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Letters

Identification of a Selective Inverse Agonist for the Orphan Nuclear Receptor Estrogen-Related Receptor α

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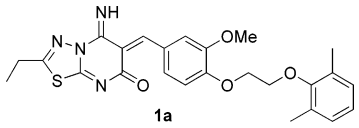
Abstract: The estrogen-related receptor α (ERRα) is an orphan receptor belonging to the nuclear receptor superfamily. The physiological role of ERRα has yet to be established primarily because of lack of a natural ligand. Herein, we describe the discovery of the first potent and selective inverse agonist of ERRα. Through in vitro and in vivo studies, these ligands will elucidate the endocrine signaling pathways mediated by ERRα including association with human disease states.

The estrogen-related receptors (ERRs α,β,γ) structurally and functionally belong to the subfamily of estrogen receptors (ERs), and recent discoveries suggest that the ERR and ER families may be more closely related than previously thought. Such evidence includes findings that ERRs have a role in controlling proliferation and differentiation of cells including osteoclasts/osteoblasts and cells implicated in mammary carcinoma.[1] ERRα is principally expressed in tissues involved in fatty acid metabolism, and ERR target genes include genes involved in energy metabolism.[2,3] Recent experiments have also demonstrated that deletion of ERRα results in mice that are lean.[4]

Symptoms of type II diabetes (non-insulin-dependent diabetes mellitus (NIDDM)) include a reduction in energy metabolism that is in part due to a decrease in muscle mitochondria and whole-body oxygen consumption. In contrast, exercise improves mitochondrial proliferation. In recent publications, changes in the expression of genes involved in oxidative phosphorylation have been found in individuals with NIDDM and it has been suggested that a nuclear receptor coactivator, peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1), is responsible for orchestrating these genetic modifications.[5] This implicates PGC-1 as an important regulator of energy metabolism through metabolic regulation in skeletal muscle and the liver. PGC-1 is a potent regulator of ERRα,[6−8] and it is postulated that ligands modulating ERRα activity should regulate oxidative phosphorylation and increase mitochondrial respiration, which is severely compromised in type II diabetes.[9]



1a

Figure 1. Reported structure of the HTS lead compound.

ERRα is a constitutively active receptor because it can function as a transcriptional activator in the absence of any added ligand.[10] Despite its high homology with ERs, ERRα does not respond to estradiol and no endogenous ligands have been reported to date. While ERRα was the first orphan nuclear receptor identified over a decade ago,[11] the lack of a chemical tool has severely hampered the ability to associate its function with endocrine physiology, especially in the regulation of energy metabolism and type II diabetes. Our goal was to identify a potent and selective ligand for ERRα and establish the biology and physiological relevance of this receptor via reverse endocrinology.[12]

Our work was initiated by the high-throughput screening (HTS) of our compound library and the identification of thiadiazolopyrimidinone **1a** as an ERRα inverse agonist.[13] Compound **1a** was found using a fluorescence polarization (FP) assay, which measures the ligand-dependent displacement of a labeled steroid receptor coactivator-1 (SRC-1) fragment peptide from ERRα. The selection of the SRC-1 peptide followed the

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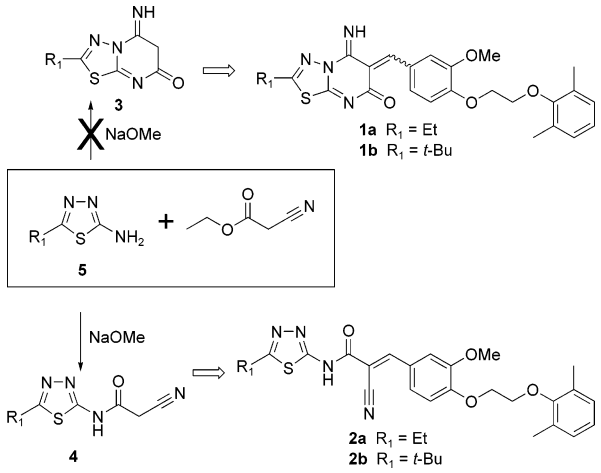
Scheme 1



FP assay of 15 peptides comprising individual LxxLL motifs from nuclear receptor coactivators including SRC-1, SRC-2, SRC-3, TIF1, and CBP. The greatest signal-to-noise was achieved with the ILRKLLQE peptide motif from SRC-1. By use of the FP assay, **1a** was found to exhibit an IC_{50} of 1.4 μM. The activity of the compound was confirmed in a cell-based cotransfection assay using the Gal4-ERR format and displayed an IC_{50} of 2.3 μM.

