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Diversity synthesis yields novel multistage antimalarial inhibitors

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Supplementary Methods

Chemical Synthesis and Analytical Data

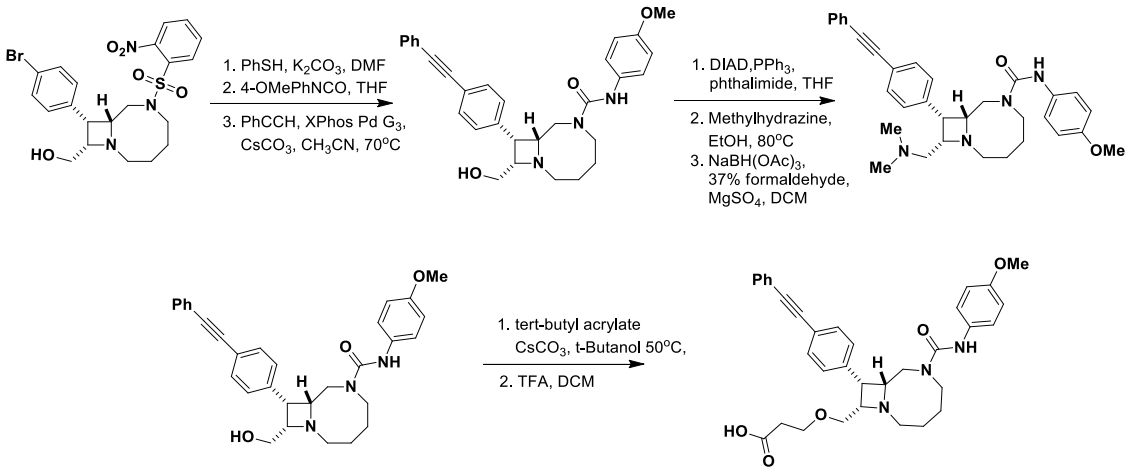
Abbreviations

DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethylsulphoxide
EtOAc	ethyl acetate
HPLC	high performance liquid chromatography
MeOH	methanol
PBS	phosphate buffered saline
THF	tetrahydrofuran
TFA	trifluoroacetic acid
TLC	thin layer chromatography
UPLC	ultra performance liquid chromatography
XPhos Pd G3	(2-Dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II) methanesulfonate

Chemical Synthesis of BRD3444, BRD1095, BRD3316 and BRD7929

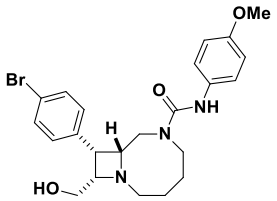
All compounds screened in this study are derived from a synthetic process performed at the Broad institute based on the principles of diversity-oriented synthesis. The electronic descriptions of these compounds using SMILES descriptors have been deposited in the public database PubChem (https://pubchem.ncbi.nlm.nih.gov/). These include the primary screening hits, which include BRD3444, BRD73842, BRD0026 and BRD7539. All reactions were carried out under N_2 or argon atmosphere. All reagents and solvents were purchased from commercial vendors and used as received, or synthesized according to the footnoted references. NMR spectra were recorded on a Bruker 300 (300 MHz ^{1}H, 75 MHz ^{13}C), Bruker 400 (400 MHz ^{1}H, 100 MHz ^{13}C), and Varian 400 (400 MHz ^{1}H, 100 MHz ^{13}C) spectrometer. Proton chemical shifts are reported in ppm (δ) referenced to the NMR solvent[74]. Data are reported as follows: chemical shifts, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet; coupling constant(s) in Hz; integration). Unless otherwise indicated NMR data were collected at 25°C. Flash chromatography was performed using 40-60 μm Silica Gel (60 Å mesh) on a Teledyne Isco Combiflash Rf. For purity analysis one of two general methods was used. For method one, purity was measured by LC-MS on a Waters 2795 separations module by UV absorbance at 210 nm and identity was determined on a SQ mass spectrometer by positive electrospray ionization. Mobile phase A consisted of 0.01% formic acid in water, while mobile phase B consisted of 0.01% formic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 2.5, 5 or 7.5 minutes at 1.75 mL/min. An Agilent Poroshell 120 EC-C18, 2.7 um, 3.0x30 mm column was used with column temperature maintained at 40°C. For method two, purity was measured by LC-MS on an Agilent 1200 separations module by UV absorbance at 220 nm and identity was determined on a 6120 mass spectrometer by positive electrospray ionization. Mobile phase A consisted of 0.037% trifluoroacetic acid in water, while mobile phase B consisted of 0.018% trifluoroacetic acid in acetonitrile. The gradient ran from 10% to 100% mobile phase B over 4.5 minutes at 0.8 mL/min. A Luna-C18(2), 5 um, 2.0x50 mm column was used with column temperature maintained at 40°C. Analytical TLC was performed on EM Reagent 0.25 mm silica gel 60-F plates. Visualization was accomplished with UV light and aqueous potassium permanganate ($KMnO_4$) stain followed by heating. Accurate mass measurements were obtained on an Agilent 6230 Time-of-Flight mass spectrometer as the $(M+H)^+$. Compound purity and identity were also determined by UPLC-MS. Purity was measured by UV absorbance at 210 nm. Identity was determined on a SQ mass spectrometer by positive and negative electrospray ionization. Mobile phase A consisted of either 0.1% ammonium hydroxide or 0.05% trifluoroacetic acid in water, while

mobile phase B consisted of either 0.1% ammonium hydroxide or 0.06% trifluoroacetic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 2.65 min at 0.9 mL/min. An Acquity BEH C18, 1.7 µm, 2.1x50 mm column was used with column temperature maintained at 65°C. Compounds were dissolved in DMSO at a nominal concentration of 1 mg/mL, and 1.0 uL of this solution was injected. Chiral separations were performed by SFC-MS. A Berger G600 supercritical fluid chromatograph was coupled with a Waters ZQ single quadrupole mass spectrometer operating in positive APCI mode. Using liquefied CO_2 modified with 20% isopropanol, an isocratic separation was performed for 5.0 minutes at 4.0 mL/min on a 4.6x100 mm Chiralpak AD-H column maintained at 40°C. Compounds were dissolved in methanol at a nominal concentration of 1 mg/mL, and 10 µL of this solution was injected.



Scheme 1. Synthetic route to BRD3444, BRD1095, BRD3316 and BRD7929

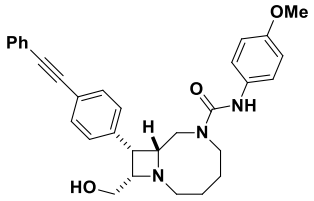
(8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide



To a solution of ((8R,9R,10S)-9-(4-bromophenyl)-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methanol (715 mg, 1.401 mmol) (prepared according to the method of Lowe *et al.*[37]) in DMF (7 mL) was added potassium carbonate (1.96 g, 14.01 mmol) and thiophenol (0.721 mL, 7.00 mmol) at room temperature. The reaction was stirred for 16 hours. The reaction was diluted with water and extracted with EtOAc. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was dissolved in DCM and passed over a plug of acidic resin (10 g of 0.76 mmol/g, Si-Tosic acid; Silicycle) and rinsed first with DCM and then with 1M NH_3 in MeOH to afford ((8R,9R,10S)-9-(4-bromophenyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methanol that was carried onto the subsequent step without further purification. This crude material was dissolved in THF (14 mL). To this solution was added 4-methoxyphenylisocyanate (0.173 mL, 1.332 mmol) in 6 portions over a 2 hours period and the resulting solution was stirred for 16 hours at room temperature. The reaction was concentrated and purified by flash column chromatography and eluting with DCM/MeOH to give the desired compound as a white solid (513 mg, Yield: 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.40 (m, 2H), 7.39–7.32 (m, 2H), 7.30–7.20 (m, 2H), 6.90–6.75 (m, 2H), 6.11 (s, 1H), 3.90–3.78 (m, 1H), 3.77 (s, 3H), 3.69–3.54 (m, 5H), 3.54–3.39 (m, 1H), 3.37–3.20 (m, 1H),

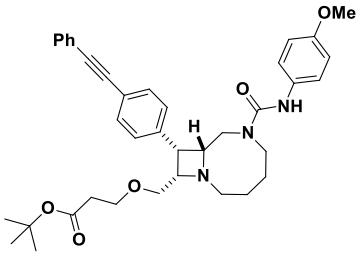
3.11–2.93 (m, 1H), 2.91–2.75 (m, 1H), 2.54–2.35 (m, 1H), 1.91–1.71 (m, 2H), 1.72–1.54 (m, 2H). Purity by LCMS (UV Chromatogram, 210nm, 5 min run) 97% by UV, rt = 1.33 min, m/z 474 $(M+H)^+$.

