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Chemistry & Drug Discovery

Supporting Information

Estrogenic analogues synthesized via click chemistry

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1. Experimental Section

Chemistry: Commercially available reagents (aminophenols and iodophenols) and solvents were purchased from Fluka-Aldrich or Lancaster and used without further purification. Tetrahydrofuran (THF) and diethylether were distilled immediately before use from Na/benzophenone under a slight positive atmosphere of N_2. Toluene was purified by distillation on sodium and stored on activated molecular sieves (4 Å). When needed, the reactions were performed in flame- or oven-dried glassware under a positive pressure of dry N_2.

Melting points were determined in open glass capillary with a Stuart scientific SMP3 apparatus and are uncorrected. All the compounds were checked by IR (FT-IR THERMO-NICOLET AVATAR); ^{1}H and ^{13}C NMR (JEOL ECP 300 MHz) and mass spectrometry (Thermo Finningan LCQ-deca XP-*plus*) equipped with an ESI source and an ion trap detector. Chemical shifts are reported in parts per million (ppm). Column chromatography was performed on silica gel (Merck Kieselgel 70-230 mesh ASTM) using the eluants indicated. Thin layer chromatography (TLC) was carried out on 5 x 20 cm plates with a layer thickness of 0.25 mm (Merck Silica gel 60 F_{254}). Elemental Analysis (C, H, N) of the target compounds were performed by Università dell'Insubria (Como, Italy) and are within ± 0.4 % of the calculated values unless otherwise noted.

General procedure for the synthesis of azides (1-3)

To 2 g of the corresponding aminophenol derivative (1 eq) were added 50 mL of water. To the resulting suspension 4.48 mL of HCl conc. were added. Upon cooling to 0 °C and addition of a solution of $NaNO_2$ (1 eq) in 5 mL of water, the reaction mixture was stirred for 10 minutes at 0-5°C. Sodium azide (1.2 eq) added was portionwise and the mixture was stirred at room temperature for 1 h. The reaction was worked up by dilution with EtOAc. The organic layer was washed with water (x1) and brine (x1) and dried over sodium sulphate. All the azides synthesized were stored at -20°C.

2-azidophenol (1). IR (film) 3300, 2120, 1494, 1294, 745 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 273 K) δ 7.70 - 6.90-6.75 (m, 4-H) ; ^{13}C-NMR (75 MHz, $CDCl_3$, 273 K) δ 161.4, 126.4, 125.7, 120.3, 119.7, 116.6; MS (ESI) *m/z* 158 (100%) $(M+Na)^+$ (yield = 50%)

3-azidophenol (2). IR (film) 3300, 2109, 1591, 1300, 1213, 765 cm^{-1};^{1}H-NMR (300 MHz, $CDCl_3$, 273 K) δ 7.12 (t, J = 8.2 Hz, 1-H), 6.61 (d, J = 8.2 Hz, 1-H), 6.52 (s, 1-H), 6.50 (d, J = 8.2 Hz, 1-H); ^{13}C-NMR (75 MHz, $CDCl_3$, 273 K) δ 157.9, 141.2, 130.6, 112.5, 110.7, 106,4; MS (ESI) *m/z* 158 (40%) $(M+Na)^+$ (yield = 70.5 %)

4-azidophenol (3). IR (film) 3348, 2359, 2109, 1504, 1233, 826 cm^{-1};^{1}H-NMR (300 MHz, $CDCl_3$, 273 K) δ 6.90 (d, J = 8.8 Hz, 2-H), 6.81 (d, J = 8.8 Hz, 2-H); ^{13}C-NMR (75 MHz, $CDCl_3$, 273 K) δ 154.7, 130.4, 119.8, 116.6; MS (ESI) *m/z* 158 (40%) $(M+Na)^+$ (yield = 55%)

General procedure for the synthesis of trimethylsilylethynylphenols

2 g of the corresponding iodophenol (1 eq) were dissolved in 20 mL of dry toluene. To the resulting solution 190 mg of $PdCl_2(PPh_3)_2$ (0.03 eq), 152 mg of freshly prepared copper (I) iodide (0.1 eq), 1.55 ml of DIPEA (1 eq) and 1.17 ml of trimethylsylilacetilene (1 eq) were subsequently added. The reaction was stirred at room temperature for 24 h. Evaporation of the solvent gave a crude product which was purified by column cromatography using Petroleum Ether/EtOAc 98:2 as the eluant to give the corresponding trimethylsilylacetylene derivative as a brown oil.

