Steroids 77 (2012) 1123–1132

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Novel 7α-alkoxy-17α-(4′-halophenylethynyl)estradiols as potential SPECT/PET imaging agents for estrogen receptor expressing tumours: Synthesis and binding affinity evaluation

Carina Neto [a], Maria Cristina Oliveira [a,*], Lurdes Gano [a], Fernanda Marques [a], Takumi Yasuda [b], Thies Thiemann [b,c], Torsten Kniess [d], Isabel Santos [a]

[a] Unidade de Ciências Químicas e Radiofarmacêuticas, Instituto Tecnológico e Nuclear, Instituto Superior Técnico, Universidade Técnica de Lisboa, Estrada Nacional 10, 2686-953 Sacavém, Portugal
[b] Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, 6-1, Kasuga-koh-en, Kasuga-shi, Fukuoka 816-8580, Japan
[c] Department of Chemistry, Faculty of Science, United Arab Emirates University, United Arab Emirates
[d] Institute of Radiopharmacy, Helmholtz-Zentrum Dresden-Rossendorf, e.V., POB 510119, D-01314 Dresden, Germany

ARTICLE INFO

Article history:
Received 9 February 2012
Received in revised form 7 May 2012
Accepted 16 May 2012
Available online 24 May 2012

Keywords:
Breast cancer
Estrogen receptor (ER)
Estradiol
Positron emission tomography (PET)
Single photon emission computerized tomography (SPECT)
Imaging

ABSTRACT

In order to develop potential radiolabelled probes for imaging estrogen receptor (ER) positive tumours, we have synthesized and characterized a series of novel 7α-alkoxy-17α-(4′-iodophenylethynyl)estra-1,3,5(10)-triene-3,17β-diols and 7α-alkoxy-17α-(4′-fluorophenylethynyl)estra-1,3,5(10)-triene-3,17β-diols. The fluoro-substituted compounds showed a higher ER binding affinity than the corresponding iodo-derivatives, where 7α-methoxy- and 17α-(4′-fluorophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol showed the highest ER binding affinities (RBA = 80.9% and 78.9%, respectively), among the halophenylethynyl compounds studied and should be further explored as potential PET biomarkers for imaging of ER expressing tumours.

1. Introduction

Malignant tumours overexpressing estrogen receptors (ER) have a great impact on human health and welfare, regardless of the progress in their early detection and treatment. The role of the interaction of estrogens and ER in breast cancer has been investigated extensively, since many of these carcinoma are ER+, although the exact processes remain far from clear, mechanistically. However, there is ample evidence for the involvement of estrogens and their receptors in the development of gynaecological and endocrine cancers [1–5]. Such cancers are often hormonally regulated and may be associated with high levels of progesterone and epidermal growth factor receptor (EGFR) expression. The biological effects of estrogens are exerted through two subtypes of ER (ERα and ERβ), ligand-transcription factors whose activity as inducer or repressor of gene transcription depends on the nature of the bound ligand. ER is a relevant tumour biomarker, and drugs based on ER-ligands are valuable tools for diagnosis and oriented cancer therapy. Hence the search for novel ligands to specifically target ER in tumours of different etiology continues to be a very important task. Both gamma- and positron-emitting estradiol derivatives have been developed to visualize ER in breast tumours through radionuclide guided imaging techniques such as single-photon emission computed tomography (SPECT) or positron emission tomography (PET), but few of these agents have reached the clinical stage [6–8].

During the last decades, several efforts have been made to synthesize estradiol derivatives that could be labelled with gamma-emitters. Several radioiodinated estradiol derivatives have been studied [6,9,10]. Among them, both isomers of 11β-methoxy-(17α,20*E*/*Z*)-[^{123}I]iodovinylestradiol have been clinically assessed, with the 20*Z* isomer giving the better images of ER-positive human breast tumors. While both primary and metastatic tumours were detected with good sensitivity and selectivity, extensive correlations between imaging and clinical outcome have not been provided so far [11–15]. Efforts have also been directed to the design of steroidal estrogen derivatives labelled with ^{99m}Tc, due to its favourable nuclear decay properties, low cost and availability

* Corresponding author. Tel.: +351 219946184; fax: +351 219550117.
E-mail address: cmelo@itn.pt (M.C. Oliveira).

0039-128X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.steroids.2012.05.004

[16–18]. While most of the emphasis has been placed on the imaging potential of these radioligands, the presence of Auger electrons from the decay of $^{123/125}$I has initiated interest in their radiotherapeutic applications, too. Studies have shown that estrogens labelled with Auger emitters can destroy ER+ cells, while sparing ER− cells [19–21]. Concerning PET-imaging, fluorine-18, a cyclotron-produced radionuclide, has been the most explored radiohalogen for *in vivo* imaging studies of estrogen receptors [22–26]. To date, 16α-[^{18}F]-estradiol (^{18}F-FES) has been the most successful candidate for *in vivo* imaging of estrogen receptor-positive tumours and is currently in phase II of a study to predict response to first line hormone therapy in women with ER(+) metastatic breast cancer [27–29]. 4-Fluoro-11β-methoxy-16α-[^{18}F]-fluoroestradiol (4FMFES) is a newly developed radiolabeled estradiol analogue for ER imaging with PET [30]. This tracer shows favourable biodistribution in small-animal experiments and achieves higher target-to-nontarget uptake ratios than does FES [31,32]. Subsequent studies of human biodistribution and dosimetry using serial whole-body PET/CT have highlighted the potential of 4FMFES for ER imaging in patients with breast cancer [33].

However, as many of the radioligands developed till date still exhibit low receptor binding affinities (RBA) and non ER-regulated tissue uptake, our team is continuing to work on the development of new radiohalogenated steroids as ER-based radiopharmaceuticals. In line with known data on structure–activity relationships between steroidal ligands and ER, based on the ER affinity of compounds investigated to-date, their cell binding studies and biological behaviour in animal models [34–36], the authors decided to study the effect of a heteroatom with lone electron pairs directly connected to C7α of the steroidal frame on the receptor binding affinity and the efficacy of the compound. Estradiol derivatives with a heteroatom or a heteroatom functionality linked to C7 in close proximity to the steroidal skeleton have been investigated previously in form of 7-cyano containing estradiols [37]. There, the hetero-functionality exerts an electron-withdrawing effect on its bonding partner. In the present study, alkoxy functional groups are directly linked to C7 of the estradiol frame, which would allow a (receptor) H···O interaction and then would increase the availability of the protons at C6 for close contacts with the receptor through the stabilisation of a partial positive charge at C7. Within this context it must be noted that, apart from 4FMFES mentioned above, a number of 11β-methoxy substituted estradiols have been found to be good ligands for ERα [14,38]. Previously, it has been shown that 11β- and 7α-substituted estradiols often exhibit similar trends in RBA within a series. It is known that C17α can accommodate larger substituents without an appreciable deterioration in RBA. S. Top, G. Jaouen et al. have shown that in the 11β-substituted estradiol series, larger substituents were reasonably tolerated by ERα and that 11β-methoxy substituted derivatives with larger 17α-substituents show better RBA than their at C11 non-substituted analogues [38]. It must be noted in this respect, though, that in the 7*a*-series a potential synergy between the 7α substituents and 17α-substituents in the binding of the ligand to the protein can be expected for relatively small substituents, only. As 17α-ethynylation often not only increases the RBA of the respective compounds, but also renders them more resistant towards rapid metabolisation, it was planned to attach the radioisotope on an ethynylated substituent at C17α of the target compound. As haloethynyl-substituted compounds are less stable *in vitro* and *in vivo*, a halophenylethynyl substituent was chosen [39]. Therefore, 7α-alkoxy-17α-(4′-halophenylethynyl)estra-1,3,5(10)-triene-3,17β-diols of type **14**/**15** became the synthetic targets (Scheme 1). A radiofluorinated analogue of **14c** has already been successfully prepared by Wüst et al. but no biological data was presented by the authors [40]. After the evaluation of the relative binding affinities of the individual compounds in non-radiolabelled form towards ERα, it was understood that subsequently only the most promising compound(s) would be resynthesized in radiolabelled form.

2. Experimental

2.1. General methods and equipment

Unless stated otherwise, all commercial reagents and solvents were of analytical grade and were used without further purification. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were recorded at room temperature in a Varian Unity 300 MHz spectrometer or a JEOL 270 MHz spectrometer. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR chemical shifts are reported relative to residual solvent signals or TMS as reference. Mass spectra were obtained in a Bruker HCT mass spectrometer or a Finnigan MAT 312 mass spectrometer. Chemical reactions were monitored by thin-layer chromatography (TLC) on Merck plates pre-coated with silica gel F_{254}. Column chromatography was performed on silica gel 60 (Merck).

In the receptor-binding affinity assay a human estrogen receptor-α (ERα, Pan Vera, Invitrogen Corporation, CA, USA) isolated from recombinant baculovirus-infected insect cells was used and maintained at −80 °C as indicated by the manufacturer. Tritiated estradiol, [2,4,6,7-^{3}H]E_2 (specific activity of 84.0 Ci/mmol) was obtained from Amersham, GE Healthcare, UK. Hydroxyapatite (HAP) was obtained from Calbiochem (San Diego, CA, USA).

