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Small molecule tertiary amines as agonists of the nuclear hormone receptor Rev-erbα

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ABSTRACT

The structure–activity relationship study of a small molecule Rev-erbα agonist is reported. The potency and efficacy of the agonists in a cell-based assay were optimized as compared to the initial lead. Modest mouse pharmacokinetics coupled with an improved in vitro profile make **12e** a suitable in vivo probe to interrogate the functions of Rev-erbα in animal models of disease

Rev-erbα was originally identified as an orphan nuclear hormone receptor based on its canonical domain structure.[1] Rev-erbβ was identified based on its homology to other nuclear receptors (NR) and has an overlapping pattern of expression with Rev-erbα. Rev-erbs have particularly high expression in the liver, adipose tissue, skeletal muscle and brain[2–4] and are expressed in a circadian manner in these tissues.[5–8] The Rev-erbs are unique within the NR superfamily in that they lack the typical C-terminal AF2 domain (helix 12), which is required for coactivator protein binding. Although these receptors lack the ability to activate transcription of target genes due to their inability to recruit transcriptional coactivator proteins, both have been shown to be effective repressors of transcription due to their ability to recruit transcriptional corepressor proteins such as NCoR and HDAC3.[9,10] It has been recently demonstrated that the porphyrin heme functions as a ligand for Rev-erbα and Rev-erbβ.[9–12] Heme binds reversibly and specifically to the ligand binding domain (LBD) of Rev-erb. Binding induces a conformational change in the LBD that results in the ability of the receptor to recruit NCoR and thus repress target gene transcription. The nuclear hormone receptors, Rev-erbα and Rev-erbβ, regulate a number of physiological functions including the circadian rhythm, glucose and lipid metabolism, adipogenesis, and cellular differentiation.[13,14] The observation that these NRs are ligand regulated suggests that development of synthetic ligands may be possible.

Recently, the first nonporphyrin synthetic ligand for Rev-erbα, GSK4112/SR6452/**1** (Fig. 1) was identified.[15,16] This ligand acts as an agonist, mimicking the action of heme and resets the circadian rhythm in a phasic manner. It also represses expression of gluconeogenic genes in liver cells and reduces glucose output in primary hepatocytes. **1** was identified in a fluorescence resonance energy transfer (FRET) assay that significantly and specifically enhances the Rev-erbα-NCoR interaction with an EC_{50} value of 0.40 μM. We recapitulated this data showing that SR6452 was able to modulate the interaction of either Rev-erbα or Rev-erbβ with an NCoR CoRNR box peptide using Luminex technology.[17,18] SR6452 dose-dependently increased the interaction of both Rev-erbα and Rev-erbβ with the NCoR peptide, indicating that the ligand modulates the activity of both Rer-erb subtypes. Direct binding of an analog (**12e**) to Rev-erbα was also confirmed by circular dichroism analysis.[18]

The compound was reported to show no activity on related nuclear hormone receptors (LRH1, SF1, FXR, or RORα) using the same FRET assay and no activity on LXRα or LXRβ in reporter-gene assays. Unfortunately, the pharmacokinetic profile of **1** in rodents was poor hampering its use as an in vivo tool. Additionally, **1** had modest potency and limited efficacy in a cellular assay (in-house data). With the goal of interrogating the function of Rev-erbα in animal models of disease, we needed a more potent compound with improved potency and efficacy and an adequate in vivo profile. Based on trisubstituted amine **2**, we initiated the structure–activity relationships (SAR) study described herein.

Based on the lead structure **1**, the three portions of the molecule were individually modified in a step-wise fashion investigating R, R^1,

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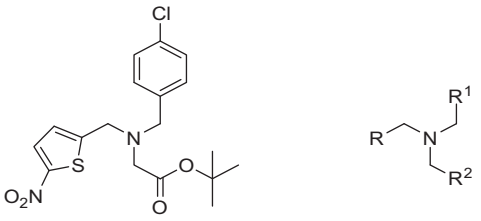


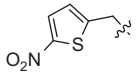
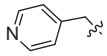
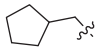
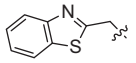
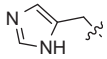
Figure 1. GSK4112/SR6452 lead.

and R^2 as in **2**. The analogs **4**–**11** were synthesized in straightforward fashion starting from commercially available starting materials (Scheme 1). In one instance, reductive amination of *t*-butyl glycine (**3**) with *p*-chlorobenzaldehyde afforded secondary amine **4**. Functionalization of the third amine substituent was carried out by a second reductive amination or sulfonylation or acylation as described to give products **5a**–**e**. Alternatively, reductive amination of *t*-butyl glycine and 5-nitrothiophenecarboxaldehyde (**6**) uneventfully afforded secondary amine **7**. This could then be converted to final products **8** in a similar fashion. Lastly, the 5-nitrothiophenecarboxaldehyde and *p*-chlorobenzylamine (**9**) could be condensed to yield amine **10**, which was converted to final products **11**.