2-[2-(trimethylsilyl)ethynyl]phenol. IR (film) 3509, 2360, 1483, 1249, 838 cm^{-1};^{1}H-NMR (300 MHz, $CDCl_3$, 273 K) δ 7.34 (d, J = 7.7 Hz, 1-H), 7.22 (t, J = 7.4 Hz, 1-H), 6.92 (d, J = 8.2 Hz, 1-H), 6.83 (t, J = 7.7 Hz, 1-H), 0.24 (s, 9-H); ^{13}C-NMR (75 MHz, $CDCl_3$, 273 K) δ 157.2, 131.7, 130.7, 120.3, 114.6, 109.6, 102.3, 99.1, 0.03; MS (ESI) *m/z* 189 (100%) $(M-H)^-$ (yield = 87 %)

3-[2-(trimethylsilyl)ethynyl]phenol. IR (film) 3382, 2359, 1578, 1249, 839 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 273 K) δ 7.20 – 6.80 (m, 4-H), 0.28 (s, 9-H); ^{13}C-NMR (75 MHz, $CDCl_3$, 273 K) δ

155.5, 129.8, 124.6, 124.3, 116.4, 115.2, 105.3, 94.4, 0.09; MS (ESI) *m/z* 189 (100%) $(M-H)^-$ (yield = 90 %)

4-[2-(trimethylsilyl)ethynyl]phenol. IR (film) 3381, 2360, 1507, 1249, 832 cm^{-1};^{1}H-NMR (300 MHz, $CDCl_3$, 273 K) δ 7.29 (d, J = 8.2 Hz, 2-H), 6.73 (d, J = 8.2 Hz, 2-H), 0.19 (s, 9-H); ^{13}C-NMR (75 MHz, $CDCl_3$, 273 K) δ 156.4, 133.7, 115.5, 114.9, 105.5, 92.4, 0.14; MS (ESI) *m/z* 189 (100%) $(M-H)^-$ (yield = 92 %)

General procedure for the synthesis of ethynylphenols (4-6)

9.4 mL of TBAF sol. 1 M in THF (1.2 eq,) were added slowly to a solution containing 1.5 g of the trimethylsilyl(ethynyl)phenol derivative (1 eq) dissolved in dry THF (15 mL) cooled at 0°C. The resulting solution was stirred at this temperature for 30 minutes. The reaction was quenched by addition of sat. aq. NH_4Cl and the mixture extracted with EtOAc. The organic layer was washed with water (x1) and brine (x1). After drying over sodium sulphate and evaporation of the solvent, the crude product was purified by column chromatography using PE/EtOAc 95:5 as eluant to give the corresponding ethynylphenol as a brown oil.

2-ethynylphenol (4). IR (film) 3285, 2359, 1705, 1484, 1224, 753 cm^{-1};^{1}H-NMR (300 MHz, $CDCl_3$, 273 K) δ 7.37 (dd, J = 7.7/1.6 Hz, 1-H), 7.27 (t, J = 7.4 Hz, 1-H), 6.94 (d, J = 8.2 Hz, 1-H), 6.83 (td, J = 7.7/1.1 Hz, 1-H), 5.90 (br s, -OH), 3.46 (s, 1-H), 0.24 ; ^{13}C-NMR (75 MHz, $CDCl_3$, 273 K) δ 157.4, 132.3, 131.1, 120.5, 115.0, 108.4, 84.5, 78.5; MS (ESI) *m/z* 117 (100%) $(M-H)^-$ (yield = 70 %)

3-ethynylphenol (5). IR (film) 3292, 2360, 1703, 1275, 784 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, 273 K) δ 7.67 (br s, -OH), 7.30-6.80 (m, 4-H), 3.14 (s, 1-H); ^{13}C-NMR (75 MHz, $CDCl_3$, 273 K) δ 155.6, 129.8, 124.7, 123.4, 119.1, 116.7, 83.7, 77.7; MS (ESI) *m/z* 117 (20%) $(M-H)^-$ (yield = 91 %)

