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Synthesis and evaluation of
**2-Halogenated-1,1-Bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)-ethylenes as potential estrogen receptor-
targeted radiodiagnostic and radiotherapeutic agents**

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Abstract

A series of three 1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)-ethylene derivatives was prepared and evaluated as potential estrogen receptor imaging agents. The compounds display high binding affinity compared to estradiol, with the 2-iodo and 2-bromo-derivatives expressing higher affinity than the parent 2-nonhalogenated derivative. Evaluation in immature female rats also indicate that the compounds were all full estrogenic agonists with potencies in the same order of activity (I~Br>H). Computational analysis of the interactions between the ligands and ER α -LBD demonstrated positive contribution of halide to binding properties. In preparation for studies using the radiohalogenated analogs, the corresponding protected 2-(tributylstannyl) derivative was prepared and converted to the corresponding 2-iodo-product.

Key words

Nonsteroidal estrogens, relative binding affinity, uterotrophic growth, Auger, synthesis

1. Introduction

As part of our program to develop new agents for the diagnosis and treatment of hormone responsive breast and ovarian cancer, we have focused on the preparation and evaluation of radio-iodinated estrogens. Our strategy is based on the mechanism of action of the estrogens that involves selective binding of ligands to the hormone receptor within the nuclear compartment of the cell.^[1-6] The observation that the resultant complex is long-lived provides the basis for imaging estrogen receptor expressing tissues with appropriate radiohalogens.^[7-12] Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are capable of non-invasively detecting ER-overexpressing tissues, e.g., certain breast cancers, from non-expressing tissue. That the ligand-receptor complexes are intimately associated with the nuclear DNA also makes the process amenable to the delivery of Auger-emitting radionuclides, such as iodine-123,-125, and bromine-80m,

as potential radiotherapeutic agents. The deposition of low energy Auger electrons in the vicinity of the nuclear DNA results in localized and potentially lethal defects to the target cancer cells without significant effects on adjacent normal cells.^[13-21] Therefore, one can generate cell death within a specific group of cells while leaving the larger population of normal cells intact. Estrogen responsive tumors, such as breast and ovarian carcinoma, over-express ER, thereby making them significantly more susceptible to the effects of estrogens that are labeled with an Auger-emitting radionuclide. Initial studies with radio-iodinated derivatives of steroidal and nonsteroidal estrogen receptor ligands have demonstrated this effect in estrogen receptor-containing cells but not in cells deficient in the estrogen receptor protein.^[22-30]

Although many high affinity estrogen receptor-targeted ligands have been prepared and evaluated, relatively few are structurally amenable to labeling with radiohalogens. Within both the steroidal and nonsteroidal classes of estrogens, introduction of iodine on the phenolic ring results in a substantial reduction of binding affinity as well as increased binding to nontarget proteins.^[31-34] [Figure 1] Incorporation of

[Insert Figure 1]

radioiodine or radiobromine at an alkyl (sp^3) position, such as the $16\alpha/\beta$ site, while tolerated by the receptor, is unstable *in vivo* due to the weak C-I/Br bond. Therefore, placement of the radioiodine on an olefinic (sp^2) bond imparts greater (bio)chemical stability as well as retention of high receptor binding properties. These observations were exploited in our preparation and evaluation of the 17α -iodovinyl estradiol derivatives.^[35-38] In their radioactive form, these compounds demonstrated both an avidity for and cytotoxicity to ER- expressing cells.

In parallel to our work on steroidal estrogens, we also undertook the evaluation of radiohalogenated nonsteroidal estrogens.^[23, 39-43] [Figure 2] Initial studies indicated that 2- iodo-1,1-[bis(4-hydroxyphenyl)]-2-

[Insert Figure 2]

phenyl ethylene (I-BHPPE) possessed significant affinity for estrogen receptors and, in its radioactive form, avidity for estrogen receptor containing tissues. However, we considered that receptor affinity needed to be

enhanced in order to achieve sufficient delineation of the target tissues. Although 2-halogenated tris-1,1,2-(4-hydroxyphenyl) ethylenes had been reported, they are particularly susceptible to metabolic oxidation, generating quinoid intermediates that undergo dehalogenation. As a result, the 2-(3-hydroxyphenyl)-analog of I-BHPPE was prepared, which demonstrated better affinity for the estrogen receptor.^[40] Preliminary results were highly encouraging and therefore it was necessary to prepare more material not only for further biological assays, but to provide precursors for its use in radiological studies.

The synthetic strategy was based upon the general synthesis of triarylethylenes.^[44, 45] (Scheme 1) It was of further interest because the preparation of 1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylenes, unlike the corresponding 2-(4-hydroxyphenyl) isomers, had not been well described.^[46] Although one can halogenate the internal olefinic position and then demethylate the protecting ether groups to yield the target compound, this procedure would be unacceptable for “no-carrier-added” synthesis of the radiolabeled product. The need for high specific activity and mild deprotection methods requires the use of the trialkylstannyl substituent to introduce the radiohalogen and trialkylsilyl ethers to protect the nascent phenolic hydroxyls. As we envisioned the process, the synthesis of the ultimate radiolabeled compound would proceed via the intermediary 2-bromo derivative which could be transmetallated to give the stannyl precursor which in turn would undergo radiohalogenation. In this study we describe the synthesis of the 2-bromo- and 2-iodo-1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylenes, their binding affinity for the estrogen receptor alpha ligand binding domain (ER α -LBD), and *in vivo* uterotrophic growth activity. In anticipation of subsequent radiochemical studies, the preparation of the corresponding tributylstannylated precursor for the I-123/125-analogs was developed. In addition we describe a computational analysis of the complexes of 1,1-bis(hydroxyphenyl)-2-phenyl and 1,1,2-tris(4-hydroxyphenyl) ethylenes, diethyl stilbestrol, estradiol, an 11 β -(4-hydroxyphenyl) estradiol and our compounds with the ER α -LBD to provide a more rational explanation for the observed results which would permit subsequent modifications, if necessary, in the structures.

2. Experimental Section

General Methods. All reagents and solvents were purchased from Aldrich Chemicals or Fisher Scientific. THF and toluene were distilled from sodium-benzophenone. Reactions were monitored by TLC on 0.2 mm silica gel plastic backed sheets containing F-254 indicator. Visualization on TLC was achieved using UV light, iodine vapor and/or phosphomolybdic acid reagent. Column chromatography was performed with 32-63 μ m silica backing or by automated flash chromatography [Flashmaster Solo, Argonaut]. Melting points were determined using a Thermo Scientific Meltemp capillary melting point apparatus and are uncorrected. NMR spectra chemical shifts are reported in part per million downfield from TMS and referenced either to TMS internal standard for deuteriochloroform (DCCl_3) or hexadeuteroacetone (CD_3COCD_3) solvent peak. Coupling constants are reported in hertz. High resolution mass spectra were obtained from University of Massachusetts, Amherst. LC-MS were obtained at Northeastern University. Compounds analyzed by MS were >95% pure by HPLC. All other compounds gave satisfactory elemental analyses, $\pm 0.4\%$, (Desert Analytics Laboratory, Tucson, AZ) unless otherwise stated.

2.1. Chemistry.

1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene 2 (BMPMPE)

Mg turnings (5.17 g, 0.21 mol) were added to anhydrous diethyl ether (freshly distilled and dried, 30 mL), followed by 5 drops of 3-methoxy benzyl bromide and a crystal of iodine. The reaction mixture was heated at 40 $^{\circ}\text{C}$ for 15 min at which time a cloudy, metallic precipitate appeared. 3-Methoxy benzyl bromide (0.030 mol in 50 mL anhydrous Et_2O) was added dropwise over 2 h. 4,4'-Dimethoxybenzophenone **1** (5.55 g, 0.023 mol) in anhydrous THF (freshly distilled and dried, 80 mL) was added dropwise to the solution over 30 min and the reaction mixture was stirred for 12 h. Saturated NH_4Cl (30 mL) was added and the resulting solution was filtered to remove unreacted magnesium salts. The aqueous solution was extracted with EtOAc (250 mL) and the organic phase was washed with water (10 X 100 mL), dried (anh MgSO_4) and evaporated to dryness to yield a colorless, viscous oil (8.3 g). Recrystallization from methanol yielded white needle crystals (6.65 g, 0.018 mol, 79%).

