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Design, synthesis and biological evaluation of estradiol-PEG-linked platinum(II) hybrid molecules: Comparative molecular modeling study of three distinct families of hybrids

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ABSTRACT

The synthesis of a series of 17β-estradiol-platinum(II) hybrid molecules is reported. The hybrids are made of a PEG linking chain of various length and a 2-(2′-aminoethyl)pyridine ligand. They are prepared from estrone in only 5 chemical steps with an overall yield of 22%. The length of the PEG chain does not influence the solubility of the compounds as it remains relatively constant throughout the series. MTT assays showed that the derivative with the longest PEG chain showed the best activity against two human breast cancer cell lines (MCF-7 and MDA-MB-231). The novel PEG-hybrids are also compared in terms of activities with two other families of 17β-estradiol-platinum(II) hybrids that we reported in previous studies. Molecular modeling study performed on a representative member of each family of hybrids reveals distinct molecular interactions with the estrogen receptor α which further corroborates their notably contrasting cytocidal activities on breast cancer cell lines. This study also shows that lipophilicity and the orientation of the tether chain between the estrogenic portion and the platinum(II) core contribute markedly to the biological activity of the various families of hybrids. The most active hybrids are those possessing an alkyl tether chain at position 16β of the steroid nucleus. For example, derivative **3** ($p = 6$) is about 16 times more potent on MCF-7 breast cancer cells than the corresponding 16α-PEG-hybrids (**2b**) made in this study.

1. Introduction

The platinum anticancer drugs have attracted considerable interest because of their great therapeutic value in treating a number of cancers [1,2]. They have been mainly used for the treatment of solid tumors, particularly small cell lung, ovarian, testicular, head and neck tumors. Unfortunately, their use has been limited by a number of severe side effects, particularly nephrotoxicity and neurotoxicity [3,4] and also by the emergence of resistance to the drug [5,6]. The major mechanisms of resistance to cisplatin (and analogs) are (1) inactivation by glutathione, metallothionein or other sulfur-containing molecules, (2) increased DNA repair, (3) reduced accumulation by alteration of drug uptake/reflux and (4) increased platinum adducts tolerance and failure of apoptotic pathways [7,8].

Even if thousands of platinum analogs have been made to date and tested for anticancer activity, there are only a few selected compounds used today in clinics. They are cisplatin, carboplatin, oxaliplatin, nedaplatin, iobaplatin and heptaplatin [5,9,10]. The specific uses of these drugs vary from one to another. However, the most recent platinum drugs have not shown any distinct and significant advantages over cisplatin and carboplatin [5,9,10].

Recent literature reviews present a broad overview of the actual knowledge of platinum-based antitumor agents as well as their action mechanisms [5,9–11]. The antitumor activity of platinum drugs is a consequence of their interaction with DNA. Cisplatin binds readily to the N7 position of the guanine bases of DNA molecules to form mainly 1,2- or 1,3-intrastrand crosslinks, and, to a lesser extent, interstrand crosslinks. These cisplatin-DNA lesions cause various cellular responses such as inhibition of replication and/or transcription, cell cycle arrest, DNA repair and ultimately induction of apoptosis [3,5,7,10]. Other lesions to the cell are also occurring and contribute to the global cisplatin activity [4,7].

Targeting platinum anticancer drugs is a subject of intense research. Many strategies are used to reduce systemic toxicity [12].

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Several research groups have been investigating the combination of a platinum complex to an estrogenic moiety in order to target the estrogen receptor (ER) in hormone-dependent diseases, particularly breast cancer [13–15]. Obviously, the main goal is to improve the selectivity and efficacy of this type of drug and, consequently, to minimize its toxic side effects. Three recent reviews have presented the many studies on the use of platinum complexes for hormone-dependent cancers treatment [13–15].

The estrogen receptor is a biological target that has attracted considerable attention over the years. It is expressed by several type of cancers; breast (60–70%), uterus (70–73%) as well as ovarian (61%) [16–18]. Together, they represent 36% of all cancers diagnosed in women and display a 22% mortality rate [19]. The biological affinity between 17β-estradiol and its cognate receptor can theoretically be used to direct a cytotoxic agent to the target cells.

This manuscript gives the detailed description of the synthesis of five 17β-estradiol-PEG-platinum(II) (E_2-Pt(II)) hybrid molecules **1** (Fig. 1) succinctly reported in an earlier communication [20]. The novel platinum complexes are linked with a polyethylene glycol (PEG) chain at position 16α of the steroid nucleus and bear a 16β-hydroxymethyl side chain. They are made from estrone in only five chemical steps with an overall yield of 22%. The PEG-chain was used in order to obtain compounds with relatively constant solubility throughout the series and to study more accurately the true influence of the length of the chain on the biological activity. This class of compounds completes our previous work done on similar derivatives; see compound **2** and **3**, which used a carbon chain tether (Fig. 1) [21–25]. This manuscript also analyses and compares, using molecular modeling calculations, the three class of estrogen-platinum(II) hybrids studied in our laboratory.

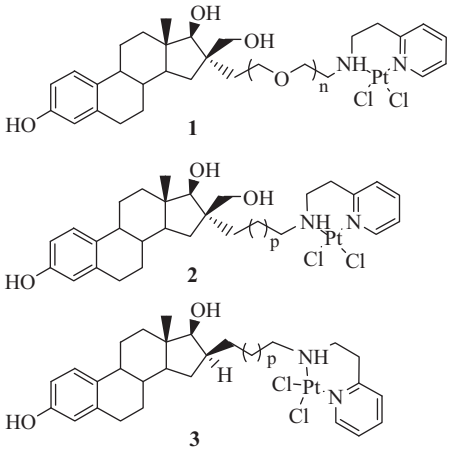
The objective of the present study was also to determine the cytotoxic effect of these novel molecules using estrogen dependent (estrogen receptor positive; ER^+) and independent (estrogen receptor negative; ER^-) human breast cancer cells. The biological activity of these compounds was evaluated *in vitro* using an MTT cell proliferation assay [26,27]. The MTT assay was performed over an incubation period of 72 h. The affinity for the estrogen receptor alpha (ERα) was also determined for derivative **1b**.

2. Experimental

2.1. Chemistry

All reactions were performed with ACS Fisher solvents. In some cases, solvent, as well as starting materials and reactants, were first purified and dried by standard means [28]. Anhydrous reactions required an inert atmosphere of dry nitrogen. Estrone was purchased from Steraloids Inc., Wilton, NH, USA and the diverse polyethylene glycol chains were purchased from Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada. All reactions were monitored by UV fluorescence or staining with iodine on Sigma T 6145 commercial TLC plates (polyester silica gel 60 Å, 0.25 mm). Purifications were done using flash column chromatography according to the method of Still et al. [29] on Silicycle UltraPure Flash Silica Gel, 40–63 μm mesh. Hexanes and acetone were distilled before their use as chromatography eluant.

The infrared spectra were taken on a Nicolet Impact 420 FT-IR. Sodium chloride or potassium bromide pellets were used for analysis. Mass spectral assays were obtained using a VG Micromass 7070 HS instrument using ionization energy of 70 eV (University of Sherbrooke). Chemical ionization (NH_3) was required for a few compounds. The platinum(II) complexes were analyzed using a MS model 6210, Agilent technology instrument. The high resolution mass spectra (HRMS) were obtained by TOF (time of flight) using ESI



p = 2 to 12 carbon atoms
n = 1(a), 2(b), 3(c), 4(d) or 5(e)

Fig. 1. Structure of the 17β-estradiol-platinum(II) complexes studied in our laboratory.

(electrospray ionization) using the positive mode (ESI+) (Université du Québec à Montréal).

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 200 MHz NMR apparatus. Samples were dissolved in deuterochloroform ($CDCl_3$) or in deuteroacetone (acetone-d_6) for data acquisition using tetramethylsilane as internal standard (TMS, δ 0.0 ppm for ^{1}H NMR and ^{13}C NMR). If necessary, carbon identification was clarified by using DEPT (Distortionless Enhancement by Polarization Transfert) technique. 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.

2.1.1. Preparation of the diiodo PEG-chains

*2.1.1.1. Synthesis of α,ω-diiodo-PEG (**7a**).* Sodium iodide (3.14 g, 21.0 mmol) was dissolved in a flask with 15 mL of anhydrous acetone (dried by $MgSO_4$) and then 2-chloroethyl ether **5** (0.82 mL, 7.0 mmol) was added. The flask was covered with an aluminum paper in order to protect from daylight. The mixture was heated to reflux and stirred for 3 days. The solvent was evaporated and the product was dissolved with diethyl ether. The resulting organic phase was first washed with water (100 mL) to which was added 6 mL of 5% sodium thiosulfate solution and after with water (4× 100 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated that gave the product with 81% yield.

*2.1.1.2. Synthesis of α,ω-diiodo-PEG (**7b-e**).* Each of the ethylene glycol **6b-e** (tri, tetra-, penta-, hexa-; 6.8 mmol, 1 equiv.) was dissolved in diethyl ether (50 mL) in presence of triethylamine (2.38 mL, 1.7 mmol) under nitrogen atmosphere. The resulting solution was cooling down with an ice bath; afterwards methanesulfonyl chloride (CH_3SO_2Cl, 1.1 mL, 13.6 mmol) was added drop-wise to the mixture. The mixture was stirrred at 0 °C for 1 h and then at room temperature for 5 h. After 5 h the solvent was evaporated and then 70 mL of dry acetone (dried with $MgSO_4$) was added and stirred for 5 min. The ammonium chloride salt was filtered (rinsed with 30 mL of acetone). Then, sodium iodide NaI (4.1 g, 27.0 mmol) was added to the filtrate (8b-e) and the mixture was heated to reflux with stirring for 20 h under a nitrogen atmosphere. Afterwards the mixture was cooled down; sodium

thiosulfate was added and stirred for 5 min, dried with anhydrous magnesium sulfate. The mixture was filtered on a silica gel (2.5 cm) and celite (0.5 cm) pad using hexanes (200 mL) mixed with a little amount of dichloromethane as the eluent. The solvent was evaporated and the yield of diiodo-PEG chain **7b-e** was 79%.

2.1.1.3. Spectral data for 1,5-diiodo-3-oxapentane (7a). **IR (NaCl, ν_{max}, cm^{-1})**: 1465 (CH_2, symmetric deformation) and 1102 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 3.72 (4H, t, J = 6.9 Hz, CH_2O), 3.21 (4H, t, J = 6.9 Hz, CH_2I). **^{13}C NMR ($CDCl_3$, δ ppm)**: 71.6 (2 × CH_2O), 2.8 (2 × CH_2I). **MS (m/e), $C_4H_8I_2O_1$**: 326 ($M^{+\bullet}$). **Exact mass**: calculated for $C_4H_8I_2O_1$ = 325.8665; found = 325.8673.