(8R,9R,10S)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD3444)



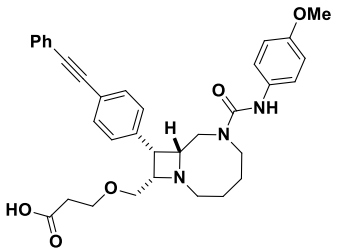
To a solution of (8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (326 mg, 0.687 mmol) in degassed acetonitrile (4.6 mL) was added ethynylbenzene (226 µL, 2.062 mmol) and XPhos Pd G3 (58.1 mg, 0.069 mmol). Cesium carbonate (896 mg, 2.75 mmol) was added to the reaction and then heated at 70°C for 1.5 hours. The reaction was cooled to room temperature, diluted with EtOAc and washed with water and brine. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was purified by flash column chromatography eluting with DCM/MeOH to give the desired compound (238 mg, Yield: 78%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.55–7.33 (m, 6H), 7.34–7.22 (m, 3H), 7.22–7.15 (m, 2H), 6.81–6.69 (m, 2H), 6.00 (s, 1H), 3.83–3.73 (m, 1H), 3.70 (s, 3H), 3.65–3.50 (m, 4H), 3.46-3.37 (m, 2H), 3.31 – 3.19 (m, 1H), 3.01–2.91 (m, 1H), 2.88–2.76 (m, 1H), 2.44–2.33 (m, 1H), 1.81–1.68 (m, 3H), 1.64–1.54 (m, 1H). Purity by LCMS (UV Chromatogram, 210nm, 2.5 min run) 99% by UV, rt = 0.87 min, m/z 496 $(M+H)^+$. HRMS (ESI) calcd for $C_{31}H_{33}N_3O_3$ $[M+H]^+$: 496.2600. Found: 496.2604.

tert-butyl 3-(((8R,9R,10S)-6-((4-methoxyphenyl)carbamoyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methoxy)propanoate



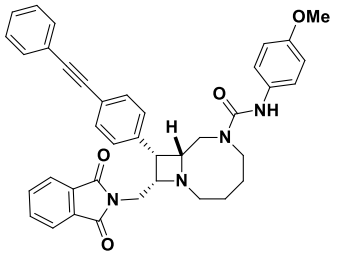
To a solution of (8R,9R,10S)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (50 mg, 0.101 mmol) in tert-butanol (504 µL) were added tert-butyl acrylate (588 µl, 4.04 mmol) and cesium carbonate (65.7 mg, 0.202 mmol). The solution was stirred at 50°C for 18 hours upon which further cesium carbonate (16 mg, 0.049 mmol) was added. After 23 hours the solution was cooled and extracted with EtOAc and saturated ammonium chloride. The organic layer was dried with $NaSO_4$, filtered and concentrated. The crude material was purified by flash column chromatography eluting with EtOAc/Hexanes to give the desired compound (61 mg, Yield: 97%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.59–7.52 (m, 2H), 7.52–7.44 (m, 3H), 7.41–7.33 (m, 3H), 7.32–7.22 (m, 3H), 6.85 (d, J = 8.4 Hz, 2H), 6.10 (s, 1H), 3.93–3.82 (m, 1H), 3.79 (s, 3H), 3.71–3.38 (m, 7H), 3.37–3.24 (m, 2H), 3.16–3.03 (m, 1H), 2.97–2.85 (m, 1H), 2.44–2.28 (m, 2H), 1.94–1.75 (m, 3H), 1.71–1.59 (m, 2H), 1.44 (s, 9H). Purity by LCMS (UV Chromatogram, 210nm, 2.5 min run) 97% by UV, rt = 1.44 min, m/z 624 $(M+H)^+$.

3-(((8R,9R,10S)-6-((4-methoxyphenyl)carbamoyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methoxy)propanoic acid (BRD3316)



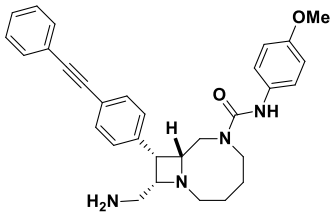
To a solution of tert-butyl 3-(((8R,9R,10S)-6-((4-methoxyphenyl)carbamoyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methoxy)propanoate (31mg, 0.050 mmol) in DCM (1988 µL) was added trifluoroacetic acid (153 µl, 1.988 mmol). The reaction was stirred at room temperature for 19 hours. The reaction was concentrated and the crude material was purified by flash column chromatography eluting with DCM/MeOH to give the desired compound (28 mg, Yield: 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72–7.47 (m, 4H), 7.43–7.33 (m, 2H), 7.31–7.20 (m, 5H), 6.85 (d, J = 8.6 Hz, 2H), 6.39 (s, 1H), 4.56–4.35 (m, 1H), 4.36–4.21 (m, 1H), 4.23–4.07 (m, 1H), 4.03–3.82 (m, 3H), 3.79 (s, 3H), 3.71–3.44 (m, 5H), 3.41–3.27 (m, 1H), 3.02–2.80 (m, 1H), 2.78–2.59 (m, 1H), 2.59–2.40 (m, 1H), 2.24–2.10 (m, 1H), 2.10–1.97 (m, 1H), 1.97–1.85 (m, 1H), 1.85–1.68 (m, 1H). Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 99% by UV, rt = 2.65 min, m/z 568 $(M+H)^+$. HRMS (ESI) calcd for $C_{34}H_{37}N_3O_5$ $[M+H]^+$: 568.2811. Found: 568.2818.

(8R,9S,10S)-10-((1,3-dioxoisoindolin-2-yl)methyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide



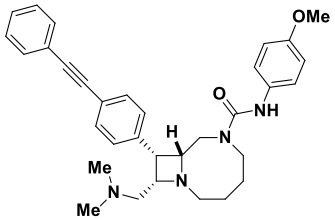
To a solution of triphenylphosphine (772 mg, 2.94 mmol) in THF (14.7 mL) at 0°C was slowly added (E)-diisopropyl diazene-1,2-dicarboxylate (572 µl, 2.94 mmol) under argon. After 10 minutes the mixture became milky yellow. Then 3.98 mL of this prepared mixture (0.796 mmol) was added to a flask containing (8R,9R,10S)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (112 mg, 0.226 mmol) and phthalimide (49.9 mg, 0.339 mmol) in THF (500 mL) at 0°C. The mixture was stirred at room temperature for 1.5 hours. The reaction was diluted with EtOAc and washed with water and brine. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was purified by flash column chromatography eluting with EtOAc/Hexanes to give the desired compound (103 mg, Yield: 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81–7.66 (m, 2H), 7.66–7.58 (m, 2H), 7.55–7.42 (m, 4H), 7.42–7.33 (m, 1H), 7.33–7.21 (m, 3H), 7.23–7.12 (m, 3H), 6.79–6.68 (m, 2H), 6.00 (s, 1H), 3.91–3.72 (m, 2H), 3.69 (s, 3H), 3.65–3.55 (m, 2H), 3.54–3.41 (m, 3H), 3.33–3.19 (m, 1H), 2.91–2.77 (m, 2H), 2.29–2.19 (m, 1H), 1.77–1.61 (m, 3H), 1.60–1.47 (m, 1H). Purity by LCMS (UV Chromatogram, 210nm, 2.5 min run) 91% by UV, rt = 1.63 min, m/z 625 $(M+H)^+$.

(8R,9S,10S)-10-(aminomethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD1095)



(8R,9S,10S)-10-((1,3-dioxoisoindolin-2-yl)methyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (103 mg, 0.165 mmol) was dissolved in ethanol (1649 µL) and to this was added methylhydrazine (87 µl, 1.649 mmol) and the reaction mixture was stirred at 80°C for 3 hours. The reaction mixture was concentrated. The crude material was purified by flash column chromatography eluting with DCM/MeOH to give the desired compound (44mg, Yield: 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51–7.34 (m, 6H), 7.32–7.22 (m, 3H), 7.21–7.12 (m, 2H), 6.78–6.72 (m, 2H), 6.05 (s, 1H), 3.83–3.72 (m, 1H), 3.69 (s, 3H), 3.63–3.52 (m, 2H), 3.52–3.42 (m, 1H), 3.41–3.33 (m, 1H), 3.28–3.14 (m, 2H), 2.99–2.87 (m, 1H), 2.88–2.76 (m, 1H), 2.74–2.65 (m, 1H), 2.36–2.25 (m, 1H), 1.81 –1.63 (m, 3H), 1.64–1.46 (m, 1H). ^{13}C NMR NMR (101 MHz, $CDCl_3$) δ 155.78, 154.85, 136.56, 132.10, 131.60, 131.23, 130.83, 128.35, 128.28, 123.28, 122.15, 121.80, 114.09, 89.66, 89.17, 69.84, 65.63, 58.61, 55.54, 50.50, 48.69, 42.59, 40.74, 27.84, 27.07. Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 99% by UV, rt = 2.61 min, m/z 495 $(M+H)^+$. HRMS (ESI) calcd for $C_{31}H_{34}N_4O_2$ $[M+H]^+$: 495.276. Found: 495.2764.

