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STRUCTURE-ACTIVITY STUDY OF ESTROGENIC AGONISTS BEARING DICARBA-*CLOSO*-DODECABORANE. EFFECT OF GEOMETRY AND SEPARATION DISTANCE OF HYDROXYL GROUPS AT THE ENDS OF MOLECULES

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Abstract: Dicarba-*closo*-dodecaboranes (carboranes), which have spherical geometry and hydrophobicity, are applicable as a hydrophobic pharmacophore of biologically active molecules. We have investigated structure-activity relations based on the structure of the potent estrogenic agonist, 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane, which we have previously reported. The geometry and separation distance of the phenolic and alcoholic hydroxyl groups play a critical role in the appearance of biological activity.

Applications of the unique structural and chemical properties[1] offered by icosahedral carboranes (dicarba-*closo*-dodecaboranes) in the field of biomedical sciences, especially in boron neutron capture therapy (BNCT), have received increasing attention over the past 30 years.[2] We have focused on the possibility of using carboranes as a hydrophobic component in biologically active molecules which interact hydrophobically with receptors. We reasoned that the remarkable thermal and chemical stability, the exceptionally hydrophobic character and the spherical geometry of carboranes make them interesting candidates for use as a hydrophobic pharmacophore. Recently, we have reported examples of the design, synthesis and biological evaluation of nuclear receptor ligands (retinoids)[3] and of protein kinase C modulators[4] containing a carborane cage as a hydrophobic pharmacophore. Recently, we have also reported potent estrogenic agonists bearing dicarba-*closo*-dodecaborane as a hydrophobic pharmacophore.[5] Among the tested compounds, 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2c**) exhibited potent transcriptional activity for estrogen receptor α (ERα) at the concentration of 10^{-10} M; its potency is at least 10-fold greater than that of 17β-estradiol (**1**). We also found that 1-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2a**), which lacks the alcoholic hydroxyl group, exhibited potent activity comparable to that of **1**. The remarkable activity of **2c** suggested that the carborane cage works as a hydrophobic group for binding to the hydrophobic cavity of ER, and the spherical carborane cage produces a stronger interaction than that in the case of 17β-estradiol.[5] This raised the possibility that structure-function studies of the phenylcarborane skeleton could

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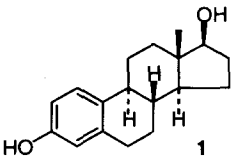
lead to the development of more selective estrogen agonists and antagonists. We report herein the synthesis and biological evaluation of phenylcarboranes with two hydroxyl groups at various positions, and a computational simulation of the docking of these molecules to the human estrogen receptor-α ligand binding domain (hERαLBD).

Figure 1.



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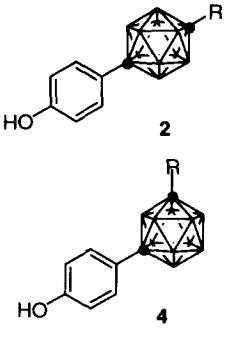

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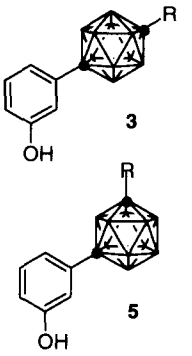


