Synthesis, antiproliferative activity and estrogen receptor α affinity of novel estradiol-linked platinum(II) complex analogs to carboplatin and oxaliplatin. Potential vector complexes to target estrogen-dependent tissues

Pijus Saha, Caroline Descôteaux, Kevin Brasseur, Sébastien Fortin, Valérie Leblanc, Sophie Parent, Éric Asselin, Gervais Bérubé*

Département de Chimie-Biologie, Groupe de Recherche en Oncologie et Endocrinologie Moléculaires, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec, Canada G9A 5H7, e-mail: Pijus.Saha@uqtr.ca, Caroline.Descoteaux@uqtr.ca, Kevin.Brasseur@uqtr.ca, Sebastien.Fortin1@uqtr.ca, valerie.leblanc@uqtr.ca, Sophie.Parent@uqtr.ca, Eric.Asselin@uqtr.ca, Gervais.Berube@uqtr.ca

***Corresponding author:** Gervais Bérubé; Phone: 819-376-5011 ext. 3353, Fax: 819-376-5084, e-mail: Gervais.Berube@uqtr.ca. Département de Chimie-Biologie, Groupe de Recherche en Oncologie et Endocrinologie Moléculaires, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec, Canada G9A 5H7

Abbreviations List: estrogen-receptor positive, ER+; estrogen-receptor negative, ER-; estradiol-linked platinum(II) complex analog to cisplatin; E-CDDP; estradiol-linked platinum(II) complex analog to carboplatin, E-CarboP; estradiol-linked platinum(II) complex analog to oxaliplatin, E-OxaP.

Abstract

In the course of efforts to develop 17β-estradiol-linked to anticancer agents targeting estrogen-dependent tissue, we identified three estradiol-linked platinum(II) complex analogs to cisplatin (E-CDDP) derivatives namely: VP-128 (**1**), CD-38 (**2**) and JMP-39 (**3**) that exhibit potent *in vitro* and *in vivo* (for derivative VP-128) activity along with interaction with the estrogen receptor α (ERα). In this study, we prepared and biologically evaluated two novel classes of estradiol-linked platinum(II) complex analogs to carboplatin (E-CarboP, **1a**-**3a**) and oxaliplatin (E-OxaP, **1b**-**3b**). E-CarboP and E-OxaP were designed and based on the estradiol-linker scaffold of E-CDDP derivatives previously identified. Consequently, we assessed the importance of the nature of platinum(II) salt on the antiproliferative activity on MCF-7 and MDA-MB-231 human mammary carcinoma cell lines together with affinity for the ERα by replacing the dichloroplatinum(II) moiety by a cyclobutane-1,1-dicarboxylateplatinum(II) or an oxalateplatinum(II) moiety. Except for compound **3b** which is inactive at the concentration tested, the antiproliferative activity of all compounds on both human mammary carcinomas cell lines are in micromolar range and are more active than carboplatin and oxaliplatin alone but less active that their E-CDDP counterparts (**1**-**3**). In addition, E-CarboP derivatives **1a**-**3a** show very low affinity for ERα whereas E-OxaPs **1b** and **2b** show higher affinity for ERα than their parents E-CDDPs (**1**-**2**), suggesting that the nature of the platinum(II) salt involved in the vector complexes is extremely important to both retain significant antiproliferative activity and selectivity for the ERα and possibility to target estrogen-dependent tissues. Finally, E-OxaPs **1b** and **2b** are potentially promising alternatives vector complexes to target estrogen-dependent tissues.

Keywords. Estradiol-carboplatin analogs; Estradiol-oxaliplatin analogs; Vectorization therapy; Anticancer agents; Estrogen receptor; Breast cancer.

1. Introduction

Cisplatin (cis-diamminedichloroplatinum(II)) is an effective anticancer drug widely used against ovarian carcinoma, lung, head and neck cancers. Its discovery has triggered numerous research programs to find platinum(II) derivatives with enhanced biological activities, decreased toxicities and not triggering chemoresistance of cancer cells. Hence, the screening of hundreds of platinum(II) drugs has resulted in the discovery of carboplatin (cis-diammine(cyclobutane-1,1-dicarboxylato)-platinum(II)) and oxaliplatin (1,2-diaminocyclohexaneoxalato platinum(II)) [1, 2]. The latter is in use for the treatment of metastatic colorectal cancer and is less prone to side effects than cisplatin [2, 3]. Another new drug, nedaplatin (cis-diammine-glycoloato-O,O-platinum(II)) that is tenfold more water soluble than cisplatin, and is significantly less nephrotoxic than cisplatin and carboplatin has been recently approved in Japan for the treatment of non-small-cell lung carcinoma, small cell lung cancer, oesophageal and head and neck cancers (Fig. 1) [2, 3].

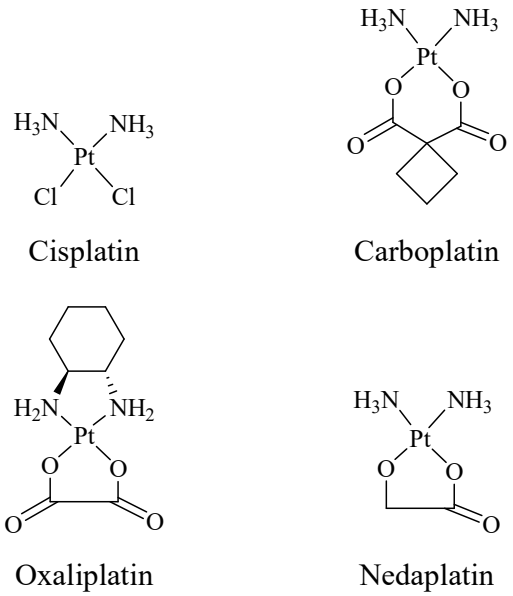


Fig. 1. Figure 1. Structure of cisplatin, carboplatin, oxaliplatin and nedaplatin.