(8R,9S,10S)-10-((dimethylamino)methyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD7929)



(8R,9S,10S)-10-(aminomethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (39 mg, 0.079 mmol) was dissolved in DCM (1,314 µL) and to this was added magnesium sulfate (95 mg, 0.788 mmol) followed by a solution of 37 wt.% formaldehyde in water (35.2 µL, 0.473 mmol). To this suspension was added anhydrous sodium triacetoxyhydroborate (234 mg, 1.104 mmol). The reaction was stirred for 2 hours upon which saturated sodium bicarbonate solution was added and the mixture was stirred for 15 minutes. The organic components were extracted with DCM and washed with water and brine. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was purified by flash column chromatography eluting with DCM/Methanol to give the desired compound (28 mg, Yield: 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60–7.45 (m, 6H), 7.41–7.34 (m, 3H), 7.31–7.24 (m, 2H), 6.88–6.82 (m, 2H), 6.08 (s, 1H), 3.94–3.82 (m, 1H), 3.80 (s, 3H), 3.71–3.50 (m, 4H), 3.34–3.20 (m, 1H), 3.14–3.04 (m, 1H), 2.94–2.84 (m, 1H), 2.58–2.43 (m, 2H), 2.43–2.32 (m, 1H), 2.08 (s, 6H), 1.93–1.58 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.71, 154.84, 137.22, 132.19, 131.57, 131.10, 131.05, 128.34, 128.22, 123.29, 122.17, 121.49, 114.03 89.51, 89.32, 66.97, 66.71, 57.89, 57.75, 55.50, 50.63, 48.78,

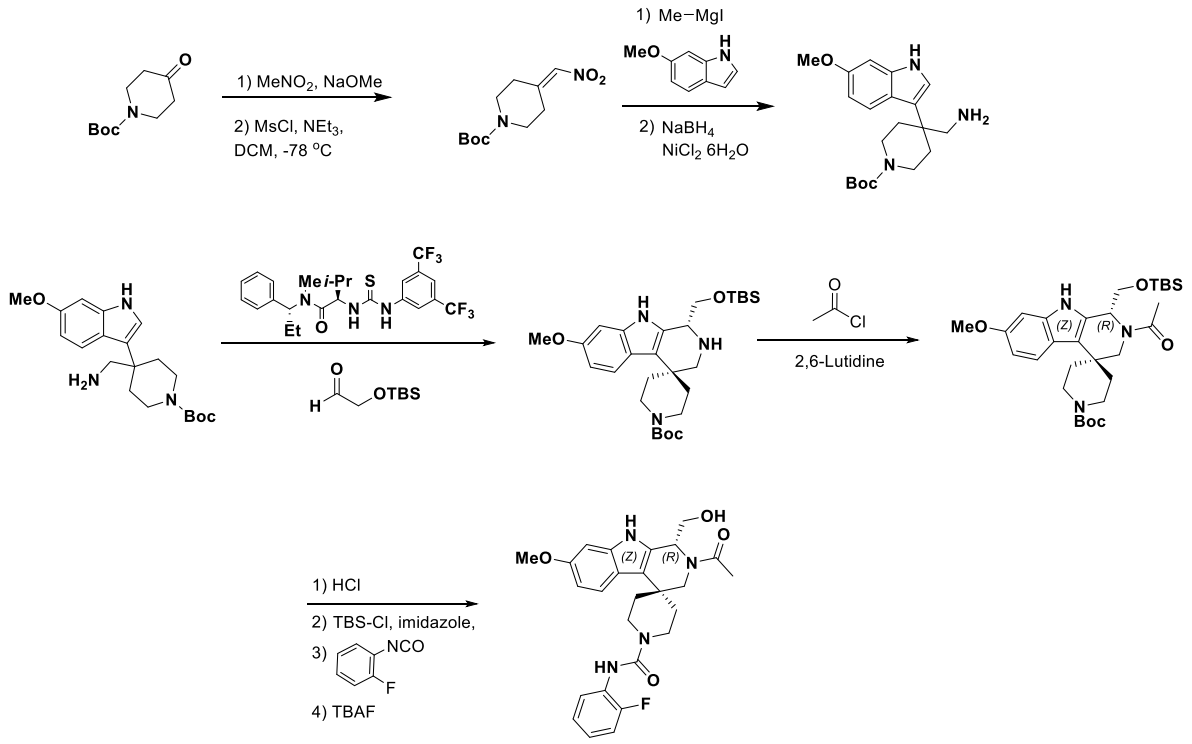
45.85, 44.82, 27.94, 27.46. Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 99% by UV, rt = 2.58 min, m/z 523 $(M+H)^+$. HRMS (ESI) calcd for $C_{33}H_{38}N_4O_2$ $[M + H]^+$: 523.3073. Found: 523.3074.

Chemical Synthesis of BRD0026

The synthesis of BRD0026 was performed by adapting the synthetic methods reported by Lowe *et al.*[37] Purity by LCMS (UV Chromatogram, 210nm, 1.2 min run) 78% by UV, rt = 0.76 min, m/z 391 $(M+H)^+$.

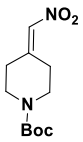
Chemical Synthesis of BRD73842

The synthesis of BRD73842 was performed by adapting the synthetic methods reported by Klausen *et al.*[75]



Scheme 2. Synthetic route to BRD73842

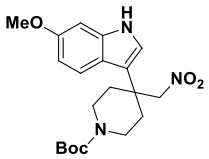
tert-butyl 4-(nitromethylene)piperidine-1-carboxylate



To a solution of 1-Boc-4-piperidinone (15 g, 75 mmol) in MeOH (151 mL) was added nitromethane (41 mL, 753 mmol) and sodium methanolate (2.034 g, 37.6 mmol) at room temperature. The reaction was stirred for 16 hours, then saturated $NaHCO_3$ was added and the mixture was extracted with ethyl acetate. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated to afford crude tert-butyl 4-hydroxy-4-(nitromethyl)piperidine-1-carboxylate that was used directly in the subsequent step. To a solution of this crude material in DCM at -78°C was added triethylamine (46

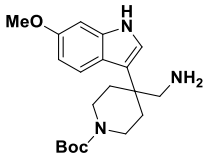
mL, 331 mmol) and methanesulfonyl chloride (18.73 mL, 241 mmol). The reaction mixture was allowed to stir at -78°C for 30 minutes. The temperature was then increased to -20°C for 4 hours upon which TLC analysis indicated complete reaction. The mixture was carefully poured into a cold ammonium chloride solution and extracted with DCM. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was purified by flash column chromatography eluting with EtOAc/Hexanes to give the desired compound (10 g, Yield: 55%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (s, 1H), 3.58 (m, 4H), 3.01 (m, 2H), 2.39–2.29 (m, 2H), 1.50 (s, 9H). Purity by LCMS (UV Chromatogram, 210nm, 2.5 min run) 99% by UV, rt = 1.29 min, m/z 241 $(M-H)^-$.

tert-butyl 4-(6-methoxy-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate



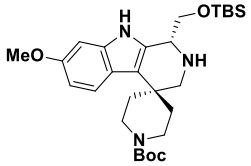
To a solution of 6-methoxy-1H-indole (3.39 g, 23.03 mmol) in THF (57.6 ml) was added methylmagnesium iodide (3.0 M in Et_2O, 8.83 mL, 26.5 mmol) dropwise. The mixture was stirred at room temperature for 20 minutes and then cooled to 0°C. tert-butyl 4-(nitromethylene)piperidine-1-carboxylate (5.58 g, 23.03 mmol) in THF (173 mL) was then added at 0°C dropwise. The reaction mixture was warmed slowly to room temperature and stirred for 16 hours. Saturated ammonium chloride solution was then added was and the reaction was stirred at room temperature for 30 minutes. The organic components were extracted with EtOAc and washed with water and brine. The crude material was purified by flash column chromatography eluting with EtOAc/Hexanes to give the desired product (6.91 g, Yield: 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.54 (d, J = 8.8 Hz, 1H), 6.85 (m, 3H), 4.71 (s, 2H), 3.85 (m, 5H), 3.13 (m, 2H), 2.45 (m, 2H), 1.95 (ddd, J = 13.7, 11.0, 4.2 Hz, 2H), 1.47 (s, 9H). Purity by LCMS (UV Chromatogram, 210nm, 2.5 min run) 96% by UV, rt = 1.46 min, m/z 390 $(M+H)^+$.

tert-butyl 4-(aminomethyl)-4-(6-methoxy-1H-indol-3-yl)piperidine-1-carboxylate



To a solution of tert-butyl 4-(6-methoxy-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate (7.7 g, 19.77 mmol) in THF (316 mL) at room temperature was added $NiCl_2.6H_2O$ (4.70 g, 19.77 mmol) and $NaBH_4$ (2.99 g, 79 mmol). The reaction was cooled to 0°C and then MeOH (79 mL) was added very slowly (caution, vigorous reaction). The reaction was warmed to room temperature and after 3 hours, diethylenetriamine (29.9 mL, 277 mmol) was added. After a further 1 hour the reaction mixture was concentrated. A saturated sodium bicarbonate solution was added and the organic components were extracted with EtOAc. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was purified by flash column chromatography eluting with EtOAc/Hexanes to give the desired compound (6.37 g, Yield: 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.59 (d, J = 8.8 Hz, 1H), 6.90 (dd, J = 6.2, 2.3 Hz, 2H), 6.77 (dd, J = 8.8, 2.4 Hz, 1H), 3.86 (s, 3H), 3.84 – 3.65 (m, 2H), 3.20 – 3.11 (m, 2H), 2.97 (s, 2H), 2.30 – 2.20 (m, 2H), 1.77 – 1.68 (m, 2H), 1.46 (s, 9H). Purity by LCMS (UV Chromatogram, 210nm, 2.5 min run) 97% by UV, rt = 1.28 min, m/z 360 $(M+H)^+$.

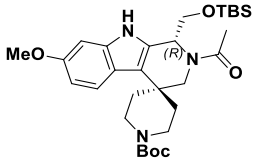
(R)-tert-butyl 1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate.



