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Synthesis and biological evaluation of *p*-carborane bisphenols and their derivatives: Structure–activity relationship for estrogenic activity

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ABSTRACT

p-Carborane bisphenols and their derivatives were prepared and evaluated for binding affinity to estrogen receptor α. Their estrogenic activity was evaluated by means of transcriptional assay and cell proliferation assay using MCF-7 cell lines. 1,12-Bis(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane **4a** showed potent estrogenic activity, approaching that of 17β-estradiol, in transactivation assay. The activity of isomers **5a** and **6a** was drastically affected by the change in the position of one of the hydroxyl groups; **6a** (*ortho*-OH in one ring) was about 1000 times less potent than **4a**. Modification of this hydroxyl group with alkyl groups decreased the estrogenic activity in all isomers. Compound **4a** also showed potent MCF-7 cell proliferation-enhancing activity.

1. Introduction

The carboranes (dicarba-*closo*-dodecaboranes) are chemical building blocks of remarkable thermal stability and high boron content; they are resistant to attack by most types of reagent, and are generally inactive toward biological systems.[1] One of their most striking features is the ability of the two carbon atoms and 10 boron atoms to adopt icosahedral geometry in which the carbon and boron atoms are hexa-coordinated. This feature of the structure gives rise to the unusual properties of such molecules and their carbon and boron derivatives. Their properties make them uniquely suitable for various specialized applications, including synthesis of polymers for high temperature use and neutron shielding.[2] In the fields of medicinal chemistry and pharmaceutical sciences, incorporation of large numbers of boron atoms into tumor calls for boron neutron capture therapy (BNCT) has become of interest in recent years.[3] Most carborane-containing compounds that have so far been synthesized are composed of cellular building blocks (nucleic acids,[4] amino acids,[5] sugars,[6] and so on), to which carborane units are added.

The steroid hormone estrogen influences the growth, differentiation, and functioning of many target tissues.[7] 17β-Estradiol (E2) is an endogenous estrogen that plays important roles in the female and male reproductive systems, as well as in bone maintenance, in the central nervous system, and in the cardiovascular system. (Fig. 1)[7] The first step in the appearance of estrogenic activity is mediated by the binding of agonist ligands to estrogen receptors (ER) α and β, resulting in a conformational change. The ligand-bound ER dimerizes, forms huge complexes with various cofactors, and binds to specific promoter elements of DNA to initiate gene transcription.[7] Compounds that either induce or inhibit cellular estrogen responses have potential value as biochemical tools and candidates for drug development. Since the discovery of the non-steroidal estrogens, many estrogen agonists and antagonists have been developed as agents for regulating fertility, preventing and controlling hormone-responsive breast cancer, and post-menopausal hormone replacement.[8]

Binding of ligands to the ER ligand binding domain (LBD) primarily requires a phenolic ring, which hydrogen-bonds with Glu353 and Arg394 amino acid residues of the hER LBD.[9] It is also well-known that the secondary alcohol group of E2 interacts with His524 of hER.[9] The hydrophobic group should closely match the hydrophobic surface of the ER, so as to increase the binding affinity. The hydrophobic structure also plays a role as a scaffold, fixing the spatial positions of the hydrogen-bonding functional groups. In our studies to develop new hydrophobic structures for use in drug design, we have focused on the exceptional hydrophobic character and spherical geometry of carboranes, and utilized them as a hydrophobic component of biologically active molecules.[10]

We have reported a potent estrogen agonist bearing a carborane, 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodeca-

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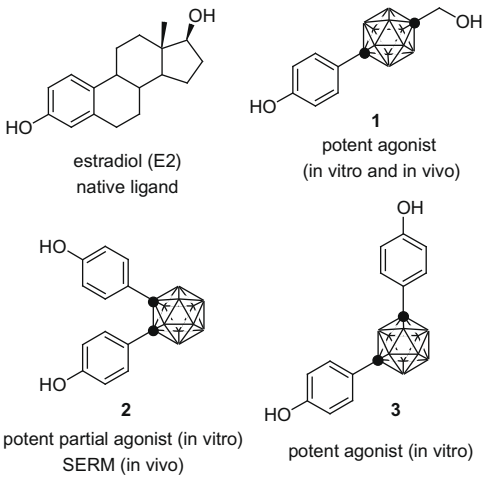


Figure 1. Structure and properties of estradiol (E2) and carborane derivatives as estrogen receptor modulators. Compounds **2** and **3** have carborane bisphenol structure.

borane (**1**),[11] which has an activity greater than that of E2 in ERα transcriptional assay and ERα binding assay. (Fig. 1) [12] The compound also showed potent in vivo effects on the recovery of uterine weight and bone loss in OVX mice.[12] These results suggested that hydrophobic interaction along the spherical carborane cage produces a stronger interaction with the receptor than that in the case of E2. We have utilized the strong binding of the spherical carborane cage to the cavity of ER LBD to design and synthesize various carborane bisphenol derivatives as candidate ER modulators. We found that bis(4-hydroxyphenyl)-*o*-carborane (**2**) exhibited antiestrogenic activity.[13] Furthermore, in an in vivo evaluation, compound **2** exhibited estrogenic action in bone, preventing bone loss without inducing estrogenic action in the uterus; it is therefore a candidate for application as a new type of selective estrogen receptor modulator (SERM) to treat osteoporosis.[13] We also found that bis(4-hydroxyphenyl)-*m*-carborane (**3**) has estrogenic activity somewhat weaker than that of E2 in transcriptional assay, and its binding affinity for ERα is similar to that of E2.[14] It is a remarkable result that *o*- and *m*-carborane derivatives exhibited different activities. In other words, the carborane cage appears to be a suitable hydrophobic pharmacophore for ER, with a great deal of potential for modulating the activity.

Therefore, we were interested in the activity of bisphenol derivatives bearing *p*-carborane, another isomer of carboranes. We designed and synthesized *p*-carborane bisphenol derivatives **4–6** Fig. 2. Compounds **7a** and **7b** were also synthesized in order to understand the effects of hydroxyl or alkoxy substituents on the activity and the relationship between the electronic properties of benzene ring and the activity. In this article, we describe the biological activities and the structure–activity relationships of these designed molecules **4–7**.

