Supplemental Information

Driving Potency with Rotationally Stable Atropisomers: Discovery of Pyridopyrimidinedione-Carbazole Inhibitors of BTK

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S1. Experimental procedures, characterization data, and single crystal X-ray supporting information.

All experiments involving animals were performed as per institutional guidelines as defined by the Institutional Animal Care and Use Committee for US Institutions.

Commercially available anhydrous solvents were used for reactions. All other reagents and solvents were reagent grade and used as received from commercial sources. Column chromatography was performed on a Teledyne ISCO system with commercially available silica gel columns. Final compounds were purified by reversed phase HPLC. Proton magnetic resonance spectra were recorded on a Bruker Spectrometer (operating at 400 MHz or 500 MHz). All mass spectra were recorded using electrospray ionization (ESMS) in positive mode unless otherwise noted. All assayed compounds have a purity of >90% as assessed by analytical HPLC.

Method of analytical HPLC: Column: Waters Acquity UPLC BEH C_{18} (2.1 x 50 mm, 1.7 μm); mobile phase A: water with 0.05% TFA; mobile phase B: MeCN with 0.05% TFA; temperature: 50 °C; flow rate 0.80 mL/min; gradient: 2-98% B over 1 min, then 0.5 min isocratic at 98% B.

Preparative high performance liquid chromatography (HPLC) was performed using a reverse phase column (Waters SunFire C18, Waters XBridge C18, PHENOMENEX®Axia C18, YMC S5 ODS or the like) of a size appropriate to the quantity of material being separated, generally eluting with a gradient of increasing concentration of methanol or acetonitrile in water, also containing 0.05% or 0.1% trifluoroacetic acid or 10 mM ammonium acetate, at a rate of elution suitable to the column size and separation to be achieved.

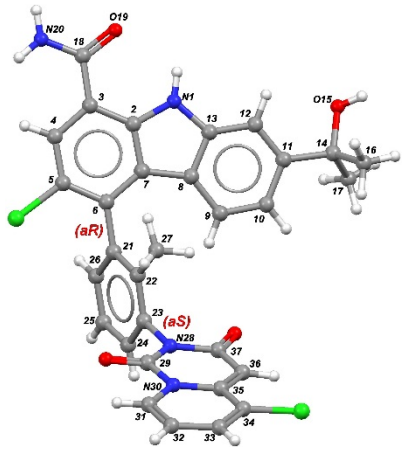
3-Chloro-4-(*R*)-(3-(*S*)-(5-chloro-1,3-dioxo-1H-pyrido[1,2-c]pyrimidin-2(3H)-yl)-2-methylphenyl)-7-(2-hydroxypropan-2-yl)-9H-carbazole-1-carboxamide (6d) (single atropisomer).[32] A mixture of 4-bromo-3-chloro-7-(2-hydroxypropan-2-yl)-9H-carbazole-1-carboxamide[28] (**7**, 50.5 g, 132 mmol), 5-chloro-2-(*S*)-(2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrido[1,2-c]pyrimidine-1,3(2H)-dione (**10**, 60.1 g, 146 mmol) and Cs_2CO_3 (86 g, 265 mmol) in THF (342 mL) and water (85 mL) was bubbled with nitrogen for 5 min, and was then treated with $PdCl_2$(dppf) DCM adduct (11.9 g, 14.6 mmol). Bubbling with nitrogen was continued for an additional 5 min, and the mixture was heated at 62 °C under nitrogen for 20 h. The reaction mixture was cooled to room temperature, and MeOH (300 mL) was added with stirring. After 15 min, water (2 L) was added, resulting in a rusty brown gum. The

supernatant was removed, and the gummy residue was washed with water (2x) and then suspended in EtOAc (2 L) and stirred for 1 h. The mixture was filtered under reduced pressure, and the filtrate was concentrated to a volume of about 1-1.5 L and treated with heptane (3 L). The mixture was stirred for two days, and the precipitate was collected by vacuum filtration, washed with heptane, and dried under reduced pressure to give a yellow solid (104 g). The solid was dissolved in THF, absorbed on Celite, dried under reduced pressure, placed on a silica gel plug, and eluted with heptane/EtOAc (10:90) to give an orange-yellow oil (74.9 g). The material was subjected to column chromatography on silica gel (3 kg), eluting with EtOAc-hexanes (gradient from 40-90%), to give 3-chloro-4-(3-(*S*)-(5-chloro-1,3-dioxo-1H-pyrido[1,2-c]pyrimidin-2(3H)-yl)-2-methylphenyl)-7-(2-hydroxypropan-2-yl)-9H-carbazole-1-carboxamide (**5h**, mixture of two atropisomers at carbazole C4) as a yellow foam (44 g, 51% yield).

