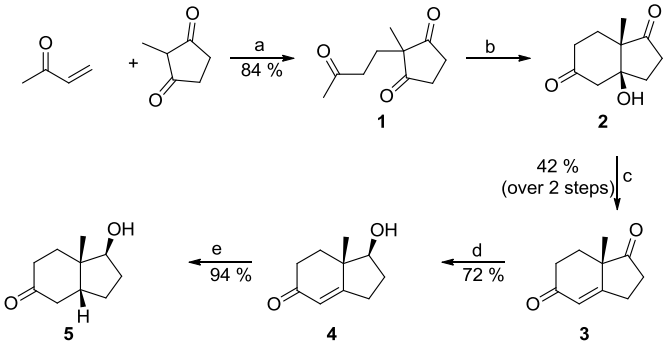
Supplemental data for "The A-CD analog of 16β,17α-estriol is a potent and highly selective estrogen receptor β agonist"

Synthesis

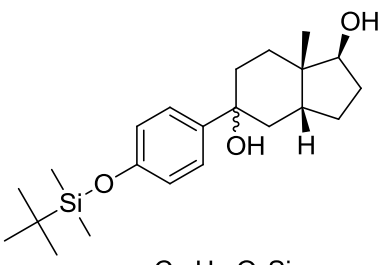
Synthesis of the Hajos-Parrish ketone:



Reagents and conditions for Hajos-Parrish-ketone synthesis. a: acetic acid, H_2O, 16 h, 70 °C; b: L-proline, DMF, 16 h, rt; c: p-TsOH, DCM, 16 h, rt; d: $NaBH_4$, DCM:MeOH (1:1), 30 min, -78 °C; e: Pd/C 10 %, MeOH, 50°C, Hcube.

The Michael addition of 2-methylcyclopentane-1,3-dione with methylvinyl ketone leads to the formation of compound **1**. The ring closure to compound **2** was then performed using L-proline as a catalyst. Elimination of the tertiary alcohol with a catalytic amount of p-TsOH yielded **3** followed by the reduction of the ketone using sodium borohydride gives the Hajos-Parrish ketone **4**. The double bond was then reduced with hydrogen and Pd/C 10% to form compound **5**.

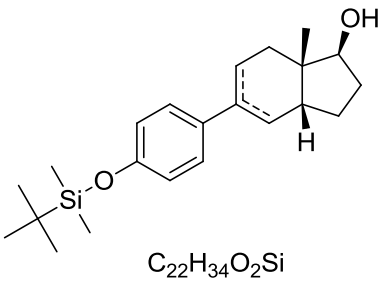
(1S,3aR,7aS)-5-(4-((Tert-butyldimethylsilyl)oxy)phenyl)-7a-methyloctahydro-1H-indene-1,5-diol (**6**)



$C_{22}H_{36}O_3Si$
Mol. Wt.: 376,6

To a solution of O-protected bromophenol(176 mg, 1.04 mmol) in dry THF (1 mL) cooled at -78 °C was added BuLi (550 µl, 1.04 mmol) dropwise and the solution was stirred 1h. Then a solution of Hajos-Parrish ketone **5** (100 mg, 0.35 mmol) in dry THF (1 mL) was added dropwise at -78 °C and the reaction was allowed to reach rt. After 30 min stirring at rt, the reaction was quenched with saturated NH_4Cl solution (3 mL) and water (3 mL). The mixture was extracted with EA (4x), dried over $MgSO_4$, filtered and evaporated. The residue was purified using flash chromatography (SiO_2; Heptane:EA, 1:0 → 7:3) to give the title compound (80 mg, 0.21 mmol, 61 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.7 Hz) and 7.23 (d, *J* = 8.7 Hz) (2H), 6.79 (d, *J* = 8.7 Hz), 6.76 (d, *J* = 8.7 Hz) (2H), 4.33 (t, *J* = 9.0 Hz, 1H), 3.66 (t, *J* = 6.1 Hz, 1H), 2.39 – 2.29 (m, 1H), 2.27 – 2.18 (m, 1H), 2.14 – 2.04 (m, 1H), 2.03 – 1.94 (m, 1H), 1.84 – 1.63 (m, 3H), 1.55 (s, 3H), 1.51 – 1.38 (m, 2H), 1.30 – 1.11 (m, 2H), 0.98 (s) and 0.97 (s) (9H), 0.19 (s) and 0.18 (s), (6H).

