Articles

Synthesis and Evaluation of Estrogen Receptor β-Selective Ligands: Fluoroalkylated Indazole Estrogens

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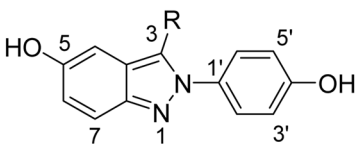
It is important to identify selective ligands for the estrogen receptor subtypes ERα or ERβ to evaluate them as pharmaceutical targets in breast cancer. To develop ERβ-selective ligands as PET imaging agents, a series of aryl indazole estrogen analogues substituted at the C3 position with fluoroethyl and fluoropropyl groups were synthesized and evaluated for their relative binding affinities and selectivities for ERα *vs* ERβ. The fluoroethylated indazole estrogen (FEIE, **1i**) and fluoropropylated indazole estrogen (FPIE, **1h**) showed 41-fold and 17-fold ERβ/ERα selectivity, respectively. However, their binding affinities to ERα and ERβ were very low.

Key Words : Estrogen receptor β, Indazole estrogen analogues, PET

Introduction

Estrogens play an essential role in the growth, development, and homeostasis of various reproductive and non-reproductive target tissues.[1] Estrogens exert many of their actions *via* estrogen receptors (ERs), which function as ligand-regulated transcription factors.[2] The discovery of the ERs (ERα was cloned in 1986[3] and ERβ in 1996[4]) prompted intensive studies on the respective functions of the two receptor subtypes. Most estrogen target tissues contain both ERα and ERβ, although in varying ratios; ERα is widely expressed in breast and uterine tissue, whereas ERβ is found at higher levels in lung, ovary, prostate, bone, vascular epithelium and certain brain regions. While it is difficult to generalize, ERβ is usually less active as a transcription factor and exerts a restraining effect on the more active ERα.[5] Although normal breast tissue contains both ERα and ERβ, in breast tumors, the level of ERβ relative to ERα declines with disease progression, as one might expect, with transition of the cancer to a more proliferative and malignant state.[6] Thus, if the independent quantification of ERα and ERβ levels in breast cancer could be achieved by imaging non-invasively, using radioisotope-labeled ER subtype-selective ligands with positron emission tomography (PET) or single photon emission computed tomography (SPECT), it might provide predictive information for disease staging and tumor response to hormone therapies.

The two ERs subtypes share about 60% amino acid identity in their ligand binding domains (LBD),[7] and the volume of the ERβ ligand binding pocket is smaller than that of ERα.[8,9] Otherwise, the ligand-binding pockets are very similar, with the amino acids lining the ERβ pocket differing from those of ERα by only 2 out of 24 residues, Leu_{384} and Met_{421} in ERα being replaced by Met_{336} and Ile_{373} in ERβ, respectively.[9] Despite this similarity, it has proved possible to develop ER ligands that bind to and activate either ERα or ERβ with a high level of selectivity, and a reasonable pharmacophore model has been advanced to guide the design of subtype-selective ligands. Compounds with good selectivity for ERα have been reported in the literature, including pyrazole-, pyrrole-, and furan-core derivatives,[10] and ERβ-selective ligands with varying core structures have also been reported, such as tetrahydrochrysenes, diarypropionitriles, cyclofenils, benzothiophene, benzothiazoles, benzimidazoles, benzoxazoles and benzofused heterocyclic analogues.[11] Various estrogen derivatives have been developed as radioisotope-labeled PET or SPECT imaging agents for the diagnosis, treatment and monitoring response to hor-



1a, R = Cl; ERα = 0.30, ERβ = 32.1, β/α = 107
1b, R = Br; ERα = 0.18, ERβ = 18.4, β/α = 102
1c, R = I; ERα = 0.17, ERβ = 8.50, β/α = 50
1d, R = CF_3; ERα = 3.90, ERβ = 69.0, β/α = 18
1e, R = CN; ERα = 1.40, ERβ = 30.1, β/α = 22
1f, R = CH_2CH_3; ERα = 0.08, ERβ = 3.24, β/α = 41
1g, R = $CH_2CH_2CH_3$; ERα = 0.04, ERβ = 0.48, β/α = 12

Figure 1. Relative binding affinity of indazole estrogen ligands for ERβ.

mone therapy in breast cancer. Thus far, however, success in developing imaging agents selective for ERα[12] or ERβ[13] that performs well *in vivo* has been limited. Therefore, differential imaging of the two ER subtypes remains a major challenge.

Recently, Katzenellenbogen *et al*. reported that compounds having a phenyl-2*H*-indazole core structure showed good selectivity for ERβ in terms of receptor binding and potency in transcription assay (Figure 1).[14] These compounds appeared to be promising agents for the selective interaction with and activation of ERβ. Another attractive feature of this heterocyclic system is that it is possible to introduce various groups (halogen, alkyl, cyano, etc., **1a-f**, Figure 1) at an internal position of the heterocyclic system (C3). Thus, on the basis of these biological results, we designed fluoroalkylated indazole core estrogens as potential ERβ-selective imaging agents. Herein, we describe the synthesis and biological evaluation of fluoroethyl and fluoropropyl C3 substituted indazole estrogens, based on the phenyl-2*H*-indazole pharmacophore, as ERβ-selective PET tracer candidates.

