Published in final edited form as:

Bioorg Med Chem Lett. 2017 April 01; 27(7): 1608–1610. doi:10.1016/j.bmcl.2017.02.014.

Synthesis of dihydroimidazole tethered imidazolinethiones and their activity as novel antagonists of the nuclear retinoic acid receptor-related orphan receptors (RORs)

Adel Nefzi^{a,*}, Guya D. Marconi^b, Maria A. Ortiz^b, Jennifer C. Davis^a, and F. Javier Piedrafita^{b,*}

^aTorrey Pines Institute for Molecular Studies, Port Saint Lucie, FL, United States

^bTorrey Pines Institute for Molecular Studies, San Diego, CA, United States

Abstract

Targeting the transcriptional activity of nuclear hormone receptors has proven an effective strategy to treat certain human diseases, and they have become a major focus point to develop novel therapies for the treatment of cancer, inflammation, autoimmune diseases, metabolic disorders, and others. One family of nuclear receptors that has attracted most interest in recent years is the retinoic acid receptor-related orphan receptors (RORs), in particular ROR γ . ROR γ is a critical regulator of the immune system and ROR γ antagonists have shown activity in animal models of inflammatory autoimmune diseases. Here we present the synthesis and biological evaluation of dihydroimidazole tethered imidazolinethiones. We have identified several dual ROR γ / α and pan-ROR antagonists with significant activity in cellular assays that could serve as starting points for future optimization efforts to generate potent and selective ROR γ modulators.

The nuclear retinoic acid receptor-related orphan receptors (RORs) (NR1F1, NR1F2, and NR1F3), in particular ROR-gamma (ROR γ), have recently been the focus of attention to develop novel treatments of metabolic, inflammatory, and autoimmune diseases. Genetic studies demonstrated a key role for ROR γ t (a truncated form of ROR γ expressed exclusively in the thymus) in the differentiation of pro-inflammatory Th17 cells¹; ROR α was subsequently shown to also be essential for Th17 cell differentiation.² A growing body of evidence suggests a pathogenic role for Th17 in several autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, type I diabetes, and psoriasis.³ The discovery that the ROR γ inverse agonists SR1001, digoxin, and ursolic acid could suppress Th17 differentiation and delay the onset of disease in an experimental autoimmune encephalomyelitis mouse model of multiple sclerosis,^{4–6} demonstrated that targeting ROR γ and/or ROR α might be an attractive strategy to treat inflammatory autoimmune diseases. During the last decade, numerous compounds have been discovered that modulate ROR γ transcriptional activity, and are the focus of therapeutic developments by several pharmaceutical companies (reviewed in⁷). Here we report the synthesis and screening results of dihydroimidazole tethered imidazolidinethiones to identify novel antagonists of RORs. We generated CHO-K1 stable cell lines expressing a 5x-UAS-

*Corresponding authors: adeln@tpims.org (A. Nefzi), jpiedrafita@tpims.org (F.J. Piedrafita).

luciferase reporter and a Gal4-ROR-LBD for all three RORs (α , β , and γ) under the control of a tetracycline repressor. These cell lines were used for the screening of all synthesized compounds and we identified several molecules with micromolar activity as ROR γ / α dual antagonists, which will serve as starting point for future computer-guided combinatorial chemistry approaches to generate potent and selective ROR γ inhibitors.

The synthesis of dihydroimidazole tethered imidazolidinethione compounds is outlined in Scheme 1.⁹ Starting from resin-bound orthogonally protected Fmoc-Lys(Boc)-OH **1**, the Boc group was deprotected with a solution of trifluoroacetic (TFA) acid in dichloromethane (DCM) and the free ϵ -amine was acylated with a variety of Boc-amino acids (diversity R¹) in the presence of diisopropylcarbodiimide (DICl) and benzotriazole (HOBt). The Boc group was deprotected and the generated amine was acylated with different commercially available carboxylic acids (diversity R²). The resin-bound polyamides **2** were treated with excess borane in tetrahydrofuran to generate the corresponding resin-bound polyamines **3**. The primary amine of the tetraamines **3** was selectively protected with 2-acetyldimedone (Dde-OH) and the resin-bound polyamines was treated with thiocarbonyldiimidazole to afford the corresponding imidazolidinethione **4**. Following Dde deprotection with a hydrazine in DMF, the free amine was acylated with different carboxylic acids (diversity R³) and the generated compound **5** was treated with phosphorous oxychloride (POCl₃) to yield following cleavage of the solid support the corresponding dihydroimidazole tethered imidazolidinethione compounds **6**.

We performed the synthesis of 116 individual compounds and evaluated them on TRex-CHO-luciferase-ROR γ cells at 2 μ g/ml; 8 compounds elicited significant toxicity as determined by resazurin assay (data not shown) and were discarded for further analysis. 30 compounds that inhibited doxycycline-dependent luciferase stimulation by 70% or more were titrated in a confirmatory assay against ROR γ and the top 15 compounds were followed up with a second dose response with a 6 point 2-fold serial dilutions starting at 8 μ g/ml, which corresponds to 15–18.7 μ M as maximum concentration. This secondary titration experiment was performed in all Gal4-ROR γ , ROR α , and ROR β stable cell lines and the IC₅₀ values obtained are shown in Fig. 1.