3

4

5

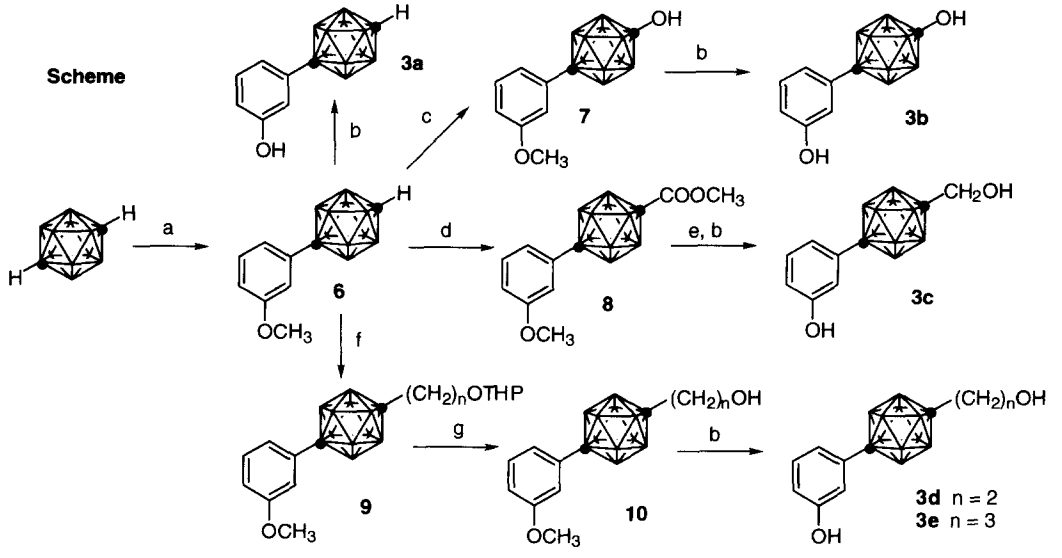
Compounds **2** - **5**

- **a** R = H
- **b** R = OH
- **c** R = CH_2OH
- **d** R = CH_2CH_2OH
- **e** R = $CH_2CH_2CH_2OH$

In icosahedral cage structure, ● represent carbon atoms and other vertices represent BH units.

Previously, we reported that the activity of the compound bearing a *meta*-substituent on the carborane cage (**4c**) was weaker than that of the *para*-substituted compound (**2c**); however, the potency of **4c** is close to that of 17β-estradiol.[5] Now, we have designed other isomers with a phenolic hydroxyl group on the aromatic nucleus (**3** and **5**). We also designed various compounds with inserted methylene group (Figure 1), to investigate the relation between the activity and the geometry and separation distance of the two hydroxyl groups on the phenylcarborane skeleton. Examples of the syntheses of the designed molecules are summarized in the Scheme. 1-(3-Methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**6**), which was prepared by coupling of the C-copper (I) derivative of 1,12-dicarba-*closo*-dodecaborane (*p*-carborane) with 3-

Scheme



Key: (a) 1) *n*-BuLi, CuCl/ DME 2) 3-iodoanisole, pyridine; (b) BBr_3/ CH_2Cl_2 ; (c) n-BuLi, $(C_6H_5COO)_2$/ benzene-Et_2O; (d) 1) *n*-BuLi/ benzene-Et_2O 2) $ClCOOCH_3$; e) $LiAlH_4$/ THF; (f) 1) n-BuLi/ benzene-Et_2O 2) $Br(CH_2)_nOTHP$; (g) *p*-TsOH·H_2O/ MeOH.

iodoanisole in dimethoxyethane in the presence of pyridine,[6] was demethylated with boron tribromide to afford compound **3a** in 93% yield. 1-Hydroxy-12-(3-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**3b**) was prepared by oxidation of the lithiate of **6** with benzoyl peroxide followed by demethylation (75%). The methoxyphenylcarborane **6** was converted to the *C*-methoxycarbonyl derivative **8** by reaction of the lithiate of **6** with methyl chloroformate (98%). After reduction of **8** with $LiAlH_4$, demethylation gave 1-hydroxymethyl-12-(3-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**3c**) in 92% yield. Compound **6** was converted to **9d** by reaction of the lithiate of **6** with 2-(2-bromoethoxy)tetrahydro-2*H*-pyran (45%). After deprotection of the THP group of **9d** with *p*-toluenesulfonic acid, demethylation of the methoxy group gave 1-hydroxyethyl-12-(3-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**3d**) in 88% yield. Similarly, introduction of a C3 unit into **6** afforded 1-hydroxypropyl-12-(3-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**3e**). Compounds bearing a *para*-substituent on the carborane cage and a 4-hydroxyl group on the benzene nucleus (**2**), compounds bearing a *meta*-substituent on the carborane cage and a 4-hydroxyl group on the benzene nucleus (**4**) and compounds bearing a *meta*-substituent on the carborane cage and a 3-hydroxyl group on the benzene nucleus (**5**) were prepared in the same manner as described for **3a**-**3e**.

