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Boron clusters for medicinal drug design: Selective estrogen receptor modulators bearing carborane*

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Abstract: The molecular shape and hydrophobicity of dicarba-*closo*-dodecaboranes may allow a new medical application as biologically active molecules. Recently, we have developed potent estrogen receptor (ER) agonists bearing carborane cage. The most potent compound (BE120) exhibited activity at least several times greater than that of 17β-estradiol in luciferase reporter gene assay and ER α binding. We also designed and synthesized estrogen antagonists on the basis of the structure of BE120, and we noticed the characteristic features of compound (BE360) having carborane cage and two phenols. The ER binding affinity of BE360 is comparable to that of estradiol. To examine in vivo estrogenic activity of the compound in bone, ovariectomized (OVX) mice were given BE360 or estradiol subcutaneously for 4 weeks. Treatment with BE360 at 1–30 μg/day dose-dependently restored bone loss in OVX mice to sham level without estrogenic action in uterus. These results suggest its possible application to osteoporosis as a new type of selective ER modulator.

INTRODUCTION

The icosahedral carboranes (dicarba-*closo*-dodecaboranes) have characteristic properties, such as high boron content, remarkable thermal and chemical stability, spherical geometry, and exceptional hydrophobic character. Their unusual properties have been utilized in materials chemistry in the preparation of materials for liquid crystals and nonlinear optics and in medicinal chemistry in the field of boron neutron capture therapy (BNCT) [1]. We have focused on the possibility of using carboranes as a hydrophobic component in biologically active molecules which interact hydrophobically with receptors, because the goodness of fit between the shape of a ligand molecule and that of a receptor (in other words, interaction between the hydrophobic structure of a ligand molecule and a hydrophobic domain of the receptor) plays an important role in determining the affinity of the ligand for the receptor. The difference of binding constants between a ligand having a suitable hydrophobic group and a ligand without such a group sometimes reaches over 10^3-fold. The hydrophobic nature of carboranes has also been noted by peptide chemists, who have synthesized several biologically active peptides, in which phenylalanine residues were replaced with (*o*-carboranyl)alanine [2]. However, this concept has not been extended to the possible use of carborane as a hydrophobic skeletal structure in the field of drug design. To evaluate the utility of icosahedral carborane as a hydrophobic component for drug design, we focused on the design and synthesis of estrogen agonists bearing a carborane cage as a hydrophobic skeletal structure. The steroid hormone estrogen influences the growth, differentiation, and functioning of many target tissues. Estrogens play an important role in the female and male reproductive

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systems, as well as in bone maintenance, in the central nervous system, and in the cardiovascular system. The initial step in the appearance of these activities is the binding of hormonal ligands to the estrogen receptors (ERs) α and β (ER α and β) [3,4]. The hormone-bound ER undergoes a conformational change, allowing the receptor to dimerize. The dimer functions as a transcription factor that mediates biological response by binding to specific promoter elements of DNA to initiate gene transcription. Compounds that either induce or inhibit cellular estrogen responses have potential value as biochemical tools and candidates for drug development. Here we describe the synthesis and biological evaluation of novel carborane-containing estrogenic agonists and antagonists, which act as selective estrogen receptor modulators (SERMs).

METHOD AND RESULTS

Application of boron clusters as a hydrophobic pharmacophore for estrogen agonists

Ligand design and synthesis

Since the discovery of the nonsteroidal estrogen, diethylstilbestrol, many stilbenes and other aromatic compounds have been synthesized and shown to possess estrogenic activity. These agonists and antagonists have been developed as agents for regulating fertility, preventing and controlling hormone-responsive breast cancer, and post-menopausal hormone replacement [5]. On the other hand, the health risks of estrogenic compounds that are either present in the environment or used in industry have become of interest in recent years [6]. One of the reasons for the estrogenic activity of such a wide variety of organic chemicals appears to be that ER binding is primarily the result of interaction between the receptor and a phenolic residue. Furthermore, the estrogenic activity is known to depend on the shape of the alkyl substituent in simple phenols having a bulky alkyl group at the 4-position. Therefore, high binding affinity for ER and the appearance of substantial estrogenic activity require an appropriate hydrophobic group adjacent to the phenolic ring, other than two hydrogen-bonding groups, such as a phenolic hydroxyl group and another hydroxyl group, located at a suitable position on the molecule. The C, D rings on the natural hormone, 17β-estradiol (**1**), play an important role in stabilizing the ligand-receptor complex by hydrophobic interactions. The size of the carborane cage seems to be appropriate for a hydrophobic skeletal structure in place of the C, D ring structure of **1**. Substitution of the two carbon atoms of the carborane isomers should allow suitable fixation of the direction of the functional groups. On the basis of these considerations, we designed the compound BE120 (**2**), which seems to contain all the essential molecular recognition components for ER. Figure 1 shows a structural comparison of 17β-estradiol (**1**) and **2** [7,8].

