Bioorganic & Medicinal Chemistry 19 (2011) 3540–3548

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Crystal structure, docking study and structure–activity relationship of carborane-containing androgen receptor antagonist 3-(12-hydroxymethyl-1,12-dicarba-*closo*-dodecaboran-1-yl)benzonitrile

Kiminori Ohta [a], Tokuhito Goto [a], Shinya Fujii [b], Masatoshi Kawahata [c], Akifumi Oda [a], Shigeru Ohta [d], Kentaro Yamaguchi [c], Shuichi Hirono [e], Yasuyuki Endo [a,*]

[a] *Faculty of Pharmaceutical Sciences, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan*
[b] *Graduate School of Biomedical Science, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan*
[c] *Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, 1314-1 Shido, Sanuki, Kagawa 769-2193, Japan*
[d] *Graduate School of Medical Sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima 734-8551, Japan*
[e] *School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan*

ARTICLE INFO

Article history:
Received 3 March 2011
Revised 6 April 2011
Accepted 8 April 2011
Available online 20 April 2011

Keywords:
Androgen receptor
Antagonist
Carborane
X-ray crystallography
Docking study
Structure–activity study

ABSTRACT

A potent androgen receptor (AR) antagonist, 3-(12-hydroxymethyl-1,12 dicarba-*closo*-dodecaboran-1-yl)benzonitrile (**3a**, BA341), contains a *p*-carborane cage as a hydrophobic pharmacophore. We elucidated the structural properties of **3a** by means of single-crystal X-ray diffraction analysis and conducted a docking study of **3a** with hAR LBD. The cyano group of **3a** formed hydrogen bonds with Gln711 and Arg752 and the hydroxymethyl group did so with Asn705 and Thr877 in hAR LBD. The bulky *p*-carborane cage was accommodated in the hydrophobic pocket of hAR LBD. To understand the structure–activity relationship around the hydroxymethyl group of **3a**, we designed, synthesized, and evaluated the biological activities of various novel AR ligands. Since the biological activities of carbonyl compounds **8a**, **8b**, and **8c** were similar to or weaker than those of the parent hydroxyl compounds **3a**, **7a**, and **7b**, it seems to be necessary to have not only a hydrogen bonding acceptor, but also a hydrogen bonding donor adjacent to the hydroxymethyl group of **3a** for efficient interaction with hAR LBD.

1. Introduction

Dicarba-*closo*-dodecaboranes (carboranes) are chemical building blocks of remarkable thermal stability and high boron content; they are resistant to attack by most types of reagent, and are generally inactive towards biological systems.[1] One of their most striking features is the ability of the two carbon atoms and ten boron atoms to adopt icosahedral geometry, in which the carbon and boron atoms are hexa-coordinated (Fig. 1a).[2] This feature gives rise to the unusual properties of such molecules and their derivatives. Their properties make them uniquely suitable for various specialized applications, including polymers for high temperature use, neutron shielding, and optical signaling.[3] In the fields of medicinal chemistry and pharmaceutical sciences, incorporation of large numbers of boron atoms into tumor cells for boron neutron capture therapy (BNCT) has become of interest in the last few decades.[4] Most carborane-containing compounds that have so far been synthesized are composed of cellular building blocks (nucleic acids,[5] amino acids,[6] sugars,[7] and so on), to which carborane units have been added. On the other hand, we have focused on the exceptional hydrophobic character and spherical geometry of carboranes to develop novel and useful hydrophobic structures for application as hydrophobic pharmacophores in biologically active molecules that interact with receptors and regulate the function of the receptors.[8] Especially, we have developed carborane-containing ligands for nuclear receptors, such as estrogen receptor, retinoic acid receptor, and retinoid X receptor, and demonstrated by means of computational docking studies that the spherical hydrophobic surface of the carborane cage interacts effectively with the hydrophobic pocket of the nuclear receptor ligand binding domain (LBD).[9]

Androgens regulate diverse physiological processes involving both reproductive and non-reproductive functions.[10] Most of the signaling effects of androgens are mediated through the androgen receptor (AR), a member of the nuclear receptor superfamily of transcription factors.[11] Inadequate circulating levels of the natural AR ligands, testosterone and dihydrotestosterone (DHT), which is the active metabolite of testosterone generated by 5α-reductase, in hypogonadal men can be compensated by administration of

* Corresponding author. Tel.: +81 22 727 0142; fax: +81 22 275 2013.
E-mail address: yendo@tohoku-pharm.ac.jp (Y. Endo).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.04.017

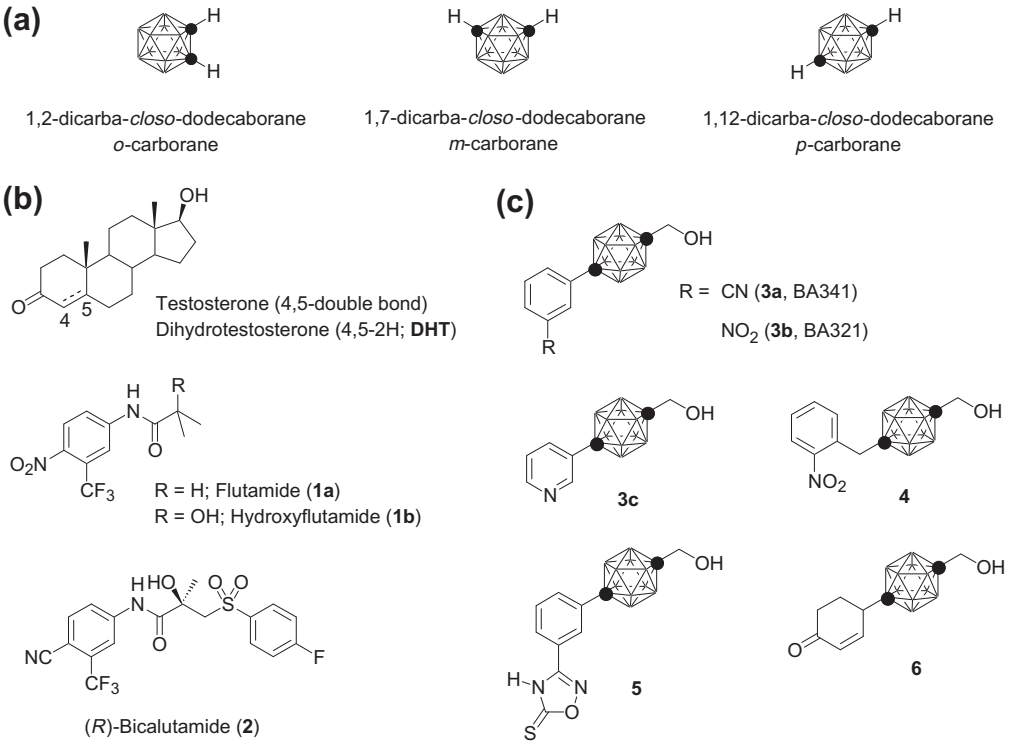


Figure 1. (a) Structures of icosahedral carboranes. (b) Structures of native androgens and androgen receptor antagonists, including pharmaceutical drugs. (c) Structures of carborane-containing inorganic AR antagonists developed in our previous studies.

exogenous androgens (Fig. 1b).[12] For decades, AR has been a target for drug development focused upon the treatment of pathological conditions arising from abnormal androgen levels or altered target tissue responsiveness, including improvement of physical performance, and regulation of male fertility. On the other hand, excessive stimulation of AR is thought to be closely related to prostate cancer.[13] Prostate cancer is the most common type of cancer and the second leading cause of cancer deaths in males.[14] The primary focus for drug discovery in this area is the synthesis of novel ligands to regulate the transcriptional activity of AR based on structural (steroidal or non-steroidal) and functional (androgenic, antiandrogenic, or anabolic) characteristics.[15] A non-steroidal ligand, flutamide (**1a**),[16] which is metabolized to a more potent antiandrogen, hydroxyflutamide (**1b**),[17] and bicalutamide, which is a racemate of a more potent (*R*)-bicalutamide (**2**)[18] and a less potent (*S*)-bicalutamide, are well-known AR antagonists used for the treatment of prostate cancer during its early stage (Fig. 1b).[19]

Binding of ligands to the AR ligand binding domain (LBD) primarily requires a nitro or cyano group, which forms a direct hydrogen bond with Arg752 of the hAR LBD.[20] This group is also hydrogen-bonded to Gln711 and Met745 via one H_2O molecule.[20] An X-ray co-crystal structure analysis of the complex of AR LBD with DHT indicated that the secondary alcohol group of DHT is hydrogen-bonded to Asn705 and Thr877 of hAR.[21] The hydrophobic group should be closely matched to the hydrophobic surface of the AR in order to increase the binding affinity. In the ligand-dependent control of nuclear receptors, a general and important structural feature has been elucidated by means of X-ray crystal analysis of complexes of several nuclear receptor LBDs with agonists and antagonists, that is, it is considered that the orientation of helix-12 is responsible for the agonist/antagonist function of nuclear receptors.[22] Therefore, for efficient design of nuclear receptor ligands, an understanding of the spatial relations between the ligand and helix-12 is absolutely essential.