2.2. Chemical synthesis

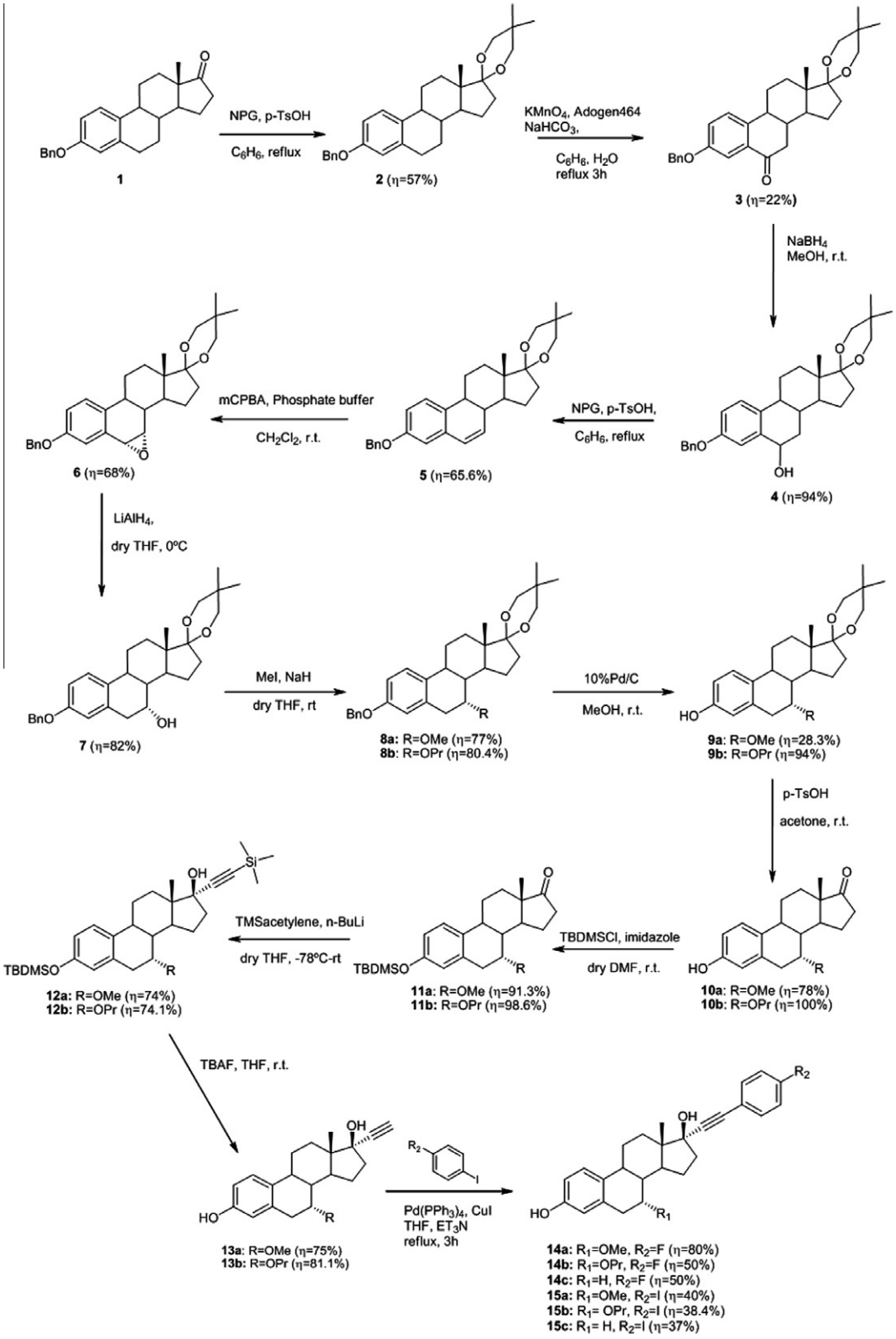
2.2.1. 3-*O*-Benzyl-estra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**2**)

A solution of 3-*O*-benzylestra-1,3,5(10)-trien-3-ol-17-one (**1**) (4.1 g, 11.4 mmol), neopentylglycol (NPG) (1.9 g, 18.3 mmol) and *p*-toluenesulfonic acid monohydrate (*p*-TsOH, 225 mg, 1.2 mmol) in benzene (78 mL) was stirred for 90 min under reflux with continuous azeotropic removal of water (Dean–Stark condenser). After cooling to room temperature, a saturated $NaHCO_3$ aqueous solution was added and the mixture was extracted with diethyl ether (3 × 50 mL). The organic phase was washed with water (50 mL), dried over $MgSO_4$ and evaporated. The residue was then purified by column chromatography on silica gel (diethyl ether:*n*-hexane:chloroform, 1:1:0.5) to give 3-*O*-benzyl-estra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**2**) as a colourless solid (2.8 g, 57%), mp. 78–81 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.71 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.3–1.94 (m, 11H), 2.27 (m, 2H), 2.82 (m, 2H), 3.37 (m, 2H), 3.47 (d, 1H, ^2J = 11.1 Hz), 3.65 (d, 1H, ^2J = 11.1 Hz), 5.01 (s, 2H), 6.69 (d, 1H, ^4J = 2.4 Hz), 6.75 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.4 Hz), 7.20 (d, 1H, ^3J = 8.4 Hz), 7.36 (m, 5H).

2.2.2. 3-*O*-Benzyl-6-oxoestra-1,3,5(10)-triene-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**3**)

A mixture of 3-*O*-benzyl-estra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**2**) (3.4 g, 7.5 mmol), $KMnO_4$ (4.9 g, 0.03 mol), Adogen 464 (0.6 g) and $NaHCO_3$ (231 mg, 2.75 mmol) in benzene (30 mL) and water (30 mL) was stirred for 3 h at reflux temperature. Thereafter, the reaction mixture was cooled to room temperature and the organic phase was extracted with diethyl ether (3 × 50 mL), washed with water (100 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. The resulting crude was purified by column chromatography on silica gel (diethyl ether:chloroform:hexane, 1:1:7) to give 3-*O*-benzyl-6-oxoestra-1,3,5(10)-triene-3-ol-17-one 17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**3**) as a colourless solid (770 mg, 22%), mp. 120–123 °C.



Scheme 1. Synthesis of 7α-methoxy-, 7α-propoxy- and 17α-(4′-halophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol derivatives (**14a**–**14c** and **15a**–**15c**).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.72 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.24–2.49 (m, 12H), 2.71 (dd, 1H, ^3J = 3.3 Hz, ^2J = 18.0 Hz), 3.37 (m, 2H), 3.46 (d, 1H, ^2J = 11.1 Hz), 3.65 (d, 1H, ^2J = 11.1 Hz), 5.08 (s, 2H), 7.16 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.4 Hz), 7.27–7.40 (m, 6H), 7.63 (d, 1H, ^4J = 2.4 Hz).

*2.2.3. 3-O-Benzyl-estra-1,3,5(10)-triene-3,6-diol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**4**)*

A solution of 3-*O*-benzyl-6-oxoestra-1,3,5(10)-triene-3-ol-17-one 17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**3**) (1.7 g, 3.7 mmol) in a mixture of methanol (24.7 mL) and diethyl ether (8.2 mL)

was cooled to 0 °C and $NaBH_4$ (0.84 g, 22.1 mmol) was slowly added. Next, the mixture was stirred for 1 h at room temperature. The solvent was removed *in vacuo* and water (30 mL) was slowly added to the residue. The resulting mixture was extracted with diethyl ether (3 × 30 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (n-hexane:diethyl ether:chloroform, 2:1:1) to give 3-*O*-benzyl-estra-1,3,5(10)-triene-3,6-diol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**4**) as a colourless solid (1.6 g, 94%), mp. 107–109 °C.

^{1}H NMR (300 MHz, $CDCl_3$,) δ: 0.71 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.24–1.73 (m, 9H), 1.89–1.95 (m, 1H), 2.22–2.33 (m, 4H), 3.37 (m, 2H), 3.46 (d, 1H, ^2J = 11.1 Hz), 3.65 (d, 1H, ^2J = 11.1 Hz), 4.81 (m, 1H), 5.04 (s, 2H), 6.84 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.4 Hz), 7.18–7.43 (m, 7H).

*2.2.4. 3-O-Benzyl-estra-1,3,5(10),6(7)-tetraen-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′- dioxane]) (**5**)*

To a solution of 3-*O*-benzyl-estra-1,3,5(10)-triene-3,6-diol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**4**) (1.6 g, 3.4 mmol) in benzene (39 mL) was added neopentylglycol (NPG, 1.55 g, 0.02 mol) and *p*-TsOH (87 mg, 0.46 mmol). The resulting mixture was heated for 4 h under reflux with continuous azeotropic removal of water (Dean–Stark condenser). The solution was cooled to room temperature, poured into a 5% aqueous Na_2CO_3 solution (30 mL), and the organic phase was separated. The organic phase was washed with water (30 mL) as well as with a saturated NaCl solution (30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane:ethyl acetate, 5:2) to afford 3-*O*-benzyl-estra-1,3,5(10),6(7)-tetraen-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**5**) as a colourless solid (1.01 g, 66%), mp. 122–124 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.79 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.27–2.43 (m, 11H), 3.44–3.53 (m, 3H), 3.73 (m, 1H), 5.08 (s, 2H), 6.04 (d, 1H, J = 9.6 Hz), 6.48 (d, 1H, J = 9.6 Hz), 6.79 (s, 1H), 6.85 (d, 1H, ^3J = 8.4 Hz), 7.22 (d, 1H, ^3J = 8.4 Hz), 7.36–7.50 (m, 5H).

*2.2.5. 3-O-Benzyl-6α,7α-epoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**6**)*

To a mixture of 3-*O*-benzyl-estra-1,3,5(10),6(7)-tetraen-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**5**) (1.0 g, 2.2 mmol) in a mixture of CH_2Cl_2 (44 mL) and phosphate buffer (0.1 M, pH 8) (44 mL) was slowly added *m*-chloroperbenzoic acid (380 mg, 2.2 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 5 h. Next, the organic layer was separated, washed with saturated sodium thiosulfate (40 mL) and water (40 mL), dried over anhydrous $MgSO_4$ and the solvent was removed. The residue was purified by column chromatography on silica gel (*n*-hexane:ethyl acetate, 5:1) to afford 3-*O*-benzyl-6α,7α-epoxyestra-1,3,5(10)-trien-3-ol-17-one 17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**6**) as a colourless solid (0.7 g, 68%), mp. 110–112 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ:0.75 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.51–1.66 (m, 5H), 1.73 (t, 1H), 1.96–2.09 (m, 3H), 2.26–2.45 (m, 3H), 3.40–3.54 (m, 4H), 3.69 (d, 1H, ^2J = 11.1 Hz), 3.82 (d, 1H, J = 4.2 Hz), 5.06 (s, 2H), 6.91 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.4 Hz), 7.05 (d, 1H, ^4J = 2.4 Hz), 7.17 (d, 1H, ^3J = 8.4 Hz), 7.32–7.46 (m, 5H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 13.5, 22.0, 22.5, 23.3, 24.1, 27.1, 28.8, 30.4, 36.0, 37.6, 44.7, 47.9, 53.8, 56.1, 70.1, 70.6, 72.7, 108.2, 109.9, 114.1, 116.4, 125.2, 127.4, 127.9, 128.6, 132.7, 133.6, 137.0, 156.9. HRMS: calculated for $C_{30}H_{37}O_4$: 461.2692; found: 461.2699.

*2.2.6. 3-O-Benzyl-estra-1,3,5(10)-trien-3,7α-diol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**7**)*

To a suspension of $LiAlH_4$ (116 mg, 3.1 mmol) in dry THF (5 mL) was added 3-*O*-benzyl-6α,7α-epoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**6**) (704 g, 1.53 mmol) at 0 °C and under an argon atmosphere, and the solution was stirred for 3 h at room temperature. Ethyl acetate (2 mL) was slowly added at 0 °C to quench the remaining $LiAlH_4$. Thereafter, aqueous NH_4Cl was added until a pH of 3 was reached, and the mixture was extracted with diethyl ether (3 × 20 mL). The organic phase was washed with water (20 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give 3-*O*-benzyl-estra-1,3,5(10)-trien-3,7α-diol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (7) as a colourless solid (578 mg, 82%), mp. 135–137 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.71 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.37–1.97 (m, 9H), 2.26–2.38 (m, 3H), 2.58 (m, 1H), 2.85–2.94 (m, 1H), 3.07–3.13 (m, 1H), 3.35–3.48 (m, 3H), 3.63 (d, 1H, ^2J = 11.1 Hz), 4.10 (m, 1H), 5.01 (s, 2H), 6.70 (d, 1H, ^4J = 2.4 Hz), 6.78 (d, 1H, ^3J = 8.4 Hz), 7.22–7.41 (m, 6H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 13.9, 22.0, 22.5, 26.1, 27.0, 29.3, 30.4, 35.5, 38.6, 42.6, 43.2, 47.4, 69.9, 70.6, 72.6, 108.4, 112.9, 115.5, 126.7, 127.4, 127.8, 128.5, 134.5, 156.9. HRMS: calculated for $C_{30}H_{39}O_4$: 463.2848; found: 463.2849.

