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Synthesis of 17β-estradiol-platinum(II) hybrid molecules showing cytotoxic activity on breast cancer cell lines

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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 five 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 breast cancer cell lines (MCF-7 and MDA-MB-231). Molecular modeling study rationalized the results.

Platinum(II)-based anticancer therapies remain, to this day, a useful and effective methodology for the management of several types of cancers.[1,2] Even if much work has been done in order to improve the platinum(II) anticancer drugs, only a few selected compounds are used today in clinics. They are cisplatin (*cis*-diamminedichloroplatinum(II)) and carboplatin (diamine[1,1-cyclobutanedicarboxylato]-*O*,*O*′-platinum(II)), the first and second platinum(II) derivatives to hit the market, and more recently oxaliplatin, nedaplatin, iobaplatin, and heptaplatin (SK12053R).[3–5] The first three platinum(II) complexes are used worldwide but the last three are used regionally, primarily in Asian countries. The platinum complexes have been mainly used for the treatment of solid tumors, particularly small cell lung, ovarian, testicular, head, and neck tumors. The specific uses of these drugs vary from one to another. However, oxaliplatin, nedaplatin, iobaplatin and heptaplatin have not shown any distinct and overwhelming advantages over cisplatin and carboplatin.[3–5] In general, the platinum-based drugs suffer from two main disadvantages: chemoresistance to the drugs can occur[4] and second they are non selective toward cancer cells which lead to severe toxic side effects, primarily kidney toxicity and neurotoxicity.[6,7]

Recent literature reviews present a broad overview of the actual knowledge of platinum-based antitumor agents as well as their action mechanisms.[3–5,8] 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 thereby blocking replication and/or transcription, and ultimately inducing apoptosis.[4–6]

Several research groups, including ours, 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.[9] Manifestly, the overall goal is to improve the selectivity and efficacy of this type of drug and, more importantly, to minimize its toxic side effects. A recent review has recently presented various preclinical and clinical studies on the use of platinum complexes for breast cancer treatment.[9]

Thus, the estrogen receptor is a biological target that has attracted considerable attention over the years. It is expressed by several types of cancers; breast (60–70%),[10] uterus (70–73%)[11] as well as ovarian (61%).[12] Together,

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they represent 40% of all cancers diagnosed in women and display a 25% mortality rate.[13] 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 describes the straightforward synthesis of a new family of 17β-estradiol-platinum(II) (E_2-Pt(II)) hybrid molecules **1** (Scheme 1). 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 (Scheme 1).[14–17]

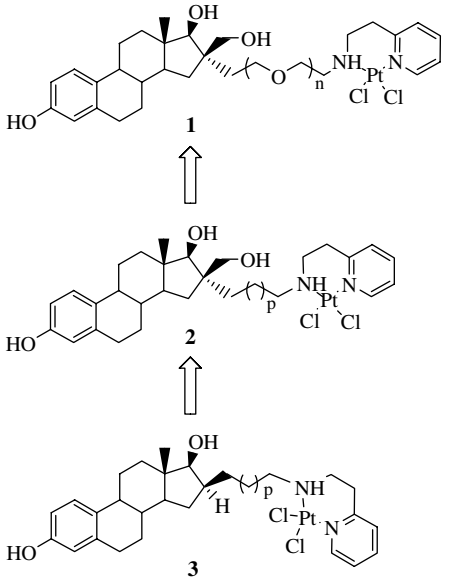
The objective of the present study was also to determine the cytotoxic effect of these novel molecules using estrogen dependent (estrogen receptor positive; ER^+) and independent (estrogen receptor negative; ER^-) human breast cancer cells. The biological activity of these compounds was evaluated in vitro using an MTT cell proliferation assay.[18,19] 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**.

As anticipated, the calculated log *P* of the aminopyridine 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 clog *P* of 4.00. The final E_2-Pt(II) hybrids **1a–e** should also possess relatively constant solubility. The clog *P* 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.

