Supporting Information

Discovery of the Selective CYP17A1 Lyase Inhibitor BMS-351 for the Treatment of Prostate Cancer

Audris Huang,* Lata Jayaraman, Aberra Fura, Gregory D. Vite, George L. Trainor, Marco M. Gottardis, Thomas E. Spires, Vanessa M. Spires, Cheryl A. Rizzo, Mary T. Obermeier, Paul A. Elzinga, Gordon Todderud, Yi Fan, John A. Newitt, Sophie M. Beyer, Yongxin Zhu, Bethanne M. Warrack, Angela K. Goodenough, Andrew J. Tebben, Arthur M. Doweyko, David L. Gold, and Aaron Balog

Bristol-Myers Squibb Research and Development, Princeton, New Jersey 08543-4000

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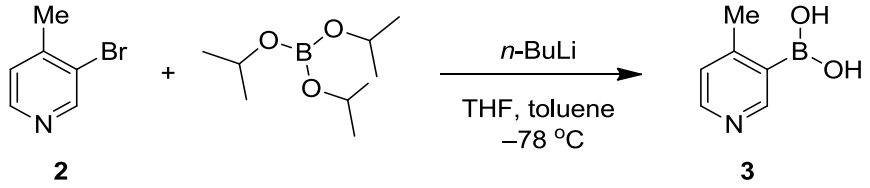
General Methods. Reagents and solvents were used as obtained from commercial suppliers without further purification. All reactions were carried out under a nitrogen atmosphere in a roundbottom flask or in a Chemglass vial fitted with a pressure relief cap. NMR spectra were recorded on Bruker 400 MHz spectrometer. Splitting patterns were designated as "s, d, t, q, m, and br" indicating "singlet, doublet, triplet, quartet, multiplet, and broad," respectively. Chemical shifts (δ) are reported with CD_3OD (δ = 3.31 ppm), DMSO (δ = 2.50 ppm), or $CDCl_3$ (δ = 7.27 ppm) as internal standard. All *J* values are given in Hz. Reaction progress was monitored by LC-MS on a Waters ZQ 2000 single quadrupole mass spectrometer (Milford, MA) interfaced to a Shimadzu Discovery VP LC (Columbia, MD). Chromatographic separations were achieved employing a 2.1 x 30 mm, 5 μm, 100Å, Phenomenex Luna C18(2) column (Torrance, CA) with gradient elution at 1.2 mL/min. Mobile phase A was 90:10 water:acetonitrile with 10 mM NH_4OAc and mobile phase B was 10:90 water:acetonitrile with 10 mM NH_4OAc, wavelength 220 nM. A linear gradient was formed from 0% to 100% mobile phase B over 2 minutes, followed by a 0.5 min hold at 100% mobile phase B. A 2 μL injection was used. Positive ESI MS data were acquired from *m/z* 125 to *m/z* 1000. The instrument was operated at unit resolution. Electrospray ionization (ESI) high resolution mass spectrometry (HRMS) was obtained on a Thermo Fisher Finnigan LTQ-FT instrument. Column chromatography was carried out using an ISCO system, and performed using pre-packed silica gel (SiO_2) columns. Preparative HPLC was performed using a Waters system with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-μm particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile:water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10-mM ammonium acetate; Gradient: 15–50% B over 30 minutes, then a 15-minute hold at 100% B; Flow: 20 mL/min. The purity of all new compounds was determined by reverse phase HPLC and was >95%.

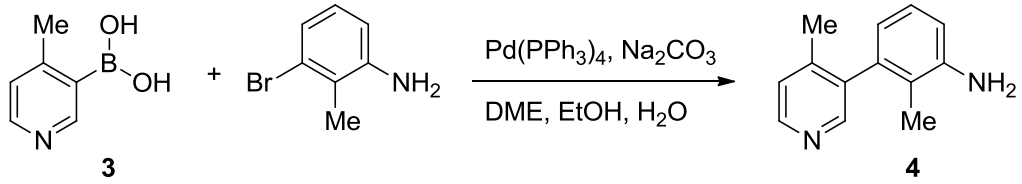
Experimental Procedures and Characterization Data for Compounds 1, 6–11, and 14–18

Synthesis of Compound 1:

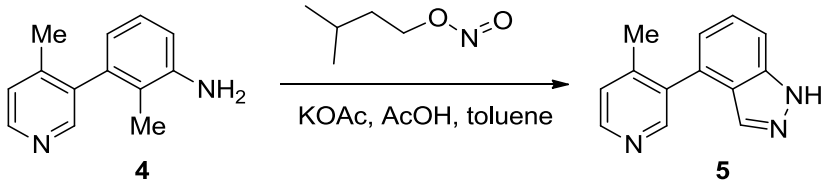


(4-Methylpyridin-3-yl)boronic acid, 3. To a stirring solution of 3-bromo-4-methylpyridine (9.68 mL, 87 mmol) and triisopropylborate (26.3 mL, 113 mmol) in toluene (77 mL) and THF (19.1 mL) cooled to –78 °C was added *n*-butyllithium (45.3 mL, 113 mmol) over the course of 2 h with a syringe pump (approx. 0.25 mL/min). During this time period, the reaction color changed from yellow to bright red. The reaction was monitored by HPLC and upon consumption of starting material, the dry/ice acetone bath was removed and 2 N HCl (60 mL) was added. The solution became yellow and an insoluble solid precipitated. The solid was filtered (crop 1). The biphasic filtrate was transferred to a 500 mL separatory funnel and the layers were separated. The aqueous layer was then neutralized to pH = 7 with a saturated aqueous solution of $NaHCO_3$ (100 mL) followed by extraction with THF (3X). All organic

phases were combined, dried over Na_2SO_4, filtered, and concentrated. As the concentration progressed, the material was periodically diluted with CH_3CN causing a precipitate to form. The resultant solid was then vigorously stirred with CH_3CN and filtered to afford 4-methylpyridin-3-ylboronic acid (9.67 g, 70.6 mmol, 81 % yield) as an off-white solid (crop 2), which was further dried under high vacuum overnight. ^{1}H NMR (400 MHz, CD_3OD) δ 8.45 (1 H, s), 8.33 (1 H, d, *J*=5.77 Hz), 7.50 (1 H, d, *J*=5.27 Hz), 2.62 (3 H, s). MS (ESI): *m/z* = 138.0 $[M+H]^+$.

