New Estrogenic Antagonists Bearing Dicarba-*closo*-dodecaborane as a Hydrophobic Pharmacophore

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We have designed and synthesized estrogen antagonists bearing dicarba-*closo*-dodecaborane (carborane) as a hydrophobic pharmacophore based on the structure of 1-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane, a potent estrogen agonist that we reported previously. Compounds with a long alkyl chain bearing an amide moiety on the carborane skeleton (6, 7) showed estrogen antagonistic activity in a luciferase reporter gene assay using COS-1 cells transfected with a rat ERα-expression plasmid and as an appropriate reporter plasmid.

Key words carborane; dicarba-*closo*-dodecaborane; estrogen; antagonist; hydrophobic moiety

The carborane (dicarba-*closo*-dodecaborane)[1] skeleton is stabilized by 26 delocalized skeletal electrons and exhibits remarkable thermal and chemical stability. The 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] and medicinal chemistry.[3] 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 made them interesting candidates for use as a hydrophobic pharmacophore. Recently, we have reported a potent estrogen agonist bearing a carborane, 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (Chart 1, **1**),[4] which exhibits greater activity than that of 17β-estradiol (**3**). We also designed and synthesized novel estrogen antagonists based on the structure of tamoxifen.[5] In the present article, we describe the synthesis and biological evaluation of new estrogen antagonists based on the phenylcarborane skeleton.

Since the discovery of the estrogen antagonist tamoxifen,[6] many stilbene derivatives and triarylethylenes have been synthesized and shown to possess activity, and some have been developed for clinical use.[7] Steroidal estrogen antagonists have also been developed, and although substitutions at various carbon atoms of estradiol have been tried, one of the most potent classes of antagonists consists of compounds that are 7α-substituted with an alkyl chain bearing an amide (ICI 164,384, **4**)[8] or sulfoxide moiety (ICI 182,780, **5**).[9]

The high agonistic activity of compound **1** suggested that the carborane cage works as a hydrophobic group binding to the hydrophobic cavity of the estrogen receptor (ER), and the hydrophobic and van der Waals contacts along the spherical carborane cage produce a stronger interaction than that in the case of 17β-estradiol. Further, 1-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2**), which lacks a hydroxymethyl group on the carborane cage, also exhibits potent estrogenic activity. Therefore, we set out to design new estrogen antagonists based on the carborane skeleton. Substitution of an alkyl group at the 2-position of the carborane cage might correspond to substitution at the 7-position of the steroidal skeleton. Therefore, we synthesized and biologically evaluated compounds having an *o*- or *m*-carborane skeleton with an alkyl chain bearing an amide and a hydroxyl group at the *para*-position of the aromatic nucleus (**6**—**9**), as shown in Chart 2. In icosahedral cage structures throughout this paper, closed circles (•) represent carbon atoms and other vertices represent BH units.

The syntheses of the designed molecules are summarized in Chart 3. Compounds **6** and **7** were prepared from 1-(4-methoxyphenyl)-1,2-dicarba-*closo*-dodecaborane (**10**), which was prepared by construction of the *o*-carborane cage from 4-ethynylanisole and *nido*-decaborane. Compound **10** was converted to **11** by reaction of the lithiate of **10** with 2-(11-bromoundecyloxy)tetrahydro-2*H*-pyran (50%). Deprotection of the THP group of **11** with *p*-toluenesulfonic acid gave the alcohol **12** (92%). Oxidation of **12** with chromium trioxide gave the acid **13** (15%) and the ester **14** (48%). The ester **14** was hydrolyzed with sulfuric acid-1,4-dioxane to give the acid **13** (70%). Demethylation of the methoxy group of **13** with boron tribromide followed by coupling with *n*-butylamine or *N*-*n*-butyl-*N*-methylamine afforded **6** (38%) or **7** (26%), respectively. Compounds **8** and **9** were prepared from 1-(4-methoxyphenyl)-1,7-dicarba-*closo*-dodecaborane[10] by means of the same procedures as used in the synthesis of **6** and **7**. The structures of the carborane-containing molecules (**6**—**9**) were confirmed by spectroscopic data including ^{1}H-NMR and HRMS.[11]

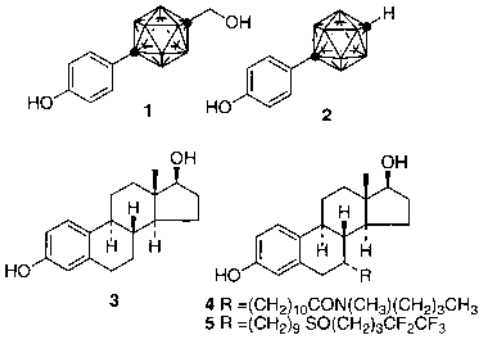

Chart 1. Structures of Potent Estrogen Agonists Bearing a Carborane Skeleton, and Conventional Steroidal Estrogen Agonist and Antagonists

