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Biological evaluation of novel estrogen–platinum(II) hybrid molecules on uterine and ovarian cancers—molecular modeling studies

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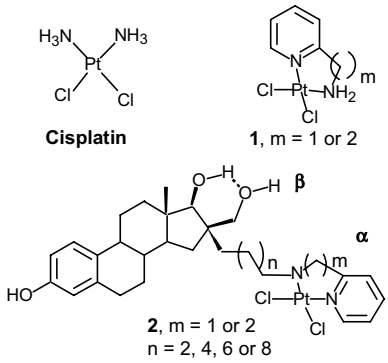
Abstract—We have recently reported the synthesis of a series of original 17β-estradiol-linked platinum(II) hybrid molecules. The biological activity of these compounds was evaluated in vitro on estrogen dependent and independent (ER^+ and ER^-) human uterine and ovarian cancers. The hybrid molecules present higher affinity than that of 17β-estradiol for the estrogen receptor alpha (ERα). The cytotoxicity and the affinity of the hybrid molecules are explained using molecular modeling analysis. This study further confirms that the derivatives made of a 2-(2′-aminoethyl)pyridine ligand displayed superior activity against the cell lines particularly when the connecting arm is 8–10 carbon atoms long. Molecular modeling shows that a long side chain can facilitate the access of the platinum(II) moiety to DNA. The novel compounds also prove to be moderately cytotoxic against platinum resistant endometrial and ovarian cancer cell lines.

Cisplatin (*cis*-diamminedichloroplatinum(II)) is still a very useful anticancer agent that was discovered nearly 40 years ago (see Scheme 1).[1,2] It has been mainly used for the treatment of solid tumors, particularly small cell lung, ovarian, testicular, head, and neck tumors. Recent literature reviews present a broad overview of the actual knowledge of platinum-based antitumor agents as well as their action mechanisms.[3,4] The antitumor activity of platinum drugs is a consequence of their interaction with DNA. Cisplatin binds readily to the N7-position of guanine bases of DNA molecules thereby blocking replication and/or transcription, and ultimately inducing apoptosis.[4,5] Three other platinum(II) drugs are presently on the market: carboplatin, oxaliplatin, and nedaplatin.[3] However, oxaplatin and nedaplatin have not shown any distinct advantages over cisplatin and carboplatin. In general, the platinum-based drugs suffer from two main disadvantages: chemoresistance to the drugs can occur[4] and they are nonselective toward cancer cells leading to severe toxic side effects, primarily kidney toxicity and neurotoxicity.[5]



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

Keywords: Estrogen–platinum(II) hybrids; Cytotoxic agents; Endometrial and ovarian cancers; Estrogen receptor; Molecular modeling.
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Drug targeting will be the key to improve selectivity of this type of drug. The aim of drug targeting is to deliver drugs only to those sites needing treatment. When this objective is met, not only will the efficacy of the treatment be improved, but toxic side effects will also be greatly minimized.

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%),[6] uterus (70–73%)[7] as well as ovarian (61%).[8] Together, they represent 40% of all cancers diagnosed in women and display a 25% mortality rate.[9] The biological affinity between 17β-estradiol and its cognate receptor can theoretically be used to direct a cytotoxic agent to the target cells. Consequently, over the years various estrogen-anticancer hybrids were investigated for the treatment of hormono-dependent cancers.[10–17] It is noteworthy that the main prerequisite for targeting is a good receptor binding affinity (RBA) of the hybrid molecules.

In order to illustrate this type of targeted approach, only the most recent work will be presented herein. Doxorubicin was coupled to *E*/*Z*-4-hydroxytamoxifen via a tether link of various length.[10] The lead derivative with a triethylene glycol tether show a RBA of 2.5% relative to *E*/*Z*-4-hydroxytamoxifen and inhibits the growth of four breast cancer cell lines with 4-fold up to 140-fold enhanced activity related to doxorubicin. Taxol was also successfully linked to estradiol through ester linkers. Unfortunately, the conjugates were in general less toxic than taxol itself.[11] However, one of the conjugates showed some selectivity for the ERα positive breast cancer cells; MCF-7.

