Supporting Information

Aminopyrazole Carboxamide Bruton's Tyrosine Kinase Inhibitors. Irreversible to Reversible Covalent Reactive Group Tuning

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1. Characterization of MS adduct observed between BTK and compound 9a

For LC-MS analysis of Btk inhibitor **9a** with Btk protein, 23 μM of purified Btk kinase domain protein (in TBS) was incubated with 35 μM of the compound **9a** for 20 min at 4 °C. At the end of the incubation, 10 μL of the reaction mixture was injected onto an Agilent 1100 HPLC system equipped with a reverse-phase C4 column. The ESI-MS data were acquired using a Waters-Micromass LCT mass spectrometer. The cone voltage was set to 35 V, and the HPLC eluate for the first 1 min, containing salts and buffer, was diverted to waste. The acquired protein data were smoothed, background subtracted, centroided, and transformed for molecular weight determination using the Micromass component analysis software. The observed components were confirmed using the Micromass maximum entropy algorithm.

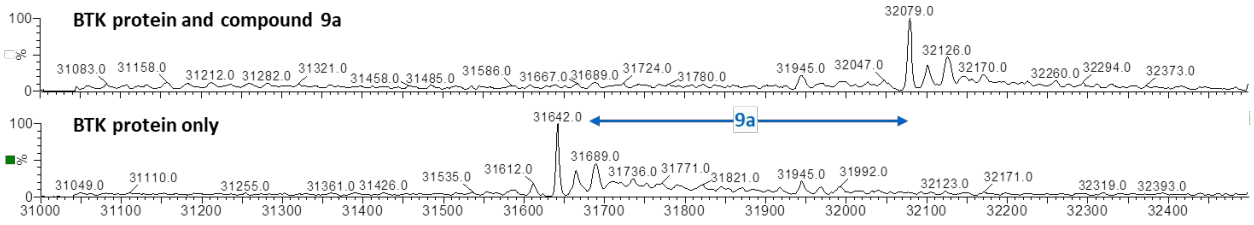


Figure S1. Mass spectroscopic analysis of recombinant human Btk kinase domain either in the present or absence of compound **9a**.

2. ^{13}C NMR analysis of adduct formation between ^{13}C-labeled 9a and BTK

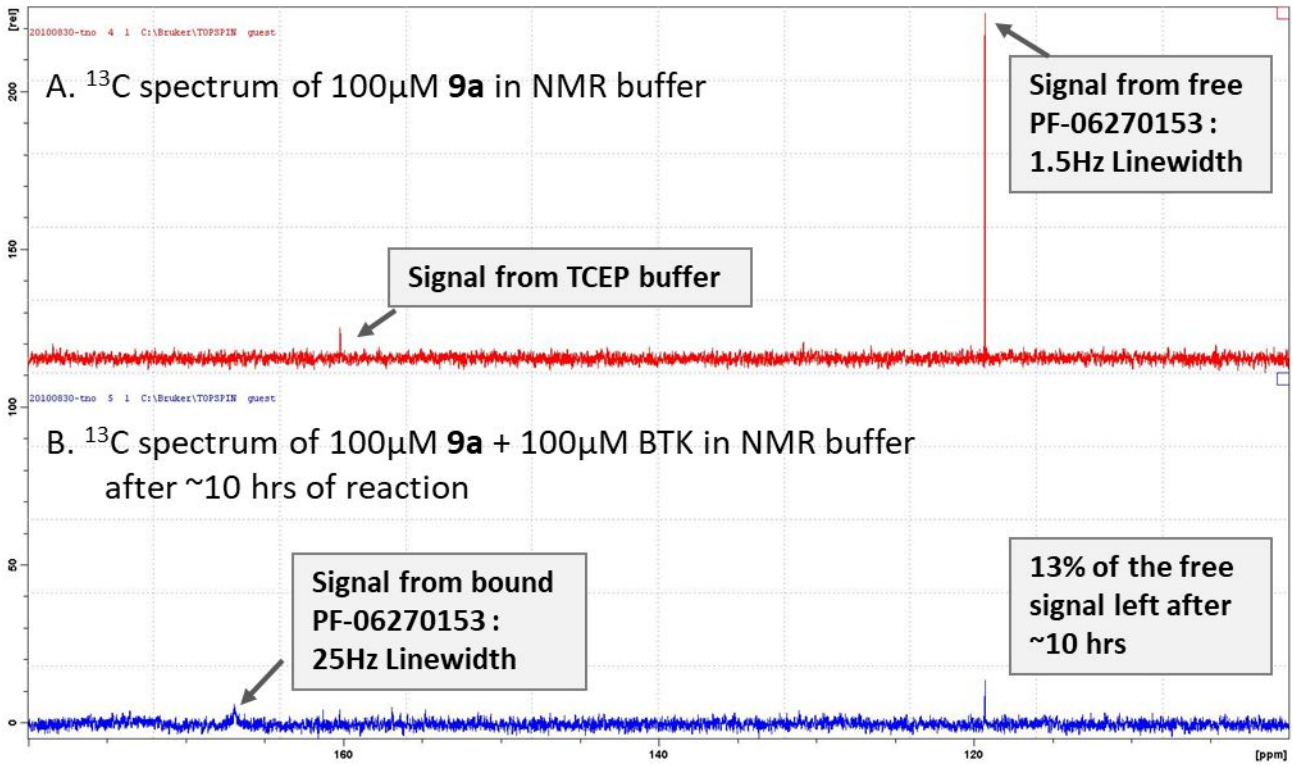


Figure S2. a) ^{13}C NMR spectra of compound **9a** in the absence of Btk protein. b) ^{13}C NMR spectra of compound **9a** in the presence of Btk protein (1:1 ratio) after 10 h incubation at 27 °C. All 1D ^{13}C NMR spectra were obtained on a Bruker Avance 500 MHz NMR spectrometer at 27 °C using a 30 degree flip angle. Broad band decoupling was preformed using a WALTZ-16 program, supplied by Bruker. NMR data was processed using Bruker Topspin software.

3. Pharmacokinetics of cyanamides in rat

Table S1. Pharmacokinetics of cyanamides in rat[a]

Cmpd	CL (mL/min/kg)	T½ (h)	V_{dss} (L/kg)	Cmax (nM)	AUC (nM·h)	F (%)
9a	10	2.5	2.1	267	2,965	76
10	17	2.8	4.2	169	1,904	86
20a	27	1.2	2.9	365	1,105	79
20b	25	0.7	1.5	295	1,296	93
20c	17	1.6	2.4	228	1,312	62

[a]Dosed IV at 1 mg/kg (n=2) and PO at 1 mg/kg (n=2).

4. Kinome profiling for 9a

Table S2. Broad kinome profiling for compound **9a** (1 mM ATP, 1 μM compound)[a]

Kinase	% Inh	Kinase	% Inh	Kinase	% Inh
ABL	-7.8	FLT1	6.6	MST2	14.1
AKT	-3.3	GSK3b	-6.8	MST4	16.7
AURA	4.0	HGFR	-1.7	NEK2	5.9
BMX	101.6	IGF1R	1.9	p38	9.1
BTK	98.5	IRAK4	-7.1	PAK4	9.2
CaMKIIa	14.5	IRK	2.9	PDK1	-1.5
CDK2	2.1	ITK	3.4	PIM2	6.0
CHK1	5.2	JAK2	-2.0	PKACa	-6.2
CHK2	2.9	JAK3	-10.0	PRKCB2	3.6
CKIa	1.9	KDR	5.2	ROCKI	9.9
CKIIa'	4.6	KIT	4.9	SGK	1.2
CSK	-13.0	LCK	16.7	SRC	11.0
EGFR	3.1	MAP4K4	0.7	TAO2	4.4
EphA2	0.6	MAPK3	-2.4	TEC	92.8
ERK2	6.0	MAPKAPK2	-2.1	TIE2	14.8
FGFR1	15.8	MARK1	4.4	TRKA	9.7
FGR	7.0	MYLK2	8.0	TXK	73.8

[a]Assays performed at Invitrogen using Z'-LYTE assay format.

5. Kinome profiling for 9a

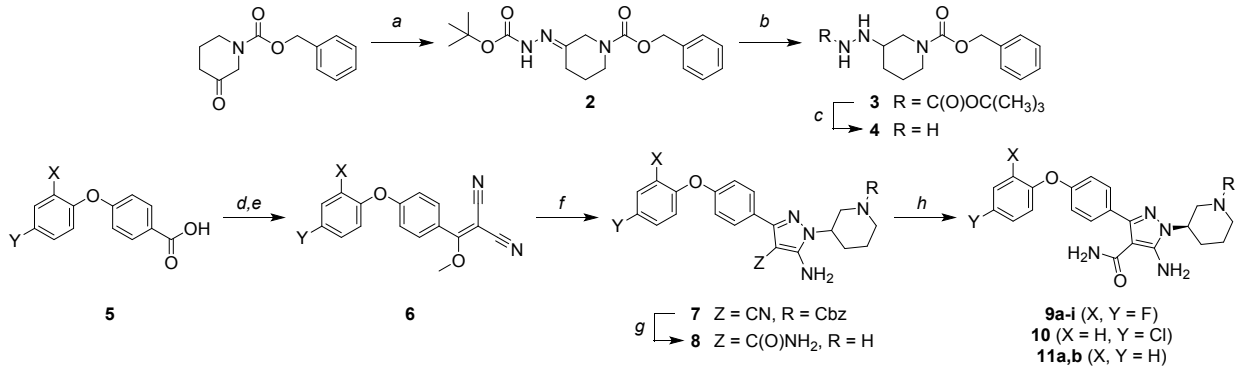
Table 4. Kinome selectivity of compounds 9a against SRC kinases and the BTK cysteine homolog family (1 mM ATP)[a]

Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)
BLK[b,d]	3,670	JAK3[b,d]	>10,000
BMX[b,c]	53	LCK	6,120
BTK[c]	10	SRC	>10,000
EGFR[b,d]	>10,000	TEC[b,c]	52
HER2[b,d]	>10,000	TXK[b,c]	291
HER4[b,e]	>10,000	MAP2K7[b,f]	>10,000
ITK[b,d]	>10,000		

[a]Assays performed through Carna Bioscience. [b]Cysteine kinase homolog to BTK. Cys+3 residue = [c]Asn, [d]Asp, [e]Glu, [f]Lys

