

# Minor Structural Change to Tertiary Sulfonamide RORc Ligands Led to Opposite Mechanisms of Action

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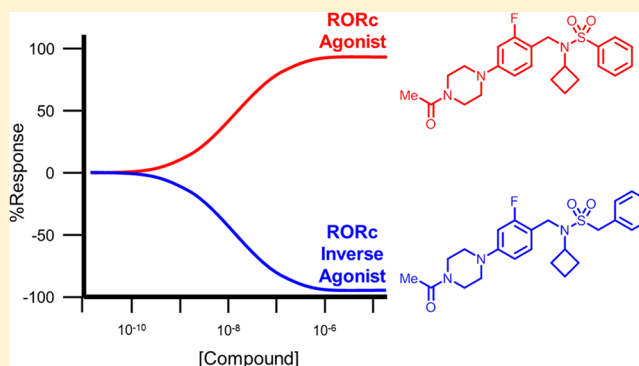
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## Supporting Information

**ABSTRACT:** A minor structural change to tertiary sulfonamide RORc ligands led to distinct mechanisms of action. Co-crystal structures of two compounds revealed mechanistically consistent protein conformational changes. Optimized phenylsulfonamides were identified as RORc agonists while benzylsulfonamides exhibited potent inverse agonist activity. Compounds behaving as agonists in our biochemical assay also gave rise to an increased production of IL-17 in human PBMCs whereas inverse agonists led to significant suppression of IL-17 under the same assay conditions. The most potent inverse agonist compound showed >180-fold selectivity over the ROR isoforms as well as all other nuclear receptors that were profiled.

**KEYWORDS:** RORc, ROR $\gamma$ , T<sub>H</sub>17, IL-17, PBMC, agonist, inverse agonist



The retinoic acid receptor-related orphan receptor gamma (ROR $\gamma$  or RORc)<sup>1</sup> is a key nuclear receptor (NR) of the ROR family that has been implicated in the production and regulation of interleukin-17 (IL-17).<sup>2</sup> This cytokine is required for the differentiation of naïve pro-inflammatory CD4<sup>+</sup> T cells and for the production of T helper-17 (T<sub>H</sub>17) cells,<sup>2</sup> which results in the expression of diverse pro-inflammatory cytokines such as IL-17,<sup>2</sup> IL-22,<sup>3</sup> and GM-CSF.<sup>4</sup> Interleukin-17 is a critical driver of autoimmune diseases such as psoriasis,<sup>5</sup> rheumatoid arthritis (RA),<sup>6</sup> multiple sclerosis (MS),<sup>7</sup> and inflammatory bowel disease (IBD).<sup>8</sup> Therefore, the modulation of IL-17 expression through small molecule inhibitors of RORc<sup>9–11</sup> has gained significant momentum as a molecular target since the first disclosure of a direct link between RORc signaling and IL-17 production by Littman et al. in 2006.<sup>2</sup>

Small molecule NRs may modulate transcription through several mechanisms.<sup>12,13</sup> Ligands can induce a conformational change in the NR that results in the reduction of coactivator protein recruitment to the NR. Such ligands are termed inverse agonists.<sup>14</sup> Inverse agonists decrease gene transcription, and in the case of RORc, this results in a reduction of IL-17 production. Conversely, ligands that promote coactivator protein recruitment and formation of the [NR-coactivator] complex generally result in increased gene transcription and are termed agonist ligands.<sup>14</sup> A third method of modulation involves ligands that bind to the ligand-binding domain (LBD)

