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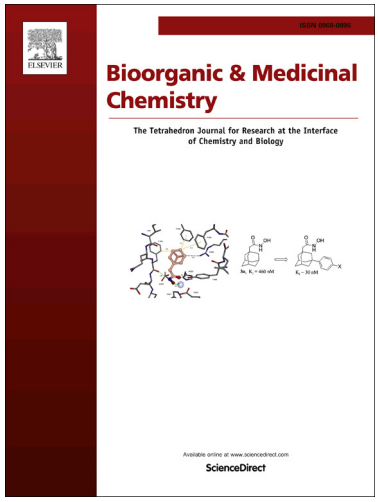

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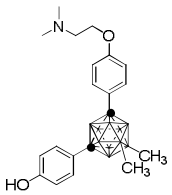

ER partial agonist candidate

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ABSTRACT

We previously discovered *m*-carborane-containing estrogen receptor (ER) modulator **4**, which exhibits weak ER-agonistic and antagonistic activities in transactivation assays. With the aim of developing novel ER partial agonists, we designed and synthesized various analogues of **4** with a bent-core structure, i.e., pseudo cyclic structure (**5**), tetrahydropyrimidinone (**6**), *m*-benzene (**7**), adamantane (**8**), and 9,10-dimethyl-*m*-carborane (**9**), in place of the *m*-carborane moiety. Compound **9** showed greater binding affinity than **4** in ER-binding assay using [6,7-^{3}H]-17β-estradiol and was a more effective partial agonist than **4** in MCF-7 cell proliferation assay. It appears to be a promising candidate as a selective ER modulator (SERM).

1. Introduction

We have shown that the carborane cage is a novel hydrophobic pharmacophore with potential for drug development,[1] and since then, various carborane-containing bioactive compounds have been reported.[2] Prior to that, carboranes had been used only as boron carriers for boron neutron capture therapy (BNCT), based on their high boron content.[3] We also designed and synthesized various carborane-containing bioactive compounds as candidate nuclear receptor ligands.[4] One derivative, 1-(4-hydroxyphenyl)-12-hydroxymethyl-*p*-carborane (BE120, **1**), shows more potent estrogenic activity than the endogenous female hormone estrogen, estradiol (E2, **2**, Figure 1).[1,5] Subsequently, many carboranyl phenol derivatives were synthesized and their estrogen-related biological activities examined.[6]

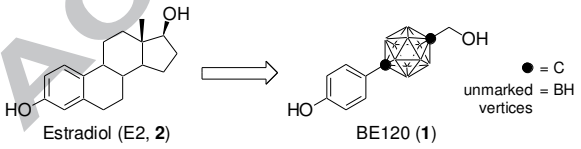

Figure 1. Development of potent ER agonist **1** based on the concept of a spherical hydrophobic pharmacophore.

Estrogens are known to play important roles in the development and maintenance of reproductive and non-reproductive tissues in both women and men.[7] However, while estrogens have essential functions in the reproductive, skeletal, cardiovascular, and central nervous systems, the proliferative effect of estrogens can be pathological, promoting cancer of the breast and uterus.[7] These multiple functions result in remarkable tissue-selective effects of estrogens, and have led to interest in for the feasibility of using selective estrogen receptor modulators (SERMs), to treat breast cancer, osteoporosis, and female postmenopausal syndrome.[8] However, these drugs involve some risk of cancer of the female reproductive organs.[8] Therefore, researchers are seeking to develop third- or fourth-generation SERMs to circumvent this risk.[8] The most noteworthy feature of these SERMs is the *N*,*N*-dialkylaminoethyl substituent, which serves to inhibit the binding of co-activators by moving helix-12 of the receptor to an unfavorable position.[9] Tamoxifen (**3a**), which is metabolized in vivo to an active derivative, 4-hydroxytamoxifen (**3b**), is a first-generation SERM.

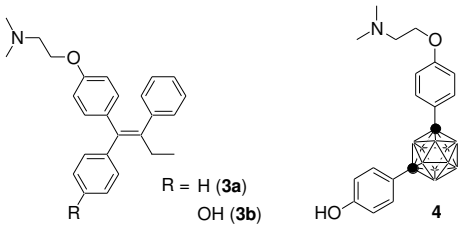

Figure 2. Structures of clinically used tamoxifen (**3a**), its metabolite (**3b**), and carborane-containing ER partial agonist **4**.

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During our studies of carborane-containing ER modulators, we found that *m*-carborane derivative (**4**) showed ER partial agonistic activity in ERα transactivation assays,[10] and we suggested that the central core structure of **4** might play an important role in expressing partial agonistic activity, as well as in the hydrophobic interaction between the ERα ligand-binding domain (LBD) and **4**. Therefore, to investigate the role of the hydrophobic core structure of in the partial agonistic activity, we designed various derivatives with a bent-core structure, i.e., pseudo cyclic structure containing an intramolecular hydrogen bond (**5**), tetrahydropyrimidinone ring (**6**), benzene (**7**), adamantane (**8**), and 9,10-dimethyl-*m*-carborane cage (**9**), in place of the *m*-carborane cage of **4** and we measured their activity in ERα transactivation assays.

