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Estrogenic activity of bis(4-hydroxyphenyl)methanes with cyclic hydrophobic structure

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ABSTRACT

Monoalkylated bis(4-hydroxyphenyl)methanes (e.g., **1**) are reported to show weak binding affinity for estrogen receptor (ER). We hypothesized that introduction of appropriately located hydrophobic substituents in these compounds would increase the binding affinity. Indeed, we found that bis(4-hydroxyphenyl)methane bearing a 3,3-dimethylcyclohexyl group (**7**) shows potent ERα binding affinity, comparable to that of estradiol. Bulkier substituents could be introduced at the 3,3-position without decreasing the affinity. However, the position of the substituents was critical: the 4,4-dimethylcyclohexyl derivative (**2**) showed very weak binding affinity. The compounds with high ER-binding affinity showed predominantly agonistic activity, together with weak antagonistic activity at high concentration, in cell proliferation assay with human breast cancer cell line MCF-7. Further structure–function studies of these compounds and their derivatives might lead to the development of more selective and potent estrogen receptor modulators.

1. Introduction

Estrogens, such as 17β-estradiol, play an important role in the female and male reproductive systems,[1] as well as in bone maintenance, in the central nervous system[2] and in the cardiovascular system.[3] The first step in the appearance of these activities is binding of the ligands to estrogen receptors α and β (ERα[4] and β[5]). This binding results in a conformational change of the receptor, inducing dimerization. The dimer functions as a transcription factor, which causes biological responses by binding to specific promoter elements of DNA to initiate gene transcription. It has been reported that ERα and ERβ have different tissue distributions[6] and biological roles, and they sometimes act in opposition to each other. Numerous subtype-selective ligands have been reported and ERβ-selective ligands are of potential clinical interest.[7] However, the nature of the differences between the two ER subtypes has not been fully established, possibly because ERα is predominant as a transcription factor, compared with ERβ.[8]

From a clinical point of view, there is great interest in selective estrogen receptor modulators (SERMs), which are tissue-selective ER agonists and antagonists. The major factor determining tissue selectivity is considered to be quantitative and qualitative differences of co-regulatory proteins in the ER-mediated transcriptional systems of each target tissue.[9] These co-regulatory proteins alter the conformational state of the ER–ligand complex to influence the transcriptional action. Similarly, the agonist/antagonist balance of SERMs is determined by the conformational state of the ER–ligand complex. These complex macromolecular systems can be controlled by low-molecular-weight ligands. Typical SERMs such as tamoxifen[10] and raloxifene[11] were found to be agonistic in bone and antagonistic in breast, but showed varying activity in uterus. The agonist/antagonist balances of the two SERMs are different: tamoxifen is more antagonistic and raloxifene is more agonistic. Therefore, elucidation of the structure–activity relationships of partial agonist/antagonists is important for elucidating ER activation mechanisms and for developing useful clinical medicines.

Based on the structure of the complex formed by estradiol and the human ER-α LBD (hERαLBD),[12] the phenolic hydroxyl group is hydrogen-bonded to Glu-353 and Arg394 of hERαLBD and the 17β-hydroxyl group is hydrogen-bonded to the δ-nitrogen of His-524. The importance of these two hydrogen-bondings is very different: the phenolic hydrogen-bonding is indispensable, whereas the 17-hydroxyl hydrogen-bonding can be replaced by hydrophobic interaction. Hydrophobic interaction of the steroidal skeleton of the ligand with the LBD also plays an important role for stabilization

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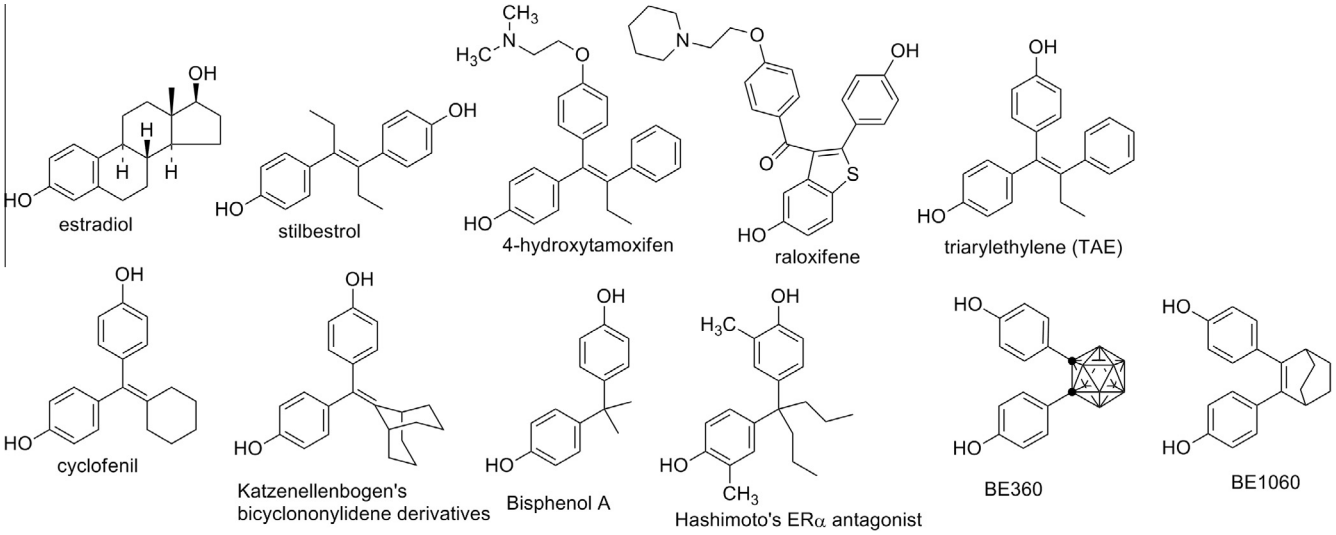
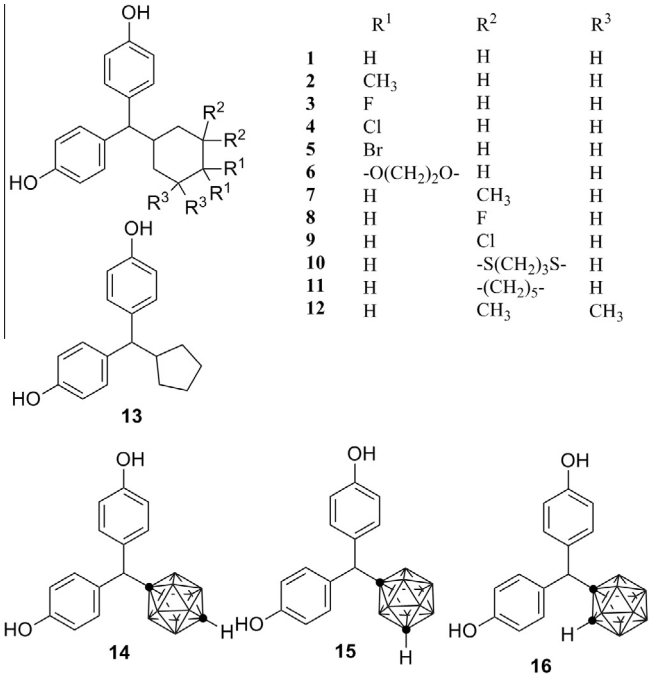


