Discovery of 3-Cyano-N-(3-(1-isobutyrylpiperidin-4-yl)-1-methyl-4-(trifluoromethyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)benzamide: A Potent, Selective, and Orally Bioavailable Retinoic Acid Receptor-Related Orphan Receptor C2 Inverse Agonist


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ABSTRACT: The nuclear hormone receptor retinoic acid receptor-related orphan receptor C2 (RORC2, also known as RORyt) is a promising target for the treatment of autoimmune diseases. A small molecule, inverse agonist of the receptor is anticipated to reduce production of IL-17, a key proinflammatory cytokine. Through a high-throughput screening approach, we identified a molecule displaying promising binding affinity for RORC2, inhibition of IL-17 production in Th17 cells, and selectivity against the related RORA and RORB receptor isoforms. Lead optimization to improve the potency and metabolic stability of this hit focused on two key design strategies, namely, iterative optimization driven by increasing lipophilic efficiency and structure-guided conformational restriction to achieve optimal ground state energetics and maximize receptor residence time. This approach successfully identified 3-cyano-N-(3-(1-isobutyrylpiperidin-4-yl)-1-methyl-4-(trifluoromethyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)benzamide as a potent and selective RORC2 inverse agonist, demonstrating good metabolic stability, oral bioavailability, and the ability to reduce IL-17 levels and skin inflammation in a preclinical in vivo animal model upon oral administration.

INTRODUCTION

Interleukin-17 (IL-17) is a proinflammatory cytokine that plays a dominant role in inflammation, autoimmunity, and host defense. IL-17 drives an amplification mechanism in inflammatory disease. At a site of inflammation, IL-17 stimulates resident cells to secrete chemokines and other proinflammatory mediators that recruit additional inflamma-
Methylindole analogue was possible by alkylation of the indole provided compound followed by acylation with cyclopentane carbonyl chloride and motor functions. In other members of the same nuclear receptor family, RORA and rheumatoid arthritis and multiple sclerosis. IL-17 is produced by a specific subset of T cells and other immune cells, of which T helper 17 (Th17) cells are believed to play a dominant role in disease pathogenesis. In these cells, the nuclear hormone receptor RORC2, a member of the retinoic acid receptor-related orphan receptor family and the immune nuclear hormone receptor RORC2, a member of the retinoic acid receptor family and the immune system, is required for the expression of IL-17 and the differentiation of Th17 cells from naive CD4+ T cells. Human subjects displaying a homozygous loss-of-function mutation in the RORC gene lack IL-17 producing cells in their peripheral blood, suggesting a critical role for RORC2 in IL-17 production. Although the endogenous ligand of RORC2 remains to be firmly established, evidence suggests that one or more intermediates along the cholesterol biosynthesis pathway may play this role. Two other members of the same nuclear receptor family, RORA and RORB, are believed to contribute to the regulation of circadian and motor functions.

In addition to the large molecule IL-17 (or IL-17R) monoclonal antibody approach to modulate the cytokine pathway, targeting RORC2 with a small molecule approach is also an attractive therapeutic strategy. An inverse agonist of the receptor is anticipated to attenuate production of IL-17 not only in the presence of agonist but also in the case where the receptor has a constitutive level of transcriptional activity. Indeed, a diverse range of chemotypes have been reported in the literature by industry and academia that display pharmacology consistent with inverse agonism of the receptor in vitro and in some cases in vivo. Most of the RORC2 ligands reported to date have been found to bind to a large, hydrophobic pocket within the ligand binding domain (LBD), presumably the same binding site of the endogenous ligand. The nature of this pocket to favor lipophilic ligands has presented a specific challenge toward the identification of pharmaceutical agents suitable for oral delivery as favorable ligands tend to have poor metabolic stability and modest oral bioavailability. In spite of the anticipated challenges in candidate optimization, we initiated a small molecule program targeting RORC2 based on its strong linkage to inflammatory diseases. Herein, we describe our approach to lead optimization of RORC2 inverse agonists originating from the identification of a high-throughput screening (HTS) hit. The combined approach of structure-guided ligand conformational restriction and lipophilic efficiency focused optimization has led to the identification of a potent, selective, and orally bioavailable inverse agonist of RORC2.

**CHEMISTRY**

The synthesis of 5-amino-3-piperidine substituted indoles has been previously described in the literature and intermediate 1 served as the precursor for the preparation of the initial HTS hit 3 and related analogues, Scheme 1. Differentiation of the two amine functionalities was realized through benzamide formation with the 5-amino substituent of the indole ring to afford compound 2. Subsequent piperidine N-deprotection followed by acylation with cyclopentane carbonyl chloride provided compound 3. Access to the corresponding 1-methylindole analogue was possible by alkylation of the indole nitrogen to provide compound 4, Scheme 1. However, to enable broader structure–activity relationship (SAR) studies, installation of the methyl group prior to amide formation was more advantageous, Scheme 2. 1-Methylindole 5 allowed for the sequential amide bond formation steps suitable for a parallel medicinal chemistry approach to yield analogues such as 7, 8a, and 8b. The representative 1-ethylindole analogue 11 was also prepared from intermediate 1 by alkylation of the indole nitrogen prior to piperidine amide formation, Scheme 3.

**Scheme 1. Synthesis of Screening Hit 3 and 1-Methylindole Analogue 4**

**Scheme 2. Representative Approach to 1-Methylindole Analogues, Including 7, 8a, and 8b**
Synthesis efforts next focused on the preparation of pyrrolopyridine and pyrrolopyrimidine scaffolds, Schemes 4 and 5. In these cases, installation of the piperidine substituent was made possible through a Suzuki cross-coupling reaction between vinyl boronate and the corresponding 3-iodopyrrolopyridine or 3-iodopyrrolopyrimidine scaffold intermediate. The pyrrolo[2,3-b]pyridine iodide was prepared from S-nitro-1H-pyrrolo[2,3-b]pyridine (13) by a one-pot N-methylation/iodination reaction to provide compound 14, Scheme 4. Subsequent cross-coupling with 12 afforded compound 15. Reduction of the olefin and nitro group concurrently under transfer hydrogenation conditions afforded amine 16, which was then coupled with the appropriate acid chlorides to provide analogues 17 and 18. Pyrrolo[3,2-b]- and pyrrolo[2,3-c]-pyridine scaffolds were provided through cross-coupling of 12 with 21a and 21b, respectively, Scheme 5. Each was prepared through iodination of either 19a or 19b followed by N-methylation of the iodinated heterocycle. The cross-coupling reaction occurred with advantageous deprotection of the formamide protecting group during workup to provide compounds 22a and 22b. Subsequent reduction of the olefin by transfer hydrogenation and amide formation afforded analogues 23 and 24. The required coupling partner 28 for the pyrrolo[3,2-d]pyrimidine scaffold arose from initial regioselective reductive dehalogenation of 2,4-dichloro-SH-pyrrolo[3,2-d]pyrimidine (25) followed by iodination and N-methylation, Scheme 5. The cross-coupling of iodo heterocycle 28 with boronate 12 provided compound 29. Installation of the 5-amino functionality was achieved through a catalytic amination reaction between 29 and benzophenone imine followed by transfer hydrogenation. The resulting amine was then reacted with 4-cyanopicolinyl chloride to provide analogue 31.

The synthesis of analogues bearing substitution at the 4-position of either the indole or pyrrolo[2,3-b]pyridine scaffold was undertaken as follows. The 4-methylindole analogue 36 was prepared through cross-coupling of 12 with 3-iodo-4-methylindole 33, which originated from 4-methyl-S-nitroindole (32) through a one-pot iodination/N-methylation, Scheme 6. Variation of the 4-position substituent to the pyrrolo[2,3-b]pyridine scaffold was challenging to introduce through parallel synthetic methods. Therefore, the corresponding substituted 3-iodo-pyrrolo[2,3-b]pyridines bearing either methyl, isopropyl, trifluoromethyl, or methoxy substitution each required unique synthetic approaches depending on the substituent, Schemes 7–10. The 4-methyl substituent was introduced through a Kumada coupling between 4-chloro-1H-pyrrolo[2,3-b]pyridine (37) and methylmagnesium bromide to provide precursor 38, Scheme 7. To achieve clean nitration at the 5-position of the ring, it was necessary to convert 38 to the corresponding benzenesulfonylamine derivative 39. In the absence of N-sulfonylation, competitive nitration at the C3-position led to complex mixtures of products. Subsequent nitration of 39 with tetramethylammonium nitrate selectively afforded 5-nitro-pyrrolo[2,3-b]pyridine 40. Removal of the phenylsulfonyl protecting group was accomplished by heating 40 with morpholine under basic conditions to afford compound 41. Subsequent N-methylation and C3-iodination provided the desired 4-methyl-3-iodopyrrolo[2,3-b]pyridine coupling partner 43. For the corresponding 4-isopropyl intermediate 49, a similar approach was adopted, Scheme 8. Suzuki cross-coupling with isopropenyl boronic acid pinacol ester proceeded cleanly with benzene-sulfonamide ester proceeding cleanly with benzene-sulfonamide 44 to afford the isoprenyl intermediate 45. Hydrogenation of the exocyclic olefin then provided the corresponding isopropylpyrrolo[2,3-b]pyridine 46. Following the same sequence of reactions (nitration, iodination and N-methylation) as with the methyl analogue, the isopropyl substituted coupling partner 49 was prepared. Preparation of 4-methoxy-3-iodopyrrolo[2,3-b]pyridine 53 relied on a different approach to introduce the 4-position substituent, Scheme 9. Compound 50, which was obtained by nitration of chloroheterocycle 44, was found to be sufficiently electron-deficient to effect the cross-coupling reaction with boronate 12.

Scheme 4. Synthesis of Pyrrolo[2,3-b]pyridine Analogues 17 and 18

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Reagents and conditions: (a) KOH, I2, DMF; K2CO3, CH3I; (b) PdCl2(PPh3)2, K2CO3, DME, EtOH, H2O, 120 °C; (c) NH4HCO3, Pd, THF:NMP (9:1), 150 °C; (d) pyridine, CH3Cl2; 3-cyanobenzoyl chloride or 4-cyanopicolinyl chloride.
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deficient to facilitate clean introduction of nucleophiles at the 4-position. Therefore, condensation of 50 with sodium methoxide afforded ether 51 which proceeded with concomitant desulfonylation. Subsequent N-methylation and iodination afforded coupling partner 53.

The desired 4-trifluoromethyl substituted intermediate for the coupling reaction was particularly challenging to prepare, Scheme 10. Starting from 4-chloro-1H-pyrrolo[2,3-b]pyridine (37), in situ protection of the N1 nitrogen atom as the acetyl derivative was required prior to a Finkelstein reaction, which yielded iodide 54. Next, N-phenylsulfonylation followed by ring nitration with trimethylammonium nitrate afforded the 4-iodo-5-nitropyrrolo[2,3-b]pyridine 56. Copper-mediated trifluoromethylation of 56 with methyl 2,2-difluoro-2-(fluorosulfonyl)acetate provided compound 57. Although it was found that the corresponding bromo- and chloro-analogues of 56 could facilitate introduction of the trifluoromethyl group, the iodide was found to be the most efficient. Furthermore, it was also found that the nitro and phenylsulfonyl groups were both critical to the success of the reaction, as all attempts to introduce a trifluoromethyl group onto substrates lacking either one of these functionalities failed to yield synthetically useful product. Compound 57 was then subjected to a one-pot iodination/desulfonylation and pyrrolopyridine N-methylation to afford coupling partner 58.

Iodides 43, 49, 53, and 58 were subjected to Suzuki cross-coupling conditions with N-Boc-tetrahydropyridine-4-boronic anhydride.
acid pinacol ester to provide compounds 59a—d, respectively, Scheme 11. Hydrogenation afforded piperidinyl intermediates 60a—d, which were then converted to the corresponding 3-cyanobenzamides 61a—d. Subsequent deprotection provided piperidine amines 62a—d. In the case of 4-methyl substituted intermediate 62a, a variety of amides 63a—I were prepared to explore the SAR of this substituent. On the other hand, only isobutyrate amides were prepared from 62b—d, resulting in analogues 64 (4-iPr), 65 (4-OMe), and 66 (4-CF3), respectively. As shown in Scheme 12, compound 59a was also transformed to amine 68 to allow for diversification of the benzamide substituent as in analogues 69a—d.

**RESULTS AND DISCUSSION**

**Screening Strategy.** A cell-based GAL4 luciferase reporter assay is uniquely suited as a screening approach to identify small molecule modulators of nuclear hormone receptors because the intrinsic transcriptional activity of the receptor is the functional readout. As opposed to a radioligand binding assay based on an endogenous ligand of the receptor, the reporter assay approach allows for the identification of pharmacologically diverse hits that are either competitive with the endogenous ligand or function by an allosteric binding site mechanism. A notable limitation of the reporter assay, however, is the requirement that the modulator is cell permeable. Nonetheless, as an initial screening strategy for a new target, an approach that quickly delivers a cell permeable hit can greatly facilitate validation of a chemotype or mechanism through functional activity in a disease-relevant pharmacological system.