2. Results and discussion

2.1. Chemistry

The designed molecules **4a**–**4d** and **5a**–**5d** were synthesized from *p*-carborane as summarized in Scheme 1. *p*-Carborane was treated with *n*-BuLi and Cu(I)Cl, and reacted with 4-iodo-1-(*tert*-butyldimethylsiloxy)benzene,[15] which was prepared from 4-iodophenol, under Ullmann coupling conditions, to afford mono-substituted compound **8** and di-substituted compound **9** in 31% and 36% yield, respectively.[16] Compound **4a**[17] was synthesized by deprotection of **9** with tetrabutylammonium fluoride (TBAF) in quantitative yield, and then treated with alkyl iodide to afford compound **4b**–**4d**. Compound **8** was easily transformed into diaryl *p*-carborane **10** by Ullmann coupling with 3-iodoanisole in 74% yield.[16] Compound **10** was treated with BBr_3 to afford demethylated compound **11** as a major product in 42% yield, bisphenol derivative **5a** in 30% yield, and desilylated compound **5b** as a by-product in 16% yield, respectively. The phenol group of intermediate **11** was reacted with alkyl iodide, followed by desilylation with 10% HCl aqueous solution to afford compounds **5c** and **5d**. The other designed molecules **6a**–**6d** and **7** were synthesized in line with Scheme 2. Compound **13** was synthesized by a stepwise Ullmann coupling via intermediate **12**[11] from *p*-carborane.[16] Demethylation of compound **13** with BBr_3 afforded bisphenol derivative **6a** in 78% yield and methoxy derivative **6b** in 5% yield, respectively, because there was a small steric effect with the *p*-carborane cage. The steric effect made it easy to selectively protect one of the two phenol groups of compound **6a** with *tert*-butyldimethylsilyl (TBS) chloride to afford compound **14** in 44% yield, and this was easily transformed into compounds **6c** and **6d** by usual alkylation and the subsequent desilylation. Compound **8** was reacted with iodobenzene under Ullmann coupling conditions, followed by desilylation with TBAF to afford compound **7a**. Compound **7b** was prepared by means of aromatic nucleophilic substitution reaction of compound **12** with hexafluorobenzene to afford **15**,[18] followed by demethylation with BBr_3.

2.2. Competitive binding assay with ERα

A competitive binding assay using [6,7-^{3}H]17β-estradiol (K_d = 0.4 nM) and human recombinant ERα was employed for initial screening of the synthesized compounds.[12] Table 1 summarizes the binding affinity data for tested compounds as relative values to that of estradiol. The bisphenol derivatives, **4a**, **5a**, and **6a** showed strong binding affinity for ERα (stronger than E_2), and compound **7a** also strongly bound to the ERα LBD. In the

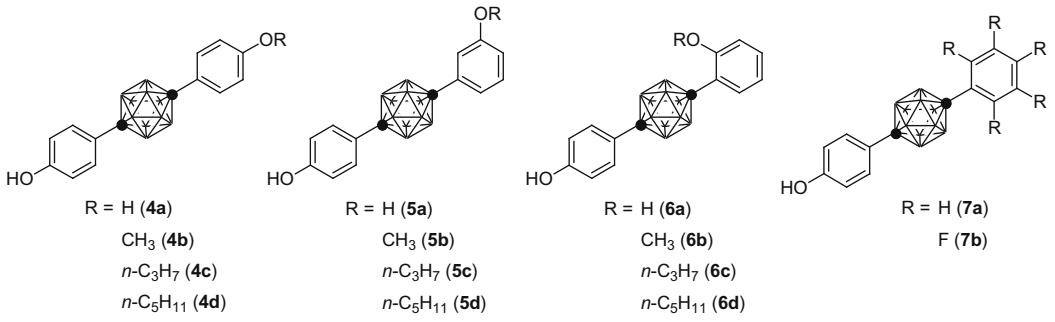
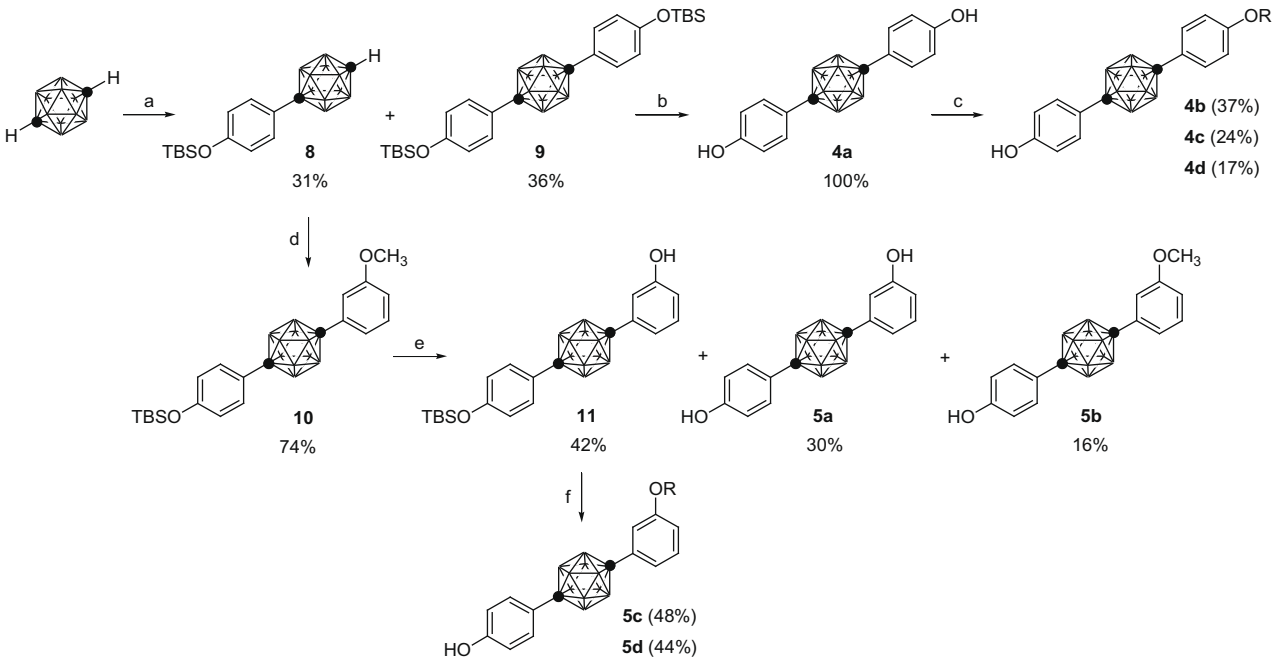
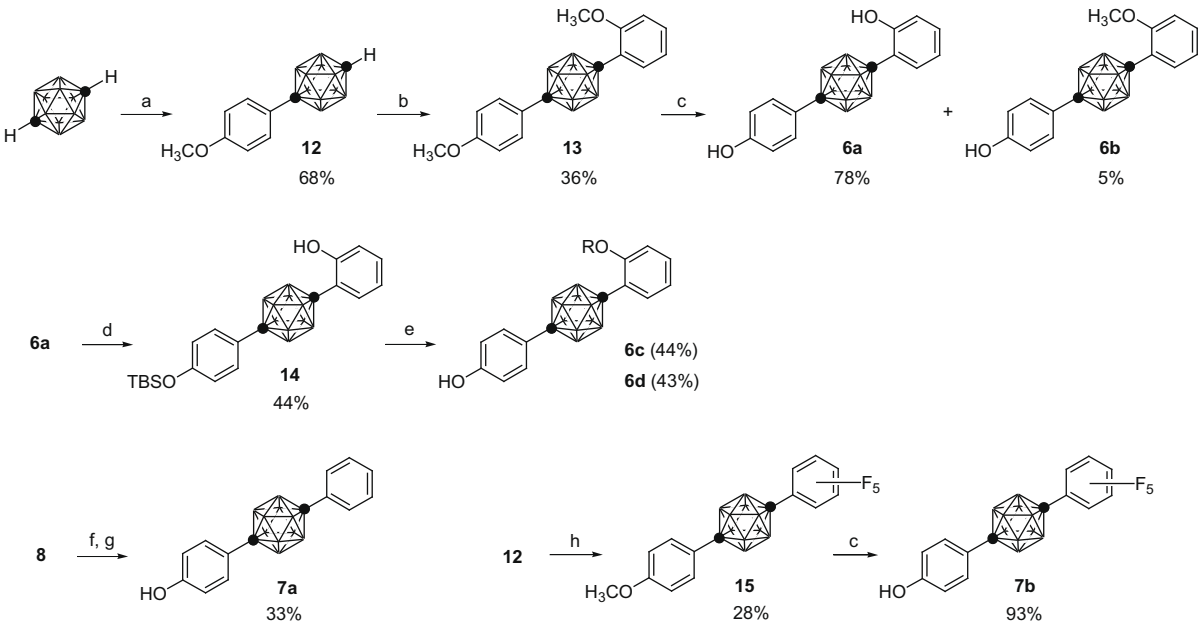


Figure 2. Molecules designed as candidate estrogen receptor ligands based upon *p*-carborane bisphenol structure.