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

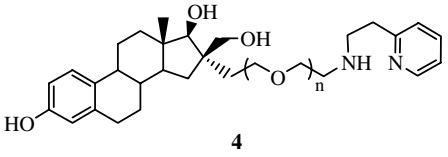
Compound[a]	clog *P*[b]	Compound[a]	clog *P*[b]
4a	3.98	**1a**	4.40
4b	3.81	**1b**	4.24
4c	3.64	**1c**	4.07
4d	3.48	**1d**	3.91
4e	3.32	**1e**	3.74
17β-Estradiol	4.00[c]		

[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.[20]



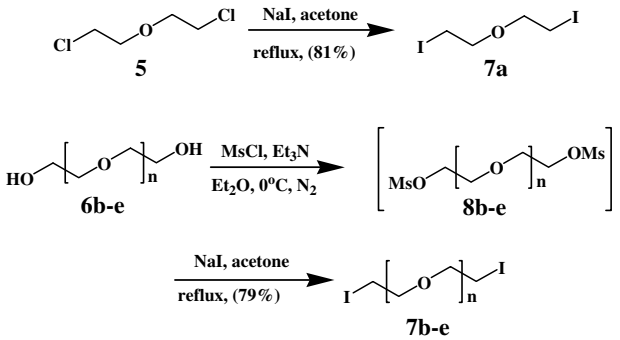
p = 2 to 12 carbon atoms
n = 1(a), 2(b), 3(c), 4(d) or 5(e)

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



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.

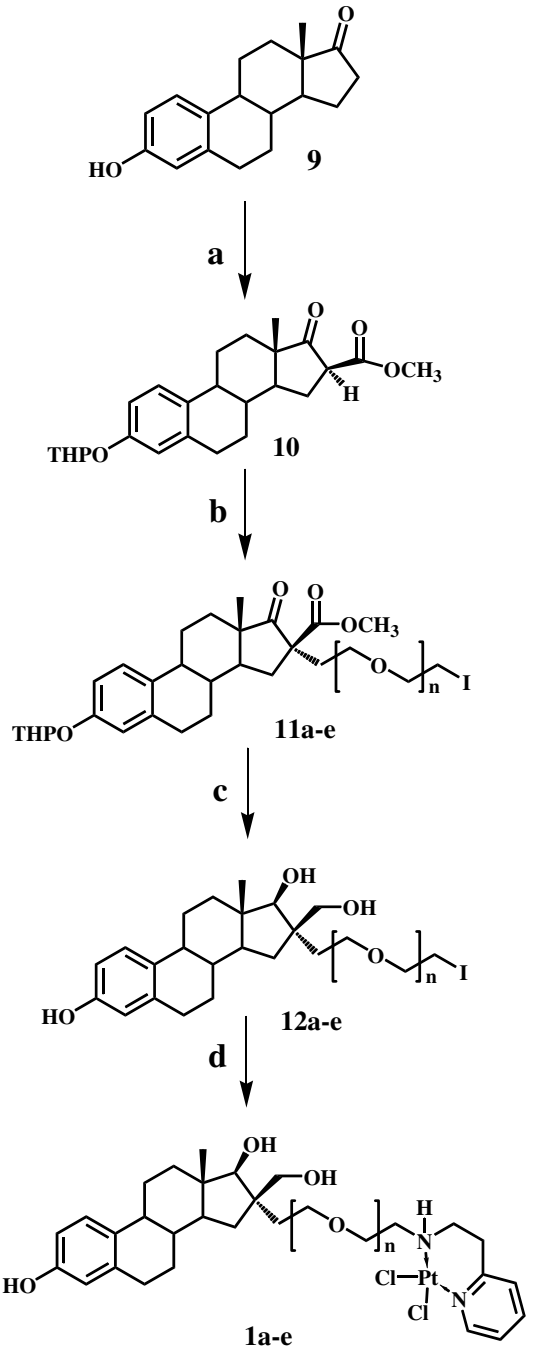
First, the preparation of the PEG chains was accomplished from commercially available 2-chloroethyl ether (**5**) and from PEG of various lengths (**6b–e**) (Scheme 2). Thus, treatment of 2-chloroethyl ether with sodium iodide in refluxing acetone for 3 days gave 2-iodoethyl ether (**7a**) in 81% yield. 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 **8a–e** were subsequently treated, with-



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

out purification, with excess sodium iodide in refluxing acetone to give the diiodo-PEG chains **7b–e** in 79% average yield.

