Supporting Information: Discovery of Highly Potent LXRβ Agonists

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SI Table 1. In vitro LXR assay data and standard deviations.

|   | LXRβ Binding Ki, nM<sup>a</sup> | LXRα Binding Ki, nM<sup>a</sup> | LXRβ EC<sub>50</sub> nM (%Eff)<sup>b</sup> | LXRα EC<sub>50</sub> nM (%Eff)<sup>b</sup> | ABCA1 HeLa EC<sub>50</sub> nM (%Eff)<sup>b</sup> | hWBA EC<sub>50</sub> nM (%Eff)<sup>c</sup> |
|---|-------------------------------|-------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| 5 | 14 ± 6                        | 68 ± 10                       | 250 ± 42 (72 ± 10%)             | 220 ± 94 (38 ± 7%)              | 33 ± 7 (50 ± 8%)                | 1200 ± 300 (55 ± 9%)            |
| 6 | 11                            | 16                            | 170 (41%)                       | 99 (20%)                        | 18 (42%)                        | 380 (32%)                       |
| 7 | 5                             | 31                            | 60 (50%)                        | 100 (39%)                       | 9 (54%)                         | 300 (72%)                       |
| 8 | 13                            | 74                            | 170 (74%)                       | 110 (46%)                       | 12 (56%)                        | 300 (46%)                       |
| 9 | 40                            | 180                           | 220 (93%)                       | 120 (47%)                       | 21 (68%)                        | 870 ± 470 (47 ± 12%)            |
| 10| 10 ± 2                        | 53 ± 17                       | 72 ± 9 (83 ± 5%)                | 76 ± 14 (29 ±3 %)               | 8 ± 2 (43 ± 1 %)                | 57 ± 14 (47 ± 9 %)              |
| 11| 14 ± 4                        | 81 ± 19                       | 160 ± 35 (68 ± 6%)              | 130 ± 16 (13 ± 2 %)             | 12 ± 3 (15 ± 3%)                | 43 (28 %)                       |
| 12| 6                             | 38                            | 42 (72%)                        | 30 (25%)                        | 3 (23%)                         | 15 (43%)                       |
| 13| 14 ± 11                       | 53 ± 19                       | 72 ± 14 (68 ± 7%)               | 72 ± 28 (12 ± 2 %)              | 8 ± 2 (16 ±4 %)                 | 76 ± 49 (34 ± 6 %)              |
| 14| 18                            | 9                             | 50 (79%)                        | 57 (25%)                        | 2 (29%)                         | 46 ± 12 (35 ± 8 %)              |
| 15| 12 ± 7                        | 19 ± 16                       | 24 ± 9 (88 ± 7 %)               | 8 ± 1 (20 ± 2 %)                | 0.6 ± 0.2 (29 ± 4%)             | 9 ± 3 nM (26 ± 4 %)             |
| 16| 14                            | 70                            | 20 (86%)                        | 11 (15%)                        | 1 (30%)                         | 41 (33%)                       |
| 17| 48                            | 50                            | 27 (51%)                        | 8 (6%)                         | 2 (12%)                         | 5 (16%)                        |
| 18| 11                            | 75                            | 140 (54%)                      | 69 (17%)                       | 5 (43%)                         | 42 (51%)                       |
| 19| 13                            | 17                            | 25 (67%)                       | 12 (18%)                       | 2 (9%)                         | 23 (17%)                       |

<sup>a</sup> Mean values are reported for two independent experiments. The mean and standard deviation are reported when three or more experiments were run.<br>
<sup>b</sup>EC<sub>50</sub> values determined from dose response curves with four points at each test concentration. The mean and standard deviation are reported when three or more experiments were run.<br>
<sup>c</sup>The mean and standard deviation are reported when three or more experiments have been run, all other data was n = 1.

SI Table 2. PK in mice after p.o. dose and cynomolgus monkeys (cyno) after p.o. and i.v. dose.
**Table 3. Antagonist and WBA data for leads 5 and 15.**

| Assay                        | 5 (BMS-779788) | 15 (BMS-852927) |
|------------------------------|---------------|-----------------|
| LXRα Antagonist Assay IC₅₀ (nM) | 2900 (62%), n = 1 | 69 ± 25 (83%)   |
| LXRβ Antagonist Assay IC₅₀ (nM) | >10000        | >10000          |
| Human WBA (ABCA1) EC₅₀ (nM)  | 1200 ± 300 (55 ± 9%) | 9 ± 3 (26 ± 4%) |
| Human WBA (ABCG1) EC₅₀ (nM)  | 1100 ± 200 (60 ± 2%) | 10 ± 2 (33 ± 11%) |
| Cyno WBA (ABCG1) EC₅₀ (nM)   | 140 (41%), n=1 | 5 ± 4 (32 ± 7%) |

*Means reported with standard deviations.

**Experimental Methods**

**Abbreviations:** α-1-acid glycoprotein (α–GP); acetonitrile (ACN); ATP binding cassette transporters (ABCA1, ABCG1, ABCG5 or ABCG8); acetate (Ac); dimethoxyethane (DME); 4-dimethylaminopyridine (DMAP); dimethyl sulfoxide (DMSO); 1,1’-bis(diphenylphosphanyl)ferrocene (dppf); ethyl (Et); ethyl acetate (EtOAc); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI); ethylenediaminetetraacetic acid (EDTA); flame ionization detector (FID); gas chromatogram (GC); high performance liquid chromatography (HPLC); high resolution mass spectra (HRMS); lithium hexamethyldisilazide (LHMDS); liver X receptor (LXR); methyl (Me); 3-chloroperbenzoic acid (mCPBA); pregnane X receptor, PXR; retinoid X receptor (RXR); RT = retention time; room temperature (rt); tetrahydrofuran (THF); ultra performance liquid chromatography (UPLC); WBA (whole blood assay).

**Synthesis**
All reactions were carried out under a static atmosphere of argon or nitrogen and stirred magnetically unless otherwise stated. All reagents used were of commercial quality. All solvents were removed by rotary evaporation under vacuum using a standard rotary evaporator equipped with a dry ice condenser. $^1$H NMR, $^{13}$C NMR and spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer for proton and 100 MHz for carbon or on a Bruker 500 MHz system spectrometer for proton and 126 MHz for carbon unless otherwise noted. $^{19}$F NMR spectra were recorded on a Bruker Avance 400 MHz NMR or a JEOL spectrometer 500 MHz and referenced to CFCl₃ at 0.0 ppm. Chemical shifts are given in parts per million (ppm), and coupling constants (J values) are given in hertz (Hz). Selected data are reported in the following manner: chemical shift, multiplicity, coupling constants, proton number. Low resolution electrospray ionization (ESI) mass spectra were recorded on a Perkin-Elmer SCIEX HPLC/MS instrument using reverse-phase conditions (acetonitrile/water, 0.05% TFA) unless otherwise noted. Unless otherwise noted HPLC were run on a Shimadzu Analytical HPLC system with conditions reported. The HRMS was determined on a Thermo LTQ-Discovery Orbitrap. Residual solvents were determined with an Agilent 6890 Plus GC/ FID Thermo Trace GC Ultra/DSQ MS instrument.

HPLC Purity Method A was determined on a Waters Acquity UPLC with SQD mass spectrometer with a Waters Acquity UPLC BEH C18, 1.7 μm, 2.1 x 50 mm column. Solvent A: 95% water, 5% Acetonitrile with 10 mM Ammonium Acetate; Solvent B: 5% water, 95% acetonitrile with 10 mM Ammonium Acetate. Gradient hold 100% A for 0.5 min. with gradient 0-100 % B over 4 minutes. Flow rate 1.0 mL/min.

HPLC Purity Method B was determined on a Shimadzu Analytical HPLC system running Prominence MD software. Solvent A: 5% acetonitrile, 95% water, 0.05% TFA. Solvent B: 5% water, 95% acetonitrile, 0.05% TFA. Linear gradient of 10 to 100% B over 12 min, with 3 min hold at 100% B. Flow rate 1.0 mL/min. Detector 1: Column: Sunfire C18; 3.5 μm; 4.6 x 150 mm. Detector 2: Column: XBridge Phenyl 3.5 μm; 4.6 x 150 mm.
SI Scheme 1: Synthesis of boronates S1-6 and S1-11.

![Reaction diagram]

Synthesis of boronates S1-6 and S1-11. Reagent and conditions: a) EDCI, DMAP, EtOH, CH₂Cl₂, 40-45 °C, 90% yield; b) NaSMe, THF, 80-85 °C, 99% crude yield; c) LiBH₄, THF, 80-85 °C; d) mCPBA, CH₂Cl₂, 0 °C - rt, 50-65% yield 2-3 steps; e) KOAc, PdCl₂(dppf), bis(pinacolato)diboron, DMSO or toluene, 100 °C, 43-45% yield; f) i) LHMDS, THF, hexanes, 0 °C - rt; ii) NaSCH₃, reflux, 92% crude yield; (g) (CH₃)₂SBH₃, THF, 80 °C, 100% crude yield.

SI Scheme 2. Synthesis of imidazole biphenyl agonists.
Synthesis of biphenyl imidazole agonists: (a) CH$_3$I, KOTBu, THF, 5-25 °C, 75-98% yields; (b) substituted 4-bromoaniline, (CH$_3$)$_3$Al, toluene, 60-90 °C; (c) (EtO)OCCOCH$_2$Br, NaHCO$_3$, THF, 80 °C; (d) TFA, 80 °C, 19-38% yield for 3 steps; (e) CH$_3$MgBr, toluene / CH$_2$Cl$_2$, 60-80 °C, 75-85% yield; (f) substituted phenyl boronate, PdCl$_2$(dppf), K$_2$CO$_3$, dimethoxyethane: H$_2$O (9:1), 80 °C, 20-78% yields.

Preparation of 4-Bromo-2-fluoro-benzoic acid ethyl ester (S1-2). Into a 1 L flask was weighed 24.7 g (113 mmol) of acid S1-1, 26.5 g (138 mmol) of EDCI, 1.7 g of DMAP, 425 mL of CH$_2$Cl$_2$, and 25 mL of ethanol. The resulting solution was heated at 40-45 °C for 24 h, and then was concentrated in vacuo to remove CH$_2$Cl$_2$. The residue was washed into a separatory funnel with ethyl acetate and 1 M HCl. The ethyl acetate was separated, washed with brine, dried with Na$_2$SO$_4$ and was concentrated in vacuo. The ester was recovered as a colorless oil (25 g, 90% yield).

Preparation of 4-Bromo-2-methylsulfanyl-benzoic acid ethyl ester (S1-3). The ester isolated above (25 g) was treated with 12.2 g of sodium thiomethoxide and 200 mL of THF, and the resulting suspension was heated to 80-85 °C for 5 h. The reaction mixture was concentrated to remove THF and was washed into a separatory funnel with ethyl acetate and 1 M HCl. The ethyl acetate was separated, washed with brine, dried with Na$_2$SO$_4$, and concentrated in vacuo to afford intermediate S1-3 as a light gray solid (27.5 g, 99% yield). $^1$H NMR (400 MHz, CDCl$_3$): δ 7.86 (d, J = 8 Hz, 1H), 7.36 (s, 1H), 7.28 (d, J = 8 Hz, 1H), 4.38 (q, J = 7 Hz, 2H), 2.45 (s, 3H), 1.39 (t, J = 7 Hz, 3H).
**Preparation of (4-Bromo-2-methylsulfanyl-phenyl)-methanol (S1-4).** Into a 1 L flask was weighed 27.5 g of ester S1-3 (99.9 mmol) and 150 mL of THF. A solution of 2.0 M LiBH₄ in THF (50 mL, 100 mmol) was added, and the reaction mixture was heated to 80-85 °C for 23h. The reaction mixture was cooled in an ice bath as acetone was added to quench the reaction. The mixture was then concentrated *in vacuo* and was washed into a separatory funnel with ethyl acetate and 1 M HCl. The ethyl acetate was separated, washed with brine, dried with Na₂SO₄, and concentrated *in vacuo*. The intermediate S1-4 was recovered as a colorless oil that solidified on standing (crude 25.5 g). ¹H NMR (400MHz, CDCl₃): δ 7.24-7.34 (m, 3H), 4.69 (s, 2H), 2.50 (s, 3H).