Figure 1. Various estrogen receptor modulators.

and binding activity of the ER–ligand complex. Since the discovery of the potent ER agonist diethylstilbestrol and SERM tamoxifen, triphenylethylene and diphenylethylene skeletons have attracted considerable attention as basic structures for ER ligands.[13] Katzenellenbogen et al. have reported that 1,1-diarylethylene derivatives with bridged bicyclic structures have high ER-binding activity, and these compounds mainly show ER antagonistic activity.[14] Jordan et al. reported that 1,1,2-triphenylethylenes with two or three hydroxyl groups in various phenyl rings showed ER agonistic activity.[15] Another important compound is bisphenol A, which has weak estrogenic activity but has been manufactured on a considerable scale as an industrial material for use in plastic products. Recently, Hashimoto et al. reported that bisphenol A derivatives with extended alkyl chains show selective ERα antagonistic activity[16] (see Fig. 1).



	R^1	R^2	R^3
1	H	H	H
2	CH_3	H	H
3	F	H	H
4	Cl	H	H
5	Br	H	H
6	$-O(CH_2)_2O-$	H	H
7	H	CH_3	H
8	H	F	H
9	H	Cl	H
10	H	$-S(CH_2)_3S-$	H
11	H	$-(CH_2)_5-$	H
12	H	CH_3	CH_3

Figure 2. ER Ligands with cyclic hydrophobic structures. In the icosahedral cage structure, ● represent carbon atoms and other vertices represent BH units.

We have focused on the application of three-dimensional hydrophobic structural units, such as icosahedral boron clusters (carboranes[17]), that fit in the hydrophobic cavity of the ER LBDs.[18] In our design and biological activity studies of bisphenols with a hydrophobic core structure, we have found that the hydrophobic core structure has the ability to regulate agonist/antagonist balance. For example, BE360, bis(4-hydroxylphenyl)-*o*-carborane, showed partial antagonist activity towards ER.[19] However, BE1060, in which the carborane cage of BE360 is replaced with a hydrocarbon core, bicyclo[2,2,2]octene, exhibited potent ER agonistic activity, even though the two phenolic groups appear to be similarly directed.[20] Thus, differences in receptor–ligand complex structures arising from the presence of different hydrophobic structures in the ligand can influence biological activity. 1,1-Diarylethylene derivatives such as cyclofenil have potent binding affinity for ER, and show mixed agonist/antagonist activities.[21] On the other hand, hydrogenated 1,1-diarylmethane derivatives[22] have not received much attention because of their lower binding affinity.[14] However, we hypothesized that addition of appropriate hydrophobic substituents would improve the binding affinity and provide a means to regulate the agonist/antagonist activity balance. In this article, we describe the synthesis and biological evaluation of simple bisphenols, bis(4-hydroxyphenyl)methanes, bearing cyclic hydrophobic structures.

2. Results

2.1. Chemistry

We presumed that the first phenolic hydroxyl group would act as an anchor at the hydrogen-bonding site of ER, while the three-dimensional hydrophobic core would fill the hydrophobic cavity of ER. The steric and electronic effects of substituents on the hydrophobic core were expected to determine the nature of the estrogenic action. Therefore, we designed bis(4-hydroxyphenyl) methanes with a cyclohexyl group bearing various substituents (**1**–**12**), as shown in Figure 2. We also designed bis(4-hydroxyphenyl)methanes with a cyclopentyl group (**13**) and a carboranyl group (**14**–**16**).

Synthesis of unsubstituted and 4,4-disubstituted cyclohexyl derivatives is summarized in Scheme 1. Cyclohexyl (**1**) and 4,4-dimethylcyclohexyl (**2**) derivatives were prepared by McMurry coupling reaction of 4,4-dimethoxybenzophenone with