

Supporting Information

DESIGN AND SYNTHESIS OF 4-(2-PYRROLYL)-4-PHENYLHEPTANE DERIVATIVES AS ESTROGEN RECEPTOR ANTAGONISTS

EXPERIMENTAL

General

Materials and Methods

All chemicals were purchased from Sigma-Aldrich, FUJIFILM Wako Pure Chemicals, and Tokyo Chemical Industry and were used without further purification. TLC analysis was conducted using Merck silica gel 60 F254 pre-coated plates and visualized using a 254 nm/365 nm UV lamp, and iodine stain. Column chromatography was performed using silica gel (spherical, neutral) purchased from Kanto Chemical. ^1H and ^{13}C NMR spectra were recorded on a JEOL ECZ600 spectrometer, and measurements were carried out in CDCl_3 with 0.03% tetramethylsilane or MeOD-d_4 . High-resolution mass spectra were measured using a Shimadzu IT-TOF MS equipped with an electrospray ionization source.

4-(4-Hydroxyheptan-4-yl)methylphenol (**6**).

To a stirred solution of **5** (4.15 g, 25.0 mmol) in THF (25 mL) at $-78\text{ }^\circ\text{C}$ was added dropwise *n*-propylmagnesium bromide (1M in THF, 50 mL, 0.10 mol) over 15 min. The reaction mixture was stirred at room temperature for 16 h. After cooling to $0\text{ }^\circ\text{C}$, the reaction mixture was quenched with sat. NH_4Cl aq. then concentrated under reduced pressure to remove a majority of THF. The residue was added water (25 mL), and extracted with EtOAc (100 mL) x 2, the combined organic extracts were washed with brine, dried over Na_2SO_4 , filtered, concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexanes/EtOAc = 9 : 1 to 8 : 2) to afford **6** as a white solid (4.8 g, 86%).

NMR spectrum was in agreement with the reported one.¹⁴

4-[4-(Benzyloxy)-3-methylphenyl]heptan-4-ol (**7**).

To a solution of **6** (0.22 g, 1.0 mmol) in acetone (2 mL) was added benzyl chloride (173 μL , 1.5 mmol), followed by K_2CO_3 (415 mg, 3.0 mmol) at room temperature. The reaction mixture was stirred at $60\text{ }^\circ\text{C}$ for 12 h. The reaction mixture was diluted with EtOAc, washed with water, brine, dried over Na_2SO_4 , filtered, concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexanes/EtOAc = 95 : 5 to 80 : 20) to afford **7** as a colorless oil (270 mg, 86%).

^1H NMR (600 MHz, CDCl_3) δ 7.45 (d, $J = 7.8$ Hz, 2H), 7.32 (dd, $J = 7.8, 7.8$ Hz, 2H), 7.32 (t, $J = 7.8$ Hz,

1H), 7.16 (d, $J = 2.4$ Hz, 1H), 7.12 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.83 (d, $J = 9.0$ Hz, 1H), 5.06 (s, 2H), 2.31 (s, 3H), 1.79 – 1.70 (m, 4H), 1.31 – 1.25 (m, 2H), 1.13 – 1.08 (m, 2H), 0.85 (t, $J = 7.8$ Hz, 6H).

General procedure for preparation of compounds 2-4.

To a mixture of **1** (0.50 mmol, 1 eq.) and amines (2 eq.) in DMF (1 mL) was added EDC-HCl (2 eq.), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc, washed with 1M HCl aq., sat. NaHCO₃ aq., brine. The organic layer was dried over Na₂SO₄, filtered, concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the desired amide compounds.

