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Estrogenic and antiestrogenic activities of 2,4-diphenylfuran-based ligands of estrogen receptors α and β☆

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Abstract

The estrogen receptor (ER) exists in two isoforms ERα and ERβ with a different distribution in the body and different functions which are not clearly identified yet. Thus, it is desirable to have both agonists and antagonists with selectivity for one or the other ER isoform available. In a previous study we showed that 2,5-diphenylfurans can be converted into pure antiestrogens with preference for ERα. When the arrangement of the phenyl rings was altered to a 2,4-substitution, the α-selectivity was lost as demonstrated by comparative assays using recombinant human ERα and ERβ. 3,5-Dialkyl-2,4-bis(4-hydroxyphenylfurans) were shown to act as agonists with preference for ERβ. Replacement of one of the alkyl groups by the [(pentylsulfanyl)propyl]aminohexyl side chain afforded estrogen antagonists without receptor selectivity. These derivatives were characterized as pure antiestrogens in transcription and proliferation assays in ER+ MCF-7 breast cancer cells. The most potent antagonists displayed IC_{50} values of ca. 20 nM (fulvestrant 4 nM). The data showed that the 2,4-arrangement of the phenyl rings in the furan structure increases the binding affinity for ERβ in comparison to the isomeric 2,5-diphenylfurans but does not lead to a pure antagonist with selectivity for ERβ.

Keywords: Non-steroidal estrogen; Antiestrogen; Hormone-dependent breast cancer; Diphenylfuran; Estrogen receptor β

1. Introduction

Estrogens, mainly 17β-estradiol (E2, **1**, Fig. 1), exert numerous pharmacological effects in a large number of targets, including the breast, uterus, bone, brain, and cardiovascular tissues. Most of their actions are mediated by the estrogen receptor (ER) which exists in two forms, ERα and ERβ, which reside predominantly in the nucleus of target cells [1]. Recently, estrogen receptors have also been detected in the cellular membrane [2,3]. However, their functions are not yet well understood. The discovery of ERβ as a second form of the estrogen receptor initiated an intensive search for ligands with subtype selectivity [4–7]. Though ERα and ERβ display a different distribution in the body, the actions of estradiol in various tissues cannot be clearly assigned to one or the other isoform of the estrogen receptor yet. Therefore, it is desirable to have selective ligands with agonist or antagonist activity available to investigate the role of ER subtypes in various tissues.

In a previous paper we reported on the development of 2,5-diphenylfuran-based pure antiestrogens with preference for ERα (**4**, Fig. 1) [8]. These agents have the potential of inhibiting the growth of estrogen-dependent breast tumors with resistance to tamoxifen for two reasons: (i) increased levels of ERα have been detected in resistant tumors following the treatment with tamoxifen [9] and (ii) antiestrogens such as fulvestrant which are devoid of any estrogenic side effects were shown to overcome resistance to the partial antagonist tamoxifen both *in vitro* and *in vivo* [10,11]. The diphenylfuran structure was applied after John Katzenellenbogen and his coworkers have identified triphenylfurans as ERα-selective ligands [12]. Their studies, however, did not show which phenyl rings are responsible for the preference for ERα. From our data it appears that the 2,5-arrangement of two 4-hydroxyphenyl groups is essential for this preference [8]. In order to proof this assumption, the