The estrogenic activities of the synthesized compounds were examined by luciferase reporter gene assay,[7] in which rat ERα-expression plasmid[8] and a reporter plasmid, which contains 5 copies of estrogen response elements, are transiently transfected into COS-1 cells. 17β-Estradiol and the carborane-containing estrogen **2c** at $1x10^{-10}$-$1x10^{-8}$ M induced the expression of luciferase in a dose-dependent manner.[5] The results of this assay system are consistent with the results of ERα binding assay.[5] The transcriptional activity of compounds **2** and **3** is summarized in Figure 2. The compound bearing *p*-carboranyl at the 4-position of

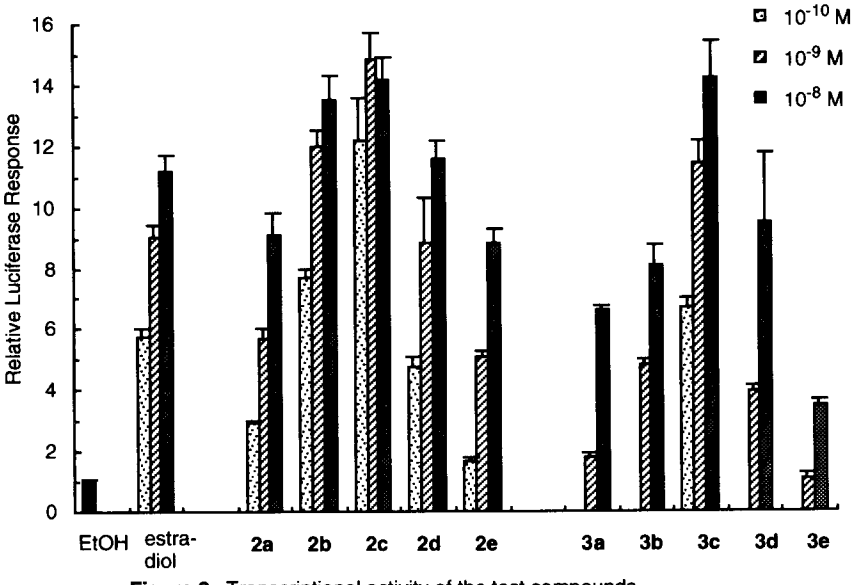


Figure 2. Transcriptional activity of the test compounds.
COS-1 cells were transfected with ERE x 5-pGL-TK and pCI-rERα and incubated with the compounds (10^{-10}-10^{-8} M).

phenol (**2a**) exhibited a potent transcriptional activity in the concentration range of $1x10^{-10}$-$1x10^{-8}$ M; its potency is comparable to that of 17β-estradiol. The activity was increased by the introduction of a hydroxyl group onto carbon of the carborane cage; the potency of the resultant compound, **2b** was greater than that of 17β-estradiol. Compound **2c**, which has a hydroxymethyl group on the carborane cage, afforded the most potent activity. On the other hand, further insertion of methylene units in the substituent at the carbon of the carborane (**2d** and **2e**) cage decreased the activity. The activity of **2d** was similar to that of 17β-estradiol, and **2e** exhibited moderate activity. The activity of the compounds bearing a *para*-substituent on the carborane cage and a 3-hydroxyl group on the benzene nucleus (**3**) was somewhat weaker than that of **2**, and showed the same tendency upon insertion of methylene groups as in the series of **2**. However, the potency of **3c** was still greater than that of 17β-estradiol. The transcriptional activity of compounds **4** and **5** is summarized in Figure 3. The activity of the compounds bearing a *meta*-substituent on the carborane cage and a 4-hydroxyl group on the benzene nucleus (**4**) was weaker than that of **2**. Further, the activity of the compounds bearing a *meta*-substituent on the carborane cage and a 3-hydroxyl group on the benzene nucleus (**5**) decreased. Series **4** and **5** showed the same tendency upon insertion of methylene groups as series of **2**; however, the effects of the hydroxymethyl group were not remarkable.

