

Supporting information

From ROR γ t Agonist to Two Types of ROR γ t Inverse Agonists

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Contents

1. Experimental in general
2. Synthesis of compounds **5a-5e**, **6**, **7a-7f**, **8**, **9**
3. Crystallography study and data of **5d**, **6**
4. Assay description
5. Docking studies

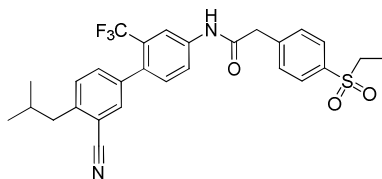
1. Experimental in General

^1H NMR spectral data were recorded on a Bruker 400 NMR spectrometer operating at 400 MHz. CDCl_3 is deuteriochloroform, $\text{DMSO}-d_6$ is hexadeuteriodimethylsulfoxide. Chemical shifts are given in parts per million (δ) downfield from the NMR solvent. Abbreviations for NMR data are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in Hertz. LCMS (Agilent 1200SL-6110) analysis was conducted for all assayed compounds on either acidic or basic conditions: 1) acidic condition refers to water containing 0.05 % TFA / acetonitrile as mobile phase on Agilent SB-C18 column (1.8 μm , 4.6 x 30 mm), with MS and photodiode array detector (PDA). The following conditions were used: a gradient from 5 to 95% in 4 min (or 6 min) and held at 95% for 1 min; UV detection at 214 and 254 nm; a flow rate of 1.5 ml/min; full scan; mass range from 100 to 1000 amu; 2) basic condition refers to water containing 10 mM NH_4HCO_3 aqueous/acetonitrile as mobile phase on Waters XBridge C18 column (3.5 μm , 4.6 x 50 mm) with MS and photodiode array detector (PDA). The following conditions were used: a gradient from 5 to 95% in 5 min and held at 95% for 1 min; UV detection at 214 and 254 nm; a flow rate of 2 ml/min; full scan; mass range from 100 to 1000 amu. All the assayed compounds possess $\geq 95\%$ purity determined using LC/MS analysis. Flash column chromatography was performed on ISCO or Biotage using a pre-packed silica gel column, a detector with UV wavelength at 254 nm and 280 nm. MDAP equipped with 2489 UV detector, 2767 sample manager, 2545 pump, and 3100 single quadrupole mass spectrometer was performed on Sunfire Prep C18 column (5 μm , 19 mm x 50 mm) using water containing 0.05% TFA/acetonitrile as mobile phase. The following conditions were used: a gradient from 5% to 95% in 15 min and held in 95% for 3 min; a flow rate of 30 mL/min.

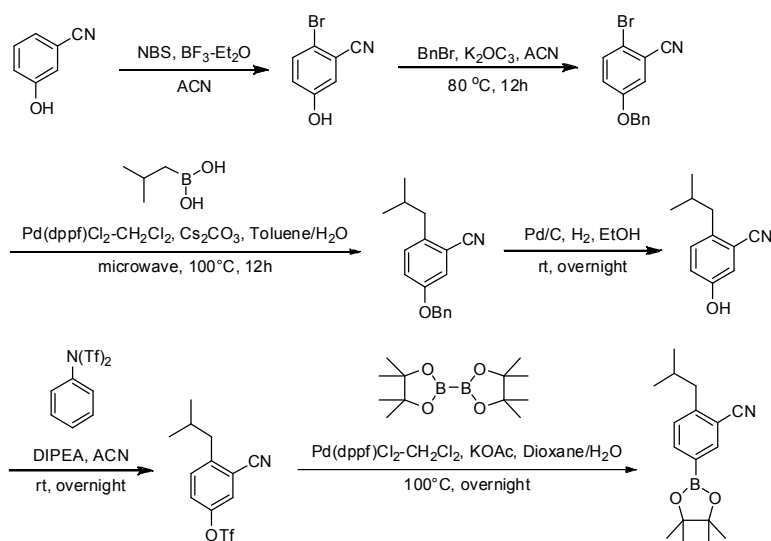
2. Synthesis of compounds **5a-5e**, **6**, **7a-7f**, **8**, **9**

Compound **5a**: see ref. 27 (a), supporting material; Compound **5b**: see ref. 27 (b), WO2012027965A1; Compound **5c-5e**: similar synthetic procedure to **5b**; Compound **7b**, **7c**, **8**, **9**: see ref. 33, WO2013029338A1

N-(3'-cyano-4'-isobutyl-2-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (**6**)



Intermediate 1: 2-isobutyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile



Step 1: 3-hydroxybenzonitrile (30 g, 252 mmol) was dissolved in Acetonitrile (300 ml) and cooled to -20°C . Boron trifluoride diethyl etherate (31.0 mL, 252 mmol) followed by NBS (44.8 g, 252 mmol) were added and the mixture allowed to warm to ambient temperature. The resulting mixture was stirred at this temperature for 12 h. The mixture treated with aqueous NH_4Cl solution (100 ml) and Water (400 ml), then extracted with EtOAc (500 ml \times 2). The organic extracts were combined, washed with brine (300 ml), dried over anhydrous Na_2SO_4 . After filtration, the solvent was removed *in vacuo* to give 2-bromo-5-hydroxybenzonitrile (56g, 52.8 % yield) as a light yellow solid. MS (ESI): m/z calcd for $\text{C}_7\text{H}_4\text{BrNO}$ $[\text{M}+\text{H}]^+$, 196.9; found, 197.9.

Step 2: (bromomethyl)benzene (47.5 g, 278 mmol) was added to a mixture of 2-bromo-5-hydroxybenzonitrile (50 g, 253 mmol) and K_2CO_3 (69.8 g, 505 mmol) in Acetone (500 ml) at rt. Then the reaction mixture was stirred at 80°C for 12 h. After cooled to rt, the reaction mixture was filtered, the filtrate was evaporated and the residue was added to a silica gel column and was eluted with Petroleum ether/EtOAc (100/1) then DCM to afford 5-(benzyloxy)-2-bromobenzonitrile (60g, 63.5 % yield) as a white solid.