Consequently, a subset of the Pfizer screening collection (ca. 150 000 compounds) was screened at 5 μM compound concentration using a GAL4-RORC2 luciferase reporter assay. HEK293 cells were transiently transfected with a mammalian expression vector containing GAL4-RORC2 LBD and a GAL4-responsive reporter gene containing firefly luciferase. An RORC2 inverse agonist would be expected to suppress the constitutively active, transcriptionally derived luminescence. Compounds demonstrating inhibition of greater than 50% were then counter-screened in a modified reporter assay, employing a GAL4-P65 expression vector instead of RORC2. Undesired activity under these assay conditions would suggest nonspecificity for RORC2, transcriptional inhibition through a general cytotoxicity mechanism, or compound specific interference with the luminescence readout. A dose—response curve for compounds demonstrating satisfactory differentiation was next acquired using comparable luciferase reporter assays in murine Neuro2A cells transfected by either GAL4-RORC2 LBD, GAL4-RORA LBD, or GAL4-RORB LBD to characterize isoform selectivity. As a result of this screening cascade, indole benzamide 3 was identified as a moderately potent RORC2 inverse agonist (IC50 = 2.7 μM), which lacked significant potency against RORA or RORB (IC50 > 30 μM), Table 1.

To further confirm that the functional changes in gene transcription observed with compound 3 resulted from specific ligand interactions with RORC2, we characterized compound 3 in a radioligand SPA binding assay and a time-resolved-fluorescence resonance energy transfer (TR-FRET) assay, Table 1. Compound 3 displaced [3H]-25-hydroxycholesterol, a putative in vitro agonist of RORC2,57 from purified human RORC2 LBD with a Ki of 1.2 μM, comparable to the functional response in the reporter assay. The competitive behavior with 25-hydroxycholesterol was the first suggestion that this new chemotype might bind within the endogenous ligand binding site of the receptor. The TR-FRET assay monitors the ability of the histidine-tagged RORC2-LBD to bind a biotinylated coactivator peptide (SRC1-2) by
fluorescence transfer mediated through association of a europium (Eu)-labeled anti-His antibody. An RORC2 inverse agonist that displaces the endogenous ligand would be expected to decrease the affinity of the coactivator peptide toward the RORC2 LBD and thus decrease the resulting fluorescence intensity. Indeed, compound 3 demonstrated potent suppression of SRC1-2 binding in the RORC2 TR-FRET assay (IC50 = 54 nM, 96% maximum suppression) while again not showing significant inverse agonism toward RORA or RORB (IC50 > 3 μM) when probed in a similar assay format. Most importantly, compound 3 inhibited the production of IL-17 in human primary Th17 cells (IC50 = 2.9 μM). Unfortunately, compound 3 demonstrated poor metabolic stability (HLM CL = 142 μL/min/mg), precluding its utility in in vivo experiments. Nonetheless, encouraged by the evidence that compound 3 could functionally inhibit production of IL-17 in a disease relevant human cell, demonstrate competent linkage of functional activity to binding of the RORC2 receptor, and display promising isoform selectivity, we undertook efforts to further optimize the chemotype for both potency and pharmacokinetic properties.

**Lead Optimization.** The topology of indole benzamide 3 provides three clear vectors for structure-activity exploration, namely the benzamide, the piperidine amide, and the indole nitrogen substituent. Diversification of the core heterocycle was also considered in our optimization strategy. Our first venture to explore the vector derived from the indole nitrogen was immediately productive. N-Methyl analogue 4 was found to be 16-fold more potent than compound 3 in the GAL4-RORC2 reporter assay (IC50 = 0.17 μM), Table 1. A similar significant improvement in potency was observed in the RORC2 TR-FRET assay (IC50 = 7 nM) and in binding affinity based on the SPA assay (Kd = 50 nM). High isoform selectivity was maintained as evident from no significant inverse agonism of RORA or RORB in either the TR-FRET or reporter assays (IC50 > 30 μM). Compound 4 was also a significantly more potent inhibitor of IL-17 production in Th17 cells (IC50 = 67 nM, 90% maximum inhibition). Although the lipophilicity of 4 is slightly higher than compound 3 (log D = 4.4 and 4.2, respectively), the addition of a single methyl resulted in a net positive improvement to lipophilic efficiency (ΔLIPE = 1.4).

From compound 4, exploration of potency and ADME space for the benzamide substituent and the piperidine amide was well-suited for application of parallel synthetic chemistry technology. Although this effort did not identify a compound with significantly improved properties, it did provide insight

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**Scheme 11.** Synthesis of 5-Substituted Pyrrolo[2,3-b]pyridine Analogue 63a−d

![Scheme 11](image)

“Reagents and conditions: (a) tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropryanine-1(2H)-carboxylate, K2CO3, Pd(PPh3)4, DME:EtOH:H2O, 80 °C; (b) (X = Me) triethylamine, Pd/C, H2, MeOH; (X = iPr) NH4HCO3, Pd/C, EtOH; (X = OCH3, CF3) Pd(OH)2, H2, MeOH; (c) 3-cyanobenzoyl chloride or 3-cyanobenzoic acid, various conditions; (d) CF3CO2H, CH2Cl2; or HCl, dioxane; (e) RCO2H or RC(O)Cl, various conditions.

**Scheme 12.** Synthesis of 5-Methylpyrrolo[2,3-b]pyridine Analogue 69a−d

![Scheme 12](image)

“Reagents and conditions: (a) HCl, dioxane, MeOH; (b) triethylamine, isobutyryl chloride, CH2Cl2; (c) triethylamine, Pd/C, H2, MeOH; (d) substituted 3-cyanobenzoic acid, DIPEA, HATU, CH2Cl2.
into the dominant interactions with the receptor and led to a compound suitable for cocrystallization with the receptor. The region of the receptor occupied by the piperidine amide appeared to preferentially tolerate large hydrophobic residues; however, this did not lead to favorable lipophilic efficiency improvements. On the other hand, the cyano group present in the benzamide substituent was found to play a dominant role in functional potency for the series. Compound 7, where the cyano was replaced by hydrogen, demonstrated a 10-fold reduction in RORC2 TR-FRET and IL-17 inhibition potency compared to lead compound 4, Table 1. Replacement of phenyl by pyridine as in 4-cyanopicolinamides 8a or 8b was one of the few modifications of the benzamide moiety that was tolerated. Although compound 8b neither demonstrated improved potency (IL-17 IC50 = 42 nM) nor metabolic stability (HLM CL = 173 μL/min/mg), it was a successful candidate for cocrystallization with the RORC2-LBD.

The recently reported crystal structure of apo-RORC2 receptor LBD38 and earlier structures of the receptor in complex with hydroxyl cholesters have revealed that the receptor has a large endogenous ligand binding pocket. Both of these structures display the receptor in an active conformation with helix-12 (H12) closely associated with the remainder of the receptor and forming a cleft allowing for coactivator peptide binding. A key structural element that stabilizes the positioning of H12 and enabling the active receptor conformation is a triplet residue latch consisting of His479-Tyr502-Phe506, Figure 1. The H12 residue Tyr502 forms a key hydrogen bond with His479 which resides on H11, thus bridging the two helices. In addition, Phe506 forms a favorable edge to face aromatic stacking arrangement with both His479 and Tyr502. This arrangement of residues allows the charged His479 residue to have a strongly favorable cation-π interaction and hold H12 in the active conformation.

The cocrystal structure of compound 8b with the RORC2 LBD confirmed that the ligand occupies the endogenous ligand binding pocket, Figure 2. Most notably from the structure, H12 is highly disordered along with partial unwinding of H11′. As a result, H12 no longer forms the coactivator binding cleft or the hydrogen bond between His479 and Tyr502. In place of the key latch interaction, His479 is now engaged in a hydrogen bond with the piperidine amide carbonyl of ligand 8b, Figure 2.

Table 1. Pharmacology Profile of Screening Hit 3 and 1-Alkylindole Analogues 4, 7, 8a, and 8b

| cmpd | R   | n  | K_i (μM) | RORC2 SPA | TR-FRET IC50 (μM) | luciferase IC50 (μM) | RORC2 RORA RORB | RORC2 RORA RORB | RORC2 RORA RORB | IL-17 IC50 (μM)
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All values are the mean of two or more independent assays. ND, not determined. Radioligand [3H]-25-hydroxycholesterol displacement assay. Inhibition of IL-17A production by human Th17 cells.

Figure 1. RORC2 active conformation His479-Tyr502-Phe506 triplet latch (3KYT). Figure 2. RORC2 LDB cocrystal structure with compound 8b (6CN5). Representation of ligand binding site with respect to receptor structure (H12 disordered and not shown).
3a. The cyclohexyl amide substituent occupies a hydrophobic pocket formed by H11 and the junction of H6 and H7. Compared to the active conformation of the receptor, this pocket significantly expanded in size due to a 110° torsional change in Trp317 with the vacancy only partially replaced by movement of Phe486. The rest of the binding site pocket is bordered by H3, H5, and H7 and a β-sheet underlying the indole core. The important indole N-methyl substituent was found to buttress against H7, making hydrophobic contacts with Ile400, Phe401, and Tyr369 within a small pocket, Figure 3b. Indeed, a detrimental impact on inhibition of coactivator binding and functional IL-17 production was encountered by only increasing the substituent size to ethyl as in compound 11 (IL-17 IC50 > 10 μM), Table 1. The indole ring itself is situated in a hydrophilic region of the receptor positioned between Met365 on top and Val376 from the β-sheet below, Figure 3c. The benzamide substituent occupies a noticeably more hydrophilic region of the pocket, making two key hydrogen bond contacts. First, the cyano group of the ligand makes a bifurcated hydrogen bond to Arg367 and the backbone NH of Leu287. The second contact is between the backbone carbonyl of Phe377 and the benzamide backbone amide NH of the ligand. To accommodate this hydrogen bond, the amide bond torsion (C4−NH of the ligand. To accommodate this hydrogen bond, the amide bond torsion (C4−NH of the ligand. To accommodate this hydrogen bond, the amide bond torsion (C4−NH of the ligand. To accommodate this hydrogen bond, the amide bond torsion (C4−N−C) must deviate from planarity with the indole by 113°.

An analysis of available crystallographic data for similar indole agonists through TR-FRET assay compared to the indole isostere 8a. Although compounds 17 and 23 were potent in the TR-FRET assay, they demonstrated a modest reduction in potency for suppression of IL-17 production in TH17 cells. The most interesting compound of the series was pyrrolo[2,3-b]pyridine 17. Although its cellular potency was diminished, it afforded a 0.5 unit reduction in lipophilicity and maintained comparable lipophilic efficiency compared to the indole isostere 8a. Unfortunately, this reduction in lipophilicity was not sufficient to improve metabolic stability (HLM CL = 144 μL/mg/min).

An alternative approach considered to alter the amide rotamer population was to substitute the indole ring adjacent to the amide substituent. For the two possible positions, the co crystal structure of 8b suggested that only substitution at the 4-position of the indole ring would be tolerated in the binding site.

### Table 2. Impact of Azaindole Heterocycles on Pharmacology and Lipophilicity

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*All values are the mean of two or more independent assays. ND, not determined. *TR-FRET cofactor recruitment. *Inhibition of IL-17A production by human Th17 cells. *Log D pH 7.4 measured by reverse phase HPLC (Elog D). *Calculated based on IC50 in IL-17 suppression assay and measured log D.
pocket. To test this hypothesis we pursued 4-methylindole 36,
Table 3. As shown in the calculated torsional energy profile
about the C4−C5−N−C dihedral angle of a representative
model system (Figure 4), substitution at the 4-position of the
indole ring with methyl significantly disfavors the proximal-
orientation of the carbonyl and methyl (θ = 0°). At the
dihedral angle of the observed bound conformation, the
calculation suggests that the unsubstituted analogue (R = H)
suffers a higher energetic penalty (3.4 kcal/mol) compared to
the methyl analogue (2.2 kcal/mol) with respect to the lowest
energy conformation (θ = 180° or 0°). This was indeed
reflected in the potency of 4-methylindole 36, which was 23-
fold more potent in the IL-17 suppression assay (IC50 = 3.3
nM) compared to the unsubstituted analogue 4 (IC50 = 75
nM), Table 3. A cocrystal structure of compound 36 bound to
the RORC2 LBD confirmed the same binding orientation in
the pocket that was observed with compound 8b, Figure 5. The
dihedral angle of the C−N bond (C4−C5−N−C) was
maintained at 113°, and the methyl substituent now occupies a
previously vacant cavity in the binding pocket bordered by
His323 and Phe378. A similar phenomenon was observed for
the pyrrolo[2,3-b]pyridine ring (Figure S1, see Supporting
Information) where the 4-methyl derivative 63h was 13-fold
more potent (IC50 = 21 nM) compared to the unsubstituted
analogue 18 (IC50 = 274 nM). In both cases, the addition of
the methyl substituent led to a reduction in lipophilicity,
Δlog D = −0.4 and −0.9 respectively for compound 36 and 63h.
As a result, through increased potency and reduced lipophilicity,
the combination of the pyrrolo[2,3-b]pyridine and the 4-
methyl substituent into compound 63h led to a significant
improvement in the lipophilic efficiency compared to
compound 4 (ΔLIPE = 2.1).

