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A new class of androgen receptor antagonists bearing carborane in place of a steroidal skeleton

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Abstract—A new class of androgen receptor antagonists containing dicarba-*closo*-dodecaborane (carborane) as a hydrophobic skeletal structure is described. The designed molecules bear the characteristic polar components of endogenous agonists, but have a hydrophobic carborane cage instead of the steroidal skeleton. These compounds bind to androgen receptor and show anti-androgenic activity towards androgen-dependent SC-3 cells with almost the same potency as the known anti-androgen hydroxyflutamide.

Androgen receptor (AR), a member of the nuclear receptor superfamily, plays a key role of the development and maintenance of the male reproductive system.[1] AR ligands have been applied clinically; for instance, agonists are used for treatment of aplastic anaemia, and antagonists for prostate cancer.[2] Testosterone (**1**) and dihydrotestosterone (DHT: **2**) are well known endogenous AR agonists, and metribolone (**3**) and mibolerone are synthetic agonists with a steroidal skeleton (Fig. 1). These steroidal ligands have two polar moieties, a carbonyl group and a hydroxyl group, at the ends of the hydrophobic steroid skeleton. It is reasonable to assume that the two hydrophilic functional groups, as well as the hydrophobic core structure between them, play important roles for effective interaction with the AR ligand binding domain. Design and synthesis of biologically active compounds bearing a novel structural core may afford unique characteristics, and could provide useful therapeutic agents for a wide variety of conditions.

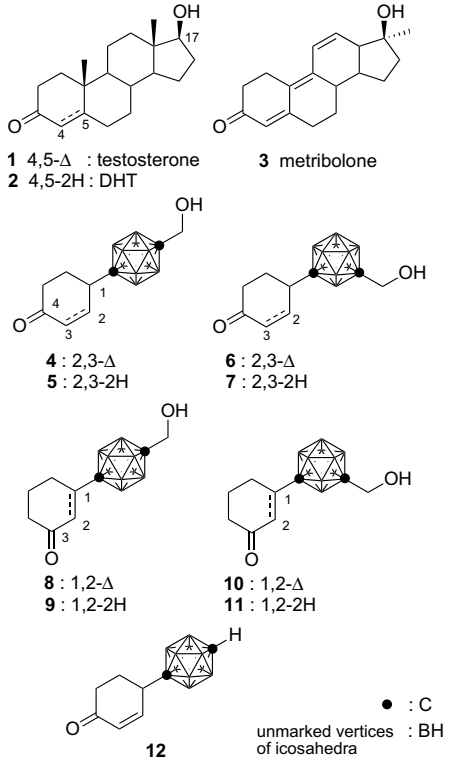

Figure 1. The structures of steroidal ligands and designed molecules.

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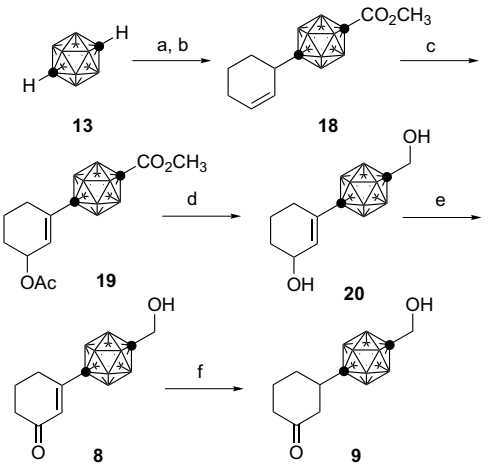
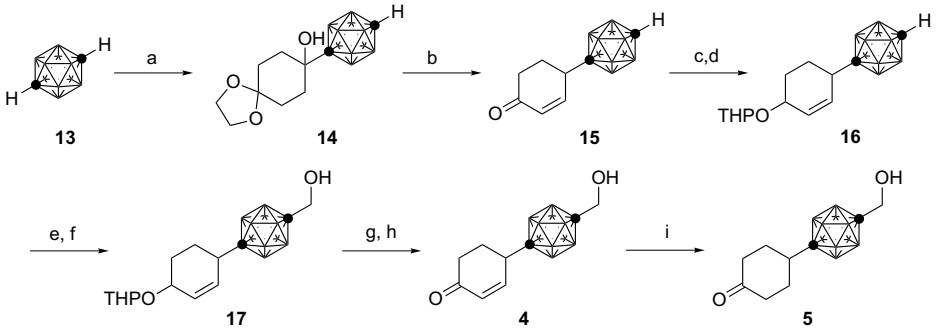
In our studies to develop new hydrophobic core structures for drug design, we have focused on dicarba-*closo*-dodecaborane (carborane)[3] as a hydrophobic component of biologically active molecules. The carboranes are icosahedral carbon-containing boron clusters with characteristic properties, such as spherical geometry and hydrophobicity. We have demonstrated that the hydrophobicity of the carboranes is comparable to that of hydrocarbon,[4] and their spherical hydrophobic surface effectively interacts with the hydrophobic surface of the ligand-binding domain of nuclear receptors.[5–7] Recently, we have reported a potent estrogen agonist bearing a carborane, 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (BE120),[5] which has an activity greater than that of 17β-estradiol in luciferase reporter gene assay using an estrogen receptor α (ERα) expression plasmid and in ERα binding assay.[5] These results suggested that the hydrophobic interaction along the spherical carborane cage produces a stronger interaction than that in the case of 17β-estradiol. The use of this new hydrophobic and spherical component, the carborane cage, for molecular drug design should make it possible to develop a wide variety of nuclear receptor ligands. In this article, we describe the synthesis and biological evaluation of androgen receptor modulators bearing carborane as a hydrophobic component.