The purified alcohol (4.00 g, 11.0 mmol) was dissolved in CH₂Cl₂ (freshly dried and distilled, 30 mL). Trifluoroacetic anhydride (5 mL, 7.43 g, 35 mmol) was added dropwise over 5 min and the reaction was heated at 50-60 °C for 3 h under Ar. The reaction solution was cooled to ambient temperature, poured into a mixture of EtOAc (250 mL) and ice-water (500 mL) and separated. The organic layer was washed with twice with 200 mL NaHCO₃ (satd), followed by water (10 X 200 mL). The organic solution was dried (anh MgSO₄) and evaporated to dryness to give a yellow oil (3.79 g). The crude product was purified by flash column chromatography (330 g of silica gel) using hexane/Et₂O (9.5:0.5) as the eluent. Fractions containing product were combined and evaporated to dryness to give a yellow solid (3.77 g, 10.9mmol, 99%). mp 69-71 °C; ¹H NMR (500 MHz, CDCl₃) 7.26 (2H, dd, *J*_d = 8.5, *J*_d = 2.0), 7.13 (2H, dd, *J*_d = 9.0, *J*_d = 2.0), 7.06 (1H, dd, *J*_d = 8.0, *J*_d = 7.5), 6.87 (2H, dd, *J*_d = 9.0, *J*_d = 2.00) 6.85 (2H, dd, *J*_d = 8.5, *J*_d = 2.00), 6.82 (1H, s, C=CH), 6.68 (1H, dd, *J*_d = 8.00, *J*_d = 2.0), 6.65 (1H, dd, *J*_d = 8.0, *J*_d = 2.0), 6.56 (1H, t, *J*_f = 2.00), 3.83 (3H, s), 3.81 (3H, s), 3.56 (3H, s) ¹³C NMR (75 MHz, CDCl₃) 159.24, 159.01, 158.94, 141.98, 139.14, 136.22, 132.75, 131.59 (2C), 128.86, 128.82 (2C), 126.05, 122.26, 114.00 (2C), 113.81, 113.53 (2C), 112.88, 55.31, 55.25, 54.82

2-Bromo-1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene 3 (BrBMPMPE)

The 1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene **2** (3.77 g, 10.9 mmol) was dissolved in CH₂Cl₂ (freshly dried and distilled, 300 mL) and cooled with an ice-bath (0 °C). Br₂ (1.76 g, 11.0 mmol) was dissolved in CH₂Cl₂ (anh, 20 mL) and added dropwise over 10 min at 0 °C. The reaction mixture was stirred at 0 °C for 20 min, poured into ice-water (500 mL) and separated. The CH₂Cl₂ layer was washed once with 200 mL satd NaHCO₃, followed by water (3 X 500 mL), dried (anh MgSO₄) and evaporated to dryness to yield a colorless oil (3.94 g). ¹H NMR and TLC (hexane/Et₂O 7:3) indicated the presence of 3 Br-BMPMPE plus trace amounts of **2** and **4** [2-bromo-1,1-bis(4-methoxyphenyl)-2-(4-bromo-3-methoxyphenyl)ethylene Br₂-BMPMPE] (*R*_f of **2** = 0.54; *R*_f of **3** = 0.51, *R*_f of **4** = 0.44). The crude product was purified by silica gel column chromatography using as the eluting solvent a mixture of hexane and Et₂O (9.7:0.3). Fractions containing **3** were combined to yield a colorless oil that crystallized on standing (3.50 g, 8.2 mmol, 76%). Fractions containing primarily **4** were rechromatographed to provide pure material.

m.p. 106-108°C; ¹H NMR (500 MHz, CDCl₃) 7.33(1H, dd, *J*_d = 8.0, *J*_d = 2.0), 7.29 (2H, ddd, *J*_d = 8.0, *J*_d = 2.0, *J*_d = 2.0), 6.91-6.85 (6H, m, *J* = 8.0), 6.70 (1H, ddd, *J*_d = 8.0, *J*_d = 2.0, *J*_d = 2.0), 6.60 (2H, ddd, *J*_d = 8.0, *J*_d = 2.0, *J*_d = 2.0), 3.84 (3H, s), 3.71 (3H, s), 3.68 (3H, s); ¹³C (75 MHz, CDCl₃) 158.89, 158.73, 158.31, 142.64, 142.63,

1 136.13, 133.67, 131.48 (2C), 130.91 (2C), 128.83, 122.72, 119.88, 115.42, 113.66, 113.22 (2C), 113.05 (2C),
2 54.97, 54.91, 54.86
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5 Anal. Calcd for C₂₃H₂₁BrO₃: C, 65.12; H, 4.88; Br, 18.69. Found: C, 64.95; H, 4.98; Br, 18.79
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7 **2-Bromo-1,1-bis(4-methoxyphenyl)-2-(4-bromo-3-methoxyphenyl)ethylene 4 (Br₂BMPMPE)**

8 m.p. 100-102°C; ¹H NMR (500 MHz, CDCl₃) 7.35(1H, d, *J_d* = 8.0)), 7.33(2H, d, *J_d* = 8.0), 6.90-6.96 (5H,
9 overlapping m), 6.63(1H, dd, *J_d* = 8.0, *J_d* = 2.0), 6.60 (1H, d, *J_d* = 8.0)), 3.83(3H, s), 3.74(3H, s), 3.69(3H, s); ¹³C
10 (75 MHz, CDCl₃) 159.26, 158.86, 158.82, 144.59, 142.95, 135.37, 133.86, 133.08, 131.18(2C), 131.02(2C),
11 117.88, 117.22, 115.99, 114.30, 113.62(SC), 113.28(2C), 55.76, 55.46, 55.27
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16 **2-Iodo-1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene 5 (IBMPMPE)**
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18 To the solution of **2** (0.57 g, 1.6 mmol) in 10 mL DMF was added N-iodosuccinimide (0.40 g, 1.8
19 mmol) and the reaction was warmed at 90°C for 1 h. As TLC indicated partial conversion to product, an
20 additional equivalent of NIS was added and the reaction was heated for 15 h. The solution was cooled,
21 partitioned between aqueous NaHSO₃ (5%) and ethyl acetate. The aqueous phase was extracted twice with ethyl
22 acetate. The organic layers were combined, washed with water, brine, and dried over magnesium sulfate,
23 filtered and evaporated to dryness. The resulting residue was separated by column chromatography, eluting with
24 hexane-ethyl acetate (4:1), Because of the small differences in mobility between the **2**, **5** and **6** products, clear
25 separations were difficult. Fractions enriched in **5** were rechromatographed and crystallized from methanol to
26 provide samples for characterization and for conversion to the corresponding demethylated product. The yield
27 of isolated product **5** was 0.28 g (0.60 mmol, 37%). Fractions enriched in **6** were also rechromatographed to
28 provide pure material and crystallized from methanol.
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44 m.p. 140-141°C; ¹H NMR (500 MHz, CDCl₃) 7.54 (1H, d, *J_d* = 8.5), 7.26 (2H, d, *J_d* = 8.5), 7.12 (2H, d, *J_d* =
45 8.5), 6.88 (2H, d, *J_d* = 8.5), 6.85(2H, d, *J_d* = 8.5), 6.77 (1H, s), 6.48(1H, dd, *J_d* = 8.5, *J_d* = 2.0), 6.43 (1H, d, *J_d* =
46 2.0), 3.83 (3H, s), 3.82(3H, s), 3.50 (3H, s); ¹³C (75 MHz, CDCl₃) 159.66, 159.35, 157.58, 143.00, 139.69,
47 138.99, 136.03, 132.73, 131.75(2C), 129.03(2C), 125.30, 124.37, 114.42, 113.85(2C), 111.77(2C), 83.55,
48 55.94, 55.56, 55.53
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52 **2-Iodo-1,1-bis(4-methoxyphenyl)-2-(4-iodo-3-methoxyphenyl)ethylene 6 (I₂BMPMPE)**