**Preparation of (4-Bromo-2-methanesulfonyl-phenyl)-methanol (S1-5).** The alcohol S1-4 (25.5 g) was dissolved in 250 mL of CH₂Cl₂, and the mixture was cooled in an ice bath, followed by portion wise addition of 44 g of 3-chloroperbenzoic acid (mCPBA, 77% max). The reaction mixture was then allowed to warm to rt where it remained for 22 h. The mixture was concentrated *in vacuo* to remove CH₂Cl₂, and the residue was washed into a separatory funnel with ethyl acetate and 1 M NaOH. The EtOAc was separated, washed with 1 M NaOH, dried with Na₂SO₄, and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (Biotage, 65 x 200 mm SiO₂, gradient elution from 100% hexanes to 100% ethyl acetate over 1 h). Appropriate fractions were combined and concentrated *in vacuo* to afford the intermediate S1-5 as a colorless, semi-crystalline solid (17.1 g, 65% yield two steps). ¹H NMR (400MHz, CDCl₃): δ 8.18 (s, 1H), 7.77 (d, J = 8 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 4.92 (s, 2H), 3.19 (s, 3H), 2.94 (br s, 1H).

**Preparation of [2-Methanesulfonyl-4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenyl]-methanol (S1-6).** Into a 1 L flask was weighed 17.1 g (64.3 mmol) of S1-5, 25 g (98.4 mmol) of bis(pinacolato)diboron, 5.0 g (6.1 mmol) of dichloro[1,1’-bis(diphenylphosphino)ferrocene]palladium (II) dicloromethane adduct, 23 g (234 mmol) of potassium acetate, and 175 mL of DMSO. The resulting suspension was heated at 100 °C for 18 h, and then was diluted with 200 mL of EtOAc and 200 mL of water. The resulting suspension was filtered through celite to remove solids, and the filtrate was transferred to a separatory funnel. The aqueous phase was separated and washed with EtOAc. The EtOAc washings were combined, washed with brine, dried with Na₂SO₄, and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (Biotage, 65 x 200 mm SiO₂, gradient elution from 100% hexanes to 40% ethyl acetate over 1 h). Appropriate fractions were combined and con-
centrated in vacuo. The partially purified product was dissolved in CH$_2$Cl$_2$ and was precipitated with hexanes. The intermediate S1-6 was recovered as an off-white powder (8.78 g, 43% yield). $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 8.45 (s, 1H), 8.04 (d, $J = 8$ Hz, 1H), 7.57 (d, $J = 8$ Hz, 1H), 4.96 (s, 2H), 3.17 (s, 3H), 1.35 (s, 6H), 1.24 (s, 6H).

*Preparation of 4-bromo-2-fluoro-6-(methylthio)benzoic acid (S1-8):*

A solution of 4-bromo-2,6-difluorobenzoic acid (300 g, 1.3 mol) in 3 L of dry THF was cooled to 0 °C, and 1.4 L of LHMDS in hexanes (1 M, 1.4 mol) was added dropwise. After the addition was complete, the reaction mixture was stirred for 30 minutes at rt, and then sodium thiomethoxide (97.4 g, 1.39 mol) was added. The mixture was heated to reflux for 3 hours. After cooling to rt, ice water was added to the mixture followed by EtOAc. The organic layer was separated and washed with 0.5N HCl, brine and dried over Na$_2$SO$_4$. Concentration on a rotary evaporator afforded S1-8 (310 g, 1.17 mol) that was >85% pure by HPLC. $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 7.39 – 7.42 (m, 1H), 7.30 (br s, 1H), 2.46 (s, 3H); HPLC (Hypersil BDS 5µ C18 4.6x50 mm, 0.8 mL/min, Solvent A: 0.1 % TFA in water, Solvent B: Acetonitrile gradient with 5 to 100 % B over 6 minutes then hold at 100 % B for 3 minutes): 5.02 minutes.

*Preparation of (4-bromo-2-fluoro-6(methylthio)phenyl)methanol (S1-9):*

To a stirring solution of S1-8 (310 g, 1.17 mol) in 3 L of anhydrous THF was slowly added borane-dimethyl sulfide (178 g, 222 mL, 2.34 mol). The mixture was brought to reflux for 3 hours at 80 °C. The mixture was cooled to rt and THF:water (1:1) was added. Ethyl acetate was added, and the organic layer was separated, washed with water, brine and dried over Na$_2$SO$_4$. Concentration on a rotary evaporator afforded S1-9 (300 g, 1.2 mol) that was 90% pure by HPLC. $^1$H-NMR (DMSO-d$_6$, 400 MHz) $\delta$ 7.29 – 7.31 (m, 1H), 7.23 (br s, 1H), 5.09 (t, $J = 5.28$, 1H), 4.43 – 4.48 (m, 2H), 2.49 (s, 3H). HPLC (Hypersil BDS 5µ C18 4.6x50 mm, 0.8 mL/min, Solvent A: 0.1% TFA in water, Solvent B: Acetonitrile gradient with 5-100% B over 6 minutes then hold at 100% B for 3 minutes): 5.07 minutes.

*Preparation of (4-bromo-2-fluoro-6(methylsulfonyl)phenyl)methanol (S1-10):*

To a solution of S1-9 (300 g, 1.2 mol) in chilled anhydrous CH$_2$Cl$_2$ was added mCPBA (825 g, 4.78 mol) portionwise. The reaction mixture was stirred overnight at rt, followed by addition of 1 N NaOH and CH$_2$Cl$_2$. The organic layer was separated and washed with water, brine, and dried with Na$_2$SO$_4$. Concentration on a rotary evaporator afforded the crude solid, which was purified by column chroma-
chromatography with petroleum ether/ethyl acetate. The desired product eluted at 20% ethyl acetate and afforded S1-10 (200 g, 0.71 mol, 54% yield for 3 steps) as a pale yellow solid that was >96% pure by HPLC. 

$^1$H-NMR (DMSO-d6, 400 MHz) $\delta$ 8.01 – 8.03 (m, 1H), 7.90 (m, 1H), 5.60 (br s, 1H), 4.86 (s, 2H), 3.43 (s, 3H). HPLC (Sunfire 3.5$\mu$m C18 4.6x50 mm, 1.0 mL/min, Solvent A: 5% Acetonitrile/water with 0.05% TFA, Solvent B: 95% Acetonitrile/water with 0.05% TFA, gradient with 10 to 100% B over 12 minutes then hold at 100% B for 3 minutes): 7.49 minutes.

Preparation of (2-fluoro-(methylsulfonyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (S1-11):

Intermediate S1-10 (200 g, 0.71 mol) and bis(pinacolato)diboron (197 g, 0.777 mol) were added to a 2 L, three neck flask, and the flask was evacuated and filled with N$_2$. Then PdCl$_2$(dpff) (28.9 g, 0.0353 mol) and KOAc (208 g, 2.12 mol) were added followed by evacuation of the flask and N$_2$ back-fill. Anhydrous toluene (2 L) that had been degassed with N$_2$ was added to the vessel followed by evacuation of the flask and subsequent N$_2$ back-fill. The reaction flask was heated to 90 °C for 16 hours. The reaction mixture was filtered through celite, and the celite was washed with CH$_2$Cl$_2$. Concentration on a rotary evaporator provided the crude material, which was purified by column chromatography using ethyl acetate and petroleum ether. The desired product eluted from the column at 20-25% ethyl acetate providing the product as a viscous liquid. The product was crystallized by dissolving the liquid in CH$_2$Cl$_2$ and petroleum ether (1:10). After two days, filtration of the crystals provided 80 g of S1-11. The filtrate was concentrated, and the residue was purified by column chromatography as described above. The product was crystallized from CH$_2$Cl$_2$ and petroleum ether as described above to afford 25 g of a second crop of the desired product. The two purifications afforded S1-11 (105 g, 0.32 mol, 45% yield) as an off-white solid that was 99% pure by HPLC. $^1$H-NMR (DMSO-d6, 400 MHz) $\delta$ 8.27 (s, 1H), 7.78 (m, 1H), 5.04 (d, $J = 7.2$, 2H); 3.22 (s, 3H); 3.00 (t, $J = 7.2$, 1H); 1.35 (s, 12H). HPLC (Sunfire 3.5$\mu$m C18 4.6x50 mm, 1.0 mL/min, Solvent A: 5% acetonitrile/water with 0.05% TFA, Solvent B: 95% Acetonitrile/water with 0.05% TFA, gradient with 10-100% B over 12 minutes then hold at 100% B for 3 minutes): 4.05 minutes.

Final compounds were prepared by using the methods described:

Preparation of 2-((1-(3-chloro-3'-fluoro-4'-(hydroxymethyl)-5'-(methylsulfonyl)biphenyl-4-yl)-2-(2-(2-fluorophenyl)propan-2-yl)-1H-imidazol-4-yl)propan-2-ol (11):
Preparation of 2-(2-fluorophenyl)-2-methylpropanenitrile.

To a 500 mL 3-neck round bottom flask with an attached addition funnel that has been purged with dry N₂, was added 2-fluorophenylacetonitrile (11.0 g, 81.4 mmol) and anhydrous THF (70 mL). The reaction solution was cooled to -10 °C prior to dropwise addition of a 1.0 M potassium tert-butoxide solution (195 mL, 2.4 molar equiv) in THF. The reaction solution was stirred at -10 °C for 20 min prior to addition of iodomethane (15.2 mL, 244 mmol). The reaction solution was allowed to stir for 4 hr while warming to rt. The reaction solution was quenched by addition of aqueous NH₄Cl and diluted with EtOAc (200 mL). The organic phase was partitioned, washed with aqueous NH₄Cl, dried over Na₂SO₄, filtered, concentrated in vacuo and chromatographed through a 240 g SiO₂ column on the Biotage SP-1 using a solvent gradient of 100 % hexanes to 50 % ethyl acetate to afford 10.1 g (76 % yield) of title product. GCMS m/z = 163 [M]+.

Preparation of N-(4-bromophenyl)-2-(2-fluorophenyl)-2-methylpropanamidamide.

To an oven dried, N₂ purged 250 mL round bottom flask attached with addition funnel was added 4-bromoaniline (7.31 g, 42.5 mmol) and anhydrous toluene (40 mL). To the reaction solution at 0 °C was added a 2.0 M Me₃Al (32 mL, 1.5 molar equiv) solution. The reaction solution was stirred at 0 °C for 30 min, then a solution of 2-(2-fluorophenyl)-2-methylpropanenitrile (7.62 g, 46.7 mmol) in toluene (25 mL) was added to the reaction flask. The reaction solution was allowed to stir at 90 °C for 5 hr. The cooled reaction solution was quenched with an aqueous sodium potassium tartrate solution. After standing 20 min, the organic phase was partitioned and washed with sodium potassium tartrate solution. The organic solution was extracted with 1N HCl (100 mL x 3). The combined aqueous HCl solution was neutralized by addition of 1N NaOH and extracted with CH₂Cl₂ (200 mL x 2). The CH₂Cl₂ product solution was dried over Na₂SO₄, filtered, and concentrated in vacuo to afford the title compound (5.5 g, 39 % yield). GCMS m/z = 334, 336 [M]+.