To a solution of tert-butyl 4-(aminomethyl)-4-(6-methoxy-1H-indol-3-yl)piperidine-1-carboxylate (2.61 g, 7.26 mmol) in toluene (132 mL) was added (R)-2-(3-(3,5-bis(trifluoromethyl)phenyl)thioureido)-N,3-dimethyl-N-((R)-1-phenylpropyl)butanamide (0.566 g, 1.089 mmol) at -20°C. The mixture was stirred for 10 minutes before 2-(tert-butyldimethylsilyloxy)acetaldehyde (1.519 g, 8.71 mmol) (freshly distilled) in toluene (1801 µL) was added. The above mixture was allowed to stir at -20°C while it was monitored by LCMS. After 89 hours further (R)-2-(3-(3,5-bis(trifluoromethyl)phenyl)thioureido)-N,3-dimethyl-N-((R)-1-phenylpropyl)butanamide 0.283 g, 0.545 mmol was added. After 135 hours saturated $NaHCO_3$ was added to the reaction mixture. The reaction was warmed to room temperature and extracted with EtOAc. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was purified by flash column chromatography eluting with EtOAc/Hexanes to give the desired compound (2.9 g, Yield: 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.13 (s, 1H), 6.67 (d, J = 2.3 Hz, 1H), 6.60 (dd, J = 8.7, 2.4 Hz, 1H), 4.05 – 3.84 (m, 3H), 3.76 (dd, J = 9.3, 5.0 Hz, 1H), 3.70 (s, 3H), 3.54 (m, 1H), 3.34 (d, J = 12.7 Hz, 1H), 2.93 – 2.61 (m, 3H), 2.40 (m, 1H), 2.07 (m, 1H), 1.67 – 1.48 (m, 2H), 1.38 (s, 9H), 0.83 (s, 9H), -0.00 (s, 3H) -0.01 (s, 3H). (mixture of rotamers by ^{1}HNMR, the major rotamer is quoted). Purity by LCMS (UV Chromatogram, 210nm, 2.5 min run) 98% by UV, rt = 1.39 min, m/z 516 $(M+H)^+$.

An ee of 91% was determined by chiral SFC analysis upon converting this material to the alloc-protected material (R)-2'-allyl 1-tert-butyl 1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-3',9'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1,2'(1'H)-dicarboxylate using the chiral SFC method detailed above.

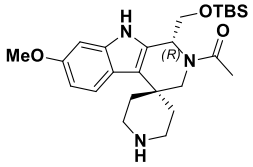
(R)-tert-butyl 2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate



To a solution of (R)-tert-butyl 1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (1 g, 1.939 mmol) in DCM (19.39 mL) was added N-ethyl-N-isopropylpropan-2-amine (1.693 mL, 9.69 mmol), and the reaction mixture was cooled to 0°C. Then acetyl chloride (0.207 mL, 2.91 mmol) was added dropwise and the reaction was allowed to warm to room temperature and stirred for 1.5 hours. At this point saturated sodium bicarbonate was added and the mixture was extracted with DCM. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was purified by flash column chromatography eluting with EtOAc/Hexanes to give the desired compound. (1.07 g, Yield: 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.49–7.38 (m, 1H), 6.75–6.69 (m, 1H), 6.69–6.60 (m, 1H), 5.45 (dd, J = 8.8, 4.6 Hz, 1H), 5.27 – 5.16 (m, 1H), 4.92 – 4.81 (m, 1H), 4.23 – 4.01 (m, 1H), 3.98 – 3.80 (m, 2H), 3.73 (s, 3H), 3.68 – 3.61 (m, 1H), 3.23 – 3.05 (m, 1H), 2.98 – 2.77 (m, 1H), 2.73 – 2.54 (m,

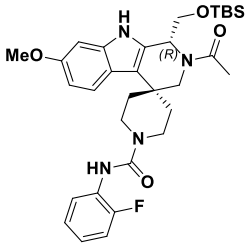
1H), 2.52 – 2.45 (m, 1H), 2.15 (s, 3H), 2.0 – 1.9 (m, 1H), 1.85 – 1.75 (m, 1H), 1.43 (s, 9H), 0.84 (s, 9H), 0.00 (s, 3H), -0.01 (s, 3H). (mixture of rotamers by ^{1}HNMR, the major rotamer is quoted). Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 99% by UV, rt = 1.83 min, m/z 558 $(M+H)^+$.

(R)-1-(1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxyspiro[piperidine-4,4'-pyrido[3,4-b]indol]-2'(1'H,3'H,9'H)-yl)ethanone



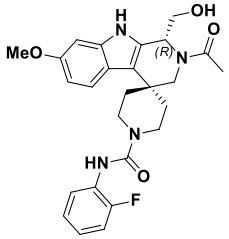
To a solution of (R)-tert-butyl 2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (1.082 g, 1.940 mmol) in DCM (24 mL) was added HCl (2.91 mL, 11.64 mmol, 4M dioxane) and the mixture was stirred at room temperature for 16 hours. The reaction material was concentrated and used directly in the next step. The crude material was dissolved in DMF (12.9 mL) and to this was added tert-butylchlorodimethylsilane (438 mg, 2.91 mmol) and imidazole (264 mg, 3.88 mmol). The reaction was stirred at 0°C for 2 hours. Then saturated ammonium chloride was added and the mixture extracted with DCM. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was purified by flash column chromatography eluting with DCM/MeOH to give the desired compound. (1.08 g, Yield: 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.68 (d, minor rotamer), 7.62 (s, minor rotamer), 7.18 (s, 1H), 7.02 (s, 1H), 6.76 – 6.64 (m, 1H), 5.46 (dd, J = 8.7, 4.9 Hz, 1H), 5.25 – 5.09 (m, 1H), 4.96 – 4.81 (m, 1H), 4.30 – 4.17 (m, 1H), 3.93 – 3.77 (m, 2H), 3.72 (s, 3H), 3.37 – 3.24 (m, 1H), 3.24 – 2.98 (m, 2H), 2.94 – 2.81 (m, 1H), 2.58 – 2.47 (m, 1H), 2.15 (s, 3H), 1.67 – 1.37 (m, 2H), 0.84 (s, 9H), 0.00 (s, 3H), -0.01 (s, 3H). (mixture of rotamers by ^{1}H NMR, the major rotamer is quoted). Purity by LCMS (UV Chromatogram, 210nm, 2.5 min run) 99% by UV, rt = 1.21 min, m/z 458 $(M+H)^+$.

(R)-2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-N-(2-fluorophenyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide



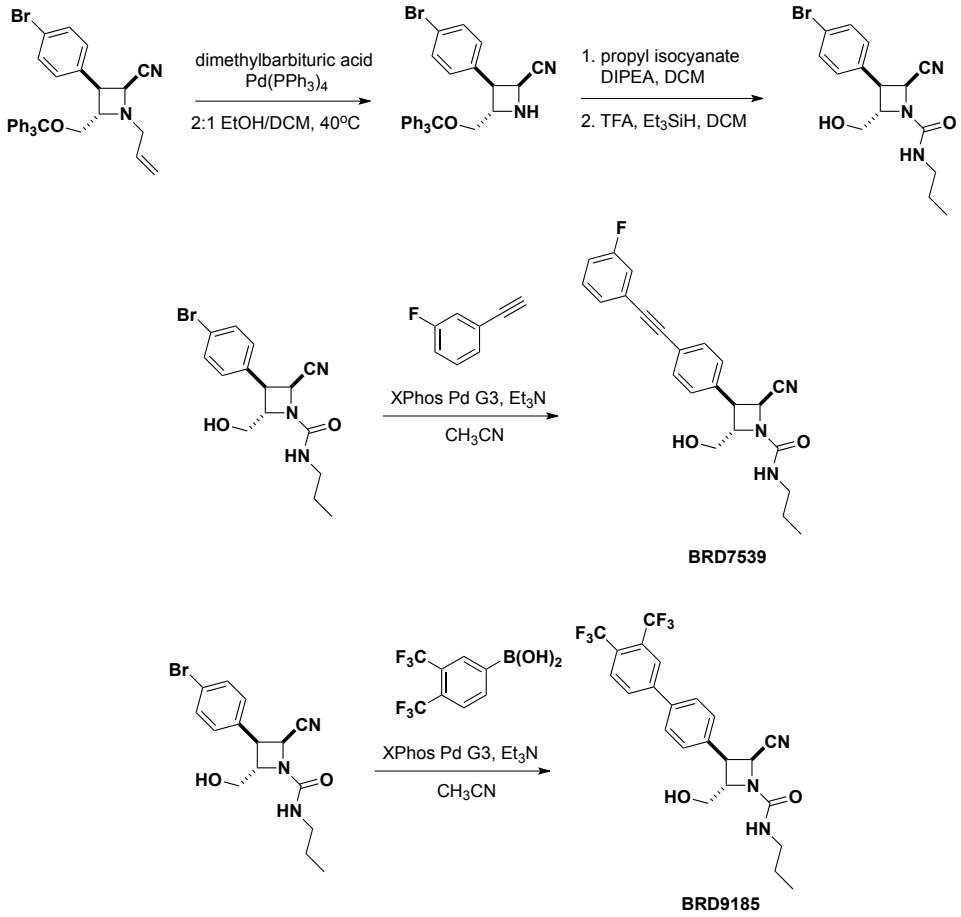
A solution of (R)-1-(1'-((tert-butyldimethylsilyloxy)methyl)-7'-methoxyspiro[piperidine-4,4'-pyrido[3,4-b]indole]-2'(1'H,3'H,9'H)-yl)ethanone (450 mg, 0.983 mmol) in DCM (18 mL) was treated with 1-fluoro-2-isocyanatobenzene (0.121 mL, 1.082 mmol) at 0°C and the reaction was stirred for 3 hours. The reaction was then concentrated under reduced pressure and purified by column chromatography eluting with EtOAc/Hexanes to give the desired compound (509 mg, Yield: 87%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.49 – 7.32 (m, 2H), 7.21 – 7.02 (m, 3H), 6.78 (s, 1H), 6.58 (m, 1H), 5.17 – 4.98 (m, 1H), 4.11 – 3.83 (m, 4H), 3.70 (s, 3H), 3.40 – 2.98 (m, 2H), 2.74 – 2.40 (m, 2H), 2.13 (s, 3H), 1.52 – 0.93 (m, 4H), 0.84 (s, 9H), 0.05 (s 3H), 0.00 (s, 3H). (mixture of rotamers by ^{1}HNMR, the major rotamer is quoted). Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 98% by UV, rt = 4.27 min, m/z 595 $(M+H)^+$.