The methyl substituent at the 4-position of the indole ring
may also have a profound effect on the torsional dynamics
between the piperidine and indole rings. In cocrystal structures
of both 8b and 36, the piperidine ring adopts a dihedral angle

Table 3. Impact of C4-Substitution on Pharmacology and Lipophilicity

<table>
<thead>
<tr>
<th>compound</th>
<th>X</th>
<th>R</th>
<th>IC50 (nM)</th>
<th>dissociation T1/2 (min)</th>
<th>IL-17 IC50 (nM)</th>
<th>log D</th>
<th>LIPE</th>
</tr>
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<tbody>
<tr>
<td>4</td>
<td>CH</td>
<td>H</td>
<td>5.5</td>
<td>106</td>
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<td>4.4</td>
<td>2.7</td>
</tr>
<tr>
<td>36</td>
<td>CH</td>
<td>CH3</td>
<td>5.0</td>
<td>1309</td>
<td>3.3</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td>18</td>
<td>N</td>
<td>H</td>
<td>7.9</td>
<td>50</td>
<td>274</td>
<td>3.8</td>
<td>2.8</td>
</tr>
<tr>
<td>63h</td>
<td>N</td>
<td>CH3</td>
<td>12.8</td>
<td>631</td>
<td>21</td>
<td>2.9</td>
<td>4.8</td>
</tr>
</tbody>
</table>

a All values are the mean of two or more independent assays. ND, not determined. b TR-FRET cofactor recruitment. c Inhibition of IL-17A production by human Th17 cells. d Log D pH7.4 measured by reverse phase HPLC (Elog D). e Calculated based on IC50 in IL-17 suppression assay and measured log D.

Figure 4. Calculated torsional energy profile (B3LYP density functional, 6-31G* basis set) 41 for model systems representing rotation of (a) the 5-benzamide substituent (C4−C5−N−C dihedral) and (b) the 3-piperidine substituent (C2−C3−C−H dihedral) to the indole ring. R substituent is hydrogen (blue), methyl (green), trifluoromethyl (red), methoxy (orange), or isopropyl (brown). Dihedral angle observed in bound conformation represented by dashed line.

Figure 5. Overlay of bound conformation for compound 8b (orange) with cocrystal structure of RORC2 LBD with compound 36 (green with gray residues) (6CN6).
of 133° (C2−C3−C−H). The torsional energy profile of this dihedral was also calculated using a simplified model system, Figure 4. To adopt the observed bound conformation, the calculations suggest that the methyl analogue suffers a minor energetic penalty (0.4 kcal/mol) compared to the unsubstituted analogue. However, the methyl group imparts a significantly higher rotational barrier (9.8 kcal/mol) compared to the unsubstituted analogue (3.6 kcal/mol). Likewise, the methyl analogue displays a greater energetic preference (ΔΔE = 2.4 kcal/mol) for the bound conformation than the next higher local minimum (ΔE = 0°) as compared to the hydrogen analogue (ΔE = −0.3 kcal/mol). Again, a similar pattern was observed for the pyrrolo[2,3-b]pyridine ring (Figure S1, see Supporting Information).

It should be noted that a similar potency enhancement as observed for inhibition of IL-17 production was not observed in the recombinant RORC2 TR-FRET assay for compounds 63e, 63f, and 63g, Table 3. To further understand this apparent disconnect, we explored the dissociation kinetics of our inverse antagonists that possess high biochemical efficiency (BE) as the capacity to induce a nonequilibrium system such as having a residence time on the target longer than the degradation rate of the receptor. The effective pseudoirreversible behavior of these compounds contributes to their high efficiency in the cellular assay. A subsequent screen of a range of inverse agonists demonstrated a strong correlation of effective dissociation half-life with IL-17 inhibitory potency (Figure S3, see Supporting Information). As a consequence, the measurement of IL-17 inhibition in Th17 cells was viewed as the most appropriate assay to rank order compound potency given the presence of the endogenous ligand in physiological concentrations and the long assay window (6 days) to achieve equilibrium.

Unfortunately, even though a significant reduction in lipophilicity had been achieved with compound 63h, metabolic clearance remained high (HLM CL = 125 µL/min/mg). To further address metabolic stability, we revisited optimization of the piperidine amide substituent in light of the advancements further address metabolic stability, we revisited optimization of the piperidine amide substituent in light of the advancements.

**Table 4. Impact of Piperidine Amide Substituent on Pharmacology and Metabolic Stability**

<table>
<thead>
<tr>
<th>compound</th>
<th>R</th>
<th>IC50 (nM)</th>
<th>RORC2</th>
<th>IL-17</th>
<th>HLM CL (µL/min/mg)</th>
<th>log DppH7.4</th>
<th>LIPH</th>
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<tbody>
<tr>
<td>63a</td>
<td>Me</td>
<td>277</td>
<td>5270</td>
<td>&lt;8</td>
<td>1.6</td>
<td>3.7</td>
<td></td>
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<tr>
<td>63b</td>
<td>Et</td>
<td>67</td>
<td>625</td>
<td>&lt;8</td>
<td>2.0</td>
<td>4.2</td>
<td></td>
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<tr>
<td>63c</td>
<td>cyclopropyl</td>
<td>16</td>
<td>242</td>
<td>&lt;8</td>
<td>2.3</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>63d</td>
<td>iPr</td>
<td>20</td>
<td>69</td>
<td>8</td>
<td>2.0</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>63e</td>
<td>cyclopropylmethyl</td>
<td>13</td>
<td>97</td>
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</tr>
<tr>
<td>63g</td>
<td>cyclobutyl</td>
<td>11</td>
<td>56</td>
<td>39</td>
<td>2.6</td>
<td>4.7</td>
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</tr>
<tr>
<td>63h</td>
<td>cyclopentyl</td>
<td>13</td>
<td>21</td>
<td>125</td>
<td>2.9</td>
<td>4.8</td>
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<tr>
<td>63i</td>
<td>cyclohexyl</td>
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<td>236</td>
<td>3.9</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>63j</td>
<td>2-fluoro-6-toluenyl</td>
<td>18</td>
<td>14</td>
<td>90</td>
<td>3.1</td>
<td>4.7</td>
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</tr>
<tr>
<td>63k</td>
<td>tetrahydrofuran-3-yl</td>
<td>74</td>
<td>3800</td>
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<td>3,5-dimethylisoxazol-4-yl</td>
<td>49</td>
<td>1310</td>
<td>&lt;8</td>
<td>2.0</td>
<td>3.9</td>
<td></td>
</tr>
</tbody>
</table>

“All values are the mean of two or more independent assays. ND, not determined. TR-FRET cofactor recruitment. Inhibition of IL-17A production by human Th17 cells. Log DpH7.4 measured by reverse phase HPLC (Elog D). Calculated based on IC50 in IL-17 suppression assay and measured log D.
substituent in 63a–j. Overall, the isopropyl analogue 63d demonstrated the best combination of potency, metabolic stability, and lipophilic efficiency (IC_{50} = 69 nM, HLM CL = 8 μL/min/mg).

Now with a better understanding of the chemical space required to achieve good metabolic stability, we pursued further optimization of potency. First, we considered additional substitution to the cyanobenzamide. Based on the cocrystal structures of 8b and 36, the 4-position of the phenyl ring appeared to provide the greatest opportunity for additional hydrophobic or hydrogen bond contacts, Table 5. For the analogues prepared (69a–d), all of them showed good metabolic stability except for the 4-chloro analogue 69c, presumably due to the significant increase in lipophilicity. Unfortunately, only the 4-methoxy analogue 69a was more potent, although only modestly (2-fold), for inhibition of IL-17

Finally, we investigated the scope of the pyrrolo[2,3-b]pyridine 4-position substituent beyond the initially identified methyl. We surveyed examples in which the substituent was sterically larger or had a different electronic character compared to methyl (64–66), Table 6. Replacing the methyl by the sterically larger isopropyl group (64) was predicted through torsional energy calculations to yield the smallest energetic penalty for the anticipated bound state, Figure 4. Nonetheless, compound 64 imparted comparable potency and metabolic stability as compound 63d, possibly due to counterbalancing steric interactions with His323 and Phe378. Compound 65 bearing an electron donating methoxy group, however, demonstrated reduced potency and higher metabolic clearance. This would be anticipated from the torsional energy profile with methoxy yielding the largest energetic penalty presumably due to a favorable intramolecular interaction between the amide hydrogen and the oxygen lone pair when the C4–C5–N–C dihedral angle is 180°. On the other hand, an electron withdrawing trifluoromethyl substituent (66) provided improved potency compared to methyl. Compound 66 achieved optimal inhibition of IL-17 production in Th17 cells (IC_{50} = 9.5 nM, 90% maximum inhibition) while affording excellent metabolic stability. Favorable metabolic stability was observed for compound 66 in spite of a significant increase in lipophilicity induced by the trifluoromethyl group (Δlog D = 1.7) which is consistent with the trend observed in reported matched molecular pair analyses. Several factors likely contribute to the enhanced potency of the trifluoromethyl analogue 66. Calculations suggest the trifluoromethyl group may further skew the C4 population toward the bound conformation through an unfavorable dipole interaction with the amide carbonyl as well as favor the bound conformation of the piperidine ring, Figure 4. Second, the electron withdrawing nature of the trifluoromethyl group now reduces the polarization of the pyrrolopyridine nitrogen, which would be better accommodated in the hydrophobic binding pocket. This is supported by the reduced negative character for the calculated electrostatic potential (ESP) of the ring nitrogen compared to methyl analogue 63d, Table 6. Lastly, the lipophilicity of compound 66 is now of the same magnitude of earlier leads with single digit nanomolar potency (e.g., 36, log D = 4.0), and therefore, the hydrophobic nature of the binding pocket may be a strong driver of potency.

### Table 5. Impact of Phenyl Amide Substitution on Pharmacology and Metabolic Stability

<table>
<thead>
<tr>
<th>compound</th>
<th>R</th>
<th>IC_{50} (nM)</th>
<th>HLM CL (μL/min/mg)</th>
<th>log D°</th>
</tr>
</thead>
<tbody>
<tr>
<td>63d</td>
<td>H</td>
<td>20</td>
<td>69</td>
<td>8</td>
</tr>
<tr>
<td>69a</td>
<td>OCH₃</td>
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<td>&lt;8</td>
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<td>69b</td>
<td>F</td>
<td>28</td>
<td>57</td>
<td>&lt;8</td>
</tr>
<tr>
<td>69c</td>
<td>Cl</td>
<td>7.6</td>
<td>61</td>
<td>20</td>
</tr>
<tr>
<td>69d</td>
<td>CH₃</td>
<td>15</td>
<td>79</td>
<td>11</td>
</tr>
</tbody>
</table>

*All values are the mean of two or more independent assays. ND, not determined. \(^{ab}\)TR-FRET cofactor recruitment. \(^{ac}\)Inhibition of IL-17A production by human Th17 cells. \(^{d}\)Log D pH7.4 measured by reverse phase HPLC (Elog D).*

### Table 6. Electronic and Steric Influence of the C4-Substituent on Pharmacology and Metabolic Stability

<table>
<thead>
<tr>
<th>compound</th>
<th>R</th>
<th>ESP-N7°</th>
<th>IC_{50} (nM)</th>
<th>HLM CL (μL/min/mg)</th>
<th>log D°</th>
</tr>
</thead>
<tbody>
<tr>
<td>63d</td>
<td>CH₃</td>
<td>–32.7</td>
<td>20</td>
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<td>8</td>
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<tr>
<td>64</td>
<td>iPr</td>
<td>–32.9</td>
<td>37</td>
<td>63</td>
<td>9</td>
</tr>
<tr>
<td>65</td>
<td>OCH₃</td>
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<td>381</td>
<td>26</td>
</tr>
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<td>66</td>
<td>CF₃</td>
<td>–25.6</td>
<td>4.1</td>
<td>9.5</td>
<td>&lt;8</td>
</tr>
</tbody>
</table>

*All values are the mean of two or more independent assays. ND, not determined. \(^{ab}\)Electrostatic potential calculated using Jaguar, Version 8.1, Schrodinger, LLC, New York, NY, 2013 (B3LYP/6-31G**). \(^{ac}\)TR-FRET cofactor recruitment. \(^{d}\)Inhibition of IL-17A production by human Th17 cells. \(^{c}\)Log D pH7.4 measured by reverse phase HPLC (Elog D).*
Figure 6. Effect of compound 66 (n = 3 replicates) against 48 nuclear receptors in HepG2 cells at 1 μM concentration and 24 h time point. HepG2 cells were transiently transfected with optimized trans-FACTORIAL library. Post-transfection (24 h), cells were washed and supplied with fresh low serum (1% FBS, charcoal stripped) culture medium and treated with inducer for 24 h. Profile of the trans-FACTORIAL activities was determined as fold of induction values versus vehicle-treated (DMSO) control cells. Graph shows average fold-induction data plotted in logarithmic scale.

Encouraged by the initial in vitro profile of compound 66, we carried out additional pharmacological characterization. The very slow dissociation rate from the RORC2 recombinant receptor seen previously with 4-substituted derivatives was also evident (T1/2 = 6 h) in compound 66. To demonstrate that compound effect was at the level of gene transcription, we showed that compound 66 inhibited mRNA production of IL-17A and other genes that have previously been described as being controlled by RORC2 such as IL-17F, IL-22, IL-26, and IL-23R (Table S1, see Supporting Information). As expected, RORC expression was not inhibited by compound 66. In addition, Th1 and Th2 lymphocyte differentiation as well as cellular viability of lymphocytes were not affected by compound 66 at the highest concentration tested (10 μM) suggesting specificity of the observed effects. High isoform selectivity was demonstrated with no significant inverse agonism toward RORA or RORB in the TR-FRET assay (IC50 > 25 μM). To more broadly explore the receptor selectivity of compound 66, we profiled the compound using the trans-FACTORIAL platform (Attagene Inc.), Figure 6. This multiplexed technology allows for the measurement of reporter RNA levels upon transfection of ligand binding domain chimeric constructs from 48 nuclear receptors with GAL4 DNA. Of the 48 receptors, only RORC2 showed a fold reduction (agonist effect) of greater than 20% of control at the same dose of 1 μM compound. Only the estrogen-related receptor alpha (ERRα) (1.28-fold, p = 0.03) and the liver X receptor alpha (LXRα) (1.30-fold, p = 0.165) showed a modest fold increase (agonist effect) greater than 20% of control at the same dose. These results clearly demonstrate the high selectivity of compound 66 for RORC2 compared to all other nuclear receptors.