Preparation of ethyl 1-(4-bromophenyl)-2-(2-(2-fluorophenyl)propan-2-yl)-1H-imidazole-4-carboxylate.

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To a 250 mL round bottom flask attached with condenser was added N-(4-bromophenyl)-2-(2-fluorophenyl)-2-methylpropanimidamide (5.0 g, 15 mmol), anhydrous THF (80 mL), NaHCO₃ (2.52 g, 30 mmol), and 90% ethyl bromopyruvate (1.90 mL, 15.1 mmol). The reaction mixture was stirred at 70 °C for 2 hr prior to analysis by LCMS. The cooled reaction mixture was decanted and concentrated in vacuo. The residue was taken into toluene (65 mL) and acetic acid (1.8 mL). The solution was stirred at reflux for 1 hr. The cooled solution was washed with H₂O (150 mL x 3), dried over Na₂SO₄, filtered, concentrated in vacuo, and purified by chromatography through a SiO₂ column using a 100 % hexanes to 30% ethyl acetate gradient to afford purified title compound (4.3 g, 67 % yield). LCMS (ESI) : m/z = 431.3, 433.3 [M+H]+.

Preparation of 2-(1-(4-bromophenyl)-2-(2-(2-fluorophenyl)propan-2-yl)-1H-imidazol-4-yl)propan-2-ol.

A 250 mL round bottom flask fitted with an addition funnel was purged with dry N₂, and a 3.0M MeMgBr (12 mL, 3.7 equiv) solution in Et₂O was added to the flask. The flask was cooled to 0 °C prior to dropwise addition of ethyl 1-(4-bromophenyl)-2-(2-(2-fluorophenyl)propan-2-yl)-1H-imidazole-4-carboxylate (4.22 g, 9.78 mmol) in a solution of anhydrous CH₂Cl₂ (80 mL). The reaction solution was allowed to stir, warming to rt over 1 hr. The reaction solution was quenched by addition of aqueous NH₄Cl. The mixture was poured to a separatory funnel and the CH₂Cl₂ layer was partitioned, dried over Na₂SO₄, filtered, concentrated and chromatographed through a 40 g SiO₂ column using a gradient of 100 % hexanes to 70% EtOAc to yield the title compound (3.19 g, 78 % yield). LCMS (ESI): m/z = 417.3, 419.3 [M+H]+.
Preparation of 2-(1-(3'-fluoro-4'-{(hydroxymethyl)-5'-(methylsulfonyl)biphenyl-4-yl})-2-(2-(fluorophenyl)propan-2-yl)-1H-imidazol-4-yl)propan-2-ol (11).

To a 50 mL round bottom flask was added 2-(1-(4-bromophenyl)-2-(2-(fluorophenyl)propan-2-yl)-1H-imidazol-4-yl)propan-2-ol (380 mg, 911 μmol), DME (25 mL) and H₂O (6 mL). The solution was sparged with N₂ for 10 min prior to addition of (2-fluoro-6-(methylsulfonyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (360 mg, 1.09 mmol), potassium carbonate (380 mg, 2.73 mmol), and dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium (II) dichloromethane adduct (74 mg, 91 μmol). The reaction mixture was allowed to stir at 80 °C for 2h. The cooled reaction solution was diluted with ethyl acetate (30 mL) and filtered through a celite padded Buchner funnel. The filtrate was washed with aqueous NH₄Cl (150 mL x 2). The organic phase was dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by silica gel flash chromatography (Biotage SP-1, 25 g SiO₂ column, gradient elution from 5 % EtOAc to 100 % EtOAc) to afford the title compound (100 mg, 20 % yield). ¹H-NMR (400 MHz, CDCl₃) δ 8.02 (d, J =2 Hz, 1H), 7.51 (dd, J₁ = 2 Hz, J₂ = 10 Hz, 1H), 7.29 (d, J = 9, 2H), 7.08-7.16 (m, 1H), 6.85-6.92 (m, 3H), 6.77-6.84 (m, 2H), 6.65 (s, 1H), 5.09 (d, J = 6 Hz, 2H), 3.35 (s, 1H), 3.30 (s, 3H), 3.02 (t, J = 6 Hz, 1H), 1.72 (s, 6H), 1.62 (s, 6H); ¹⁹F NMR (400 MHz, CDCl₃) δ -112.1, -113.5 ppm; LCMS (ESI) m/z = 541.3 [M+H]+, 563.2 [M+Na]+. HPLC Purity Method A: 93%.

Preparation of 2-(2-(2-(2-chloro-3-fluorophenyl)propan-2-yl)-1-(3'-fluoro-4'-{(hydroxymethyl)-5'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl})-1H-imidazol-4-yl)propan-2-ol (13)
Preparation of (2-chloro-3-fluorophenyl)methanol.

2-chloro-3-fluorobenzoic acid (12 g, 69 mmol) was dissolved in THF (10 mL) and borane (1 M soln. in THF, 103 mL, 1.5 eq.) added slowly at rt. The reaction mixture was heated to reflux for 10 hr (monitored by GC-MS). The mixture was cooled down to 0 °C and slowly quenched with 1N HCl. The product was extracted into CH₂Cl₂, concentrated in vacuo, and used in next step without further purification.

Preparation of 1-(bromomethyl)-2-chloro-3-fluorobenzene.

(2-chloro-3-fluorophenyl)methanol (15 g, 69 mmol) was dissolved in diethyl ether (200 mL), and the mixture was cooled down to 0 °C. PBr₃ (34.4 mmol, 0.5 eq.) was introduced to the reaction mixture drop-wise. The reaction mixture was allowed to stir overnight at rt. GC-MS showed complete conversion of alcohol. The mixture was treated with ice, and the product was extracted into ether. The ether was removed under reduced pressure, and the crude product was carried forward without further purification.

Preparation of 2-(2-chloro-3-fluorophenyl)acetonitrile.
A solution of 1-(bromomethyl)-2-chloro-3-fluorobenzene (36 g, 160 mmol) in 150 mL of ethanol was added drop-wise to a solution of sodium cyanide (20 g, 410 mmol, 2.5 eq.) in 1.5 L of (2:1) ethanol and water maintained at 60 °C. After completion of the addition, the reaction mixture was heated to 80 °C for 1.5 hr. The reaction was monitored by GC-MS. The ethanol was removed in vacuo, and the residue was diluted with water and extracted into ether to yield 19.2 g of the nitrile, which was carried forward without further purification.

Preparation of 2-(2-chloro-3-fluorophenyl)-2-methylpropanenitrile.

To a solution of potassium tert-butoxide (24 g, 210 mmol) in 300 mL of THF, which was chilled with an ice bath to 5 °C, was added 2-(2-chloro-3-fluorophenyl)acetonitrile (19 g, 97 mmol) in portions over 10 min. Iodomethane (13 mL, 210 mmol) was slowly added over 55 min to the resulting solution while keeping the temperature below 25 °C. The cooling bath was removed, and the thick slurry was stirred at rt for 1 h. The mixture was worked up by adding 300 mL of diethyl ether followed by 250 mL of a saturated ammonium chloride solution. The organic layer was further washed with 200 mL of saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated on the rotary evaporator (30 °C) to give of desired product (brown oil) in quantitative yield. This crude material was used directly in the subsequent step to form the imidazole without further purification.

The synthesis of 13 was carried out from 2-(2-chloro-3-fluorophenyl)-2-methylpropanenitrile by methods described for Example 11. $^1$H-NMR (JEOL spectrometer 500 MHz, CDCl$_3$) 7.98 (s, 1 H), 7.47 (d, $J = 9.90$ Hz, 1 H), 7.27 (d, $J = 8.25$ Hz, 2 H), 7.00 (d, $J = 8.25$ Hz, 2 H), 6.85 - 6.91 (m, 1 H), 6.80 - 6.86 (m, 1 H), 6.75 (d, $J = 7.70$ Hz, 1 H), 6.65 (s, 1 H), 5.09 (s, 2 H), 3.34 (br. s., 1 H), 3.30 (s, 3 H), 3.18 (br. s., 1 H), 1.82 (s, 6 H), 1.61 (s, 6 H). A trace amount of impurity was observed but not identified and also ACN and diethyl ether were observed. $^{13}$C-NMR (JEOL spectrometer 126 MHz, CDCl$_3$) 161.83 (d, $J = 254.3$ Hz), 158.86 (d, $J = 246.7$ Hz), 152.24, 147.61, 145.95, 142.36 (d, $J = 7.6$ Hz), 141.79, 139.00, 137.58, 128.16, 126.80, 126.98 (d, $J = 7.6$ Hz), 126.35 (d, $J = 17.8$ Hz), 123.99, 121.74 (d, $J = 17.8$ Hz), 122.50, 119.48 (d, $J = 25.4$ Hz), 116.11, 114.46 (d, $J = 22.9$ Hz), 68.81, 54.06 (d, $J = 7.6$ Hz), 45.53, 41.69, 30.17, 29.42. $^{19}$F-NMR (500 MHz, CDCl$_3$) -113.35 (d, $J = 11.5$ Hz), -112.28. HPLC (Water Xbridge Phenyl 3.5 μM 150 mm (L) x 4.6 mm (ID), 1 mL/min, Solvent A: 20 mM ammonium formate in water,
Solvent B: 90% CAN, 10% 20 mM ammonium formate. gradient with 40-95 % B over 30 minutes: 2.56 minutes, Purity, 99.8%. HRMS (m/z, Obs.): 575.15785 [M+H]+; (Calc.): 575.15774. Formula: C29H30N2O4ClF2S[M+H]+. Anal. Calcd. for C29H29N2O4SClF2 • 0.06H2O • 0.04C4H10O: C, 60.57; H, 5.08; Cl, 6.16; F, 6.61; N, 4.87; S, 5.57. Found: C, 60.41; H, 5.08; Cl, 6.25; F, 6.27; N, 4.79; S, 5.52.
The residual solvent, 0.52% diethyl ether along with trace levels (<0.05Wt %) of ethanol, acetone, acetonitrile and ethyl acetate were identified using GC/FID with internal standard quantitation. The average water content was determined to be 0.20% by KF titration.

Preparation of 2-(2-(2,6-dichlorophenyl)propan-2-yl)-1-(3,3′-difluoro-4′-(hydroxymethyl)-5′-(methylsulfonyl)biphenyl-4-yl)-1H-imidazol-4-yl)propan-2-ol (15).

Preparation of 2-(2,6-dichlorophenyl)-2-methylpropanenitrile.

To a 1 M solution of potassium tert-butoxide (403 mL, 403 mmol) at -66 °C (acetone / dry ice) was slowly added 2-(2,6-dichlorophenyl)acetonitrile (25.0 g, 134 mmol) in anhydrous THF (150 mL). The mixture was stirred at -66 °C for 20 minutes. Then, iodomethane (33.6 mL, 538 mmol) was added drop-wise over 25 minutes at -66 °C. At this stage, it was exothermic, and a large amount of light yellow precipitate was observed. The suspension was stirred at -60 °C for 30 minutes. The reaction mixture was quenched with 200 mL ice water, and extracted with ether (3 x 150 mL). The organics were combined, washed with 150 mL brine, dried over Na2SO4, and concentrated on a rotary evaporator. The crude product (30 g, yellow oil) was purified by column chromatography (ISCO, 330 g silica, 20% EtOAc in hexanes) to afford 2-(2,6-dichlorophenyl)-2-methylpropanenitrile (28.2 g, 132 mmol, 98% yield) as a light yellowish oil. 1H-NMR (CDCl3, 400 MHz) δ 7.35 (d, 2H, J =8.03 Hz ), 7.16 (t, 1H, J = 8.0 Hz),
2.09 (s, 6H); $^{13}$C-NMR (CDCl$_3$, 126 MHz) δ 134.6, 133.8, 131.4, 129.0, 124.1, 38.6, 29.2; MS m/e 214.10 (M+H+); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H$_3$PO$_4$, Solvent B: 90 % MeOH/water with 0.2 % H$_3$PO$_4$, gradient with 0-100 % B over 4 minutes): 3.16 minutes.

