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ESTROGENIC ANTAGONISTS BEARING DICARBA-*CLOSO*-DODECABORANE AS A HYDROPHOBIC PHARMACOPHORE

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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 designed and synthesized estrogenic antagonists based on the structure of the potent agonist 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane, which we have developed. The compounds showed potent antagonistic activity in luciferase reporter gene assay using COS-1 cells transfected with rat ERα-expression plasmid and an appropriate reporter plasmid.

The carboranes (dicarba-*closo*-dodecaboranes)[1] exhibit remarkable thermal stability, are resistant to attack by most types of reagent, and are generally biologically inactive. Their icosahedral geometry, in which the carbon and boron atoms are hexacoordinated, accounts for these unusual properties, which make such molecules uniquely suitable for several specialized applications, including materials chemistry.[2] In recent years, the use of carboranes in boron neutron capture therapy (BNCT) has attached much interest, on the basis of their high boron contents.[3] We, on the other hand, 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 made them interesting candidates for use as a hydrophobic pharmacophore. Recently, we have reported examples of the design, synthesis and biological evaluation of retinoids[4] containing a carborane cage as a hydrophobic pharmacophore. We have also reported the development of a potent estrogen agonist bearing a carborane, 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**1**)[5], which has an activity greater than that of 17β-estradiol (**2**). In this article, we describe the synthesis and biological evaluation of estrogen antagonists based on the structure of the above agonist.

Since the discovery of the estrogen antagonists chlomiphen[6] and tamoxifen (**3**),[7] many stilbene derivatives and triarylethylenes have been synthesized and shown to possess activity, and some have been developed for clinical use.[8] Steroidal estrogen antagonists have also been developed, and although substitutions at various carbon atoms of estradiol have been tried, the best results were obtained with two classes of compounds, namely (1) 11β-substituted with a phenyl moiety, such as RU 3941 (**5**),[9] and (2) 7 α-substituted with an alkyl chain having an amide or a sulfoxide moiety.

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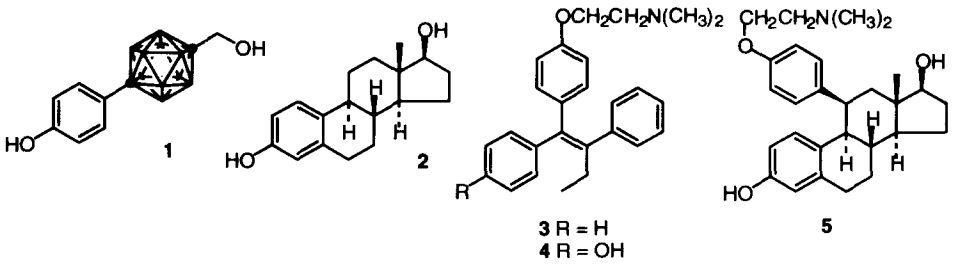


The high estrogenic activity of compound **1** suggests that the carborane cage works as a hydrophobic group for binding to the hydrophobic cavity of ER, and the hydrophobic van der Waals contacts along the spherical carborane cage produce a stronger interaction than that in the case of 17β-estradiol. Therefore, it should be possible to design new estrogen antagonists on the basis of the carborane skeleton. These considerations led us to synthesize and biologically evaluate compounds having *o*-, *m*- and *p*-carborane skeletons and a hydroxyl group at the *para*- and *meta*-position of an aromatic nucleus (**6-11**), as shown in Fig 1. In icosahedral cage structures throughout this paper, closed circles (•) represent carbon atoms and other vertices represent BH units.