53 m.p. 128-132°C; ¹H NMR (500 MHz, CDCl₃) 7.55 (1H, d, *J_d* = 8.0), 7.25 (3H, d, *J_d* = 8.0), 6.90 (2H, d, *J_d* =
54 8.0), 6.84 (2H, d, *J_d* = 8.0), 6.72 (1H, d, *J_d* = 2.0), 6.69 (1H, dd, *J_d* = 8.0, *J_d* = 2.0), 6.59 (2H, d, *J_d* = 8.0), 3.84
55 (3H, s), 3.71 (3H, s), 3.69 (3H, s); ¹³C (75 MHz, CDCl₃) 159.29, 158.73, 157.61, 149.93, 146.63, 139.79,
56 139.07, 133.24, 131.76, 130.96, 125.00, 113.70, 113.50, 113.45, 97.99, 84.96, 56.48, 55.47, 55.37
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61 **1,1-Bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene 7 (BHPHPE)**
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The 1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene **2**, (1.40g, 4.0 mmol) was dissolved in CH₂Cl₂ (freshly dried and distilled, 5 mL). BBr₃ (1.0 M in CH₂Cl₂, 25 mL, 25 mmol) was added dropwise at –78 °C over 15 min. to give a bright red solution. The reaction solution was stirred at –78 °C for 0.5 h, warmed to ambient temperature and stirred for 8 h. The solution was cooled to -78 °C and ice-water (20 mL) was added dropwise over 10 min. The resulting mixture was partitioned between ice-water (500 mL) and ethyl acetate (250 mL). The organic layer was washed with water (10 X 200 mL), dried and evaporated to dryness to yield a yellow oil. Flash chromatography on silica gel using hexane-ethyl acetate (2:1) gave the pure 1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene **7** (0.92g, 3.0 mmol, 75%).
m.p. 166-169°C; ¹H NMR (500 MHz, CD₃OD) 7.15(1H, d, *J_d* = 8.5), 7.11(2H, d, *J_d* = 8.5), 6.93 (2H, d, *J_d* = 8.5), 6.73(2H, d, *J_d* = 8.5), 6.71 (2H, d, *J_d* = 8.5), 6.64 (1H,s), 6.62 (1H, d, *J_d* = 2.0), 6.41 (1H, dd, *J_d* = 8.5, *J_d* = 2.0); ¹³C (75 MHz, CD₃OD) 158.61, 158.34, 154.88, 144.83, 140.59, 136.68, 133.44, 132.90, 132.86, 130.19, 125.26, 123.35, 118.27, 116.60, 116.10, 108.63
LC-MS (ESI)) *m/z* calcd for C₂₀H₁₆O₃ (M+1⁺) 305.11, found 305.11

2-Bromo-1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene 8 (BrBHPHPE)

The 2-bromo-1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene **4**, (1.73 g, 4.06 mmol) was dissolved in CH₂Cl₂, BBr₃ (1.0 M in CH₂Cl₂, 25 mL, 25 mmol) was added dropwise at –78 °C over 15 min. to give a bright red solution. The reaction solution was stirred at –78 °C for 0.5 h, warmed to ambient temperature and stirred for 9 h. The solution was cooled to -78 °C and ice-water (20 mL) was added dropwise over 10 min. The resulting mixture was partitioned between ice-water (500 mL) and ethyl acetate (250 mL). The organic layer was washed with water (10 X 200 mL), dried and evaporated to dryness to yield a yellow oil (1.55 g, 4.04 mmol, 99 %). TLC using hexane/EtOAc (6:4) demonstrated the presence **7** (R_f = 0.37) and **8** (R_f = 0.30). Flash chromatography on silica gel using hexane-ethyl acetate (2:1) gave the pure **8**.
m.p. 182-184°C (darkens ~ 150°C) ¹H NMR (500 MHz, CD₃OD) 7.12 (2H, d, *J_d* = 8.5), 7.00 (1H, t, *J_t* = 8.5), 6.75 (2H, d, *J_d* = 8.5), 6.73(2H, d, *J_d* = 8.5), 6.70 (1H, dd, *J_d* = 8.5, *J_d* = 2.0), 6.57 (1H, dd, *J_d* = 8.5, *J_d* = 2.0), 6.47 (2H, d, *J_d* = 8.5);
¹³C (75 MHz, CD₃OD) 158.86, 156.71, 156.37, 143.42, 135.59, 132.96, 131.62, 130.93, 128.74, 121.66, 119.26, 117.01, 114.48, 114.31

LC-MS (ESI)) m/z calcd for $C_{20}H_{16}O_3Br$ ($M+H^+$) 383.02, 385.02, found 382.95, 384.98

2-Iodo-1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene 9 (IBHPHPE)

The 2-iodo-1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene **5**, (0.28 g, 0.60 mmol) was dissolved in CH_2Cl_2 , 10 mL BBr_3 (2 mL, 1.0 M in CH_2Cl_2) was added dropwise at $-78^\circ C$ over 15 min. to give a bright red solution. The reaction solution was stirred at $-78^\circ C$ for 0.5 h, then warm to ambient temperature and stirred for 16 h. The solution was cooled to $-78^\circ C$ and ice-water (20 mL) was added dropwise over 10 min. The resulting mixture was partitioned between ice-water (100 mL) and ethyl acetate (50 mL). The organic layer was washed with water (10 X 50 mL), dried and the solvent was evaporated to dryness to yield a yellow solid. TLC using hexane/EtOAc (6:4) indicated the presence **7** ($R_f = 0.37$) and **9** ($R_f = 0.30$). Flash chromatography on silica gel using hexane-ethyl acetate (2:1) gave the pure IBHPHPE (0.22g, 0.5 mmol, 83%).

m.p. $205-207^\circ C$ (darkens at $165^\circ C$); 1H NMR (500 MHz, CD_3OD) 7.12 (2H, d, $J_d = 8.5$), 6.94 (2H, d, $J_d = 8.5$), 6.93 (1H, t, $J_t = 8.5$), 6.75 (2H, d, $J_d = 8.5$), 6.72 (2H, d, $J_d = 8.5$), 6.69 (1H, s), 6.48-6.52 (2H, overlapping m); ^{13}C (75 MHz, d_6 -DMSO) 157.74, 157.48, 157.29, 142.33, 139.61, 134.83, 131.59, 131.29, 129.22, 129.09, 125.29, 120.75, 116.60, 116.13, 115.61, 113.98

HRMS (FAB) m/z calcd for $C_{20}H_{16}O_3I$ (M^+) 430.0066, found 430.0072

2-Tri-n-butylstannyl-1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene 10 (SnBMPMPE)

2-Bromo-1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene **3** (0.36 g, 0.84 mmol) was dissolved in THF (distilled, 10 mL) and cooled to $-78^\circ C$. Phenyllithium (1.8 M in cyclohexane-ether 70-30, 0.47 mL, 0.85 mmol) was added at $-78^\circ C$; the pale green reaction solution was slowly warmed to $-20^\circ C$, and stirred at $-20^\circ C$ for 1 h. The reaction solution was cooled to $-78^\circ C$ and tri-n-butyltin chloride (0.23 mL, 0.276 g, 0.85 mmol) was added. The reaction solution was warmed to ambient temperature, ice water (5 mL) was added and the resulting mixture was partitioned between ether (100 mL) and ice water (100 mL). The ether layer was washed sequentially with water (100 mL), saturated NH_4Cl (100 mL) and water (3 X 100 mL). The ether solution was dried and evaporated to dryness to give the crude product (0.95 g). The crude product was purified

by flash chromatography using hexane (125 mL) to remove $\text{PhSn}(\text{n-Bu})_3$, followed by elution with 0.25% Et_2O in hexane to give Sn-TMPE . The combined fractions were evaporated to dryness to give a colorless oil (0.10 g, 0.16 mmol, 19%).