As shown in Scheme 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.[21] 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 KH in dry tetrahydrofuran.[22,23] 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 was added to the less hindered α face of the molecule as shown by the presence of a single peak for the 18-CH_3 at δ 0.90 in the ^{1}H NMR spectrum and at δ 14.2 ppm in the ^{13}C NMR spectrum. Reduction of the β-keto-ester moiety with lithium borohydride in dry ether at 0 °C followed by the cleavage of the tetrahydropyranyl ether gave the triol **12a–e**.[14,21] 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.[24]



Scheme 3. Synthesis of 17β-estradiol-linked platinum(II) complexes. Reagents and conditions: (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%.

The final 17β-estradiol-linked Pt(II) complexes **1a–e** were obtained in a two-step chemical sequence.[14] First, the triol **12a–e** was treated with excess 2-aminoalkylpyridine 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 is used.[14,16] Second, 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.[25]

As shown by the MTT assays, the new Pt(II) complexes present no specific toxicity toward ER^+ breast cancer cells (Table 2). However, it is important to indicate that the desired selectivity toward ER^+ cancer cells might be

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}[a] (μM)	MDA-MB-231 (ER^-) IC_{50}[a] (μM)	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 means ± SEM of three independent experiments. The cells were incubated for a period of 72 h.

expressed more clearly (and possibly only) in vivo as it was previously demonstrated for similar types of derivatives.[26,27] A typical dose–response curve for derivative **1e** is shown in Figure 1.

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, 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.

The estrogen receptor alpha (ERα) affinity assay was performed using the HitHunter™ EFC Estrogen Fluorescence assay kit (Discoverx, Fremont, CA) according to the manufacturer's instructions.[28]

The estrogen receptor binding studies showed good affinity for derivative **1b** to the estrogen receptor alpha. The reference derivative, that is, cisplatin present, as expected, no affinity for the ERα. 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 class of derivatives **2** and **3** reported earlier.

Molecular modeling: All calculations (molecular mechanics, MM2) and modeling were performed on CAChe WorkSystem Pro.[29] In order to better understand the biological activity of these molecules they were docked into the active site of the estrogen receptor (PDB 1ERE).[30]

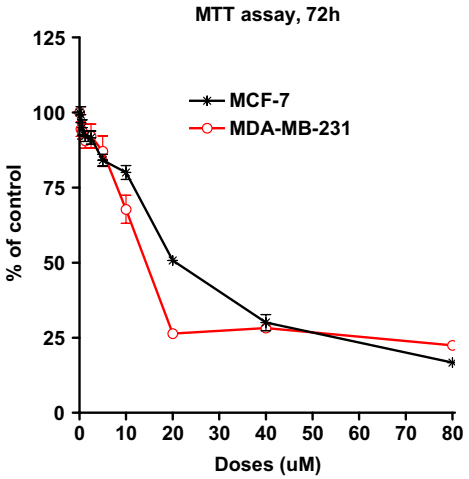


Figure 1. Typical dose–response curves for E_2-PEG-Pt(II) complex **1e** as obtained by the MTT assay for 72 h treatment.

As mentioned earlier, the new estrogen-PEG-Pt(II) hybrid molecules **1a**–**e** are relatively less potent than the previous analogous platinum complexes linked with an alkyl chain (see structure **2**). To understand the differences in potency between these two series, we performed molecular modeling and docking experiments using the optimized structures of these classes of compounds and the crystal structure of estrogen receptor alpha (ERα). Thus, we superimposed two of the most active compounds from each class (Fig. 2). Figure 2 shows that the orientation of the reactive site, $PtCl_2$ portion, is significantly different between the two compounds (**1e** and **CD-38** (compound **2**, $p = 8$)) despite their significant planarity between the estradiol moieties. Similar differences were observed when these two compounds were docked to the active site of the ERα (Figs. 3 and 4). According to 3D model based on X-ray structure of 17β-estradiol bound to the estrogen receptor, the OH group at position 3 (steroid numbering) of all compounds form three hydrogen bonds with proteins, ARG 394 and GLU 353 and the other with a second hydroxyl group of the estra-