(R)-2'-acetyl-N-(2-fluorophenyl)-1'-(hydroxymethyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (BRD73842)



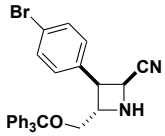
To a solution of (R)-2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-N-(2-fluorophenyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (496 mg, 0.834 mmol) in THF (8,339 µL) was added tetrabutylammonium fluoride (1M in THF, 2085 µl, 2.085 mmol). After 1 hour saturated ammonium chloride was added and the mixture was extracted with EtOAc. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude material was purified by flash column chromatography eluting with DCM/MeOH to give the desired compound (407 mg, Yield: 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.39 (s, 1H), 7.52 – 7.33 (m, 2H), 7.30 – 7.10 (m, 3H), 6.91-6.77 (m, 1H), 6.69-6.55 (m, 1H), 5.50 – 5.30 (m, 1H), 5.19 – 5.07 (m, 1H), 5.03 – 4.91 (m, 1H), 4.16 – 3.90 (m, 2H), 3.74 (s, 4H), 3.39 – 3.28 (m, 1H), 3.29 – 2.97 (m, 3H), 2.77 – 2.54 (m, 1H), 2.18 (s, 3H), 1.56 – 1.20 (m, 2H). (mixture of rotamers by ^{1}H NMR, the major rotamer is quoted). ^{13}C NMR (101 MHz, DMSO) δ 169.92, 169.29, 157.18, 155.91, 155.86, 155.55, 155.42, 154.75, 137.75, 137.55, 131.44, 130.33, 130.18, 128.42, 128.31, 126.68, 126.56, 125.30, 124.48, 124.45, 119.99, 119.76, 119.11, 115.05, 108.72, 108.49, 95.58, 95.45, 62.52, 56.27, 55.68, 50.81, 48.47, 41.82, 41.59, 40.96, 35.49, 35.30, 34.63, 34.14, 32.80, 32.17, 32.15, 22.23. (Mixture of rotamers by ^{13}C NMR). Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 99% by UV, rt = 2.46 min, m/z 481 $(M+H)^+$. HRMS (ESI) calcd for $C_{26}H_{29}FN_4O_4$ $[M+H]^+$: 481.2251. Found: 481.2255.

Chemical Synthesis of BRD7539



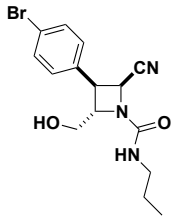
Scheme 3. Synthetic route to BRD7539 and BRD9185.

(2S,3S,4S)-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile.



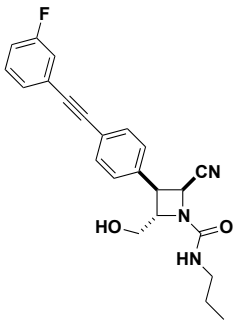
(2S,3S,4S)-1-allyl-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (1.04 g, 1.845 mmol) (prepared according to the method of Lowe *et al*[37] was dissolved in 2:1 EtOH (12.3 mL) and DCM (6.15 mL). Dimethylbarbituric acid (432 mg, 2.77 mmol) and $Pd(PPh_3)_4$ (213 mg, 0.185 mmol) were then added, and the mixture was heated to 40°C. After 2 hours, the solvent was removed *in vacuo*, and the resulting residue was purified by flash column chromatography (hexanes/EtOAc) to afford the product as a light yellow foaming solid. (900 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.51 (m, 2H), 7.45 – 7.40 (m, 6H), 7.35 – 7.26 (m, 12H), 4.73 (d, *J* = 8.5 Hz, 1H), 4.33 (dt, *J* = 7.1, 4.9 Hz, 1H), 3.89 (dd, *J* = 8.4, 7.2 Hz, 1H), 3.40 (dd, *J* = 10.2, 5.1 Hz, 1H), 3.36 – 3.29 (m, 2H), 2.30 (s, 1H). Purity by LCMS (UV Chromatogram, 210nm, 7.5 min run) 99% by UV, rt = 5.06 min, m/z 557 $(M+HCOOH)^+$.

(2S,3S,4S)-3-(4-bromophenyl)-2-cyano-N-propyl-4-((trityloxy)methyl)azetidine-1-carboxamide.



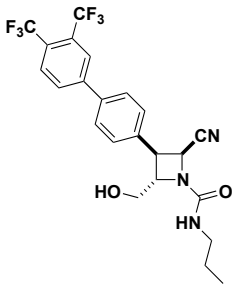
(2S,3S,4S)-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (415 mg, 0.815 mmol) was dissolved in DCM (8.2 mL), followed by the dropwise addition of *N,N*-diisopropylethylamine (0.6 mL, 4.08 mmol) and propyl isocyanate (115 mL, 1.222 mmol). After 30 minutes, the reaction was quenched by the addition of methanol (1 mL) and stirred for 15 minutes, after which point the solvent was removed *in vacuo*. The resulting residue was redissolved in DCM (8.2 mL), and trifluoroacetic acid (624 µL, 8.15 mmol) and triethylsilane (195 µL, 1.222 mmol) were added dropwise. The mixture was allowed to stir for 30 minutes before the solvent was once again removed *in vacuo* and resulting residue purified via flash column chromatography (MeOH/DCM) to afford the product as white foaming solid (185 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.8 Hz, 2H), 7.20 (d, *J* = 7.7 Hz, 2H), 6.54 (s, 1H), 4.97 (d, *J* = 8.4 Hz, 1H), 4.89 – 4.70 (m, 1H), 4.70 – 4.61 (m, 1H), 3.96 – 3.62 (m, 3H), 3.13 (m, 2H), 1.57 – 1.40 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). Purity by LCMS (UV Chromatogram, 210nm, 7.5 min run) 99% by UV, rt = 5.06 min, m/z 353 $(M+H)^+$.

(2S,3S,4S)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (BRD7539).



(2S,3S,4S)-3-(4-bromophenyl)-2-cyano-N-propyl-4-((trityloxy)methyl)azetidine-1-carboxamide (105 mg, 0.298 mmol) was dissolved in degassed CH_3CN (3 mL), followed by the addition of 1-ethynyl-3-fluorobenzene (172 µL, 1.491 mmol) and XPhos Pd G3 (38 mg, 0.045 mmol). Finally, Et_3N (1.04 mL, 7.45 mmol) was added, and the reaction was heated at 70°C for 2 hours. The reaction was cooled to room temperature, diluted with pH 7 buffer, and extracted 3x with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and removed *in vacuo*. The resulting mixture was purified by flash column chromatography (MeOH/DCM) to afford the product as a pale yellow powder/solid (106 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 7.7 Hz, 2H), 7.38 – 7.28 (m, 4H), 7.21 (dd, *J* = 9.5, 3.0 Hz, 1H), 7.04 (dq, *J* = 7.8, 3.7 Hz, 1H), 6.27 (s, 1H), 5.01 (d, *J* = 8.4 Hz, 1H), 4.74 (t, *J* = 7.8 Hz, 1H), 4.02 (s, 1H), 3.92 (d, *J* = 10.9 Hz, 1H), 3.88 – 3.80 (m, 1H), 3.74 (t, *J* = 8.1 Hz, 1H), 3.18 (m, 2H), 1.51 (d, *J* = 7.2 Hz, 2H), 0.92 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.73, 161.28, 158.06, 134.35, 132.25, 130.10 (d, *J* = 8.6 Hz), 129.48, 128.51, 127.66 (d, *J* = 3.0 Hz), 124.92 (d, *J* = 9.5 Hz), 123.45, 118.62, 118.39, 116.05, 115.79 (d, *J* = 10.1 Hz), 89.59, 89.29 (d, *J* = 3.4 Hz), 68.12, 65.81, 52.73, 42.29, 39.54, 29.82, 23.27, 11.46. Purity by LCMS (UV Chromatogram, 210nm, 7.5 min run) 99% by UV, rt = 3.49 min, m/z 392 $(M+H)^+$. HRMS (ESI) calcd for $C_{23}H_{22}FN_3O_2$ $[M+H]^+$: 392.1774. Found: 392.1781.

(2S,3S,4S)-3-(3',4'-bis(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide.