5-[1-(4-Hydroxy-3-methylphenyl)-1-propylbutyl]-1H-pyrrole-2-carboxamide (2):

Prepared using HOBt-NH₃ as an amine source, and purified by silica gel column chromatography (Hexane/EtOAc = 80 : 20 to 30 : 70) to give a white solid (Yield : 10%)

¹H NMR (600 MHz, CDCl₃) δ 9.31 (s, 0.4H), 8.83 (s, 0.6H), 6.97 – 6.87 (m, 2H), 6.94 – 6.78 (m, 1H), 6.75 (s, 0.6 H), 6.67 – 6.60 (m, 2H), 6.56 – 6.55 (m, 0.4H), 6.27 (s, 0.4H), 6.13 – 6.12 (m, 0.6H), 5.55 (br. s, 2H), 2.21 – 2.17 (m, 0.6H), 1.93 – 1.83 (m, 4H), 1.05 – 1.00 (m, 4H), 0.85 (t, $J = 7.2$ Hz, 6H).

HRMS (ESI) m/z calculated for C₁₉H₂₇N₂O₂⁺ 315.2067 [M + H]⁺, found 315.2042 [M + H]⁺

N-Ethyl-5-[1-(4-hydroxy-3-methylphenyl)-1-propylbutyl]-1H-pyrrole-2-carboxamide (3):

Prepared using ethylamine in THF (2M) as an amine, and purified by silica gel column chromatography (Hexane/EtOAc = 80 : 20 to 50 : 50) to give a white foam (Yield : 15%)

¹H NMR (600 MHz, CDCl₃) δ 8.99 (s, 1H), 7.13 (br, 1H), 6.85 (d, $J = 1.8$ Hz, 1H), 6.72 (d, $J = 7.8, 1.8$ Hz, 1H), 6.55 (d, $J = 7.8$ Hz, 1H), 6.47 (dd, $J = 3.0$ Hz, 1H), 6.11 (dd, $J = 3.0$ Hz, 1H), 5.82 (brt, $J = 6.0$ Hz, 1H), 3.41 (d, $J = 7.8$ Hz, 2H), 2.14 (s, 3H), 1.92 – 1.89 (m, 4H), 1.20 (t, $J = 7.8$ Hz, 3H), 1.03 – 1.01 (m, 4H), 0.84 (t, $J = 7.2$ Hz, 6H).

¹³C NMR (151 MHz, CDCl₃) δ 161.58, 152.40, 144.51, 137.22, 129.48, 125.73, 124.07, 123.28, 114.35, 108.58, 107.50, 45.83, 40.18, 34.31, 17.20, 16.24, 15.06, 14.60.

HRMS (ESI) m/z calculated for C₂₁H₃₁N₂O₂⁺ 343.2380 [M + H]⁺, found 343.2417 [M + H]⁺

N-Benzyl-5-[1-(4-hydroxy-3-methylphenyl)-1-propylbutyl]-1H-pyrrole-2-carboxamide (4):

Prepared using benzylamine as an amine, and purified by silica gel column chromatography (CH₂Cl₂/MeOH = 100 : 0 to 95 : 5) to give a light brown solid (Yield : 9%)

¹H NMR (600 MHz, CDCl₃) δ 9.07 (s, 1H), 7.35 – 7.27 (m, 6H), 6.86 (d, $J = 2.4$ Hz, 1H), 6.72 (dd, $J = 8.4, 2.4$ Hz, 1H), 6.55 (d, $J = 8.4$ Hz, 1H), 6.49 (dd, $J = 3.0, 1.8$ Hz, 1H), 6.13 (t, $J = 3.0$ Hz, 1H), 6.11

(dd, $J = 3.0, 3.0$ Hz, 1H), 4.56 (d, $J = 6.0$ Hz, 2H), 2.14 (s, 3H), 1.93 – 1.90 (m, 4H), 1.05 – 1.01 (m, 4H), 0.85 (t, $J = 7.2$ Hz, 6H).

^{13}C NMR (151 MHz, CDCl_3) δ 161.54, 152.43, 144.95, 138.26, 137.11, 129.46, 128.70, 127.88, 127.52, 125.73, 123.71, 123.33, 114.43, 109.15, 107.65, 45.88, 43.44, 40.21, 17.20, 16.25, 14.60.