Preparation of $N$-(4-bromo-2-fluorophenyl)-2-(2,6-dichlorophenyl)-2-methylpropanimidamide.

Preparation of ethyl 1-(4-bromo-2-fluorophenyl)-2-(2,6-dichlorophenyl)propan-2-yl)-4-hydroxy-4,5-dihydro-1H-imidazole-4-carboxylate.
To a mixture of N-(4-bromo-2-fluorophenyl)-2-(2,6-dichlorophenyl)-2-methylpropanimidamide (48.0 g, 119 mmol), K₂CO₃ (41.0 g, 297 mmol) in toluene (180 mL) and THF (180 mL) at 55 °C was added slowly a solution of ethyl 3-bromo-2-oxopropanoate (23.3 mL, 166 mmol) in 24 mL of THF over 50 minutes. The reaction mixture was kept at 55 °C for 1.5 hours. A white slurry was observed. The reaction mixture was cooled to 5 °C. HCl (0.5N, 450 mL) was added drop-wise (end point pH = 9–10). After addition, the suspension was cooled to 0 °C. The solid was collected by filtration, washed with water (2 x 50 mL), and then dried in a vacuum oven at 60 °C overnight. Ethyl 1-(4-bromo-2-fluorophenyl)-2-(2-(2,6-dichlorophenyl)propan-2-yl)-4-hydroxy-4,5-dihydro-1H-imidazole-4-carboxylate (59 g, 114 mmol, 96% yield) was obtained as a white solid. ¹H-NMR (CDCl₃, 400 MHz) δ 7.11 (m, 3H), 6.96 (m, 2H), 6.72 (t, 1H, J = 8.28 Hz), 4.35 (m, 2H), 4.25 (d, 1H, J = 10.5 Hz), 3.80 (d, 1H, J = 10.8 Hz), 1.98 (s, 3H), 1.93 (s, 3H), 1.38 (t, 3H, J = 7.03 Hz); ¹³C-NMR (CDCl₃, 126 MHz) δ 173.0, 171.5, 159.8, 157.8, 137.3, 135.7, 132.1, 131.1, 128.1, 127.4, 125.6, 122.2, 120.1, 93.5, 62.5, 45.5, 30.2, 14.0; MS m/e 517.05 (M+H⁺); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H₃PO₄, Solvent B: 90 % MeOH/water with 0.2 % H₃PO₄, gradient with 0-100 % B over 4 minutes): 2.74 minutes.

Preparation of ethyl 1-(4-bromo-2-fluorophenyl)-2-(2-(2,6-dichlorophenyl)propan-2-yl)-1H-imidazole-4-carboxylate.

To a mixture of ethyl 1-(4-bromo-2-fluorophenyl)-2-(2-(2,6-dichlorophenyl)propan-2-yl)-4-hydroxy-4,5-dihydro-1H-imidazole-4-carboxylate (38 g, 73 mmol) in EtOH (200 mL) was added TFA (25.0 g, 220 mmol). The mixture was subsequently heated to 95 °C. HPLC analysis after 2.5 hours showed < 1% of alcohol intermediate remaining. The mixture was diluted with 300 mL of CH₂Cl₂ and cooled to approximately 5 °C with an ice bath. The mixture was neutralized with 1N NaOH (120 mL) and the organ-
ic layer was separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (2 x 100 mL). The combined organic layers were concentrated on a rotary evaporator to give crude material. Recrystallization in EtOH (5 mL / 1 g) provided 32 g of ethyl 1-(4-bromo-2-fluorophenyl)-2-(2-(2,6-dichlorophenyl)propan-2-yl)-1H-imidazole-4-carboxylate as an off-white solid (86% yield). $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ 7.92 (s, 1H), 7.16 (d, 1H, $J = 8.0$ Hz), 7.22 (m, 3H), 7.11 (m, 1H), 7.04 (t, 1H, $J = 12.0$ Hz), 4.25 (q, 2H, $J = 8.0$ Hz), 1.94 (s, 6H), 1.27 (t, 3H, $J = 8.0$ Hz); MS m/e 502.68 (M+H+); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10% MeOH/water with 0.2% H$_3$PO$_4$, Solvent B: 90% MeOH/water with 0.2% H$_3$PO$_4$, gradient with 0-100% B over 4 minutes): 3.87 minutes.

**Preparation of 2-(1-(4-bromo-2-fluorophenyl)-2-(2-(2,6-dichlorophenyl)propan-2-yl)-1Himidazol-4-yl)propan-2-ol.**

To a mixture of methylmagnesium bromide (60 mL, 180 mmol, 3M in ether) in 120 mL of THF cooled with an ice/salt bath (-15 to -17 °C) was added slowly a solution of ethyl 1-(4-bromo-2-fluorophenyl)-2-(2-(2,6-dichlorophenyl)propan-2-yl)-1H-imidazole-4-carboxylate (30 g, 60 mmol) in 65 mL of CH$_2$Cl$_2$ and 87 mL of THF over 45 minutes. The internal temperature was carefully kept below 0 °C. A further 2 X 20 mL of CH$_2$Cl$_2$ was used to wash forward the residual material. The reaction mixture temperature was maintained below 0 °C for 1 hour with stirring. Then the reaction mixture was diluted with 100 mL of CH$_2$Cl$_2$, and saturated NH$_4$Cl was added slowly. The resulting mixture was extracted with CH$_2$Cl$_2$ (2 X 80 mL). Organics were combined, washed with brine, dried with Na$_2$SO$_4$, and concentrated on a rotary evaporator to afford 2-(1-(4-bromo-2-fluorophenyl)-2-(2-(2,6-dichlorophenyl)propan-2-yl)-1H-imidazol-4-yl)propan-2-ol (28.5 g, 58.6 mmol, 98% yield) as a white solid. $^1$H-NMR (CDCl$_3$, 400 MHz) δ 7.13 (dd, 1H, $J = 9.03$, 2.01 Hz), 7.09 (s, 1H), 7.07 (s, 1H), 6.93 (m, 2H), 6.75 (t, 1H, $J = 8.16$ Hz), 6.55 (s, 1H), 3.18 (s, 1H), 2.00 (s, 6H), 1.58 (s, 6H); $^{13}$C-NMR (CDCl$_3$, 126 MHz) δ 158.1, 156.1, 154.5, 147.8, 139.3, 135.7, 131.3, 130.3, 127.8, 126.9, 122.7, 119.8, 115.1, 68.7, 44.8, 31.1, 29.9; MS m/e 485.05 (M+H+); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10% MeOH/water with 0.2% H$_3$PO$_4$, Solvent B: 90% MeOH/water with 0.2% H$_3$PO$_4$, gradient with 0-100% B over 4 minutes): 2.78 minutes.
Preparation of 2-(2-(2,6-dichlorophenyl)propan-2-yl)-1-(3,3'-difluoro-4'-(hydroxymethyl)-5'-(methylsulfonyl)biphenyl-4-yl)-1H-imidazol-4-yl)propan-2-ol (15).

To a 1 L 3-necked round bottom flask under nitrogen was added 2-(1-(4-bromo-2-fluorophenyl)-2-(2,6-dichlorophenyl)propan-2-yl)-1H-imidazol-4-yl)propan-2-ol (12.0 g, 24.7 mmol), [2-fluoro-6-methanesulfonyl-4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenyl]-methanol (9.78 g, 29.6 mmol), K$_2$CO$_3$ (10.2 g, 74 mmol), DME (120 mL) and water (12 mL). The mixture was heated to 60 °C, and then 1,1'-bis(diphenylphosphino)ferrocene palladium (II) chloride complex (4.06 g, 4.94 mmol) was added under nitrogen. The reaction mixture was heated to 80 °C for 30 minutes. The resulting darkly colored mixture was cooled with an ice bath, and partitioned in 200 mL of CH$_2$Cl$_2$ and 200 mL of water. The organic layers were combined and dried with Na$_2$SO$_4$. After concentration, the crude product was purified by flash chromatography (ISCO, 330 g silica, 0% to 100% EtOAc in hexanes) to afford 12.8 g of crude product (85% yield) as a light yellow solid. Recrystallization was carried out by dissolving 9.5 g of crude product in acetone (80 mL) at 65 °C. The resulting solution was cooled slowly to 25 °C over 5 hours, and then cooled to 0 °C for an additional 30 minutes. Crystals began to form at 45 °C. The solid was collected by filtration and rinsed with cold acetone. After drying in an oven at 45 °C under vacuum for 14 hours, 4.9 g of pure product was obtained. To recover additional crystalline product, the mother liquid was concentrated to approximately 10 mL and passed through a silica pad. EtOAc (100 mL) was used to elute the compound. The filtrate was concentrated under vacuum to give a crude solid. The crude solid was recrystallized in acetone following the procedure above to afford an additional 2.5 g of product. The combined recovery for the two crops after recrystallization was a 78% yield.

$^1$H-NMR (DMSO-d$_6$, 400 MHz) δ 7.94 (m, 2H), 7.63 (dd, 1H, J = 11.29, 1.51 Hz), 7.34 (d, 1H, J = 9.54 Hz), 7.14 (m, 3H), 7.05 (m, 1H), 6.83 (s, 1H), 5.58 (t, 1H, J = 5.27 Hz), 4.96 (d, 2H, J = 4.27 Hz), 4.70 (s, 1H), 3.46 (s, 3H), 1.96 (s, 6H), 1.45 (s, 6H); MS m/e 609.16 (M+H+); $^{13}$CNMR (DMSO-d$_6$, 400 MHz) 161.42 (d, J=249.49 Hz), 156.85 (d, J=250.25 Hz), 153.18, 148.39, 141.69 (d, J=3.05 Hz), 139.45 (dd, J=9.16, 1.53 Hz), 139.32 (dd, J=8.39, 1.53 Hz), 138.58, 134.68, 131.39, 129.96, 128.40,
127.12 (d, $J$=17.55 Hz), 125.72 (d, $J$=12.97 Hz), 123.15 (d, $J$=2.29 Hz), 122.49 (d, $J$=3.05 Hz), 119.04 (d, $J$=25.18 Hz), 116.30, 114.52 (d, $J$=22.13 Hz), 68.11, 51.97 (d, $J$=5.34 Hz), 45.53, 44.78, 44.29, 31.01, 30.53. $^{19}$F-NMR (JEOL 500 MHz, CDCl$_3$) -113.55, -116.73. HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H3PO4, Solvent B: 90 % MeOH/water with 0.2 % H3PO4, gradient with 0-100 % B over 4 minutes): 2.56 minutes, Purity, 99.7%. HRMS (m/z, Obs.): 609.12065 [M+H$^+$]; (Calc.): 609.11877. Formula: C$_{29}$H$_{29}$Cl$_2$F$_2$N$_2$O$_4$S. Anal. Calcd. for C$_{29}$H$_{28}$N$_2$O$_4$SCl$_2$F$_2$: C, 57.05; H, 4.75; Cl, 11.42; F, 6.10; N, 4.50; S, 5.15. Found: C, 57.14; H, 4.54; Cl, 11.57; F, 5.94; N, 4.36; S, 5.07. The residual solvents, ethyl acetate (1.39 weight %), ethanol (0.74 weight %), dichloromethane (0.05 weight %), and heptane (< 0.05 weight %) were identified in the sample by GC/MS and the retention times were matched with the reference standards.