The material was separated by chiral super-critical fluid chromatography as follows: column: Chiralpak AS-H (2 x 50 cm, 10 μm); mobile phase: CO_2-MeOH (55:45) at 140 mL/min, 40 °C, 100 bar; sample preparation: 56 mg/mL in MeOH-DCM (1:1); injection: 3.33 mL. The first peak eluting from the column provided 3-chloro-4-(*R*)-(3-(*S*)-(5-chloro-1,3-dioxo-1H-pyrido[1,2-c]pyrimidin-2(3H)-yl)-2-methylphenyl)-7-(2-hydroxypropan-2-yl)-9H-carbazole-1-carboxamide (**6d**) as a yellow solid (18.3 g, 24% yield).

HPLC purity: 98.5%; t_r = 23.04 min. (Instrument: Agilent 11--/DAD/MSD; Column: Waters Sunfire C18 150 mm (L) x 4.6 mm (ID), 3.5 μ); Temp. 35ºC; Flow: 1.0 mL/min; Mobile Phase: A = 0.05% TFA in water, B = 0.05% TFA in MeCN; Gradient time (min)/%B: 0/20, 25/55, 30/95; Sample Injection: 2 μL of 1.0 mg/mL sample in MeCN). Chiral purity: 99.4% ie; Optical rotation: $[\alpha]_D^{20}$ (c = 2.10, $CHCl_3$) = +332.34º. Mass spectrum m/z 569 $(M+H-H_2O)^+$. Anal. Calcd. for $C_{31}H_{25}ClN_4O_4$: C, 67.33; H, 4.56; N, 10.13; Found: C 65.92, H 5.43, N 9.48. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 11.50 (s, 1H), 8.27 (br d, *J* = 7.5 Hz, 2H), 8.14 (s, 1H), 7.84 (d, *J* = 1.1 Hz, 1H), 7.60 (d, *J* = 7.0 Hz, 1H), 7.60 - 7.56 (m, 1H), 7.56 - 7.51 (m, 1H), 7.49 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.28 (dd, *J* = 7.4, 1.4 Hz, 1H), 7.00 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.73 (d, *J* = 8.3 Hz, 1H), 6.57 (t, *J* = 7.4 Hz, 1H), 5.99 (s, 1H), 4.99 (s, 1H), 1.72 (s, 2H), and 1.44 (d, *J* = 2.3 Hz, 6H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 167.8, 159.5, 149.4, 148.4, 144.7, 140.8, 138.1, 137.5, 135.6, 135.5, 134.1, 133.6, 129.3, 128.8, 128.0, 127.1, 124.5, 124.1, 123.6, 121.1, 120.7, 118.7, 116.9, 116.1, 109.7, 107.9, 89.8, 70.9, 32.3, 32.0, and 13.9.

The absolute configuration of **6d** was confirmed by single crystal X-ray analysis (CDCC # 1501157) of crystals prepared by dissolving the compound in excess methanol and slowly evaporating the solvent at room temperature to provide a di-methanol solvate. Unit cell dimensions: a = 7.41 Å, b = 9.74 Å, c = 44.55 Å, α = 90.0°, β = 90.0°, γ = 90.0°; Space group: $P2_12_12_1$; Molecules of **6d**/asymmetric unit: 1; Volume/Number of molecules in the unit cell = 3214 $Å^3$; Density (calculated) = 1.346 g/cm^3. Fractional atomic coordinates at 173 K are below, and a depiction of the structure is:



FractionalAtomic Coordinates of **6d**, Form M2-1 Calculated at a Temperature of about 173K; Atomic Coordinates (xlO4)

