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Design and synthesis of estrogen receptor ligands with a 4-heterocycle-4-phenylheptane skeleton

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ABSTRACT

The estrogen receptor (ER), a member of the nuclear receptor (NR) family, is involved in the regulation of physiological effects such as reproduction and bone homeostasis. Approximately 70% of human breast cancers are hormone-dependent and ERα-positive, and, thus, ER antagonists are broadly used in breast cancer therapy. We herein designed and synthesized a set of ER antagonists with a 4-heterocycle-4-phenylheptane skeleton.

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1. Introduction

Estrogen receptors (ER), a member of the nuclear receptor (NR) family, are involved in the regulation of a number of physiological effects such as reproduction and bone homeostasis.[1,2] Binding of the hormone estrogen (17β-estradiol) to ER induces the translocation of ER into the nucleus, and this is followed by transactivation of the target gene. There are two different forms of ER, referred to as ERα and ERβ, each of which is encoded by a separate gene.[3,4] Approximately 70% of human breast cancers are hormone-dependent and ERα-positive.[5,6] Therefore, several ERα antagonists such as tamoxifen (an ERα antagonist)[7] are broadly used in ERα-positive breast cancer therapy (Fig. 1). Tamoxifen is metabolized to 4-hydroxytamoxifen (4-OHT), which has a markedly stronger binding affinity for ERα, and 4-OHT exerts its anti-estrogenic effects.[8,9] Hashimoto and coworkers previously reported that the diphenylmethane (DPM) skeleton functioned as a steroid mimic, and is a key structure in the development of ligands for several steroid receptors with the appropriate introduction of substituents.[10,11] Based on these findings, an ER antagonist (**1**: **PBP**) based on the diphenylheptane skeleton has been reported to exhibit potent ER antagonistic activity with an IC_{50} value of 4.5 nM in a reporter gene assay.[12] We previously developed an ER-selective degradation inducer (**PBP-NC10**) based on the diphenylheptane skeleton that directly binds to ERα and induces its degradation via the ubiqui-

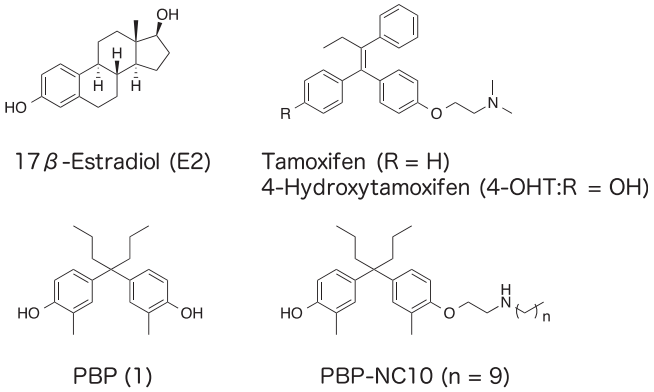

Fig. 1. Chemical structures of 17 β-estradiol, anti-estrogenic compounds, and ER-degradation inducers.

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tin-proteasome system (UPS).[13] We also reported an efficient synthetic method to construct a multi-substituted DPM skeleton and demonstrated that the synthesized compound (**2**) based on our synthetic method exhibited moderate ERα antagonistic activity.[14] In order to demonstrate the utility of our synthetic method, we continued the structural development of diphenylheptane derivatives using our synthetic method and evaluated their ERα binding affinities and ERα transcriptional activities. In the present study, we introduced several heterocycles instead of the phenyl ring of the diphenylheptane skeleton. Heterocycles are the core elements of biomolecules such as nucleic acids and amino acids, and are broadly utilized in marketed drugs as well as in the medicinal chemistry field. A previous study reported that some drug properties such as potency, hydrophobicity, and solubility may be modulated by the introduction of heterocycles into molecules.[15,16] Therefore, we expected that the introduction of heterocycles into the diphenylheptane skeleton using our method to lead to the development of a large number of ERα modulators.

2. Results and discussion

We designed and synthesized 4-heterocycle-4-phenylheptane derivatives containing several heterocycles such as thiophene (**3**, **4**), pyrrole (**5**–**8**), and indole (**9**, **10**) (Fig. 2). The synthetic routes of the designed compounds (**3**–**10**) are shown in Scheme 1. Briefly, methyl 4-hydroxy-3-methylbenzoate was treated with *n*PrMgBr to obtain compound **11**. The hydroxy group on the aromatic ring of **11** was acetylated with acetic anhydride to afford compound **12**. The corresponding heterocycles were introduced using BF_3-Et_2O and the subsequent deprotection of the acetyl group gave compounds **3**–**6** and **9**–**10**. The ethyl esters of **5** and **6** were hydrolyzed with aqueous NaOH to afford compounds **7** and **8**, respectively. Compound structures were determined referring to previous reports.[17,18]