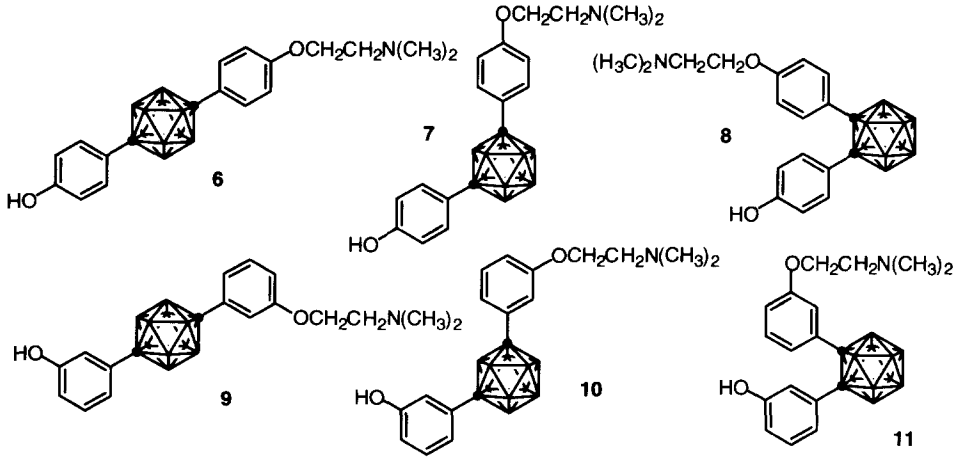
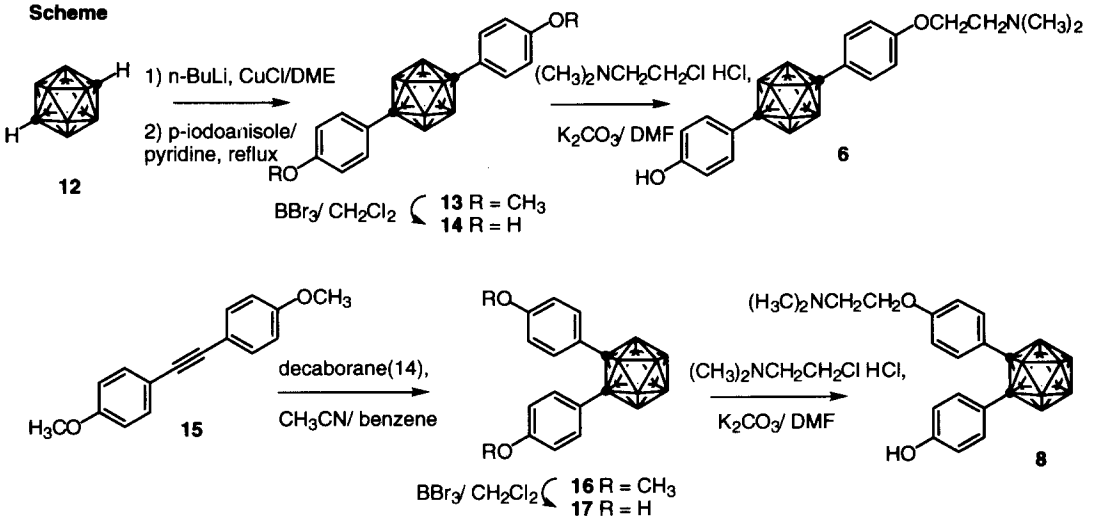


Figure1. Designed carborane-containing molecules (**6-11**)

The syntheses of the designed molecules are summarized in the Scheme. Compound **6** was prepared from 1,12-dicarba-*closo*-dodecaborane (**12**). Coupling of the C-copper (I) derivative of **12**, prepared from the corresponding lithiocarborane, with 4-methoxyiodobenzene in dimethoxyethane in the presence of pyridine gave the bis-C-arylated product (**13**) in 60% yield.[10] Demethylation of the methoxy group of **13** with boron tribromide afforded 1,12-bis(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**14**) in 97% yield. The bisphenol **14** was converted to the monoalkylated derivative (**6**) by reaction with 2-chloroethyldimethylamine hydrochloride (32%).[11] Compound **7** was prepared from 1,7-dicarba-*closo*-dodecaborane in the same manner as described for **6**. The coupling of the C-copper (I) derivative of 1,2-dicarba-*closo*-dodecaborane with 4-

methoxyiodobenzene did not afford the bis-C-arylated product because of steric hindrance to 1,2-substitution of the carborane cage. Therefore, compound **8** was prepared by construction of the *o*-carborane cage from diarylalkyne with *nido*-decaborane(14). Bis(4-methoxyphenyl)acetylene (**15**), which was prepared from 4-methoxyethynylbenzene with 4-methoxyiodobenzene, was converted to 1,2-bis(4-hydroxyphenyl)-1,2-dicarba-*closo*-dodecaborane (**16**) in 31% yield. Demethylation of the methoxy group of **16** with boron tribromide afforded 1,12-bis(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**17**) in 99% yield. The bisphenol **17** was converted to the monoalkylated derivative (**8**) by reaction with 2-chloroethyldimethylamine hydrochloride (12%).[11] Compounds bearing a *meta*-hydroxyl group, **9**, **10** and **11**, were prepared from 3-methoxyiodobenzene and the corresponding carborane in the same manner as described for the *para*-hydroxyl isomers.