Atom	X	Y	Z Atom	X	Y	Z
CU	0.4450	-0.0974	0.0917 03	0.1923	0.6096	0.0807
C12	0.2679	0.9390	-0.0104 04	0.2649	0.3023	0.0050
NI	0.7146	0.2778	0.1831 05	-0.0568	0.6980	0.1220
Cl	0.6840	-0.0285	0.1346 C32	-0.1535	0.5826	0.1313
C2	0.4628	0.1463	0.1208 06	0.9895	0.0341	0.2613
C3	0.7574	0.0528	0.1572 C33	0.9227	-0.0982	0.2682
C4	0.5932	0.3851	0.1807 Hl	0.8003	0.2727	0.1961
C5	0.4797	0.3617	0.1559 H2	0.7322	-0.1151	0.1310
C6	0.4364	0.5908	0.1933 H3	0.2399	0.6362	0.1632
Cl	0.5330	0.2289	0.1438 H4	0.6524	0.5129	0.2154
C8	0.3277	0.5712	0.1678 H5	0.2733	0.4474	0.1327
C9	0.5744	0.4989	0.1993 H6	0.1580	0.6793	0.2204
CIO	0.3475	0.4586	0.1493 H7	0.4410	0.7750	0.2571
cii	0.6778	0.1810	0.1617 H8	0.5965	0.6803	0.2452
C12	0.5397	0.0174	0.1173 H9	0.4092	0.6166	0.2538
C13	0.4025	0.7166	0.2133 HlO	0.4409	0.8558	0.1796
Ol	0.2131	0.7465	0.2141 Hll	0.6171	0.8286	0.1982
C14	0.9038	0.0063	0.1779 H12	0.4636	0.9209	0.2116
C15	0.4684	0.6951	0.2453 H13	1.0747	-0.1382	0.1834
C16	0.4889	0.8419	0.1994 H14	0.9653	-0.1543	0.1555

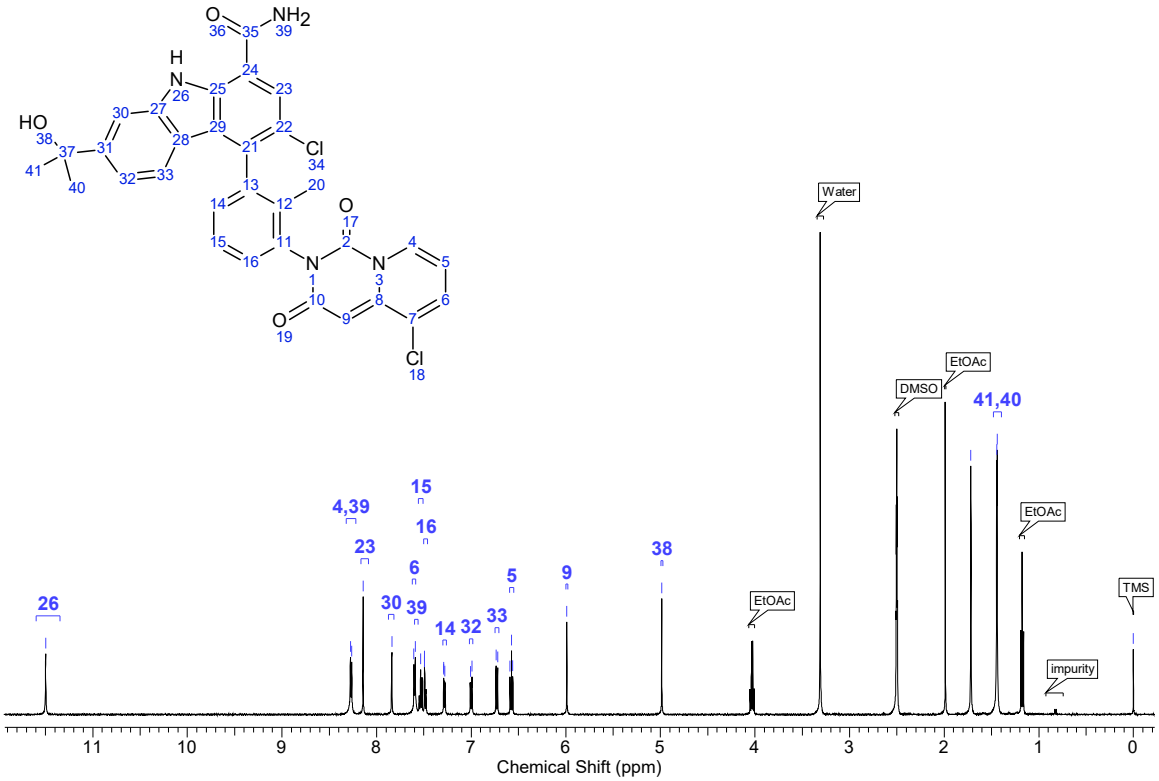
02	0.9372	0.0751	0.2007 H15	0.1144	0.0986	0.1246
N2	0.9916	-0.1089	0.1715 H16	-0.0771	0.3508	0.0613
C17	0.3100	0.1985	0.1022 H17	-0.1264	0.1899	0.0990
C18	0.1348	0.1615	0.1093 H18	0.5554	0.3096	0.0504
C19	0.1945	0.3479	0.0644 H19	0.6156	0.2937	0.0840
C20	0.0197	0.3118	0.0715 H20	0.5405	0.4346	0.0725
C21	-0.0095	0.2166	0.0941 H21	0.2431	0.7886	0.0381
C22	0.3427	0.2931	0.0791 H22	0.3851	0.5712	-0.0762
C23	0.5305	0.3367	0.0708 H23	0.3524	0.4052	-0.0410
N3	0.2226	0.4569	0.0426 H24	0.3306	0.8009	-0.0641
C24	0.2578	0.4191	0.0134 H25	0.0254	0.6742	0.1108
N4	0.2870	0.5312	-0.0066 H26	-0.0765	0.5242	0.1430
C25	0.2192	0.5925	0.0539 H27	-0.1958	0.5332	0.1140
C26	0.2741	0.6673	0.0027 H28	-0.2548	0.6110	0.1432
C27	0.2455	0.6972	0.0321 H29	0.9836	0.0467	0.2431
C28	0.2898	0.7686	-0.0207 H30	0.8087	-0.1118	0.2584
C29	0.3530	0.5950	-0.0567 H31	0.9071	-0.1064	0.2896
C30	0.3340	0.4967	-0.0359 H32	1.0070	-0.1664	0.2614
C31	0.3249	0.7337	-0.0493 —	—	—	—