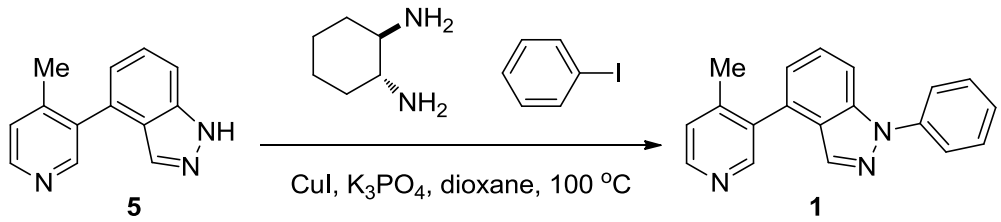


2-Methyl-3-(4-methylpyridin-3-yl)aniline, 4. To a solution of 3-bromo-2-methylaniline (4.00 g, 21.5 mmol) and 4-methylpyridin-3-ylboronic acid (3.93 g, 21.5 mmol) in a mixture of DME (80 mL), water (40 mL) and EtOH (40 mL) combined in a sealed tube was added Na_2CO_3 (9.11 g, 86.0 mmol). This suspension was degassed with a stream of N_2 for 10 minutes and then tetrakis (triphenyl phosphine) palladium (0) (1.24 g, 1.08 mmol) was added followed by degassing for 10 minutes. The tube was sealed and heated at 90 °C for 16 hours. Upon cooling, the reaction mixture was diluted with copious amounts of MeOH and filtered, washing with MeOH. The filtrate was concentrated under reduced pressure. The crude material was purified by silica gel chomatography using a 220 g ISCO column eluting with 100% CH_2Cl_2 to 5% MeOH at 150 mL/min. Concentration of appropriate fractions provided the title compound as a tan solid (4.15 g, 19.7 mmol, 92 % yield). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.37 (1 H, d, *J*=5.04 Hz), 8.18 (1 H, s), 7.36 (1 H, d, *J*=5.29 Hz), 7.05 (1 H, t, *J*=7.68 Hz), 6.81 (1 H, dd, *J*=7.93, 0.88 Hz), 6.47 (1 H, dd, *J*=7.55, 1.01 Hz), 2.12 (3 H, s), 1.84 (3 H, s). MS (ESI): *m/z* = 199.1 $[M+H]^+$.



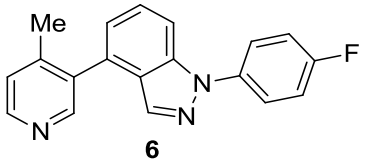
4-(4-Methylpyridin-3-yl)-1H-indazole, **5**. A stirring solution of 2-methyl-3-(4-methylpyridin-3-yl)aniline (2.20 g, 11.1 mmol) in acetic acid (6.35 mL, 111 mmol) and toluene (20 mL) was cooled to 0 °C. At this time isoamyl nitrite (2.24 mL, 16.6 mmol) was added dropwise and the reaction was stirred for an additional 15 min. Then, potassium acetate (0.109 g, 1.11 mmol) was added and the reaction was allowed to warm to ambient temperature for 2 h. The reaction was monitored by HPLC and upon completion, the reaction was diluted with water (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organics where washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by silica gel chomatography using a 40 g ISCO

column eluting with 100% CH_2Cl_2 to 5% MeOH at 40 mL/min. Concentration of appropriate fractions provided the title compound as a dark red solid (1.69 g, 72.8% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.46 (1 H, d, *J*=5.22 Hz), 8.42 (1 H, s), 7.73 (1 H, s), 7.73 (1 H, s), 7.63 (1 H, d, *J*=8.52 Hz), 7.51 (1 H, t, *J*=7.70 Hz), 7.46 (1 H, d, *J*=5.22 Hz), 7.08 (1 H, d, *J*=6.87 Hz), 2.25 (3 H, s). MS (ESI): *m/z* = 210.1 $[M+H]^+$.



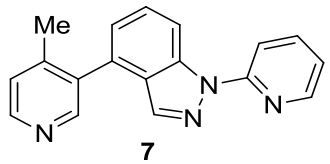
4-(4-Methylpyridin-3-yl)-1-phenyl-1H-indazole, 1. To a solution of 4-(4-methylpyridin-3-yl)-1H-indazole (0.050 g, 0.239 mmol) in dioxane (0.239 mL) at ambient temperature was added potassium phosphate (0.103 g, 0.478 mmol), iodobenzene (0.049 g, 0.239 mmol) and (*1R,2R*)-cyclohexane-1,2-diamine (0.014 g, 0.119 mmol). The vial was then purged with nitrogen followed by addition of copper(I)iodide (2.00 mg, 0.012 mmol). The vial was sealed and heated at 100 °C for 4 hours. The reaction was allowed to cool to rt, diluted with EtOAc, filtered and concentrated *in vacuo*. The crude material was purified by silica gel chomatography using a 4 g ISCO column eluting with 100% CH_2Cl_2 to 15% acetone at 18 mL/min. Concentration of appropriate fractions provided the title compound as a clear viscous oil (0.051 g, 74.1% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (1 H, s), 8.56 (1 H, d, *J*=4.95 Hz), 7.93 (1 H, d, *J*=0.82 Hz), 7.80 (1 H, d, *J*=8.52 Hz), 7.77 (2 H, dd, *J*=8.39, 0.96 Hz), 7.57 (2 H, t, *J*=7.84 Hz), 7.52 (1 H, dd, *J*=8.52, 6.87 Hz), 7.41 (1 H, t, *J*=7.42 Hz), 7.31 (1 H, d, *J*=4.95 Hz), 7.13 (1 H, d, *J*=7.15 Hz), 2.28 (3 H, s); ^{13}C NMR (126 MHz, CD_3OD) δ 150.3, 149.4, 148.3, 141.3, 140.4, 137.3, 135.6, 132.5, 130.9, 129.0, 128.6, 127.2, 126.1, 124.4, 124.1, 111.6, 20.0; HRMS (ESI) *m/z* calcd for $C_{19}H_{16}N_3$ $[M+H]^+$ 286.13387, found 286.13356. MS (ESI): *m/z* = 286.3 $[M+H]^+$.