Experimental

Materials. All commercial reagents and solvents were used without further purification unless otherwise specified. Solvents and reagents were commercially purchased from Aldrich (USA) and Merck Co. (Germany). Thin layer chromatography (TLC) was performed on Merck 60 F-254 silica plates and visualized by UV. Flash column chromatography was performed on silica gel (Merck, 230-400 mesh ASTM). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker-300 and are reported in parts per million downfield from internal tetramethylsilane. Mass spectra were obtained on a JMS700 spectrometer (JEOL Co., Japan). Indazole core and known compound were synthesized following the reported procedure with a little modification and characterized by ^{1}H and ^{13}C NMR.[14,15]

1-(2-Bromo-5-methoxybenzyl)-1-(4-methoxyphenyl)-hydrazine (4). To a solution of NaHMDS (45.8 mL, 1.0 M/THF, 45.8 mmol) at 0 °C was added 4-methoxyphenylhydazine hydrochloride (**3**) (4.0 g, 22.9 mmol) under an Ar atmosphere. After 15 min, the ice bath was removed and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was re-cooled to 0 °C, and then the 2-bromo-5-methoxybenzylbromide (**2**, 6.4 g, 22.9 mmol) was slowly added. After the reaction mixture was stirred at room temperature for 1 h, it was then quenched with water. The mixture was extracted with ethyl acetate and the organic layer was dried over sodium sulfate and purified by flash column chromatography (30% EtOAc/hexane) to give **4** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (d, J = 8.7 Hz, 1H), 7.06 (d, J = 6.9 Hz, 2H), 6.98 (d, J = 3.0 Hz, 1H), 6.88 (d, J = 6.9 Hz, 2H), 6.74 (dd, J = 8.7, 3.0 Hz, 1H), 4.54 (s, 2H), 3.80 (s, 3H), 3.76 (s, 3H), 3.66 (brs, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.3, 153.3, 146.2, 137.9, 133.5, 115.6, 115.0, 114.50, 114.47, 114.0, 62.6, 55.7, 55.5.

5-Methoxy-2-(4-methoxyphenyl)-2*H*-indazole (5). The purified **4** (6.5 g 19.3 mmol) was dissolved in anhydrous toluene. $Pd(OAc)_2$ (217 mg, 5 mol%,), dppf (804 mg, 7.5 mol%), and NaO^{t}Bu (2.79 g, 29 mmol) were added under Ar atmosphere. The reaction mixture was stirred at 90 °C for 15 h. The reaction mixture was filtered and the crude product purified by flash column chromatography to give the desired product **5** as a brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, J = 0.6 Hz, 1H), 7.80 (d, J = 6.9 Hz, 2H), 7.70 (d, J = 9.3 Hz, 1H), 7.03-7.07 (m, 3H), 6.93 (d, J = 2.4 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1, 155.5, 146.5, 134.3, 122.7, 122.1, 121.6, 119.24, 119.17, 114.6, 96.4, 55.6, 55.4.

3-Bromo-5-methoxy-2-(4-methoxyphenyl)-2*H*-indazole (6a). Compound 5 (2.36 g, 9.29 mmol) was dissolved in acetic acid and *N*-bromosuccinimide (1.74 g, 9.75 mmol) was added. The reaction mixture heated under reflux for overnight. The reaction mixture was quenched with water in an ice bath and extracted with ethyl acetate. The organic layer was washed with 1 N NaOH, dried over sodium sulfate. The crude product was purified by flash chromatography (20% EtOAc/hexane) to give **6a** (62%) as a pale yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, J = 0.9 Hz, 1H), 7.83 (d, J = 6.9 Hz, 2H), 7.75 (dd, J = 9.3, 0.9 Hz, 1H), 7.21 (d, J = 9.3 Hz, ^{1}H), 7.06 (d, J = 6.9 Hz, 2H), 4.00 (s, 3H), 3.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 151.1, 146.1, 133.9, 125.4, 122.3, 120.7, 118.3, 117.6, 114.7, 98.5, 58.1, 55.6.

3-Allyl-5-methoxy-2-(4-methoxyphenyl)-2*H*-indazole (7a). Compound **6a** (300 mg, 0.90 mmol) was dissolved in anhydrous 1,4-dioxane and the following reagents were added: $Pd_2(dba)_3$ (36 mg, 4.5 mol%), $PtBu_3$ (32 mg, 18 mol%), CsF (301 mg, 2.20 mmol), and allyltributyltin (241 μL, 1.08 mmol). The reaction mixture was heated at 100 °C for 2 h under an Ar atmosphere. The reaction mixture was collected by filtration, the solvent removed in a vacuum and the crude product purified by flash chromatography (30% EtOAc/hexanes) to give **7a** (65%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, J = 0.9 Hz, 1H), 7.82 (d, J = 6.9 Hz, 2H), 7.73 (dd, J = 9.3 Hz, 0.9 Hz, 1H), 7.25 (d, J = 9.3 Hz, 1H), 7.07 (d, J = 6.9 Hz, 2H), 6.11-6.01 (m, 1H), 5.17-5.06 (m, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.71-3.68 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 151.5, 145.7, 136.5, 133.6, 124.0, 122.5, 119.6, 118.7, 116.9, 116.6, 115.4, 114.7, 58.3, 55.6, 31.5.

3-(3-Hydroxypropoxy)-5-methoxy-2-(4-methoxyphenyl)-2*H*-indazole (8a). To an anhydrous tetrahydrofuran solution of **7a** (180 mg, 0.61 mmol) was added dropwise 1 M BH_3-THF (0.92 mL, 0.92 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 4 h under an Ar atmosphere. The mixture was quenched with water (2 mL). Sodium hydroxide (4 M, 1 mL) and 30% H_2O_2 (1.2 mL) were added and the resulting reaction mixture was stirred for 20 min more in ice bath. The reaction mixture was extracted with ethyl acetate and the combined organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (50% EtOAc/hexane) to give **8a** (36%) as a colorless oil: ^{1}H NMR (300

MHz, $CDCl_3$) δ 8.29 (d, J = 0.9 Hz, 1H), 7.82 (d, J = 9.0 Hz, 2H), 7.66 (dd, J = 9.3, 0.9 Hz, 1H), 7.20 (d, J = 9.3 Hz, 1H), 7.04 (d, J = 9.0 Hz, 2H), 3.93 (s, 3H), 3.89 (s, 3H), 3.60-3.58 (m, 2H), 3.04 (t, J = 6.9 Hz, 2H), 2.41 (brs, 1H), 1.99-1.91 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.2, 151.2, 146.7, 134.2, 124.2, 122.2, 118.8, 118.2, 117.5, 116.8, 114.6, 61.3, 58.1, 55.6, 31.9, 22.8; MS (CI) m/z: 313 (MH^+). HRMS (CI) calcd for $C_{18}H_{21}N_2O_3$ (MH^+) 313.1552, found 313.1552.