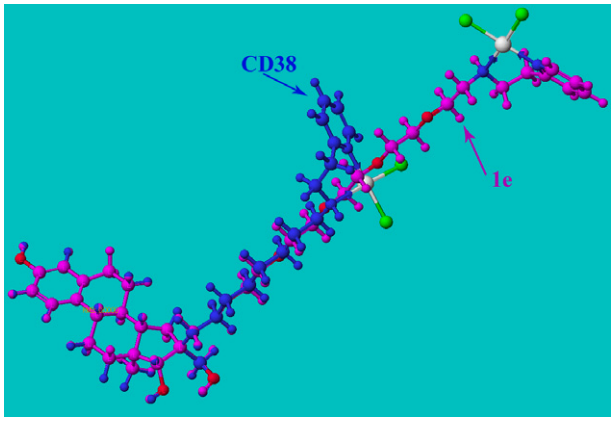


Figure 2. Superimposition of the most stable conformation of compounds **1e** (purple) and **CD-38** (blue) showing the difference between the two compounds.

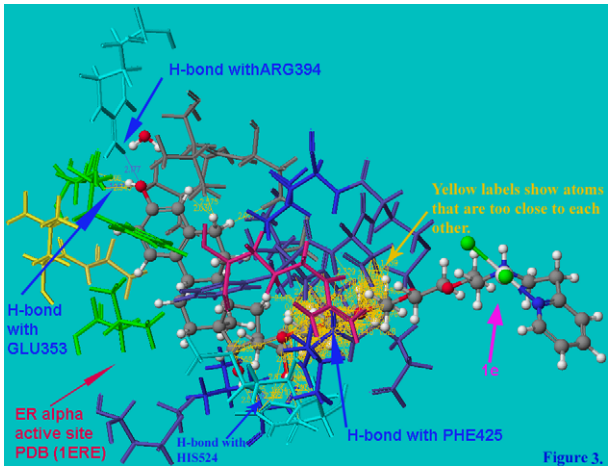


Figure 3. Docking view of compound **1e** to the active site (within 3 Å) of ERα (PDB, Code: 1ERE). All Hydrogen bond are shown using blue arrows and labels. Yellow labels indicate atoms that are too close together. The $PtCl_2$ moiety is pointing toward the active site.

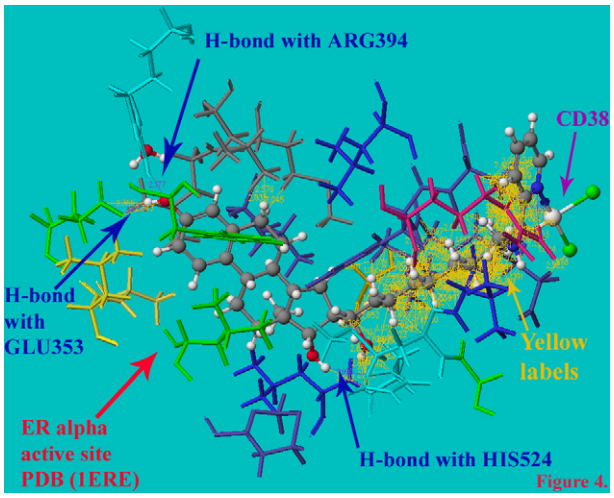


Figure 4. Docking view of compound **CD-38** to the active site (within 3 Å) of ERα (PDB, Code: 1ERE). All Hydrogen bond are shown using blue arrows and labels. Yellow labels indicate atoms that are too close together. The $PtCl_2$ core is oriented outward of the active site.

diol moiety (blue labels in Figs. 3 and 4). Both classes of compounds have very similar bonding interactions with the active site of ERα but the orientation of the platinum moiety is quite different between the two compounds (Figs. 3 and 4). In PEG derivative, the $PtCl_2$ moiety is oriented toward the ERα and may not be freely available for further interaction with its targets. However, the $PtCl_2$ is oriented outside the ERα pocket and the reactive site ($PtCl_2$) maybe readily available for its targets. Despite the extra hydrogen bond between PHE 425 and **1e** (Fig. 3), **CD-38** is the most potent E_2-Pt(II) hybrid. Therefore, the orientation of the reactive site is an important factor for biological activity.