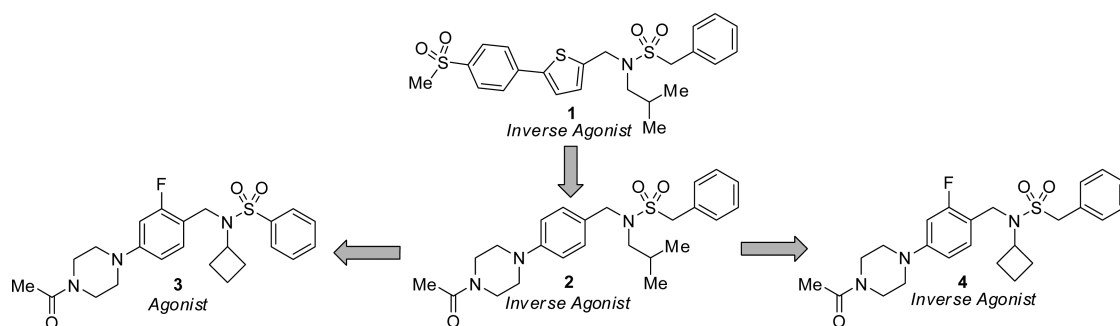
of the NR but do not affect the basal transcriptional activity of the protein. Such molecules may be termed silent ligands or neutral antagonists.<sup>15</sup>

In addition to numerous small molecule RORc modulators previously reported,<sup>9–11</sup> compound **1** was recently disclosed as a potent biaryl sulfonamide inverse agonist that was identified in a biochemical screen of the Genentech/Roche corporate collection (Figure 1).<sup>16</sup> Co-crystallization of **1** with the human RORc-LBD [PDB: 4QM0]<sup>17</sup> revealed that the benzylic phenyl ring of **1** interacted with His479, thus preventing His479 from forming a crucial hydrogen bond with Tyr502 in the protein. The interaction between His479 and Tyr502 is required to stabilize the secondary structure of helices 11–12 and induce coactivator recruitment.<sup>18</sup> The disruption of this interaction may account for the inverse agonist mechanism of action (MOA)<sup>19,20</sup> observed in our biochemical coactivator recruitment assay with compound **1**. Further replacement of the biaryl motif in **1** with an acylpiperazine (**2**) led to significant improvement of the physicochemical properties, plasma-protein binding, and permeability of the series while preserving RORc inverse agonist potency.<sup>17</sup>

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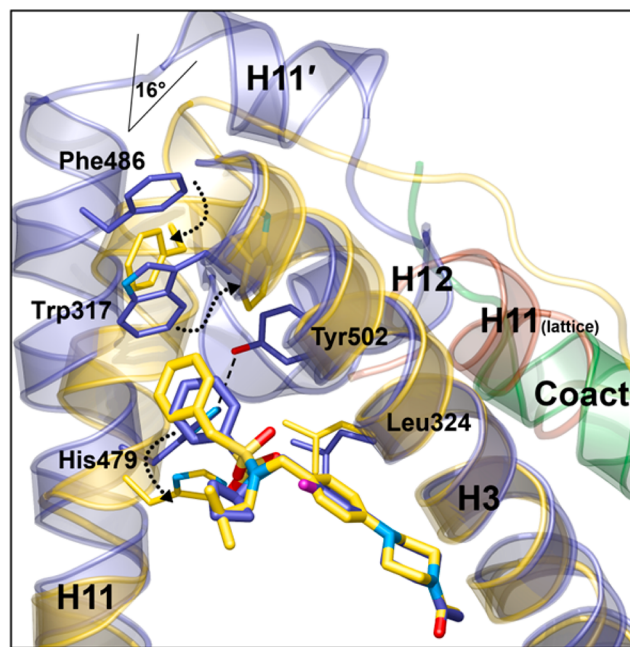


**Figure 1.** Evolution of Genentech's tertiary sulfonamide RORc modulators.

Additional optimization of benzy sulfonamide replacements was explored using parallel synthesis and resulted in the discovery of the matched pair of phenyl sulfonamide **3** and benzyl sulfonamide **4**. While benzyl analog **4** showed the typical inverse agonist behavior for the series, phenyl sulfonamide **3** was a high affinity ligand of RORc that showed strong agonist efficacy in the biochemical coactivator recruitment assay. Herein, we disclose the discovery of additional phenyl-sulfonamide and benzy sulfonamide pairs that demonstrate that a minor change in the structure of the ligand (i.e., one methylene group) can lead to agonist and inverse agonist MOAs, respectively.