Step 3: To the mixture of 5-(benzyloxy)-2-bromobenzonitrile (12.97 g, 45 mmol), isobutylboronic acid (5.05g, 49.5 mmol) and Cs_2CO_3 (29.3 g, 90 mmol) in toluene (250 ml) and H_2O (25 ml) was added $\text{PdCl}_2(\text{dppf}) \cdot \text{CH}_2\text{Cl}_2$ (3.67 g, 4.5 mmol) under N_2 . The reaction mixture was stirred at 100°C for 12 h. After cooled to room temperature, the mixture was concentrated under reduced pressure, diluted with EtOAc (300 ml), washed with water and brine, dried over Na_2SO_4 , further concentrated and purified by a silica gel column eluted with Petroleum ether/EtOAc (19/1) to afford 5-(benzyloxy)-2-isobutylbenzonitrile (9.8 g, 80%).

Step 4: To a solution of 5-(benzyloxy)-2-isobutylbenzonitrile (9.82 g, 37 mmol) in EtOH (250 ml) was added Pd/C (0.984 g, 9.25 mmol) and the reaction mixture was stirred under H_2

at room temperature overnight. The mixture was filtered and the filtrate was concentrated *in vacuo* to afford 5-hydroxy-2-isobutylbenzonitrile (6.4 g, 99% yield).

Step 5: To a solution of 5-hydroxy-2-isobutylbenzonitrile (6.4 g, 36.6 mmol) in Acetonitrile (250 ml) was added DIPEA (5.2g, 4.03mmol) and trifluoro-*N*-phenyl-*N*-(trifluoromethylsulfonyl)methanesulfonamide (15.69 g, 43.9 mmol) at room temperature. The mixture was stirred at room temperature overnight under N₂. The mixture was concentrated and the residue was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc for 2 times (250 ml × 2). The combined organic layers were washed with brine, dried over Na₂SO₄, and concentrated to give a crude product which was purified by a silica gel column eluting with Petroleum ether/EtOAc (100/1) to afford 3-chloro-4-isobutylphenyl trifluoromethanesulfonate (11 g, 96% yield).

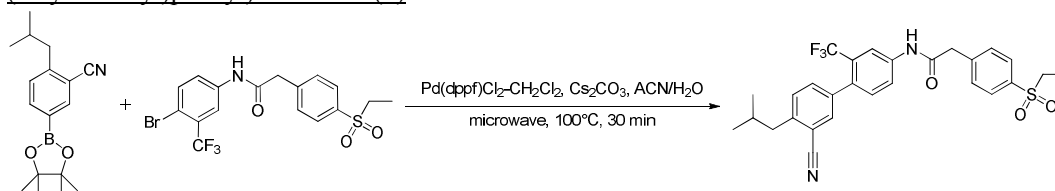
Step 6: 3-cyano-4-isobutylphenyl trifluoromethanesulfonate (11 g, 35.8 mmol), PdCl₂(dppf)-CH₂Cl₂ (2.339 g, 2.86 mmol), and KOAc (10.54 g, 107 mmol) were mixed in 1,4-dioxane (250 ml) and H₂O (2 ml), to which 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (10.91 g, 43 mmol) was added. The resulting mixture was degassed for several minutes and refluxed at 100 °C overnight under N₂. After cooled to room temperature, the mixture was filtered and the filtrate was concentrated under reduced pressure to give a crude product, which was purified by a silica gel column eluting with Petroleum ether/EtOAc (200/1~50/1) to give 2-isobutyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (2 g, 19.59% yield) as a white solid.

Intermediate 2: *N*-(4-bromo-3-(trifluoromethyl)phenyl)-2-(4(ethylsulfonyl)phenyl)acetamide



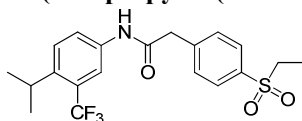
4-Bromo-3-(trifluoromethyl)aniline (600 mg, 2.5 mmol), 2-(4-(ethylsulfonyl)phenyl)acetic acid (570 mg, 2.5 mmol) and HATU (564 mg, 3.0 mmol) were dissolved in dry dichloromethane (DCM) (20 ml). Then DIPEA (648 mg, 5.0 mmol) was added gradually to this stirred solution. The solution was stirred at room temperature under N₂ overnight. The reaction mixture was washed with 1M HCl, saturated NaHCO₃ and brine successively. The organic layer was dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 1:10 to 1:1) to give *N*-(4-bromo-3-(trifluoromethyl)phenyl)-2-(4-(ethylsulfonyl)phenyl)acetamide as a white solid (855 mg, 76.1% yield). MS(ESI) *m/z* 451.8 (M+H)⁺.

Preparation of *N*-(3'-cyano-4'-isobutyl-2-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (6)

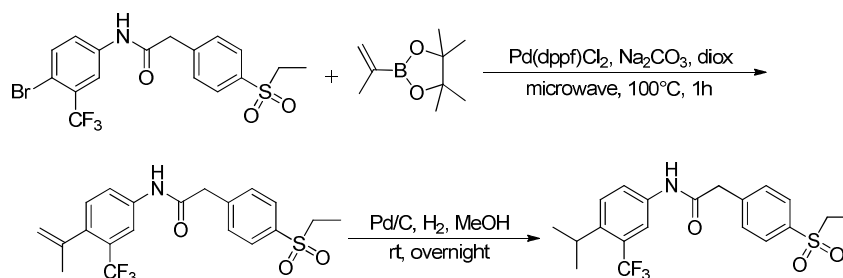


The mixture of 2-(2-methylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (41.8 mg, 0.147 mmol), *N*-[4-bromo-3-(trifluoromethyl)phenyl]-2-[4-(ethylsulfonyl)phenyl]acetamide (60 mg, 0.133 mmol), PdCl₂(dppf)-CH₂Cl₂ (10 mg, 0.012 mmol) and Cs₂CO₃ (52.1 mg, 0.160 mmol) in Acetonitrile (1.5 mL)/Water (0.500 mL) was sealed in vessel and heated in a Biotage initiator using initiation high to 100 °C for 30 mins. After cooled to room temperature, the reaction mixture was filtered through a short silica pad and the filtrate was concentrated under reduced pressure, the residue was purified by MDAP to afford *N*-[3'-cyano-4'-(2-methylpropyl)-2-(trifluoromethyl)-4-biphenyl]-2-[4-(ethylsulfonyl)phenyl]acetamide (14 mg, 19.68 % yield) as white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.65 (s, 1 H), 8.10 (d, *J* = 2.0 Hz, 1H), 7.81-7.79 (m, 3 H), 7.64 (d, *J* = 1.6 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.50-7.43 (m, 2 H), 7.35 (d, *J* = 8.4 Hz, 1H), 3.80 (s, 2 H), 3.28 (q, *J* = 7.2 Hz, 2H), 1.93-1.87 (m, 1 H), 2.67-2.60 (m, 2 H), 1.10 (t, *J* = 7.2 Hz, 3H), 0.86 (d, *J* = 6.4 Hz, 6H). MS (ESI): *m/z* calcd for C₂₈H₂₇F₃N₂O₃S [M+H]⁺, 528.2; found, 529.1.