Recently, we have reported potent AR antagonists bearing a carborane moiety, 3-(12-hydroxymethyl-1,12-dicarba-*closo*-dodecaboran-1-yl)benzonitrile (**3a**, BA341) and 3-(12-hydroxymethyl-1,12-dicarba-*closo*-dodecaboran-1-yl)nitrobenzene (**3b**, BA321), which are more potent than **1b** in AR-binding assay and cell proliferation assay (Fig. 1c).[23] Novel AR antagonists **3c**,[24] **4**,[25] and **5**[26] based on the structure of **3a** were also developed in our medicinal-chemical studies (Fig. 1c). Surprisingly, compound **6** acted as a potent AR antagonist, although it was designed based upon the structure of an AR agonist, testosterone (Fig. 1c).[27] We have not found any carborane-containing ligand that acts as an agonist for wild-type AR. These results support the idea that the bulky carborane cage favors expression of AR antagonistic activity. We have suggested that the carborane structure interacts sterically with Met895 and its surrounding amino acid residues in the hydrophobic pocket of hAR-LBD in such a way that helix-12 is moved away from the location required for agonist activity.[27]

We have been investigating the structure–activity relationships of **3a** around the cyanophenyl moiety and have explored novel bioisosteres of the cyano group. A wide range of the structure–activity relationship studies on **3a** is needed to aid the development of useful carborane-containing ligands, and docking simulation studies of **3a** with hAR LBD are also expected to be helpful for understanding the significance of the carborane cage in AR ligands. Here, we describe X-ray crystal structure analysis, docking studies with hAR LBD, and the structure–activity relationships of a number of novel analogues of **3a**.

2. Results and discussion

2.1. X-ray crystal structure of 3a

The potent AR antagonist, **3a**, was obtained according to the reported procedure.[22] Single crystals of **3a** were grown from a

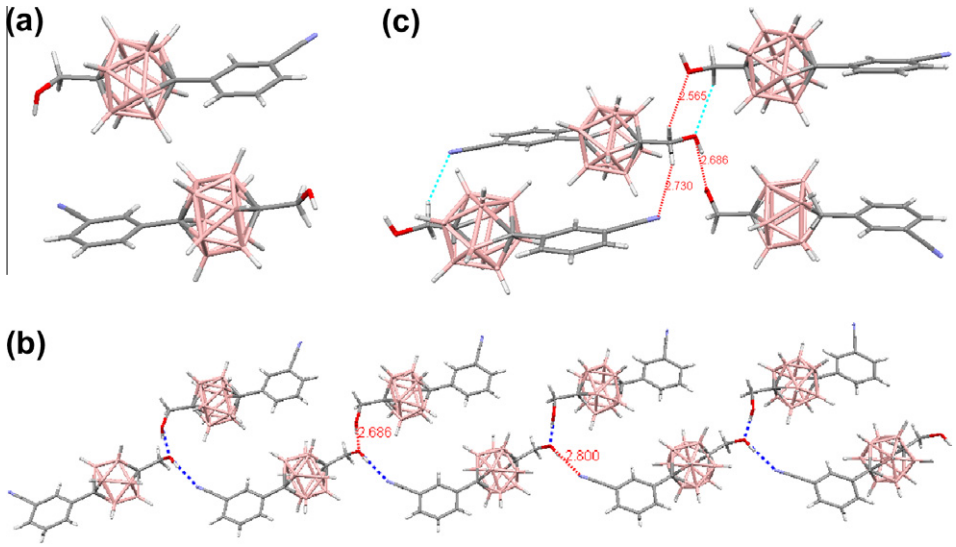


Figure 2. Various views of the X-ray structure of **3a**; Atom colors are C (gray), H (white), B (pink), N (blue), and O (red) (a) Ortep structure of **3a**. (b) Structure of OH···O and OH···N hydrogen bond networks; the distances between hetero atoms, O(H)···O and O(H)···N, are 2.686 and 2.800 Å, respectively. (c) Structure of interaction networks via weak hydrogen bonds, such as C–H···O and C–H···N; the distances of C–H···O and C–H···N, are 2.565 and 2.730 Å, respectively.

solution in $CHCl_3$ by slow evaporation of the solvent, and the solid-state structure was confirmed by single-crystal X-ray diffraction analysis. Compound **3a** crystallizes in the triclinic space group *P*−1. The crystal included two different structures of **3a** (Fig. 2a), which were designated as structure A and structure B (C(1)–C(10) and C(11)–C(20), respectively). Selected geometrical parameters of each structure are shown as Table 4 in Experimental section. All of the bond distances of structure A, except for C(8)–O(1), are shorter than those of structure B. The bond length of C(8)–O(1) of structure A is longer than the corresponding bond length of structure B owing to a more obtuse angle of C(9)–C(10)–O(1). The distance between the two carbon atoms, C(8) and C(9), of the *p*-carborane cage is 3.119 Å, which is 0.3 Å longer than the diameter (2.810 Å) of the benzene ring, C(3)–C(6). The distances between the hydrogen bonding acceptors, N(1)–O(1) and N(2)–O(2), which are essential for ligand-receptor binding, are 11.013 and 11.116 Å, respectively.

In the crystal packing of **3a**, the primary hydroxyl group forms two hydrogen bonds with a hydroxyl group and a cyano group of neighboring **3a** (Fig. 2b). The hydrogen of the hydroxyl group is used for formation of an O(H)···N hydrogen bond with a cyano group, and the oxygen of the hydroxyl group interacts with a hydroxyl group of neighboring **3a**, forming an O···(H)O hydrogen bond. The O···O and O···N distances are 2.686 and 2.800 Å, respectively. The methylene C–H hydrogen is activated by the electron-withdrawing character of the *p*-carborane cage and can behave as a hydrogen-bonding donor. Figure 2c shows a set of hydrogen-bonding networks, including classical and non-classical hydrogen bonds, in the crystal structure of **3a**. Both C–H hydrogens of a methylene group interact with the oxygen atom of the hydroxyl group and the cyano group of a neighboring **3a** via non-classical hydrogen bonds with distances of 2.565 and 2.730 Å, respectively. The steric bulkiness of the *p*-carborane cage has no effect on intermolecular hydrogen bond formation by the cyano and the hydroxymethyl groups of **3a**.