$^1\text{H-NMR}$ (500 MHz, CDCl_3) 7.19 (2H, ddd, $J_d = 8.55$, $J_d = 1.81$), 7.05 (1H, dd, $J_d = 7.95$, $J_d = 7.95$), 6.86 (4H, dd, $J_d = 8.55$, $J_d = 1.81$), 6.51 (2H, dd, $J_d = 7.95$, $J_d = 1.50$), 6.47 (1H, dd, $J_d = 2.40$, $J_d = 1.50$), 3.82 (2H, s), 3.71 (3H, s), 3.67 (3H, s), 1.24-1.33 (6H, m), 1.18 (6H, m), 0.81 (9H, t, $J_t = 7.2$), 0.43-0.61 (6H, m)

$^{13}\text{C-NMR}$ (75 MHz, CDCl_3) 159.07, 158.80, 157.57, 151.36, 148.03, 146.54, 138.96, 135.27, 131.17 (2C), 130.57 (2C), 128.67, 120.78, 113.44, 113.38 (2C), 112.57 (2C), 110.08, 55.28, 55.05, 54.97, 28.99 (3C), 27.32 (3C), 13.62 (3C), 11.71 (3C)

Anal. Calcd for $\text{C}_{35}\text{H}_{48}\text{O}_3\text{Sn}$: C, 66.15; H, 7.71; Sn, 18.68. Found: C, 66.33; H, 7.74; Sn, 18.82

Tris(tert-butyldimethylsilyl)-2-bromo-1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene 11 (TBSi₃-BrBHPHPE)

2-Bromo-1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene **8**, (1.01 g, 2.6 mmol) was dissolved in THF (anhydrous, 20 mL) followed by the addition of sodium hydride (0.19 g, 7.9 mmol) and TBS-Cl (7.9 mmol). The reaction solution was stirred at 0 °C for 16 h under Ar, warmed to ambient temperature and then poured into a mixture of EtOAc (500 mL) and iced-water (500 mL). The EtOAc layer was washed with iced-water (10 X 200 mL), dried and was evaporated to give a yellowish oil. The ^1H NMR of the crude product indicated the presence of the 2-Br- and 2-H-tri-TBSi-1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene. The mixture was purified by flash column chromatography. Fractions containing the pure product were combined and evaporated to give a colorless oil, 1.35 g (1.8 mmol, 69% yield) which was used for the lithiation reaction without further purification.

^1H NMR (500 MHz, CDCl_3) 7.35-6.38 (12H, m), 0.99 (9H, s), 0.93 (9H, s), 0.92 (9H, s), 0.23 (6H, s), 0.12 (6H, s), 0.08 (6H, s).

Tris(tert-butyldimethylsilyl)-2-Tri-n-butylstannyl-1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene

12 (TBSi₃-SnBHPHPE)

Crude TBSi₃-Br-BHPHPE (1.09 g, 1.50 mmol) was dissolved in dry THF (20 mL) under Ar. The solution was cooled to -78 °C and sec-butyllithium (1.4 M in cyclohexane, 1.5 mL, 2.1 mmol) was added. The reaction solution was stirred at -78 °C for 1 h and tri-n-butyltin chloride (0.5 mL, 0.60 g, 1.85 mmol) was added, whereupon the reaction immediately turned yellow. The reaction solution was warmed to ambient temperature and ice water (10 mL) was added. The resulting mixture was partitioned between EtOAc (300 mL) and ice water (500 mL). The EtOAc layer was washed with water (10 X 200 mL times), dried (anh MgSO₄) and evaporated to dryness to give a yellow oil (1.41 g). The ¹H NMR of the crude product showed the presence of the desired product in approximately 50% conversion.

Because a TLC of the crude product in 100% hexane demonstrated that TBSi₃-Sn-BHPHPE had *R_f* of 0.33, hexane was chosen as the eluting solvent. Silica gel (200 g) was packed in a column using a mixture of hexane (1000 mL) and triethylamine (2 mL) as a packing solvent (size of silica gel column, 6.2 cm OD, 7 inch long). The silica gel column was eluted with 100% hexane to eliminate the excess triethylamine (8 L of hexane). The crude material was applied to the column and hexane was used to begin the elution. Sn(n-Bu)₃(sec-Bu) eluted with the initial 0.5 L. After 6 L of hexane, the polarity of the eluting solvent was increased to 0.1% Et₂O in hexane was using for 1 L, followed by 0.2% Et₂O in hexane. TBSi₃-Sn-BHPHPE was eluted after 9 L and was isolated (0.073 g, 5% yield). The bulk of the TBS-Sn-THPE appeared to have decomposed on the column as a large red band appeared at the top of the column.

¹H NMR (CDCl₃, 500 MHz) 7.12 (2H, ddd, *J_d* = 8.5, *J_d* = 2.0, *J_d* = 2.0), 6.98, (1H, dd, *J_d* = 8.3, *J_d* = 8.0), 6.78 (2H, ddd, *J_d* = 8.5, *J_d* = 2.0, *J_d* = 2.0), 6.77 (2H, ddd, *J_d* = 8.5, *J_d* = 2.0, *J_d* = 2.0), 6.51 (1H, dd, *J_d* = 8.0, *J_d* = 2.0), 6.47 (1H, dd, *J_d* = 8.3, *J_d* = 2.0), 6.45 (2H, ddd, *J_d* = 8.5, *J_d* = 2.0, *J_d* = 2.0), 6.37 (1H, dd, *J_d* = 2.0, *J_d* = 2.0), 1.33-1.25 (6H, m), 1.17 (6H, hex, *J_{hex}* = 7.50), 1.00 (9H, s); 0.94 (9H, s); 0.90 (9H, s); 0.815 (9H, t, *J_t* = 7.50), 0.60-0.47 (6H, m, Sn-CH₂), 0.21 (6H, s, Si-CH₃); 0.09 (6H, s, Si-CH₃); 0.07 (6H, s, Si-CH₃); ¹³C NMR (CDCl₃, 75 MHz) 155.15, 154.82, 154.68, 153.64, 151.69, 148.10, 146.36, 139.50, 135.77, 131.14, 130.60, 130.36, 130.01, 128.50, 121.64, 119.65, 119.52, 118.77, 118.62, 116.26, 29.03 (3C, Sn-nBu₃), 27.35, 27.32 (3C, Sn-nBu₃), 25.72 (2C), 25.70 (2C), 25.65 (2C), 25.62 (2C), 18.20, 18.18, 18.10, 13.66 (3C, Sn-nBu₃), 11.65 (3C, Sn-nBu₃), -4.40 (2C, C-Si), -4.71 (4C, C-Si)

HRMS (FAB) *m/z* calcd for C₄₆H₇₅O₃Si₃Sn (M⁺-n-Bu) 879.4046, found 879.4041

2.2. Biological Assays.

2.2.1. Receptor Binding Studies. In vitro competitive binding assay.

The compounds were screened for their affinity for the ER α -HBD isolated from BL 21 cells that over-expressed the 33kDa pET-23d ERG vector.^[47] The cells were induced with 0.6 mM isopropyl- β -thiogalactopyranoside for 3h at RT, pelleted by centrifugation, frozen and stored at -75°C . The cells were thawed, and lysed by sonication (4 X 20 sec) in four volumes of lysis buffer (50 mM Tris, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 M urea, pH 7.4) several times. Clarified fractions, obtained at 30,000 x g for 30 min were pooled, assayed for receptor binding, diluted to 50 nM in ER and 100 μL aliquots were frozen and stored at -75°C until ready for use. Then 80 μL of the ER α -LBD- containing extract was incubated with 10 μL of 10 nM 6,7-[H-3]-estradiol (specific activity = 51 Ci/mmol) and 10 μL of either buffer, unlabeled estradiol or test ligand in 100 μL total volume. The final concentrations were 1 nM 6,7-[H3]- estradiol, 2 nM unlabeled estradiol, (using 200 nM estradiol to define specific binding) and 0.5-5000 nM of the test ligand. In all cases, 10 μL of each incubation solution was removed for assay of the actual initial concentration of [H3]-estradiol and the remainder was incubated at 2°C or 25°C for 18 hours. After incubation, 100 μL of dextran coated charcoal suspension (fines removed) was added to adsorb the unbound [H3]-estradiol, incubated for 10 min, centrifuged, and 100 μL samples were taken from the supernatant fraction for assay of radioactivity. The results were calculated and plotted as % specific binding as a function of log of competitor concentration using the best fit equation for the binding inhibition to define 50% inhibition level. The relative binding affinity (RBA) was calculated as 100 times $[E]/[C]$, where [E] was the concentration of unlabeled estradiol needed to reduce the specific binding of [H-3]-estradiol by 50% and [C] was the concentration of test ligand needed to reduce the specific binding by 50%.

