 Hz, NHCH$_x$**H**$_y$pyridyl), 4.28 (1H, d, J = 16.3 Hz, NHC**H**$_x$H$_y$pyridyl) 3.80 (1H, d, J = 9.8 Hz, C**H**OH, isomer 16β), 3.32 (1H, d, J = 7.4 Hz, C**H**OH, isomer 16α), 3.16 and 2.83 (2H, 2m, (CH$_2$)$_{10}$C**H$_2$**NH), 2.88 (2H, m, 6-CH$_2$), 2.34–1.05 (32H, #m, 3× CH, 14× CH$_2$, 17-OH), 0.88, and 0.86 (3H, 2s, 18-CH$_3$, 16α, β (1:3.7)). ^{13}C NMR (CDCl$_3$: Acetone-d_6 (9:1), δ ppm), major isomer 16β: 161.6 (pyridyl-C), 154.0 (3-C), 147.4 (a′-C), 137.8 (5-C), 136.9 (c′-C), 130.7 (10-C), 125.4 (1-C), 123.4 (d′-C), 121.2 (b′-C), 114.4 (4-C), 112.0 (2-C), 81.1 (CHOH, 16β), 59.9, 54.8, 47.9, 43.3, 42.7, 39.6, 37.8, 37.1, 35.3, 31.7, 30.9, 30.1, 28.0, 26.8, 26.7, 25.8, 25.6, 11.7 (18-C).

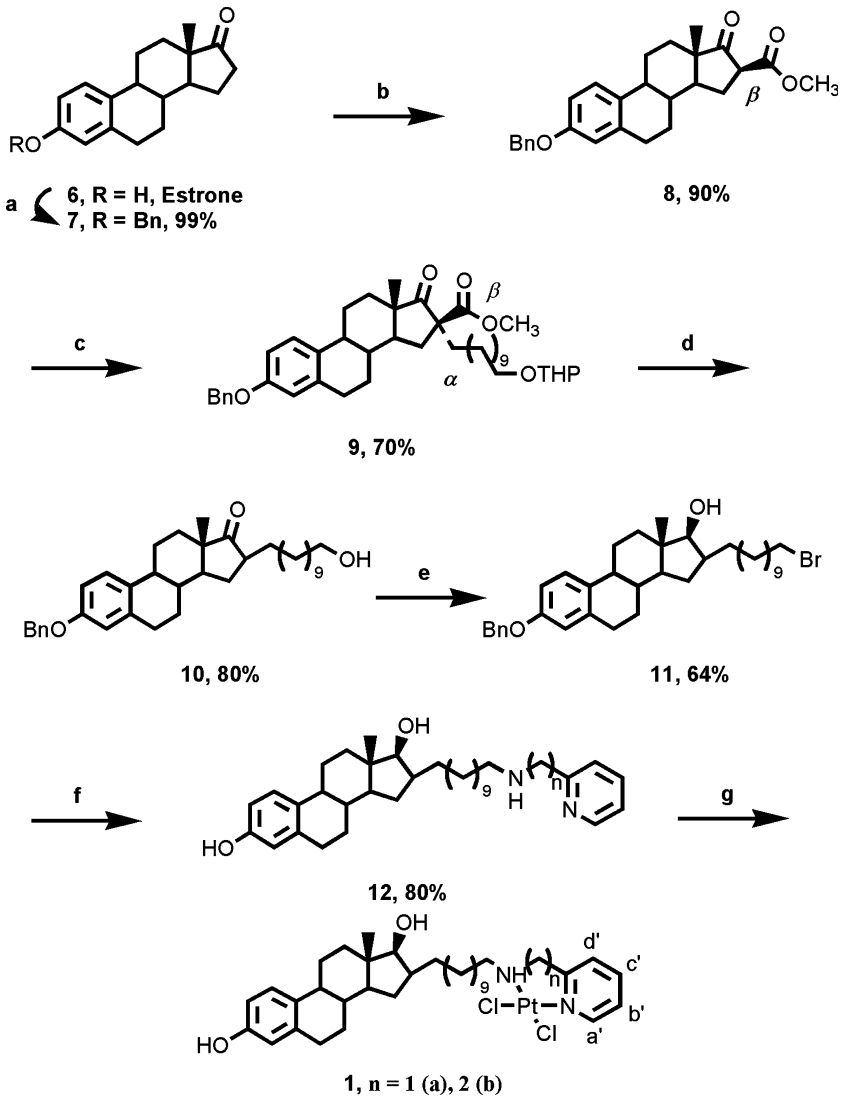
*3.11. Synthesis of 16α,β-[11′-(2″-pyridylethylamino)undecanyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinate (II) (**1b**)*