2.2. Docking study of 3a with hAR

Several studies on the three-dimensional structure of the complex formed by AR ligand and the hAR LBD have revealed the structural requirements for the appearance of agonist/antagonist activity. Docking simulation study of the potent AR antagonist **3a** is likely to be of great significance for the development of carborane-containing AR ligands with various biological profiles, such as partial agonists, selective androgen receptor modulators (SARMs), and pure AR antagonists for numerous mutated ARs. However, there is no report of docking simulation studies of carborane-containing AR ligand with hAR LBD. Initially, a docking simulation of wild-type hAR LBD with the carborane-containing ligand, **3a**, using the co-crystal structure of hAR LBD with the AR antagonist, metribolone (PDB: 1E3G), was performed with an automatic docking program (FlexX). Arg752 and Gln711 in hAR LBD, which are essential amino acid residues for receptor-ligand binding, interact directly with the cyano group of **3a**, with N···N distances of 2.49 and 2.86 Å, respectively (Fig. 3). The primary hydroxyl group of **3a** interacts with the carbonyl group of Asn705 and the hydroxyl group of Thr877, with O···O distances of 3.09 and 2.78 Å, respectively. Two C–H hydrogens of the methylene group of **3a** showed no clear interaction with amino acid residues of hAR LBD. The highly hydrophobic *p*-carborane cage is located in the hydrophobic pocket of the receptor. Four hydrogen bonds and the hydrophobic interaction in the ligand-receptor complex appear to be essential for high binding affinity of **3a** to the hAR. The *p*-carborane cage of **3a** is close to Met895, which is a part of helix-12, and it seems that the steric bulkiness of the *p*-carborane cage causes helix-12 to move from the agonist to the antagonist form. In our previous study, compounds with a hydroxyl group and a hydroxyethyl group instead of the hydroxymethyl group of **3a** showed much weaker binding affinity than that of **3a**.[22] It would appear that the spatial arrangement among the hydrogen-bonding functional groups and the *p*-carborane cage of **3a** is the most appropriate for forming the necessary hydrogen bonds and hydrophobic interaction with hAR LBD.

2.3. Design and synthesis of novel AR ligands

It is of great interest to examine the structure–activity relationships around the hydroxymethyl group of **3a** since this group lies near helix-12, according to the result of the docking study. Thus, we designed novel AR ligands, compounds **7a**–**7d** with a secondary or tertiary hydroxyl group, to examine the role of the region around Asn705, Thr877, and Met895 in hAR LBD (Fig. 4). We also designed

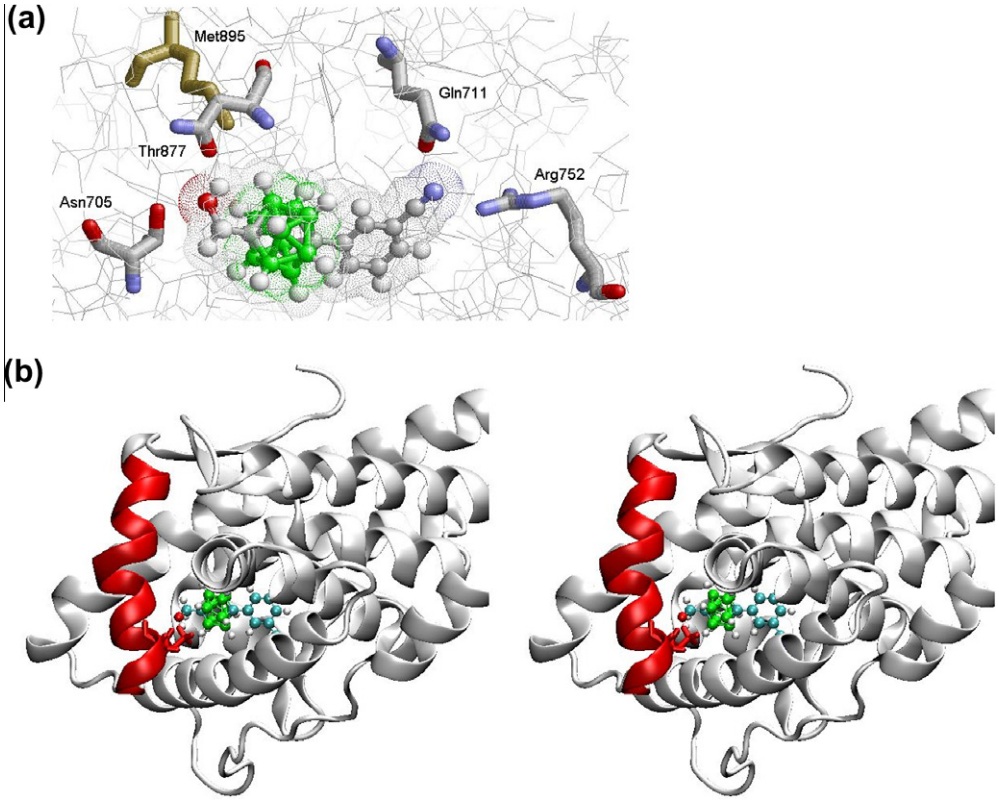
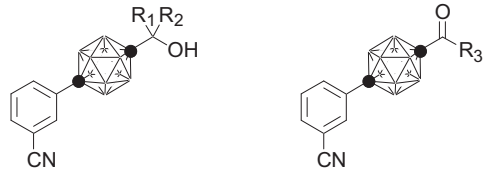
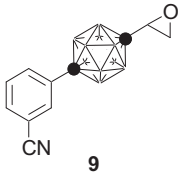


Figure 3. Docking model of **3a** to the hAR LBD. (a) Hydrogen bondings of **3a** with four amino acid residues in hAR LBD and the arrangements of Met895 (gold) and the *p*-carborane cage (green) of **3a**. (b) Stereoview of the overall picture of the docking model. Met895 and helix-12, carbon, and boron atoms are colored red, blue, and green, respectively.



7a (R_1 = H, R_2 = CH_3)
7b (R_1 = H, R_2 = Ph)
7c (R_1 = CH_3, R_2 = CH_3)
7d (R_1 = CH_3, R_2 = Ph)

8a (R_3 = H)
8b (R_3 = CH_3)
8c (R_3 = Ph)



9

Figure 4. Design of new androgen receptor antagonists **7**–**9**.

carbonyl compounds **8a**–**8c** and epoxide **9**, with hydrogen-bonding acceptors instead of the primary hydroxyl group of **3a**.

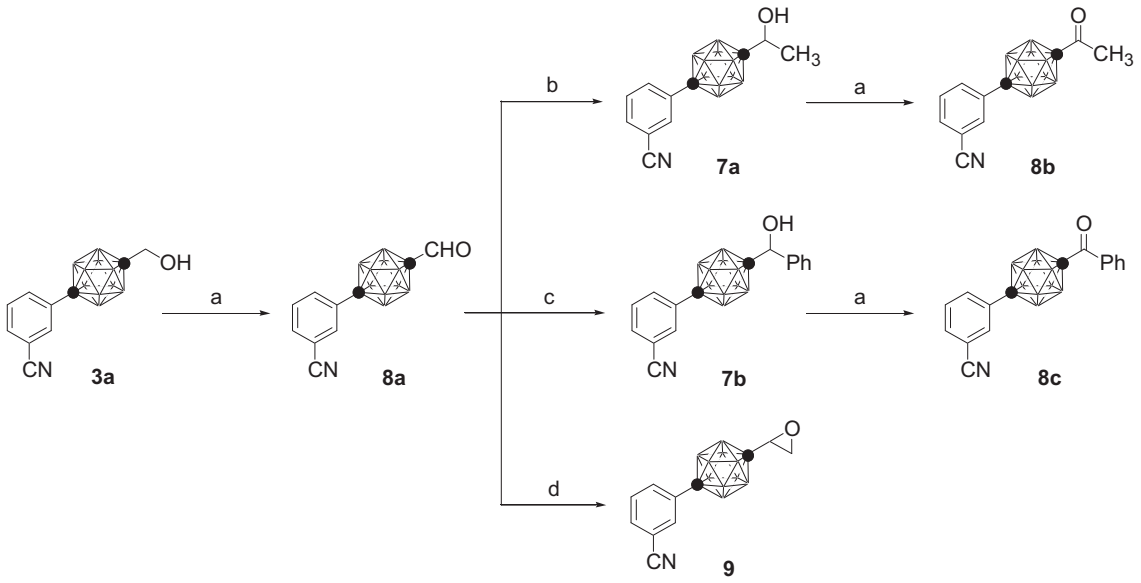
The synthesis of the designed compounds **7**–**9** is summarized in Scheme 1. The potent AR antagonist, **3a**, was treated with Dess–Martin reagent in CH_2Cl_2 to afford the key intermediate **8a** in 91% yield.[28] Compound **8a** was reacted with Grignard reagents, methylmagnesium bromide (CH_3MgBr) and phenylmagnesium bromide (PhMgBr), in THF at −78 °C to afford the secondary alcohols **7a** and **7b** in 84% and 77% yields, respectively, followed by oxidation reaction with Dess–Martin reagent in CH_2Cl_2 to afford the corresponding carbonyl compounds **8b** and **8c** in 90% and 93% yields, respectively. Transformation of the aldehyde **8a** into epoxide **9** was accomplished by Simmons-Smith reaction using diethylzinc, chloroiodomethane and tetrahydrothiophene in 71% yield.[29]

The synthesis of the tertiary alcohols **7c** and **7d** is summarized in Scheme 2. First, we tried the reaction of ketones **8b** and **8c** with CH_3MgBr but the starting materials were recovered, and the desired products were not obtained. Next, the reactions of **8b** and **8c** with methyllithium (CH_3Li) reagent were performed in THF at −78 °C. The yields of the products **7c** and **7d** in this reaction are summarized in Table 1.