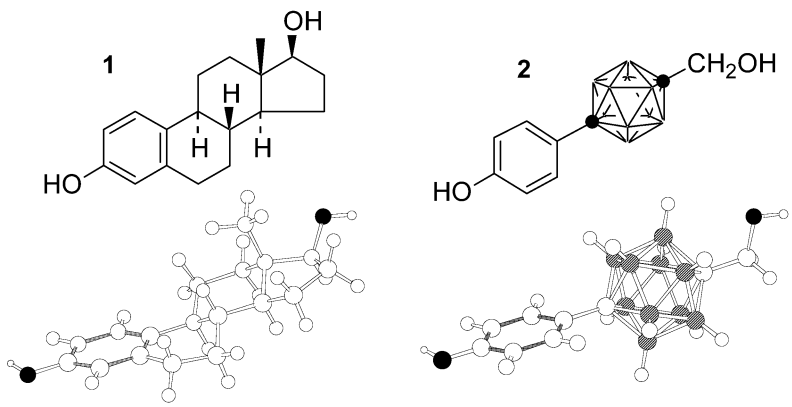

Fig. 1 Structural comparison of 17β-estradiol (**1**, left) and BE120 (**2**, right).

The designed molecules **2** were synthesized from 1,12-dicarba-*closo*-dodecaborane (*p*-carborane (**3**). 1-(4-Methoxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**4**), which was prepared by coupling of the C-copper (I) derivative of **3** with 4-iodoanisole in dimethoxyethane in the presence of pyridine in 60 % yield, was converted to the *C*-methoxycarbonyl derivative **5** by reaction of the lithiate of **4** with methyl chloroformate (91 %). After reduction of **5** with $LiAlH_4$, demethylation gave 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (BE120, **2**) in 98 % yield. The compound **6**, which is a simple phenol bearing a *p*-carboranyl moiety at the 4-position (without a hydroxymethyl group on the carborane cage) was prepared from **4** by demethylation (93 %). The related compound **7**, which has an amino group on the carborane cage, was also prepared from **5** by means of the modified Curtius rearrangement (41 %).

