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Fluorous tolerance of the estrogen receptor alpha as probed by 11-polyfluoroalkylestradiol derivatives

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ABSTRACT

The concern of this work was to try to delineate factors, inherent to fluorination, susceptible to influence estradiol binding to the estrogen receptor alpha (ERα). For this purpose, fluorinated chains were linked at 11β position of the steroid (i.e., C_6F_{13}, $CH_2CH_2C_4F_9$, $CH_2CH_2C_8F_{17}$). Relative binding affinity (RBA) for ERα of these compounds and of other related fluorinated derivatives was compared to those of non-fluorinated analogs. Despite being relatively well accepted by the receptor, investigated compounds exhibited lower RBA values at 0 °C than their non-fluorinated counterparts. Nevertheless, heavily fluorinated chains were tolerated in so far as they are not too long (C-4) and insulated from the steroidal core by a two methylene spacer unit. Increase of the temperature of our binding assay (25 °C) failed to change the RBA values of two selected polyfluorohexyl derivatives while it drastically enhanced the value of the corresponding non-fluorinated analogs. Rigidity of the chain induced by fluorination as well as the oleophilic (fluorophobic) nature of the estradiol binding cavity of ERα is proposed to explain these properties.

1. Introduction

Fluorinated molecules are practically unknown in nature [1]. However the number of fluorinated compounds synthesized for industrial means has been constantly increasing during the last decade [2]. In the field of medicinal chemistry interest for fluorinated substituents (besides mono- and difluorinated compounds) mainly focused on short perfluorinated chains (CF_3, C_2F_5) [3]. Emphasis especially focused on enhanced hydrophobic properties brought by fluorinated groups to a host molecule as measured by the π Hansch–Leo parameter [4], as well as to the diminished rate of metabolic inactivation expected for fluorinated molecules [5]. Numerous drugs bearing these groups were shown to have an increased capacity to cross lipid membranes [6]. Concomitant oleophobic nature of longer perfluoroalkylated chains emerged also as a powerful tool for catalysis, materials engineering and separation techniques suitable for biotechnology [7–9].

As yet, only a few reports on perfluoroalkylated steroids appeared in the literature outside the patent area [10–13]. None of these reports discuss the influence of grafting fluorinated chains onto the biological properties of these steroids. This point stresses some interest for our own investigations devoted to the study of the binding properties to the estrogen receptor alpha (ERα) of estradiol (E_2) derivatives bearing perfluorinated chains at the 7α [14] or 11β [15] positions. These

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two positions considered as almost equivalent with regard to receptor binding (potential rotation of the steroid moiety along a C3–C17 axis within the ligand binding pocket) [16,17,21] were selected because they appear especially appropriate for the synthesis of active compounds. Indeed, as stated by Anstead et al. [18] "The ER is remarkably tolerant of relatively large, non-polar substituents in the 11β-position". The general trend values upon introduction of alkyl chains is usually an increase of binding affinities for short alkyl chains (Me, Et) and a decrease for longer side chains (efficiency of this effect seems species dependent) [18,19]. The known strong antiestrogenicity of E_2 bearing 7α or 11β functionalized alkyl side chains [20–27] was another argument for the choice of these grafting positions.

We report here the synthesis of a few 11β-perfluoroalkylestradiols and some related molecules as well as their non-fluorinated counterparts. Binding affinities of all these compounds were measured and critically analyzed to provide guidelines for the design of new estrogen and antiestrogen derivatives with high therapeutic activities [3,28].

2. Experimental

2.1. General

NMR spectra were recorded on a Bruker AC-300 spectrometer. Reported coupling constants and chemicals shifts were based on a first order analysis. Internal reference was the residual peak of $CHCl_3$ (7.27 ppm) for ^1H (300 MHz), central peak of $CDCl_3$ (77 ppm) for ^{13}C (75 MHz) spectra and internal $CFCl_3$ (0 ppm) for ^{19}F (282 MHz) NMR spectra. Optical rotations were measured on a PerkinElmer 341 digital polarimeter operating at 589 nm and 25 °C. Low-resolution mass spectra were recorded on a HP–MS engine 5989B. High-resolution electrospray mass spectra in the positive ion mode were obtained on a Q-TOF Ultima Global hybrid quadrupole/time-of-flight instrument (Waters-Micromass, Manchester, U.K.), equipped with a pneumatically assisted electrospray (Z-spray) ion source and an additional sprayer (Lock Spray) for the reference compound. Melting points were determined on a Mettler FP61 melting point apparatus.

2.2. Chemical synthesis

2.2.1. General procedure for alkylation of 11-ketoestradiol derivatives as exemplified by the preparation of 17β-bisbenzyloxy-11α-hexyl-estra-1,3,5(10)-trien-11β-ol (6b)

Hexylmagnesium bromide in diethylether (1.2 M, 7.1 mL, 8.5 mmol, 10 equiv.) was carefully added to a solution of 11-ketoestradiol **5** (400 mg, 0.86 mmol) in distilled THF (4 mL). After 3 h stirring at room temperature, the crude mixture was quenched by an aqueous solution of ammonium chloride (5 mL). The solution was extracted with dichloromethane (3× 10 mL). Organic layers were then washed with water (2× 5 mL), dried over $MgSO_4$ and evaporated to dryness. Purification by column chromatography (pure CH_2Cl_2) finally afforded the desired product **6b** as a glass.