(2S,3S,4S)-3-(4-bromophenyl)-2-cyano-N-propyl-4-((trityloxy)methyl)azetidine-1-carboxamide (7.5 mg, 0.021 mmol) was dissolved in degassed 2:1 THF (300 µL) and 0.5 M K_3PO_4 (150 µL). Finally, XPhos Pd G3 was added, and the reaction was stirred at room temperature for 2.5 hours. The mixture was quenched with pH 7 buffer and extracted 3x with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and removed *in vacuo*. The resulting mixture was purified by flash column chromatography (MeOH/DCM) to afford the product as a white solid (5.5 mg, 53%). ^{1}H NMR (400 MHz, DCM-d_2) δ 7.98 (d, *J* = 1.9 Hz, 2H), 7.81 (s, 1H), 7.65 – 7.60 (m, 2H), 7.47 – 7.42 (m, 2H), 6.01 (d, *J* = 5.9 Hz, 1H), 4.97 (d, *J* = 8.6 Hz, 1H), 4.71 (td, *J* = 8.1, 2.3 Hz, 1H), 3.92 – 3.70 (m, 3H), 3.11 (m, 2H), 1.46 (m, 2H), 0.85 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DCM-d_2) δ 157.72, 142.39, 138.36, 135.04 (d, *J* = 7.3 Hz), 132.12, 131.79, 130.79, 129.31, 127.44 (d, *J* = 26.5 Hz), 124.78, 122.07, 121.66 – 121.08 (m), 115.75, 67.89, 65.81, 53.33, 53.13, 52.86, 52.55, 42.11, 39.13, 23.21, 11.07. Purity by LCMS (UV Chromatogram, 210nm, 7.5 min run) 99% by UV, rt = 3.92 min, m/z 486 $(M+H)^+$. HRMS (ESI) calcd for $C_{23}H_{21}F_6N_3O_2$ $[M+H]^+$: 486.1608. Found: 486.1618.

Analytical data for compounds in Extended Data Table 2

The synthesis of these tool compounds were performed using the general synthetic methods outlined in Lowe *et al*.[37] and above for BRD7929

(8R,9S,10S)-10-((dimethylamino)methyl)-N-(4-(2-fluoroethoxy)phenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (Formic acid salt) (BRD8805)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.41 (m, 4H), 7.41 – 7.33 (m, 2H), 7.32 – 7.23 (m, 3H), 7.23 – 7.14 (m, 2H), 6.83 – 6.71 (m, 2H), 6.05 (s, 1H), 4.76 – 4.68 (m, 1H), 4.63 – 4.54 (m, 1H), 4.18 – 4.10 (m, 1H), 4.10 – 4.02 (m, 1H), 3.86 – 3.72 (m, 1H), 3.63 – 3.46 (m, 4H), 3.21 – 3.10 (m, 1H), 3.05 – 2.92 (m, 1H), 2.84 – 2.73 (m, 1H), 2.64 – 2.52 (m, 2H), 2.33 – 2.23 (m, 1H), 2.08 (s, 6H), 1.84 – 1.64 (m, 3H), 1.64 – 1.47 (m, 1H). Purity by LCMS (UV Chromatogram, 210nm, 4.5 min run) 95% by UV, rt = 2.64 min, m/z 555 $(M+H)^+$.

(8R,9S,10S)-10-((isopropyl(methyl)amino)methyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (HCl salt) (BRD4716)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.36 (m, 6H), 7.33 – 7.23 (m, 3H), 7.24 – 7.10 (m, 2H), 6.80 – 6.69 (m, 2H), 5.99 (s, 1H), 3.84 – 3.73 (m, 1H), 3.70 (s, 3H), 3.66 – 3.47 (m, 4H), 3.20 – 3.10 (m, 1H), 3.10 – 2.98 (m, 1H), 2.89 – 2.76 (m, 1H), 2.43 – 2.29 (m, 2H), 2.12 – 1.93 (m, 2H), 1.85 – 1.67 (br s, 3H), 1.18 (s, 6H), 1.09 – 0.84 (m, 2H), 0.82 – 0.67 (m, 2H). Purity by LCMS (UV Chromatogram, 210nm, 4.5 min run) 96% by UV, rt = 2.29 min, m/z 551 $(M+H)^+$.

(8R,9S,10S)-10-(((2-fluoroethyl)(methyl)amino)methyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (HCl salt) (BRD2132)
^{1}H NMR (400 MHz, CH_3OD) δ 7.79 – 7.67 (m, 2H), 7.67 – 7.56 (m, 2H), 7.55 – 7.43 (m, 2H), 7.42 – 7.29 (m, 3H), 7.24 – 7.16 (m, 2H), 6.87 – 6.76 (m, 2H), 4.76 – 4.68 (m, 1H), 4.65 – 4.53 (m, 1H), 4.49 – 4.33 (m, 1H), 4.25 – 4.15 (m, 1H), 4.10 – 3.98 (m, 1H), 3.89 – 3.77 (m, 1H), 3.74 (s, 3H), 3.67 – 4.60 (m, 1H), 3.54 – 3.32 (m, 4H), 3.30 – 3.19 (m, 3H), 3.08 – 2.92 (m, 1H), 2.72 (s, 3H), 1.97 – 1.85 (m, 4H). Purity by LCMS (UV Chromatogram, 210nm, 4.5 min run) 99.5% by UV, rt = 2.25 min, m/z 555 $(M+H)^+$.

(9R,10R,11S)-11-(hydroxymethyl)-N-(4-methoxyphenyl)-10-(4-(phenylethynyl)phenyl)-1,7-diazabicyclo[7.2.0]undecane-7-carboxamide (BRD0185)
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 – 7.68 (m, 1H), 7.63 – 7.46 (m, 3H), 7.41 – 7.31 (m, 3H), 7.33 – 7.20 (m, 4H), 6.93 – 6.79 (m, 2H), 6.38 – 6.18 (m, 1H), 5.11 – 4.82 (m, 1H), 4.52 – 4.29 (m, 1H), 4.22 – 3.87 (m, 2H), 3.76 (s, 3H), 3.72 – 3.58 (m, 1H), 3.48 – 3.29 (m, 2H), 3.18 – 2.80 (m, 2H), 2.57 – 2.28 (m, 2H), 1.97 – 1.64 (m, 6H). Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 96% by UV, rt = 2.97 min, m/z 510 $(M+H)^+$.

(8R,9S,10S)-10-(azetidin-1-ylmethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD8493)
^{1}H NMR (400 MHz, CH_3OD) δ 7.56 – 7.46 (m, 6H), 7.41 – 7.30 (m, 3H), 7.25 – 7.14 (m, 2H), 6.88 – 6.76 (m, 2H), 3.91 – 3.78 (m, 1H), 3.75 (s, 3H), 3.60 – 3.47 (m, 2H), 3.46 – 3.33 (m, 2H), 3.26 – 3.17 (m, 3H), 3.25 – 3.21 (m, 1H), 3.19 – 2.96 (m, 5H), 2.90 – 2.80 (m, 1H), 2.75 – 2.53 (m, 2H), 2.07 – 1.89 (m, 2H), 1.81 – 1.63 (m, 2H). Purity by LCMS (UV Chromatogram, 210nm, 4.5 min run) 94% by UV, rt = 2.04 min, m/z 535 $(M+H)^+$.

(8R,9S,10S)-N-(4-methoxyphenyl)-10-((4-methylpiperazin-1-yl)methyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD6479)
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52-7.55 (m, 2H), 7.45 (d, J = 8.3 Hz, 2H), 7.40 – 7.31 (m, 4H), 7.24-7.27 (m, 3H), 6.83 (d, J = 8.9 Hz, 2H), 6.11 (s, 1H), 3.78 (s, 3H), 3.67 (bd s, 1H), 3.65 – 3.55 (m, 2H), 3.49 – 3.36 (m, 2H), 3.18 – 3.00 (m, 2H), 2.98 (d, J = 6.1 Hz, 1H), 2.92 (d, J = 8.7 Hz, 1H), 2.90 – 2.75 (m, 2H), 2.36 – 2.18 (m, 8H), 2.15 (s, 3H), 1.93 – 1.76 (m, 4H). Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 94% by UV, rt = 2.91 min, m/z 578 $(M+H)^+$.

(8R,9R,10S)-9-(4-((2-cyanophenyl)ethynyl)phenyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD4873)
^{1}H NMR (400 MHz, CH_3OD) δ 7.84 – 7.77 (m, 1H), 7.77 – 7.65 (m, 2H), 7.66 – 7.50 (m, 5H), 7.28 – 7.18 (m, 2H), 6.90 – 6.81 (m, 2H), 4.06 – 4.08 (m, 1H), 3.80-3.73 (m, 4H), 3.69 – 3.62 (m, 1H), 3.62 – 3.42 (m, 4H), 3.27 – 3.14 (m, 2H), 3.08 – 3.00 (m, 1H), 2.48 – 2.39 (m, 1H), 1.89 – 1.64 (m, 4H). Purity by LCMS (UV Chromatogram, 210nm, 4.5 min run) 98.5 % by UV, rt = 2.47 min, m/z 521 $(M+H)^+$.

(7R,8R,9S)-9-(hydroxymethyl)-N-(4-methoxyphenyl)-8-(4-(phenylethynyl)phenyl)-1,5-diazabicyclo[5.2.0]nonane-5-carboxamide (BRD9599)
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 – 7.44 (m, 5H), 7.40 – 7.30 (m, 3H), 7.29 – 7.17 (m, 3H), 6.86 – 6.76 (m, 2H), 6.05 (s, 1H), 4.20 – 4.04 (m, 1H), 3.81 – 3.73 (m, 3H), 3.71 – 3.41 (m, 6H), 3.24 – 3.13 (m, 1H), 3.12 – 2.94 (m, 2H), 2.32 – 2.17 (m, 1H), 1.97 – 1.72 (m, 2H). Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 96 % by UV, rt = 2.32 min, m/z 483 $(M+H)^+$.