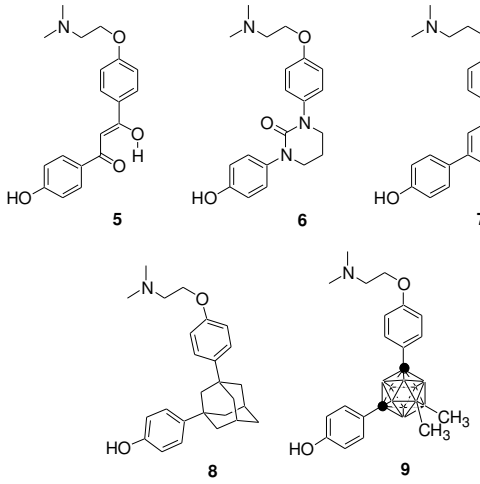
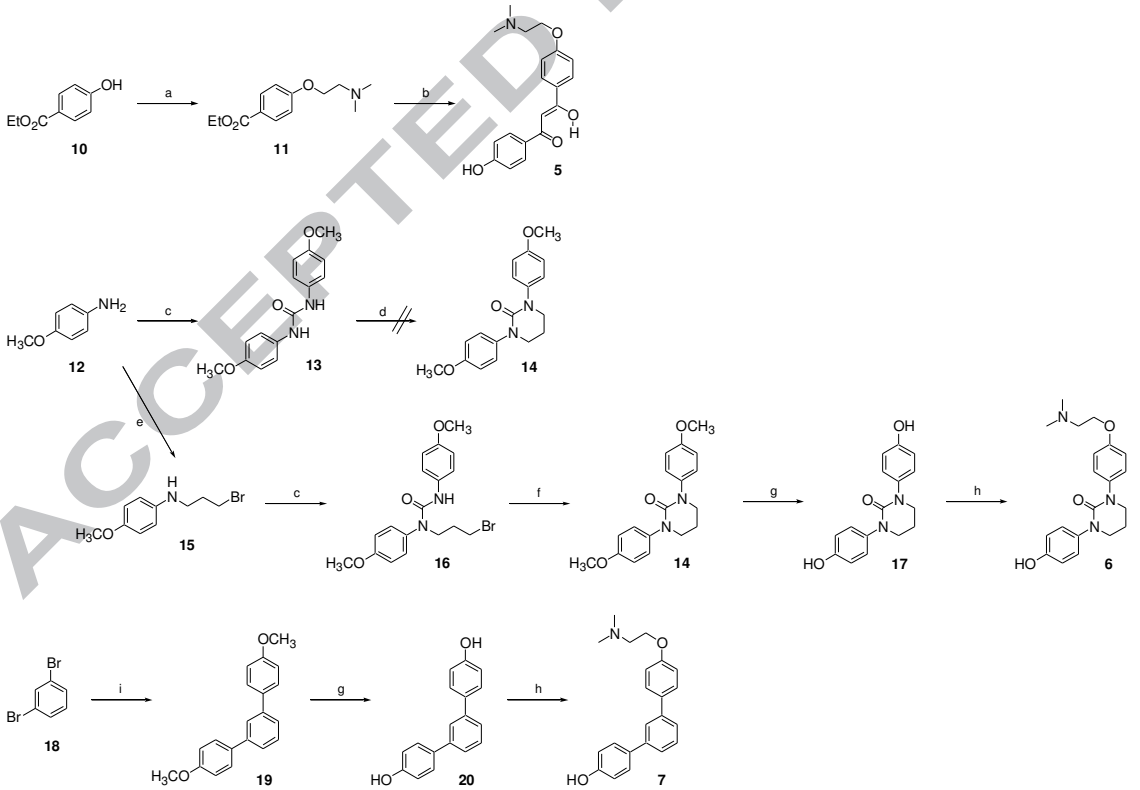


Figure 3. Target compounds **5**-**9**.