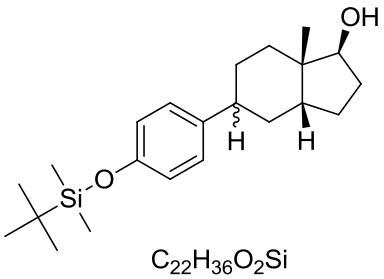
(1S,3aR,7aS)-5-(4-((Tert-butyldimethylsilyl)oxy)phenyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1H-inden-1-ol (**7**)



$C_{22}H_{34}O_2Si$
Mol. Wt.: 358,59

A solution of compound **6** (650 mg, 1.72 mmol) and *p*-TsOH (20 mg, 0.10 mmol) in DCM (20 mL) was stirred at rt overnight. EA was added and the solution was washed with $NaHCO_3$ (1x) and saturated brine (1x), dried over Na_2SO_4, filtered and solvent removed in vacuo. The residue was purified using column chromatography (SiO_2; Heptane:EA, 1:0 → 1:1) to give **7** (120 mg, 0.34 mmol, 19 %) and **7a** (140 mg, 0.57 mmol, 33 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.7 Hz, 2H), 6.87 – 6.69 (m, 2H), 6.03 – 5.98 (m) and 5.97 – 5.92 (m) (1H), 3.89 (t, *J* = 5.6 Hz) and 3.81 (dd, *J* = 6.5, 2.2 Hz) (1H), 2.45 – 2.33 (m, 2H), 2.29 – 2.07 (m, 2H), 1.96 – 1.76 (m, 1H), 1.68 – 1.53 (m, 2H), 1.54 – 1.45 (m, 1H), 1.46 – 1.32 (m, 1H), 1.04 (s) and 1.02 (s) (3H), 0.99 (s, 9H), 0.20 (s, 6H).

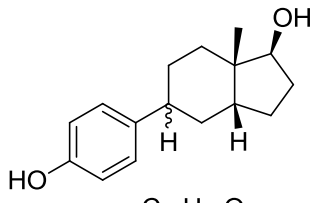
(1S,3aR,7aS)-5-(4-((Tert-butyldimethylsilyl)oxy)phenyl)-7a-methyloctahydro-1H-inden-1-ol (**8**)



$C_{22}H_{36}O_2Si$
Mol. Wt.: 360,61

A 0.05 M solution of compound **7** (780 mg, 2.18 mmol) in methanol (43 mL) was hydrogenated on the H-Cube. A cartouche of 5 g Pd/C (10 %) was used, a temperature of 25 °C in full H_2 mode was settled. The methanol was removed in vacuo to give **8** (715 mg, 1.98 mmol, 91 %) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, *J* = 8.5 Hz, 2H), 7.03 (d, *J* = 8.5 Hz, 2H), 6.77 (d, *J* = 4.1 Hz, 2H), 6.75 (d, *J* = 4.1 Hz, 2H), 4.38 (t, *J* = 8.6 Hz, 1H), 3.73 (d, *J* = 5.7 Hz, 1H), 2.66 (tt, *J* = 12.1, 3.9 Hz, 1H), 2.41 (tt, *J* = 12.3, 3.0 Hz, 1H), 2.33 – 2.15 (m, 2H), 2.15 – 2.00 (m, 2H), 1.94 – 1.49 (m, 13H), 1.41 (td, *J* = 13.2, 4.8 Hz, 1H), 1.32 – 1.25 (m, 2H), 1.24 – 1.15 (m, 2H), 1.13 (s, 3H), 0.99 (s, 18H), 0.93 (s, 3H), 0.19 (s, 12H).^{13}C NMR (101 MHz, $CDCl_3$) δ 158.00, 144.44, 132.04, 131.79, 124.13, 124.12, 86.96, 78.09, 49.52, 48.46, 47.19, 47.03, 45.93, 43.53, 41.72, 37.51, 36.62, 36.48, 34.38, 33.93, 33.87, 31.26, 30.95, 30.11, 26.55, 22.76, 22.59, -0.00.