5-Methoxy-2-(4-methoxyphenyl)-3-(3-*p*-toluenesulfonyloxypropyl)-2*H*-indazole (9a). Compound **8a** (80 mg, 0.26 mmol) was dissolved in dichloromethane. To a stirred mixture, *p*-toluenesulfonic anhydride (169 mg, 0.52 mmol) and pyridine (63 μL, 0.78 mmol) were added and stirred at room temperature for 2 h. The reaction mixture was extracted with ethyl acetate and combined organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (30% EtOAc/hexane) to give **9a** (93%) as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, J = 0.9 Hz, 1H), 7.85 (d, J = 9.0 Hz, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.66 (d, J = 9.3, 0.9 Hz, 1H), 7.28 (d, J = 8.1 Hz, 2H), 7.06 (d, J = 9.0 Hz, 2H), 4.08 (t, J = 6.3 Hz, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.00 (t, J = 7.2 Hz, 2H), 2.42 (s, 3H), 2.08-2.02 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.2, 151.3, 146.4, 144.7, 134.2, 133.1, 129.8, 127.8, 124.4, 122.2, 118.7, 117.1, 117.0, 116.9, 114.6, 70.3, 57.5, 55.6, 28.8, 23.4, 21.6; MS (CI) m/z: 467 (MH^+). HRMS (CI) calcd for $C_{25}H_{27}N_2O_5S$ (MH^+) 467.1640, found 467.1644.

3-(3-Fluoropropyl)-5-methoxy-2-(4-methoxyphenyl)-2*H*-indazole (10a). Tosylate **9a** (80 mg, 0.17 mmol) and TBAF·$3H_2O$ (108 mg, 0.34 mmol) were dissolved in anhydrous acetonitrile and stirred at 90 °C for 30 min. The reaction mixture was extracted with ethyl acetate and combined organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (20% EtOAc/hexane) to give **10a** (83%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, J = 0.6 Hz, 1H), 7.83 (d, J = 9.0 Hz, 2H), 7.68 (d, J = 9.3, 0.6 Hz, 1H), 7.21 (d, J = 9.3 Hz, 1H), 7.04 (d, J = 9.0 Hz, 2H), 4.50 (dt, J = 47.1, 5.7 Hz, 2H), 3.03 (t, J = 7.2 Hz, 2H), 2.12 (dp, J = 18.9, 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1, 151.3, 146.5, 134.2, 124.5, 122.2, 118.6, 117.7, 117.4, 116.8, 114.5, 83.6 (d, J = 163.0 Hz), 57.7, 55.6, 30.0 (d, J = 19.6 Hz), 23.0 (d, J = 5.4 Hz); MS (CI) m/z: 315 (MH^+). HRMS (CI) calcd for $C_{18}H_{20}N_2O_2F$ (MH^+) 315.1509, found 315.1513.

3-Bromo-2-(4-hydroxyphenyl)-2*H*-indazol-5-ol (1b). To a solution of **6a** (4.13 g, 12.4 mmol) in anhydrous dichloromethane was added 1 M BBr_3 (49.7 mL) in portions at 0 °C. The solution was allowed to warm to room temperature slowly and stirred overnight. The reaction was quenched with water at 0 °C. The reaction mixture was diluted by ethyl acetate and then washed by brine solution. The organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (70% EtOAc/hexane) to give **1b** (94%) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.42 (d, J = 0.6 Hz, 1H), 7.75 (d, J = 9.0 Hz, 2H), 7.55 (dd, J = 9.0 Hz, 0.6 Hz, 1H), 7.09 (d, J = 9.0 Hz, 1H), 6.97 (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, MeOD-d4) δ 157.6, 148.9, 145.2, 132.6, 125.0, 122.2, 120.7, 120.6, 117.0, 115.6, 93.5.

3-Bromo-5-(methoxymethoxy)-2-(4-methoxymethoxyphenyl)-2*H*-indazole (6b). To a solution of **1b** (3.6 g, 11.8 mmol) in anhydrous THF was added chloromethyl methyl ether (MOM chloride, 5.4 mL, 70.8 mmol) and NaH (60%, 1.42 g, 35.4 mmol) in several portions over 5 min. The reaction mixture was refluxed for 2 h and then quenched with water in an ice bath. The solution was diluted with ethyl acetate and then washed by water, dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (25% EtOAc/hexane) to give **6b** (70%) as a pale red oily solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (d, J = 0.6 Hz, 1H), 7.83 (d, J = 9.0 Hz, 2H), 7.72 (dd, J = 9.3 0.6 Hz, 1H), 7.31 (d, J = 9.3 Hz, 1H), 7.21 (d, J = 9.0 Hz, 2H), 5.29 (s, 2H), 5.27 (s, 2H), 3.63 (s. 3H), 3.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.1, 149.0, 146.5, 134.7, 125.2, 122.2, 121.1, 120.7, 118.3, 117.1, 100.7, 96.7, 94.6, 56.7, 56.2; MS (CI) m/z: 393 (MH^+). HRMS (CI) calcd for $C_{17}H_{18}N_2O_4Br$ (MH^+) 393.0449, found 393.0451.