Based on the above considerations, we designed carborane-containing AR ligands in which a cyclohexanone ring and a hydroxyl group are placed at the appropriate contralateral vertices of the hydrophobic carborane cage, as shown in Figure 1. Syntheses of the designed molecules are summarized in Schemes 1 and 2. For synthesis of the target compounds **4** and **5**, the construction of the cyclohexenone moiety corresponding to the A-ring of testosterone was a key process. We found that the lithiated *p*-carborane (**13**) reacts with the 1,4-cyclohexanedione-*mono*-ethyleneketal to give compound **14** in 87% yield. Deprotection of ketal followed by dehydration and isomerization of the double bond with concentrated sulfuric acid, readily converted **14** into the α,β-unsaturated ketone **15** in one pot with a yield of 86%. Then, the unsaturated ketone **15** was converted to the tetrahydropyranyl ether **16** by reduction with DIBAH followed by protection of the alcohol in 79% yield. To introduce the hydroxyl group on the contralateral vertex of the carborane cage, the reaction between the lithiated **16** and methyl chloroformate was employed, followed by reduction with $LiAlH_4$, affording the alcohol **17** in 88% yield. Deprotection of the THP group of **17** with TsOH followed by oxidation with MnO_2, afforded the enone form of **4** in 60% yield. Compound **5** was obtained from **4** by catalytic hydrogenation in 75% yield. The *m*-carborane derivatives **6** and **7** were prepared similarly, using *m*-carborane as a starting material. Synthesis of compounds with a carboranyl group at the 3-position of the cyclohexanone ring, such as **8** and **9**, is illustrated in Scheme 2. Condensation of lithiated carborane with cyclohexenone, and then introduction of a methoxycarbonyl group into the other C-vertex gave compound **18** in a yield of 46%. Oxidation of **18** with selenium oxide in acetic acid caused isomerization of the double bond to give compound **19** as a major product (34%). Reduction of both ester groups of **19** with $LiAlH_4$ (81%) followed by oxidation of the allylic hydroxyl group with MnO_2 afforded compound **8** in 82% yield. Compound **9** was obtained from **8** by catalytic hydrogenation (78%). *m*-Carborane derivatives



Scheme 2. Synthesis of designed molecules **8** and **9**. (a) *n*-BuLi, 3-bromocyclohexene/THF, 0 °C then 80 °C; (b) *n*-BuLi, $ClCO_2Me/Et_2O$, 0 °C then rt, 46% from *p*-carborane; (c) SeO_2/AcOH, 80 °C, 34%; (d) $LiAlH_4/Et_2O$, reflux, 81%; (e) MnO_2/CH_2Cl_2, rt, 82%; (f) H_2, Pd/C/MeOH, rt 78%.