The platinum(II)-based anticancer agents cause damages to tumors by the formation of reactive platinum species that bind to nucleophillic groups such as purine present in DNA, resulting in intrastrand [4] and interstrand [5] cross-links that ultimately lead to apoptosis [1, 2]. Apoptosis resulting from platinum salts cytotoxicity is also responsible for the characteristic nephrotoxicity, ototoxicity and other toxicities described for platinum(II)–based drugs, that are a direct consequence of their systemic effects and lack of selectivity toward cancer cells. Another major drawback of platinum(II)-based drugs treatments is the triggering of tumor cell chemoresistance notably: (1) reduction of drug uptake and/or increased drug efflux, (2) degradation and deactivation by intracellular thiols and (3) improvement of repair or tolerance of DNA–cisplatin adducts [1-3].

Several strategies have been used to increase tumor cell selectivity of platinum drugs, including their conjugation to potentially site-directing molecules, such as folate, porphyrins, adenine, terpenoids, peptides and many others [6-14]. The approach used by our research group and few others is based on their potential site-direction through their conjugation to estrogen analogs and derivatives to target estrogen-dependent tissues such as breast, ovarian and uterus [15-20]. In the course of our research program, we have selected three potent derivatives amongst three different series of estradiol-linked platinum(II) complex analogs to cisplatin (E-CDDP) exhibiting favorable *in vitro* biological activity compared to the corresponding cisplatin against breast cancer cell lines [16-19]. These derivatives selected are namely: 16α,β-[11-(2-pyridylethylamino)undecanyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinum(II) (VP-128, **1**), 16β-hydroxymethyl-16α-[10-(2-pyridylethylamino)decyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinum(II) (CD-38, **2**) and 16β-hydroxymethyl-16α-[8-(2-pyridylethylamino)-3,6-dioxaoctyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinum(II) (JMP-39, **3**) (Fig. 2) [16-19]. E-CDDP derivatives **1**, **2** and **3** showed better *in vitro* activity than cisplatin against breast cancer cell lines along with higher affinity with the estrogen

receptor α (ERα), RNA and DNA [21-24]. In addition, a human breast cancer tumors estrogen-receptor positive (ER+) xenograft model using MCF-7 cells on mice showed that E-CDDP derivative **1** achieved a better tumor regression than cisplatin [24]. This demonstrates that E-CDDP is a good approach to increase both tumor cell selectivity of platinum(II) drugs and selectively target estrogen-dependent tissues.

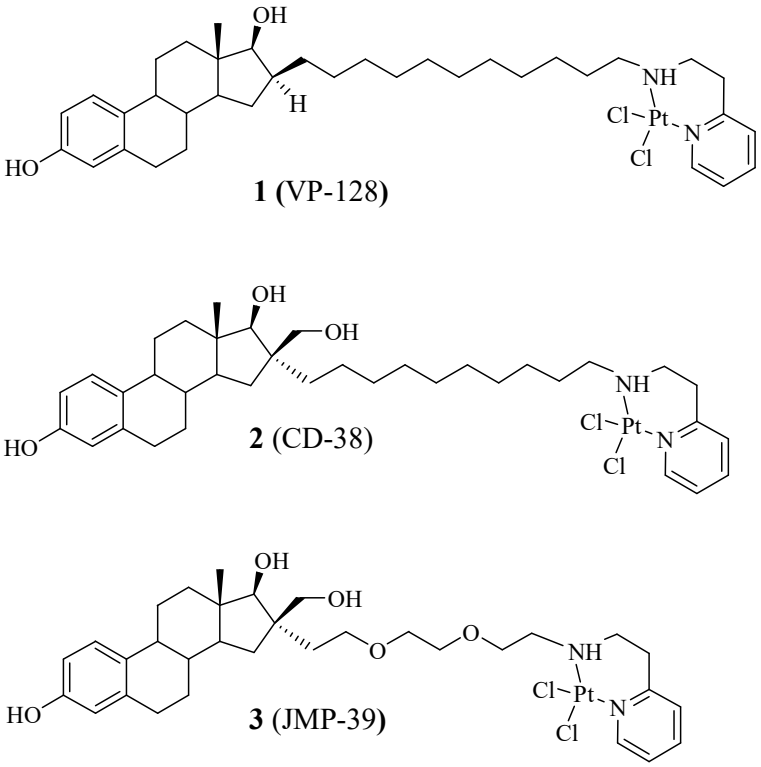


Fig. 2. Structure of the E-CDDP selected to make E-CarboP and E-OxaP.

In the aim to improve selectivity and efficacy while minimizing the potential deleterious effects of our E-CDDP, we prepared and assessed the biological activity of two novel classes of estradiol-linked platinum(II) complex analogs to carboplatin (E-CarboP**, 1a**-**3a**) and oxaliplatin (E-OxaP, **1b**-**3b**). These two novel classes were designed and based on the estradiol-linker scaffold of E-CDDP derivatives previously identified. In this study, we assessed the importance of the nature of platinum(II) salt by replacing the

