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Identification of an agonist ligand for estrogen-related receptors ERRβ/γ

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Abstract—In order to develop agonist ligands that are specific for the estrogen-related receptors ERRβ/γ, a hydrazone with a 4-hydroxy group at one phenyl ring and a 4-diethylamino moiety at the other phenyl ring was synthesized. We demonstrate that compound **3** (DY131; N′-{(1*E*)-[4-(diethylamino)phenyl]methylene}-4-hydroxybenzohydrazide) effectively and selectively activates ERRβ/γ. DY131 had no effect on the structurally related receptors ERRα or the estrogen receptors α and β (ERα/β). This work defines a convenient synthesis for a novel and selective pharmacologic tool that can be used to elucidate the biological activities of ERRβ/γ.

Metabolic disease, including obesity, diabetes and atherosclerosis is the leading cause of mortality in industrialized nations. It is estimated that over one-third of the United States population is obese and these individuals are at risk for developing diabetes and atherosclerosis. These disorders are responsible for over 500,000 deaths in the United States each year. The growing incidence of metabolic disease has led to an intense interest in identifying new molecular targets and new pharmacologic agents to treat and/or prevent these disorders.

Orphan nuclear receptors provide an example of an important class of molecular targets for the treatment of metabolic disease. Nuclear receptors are ligand-dependent transcription factors that regulate gene expression in response to small-molecule ligands. The orphan receptor ERRα (estrogen-related receptor α) is known to be a key regulator of lipid homeostasis. For example, mice lacking ERRα are lean and are resistant to developing obesity when challenged with a high-fat diet.[1] ERRα is expressed in tissues with a high capacity for β-oxidation of fatty acids including the heart, kidneys, brown adipose tissue and skeletal muscle.[2] ERRα expression is stimulated by the coactivator PGC-1α[3] and both proteins form a complex that stimulates the expression of genes involved in mitochondrial fatty acid oxidation (medium-chain acyl coenzyme A dehydrogenase,[4] peroxisome proliferator activated receptor α[5]) and oxidative phosphorylation.[6,7] ERRα has also been shown to regulate genes involved in intestinal lipid absorption, satiety signals (apolipoprotein A-IV[8]) and vascular relaxation (endothelial nitric oxide synthase[9]).

An important advance in the elucidation of orphan receptor biology is the identification of synthetic ligands that can be used to modulate specific receptors in cells and in vivo. For example, a synthetic ERRα antagonist known as XCT790 (Fig. 1) has recently been described.[10,11] This chemical reagent facilitated the discovery of ERRα as a regulator of oxidative phosphorylation[6] and of monoamine oxidase B expression.[10] XCT790 has no activity on ERRγ; its activity on the related receptors ERRβ, ERα and ERβ has not been reported.

Although there has been significant progress in understanding the physiologic activity of ERRα, little is known about the biological activity of ERRβ and ERRγ. A major factor that is limiting progress in this field is the lack of selective ERRβ/γ ligands that can be utilized in cell culture and in vivo studies. For example, while (*Z*)-4-hydroxytamoxifen has been reported to be an antagonist of both ERRβ and ERRγ,[12,13] this compound is not useful experimentally because it is several orders of magnitude more potent as an ERα

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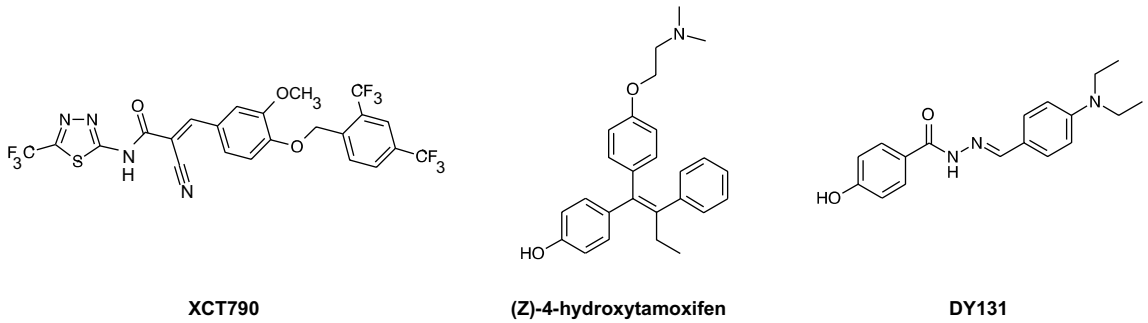