Alternative preparation of 6d:

A mixture of 4-bromo-3-chloro-7-(2-hydroxypropan-2-yl)-9H-carbazole-1-carboxamide[28] (**7**, 1.11 g, 2.91 mmol), 5-chloro-2-(2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrido[1,2-c]pyrimidine-1,3(2H)-dione (**8**, 1.00 g, 2.42 mmol), and Cs_2CO_3 (1.58 g, 4.85 mmol) in THF (8 mL) and water (2 mL) was bubbled with argon for 3 min. The mixture was treated with $PdCl_2$(dppf) DCM adduct (0.099 g, 0.121 mmol) and heated at 60 °C overnight. The cooled mixture was diluted with EtOAc and washed sequentially with water and brine. The combined aqueous layers were extracted with DCM containing a small amount of MeOH. The combined organic layers were dried and concentrated until a tan precipitate formed, which was removed by filtration. The filtrate was concentrated and purified by column chromatography on silica gel (120 g), eluting with EtOAc-DCM (sequentially 70%, 80%, and 100%), to give 3-chloro-4-(3-(5-chloro-1,3-dioxo-1H-pyrido[1,2-c] pyrimidin-2(3H)-yl)-2-methylphenyl)-7-(2-hydroxypropan-2-yl)-9H-carbazole-1-carboxamide (**9,** mixture of four atropisomers) as a yellow solid (993 mg, 69% yield). The material was separated by chiral super-critical fluid chromatography as follows: column: Chiralpak AD-H (3 x 25 cm, 5 µm); mobile phase: CO_2-IPA (50:50) at 150 mL/min, 45 °C, 100 bar; sample preparation: 5.6 mg/mL in MeOH-DCM (1:1); injection: 3 mL. The fourth peak eluting from the column provided 3-chloro-4-(*R*)-(3-(*S*)-(5-

chloro-1,3-dioxo-1H-pyrido[1,2-c]pyrimidin-2(3H)-yl)-2-methylphenyl)-7-(2-hydroxypropan-2-yl)-9H-carbazole-1-carboxamide (**6d**).

Proton Spectrum of 6d in d_6-DMSO at 27°C with TMS. Experimental: 10 mg sample dissolved in 650µl d_6-DMSO, 500 MHz NMR. Proton resonance frequency 499.50 MHz, 16 scans, delay = 1.5 sec, 32K points at 27°C. ^{1}H-NMR (500MHz, DMSO-d_6) δ 11.50 (s, 1H), 8.27 (d, *J*=7.5 Hz, 2H), 8.14 (s, 1H), 7.84 (d, *J*=1.1 Hz, 1H), 7.60 (d, *J*=7.0 Hz, 1H), 7.60 - 7.56 (m, 1H), 7.56 - 7.51 (m, 1H), 7.49 (dd, *J*=7.7, 1.1 Hz, 1H), 7.28 (dd, *J*=7.4, 1.4 Hz, 1H), 7.00 (dd, *J*=8.4, 1.6 Hz, 1H), 6.73 (d, *J*=8.3 Hz, 1H), 6.57 (t, *J*=7.4 Hz, 1H), 5.99 (s, 1H), 4.99 (s, 1H), 1.72 (s, 3H), 1.45 (s, 3H), and 1.44 (s, 3H).



