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

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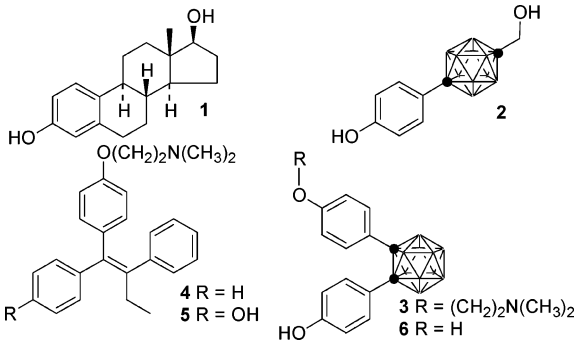
Abstract: Candidate estrogen receptor (ER) ligands with two phenolic residues on a three-dimensional hydrophobic core structure (carborane, bicyclo[2.2.2]octene, or adamantane) were synthesized and biologically evaluated. The biological properties of the ligands were markedly dependent on the nature of the hydrophobic core structure. Bis(4-hydroxyphenyl)-*o*-carborane (**6**) was a partial agonist/antagonist for ER. 1,2-Bis(4-hydroxyphenyl)bicyclo[2.2.2]octene (**10**) exhibited potent agonist activity for ER, even though the two phenolic groups are located similarly to those of **6**.

The estrogen receptor (ER) is a member of the superfamily of ligand-dependent transcriptional factors. Endogenous estrogen, 17β-estradiol (**1**, E2), plays an important role in the female and male reproductive systems and also in bone maintenance, in the central nervous system, and in the cardiovascular system. The first step in the appearance of estrogenic activity is the binding of agonist ligands to ERα[1] and β[2], resulting in a conformational change. The resulting ligand-bound ER then dimerizes, forms complexes with various cofactors, and binds to specific promoter elements of DNA to initiate gene transcription. Antagonist ligands form ER−ligand complexes with different conformations, thereby inhibiting the interactions with cell- and promoter-specific factors. Differences of distribution and function of these factors among tissues seems to be connected with the tissue selectivity of certain ER ligands, which are called selective estrogen receptor modulators (SERM).[3] Therefore, even minor differences in the conformation of ER−ligand complexes depending on the structure of the ligand may be important in determining whether the ligand exhibits agonist or antagonist effects in a certain tissue.[4]

Binding of ligands to the ER ligand binding domain (LBD) primarily requires a phenolic ring, with an appropriate hydrophobic group adjacent to the phenolic ring. The hydrophobic group should closely match the hydrophobic surface of the ER, serving to increase the binding affinity. The hydrophobic structure also plays a role as a scaffold, fixing the spatial positions of hydrogen-bonding functional groups. In our studies to develop new hydrophobic core structures for drug design, we have focused on dicarba-*closo*-dodecaborane

Chart 1. Structures of ER Ligands. in Icosahedral Cage Structures throughout This Paper, Closed Circles Represent Carbon Atoms and Other Vertices Represent BH Units



(carborane)[5] as a hydrophobic component of biologically active molecules. The carboranes are icosahedral carbon-containing boron clusters with characteristic properties, such as spherical geometry and hydrophobicity. We have demonstrated that the hydrophobicity of the carboranes is comparable with that of hydrocarbons,[6] and their spherical hydrophobic surface effectively interacts with the hydrophobic surface of the ligand-binding domain of nuclear receptors.[7−10] Recently, we have reported a potent estrogen agonist bearing a carborane, 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane, BE120 (**2**),[9] which has an activity greater than that of E2 in transcriptional assay using an ERα and in ERα binding assay.[10] The compound also showed potent in vivo effects on the recovery of uterine weight and bone loss in OVX mice.[10] These results suggested that the hydrophobic interaction along the spherical carborane cage produces a stronger interaction than that in the case of E2. We have utilized the strong binding of the spherical carborane cage to the cavity of ERαLBD to develop a potent ER antagonist, BE361 (**3**),[11] which has two hydroxyphenyl groups and a basic side chain, like that of tamoxifen (**4**) and 4-hydroxytamoxifen (**5**). During our studies of ER antagonists, we also found that the simple bis(hydroxyphenyl)-*o*-carborane, BE360 (**6**) exhibited antiestrogenic activity, although the antagonist activity was somewhat weaker than that of **3** bearing the basic side chain. Furthermore, in an in vivo evaluation, compound **6** exhibited estrogenic action in bone, preventing bone loss without inducing estrogenic action in the uterus,[12] suggesting its possible application as a new type of SERM to treat osteoporosis.