Compound **6a** showed the lowest IC₅₀ towards ROR γ (5.1 μ M), but it lacked selectivity as it also inhibited transcriptional activity by ROR α with comparable efficacy (6.4 μ M) and ROR β with slightly higher IC₅₀ (8.3 μ M). Compounds **6b** and **6c** also inhibited ROR γ with similar potency (5.6 and 5.8 μ M, respectively); whereas **6b** was a dual ROR γ / α antagonist with lower potency against ROR β , compound **6c** showed a slight selectivity towards ROR γ with a 2-fold lower IC₅₀. Interestingly, all these compounds share the same R1 (L-Phe) and R2 (phenylacetic acid) groups, varying only in the R3 position. In general, most of the active compounds in this class could be categorized as dual ROR γ / α antagonists, with a moderately higher IC₅₀ values against ROR β cells. Compounds **6n** and **6o** showed some selectivity towards ROR γ , but their potency was compromised.

Cell lines and luciferase assays

TRex-CHO-K1 cells (Thermo Scientific), which express the tetracycline repressor, were used to generate stable cell lines expressing a luciferase reporter gene under the control of a 5x-UAS response element (pGL-4.31 [luc2P/Gal4UAS/Hygro], Promega). Following transfection and a two weeks selection with hygromycin, TRex-CHO-UAS-luc cells were transfected with a pcDNA4/TO expression vector (Thermo Scientific) encoding the Gal4-DBD-ROR-LBD fusion proteins containing the following ROR LBDs: mROR γ (NR1F1) (Pro-261-end), hROR α (NR1F2) (Pro-211-end), or rROR β (Pro 205-end). The pcDNA4/TO expression vector allows for tetracycline controlled expression of the corresponding Gal4-ROR proteins as well as for selection of stably transfected cells with Zeocin. Individual clones for each of the Gal4-ROR γ , Gal4-ROR α , and Gal4-ROR β cell lines were isolated using a standard serial dilution protocol. Clones with the highest doxycycline-dependent stimulation of luciferase activity were selected for all functional experiments described below. For TRex-CHO-Gal4-ROR γ -Luc cells, we also selected for clones that showed sensitivity to SR2211 as reported in the literature.⁸ All cell lines were maintained in complete Ham's F12 medium containing 10% fetal bovine serum (FBS), 10 μ g/ml blasticidine, 100 μ g/ml hygromycin, and 100 μ g/ml Zeocin, and were used within 2 months of culture without any loss of doxycycline-dependent stimulation of luciferase activity.

For screening dihydroimidazole tethered imidazolinethione compounds, cells were resuspended in Ham's F12 assay medium containing 2% charcoal-treated FBS and no selection drugs. Cells were seeded in 30 μ l of assay medium at a density of 10,000 cells per well in 384 white plates with clear bottom (Greiner). Cells were allowed to attach for 2 h, followed by compound treatment and stimulation with 20 ng/ml doxycycline for an additional 16–20 h. Appropriate solvent controls, negative controls with no doxycycline, and blanks with no cells were included in every plate. Prior to measuring luciferase activity, 6 μ l of 0.15 mg/ml resazurin (Cayman Chem.) in PBS were added and cells were incubated for 1 h at 37 °C. Fluorescence intensity was measured at 545 nm excitation/590 nm emission using a CLARIOstar reader (BMGLabtech) as a measure of cell viability, to detect any potential cytotoxic effect of the test compounds. Following the resazurin assay, the medium was removed and 20 μ l of BriteLite-Plus luciferase reagent (Perkin Elmer) were added; luminescence was measured in a CLARIOstar reader using a 384 aperture spoon and a 0.1 s measuring interval. Luminescence was normalized by the fluorescence intensity and the percentage of inhibition was calculated with respect to control cells stimulated with doxycycline in the presence of solvent control (0.1% DMF). All primary screens were performed in triplicate with a final concentration of 2 μ g/ml. Active compounds were subjected to a dose response confirmatory assay followed by a second 6 point titration experiment with quadruplicate data points. IC₅₀ values were calculated using GraphPrism (GraphPad software).

Acknowledgments

This work was funded in part through the Florida Drug Discovery Acceleration Program by the State of Florida, Department of Health. NIH grant 1R01AI105836-01A1 (Piedrafita/Nefzi).

References

1. Ivanov II, McKenzie BS, Zhou L, et al. Cell. 2006; 126:1121–1133. [PubMed: 16990136]
2. Yang XO, Pappu BP, Nurieva R, et al. Immunity. 2008; 28:29–39. [PubMed: 18164222]
3. Waite JC, Skokos D. Int J Inflam. 2012; 2012:819467. [PubMed: 22229105]
4. Huh JR, Leung MW, Huang P, et al. Nature. 2011; 472:486–490. [PubMed: 21441909]
5. Solt LA, Kumar N, Nuhant P, et al. Nature. 2011; 472:491–494. [PubMed: 21499262]
6. Xu T, Wang X, Zhong B, Nurieva RI, Ding S, Dong C. J Biol Chem. 2011; 286:22707–22710. [PubMed: 21566134]
7. Fauber BP, Magnuson S. J Med Chem. 2014
8. Kumar N, Lyda B, Chang MR, et al. ACS Chem Biol. 2012; 7:672–677. [PubMed: 22292739]
9. Acharya AN, Ostresh JM, Houghten RA. J Comb Chem. 2001; 3:612–623. [PubMed: 11703159]

Fig. 1.
The IC_{50} values (in μ M) for each cell line.

Scheme 1.

Parallel solid-phase synthesis of dihydroimidazole tethered imidazolinethiones.