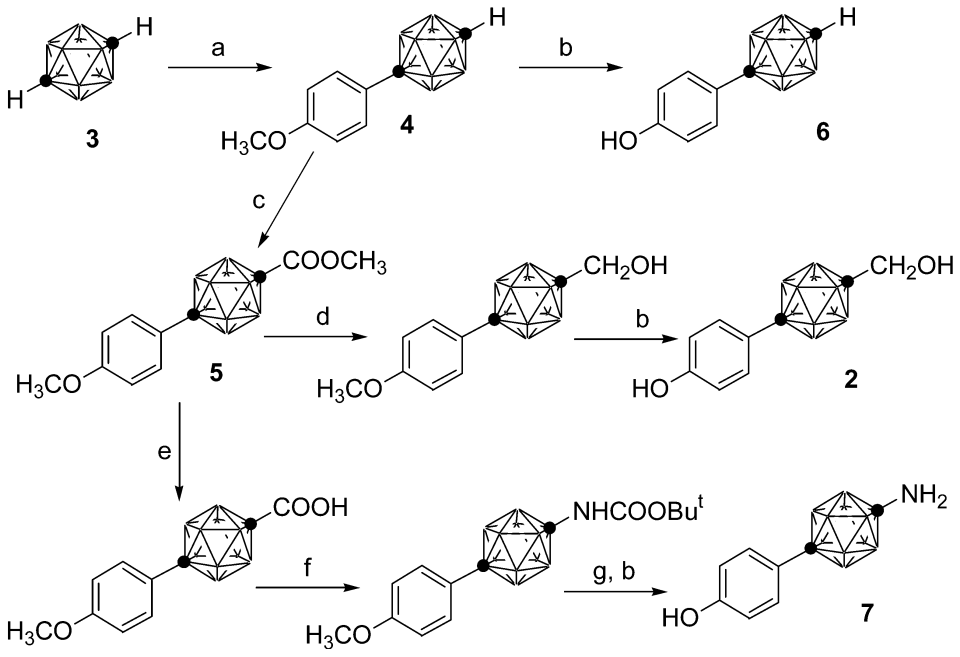

Fig. 2 Synthetic route for the preparation of estrogen agonists bearing carborane. Key: (a) (1) *n*-BuLi, CuCl/DME (2) *p*-iodoanisole/pyridine, reflux; (b) BBr_3/CH_2Cl_2; (c) (1) *n*-BuLi/benzene-Et_2O (2) ClCOOCH3; (d) $LiAlH_4$/THF; (e) KOH/H_2O-THF; (f) DPPA, Et_3N, DMAP/*t*-BuOH reflux; (g) CF_3COOH/CH_2Cl_2.

Transactivational properties and ER-binding affinity of the designed carborane-containing molecules [8,9]

The estrogenic activities of the synthesized compounds were examined by luciferase reporter gene assay, in which a rat ERα-expression plasmid and a reporter plasmid, which contains five copies of estrogen response elements, are transiently transfected into COS-1 cells. 17β-Estradiol at 1×10^{-10}–1×10^{-8} M induced the expression of luciferase in a dose-dependent manner. Compound **2** also exhibited potent transcriptional activity in the concentration range of 1×10^{-10}–1×10^{-8} M; its potency is higher than that of 17β-estradiol. Surprisingly, compound **6**, which is a simple phenol bearing *p*-carboranyl at the 4-position, exhibited potent transcriptional activity in the concentration range of 1×10^{-10}–1×10^{-8} M; its potency is much higher than that of 4-alkylphenols. ERα binding assays were performed on the three active compounds (**2**, **6**, **7**) and 17β-estradiol to confirm that the gene-regulatory activity correlated with the binding affinity for the ERα. The assays were done by measurement of inhibition of [6,7-^{3}H]17β-estradiol binding (K_d = 0.4 nM) to human recombinant ERα (PanVera), using

the nitrocellulose filter binding assay method. The ERα-binding data for these compounds are consistent with the results of the above-mentioned reporter gene assay. Compounds **6** and **7** showed strong affinity for ERα; their potency was almost the same as that of 17β-estradiol. The most active compound in the reporter gene assay, **2**, showed the highest affinity for the ERα, and its affinity was higher than that of 17β-estradiol. The K_i's of **6**, **2**, and **7** for ERα were 0.40 nM, 0.10 nM, and 0.65 nM, respectively.

*Effects of the carborane-containing estrogen agonist **2** on the whole animal [8]*

Estrogen deficiency results in uterine atrophy and marked bone loss, and these changes can be reversed by estrogen administration. Using ovariectomized (OVX) mice, we compared the effects of 17β-estradiol and **2** on the uterus. The uterine weight decreased markedly in OVX mice, indicating that the mice were estrogen-deficient, as shown in Fig. 3. As reported previously, 17β-estradiol (100 ng/day) restored the decreased uterine weight in OVX mice to a level higher than that of the sham-operated mice. Compound **2** (100 ng/day) also restored the uterine weight of OVX mice, and its potency was similar to that of **1**. This indicates that **2** has a potent estrogenic action in the uterus in vivo. To determine the effects of **2** on bone mass, OVX mice were treated for 4 weeks with 100 ng/day of **2** and the femurs were subjected to radiographic analysis. X-ray analysis showed marked loss of mineralized cancellous bone, especially in the distal metaphysis of the femur, in OVX mice. Treatment with 17β-estradiol or **2**

