Supplementary Materials

Potent Estrogen Receptor Ligands Based on Bisphenols with a Globular Hydrophobic Core

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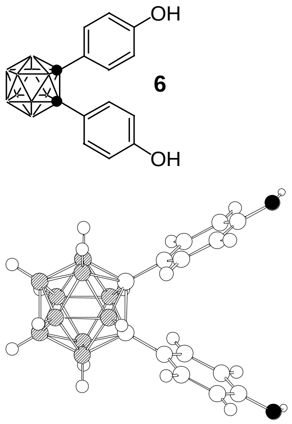
Comparison of structures for compounds with carborane core (**6**, **7**, **8**) and compounds with hydrocarbone cores (**9**, **10**, **11**, **12**)



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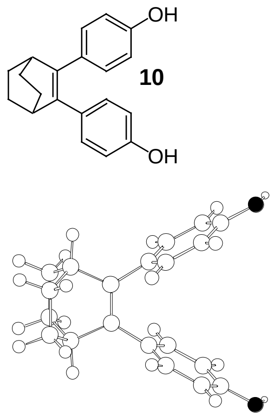

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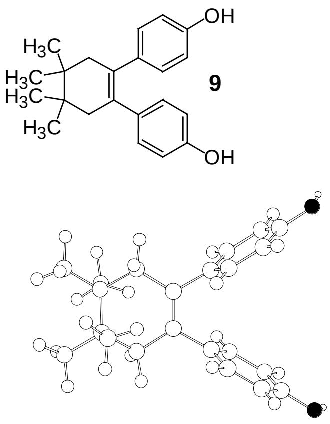

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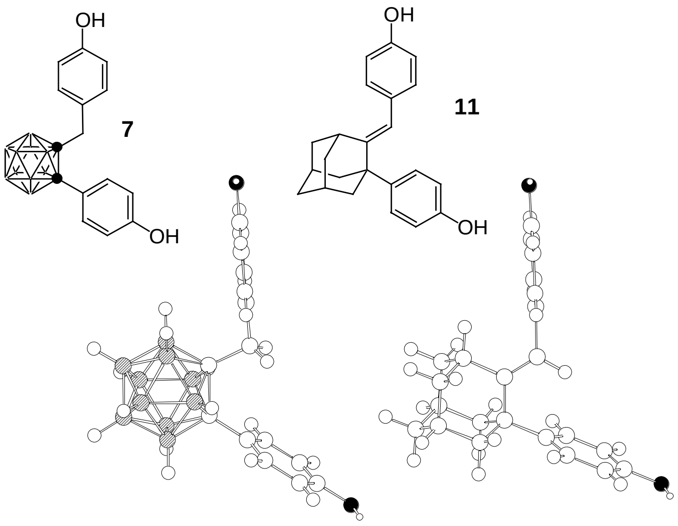

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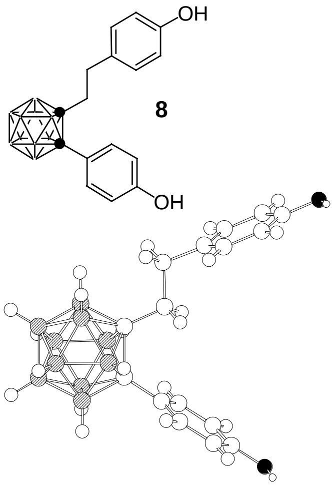

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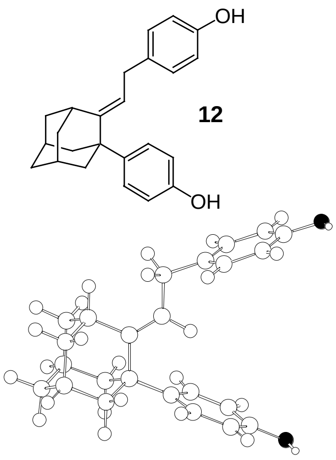