*2.2.7. 3-O-Benzyl-7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**8a**)*

To a solution of 3-*O*-benzyl-estra-1,3,5(10)-trien-3,7α-diol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**7**) (578 mg, 1.25 mmol) in dry THF (20 mL) was added NaH (1.8 g, 75 mmol) and then methyl iodide (1.78 g, 0.78 mL, 0.01 mol). The reaction mixture was stirred at room temperature for 20 h. Thereafter, the mixture was poured into cold water and extracted with diethyl ether (3 × 30 mL). The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed. The residue was purified by column chromatography on silica gel (hexane:diethyl ether:chloroform, 5:1:1) to give 3-*O*-benzyl-7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**8a**) as a colourless solid (458 mg, 77%), mp. 124–126 °C.

^{1}H NMR (300 MHz, $CDCl_3$,) δ: 0.71 (s, 3H), 0.80 (s, 3H), 1.15 (s, 3H), 1.25–2.05 (m, 6H), 2.35 (m, 2H), 2.65 (m, 1H), 2.83 (m, 1H), 3.09 (d, 1H, J = 18 Hz), 3.34 (s, 3H), 3.36–3.53 (m, 4H), 3.62–3.75 (m, 3H), 5.01 (s, 2H), 6.70 (d, 1H, ^4J = 2.4 Hz), 6.77 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.4 Hz), 7.21–7.42 (m, 6H). HRMS: calculated for $C_{31}H_{40}O_4$: 477.3005; found: 477.3003.

*2.2.8. 3-O-Benzyl-7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**8b**)*

To a solution of 3-*O*-benzyl-estra-1,3,5(10)-trien-3,7α-diol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**7**) (300 mg, 0.65 mmol) in dry THF (10 mL) was added NaH (519 mg, 13.0 mmol) and n-propyl iodide (1.10 g, 6.5 mmol) and the reaction mixture was stirred at reflux temperature for 20 h. Then, the mixture was poured into cold water and extracted with diethyl ether (3 × 20 mL). The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed. The residue was purified by column chromatography on silica gel (hexane:diethyl ether:chloroform, 8:1:1) to give 3-*O*-benzyl-7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**8b**) as a colourless solid (263 mg, 80%), mp. 95–98 °C.

^{1}H NMR (270 MHz, $CDCl_3$) δ: 0.71 (s, 3H, CH_3), 0.79 (s, 3H, CH_3), 0.83 (t, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.31–1.72 (m, 9H), 1.89–2.13 (m, 2H), 2.23–2.38 (m, 2H), 2.65–2.89 (m, 2H), 3.03 (d, 1H, J = 17 Hz), 3.21–3.29 (m, 1H), 3.35–3.67 (m, 6H), 5.01 (s, 1H), 6.68 (d, 1H, ^4J = 2.4 Hz), 6.76 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.20–7.43 (m, 6H). HRMS: calculated for $C_{33}H_{44}O_4$: 504.3240; found: 504.3246.

*2.2.9. 7α-Methoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**9a**)*

To a solution of 3-*O*-benzyl-7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**8a**) (458 mg, 0.96 mmol) in THF (20 mL) was added 10w% Pd/C (25 mg). Hydrogen was bubbled into the reaction mixture, and hydrogenolysis was performed for 24 h at room temperature under 1 atmosphere of hydrogen. Then, the solution was filtered over Celite, washed with THF and the solvent removed. The residue was purified by column chromatography on silica gel (hexane:diethyl ether:chloroform, 4:1:1) to give 7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**9a**) as a colourless solid (105 mg, 28%), mp. 170–171 °C.

^{1}H (300 MHz, $CDCl_3$) δ: 0.72 (s, 3H, CH_3); 0.81 (s, 3H, CH_3); 1.15 (s, 3H, CH_3); 1.20–1.55 (m, 3H); 1.6–2.07 (m, 2H); 2.28–2.87 (m, 2H), 2.62–2.64 (m, 1H), 2.74–2.81 (m, 1H), 3.00–3.07 (m, 1H), 3.34 (s, 3H, OCH_3), 3.37–3.77 (m, 8H), 5.26 (s, 1H), 6.51 (dd, 1H, ^4J = 2.4 Hz), 6.59 (d, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.15 (d, 1H, ^3J = 8.4 Hz). HRMS: calculated for $C_{24}H_{35}O_4$: 387.2535; found: 387.2538.

*2.2.10. 7α-Propoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**9b**)*

To a solution of 3-*O*-benzyl-7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**8b**) (263 mg, 0.52 mmol) in THF (5 mL) was added 10w% Pd/C (27 mg). Hydrogen was bubbled into the reaction mixture, and hydrogenolysis was performed for 24 h at room temperature under 1 atmosphere of hydrogen. Then, the solution was filtered and the solvent removed. The residue was purified by column chromatography on silica gel (hexane:ether:chloroform, 4:1:1) to 33give 7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**9b**) as a colourless solid (203 mg, 94%), mp. 167–169 °C.

^{1}H NMR (270 MHz, $CDCl_3$) δ: 0.73 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 0.87 (t, 3H, CH_3), 1.16 (s, 3H, CH_3), 1.23–1.74 (m, 8H), 1.90–2.14 (m, 2H), 2.28–2.40 (m, 2H), 2.66–2.82 (m, 2H), 2.98–3.05 (m, 1H), 3.22–3.28 (m, 1H), 3.37–3.69 (m, 6H), 4.51 (s, 1H), 6.54 (d, 1H, ^4J = 2.4 Hz), 6.62 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.4 Hz), 7.18 (d, 1H, ^3J = 8.4 Hz). HRMS: calculated for $C_{26}H_{38}O_4$: 414.2770; found: 414.2767.

*2.2.11. 7α-Methoxyestra-1,3,5(10)-trien-3-ol-17-one (**10a**)*

A solution of 7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**9a**) (410 mg, 1.1 mmol) and *p*-TsOH (83 mg, 0.43 mmol) in acetone (83 mL) was stirred at room temperature for 15 h. Then, the reaction mixture was poured into an aqueous solution of Na_2CO_3 (80 mL). The mixture was extracted with diethyl ether (3 × 50 mL) and the organic phase was washed with water (100 mL), dried over anhydrous $MgSO_4$ and the solvent was removed. The residue was purified by column chromatography on silica gel (hexane:diethyl ether:chloroform, 4:1:1) to give 7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one (**10a**) as a colourless solid (242 mg, 78%), mp. 205–207 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (s, 3H, CH_3), 1.24–1.68 (m, 6H), 1.83–2.24 (m, 3H), 2.39–2.54 (m, 2H), 2.63–2.69 (m, 1H), 2.79–2.86 (m, 1H), 3.11 (d, 1H), 3.38 (s, 3H, OCH_3), 3.66 (m, 1H), 6.57 (d, 1H, ^4J = 2.4 Hz), 6.63 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.13 (d, 1H, ^3J = 8.4 Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 13.6, 21.4, 26.0, 31.4, 32.74, 35.8, 36.3, 42.0, 45.8, 47.8, 56.8, 74.2, 113.2, 115.8, 126.7, 131.7, 134.9, 153.6, 220.9. HRMS: calculated for $C_{19}H_{24}O_3$: 300.1725; found: 300.1725.

*2.2.12. 7α-Propoxyestra-1,3,5(10)-trien-3-ol-17-one (**10b**)*

A solution of 7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one-17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) (**9b**) (283 mg, 0.68 mmol) and *p*-TsOH (51 mg, 0.27 mmol) in acetone (51 mL) was stirred at room temperature for 15 h. Then, the reaction mixture was poured into an aqueous solution of Na_2CO_3 (50 mL). The mixture was extracted with diethyl ether (3 × 50 mL) and the organic phase was washed with water (100 mL), dried over anhydrous $MgSO_4$ and the solvent was removed. The residue was purified by column chromatography on silica gel (hexane:diethyl ether:chloroform, 4:1:1) to give 7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one (**10b**) as a colourless solid (227 mg, quant.), mp. 210–212 °C.

^{1}H NMR (270 MHz, $CDCl_3$) δ: 0.83–0.89 (s, t, 6H, 2 CH_3), 1.51–2.20 (m, 10H), 2.40–2.55 (m, 2H), 2.73–2.86 (m, 2H), 3.05–3.12 (m, 1H), 3.23–3.31(m, 1H), 3.57–3.65 (m, 1H), 3.76 (m, 1H), 4.73 (s, 1H), 6.57 (d, 1H, ^3J = 2.4 Hz), 6.63–6.66 (dd, 1H, ^4J = 8.4 Hz, ^3J = 2.4 Hz), 7.16 (d, 1H, ^4J = 8.4 Hz) ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 10.7, 13.6, 21.4, 23.3, 25.9, 31.4, 33.6, 35.8, 36.2, 42.1, 45.8, 47.8, 70.7, 72.4, 113.0, 115.8, 126.6, 132.0, 135.4, 153.5, 221.0. HRMS: calculated for $C_{21}H_{28}O_3$: 328.2038; found: 328.2036.

*2.2.13. 3-O-(tert-Butyldimethylsilyl)-7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one (**11a**)*

A solution of *tert*-butyldimethylsilyl chloride (146 mg, 0.97 mmol) solution in anhydrous DMF (1.7 mL) was added to a solution of 7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one (**10a**) (242 mg, 0.80 mmol) and imidazole (137 mg, 2.02 mmol) in anhydrous DMF (3 mL) at 0 °C. The mixture was stirred for 16 h at room temperature. Then, the solvent was removed and the residue was purified by column chromatography on silica gel (hexane:diethyl ether:chloroform, 4:1:1) to afford 3-*O*-(*tert*-butyldimethylsilyl)-7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one (**11a**) as a slowly crystallizing, colourless solid (305 mg, 91%).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.05 (s, 6H, 2 CH_3), 0.87 (s, 3H, CH_3), 0.96 (s, 9H, Bu^t), 1.45–1.68 (m, 5H), 1.86–2.20 (m, 3H), 2.40–2.53 (m, 2H), 2.65 (m, 1H), 2.78–2.85 (m, 1H), 3.11 (d, 1H, ^2J = 18 Hz), 3.36 (s, 3H), 3.66 (m, 1H), 6.56 (d, 1H, ^4J = 2.4 Hz), 6.61 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.12 (d, 1H, ^3J = 8.4 Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 4.4, 13.6, 18.2, 21.4, 25.7, 26.0, 31.5, 32.7, 35.8, 36.4, 42.0, 45.8, 47.8, 56.7, 74.3, 117.6, 120.5, 126.3, 132.0, 134.6, 153.6, 220.7. HRMS: calculated for $C_{25}H_{38}O_3Si$: 414.2590; found: 414.2594.