3-Allyl-5-(methoxymethoxy)-2-(4-methoxymethoxyphenyl)-2*H*-indazole (7b). To a solution of **6b** (200 mg, 0.51 mmol) in anhydrous 1,4-dioxane was added allyltributyltin (0.17 mL, 0.77 mmol), CsF (170 mg, 1.12 mmol) and $Pd(P(t\text{-}Bu)_3)_2$ (7.8 mg, 3 mol%). After 4 h at 100 °C, reaction mixture was extracted with ethyl acetate and dried over sodium sulfate. The combined organic layer was purified by flash column chromatography (25% EtOAc/hexane) to give **7b** (75%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.80 (d, J = 9.0 Hz, 2H), 7.65 (d, J = 9.3 Hz, 1H), 7.30-7.18 (m, 3H), 6.11-6.01 (m, 1H), 5.26 (s, 2H), 5.18 (s, 2H), 5.13-5.07 (m, 2H), 3.72 (d, J = 6.0 Hz, 2H), 3.58 (s, 3H), 3.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.8, 148.9, 147.1, 136.5, 135.1, 124.0, 122.2, 120.7, 119.3, 118.2, 117.2, 117.0, 115.5, 96.8, 94.5, 56.4, 56.1, 31.8; MS (CI) m/z: 355 (MH^+). HRMS (CI) calcd for $C_{20}H_{23}N_2O_4$ (MH^+) 355.1658, found 355.1655.

5-(Methoxymethoxy)-2-(4-(methoxymethoxy)phenyl)-3-(3-*p*-toluenesulfonyloxypropyl)-2*H*-indazole (9b). This material was prepared by a procedure similar to that used for the preparation of compound **8a** described above: 120 mg (0.34 mmol) of olefin **7b** was added to 0.68 mL (0.68 mmol) of 1 M BH_3-THF in dried THF at 0 °C. After 2 h at 0 °C, water was added cautiously to decompose excess hydride. Oxidation was carried out by adding 0.60 mL of 4 N NaOH, followed by dropwise addition of 0.70 mL of 30% hydrogen peroxide. The resulting mixture was stirred for 20 min in ice bath. The solution was diluted by ethyl acetate, then washed by water and dried over sodium sulfate. The crude product was purified by flash column chromatography (50% EtOAc/hexane) to give **8b** (43%, about 85% purity) as a yellow oil: MS (CI) m/z: 373 (MH^+). HRMS (CI) calcd for $C_{20}H_{25}N_2O_5$ (MH^+) 373.1763, found 373.1765.

The collected product **8b** was dissolved in dichloromethane. To a stirred mixture, *p*-toluenesulfonic anhydride

(222 mg, 0.68 mmol) and pyridine (82 μL, 1.02 mmol) were added and stirred at room temperature for 2 h. The reaction mixture was extracted with ethyl acetate and combined organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (40% EtOAc/hexane) to give **9b** (88%) as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.85 (d, J = 9.0 Hz, 2H), 8.32 (s, 1H), 7.76 (d, J = 8.1 Hz, 2H), 7.34-7.19 (m, 5H), 5.26 (s, 2H), 5.18 (s, 2H), 4.10 (t, J = 6.0 Hz, 2H), 3.54 (s, 3H), 3.53 (s, 3H), 3.00 (t, J = 7.2 Hz, 2H), 2.43 (s, 3H), 2.11-2.07 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.8, 149.2, 146.8, 144.7, 135.1, 133.0, 129.8, 127.8, 124.2, 122.2, 119.0, 118.5, 117.1, 117.0, 96.2, 94.6, 70.3, 56.3, 56.1, 28.9, 23.6, 21.6; MS (FAB) m/z: 527 (MH^+). HRMS (CI) calcd for $C_{27}H_{31}N_2O_7S$ (MH^+) 527.1852, found 527.1849.

3-(3-Fluoropropyl)-5-hydroxy-2-(4-hydroxyphenyl)-2*H*-indazole (1h, FPIE). This material was prepared by a procedure similar to that used for the preparation of compound **10a**, described above: tosylate **9b** (60 mg, 0.11 mmol) and TBAF·3H_2O (72 mg, 0.23 mmol) were dissolved in anhydrous acetonitrile and stirred at 90 °C for 30 min. The reaction mixture was extracted with ethyl acetate and combined organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (20% EtOAc/hexane) to give **10b** (86%) as a pale yellow oil. The collected product **10b** was dissolved in CH_3CN. To a stirred mixture, 1 N HCl (0.3 mL) was added and stirred at 80 °C for 30 min. The reaction mixture was extracted with ethyl acetate and combined organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (70% EtOAc/hexane) to give **1h** (93%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (d, J = 0.6 Hz, 1H), 7.70 (d, J = 9.0 Hz, 2H), 7.42 (dd, J = 9.3 Hz, 0.6 Hz, 1H), 7.03 (d, J = 9.3 Hz, 1H), 6.95 (d, J = 9.0 Hz, 2H), 4.48 (dt, J = 47.4, 6.3 Hz, 2H), 2.99 (t, J = 7.5 Hz, 2H), 2.09 (dp, J = 24.6, 7.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.3, 148.3, 145.9, 132.9, 124.5, 122.2, 121.0, 119.2, 115.6, 115.1, 113.4, 83.2 (d, J = 162.5 Hz), 29.8 (d, J = 19.4 Hz), 22.3 (d, J = 6.2 Hz); MS (CI) m/z: 287 (MH^+). HRMS (CI) calcd for $C_{16}H_{16}N_2O_2F$ (MH^+) 287.1196, found 287.1197.

