Bioorganic & Medicinal Chemistry 16 (2008) 8022–8028

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design and synthesis of carborane-containing androgen receptor (AR) antagonist bearing a pyridine ring

Kiminori Ohta [a], Tokuhito Goto [a], Shinya Fijii [b], Tomoharu Suzuki [c], Shigeru Ohta [c], Yasuyuki Endo [a,*]

[a] Faculty of Pharmaceutical Sciences, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan
[b] School of Medical Science, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10, Kanda-Surugadai, Chiyada-ku, Tokyo 101-0062, Japan
[c] Graduate School of Medical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

ARTICLE INFO

Article history:
Received 20 June 2008
Revised 19 July 2008
Accepted 22 July 2008
Available online 24 July 2008

Keywords:
Androgen receptor ligand
Binding
Antagonist
Transcriptional activation

ABSTRACT

We previously developed carborane-containing potent AR antagonists, BA321 and BA341, on the basis of our hypothesis that the carborane cage would be an excellent hydrophobic pharmacophore in place of steroidal C and D rings. As an extension of that work, we designed and synthesized carborane-containing AR antagonist candidates with a pyridine ring. Compound **6b**, which has a pyridine ring directly bound to the *p*-carborane cage at the 3-position, exhibited potent AR-antagonistic activity in transcriptional activation assay using NIH3T3 cells transfected with a hAR-expression plasmid. In addition, it showed more potent antiandrogenic activity than that of the well-known antiandrogen flutamide and comparable activity to that of (*R*)-bicalutamide in SC-3 cell proliferation assay.

1. Introduction

Androgens regulate diverse physiological process involving both reproductive and non-reproductive functions.[1] Most of the signaling effects of androgens are mediated through the androgen receptor (AR), a member of the nuclear receptor superfamily of transcription factors.[2] Deficiencies in 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 exogenous androgens.[3] 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. The primary focus for drug discovery is the synthesis of novel compounds to regulate the transcriptional activity of AR based on the structural (steroidal or non-steroidal) and functional (androgenic, antiandrogenic, or anabolic) properties of ligands.[4–6] Non-steroidal ligand, flutamide (**1a**)[7,8] which is metabolized to a more potent antiandrogen, hydroxyflutamide (**1b**),[9,10] is a well-known AR antagonist used for the treatment of prostate cancer. On the other hand, (*R*)-bicalutamide (**2**) is used for the treatment of D2 stage metastatic prostate cancer (Fig. 1).[11]

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 amino acid residue of the hAR-LBD.[12] This group is also hydrogen-bonded to Gln711 and Met745 amino acid residues via one H_2O molecule.[12] 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 Thr877 amino acid residue of hAR.[13] The hydrophobic group should closely match the hydrophobic surface of the AR, in order to increase the binding affinity. The hydrophobic structure also plays a role as a scaffold, fixing the spatial positions of hydrogen-bonding functional groups. To develop new hydrophobic structures useful for drug design, we have focused on the exceptional hydrophobic character and spherical geometry of dicarba-*closo*-dodecaboranes (carboranes), and utilized them as a hydrophobic component of biologically active molecules.[14–24]

The carboranes are icosahedral carbon-containing boron clusters with characteristic properties, such as spherical geometry and hydrophobicity.[25] We have demonstrated that the hydrophobicity of carboranes is comparable with that of hydrocarbons, and their spherical hydrophobic surface effectively interacts with the hydrophobic surface of the ligand-binding domain of nuclear receptors. Recently, we have reported potent AR antagonists bearing a carborane moiety, BA321 (**3a**) and BA341 (**3b**), which are 10 times more potent than hydroxyflutamide in transcriptional and cell proliferation assay and in AR-binding assay.[26] Moreover, we have reported the design and synthesis of AR antagonists **4**,[27] **5a**, and **5b**[28] based on the structures of DHT and BA341, respectively. These results confirmed that the carborane ring is suitable for the expression of AR-antagonistic activity, because Met895 and

* 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 © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.07.055

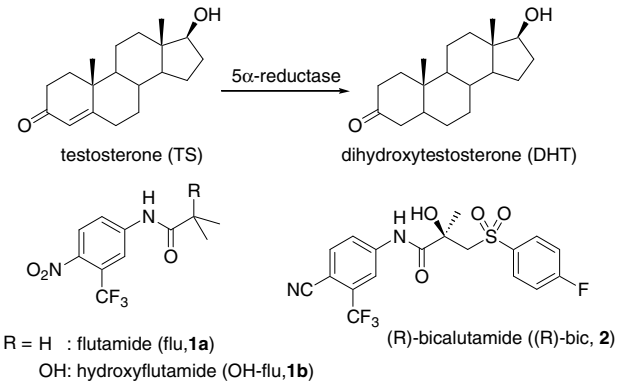


Figure 1. Structures of natural androgens and well-known synthetic androgens.

its surrounding amino acid residues in the hAR-LBD repel the bulky carborane structure to move Helix-12 away from the agonist expression form (Fig. 2).

Therefore, we focused on the development of novel carborane-containing AR antagonists based on the structures of **3** and **5**, and designed several novel AR antagonist candidates, **6**, **7**, and **8**, which have a pyridine ring instead of a cyano- or nitrobenzene moiety, taking into account the interaction between the ligands and Arg752 amino acid residue of hAR (Fig. 3). In this article, we describe the syntheses, biological activities, and the structure–activity relationships of these designed molecules.

2. Results and discussion

2.1. Chemistry

The synthesis of the designed compounds **6a**–**6c** and **7a**–**7c** is summarized in Scheme 1.