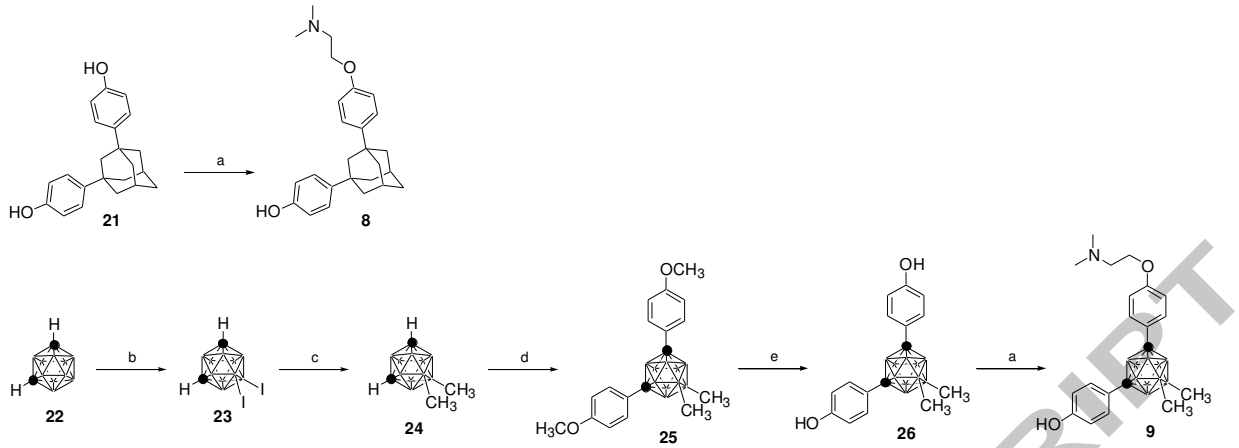
2. Results and discussion

2.1. Synthesis of bisphenol derivatives

Scheme 1 summarizes the synthesis of non-spherical hydrophobic bent-structure derivatives **5**-**7**. Ethyl 4-hydroxybenzoate **10** was reacted with *N*,*N*-dimethylaminoethanol under Mitsunobu reaction conditions to afford the corresponding alkylated compound **11**. Claisen condensation of **11** with *tert*-butyldimethylsilyl (TBDMS)-protected 4-hydroxyacetophenone afforded 1,3-diketone **5** in 6% yield over 2 steps. We tried to synthesize tetrahydropyrimidinone derivative **6** from *p*-anisidine **12**, which was first reacted with 4-methoxyphenyl isocyanate to afford symmetric urea **13**. Cyclization reaction of **13** with 1,3-dibromopropane, however, gave a complex mixture, which did not contain the desired compound. Therefore, the propane unit was incorporated first into *p*-anisidine **12**, followed by reaction with 4-methoxyphenyl isocyanate, and intramolecular *N*-alkylation to afford cyclic urea derivative **14**. Demethylation of both methoxy groups of **14** afforded the corresponding bisphenol **17**, which was alkylated with *N*,*N*-dimethylaminoethyl chloride on one side to afford tetrahydropyrimidinone derivative **6** in 5% yield over 5 steps. 1,3-Dibromobenzene was reacted with 4-methoxyphenyl boronic acid under Suzuki-Miyaura cross-coupling conditions to afford *m*-terphenyl derivative **19**.[11] Deprotection of the methoxy group with BBr_3 was followed by *O*-alkylation with *N*,*N*-dimethylaminoethyl chloride to give terphenyl derivative **7** in 5% yield over 3 steps.



Scheme 1. Syntheses of target compounds **5**-**7** with a non-spherical hydrophobic bent core structure. Reagents and conditions; a) *N*,*N*-dimethylaminoethanol, diethyl azodicarboxylate Ph_3P, THF; b) NaH, DME; c) 4-methoxyphenyl isocyanate, triethylamine, toluene; d) NaH, 1,3-dibromopropane, DMF; e) 1,3-dibromopropane, CH_3CN; f) NaH, THF; g) BBr_3, CH_2Cl_2; h) *N*,*N*-dimethylaminoethyl chloride hydrochloride, K_2CO_3, DMF/acetone; i) 4-methoxyphenylboronic acid, $Pd(PPh_3)_4$, 1M Na_2CO_3 aq, toluene



Scheme 2. Syntheses of compounds **8** and **9** with a bulky hydrophobic core structure. Reagents and conditions; a) *N*,*N*-dimethylaminoethyl chloride hydrochloride; b) I_2, $AlCl_3$, CH_2Cl_2; c) CH_3MgBr, $PdCl_2(PPh_3)_2$, DME; d) *n*-BuLi, CuCl, DME, then pyridine, 4-iodoanisole, DME; e) BBr_3, CH_2Cl_2.

Compound **8** was obtained through alkylation of one phenol group of commercially available **21** in 24% yield. Compound **23**, which was prepared from **22** by treat with iodine in the presence of $AlCl_3$, was transformed into 9,10-dimethyl-*m*-carborane **24** by reaction with methylmagnesium chloride under Pd-catalyzed coupling conditions.[12] Compound **24** was treated with 2 equivalent of *n*-BuLi, followed by CuCl to afford the di-Cu salt, which was reacted with 4-iodoanisole in the presence of pyridine as a ligand of Cu to afford diaryl-*m*-carborane intermediate **25**.[13] Deprotection of the methoxy group with BBr_3, followed by *O*-alkylation with *N*,*N*-dimethylaminoethyl chloride, afforded *m*-carborane-containing derivative **9** in 0.3% yield over 5 steps.