(8R,9S,10S)-8,10-bis(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD2936)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.47 (m, 4H), 7.41 – 7.33 (m, 2H), 7.30 – 7.27 (m, 5H), 6.91 – 6.82 (m, 2H), 4.11 – 4.02 (m, 1H), 4.02 – 3.90 (m, 3H), 3.86 – 3.66 (m, 7H), 3.48 – 3.31 (m, 1H), 2.98 – 2.79 (m, 2H), 2.68 – 2.55 (m, 1H), 1.86 – 1.49 (m, 4H). Purity by LCMS (UV Chromatogram, 210nm, 7 min run) 97 % by UV, rt = 3.68 min, m/z 527 $(M+H)^+$.

((8R,9R,10S)-6-(benzo[d]thiazol-2-yl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methanol (BRD5349)

^{1}H NMR (400 MHz, CH_3OD) δ 7.68 – 7.59 (m, 4H), 7.61 – 7.48 (m, 4H), 7.49 – 7.44 (m, 1H), 7.42 – 7.37 (m, 2H), 7.31 – 7.23 (m, 1H), 7.14 – 6.99 (m, 1H), 4.08 – 3.93 (m, 1H), 3.87 – 3.71 (m, 1H), 3.70 – 3.57 (m, 3H), 3.59 – 3.38 (m, 4H), 3.23 – 3.09 (m, 1H), 2.42 – 2.31 (m, 1H), 2.01 – 1.65 (m, 4H). Purity by LCMS (UV Chromatogram, 210nm, 4.5 min run) 98.7% by UV, rt = 2.81 min, m/z 480 $(M+H)^+$.

(8R,9R,10S)-9-(4-(cyclopropylethynyl)phenyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD5774)

^{1}H NMR (400 MHz, CH_3OD) δ 7.47 – 7.36 (m, 2H), 7.31 – 7.23 (m, 2H), 7.22 – 7.11 (m, 2H), 6.88 – 6.73 (m, 2H), 4.06 – 3.92 (m, 1H), 3.75 (s, 3H), 3.68 – 3.56 (m, 2H), 3.55 – 3.33 (m, 4H), 3.23 – 3.06 (m, 2H), 3.03 – 2.91 (m, 1H), 2.44 – 2.33 (m, 1H), 1.85 – 1.59 (m, 4H), 1.50 – 1.37 (m, 1H), 0.92 – 0.79 (m, 2H), 0.77 – 0.66 (m, 2H). Purity by LCMS (UV Chromatogram, 210nm, 4.5 min run) 96% by UV, rt = 2.35 min, m/z 460 $(M+H)^+$.

(8R,9R,10S)-N-cyclobutyl-10-(hydroxymethyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD8260)

^{1}H NMR (400 MHz, CH_3OD) δ 7.59 – 7.45 (m, 6H), 7.45 – 7.32 (m, 3H), 4.28 – 4.16 (m, 1H), 3.94 – 3.84 (m, 1H), 3.75 – 3.58 (m, 2H), 3.51 – 3.39 (m, 4H), 3.19 – 3.02 (m, 2H), 2.97 – 2.85 (m, 1H), 2.43 – 2.31 (m, 1H), 2.31 – 2.14 (m, 2H), 2.04 – 1.89 (m, 2H), 1.84 – 1.54 (m, 6H). Purity by LCMS (UV Chromatogram, 210nm, 4.5 min run) 99 % by UV, rt = 2.48 min, m/z 444 $(M+H)^+$.

X-ray crystal structure of BRD7929

BRD7929 was crystallized as a salt with two equivalents of L-tartaric acid. Crystallographic studies were preformed Crystallographic Resources, inc. Dewitt, MI, 48820. The crystallographic data has been deposited in the Cambridge Structural Database (http://www.ccdc.cam.ac.uk) and the data has been assigned the deposition number CCDC 1429949.

Supplementary Figure 4 | Chemical and X-ray crystal structures of BRD7929.

Supplementary Tables

Assay	EC_{50} (nM)				
	BRD0026	BRD7539	BRD73842	BRD3444	Common antimalarial [drug]
Pf, Dd2 [CQ, QN, PY, SDX]	1,010.6 (34.4)*	10.8 (5.1)*	80.1 (2.8)*	9.2 (0.7)*	148.7 (32.3)* [CQ]
*Pfsc*DHODH	368	8,917	26	3	-
*Pf*NITD609^{R}	1,778	6	16	3	-
HepG2	> 26,000	> 26,000	> 26,000	> 26,000	-
Pf, TM90C6B [CQ, PY, MQ, ATV]	-	11	-	-	18,800 [PY]
*Pf*CYTb:G33V [IDI5994]	-	3	-	-	-
*Pf*DHODH:E182D [Genz-666136]	-	637	-	-	-
*Pf*DHODH (enzyme)	-	33	-	-	-
*Pf*PI(4)K (enzyme)	-	-	21	-	-
*Pfc*PheRS (enzyme)	-	-	-	21	-
Pf, 3D7 [SDX]	1,004.7 (82.9)*	10.2 (2.9)*	100.1 (4.6)*	12.3 (1.5)*	-
Pf, D6 [MQ]	1,414.3 (156.4)*	7.1 (3.0)*	68.2 (3.2)*	11.5 (3.2)*	18.8 (4.5)* [MQ]
Pf, K1 [CQ, SDX, PY, CYC]	937.9 (76.2)*	10.3 (3.6)*	58.9 (8.7)*	13.2 (1.2)*	12,100* [PY]
Pf, NF54	1,213.3 (40.3)*	5.4 (1.3)*	53.4 (5.0)*	9.4 (1.4)*	16.2 (1.1)* [MQ]
Pf, V1/3 [CQ, SDX, PY, CYC]	1,560.7 (107.9)*	20.7 (7.6)*	67.7 (4.2)*	16.2 (2.2)*	19,200* [PY]
Pf, HB3 [PY]	889.2 (204.2)*	5.3 (1.6)*	56.8 (4.3)*	11.5 (2.2)*	2,250 (489)* [PY]
Pf, 7G8 [CQ, PY, CYC]	1,330.7 (118.3)*	10.6 (4.0)*	79.4 (0.0)*	13.1 (4.0)*	9,810* [PY]
Pf, FCB [CQ, CYC]	787.0 (181.0)*	13.0 (4.3)*	93.8 (12.2)*	10.4 (2.0)*	174.0 (52.7)* [CQ]
Pf gametocyte (stage IV-V)	-	-	643	663	-
Pb hepatic stage	-	-	459	339	-
Pc hepatic (small form)	-	-	344	3,300	-
Pc hepatic (large form)	-	-	832	2,860	-

CQ, chloroquine; PY, pyrimethamine; MQ, mefloquine; ATV, atovaquone; SDX, sulphadoxine; CYC, cycloguanil.
*Values shown are the mean of three technical and three biological replicates (standard deviations shown in parentheses). Slight differences in values from those reported in the main text reflect measurements made in different experiments.

Supplementary Table 1 | Summary table of four screening hit compounds. For strains harboring drug resistance (Dd2, 3D7, D6, K1, NF54, V1/3, HB3, 7G8, and FCB), the appropriate controls are indicated on the right, including the resistance drug and activity of that drug against a non-resistant control strain.

Strain name	Original strain name	Generated by	Parental strain	Resistant against	Resistant gene	Mutation	Reference (PMID)
*Pf*scDHODH	D10+ DHOD-GFP	Transgenic	D10	ECT inhibitors	*scdhodh*	-	17330044
*Pf*NITD609^{R}	*Pf*Cam-D1247Y	Transgenic	Dd2	NITD609	*pfatp4*	D1247Y	20813948
TM90C6B	TM90C6B	Field isolate	-	Atovaquone	*pfcytb*-Qo site	Y268S	22430961
*Pf*CYTb:G33V	*Pf*CYTb:G33V	Selection	Dd2	IDI 5994	*pfcytb*-Qi site	G33V	25336726
*Pf*DHODH:E182D	3D7: E182D	Selection	3D7	Genz-666136	*pfdhodh*	E182D	24381157
*Pf*BRD73842^{R}-A	-	Selection	Dd2	BRD73842	*pfpi4k*	Y1356N	-
*Pf*BRD73842^{R}-B	-	Selection	Dd2	BRD73842	*pfpi4k*	L1418F	-
*Pf*KAI407^{R}	*Pf*PI(4)K mutant	Transgenic	Dd2	KAI407	*pfpi4k*	H1484Y	24284631
*Pf*MMV048^{R}	-	Transgenic	Dd2	MMV048	*Pfpfpi4k*	-	-

Supplementary Table 2 | Genotypes of *Plasmodium* strains used to characterize phenotypic responses of screening hits.

	Flask 2*		Flask 3*	
EC_{50} (nM) / fold Δ	Clone #1	Clone #2	Clone #3	Clone #4
	1,500 / 21	817 / 11	511 / 7	560 / 7.7
Genome coverage (x)	68	108	90	72
% covered by 15 or more reads	85.3	94.4	89.5	87.2
SNVs identified				
Raw†	76064	84633	80109	76128
Quality‡	4713	7852	6364	5603
Unique prior to IGV¶	157	151	175	169
Unique post IGV¶	8	9	3	5
Intergenic	1	2	1	1
Intronic	0	2	0	1
Synonymous	0	0	1	2
Total nonsynonymous	7	5	1	1
Genes mutated in all samples				
Locus	PF3D7_0509800	PF3D7_0509800	PF3D7_0509800	PF3D7_0509800
Annotation	PI(4)K	PI(4)K	PI(4)K	PI(4)K
Mutation	Y1356N	Y1356N	L1418F	L1418F

*See Supplementary Information for methods.
†After alignment to *P. falciparum* 3D7 reference genome.
‡Quality filters based on parameters defined in Methods.
¶Compared to Dd2 parent.