Scheme



The estrogenic activities of the synthesized compounds were examined by luciferase reporter gene assay,[12] in which rat ERα-expression plasmid[13] and a reporter plasmid, which contains 5 copies of estrogen response elements, are transiently transfected into COS-1 cells. 17β-Estradiol at $1x10^{-10}$-$1x10^{-8}$ M induced the expression of luciferase in a dose-dependent manner. This activation by 17β-estradiol was dependent upon the expression of ER and was completely inhibited by estrogen antagonists (tamoxifen and ICI 182,780). Therefore, the assay system is ER-dependent and sufficiently sensitive to identify estrogenic compounds and antiestrogens. The results on the inhibition of transcriptional activity of 17β-estradiol at concentration of 10^{-9} M by our carborane-containing molecules (**6**-**11**) are summarized in Figure 2. The compound based on *para*-carborane (**6**) exhibited antiestrogenic activity toward 17β-estradiol at 1 x 10^{-8} M. However, **6** did not inhibit the activity to the control level even at the concentration of 1 x 10^{-6} M. On the other hand, the compound based on *meta*-carborane (**7**) inhibited the activity of 17β-estradiol in the concentration range of 1 x 10^{-7}-10^{-6} M in a dose-dependent manner. The antagonistic activity was increased in the case of the compound based on *ortho*-carborane (**8**), which inhibited 70% of the transcriptional response to 17β-estradiol at the concentration of 1 x 10^{-7} M, and almost completely inhibited it at 1 x 10^{-6} M. The antagonistic activity of the compounds

bearing a *meta*-hydroxyl group, **10** and **11**, was somewhat weaker than that of *para*-hydroxyl compounds; however, compound **11** almost completely inhibited the transcriptional response to 17β-estradiol at the concentration of 1 x 10^{-6} M.

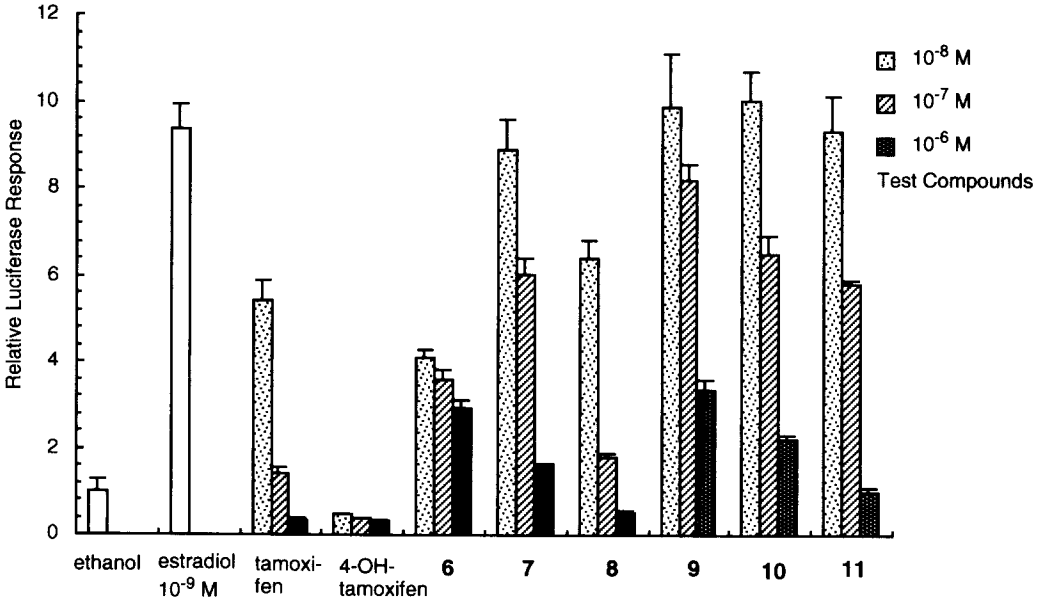


Figure 2. Inhibition of transcriptional activation of 17β-estradiol by the test compounds. COS-1 cells were transfected with ERE x 5-pGL-TK and pCI-rERα and incubated with no agonist (ethanol), with 17β-estradiol (10^{-9}M) or test compounds (10^{-8}-10^{-6} M) plus 17β-estradiol (10^{-9}M). Results are shown as means ± SD for triplicate transfections.