2-(4-(ethylsulfonyl)phenyl)-*N*-(4-isopropyl-3-(trifluoromethyl)phenyl)acetamide (7a)



Preparation of 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-isopropyl-3-(trifluoromethyl)phenyl)acetamide (7a)

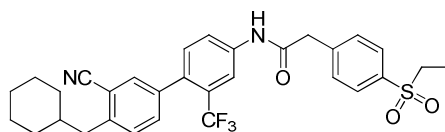


Step 1: Pd(dppf)Cl₂ (5 mg, 0.007 mmol) was added to the solution of *N*-(4-bromo-3-(trifluoromethyl)phenyl)-2-(4-(ethylsulfonyl)phenyl)acetamide (60 mg, 0.133 mmol), 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (27 mg, 0.160 mmol) and 2M aqueous Na₂CO₃ (0.14 mL) in 1,4-dioxane (2 mL). The mixture was bubbled with argon. Then the reaction vessel was sealed and heated in the microwave at 100 °C for 1 h. After completion of

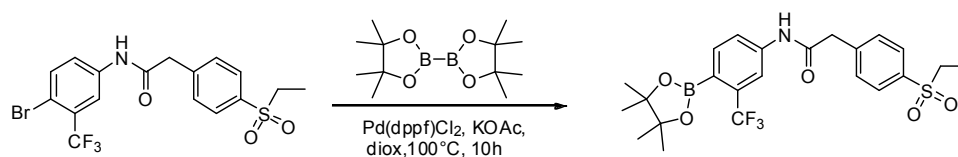
the reaction as indicated by TLC, the mixture was filtered through celite and the filtrate was evaporated *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 1:3) to give 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-(prop-1-en-2-yl)-3-(trifluoromethyl)phenyl)acetamide as a yellow oil (54 mg, 98.5% yield).

Step 2: 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-(prop-1-en-2-yl)-3-(trifluoromethyl)phenyl)acetamide (54 mg, 0.131 mmol) was dissolved in methanol (10 mL). Pd/C (14 mg, 0.131 mmol, 10%) was added and the resulting mixture was stirred under H₂ (excess) at room temperature overnight. After completion of the reaction as indicated by LC-MS, the mixture was filtered through celite and the filtrate was evaporated *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 1:3) to give 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-isopropyl-3-(trifluoromethyl)phenyl)acetamide as a yellowish solid (48 mg, 88.9% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.20 (s, 1H), 7.78 (d, *J* = 8.0 Hz, 2H), 7.75 – 7.68 (m, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 1H), 3.76 (s, 2H), 3.27 (dt, *J* = 13.0, 6.6 Hz, 1H), 3.10 (q, *J* = 7.4 Hz, 2H), 1.28 – 1.19 (m, 9H). MS (ESI): *m/z* calcd for C₂₀H₂₂F₃NO₃S [M+H]⁺, 414.1; found, 413.9.

***N*-(3'-cyano-4'-(cyclohexylmethyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (7d)**

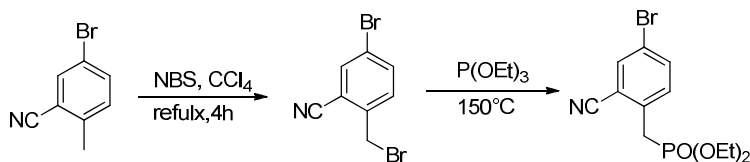


Intermediate 3: 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)phenyl)acetamide



Pd(dppf)Cl₂ (54 mg, 0.073 mmol) was added to the mixture of *N*-(4-bromo-3-(trifluoromethyl)phenyl)-2-(4-(ethylsulfonyl)phenyl)acetamide (655 mg, 1.455 mmol), 4,4,4',5,5,5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (443 mg, 1.746 mmol) and potassium acetate (428 mg, 4.364 mmol) in 1,4-dioxane (10 mL). Then the reaction mixture was heated to 100 °C for 10 h under argon. After completion of the reaction as indicated by TLC, the mixture was filtered through celite and the filtrate was evaporated *in vacuo* to afford yellow oil, which was purified by column chromatography on silica gel (EtOAc:PE = 1:5) to give 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)phenyl)acetamide crude as a yellow solid (725 mg, 48% of the crude).

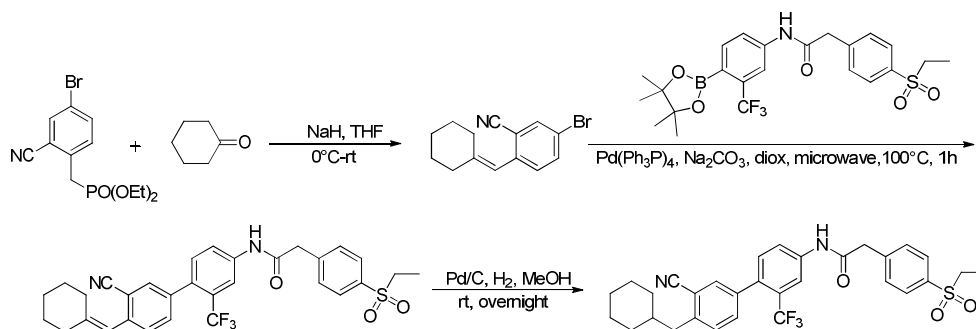
Intermediate 4: diethyl 4-bromo-2-cyanobenzylphosphonate.