6. Compound Synthesis

Scheme S1. Synthesis of biarylethers 9-11.[a]

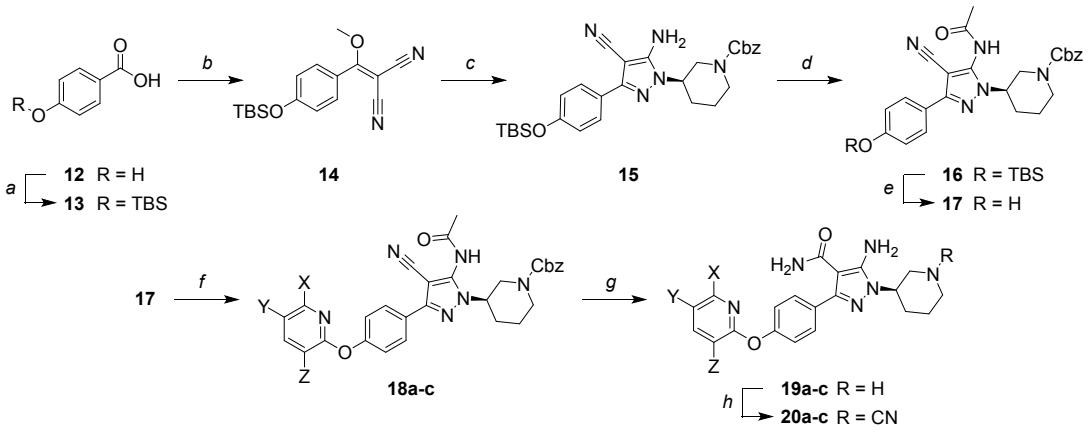


[a]Reagents and conditions: a) *tert*-butyl carbazate, CH_2Cl_2, 100%; b) $NaBH_3CN$, THF; pTSA, 73%; c) HCl, MeOH, 64%; d) $SOCl_2$, reflux; e) malononitrile, NaH, THF; $(CH_3O)_2SO_2$, reflux, 48-52% (2 steps); f) **4**, TEA, EtOH, 40-95%; g) NaOH, EtOH, 140 °C, 100%; h) K_2CO_3, BrCN, DMF; chiral separation; or chiral separation; RCO_2H/RCOCl, various conditions (See Supporting Information).

The desired aminopyrazole carboxamides were prepared through a convergent route employing condensation of hydrazine **4** (Scheme S1) and an appropriately substituted malononitrile (Schemes S1 and S2). Benzyl 3-oxo-piperidine-1-carboxylate was condensed with tert-butyl carbazate to afford the corresponding hydrazone **2**, Scheme S1. Subsequent hydride reduction and acidic deprotection afforded the desired hydrazine **4** as a racemic mixture. Optically pure **(*R*)-4** was available through chiral chromatographic resolution of **3** followed by deprotection. Substituted aryloxybenzoic acids (**5**) were converted to their corresponding acid chlorides and then condensed with sodium malononitrile, Scheme S1. The resulting enol was alkylated with dimethyl sulfate to afford compound **6**. 4-Cyano-5-aminopyrazole **7** was provided after reaction of **6** with hydrazine **4**. Hydrolysis of **7** under basic conditions provided carboxamide **8** with concomitant deprotection of the piperidine amine. The resulting amine was

then cyanated or acylated to afford the final analogs with the CRG of interest (**9a-i**, **10**, and **11a-b**). Pyridinyl ethers **20a-c** were prepared by ether formation with an advanced phenol intermediate, Scheme 2. 4-Cyano-5-aminopyrazole **15** was prepared in a similar manner to the aryl derivatives from 4-hydroxybenzoic acid. Transient acylation of the amino group was required to prevent cross-reactivity in the ether formation step. The common phenol coupling partner **17** was provided after silyl deprotection. Compound **17** was heated with substituted 2-halopyridines in the presence of cesium carbonate to provide ethers **18a-c**. The desired cyanamides **20a-c** were provided after acid hydrolysis and reaction with cyanogen bromide.

Scheme S2. Synthesis of pyridine aryl ethers 20a-c.[a]



[a]Reagents and conditions: a) TBDMSCl, imidazole, DMF, 0 °C, 47%; b) malononitrile, NaH, THF, 0 °C; NMM, isobutyl chloroformate; dimethyl sulfate, 61%; c) **(*R*)-4**, TEA, EtOH, reflux, 98%; d) TEA, acetyl chloride, CH_2Cl_2, 88%; e) LiOH, MeOH, water; 0 °C, 83%; f) 2-halopyridine, Cs_2CO_3, DMSO, 80 °C, 79-94%; g) 80% H_2SO_4, 40 °C, 68-94%; h) BrCN, Cs_2CO_3, DMF, 54-67%.

7. Experimental Section

7.1. Chemistry

General Chemistry. All reagents and solvents were used as purchased without further purification. The purity of the final compounds was characterized by high-performance liquid chromatography (HPLC) using a gradient elution program (e.g. C18, acetonitrile:water, 0.1% formic acid, 5:95–95:5) and UV-detection (220 nM). The purity of all final compounds was 95% or greater. Proton (^{1}H) NMR chemical shifts are referenced to a residual solvent peak.

Benzyl 3-(2-(*tert*-butoxycarbonyl)hydrazinylidene)piperidine-1-carboxylate (2). To a solution of 3-oxo-piperidine-1-carboxylic acid benzyl ester (150 g, 0.64 mol) in THF (1.5 L) was added *tert*-butyl carbazate (85 g, 0.64 mol). The solution was heated to reflux for 2 h, after which it was cooled to ambient temperature and concentrated in vacuo to afford 230 g (100%) of **2** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.41–7.28 (m, 5H), 5.16 (s, 0.6H), 5.14 (s, 1.4H), 4.25 (s, 1.4H), 4.14 (s, 0.6H), 3.78–3.73 (m, 0.6H), 3.61–3.53 (m, 1.4H), 2.54 (t, J = 6.4, 0.7H), 2.35 (t, J = 6.5 Hz, 1.3H), 1.91-1.82 (m, 2H), 1.58–1.40 (m, 9H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{18}H_{26}N_3O_4$ 348.2; Found 348.2.

Benzyl 3-(2-(*tert*-butoxycarbonyl)hydrazinyl)piperidine-1-carboxylate (3). To a solution of **2** (230 g, 0.66 mol) in THF (1.5 L) was added sodium cyanoborohydride (41.6 g, 0.66 mol). A solution of para-toluenesulfonic acid monohydrate (126 g, 0.66 mol) in THF (590 mL) was then added dropwise over 1.5 h, ensuring that the temperature did not exceed 21°C. The reaction was then allowed to stir for 16 h. The reaction mixture was concentrated. The resulting residue was dissolved in EtOAc (2.0 L) and washed with saturated aqueous sodium bicarbonate (1 L). The organic layer was then added to 1N sodium hydroxide (1.5 L) and allowed to stir for 1 h. The organic layer was separated, washed with brine, dried over sodium sulfate, and filtered. The

filtrate was concentrated and the crude residue was purified by silica gel column chromatography (CH_2Cl_2/methanol, 100/0–97/3) to afford 169 g (73%) of **3** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.29 (m, 5H), 6.33 (br. s., 0.6H), 5.88 (br. s., 0.4H), 5.20–5.00 (m, 2H), 3.64–2.90 (m, 6H), 1.74–1.80 (m, 2H), 1.50–1.35 (s, 11H).

Benzyl 3-hydrazinylpiperidine-1-carboxylate hydrochloride (4). To a solution of **3** (50 g, 0.143 mol) in methanol (180 mL) was added a solution of 4N hydrochloric acid in dioxane (180 mL) dropwise, ensuring that the temperature did not exceed 10 °C. The reaction mixture was allowed to stir at room temperature for 16 h. The resulting precipitate was filtered. The solids were stirred in EtOAc (700 mL) at room temperature for 16 h and then filtered. The solids were dried under vacuum to afford 26 g (64%) of **4** as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.41–7.28 (m, 5H), 5.08 (s, 2H), 4.08 (d, J = 9.5 Hz, 1H), 3.72 (d, J = 13.3 Hz, 1H), 3.38 (br. s., 4H), 2.95 (br. s., 3H), 2.02–1.90 (m, 1H), 1.75–1.65 (m, 1H), 1.45–1.29 (m, 2H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{13}H_{20}N_3O_2$ 250.2; Found 250.2.

2-((4-(2,4-Difluorophenoxy)phenyl)(methoxy)methylene)malononitrile (6). 4-(2,4-Difluorophenoxy)benzoic acid (**5**) (3.0 g, 30 mmol) in thionyl chloride (80 mL) was refluxed overnight. The volatiles were evaporated to afford 4-(2,4-difluorophenoxy)benzoyl chloride. A solution of malononitrile (1.0 g, 15.52 mmol) in THF (10 mL) was added dropwise to a stirred suspension of NaH (574 mg, 23.9 mmol) in THF (50 mL) at 0 °C under a nitrogen atmosphere. After stirring for 30 min, 4-(2,4-difluorophenoxy)benzoyl chloride (3.2 g, 11.94 mmol) in THF (15 mL) was added dropwise. The reaction mixture was brought to room temperature and stirred for 3 h. The reaction mixture was then heated to reflux and dimethyl sulfate (7.7 mL, 83.6 mmol) was added dropwise. The mixture was refluxed for 18 h. After cooling to room temperature, the mixture was quenched with ice water (100 mL) and extracted with EtOAc (2 ×

100 mL). The combined organic layers were dried over sodium sulfate, concentrated and filtered. The filtrate was concentrated and the residue was purified by silica gel chromatography (petroleum ether/EtOAc, 88/12) to afford 1.8 g (52%) of **6** as a liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 (d, J = 7.9 Hz, 2H), 7.60–7.52 (m, 1H), 7.52–7.43 (m, 1H), 7.25–7.18 (m, 1H), 7.16 (d, J = 7.9 Hz, 2H), 3.93 (s, 3H). MS (ES+) *m/z* $[M\text{-}CH_3]^+$ Calcd for $C_{16}H_7F_2N_2O_2$ 297.1; Found 297.1.