The pioneering work of the following groups describing steroid-linked platinum(II) complexes must be cited. In this early work the platinum(II) group was attached to the estradiol skeleton either at the 3- or 17-hydroxyl position, which was unfavorable to receptor detection.[12–14] Nowadays, the platinum moiety is attached in such a fashion that the hydroxyls groups of estradiol remain unaltered for optimal RBA. For example, platinum(II) and technetium(I) moieties were anchored to ethynylestradiol with success.[15] The RBA values were disappointingly less than 1% possibly due to a partial protonation of the amino groups of the ligands at physiological pH as explained by the authors.[15] Some earlier work on ethynylestradiol yielded very interesting metal complexes associated to 2,6-bis-[(alkylthio)methyl]pyridine ligands with, in some cases, higher RBA than the free (uncomplexed) ligands.[16] Several estrogen-tethered orally active platinum(IV) complexes were designed to release the toxic moiety by intracellular esterases.[17] The reducing environment of the cell converts the platinum(IV) to platinum(II), in this case cisplatin itself. The design rationale was inspired by the observation that ER^+ cells exposed to the hormone are sensitized to cisplatin.[17]

This manuscript presents a new family of 17β-estradiol-platinum(II) molecules **2**, which are linked with an alkyl chain at position 16α of the steroid nucleus and bear a 16β-hydroxymethyl side chain. They are made from estrone in only five chemical steps.[18]

The objective of the present study was to determine the cytotoxic effect of these novel molecules using estrogen dependent (estrogen receptor positive; ER^+) and independent (estrogen receptor negative; ER^-) human uterine and ovarian cancer cells. The biological activity of these compounds was evaluated in vitro using an MTT cell proliferation assay.[19,20] The MTT assay was performed over an incubation period of 72 h. The affinity for the estrogen receptor alpha (ERα) was also determined.

As shown by the MTT assays, the new Pt(II) complexes do not present any apparent specific toxicity toward ER^+ uterine and ovarian cancer cells (Tables 1 and 2). The desired selectivity toward ER^+ cancer cells might only be observed during the in vivo biological tests as noticed previously by other researchers. The reference derivatives **1** ($m = 1$ or 2) were generally less toxic than the estrogen–Pt(II) hybrid molecules particularly with **2**, $m = 2$ and $n = 6$ or 8.[21] The same observations were made previously with breast cancer cell lines.[18] It was speculated that a large organic portion would enhance the cellular penetration of the membranes to the nucleus thus increasing cytotoxicity.[18] The hybrids **2**, $m = 1$ are, in general, less toxic than those where $m = 2$. The length of the side chain seems to be optimal at $n = 6$ or 8 for both types of aminopyridine analogs ($m = 1$ or 2). The derivatives with short side chains ($n = 2$ or 4) are generally less toxic than cisplatin. The estrogen–Pt(II) hybrid molecules **2**, $m = 2$ and $n = 6$ or 8 are the most interest-

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

Cell lines	Type	ER	Cisplatin	**1**	**2**, $n = 2$	**2**, $n = 4$	**2**, $n = 6$	**2**, $n = 8$
HeLa	Uterus	–	3.6 ± 0.2	13.0 ± 3.1	NR	20.2 ± 3.5	16.3 ± 0.2	18.8 ± 2.7
HEC-1A	Uterus	–	11.7 ± 1.6	27.0 ± 2.1	31.4 ± 6.7	14.7 ± 1.2	6.9 ± 0.2	7.8 ± 0.4
KLE	Uterus	–	12.5 ± 2.8	NR	NR	21.6 ± 4.2	12.8 ± 2.0	9.0 ± 2.0
RL-95-2	Uterus	+	10.3 ± 0.9	31.0 ± 2.5	15.6 ± 0.9	10.9 ± 1.9	5.7 ± 1.4	9.7 ± 0.8
Ishikawa	Uterus	+	7.9 ± 0.7	25.2 ± 2.9	32.5 ± 0.0	19.7 ± 1.9	16.3 ± 1.9	12.2 ± 1.6
A2780wt	Ovary	–	0.7 ± 0.2	4.2	26.6 ± 6.7	15.0 ± 3.3	4.5 ± 0.3	7.5 ± 1.0
A2780cp	Ovary	–	6.5 ± 3.3	NR	27.5 ± 0.2	18.4 ± 3.1	7.8 ± 0.4	13.1 ± 2.1
OVCAR-3	Ovary	+	2.5 ± 0.1	29.0	36.3 ± 2.2	NR	12.2 ± 3.7	5.0 ± 0.1
SKOV-3	Ovary	+	5.8 ± 1.2	24.5	NR	NR	31.1 ± 3.3	15.9 ± 3.3

[a] Inhibitory concentration (IC_{50}, μM) as obtained by the MTT assay. Experiments were performed in duplicates and the results represent the mean ± SEM of three independent experiments. NR (not reached) = $IC_{50} > 40$ μM.