(1S,3aR,7aS)-5-(4-Hydroxyphenyl)-7a-methyloctahydro-1H-inden-1-ol (**9**)



$C_{16}H_{22}O_2$
Mol. Wt.: 246,34

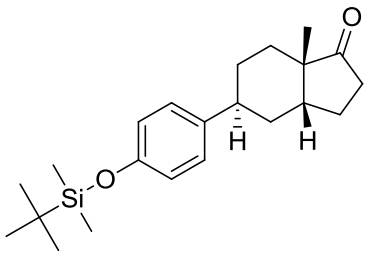
To a mixture of compound **8** (115 mg, 0.32 mmol) in THF (5 mL) was added 1 M TBAF (366 µl, 0.37 mmol). The solution was stirred at rt over 3h. Brine (5 mL) was added and the solution was extracted with EA, dried over $MgSO_4$, filtered and evaporated. Flash chromatography of the residue (SiO_2; Heptane:EA, 1:0 → 1:1) gave **9** (65 mg, 0.26 mmol, 83 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, *J* = 8.6 Hz, 2H), 7.03 (d, *J* = 8.5 Hz, 2H), 6.78 (d, *J* = 5.2 Hz, 2H), 6.76 (d, *J* = 5.2 Hz, 2H), 4.40 (t, *J* = 8.6 Hz, 1H), 3.76 (d, *J* = 5.7 Hz, 1H), 2.64 (tt, *J* = 12.1, 4.1 Hz, 1H), 2.40 (tt, *J* = 12.1, 3.0 Hz, 1H), 2.31 – 2.16 (m, 2H), 2.14 – 2.01 (m, 2H), 1.92 – 1.48 (m, 14H), 1.46 – 1.35 (m, 1H), 1.32 – 1.24 (m, 2H), 1.24 – 1.14 (m, 1H), 1.13 (s, 3H), 0.93 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 153.91, 153.90, 139.29, 127.90, 127.64, 115.21, 115.19, 82.82, 73.92, 45.03, 44.04, 42.69, 42.59, 41.52, 39.08, 37.24, 33.04, 32.25, 32.00, 31.89, 29.76, 29.51, 29.50, 26.82, 26.50, 22.18, 18.37. GCMS: M=247 g.mol^{-1}.

(3aR,7aS)-5-(4-((tert-butyldimethylsilyl)oxy)phenyl)-7a-methyloctahydro-1H-inden-1-ones **10a** and **10b**

A mixture of Dess-Martin periodinane (765 mg, 1.81 mmol) and compound **9** (500 mg, 1.39 mmol) in DCM (10 mL) was stirred in the microwave reactor for 1h at 140 °C. The reaction mixture was diluted with ether, and poured into 10 mL of 1M NaOH solution. The mixture was stirred for 10 min and

the ether layer was extracted and washed with 1 M NaOH and water. The organic layer was dried over $MgSO_4$, filtered and evaporated. Flash chromatography of the residue (SiO_2; Hexane:EA, 1:0 → 20:1) allowed the separation of the two estrone analogue diastereomers, **10a** (180 mg, 0.50 mmol, 36 %) and **10b** (120 mg, 0.33 mmol, 24 %).

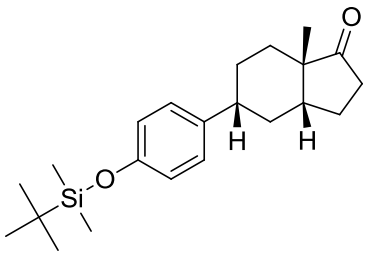
(3aR,5S,7aS)-5-(4-((Tert-butyldimethylsilyl)oxy)phenyl)-7a-methyloctahydro-1H-inden-1-one (**10a**)



$C_{22}H_{34}O_2Si$
Mol. Wt.: 358,59

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, *J* = 8.4 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 2.75 – 2.62 (m, 1H), 2.59 – 2.46 (m, 1H), 2.29 – 2.15 (m, 1H), 2.15 – 1.97 (m, 1H), 1.94 – 1.69 (m, 2H), 1.63 – 1.47 (m, 1H), 1.37 – 1.25 (m, 1H), 1.17 (s, 1H), 0.98 (s, 4H), 0.19 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 227.24, 158.26, 143.64, 131.99, 124.26, 51.05, 47.69, 41.75, 40.81, 36.93, 33.26, 33.13, 30.11, 27.49, 23.30, 22.60, 0.00.