Compounds **6-11** were prepared by the procedure described for compound **1** using indazole **5** and the appropriate aryl iodide or aryl bromide. The characterization data for compounds **6-11** is provided below.



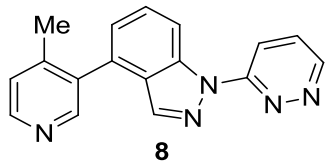
1-(4-Fluorophenyl)-4-(4-methylpyridin-3-yl)-1H-indazole, 6. Yield: 7.6 mg, 33%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.60 (br. s., 2H), 7.98 (d, *J*=0.9 Hz, 1H), 7.84 (d, *J*=8.7 Hz, 1H), 7.81 - 7.75 (m, 2H), 7.70 (d, *J*=4.6 Hz, 1H), 7.63 (dd, *J*=8.6, 7.0 Hz, 1H), 7.41 - 7.33 (m, 2H), 7.24 (dd, *J*=7.0, 0.6 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 163.2 (d, *J*=245.4 Hz, 1C), 155.5 (br. s., 1C), 145.8 (br. s., 1C), 144.7 (br. s., 1C), 140.6, 139.0 (br. s., 1C), 137.4 (d, *J*=2.9 Hz, 1C), 135.4, 130.3, 129.1, 126.6 (d,

J=8.4 Hz, 2C), 125.7, 124.3, 117.7 (d, *J*=22.4 Hz, 2C), 112.3, 20.8. HRMS (ESI) *m/z* calcd for $C_{19}H_{15}FN_3$ $[M+H]^+$ 304.12445, found 304.12424.



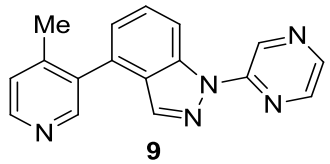
7

4-(4-Methylpyridin-3-yl)-1-(pyridin-2-yl)-1H-indazole, 7. Yield: 20 mg, 51%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.88 (1 H, d, *J*=8.53 Hz), 8.56 (1 H, dt, *J*=4.83, 0.85 Hz), 8.48 (1 H, d, *J*=5.02 Hz), 8.45 (1 H, s), 8.04 (1 H, d, *J*=8.28 Hz), 7.89 – 7.97 (2 H, m), 7.63 (1 H, dd, *J*=8.53, 7.03 Hz), 7.47 (1 H, d, *J*=5.02 Hz), 7.24 – 7.30 (1 H, m), 7.22 (1 H, d, *J*=7.03 Hz), 2.27 (3 H, s); ^{13}C NMR (101 MHz, CD_3OD) δ 155.6, 150.4, 149.4, 149.1, 148.4, 140.3, 140.0, 137.2, 137.0, 131.9, 129.2, 127.2, 126.9, 124.9, 121.9, 116.3, 114.8, 20.0. HRMS (ESI) *m/z* calcd for $C_{18}H_{15}N_4$ $[M+H]^+$ 287.12912, found 287.12875.



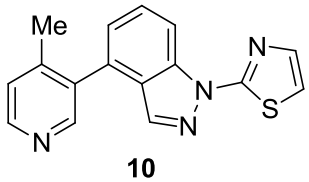
8

4-(4-Methylpyridin-3-yl)-1-(pyridazin-3-yl)-1H-indazole, 8. Yield: 6.2 mg, 22%; ^{1}H NMR (400 MHz, CD_3OD) δ 9.11 (dd, *J*=4.8, 1.3 Hz, 1H), 8.96 (d, *J*=8.5 Hz, 1H), 8.51 (d, *J*=5.0 Hz, 1H), 8.47 (s, 1H), 8.44 (dd, *J*=9.0, 1.5 Hz, 1H), 8.12 – 8.06 (m, 1H), 7.89 (dd, *J*=9.0, 4.8 Hz, 1H), 7.75 (dd, *J*=8.5, 7.3 Hz, 1H), 7.50 (d, *J*=5.0 Hz, 1H), 7.32 (d, *J*=6.8 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 159.1, 150.5, 150.3, 149.5, 148.4, 140.2, 138.9, 136.9 (br. s., 1C), 132.3, 131.2, 130.1, 127.4, 125.8, 120.4, 116.6, 20.0. HRMS (ESI) *m/z* calcd for $C_{17}H_{14}N_5$ $[M+H]^+$ 288.12437, found 288.12407.



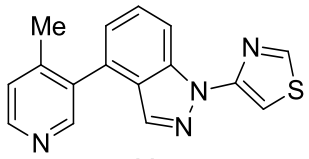
9

4-(4-Methylpyridin-3-yl)-1-(pyrazin-2-yl)-1H-indazole, 9. Yield: 31 mg, 25%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (d, *J*=1.3 Hz, 1H), 8.84 – 8.71 (m, 3H), 8.68 (dd, *J*=2.6, 1.4 Hz, 1H), 8.61 (d, *J*=2.5 Hz, 1H), 8.36 (s, 1H), 7.87 – 7.72 (m, 2H), 7.42 (d, *J*=6.8 Hz, 1H), 2.34 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 151.9, 148.3, 147.4 (br. s., 1C), 143.4, 141.5, 140.5, 138.4, 137.7, 137.2, 131.4, 129.9, 128.1, 127.0, 125.7, 116.5, 20.4. HRMS (ESI) *m/z* calcd for $C_{17}H_{14}N_5$ $[M+H]^+$ 288.12437, found 288.12396.



