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m-Carborane bisphenol structure as a pharmacophore for selective estrogen receptor modulators

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Abstract—A series of *m*-carborane derivatives was prepared based upon the structures of antiestrogenic drugs and their activities were evaluated by estrogen receptor alpha (ERα) binding assay and transactivation assay using human breast cancer cell line, MCF-7 cells. The *m*-carborane bisphenol **5** exhibited about a thousand times more potent ER agonistic activity than the *o*-carborane bisphenol **11**. The *m*-carborane bisphenol structure appears to be a favorable hydrophobic pharmacophore for the development of novel selective estrogen receptor modulators (SERMs).

Estradiol (E2, **1**) plays important roles in the regulation of the female and male reproductive systems, bone metabolism, and the cardiovascular system as well as the central nervous system, through binding to and activating a specific nuclear receptor, the estrogen receptor (ER).[1] ER is activated by ligand binding to form large complexes with various cofactors, followed by gene transcription through binding to target enhancer of DNA.[1] Differences of distribution and function of cofactors among tissues seem to be connected with the tissue selectivity of certain ER ligands, which are called selective estrogen receptor modulators (SERMs).[2] SERMs can act as agonists for the bone system and as antagonists for cancers, and have been extensively studied for the treatment of reproductive disorders, estrogen-responsive cancers, and osteoporosis. The relative stability of ER-ligand–cofactors complexes, agonist form or antagonist form, in tissues seems to determine whether or not certain ER ligands can act as SERMs.[3]

Tamoxifen (**2a**), which is metabolized in vivo to an active derivative, 4-hydroxytamoxifen (**2b**), is a first-generation SERM, while raloxifen (**3**) is a second-generation SERM. However, these drugs involve a risk of cancers of the female reproductive organs. Currently, researchers are seeking to develop third- or fourth-generation SERMs to circumvent this risk.[4] For this purpose, it is important to understand the structure–activity relationships of existing SERMs. The most noteworthy substituent in these SERMs is the *N*,*N*-dialkylaminoethyl group, which inhibits the binding of coactivators by moving helix-12 to an unfavorable position.[5] (see Chart 1)

Recently, we have reported that dicarba-*closo*-dodecaboranes (carboranes) can act as a hydrophobic structure of various biologically active molecules, including

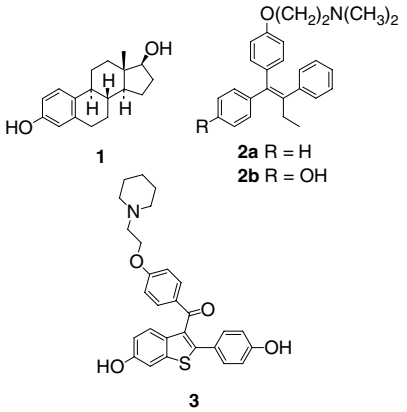

Chart 1. The structures of native ER ligand and SERMs.

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ER modulators.[6] Their spherical structures and hydrophobic surface make easy for them to interact with hydrophobic residues of the ligand binding pocket of receptors.[7] We have also reported that the *m*-carborane bisphenol derivative **4** with a basic side chain, the *N*,*N*-dimethylethylamino group, acted as an ERα antagonist in luciferase reporter gene assay (Chart 2).[8] However, we did not examine in detail its activity profile, binding affinity to ERα, and agonistic activity in transcriptional assay.[8]

Therefore, we have focused on the development of SERMs with *m*-carborane bisphenol structure. We designed and synthesized *m*-carborane bisphenol derivatives **4**–**10** and *o*-carborane bisphenol **11**, which have similar geometry to *m*-carborane bisphenol (Chart 3). In this paper, we described the structure–activity relationships at ERα and the biological activities of these designed derivatives.

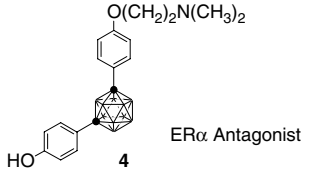


Chart 2. *m*-Carborane derivative having ER antagonistic activity.