Step 1: 5-Bromo-2-methylbenzonitrile (1 g, 5.1 mmol), NBS (1 g, 5.6 mmol) and AIBN (25 mg, 0.15 mmol, 3%) were dissolved in CCl_4 (10 mL). The reaction mixture was heated under reflux for 4 h and cooled at rt. The mixtures were filtered, and the filtrate was dried *in vacuo*, and chromatographed over a silica column (EtOAc:PE = 0:1) to obtain 5-bromo-2-(bromomethyl)benzonitrile as a white solid (911 mg, 65.1% yield).

Step 2: The mixture of 5-bromo-2-(bromomethyl)benzonitrile (600 mg, 2.18 mmol) and $\text{P}(\text{OEt})_3$ (725 mg, 6.22 mmol), and the mixture was heated at 150 °C for 2 h. After the reaction was complete, the mixture was purified by column chromatography on silica gel (EtOAc:PE = 1:10 to 1:2) to give diethyl 4-bromo-2-cyanobenzylphosphonate as a colorless solid (710 mg, 97.9% yield).

Preparation of *N*-(3'-cyano-4'-(cyclohexylmethyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (7d)



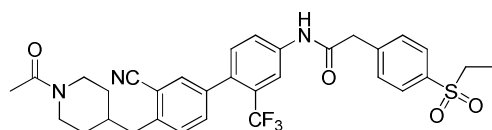
Step 1: To a solution of 4-bromo-2-cyanobenzylphosphonate (220 mg, 0.662 mmol) in tetrahydrofuran (THF) (5 mL) cooled at 0 °C was added NaH (60% in mineral oil, 79 mg, 1.986 mmol). The resulting mixture was stirred at room temperature for 30 mins. Cyclohexanone (65 mg, 0.662 mmol) was added. After stirring at room temperature overnight, the reaction was quenched with water, and then extracted with EtOAc for 3 times. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. After filtration, solvent was removed *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 0:1) to give 5-bromo-2-(cyclohexylidenemethyl)benzonitrile as a colorless oil (135 mg, 73.8% yield).

Step 2: $\text{Pd}(\text{Ph}_3\text{P})_4$ (5 mg, 0.004 mmol) was added to the solution of 5-bromo-2-(cyclohexylidenemethyl)benzonitrile (29 mg, 0.106 mmol), 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)phenyl)acetamide (44 mg pure material via pre-TLC) and 2M aqueous Na_2CO_3 (0.10 mL) in 1,4-dioxane (2 mL). The

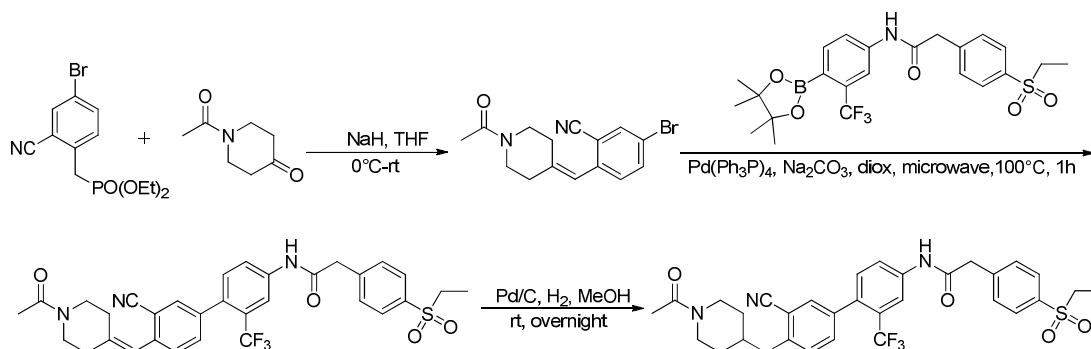
mixture was bubbled with argon. Then the reaction vessel was sealed and heated in the microwave at 100 °C for 1 h. After completion of the reaction as indicated by TLC, the mixture was filtered through celite and the filtrate was evaporated *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 0:1 to 1:2) to give *N*-(3'-cyano-4'-(cyclohexylidenemethyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide as a yellow oil (45 mg, 89.6% yield).

Step 3: *N*-(3'-cyano-4'-(cyclohexylidenemethyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (45 mg, 0.079 mmol) was dissolved in methanol (5 mL). Pd/C (9 mg, 0.079 mmol, 10%) was added and the resulting mixture was stirred under H₂ (excess) at room temperature overnight. After completion of the reaction as indicated by LC-MS, the mixture was filtered through celite and the filtrate was evaporated *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 1:2) to give *N*-(3'-cyano-4'-(cyclohexylmethyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide as a colorless oil (32 mg, 71.1% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.34 (s, 1H), 7.92 (s, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.57 – 7.48 (m, 3H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 3.84 (s, 2H), 3.13 (q, *J* = 7.4 Hz, 2H), 2.75 (d, *J* = 6.7 Hz, 2H), 1.75 – 1.62 (m, 6H), 1.29 (t, *J* = 7.4 Hz, 3H), 1.25 – 1.16 (m, 3H), 1.11 – 1.00 (m, 2H). MS (ESI): *m/z* calcd for C₃₁H₃₁F₃N₂O₃S [M+H]⁺, 569.2; found, 568.8.

***N*-(4'-((1-acetylpiperidin-4-yl)methyl)-3'-cyano-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (7e)**



Preparation of *N*-(4'-((1-acetylpiperidin-4-yl)methyl)-3'-cyano-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (7e)



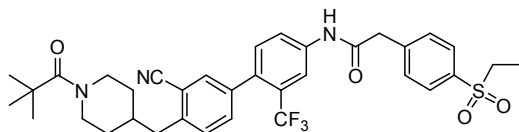
Step 1: To a solution of 4-bromo-2-cyanobenzylphosphonate (250 mg, 0.753 mmol) in

tetrahydrofuran (THF) (5 mL) cooled at 0 °C was added NaH (60% in mineral oil, 91 mg, 2.258 mmol). The resulting mixture was stirred at room temperature for 30 mins. 1-Acetylpiperidin-4-one (106 mg, 0.753 mmol) was added. After stirring at room temperature overnight, the reaction was quenched with water, and then extracted with EtOAc for 3 times. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. After filtration, solvent was removed *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 1:5 to 1:1) to give 2-((1-acetylpiperidin-4-ylidene)methyl)-5-bromobenzonitrile as a yellow solid (176 mg, 73.3% yield).