4-ethynylphenol (6). IR (film) 3287, 2360, 1705, 1239, 1043 cm^{-1};^{1}H-NMR (300 MHz, $CDCl_3$, 273 K) δ 7.37 (d, J = 8.8 Hz, 2-H), 7.07 (br s, -OH), 6.78 (d, J = 8.8 Hz, 2-H), 3.01 (s, 1-H); ^{13}C-

NMR (75 MHz, $CDCl_3$, 273 K) δ 156.4, 131.5, 115.3, 114.1, 83.8, 75.9; MS (ESI) *m/z* 117 (40%) $(M-H)^-$ (yield = 90 %)

General procedure for the synthesis of triazoles (7-15)

1 eq of the corresponding ethynylphenol and 1 eq of the corresponding azidophenol were suspended in a solution of water / *tert*-butanol 1:1. Sodium ascorbate (0.1 eq) of a freshly prepared 1 M solution in water was added, followed by the addition of copper (II) sulfate pentahydrate (0.01 eq). The resulting reaction was heated to 60 °C for 24 h in a nitrogen atmosphere. The reaction mixture was then diluted with water, cooled on ice, and the precipitate was collected by filtration. When addition of water failed to precipitate the desired triazole, evaporation of the solvent and purification by column chromatography was used.

2-[4-(2-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenol (7). Eluant: PE/EtOAc 9:1; Brown solid; m.p. 194.7-195.6 °C; IR (KBr) 3174, 1613, 1504, 1418, 1059, 751 cm^{-1};^{1}H-NMR (300 MHz, CD_3OD, 273 K) δ 8.79 (s, 1-H), 7.98 (dd, J = 8.2/1.9 Hz, 1-H), 7.69 (dd, J = 8.0/1.6 Hz, 1-H), 7.43 (td, J = 8.0/1.6 Hz, 1-H), 7.19 (td, J = 8.2/1.9 Hz, 1-H), 7.08 (dd, J = 8.2/1.9 Hz, 1-H), 7.02 (td, J = 8.2/1.9 Hz, 1-H), 6.96 – 6.90 (m; 2-H) ; MS (ESI) *m/z* 252 (100%) $(M-H)^-$; (yield = 22 %); anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

2-[4-(3-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenol (8). Eluant: PE/EtOAc 9:1; Brown solid; m.p. 208-209 °C; IR (KBr) 3398, 1603, 1511, 1235, 890 cm^{-1};^{1}H-NMR (300 MHz, CD_3OD, 273 K) δ 8.65 (s, 1-H); 7.68 (d, J = 7.9 Hz), 7.35 (m, 2-H), 7.26 (t, J = 7.9 Hz, 1-H), 7.10 (s, 1-H), 7.08 (d, J = 7.9 Hz, 1-H), 7.02 (t, J = 7.7 Hz, 1-H), 6.78 (d, J = 7.7 Hz, 1-H); MS (ESI) *m/z* 252 (100%) $(M-H)^-$; (yield = 37 %); anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

2-[4-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenol (9). Brown solid; m.p. 234.5-234.9 °C; IR (KBr) 3372, 3044, 1614, 1498, 1244, 834, 806 cm^{-1};^{1}H-NMR (300 MHz, CD_3OD, 273 K) δ 8.59 (s, 1-H), 7.72 (d, J = 8.5 Hz, 2-H), 7.67 (d, J = 8.0 Hz, 1-H), 7.35 (t, J = 8.2 Hz, 1-H), 7.09 (d, J = 8.2

Hz, 1-H), 7.02 (t, J = 8.2 Hz, 1-H), 6.87 (d, J = 8.5 Hz, 2-H); MS (ESI) *m/z* 252 (100%) $(M-H)^-$; (yield = 45 %); anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

2-[1-(3-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl]phenol (10). Eluant: PE/EtOAc 9:1; Brown solid; m.p. 218.1-219.1 °C; IR (KBr) 3301, 1610, 1481, 1255, 1066, 768 cm^{-1};^{1}H-NMR (300 MHz, CD_3OD, 273 K) δ 8.81 (s, 1-H), 7.97 (dd, J = 8.5/1.6 Hz, 1-H), 7.42 – 7.39 (m; 4-H), 7.21 (t, J = 8.5 Hz, 1-H), 6.96 (d, J = 7.7 Hz, 1-H), 6.92 (m, 1-H) ; MS (ESI) *m/z* 252 (100%) $(M-H)^-$; (yield = 72 %); anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