In addition to low in vitro human microsomal and hepatocyte clearance, compound 66 also demonstrated high passive permeability (Papp = 13.2 × 10−6 cm/sec, RRCK cell monolayer). Our primary concern with regard to predicted human pharmacokinetics was the relatively low thermodynamic solubility of compound 66 (1.0 μM, pH 6.5). In a rat pharmacokinetic study, compound 66 demonstrated low in vivo clearance (CL = 3.4 mL/min/kg) and a moderate half-life (6 h) consistent with the low human (8 μL/min/mL) and rat (<14 μL/min/mL) microsomal clearance, Table 7. Clearance was also very low in dog and mouse (0.46 and 1.2 mL/min/kg, respectively) with the elimination half-life in the dog being exceptionally long (39 h). Upon oral dosing of crystalline solid (3 mg/kg) in rats, oral bioavailability was moderate (21%), consistent with a highly permeable but low solubility compound. Unfortunately, due to the decreased basicity of the pyrrolopyridine nitrogen, salt formation was not a viable approach for solubility enhancement. In hopes of improving the compound dissolution rate and ultimately the oral bioavailability, we formulated the compound as a spray-dried dispersion (SDD) (25%, HPMCAS-H polymer). This approach was highly effective resulting in an increased oral bioavailability in rat of 83%. Oral bioavailability in dog remained high with the SDD formulation (73%, 3 mg/kg). Oral bioavailability in mouse was also acceptable at higher doses using the SDD formulation (30%, 30 mg/kg). Overall, compound 66 demonstrated excellent in vivo pharmacokinetics consistent with in vitro parameters, and the SDD formulation approach was very effective in overcoming the low aqueous solubility of the molecule.

Compound 66 also showed potent inhibition of IL-17 production in mouse derived Th17 cells (IC50 = 32 nM, 92% maximum inhibition) which supported assessment of in vivo efficacy in the mouse imiquimod-induced skin inflammation model. Topical administration of imiquimod can induce a psoriasis-like skin inflammation resulting in epidermal thickening. This clinical outcome is at least in part mechanistically dependent on the IL-23/IL-17 pathway. Mice were challenged with topical imiquimod cream (5%) applied to their back and ear for 3 days (days 1–5). Compound 66 was dosed orally at three dose levels (10, 30, and 100 mg/kg) once a day over 5 days (days 1–5). Compound administration was well tolerated without any compound-related adverse events.

Table 7. Pharmacokinetic Profile of 66 in Rat, Mouse, and Dog

<table>
<thead>
<tr>
<th>Species</th>
<th>CL (mL/min/kg)</th>
<th>T1/2 (h)</th>
<th>Vss (L/kg)</th>
<th>F (%)</th>
<th>Fdilumina</th>
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<tr>
<td>rat</td>
<td>3.4</td>
<td>6</td>
<td>1.4</td>
<td>83</td>
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<td>dog</td>
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<td>39</td>
<td>1.5</td>
<td>73</td>
<td>0.065</td>
</tr>
<tr>
<td>mouse</td>
<td>1.2</td>
<td>10</td>
<td>0.8</td>
<td>30</td>
<td>0.021</td>
</tr>
</tbody>
</table>

For formulation conditions, see Experimental Section. Dosed IV at 1 mg/kg (n = 2) and PO at 3 mg/kg (n = 2). Dosed IV at 1 mg/kg (n = 3) and PO at 30 mg/kg (n = 3). Oral bioavailability following dosing of compound 66 as a 25% spray-dried dispersion or (crystalline solid). Calculated based on AUC0−10426.
Compound plasma AUC and \(C_{\text{max}}\) increased with dose although not proportionally, Figure 7. An anti-IL-17 monoclonal antibody and an anti-p40 (IL-23 and IL-12 subunit) antibody were administered by intraperitoneal injection as controls. At the conclusion of the dosing, ear thickness was measured and IL-17A protein levels in the ear were assessed, Figure 8. Compound 66 demonstrated a dose dependent inhibition of ear swelling in the model. A maximum inhibition of 46% (\(p < 0.0001\)) was achieved at a dose of 100 mg/kg which was comparable to the reduction observed with the anti-IL-17A Ab (45%, \(p = 0.0005\)) compared to the isotype control. The degree of inhibition correlated with the time period in which compound concentration exceeded the IC\(_{80}\) throughout the dosing interval, Figure 7. Consistent with the clinical outcome and the role RORC2 plays, a significant reduction in the level of IL-17A protein in the ear was also observed at all doses of compound 66 compared to the vehicle control, Figure 8. Similar reductions of IL-17A protein in the ear were seen at both the 30 and 100 mg/kg doses (70 and 72%, respectively) (\(p < 0.0001\)).

**CONCLUSION**

Through a combination of de novo and structure-guided design, we optimized a novel high-throughput screening hit against RORC2 with modest potency and poor metabolic stability into a highly potent and orally bioavailable lead. To align the two properties of potency and metabolic stability into a single molecule, it was important to exploit two key design strategies, namely, iterative optimization through lipophilic efficiency and conformational restriction to achieve optimal ground state energetics and maximize receptor residence time. The introduction of two “magic methyl” substituents\(^48,49\) profoundly impacted the potency characteristics of the chemotype. The first example was transformation of compound 3 to 1-methylindole 4 resulting in a 43-fold improvement in potency. As revealed by the subsequent cocrystal structure of compound 8b, this methyl group occupies a favorable hydrophobic pocket in the receptor, yielding a beneficial lipophilic efficiency (\(\Delta LIPE = 1.4\)). The second example was the transformation of compound 4 to 4-methylindole 36, resulting in an additional 20-fold potency improvement and further lipophilic efficiency improvement (\(\Delta LIPE = 1.7\)). In this case, the methyl substituent is believed to restrict the conformational dynamics of the ligand, leading to a lower solution to bound state energetic penalty as well as imparting a profound resonance time enhancement to the ligand in the binding pocket. Although largely neutral toward lipophilic efficiency, the next two prominent changes to the molecule provided an important lowering of the intrinsic lipophilicity. These changes included the introduction of the pyrrolo[2,3-b]pyridine ring system (63h, \(\Delta \log \delta D = -1.1\)) and replacement of the cyclopentylamide by isopropylamide (63d, \(\Delta \log \delta D = -0.9\)). Effectively, this series of transformations achieved a comparable level of potency to the early lead compound 4, but in a substantially lower lipophilic space (\(\Delta \log \delta D = -2.4\)) and now with excellent metabolic stability. The reduction in lipophilicity was crucial to allow for the final potency enhancement through introduction of the metabolically stable but lipophilic 4-trifluoromethyl group found in compound 66. Indeed, compound 66 was found to be a potent and selective RORC2 inverse agonist with excellent pharmacokinetic properties in preclinical species. The ability of compound 66 to reduce levels of IL-17A in vivo after oral dosing in mice, and corresponding reduction in skin inflammation further supports the potential of small molecule RORC2 modulation as a therapeutic target for the treatment of inflammatory diseases.

**EXPERIMENTAL SECTION**

**Chemistry.** All reagents and solvents were used as purchased without further purification. The purity of the final compounds was characterized by high-performance liquid chromatography (HPLC) using a gradient elution program (e.g., C18, acetonitrile-water, 0.1% formic acid, 5:95–95:5) and UV-detection (220 nm). The purity of all final compounds was 95% or greater. Proton (\(^1H\)) NMR chemical shifts are referenced to a residual solvent peak.

tert-Butyl 4-[(3-Cyanobenzamido)-1H-indol-3-yl)piperidine-1-carboxylate (2). DIPEA (0.45 mL, 2.6 mmol) was added to a solution of 3-cyanobenzoic acid (0.367 g, 2.5 mmol) and TBTU (0.881 g, 2.6 mmol) in DMF (10 mL). After stirring at room temperature for 30 min, a solution of tert-butyl 4-(5-amino-1H-indol-3-yl)piperidine-1-carboxylate \(^1\) (1, 0.787 g, 2.5 mmol) in DMF (5 mL) was added, and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (MeOH/CH\(_2\)Cl\(_2\), 2/98) to afford 0.8 g (72%) of 2. \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta 9.45\) (brs, 1H), 9.10 (brs, 1H), 8.25 (s, 1H), 8.17 (d, \(J = 8.2\ Hz\), 1H), 7.95 (s, 1H), 7.65 (d, \(J = 8.0\ Hz\), 1H), 7.45 (t, \(J = 7.8\ Hz\), 1H), 7.28 (d, \(J = 8.2\ Hz\), 1H), 7.20 (d, \(J = 8.2\ Hz\), 1H), 6.80 (s, 1H), 4.20–4.05 (m, 2H), 2.80–2.60 (m, 3H), 1.95–1.80 (m, 2H), 1.55–1.28 (s, 11H).

![Figure 7](image-url) Unbound plasma concentrations of compound 66 following last dosing in the mouse imiquimod-induced skin inflammation model. Compound dosed at 10 mg/kg (blue), 30 mg/kg (green), and 100 mg/kg (red) once a day by oral gavage. For reference, the respective mouse IL-17 IC\(_{50}\) and IC\(_{80}\) (\(4 \times 100\) mg/kg) once a day by oral gavage. For reference, the respective mouse IL-17 IC\(_{50}\) and IC\(_{80}\) (\(4 \times 100\) mg/kg) adjusted for fraction unbound in the in vitro assay media (\(F_u = 0.62\)) are depicted (IC\(_{50}\) = 20 nM, IC\(_{80}\) = 80 nM).

![Figure 8](image-url) End of study magnitude of ear swelling (\(\Delta \mu m\)) (a) and protein levels of IL-17A (pg/mL) (b) in the ear following mouse imiquimod-induced skin inflammation model treated with compound 66, anti-IL-17 Ab (20 mg/kg), or anti-p40 Ab (20 mg/kg) (mean \(\pm 95\%\) CI).
3-Cyano-N-(3-(1-(cyclopentanecarbonyl)piperidin-4-yl)-1H-indol-5-yl)benzamide (3). A solution of 4 N HCl in dioxane (0.9 mL) was added to a solution of 2 (201 mg, 0.45 mmol) in methanol (2 mL). The mixture was stirred at room temperature for 2.5 h and then concentrated under reduced pressure at 30 °C. The residue was suspended in toluene and concentrated. The resulting crude amine was dissolved in DMF (1 mL), and DIPEA (0.37 mL, 2.24 mmol) was added. The solution was stirred at room temperature for 30 min, and then a solution of cyclohexane-1,2-dicarboxylate (86 mg, 0.65 mmol) in dichloroethane (1.5 mL) was added. The mixture was stirred at room temperature overnight and then quenched with saturated aqueous NaHCO3 solution. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried (Na2SO4), filtered and then concentrated. The residue was purified by silica gel column chromatography (MeOH/CH2Cl2, 0/100–10/90). The crude product was dissolved in EtOAc, washed with water, and concentrated to afford 180 mg (91%) of 3 as a tan solid. MS (ES+) m/z 441 (M+H).

1H NMR (300 MHz, CDCl3) δ 7.98 (s, 1H), 7.96–7.82 (m, 2H), 7.64–7.44 (m, 2H), 7.37–7.26 (m, 4H), 6.83 (s, 1H), 4.80 (d, δ = 13.3 Hz, 1H), 4.09 (d, δ = 13.7 Hz, 1H), 3.76 (s, 2H), 3.21 (t, δ = 12.1 Hz, 1H), 3.11 (t, δ = 12.1 Hz, 1H), 2.95 (q, δ = 8.1 Hz, 1H), 2.75 (t, δ = 13.1 Hz, 1H), 2.27–1.98 (m, 2H), 1.92–1.45 (m, 9H), 1.28 (br. s., 1H), 0.89 (t, δ = 6.6 Hz, 1H).

General Method for the Synthesis of 8a and b. A solution of 4 M HCl in dioxane (3 mL) was added to a cold (0 °C) solution of 6b (3.7 g, 8.04 mmol) in methanol (15 mL). The mixture was allowed to warm to room temperature and stir overnight. The reaction mixture was then concentrated, and the residue was purified by silica gel column chromatography (heptane/EtOAc, 100/0–10/90) to afford 39% (78%) of a as a white solid. MS (ES+) m/z 430 (M+H).

1H NMR (400 MHz, CDCl3) δ 8.08 (s, 1H), 7.96–7.82 (m, 2H), 7.64–7.44 (m, 2H), 7.37–7.26 (m, 4H), 6.83 (s, 1H), 4.80 (d, δ = 13.3 Hz, 1H), 4.09 (d, δ = 13.7 Hz, 1H), 3.76 (s, 2H), 3.21 (t, δ = 12.1 Hz, 1H), 3.11 (t, δ = 12.1 Hz, 1H), 2.95 (q, δ = 8.1 Hz, 1H), 2.75 (t, δ = 13.1 Hz, 1H), 2.27–1.98 (m, 2H), 1.92–1.45 (m, 9H), 1.28 (br. s., 1H), 0.89 (t, δ = 6.6 Hz, 1H).

4-Cyano-N-(3-(1-(cyclopentanecarbonyl)piperidin-4-yl)-1H-indol-5-yl)benzamide (8a). Cyclohexancarboxylic acid; 40 mg (4%), yellow solid. MS (ES+) m/z 456 (M+H).