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Synthesis, spectral data for compounds **7-12** and experimental procedures of biological evaluations

*1-{4-[(2-Methoxyethoxy)methoxy]phenyl}-1,2-dicarba-closo-dodecaborane (**14**).* A solution of decaborane (14) (4.65 g, 38.0 mmol), 1-ethynyl-4-[(2-methoxyethoxy)methoxy]benzene (7.84 g, 38.0 mmol) and acetonitrile (15 ml) in dry benzene (150 ml) was refluxed under an argon atmosphere for 45 h. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel (eluent: hexane/ethyl acetate, 2:1) to afford **14** as a colorless solid (4.50 g, 37%). Colorless plates (AcOEt-hexane); mp 46-47□. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.50-3.20 (brm, 10H), 3.37 (s, 3H), 3.54 (m, 2H), 3.80 (m, 2H), 3.88 (brs, 1H), 5.26 (s, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 9.0 Hz, 2H). HRMS Calcd for $C_{12}H_{24}{}^{10}B_2{}^{11}B_8O_3$: 324.2729, Found: 324.2725.

*1-[4-(tert-Butyldimethylsiloxy)benzyl]-2-{4-[(2-methoxyethoxy)methoxy]phenyl}-1,2-dicarba-closo-dodecaborane (**15**).* To a solution of **14** (2.18 g, 6.73 mmol) in benzene/Et_2O (2:1, 30 ml) was added dropwise a 1.6 M solution of *n*-BuLi in hexane (5.05 mL, 8.08 mmol) at 0 °C under Ar. The mixture was stirred at room temperature for 30 min. The solution was cooled at 0°C and 4-(*tert*-butyldimethylsiloxy)benzyl bromide (3.04 g, 10.1 mmol) in Et_2O (5 ml) was added dropwise, then the mixture was refluxed for 18 h. The reaction was quenched with water and the mixture was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 4:1) gave **15** (1.31 g, 36%) as a viscous liquid. The starting material **14** was recovered in 58% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.18 (s, 6H), 0.96 (s, 9H), 1.50-3.10 (brm, 10H), 3.00 (s, 2H), 3.37 (s, 3H), 3.56 (m, 2H), 3.84 (m, 2H), 5.32 (s, 2H), 6.70 (s, 4H), 7.08 (d, *J* = 9.0 Hz, 2H), 7.59 (d, *J* = 9.0 Hz, 2H). HRMS Calcd for $C_{25}H_{44}{}^{10}B_2{}^{11}B_8O_4Si$: 544.4012, Found: 544.4022.

*1-(4-Hydroxybenzyl)-2-(4-hydroxyphenyl)-1,2-dicarba-closo-dodecaborane (**7**: BE380).* A solution of **15** (153 mg, 0.281 mmol) in methanol (5 ml) was refluxed with 0.2 ml of conc. hydrochloric acid for 30 min. After addition of water, the mixture was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 1:1) gave **7** (96 mg, quant.) as a colorless solid. Colorless prisms (CH_2Cl_2-hexane); mp 176-178°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.40-3.10 (brm, 10H), 3.02 (s, 2H), 4.70 (br, 1H), 5.12 (br, 1H), 6.69 (d, J = 9.0 Hz, 2H), 6.72 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 9.0 Hz, 2H). HRMS Calcd for $C_{15}H_{22}{}^{10}B_2{}^{11}B_8O_2$: 342.2623, Found: 342.2600. Anal. Calcd for $C_{15}H_{22}B_{10}O_2$: C, 52.61; H, 6.48. Found: C, 52.65; H, 6.49.