Compound **9**,[28] which was obtained from 1-hydroxymethyl *p*-carborane, was treated with *n*-BuLi in dimethoxyethane (DME) followed by addition of CuCl(I) to afford carboranyl copper reagent, which was reacted with 2-bromopyridine under Ullmann coupling condition to afford compound **10a** in 52% yield (Table 1: method A).[29,30] However, the reaction of 3-bromopyridine with carboranyl copper reagent was unsuccessful under this condition. Therefore, 3-iodopyridine was used for the coupling reaction to afford compound **10b** in 58% yield (Table 1: method B). The protecting group, *tert*-butyldimethylsilyl (TBS), was deprotected with tetra-*n*-butylammonium fluoride (TBAF) to afford the desired compounds **6a**–**6c** in high yield. Compounds **7a**–**7c** were synthesized by the reaction of **9** with the corresponding bromomethylpyridine and followed by deprotection with TBAF.

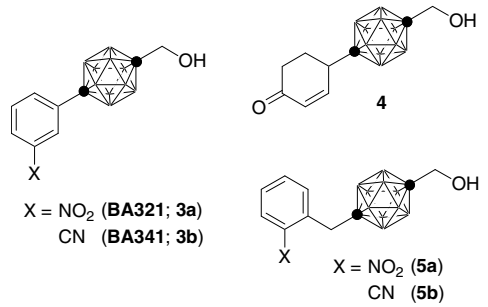


Figure 2. Structures of *p*-carborane-containing AR antagonists developed by us.

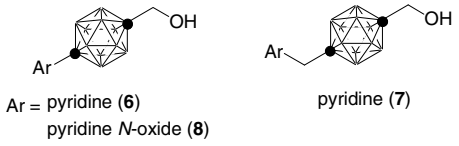


Figure 3. Novel AR antagonist candidates with a pyridine ring.

The designed *N*-oxide derivatives were synthesized from intermediates **10b** and **10c** as shown in Scheme 2. Compounds **10b** and **10c** were treated with *m*-chloroperbenzoic acid (*m*-CPBA) to afford *N*-oxides **12a** and **12b** in excellent yield, and these were deprotected with TBAF to afford **8a** and **8b** in 47% and 88% yield, respectively.

2.2. Competitive-binding assay of the synthesized compounds with hAR

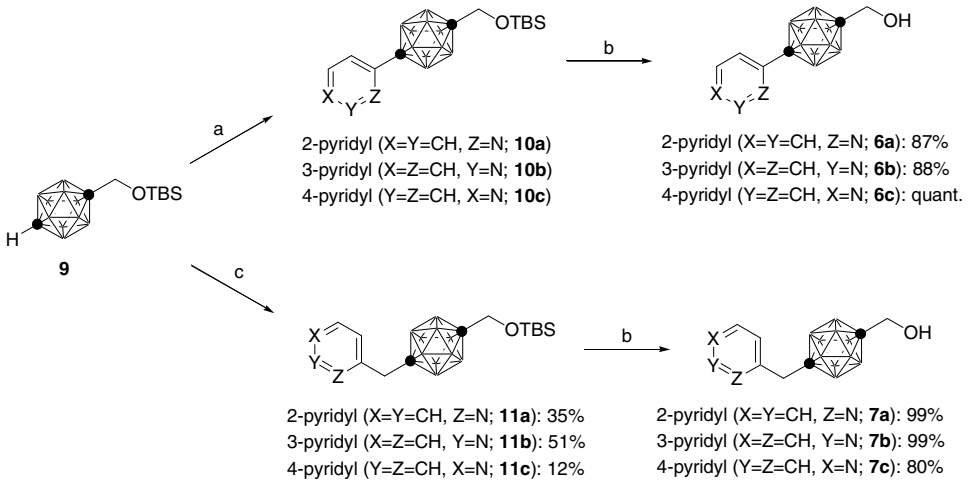
The binding affinity of the new carborane-containing molecules for AR was evaluated by means of competitive-binding assay using [1,2-^{3}H]dihydrotestosterone ([^{3}H]DHT) and hAR.[26,31] Table 2 summarizes the binding affinity data of the synthesized compounds, and the values indicate the percent displacement of specific [^{3}H]DHT binding to hAR by each compound at the concentration of 10 μM. All compounds showed weaker-binding affinity to hAR than that of **1b**. Compound **6b**, which has a 3-pyridine ring, exhibited the strongest-binding affinity among the synthesized compounds, and the binding affinity of **6b** was similar to that of **1a**. The binding affinities of *N*-oxides, **8a** and **8b**, were markedly decreased in comparison with those of the parent compounds, **6b** and **6c**, respectively.

2.3. Transcriptional activation assay of synthesized compounds

To evaluate the activity of the synthesized compounds as agonists and antagonists, transcriptional assay was done with hAR-expression plasmid, ARE/Luci (firefly luciferase) and pRL/CMV (*Renilla* luciferase) plasmids, which were transiently transfected into NIH3T3 cells.[26,32] None of the synthesized compounds exhibited androgenic activity (data not shown). These compounds did dose-dependently inhibit the transcriptional activation in the presence of 1×10^{-10} M DHT. Table 2 summarizes the IC_{50} values of the pyridine derivatives in transactivation assay. Among the synthesized compounds, compound **6b** exhibited the most potent inhibitory activity for the transactivation induced by DHT. The potency of the antagonistic activity of **6b** was comparable to that of **1a**, and the results of transcriptional assay were well correlated with those of binding assay.