5-(Methoxymethoxy)-2-(4-methoxymethoxyphenyl)-3-vinyl-2*H*-indazole (7c). This material was prepared by a procedure similar to that used for the preparation of compound **7b**, described above: solution of **6b** (300 mg, 0.77 mmol) in anhydrous 1,4-dioxane was added vinyltributyltin (0.40 mL, 1.38 mmol), CsF (258 mg, 1.7 mmol) and Pd(P(*t*-Bu)$_3$)$_2$ (12 mg, 3 mol%). After 4 h at 100 °C, reaction mixture was extracted with ethyl acetate and dried over sodium sulfate. The combined organic layer was purified by flash column chromatography (25% EtOAc/hexane) to give **7c** (78%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.82 (d, J = 6.9 Hz, 2H), 7.72 (d, J = 9.3 Hz, 1H), 7.29 (d, J = 9.3 Hz, 1H), 7.19 (d, J = 6.9 Hz, 2H), 5.92 (dd, J = 18.0, 1.2 Hz, 1H), 5.55 (dd, J = 11.7, 1.2 Hz, 1H), 5.27 (s, 2H), 5.25 (s, 2H), 3.58 (s, 3H), 3.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 149.8, 147.3, 135.0, 130.8, 122.3, 121.0, 120.1, 120.0, 119.1, 117.6, 117.0, 116.4, 96.5, 94.6, 56.5, 56.1; MS (CI) m/z: 341 (MH^+). HRMS (CI) calcd for $C_{19}H_{21}N_2O_4$ (MH^+) 341.1501, found 341.1498.

3-(2-Hydroxyethoxy)-5-(methoxymethoxy)-2-(4-methoxymethoxyphenyl)-2*H*-indazole (8c). This material was prepared by a procedure similar to that used for the preparation of compound **8a**, described above: to 250 mg (0.34 mmol) of olefin **7c** was added to 0.88 mL (0.88 mmol) of 1 M BH3-THF in dried THF at 0 °C. The reaction mixture was allowed to warm to room temperature slowly and stirred for 12 h under an Ar atmosphere. The mixture was cooled to 0 °C again and added cautiously to decompose excess hydride. Oxidation was carried out by adding 0.78 mL of 4 N NaOH, followed by dropwise addition of 0.90 mL of 30% hydrogen peroxide. The resulting mixture was stirred for 1 h in ice bath. The solution was diluted by ethyl acetate and then washed by water and dried over sodium sulfate. The crude product was purified by flash column chromatography (50% EtOAc/hexane) to give **8c** (48%) as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.81 (d, J = 6.9 Hz, 2H), 7.66 (d, J = 9.3 Hz, 1H), 7.29 (d, J = 9.3 Hz, 1H), 7.19 (d, J = 6.9 Hz, 2H), 5.25 (s, 2H), 5.22 (s, 2H), 4.00 (t, J = 6.3 Hz, 2H), 3.57 (s, 3H), 3.54 (s, 3H), 3.23 (t, J = 6.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 149.7, 146.9, 135.0, 124.5, 122.2, 120.0, 119.3, 117.4, 117.0, 116.9, 96.5, 94.6, 62.6, 56.4, 56.2, 31.3; MS (CI) m/z: 359 (MH^+). HRMS (CI) calcd for $C_{19}H_{23}N_2O_5$ (MH^+) 359.1607, found 359.1601.

5-(Methoxymethoxy)-2-(4-methoxymethoxyphenyl)-3-(3-*p*-toluenesulfonyloxypropyl)-2*H*-indazole (9c). According to the procedure for the preparation of compound **9a** described above, tosylate **8c** (110 mg, 0.31 mmol) was dissolved in dichloromethane. To a stirred mixture, *p*-toluenesulfonic anhydride (200 mg, 0.61 mmol) and pyridine (73 μL, 0.9 mmol) were added and stirred at room temperature for 2 h. The reaction mixture was extracted with ethyl acetate and combined organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (40% EtOAc/hexane) to give **9c** (91%) as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, J = 0.9 Hz, 1H), 7.79 (d, J = 9.0 Hz, 2H), 7.63 (dd, J = 9.3 Hz, 0.9 Hz, 1H), 7.54 (d, J = 8.4 Hz, 2H), 7.23-7.13 (m, 3H), 7.14 (d, J = 8.1 Hz, 2H), 5.27 (s, 2H), 5.14 (s, 2H), 4.36 (t, J = 6.9 Hz, 2H), 3.55 (s, 3H), 3.50 (s, 3H), 3.29 (t, J = 6.9 Hz, 2H), 2.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 149.8, 146.6, 144.5, 134.9, 132.7, 129.5, 127.5, 124.2, 122.1, 119.3, 119.0, 117.9, 117.0, 114.1, 96.1, 94.6, 69.5, 56.3, 56.2, 27.7, 21.6; MS (CI) m/z: 513 (MH^+). HRMS (CI) calcd for $C_{26}H_{29}N_2O_7S$ (MH^+) 513.1695, found 513.1697.

3-(3-Fluoroethyl)-5-hydroxy-2-(4-hydroxyphenyl)-2*H*-indazole (1i, FEIE). Tosylate **9c** (80 mg, 0.16 mmol) and CsF (71 mg, 0.47 mmol) were dissolved in anhydrous *t*-butanol and stirred at 100 °C for 18 h. The reaction mixture was extracted with ethyl acetate and combined organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (25% EtOAc/hexane) to give **10c** (68%) as a pale yellow oil. The

collected product **10c** was dissolved in CH_3CN. To a stirred mixture, 1 N HCl (0.3 mL) was added and stirred at 80 °C for 30 min. The reaction mixture was extracted with ethyl acetate and combined organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (70% EtOAc/hexane) to give **1i** (91%) as a yellow solid: ^{1}H NMR (300 MHz, MeOD-d4) δ8.47 (d, J = 0.9 Hz, 1H), 7.68 (d, J = 6.9 Hz, 2H), 7.42 (dd, J = 9.3 Hz, 0.9 Hz, 1H), 7.00 (d, J = 9.3 Hz, 1H), 6.93 (d, J = 6.9 Hz, 2H), 4.65 (dt, J = 47.4, 6.9 Hz, 2H), 3.29 (m, 2H); ^{13}C NMR (75 MHz, MeOD-d4) δ 157.3, 149.0, 145.7, 132.9, 124.8, 122.2, 120.8, 119.5, 115.8, 115.6, 108.9, 82.3 (d, J = 167.3 Hz), 28.3 (d, J = 21.5 Hz); MS (CI) m/z: 273 (MH^+). HRMS (CI) calcd for $C_{15}H_{14}N_2O_2F$ (MH^+) 273.1039, found 273.1039.