2.2.2. Immature rat uterotrophic growth assay

Test ligands were evaluated using the uterotrophic growth assay.^[48] Groups of immature female rats (at least 5 per group) were injected subcutaneously starting with either peanut oil vehicle (control), or part or all of the range of 0.04, 0.156, 0.625, 2.5, 10, 40, 160 or 640 nmoles of test ligand in 0.1 mL peanut oil (with

less than 5% ethanol) and the uterine weights were compared to that of rats receiving estradiol for 3 days. Animals were sacrificed 24h after the last injection, uteri were removed, stripped free of fat and connective tissue, weighed wet, dried in vacuo and weighed to dry weight. Curves of uterine weight (wet and dry) vs. amount of compound injected were compared to assess the potency of the test compound vs. estradiol. The relative estrogenicity of the test ligands to that of estradiol was assessed by determining the dose at which the compound or estradiol gave a uterine growth response equal to 50% of that of 10 nmoles of estradiol.

2.3. Molecular Modeling.

Receptor crystal structure sources:

Two crystal structures of the estrogen receptor ER α -LBD were used in this study: 3ERD^[49] (receptor complexed with diethylstilbestrol) and 1A52^[50] (receptor complexed with 17 β -estradiol). Both files were downloaded from the Protein Data Bank^[51] (<http://www.rcsb.org/pdb/home/home.do>). A list of ligand-protein contacts for both starting complexes (3ERD and 1A52) were generated with the Ligand Protein Contact (LPC) server^[52] (<http://lgin.weizmann.ac.il/cgi-bin/lpccsu/LpcCsu.cgi>).

Modeling ligand-receptor interactions:

Two different docking programs were used to study ligand binding to the receptors, the AUTODOCK^[53] module embedded in the YASARA^[54] suite of programs and GLIDE^[55-57], supplied by Schrodinger, Inc.

YASARA: *Ligand Preparation and Minimization:*

Ligands were first prepared using the YASARA suite of programs^[54]. Each nonsteroidal ligand other than diethylstilbestrol was built *in silico* starting with the structures of the bound ligands in the PDB files, modifying atom types and bond orders as necessary. All C-C bonds in the aromatic rings were given bond orders of 1.5. The olefinic (C=C) bond's order was 2. All other bonds had an order of 1. 11- β -(4-Hydroxyphenyl) estradiol was created by modifying the estradiol residue from 1A52 in the same way. Each ligand had its energy minimized to eliminate awkward bond angles. The resulting structures were minimized

again from three different starting positions. The minimized conformation with the lowest energy for each ligand was used in the docking experiments. Solvation of the ligands prior to minimization was carried out at physiological pH (7.4) with 0.9% NaCl concentration. Minimization was performed using PM3 quantum mechanics.^[58] The simulation cell was defined as extending 10 Å around all atoms, and the Yamber 3 force field was used.

YASARA: *Receptor preparation:*

The crystal structures 3ERD and 1A52 were imported into YASARA from the Protein Data Bank ^[51] (<http://www.rcsb.org/pdb/home/home.do>). All chains except for the “A” chain were deleted. 1A52 contains three gold ions which were deleted because YASARA considers them to be too exotic to include in its calculations. Water molecules and 3ERD's chloride ion were left intact. The simulation cell was defined as extending 6 Å around the following atoms in the receptor: Asp 351 OD1 and OD2 (carboxylate oxygens), Glu 353 OE1 and OE2 (carboxylate oxygens), Trp 383 CB (side-chain-bearing carbon), Leu 387 O (backbone carbonyl), Arg 394 NH2 (sidechain nitrogen), Phe 425 CB (side-chain-bearing carbon), His 524 ND1 (imidazole nitrogen), and HOH 11/HOH 558 (water molecule near Glu 353 and Arg394 in 3ERD/1A52, respectively). All of the residues listed were identified as ligand –contacting residues by the LPC server with the exception of Asp 351, which we included in the simulation so as not to exclude *a priori* the possibility of an antagonistic interaction.^[49,50] However, it should be noted that the ligands we investigated are unlikely to engage in antagonistinc binding due to their lack of a long, hydrophilic side chain capable of forming a hydrogen bond with Asp 351.

AUTODOCK/YASARA: *Docking:*

For the Autodock calculations, each ligand was manually pre-docked into the empty receptor in four different starting positions, defined as A, B, C, and D (Figure 5). Starting positions were obtained using the crystal structure complexes as guides; the nonsteroidal ligands were superimposed onto the diethylstilbestrol of

the 3ERD structure, while the steroidal ligands were superimposed onto the estradiol of the 1A52 structure.

Superposition was obtained using the “superpose” function in YASARA, which minimizes the RMSD.

Schrodinger / Maestro/LigPrep: *Ligand Preparation:*

The ligands were built using LigPrep. Each ligand's dihedral angles were adjusted so that they matched those of the lowest-energy conformations that were used in the YASARA-based docking experiments.

Schrodinger/ProteinPrepWiz: *Receptor Preparation:*

The “A” chains of 3ERD and 1A52 were imported and prepared using the Protein Preparation Wizard^[59] in the Schrodinger software suite. The gold atoms in 1A52 were deleted, but waters were left intact. Restrained minimization of the protein was performed to relieve steric clashes. The prepared receptor files were used to generate the receptor grids. Grid generation was performed using default settings and no constraints were used.

Schrodinger GLIDE^[55-57]: *Docking:*

Docking was performed using GLIDE with Standard Precision and default settings. Since GLIDE docking does not allow for pre-positioning of the ligand within the receptor, there was only one docking run for each ligand-receptor pair in GLIDE.

Analysis of results:

The best pose generated for each ligand-receptor pair by each program was classified as “A”, “B”, “C”, or “D” according to the scheme used to define the starting positions (Figure 5). The predicted binding energies (YASARA) or GLIDEScores (GLIDE) were recorded. When inspecting ligand-receptor contacts, hydrogen bonds as well as contacts between the halogen atom (or vinyl proton) in the nonsteroidal ligands and the receptor were considered. GLIDE defines “good” contacts as those whose cutoff ratios (defined by the equation $C = D_{1,2}/(R_1+R_2)$, where C = cutoff ratio, $D_{1,2}$ = distance between atomic centers, R_1 = Van der Waals radius of

first atom and R_2 = Van der Waals radius of second atom) are between 1.3 Å and 0.89 Å; shorter distances qualify a contact as “bad” or “ugly”. YASARA simply calculates contacts based on distance; all atoms within 5 Å of the halogen which are not occluded by another atom in between are considered contacts.

3. Results

3.1. Chemistry.

The synthesis of the target compounds is shown in Scheme 1. The construction of the 1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl) ethylene (BMPMPE) scaffold **2** proceeded in high yields using

[Insert Scheme 1]

Reagents: (i) 3-methoxybenzyl bromide, Mg turnings; (ii) trifluoroacetic anhydride, CH_2Cl_2 ; (iii) Br_2 , CH_2Cl_2 ; (iv) NIS, DMF; (v) BBr_3 , CH_2Cl_2

Scheme 1. Synthesis of 1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene and the 2-bromo- and 3-iodo-derivatives.