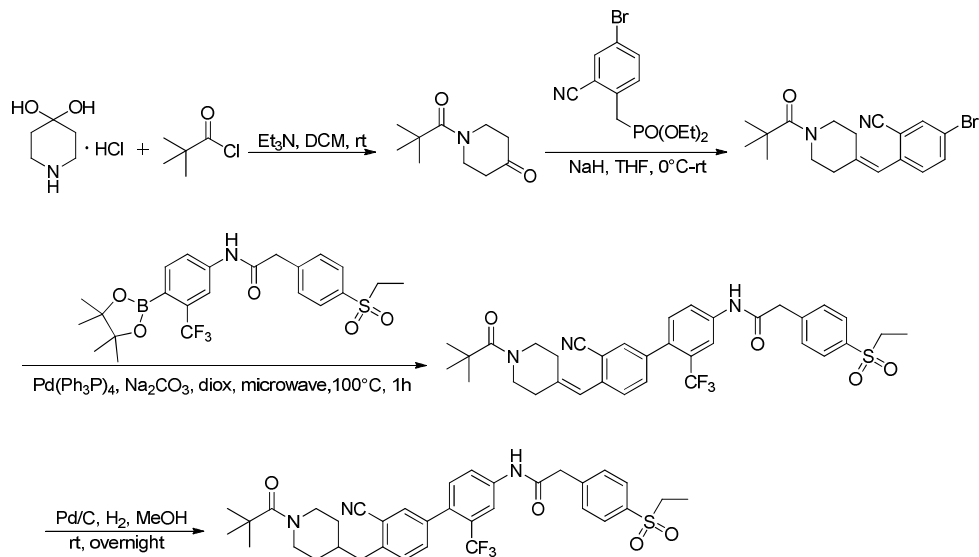
Step 2: Pd(Ph₃P)₄ (9 mg, 0.008 mmol) was added to the solution of 2-((1-acetylpiperidin-4-ylidene)methyl)-5-bromobenzonitrile (65 mg, 0.20 mmol), 2-(4-(ethylsulfonyl)phenyl)-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)phenyl)acetamide (158 mg crude) and 2M aqueous Na₂CO₃ (0.16 mL) in 1,4-dioxane (2 mL). The mixture was bubbled with argon. Then the reaction vessel was sealed and heated in the microwave at 100 °C for 1 h. After completion of the reaction as indicated by TLC, the mixture was filtered through celite and the filtrate was evaporated *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 1:2 to 3:1) to give N-(4'-((1-acetylpiperidin-4-ylidene)methyl)-3'-cyano-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide as a yellow solid (90 mg) and 2-(4-(ethylsulfonyl)phenyl)-N-(3-(trifluoromethyl)phenyl)acetamide as a white solid (83 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.91 (d, *J* = 8.3 Hz, 2H), 7.82 (s, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 2H), 7.44 (t, *J* = 8.1 Hz, 1H), 7.38 (d, *J* = 7.5 Hz, 2H), 3.84 (s, 2H), 3.13 (q, *J* = 7.5 Hz, 2H), 1.30 (t, *J* = 7.4 Hz, 3H). MS (ESI): *m/z* calcd for C₁₇H₁₆F₃NO₃S [M+H]⁺, 372.1; found, 371.9.

Step 3: N-(4'-((1-Acetylpiperidin-4-ylidene)methyl)-3'-cyano-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (90 mg, 0.148 mmol) was dissolved in methanol (10 mL). Pd/C (16mg, 0.148mmol, 10%) was added and the resulting mixture was stirred under H₂ (excess) at room temperature overnight. After completion of the reaction as indicated by LC-MS, the mixture was filtered through celite and the filtrate was evaporated *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE= 2:1) to give N-(4'-((1-acetylpiperidin-4yl)methyl)-3'-cyano-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide as a yellowish solid (85 mg, 94.4% yield). ¹H NMR (400 MHz, CDCl₃) δ 9.10 (s, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.90 (s, 1H), 7.84 (d, *J* = 8.1 Hz, 2H), 7.60 – 7.51 (m, 3H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 8.8 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 1H), 4.62 (d, *J* = 12.8 Hz, 1H), 3.91 – 3.79 (m, 3H), 3.15 – 3.01 (m, 3H), 2.91 – 2.74 (m, 2H), 2.53 (t, *J* = 12.1 Hz, 1H), 2.11 (s, 3H), 1.97 – 1.90 (m, 1H), 1.78 (d, *J* = 12.4 Hz, 1H), 1.66 (d, *J* = 12.9 Hz, 1H), 1.30 – 1.23 (m, 5H). MS (ESI): *m/z* calcd for C₃₂H₃₂F₃N₃O₄S [M+H]⁺, 612.2; found, 611.8.

***N*-(3'-cyano-4'-((1-pivaloylpiperidin-4-yl)methyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (7f)**



Preparation of *N*-(3'-cyano-4'-((1-pivaloylpiperidin-4-yl)methyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (7f)



Step 1: Pivaloyl chloride (942 mg, 7.8 mmol) was dissolved in DCM (5 mL) and was added dropwise to a suspension of 4-piperidone monohydrate hydrochloride (600 mg, 3.9 mmol) and dry triethylamine (1.6 mL, 11.7 mmol) in dry DCM (10 mL). The solution was stirred at room temperature for 4 h. The organic phase was washed with water (2 × 10 mL) and dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated *in vacuo* and the crude product was purified by recrystallization in petroleum ether/ethyl acetate to yield 1-pivaloylpiperidin-4-one as a white lamellar crystal (495 mg, 69.1% yield). MS(ESI) m/z 184.1 (M+H)⁺.

Step 2: To a solution of 4-bromo-2-cyanobenzylphosphonate (210 mg, 0.632 mmol) in tetrahydrofuran (THF) (5 mL) cooled at 0 °C was added NaH (60% in mineral oil, 76 mg, 1.896 mmol). The resulting mixture was stirred at room temperature for 30 mins. 1-Pivaloylpiperidin-4-one (116 mg, 0.632 mmol) was added. After stirring at room temperature overnight, the reaction was quenched with water, and then extracted with EtOAc for 3 times. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. After filtration, solvent was removed *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 1:15) to give 5-bromo-2-((1-

pivaloylpiperidin-4-ylidene)methyl)benzonitrile as a yellow oil (133 mg, 61.0% yield).

