Supporting Information

Discovery of (*R*)-8-(6-Methyl-4-oxo-1,4,5,6-tetrahydropyrrolo[3,4-*b*]pyrrol-2-yl)-3-(1-methylcyclopropyl)-2-((1-methylcyclopropyl)amino)quinazolin-4(3*H*)-one, a Potent and Selective Pim- 1/2 Kinase Inhibitor for Hematological Malignancies

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Table SI-1. KINOMEscan data of 28

The compound **28** were screened at 1000 nM concentration, and results for primary screen binding interactions are reported as '% Ctrl', where lower numbers indicate stronger hits in the matrix.

Compound	**28**	**28**
Replicate	1	2
# of Kinases with a POC <10	2	1
ABL1-nonphosphorylated	100	100
ABL1-phosphorylated	100	100
AKT1	85	100
ALK	90	96
AMPK-alpha1	100	98
AURKA	92	74
BMPR1A	100	85
BRAF	78	72
BRK	100	100
BTK	100	100
CAMK2D	100	100
CAMK4	80	74
CDK2	100	93
CDK4-cyclinD1	74	100
CDK8	100	66
CHEK1	100	100
CLK4	38	30
CSNK1D	76	62
CSNK1G2	46	54
CSNK2A1	100	73
DAPK1	100	100
DDR1	100	100
DMPK	100	100
DYRK1A	49	53
EGFR	100	100
EPHA2	100	100
EPHB3	100	100
ERK2	100	89
ERK4	71	80
ERN1	100	100
FGFR1	92	100
FLT3	92	100
FYN	100	100
GAK	85	92
GRK1	79	83

GSK3B	89	90
HIPK1	68	82
HPK1	88	100
IGF1R	95	100
INSR	100	86
IRAK4	91	85
JAK2(JH1domain-catalytic)	83	72
JNK3	9.9	11
KIT	93	99
LCK	81	100
LIMK1	83	100
LYN	100	100
MAP3K1	100	97
MAP4K4	100	100
MAPKAPK2	87	100
MARK1	100	100
MEK3	33	28
MEK5	87	83
MET	100	100
MKNK1	82	75
MLK1	78	96
MST2	100	92
MTOR	86	98
NEK4	73	90
NEK6	78	100
OSR1	100	92
p38-alpha	100	100
p38-gamma	60	60
PAK2	77	70
PCTK3	90	50
PDGFRB	90	95
PIK3CA	100	100
PIK3CD	100	100
PIM1	1.4	4.1
PKAC-alpha	100	100
PLK1	85	74
PLK4	100	100
PRKCE	83	88
PRKD2	83	84
PRKG1	86	100
PRKR	86	82
RIPK2	100	100

ROCK2	81	86
RPS6KA5(Kin.Dom.1-N-terminal)	100	100
RSK1(Kin.Dom.1-N-terminal)	96	82
S6K1	100	100
SRC	90	100
STK33	76	78
SYK	100	88
TAK1	59	61
TAOK2	100	100
TBK1	100	100
TGFBR1	88	100
TGFBR2	100	99
TIE2	100	100
TNIK	66	90
TRKA	100	100
TSSK1B	78	68
TTK	80	84
TYK2(JH1domain-catalytic)	92	92
VEGFR2	85	91
WEE1	100	98
YANK2	100	100
YSK4	41	59
ZAK	100	84

PDB: 6MT0 Crystal structure of 28 in complex with human Pim-1 kinase

Crystal	Pim-1 + compound **28 (6MT0)**
Data Collection	
Wavelength	1.5418
Space group	$P6_5$
Unit cell parameters (Å)	a= 98.181, b= 98.181, c=80.827
	$\alpha=90^o$, $\beta=90^o$, $\gamma=120^o$
Resolution (Å)	30-2.20 (2.28-2.20)
Unique reflections	22588
Completeness (%)	99.8 (99.6)
R_{merge}*	8.3 (45.2)
I/σ (I)	8.7 (2.5)
Redundancy	3.4 (3.3)
Refinement	
Resolution (Å)	29.86-2.20
Completeness (%)	99.81
Reflections used	21363
R / R_{free} (%)	17.9 / 20.8
Average B overall ($Å^2$)	37.02
r.m.s.d bonds (Å)	0.006
r.m.s.d angles (°)	1.026
Total number of atoms	2441
Ramachandran Plot	
Preferred (%)	98.5
Allowed (%)	1.5
Outliers (%)	0

Values in parentheses are for highest resolution shell.

*$R_{merge} = \Sigma(|(I - < I >)|/\Sigma (I)$.

Recombinant human Pim-1 kinase residues 33-305, containing a caspase cleavable N- terminal poly-histidine tag, was subcloned into pET28 vector (Novagen) and expressed

in *E. coli*. Cells were lysed by sonication, and after centrifugation, the supernatant (containing 50mM HEPES, pH 8.0, 500mM NaCl, 10% glycerol, 10mM β-Mercaptoethanol) was loaded onto Ni-NTA SF resin (Qiagen). The protein was eluted with a gradient of 0-400mM imidazole. It was further purified by SEC onto Superdex 75 column (GE Healthcare Life Sciences), equilibrated in 20mM HEPES pH8.0, 100mM NaCl, 5mM DTT, 5% glycerol. His-tagged Pim-1 was concentrated to 10mg/ml and stored at -80^{o}C prior to crystallization.

Purified human Pim-1 was crystallized using hanging drop vapor diffusion method. Protein and crystallization buffer (1.0M LiCl, 0.1M TRIS pH8.0, 20% PEG6000) were mixed 1:1, spin filtered and dispensed in 2ul hanging drops over a reservoir solution of 80% crystallization buffer at 4^{o}C. Rod-shaped crystals appeared in 1 day, and were stable for soaking within 1 week. Apo crystals were transferred to a 10ul hanging drop of crystallization buffer. Compound **28** was soaked into individual apo crystals for 1 day at a final concentration of 2.5mM.

A complexed crystal was equilibrated in buffer containing 30% glycerol as cryoprotectant, and was frozen directly in the cryogenic stream. The data set was collected using CrystalClear data collection software on a Rigaku Saturn92 CCD detector / FR-E SuperBright rotating anode generator. The data were integrated and scaled using HKL2000 [1]. The crystals belong to the hexagonal space group $P6_5$ with unit cell dimensions of a = 98.1Å, b = 98.1Å, c = 80.8Å, α=90^{o}, β= 90^{o}, γ=120^{o}. The structure was solved by molecular replacement using MolRep [2,3], with an apo Pim-1 structure as a search model. There is 1 molecule in the asymmetric unit. The structure was refined using Refmac5 [2,4], and model building was performed using the graphics program Coot [5]. The ligand was generated using the PHENIX module eLBOW [6,7]. The structure of Pim-1 and compound **28** was refined to 2.20Å with an R-factor of 17.9% and R_{free} of 20.8%. The N-terminal tag was unresolved in the crystal structure. The atomic coordinates and structure factors have been deposited in the Protein Data Bank (PDB ID code: **6MT0**).

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