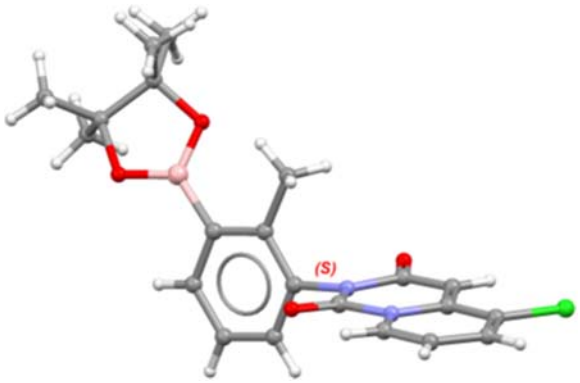
5-Chloro-2-(2-methyl-3-(4,4,5, 5-tetramethyl-l,3,2-dioxaborolan-2-yl)phenyl)-lH-pyrido [l,2-c]pyrimidine-l,3(2H)-dione (8).[31,32] A mixture of 2-(3-chloropyridin-2-yl)-N-(2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetamide (**15**, 0.192 g, 0.497 mmol) and CDI (0.322 g, 1.986 mmol) in toluene (2 mL) was heated at 110 °C. After 5 h, the cooled mixture was diluted with EtOAc and washed sequentially with water and brine. The combined aqueous layers were extracted with EtOAc, and the combined organic phases were dried and concentrated. The residue was purified by column chromatography on silica gel, eluting with EtOAc-hexanes, to provide racemic 5-chloro-2-(2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-

1H-pyrido[1,2-c]pyrimidine-1,3(2H)-dione as a bright yellow solid (0.133 g, 65% yield). Mass spectrum m/z 413 $(M+H)^+$. ^{1}H-NMR (400 MHz, chloroform-d) δ 8.26 (dt, *J*=7.6, 0.9 Hz, 1H), 7.94 (dd, *J*=7.5, 1.3 Hz, 1H), 7.36 (t, *J*=7.6 Hz, 1H), 7.27-7.18 (m, 2H), 6.36 (t, *J*=7.3 Hz, 1H), 6.31 (s, 1H), 1.57 (s, 3H), and 1.36 (s, 12H).

5-Chloro-2-(*S*)-(2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrido[1,2-c]pyrimidine-1,3(2H)-dione (10).[32] A sample of racemic 5-chloro-2-(2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrido[1,2-c]pyrimidine-1,3(2H)-dione (**8**) was separated by chiral super-critical fluid chromatography as follows: column: Whelko RR (3 x 25 cm, 5 μm); mobile phase: CO_2-MeOH (55:45) at 200 mL/min, 100 bar, 35 °C; sample preparation: 96 mg/ mL in MeCN-DCM (1:4); injection: 5 mL. The second peak eluting from the column provided 5-chloro-2-(*S*)-(2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrido[1,2-c]pyrimidine-1,3(2H)-dione (**10**). The mass spectrum and ^{1}H NMR for each enantiomeric atropisomer were the same as those for (**10**).

Alternatively, a sample of racemic 5-chloro-2-(2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrido[1,2-c]pyrimidine-1,3(2H)-dione (**8**) was separated by chiral super-critical fluid chromatography as follows: column: Whelko RR (3 x 25 cm, 5 μm); mobile phase: CO_2-CH_3CN (55:45) at 200 mL/min, 100 bar, 35 °C; sample preparation: 96 mg/ mL in MeCN-DCM (1:4); injection: 5 mL. The first peak eluting from the column provided 5-chloro-2-(*S*)-(2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrido[1,2-c] pyrimidine-1,3(2H)-dione (**10**). The material could be further purified by dissolving in THF, diluting with hexanes and collecting the precipitate by filtration.

The absolute configuration of **10** was confirmed by single crystal x-ray analysis (CDCC # 1501156) of crystals prepared by dissolving the compound in excess acetone and slowly evaporating the solvent at room temperature. Unit cell dimensions: a = 19.6161(8) Å, b = 9.1411(4) Å, c = 12.7541(6) Å, α = 90°, β = 113.165(2)°, γ = 90°; Space group: C2; Molecules of Intermediate 35/asymmetric unit (Z'): 1; Density, calc g-cm^{-3}: 1.304.



Fractional Atomic Coordinates for **10** at Room Temperature.