Benzyl 3-(5-amino-4-cyano-3-(4-(2,4-difluorophenoxy)phenyl)-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (7). Triethylamine (2.2 mL 14.4 mmol) was added to a stirred mixture of **6** (1.5 g, 4.8 mmol) and **4** (1.4 g, 4.8 mmol) in ethanol (30 mL) at room temperature. After stirring for 3 h the precipitate was filtered. The resulting solid was washed with ethanol and dried under vacuum to afford 1.8 g (40%) of compound **7**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.78 (d, J = 9.1 Hz, 2H), 7.55–7.45 (m, 1H), 7.40–7.28 (m, 6H), 7.20–7.10 (m, 1H), 7.05 (d, J = 9.1 Hz, 2H), 6.78 (s, 2H), 5.06 (br. s., 2H), 4.32–4.20 (m, 1H), 4.08–3.85 (m, 2H), 3.35–3.25 (m, 1H), 2.97 (t, J = 10.9 Hz, 1H), 2.00–1.90 (m, 3H), 1.60–1.42 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{29}H_{26}F_2N_5O_3$ 530.2; Found 530.2.

5-Amino-3-(4-(2,4-difluorophenoxy)phenyl)-1-(piperidin-3-yl)-1*H*-pyrazole-4-carboxamide (8). A cold 2.5M aqueous sodium hydroxide solution (20 mL) was added to a mixture of **7** (1.8 g, 3.39 mmol) in ethanol (20 mL) in a 100 mL sealed tube. The mixture was heated with stirring at 140 °C for 24 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with EtOAc. The combined organic layers were dried over sodium sulfate and filtered. The filtrate was concentrated to afford 1.4 g (100%) of **8**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55–7.47 (m, 1H), 7.46 (d, J = 8.2 Hz, 2H), 7.39–7.32 (m, 1H), 7.19–7.12 (m, 1H), 7.20–7.10 (m, 1H), 7.01 (d, J = 8.8 Hz, 2H), 6.30 (s, 2H), 4.10–4.00 (m, 1H), 2.98

(d, *J* = 9.6 Hz, 1H), 2.85 (d, *J* = 11.4 Hz, 1H), 2.77 (t, *J* = 11.3 Hz, 1H), 2.50–2.38 (m, 1H), 1.95–1.80 (m, 2H), 1.74–1.65 (m, 1H), 1.55–1.40 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{21}H_{22}F_2N_5O_2$ 414.2; Found 414.2.

(*R*)-5-Amino-1-(1-cyanopiperidin-3-yl)-3-(4-(2,4-difluorophenoxy)phenyl)-1*H*-pyrazole-4-carboxamide (9a). Potassium carbonate (450 mg, 3.3 mmol) was added to a solution of **8** (0.9 g, 2.2 mmol) in DMF (10 mL). After stirring for 5 min, cyanogen bromide (260 mg, 2.42 mmol) was added and the resulting mixture was stirred at 60 °C for 2 h. The reaction mixture was cooled to room temperature and water was added. The resulting precipitate was filtered. The crude product was purified by silica gel column chromatography (EtOAc/hexane, 1/1). The racemic product was resolved by preparative chiral HPLC (ChiralPak IA, 4.6 × 250 mm, 5 μm, hexane/ethanol, 30/70, 0.8 mL/min flow rate). Isolation of the second eluting isomer afforded 147 mg (15%) of compound **9a**. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.54–7.44 (m, 3H), 7.35 (td, *J* = 9.2, 5.6 Hz, 1H), 7.19-7.12 (m, 1H), 7.02 (d, *J* = 8.7 Hz, 2H), 6.43 (br. s., 2H), 4.41–4.31 (m, 1H), 3.49 (dd, *J* = 12.3, 4.4 Hz, 1H), 3.35–3.29 (m, 2H), 3.06 (td, *J* = 12.5, 2.8 Hz, 1H), 1.99–1.78 (m, 3H), 1.78–1.62 (m, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 166.05, 157.20, 156.06 (d, *J* = 242 Hz), 153.68 (d, *J* = 244 Hz), 150.29, 147.56, 138.83, 130.48 (2C), 128.65, 123.59, 117.58, 116.26 (2C), 112.20 (dd, *J* = 22.9, 3.9 Hz), 105.80 (dd, *J* = 27.5, 22.4 Hz), 94.61, 51.44, 50.16, 48.52, 28.47, 23.46. MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{22}H_{21}F_2N_6O_2$ 439.2; Found 439.2. HRMS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{22}H_{21}F_2N_6O_2$ 439.1694; Found 439.1685. $[\alpha]^{20}_D$ = -52.4° (c=0.5, MeOH). Absolute configuration was established by conversion or **(*R*)-8** into **9a** and **21** for which stereochemistry was established by single crystal X-ray diffraction.

Chiral resolution of 5-amino-3-(4-(2,4-difluorophenoxy)phenyl)-1-(piperidin-3-yl)-1*H*-pyrazole-4-carboxamide (8). Racemic compound **8** was resolved by steady state recycling

chromatography (Varian 2K) (Chiralpak OD, 20 μm, 15 cm × 20 cm, CH_3CN/MeOH/TEA, 60/40/0.1, 20 g/L, 1084 mL/min). Isolation of the first eluting isomer afforded enantiomer **(*R*)-8**. $[\alpha]^{20}_D$ = -3.9° (c=1.0, MeOH). Absolute configuration was established by conversion into **21** for which stereochemistry was established by single crystal X-ray diffraction.

(*R*)-1-(1-Acryloylpiperidin-3-yl)-5-amino-3-(4-(2,4-difluorophenoxy)phenyl)-1*H*-pyrazole-4-carboxamide (9b). Acrylyl chloride (16, mg, 0.17 mmol) and DMAP (50 mg, 0.41 mmol) was added to a solution of **(*R*)-8** (63 mg, 0.15 mmol) in a mixture of CH_2Cl_2:DMF (2 mL, 1:1). The reaction mixture was stirred at room temperature for 16 h and then purified by reverse phase preparative HPLC to afford 55 mg (77%) of **9b** as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.50 (d, *J* = 7.6 Hz, 2H), 7.24 (dt, *J* = 5.0, 8.6 Hz, 1H), 7.16 (ddd, *J* =10.2, 8.4, 2.6 Hz, 1H), 7.08–6.98 (m, 5H), 6.77 (ddd, *J* = 21.4, 16.8, 9.9 Hz, 1H), 6.20 (t, *J* = 16.5 Hz, 1H), 5.74 (dd, *J* = 20.1, 11.1 Hz, 1H), 4.65 (d, *J* = 10.0 Hz, 0.5H), 4.42 (d, *J* = 12.2 Hz, 0.5H), 4.26–4.08 (m, 2H), 3.62 (t, *J* = 9.3 Hz, 0.5H), 3.15 (t, *J* = 14.1 Hz, 1H), 2.88 (t, *J* = 15.7 Hz, 0.5H), 2.22–2.05 (m, 2H), 2.00–1.90 (m, 1H), 1.70–1.55 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{24}H_{24}F_2N_5O_3$ 468.2; Found 468.2.

(*R*)-5-Amino-3-(4-(2,4-difluorophenoxy)phenyl)-1-(1-methacryloylpiperidin-3-yl)-1*H*-pyrazole-4-carboxamide (9c). DIPEA (0.1 mL, 0.56 mmol) was added to a solution of **(*R*)-8** (69 mg, 0.17 mmol), methacrylic acid (15 mg, 0.17 mmol) and HBTU (67 mg, 0.17 mmol) in DMF (2 mL). The reaction mixture was stirred at room temperature for 16 h and then was purified by reverse phase HPLC to afford 56 mg (84%) of **9c** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54–7.46 (m, 3H), 7.35 (td, *J* = 9.1, 5.6 Hz, 1H), 7.16 (t, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 8.5 Hz, 2H), 6.40 (br.s.,2H), 5.17 (br. s., 1H), 5.01 (s, 1H), 4.45–4.15 (m, 2H), 3.97–

3.80 (m, 1H), 3.30 (m, 1H), 3.10 (m, 1H), 2.05–1.80 (m, 6H), 1.58–1.43 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{25}H_{26}F_2N_5O_3$ 482.2; Found 482.2.

(*R*)-5-Amino-1-(1-((E)-but-2-enoyl)piperidin-3-yl)-3-(4-(2,4-difluorophenoxy)phenyl)-1*H*-pyrazole-4-carboxamide (9d). BOP (118 mg, 0.27 mmol) and DIPEA (0.1 mL, 0.61 mmol), and (*E*)-but-2-enoic acid (22.9 mg, 0.27 mmol) were added to a solution of **(*R*)-8** (100 mg, 0.24 mmol) in DMF (0.5 mL) at 0 °C, and the mixture was stirred for 15 min. The reaction mixture was quenched with ice water (10 mL) and extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with water (2 × 10 mL) followed by brine (2 × 10 mL), dried over sodium sulfate and filtered. The filtrate was concentrated and the crude residue was purified by silica gel column chromatography (EtOAc/methanol, 99/1) followed by trituration with CH_2Cl_2/hexane, (1/5, 12 mL) to afford 55 mg (47%) of **9d** as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55–7.45 (m, 3H), 7.35 (dt, *J* = 5.6, 9.2 Hz, 1H), 7.17–7.13 (m, 1H), 7.02 (d, *J* = 8.6 Hz, 2H), 6.75–6.60 (m, 1H), 6.60–6.50 (m, 1H), 6.42–6.35 (m, 2H), 4.50–4.00 (m, 3H), 3.50–3.35 (m, 1H), 3.10–2.90 (m, 1H), 2.00–1.75 (m, 6H), 1.52–1.38 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{25}H_{26}F_2N_5O_3$ 482.2; Found 482.2.