10

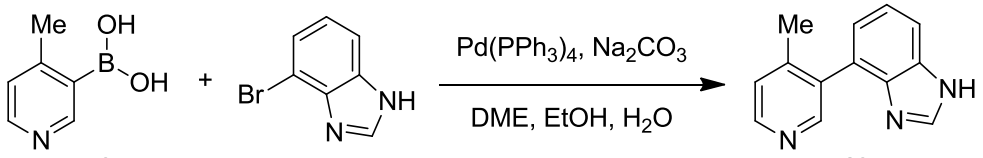
2-(4-(4-Methylpyridin-3-yl)-1H-indazol-1-yl)thiazole, 10. Yield: 14 mg, 14%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.87 (br. s., 1H), 8.82 (br. s., 1H), 8.80 (d, *J*=8.8 Hz, 1H), 8.12 (s, 1H), 8.11 (s, 1H), 7.79 (dd, *J*=8.7, 7.2 Hz, 1H), 7.70 (d, *J*=3.5 Hz, 1H), 7.42 (dd, *J*=7.3, 0.8 Hz, 1H), 7.34 (d, *J*=3.8 Hz, 1H), 2.53 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 163.9, 152.6, 147.7, 146.8, 141.6, 139.7, 138.1, 137.9, 131.2, 130.3, 128.3, 126.6, 125.9, 116.3, 115.4, 20.5. HRMS (ESI) *m/z* calcd for $C_{16}H_{13}N_4S$ $[M+H]^+$ 293.08554, found 293.08525.



11

4-(4-(4-Methylpyridin-3-yl)-1H-indazol-1-yl)thiazole, 11. Yield: 17 mg, 57%; ^{1}H NMR (400 MHz, CD_3OD) δ 9.13 (d, *J*=2.3 Hz, 1H), 8.53 – 8.45 (m, 3H), 7.94 (d, *J*=1.0 Hz, 1H), 7.67 (d, *J*=2.5 Hz, 1H), 7.64 (dd, *J*=8.5, 7.0 Hz, 1H), 7.49 (d, *J*=5.0 Hz, 1H), 7.22 (dd, *J*=7.2, 0.6 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 154.8, 153.2, 150.3, 149.4, 148.4, 140.3, 137.2, 136.6, 132.1, 129.1, 127.2, 125.9, 124.5, 114.4, 104.8, 20.0. HRMS (ESI) *m/z* calcd for $C_{16}H_{13}N_4S$ $[M+H]^+$ 293.08554, found 293.08511.

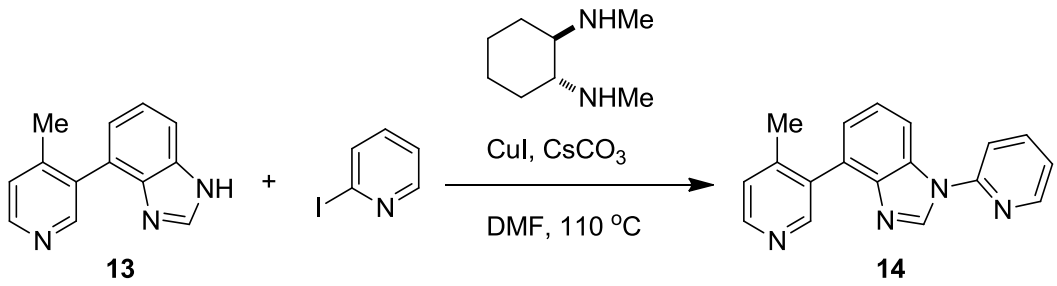
Synthesis of Compound 14:



3 **13**

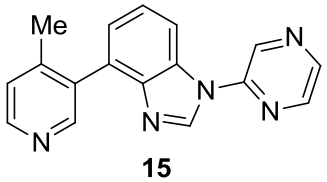
4-(4-Methylpyridin-3-yl)-1H-benzo[d]imidazole, 13. A vial was charged with tetrakis(triphenylphosphine) palladium(0) (0.309 g, 0.267 mmol), 4-bromo-1H-benzo[d]imidazole (1.05 g, 5.35 mmol), sodium carbonate (2.83 g, 26.7 mmol), and 4-methylpyridin-3-ylboronic acid (1.10 g, 8.02 mmol). The mixture was stirred at room temperature for 10 min under N_2, then water (20.0 mL), DME (9.98 mL), and EtOH (9.98 mL) were added sequentially. The resultant mixture was stirred under N_2 for 15 min, then heated at 90 °C overnight. After 23 h, the reaction mixture was allowed to cool to room temperature, then quenched with water and diluted with EtOAc. The layers were separated and the aqueous phase extracted with EtOAc (3X). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated to afford a brown residue. The crude material was dissolved in a minimal

amount of CH_2Cl_2 and purified by silica gel chromatography using an ISCO instrument (80 g column, 60 mL/min, 1–20% MeOH in CH_2Cl_2 over 30 min, t_r = 24 min) gave the title compound (1.04 g, 4.97 mmol, 93 % yield) as an off- white foam. ^{1}H NMR (400 MHz, CD_3OD) δ 9.13 (d, *J*=2.3 Hz, 1H), 8.53 – 8.45 (m, 3H), 7.94 (d, *J*=1.0 Hz, 1H), 7.67 (d, *J*=2.5 Hz, 1H), 7.64 (dd, *J*=8.5, 7.0 Hz, 1H), 7.49 (d, *J*=5.0 Hz, 1H), 7.22 (dd, *J*=7.2, 0.6 Hz, 1H), 2.28 (s, 3H); ESI MS $[M+H]^+$ = 210.0.