3-methoxybenzyl magnesium bromide and 4,4'-dimethoxybenzophenone **1**, followed by dehydration with trifluoroacetic anhydride. Bromination of 1,1-tris(4-methoxyphenyl)ethylene was reported to proceed readily,^[44, 45] however, when the methoxy on the 2-phenyl group is meta- rather than para-, reagent stoichiometry and temperature were key factors in determining the formation of the desired product. Initial halogenation occurs at the olefinic 2-position, however, halogenation on the meta-substituted aryl ring becomes a competing reaction as the concentration of starting material decreases. As a result, use of one equivalent of bromine at room temperature gave a reaction mixture consisting of primarily of the desired mono-brominated product **3**, but also lesser amounts of unreacted starting material **2** and di-brominated by-product **4**. Because the brominated product is slightly more polar than the starting material, it was difficult to obtain pure product by (flash) column

1 chromatography. Increasing the bromine to starting material ratio to 1.2:1 resulted in complete consumption of
2
3 starting material but also reduced the yield of the desired mono-brominated product. Ultimately, a careful
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5 addition of exactly one equivalent of bromine to 1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene at
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7 0°C, over 20 min, followed by a rapid work-up and flash chromatographic separation gave the desired mono-
8
9 brominated product **3** (Br-BMPMPE) in >90% isolated yield. Isolation and analysis of the ¹H-NMR spectrum of
10
11 the di-brominated material **4** indicated that the site of the second bromo-group was probably at the para (4-)
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13 position of the 2-(3-methoxyphenyl) moiety.
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18 Selective iodination of 1,1-bis(4-methoxyphenyl)-2-(3-methoxyphenyl)ethylene **2** was more difficult.
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20 The literature method for iodination of the 1,1,2-tris(4-methoxyphenyl)ethylene used metallation of the bromo-
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22 derivative followed by addition of iodine.^[44] N-iodosuccinimide (2.5 equivalents) in DMF at 90-100°C, in
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24 contrast to N-bromosuccinimide or bromine, gave a complex mixture consisting of starting material **2**, mono-
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26 iodinated and di-iodinated products (**5** and **6** respectively) from which enriched fractions of the mono-
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28 iodinated product **5** were isolated by flash chromatography. Rechromatography followed by crystallization from
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30 methanol gave the pure 2-iodinated products **5** and **6**.
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35 Demethylation of the derivatives **2**, **3** and **5** using boron tribromide^[47] gave the desired products **6**, **7**,
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37 and **8**. Reactions with the halogenated intermediates yielded a small amount of the dehalogenated material **6**,
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39 however, careful flash chromatography followed by crystallization from dichloromethane gave the final
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41 products which were stable for extended periods of time if kept in the dark at 4°C or under argon.
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45 The synthesis of the precursor for radiohalogenation used **8** (Br-BHPHPE) as the starting material
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47 (Scheme 2). As a trial for the actual synthesis, 2-(tri-n-butylstannyl)- 1,1-bis(4-methoxyphenyl)-2-(3-
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49 methoxyphenyl)ethylene **10** (Sn-BMPMPE) was prepared first. Attempts to transmetallate at -78°C with
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51 phenyllithium were unsuccessful and gave only tributylstannyl benzene. Warming the reaction to -20°C was
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53 necessary to achieve full transmetallation. Recooling of the reaction to -78°C followed by addition of the tri-n-
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55 butylstannyl chloride
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[Insert Scheme 2]

Reagents. i. sec-Butyllithium, -78°C to -20°C to -78°C. ii. Tri-n-butylstannyl chloride. iii. N-iodosuccinimide, THF, 25°C. iv. NaH, TBDMSiCl. v. TBAF, THF

Scheme 2. Synthesis of 2- stannylated derivatives and conversion to corresponding 2-iodo products.

gave a good yield of **10**. Reaction with NIS in THF then gave an immediate conversion to **5** (I-BMPMPE), which had been previously prepared. The eventual preparation of radioiodinated I-BHPHPE would require silyl protection for the phenolic groups that could be removed following radiohalogenation. Therefore, the phenolic groups of **8** were initially protected as silyl ethers. Use of imidazole as the base in this reaction, however, gave inconsistent yields. Use of sodium hydride and tert-butyldimethylsilyl chloride (TBDMS-Cl) provided the tris-TBDMS-BrBHPHPE **11** in better yields. Metallation of the **11** was achieved with sec-butyllithium, instead of phenyllithium, at -78°C, followed by slow addition of tri-n-butylstannyl chloride. Work-up of the reaction indicated the presence of both 2-stannyl- and 2-protio- products. Careful chromatographic separation using 1% ether in hexane gave fractions containing pure TBDMSi₃-Sn-BHPHPE **12** as well as a mixture of the 2-stannylated and 2-protio-materials. The pure fractions were combined, characterized and reserved for future radiohalogenation experiments. The fractions containing the mixture of products were combined. Addition of NIS followed by deprotection with tetrabutylammonium fluoride gave the expected mixtures of **7** and **9**, as determined by comparison with authentic samples previously obtained by an alternate method. The difference in polarity between the 2- iodo- and 2-protio-derivatives (iodo- more polar than protio-) facilitated the isolation of the products.

3.2. Biological Assays.

The halogenated products, **8** and **9**, as well as **7** were evaluated as competitive ligands for the ER α -LBD and as *in vivo* ER agonists in the immature female rat uterotrophic growth assay. The competitive binding assay indicated that the introduction of the halogen significantly increased the affinity of the ligand for the ER α -LBD.

While the relative binding affinity (RBA) of 5.5% for **7** (BHPHPE) was much less than that for estradiol (RBA = 100%), the RBA values for **8** (Br-BHPHPE) and **9** (I-BHPHPE) were 53% and 79% respectively. These values are somewhat higher than those reported for the corresponding Br/I-BHPPE and 2- alkyl-THPE analogs.^[23, 39-41, 46] The same order of responses was observed *in vivo* where **9** (I-BHPHPE) and **8** (Br-BHPHPE) were essentially equipotent (ED₅₀ ~ 2 nmole), and **7** (BHPHPE) was an order of magnitude less active (Figure 3). The maximal response of each compound was essentially identical to that of 10 nmoles of estradiol, indicating that all three compounds were full estrogenic agonists in this assay.

Insert Figure 3

3.3. Molecular modeling studies.

The binding modes of 2-substituted-1,1-bis(4-hydroxyphenyl)-2-aryl(alkyl)-ethylenes as ER ligands have been examined,^[46, 60-62] however, there had not been a comparable computational analysis of 2-substituted-1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylenes (BHPHPEs). Docking experiments were carried out to determine the preferred binding modes of these compounds and analogs (Figure 4) using

Insert Figure 4

YASARA and Schrodinger GLIDE modeling programs. The computationally derived 2-(4-hydroxyphenyl) analogs of compounds **7-9** were included to clarify the importance of the placement of the hydroxyl of the 2-phenyl moiety. Diethylstilbestrol (DES) and estradiol (E2) were included in the ligand set as standards to verify whether the binding pose predicted by YASARA matched the empirically determined poses, as the crystal structures of these two complexes, 3ERD (diethylstilbestrol) and 1A52 (estradiol), were used as the ligand-receptor complexes. 11 β -(4-Hydroxyphenyl) estradiol, previously reported, was included in the study as it constituted the steroidal analog of the target compounds.^[63] Based upon the structural similarities, it was hypothesized that the 11 β -(4-hydroxyphenyl) and the 1-(4-hydroxyphenyl) *trans* to the ethylene-halogen/hydrogen of the nonsteroidal ligands would occupy similar binding pockets within the preferred poses. Comparison of the experimentally determined relative binding affinities with the predicted binding energies obtained with YASARA would provide further support for the computational analysis and allow for a more