(*R*)-5-Amino-3-(4-(2,4-difluorophenoxy)phenyl)-1-(1-(2-fluoroacryloyl)piperidin-3-yl)-1*H*-pyrazole-4-carboxamide (9e). BOP (235 mg, 0.53 mmol), DIPEA (0.22 mL, 1.21 mmol) and 2-fluoroacrylic acid (43.6 mg, 0.48 mmol) were added to a solution of **(*R*)-8** (200 mg, 0.48 mmol) in DMF (3 mL) at 0 °C, and the mixture was stirred for 30 min. The reaction mixture was quenched with ice water (10 mL) and extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over sodium sulfate and filtered. The filtrate was concentrated, and the crude product was purified by reverse-phase HPLC to afford 31 mg (13%) of **9e**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.58–7.52 (m, 3H), 7.40 (td, *J* = 9.2, 5.6 Hz, 1H), 7.20 (ddt, *J* = 11.8, 8.8, 1.5

Hz, 1H), 7.07 (d, *J* = 8.7 Hz, 2H), 6.45 (br.s., 2H), 5.36–5.28 (m, 1H), 5.20 (dd, *J* = 50.2, 4.1 Hz, 1H), 4.41–4.12 (m, 2H), 4.03–3.88 (m, 1H), 3.66–3.56 (m, 0.5H), 3.28–3.16 (m, 1H), 3.00–2.91 (m, 0.5H), 2.09–1.92 (m, 3H), 1.62–1.51 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{24}H_{23}F_3N_5O_3$ 486.2; Found 486.2.

(*R*)-5-Amino-3-(4-(2,4-difluorophenoxy)phenyl)-1-(1-((2E)-4-hydroxybut-2-enoyl)piperidin-3-yl)-1*H*-pyrazole-4-carboxamide (9f). DIEA (0.07 mL, 0.39 mmol) was added dropwise to a solution of **(*R*)-8** (54 mg, 0.13 mmol), HBTU (61 mg, 0.16 mmol), (*E*)-4-hydroxybut-2-enoic acid (54 mg, 0.52 mmol) in DMF (2 mL). The mixture was stirred 16 h at room temperature. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (2 × 10 mL). The combined organic extract was dried over sodium sulfate and filtered. The filtrate was concentrated and the crude product was purified by reverse-phase HPLC to afford 45 mg (69%) of **9f** as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.50 (d, *J* = 8.3 Hz, 2H), 7.25 (td, *J* = 9.1, 5.5 Hz, 1H), 7.16 (ddd, *J* = 10.8, 8.6, 3.0 Hz, 1H), 7.06–6.98 (m, 3H), 6.93–6.75 (m, 1 H), 6.72–6.59 (m, 1H), 4.68–4.60 (m, 0.5H), 4.47–4.39 (m, 0.5H), 4.30–4.10 (m, 4H), 3.67–3.59 (m, 0.5H), 3.22–3.10 (m, 1H), 2.92–2.83 (m, 0.5H), 2.21–2.06 (m, 2H), 1.97 (dt, *J* = 12.6, 3.1 Hz, 1H), 1.71–1.56 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{25}H_{26}F_2N_5O_4$ 498.2; Found 498.2.

(*R*)-5-Amino-3-(4-(2,4-difluorophenoxy)phenyl)-1-(1-((2E)-4-fluorobut-2-enoyl)piperidin-3-yl)-1*H*-pyrazole-4-carboxamide (9g). BOP (176 mg, 0.40 mmol), DIPEA (0.16 mL, 0.91 mmol) and (*E*)-4-fluorobut-2-enoic acid (41.5 mg, 0.40 mmol) were added to a solution of **(*R*)-8** (150 mg, 0.36 mmol) in DMF (0.5 mL) at 0 °C, and the mixture was stirred for 15 min. The reaction mixture was quenched with ice water (10 mL) and was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with water (2 × 10 mL) followed by brine (2 ×

10 mL), dried over sodium sulfate and filtered. The filtrate was concentrated and the crude residue was purified by silica gel column chromatography (EtOAc/methanol, 99/1) followed by trituration with CH_2Cl_2/hexane, (1/5, 12 mL) to afford 60 mg (33%) of **9g** as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55–7.45 (m, 3H), 7.35 (dt, J = 5.6, 6.0 Hz, 1H), 7.18–7.13 (m, 1H), 7.02 (d, J = 8.4 Hz, 2H), 6.80–6.70 (m, 2H), 6.43–6.35 (m, 2H), 5.15 (d, J = 18.8 Hz, 1H), 5.02 (d, J = 18.8 Hz, 1H), 4.60–4.00 (m, 3H), 3.48 (t, J = 12.4 Hz, 0.5H), 3.07 (q, J = 10.8 Hz, 1H), 2.76 (t, J = 13.6 Hz, 0.5H), 1.98 (br. s., 2H), 2.05–1.75 (m, 3H), 1.52–1.35 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{25}H_{25}F_3N_5O_3$ 500.2; Found 500.2.

(*R*)-5-Amino-1-(1-((2E)-4,4-difluorobut-2-enoyl)piperidin-3-yl)-3-(4-(2,4-difluorophenoxy)phenyl)-1*H*-pyrazole-4-carboxamide (9h). BOP (176 mg, 0.40 mmol), DIPEA (0.16 mL, 0.91 mmol) and (*E*)-4,4-difluorobut-2-enoic acid [McBee, E. T.; Keogh, M. J.; Levek, R. P.; Wesseler, E. P. J. Stereochemistry of the Diels-Alder Reaction. V. Fluorinated trans-olefinic acids and derivatives with cyclopentadiene. *Org. Chem.* **1973**, *38*, 632-636.] (48.7 mg, 0.40 mmol) were added to a solution of **(*R*)-8** (150 mg, 0.36 mmol) in DMF (0.5 mL) at 0 °C, and the mixture was stirred for 15 min. The reaction mixture was quenched with ice water (10 mL) and was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with water (2 × 10 mL) followed by brine (2 × 10 mL), dried over sodium sulfate and filtered. The filtrate was concentrated and the crude residue was purified by silica gel column chromatography (EtOAc/methanol, 99/1) followed by trituration with CH_2Cl_2/hexane (1/5, 12 mL) to afford 70 mg (37%) of **9h** as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55–7.45 (m, 3H), 7.35 (dt, J = 5.7, 5.8 Hz 1H), 7.23–7.11 (m, 2H), 7.02 (d, J = 8.6 Hz, 2H), 6.74–6.33 (m, 4H), 4.50–4.12 (m, 2H), 4.10–3.95 (m, 1H), 3.53 (t, J = 11.3 Hz, 0.5H), 3.12 (q, J =

11.6 Hz, 1H), 2.81 (t, *J* = 11.3 Hz, 0.5H), 2.05–1.80 (m, 3H), 1.58–1.40 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{25}H_{24}F_4N_5O_3$ 518.2; Found 518.2.

(*R*)-5-Amino-3-(4-(2,4-difluorophenoxy)phenyl)-1-(1-((2E)-4-(dimethylamino)but-2-enoyl)piperidin-3-yl)-1*H*-pyrazole-4-carboxamide (9i). DIEA (0.14 mL, 0.81 mmol) was added dropwise to a solution of **(*R*)-8** (56 mg, 0.14 mmol), HBTU (68 mg, 0.18 mmol), (*E*)-4-(dimethylamino)but-2-enoic acid (29 mg, 0.18 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 16 h and was then purified by reverse phase HPLC to afford 65 mg (92%) of **9i** as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.53–7.47 (m, 2H), 7.24 (td, *J* = 9.1, 5.4 Hz, 1 H), 7.16 (ddd, *J* = 11.0, 8.4, 3.0 Hz, 1H), 7.07–6.97 (m, 3H), 6.83–6.55 (m, 2H), 4.63 (d, *J* = 12.0 Hz, 1H), 4.34 (d, *J* = 13.0 Hz, 1H), 4.27–4.09 (m, 4H), 3.65 (dd, *J* = 13.1, 10.0 Hz, 1H), 3.22–3.06 (m, 3H), 3.01–2.90 (m, 1H), 2.27 (s, 3H), 2.24 (s, 3H), 2.18–1.93 (m, 3H), 1.70–1.56 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{27}H_{31}F_2N_6O_3$ 525.2; Found 525.2.

(*R*)-5-Amino-3-(4-(4-chlorophenoxy)phenyl)-1-(1-cyanopiperidin-3-yl)-1*H*-pyrazole-4-carboxamide (10). Prepared analogous to **9a** starting from 4-(4-chlorophenoxy)benzoic acid. The resulting racemic product was resolved by preparative chiral HPLC (ChiralPak IA, 4.6 × 250 mm, 5 μm, hexane/ethanol, 50/50, 0.8 mL/min flow rate). Isolation of the second eluting isomer afforded compound **10**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 9.0 Hz, 2H), 7.10 (d, *J* = 9.0 Hz, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 6.46 (br. s., 2H), 4.41–4.32 (m, 1H), 3.50 (dd, *J* = 12.2, 4.4 Hz, 1H), 3.37–3.30 (m, 2H), 3.07 (td, *J* = 12.4, 2.8 Hz, 1H), 2.00–1.78 (m, 3H), 1.76–1.63 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.06, 156.34, 155.40, 150.31, 147.58, 130.55 (2C), 129.89 (2C), 129.21, 127.38, 120.44 (2C), 118.60 (2C), 117.59, 94.62, 51.44, 50.17, 48.52, 28.48, 23.47. MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{22}H_{22}ClN_6O_2$ 437.2;

Found 437.2. HRMS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{22}H_{22}ClN_6O_2$ 437.1493; Found 437.1484. $[\alpha]^{20}_D$ = -56.8 (c=0.5, MeOH).

(*R*)-5-Amino-1-(1-cyanopiperidin-3-yl)-3-(4-phenoxyphenyl)-1*H*-pyrazole-4-carboxamide (11a). Prepared analogous to **9a** starting from 4-phenoxybenzoic acid. The resulting racemic product was resolved by preparative chiral supercritical fluid chromatography (OJ-H, 30 × 250 mm col, 50% methanol, 70 mL/min). Isolation of the first eluting isomer afforded compound **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (d, J = 8.8 Hz, 2H), 7.39 (t, J = 7.9 Hz, 2H), 7.14 (t, J = 7.3 Hz, 1H), 7.08 (d, J = 9.1 Hz, 2H), 7.06 (d, J = 8.9 Hz, 2H), 6.44 (br. s., 2H), 4.41–4.32 (m, 1H), 3.50 (dd, J = 12.3, 4.4 Hz, 1H), 3.38–3.30 (m, 2H), 3.07 (td, J = 12.3, 2.2 Hz, 1H), 2.00–1.78 (m, 3H), 1.76–1.63 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{22}H_{23}N_6O_2$ 403.2; Found 403.2. $[\alpha]^{20}_D$ = -64.5 (c=1.0, MeOH).

