Design, synthesis, cytocidal activity and estrogen receptor α affinity of doxorubicin conjugates at 16α-position of estrogen for site-specific treatment of estrogen receptor positive breast cancers

Pijus Saha[†], Sébastien Fortin[†], Valérie Leblanc, Sophie Parent, Éric Asselin, Gervais Bérubé*

Groupe de Recherche en Oncologie et Endocrinologie Moléculaires, Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec, Canada G9A 5H7

Pijus Saha, Ph.D., Post-doctoral research fellow (Pijus.Saha@uqtr.ca)

Sébastien Fortin, Ph.D., Post-doctoral research fellow (Sebastien.Fortin1@uqtr.ca)

Valérie Leblanc, M.Sc., Research assistant (Valerie.Leblanc@uqtr.ca)

Sophie Parent, M.Sc., Research assistant (sophie.parent@uqtr.ca)

Éric Asselin, Ph.D., Professor (Eric.Asselin@uqtr.ca)

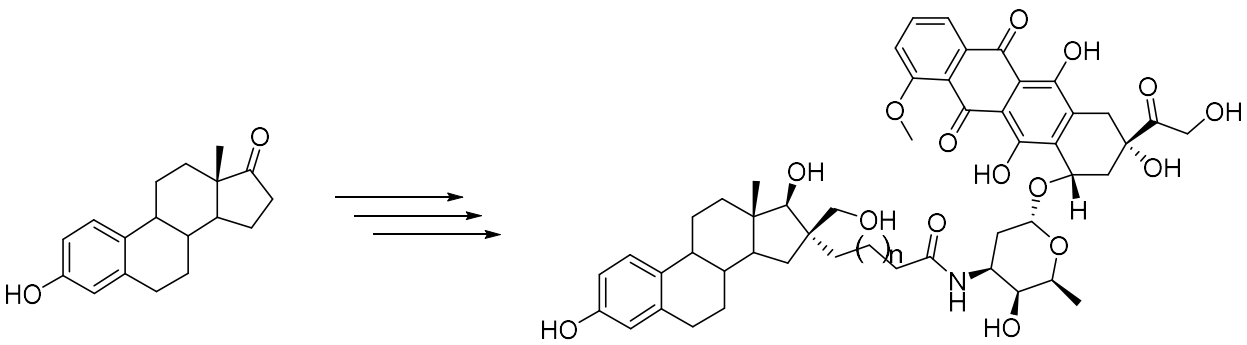
Gervais Bérubé, Ph.D., Professor (Gervais.Berube@uqtr.ca)

*Corresponding author. Département de Chimie-Biologie, Université du Québec à Trois-Rivières (UQTR), 335-boul. des Forges, C.P. 500, Trois-Rivières, Québec, G9A 5H7, Canada, Phone: 1-819-376-5011 Ext. 3353, Fax : 1-819-376-5084, E-mail: Gervais.Berube@uqtr.ca

[†]These authors contributed equally to this work.

Abbreviations: doxorubicin, DOX; estrogen receptor, ER; estrogen-doxorubicin conjugate, E-DOX; daunorubicin, DNR.

Graphical abstract



8a: n = 1, IC_{50} = 14 μM, EC_{50} = 72 nM
8b: n = 3, IC_{50} > 50 μM, EC_{50} = 79 nM
8c: n = 5, IC_{50} > 50 μM, EC_{50} = 100 nM
8d: n = 7, IC_{50} > 50 μM, EC_{50} > 215 nM

Highlights

- First reported doxorubicin conjugates at 16α-position of estrogen
- E-DOX **8a** is active in the micromolar range on HT-29 and MCF7 cell lines
- E-DOX **8a** exhibiting selectivity ratio (ER^+/ER^- cell lines) > 3.5-fold
- The affinity of E-DOX **8a-8c** are in the hundred nanomolar range
- E-DOX **8a** is a potential conjugates to target ER^+ breast cancers

Abstract

Doxorubicin (DOX) is an important medicine for the treatment of breast cancer, which is the most frequently diagnosed and the most lethal cancers in women worldwide. However, the clinical use of DOX is impeded by serious toxic effects such as cardiomyopathy and congestive heart failure. Covalently linking DOX to estrogen to selectively deliver the drug to estrogen receptor-positive (ER^+) cancer tissues is one of the strategies under investigation for improving the efficacy and decreasing the cardiac toxicity of DOX. However, conjugation of drug performed until now was at 3- or 17-position of estrogen, which is not ideal since the hydroxyl groups at this position are important for receptor binding affinity. In this study, we designed, prepared and evaluated *in vitro* the first estrogen-doxorubicin conjugates at 16α-position of estradiol termed E-DOXs (**8a**-**d**). DOX was conjugated using a 3 to 9 carbon atoms alkylamide linking arm. E-DOXs were prepared from estrone using a seven-step procedure to afford the desired conjugates in low to moderate yields. The antiproliferative activities of the E-DOX **8a** conjugate through a 3-carbon spacer chain on ER^+ MCF7 and HT-29 are in the micromolar range while inactive on M21 and the ER^- MDA-MB-231 cells (> 50 μM). Compound **8a** exhibits a selectivity ratio (ER^+/ER^- cell lines) of > 3.5. Compounds **8b**-**8d** bearing alkylamide linking arms ranging from 5 to 9 carbon atoms were inactive at the concentrations tested (> 50 μM). Interestingly, compounds **8a**-**8c** exhibited affinity for the estrogen receptor α (ERα) in the nanomolar range (72-100 nM) whereas compound **8d** exhibited no activity at concentrations up to 215 nM. These results indicate that a short alkylamide spacer is required to maintain both antiproliferative activity toward ER^+ MCF7 and affinity for the ERα of E-DOX conjugates. Compound **8a** is potentially a promising conjugate to target ER^+ breast cancers and might be useful also for the design of more potent E-DOX conjugates.

Keywords

Doxorubicin; Estrogen; Estradiol conjugate; Breast cancer; Estrogen receptor alpha; Biological activity

1. Introduction

Breast cancer is the most frequently diagnosed cancer in women in the US. An estimated of 230480 invasive and 57650 in situ new cases of breast cancer are expected to occur among women during 2011 [1]. Seventy eight percent of invasive breast cancers are estrogen receptor-positive (ER^+) and if the current trends continue, the incidence of ER^+ breast cancers will increase through the year 2016 [2]. Despite the fact that death rates for breast cancer have steadily decreased in women since 1990, an estimated of 39520 breast cancer deaths are expected in 2011 in US. Worldwide, it is also by far the most frequent cancer among women with an estimated 1.38 million new cancer cases diagnosed in 2008 (23% of all cancers) and the most frequent cause of cancer death in women (12.7% of all cancers) [3, 4]. In this context, new treatments for breast cancer improving survival and quality of life together with reducing morbidity are greatly sought by investigators today.

Anthracyclines such as doxorubicin (DOX, **1**) and daunorubicin (DNR, **2**) are members of a class of drugs ranked among the most effective anticancer drugs developed so far (Fig.1) [5]. The side chain at 9-position of DOX is constituted of a primary alcohol while DNR is bearing a methyl group at the same position. That minor structural difference impacts significantly on the clinical use of DOX and DNR. On one hand, DOX is an essential component of the treatment of breast cancer, childhood solid tumors, soft tissue sarcomas, and aggressive lymphomas. On the other hand, DNR is mainly used for the treatment of acute lymphoblastic or myeloblastic leukemias [5]. Anthracyclines act by two main mechanisms of action: 1) the formation of free radicals occurring through the Fenton and the Haber-Weiss reactions in the quinone moiety and 2) the intercalation in DNA interfering with the activity of topoisomerase II and resulting in DNA strand breakage [6]. Anthracyclines are not tissue-selective and therefore they are toxic to healthy tissues which trigger deleterious effects notably nausea, vomiting, neutropenia, alopecia, heart arrhythmias, chronic cardiomyopathy and congestive heart failure [5]. The latter are the most serious adverse effects occurring in clinics using anthracyclines. Heart toxicity of anthracyclines is dose-dependent and is limited at cumulative doses that do not exceed 500 mg/m^2 [7]. The natural tropism of anthracyclines for cardiac tissues results in the accumulation of the drug and to the generation of high levels of toxic free radicals in this specific tissue [8, 9]. Several strategies for improving the efficacy and to decrease the cardiotoxicity of anthracyclines

are currently under investigation. One of the strategies consists in covalently linking anthracyclines to drug-carriers such as estrogen, which exhibit selectivity towards cancer tumors [10].

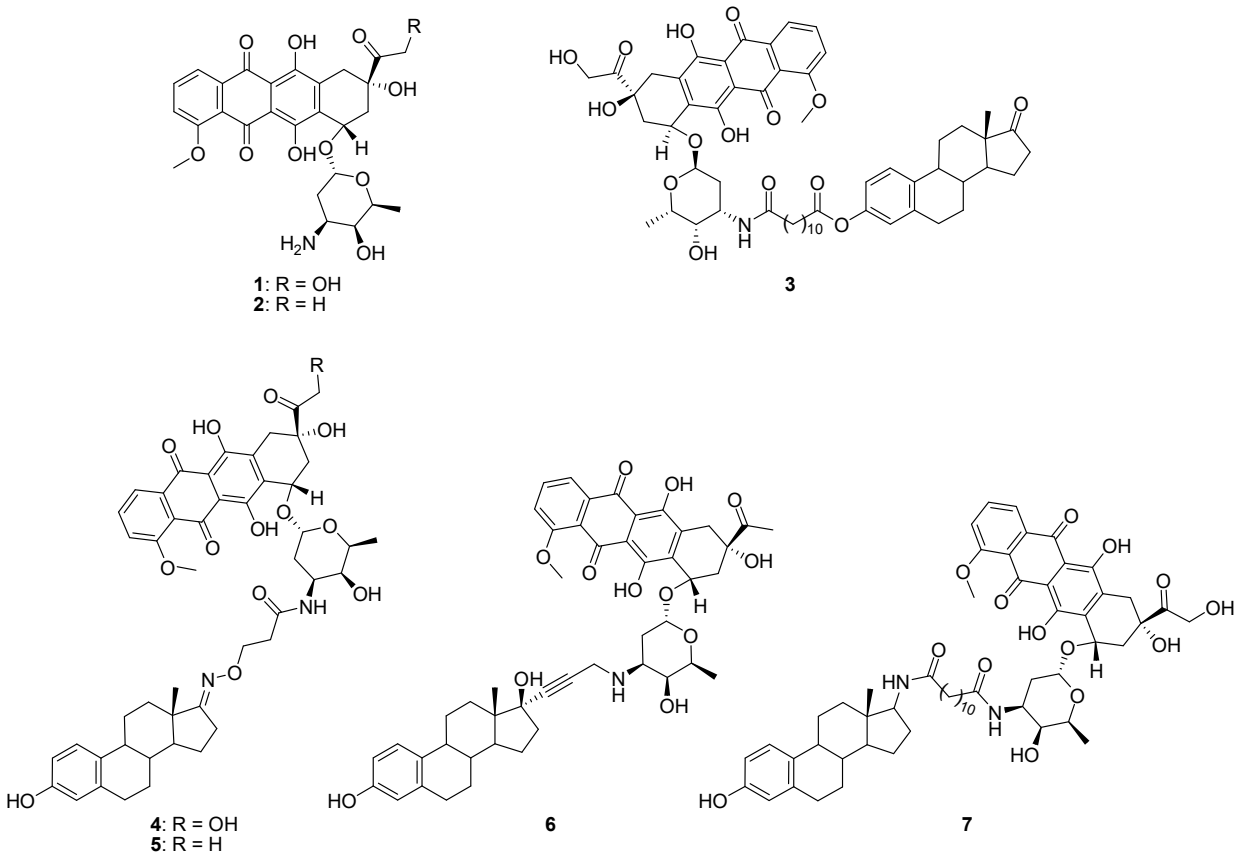


Fig. 1. Structure of DOX (**1**), DNR (**2**) and estrogen-anthracycline conjugates (**3**-**7**).