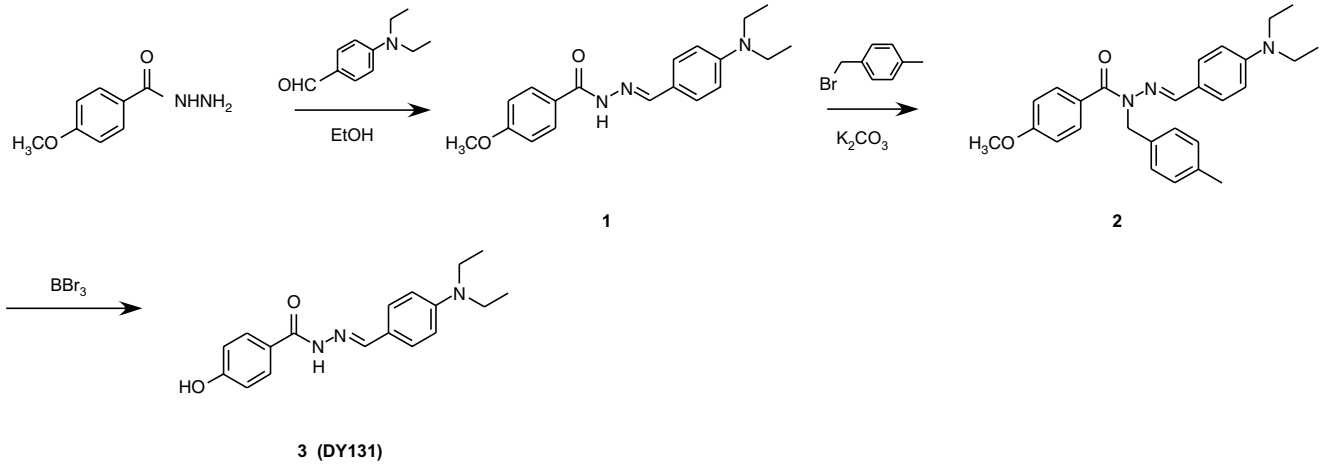
Figure 1. Chemical structures of ERR ligands. XCT790 is an ERRα antagonist. (*Z*)-4-Hydroxytamoxifen is a high affinity antagonist for ERα/β and a low affinity antagonist for ERRβ/γ. DY131 is an ERRβ/γ agonist.

and ERβ antagonist. Moreover, no agonist or activating ligands have been reported for any of the ERRs; only antagonists have been identified. We therefore sought to develop a simple and efficient synthesis for compounds that could selectively activate ERRβ/γ with little activity on ERRα and ERα/β. In a recent symposia, GlaxoSmithKline suggested acyl hydrazone derivatives as potential ligands for ERRγ.[14] As part of our ongoing programme, we developed two alternative routes for the synthesis of the acyl phenylhydrazone derivative DY131 (Fig. 1) and evaluated its potential to act as a selective ERRβ/γ agonist.