Compounds were screened in a cell-based luciferase assay in a two-step format.[18–20] Cells were co-transfected with an expression plasmid harboring full-length Rev-erbα and a luciferase reporter driven by the *Bmal1* promoter. Compounds were first screened at two concentrations (1 μM and 10 μM) to determine the effect on repression of *Bmal1* transcription. Rev-erb is a transcriptional repressor. Rev-erb agonists lead to recruitment of co-repressors, which leads to repression of transcription. Maximum inhibition at 10 μM is reported.[21] The lower the value, the more efficacious the agonist is at repressing transcription. A value of 1.0 effectively means no repression. Compounds that appeared efficacious at 10 μM were then fully titrated in an eleven-point dose–response format to generate EC_{50} values. In our in-house cell-based assay, GSK4112/SR6452/**1** showed only modest potency and minimal efficacy (Table 1).

Given the potential for issues with the nitrothiophene residue in vivo, we assessed replacement of this group first. Several small heterocycles and carbocycles were tried as nitrothiophene isosteres, however none of them showed any improvement with regards to efficacy (Table 1). One might argue that the 4-pyridyl analog (**5a**) and the benzothiazole analog (**5c**) were equally efficacious as **1**, however these early compounds were not fully titrated. Compounds **4**, **5b** and **5d** showed no repression. Temporarily unsuccessful in replacing the nitrothiophene ring, we moved on to investigate the other two portions of the molecule.

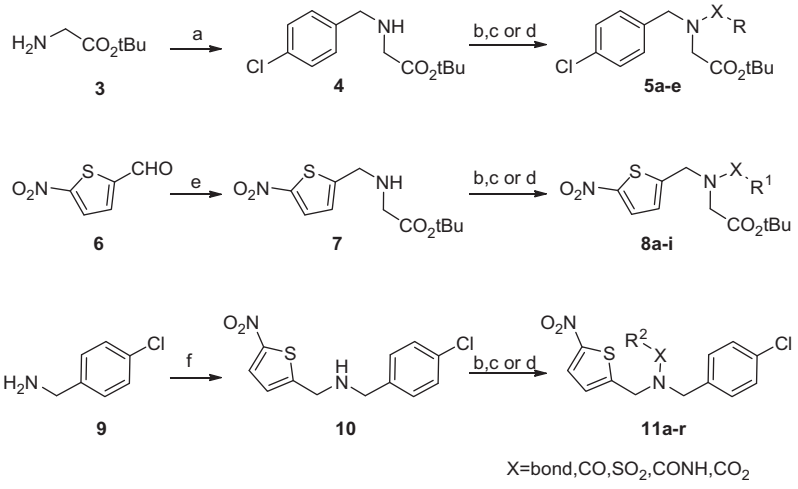
Table 1
Nitrothiophene analogs

Compound	R	[a]Max Inh	EC_{50} (μM)
1		0.82	2.3
4	H	1.2	[b]NT
5a		0.81	NT
5b		1.1	NT
5c		0.82	NT
5d		0.93	NT
5e		0.88	NT

[a] Results are average of two or more experiments. Value = fold change relative to DMSO control at 10 μM compound.
[b] NT = not tested. All standard deviations ⩽25%.

Efforts were then focused on replacing the *p*-chlorobenzyl group (Table 2). The compounds shown are only a subset of those actually made however they are representative of the group. Substitutions on the benzyl group had modest effects on efficacy (**8a**–**d**) as did the naphthyl analogs (**8e**–**f**), however this did not translate into an improved EC_{50}. Converting the amine to an amide or sulfonamide showed improved efficacy, and **8i** was the first compound synthesized with an EC_{50} <1 μM. This represented a nice improvement over **1**. Unfortunately, we were unable to assess the in vivo characteristics of **8i** as this analog could not be detected in the mass spectrometer due to poor ionization under a number of conditions.

Finally, we began to modify the third segment of **1** and looked to modify the acetic ester side chain (Table 3). We found that the



Scheme 1. Reagents and conditions: (a) $NaBH(OAc)_3$, HOAc, $Cl(CH_2)_2Cl$, 4-Cl-PhCHO; (b) RCHO, $NaBH(OAc)_3$, HOAc, $Cl(CH_2)_2Cl$; (c) RCOCl, TEA; (d) RSO_2Cl, TEA; (e) $NaBH(OAc)_3$, HOAc, $Cl(CH_2)_2Cl$, $H_2NCH_2CO_2tBu$; (f) 5-nitrothiophenecarboxaldehyde, $NaBH(OAc)_3$, HOAc, $Cl(CH_2)_2Cl$.