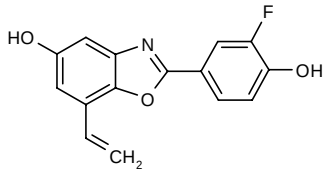
Drugs of the Future 2005, 30(4): 333-336

CCC: 0377-8282/2005
DOI: 10.1358/dof.2005.030.04.893667

ERB-041

Estrogen Receptor (ER) β Agonist
Treatment of Endometriosis
Treatment of Rheumatoid Arthritis

2-(3-Fluoro-4-hydroxyphenyl)-7-vinylbenzoxazol-5-ol



$C_{15}H_{10}FNO_3$
Mol wt: 271.246
CAS: 524684-52-4
EN: 343125

Abstract

ERB-041 is a potent and highly selective estrogen receptor (ER) β agonist that has comparable binding affinity for ERβ as the natural ligand (17β-estradiol), but is > 200-fold selective over ERα. The development of ERB-041 followed a traditional SAR approach, but those efforts were significantly strengthened by structural information in the form of X-ray co-crystals and molecular modeling. ERB-041 has proven invaluable in validating ERβ as a drug target, and in combination with the ERα-selective ligand PPT, has allowed us to dissect the *in vivo* roles of these two ERs. ERB-041 is inactive in models of classic estrogen action but has dramatic beneficial activity in certain *in vivo* models of human disease. These include two models of chronic inflammation (HLA-B27 transgenic rats, Lewis rat model of adjuvant-induced arthritis) and the nude mouse model of endometriosis. ERB-041 is in clinical development for endometriosis and rheumatoid arthritis.

Synthesis

ERB-041 can be prepared according to Scheme 1:

Phenol (I) is first brominated with Br_2/NaOAc in acetic acid to produce bromophenol (II), which is reduced with H_2 over Ra-Ni in EtOAc to afford aniline (III). Coupling of (III) with 3-fluoro-4-methoxybenzoyl chloride (IV) in the presence of pyridine produces the amide ester (V). Conversion of (V) to benzoxazole (VI) is accomplished under acidic conditions (*p*-toluenesulfonic acid) at high temperature (150 °C) in *p*-xylenes. Demethylation of (VI) with boron tribromide affords the diphenolic benzoxazole (VII). Palladium-catalyzed cross-coupling reaction (7) of benzoxazole (VII) with tributyl(vinyl)tin in the presence of dichlorobis(tri-*o*-tolylphosphine)palladium(II) 1,2-diethoxyethane gives ERB-041.

Introduction

Although long known to play a critical role in sexual development and reproductive function, estrogens are now appreciated to affect essentially every organ system in the body. Estrogens exert their effects by interacting with receptors, the first of which was cloned in 1986 (1). For 10 years, many physiologists believed that what we now call ERα was the sole estrogen receptor (ER). However in 1996, a second form (ERβ) was unexpectedly discovered during a search for novel androgen receptors in the rat prostate (2). Because the mRNA expression of ERβ was distinct from ERα (although many tissues express both ERs) (3), its discovery led to a re-examination of estrogen actions throughout the body. The expectation was that ERβ-selective ligands would exhibit a different pharmacological profile than compounds such as 17β-estradiol and 17α-ethinyl-17β-estradiol because these ER ligands bind equally well to both subtypes. Moreover, the fact that ERβ is not the dominant ER in the uterus or breast makes it a very attractive drug target.

In the 9 years since its discovery, a large body of information has been published on ERβ. However, dissecting the biological functions of ERα and ERβ became possible only recently with the development of highly selective ligands for both ER subtypes. Preclinical studies in rodents have clearly shown that ERα mediates the "classic"