Step 3: Pd(Ph₃P)₄ (11 mg, 0.009 mmol) was added to the solution of 5-bromo-2-((1-pivaloylpiperidin-4-ylidene)methyl)benzonitrile (78 mg, 0.22 mmol), 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)phenyl)acetamide (180 mg crude, 48%) and 2M aqueous Na₂CO₃ (0.18 mL) in 1,4-dioxane (2 mL). The mixture was bubbled with argon. Then the reaction vessel was sealed and heated in the microwave at 100 °C for 1 h. After completion of the reaction as indicated by TLC, the mixture was filtered through celite and the filtrate was evaporated *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 1:2) to give *N*-(3'-cyano-4'-((1-pivaloylpiperidin-4-ylidene)methyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide as a yellow oil (108 mg).

Step 4: *N*-(3'-cyano-4'-((1-pivaloylpiperidin-4-ylidene)methyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (108 mg, 0.166 mmol) was dissolved in methanol (10 mL). Pd/C (18mg, 0.166mmol, 10%) was added and the resulting mixture was stirred under H₂ (excess) at room temperature overnight. After completion of the reaction as indicated by LC-MS, the mixture was filtered through celite and the filtrate was evaporated *in vacuo* to afford the crude, which was purified by column chromatography on silica gel (EtOAc:PE = 1:2) to give *N*-(3'-cyano-4'-((1-pivaloylpiperidin-4-yl)methyl)-2-(trifluoromethyl)biphenyl-4-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide as a yellowish solid (98 mg, 90.7% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.01 (s, 1H), 7.92 – 7.83 (m, 4H), 7.56 (d, *J* = 9.8 Hz, 3H), 7.43 (d, *J* = 7.0 Hz, 1H), 7.28 (d, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 1.3 Hz, 1H), 4.44 (d, *J* = 14.0 Hz, 2H), 3.87 (s, 2H), 3.13 (q, *J* = 7.5 Hz, 2H), 2.83 (d, *J* = 7.0 Hz, 2H), 2.80 – 2.68 (m, 2H), 2.03 – 1.89 (m, 1H), 1.71 (d, *J* = 12.5 Hz, 2H), 1.65 (s, 2H), 1.32 – 1.26 (m, 12H). MS (ESI): *m/z* calcd for C₃₅H₃₈F₃N₃O₄S [M+H]⁺, 654.2; found, 653.9.

3. Crystallography study and data for 5d and 6

X-ray study: protein preparation, crystallization and structure determination:

Human RORγt 263-509 was expressed from a modified pET21d vector in *E. Coli* BL21(DE3) cells with a TEV- cleavable N-terminal hexa-histidine tag. The cells were harvested after 12-16 hours of protein expression at 15°C and lysed in Nickel Buffer A (30 mM Imidazole, pH 8.0, 150 mM NaCl).

Cleared lysate was applied to a 5ml HisTrap column (GE healthcare) at 2ml/min. The column was washed with 5% Nickel Buffer B (500 mM Imidazole, pH 8.0, 150 mM NaCl) and eluted with 5% to 100% gradient Nickel Buffer B. The protein was further purified with a Superdex 200 16/60 column (GE Healthcare) in the storage buffer (20mM Hepes, pH 7.4, 50 mM NaCl). 5-fold excess of RORγt activator compound and 2-fold excess of the SRC2-2 peptide

(KEKHKILHRLQDSS, China Peptide) were preincubated with purified ROR γ t LBD on ice for 30 minutes before filter concentrated to 10 mg/ml in storage buffer.

Crystals of ROR γ t LBD complexed with activator compound were grown at 20°C in hanging drops by mixing 1 μ l of protein (at 10 mg/ml) and 1 μ l of a reservoir solution (0.2M Ammonium Sulfate, 0.1M Bis-Tris, pH 6.5, 14% (w/v) PEG 8000). Crystals were quickly transferred to reservoir solution supplemented with 20% (v/v) glycerol before flash-frozen in liquid nitrogen. X-ray diffraction data were collected at the 17U beamline of the Shanghai Synchrotron Radiation Facility (SSRF). All data were processed with HKL2000 denzo/scalepack package (Otwinowski and Minor, 1997).

Molecular replacement using PHASER (Read et al., 2001) was done with a previously solved ROR LBD structure (PDB code: 3L0J) as initial search model. Further refinement used REFMAC5 (Murshudov et al., 1997) and manual modeling built with COOT (Emsley and Cowtan, 2004). Structure validation was checked with PROCHECK (Laskowski et al., 1993) and MOLPROBITY (Davis et al., 2007).

Crystallographic data and refinement statistics:

For details of the data collection, and refinement statistics, see Table S-1 and Table S-2.

Table S-1. Crystallographic data and refinement statistics for ROR γ t LBD with **5d**

Data collection	
Synchrotron	SSRF ^a
Beamline	17U
Space group	P4 ₁ 2 ₁ 2
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	<i>a</i> =62.1, <i>b</i> =62.1, <i>c</i> =154.8
α , β , γ (°)	α = β = γ =90
Wavelength (Å)	0.9792
Resolution (Å) ^b	57.6-2.65
<i>R</i> _{sym} (%) ^b	10.5 (41.5)
$\langle I / \sigma I \rangle$ ^b	23.4 (5.9)
Completeness (%) ^b	92.6 (95.0)
Redundancy ^c	7.3 (7.4)
Refinement	
Resolution (Å)	57.6-2.65
No. reflections	8323

Test set	417 (4.8 %)
$R_{\text{work}} / R_{\text{free}}$ (%)	20.6 / 25.3
R.m.s deviations	
Bond lengths (Å)	0.0163
Bond angles (°)	1.605

^aSSRF, Shanghai Synchrotron Radiation Facility

^bHighest-resolution shell is shown in parentheses.