*2.2.14. 3-O-(tert-Butyldimethylsilyl)-7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one (**11b**)*

A solution of *tert*-butyldimethylsilyl chloride (109 mg, 0.72 mmol) in anhydrous DMF (2 mL) was added to a solution of 7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one (**10b**) (197 mg, 0.60 mmol) and imidazole (102 mg, 1.5 mmol) in anhydrous DMF (3 mL) at 0 °C. The mixture was stirred for 10 h at room temperature. Then, the solvent was evaporated and the residue was purified by column chromatography on silica gel to afford 3-*O*-(*tert*-butyldimethylsilyl)-7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one (**11b**) as a slowly crystallizing, colourless solid (262 mg, 99%).

^{1}H NMR (270 MHz, $CDCl_3$) δ: 0.19 (s, 6H, $2CH_3$), 0.83–0.88 (s, t, 6H, 2 CH_3) 0.98 (s, 9H, But), 1.46–1.68 (m, 8H), 1.89–2.20 (m, 4H), 2.41–2.55(m, 2H), 2.72–2.86 (m, 2H), 3.04–3.11 (m, 1H), 3.23–3.32 (m, 1H), 3.57–3.65 (m, 1H), 3.75 (m, 1H), 6.56 (d, 1H, ^4J = 2.4 Hz), 6.61–6.65 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.20 (d, 1H, ^3J = 8.4 Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 4.4, 10.7, 13.6, 18.2, 23.3, 25.7, 25.9, 31.5, 33.6, 35.8, 36.2, 42.1, 45.8, 47.8, 70.6, 72.4, 117.5, 120.5, 126.2, 132.4, 135.0, 153.5, 220.9. HRMS: calculated for $C_{27}H_{42}O_3Si$: 422.2903; found: 442.2904.

*2.2.15. 3-O-(tert-Butyldimethylsilyl)-7α-methoxy-17α-trimethylsilylethynylestra-1,3,5(10)-trien-3,17β-diol (**12a**)*

To a solution of trimethylsilylacetylene (216 mg, 2.20 mmol) in dry THF (8 mL) was added under nitrogen at −78 °C *n*-butyl lithium (solution in *n*-hexane, 1.58 M, 1.1 mL), and the resulting mixture was stirred at −78 °C for 30 min and for further 30 min at 0 °C.

Then, the reaction mixture was re-cooled to −78 °C and a solution of 3-*O*-(*tert*-butyldimethylsilyl)-7α-methoxyestra-1,3,5(10)-trien-3-ol-17-one (**11a**) (305 mg, 0.74 mmol) in dry THF (8 mL) was added. The mixture was allowed to warm slowly to room temperature and was stirred for 15 h. Then, a saturated NH_4Cl solution (2 M, 40 mL) was added and the mixture was extracted with diethyl ether (3 × 50 mL). The organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed. The residue was purified by column chromatography on silica gel (hexane:diethyl ether:chloroform, 4:1:1) to give 3-*O*-(*tert*-butyldimethylsilyl)-7α-methoxy-17α-trimethylsilylethynylestra-1,3,5(10)-trien-3,17*β*-*diol* (**12a**) as a slowly crystallizing, colourless solid (280 mg, 74%).

^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 0.16 (2s, 15H, 5 CH_3), 0.83 (s, 3H, CH_3), 0.95 (s, 9H, Bu^t), 1.40–2.81 (m, 13H), 3.20 (m, 1H), 3.36 (s, 3H, OCH_3), 3.42 (m, 1H), 6.53 (d, 1H, ^4J = 2.4 Hz), 6.60 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.14 (d, 1H, ^3J = 8.4 Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$) *δ*: −4.4, 0.0, 12.5, 18.2, 22.7, 25.7, 26.5, 32.7, 33.4, 36.2, 38.9, 43.1, 44.9, 47.3, 57.2, 75.1, 80.1, 90.3, 109.6, 117.6, 120.6, 126.5, 132.4, 134.8, 153.5. HRMS: calculated for $C_{30}H_{48}O_3Si_2$: 512.3142; found: 512.3143.

2.2.16. 3-O-(tert-Butyldimethylsilyl)-7α-propoxy-17α-trimethylsilylethynylestra-1,3,5(10)-trien-3,17β-diol (*12b*)

To a solution of trimethylsilylacetylene (162 mg, 1.65 mmol) in dry THF (6 mL) was added at −78 °C lithium diisopropylamide (solution in THF/ethylbenzene/heptane, 2 M, 0.9 mL, 1.8 mmol), and the resulting mixture was stirred for 30 min at −78 °C and for an additional 30 min at 0 °C. Then, the reaction mixture was re-cooled to −78 °C, and a solution of 3-*O*-(*tert*-butyldimethylsilyl)-7α-propoxyestra-1,3,5(10)-trien-3-ol-17-one (**11b**) (243 mg, 0.55 mmol) in dry THF (6 mL) was added. The mixture was allowed to warm overnight (15 h). Then, NH_4Cl (2 M, 30 mL) was added, and the mixture was extracted with ether (3 × 30 mL). The organic phase was dried over anhydrous $MgSO_4$ and the solvent evaporated. The residue was purified by column chromatography on silica gel (hexane:diethyl ether:chloroform, 4:1:1) to give 3-*O*-(*tert*-butyldimethylsilyl)-7α-propoxy-17α-trimethylsilylethynylestra-1,3,5(10)-trien-3,17β-diol (**12b**) as a slowly crystallizing, colourless solid (220 mg, 74%).

^{1}H NMR (270 MHz, $CDCl_3$) *δ*: 0.16–0.18 (2s, 15H, 5 CH_3), 0.97 (s, 3H, CH_3), 1.40–2.38 (m, 25H), 2.67–2.83 (m, 2H), 2.97–3.04 (m, 1H), 3.18–3.24 (m, 1H), 3.61–3.64 (m, 2H), 6.55 (d, 1H, ^4J = 2.4 Hz), 6.61 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.16 (d, 1H, ^3J = 8.4 Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$) *δ*: −4.3, 0.0, 11.0, 12.4, 22.6, 23.4, 25.8, 26.3, 32.6, 33.9, 35.9, 38.9, 43.1, 44.6, 47.1, 70.9, 73.3, 80.1, 90.0, 109.3, 117.4, 120.4, 120.5, 126.2, 132.8, 135.2, 153.3. HRMS: calculated for $C_{31}H_{52}O_3Si_2$: 540.3455; found: 540.3451.

2.2.17. 7α-Methoxy-17α-ethynylestra-1,3,5(10)-trien-3,17β-diol (*13a*)

To a solution of 3-*O*-(*tert*-butyldimethylsilyl)-7α-methoxy-17α-trimethylsilylethynylestra-1,3,5(10)-trien-3,17*β*-*diol* (**12a**) (280 mg, 0.54 mmol) in THF (10 mL) was added at −10 °C tetrabutylammonium fluoride (TBAF, 565 mg, 2.16 mmol). The reaction mixture was stirred at room temperature for 2 h. Then, diethyl ether (30 mL) was added, and the organic phase was extracted. The organic phase was dried over anhydrous $MgSO_4$ and the solvent evaporated. The residue was purified by column chromatography on silica gel (hexane:ether:chloroform, 4:1:1) to give 7α-methoxy-17α-ethynylestra-1,3,5(10)-trien-3,17*β*-*diol* (**13a**) as a colourless solid (133 mg, 75%), mp. 193–196 °C.

^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 0.86 (s, 3H, CH_3), 1.27–2.53 (m, 10H), 2.61 (s, 1H), 2.59–2.70 (m, 1H), 2.76 (m, 1H); 2.85 (m, 1H), 3.07 (d, 1H, ^2J = 17.5 Hz), 3.36 (s, 3H, OCH_3), 3.53 (m, 1H), 4.62–4.66 (m, 1H), 6.56 (d, 1H, ^4J = 2.4 Hz), 6.64 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.17 (d, 1H, ^3J = 8.4 Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$) *δ*:12.4, 22.7, 26.6, 32.6, 33.2, 35.9, 38.9, 43.2, 44.8, 47.2, 56.1, 74.2, 74.9, 79.9, 87.5, 113.2, 115.9, 126.8, 132.2, 135.2, 153.4. HRMS: calculated for $C_{21}H_{26}O_3$: 326.1882; found: 326.1879.

2.2.18. 7α-Propoxy-17α-ethynylestra-1,3,5(10)-trien-3,17β-diol (*13b*)

To a solution of 3-*O*-(*tert*-butyldimethylsilyl)-7α-propoxy-17α-trimethylsilylethynylestra-1,3,5(10)-trien-3,17β-diol (**12b**) (216 mg, 0.40 mmol) in THF (10 mL) was added at −10 °C tetrabutylammonium fluoride (TBAF, 418 mg, 1.6 mmol). The reaction mixture was stirred at room temperature for 2 h. Then, diethyl ether (30 mL) was added, and the organic phase was extracted. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography on silica gel (hexane:diethyl ether:chloroform, 4:1:1) to give 7α-propoxy-17α-ethynylestra-1,3,5(10)-trien-3,17β-diol (**13b**) as a colourless solid (115 mg, 81%), mp. 196–198 °C.

^{1}H NMR (270 MHz, $CDCl_3$) *δ*: 0.86 (s, 3H, CH_3), 0.88 (t, 3H, CH_3), 1.18–2.50 (m, 13H), 2.59 (s, 1H), 2.69–2.84 (m, 2H), 2.99–3.05 (m, 1H), 3.21–3.29 (m, 1H), 3.56–3.64 (m, 2H), 4.63 (s, 1H), 6.55 (d, 1H, ^4J = 2.4 Hz), 6.61–6.64 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.17 (d, 1H, ^3J = 8.4 Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$) *δ*: 10.7, 12.4, 22.6, 23.3, 26.4, 32.5, 34.0, 35.7, 38.9, 43.1, 44.6, 47.0, 70.9, 73.0, 74.0, 79.9, 87.2, 93.6, 112.9, 115.7, 126.6, 132.5, 135.6, 153.3. HRMS: calculated for $C_{23}H_{30}O_3$: 354.2195; found: 354.2193.