Therefore, control of the geometry of the two hydroxyphenyl groups and modification of the three-dimensional hydrophobic core structure should provide a basis for developing various ER ligands with agonist or antagonist activities. These considerations led us to design, synthesize, and biologically evaluate compounds having various three-dimensional hydrophobic cores bearing hydroxyphenyl groups (**7**−**12**), as shown in Figure 1. We presumed that the first phenolic hydroxyl group acts as an anchor at the hydrogen-bonding site of ER, while the three-dimensional hydrophobic core fills the hydrophobic cavity of ER. The position and angle of the second

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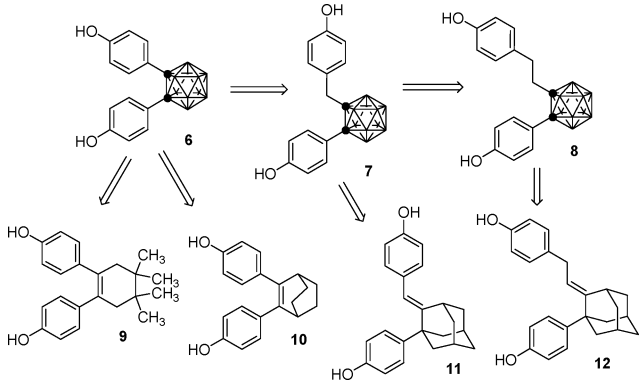
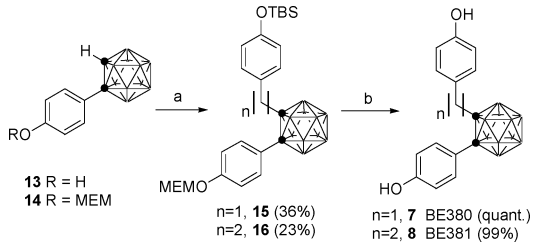


Figure 1. Designed bisphenolic ER ligands (**6**−**12**) with carborane and globular hydrocarbon cores.

Scheme 1[a]



[a] Reagents: (a) (1) n-BuLi/benzene−Et_2O, (2) 4-TBSO-benzyl bromide or 4-TBSO-phenylethyl bromide; (b) HCl/MeOH.

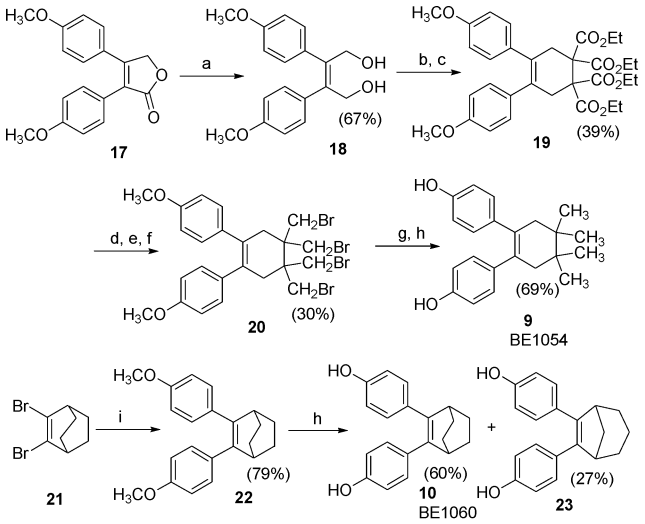
hydroxyphenyl group are presumed to determine the nature of the estrogenic action. Therefore, we designed BE380 (**7**) and BE381 (**8**), which have one or two methylene groups inserted between the second hydroxyphenyl group and the carborane cage of **6**. We also designed BE1054 (**9**) and BE1060 (**10**) bearing a three-dimensional hydrocarbon unit, 4,4,5,5-tetramethylcyclohexene or bicyclo[2.2.2]octene, in place of the carborane cage of **6**, while retaining similar geometry of the two hydroxyphenyl groups.[13] BE1080 (**11**) and BE1081 (**12**) with an adamantane unit are analogues of **7** and **8**, respectively.