2.4. Cell growth inhibition assay using SC-3 cell line

The antiandrogenic activity of the synthesized compounds was evaluated in cell proliferation assay using Shionogi Carcinoma-3 (SC-3) cell line, which shows androgen-dependent growth.[26,33,34] Compounds **1a**, **1b**, **2**, and **3b**, well-known potent antiandrogens, were used as comparative compounds in this assay system. None of the synthesized compounds exhibited androgenic activity on the androgen-dependent cell line SC-3 (data not shown). The antiandrogenic activity of the synthesized compounds is shown in Figure 4. In the concentration range of 1 nM to 10 μM, all of the test compounds, except for **8a** and **8b**, dose-dependently inhibited cell proliferation induced by 1 nM testosterone. Compounds **6a**–**6c**, which have a pyridine ring directly bound to the *p*-carborane cage, exhibited more potent antiandrogenic activity than **7a**–**7c**, **8a** and **8b**. The antiandrogenic activity was in the following order: **6b** > **6c** > **6a**. The antiandrogenic activity of **6b** was more potent than that of **1a** and comparable to that of **2**. Compounds **7a**–**7c**, which have a methylene bridge between the *p*-carborane cage and the pyridine ring, exhibited weak antiandrogenic activity. In our previous studies,[28] the antiandrogenic activity of **5b** (designed

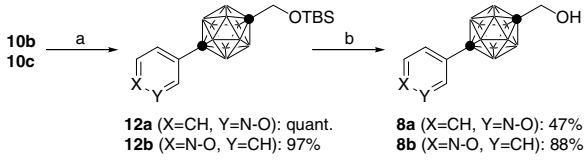


Scheme 1. Synthesis of compounds **6a**–**6c** and **7a**–**7c**. Reagents: (a) *n*-BuLi, DME then Cu(I)Cl, bromopyridine (method A) or iodopyridine (method B), pyridine; (b) TBAF, THF; (c) *n*-BuLi, bromomethylpyridine, DMF.

Table 1
Yield of the Ullmann coupling products using the two methods

Compound	Substituent	Yield (%)	
		Method A	Method B
10a	X = Y = H, Z = N	52	n.t.[a]
10b	X = Z = H, Y = N	0	58
10c	Y = Z = H, X = N	n.t.[a]	65

[a] n.t., indicate not tested.



Scheme 2. Synthesis of *N*-oxide derivatives. Reagents: (a) *m*-chloroperbenzoic acid, CH_2Cl_2; (b) TBAF, THF.

Table 2
Binding affinity of the pyridine compounds to hAR and IC_{50} values in transactivation assay

Compound	Binding affinity[a] (%)	Transactivation assay IC_{50}[b] (M)
6a	2.8	2.6×10^{-6}
6b	10.0	5.2×10^{-7}
6c	4.9	1.5×10^{-6}
7a	<1.0	4.1×10^{-6}
7b	8.9	6.0×10^{-6}
7c	8.6	2.8×10^{-6}
8a	<1.0	n.t.[c]
8b	2.7	n.t.[c]
Flutamide (**1a**)	13.7	5.8×10^{-7}
Hydroxyflutamide (**1b**)	50.3	6.7×10^{-8}

[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 were treated with the test compounds (1×10^{-8} to 1×10^{-5} M) in the presence of DHT (1×10^{-10} M). All transactivation assays were performed in triplicate ($n = 3$). IC_{50} values of the test compounds were estimated from the sigmoidal dose–response curves using GraphPad Prism 4 software.

[c] n.t., indicates not tested.

based on the potent AR antagonist **3b)** was remarkably decreased as compared with that of the parent compound **3b**, suggesting that an increase of flexibility by the introduction of a methylene bridge might have a deleterious effect on the antiandrogenic activity. The decreased activity of **7a**–**7c** compared with **6a**–**6c** is consistent with our previous results. A fixation of aromatic ring with *p*-carborane cage is an important factor for the expression of the potent antiandrogenic activity. *N*-Oxide derivatives, **8a** and **8b**, were inactive, as expected from the results of the binding assay.

Compound **6b** showed moderate antiandrogenic activity in biological evaluation. All the biological activities of compound **6b** paralleled the binding of **6b** to hAR-LBD. Since the *N*-oxide derivative **8a** was inactive in cell proliferation assay, the nitrogen atom of the pyridine ring plays an important role in the expression of antiandrogenic activity, and it may interact with the amino acid residues such as Gln711, Met745, or Arg752 of hAR-LBD. Thus, a pyridine ring can be used in place of a nitro- or cyanobenzene ring in carborane-containing AR antagonists. The distance between the nitrogen atom and hydroxymethyl group of **6b** was shorter than that of **3b**. We speculate that the hydroxymethyl group of **6b** might not readily form a hydrogen bond with Thr877 amino acid residue, and this may account for the decreased antiandrogenic activity of **6b** relative to that of **3b**. Although the distance between the nitrogen atom and the hydroxymethyl group of compounds **7a**–**7c** is longer than that of **6b**, the biological activities of them are less than that of **6b**, because of the flexibility between the pyridine ring and the *p*-carborane cage. Further investigation of the structure–activity relationships around the hydroxymethyl group of **6b** may yield even more potent AR ligands.