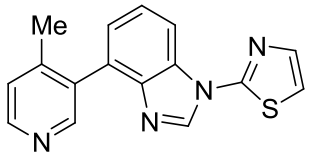
4-(4-Methylpyridin-3-yl)-1-(pyridin-2-yl)-1H-benzo[d]imidazole, 14. A vial was charged with 4-(4-methylpyridin-3-yl)-1H-benzo[d]imidazole (25.0 mg, 0.119 mmol), copper (I) iodide (1.14 mg, 5.97 µmol), and cesium carbonate (82.0 mg, 0.251 mmol). The reaction vessel was fitted with a rubber septum, evacuated, and back-filled with N_2. This evacuation/back-fill sequence was repeated one additional time. To this mixture were added 2-iodopyridine (25.4 µl, 0.239 mmol), (*1R,2R*)-N1,N2-dimethylcyclohexane-1,2-diamine (3.77 µl, 0.024 mmol), and DMF (119 µl) under N_2. The reaction vial was quickly sealed and the contents were stirred while heating in a metal pie block at 110 °C. After 21 h, the reaction was allowed to cool to rt. The heterogeneous mixture was filtered through a disposable fritted funnel and the filter cake washed with CH_2Cl_2 (10 mL). The filtrate was concentrated and purified by silica gel chromatography using an ISCO instrument (12 g column, 30 mL/min, 0–20% MeOH in CH_2Cl_2 over 20 min, t_r = 11.0 min) to afford 4-(4-methylpyridin-3-yl)-1-(pyridin-2-yl)-1H-benzo[d]imidazole (24 mg, 0.081 mmol, 68.1 % yield) as a colorless film. ^{1}H NMR (400 MHz, CD_3OD) δ 8.81 (s, 1H), 8.69 – 8.63 (m, 1H), 8.47 (br. s., 2H), 8.31 (dd, *J*=8.4, 1.1 Hz, 1H), 8.13 – 8.04 (m, 1H), 7.87 (d, *J*=8.1 Hz, 1H), 7.54 (dd, *J*=8.1, 7.5 Hz, 1H), 7.46 (ddd, *J*=7.4, 5.0, 0.8 Hz, 2H), 7.30 (dd, *J*=7.5, 0.9 Hz, 1H), 2.26 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 151.3, 150.63, 150.60, 149.14, 149.07, 143.7, 143.5, 141.0, 136.8, 133.9, 130.6, 127.0, 125.82, 125.79, 124.0, 116.7, 115.0, 20.3. HRMS (ESI) *m/z* calcd for $C_{18}H_{15}N_4$ $[M+H]^+$ 287.12912, found 287.12929.

Compounds **15–17** were prepared by the procedure described for compound **14** using benzimidazole **13** and the appropriate aryl iodide or aryl bromide. The characterization data for compounds **15–17** is provided below.



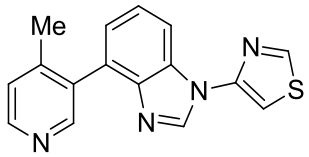
15

4-(4-Methylpyridin-3-yl)-1-(pyrazin-2-yl)-1H-benzo[d]imidazole, 15. Yield: 24 mg, 58%; ^{1}H NMR (400 MHz, CD_3OD) δ 9.21 (d, *J*=1.3 Hz, 1H), 8.93 (s, 1H), 8.70 - 8.61 (m, 2H), 8.44 (br. s., 2H), 8.37 (dd, *J*=8.4, 0.7 Hz, 1H), 7.54 (t, *J*=7.8 Hz, 1H), 7.44 (d, *J*=4.8 Hz, 1H), 7.31 (dd, *J*=7.5, 0.9 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 150.6, 149.1, 148.5, 144.7, 143.9, 143.6, 138.3, 136.6, 133.6, 130.8, 127.0, 126.3, 126.2, 115.3, 20.3. HRMS (ESI) *m/z* calcd for $C_{17}H_{14}N_5$ $[M+H]^+$ 288.12437, found 288.12447.



16

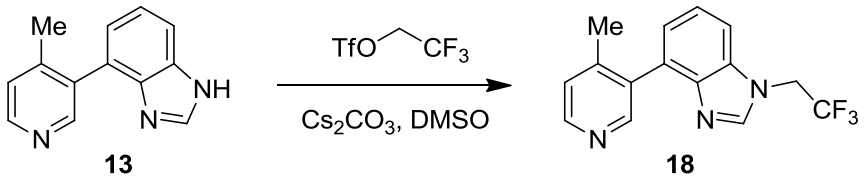
2-(4-(4-Methylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)thiazole, 16. Yield: 23 mg, 64%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.78 (s, 1H), 8.52 - 8.40 (m, 2H), 8.29 (dd, *J*=8.3, 1.0 Hz, 1H), 7.80 (d, *J*=3.5 Hz, 1H), 7.63 - 7.57 (m, 2H), 7.45 (d, *J*=5.1 Hz, 1H), 7.36 (dd, *J*=7.4, 1.0 Hz, 1H), 2.26 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 158.5, 150.6, 149.2, 149.1, 143.8, 143.2, 141.8, 136.4, 133.6, 131.1, 127.0, 126.6, 126.5, 118.2, 114.2, 20.3. HRMS (ESI) *m/z* calcd for $C_{16}H_{13}N_4S$ $[M+H]^+$ 293.08554, found 293.08586.



17

4-(4-(4-Methylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)thiazole, 17. Yield: 4.2 mg, 14%; ^{1}H NMR (400 MHz, CD_3OD) δ 9.18 (d, *J*=2.3 Hz, 1H), 8.72 (s, 1H), 8.49 (br. s., 2H), 8.10 (dd, *J*=8.3, 1.0 Hz, 1H), 7.94 (d, *J*=2.3 Hz, 1H), 7.61 - 7.53 (m, 1H), 7.52 (d, *J*=5.3 Hz, 1H), 7.32 (dd, *J*=7.4, 0.9 Hz, 1H), 2.29 (s, 3H). MS (ESI): *m/z* = 293.0 $[M+H]^+$.

