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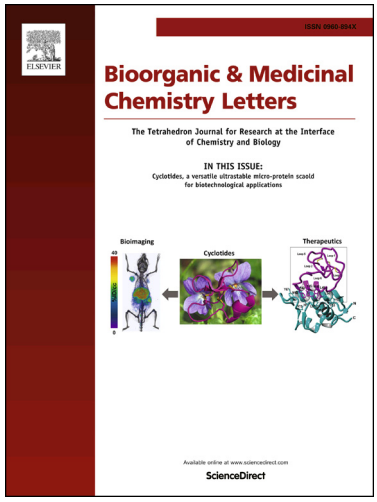

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Identification of an aminothiazole series of RORβ modulators

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ABSTRACT

Crystallography has identified stearic acid, ALRT 1550 and ATRA as ligands that bind RORβ, however, none of these molecules represent good starting points to develop optimized small molecule modulators. Recently, Compound 1 was identified as a potent dual RORβ and RORγ inverse agonist with no activity towards RORα (Figure 1). To our knowledge, this is one of only two small molecule RORβ inverse agonists identified in the primary literature from a tractable chemical series and represents an ideal starting point from which to design RORβ-selective modulators. Herein we describe our SAR optimization efforts that led to a series of potent neutral antagonists of RORβ.

The nuclear receptor (NR) superfamily of ligand-regulated transcription factors has proven to be a rich source of targets for the development of therapeutics for a wide range of human diseases. Retinoic acid receptor-related orphan receptors (RORs) are transcription factors that belong to the steroid hormone nuclear receptor super family. Several nuclear receptors are characterized as orphan receptors because the ligands for these receptors are still unknown or controversial. The NR1F subfamily, known as the retinoic acid receptor-related orphan receptors (RORα [NR1F1], RORβ [NR1F2] and RORγ [NR1F3]), regulate several physiological processes, including the circadian rhythm, glucose and lipid metabolism, and immune functions. RORα is expressed in the liver, skeletal muscle, skin lungs, adipose tissue, kidney, thymus and brain.[1,2] RORγ is most highly expressed in the thymus, but significant expression is also found in the liver, skeletal muscle, adipose tissue and kidney.[3] RORβ has a more restricted expression pattern and is found in regions of the central nervous system (CNS) that are involved in processing of sensory information and components of the mammalian timing system (circadian clock), including the suprachiasmatic nuclei (SCN), retina, pineal gland and bone.[4,5] RORβ-/- mice show defects in circadian rhythmicity.[4] Aberrant circadian rhythms are associated with numerous ailments in humans including bipolar disorder, schizophrenia, major depressive disorder and seasonal affective disorder.[6-12] RORβ is expressed in the retina and genetic deletion of RORβ results in retina degeneration, implicating its role in vision development. RORβ null mice suffer from retinal degeneration, and are born blind.[13] Most recently, it was discovered that RORβ plays a role in osteogenesis by impacting Runx2 expression.[14]

Levels of RORβ inversely correlate with osteogenic potential, suggesting that suppression of RORβ may drive osteoblast mineralization. While RORα-/- mice displayed bone abnormalities, the bone phenotype in RORβ-/- mice has not been characterized. RORβ and a subset of RORβ-regulated genes were found to be overexpressed in bone biopsies from post-menopausal women, suggesting a role for RORβ in human age-related bone loss.[15]

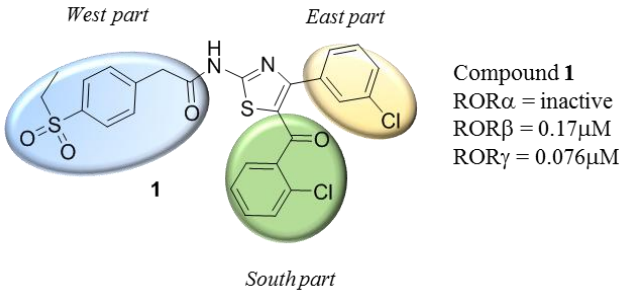


Figure 1. Lead dual RORβ/RORγ antagonist

While the *in vivo* functions of RORα and RORγ have been widely explored over the past decade, the exact roles for RORβ still remain elusive, in part due to lack of specific molecular probes that would enable investigation of its function. Previously it was shown that all trans retinoic acid

(ATRA) and a synthetic analog (ALRT 1550) bind to RORβ and are functional inverse agonists, but these ligands lack potency and bind other nuclear receptors including the RXRs and RARs.[16,17] Stearic acid was also shown to bind RORβ during expression and purification, but does not activate the receptor.[18,19]