2.2.19. 7α-Methoxy-17α-(4′-fluorophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (*14a*)

A solution of 7α-methoxy-17α-ethynylestra-1,3,5(10)-trien-3,17β-diol (**13a**) (80 mg, 0.24 mmol), 1,4-fluoro-iodobenzene (27 μL, 0.24 mmol), copper (I) iodide (CuI, 1.8 mg, 9.6×10^{-3} mmol) and $Pd[PPh_3]_4$ (5.54 mg, 4.8×10^{-3} mmol) was refluxed in a mixture of dry THF (5 mL) and dry triethylamine (5 mL) for 3 h under a nitrogen atmosphere. After cooling to room temperature, the solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate:n-hexane, 1:1) to give 7α-methoxy-17α-(4′-fluorophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (**14a**) as a light brown solid (82 mg, 80%), mp. 109–110 °C.

^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 0.89 (s, 3H, CH_3), 1.27–2.24 (m, 11H), 2.37–2.47 (m, 1H), 2.58–2.66 (m, 1H), 2.74–2.81 (m, 2H), 3.03 (d, 1H, ^2J = 17.7 Hz), 3.34 (s, 3H, OCH_3), 3.54 (m, 1H), 5.65 (s, 1H), 6.53 (d, 1H, ^4J = 2.4 Hz), 6.60 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 6.98 (m, 2H), 7.13 (d, 1H, ^3J = 8.4 Hz), 7.41 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 13.0, 23.0, 27.0, 33.2, 33.4, 36.3, 39.2, 43.4, 45.4, 48.0, 57.3, 75.3, 80.6, 85.3, 113.4, 115.7 (d, J_{CF} = 22.0 Hz), 116.1, 119.3, 127.0, 131.9, 133.7, 133.8 (d, J_{CF} = 9.3 Hz), 135.3, 154.0, 162.7 (d, J_{CF} = 247.7 Hz). ^{19}F-RMN (282 MHz, $CDCl_3$) *δ*: −111.46 (quint.). ESI/MS: calculated for $C_{27}H_{29}O_3F$: 419.2 $[M-H]^-$; found: 419.1 $[M-H]^-$. $C_{27}H_{29}O_3F$: calcd. C, 77.11; H, 6.95. Found. C, 77.20; H, 6.64.

2.2.20. 7α-Propoxy-17α-(4′-fluorophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (*14b*)

A solution of 7α-propoxy-17α-ethynylestra-1,3,5(10)-trien-3,17β-diol (**13b**) (40 mg, 0.11 mmol), 1,4-fluoro-iodobenzene (12.7 μL, 0.11 mmol), CuI (1.0 mg, 4.4×10^{-3} mmol) and $Pd[PPh_3]_4$ (5.54 mg, 4.8×10^{-3} mmol) was refluxed in a mixture of dry THF (5 mL) and dry triethylamine (5 mL) for 3 h under nitrogen atmosphere. After cooling to room temperature, the solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate:*n*-hexane, 1:1) to give 7α-methoxy-17α-(4′-fluorophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (**14b**) as a light brown solid (25 mg, 50%), mp. 164–166 °C.

^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 0.82 (s, 3H, CH_3), 0.86 (t, 3H, CH_3), 1.22–2.44 (m, 13H), 2.58 (m, 1H), 2.69–2.84 (m, 2H), 3.00 (m, 1H),

3.21–3.26 (m, 1H), 3.58–3.65 (m, 2H), 5.06 (s, 1H), 6.53 (d, 1H, ^4J = 2.4 Hz), 6.61 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 6.97 (m, 2H), 7.15 (d, 1H, ^3J = 8.4 Hz), 7.38 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 9.7, 11.4, 21.6, 22.3, 25.4, 31.8, 33.0, 34.8, 37.9, 42.2, 43.8, 46.5, 65.9, 72.2, 79.4, 84.0, 91.3, 111.9, 114.5 (d, J_{CF} = 22 Hz), 114.8, 118.1, 125.6, 131.2, 132.4 (d, J_{CF} = 8.3 Hz), 132.4, 134.5, 152.4, 161.4 (d, J_{CF} = 247.7 Hz). ^{19}F-RMN (282 MHz $CDCl_3$) δ: −111.44 (quint.). ESI/MS: calculated for $C_{29}H_{33}O_3F$: 449.2 $[M+H]^+$; found: 449.4 $[M+H]^+$. $C_{29}H_{33}O_3F$: calcd. C, 77.65; H, 7.42. Found. C, 77.73; H, 7.71.

2.2.21. 17α-(4′Fluorophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (**14c**)

Preparation of **14c** followed the general literature procedure [35]. A solution of 17α-ethynylestradiol (**13c**) (100 mg, 0.34 mmol), 1,4-fluoro-iodobenzene (40μL, 0.34 mmol), CuI (2.7 mg, 1.4×10^{-2} mmol) and $Pd[PPh_3]_4$ (7.9 mg, 6.8×10^{-3} mmol) was refluxed in a mixture of dry THF (5 mL) and dry triethylamine (5 mL) for 3 h under nitrogen atmosphere. After cooling to room temperature, the solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate:*n*-hexane, 1:1) to give 17α-(4′-fluorophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (**14c**) as a light brown solid (66 mg, 50%), mp. 85–88 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.92 (s, 3H, CH_3), 1.22–2.40 (m, 12H), 2.88 (m, 3H), 6.56 (d, 1H, ^4J = 2.4 Hz), 6.63 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 6.99 (m, 2H), 7.14 (d, 1H, ^3J = 8.4 Hz), 7.41 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.0, 23.0, 26.6, 27.4, 29.9, 33.0, 39.2, 39.6, 43.7, 47.4, 49.7, 74.4, 80.2, 87.7, 113.0, 115.5, 115.8 (d, J_{CF} = 22 Hz), 126.8, 132.7, 133.8 (d, J_{CF} = 8.2 Hz), 138.5, 153.6, 162.7 (d, J_{CF} = 248.3 Hz). ^{19}F-RMN (282 MHz $CDCl_3$) δ: −111.41 (quint.). ESI/MS: calculated for $C_{26}H_{27}O_2F$: 391.2 $[M+H]^+$; found: 391.2 $[M+H]^+$. $C_{26}H_{27}O_2F$: calcd. C, 79.97; H, 6.97. Found. C, 80.06; H, 6.84.

2.2.22. 7α-Methoxy-17α-(4′-iodophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (**15a**)

A solution of 7α-methoxy-17α-ethynylestra-1,3,5(10)-triene-3,17β-diol (**13a**) (30 mg, 0.09 mmol), 1,4-diiodobenzene (21.6 mg, 0.09 mmol), CuI (1 mg, 3.6×10^{-3} mmol) and $Pd[PPh_3]_4$ (2.1 mg, 1.8×10^{-3} mmol) was refluxed in a mixture of dry THF (5 mL) and dry triethylamine (5 mL) for 3 h under nitrogen atmosphere. After cooling to room temperature, the solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate:*n*-hexane, 1:1) to give 7α-methoxy-17α-(4′-iodophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (**15a**) as a colourless solid (19 mg, 40%), mp. 180–182 °C.

^{1}H RMN (300 MHz, $CDCl_3$) δ: 0.83 (s, 3H, CH_3), 1.22–2.47 (m, 10H), 2.59–2.65 (m, 1H), 2.76–2.81 (m, 1H), 3.05 (d, 1H, ^2J = 17.5 Hz), 3.34 (s, 1H), 3.54 (m, 1H), 4.80 (s, 1H), 6.54 (d, 1H, ^4J = 2.4 Hz), 6.61 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.4 Hz), 7.15 (m, 3H), 7.62 (d, 2H, ^3J = 8.4 Hz). ^{13}C RMN (75 MHz, $CDCl_3$) δ: 12.9, 23.0, 26.9, 33.2, 33.3, 36.4, 39.2, 43.4, 47.9, 57.3, 75.1, 80.6, 85.5, 94.4, 113.3, 116.1, 122.7, 127.1, 132.2, 132.2, 135.4, 137.7, 153.7. ESI/MS: calculated for $C_{27}H_{29}O_3I$: 527.1 $[M\text{-}H]^-$; found: 527.3 $[M-H]^-$. $C_{27}H_{29}O_3I$: calcd. C, 61.37; H, 5.53. Found. C, 61.69; H, 5.23.

2.2.23. 7α- Propoxy-17α-(4′-iodophenylethynyl)estra-1,3,5(10)-triene-3,17βdiol (**15b**)

A solution of 7α-propoxy-17α-ethynylestra-1,3,5(10)-triene-3,17β-diol (**13b**) (30 mg, 0.08 mmol), 1,4-diiodobenzene (17.8 mg, 0.08 mmol), CuI (0.6 mg, 3.2×10^{-3} mmol) and $Pd[PPh_3]_4$ (1.8 mg, 1.6×10^{-3} mmol) was refluxed in a mixture of dry THF (5 mL) and dry triethylamine (5 mL) for 3 h under nitrogen atmosphere. After cooling to room temperature, the solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate:*n*-hexane, 1:1) to give 7α-propoxy-17α-(4′-iodophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (**15b**) as a colourless solid (18.1 mg, 38%), 190–193 °C.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.82 (s, 3H, CH_3), 0.86 (t, 3H, CH_3), 1.21–2.43 (m, 13H), 2.58 (s, 1H), 2.66–2.82 (m, 2H), 2.99 (m, 1H), 3.22 (m, 1H), 3.55 (m, 2H), 4.72 (s, 1H), 6.55 (d, 1H, ^4J = 2.4 Hz), 6.61 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.14 (m, 3H), 7.61 (d, 2H, ^3J = 8.4 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 9.7, 11.4, 21.6, 22.4, 25.3, 31.5, 33.0, 34.6, 37.9, 42.2, 43.6, 46.0, 69.9, 72.0, 78.9, 84.1, 93.1, 111.9, 114.3, 121.5, 125.6, 130.3, 131.5, 134.6, 136.4, 152.3. ESI/MS: calculated for $C_{29}H_{33}O_3I$: 557.1 $[M+H]^+$; found: 557.2 $[M+H]^+$. $C_{29}H_{33}O_3I$: calcd. C, 62.59; H, 5.98. Found. C, 62.36; H, 5.89.

2.2.24. 17α-(4′-Iodophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (**15c**)

Preparation of **15c** followed the general literature procedure [40]. A solution of 17α-ethynylestradiol (**13c**) (50 mg, 0.17 mmol), 1,4-diiodobenzene (56.1 mg, 0.17 mmol), CuI (1.3 mg, 6.8×10^{-3} mmol) and $Pd[PPh_3]_4$ (9.4 mg, 3.4×10^{-3} mmol) was refluxed in a mixture of dry THF (5 mL) and dry triethylamine (5 mL) for 3 h under nitrogen atmosphere. After cooling to room temperature, the solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate:*n*-hexane, 1:1) to give 17α-(4′-iodophenylethynyl)estra-1,3,5(10)-triene-3,17β-diol (**15c**) as a colourless solid (31 mg, 37%), mp. 219–222 °C.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.83 (s, 3H, CH_3), 1.24–2.42 (m, 12H), 2.79 (s, 1H), 6.54 (d, 1H, ^4J = 2.4 Hz), 6.60 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.4 Hz), 7.14 (m, 3H), 7.61 (d, 2H, ^3J = 8.4 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 12.9, 22.6, 26.4, 27.2, 29.4, 33.1, 39.4, 39.9, 43.6, 47.7,49.8, 79.0, 83.9, 95.0, 112.7, 115.0, 122.5, 126.6, 129.5, 133.2, 137.4, 139.3, 154.9. ESI/MS: calculated for $C_{26}H_{27}O_2I$: 499.1 $[M+H]^+$; found: 498.9 $[M+H]^+$. $C_{26}H_{27}O_2I$: calcd. C, 62.66; H, 5.46. Found. C, 62.61; H, 5.55.