3-[1-(3-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl]phenol (11). Eluant: PE/EtOAc 9:1; Brown solid; m.p. 207.1-207.6 °C; IR (KBr) 3397, 1613, 1590, 1235, 890, 683 cm^{-1};^{1}H-NMR (300 MHz, CD_3OD, 273 K) δ 8.76 (s, 1-H), 7.41 – 7.24 (m, 6-H), 6.92 (d, J = 7.8 Hz, 1-H), 6.81 (d, J = 7.7 Hz, 1-H) ; MS (ESI) *m/z* 252 (100%) $(M-H)^-$; (yield = 56 %); anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

3-[4-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenol (12). Brown solid; m.p. 309.6-310.2 °C; IR (KBr) 3292, 1615, 1496, 1239, 1069, 789 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD, 273 K) δ 9.44 (s, 1-H), 8.43 (d, J = 8.8 Hz, 2-H), 8.03 (m, 3-H), 7.57 (m, 3-H); MS (ESI) *m/z* 252 (100%) $(M-H)^-$; (yield = 55 %); anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

2-[1-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl]phenol (13). Eluant: PE/EtOAc 9:1; Brown solid; m.p. 205.5-206.6 °C; IR (KBr) 3259, 1602, 1519, 1240, 829 cm^{-1};^{1}H-NMR (300 MHz, CD_3OD, 273 K) δ 8.68 (s, 1-H), 7.93 (d, J = 8.0 Hz, 1-H), 7.65 (m; 3-H), 7.19 (t, J = 8.0 Hz, 1-H), 6.95 (m, 3-H), 4.85 (br s, -OH); MS (ESI) *m/z* 252 (100%) $(M-H)^-$; (yield = 56 %); anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

3-[1-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl]phenol (14). Brown solid; m.p. 244.2-245.2 °C; IR (KBr) 3353, 1579, 1519, 1239, 830 cm^{-1} ;^{1}H-NMR (300 MHz, CD_3OD, 273 K) δ 8.65 (s, 1-H), 7.65

(d, J = 8.8 Hz, 2-H), 7.33 (m, 1-H), 7.25 (t, J = 7.8 Hz, 1-H), 6.96 (d, J = 8.8 Hz, 2-H), 6.80 (m, 2-H); MS (ESI) *m/z* 252 (100%) $(M-H)^-$; (yield = 72 %); anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

4-[4-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenol (15). Brown solid; m.p. 274.8-275.5 °C; IR (KBr) 3313, 1613, 1520, 1247, 839 cm^{-1} ;^{1}H-NMR (300 MHz, CD_3OD, 273 K) δ 8.61 (s, 1-H), 7.71 (d, J = 8.9 Hz, 2-H), 7.67 (d, J = 8.8 Hz. 2-H), 6.95 (d, J = 8.9 Hz, 2-H), 6.86 (d, J = 8.8 Hz, 2-H); MS (ESI) *m/z* 252 (100%) $(M-H)^-$; (yield = 33 %); anal. ($C_{14}H_{11}N_3O_2$) C, H, N.

2. Effects of the synthesised compounds on the MTT assay

Table S1.

