HETEROCYCLES, Vol. 101, No. 2, 2020, pp. 429 - 434. © 2020 The Japan Institute of Heterocyclic Chemistry
Received, 28th June, 2019, Accepted, 26th July, 2019, Published online 29th August, 2019
DOI: 10.3987/COM-19-14121

DESIGN AND SYNTHESIS OF 4-(2-PYRROLYL)-4-PHENYLHEPTANE DERIVATIVES AS ESTROGEN RECEPTOR ANTAGONISTS

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We dedicate this paper to Professor Dr. Kaoru Fuji on the celebration of his 80th birthday.

Abstract – The estrogen receptor (ER) has been recognized as a potential target for the treatment of breast cancer, which is the most common malignancy found in woman. In this study, a series of 4-(2-pyrrolyl)-4-phenylheptane derivatives as ER antagonists were designed and synthesized. The ER antagonistic activity of these compounds was evaluated to study their structure-activity relationships.

Estrogen receptors (ERs), which are a member of the nuclear receptor family, are involved in the regulation of numerous physiological functions, such as reproduction and bone homeostasis.[1] Binding of estrogen (17β-estradiol) to ERs induces the transcription of the ERs into the nucleus, which leads to transactivation of the target gene. It has been reported that two different forms of ER, referred to as ERα and ERβ, are encoded by a separate gene.[2] Approximately 70% of human breast cancers are hormone-dependent and ERα-positive,[3] hence several ERα antagonists including tamoxifen have been widely used as therapeutic agents against ERα-positive breast cancer.[4] However, it has been reported that 30% of patients suffering from estrogen-sensitive breast cancer will acquire tolerance to tamoxifen upon its long-term use.[5,6] Hence, the development of novel ERα antagonistic ligands as potential breast cancer therapeutic agents is urgently required.[7,8] The Hashimoto group has reported that diphenylmethane

(DPM) derivatives behave as steroid mimics and bind to nuclear receptors, such as the farnesoid X receptor (FXR), peroxisome proliferator-activated receptor (PPARα),[9] vitamin D receptor (VDR), and androgen receptor (AR).[10] Among them, their diphenylheptane derivative (PBP) displays potent ER antagonistic activity at nanomolar concentrations (Figure 1).[11] Inspired by the PBP skeleton, we have previously reported an efficient synthetic method to construct a multi-substituted diphenylmethane skeleton that exhibits ERα antagonistic activity,[12] and the further development of PBP-based compounds containing a hydrophobic side chain that exhibit ER-selective degradation activity via the ubiquitin-proteasome system.[13] From these studies, the diphenylmethane skeleton has been identified as a potent structure in the therapeutic agents used to treat ER-related disease, such as breast cancer. However, further research efforts should be focused on the molecular design and structure-activity relationships toward the development of more efficient ER antagonists. For instance, the introduction of heterocycles into the molecules may provide several benefits, such as the addition of hydrogen bonding sites, enhanced water-solubility, and changes in the hydrophilic/hydrophobic properties. From this viewpoint, we have recently developed ER binding ligands containing a 4-heterocycle-4-phenylheptane skeleton, in which a heteroaromatic ring was introduced into the PBP skeleton instead of a phenyl ring.[14] A reporter gene assay showed one of the ligands, 4-(2-pyrrolyl)-4-phenylheptane, exhibited moderate ER antagonistic activity (IC_{50} = 450 nM). Pyrrole is known as a biologically active scaffold, which is frequently found in bioactive natural products, such as alkaloids and porphyrins.[15,16] Furthermore, pyrrole and its derivatives have been widely used as pharmaceutically active functional groups in medicinal chemistry.[17] Therefore, we envisaged that the 4-(2-pyrrolyl)-4-phenyl skeleton is an appropriate structure for derivatization toward the development of efficient ER antagonistic agents. In this study, we have focused on the potency of the 4-(2-pyrrolyl)-4-phenyl skeleton as an ER antagonist, and we have designed and synthesized its derivatives to study their structure-activity relationships to obtain further insight on the role of the pyrrole moiety in their ER antagonistic activity.