2-(2-(1-(2,6-dichlorophenyl)ethyl)-1-(3,3'-difluoro-4'-(hydroxymethyl)-5'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)-1H-imidazol-4-yl)propan-2-ol (19).

**Preparation of 2-(2,6-dichlorophenyl)propanenitrile.**

To a solution of 2-(2,6-dichlorophenyl)acetonitrile (10.0 g, 53.8 mmol) in THF (100 mL) at -78°C was added a solution of potassium tert-butoxide (6.03 g, 53.8 mmol) in THF (54 mL) dropwise via an addition funnel. Iodomethane (3.36 mL, 53.8 mmol) was added, and the reaction mixture was stirred for 1 hour at -78 °C, and then at rt for 3 hours. The reaction was concentrated in vacuo to remove the THF, and the residue was diluted with ethyl acetate and washed with 1 M HCl. The ethyl acetate extracts
were washed with brine and dried over Na₂SO₄ and concentrated to give the crude product. The crude product was purified by column chromatography (0-10% EtOAc/Hexanes) to give 2-(2,6-dichlorophenyl)propanenitrile (5.70 g, 28.5 mmol). MS (ESI) 200.1 [M+H]+.

**Preparation of N-(4-bromo-2-fluorophenyl)-2-(2,6-dichlorophenyl)propanimidamide.**

![Chemical structure](attachment:image1)

To a solution of 4-bromo-2-fluoroaniline (4.87 g, 25.7 mmol) in toluene (28.5 mL) at 0 °C, was added trimethylaluminum (21.4 mL, 42.8 mmol) dropwise, and the reaction solution was stirred for 30 mins at 0 °C. A solution of 2-(2,6-dichlorophenyl)propanenitrile (5.70 g, 28.5 mmol) in toluene (15 mL) was added to the reaction mixture, and the solution was heated to 80 °C for 18 h. The mixture was cooled to rt and poured over a slurry of silica in CHCl₃/MeOH (4/1), stirred for 40 mins, filtered and washed with CHCl₃/MeOH (4/1). The filtrate was concentrated to give the crude product, N-(4-bromo-2-fluorophenyl)-2-(2,6-dichlorophenyl)propanimidamide (10.1 g, 25.8 mmol) as an orange solid. MS (ESI) 389.0 [M+H]+.

**Preparation of ethyl 1-(4-bromo-2-fluorophenyl)-2-(1-(2,6-dichlorophenyl)ethyl)-1H-imidazole-4-carboxylate.**

![Chemical structure](attachment:image2)

To a solution of N-(4-bromo-2-fluorophenyl)-2-(2,6-dichlorophenyl)propanimidamide (10.1 g, 25.8 mmol) in THF (140 mL) was added NaHCO₃ (4.33 g, 51.5 mmol) and 90% ethyl bromopyruvate (3.96 mL, 28.3 mmol). The reaction mixture was heated to 70 °C for 2 h, then was cooled, decanted and concentrated in vacuo. The residue was brought up in toluene (112 mL) and acetic acid (3.69 mL, 64.4 mmol). The solution was stirred at reflux for 1 h. The cooled solution was washed with H₂O, dried over Na₂SO₄, filtered and concentrated in vacuo to give the crude product. The crude product was purified by column chromatography (45 % EtOAc/Hexanes) to give the title compound (5.22 g, 10.7 mmol). MS (ESI) 485.0 [M+H]+.
Preparation of 2-(1-(4-bromo-2-fluorophenyl)-2-(1-(2,6-dichlorophenyl)ethyl)-1H-imidazol-4-yl)propan-2-ol.

To a 1.4 M solution of methyl magnesium bromide (26.0 mL, 36.4 mmol) in toluene/THF at 0 °C, was added dropwise a solution of ethyl 1-(4-bromo-2-fluorophenyl)-2-(1-(2,6-dichlorophenyl)ethyl)-1H-imidazole-4-carboxylate (5.22 g, 10.7 mmol) in THF (100 mL). The reaction mixture was stirred at rt for 1.5 h. The reaction solution was quenched with a saturated aqueous solution of NH₄Cl and extracted with ethyl acetate. The combined organics were washed with brine, dried over Na₂SO₄, then concentrated to give the crude product. The crude product was purified by column chromatography (50 % EtOAc/Hexanes) to give the title compound (4.36 g, 9.25 mmol). MS (ESI) 471.0 [M+H]⁺.

Preparation of 19.

To a solution of S1-11 (1.12 g, 3.39 mmol) and 2-(1-(4-bromo-2-fluorophenyl)-2-(1-(2,6-dichlorophenyl)ethyl)-1H-imidazol-4-yl)propan-2-ol (1.60 g, 3.39 mmol) in DME (18.0 mL) and H₂O (1.30 mL), was added K₂CO₃ (1.41 g, 10.2 mmol) and PdCl₂(dppf)-CH₂Cl₂ adduct (138 mg, 0.169 mmol). The reaction mixture was heated in a microwave at 120 °C for 20 mins. The reaction mixture was filtered over celite, then diluted with ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined organics were washed with brine, dried over Na₂SO₄, then concentrated to give the crude product. The crude product was purified by column chromatography (90 % EtOAc
hexanes) to give the title compound (1.15 g, 1.89 mmol). \(^1\)H NMR (400 MHz, DMSO-d6) \(\delta\) 7.93 - 7.85 (m, 2H), 7.58 (d, \(J=11.0\) Hz, 1H), 7.41 - 7.36 (m, 1H), 7.12 - 7.05 (m, 2H), 7.02 - 6.95 (m, 2H), 5.57(t, \(J=5.2\) Hz, 1H), 5.00 - 4.88 (m, 2H), 4.74 (s, 1H), 3.46 (s, 3H), 1.67 (d, \(J=7.1\) Hz, 3H), 1.47 (s, 6H). MS (ESI) 595.3 [M+H]\(^+\). Orthogonal HPLC indicates a purity of 98% (Detector 1, RT = 5.62 min., Detector 2, RT = 6.77 min.) (Detector 1: [Sunfire 3.5 mm 4.6 x 150 mm, (15 min, 10-100%) 95% ACN/ 5% H\(_2\)O/ 0.05% TFA], Detector 2: [Xbridge Phenyl 3.5 mm 4.6 x 150 mm, (15 mins, 10-100%) 95% ACN / 5% H\(_2\)O / 0.05% TFA]).

The compounds below were prepared by the methods described herein.

2-(2-(2,6-dichlorophenyl)propan-2-yl)-1-(3′-(methylsulfonyl)-[1,1′-biphenyl]-4-yl)imidazol-4-yl)propan-2-ol (6). Prepared as describe for Example 11 using reagents 2-(2,6-dichlorophenyl)-2-methylpropanenitrile, 4-bromo aniline and [(3-methylsulfonyl)phenyl]boronic acid.

![Chemical structure](image)

\(^1\)H-NMR (400 MHz, CDCl\(_3\)) - \(\delta\) 8.03 (t, \(J=1.7\) Hz, 1H), 7.95 (dt, \(J=7.6, 1.5\) Hz, 1H), 7.77 - 7.71 (m, 1H), 7.70 - 7.63 (m, 1H), 7.35 - 7.31 (m, 2H), 7.17 - 7.11 (m, 2H), 7.00 (d, \(J=7.9\) Hz, 2H), 6.85 - 6.78 (m, 1H), 6.66 (s, 1H), 3.17 - 3.08 (m, 3H), 2.11 - 1.99 (m, 6H), 1.61 (s, 6H). LCMS (ESI) m/z = 543.3 [M+H]+. HPLC Purity Method B: 95% (Detector 1, RT = 6.09 min, Detector 2, RT = 7.29 min).

2-(2-(2-chlorobenzyl)-1-(4′-(hydroxymethyl)-3′-(methylsulfonyl)-[1,1′-biphenyl]-4-yl)imidazol-4-yl)propan-2-ol (7). Prepared as describe for Example 11 using reagents 2-(2-chlorophenyl)acetonitrile, 4-bromo aniline and S1-6, except 2-(2-chlorophenyl)acetonitrile was taken directly to form N-(4-bromophenyl)-2-(2-chlorophenyl)acetimidamide.
1H NMR (500MHz, ACETONITRILE-d3) δ 8.19 (d, J=1.9 Hz, 1H), 7.97 (dd, J=8.1, 2.1 Hz, 1H), 7.82 (d, J=8.0 Hz, 1H), 7.76 (d, J=4.7 Hz, 2H), 7.38 (d, J=8.5 Hz, 2H), 7.34 - 7.29 (m, 1H), 7.22 - 7.18 (m, 2H), 7.13 - 7.09 (m, 1H), 7.06 (s, 1H), 5.00 (d, J=5.5 Hz, 2H), 4.14 (s, 2H), 3.53 (t, J=5.8 Hz, 1H), 3.19 (s, 3H), 3.05 (s, 1H), 1.49 (s, 6H). MS (ESI) m/z = 511.4 [M+H]+. HPLC Purity Method B: 95 %.

2-(2-(2-chlorophenyl)propan-2-yl)-1-(4'-hydroxymethyl)-3'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)-1H-imidazol-4-yl)propan-2-ol (8). Prepared as describe for Example 11 using reagents 2-(2-chlorophenyl)acetonitrile, 4-bromo aniline and S1-6.

1H NMR (400MHz, CDCl3) δ 8.14 (d, J=1.8 Hz, 1H), 7.75 - 7.64 (m, 2H), 7.24 (d, J=8.4 Hz, 2H), 7.20 (dd, J=7.9, 1.3 Hz, 1H), 7.01 (td, J=7.5, 1.5 Hz, 1H), 6.98 - 6.91 (m, 3H), 6.89 - 6.80 (m, 1H), 6.65 (s, 1H), 5.02 (s, 2H), 3.24 (s, 3H), 1.81 (s, 6H), 1.62 (s, 6H). LCMS (ESI) m/z = 539.3 [M+H]+. HPLC Purity Method B: 97 %.

2-(2-(2-chlorophenyl)propan-2-yl)-1-(4'-1-hydroxyethyl)-3'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)-1H-imidazol-4-yl)propan-2-ol (9). Prepared as describe for Example 11 using reagents 2-(2-chlorophenyl)acetonitrile, 4-bromo aniline and 1-(2-(methylsulfonyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethan-1-ol, shown below.
$^1$H NMR (500 MHz, ACETONITRILE-d$_3$) δ 8.01 (d, $J=1.9$ Hz, 1H), 7.93 (d, $J=8.3$ Hz, 1H), 7.83 (dd, $J=8.3$, 1.9 Hz, 1H), 7.31 (d, $J=8.5$ Hz, 2H), 7.19 (dd, $J=7.8$, 1.2 Hz, 1H), 7.08 - 6.95 (m, 4H), 6.93 - 6.82 (m, 1H), 6.77 (s, 1H), 5.72 - 5.57 (m, 1H), 3.54 (d, $J=3.9$ Hz, 1H), 3.20 (s, 3H), 1.77 (s, 6H), 1.51 (s, 6H), 1.49 (d, $J=6.3$ Hz, 3H). MS (ES) m/z = 553.5 [M+H]+. HPLC Purity Method A: >98%.

