VX-509 (Decernotinib) is a potent and selective Janus kinase 3 (JAK3) inhibitor that attenuates inflammation in animal models of autoimmune disease

Sudipta Mahajan, James K. Hogan, Dina Shlyakhter, Luke Oh, Francesco G. Salituro, Luc Farmer and Thomas C. Hoock

Supplemental Table 1. ATP and peptide substrate concentrations used in kinase assays of VX-509.

Kinase	[ATP], μM	[peptide]	Peptide	Assay Format
AURA (AUR2)	50	800 μM	LRRASLG	Spectrophotometric
CDK2/cyclin A	200	300 μM	MAHHRSPRKRAKKK	Spectrophotometric
CIT	10	0.2 mg/mL	KKRNRTLSV	Radiometric
COT	10	1 μM	inactive MEK1	Radiometric
cMET	200	10 μM	polyE4Y	Radiometric
ERK2	65	800 μM	ATGPLSPGPFGRR	Spectrophotometric
FAK	70	0.5 mg/mL	polyE4Y	Radiometric
FLT3	90	0.5 mg/mL	polyE4Y	Radiometric
GCK	500	1.2 mg/mL	MBP	Spectrophotometric
GSK3β	20	300 μM	HSSPHQ(Sp)EDEEE	Spectrophotometric
IRAK4	800	300 μM	HMRSAMSGLHLVKRR	Spectrophotometric
ITK	15	2 μM	SAM68	Radiometric
JAK1	27	0.25 mg/mL	polyE4Y	Radiometric
JAK2	12	0.5 mg/mL	polyE4Y	Radiometric
JAK3	5	0.25 mg/mL	polyE4Y	Radiometric
JNK3	20	200 μM	KRELVETLTPSGEAPNQALLR	Spectrophotometric
KDR	300	10 μM	polyE4Y	Spectrophotometric
KIT	700	0.5 mg/mL	polyE4Y	Radiometric
P38α	200	400 μM	KRELVETLTPSGEAPNQALLR	Spectrophotometric
PI3Kγ	15	30 μM	Phosphatidylinositol bisphosphate	Radiometric
PKA	5	50 μM	LRRASLG	Spectrophotometric
PknA	15	6 μM	GarA	Radiometric
PknB	3	4 μM	GarA	Radiometric
PknG	6	6 μM	GarA	Radiometric
PLK1	20	4 μM	SAM68	Radiometric
ROCKI	15	45 μM	KKRNRTLSV	Radiometric
Src	50	0.3 mg/mL	polyE4Y	Spectrophotometric
SYK	50	0.1 mg/mL	polyE4Y	Radiometric
TAK1	50	18 μM	MBP	Radiometric
TYK2	14	0.25 mg/mL	polyE4Y	Radiometric

Supplemental Table 2. ATP, peptide, and protein concentrations used in assays of VX-509.

Kinase Assay	ATP (μM)	ATP K_M (μM)	[peptide substrate]	Peptide Substrate
ABL	45	48	50 μM	EAIYAAPFAKKK
ALK	200	229	250 μM	KKKSPGEYVNIEFG
AMPK	90	80	200 μM	AMARAASAAALARRR
ASK1	200	371	0.33 mg/mL	MBP
AXL	90	80	250 μM	KKSRGDYMTMQIG
BLK	120	125	0.1 mg/mL	polyE4Y
CAMKII	15	22	30 μM	KKLNRTLSVA
CDK1/CycB	45	51	0.1 mg/mL	Histone H1
CDK5/P35	15	30	0.1 mg/mL	Histone H1
CHK1	90	84	200 μM	KKKVSRSGLYRSPSMPENLNRPR
CK1	45	38	200 μM	KRRRALS(p)VASLPGL
EGFR	10	3	0.1 mg/mL	polyE4Y
EphB4	10	11	0.1 mg/mL	polyE4Y
ERK1 (MAPK1)	70	67	250 μM	RRELVEPLTPSGEAPNQALLR
FES	45	50	0.1 mg/mL	polyE4Y
FGFR3	15	28	0.1 mg/mL	polyE4Y
Flt1 (VEGFR1)	200	2118	250 μM	KKKSPGEYVNIEFG
FYN	70	64	250 μM	KVEKIGEGTYGVVYK
IKKα	10	6	200 μM	KKKKERLLDDRHDSGLDSMKDEE
IKKβ	10	7	100 μM	KKKKERLLDDRHDSGLDSMKDEE
IR	200	447	250 μM	KKSRGDYMTMQIG
JNK1 (SAPK1γ)	45	41	3 μM	ATF2
LCK	90	95	250 μM	KVEKIGEGTYGVVYK
LYN	70	79	0.1 mg/mL	polyE4Y
MEK1	10	ND	1 μM	inactive MAPK2
MKK6	10	ND	1 μM	inactive SAPK2a
MKK7	10	ND	2 μM	inactive JNK1a1
MSK1	90	76	30 μM	GRPRTSSFAEGKK
MST2	155	165	0.33 mg/mL	MBP
NEK2	120	120	0.33 mg/mL	MBP
P38β (SAPK2β)	45	58	0.33 mg/mL	MBP
PAK2	90	89	30 μM	KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK
PDGFRα	120	106	0.1 mg/mL	polyE4Y
PKCβII	70	58	0.1 mg/mL	Histone H1
PKCι	155	170	50 μM	ERMRPRKRQGSVRRRV
PKCθ	15	16	0.1 mg/mL	Histone H1
PLK3 (FNK)	70	70	2 mg/mL	casein
PRAK	15	18	30 μM	KKLRRTLSVA
PRK2	15	10	30 μM	AKRRRLSSLRA
RAFc	45	49	0.66 mg/mL	MBP
RET	70	58	250 μM	KKKSPGEYVNIEFG
RIPK2 (RICK)	120	133	0.33 mg/mL	MBP
ROCK II	15	22	30 μM	KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK
RON	70	74	250 μM	KKSRGDYMTMQIG
ROS (KROS)	200	563	250 μM	KKKSPGEYVNIEFG
RSK3	10	7	30 μM	KKKNRTLSVA
SGK	90	93	30 μM	GRPRTSSFAEGKK
TIE2	200	439	0.1 mg/mL	polyE4Y
TrkA	120	129	250 μM	KKKSPGEYVNIEFG
ZIPK	15	14	250 μM	KKLNRTLSFAEPG