2.1.1.4. Spectral data for 1,8-diiodo-3,6-dioxaoctane (7b). **IR (NaCl, ν_{max}, cm^{-1})**: 1465 (CH_2, symmetric deformation) and 1102 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 3.72 (4H, t, J = 6.6 Hz, OCH_2CH_2I), 3.61 (8H, m, OCH_2CH_2O), 3.21 (4H, t, J = 6.6 Hz, CH_2I). **^{13}C NMR ($CDCl_3$, δ ppm)**: 72.1 (2 × OCH_2CH_2I), 70.3 (OCH_2CH_2O), 3.5 (2 × CH_2I). **MS (m/e), $C_6H_{12}I_2O_2$**: 370 ($M^{+\bullet}$). **Exact mass**: calculated for $C_6H_{12}I_2O_2$ = 369.8927; found = 369.8930.

2.1.1.5. Spectral data for 1,11-diiodo-3,6,9-trioxaundecane (7c). **IR (NaCl, ν_{max}, cm^{-1})**: 1465 (CH_2, symmetric deformation) and 1112 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 3.77 (4H, t, J = 6.8 Hz, OCH_2CH_2I), 3.67 (8H, m, OCH_2CH_2O), 3.21 (4H, t, J = 6.9 Hz, CH_2I). **^{13}C NMR ($CDCl_3$, δ ppm)**: 72.2 (2 × OCH_2CH_2I), 70.9 and 70.5 (2 × OCH_2CH_2O), 3.3 (2 × CH_2I). **MS (m/e), $C_8H_{16}I_2O_3$**: 415 $(M+H)^+$, 432 $(M+NH_4)^+$. **Exact mass**: calculated for $C_8H_{16}I_2O_3$ + H = 414.9267; found = 414.9275.

2.1.1.6. Spectral data for 1,14-diiodo-3,6,9,12-tetraoxatetradecane (7d). **IR (NaCl, ν_{max}, cm^{-1})**: 1465 (CH_2, symmetric deformation) and 1107 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 3.67 (4H, t, J = 7.0 Hz, OCH_2CH_2I), 3.57 (12H, m, OCH_2CH_2O), 3.17 (4H, t, J = 7.0 Hz, CH_2I). **^{13}C NMR ($CDCl_3$, δ ppm)**: 71.9 (2 × OCH_2CH_2I), 70.7, 70.6 and 70.2 (2 × CH_2-OCH_2CH_2O), 3.3 (2 × CH_2I). **MS (m/e), $C_{10}H_{20}I_2O_4$**: 459 $(M+H)^+$, 476 $(M+NH_4)^+$. **Exact mass**: calculated for $C_{10}H_{20}I_2O_4$ + H = 458.9529; found = 458.9525.

2.1.1.7. Spectral data for 1,17-diiodo-3,6,9,12,15-pentaoxaheptadecane (7e). **IR (NaCl, ν_{max}, cm^{-1})**: 1460 (CH_2, symmetric deformation) and 1102 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 3.70 (4H, t, J = 6.8 Hz, OCH_2CH_2I), 3.60 (16H, m, OCH_2CH_2O), 3.20 (4H, t, J = 7.0 Hz, CH_2I). **^{13}C NMR ($CDCl_3$, δ ppm)**: 72.1 (2 × OCH_2CH_2I), 70.8 (2C), 70.7 (4C) and 70.4 (2C) (4 × OCH_2CH_2O), 3.3 (2 × CH_2I). **MS (m/e), $C_{12}H_{24}I_2O_5$**: 503 $(M+H)^+$, 520 $(M+NH_4)^+$. **Exact mass**: calculated for $C_{12}H_{24}I_2O_5$ + H = 502.9791; found = 502.9780.

2.1.2. Synthesis of platinum complexes 1a–e

*2.1.2.1. Synthesis of 3-tetrahydropyrannyloxy-16β-(methoxycarbonyl)-1,3,5(10)-estratrien-17-one (**10**): Step 1 (Protection): synthesis of 3-(tetrahydropyran-2-yloxy)-1,3,5(10)-estratrien-17-one.* To a solution of estrone (**9**) (4.40 g, 16.2 mmol) in dichloromethane (45 mL), pyridinium *p*-toluenesulfonate (100 mg, 0.4 mmol) and 3,4-dihydro-2*H*-pyran (3.7 mL, 40.5 mmol), were added. The resulting mixture was stirred at room temperature (22 °C) for 24 h. Afterwards, solution was neutralised with a small amount of solid sodium bicarbonate. Magnesium sulfate was added for drying and the mixture was stirred vigorously. The mixture was filtered on a silica gel (3 cm) and celite (1 cm) pad using a mixture of dichloromethane/diethyl ether (50:50) as the eluant. The resulting filtrate was evaporated and set to vacuum for an hour to give a beige solid, 100% yield.

2.1.2.2. Spectral data for Step 1 (3-(tetrahydropyran-2-yloxy)-1,3,5(10)-estratrien-17-one): IR (NaCl, ν_{max}, cm^{-1}). 1738 (C=O), 1611 and 1505 (C=C, aromatic), 1247 and 1043 (C–O–C, aryl alkyl) and 1127 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 7.19 (1H, d, J = 8.6 Hz, 1-CH), 6.84 (2H, m, 2-CH and 4-CH), 5.39 (1H, t, J = 2.93 Hz, CH, THP), 3.92 and 3.58 (2H, 2 m, CH_2O, THP), 2.87 (2H, m, 6-CH_2), 2.55-1.36 (19 H, several m, 3 × CH and 8 × CH_2), 0,90 (3H, s, 18-CH_3). **^{13}C NMR ($CDCl_3$, δ ppm)**: 220.9 (C-17), 155.3 (C-3), 137.9 (C-5), 133.2 (C-10), 126.4 (C-1), 116.8 (C-4), 114.4 (C-2), 96.6 (CH, THP), 62.2 (CH_2O, THP), 50.7, 48.2, 44.3, 38.6, 36.1, 31.8, 30.7, 29.8, 26.8, 26.1, 25.5, 21.8, 19.0, 14.1 (C-18). **MS (m/e), $C_{23}H_{30}O_3$**: 354 ($M^{+\bullet}$), 271 (M-C_5H_7O)$^{+\bullet}$. **Exact mass**: calculated for $C_{23}H_{30}O_3$ = 354.2195; found = 354.2200.

2.1.2.3. Step 2 (activation). In a double necked flask, anhydrous tetrahydrofuran (30 mL) was added to potassium hydride (30% in oil) (KH pure: 1.95 g, 48.6 mmol, with oil: 6.5 g) under nitrogen atmosphere and the suspension was stirred at room temperature. Dimethylcarbonate (3.35 mL, 40.5 mmol) was then added to the potassium hydride suspension. Separately, the protected estrone (from Step 1) (5.74 g, 16.2 mmol) was dissolved in tetrahydrofuran (45 mL) under nitrogen atmosphere. The estrone solution was then added to reaction mixture and the overall mixture was refluxed for 3–5 h. Afterwards, the mixture was cooled down. Tert-butanol (2 mL), methyl alcohol (2 mL) and 20 mL of water were added successively and the resulting solution was stirred for 20 min between each addition. The mixture was then diluted with ethyl acetate (100 mL) and the organic phase was washed with a saturated NH_4Cl solution and then with water (5× 100 mL). The organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated to give the crude product. The residue was purified by flash chromatography with a mixture of hexanes and acetone (90:10) to give the activated estrone in 90% yield.

2.1.2.4. Spectral data for Step 2 (3-tetrahydropyrannyloxy-16β-(methoxycarbonyl)-1,3,5(10)-estratrien-17-one) (10). **IR (NaCl, ν_{max}, cm^{-1})**: 1757 (C=O, ester), 1731 (C=O, ketone), 1619 and 1496 (C=C aromatic), 1224 and 1035 (C–O–C, aryl alkyl) and 1143 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 7.17 (1H, d, J = 8.6 Hz, 1-CH), 6.81 (2H, m, 2-CH and 4-CH), 5.36 (1H, m, CH, THP), 3.92 and 3.58 (2H, 2 m, C**H$_2$**O, THP), 3.76 (3H, s, COOC**H$_3$**), 3.20 (1H, t, J = 9.2 Hz, 16-CH), 2.88 (2H, m, 6-CH_2), 2.40–1.41 (17H, several m, 3 × CH and 7 × CH_2), 0.95 (3H, s, 18-CH_3). **^{13}C NMR ($CDCl_3$, δ ppm)**: 212.2 (C-17), 170.1 (**C**$OOCH_3$), 155.3 (C-3), 137.7 (C-5), 132.9 (C-10), 126.4 (C-1), 116.8 (C-4), 114.3 (C-2), 96.5 (CH, THP), 62.1 (CH_2O, THP), 54.3 (COO**C**H_3), 52.7, 49.1, 48.0, 44.2, 38.1, 32.1, 30.6, 29.8, 26.8, 26.6, 26.0, 25.5, 19.0, 13.5 (C-18). **MS (m/e), $C_{25}H_{32}O_5$**: 413 $(M+H)^+$, 381 $(M-CH_3O)^+$, 328 $(M-C_5H_7O)^+$. **Exact mass**: calculated for $C_{25}H_{32}O_5$ + H = 413.2328; found = 413.2319.

*2.1.2.5. General procedure for the synthesis of 16α-[ω-iodo-PEG]-16β-(methoxycarbonyl)-3-(tetrahydropyran-2-yloxy)-1,3,5(10)-estratrien-17-one (**11a-e**).* The activated product **10** (1.2 mmol) and the diiodo chains 7 (a, b, c, d or e) (4.8 mmol) were dissolved with 20 mL of dry tetrahydrofuran (THF) in a flask. Cesium carbonate (0.43 g, 1.3 mmol) was then added to the solution and the resulting mixture was stirred at reflux, under nitrogen atmosphere, the reaction vessel sheltered from daylight, for 5 h. Afterwards, the solvent was evaporated. The mixture was diluted with diethyl ether (100 mL) and washed initially with a saturated ammonium chloride solution (100 mL) and then with distilled water (5× 100 mL). The organic phase was dried with magnesium sulfate, filtered and evaporated to give the crude product. The final product was purified by flash chromatography with a mixture of hexanes/acetone of 90:10 for the compounds **11a**, **11b** and **11c** (n = 1–3) and of 85:15 for **11d** and **11e** (n = 4 and 5). The optimized output of the pure product was obtained with 63% yield.