Table S-2. Crystallographic data and refinement statistics for RORγt LBD with **6**

Data collection

Synchrotron	SSRF ^a
Beamline	17U
Space group	P4 ₁ 2 ₁ 2
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	<i>a</i> =62.2, <i>b</i> =62.2, <i>c</i> =154.8
α , β , γ (°)	α = β = γ =90
Wavelength (Å)	0.9792
Resolution (Å)	57.7-2.2
R_{sym} (%) ^b	11.7 (44.9)
$\langle I / \sigma I \rangle$ b	24.19 (7.79)
Completeness (%) ^b	100 (100)
Redundancy ^b	12.8 (13.2)

Refinement

Resolution (Å)	57.7-2.2
No. reflections	15356
Test set	813(5%)
$R_{\text{work}} / R_{\text{free}}$ (%)	20.8/ 24.7
R.m.s deviations	
Bond lengths (Å)	0.0044
Bond angles (°)	0.8253

^aSSRF, Shanghai Synchrotron Radiation Facility

^bHighest-resolution shell is shown in parentheses.

4. Assay description

a) ROR γ FRET Assay

The assays were performed in an assay buffer consisting of 50 mM NaF, 50 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.4, 50 μ M 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate, 0.1 mg/ml bovine serum albumin, and 10 mM dithiothreitol in 384-well plates (Greiner 784076, Longwood, FL). The total volume was 10 μ l/well. The europium-labeled SRC1 solution was prepared by adding an appropriate amount of biotinylated SRC and europiumlabeled streptavidin (PerkinElmer Life and Analytical Sciences, Waltham, MA) into assay buffer, with final concentrations of 27 and 3.3 nM, respectively. The APC-labeled-LBD solution was prepared by adding an appropriate amount of biotinylated ROR γ -LBD and APC-labeled streptavidin (CR130-100; PerkinElmer Life and Analytical Sciences) at a final concentration of 33 nM each. After 15 min of incubation at room temperature, a 20-fold excess of biotin was added to block the remaining free streptavidin. Equal volumes of europium-labeled SRC- and APC-labeled ROR γ -LBD were then mixed with 0.2 μ M surrogate agonist *N*-(2-chloro-6-fluorobenzyl)-*N*-((2'-methoxy-[1,1'-biphenyl]-4-yl)methyl)benzenesulfonamide and dispensed into 384-well assay plates at 10 μ l volume/well. The 384-well assay plates had 25 nl of test compound in DMSO predisposed into each well. The plates were incubated for 1 h at room temperature and then read on ViewLux (PerkinElmer Life and Analytical Sciences) in LANCE mode configured for europium-APC labels.

b) ROR γ dual FRET assay

Materials: RAR-related orphan receptor gamma (ROR γ) protein was made at GSK. Proteins were chemically biotinylated using standard methods. Typically proteins have between 1-5 biotins. Biotinylated-Peptide was purchased from CPC scientific. Streptavidin-labeled APC (CR130-150) and Eu-W1024 labeled Streptavidin (AD0063) were purchased from Perkin Elmer. DMSO purchased from EMD (MX14561). MOPS (M1254), Sodium Fluoride (S6521) and CHAPS (C3023) were purchased from Sigma. DTT (F780-01) was purchased from JT Baker. Immunopure D-biotin (29129) was purchased from Pierce. BSA (100350), Frac V, fatty acid free was purchased from Boehringer Mannheim.

Compound preparation: Compounds were diluted in 100% neat DMSO at 10 mM. The compounds were then dispensed into an intermediate plate (polypropylene Greiner PP V-bottom: 781280) to make serial dilutions in 100% neat DMSO. Approximately 100 nL of the serial dilution was added to the assay plate (Costar 3573) using a Hummingbird (Genomic Solutions).

Stock Buffer: A 0.5 M solution of MOPS was made by adding 104 grams of MOPS to 800 mL H₂O in a graduated cylinder, using a calibrated pH meter, add increasing amounts NaOH to give a final pH of 7.5. This solution was filtered using a Costar 0.2 um filtering apparatus and stored in the refrigerator until ready to use.

Assay buffer: Add 100 mL of 10x MOPS stock solution to graduated cylinder bring up to 800 mL. Add 2.09 grams of NaF, 0.03 grams of CHAPS to the flask, 0.1 grams of BSA to the flask. Make sure all components are dissolved. Add dH₂O to give final volume of 1L. The assay buffer was filtered with a Costar 0.2 um filtering apparatus. On assay day, DTT was added to the assay buffer to a final concentration of 10 mM. Fresh DTT solid should be used.

Assay: ROR γ were assayed using the generic protocol described. To polypropylene costar conical centrifuge tubes, add assay buffer, an appropriate amount of biotinylated-SRC1(2) from the 1E-4 M (100 uM) stock solution to give a final concentration of 4E-8 M (40 nM). To the above biotinylated SRC1(2) solution, add an appropriate amount of Europium-labeled streptavidin to give a final concentration of 1E-8 M (10 nM). Invert gently to mix. Incubate 15 minutes at room temperature. At the same time, but in another polypropylene tube add an appropriate amount of biotinylated-ROR protein from the stock solution to give a final concentration of 4E-8 M (40 nM). To the biotinylated-ROR solution, add an appropriate amount of APC-labeled streptavidin to give a final concentration of 2E-8 M (20 nM). Invert gently to mix. Incubate 15 minutes at room temperature. Following the 15 minute incubations, add 20-fold excess biotin from the 1E-2 M (10 mM) stock solution. Invert gently to mix. Incubate 10 minutes at room temperature. Gently mix the above solutions together to give a final solution containing 20 nM ROR_10 nM APC and 20 nM SRC1(2)_5nM SA_EU. Incubate 5 min and use a Thermo Combi Multidrop to add 25 uL peptide/ROR solution to assay plates containing 100 nL of test compound. Incubate plates for 1 hr at room temperature, then read on ViewLux in Lance mode for EU/APC.

Data analysis: Raw data was analyzed using ABASE (IBDS) software. The data was normalized initially using the following equation: Normalization = 100*((Basal HTRF –

value)/(Basal HTRF – Minimal HTRF). The normalized data was then fit to a 4 – parameter logistic equation.

c) ROR γ peptide profiling assay

Materials. RAR-related orphan receptor gamma (ROR γ) protein was made at GSK. Protein was chemically biotinylated using standard methods. Typically proteins have between 1-5 biotins. Biotinylated-Peptides were purchased from CPC scientific. Streptavidin-labeled APC (CR130-150) and Eu-W1024 labeled Streptavidin (AD0063) were purchased from Perkin Elmer. DMSO purchased from EMD (MX14561). MOPS (M1254), Sodium Fluoride (S6521) and CHAPS (C3023) were purchased from Sigma. DTT (F780-01) was purchased from JT Baker. Immunopure D-biotin (29129) was purchased from Pierce. BSA (100350), Frac V, fatty acid free was purchased from Boehringer Mannheim.