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

Cell lines	Type	ER	Cisplatin	**1**	**2**, $n = 2$	**2**, $n = 4$	**2**, $n = 6$	**2**, $n = 8$
HeLa	Uterus	–	3.6 ± 0.2	20.0 ± 2.5	10.8 ± 2.7	4.8 ± 0.1	2.3 ± 0.2	5.2 ± 1.7
HEC-1A	Uterus	–	11.7 ± 1.6	35.2 ± 0.2	8.8 ± 0.6	3.9 ± 0.5	1.7 ± 0.2	2.3 ± 0.8
KLE	Uterus	–	12.5 ± 2.8	NR	12.3 ± 3.4	6.0 ± 2.0	2.3 ± 0.3	3.5 ± 1.3
RL-95-2	Uterus	+	10.3 ± 0.9	30.9 ± 3.0	8.1 ± 0.5	3.3 ± 0.5	1.7 ± 0.2	2.7 ± 1.2
Ishikawa	Uterus	+	7.9 ± 0.7	29.8 ± 3.9	12.3 ± 2.2	2.8 ± 1.5	2.3 ± 0.3	3.3 ± 1.3
A2780wt	Ovary	–	0.7 ± 0.2	7.0	12.0 ± 3.7	3.3 ± 0.2	2.4 ± 0.5	1.7 ± 0.7
A2780cp	Ovary	–	6.5 ± 3.3	NR	18.9 ± 5.8	4.2 ± 0.2	2.7 ± 0.7	2.3 ± 0.6
OVCAR-3	Ovary	+	2.5 ± 0.1	25.0	27.3 ± 4.9	9.6 ± 3.6	3.0 ± 0.7	2.9 ± 0.8
SKOV-3	Ovary	+	5.8 ± 1.2	27.8	26.7 ± 4.6	8.1 ± 1.0	3.1 ± 1.0	5.2 ± 1.5

[a] Inhibitory concentration (IC_{50}, μM) as obtained by the MTT assay. Experiments were performed in duplicates and the results represent the mean ± SEM of three independent experiments. NR (not reached) = $IC_{50} > 40\,\mu M$.

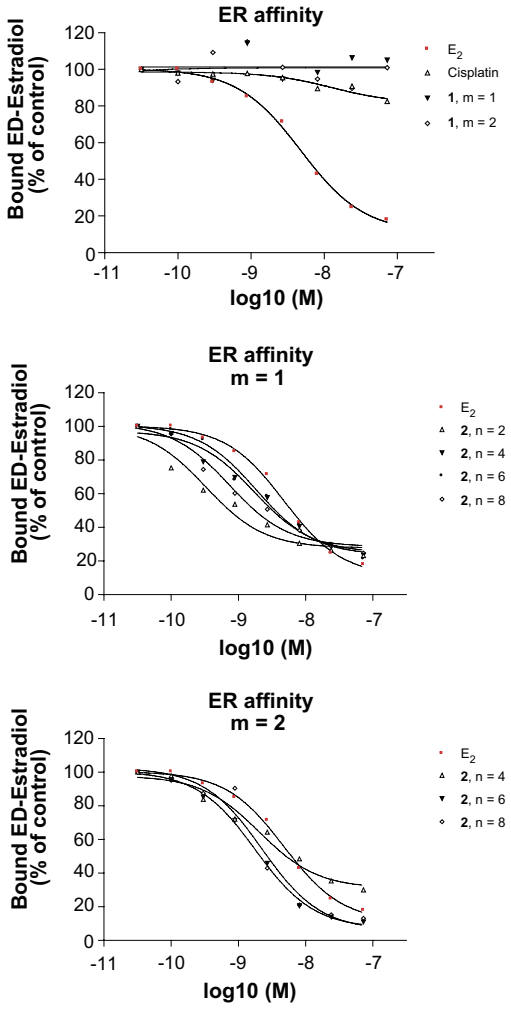

Figure 1. Estrogen receptor binding affinity (ERα) ED-estradiol: enzyme donor-estradiol; E_2: 17β-estradiol. Cisplatin, **1** ($m = 1$ or 2) present no affinity for the ER.