Atom	X	Y	Z	Atom	X	Y	Z
Cl1	-0.1755	-0.1003	0.3365	C21	0.0166	-0.2724	0.3622
O1	0.2261	0.6937	0.2037	O3	-0.0487	0.3988	0.3318
C1	0.2132	0.8050	0.1156	O4	0.1356	0.0809	0.3596
C2	0.1347	0.7655	0.0313	H1	0.1356	0.3551	0.5384
O2	0.1028	0.6968	0.1045	H2	0.2203	0.5422	0.5706
B1	0.1597	0.6467	0.1980	H3	0.2313	0.6587	0.4179
C3	0.1403	0.4028	0.4772	H4	0.0388	0.8624	-0.0807
C4	0.1906	0.5142	0.4966	H5	0.1101	0.9509	-0.0687
C5	0.1012	0.4308	0.2727	H6	0.0796	0.9586	0.0279
C6	0.1966	0.5843	0.4048	H7	0.0613	0.2788	0.1461
C7	0.0966	0.3615	0.3668	H8	0.0655	0.4356	0.0994
C8	0.1517	0.5466	0.2926	H9	0.0019	0.3967	0.1400
C9	0.0863	0.8966	-0.0281	H10	0.1556	0.5689	-0.0231
C10	0.0532	0.3809	0.1539	H11	0.1519	0.6967	-0.1073
C11	0.1298	0.6565	-0.0585	H12	0.0787	0.6339	-0.1029
C12	0.2226	0.9474	0.1724	H13	0.1871	0.9571	0.2066
C13	0.2710	0.7829	0.0643	H14	0.2151	1.0241	0.1175
N1	0.0457	0.2404	0.3528	H15	0.2718	0.9545	0.2304
C14	0.0746	0.1035	0.3564	H16	0.3176	0.8231	0.1147
N2	0.0270	-0.0148	0.3551	H17	0.2550	0.8312	-0.0083
C15	-0.0287	0.2712	0.3397	H18	0.2769	0.6801	0.0543

C16	-0.0453	0.0086	0.3467	H19	-0.1208	0.1615	0.3296
C17	-0.0720	0.1477	0.3375	H20	0.1039	-0.1652	0.3637
C18	0.0561	-0.1540	0.3608	H21	-0.0838	-0.3378	0.3584
C19	-0.0563	-0.2557	0.3562	H22	0.0370	-0.3652	0.3671
C20	-0.0863	-0.1218	0.3472	-	-	-	-

Diethyl 2-(3-chloropyridin-2-yl)malonate (11).[31,32] A mixture of 3-chloro-2-fluoropyridine (5.00 g, 38.0 mmol), diethyl malonate (14.6 g, 91 mmol) and Cs_2CO_3 (29.7 g, 91 mmol) in DMSO (42 mL) was heated at 100 °C for 7 h. After stirring overnight at room temperature, the mixture was diluted with EtOAc, washed with water (2x), and washed with brine. The combined aqueous layers were extracted with EtOAc, and the combined organic phases were dried and concentrated to give crude diethyl 2-(3-chloropyridin-2-yl) malonate as a colorless oil, which was used without further purification. Mass spectrum m/z 272 $(M+H)^+$.

Ethyl 2-(3-chloropyridin-2-yl)acetate (12).[31,32] A mixture of diethyl 2-(3-chloropyridin-2-yl) malonate (**11**, 10.3 g, 38 mmol), sodium chloride (5.55 g, 95 mmol) and water (3.42 mL, 190 mmol) in DMSO (40 mL) was heated at 145 °C for 8 h. The mixture was cooled to room temperature, diluted with EtOAc, washed with water (2x), and washed with brine. The organic phase was dried and concentrated to provide crude ethyl 2-(3-chloropyridin-2-yl)acetate, which was used without further purification. Mass spectrum m/z 200 $(M+H)^+$.

Sodium 2-(3-chloropyridin-2-yl)acetate (13).[31,32] A solution of ethyl 2-(3-chloropyridin-2-yl)acetate (**12**, 7.59 g, 38 mmol) in THF (76 mL) was treated at room temperature with 3 M aqueous NaOH (25.3 mL, 76 mmol). The mixture was stirred at room temperature overnight and concentrated to remove the THF. The aqueous residue was frozen on dry ice and lyophilized to give sodium 2-(3-chloropyridin-2-yl)acetate as an off white solid, which was used without further purification. Mass spectrum m/z 172 $(M+H)^+$.