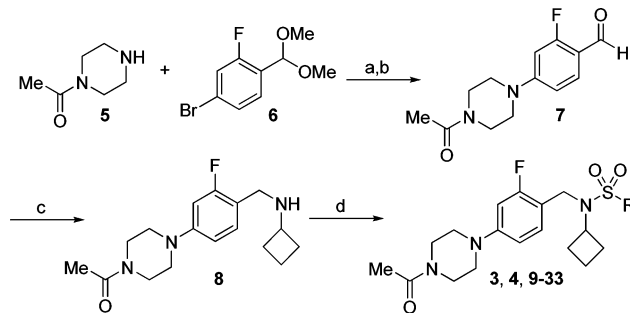
To better understand how the changes to the sulfonamide groups affected the binding mode of the ligands and resulted in divergent MOAs, we cocrystallized inverse agonist ligand **2** and agonist ligand **3** with the human RORc-LBD and obtained 2.0 and 2.2 Å resolution X-ray structures, respectively.<sup>21</sup> In both cases, the ligands were bound within the ligand binding pocket of RORc (Figure 2), and approximately one-third of the ligands (from the acetyl moiety through the piperazine ring) superimposed nearly identically. The trajectory of the compounds then diverged slightly, projecting their terminal phenyl rings from diverging vectors toward helix 11. The agonist ligand **3** (Figure 2, purple ribbon) packed against Trp317, His479, and Tyr502, stabilized the agonist conformation of helix 12, and recruited the coactivator peptide. On the other hand, the benzyl moiety of the inverse agonist ligand **2** (Figure 2, yellow ribbon) displaced His479, and prevented formation of the hydrogen bond with Tyr502 of helix 12. The disruption of the hydrogen bond between His479 and Tyr502 dislodged helix 12 entirely in the costructure with **2** and eliminated the binding site for coregulator proteins. This observation was consistent with the RORc inverse agonist activity of **2**. The C-terminal half of helix 11 bent 16° inward and filled in the gap created while multiple aromatic side chains reoriented in a switch-like fashion, and repacked the apex of the pocket in the absence of helix 12. Similarly, concerted motions of Trp317 and Phe486 rotated, and filled pockets created by these changes. Both **2** and **3** formed a single hydrogen bond from the oxygen of their terminal acetyl groups to an ordered water molecule at the opposite end of the pocket from helix 12 (Figure S2). The rest of the contacts made to the compounds were van der Waals interactions with the predominantly hydrophobic binding site residues.

To further evaluate the effects of different sulfonamide substituents on the MOA, we synthesized a series of benzy- and arylsulfonamides using a late-stage diversification synthetic approach (Scheme 1). Benzaldehyde intermediate **7** was obtained using a Buchwald–Hartwig amination reaction<sup>22</sup>



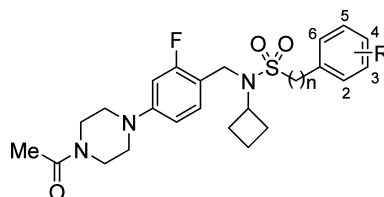
**Figure 2.** Binding modes of agonist ligand **3** and inverse agonist ligand **2** within the ligand binding pocket of RORc. The crystal structure of the agonist ligand **3** (purple ribbon) stabilized helix 12 and revealed a coactivator peptide (green) bound to the receptor. The crystal structure of the inverse agonist ligand **2** (yellow ribbon) did not have resolved density for helices 11–12. Concerted motions of Trp317, Phe486 and His479 are depicted with arrows.