Estrogens are a group of compounds known to be involved in the development and the proliferation of breast cancer [11, 12]. These molecules exhibit appropriate physicochemical properties enabling their diffusion into tumoral tissues and cancer cells. Moreover, 70% of breast cancer cells overexpress the ERα whereas normal mammary gland predominantly express the ERβ and a low expression of ERα. [13]. ER^+ breast cancers have an estimated range of 5000 to 50 000 estrogen ERα receptor units per cancer cell [14]. This biochemical difference between cancer and healthy tissues together with the proper physicochemical properties of estrogens suggest that these molecules could be candidates as drug-carriers for the specific targeting of anthracyclines to ER^+ human breast cancers [15-17].

The first estrogenic-anthracycline conjugates were prepared by linking the drug either at position 3- or 17-position of estrogen. However, these positions on estrogen are not ideal since these hydroxyl groups are important haptophore groups for receptor binding affinity; the phenolic hydroxyl group at position 3 contributes for roughly 1.9 kcal/mol and the hydroxyl group at 17β-position contributes for approximately 0.6 kcal/mol to the binding free energy [18]. Consequently, estrogen-doxorubicin conjugate (E-DOX **3**, Fig. 1) substituted at position 3 with an ester group was relatively inactive and nonselective against ER^+ MCF7 cells [19]. In addition, estrogen-anthracycline conjugates (E-DOX **4** and E-DNR **5**) substituted at position 17 by an imine group showed also negligible selectivity for MCF7 [20, 21]. Other E-DNRs (**6**) bearing an alkyne chain at position 17α of estrogen exhibited no selectivity towards MCF7 cells [10]. The only exception was E-DOX **7** bearing an amide group that exhibited both efficacy and selectivity against MCF7 cells [19]. On one hand, these results show that a free hydroxyl group at position 3 of estrogen of estrogen-anthracycline conjugates is essential for activity and selectivity towards ER^+ MCF7 cells. However, the role and the nature of the group substituting position 17 are not as conclusive. A weak hydrogen bond acceptor or donor such as an amide (e.g., compound **7**) maintains a good affinity and selectivity towards MCF7 cells whereas imine and alkyne group at that position exhibits weak or no selectivity for MCF7 cells as exemplified by compounds **4** to **6**. On the other hand, in the course of our research program, we have identified 3 different series of estrogen derivatives substituted at position 16 with platinum(II) complexes exhibiting potent antiproliferative activity against ER^+ MCF7 cells compared to the corresponding cisplatin complex alone and exhibited good affinity for the ERα [22-25]. In addition, selected 16α,β-[11-(2-pyridylethylamino)undecanyl]-1,3,5(10)-estratrien-3,17β-diol dichloroplatinum(II) (VP-128) was more potent than cisplatin to suppress tumor growth towards ER^+ xenograft model using MCF7 cells [26]. In this context, the conjugation of DOX to the estrogen at position 16 seems promising for the design of series of novel E-DOX derivatives.

In the aim of improving selectivity and efficacy while minimizing cardiac toxicity of anthracyclines, we prepared and assessed the biological activity of a novel series of E-DOX (**8a-d**, Scheme 2) for site-specific treatment of ER^+ breast cancers. In this study, DOX is coupled to position 16α of the estradiol carrier via an alkylamide linking arm having 3, 5, 7 or 9 carbon atoms. We assessed the importance of the length of the linker arm on the antiproliferative

activity of the drugs on HT-29 human colon carcinoma, M21 human skin melanoma together with ER^+ MCF7 and ER^- MDA-MB-231 breast carcinoma cell lines and on the binding affinity to ERα.

2. Experimental

2.1. Chemistry

All reactions were performed with ACS Fisher solvents. In some cases, solvents and reagents were purified and dried according to standard procedures [27]. Estrone was purchased from Steraloids Inc. (Wilton, NH, USA) and doxorubicin hydrochloride was generously provided by Farmitalia Carlo Erba (Italy). Chemicals were supplied by Sigma-Aldrich Canada Ltd., (Oakville, Ontario, Canada) and were used as received unless specified. All reactions were conducted under dry nitrogen atmosphere and were monitored by UV fluorescence or staining with iodine on Glass Backed TLC Extra Hard Layer, 60Å (thickness 250 μm, 2.5 x 10 cm, ultrapure silica gel, Silicycle Inc., Quebec, Canada). Purifications were done using flash column chromatography according to the method of Still et al. [28] on Silicycle UltraPure Flash Silica Gel, 40–63 μm mesh. The infrared spectra were taken by Thermo Scientific Nicolet 420 FT-IR spectrometer. NMR spectra were recorded by Varian 200 MHz NMR device. Deuterochloroform ($CDCl_3$), deuteroacetone (acetone-d_6) or deuteromethanol (methanol-d_4) were used as NMR solvents (purchased from CDN Isotopes, Canada). Chemical shifts (δ) are expressed in ppm and coupling constants (*J*) are in Hertz (Hz). Multiplicities are described by s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, m for multiplet and bs for broad singlet. Mass spectral assays were carried out using a MS model 6210, Agilent technology instrument and the high-resolution mass spectra (HRMS) were obtained by TOF (time-of-flight) using ESI (electrospray ionization) in the positive mode (ESI+) at the Université du Quebec à Montreal (Plateforme analytique pour molécules organiques de l'Université du Québec à Montréal).

Note: The nomenclature of the various estrogen derivatives reported in this manuscript was based on the estrogen skeleton for better clarity to the reader in the field.

2.1.1. Preparation of 1-(ω-iodoalkyl)-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane (11a-d)

Preparation of 1-(ω-iodoalkyl)-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane (**11a-d**) was achieved using the method described by Petroski et al. [29]. 8-Bromooctanoyl chloride and 10-bromodecanoyl chloride used as starting materials were not commercially available. They were initially prepared using the method described by Zhang et al. [30] and Hoarwood and Moody [31]. Briefly, a mixture of thionyl chloride (13 mmol) and the appropriate ω-bromoalkanoic acid was refluxed for 1 h. The excess of thionyl chloride was evaporated under reduced pressure to afford the desired ω-bromoalkanoyl chloride in quantitative yields and were used without further purification at the next step.

2.1.1.1. Preparation of (3-methyloxetan-3-yl)methyl ω-bromoalkanoate (9a-d). Pyridine (10.5 mmol) was dissolved into dry tetrahydrofuran (18 mL) and the solution cooled to 0 °C in an ice bath. 3-Methyl-3-oxetanemethanol (8.75 mmol) was added and then the appropriate ω-bromoalkanoyl chloride (9.75 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for an additional 1 h. The reaction mixture was poured into an ice-water mixture and then extracted with methylene chloride (5x 30 mL). The aqueous phase was concentrated under reduced pressure to remove remaining tetrahydrofuran and methylene chloride, and then extracted with methylene chloride (2 x 100 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to afford the desired (3-methyloxetan-3-yl)methyl ω-bromoalkanoates (**9a-d**) in quantitative yields that were used without further purification at the next step.

2.1.1.2. (3-Methyloxetan-3-yl)methyl 4-bromobutanoate (9a). IR (ν_{max}, cm^{-1}): 1742 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 4.52-4.49 (m, 2H, OCH_2), 4.40-4.37 (m, 2H, OCH_2), 4.17 (s, 2H, OCH_2), 3.47 (t, J = 6.4 Hz, 2H, CH_2Br), 2.56 (t, J = 7.2 Hz, 2H, CH_2CO), 2.25-2.15 (m, 2H, CH_2), 1.33 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 172.6, 79.5, 68.8, 39.0, 32.6, 32.3, 27.6, 21.2.

2.1.1.3. (3-Methyloxetan-3-yl)methyl 6-bromohexanoate (9b). IR (ν_{max}, cm^{-1}): 1735 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 4.52-4.49 (m, 2H, OCH_2), 4.38-4.35 (m, 2H, OCH_2), 4.15 (s, 2H, OCH_2), 3.39 (t, J = 6.7 Hz, 2H, CH_2Br), 2.36 (t, J = 7.2 Hz, 2H, CH_2CO), 1.96-1.83 (m, 2H,

CH_2), 1.74-1.62 (m, 2H, CH_2), 1.54-1.42 (m, 2H, CH_2), 1.31 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 173.5, 79.5, 68.5, 39.0, 33.9, 33.4, 32.3, 27.6, 24.1, 21.2.

2.1.1.4. (3-Methyloxetan-3-yl)methyl 8-bromooctanoate (9c). IR (ν_{max}, cm^{-1}): 1734 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 4.52-4.49 (m, 2H, OCH_2), 4.39-4.36 (m, 2H, OCH_2), 4.15 (s, 2H, OCH_2), 3.39 (t, J = 6.8 Hz, 2H, CH_2Br), 2.34 (t, J = 7.6 Hz, 2H, CH_2CO), 1.92-1.77 (m, 2H, CH_2), 1.71-1.56 (m, 2H, CH_2), 1.52-1.41 (m, 6H, 3xCH_2),1.32 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 173.8, 79.6, 68.4, 39.0, 34.1, 33.8, 32.7, 28.9, 28.3, 27.9, 24.8, 21.2.

2.1.1.5. (3-Methyloxetan-3-yl)methyl 10-bromodecanoate (9d). IR (ν_{max}, cm^{-1}): 1735 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 4.52-4.48 (m, 2H, OCH_2), 4.41-4.35 (m, 2H, OCH_2), 4.14 (s, 2H, OCH_2), 3.39 (t, J = 6.9 Hz, 2H, CH_2Br), 2.34 (t, J = 7.4 Hz, 2H, CH_2CO), 1.90-1.77 (m, 2H, CH_2), 1.68-1.55 (m, 2H, CH_2), 1.45-1.29 (m, 13H, 5xCH_2 and CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 173.9, 79.6, 68.4, 39.1, 34.2, 34.0, 32.8, 29.2, 29.1, 29.0, 28.6, 28.1, 24.9, 21.2.