*1-[4-(tert-Butyldimethylsiloxy)phenethyl]-2-{4-[(2-methoxyethoxy)methoxy]phenyl}-1,2-dicarba-closo-dodecaborane (**16**).* Compound **16** was prepared from **14** by the same method as that used for preparation of **15**, with 4-(*tert*-butyldimethylsiloxy)phenethyl bromide. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 4:1) gave **16** (23 %). The starting material **14** was recovered in 75% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.14 (s, 6H), 0.94 (s, 9H), 1.40-3.00 (brm, 10H), 2.03 (m, 2H), 2.58 (m, 2H), 3.35 (s, 3H), 3.54 (m, 2H), 3.81 (m, 2H), 5.29 (s, 2H), 6.64 (d, J = 8.4 Hz, 2H), 6.73 (d, J = 8.4 Hz, 2H), 7.03 (d, J = 9.2 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H). HRMS Calcd for $C_{26}H_{46}{}^{10}B_2{}^{11}B_8O_4Si$: 558.4168, Found: 558.4164.

*1-(4-Hydroxyphenethyl)-2-{4-[(2-methoxyethoxy)methoxy]phenyl}-1,2-dicarba-closo-dodecaborane (**8**, BE381).* Compound **8** was prepared from **16** by the same method as that used for preparation of **7**. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 1:1) gave **8** (99 %). Colorless prisms (CH_2Cl_2-hexane); mp 173-175□. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.40-3.00 (brm, 10H), 2.01 (m, 2H), 2.58 (m, 2H), 4.58 (brs, 1H), 5.10 (brs, 1H), 6.66 (d, J = 8.6 Hz, 2H), 6.75 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 9.0 Hz, 2H), 7.51 (d, *J* = 9.0 Hz, 2H). HRMS Calcd for $C_{16}H_{24}{}^{10}B_2{}^{11}B_8O_2$: 356.2779, Found: 356.2810. Anal. Calcd for $C_{16}H_{24}B_{10}O_2$: C, 53.91; H, 6.79. Found: C, 53.69; H, 6.76.

(Z)-2,3-Bis(4-methoxyphenyl)but-2-ene-1,4-diol ***(18)****.* To a solution of 3,4-bis(4-methoxyphenyl)-*5H*-furan-2-one[15] (**17**, 7.22 g, 24.4 mmol) in THF (250 ml) was added diisobutylaluminum hydride (0.95 M in hexane, 100 ml, 95.0 mmol). The mixture was stirred for 30 min at 0°C, then for 2 h at room temperature under an argon atmosphere. The mixture was poured into 10% aqueous hydrochloric acid and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 1:1 to AcOEt) gave **18** (4.90 g, 46%) as a colorless viscous liquid. ^1H-NMR (270 MHz, $CDCl_3$) δ: 3.73 (s, 6H), 4.62 (s, 4H), 6.68 (d, *J* = 8.9 Hz, 4H), 6.99 (d, *J* = 8.9 Hz, 4H). LRMS: *m/z* 300 (M^+).

Tetraethyl 4,5-bis(4-methoxyphenyl)cyclohex-4-ene-1,1,2,2-tetracarboxylate ***(19)****.* To a solution of **18** (4.90 g, 16.3 mmol) in Et_2O (100 ml) was added pyridine (0.5 ml) and PBr_3 (4.41 g, 16.3 mmol). The mixture was refluxed for 90 min. The mixture was poured into ice water and extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to afford a 3:4 mixture of *E*-, *Z*-1,4-dibromo-2,3-bis(4-methoxyphenyl)but-2-ene. *Z*-form: ^1H-NMR (270 MHz, $CDCl_3$) δ: 3.74 (s, 6H), 4.50 (s, 4H), 6.69 (d, *J* = 8.9 Hz, 4H), 7.00 (d, *J* = 8.9 Hz, 4H). *E*-form: ^1H-NMR (270 MHz, $CDCl_3$) δ: 3.86 (s, 6H), 4.07 (s, 4H), 6.98 (d, *J* = 8.7 Hz, 4H), 7.38 (d, *J* = 8.9 Hz, 4H). The bromide was used for subsequent reaction without further purification, because isomerization of the *Z*-form to the undesired *E*-form occurs during purification. Sodium hydride (60% in oil, 1.29 g, 32.3 mmol) was washed with *n*-hexane, dried under reduced pressure and suspended in 90 ml of THF under an Ar atmosphere. A solution of 4.66 g (14.6 mmol) of tetraethylethane-1,1,2,2-tetracarboxylate and was added to the above solution at 0°C. the mixture was stirred for 15 min, then a solution of *E*-, *Z*- mixture of the bromide (5.65 g, 13.3 mmol) in THF (60 ml) was added. The resulting solution was refluxed for 14 h, then poured into water, and the whole was extracted with AcOEt. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated. Purification by column chromatography on silica gel (eluent: *n*-hexane/ethyl acetate 3:1) afforded 3.42 g (39 %, 2 steps) of **19** as a pale yellow viscous liquid. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.29 (t, *J* = 7.1 Hz, 12H), 3.18 (s, 4H), 3.72 (s, 6H), 4.26 (q, *J* = 7.1 Hz, 8H), 6.65 (d, *J* = 8.9 Hz, 4H), 6.89 (d, *J* = 8.7 Hz, 4H). LRMS: *m/z* 582 (M^+).

