

SUPPORTING INFORMATION.

Allosteric Inhibition of SHP2: Identification of a Potent, Selective, and Orally Efficacious Phosphatase Inhibitor

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Contents: selectivity assays, Xray data table, pharmacology.

Selectivity assays.

Eurofins Phosphatase	Species	Native phosphatase sequence present in recombinant protein	IC₅₀, μM
CD45(h)	Human	598-end	>100
DUSP22(h)	Human	Full length	>100
HePTP(h)	Human	22-end	>100
LMPTP-A(h)	Human	Full length; Q106R	>100
LMPTP-B(h)	Human	Full length	>100
MKP5(h)	Human	320-end	>100
PP1a(h)	Human	Full length	>100
PP2A(h)	Human	Native enzyme	>100
PP5(h)	Human	Full length	>100
PTPb(h)	Human	1643-end	>100
PTP-1B(h)	Human	1-321	>100
PTP MEG1(h)	Human	423-end	>100
PTP MEG2(h)	Human	283-end	>100
PTPN22(h)	Human	1-312	>100
RPTPm(h)	Human	879-1184	>100
SHP-1(h)	Human	Full length	>100

SHP-2(h)	Human	230-545	>100
TCPTP(h)	Human	1-341	>100
TMDP(h)	Human	Full length	>100
VHR(h)	Human	Full length	>100
YopH(Yersinia)	Yersinia	Full length; R211A	>100

Kinases	IC₅₀, μM	Kinases	IC₅₀, μM
CE ABL1 (64-515) nonphos v2	>10	CE MAPK1	>10
CE ACVR1 (172-499)	>10	CE MAPK10	>10
CE AKT1	>10	CE MAPK14	>10
CE ALK (1066-1459)	>10	CE MAPKAPK2	>10
CE AURKA	>10	CE MAPKAPK5 (2-472)	>10
CE BTK	>10	CE MET (956-1390)	>10
CE CAMK2D	>10	CE MKNK1	>10
CE CDK1B	>10	CE MKNK2	>10
CE CDK2A	>10	CE PAK2	>10
CE CDK4D1	>10	CE PDGFRa (551-V561D-1089)	>10
CE CSK	>10	CE PDPK1	>10
CE CSNK1G3 (35-362)	>10	CE PIM2	>10

CE EGFR (668-1210)	>10	CE PKN1	>10
CE EPHB4 (566-987)	>10	CE PKN2	>10
CE ERBB4 (673-1308)	>10	CE PLK1	>10
CE FGFR1 (407-822)	>10	CE PRKACA	>10
CE FGFR2 (406-821)	9.7	CE PRKCA	>10
CE FGFR3 (411-806)	>10	CE PRKCQ	>10
CE FGFR3 (411-K650E-806)	>10	CE RET (658-1072)	>10
CE FGFR4 (388-802)	>10	CE ROCK2 (6-553)	>10
CE FLT3 (563-D835Y-993)	>10	CE RPS6KB1 (1-421)	>10
CE GSK3B	9.9	CE SRC (1-535)	>10
CE INSR (871-1343)	>10	CE STK17B	>10
CE IRAK1 (184-712)	>10	CE SYK (2-635) nonphos	>10
CE IRAK4 (1-460)	>10	CE WNK1 (2-491)	>10
CE JAK1 (866-1154)	>10	CE ZAP70	>10
CE JAK2 (808-1132)	>10	ADP-FRET PIK3CD	>10
CE KDR (807-1356)	>10	ADP-FRET PIK3CG	>10
CE KIT (544-976)	>10	ATP-binding MTOR(1360-2549)	>10
CE LCK (1-508)	>10	KGlo PIK3C3	>10
CE LYN (1-512)	>10	KGlo PIK3CA	>10

CE MAP3K8 (30-404)	>10	KGlo PIK3CB	>10
CE MAP4K4	>10	KGlo PIK4CB	>10

Extended Data Table 1. Crystallographic Data and Refinement Statistics

Parameters	SHP2/SHP836 complex
Space group	P2 ₁
Unit Cell (Å)	a=46.31, b=213.73, c=55.98
Resolution range (Å)	27.09 – 1.85 (1.90 – 1.85)
Total observations	282590
Unique reflections	87497
Completeness (%) ^a	95.9 (76.4)
Multiplicity	3.2 (2.4)
$\langle I/\sigma(I) \rangle$ ^a	15.9 (2.1)
R_{merge} ^{a,b}	0.044 (0.362)
$R_{\text{cryst}}/R_{\text{free}}$ ^c	0.175/0.208 (0.223/0.264)
Protein atoms	7760
Heterogen atoms	86
Solvent molecules	654
Average <i>B</i> -factor (Å ²)	34.2
R.m.s.d. bond lengths (Å)	0.01
R.m.s.d. bond angle (°)	1.00
<u>Ramachandran Plot (%)</u>	

Favored	96.8
Allowed	2.8
Outliers	0.4

^a Numbers in parenthesis are for the highest resolution shell.

^b $R_{\text{merge}} = \Sigma |I_h - \langle I_h \rangle| / \Sigma I_h$ over all h , where I_h is the intensity of reflection h .

^c R_{cryst} and $R_{\text{free}} = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$, where F_o and F_c are observed and calculated amplitudes, respectively. R_{free} was calculated using 5% of data excluded from the refinement.

Pharmacology. SHP099 (**1**) and erlotinib were evaluated in the EGFR amplified human esophageal squamous cell carcinoma KYSE-520 xenograft model subcutaneously implanted into immunocompromised mice. Tumor stasis was achieved in both groups (100 mg/kg SHP099 and 80 mg/kg erlotinib), with no significant change in body weight.