Scheme 1. Synthesis of compounds **4a**–**4d** and **5a**–**5d**. Reagents: (a) *n*-BuLi, DME then Cu(I)Cl, 4-iodo-1-(*tert*-butyldimethylsiloxy)benzene, pyridine; (b) TBAF, THF; (c) alkyl iodide, K_2CO_3, DMF; (d) *n*-BuLi, DME then Cu(I)Cl, 3-iodoanisole, pyridine; (e) BBr_3, CH_2Cl_2; (f) alkyl iodide, K_2CO_3, DMF, then 10% HCl.



Scheme 2. Synthesis of compounds **6a**–**6d**, **7a**, and **7b**. Reagents: (a) *n*-BuLi, DME then CuCl(I), 4-iodoanisole, pyridine; (b) *n*-BuLi, DME then CuCl(I), 2-iodoanisole, pyridine; (c) BBr_3, CH_2Cl_2; (d) TBSCl, triethylamine, CH_2Cl_2; (e) alkyl iodide, K_2CO_3, DMF, then 10% HCl; (f) *n*-BuLi, DME then CuCl(I), iodobenzene, pyridine; (g) TBAF, THF; (h) *n*-BuLi, ether, then C_6F_6.

derivatives with an alkoxy group, the binding affinity to ERα decreased as the carbon chain of the alkoxy group was lengthened, except for *p*-substituted derivatives. The binding affinity of the compounds with a *n*-pentyl group was markedly decreased (**4d**, **5d**, and **6d**). The binding of the derivatives to the ERα ligand binding pocket is impaired by the steric effect of the alkyl side chains. However, it is not known exactly why compounds **4b** and **4c** exhibited higher binding affinity for ERα than the parent compound **4a**. Introduction of the pentafluorophenyl group led to a decrease of the binding affinity for ERα. The electrostatic character of the pentafluorophenyl group apparently impairs the binding of **7b** to the ERα ligand binding pocket.

2.3. Transcriptional activation assay of synthesized compounds

To evaluate the activity of the synthesized compounds as ER agonists and antagonists, transcriptional assay was done with ERE/Luci (firefly luciferase) and phRL/CMV (*Renilla* luciferase) plasmids, which were transiently transfected into MCF-7 cells.[19] Figure 3 summarizes the results of the transcriptional activation

Table 1
Binding affinity of test compounds to human ERα[a]

Compound		R	Binding affinity[b]
4a	*p*-	OH	149
4b		OCH_3	212
4c		O-*n*-C_3H_7	165
4d		O-*n*-C_5H_{11}	38
5a	*m*-	OH	144
5b		OCH_3	95
5c		O-*n*-C_3H_7	73
5d		O-*n*-C_5H_{11}	45
6a	*o*-	OH	126
6b		OCH_3	125
6c		O-*n*-C_3H_7	112
6d		O-*n*-C_5H_{11}	61
7a		H	130
7b		F(5)	61

[a] All binding assays were performed in duplicate ($n = 2$).
[b] Values are average of percentage displacement of [^{3}H]estradiol (4 nM) specific binding to human recombinant ERα by each compound at 4 nM. The binding affinity of estradiol is taken as 100.

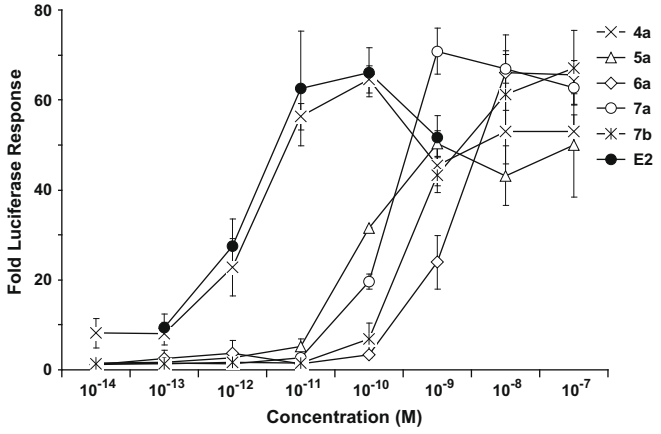


Figure 3. Transcriptional activation by the bisphenol derivatives **4a**, **5a**, and **6a**, and **7a** and **7b**. MCF-7 cells were transfected with ERE (SV-40)-LUC and phRL/CMV, and incubated with test compounds (1×10^{-7}–1×10^{-14}). EtOH was used as a standard (control). Results are shown as means ± SD for triplicate transfections.

assay of bisphenol compounds **3a, 4a**, and **5a**, and compounds **6** and **7**, which have no anti-estrogenic activity (data not shown). 17β-Estradiol and the test compounds induced the expression of luciferase in dose-dependent manner at 1×10^{-7}–1×10^{-14} M. *p*-Carborane bisphenol **4a** showed by far the most potent estrogenic activity, which was similar to that of E2. The activity of other bisphenol isomers **5a** and **6a** was strongly dependent on the position of the hydroxyl group. Interestingly, the activity of **6a** was about 1000 times weaker than that of **4a**. The order of estrogenic activity was **4a** > **5a** > **6a**, which is the same as that of the binding affinity for ERα. The estrogenic activity of non-substituted compound **7a** is higher than that of **6a** and similar to that of **5a**. The activity of the pentafluorophenyl derivative, **7b**, is weaker than that of **7a**. It seems that the electrostatic character of the pentafluoro group or the electron-deficient benzene ring of **7b** negatively affects estrogenic activity, in accordance with the binding assay.

In view of the very high estrogenic activity of **4a** compared to that of **5a** and **6a**, both hydroxyl groups of **4a** are likely to interact strongly with the primary amino acid residues of the ERα ligand binding pocket, as the phenolic hydroxyl group and the secondary alcoholic hydrogen of E2 interact with Glu353 and Arg394, and His524.