2.1.2.6. Spectral data for 16α-[5-iodo-3-oxapentyl]-16β-(methoxycarbonyl)-3-(tetrahydropyran-2-yloxy)-1,3,5(10)-estratrien-17-one (11a). **IR (NaCl, ν_{max}, cm^{-1})**: 1757 (C=O, ester), 1726 (C=O, ketone), 1609 et 1501 (C=C, aromatic), 1240 (C–O–C, aryl alkyl) and 1117 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 7.18 (1H, d, *J* = 8.6 Hz, 1-CH), 6.83 (2H, m, 2-CH and 4-CH), 5.39 (1H, t, *J* = 3.1 Hz, CH, THP), 3.92 (1H, m, C**H**$_a$H$_b$O on THP), 3.72 (3H, s, COOC**H**$_3$), 3.62 (5H, m, CH$_a$**H**$_b$O on THP and 2 × OCH_2 on PEG chain), 3.20 (2H, t, *J* = 6.6 Hz, C**H$_2$**I), 2.88 (2H, m, 6-CH_2), 2.45–1.26 (19H, several m, 3 × CH et 8 × CH_2), 0.95 (3H, s, 18-CH_3). **^{13}C NMR ($CDCl_3$, δ ppm)**: 214.1 (C-17), 171.9 (**C**$OOCH_3$), 155.2 (C-3), 137.6 (C-5), 132.8 (C-10), 126.3 (C-1), 116.7 (C-4), 114.2 (C-2), 96.4 (CH, THP), 71.5 and 67.5 (2 × O**C**H_2 on PEG chain), 62.0 (**C**H_2O, THP), 58.3 (COO**C**H_3), 52.8, 49.5, 46.0, 44.2, 37.9, 34.9, 32.3, 31.3, 30.5, 29.6, 26.7, 25.8, 25.4, 18.9, 14.3 (C-18), 3.0 (CH_2I). **MS (m/e), $C_{29}H_{39}I_1O_6$**: 610 ($M^{+\bullet}$), 526 ($M–C_5H_8O_1)^{+\bullet}$. **Exact mass**: calculated for $C_{29}H_{39}I_1O_6$ = 610.1791; found = 610.1780.

2.1.2.7. Spectral data for 16α-[8-iodo-3,6-dioxaoctyl]-16β-(methoxycarbonyl)-3-(tetrahydropyran-2-yloxy)-1,3,5(10)-estratrien-17-one (11b). **IR (NaCl, ν_{max}, cm^{-1})**: 1752 (C=O, ester), 1721 (C=O, ketone), 1609 and 1496 (C=C, aromatic), 1240 (C–O–C, aryl alkyl) and 1117 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 7.12 (1H, d, *J* = 8.6 Hz, 1-CH), 6.75 (2H, m, 2-CH and 4-CH), 5.3 (1H, m, CH, THP), 3.86 (1H, m, C**H$_a$**H$_b$O on THP), 3.67 (3H, s, COOC**H$_3$**), 3.52 (9H, m, CH$_a$**H$_b$**O on THP and 4 × OCH_2 on PEG chain), 3.19 (2H, t, *J* = 6.8 Hz, C**H$_2$**I), 2.83 (2H, m, 6-CH_2), 2.40–1.38 (19H, several m, 3 × CH and 8 × CH_2), 0.90 (3H, s, 18-CH_3). **^{13}C NMR ($CDCl_3$, δ ppm)**: 213.9 (C-17), 171.8 (**C**$OOCH_3$), 155.0 (C-3), 137.4 (C-5), 132.7 (C-10), 126.1 (C-1), 116.5 (C-4), 114.0 (C-2), 96.2 (CH, THP), 71.9, 70.1 and 67.8 (4 × O**C**H_2, 70.1 represents 2C), 61.8 (**C**H_2O, THP), 58.2 (COO**C**H_3), 52.6, 49.4, 45.8, 44.0, 37.8, 34.7, 32.1, 31.0, 30.4, 29.5, 26.5, 25.7, 25.2, 18.8, 14.2 (C-18), 3.1 (**C**H_2I). **MS (m/e), $C_{31}H_{43}I_1O_7$**: 654 ($M^{+\bullet}$), 570 ($M–C_5H_8O_1)^{+\bullet}$. **Exact mass**: calculated for $C_{31}H_{43}I_1O_7$ = 654.2053; found = 654.2042.

2.1.2.8. Spectral data for 16α-[11-iodo-3,6,9-trioxaundecanyl]-16β-(methoxy-carbonyl)-3-(tetrahydropyran-2-yloxy)-1,3,5(10)-estratrien-17-one (11c). **IR (NaCl, ν_{max}, cm^{-1})**: 1757 (C=O, ester), 1726 (C=O, ketone), 1614 and 1511 (C=C, aromatic), 1240 (C–O–C, aryl alkyl) and 1112 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 7.18 (1H, d, *J* = 8.6 Hz, 1-CH), 6.83 (2H, m, 2-CH et 4-CH), 5.39 (1H, t, *J* = 3.1 Hz, CH, THP), 3.92 (1H, m, C**H$_a$**H$_b$O on THP), 3.71 (3H, s, COOC**H$_3$**), 3.62 (13H, m, CH$_a$**H$_b$**O on THP and 6 × OCH_2 on PEG chain), 3.21 (2H, t, *J* = 7.0 Hz, C**H$_2$**I), 2.88 (2H, m, 6-CH_2), 2.45–1.25 (19H, several m, 3 × CH and 8 × CH_2), 0.94 (3H, s, 18-CH_3). **^{13}C NMR ($CDCl_3$, δ ppm)**: 214.2 (C-17), 172.0 (**C**$OOCH_3$), 155.2 (C-3), 137.7 (C-5), 132.9 (C-10), 126.3 (C-1), 116.7 (C-4), 114.3 (C-2), 96.4 (CH, THP), 72.1, 70.7, 70.3 and 68.0 (6 × O**C**H_2 on PEG chain, 70.7 and 70.3 represent 2C), 62.1 (**C**H_2O, THP), 58.4 (COO**C**H_3), 52.8, 49.6, 46.0, 44.2, 38.0, 34.9, 32.3, 31.2, 30.5, 29.7, 26.7, 25.8, 25.4, 18.9, 14.3 (C-18), 3.1 (**C**H_2I). **MS (m/e), $C_{33}H_{47}I_1O_8$**: 698 ($M^{+\bullet}$), 614 ($M–C_5H_8O_1)^{+\bullet}$. **Exact mass**: calculated for $C_{33}H_{47}I_1O_8$ = 698.2315; found = 698.2303.

2.1.2.9. Spectral data for 16α-[14-iodo-3,6,9,12-tetraoxatetradecanyl]-16β-(methoxycarbonyl)-3-(tetrahydropyran-2-yloxy)-1,3,5(10)-estratrien-17-one (11d). **IR (NaCl, ν_{max}, cm^{-1})**: 1752 (C=O, ester), 1731 (C=O, ketone), 1614 and 1501 (C=C, aromatic), 1245 (C–O–C, aryl alkyl) and 1112 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 7.16 (1H, d, *J* = 8.6 Hz, 1-CH), 6.80 (2H, m, 2-CH and 4-CH), 5.37 (1H, t, *J* = 3.1 Hz, CH, THP), 3.92 (1H, m, C**H$_a$**H$_b$O on THP), 3,69 (3H, s, COOC**H$_3$**), 3.63 (17H, m, CH$_a$**H$_b$**O on THP and 8 × OCH_2 on PEG chain), 3.23 (2H, t, *J* = 6.8 Hz, C**H$_2$**I), 2.88 (2H, m, 6-CH_2), 2.45-1.25 (19H, several m, 3 × CH et 8 × CH_2), 0.92 (3H, s, 18-CH_3). **^{13}C NMR ($CDCl_3$, δ ppm)**: 214.0 (C-17), 171.9 (**C**$OOCH_3$), 155.2 (C-3), 137.6 (C-5), 132.9 (C-10), 126.3 (C-1), 116.7 (C-4), 114.2 (C-2), 96.4 (CH, THP), 72.1, 70.8, 70.7, 70.6, 70.3 and 68.0 (8 × O**C**H_2 on PEG chain, 70.8 and 70.7 represent 2C), 62.0 (**C**H_2O, THP), 58.3 (COO**C**H_3), 52.7, 49.5, 46.0, 44.2, 38.0, 34.9, 32.3, 31.1, 30.5, 29.7, 26.6, 25.8, 25,4, 18.9, 14.3 (C-18), 3.2 (**C**H_2I). **MS (m/e), $C_{35}H_{51}I_1O_9$**: 743 $(M+H)^+$, 760 $(M+NH_4)^+$. **Exact mass**: calculated for $C_{35}H_{51}I_1O_9$ + H = 743.2656; found = 743.2642.

2.1.2.10. Spectral data for 16α-[17-iodo-3,6,9,12,15-pentaoxaheptadecanyl]-16β-(methoxycarbonyl)-3-(tetrahydropyran-2-yloxy)-1,3,5(10)-estratrien-17-one (11e). **IR (NaCl, ν_{max}, cm^{-1})**: 1752 (C=O, ester), 1721 (C=O, ketone), 1614 and 1496 (C=C, aromatic), 1240 (C–O–C, aryl alkyl) and 1107 (C–O–C, aliphatic). **^{1}H NMR ($CDCl_3$, δ ppm)**: 7.15 (1H, d, *J* = 8.6 Hz, 1-CH), 6.78 (2H, m, 2-CH and 4-CH), 5.36 (1H, t, *J* = 3.1 Hz, CH, THP), 3.92 (1H, m, CH$_a$H$_b$O on THP), 3.68 (3H, s, $COOCH_3$), 3.62 (21H, m, CH$_a$**H$_b$**O on THP and 10 × OCH_2 on PEG chain), 3.22 (2H, t, *J* = 6.8 Hz, C**H$_2$**I), 2.88 (2H, m, 6-CH_2), 2.45–1.25 (19H, several m, 3 × CH and 8 × CH_2), 0.91 (3H, s, 18-CH_3). **^{13}C NMR ($CDCl_3$, δ ppm)**: 214.2 (C-17), 172.0 (**C**$OOCH_3$), 155.2 (C-3), 137.7 (C-5), 132.9 (C-10), 126.3 (C-1), 116.7 (C-4), 114.3 (C-2), 96.4 (CH, THP), 72.2, 70.8, 70.8, 70.7, 70.4, 70.3 and 68.0 (10 × O**C**H_2 on PEG chain, 2 signals of 70.8 represent 5C), 62.1 (**C**H_2O, THP), 58.4 (COO**C**H_3), 52.8, 49.6, 46.1, 44.2, 38.0, 35.0, 32.4, 31.2, 30.6, 29.7, 26.7, 25.9, 25.4, 18.9, 14.3 (C-18), 3.2 (CH_2I). **MS (m/e), $C_{37}H_{55}I_1O_{10}$**: 804 $(M+NH_4)^+$. **Exact mass**: calculated for $C_{37}H_{55}I_1O_{10}$ + NH_4 = 804.3183; found = 804.3190.