**Preparation of 4-bromo-2-(methylthio)benzoic acid.**

To a solution of methyl 4-bromo-2-(methylthio)benzoate (250 mg, 0.96 mmol) in MeOH (4.8 mL) was added 1N NaOH (3.8 mL, 3.8 mmol), and the reaction solution was stirred for 18 h at room temperature. The reaction solution was concentrated in vacuo, then diluted with EtOAc and 1N HCl. The aqueous layer was extracted twice with EtOAc, and the combined organics were washed with brine, dried with Na$_2$SO$_4$ and was concentrated in vacuo to give the title compound, (225 mg, 95% yield). MS (ES) m/z = 247.0 [M+H]+.

**Preparation of 4-bromo-N-methoxy-N-methyl-2-(methylthio)benzamide.**

A mixture of 4-bromo-2-(methylthio)benzoic acid (220 mg, 0.91 mmol), N,O-dimethylhydroxylamine hydrochloride (110 mg, 1.1 mmol), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (480 mg, 1.1 mmol), DMAP (11 mg, 0.091 mmol) and triethyl amine (380 µL, 2.7 mmol) in THF (4.5 mL) was stirred at room temperature for 18 h. The mixture was diluted with EtOAc and was washed with a solution of aqueous saturated sodium bicarbonate, followed by aqueous 1.0 M HCl and
then brine. The organic layer was dried with Na$_2$SO$_4$ and was concentrated in vacuo. The crude product was purified by silica gel flash chromatography (0-100 % gradient of EtOAc/Hexanes). Appropriate fractions were combined and concentrated in vacuo to give the title compound, (264 mg, 100% yield). MS (ES) m/z = 290.1 [M+H]+-triethyl amine

Preparation of 1-(4-bromo-2-(methylthio)phenyl)ethan-1-one.

To a cold (0°C) solution of 4-bromo-N-methoxy-N-methyl-2-(methylthio)benzamide (260 mg, 0.91 mmol) in THF (6 mL) was added a 3M solution of methylmagnesium bromide in Et$_2$O (790 µL, 2.4 mmol). The reaction mixture was stirred for 3 h, and then quenched with 1N HCl. The reaction solution was extracted with EtOAc twice, and the combined organics were washed with brine, dried with Na$_2$SO$_4$ and was concentrated in vacuo to give the title compound, (223 mg, 100% yield). MS (ES) m/z = 245.0 [M+H]+.

Preparation of 1-(4-bromo-2-(methylthio)phenyl)ethan-1-ol.

At 0°C, NaBH$_4$ (45 mg, 1.2 mmol) was added to a solution of 1-(4-bromo-2-(methylthio)phenyl)ethanone (223 mg, 0.91 mmol) in MeOH (9.1 mL). The reaction solution was stirred for 1 h at room temperature, and then quenched with 1N HCl. The reaction solution was extracted with EtOAc twice, and the combined organics were washed with brine, dried with Na$_2$SO$_4$ and was concentrated in vacuo to give the title compound, (205 mg, 91 % yield). MS (ES) m/z = 239.0 [M-OH]+.

Preparation of 1-(4-bromo-2-(methylsulfonyl)phenyl)ethan-1-ol.
To a cold (0°C) solution of 1-(4-bromo-2-(methylthio)phenyl)ethanol (205 mg, 0.83 mmol) in CH₂Cl₂ (5.5 mL) was added mCPBA (570 mg, 3.30 mmol) portionwise. The reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with 1N NaOH and extracted with CH₂Cl₂ twice. The combined organics were washed with brine, dried with Na₂SO₄ and was concentrated in vacuo. The crude product was purified by silica gel flash chromatography (0-100 % gradient of EtOAc/Hexanes). Appropriate fractions were combined and concentrated in vacuo to give the title compound, (201 mg, 87 % yield). MS (ES) m/z = 261.0 [M-OH]+.

Preparation of 1-(2-(methylsulfonyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethan-1-ol.

To a solution of 1-(4-bromo-2-(methylsulfonyl)phenyl)ethanol (200 mg, 0.72 mmol) and bis(pinacolato) diboron (200 mg, 0.79 mmol) in toluene (2.9 mL) was added PdCl₂(dppf) (26 mg, 0.036 mmol) and potassium acetate (210 mg, 2.2 mmol) under a N₂ atm. The reaction solution was heated to 90 °C for 18 hours. The reaction solution was diluted with EtOAc, washed with water, then brine, dried over Na₂SO₄ and concentrated. The crude product was purified by silica gel flash chromatography (0-100 % gradient of EtOAc/Hexanes). Appropriate fractions were combined and concentrated in vacuo to give the title compound, (160 mg, 68 % yield). MS (ES) m/z = 309.3 [M-OH]+.

2-(2-(2-(2-chlorophenyl)propan-2-yl)-1-(3'-fluoro-4'-(hydroxymethyl)-5'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)-1H-imidazol-4-yl)propan-2-ol (10). Prepared as describe for Example 11 using reagents 2-(2-chlorophenyl)acetonitrile, 4-bromo aniline and S1-11.
$^1$H NMR (400MHz, CDCl$_3$) δ 7.98 (d, $J$=0.9 Hz, 1H), 7.48 (dd, $J$=10.1, 2.0 Hz, 1H), 7.23 (d, $J$=8.6 Hz, 2H), 7.19 (dd, $J$=7.9, 1.3 Hz, 1H), 7.04 - 6.96 (m, 3H), 6.95 - 6.92 (m, 1H), 6.88 - 6.82 (m, 1H), 6.65 (s, 1H), 5.09 (d, $J$=5.5 Hz, 2H), 3.29 (s, 3H), 2.88 (s, 1H), 1.81 (s, 6H), 1.62 (s, 6H). LCMS (ESI) m/z = 557.3[M+H]+. HPLC Purity Method B: 100 % ( Detector 1, RT = 6.80 min, Detector 2, RT = 5.78 min).

2-(2-(2,6-dichlorophenyl)propan-2-yl)-1-(3’fluoro-4’-(hydroxymethyl)-5’-(methylsulfonyl)biphenyl-4-yl)-1H-imidazol-4-yl)propan-2-ol (12). Prepared as describe for Example 11 from intermediate 2-(2,6-dichlorophenyl)-2-methylpropanenitrile, 4-bromo aniline and S1-11.

`![Chemical structure image]`

$^1$H-NMR (400 MHz, DMSO-d6) δ 7.89-7.90 (m, 1H), 7.82-7.85 (m, 1H), 7.52 (d, $J$ = 8.6 Hz, 2H), 7.16 (d, $J$ =8.6 Hz, 2H), 7.07-7.09 (m, 2H), 6.94-6.98 (m, 1H), 6.80 (s, 1H), 5.55 (t, $J$ = 5.2 Hz, 1H), 4.93-4.95 (m, 2H), 4.65 (s, 1H), 3.45 (s, 3H), 1.96 (s, 6H), 1.45 (s, 6H). LCMS (ESI) m/z = 591.5 [M+H]+. HPLC Purity Method A: 93%.

2-(2-(2-Chlorophenyl)propan-2-yl)-1-(3,3’-difluoro-4’-hydroxymethyl-5’-(methylsulfonyl)biphenyl-4-yl)-1H-imidazol-4-yl)-propan-2-ol (14). Prepared as describe for Example 11 from reagents 2-(2-chlorophenyl)acetonitrile, 4-bromo-2-fluoroaniline and S1-11.
1H NMR (400 MHz, CDCl₃) δ 7.97 (s, 1H), 7.46 (dd, J = 9.9, 1.8, 1H), 7.23 – 7.18 (m, 1H), 7.12 (dd, J = 10.3, 1.9, 1H), 6.97 (ddd, J = 23.4, 9.0, 4.0, 2H), 6.88 – 6.79 (m, 2H), 6.61 (s, 1H), 5.08 (d, J = 5.4, 2H), 3.30 (s, 3H), 3.27 – 3.23 (m, 1H), 2.92 (t, J = 6.9, 1H), 1.61 (s, 12H). LCMS (ESI): 575.3 [M+H]+. HPLC Purity Method A: >98 %.

2-(2-(2-chloro-6-fluorophenyl)propan-2-yl)-1-(3,3'-difluoro-4'-(hydroxymethyl)-5'-(methylsulfonyl)biphenyl-4-yl)-1H-imidazol-4-yl)propan-2-ol (16). Prepared as describe for Example 11 from reagents 2-(2-chloro-6-fluorophenyl)acetonitrile, 4-bromo-2-fluoraniline and S1-11.

1H-NMR (400 MHz, DMSO-d6) δ 7.95-7.90 (m, 2H), 7.62 (dd, J = 11, 1.5 Hz, 1H), 7.33 (dd, J = 9.5, 1.5 Hz, 1H), 7.13-7.08 (m, 3H), 6.85 (s, 1H), 6.80-6.70 (m, 1H), 5.57 (t, J = 5.3 Hz, 1H), 4.95 (d, J= 4.3Hz, 2H), 4.71 (s, 1H), 3.47 (s, 3H), 1.85 (s, 6H), 1.46 (s, 6H). LCMS (ESI) m/z = 593.3, 595.3 [M+H]+. HPLC Purity Method A: 91 %.

2-(1-(3-chloro-3'-fluoro-4'-(hydroxymethyl)-5'-(methylsulfonyl)biphenyl-4-yl)-2-(2,6-dichlorophenyl)propan-2-yl)-1H-imidazol-4-yl)propan-2-ol (17). Prepared as describe for Example 11 from intermediate 2-(2,6-dichlorophenyl)-2-methylpropanenitrile, 4-bromo-2-chloroaniline and S1-11.
1H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 8.00 (m, 1H), 7.57 (d, \( J = 2.1 \), 1H), 7.55 – 7.49 (m, 1H), 7.13 (s, 1H), 7.11 (s, 1H), 7.07 (dd, \( J = 8.3 \), 2.1, 1H), 7.01 – 6.95 (m, 1H), 6.81 (d, \( J = 8.3 \), 1H), 6.59 (s, 1H), 5.09 (d, \( J = 5.4 \), 2H), 3.30 (s, 3H), 3.26 (m, 1H), 2.89 (t, \( J = 7.0 \), 1H), 2.06 (s, 3H), 1.92 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H). LCMS (ESI): 627.2 [M+H]+. HPLC Purity Method A: > 98%.

2-(2-(2,6-dichlorobenzyl)-1-(3'-fluoro-4'-(hydroxymethyl)-5'-(methylsulfonyl)biphenyl-4-yl)-1H-imidazol-4-yl)propan-2-ol (18). Prepared as describe for Example 11 from reagent 2-(2,6-dichlorophenyl)acetonitrile, 4-bromo aniline and S1-11, except the first alkylation step with methyl iodide was not performed.

1H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 8.12 (s, 1H), 7.70 – 7.59 (m, 3H), 7.45 (d, \( J = 8.4 \), 2H), 7.24 (d, \( J = 8.0 \), 2H), 7.16 – 7.07 (m, 1H), 6.85 (s, 1H), 5.10 (d, \( J = 5.6 \), 2H), 4.31 (s, 2H), 3.30 (s, 3H), 3.07 (s, 1H), 2.94 (t, \( J = 7.0 \), 1H), 1.55 (s, 6H). LCMS (ESI): 586.5 [M+H]+. HPLC Purity Method A: 98%
SI Figure 1. PK Agonists used as controls in in vitro experiments.