N-(3-Bromo-2-methylphenyl)-2- (3 -chloropyridin-2-yl)acetamide (14).[31,32] A mixture of sodium 2-(3-chloropyridin-2-yl)acetate (**13**, 7.39 g, 38 mmol), 3-bromo-2-methylaniline (4.7 mL, 38.4 mmol), DIPEA (13.3 mL, 76 mmol), and HATU (14.6 g, 38.4 mmol) in DMF (127 mL) was stirred at room temperature. After 90 min, the mixture was diluted with EtOAc and washed with 10% LiCl (2x), followed by brine. The combined aqueous layers were extracted with EtOAc, and the combined organic phases were dried and concentrated to a smaller volume. The solution was

seeded with a crystal from an earlier batch and allowed to stand overnight, resulting in a precipitate which was collected by filtration and washed with 50% EtOAc-hexanes to provide a white solid. The filtrate was concentrated and recrystallized, in a similar manner, three times to give additional solid. The solids were combined to give N-(3-bromo-2-methylphenyl)-2-(3-chloropyridin-2-yl)acetamide as a white solid (11.4 g, 89% yield). Mass spectrum m/z 339, 341 $(M+H)^+$. ^{1}H-NMR (400 MHz, chloroform-d) δ 9.76 (br. s., 1H), 8.52 (d, *J*=3.5 Hz, 1H), 7.92 (d, *J*=7.9 Hz, 1H), 7.80 (dd, *J*=8.1, 1.1 Hz, 1H), 7.36 (d, *J*=7.9 Hz, 1H), 7.32-7.23 (m, 1H), 7.06 (t, *J*=8.0 Hz, 1H), 4.16 (s, 2H), and 2.39 (s, 3H).

2-(3-Chloropyridin-2-yl)-N-(2-methyl-3-(4,4,5,5-tetramethyl-l,3,2-dioxaborolan-2-yl)phenyl)acetamide (15).[31,32] A mixture of N-(3-bromo-2-methylphenyl)-2-(3-chloro- pyridin-2-yl)acetamide (**14**, 4.0 g, 11.8 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(l,3,2-dioxaborolane) (3.29 g, 13.0mmol) in DMSO (5 mL) and dioxane (25 mL) was bubbled with argon for 7 min, followed by the addition of potassium acetate (2.89 g, 29.4 mmol). Argon bubbling was continued for 7 min, after which $PdCl_2$(dppf)-DCM adduct (0.481 g, 0.589 mmol) was added. The mixture was heated at 90° C. for 7 h. The cooled mixture was diluted with EtOAc and filtered through CELITE®. The filtrate was washed sequentially with water and brine. The combined aqueous layers were extracted with EtOAc, and the combined organic phases were dried and concentrated. The residue was recrystallized from EtOAc to provide a white solid. The mother liquor was concentrated, and the residue was recrystallized from EtOAc. The two solids were combined to provide 2-(3-chloropyridin-2-yl)-N-(2-methyl-3-(4,4,5,5-tetramethyl-l,3,2-dioxaborolan-2-yl)phenyl)acetamide as a white solid (3.88 g, 85% yield). Mass spectrum m/z 387, 389. ^{1}H-NMR (400 MHz, CHLOROFORM-d) δ 9.44 (br s, 1H), 8.52 (dd, J=4.7, 1.2 Hz, 1H), 8.11 (d, J=7.9 Hz, 1H), 7.78 (dd, J=8.1, 1.1 Hz, 1H), 7.56 (d, J=7.0 Hz, 1H), 7.26 - 7.16 (m, 2H), 4.16 (s, 2H), 2.47 (s, 3H), and 1.35 (s, 12H).

S2. In vitro and in vivo Biological assay/study procedures.

Human recombinant BTK enzyme assay: To V-bottom 384-well plates were added test compounds, human recombinant BTK (1 nM, Invitrogen Corporation), fluoresceinated peptide (1.5 μM), ATP (20 μM), and assay buffer (20 mM HEPES pH 7.4, 10 mM $MgCl_2$, 0.015% Brij 35 surfactant and 4 mM DTT in 1.6% DMSO), with a final volume of 30 μL. After incubating at

room temperature for 60 min, the reaction was terminated by adding 45 µL of 35 mM EDTA to each sample. The reaction mixture was analyzed on the Caliper LabChip 3000 (Caliper, Hopkinton, MA) by electrophoretic separation of the fluorescent substrate and phosphorylated product. Inhibition data were calculated by comparison to control reactions with no enzyme (for 100% inhibition) and controls with no inhibitor (for 0% inhibition). Dose response curves were generated to determine the concentration required for inhibiting 50% of BTK activity (IC_{50}). Compounds were dissolved at 10 mM in DMSO and evaluated at eleven concentrations.