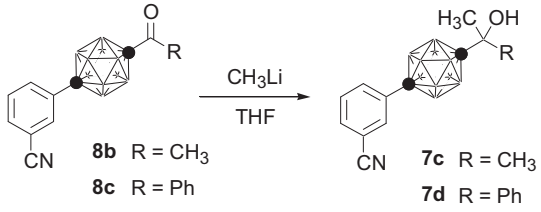
Compound **8b** was treated with 3.0-fold molar excess of CH_3Li to afford only the desired product **7c** in 58% yield (Entry 2). Use of 13.4-fold molar excess of CH_3Li afforded not only the corresponding tertiary alcohol **7c** in 14% yield, but also the 3-acetylphenyl derivatives **10** in 42% yield, which is generated by the reaction of the benzonitrile moiety with CH_3Li (Entry 3, Fig. 5). Since the steric bulkiness of the carborane cage hindered nucleophilic attack of the lithium reagent on the aldehyde, an excess of lithium reagent was necessary to obtain the desired compounds. When the sterically crowded ketone **8c** was used as a starting material, no reaction was occurred with 3.0-fold molar excess of CH_3Li (Entry 4). Finally, the desired product **7b** was obtained by the use of 8.9-fold molar excess of CH_3Li, in 81% yield (Entry 5). The corresponding acetophenone derivative was not observed in this reaction. Compounds **7a**, **7b**, **7d**, and **9** have an asymmetric carbon and were used for biological assays in racemic form.

2.4. Biological evaluations

The binding affinity of the new carborane-containing compounds for AR was evaluated by means of competitive-binding assay using [1,2-^{3}H]dihydrotestosterone ([^{3}H]DHT) and hAR.[30]



Scheme 1. Synthesis of analogues of **3a**. Reagents: (a) Dess–Martin reagent, CH_2Cl_2; (b) CH_3MgBr, THF; (c) PhMgBr, THF; (d) Et_2Zn, $ClCH_2I$, tetrahydrothiophene CH_2Cl_2.



Scheme 2. The reaction of *C*-acylcarborane derivatives **7c** and **7d** with methyllithium.

Table 1
Yields of the desired products **7** in the reaction of **8** with methyl lithium[a]

Entry	Compound	R	CH_3Li (equiv)	Yield (%)
1	**8b**	CH_3	1.0	0 (**7c**)
2	**8b**	CH_3	3.0	58 (**7c**)
3	**8b**	CH_3	13.4	14 (**7c**)[b]
4	**8c**	Ph	3.0	0 (**7d**)
5	**8c**	Ph	8.9	81 (**7d**)

[a] The reactions were performed at -78 °C for 30 min.
[b] 3-Acetylphenyl derivatives **10** was obtained in 42% yield.

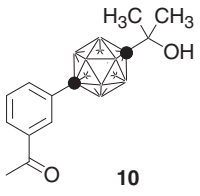


Figure 5. Structure of the by-product **10** obtained from the reaction of **8b** with methyl lithium.

Table 2 summarizes the binding affinity data of the synthesized compounds: the values indicate the percent displacement of specific [^{3}H]DHT binding to hAR by each compounds at the concentration of 10 μM. Compounds **7a**, **7b** and **8a** showed high binding affinity for hAR but were less potent binders than **3a**. The binding affinity of the synthesized compounds was affected by the hydrogen-bonding groups with Asn705 and Thr877 of AR LBD and the size of the substituents R_1, R_2, and R_3.

Table 2
Biological properties of the designed compounds **7**–**10**

Compound	Binding affinity[a] (%)	IC_{50}[b] (μM)
7a	78	0.32
7b	71	1.80
7c	52	0.33
7d	54	0.61
8a	78	0.17
8b	16	0.48
8c	24	1.67
9	33	nt[c]
10	44	5.39
3a (BA341)	95	0.15

[a] Values are percentage displacement of [^{3}H]DHT (4 nM) specific binding to hAR by each compound at 10 μM. All binding assays were performed in duplicate (n = 2), and the average value is indicated as the binding affinity.
[b] NIH3T3 cells were transiently transfected with hAR-expression plasmid, ARE/Luci (*firefly* luciferase) and pRL/CMV (*Renilla* luciferase) plasmids, and treated with the test compounds in the presence of DHT (1×10^{-10} M). IC_{50} values of the test compounds were estimated from the sigmoidal dose–response curves using GraphPad Prism 4 software.
[c] nt indicates not tested.

To evaluate the functional activity, agonist or antagonist, of the synthesized compounds, except for **9**, transcriptional assay was done with hAR-expression plasmid, ARE/Luci (*firefly* luciferase) and pRL/CMV in NIH3T3 cells.[31] None of the synthesized compounds exhibited AR-agonistic activity at the concentration of 1×10^{-5}–1×10^{-8} M. These compounds dose-dependently inhibited the transcriptional activation induced by 1×10^{-10} M DHT and acted as AR antagonists. Table 2 summarizes the IC_{50} values of **3a** analogues **7–10** in AR transactivation assay. Compounds **7a** and **7c**, which have methyl groups on the methylene carbon, showed potent inhibitory activity for DHT-induced AR transactivation, and their IC_{50} values were 0.32 and 0.33 μM, respectively. Although their inhibitory activities are two-fold weaker than that of the parent compound **3a**, methyl groups seem to be allowed at that position. Meanwhile, introduction of a phenyl group on the methylene carbon led to a marked decrease of DHT-induced AR transactivation (Table 2; **7b** and **7d**).

Carbonyl-containing compounds **8a**–**8c** showed similar structure–activity relationships to the alcohol derivatives, and their activity was markedly decreased with increasing size of substituents connected with the carbonyl group. Compound **10** with an acetyl

group instead of the cyano group showed weak AR antagonistic activity and the highest IC_{50} value, 5.39 μM, among the evaluated compounds. Compound **8a** exhibited the most potent inhibitory activity for DHT-induced AR transactivation (IC_{50} = 0.17 μM), and its IC_{50} value is similar to that of **3a** (IC_{50} = 0.15 μM). As regards the biological activities of these derivatives, the alcohol derivatives showed better biological profiles than the carbonyl derivatives (**3a** vs **8a** and **7a** vs **8b**). This can be explained in terms of the existence or not of hydrogen bonding between the hydroxyl group of the ligands and the carbonyl group of Asn705 in hAR LBD. From the SAR studies of these compounds, it seems that Asn705 and Thr877 in hAR LBD require hydrogen-bonding donors and acceptors, respectively, in the carborane-containing ligands for receptor-ligand binding. In addition, a large substituent, such as phenyl group, around Thr877 is unfavorable for AR antagonistic activity.