(3aR,5R,7aS)-5-(4-((Tert-butyldimethylsilyl)oxy)phenyl)-7a-methyloctahydro-1H-inden-1-one (**10b**)



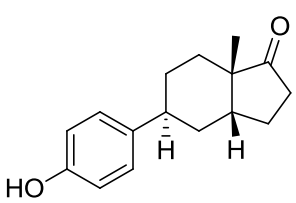
$C_{22}H_{34}O_2Si$
Mol. Wt.: 358,59

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (d, *J* = 8.5 Hz, 2H), 6.73 (d, *J* = 8.5 Hz, 2H), 2.45 – 2.33 (m, 2H), 2.33 – 2.16 (m, 3H), 2.14 – 2.07 (m, 1H), 1.88 (ddt, *J* = 13.5, 5.3, 2.5 Hz, 1H), 1.73 – 1.66 (m, 1H), 1.63 – 1.54 (m, 1H), 1.37 (td, *J* = 13.5, 4.2 Hz, 1H), 1.20 (ddd, *J* = 25.8, 13.0, 3.7 Hz, 1H), 1.13 – 1.05 (m, 1H), 1.03 (s, 3H), 0.97 (s, 9H), 0.17 (s, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 226.22, 158.14, 143.89, 131.76, 124.20, 53.99, 48.16, 46.32, 43.22, 37.68, 36.52, 35.51, 30.11, 28.86, 28.34, 22.58, 0.00.

(3aR,7aS)-5-(4-Hydroxyphenyl)-7a-methyloctahydro-1H-inden-1-ones **11a** and **11b**

To a mixture of compound **10a** or **10b** (110 mg, 0.31 mmol) in THF (5 mL) was added 1 M TBAF (338 µl, 0.34 mmol). The solution was stirred at rt over 3h. Brine (5 mL) was added and the solution was extracted with EA, dried over $MgSO_4$, filtered and evaporated. Flash chromatography of the residue (SiO_2; Heptane:EA, 1:0 → 1:1) gave **11a** or **11b** (75 mg, 0.31 mmol, 100 %).

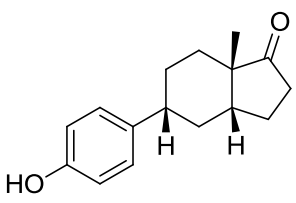
(3aR,5S,7aS)-5-(4-Hydroxyphenyl)-7a-methyloctahydro-1H-inden-1-one (**11a**)



$C_{16}H_{20}O_2$
Mol. Wt.: 244,33

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 8.6 Hz, 2H), 4.61 (s, OH), 2.70 (m, 1H), 2.54 (m, 1H), 2.27-2.17 (m, 1H), 2.15 – 1.95 (m, 2H), 1.93-1.70 (m, 4H), 1.60-1.47 (m, 2H), 1.30 (m, 1H), 1.17 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 222.40, 153.86, 138.94, 127.61, 115.14, 49.65, 43.70, 41.81, 38.83, 33.28, 32.03, 31.09, 24.39, 23.89. HRMS (ESI) calc'd for $C_{16}H_{20}O_2$ $[M+Na]^+$: 267.1356, found: 267.1344.

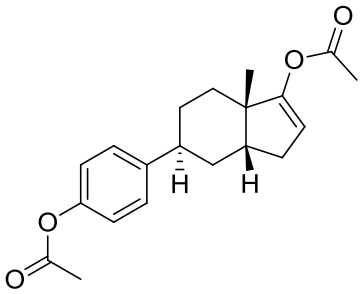
(3aR,5R,7aS)-5-(4-Hydroxyphenyl)-7a-methyloctahydro-1H-inden-1-one (**11b**)



$C_{16}H_{20}O_2$
Mol. Wt.: 244,33

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (d, *J* = 8.4 Hz, 2H), 6.75 (d, *J* = 8.6 Hz, 2H), 5.55 (s, OH), 2.49 – 2.16 (m, 5H), 2.12 (dt, *J* = 11.6, 6.2 Hz, 1H), 1.87 (ddt, *J* = 13.6, 5.5, 2.7 Hz, 1H), 1.73 – 1.65 (m, 1H), 1.63 – 1.54 (m, 1H), 1.38 (td, *J* = 13.6, 4.2 Hz, 1H), 1.32 – 1.14 (m, 2H), 1.05 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 222.93, 154.01, 138.79, 127.59, 115.16, 49.72, 43.70, 41.77, 38.85, 33.32, 32.01, 31.08, 24.37, 23.88. GCMS: M=246 g.mol^{-1}.