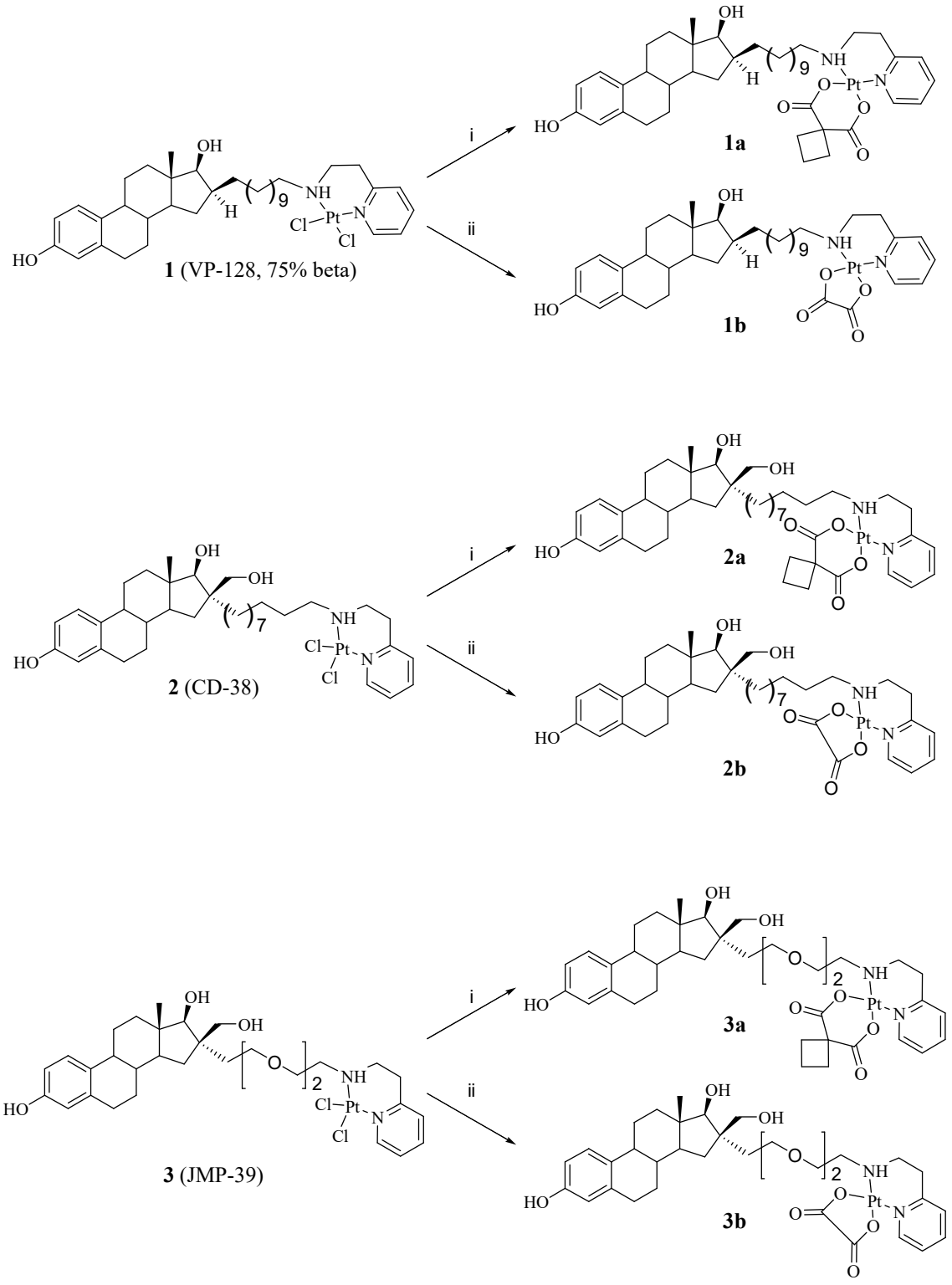
dichloroplatinum(II) moiety of our E-CDDP derivatives by a cyclobutane-1,1-dicarboxylateplatinum(II) or a oxalateplatinum(II) moiety on the antiproliferative activity on ER+ (MCF-7) and ER- (MDA-MB-231) human mammary carcinomas cells together with their affinity for the ERα.

2. Results and discussion

2.1. Chemistry

Cisplatin, carboplatin and oxaliplatin were linked to the estradiol framework at position 16 (α or β) of the steroid nucleus. The starting E-CDDP derivatives **1**, **2** and **3** material were obtained from estrone with overall yields of 21%, 28% and 22%, respectively using procedures described previously [16-19]. The different platinum complexes were linked to estrone using either an eleven (derivative **1**) or a ten carbon atom alkyl chain (derivative **2**) or a triethylene glycol chain (derivative **3**). For compound **1**, the chain substitutes position 16β of the steroid nucleus whereas for compounds **2** and **3** the substitution is at position 16α of the steroid nucleus and bears a 16β-hydroxymethyl side chain (Fig. 2).

The method used for the preparation of E-CarboP derivatives (**1a**-**3a**) and E-OxaP derivatives (**1b**-**3b**) is described in Scheme 1. Briefly, compounds **1a**-**3b** were prepared by treating compounds **1**, **2** or **3** with silver cyclobutane-1,1-dicarboxylate or silver oxalate in a mixture of acetone/H_2O (2:1) at room temperature for 1 to 15 days to afford the desired material in yields ranging from 34% to 67%. It was observed that the reaction time was significantly different following the solubility of the starting material. The completion of the reaction took 7-15 days for compounds **1**, **2** and only 1 day for compound **3**.



Scheme 1. Reagents: (i) Silver cyclobutane-1,1-dicarboxylate, acetone, H_2O; (ii) Silver oxalate, acetone, H_2O.

2.2. Antiproliferative activity

The antiproliferative activity of E-CarboP derivatives (**1a**-**3a**) or E-OxaP derivatives (**1b**-**3b**) was evaluated on the human ER+ MCF-7 and ER- MDA-MB-231 mammary carcinomas using the MTT colorimetric assay [25]. The antiproliferative activity of compounds **1a**-**3b** was tested along with E-CDDP derivatives (**1**-**3**), cisplatin, carboplatin and oxaliplatin. As shown in Table 1, compound **3b** and carboplatin is inactive at the concentration evaluated (80 μM) whereas antiproliferative activities of E-CarboP derivatives (**1a**-**3a**) and E-OxaP derivatives (**1b**-**2b**) are in the micromolar range on both human mammary carcinomas cell lines. They are more active than carboplatin and oxaliplatin but less active that their parent E-CDDP derivatives (**1**-**3**). Consequently, the general order of activities is as follow: E-CDDP > E-OxaP > E-CarboP. In general, the antiproliferative activity trend into two different classes of E-CarboP and E-OxaP is similar to parent E-CDDP and the general order of antiproliferative activities is as follow: compound **1 >** compound **2 >** compound **3**. For that reason, the importance of the nature of the platinum(II) salts used seems to affect only slightly the antiproliferative activity of E-OxaP derivatives whereas more significantly E-CarboP derivatives, decreasing activity of 1.2 (**1b**/**1**) to 2.6-fold (**2b**/**2**) and 2.2 (**3a**/**3**) to 7.8-fold (**2a**/**2**), respectively, comparatively to the E-CDDP derivatives. As expected the nature of platinum(II) salts in the vector complexes seems to play an important role in the antiproliferative activity. This difference activity could be related to different biological and biophysical properties of platinum salt together with different conformations and steric hindrance for the binding to DNA.