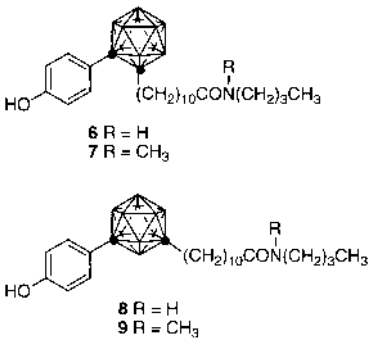

Chart 2. The Designed Carborane-containing Molecules (**6**—**9**)

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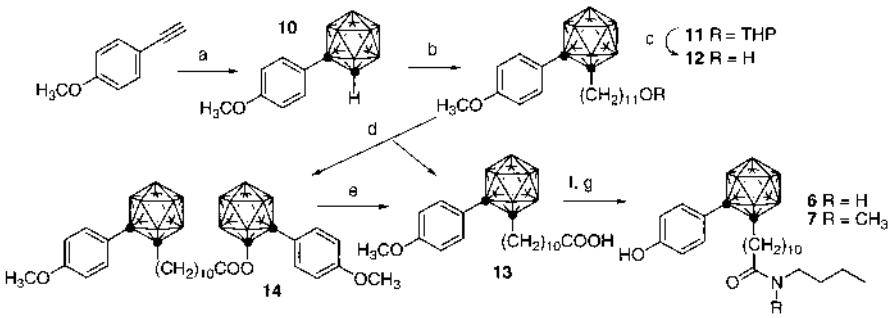

a) decaborane(14), acetonitrile/ benzene; b) 1) *n*-BuLi/ DME 2) CuCl 3) 2-(11-bromo-*n*-undecyloxy) tetrahydro-2*H*-pyran, pyridine; c) *p*-toluenesulfonic acid/ CH_3OH; d) CrO_3/ 20% H_2SO_4, acetone; e) H_2SO_4/ dioxane-H_2O; f) BBr_3/CH_2Cl_2; g) *n*-butylamine or *N*-*n*-butyl-*N*-methylamine, DCC/ acetonitrile.

Chart 3

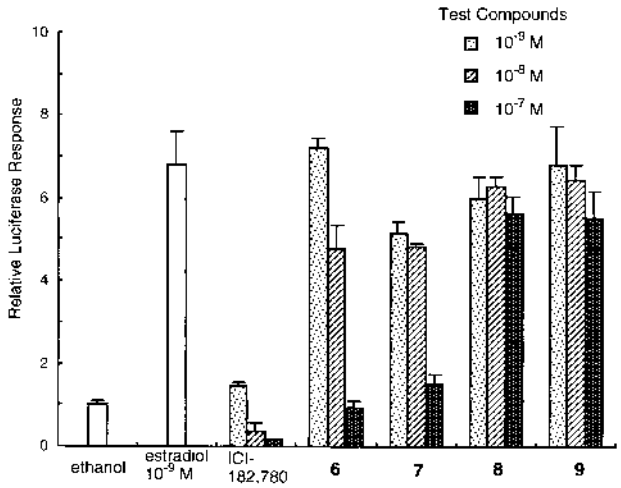

Fig. 1. Inhibition of Transcriptional Activation of 17β-Estradiol by the Test Compounds

COS-1 cells were transfected with ERE×5-pGL-TK and pCI-rERα (see text) and incubated with no agonist (ethanol), with 17β-estradiol (10^{-9} M) or with a test compound (10^{-9}—10^{-7} M) plus 17β-estradiol (10^{-9} M). Results are shown as means±S.D. for triplicate transfections.

The estrogenic activities of the synthesized compounds were examined by luciferase reporter gene assay,[12] in which a rat ERα-expression plasmid[13] and a reporter plasmid, which contains 5 copies of an estrogen response element, are transiently transfected into COS-1 cells. 17β-Estradiol at 1×10^{-10}—1×10^{-8} M induced the expression of luciferase in a dose-dependent manner. The results of inhibition of transcriptional activity of 17β-estradiol at a concentration of 10^{-9} M by our carborane-containing molecules (**6**—**9**) are summarized in Fig. 1. Compounds based on *ortho*-carborane (**6** and **7**) inhibited the activity of 17β-estradiol in the concentration range of 1×10^{-8}—10^{-7} M. The potency of **6** is less than that of ICI 182,780, which is the most potent full estrogen agonist currently known. However, compound **6** at 1×10^{-7} M inhibited 85% of the transcriptional response to 10^{-9} M 17β-estradiol. Compounds based on *meta*-carborane (**8** and **9**) did not exhibit antagonistic activity.