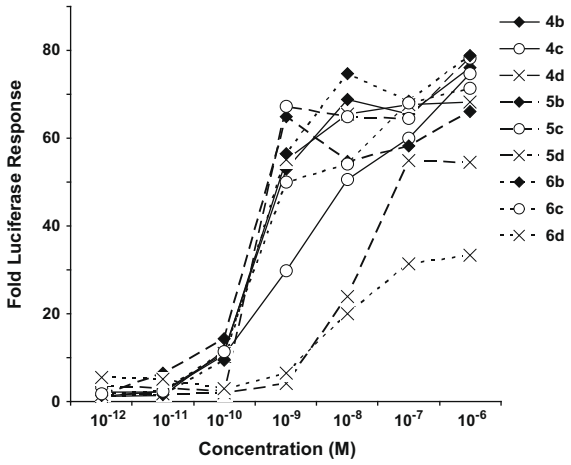


Figure 4. Transcriptional assay with the alkoxy derivatives, **4b**–**4d**, **5b**–**5d**, and **6b**–**6d**. All experiments were performed using the same method as described in Figure 3. Solid lines (**4b**–**4d**) indicate *para*-substituted compounds. Wide dashed lines (**5b**–**5d**) and fine dashed lines (**6b**–**6d**) indicate *meta*- and *ortho*-substituted compounds, respectively.

The estrogenic activity of alkylated compounds is summarized in Figure 4. None of the alkylated compounds exhibited anti-estrogenic activity (data not shown). Introduction of an alkyl group onto the hydroxyl group led to a decrease of estrogenic activity. However, *para*-substituted compounds retained moderate estrogenic activity regardless of the length of the alkyl chain (Fig. 4, **4b**–**4d**). Among *meta*- and *ortho*-substituted compounds, methyl and *n*-propyl groups were compatible with estrogenic activity, but the *n*-pentyl group abrogated both estrogenic activity and binding to ERα LBD (Fig. 4, **5b**–**5d** and **6b**-**6d**). The activities of compounds **5d** and **6d**, which have an *n*-pentyl group at the *meta* and *ortho* position, respectively, were markedly decreased compared with those of the methyl derivatives **5b** and **6b**, respectively. Sterically bulky substituents around the phenol group adversely affect the estrogenic activity, and these results are in good accordance with the effects on binding to ERα.

2.4. Cell proliferation assay using MCF-7 cell line

The estrogenic activities of bisphenol compounds **4a, 5a**, and **6a** and monophenol **7a** were evaluated by cell proliferation assay with human breast cancer cell lines, MCF-7 (Table 2).[20] The order of cell proliferation-inducing activity, **4a** > **5a** > **6a**, was consistent with those of binding and transactivation assays. In addition, the activities of the potent estrogen agonist **1** and *o*- and *m*-carborane-based bisphenol derivatives **2** and **3**, which were developed in our previous studies, were evaluated and compared with that of **4a**. The cell proliferation-inducing activity of **4a** was less than

Table 2
EC_{50} values of bisphenol and monophenol derivatives in cell proliferation assay using MCF-7 cells[a]

Compound	EC_{50}[b] (M)
4a	$2.57 \pm 1.61 \times 10^{-11}$
5a	$1.95 \pm 0.59 \times 10^{-10}$
6a	$2.71 \pm 0.95 \times 10^{-10}$
7a	$1.09 \pm 0.40 \times 10^{-10}$
E2	$5.42 \pm 1.50 \times 10^{-13}$
1	$3.04 \pm 1.72 \times 10^{-12}$
2	$9.44 \pm 3.98 \times 10^{-10}$
3	$3.78 \pm 1.41 \times 10^{-9}$

[a] MCF-7 cells were incubated with the test compounds for 5 days. Cell proliferation assays were performed in triplicate ($n = 3$).
[b] EC_{50} values of the test compounds were estimated from the sigmoidal dose–response curves using GraphPad Prism 4 software.

that of **E2** and **1**, but greater than that of bisphenol derivatives **2** and **3**.

The activities of other bisphenol isomers **5a** and **6a** were drastically affected by the position of the hydroxyl group, and the estrogenic activity of **6a** was about 1000 times weaker than that of **4a**. One phenolic OH group, which is common to all of designed derivatives, most probably interacts with Glu353 and Arg394 of hER LBD, with which E2 and **1** form hydrogen bonds. The other phenolic OH group seems to modulate the potency of the estrogenic activity. Compound **4a** showed potent binding affinity, more potent estrogenic activity than any of the other designed compounds in transactivation assay, and the most potent MCF-7 cell proliferation-inducing activity. The potency was markedly decreased by the introduction of an alkyl group onto the phenolic hydroxyl group. Since the estrogenic activity of *o*-phenol derivative **6a** was less than that of *m*-phenol derivative **5a** or the unsubstituted benzene derivative **7a** in both cell assays, the OH group of **6a** may disturb the interaction with the hER LBD. The position of the OH group thus appears to be very important for modulating the potency of estrogenic activity. The OH group of **4a** presumably has a significant interaction with the hER LBD. The His524 amino acid residue of hER interacts with the secondary alcohol of E2 and the primary alcohol of **1**, and probably compound **4a** would bind to the hER LBD with the same binding mode as compound **1**.[12] However, there is a possibility that the phenolic OH of **4a** interacts with some other amino acid residue located further in the binding pocket because the distance between two phenolic OH groups of **4a** is greater than that between the phenolic OH group and the alcoholic groups of E2 or **1**. If this is so, it will be an important consideration in the design of novel ER modulators.

3. Conclusion

In conclusion, we designed and synthesized a series of *p*-carborane bisphenol derivatives utilizing *p*-carborane as a hydrophobic pharmacophore. ERα binding ability was assessed by means of receptor binding assay and estrogenic activity by means of transactivation and cell proliferation assays. *p*-Carborane bisphenol **4a** showed high binding ability to ERα and potent estrogenic activity. The activities of other bisphenol isomers **5a** and **6a** were drastically influenced by the position of the second hydroxyl group: the estrogenic activity of **6a** in transactivation assay was about 1000 times weaker than that of **4a**. Conversion of the hydroxyl group to alkoxy decreased the estrogenic activity. However, the *para*-substituted compounds retained moderate estrogenic activity regardless of the length of the alkyl chain. Both hydroxyl groups of **4a** appear to interact strongly with amino acid residues of the ERα ligand binding pocket. The cell proliferation-inducing activity of **4a** was less than that of **E2** and **1**, but greater than that of bisphenol derivatives **2** and **3**.

4. Experimental

4.1. General consideration

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, JNM-LA-400 and JNM-LA-600 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 designated as follows: s (singlet), d (doublet), t (triplet) 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 Merck Silica Gel 60 (0.063–0.200 μm) 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 commercial products of reagent grade, and were used without further purification.

4.2. Synthesis

4.2.1. 4-Iodo-1-(*tert*-butyldimethylsiloxy)benzene[14]

A mixture of 4-iodophenol (10.0 g, 45.5 mmol), *tert*-butyldimethylsilyl chloride (TBSCl) (8.22 g, 54.5 mmol) and triethylamine (7.6 mL, 54.5 mmol) in 20 mL of CH_2Cl_2 was stirred for 1.5 h at room temperature. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane to give the title compound (15.4 g, 100%) as a colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.18 (6H, s), 0.97 (9H, s), 6.61 (2H, dd, J = 2.1, 6.8 Hz), 7.50 (2H, dd, J = 2.1, 6.6 Hz); MS (EI) *m/z* 334 (M^+), 227 (100%); HRMS calcd for $C_{12}H_{19}IOSi$: 334.0250, found: 334.0231.