3. Conclusions

We have established the molecular structure of the carborane-containing potent AR ligand **3a** by means of X-ray crystal structure analysis, and examined the binding mode of **3a** with hAR LBD by means of computational docking study using the FlexX program. As expected, the cyano group of **3a** interacts with Arg752 and Gln711, and the hydroxymethyl group forms hydrogen bonds with Thr877 and Asn705. The *p*-carborane cage is located along the hydrophobic surface of hAR LBD and lies close to Met895, which is associated with helix-12. Compound **3a** derivatives designed based upon the docking study showed potent AR-binding affinity and AR antagonistic activity. The hydrogen bonds between the carborane-containing ligands and the four amino acid residues in hAR LBD are important for the receptor-ligand binding, and the steric repulsion of *p*-carborane cage with helix-12 results in the AR antagonistic activity of carborane-containing AR ligands. These findings are expected to be helpful for the development of novel carborane-containing anti-androgens, having a different spectrum of ligand responsiveness from other therapeutic agents.

4. Experimental

4.1. General considerations

Melting points were determined with a Yanagimoto micro melting point apparatus without correction. ^{1}H NMR, ^{13}C NMR and ^{10}B NMR spectra were recorded with JEOL JNM-LA-400 and JNM-FX-400 spectrometers. Chemical shifts for ^{1}H NMR spectra were referenced to tetramethylsilane (0.0 ppm) as an internal standard. Chemical shifts for ^{13}C NMR spectra were referenced to residual ^{13}C present in deuterated solvents. Chemical shift values for ^{11}B spectra were referenced to external BF_3•OEt (0.0 ppm, with negative values upfield). The chemical shifts are reported in ppm (δ scale) and all coupling constant (J) values are given in hertz (Hz). The splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). Mass spectra were recorded on a JEOL JMS-DX-303 spectrometer. Elemental analyses were performed with a Perkin Elmer 2400 CHN spectrometer. Unless otherwise noted, the reagents and solvents were purchased from Aldrich Chemical Co., TCI, Kanto Chemicals, or Wako Chemicals, Inc. and were used as received. *p*-Carborane was purchased from Katchem s.r.o. (Prague, Czech Republic). Compound **3a** was prepared according to the literature.[23]

4.2. X-ray crystallography

Details of data collection and structure refinement for **3a** are given in Table 3. Diffraction data were obtained with a Rigaku AFC7S four-circle diffractometer with graphitemonochromated Mo Ka radiation (λ = 0.71073 Å). Generally, indexing was performed from three oscillation images exposed for 4.0 min, and a total of 15 oscillation images within the 2θ value of 50.0° were collected with the imaging plate area detector. The structure was solved by direct methods using SHELXS-97[32] and refined by the full matrix least-squares technique on F2 using SHELXL-97.

4.3. Docking study

The most stable model for **3a** was estimated using the automatic docking program FlexX version 1.2[33] implemented in SYBYL 6.91 (Tripos, Inc., St. Louis, MO). For the docking calculation, the quantum-chemically optimized structure of ligand **3a** was used as the initial structure. The optimization of the crystal structure of **3a** was carried out with B3LYP/6-31G(d,p) using GAUSSIAN 98 (Gaussian, Inc., Wallingford, CT). The structure of the hAR LBD was obtained from the crystal structure of the hAR LBD-metribolone complex (PDB code: 1E3G) by removing the metribolone molecule. All hydrogen atoms that are missing in the crystal structure but should exist in the protein structure were computationally located at appropriate positions by use of the Biopolymer module of SYBYL 6.91. The active site of hAR LBD was defined as the collection of amino acids for which at least one atom was closer than 6.5 Å to any atom of the bound metribolone. For FlexX calculation, the maximum number of generated poses was set to 500 and the parameter MAX_OVERLAP_VOL was expanded to 8.0 $Å^3$. Default values were used for other parameters, and the treatment of the ligand molecule was the same as in a previous study. [21] The generated docking poses were evaluated by means of a consensus scoring scheme using the CScore module of SYBYL 6.91, and the top-ranked pose which won the highest number of votes was selected.

4.4. Synthesis

4.4.1. 3-(12-Formyl-1,12-dicarba-*closo*-dodecaboran-1-yl)benzonitrile (8a)

To a solution of **3a** (146 mg, 0.53 mmol) in 3 mL of dry CH_2Cl_2 was added Dess–Martin periodinate (450 mg, 1.06 mmol). The reaction mixture was stirred for 8 h at room temperature under

Table 3
Crystal data of **3a**

Compound	**3a**
Formula	$C_{10}H_{17}B_{10}NO$
M_r	275.35
Recryst. solvent	$CHCl_3$
Crystal system	Triclinic
Lattice parameter	
a (A)	11.5045 (13)
b (A)	12.7194 (15)
c (A)	13.1240 (15)
α (°)	107.2910 (10)
β (°)	109.8900 (10)
γ (°)	107.4630 (10)
V ($Å^3$)	1542.5 (3)
Space group	P−1
Z value	4
P_{calc} (Mg/m^3)	1.186
Absorption coefficient (mm^{-1})	0.063
Crystal size (mm^3)	0.50 × 0.50 × 0.50
Temperature (K)	150
2θ max (°)	56.5
Reflections collected	15282
Independent reflections	6589
Data/restraints/parameters	6589/0/483
Goodness-of-fit on F^2	0.94
Residuals: R, Rw	0.0593, 0.1540
CCDC reference number	787463

Table 4
Comparison of selected geometrical parameters of each structure of **3a**

Distance and angle	Structure A	Structure B
C(1)–C(7)	1.435	1.440
C(3)–C(6)	2.810	2.804
C(3)–C(8)	1.506	1.509
C(8)–C(9)	3.119	3.130
C(9)–C(10)	1.534	1.541
C(8)–O(1)	5.401	5.386
C(10)–O(1)	1.400	1.424
C(7)–N(1)	1.142	1.146
C(10)–N(1)	10.054	10.077
B(1)–B(9)	3.373	3.373
N(1)–O(1)	11.013	11.116
C(9)–C(10)–O(1)	113.43	111.72

an Ar atmosphere, then filtered through Celite and the filtrate was concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/AcOEt 20:1) to afford 129 mg (89%) of compound **8a** as a colorless solid; Colorless cubes (CH_2Cl_2/*n*-hexane); mp 127.5–128.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 7.33 (m, 1H), 7.44 (dt, J = 1.3 Hz, 8.2 Hz, 1H), 7.48 (m, 1H), 7.55 (dt, J = 1.3 Hz, 7.6 Hz, 1H), 8.87 (s, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 81.9, 84.1, 112.6, 117.7, 129.1, 130.4, 131.1, 132.1, 137.2, 185.4; MS (EI) *m/z* 273 (M^+, 100%); Anal. Calcd for $C_{10}H_{15}B_{10}NO$: C, 43.94; H, 5.53; N, 5.12. Found: C, 44.19; H, 5.61; N, 5.26.

4.4.2. 3-[12-(1-Hydroxyethyl)-1,12-dicarba-*closo*-dodecaboran-1-yl]benzonitrile (7a)

To a solution of **8a** (100 mg, 0.37 mmol) in 4 mL of dry THF was added CH_3MgBr (2.8 mL, 2.59 mmol) at -78 °C under an Ar atmosphere. The reaction mixture was stirred for 21 h at the same temperature, then quenched with saturated aqueous NH_4Cl solution, and extracted with AcOEt. The organic solution was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/CH_2Cl_2 1:2 to *n*-hexane/AcOEt 10:1) to afford 58 mg (54%) of compound **7a** as a colorless solid; Colorless cubes (CH_2Cl_2/*n*-hexane); mp 114–115 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 1.12 (d, J = 6.3 Hz, 3H), 1.73 (br s, 1H), 3.75 (quint, J = 6.3 Hz, 1H), 7.30 (m, 1H), 7.45 (m, 1H), 7.50 (m, 1H), 7.51 (m, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 22.9, 69.3, 81.0, 87.7, 112.6, 118.1, 129.1, 130.8, 131.5, 131.9, 137.6; MS (EI) *m/z* 289 (M^+, 100%); Anal. Calcd for $C_{11}H_{19}B_{10}NO$: C, 45.65; H, 6.62; N, 4.84. Found: C, 45.79; H, 6.64; N, 4.93.