	10 pM	100 pM	1 nM	10 nM	100 nM	1 µM	10 µM	100 µM
Res	95±17.5	84±13.9	80±13.7	100±18.9	124±7.1	106±16.3	91±14.6	8±3.2
	114±22	117±25.1	122±14.9	102±14.9	105±18.6	81±14.1	89±15.6	9±1.1
E2	-	90±28.8	120±21.3	136±18.4	134±10.6	116±12.2	104±10.2	-
	-	115±13.7	104±9.9	104±14.5	102±34.8	111±9.9	-	-
7	-	-	88±2.4	95±14.2	92±13.4	88±16.4	61±12.9	13±3.1
	-	-	104±4.6	110±15.5	95±14.1	92±20.4	77±12.7	4±2
8	-	-	96±10.6	97±5.9	75±18.8	112±8.1	77±14.1	21±8.6
	-	-	88±15.7	86±11.8	74±8.5	67±12.7	69±16.6	3±4.4
9	-	-	53±1.4	41±8.2	54±13.2	41±10	65±9.1	-14±4.6
	-	-	90±3.6	112±18	99±9.2	107±12.1	79±13.1	21±16.7
10	86±11.3	66±16	76±12.8	99±9	109±13.5	119±8.4	53±6.4	2±1.2
	83±25	105±13.1	65±16.4	73±19.5	55±10.5	66±4.8	53±3.7	0±0.6
11	102±6.5	162±18.4	132±20.7	146±10.2	118±7.8	138±13.1	93±11	52±3.1
	75±6.5	96±5.6	90±10.1	87±11.2	80±8.4	111±15.3	124±31.3	7±13.8
12	-	-	86±17.7	118±26.4	95±18.2	65±10	67±9.8	30±2.1
	-	-	113±24.3	104±11.5	77±0	78±13.8	70±8.5	52±16.1
13	-	-	97±9.2	92±9.9	96±24.1	98±9.3	94±10.2	6±1.7
	-	-	89±18.5	136±58.1	101±34.7	81±8.3	112±20.4	4±3.4
14	-	-	115±13.2	122±5.6	95±21.4	96±16.6	82±10.9	17±5.7
	-	-	59±1.1	96±1.7	72±10.5	72±7.2	74±10.5	28±16
15	-	-	116±6.8	91±17.3	79±0.9	59±24.7	31±3.7	20±2.3
	-	-	90±21.9	119±5.9	93±4.2	120±27.9	111±22	48±4.7

Table 1. Effect of resveratrol, E2 or click-estrogens on proliferation/toxicity in MCF-7 (white boxes) or MDA-MB-231 (shaded boxes) cells. Numbers represent % of control ± S.D.

3. Calculated distances between the two hydroxyl groups

Table S2.

Compound	MNDO, MMFF (Å)	PM_3, MMFF (Å)
7	6.933	6.509
8	9.745	9.490
9	10.610	10.381
10	9.697	9.371
11	11.523	11.243
12	11.531	11.080
13	10.585	10.328
14	11.538	11.117
15	13.260	12.952

4. Biological Materials and methods

Reagents and antibodies

Polyclonal antibodies to Erk 1/2 MAP Kinase, cyclin D1 and actin were from Santa Cruz Biotecnology (Santa Cruz, CA). Monoclonal antibody anti Akt and phospho-specific polyclonal antibodies to Erk 1/2 MAP Kinase (Thr185 and Tyr187) and anti-Akt (Ser473 and Thr308) were from Cell Signaling Tecnology (Beverly, MA). The ECL reagent was from Amersham Pharmacia Biotech (Uppsala, Sweden). PVDF membranes and protein assay kits were from Bio-Rad (Hercules, CA). Anti mouse and anti rabbit IgG peroxidase conjugated antibodies, human 17ß? estradiol (E2) and chemical reagent were from Sigma-Aldrich (St Louis, MO). All reagents were of analytical grade. Culture media, sera and antibiotics were from Invitrogen, Germany.

Cell culture

Human Breast cancer derived cell lines MCF-7 and MDA-MB-231 were purchased from ATCC (Manassas, VA,USA). Breast cancer cells were grown in DMEM supplemented with phenol red, L-glutamine (2mM), penicillin (100U/ml), streptomycin (100 µg/ml), gentamicin (50 µg/ml), insulin (6 ng/ml), hydrocortisone (3.75 ng/ml) and 10 % fetal calf serum (FCS). Human breast cancer MDA-MB-231 cells were grown at 37°C in DMEM supplemented with phenol red, L-glutamine (2mM), penicillin (100U/ml), steptomycin (100 µg/ml), gentamicin (50 µg/ml), insulin (0.02 U/ml) and 10 % foetal calf serum (FCS). Prior to experiments, the cells were grown in the media lacking phenol red and containing charcoal-stripped FCS. Cell viability/proliferation was assayed by the MTT assay.