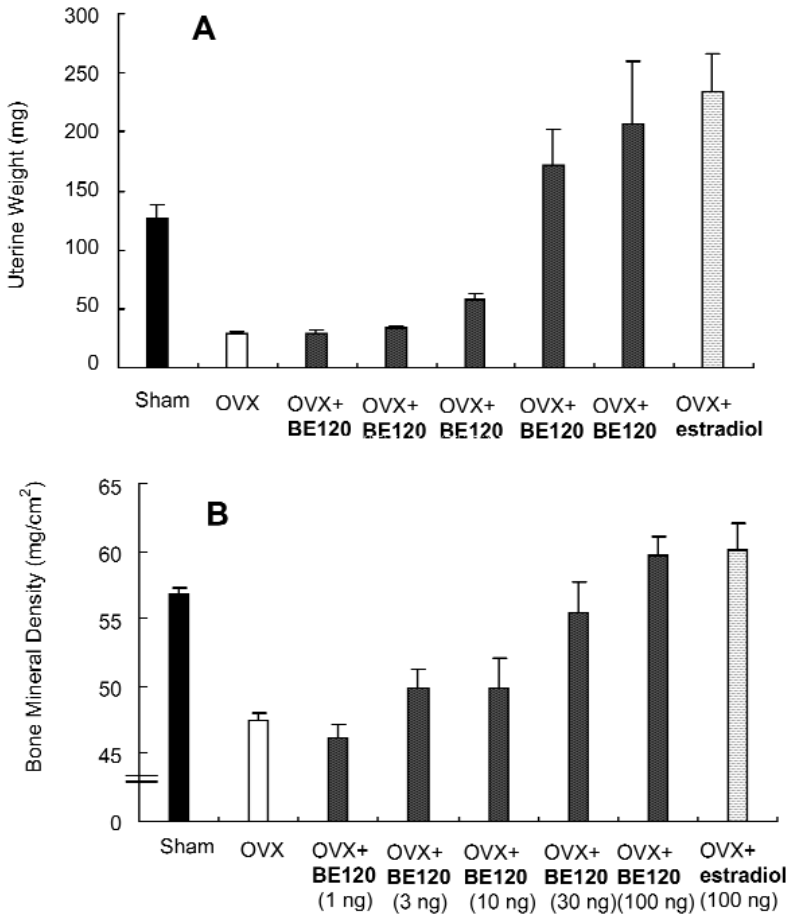

Fig. 3 Effects of **1** and **2** on the uterine weight (**A**) and bone mineral density (BMD) of femurs (**B**) in OVX mice.

at 100 ng/day markedly and similarly prevented the bone loss in the distal metaphysis in OVX mice. The effect of **2** on bone mass was determined by measuring BMD at a distal region of the femur. BMD was significantly reduced by OVX but recovered completely to the sham level on the administration of 100 ng/day of **2**. 17β-Estradiol at the same dosage also restored the BMD, as reported previously. These results suggest that **2** binds to estrogen receptor(s) present in uterus and bone, and exhibits estrogen-like effects in both tissues.

Application of boron clusters as a hydrophobic pharmacophore for estrogen antagonists and selective estrogen receptor modulators

Ligand design and synthesis

Many nonsteroidal estrogen antagonists, such as tamoxifen (**8**)-related compounds, have been synthesized and shown to possess activity, and some have been developed for clinical use. Recently, studies on the three-dimensional structure of the complexes formed by estrogen agonists (17β-estradiol, [10] stilbestrol) and antagonists (4-hydroxytamoxifen (**9**) [11] and raloxifene (**10**)) with the human estrogen receptor-α ligand-binding domain (hERαLBD) have been reported. It is suggested that an agonist-induced conformational change involving helix 12, the most C-terminal helix of LBD, is essential for activation function (AF-2) activity and the appearance of estrogenic action. 4-Hydroxytamoxifen is oriented in the hERα ligand-binding pocket in such a way that the phenolic hydroxyl group is hydrogen-bonded to glutamate (Glu-353) and arginine (Arg-394) of hERαLBD in approximately the same manner as the phenolic hydroxyl group of 17β-estradiol. However, the bulky $(CH_3)_2NCH_2CH_2O$-phenyl group of the antagonist extends towards helices 3, 8, and 11 of hERαLBD compared to the case of the agonist-hERaLBD complex. This may result in conformational change of helix 12 and the appearance of antiestrogen activity. On the other hand, the high estrogenic activity of BE120 (**2**) suggests that the carborane cage works as a hydrophobic group for binding to the hydrophobic cavity of ER, and the hydrophobic van der Waals contacts along the spherical carborane cage produce a stronger interaction than that in the case of 17β-estradiol. Therefore, it should be possible to design new estrogen antagonists on the basis of the carborane skeleton. These considerations led us to synthesize and biologically evaluate compounds having *o*-, *m*-carborane skeletons and a hydroxyl group at the *para*- and *meta*-position of an aromatic nucleus (**12**–**16**), as shown in Fig. 4 [12].