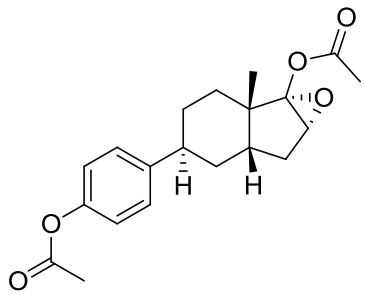
4-((3aS,6S,7aS)-3-Acetoxy-3a-methyl-3a,4,5,6,7,7a-hexahydro-1H-inden-6-yl)phenyl acetate (**12**)



$C_{20}H_{24}O_4$
Mol. Wt.: 328,4

To a solution containing compound **11a** (75 mg, 0.31 mmol) in 5 mL. of isopropenyl acetate was added 0.2 mL of catalyst solution (5 mL. of isopropenyl acetate and 0.1 mL. of concentrated sulfuric acid). The solution was heated at 70 °C for 16h. The solution was then cooled and diluted with ether. The ether solution was washed with cold sodium carbonate solution and with water, dried over $MgSO_4$ and the solvent was evaporated. The residue was purified over column chromatography (SiO_2; Heptane:EA, 1:0 →7:3) to give **12** (23 mg, 0.07 mmol, 22 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 – 7.13 (m, 2H), 6.99 – 6.85 (m, 2H), 5.44 – 5.38 (m, 1H), 2.64 – 2.52 (m, 1H), 2.35 – 2.15 (m, 2H), 2.21 (s, 3H), 2.10 (s, 3H), 2.13 – 2.01 (m, 1H), 1.74 – 1.56 (m, 4H), 1.50 (td, *J* = 13.1, 3.2 Hz, 1H), 1.42 – 1.29 (m, 1H), 1.06 (s, 3H).

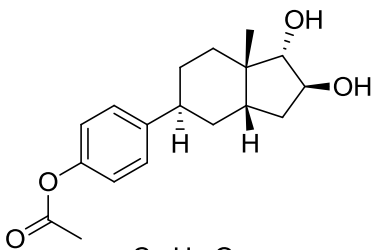
4-((1aR,1bS,4S,5aS,6aR)-1a-Acetoxy-1b-methyloctahydro-1aH-indeno[1,2-b]oxiren-4-yl)phenyl acetate (**13**)



$C_{20}H_{24}O_5$
Mol. Wt.: 344,4

A solution of compound **12** (23 mg, 0.07 mmol) in 5 mL of 0.05 M *m*-CPBA in toluene was allowed to stir for 20 hours at room temperature. The solution was diluted with ether and washed with a solution of sodium hydroxide containing ice and then with water. After drying the organic layer with $MgSO_4$ and evaporation of the solvent, crude compound **13** (15 mg, 0.04 mmol, 62 %) was obtained. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.03 (d, *J*=8.2 Hz, 2 H) 6.69 (d, *J*=8.2 Hz, 2 H) 4.17 - 4.09 (m, 1 H) 3.52 (d, *J*=6.7 Hz, 1 H) 2.60 (ddt, *J*=16.24, 8.12, 3.86, 3.86 Hz, 1 H) 2.25 (s, 3 H) 2.09-1.95 (m, 5 H) 1.20 - 1.81 (m, 7 H) 1.12 (s, 3 H).

4-((1S,2S,3aS,5S,7aS)-1,2-Dihydroxy-7a-methyloctahydro-1H-inden-5-yl)phenyl acetate (**14**)

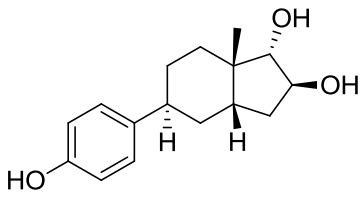


$C_{18}H_{24}O_4$
Mol. Wt.: 304,38

To a stirred solution of $LiAlH_4$ (10 mg, 0.26 mmol) in dry THF (4 mL) was added a solution of epoxide **13** (15 mg, 0.04 mmol) in dry THF (1 mL). The solution was stirred at rt for 2h and quenched at 0°C with EA and water. Organic layer was separated and aqueous layer was extracted with EA. Combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated to give crude **14** (10 mg, 0.03 mmol, 75 %).

^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.15 (d, *J*=8.2 Hz, 2 H), 6.89 (d, *J*=8.2 Hz, 2 H) 4.05 (m, *J*=3.91 Hz, 1 H) 3.43 (d, *J*=6.3 Hz, 1 H) 2.63 (tt, *J*=12.37, 3.28 Hz, 1 H) 2.15 (s, 3 H) 2.00 – 1.88 (m, 2 H) 1.70 - 1.25 (m, 7H) 1.05 (s, 3 H).