1H NMR (400 MHz, DMSO-d6) δ 10.65 (s, 1H), 9.01 (d, δ = 4.8 Hz, 1H), 8.51 (s, 1H), 8.23 (d, δ = 1.6 Hz, 1H), 8.18 (dd, δ = 4.8, 1.2 Hz, 1H), 7.65 (dd, δ = 8.8, 1.6 Hz, 1H), 7.70 (d, δ = 8.8 Hz, 1H), 7.15 (s, 1H), 6.40–4.52 (m, 1H), 4.18–4.09 (m, 1H), 3.75 (s, 3H), 3.28–3.16 (m, 1H), 3.10–2.98 (m, 1H), 2.78–2.68 (m, 2H), 2.10–1.95 (m, 2H), 1.76–1.40 (m, 4H).

4-Cyano-N-(3-(1-(cyclopentanecarbonyl)piperidin-4-yl)-1H-indol-5-yl)picolinamide (8b). Cyclohexancarboxylic acid, 40 mg (8%), yellow solid. MS (ES+) m/z 470 (M+H).

1H NMR (400 MHz, DMSO-d6) δ 10.62 (s, 1H), 8.99 (d, δ = 4.8 Hz, 1H), 8.49 (s, 1H), 8.21 (d, δ = 1.6 Hz, 1H), 8.16 (dd, δ = 4.8, 1.6 Hz, 1H), 7.63 (d, δ = 8.8, 1.6 Hz, 1H), 7.38 (d, δ = 8.8 Hz, 1H), 7.14 (s, 1H), 5.77 (s, 1H), 4.58–4.52 (m, 1H), 4.10–4.04 (m, 1H), 3.78 (s, 3H), 3.28–3.16 (m, 1H), 3.06–2.95 (m, 1H), 2.72–2.58 (m, 2H), 2.08–1.95 (m, 2H), 1.76–1.40 (m, 12H).

Cyclopentyl 4-(5-(cyanoethyl)carboxamidino)-1H-indol-5-yl)piperidine-1-carboxylate (9). Triethylamine (14.6 mL, 105 mmol) was added to a suspension of tert-butyl 4-(5-amino-1H-indol-3-yl)piperidine-1-carboxylate (3.01 mL, 205 mmol), and then the mixture was cooled to 0 °C. A solution of 4-cynopicolinol chloride (4.49 g, 26.9 mmol) in CH2Cl2 (60 mL) was slowly added to the mixture. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 20 h. Saturated aqueous sodium chloride and the mixture was extracted with CH2Cl2 and EtOAc. The combined organic layers were concentrated. The residue was triturated with ethanol (150 mL) and filtered. The resulting solid was washed with ethanol (50 mL) and dissolved in a mixture of acetone and CH2Cl2. The solution was filtered and concentrated to afford 12.16 g (95%) of 9 as a yellow solid. 1H NMR (400 MHz, DMSO-d6) δ 10.82 (s, 1H), 10.55 (s, 1H), 8.95 (d, δ = 5.2 Hz, 1H), 8.45 (s, 1H), 8.15–8.10 (m, 1H).
2H), 7.54 (dd, J = 9.1, 1.0 Hz, 1H), 7.3 (d, J = 9.1 Hz, 1H), 7.12 (d, J = 1.0 Hz, 1H), 4.10−4.00 (m, 2H), 2.95−2.85 (m, 3H), 1.90−1.80 (m, 2H), 1.60−1.46 (m, 2H), 1.40 (s, 9H).

tert-Butyl 4-(4-Cyanopicolinamido)-1-ethyl-1H-indol-3-yl-piperidine-1-carboxylate (10). A solution of sodium hydroxide (2.5 M, 6.28 mL, 15.7 mmol), ethyl iodide (1.08 mL, 13.5 mmol), and a catalytic amount of Alq3 336 was added to a solution of 9 (200 mg, 0.45 mmol) in CH2Cl2 (6 mL). The reaction mixture was stirred at room temperature for 48 h and then partitioned with water. The aqueous layer was extracted with CH2Cl2. The combined organic layers were concentrated and the residue was purified by preparative HPLC to afford 110 mg (52%) of a yellow solid. 1H NMR (400 MHz, CDCl3) δ 8.98 (s, 1H), 8.82 (d, J = 4.5 Hz, 1H), 8.54 (s, 1H), 8.13 (s, 1H), 7.70 (dd, J = 4.8, 1.0 Hz, 1H), 7.46 (dd, J = 7.2, 1.0 Hz, 1H), 7.31 (d, J = 7.2 Hz, 1H), 6.91 (s, 1H), 4.30−4.15 (m, 2H), 4.12 (q, J = 5.9 Hz, 2H), 3.00 (t, J = 10.5 Hz, 1H), 2.95−2.85 (m, 2H), 2.08−2.01 (m, 2H), 1.70−1.60 (m, 2H), 1.49 (s, 9H), 1.45 (t, J = 5.4 Hz, 3H).

4-Cyano-N-(3-(1-cyclopentanecarbonylpiperidin-4-yl)-1-ethyl-1H-indol-5-yl)picolinamide (11). A solution of 4 M HCl in dioxane (0.9 mL) was added to 10 (110 mg, 0.23 mmol) and the mixture was stirred at room temperature for 12 h. The reaction mixture was partitioned between saturated aqueous NaHCO3 and CH2Cl2, and the organic layer was concentrated. Triethylamine (32 μL, 0.23 mmol) and cyclopentanecarbonyl chloride (28 μL, 0.23 mmol) were added to a solution of the residue in CH2Cl2 (5 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was partitioned between 1 M HCl (20 mL) and CH2Cl2 (50 mL). The organic layer was concentrated, and the residue was purified by preparative HPLC to afford 60 mg (64%) of 11 as a white solid. MS (ES+) m/z 470 (M+H). 1H NMR (400 MHz, DMSO-d6) δ 10.60 (s, 1H), 9.00 (d, J = 4.7 Hz, 1H), 8.50 (s, 1H), 8.20 (d, J = 1.6 Hz, 1H), 8.16 (dd, J = 1.4, 4.9 Hz, 1H), 7.62 (dd, J = 1.6, 9.0 Hz, 1H), 7.43 (d, J = 8.6 Hz, 1H), 7.21 (s, 1H), 4.57 (d, J = 12.5 Hz, 1H), 4.19−4.08 (m, 3H), 3.22 (t, J = 12.5 Hz, 1H), 3.09−3.00 (m, 2H), 2.73 (t, J = 12.3 Hz, 1H), 2.09−1.97 (m, 2H), 1.86−1.44 (m, 10H), 1.36 (t, J = 7.2 Hz, 3H).

Cyclopentyl(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridin-1(2H)-yilmethanone (12). Trifluoroacetic acid (472 g, 4.14 mol) was added dropwise to a cold (0 °C) solution of tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-y-carboxylate (160 g, 0.17 mmol) in CH2Cl2 (640 mL). The mixture was allowed warm to 20 °C and was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and then azeotroped with CH2Cl2 (4 × 200 mL). The residue was divided into two equal portions and each dissolved into CHCl3 (1.5 L). Triethylamine (209.6 mL) and then cyclopentanecarbonyl chloride (99.7 g, 751.8 mmol) were added dropwise into the reaction mixture while keeping the internal temperature at 0−5 °C. The mixture was allowed warm to 20 °C and was stirred overnight. The reaction mixture was washed with aqueous NaHCO3 (2 × 750 mL). The organic layer was washed with brine, dried (Na2SO4) and concentrated. The crude product was purified by silica gel column chromatography (PE/EtOAc, 50/1−1/1) to afford 107.98 g (68%) of 12 as a yellow oil. 1H NMR (400 MHz, CDCl3) δ 6.84 (m, 1H), 4.10 (m, 2H), 3.65 (m, 1H), 3.52 (m, 1H), 2.95−2.80 (m, 1H), 2.30−2.20 (m, 2H), 1.90−1.65 (m, 6H), 1.60−1.50 (m, 2H), 1.26 (s, 12H).

3-Iodo-1-methyl-5-nitro-1H-pyrrolo[2,3-b]pyridine (14). Potassium hydroxide (241 mg, 4.29 mmol, pellets) was added to a suspension of 5-nitro-1H-pyrrolo[2,3-b]pyridine (500 mg, 3.06 mmol) in DMF (15 mL), and the mixture was stirred at room temperature for 10 min. Iodine (856 mg, 3.37 mmol) was then added, and stirring was continued for 1.5 h. K2CO3 (974 mg, 7.05 mmol) followed by isodomethane (1.14 mL, 18.4 mmol) was added and stirring was continued at room temperature for 2.5 h. The mixture was diluted with water (50 mL), treated with NaHSO3 until yellow, and was then stirred for 30 min. The precipitate was collected by filtration, washed with water, and dried in vacuo to provide 845 mg (91%) of 14 as a yellow solid. MS (ES+) m/z 304 (M+H).

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(E)-N-[3-iodo-1-methyl-1H-pyrrolo[3,2-b]pyridin-5-yl]-N,N-dimethylformimidamide (21a). Tetrazylationmonium bromide (80 mg, 0.25 mmol), NaOH (4 mL, 2M), and iodomethane (200 μL, 3.25 mmol) were added to a stirred solution of 20a (785 mg, 2.5 mmol) in CH2Cl2 (22 mL), and the mixture was stirred for 16 h at room temperature. The reaction mixture was partitioned between water and CH2Cl2/EtOAc. The organic layer was concentrated, and the residue was purified by neutral alumina chromatography to provide 686 mg (84%) of 21a. MS (ES+) m/z 329 (M+H). 1H NMR (500 MHz, acetone-d6) δ 8.93 (s, 1H), 8.04 (s, 1H), 4.05 (s, 3H).

(4-Chloro-5-methyl-5H-pyrrolo[3,2-b]pyridin-7-yl)-5,6-dihydropyridin-1(2H)-yl)cyclopentanecarboxylic acid (22a). A mixture of 21a (40 mg, 0.12 mmol), 12 (89 mg, 0.29 mmol), Pd(PPh3)4 (14 mg, 0.02 mmol), and K2CO3 (50 mg, 0.37 mmol) in DMF/water (8:1, 1.5 mL) was degassed and flushed with nitrogen gas. The mixture was stirred at 100 °C for 16 h. The reaction mixture was partitioned between CHCl3/EtOAc and water. The organic layer was dried and the solvent was evaporated. The residue was purified by preparative HPLC to provide 110 mg (67%) of 22a. MS (ES+) m/z 345 (M+H)+.

Cyclopropyl(4-[(4-diphenylmethylenemino)5-methyl-5H-pyrrolo[3,2-b]pyridin-7-yl]-5,6-dihydropyridin-1(2H)-yl)methanone (30). Pd(OAc)2 (1 mg, 0.015 mmol) and BINAP (4 mg, 0.02 mmol) were dissolved in degassed dioxane (0.5 mL) and stirred for 5 min. This solution was added to a mixture of 29 (10 mg, 0.03 mmol), diphenylmethanamine (16 mg, 0.09 mmol), and sodium tert-butoxide (6 mg, 0.06 mmol) in degassed dioxane (0.5 mL). The mixture was heated under microwave irradiation for 30 min at 140 °C. CH2Cl2/EtOAc and a small amount of water were added, and the phases were separated. The organic layer was concentrated, and the crude product was purified by preparative HPLC to afford 10 mg (71%) of 30. MS (ES+) m/z 490 (M+H)+.

3-Iodo-1,4-dimethyl-5-nitro-1H-indole (23a). A mixture of 12 (35%) of 21a and 12 (40 mg, 0.12 mmol), Pd(OAc)2 (1 mg, 0.015 mmol) and BINAP (4 mg, 0.02 mmol) was dissolved in degassed dioxane (0.5 mL) and stirred at room temperature for 30 min, whereupon the solvents were evaporated. The residue was purified by preparative HPLC to provide 10 mg (59%, 2 steps) of 23a. MS (ES+) m/z 457 (M+H)+. 1H NMR (500 MHz, DMSO-d6) δ 9.02 (d, J = 5.0, 1.0, 1H), 8.25 (s, 1H), 8.54 (s, 1H), 8.79 (d, J = 9.0, 1H), 7.31 (s, 1H), 4.75–4.68 (m, 1H), 4.28–4.23 (m, 1H), 3.82 (s, 3H), 3.20–3.13 (m, 1H), 2.90–2.82 (m, 1H), 2.25–2.11 (m, 2H), 1.94–1.60 (m, 12H).

3-Iodo-1,4-dimethyl-5-nitro-1H-indole (23). A mixture of 3-Iodo-1,4-dimethyl-5-nitro-1H-indole (23a) (10 mg, 0.03 mmol) and Pd(PPh3)4 (1 mg, 0.015 mmol) was dissolved in degassed dioxane (0.5 mL) and stirred at room temperature for 30 min, whereupon the solvents were evaporated. The residue was purified by preparative HPLC to provide 0.4 mg (4%, 2 steps) of 31. MS (ES+) m/z 458 (M+H)+. 1H NMR (500 MHz, acetone-d6) δ 9.02 (d, J = 5.0, 1.50, 1H), 8.54 (s, 1H), 8.11 (dd, J = 5.0, 1.8, 1H), 7.58 (s, 1H), 4.72–4.65 (m, 1H), 4.22–4.15 (m, 1H), 3.96 (s, 3H), 3.30–3.18 (m, 1H), 3.10–3.05 (m, 1H), 2.30–2.20 (m, 1H), 1.90–1.50 (m, 12H).