Synthesis of Compound 18:



13 **18**

4-(4-Methylpyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-benzo[d]imidazole, 18. To a solution of 4-(4-methylpyridin-3-yl)-1H-benzo[d]imidazole (0.300 g, 1.43 mmol) in DMSO (10.0 mL) was added cesium carbonate (0.934 g, 2.87 mmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (0.215 mL,

1.51 mmol). After 17 h, the reaction was quenched with H_2O and diluted with EtOAc. The layers were separated and the aqueous phase was extracted with EtOAc (3X). The combined organic phases were washed with H_2O (2X) and brine (1X), then dried over Na_2SO_4, filtered, and concentrated to afford a yellow residue. The crude material was dissolved in a minimal amount of CH_2Cl_2 and purified by silica gel chromatography using an ISCO instrument (80 g column, 60 mL/min, 1–10% MeOH in CH_2Cl_2 over 30 min, t_r = 23 min) to give the title compound (340 mg, 1.17 mmol, 81 % yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 8.44 (1 H, d, *J*=5.3 Hz), 8.42 (1 H, s), 8.27 (1 H, s), 7.75 (1 H, d, *J*=8.3 Hz), 7.48 – 7.54 (1 H, m), 7.42 (1 H, d, *J*=5.0 Hz), 7.22 – 7.28 (1 H, m), 5.24 (2 H, q, *J*=8.9 Hz), 2.22 (3 H, s); ^{13}C NMR (126 MHz, CD_3OD) δ 150.5, 149.0, 146.0, 142.3, 136.5, 135.5, 130.5, 126.8, 125.3, 125.2, 125.3, 111.8, 46.6, 20.1; HRMS (ESI) *m/z* calcd for $C_{15}H_{13}F_3N_3$ $[M+H]^+$ 292.10561, found 292.10620. Anal. Calcd for $C_{15}H_{12}F_3N_3$: C, 61.85; H, 4.16; N, 14.42. Found C, 61.79; H, 4.05; N, 14.35.

Biological Assays

CYP17A1 Total SPA assay

The assay was performed in a U-bottom 384-well optiplate. The final assay volume was 15 μL prepared from 7.5 μL additions of microsomes (prepared as a high-speed pellet from homogenized HEK293 cells stably transfected with CYP17A1), substrates (3H-Pregnenolone and NADPH) and test compounds in assay buffer (50 mM Potassium phosphate pH 7.2, 10% glycerol). The reaction was initiated by the combination of the microsomes and substrates in wells containing compound. The reaction was incubated at room temperature for 45 minutes and terminated by adding 7.5 μL of 0.2 N HCl to each well. Following an incubation period of 10 minutes, anti-DHEA-coated SPA beads were added to the terminated reaction. The plate was sealed and incubated overnight with shaking at 4°C. The beads were allowed to settle in the plate for 1 hour and the plate read on a Topcount (Perkin-Elmer) plate reader.

Inhibition data were calculated by comparison to no enzyme control reactions for 100% inhibition and vehicle-only reactions for 0% inhibition. The final concentration of reagents in the assays are NADPH, 2 mM; 3H-Pregnenolone, 1 μM; microsomes, 1.25 μg/ml; Anti-DHEA-SPA beads (0.125 mg/well) in 0.5% Triton X-100 and DMSO, 0.05%. Dose response curves were generated to determine the concentration required inhibiting 50% of enzyme activity (IC_{50}). Compounds were dissolved at 10 mM in dimethylsulfoxide (DMSO) and evaluated at eleven concentrations, each in duplicate. IC_{50} values were derived by non-linear regression analysis. The % conversion of [3H]-pregnenolone to DHEA is 4.8% in the control experiment.

CYP17A1 Hydroxylase Assay

E. coli were transformed to express human CYP17A1 and membrane preparations were subsequently made and used as the source of enzyme in the assay. The reaction was carried out in a 50 μL final volume containing 200 nM hCYP17A1 membranes, 25 μM Pregnenolone (Sigma), 7 mM NADPH (CalBiochem), 1 μM cytochrome P450 reductase (Invitrogen), and 50 mM sodium phosphate buffer, pH 7.3. The IC_{50} determination of compounds dissolved in 100% DMSO was done by serial dilution into the assay buffer to a final concentration of 0.2% DMSO. The reaction was incubated at 37 °C for 120 minutes and stopped by the addition of 200 μL of 0.02 N HCl in acetonitrile. Samples were then

centrifuged at 2000 rpm and 200 μL of the supernatant was transferred to low binding plates (Costar). The product of the reaction, 17-alpha pregnenolone, was analyzed via LC/MS. The % conversion to 17-alpha pregnenolone in the control experiment was approximately 4% and no appreciable amount of DHEA was detected in this assay without addition of Cytochrome b5, the cofactor necessary to facilitate the lyase reaction potential of CYP17A1.

CYP17A1 Lyase Assay

Human CYP17A1 was expressed in HEK293 cells and microsomal preparations were made and subsequently used as the source of enzyme in the lyase assay. The reaction consists of 200 nM [3H]-hydroxypregnenolone (ARC), 200 nM 17-hydroxypregnenolone (Sigma), 2 mM NADPH (CalBiochem), and CYP17A1-HEK293 microsomes which were incubated in the presence of DMSO or test compounds for 20 minutes at room temperature. Compounds were dissolved in DMSO and serially diluted. The reaction was stopped by the addition of 0.2 N HCl and the product was captured using anti-mouse YSi SPA beads (GE) conjugated to an anti-DHEA monoclonal antibody (Abcam). Signal intensity determined by a Packard Top Count was used to calculate percent inhibition and IC_{50} values.