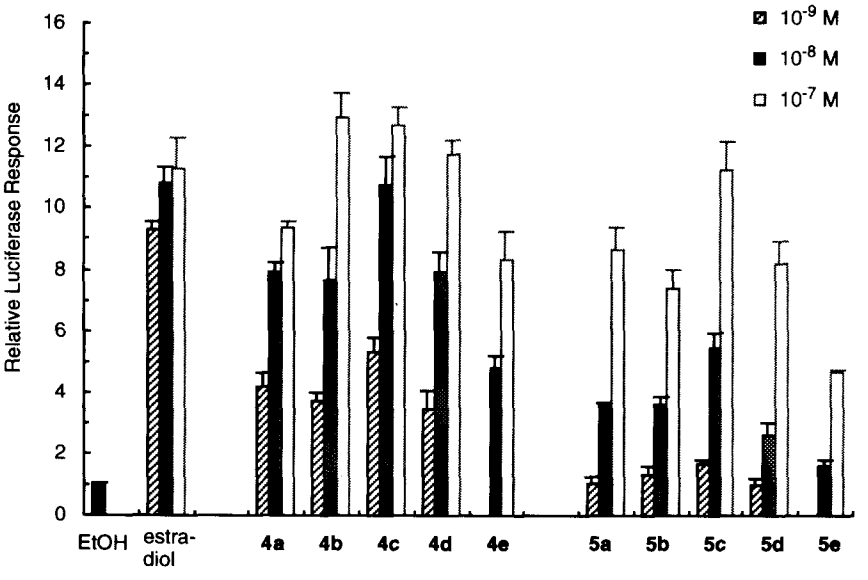


Figure 3. Transcriptional activity of the test compounds.
COS-1 cells were transfected with ERE x 5-pGL-TK and pCI-rERα and incubated with the compounds (10^{-9}-10^{-7} M).

The first step in the appearance of the estrogenic activities is mediated by the binding of hormonal ligands to the estrogen receptors α and β (ER α and β).[8,9] The ER undergoes a conformational change, allowing the receptor to dimerize. The dimer binds with high affinity to chromatin to modulate the transcription of target genes. In 1997, the crystal structure of the hERαLBD was elucidated in the complex with 17β-estradiol.[10] Docking simulations to this structure would be useful to clarify the relation between the

three-dimensional structures and high activity of carborane-containing estrogens. Docking simulations in this study were performed using an automatic docking program (ADAM).[11] The most potent carborane-containing estrogen **2c** was well-fitted to the cavity of the X-ray structure of hERαLBD. **2c** is oriented in the cavity as shown in Figure 4.**B** by two types of contacts: hydrogen bonding at both ends and hydrophobic van der Waals contacts along the body of the skeleton, as found for 17β-Estradiol (**1**) in the X-ray structure (Figure 4.**A**). Two functional groups hydrogen-bonded to amino acid residues of the cavity: the phenolic hydroxyl to both glutamate (Glu-353) and guanydyl (Arg-394),

Table 1. Heteroatom-Heteroatom Distances in Å (Hydrogen-Bond Distances) in the Most Stable Docking Models.

	phenolic-OH/Arg	phenolic-OH/Glu	alcoholic-OH/His
2b	3.11	2.59	3.11
2c	2.93	2.43	2.73
3c	3.67	2.70	2.95
4c	2.25	2.79	3.37
5c	3.72	2.44	3.96
estradiol	3.15	2.34	2.80