2.2. ERα binding

Binding affinity of compounds **5**-**9** for ERα was evaluated by means of a competitive binding assay using [6,7-^{3}H]17β-estradiol and human recombinant ERα.[14] The binding affinity data are summarized in Figure 3. Although compounds **5**, **6**, and **7** with non-spherical central core structure showed little binding affinity for ERα, compounds **4**, **8**, and **9**, which have three-dimensional hydrophobic core structure, bound to ERα in a dose-dependent manner. The hydrophobic ER LBD could not accept the benzene ring of **5** as a core structure, or the polar functional groups of **6** and **7**. Compound **8** with adamantane core structure showed about 10 times weaker binding affinity than **4**. We previously reported that the hydrophobic parameter π values of 4-(*m*-carboran-1-yl)phenol and 4-(1-adamantyl)phenol were 4.26 and 4.04, respectively.[15] Thus, adamantane is a less hydrophobic structure than the *m*-carborane cage and interacts less efficiently with the hydrophobic ER LBD. This idea is strongly supported by the binding affinity of **9**. Compound **9**, containing 9,10-dimethyl-*m*-carborane cage as a hydrophobic core structure, showed a greater ER-binding affinity than **4**. This result is consistent with the hydrophobicity of the central core structure, because introduction of two methyl groups into the *m*-carborane cage causes an increase of π value.

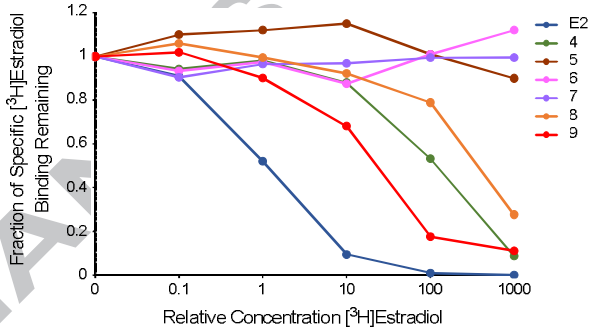


Figure 3. Competitive binding assay of compounds **4**-**9** with [^{3}H]E2 (4 nM) at ERα. Binding assays were conducted with the test compounds (0.4 nM–4 μM) in the presence of [6,7-^{3}H]17β-estradiol (4 nM) and performed in duplicate (n = 2).

2.3. MCF-7 cell proliferation assay

The biological activities of selected compounds, **4**, **8** and **9**, were evaluated by means of cell proliferation assay using human breast cancer MCF-7 cells.[14] EC_{50} or IC_{50} values of the test compounds were estimated from dose-response curves of MCF-7 cell proliferation in the absence or presence of 0.1 nM estradiol, respectively. The E_{max} value is the maximal efficacy of each compound in cell proliferation and is an index of ER partial agonistic activity. This value was estimated based on that of estradiol taken as 100. Compound **4** showed an EC_{50} value of 39 nM and its E_{max} value (100%) was equal to that of E2, but it did not show ER-antagonistic activity.[10] Unexpectedly, **4** was not an ER partial agonist in MCF-7 cell proliferation assay. Compounds **8** and **9** showed more potent estrogenic activity than that of E2, with the EC_{50} values of 16 nM and 15 nM, respectively. Interestingly, they showed low E_{max} values of 17% and 23%, respectively, and acted as ER partial agonists, in spite of containing spherical hydrophobic core structures similar to that of **4**. The difference of ER-agonistic activity among them might be caused by subtle differences of their binding forms in the ERα LBD. It is noteworthy that the biological activities of **4** and **9** are dramatically different, in spite of the very similar geometry. The two methyl substituents of **9** play a significant role in the expression of ER partial agonistic activity. The IC_{50} values of **8** and **9** were estimated to be 1.06 μM and 0.88 μM, respectively. These compounds effectively inhibit the estrogenic activity of estradiol, and appear to be promising SERM candidates.

Table 1. Biological activities of **4**, **8**, and, **9** on MCF-7 cell proliferation.

Compound	EC_{50} (μM)[a]	E_{max} (%)[b]	IC_{50} (μM)[a]
4	0.039	100	inactive
8	0.016	17	1.06
9	0.015	23	0.88

[a] MCF-7 cells were treated with the test compounds (1 x 10^{-13} to 1 x 10^{-5} M) alone or in the presence of 0.1 nM E2. EC_{50} and IC_{50} values were estimated from the sigmoidal dose-response curves using GraphPad Prism 4 software. [b] E_{max} values indicate the efficacy for cell proliferation, based on the value for E2 taken as 100.

3. Conclusion

In conclusion, we have designed and synthesized estrogen receptor modulators with various bent-core structures and examined their biological activities. Compounds **5**, **6**, and **7**, which contain non-spherical bent-core structures, did not bind to ERα. In contrast, spherical hydrophobic bent-core derivatives **4**, **8**, and **9** bound to the ERα LBD. Compounds **8** and **9** showed ERα-agonistic activity with very low E_{max} values of 17% and 23%, respectively, and partial agonistic activity, inhibiting MCF-7 cell proliferation at the concentrations of 1.06 and 0.88 μM, respectively. The two methyl groups of compound **9** have an important role in the expression of ER partial agonistic activity. Thus, as observed in the case of **4** and **9**, modification at the carborane cage causes marked changes of biological activities as well as chemical properties. These findings should be helpful in the molecular design of novel carborane-containing bioactive compounds, as well as ER partial agonists and SERMs.