Adrenal SPA Assay

Human and cyno adrenal microsomes were prepared and isolated by standard methods using homogenization and high speed (100,000xg) centrifugation, aliquoted and stored at –80 ^{o}C. The human adrenal microsome assays were run in 30 μL reaction volumes consisting of 1uM [3H]-pregnenolone (PerkinElmer), 2 mM NADPH (CalBiochem), and 2 μg/well of adrenal microsomes and incubated in the presence of DMSO or test compounds for 90 minutes at room temperature. Cyno adrenal microsome assays were run similarly except the reactions were conducted for 45 minutes and contained 0.25ug/well of adrneal microsomes and 75 nM [3H]-pregnenolone (PerkinElmer). Compounds were dissolved in DMSO and serially diluted. The reactions were stopped with the addition of 0.2 N HCl and the product was captured using anti-mouse Ysi SPA beads (GE) conjugated to an anti-DHEA monoclonal antibody (Abcam). Signal intensity determined by a Packard Top Count was used to calculate percent inhibition and IC_{50} values.

CYP21A2 Assay

H295R cells were plated in 96 well Microtest plates (Falcon) and allowed to attach overnight at 37 ^{o}C with 5% CO_2 atmosphere. Culture medium was then removed and 50 mL of 10 mM Hydroxy Progesterone (Sigma) in Dulbecco's Modified Eagle's Medium and Ham's F12 Kaighn's Modification (Gibco), 1:1 ratio, + 2.5% Charcoaled Striped Fetal Bovine Serum (Hyclone) + ITS Premix (BD Bioscience) was added. Cells were incubated for 120 minutes at 37 ^{o}C with 5% CO_2 atmosphere. The reaction was terminated with the addition of 200 mL Acetonitrile (EMD) + 0.5 mM Pregnenolone-17α,21,21,21-d_4 per well. The plate was centrifuged at 2000 RPM for 15 minutes and 200 mL was transferred to low binding plates (Costar). Samples were analyzed for 11-deoxycortisol synthesis via LC-MS methods.

CYP11B1 Assay

H295R cells were plated in a total volume of 100 mL growth medium with 50 nM Angiotensin II and 250 mM Br cAMP (Sigma) per well in 96 well Microtest plates (Falcon). Cells were allowed to attach for 24 hours at 37 ^{o}C with 5% CO_2 atmosphere. Culture medium was removed and 50 mL of 40 mM Deoxycorticosterone (Sigma) in Dulbecco's Modified Eagle's Medium and Ham's F12 Kaighn's Modification (Gibco), 1:1 ratio, + 2.5% Charcoaled Striped Fetal Bovine Serum (Hyclone) + ITS Premix (BD Bioscience) was added. Cells were incubated for 48 hours at 37 ^{o}C with 5% CO_2 atmosphere and the reaction was terminated with the addition of 200 mL Acetonitrile (EMD) + 0.5 mM Pregnenolone-17α,21,21,21-d_4 per well. The plates were centrifuged at 2000 RPM for 15 minutes and 200 mL was transferred to low binding plates (Costar). Samples were analyzed for corticosterone synthesis via LC-MS methods.

Statistical Analysis of Compounds Tested in the hCYP17A1 Assay[a]

Cmpd	**1**	**9**	**10**	**11**	**16**	**17**	**18**
# values	3	2	2	2	2	2	12
Mean (nM)	3.667	4.95	3.5	5.1	8.25	7.55	19.4
SD	1.102	0.7778	0.9899	0.4243	2.475	2.475	10.81
SEM	0.636	0.55	0.7	0.3	1.75	1.75	3.119

[a] IC_{50} values of cmpds **6**–**8** and **14**–**15** were determined from one experiment

Xenobiotic CYP Activity of BMS-351

Enzyme	IC_{50} (μM)
1A2	9
2C19	>40
2C9	>40
2D6	>40
3A4	13
2C8	>40
2B6	>40

Pharmacokinetic Data and 1-Day Castrated Cyno PK/PD Study

1-Day Cyno PK/PD and Castrated Cyno Study Protocol:

Animals: All procedures involving animals and their care were conducted in conformity with the guidelines that are in compliance with the Bristol-Myers Squibb Institutional Animal Care and Use Committee. Fully mature male cynomolgus monkeys (>4 yrs of age; 5–6 kg) were from an in-house colony. All the monkeys used had chronically implanted femoral vein access ports. For oral studies, all animals were fasted overnight prior to dosing and were fed 4 hr after dosing. All animals had free access to water and were conscious throughout the study.

Castration: For the 1-day castrated cyno PK/PD study, the animals were castrated by pre-dosing the cynomolgus monkeys with 15 mg leuprolide acetate once every 28 days until the baseline for testosterone reached < 20 ng/dL. This took 4 months to achieve.

Drug: For all oral pharmacokinetic studies in cynomolgus monkeys, the tested compound was formulated in polyethylene glycol (PEG 400): water (80:20, v:v) at concentrations of 1–5 mg/mL.
Drug Treatment: The tested compound was administered by oral gavage to cynomolgus monkeys.
Sampling: Blood samples were collected from the femoral port, at 15, 30, and 45 min, and 1, 2, 4, 6, 8, 12, 24, 30, and 48 hr after oral administration. All blood samples were collected into syringes containing sodium heparin. The plasma fraction was immediately separated by centrifugation (14,000 rpm, 10 min, 4 °C), frozen on dry ice, and stored at –20°C until the samples were analyzed.
Analysis of Tested Compound: Plasma samples were thawed and treated with two volumes of acetonitrile containing internal standard. After centrifugation to remove precipitated proteins, an aliquot of supernatant was analyzed by LC/MS/MS.
Analysis of Steroids. Plasma samples were thawed, and assayed in accordance with package insert instructions for the following kits: Coat-A-Count total testosterone solid phase RIA kit, Coat-A-Count total progesterone solid phase RIA kit, and Coat-A-Count total cortisol solid phase RIA kit (Diagnostic Product Corp, Siemens Healthcare Diagnostics, Deerfield, IL).