(1S,2S,3aS,5S,7aS)-5-(4-Hydroxyphenyl)-7a-methyloctahydro-1H-indene-1,2-diol (**15**)



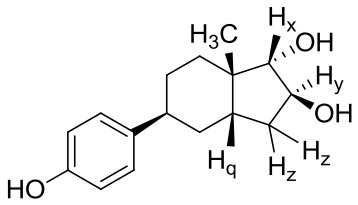
$C_{16}H_{22}O_3$
Mol. Wt.: 262,34

To a solution of compound **14** (194 mg, 1.0 mmol) in aqueous MeOH (1:4, 5 mL), NH_4OAc (618 mg, 8.0 mmol) was added. The resulting mixture was stirred at room temperature and the progress of the reaction was monitored by TLC. After 2 h the mixture was concentrated and the residue was extracted with EA, dried over $MgSO_4$ and evaporated. The crude mixture was purified over column chromatography (SiO_2; Heptane:EA, 1:1 →0:1) to give pure desired compound **15** (2.0 mg, $5.70.10^{-3}$ mmol, 23 %). ^{1}H NMR (400 MHz, CD_3OD) δ 6.93 (d, *J* = 8.5 Hz, 2H), 6.59 (d, *J* = 8.5 Hz, 2H), 4.03 (ddd, *J* = 10.2, 6.6, 3.8 Hz, 1H), 3.42 (d, *J* = 8 Hz, 1H), 2.50 (tt, *J* = 12.3, 3.6 Hz, 1H), 2.25 – 2.10 (m, 1H), 2.00 – 1.85 (m, 1H), 1.68 – 1.23 (m, 7H), 1.03 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 154.96, 138.16, 127.27, 114.60, 89.56, 76.11, 41.20, 40.28, 37.44, 33.56, 32.45, 29.05, 27.79, 21.69. $[\alpha]_D^{20}$ = -9.0 (c 0.2, methanol). HRMS (ESI) calc'd for $C_{16}H_{22}O_3$ $[M+Na]^+$: 285.1462, found: 285.1477.

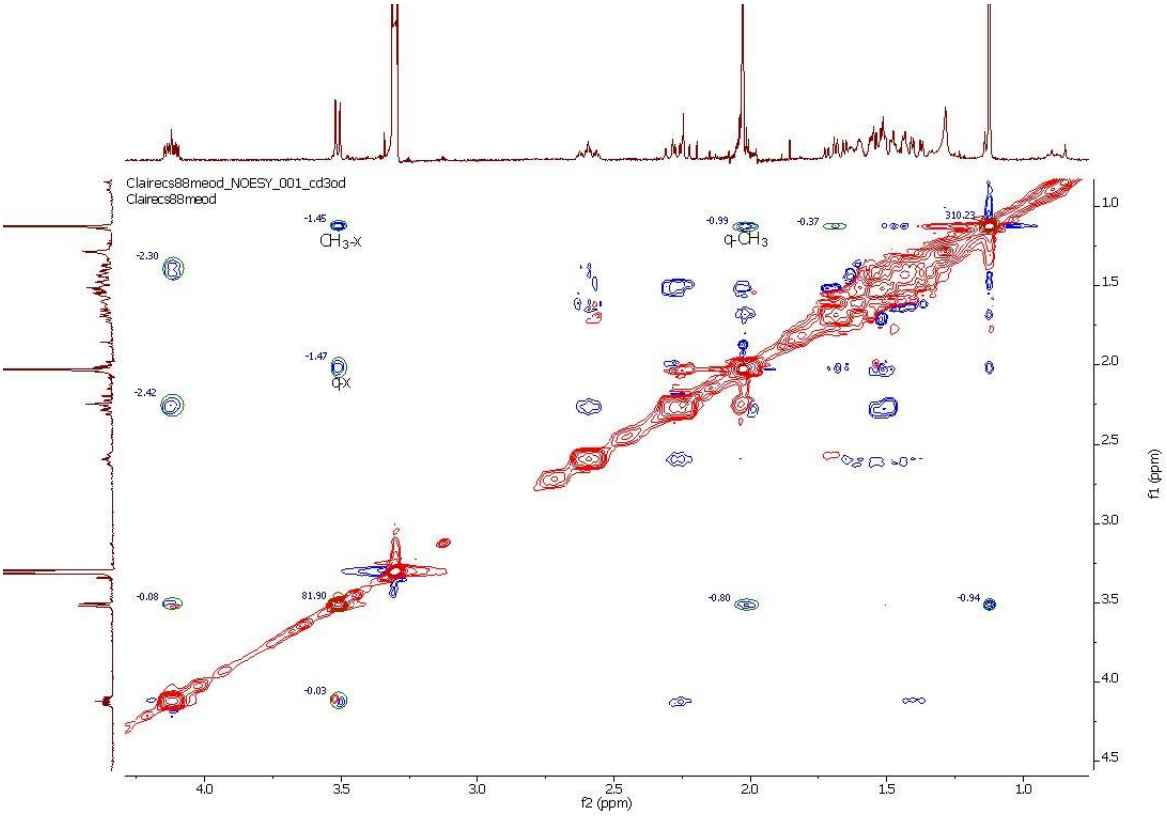
Structural assignment of compound **15**:

Protons x and y are trans: The coupling constant between Hx (3.4 ppm) and Hy (4.0 ppm) is 8 Hz, which is indicative of a trans coupling. In addition, there is no NOE between Hx and Hy, but there is a COSY.

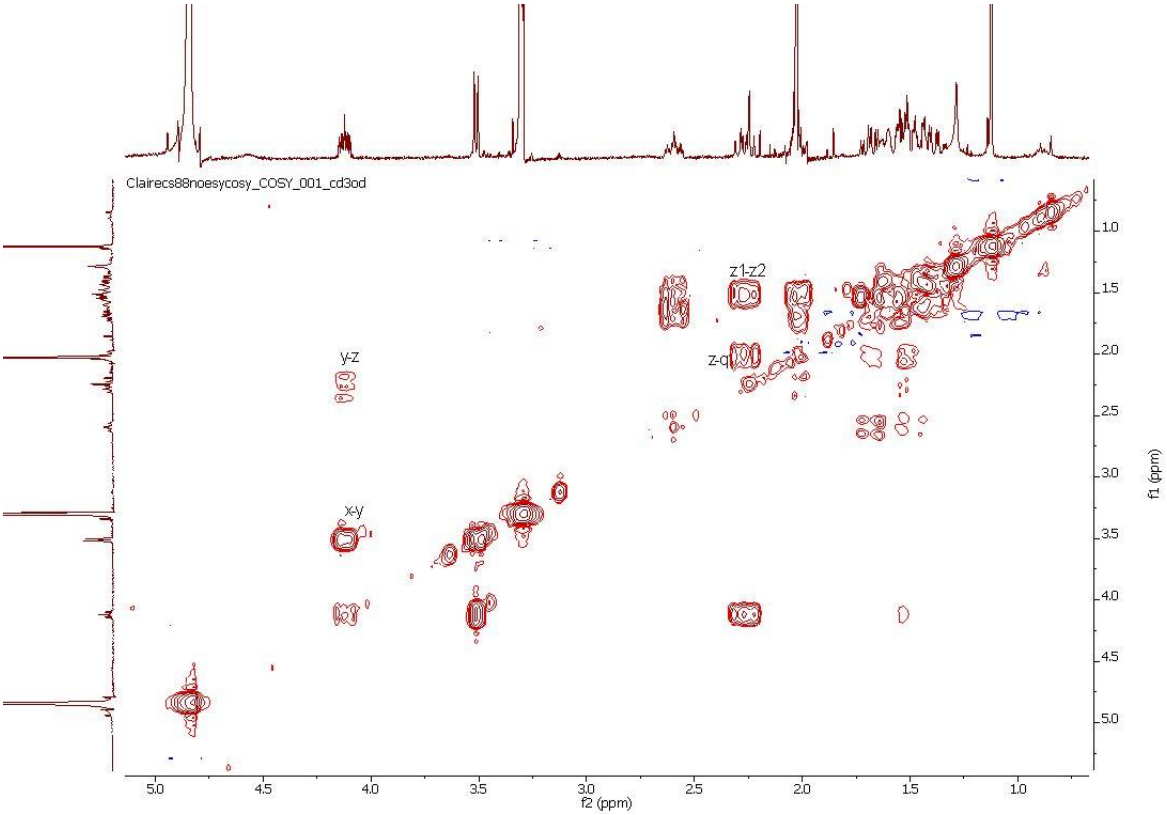
Proton x is facing upwards just like the CH_3 group (1.0 ppm) and proton q (2.0-1.9 ppm): The CH_3 group has a strong NOE to proton x and proton q. Proton q has COSY with z proton, they have a COSY with proton y and y has COSY with proton x, so they must be respective neighbors.