4.4.3. 3-[12-(1-Hydroxybenzyl)-1,12-dicarba-*closo*-dodecaboran-1-yl]benzonitrile (7b)

To a solution of **8a** (100 mg, 0.37 mmol) in 4 mL of dry THF was added PhMgBr (0.72 mL, 0.74 mmol) at -78 °C under an Ar atmosphere. The reaction mixture was stirred for 18 h at the same temperature, quenched with saturated aqueous NH_4Cl solution, and extracted with AcOE. The organic solution was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/AcOEt 10:1) to afford 100 mg (77%) of compound **7b** as a colorless solid; Colorless prisms (CH_2Cl_2/*n*-hexane); mp 135.5–137.0 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 1.59 (s, 1H), 4.67 (s, 1H), 7.13–7.53 (m, 9H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 75.7, 81.5, 86.9, 112.5, 118.1, 126.7, 128.1, 128.8, 129.0, 130.8, 131.5, 131.9, 137.6, 139.9; MS (EI) *m/z* 351 (M^+), 107 (100%); Anal. Calcd for $C_{16}H_{21}B_{10}NO$: C, 54.68; H, 6.02; N, 3.99. Found: C, 54.60; H, 6.10; N, 4.09.

4.4.4. 3-(12-Acetyl-1,12-dicarba-*closo*-dodecaboran-1-yl)benzonitrile (8b)

Compound **8b** was prepared by the same method as described for the synthesis of **8a**; 90% yield; Colorless prisms (CH_2Cl_2/*n*-hexane); mp 108.0–108.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 2.12 (s, 3H), 7.30 (dd, J = 7.5 Hz, 8.6 Hz, 1H), 7.45 (ddd, J = 1.2 Hz, 2.0 Hz, 8.1 Hz, 1H), 7.50 (t, J = 1.5 Hz, 1H), 7.54 (ddd, J = 1.3 Hz, 1.5 Hz, 7.6 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 27.3, 83.1, 84.6, 112.6, 117.8, 129.1, 130.4, 131.2, 132.0, 137.2, 192.2; MS (EI) *m/z* 287 (M^+, 100%); Anal. Calcd for $C_{11}H_{17}B_{10}NO$: C, 45.97; H, 5.96; N, 4.87. Found: C, 45.75; H, 5.81; N, 4.78.

4.4.5. 3-(12-Benzoyl-1,12-dicarba-*closo*-dodecaboran-1-yl)benzonitrile (8c)

Compound **8c** was prepared by the same method as described for the synthesis of **8a**; 93% yield; Colorless cotton (CH_2Cl_2/*n*-hexane); mp 143.5–144.0 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 7.29–7.55 (m, 9H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 84.3, 112.6, 117.8, 127.8, 128.0, 129.0, 130.4, 130.7, 131.1, 131.9, 132.2, 135.9, 137.2, 189.1; MS (EI) *m/z* 349 (M^+), 105 (100%); Anal. Calcd for $C_{16}H_{19}B_{10}NO$: C, 54.99; H, 5.48; N, 4.01. Found: C, 54.77; H, 5.45; N, 3.90.

4.4.6. 3-[12-(1-Hydroxy-1-methyl)ethyl-1,12-dicarba-*closo*-dodecaboran-1-yl]benzonitrile (7c)

To a solution of **8b** (100 mg, 0.35 mmol) in 5 mL of dry ether was added CH_3Li (1.02 mL, 1.05 mmol) at −78 °C under an Ar atmosphere. The reaction mixture was stirred for 30 min at the same temperature, quenched with saturated aqueous NH_4Cl solution, and extracted with AcOEt. The organic solution was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/AcOEt 20:1) to afford 61 mg (58%) of compound **7c** as a colorless solid; Colorless needles (CH_2Cl_2/*n*-hexane); mp 172.5–174.0 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 1.23 (s, 6H), 2.05 (s, 3H), 7.30 (m, 1H), 7.45 (ddd, J = 1.3 Hz, 2.1 Hz, 8.4 Hz, 1H), 7.50 (m, 1H), 7.52 (ddd, J = 1.3 Hz, 1.5 Hz, 7.1 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 31.6, 70.9, 81.6, 92.8, 112.5, 118.0, 128.9, 130.7, 131.4, 131.8, 137.5; MS (EI) *m/z* 305 (M^++2), 288 (100%); Anal. Calcd for $C_{12}H_{21}B_{10}NO$: C, 47.50; H, 6.98; N, 4.62. Found: C, 47.21; H, 6.85; N, 4.48.

4.4.7. 3-[12-(1-Hydroxy-1-phenyl)ethyl-1,12-dicarba-*closo*-dodecaboran-1-yl]benzonitrile (7d)

To a solution of **8c** (50 mg, 0.14 mmol) in 3 mL of dry THF was added CH_3Li (1.2 mL, 1.25 mmol) at −78 °C under an Ar atmosphere. The reaction mixture was stirred for 30 min at the same temperature, quenched with saturated aqueous NH_4Cl solution, and extracted with AcOEt. The organic solution was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/AcOEt 10:1) to afford 42 mg (81%) of compound **7d** as a colorless solid; Colorless leaflets (CH_2Cl_2/*n*-hexane); mp 199.5–201.0 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 1.65 (s, 3H), 7.2–7.6 (m, 9H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 44.4, 74.0, 82.0, 82.9, 112.4, 118.0, 121.6, 125.5, 127.7, 128.9, 130.6, 131.4, 131.7, 131.8, 137.5; MS (EI) *m/z* 366 (M^++1), 121 (100%); Anal. Calcd for $C_{15}H_{23}B_{10}NO$: C, 55.87; H, 6.34; N, 3.83. Found: C, 55.57; H, 6.36; N, 3.64.

4.4.8. 3-[12-(1,2-Epoxyethane)-1,12-dicarba-*closo*-dodecaboran-1-yl]benzonitrile (9)

To a solution of tetrahydrothiophene (727 mg, 8.24 mmol) in 50 mL of dry CH_2Cl_2 was added chloroiodomethane (0.6 mL, 8.24 mmol), and the mixture was stirred for 30 min at room tem-

perature under an Ar atmosphere. To the reaction mixture was added **8a** (1.13 g, 4.12 mmol), followed by a solution of Et_2Zn (1.0 M in hexane, 4.12 mL, 4.12 mmol). The reaction mixture was stirred for 6.5 h at the same temperature, quenched with water, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/AcOEt 10:1) to afford 843 mg (71%) of compound **9** as a colorless solid; Colorless cotton (CH_2Cl_2/*n*-hexane); mp 124–125 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 2.46 (dd, J = 2.3 Hz, 4.9 Hz, 1H), 2.64 (dd, J = 3.4 Hz, 4.8 Hz, 1H), 2.88 (dd, J = 2.3 Hz, 3.8 Hz, 1H), 7.31 (dt, J = 0.7 Hz, 7.6 Hz, 1H), 7.43 (ddd, J = 1.2 Hz, 2.1 Hz, 8.1 Hz, 1H), 7.48 (t, J = 1.5 Hz, 1H), 7.52 (ddd, J = 1.3 Hz, 1.5 Hz, 7.6 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 48.1, 52.7, 79.3, 81.3, 112.8, 118.0, 129.4, 130.7, 131.6, 132.2, 137.5; MS (EI) *m/z* 287 (M^+, 100%); Anal. Calcd for $C_{11}H_{17}B_{10}NO$: C, 45.97; H, 5.96; N, 4.87. Found: C, 45.81; H, 5.82; N, 4.80.