The designed molecules **7** and **8** were synthesized from 1-(4-MEMO-phenyl)-*o*-carborane (**14**) as shown in Scheme 1. Compound **14**, which was prepared by transformation of 1-ethynyl-4-MEMO-benzene with decaborane (**14**)[5] in 37% yield, was treated with *n*-BuLi and then reacted with an electrophile,[14] 4-TBSO-benzyl bromide or 4-TBSO-phenethyl bromide, to afford **15** or **16**, respectively, in 23−36% yield. The O-protected groups of **15** and **16** were deprotected under an acidic condition to give **7** and **8**, respectively.

The synthesis of the designed compounds **9** and **10** is summarized in Scheme 2. Reduction of 3,4-bis(4-methoxyphenyl)-*5H*-furan-2-one (**17**)[15] with DIBAL in THF afforded the diol **18** in 67% yield. Replacement of the hydroxyl groups of the diol **18** with bromines, followed by reaction with tetraethyl ethane-1,1,2,2-tetracarboxylate in the presence of NaH in THF, gave the cyclohexene tetracarboxylate **19** in 39% yield.

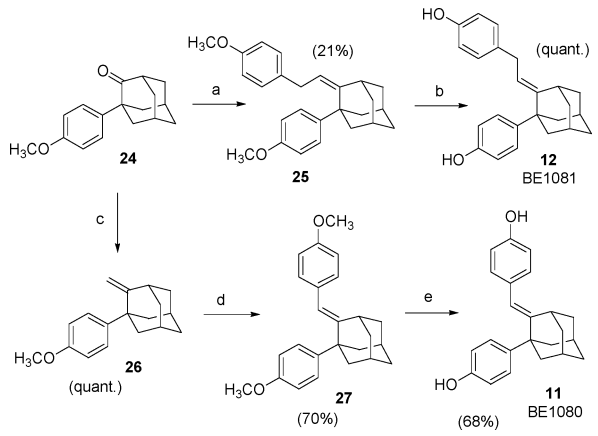
The tetracarboxylate **19** was converted into the tetrabromide **20**[16] in 30% yield by reduction with $LiAlH_4$, methanesulfonylation employing mesyl chloride, and then substitution of the mesyl groups with bromo groups. Reduction of the bromide[19] with $NaBH_4$ in HMPA followed by demethylation afforded **9** in 69% yield. Compound **10** was prepared by Miyaura−Suzuki coupling of 1,2-dibromobicyclo[2.2.2]octene (**21**)[17] with 4-methoxyphenylboronic acid in the presence of $Pd(PPh_3)_4$, followed by demethylation using BBr_3 (47%, two steps). In the demethylation, formation of 6,7-bis(4-hydroxyphenyl)bicyclo[3.2.1]oct-6-ene (**23**) as a rearranged byproduct was observed. The synthesis of the designed compounds **11** and **12** was started from 1-(4-methoylphenyl)adamantan-2-one (**24**)[18] and is summarized in Scheme 3. The Wittig reaction of the adamantanone **24** with 4-methoxyphenethyltriphenylphosphorus ylide afforded an (*E*) and (*Z*) mixture of 1-(4-methoxyphenyl)-2-[2-(4-methoxyphenyl)ethylidene]adamantane (**25**) (21%). After isolation of the (*Z*)-isomer of **25** by recrystallization, treatment with BBr_3 resulted in isomerization of the double bond to give the (*E*)-isomer of **12** in a quantitative yield. In the synthesis of **12**, the ketone of **24** was transformed into a methylene group by Wittig reaction with methyltriphenylphosphorus ylide, followed by Heck reaction with 4-iodo-

Scheme 2[a]



[a] Reagents: (a) DIBAL/THF; (b) PBr_3, pyridine/Et_2O; (c) $[CH(CO_2Et)_2]_2$, NaH/THF; (d) $LiAlH_4$/THF; (e) MsCl/pyridine;(f) LiBr/2-ethoxyethanol; (g) $NaBH_4$/HMPA; (h) BBr_3/CH_2Cl_2; (i) 4-methoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3/toluene−EtOH−H_2O.

Scheme 3[a]



[a] Reagents: (a) 4-methoxyphenylethyltriphenylphosphonium bromide, n-BuLi/Et_2O; (b) BBr_3/CH_2Cl_2; (c) methyltriphenylphosphonium bromide, NaHMDS/THF; (d) 4-iodoanisole, $Pd(OAc)_2$,Ph_3P, Ag_2CO_3/DMF; (e) C_2H_5SNa/DMF.