*4,4,5,5-Tetrakis(bromomethyl)-1,2-bis(4-methoxyphenyl)cyclohex-1-ene (**20**).* To a solution of **19** (2.47 g, 4.24 mmol) in THF (100 mL) was added portionwise $LiAlH_4$ (644 mg, 17.0 mmol). The mixture was refluxed for 20 h under an Ar atmosphere, then poured into 10% aqueous hydrochloric acid, and the whole was extracted with AcOEt. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated. The resulting tetrol was dissolved in pyridine (16 mL) and this solution was added to methanesulfonyl chloride (2.91 g, 25.4 mmol) at -15°C. The mixture was stirred for 24 h at room temperature under an Ar atmosphere, poured into 10% aqueous hydrochloric acid and extracted with AcOEt. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated. The mesylate was dissolved in 2-ethoxyethanol (60 mL) and LiBr (4.42 g, 50.9 mmol) was added at room temperature. The mixture was heated to reflux for 66 h. Water was added, and the whole was extracted with Et_2O. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 10:1) gave **20** (838 mg, 30%) as a pale yellow viscous liquid. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 2.72 (s, 4H), 3.73 (s, 6H), 3.82 (d, *J* = 10.6 Hz, 4H), 4.24 (d, *J* = 10.9 Hz, 4H), 6.68 (d, *J* = 8.7 Hz, 4H), 6.90 (d, *J* = 8.9 Hz, 4H).

*1,2-Bis(4-hydroxyphenyl)-4,4,5,5-tetramethylcyclohex-1-ene (**9**, BE1054).* To a solution of **20** (319 mg, 0.479 mmol) in HMPT (10 ml) was added $NaBH_4$ (145 mg, 3.83 mmol), and the mixture was heated at 80°C for 45 h under an Ar atmosphere. The mixture was poured into water and extracted with AcOEt. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated. Purification by silica gel flash column chromatography (eluent: hexane/AcOEt, 10:1) gave 1,2-bis(4-methoxyphenyl)-4,4,5,5-tetramethylcyclohex-1-ene (116 mg, 69%). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.99 (s, 12H), 2.24 (s, 4H), 3.72 (s, 6H), 6.64 (d, J = 8.9 Hz, 4H), 6.89 (d, J = 8.7 Hz, 4H). To a solution of the product described above (116 mg, 0.331 mmol) in CH_2Cl_2 (1 ml) was added dropwise a 1 M solution of BBr_3 in CH_2Cl_2 (0.83 ml) at -78°C. The mixture was stirred at room temperature for 12 h, then poured into ice water, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. Purification by silica gel column chromatography (eluent: hexane/AcOEt, 2:1) gave **9** (107 mg, 99%). **9**: colorless powder (CH_2Cl_2-hexane); mp 70-71°C. ^{1}H-NMR (270 MHz, CD_3OD) δ: 1.00 (s, 12H), 2.20 (s, 4H), 6.50 (d, J = 8.7 Hz, 4H), 6.75 (d, J = 8.6 Hz, 4H). ^{13}C-NMR (67.5 MHz, CD_3OD) δ: 24.2, 35.6, 46.9, 115.3, 131.0, 133.5, 136.3, 155.9. HRMS Calcd for $C_{22}H_{26}O_2$: 322.1933, Found 322.1905. Anal. Calcd for $C_{22}H_{26}O_2 \cdot 1/4H_2O$: C, 80.82; H, 8.17. Found C, 80.54; H, 8.26.