*2.2.1.1. 3,17β-Bisbenzyloxy-11α-hexyl-estra-1,3,5(10)-trien-11β-ol (**6b**).* 22% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, 3H, J = 5.6 Hz), 1.11 (s, 3H), 1.33–1.81 (m, 18H), 2.02–2.32 (m, 4H), 2.75 (m, 2H), 3.48 (t, 1H, J = 7.6 Hz), 4.59 (s, 2H), 5.07 (s, 2H), 6.80 (m, 2H), 7.26–7.48 (m, 10H), 7.72 (d, 1H, J = 9.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 14.1, 23.8, 24.4, 26.3, 27.6, 29.5, 29.8, 31.4, 31.8, 34.3, 43.1, 44.3, 50.3, 50.9, 51.4, 69.9, 71.7, 75.7, 89.5, 111.4, 114.8, 127.4, 127.5, 127.8, 128.3, 128.5, 130.6, 137.3, 139.1, 142.3, 156.5; MS (pos. ESI) (*m*/z): 575 $(M+Na)^+$; 553 $(M+H)^+$; $[\alpha]_D^{25}$ −16,4 (c 0.31, chloroform).

*2.2.1.2. 3,17β-Bisbenzyloxy-11α-(3,3,4,4,5,5,6,6,6-nonafluorohexyl)-estra-1,3,5(10)-trien-11β-ol (**6c**).* From 1.07 mmol of ketone **5** (500 mg) using 5 equiv. of perfluorinated Grignard reagent (1 M).

46% yield (355 mg); glass; ^1H NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 3H), 1.29–1.89 (m, 9H), 2.10–2.76 (m, 9H), 3.51 (t, 1H, J = 7.9 Hz), 4.55–4.59–4.65–4.69 (AB system, 2H, J = 12.2 Hz), 5.10 (s, 2H), 6.88 (m, 2H), 7.35–7.51 (m, 10H), 7.69 (d, 1H, J = 9.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 23.7, 25.4 (t, J = 22 Hz), 26.0, 27.4, 28.8, 33.1, 34.3, 42.6, 50.3, 50.7, 51.3, 69.8, 71.6, 74.0, 88.9, 111.3, 115.2, 127.4, 127.5, 127.8, 128.2, 128.5, 129.6, 137.1, 139.0, 142.3, 156.7; ^{19}F NMR (188 MHz, $CDCl_3$) δ −81.5 (t, 3F, J = 10 Hz), −114.8 (m, 2F), −124.4 (m, 2F), −126.4 (m, 2F); MS (pos. ESI) (m/z): 737 $(M+Na)^+$, 753 $(M+K)^+$.

*2.2.1.3. 3,17β-Bisbenzyloxy-11α-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-decyl)-estra-1,3,5(10)-trien-11β-ol (**6d**).* From 1.2 mmol of ketone **5** (600 mg) using 5 equiv. of perfluorinated Grignard reagent (0.8 M).

36% yield (433 mg); glass; ^1H NMR (200 MHz, $CDCl_3$) δ 1.10 (s, 3H), 1.26–1.77 (m, 10H), 2.11–2.33 (m, 4H), 2.67–2.75 (m, 2H), 3.49 (t, 1H, J = 7.5 Hz), 4.51–4.57–4.63–4.69 (AB system, 2H, J = 12.2 Hz), 5.09 (s, 2H), 6.87 (m, 2H), 7.32–7.46 (m, 10H), 7.67 (d, 1H, J = 9.4 Hz): ^{19}F NMR (188 MHz, $CDCl_3$) δ −81.5 (t, 3F, J = 10 Hz), −115.7 (m, 2F), −122.5 (m, 6F), −123.4 (m, 2F), −124.1 (m, 2F), −126.8 (m, 2F); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.5, 23.7, 25.6 (t, J = 21 Hz), 27.5, 28.9, 33.2, 34.4, 42.6, 50.4, 50.8, 51.4, 69.9, 71.7, 73.2, 89.0, 111.4, 115.3, 127.4, 127.6, 127.8, 128.3, 128.5, 129.6, 137.2, 139.1, 142.3, 156.8; MS (pos. ESI) (m/z): 935 $(M+Na)^+$.

*2.2.1.4. 3,17β-Bisbenzyloxy-11β-decyl-estra-1,3,5(10)-triene-11β-ol (**6e**).* From 0.85 mmol of 11-keto-bisbenzyloxyestradiol **5** (400 mg) using 5 equiv. of *n*-decylmagnesium bromide (1.05 M).