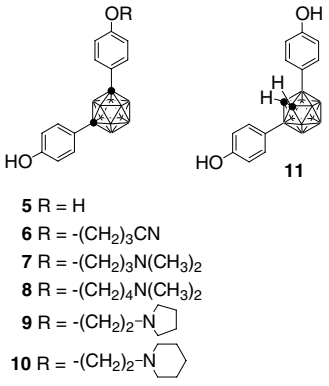
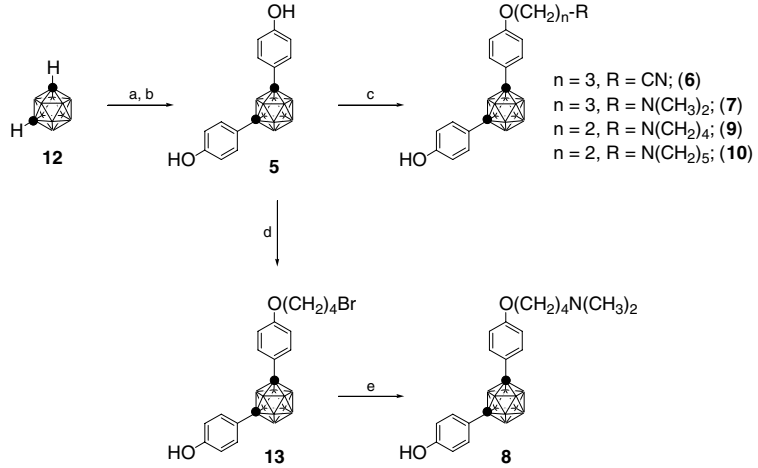


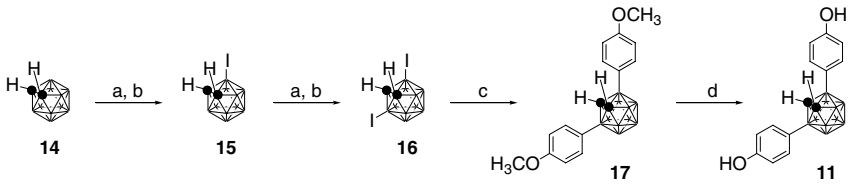
Chart 3. Designed molecules as candidate SERMs.

The designed molecules **6**–**10** were synthesized from *m*-carborane **12** as shown in Scheme 1. Compound **12** was treated with *n*-BuLi and CuCl (I), and reacted with 4-iodoanisole under Ullmann coupling conditions,[9] followed by demethylation with BBr_3 to afford a bisphenol **5** in 62% yield. Compound **5** was reacted with various alkyl halides to afford the corresponding monophenol derivatives **6**–**10** (except for **8**) in 20–30% yield.[10] Compound **8** was synthesized stepwise from **5** through the intermediate **13** in 10% yield since *N*,*N*-dimethylaminobutyl chloride did not react with **5** and was decomposed under the reaction conditions. The synthesis of the *o*-carborane bisphenol **11** is summarized in Scheme 2. Iodine atoms were introduced onto the boron atoms at the 3 and 6 positions of *o*-carborane **14** through decomposition and reconstruction of the *o*-carborane cage. The *o*-carborane cage was easily transformed into a *nido*-compound under basic conditions, and reaction with BI_3 gave 3-iodo-*o*-carborane **15** in 74% yield.[11] Compound **15** was also deboronated to afford the corresponding *nido*-compound, and the synthesis of 3,6-diiodo-*o*-carborane **16** was achieved in 52% yield under same conditions as those used for **15**.[10] Compound **16** reacted with 4-methoxyphenyl magnesium bromide under Pd-catalyzed cross-coupling conditions to afford compound **17** in 42% yield,[12] and this was easily transformed into the *o*-carborane bisphenol **11** by treatment with BBr_3 in quantitative yield.

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.[13] Table 1 summarizes the binding affinity data. All the test compounds competed with ^{3}H-labeled E2 and bound to the ERα ligand binding pocket in a concentration-dependent manner. The binding affinities of **4** and **7**–**10**, linked with an aminoalkyl group, were



Scheme 1. Synthetic scheme of *m*-carborane derivatives **6**–**10**. Reagents: (a) *n*-BuLi, DME, then CuCl(I), 4-iodoanisole, pyridine; (b) BBr_3, CH_2Cl_2; (c) alkyl halides, K_2CO_3, acetone/DMF (1:1); (d) 1,4-dibromobutane, K_2CO_3, acetone/DMF (1:1); (e) 50% dimethylamine in water, THF.