*2,3-Bis(4-methoxyphenyl)bicyclo[2.2.2]oct-2-ene (**22**).* A mixture of 2,3-dibromobicyclo[2.2.2]oct-2-ene[17] (**21**, 330 mg, 1.24 mmol), 4-methoxyphenylboronic acid (471 mg, 3.10 mmol), $Pd(PPh_3)_4$ (143 mg, 0.124 mmol) and Na_2CO_3 (1.97 g, 18.6 mmol) in 170 ml of toluene/EtOH/water (5:5:2) was refluxed for 24 h under an Ar atmosphere. After removal of the solvent under reduced pressure, the mixture was added to water and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. Purification by silica gel column chromatography (eluent: hexane/AcOEt, 30:1) gave **22** (315 mg, 79%). **22**: ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.50-1.70 (m, 8H), 2.87 (brs, 2H), 3.76 (s, 6H), 6.71 (d, J = 8.7 Hz, 4H), 7.02 (d, J = 8.9 Hz, 4H). LRMS: *m/z* 320 (M^+).

*2,3-Bis(4-hydroxyphenyl)bicyclo[2.2.2]oct-2-ene (**10**: BE1060)* To a solution of **22** (310 mg, 0.967 mmol) in CH_2Cl_2 (3 ml) was added dropwise a 1 M solution of BBr_3 in CH_2Cl_2 (2.43 ml) at -78 °C. The mixture was stirred at room temperature for 16 h, then poured into ice water, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. Purification by silica gel column chromatography (eluent: hexane/AcOEt, 4:1) gave a 9:4 mixture of **10** and 6,7-bis(4-hydroxyphenyl)bicyclo[3.2.1]oct-6-ene (245 mg, 87%). Purification by recrystallization (three times) afforded **10**. Data for the rearranged isomer: ^{1}H-NMR (270 MHz, CD_3OD) δ: 1.50-1.80 (m, 7H), 2.25 (m, 1H), 2.80 (m, 2H), 6.62 (d, J = 8.7 Hz, 4H), 7.06 (d, J = 8.7 Hz, 4H). ^{13}C-NMR (150 MHz, CD_3OD) δ: 20.4, 26.7, 45.1, 47.4, 115.9, 130.2, 132.1, 137.8, 156.8. **10**: colorless prisms (AcOEt-hexane); mp 157-159°C. ^{1}H-NMR (270 MHz, CD_3OD) δ: 1.50-1.75 (m, 8H), 2.81 (m, 2H), 6.56 (d, J = 8.7 Hz, 4H), 6.88 (d, J = 8.7 Hz, 4H). ^{13}C-NMR (150 MHz, CD_3OD) δ: 27.3, 38.8, 115.7, 130.6, 134.0, 139.8, 156.5. HRMS Calcd for $C_{20}H_{20}O_2$: 292.1463, Found 292.1435. Anal. Calcd for $C_{22}H_{26}O_2$□$1/2H_2O$: C, 79.71; H, 7.02. Found C, 79.74; H, 7.33.