29% yield (102 mg); glass; ^1H NMR (200 MHz, $CDCl_3$) δ 0.92 (s, 3H, J = 6.48 Hz), 1.12 (s, 3H), 1.31–2.32 (m, 31H), 2.73 (m, 2H), 3.49 (t, 1H, J = 7.68 Hz), 4.60 (s, 2H), 5.07 (s, 2H), 6.80 (m, 2H), 7.34–7.44 (m, 10H), 7.72 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.5, 14.1, 22.7, 23.8, 24.3, 26.2, 27.6, 29.3, 29.4, 29.6, 30.1, 31.9, 34.3, 43.0, 44.3, 50.3, 50.8, 51.4, 69.8, 71.6, 75.6, 89.4, 111.3, 114.8, 127.3, 127.4, 127.8, 128.2, 128.4, 128.5, 130.5, 137.3, 139.2, 142.2, 156.5.

2.2.2. General procedure for the deoxygenation of 11β-alcohols as exemplified by the preparation of 3,17β-bisbenzyloxy-11β-hexyl-estra-1,3,5(10)-triene (7b)

Triethylsilane (0.76 mL, 35 equiv.) and boron trifluoride (1.524 mL, 90 equiv.) were added at 0 °C to a solution of alcohol **6b** (80 mg, 0.144 mmol) in dichloromethane (7.5 mL). After 30 min stirring at room temperature, the mixture

was quenched with a saturated aqueous solution of $NaHCO_3$ (3 mL). Steroidal compounds were extracted with dichloromethane (2× 5 mL). Organic layers were washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography using CH_2Cl_2/pentane (2:3) to yield the deoxygenated product as a glass.

*2.2.2.1. 3,17β-Bisbenzyloxy-11β-hexyl-estra-1,3,5(10)-triene (**7b**).* 77% yield (77 mg); glass; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 6.3 Hz), 1.03 (s, 3H), 1.22–1.72 (m, 17H), 1.88 (d, 1H, J = 12.3 Hz), 2.02 (m, 1H), 2.34–2.45 (m, 2H), 2.53 (dd, 1H, J = 10.4 Hz and 4.2 Hz), 2.70–2.86 (m, 2H), 3.50 (t, 1H, J = 7.5 Hz), 4.61 (s, 2H), 5.05 (s, 2H), 6.72 (d, 1H, J = 2.3 Hz), 6.72 (dd, 1H, J = 8.7 Hz and 2.3 Hz), 7.08 (d, 1H, J = 8.7 Hz), 7.32–7.47 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 15.1, 22.7, 23.1, 27.1, 28.0, 28.2, 28.3, 29.4, 30.4, 31.9, 34.2, 36.4, 39.6, 43.7, 49.6, 52.2, 69.9, 71.6, 89.6, 112.8, 114.5, 127.3, 127.7, 127.8, 128.2, 128.5, 130.7, 137.3, 138.9, 139.3, 156.2; MS (pos. ESI) (m/z): 575 $(M+K)^+$, 559 $(M+Na)^+$; $[\alpha]_D^{25}$ 55.2 (c 0.5, chloroform).

*2.2.2.2. 3,17β-Bisbenzyloxy-11β-(3,3,4,4,5,5,6,6,6-nonafluorohexyl)-estra-1,3,5(10)-triene (**7c**).* From 0.501 mmol of alcohol **6c** (350 mg) in the presence of 15 equiv. of Et_3SiH (2.6 mL) and 30 equiv. of $BF_3.Et_2O$ (5.1 mL). 80% yield (280 mg); glass; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (s, 3H), 1.19–1.71 (m, 9H), 1.92–2.48 (m, 6H), 2.63 (dd, 1H, J = 10.4 Hz and 3.4 Hz), 2.76–2.93 (m, 2H), 3.53 (t, 1H, J = 7.5 Hz), 4.57–4.61–4.66–4.70 (AB system, 2H, J = 12.1 Hz), 5.08 (s, 2H), 6.77 (s, 1H), 6.86 (d, 1H, J = 8.6 Hz), 7.07 (d, 1H, J = 8.6 Hz), 7.34–7.43 (m, 10H); ^{19}F NMR (188 MHz, $CDCl_3$) δ −81.5 (t, 3F, J = 10 Hz), −114.9 (m, 2F), −124.7 (m, 2F), −126.5 (m, 2F); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.2, 19.1, 23.0, 26.9, 27.9, 29.7 (t, J = 22 Hz), 30.3, 34.1, 36.1, 39.3, 43.5, 49.4, 51.9, 69.9, 71.6, 89.4, 113.0, 114.9, 127.1, 127.3, 127.4, 127.5, 127.9, 128.3, 128.5, 129.6, 137.2, 139.0, 139.2, 156.5; MS (pos. ESI) (m/z): 721 $(M+Na)^+$.

