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PAPER

Towards β-selectivity in functional estrogen receptor antagonists†

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Based on the benzo[*b*]naphtho[1,2-*d*]furan and benzo[*b*]naphtho[1,2-*d*]thiophene frameworks, a series of ligands with different basic side chains (BSCs) has been synthesized and pharmacologically evaluated. Also, their binding modes have been modelled using docking techniques. It was found that the introduction of a BSC in these systems brings about a decrease of affinity for both estrogen receptors α and β in an *in vitro* competitive binding assay. However, two full antagonists of the estrogen receptor β (**9c** and **9f**) have been discovered, with potency in the low micromolar concentration in a cell-based luciferase reporter assay, and completely devoid of activity against the α receptor at the same concentration range. Differences in the ERα/ERβ binding modes have also been rationalized with the help of molecular modelling techniques. This interesting functional profile could be used to elucidate the physiological role of each ER subtype.

Introduction

The biological actions of estrogens are manifested through two genetically distinct estrogen receptors (ERα and ERβ) that display nonidentical expression patterns in target tissues. Interesting differences have been found in the physiological role of both receptor subtypes. ERα is predominantly involved in the development and function of the mammary gland and uterus, and in the maintenance of metabolic and skeletal homeostasis. ERβ has more pronounced effects on the central nervous system and on cellular hyperproliferation.[1]

Since the discovery of ERβ in 1996, compounds that are selective in activating or inhibiting both ER subtypes are intensively sought after.[2] The data obtained suggest that the discovery of compounds that selectively bind ERα or ERβ is of great interest for the development of more efficient drugs for the treatment of several disorders, such as cancer, cardiovascular disease, multiple sclerosis and Alzheimer's disease.[3,4] The use of the ERα selective agonist propylpyrazoletriol (PPT) (Fig. 1) has shown that several classical estrogen-induced tissue responses can be effectively evoked *via* ERα alone.[5] On the other hand, the design of highly ERβ selective ligands has proved to be quite challenging, and several groups have reported attempts to design this kind of compound using different scaffolds. The highly ERβ selective agonist ERB-041 (Fig. 1) has been used to demonstrate that this receptor may be a useful target for certain inflammatory diseases. This compound has a dramatic beneficial effect in the HLA-B27 transgenic model of inflammatory bowel disease and the Lewis rat adjuvant-induced arthritis model, while it is inactive in several classic models of estrogen action.[6] Other non-steroidal scaffolds which have been developed as ERβ ligands are diarylpropanenitriles,[7] 2-phenylnaphthalenes,[8,9] and phenyl-2*H*-indazoles.[10]

Interestingly, some substituted tetrahydrochrysene ligands such as *cis*-(*R*,*R*)-diethyl (THC) (Fig. 1) have been described as potent agonists on ERα, but more potent antagonists on ERβ,[11,12] in contrast with tamoxifen and raloxifene which are partial antagonists on both ERα and ERβ (Fig. 1). The structure of these ERβ antagonists is also different from the structure of tamoxifen and raloxifene, where the bulky basic side chain (BSC) is responsible for their antagonist activity through the blockage of the ER helix-12 movement by interaction with Asp351 carboxylate (ERα numbering).[13] Crystallographic structures of the ERα ligand binding domain (LBD) bound to both THC and a fragment of the transcriptional coactivator GRIP1, and ERβ LBD bound to THC show that this compound antagonizes ERβ through a novel mechanism termed "passive

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