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Synthesis and biological evaluation of novel *m*-carborane-containing estrogen receptor partial agonists as SERM candidates

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ABSTRACT

We designed and synthesized novel *m*-carborane-containing selective estrogen receptor modulator (SERM) candidates using previously reported *m*-carborane-containing ER partial agonist **1** as the lead compound. Biological activities were evaluated by means of ERα competitive binding assay and MCF-7 cell proliferation assay. Re-positioning the *N*,*N*-dimethylaminoethyloxy group at the *para* position of **1** to the *meta* position enhanced the ERα-binding affinity, and **4c** showed the highest relative binding affinity (RBA: 83 vs 17β-estradiol = 100) among the tested compounds. Compound **4b** showed the most potent ER-agonist activity (EC_{50}: 1.4 nM) and the lowest maximal efficacy (E_{max}: 50%) in MCF-7 cell proliferation assay. Inhibition of 0.1 nM 17β-estradiol-induced MCF-7 cell proliferation by **4b** (IC_{50}: 0.4 μM) was at least 10 times more potent than that of the lead compound **1**.

Estrogens are involved in regulation of the female and male reproductive systems, bone metabolism, and the cardiovascular system, as well as the central nervous system, and these activities are expressed through binding to and activation of nuclear estrogen receptor (ER).[1] ER has two subtypes (α and β), which show different patterns of tissue expression and mediate two different signaling pathways: transcriptional regulation and non-genomic membrane-associated transduction.[1] Many of the physiological effects of ER are subtype-specific. Non-steroidal and non-hormonal ER antagonists, such as tamoxifen[2] and raloxifene,[3] are widely used for treatment of breast cancer (Fig. 1). Tamoxifen and its active metabolite, 4-hydroxytamoxifen, show ER agonism in endometrium and bone, whereas raloxifene acts as an antagonist in endothelium and as an agonist in bone.[4] Therefore, raloxifene has no risk for cancer of the female reproductive system, and is used as a protective agent against osteoporosis in post-menopausal women.[5] Compounds having tissue-specific ER agonist or antagonist activity are called selective estrogen receptor modulators (SERMs).[6] They exhibit considerable functional diversity: for example, bazedoxifene contains an alkylamino side chain, which is critical for agonist and antagonist activities of SERMs, but its biological activities are different from those of tamoxifen and raloxifene.[7] In addition, the binding mode of bazedoxifene to ERα is different from that of 4-hydroxytamoxifen, as evaluated by docking simulation study.[8] The relative agonist/antagonist activities of SERMs, including ER partial agonists, seem to be controlled by the overall shape of the ER homodimer formed after ER-ligand binding. Therefore, binding of different ligands to ER can facilitate or impede the interaction of ER homodimer with various co-regulators.[9] The hydrophobic core structure of SERMs plays an important role in determining their elaborate biological and pharmacokinetic profiles. Therefore, development of ER modulators with novel hydrophobic core structures is expected to afford unique SERMs with distinctive biological properties.

We have developed several ER modulators having a carborane cage as a novel hydrophobic pharmacophore.[10] They exhibit unique estrogen-related biological activities, different from those of the endogenous estrogen 17β-estradiol (E2), or the above-mentioned SERMs. BE360, an *o*-carborane-containing ER modulator without an alkylamino side chain, showed partial agonist activity in MCF-7 cell proliferation assay, and it increased bone density with no effect on the uterus in ovariectomized mice (Fig. 2).[11] That is, BE360 is a carborane-containing SERM. We have also reported that *m*-carborane derivative **1** bearing an alkylamino group acted as a potent ER partial agonist in ER transactivation assays (Fig. 2).[12] However, the maximal efficacy of **1** is not low, and so there is a risk that **1** might induce breast cancer. SAR studies of the alkylamino chain of *m*-carborane derivative **2** revealed that compounds with an alkyl, carbamate, or thiocarbamate group instead of the alkylamino group acted as ER full agonists.[13] Thus, the alkylamino group of **1** is essential for expression of ER partial agonist activity, as has been observed with other SERM candidates (Fig. 2).[14] Recently, we have reported that the

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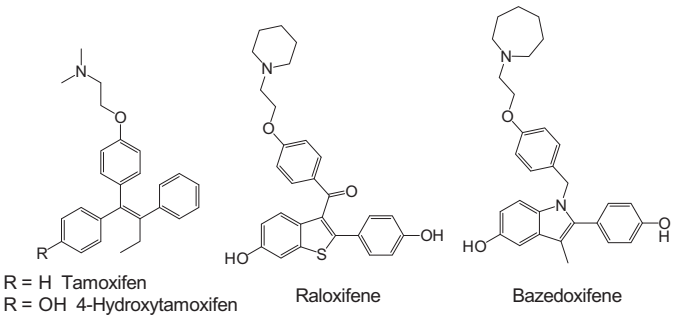


Figure 1. Structures of clinically used SERMs.