*2.2.2.3. 3,17β-Bisbenzyloxy-11β-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-decyl)-estra-1,3,5(10)-triene (**7d**).* From 0.332 mmol of alcohol **6d** (300 mg) in the presence of 15 equiv. of Et_3SiH (0.8 mL) and 30 equiv. of $BF_3.Et_2O$ (1.52 mL). 51% yield (151 mg); glass; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01 (s, 3H), 1.27—1.67 (m, 10H), 1.87–2.43 (m, 4H), 2.60 (dd, 1H, J = 10.0 Hz and 3.6 Hz), 2.80 (m, 2H), 3.49 (t, 1H, J = 7.5 Hz), 4.51–4.57–4.60–4.66 (AB system, 2H, J = 12.3 Hz), 5.04 (s, 2H), 6.72 (s, 1H), 6.80 (d, 1H, J = 8.5 Hz), 7.03 (d, 1H, J = 8.5 Hz), 7.29–7.43 (m, 10H); ^{19}F NMR (188 MHz, $CDCl_3$) δ −81.2 (t, 3F, J = 10 Hz), −114.8 (m, 2F), −122.4 (m, 6F), −123.2 (m, 2F), −123.7 (m, 2F), −126.6 (m, 2F); ^{13}C NMR (50 MHz, $CDCl_3$) δ 15.2, 19.1, 23.1, 26.9, 27.9, 29.7 (t, J = 16 Hz), 29.8, 30.3, 34.1, 36.2, 39.4, 43.5, 49.4, 52.0, 69.9, 71.7, 89.4, 113.0, 114.9, 127.2, 127.4, 127.5, 127.9, 128.3, 128.5, 129.7, 137.2, 139.0, 139.2, 156.5; MS (ESI pos.) (m/z): 921 $(M+Na)^+$, 899 $(M+H)^+$.

*2.2.2.4. 3,17β-Bisbenzyloxy-11β-decyl-estra-1,3,5(10)-triene (**7e**).* From 0.167 mmol of alcohol **6e** (102 mg). 51% yield (56 mg); glass; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (s, 3H, J = 6.37 Hz), 1.02 (s, 3H), 1.27–1.68 (m, 26H), 1.85–2.05 (m, 2H), 2.32–2.55 (m, 2H), 2.76–2.84 (m, 2H), 3.48 (t, 1H, J = 7.56 Hz), 4.61 (s, 2H), 5.04 (s, 2H), 6.71–6.82 (m, 2H), 7.08 (d, 1H, J = 8.28 Hz), 7.34–7.44 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 15.3, 22.7, 23.1, 27.1, 28.0, 28.2, 28.4, 29.4, 29.6, 29.7, 29.8, 30.4, 31.9, 34.2, 36.4, 39.6, 43.7, 49.6, 52.2, 69.8, 71.6, 89.6, 112.8, 114.5, 127.2, 127.3, 127.5, 127.7,127.8,128.3, 128.5,130.7, 137.4, 138.9, 139.2, 156.2

*2.2.3. General procedure for catalytic hydrogenations as exemplified by the preparation of 3,17β-dihydroxy-11β-hexyl-estra-1,3,5(10)-triene (**8b**)*

To a solution of the bisbenzylated steroid **7b** (0.045 mmol) in MeOH (2 mL) was added 10% Pd/C (6 mg). The substrate was hydrogenated overnight under 1 atm. The mixture was then filtered on celite® and the filtrate was evaporated to afford the product.

*2.2.3.1. 3,17β-Dihydroxy-11β-hexyl-estra-1,3,5(10)-triene (**8b**).* 98% yield (32 mg); white solid: mp 157.2–158.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79–0.92 (m, 6H), 1.16–1.68 (m, 18H), 1.80 (d, 1H, J = 12.3 Hz), 1.97 (m, 1H), 2.19 (d, 1H, J = 13.5 Hz), 2.45–2.54 (m, 2H), 2.65–2.85 (m, 2H), 3.71 (t, 1H, J = 7.3 Hz), 6.55 (s, 1H), 6.64 (d, 1H, J = 8.3 Hz), 7.01 (d, 1H, J = 8.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.3, 22.7, 23.1, 27.0, 28.1, 28.2, 28.4, 30.2, 30.5, 31.9, 34.4, 36.2, 38.3, 43.5, 49.6, 52.0, 83.3, 113.1, 115.2, 127.9, 130.4, 139.2, 152.7; MS pos. ESI (m/z): 379 $(M+Na)^+$; $[\alpha]_D^{25}$ 67.4 (c 0.54, chloroform); (HRMS ESI Neg.) obsd 355.2637, calcd 355.2649 (+ 3.5 ppm) ($C_{24}H_{35}O_2$).