2.1.1.6. Preparation of (3-methyloxetan-3-yl)methyl ω-iodoalkanoate (10a-d). A mixture of the appropriate (3-methyloxetan-3-yl)methyl ω-bromoalkanoate **9a-d** (11.95 mmol), acetone (90 mL), NaI (60 mmol) and Na_2SO_4 (60 mmol) was stirred for 5 h at room temperature. The mixture was filtered and the filtrate was evaporated under reduced pressure. The oily residue was dissolved in methylene chloride and filtered again. The filtrate was evaporated under reduced pressure to afforded the desired (3-methyloxetan-3-yl)methyl ω-iodoalkanoates (**10a-d**) in 97% to quantitative yield (homogeneous by TLC) and used without further purification in the next step.

2.1.1.7. (3-Methyloxetan-3-yl)methyl 4-iodobutanoate (10a). Yield: 98%. IR (ν_{max}, cm^{-1}): 1742 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 4.52-4.49 (m, 2H, OCH_2), 4.40-4.37 (m, 2H, OCH_2), 4.17 (s, 2H, OCH_2), 3.24 (t, J = 6.6 Hz, 2H, CH_2I), 2.51 (t, J = 7.2 Hz, 2H, CH_2CO), 2.21-2.06 (m, 2H, CH_2), 1.33 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 172.4, 79.5, 68.8, 39.0, 34.7, 28.3, 21.2, 5.4.

2.1.1.8. (3-Methyloxetan-3-yl)methyl 6-iodohexanoate (10b). Yield: quantitative. IR (ν_{max}, cm^{-1}): 1735 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 4.52-4.49 (m, 2H, OCH_2), 4.39-4.36 (m, 2H, OCH_2),

4.15 (s, 2H, OCH_2), 3.17 (t, J = 7.0 Hz, 2H, CH_2I), 2.37 (t, J = 7.4 Hz, 2H, CH_2CO), 1.95-1.78 (m, 2H, CH_2), 1.72-1.60 (m, 2H, CH_2), 1.52-1.41 (m, 2H, CH_2), 1.32 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 173.5, 79.6, 68.6, 39.0, 33.9, 33.0, 29.9, 23.9, 21.2, 6.5.

2.1.1.9. (3-Methyloxetan-3-yl)methyl 8-iodooctanoate (10c). Yield: 97%. IR (ν_{max}, cm^{-1}): 1733 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 4.52-4.49 (m, 2H, OCH_2), 4.39-4.36 (m, 2H, OCH_2), 4.15 (s, 2H, OCH_2), 3.17 (t, J = 7.1 Hz, 2H, CH_2I), 2.34 (t, J = 7.4 Hz, 2H, CH_2CO), 1.88-1.74 (m, 2H, CH_2), 1.70-1.55 (m, 2H, CH_2), 1.45-1.33 (m, 6H, 3xCH_2), 1.32 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 173.8, 79.6, 68.4, 39.0, 34.1, 33.4, 30.3, 28.9, 28.1, 24.8, 21.2, 7.1.

2.1.1.10. (3-Methyloxetan-3-yl)methyl 10-iododecanoate (10d). Yield: 97%. IR (ν_{max}, cm^{-1}): 1735 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 4.52-4.49 (m, 2H, OCH_2), 4.41-4.35 (m, *2*H, OCH_2), 4.14 (s, 2H, OCH_2), 3.17 (t, J = 6.9 Hz, 2H, CH_2I), 2.34 (t, J = 7.5 Hz, 2H, CH_2CO), 1.87-1.73 (m, 2H, CH_2), 1.68-1.57 (m, 2H, CH_2), 1.45-1.29 (m, 13H, 5xCH_2 and CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 173.9, 79.6, 68.4, 39.1, 34.2, 33.5, 30.4, 29.2, 29.1, 29.0, 28.4, 24.9, 21.2, 7.2.

2.1.1.11. Preparation of 1-(ω-iodoalkyl)-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane (11a-d). The appropriate (3-methyloxetan-3-yl)methyl ω-iodoalkanoate **10a-d** (11.74 mmol) was dissolved in methylene chloride (20 mL) and cooled to 0 °C in an ice bath. Boron trifluoride etherate (4.4 mmol) was added dropwise and the solution was stirred for 4 h at 0 °C. The reaction mixture was quenched by the addition of 2.5 mL of triethylamine and afterwards 20 mL of ether were added. The mixture was stirred for five minutes at 0 °C, filtered and the filtrate was kept at -20 °C overnight. The solution was filtered again and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (pre-neutralised with 5% triethylamine in hexanes) using hexanes and acetone (7:3) as eluent and the yields ranged from 35% to 65 %.

2.1.1.12. 1-(3-Iodopropyl)-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane (11a). Yield: 65%. IR (ν_{max}, cm^{-1}): 1735 (C-O). ^1H NMR ($CDCl_3$, δ ppm): 3.87 (s, 6H, 3xOCH_2), 3.20 (t, J = 6.8 Hz, 2H, CH_2I), 2.06-1.92 (m, 2H, CH_2), 1.79-1.71 (m, 2H, CH_2), 0.79 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 108.5, 72.6, 37.3, 30.3, 27.7, 14.5, 6.9. HRMS (ESI+): *m/z* found 299.0130; $C_9H_{16}IO_3$ $[M + H]^+$ requires 299.0139.

2.1.1.13. 1-(5-Iodopentyl)-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane (11b). Yield: 49%. IR (ν_{max}, cm^{-1}): 1735 (C-O). ^1H NMR ($CDCl_3$, δ ppm): 3.88 (s, 6H, 3xOCH_2), 3.17 (t, J = 7.1 Hz, 2H, CH_2I), 1.89-1.78 (m, 2H, CH_2), 1.74-1.62 (m, 2H, CH_2), 1.49-1.37 (m, 4H, 2xCH_2), 0.79 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 108.9, 72.6, 36.4, 33.4, 30.3, 30.2, 22.1, 14.5, 7.0. HRMS (ESI+): *m/z* found 327.0446; $C_{11}H_{20}IO_3$ $[M + H]^+$ requires 327.0452.

2.1.1.14. 1-(7-Iodoheptyl)-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane (11c). Yield: 51%. IR (ν_{max}, cm^{-1}): 1733 (C-O). ^1H NMR ($CDCl_3$, δ ppm): 3.88 (s, 6H, 3xOCH_2), 3.16 (t, J = 7.0 Hz, 2H, CH_2I), 1.87-1.72 (m, 2H, CH_2), 1.69-1.60 (m, 2H, CH_2), 1.45-1.23 (m, 8H, 4xCH_2), 0.79 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 109.0, 72.6, 36.6, 33.5, 30.4, 30.2, 29.2, 28.3, 23.0, 14.6, 7.2. HRMS (ESI+): *m/z* found 355.0756; $C_{13}H_{24}IO_3$ $[M + H]^+$ requires 355.0765.

2.1.1.15. 1-(9-Iodononyl)-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane (11d). Yield: 34%. IR (ν_{max}, cm^{-1}): 1735 (C-O). ^1H NMR ($CDCl_3$, δ ppm): 3.88 (s, 6H, 3xOCH_2), 3.17 (t, J = 7.1 Hz, 2H, CH_2I), 1.87-1.73 (m, 2H, CH_2), 1.68-1.61 (m, 2H, CH_2), 1.45-1.26 (m, 12H, 6xCH_2), 0.79 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 109.0, 72.6, 36.7, 33.6, 30.5, 30.2, 29.4, 29.3, 29.2, 28.5, 23.1, 14.6, 7.3. HRMS (ESI+): *m/z* found 383.1077; $C_{15}H_{28}IO_3$ $[M + H]^+$ requires 383.1078.

2.1.2. Preparation of E-DOXs (8a-8d)

2.1.2.1. Preparation of 3-tetrahydropyrannyloxy-1,3,5(10)-estratrien-17-one (13). The synthesis of compounds **12** and **13** was achieved using the method described by Descoteaux et al. [22]. Briefly, to a solution of estrone (37.43 mmol) in methylene chloride, pyridinium *p*-toluenesulfonate (4.0 mmol) and 3,4-dihydro-2*H*-pyran (DHP, 93.6 mmol), were added dropwise. The resulting mixture was stirred at room temperature (21 °C) for 20 h. Afterwards, the solution was neutralized by the addition of a small amount of sodium bicarbonate, dried with $MgSO_4$ and filtered on a silica gel (3 cm) and celite (1 cm) using hexanes as eluent. The filtrate was evaporated under reduced pressure to afford 3-tetrahydropyrannyloxy-1,3,5(10)-estratrien-17-one (**12**) in 100% yield. Thereafter, the protected estrone (**12**) (37.6 mmol) dissolved in tetrahydrofuran (70 mL) was added to a mixture of potassium hydride (30% in oil, 112.7 mmol) and dimethylcarbonate (93.9 mmol) in tetrahydrofuran (30 mL) and heated to reflux for 2.5 h. Afterward, the mixture was cooled down at room temperature. *Tert*-butanol (10 mL), methanol

(20 mL) and water were successively added and the solution was stirred for 15 minutes between each solvent addition. The solution was then diluted with ethyl acetate (80 mL) and the organic phase was washed twice with saturated NH_4Cl solution and four times with water. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the crude compound **13**. The residue was first triturated with hexanes and purified by flash chromatography using a mixture of hexanes and acetone (92:8) to give 3-tetrahydropyrannyloxy-1,3,5(10)-estratrien-17-one (**13**) in 90% yield.

2.1.2.2. 16β-Methoxycarbonyl-16α-(ω-[4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octan-1-yl]alkyl)-3-(tetrahydropyrannyloxy)-1,3,5(10)-estratrien-17-one (14a-d). The appropriate 1-(ω-iodoalkyl)-4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octane derivative **11a-d** (1.82 mmol) and compound **13** (1.21 mmol) were dissolved in anhydrous tetrahydrofuran (12 mL). Cs_2CO_3 (790 mg, 2.42 mmol) was added and the reaction mixture was refluxed for 6 h. The reaction mixture was diluted with ether, washed with saturated ammonium chloride solution and then washed four times with water; dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash chromatography using hexanes and acetone (7:3) to afford **14a-d** in 48-56% yield.

2.1.2.3. 16β-Methoxycarbonyl-16α-(3-[4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octan-1-yl]propyl)-3-(tetrahydropyrannyloxy)-1,3,5(10)-estratrien-17-one (14a). Yield: 56%. IR (ν_{max}, cm^{-1}): 1747 and 1724 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 7.19-7.15 (m, 1H, Ar), 6.87-6.79 (m, 2H, Ar), 5.40 (t, J = 2.90 Hz, 1H, OCHO), 3.91-3.77 and 3.61-3.43 (two m, partly hidden, 2H, OCH_2), 3.87 (s, 6H, 3xOCH_2), 3.72 (s, 3H, OCH_3), 2.90-2.86 (m, 2H, CH_2), 2.44-1.18 (several m, 23H, 3xCH and 10xCH_2), 0.91 (s, 3H, CH_3), 0.79 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 213.9, 171.6, 155.1, 137.6, 132.8, 126.2, 116.6, 116.5, 114.1, 108.7, 96.3, 72.6, 61.9, 60.4, 52.7, 49.5, 45.9, 44.1, 37.9, 36.7, 35.0, 32.1, 30.4, 30.2, 30.0, 29.6, 26.6, 25.7, 25.3, 19.3, 18.8, 14.5, 14.0. HRMS (ESI+): *m/z* found 583.3265; $C_{34}H_{47}O_8$ $[M + H]^+$ requires 583.3271.