3. Conclusion

In conclusion, novel carborane-containing AR antagonist with a pyridine ring was designed and synthesized based upon the structure of potent AR antagonists **3a** and **3b**. These compounds were evaluated by their AR-binding ability in a competitive-binding assay and antiandrogenic activity in a transcriptional assay using NIH3T3 cell transfected with hAR-expression plasmid and cell proliferation assay using SC-3 cells. Compound **6b** exhibited more potent antiandrogenic activity than that of **1a** in SC-3 cell proliferation assay. Since their *N*-oxide derivatives **8a** and **8b** were inactive in cell proliferation assay, a nitrogen atom of the pyridine ring plays an important role for the expression of antiandrogenic activity and might well be interacted with the amino acid residues

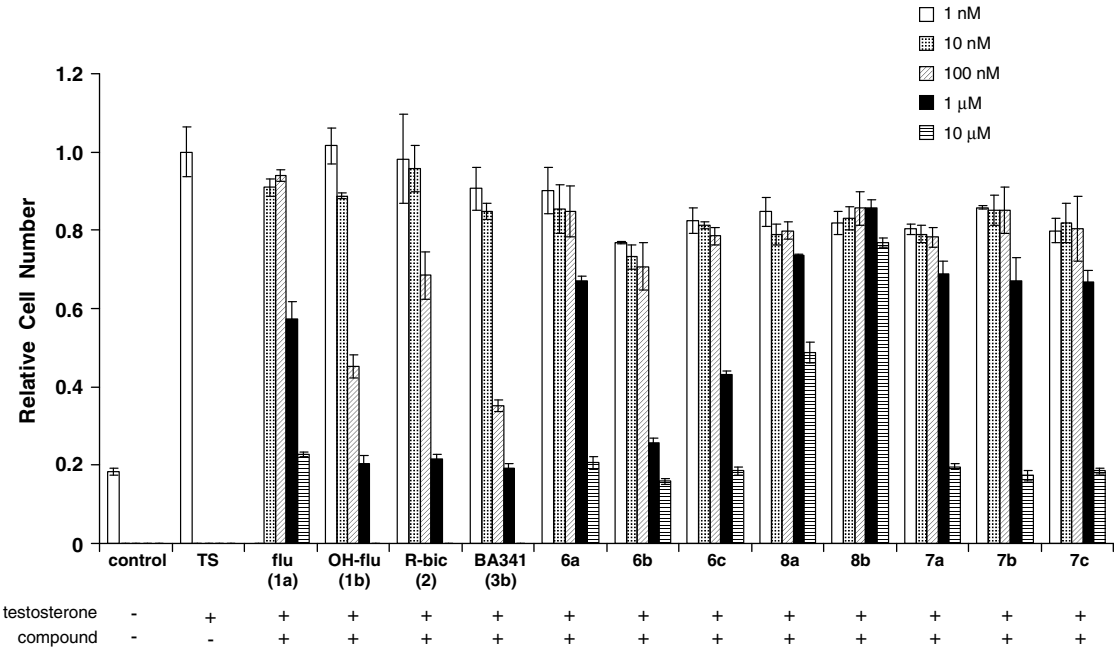


Figure 4. SC-3 cell growth inhibition by the synthesized compounds. The symbol of + and − at the bottom of graph means 'in the presence' and 'the absence', respectively. The control means the absence of testosterone and was 0.5% DMSO, which was included in all test compound solutions. SC-3 cells were incubated with the test compounds (1×10^{-8} to 1×10^{-5} M) in the presence of testosterone (1×10^{-9} M) for 3 days, and the results are shown as relative cell number, with the value for testosterone (1 nM) taken as 1. Cell proliferation assay was performed in triplicate. Values are means ± SD for separate experiments ($n = 3$).

of AR-LBD. We found that a pyridine ring is also suitable as an aromatic ring of AR ligand, while further investigation of structure–activity relationship will be required.

4. Experimental

4.1. General considerations

Melting points were determined with a Yanaco micro melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with JEOL JNM-EX-270 and JNM-LA-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. The splitting patterns are designed as follows: s (singlet), d (doublet), t (triplet), and m (multiplet). Mass spectra were recorded on a JEOL JMS-DX-303 spectrometer. Elemental analyses were performed with a Perkin-Elmer 2400 CHN spectrometer. Column chromatography was carried out using Merck silica gel 60 (0.063–0.200 μm), and TLC was performed on Merck silica gel F_{254}. *p*-Carborane was purchased from Katchem s.r.o. (Prague, Czech Republic). Other reagents were purchased from Wako Pure Chemical Industries, Ltd, Sigma–Aldrich Co., and Tokyo Chemical Industry, Ltd (TCI). All solvents were of reagent quality, purchased commercially, and were used without further purification. 1-(*tert*-Butyldimethylsiloxymethyl)-*p*-carborane (**9**) was prepared according to the literature procedure.[28]

4.2. Synthesis

4.2.1. 1-(*tert*-Butyldimethylsiloxymethyl)-12-(2-pyridyl)-1,12-dicarba-*closo*-dodecaborane (10a)

To a solution of **9** (300 mg, 1.04 mmol) in 1,2-dimethoxyethane (1.5 mL) was added dropwise a 1.58 M solution of *n*-BuLi in hexane (0.75 mL, 1.19 mmol) at 0 °C under an Ar atmosphere. The mixture was stirred for 30 min at the same temperature, and CuCl (130 mg, 1.31 mmol) was added in one portion. Stirring was continued at room temperature for 1 h. Pyridine (0.6 mL, 7.63 mmol) and 2-bromopyridine (113 μL, 1.19 mmol) were added in one portion, and the mixture was heated at 80 °C for 31 h. After cooling, the reaction mixture was diluted with Et_2O and stirred at room temperature for 3 h. Insoluble solids were filtered off through Celite, and the filtrate was washed with 10% HCl aqueous solution, water and brine, dried over $MgSO_4$, and then concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane to give **10a** (199 mg, 52%) as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) −0.01 (s, 6H), 0.86 (s, 9H), 1.0–3.5 (br m, 10H), 3.50 (s, 2H), 7.14 (ddd, $J = 1.2$, 4.9, 7.7 Hz, 1H), 7.23 (d, $J = 8.1$ Hz, 1H), 7.52 (ddd, $J = 2.0$, 7.7, 8.1 Hz, 1H), 8.41 (m, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) −5.7, 18.1, 25.6, 66.1, 82.3, 82.5, 121.2, 123.0, 136.4, 148.5, 153.5; MS (EI) $m/z = 365$ (M^+), 308 (100%).