*2.2.3.2. 3,17β-Dihydroxy-11β-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-decyl)-estra-1,3,5(10)-triene (**8c**).* From 0.064 mmol of bisbenzylated steroid **7c** (58 mg). 91% yield (37 mg); white solid: mp 189.9–190.5 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 0.91 (s, 3H), 1.09–2.07 (m, 14H), 2.49–2.80 (m, 4H), 3.30 (m, 2H), 3.65 (t, 1H, J = 7.89 Hz), 6.50 (s, 1H), 6.58 (dd, 1H, J = 8.31 Hz and 2.20 Hz), 6.98 (d, 1H, J = 8.31 Hz); ^{19}F NMR (188 MHz, CD_3OD) δ −78.9 (t, 3F, J = 10 Hz), −111.9 (m, 2F), −119.5 (m, 6F), −120.3 (m, 2F), −121.1 (m, 2F), −123.8 (m, 2F); ^{13}C NMR (50 MHz, CD_3OD) δ 17.6, 22.8, 26.5, 30.7, 33.2, 33.4 (t, J = 21Hz), 33.8, 38.5, 39.8, 42.1, 47.1, 53.2, 55.5, 86.2, 116.9, 118.9, 130.9, 131.9, 142.7, 158.3; $[\alpha]_D^{25}$ 75.2 (c 0.38, methanol); HRMS (ESI Neg.) obsd 717.1627, calcd 717.1661 (−4.8 ppm) ($C_{28}H_{26}O_2F_{17}$).

*2.2.3.3. 3,17β-Dihydroxy-11β-(3,3,4,4,5,5,6,6,6-nonafluorohexyl)-estra-1,3,5(10)-triene (**8d**).* From 0.329 mmol of bisbenzylated steroid **7d** (230 mg). 72% yield (122 mg); white solid: mp 213.0–213.6 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 0.93 (s, 3H), 1.18–1.71 (m, 12H), 1.87 (d, 1H, J = 12.3 Hz), 2.02–2.56 (m, 5H), 2.63–2.84 (m, 2H), 3.68 (t, 1H, J = 7.9 Hz), 6.53 (s, 1H), 6.62 (dd, 1H, J = 8.46 Hz and 2.49 Hz), 6.98 (d, 1H, J = 8.46 Hz); ^{19}F NMR (188 MHz, CD_3OD) δ −79.2 (t, 3F, J = 10 Hz), −112.1 (m, 2F), −122.1 (m, 2F), −123.8 (m, 2F); ^{13}C NMR (75 MHz, CD_3OD) δ 15.2, 20.2, 24.0, 28.2, 30.7, 30.8 (t, J = 22 Hz), 31.3, 35.9, 37.3, 39.6, 44.6, 50.7, 53.0, 83.7, 114.4, 116.4, 128.4, 129.5, 140.2, 155.8; MS pos. ESI (m/z): 541 $(M+Na)^+$; $[\alpha]_D^{25}$ 75.2 (c 0.38, methanol); HRMS (ESI Neg.) obsd 517.1772, calcd 517.1789 (−3.3 ppm) ($C_{24}H_{26}O_2F_9$).

*2.2.3.4. 3,17β-Dihydroxy-11β-decyl-estra-1,3,5(10)-triene (**8e**).* From 0.094 mmol of bisbenzylated steroid **7e** (56 mg). 80% yield (37 mg); white solid: mp 146.4–147.2 °C; ^{1}H NMR (200 MHz,

$CDCl_3$) δ 0.86 (s, 3H, J = 6.58 Hz), 0.90 (s, 3H), 1.25–2.93 (m, 34H), 3.67 (t, 1H, J = 7.56 Hz), 6.53 (s, 1H), 6.58 (d, 1H, J = 8.34 Hz), 6.97 (d, 1H, J = 8.34 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0, 14.2, 22.6, 23.0, 27.0, 28.0, 28.2, 29.2, 29.5, 29.6, 29.7, 30.1, 30.2, 34.4, 36.1, 38.2, 43.4, 48.4, 48.9, 50.2, 51.9, 83.0, 113.0, 115.1, 127.7, 129.5, 138.9, 153.3; MS-CI CH_4 (m/z) 413 $[M+H]^+$, 395 $[MH–H_2O]^+$; $[\alpha]_D^{25}$ 70.1 (c 0.4, chloroform); HRMS (ESI Neg.) obsd 411.3268, calcd 411.3263 (+1.3 ppm) ($C_{28}H_{43}O_2$).