4.2.2. 1-{4-(*tert*-Butyldimethylsiloxy)phenyl}-1,12-dicarba-*closo*-dodecaborane (8) and 1,12-bis{4-(*tert*-butyldimethylsiloxy) phenyl}-1,12-dicarba-*closo*-dodecaborane (9)

To a solution of *p*-carborane (4.0 g, 27.7 mmol) in 15 mL of DME was added dropwise 1.54 M *n*-BuLi in hexane (37.8 mL, 58.2 mmol) at 0 °C under an Ar atmosphere, and the mixture was stirred for 30 min at the same temperature. CuCl (5.77 g, 58.2 mmol) was added in one portion and the mixture was stirred at room temperature for 1 h. Pyridine (33.6 mL, 416 mmol) and 4-iodo-1-(*tert*-butyldimethylsiloxy)benzene (13.9 g, 41.6 mmol) were added in one portion, and the mixture was heated at 110 °C for 21 h. After cooling to room temperature, the mixture was diluted with AcOEt and insoluble materials were removed by filtration through Celite. The filtrate was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane to give **8** (3.05 g, 31%) as the first eluted compound and **9** (5.56 g, 36%) as the second eluted compound; Compound **8**: ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.15 (6H, s), 0.94 (9H, s), 1.60–3.60 (10H, br m), 2.73 (1H, br m), 6.60 (2H, d, J = 8.9 Hz), 7.04 (2H, d, J = 8.9 Hz); MS (EI) *m/z* 350 (M^+), 293 (100%); Compound **9**: ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.16 (12H, s), 0.95 (18H, s), 1.60–3.60 (10H, br m), 6.61 (4H, d, J = 8.7 Hz), 7.07 (4H, d, J = 8.9 Hz); MS (EI) *m/z* 556 (M^+), 500 (100%).

4.2.3. 1,12-Bis(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (4a)

To a solution of **9** (4.85 g, 8.7 mmol) in 20 mL of THF was added 1 M tetrabutylammonium fluoride (TBAF) in THF (18.3 mL, 18.3 mmol) at room temperature, and the mixture was stirred for 30 min at the same temperature. The mixture was poured into water and extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:5 AcOEt/*n*-hexane to give **4a** (2.85 g, 100%) as a colorless solid; Colorless needles (AcOEt/*n*-hexane); mp 292–294 °C; ^{1}H NMR (270 MHz, DMSO-d_6) δ (ppm) 1.60–3.60 (10H, br m), 6.60 (4H, d, J = 8.7 Hz), 7.00 (4H, d, J = 8.9 Hz), 9.71 (2H, br s); ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 83.7, 115.7, 128.6, 129.3, 159.0; MS (EI) *m/z* 328 (M^+,

100%); Anal. Calcd for $C_{14}H_{20}B_{10}O_2$: C, 51.20; H, 6.14. Found: C, 51.23; H, 6.12.

4.2.4. 1-(4-Hydroxyphenyl)-12-(4-methoxyphenyl)-1, 12-dicarba-*closo*-dodecaborane (4b)

To a mixture of **4a** (200 mg, 0.61 mmol) and K_2CO_3 (110 mg, 0.79 mmol) in 3 mL of DMF was added iodomethane (0.049 mL, 0.73 mmol), and the mixture was stirred for 2.5 h at room temperature. The mixture was poured into water and extracted with Et_2O. The organic layer was washed with 10% $Na_2S_2O_3$ aqueous solution, water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:10 AcOEt/*n*-hexane to give **4b** (77 mg, 37%) as a colorless solid; Colorless cubes (AcOEt/*n*-hexane); mp 197–198.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.60–3.60 (10H, br m), 3.75 (3H, s), 4.87 (1H, br s), 6.63 (2H, d, J = 8.7 Hz), 6.70 (2H, d, J = 9.1 Hz), 7.10 (2H, d, J = 8.9 Hz), 7.15 (2H, d, J = 9.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 55.3, 82.0, 82.1, 113.3, 114.8, 128.3, 128.6, 128.8, 129.0, 155.7, 159.6; MS (EI) *m/z* 342 (M^+, 100%); Anal. Calcd for $C_{15}H_{22}B_{10}O_2$: C, 52.61; H, 6.48. Found: C, 52.53; H, 6.39.

4.2.5. 1-(4-Hydroxyphenyl)-12-{4-*n*-propoxyphenyl)-1, 12-dicarba-*closo*-dodecaborane (4c)

Compound **4c** was prepared by the same method as described for the synthesis of **4b**, but with *n*-propyl iodide in place of iodomethane; 24% yield; colorless cubes (AcOEt/*n*-hexane); mp 184.5–186 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.99 (3H, t, J = 7.4 Hz), 1.60–3.60 (10H, br m), 1.76 (2H, sextet, J = 7.4 Hz), 3.85 (2H, t, J = 6.6 Hz), 5.52 (1H, br s), 6.63 (2H, d, J = 8.9 Hz), 6.68 (2H, d, J = 9.1 Hz), 7.10 (2H, d, J = 9.1 Hz), 7.13 (2H, d, J = 9.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 10.5, 22.5, 69.5, 80.2, 82.0, 113.8, 114.8, 128.2, 128.5, 128.7, 129.1, 155.6, 159.2; MS (EI) *m/z* 370 (M^+), 328 (100%); Anal. Calcd for $C_{17}H_{26}B_{10}O_2$: C, 55.11; H, 7.07. Found: C, 55.19; H, 7.09.

4.2.6. 1-(4-Hydroxyphenyl)-12-(4-*n*-pentoxyphenyl)-1, 12-dicarba-*closo*-dodecaborane (4d)

Compound **4d** was prepared by the same method as described for the synthesis of **4b**, but with *n*-pentyl iodide in place of iodomethane; 17% yield; colorless leaflets (AcOEt/*n*-hexane); mp 137–139.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.99 (3H, t, J = 6.9 Hz), 1.30–1.45 (4H, m), 1.60–3.60 (10H, br m), 1.74 (2H, quintet, J = 6.6 Hz), 3.88 (2H, t, J = 6.4 Hz), 5.37 (1H, br s), 6.62 (2H, d, J = 8.6 Hz), 6.68 (2H, d, J = 8.9 Hz), 7.10 (2H, d, J = 8.6 Hz), 7.13 (2H, d, J = 8.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.0, 22.4, 28.1, 28.8, 68.0, 81.9, 82.2, 113.8, 114.8, 128.2, 128.5, 128.6, 129.0, 155.6, 159.1; MS (EI) *m/z* 398 (M^+), 328 (100%); Anal. Calcd for $C_{19}H_{30}B_{10}O_2$: C, 57.26; H, 7.59. Found: C, 57.26; H, 7.69.