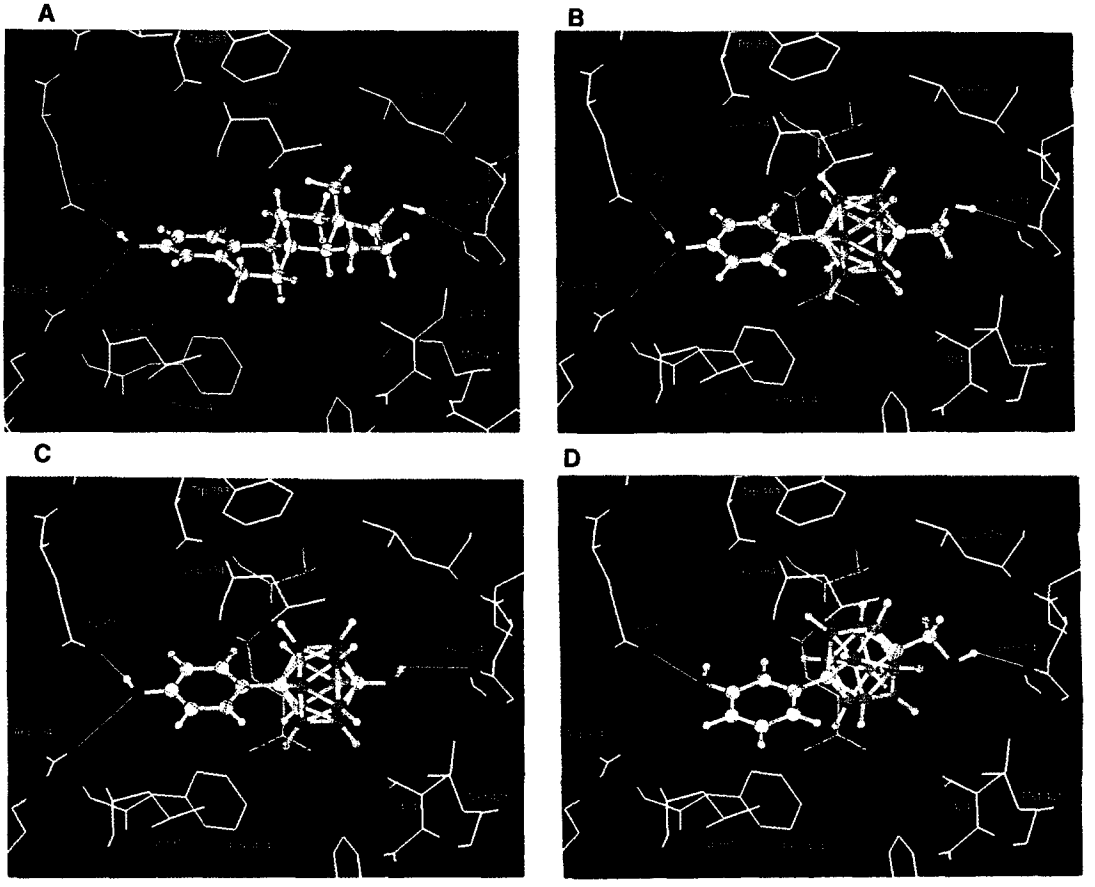


Figure 4. Drawings of the Crystalline Structure of 17β-Estradiol Complex (**A**) and the Stable Docking Models of **2c** (**B**), **2b** (**C**), **3c** (**D**) in the ERα cavity.

and alcoholic hydroxyl to the δ-nitrogen of His-524. The hydrophobic interaction along the spherical carborane cage seems to produce a stronger interaction than that in the case of 17β-estradiol. This may be the reason for the high activity of compound **2a**, which lacks a hydroxyl group for hydrogen-bonding. Compound **2b**, with a hydroxyl group directly substituted on the carborane cage, and the 3-hydroxy isomer **3c** were also well-fitted to the cavity of hERαLBD (Figure 4.**C**, **D**). Table 1 shows the heteroatom-heteroatom distances of compounds **2b**, **2c**, **3c**, **4c** and **5c** in the most stable docking models, and that in the crystal structure of **1**. The results appear to account well for the order of the estrogenic activity.[12]

Potent carborane-containing estrogenic agonists having a new skeletal structure and unique characteristics should be helpful in the design of further compounds as therapeutic agents, especially selective estrogen receptor modulators. Furthermore, the suitability of the spherical carborane cage for binding to the cavity of ERαLBD should provide a basis for a similar approach to other steroid receptor ligands.

References and Notes

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12. Relation between heteroatom-heteroatom distances and biological activity suggested that the three distances within 3.2Å seem to be effective for hydrogen-bondings. However, the interactions of ligand to the receptor cannot be explained only the hydrogen-bonding but hydrophobic interaction. The lack of one hydrogen-bonding would probably compensate by an effective hydrophobic interaction.