NOESY of compound **15**:



COSY of compound **15**:



Biological evaluation

Luciferase reporter gene assay

The luciferase reporter gene assay were performed essentially as described (Piu et al., 2008) with the following modifications. HEK293T cells were seeded in DMEM containing 1% PSG, 10% fetal calf serum (Hyclone) at 5.5 e6 cells per 10 cm dish the day prior to transfection(approximately 70% confluency). On the day of transfection (day 1), Transient transfections were performed with 2 µg of receptor DNA, and 6 µg reporter construct (4x estrogen response element (ERE) upstream of the luciferase gene, pTranslucent vector, Panomics) per 10 cm dish using the transfection reagent FuGENE (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. The day after transfection, the cells were harvested, seeded at a density of 20,000 cells/well in white 96-well plates (COSTAR, part #3917), and incubated overnight in DMEM containing 1% PSG, 10% fetal calf serum. Subsequently, the medium was removed, cells washed with serum free phenol-red free DMEM containing 0.5% BSA, and then the indicated concentrations of ligands combined with 5% charcoal-treated fetal calf serum in phenol-red free DMEM were added in a volume of 200 µl/well, and incubated for an additional 24 hours. Luciferase activity was measured on a TopCount using a Steady-Glo luciferase assay kit (part #E2520) from Promega (Madison, WI), according to the manufacturer's instructions.

Molecular modeling

Methods: All modeling was performed using Schrödinger Suite 2012 with the Maestro[1] interface for the windows 7 platform.

Protein preparation: Crystal structure of ERβ and 17-epiestriol and ER with estriol (2J7Y.pdb and 3Q95.pdb, respectively) were obtained from the protein databank.[2] The protein was prepared using the "Protein Preparation Wizard"[3] workflow in Maestro with default settings. A Grid file was generated for the receptor ligand pocket defined around the co-crystallized ligands. The two water molecules were kept during all the steps including the docking studies.

Ligand Preparation: The structure of compound 15 was built and prepared in MacroModel 9.9[4] and conformational searches were performed using default parameters, and the low energy conformations within 5 kcal/mol were used in the docking studies.

Docking: Glide 5.8[5-8] was used with standard precision (SP) mode and the default settings. The top scored poses of compound 15 were analyzed and inspected and the poses where the phenolic moiety is positioned in the area of Glu305, Arg346 and the two water molecules were selected for further minimization and scoring. Theses poses were post processed in the Prime 3.1 MM/GBSA module[9-11] to allow the compound to be minimized within the receptor cavity and allowing side chains within 4 Å of the ligands to be optimized.

1. Schrödinger Suite 2012, the Maestro, version 9.3, Schrödinger, LLC, New York, NY, 2012.

2. RCSB Protein Data Bank. http://rcsb.org/pdb

3. Protein Preparation Wizard; Epik version 2.3, Schrödinger, LLC, New York, NY, 2012; Impact version 5.8, Schrödinger, LLC, New York, NY, 2012; Prime version 3.1, Schrödinger, LLC, New York, NY, 2012.

4. MacroModel, version 9.9, Schrödinger, LLC, New York, NY, 2012.

5. Glide Version 5.8 Schrödinger, LLC, New York, NY, 2012.

6. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shaw, D. E.; Shelley, M.; Perry, J. K. ; Francis, P.; Shenkin, P. S., Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessmentof Docking Accuracy, J. Med. Chem., 2004, 47, 1739–1749.

7. Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L., Glide: A New Approach for Rapid, Accurate Docking and Scoring. 2. Enrichment Factors in Database Screening. J. Med. Chem., 2004, 47, 1750–1759.

8. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren,T. A.; Sanschagrin, P. C.; Mainz, D. T., Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes. J. Med. Chem., 2006, 49, 6177–6196.

9. Prime, version 3.1, Schrödinger, LLC, New York, NY, 2012.

10. Lyne, P. D.; Lamb, M. L.; Saeh, J. C., Accurate Prediction of the Relative Potencies of Members of a Series of Kinase Inhibitors Using Molecular Docking and MM-GBSA Scoring, J. Med. Chem., 2006, 49, 4805-4808.

11. Nu, H.; Kalyanaraman, C.; Irwin, J. J.; Jacobson, M. P. Physics-based scoring of protein-ligand complexes: enrichment of known inhibitors in large-scale virtual screening. J. Chem. Inf. Model. 2006, 46, 243-253.