We also examined the quantitative structure–activity relationship between IC_{50} and the solvent accessible surface area, a known molecular descriptor. The surface area of the electron density isosurface is determined after optimizing the molecular geometry using MOPAC with PM5 parameters. Figure 5 shows the plots of solvent accessible surface area versus IC_{50}. A linear relationship between this molecular descriptor and IC_{50} was observed. The value of correlation coefficient (r^2) between solvent accessible surface area and IC_{50} is 0.730 for MCF-7 and 0.64 for MDA-MB-231 cell lines, respectively. As shown in Figure 5, the potency is greater for larger value of solvent accessible surface area.

In summary, 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 five chemical steps with excellent yields (22% overall). The biological activity is lower than that of the hybrids of first and second family, see derivatives **2** and **3**. The most promising 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 prototypes (**2** and **3**). Molecular modeling studies show that the orientation of the platinum core is different when the E_2-PEG-Pt(II) hybrid is compared to the best of the platinum complexes of family 2 (**CD-38** (**2**, $p = 8$)), which bears a 10 carbon atoms alkyl chain. However, we show that the platinum core is outside the ERα pocket for the novel E_2-PEG-Pt(II) hybrid **1e**, which could account for its cytocidal activity. Further biological investigation of this type of compounds is warranted and is in progress in our laboratory.

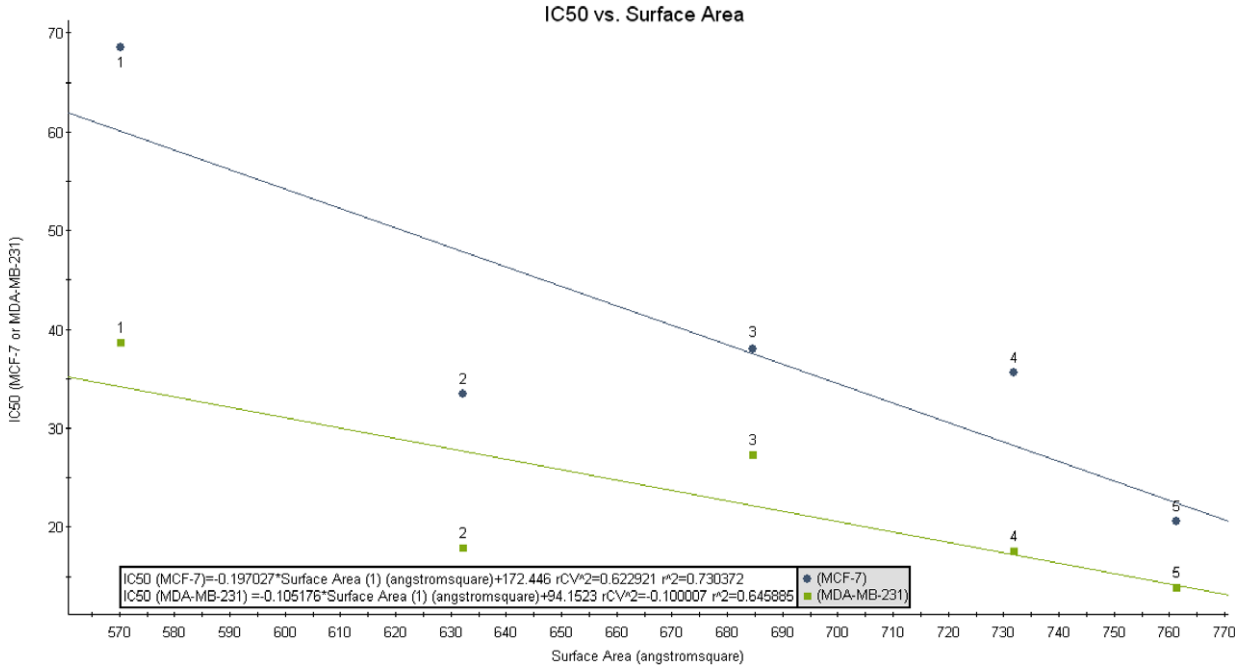


Figure 5. Regression analysis showing the relationship between the surface area and the IC_{50} values of MCF-7 cells (black circles on the line, $r^2 = 0.73$) and for MDA-MB-231cells (green rectangles and line, $r^2 = 0.65$).