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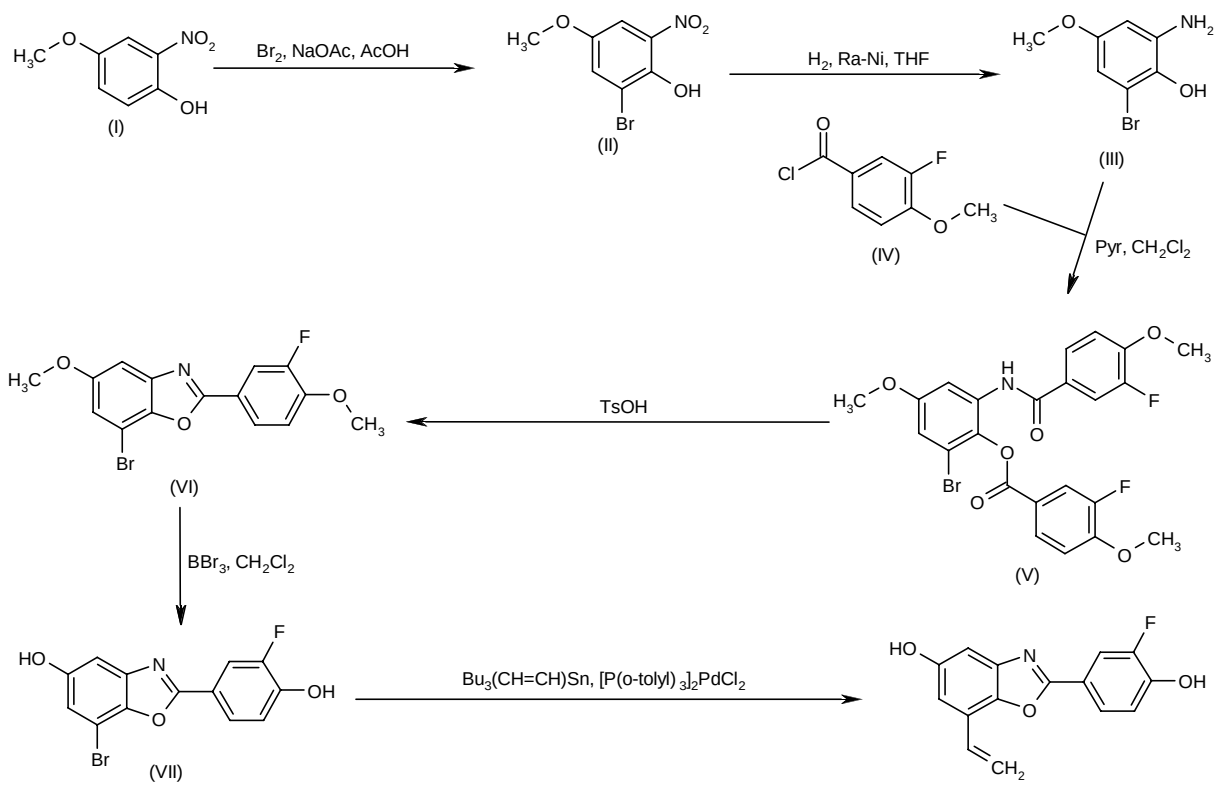
Scheme 1: Synthesis of ERB-041



effects of estrogens, such as stimulation of the uterus, prevention of bone loss following ovariectomy and prevention of vasomotor instability (4). Conversely, ERβ-selective compounds are inactive in these models, and in addition, do not stimulate mammary gland development or inhibit ovulation (5).

This review concerns a potent and selective ERβ agonist, ERB-041, that was developed by Wyeth scientists (6). The development of this clinical candidate followed a traditional structure-activity relationship (SAR) approach, but those efforts were significantly strengthened by structural information in the form of X-ray co-crystals and molecular modeling. The combination of these approaches helped expedite the discovery of highly potent and selective ligands. We primarily concentrated our synthetic efforts on the α-face of the ERβ binding pocket, utilizing only 1 of the amino acid differences (ERα Met421 → ERβ Ile373) between this ER subtype and ERα. This compound is in clinical development for endometriosis and rheumatoid arthritis.

Pharmacological Actions

A competitive radioligand binding assay (8) was used to determine the relative binding affinity (IC_{50}) of ERB-041 for the ligand binding domain (LBD) of human, rat and mouse ERα and ERβ. ERB-041 binds to ERβ with an IC_{50} of 3-5 nM (similar to 17β-estradiol) and is > 200-fold selective relative to ERα (5). Binding to full-length ERβ and ERα was also evaluated, and the compound proved to be similarly potent and selective for ERβ (6). ERB-041 was tested for its ability to bind to a diverse set of 65 other receptors (*i.e.*, Novascreen analysis), and no high-affinity interactions were found (unpublished observations).