Potassium hydroxide pellets (0.07 g, 14 mmol) were added to a cooled solution (ice-water bath) of 4-methyl-5-nitroindole (1.76 g, 10 mmol) in DMF (30 mL). The mixture was stirred at room temperature for 10 min. Iodine (2.79 g, 11 mmol) was added, and the stirring was continued for 5 h at room temperature. Potassium carbonate (3.17 g, 23 mmol) and methyl iodide (3.1 mL, 50 mmol) were added, and stirring was continued at room temperature for 16 h. The mixture was diluted with water (150 mL) and treated with solid NaHSO4 with stirring until all excess iodine was quenched. The crude product was collected by filtration, washed with water, and dried. The solid was suspended in 96% EtOH. After the solution was stirred for 15 min, the precipitate was collected and washed with two small portions of EtOH to provide 2.78 g (88%) of 33 as a golden-brown solid. MS (ES+) m/z 316 (M+H)+. 1H NMR (500 MHz, DMSO-d6) δ 7.79 (s, 1H), 7.75 (d, J = 9.1 Hz, 1H), 7.53 (d, J = 9.2 Hz, 1H), 4.381 (s, 3H), 2.92 (s, 3H).

Cyclopropyl(4,1-dimethyl-5-nitro-1H-indole-3-yl)-5,6-dihydropyridin-1(2H)-yl)methanone (34). Compound 33 (1.24 g, 3.9 mmol), 12 (1.56 g, 5.1 mmol), Pd EnCat TPP30 (palladium acetate/PPPh3, encapsulated, Aldrich 644706, 200 mg), and K2CO3 (1.08 g, 7.99 mmol) were suspended in DME:EtOH:H2O (4:1-1, 24 mL). The mixture was degassed with nitrogen and then heated at 70 °C for 18 h. The reaction mixture was partitioned between CH2Cl2 and water. The aqueous layer was extracted with CH2Cl2. The combined organic layers were concentrated, and the residue was purified by silica gel column chromatography (CH2Cl2/EtOAc, 100/0–80/20) to afford 1.14 g (79%) of 34 as a yellow solid. MS (ES+) m/z 368 (M+H)+. 1H NMR (500 MHz, acetone-d6) δ 7.75 (d, J = 9.0 Hz, 1H), 7.42 (d, J = 11.3 Hz, 1H), 7.3 (s, 1H), 5.8 (s, 1H), 4.30–4.20 (m, 2H), 3.90–3.80 (s, 2H), 3.20–3.05 (m, 1H), 2.75 (s, 3H), 2.60–2.40 (m, 2H), 1.90–1.55 (m, 8H).
(4-(5-Amino-1,4-dimethyl-1H-indol-3-yl)pyridin-1-yl) (cyclopentyl)methane) (35). A mixture of 34 (1.37 g, 3.69 mmol), ammonium formate (2.79 g, 44.3 mmol), 5% Pd/C (270 mg), and 96% EtOH (50 mL) was heated at 85 °C for 90 min under an atmosphere of nitrogen. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was diluted with water and treated with CHCl₃. The combined organic layers were dried (Na₂SO₄) and concentrated to yield 1.15 g (92%) of 35 as an off-white foam. MS (ES+) m/z 340 (M+H). 1H NMR (400 MHz, CDCl₃) δ 7.47 (s, 1H), 7.36 (d, J = 8.3 Hz, 1H), 3.93 (q, J = 6.8 Hz, 2H), 2.49 (s, 3H), 1.45 (m, 10H).

A mixture of DMF/H₂O (10/1, 11 mL) was added to a vial containing 4-chloro-1H-pyrrolo[2,3-b]pyridine (300 mg, 1.97 mol) and Pd(dppf)Cl₂ (28.77 g, 39.32 mmol) in toluene (6 L) under nitrogen. After addition, the reaction mixture was heated at 120 °C for 4 h. The reaction mixture was then poured into water (5 L) followed by brine (3.5 L), dried (Na₂SO₄), and filtered. The filtrate was concentrated, and the crude product was triturated with a mixture of CH₂Cl₂ and MTBE (3 L, 1:3) to afford 141 g (65%) of 36 as a yellow solid. 1H NMR (400 MHz, CDCl₃) δ 7.99 (s, 1H), 7.78 (t, J = 7.7 Hz, 1H), 7.68 (d, J = 3.6 Hz, 1H), 6.84 (d, J = 3.6 Hz, 1H), 2.81 (s, 3H).

1,4-Dimethyl-5-nitro-1H-pyrrolo[2,3-b]pyridine (41). To a stirred suspension of 40 (300 g, 0.95 mol) in MeOH (1.1 L) was sequentially added K₂CO₃ (220.75 g, 1.6 mol) and morpholine (824 g, 9.5 mol) at 25 °C. The mixture was heated at 65 °C for 20 min. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in CH₂Cl₂ (6 L), and to this mixture was added saturated aqueous NH₄Cl (6 L) and water (3 L). The mixture was stirred at room temperature overnight and then filtered. The solids were rinsed with water followed by CH₂Cl₂, and then concentrated (304 mg) of 41 as an off-yellow solid. 1H NMR (400 MHz, DMSO-d₆) δ 12.34 (br. s., 1H), 8.90 (s, 1H), 7.68 (d, J = 3.6 Hz, 1H), 6.84 (d, J = 3.6 Hz, 1H), 2.81 (s, 3H).

2-Methyl-1H-indol-5-ylbenzamide (36). Trifluoroacetic anhydride (485.89 g, 2.31 mol) was then added dropwise to the mixture maintaining a temperature of ~5 °C. The mixture was then stirred at 30 °C for 16 h and then was partitioned into water (5 L). The organic layer was dried (Na₂SO₄) and filtered. The filtrate was concentrated, and the crude product was triturated with MTBE (3 L) to afford 10431 (g, 0.615 mol) in DMF (1.9 L). Iodomethane (489 g, 3.5 mol) was added dropwise over 1 h. The reaction mixture was stirred at room temperature for 2 h and then was poured into water (4 L). The resulting mixture was stirred for 20 min and then filtered. The solids were washed with EtOAc followed by water and then dried under vacuum. The filtrate was extracted with EtOAc (1 L). The organic layer was washed with water (3 × 0.5 L) followed by brine (dried, Na₂SO₄), filtered, and concentrated. The residue was combined with the solids, and purified by silica gel column chromatography (CHCl₃/MeOH 95:5) to afford 44 mg (90%) of 36 as a yellow solid. 1H NMR (400 MHz, CDCl₃) δ 7.47 (s, 1H), 7.29 (d, J = 3.6 Hz, 1H), 6.67 (d, J = 3.6 Hz, 1H), 3.92 (s, 3H), 2.86 (s, 3H).

3-ido-1,4-dimethyl-5-nitro-1H-pyrrolo[2,3-b]pyridine (43). N-Lodosuccinimide (141 g, 627 mmol) was added to a stirred suspension of 42 (100 g, 523 mmol) in DMF (1.14 L) at room temperature. The mixture was stirred for 2 h. The reaction mixture was then poured into water (5 L), and the resulting mixture was stirred at room temperature for 20 min. The mixture was filtered and the collected solids were dissolved in CH₂Cl₂ (20 L). The mixture was washed with water (2 × 5 L) followed by brine (3.5 L), dried (Na₂SO₄), and filtered. The filtrate was concentrated, and the crude product was triturated with a mixture of CH₂Cl₂ and MTBE (3 L, 1:3) to afford 143 g (86%) of 43 as a yellow solid. 1H NMR (400 MHz, DMSO-d₆) δ 8.88 (s, 1H), 8.00 (s, 1H), 3.85 (s, 3H), 3.00 (s, 3H).

1-Phenyl-1H-pyrrolo[2,3-b]pyridine (45). A mixture of DMF/H₂O (10/1, 11 mL) was added to a vial containing 1-phenyl-1H-pyrrolo[2,3-b]pyridine (500 mg, 1.7 mmol), 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (373 mmg, 2.2 mol), Pd(PPh₃)₄ (197 mg, 0.2 mol), and K₂CO₃ (472 mg, 3.4 mmol). The vial was purged with nitrogen and heated at 120 °C by microwave irradiation for 1.5 h. After cooling, the reaction mixture was poured into water with extracted with CH₂Cl₂. The combined organic layers were concentrated and the crude product was purified by preparative HPLC to afford 448 mg (88%) of 45 as a colorless glass. MS (ES+) m/z 299 (M+H). 1H NMR (400 MHz, CDCl₃) δ 8.40 (d, J = 3.8 Hz, 1H), 2.83 (d, J = 7.3 Hz, 2H), 7.75 (d, J = 3.8 Hz, 1H), 7.65–7.70 (m, 1H), 7.55–7.49 (m, 2H), 7.10 (d, J = 3.8, 1H), 6.78 (d, J = 3.8 Hz, 1H), 5.42 (s, 2H), 2.20 (s, 3H).

4-Isopropyl-1-phenyl-1H-pyrrolo[2,3-b]pyridine (46). A mixture of 45 (445 mg, 1.55 mmol) and 5% Pd/C (45 mg) in EtOH (5 mL) was stirred at room temperature under a hydrogen atmosphere (1 bar) for 18 h. The mixture was filtered through Celite, and the filtrate was concentrated to afford 436 mg (97%) of 46 as a colorless glass. MS (ES+) m/z 301 (M+H). 1H NMR (400 MHz, CDCl₃) δ 8.48 (m, 8.8), 8.23 (d, J = 7.6 Hz, 2H), 7.72 (d, J = 3.8 Hz, 1H), 7.58 (t, J = 7.6 Hz, 1H), 7.50 (t, J = 6.9 Hz, 1H), 7.06 (m, 1H), 6.70 (d, J = 4.4 Hz, 1H), 3.30–3.20 (m, 1H), 1.32 (d, J = 5.7 Hz, 6H).

4-Isopropyl-5-nitro-1-phenyl-1H-pyrrolo[2,3-b]pyridine (47). Tetraethylammonium nitrate (795 mg, 2.61 mmol) followed by trifluoroacetic anhydride (0.37 mL, 2.61 mmol) were added to a cold
(0 °C) solution of 46 (436 mg, 1.45 mmol) in CH2Cl2 (25 mL), and the mixture was then stirred at 0 °C for 30 min. The reaction mixture was poured into water and extracted with CH2Cl2. The combined organic layers were concentrated, and the crude product was purified by preparative HPLC to afford 417 mg (83%) of 47 as a yellow solid. MS (ES+) m/z 346 (M+H)+. 1H NMR (400 MHz, CDCl3) δ 8.73 (s, 1H), 8.25 (d, J = 6.3 Hz, 2H), 7.9 (s, 1H), 7.68 (t, J = 4.5 Hz, 1H), 7.58 (t, J = 6.6 Hz, 2H), 6.92 (d, J = 4.5 Hz, 1H), 3.65–3.55 (m, 1H), 1.48 (d, J = 6.3 Hz, 6H).

4-Isopropyl-5-nitro-1H-pyrrolo[2,3-b]pyridine (48). An aqueous 2N NaOH solution (10 mL) was added to a solution of 47 (417 mg, 1.21 mmol) in THF (20 mL). The mixture was then stirred at room temperature for 16 h. The reaction mixture was neutralized with acetic acid, and the THF solvent was removed under reduced pressure. The aqueous mixture was extracted with CH2Cl2. The combined organic layers were concentrated to a 348 g (66%) of 48.

A solution of 4-chloro-1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridine (50) and NaOH (344 g, 8.61 mol) in CH2Cl2 (4 L) while maintaining an internal reaction temperature of 0 °C. The mixture was stirred for 5 min, and then the resulting mixture was stirred at room temperature overnight. The mixture was then triturated with EtOAc. The solids were washed with water (3 × 500 mL) and dried under reduced pressure. The crude product was triturated with EtOAc (600 mL) overnight, filtered, and dried under reduced pressure to provide 151 g (63%) of 53 as a yellow solid. MS (ES+) m/z 334 (M+H)+. 1H NMR (400 MHz, CDCl3) δ 8.94 (s, 1H), 7.35 (s, 1H), 4.17 (s, 3H), 3.91 (s, 3H).

4-Iodo-1H-pyrrolo[2,3-b]pyridine (54). Acetyl chloride (140 mL, 1.96 mol) was added slowly to a mixture of 4-chloro-1H-pyrrolo[2,3-b]pyridine (75 g, 0.49 mol) and NaN (442 g, 2.95 mol) in acetonitrile (2 L). The mixture was heated at 100 °C for 24 h. After cooling, the reaction mixture was poured into chilled ice with vigorous stirring. Saturated aqueous solutions of NaHCO3 and NaHSO3 were added sequentially to the biphasic solution, which was stirred vigorously for 30 min. The reaction mixture was then extracted with EtOAc. The combined organic layers were concentrated, and the resulting residue was dissolved in MeOH (2 L). Aqueous 2N NaOH (1 L) was added, and the mixture was stirred at room temperature overnight. The methanol was removed under reduced pressure, and the residue was partitioned between water and CH2Cl2. The organic layer was dried (Na2SO4), filtered, and concentrated to afford 100 g (84%) of 54 as an off-white solid. 1H NMR (400 MHz, CDCl3) δ 11.21 (br. s., 1H), 7.95 (d, J = 4.8 Hz, 1H), 7.52 (d, J = 4.8 Hz, 1H), 7.43 (d, J = 4.0 Hz, 1H), 6.42 (d, J = 4.0 Hz, 1H).