*1-(4-Methoxyphenyl)-2-methyleneadamantane (**26**).* To a stirred solution of methyltriphenylphosphonium bromide (6.97 g, 19.5 mmol) in THF (15 mL), sodium bis(trimethylsilyl)amide (1.0 M in THF, 19.5 mL, 19.5 mmol) was added at -60°C under an Ar atmosphere, and the solution was stirred for 1 h. A solution of 1-(4-methoxyphenyl)adamantan-2-one[18] (**24**, 2.00 g, 7.80 mmol) in THF (40 mL) was added to the solution, and the mixture was stirred for 2 h at room temperature. It was then diluted with hexane, and filtered though Celite. The filtrate was concentrated and chromatographed on silica gel (eluent: hexane/AcOEt, 20:1) to give **26** (1.98 g, quant.). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.75-2.41 (m, 12H), 2.64 (m, 1H), 3.81 (s, 3H), 3.84 (d, J = 2.0 Hz, 1H), 4.59 (d, J = 2.0 Hz, 1H), 6.88 (d, J = 9.0 Hz, 2H), 7.27 (d, J = 9.0 Hz, 2H).

*(E)-2-(4-Methoxybenzylidene)-1-(4-methoxyphenyl)adamantane (**27**).* To a solution of **26**

(136 mg, 0.535 mmol) and 4-iodoanisole (250 mg, 1.07 mmol) in DMF (6 mL), was added $Pd(OAc)_2$ (8 mg, 0.036 mmol), triphenylphosphine (19 mg, 0.072 mmol) and Ag_2CO_3 (295 mg, 1.07 mmol) at room temperature. The mixture was stirred for 72 h at 80°C, then poured into water and extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. Purification of the residue by silica gel column chromatography (eluent: hexane/AcOEt, 30:1) gave **27** (134 mg, 70%). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.80-2.45 (m, 12H), 3.24 (m, 1H), 3.76 (s, 3H), 3.82 (s, 3H), 5.42 (s, 1H), 6.78 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.9 Hz, 2H), 6.96 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 9.1 Hz, 2H).

*(E)-2-(4-Hydroxybenzylidene)-1-(4-hydroxyphenyl)adamantane (**11**: BE1080).* A mixture of **27** (130 mg, 0.361mmol) and EtSNa (152 mg, 1.80 mmol) in DMF (5 mL) was refluxed for 3 h under an Ar atmosphere. The mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. Purification of the residue by silica gel column chromatography (eluent: hexane/AcOEt, 2:1) afforded **11** (81 mg, 68%). colorless prisms (AcOEt-hexane); mp 230-231°C. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 1.79-2.36 (m, 12H), 3.16 (m, 1H), 5.24 (s, 1H), 6.62 (d, J = 8.2 Hz, 2H), 6.73 (d, J = 8.6 Hz, 2H), 6.76 (d, J = 8.2 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 9.19 (s, 1H), 9.23 (s, 1H). ^{13}C-NMR (67.5 MHz, CD_3OD) δ: 30.6, 34.0, 37.5, 40.1, 45.4, 46.0, 115.5, 115.6, 121.3, 128.9, 130.5, 131.2, 139.1, 153.3, 156.1, 156.2. HRMS Calcd for $C_{23}H_{24}O_2$: 332.1776, Found 332.1739. Anal. Calcd for $C_{23}H_{24}O_2$□1/10H_2O: C, 82.65; H, 7.30. Found C, 82.50; H, 7.26.

*(Z)-1-(4-Methoxyphenyl)-2-[2-(4-methoxyphenyl)ethylidene]adamantane (**25**).* To a stirred solution of 4-methoxyphenethyltriphenylphosphonium bromide (2.37 g, 4.97 mmol) in Et_2O (10 ml), *n*-BuLi (1.59 M in hexane, 3.13 ml, 4.97 mmol) was added at 0°C under an Ar atmosphere, and the mixture was stirred for 2.5 h. A solution of **24**[18] (769 mg, 3.00 mmol) in THF (5 mL) was added to the solution, and the mixture was stirred for 44 h at 50°C. The mixture was diluted with hexane, and filtered though Celite. The filtrate was concentrated