Scheme 1. Synthesis of designed molecules **4** and **5**. (a) *n*-BuLi, 1,4-cyclohexanedione-*mono*-ethyleneketal/benzene, Et_2O, 0 °C then rt, 87%; (b) concd H_2SO_4, 80 °C, 86%; (c) DIBAH/benzene, rt, 99%; (d) 3,4-dihydro-2*H*-pyran, TsOH/CH_2Cl_2, rt, 80%; (e) *n*-BuLi, $ClCO_2Me/Et_2O$, 0 °C then rt, 88%; (f) $LiAlH_4/Et_2O$, 40 °C, q.y.; (g) TsOH/MeOH, rt, 86%; (h) MnO_2/CH_2Cl_2, rt, 70%; (i) H_2, Pd/C/MeOH, rt, 75%.

10–11 were synthesized in the same manner using *m*-carborane as a starting material.

For initial screening of the designed compounds, binding affinity to hAR was evaluated by competitive binding assay using [^{3}H]DHT and hAR.[8,9] Table 1 shows the synthesized molecules and their binding activity. The values indicate the percent displacement of specific [^{3}H]DHT binding to hAR by each compound at the concentration of 10 μM. Among the synthesized molecules, compounds **4** and **6**, bearing a carboranyl group at the 4-position of the cyclohexenone ring, exhibited binding activity to hAR. However, the other derivatives with a carboranyl group at the 3-position or with a saturated cyclohexanone ring, did not exhibit significant binding affinity.

To evaluate the activity of these carborane-containing compounds as transcriptional agonists and antagonists, co-transfection assay was conducted in mouse fibroblast cells NIH3T3, using expression plasmid for hAR and reporter plasmids, ARE/Luci (firefly luciferase), pRL/CMV (*Renilla* luciferase).[10] DHT at 1×10^{-12}–1×10^{-9} M induced the expression of luciferase in a dose-dependent manner, while none of the test compounds induced the expression at 1×10^{-7}–1×10^{-5} M. However, all of the test compounds, except for compound **5**, inhibited the activity of DHT in the concentration range of 1×10^{-7}–10^{-5} M in a dose-dependent manner. The results on the inhibition of transcriptional activity of DHT at the concentration of 10^{-10} M by the carborane-containing molecules (**4–11**) are summarized in Figure 2. In addition, compound **12**, which lacks a hydroxylmethyl group, also exhibited considerable transcriptional activity.[11]

Table 1. Binding affinity of compounds **4–11**

Compound	Binding affinity[a]
4	81
5	20
6	84
7	16
8	31
9	23
10	41
11	39

[a] Values are percentage displacement of [^{3}H]DHT (4 nM) specific binding to hAR by each compound at 10 μM.

The anti-androgenic activity of the representative *p*-carborane derivative **4** and *m*-carborane derivative **6** were confirmed by growth promotion/inhibition assay using androgen-dependent SC-3 cells.[8,12] None of the compounds showed growth-promoting activity in the absence of testosterone. On the other hand, these compounds inhibited testosterone-promoted cell growth of SC-3 (Fig. 3A). The potency of the antagonistic activity of **4** and **6** was comparable to that of hydroxyflutamide,

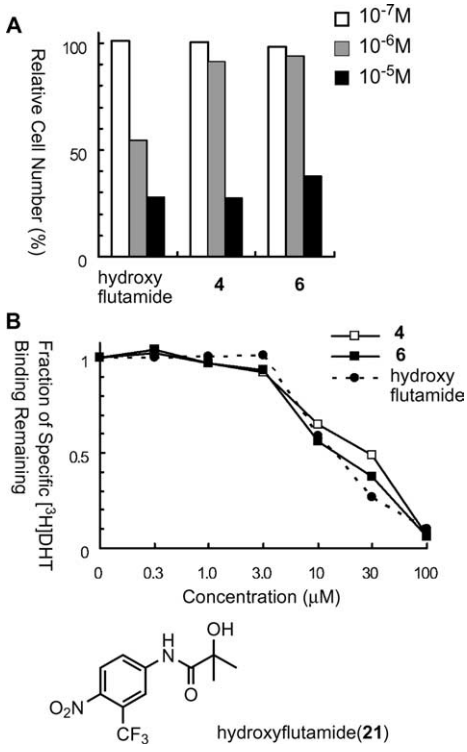


Figure 3. The antagonistic activities of **4** and **6**. (A) Each compound inhibited testosterone-promoted cell growth of SC-3. Cell number was normalized in the number in the absence of compound. Testosterone concentration was 10 nM ($n = 2$). (B) Results of competitive binding assay. The binding potencies of **4** and **6** were the same as that of hydroxyflutamide **21** ($n = 2$).