We initially investigated the binding affinities of synthesized compounds **3**–**10** to ERα (Table 1). In order to evaluate the binding abilities of these compounds to ERα, a fluorescence polarization (FP)-based competitive binding assay was conducted using the PolarScreen nuclear receptor competitor assay kit (Life Technologies).[13] As reported previously, **PBP** strongly bound to ERα with an IC_{50} value of 7.3 nM. Compound **5** with a pyrrole ring exhibited potent activity (ERα binding affinity: **5**; IC_{50} = 195 nM). Compounds **3**–**4** and **9**–**10**, with a thiophene ring and indole ring, respectively, exhibited moderate or weak binding affinities (ERα binding affinities: **3**; IC_{50} = 4677 nM, **4**; IC_{50} = 1129 nM, **9**; IC_{50} >10,000 nM, **10**; IC_{50} = 613 nM). Moreover, methylation of the N atom of the pyrrole ring decreased binding affinity (ERα binding affinity: **6**; IC_{50} = 1040 nM), and the hydrolysis of compounds **5** and **6** significantly diminished their ERα binding affinities (ERα binding affinities: **3**, **4**; IC_{50} > 10,000 nM). These results indicate that the H atom on the pyrrole ring plays a pivotal role in binding to the ligand binding domain (LBD) of ERα. Furthermore, the benzyl group at the 6-position of compound **10** is accepted to exert its binding affinity, while the hydrolysis of compound **5** reduced its binding affinity. These results suggest that the hydrophobic binding pocket around the 2-position of pyrrole or 6-position of indole exists to accommodate a benzyl group. It has been reported that two hydroxy groups of compound **1** play an important role to exert their ER binding affinity, and the alkylation of the hydroxy group decreased ER binding affinity.[13] We assumed that substitution of a phenyl group of **1** with heterocycles attenuate their ER binding affinity because of a lacking functional hydroxy group.

ERα agonistic transcriptional activity was then evaluated using a reporter gene assay with CMX-GAL4N-hERα as the recombinant receptor gene, TK-MH100x4-LUC as the reporter gene, and the CMX-β-galactosidase gene for normalization, as reported previously.[12] Human Embryonic Kidney 293 (HEK293) cells were incubated with the synthesized compounds in the absence of E2 (0.3 nM). After the incubation, cells were assayed for reporter gene and β-galactosidase activities. ERα antagonistic activity was evaluated using a firefly reporter gene assay with an estrogen-responsible element (ERE) and control *Renilla* luciferase plasmid-SV40. Human breast carcinoma MCF-7 cells were seeded with the synthesized compounds. After the incubation, cells were assayed for the reporter gene using the dual luciferase reporter assay system kit (Promega). As shown in Table 2, the synthesized compounds exhibited weak or no ERα agonistic activity at 30 μM. Moreover, compound **5** exhibited ERα antagonistic activity, whereas the other compounds showed weak or no ERα antagonistic activity (IC_{50} = 450 nM). These results show that the ERα antagonistic activity of the tested compounds positively correlated with their binding affinities.

We performed a computational docking analysis to investigate the binding mode of compound **5** to ERα. The binding mode is of interest because it may help explain why compound **5** exhibits ERα antagonistic activity. The X-ray co-crystal structure of ERα bound to 4-OHT was obtained from the Protein Data Bank (PDB: 3ERT) and used in docking studies.[19] Docking models of compound **5** bound to ERα were constructed via a conformational analysis using the Molecular Operating Environment (MOE). Docking studies revealed that the hydroxy group of compound **5** hydrogen-bonded with the Leu387 and Arg394 residues, while the carbonyl group hydrogen-bonded with the Thr347 residue (Fig. 3a). Furthermore, compound **5** bound to ERα in a similar conformation to 4-OHT (Fig. 3b), resulting in the inhibition of ERα transcriptional activity. Although the amine group of pyrrole ring did not relate to hydrogen bond formation, methylation of the amine in pyrrole ring of compound **5** lowered the ER antagonistic activity (ERα antagonistic activity: **5**; IC_{50} = 450 nM, **6**; 41% inhibition at 10 μM treatment). We speculated that the amine of pyrrole ring would form a hydrogen bond with the vicinal carbonyl group and fixed the orientation of the ethyl ester, resulting in hydrogen bond formation with Thr347 and increase their ER antagonistic activity.

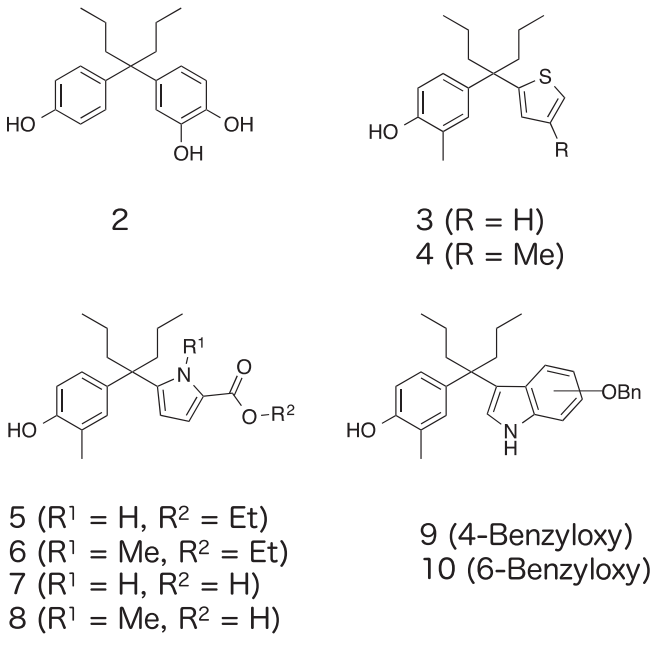

Fig. 2. Chemical structures of compounds **2** and **3**–**10** in the present study.

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