#### Scheme 1. Synthesis of Sulfonamides **3**, **4**, and **9–33**<sup>a</sup>



<sup>a</sup>Reagents and conditions: (a) Pd(OAc)<sub>2</sub>, RuPhos, NaOt-Bu, 1,4-dioxane, 100 °C; (b) HCl, H<sub>2</sub>O, THF, 23 °C, 60% (2 steps); (c) cyclobutylamine, NaBH(OAc)<sub>3</sub>, AcOH, 1,2-DCE, 23 °C, quant.; (d) R-SO<sub>2</sub>Cl, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, 23 °C, 29–57%.

between acetyl piperazine **5** and acetal-protected bromoarene **6**, followed by acetal removal under acidic conditions.

Table 1. Structure–Activity Relationships of Phenyl- and Benzylsulfonamide Matched Pairs<sup>a</sup>

Compd	<i>n</i>	R	RORc IC <sub>50</sub> <sup>b</sup> (μM)	RORc SRC1 EC <sub>50</sub> <sup>c</sup> (μM) [% efficacy]	cLogP <sup>d</sup>	LLE <sup>e</sup>	MOA <sup>f</sup>
3	0	H	0.25	0.069 [+35%]	3.7	3.5	Agonist
4	1	H	0.015	0.011 [-99%]	3.3	4.7	Inverse Agonist
9	2	H	0.11	0.31 [-64%]	3.7	2.8	Inverse Agonist
10	0	2-F	0.076	0.063 [+46%]	3.9	3.3	Agonist
11	1	2-F	0.010	0.003 [-98%]	3.4	5.1	Inverse Agonist
12	0	3-F	0.065	0.041 [+46%]	3.9	3.5	Agonist
13	1	3-F	0.025	0.010 [-98%]	3.5	4.5	Inverse Agonist
14	0	4-F	0.22	>10 [-37%]	3.9	-	Inverse Agonist
15	1	4-F	0.017	0.007 [-97%]	3.5	4.7	Inverse Agonist
16	0	2-Cl	0.055	0.047 [+33%]	4.4	2.9	Agonist
17	1	2-Cl	0.009	0.004 [-96]	3.9	4.5	Inverse Agonist
18	0	3-Cl	0.022	0.050 [+53%]	4.4	2.9	Agonist
19	1	3-Cl	0.019	0.009 [-98%]	4.1	4.0	Inverse Agonist
20	0	4-Cl	0.092	0.19 [-63%]	4.4	2.3	Inverse Agonist
21	1	4-Cl	0.037	0.033 [-97%]	4.1	3.4	Inverse Agonist
22	0	3,5-diCl	0.006	0.006 [+65%]	5.1	3.4	Agonist
23	1	3,5-diCl	0.029	0.040 [-92%]	4.8	2.6	Inverse Agonist
24	0	3,5-diF	0.049	0.014 [+43%]	4.1	3.7	Agonist
25	0	3-OMe	0.036	0.026 [+54%]	3.8	3.8	Agonist

<sup>a</sup>All assay results are reported as the geometric mean of at least two separate runs.<sup>24</sup> <sup>b</sup>Biochemical inhibition of the RORc-LBD and [<sup>3</sup>H<sub>2</sub>]25-hydroxycholesterol interaction. <sup>c</sup>Activation or inhibition of RORc-LBD recruitment of the SRC1 coactivator; positive “%eff.” denotes agonism and negative “%eff.” denotes inverse agonism relative to the basal assay signal for apo-RORc LBD in this assay format. <sup>d</sup>Calculated logP (cLogP) value.<sup>27</sup> <sup>e</sup>Ligand-lipophilicity efficiency (LLE)<sup>25</sup> was calculated using RORc SRC1 EC<sub>50</sub> and the cLogP. <sup>f</sup>Mechanism of action (MOA) reported as agonist, inverse agonist, or silent ligand.