4.4.9. 3-[12-(1-Hydroxy-1-methyl)ethyl-1,12-dicarba-*closo*-dodecaboran-1-yl]acetophenone (10)

To a solution of **8b** (150 mg, 0.52 mmol) in 7 mL of dry THF was added CH_3Li (6.71 mL, 6.98 mmol) at −78 °C under an Ar atmosphere. The reaction mixture was stirred for 30 min at the same temperature, quenched with saturated aqueous NH_4Cl solution, and extracted with AcOEt. The organic solution was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/AcOEt 20:1) to afford 70 mg (42%) of compound **10** as a colorless solid and 19 mg (14%) of compound **7c** as a colorless solid; Colorless cotton (CH_2Cl_2/*n*-hexane); mp 108.5–109.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 2.12 (s, 3H), 7.30 (dd, J = 7.5 Hz, 8.6 Hz, 1H), 7.45 (ddd, J = 1.2 Hz, 2.0 Hz, 8.1 Hz, 1H), 7.50 (t, J = 1.5 Hz, 1H), 7.54 (ddd, J = 1.3 Hz, 1.5 Hz, 7.6 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 26.6, 31.5, 70.7, 82.8, 92.1, 126.7, 128.1, 128.2, 131.4, 136.6, 136.7, 196.9; MS (EI) *m/z* 320 (M^+), 305 (100%); Anal. Calcd for $C_{13}H_{24}B_{10}O_2$: C, 48.73; H, 7.55. Found: C, 48.43; H, 7.68.

4.5. Biological evaluation

4.5.1. Competitive-binding assay with hAR[30]

Binding affinities of test compounds for hAR (human androgen receptor) were measured in competition experiments using [^{3}H]DHT and cytosolic fraction of hAR-LBD (hAR ligand-binding domain)-transformed *Escherichia coli*. hAR-LBD expression plasmid vector encoding GST-hARLBD (627–919 aa, EF domain) fusion protein under the *lac* promoter (provided by Prof. S. Kato, University of Tokyo) was transfected into *E. coli* strain *HB-101*. An overnight culture (10 mL) of the bacteria was added to 1 L of LB medium and incubated at 27 °C until its optical density reached 0.6–0.7 at 600 nm. Following the addition of IPTG to a concentration of 1 mM, incubation was continued for an additional 4.5 h. Cells were harvested by centrifugation at 4000*g* at 4 °C for 15 min and stored at −80 °C until use. All subsequent operations were performed at 4 °C. The bacterial pellet obtained from 40 mL of culture was resuspended in 1 mL of ice-cold TEGDM buffer (10 mM Tris–HCl pH 7.4, 1 mM EDTA, 10% glycerol, 10 mM DTT, 10 mM sodium molybdate). This suspension was subjected to sonication (10 × 10 s bursts) on ice, and crude GST-hARLBD fraction was prepared by centrifugation of the suspension at 12,000*g* for 30 min at 4 °C. This crude receptor fraction was diluted with buffer (20 mM Tris–HCl pH 8.0, 0.3 M KCl, 1 mM EDTA) to a protein concentration of 0.3–0.5 mg/mL and used in binding assays as hAR-LBD fraction. Aliquots of the hAR-LBD fraction were incubated in the dark at 4 °C with [^{3}H]DHT (NEN, 4 nM final concentration), triamcinolone acetonide (1 μM final concentration), and reference or test compounds (dissolved in DMSO). Nonspecific binding was assessed by addition of a 200-fold excess of nonradioactive DHT. After 15 h, a Dextran T-70/γ-globulin-coated-charcoal suspension was added to the ligand/protein mixture (1% Norit A, 0.05% γ-globulin, 0.05% Dextran T-70 final concentration each) and the whole was incubated at 4 °C for 10 min. The charcoal was removed by centrifugation for 5 min at 1300*g*, and the radioactivity of the supernatant was measured in Atomlight (NEN) by using a liquid scintillation counter.

4.5.2. Transcriptional activation assays[31]

Assay of androgenic activity was performed by means of ARE-luciferase reporter assay using NIH3T3 cells. Culture was conducted in phenol red-free DMEM (SIGMA), supplemented with dextran-coated charcoal-stripped FBS (sFBS) to deplete internal stores of steroids, penicillin, and streptomycin for 2–3 days. Transient transfections of NIH3T3 cells were performed using Transfast™ (Promega) according to the manufacturer's protocol. Transfections were done in 48-well plates at 2×10^4 cells/well with 50 ng of pSG5-hAR, 300 ng of p(ARE)$_2$-luc and 10 ng of pRL/CMV (Promega) as an internal standard. Twenty-four hours after addition of the sample (final concentration, 10^{-5}–10^{-7} M) and 1×10^{-10} DHT, cells were harvested with 25 μL of cell lysis buffer (Promega), and the *firefly* and *Renilla* luciferase activities were determined with a Dual Luciferase Asaay Kit (Promega) by measuring luminescence with a Wallac Micro-Beta scintillation counter (Perkin-Elmer Life Sciences). *Firefly* luciferase reporter activity was normalized to *Renilla* luciferase activity from pRL/CMV.

Acknowledgments

This research was supported by a Grant-in-Aid for High Technology Research Program, a Grant-in-Aid for Scientific Research (B) (No. 20390035) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.04.017.

References and notes

1. Hawthorne, M. F.; Zheng, Z. *Acc. Chem. Res.* **1997**, *30*, 267; Kaszynski, P. *Collect Czech. Chem. Commun.* **1999**, *64*, 895; Valliant, J. F.; Guenther, K. J.; King, A. S.; Morel, P.; Schaffer, P.; Sogbein, O. O.; Stephenson, K. A. *Coord. Chem. Rev.* **2002**, *232*, 173; Lesnikowski, Z. J. *Collect Czech. Chem. Commun.* **2007**, *72*, 1646.
2. Bregradze, V. I. *Chem. Rev.* **1992**, *92*, 209; Fox, M. A.; Hughes, A. K. *Coord. Chem. Rev.* **2004**, *248*, 457.
3. Plešek, J. *Chem. Rev.* **1992**, *92*, 269; Peterson, J. J.; Simon, Y. C.; Coughlin, E. B.; Carter, K. R. *Chem. Commun.* **2009**, 4950; Kokado, K.; Tokoro, Y.; Chujo, Y. *Biomacromolecules* **2009**, *42*, 2925.
4. Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515; Hawthorne, M. F.; Maderna, A. *Chem. Rev.* **1999**, *99*, 3421; Crossley, E. L.; Ziolkowski, E. J.; Coderre, J. A.; Rendina, L. M. *Mini-Rev. Med. Chem.* **2007**, *7*, 303; Armstrong, A. F.; Valliant, J. F. *Dalton Trans.* **2007**, 4240; Yamamoto, T.; Nakai, K.; Matsumura, A. *Cancer Lett.* **2008**, *262*, 143.
5. Lesnikowski, Z. J.; Shi, J.; Schinazi, R. F. *J. Organomet. Chem.* **1999**, *581*, 156; Todd, J. A.; Rendina, L. M. *Inorg. Chem.* **2002**, *41*, 3331; Lesnikowski, Z. J. *Eur. J. Org. Chem.* **2003**, 4489; Olejniczak, A. B.; Semenuc, A.; Kwiatkowski, M.; Lesnikowski, Z. J. *J. Organomet. Chem.* **2003**, *680*, 124; Tjarks, W.; Tiwari, R.; Byun, Y.; Narayanasamy, S.; Barth, R. F. *Chem. Commun.* **2007**, 4978.
6. Wyzlic, I. M.; Soloway, A.; A.H. *Tetrahedron Lett.* **1992**, *33*, 7489; Kahl, S. B.; Kasar, R. A. *J. Am. Chem. Soc.* **1996**, *118*, 1223; Naeslund, C.; Ghirmal, S.; Sjoeberg, S. *Tetrahedron* **2005**, *61*, 1181.
7. Thimon, C.; Panza, L.; Morin, C. *Synlett* **2003**, 1399; Morandi, S.; Ristori, S.; Berti, D.; Panza, L.; Becciolini, A.; Martini, G. *Biochim. Biophys. Acta* **2004**, *1664*, 53; Bonechi, C.; Ristori, S.; Martini, S.; Panza, L.; Martini, G.; Rossi, C.; Donati, A. *Biophys. Chem.* **2007**, *125*, 320.
8. Endo, Y.; Iijima, T.; Yamakoshi, Y.; Yamaguchi, M.; Fukasawa, H.; Shudo, K. *J. Med. Chem.* **1999**, *42*, 1501; Iijima, T.; Endo, Y.; Tsuji, M.; Kawachi, E.;

Kagechika, H.; Shudo, K. *Chem. Pharm. Bull.* **1999**, *47*, 398; Ohta, K.; Iijima, T.; Kawachi, E.; Kagechika, H.; Endo, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5913; Endo, Y.; Yoshimi, T.; Ohta, K.; Suzuki, T.; Ohta, S. *J. Med. Chem.* **2005**, *48*, 3941; Calléja, C.; Messaddeq, N.; Chapellier, B.; Yang, H.; Krezel, W.; Li, M.; Metzger, D.; Mascrez, B.; Ohta, K.; Kagechika, H.; Endo, Y.; Mark, M.; Ghyselinck, N. B.; Chambon, P. *Gene Dev.* **2006**, *20*, 1525; Hirata, M.; Inada, M.; Matsumoto, C.; Takita, M.; Ogawa, T.; Endo, Y.; Miyaura, C. *Biochem. Biophys. Res. Commun.* **2009**, *380*, 218.