*2.1.2.11. General procedure for the synthesis of 16β-hydroxymethyl-16α-[ω-iodo-PEG]-1,3,5(10)-estratrien-3,17β-diol (**12a-e**).*. Step 1 (Reduction): Synthesis of 16β-16-hydroxymethyl-16α-[ω-iodo-PEG]-3-(tetrahydro-pyran-2-yloxy)-1,3,5(10)-estratrien-17β-ol. The alkylated product **11** (0.6 mmol) was dissolved in anhydrous diethyl ether (10 mL), under nitrogen atmosphere. The resulting solution was cooled down in an ice bath. After a few minutes, the lithium borohydride $LiBH_4$ (82 mg, 3.8 mmol) was added in two portions separated by approximately 5 min and the reaction vessel sheltered from daylight. The mixture was kept at 0 °C and under nitrogen atmosphere for 1 h and then at room temperature for 11 h. Afterwards, the solution was neutralized with a small amount of sodium sulfate decahydrate. The product was extracted with ethyl acetate (100 mL) and washed first with a saturated NH_4Cl solution (100 mL) and then with water (5× 100 mL). The organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated to give the crude product. The compound was then used as such for the next step; the removal of the protective group at C-3.

2.1.2.12. Step 2 (deprotection): to a solution of crude product (0.6 mmol) in 10 mL of ethanol, pyridinium p-toluenesulfonate (50 mg, 0.2 mmol) was added. The resulting mixture was stirred at reflux, the reaction vessel sheltered from daylight, for 4 h. When the reaction was completed, 5 mL of saturated ammonium chloride solution (NH_4Cl sat.) was added and stirred for 5 min. The product was extracted with ethyl acetate (100 mL) and washed first with a saturated NH_4Cl solution (100 mL) and then with water (5× 100 mL). The organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated to give the crude product. The compound was finally purified by flash chromatography while using a mixture of hexanes and acetone 70:30 for the product **12a**, **12b**, **12c** and **12d** and of 60:40 for the product **12e**. The optimized output of the reaction was of 70% yield.

2.1.2.13. Spectral data for 16β-hydroxymethyl-16α-[5-iodo-3-oxapentyl]-1,3,5(10)-estratrien-3,17β-diol (12a). **IR (NaCl, ν_{max}, cm^{-1})**: 3365 (O–H), 1609 and 1501 (C=C, aromatic) and 1102 (C–O–C, aliphatic). **^{1}H NMR (acetone-d_6, δ ppm)**: 7.93 (1H, s,

phenol-O**H**), 7.09 (1H, d, *J* = 8.6 Hz, 1-CH), 6.59 (1H, dd, *J* = 8.6 Hz and 2.7 Hz, 2-CH), 6.52 (1H, d, *J* = 2.7 Hz, 4-CH), 4.25 (1H, d, *J* = 4.3 Hz, C**H$_a$**H$_b$OH), 3.80–3.39 (6H, m, C**H**OH, CH$_a$**H$_b$**OH and 2 × OCH$_2$ on PEG chain), 3.35 (2H, t, *J* = 6.4 Hz, C**H$_2$**I), 2.75 (2H, m, 6-CH$_2$), 2.25–1.11 (15H, several m, 2 × OH, 3 × CH and 5 × CH$_2$), 0.88 (3H, s, 18-CH$_3$). **^{13}C NMR (acetone-d$_6$, δ ppm)**: 155.1 (C-3), 138.5 (C-5), 132.2 (C-10), 127.1 (C-1), 116.1 (C-4), 113.7 (C-2), 90.4 (17-C), 72.3, 68.9 and 67.6 (**C**H$_2$OH and 2 × O**C**H$_2$ on PEG chain), 48.4, 46.9, 45.8, 45.0, 39.9, 39.3, 39.0, 35.4, 30.5, 28.5, 27.3, 12.6 (C-18), 4.8 (**C**H$_2$I). **MS (m/e), C$_{23}$H$_{33}$I$_1$O$_4$**: 500 (M$^{+\bullet}$). **Exact mass**: calculated for C$_{23}$H$_{33}$I$_1$O$_4$ = 500.1423; found = 500.1419.

2.1.2.14. Spectral data for 16β-hydroxymethyl-16α-[8-iodo-3,6-dioxaoctyl]-1,3,5(10)-estratrien-3,17β-diol (12b). **IR (NaCl, ν_{max}, cm^{-1})**: 3369 (O–H), 1616 and 1506 (C=C, aromatic) and 1099 (C–O–C, aliphatic). **^{1}H NMR (acetone-d$_6$, δ ppm)**: 7.92 (1H, s, phenol-O**H**), 7.09 (1H, d, *J* = 8.6 Hz, 1-CH), 6.59 (1H, dd, *J* = 8.6 Hz et 2.5 Hz, 2-CH), 6.52 (1H, d, *J* = 2.3 Hz, 4-CH), 4.20 (1H, d, *J* = 4.3 Hz, C**H$_a$**H$_b$OH), 3.78–3.39 (10H, m, C**H**OH, CH$_a$**H$_b$**OH and 4 × OCH$_2$ on PEG chain), 3.34 (2H, t, *J* = 6.4 Hz, C**H$_2$**I), 2.75 (2H, m, 6-CH$_2$), 2.31–1.11 (15H, several m, 2 × OH, 3 × CH and 5 × CH$_2$), 0.88 (3H, s, 18-CH$_3$). **^{13}C NMR (acetone-d$_6$, δ ppm)**: 155.9 (C-3), 138.4 (C-5), 132.1 (C-10), 127.0 (C-1), 115.9 (C-4), 113.6 (C-2), 90.2 (C-17), 72.5, 70.9, 70.6, 69.1 and 67.4 (**C**H$_2$OH and 4 × O**C**H$_2$ on PEG chain), 48.2, 46.9, 45.7, 44.8, 40.0, 39.1, 38.9, 35.8, 30.2, 28.3, 27.2, 12.5 (C-18), 4.1 (**C**H$_2$I). **MS (m/e), C$_{25}$H$_{37}$I$_1$O$_5$**: 544 (M$^{+\bullet}$), 526 (M–H$_2$O)$^{+\bullet}$. **Exact mass**: calculated for C$_{25}$H$_{37}$I$_1$O$_5$ = 544.1686; found = 544.1675.

2.1.2.15. Spectral data for 16β-hydroxymethyl-16α-[11-iodo-3,6,9-trioxaundecanyl]-1,3,5(10)-estratrien-3,17β-diol (12c). **IR (NaCl, ν_{max}, cm^{-1})**: 3380 (O–H), 1619 and 1506 (C=C, aromatic) and 1107 (C–O–C, aliphatic). **^{1}H NMR (acetone-d$_6$, δ ppm)**: 7.99 (1H, s, phenol-O**H**), 7.08 (1H, d, *J* = 8.6 Hz, 1-CH), 6.59 (1H, dd, *J* = 8.2 Hz and 2.7 Hz, 2-CH), 6.52 (1H, d, *J* = 2.4 Hz, 4-CH), 4.26 (1H, d, *J* = 3.9 Hz, C**H$_a$**H$_b$OH), 3.76–3.42 (14H, m, C**H**OH, CH$_a$**H$_b$**OH and 6 × OCH$_2$ on PEG chain), 3.32 (2H, t, *J* = 6.6 Hz, C**H$_2$**I), 2.74 (2H, m, 6-CH$_2$), 2.30–1.10 (15H, several m, 2 × OH, 3 × CH and 5 × CH$_2$), 0.87 (3H, s, 18-CH$_3$). **^{13}C NMR (acetone-d$_6$, δ ppm)**: 155.9 (C-3), 138.3 (C-5), 132.0 (C-10), 127.0 (C-1), 116.0 (C-4), 113.6 (C-2), 90.2 (C-17), 72.5, 71.2, 71.1, 70.9, 70.8, 69.1 and 67.4 (**C**H$_2$OH and 6 × O**C**H$_2$ on PEG chain), 48.2, 46.9, 45.8, 44.8, 40.0, 39.1, 38.9, 35.9, 30.3, 28.3, 27.2, 12.6 (C-18), 4.5 (**C**H$_2$I). **MS (m/e), C$_{27}$H$_{41}$I$_1$O$_6$**: 588 (M$^{+\bullet}$). **Exact mass**: calculated for C$_{27}$H$_{41}$I$_1$O$_6$ = 588.1948; found = 588.1940.

2.1.2.16. Spectral data for 16β-hydroxymethyl-16α-[14-iodo-3,6,9,12-tetraoxatetradecanyl]-1,3,5(10)-estratrien-3,17β-diol (12d). **IR (NaCl, ν_{max}, cm^{-1})**: 3370 (O–H), 1614 and 1506 (C=C, aromatic) and 1102 (C–O–C, aliphatic). **^{1}H NMR (acetone-d$_6$, δ ppm)**: 7.95 (1H, s, phenol–O**H**), 7.08 (1H, d, *J* = 8.6 Hz, 1-CH), 6.59 (1H, dd, *J* = 8.2 Hz and 2.7 Hz, 2-CH), 6.52 (1H, d, *J* = 2.4 Hz, 4-CH), 4.24 (1H, d, *J* = 4.3 Hz, C**H$_a$**H$_b$OH), 3.76–3.41 (18H, m, C**H**OH, CH$_a$**H$_b$**OH and 8 × OCH$_2$ on PEG chain), 3.32 (2H, t, *J* = 6.6 Hz, C**H$_2$**I), 2.75 (2H, m, 6-CH$_2$), 2.23–1.10 (15H, several m, 2 × OH, 3 × CH and 5 × CH$_2$), 0.87 (3H, s, 18-CH$_3$). **^{13}C NMR (acetone-d$_6$, δ ppm)**: 156.0 (C-3), 138.4 (C-5), 132.1 (C-10), 127.0 (C-1), 116.0 (C-4), 113.7 (C-2), 90.2 (C-17), 72.6, 71.3, 71.2, 71.1, 70.9, 70.8, 69.1 and 67.5 (**C**H$_2$OH and 8 × O**C**H$_2$ on PEG chain, 71.2 represents 2 C), 48.3, 47.0, 45.8, 44.9, 40.0, 39.2, 39.0, 36.0, 30.3, 28.4, 27.2, 12.6 (C-18), 4.6 (**C**H$_2$I). **MS (m/e), C$_{29}$H$_{45}$I$_1$O$_7$**: 633 (M+H)$^+$. **Exact mass**: calculated for C$_{29}$H$_{45}$I$_1$O$_7$ + H = 633.2288; found = 633.2278.