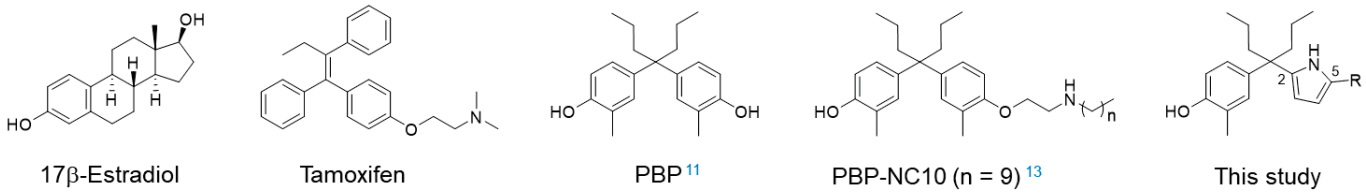
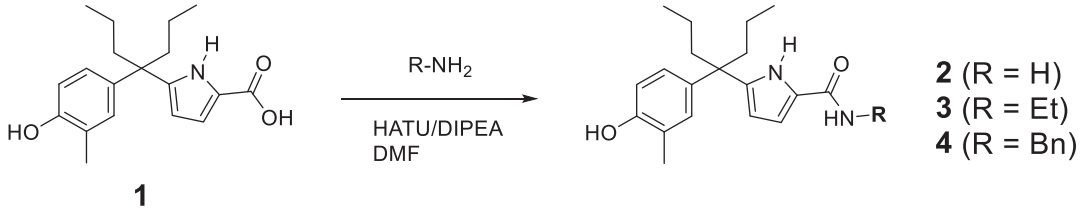


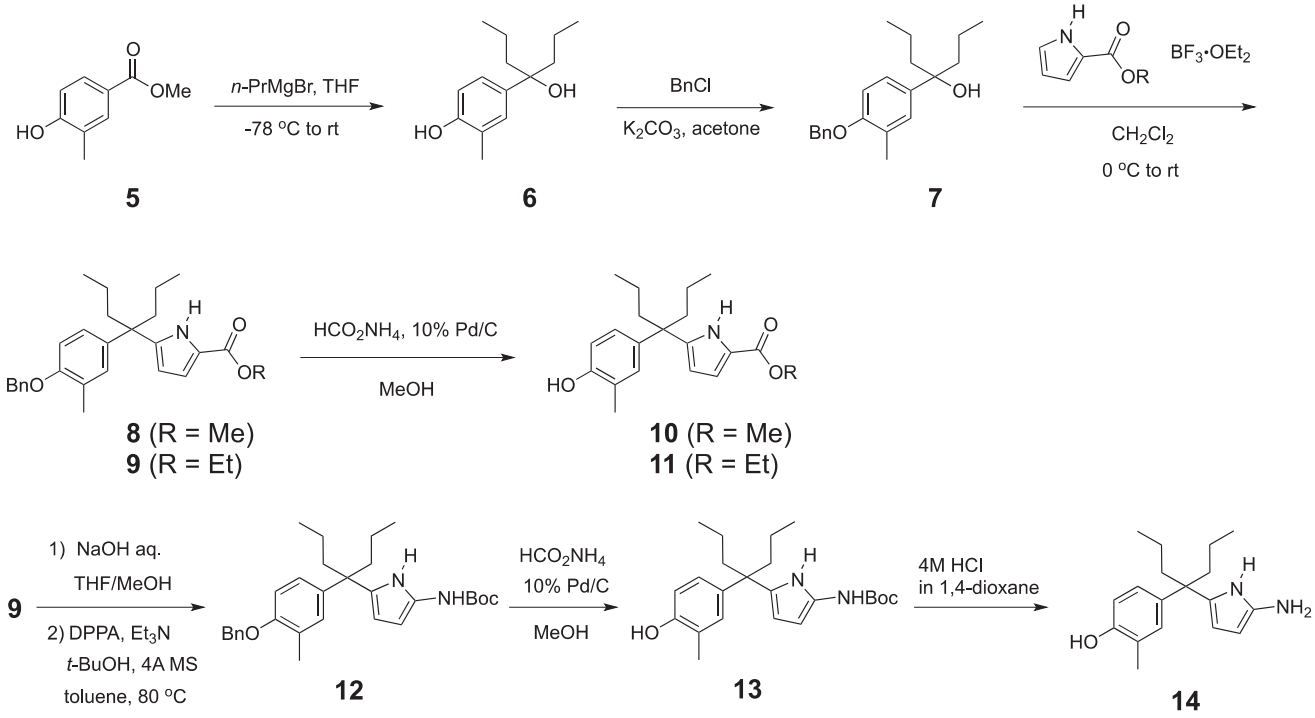
Figure 1. Chemical structures of representative ER ligands