Supplemental Table 3. K_i, $IC_{50,}$ or percent inhibition values for VX-509 in kinase assays.

Enzyme	Result Value[a]	Result Type	Enzyme	Result Value[a]	Result Type
AURA (AUR2)	> 0.8	Ki (μM)	KDR (VEGFR)	> 4	Ki (μM)
CDK2/CycA	2.6 ± 0.8	Ki (μM)	KIT	> 2.5	Ki (μM)
CIT	> 10	IC_{50} (μM)	cMET	> 4	Ki (μM)
COT	> 4	Ki (μM)	P38α(SAPK2α)	> 4	Ki (μM)
ERK2 (MAPK2)	> 4	Ki (μM)	PI3Kγ	> 4	Ki (μM)
FAK	>10	IC_{50} (μM)	PKA	> 4	Ki (μM)
FLT3	1.0 ± 0.3	Ki (μM)	PknA	> 10	IC_{50} (μM)
GCK	71 ± 16	% inhibition @ 3 uM	PknB	8	IC_{50} (μM)
GSK3β	1.8 ± 0.6	Ki (μM)	PknG	> 10	IC_{50} (μM)
IRAK4	> 4	Ki (μM)	PLK1	> 4	Ki (μM)
ITK	> 4	Ki (μM)	ROCK I	1.5 ± 0.4	Ki (μM)
JAK1	0.011 ± 0.002	Ki (μM)	SRC	> 4	Ki (μM)
JAK2	0.013 ± 0.004	Ki (μM)	SYK	> 4	Ki (μM)
JAK3	0.0025 ± 0.0007	Ki (μM)	TAK1	> 4	Ki (μM)
JNK3 (SAPK1β)	> 4	Ki (μM)	TYK2	0.011	Ki (μM)

Supplemental Table 4. Selectivity profile of VX-509 for kinases from the KinaseProfiler™ assay.

Enzyme	% inh @ 2 μM	Enzyme	% inh @ 2 μM	Enzyme	% inh @ 2 μM
ABL	28	FYN	4	PKCι	0
ALK	0	IKKα	0	PKCθ	21
AMPK	46	IKKβ	1	PLK3 (FNK)	0
ASK1	0	IR	3	PRAK	0
AXL	0	JNK1 (SAPK1c)	0	PRK2	71
BLK	14	LCK	4	RAFc	12
CAMKII	31	LYN	28	RET	0
CDK1/CycB	22	MEK1	0	RIPK2 (RICK)	0
CDK5/P35	55	MKK6	0	ROCK II	55
CHK1	0	MKK7	0	RON	0
CK1	0	MSK1	8	ROS (KROS)	0
EGFR	0	MST2	64	RSK3	27
EphB4	20	NEK2	0	SGK	0
ERK1 (MAPK1)	0	P38β (SAPK2β)	3	TIE2	37
FES	42	PAK2	0	TrkA	48
FGFR3	26	PDGFRα	0	ZIPK	0
Flt1 (VEGFR1)	19	PKCβII	30		