2.3. Estimation of lipophilicity

The lipophilicity, which is referred to as $\log P_{o/w}$ (where $\log P_{o/w}$ = octanol/water partition coefficient), of the compounds **14a/b** and **15a/b** was determined from the log K'_w values (K'_w = chromatographic capacity factor at 100% aqueous solution). The log K'_w values were determined by reversed-phase HPLC on a C-18 column according to the method described by Minick [41].

Measurement of the chromatographic capacity factors (k') for each compound was carried out at various concentrations in the range 85–70% methanol (containing 0.25% octanol) and an aqueous phase consisting of 0.15% *n*-decylamine in 0.02 M MOPS (3-morpholinopropanesulfonic acid) buffer pH 7.4 (prepared in 1-octanol-saturated water). The compounds were dissolved in methanol (1 mg/mL) and about 5 μg were injected onto the column. The k' values are defined as $(t_r - t_0)/t_0$, where t_r and t_0 are the retention times of the compounds and non-retained species (solvent), respectively. The log k'_w values were obtained from linear extrapolation of log k' *vs* φmethanol (φ = volume fractions methanol) data acquired in the region $0.70 \leqslant \varphi\text{methanol} \leqslant 0.85$. The log $P_{o/w}$ values of the compounds were determined from a standard curve (log $P_{o/w}$ *vs* log k'_w) constructed with data of standard compounds.

2.4. Receptor-binding affinity

The ERα competitive binding assay was performed according to a described method with minor modifications [42]. The ERα binding buffer used for dilution of the receptor preparations consisted of 10% glycerol, 2 mM dithiothreitol, 1 mg/mL BSA and 10 mM Tris–HCl (pH 7.5). The ERα washing buffer contained 40 mM Tris–HCl and 100 mM KCl (pH 7.4). The hydroxyapatite (HAP) slur-

ry was adjusted to a final concentration of 50% (v/v) by using a 50 mM Tris–HCl solution (pH 7.4). The reaction mixture contained 50 μL of varying concentrations of the test compound in the ERα binding buffer, 45 μL of a solution of tritiated estradiol (23.8 nM) and 5 μL (0.25 pmol) of ERα protein solution. Non-specific binding by the tritiated estradiol was determined by the addition of a 50 μM concentration of the nonradioactive estradiol (E2). The binding mixture was incubated at 4 °C for 16–18 h. At the end of the incubation, 200 μL of the HAP slurry was added, and tubes were incubated on ice and vortexed three times for 15 min. An aliquot of 1 mL of washing buffer was added, mixed and centrifuged at 10,000*g* for 10 min, and the supernatants were discarded. This wash-step was repeated twice. The HAP pellets were then re-suspended in 750 μL cold ethanol, vortexed three times for 20 min, centrifuged and the supernatants were transferred to scintillation vials for measurement of ^{3}H radioactivity in a liquid scintillation counter (Packard Tri-CARB 3170 TR/SL). The data obtained from triplicate measurements were expressed as the percent specific binding of [^{3}H]E2 *vs* the log molar concentration of the competing compound. The IC_{50} values (calculated using GraphPad Prism software) represent the concentration of the test compound required to reduce the [^{3}H]E2 binding by 50%. Data were then expressed as a relative binding affinity percentage (RBA) determined in comparison with E2.

3. Results and discussion

3.1. Chemistry

Initially, the synthesis of the 7α-alkoxyestradiol derivatives **10** followed the general route published earlier, albeit with a different protective group at C3 [43]. The synthetic route is outlined in Scheme 1.

The C3 phenol function of estrone was protected as its benzyl ether, according to a described procedure, to give 3-*O*-benzylestra-1,3,5(10)-trien-3-ol-17-one, **1** [44]. The acetalization of the C17 keto group in **1** was carried out with neopentyl glycol (NPG) under standard conditions to give the fully protected **2** [45].

In order to access the C7-position in estranes of type **2** it was necessary to activate C6. For this purpose, C6 was oxidized. The direct oxidation of estrane derivatives to 6-ketoestranes has been previously reported and a variety of reagents has been used namely, CrO_3, CH_2Cl_2, 2,5-dimethylpyrazole [46], AcOH, CrO_3, H_2O [47–49], pyridinium chlorochromate (PCC), benzene [50] and CrO_3, H_2SO_4 [51]. However, in our hands, the direct oxidation of fully protected **3** could best be achieved with $KMnO_4$ under PTC conditions (Adogen 464, benzene, aq. $NaHCO_3$) [45,52]. The outcome of the reaction is dependent on reaction temperature and reaction time. Nevertheless, the yield of **3** is similar, when the reaction is run at 80 °C for 2 h or when it is run at 50 °C for 12 h.

Next, **3** was reduced with $NaBH_4$ in a mixture of MeOH/Et_2O to give **4**. NOE experiments have shown that the stereochemistry of **4** at C6 was α-(hydroxyl). A small amount of β-isomer was also formed. **4** was subjected to dehydration with *p*-TsOH (benzene, reflux) to give 3-*O*-benzyl-estra-1,3,5(10),6(7)-tetraen-3-ol-17-one 17,17-(2′-[5′,5′-dimethyl-1′,3′-dioxane]) **5**. In the reaction, small amounts of neopentylglycol were added in order to maintain the protective group at C17. **5** was then reacted with *m*-chloroperbenzoic acid in a phosphate buffer. The reaction is stereoselective and gives 6α,7α-epoxyestra-1,3,5(10)-trien-3-ol-17-one **6**. Again, the stereochemistry at C6/C7 was determined by NOE experiments. In order to better attribute the proton signals, a ^{1}H–^{1}H COSY experiment was carried out beforehand.

Benzocycloalkene 1,2-oxides are known to undergo regioselective reductive ring opening with complex hydrides [53–55]. While reactions of **7** with carbon nucleophiles such as with (n-Bu)$_2$-Cu(CN)Li_2 were found to be not completely regioselective, the reaction of **6** with $LiAlH_4$ led exclusively to **7** [56]. Again, the stereochemistry of **7** at C7 was determined by NOE experiments, and the compound was confirmed to be the 7α-hydroxyl derivative [57]. Additionally, the coupling constants ^3J of H(C7) at δ 4.13 (in $CDCl_3$) with Hα/Hβ(C6) and H(C8) are very small, indicative of H(C7) at an equatorial position as one would expect larger coupling constants between neighbouring axially positioned protons [58].

Etherification of **7** was carried out using NaH as base and alkyl halides such as methyl iodide and *n*-propyl iodide as alkylating agents. In the next step the steroidal esters **8a/8b** were debenzylated at O–C3 by catalytic hydrogenation. Steroidal ethers **9a** and **9b** were deprotected at C17 by transacetalisation (acetone, *p*-TsOH, rt) to give **10a/10b**. The phenolic function at C3 of **10a/10b** was protected once again as its TBDMS ether by treatment with imidazole in dry DMF, followed by silylation of the phenoxide with *tert*-butyldimethylsilyl chloride (TBDMSCl) to give **11a** and **11b**, respectively.

The C7-substituted estratetraene derivatives **11a/11b** were reacted with lithium trimethylsilylacetylide to give **12a** and **12b**, after column chromatographic separation on silica gel. Subsequently, **12a** and **12b** were reacted with tetra-*n*-butyl ammonium fluoride in THF for 1 h for complete desilylation, giving compounds **13a** and **13b** in high yield.

For the preparation of fluorinated **14a**–**14c** and the iodinated estradiol derivatives **15a**–**15c** to be used for the *in vitro* biological evaluation studies, a Sonogashira coupling reaction of **13** with 1,4-diiodobenzene and with 1,4-fluoroiodobenzene, respectively, was used [59–61].

The desired coupling products **14a/14b** were obtained in high chemical purity and good chemical yield (80% and 50%, respectively). Also, the iodinated derivatives, **15a/15b** were obtained in high chemical purity and fair chemical yield (40% and 39%, respectively). These compounds were used for the subsequent biological studies. Also, the non 7α-substituted fluorinated and iodinated derivatives (**14c** and **15c**) were prepared under the same conditions using 17α-ethynylestradiol as starting material and were obtained in 50 and 37% yield, respectively.

3.2. In vitro studies

3.2.1. Estimation of lipophilicity

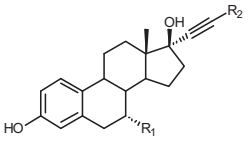
The lipophilicity of compounds can affect their tissue permeability, which can impact their localization in target tissues. Lipophilicity may also affect binding to low affinity, nonspecific sites that can compromise target tissue to background tissue ratios. To anticipate the potential of the synthesized compounds to be studied further *in vivo* (cell lines, animal experiments) their octanol/water partition coefficients were estimated by a reversed-phase HPLC method described by Minick [41]. As expected on the basis of their chemical structures, the estimated values (Table 1) indicated that the novel 17α-(4′-halophenylethynyl)estradiol derivatives are more lipophilic than estradiol ($\log P_{o/w.} = 3.82 \pm 0.09$) suggesting their ability to penetrate cell membranes.

3.2.2. Receptor binding affinity studies

The *in vitro* ERα binding affinity was determined by competitive radiometric binding assay using [^{3}H]estradiol as tracer. Incubations were done overnight at 4 °C and hydroxylapatite was used to separate bound receptor–ligand complex. The relative binding affinity (RBA) for each compound was calculated against estradiol (E2) by using the following equation: *RBA = IC_{50} for E2/IC_{50} for each compound x 100* and are presented in Table 1.

To investigate whether the replacement of the terminal proton in the acetylene unit by a 4′-halophenyl group would lead to com-

Table 1
Relative binding affinities (RBA) and log *P* values for the estradiol derivatives.