4.2.7. 1-{4-(*tert*-Butyldimethylsiloxy)phenyl}-12-(3-methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (10)

To a solution of **8** (1.0 g, 2.85 mmol) in 5 mL of DME was added dropwise 1.54 M *n*-BuLi in hexane (2.2 mL, 3.42 mmol) at 0 °C under an Ar atmosphere, and the mixture was stirred for 30 min at the same temperature. CuCl (311 mg, 3.14 mmol) was added in one portion and the mixture was stirred at room temperature for 1 h. Pyridine (3.5 mL, 42.7 mmol) and 3-iodoanisole (0.38 mL, 2.85 mmol) were added in one portion, and the mixture was heated at 110 °C for 24 h. After cooling to room temperature, the mixture was diluted with AcOEt and insoluble materials were removed by filtration through Celite. The filtrate was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane to 1:20 AcOEt/*n*-hexane to give **10** (960 mg, 74%) as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.16 (6H, s), 0.95 (9H, s), 1.60–3.60 (10H, br m), 3.76 (3H, s), 6.62 (2H, d, J = 8.9 Hz), 6.75 (1H, dd, J = 1.1, 2.3 Hz), 6.76–6.82 (1H, m), 6.84 (1H, dd, J = 1.1, 1.9 Hz), 7.07 (2H, d, J = 8.9 Hz), 7.09 (1H, dd, J = 7.7 Hz, 8.6 Hz); MS (EI) *m/z* 457 (M^+), 400 (100%).

4.2.8. 1-{4-(*tert*-Butyldimethylsiloxy)phenyl}-12-(3-methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (11), 1-(3-hydroxyphenyl)-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (5a) and 1-(4-hydroxyphenyl)-12-(3-methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (5b)

To a solution of **10** (900 mg, 1.97 mmol) in 5 mL of CH_2Cl_2 was added dropwise 1 M BBr_3 in CH_2Cl_2 (2.52 mL, 2.52 mmol) at 0 °C under an Ar atmosphere, and the mixture was stirred for 1 h at room temperature. The mixture was poured onto ice and extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:5 AcOEt/*n*-hexane to give **11** (365 mg, 42%) as the first eluted compound, **5b** (104 mg, 16%) as the second eluted compound and **5a** (197 mg, 42%) as the third eluted compound; Compound **11**: ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.16 (6H, s), 0.94 (9H, s), 1.60–3.60 (10H, br m), 5.16 (1H, br s), 6.62 (2H, d, J = 8.7 Hz), 6.68 (1H, dd, J = 0.8, 2.3 Hz), 6.71 (1H, ddd, J = 0.8, 2.3, 8.4 Hz), 6.80 (1H, ddd, J = 0.8, 1.8, 7.3 Hz), 7.04 (1H, dd, J = 7.9, 8.1 Hz, 1H), 7.07 (2H, d, J = 8.9 Hz); MS (EI) *m/z* 442 (M^+), 386 (100%); Compound **5b**: colorless flakes (AcOEt/*n*-hexane); mp 161–163 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.60–3.60 (10H, br m), 3.76 (3H, s), 5.73 (1H, s), 6.64 (2H, d, J = 8.9 Hz), 6.74–6.85 (3H, m), 7.09 (1H, t, J = 7.9 Hz), 7.10 (2H, d, J = 8.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 55.23, 82.0, 82.7, 113.4, 113.6, 114.8, 119.6, 128.5, 128.8, 129.0, 137.7, 155.8, 159.0; MS (EI) *m/z* 342 (M^+, 100%); Anal. Calcd for $C_{15}H_{22}B_{10}O_2$: C, 52.61; H, 6.48. Found: C, 52.27; H, 6.15; Compound **5a**: colorless cubes (AcOEt/*n*-hexane); mp 262–263.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.60–3.60 (10H, br m), 4.77 (1H, s), 4.81 (1H, s), 6.63 (2H, d, J = 8.9 Hz), 6.68 (1H, dd, J = 0.7 Hz, 2.3 Hz), 6.70–6.75 (1H, m), 6.81 (1H, ddd, J = 0.8, 1.8, 8.6 Hz), 7.05 (1H, dd, J = 7.4, 7.7 Hz), 7.10 (2H, d, J = 8.9 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 83.3, 84.5, 115.4, 115.8, 116.4, 119.3, 128.5, 129.3, 130.2, 138.7, 158.3, 159.1; MS (EI) *m/z* 328 (M^+, 100%); Anal. Calcd for $C_{14}H_{20}B_{10}O_2$: C, 51.20; H, 6.14. Found: C, 50.95; H, 5.95.

4.2.9. 1-(4-Hydroxyphenyl)-12-{3-*n*-propoxyphenyl)-1, 12-dicarba-*closo*-dodecaborane (5c)

To a mixture of **11** (15 mg, 0.034 mmol) and K_2CO_3 (5.2 mg, 0.037 mmol) in 0.5 mL of DMF was added *n*-propyl iodide (6 mg, 0.037 mmol), and the mixture was stirred for 1.5 h at room temperature. Then, 1 mL of 10% HCl aqueous solution was added, and stirring was continued for 30 min at the same temperature. The mixture was extracted with Et_2O, and the organic layer was washed with 10% $Na_2S_2O_3$ aqueous solution, water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:7 Et_2O/*n*-hexane to give **5c** (6 mg, 48%) as a colorless solid; colorless cubes (AcOEt/*n*-hexane); mp 145.5–147 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.02 (3H, t, J = 7.4 Hz), 1.60–3.60 (10H, br m), 1.78 (2H, quintet, J = 7.4 Hz), 3.86 (2H, t, J = 6.6 Hz), 5.52 (1H, br s), 6.64 (2H, d, J = 8.9 Hz), 6.68–6.83 (3H, m), 7.04–7.15 (1H, m), 7.10 (2H, d, J = 8.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 10.5, 22.4, 69.5, 81.6, 82.8, 113.8, 114.6, 115.3, 119.7, 128.2, 128.5, 129.2, 138.0, 155.1, 159.1; MS (EI) *m/z* 370 (M^+), 328 (100%); HRMS calcd for $C_{17}H_{26}B_{10}O_2$: 370.2936, found: 370.2946.

4.2.10. 1-(4-Hydroxyphenyl)-12-(3-*n*-pentoxyphenyl)-1, 12-dicarba-*closo*-dodecaborane (5d)

Compound **5d** was prepared by the same method as described for the synthesis of **5c**, but with *n*-pentyl iodide in place of *n*-propyl iodide; 44% yield; colorless cubes (AcOEt/*n*-hexane); mp 116–

117 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.86 (3H, t, J = 7.1 Hz), 1.60–1.71 (6H, m), 1.60–3.60 (10H, br m), 3.82 (2H, t, J = 6.6 Hz), 4.78 (1H, br s), 6.55 (2H, d, J = 8.6 Hz), 6.55–6.80 (3H, m), 6.90–7.10 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.0, 22.4, 28.1, 28.8, 68.0, 81.6, 82.9, 113.8, 114.6, 115.3, 119.8, 128.2, 128.5, 129.3, 138.0, 155.0, 159.2; MS (EI) m/z 398 (M^+), 328 (100%); HRMS calcd for $C_{19}H_{30}B_{10}O_2$: 398.3249, found: 398.3251.