2.1.2.17. Spectral data for 16β-hydroxymethyl-16α-[17-iodo-3,6,9,12,15-pentaoxaheptadecanyl]-1,3,5(10)-estratrien-3,17β-diol (12e). **IR (NaCl, ν_{max}, cm^{-1})**: 3385 (O–H), 1619 and 1505 (C=C, aromatic) and 1102 (C–O–C, aliphatic). **^{1}H NMR (acetone-d$_6$, δ ppm)**: 7.93 (1H, s, phenol–O**H**), 7.09 (1H, d, *J* = 8.6 Hz, 1-CH), 6.59 (1H, dd, *J* = 8.4 Hz and 2.5 Hz, 2-CH), 6.52 (1H, d, *J* = 2.4 Hz, 4-CH), 4.20 (1H, d, *J* = 4.3 Hz, C**H$_a$**H$_b$OH), 3.76–3.43 (22H, m, C**H**OH, CH$_a$**H$_b$**OH and 10 × OCH$_2$ on PEG chain), 3.32 (2H, t, *J* = 6.4 Hz, C**H$_2$**I), 2.75 (2H, m, 6-CH$_2$), 2.23-1.10 (15H, several m, 2 × OH, 3 × CH and 5 × CH$_2$), 0.87 (3H, s, 18-CH$_3$). **^{13}C NMR (acetone-d$_6$, δ ppm)**: 156.1 (C-3), 138.5 (C-5), 132.2 (C-10), 127.1 (C-1), 116.1 (C-4), 113.7 (C-2), 90.3 (C-17), 72.7, 71.4, 71.4, 71.2, 71.0, 71.0 69.2 and 67.5 (**C**H$_2$OH and 10 × O**C**H$_2$ on PEG chain, two signals of 71.4 represents 5C), 48.4, 47.1, 45.9, 45.0, 40.1, 39.3, 39.1, 36.1, 30.4, 28.4, 27.3, 12.7 (C-18), 4.6 (**C**H$_2$I). **MS (m/e), C$_{31}$H$_{49}$I$_1$O$_8$**: 677 (M+H)$^+$. **Exact mass**: calculated for C$_{31}$H$_{49}$I$_1$O$_8$ + H = 677.2550; found = 677.2540.

*2.1.2.18. General procedure for the synthesis of 16β-hydroxymethyl-16α-[ω-(2-pyridylethylamino)-PEG]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinate(II) (**1a-e**). Step 1 (Addition of aminoethylpyridine).* To a solution of derivative 12 in methanol (4 mL) was added 2-aminoethylpyridine (0.64 mL, 5.4 mmol) under nitrogen atmosphere. The resulting mixture was stirred at reflux, the reaction vessel sheltered from daylight, for 4 h. Then, the methanol was evaporated and the residue was diluted with ethyl acetate (50 mL) and washed with distilled water (5× 50 mL). The organic phase was dried with magnesium sulfate, filtered and evaporated to yield the crude product **4a-e** which was used as such at the next step.

2.1.2.19. Step 2 (complexation). A solution of crude aminopyridine (0.37 mmol) in DMF (6 mL) was treated with a solution of potassium tetrachloroplatinate(II) (0.187 g, 0.5 mmol) in water (2 mL) and the mixture was stirred at room temperature (22 °C), the reaction vessel kept in the dark. The pH was measured and it usually varied between pH 8–9. After 3 days of stirring in the darkness, pH was reached to about 5. Then, 4–5 drops of DMSO were added to the resulting mixture to destroy the excess of K_2PtCl_4 and the solution was further stirred for 3 h. Next, a saturated KCl solution (4–5 mL) was added to the mixture and stirred for 1 h. A small amount of solid KCl was then added and the mixture was strongly stirred for 18 h in order to pulverize the lumps of the precipitated Pt(II) complex. The solid suspension was then filtered; the product was washed with water and then with diethyl ether to accelerate the drying process. Of note, only a small amount of diethyl ether should be used as the platinum complex is slightly soluble. The solid is dried in desiccators for 1 day. The residue was finally dissolved in a mixture of acetone and methanol and adsorbed on silica gel. Then, it was purified by flash chromatography with hexanes and acetone (45:55) for **1a**, 40:60 for **1b**, **1c** and **1d** and 35:65 for **1e**. The optimized output of the pure product **1** was obtained 55% yield.

2.1.2.20. Spectral data for 16β-hydroxymethyl-16α-[5-(2-pyridin-2-yl-ethylamino)-3-oxapentyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinum(II) (1a). **IR (KBr, ν_{max}, cm^{-1})**: 3430–3170 (O–H and N–H), 1611 and 1499 (C=C, aromatic) and 1109 (C–O–C, aliphatic). **^{1}H NMR (acetone-d$_6$, δ ppm)**: 9.16 (1H, d, *J* = 5.9 Hz, a-CH), 8.04 (1H, t, *J* = 7.2 Hz, c-CH), 7.93 (1H, s, phenol-O**H**), 7.54 (1H, d, *J* = 7.4 Hz, d-CH), 7.43 (1H, t, *J* = 6.6 Hz, b-CH), 7.08 (1H, d, *J* = 8.2 Hz, 1-C), 6.59 (1H, dd, *J* = 8.1 Hz and 2.5 Hz, 2-CH), 6.53 (1H, d, *J* = 2.3 Hz, 4-CH), 6.12 (1H, s, NH) 4.21 (1H, m, C**H$_a$**H$_b$OH), 4.12–2.90 (12H, several m, C**H**OH, CH$_a$**H$_b$**OH, 2 × OCH$_2$ on PEG chain and OCH$_2$C**H$_2$**NHC**H$_2$**C**H$_2$**-pyridine), 2.75 (2H, m, 6-CH$_2$), 2.69–1.16 (15H, several m, 2 × OH, 3 × CH and 5 × CH$_2$), 0.86 (3H, s, 18-CH$_3$). **^{13}C NMR (acetone-d$_6$, δ ppm)**: 159.9 (C-e), 155.3 (C-3), 153.7 (C-a), 139.4 (C-c), 137.8 (C-5), 131.5 (10-C), 126.4 (C-1), 124.8 (C-d), 123.9 (C-b), 115.3 (C-4), 113.0 (C-2), 89.3 (17-C), 69.0, 68.4 et 66.8 (**C**H$_2$OH and 2 × O**C**H$_2$ on PEG chain), 55.1, 47.6, 46.5, 46.0, 45.1, 44.2, 39.8,

38.9, 38.5, 38.2, 34.7, 29.6, 27.7, 26.5, 11.9 (C-18). **ESI + HRMS**: $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{30}H_{43}Cl_2N_2O_4Pt$ and $C_{30}H_{42}Cl_2N_2NaO_4Pt$: 761.2233 and 783.2052; found = 761.2220 and 783.2047, respectively.

2.1.2.21. Spectral data for 16β-hydroxymethyl-16α-[8-(2-pyridin-2-yl-ethylamino)-3,6-dioxaoctyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinum(II) (1b). **IR (KBr, ν_{max}, cm^{-1})**: 3430–3170 (O–H and N–H), 1611 and 1502 (C=C, aromatic) and 1096 (C–O–C, aliphatic). **^{1}H NMR (acetone-d_6, δ ppm)**: 9.12 (1H, d, *J* = 5.9 Hz, a-CH), 8.04 (1H, t, *J* = 7.6 Hz, c-CH), 7.96 (1H, s, phenol-O**H**), 7.55 (1H, d, *J* = 7.8 Hz, d-CH), 7.42 (1H, t, *J* = 6.6 Hz, b-CH), 7.08 (1H, d, *J* = 8.6 Hz, 1-C), 6.60 (1H, dd, *J* = 8.4 Hz and 2,5 Hz, 2-CH), 6.53 (1H, d, *J* = 2.7 Hz, 4-CH), 6.12 (1H, s, NH), 4.24 (1H, m, C**H$_a$**H$_b$OH), 3.10–2.81 (16H, several m, C**H**OH, CH$_a$**H$_b$**OH, 4 × OCH_2 on PEG chain and $OCH_2C\mathbf{H_2}NHC\mathbf{H_2}C\mathbf{H_2}$-pyridine), 2.75 (2H, m, 6-CH_2), 2.57–1.21 (15H, several m, 2 × OH, 3 × CH and 5 × CH_2), 0.89 (3H, s, 18-CH_3). **^{13}C NMR (acetone-d_6, δ ppm)**: 159.9 (C-e), 155.3 (C-3), 153.7 (C-a), 139.4 (C-c), 137.8 (C-5), 131.5 (C-10), 126.4 (C-1), 124.8 (C-d), 123.8 (C-b), 115.3 (C-4), 113.0 (C-2), 89.6 (C-17), 70.0, 68.8, 68.7 and 66.7 (**C**H_2OH and 4 × O**C**H_2 on PEG chain, 70.0 represents 2C), 55.7, 47.6, 46.6, 46.4, 45.1, 44.2, 39.7, 39.4, 38.5, 38.4, 35.6, 29.6, 27.7, 26.6, 12.1 (C-18). **ESI + HRMS**: $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{32}H_{47}Cl_2N_2O_5Pt$ and $C_{32}H_{46}Cl_2N_2NaO_5Pt$:804.25043 and 826.23237; found = 804.25045 and 826.23244, respectively.