Compound	Substitution	RBA (%)	Log $P_{o/w}$
Estradiol		100	3.82 ± 0.09
Ethynylestradiol		154.68 ± 0.36	3.74 ± 0.13
13a	$R_1 = OCH_3$; $R_2 = H$	150.08 ± 0.01	2.93 ± 0.09
13b	$R_1 = OC_3H_7$; $R_2 = H$	15.60 ± 0.01	3.83 ± 0.13
14a	$R_1 = OCH_3$; $R_2 = PhF$	78.88 ± 0.01	4.36 ± 0.09
14b	$R_1 = OC_3H_7$; $R_2 = PhF$	15.82 ± 0.01	5.20 ± 0.17
14c	$R_1 = H$; $R_2 = PhF$	80.87 ± 0.02	5.01 ± 0.15
15a	$R_1 = OCH_3$; $R_2 = PhI$	0.42 ± 0.01	5.12 ± 0.09
15b	$R_1 = OC_3H_7$; $R_2 = PhI$	n.d.	6.15 ± 0.15
15c	$R_1 = H$; $R_2 = PhI$	4.56 ± 0.01	5.65 ± 0.11

pounds that still retain ER binding affinity, the RBA values were compared for both the estradiol and the 7α-alkoxy-estradiol series.

The binding affinity enhancing effect of the ethynyl substituent itself is evident in comparing RBA values of estradiol and 17α-ethynylestradiol (100% *vs* 154%). Both in the estradiol (**14c**) and in the 7α-methoxy series (**14a**) the introduction of a 4′-fluorophenylethynyl moiety is well tolerated by the ER (80% *vs* 79%). However, in both series, the introduction of a 4′-iodophenylethynyl group (**15c** and **15a**) drastically diminishes the binding affinity, rendering these derivatives unlikely candidates for SPECT imaging. This lack of binding affinity might be explained by the presence of the bulky iodine atom, not very far away from the steroidal frame.

In this study, we have also compared the effect of the 7α-methoxy *vs* 7α-propoxy group on the ethynyl (**13a** and **13b**) and 4′-fluorophenylethynyl (**14a**, **14b**, **14c**) series. Addition of the 7α-methoxy group to ethynylestradiol or to **14c**, leading to **13a** or **14a** still maintains the high binding affinity of the compounds. In contrast, adding a 7α-propoxy group to ethynylestradiol, leading to **14b**, did decrease the ER binding affinity to values of about 15% of that of estradiol, which, however, can still be considered an adequate binding constant. This data suggests that an elongation of the alkyl chain of C7α-alkoxyestradiols interferes to some extent with the ER binding process. Interestingly, within the 11β-series a similar trend has been reported, where 11β-butoxyestradiol [62] was shown to exhibit little binding affinity to ERα, in contrast to 11β-methoxyestradiol.

As we can see from Table 1 the order of increasing lipophilicity and steric hindrance of the substituents at C-17α is $C{\equiv}CH < C_6H_4F < C_6H_4I$. The encountered RBA values decrease with enhanced lipophilicity and bulkiness of the substituent, which is in agreement with observations made by others [63]. The same trend is observed in the 7α-methoxy and in the 7α-propoxy series. For the *p*-fluorophenyl substituted estradiols **14a** and **14c** a hydrogen bonding can be envisaged between water molecules within the estrogen receptor, leading to stabilization of the ligand receptor complex. Indeed, an X-ray crystal structure of the 2-trifluorophenylvinylestradiol with the estrogen receptor ERa has identified two water molecules in the region where the *p*-fluorophenyl substituent of **14a** and **14c** is expected to reside. This interaction is not possible for the iodophenyl-substituted estradiols [64].

4. Conclusions

In our quest for novel based ligands for the ERα that could ultimately be radiolabelled with PET/SPECT radionuclides, we have synthesized novel 7α-alkoxy-17α-(4′-halophenylethynyl)estradiols and have evaluated their ER binding affinity. Binding affinity data indicate that the introduction of a methoxy group into the C7 position of the ethynylestradiol framework, as in **13a**, maintained a high ER binding affinity of the molecule (150% *vs* 154%). Also, the introduction of a 4′-fluorophenylethynyl group on the estradiol framework, as in **14c**, is well tolerated by the receptor (81%). Moreover, the simultaneous addition of a 7α-methoxy and a 4′-fluorophenyl on the ethynylestradiol, as in **14a**, did not significantly diminish the ER binding affinity (79%). In contrast, the presence of 4′-iodophenylethynyl substituents is not tolerated by the estrogen receptor, excluding the possibility of using their ^{123}I-labelled analogues as SPECT imaging agents for estrogen receptor densities in tumours.

While the 17α-(4′-fluorophenylethynyl)estradiol derivatives may be explored further as potential PET biomarkers for imaging of ER expressing tumours, the more important consequence of the study is that as 7α-methoxyestradiols such as **13a** show high binding affinity to ERα, these molecules could potentially be used as a platform for radiolabeled steroids, where the radiohalogen is introduced in either the steroidal frame or the alkoxy side-chain. Hence, studies in this direction are currently underway.

Acknowledgements

The authors gratefully acknowledge to DAAD/FCT (0811983/441.00) for financial support. C. Neto thanks Fundação para a Ciência e Tecnologia (FCT) for a Ph.D. grant (SFRH/BD/31319/2006). The authors thank Dr. Joaquim Marçalo and Dr Vânia Sousa for performing mass spectra analysis and elemental analysis, respectively. The authors are grateful to Dr João Abrantes for β measurement in the RBA assays. Yosef Al Jasem, UAE University, is thanked for discussions regarding ligand–receptor interactions. The QITMS instrument was acquired with the support of the Programa Nacional de Reequipamento Científico (Contract REDE/1503/REM/2005-ITN) of FCT and is part of Rede Nacional de Espectrometria de Massa (RNEM).

References

[1] Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. New Engl J Med 2006;354:270–82.
[2] Latil A, Biache I, Vidaud D, Lidereau R, Berthon P, Cussenot O, et al. Evaluation of androgen, estrogen (ERa and ERb), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse transcription-polymerase chain reaction assays. Cancer Res 2001;61:1919–26.
[3] Chan KKL, Wei N, Liu SS, Xiao-Yun L, Cheung AN, Ngan HYS. Estrogen receptor subtypes in ovarian cancer: a clinical correlation. Obstet Gynecol 2008;111:144–51.
[4] Smith HO, Leslie KK, Singh M, Qualls CR, Revankar CM, Joste NE, et al. GPR30: a novel indicator of poor survival for endometrial carcinoma. Am J Obstet Gynecol 2007;196(386):e1–e11.
[5] Nacusi LP, Debes JD. Primers on molecular pathways: nuclear receptors in pancreatic cancer. The ligand-independent way. Pancreatology 2008;8:422–4.
[6] Van de Wiele C, De Vos F, Slegers G, Van Belle S, Dierckx RA. Radiolabeled estradiol derivatives to predict response to hormonal treatment in breast cancer: a review. Eur J Nucl Med Mol Imaging 2000;27:1421–33.
[7] Katzenellenbogen JA. Receptor imaging of tumors (non-peptide). In: Welch MJ, Redvanly CS, editors. Handbook of Radiopharmaceuticals Radiochemistry and Applications. Chichester, UK: John Wiley & Sons, Ltd.; 2003. p. 715–30.
[8] Bénard F, Turcotte E. Imaging in breast cancer: single-photon computed tomography and positron-emission tomography. Breast Cancer Res 2005;7:153–62.
[9] Cummins CH. Radiolabeled steroidal estrogens in cancer research. Steroids 1993;58:245–59.
[10] Oliveira MC, Neto C, Gano L, Marques F, Santos I, Thiemann T, et al. Estrogen receptor ligands for trageting breast tumours: a brief outlook on radioiodination strategies. Curr Radiopharm 2012;5:124–41 (and references therein).
[11] Pavlik EJ, Nelson K, Gallion HH, van Nagell JR, Donaldson ES, Shih WJ, et al. Characterization of high specific activity [16a-^{123}I]Iodo-17b-estradiol as an estrogen receptor-specific radioligand capable of imaging estrogen receptor-positive tumors. Cancer Res 1990;50:7799–805.
[12] Ribeiro-Barras MJ, Foulon C, Baulieu JL, Guilloteau D, Bougnoux P, Lansac J, et al. Estrogen receptor imaging with 17a-[^{123}I]iodovinyl-11b-