HRMS (ESI) m/z calculated for $\text{C}_{26}\text{H}_{33}\text{N}_2\text{O}_2^+$ 405.2537 $[\text{M} + \text{H}]^+$, found 405.2556 $[\text{M} + \text{H}]^+$

Ethyl 5-{1-[4-(benzyloxy)-3-methylphenyl]-1-propylbutyl}-1H-pyrrole-2-carboxylate (9).

To a stirred mixture of **7** (0.62 g, 2.0 mmol) and ethyl pyrrole-2-carboxylate (0.33 g, 2.4 mmol) in CH_2Cl_2 (8 mL) at 0 °C was added dropwise a solution of $\text{BF}_3 \cdot \text{OEt}_2$ (0.6 mL, 4.8 mmol) in CH_2Cl_2 (2 mL). After addition, the reaction mixture was allowed to warm up to room temperature, and kept stirred for 12 h. After cooling to 0 °C, the reaction mixture was quenched with sat. NaHCO_3 aq., and extracted with CH_2Cl_2 . The organic layer was dried over Na_2SO_4 , filtered, concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexanes/EtOAc = 90 : 10 to 80 : 20) to afford **7** as a colorless oil (459 mg, 53%).

^1H NMR (600 MHz, CDCl_3) δ 8.40 (s, 1H), 7.44 (d, $J = 7.2$ Hz, 2H), 7.38 (dd, $J = 7.2, 7.2$ Hz, 2H), 7.32 (dd, $J = 7.2, 7.2$ Hz, 1H), 6.94 – 6.93 (m, 2H), 6.82 (dd, $J = 3.0, 3.0$ Hz, 1H), 6.77 (d, $J = 7.2$ Hz, 1H), 6.14 (dd, $J = 3.0, 3.0$ Hz, 1H), 5.05 (s, 2H), 4.25 (q, $J = 7.2$ Hz, 2H), 2.23 (s, 3H), 1.95 – 1.91 (m, 4H), 1.31 (t, $J = 7.9$ Hz, 3H), 1.04 – 0.99 (m, 4H), 0.85 (t, $J = 7.2$ Hz, 6H).

HRMS (ESI) m/z calculated for $\text{C}_{26}\text{H}_{33}\text{N}_2\text{O}_2^+$ 434.2690 $[\text{M} + \text{H}]^+$, found 434.2721 $[\text{M} + \text{H}]^+$

Methyl 5-{1-[4-(benzyloxy)-3-methylphenyl]-1-propylbutyl}-1H-pyrrole-2-carboxylate (8).

Prepared by the similar procedure described in synthesis of compound **9**.

^1H NMR (600 MHz, CDCl_3) δ 8.39 (s, 1H), 7.45 (d, $J = 7.8$ Hz, 2H), 7.39 (dd, $J = 7.8, 7.8$ Hz, 2H), 7.32 (t, $J = 7.8$ Hz, 1H), 6.94 – 6.93 (m, 2H), 6.84 (dd, $J = 3.0, 3.0$ Hz, 1H), 6.78 (d, $J = 9.0$ Hz, 1H), 6.15 (dd, $J = 3.0, 3.0$ Hz, 1H), 5.05 (s, 2H), 3.78 (s, 3H), 2.24 (s, 3H), 1.96 – 1.90 (m, 4H), 1.06 – 1.00 (m, 4H), 0.85 (t, $J = 7.2$ Hz, 6H).

HRMS (ESI) m/z calculated for $\text{C}_{27}\text{H}_{34}\text{NO}_3^+$ 420.2533 $[\text{M} + \text{H}]^+$, found 420.2557 $[\text{M} + \text{H}]^+$

Methyl 5-[1-(4-hydroxy-3-methylphenyl)-1-propylbutyl]-1H-pyrrole-2-carboxylate (10).

To a solution of **8** (42 mg, 0.10 mmol) in MeOH (2 mL) was added 10% Pd/C (10 mg), followed by ammonium formate (6.3 mg, 1.0 mmol), and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of Celite to remove the insoluble materials. The filtrate was concentrated under reduced pressure to afford **10** as a colorless solid (32 mg, 97%).