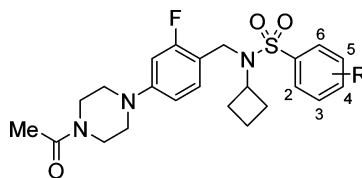
Subsequent reductive amination of aldehyde **7** with cyclobutylamine gave the advanced intermediate amine **8**. This late-stage intermediate allowed for the incorporation of a diverse set of sulfonyl chlorides and gave sulfonamides **3**, **4**, and **9–33**.<sup>23</sup>

The analogs were then evaluated in two RORc biochemical assays.<sup>24</sup> The affinity of the analogs for the RORc-LBD was measured using a radiometric binding assay that monitored the displacement of [<sup>3</sup>H<sub>2</sub>]25-hydroxycholesterol from the RORc-LBD. Compounds were also evaluated in a time-resolved fluorescence biochemical assay that monitored the ability of the human RORc-LBD to bind to a coactivator peptide derived from steroid receptor coactivator-1 (SRC1). Compounds disrupting the recruitment of the SRC1 peptide were determined to be inverse agonists whereas compounds that enhanced peptide recruitment were agonists. Alternatively, compounds that did not modulate the level of recruitment but showed affinity in the radiometric binding assay were identified as silent ligands. Full inverse agonism (−100% efficacy) was experimentally measured and corresponded to complete suppression of basal activity. Full agonism (+100% efficacy) was calculated as the additive inverse of a complete suppression of basal activity, since the experimental maximum % efficacy of a RORc full physiological agonist is currently undefined.

Matched pair analysis of the aryl- and benzylsulfonamides (Table 1) revealed that phenylsulfonamide **3** was a RORc agonist (EC<sub>50</sub> = 69 nM, +35% efficacy) while the corresponding benzyl analog **4** was a potent full inverse agonist (EC<sub>50</sub> = 11 nM, −99% efficacy). The phenethylsulfonamide (**9**)

was also an inverse agonist, albeit with a significantly lower potency and efficacy (EC<sub>50</sub> = 310 nM, −64% efficacy). We then sought to improve the potency of our ligands by introducing small nonpolar substituents around the arene to provide favorable van der Waals interactions with the ligand-binding pocket. The ligand-lipophilicity efficiency (LLE)<sup>25,26</sup> was used as a metric to ensure the effective use of nonpolar interactions. Incorporation of a 2-fluorine substituent in compound **10** resulted in an equipotent and equi-efficacious agonist (EC<sub>50</sub> = 63 nM, +46% efficacy) when compared to the phenyl analog **3**. However, the same 2-fluoro substituent provided a more substantial potency improvement with inverse agonist benzyl compound **11** (EC<sub>50</sub> = 3 nM, −98% efficacy). The LLE of compound **11** was also improved compared to compound **4**. Similar trends were observed with 3-fluorophenylsulfonamide **12** and 3-fluorobenzylsulfonamide **13**, with **12** being a slightly more potent agonist (EC<sub>50</sub> = 41 nM, +46% efficacy) than **10**. Interestingly, 4-fluoro substitution (**14**) led to a loss of effect on peptide recruitment and weak inverse agonism (EC<sub>50</sub> > 10 μM, −37% efficacy), although this ligand demonstrated significant affinity for the receptor in the radioligand binding assay (RORc IC<sub>50</sub> = 220 nM). The 4-fluorobenzylsulfonamide **15** was a potent inverse agonist (EC<sub>50</sub> = 7 nM, −97% efficacy).

A similar scan was performed with 2-, 3-, and 4-chloro substituted phenyl- and benzylsulfonamides **16–21**. The 2- and 3-chlorophenylsulfonamides (**16** and **18**, respectively) were both similarly potent agonists (EC<sub>50</sub> = 47 nM, +33% efficacy and 50 nM, +53% efficacy, respectively), and their benzylsulfo-

Table 2. Structure–Activity Relationship of 4-Substituted Arylsulfonamides<sup>a</sup>