4. Experimental

4.1. General Considerations

Melting points were determined with a Yanaco micro melting point apparatus and were not corrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with JEOL JNM-EX-270 and JNM-LA-400 spectrometers. Chemical shifts for ^{1}H NMR spectra were referenced to tetramethylsilane (0.0 ppm) as an internal standard. Chemical shifts for ^{13}C NMR spectra were referenced to residual ^{13}C present in deuterated solvents. The splitting patterns are designed as follows: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra were recorded on a JEOL JMS-DX-303 spectrometer. Elemental analyses were performed with a Perkin Elmer 2400 CHN spectrometer. Column chromatography was carried out using Fuji Silysia silica gel BW-80S and TLC was performed on Merck silica gel F_{254}. *p*-Carborane was purchased from Katchem s.r.o. (Prague, Czech Republic). Other reagents were purchased from Wako Pure Chemical Industries, Ltd., Sigma-Aldrich Co., and Tokyo Chemical Industry, Ltd. (TCI). All solvents were of reagent quality, purchased commercially, and were used without further purification.

*4.1.1. Ethyl 4-(dimethylaminoethoxy)benzoate (**11**)*

To a mixture of ethyl 4-hydroxybenzoate **10** (2.0 g, 12.0 mmol), dimethylaminoethanol (1.33 mL, 13.2 mmol), and triphenylphosphine (3.47 g, 13.2 mmol) in 20 mL of THF was added a 40% solution of DEAD in toluene (5.76 mL, 13.2 mmol) at room temperature. The mixture was stirred at room temperature for 24 h, then concentrated, and the residue was purified by silica gel column chromatography (AcOEt:*n*-hexane = 1:1) to give the title compound as a colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.37 (t, *J* = 7.1 Hz, 3H), 2.34 (s, 6H), 2.74 (t, *J* = 5.8 Hz, 2H), 4.11 (t, *J* = 5.6 Hz, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 6.93 (dd, *J* = 2.1 Hz, 6.9 Hz, 2H), 7.99 (dd, *J* = 2.0 Hz, 6.8 Hz, 2H); MS (EI) *m/z* 237 (M^+, 100%).

*4.1.2. 1-[4-(2-Dimethylaminoethoxy)phenyl]-3-(4-hydroxyphenyl)propane-1,3-dione (**5**)*

To a solution of **11** (500 mg, 2.0 mmol) and 4-(*tert*-butyldimethylsilyloxy)acetophenone (580 mg, 2.20 mmol) in 5 mL of dry DME was added sodium hydride (50%, 121 mg, 2.34 mmol) at 0°C, and the mixture was refluxed for 4 h. A further amount of sodium hydride (50%, 121 mg, 2.34 mmol) was added, and the mixture was refluxed for an additional 1 h, then poured onto ice and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:*n*-hexane = 1:7 to 1:5) to give 183 mg (27%) of the title compound as yellow needles; mp 169.5-170.5 °C (MeOH-AcOEt); ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 2,41 (s, 6H), 2.83 (t, *J* = 5.4 Hz, 2H), 4.15 (t, *J* = 5.4 Hz, 2H), 6.65 (s, 1H), 6.83 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 7.83 (d, *J* = 8.7 Hz, 2H), 7.88 (d, *J* = 8.9 Hz, 2H); MS (EI) *m/z* 327 (M^+, 100%); HRMS Calcd for $C_{19}H_{21}NO_4$: 327.1471, Found: 327.1477.

*4.1.3. N-(3-Bromopropyl)anisidine (**15**)*

A solution of anisidine **12** (1.0 g, 8.1 mmol) and 1,3-dibromopropane (1.25 mL, 12.2 mmol) in 10 mL of acetonitrile was stirred at 85°C for 5 h. Saturated $NaHCO_3$ aqueous solution was added to it, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:*n*-hexane = 1:7 to 1:5) to give 470 mg (24%) of the title compound as a colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 2.10 (quint, *J* = 6.6 Hz, 2H), 3.25 (t, *J* = 6.6 Hz, 2H), 3.48 (t, *J* = 6.4 Hz, 2H), 3.54 (brs, 1H), 3.73 (s, 3H), 6.59 (dd, *J* = 2.3 Hz, 6.6 Hz, 2H), 6.78 (dd, *J* = 2.3 Hz, 6.6 Hz, 2H); MS (EI) *m/z* 243:245 (1:1, M^+), 136 (100%).

*4.1.4. N'-(4-Methoxyphenyl)-N-(4-methoxyphenyl)-N-(3-bromopropyl)urea (**16**)*

A solution of **15** (470 mg, 1.93 mmol), 4-methoxyphenyl isocyanate (299 μg, 2.31 mmol), and triethylamine (5 mg) in 10 mL of toluene was refluxed for 3 h at room temperature, and then concentrated. The residue was purified by silica gel column chromatography with 1:2 AcOEt:*n*-hexane to give 598 mg (79%) of the title compound as a colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 2.16 (quint, *J* = 6.9 Hz, 2H), 3.45 (t, *J* = 6.9 Hz, 2H), 3.75 (s, 3H), 3.81 (t, *J* = 6.9 Hz, 2H), 3.86 (s, 3H), 6.00 (brs, 1H), 6.78 (d, *J* = 9.1 Hz, 2H), 6.99 (d, *J* = 8.9 Hz, 2H), 7.17 (d, *J* = 9.1 Hz, 2H), 7.23 (d, *J* = 8.9 Hz, 2H); MS (EI) *m/z* 392:394 (1:1, M^+), 136 (100%).