1-[(3*R*)-1-Acryloylpiperidin-3-yl]-5-amino-3-(4-phenoxyphenyl)-1*H*-pyrazole-4-carboxamide (11b). Prepared analogous to **9b** starting from 4-phenoxybenzoic acid. Racemic amine **8** (X = Y = H) was chirally separated by supercritical fluid chromatography (Chiralpak IC, 30 x 250 mm col, 50/50, CO_2/1% triethylamine in ethanol, 100 mL/min). Isolation of the second eluting isomer afforded **(*R*)-8** (X = Y = H). To a solution of the resulting amine (377 mg, 1.0 mmol) in DMF (4 mL) was added BOP (486 mg, 1.1 mmol) and DIPEA (323 mg, 2.5 mmol). The reaction mixture was cooled to 0 °C and a solution of acrylic acid (79.3 mg, 1.1 mmol) in DMF (1.0 mL) was added dropwise over 5 min. The mixture was gradually warmed to room temperature and stirred for 10 min, after which water was added. The reaction mixture was extracted into EtOAc. The combined organic layers were dried over sodium sulfate and filtered the filtrate was concentrated in vacuo and the residue was purified by silica gel column chromatography (EtOAc/MeOH, 90/10) to afford 150 mg (35%) of **11b** as a white solid. ^{1}H

NMR (400 MHz, DMSO-d_6) δ 7.55–7.47 (m, 4H), 7.27 (m, 1H), 7.2 - 7.0 (m, 4H), 6.91–6.77 (m, 1H), 6.41 (br. s., 2H), 6.19–6.04 (m, 1H), 5.77–5.61 (m, 1H), 4.53–4.03 (m, 3H), 3.53–3.43 (m, 1H), 3.13–2.97 (m, 1H), 2.85–2.65 (m, 1H), 2.08–1.92 (m, 1H), 1.90–1.78 (m, 1H), 1.55–1.45 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{24}H_{26}N_5O_3$ 432.2; Found 432.3. $[\alpha]^{20}_D$ = -97.2 (c=1.0, MeOH).

4-((tert-Butyldimethylsilyl)oxy)benzoic acid (13). To a stirred solution of 4-hydroxybenzoic acid (**12**) (200 g, 1.45 mol) in DMF (3.25 L), was added imidazole (595 g, 8.67 mol) followed by *tert*-butyl dimethylsilyl chloride (327 g, 2.17 mol) at 0 °C. The resulting mixture was stirred at room temperature for 16 h. The reaction mixture was poured onto crushed ice and extracted with EtOAc (2 × 2 L). The combined organic layers were washed with water (2 × 1 L) followed by brine (1 L), dried over sodium sulfate and filtered. The filtrate was concentrated, and the crude product was purified by silica gel column chromatography (hexane) to afford 170 g (47%) of **13** as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 0.98 (s, 9H), 0.23 (s, 6H).

2-((4-((*tert*-Butyldimethylsilyl)oxy)phenyl)(methoxy)methylene)malononitrile (14). A solution of malononitrile (31.4 g, 0.47 mol) in THF (600 mL) was added to a stirred suspension of sodium hydride (60%, 22.8 g, 0.95 mol) in THF (600 mL) at 0 °C. The resulting suspension was stirred at 0 °C for 1 h. To another 3 necked round bottom flask, *N*-methylmorpholine (52.9 mL, 0.47 mol) and a solution of isobutyl chloroformate (61.94 mL, 0.47 mol) in THF (600 mL) was added to a solution of **13** (120 g, 0.47 mol) dissolved in THF (1.2 L) at -30 °C. The resulting white suspension was stirred at -30 °C for 1 h. This acid chloride suspension was slowly added (through cannula) at 0 °C to the stirred suspension of NaH. The resulting suspension was stirred at room temperature for 3 h. Dimethyl sulfate (135.9 mL, 1.4 mol) was

added to the suspension at room temperature and the resulting reaction mixture was heated at reflux for 16 h. The reaction mixture was poured onto crushed ice and extracted with EtOAc (2 × 2 L). The combined organic layers were washed with water (2 × 1 L) followed by brine (1 L), dried over sodium sulfate and filtered. The filtrate was concentrated, and the crude product was purified by silica gel column chromatography to afford 76 g (61%) of compound **14** as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 11.4 Hz, 2H), 3.95 (s, 3H), 0.98 (s, 9H), 0.24 (s, 6H).

Chiral resolution of benzyl 3-hydrazinylpiperidine-1-carboxylate (4). Racemic compound **3** was resolved by chiral multi-column chromatography (Chiralpak AD, 20 µm, 5 cm × 10 cm, CH_3CN/EtOH, 90/10, 10 g/L, 100 mL/min). Isolation of the first eluting isomer afforded enantiomer **(*R*)-3**. $[\alpha]^{20}_D$ = -12.5 (c=1.0, MeOH). **(*R*)-3** was converted as above to afford **(*R*)-4**. $[\alpha]^{20}_D$ = +7.6 (c=1.0, MeOH). Absolute configuration was established by conversion of **(*R*)-4** into **9a**.

Benzyl (*R*)-3-(5-amino-3-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-4-cyano-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (15). To a stirred solution of **14** (9.0 g, 28.6 mmol) in ethanol (100 mL) was added **(*R*)-4** (8.15 g, 28.6 mmol) followed by addition of triethylamine (19.9 mL, 143 mmol) at room temperature. The resulting reaction mixture was heated to reflux for 16 h and then concentrated under reduced pressure. The residue was diluted with water (50 mL) and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with water (50 mL) followed by brine (50 mL), dried over sodium sulfate and filtered. The filtrate was concentrated and the crude product was purified by silica gel column chromatography (hexane/EtOAc, 5/1) to afford 15 g (98%) of compound **15** as off white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.5 Hz, 2H), 7.39–7.32 (m, 5H), 6.86 (d, *J* = 8.5 Hz, 2H), 5.18–5.13

(m, 2H), 4.16–4.52 (m, 3H), 3.82 (m, 1H), 3.17 (m, 1H), 2.86 (t, *J* = 12.0 Hz, 1H), 2.25 (m, 1H), 2.12–2.09 (m, 1H), 1.88 (m, 1H), 0.97 (s, 9H), 0.20 (s, 6H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{29}H_{38}N_5O_3Si$ 532.3; Found 532.5.

Benzyl (*R*)-3-(5-acetamido-3-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-4-cyano-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (16). To a stirred solution of **15** (14.5 g, 27.3 mmol) in CH_2Cl_2 (150 mL) was added triethylamine (19 mL, 136.3 mmol) followed by dropwise addition of acetyl chloride (19.4 mL, 273 mmol) at 0 °C. The resulting reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 16 h. The reaction mixture was diluted with cold water (50 mL). The resulting aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with water (2 × 20 mL) followed by brine (2 × 20 mL), dried over sodium sulfate and filtered. The filtrate was concentrated, and the crude product was purified by silica gel column chromatography (hexane/EtOAc, 7/3) to afford 13.85 g (88%) of **16**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (br. s., 1H), 7.79 (d, *J* = 8.5 Hz, 2H), 7.33 (m, 5H), 6.88 (d, *J* = 8.5 Hz, 2H), 5.11 (s, 2H), 4.30–4.01 (m, 4H), 3.30–3.15 (m, 1H), 2.90 (t, *J* = 12.0 Hz, 1H), 2.35–2.05 (m, 5H), 1.95–1.85 (m, 1H), 0.98 (s, 9H), 0.20 (s, 6H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{31}H_{40}N_5O_4Si$ 574.3; Found 574.4.

Benzyl (*R*)-3-(5-acetamido-4-cyano-3-(4-hydroxyphenyl)-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (17). To a stirred solution of **16** (12.7 g, 22.1 mmol) in methanol:water (4:1, 225 mL) was added $LiOH·H_2O$ (2.8 g, 66.4 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h and then was concentrated under reduced pressure. The residue was dissolved in water (20 mL) and neutralized with 1N hydrochloric acid solution to pH 6.5. The precipitated solid was filtered, washed with water (2 × 50 mL) and dried under vacuum to afford 8.5 g (83%) of **17** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.48 (s, 1H), 9.84 (s, 1H), 7.67 (d, *J* = 8.5 Hz, 2H),

7.34 (br. s., 5H), 6.88 (d, *J* = 8.5 Hz, 2H), 5.06 (br. s., 2H), 4.23 (br. s., 1H), 4.10–3.95 (m, 1H), 3.95–3.85 (m, 1H), 3.00 (t, *J* = 11.0 Hz, 1H), 2.12 (s, 3H), 2.10–1.95 (m, 2H), 1.95–1.82 (m, 1H), 1.60–1.48 (m, 1H). $[\alpha]^{20}_{D}$ = -65.5 (c=1.0, MeOH). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{25}H_{26}N_5O_4$ 460.2; Found 460.2.

Benzyl (*R*)-3-(5-acetamido-3-(4-((5-chloropyridin-2-yl)oxy)phenyl)-4-cyano-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (18a). 5-Chloro-2-fluoro-pyridine (0.72 mL, 7.2 mmol) was added to a mixture of **17** (3.0 g, 6.5 mmol) and Cs_2CO_3 (4.25 g, 13.1 mmol) in DMSO (15 mL) at room temperature. The reaction mixture was heated at 80 °C for 24 h. After cooling to room temperature, the mixture was diluted with water and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over sodium sulfate and filtered. The filtrate was concentrated to afford 3.5 g (94%) of **18a** as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 8.24 (d, *J* = 2.7 Hz, 1H), 8.00 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.40–7.25 (m, 7H), 7.17 (d, *J* = 8.7 Hz, 1H), 5.06 (s, 2H), 4.29 (br. s., 1H), 4.09–4.05 (m, 2H), 3.89 (br. s., 1H), 3.03 (t, *J* = 11.7 Hz, 1H), 2.14 (s, 3H), 2.10–2.00 (m, 2H), 1.88 (br. s., 1H), 1.70–1.45 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{30}H_{28}ClN_6O_4$ 571.2; Found 571.4.