Compd	R	RORc IC <sub>50</sub> <sup>b</sup> (μM)	RORc SRC1 EC <sub>50</sub> <sup>c</sup> (μM) [% efficacy]	cLogP <sup>d</sup>	LLE <sup>e</sup>	MOA <sup>f</sup>
26	2,4-diMe	0.024	>10 [+9%]	4.7		Silent
27	4-CN	0.28	>10 [-29%]	3.4		Inverse Agonist
28	4-OMe	0.12	3.3 [-52%]	3.8	1.7	Inverse Agonist
29	2,4-diCl	0.076	0.22 [-37%]	5.2	1.5	Inverse Agonist
30	4-OCF <sub>2</sub> H	0.031	0.15 [-75%]	4.1	2.7	Inverse Agonist
31	4-OCF <sub>3</sub>	0.29	0.16 [-82%]	4.9	1.9	Inverse Agonist
32	4-CF <sub>3</sub>	0.050	0.30 [-39%]	4.6	1.9	Inverse Agonist
33	4- <i>t</i> -Bu	0.018	0.12 [-55%]	5.3	1.6	Inverse Agonist

<sup>a</sup>All assay results are reported as the geometric mean of at least two separate runs. <sup>b</sup>Biochemical inhibition of the RORc-LBD and [<sup>3</sup>H<sub>2</sub>]25-hydroxycholesterol interaction. <sup>c</sup>Activation or inhibition of RORc-LBD recruitment of the SRC1 coactivator; positive “%eff.” denotes agonism, and negative “%eff.” denotes inverse agonism relative to the basal assay signal for apo-RORc LBD in this assay format. <sup>d</sup>Calculated logP (cLogP) value.<sup>27</sup> <sup>e</sup>Ligand-lipophilicity efficiency (LLE)<sup>25</sup> was calculated using RORc SRC1 EC<sub>50</sub> and the cLogP. <sup>f</sup>Mechanism of action (MOA) reported as agonist, inverse agonist, or silent ligand.

namide counterparts **17** and **19** displayed full inverse agonist MOA with RORc SRC1 EC<sub>50</sub> values of 4 nM and 9 nM, respectively. Substitution at the 4-position of arylsulfonamide **20** with the chloride atom led to a reversal of MOA, giving rise to an inverse agonist (EC<sub>50</sub> = 190 nM, -63% efficacy), when compared to **3**. The 4-chlorobenzyl (**21**) matched pair to **20** also showed inverse agonism (EC<sub>50</sub> = 33 nM, -97% efficacy), although not as potent as other substituted benzylsulfonamides. Finally, the 3,5-dichloroarylsulfonamide **22** gave rise to our most potent agonist (EC<sub>50</sub> = 6 nM, +65% efficacy), albeit with a poor LLE of 3.4 resulting from the high lipophilicity of the dichloroarene. Conversely, the 3,5-dichlorobenzyl analog **23** was a potent inverse agonist (EC<sub>50</sub> = 40 nM, -92% efficacy). Replacing the chlorine atoms of **22** with less lipophilic fluorines (**24**) led to a higher LLE agonist (EC<sub>50</sub> = 14 nM, +43% efficacy, LLE = 3.7). A further reduction in lipophilicity was accomplished by appropriately incorporating a 3-methoxy substituent (**25**), which led to further improvement of the LLE (EC<sub>50</sub> = 26 nM, +54% efficacy, LLE = 3.8). This initial SAR on the right-hand side (RHS) identified single-digit nanomolar analogs with both agonist and inverse agonist profiles. Phenylsulfonamides generally behaved as agonists of RORc, while the benzylsulfonamides acted as inverse agonists.