methoxyestradiol ($MIVE_2$)-part II. Preliminary results in patients with breast carcinoma. Nucl Med Biol 1992;19:263–7.
[13] Rijks LJM, Boer GJ, Endert E, Bruin K, Bos JC, Doremalen PAPM, et al. The stereoisomers of 17α-[^{123}I]iodovinyloestradiol and its 11b-methoxy derivative evaluated for their oestrogen receptor binding in human MCF-7 cells and rat uterus, and their distribution in immature rats. Eur J Nucl Med Mol Imaging 1996;23:295–307.
[14] Rijks LJM, Boer GJ, Endert E, de Bruin K, Janssen AGM, van Royen EA. The Z-isomer of 11b-methoxy-17a-[^{123}I]iodovinylestradiol is a promising radioligand for estrogen receptor imaging in human breast cancer. Nucl Med Biol 1997;24:65–75.
[15] Bennink RJ, Rijks LJ, van Tienhoven G, Noorduyn LA, Janssen AG, Sloof GW. Estrogen receptor status in primary breast cancer: iodine ^{123}I-labeled cis-11b-methoxy-17a-iodovinyl estradiol scintigraphy1. Radiology 2001;220:774–9.
[16] Arterburn JB, Corona C, Rao KV, Carlson KE, Katzenellenbogen JA. Synthesis of 17-a-substituted estradiol-pyridin-2-yl hydrazine conjugates as effective ligands for labeling with alberto's complex fac-$[Re(OH_2)_3(CO)_3]^+$ in water. J Org Chem 2003;68:7063–70.
[17] Ramesh C, Bryant B, Nayak T, Revankar CM, Anderson T, Carlson KE, et al. Linkage effects on binding affinity and activation of GPR30 and estrogen receptors ERa/b with tridentate pyridin-2-yl hydrazine tricarbonyl Re/^{99m}Tc(I) chelates. J Am Chem Soc 2006;128:14476–7.
[18] Nayak TK, Hathaway HJ, Ramesh C, Arterburn JB, Dai D, Sklar LA, et al. Preclinical development of a neutral, estrogen receptor-targeted, tridentate Tc-99m(I)-estradiol-pyridin-2-yl hydrazine derivative for imaging of breast and endometrial cancers. J Nucl Med 2008;49:978–86.
[19] DeSombre ER, Hughes A, Hanson RN, Kearney T. Therapy of estrogen receptor-positive micrometastases in the peritoneal cavity with Auger electron-emitting estrogen. Acta Oncologica 2000;39:659–66.
[20] McLaughlin WH, Milius RA, Pillai KMR, Edasery JP, Blumenthal RD, Bloomer WD. Cytotoxicity of receptor-mediated 16a-[^{125}I]iodo-estradiol in cultured MCF-7 human breast cancer cells. J Natl Cancer Inst 1989;81:437–40.
[21] DeSombre ER, Shafii B, Hanson RN, Kuivanen PC, Hughes A. Estrogen receptor-directed radiotoxicity with auger electrons: specificity and mean lethal dose. Cancer Res 1992;52:5752–8.
[22] Pomper MG, VanBrocklin H, Thieme AM, Thomas RD, Kiesewetter DO, Carlson KE, et al. 11b-Methoxy-, 11b-ethyl, and 17a-ethynyl-substituted 16a-fluoroestradiols: receptor-based imaging agents with enhanced uptake efficiency and selectivity. J Med Chem 1990;33:3143–55.
[23] Kiesewetter DO, Kilbourn MR, Landvatter SW, Heiman DF, Katzenellenbogen JA, Welch MJ. Preparation of four fluorine-18-labeled estrogens and their selective uptakes in target tissues of immature rats. J Nucl Med 1984;25: 1212–21.
[24] VanBrocklin HF, Carlson KE, Katzenellenbogen JA, Welch MJ. 16b-([^{18}F]Fluoro)estrogens: systematic investigation of a new series of fluorine-18-labeled estrogens as potential imaging agents for estrogen-receptor-positive breast tumors. J Med Chem 1993;36:1619–29.
[25] VanBrocklin HF, Liu A, Welch MJ, O'Neil JP, Katzenellenbogen JA. The synthesis of 7a-methyl-substituted estrogens labeled with fluorine-18: potential breast tumor imaging agents. Steroids 1994;59:34–45.
[26] Seimbille Y, Rousseau J, Bénard F, Morin C, Ali H, Avvakumov G, et al. ^{18}F-labeled difluoroestradiols: preparation and preclinical evaluation as estrogen receptor-binding radiopharmaceuticals. Steroids 2002;67:765–75.
[27] Linden HM, Stekhova SA, Link JM, Gralow JR, Livingston RB, Ellis GK, et al. Quantitative fluoroestradiol positron emission tomography imaging predicts response to endocrine treatment in breast cancer. J Clin Oncol 2006;24:2793–9.
[28] Mortimer JE, Dehdashti F, Siegel BA, Katzenellenbogen JA, Fracasso P, Welch MJ. Positron emission tomography with 2-[^{18}F]fluoro-2-deoxy-D-glucose and 16a-[^{18}F]fluoro-17b-estradiol in breast cancer: correlation with estrogen receptor status and response to systemic therapy. Clin Cancer Res 1996;2:933–9.
[29] Sundararajan L, Linden HM, Link JM, Krohn KA, Mankoff DA. ^{18}F-Fluoroestradiol. Sem Nucl Med. 2007;37:470–6.
[30] Seimbille Y, Ali H, van Lier JE. Synthesis of 2,16α- and 4,16α-difluoroestradiols and their 11β-methoxy derivatives as potential estrogen receptor-binding radiopharmaceuticals. J Chem Soc Perk Trans 2002;1:657–63.
[31] Seimbille Y, Rousseau J, Bénard F, et al. 18F-labeled difluoroestradiols: preparation and preclinical evaluation as estrogen receptor-binding radiopharmaceuticals. Steroids 2002;67. 765–5..
[32] Bénard F, Ahmed N, Beauregard JM, et al. [18F]Fluorinated estradiol derivatives for oestrogen receptor imaging: impact of substituents, formulation and specific activity on the biodistribution in breast tumour-bearing mice. Eur J Nucl Med Mol Imaging. 2008;35:1473–9.
[33] Beauregard J-M, Croteau E, Ahmed N, van Lier JE, Bénard F. Assessment of human biodistribution and dosimetry of 4-fluoro-11*b*-methoxy-16*a*-^{18}F-fluoroestradiol using serial whole body PET/CT. J Nucl Med 2009;50:100–7.
[34] Oliveira MC, Videira M, Almeida A, Gano L, Watanabe M, Thiemann T, et al. Synthesis and biodistribution studies of two novel radioiodinated arenoannelated estra-1,3,5(10),16-tetraene-3-ols as promising estrogen receptor radioligands. J Labelled Comp Radiopharm 2006;49:559–69.
[35] Neto C, Oliveira MC, Gano L, Marques F, Santos I, Morais GR, et al. Radioiodinated ligands for the estrogen receptor: effect of different 7-cyanoalkyl chains on the binding affinity of novel iodovinyl-6-dehydroestradiols. Appl Radiat Isot 2009;67:301–7.
[36] Neto C, Oliveira MC, Gano L, Marques F, Santos I, Morais G, et al. Evaluation of novel radioiodinated C7-substituted 6,7-estradiol derivatives for molecular recognition of ER-positive breast tumours. Curr Radiopharm 2009;2:83–91.
[37] Ali H, Rousseau J, Paquette B, Dubã C, Marko B, van Lier JE. Synthesis and biological properties of 7a-cyano derivatives of the (17a,20*E*/*Z*)-[^{125}I]iodovinyl- and 16a-[^{125}I]iodo-estradiols. Steroids 2003;68:1189–200.
[38] Top S, El Hafa H, Vessieres A, Huché M, Vaissermann J, Jaouen G. Novel estradiol derivatives with Ru, W, and Co complexes. Influence on hormone-receptor affinity of several organometallic groups at the 17*a* position. Chem Eur J 2002;8:5241–9.
[39] Wang J, Watanabe M, Mataka S, Thiemann T, Ribeiro Morais G, Roleira F, et al. Synthesis and radiosynthesis of 17a-[p-(iodophenylethynyl)]estra-3,17b-diols. Z Naturforsch 2003;58b:799–804.
[40] Wüst FR, Kniess T. Synthesis of 4-[^{18}F]fluoroiodobenzene and its application in sonogashira cross-coupling reactions. J Labelled Comp Radiopharm 2003;46:699–713.
[41] Minick DJ, Frenz JH, Patrick MA, Brent DA. A comprehensive method for determining hydrophobicity constants by reversed-phase high-performance liquid chromatography. J Med Chem 1988;31:1923–33.
[42] Jiang X-R. Walter Sowell J, Zhu BT. Synthesis of 7a-substituted derivatives of 17β-estradiol. Steroids 2006;71:334–42.
[43] Yasuda T, Shima Y, Ideta K, das Neves Oliveira C, Thiemann T. 7-Alkoxyestra-1,3,5(10)-trienes. J Chem Res 2008;2008:32–7.
[44] Bérubé M, Kamal F, Roy J, Poirier DA. Dehydrohalogenation methodology for synthesizing terminal olefins under mild conditions. Synthesis 2006;2006(3085):91.
[45] Inohae E, Thiemann T, Mataka S, Melo e Silva MC, Catela LP. Synthesis of 7-w-amidoalkyl-subtituted 6,7-dehydroestra-3,17b-diols. Rep Inst Adv Mater Study 1999;13:31–6.
[46] Tremblay MR, Boivin RP, Luu-The V, Poirier D. Inhibitors of type 1 17b-hydroxysteroid dehydrogenase with reduced estrogenic activity: modifications of the positions 3 and 6 of estradiol. J Enzym Inhib Med Chem 2005;20:153–63.
[47] Weber G, Schaumann J, Carl C, Schwarz S, Leisner R. A novel efficient approach to the synthesis of 6-oxo ethinylestradiol and its 3-isopropanesulfonate. J Prakt Chemie 1989;331:223–30.
[48] Banerjee S, Das T, Chakraborty S, Samuel G, Korde A, Venkatesh M, et al. An estradiol-conjugate for radiolabelling with ^{177}Lu: an attempt to prepare a radiotherapeutic agent. Bioorg Med Chem. 2005;13:4315–22.
[49] Sakac MN, Penov-Gasi KM, Popsavin M, Djurendic EA, Andric S, Kovacevic RM. Synthesis and estrogenic activity screening of some 6,9-disubstituted estradiol derivatives. Coll Czech Chem Commun 2005;70:479–86.
[50] Mons S, Lebeau L, Mioskowski C. An efficient synthesis of 6-oxo-17-b-estradiol and its *O*-carboxymethyl oxime. Synth Commun 1998;28:213–8.
[51] Dorobantu I, Iancu EI. Synthesis and characterization of immunogenic conjugate 17b-oestradiol-6-carboxymethyl-oxime-BSA and 17b-oestradiol-6-carboxymethyl-oxime-tyramine-125I. Revue Romaine de Biochimie 1993;30:15–9.
[52] Melo e Silva MC, Patricio L, Gano L, Sá e Melo ML, Inohae E, Mataka S, et al. Synthesis and biological evaluation of two new radiolabelled estrogens: [125I](E)-3-methoxy-17a-iodovinylestra-1,3,5(10),6-tetraen-17b-ol and [125I](Z)-3-methoxy-17a-iodovinylestra-1,3,5(10),6-tetraen-17b-ol. Appl Radiat Isot 2001;54:227–39.
[53] Talekar DG, Rao AS. A Novel Route for the Synthesis of 3,4-Dihydro-1H-2-benzopyrans and 1,3,4,5-Tetrahydro-2-benzoxepins. Synthesis 1983;1983:595–7.
[54] Boyd DR, Greene RME. Synthesis, resolution and racemization studies of 1,2-epoxy-1,2-dihydrochrysene. J Chem Soc Perkin Trans 1982;1:1535–9.
[55] Marshall P, Prager R. Hydroboration–oxidation of benzocycloalkenes. Aust J Chem. 1979;32:1251–60.
[56] Shima Y. Synthesis of C7-substituted estrane derivatives with unsaturations in the steroidal framework. MSc Thesis, Kyushu University; 2002.
[57] Plate R, van Wuijtswinkel RCAL, Jans CGJM, Groen MB. Synthesis of (3a,7b,17a)-7-methyl-19-norpregn-5(10)-en-20-yne-3,7,17-triol, a metabolite of ORG OD14, and its 7-epimer. Steroids 2000;65:497–504.
[58] Hesse M, Meier H, Zeeh B. Spektroskopische Methoden in der Organischen Chemie. Stuttgart, New York: Georg Thieme Verlag; 1979.
[59] Kenkichi S. Development of Pd/Cu catalyzed cross-coupling of terminal acetylenes with sp2-carbon halides. J Organometal Chem 2002;653:46–9.
[60] Negishi E-i, Anastasia L. Palladium-catalyzed alkynylation. Chem Rev 2003;103:1979–2018.
[61] Nicolaou KC, Bulger PG, Sarlah D. Palladium-catalyzed cross-coupling reactions in total synthesis. Angew Chem Int Ed 2005;44:4442–89.
[62] Zhang J, Labaree DC, Hochberg RB. Nonpolar and short side chain groups at C-11b of estradiol result in antiestrogens. J Med Chem 2005;48:1428–47.
[63] Da Silva JN, van Lier JE. In vivo evaluation of 7a-[11-(4-[^{125}I]Iodophenoxy)undecyl]-17b-estradiol: a potential vector for therapy of adrenal and estrogen receptor-positive cancers. J Steroid Biochem Mol Biol 1990;37:77–83.
[64] Nettles KW, Bruning JB, Gil G, O'Neill EE, Nowack J, Guo Y, Kim Y, DeSombre ER, Dilis R, Hanson RN, Joachimiak A, Greene GL. Structural plasticity in the estrogen receptor–ligand binding domain. EMBO Rep 2007;8:563–8.