We synthesized several batches of pyrimidinone **1a** using the reported methodology shown in Scheme 1[14] and several closely related analogues where the R^1 group of the thiadiazole ring was varied. We were able to obtain crystals of the *tert*-butyl analogue **1b** for X-ray diffraction. To our surprise, the structure indicated that the product obtained from our synthetic sequence was the monocyclic thiadiazoloacrylamide **2b** and not the thiadiazolopyrimidinone **1b** (Scheme 1). Subsequent IR analyses of other thiadiazoleacrylamides showed the characteristic nitrile stretch in the region 2260−2220 cm^{-1}.

We believe that the reported methodology[15] to prepare the thiadiazolopyrimidinone precursor **3** from the 2-alkyl-5-amino[1,3,4]thiadiazole derivatives **5** provides only the transamidation product **4**. The spectral data for the resynthesized HTS hit **1a** were identical to the commercially obtained compound, suggesting that the structure of the commercial compound was also assigned incorrectly. Indeed, our thorough perusal of literature data on the synthesis and characterization of these compounds led us to the conclusion that our assignments are consistent with the open acrylamide structures **2a**,**b**. With the correct lead structure elucidated, we initiated lead optimization to develop potent, selective, and orally bioavailable ERRα inverse agonists.

Thiadiazoloacrylamide analogues reported in this work were prepared by the synthetic route shown in Scheme 2. The 2-alkyl-5-amino[1,3,4]thiadiazole derivatives **5** were commercially available or readily prepared from acid chlorides and thiosemicarbazide, via cyclodehydration of the acylthiosemicarbazide intermediate under acidic conditions.[16] The thiadiazoloacetamides **4** were prepared from the amino thiadiazoles **5** and ethyl cyanoacetate in a refluxing MeONa/MeOH mixture. Standard alkylation conditions were used to prepare the chloroethyl ether derivative **6**, which was reacted with

Scheme 2

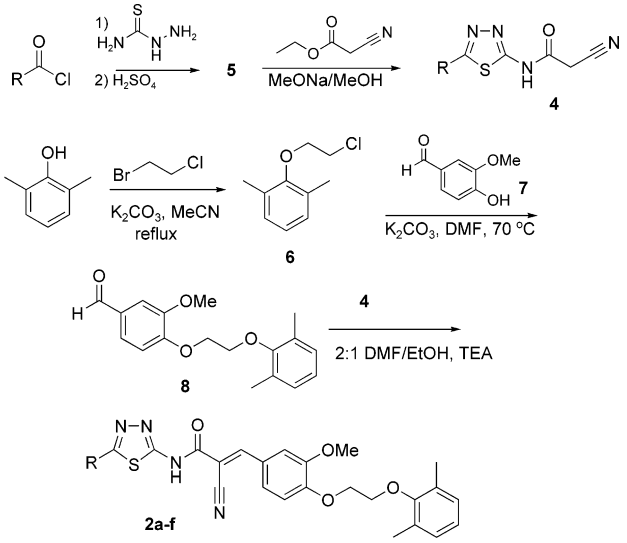
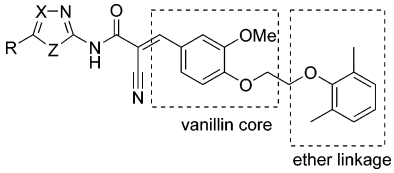


Table 1. Analogues of the ERRα Inverse Agonist Lead



compd	R	X	Z	ERRα Gal4 IC_{50} (μM)[a]	% inhibition (10 μM)
2a	Et	N	S	2.0	96
2b	^{t}Bu	N	S	1.7	104
2c	MeS	N	S	2.6	92
2d	CF_3	N	S	1.5	112
2e	*p*-Me_2NPh	N	S	2.0	74
2f	H	N	S	1.4	103
2g	H	CH	S	NA[b]	
2h	Et	N	O	3.3	88