9,10-dimethyl-*m*-carborane cage is effective for obtaining ER partial agonist activity with very low maximal efficacy, as well as for increasing ER-binding affinity (compound **3**, Fig. 2).[15] This may be due to a geometry change of the alkylamino group remotely induced by steric repulsion between the bulky hydrophobic structure and amino acid residues surrounding the ER ligand-binding domain (LBD). Although the 9,10-dimethyl-*m*-carborane cage of **3** seems to be more promising as a hydrophobic structure for ER partial agonist discovery than *m*-carborane, preparation of 1,7-diaryl-9,10-dimethyl-*m*-carborane derivatives is synthetically difficult in that coupling reaction of 9,10-dimethyl-*m*-carborane with aryl iodides affords the products in very low yield.

Therefore, we selected the *m*-carborane cage as a hydrophobic structure for the preparation of ER partial agonists and designed derivatives **4** in which the alkylamino side chain of **1** is transferred to a neighboring carbon (Fig. 3).

Scheme 1 summarizes the synthesis of *m*-carborane-containing ER partial agonist candidates **4**. *m*-Carborane **5** was treated with *n*-BuLi, and then transformed into *C*-copper-*p*-carborane, which was reacted with 4-iodoanisole in the presence of pyridine as a ligand of copper to afford 4-methoxyphenyl-*m*-carborane **6** in 71% yield.[16] Next, coupling reaction of **6** with TBS-protected iodophenol under the same conditions, followed by deprotection of the TBS group, afforded key intermediate diaryl-*m*-carborane **7** in 71% yield. Dimethylaminoethyl and dimethylaminopropyl groups were introduced into **7** by using the corresponding alkyl halides in 33% and 24% yields, respectively. Demethylation of **8** with BBr_3 afforded the desired compounds **4a** and **4b** in 45% and 74% yields, respectively. Compound **7** was reacted with BBr_3 to afford bisphenol **9**

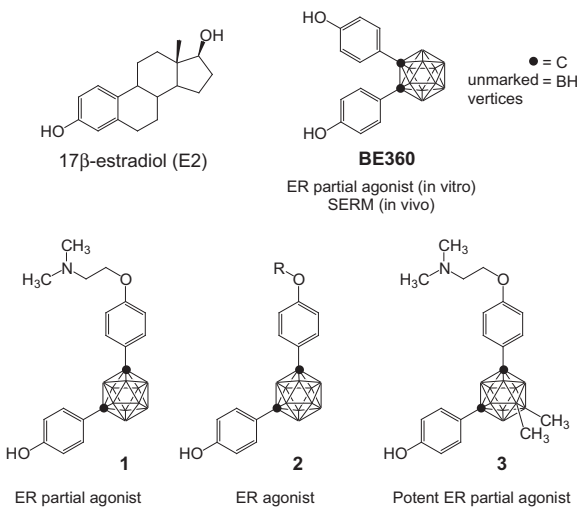


Figure 2. Structures of 17β-estradiol (E2) and *m*-carborane-containing ER modulators.

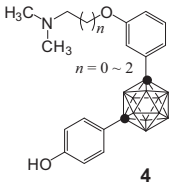
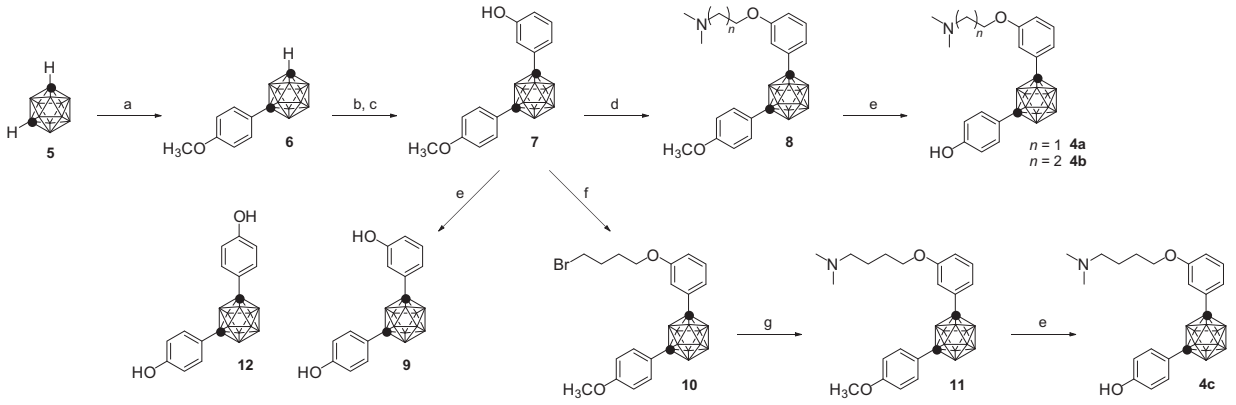