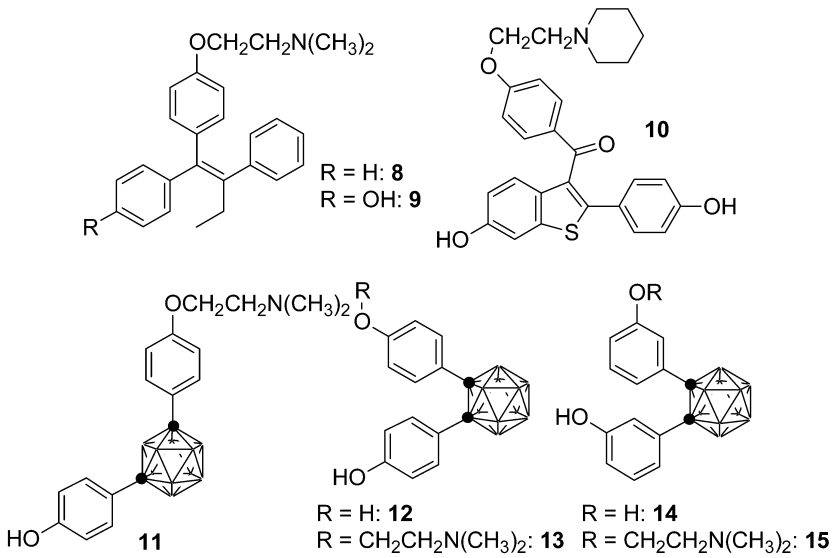

Fig. 4 Structures of typical nonsteroidal estrogen antagonists and designed molecules bearing carborane.

The synthetic routes to the designed molecules are summarized in Fig. 5. Compound **11** was prepared from 1,7-dicarba-*closo*-dodecaborane (**16**). Coupling of the C-copper (I) derivative of **16**, prepared from the corresponding lithiocarborane, with 4-methoxyiodobenzene in dimethoxyethane in the presence of pyridine gave the bis-C-arylated product (**17**) in 60 % yield. Demethylation of the methoxy group of **17** with boron tribromide followed by reaction with 2-chloroethyldimethylamine hydrochloride gave **11** (BE262) (32 %). The coupling of the C-copper (I) derivative of 1,2-dicarba-*closo*-dodecaborane with 4-methoxyiodobenzene did not afford the bis-C-arylated product because of steric hindrance to 1,2-substitution of the carborane cage. Therefore, compounds **12** and **13** were prepared by construction of the *o*-carborane cage from diarylalkyne with *nido*-decaborane(14). Bis(4-methoxyphenyl)acetylene (**18**), which was prepared from 4-methoxyethynylbenzene with 4-methoxyiodobenzene, was converted to 1,2-bis(4-hydroxyphenyl)-1,2-dicarba-*closo*-dodecaborane (**19**) in 31 % yield. Demethylation of the methoxy group of **19** with boron tribromide afforded 1,12-bis(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (BE360, **12**) in 99 % yield. The bisphenol **12** was converted to the monoalkylated derivative (BE362, **13**) by reaction with 2-chloroethyldimethylamine hydrochloride (12 %). Compounds bearing a *meta*-hydroxyl group, **14** and **15**, were prepared from 3-methoxyiodobenzene and the corresponding carborane in the same manner as described for the *para*-hydroxyl isomers.