2.1.2.4. 16β-Methoxycarbonyl-16α-(5-[4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octan-1-yl]pentyl)-3-(tetrahydropyrannyloxy)-1,3,5(10)-estratrien-17-one (14b). Yield: 61%. IR (ν_{max}, cm^{-1}): 1747 and 1721 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 7.20-7.16 (m, 1H, Ar), 6.87-6.79 (m, 2H, Ar), 5.39 (t, J = 2.90 Hz, 1H, OCHO), 3.91-3.77 and 3.69-3.50 (two m, partly hidden,

2H, OCH_2), 3.88 (s, 6H, 3xOCH_2), 3.71 (s, 3H, OCH_3), 2.88-2.86 (m, 2H, CH_2), 2.43-1.20 (several m, 27H, 3xCH and 12xCH_2), 0.91 (s, 3H, CH_3), 0.79 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 214.0, 171.8, 155.1, 137.6, 132.8, 126.2, 116.6, 116.5, 114.1, 109.0, 96.3, 72.6, 61.9, 60.2, 52.6, 49.5, 45.9, 44.1, 37.9, 36.6, 35.4, 32.0, 30.4, 30.2, 29.6, 29.5, 26.5, 25.7, 25.3, 25.3, 22.9, 18.8, 14.5, 14.0. HRMS (ESI+): *m/z* found 611.3568; $C_{36}H_{51}O_8$ $[M + H]^+$ requires 611.3578.

2.1.2.5. 16β-Methoxycarbonyl-16α-(7-[4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octan-1-yl]heptyl)-3-(tetrahydropyrannyloxy)-1,3,5(10)-estratrien-17-one (14c). Yield: 50%. IR (ν_{max}, cm^{-1}): 1749 and 1722 (C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 7.19-7.15 (m, 1H, Ar), 6.82-6.79 (m, 2H, Ar), 5.38 (t, *J* = 2.90 Hz, 1H, OCHO), 3.91-3.77 and 3.69-3.48 (two m, partly hidden, 2H, OCH_2), 3.88 (s, 6H, 3xOCH_2), 3.71 (s, 3H, OCH_3), 2.88-2.84 (m, 2H, CH_2), 2.43-1.12 (several m, 31H, 3xCH and 14xCH_2), 0.91 (s, 3H, CH_3), 0.78 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 214.1, 171.9, 155.1, 137.6, 132.8, 126.2, 116.6, 116.5, 114.1, 109.0, 96.3, 72.6, 61.9, 60.2, 52.6, 49.5, 45.9, 44.0, 37.9, 36.7, 35.5, 32.1, 30.4, 30.2, 29.6, 29.5, 29.3, 29.2, 26.5, 25.7, 25.4, 25.3, 23.0, 18.8, 14.5, 14.0. HRMS (ESI+): *m/z* found 639.3884; $C_{38}H_{55}O_8$ $[M + H]^+$ requires 639.3891.

2.1.2.6. 16β-Methoxycarbonyl-16α-(9-[4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octan-1-yl]nonyl)-3-(tetrahydropyrannyloxy)-1,3,5(10)-estratrien-17-one (14d). Yield: 56%. IR (ν_{max}, cm^{-1}): 1748 and 1721 (C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 7.20-7.16 (m, 1H, Ar), 6.87-6.80 (m, 2H, Ar), 5.39 (t, *J* = 2.90 Hz, 1H, OCHO), 3.91-3.77 and 3.69-3.48 (two m, partly hidden, 2H, OCH_2), 3.88 (s, 6H, 3xOCH_2), 3.72 (s, 3H, OCH_3), 2.92-2.86 (m, 2H, CH_2), 2.43-1.12 (several m, 35H, 3xCH and 16xCH_2), 0.91 (s, 3H, CH_3), 0.79 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 214.1, 171.9, 155.1, 137.6, 132.9, 126.2, 116.6, 116.5, 114.1, 109.1, 96.3, 72.6, 61.9, 60.2, 52.6, 49.5, 45.9, 44.1, 37.9, 36.7, 35.6, 32.1, 30.5, 30.4, 30.2, 29.8, 29.5, 29.4, 29.4, 29.3, 26.5, 25.7, 25.4, 25.3, 23.1, 18.8, 14.6, 14.0. HRMS (ESI+): *m/z* found 667.4198; $C_{40}H_{59}O_8$ $[M + H]^+$ requires 667.4204.

2.1.2.7. 16α-(Ethoxycarbonylalkyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (15a-c). The appropriate compound **14a-c** (0.31 mmol) was dissolved in dry ether (7 mL) and cooled to 0 °C. $LiBH_4$ (2.83 mmol) was added in two portions 30 minutes apart. The mixture was stirred to 0 °C for two hours and then kept at room temperature for 24 h. The reaction mixture was

quenched by the addition of a saturated ammonium chloride solution (25 mL). The mixture was diluted with ether and then washed with water four times. The organic phase was dried over anhydrous $MgSO_4$, filtered and the filtrate evaporated under reduced pressure. Thereafter, the appropriate crude products (0.27 mmol) were dissolved in ethanol and pyridinium *p*-toluenesulfonate (PPTs, 0.10 mmol) was added and the solution refluxed for 8 h. The reaction mixture was diluted with ethyl acetate, washed with a saturated ammonium chloride solution and water four times. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography using hexanes and acetone as eluents (7:3) to afford the pure product in 18 to 29% yields.

2.1.2.8. 16α-(Ethoxycarbonylpropyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (15a). Yield: 29%. IR (ν_{max}, cm^{-1}): 3396 (OH), 1711 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 7.13-7.08 (m, 1H, Ar), 6.65-6.55 (m, 2H, Ar), 6.22 (bs, 1H, OH), 4.15 (q, J = 7.17 Hz, 2H, OCH_2), 3.87-3.82 (m, 1H, CH), 3.56-3.45 (m, 2H, CH_2), 3.26 (bs, 1H, OH), 3.05 (bs, 1H, OH), 2.79-2.77 (m, 2H, CH_2), 2.36-1.10 (several m, 20H, 3xCH, 7xCH_2 and CH_3), 0.86 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 174.4, 153.8, 138.0, 132.1, 126.4, 115.3, 112.8, 90.4, 66.7, 60.6, 47.6, 46.7, 44.9, 43.7, 38.2, 37.9, 34.5, 33.0, 29.6, 27.4, 26.2, 19.9, 14.3, 11.9. HRMS (ESI+): *m/z* found 417.2635; $C_{25}H_{37}O_5$ $[M + H]^+$ requires 417.2636.

2.1.2.9. 16α-(Ethoxycarbonylpentyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (15b). Yield: 18%. IR (ν_{max}, cm^{-1}): 3396 (OH), 1711 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 7.15-7.10 (m, 1H, Ar), 6.65-6.55 (m, 2H, Ar), 5.60 (bs, 1H, OH), 4.13 (q, J = 7.17 Hz, 2H, OCH_2), 3.83-3.77 (m, 1H, CH), 3.52-3.46 (m, 2H, CH_2), 2.98 (bs, 1H, OH), 2.81-2.79 (m, 2H, CH_2), 2.58 (bs, 1H, OH), 2.31-1.10 (several m, 24H, 3xCH and 9xCH_2 and CH_3), 0.87 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 174.1, 153.6, 138.1, 132.3, 126.4, 115.3, 112.7, 90.7, 66.9, 60.3, 47.6, 46.8, 44.9, 43.8, 39.0, 37.9, 34.4, 33.1, 30.0, 29.6, 27.4, 26.2, 24.9, 24.4, 14.3, 11.9. HRMS (ESI+): *m/z* found 445.2943; $C_{27}H_{41}O_5$ $[M + H]^+$ requires 445.2949.

2.1.2.10. 16α-(Ethoxycarbonylheptyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (15c). Yield: 21%. IR (ν_{max}, cm^{-1}): 3361 (OH), 1709 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 7.14-7.10 (m, 1H, Ar), 6.66-6.56 (m, 2H, Ar), 5.90 (bs, 1H, OH), 4.13 (q, J = 7.17 Hz, 2H, OCH_2), 3.83-3.77 (m, 1H, CH), 3.52-3.46 (m, 2H, CH_2), 3.03 (bs, 1H, OH), 2.79-2.65 (m, 3H, CH_2 and OH),

2.29-1.00 (several m, 28H, 3xCH, 11xCH_2 and CH_3), 0.87 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 174.2, 153.7, 138.0, 132.3, 126.4, 115.3, 112.8, 90.7, 66.9, 60.3, 47.6, 46.8, 44.9, 43.8, 39.2, 37.9, 34.4, 33.1, 30.3, 29.6, 29.2, 29.1, 27.4, 26.2, 24.9, 24.6, 14.3, 11.9. HRMS (ESI+): *m/z* found 473.3257; $C_{29}H_{45}O_5$ $[M + H]^+$ requires 473.3262.

2.1.2.11. 16α-(2,2-Bis(hydroxymethyl)propyloxycarbonylnonyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (15d). Compound **14d** (0.31 mmol) was dissolved in dry ether (7 mL) and the solution cooled to 0 °C. $LiBH_4$ was added in two portions added 30 minutes apart. The mixture was stirred for two hours at 0 °C and then for 24 h at room temperature. The reaction mixture was quenched by the addition of a saturated ammonium chloride solution (25 mL), diluted with ether and then washed four times with water. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated. Afterward, the crude product was dissolved in 3 mL of methanol/water solution (40:1), pyridinium *p*-toluenesulfonate (0.04 mmol) was added and the mixture refluxed for 5 h. The reaction mixture was diluted with ethyl acetate, washed first with a $NaHCO_3$ solution followed by water (three times). The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography using hexanes and acetone as eluents (6:4). Yield: 51%. IR (ν_{max}, cm^{-1}): 3405 (OH), 1711 (C=O). ^{1}H NMR (acetone-d_6, δ ppm): 7.91 (s, 1H, OH), 7.10-7.06 (m, 1H, Ar), 6.61-6.51 (m, 2H, Ar), 4.28 (d, J = 4.70, Hz, 1H, OH), 4.03 (s, 2H, OCH_2), 3.77-3.43 (several m, 7H, CH and 3xCH_2), 2.89-2.75 (m, 3H, CH_2 and OH), 2.35–1.15 (several m, 31H, 3xCH, 13xCH_2 and 2xOH), 0.88 (s, 6H, 2xCH_3). ^{13}C NMR (acetone-d_6, δ ppm): 173.1, 155.1, 137.5, 131.2, 126.1, 115.1, 112.7, 89.7, 66.3, 65.9, 65.1, 47.5, 46.6, 44.9, 44.0, 40.6, 39.4, 38.3, 38.0, 33.8, 33.7, 30.5, 27.4, 26.3, 24.8, 24.4, 16.1, 11.6. HRMS (ESI+): *m/z* found 575.3937; $C_{34}H_{55}O_7$ $[M + H]^+$ requires 575.3942.