**In vitro Assay Methods:**

*Ligand Binding Assays.* Baculovirus encoding human RXRa and human LXRα or human LXRβ were used to co-infect Sf9 cells. Infected cell lysates were prepared and supernatants containing soluble RXRa-LXRα or RXRa-LXRβ heterodimers were used in scintillation proximity ligand binding assays in which compounds were tested for the ability to compete for binding with 50 nM $^{3}$H-24,25-epoxycholesterol (NEN Life Science Products / Perkin Elmer). The determined Ki represents the average of at least two independent dose response experiments. The binding affinity for each compound was be determined by non-linear regression analysis using the one site competition formula to determine the IC$_{50}$ where: Y = Bottom + (Top - Bottom)/(1+10$^{X-\log IC_{50}}$). The Ki was then calculated: Ki = IC$_{50}$/(1 + [Concentration of Ligand]/Kd of Ligand). For this assay, typically the Concentration of Ligand = 50 nM and the Kd of ligand for the receptor was 200 nM as determined by saturation binding.

*Transient Transfection Reporter Assays.*

**LXRα & LXRβ LXRE assays (Isotype-specific).** For agonist assays CV-1 cells were co-transfected with pCXM-hLXRα or pCMX-hLXRβ, and LXREx1-tk-luc plasmids in bulk and replated at a density of 8,000 cells/well in 384-well plates containing 5 µL media with 0.5% DMSO or the test agonist. LXR antagonist assays were carried out the same way but in presence of 60 nM of the agonist Pan Agonist A. 100% inhibition was calculated based on values obtained in the absence of agonist. The first two columns of each plate contained Pan Agonist A, to determine the 0% inhibition level.

Cells were incubated for 18-20 hours, lysed and assayed for luciferase activity using a Northstar HTS Workstation (Applied BioSystems). The dose response curves are generated from a 10 point curve with concentrations differing by $\frac{1}{2}$ log units. Each point represented the average of 4 wells of data from a
384 well plate. The data from this assay was fitted to the following equation, from which the EC\textsubscript{50} value may be solved: \( Y = \text{Bottom} + \frac{(\text{Top}-\text{Bottom})}{(1+10^{((\log\text{EC}_{50}-X)*\text{HillSlope})})} \).

The EC\textsubscript{50} is defined as the concentration at which an agonist elicits a response that is half way between the Top (maximum) and Bottom (baseline) values. The EC\textsubscript{50} values represented are the averages of at least 2 independent experiments. The determination of the relative efficacy or % efficacy for an agonist was by comparison to the maximum response achieved by Pan Agonist A (Supporting Information Figure 1), which was measured individually in each dose response experiment.

\textit{ABCA1 HeLa Assay (Endogenous Receptors).} HeLa cells were transfected with ABCA1x3-tk-luc reporter and pCMX-β-galactosidase plasmids and assayed for luciferase activity as described above.

\textit{Cynomolgus Monkey and Human Whole Blood Assays.} Cynomolgus monkey or human whole blood was collected in EDTA containing tubes and 0.5 mL aliquots were immediately mixed in a 96 well block with the appropriate dilution of test compound, in 0.5% DMSO. Samples were incubated at 37 °C with constant rotation for 4 hours. Incubation for 4 hours was found to be optimal for maximal RNA inductions \textit{in vitro} and it was also close in time to the 5 hr time point chosen for \textit{in vivo} blood cell RNA induction determinations. Following cell lysis, total RNA was purified, cDNAs were synthesized, and mRNAs were quantitated using SYBR-Green Quantitative PCR (Q-PCR) on an ABI Prism 7900HT Sequence Detection System. The quantity of each mRNA was determined by the \( \Delta\Delta CT \) method and normalized to the quantity of two control mRNAs, i.e. ribosomal protein L30 (L30) and beta-2-microglobulin (B2M). The induction of ABCA1 and ABCG1 by the test compound was graphed as a percent of the LXR full agonist reference standard Pan Agonist B (Supporting Information Figure 1), and potency (EC\textsubscript{50}) and efficacy (% MAX) were calculated by fitting a sigmoidal response curve as a function of log transformed compound concentration.

\textit{Primers used for RT-PCR}

\textit{Human primers:}

ABCA1: 5’-GGTGATGTTTCTGACCAATGTGA-3’ (forward)

5’–TGTCCTCATACCAGTTGAGAC-3’ (reverse);

ABCG1: 5’-GACTGCGTGTCTGCAAAAATC-3’ (forward)
L30: 5'-GCTGGAGTCGATCAACTCTAGG-3’ (forward) and
5’-CCAATTTCGCTTTGCCTTGTC-3' (reverse).

B2M: 5’-GGCTATCCAGCGTACTCCAAA-3’ (forward) and
5’-CGGCAGGCACATCTCATTTTT-3’ (reverse).

Monkey primers:

ABCA1: 5’-GGTGATGTTTCTGACCAATGTGA-3’ (forward) and
5’–TGTCCTCATACCAGTTGAGAG-3’ (reverse);

ABCG1: 5’-ACGCAGACAGCAGCTGGTGA-3’ (forward) and
5’-CTTCCCTCCACCTGGAACCT-3’ (reverse);

L30: 5’-GCTGGAGTCGATCAACTCTAGG-3’ (forward) and
5’-CCAATTTCGCTTTGCCTTGTC-3’ (reverse);

B2M: 5’-GGCTATCCAGCGTACTCCAAA-3’ (forward) and
5’-CGGCAGGCACATCTCATTTTT-3’ (reverse).

PXR Transactivation.

The cell culture medium used is DMEM (Dulbecco’s Modified Eagle’s Medium). Lipofectamine 2000, PBS, heat-inactivated fetal bovine serum (FBS), trypsin-EDTA (0.25%), and penicillin-streptomycin were purchased from GIBCO/Invitrogen (Carlsbad, CA). Charcoal/dextran treated fetal bovine serum (FBS) was purchased from Hyclone (Logan, UT). HepG2 cells were obtained from ATCC (Manassas, VA). Human PXR-pcDNA3 and luciferase reporter containing CYP3A4 promoter, CYP3A-Luc, were generated at Bristol-Myers Squibb. White tissue culture (TC)-surface 384-well plates were purchased from Perkin Elmer (Boston, MA). Luciferase substrate (Steady-Glo) was purchased from Promega (Madison, WI). Control compounds rifampicin, mifepristone, and sulfinpyrazone were purchased from Sigma (St. Louis, MO).

Cell Culture, Transient Transfection, and Assay.
Culture of HepG2 cells is performed in T175 flasks using DMEM containing 10% FBS. The transfection mixture contains 1 µg/ml of PXR-pcDNA3 plasmid DNA, 20 µg/ml of Cyp3A-Luc plasmid DNA, 90 µl/ml of Lipofectamine 2000, and serum-free medium. After incubating at room temperature for 20 minutes, the transfection mixture (1 ml per flask) is applied to the cells in fresh medium (20 ml per flask), and flasks incubated at 37°C (5% CO₂) overnight.

Cells in each flask are washed with PBS and 2 mL of Trypsin-EDTA (0.25%) is added and incubated for five minutes at 37 °C, 5% CO₂. The flasks are then tapped vigorously to break up cell aggregates. After the addition of 8 ml of DMEM containing 5% charcoal/dextran-treated FBS, the entire mixture is transferred to conical tubes. Cells are then centrifuged at 1000 rpm for 5 minutes. Cell pellets are resuspended to a final count of ~7 x 10⁶ cells/ml in freezing media (DMEM containing 20% serum and 10% DMSO). The cell suspension is aliquoted into 15-ml polypropylene tubes, 5 ml per tube. Cells are slowly frozen by placing in a Styrofoam-insulated container at -80 °C overnight. Vials are transferred to an Ultracold (-140 °C) freezer after 24 hours for long-term storage.

Vials of cryopreserved cells are thawed rapidly in a warm water bath for five minutes. Cells are pooled and diluted to 50 ml in a 50-ml conical vial. The thawed cells are centrifuged at 1500 rpm for 5 minutes to collect the cells and the supernatant discarded. Cells are then resuspended in fresh Media II (DMEM containing 5% charcoal/dextran-treated FBS, 1% Penicillin/Streptomycin, 100 µM Non-essential Amino Acids, 1 mM Sodium Pyruvate, and 2 mM L-Glutamine), counted using the Guava Cell Counter, and diluted to 1.6 x 10⁵ cells/ml in the same media.

Fifty microliters of cell mixture is added to wells in columns 1-23 of white tissue-culture treated 384-well plates containing 0.25 µl of test compound dissolved in 100% DMSO. Fifty microliters of Media II is added to wells in column 24. The plates are incubated at 37°C (5% CO₂) for 24 hours, then 5 µl of Alamar Blue reagent (Trek Diagnostics, Cat #00-100) is added to each well. Plates are then incubated an additional two hours at 37 °C, 5% CO₂ and then one hour at room temperature. Fluorescence is read at Ex525/Em598. After the fluorescence is measured, 25 µl of luciferase substrate (Steady-Glo, Promega) is added to each well. The plates are incubated for fifteen minutes at room temperature, after which the luminescence is read on a PheraStar (BMG Labtech) plate reader.

Data Analysis
Rifampicin (10 μM), a well-known agonist of PXR, is included in each plate as an internal standard and positive control. The data is then expressed as percent control (% CTRL), where the control signal is the signal from the 10 μM rifampicin and the blank signal is that from the DMSO vehicle.

\[
%\text{CTRL} = \left( \frac{\text{Compound signal} - \text{Blank signal}}{\text{Control signal} - \text{Blank signal}} \right) \times 100
\]

Compounds are tested at ten concentrations (2.5 nM - 50 μM, 1:3 serial dilution). Assay results are reported as EC\textsubscript{50}, the concentration of compound at which 50% of the maximal response is observed, and as YMAXOBS, the maximal response (highest percent CTRL) observed for that compound. The EC\textsubscript{50} is defined as the concentration corresponding to half of the maximal response derived from the fitted 20-point curve as determined using a four-parameter logistic regression model.

**Crystal Structure with 15 complexed to LXRβ.**

Crystals were grown in a custom butane-diol/lithium sulfate random screen with 15 that diffract to 2.4 Å. Despite a large number of crystallization attempts, no diffraction quality crystals were obtained, and required a combination of multiple rounds of seeding into large numbers of pre-equilibrated drops to yield the crystals. Data were collected and processed with Exprocess. Data were collected on a Rigaku R-AXIS IV detector and MicroMax generator (Rigaku, The Woodlands, Texas). Data were then reduced in XDS\textsuperscript{3} and phasing performed in Phaser.\textsuperscript{4} The model was rebuilt to a final free R value (R\text{free}) and working set R value (R\text{cryst}) of 0.26783 and 0.22541, respectively, through several rounds of refinement in REFMAC,\textsuperscript{5} and model building in Coot.\textsuperscript{6} The complex crystallized in space group C2 with four independent subunits in the asymmetric unit, labeled A, B, C and D. A and B subunits form a canonical dimer, as do C and D. Helix 12 from subunit A is bound in the co-activator binding pocket of subunit B; and helix 12 from subunit C is bound in the co-activator binding pocket of subunit D. The coordinates have been deposited (PDB code: 5JY3).

**In vivo Methods**

*In vivo* pharmacokinetic and pharmacodynamic studies were performed according to American Association for Accreditation of Laboratory Animal Care guidelines and protocols were approved by the Bristol-Myers Squibb Animal Care and Use Committee.