Scheme 2. Synthesis of *o*-carborane bisphenol **11**. Reagents: (a) KOH, EtOH, trimethylammonium chloride; (b) *n*-BuLi, ether, then BI_3, toluene; (c) $Pd(PPh_3)_2Cl_2$, CuI(I), 4-methoxyphenylmagnesium bromide, THF; (d) BBr_3, CH_2Cl_2.

Table 1. Relative binding affinity (RBA) of test compounds versus specific [^{3}H]estradiol (4 nM) binding with human recombinant ERα

Compound	RBA[a]
2a	2.1
4	1.1
5	106
6	25
7	1.5
8	1.7
9	1.5
10	1.8

[a] The relative binding affinity of estradiol is taken as 100. Values represent the average range of duplicate experiments.

several tens of times weaker than that of E2, and were equivalent to that of **2a**. The potency of **6**, which has a cyanopropyl group, was several times weaker than that of E2. Interestingly, the affinity of **5**, which is an intermediate in the synthesis of the derivatives **4** and **6–10**, was similar to that of E2. It is noteworthy that the affinity of **4** was unexpectedly weak, though the intermediate **5** bound to ERα with an affinity almost same as that of E2. The result for **5** indicates that *m*-carborane bisphenol structure fits the ligand binding pocket of ERα. It seems that the binding of the derivative to the ERα ligand binding pocket is impaired by the basicity and the steric effect of the alkyl side chains.

The biological activities were evaluated by transcriptional assay using MCF-7 cells co-transfected with ERE/Luci (firefly luciferase) and phRL/CMV (*Renilla* luciferase) plasmids.[14] Figures 1 and 2 summarize the results of the transcriptional activation and inhibition assays, respectively. All the tested compounds, except for **5**, exhibited agonistic activity at concentrations of more than 10^{-8} M.

Though the agonistic activity of **5** was about ten times weaker than that of E2, its EC_{50} value was very low, 0.1 nM. The activity of **6** was 10–100 times more potent than that of the alkylamino derivatives **4** and **7–10**. The agonistic activities of **9** and **10**, having a cyclic alkylamino group, plateaued at above 10^{-7} M. On the other hand, compounds **4**, **9**, and **10**, which have an ethylene linker between the phenolic oxygen and the basic amino part, exhibited antagonistic activity equal to that of **2a**. Compound **8** was a weaker antagonist than **4**, **9**, and **10**, and **5–7** did not exhibit antagonistic activity. Thus, compounds **4** and **8** were moderate ER partial agonists with both ER agonistic and antagonistic activities, and **9** and **10** were quite potent ER partial agonists with the best agonistic–antagonistic activity balance. The antagonistic activity of these compounds seems to be related to moderate ER agonistic activity. We suggest that these compounds can act as moderate agonists for apo-ERα and moderate antagonists for E2-activated ERα by the replacement of E2 inside the ERα ligand binding pocket. That is, compounds **4** and **8–10** displace E2 from the ligand binding pocket and negate the agonistic activity of E2 for activated ERα. Thus, compounds

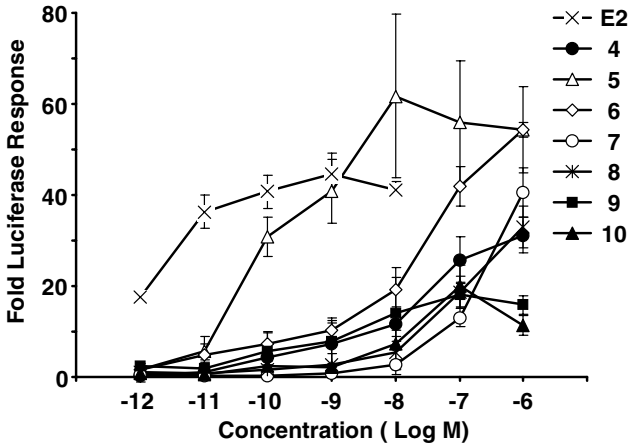


Figure 1. Transcriptional activation by the test compounds. MCF-7 cells were transfected with ERE (SV-40)-LUC and phRL/CMV, and incubated with test compounds (10^{-12}–10^{-6} M). EtOH was used as a standard (control). Results are shown as means ± SD for triplicate transfections.