Our goal was to identify, characterize and develop potent RORβ selective modulators with sufficient ADME properties to facilitate their use as *in vivo* probes. Recently, Phenex Pharmaceuticals identified a potent dual RORβ and RORγ inverse agonist with no activity toward RORα (Compound **1**, Figure 1).[20] To our knowledge, this is one of only two small molecule RORβ inverse agonists identified in the primary literature from a tractable chemical series and represents an ideal starting point from which to design RORβ selective modulators.[21]

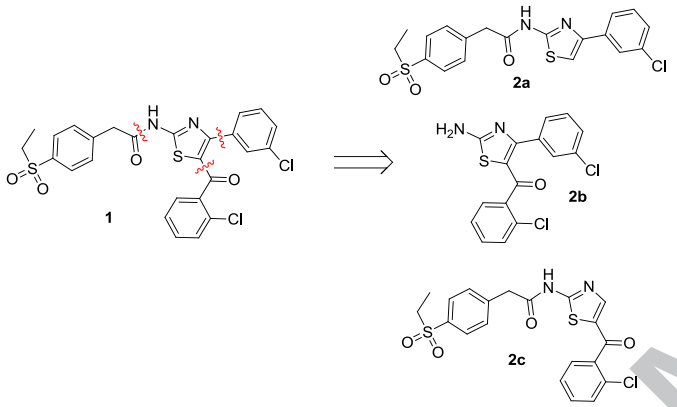


Figure 2. Truncations of compound **1**

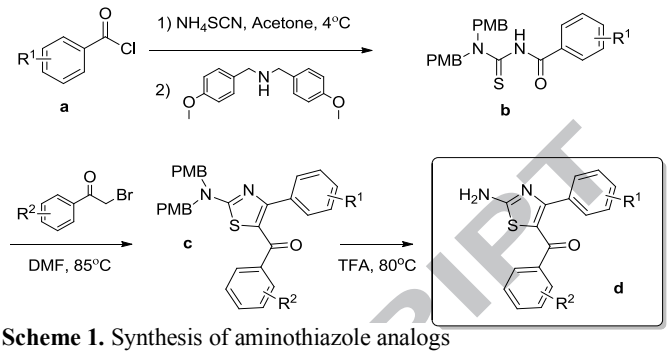
Table 1. Binding affinity of truncated analogs

Compound	[a]RORβ %inhib	[a]RORγ %inhib	[b]RORβ IC_{50} μM	[c]RORγ IC_{50} μM
1	80	85	0.059±0.02	0.013±0.001
2a	30	n.t.		
2b	50	2	0.24±0.05	>40
2c	35	n.t.		

[a] Percent displacement of [^{3}H]-T09 at 1 μM; [b] Displacement of [^{3}H]-T09 from human RORβ LBD. Values are the mean ± SEM of at least three replicates; [c] Displacement of [^{3}H]-T09 from human RORγ LBD. Values are the mean ± SEM of at least three replicates; n.t: not tested.

Compound **1** can be dissected into three fragments of equal size and complexity (Figure 2). We were curious to see which portions of the molecule were absolutely required for activity, so we synthesized three compounds each lacking one portion of the parent molecule (Figure 2, **2a-c**). The binding potency of the analogs was determined in a scintillation proximity assay (SPA) using ^{3}H T0901317 and recombinant human RORβ and RORγ LBDs.[22] This assay measures the affinity of the compounds for RORβ vs RORγ and is tabulated in Table 1.

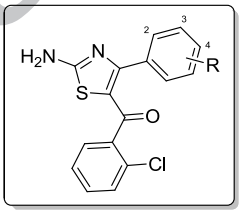
Removal of the benzoyl portion led to **2a** and a significant reduction in activity against RORβ as did removal of the C-4 aryl group (**2c**, Table 1). Much to our surprise, truncation of the west part afforded aminothiazole **2b**, which was selective for RORβ vs RORγ. This was unexpected as the ligand binding pocket in RORβ is actually larger than it is in RORγ (766^{3}Å versus 705^{3}Å, respectfully).[23] Despite a ten-fold drop in potency vs compound **1**, this was the first RORβ modulator identified devoid of RORγ activity, and served as the basis for our SAR campaign described herein.