*Pharmacokinetics in Mice*
The pharmacokinetics were characterized in male C57BL6 mice (21.6-36 g). Mice received compound by oral gavage (fasted overnight) as a suspension in 0.75% CMC/0.1% Tween 80 in water. Blood samples (~0.2 mL) were obtained from 3 mice per time point at 0.25, 0.5, 1, 3, 6, 8 and 24 h post dose resulting in a composite pharmacokinetic profile (three blood sample were collected from each mouse). Blood samples were allowed to coagulate and centrifuged at 4 °C (1500-2000xg) to obtain serum. Serum samples were stored at -20 °C until analysis by LC/MS/MS.

*Distribution Into Mice Brain*

Distribution of 15 into the brain was studied following oral administration (10 mg/kg) to C57BL6 mice (N = 12). Brain and blood samples were collected at 1, 4, 8 and 24 h post dose (N = 3 animals per time point). Brain tissues were blotted dry, weighed, and homogenized (1:6 v/v) with 50% acetonitrile/water and centrifuged (10,000xg at 4°C for 10 min). Plasma samples were obtained from blood by centrifugation at 4°C (1500-2000xg). All samples were stored at -20°C until analysis by LC/MS/MS.

*Cynomolgus Monkey Single-dose Pharmacokinetics*

The pharmacokinetics to evaluate clearance in cynomolagus monkeys (*Table 2*) was performed either by i.v. dosing of a single compound or i.v. dosing at 0.2 mg/kg in a cassette of 5 compounds that have different molecular weights. The i.v. infusion was done over 10 minutes (vehicle: 10% EtOH; 50% PEG400; 40% saline) two monkeys by similar procedures described below.

The pharmacokinetics were evaluated in male cynomolagus monkeys in a crossover design (*SI Table 1*). Following an overnight fast, 3 animals (4.5 to 5.7 kg) received drug by IV infusion (1 mg/kg over 10 min) via a femoral vein and by oral gavage (3 mg/kg), with a 1-week washout between treatments. Serial blood samples (~0.3 mL) were collected from a femoral artery pre-dose and at 0.17 (IV only), 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 48 and 72 h post dose, and centrifuged at 4 °C (1500-2000xg) to obtain plasma. Plasma samples were stored at -20 °C until analysis by LC/MS/MS.

*Cynomolgus Monkey Pharmacodynamics Studies.*

Male cynomolgus monkeys (Macaca fascicularis) were obtained from the Bioculture Group (Mauritius) and pair-housed for 3 weeks for acclimation and animals were subsequently transferred to single housing in standard non-human primate cages at the start of the study. Water and standard primate chow
(Harlan Teklad Global 2050, Frederick, MD) were provided ad libitum. Food was provided daily in the amount of 120 grams. When fasting was required, food was removed at 3 PM and returned on the following day at 9 AM, 2 hours after dosing. For the 14 day PD studies, animals were randomized into treatment groups (N=3/group; 3-6 kg) and received the following treatments at 7 am, q.d. for 14 days by oral gavage: vehicle (0.5% carboxymethyl cellulose and 2% Tween 80 (Sigma-Aldrich, St. Louis, MO)), 10 mg/kg/d T0901317, and 0.1, 0.3, 1, or 3 mg/kg/d BMS-852927 or 0.3, 1, 3, 10, 30 mg/kg/d BMS-832878. An initial venous blood sample was obtained on the day prior to the start of dosing (day -1) for baseline RNA and lipid measurements, followed by samples taken on days 1, 4, 7, and 14 of dosing for the PD study. Blood samples for RNA and compound exposure determinations were collected at 5-6 hours post-dose, and those for plasma lipids were collected 24 hours post-dose. Plasma triglycerides were determined using a kit for triglycerides from Roche Diagnostics (Indianapolis, IN). The values reported are the mean. Statistical analysis was done by ANOVA, using Dunnett’s post-hoc test. The reported inductions of ABCG1 in the text were statistically significant compared to baseline levels by ANOVA, using Dunnett’s post-hoc test with p < 0.05 or better.

Data Analysis of Pharmacokinetic Samples.

The pharmacokinetic parameters were obtained by non-compartmental analysis of plasma concentration vs. time data (KINETICA™ software, Version 4.2, InnaPhase Corporation, Philadelphia, PA). The peak concentration (Cmax) and time for Cmax (Tmax) were recorded directly from experimental observations. The area under the curve from time zero to the last sampling time (AUC(0-T)) and the area under the curve from time zero to infinity (AUC(INF)) were calculated using a combination of linear and log trapezoidal summations. The total plasma clearance (CL), steady-state volume of distribution (Vss), and apparent elimination half-life (T1/2) were estimated after i.v. administration. Estimations of AUC and T1/2 were made using a minimum of 3 time points with quantifiable concentrations. The absolute oral bioavailability (F) was estimated as the ratio of dose-normalized AUC values following oral and i.v. doses.

Compound Quantification in Biological Samples.

The quantification of LXR agonists in biological samples was carried out by methods as described for Example 15. The following section details the liquid chromatography with tandem mass spectrometry (LC/MS/MS)-based bioanalytical methods developed to support the analysis of reported agonists in in
*vivo* biological samples from pharmacokinetic, and pharmacodynamic studies in mouse and cynomolgus monkey. Similar methods were used to quantitate Example 13.

**Standard Curve Preparation**

Standard curves and quality control (QC) samples defining the dynamic range of the bioanalytical method were prepared in the respective biological matrix and processed in the same fashion as the test samples, unless otherwise noted. Standards were analyzed in singlicates. Quality control samples were prepared in blank biological matrix at 3 concentration levels within the range of the standard curve and were analyzed as replicates (N=2) within each analytical set. The predicted concentrations of more than 75% of the QCs from different matrices were within 20% of their nominal values, indicating acceptable assay performance.

**Sample Preparation: Blood, Plasma, Serum**

Plasma, serum, and blood samples were prepared as described below. If dilutions were required, an aliquot of the sample was diluted into the respective matrix. Sample preparation was conducted either manually, on a Janus 8-tip automated liquid handler, or on a Janus Mini 96-tip automated liquid handler. Aliquots (50 µL) of the biological matrix from in vivo studies and standard/QC samples were treated with 1 M ammonium carbonate in water (pH 9.2, unadjusted) (50 µL) containing internal standard (IS), followed by 300 µL methyl t-butyl ether (MTBE) and partitioned by liquid-liquid extraction (LLE) using forty fill-expel tip repetitions for approximately 3 minutes. The aqueous and organic layers was then centrifuged for 2-min at 3900 rpm. The top organic layer extraction solvent MTBE (250 µL) was removed to another clean 96-well plate and placed in a nitrogen evaporator for 15 min at 40°C to dryness. Aliquots (100 µL) were used to reconstitute the dry extracts with mobile phase consisting of 1:1 acetonitrile/water. An aliquot (10 µL) was first injected onto the high-Turboflow performance liquid chromatography (HTLC) extraction column, then eluted onto a second high-performance liquid chromatography (HPLC) column for LC/MS/MS-based analysis.

**Instrumentation:** The Aria TX-2 (Thermo-Fisher Scientific, Franklin, MA) HPLC system consisted of 8 Shimadzu LC10AD pumps with 2 SCL-10AVP System Controllers (Columbia, MD) and a dual arm CTC Analytics HTS PAL autosampler (Switzerland) equipped with cooling stack that maintained samples at 10°C during analysis. The HTLC on-line extraction column was a Cyclone-P mixed polymer (0.5 x 50 mm, 50 µM particle, Thermo-Fisher Scientific, Franklin, MA) kept at room temperature. The HPLC C18 analytical column used was an XBridge C18 (2.1 mm x 50 mm, 5 µM particle, Waters Cor-
poration, Milford, MA) kept at room temperature. The mobile phase, which consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), was delivered at a flow rate of 1.5 mL/min to the HTLC on-line extraction column and at 0.5 mL/min to the HPLC C18 analytical column. These flow rates change during the transfer step between 0.5 to 1.0 min when analyte is eluted off the HTLC on-line extraction column onto the HPLC C18 analytical column. The gradients are summarized in the following tables.

### Table S2: Mobile Phase Gradient for HPLC C18 Analytical Column LC/MS/MS Sample Analysis

| Time (min) | % A  | % B  | Flow Rate (mL/min) | Curve   |
|------------|------|------|-------------------|---------|
| 0 (Initial)| 95   | 5    | 0.5               | Isocratic|
| 0.50       | 95   | 5    | 0.3               | Isocratic|
| 1.00       | 95   | 5    | 0.3               | Isocratic|
| 1.10       | 95   | 5    | 0.5               | Isocratic|
| 2.00       | 5    | 95   | 0.5               | Linear  |
| 2.50       | 5    | 95   | 0.5               | Isocratic|
| 2.60       | 95   | 5    | 0.5               | Step    |
| 5.00       | 95   | 5    | 0.5               | Isocratic|

### Table S3: Mobile Phase Gradient for HTLC On-line Extraction Column LC/MS/MS Sample Analysis

| Time (min) | % A  | % B  | Flow Rate (mL/min) | Curve   |
|------------|------|------|-------------------|---------|
| 0 (Initial)| 100  | 0    | 1.5               | Isocratic|
| 0.50       | 100  | 0    | 0.2               | Isocratic|
| 1.00       | 100  | 0    | 0.2               | Isocratic|
| 1.10       | 0    | 100  | 1.5               | Step    |
| 2.00       | 0    | 100  | 1.5               | Isocratic|
| 2.10       | 50   | 50   | 1.5               | Step    |
| 2.50       | 50   | 50   | 1.5               | Isocratic|
| 2.60       | 100  | 0    | 1.5               | Step    |
| 5.00       | 100  | 0    | 1.5               | Isocratic|

Using this method the retention times for both 15 and the Internal Standard were 1.66 min. The retention time of the metabolite 19 was 1.69 min. The total analysis time was 5.0 min.

The HPLC was interfaced to a Finnigan Quantum Ultra LC/MS/MS tandem mass spectrometer (Thermo Electron Corporation, San Jose, CA) equipped with a heated electrospray interface operating in the positive ionization mode. Ultra-high-purity (UHP) nitrogen was used as the sheath and aux gases at flow
rates of 55 psi for sheath and 25 units for aux. The desolvation temperature was 350°C and the source temperature was 350°C. Detection of each analyte was achieved through selected reaction monitoring.

**Equilibrium Dialysis Studies with α1 AGP and HSA.**

The method involved equilibrium dialysis using 0.134 M phosphate buffer (pH 7.4) and dialysis membranes (HTDialysis LLC, Gales Ferry, CT) with a 12,000- to 14,000-dalton molecular weight cutoff. The dialysis cells (Spectrum, Laguna Hills, CA) were rotated at 20 rpm in a water bath maintained at 37 °C. The extent of binding of test compound was determined at a nominal concentration of 1 µM (N = 3, 0.1% α1 AGP and 4% HSA). Aliquots from the buffer and protein side (4% HSA or 0.1% hAGP) were taken at 4 h and collected in the following manner. Buffer samples (100 µL) were collected in a 96-well plate that contained 100 µL of the protein solution. Protein solutions (20 µL) were collected in a 96-well plate that contained a mixed-matrix solution (180 µL). The mixed-matrix solution contained 50% protein binding buffer and 50% blank hAGP or HSA solution of the species under investigation. Samples were analyzed for test compound. Percent protein binding was calculated from the ratio of the measured concentration in buffer to that in protein solution using the following equation:

\[ \% \text{Bound} = 100 \times \frac{C_{\text{serum}} - C_{\text{buffer}}}{C_{\text{serum}}} \]

where \( C_{\text{serum}} \) is the concentration of test compound in the protein solutions (HSA, hAGP) and \( C_{\text{buffer}} \) is the concentration of test compound in buffer.

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