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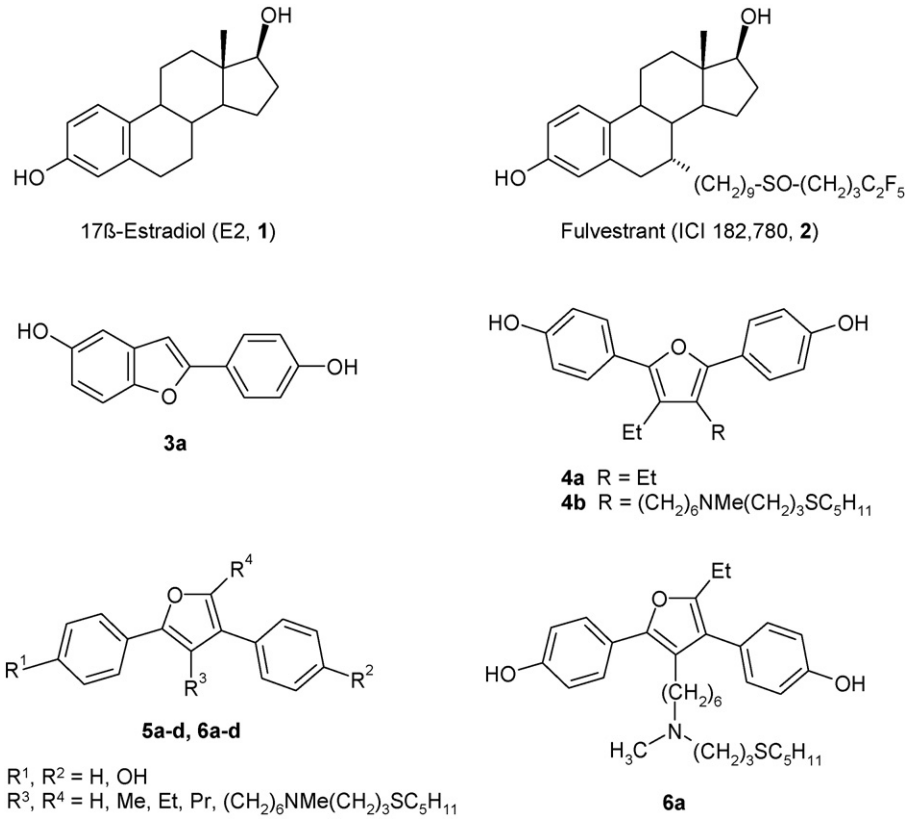

Fig. 1. Chemical structures of 17β-estradiol (**1**), fulvestrant (**2**), 5-hydroxy-2-(4-hydroxyphenyl)benzo[b]furan (**3a**), and diphenylfuran-based ligands of the estrogen receptor.

isomeric 2,4-diphenylfuran derivatives **5** and **6** were synthesized and investigated. The substituents at positions 3 and 5 were selected on the basis of previous investigations which revealed that short alkyl groups are favorable for the binding to ERα, whereas longer aliphatic side chains reduce the binding affinity unless they are furnished with an appropriate functional group that is capable of interacting with Asp351 in ERα. The long aliphatic side chain with both an amino function and a thioether element used in this study has been shown to exert full estrogen antagonism in the 2,5-diphenylfuran series [8]. All derivatives were tested for binding affinity for the bovine ER and for the recombinant human ERα and ERβ isoforms. In addition, all compounds were evaluated for estrogenic and antiestrogenic activity, and for antiproliferative activity in both estrogen-sensitive and hormone-independent breast cancer cells.

2. Materials and methods

2.1. General methods

^{1}H NMR spectra were recorded on a Bruker AC-250 or AVANCE 300 spectrometer with TMS as an internal standard and were in accord with the assigned structures. Elemental analyses of crystalline compounds were performed by the Mikroanalytisches Laboratorium, University of Regensburg, and were within 0.4% of the calculated values. Non-crystalline compounds were analyzed by HRMS (MAT 95, Finnegan) and gave the correct composition. The starting compounds **7** with the complete side chains are commercially available or were synthesized by established methods. 1-(4-Methoxyphenyl)-8-[*N*-methyl-*N*-[3-(pentylsulfanyl)propyl]amino]octan-1-one (**7f**) was prepared by the reaction of 8-bromo-1-(4-methoxyphenyl)octan-1-one with the corresponding amine. The second component (**8**) was obtained by bromination of the respective alkyl-phenylketone. The synthesis of the 2,4-diphenylfurans **5** and **6** followed a common route via the epoxides **9**.

2.2. Preparation of the epoxides *9b–h*

Under nitrogen atmosphere at −78 °C, a solution of alkylarylketone (1.0 equiv.) in dry THF was added dropwise to LDA (2 M in THF, 1.0 equiv.) and the mixture was stirred for 0.5 h. Then, the respective α-bromoketone (1.0 equiv.) in dry THF was added and stirring continued at this temperature for 1.5 h. Subsequently, the reaction mixture was warmed to −10 °C and kept stirring for another 1.5 h. The mixture was hydrolyzed by the addition of water (75 ml) and the aqueous phase extracted with ethyl acetate (3× 75 ml). The combined organic layers were washed with water and brine. After drying over Na_2SO_4 the solvent was evaporated *in vacuo*.