*4.1.5. 1,3-Bis(4-methoxyphenyl)tetrahydropyrimidinone (**14**)*

To a solution of **16** (70 mg, 188 μmol) in 10 mL of THF was added sodium hydride (50%, 26 mg, 534 μmol) at 0°C. The mixture was stirred for 1.5 h at room temperature, then poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:*n*-hexane = 1:1) to give 51 mg (91%) of the title compound as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 2.24 (quint, *J* = 5.9 Hz, 2H), 3.77 (t, *J* = 5.8 Hz, 4H), 3.78 (s, 6H), 6.86 (dd, *J* = 2.3 Hz, 6.8 Hz, 4H), 7.24 (dd, *J* = 2.1 Hz, 6.6 Hz, 4H); MS (EI) *m/z* 312 (M^+, 100%).

*4.1.6. 1,3-Bis(4-hydroxyphenyl)tetrahydropyrimidinone (**17**)*

To a solution of **14** (25 mg, 80 μmol) in 2 mL of CH_2Cl_2 was added 1 M BBr_3 in CH_2Cl_2 (0.18 mL, 180 μmol) at -78°C. The mixture was stirred for 3 h at room temperature, then poured onto ice and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt) to give 19 mg (83%) of the title compound as a colorless solid; ^{1}H NMR (270 MHz, CD_3OD) δ (ppm) 2.13 (quint, *J* = 5.9 Hz, 2H), 3.63 (t, *J* = 5.9 Hz, 4H), 6.66 (dd, *J* = 8.9 Hz, 4H), 7.00 (d, *J* = 8.9 Hz, 4H); MS (EI) *m/z* 284 (M^+, 100%).

*4.1.7. 1-[4-(2-Dimethylaminoethoxy)phenyl]-3-(4-hydroxyphenyl)tetrahydropyrimidinone (**6**)*

To a solution of **17** (65 mg, 0.22 mmol) and 2-dimethylaminoethylchloride hydrochloride (42.8 mg, 0.3 mmol) in 1 mL of DMF and 1 mL of acetone was added K_2CO_3 (94.8 mg, 0.69 mmol), and the mixture was heated at 80°C for 5 h. Saturated $NaHCO_3$ aqueous solution was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (MeOH:$CHCl_3$ = 1:3 to 1:1) to give 25 mg (31%) of the title compound as colorless needles; mp 198.0-200.0 °C (MeOH); ^{1}H NMR (270 MHz, CD_3OD) δ (ppm) 2.14 (quint, *J* = 5.9 Hz, 2H), 2.32 (s, 6H), 2.77 (t, *J* = 5.4 Hz, 2H), 3.65 (q, *J* = 5.4 Hz, 4H), 4.02 (t, *J* = 5.4 Hz, 2H), 6.66 (d, *J* = 8.9 Hz, 2H), 6.84 (d, *J* = 8.9 Hz, 2H), 7.00 (d, *J* = 8.9 Hz, 2H), 7.12 (d, *J* = 8.9 Hz, 2H); MS (EI) *m/z* 355 (M^+, 100%); HRMS Calcd for $C_{20}H_{25}N_3O_3$: 355.1896, Found: 355.1904.

*4.1.8. 1,3-Bis(4-methoxyphenyl)benzene (**19**)*

A solution of 1,3-dibromobenzene **18** (110 mg, 0.47 mmol), 4-methoxyphenylboronic acid (156 mg, 1.03 mmol) and $Pd(PPh_3)_4$ (54 mg, 47 μmol) in 5 mL of toluene and 3 mL of saturated Na_2CO_3 aqueous solution was refluxed for 30 h. The mixture was extracted with AcOEt, washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by silica gel column chromatography (AcOEt:*n*-hexane = 1:30 to 1:20) to give 22 mg (16%) of the title compound as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 3.86 (s, 6H), 7.00 (dd, *J* = 2.0 Hz, 6.6 Hz, 4H), 7.45-7.51 (m, 3H), 7.58 (dd, *J* = 2.0 Hz, 6.6 Hz, 4H), 7.71-7.74 (m, 1H).

*4.1.9. 1,3-Bis(4-hydroxyphenyl)benzene (**20**)*

To a solution of **19** (22 mg, 76 μmol) in 2 mL of CH_2Cl_2 was added 1 M BBr_3 in CH_2Cl_2 (0.17 mL, 170 μmol) at -78°C. The mixture was stirred for 3 h at room temperature, then poured onto ice and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:*n*-hexane = 1:2) to give 19 mg (95%) of the title compound as a colorless solid; ^{1}H NMR (270 MHz, CD_3OD) δ (ppm) 4.85 (brs, 2H), 6.92 (dd, *J* = 2.1 Hz, 6.6 Hz, 4H), 7.45-7.50 (m, 3H), 7.53 (dd, *J* = 2.1 Hz, 6.6 Hz, 4H), 7.68-7.72 (m, 1H); MS (EI) *m/z* 284 (M^+, 100%).