To a solution of 16α, β-[11′-(2″-pyridylethylamino)undecanyl]-1,3,5(10)-estratrien-3, 17β-diol (**12b**, 0.13 g, 0.24 mmol) in DMF (1 mL) at 23 °C was added potassium tetrachloroplatinate (II) (0.11 g, 0.26 mmol) dissolved in a mixture of DMF/H_2O (2: 1.6 ml). The resulting mixture (pH 8–9) was stirred in the dark for 2–3 days until the pH value reached 4–5. Then, a drop of dimethylsulfoxide was added to destroy the excess K_2PtCl_4 and the stirring was continued for 2–3 h. The solvent was evaporated and the residue was stirred vigorously in a saturated aqueous potassium chloride solution (5 mL) for 15 min. A vigorous stirring was essential in order to pulverize the lumps of precipitated platinum(II) complex. The resulting suspension was filtered, washed with water (100 mL), and dried in a desiccator for a 1 day. The product was further purified by flash column chromatography (hexanes: acetone, 3:2) to give the title compound in the form of yellow crystals (52% yield). M.P.: >148 °C decomposed. IR (NaCl, ν_{max}, cm^{-1}): 3600–3100 (O—H and N—H), 1610 (C=C), 1211, and 1063 (C—O). ^{1}H NMR (Acetone-d_6, 310 K, δ ppm): 9.13 (1H, d, J = 5.9 Hz, a′-CH), 8.02 (1H, t, J = 7,8 Hz, c′-CH), 7,53 (1H, d, J = 7,8 Hz, d′-CH), 7,41 (1H, t, J = 6,7 Hz, b′-CH), 7.08 (1H, d, J = 8.5 Hz, 1-CH), 6.59 (1H, dd, J = 1.9 Hz and J = 8.5 Hz, 2-CH), 6.53 (1H, s, 4-CH), 5.87 (1H, br s, NH), 3.72 (1H, d, J = 9.8 Hz, C**H**OH, isomer 16β), 3.66–3.61, 3.23–3.17, 3.00–2.90, and 2.90–2.80 (7H, 4m, RC**H$_2$**NHC**H$_2$C H$_2$**pyridyl and C**H**OH, isomer 16α), 2.80–2.72 (2H, m, 6-CH$_2$), 2.40–1.00 (34H, #m, 4× CH, 14× CH$_2$, 2× OH), 0.81 and 0.78 (3H, 2s, 18-CH$_3$, 16α,β (1:3.8)). ^{13}C NMR (Acetone-d_6, δ ppm), major isomer 16β: 160.5 (pyridyl-C), 156.0 (a′-C), 154.4 (3-C), 140.0 (c′-C), 138.5 (5-C), 132.3 (10-C), 127.1 (1-C), 125.5 (d′-C), 124.6 (b′-C), 116.0 (4-C), 113.7 (2-C), 82.4 (CHOH, 16β), 57.3, 49.7, 46.8, 45.1, 41.5, 40.4, 39.6, 38.8, 33.5, 32.8, 30.8, 30.7, 28.7, 28.5, 27.3, 13.2 (18-C).

4. Results and discussion

4.1. Synthesis of 17β-estradiol-linked Pt(II) complexes

As shown in Scheme 1, the synthesis involves nine chemical steps starting from estrone (**6**) as the steroid template. The 17β-estradiol Pt(II) complexes **1a** and **1b** were obtained using a straightforward reaction sequence.



Scheme 1. Reagents: (a) $BnBr,Bu_4N^+HSO_4^-$, NaOH 10% p/v, CH_2Cl_2, reflux, 24 h, 99%; (b) KH, dimethyl carbonate, THF, reflux, 3 h, 90%; (c) $Br(CH_2)_{11}OTHP$, $Et_3N^+BnCl^-$, NaOH 10% p/v, CH_2Cl_2, reflux, 20 h, 70%; (d) LiCl, DMF: H_2O, reflux, 20 h, 80%; (e) (1) $LiAlH_4$ 1 M/THF, −78 °C, 1 h, 78%; (2) CBr_4, PPh_3, CH_2Cl_2, 22 °C, 1–2 h, 64%; (f) (1) H_2, Pd/C, 10% p/p, THF, 22 °C, 3–6 h, 90%; (2) 2-aminomethyl pyridine ($n = 1$) or 2-(2′-aminoethyl)pyridine ($n = 2$), CH_3OH, reflux, 3 days, 90%; (g) K_2PtCl_4, DMF, H_2O (2:1), 22 °C, 2–3 days, 57%.