Figure 3. Structures of novel ER partial agonist candidates **4**.

in 94% yield; its biological activity was evaluated and compared with that of bisphenol **12**, an intermediate of **1** (Scheme 1). Compound **4c** was obtained by stepwise synthesis involving two S_N2 reactions of dibromobutane. One bromine atom was changed to a phenolic hydroxyl group (**7**) in 62% yield, and the other was reacted with dimethylamine to afford **11** in 78% yield. Demethylation of **11** with BBr_3 afforded **4c** in 34% yield.

ER-binding affinity was evaluated by means of competitive binding assay using human recombinant ERα and [6,7-^{3}H] 17β-estradiol. Relative binding affinity (RBA) values of the test compounds are summarized in Table 1.[17] Compounds **1** and **12** showed low and high RBA values of 1.5 and 110, respectively, which are close to the previously reported values of 1.1 and 106, respectively.[12] Although the binding affinity of the parent bisphenol **9** is similar to that of **12**, compound **4a** with an *N*,*N*-dimethylaminoethoxy side chain showed 5 times more potent ERα-binding than the corresponding *p*-substituted derivative **1**. Our previous results showed that extension of the alkylamino chain of **1** has little influence on RBA values, but the same modification of the alkylamino chain of **4** led to a remarkable enhancement of RBA value, and the RBA of **4c** was 83.[18] These results suggest that an alkylamino side chain at the *meta* position fits well into the cavity of the ERα LBD, and the terminal tertiary amino group of **4c** forms hydrogen bonds with amino acid residues of the ERα LBD.

Next, the functional activities of the test compounds were evaluated by means of cell proliferation assays using MCF-7 cell lines that show ER-dependent growth.[17] Table 2 summarizes EC_{50} and IC_{50} values as parameters of the agonist and antagonist activities of the test compounds, respectively. Agonist activity is also shown as relative maximal efficacy (E_{max}), based on estradiol as 100%. Bisphenol **9** showed similar EC_{50} and E_{max} values to **12**. Both bisphenols showed no ER-antagonist activity and acted as ER full agonists, not as side-chainless partial agonists like BE360. The lead compound **1** showed moderate agonist activity and its E_{max} value was 78%, which means it has lower maximal efficacy than E2. Compound **1** antagonized MCF-7 cell proliferation induced by 0.1 nM of E2 with an IC_{50} value of 4.4 μM. Compound **4a**, which has the same side chain as the lead compound **1**, but at the *meta* position, showed potent ER agonist activity (EC_{50} = 4.7 nM) and a low E_{max} value of 63%. The IC_{50} value of **4a** was 6.5 μM, which is similar to that of **1**. Compound **4b** with a dimethylaminopropyl group showed the lowest EC_{50} value and the lowest E_{max} value among the tested compounds. In addition, compound **4b** showed 10 times more potent ER antagonist activity than the lead compound **1**. Although dimethylaminobutyl derivative **4c** showed the greatest ERα-binding affinity, its biological activities parameters, EC_{50}, E_{max}, and IC_{50}, are similar to those of compound **4a**. These results confirm that the dimethylaminopropyl group is the most suitable ER partial agonist activity-inducing substituent in the series of *m*-carborane-containing *m*-substituted derivatives **4**. The ER-antagonist activity of **4b** was more potent than that of **3**, which contains the 9,12-dimethyl-*m*-carborane cage (IC_{50} of **3** = 0.88 μM). The low IC_{50} value of **4b** suggested that the side chain serves to inhibit binding of co-activators by moving helix-12 of ER to an unfavorable position. However, compound **4b** has a higher



Scheme 1. Synthesis of novel ER modulators **4**. Reagents and conditions: (a) *n*-BuLi, DME, then CuCl, pyridine, 4-iodoanisole, 71%; (b) *n*-BuLi, DME, then CuCl, pyridine, 3-*tert*-butyldimethylsiloxyiodobenzen; (c) TBAF, THF, 71% over 2 steps; (d) *N*,*N*-dimethylaminoalkyl chloride, K_2CO_3, acetone, 24–38%; (e) BBr_3, CH_2Cl_2, 34–94%; (f) 1,4-dibromobutane, K_2CO_3, acetone, 62%; (g) dimethylamine, THF, 78%.