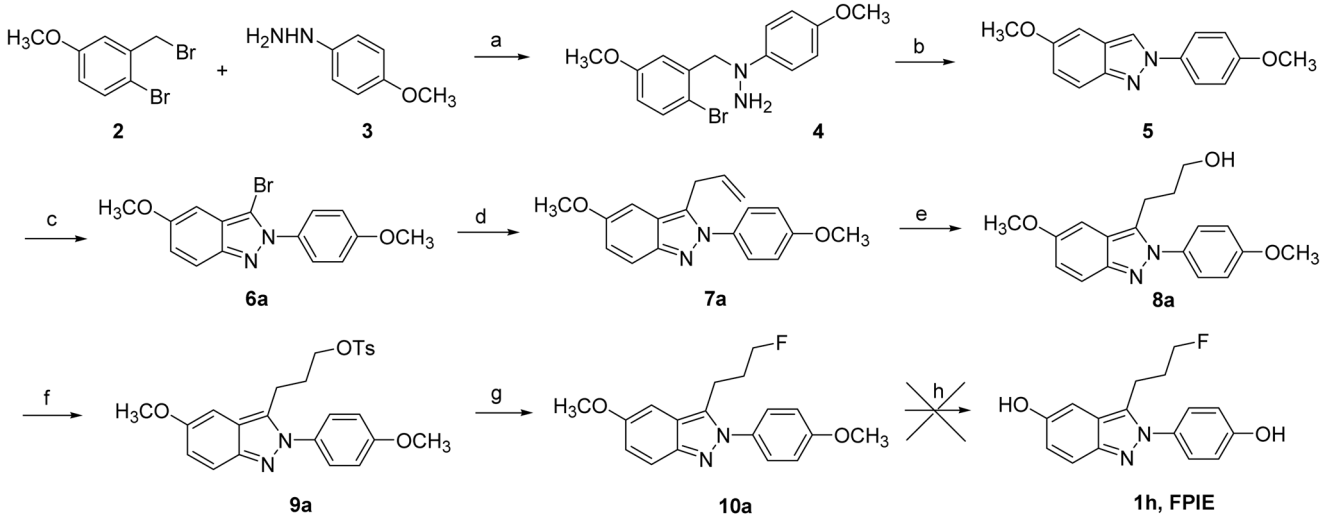
Estrogen receptor binding affinity assays. Relative binding affinities were determined by a competitive radiometric binding assay as previously described,[16] using 10 nM [^{3}H]estradiol as tracer ([6,7-^{3}H]estra-1,3,5,(10)-triene-3,17β-diol, 51-53 Ci/mmol, Amersham BioSciences, Piscataway, NJ), and purified full-length human ERα and ERβ were purchased from PanVera/Invitrogen (Madison, WI). Incubations were for 18-24 h at 0 °C. Hydroxyapatite (Bio-Rad, Hercules, CA) was used to absorb the receptor-ligand complexes, and free ligand was washed away. The binding affinities are expressed as relative binding affinity (RBA) values with the RBA of estradiol set to 100%. The values given are the average ± range or SD of three independent determinations. Estradiol binds to ERα with a K_d of 0.2 nM and to ERβ with a K_d of 0.5 nM.

Results and Discussion

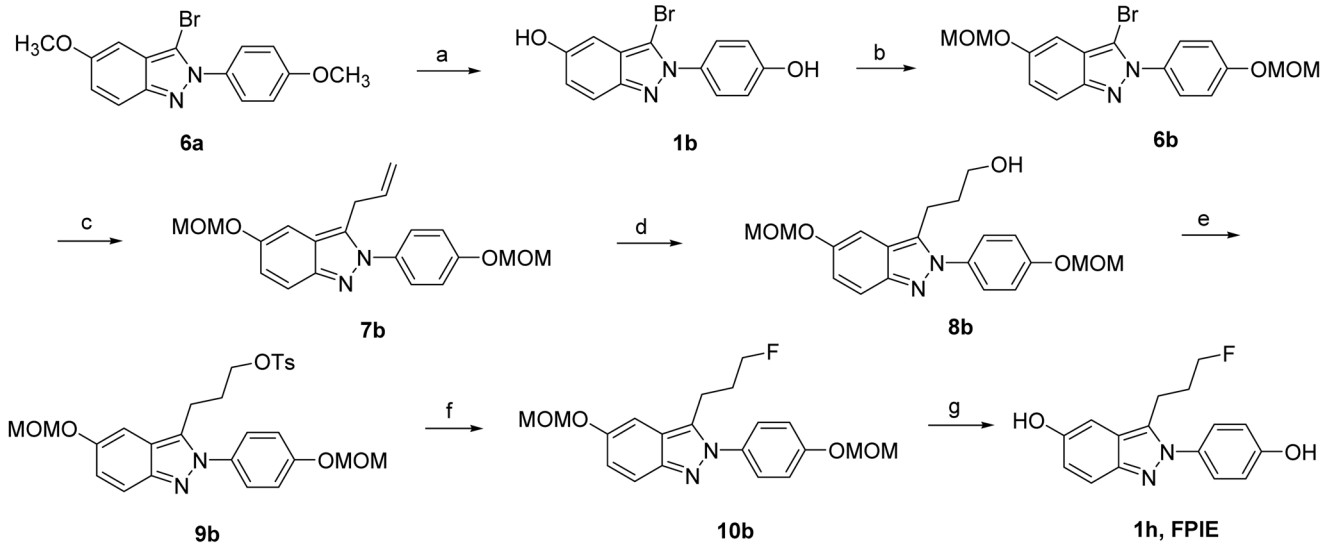
Synthesis. Synthesis of the indazole core structure was accomplished following a known procedure.[15] Treatment of phenylhydrazine **3** with sodium hexamethyl disilyl amide followed by the *o*-bromobenzyl bromide **2** provided the alkylated derivative **4** (Scheme 1). This was cyclized in a palladium-catalyzed coupling reaction and was then oxidized *in situ* to furnish the protected indazole derivative **5**. To prepare the desired fluoroalkyl analogues, we developed methods to introduce substituents at C3 that would contribute a vinyl or allyl moiety from which the fluoroethyl and fluoropropyl groups could be prepared. Since the C3 position can be easily halogenated, bromo-indazole derivative **6a** was obtained by treatment of the core system **5a** with NBS.