Intrigued by the reversal of MOA of 4-substituted arylsulfonamides **14** and **20**, we sought to rationalize this phenomenon by further examining the X-ray costructure of agonist **3**. The unsubstituted phenyl ring of **3** was directed at Trp317 and formed an edge-to-face  $\pi$ - $\pi$  interaction (3.6 Å),<sup>28</sup> which in turn formed a  $\pi$  stacking interaction with Phe486 (4.2 Å), stabilizing helix 11 and the bound ligand. Substitution at the 4-position on the phenyl ring in this binding mode would clash with Trp317. This could cause the pocket to adopt a different conformation and ultimately disrupt helices 11–12. We hypothesized that while no substitution at the 4-position (R = H, **3**) led to the observed agonist binding mode in the RORc SRC1 assay, changes to a small substituent at the 4-position (R = F, **14**) would begin destabilizing the C-terminus and prevent SRC1 peptide recruitment beyond the basal level, leading to low inverse agonist efficacy or silent MOA ligands. Further steric bulk directed toward the C-terminal region (R =

Cl, **20**) would fully disrupt the secondary structure of that region of the protein and prevent RORc interaction with the coactivator peptide, resulting in a stronger inverse agonist MOA.<sup>29</sup>

With this consideration in mind, we synthesized additional analogs bearing incrementally bulkier functionalities at the *para* position of the arylsulfonamide (Table 2) to further explore the level of efficacy of inverse agonism that could be achieved. Compound **26**, bearing a 4-methyl substituent, showed significant affinity for RORc in the binding assay (RORc IC<sub>50</sub> = 24 nM) but behaved as silent ligand in the RORc SRC1 biochemical assay. Additionally, a more polarized 4-cyano substituent<sup>30</sup> possessed moderate RORc binding affinity, as exemplified with compound **27** (RORc IC<sub>50</sub> = 280 nM). However, the steric bulk of the nitrile group was not sufficient to impart full RORc inverse agonism in the RORc SRC1 biochemical assay (EC<sub>50</sub> > 10 μM, -29% efficacy). Substitution with a 4-methoxy group ultimately provided enough steric bulk and gave rise to stronger RORc inverse agonist **28** (EC<sub>50</sub> = 3.3 μM, -52% efficacy). Further increasing the steric demand of the ligand with dichloro substitution (**29**) or with 4-difluoromethoxy (**30**), 4-trifluoromethoxy (**31**), 4-trifluoromethyl (**32**), or 4-*tert*-butyl (**33**) groups afforded similarly potent RORc inverse agonists with ranging efficacy of -37%, -75%, -82%, -39%, and -55%, respectively, with **33** being the most potent (EC<sub>50</sub> = 120 nM). In summary, we demonstrated that arylsulfonamide agonists could be transformed into silent ligands by adding steric bulk to the 4-position of the arene. Additional steric hindrance at the 4-position eventually led to a RORc inverse agonist MOA.

We also explored the effect of replacing the sulfonamide moiety in **3** and **4** with an amide functional group. Both phenyl- and benzylamide compounds showed <50% inhibition in the SRC1 peptide recruitment assay at a 10 μM concentration. Sulfonamides typically adopt a nonplanar conformation<sup>31</sup> that was also observed in the cocrystal structures of compounds **2** and **3** with the RORc-LBD. Presumably, the planar conformation of the amide did not allow for the amide analogs to effectively bind to RORc-LBD and affect the RORc SRC1 peptide recruitment assay.



We progressed two leading agonist/inverse agonist matched pairs (3–4 and 10–11) into a human peripheral blood mononuclear cell (PBMC) cytokine production assay to evaluate their effects on IL-17 production (Table 3).<sup>24</sup> RORc

**Table 3. Potency and Mechanism of Action of Phenyl- and Benzylsulfonamides in hPBMC IL-17 Production Assay<sup>a</sup>**

Compd	IL-17 PBMC EC <sub>50</sub> (μM)	IL-17 PBMC efficacy (%)	IFN $\gamma$ EC <sub>50</sub> (μM)	CTG EC <sub>50</sub> (μM)
3	0.78	+77	>10	>20
4	0.35	-79	>10	>20
10	0.80	+71	>10	>20
11	0.32	-72	>10	>20