BCR-Stimulated calcium flux in Ramos B cells: Human Ramos (RA1) B cells (ATCC CRL-1596) at a density of 2 x 106 cells/mL in RPMI minus phenol red (Invitrogen 11835-030) and 50 mM HEPES (Invitrogen 15630-130) containing 0.1% BSA (Sigma A8577) were added to one half volume of calcium loading buffer (BD bulk kit for probenecid sensitive assays, # 640177) and incubated at room temperature in the dark for 1 hour. Dye-loaded cells were pelleted (Beckmann GS-CKR, 1200 rpm, room temperature, 5 min) and resuspended at room temperature in RPMI minus phenol red with 50 mM HEPES and 10% FBS to a density of 1 x 106 cells/mL. 150 µL aliquots (150,000 cells/well) were plated into 96 well poly-D-lysine coated assay plates (BD 35 4640) and briefly centrifuged (Beckmann GS-CKR 800 rpm, 5 min, without brake). Next, 50 µL compound dilutions in 0.4% DMSO/RPMI minus phenol red + 50 mM HEPES + 10% FBS were added to the wells and the plate was incubated at room temperature in the dark for 1 hour. The assay plate was briefly centrifuged as above prior to measuring calcium levels. Using the FLIPR1 (Molecular Devices), cells were stimulated by adding goat anti-human IgM (Invitrogen AHI0601) to 2.5 µg/mL. Changes in intracellular calcium concentrations were measured for 180 seconds and percent inhibition was determined relative to peak calcium levels seen in the presence of stimulation only.

Whole blood assays of BCR-stimulated CD69 expression on B cells: To measure BCR-stimulated B cells, heparanized human whole blood was added with various concentrations of test compound and stimulated with 30 µg/mL AffiniPure F(ab')2 fragment goat anti human IgM (Jackson 109-006-1299 – endotoxin cleared) and 10 ng/mL human IL-4 (Peprotech 200-04) for 18 h at 37ºC with agitation. The cells were stained with FITC-conjugated mouse anti-human CD20 (BD Pharmingen 555622) and PE-conjugated mouse anti-human CD69 monoclonal antibody (BD Pharmingen 555531), lysed and fixed, then washed. The amount of CD69 expression was quantitated by the mean fluorescence intensity (MFI) after gating on the CD20-positive B cell

population as measured by FACS analysis. B cells in mouse whole blood were stimulated in an similar way, using AffinPure F(ab')2 Fragment goat anti mouse IgG + IgM (Jackson Cat#115-006-068) at 100 µg/mL to stimulate, and staining with allophycocyanin (APC) rat anti-mouse CD19 antibody (BD Biosciences 550992) to identify the B cells and CD69 quantitation with FITC-conjugated anti-mouse CD69 monoclonal antibody (BD Biosciences 553236).

Collagen-induced arthritis in mice: DBA/1 male mice (8-10wk of age; Harlan) were immunized subcutaneously at the base of the tail on Day 0 and again on Day 21 with 200 µg bovine type II collagen admixed with reconstituted Sigma Adjuvant System (SAS; Sigma-Aldrich). For "preventative" dosing, mice were dosed daily (beginning on Day 0) by oral gavage with vehicle (EtOH:TPGS:PEG300; 5:5:90) or compound **6d**. For "pseudo-established" dosing, the start of dosing was delayed until the Day 21 booster immunization. Following the booster immunization, mice were monitored 3 times per week for the development and severity of paw inflammation. Each paw was visually scored by the following scheme: +0 = normal. +1 = one (or more) joints inflamed on digits. +2 = mild-moderate inflammation of plantar surface of paw and paw thickness modestly increased. +3 = moderate-severe inflammation of plantar surface of paw and paw thickness significantly increased. +4 = ankylosis of ankle joint (significantly reduced hock joint motion on flexion/extension). Unblinded clinical paw scores for all four paws were summed for each mouse, and mean ± SEM was calculated for each treatment group.

Collagen antibody-induced arthritis in mice: Female BALB/c mice (8-10 weeks of age; Harlan) were injected IP with a mixture of four monoclonal anti-mouse type II collagen antibodies (1mg of each). Daily oral dosing was immediately started with vehicle (EtOH:TPGS:PEG300; 5:5:90), compound **6d** (10 or 30 mg/kg) or dexamethasone (1mg/kg). Three days later, the mice were injected IP with 1.25 mg/kg LPS (E. coli O111:B4; Sigma). Thereafter, mice were monitored 3X/wk for the development and severity of paw inflammation. Each paw was visually scored by the following scheme: +0 = normal. +1 = one (or more) joints inflamed on digits. +2 = mild-moderate inflammation of plantar surface of paw and paw thickness modestly increased. +3 = moderate-severe inflammation of plantar surface of paw and paw thickness significantly increased. +4 = ankylosis of ankle joint (significantly reduced hock joint motion on flexion/extension). Clinical paw scores for all four paws were summed for each mouse, and mean ± SEM was calculated for each treatment group.