ing derivative of the series. These hybrid molecules carry a 2-(2′-aminoethyl)pyridine ligand. They were either equivalent or up to ~7 times more efficient at killing cells when compared to the IC_{50} of cisplatin. Interestingly, they are also active on the cisplatin resistant ovarian cancer A2780cp showing an IC_{50} of 2.7 μM (**2**, $m = 2$, $n = 6$) and 2.3 μM (**2**, $m = 2$, $n = 8$) as compared to 6.5 μM for cisplatin. Furthermore, they present activity on cisplatin resistant KLE endometrial cancer with an IC_{50} of 2.3 and 3.5 μM, respectively as compared to 12.5 μM for cisplatin.[22] Derivatives **2**, $m = 2$ and $n = 6$ or 8 might become an alternative of choice for the treatment of such resistant endometrial and ovarian cancers.

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]

The estrogen receptor binding studies showed a strong affinity of these molecules to the estrogen receptor alpha (see Fig. 1). The reference derivatives, that is, cisplatin and **1** ($m = 1$ and $m = 2$) present, as expected, no affinity for the ERα. The estrogen–Pt(II) hybrid molecules have an IC_{50} ranging from 0.33 to 2.26 nM compared to 4.79 nM for 17β-estradiol, the natural ligand (Table 3). It has exceptionally high affinity. It is believed to be due to the presence of the 16β-hydroxymethyl group allowing additional hydrogen bonding to the estrogen receptor. Molecular modeling studies confirm this hypothesis as described below.

Molecular modeling: All calculations (molecular mechanics, MM2) and modeling were performed on CACheWorkSystem Pro.[24] CD-38 (**2**, $m = 2$, $n = 8$) is one of the most cytotoxic derivatives in this series of

Table 3. Affinity for the estrogen receptor α (ERα)

Compounds	IC_{50} (nM)
17β-Estradiol	4.79
Cisplatin	ND
1, $m = 1$	ND
1, $m = 2$	ND
Hybrids, m = 1	
2, $n = 2$ (CD-50)	0.33
2, $n = 4$	1.72
2, $n = 6$	1.86
2, $n = 8$	0.74
Hybrids, m = 2	
2, $n = 2$	NA
2, $n = 4$	2.05
2, $n = 6$	1.79
2, $n = 8$ (CD-38)	2.26

ND = not determined, NA = not available.

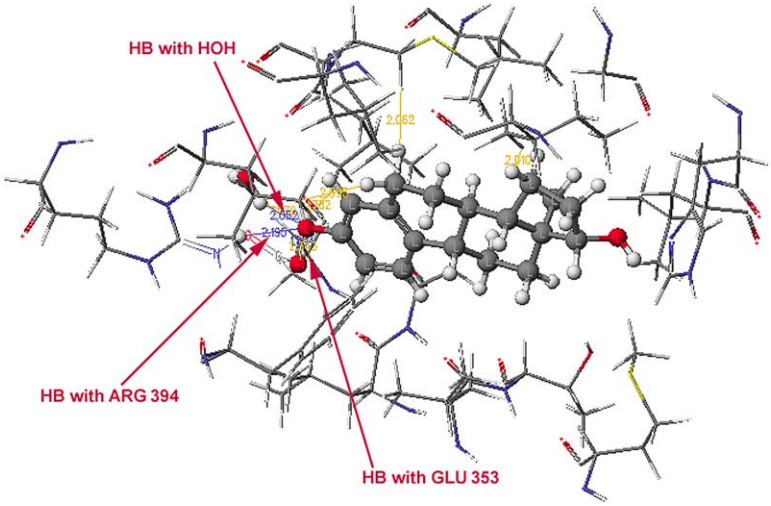

Figure 2. 17β-Estradiol within the active site of the ER (PDB, 1ERE). The main interactions are occurring on ring A of the steroid nucleus, there are three hydrogen bonds (HB).

compounds. However, the affinity for the estrogen receptor (ER) of CD-50 (**2**, $m = 1$, $n = 2$) is much greater than all other derivatives, including 17β-estradiol, the natural ligand. Surprisingly, CD-50 has significantly lower activity despite its excellent affinity for the ER. In order to understand such differences in cytotoxicity, CD-38 and CD-50 were docked into the active site of the estrogen receptor (PDB 1ERE).[25] 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 of which two are with proteins, ARG 394 and GLU 353 and the other with a water molecule (Figs. 3 and 4, blue labels) in a comparable fashion to 17β-estradiol itself (Fig. 2).