^1H NMR (600 MHz, CDCl_3) δ 8.61 (s, 1H), 6.87 (s, 1H), 6.85 (dd, $J = 3.3, 3.3$ Hz, 1H), 6.82 (dd, $J = 8.4, 1.8$ Hz, 1H), 6.62 (d, $J = 8.4$ Hz, 1H), 6.15 (dd, $J = 3.3, 3.3$ Hz, 1H), 3.79 (s, 3H), 2.17 (s, 3H), 1.92 (t, $J = 8.4$ Hz, 4H), 1.05 – 1.00 (m, 4H), 0.85 (t, $J = 7.2$ Hz, 6H).

^{13}C NMR (151 MHz, CDCl_3) δ 162.09, 152.17, 145.92, 137.60, 129.81, 125.93, 123.28, 120.91, 115.40, 114.46, 108.56, 51.37, 46.00, 40.23, 17.17, 16.10, 14.56.

HRMS (ESI) m/z calculated for $\text{C}_{20}\text{H}_{28}\text{NO}_3^+$ 330.2064 $[\text{M} + \text{H}]^+$, found 330.2024 $[\text{M} + \text{H}]^+$

2-[(*tert*-Butoxycarbonyl)amino]-5-{1-[4-(benzyloxy)-3-methylphenyl]-1-propylbutyl}-1*H*-pyrrole (12).

To a solution of **9** (500 mg, 1.15 mmol) in THF (10 mL)/EtOH (10 mL) was added 4M LiOH aq. (10 mL), and the reaction mixture was stirred at 80 °C for 6 h. After cooling to room temperature, the reaction mixture was acidified by the addition of 10% HCl aq., and extracted with EtOAc (20 mL x 2). The combined organic extracts were dried over Na_2SO_4 , filtered, concentrated under reduced pressure to afford the corresponding carboxylic acid as a pale brown foam (240 mg, 51%). This product was used in next reaction without further purification. The above product was dissolved in toluene (2 mL)/*t*-BuOH (1 mL), and added the activated 4Å molecular sieve (60 mg). After being stirred at room temperature for 15 min, the mixture was added diphenylphosphoryl azide (86 μL , 0.40 mmol), and the reaction mixture was stirred at 90 °C for 16 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc, filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexanes/EtOAc = 95 : 5 to 80 : 20) to afford **12** as a colorless oil (90 mg, 57%).

^1H NMR (600 MHz, CDCl_3) δ 8.34 (s, 1H), 7.45 (d, $J = 7.8$ Hz, 2H), 7.38 (dd, $J = 7.5, 7.5$ Hz, 2H), 7.31 (dd, $J = 7.2, 7.2$ Hz, 1H), 6.95 – 6.94 (m, 2H), 6.77 (d, $J = 9.6$ Hz, 1H), 6.73 (dd, $J = 3.0, 3.0$ Hz, 1H), 6.11 (dd, $J = 3.0, 3.0$ Hz, 1H), 5.04 (s, 2H), 2.24 (s, 3H), 1.94 – 1.91 (m, 4H), 1.52 (s, 9H), 1.06 – 1.01 (m, 4H), 0.85 (t, $J = 7.2$ Hz, 6H).

^{13}C NMR (151 MHz, CDCl_3) δ 160.97, 155.25, 144.81, 137.69, 137.51, 129.70, 128.44, 127.66, 127.09, 126.45, 125.47, 122.77, 114.07, 110.72, 108.06, 80.29, 69.80, 45.82, 40.07, 28.35, 17.14, 16.67, 14.58.

HRMS (ESI) m/z calculated for $\text{C}_{30}\text{H}_{41}\text{N}_2\text{O}_3^+$ 477.3112 $[\text{M} + \text{H}]^+$, found 477.3121 $[\text{M} + \text{H}]^+$

2-[(*tert*-Butoxycarbonyl)amino]-5-[1-(4-hydroxy-3-methylphenyl)-1-propylbutyl]-1*H*-pyrrole (13).