Table 1. Relative Binding Affinity (RBA) of Test Compounds versus Specific [^{3}H]Estradiol (4 nM) Binding with Human Recombinant ERα.

compound	RBA[a]	compound	RBA[a]
6	48 ± 5	**9**	20 ± 6
7	45 ± 8	**10**	47 ± 5
8	15 ± 3	**11**	9.3 ± 0.8
tamoxifen	2.1 ± 0.2	**12**	1.8 ± 0.3

[a] The relative binding affinity of estradiol is taken as 100. Values represent the average range or SD of two or three independent experiments.

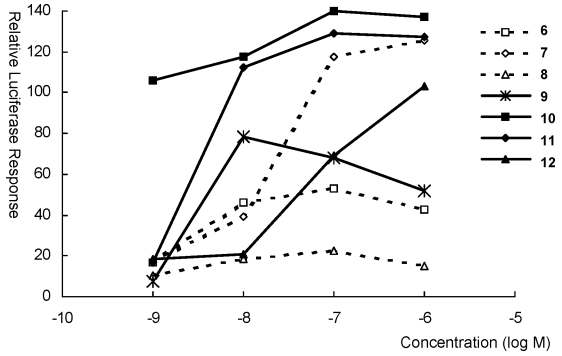


Figure 2. Transcriptional activation by the test compounds. MCF-7 cells were transfected with ERE (SV-40)-LUC and phRL/CMV and incubated with test compounds (10^{-9}–10^{-6} M). The values given are averages for duplicate transfections and are expressed as a percent of the response with 10^{-10} M estradiol.

anisole to give (*E*)-2-(4-methoxybenzylidene)-1-(4-methoxyphenyl)adamantane (**27**) in 70% yield. Demethylation of **27** employing BBr_3 failed, because of formation of the benzyl cation and rearrangement of the adamantane skeleton. Therefore, the demethylation was performed with C_2H_5SNa in DMF to afford **11** in 68% yield.

A competitive binding assay using [6,7-^{3}H]17β-estradiol (K_d = 0.4 nM) and human recombinant ERα (PanVera) was employed for initial screening of the synthesized compounds.[10] The binding affinity data are summarized in Table 1. Insertion of a methylene group into **6** afforded **7**, which retained potent ER affinity. The potencies of **6** and **7** were equivalent and were somewhat weaker than that of E2. The affinity of **8**, into which two methylene units had been inserted, was 10 times weaker than that of E2. Alteration of the hydrophobic core from carborane to bicyclo[2.2.2]octene (**10**) or 4,4,5,5-tetramethyl-cyclohexene (**9**) resulted in equivalent affinity to that of **6**. On the other hand, the affinities of **11** and **12**, bearing adamantane, were decreased 10-fold compared with those of **7** and **8** bearing a carborane cage.

To evaluate the activity of the synthesized compounds as agonists and antagonists, transcriptional assay was done with ERE/Luci (firefly luciferase) and phRL/CMV (Renilla luciferase) plasmid-cotransfected MCF-7 cells.[19] The results of the transcriptional activation and inhibition are summarized in Figure 2 (agonist) and Figure 3 (antagonist). A remarkable difference of activity among the three analogues bearing a carborane cage (**6**, **7**, and **8**) was observed. The compound **6** exhibited partial agonist/antagonist activity, while **7**, with an additional methylene group, showed weak agonist activity. Compound **8**, with two additional methylene groups, was an antagonist with a higher potency than that of **6**. ER

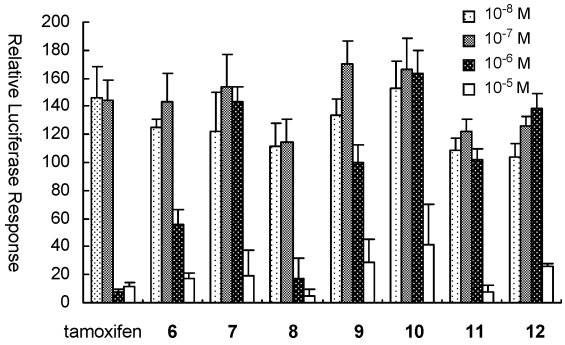


Figure 3. Inhibition of transcriptional activation of estradiol by the test compounds. MCF-7 cells were transfected with ERE (SV-40)-LUC and phRL/CMV and incubated with estradiol (10^{-10} M) and test compounds (10^{-8}–10^{-5} M). Results are shown as means ± SD for triplicate transfection. The values given are expressed as a percent of the response with 10^{-10} M estradiol.