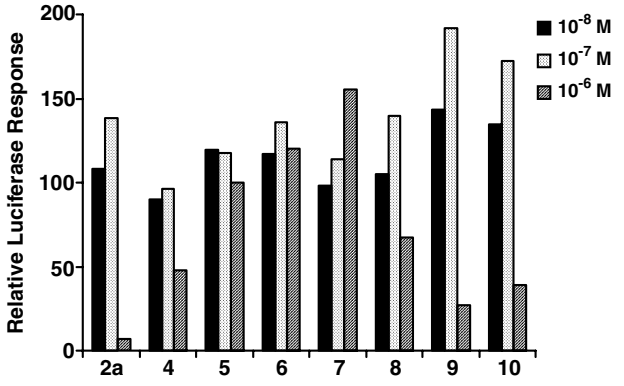


Figure 2. Inhibition of transcriptional activation of E2 by the test compounds. MCF-7 cells were transfected with ERE (SV-40)-LUC and phRL/CMV, and incubated with E2 (10^{-10} M) and test compounds (10^{-8}–10^{-6} M). The values given are averages for duplicate transfections and are expressed as a percent of the response with 10^{-10} M E2.

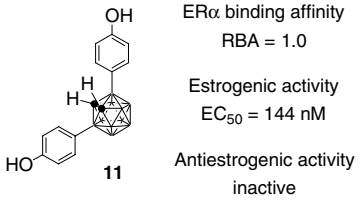


Figure 3. Biological profile of compound **11**.

4 and **8** exhibit moderate antagonistic activity because their activation level is weaker than that of E2. In the cases of compounds **9** and **10**, since the activation level reaches a plateau of half that of E2 at the concentration of 10^{-7} M, the antagonistic activity is more potent than those of **4** and **8**. We concluded that compounds **5**–**7** did not exhibit antagonistic activity because their activation levels are equivalent to that of E2. Thus, we have found candidate SERMs, the partial agonists **9** and **10**. Presumably the bulky cyclic amino group of these candidates moves helix-12 to a partial agonist position in a similar manner to the known SERM, **3**.

To examine the potency of the *m*-carborane cage as a hydrophobic component, we compared the binding affinity and biological activities of the *m*-carborane bisphenol **5** with those of the *o*-carborane bisphenol **11**.

Both compounds have similar geometry of the two hydroxyl groups and a spherical structure. The biological profile of **11** is summarized in Figure 3. Interestingly, the binding affinity of **11** was about a hundred times weaker than that of **5**. Further, the EC_{50} of **11** was 144 nM, that is, about 1000 times weaker than that of **5** (IC_{50} = 0.10 nM). Neither of these compounds exhibited ERα antagonistic activity. From the results, it seems that both compounds bind to the ERα ligand binding pocket in a similar mode. We think that the significant differences in the binding affinity and the agonistic activity may arise from differences in the hydrophobicity of the molecules and the acidic C–H hydrogens of the *o*-carborane cage. Previously, we showed that the binding affinity of various carboranyl phenols to ERα depends not on the pK_a of the phenolic proton, but rather on the hydrophobicity of the molecule, log *P*.[15] Since the acidic C–H hydrogen reduces the hydrophobicity, compound **11** having two acidic C–H hydrogens would be at a clear disadvantage for binding to ERα. Therefore, a C,C-substituted *m*-carborane cage, not having any naked C–H hydrogens, should be a suitable hydrophobic structure for the ligand binding pocket of ERα.

In summary, we have synthesized and biologically evaluated novel ER ligands bearing an *m*-carborane cage **4**–**10** and 3,6-bis(4-hydroxyphenyl)-*o*-carborane **11** with a similar geometry to the *m*-carborane bisphenol **5**. We found that compounds **9** and **10** behave as partial agonists for ERα. Therefore, they are good candidates for SERMs, because a good balance between agonistic and antagonistic activities is important for the development of SERMs. Comparison of the biological activities of the designed molecules indicated that the biological activities of ER modulators based on the carborane bisphenol structure are strongly controlled by not only the spherical carborane structure and the basic side chains, but also the hydrophobicity of the molecules. *m*-Carborane bisphenol structure seems to be a suitable hydrophobic pharmacophore for the development of novel SERMs.

Acknowledgments

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