Table 1
Relative binding affinity (RBA) of test compounds versus specific [^{3}H]estradiol (4 nM) binding with human recombinant ERα

Compound	Position	Substituent	RBA[a]
1	*p*-	–O–$(CH_2)_2$–$N(CH_3)_2$	1.5
4a	*m*-	–O–$(CH_2)_2$–$N(CH_3)_2$	7.4
4b	*m*-	–O–$(CH_2)_3$–$N(CH_3)_2$	8.4
4c	*m*-	–O–$(CH_2)_4$–$N(CH_3)_2$	83
9	*m*-	–OH	131
12	*p*-	–OH	110

[a] All binding assay were treated with the test compounds (0.4 nM to 4 μM) in the presence of [6,7-^{3}H]17β-estradiol (4 nM). The relative binding affinity is calculated from IC50 values of E2 and test compounds taking that of E2 as 100%. Values represent the average of duplicate experiments.

E_{max} value than compound **3** (E_{max} of **3** = 23%), and might show moderate estrogenic activity in breast tissue. A terminal cyclic alkylamino group, such as piperidine or azepane (used in raloxifene and bazedoxifene, respectively), can often enhance ER-antagonist activity, and thus introduction of these substituents might afford better partial agonists or SERM candidates. Carborane cages are promising hydrophobic core structure for the development of ER modulators,[10–13,15,17] and carborane-containing ER modulators may show unique biological properties.

Table 2
Biological activities of the test compounds on MCF-7 cell proliferation

Compound	Position	Substituent	EC_{50}[a] (nM)	E_{max}[b] (%)	IC_{50}[c] (μM)
1	*p*-	–O–$(CH_2)_2$–$N(CH_3)_2$	8.2	78	4.4
4a	*m*-	–O–$(CH_2)_2$–$N(CH_3)_2$	4.7	63	6.5
4b	*m*-	–O–$(CH_2)_3$–$N(CH_3)_2$	1.4	50	0.4
4c	*m*-	–O–$(CH_2)_4$–$N(CH_3)_2$	2.6	62	4.3
9	*m*-	–OH	1.8	107	Inactive
12	*p*-	–OH	1.4	90	Inactive

[a] MCF-7 cells were treated with the test compounds (1×10^{-13} to 1×10^{-5} M) alone. EC_{50} values were estimated from the sigmoidal dose-response curves using GraphPad Prism software.

[b] E_{max} values indicate efficacy for cell proliferation, based on the value for E2 taken as 100%.

[c] MCF-7 cells were treated with the test compounds (1×10^{-11} to 1×10^{-5} M) in the presence of 0.1 nM E2. IC_{50} values were estimated from the sigmoidal dose-response curves for competitive antagonism against cell proliferation activity induced by 0.1nM E2, using GraphPad Prism software.

Novel ER modulator **3** showed better partial agonist activity than **1**, even though it contains the same alkylamino chain and has the same substitution position.[15] Further syntheses of a series of diaryl-*m*-carborane derivatives, docking simulation studies, and biological investigations with other ER-expressing tissues, determination of ER expression levels, and examination of the influence of 9,12-dimethyl-*m*-carborane structure on ER partial agonist activity, as well as in vivo experiments, are in progress.

In conclusion, novel *m*-carborane-containing ER partial agonists were synthesized and their biological activities were evaluated by means of competitive ER binding assay and MCF-7 cell proliferation assay. All tested compounds **4** showed higher RBA values than the corresponding *para*-substituted derivatives, suggesting that an alkylamino side chain at the *meta* position fits well into the cavity of the ERα LBD. Compound **4b** showed the most potent ER-agonist activity and the lowest E_{max} value among the tested compounds. Moreover, the ER-antagonist activity of **4b** was 10 times more potent than that of the lead compound **1**. As observed in the case of bazedoxifene, the unique hydrophobic carborane cage structure provide an entry into carborane-containing ER modulators with various characteristics of ER up- or down-regulation, as well as distinctive pharmacokinetic properties. These findings should be helpful for molecular design of further carborane-containing ER modulators, including agonists, partial agonists, antagonists, and SERMs as biological tools or candidate therapeutic agents for ER-related diseases.

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18. RBA values of the corresponding *m*-carborane-containing *p*-substituted derivatives are as follows: *N*,*N*-dimethylaminoethyl = 1.1, *N*,*N*-dimethylaminopropyl = 1.5, and *N*,*N*-dimethylaminobutyl = 1.7.