Table 1

Antiproliferative activity on both ER+ MCF-7 and ER- MDA-MB-231 human mammary carcinoma cell lines and affinity for the ERα of novel E-CarboP derivatives (compounds **1a**, **2a** and **3a**) and E-OxaP derivatives (compounds **1b**, **2b** and **3b**) together with parent E-CDDP derivatives (compounds **1**, **2**, **3)**, cisplatin, carboplatin, oxaliplatin and 17β-estradiol.

Compounds	**IC_{50} (μM)[a]**		**EC_{50} (nM)** [26]
	MCF-7 (ER^+)	**MDA-MB-231 (ER^-)**	
17β-estradiol [16, 17]	NT[b]	NT[b]	0.40 - 0.66
Cisplatin [16]	19.0 ± 0.4	17 ± 2	NA[c]
1 [16]	3.81 ± 0.05	1.88 ± 0.04	2.75
2 [17]	4.0 ± 0.6	5 ± 3	2.26
3 [18, 19]	33 ± 2	18 ± 1	4.25
Carboplatin	NR[d]	NR[d]	NA[c]
1a	21 ± 2	13.1 ± 0.7	59500
2a	31 ± 1	16.0 ± 0.4	299400
3a	74 ± 3	53 ± 4	3000
Oxaliplatin	21 ± 3	19 ± 1	NA[c]
1b	8.7 ± 0.6	2.2 ± 0.1	2.73
2b	10.5 ± 0.9	7.6 ± 0.1	1.30
3b	NR[d]	NR[d]	NT[b]

[a] Inhibitory concentration (IC_{50}, μM) as obtained by the MTT assay and expressed as the concentration of drug inhibiting cell growth by 50%. Experiments were performed in duplicates and the results represent the mean ± SEM of three independent experiments; [b] NT = Not tested; [C] NA = No affinity; [d] NR = Not reached.

2.3. Estrogen receptor binding affinity

E-CDDPs (**1-3)** were linked to the estradiol framework at position 16 (α or β) of the steroid nucleus (Fig. 2). Although this position is close to the estrogenic binding site, its substitution does not affect drastically its binding to the estrogen receptor α. As depicted in Table 1, we observed that the use of estradiol as a drug-carrier moiety for E-CDDP (**1-3**) provides hybrids exhibiting good to excellent receptor binding affinity [16-19]. In this study, we showed that

all E-CarboP derivatives (**1a**-**3a**) exhibit also some affinity for the ERα, albeit very low. More interestingly, E-OxaP derivatives **1b** and **2b** had excellent affinities for ERα with EC_{50} of 2.73 nM and 1.30 nM, respectively, which are higher than their E-CDDP counterparts. Compound **3b** was devoid of antiproliferative activity and therefore its affinity for ERα was not assessed. As expected, cisplatin, carboplatin and oxaliplatin had no affinity for ERα [16]. As shown in our previous study, the estrogen analogs and linker moieties of 17β-estradiol-linked to platinum(II) salts play an important role for the antiproliferative activity and the affinity of the drug for the ERα [16-19]. Unexpectedly, the nature of platinum(II) salts in the vector complexes seems to play also a key role in the affinity of the drugs for the ERα and the possibility to target estrogen-dependent tissues. We hypothesized that the final folding conformation of the vector complexes obtain with different platinum(II) salts could interfere in the interaction occurring between the estrogen analog moiety and ERα. Therefore, further SAR studies must be conducted to assess the relationship between affinity of the estrogen analog for the ERα and the structure of the platinum salt involved in the complex.

3. Conclusions

This manuscript describes the synthesis, the *in vitro* biological evaluation and the affinity for the ERα of two novel classes of E-CarboP and E-OxaP derivatives. The aim of this study was to evaluate the effect of converting the dichloroplatinum(II) moiety into cyclobutane-1,1-dicarboxylateplatinum(II) and oxalateplatinum(II) moieties on the antiproliferative activity and the affinity of the drugs for the ERα to develop alternative vectors for the targeting of anticancer drugs to estrogen-dependent tissues. The results showed that the modification of dichloroplatinum(II) moiety by oxalateplatinum(II) (E-OxaP, derivatives **1b** and **2b**) seems to affect only slightly the antiproliferative activity whereas modification of dichloroplatinum(II)

moiety by cyclobutane-1,1-dicarboxylateplatinum(II) (E-CarboP, derivatives **1a**-**3a**) affect more significantly antiproliferative activity. In addition, E-CarboP derivatives are losing their affinity for ERα whereas E-OxaP derivatives **1b** and **2b** maintain an excellent affinity for the ERα. Consequently, the nature of platinum(II) salt in the vector complexes is extremely important to retain significant antiproliferative activity and selectivity for the ERα and possibility to target estrogen-dependent tissues. Finally, E-OxaP derivatives **1b** and **2b** will be further studied for their *in vitro* and *in vivo* biological potential on animal models and potentially be promising alternatives vector complexes to target estrogen-dependent tissues.

4. Experimental protocols

4.1 Biological Methods

Human breast carcinoma cell lines MCF-7 (ER+) and MDA-MB-468 (ER-) were generously provided by Dr René C.-Gaudreault (CHUQ Research Center, Québec, Canada) and maintained in RPMI medium containing 10% bovine growth serum containing 50 mg/ml gentamycin. The cells were maintained at 37 ºC in a moisture-saturated atmosphere containing 5% CO_2.