For immunoblotting, cells were extracted in The following buffer: 1% NP-40, 150 mM NaCl, 50 mM Tris-HCl pH 8, 5 mM EDTA, 10 mM NaF, 10 mM $Na_4P_2O_7$, 0.4 mM Na_3VO_4, 10 mg/ml leupeptin, 4 mg/ml pepstatin and 0.1 U/ml aprotinin. Cell lysates were centrifuged at 13.000 g at 4

°C for 10 min and the supernatant was collected and assayed for protein concentration. Proteins were run on 8 % and 10 % SDS-PAGE under reducing conditions. Proteins were transferred to a PVDF membrane and incubated with specific antibodies overnight. The proteins were visualised with peroxidase-conjugate secondary antibodies. For cyclin D1 expression, cells were extracted in RIPA BUFFER (1 % Triton X-100, 0,1 % SDS, 1 % Na-deoxycholate, 150 mM NaCl, 50 mM Tris-Hcl pH 7, 0.4 mM Na_3VO_4, 10 µg/ml leupeptin, 4 µg/ml pepstatin and 0.1 U/ml aprotinin) and analysed as indicated above.

For transcriptional assays, HeLa cells were cultured in 96 well-plates for 24 hours in phenol-red media. Cells were then transfected (Lipofectamine, Invitrogen) with a luciferase reporter gene driven by estrogens (pGL3-ERE; 0.075 µg/well) and a control renilla reporter gene (pRL-TK, Promega; 0.075 µg/well). After 5 hours of transfection, cells were washed, treated to the compounds, and cultured for 48 hours in phenol red-free media. The light signal by luciferase and renilla was then assessed using a commercial kit (dual-luciferase, Promega) using a plate reader (Victor, Perkin Elmer).

Estrogen binding assay

Porcine uterine cytosol was prepared as follows: 20 g of pig uterus was minced and homogenised in 200 ml ice-cold TEGD buffer (10 mM Tris, pH 7.4, 1.5 mM EDTA, 10% glycerol, 1 mM DTT) and centrifuged at 500 g for 5 minutes to remove tissue fragments. The supernatant was then centrifuged at 100,000g for 1 hour. The resulting supernatant, corresponding to the cytosolic fraction, was then treated with activated charcoal in order to remove contaminating estrogen. Briefly, 250 mg of activated charcoal and 5 mg of dextran were added to 100 ml of 10 mM Tris-HCl, pH 8. The solution was agitated for 30 minutes at room temperature and then centrifuged at 2000 rpm for 25 minutes. 12 ml of the uterine cytosol was added to the resulting pellet and agitated for 30 minutes before centrifugation at 2000 rpm for 25 minutes. The supernatant was aliquoted and stored at -80^{o}C for use in the binding assay.

Competition binding assays were carried using 40 µg of uterine cytosol, 300 pM [^{3}H]-estrogen (Perkin-Elmer) in TEGD buffer (final volume 500 µl) with the appropriate concentrations of authentic, unlabelled estrogen, the synthesised compounds or ethanol as the vehicle control. Reactions were incubated for 18 hours on a rotating wheel at 4^{o}C and all subsequent steps were carried out on ice in pre-chilled racks. [^{3}H]-estrogen bound to the estrogen receptor was separated from free [^{3}H]-estrogen by the addition of 250 µl of hydroxyapatite slurry (HAP, 60% in TEGD). The reaction with HAP was incubated for 15 minutes on the rotating wheel before the addition of 500 µl ice-cold TEGD. The reaction was centrifuged at 2500 g for 5 minutes, the supernatant decanted and the HAP pellet was resuspended in 1.25 ml TEGD. This wash step was repeated and the final HAP pellet was resuspended in ethanol and vortexed for 20 minutes at room temperature before centrifugation at 2500 g for 5 minutes. 250 µl of the supernatant were added to 5 ml scintillation fluid and counted using a standard counting procedure.

5. **Displacement of estrogen binding in porcine uterus cytosol by the synthesised compounds.**

Table S3.

	[^{3}H]E2 binding (% of control)
Control	100 ± 3.3
11 1 nM	80.1 ± 6.5
E2 1 nM	74.4 ± 1.5
7 1 nM	43.4 ± 2.0
8 1 nM	40.7 ± 1.0
10 1 nM	42.3 ± 1.3
12 1 nM	34.7 ± 1.1
14 1 nM	39.3 ± 1.9
15 1 nM	47.8 ± 2.5
11 100 nM	72.3 ± 8.7
E2 100 nM	31.3 ± 2.6

Results expressed as % of total binding (control) in the absence of competing ligand. Mean ± S.E.M, n = 3-6.