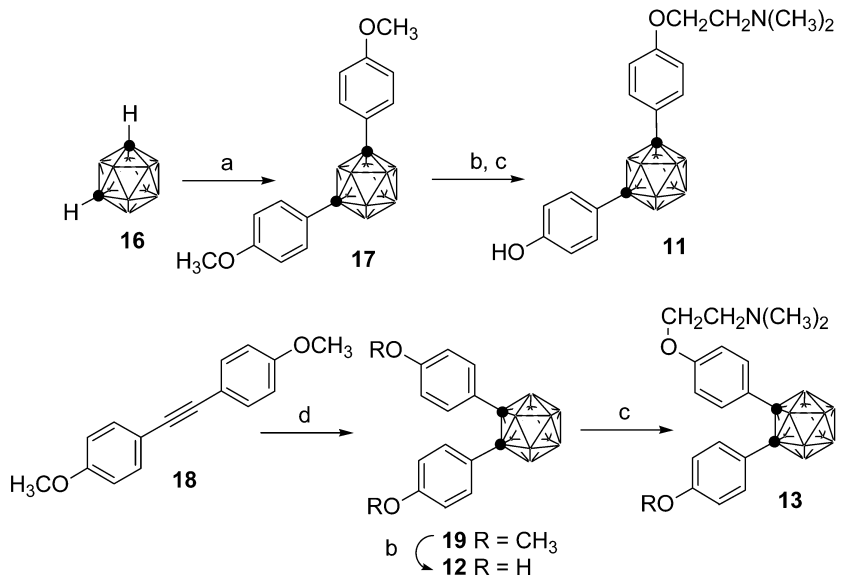

Fig. 5 Synthetic route used for the preparation of estrogen antagonists bearing carborane. Key: (a) 1) *n*-BuLi, CuCl/DME 2) *p*-iodoanisole/pyridine, reflux; (b) BBr_3/CH_2Cl_2; (c) $(CH_3)_2NCH_2CH_2Cl$ HCl, K_2CO_3/DMF; (d) decaborane(14), CH_3CN/benzene, reflux.

Transactivational properties and ER-binding affinity of the designed carborane-containing molecules [12]

The results on the inhibition of transcriptional activity of 17β-estradiol at the concentration of 10^{-9} M by our carborane-containing molecules (**11**–**15**) are summarized in Fig. 6. The compound based on *meta*-carborane (**11**) exhibited antiestrogenic activity toward 17β-estradiol in the concentration range of 1×10^{-7}–10^{-6} M in a dose-dependent manner. The antagonistic activity was increased in the case of the compound based on *ortho*-carborane (**13**), which inhibited 70 % of the transcriptional response to 17β-estradiol at the concentration of 1×10^{-7} M, and almost completely inhibited it at 1×10^{-6} M. The antagonistic activity of the compounds bearing a *meta*-hydroxyl group, **15**, was weaker than that of *para*-hydroxyl compounds; however, compound **15** almost completely inhibited the transcriptional re-

sponse to 17β-estradiol at the concentration of 1×10^{-6} M. The simple bis(hydroxylphenyl)-*o*-carboranes (**12** and **14**) also exhibited antiestrogenic activity. The activities were somewhat weaker than that of compounds **11**, **13**, and **15**, with a dimethylaminoethyl group.

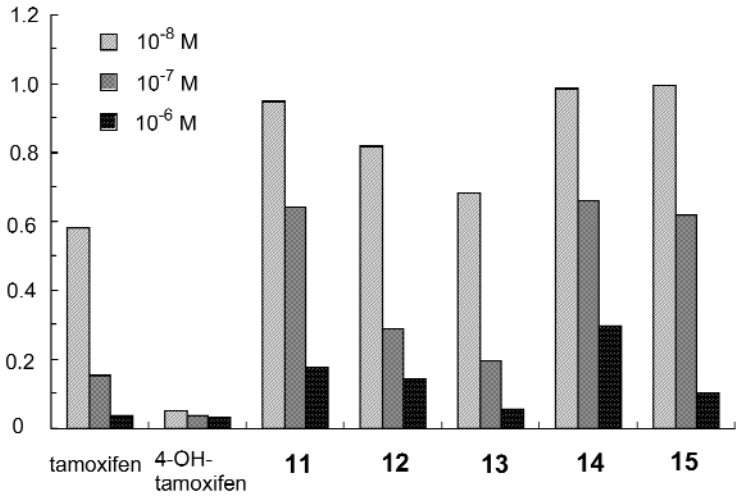

Fig. 6 Luciferase reporter gene assay of estrogen antagonists bearing carboranes (**11**–**15**). The values indicate the inhibition of the effect of 10^{-9} M 17β-estradiol (taken as 1.0).