To prepare the allyl-substituted product **7a**, we used a Stille coupling reaction with allyltributyltin, $Pd_2(dba)_3$, P(*t*-Bu)$_3$, and CsF (Scheme 1). Although the corresponding iodo-indazole derivative could also be prepared by treating compound **5** with I_2, the only compound obtained under diverse Pd catalyst-mediated Stille coupling reaction conditions was the deiodinated compound **5**, with no formation of the allylated compound **7a** being evident. Hydroboration of alkene **7a** using borane-THF complex in THF at 0 °C for 2 h and subsequent oxidation with alkaline peroxide gave the aliphatic alcohol **8a**, which was converted the corresponding tosylate **9a**. The ether-protected fluoropropyl compound **10a** was prepared from the tosylated precursor **9a**, using TBAF·$3H_2O$. However, when we tried to deprotect methyl ether groups using various demethylation reagents such as BF_3·SMe_2, BBr_3, BF_3, $AlCl_3$, CH_3SO_3H, [bmim][Br]+TsOH,[17] and [bmim][Br]+HBr,[17] as well as others (Scheme 1), we were unable to obtain the desired fluorobisphenol without also cleaving the C-F bond. This necessitated a change to a more easily cleaved protecting group.

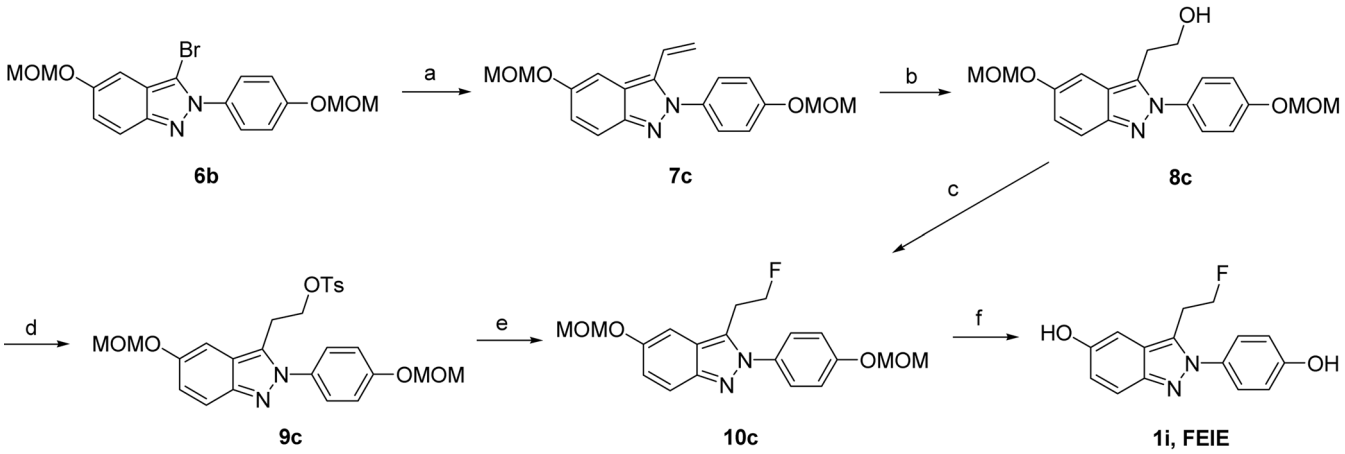
As a new protecting group, we selected the MOM group because it should be stable during the fluorination step, which is conducted under basic conditions, yet can be rapidly removed under relatively mild acidic conditions. Our second synthesis began with deprotection of the methyl



Scheme 1. Reagents and Conditions: a) NaHMDS, THF, 0 °C to rt, 1 h; b) $Pd(OAc)_2$, dppf, NaO^{t}Bu, toluene, 90 °C, 15 h; c) NBS, AcOH, reflux, 12 h; d) allyltributyltin, CsF, $Pd_2(dba)_3$, P(*t*-Bu)$_3$, 1,4-dioxane, 100 °C, 2 h; e) BH_3-THF complex, 4 N NaOH, 30% H_2O_2, THF, 0 °C, 4 h; f) Ts_2O, pyridine, CH_2Cl_2, rt, 2 h; g) TBAF·$3H_2O$, CH_3CN, 90 °C, 30 min; h) BF_3·SMe_2, BBr_3, BF_3, $AlCl_3$, [bmim][Br]+*p*-TsOH or [bmim][BF_4]+HBr and others.



Scheme 2. Reagents and Conditions: a) BBr_3, CH_2Cl_2, 0 °C to rt 12 h; b) MOMCl, NaH, THF, 0 °C to reflux, 2 h; c) allyltributyltin, $Pd(P(t\text{-}Bu)_3)_2$, CsF, 1,4-dioxane, 100 °C, 4 h; d) BH_3-THF complex, 4 N NaOH, 30% H_2O_2, THF, 0 °C, 2 h; e) Ts_2O, pyridine, CH_2Cl_2, rt, 2 h; f) TBAF·$3H_2O$, CH_3CN, 90 °C, 30 min; g) 1 N HCl, CH_3CN, 80 °C, 30 min.