Recently, studies on the three-dimensional structure of the complexes formed by raloxifene and the human estrogen receptor-α ligand binding domain (hERαLBD),[14] and by 4-hydroxytamoxifen (**4**) and hERαLBD have been reported.[15] It is suggested that an agonist-induced conformational change involving helix 12, the most C-terminal helix of LBD, is essential for activation function (AF-2) activity and the appearance of estrogenic action. 4-Hydroxytamoxifen is oriented in the hERα ligand binding pocket in such a way that the phenolic hydroxyl group is hydrogen-bonded to glutamate (Glu-353) and arginine (Arg-394) of hERαLBD in approximately the same manner as the phenolic hydroxyl group of 17β-estradiol.[14] However, the bulky $(CH_3)_2NCH_2CH_2O$-phenyl group of the antagonist extends towards helices 3, 8 and 11 of hERαLBD compared to the case of agonist-hERαLBD complex. This may result in conformational change of helix 12 and the appearance of anti-estrogen activity. Figure 3 shows the crystal structure of 4-hydroxytamoxifen (**4**) and hERαLBD complex (left) and the most stable docking model of **8** (right) to the crystal structure as seen in the complex, obtained by using advanced computational docking.[16] The molecule of **8** forms a stable complex with the same hydrogen-bonding pattern as in the case of **4**, and the bulky $(CH_3)_2NCH_2CH_2O$-phenyl group is located in the narrow cavity as in the case of **4**. The dihedral angle between the two phenyl groups of **8** does

not seem to be the most appropriate for fitting the cavity, and this may account the relatively low activity of **8** compared to **4**. Although the antagonistic activity of the carborane-containing molecules is moderate, optimization of the structure may allow the development of selective estrogen receptor modulators.

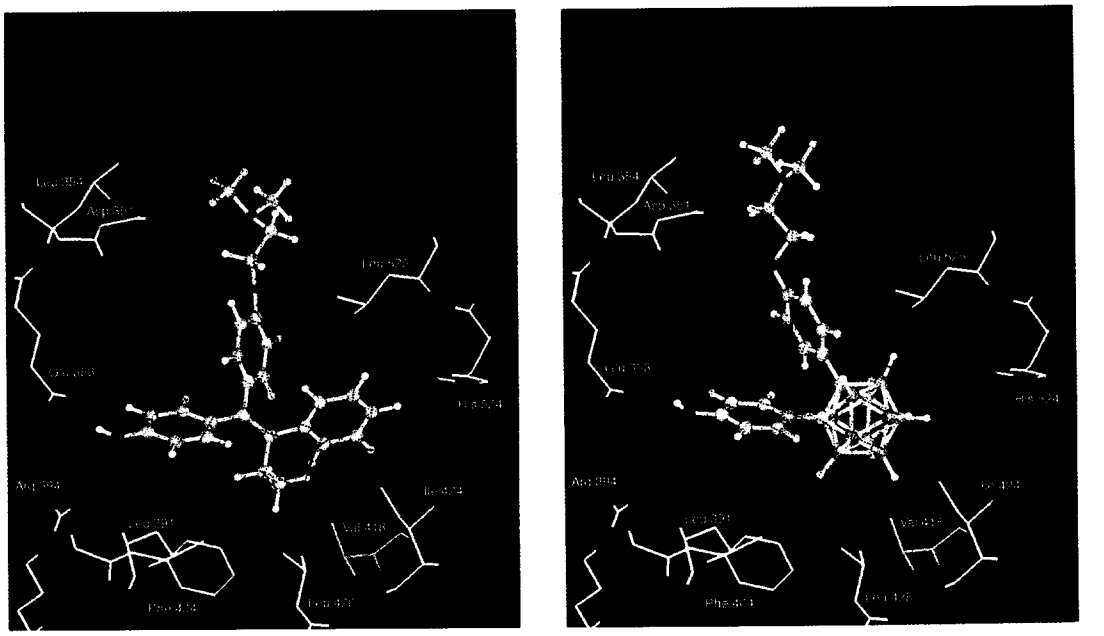


Figure 3. Drawings of the crystalline structure of ERα-4-hydroxytamoxifen complex (left) and a stable docking model of **8** in the ERα cavity (right).

In summary, we have developed novel carborane-containing molecules with estrogen-antagonistic activity. These carborane-containing estrogenic antagonists having a new skeletal structure and unique characteristics, should provide a basis for the design of superior compounds as therapeutic agents.

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