Figure 5 and 6 show the results of a 1-day castrated cyno PK/PD study in NHP cynomolgus monkeys with BMS-351. BMS-351 was formulated in 80% PEG-400/water at a volume of 1 mL/kg of monkey and administered orally at 1.5 mg/kg. The formulation was then dosed orally at time = 0 hours and blood samples were taken over a 24 hour period to monitor for drug exposure and testosterone, progesterone, and cortisol levels.

Statistical Analysis for 1-Day Castrated Cyno PK/PD Data

Percent changes from baseline for T, P, and C were visualized by sample average ± standard deviation (nM) at each sampling time point on day 1, 1–24 hours, with n = 4 cyno monkeys. Hormone measurements below the LLQ were imputed as LLQ/2, and vehicle measurements at 24 h imputed as baseline. Consequently percentages changes from baseline were truncated below by –80%. The log fold changes (FC)'s in T, P, and C from baseline to 5 h post-treatment were compared, denoted as vehicle X and treatment Y, or between treatments, by testing the null hypothesis that the probability $Pr(X>Y)=\int Pr(X>y)Pr(Y=y)dy=0.50$ versus $Pr(X>Y)>0.50$ for the one-sided, or $Pr(X>Y) \neq 0.5$ for the two-sided test. Measurements below the lower limit of quantification were treated as left censored, and above the upper limit of quantification as right censored. The p-values were derived by Gaussian parametric model-based bootstrap, or with non-parametric methods in the event that censoring impeded parameter estimation.

Computational Chemisty

CYP17A1A Homology Modeling

Homology model building was carried out using the Prime Structure Prediction application[1] within Maestro version 8.5[2]. The human steroid 17-alpha-hydroxylase/17,20 lyase (CYP17A1A, Uniprot ID P05093) was aligned with the crystal structure of CYP2R1 (vitamin D 25-hydroxylase) in complex with

[1] Prime version 2.0, Schrodinger LLC, New York, NY, 2008.
[2] Maestro, version 8.5, Schrodinger, LLC, New York, NY, 2008.

vitamin D3 (RCSB ID, 3C6G)[3] using the Align module. The resulting alignment is included as Figure 7. The model was then constructed using the Build Structure module with the default options and including heme and vitamin D_3 from the crystal structure. A Glide[4] grid was built within the ligand binding site using vitamin D_3 to center the enclosing box with a metal constraint to the heme iron requiring a hydrogen bond acceptor atom within 2.5Å of the iron atom. Compound **1** was prepared for docking using the LigPrep[5] application starting from a 2D sd file with the molecule neutralized. The prepared ligand was docked using Glide[4] and the top scoring pose selected.

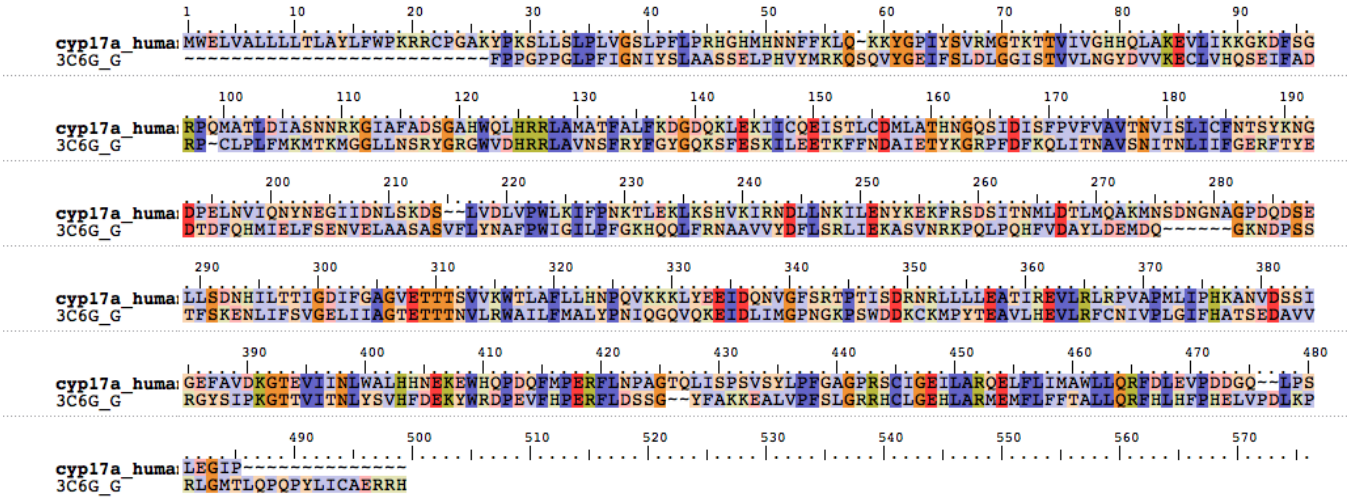


Figure 7. Alignment of CYP17A1A with CYP2R1 (RCSB ID 3RUK) used for homology model building.

BMS-351 Docking into the CYP17A1A crystal structure

The crystal structure of CYP17A1A in complex with abiraterone (RCSB ID 3RUK) was extracted from the RCSB database and prepared using the ProteinPrep workflow within Maestro 8.5 with the default options. A Glide grid was then prepared using abiraterone to center the enclosing box and including a metal constraint to the heme iron requiring a hydrogen bond acceptor within 2.5Å of the heme iron. BMS-351 was prepared using the LigPrep application starting from a 2D sd file with the molecule neutralized. The prepared ligand was docked using Glide and the top scoring pose selected.

[3] Strushkevich, N.; Usanov, S. A.; Plotinkov, A. N.; Jones, G.; Park, H-W. Structural Analysis of CYP2R1 in Complex with Vitamin D3. *J. Mol. Biol.* **2008**, *380*, 95–106.
[4] Glide version 5.0, Schrodinger LLC, New York, NY, 2008.
[5] LigPrep version 2.2, Schrodinger LLC, New York, NY 2008.