2.1.2.12. 16α-(ω-Carboxyalkyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (16a-d). The appropriate compound **15a-d** (0.25 mmol) was dissolved in tetrahydrofuran (3 mL), 1 M NaOH solution (3 mL) was added and the solution stirred overnight at room temperature. Tetrahydrofuran was evaporated under reduced pressure. The aqueous layer was washed with ethyl acetate, acidified with 10 % HCl solution (pH 1) and then extracted three times with ether. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced

pressure. The product was purified by flash chromatography using hexanes and acetone (6:4) as eluent to afford **15a-d** in 12 to 46% yields.

2.1.2.13. 16α-(3-Carboxypropyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (16a). Yield: 46%. IR (ν_{max}, cm^{-1}): 3368 (OH), 1704 (C=O). ^{1}H NMR (acetone-d_6, δ ppm): 7.90 (bs, 1H, OH), 7.10-7.06 (m, 1H, Ar), 6.61-6.51 (m, 2H, Ar), 4.33 (bs, 1H, OH), 3.79-3.73 (m, 1H, CH), 3.49-3.42 (m, 2H, CH_2), 2.81-2.75 (m, 2H, CH_2), 2.33-1.11 (several m, 18H, 3xCH, 7xCH_2 and OH), 0.89 (s, 3H, CH_3). ^{13}C NMR (acetone-d_6, δ ppm): 174.0, 155.0, 137.5, 131.2, 126.1, 115.1, 112.7, 89.4, 66.2, 47.5, 46.6, 45.0, 43.9, 38.7, 38.3, 37.9, 34.1, 33.7, 29.8, 27.4, 26.3, 20.1, 11.6. HRMS (ESI+): *m/z* found 389.2321; $C_{23}H_{33}O_5$ $[M + H]^+$ requires 389.2323.

2.1.2.14. 16α-(5-Carboxypentyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (16b). Yield: 35%. IR (ν_{max}, cm^{-1}): 3331 (OH), 1705 (C=O). ^{1}H NMR (acetone-d_6, δ ppm): 7.10-7.06 (m, 1H, Ar), 6.61-6.51 (m, 2H, Ar), 4.30 (bs, 1H, OH), 3.77-3.71 (m, 1H, CH), 3.47-3.42 (m, 2H, CH_2), 2.82-2.73 (m, 2H, CH_2), 2.34-1.12 (several m, 23H, 3xCH, 9xCH_2 and 2xOH), 0.89 (s, 3H, CH_3). ^{13}C NMR (acetone-d_6, δ ppm): 173.8, 155.0, 137.5, 131.2, 126.1, 115.0, 112.7, 89.6, 66.2, 47.5, 46.6, 44.9, 43.9, 39.2, 38.3, 38.0, 33.7, 33.3, 29.9, 27.4, 26.3, 24.8, 24.1, 11.6. HRMS (ESI+): *m/z* found 417.2628; $C_{25}H_{37}O_5$ $[M + H]^+$ requires 417.2636.

2.1.2.15. 16α-(7-Carboxyheptyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (16c). Yield: 12%. IR (ν_{max}, cm^{-1}): 3364 (OH), 1796 (C=O). ^{1}H NMR (acetone-d_6, δ ppm): 7.10-7.06 (m, 1H, Ar), 6.60-6.51 (m, 2H, ArH), 4.30 (bs, 1H, OH), 3.76-3.71 (m, 1H, CH), 3.47-3.44 (m, 2H, CH_2), 2.82-2.77 (m, 2H, CH_2), 2.33-1.20 (several m, 27H, 3xCH, 11xCH_2 and 2xOH), 0.89 (s, 3H, CH_3). ^{13}C NMR (acetone-d_6, δ ppm): 173.8, 155.0, 137.5, 131.2, 126.1, 115.0, 112.7, 89.7, 66.3, 47.5, 46.7, 44.9, 43.9, 39.4, 38.3, 38.0, 33.7, 33.3, 30.4, 27.4, 26.3, 24.8, 24.3, 11.6. HRMS (ESI+): *m/z* found 445.2942; $C_{27}H_{41}O_5$ $[M + H]^+$ requires 445.2949.

2.1.2.16. 16α-(9-Carboxynonyl)-16β-hydroxymethyl-1,3,5(10)-estratrien-3,17-diol (16d): Yield: 31%. IR (ν_{max}, cm^{-1}): 3320 (OH), 1704 (C=O). ^{1}H NMR (acetone-d_6, δ ppm): 7.10-7.06 (m, 1H, Ar), 6.61–6.51 (m, 2H, Ar), 4.30 (bs, 1H, OH), 3.76-3.71 (m, 1H, CH), 3.47-3.43 (m, 2H, CH_2), 2.78-2.73 (m, 2H, CH_2), 2.38-1.20 (several m, 31H, 3xCH, 13xCH_2 and 2xOH), 0.89 (s, 3H, CH_3). ^{13}C NMR (acetone-d_6, δ ppm): 173.8, 155.0, 137.5, 131.2, 126.1, 115.0, 112.7,

89.7, 66.3, 47.5, 46.6, 44.9, 43.9, 39.4, 38.3, 38.0, 33.7, 33.3, 30.5, 27.4, 26.3, 24.8, 24.4, 11.6. HRMS (ESI+): *m/z* found 473.3258; $C_{29}H_{45}O_5$ $[M + H]^+$ requires 473.3262.

2.1.2.17. 16β-Hydroxymethyl-1,3,5(10)-estratrien-3,17-diol-16α-(ω-alkanoic acid *N*-doxorubicinamides (E-DOXs 8a-d). The appropriate compound **16a-d** (0.06 mmol) was dissolved in DMF (1.5 mL). DCC (13 mg, 0.07 mmol) and HOBt (9 mg, 0.07 mmol) were added and the mixture was stirred for 30 minutes at room temperature. In a separate flask doxorubicin hydrochloride (37 mg, 0.063 mmol), triethylamine (0.25 mmol) were dissolved in 3.5 mL of DMF and the mixture was added to the reaction mixture. The reaction mixture was stirred for 24 h, diluted with ethyl acetate, washed with a saturated solution of $NaHCO_3$, brine and water. The aqueous layer was extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified with flash chromatography using methylene chloride/methanol (9:1) as eluent to afford the desired conjugates in 15 to 32% yields.

2.1.2.18. 16β-Hydroxymethyl-1,3,5(10)-estratrien-3,17-diol-16α-butanoic acid *N*-doxorubicinamide (E-DOX 8a). Yield: 15%. IR (ν_{max}, cm^{-1}): 3300 (OH and NH), 1724 (C=O), 1614 and 1580 (C=O, quinone). ^1H NMR ($CDCl_3$ and CD_3OD, δ ppm): 7.97-7.90 (m, 1H, Ar, DOX1), 7.77-7.69 (m,1H, Ar, DOX2), 7.38-7.32 (m, 1H, Ar, DOX3), 7.09-6.78 (m, 2H, Ar and NH), 6.78-6.44 (m, 2H, Ar), 5.43 (s, 1H, DOX1'-CH), 5.17 (s, 1H, DOX7-CH), 4.71 (s, 3H, DOX14-CH_2 and DOX4'-CH), 4.18-4.02 (m, 5H, DOX4-OCH_3, DOX3'-CH and DOX5'-CH), 3.61-2.81 (several m, 5H, CH, CH_2 and DOX10-CH_2), 2.67 (m, 2H, CH_2), 2.27-1.00 (several m, 32H, 3xCH, 7xCH_2, 8xOH, DOX8-CH_2, DOX2'-CH_2 and DOX5'-CH_3), 0.76 (s, 3H, CH_3). HRMS (ESI+): *m/z* found 936.3768; $C_{50}H_{59}NNaO_{15}$ $[M + Na]^+$ requires 936.3782.

2.1.2.19. 16β-Hydroxymethyl-1,3,5(10)-estratrien-3,17-diol-16α-hexanoic acid *N*-doxorubicinamide (E-DOX 8b). Yield: 34% IR (ν_{max}, cm^{-1}): 3322 (OH and NH), 1724 (C=O), 1613 and 1581 (C=O, quinone). ^1H NMR ($CDCl_3$ and CD_3OD, δ ppm): 7.97-7.80 (m, 1H, Ar, DOX1), 7.75-7.60 (m,1H, Ar, DOX2), 7.38-7.32 (m, 1H, Ar, DOX3), 7.10-6.80 (m, 2H, Ar and OH), 6.72 (2H, NH and OH), 6.60-6.40 (m, 2H, Ar), 5.40 (s, 1H, DOX1'-CH), 5.16 (s, 1H, DOX7-CH), 4.68 (s, 2H, DOX14-CH_2), 4.18-3.90 (m, 5H, DOX4-OCH_3, DOX3'-CH and DOX5'-CH), 3.61-2.81 (several m, 5H, CH, CH_2 and DOX10-CH_2), 2.65 (m, 2H, CH_2), 2.27-

1.00 (several m, 34H, 3xCH, 9xCH_2, 6xOH, DOX8-CH_2, DOX2'-CH_2 and DOX5'-CH_3), 0.74 (s, 3H, CH_3). HRMS (ESI+): *m/z* found 964.4089; $C_{52}H_{63}NNaO_{15}$ $[M + Na]^+$ requires 964.4090.

2.1.2.20. 16β-Hydroxymethyl-1,3,5(10)-estratrien-3,17-diol-16α-octanoic acid *N*-doxorubicinamide (E-DOX 8c). Yield: 27%. IR (ν_{max}, cm^{-1}): 3350 (OH and NH), 1724 (C=O), 1614 and 1581 (C=O, quinone). ^1H NMR ($CDCl_3$ and CD_3OD, δ ppm): 8.01-7.97 (m, 1H, Ar, DOX1), 7.78-7.70 (m,1H, Ar, DOX2), 7.36-7.32 (m, 1H, Ar, DOX3), 7.08-7.04 (m, 1H, Ar), 6.78-6.50 (m, 2H, Ar), 6.31 (d, 1H, *J* = 8.60 Hz, NH), 5.44 (s, 1H, DOX1'-CH), 5.23 (s, 1H, DOX7-CH), 4.72 (s, 3H, DOX14-CH_2 and DOX4′-CH), 4.15-4.02 (m, 5H, DOX4-OCH_3, DOX3'-CH and DOX5'-CH), 3.73-2.95 (several m, 5H, CH, CH_2 and DOX10-CH_2), 2.73 (m, 2H, CH_2), 2.27-0.96 (several m, 40H, 3xCH, 11xCH_2, 8xOH, DOX8-CH_2, DOX2'-CH_2 and DOX5'-CH_3), 0.80 (s, 3H, CH_3). HRMS (ESI+): *m/z* found 992.4401; $C_{54}H_{67}NNaO_{15}$ $[M + Na]^+$ requires 992.4403.