4.2.2. 1-(*tert*-Butyldimethylsiloxymethyl)-12-(3-pyridyl)-1,12-dicarba-*closo*-dodecaborane (10b)

Compound **10b** was prepared by the same method as described for the synthesis of **10a**. 3-Iodopyridine was used instead of 3-bromopyridine; 58% yield; colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) −0.01 (s, 6H), 0.86 (s, 9H), 1.0–3.5 (br m, 10H), 3.50 (s, 2H), 7.12 (dd, $J = 4.5$, 8.2 Hz, 1H), 7.50 (d, $J = 8.1$ Hz, 1H), 8.47 (m, 2H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) −5.7, 18.1, 25.6, 66.0, 78.5, 82.5, 122.7, 132.3, 134.6, 148.0, 149.4; MS (EI) $m/z = 365$ (M^+), 311 (100%).

4.2.3. 1-(*tert*-Butyldimethylsiloxymethyl)-12-(4-pyridyl)-1,12-dicarba-*closo*-dodecaborane (10c)

Compound **10c** was prepared by the same method as described for the synthesis of **10a**. 4-Iodopyridine was used instead of 4-bromopyridine; 65% yield; colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) −0.01 (s, 6H), 0.86 (s, 9H), 1.0–3.5 (br m, 10H), 3.49 (s, 2H), 7.10 (d, $J = 6.3$ Hz, 2H), 8.42 (d, $J = 6.3$ Hz, 2H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) −5.7, 18.1, 25.6, 66.0, 79.2, 82.8, 121.8, 144.6, 149.6; MS (EI) $m/z = 365$ (M^+), 311 (100%).

4.2.4. 1-Hydroxymethyl-12-(2-pyridyl)-1,12-dicarba-*closo*-dodecaborane (6a)

To a solution of **10a** (199 mg, 0.54 mmol) in THF (2 mL) was added a solution of 1 M TBAF in THF (0.82 mL, 0.82 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was quenched with water and the mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and then concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane/AcOEt 10:1 to 3:1 to give **6a** (119 mg, 87%) as a colorless solid; colorless prisms (CH_2Cl_2/*n*-hexane); mp 106–106.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 1.72 (t, J = 7.1 Hz, 1H), 3.56 (d, J = 7.1 Hz, 2H), 7.16 (ddd, J = 1.0, 4.8, 7.4 Hz, 1H), 7.23 (ddd, J = 0.8, 1.0, 8.1 Hz, 1H), 7.52 (m, 1H), 8.42 (ddd, J = 0.8, 1.8, 4.8 Hz, 1H); ^{13}C NMR (68 MHz, CD_3OD) δ (ppm) 66.3, 83.7, 84.9, 122.6, 124.7, 138.3, 149.6, 154.5; MS (EI) m/z = 251 (M^+, 100%); HRMS Calcd for $C_8H_{17}B_{10}NO$: 251.2313. Found: 251.2296.

4.2.5. 1-Hydroxymethyl-12-(3-pyridyl)-1,12-dicarba-*closo*-dodecaborane (6b)

Compound **6b** was prepared by the same method as described for the synthesis of **6a**; 88% yield; colorless needles (CH_2Cl_2/*n*-hexane); mp 186–187 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 2.09 (br s, 1H), 3.56 (s, 2H), 7.14 (dd, J = 4.8, 8.1 Hz, 1H), 7.51 (ddd, J = 1.5, 2.5, 8.1 Hz, 1H), 8.45 (m, 2H); ^{13}C NMR (68 MHz, CD_3OD) δ (ppm) 66.2, 79.7, 85.1, 124.7, 134.3, 136.8, 148.1, 150.2; MS (EI) m/z = 251 (M^+, 100%); HRMS Calcd for $C_8H_{17}B_{10}NO$: 251.2313. Found: 251.2320.

4.2.6. 1-Hydroxymethyl-12-(4-pyridyl)-1,12-dicarba-*closo*-dodecaborane (6c)

Compound **6c** was prepared by the same method as described for the synthesis of **6a**; quant.; colorless cubes (CH_2Cl_2/*n*-hexane); mp 200–201 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 3.53 (s, 2H), 4.29 (br s, 1H), 7.13 (d, J = 6.3 Hz, 2H), 8.38 (d, J = 6.3 Hz, 2H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 65.5, 79.1, 83.5, 122.1, 145.1, 149.2; MS (EI) m/z = 251 (M^+, 100%); Anal. Calcd for $C_8H_{17}B_{10}NO$: C, 38.23; H, 6.82; N, 5.57. Found: C, 38.28; H, 6.66; N, 5.57.