2.1.2.22. Spectral data for 16β-hydroxymethyl-16α-[11-(2-pyridin-2-yl-ethylamino)-3,6,9-trioxaundecanyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinum(II) (1c). **IR (KBr, ν_{max}, cm^{-1})**: 3430–3170 (O–H and N–H), 1614 and 1499 (C=C, aromatic) and 1100 (C–O–C, aliphatic). **^{1}H NMR (acetone-d_6, δ ppm)**: 9.13 (1H, d, *J* = 5.5 Hz, a-CH), 8.03 (1H, t, *J* = 7.6 Hz, c-CH), 7.92 (1H, s, phenol-O**H**), 7.54 (1H, d, *J* = 7.0 Hz, d-CH), 7.41 (1H, t, *J* = 6.8 Hz, b-CH), 7.09 (1H, d, *J* = 8.2 Hz, 1-C), 6.58 (1H, dd, *J* = 8.2 Hz and 2.7 Hz, 2-CH), 6.53 (1H, d, *J* = 2.7 Hz, 4-CH), 6.05 (1H, s, NH), 4.17 (1H, m, C**H$_a$**H$_b$OH), 4.08–2.86 (20H, several m, C**H**OH, CH$_a$**H$_b$**OH, 6 × OCH_2 on PEG chain and $OCH_2C\mathbf{H_2}NHC\mathbf{H_2}C\mathbf{H_2}$-pyridine), 2.75 (2H, m, 6-CH_2), 2.66–1.11 (15H, several m, 2 × OH, 3 × CH and 5 × CH_2), 0.87 (3H, s, 18-CH_3). **^{13}C NMR (acetone-d_6, δ ppm)**: 160.0 (C-e), 155.3 (C-3), 153.7 (C-a), 139.4 (C-c), 137.8 (C-5), 131.5 (C-10), 126.4 (C-1), 124.8 (C-d), 123.8 (C-b), 115.3 (C-4), 112.9 (C -2), 89.5 (C-17), 70.4, 70.3, 70.2, 69.0, 68.5 and 66.7 (**C**H_2OH and 6 × O**C**H_2 on PEG chain, 70.2 represents 2C), 55.4, 47.6, 46.4, 46.3, 45.1, 44.2, 39.7, 39.4, 38.5, 38.3, 35.3, 29.6, 27.7, 26.5, 12.0 (C-18). **ESI + HRMS**: $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{34}H_{51}Cl_2N_2O_6Pt$ and $C_{34}H_{50}Cl_2N_2NaO_6Pt$: 849.2758 and 871.2578; found = 849.2747 and 871.2574, respectively.

2.1.2.23. Spectral data for 16β-hydroxymethyl-16α-[14-(2-pyridin-2-yl-ethylamino)-3,6,9,12-tetraoxatetradecanyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinum(II) (1d). **IR (KBr, ν_{max}, cm^{-1})**: 3430–3170 (O–H and N–H), 1615 and 1502 (C=C, aromatic) and 1106 (C–O–C, aliphatic). **^{1}H NMR (acetone-d_6, δ ppm)**: 9.13 (1H, d, *J* = 5.8 Hz, a-CH), 8.03 (1H, t, *J* = 7.8 Hz, c-CH), 7.92 (1H, s, phenol-O**H**), 7.54 (1H, d, *J* = 6.6 Hz, d-CH), 7.42 (1H, t, *J* = 6.8 Hz, b-CH), 7.09 (1H, d, *J* = 8.6 Hz, 1-C), 6.59 (1H, dd, *J* = 8.6 Hz and 2.7 Hz, 2-CH), 6.52 (1H, d, *J* = 2.7 Hz, 4-CH), 5.95 (1H, s, NH), 4.18 (1H, d, *J* = 4.3 Hz, C**H$_a$**H$_b$OH), 4.15–2.86 (24H, several m, C**H**OH, CH$_a$**H$_b$**OH, 8 × OCH_2 on PEG chain and $OCH_2C\mathbf{H_2}NHC\mathbf{H_2}C\mathbf{H_2}$-pyridine), 2.75 (2H, m, 6-CH_2), 2.70-1.07 (15H, several m, 2 × OH, 3 × CH and 5 × CH_2), 0.87 (3H, s, 18-CH_3). **^{13}C NMR (acetone-d_6, δ ppm)**: 160.0 (C-e), 155.3 (C-3), 153.7 (C-a), 139.4 (C-c), 137.8 (C-5), 131.5 (C-10), 126.4 (C-1), 124.8 (C-d), 123.8 (C-b), 115.3 (C-4), 112.9 (C-2), 89.5 (C-17), 70.5, 70.4, 70.3, 70.2, 69.1, 68.5 and 66.8 (**C**H_2OH and 8 × O**C**H_2 on PEG chain, 70.4 and 70.2 represents 2 C), 55.3, 47.6, 46.4, 46.3, 45.1, 44.2, 39.7, 39.4, 38.5, 38.3, 35.3, 29.6, 27.7, 26.5, 11.9 (C-18). **ESI + HRMS**: $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{36}H_{55}Cl_2N_2O_7Pt$ and $C_{36}H_{54}Cl_2N_2NaO_7Pt$: 893.3021 and 915.2841; found = 893.3018 and 915.2837, respectively.

2.1.2.24. Spectral data for 16β-hydroxymethyl-16α-[17-(2-pyridin-2-yl-ethylamino)-3,6,9,12,15-pentaoxaheptadecanyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinum(II) (1e). **IR (KBr, ν_{max}, cm^{-1})**: 3430–3170 (O–H and N–H), 1611 and 1498 (C=C, aromatic) and 1100 (C–O–C, aliphatic). **^{1}H NMR (acetone-d_6, δ ppm)**: 9.13 (1H, d, *J* = 6.1 Hz, a-CH), 8.04 (1H, t, *J* = 6.6 Hz, c-CH), 7.96 (1H, s, phenol–O**H**), 7.55 (1H, d, *J* = 7.8 Hz, d-CH), 7.42 (1H, t, *J* = 6.6 Hz, b-CH), 7.09 (1H, d, *J* = 8.6 Hz, 1-C), 6.59 (1H, dd, *J* = 8.4 Hz and 2.5 Hz, 2-CH), 6.53 (1H, d, *J* = 2.7 Hz, 4-CH), 5.95 (1H, s, NH), 4.20 (1H, d, *J* = 3.9 Hz, C**H$_a$**H$_b$OH), 4.10–2.86 (28H, several m, C**H**OH, CH$_a$**H$_b$**OH, 10 × OCH_2 on PEG chain and $OCH_2C\mathbf{H_2}NHC\mathbf{H_2}C\mathbf{H_2}$-pyridine), 2.75 (2H, m, 6-CH_2), 2.70–1.06 (15H, several m, 2 × OH, 3 × CH and 5 × CH_2), 0.87 (3H, s, 18-CH_3). **^{13}C NMR (acetone-d_6, δ ppm)**: 160.0 (C-e), 155.3 (C-3), 153.7 (C-a), 139.4 (C-c), 137.8 (C-5), 131.5 (C-10), 126.4 (C-1), 124.8 (C-d), 123.8 (C-b), 115.3 (C-4), 112.9 (C-2), 89.5 (C-17), 70.5, 70.3, 70.2, 69.2, 68.5 and 66.8 (**C**H_2OH and 10 × O**C**H_2 on PEG chain, 70.5, 70.3 and 70.2 represents 8C), 54.8, 47.6, 46.4, 46.3, 45.1, 44.2, 39.6, 39.3, 38.5, 38.3, 35.3, 29.6, 27.7, 26.5, 11.9 (C-18). **ESI + HRMS**: $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{38}H_{59}Cl_2N_2O_8Pt$ and $C_{38}H_{58}Cl_2N_2NaO_8Pt$: 937.3284 and 959.3104; found = 937.3281 and 959.3099, respectively.

2.2. Biology

2.2.1. In vitro cytotoxic activity

The cytotoxicity of the 17β-estradiol-PEG-platinum(II) complexes (**1a**–**e**) was evaluated on MCF-7 (ER^+) and MDA-MD-231 (ER^-) breast cancer cell lines. MTT (3-(4,5-dimethylthiazol-2-yl)-phenyl-tetrazolium bromide) assay, a standard colorimetric test, was used for measuring cellular proliferation [27]. Tumor cell lines were added into 96-well tissue culture plates in culture medium and incubated at 37 °C in a 5% CO_2 atmosphere. Dilutions were done using chromophore:ethanol (1:1) solution. Cells were incubated with or without drugs for 72 h. Culture plates were processed using MTT for 3.5 h. Afterwards SDS solubilisation solution (HCl 0.010 M, sodium dodecyl solution 10%) was added. The absorbance was read using a scanning multiwell spectrophotometer (FLUOStar OPTIMA) at 565 nm. All measurements were carried out in triplicate. The results were compared with those of a control reference plate fixed on the treatment day, and the growth inhibition percentage was calculated for each drug contact period.

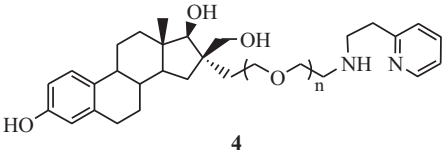
2.2.2. Estrogen receptor binding affinity

The estrogen receptor alpha (ERα) affinity assay was performed using recombinant hERα (Calbiochem/EMD BioSciences, Darmstadt, Germany) and the HitHunter™ Enzyme Fragment Complementary (EFC) Estrogen Receptor Assay kit (Discoverex Corporation, Fremont, CA) according to manufacturer's protocol [30]. HitHunter™ EFC technology is based on a genetically engineered β-galactosidase enzyme that consists of two fragments termed enzyme acceptor (EA) and enzyme donor (ED). Briefly, different concentrations of estrogen analog (**1b**) were added to wells containing ES (Estrogen Steroid) Receptor + ED in a 96-well black plate. Incubation provided competition for the estrogen receptor binding against labelled Enzyme Donor-Estrogen Steroid hormone conjugate (ED-ES conjugate), a small peptide fragment of β-galactosidase (β-gal). Then, EA, an inactive β-gal protein fragment, and a Fluorescent substrate were added to each well. Unbound ED-ES bind to EA to form an active enzyme, which subsequently hydrolyse the fluorescent substrate for EFC detection by a microplate reader (FLUOStar OPTIMA). The excitation wave is 530 nm and luminosity is detected at 620 nm. The amount of free ED conjugate in the assay

Table 1
Calculated Log *P* of the aminopyridine ligands **4a–e**, of the final E_2-Pt(II) hybrids **1a–e** and of 17β-estradiol.

Compounds[a]	c Log *P*[b]	Compounds[a]	c Log *P*[b]
4a	3.98	**1a**	4.40
4b	3.81	**1b**	4.24
4c	3.64	**1c**	4.07
4d	3.48	**1d**	3.91
4e	3.32	**1e**	3.74
17β-Estradiol	4.00[c]		

[a] Chain length: **a** (*n* = 1), **b** (*n* = 2), **c** (*n* = 3), **d** (*n* = 4) and **e** (*n* = 5).
[b] Calculated Log *P* as obtained with CaChe work system pro, 2006.
[c] Reported literature value for 17β-estradiol is 3.90 [33]



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is proportional to the concentration of estrogen analogs bound to the estrogen receptor [30]. A standard curve of 17β-estradiol was run in parallel. All assays were done in triplicate.