1 detailed dissection of the factors contributing to biological activity of this class of ER ligands. In particular,
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3 analysis focused on the specific contribution of the 2-halogen to binding affinity.
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6 The initial docking orientations of the ligands were based on the orientation of DES in the 3ERD crystal
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8 structure, and estradiol in the 1A52 crystal structure. Four starting poses (A-D) were generated for each ligand
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10 which corresponded to the orientation of the phenolic groups within the binding pocket (Figure 5).
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13 Insert Figure 5
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18 Each of the ten ligands was docked using the YASARA program with starting poses A-D in both
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20 receptor crystal structures (3ERD and 1A52). In each docking run, YASARA tried 250 possible positions for
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22 the ligand, then collected similar results into “clusters”. The cluster with the best predicted binding energy out
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24 of all four or five docking runs performed for each ligand/receptor pair is reported in Table 1. The results of the
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28 Insert Table 1
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30 docking experiments were very consistent. The most common pose for the nonsteroidal ligands when docked
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32 with 3ERD was Pose A, while the steroids preferred Pose B. Although the result with the highest predicted
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34 binding energy for the 2-(4-hydroxyphenyl) or *all para* analog of 9 was Pose C, two of the other YASARA
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36 docking runs and the GLIDE run resulted in Pose A (Supporting Info). Similarly, two of the other YASARA
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38 runs for DES resulted in Pose B. This finding suggests that because of the symmetry of DES, its binding mode
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40 has less of an impact on its binding energy than is the case for the other ligands. With crystal structure 1A52,
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42 the preferred pose for all ligands in YASARA, except for DES, was Pose B. These results were consistent with
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44 the orientations determined in the previously cited studies.^[46,60-62]
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49 For comparison, the ten ligands were evaluated with both 3ERD and 1A52 using Schrodinger's GLIDE
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51 program, which, unlike YASARA, does not allow the ligand to be prepositioned in the binding site before
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53 docking. The best binding poses determined in this process generally agreed with the previous results (Table 1).
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55 Pose A was optimal for all nonsteroidal ligands when docked with 3ERD. Pose B was optimal for all ligands
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57 when docked with 1A52, except for the 2-(4-hydroxyphenyl) analog of 8, for which Pose D was optimal.
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1 However, it should be noted that both poses B and D place the phenol *trans* to the halogen in the 11 β -pocket of
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3 the receptor. The pose B was optimal for the steroidal ligands when dock in either crystal structure. Therefore,
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5 results support the contention that these nonsteroidal derivatives were aligned within the binding pocket in a
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7 manner similar to that described for other high affinity nonsteroidal agonists.^[46,60-62]
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10 An analysis of the binding modes provided significant details regarding the interactions between the
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12 ligand and specific residues within the ligand binding pocket of the receptor. (Figure 6) In particular, the 2-(3-
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18 hydroxyphenyl) moiety establishes hydrogen bonding interactions with His-524 comparable to the 17 β -
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20 hydroxyl of estradiol and the 4-hydroxyphenyl of DES. Although the hydrogen bonding interaction is not
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22 critical for affinity, as noted by the high RBA value associated with the BHPPE derivatives, it may contribute to
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24 facilitating subsequent coregulatory protein recruitment and the estrogenic responses. In both complexes
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26 (Figure 6A and 6B), the 4-hydroxyphenyl group cis to the ethylene-H/Br/I moiety establishes strong interactions
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28 with the Glu-Arg-water triad associated with estrogen binding. The 2-halo group for **8** and **9** occupies the same
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30 site as the C-7 of 11 β -(4-HP)E2 and the dipole of the halogen is oriented toward the 7 α -pocket. This effect is
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32 comparable to the 2-alkyl substituent present on previously evaluated nonsteroidal ligands.^[46] The van der
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34 waals radii and hydrophobic constants for Br- and I- are similar to those for a methyl group^[66] In this pose, the
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36 1-(4-hydroxyphenyl) group *trans* to the 2-halo moiety also occupies the hydrophobic pocket similar to, but not
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38 identical with, 11 β -(4-hydroxyphenyl) estradiol [Figure 6C]. The 11 β phenolic group in this posed is in the
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40 proximity of Asp-351 but does not appear to establish any direct interactions that would lead to antagonist
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42 generation conformations.
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50 The results from the docking studies were used to investigate whether a correlation could be established
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52 between the calculated binding energies for the preferred poses and the RBA values obtained for the compounds
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54 **7-9**. The corresponding 2-(4-hydroxyphenyl) isomers were considered, however, they were not included
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56 because of the slight but significant differences in binding modes, plus the fact that no actual RBA values for
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58 them existed. The results, shown in Table 2, indicated that the 2-bromo and 2-iodo derivatives, which have the
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higher RBA values, expressed higher calculated binding energies than the 2-unsubstituted (protio-) analog with both receptor models. A precise correlation between the RBA values and the calculated binding energy could not be made given the limited sampling size.

Insert Table 2

4. Discussion

The objectives of this study were to prepare and characterize the previously reported 2-halogenated derivatives of 1,1-bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene (BHPHPE), to prepare and characterize the stannylated precursor for the radiohalogenated analogs and to evaluate the interactions of the ligands with ER α -LBD using computational methods. Although the literature cites many examples of 1,1,2-triphenylethenes, there were no previous examples in which the 2-phenyl moiety contained a 3-hydroxy substituent.^[67] Similarly, there are many examples of 2-chloro- and 2-bromo-triarylethenes, but very few 2-iodo derivatives.^[68-74] In part this may be due to the susceptibility of the intermediate 2-iodo-tris(methoxyphenyl)ethylenes to undergo photochemical cleavage of halo-vinyl bond^[75-77] Our interest in the title compounds revolved around their potential use as therapeutic agents for breast or ovarian carcinoma when prepared as their Br-80m or I-123 analogs. For such therapy with our agents to be effective, the carrier molecule must possess sufficiently high affinity for the ER and be sufficiently stable chemically to reside within the nucleus of the target cell long enough for the radionuclide to undergo the decay process. Therefore, it was necessary to ensure that the chemical and biological criteria could be satisfied, that the requisite precursor could be prepared, and the binding process be understood sufficiently to permit development of improved analogs.

The general synthetic strategy employed in the study was similar to that reported for chloroanisene, however, the presence of 3-methoxy group instead of a 4-methoxy group in the intermediate introduced synthetic complications. In the preparation of chloroanisene, polychlorination is not a major problem because, after halogenation of the 2-position of the ethylene, the remaining positions on the aromatic rings are not particularly activated (either ortho to methoxy and meta to the remaining conjugated system or meta to methoxy and ortho to the remaining conjugated system). In our case, the 2-position of the ethylene remains the most

1 reactive site in the molecule, however, the 4-position on the 2-(3-methoxyphenyl)-group is also reactive (ortho
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3 to the methoxy and para to the conjugated system). Consequently, as the concentration of the starting material **2**
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5 decreases, para-bromination of **3** (Br-BMMPE) becomes a competing reaction, particularly at ambient
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7 temperature. Chromatographic separation of a mixture of **2**, **3** and **4** was complicated by the similarity in
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9 physicochemical properties. Ultimately, the optimal conditions involved performing the reaction at 0-4°C with
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11 0.99 equivalents of bromine added over 20 min followed by immediate work-up. These conditions suppressed
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13 the 4-bromination of the 2-(3-methoxyphenyl) group while permitting facile bromination of the ethylene.
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15 Although iodination presented different problems, because of its lower reactivity, the experience with bromine
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17 permitted the development of a procedure that gave sufficient quantities of the pure **5**.
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23 Demethylation of the BMPMRE derivatives **2**, **3**, **5** was achieved using boron tribromide in
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25 dichloromethane. While this is a relatively standard procedure for aryl methyl ethers, at room temperature, with
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27 an excess of reagent or when allowed to react for more than 1 hour, significant dehalogenation of the product
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29 was observed. Consequently, as in the case of halogenation, careful control of stoichiometry (1 B-Br equivalent
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31 per ether group), temperature (-20 - 0°C) and time (1 h) was necessary to give complete demethylation while
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33 suppressing dehalogenation. However, if the products were pure and crystalline, they were stable for long
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35 periods in the dark at <4°C. The photochemical instability of the final products was observed as bromo- and
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37 iodo-derivatives underwent gradual darkening (dehalogenation) upon exposure to ambient light.
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42 We successfully developed the process for silylated derivative of Sn-BHPHPE. An unpublished
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44 procedure suggested that the phenolic groups could be protected as trimethylsilyl ethers and that metallation
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46 could be achieved at -78°C with phenyllithium followed by addition of the tributylstannyl chloride. In actuality,
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48 the procedure required a more stable silyl group (TBDMSi) and a different transmetallation method. The
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50 metallation reaction required warming the solution of the brominated ethylene and sec-butyllithium from -78°C
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52 to -20°C and then recooling the solution before adding the tri-n-butylstannyl chloride. Evaluation of the reaction
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54 indicated complete metallation, but mixture of stannyl and protio-products. The stannylated product was highly
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56 sensitive to proton sources as exposure to weak acids, including silica gel, resulted in formation of the proteo-
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derivative. Therefore careful handling was required for characterization and storage. Nevertheless, an experiment using the mixed fractions for iodination gave rapid and complete conversion to the iodinated silylated intermediate which, after deprotection with TBAF, yielded authentic **9**.