4.2.7. 1-(*tert*-Butyldimethylsiloxymethyl)-12-(2-pyridylmethyl)-1,12- dicarba-*closo*-dodecaborane (11a)

To a solution of **9** (100 mg, 0.35 mmol) in THF (1 mL) was added dropwise a 1.56 M solution of *n*-BuLi in hexane (0.56 mL, 0.87 mmol) at 0 °C under an Ar atmosphere. The mixture was stirred at the same temperature for 30 min, and 2-(bromomethyl)pyridine hydrobromide (105 mg, 0.42 mmol) was added. Stirring was continued at room temperature for 27 h. The reaction mixture was poured into saturated $NaHCO_3$ aqueous solution and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and then concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane/AcOEt 20:1 to give **11a** (46 mg, 35%) as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) −0.06 (s, 6H), 0.82 (s, 9H), 1.0–3.5 (br m, 10H), 3.08 (s, 2H), 3.41 (s, 2H), 6.98 (dt, J = 1.1, 7.7 Hz, 1H), 7.15 (ddd, J = 1.2, 4.9, 7.6 Hz, 1H), 7.58 (ddd, J = 1.8, 7.6, 7.7 Hz, 1H), 8.51 (ddd, J = 1.0, 1.8, 4.9 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) −5.7, 18.1, 25.6, 45.9, 65.9, 78.3, 80.5, 122.1, 124.3, 136.1, 149.2, 156.6; MS (EI) m/z = 379 (M^+), 322 (100%).

4.2.8. 1-(*tert*-Butyldimethylsiloxymethyl)-12-(3-pyridylmethyl)-1,12- dicarba-*closo*-dodecaborane (11b)

Compound **11b** was prepared by the same method as described for the synthesis of **11a**; 51% yield; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) −0.06 (s, 6H), 0.82 (s, 9H), 1.0–3.5 (br m, 10H), 2.90 (s, 2H), 3.41 (s, 2H), 7.20 (dd, J = 4.8, 7.9 Hz, 1H), 7.30 (ddd, J = 1.8, 2.0, 7.9 Hz, 1H), 8.23 (d, J = 2.0 Hz, 1H), 8.41 (dd, J = 1.8, 4.8 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) −5.8, 18.0, 25.5, 40.8, 65.8, 78.7, 80.5, 122.9, 132.4, 137.0, 148.6, 150.5; MS (EI) m/z = 379 (M^+), 325 (100%).

4.2.9. 1-(*tert*-Butyldimethylsiloxymethyl)-12-(4-pyridylmethyl)-1,12-dicarba-*closo*-dodecaborane (11c)

Compound **11c** was prepared by the same method as described for the synthesis of **11a**; 16% yield; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) −0.06 (s, 6H), 0.83 (s, 9H), 1.0–3.5 (br m, 10H), 2.87 (s, 2H), 3.41 (s, 2H), 6.70 (d, J = 6.0 Hz, 2H), 8.51 (d, J = 6.0 Hz, 2H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) −5.7, 18.1, 25.6, 43.0, 65.8, 77.7, 80.7, 124.9, 145.1, 149.7; MS (EI) m/z = 379 (M^+), 324 (100%).

4.2.10. 1-Hydroxymethyl-12-(2-pyridylmethyl)-1,12-dicarba-*closo*-dodecaborane (7a)

Compound **7a** was prepared by the same method as described for the synthesis of **6a**; 99% yield; colorless leaflets (CH_2Cl_2/*n*-hexane); mp 131–132 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 3.09 (s, 2H), 3.46 (s, 2H), 6.99 (d, J = 7.7 Hz, 1H), 7.17 (dd, J = 4.9, 7.6 Hz, 1H), 7.60 (ddd, J = 1.8, 7.6, 7.7 Hz, 1H), 8.52 (m, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 45.5, 65.4, 78.3, 80.8, 122.4, 124.6, 136.5, 148.9, 156.2; MS (EI) m/z = 265 (M^+, 100%); HRMS Calcd for $C_9H_{19}B_{10}NO$: 265.2470. Found: 265.2494.

4.2.11. 1-Hydroxymethyl-12-(3-pyridylmethyl)-1,12-dicarba-*closo*-dodecaborane (7b)

Compound **7b** was prepared by the same method as described for the synthesis of **6a**; 99% yield; colorless prisms (CH_2Cl_2/*n*-hexane); mp 113–114 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 2.91 (s, 2H), 3.47 (s, 2H), 7.21 (dd, J = 4.8, 7.7 Hz, 1H), 7.32 (m, 1H), 8.19 (d, J = 1.6 Hz, 1H), 8.44 (dd, J = 1.8, 4.8 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) 40.8, 65.3, 78.6, 81.3, 123.3, 132.8, 137.6, 148.0, 150.0; MS (EI) m/z = 265 (M^+, 100%); HRMS Calcd for $C_9H_{19}B_{10}NO$: 265.2470. Found: 265.2466.

4.2.12. 1-Hydroxymethyl-12-(4-pyridylmethyl)-1,12-dicarba-*closo*-dodecaborane (7c)

Compound **7c** was prepared by the same method as described for the synthesis of **6a**; 80% yield; colorless needles (CH_3OH); mp 163–165 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 2.88 (s, 2H), 3.45 (s, 2H), 5.00 (br s, 1H), 6.92 (d, J = 5.8 Hz, 2H), 8.41 (d, J = 5.8 Hz, 2H); ^{13}C NMR (68 MHz, CD_3OD) δ (ppm) 43.6, 66.1, 79.2, 83.0, 126.8, 148.0, 150.0; MS (EI) m/z = 265 (M^+, 100%); Anal. Calcd for $C_8H_{17}B_{10}NO$: C, 40.74; H, 7.22; N, 5.28. Found: C, 41.14; H, 7.04; N, 5.32.