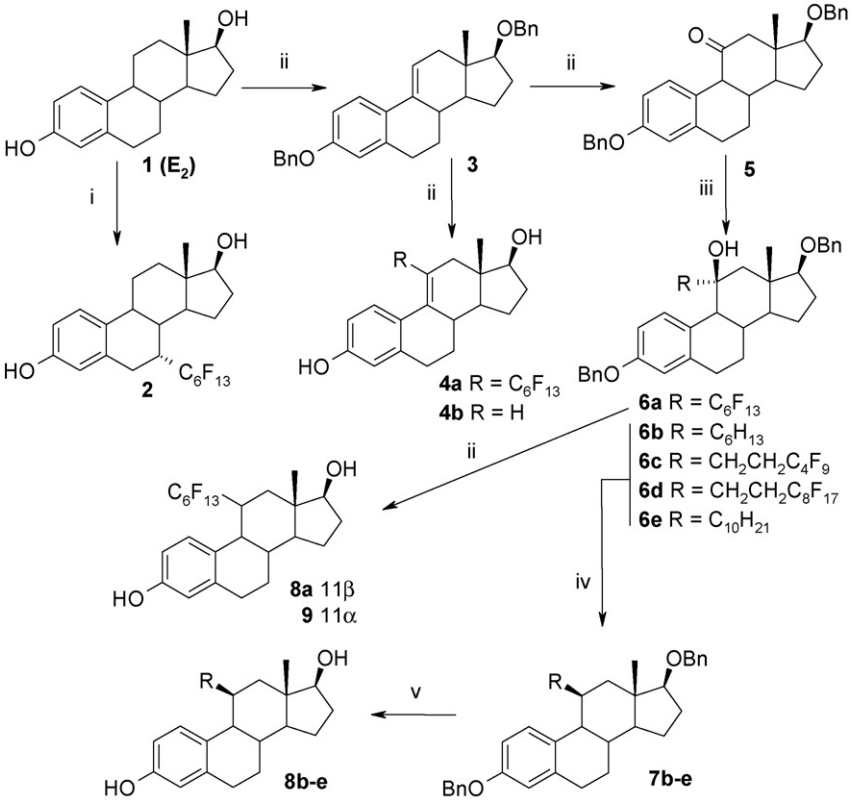
2.3. *Binding affinity for ERα*

Binding affinity of investigated compounds was performed in a semi-solid radiometric assay based on the ability of ERα to adsorb on hydroxylapatite (HAP) at low ionic strength [29]. For that purpose, highly purified recombinant hERα (Calbiochem, Euro-Biochem, Bierges, Belgium) diluted in a buffered bovine serum albumin solution was adsorbed onto HAP (hERα dilution: 1:500 in 10^{-2} M Tris–HCl pH 8 containing 1 mg/mL BSA) [29]. After removal of unbound material by centrifugation, HAP was incubated overnight at 0 °C with 10^{-9} M $[^3H]E_2$ (GE Healthcare Biosciences, NL) in the presence or absence of increasing amounts of unlabeled E_2 (Sigma, St. Louis, MO) or investigated steroid derivatives. Radioactivity adsorbed onto HAP was then extracted with ethanol and measured by liquid scintillation counting. Relative concentrations of E_2 and investigated compound required to reduce the binding of $[^3H]E_2$ by 50% gave its RBA value; RBA = ($[I_{50}]E_2/[I_{50}]$ compound) × 100. For key compounds, incubation with $[^3H]E_2$ was also performed at 25 °C in order to assess the influence of the temperature on RBA values. Assays were performed at least twice, each time in duplicates. In each competition curve, variations between bound $[^3H]E_2$ values were extremely low (mean ± S.D = 3), indicating a very high reproducibility of our data.

3. Results and discussion

3.1. *Synthesis*

We earlier described the synthesis of 7α-perfluorohexylestradiol (**2**) (Scheme 1) [14] starting from estradiol (**1**). This compound was obtained through perfluoroalkylation of an enolsilylether derived from 6-ketoestradiol [30] with FITS-6 (tridecafluorohexyl-phenyliodonium trifluoromethanesulfonate) reagent [31]. Compound **4** resulted from the perfluoroalkylation of 9(11)-dehydroestradiol (**3**) [32] with the same FITS-6 reagent [15]. All other estradiol derivatives reported herein were issued from the same key ketone **5** [15] prepared via **3** essentially by adaptation of published methods [32,33]. Thus, following a route already devised by Napolitano et al. [33], condensation of ketone **5** with the corresponding Grignard reagent led to the 11β-alcohols **6a–e**. It is worth noting that condensation yields were closely related to the stability of the Grignard reagent used. The less stable perfluoroalkylated organometallic reagent [34] afforded compound **6a** in 51% yield. Partially fluorinated chain derived reagents gave slightly lower results (36 and 46%) whereas



Scheme 1 – Synthetic routes leading to compounds 2, 4, 8a–d, and 9. (i) See ref. [14]; (ii) See ref. [15] and text; (iii) RMgBr, THF 22–51%; (iv) Et_3SiH, $BF_3.Et_2O$, CH_2Cl_2, 0 °C, 5 mn 51–80%; (v) H_2, Pd/C, MeOH 72–98%.

simple alkyl Grignard species gave consistently lower yields in this reaction (22 and 29%).

Further ionic deoxygenation of tertiary alcohols **6b–e** with triethylsilane and boron trifluoride etherate was achieved with total inversion of configuration at the C-11 center to the corresponding 11β-alkylsteroids **7b–e**. Hydrogenolysis of the benzyl protecting groups readily afforded the 11β-alkylestradiol derivatives **8b-e**. Because perfluoroalkylated groups are known to show a strong destabilizing effect on adjacent incipient carbocations [35,36], the perfluorohexyl alcohol **6a** could not be deoxygenated by the ionic procedure already used for the other molecules. For this particular alcohol, we had to resort to a radical deoxygenation [15,37], leading, after uneventful deprotection, to a 50:50 mixture of the 11β (**8a**) and 11α (**9**) isomeric perfluorohexylestradiols [15]. The stereochemical outcome of this reduction is reminiscent to the conformational memory effect observed in the tetrahydropyran series [38].