2.3. Molecular modeling

All calculations (molecular mechanics, MM2) and modeling were performed on Scigress Explorer Ultra Version 7.7.0.47, Fujitsu, USA [31]. The active site of the estrogen receptor (PDB 1ERE) was available on the Protein Data Bank web site [32].

3. Results and discussion

3.1. Synthesis of 17β-estradiol-platinum(II) complexes (7a–e)

As shown in Table 1, the calculated Log *P* (c Log *P*) of the aminoethylpyridine ligands **4a–e** is relatively constant throughout the series varying from 3.98 for **4a** to 3.32 for **4e** (Table 1). Estradiol itself has a c Log *P* of 4.00. The final E_2-Pt(II) hybrids **1a–e** should also possess relatively constant solubility. The cLogP of the final platinum(II) complexes vary from 4.40 for **1a** to 3.74 for **1e**. Thus, a slight increase of solubility is predicted for derivatives **1** as the chain is lengthening. Consequently, we speculated that the difference in cytotoxicity, if any, should only reflect the influence of the length of the PEG tether chain on the final hybrid molecules.

The synthesis involves only five chemical steps starting from estrone as the steroid template. The 17β-estradiol-Pt(II) complexes **1a–e** were obtained efficiently in high yield (22% overall) using an efficient reaction sequence. First, the preparation of the PEG chains was accomplished from commercially available 2-chloroethyl ether (**5**) and from PEG of various lengths (**6b–e**) (Fig. 2). Thus, treatment of 2-chloroethyl ether with sodium iodide in refluxing acetone for 3 days gave 2-iodoethyl ether (**7a**) in 81% yield. Derivatives **7b–e** were obtained in a two-step reaction sequence. The PEG chains were treated with mesyl chloride and triethylamine in diethyl ether at 0 °C to give the bis-mesylate intermediates **8b–e**. The bis-mesylates **8b–e** were subsequently treated, without purification, with excess sodium iodide in refluxing acetone to give the diiodo-PEG chains **7b–e** in 79% average yield.

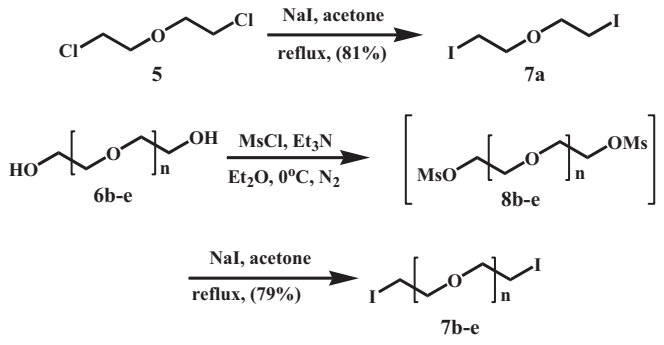

Fig. 2. Preparation of bis-diodo-PEG chains **7a–e**.

Table 2
Inhibitory concentration[a] of cisplatin and **1a–e** on both ER$^+$ and ER$^-$ breast cancer cell lines.

Compounds	MCF-7 (ER$^+$) IC_{50}, μM[a]	MDA-MB-231 (ER$^-$) IC_{50}, μM[a]	Chain length *n*
Cisplatin	18.97 ± 0.43	17.33 ± 2.28	–
1a	68.50 ± 3.01	38.67 ± 4.15	1
1b	33.44 ± 1.79	17.86 ± 1.33	2
1c	38.04 ± 2.02	27.29 ± 4.61	3
1d	35.64 ± 0.84	17.52 ± 1.47	4
1e	20.61 ± 0.94	13.90 ± 1.87	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. The cells were incubated for a period of 72 h.

As shown in Fig. 3, estrone (**9**) was initially 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 [34]. The yield of the protection reaction is 99%. The intermediate was further transformed into the β-keto-ester **10** upon treatment with dimethyl carbonate in the presence of a mixture of KH in dry tetrahydrofuran [35,36]. Derivative **10** was obtained with 90% yield. Treatment of derivative **10** with a suitable diiodo-PEG chains **7a–e** and with cesium carbonate in tetrahydrofuran gave compounds **11a–e** in 63% yield. The iodo-PEG chain were added to the less hindered α face of the molecule as shown by the presence of a single peak for the 18-**C**H_3 at δ 0.90 in the ^{1}H NMR spectrum and at δ 14.2 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 gave the triol **12a–e** [21,34]. It was obtained in 70% overall yield as a single 17β-hydroxy isomer as shown by a sole signal for the 18-CH_3 at δ 0.88 in the ^{1}H 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 [37].

The final 17β-estradiol-linked Pt(II) complexes **1a-e** were obtained in a two-step chemical sequence [21]. Initially, the triol **12a–e** was treated with excess 2-(2′-aminoethyl)pyridine to give derivatives **4a–e** for a yield of 80–100%. It is noteworthy that a short reaction period is necessary for this reaction in order to avoid the decomposition of the PEG tether chain. Thus, this reaction is performed in only 4 h instead of about 20 h when α,ω-dibromoalkane chain are used [21,23,25]. Then, the triol-aminopyridine intermediates **4a–e** were treated with potassium tetrachloroplatinate in a mixture of dimethylformamide and water to give the corresponding 17β-estradiol-PEG-linked Pt(II) complexes **1a–e** with *n* = 1, 2, 3, 4 and 5. The yield of this two-step sequence was 55%. As a result, the new cytotoxic molecules possess a PEG tether chain varying from 5 to 17 atoms long. All new compounds synthesized were characterized by IR, NMR spectroscopy and mass spectrometry.

3.2. In vitro cytotoxic activity

As shown by the MTT assays, the new Pt(II) complexes present no specific toxicity towards ER$^+$ breast cancer cells (Table 2). How-

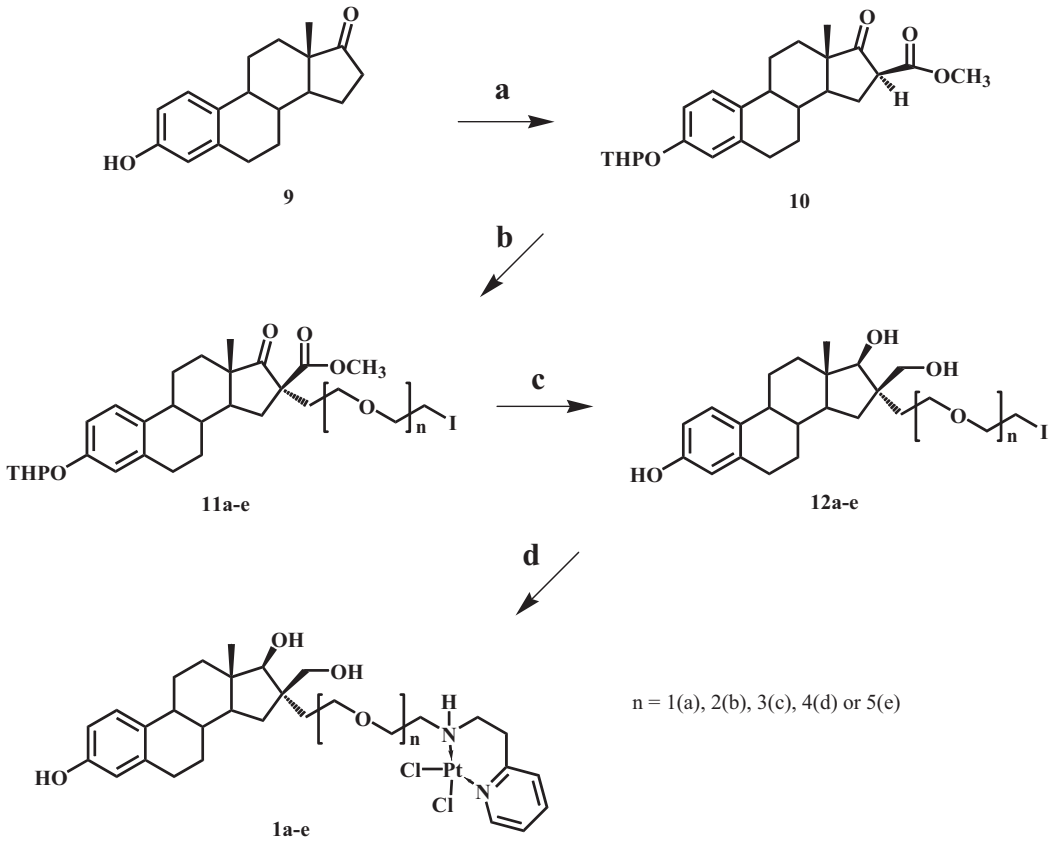

Fig. 3. Synthesis of 17β-estradiol-linked platinum(II) complexes. Reagents. **(a)** (1) DHP, PPTs, CH_2Cl_2, 22 °C, 24 h; (2) KH, dimethyl carbonate, THF, reflux, 3 h, 90%; **(b) 7a–e**, Cs_2CO_3, THF, reflux, 4 h, 63%; **(c)** (1) $LiBH_4$, Et_2O, 0 °C, 1 h and 22 °C, 11 h; (2) PPTs, EtOH, 22 °C, 4 h, 70%; **(d)** (1) 2-(2′-aminoethyl) pyridine, CH_3OH, reflux, 4 h; (2) K_2PtCl_4, DMF: H_2O (2:1), 22 °C, 2 days, 55%.

ever, it is important to indicate that the desired selectivity towards ER^+ cancer cells might be expressed more clearly (and possibly only) *in vivo* as it was previously demonstrated for similar types of derivatives [38,39].

The estrogen-PEG-Pt(II) hybrid molecules carry a 2-(2′-aminoethyl)pyridine ligand which was found to be the best ligand for biological activity for hybrids of formulae **2** and **3**. However, the hybrids **1a**–**e** were generally less cytotoxic than cisplatin itself. The length of the side chain seems to be optimal at $n = 5$ where we observed an IC_{50} of 20.6 and 13.9 μM, respectively for the MCF-7 and MDA-MB-231 cancer cells. The derivative with shorter side chains ($n = 1$) is the least active estradiol-PEG-hybrid of the series. Moreover, it is observed that the cytocidal activity is generally more important on the hormone-independent breast cancer cells. If selectivity can be achieved in animal models, derivative **1e** might become an alternative of choice for site-specific treatment of hormone-dependent breast cancer.

3.3. Estrogen receptor binding affinity

The estrogen receptor binding studies showed good affinity for derivative **1b** to the estrogen receptor alpha. The reference derivative, i.e. cisplatin presents, as expected, no affinity for the ERα (see Table 3). The estrogen-PEG-Pt(II) hybrid molecule **1b** has an EC_{50} of 4.25 nM compared to 0.66 nM for 17β-estradiol, the natural ligand. This type of molecule presents lower affinity than the previous classes of derivatives **2** and **3** reported [21–24]. As indicated in Table 3, derivatives **2** ($p = 6$) and **3** ($p = 6$) have an EC_{50} of 1.79 and 1.52 respectively.