4.2.13. 3-(12-*tert*-Butyldimethylsiloxymethyl-1,12-dicarba-*closo*-dodecaboran-1-yl)pyridine *N*-oxide (12a)

To a solution of **10b** (671 mg, 1.84 mmol) in CH_2Cl_2 (10 mL) was added slowly *m*-chloroperbenzoic acid (633.4 mg, 3.67 mmol) at 0 °C under an Ar atmosphere. The reaction mixture was stirred at room temperature for 5 h, then poured into water and extracted with 10% CH_3OH-containing $CHCl_3$, The extract was dried over $MgSO_4$, and then concentrated. The residue was purified by column chromatography on silica gel with $CHCl_3/CH_3OH$ 10:1 to give **12a** (699 mg, quant.) as a colorless solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) −0.02 (s, 6H), 0.86 (s, 9H), 1.0–3.5 (br m, 10H), 3.48 (s, 2H), 7.07–7.09 (m, 2H), 8.03–8.09 (m, 2H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) −5.7, 18.1, 25.6, 65.9, 76.1, 83.3, 124.6, 125.0, 136.2, 138.2, 138.5; MS (EI) m/z = 381 (M^+), 308 (100%).

4.2.14. 4-(12-*tert*-Butyldimethylsiloxymethyl-1,12-dicarba-*closo*-dodecaboran-1-yl)pyridine *N*-oxide (12b)

Compound **12b** was prepared by the same method as described for the synthesis of **12a**; 97% yield; ^{1}H NMR (270 MHz, $CDCl_3$) δ

(ppm) −0.02 (s, 6H), 0.86 (s, 9H), 1.0–3.5 (br m, 10H), 3.49 (s, 2H), 7.15 (d, J = 7.4 Hz, 2H), 8.19 (d, J = 7.4 Hz, 2H); ^{13}C NMR (68 MHz, $CDCl_3$) δ (ppm) −5.8, 18.0, 25.5, 65.8, 77.9, 82.7, 124.4, 134.2, 138.3; MS (EI) m/z = 381 (M^+), 309 (100%).

4.2.15. 3-(12-Hydroxymethyl-1,12-dicarba-*closo*-dodecaboran-1-yl) pyridine *N*-oxide (8a)

Compound **8a** was prepared by the same method as described for the synthesis of **6a**; 47% yield; colorless plates ($CHCl_3$/AcOEt/CH_3OH); mp 201.5–203 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 3.54 (s, 2H), 7.08–7.11 (m, 2H), 8.05 (ddd, J = 1.8, 2.4, 5.3 Hz, 1H), 8.08 (dd, J = 1.2, 1.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 66.2, 77.4, 86.0, 127.6, 129.1, 137.9, 138.7, 140.2; MS (EI) m/z = 267 (M^+, 100%); HRMS Calcd for $C_8H_{17}B_{10}NO_2$: 267.2263. Found: 267.2266.

4.2.16. 4-(12-Hydroxymethyl-1,12-dicarba-*closo*-dodecaboran-1-yl) pyridine *N*-oxide (8b)

Compound **8b** was prepared by the same method as described for the synthesis of **6a**; 88% yield; colorless prisms ($CHCl_3$/AcOEt/CH_3OH); mp 265–266 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ (ppm) 1.0–3.5 (br m, 10H), 1.86 (t, J = 7.3 Hz, 1H), 3.55 (d, J = 7.3 Hz, 2H), 7.06 (d, J = 7.3 Hz, 2H), 7.98 (d, J = 7.3 Hz, 2H); ^{13}C NMR (68 MHz, 10% CD_3OD in $CDCl_3$) δ (ppm) 65.1, 77.2, 83.6, 124.8, 136.6, 138.3; MS (EI) m/z = 267 (M^+), 251 (100%); Anal. Calcd for $C_8H_{17}B_{10}NO_2$: C, 35.94; H, 6.41; N, 5.24. Found: C, 36.22; H, 6.33; N, 5.25.

4.3. Competitive-binding assay with hAR

A hAR-LBD-expression plasmid vector which encodes GST-hAR-LBD (627–919 aa, EF domain) fusion protein under the *lac* promoter (provided by Prof. S. Kato, University of Tokyo) was transfected into *Escherichia 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 4000g 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 using 10 × 10 s bursts on ice, and crude GST-hARLBD fraction was prepared by centrifugation of the suspension at 12,000g 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). Non-specific binding was assessed by addition of a 200-fold excess of non-radioactive 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 concentrations) and the whole was incubated at 4 °C for 10 min. The charcoal was removed by centrifugation for 5 min at 1300g, and the radioactivity of the supernatant was measured in Atomlight (NEN) by using a liquid scintillation counter.

4.4. Transfection and transactivation assay

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 Chemical Co.) containing penicillin, streptomycin, and dextran-charcoal-treated calf serum for 2–3 days. Transient transfections of NIH3T3 cells were performed using Transfast™ (Promega Co., Madison, WI), 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 Co.), 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 Co.) and the firefly and *Renilla* luciferase activities were determined with a Dual Luciferase Assay Kit (Promega Co.) by measuring luminescence with a Wallac Micro-Beta scintillation counter (Perkin-Elmer Life Sciences, Boston, MA). Firefly luciferase reporter activity was normalized to *Renilla* luciferase activity from pRL/CMV.

4.5. SC-3 growth inhibition assay

SC-3 cells were cultured in the presence of MEMα (Wako Co.) supplemented with 2% FBS, penicillin, streptomycin, and 10 nM testosterone at 37 °C under 5% CO_2. All experiments were performed in triplicate or more. Cells were trypsinized and diluted to 2×10^{-4} cell/mL with MEMα supplemented with 2% stripped-FBS, penicillin, and streptomycin. This cell suspension was seeded in 96-well plates at the volume of 100 μL and incubated at 24 h. After removal of 10 μL of medium from each well, 10 μL of the drug solution, which was supplemented with serial dilutions of the test compounds or DMSO as dilution control in the presence or absence of 1 nM testosterone, was added. Then the plates were incubated at 37 °C under 5% CO_2 for 3 days, and the cell number was determined using the Cell Counting Kit-8 (DOJINDO). 10 μL of WST-8 was added to each well of microcultures, and the cells were incubated for 2–4 h. The absorbance at 450 nm was measured with a Model-680 micro plate reader (Bio-Rad). This parameter is considered to be a measure of the number of living cells in the culture.