[a] Unless otherwise specified, IC_{50} values were generated by nonlinear regression from titration curves of compounds from 10 doses and reported as an average of at least two experiments. Standard error of the mean was typically less than 30% for each experiment. [b] NA: not active.

vanillin **7** to provide the benzaldehyde **8**. Thiadiazoloacrylamides **2a**−**f** were synthesized by Knoevenagel condensation of the benzaldehyde analogue **8** with the cyanoacetamide **4** under basic or acidic conditions using triethylamine or acetic acid, respectively. The thiazole analogue **2g** and the oxadiazole analogue **2h** were prepared from a similar sequence using commercially available 2-ethyl-5-aminothiazole and 2-ethyl-5-aminooxadiazole, respectively. On the basis of the X-ray structure of **2b**, only the *E* regioisomer is observed under our condensation and purification conditions. The screening results for synthetic compounds are shown in Table 1.

Variation of the R group had little effect on ERRα potency. For example, **2b**, R = ^{t}Bu (IC_{50} = 1.7 μM), exhibited potency similar to that of **2f**, R = H (IC_{50} = 1.4 μM). Compound **2g** was found to be inactive, potentially showing the importance of the endocyclic 3-postion nitrogen to ERRα activity. The oxadiazole **2h** was comparable in activity to the corresponding thiadiazole **2a**.

In earlier efforts toward lead validation, we had established that the vanillin core is beneficial for ERRα

Scheme 3

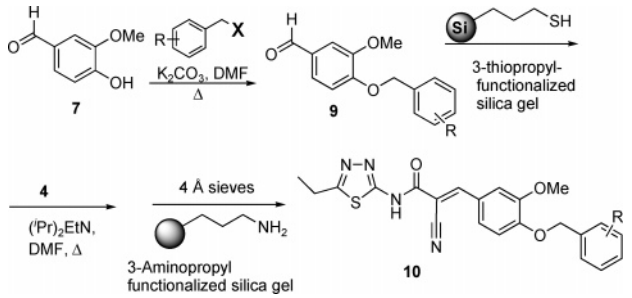


Table 2. Data for Selected Library Analogues

compd	R	ERRα Gal4 IC_{50} (μM)[a]	% inhibition (10 μM)
10a	2,4-CF_3	0.4	106
10b	5-F, 2-CF_3	0.80	112
10c	4-F, 2-CF_3	0.92	109
10d	2-OCF_3	1.8	101
10e	1-naphthyl	1.8	90
10f	2-Br	2.2	57

[a] Unless otherwise specified, IC_{50} values were generated by nonlinear regression from titration curves of compounds from 10 doses and reported as an average of at least two experiments. Standard error of the mean was typically less than 30% for each experiment.

activity. We therefore decided to explore the SAR around the ether linkage via a focused library derived from an alkylation−condensation sequence as shown in Scheme 3.

The target compounds **10** were synthesized via a two-step procedure starting from vanillin **7**. Vanillin was alkylated with commercially available alkyl and benzyl halides (185 reagents including α-halo ketones, esters, and acetamides) to provide aldehydes **9**, which were then condensed with the thiadiazoloacetamide **4** to provide the target compounds **10**. Excess alkylating agents and excess aldehydes **9** were scavenged using silica gel based thiol and amino resins, respectively.

The samples were purified using reverse-phase HPLC and were quantified using evaporative light scattering detection to afford 2−5 mg of 100 discrete vanillin ethers with a purity criteria of >85% pure by HPLC−MS. The samples were formatted to a 10 μM concentration in DMSO and assayed at a single 3 μM concentration in the Gal4-ERRα assay. Dose response was carried out on any compound exhibiting >50% inhibition at 3 μM. Table 2 shows data for selected library compounds.

The three most potent compounds identified from the library were the 2-(trifluoromethy)benzyl derivatives **10a**−**c**, and these were resynthesized on a 10 mg scale for full analytical and biological characterization. In addition, two related trifluoromethyl analogues **10g** and **10h** (not originally synthesized in the library) were also prepared. ERRα inverse agonist activity was measured in the Gal4-ERRα assay, and the data are shown in Table 3.

The bis-trifluoromethyl analogue **10a** was reconfirmed as the most potent analogue from the ether library with a 4-fold improvement in potency over the initial lead **2a**. Analogues **10b** and **10c** were weaker as resynthesized compounds, while **10g** and **10h** were inactive.