and chromatographed on silica gel (eluent: hexane/AcOEt, 20:1) to give a mixture of *Z*-**26** and its *E*-isomer (380 mg, 34%). Purification of the mixture by recrystallization from hexane gave **26** (235mg, 21%) **26**: colorless needles (hexane); mp 132-134□. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.72-2.18 (m, 10H), 2.15 (d, *J* = 7.3 Hz, 2H), 2.39-2.44 (m, 3H), 3.74 (s, 3H), 3.78 (s, 3H), 5.13 (t, *J* = 7.3 Hz, 1H), 6.72 (d, *J* = 8.8 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 4H), 7.34 (d, *J* = 9.0 Hz, 2H). HRMS Calcd for $C_{26}H_{30}O_2$: 374.2246, Found 374.2268.

*(E)-1-(4-Hydroxyphenyl)-2-[2-(4-hydroxyphenyl)ethylidene]adamantane (**12**: BE1081).* To a solution of **26** (38 mg, 0.10 mmol) in CH_2Cl_2 (1 ml) was added dropwise a 1 M solution of BBr_3 in CH_2Cl_2 (0.83 ml) at -78°C. The mixture was stirred at room temperature for 15 h, then poured into ice water, and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel column chromatography (eluent: hexane/AcOEt, 3:1) gave **12** (35 mg, quant.). **12**: colorless prisms ($CHCl_3$); mp 184-186°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.75-2.33 (m, 12H), 3.07 (m, 1H), 3.19 (d, *J* = 7.5 Hz, 2H), 4.49-4.55 (br, 2H), 4.55 (t, *J* = 7.5 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 2H), 6.76 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 7.19 (d, *J* = 9.0 Hz, 2H). ^{13}C-NMR (67.5 MHz, CD_3OD) δ: 30.6, 33.0, 33.8, 37.5, 39.9, 45.3, 45.9, 115.4, 115.9, 119.9, 128.8, 129.8, 133.9, 139.2, 148.5, 151.4, 155.9. HRMS Calcd for $C_{24}H_{26}O_2$: 346.1933, Found 346.1952. Anal. Calcd for $C_{24}H_{26}O_2$: C, 83.20; H, 7.56. Found C, 82.93; H, 7.79.

Estrogen receptor α ligand binding assay

The ligand binding activity of estrogen receptor α (ERα) was determined by the nitrocellulose filter binding assay method. ERα(0.5 µg/tube, PanVera Co. Ltd.) was diluted with a binding assay buffer (20 mM Tris-HCl pH 8.0, 0.3 M NaCl, 1 mM EDTA pH 8.0, 10 mM 2-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride) and incubated with 4 nM [6,7-^{3}H]17β-estradiol in the presence or absence of an unlabeled competitor at 4°C for 16 h. The incubation mixture was absorbed by suction onto a nitrocellulose membrane that had been soaked in binding assay buffer. The membrane was washed twice with buffer (20 mM Tris-HCl pH 8.0, 0.3 M NaCl) and then with 25% ethanol in distilled water. Radioactivity that remained on the membrane was measured in Atomlight (NEN) by using a liquid scintillation counter.

Transfection and luciferase assays

Human breast cancer cell-line MCF-7 cells were maintained in DMEM (Sigma Chemical Co.) containing penicillin and streptomycin with 5 % fetal bovine serum (FBS; Life Technologies, Rockville, MD). ERE-luciferase reporter assay using MCF-7 cells was performed according to the previously reported method. Briefly, transient transfections in MCF-7 cells were performed using Transfast^{TM} (Promega Co., Madison, WI), according to the manufacturer's protocol. Transfections were done in 96-well plates at 8000 cells/well with 0.1 μg of p(ERE)$_3$-SV40-luc and 0.3 ng of phRL/CMV (Promega Co.) as an internal standard. Twenty-four hours after addition of the sample, the assay was performed with a Dual Luciferase assay kit^{TM} (Promega Co.). For the assay of anti-estrogens, the inhibitory effect of test compounds on the estrogenic activity of E2 at the concentration of 1×10^{-10} M was examined.