4.1.1 Antiproliferative activity MTT assay

Cells were plated in 96-well plates 48 h before the assay. Stock solutions of the compounds were prepared by dissolving them in cremophor EL™:ethanol (1:1). Cells were treated for 72 h with serial dilution of the drugs between 80 and 1 μM in a total volume of 100 μL per well. The final concentration of cremophor:ethanol (1:1) in the culture media was 0.1% and was kept constant in all experiment conditions. After 68 h of incubation, 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) was added to each

well. 4 h later, 100 μL of the solubilization solution (10% SDS in 0.01 M HCl) was added and the plate was incubated overnight (37 °C, 5% CO_2). The optical density was read with Fluostar OPTIMA BMG (BMG LABTECH inc., Durham, NC) at 550 nm.

4.1.2 Estrogen receptor α binding affinity

The ERα affinity assay was performed using recombinant hERα (Calbiochem/EMD BioSciences, Darmstadt, Germany) and the HitHunterTM Enzyme Fragment Complementary (EFC) Estrogen Receptor Assay kit (Discoverex Corporation, Fremont, CA) according to manufacturer's protocol [26]. HitHunterTM EFC technology is based on a genetically engineered β-galactosidase enzyme that consists of two fragments termed Enzyme Acceptor and Enzyme Donor. Briefly, different concentrations of drugs were added to wells containing Estrogen Steroid Receptor + Enzyme Donor in a 96-well black plate. Incubation provided competition for the estrogen receptor binding against labeled Enzyme Donor-Estrogen Steroid hormone conjugate, a small peptide fragment of β-galactosidase. Then, Enzyme Acceptor, an inactive β-galactosidase protein fragment, and a Fluorescent substrate were added to each well. Unbound Donor-Estrogen Steroid hormone conjugate bind to Enzyme Acceptor to form an active enzyme, which subsequently hydrolyses 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 Enzyme Donor conjugate in the assay is proportional to the concentration of drugs bound to the estrogen receptor [26]. A standard curve of 17β-estradiol was run in parallel. All assays were done in triplicate.

4.2 Chemistry

Proton NMR spectra were recorded on a Varian 200 MHz NMR apparatus and carbon NMR spectra were recorded on a Varian 50 MHz NMR apparatus. Chemical shifts (δ) are reported

in parts per million. IR spectra were recorded on a Nicolet Impact 420 FT-IR spectrophotometer (Nicolet instrument corporation, Madison, WI, USA). All reactions were conducted under a dried nitrogen atmosphere. Chemicals were supplied by Aldrich Chemicals (Milwaukee, WI, USA) or VWR International (Mont-Royal, Qc, Canada). Liquid flash chromatography was performed on silica gel Merck grade 60 Silica Gel, 230–400 mesh and solvent mixture expressed as volume/volume ratios. The progress of all reactions was monitored using commercial TLC plates from Sigma T6145 (polyester silica gel 60 Å plates, 0.25 mm). The chromatograms were viewed under UV light at 254 and/or 265 nm. The platinum(II) complexes were analyzed using a MS model 6210, Agilent technology instrument. High resolution mass spectra (HRMS) were obtained by TOF (time of flight) using ESI (electrospray ionization) using the positive mode (ESI+). The syntheses of estradiol-linked cisplatin **1**, **2** and **3** have been described previously [16-19].

4.3 General procedure for the synthesis of E-CarboP derivatives (compounds 1a, 2a and 3a).

Compounds **1**, **2**, or **3** (0.06 mmol) was taken in a flask and dissolved in acetone (2 mL) and water (1 mL). Silver cyclobutane-1,1-dicarboxylate (0.06 mmol) was added slowly to the solution. The reaction mixture was stirred in the dark until the disappearance of the starting material (**1**, **2** or **3**) as indicated by thin layer chromatography. The reaction mixture was filtered and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography using a mixture of dichloromethane/methanol (92:8) as the eluent.

Note: The silver salt was initially prepared from cyclobutane-1,1-dicarboxylic acid (1.0 g, 6.93 mmol) upon treatment with NaOH (555 mg, 13.87 mmol) in water (10 mL), followed by addition of $AgNO_3$ (2.47 g, 14.57 mmol) in H_2O (2 mL). The reaction mixture was stirred vigorously for 2 h and the precipitate silver salt was filtered, washed with water (100 mL) and dried in a desiccator before use. This preparation yielded 2.32 g (94% yield) of silver

cyclobutane-1,1-dicarboxylate. The same procedure was used for the preparation of silver oxalate using oxalic acid as the starting material instead of cyclobutane-1,1-dicarboxylic acid.

4.3.1 16α,β-[11-(2-Pyridylethylamino)undecanyl]-1,3,5(10)-estratrien-3,17β-diol cyclobutane-1,1-dicarboxylateplatinum(II) (1a): Yield: 46% (reaction time 14 days); IR: 3339, 1644 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.67 (d, J = 5.90 Hz, 1H), 7.98 (t, J = 7.05 Hz, 1H), 7.47 (d, J = 7.80 Hz, 1H), 7.40 (t, J = 6.85 Hz, 1H), 7.06 (d, J = 8.60 Hz, 1H, ArH), 6.84 (bs, 1H, NH), 6.55-6.46 (m, 2H, ArH), 3.65 (m apparent, 1H, OH), 3.30 (m apparent, 1H, CHOH), 3.16-2.68 (several m, 13H, CH_2-NCH_2CH_2Pyridyl, 6-CH_2, 9-CH, 2x CH_2-carboPt), 2.29-1.30 (several m, 34H, 15xCH_2, 3xCH, 1xOH), 0.79 and 0.75 (2s, 3H, 18-CH_3, 16α,β (1: 3)); ^{13}C NMR (50 MHz, CD_3OD) δ 180.3, 179.8, 159.5, 154.5, 151.2, 139.5, 137.4, 131.3, 125.8, 124.9, 123.5, 114.7, 112.3, 81.9, 55.9, 53.4, 48.6, 48.2, 45.9, 44.0, 43.8, 40.2, 38.6, 37.8, 37.7, 32.2, 31.6, 30.8, 30.4, 29,6, 29,4, 29.2, 29.1, 28.5, 27.4, 26.5, 26.2, 15.1, 11.9; HRMS (ES+) *m/z* $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{42}H_{61}N_2O_6Pt$ and $C_{42}H_{60}N_2NaO_6Pt$: 884.4176 and 906.3995; found = 884.4154 and 906.3982, respectively.