Compound preparation: Compounds were diluted in 100% neat DMSO at 10 mM. The compounds were then dispensed into an intermediate plate (polypropylene Greiner PP V bottom: 781280) to make serial dilutions in 100% neat DMSO. Approximately 100 nL of the serial dilution was added to the assay plate (Costar 3573) using a Hummingbird (Genomic Solutions).

Stock Buffer: A 0.5M solution of MOPS was made by adding 104 grams of MOPS to 800 mL H₂O in a graduated cylinder, using a calibrated pH meter, add increasing amounts NaOH to give a final pH of 7.5. This solution was filtered using a Costar 0.2 μ m filtering apparatus and stored in the refrigerator until ready to use.

Assay buffer: Add 100 mL of 10x MOPS stock solution to graduated cylinder bring up to 800 mL. Add 2.09 grams of NaF, 0.03 grams of CHAPS to the flask, 0.1 grams of BSA to the flask. Make sure all components are dissolved. Add dH₂O to give final volume of 1 L. The assay buffer was filtered using a Costar 0.2 μ m filtering apparatus. On assay day, DTT was added to the assay buffer to a final concentration of 10 mM. Fresh DTT solid should be used.

Assay: Peptide interactions with ROR γ were determined using the generic protocol described. The following peptides were used in this experiment. SRC1(676-700), RIP140 (366-390), SMRT2 (2329-2354) and NCOR2 (2251-2257). Each peptide was made in individual polypropylene costar conical centrifuge tubes. To each tube assay buffer was added, an appropriate amount of biotinylated peptide from the 1E-4 M (100 μ M) stock solution to give a

final concentration of 4E-8 M (40 nM). To the above biotinylated peptide solution, add an appropriate amount of Europium-labeled streptavidin to give a final concentration of 1E-8 M (10 nM). Invert gently to mix. Incubate 15 minutes at room temperature. At the same time, but in another polypropylene tube add an appropriate amount of biotinylated-ROR γ protein from the stock solution to give a final concentration of 4E-8 M (40 nM). To the biotinylated ROR γ solution, add an appropriate amount of APC-labeled streptavidin to give a final concentration of 2E-8 M (20 nM). Invert gently to mix. Incubate 15 minutes at room temperature. Following the 15 minute incubations, add 20-fold excess biotin from the 1E-2 M (10 mM) stock solution. Invert gently to mix. Incubate 10 minutes at room temperature. Gently mix the above solutions together to give a final solution containing 20 nM ROR_10 nM APC and 20 nM Peptide_5 nM SA_EU. Incubate 5 min and use a Thermo Combi Multidrop to add 25 μ L peptide/ROR γ solution to assay plates containing 100 nL of test compound. Incubate plates for 1 hr at room temperature, then read on ViewLux in Lance mode for EU/APC.

Data analysis: The data was normalized initially using the following equation: $\text{Normalization} = 100 * [(\text{Unk} - \text{Low_Ctl}) / (\text{Hi_Ctl} - \text{Low_Ctl})]$. The normalized data was then was fit to a 4 – parameter non-linear equation.

d) Mouse Th17 cell differentiation assay

CD4⁺T cells were purified from spleens using anti-CD4 magnetic microbeads (Miltenyi Biotec) and MACS columns (purity was >95%). CD4⁺ cells were resuspended in RPMI 1640 complete medium and added to 96-well plates pre-coated with anti-mCD3 (5 μ g/ml) at 10⁵ cell/well in a total volume of 80 μ L. One hundred microliters of a 2 \times cytokine cocktail and 20 μ L of compounds (100 \times) were added to the well. The final concentrations of antibodies and cytokines (all from R&D Systems, Minneapolis, MN) were as follows: anti-mCD28 (5 μ g/ml); anti-mIFN- γ (10 μ g/ml); anti-mIL4 (10 μ g/ml); mIL-6 (20 ng/ml); hTGF- β 1 (5 ng/ml). The culture was incubated in 37°C for 3 days, and supernatants were collected for ELISA. The mouse IL-17 ELISA was performed according to the manufacturer's instruction (R&D Systems). The results were analyzed using Prism software with nonlinear regression to determine the IC₅₀ value.

5. Docking studies

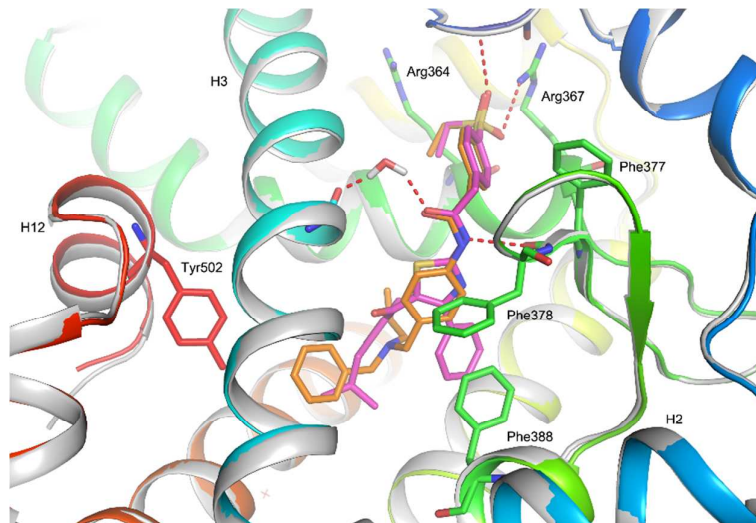


Figure S1. Overlay of agonist **5d** (magenta) and **3a** (brown) bound to ROR γ t LBD. Co-crystal structures of agonist **5d** (PDB 5YP5) and **3a** (PDB 4NIE) bound to ROR γ t LBD were superposed together, with ROR γ t LBD shown in rainbow and grey cartoons, respectively.