On the other hand, the ERα-binding data for these compounds are not consistent with the results of the above-mentioned reporter gene assay. Compounds **12** showed strong affinity for ERα; its potency was comparable to that of 17β-estradiol. The affinities of the compounds with a dimethylaminoethyl group, **11** and **13** were greatly reduced. The K_i's of **12**, **11**, and **13** for ERα were 1.0 nM, 5.0 nM, and 60 nM, respectively.

Effects of carborane-containing estrogen antagonists on the whole animal

Among the estrogen antagonists bearing carborane, we focused on BE120 and BE360. To examine the estrogenic activity of these compounds in bone, OVX mice were given BE120 (**2**), BE360 (**12**), or estradiol (**1**) subcutaneously for 4 weeks using a miniosmotic pump. Reduced uterine weight in OVX mice was restored completely by **2**, as mentioned above. However, 1000-fold higher doses of **12** did not increase the uterine weight of OVX mice. Histological analysis confirmed that **12** exhibited no estrogenic activity in uterus. In OVX mice, the trabecular bone volume of the femoral distal metaphysis was reduced markedly, when measured by DEXA and histological analysis. Treatment with **2** at 1–30 ng/head/day markedly prevented the bone loss in OVX mice, and the effective doses in bone and uterus were similar to those of **1**. At the 1000-fold higher dose of 1–30 mg/head/day, compound **12** dose-dependently restored bone loss in OVX mice to the sham level without estrogenic action in the uterus. These results indicate that BE360 (**12**) binds to ERα and exhibits estrogenic action in bone to prevent bone loss *without* inducing estrogenic action in the uterus, suggesting its possible application to treat osteoporosis, as a new type of selective estrogen receptor modulator.

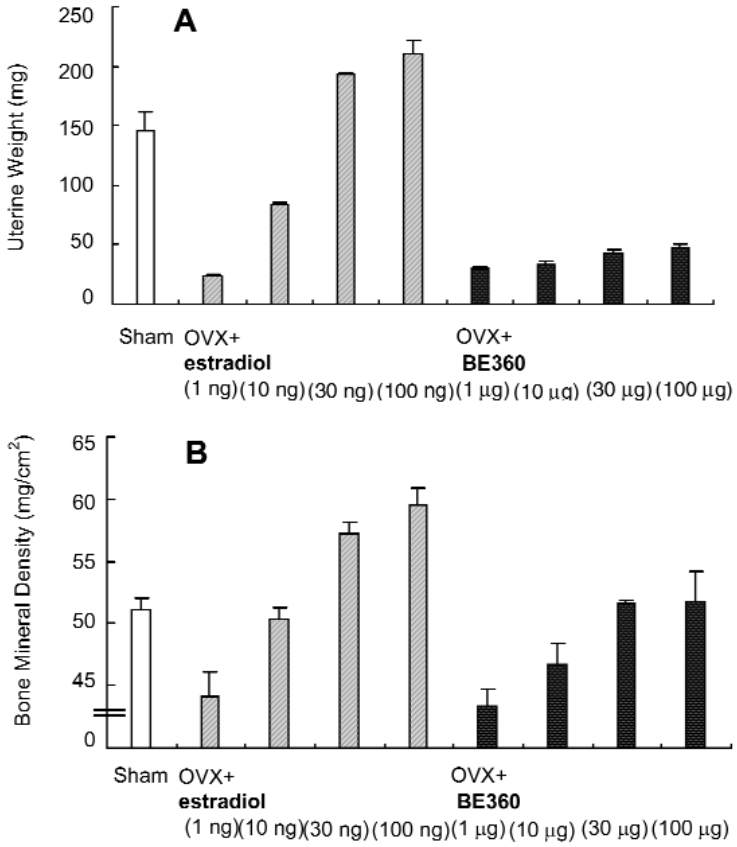

Fig. 7 Effects of **1** and **12** on the uterine weight (**A**) and BMD of femurs (**B**) in OVX mice.

CONCLUSION

In summary, we have developed novel carborane-containing molecules with potent estrogenic activities. The unique character of biologically active molecules containing a carborane skeleton may give rise to unusual membrane transportation properties and metabolism, compared with conventional active molecules. The selective effect of the carborane-containing compounds toward different organs raises the possibility of the development of SERMs, which could be useful as therapeutic agents.

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