To a solution of **12** (48 mg, 0.10 mmol) in MeOH (2 mL) was added 10% Pd/C (10 mg), followed by ammonium formate (63 mg, 1.0 mmol), and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of Celite to remove the insoluble materials. The filtrate was concentrated under reduced pressure to afford **13** as a colorless foam (40 mg, quant.).

¹H NMR (600 MHz, CDCl₃) δ 8.75 (s, 1H), 6.87 (brs, 1H), 6.76 – 6.75 (m, 2H), 6.55 (d, *J* = 7.8 Hz, 1H), 6.12 (dd, *J* = 2.4, 2.4 Hz, 1H), 2.15 (s, 3H), 1.91 (t, *J* = 8.1 Hz, 4H), 1.53 (s, 9H), 1.04 – 1.00 (m, 4H), 0.85 (t, *J* = 7.2 Hz, 6H).

¹³C NMR (151 MHz, CDCl₃) δ 161.81, 152.17, 145.60, 137.53, 129.62, 125.88, 123.21, 122.54, 114.74, 114.42, 108.10, 80.80, 45.97, 40.21, 28.36, 17.17, 16.16, 14.59.

2-Amino-5-[1-(4-hydroxy-3-methylphenyl)-1-propylbutyl]-1H-pyrrole (14).

13 (26 mg, 0.07 mmol) was treated with 4M HCl in 1,4-dioxane (1 mL) at room temperature for 30 min. The volatiles were removed by evaporation to dryness. The residue was diluted with EtOAc (20 mL), washed with sat. NaHCO₃ aq. (10 mL x 2), and the organic layer were dried over Na₂SO₄, filtered, concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexanes/EtOAc = 2 : 1) to afford **14** as a colorless solid (14 mg, 74%).

¹H NMR (600 MHz, MeOD-*d*₄) δ 9.86 (brs, 1H), 6.78 (s, 1H), 6.72 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.70 (d, *J* = 3.6 Hz, 1H), 6.55 (d, *J* = 8.4 Hz, 1H), 6.00 (dd, *J* = 3.6, 3.6 Hz, 1H), 2.04 (s, 3H), 2.04 – 1.83 (m, 4H), 0.95 – 0.89 (m, 4H), 0.76 (t, *J* = 7.2 Hz, 6H).

¹³C NMR (151 MHz, MeOD-*d*₄) δ 154.60, 147.52, 138.52, 130.84, 126.69, 124.81, 122.75, 116.82, 115.00, 109.44, 47.07, 41.02, 18.30, 16.52, 14.92.

HRMS (ESI) *m/z* calculated for C₁₈H₂₆N₂NaO⁺ 309.1937 [M + Na]⁺, found 309.1946 [M + Na]⁺

Cell culture

Human breast carcinoma MCF-7 cells were maintained in RPMI1640 medium (R8758, SIGMA Life Science) containing 10% FBS. Tested compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution (10 mM). The cells were treated with a set of concentration of compounds (Final volume of DMSO in cell lysates was less than 0.1%).

Fluorescence polarization assay

The relative binding affinities of tested compounds for ERα were determined by fluorescence polarization-based competition binding assay using commercially available kits (P2698, Life Technologies, Inc.) according to the manufacture's instruction. After 16 h, fluorescence polarization signals (mP values) were measured using the plate reader (EnVision 2105, Perkin Elmer) with a 480 nm excitation/535 nm emission filter. The fraction of compounds bound to ERα was correlated to the mP value and plotted against values of competitor concentrations.

Reporter gene assay

ER-antagonistic activities of compounds were evaluated by means of reporter assay using Dual-Luciferase[®] Reporter Assay system (Promega). MCF-7 cells were transfected with *firefly* luciferase reporter plasmid containing three tandem copies of estrogen-response element and control *Renilla* luciferase plasmid-SV40 using Lipofectamine LTX (Life Technologies, Inc.). After 16 h, cells were treated with the indicated concentrations of tested compounds in the presence of 17 β -estradiol (0.3 nM). The firefly luciferase activity in cell lysates was determined based on fluorescence intensity using a plate reader (ARVO SX1420, Perkin Elmer), and normalized with *Renilla* luciferase.