2.1.2.21. 16β-Hydroxymethyl-1,3,5(10)-estratrien-3,17-diol-16α-decanoic acid *N*-doxorubicinamide (E-DOX 8d). Yield: 32%. IR (ν_{max}, cm^{-1}): 3346 (OH and NH), 1723 (C=O), 1613 and 1581 (C=O, quinone). ^1H NMR ($CDCl_3$ and CD_3OD, δ ppm): 7.98-7.94 (m, 1H, Ar, DOX1), 7.76-7.68 (m,1H, Ar, DOX2), 7.35-7.31 (m, 1H, Ar, DOX3), 7.05-7.01 (m, 1H, Ar), 6.78-6.48 (m, 2H, Ar), 6.36 (d, 1H, *J* = 8.20 Hz, NH), 5.42 (s, 1H, DOX1'-CH), 5.20 (s, 1H, DOX7-CH), 4.71 (s, 3H, DOX14-CH_2 and DOX4'-CH), 4.20-4.01 (m, 5H, DOX4-OCH_3, DOX3'-CH and DOX5'-CH), 3.72-2.91 (several m, 5H, CH, CH_2 and DOX10-CH_2), 2.71 (m, 2H, CH_2), 2.38-1.00 (several m, 44H, 3xCH, 13xCH_2, 8xOH, DOX8-CH_2, DOX2'-CH_2 and DOX5'-CH_3), 0.79 (s, 3H, CH_3). HRMS (ESI+): *m/z* found 1020.4704; $C_{56}H_{71}NNaO_{15}$ $[M + Na]^+$ requires 1020.4716.

2.2 Biology

2.2.1. Cell Lines Culture. HT-29 human colon carcinoma, MCF7 human breast carcinoma and MDA-MB-231 human breast carcinoma were purchased from the American Type Culture Collection (Manassas, VA). M21 human skin melanoma cells were provided by Dr. David Cheresh (University of California, San Diego School of Medicine, CA). Cells were cultured in DMEM medium containing sodium bicarbonate, high glucose concentration, glutamine, and

sodium pyruvate (Hyclone, Logan, UT) supplemented with 5% of calf serum. The cells were maintained at 37 °C in a moisture saturated atmosphere containing 5% CO_2.

2.2.2. Antiproliferative Activity Assay. The antiproliferative activity of E-DOXs (**8a**-**8d**) was assessed using the procedure described by the National Cancer Institute for its drug screening program with slight modifications [32]. Ninety-six-well microtiter plates were seeded with 75 μL of a tumor cell suspension (for HT-29, 5000 cells; M21, 3500 cells MCF7, 7500 cells; MDA-MB-231, 3000 cells) in the appropriate culture medium. Plates were incubated at 37 °C, 5% CO_2 for 24 h. Freshly solubilized drugs in DMSO were diluted in fresh medium, and 75 μL aliquots containing escalating concentrations (0.4 - 50 μM for E-DOXs and 0.004 – 1 μM for DOX) of the drug were added. Plates were incubated for 48 h. Plates containing attached cells were then stained with sulforhodamine B. Briefly, cells were fixed by addition of cold trichloroacetic acid to the wells (10% (w/v) final concentration), for 30 minutes at 4 °C. Plates were washed five times with tap water and dried. Sulforhodamine B solution (50 μL) at 0.1% (w/v) in 1% acetic acid was added to each well, and the plates were incubated for 15 minutes at room temperature. Unbound dye was removed by washing five times with 1% acetic acid. Bonded dye was solubilized in 10 mM Tris base, and the absorbance was read using a μQuant Universal microplate spectrophotometer (Biotek, Winooski, VT) at a wavelength between 530 and 565 nm according to color intensity. The experiments were performed thrice in triplicate. The IC_{50} assay was considered valid when the relative standard deviation was less than 10%.

2.2.3. ERα binding affinity. The ERα affinity assay was performed using the HitHunter^{TM} ER Enzyme Fragment Complementary (EFC) chemiluminescent detection Assay kit (DiscoveRx, Fremont, CA) according to manufacturer's protocol [33]. HitHunter^{TM} 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, escalating concentrations of E-DOX (**8a**-**d**) ranging from 0.098 to 215 nM were added to wells containing ES (Estrogen Steroid) Receptor + ED in a 96-well plate. Incubation provided competition for the ERα binding against labeled 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 chemiluminescent substrate were added to each well. Unbound ED-ES bind to EA to form an active enzyme, which subsequently hydrolyses the chemiluminescent substrate for EFC detection

by reading the emitted luminescence with the FluoStar Optima Instrument (BMG labtech, Ortenberg, Germany). The amount of free ED conjugate in the assay is proportional to the concentration of the estrogen analog bound to ERα [33]. A standard curve of 17β-estradiol was also assessed in parallel. All assays were done in duplicates.

3. Results and discussion

3.1. Design of E-DOX

In this study, DOX was conjugated to position 16α of the estradiol carrier via an alkylamide linking arm for site-specific treatment of ER^+ breast cancer in the aim to improve selectivity and efficacy of DOX towards cancer tumors together with reducing cardiac toxicity by preventing its accumulation in cardiac tissue. Estradiol was chosen as drug carrier because it exhibits proper physicochemical properties suitable for targeting the estrogen-dependent tissues. Moreover, we have already obtained promising results when using estradiol-linked platinum(II) complex (VP-128) that achieved a better tumor regression than cisplatin (CDDP) alone in estrogen receptor positive (ER+) xenograft model using MCF-7 cells on mice [26]. Finally, we could benefit in the case that our compounds are acting as ER agonists, that E-DOX could be sent directly to the nuclei of cells, that is one of the main target of DOX and in addition we could also benefit from the fact that proliferating cells are more sensitive to DOX than non-proliferating cells [34]. Estradiol was substituted at position 16α rather than at position 3 or 17 because the hydroxyl groups at these positions (3 and 17) are important for receptor binding affinity [18]. In addition, position 16α has already shown promising results when estrogen was conjugated to platinum complexes [22-26, 35-39]. Moreover, the length of the alkyl linking arm between estrogen substituted at position 16α and the platinum complexes is an important parameter for the biological activity and the affinity of the conjugate to the receptor. The biological activity and the affinity for the receptor are both optimal when the alkyl spacer has 6 to 10 carbon atoms in length [22, 23, 35, 38]. We therefore decided to investigate the same parameter on our novel E-DOXs by varying the length of the alkylamide linking arm between estrogen and DOX from 3 to 9 carbon atoms (E-DOX **8a**-**d**). Finally, we have conjugated estrogen to DOX using an amide bond because it is easily formed with DOX, which contains a single amino group precursor. Amide linkage on the carbohydrate moiety of doxorubicin is known to generally reduce the

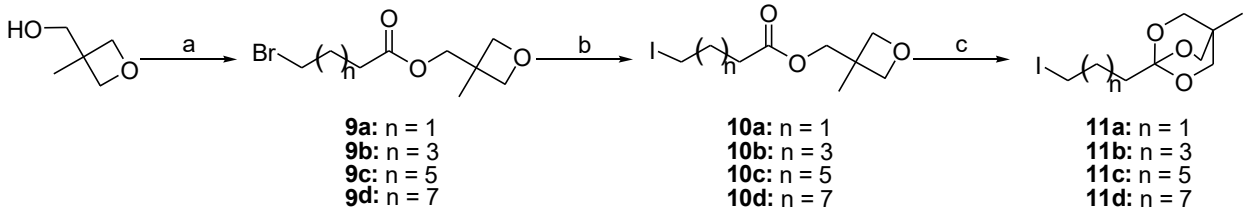
activity of the anthracycline [40]. Nonetheless, amide bond also resists to hydrolysis and confers structural rigidity to E-DOXs. Additionally, the amino group on DOX is located on the oxanyloxy moiety, which is remote from the quinone moiety which is essential for biological activity. In addition, the planar portion of DOX is important in the mechanism of cytotoxicity of DOX (generation of free radicals and intercalate into double-stranded DNA) [6]. All these factors led to the design and synthesis of the first E-DOXs at 16α-position of estrogen (E-DOX **8a-d**).

Iodoalkyl bridged orthoester derivatives (compounds **11a-d**) are the key intermediates to access the carboxylic acid estrogen substituted at position 16α and precursor to E-DOX **8a-d**. The orthoester protecting group is a rational choice to protect the acid group of the alkyl linking arms because it is inert under basic condition ($CsCO_3$) used to conjugate the linking arm to the position 16α of estrogen (compounds **14a**-d) and under nucleophilic condition ($LiBH_4$) used to reduce the carbonyl at the 17-position and the methyl ester at the 16β-position of the 1,3,5(10)-estratrien intermediates (compounds **15a-d**). Moreover, cleavage of the orthoester protective group to regenerate the carboxylic acid function is easily accomplished under mild acidic conditions, which is well tolerated by the estrogen moiety.

3.2. Chemistry

The synthesis of iodoalkyl bridged orthoester derivatives (compounds **11a-d**) are depicted in scheme 1. Iodoalkyl bridged orthoester derivatives bearing different chain length (compounds **11a-d**) were synthesized using modifications of the method previously described by Petroski et al. [29]. Briefly, compounds **9a-d** were prepared by esterification of the appropriate bromoalkanoyl chloride with 3-methyl-3-oxetanemethanol in a mixture of tetrahydrofuran with and pyridine. Then, the synthesis of compounds **10a-d** was achieved by treating the bromo-ester (**9a-d**) with NaI in acetone in presence of anhydrous Na_2SO_4. Finally, the key iodoalkyl bridged orthoester intermediates (compounds **11a-d**) were prepared by treating iodo-ester (**10a-d**) with boron trifluoride etherate in methylene chloride leading to rearrangement of the corresponding iodo-ester into bridged orthoesters. This synthetic route is easily performed in high yields and does not require tedious purification before the final step. Compounds **11a-d** were purified by flash chromatography using hexanes/acetone (7:3) as eluent that gave the desired orthoesters in

35 to 65% yields. The silica gel was neutralized with triethylamine prior to chromatography as the bridged orthoesters are labile under the mild acidic conditions encountered in typical silica gel chromatography [29].