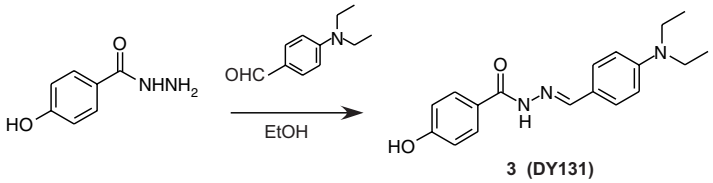
The simplest synthesis of DY131 would be a direct condensation of 4-hydroxybenzoic acid hydrazide with 4-diethylaminobenzaldehyde. However, because of the uncertainty in using an unprotected hydroxyl, we initially used 4-methoxybenzoic acid hydrazide. 4-Diethylaminobenzaldehyde was condensed with 4-methoxybenzoic acid hydrazide by refluxing in absolute ethanol with a catalytic amount of acetic acid according to the method of Bahadur et al.[15] This resulted in a 76% yield of compound **1**. We found that direct conversion of **1** to **3** with boron tribromide gave low yields. Thus, a two-step procedure was explored whereby the amine of **1** was protected prior to conversion to **3**. Specifically, the amino group of **1** was protected by reacting with α-bromo-*p*-xylene to give compound **2**. This was followed by the simultaneous demethylation and deprotection of the *N*-benzyl group of **2** by ether cleavage with boron tribromide. This produced compound **3** (DY131, 4-hydroxy-benzoic acid (4-diethylamino-benzylidene)-hydrazide), with an overall yield of 27%.

We next sought a more efficient procedure for the synthesis of DY131 (**3**), for example, one that replaced the three-step procedure of Scheme 1 with a single step synthesis. To do so, we synthesized DY131 by direct condensation of 4-hydroxybenzoic acid hydrazide with 4-diethylaminobenzaldehyde (Scheme 2). The phenolic hydroxyl and the hydrogen of the secondary amine remained unchanged during the reaction and DY131 (**3**) was successfully synthesized in this one-step reaction with a yield of 65%.

DY131 (**3**) was evaluated for selectivity and efficacy in modulating the transcriptional activity of ERRα/β/γ and ERα/β. CV-1 cells were transfected with appropriate reporter constructs and expression vectors and the fold activation of the reporter construct was determined at several concentrations of DY131 (Table 1). DY131 failed to activate the reporter construct or ERRα at any of the concentrations tested. In contrast, DY131 resulted in three- to fourfold activation of ERRβ at concentrations of 10–30 μM. Activity on ERRγ was even more pronounced with fivefold activation at 3 μM and



Scheme 1. Three-step synthesis of **3** (DY131) with an overall yield of 27%.



Scheme 2. One-step synthesis of **3** (DY131) with an overall yield of 65%.

Table 1. Cell-based reporter gene assays examining the fold activation of ERR and ER in response to DY131 and β-estradiol

Receptor[a]	DY131 (3)			β-Estradiol
	3 μM	10 μM	30 μM	100 nM
—	0.8	0.7	0.9	1.1
ERRα	1.5	1.7	1.6	1.1
ERRβ	1.9	3.1	3.8	1.0
ERRγ	5.0	5.9	6.6	0.8
—	1.0	1.1	1.0	1.0
ERα	1.1	1.0	0.9	5.2
ERβ	1.1	1.0	1.2	6.2

[a] Cell based transactivation assays were performed in CV-1 cells as described.[16] ERR activity was assayed with a GAL4 reporter construct and fusion proteins containing the ligand binding domains of human ERRα, human ERRβ and mouse ERRγ linked to the DNA binding domain of yeast GAL4. Human ERα and ERβ were examined as full-length proteins using an estrogen receptor responsive reporter construct. Fold activation is reported.

maximal 6.6-fold activity observed at 30 μM. Thus, DY131 is an ERRβ/γ-specific ligand that displays preferential selectivity for ERRγ at lower concentrations.

Due to the overlap in recognition of (*Z*)-4-hydroxytamoxifen by both ERR and ER, we also examined the activity of DY131 on ERα and ERβ. DY131 failed to activate or inhibit either of these receptors whereas the control ligand, β-estradiol, resulted in the expected five- to sixfold activation. These data demonstrate that DY131 is a selective agonist of ERRβ/γ with no activity on the related receptors ERRα, ERα and ERβ. This selective activity establishes DY131 as a novel pharmacologic tool to study the biological activities of ERRβ/γ.

In summary, we designed a simple, one-step procedure for the synthesis of DY131, a novel ERRβ/γ selective agonist. This compound provides a unique chemical tool for studying ERRβ/γ action in human cells to elucidate the physiological activities of ERRβ/γ.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.01.025. The supplementary data provide an experimental description of the preparation of the compounds as well spectral, physical and elemental analyses.

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