Supporting Information

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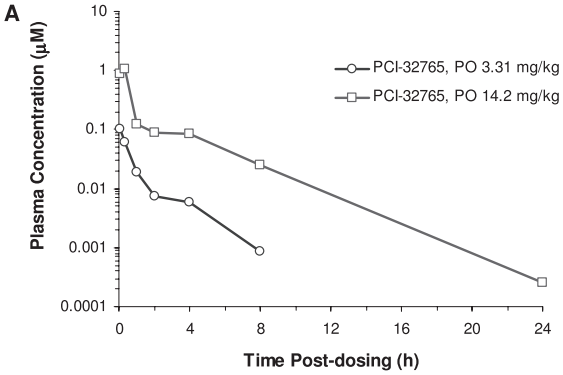
SI Materials and Methods

Kinase Assays. In vitro kinase IC_{50}s were measured using ^{33}P filtration binding assay after 1 h incubation of kinase, ^{33}P-ATP, inhibitor, and substrate [0.2 mg/mL poly(EY)(4:1]. Assays were performed at Reaction Biology (Malvern, PA).

PK. Female CD-1 mice were administered a single 3.31- or 14.2-mg/kg dose of formulated PCI-32765 by oral gavage. Dose volumes were adjusted based on body weight data collected immediately before dosing. Blood samples were collected at 0.0833 (5 min), 0.333 (20 min), 1, 2, 4, 8, and 24 h after dosing and processed to plasma. Plasma samples prepared as 75-μL aliquots were mixed with 10 μL of an internal standard solution (1 μg/mL). The internal standard was PCI-31431, a Btk inhibitor with structural similarity to PCI-32765. Soluble proteins were precipitated by the addition of 200 μL of acetonitrile, followed by centrifugation (20 min at 16,000 × *g*). The samples were evaporated to dryness and reconstituted in 200 μL of water containing 0.2% formic acid and 10% methanol. All samples were loaded onto an autosampler maintained at approximately 6 °C and evaluated for concentrations of test compound by LC-MS/MS as follows. Reverse-phase HPLC was performed under gradient conditions using water containing 0.2% formic acid and acetonitrile as mobile phases at a flow rate of 0.7 mL/min. A Phenomenex C-18 guard column and a Varian Metasil 3-μm HPLC column were used. Both PCI-32765 and the internal standard had a retention time of 6.80 min. Analytes were ionized for MS using an electrospray ion source (API-3200 mass spectrometer) and quantified by multiple-reaction monitoring using an *m*/*z* transition of 441.0 → 138.1. The quantifiable range of PCI-32765 concentrations in mouse plasma was 1 to 1,000 ng/mL. Pharmacokinetic parameters were calculated from plasma concentrations of PCI-32765 using WinNonlin 5.01 (Pharsight). The analyses were performed using nominal sample times and a noncompartmental method with uniform weighting. The $AUC_{0\text{-}\infty}$ for mice administered the lower dose of PCI-32765 was extrapolated from $AUC_{0\text{–}8h}$.



B

	Dosage	
	3.31 mg/kg	14.2 mg/kg
$C_{max,obs}$ (μM)	0.101	1.07
T_{max} (h)	0.83	0.33
CL/F (L/h/kg)	79.8	24.8
$AUC_{0\text{-}\infty}$ (μM•h)	0.09	1.29
Terminal $t_{1/2}$ (h)	1.71	3.10

Fig. S1. Pharmacokinetics of PCI-32765 in mice. (*A*) Mean plasma concentrations following a single oral dose of PCI-32765 in female CD-1 mice, *n* = 3–5 per time point. (*B*) Summary of pharmacokinetic parameters calculated from data shown in *A*.

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Table S1. IC_{50} values and fold selectivity for inhibition of enzymatic activity by PCI-32765

Kinase	IC_{50}, nM	Btk selectivity, fold
BTK	0.5	—
BLK*	0.5	1
BMX*	0.8	1.6
CSK	2.3	4.6
FGR	2.3	4.6
BRK	3.3	6.6
HCK	3.7	7.4
EGFR*	5.6	11.2
YES	6.5	13
ErbB2*	9.4	18.8
ITK*	10.7	21.4
JAK3*	16.1	32.2
FRK	29.2	58.4
LCK	33.2	66.4
RET	36.5	73
FLT3	73	146
TEC*	78	156
ABL	86	172
FYN	96	192
RIPK2	152	304
c-SRC	171	342
LYN	200	400
PDGFRα	718	1436
FMS	5545	>10,000
FER	8070	>10,000
JAK1	>10,000	>10,000
JAK2	>10,000	>10,000
NEK2	>10,000	>10,000
p38	>10,000	>10,000
PI3K	>10,000	>10,000
PLK1	>10,000	>10,000
RSK1	>10,000	>10,000
SYK	>10,000	>10,000

*Kinases that contain a cysteine residue aligning with Cys-481 in Btk.