Scheme 1. Synthesis of aminothiazole analogs

We decided to continue our SAR exploration of **2b** and begin with modifications to the East part of the structure (Table 2). Synthesis of these aminothiazole analogs was straightforward following chemistry as described in the primary literature and is shown in Scheme 1.[24]

Table 2. East part SAR – Substituted aryl rings

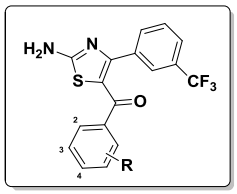


Cmpd	R	[a]RORβ IC_{50} μM	[b]RORγ IC_{50} μM
2b	3-Cl	0.24±0.05	>40
3a	2-Cl	(50%)	n.t.
3b	4-Cl	(30%)	n.t.
3c	2-Br	(15%)	(20%)
3d	3-Br	0.41±0.13	(15%)
3e	4-Br	(25%)	n.t.
3f	3-OMe	(10%)	n.t.
3g	3-CF_3	0.61±0.06	>40
3h	3-NO_2	(15%)	n.t.
3i	3-CN	(5%)	n.t.
3j	3-SO_2Me	(30%)	n.t.
3k	3-Ph	1.4±0.55	nt
3l	3-Cl, 5-CF_3	0.14±0.018	(10%)
3m	3-Br, 5-CF_3	0.063±0.01	>40
3n	3,5-CF_3	0.31±0.074	13.4
3o	3,5-Br	(60%)	n.t.
3p	3,5-Dimethyl	(55%)	n.t.
3q	3-CF_3, 4-Br	0.29±0.076	(10%)
3r	3-CF_3, 4-Cl	0.12±0.048	>40

[a] Displacement of [^{3}H]-T09 from human RORβ LBD. Values are the mean ± SEM of at least three replicates. IC_{50} or displacement of [^{3}H]-T09 at 1 μM); [b] Displacement of [^{3}H]-T09 from human RORγ LBD. Values are the mean ± SEM of at least three replicates. IC_{50} or displacement of [^{3}H]-T09 at 1 μM); n.t. = not tested.

The chlorine substitution was best tolerated at the 3-position in the phenyl ring, but was not tolerated at the 2- and 4-positions (**2b**, **3a**, **3b**, respectively, Table 2). A scan of the ortho-(**3c**), meta-(**3d**), and para-positions (**3e**) of the phenyl ring with a bromine atom showed a similar trend, with the 3-position optimal for potency. Incorporation of additional substituents at the 3-position revealed a striking preference for hydrophobic groups (**3g**, **3k**), whereas polar residues led to loss of affinity (**3f**, **3h**, **3i**, **3j**). Given the bias for meta substitution, we synthesized several 3,5-disubstituted analogs which led to our most potent compounds to date (**3l**, **3m**). Interestingly, while 4-substitution wasn't well tolerated alone (**3b**, **3e**), inclusion of a 3-CF_3 group afforded potent analogs (**3q**, **3r**). None of the compounds had much affinity for RORγ.

Table 3. South part SAR – Substituted benzoyl rings

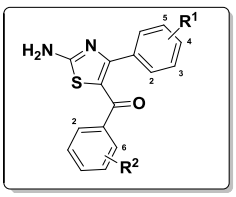


Cmpd.	R	[a]RORβ IC_{50} μM	[b]RORγ IC_{50} μM
3g	2-Cl	0.61±0.06	>40
4a	3-Cl	(60)	n.t.
4b	4-Cl	(40)	n.t.
4c	2-CF_3	0.09±0.05	>40
4d	3-CF_3	(60)	n.t.
4e	4-CF_3	(45)	n.t.
4f	2-Br	0.11±0.012	>40
4g	2-OMe	0.37±0.11	>40
4h	2-Me	(60)	n.t.
4i	2-OCF_3	(45)	n.t.
4j	2-F, 6-CF_3	0.13±0.028	>40
4k	2-F, 6-Cl	0.41±0.12	>40

[a] Displacement of [^{3}H]-T09 from human RORβ LBD. Values are the mean ± SEM of at least three replicates. IC_{50} or displacement of [^{3}H]-T09 at 1 μM); [b] Displacement of [^{3}H]-T09 from human RORγ LBD. Values are the mean ± SEM of at least three replicates. IC_{50} or displacement of [^{3}H]-T09 at 1 μM); n.t. = not tested.