Acknowledgments

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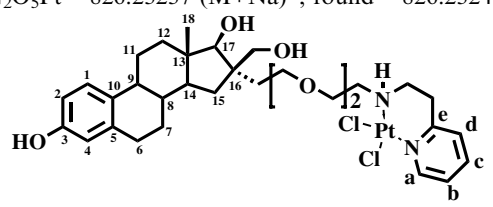
References and notes

1. Kelland, L. R.; Farrell, N. P. In *Platinum-Based Drugs in Cancer Therapy*; Humana Press: Totowa, New Jersey, 2000.
2. Rosenberg, B.; Van Camp, L.; Krigas, T. *Nature* **1965**, *205*, 698.
3. Wong, E.; Giandomenico, C. M. *Chem. Rev.* **1999**, *99*, 2451.
4. Jamieson, E. R.; Lippard, S. J. *Chem. Rev.* **1999**, *99*, 2467.
5. Galanski, M.; Keppler, B. K. *Anticancer Agents Med. Chem.* **2007**, *7*, 55.
6. (a) Reedijk, J. *Inorg. Chim. Acta* **1992**, *198*, 873; (b) Lemaire, D.; Fouchet, M.-H.; Kozelka, J. *J. Inorg. Biochem.* **1994**, *53*, 261; (c) Hydes, P. C.; Russell, M. J. H. *Cancer Metastasis Rev.* **1988**, *7*, 67.
7. Cepeda, V.; Fuertes, M. A.; Castilla, J.; Alonso, C.; Quevedo, C.; Pérez, J. M. *Anticancer Agents Med. Chem.* **2007**, *7*, 3.
8. Kostova, I. *Recent Patents Anticancer Drug Disc.* **2006**, *1*, 1.
9. Gust, R.; Ott, I. *Anticancer Agents Med. Chem.* **2007**, *7*, 95.
10. von Angerer, E. In *Metal Complexes in Cancer Chemotherapy*; Keppler, B. K., Ed.; VCH: Weinheim, 1993; pp 75–83.
11. Kounelis, S.; Kapranos, N.; Kouri, E.; Coppola, D.; Papadaki, H.; Jones, M. W. *Mod. Pathol.* **2000**, *13*, 379.
12. Rao, B. R.; Slotman, B. J. *Endocr. Rev.* **1991**, *12*, 14.
13. Canadian Cancer Society, Statistics for 2006 at http://www.cancer.ca.
14. Descôteaux, C.; Provencher-Mandeville, J.; Mathieu, I.; Perron, V.; Mandal, S. K.; Asselin, É.; Bérubé, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3927.
15. Gagnon, V.; St-Germain, M.-È.; Descôteaux, C.; Provencher-Mandeville, J.; Parent, S.; Mandal, S. K.; Asselin, É.; Bérubé, G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5919.
16. Perron, V.; Rabouin, D.; Asselin, É.; Parent, S.; Gaudreault, R. C.; Bérubé, G. *Bioorg. Chem.* **2005**, *33*, 1.
17. Bérubé, G. US Patent 7,153,844, 2006.
18. Carmichael, J.; DeGrapp, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
19. Ford, C. H. J.; Richardson, V. J.; Tsaltas, G. *Cancer Chemother. Pharmacol.* **1989**, *24*, 295.
20. Zhao, Y.; Jona, J.; Chow, D. T.; Rong, H.; Semin, D.; Xia, X.; Zanon, R.; Spancake, C.; Maliski, E. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1548.
21. Greene, T. W.; Wuts, P. G. M. In *Protective Groups in Organic Synthesis*, 3rd ed.; John Wiley & Sons: New York, 1999.
22. Ruest, L.; Blouin, G.; Deslongchamps, P. *Synth. Commun.* **1976**, *6*, 169.
23. Tremblay, R.; Auger, S.; Poirier, D. *Synth. Commun.* **1995**, *25*, 2483.
24. Dionne, P.; Ngatcha, B. T.; Poirier, D. *Steroid* **1997**, *62*, 674.