4.3.2 16β-Hydroxymethyl-16α-[10-(2-pyridylethylamino)decyl]-1,3,5(10)-estratrien-3,17β-diol cyclobutane-1,1-dicarboxylateplatinum(II) (2a): Yield: 61% (reaction time 7 days); IR: 3342, 1644 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.70 (d, J = 5.50 Hz, 1H), 8.00 (t, J = 7.50 Hz, 1H), 7.49 (d, J = 7.50 Hz, 1H), 7.40 (t, J = 6.50 Hz, 1H), 7.10 (d, J = 8.40 Hz, 1H, ArH), 6.83 (bs, 1H, NH), 6.59-6.41 (m, 2H, ArH), 3.72 (2d, 2H, parts A and B of an AB system, J_{AB} = 7.40 Hz, C$\underline{H}_2$OH), 3.40 (s, 1H, C$\underline{H}$OH), 3.25-2.50 (several m, 13H, C$\underline{H}_2$-NC$\underline{H}_2$C$\underline{H}_2$Pyridyl, 6-CH_2, 9-CH, 2x CH_2-carboPt), 2.40-1.18 (several m, 33H, 14xCH_2, 2xCH, 3xOH), 0.87 (s, 3H, 18-CH_3); ^{13}C NMR (CD_3OD) δ 180.3, 179.8, 159.5, 154.5, 151.1, 139.5, 137.3, 131.2, 125.8, 124.9, 123.5, 114.6, 112.3, 89.1, 66.0, 55.9, 54.6, 46.0, 44.8, 44.0, 43.7, 39.1, 38.3, 37.8, 34.2, 30.9, 30.3, 29.3, 29.2, 29.0, 27.3, 26.4, 26.2, 24.0, 15.1, 11.3; HRMS (ES+) *m/z*

$(M+H)^+$ and $(M+Na)^+$ calculated for $C_{42}H_{61}N_2O_7Pt$ and $C_{42}H_{60}N_2NaO_7Pt$: 900.4125 and 922.3944; found = 900.4115 and 922.3931, respectively.

4.3.3 16β-Hydroxymethyl-16α-[8-(2-pyridylethylamino)-3,6-dioxaoctyl]-1,3,5(10)-estratrien-3,17β-diol cyclobutane-1,1-dicarboxylateplatinum(II) (3a): Yield: 65% (reaction time 24 hours); IR: 3362, 1610 cm^{-1}; ^1H NMR (CD_3OD) δ 8.68 (d, J = 5.80 Hz, 1H), 8.00 (t, J = 7.80 Hz, 1H), 7.55 (d, J = 7.40 Hz, 1H), 7.40 (t, J = 6.65 Hz, 1H), 7.06 (d, J = 8.20 Hz, 1H, ArH), 6.82 (bs, 1H, NH), 6.55-6.46 (m, 2H, ArH), 3.94 (m, 1H, $C\underline{H}_aH_bOH$), 3.75-2.70 (several m, 23H, $C\underline{H}OH$, $CH_a\underline{H}_bOH$, 4xOCH_2 on PEG chain, $C\underline{H}_2$-$NC\underline{H}_2C\underline{H}_2$Pyridyl, 6-$CH_2$, 9-CH, 2x$CH_2$-carboPt), 2.50-1.18 (several m, 17H, 6xCH_2, 2xCH, 3xOH), 0.84 (s, 3H, 18-CH_3); ^{13}C NMR (CD_3OD) δ 180.2, 179.9, 159.6, 154.5, 151.1, 139.6, 137.3, 131.1, 125.8, 124.8, 123.4, 114.6, 112.4, 89.1, 69.7, 68.1, 67.9, 67.6, 66.1, 55.9, 53.8, 44.8, 44.0, 38.8, 38.7, 38.3, 38.0, 35.3, 31.3, 31.2, 30.0, 29.8, 29.3, 28.1, 27.3, 26.1, 15.1, 11.4; HRMS (ES+) *m/z* $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{38}H_{53}N_2O_9Pt$ and $C_{38}H_{52}N_2NaO_9Pt$: 876.3397 and 898.3216; found = 876.3377 and 898.3206, respectively.

4.4 General procedure for the synthesis of E-OxaP derivatives (compounds 1b, 2b and 3b).

Compounds **1**, **2**, or **3** (0.10 mmol) was taken in a flask and dissolved in acetone (2 mL) and water (1 mL). Silver oxalate (0.10 mmol) was added slowly to the solution. The reaction mixture was stirred in the dark until the disappearance of the starting material (**1**, **2** or **3**) as indicated by thin layer chromatography. The reaction mixture was filtered and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography using a mixture of dichloromethane/methanol (92:8) as the eluent.

4.4.1 16α,β-[11-(2-Pyridylethylamino)undecanyl]-1,3,5(10)-estratrien-3,17β-diol oxalateplatinum(II) (1b): Yield: 46% (reaction time 15 days); IR: 3361, 1695 cm^{-1}; ^1H NMR (CD_3OD) δ 8.70 (d, J = 6.20 Hz, 1H), 8.01 (t, J = 7.40 Hz, 1H), 7.50 (d, J = 7.80 Hz, 1H), 7.39 (t, J = 6.80 Hz, 1H), 7.07 (d and bs overlapped, J = 8.20 Hz, 2H, ArH and NH), 6.54-6.47 (m, 2H, ArH), 3.62 (m, 1H, OH), 3.30 (m, 1H, C<u>H</u>OH), 3.16-2.67 (several m, 9H, C<u>H</u>$_2$-NC<u>H</u>$_2$C<u>H</u>$_2$Pyridyl, 6-CH_2, 9-CH), 2.31-1.29 (several m, 32H, 14xCH_2, 3xCH, 1xOH), 0.79 and 0.76 (2s, 3H, 18-CH_3, 16α,β (1: 3)); ^{13}C NMR (CD_3OD) δ 168.4, 167.5, 159.1, 154.5, 151.3, 139.6, 137.4, 131.3, 125.8, 125.4, 123.6, 114.6, 112.3, 81.9, 43.8, 38.6, 37.7, 37.2, 31.5, 29.3, 28.9, 28.4, 27.4, 27.2, 26.4, 26.2, 16.9, 11.8; HRMS (ES+) *m/z* $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{38}H_{55}N_2O_6Pt$ and $C_{38}H_{54}N_2NaO_6Pt$: 830.3706 and 852.3525; found = 830.3698 and 852.3514, respectively.