Recently, studies on the three-dimensional structures of the complexes formed by raloxifene and the human estrogen receptor-α ligand binding domain (hERαLBD),[14] and by 4-hydroxytamoxifen and hERαLBD have been reported.[15] The structural studies suggest 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.[14] Although the binding mode of 7-substituted steroidal antagonists to ER has not been clarified, the role of a linear alkyl substituent seems to be similar to that in the case of 4-hydroxytamoxifen. The alkyl group is considered to fit in a narrow corridor in the receptor cavity. Although the linear alkyl substituents of these antagonists are flexible, the direction of the substituents plays a critical role in the antagonistic activity. Chart 4 shows the core structures of compounds bearing an *o*- or *m*-carborane skeleton. The great difference in activity between **6** and **8** may be interpreted in terms of the direction of the alkyl substituent. The antagonistic activity of these carborane-containing molecules is moderate, but optimization of the structure may afford more potent and selective antagonists.

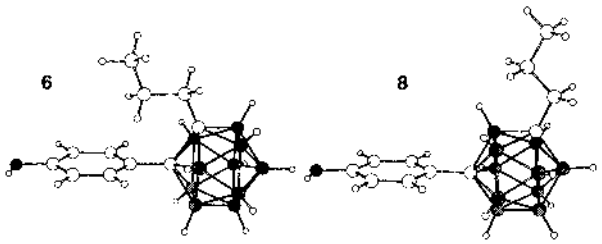

Chart 4. Core Structures of **6** and **8**

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

References and Notes

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11) Compound **6**: Colorless viscous liquid, ^{1}H-NMR ($CDCl_3$) 0.85—1.40 (m, 18H), 0.95 (t, J=7.3 Hz, 3H), 1.50—1.60 (m, 2H), 1.5—3.2 (br m, 10H), 1.80 (m, 2H), 2.22 (t, J=7.3 Hz, 2H), 3.31 (dt, J=5.8, 7.1 Hz, 2H), 5.60 (br s, 1H), 6.86 (d, J=8.8 Hz, 2H), 7.44 (d, J=8.8 Hz, 2H), 9.50 (br s, 1H). HRMS: Calcd for $C_{23}H_{45}{}^{10}B_2{}^{11}B_8NO_2$, 475.4453; Found, 475.4450. Compound **7**: Colorless viscous liquid, ^{1}H-NMR ($CDCl_3$) 0.85—1.40 (m, 18H), 0.94, 0.97 (t×2, J=7.3 Hz, 3H), 1.50—1.65 (m, 2H), 1.5—3.2 (br m, 10H), 1.79 (m, 2H), 2.35, 2.36 (t×2, J=7.3 Hz, 3H), 2.98, 3.02 (s×2, 3H), 3.30, 3.42 (t×2, J=7.5 Hz, 3H), 6.87, 6.88 (d×2, J=8.8 Hz, 2H), 7.43 (d, J=8.8 Hz, 2H), 9.79, 9.82 (br s×2, 1H) (conformational mixture of *cis*- and *trans*-amide (1 : 1) in $CDCl_3$). HRMS: Calcd for $C_{24}H_{47}{}^{10}B_2{}^{11}B_8NO_2$, 489.4610; Found, 489.4607. Compound **8**: Colorless viscous liquid, ^{1}H-NMR ($CDCl_3$) 0.92 (t, J=7.3 Hz, 3H), 1.05—1.42 (m, 18H), 1.48 (quint, J=7.6 Hz, 2H), 1.6—3.1 (br m, 10H), 1.95 (m, 2H), 2.16 (t, J=7.6 Hz, 2H), 3.25 (dt, J=5.9, 7.0 Hz, 2H), 5.44 (br s,1H), 6.30 (br s,1H), 6.71 (d, J=8.8 Hz, 2H), 7.26 (d, J=8.8 Hz, 2H). HRMS: Calcd for $C_{23}H_{45}{}^{10}B_2{}^{11}B_8NO_2$, 475.4453; Found, 475.4460. Compound **9**: Colorless viscous liquid, ^{1}H-NMR ($CDCl_3$) 0.91, 0.95 (t×2, J=7.3 Hz, 3H), 1.05—1.42 (m, 18H), 1.45—1.60 (m, 2H), 1.6—3.1 (br m, 10H), 1.95 (m, 2H), 2.29, 2.31 (t×2, J=7.4 Hz, 3H), 2.92, 2.98 (s×2, 3H), 3.26, 3.36 (t×2, J=7.5 Hz, 3H), 6.72 (d, J=8.8 Hz, 2H), 7.25 (d, J=8.8 Hz, 2H). (conformational mixture of *cis*- and *trans*-amide (1 : 1) in $CDCl_3$). HRMS: Calcd for $C_{24}H_{47}{}^{10}B_2{}^{11}B_8NO_2$, 489.4610; Found, 489.4613.
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