Supplementary Table 3 | BRD73842 targets *Pf*PI(4)K. Two independent BRD73842-resistant strains were selected and assessed via whole-genome sequencing. Both lines (two clones each) contained mutations within PF3D7_0509800, which encodes the *Pf*PI(4)K. EC_{50} values and fold change (compared to wild type Dd2) are indicated underneath clone IDs.

EC_{50} (nM) / fold Δ	Flask 5*		Flask 7*		Flask 8*		
	Clone #1	Clone #2	Clone #3	Clone #4	Clone #5	Clone #6	Clone #7
	1,200 / 84	1,200 / 84	65 / 4	59 / 4	376 / 25	138 / 9	168 / 11
Genome coverage (x)	77.37	81.92	76.14	73.36	76.15	88.76	61.79
% covered by 15 or more reads	91.2	91.6	91.6	91.7	89.8	90.5	90
SNVs identified							
Raw†	70316	70509	69781	69653	69369	73042	72068
Quality‡	8628	8857	8640	8720	8418	8591	6795
Unique prior to IGV¶	285	324	281	290	269	252	181
Unique post IGV¶	4	5	6	5	4	6	2
Intergenic	0	2	1	1	0	3	0
Intronic	0	0	1	0	1	0	0
Synonymous	1	1	2	2	1	1	0
Total nonsynonymous	3	2	2	2	2	2	2
Genes mutated in all samples							
Locus	PF3D7_0109800	PF3D7_0109800	PF3D7_0109800	PF3D7_0109800	PF3D7_0109800	PF3D7_0109800	PF3D7_0109800
Annotation	PheRSα	PheRSα	PheRSα	PheRSα	PheRSα	PheRSα	PheRSα
Mutation	L550V	L550V	M316I	M316I	G512E; V545I	G512E; V545I	G512E; V545I

*See Supplementary Information for methods.
†After alignment to *P. falciparum* 3D7 reference genome.
‡Quality filters based on parameters defined in Methods.
¶Compared to Dd2 parent.

Supplementary Table 4 | BRD1095 targets *Pf*PheRS. Whole-genome sequencing of BRD1095-resistant clones reveals mutations within the putative cytoplasmic *pfpheRS*. Three independent BRD1095-resistant strains were selected and assessed via whole-genome sequencing. EC_{50} values and fold change (compared to wild type Dd2) are indicated underneath clone IDs. All three lines contained mutations within PF3D7_0109800, which is annotated as encoding the alpha subunit of cytoplasmic PheRS. The resistant clones isolated from two of the flasks contained single SNV, while the clones from the third flask contained two SNVs.

Supplemented amino acid		Average EC_{50} (nM) ± SD				
		BRD1095	BRD7929	Atovaquone	Mefloquine	Dihydro-artemisinin
Control	-	16.4 (2.4)	10.2 (2.0)	0.30 (0.08)	17.2 (3.3)	8.6 (2.3)
L-Phenylalanine	10x	135.6 (5.8)	101.1 (7.7)	0.32 (0.05)	33.9 (0.6)	2.8 (0.5)
	20x	270.4 (4.9)	220.4 (15.1)	0.26 (0.01)	35.1 (0.2)	2.4 (0.1)
	50x	677.1 (73.6)	477.7 (21.6)	0.26 (0.02)	30.2 (0.8)	2.2 (0.1)
D- Phenylalanine	10x	20.8 (2.6)	15.1 (0.3)	0.27 (0.02)	34.9 (0.8)	2.3 (0.1)
	20x	19.1 (1.5)	14.5 (0.9)	0.36 (0.04)	34.3 (0.4)	2.9 (0.2)
	50x	18.6 (1.8)	15.1 (0.7)	0.20 (0.02)	28.9 (0.4)	2.7 (0.4)
L-Aspartic acid	10x	16.9 (2.9)	8.5 (1.7)	0.43 (0.02)	23.7 (1.0)	13.0 (0.4)
	20x	13.7 (2.2)	7.5 (0.6)	0.42 (0.05)	23.7 (3.5)	12.6 (0.4)
	50x	19.1 (2.5)	10.3 (0.6)	0.38 (0.02)	13.9 (6.1)	11.1 (0.6)
L-Threonine	2x	19.2 (5.1)	6.3 (1.1)	0.37 (0.07)	20.0 (2.1)	12.5 (1.2)
	4x	15.0 (1.3)	10.1 (2.3)	0.45 (0.05)	19.0 (0.7)	9.4 (2.3)
	10x	20.3 (3.1)	9.0 (1.5)	0.32 (0.03)	15.4 (1.1)	10.0 (1.7)
L-Tyrosine	10x	16.4 (0.8)	10.7 (1.9)	0.33 (0.02)	17.4 (4.2)	7.1 (0.8)
	20x	17.6 (4.1)	8.6 (0.6)	0.47 (0.13)	15.7 (3.4)	11.0 (1.0)
	50x	17.2 (1.8)	10.1 (2.5)	0.43 (0.10)	21.5 (0.1)	11.3 (1.0)

Supplementary Table 5 | Supplementation with exogenous amino acids to the *in vitro* culture medium increased the EC_{50} values. *In vitro* potency of BRD1095, BRD7929 and other antimalarials were determined by growth inhibition assay with the presence of 5 different amino acids at 3 doses. The activity of BRD1095 and BRD7929 could be effectively reduced by the addition of exogenous L-phenylalanine to the growth medium (RPMI) but exogenous D-phenylalanine L-aspartic acid, L-threonine and L-tyrosine had no effect on the activity of BRD1095 and BRD7929. Values shown are the average of three technical and three biological replicates (standard deviations shown in parentheses).

		Dose (μg/plate)	Number of revertants per plate, Mean (individual data)									
			TA100		TA1535		TA98		TA1537		WP2uvrA	
Without S9 mix	DMSO	0	127 (141, 112)		10 (12, 8)		26 (20, 31)		28 (26, 30)		28 (24, 31)	
	ER-0011 84635-000	6.86	139 (135, 142)		11 (14, 8)		36 (24, 47)		24 (20, 28)		40 (45, 34)	
		20.6	125 (131, 118)		9 (11, 6)		23 (21, 24)		18 (18, 17)		38 (37, 39)	
		61.7	91 (94, 87)	T	2 (2, 1)	T	22 (23, 20)	T	1 (1, 0)	T	41 (33, 48)	T
		185	0 (0, 0)	T	0 (0, 0)	T	0 (0, 0)	T	0 (0, 0)	T	29 (33, 25)	T
		556	0 (0, 0)	T	0 (0, 0)	T	0 (0, 0)	T	0 (0, 0)	T	15 (12, 17)	T
		1667	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T
		5000	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T
	AF2	0.01	476 (466, 486)		NT		NT		NT		135 (125, 144)	
		0.1	NT		NT		435 (419, 451)		NT		NT	
	9AA	80	NT		NT		NT		377 (342, 411)		NT	
	SA	0.5	NT		559 (568, 550)		NT		NT		NT	
With S9 mix	DMSO	0	136 (131, 140)		11 (10, 11)		41 (37, 44)		20 (13, 26)		40 (48, 32)	
	ER-0011 84635-000	6.86	153 (161, 145)		10 (11, 8)		45 (40, 50)		29 (30, 28)		38 (30, 45)	
		20.6	175 (174, 176)		12 (13, 11)		51 (40, 62)		31 (35, 26)		41 (37, 45)	
		61.7	114 (105, 123)	T	9 (12, 6)	T	32 (34, 30)		9 (10, 8)	T	36 (43, 29)	
		185	0 (0, 0)	T	0 (0, 0)	T	0 (0, 0)	T	0 (0, 0)	T	31 (32, 30)	
		556	0 (0, 0)	T	0 (0, 0)	T	0 (0, 0)	T	0 (0, 0)	T	11 (10, 11)	T
		1667	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T
		5000	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T	0 (0, 0)	P,T
	2AA	0.5	NT		NT		581 (605, 556)		NT		NT	
		1	1144 (1137, 1150)		NT		NT		NT		NT	
		2	NT		278 (278, 277)		NT		108 (92, 124)		NT	
		10	NT		NT		NT		NT		602 (613, 591)	

NT, Not tested; T, Toxic (growth inhibition); P, Precipitation; 9AA, 9-aminoacridine hydrochloride monohydrate; 2AA, 2-aminoanthracene; DMSO, dimethyl sulfoxide; AF2, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; SA, sodium azide.

Supplementary Table 6 | Reverse mutation assay in bacteria with BRD7929. BRD7929 was tested for mutagenicity. The Ames test, with preincubation at 37°C for 20 minutes, was conducted in duplicate in the presence or absence of S9 mix using bacterial strains of *Salmonella typhimurium* TA100, TA1535, TA98, TA1537, and *Escherichia coli* WP2uvrA. The S9 mix included cofactors and S9 fraction from liver homogenate of male Sprague Dawley rats treated with phenobarbital and 5,6-benzoflavone. Dimethyl sulfoxide was used as a vehicle. BRD7929 is judged to be negative in the reverse mutation assay in bacteria under the experimental conditions employed in this study.

Supplementary References

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