25. 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). ^1H NMR (200 MHz, $CDCl_3$): δ 7.12 (1H, d, J = 8.6 Hz, 1-CH), 6.75 (2H, m, 2-CH and 4-CH), 5.3 (1H, m, CH), 3.86 (1H, m, CH_aH_bO (on THP)), 3.67 (3H, s, $COOCH_3$), 3.52 (9H, m, CH_bH_aO (on THP) and 4× OCH_2), 3.19 (2H, t, J = 6.8 Hz, CH_2I), 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). RMN-^{13}C (50 MHz, $CDCl_3$): δ 213.9 (C-17), 171.8 ($COOCH_3$), 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 for THP), 71.9, 70.1 and 67.8 (4× OCH_2, 70.1 represents 2C), 61.8 (CH_2O for THP), 58.2 ($COOCH_3$), 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 (CH_2I). MS (*m/e*), $C_{31}H_{43}I_1O_7$: 654 ($M^{+\cdot}$), 570 $(M-C_5H_8O_1)^{+\cdot}$. *Exact mass:* Calcd for $C_{31}H_{43}I_1O_7$ = 654.2053; found = 654.2042.
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). ^1H NMR (200 MHz, acetone-d_6): δ 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 and 2.5 Hz, 2-CH), 6.52 (1H, d, J = 2.3 Hz, 4-CH), 4.20 (1H, d, J = 4.3 Hz, CH_aOH), 3.78–3.39 (10H, m, CHOH, $CH_{a'}OH$ and 4× OCH_2), 3.34 (2H, t, J = 6.4 Hz, CH_2I), 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 (50 MHz, acetone-d_6): δ 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 (CH_2OH and 4× OCH_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(CH_2I). MS (*m/e*), $C_{25}H_{37}I_1O_5$: 544 ($M^{+\cdot}$), 526 $(M-H_2O)^{+\cdot}$. *Exact mass:* Calcd for $C_{25}H_{37}I_1O_5$ = 544.1686; found = 544.1675. Spectral data for 16β-hydroxymethyl-16α-[8-(2-pyridine-2-yl-ethylamino)-3,6-dioxaoctyl]-1,3, 5(10)-estratrien-3,17β-diol dichloroplatinum(II) (**1b**, n = 2): IR (KBr, ν_{max}, cm^{-1}): 3430–3170 (O–H and N–H), 1611 and 1502 (C=C aromatic). ^1H NMR (200 MHz, acetone-d_6): δ 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-CH), 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, CH_aOH), 3.10–2.81 (16H, several m, CHOH, $CH_{a'}OH$, 4× OCH_2 on PEG chain and OCH_2CH_2 $NHCH_2CH_2$-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 (50 MHz, acetone-d_6): δ 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 (CH_2OH and 4× OCH_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). MS (*m/e*), $C_{32}H_{46}Cl_2N_2O_5Pt$: 804.2 $(M+H)^+$. *Exact mass:* Calcd for $C_{32}H_{46}Cl_2N_2O_5Pt$ = 804.25043 $(M+H)^+$; found = 804.25045 and calcd for $C_{32}H_{46}Cl_2N_2O_5Pt$ = 826.23237 $(M+Na)^+$; found = 826.23244.



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26. Otto, A. M.; Faded, M.; Schonenberger, H. *Cancer Res.* **1991**, *51*, 3217.
27. Karl, J.; Gust, R.; Spruss, T.; Schneider, M. R.; Schonenberger, H.; Engel, J.; Wrobel, K. H.; Lux, F.; Haeberlin, S. T. *J. Med. Chem.* **1988**, *31*, 72.
28. The HitHunter™ EFC Estrogen Fluorescence assay kit was obtained from Discoverx, Fremont, CA (http://www.discoverx.com).
29. CACheWorkSystem Pro, version 6.1.12, Fujitsu, USA, 2006.
30. Available from: http://www.rcsb.org/pdb/.