*4.1.10. 1-[4-(2-Dimethylaminoethoxy)phenyl]-3-(4-hydroxyphenyl)benzene (**7**)*

To a solution of **20** (19 mg, 72 μmol) and 2-dimethylaminoethylchloride hydrochloride (14 mg, 94.2 μmol) in 1 mL of DMF and 1 mL of acetone was added K_2CO_3 (30 mg, 0.22 mmol), and the mixture was heated at 80°C for 8 h. Saturated $NaHCO_3$ aqueous solution was added, and the mixture was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (MeOH:$CHCl_3$ = 1:15) to give 8 mg (35%) of the title compound as a colorless powder; mp 154.0-155.0 °C (AcOEt-*n*-hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 2.46 (s, 6H), 2.90 (t, *J* = 5.4 Hz, 2H), 4.16 (t, *J* = 5.5 Hz, 2H), 5.53 (brs, 1H), 6.90 (d, *J* = 8.2 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 7.40-7.55 (m, 7H), 7.65-7.68 (m, 1H); MS (EI) *m/z* 333 (M^+, 100%); HRMS Calcd for $C_{22}H_{23}NO_2$: 333.1729, Found: 333.1729.

*4.1.11. 1-[4-(2-Dimethylaminoethoxy)phenyl]-3-(4-hydroxyphenyl)-1,3-adamantane (**8**)*

To a solution of 1,3-bis(4-hydroxyphenyl)-1,3-adamantane **21** (200 mg, 0.64 mmol) and 2-dimethylaminoethylchloride hydrochloride (117 mg, 0.81 mmol) in 2 mL of DMF was added K_2CO_3 (259 mg, 1.87 mmol), and the mixture was heated at 80°C for 15 h. Saturated $NaHCO_3$ aqueous solution was added, and the mixture was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (MeOH:$CHCl_3$ = 1:15 to 1:10) to give 58 mg (24%) of the title compound as colorless cubes; mp 172.0-173.0 °C (THF-*n*-hexane); ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 1.80 (brs, 2H), 1.91-1.93 (m, 10H), 2.26 (brs, 2H), 2.35 (s, 6H), 2.78 (t, *J* = 5.6 Hz, 2H), 4.08 (t, *J* = 5.6 Hz, 2H), 6.72 (t, *J* = 8.8 Hz, 1H), 6.88 (d, *J* = 8.8 Hz, 2H), 7.20 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 9.0 Hz, 2H); MS (EI) *m/z* 391 (M^+, 100%); HRMS Calcd for $C_{26}H_{33}NO_2$: 391.2511, Found: 391.2504.

*4.1.12. 9,10-Diiodo-m-carborane (**23**)*

To a solution of 1,7-dicarba-*closo*-dodecaborane **22** (3.0 g, 20.8 mmol) in 50 mL of CH_2Cl_2 was added iodine (2.64 g, 20.8 mmol) and aluminum trichloride (277 mg, 2.1 mmol) and the mixture was refluxed under an Ar atmosphere. After 1 h, a second portion of iodine (2.64 g, 20.8 mmol) was added and refluxing was continued for 27 h. After cooling, the resultant mixture was poured into water with ice. The organic phase was separated and the aqueous phase was extracted with AcOEt. The combined organic phase was washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was recrystallized from CH_2Cl_2 to afford 3.89 g (47%) of the title compound as a colorless solid; ^{1}H NMR (270 MHz, acetone-d_6) δ (ppm) 1.50-3.80 (brm, 8H), 4.08 (brs, 2H), MS (EI) *m/z* 396 (M^+, 100%).

*4.1.13. 9,10-Dimethyl-m-carborane (**24**)*

A solution of 3 M methyl magnesium bromide in diethyl ether (6.3 mL, 19.0 mmol) was added dropwise to 10 mL of a dry 1,2-dimethoxyethane solution of **23** (1.0 g, 2.53 mmol) cooled with ice under an Ar atmosphere, and the slurry was stirred at room temperature for 30 min. Bis(triphenylphosphine)palladium (II) dichloride (178 mg, 0.25 mmol) and cupper (I) iodide (48 mg, 0.25 mmol) were added, and the mixture was refluxed for 24 h. After removal of the solvent, diethyl ether was added to the residue and the excess Grignard reagent was destroyed by slow addition of 10 % HCl. The organic layer was separated from the mixture, and the aqueous layer was extracted with diethyl ether. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane to afford 427 mg (98%) as a white solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.29 (s, 6 H), 1.50-3.80 (brm, 8H), 2.79 (brs, 2H); MS (EI) *m/z* 172 (M^+), 157 (100%).