The synthetic route used to prepare the designed compounds is shown in Schemes 1 and 2. Intermediate **1** was prepared according to a literature method.[14] Compounds **2**–**4** were obtained via a condensation

reaction between carboxylic acid **1** and a variety of amines using EDC•HCl (Scheme 1). Introduction of a 2-pyrrolyl unit into the structure of intermediate **7** using BF_3•OEt_2 afforded compound **8**, which was used for the synthesis of a variety of derivatives containing the 4-(2-pyrrolyl)-4-phenyl skeleton. Compound **12**, a *tert*-butyl carbamate derivative, was obtained from **9** via hydrolysis and a Curtius rearrangement of the resulting carboxylic acid using diphenylphosphoryl azide (DPPA) in the presence of *t*-BuOH. Deprotection of the benzyl group in **12** provided **13**, and deprotection of the Boc group afforded compound **14** (Scheme 2).



Scheme 1. Synthesis of 4-(2-pyrrolyl)-4-phenylheptane ligands **2**–**4**



Scheme 2. Synthesis of 4-(2-pyrrolyl)-4-phenylheptane ligands **10**, **11**, and **14**

We initially investigated the binding affinity of the as-obtained ligands toward ERα using a fluorescence polarization-based competition assay, which was carried out using a commercially available assay kit (Table 1). The results show that compounds **2**–**4**, which contain amide moieties at the 5-position of the

pyrrole ring, display some binding affinity toward ERα with IC_{50} values of 101, 128, and 488 nM, respectively.

Table 1. ERα binding affinity of the as-synthesized compounds

Compounds	ERα binding affinity IC_{50} (nM)
1	>10,000 [a]
2	101
3	128
4	448
10	108
11	195 [a]
14	31.3

[a] Reference 14

These results indicate that alkylation of the amide N atom decreases the ER binding affinity. Moreover, this tendency was also observed in the results obtained for the ester ligands (IC_{50} = 108 and 195 nM for **10** and **11**, respectively). In this family of ligands, compound **14** exhibited the best IC_{50} value (31.3 nM) in the ERα binding assay. We assumed that the amino group at the 5-position of the pyrrole ring may play a key role in interacting with the binding domain of ERα. Consequently, we performed a luciferase reporter gene assay in the presence of 17β-estradiol to confirm the ERα antagonistic activity of the ligands studied. The results revealed that **3** and **10** possess moderate ERα antagonistic activity (Table 2).

Table 2. ERα transcriptional activity of the as-synthesized compounds

Compounds	ERα antagonistic activity EC_{50} (nM)
2	N.A.[a]
3	33%[b]
10	972
11	450
14	N.A.[a]

[a] No activity at 1 μM
[b] Inhibition rate at 1 μM

In contrast, ERα antagonistic activity was not observed for **2** or **14** at a ligand concentration of 1 μM. The cause of these results was obscure; these ligands contain no functional groups, such as an alkyl side chain

on the N atoms of the amine or amide groups at the 5-position of the pyrrole ring, which causes their hydrophobicity to be lower than that of the other ligands studied. Hence, their lower hydrophobicity decreases their membrane permeability, resulting in no detectable ERα antagonistic activity. The difference in the ERα antagonistic activity observed between **10** and **11** can also be attributed to this (Table 2).

In conclusion, we have developed a series of 4-(2-pyrrolyl)-4-phenylheptane derivatives with functional groups at the 5-position of the pyrrole ring and demonstrated their structure-activity relationships for their ERα binding affinity and transcriptional activity. Compounds **3** and **10**, which contain an amide and ester moiety, respectively show moderate ERα binding affinities. On the other hand, our ERα transcriptional assay indicated that compound **10** displays enhanced antagonistic activity toward ERα. We expect this study will be helpful toward the design of this type of ER antagonist.

ACKNOWLEDGEMENTS

This work was supported in part by the Japan Society for Promotion of Science KAKENHI Grant Number JP17K08385 (YD)/JP19K16333 (GT).

SUPPORTING INFORMATION

Supplementary (synthesis of the starting azides, HPLC chromatograms, IR, ^{1}H and ^{13}C NMR, MS spectra, etc.) data associated with this article can be found, in the online version, at URL: https://www.heterocycles.jp/newlibrary/downloads/PDFsi/26396/101/2.

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