Benzyl (*R*)-3-(5-acetamido-4-cyano-3-(4-((6-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (18b). Prepared analogous to compound **18a** employing **17** and 2-chloro-6-trifluoromethyl pyridine to afford a white solid (79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (br. s., 1H), 8.15 (t, J = 7.5 Hz, 1H), 7.92 (d, J = 8.3 Hz, 2H), 7.68 (d, J = 6.9 Hz, 1H), 7.42–7.25 (m, 8H), 5.08 (br. s., 2H), 4.35–4.25 (m, 1H), 4.15–4.05 (m, 1H), 3.95–3.85 (m, 1H), 3.50–3.35 (m, 1H), 3.05 (t, J = 11.6 Hz, 1H), 2.20–1.85 (m, 6H), 1.60–1.50 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{31}H_{28}F_3N_6O_4$ 605.2; Found 605.1.

Benzyl (*R*)-3-(5-acetamido-3-(4-((5-chloro-3-fluoropyridin-2-yl)oxy)phenyl)-4-cyano-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (18c). Prepared analogous to compound **18a** employing **17** and 5-chloro-2,3-difluoropyridine to afford a white solid (88%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.55 (br. s., 1H), 8.27 (dd, J = 10.0, 1.0 Hz, 1H), 8.10 (d, J = 1.0, 1H), 7.88 (d, J = 9.4 Hz, 2H), 7.40–7.25 (m, 7H), 5.08 (br. s., 2H), 4.35–4.22 (m, 1H), 4.15–4.05 (m, 1H), 3.95–3.85 (m, 1H), 3.45–3.30 (m, 1H), 3.10–2.98 (m, 1H), 2.20–1.85 (m, 6H), 0.65–0.45 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{30}H_{27}ClFN_6O_4$ 589.2; Found 589.3.

(*R*)-5-Amino-3-(4-((5-chloropyridin-2-yl)oxy)phenyl)-1-(piperidin-3-yl)-1H-pyrazole-4-carboxamide (19a). Compound **18a** (3.5 g, 6.12 mmol) was treated with 80% aqueous H_2SO_4 (20 mL) and the resulting mixture was heated at 40 °C for 2 h. After cooling to room temperature, the reaction mixture was poured into crushed ice and rendered basic with aqueous ammonia. The mixture was extracted with EtOAc (5 × 50 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over sodium sulfate and filtered. The filtrate was concentrated, and the residue triturated with diethyl ether to afford 2.5 g (94%) of **19a** as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, *J* = 2.8 Hz, 1H), 7.97 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.20 (d, *J* = 8.6 Hz, 2H), 7.15 (d, *J* = 8.8 Hz, 1H), 7.05 (br. s., 2H), 6.32 (s, 2H), 4.20–4.00 (m, 1H), 3.71 (br. s., 1H), 3.01 (dd, *J* = 11.8, 4.1 Hz, 1H), 2.86 (d, *J* = 12.5 Hz, 1H), 2.77 (dd, *J* = 11.8, 10.1 Hz, 1H), 2.41 (td, *J* = 12.1, 2.8 Hz, 1H), 1.95–1.85 (m, 2H), 1.75–1.65 (m, 1H), 1.55–1.45 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{20}H_{22}ClN_6O_2$ 413.2; Found 413.3.

(*R*)-5-Amino-1-(piperidin-3-yl)-3-(4-((6-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-1*H*-pyrazole-4-carboxamide (19b). Prepared analogous to compound **19a** employing **18b** to afford a yellow solid (82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (t, J = 7.5 Hz, 1H), 7.68 (d, J = 8.3

Hz, 1H), 7.57 (d, J = 10.0 Hz, 2H), 7.35 (d, J = 9.0 Hz, 1H), 7.27 (d, J = 8.3 Hz, 2H), 7.05 (br. s., 2H), 6.32 (s, 2H), 4.15–4.05 (m, 1H), 3.80 (br. s., 1H), 3.05–2.98 (m, 1H), 2.92–2.78 (m, 2H), 2.50–2.40 (m, 1H), 1.95–1.85 (m, 2H), 1.75–1.65 (m, 1H), 1.58–1.45 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{21}H_{22}F_3N_6O_2$ 447.2; Found 447.1.

(*R*)-5-Amino-3-(4-((5-chloro-3-fluoropyridin-2-yl)oxy)phenyl)-1-(piperidin-3-yl)-1*H*-pyrazole-4-carboxamide (19c). Prepared analogous to compound **19a** employing **18c** to afford a white solid (68%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.25 (d, J = 10.8 Hz, 1H), 8.08 (s, 1H), 7.55 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 6.32 (s, 2H), 4.17–4.05 (m, 1H), 3.05–2.95 (m, 1H), 2.92–2.75 (m, 2H), 2.50–2.40 (m, 1H), 1.95–1.85 (m, 2H), 1.75–1.65 (m, 1H), 1.60–1.40 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{20}H_{21}ClFN_6O_2$ 431.1; Found 431.0.

(*R*)-5-Amino-3-(4-((5-chloropyridin-2-yl)oxy)phenyl)-1-(1-cyanopiperidin-3-yl)-1*H*-pyrazole-4-carboxamide (20a). Cyanogen bromide (385 mg, 3.64 mmol) was added to a solution of **19a** (750 mg, 1.82 mmol) and Cs_2CO_3 (1.78 g, 5.46 mmol) in DMF (3.5 mL) and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water and extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine (2 × 5 mL), dried over sodium sulfate and filtered. The filtrate was concentrated and the crude product was purified by silica gel column chromatography (CH_2Cl_2/methanol, 98/2) to afforded 430 mg (54%) of **20a** as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, *J* = 2.5 Hz, 1H), 7.97 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.45 (br. s., 2H), 4.42–4.32 (m, 1H), 3.55–3.45 (m, 1H), 3.40–3.25 (m, 2H), 3.06 (t, *J* = 12.2 Hz, 1H), 2.00–1.80 (m, 3H), 1.80–1.60 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.07, 161.52, 153.66, 150.26, 147.60, 145.62, 139.98, 130.29, 130.15 (2C), 125.40, 120.99 (2C), 117.59, 113.16, 94.70, 51.45, 50.18, 48.53, 28.48, 23.46. MS (ES+) *m/z* $[M+H]^+$ Calcd for

$C_{21}H_{21}ClN_7O_2$ 438.1; Found 438.2. HRMS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{21}H_{21}ClN_7O_2$ 438.1445; Found 438.1438. $[\alpha]^{20}_D$ = -49.9 (c=1.0, MeOH).

(*R*)-5-Amino-1-(1-cyanopiperidin-3-yl)-3-(4-((6-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-1*H*-pyrazole-4-carboxamide (20b). Prepared analogous to compound **20a** employing **19b** to afford a yellow solid (57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (t, *J* = 7.9 Hz, 1H), 7.66 (d, *J* = 7.1 Hz, 1H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.37 (d, *J* = 8.3 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 2H), 6.46 (br. s., 2H), 4.43–4.34 (m, 1H), 3.51 (dd, *J* = 12.2, 4.5 Hz, 1H), 3.40–3.32 (m, 2H), 3.08 (td, *J* = 12.5, 2.9 Hz, 1H), 1.99–1.64 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.06, 162.80, 153.10, 150.29, 147.53, 144.24 (d, *J* = 34 Hz), 142.37, 130.61, 130.27 (2C), 121.05 (q, -CF3), 120.91 (2C), 119.68, 115.91, 115.69, 94.75, 51.45, 50.22, 48.52, 28.48, 23.49. MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{22}H_{21}F_3N_7O_2$ 472.2; Found 472.2. HRMS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{22}H_{21}F_3N_7O_2$ 472.1709; Found 472.1700. $[\alpha]^{20}_D$ = -64.0 (c=1.0, MeOH).

(*R*)-5-Amino-3-(4-((5-chloro-3-fluoropyridin-2-yl)oxy)phenyl)-1-(1-cyanopiperidin-3-yl)-1*H*-pyrazole-4-carboxamide (20c). Prepared analogous to **20a** employing **19c** to afford a yellow solid (67%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (dd, *J* = 9.8, 1.8 Hz, 1H), 8.08 (d, *J* = 1.8 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.27 (d, *J* = 8.4 Hz, 2H), 6.44 (s, 2H), 4.44–4.32 (m, 1H), 3.50 (dd, *J* = 11.8, 3.4 Hz, 1H), 3.39–3.34 (m, 2H), 3.07 (t, *J* = 11.5 Hz, 1H), 2.02–1.67 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.03, 152.99, 150.3, 147.51, 146.75 (d, *J* = 264 Hz), 140.48, 130.62, 130.11 (2C), 126.15, 125.96, 125.1, 120.84 (2C), 117.59, 94.77, 51.45, 50.9, 48.5, 28.47, 23.45. MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{21}H_{20}ClFN_7O_2$ 456.1; Found 456.1. HRMS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{21}H_{20}ClFN_7O_2$ 456.1351; Found 456.1342. $[\alpha]^{20}_D$ = -62.8 (c=1.0, MeOH).

(*R*)-5-Amino-1-(1-(4-bromobenzoyl)piperidin-3-yl)-3-(4-(2,4-difluorophenoxy)phenyl)-1*H*-pyrazole-4-carboxamide (21). 4-Bromobenzoyl chloride (53 mg, 0.24 mmol) and potassium carbonate (103 mg, 0.73 mmol) were added to a solution of **(*R*)-8** (100 mg, 0.24 mmol) in THF (5 mL). The solution was stirred at room temperature for 1 h and then at 50 °C for 15 min. The reaction mixture was added to water (5 mL) and extracted with EtOAc (2 × 15 mL). The combined organic layers were dried over sodium sulfate and filtered. The filtrate was concentrated and the crude product was purified by silica gel column chromatography (heptane/EtOAc, 100/0–0/80) to afford 130 mg (92%) of **21** as a white solid. Crystallization of the resulting solid from methanol and water afforded crystals suitable for X-ray diffraction. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70–7.57 (m, 2H), 7.55-7.42 (m, 3H), 7.40-7.31 (m, 3H), 7.19-7.12 (m, 1H), 7.02 (d, J = 8.2 Hz, 2H), 6.41 (br. s., 1H), 6.31 (br. s., 1H), 4.51-4.20 (m, 2H), 3.61-3.39 (m, 1.5H), 3.29-2.93 (m, 1.5H), 2.06-1.91 (m, 2H), 1.83-1.75 (m, 1H), 1.62-1.48 (m, 1H). MS (ES+) *m/z* $[M+H]^+$ Calcd for $C_{28}H_{25}BrF_2N_5O_3$ 596.1; Found 596.1.