9. Endo, Y.; Iijima, T.; Yamakoshi, Y.; Fukasawa, H.; Miyaura, C.; Inada, M.; Kubo, A.; Itai, A. *Chem. Biol.* **2001**, *8*, 341; Endo, Y.; Iijima, T.; Yaguchi, K.; Kawachi, E.; Inoue, N.; Kagechika, H.; Kubo, K.; Itai, A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1307; Endo, Y.; Yamamoto, K.; Kagechika, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4089; Johnsamuel, J.; Byun, Y.; Jones, T. P.; Endo, Y.; Tjarks, W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3213; Johnsamuel, J.; Byun, Y.; Jones, T. P.; Endo, Y.; Tjarks, W. J. *Organomet. Chem.* **2003**, *680*, 223.
10. Mooradian, A. D.; Morley, J. E.; Korenman, S. G. *Endocr. Rev.* **1987**, *8*, 1; Bagatell, C. J.; Bremner, W. J. *N. Engl. J. Med.* **1996**, *334*, 707; Kaufman, J. M.; Vermeulen, A. *Endocr. Rev.* **2005**, *26*, 833.
11. Evans, R. M. *Science* **1988**, *240*, 889; MacLean, H. E.; Warne, G. L.; Zajac, J. D. *J. Steroid Biochem. Mol. Biol.* **1997**, *62*, 233; McEwan, I. J. *Biochem. Soc. Trans.* **2000**, *28*, 369; Taplin, M.-E. *Expert. Rev. Anticancer Ther.* **2008**, *8*, 1495.
12. Liu, P. Y.; Swerdloff, R. S.; Veldhuis, J. D. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 4789.
13. Debes, J. D.; Tindall, D. J. *Cancer Lett.* **2002**, *187*, 1.
14. Visakorpi, T.; Hyytinen, E.; Koivisto, P.; Tanner, M.; Keinanen, R.; Palmberg, C.; Palotie, A.; Tammela, T.; Isola, J.; Kallioniemi, O.-P. *Nat. Genet.* **1995**, *9*, 401; Taplin, M. E.; Bubley, G. J.; Shuster, T. D.; Frantz, M. E.; Spooner, A. E.; Ogata, G. K.; Keer, H. N.; Balk, S. P. *N. Engl. J. Med.* **1995**, *332*, 1393; Brinkmann, A. O.; Trapman, J. *Nat. Med.* **2000**, *6*, 628; Jemal, A.; Thomas, H.; Murray, T.; Thun, M. *CA Cancer J. Clin.* **2002**, *52*, 23; Mohler, J. L.; Gregory, C. W.; Ford, O. H., III; Kim, D.; Weaver, C. M.; Petrusz, P.; Wilson, E. M.; French, F. S. *Clin. Cancer Res.* **2004**, *10*, 440.
15. Chengalvala, M.; Oh, T.; Roy, A. K. *Expert Opin. Ther. Pat.* **2003**, *13*, 59; Gao, W.; Bohl, C. E.; Dalton, J. T. *Chem. Rev.* **2005**, *105*, 3352; Chen, J.; Kim, J.; Dalton, J. T. *Mol. Invest.* **2005**, *5*, 173.
16. Neri, R. O.; Peets, E. A. *J. Steroid Biochem.* **1975**, *6*, 815; Koch, H. *Drugs Today* **1984**, *20*, 561; Neri, R. *Urology* **1989**, *34*, 19.
17. Wakeling, A. E.; Furr, B. J.; Glen, A. T.; Hughes, L. R. *J. Steroid Biochem.* **1981**, *15*, 355; Yeh, S.; Miyamoto, H.; Chang, C. *Lancet* **1997**, *349*, 852; Bohl, C. E.; Miller, D. D.; Chen, J.; Bell, C. E.; Dalton, J. T. *J. Biol. Chem.* **2005**, *280*, 37747.
18. Tucker, H.; Chesterson, G. J. *J. Med. Chem.* **1988**, *31*, 885; Fradet, Y. *Expert. Rev. Anticancer Ther.* **2004**, *4*, 37; Kolvenbag, G. J.; Blackledge, G. R.; Gotting-Smith, K. *Prostate* **1998**, *34*, 61.
19. Boccardo, F. *Crit. Rev. Oncol. Hematol.* **2000**, *35*, 121; Suzuki, H.; Kamiya, N.; Imamoto, T.; Kawamura, K.; Yano, M.; Takano, M.; Utsumi, T.; Naya, Y.; Ichikawa, T. *Int. J. Clin. Oncol.* **2008**, *13*, 401.
20. Bohl, C. E.; Gao, W.; Miller, D. D.; Bell, C. E.; Dalton, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6201.
21. Sack, J. S.; Kish, K. F.; Wang, C.; Attar, R. M.; Kiefer, S. E.; An, Y.; Wu, G. Y.; Scheffler, J. E.; Salvati, M. E.; Krystek, S. R., Jr.; Weinmann, R.; Einspahr, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4904; De Jésus-Tran, K. P.; Côté, P.-L.; Cantin, L.; Blanchet, J.; Labrie, F.; Breton, R. *Protein Sci.* **2006**, *15*, 987.
22. Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2005**, *13*, 5080.
23. Fujii, S.; Goto, T.; Ohta, K.; Hashimoto, Y.; Suzuki, T.; Ohta, S.; Endo, Y. *J. Med. Chem.* **2005**, *48*, 4654.
24. Ohta, K.; Goto, T.; Fujii, S.; Suzuki, T.; Ohta, S.; Endo, Y. *Bioorg. Med. Chem.* **2008**, *16*, 8022.
25. Goto, T.; Ohta, K.; Suzuki, T.; Ohta, S.; Endo, Y. *Bioorg. Med. Chem.* **2005**, *13*, 6414.
26. Fujii, S.; Ohta, K.; Goto, T.; Kagechika, H.; Endo, Y. *Bioorg. Med. Chem.* **2009**, *17*, 344.
27. Fujii, S.; Hashimoto, Y.; Suzuki, T.; Ohta, S.; Endo, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 227.
28. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155; Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.
29. Aggarwal, V. K.; Ali, A.; Coogan, M. P. *J. Org. Chem.* **1997**, *62*, 8628; Aggarwal, V. K.; Coogan, M. P.; Stenson, R. A.; Jones, R. V. H.; Fieldhouse, R.; Blacker, J. *Eur. J. Org. Chem.* **2002**, 319.
30. Ishioka, T.; Kubo, A.; Koiso, Y.; Nagasawa, K.; Itai, A.; Hashimoto, Y. *Bioorg. Med. Chem.* **2002**, *10*, 1555.
31. Kitamura, S.; Suzuki, T.; Ohta, S.; Fujimoto, N. *Environ. Health Perspect.* **2003**, *111*, 503.
32. Sheldrick, G. M. SHELXS97. Program for the Solution of Crystal Structures, 1997, University of Göttingen, Germany.
33. Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. *J. Mol. Biol.* **1996**, *261*, 470.