4.2.11. 1-(4-Methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (12)

To a solution of *p*-carborane (2.0 g, 13.7 mmol) in 10 mL of DME was added dropwise 1.54 M *n*-BuLi in hexane (19.0 mL, 29.1 mmol) at 0 °C under an Ar atmosphere, and the mixture was stirred for 30 min at the same temperature. CuCl (311 mg, 3.14 mmol) was added in one portion and the mixture was stirred at room temperature for 1.5 h. Pyridine (17 mL, 208 mmol) and 4-iodoanisole (3.89 g, 16.6 mmol) were added in one portion, and the mixture was heated at 110 °C for 18 h. After cooling to room temperature, the mixture was diluted with AcOEt and insoluble materials were removed by filtration through Celite. The filtrate was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:50 to 1:20 AcOEt/*n*-hexane to give **12** (2.37 g, 68%) as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.60–3.70 (10H, br m), 2.74 (1H, br s), 3.74 (3H, s), 6.68 (2H, d, J = 9.1 Hz), 7.12 (2H, dd, J = 9.1 Hz); MS (EI) m/z 250 (M^+, 100%).

4.2.12. 1-(2-Methoxyphenyl)-12-(4-methoxyphenyl)-1, 12-dicarba-*closo*-dodecaborane (13)

To a solution of **12** (1.5 g, 6.0 mmol) in 5 mL of DME was added dropwise 1.54 M *n*-BuLi in hexane (4.2 mL, 6.6 mmol) at 0 °C under an Ar atmosphere, and the mixture was stirred for 30 min at the same temperature. CuCl (650 mg, 6.6 mmol) was added in one portion and the mixture was stirred at room temperature for 1.5 h. Pyridine (7.3 mL, 90 mmol) and 2-iodoanisole (0.86 mL, 6.6 mmol) were added in one portion, and the mixture was heated at 110 °C for 42 h. After cooling to room temperature, the mixture was diluted with AcOEt and insoluble materials were removed by filtration through Celite. The filtrate was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:20 AcOEt/*n*-hexane to give **13** (771 mg, 36%) as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.60–3.70 (10H, br m), 3.74 (3H, s), 3.75 (3H, s), 6.70 (2H, d, J = 8.9 Hz), 6.75–6.83 (2H, m), 7.16 (2H, d, J = 8.9 Hz), 7.15–7.25 (1H, m), 7.37 (1H, dd, J = 1.5, 7.7 Hz); MS (EI) m/z 356 (M^+, 100%).

4.2.13. 1-(2-Hydroxyphenyl)-12-(4-hydroxyphenyl)-1, 12-dicarba-*closo*-dodecaborane (6a) and 1-(4-hydroxyphenyl)-12-(2-methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (6b)

To a solution of **13** (771 mg, 2.17 mmol) in 5 mL of CH_2Cl_2 was added 1 M BBr_3 in CH_2Cl_2 (3.68 mL, 3.68 mmol) at −78 °C under an Ar atmosphere, and the mixture was stirred for 2 h at room temperature. The mixture was poured onto ice and extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:5 AcOEt/*n*-hexane to give **6b** (40 mg, 5%) as the first eluted compound and **6a** (556 mg, 78%) as the second eluted compound; Compound **6b**: colorless cubes (AcOEt/*n*-hexane); mp 170.5–172 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.60–3.60 (10H, br m), 3.75 (3H, s), 4.76 (1H, br s), 6.63 (2H, d, J = 8.9 Hz), 6.75–6.83 (2H, m), 7.12 (1H, d, J = 8.7 Hz), 7.19 (2H, ddd, J = 1.6, 7.4, 7.8 Hz), 7.37 (1H, dd, J = 1.4, 7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 55.2, 79.8, 84.6, 112.6, 114.7, 120.3, 123.6, 128.5, 129.4, 129.9, 131.1, 155.5, 156.7; MS (EI) m/z 342 (M^+, 100%); Anal. Calcd for $C_{15}H_{22}B_{10}O_2$: C, 52.61; H, 6.48. Found: C, 52.69; H, 6.25; Compound **6a**: colorless plates (AcOEt/*n*-hexane); mp 201–202.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.60–3.60 (10H, br m), 4.85 (1H, s), 5.56 (1H, s), 6.64 (2H, d, J = 8.9 Hz), 6.70 (1H, dd, J = 1.3, 8.1 Hz), 6.78 (1H, ddd, J = 1.3, 7.6, 8.1 Hz), 7.11 (2H, d, J = 8.7 Hz), 7.11 (1H, ddd, J = 1.3, 7.5, 7.7 Hz), 7.31 (1H, dd, J = 1.6, 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 79.7, 84.9, 114.8, 118.1, 120.5, 120.7, 128.5, 128.9, 130.1, 130.9, 152.8, 155.7; MS (EI) m/z 328 (M^+, 100%); Anal. Calcd for $C_{14}H_{20}B_{10}O_2$: C, 51.20; H, 6.14. Found: C, 51.30; H, 5.90.

4.2.14. 1-{4-(*tert*-Butyldimethylsiloxy)phenyl}-12-(2-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (14)

A mixture of **6a** (400 mg, 1.22 mmol), TBSCl (202 mg, 1.34 mmol) and triethylamine (0.19 mL, 1.34 mmol) in 5 mL of CH_2Cl_2 was stirred for 3 h at room temperature. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:10 AcOEt/*n*-hexane to give **14** (238 mg, 44%) as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 0.17 (6H, s), 0.95 (9H, s), 1.60–3.60 (10H, br m), 5.57 (1H, s), 6.62 (2H, d, J = 8.9 Hz), 6.70 (1H, dd, J = 1.3, 8.1 Hz), 6.77 (1H, ddd, J = 1.5, 7.0, 8.3 Hz), 7.08 (2H, d, J = 8.9 Hz), 7.11 (1H, ddd, J = 1.6, 7.3, 8.9 Hz), 7.31 (1H, dd, J = 1.6, 8.2 Hz); MS (EI) m/z 442 (M^+), 386 (100%).

4.2.15. 1-(4-Hydroxyphenyl)-12-{2-*n*-propoxyphenyl)-1, 12-dicarba-*closo*-dodecaborane (6c)

Compound **6c** was prepared by the same method as described for the synthesis of **5c**; 44% yield; Colorless needles (AcOEt/*n*-hexane); mp 107.5–108.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.12 (3H, t, J = 7.4 Hz), 1.60–3.60 (10H, br m), 1.91 (2H, sextet, J = 7.1 Hz), 3.88 (2H, t, J = 6.4 Hz), 5.07 (1H, br s), 6.62 (2H, d, J = 8.7 Hz), 6.73–6.80 (2H, m), 7.11 (2H, d, J = 8.7 Hz), 7.13–7.20 (1H, m), 7.38 (1H, dd, J = 1.6 Hz, 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 11.1, 22.3, 70.4, 80.1, 84.6, 112.6, 114.7, 119.8, 123.2, 128.5, 129.4, 129.8, 131.1, 155.5, 156.1; MS (EI) m/z 370 (M^+), 328 (100%); Anal. Calcd for $C_{17}H_{26}B_{10}O_2$: C, 55.11; H, 7.07. Found: C, 55.12; H, 7.08.