7.2. Pharmacology

TR-FRET LanthaScreen assays. The BTK TR-FRET LanthaScreen assay was performed by pre-incubating compounds in serial dilution with 700 pM of human full length BTK kinase (Invitrogen, PV3587) (0.45 mg/mL) for 1 h before starting the reaction by adding 50 μM ATP and 100 nM FAM-Srctide peptide substrate (5FAM-GEEPLYWSFPAKKK-NH_2) (Molecular Devices, RP7095). Samples containing enzyme but no inhibitor were included to determine the maximal extent of reaction. Samples containing no enzyme served as the negative control. The reaction mixture was incubated at room temperature for 1 h before stopping the kinase activity with the addition of 15 mM EDTA. Peptide phosphorylation by BTK was detected using a

Terbium-conjugated anti-phospho-Tyrosine antibody (Tb-PT66 antibody, Invitrogen PV3557). Phosphorylation of peptide substrate was measured by determining the intensity ratio of 520/495 nm on an Envision Multi label Reader (Perkin Elmer) and IC_{50} values were calculated by fitting the data to a four-parameter equation. Compound **9a** demonstrated a standard error in the assay of 1.47±0.09 nM (n=168), geometric 95% confidence interval was 1.316–1.640 nM.

Full length human BTK protein in which residue 481 was mutated from Cys to Ser (BTK-C481S) was expressed in Sf9 insect cells containing a C-terminal his6 tag and purified by Pfizer. Compound inhibitory activity against phosphorylation by BTK-C481S was measured using conditions similar to those described above.

The EGFR TR-FRET LanthaScreen assay was performed as described above employing 20 μM ATP, 100 nM peptide substrate (FITC-C6-KKAEEEEYFELVAKK-NH_2 (American Peptide, 333778) and 600 pM of human EGFR kinase domain (Invitrogen, PV4190). Compound **1** demonstrated a standard error in the assay of 18.12±1.63 nM (n=125), geometric 95% confidence interval was 15.51–21.17 nM. The SRC TR-FRET LanthaScreen assay was performed as described above employing 20 μM ATP, 100 nM FAM-Srctide peptide substrate (5FAM-GEEPLYWSFPAKKK-NH_2) (Molecular Devices, RP7095), and 50 ng/mL of human full length SRC kinase (Invitrogen, P3044). Compound **1** demonstrated a standard error in the assay of 37.56±4.55 nM (n=110), geometric 95% confidence interval was 31.34–45.02 nM.

Selectivity screening against related Cys-containing kinases. The inhibitory activity of compounds were tested against a panel of kinases having a Cys residue at the homologous sequence location as BTK (Cys481) employing a mobility shift assay at Carna Biosciences. Compounds were incubated with enzyme and reaction buffer for 1 h or in the case of TXK and MAP2K7, 5 hours. Assays were run using an ATP concentration of 1 mM. Kinases screened

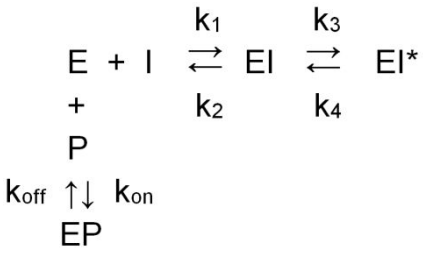
under these conditions included BLK, BMX, BTK, EGFR, HER2, HER4, ITK, JAK3, TEC, TXK, and MAP2K7.

BTK k_{inact}/K_i and $T_{½}$ measurements. A TR-FRET competition binding assay was used wherein Eu-anti-His Antibody (Invitrogen, PV5597) and fluorescent probe (KT-236, PV5592, or KT-178, PV5593, both from Invitrogen) formed a ternary signaling complex with free His-tagged BTK (Invitrogen, PV3587). The assay buffer contained 20 mM HEPES buffer, 10 mM $MgCl_2$, 0.01% w/v bovine serum albumin, 0.0005% v/v Tween 20, and 1 mM DTT, pH 7.4. The assay was performed at ambient temperature in 384-well low volume black round bottom polystyrene NBS™ microplates (product 3676, Corning, Inc.) and dynamically mixed following final reagent additions using 384-well pin tools (product VP248, V&P Scientific, Inc.). Inhibitor was prepared at 4x final concentration in 4% DMSO by first diluting in DMSO and then adding 24 volumes of assay buffer. Final concentrations in the assay were 5 nM BTK, 2 nM antibody, either 12 nM KT-236 or 50 nM KT-178, and 2% DMSO, 1% of which was contributed by the inhibitor dilution or DMSO control and the other 1% from KT-236 or KT-178. After final reagent addition and thorough mixing, the 665 nm to 615 nm TR-FRET emission ratio was monitored for approximately 3 h (99 readings, 108 s apart) using an Envision 2101 Multi-label reader (Perkin Elmer).

Two different versions of the assay were conducted. To measure k_{inact}/K_i, 1.333-fold final concentrations of enzyme, antibody and KT-236 tracer (the latter at a concentration about equal to its dissociation constant) were pre-incubated for 2 h. Upon addition of 15 µL of this mixture to wells containing 5 µL of 4x inhibitor dilution or 4% DMSO control an increasing association of inhibitor with free enzyme resulted in a loss of TR-FRET signal with time. To measure $T_{½}$, 1.333-fold final concentrations of enzyme, antibody and inhibitor were pre-incubated 2 h in a

volume of 15 µL. Then, 5 µL KT-178 tracer was added at a final concentration 100-fold greater than its dissociation constant. This effectively prevented rebinding of inhibitor and resulted in an increased association of tracer with free enzyme and resulting TR-FRET signal if the inhibitor association was reversible.

To obtain kinetic constants, TR-FRET data was fit to models of time-dependent irreversible and reversible inhibition using the program DynaFit.[1] The models were variations of the following general model:



where E was enzyme, I was inhibitor, EI was enzyme-inhibitor complex, EI* was an additional form of the complex, P was the fluorescent KT-236 or KT-178 tracer, and EP was the enzyme-probe complex that resulted in the TR-FRET signal. The kinetic constants, k_1, k_2, k_3, and k_4, were fixed at the following values for the different models: one-step irreversible model, $k_2 = k_3 = k_4 = 0$; two-step irreversible with a rapid equilibrium first step, $k_1 = 100\ \mu M^{-1}s^{-1}$, $k_4 = 0$; two-step irreversible, $k_4 = 0$; one-step reversible, $k_3 = k_4 = 0$; two-step reversible with a rapid equilibrium first step, $k_1 = 100\ \mu M^{-1}s^{-1}$; and two-step reversible, k_1, k_2, k_3, and k_4, were all allowed to vary. While the absolute values of k_{on} and k_{off} were allowed to vary in the analysis to obtain the best fit to the data, their ratio (k_{off}/k_{on}) was fixed at the appropriate dissociation constant. The dissociation constants (k_{off}/k_{on}) for KT-236 (13 nM) and KT-178 (0.5 nM) were determined in separate experiments where the data for KT-178 was treated as described by Kuzmiĉ et al.[2] to yield both dissociation constant and active enzyme concentration. In the analysis of TR-FRET data, the total enzyme concentration was fixed at this active value (1.1 nM final, or 22% of the

final nominal concentration of 5 nM). Two additional aspects of the data were taken into account in the models to improve data fitting. In the k_{inact}/K_i assay, the TR-FRET signal in the absence of inhibitor was observed to decrease with time. This loss of signal was modeled by including an exponential loss of EP complex with an appropriate 1st-order rate constant that was fit globally across all the data. In the $T_{½}$ assay, the background TR-FRET signal in the absence of enzyme was found to increase with time. This was modeled by including a no-enzyme control in the assay, fitting a zero-order rate constant to these data, and then applying this correction to the data from enzyme-containing wells. The Akaike information criterion (AIC)[3] was used to compare the likelihood of various irreversible and reversible models. The second-order inhibitor association rate constant (equivalent to k_{inact}/K_i for irreversible inhibitors) was calculated as k_1 for one-step models and as $k_1(k_3+k_4)/(k_2+k_3+k_4)$ for two-step models, while inhibitor dissociation rate was calculated as k_2 for one-step models and as $k_2k_4/(k_2+k_3+k_4)$ for two-step models.[4] $T_{½}$ was calculated as ln(2) divided by the inhibitor dissociation rate. Compound **9a** demonstrated a standard error in the k_{inact}/K_i variable of 123900±3400 1/(M·s) (n=19).

EGFR k_{inact}/K_i measurements. A Caliper LC3000 instrument (Perkin Elmer) with a LabChip EZ-Reader 12-sipper chip (Perkin Elmer, 760404) was used to separate and quantify peptide substrate (FITC-C6-Lys-Lys-Ala-Glu-Glu-Glu-Glu-Tyr-Phe-Glu-Leu-Val-Ala-Lys-Lys-NH_2, American Peptide, 333778) and phospho-peptide reaction product. Assay buffer and preparation of inhibitor dilutions was as described above for the BTK k_{inact}/K_i assay. Final assay concentrations were 5 nM EGFR (Invitrogen, PV4190), 40 μM ATP (Sigma), 1 μM peptide substrate, and 2% DMSO, of which 1% came from the inhibitor dilution and 1% from the peptide. The assay was performed at ambient temperature in 384-well round bottom polypropylene microplates (Greiner, 781280). Reactions were initiated by adding 40 μL of 2x

final enzyme to 40 µL 2x final inhibitor, peptide and ATP, and then sampled by the Caliper instrument for 210 min to quantify product as a function of time for each inhibitor concentration. These progress curves were fit to models of time-dependent inhibition using program DynaFit and k_{inact}/K_i determined as described above, except that the following general model was used:

$$\begin{array}{lcccl} & & k_1 & k_3 & \\ E + I & \rightleftarrows & EI & \rightleftarrows & EI^* \\ + & & k_2 & k_4 & \\ S & & & & \\ \downarrow k_{cat}/K_m & & & & \\ E + P & & & & \end{array}$$

where E, I, EI and EI*, were as defined previously, S was peptide substrate, P was phospho-peptide product, and k_{cat}/K_m was the apparent second-order rate constant for production of product. The mechanism assumed negligible consumption of ATP during the course of the reaction and a peptide K_m much greater than the assay concentration of 1 µM. These assumptions were justified because of the much lower concentration of peptide relative to ATP in the assay and preliminary experiments that showed a linear relationship between reaction rate and peptide concentration up to 10 µM. Compound **1** demonstrated a standard error in the k_{inact}/K_i variable of 45,040±5870 1/(M·s) (n=3).