3.4. Comparative molecular modeling studies

As mentioned earlier, the new estrogen-PEG-Pt(II) hybrid molecules **1a**–**e** are relatively less potent than the previous related platinum complexes linked with an alkyl chain (see structures **2** and **3**). Of note, the best 17β-estradiol-PEG-Pt(II) hybrid (**1e**)

Table 3
Comparison of different families of 17β-estradiol-Pt(II) complexes. Inhibitory concentration[a] of cisplatin **1b**, **2** ($p = 6$) and **3** ($p = 6$) on both ER^+ and ER^- breast cancer cell lines and binding affinity on ERα.

Compounds	Value of *n* or *p*	Total number of atoms	MCF-7 (ER^+) IC_{50}, μM[a]	Ratio CisPt/drug	MDA-MB-231 (ER^-) IC_{50}, μM[a]	Ratio CisPt/drug	ERα affinity EC_{50}, nM
Cisplatin	–	–	18.97 ± 0.43	–	17.33 ± 2.28	–	–
1b This study	$n = 2$	8	33.44 ± 1.79	0.59	17.86 ± 1.33	0.97	4.25
2 [21,22]	$p = 6$	8	5.80 ± 3.00	3.27	8.60 ± 0.1	2.01	1.79
3 [23,24]	$p = 6$	8	2.18 ± 0.11	8.70	2.16 ± 0.16	8.02	1.52

[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. The cells were incubated for a period of 72 h.

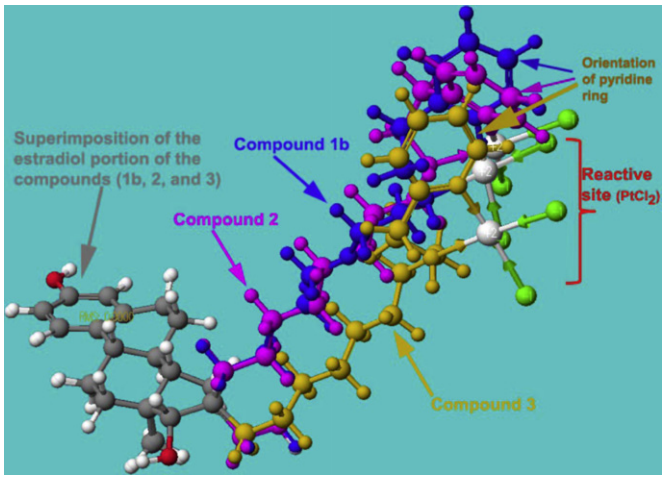

Fig. 4. Superimposition of the energy minimized structures of compounds **1b** ($n=2$) (blue), compound **2** ($p=6$) (pink), and compound **3** ($p=6$) (brown), platinum and chlorine atoms are shown as white and green colors, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

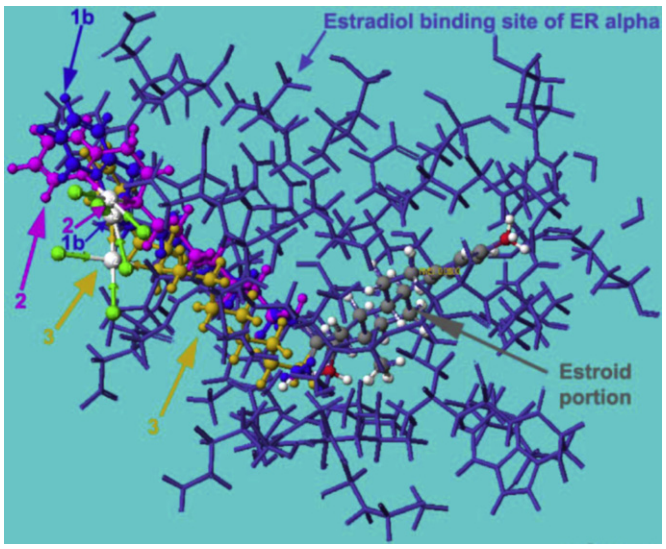

Fig. 5. Orientation of compounds **1b** ($n=2$), **2** ($p=6$), and **3** ($p=6$) in the ER alpha binding pocket: showing the positional differences in the reactive site ($PtCl_2$) portion and similarity of steroid portion.

was already analyzed by molecular modeling in a previous communication [20]. Thus, to understand the differences in potency between these three series of platinum complexes, we performed molecular modeling and docking experiments using the optimized structures of these classes of compounds (compounds **1b**, **2** and **3**) and the crystal structure of estrogen receptor alpha (ERα).

For this purpose, three specific derivatives were selected (**1b** ($n=2$), **2** ($p=6$) and **3** ($p=6$)) among the families and their structures are shown in Fig. 1. It is noteworthy that the overall length of the linking arm between the estrogenic portion and the platinum(II) moiety is, in each case, 8 atoms (Table 3). Their respective cytocidal activities on hormone-dependent (MCF-7) and hormone-independent (MDA-MB-231) breast cancer cells are presented in Table 3. Table 3 also gives the CisPt/drug ratios as well as the binding affinity of the compounds. It is quite clear that there is a substantial difference of activities among the three estrogen-platinum(II) derivatives. In fact, it becomes obvious that in both types of cells activity increases with lipophilicity of the derivatives, and the cytocidal activity follows this order; **1b** < **2** < **3**. The estradiol-platinum(II) derivative **3** ($p=6$) is about 8 times more cytotoxic than cisplatin. Derivative **2** ($p=6$) is about 2 to 3.27 times more active than cisplatin and derivative **1b** ($n=2$) is slightly less active than cisplatin. On MCF-7 cells, derivative **3** ($p=6$) is about 16 times more potent than the corresponding estrogen-PEG-Pt(II) hybrid **1b** ($n=2$). The potency of the various estradiol-platinum(II) hybrids is also in relation with the binding affinity with compound **3** ($p=6$) having the best affinity for the ERα (Table 3).

Fig. 4, the superimposition view of compounds **1b** ($n=2$), **2** ($p=6$), and **3** ($p=6$), shows that the orientation of compound **3**, bearing a beta linking arm, is significantly different from the other two derivatives (compounds **1b** and **2**) while compounds **1b** and **2** are rather similar with their alpha linking arm. However, the positions of the portion of the reactive site ($PtCl_2$) in all three compounds are different while the conformation of the steroid portion of all three molecules remains the same. These structural differences could be one of the reasons for the variations in potency among these three compounds.

Due to the structural disparities among these three compounds, their respective binding affinities to the ER alpha are naturally distinct and, to understand this binding difference, we performed the docking experiments. Fig. 5 shows the docking view of these three

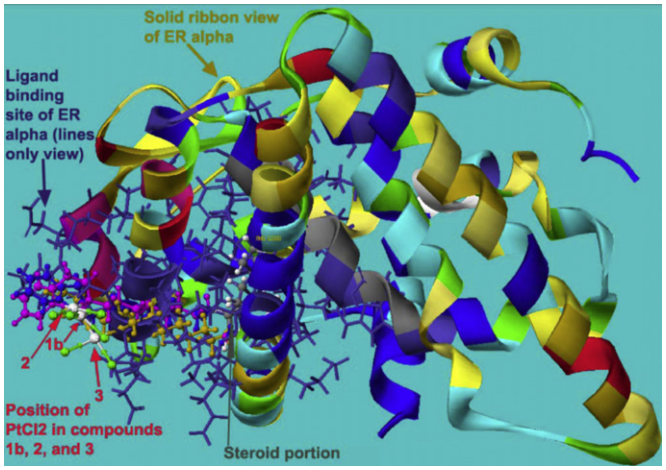

Fig. 6. Docking view of compounds **1b** ($n=2$), **2** ($p=6$), and **3** ($p=6$) to the active site of ER alpha: ER alpha binding pocket is shown with lines only (blue lines) and the folding of rest of the ER alpha molecule is shown as solid ribbon. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

compounds to the ER alpha binding pocket. In this diagram, it is clear that the binding of the steroid portion is similar while the orientation and the position of the reactive sites were significantly different. Fig. 6 shows the positions of the reactive sites of the three compounds. In compound **3** ($p=6$), the Cl groups of the reactive site is exposed outside of the ER alpha where water molecules are readily available for hydrolysis and, the Pt moiety would be freely available for binding to the target molecule. These reasons could explain the greater potency of compound **3** compared to other two derivatives. Also, this diagram shows the folding of ER alpha upon binding of these molecules.

4. Conclusion

This manuscript presents a new series of cytotoxic 17β-estradiol-PEG linked Pt(II) hybrid molecules (**1a-e**). They are readily available from estrone in only 5 chemical steps with excellent yields. The biological activity is lower than that of the hybrids of first and second family, see derivatives **2** and **3**. The most promis-

ing compound of the series is derivative **1e** which is equipotent to cisplatin itself. Despite a relatively low cytocidal activity, the novel hybrids could have interesting *in vivo* biological potential due to their enhanced solubility as compared to the first two prototype hybrids (**2** and **3**).

Three 17β-estradiol-platinum(II) complexes were selected for comparative analysis in terms of cytocidal activities and molecular modeling. Molecular modeling studies show that the orientation of the platinum core is different when the E_2-PEG-Pt(II) hybrid (**1b**) is compared to the platinum complexes **2** ($p = 6$) and **3** ($p = 6$) which bear a 8 carbon atoms alkyl chain. We show that the platinum core of **3** is oriented quite differently within the ERα pocket. It definitely shows that the chlorine atoms are exposed outside of the ER alpha which could account for its greater cytocidal activity when compared to the other two classes of derivatives. This study also demonstrates that lipophilicity plays an important role in the cytocidal activity of the various classes of hybrids. Of course, lipophilic estradiol-Pt(II) hybrids would present enhanced cellular penetration of the membranes to the nucleus and thus would show, as demonstrated herein, higher cytotoxicity.

This study further confirms that lipophilicity and the orientation of the tether chain between the estrogenic portion and the platinum(II) core contribute markedly to the biological activity of the various families of hybrids. The most active hybrids are those possessing an alkyl tether chain at position 16β of the steroid nucleus. For example, derivative **3** ($p = 6$) is about 16 times more potent on MCF-7 breast cancer cells than the corresponding 16α-PEG-hybrids (**2b**) made in this study. Further biological investigation of this type of compounds is being performed in our laboratory.

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