Acknowledgements

This research was supported by a Grant-in-Aid for High Technology Research Program, a Grant-in-Aid for Scientific Research (B) (No. 16390032), and a Grant-in-Aid for Young Scientists (B) (No. 18790089) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References and notes

1. Mooradian, A. D.; Morley, J. E.; Korenman, S. G. *Endocr. Rev.* **1987**, *8*, 1.
2. Evans, R. M. *Science* **1988**, *240*, 889.
3. Liu, P. Y.; Swerdloff, R. S.; Veldhuis, J. D. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 4789.
4. Chengalvala, M.; Oh, T.; Roy, A. K. *Expert Opin. Ther. Pat.* **2003**, *13*, 59.
5. Gao, W.; Bohl, C. E.; Dalton, J. T. *Chem. Rev.* **2005**, *105*, 3352.
6. Chen, J.; Kim, J.; Dalton, J. T. *Mol. Interventions* **2005**, *5*, 173.
7. Neri, R.; Peets, E. J. *J. Steroid Biochem.* **1975**, *6*, 815.
8. Koch, H. *Drugs Today* **1984**, *20*, 561.
9. Yeh, S.; Miyamoto, H.; Chang, C. *Lancet* **1997**, *349*, 852.
10. Bohl, C. E.; Miller, D. D.; Chen, J.; Bell, C. E.; Dalton, J. T. *J. Biol. Chem.* **2005**, *280*, 37747.
11. Fradet, Y. *Expert Rev. Anticancer Ther.* **2004**, *4*, 37.
12. Bohl, C. E.; Gao, W.; Miller, D. D.; Bell, E. C.; Dalton, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6201.
13. De Jésus-Tran, K. P.; Côté, P.-L.; Cantin, L.; Blanchet, J.; Labrie, F.; Breton, R. *Protein Sci.* **2006**, *15*, 987.
14. Endo, Y.; Iijia, T.; Yamakoshi, Y.; Yamaguchi, M.; Fukasawa, H.; Shudo, K. *J. Med. Chem.* **1999**, *42*, 1501.
15. Endo, Y.; Iijima, T.; Yamakoshi, Y.; Kubo, A.; Itai, A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3313.
16. Endo, Y.; Yoshimi, T.; Iijima, T.; Yamakoshi, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3387.
17. Endo, Y.; Iijima, T.; Yamakoshi, Y.; Fukasawa, H.; Miyaura, C.; Kubo, A.; Itai, A. *Chem. Biol.* **2001**, *8*, 341.
18. Endo, Y.; Yoshimi, T.; Ohta, K.; Suzuki, T.; Ohta, S. *J. Med. Chem.* **2005**, *48*, 3941.

19. Ogawa, T.; Ohta, K.; Yoshimi, T.; Yamazaki, H.; Suzuki, T.; Ohta, S.; Endo, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3943.
20. Endo, Y.; Iijima, T.; Kagechika, H.; Ohta, K.; Kawachi, E.; Shudo, K. *Chem. Pharm. Bull.* **1999**, *47*, 585.
21. Endo, Y.; Yaguchi, K.; Kawachi, E.; Kagechika, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1733.
22. Endo, Y.; Iijima, T.; Yaguchi, K.; Kawachi, E.; Kagechika, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1307.
23. Ohta, K.; Iijima, T.; Kawachi, E.; Kagechika, H.; Endo, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5913.
24. Calleja, 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.
25. Bregradze, V. I. *Chem. Rev.* **1992**, *92*, 209.
26. Fujii, S.; Goto, T.; Ohta, K.; Hashimoto, Y.; Suzuki, T.; Ohta, S.; Endo, Y. *J. Med. Chem.* **2005**, *48*, 4654.
27. Fujii, S.; Hashimoto, Y.; Suzuki, T.; Ohta, S.; Endo, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 227.
28. Goto, T.; Ohta, K.; Suzuki, T.; Ohta, S.; Endo, Y. *Bioorg. Med. Chem.* **2005**, *13*, 6414.
29. Coult, R.; Fox, M. A.; Gill, W. R.; Herbertson, P. L.; MacBride, J. A. H.; Wade, K. *J. Organomet. Chem.* **1993**, *462*, 19.
30. Gill, W. R.; Herbertson, P. L.; MacBride, J. A. H.; Wade, K. *J. Organomet. Chem.* **1996**, *507*, 249.
31. Ishioka, T.; Kubo, A.; Koiso, Y.; Nagasawa, K.; Itai, A.; Hashimoto, Y. *Bioorg. Med. Chem.* **2002**, *10*, 1555.
32. Kitamura, S.; Suzuki, T.; Ohta, S.; Fujimoto, N. *Environ. Health Perspect.* **2003**, *111*, 503.
33. Hiraoka, D.; Nakamura, N.; Nishizawa, Y.; Uchida, N.; Noguchi, S.; Matsumoto, K.; Sato, B. *Cancer Res.* **1987**, *47*, 6560.
34. Kasayama, S.; Saito, H.; Kouhara, H.; Sumitani, S.; Sato, B. *J. Cell Physiol.* **1993**, *154*, 254.