Both, CD-38 and CD-50 can readily form an intramolecular hydrogen bond (see Scheme 1, general structure **2**). Interestingly, unlike 17β-estradiol, CD-50 forms three additional hydrogen bonds of which two are intermolecular between HIS 524, LEU 525, and OH groups at 16- and 17-positions and one intramolecular hydrogen bond within OH groups at 16- and 17-positions of the estradiol part (Figs. 2 and 3). This is distinctly different from CD-38, which only forms three intermolecular hydrogen bonds like 17β-estradiol and one intramolecular hydrogen bond between 16- and 17-hydroxy groups (Fig. 4). Since CD-50 forms additional hydrogen bonds, the binding energy will be much greater for CD-50 than for CD-38 or even for 17β-estradiol, which explains its strong ER affinity. However, CD-38 is more potent than CD-50. To account for the difference in activity between CD-50 and CD-38, the steric hindrance (bumping atoms) between the ER and those molecules were evaluated (Fig. 5A and B yellow labels). As shown in Figure 5A and B, the yellow labels are far more intense for

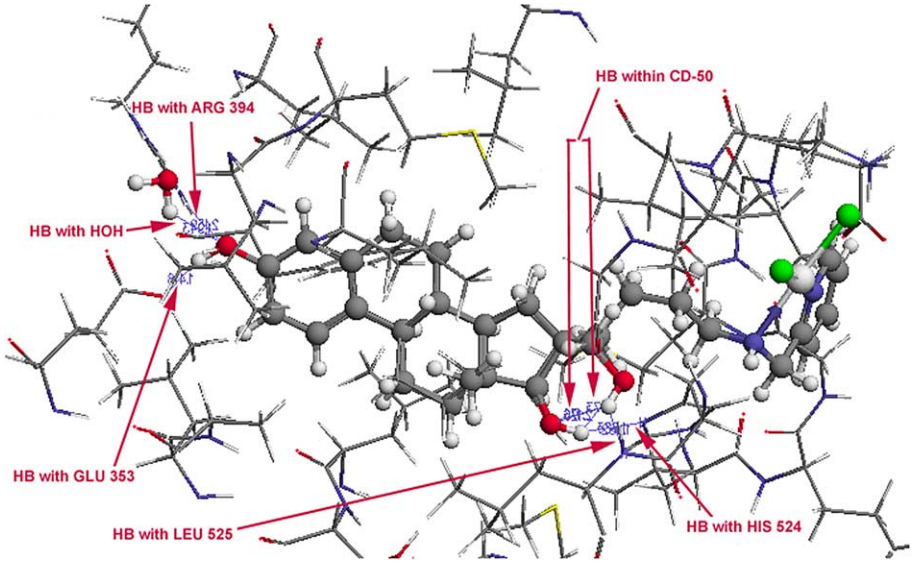

Figure 3. CD-50 in the active site showing additional HB, blue labels, when compared to CD-38 (Fig. 4) or 17β-estradiol (Fig. 2). CD-50 presents seven HB.