4.2.16. 1-(4-Hydroxyphenyl)-12-{2-*n*-pentoxyphenyl)-1, 12-dicarba-*closo*-dodecaborane (6d)

Compound **6d** was prepared by the same method as described for the synthesis of **5d**; 43% yield; colorless needles (AcOEt/*n*-hexane); mp 66–67 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.21 (3H, t, J = 7.1 Hz), 1.35–1.60 (4H, m), 1.60–3.60 (10H, br m), 1.89 (2H, quintet, J = 8.2 Hz), 3.90 (2H, t, J = 6.6 Hz), 4.97 (1H, br s), 6.62 (2H, d, J = 8.9 Hz), 6.70–6.80 (2H, m), 7.12 (2H, d, J = 9.1 Hz), 7.12–7.20 (1H, m), 7.38 (1H, dd, J = 1.6, 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.1, 22.5, 28.5, 28.6, 68.7, 80.0. 84.6, 112.6, 114.7, 119.8, 123.2, 128.5, 129.4, 129.8, 131.1, 155.5, 156.1; MS (EI) m/z 398 (M^+), 328 (100%); Anal. Calcd for $C_{19}H_{30}B_{10}O_2 \cdot 0.5\ H_2O$: C, 55.99; H, 7.67. Found: C, 55.94; H, 7.74.

4.2.17. 1-(4-Hydroxyphenyl)-12-phenyl-1,12-dicarba-*closo*-dodecaborane (7a)

To a solution of **8** (500 mg, 1.43 mmol) in 3 mL of DME was added dropwise 1.58 M *n*-BuLi in hexane (1.0 mL, 1.58 mmol) at 0 °C under an Ar atmosphere, and the mixture was stirred for 30 min at the same temperature. CuCl (155 mg, 1.57 mmol) was added in one portion and the mixture was stirred at room temperature for 1 h. Pyridine (1.7 mL, 21.4 mmol) and iodobenzene (159 μL, 1.43 mmol) were added in one portion, and the mixture was heated at 110 °C for 24 h. After cooling to room temperature,

the mixture was diluted with AcOEt and insoluble materials were removed by filtration through Celite. The filtrate was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was diluted with 3 mL of THF, and then 1 M TBAF in THF (2.14 mL, 2.14 mmol) was added. The mixture was stirred for 1 h at room temperature, 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 column chromatography on silica gel with 1:5 AcOEt/*n*-hexane to give **7a** (148 mg, 33%) as a colorless solid; colorless needles (AcOEt/*n*-hexane); mp 218–219.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.60–3.60 (10H, br m), 5.81 (1H, br s), 6.64 (2H, dd, J = 2.3, 6.1 Hz), 7.10 (2H, dd, J = 2.1, 6.6 Hz), 7.13–7.25 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 82.2, 82.6, 114.8, 127.1, 128.1, 128.3, 128.6, 129.1, 136.3, 155.6; MS (EI) *m/z* 312 (M^+, 100%); Anal. Calcd for $C_{14}H_{20}B_{10}O$: C, 53.82; H, 6.45. Found: C, 53.88; H, 6.35.

4.2.18. 1-(4-Methoxyphenyl)-12-pentafluorophenyl-1, 12-dicarba-*closo*-dodecaborane (15)

To a solution of **12** (500 mg, 2.00 mmol) in 10 mL of Et_2O was added dropwise 2.67 M *n*-BuLi in hexane (0.9 mL, 2.40 mmol) at 0 °C under Ar atmosphere, and the mixture was stirred for 30 min at the same temperature. To a reaction mixture was added hexafluorobenzene (458 μg, 3.99 mmol) in one portion, and the mixture was stirred for 48 h at room temperature. The mixture was poured into 10% HCl aqueous solution and extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:7 AcOEt/*n*-hexane to give **15** (233 mg, 28%) as colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.50–3.80 (10H, br m), 3.75 (3H, s), 6.71 (2H, dd, J = 2.1, 6.8 Hz), 7.13 (2H, dd, J = 2.1, 6.8 Hz, 2H); MS (EI) *m/z* 416 (M^+, 100%).

4.2.19. 1-(4-Hydroxyphenyl)-12-pentafluorophenyl-1, 12-dicarba-*closo*-dodecaborane (7b)

To a solution of **15** (30 mg, 0.072 mmol) in 1 mL of CH_2Cl_2 was added 1 M BBr_3 in CH_2Cl_2 (0.5 mL, 0.5 mmol) at −78 °C under an Ar atmosphere, and the mixture was stirred for 6 h at room temperature, then poured onto ice and extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on silica gel with 1:7 AcOEt/*n*-hexane to give **7b** (27 mg, 93%) as a colorless solid; Colorless needles (AcOEt/*n*-hexane); mp 233.5–235 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.50–3.80 (10H, br m), 5.37 (1H, br s), 6.64 (2H, dd, J = 2.3, 6.8 Hz), 7.08 (2H, dd, J = 2.1, 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 70.8, 88.0, 114.9, 128.3, 128.7, 156.0; MS (EI) *m/z* 402 (M^+, 100%); HRMS calcd for $C_{14}H_{15}B_{10}F_5O_2$: 402.2046, found: 402.2054.

4.3. Competitive binding assay to ERα

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.

4.4. Transcriptional activation assay using human breast cancer cell lines, MCF-7 cells

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 reported method. Briefly, transient transfections in MCF-7 cells were performed using Transfast™ (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 internal standards. Twenty-four hours after addition of the sample, the assay was performed with a Dual Luciferase assay kit™ (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.

4.5. Cell proliferation assay using MCF-7 cell lines

4.5.1. Cell culture

At 80% confluence, cells of the human breast adenocarcinoma cell line MCF-7 was trypsinized from the maintenance dish with 0.25% trypsin-EDTA and collected by centrifugation (4 °C, 1500 rpm, 5 min). The supernatant of medium was removed, 1 mL of DMEM supplemented with 10% FBS, 100 IU/mL penicillin and 100 mg/mL streptomycin was added, and cell aggregates were broken up by thorough pipetting. Cells were seeded in a dish at a concentration of 4×10^4 cell/mL or 8×10^4 cell/mL, and cultivated at 37 °C in a 5% CO_2 humidified incubator. Cells were routinely cultivated two days or three days later.

4.5.2. Cell proliferation assay

Cells of the human breast adenocarcinoma line MCF-7 were routinely cultivated in DMEM supplemented with 10% FBS, 100 IU/mL penicillin and 100 mg/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% sFBS, 100 IU/mL penicillin and 100 mg/mL streptomycin) Cells were trypsinized from the maintenance dish with phenol red-free trypsin-EDTA and seeded in a 96-well plate at a density of 1×10^3 or 2×10^3 cells per final volume of 100 μL DMEM supplemented with 5% sFBS, 100 IU/mL penicillin and 100 mg/mL streptomycin. After 24 h, the medium was replaced with 90 μL of fresh DMEM and 10 μL of drug solution, supplemented with serial dilutions of 4-OH-Tam or DMSO as the dilution control in the presence or absence of 1×10^{-10} M or 1×10^{-11} M E2, was added to triplicate microcultures. Cells were incubated for 5 days, and medium with 4-OH-Tam in the presence or absence of 1×10^{-10} M or 1×10^{-11} M E2 was replaced once after 3 days. At the end of the incubation time, proliferation was evaluated by using WST-8. 10 μM of WST-8 was added to microcultures and cells were incubated for 2 h. The absorbance at 450 nm was measured. This parameter is related to the number of living cells in the culture.

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