Initially, estrone (**6**) was protected as a benzyl ether using phase transfer catalysis (PTC) methodology. Accordingly, estrone was treated with benzyl bromide and tetrabutylammonium hydrogensulfate in dichloromethane in the presence of a 10%

aqueous sodium hydroxide solution [15]. The yield of the protection reaction is 99%. The derivative **7** was transformed into the β-ketoester **8** upon treatment with dimethyl carbonate in the presence of a mixture of NaH/KH in dry tetrahydrofuran [16,17]. Derivative **8** was obtained with 90% yield. Treatment of derivative **8** and 1-tetrahydropyranyloxy-11-bromoundecane under PTC reaction conditions gave compound **9** in 70% yield. The bromoalkane side chain was added mainly to the less hindered α face of the molecule (16α,β; 6:1) as shown by the presence of two singlets for the 18-CH_3 at δ 0.93 and 0.91 in the ^{1}H NMR spectrum. Decarboalkoxylation with simultaneous deprotection of the tetrahydropyranyl ether of derivative **9** was achieved upon treatment with lithium chloride in a mixture of *N,N*-dimethylformamide and water at reflux for 20 h. The cleavage of the tetrahydropyranyl ether under these reaction conditions is a known process [18]. The alcohol **10** was obtained in 80% yield as a mixture of α and β isomers (16α,β; 1:1.7). Reduction of derivative **10** with lithium aluminum hydride in dry THF gave the intermediate diol. The stereochemistry of the 17β-hydroxy function as well as the 16-α or 16-β side chain position was confirmed by comparison with ^{13}C NMR spectral data of known 16-α and 16-β substituted 17β-estradiol derivatives [19]. Selective bromination of the primary alcohol was performed with a mixture of carbon tetrabromide, triphenylphosphine in dichloromethane. The hydroxy-halide **11** was obtained in 64% overall yield.

The final 17β-estradiol-linked Pt(II) complexes **1a** and **1b** were obtained in three additional chemical reactions. Initially, hydrogenolysis of the benzyl ether found in compound **11** with 10% Pd/C in tetrahydrofuran gave the phenol intermediate, which was treated with an excess 2-aminoalkylpyridine to give derivative **12** in 80% yield. The diol-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**, n = 1(a), 2(b). All new compounds synthesized were characterized by their respective IR, ^{1}H NMR and ^{13}C NMR spectra.

4.2. In vitro cytotoxic activity

The toxicity of the 17β-estradiol-linked Pt(II) complexes was evaluated on two human breast tumor cell lines using the Sulforhodamine B colorimetric assay [20,21]. The cytotoxicity of the compounds was tested along with controls (cisplatin, estradiol and tamoxifen) on both estrogen-receptor positive (ER^+, MCF-7) and estrogen-receptor negative (ER^-, MDA-MB-231) human mammary carcinomas [22]. They were also tested on skin (B16-F10) and intestine (HT-29) cancers for additional comparison of activities.

As shown by the SRB assays on the two human breast cancer cell lines, the new Pt(II) complexes are very toxic toward breast cancer cells (Table 1). The Pt(II) complex **1a** is twice as toxic as tamoxifen on MCF-7 cells and five times more toxic on MDA-MB-231 cells, showing an IC_{50} of 5.9 and 4.1 μM, respectively. Moreover, it presents an activity three times greater than cisplatin itself on both types of cells. The Pt(II) complex **1b** presents a greater activity than tamoxifen; it is 22 times more toxic on MCF-7 cells and 37 times more toxic on MDA-MB-231 cells. Furthermore, it is 32 times more toxic on MCF-7 and 25 times more toxic on MDA-MB-231 than

Table 1
Inhibitory concentration of drug on breast (ER^+ and ER^-), skin, and intestine cancer cell lines

Compounds	IC_{50} (μM)[a]			
	MCF-7 (ER^+)	MDA-MB-231 (ER^-)	B16-F10	HT-29
Cisplatin	16.1	12.8	4.5	10.3
Estradiol	31.5	>100	28.9	85
Tamoxifen	11.1	18.9	7.0	11.0
1a	5.9	4.1	2.7	13.5
1b	0.5	0.5	0.5	2.1