9a: n = 1 **9b:** n = 3 **9c:** n = 5 **9d:** n = 7

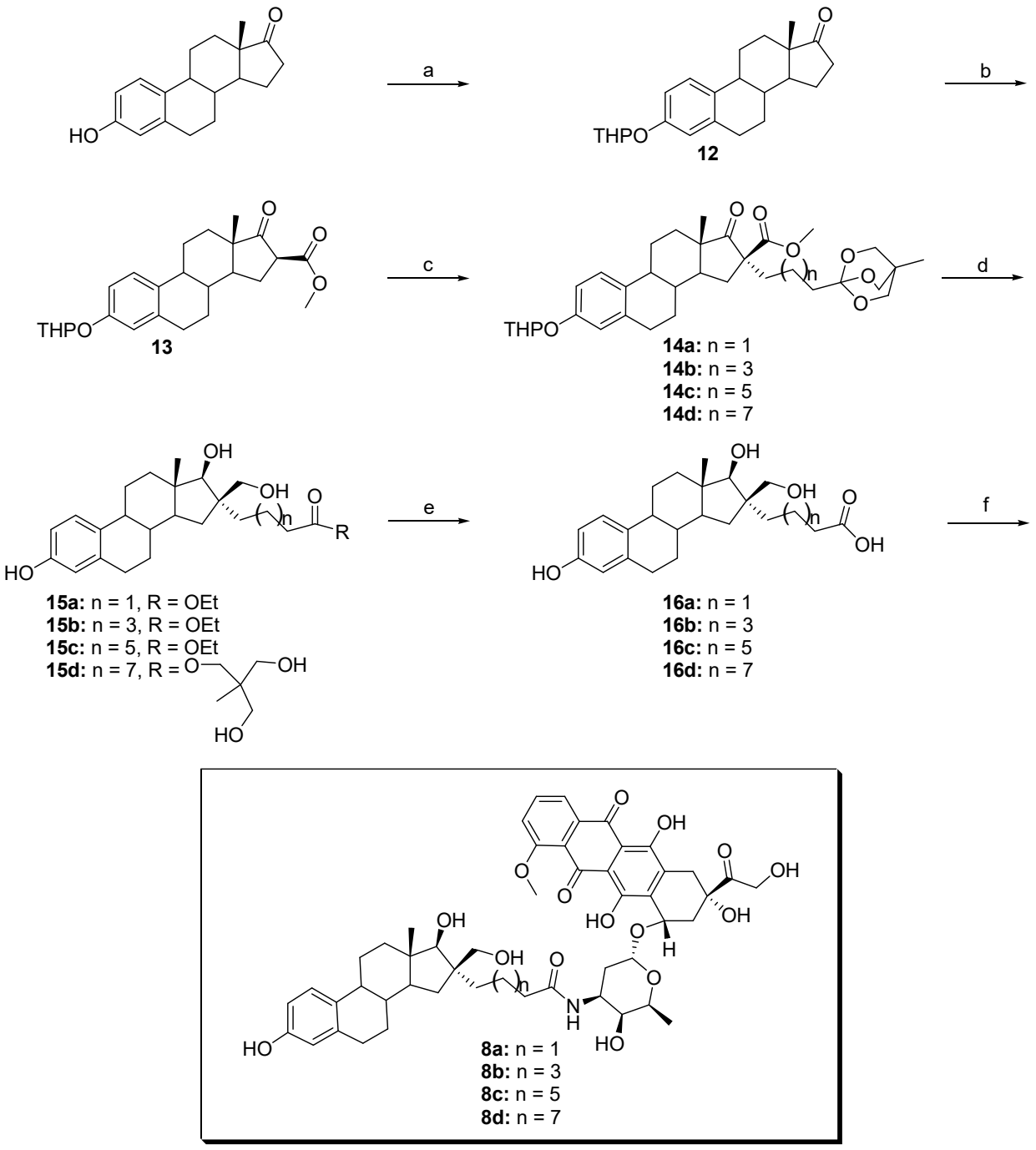
10a: n = 1 **10b:** n = 3 **10c:** n = 5 **10d:** n = 7

11a: n = 1 **11b:** n = 3 **11c:** n = 5 **11d:** n = 7

Scheme 1. Reagents and conditions: (a) THF, pyridine, ω-bromoalkanoyl chloride, 0 °C, 1h; (b) acetone, NaI, Na_2SO_4, rt, 5h; (c) CH_2Cl_2, $BF_3{\cdot}Et_2O$, 0 °C, 4h, then Et_3N.

The synthetic pathway to prepare E-DOXs **8a-d** is described in scheme 2. The conjugates have been synthesized in low to moderate yields using a seven-step procedure starting from estrone. The phenol group of estrone was protected using the method described by Descôteaux et al. [22] by treating estrone with 3,4-dihydro-2*H*-pyran in methylene chloride at room temperature to give the 3-tetrahydropyrannyloxy-1,3,5(10)-estratrien-17-one (**12**) derivative in quantitative yield. Afterwards, compound **12** was activated at position 16 with the formation of the 3-tetrahydropyrannyloxy-16α,β-(methoxycarbonyl)-1,3,5(10)-estratrien-17-one derivative (**13**) by reacting with potassium hydride (KH) and dimethyl carbonate in refluxing dry tetrahydrofuran in 90% yield. Subsequently, the alkyl bridged orthoester spacer were connected to the position 16α of compound **13** with using iodoalkyl orthoesters (**11a-d**) in refluxing tetrahydrofuran in the presence of Cs_2CO_3 to afford compounds **14a-d** in 48 to 55 % yields. The β-ketoester at position 16β and ketone group at position 17 of compounds **14a-c** were reduced into hydroxyl groups by treatment with $LiBH_4$ at 0 °C and at room temperature. Thereafter, [4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octan-1-yl] and tetrahydropyrannyloxy protecting groups were removed in presence of pyridinium *p*-toluenesulfonate in refluxing ethanol to obtain compounds **15a-c** in 19 to 41% yields. The β-ketoester at position 16β of compound **14d** was also reduced into the corresponding hydroxyl group by treating **14** with $LiBH_4$ at 0 °C and at room temperature but it was converted to **15d** by refluxing in methanol/water (40:1) in presence of pyridinium *p*-toluenesulfonate in 52% yield. Deprotection of the orthoester group is normally conducted under mild acidic conditions such as refluxing ethanol or methanol/water solution (40:1) containing

pyridinium *p*-toluenesulfonate. However, in our hand we did not obtained the expected free carboxylic acid function. We obtained instead the ester functions with deprotected hydroxyl group at position 3 of estrogen (compound **15a-d**). Thus, two deprotection steps were needed two obtain the carboxylic acid function. Compounds **15a-d** were hydrolyzed to the corresponding carboxylic acids **16a-d** by treatment with 1 M NaOH solution in tetrahydrofuran at room temperature. Finally the desired E-DOXs **8a-d** were synthesized in 15-32% yields by coupling DOX·HCl with compounds **16a-d** in presence of triethylamine, DCC and HOBt in DMF.



Scheme 2. Reagents and conditions: (a) DHP, PPTs, CH_2Cl_2, rt, 20 h; (b) dimethylcarbonate, KH, THF, reflux; (c) compounds **11a-d**, THF, Cs_2CO_3, reflux, 6 h; (d) i) $LiBH_4$, ether, 0 °C for 2 h, then 24 h at rt; ii) PPTs, EtOH, reflux, 8 h or PPTs, MeOH:H_2O (40:1), reflux, 5 h; (e) THF, NaOH 1M, rt, 12 h; (f) DOX·HCl, DCC, HOBt, DMF, Et_3N, rt, 24 h.

3.3. Biology

The antiproliferative activity of E-DOX derivatives **8a-d** was assessed on four human cancer cell lines, namely, HT-29 colon carcinoma, M21 skin melanoma, ER^+ MCF7 breast carcinoma and ER^- MDA-MB-231 breast carcinoma. These cell lines were selected as they are good representatives of tumor cells originating from the three embryonic germ layers and to assess the sensitivity and the selectivity of E-DOX on ER^+ cell line (MCF7) vs. other cell lines including ER^- cell lines such as MDA-MB-231. Cell growth inhibition was assessed according to the NCI/NIH Developmental Therapeutics Program [32]. The results are summarized in Table 1 and expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}).

Table 1. Evaluation of the antiproliferative activity of DOX and E-DOXs **8a-d** on HT-29, M21, MCF7 (ER^+) and MDA-MB-231 (ER^-) cancer cell lines.

	IC_{50} (μM)[b]			
Compds	**HT-29**	**M21**	**MCF7 (ER^+)**	**MDA-MB-231 (ER^-)**
8a	18	> 50	14	> 50
8b	> 50	> 50	> 50	> 50
8c	> 50	> 50	> 50	> 50
8d	> 50	> 50	> 50	> 50
DOX[a]	0.13	0.26	0.16	0.18

[a] DOX, doxorubicin. [b] IC_{50} is expressed as the concentration of drug inhibiting cell growth by 50%.

The IC_{50} of E-DOX **8a** on ER^+ MCF7 and HT-29 cells are 14 and 18 μM, respectively whereas it is mainly inactive (> 50 μM) on M21 and ER^- MDA-MB-231 cells. This result show that the selectivity of **8a** for ER^+ MCF7 vs. ER^- MDA-MB-231 is > 3.5 fold. E-DOX **8b-d** are mainly inactive (> 50 μM) on all cancer cell lines tested so far.

The ERα affinity assay was performed using the HitHunter™ ER (EFC) chemiluminescent detection Assay kit [33]. The results are summarized in Table 2 and expressed as the concentration of a drug inducing a response halfway between the baseline and maximum (EC_{50}).

Table 2. Affinity of E-DOXs **8a-d** for ERα.

Compds	EC_{50} (nM)[a]
17-β-Estradiol	2.2
8a	72
8b	79
8c	100
8d	NA[b]

[a] EC_{50} is expressed as the concentration of a drug inducing a response halfway between the baseline and maximum. [b] NA = No affinity at the concentration tested.

E-DOXs **8a-8c** exhibit affinity for the ERα in the nanomolar range whereas compound **8d** did not show affinity at the concentration tested (> 215 nM). Compound **8a** exhibited the highest affinity for the ER with an EC_{50} of 72 nM, followed by compound **8b** with an EC_{50} of 79 nM and compound **8c** with an EC_{50} of 100 nM. The affinity for ERα seems to increases with the chain length.

Interestingly, these results indicate that alkylamide linking arm between estrogen substituted at position 16α and DOX is also an important parameter for the antiproliferative activity and the affinity for the ERα as observed when using platinum complexes. However, the antiproliferative activity of platinum complexes increases with the lengthening of the alkyl spacer length and is optimal when the alkyl spacer bears 6 to 10 carbon atoms [22, 23, 35, 38] contrary to E-DOX, which require a shorter alkylamide spacer arm (< 5 carbon atoms) for maintain both significant antiproliferative activity and affinity with ERα. These results are underlying the fact that each element of the anticancer drug conjugates to estrogen (e.g., nature of the anticancer drugs, nature and the alkyl spacer chain length) plays an important role in the antiproliferative activity and its ability to target ER^+ breast cancers. Therefore, it is difficult to predict the antiproliferative activity of estrogen conjugates because each series of conjugate seems to behave differently towards ER^+ cells seemingly through the synergy of physicochemical properties variations, intrinsic activity and steric hindrance of the conjugates towards their affinity for the ERα. Moreover, as elegantly described by Wong et al. [41] it's extremely difficult to attempt extrapolation of *in vitro* results to *in vivo* activity. Consequently, our results give no evidenced that this new compound will target estrogen-dependent tissues in animal models. Nonetheless, the antiproliferative activity of compound **8a**, its selectivity toward ER^+ MCF7 cells and its

affinity for ERα are promising results and might be potentially useful for site-specific inhibition of the cell growth of ER^+ breast cancer cells and possibly be less cardiotoxic than DOX alone making this new compound a promising alternative conjugate to target ER^+ breast cancers.