binding of ligands is primarily the result of interaction of the receptor with a phenolic residue and an appropriate hydrophobic group adjacent to the phenolic ring. The compounds based on carborane (**6**, **7**, and **8**) showed potent binding affinity. This is reasonable, because 4-hydroxyphenyl-*o*-carborane (**13**) itself binds strongly to ERα with a K_i of 1.1 nM.[20] The high affinity suggests that the hydrophobic van der Waals contacts along the spherical carborane cage with the hydrophobic cavity of ER produce a strong interaction. In view of the expected fixation by the 4-hydroxyphenyl-*o*-carborane moiety in the cavity of ER, the notable differences of the transcriptional characteristics of **6**, **7**, and **8** can be well interpreted in terms of differences of the direction of the second hydroxyphenyl moiety and the distance between it and the carborane cage. The conformation of the ER−ligand complex determines the agonist−antagonist nature of a particular ligand in the interaction with cell- and promoter-specific factors. The partial agonist-antagonist nature of **6** is supposed that **6**−ER complex is suitable for the appearance of the activity. The insertion of one methylene group alters the direction of the second hydroxyphenyl group with respect to the 4-hydroxyphenyl-*o*-carborane moiety, so that the conformation of **7**−ER complex corresponds to the agonist conformation. The insertion of two methylene groups again alters the direction of the second hydroxyphenyl group and its distance from the 4-hydroxyphenyl-*o*-carborane moiety, producing an antagonist conformation of the **8**−ER complex.

Another remarkable change of transcriptional nature was observed upon altering the 3D hydrophobic core from carborane to bicyclo[2.2.2]octene. The compound **10** bearing bicyclo[2.2.2]octene, exhibited potent agonist activity, although **10** and **6** have a similar geometry of the two hydroxyphenyl groups, and show similar binding affinity to ERα. The change of the transcriptional nature seems to be caused by the difference of shape between the carborane with its smooth spherical surface and the bicyclooctene with an uneven surface. This difference of the molecular surface apparently leads to the formation of distinct ER−ligand complexes with opposite transcriptional effects. On the other hand, the compound **9** bearing 4,4,5,5-tetramethylcyclohexene, which has a markedly nonspherical shape, was a partial

agonist without any marked antagonist effect below 10^{-6} M.

The compound **11** bearing adamantane in place of the carborane cage of **7** exhibited agonist activity 10 times greater than that of **7** (though this may also be related to a change of flexibility in the side chain). In the case of **12** bearing adamantane in place of the carborane cage of **8**, the potent antagonist activity of **8** was changed to the weak agonist activity of **12**. Recently, ER ligands based on a 1,1-diarylethylene moiety bearing bridged bicyclic hydrocarbon cores have been developed.[21] Most of the 1,1-diarylethylenes showed activity as partial agonists and/or antagonists for ERα. Our 1-(4-hydroxyphenyl)adamantane derivatives (**11** and **12**) exhibited full agonist activity without any marked antagonist effect below 10^{-6} M.

In summary, we have synthesized and biologically evaluated novel ER ligands bearing two phenolic residues on a three-dimensional hydrophobic core structure (carborane, bicyclo[2.2.2]octene, or adamantane). Among the carborane-containing compounds (**6**, **7**, and **8**), a dramatic change of agonist/antagonist balance was observed depending on the direction of the second hydroxyphenyl moiety and the distance between it and the carborane cage. In general, replacement of the carborane cage with 3D hydrocarbon cores increases the agonist nature of the ligand. The compound **10** exhibited potent agonist activity for ER, even though the two phenolic groups appear to be similarly directed to those of the partial agonist/antagonist **6**. The results obtained with these three-dimensional hydrophobic core structures raise the possibility that structure−function studies could lead to the development of more selective estrogen agonists and antagonists, which could be useful as therapeutic agents for a wide variety of conditions.

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Supporting Information Available: Details of synthesis, spectral data for compounds **7**−**12**, stereo representations for **6**−**8**, **9**−**12**, and experimental procedures of biological evaluations. This material is available free of charge via the Internet at http://pubs.acs.org.

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