3.2. *Influence of perfluoalkylation on estradiol binding affinity to ERα*

Binding affinity of investigated compounds was determined by a conventional [^{3}H]E_2 competitive binding assay. As shown in Table 1, all compounds displayed a lower binding affinity than E_2 (RBA, E_2 = 100) when the assay was performed at 0 °C. Three factors susceptible to generate such a decrease were analyzed: the degree of fluorination of the side chain, its orientation within the ligand binding pocket and the temperature of the assay.

3.2.1. *Degree of fluorination of the side chain*

Overall, binding affinities associated with non-fluorinated chains were found to be significantly higher than their fully or highly fluorinated counterparts (compare entries for compound **8b** *vs.* **8a** and **8d** *vs.* **8e**). 7α (**2**) and 11β (**8a**) C_6F_{13} derivatives displayed RBA values of the same order of magnitude, confirming the equivalency of these positions with regard to association with ERα [16,17,21]. Increase of chain length appeared also detrimental for binding (compounds **8d** and **8e**), as already reported for long alkyl chains without a polar terminal group [18,20,39].

3.2.2. *Orientation of the side chain*

For the C_6F_{13} side chain, the lack of stereochemistry at position 11 was associated with a drastic loss of binding affinity (**4a**, RBA < 0.1; compare with **4b**, RBA = 50, and with **8a**, RBA = 3). Also noteworthy, RBA value for 11α-perfluorohexylestradiol (**9**) seemed astonishingly high for a derivative with such a chain length. Nevertheless, in view of the lack of reported data for 11α-alkylestradiol we cannot infer if this value is intrinsic to this substitution or a consequence of fluorination. As expected, the binding affinity of **9** was abolished by the additional introduction of a polar substituent in the 11β position [40] (RBA for 11β-OH E_2 ca. 3 [18]): the debenzylated derivative of **6a** did not show any binding to ERα when assayed at 1000-fold excess concentration with regard to [^{3}H] E_2.

3.2.3. *Temperature of incubation*

Assays performed at 25 °C rather than 0 °C were reported to provide higher RBA values for E_2 derivatives substituted with small hydrophobic groups [18], suggesting an increased plasticity of the receptor under higher thermal conditions. Remarkably, such a property recorded here for **8b** (C_6H_{13}) was not observed with either **8a** (C_6F_{13}) or with **8c** ($CH_2CH_2C_4F_9$), clearly indicating that perfluorination of the side chain affects ERα binding even under this especially favorable condition (Fig. 1, Table 1). Interestingly, the 7α analog (**2**) of **8a** similarly failed to show any increase of binding affinity at 25 °C reinforcing the concept of equivalency of these positions for association with the receptor.

3.3. *Specific interactions of fluorinated side chains with ERα*

Overall, these data reveal that, at equal length, a non-fluorinated alkyl chain would more easily fit within the ERα binding pocket than its fully fluorinated counterpart. This

Table 1 – Relative binding affinities (RBA) for ERα

Compound	Substituents	RBA (0 °C)	I_{1000}[a] (%)	RBA (25 °C)
1 (E_2)	–	100		100
2	7α-C_6F_{13}	1[b]		0.6
4a	9,11-Dehydro; 11-C_6F_{13}	<0.1	20	
4b	9,11-Dehydro	50		
6a[c]	11α-C_6F_{13}; 11β-OH	nb[d]	0	
8a	11β-C_6F_{13}	3		3
8b	11β-C_6H_{13}	10		100
8c	11β-$CH_2CH_2C_4F_9$	10		10
8d	11β-$CH_2CH_2C_8F_{17}$	nb[d]	0	
8e	11β-$C_{10}H_{21}$	<0.1	20	
9	11α-C_6F_{13}	0.5		

[a] [^{3}H]E_2 binding inhibition provoked by a 1000-fold excess of compound at 0 °C.
[b] The present value for **2** is about 10-fold higher than in our earlier work [14]. This difference may be ascribed to differences in experimental protocols (i.e., DCC and whole cell assays in the past *versus* HAP assay at present).
[c] Tested as its debenzylated derivative.
[d] No binding.