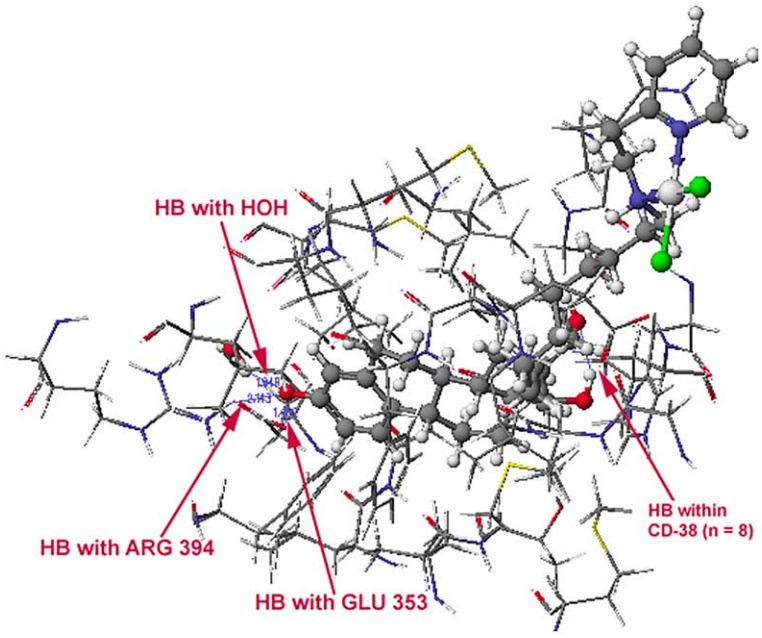

Figure 4. CD-38 in the active site of the ER. There are four HB.

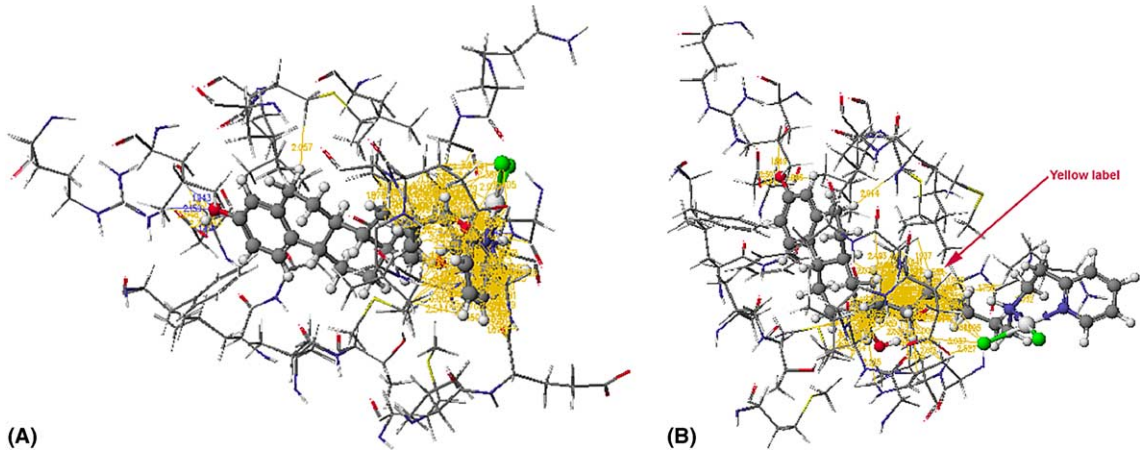

Figure 5. Surroundings of the platinum atom of CD-50 (left) and CD-38 (right) within the ER. The chlorine atoms (green) in CD-50 and CD-38 are pointing very differently. The platinum atom is more hindered for CD-50 than for CD-38 (yellow label) and thus unavailable for binding to DNA.

CD-50 than for CD-38. In CD-50, the platinum and the chlorine atoms are well buried within the ER site, the latter atoms are also pointing differently to those of CD-38 and, consequently, may not be freely available. This is not the case for CD-38; the chlorine atoms are much less crowded within the protein. In this case, they are readily available to be hydrolyzed and allow the platinum atom to bind to DNA and exert its cytotoxic activity.

In summary, this manuscript presents a series of cytotoxic 17β-estradiol–Pt(II) hybrid molecules. They are readily available from estrone in only five chemical steps with excellent yields (28% overall).[18] They present higher affinity than that of 17β-estradiol for the ERα. The affinity of the hybrid molecules was explained using molecular modeling analysis that shows additional hydrogen bonds with the ER taking place on ring D of the steroid nucleus when compared with 17β-estradiol. Furthermore, molecular modeling demonstrates that a long side chain facilitates the access of the platinum(II) moiety to DNA, accounting for the cytotoxic activity of the 17β-estradiol–Pt(II) molecules. The hybrids **2**, $m = 2$ and $n = 6$ or 8 also proved to be moderately cytotoxic against platinum resistant endometrial and ovarian cancer cell lines. These two hybrids have a remarkably high affinity that 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.

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