Human B and T cell proliferation assay. B cells were isolated from buffy coats using Rosette Sep Human B cell or CD4 T cell Enrichment Cocktail according to the manufacturer′s instructions (Stem Cell Technologies). A total of 2×10^5 B or $CD4^+$ T cells were preincubated with the BTK inhibitor or vehicle for 30 min at 37 °C. B cells were stimulated with 30 µg/mL goat anti-hIgM $F(ab')_2$ (Southern Biotech, Birmingham, AL). $CD4^+$ T cells were stimulated with 1×10^5 anti-CD3/CD28 beads. Three days later, cells were pulsed with 1 µCi [^{3}H]thymidine, harvested 18 h later, and counted. IC_{50} values were determined by the non-linear regression analysis of dose response curves. For the B cell proliferation assay, compound **9a** demonstrated

a standard error in the assay of 2.72±0.73 nM (n=24), geometric 95% confidence interval was 1.95–3.78 nM. For the T cell proliferation assay, compound **9a** demonstrated a standard error in the assay of 2.72±0.73 nM (n=24), geometric 95% confidence interval was 1.95–3.78 nM. A positive control was not routinely included in the T cell proliferation assay.

Human Whole Blood Histamine Assay. Heparinized human whole blood (200 μL) was plated in 96-well V-bottom assay plates (VWR). Compounds diluted in 100% DMSO (1 μL) were added, and the plates were incubated at 37 ºC for 2 h. Anti-human-IgE antibody (KPL) was added to a final concentration of 2 μg/mL, and the assay plates were incubated for 30 minutes at 37 ºC. The plates were spun at 2000 rpm for 8 min and then analyzed for histamine release by ELISA kits (Beckman Coulter). For each inhibitor tested, inhibition of histamine release is normalized as a percentage of control histamine based on the formula: % of Control = 100 x (A-B)/(C-B) where A is the histamine from wells containing inhibitor and anti-IgE antibody, B is the histamine from wells without anti-IgE antibody(minimum histamine) and C is the histamine from wells containing anti-IgE antibody but no inhibitor (maximum). IC_{50} values were determined by the non-linear regression analysis of dose response curves. Compound **9a** demonstrated a standard error in the assay of 64.3±11.5 nM (n=14), geometric 95% confidence interval was 44.3–93.3 nM.

Human whole-blood anti-IgD–mediated CD69 upregulation assay. Human whole blood (500 μL) and 1 μL of compound stock solution or vehicle were added to a deep well plate and incubated at 37 °C for 1 h. A aliquot of each sample (25 μL) was then stimulated with 20 μg/mL goat anti-hIgD $F(ab')_2$ (Southern Biotech, Birmingham, AL) and incubated at 37 °C for 3 h. Cells were then washed and stained with anti-CD19 APC and anti-CD69 PE Abs (BD Biosciences). RBCs were eliminated after staining by resuspending the cells in Fix/Lyse solution (BD

Biosciences). Samples were analyzed on a flow cytometer. IC_{50} values were determined by the non-linear regression analysis of dose response curves. Compound **9a** demonstrated a standard error in the assay of 52.9±8.6 nM (n=4), geometric 95% confidence interval was 32.9–85.3 nM.

In vivo rat pharmacokinetics. All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by the Pfizer Institutional Animal Care and Use Committee. All compounds were dosed to male Wistar Han rats as an IV bolus in 10% EtOH, 40% PEG400 and 50% PBS; and orally by gavage as a suspension of crystalline solid in 0.5% methyl cellulose/0.1% Tween 80. Blood was collected over a 24 h time course and following centrifugation the resulting plasma samples were precipitated with acetonitrile and analyzed for test compound concentration using an LC-MS/MS procedure. PK parameters were calculated from plasma concentration-time curves using non-compartmental analysis in Watson LIMS.

8. Single crystal X-ray structure of (*R*)-5-amino-1-(1-(4-bromobenzoyl)piperidin-3-yl)-3-(4-(2,4-difluorophenoxy)phenyl)-1*H*-pyrazole-4-carboxamide (21)

Data collection was performed on a Bruker APEX-II, diffractometer at –100 °C. Data collection consisted of 3 omega scans. The structure was solved by direct methods using SHELX software suite in the space group P2(1). The structure was subsequently refined by the full-matrix least squares method. All non-hydrogen atoms were found and refined using anisotropic displacement parameters. Because of the high residuals of the disordered solvent, assigning the hydrogen atoms on N3 and N4 was challenging. Eventually, these protons were placed in idealized positions (calculated). All remaining hydrogen atoms were placed in calculated positions and were allowed to ride on their carrier atoms. The final refinement included isotropic displacement parameters for all hydrogen atoms.

Absolute configuration based on the flack parameter = 0.03 ESD 0.016 for the configuration in Figure S3. Additionally, the Hooft parameter = 0.05 with an ESD of 0.004.

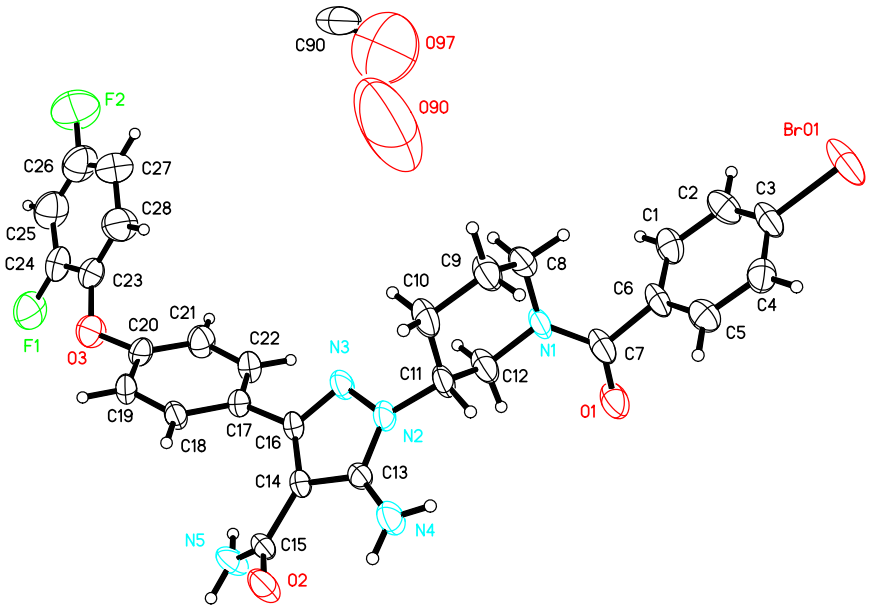


Figure S3. ORTEP with ellipsoids drawn at 50% confidence level of compound **21**.

Table S3. Crystal data and structure refinement for compound **21**.

Empirical formula	C28.50 H27 Br F2 N5 O4	
Formula weight	621.46	
Temperature	200(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 16.567(5) Å	α= 90°.
	b = 5.3608(16) Å	β= 100.604(8)°.
	c = 16.779(4) Å	γ = 90°.
Volume	1464.8(7) $Å^3$	
Z	2	
Density (calculated)	1.409 Mg/m^3	
Absorption coefficient	1.458 mm^{-1}	
F(000)	636	
Crystal size	0.59 × 0.19 × 0.18 mm^3	
Theta range for data collection	1.23 to 24.85°.	
Index ranges	-19<=h<=19, -6<=k<=6, -19<=l<=19	
Reflections collected	13118	
Independent reflections	4973 [R(int) = 0.0749]	
Completeness to theta = 24.85°	98.6 %	
Absorption correction	Empirical	
Max. and min. transmission	0.7793 and 0.4801	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4973 / 1 / 380	
Goodness-of-fit on F^2	1.027	
Final R indices [I>2sigma(I)]	R1 = 0.0693, wR2 = 0.1746	
R indices (all data)	R1 = 0.0969, wR2 = 0.2076	
Absolute structure parameter	0.033(16)	
Extinction coefficient	0.053(5)	
Largest diff. peak and hole	0.869 and -0.490 $e·Å^{-3}$	

9. Co-cystallization of 9a with mouse BTK kinase domain

His tagged murine BTK kinase domain (residues 348-659) was expressed in insect cells and purified by Nickel IMAC, anion exchange, and gel filtration chromatography. The His tag was removed during purification by TEV protease. Purified protein at 10 mg/ml in 20 mM Tris pH 8.0, 100 mM NaCl, 5 mM TCEP was incubated overnight with a 10 fold molar excess of compound **9a**. Crystals were grown by sitting drop vapor diffusion at 4 °C with 12% PEG 3350, 0.1 M Bis-Tris pH 6, 0.1 M sodium malonate. Cryo-protection was performed by a quick dip in reservoir solution plus 10% glycerol prior to freezing crystals in liquid nitrogen. X-ray diffraction data were collected at the Advanced Photon Source on the IMCA-CAT 17-ID beam line. The data sets were processed using autoPROC.

Table S4. X-ray crystallography data and refinement statistics.

Compound	**9a**
A. Data collection	
Space Group	P1
Unit Cell	a=37.9
	b= 63.5
	c=70.0
Resolution (Å)	60-2.80
(high res)	(2.86-2.80)
Completeness (%)	96.8 (96.1)
R_{sym}[a]	0.147 (0.469)
Redundancy	1.8 (1.8)
$\langle I/\sigma(I)\rangle$	6.3 (2.4)
B. Refinement	
R_{work}[b]	0.196
R_{free}[b]	0.257
Protein atoms (#)	4167
Waters (#)	185
RMSD bond length (Å)	0.01
RMSD angles (degrees)	1.10
Average B (Å^2)	31.0
[a] $R_{sym} = \Sigma_{hkl}(\lvert I_{hkl} - \langle I_{hkl}\rangle\rvert)/\Sigma_{hkl}\langle I_{hkl}\rangle$, where I_{hkl} is the intensity of reflection hkl, and $\langle I_{hkl}\rangle$ is the average intensity of multiple observations.	
[b] $R_{work} = \Sigma\lvert F_o - F_c\rvert/\Sigma F_o$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively. R_{free} is the R-factor for a randomly selected 5% of reflections which were not used in the refinement.	

10. References

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