A cell-based transcriptional assay was used to determine whether ERB-041 was an ERβ agonist or antagonist. The assay used human osteosarcoma cells (SAOS-2) engineered to express ERβ. Estradiol, acting via ERβ, upregulates insulin-like growth factor binding protein-4 (IGFBP-4) mRNA (4). ERB-041 was equieffective to 17β-estradiol, indicating that it is a full agonist (6). This conclusion is consistent with the 3-dimensional structure of the ERβ LBD when bound to ERB-041. In this structure, helix 12 is folded over the binding pocket in an agonist-like conformation, allowing for the binding of a coactivator fragment that contains an LXXLL motif known to be important for transactivation (9). Other cell-based transcriptional assays show that ERB-041 does not act as an agonist or antagonist of glucocorticoid, androgen or progesterone receptors (6).

As mentioned above, ERB-041 is inactive in models of classic estrogen action. However, this compound has dramatic beneficial activity in certain *in vivo* models of

human disease. These include two models of chronic inflammation and a model of endometriosis, as summarized below.

HLA-B27 transgenic rats express two human proteins (HLA-B27 and β_2-microglobulin) and develop several phenotypes as they age (10, 11). They first develop chronic diarrhea with accompanying intestinal lesions, and are used as a model of inflammatory bowel disease. ERB-041 (1-20 mg/kg p.o.) rapidly normalizes stool character in rats with advanced disease, and effects are seen as early as several hours after the first oral dose (5). This improvement in clinical signs is accompanied by clear histological improvement in the colonic tissue. The number of inflammatory cells, lesion size and degree of fibrosis are reduced. Additionally, there is restoration of the goblet cells and mucus secretion into the intestinal lumen. The pan-ER antagonist ICI-182780 blocks the beneficial effects of ERB-041 in this model.

Subsequent to the intestinal disorders, these rats develop arthropathy as they age. ERB-041 (10 mg/kg p.o.) is able to rapidly and significantly reduce the clinical signs of joint redness and swelling over the course of 10 days. As would be expected, histological features of disease (*e.g.*, synovitis and Mankin scores) are also significantly reduced by ERB-041 treatment (unpublished observations). These observations led to its evaluation in a second arthritis model, discussed below.

Lewis rats have a defect in the hypothalamic-pituitary-adrenal axis that makes them hypersensitive to inflammatory stimuli (12). When complete Freund's adjuvant is injected intradermally at the base of the tail, the tarsal joints swell dramatically within 8 days. This model shares features with human rheumatoid arthritis and is used as one of the standard preclinical models to develop therapeutics. When ERB-041 (1-20 mg/kg p.o.) is given after full inflammation develops (*e.g.*, day 9 after adjuvant injection), joint scores of redness and swelling are reduced to near normal over the course of about 10 days (5). Typically, improvement is first noticeable within 2-3 days of the first dose. As seen in the HLA-B27 transgenic rat, synovitis and Mankin score are also significantly improved. We are currently conducting a microarray analysis of liver, lymph node and spleen mRNA, as well as a plasma proteomics analysis, to better characterize the compound's effect. Preliminary data suggest that adjuvant treatment induced profound changes and that the majority of these changes are significantly reversed by ERB-041.

Endometriosis is a serious disease affecting reproductive-age women. The etiology is not fully understood, but the disease is thought to occur when menstrual effluent enters the peritoneal cavity and is not appropriately cleared by immune cells. As the disease occurs only in humans and nonhuman primates, it is difficult to study in the laboratory setting. However, several rodent models have been developed, including one that involves injecting human endometrium into nude mice (13, 14). These tissue explants establish lesions similar to the human disease. We tested the effects of ERB-041 in this model using mice with visibly established lesions. When administered for approximately 2 weeks, ERB-041 (10 mg/kg p.o.) caused complete lesion regression in 40-75% of the mice (15). The compound was equally effective in ovariectomized and gonad-intact mice, suggesting that endogenous estrogens do not interfere with its activity.

Summary and Future Directions

We sought to develop ERβ-selective agonists based on the hypothesis that their pharmacological profile would be more attractive than current estrogens and selective estrogen receptor modulators (SERMs). Despite the tremendous similarity in the ligand binding pocket, we were able to capitalize on one subtle difference and synthesize a potent and highly selective compound. This compound, ERB-041, has proved invaluable in validating ERβ as a drug target and, in combination with an ERα-selective ligand (*i.e.*, PPT) (4), has allowed us to dissect the *in vivo* roles of these two ERs. The next two challenges are to elucidate the mechanism of action of ERB-041 (beyond ERβ) and to determine whether its impressive preclinical activity will translate into clinical efficacy. Studies to address both questions are in progress.

Source

Wyeth Research (USA).

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