*4.1.14. 1,7-Bis(4-methoxyphenyl)-9,10-dimethyl-m-carborane (**25**)*

To a solution of **24** (394 mg, 2.29 mmol) in 3 mL of DME was added dropwise 1.56 M *n*-BuLi in hexane (3.2 mL, 5.0 mmol) under an Ar atmosphere at 0°C. The reaction mixture was

stirred for 30 min at room temperature, then CuCl (498 mg, 5.0 mmol) was added in one portion, and the mixture was stirred at room temperature for 1.5 h. Pyridine (2.8 mL, 34.3 mmol) and *p*-iodoanisole (1.18 g, 5.0 mmol) were added in one portion, and the resulting mixture was heated at 110°C for 34 h. After cooling, the mixture was washed with 10% HCl aqueous solution, 10% $Na_2S_2O_3$ aqueous solution, water, and brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (Et_2O:*n*-hexane = 1:15 to 1:10) to give 42 mg (5%) of the title compound as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.38 (s, 6H), 1.50-3.80 (brm, 8H), 3.77 (s, 6H), 6.75 (d, *J* = 9.1 Hz, 4H), 7.36 (d, J = 9.1 Hz, 4H); MS (EI) *m/z* 384 (M^+, 100%).

*4.1.15. 1,7-Bis(4-hydroxyphenyl)-9,10-dimethyl-m-carborane (**26**)*

To a solution of **25** (42 mg, 0.11 mmol) in 5 mL of CH_2Cl_2 was added 1 M BBr_3 in CH_2Cl_2 (0.24 mL, 240 μmol) at -78°C, and the mixture was stirred for 6 h at room temperature. The mixture was poured onto ice and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:*n*-hexane = 1:2) to give 36 mg (92%) of the title compound as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.40 (s, 6H), 1.50-3.80 (brm, 8H), 5.15 (brs, 2H), 6.69 (d, *J* = 8.9 Hz, 4H), 7.31 (d, *J* = 8.9 Hz, 4H); MS (EI) *m/z* 356 (M^+, 100%).

*4.1.16. 1-[4-(2-Dimethylaminoethoxy)phenyl]-7-(4-hydroxyphenyl)-9,10-dimethyl-m-carborane (**9**)*

To a solution of **26** (27 mg, 76 μmol) and 2-dimethylaminoethylchloride hydrochloride (14 mg, 99 μmol) in 0.5 mL of DMF and 0.5 mL of acetone was added K_2CO_3 (31 mg, 0.23 mmol), and the mixture was heated at 60°C for 5 h. Saturated $NaHCO_3$ aqueous solution was added, and the mixture was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (MeOH:$CHCl_3$ = 1:20 to 1:10) to give 4 mg (13%) of the title compound as a colorless needles; mp 155.0-156.0 °C (ether); ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.37 (s, 6H), 1.40-3.80 (brm, 8H), 2.39 (s, 6H), 2.80 (t, *J* = 5.4 Hz, 2H), 4.03 (t, *J* = 5.4 Hz, 2H), 6.56 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 8.9 Hz, 2H), 7.30 (d, *J* = 8.9 Hz, 2H); MS (EI) *m/z* 427 (M^+), 59 (100%); HRMS Calcd for $C_{20}H_{33}B_{10}NO_2$: 427.3514, Found: 427.3507.

4.2. Biological evaluations

4.2.1. ERα-binding assay

Ligand-binding activity to estrogen receptor α (ERα) was determined by means of the nitrocellulose filter binding assay method. ERα (0.5 μg/tube, Invitrogen) was diluted with 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 18 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, 0.15 M NaCl) and then with 25% ethanol in distilled water. Radioactivity that remained on the membrane was measured in Atomlight (Perkin Elmer) by using a liquid scintillation counter.

4.2.2. MCF-7 Cell proliferation assay

Human breast adenocarcinoma cell line MCF-7 was routinely cultivated in DMEM supplemented with 10% FBS and 100 IU/mL penicillin and 100 μg/mL streptomycin at 37°C in a 5% CO_2 humidified incubator. On the day before an assay, MCF-7 cells were switched to DMEM (low glucose, phenol red-free) supplemented with 5% dextran-coated charcoal-stripped FBS (sFBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin. Cells were trypsinized from the maintenance dish with phenol red-free 0.25% trypsin-EDTA and seeded in a 96-well plate at a density of 2000 cells per final volume of 100 μL DMEM (low glucose, phenol red-free) supplemented with 5% sFBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin. After 24 h, the medium was replaced with 90 μL of fresh DMEM (Low glucose, phenol red free) supplemented with 5% sFBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin, and 10 μL aliquots of serial dilutions of test compounds or DMSO (dilution control), were added to triplicate microcultures in the presence or absence of 1 x 10^{-10} M estradiol. The cells were incubated for 5 days, with one change of the medium after 3 days. Finally, the number of cells was counted by adding WST-8 (10 μL) to the microcultures followed by incubation for 2 h. The absorbance at 450 nm of each well was then determined as a measure of the number of living cells in the culture. EC_{50} and IC_{50} values were estimated with GraphPad Prism 4.

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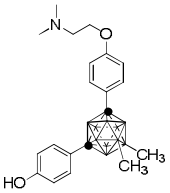

ER partial agonist candidate

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