4-Iodo-1H-pyrrolo[2,3-b]pyridine (55). Iodide 54 (700 g, 2.87 mol) was added portionwise over the course of 25 min to a mixture of tetrabutylammonium sulfate (141 mmol) and NaOH (344 g, 8.61 mol) in CH2Cl2 (4 L) while maintaining an internal reaction temperature of 0 °C. The mixture was stirred for 0.5 h at 0 °C, and then phenylsulfonyl chloride (760 g, 4.30 mol) was added dropwise to the mixture over the course of 20 min while maintaining an internal reaction temperature of 0 °C. The reaction mixture was stirred at room temperature overnight and then partitioned between CH2Cl2 and water. The organic layer was washed with water (2 × 1 L) followed by brine (10 L), dried (Na2SO4), and filtered. The filtrate was concentrated, and the crude product was triturated with MeOH (3 L) to afford 983 g (89%) of 55 as a yellow solid. 1H NMR (400 MHz, CDCl3) δ 8.18 (d, J = 1.0 Hz, 1H), 8.16 (d, J = 1.0 Hz, 1H), 8.03 (d, J = 4.8 Hz, 1H), 7.79 (d, J = 4.0 Hz, 1H), 7.60–7.57 (m, 2H), 7.51–7.46 (m, 2H), 6.51 (d, J = 4.0 Hz, 1H). MS (ES+)+ m/z 385 (M+H)+.

4-Iodo-1H-pyrrolo[2,3-b]pyridine (56). A solution of trifluoroacetic anhydride (31.2 g, 148 mmol) in CH2Cl2 (100 mL) was added dropwise to a stirred, room temperature solution of tetramethylammonium nitrate (19.1 g, 141 mmol) in CH2Cl2 (200 mL). The resulting slurry was stirred at room temperature for 1.5 h and then cooled in a dry ice/acetone bath. A solution of 55 (30.02 g, 78.1 mmol) in CH2Cl2 (100 mL) was added dropwise to the mixture while maintaining the temperature at −65 °C. The reaction mixture was allowed to slowly warm to room temperature and stir for 16 h. The stirred reaction was quenched with saturated aqueous NaHCO3. The organic layer was washed with water (5 × 200 mL). The combined aqueous layers were extracted with CH2Cl2 (3 × 200 mL). The combined organic layers were dried (Na2SO4) and filtered. The filtrate was concentrated, and the crude product was triturated with EtOAc (150 mL, 6 h) to afford 22.0 g (66%) of 56 as a yellow solid. 1H NMR (400 MHz, CDCl3) δ 8.89 (s, 1H), 8.25–8.20 (m, 2H), 7.99 (d, J = 4.1 Hz, 1H), 7.70–7.63 (m, 1H), 7.59–7.52 (m, 2H), 6.73 (d, J = 4.1 Hz, 1H).

5-Nitro-1H-pyrrolo[2,3-b]pyridine (57). Methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (43 g, 224 mmol) and CuI (35.5 g, 186 mmol) were added to a solution of 56 (80 g, 190 mmol) in DMF (480 mL). The mixture was degassed with nitrogen and then heated to 100 °C for 2 h. After cooling, the reaction mixture was filtered through Celite, and the filter cake was washed with MTBE (2 × 200 mL). The filtrate was washed with water and brine. The organic layer was dried (Na2SO4) and filtered. The filtrate was concentrated, and the crude product was triturated with CH2Cl2/PE (1:5, 300 mL) to afford 42 g (61%) of 57 as a yellow solid. 1H NMR (CDCl3) δ 8.88 (s, 1H), 8.23 (d, J = 7.6 Hz, 2H), 8.10
(d. J = 4.0 Hz, 1H), 7.70–7.66 (m, 1H), 7.59–7.55 (m, 2H), 6.96 (d, J = 2.0 Hz, 1H).

3-ido-1-methyl-5-nitro-4-(trifluoromethyl)-1H-pyrrolo[2,3-b]-pyridine (58). KOH (31.7 g, 566 mmol) was added to a cold (0 °C), stirred solution of 57 (42 g, 110 mmol) in 2-methyltetrahydrofuran (CH2Cl2, 2.1, 600 mL). The reaction mixture was allowed to warm to room temperature and stir for 1 h. Iodine (89.3 g, 352 mmol) was added, and the mixture was stirred for 1 h. The reaction mixture was concentrated, and the residue was dissolved in CH2Cl2 (2 L). The solution was washed with 10% of aqueous NaHSO3 (2 × 200 mL) and brine. The organic layer was dried (Na2SO4) and filtered. The filtrate was concentrated, and the crude product was triturated with MTBE (200 mL, 12 h) followed by water (200 mL, 12 h) to afford 27 g (66%) of 58 as a yellow solid.

1H NMR (CDCl3) δ 8.80 (s, 1H), 7.75 (s, 1H), 3.98 (s, 3H).

tert-Butyl 4-(4,4,5,5-tetramethyl-1,3-dioxaborolanyl)-3-biphenyl-3-ylcarboxylate (59a). A solution of the crude residue was purified by preparative chromatography (EtOAc/PE, 15/85) to a 59a hydrochloride (9H). MS (ES+) (M+) z 374 (M+H).

Compounds 62b–d were prepared in a similar fashion from 61b–d, respectively. (see Supporting Information).

General Methods for the Preparation of 63a–1 and 64–66.

Method A: The corresponding acid chloride (1 equiv) was added to the solution of amine (1 equiv) in pyridine (0.1 M), and the mixture was stirred at room temperature for 18 h. The reaction mixture was then concentrated, and the crude residue was purified by preparative HPLC. Method B: A mixture of amine (1 equiv), DIPF (10 equiv), HATU (1.5 equiv), and the corresponding carboxylic acid (1.5 equiv) in DMF (0.1 M) was stirred at room temperature for 18 h. The reaction mixture was diluted with CH2Cl2 and washed with saturated aqueous NaHCO3. The organic layer was dried (Na2SO4), filtered, and concentrated. The reaction mixture was then concentrated, and the crude residue was purified by preparative HPLC. Method C: A mixture of triethylamine (4 equiv) and amine (1 equiv) in CH2Cl2 (0.1M) was cooled to 0 °C and the corresponding acid chloride (1.2 equiv) was added dropwise. The mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with CH2Cl2 and was washed with a saturated aqueous NaHCO3 solution. The organic layer was washed with brine, dried (Na2SO4), and concentrated. The reaction mixture was then concentrated, and the crude residue was purified by preparative HPLC. Method D: A mixture of DIPF (20 equiv) and the corresponding acid chloride (1 equiv) were added to a solution of amine (1 equiv) in CH2Cl2 (0.1M), and the mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with CH2Cl2 and was washed with a saturated aqueous NaHCl solution. The organic layer was dried (MgSO4) and filtered. The filtrate was concentrated, and the residue was purified by preparative HPLC.

N-3-(1-Acetylpropionylidene-4-yl)-1,4-dimethyl-1H-pyrrolo[2,3-b]-pyridin-5-yl)benzamide (63a). Method B. 62a, acetic acid, 45 mg (40%), white solid. MS (ES+) m/z 416 (M+H).

1H NMR (400 MHz, DMSO-d6) δ 10.26 (s, 1H), 8.46 (t, J = 1.4 Hz, 1H), 8.32 (dt, J = 8.0 Hz, J = 1.4 Hz, 1H), 8.10–8.07 (m, 2H), 7.71 (t, J = 7.8 Hz, 1H), 7.32 (s, 1H), 4.52 (d, J = 13.2 Hz, 1H), 3.92 (d, J = 13.8 Hz, 1H), 3.75 (s, 3H), 3.33–3.16 (m, 2H), 2.71–2.62 (m, 2H), 2.52 (s, 3H), 2.03–2.19 (m, 2H), 1.62–1.48 (m, 1H), 1.48–1.35 (m, 1H).
3-Cyano-N-(1-(2-cyclopropylacetyl)piperidin-4-yl)-1,4-dimethyl-1H-pyrrole[2,3-b]pyridin-5-yl]benzamide (63e). Method B. 62a, 2-cyclopropylacetic acid, 40 mg (33%), white solid. MS (ES+) m/z 465 (M+H). 1H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 10.27 (s, 1H), 8.46 (s, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.11–8.06 (m, 2H), 7.77 (t, J = 7.8 Hz, 1H), 7.73 (s, 1H), 6.45 (d, J = 13.6 Hz, 1H), 3.75 (s, 3H), 3.12–3.33 (m, 2H), 2.67 (J = 12.1 Hz, 1H), 2.51 (s, 3H), 2.28 (d, J = 6.8 Hz, 2H), 1.97 (t, J = 10.4 Hz, 2H), 1.56–1.37 (m, 2H), 1.03–0.91 (m, 1H), 0.49–0.42 (m, 2H), 0.10–0.05 (m, 2H).

3-Cyano-N-(1,4-dimethyl-3-(1-(3-methylbutanoyl)piperidin-4-yl)-1H-pyrrole[2,3-b]pyridin-5-yl]benzamide (63f). Method C. 62a, 3-methylbutanoyl chloride, 115 mg (36%), white solid. MS (ES+) m/z 472 (M+H). 1H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 9.38 (br. s., 1H), 8.48 (s, 1H), 8.42 (d, J = 6.3 Hz, 1H), 8.10 (s, 1H), 8.05 (d, J = 6.3 Hz, 1H), 7.84 (t, J = 6.3 Hz, 1H), 7.30 (s, 1H), 4.80–4.70 (m, 1H), 4.25–4.15 (m, 1H), 3.95–3.90 (m, 1H), 3.82 (s, 3H), 3.35–3.25 (m, 2H), 3.00–2.90 (m, 1H), 2.85–2.80 (m, 1H), 2.74–2.65 (m, 1H), 2.20–2.10 (m, 1H), 1.74–1.50 (m, 2H), 1.49 (d, J = 6.5 Hz, 6H), 1.15–1.05 (m, 6H).

3-Cyano-N-(1-isobutyryl piperidin-4-yl)-1,4-dimethyl-1H-pyrrole[2,3-b]pyridin-5-yl]benzamide (63g). Method B. 62c, isobutyl chloride, 50 mg (40%), white solid. MS (ES+) m/z 460 (M+H). 1H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 10.28 (s, 1H), 8.46 (s, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.13–8.09 (m, 2H), 7.77 (t, J = 7.8 Hz, 1H), 7.23 (s, 1H), 4.56–4.53 (m, 1H), 4.07–4.04 (m, 1H), 3.92 (s, 3H), 3.73 (s, 3H), 3.09–3.05 (m, 2H), 2.95–2.85 (m, 1H), 2.70–2.55 (m, 2H), 2.05–1.90 (m, 2H), 1.55–1.35 (m, 2H), 1.03–0.99 (m, 6H).

3-Cyano-N-(1-(2-fluoro-6-methylbenzoyl)piperidin-4-yl)-1,4-dimethyl-1H-pyrrole[2,3-b]pyridin-5-yl]benzamide (63h). Method D. 62d, isobutyryl chloride, 4.6 mg (97%), white solid. MS (ES+) m/z 498 (M+H). 1H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 10.60 (s, 1H), 8.42 (s, 1H), 8.35–8.29 (m, 2H), 8.12 (d, J = 7.6 Hz, 1H), 7.88 (s, 1H), 7.80 (t, J = 7.6 Hz, 1H), 4.65–4.55 (m, 1H), 4.15–4.05 (m, 1H), 3.86 (s, 3H), 3.18–3.05 (m, 2H), 2.98–2.88 (m, 1H), 2.60–2.50 (m, 2H), 2.00–1.82 (m, 2H), 1.60–1.40 (m, 2H), 1.10–1.00 (m, 6H).

1-(1,4-Dimethyl-5-nitro-1H-pyrrole[2,3-b]pyridin-3-yl)-5,6-dihydropyridin-1(2H)yl-2-methylprop-1-anone (67). A solution of HCl (13.5 mL, 4 M in dioxane) was added to a cold (0 °C) solution of diethylamine (1.34 mmol) in MeOH. The mixture was diluted to room temperature and stirred for 3 h. Solvents were removed under reduced pressure below 30 °C. The residue was neutralized with saturated aqueous NaHCO<sub>3</sub> solution and was extracted with a mixture of CH<sub>2</sub>Cl<sub>2</sub>/MeOH (97/3). The combined organic layers were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), and filtered. The filtrate was concentrated. Triethylamine (5.3 mL, 38.5 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was added to the residue followed by isobutyl chloride (1.6 mL, 15.4 mmol) at 0 °C. The mixture was allowed to stir at room temperature for 1 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with a saturated aqueous NaHCO<sub>3</sub> solution. The organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude product was triturated with diethyl ether to afford 4.0 g (91%) of 67. 1H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.98 (s, 1H), 7.11 (s, 1H), 5.85–5.71 (m, 1H), 4.25–4.20 (m, 2H), 3.89 (s, 3H), 3.76 (t, J = 5.0 Hz, 2H), 2.49–2.43 (m, 2H), 2.93–2.85 (m, 1H), 2.81 (s, 3H), 1.19 (d, J = 6.5 Hz, 6H).

General Method for the Preparation of 69a–d. To a solution of the corresponding acid (1.2 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added DipeA (5 equiv) followed by HATU (2 equiv), and the mixture was allowed to stir at room temperature for 1 h. Amine 68 (100 mg, 0.32 mmol, 1 equiv) was then added, and the reaction mixture was further stirred for 16 h at room temperature. The reaction mixture was diluted with water and extracted using CH<sub>2</sub>Cl<sub>2</sub>. The combined
organically layers were dried (Na2SO4) and filtered. The filtrate was concentrated, and the residue was purified by preparative HPLC.