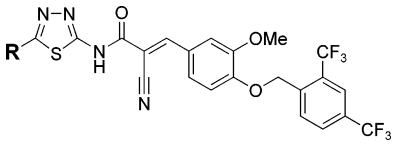
Having optimized the ether linkage on the lead compound with the bis-trifluoromethyl analogue **10a**,

Table 3. Data for Library Resynthesis Compounds

compd	R	ERRα Gal4 IC_{50} (μM)[a]	% inhibition (10 μM)
10a	2,4-CF_3	0.61	104
10b	5-F, 2-CF_3	1.7	86
10c	4-F, 2-CF_3	1.5	90
10g	2,5-CF_3	NA[b]	NA[b]
10h	2-F, 6-CF_3	NA[b]	NA[b]

[a] Unless otherwise specified, IC_{50} values were generated by nonlinear regression from titration curves of compounds from 10 doses and reported as an average of at least two experiments. Standard error of the mean was typically less than 30% for each experiment. [b] NA: not active under assay conditions.

Table 4. Functionalization of the Thiadiazole Core



compd	R	ERRα Gal4 IC_{50} (μM)[a]	% inhibition (10 μM)
10a	Et	0.54	99
11	Ph	0.50	105
12	CF_3	0.37	109
13	H	0.75	99
14	MeS	0.44	105
15	^{t}Bu	>10	43

[a] Unless otherwise specified, IC_{50} values were generated by nonlinear regression from titration curves of compounds from 10 doses and reported as an average of at least two experiments. Standard error of the mean was typically less than 30% for each experiment.

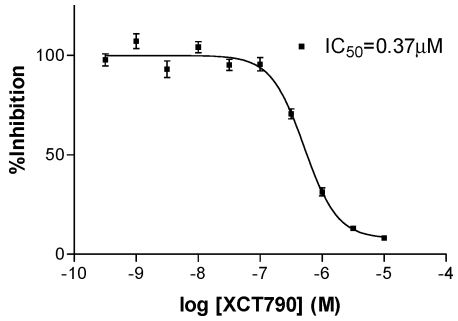


Figure 2. Inverse agonist activity of **12** in GAL4-ERRα cell-based transfection assay.

we then decided to optimize the R groups on the thiadiazole ring. To this end we selected functional groups identified in the initial set of compounds **2a**−**h** as shown in Table 1 and incorporated them into template **10** using procedures developed for the library synthesis.

As shown in Table 4, a wide range of functionality is tolerated on the thiadiazole ring. The trifluoromethyl analogue **12** displays the highest potency and inverse agonist activity in the cell-based GAL4-ERRa transfection assay (Figure 2). Additionally, **12** was inactive against ERRγ and the estrogen receptors ERα and ERβ. When profiled for cross-reactivity against a broader panel of nuclear receptors, **12** was also highly selective except for weak agonist activity against PPARγ (EC_{50} = 1.3 μM; 15% efficacy vs agonist control).

In summary, thiadiazoleacrylamide **12** (XCT790) represents the first potent inverse agonist for ERRα. Identification of a ligand for an orphan nuclear receptor

is a significant milestone that should allow for reverse endocrinology studies directed toward understanding the biology of ERRα. Using XCT790, we have demonstrated that ERRα inverse agonists alter the ERRα/PGC-1 signaling pathways and are currently validating ERRα as a target for the regulation of energy metabolism and type II diabetes (results published elsewhere).[6,17] Discovery of ligands specific to ERRα will help to elucidate the role of ERRα in the endocrine and metabolic signaling pathways. Through the identification of novel ligands such as **12** (XCT790), it is likely that new classes of selective ERRα modulators will be identified. As in the case of other orphan nuclear receptors, these ligands may eventually lead to the discovery of new drugs targeting ERRα and as potential new therapeutics for diabetes and cancer.

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Supporting Information Available: Experimental procedures and characterization data (^{1}H NMR, LC/MS, and HRMS) for cyanoacetamide **4a**, benzaldehydes **8** and **9a**, and thiadiazoloacrylamides **2a**−**h** and **11**−**16**, X-ray structure data for **2b**, general procedures for the library synthesis and resynthesis of identified active compounds from the thiadiazole library **10a**−**f**, and protocols for the ERRα FP and cotransfection assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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