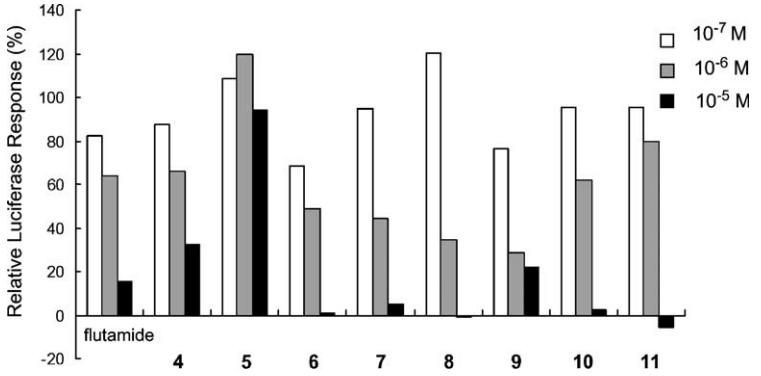


Figure 2. Inhibition of transcriptional activation of DHT by the test compounds (**4–11**). NIH3T3 cells were transfected with hAR expression vector, ARE/Luci (firefly luciferase) and pRL/CMV (*Renilla* luciferase) and incubated with the test compounds (10^{-7}–10^{-5} M) plus DHT(10^{-10} M)($n = 2$). Values are percentages of the transcriptional response of DHT(10^{-10} M).

which is a well-known active metabolite of the nonsteroidal anti-androgen flutamide. Dose–response curves in the competitive binding assay using [^{3}H]DHT and hAR were also obtained for compounds **4** and **6**, whose affinities were comparable to that of hydroxyflutamide (Fig. 3B).

The present experimental results indicate that the designed carborane-containing molecules act as androgen antagonists, even though we designed these compounds by analogy with potent carborane-containing ER agonists.[5] However, it is not unreasonable that the role of the bulky carborane cage in AR ligands differs from that in ER ligands, in view of the structural differences between the AR ligand binding domain (LBD) and the ER LBD. The ER ligand binding pocket is discriminated from those of other steroid receptors by its binding region for the planar A-ring residue and the direction of the A-ring residue.[13] Recent X-ray crystallographic analyses of AR complexed with agonists DHT (**2**)[14] and metribolone (**3**)[15] revealed the structural requirements for estrogenic activity. The carbonyl group on the A ring is hydrogen bonded to Arg-752 of hARLBD and the 17-β-hydroxyl group is hydrogen bonded to the Thr-877 residue. On the other hand, the binding mode of synthetic antagonists such as hydroxyflutamide and bicaltamide has not been clarified. Antagonism of nuclear receptors, in general, may result from conformational alteration such that helix 12 in the LBD cannot adopt an agonist-type conformation. Thus, the receptor–ligand complex may not readily interact with cellular coregulators.[16] In the case of the carborane-containing compounds, matching of the carborane structure with the hydrophobic region of the AR ligand binding pocket, and hydrogen bonding to AR may account for the binding affinity to AR. Of the two functional groups of the carborane-containing compounds, the hydroxyl group may not play an important role in hydrogen bonding, because of the activity of compound **12**, which lacks a hydroxymethyl group. In any case, the resulting conformation of the AR–ligand complex may not interact with cellular coregulators to exhibit antagonistic activity.

In summary, we have demonstrated a new class of AR antagonists bearing an icosahedral carborane skeletal and having a potency comparable to that of hydroxyflutamide. The antagonistic activities of these compounds can be attributed to introduction of the bulky and spherical carborane cage in place of the steroidal skeleton. The present results raise the possibility that structure–function studies of AR could lead to the development of more selective and potent androgen antagonists.

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