4.4.2 16β-Hydroxymethyl-16α-[10-(2-pyridylethylamino)decyl]-1,3,5(10)-estratrien-3,17β-diol oxalateplatinum(II) (2b): Yield: 67% (reaction time 10 days); IR: 3354, 1695 cm^{-1}; ^1H NMR (CD_3OD) δ 8.78 (d, J = 5.50 Hz, 1H), 8.00 (t, J = 7.50 Hz, 1H), 7.55 (d, J = 7.50 Hz, 1H), 7.40 (t, J = 6.50 Hz, 1H), 7.10 (d, J = 8.40 Hz, 1H, ArH), 7.00 (bs, 1H, NH), 6.60-6.41 (m, 2H, ArH), 3.72 (2d, 2H, parts A and B of an AB system, J_{AB} = 7.40 Hz, C<u>H</u>$_2$OH), 3.41 (s, 1H, OH), 3.30 (m apparent, 1H, C<u>H</u>OH), 3.25-2.50 (several m, 9H, C<u>H</u>$_2$-NC<u>H</u>$_2$C<u>H</u>$_2$Pyridyl, 6-CH_2, 9-CH), 2.40-1.20 (several m, 31H, 13xCH_2, 3xCH, 2xOH), 0.87 (s, 3H, 18-CH_3); ^{13}C NMR (CD_3OD) δ 168.4, 167.5, 159.0, 154.5, 151.3, 139.6, 137.3, 131.2, 125.8, 125.4, 123.6, 114.6, 112.3, 89.0, 66.0, 54.6, 45.9, 44.8, 44.0, 39.0, 38.3, 37.8, 37.2, 34.2, 30.2, 29.3, 29.1, 28.9, 27.3, 27.1, 26.4, 26.2, 24.0, 11.3; HRMS (ES+) *m/z* $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{38}H_{55}N_2O_7Pt$ and $C_{38}H_{54}N_2NaO_7Pt$: 846.3655 and 868.3474; found = 846.3647 and 868.3475, respectively.

4.4.3 16β-Hydroxymethyl-16α-[8-(2-pyridylethylamino)-3,6-dioxaoctyl]-1,3,5(10)-estratrien-3,17β-diol oxalateplatinum(II) (3b): Yield: 34% (reaction time 24 hours); IR: 3324, 1698 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.70 (d, J = 5.80 Hz, 1H), 8.00 (t, J = 7.80 Hz, 1H), 7.50 (d, J = 7.00 Hz, 1H), 7.40 (t, J = 7.40 Hz, 1H), 7.05 (d, J = 8.60 Hz, 1H, ArH), 6.55-6.45 (m, 2H, ArH), 3.92 (m, 1H, $C\underline{H}_aH_bOH$), 3.75-2.70 (several m, 19H, $C\underline{H}OH$, $CH_a\underline{H}_bOH$, 4xOCH_2 on PEG chain, $C\underline{H}_2$-$NC\underline{H}_2C\underline{H}_2$-Pyridyl, 6-$CH_2$, 9-CH), 2.50-1.21 (several m, 17H, 5xCH_2, 3xCH, 3xOH, NH), 0.85 (s, 3H, 18-CH_3); ^{13}C NMR (CD_3OD) δ 168.3, 167.5, 159.1, 154.5, 151.3, 139.6, 137.3, 131.1, 125.8, 125.4, 123.6, 114.6, 112.3, 89.1, 69.8, 69.5, 68.1, 67.1, 66.1, 54.3, 45.8, 44.7, 43.9, 38.2, 37.8, 37.3, 35.0, 29.3, 27.3, 26.1, 11.4; HRMS (ES+) *m/z* $(M+H)^+$ and $(M+Na)^+$ calculated for $C_{34}H_{47}N_2O_9Pt$ and $C_{34}H_{46}N_2NaO_9Pt$: 822.2927 and 844.2746; found = 822.2908 and 844.2735, respectively.

Acknowledgments

This work was supported by the Fonds de Recherche sur la Nature et les Technologies du Québec (FQRNT, Grant #132839). The Natural Sciences and Engineering Research Council of Canada (NSERC) student fellowship to C. Descôteaux is gratefully acknowledged. S. Fortin is recipient of a studentship from the Fonds de Recherche en Santé du Québec (FRSQ).

References

[1] T. Boulikas, M. Vougiouka, Cisplatin and platinum drugs at the molecular level. (Review), Oncol. Rep. 10 (2003) 1663-1682.

[2] E. Wong, C.M. Giandomenico, Current status of platinum-based antitumor drugs, Chem. Rev. 99 (1999) 2451-2466.

[3] N.J. Wheate, S. Walker, G.E. Craig, R. Oun, The status of platinum anticancer drugs in the clinic and in clinical trials, Dalton Trans. 39 (2010) 8113-8127.

[4] K. Stehlikova, H. Kostrhunova, J. Kasparkova, V. Brabec, DNA bending and unwinding due to the major 1,2-GG intrastrand cross-link formed by antitumor cis-diamminedichloroplatinum(II) are flanking-base independent, Nucleic Acids Res. 30 (2002) 2894-2898.

[5] H. Huang, L. Zhu, B.R. Reid, G.P. Drobny, P.B. Hopkins, Solution structure of a cisplatin-induced DNA interstrand cross-link, Sciences (New York) 270 (1995) 1842-1845.