With this east-part SAR information in hand, we turned our attention toward the south part of the structure (Table 3). A scan of the 2-(**3g**), 3-(**4a**), and 4-positions (**4b**) of the south part benzoyl ring with a chlorine atom revealed a distinct preference for substitution at the 2-position (**3g**). A complimentary scan with a trifluoromethyl group (**4c**, **4d**, **4e**) confirmed this. Inclusion of additional substitutents at the 2-position showed a range of potencies depending on the substituent. The 2-Br group was the most potent, but increasing the size of the group led to reduced binding affinity (**4g**, **4h**, **4i**). A pair of 2,6-disubstituted analogs were essentially equipotent to their mono-substituted counterparts (**4j** vs **4c**, and **4k** vs **3g**). The fluorine substitution had no significant effect. None of the analogs tested exhibited any affinity for RORγ. One of the most selective compounds **4c** shows >400-fold selectivity for RORβ vs RORγ.

Table 4. Combined SAR of both regions



Cmpd.	R^1	R^2	[a]RORβ IC_{50} μM	[b]RORγ IC_{50} μM
5a	3,5-$(CF_3)_2$	2-OMe	0.29±0.13	n.t.
5b	3,5-$(CF_3)_2$	2-F, 6-CF_3	(55%)	n.t.
5c	3-CF_3, 4-Cl	2-OMe	0.16±0.06	>40
5d	3-CF_3, 4-Cl	2-F, 6-CF_3	(55%)	n.t.
5e	3-Br, 5-CF_3	2-OMe	0.16±0.03	16.1
5f	3-Br, 5-CF_3	2-F, 6-CF_3	(60%)	n.t.

[a] Displacement of [^{3}H]-T09 from human RORβ LBD. Values are the mean ± SEM of at least three replicates. IC_{50} or displacement of [^{3}H]-T09 at 1 μM); [b] Displacement of [^{3}H]-T09 from human RORγ LBD. Values are the mean ± SEM of at least three replicates. IC_{50} or displacement of [^{3}H]-T09 at 1 μM); n.t. = not tested.

Combining some of the improvements identified in the East part SAR (R^1) with that found in the South part SAR (R^2) were investigated (Table 4). Additivity was not necessarily observed (**5d**, **5f**) wherein some compounds were actually less potent binders of RORβ. This might suggest separate modifications to each ring induce slight shifts in the pocket to accommodate substitutions. Hence, once optimal substituents are found for one phenyl ring, the SAR of the other ring would have to be reinvestigated de novo.

To further confirm pharmacology of the compounds *in vitro*, compounds **1**, **2b**, **3m**, **5c** and **5e** were screened in HEK293T cells in a Gal4-RORα::UAS-Luc, Gal4-RORβ::UAS-Luc or Gal4-RORγ::UAS-Luc reporter assay with counterscreening against Gal4-VP16::UAS-Luc (data not shown). While **1** clearly displayed inverse agonism of all three receptors (RORα IC_{50}: 0.11 μM; RORβ IC_{50}: 0.86 μM; RORγ IC_{50}: 0.066 μM), none of the new analogs displayed any significant activity. It is unlikely that there are cell permeability issues given the high hydrophobicity of the compounds and the compounds do not appear to be toxic at the doses tested (10 μM and below). However it is possible that the compounds are neutral antagonists. Despite binding to the receptor and competeing out T09, they do not induce a conformational change which can disrupt the AF2 surface and alter coregulator interactions. Additional studies will need to be completed to fully understand the phamracology.

Several analogs were investigated for stability in the presence of mouse liver microsomes (Table 5). Unfortunately, *in vitro* stability decreased significantly by removing the sulfonamide linker (**1→2b**). All other analogs tested also had poor stability as compared to the lead. This

will be something that needs to be addressed before compounds are ready for *in vivo* evaluation.

Table 5. Mouse liver microsome stability of selected analogs

Compound	Mouse ($T_{1/2}$ in min)	Compound	Mouse ($T_{1/2}$ in min)
1	34	**3n**	7.4
2b	1.9	**3q**	2.5
3g	2.9	**4k**	2.7
3l	6.1	**5a**	5.2
3m	5.2	**5e**	2.3

In summary, we have identified a series of disubstituted aminothiazoles which bind RORβ with excellent potency. Starting from a potent dual RORβ/RORγ inverse agonist **1**, removal of the sulfone-containing side chain ablated affinity for RORγ. Optimization of the 4-phenyl and 5-benzoyl groups led to improvements in RORβ potency without compromising selectivity for RORγ. Analogs **3m** and **4c** were the most potent analogs identified. Current compounds are not robustly stable in the presence of liver microsomes, and will require additional manipulation before *in vivo* probes are identified. These studies are on-going and will be reported in due course.

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