Biological evaluation of the compounds using competitive binding and *in vivo* uterotrophic growth assays indicated that the compounds were potent estrogenic substances. Prior studies using gene activation in cells provided conflicting results regarding antagonist or agonist activity of the tris(hydroxyphenyl) ethylene derivatives,^[46,60] the *in vivo* results clearly demonstrate the full agonist properties of the compounds. All generated maximal responses comparable to estradiol. Introduction of the halogen at the 2-position significantly enhanced the affinity of the ligand for the receptor compared to the protio derivative and this was translated to the *in vivo* activity. In both assays the bromo- and iodo-derivatives were essentially equipotent and an order of magnitude more potent than the protio-derivative.

Computational analysis aided in rationalizing the observed effect. Comparison of our compounds with previous studies indicated that they were probably aligned in the same manner as other 1,1-bis(4-hydroxyphenyl)ethylene derivatives within an agonist complex.^[46,60,61] In this pose, the 2-(3-hydroxyphenyl) group occupies the D-ring region of the ligand binding pocket and the 1,1-(4-hydroxyphenyl) components occupy the A-ring and 11 β -pockets of the receptor. The halogen would be oriented toward the binding pocket typically occupied by 7 α -substituents on estradiol. Docking studies indicated that small groups, such as methyl and ethyl, have the best interactions while larger alkyl groups tend to experience steric clashes with adjacent hydrophobic residues. Bromo- and iodo- groups are bioisosteres for the methyl group, however, due to their valence shells, the halogens also would contribute electronic interactions not present with the alkyl groups.^[63] The slightly higher RBA values observed for these compounds may be due to those additional interactions. Because of the differences in bioassay conditions used for the different ligands and the different computational methods used in these studies one must be careful not to over-interpret these observations.

5. Conclusions

In summary, we have prepared the 2-bromo and 2-iodo derivatives of 1,1-bis(4-hydroxyphenyl)-2-(3-

hydroxyphenyl)ethylene in good overall yields. Biological evaluation of the derivatives indicated that they retain high estrogen receptor binding affinity and exhibit potent *in vivo* estrogenic activity. Evaluation of the biological results suggested that the presence of the 2-halo group enhanced receptor affinity compared to the unsubstituted ethylene. Computational studies using two different docking methods suggested the preferred orientation of these ligands can be accommodated within the agonist conformation of the receptor. The halogen-contacting residues in the I- and Br-BHPHPE complexes provided a possible rationale for the higher RBA values observed for the halogenated ligands. We also successfully prepared the requisite stannylated precursor and demonstrated its conversion to the corresponding iodinated derivative. The chemical and biological properties of the final compounds meet the criteria established for considering the radiolabeled analogs as potential radiodiagnostic and/or radiotherapeutic agents for estrogen receptor-expressing breast and ovarian cancer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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51 **Figure legends**

52 **Figure 1** Structures of radiohalogenated steroidal estrogens for imaging or radiotherapy.

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54 **Figure 2** Iodinated bis- and tris-(Hydroxyphenyl)ethylene nonsteroidal estrogens that have been considered for
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56 imaging or radiotherapy.
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58 **Figure 3** In vivo uterotrophic growth assay in immature female rats. Compounds were injected s.c. over 3 days
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60 and sacrificed on day 4. Results are compared to peanut oil (control) and 10 nmole estradiol.
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Figure 4 Structures of the nonsteroidal and steroidal ligands used in the computational study.

Figure 5 Starting poses A-D for the nonsteroidal ligand IBHPHPE (left) and the steroidal ligand E2 (right) in the binding site of 3ERD. Screenshots were taken in YASARA.

Figure 6 Comparison of nonsteroidal ligand binding modes with key receptor residues. A) **7, 8** (foreground) and **9** in 3ERD, with the phenol *trans* to the halogen/hydrogen pointing in the 7α -direction. B) **7, 8** (foreground) and **9** in 1A52, with the phenol *trans* to the halogen/hydrogen pointing in the 11β -direction. C) **9** and 11β -(4-HP) E2 in 1A52; both the phenol at the 11β -position of the steroid and the 3-hydroxyphenyl group *trans* to the halogen/hydrogen of **9** occupy both occupy the 11β -pocket of the ER α -LBD. Images generated from YASARA results using Ligplot+. ^[62] Ligands and H-bonding residues are shown as ball-and-stick models. Hydrophobic residues are represented as a red semicircle with radiating lines. Residues which overlap between the complexes being compared are circled in red.

Table legends

Table 1 The letters in the above table refer to the general orientation of the ligand in the result with the best predicted binding energy (YASARA) or GLIDEScore (Schrodinger). In the case of the YASARA results, the pose reported has the best predicted binding energy out of the top clusters of each of the four or five docking runs for that ligand-receptor pair. A complete list of the pose results for each individual docking run can be found in the supporting information. *GLIDE did not generate any results for 11β -(4-HP) E2 in the 3ERD receptor.

Table 2 The best predicted binding energies of selected ligands, along with the relative binding affinity (RBA) as determined by competitive binding assay.

Figures

R = H, OCH₃

X = Br/I nuclides

Figure 1.

2-iodo-1,1-Bis(4-hydroxyphenyl)-ethylene (I-BHPE)

2-iodo-1,1-Bis(4-hydroxyphenyl)-2-phenylethylene (I-BHPPE)

2-iodo-1,1-Bis(4-hydroxyphenyl)-2-(3-hydroxyphenyl)ethylene (I-BHPHPE)

Figure 2.

UTERINE WET WEIGHT RESPONSES

Figure 3.

Figure 4.

Figure 5

Figure 6

Scheme 1

Scheme 2

Ligand	Preferred Binding Pose in 3ERD (YASARA)	Preferred Binding Pose in 1A52 (YASARA)	Preferred Binding Pose in 3ERD (GLIDE)	Preferred Binding Pose in 1A52 (GLIDE)
DES	A	C	A	A
I-BHPPE	A	B	A	B
7 (BHPHPE)	A	B	A	B
8 (BrBHPHPE)	A	B	A	B
9 (IBHPHPE)	A	B	A	B
2-(4-hydroxyphenyl) analog of 7	A	B	A	B
2-(4-hydroxyphenyl) analog of 8	A	B	A	D
2-(4-hydroxyphenyl) analog 9	C	B	A	B
E2	B	B	B	B
11 β -(4-HP) E2	B	B	N/A*	B

Table 1

Ligand	Best Predicted Binding Energy (kcal/mol) in 3ERD (YASARA)	Best Predicted Binding Energy (kcal/mol) in 1A52 (YASARA)	Relative Binding Affinity
DES	9.95	9.08	400 % ^[64]
7	8.86	10.21	5.5%
8	11.37	10.86	53 %
9	9.61	10.54	79 %
E2	11.37	11.00	100 %

Table 2

Graphical Abstract

X	RBA(%)
H	5.5
Br	53
I	79

Precursor for radioiodination