[6] O. Aronov, A.T. Horowitz, A. Gabizon, M.A. Fuertes, J.M. Perez, D. Gibson, Nuclear localization signal-targeted poly(ethylene glycol) conjugates as potential carriers and nuclear localizing agents for carboplatin analogues, Bioconjug. Chem. 15 (2004) 814-823.

[7] O. Aronov, A.T. Horowitz, A. Gabizon, D. Gibson, Folate-targeted PEG as a potential carrier for carboplatin analogs. Synthesis and in vitro studies, Bioconjug. Chem. 14 (2003) 563-574.

[8] C. Lottner, R. Knuechel, G. Bernhardt, H. Brunner, Distribution and subcellular localization of a water-soluble hematoporphyrin-platinum(II) complex in human bladder cancer cells, Cancer Lett. 215 (2004) 167-177.

[9] S. Mukhopadhyay, C.M. Barnes, A. Haskel, S.M. Short, K.R. Barnes, S.J. Lippard, Conjugated platinum(IV)-peptide complexes for targeting angiogenic tumor vasculature, Bioconjug. Chem. 19 (2008) 39-49.

[10] M. Galanski, B.K. Keppler, Searching for the magic bullet: anticancer platinum drugs which can be accumulated or activated in the tumor tissue, Anticancer Agents Med. Chem. 7 (2007) 55-73.

[11] S. Moradell, J. Lorenzo, A. Rovira, S. van Zutphen, F.X. Aviles, V. Moreno, R. de Llorens, M.A. Martinez, J. Reedijk, A. Llobet, Water-soluble platinum(II) complexes of diamine chelating ligands bearing amino-acid type substituents: the effect of the linked amino acid and the diamine chelate ring size on antitumor activity, and interactions with 5'-GMP and DNA, J. Inorg. Biochem. 98 (2004) 1933-1946.

[12] J.F. Arambula, J.L. Sessler, M.E. Fountain, W.H. Wei, D. Magda, Z.H. Siddik, Gadolinium texaphyrin (Gd-Tex)-malonato-platinum conjugates: synthesis and comparison with carboplatin in normal and Pt-resistant cell lines, Dalton Trans. (2009) 10834-10840.

[13] P. Starha, Z. Travnicek, I. Popa, Platinum(II) oxalato complexes with adenine-based carrier ligands showing significant in vitro antitumor activity, J. Inorg. Biochem. 104 (2010) 639-647.

[14] G. Amr Ael, K.A. Ali, M.M. Abdalla, Cytotoxic, antioxidant activities and structure activity relationship of some newly synthesized terpenoidal oxaliplatin analogs, Eur. J. Med. Chem. 44 (2009) 901-907.

[15] I. Ott, R. Gust, Preclinical and clinical studies on the use of platinum complexes for breast cancer treatment, Anticancer Agents Med. Chem. 7 (2007) 95-110.

[16] C. Descoteaux, V. Leblanc, G. Belanger, S. Parent, E. Asselin, G. Berube, Improved synthesis of unique estradiol-linked platinum(II) complexes showing potent cytocidal activity and affinity for the estrogen receptor α and β, Steroids. 73 (2008) 1077-1089.

[17] C. Descoteaux, J. Provencher-Mandeville, I. Mathieu, V. Perron, S.K. Mandal, E. Asselin, G. Berube, Synthesis of 17β-estradiol platinum(II) complexes: biological evaluation on breast cancer cell lines, Bioorg. Med. Chem. Lett. 13 (2003) 3927-3931.

[18] J. Provencher-Mandeville, C. Debnath, S.K. Mandal, V. Leblanc, S. Parent, E. Asselin, G. Berube, Design, synthesis and biological evaluation of estradiol-PEG-linked platinum(II) hybrid molecules: comparative molecular modeling study of three distinct families of hybrids, Steroids. 76 (2011) 94-103.

[19] J. Provencher-Mandeville, C. Descoteaux, S.K. Mandal, V. Leblanc, E. Asselin, G. Berube, Synthesis of 17β-estradiol-platinum(II) hybrid molecules showing cytotoxic activity on breast cancer cell lines, Bioorg. Med. Chem. Lett. 18 (2008) 2282-2287.

[20] A.R. Yaya, M. Touaibia, G. Massarweh, F.D. Rochon, L. Breau, Synthesis of 17 α-substituted ethynylestradiols: potential ligands for drug vectors, Steroids. 75 (2010) 489-498.

[21] C.N. N'Soukpoe-Kossi, C. Descoteaux, E. Asselin, J. Bariyanga, H.A. Tajmir-Riahi, G. Berube, Transfer RNA bindings to antitumor estradiol-platinum(II) hybrid and cisplatin, DNA Cell. Biol. 27 (2008) 337-343.

[22] C.N. N'Soukpoe-Kossi, C. Descoteaux, E. Asselin, H.A. Tajmir-Riahi, G. Berube, DNA interaction with novel antitumor estradiol-platinum(II) hybrid molecule: a comparative study with cisplatin drug, DNA Cell. Biol. 27 (2008) 101-107.

[23] E. Froehlich, A. Gupta, J. Provencher-Mandeville, E. Asselin, J. Bariyanga, G. Berube, H.A. Tajmir-Riahi, Study of DNA Interactions with Steroidal and Nonsteroidal Estrogen-Platinum (II)-Based Anticancer Drugs, DNA Cell. Biol. 28 (2009) 31-39.

[24] C. Van Themsche, S. Parent, V. Leblanc, C. Descoteaux, A.M. Simard, G. Berube, E. Asselin, VP-128, a novel oestradiol-platinum(II) hybrid with selective anti-tumour activity towards hormone-dependent breast cancer cells in vivo, Endocr. Relat. Cancer. 16 (2009) 1185-1195.

[25] J. Carmichael, W.G. DeGraff, A.F. Gazdar, J.D. Minna, J.B. Mitchell, Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing, Cancer Res. 47 (1987) 936-942.

[26] R.M. Eglen, Enzyme fragment complementation: a flexible high throughput screening assay technology, Assay Drug Dev. Technol. 1 (2002) 97-104.