4. Conclusion

In summary, we have designed, prepared and evaluated biologically the first DOX conjugates at the position 16α of estradiol (E-DOXs **8a-d**) instead 3- or 17-position, which is not ideal since these hydroxyl groups at this position are important for receptor binding affinity. DOX was conjugated using a 3 to 9 carbon atoms alkylamide linking arm. The novel E-DOX conjugates were prepared in low to moderate yields from estrone using a seven-step procedure. The key orthoester protecting group used to shield the carboxylic acid starting material was used to obain precursors of E-DOXs substituted at 16α-position. The antiproliferative activity of E-DOX **8a** on ER^+ MCF7 breast carcinoma and HT-29 human colon carcinoma cells are in the micromolar range whereas is mainly inactive on M21 and ER^- MDA-MB-231 cells showing a selectivity of this compound > 3.5 fold higher for ER^+ MCF7 than for ER^- MDA-MB-231. E-DOX **8b-d** derivatives are mainly inactive on all cancer cell lines tested. E-DOXs **8a-8c** exhibit affinity for the ERα in the nanomolar range (72-100 nM) whereas compound **8d** exhibited no activity at concentrations up to 215 nM. These results show that a short alkylamide linking arm (< 5 carbon atoms) is required to maintain both significant antiproliferative activity and affinity for ERα. In addition, this study suggests also that the nature of the anticancer drug conjugated to DOX together with the nature and the chain length of the alkyl spacer play important roles in the antiproliferative activity presumably through the synergy of physicochemical properties variations, intrinsic activity and steric hindrance of the conjugates towards their affinity for the ERα. Finally, antiproliferative activity, affinity for ERα and selectivity of E-DOX **8a** toward ER^+ MCF7 cells indicates that this compound might be promising useful for site specific treatment of ER^+ breast cancers and the design of more potent conjugates.

Acknowledgments

The authors wish to thank the Fonds de Recherche sur la Nature et les Technologies du Québec (FQRNT) for financial support. The FRSQ postgraduate fellowship for Sébastien Fortin is gratefully acknowledged.

References

[1] American Cancer Society, Cancer facts & figures 2011, Atlanta: American Cancer Society, 2011.

[2] Anderson WF, Katki HA, Rosenberg PS. Incidence of breast cancer in the United States: current and future trends. J Natl Cancer Inst 2011;103:1397-402.

[3] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin D. GLOBOCAN 2008 v12, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No 10, Lyon, France: International Agency for Research on Cancer, 2010 Available from: http://globocaniarcfr, [accessed on 14th, March 2012].

[4] World Health organiztion, Fact sheet on cancer no 297, Globocan 2008, IARC, February 2011, Available from: http://www.who.int/mediacentre/factsheets/fs297/en/, [accessed on 14th, March 2012].

[5] Weiss RB. The anthracyclines: will we ever find a better doxorubicin? Semin Oncol 1992;19:670-86.

[6] Keizer HG, Pinedo HM, Schuurhuis GJ, Joenje H. Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. Pharmacol Ther 1990;47:219-31.

[7] Dhingra K, Frye D, Newman RA, Walters R, Theriault R, Fraschini G, et al. Phase II clinical and pharmacological study of pirarubicin in combination with 5-fluorouracil and cyclophosphamide in metastatic breast cancer. Clin Cancer Res 1995;1:691-7.

[8] Johnson BA, Cheang MS, Goldenberg GJ. Comparison of adriamycin uptake in chick embryo heart and liver cells an murine L5178Y lymphoblasts in vitro: role of drug uptake in cardiotoxicity. Cancer Res 1986;46:218-23.

[9] Lampidis TJ, Johnson LV, Israel M. Effects of adriamycin on rat heart cells in culture: increased accumulation and nucleoli fragmentation in cardiac muscle v. non-muscle cells J Mol Cell Cardiol 1981;13:913-924.

[10] Kasiotis KM, Magiatis P, Pratsinis H, Skaltsounis A, Abadji V, Charalambous A, et al. Synthesis and biological evaluation of novel daunorubicin-estrogen conjugates. Steroids 2001;66:785-91.

[11] Khan SA, Rogers MA, Khurana KK, Meguid MM, Numann PJ. Estrogen receptor expression in benign breast epithelium and breast cancer risk. J Natl Cancer Inst 1998;90:37-42.

[12] Soderqvist G. Effects of sex steroids on proliferation in normal mammary tissue. Ann Med 1998;30:511-24.

[13] Lesniewska M, Miltyk W, Swiatecka J, Tomaszewska M, Kuzmicki M, Palka J, et al. Estrogen receptor β participate in the regulation of metabolizm of extracellular matrix in estrogen α negative breast cancer Folia. Histochem Cytobiol 2009;47:S107-12.

[14] Quivy J, Leclercq G, Deblaton M, Henrot P, Velings N, Norberg B, et al. Synthesis, structure and biological properties of Z-17α-(2-iodovinyl)-11β-chloromethyl estradiol-17β (Z-CMIV), a high affinity ligand for the characterization of estrogen receptor-positive tumors. J Steroid Biochem Mol Biol 1996;59:103-17.

[15] Devraj R, Barrett JF, Fernandez JA, Katzenellenbogen JA, Cushman M. Design, synthesis, and biological evaluation of ellipticine-estradiol conjugates. J Med Chem 1996;39:3367-74.

[16] Georgiadis M, Chondros KP, Haroutounian SA. Synthesis and biological studies of steroidal cis-platinum (II) complexes. Inorg Chim Acta 1987;138:249-52.

[17] DiZio JP, Carlson KE, Bannochie CJ, Welch MJ, Von Angerer E, Katzenellenbogen JA. Estrogen platinum-diamine complexes: preparation of a non-steroidal estrogen platinum-diamine complex labeled with platinum-191 and a study of its binding to the estrogen receptor in vitro and its tissue distribution in vivo. J Steroid Biochem Mol Biol 1992;42:363-73.

[18] Anstead GM, Carlson KE, Katzenellenbogen JA. The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site. Steroids 1997;62:268-303.

[19] Aliabadi HS, Brown JE. Synthesis, analysis and biological evaluation of novel steroidal estrogenic prodrugs for the treatment of breast cancer. Pharmaceut Biol 2004;42:367-73.

[20] Hartman NG, Angelucci F, Suarato A. United Kingdom Patent GB 2201419 A (Application Number 8704236); 1988.

[21] Hartman NG, Patterson LH, Workman P, Suarato A, Angelucci F. Doxorubicin-3'-NH-oestrone-17-oxime-ethyl-carbonyl, a doxorubicin-oestrone conjugate that does not redox cycle in rat liver microsomes. Biochem Pharmacol 1990;40:1164-7.

[22] Descoteaux C, Leblanc V, Belanger G, Parent S, Asselin E, Berube G. Improved synthesis of unique estradiol-linked platinum(II) complexes showing potent cytocidal activity and affinity for the estrogen receptor α and β. Steroids 2008;73:1077-89.

[23] Descoteaux C, Provencher-Mandeville J, Mathieu I, Perron V, Mandal SK, Asselin E, et al. Synthesis of 17β-estradiol platinum(II) complexes: biological evaluation on breast cancer cell lines. Bioorg Med Chem Lett 2003;13:3927-31.

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

[25] Provencher-Mandeville J, Descoteaux C, Mandal SK, Leblanc V, Asselin E, Berube G. Synthesis of 17β-estradiol-platinum(II) hybrid molecules showing cytotoxic activity on breast cancer cell lines. Bioorg Med Chem Lett 2008;18:2282-7.

[26] Van Themsche C, Parent S, Leblanc V, Descoteaux C, Simard AM, Berube G,et al. VP-128, a novel oestradiol-platinum(II) hybrid with selective anti-tumour activity towards hormone-dependent breast cancer cells in vivo. Endocr Relat Cancer 2009;16:1185-95.

[27] Perrin DD, Armarego WLF. Purification of laboratory chemicals. 3rd ed. Oxford: Pergamon Press; 1988.

[28] Still WC, Kahn M, Mitra A. Rapid chromatographic techniques for preparative separation with moderate resolution. J Org Chem 1978;43:2923–25.

[29] Petroski RJ. Improved preparation of halopropyl bridged carboxylic ortho esters. Org Commun 2008;1:48-53.

[30] Zhang SJ, Ge QF, Guo DW, Hu WX, Liu HZ. Synthesis and anticancer evaluation of α-lipoic acid derivatives. Bioorg Med Chem Lett 2010;20:3078-83.

[31] Harwood LM, Moody CJ. Experimental Organic Chemistry- Principles and Practice (Functional group interconversion, pp 440-538, Chapter 07) Blackwell Scientific Publications: Oxford, London; 1989.

[32] National Cancer Institute (NCI/NIH), Developmental therapeutics program human tumor cell line screen. Available from: http://dtp.nci.nih.gov/branches/btb/ivclsp.html [accessed on 14th, March 2012].

[33] Eglen RM. Enzyme fragment complementation: a flexible high throughput screening assay technology. Assay and drug dev technol 2002;1:97-104.

[34] Baral E, Auer G. In vitro effect of doxorubicin on non-proliferating and proliferating epithelial cells. Int J Radiat Oncol Biol Phys 1990;19:963-965.

[35] Perron V, Rabouin D, Asselin E, Parent S, C.-Gaudreault R, Berube, G. Synthesis of 17β-estradiol-linked platinum(II) complexes and their cytocidal activity on estrogen-dependent and -independent breast tumor cells. Bioorg Chem 2005;33:1-15.

[36] N'Soukpoe-Kossi CN, Descoteaux C, Asselin E, Tajmir-Riahi HA, Berube G. DNA interaction with novel antitumor estradiol-platinum(II) hybrid molecule: a comparative study with cisplatin drug DNA. Cell Biol 2008;27:101-7.

[37] N'Soukpoe-Kossi CN, Descoteaux C, Asselin E, Bariyanga J, Tajmir-Riahi H A, Berube G. Transfer RNA bindings to antitumor estradiol-platinum(II) hybrid and cisplatin. DNA Cell Biol 2008;27:337-43.

[38] Saha P, Descoteaux C, Brasseur K, Fortin S, Leblanc V, Parent S, et al. 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. Eur J Med Chem 2012;48:385-90.

[39] Froehlich E, Gupta A, Provencher-Mandeville J, Asselin E, Bariyanga J, Berube G, et al. Study of DNA Interactions with Steroidal and Nonsteroidal Estrogen-Platinum (II)-Based Anticancer Drugs. DNA Cell Biol 2009;28:31-9.

[40] Lau A, Bérubé G, Ford CHJ, Gallant M. Novel doxorubicin anticarcinoembryonic antigen antibody immunoconjugate activity *in vitro*. Bioorg Med Chem 1995;3:1305–12

[41] Wong CC, Cheng KW, Rigas B. Preclinical predictors of anticancer drug efficacy: critical assessment with emphasis on whether nanomolar potency should be required of candidate agents. J Pharmacol Exp Ther 2012;341:572-578.