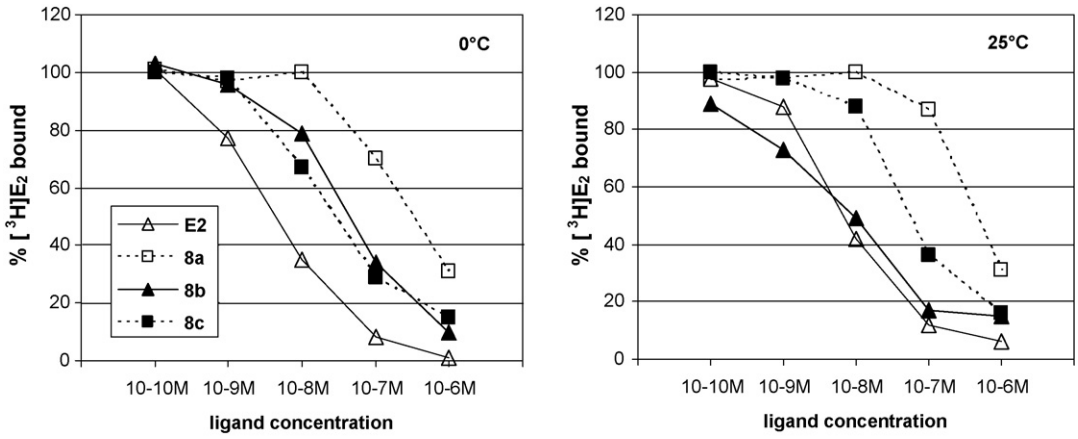


Fig. 1 – Effect of the incubation temperature upon the ability of compounds 8a-c and E_2 to compete with $[^3H]E_2$ for binding to ERα.

property may reside in bulkiness and enhanced rigidity of the fluorinated chain as well as to the intrinsic oleophobic nature of perfluoroalkyl residues. It is difficult to identify the individual contribution of these factors because steric hindrance is interrelated with the hydrophobic effect which depends either upon volume or molecular surface area [41,42]. On geometrical grounds, fluorinated substituents are considered bulkier than alkyl ones [43,44] (in fact, this is true [45]). Bulk size of perfluoroalkyl chains of our compounds would not be the main factor affecting E_2 binding as revealed by RBA values established at 0 °C, i.e., **8a** (R = C_6F_{13}, RBA = 3), **8b** (R = C_6H_{13}, RBA = 10), **8c** (R = $CH_2CH_2C_4F_9$, RBA = 10) (see Table 1). Thus, the partially fluorinated compound (**8c**) displays the same binding affinity than the non-fluorinated one (**8b**) whilst exhibiting ca. 70% (9/13) of the steric bulk of **8a** provoked by the presence of fluorine atoms. Interestingly, the presence of a two methylene spacer in the chain of **8c**, which reduces its rigidity, restores the binding ability of the non-fluorinated compound **8b** (RBA = 10). This observation highly suggests that the low flexibility of the hexafluoro side chain of **8a** [46,47] may be the clue of its low binding. Comparison of RBA values recorded at 25 °C instead of 0 °C supports this view. Thus, the value for hexylestradiol (**8b**) greatly enhanced with the increase of temperature (10 *vs.* 100), while values of perfluorohexyl (**8a**) and nonafluorobutyl (**8c**) derivatives remained constant. Hence, rigidity of the fluorinated chain would counteract relocation of the steroid within the binding pocket even under thermal conditions that would logically increase plasticity of the latter. Alternatively, rigidity may perhaps impede this putative increase of plasticity.

In fact this observation is in agreement with a study by Whitesides' group relevant to the binding of alkylated or perfluoroalkylated sulfonamides to carbonic anhydrase [41]. These authors reported higher binding affinities upon fluorination that they attributed solely to the associated increase of hydrophobicity (better affinities of the fluorinated compounds at equal chain length generated by their larger areas of hydrophobic surfaces). Such data let DiMagno to propose that "general enhancement of molecular recognition is expected to be larger if hydrophilic groups are replaced by fluorocarbon moieties or if the fluorocarbon segment is incorporated into the binding pocket" [48]. If one considers that the binding site of carbonic anhydrase possesses a fairly open structure [49], while the ER binding pocket may be envisioned as more closely packed (resulting from the reorganization in the tertiary structure of the receptor upon hormone binding) [18,50,51], an increase of the binding to ERα is expected upon fluorination. Our observation of a reverse response indicates that this favorable factor was not fully operative for our compounds and may be largely compensated by other factors intrinsic to fluorination. In this regard, besides increased stiffness, considering the bulk of present fluorinated chains, it seems that they may lie in very close vicinity of the hydrophobic envelope of binding pocket exacerbating fluorophobic interactions able to decrease binding [7]. Again, this potential effect is very difficult to quantify because it is related to the steric factors *via* the size of the substituent and thus also to hydrophobicity.

4. Conclusion

To our knowledge this work is the first attempt to delineate some factors, inherent to fluorination, susceptible to influence binding to ERα of estradiol derivatives bearing heavily fluorinated side chains. We suggested that increased stiffness of fluorinated chains and perhaps also fluorophobic interactions are crucial factors governing this binding. Based on these findings, we also demonstrated that a C-4 fluorinated segment insulated from the steroidal core by a two methylene spacer in an 11β side chain is able to mimic the behavior of the corresponding non-fluorinated analog.

With regard to the hormonal property of these new estradiol derivatives, we may refer to our previous study showing that the 7α-perfluorohexylestradiol (**2**) maintains a weak capacity to enhance ERE-dependent transcription and to stimulate cell growth in the MCF-7 breast cancer cell line, with a concomitant ability to down-regulate ERα [14]. One may anticipate that perfluorination in 11β may similarly decrease estrogenic potency of the hormone. A study is presently carried out to confirm or infirm this hypothesis. Synthesis of functionalized side chains derivatives has also been initiated

with the aim of producing compounds with potential antiestrogenicity.

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