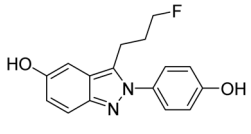
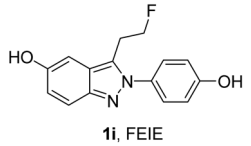
Scheme 3. a) Reagents and Conditions: $vinylSnBu_3$, $Pd(P(t\text{-}Bu)_3)_2$, CsF, 1,4-dioxane, 100 °C, 4 h; b) BH_3·THF complex, 4 N NaOH, 30% H_2O_2, THF, 0 °C to rt, 12 h; c) DAST, CH_2Cl_2, −10 °C, 2 h, < 10% yield; d) Ts_2O, pyridine, CH_2Cl_2, rt, 2 h; e) TBAF·$3H_2O$, CH_3CN, 90 °C, 30 min, < 3% yield; or CsF, *tert*-BuOH, 100 °C, 18 h, 68% yield; f) 1 N HCl, CH_3CN, 80 °C, 30 min.

groups in intermediate **6a** (Scheme 2). This was successfully achieved under typical BBr_3 conditions to give the bromobisphenol **1b**, which was then reprotected with MOMCl and NaH condition to give the MOM-protected analog **6b**. Allylated **7b** was prepared from the MOM-protected 3-bromoindazole **6b** by the Still reaction using $Pd(P(t\text{-}Bu)_3)_2$ and allyltributyltin. Hydroboration of **7b** with BH_3·THF followed by treatment with aqueous NaOH/H_2O_2 converted the alkene **7b** into the terminal alcohol **8b**, which was converted to the tosylate, giving the fluorination precursor **9b**. Subsequently, fluorination to give **10b** was achieved by employing reaction conditions similar to those shown in Scheme 1. Deprotection of the MOM groups in **10b** was easily achieved by treatment with 1 N HCl to give final product fluoropropylated indazole estrogen (FPIE, **1h**).

For the synthesis of the tosylated precursor for fluoroethylated indazole estrogen (FEIE, **1i**) (Scheme 3), Stille coupling of the MOM-protected bromoindazole **6b** with vinyltributyltin, hydroboration, followed by oxidation and tosylation of primary alcohol, were achieved under reaction conditions similar to those shown Scheme 2. To prepare an unlabeled authentic sample, the hydroxy group in compound **8c** was converted to fluorine using DAST to give, after MOM deprotection, the desired fluoroethylated indazole estrogen (FEIE, **1i**) in < 10% yield. Fluorination of **9c** with TBAF·$3H_2O$ was achieved in < 3% yield, with the vinyl compound **7c**, resulting from elimination, being the major reaction product. Despite the low yields obtained in both of these fluorination reactions, MOM-protected FEIE (**10c**) can be synthesized efficiently from the tosylated precursor **9c** in 68% yield, using CsF in *tert*-BuOH, novel fluorination conditions that we have recently described.[18]

Estrogen receptor binding assays. The fluoroethyl- and fluoropropyl indazole analogues, FPIE (**1h**) and FEIE (**1i**),

Table 1. Relative binding affinity (RBA) values for ERα and ERβ and the selectivity for ERβ as determined by β/α ratio

Compound	ERα	ERβ	β/α
 **1h**, FPIE	0.005 ± 0.001	0.087 ± 0.013	17.4
 **1i**, FEIE	0.003 ± 0.001	0.124 ± 0.007	41.3

were evaluated in competitive radiometric binding assays to determine their affinities for human ERα and ERβ. Affinities from these competitive binding assays are expressed as relative binding affinity (RBA) values, that is, relative to the affinity of [^{3}H]estradiol, which is 100% by definition. The RBA values and ERβ/ERα (β/α) ratios are summarized in Table 1.

The indazole estrogen derivatives substituted at C3 with either fluoroethyl or fluoropropyl groups were investigated. The affinity of the fluoropropylated indazole estrogen (FPIE, **1h**) for ERα is only 0.005% that of estradiol, while its affinity for ERβ is around 0.087%, giving an ERβ affinity selectivity of 17.4 fold based on comparison of RBA values. With the fluoroethylated indazole estrogen (FEIE, **1i**), the affinity for ERα (0.003%) is slightly lower than that of FPIE (**1h**), while its affinity for ERβ was 0.124%, giving it an ERβ selectivity of 41.3 fold, which is actually higher than that of the fluoropropyl-substituted analog. Thus, in terms of both ERβ binding affinity and selectivity, the fluoroethyl derivative (FEIE, **1i**) is better of two fluoroalkyl indazoles.

The substitution of the indazole system at C3 with these fluoroalkyl groups (as in FPIE, **1h** and FEIE, **1i**) rather than halogens (**1a-c**) or alkyl, nitrile, or other groups (**1d-g**), led to a very marked reduction in affinity, although strong ERβ affinity selectivity still persisted. From the binding trends shown in Figure 1, it is clear that as the length of an alkyl substituent increase, binding affinity drops (compare ethyl **1f** *vs*. propyl **1g**). In addition, although ERβ seems more tolerant of interior polarity,[11] as a general rule, polar substituents are generally not preferred in the interior of estrogen ligands.[19] Given the polar nature of a single fluorine substituent, it is therefore not surprising, in retrospect, that both FPIE (**1h**) and FEIE (**1i**) are relatively low affinity ligands for both ERs. These indazole estrogens, therefore, are not adequate as a PET imaging radiotracer and further investigation needs.

In summary, we have prepared two novel 2-phenyl-2*H*-indazoles having fluoroethyl- and fluoropropyl side chain as PET tracer candidates for the imaging of estrogen receptor b. Both fluoroalkyl derivatives showed low binding affinity values for ERα and ERβ. However, the fluoroethyl analog, FEIE (**1i**) showed good relative binding affinity selectivity for ERβ (41.3-fold ERβ selective). It is possible that modification of indazole estrogens at the C3', C6, or C7 position might lead to compounds with better estrogen receptor binding affinities than those modified at the C3 position. Further investigations of such derivatives might be fruitful.

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