<sup>a</sup>All assay results are reported as the geometric mean of at least two separate runs. All assays were conducted using peripheral blood mononuclear cells (PBMCs) isolated from human whole blood.<sup>24</sup> Positive %efficacy denotes a potentiation of IL-17 production. Negative %efficacy denotes an inhibition of IL-17 production. Interferon- $\gamma$  (INF- $\gamma$ ) and CellTiter-Glo (CTG) cell measurement assays were used as positive controls to monitor for non-T<sub>H</sub>17 cell cytokine activity and adverse off-target effects on cell health, respectively.<sup>24</sup>

agonists **3** and **10** gave rise to an increased production of IL-17 (EC<sub>50</sub> = 0.78 μM, +77% efficacy and 0.80 μM, +71% efficacy, respectively), which was in agreement with their MOA determined in the RORc SRC1 biochemical assay. Conversely, compounds **4** and **11**, which were shown to be RORc inverse agonists in the SRC1 peptide recruitment biochemical assay, both led to a suppression of IL-17 production in the human PBMC assay (EC<sub>50</sub> = 0.35 μM, -79% efficacy and 0.32 μM, -72% efficacy, respectively). In addition, none of the tested compounds affected interferon- $\gamma$  (INF- $\gamma$ ) biosynthesis or cell health as assessed by CellTiter-Glo (CTG) cellular ATP measurements, confirming that the compounds did not exhibit off-target cytokine activity or gross toxicity. These results demonstrated that closely related compounds with only minor structural differences could be ligands that increased (phenylsulfonamides) or decreased (benzylsulfonamides) IL-17 production in human PBMCs.

The potent RORc inverse agonist **11** (RORc SRC 1 EC<sub>50</sub> = 3.1 nM, IL-17 PBMC EC<sub>50</sub> = 320 nM) was profiled for its selectivity in a panel of HEK293 cell Gal4-ROR construct human NR-dependent transcriptional reporter assays. The panel included the three isoforms of ROR (RORa, RORb, and RORc) and monitored for suppression of basal transcription activity in the absence of any exogenous agonist.<sup>24</sup> In addition, the following NRs were evaluated in both agonist mode (no exogenous agonist ligand added) and antagonist mode (exogenous agonist ligand added): farnesoid X receptor (FXR), liver X receptors (LXR)- $\alpha$  and LXR $\beta$ , and pregnane X receptor (PXR).<sup>24</sup> Compound **11** was a potent inverse agonist of RORc in the Gal4 reporter assay (EC<sub>50</sub> = 15 nM) and showed no detectable activity against the RORa isoform while it was 180-fold selective against RORb (EC<sub>50</sub> = 2.7 μM). The compound also displayed favorable selectivity against other NRs in the antagonist mode. For example, it was >400-fold selective over FXR (EC<sub>50</sub> = 6.1 μM) and >600-fold selective over LXR $\alpha$  (EC<sub>50</sub> = 9.2 μM). No activity was detected against LXR $\beta$  and PXR. Additionally, **11** did not exhibit any agonist activity in all the NRs profiled.

In conclusion, we have demonstrated that a minor structural change to our tertiary sulfonamide series led to opposite

mechanisms of action with RORc in both biochemical and primary human blood cell assays. We utilized X-ray cocrystal structural data to identify and characterize two distinct binding modes consistent with these biochemical and cellular MOAs. Optimized phenylsulfonamides were identified as RORc agonists while benzylsulfonamides exhibited potent inverse agonist activity. Our most potent inverse agonist showed >180-fold selectivity over all other RORc isoforms and other nuclear receptors that were profiled. Finally, compounds behaving as agonists in our biochemical assay also gave rise to an increased production of IL-17 in human PBMCs, while inverse agonists led to significant suppression of IL-17 under the same assay conditions. Thus, we were able to observe translation of biochemical MOA into the same MOA in human primary blood cells.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Cocrystal structure data and compound characterization. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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### Notes

The authors declare no competing financial interest.

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