3-Cyano-N-(3-(1-isobutyrylpiperidin-4-yl)-4-methoxybenzamide (69a). 3-Cyano-4-methylbenzoic acid, 52 mg (35%). 1H NMR (400 MHz, MeOH-d4) δ 8.32–8.29 (m, 2H), 8.09 (s, 1H), 7.34 (d, J = 8.8 Hz, 1H), 4.69 (d, J = 12.8 Hz, 1H), 4.18 (d, J = 12.8 Hz, 1H), 4.06 (s, 3H), 3.81 (s, 3H), 3.39–3.25 (m, 2H), 3.03–2.99 (m, 1H), 2.81–2.75 (m, 1H), 2.63 (s, 3H), 2.19–2.09 (m, 2H), 1.68–1.52 (m, 2H), 1.14–1.11 (m, 6H). MS (ES+) m/z 474 (M+H+). 1H NMR (400 MHz, DMSO-d6) δ 8.73 (d, J = 8.8 Hz, 1H), 7.24 (s, 1H), 4.19 (d, J = 13.2 Hz, 1H), 3.81 (s, 3H), 3.40–3.26 (m, 2H), 3.05–2.95 (m, 1H), 2.81–2.75 (m, 1H), 2.64 (s, 3H), 2.19–2.03 (m, 2H), 1.68–1.51 (m, 2H), 1.15 (d, J = 6.8 Hz, 3H), 1.11 (d, J = 7.2 Hz, 3H). MS (ES+) m/z 462 (M)+.

3-Cyano-4-fluoro-N-(3-(1-isobutyrylpiperidin-4-yl)-1,4-dimethyl-1H-pyrrrolo[2,3-b]pyridin-5-yl)benzamide (69b). 3-Cyano-4-fluorobenzoic acid, 22 mg (15%). 1H NMR (400 MHz, MeOH-d4) δ 8.45 (d, J = 6.4, 2.4 Hz, 1H), 8.39–8.35 (m, 1H), 8.12 (s, 1H), 7.56 (t, J = 8.8 Hz, 1H), 7.24 (s, 1H), 4.39 (d, J = 13.2 Hz, 1H), 4.18 (d, J = 13.2 Hz, 1H), 3.81 (s, 3H), 3.48–3.26 (m, 2H), 2.87 (d, J = 13.2 Hz, 1H), 2.70–2.47 (m, 4H), 2.16–2.04 (m, 4H), 2.00–1.90 (m, 1H), 1.60–1.45 (m, 1H), 1.30–1.10 (m, 7H). MS (ES+) m/z 462 (M+H+).

PAINS. All compounds reported with biological activity were electronically filtered for structural attributes consistent with classification as pan assay interference compounds (PAINS) and were found to be negative. Compound 3 has undergone broad enzyme and receptor profiling and been found not to be promiscuous (4/81 targets with % inhibition >50%, 10 μM concentration) (see Supporting Information, Table S2).

Torsional Energy Calculations. All quantum mechanical calculations were performed with the PetaChem software utilizing the B3LYP density functional and the 6-31G* basis set in gas phase. Dihedral scans were performed using a series of constrained optimizations, whereby the specified dihedral angle was kept frozen at a given value, with full optimization of the remaining geometric parameters subject to the given dihedral constraint. Total energies for a given system were then converted into relative energies (kcal/mol) for graphical depiction.

Gal4-RORC2 Luciferase Reporter Assay. Neuro2A cells (murine neuroblastoma cell line obtained from HPACC) were transiently transfected with a mammalian expression vector (pM) containing Gal4-RORC2 LBD and a Gal4-responsive reporter gene containing firefly luciferase (SxGal4AUS-Luc3). Gal4-RORC2 LBD is active in the transfected Neuro2A cells in the absence of any added stimuli, resulting in a robust luciferase response signal. The growth medium was composed of MEM EBS without t-glutamine, 10% (v/v) FBS, 2 mM MgCl2, 0.5 mg/mL bovine serum albumin, and 5 mM dithiothreitol. The receptor was enzymatically biotinylated at its N-terminal (SPA).

Estimation of Compound Dissociation Rate Using TR-FRET Coactivator Recruitment Assay. A modification of the coactivator peptide recruitment assay was used to estimate the off-rate of inverse agonists. RORC2 LBD, SRC1-2, and compound (at approximately its IC50 concentration) were incubated for 3 h as indicated above. Then, 3 μM RORC2 agonist 3,5-dibromo-4-(3-isopropyl-4-methoxyphenyl)(1-[(ethoxymethyl)-1H-imidazol-2-yl]-methanol (EC50 = 9.5 nM, see Supporting Information for synthesis) was added, and the TR-FRET signal was monitored during the subsequent 9.5 h. Apparent T1/2 values of compounds were determined by analysis of the progress curves.
IL-17 Production Assay in Human Th17 Cells. Human CD4+ T cells were prepared from mouse splenocytes by negative selection using magnetic beads coated with antibodies that capture unwanted cells using Mouse CD4+ EasySep kit from StemCell Technologies and following the manufacturer’s protocol. Each mouse spleen (from 6–8 week-old Balb/c mice, Jackson Laboratories) was minced and mixed with 10% phosphate buffer saline containing 2% (v/v) fetal bovine serum and then filtered through a 70-μm cell strainer. The resulting filtrate containing splenocytes was collected into a 50 mL tube and centrifuged at 300g at room temperature for 10 minutes, and then resuspended at 10^6 cells/mL with RoboSep buffer. Then 8 mL of the cell suspension were transferred to a 14 mL polystyrene tube, and rat serum was added at 50 μL/mL of cell suspension. The following reagents were added in order: EasySep mouse CD4+ T cells enrichment cocktail (50 μL/mL of cell suspension) and EasySep D2Magnetic Particles (100 μL nanoparticle suspension/mL of cell suspension). Each addition was followed by manual mixing and incubation at 2–8 °C for 15, 15, and 5 minutes, respectively. Then a magnetic separation was carried out by placing the tube into Silver EasySep magnet (StemCell Technologies) for 5 minutes following by decantation of CD4+ cells.

CD4+ cells were suspended with X-Vivo 15 medium containing 50 μM β-mercaptoethanol at 10^6 cells/mL and placed in a tissue culture incubator at 37 °C overnight. The next day the compound plate was prepared by dispensing 1 μL/well of compound 66 dissolved in 100% DMSO at 2000-fold final concentrations in the assay or DMSO only for control wells. X-Vivo 15 medium was then added to the compound plate at 199 μL/well. CD4+ cells were activated with antimouse CD3 and antimouse CD28 and induced to differentiate into Th17 cells with a mixture of cytokines. The assay plate was prepared as follows. A cytokine cocktail at 10-fold final concentration was prepared by mixing the following reagents in X-Vivo 15 medium: human TGFβ (5 ng/mL), mouse IL-6 (20 ng/mL), mouse IL-1β (20 ng/mL), and mouse IL-23 (40 ng/mL). Then, 6.75 μL cytokine cocktail was mixed with 47.25 mL CD4+ cells (adjusted to a cell density of 7.1 × 10^5 cells/mL resulting in 50 000 cells/well in the assay), and the mixture was dispensed at 80 μL/well to a tissue culture 384-well plate. Then, Mouse T-Activator CD3/CD28 beads (5 × 10^5 beads/mL) were added at 10 μL/well. The assay plate was then incubated at 37 °C for 1 hour in a tissue culture incubator. Various concentrations of compound 66 were transferred from the compound plate to the assay plate at 10 μL/well, and the assay plate was placed in a tissue culture incubator at 37 °C for 4 days. Determination of IL-17 in the supernatant was carried out by an electrochemiluminescence assay system from Meso Scale Diagnostics following the manufacturer’s protocol as described above. Cell viability in the assay plate was determined by CellTiter-Glo method (Promega) following the manufacturer’s protocol.

Gene Expression in Human Lymphocytes. Human CD4+ cells were prepared, differentiated and treated with compound 66 as described above. At the end of the 6-day incubation, cells were lysed and RNA purified with solid-phase extraction using RNAeasy kit from Qiagen following the manufacturer’s protocol. Cells were mixed with 150 μL Buffer RLT in a 96 well plate, and then 150 μL 70% ethanol was added. The resulting cell lysate was transferred to a RNAeasy 96 well plate that contains silica-based resin to which RNA binds. The plate was washed three times with Buffer RPE by centrifugation at 5600g for 4 min at room temperature. To elute RNA, 45–70 μL RNase-free water was added to each well followed by centrifugation at 5600g for 4 min at room temperature. RNA was stored at −70 °C. RNA concentration, and purity was assessed spectrophotometrically. Quantitative PCR (qPCR) was performed using TaqMan primer/probe chemistry. Reverse transcription was carried out on a thermocycler with 5 to 10 ng/mL RNA and buffer and enzymes from TaqMan Fast Cells kit in a final volume of 20 μL with two consecutive incubations at 37 and 95 °C for 60 and 5 minutes, respectively. PCR amplification was carried out using the Biomark HD platform (Fluidigm). Gene expression values were normalized to
expression levels of housekeeping genes (RPLP0 and B2M) prior to calculating relative expression compared with DMSO vehicle.

Nuclear Receptor Selectivity. The activity of compound 66 on nuclear receptors in vitro was evaluated by a trans-FACTORIAL system (Attagene Inc.) which is a multiplexed cell-based assay technology that measures reporter RNA levels upon transfection of 48 chimeric constructs composed of nuclear receptor ligand binding domain and Gal4 DNA binding domain.

In Vivo Pharmacokinetic and Skin Inflammation Model. All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by the Pfizer Institutional Animal Care and Use Committee.

For pharmacokinetic studies in rats, compound 66 was dosed to male Wistar Han rats as an IV bolus in 10% DMSO, 50% PEG400 and 40% water; orally by gavage as a suspension of crystalline solid in 0.5% methyl cellulose; or orally by gavage as a suspension of 25% spray dried dispersion (HPMCAS-H polymer) in 0.5% methyl cellulose, 0.5% HPMCAS-HF and 20 m M Tris pH 7.4 buffer. For pharmacokinetic studies in dogs, male Beagle dogs were dosed with an IV bolus of compound 66 in 10% DMSO, 40% PEG400, 30% glycerol formal, 1% N-methyl-2-pyrrolidone, and 19% phosphate buffered saline or orally by gavage as a suspension of 25% spray dried dispersion (HPMCAS-H polymer) in 0.5% methyl cellulose, 0.5% HPMCAS-HF, and 20 m M Tris pH 7.4 buffer. For pharmacokinetic studies in mice, female Balb/c mice were dosed with compound 66 as an IV bolus in 10% DMSO, 50% PEG400, and 40% water or orally by gavage as a suspension of 25% spray dried dispersion (HPMCAS-H polymer) in 0.5% methyl cellulose, 0.5% HPMCAS-HF, and 20 m M Tris pH 7.4 buffer. Blood was collected over a 24 h time course, and following centrifugation, the resulting plasma samples were precipitated with acetonitrile and analyzed for test compound concentration using an LC-MS/MS procedure. PK parameters were calculated from plasma concentration–time curves using non-compartamental analysis in Watson LIMS.

The imiquimod-induced skin inflammation model was performed with 8–10 week old female Balb/c mice, purchased from Taconic Farms, Germantown, NY. Commercially available 5% imiquimod cream (Sandoz, Princeton, NJ) was applied to the shaved back skin and left ear for three consecutive days (days 1–3). Mice were dosed with vehicle or compound 66 as a spray dried dispersion (25%, HPMCAS-H polymer) once daily by oral gavage in 0.5% methylcellulose, 0.5% HPMCAS-HF and 20 mM Tris pH 7.4 buffer for 5 days (days 1–5). Alternatively, mice were dosed by intraperitoneal injection with either anti-p40 Ab, anti-IL-17A Ab, or isotype control (20 mg/kg) on days 1 and 3. At the end of the study (day 5), ear thickness was measured in triplicate using an engineer’s micrometer (Mitutoyo, IL) as a means of assessing swelling and epidermal hyperplasia. Also, plasma samples were collected for exposure, and ear tissues samples were collected for IL-17A protein measurements. Ears were homogenized and protein was measured using the Pierce BCA protein kit (Thermo Fisher, Rockford, IL). Samples were normalized for protein (100 μg), and then murine IL-17A cytokine was measured with the R&D Quantikine ELISA kit.

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Notes
The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADME, absorption, distribution, metabolism, and excretion; AUC, area under the curve; CLu, clearance; DIPEA, N,N-diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; ESP, electrostatic potential; F, fraction absorbed; Fub, fraction unbound; HATU, 1-[bis(dimethylamino)methyl]-1H-
1,2,3-triazol[4,5-b]pyridinium 3-oxide hexafluorophosphate; HEK, human embryonic kidney; HLM, human liver microsomes; HOBT, 1-hydroxybenzotriazole; HPMCAS, hydroxypropyl methylcellulose acetate succinate; IL-17, interleukin-17; LBD, ligand binding domain; LIPE, lipophilic efficiency; NMP, N-methyl-2-pyrrolidone; ROR, retinoic acid receptor-related orphan receptor; SDD, spray-dried dispersion; SPA, scintillation proximity assay; T1/2, half-life; TBTU, O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate; Th17, T helper 17; THF, tetrahydrofuran; TR-FRET, time-resolved fluorescence resonance energy transfer; Vdss, volume of distribution at steady state

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(33) Compound 66 (PF-06477711) is commercially available via MilliporeSigma (catalog # P20390).


(43) Compound 66 (PF-06477711) is commercially available via MilliporeSigma (catalog # P20390).


NOTE ADDED AFTER ASAP PUBLICATION

After this paper was published ASAP September 9, 2018, a correction was made to the compound number in ref 43. The corrected version was reposted September 24, 2018.