[a] Inhibitory concentration as obtained by the SRB assay. Experiments were performed in octuplicate.

cisplatin. Platinum complex **1b** shows an IC_{50} of 0.5 μM on all the cells at the exception of the HT-29 intestine cancer cells with an IC_{50} of 2.1 μM. 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. This was previously observed with the triphenylethylene Pt(II) complexes [10]. The cytotoxic activity of **1b** is remarkable as it is significantly more toxic than cisplatin. Both Pt(II) complexes **1a** and **1b** are more toxic toward the skin cancer cells (B16-F10) as compared to the intestine cancer cells (HT-29). It is also observed that estradiol is essentially inactive on all the cell lines when compared to the estradiol-linked Pt(II) complexes.

The estrogen receptor alpha (ERα) affinity assay was performed using the HitHunter EFC Estrogen Fluorescence assay kit (Discoverx, Fremont, CA) according to manufacturer's instructions [23]. Only the most promising estrogen-Pt(II) complex **1b** was tested in this experiment.

The estrogen receptor binding studies showed a strong affinity for **1b**, the most cytotoxic molecule, to the estrogen receptor α (see Fig. 1). The reference derivative

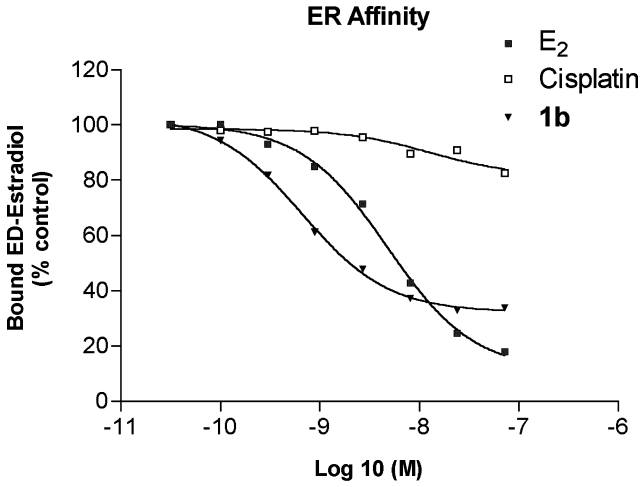


Fig. 1. Estrogen receptor binding affinity (ERα). ED-Estradiol, Enzyme Donor-Estradiol; E_2, 17β-estradiol. Cisplatin presents no affinity for the ER.

(i.e., cisplatin) presents, as expected, no affinity for the ERα. The estrogen-Pt (II) hybrid molecule **1b** shows an IC_{50} of 0.35 nM compared to 4.79 nM for 17β-estradiol, the natural ligand. It has a remarkably high affinity which is likely to lead the cytotoxic Pt(II) moiety to the ERα-expressing target cells in an in vivo study that will be performed in the near future.

5. Conclusion

In summary, this manuscript presents a facile synthesis of highly toxic 17β-estradiol Pt(II) complexes. They are made form estrone in nine chemical steps with an overall yield exceeding 10%. Using this strategy a large variety of Pt(II) complexes could be easily 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 **11**. This kind of estrogen-linked Pt(II) complexes could present several advantages over the known cisplatine analogs. Theoretically, the estrogenic portion of the molecule could direct the cytotoxic Pt(II) moiety toward the target cells via the ERα, increasing specificity and reducing systemic toxicity. The most promising estrogen-Pt(II) complexe **1b** displays high affinity for the ERα. This preliminary investigation suggests that these 17β-estradiol Pt(II) complexes are quite cytotoxic towards breast cancer cell lines as compared to cisplatin. Further research will be necessary to assess the full biological potential of these new cytotoxic agents. Particularly, the synthesis of optically pure 16α and 16β isomers is now warranted considering the biological results obtained with the mixture of epimers at position 16.

Acknowledgments

This work was supported by the Université du Québec à Trois-Rivières. We are grateful to Mr. Jacques Lacroix for biological evaluation studies. We also thank Dr. Gilles Sauvé and Mr. Nicolas LeBerre, Pharmacor Inc. for the ^{1}H and ^{13}C NMR spectra and Mr. Gaston Boulay, Université de Sherbrooke, for the mass spectra.

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