

## **SUPPORTING INFORMATION**

### **2,4,6-Triaminopyrimidine as a Novel Hinge Binder in a Series of PI3K $\delta$ Selective Inhibitors**

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## **Histopathology Scoring Criteria for Rat CIA model**

### **Ankle Inflammation**

0 = Normal.

0.5 = Minimal focal inflammation.

1 = Minimal infiltration of inflammatory cells in synovium/periarticular tissue.

2 = Mild infiltration.

3 = Moderate infiltration with moderate edema.

4 = Marked infiltration with marked edema.

5 = Severe infiltration with severe edema.

### **Ankle Pannus**

0 = Normal.

0.5 = Minimal infiltration of pannus in cartilage and subchondral bone, affects only marginal zones and affects only a few joints.

1 = Minimal infiltration of pannus in cartilage and subchondral bone, primarily affects marginal zones.

2 = Mild infiltration (<1/4 of tibia or tarsals at marginal zones).

3 = Moderate infiltration (1/4 to 1/3 of tibia or small tarsals affected at marginal zones).

4 = Marked infiltration (1/2 to 3/4 of tibia or tarsals affected at marginal zones).

5 = Severe infiltration (>3/4 of tibia or tarsals affected at marginal zones, severe distortion of overall architecture).

### **Ankle Cartilage Damage (emphasis on small tarsals)**

0 = Normal

0.5 = Very minimal = Minimal decrease in toluidine blue staining, affects only marginal zones and affects only a few joints.

1 = Minimal = Minimal to mild loss of toluidine blue staining with no obvious chondrocyte loss or collagen disruption.

2 = Mild = Mild loss of toluidine blue staining with focal mild (superficial) chondrocyte loss and/or collagen disruption.

3 = Moderate = Moderate loss of toluidine blue staining with multifocal moderate (depth to middle zone) chondrocyte loss and/or collagen disruption, smaller tarsals affected to 1/2 to 3/4 depth with rare areas of full thickness loss.

4 = Marked = Marked loss of toluidine blue staining with multifocal marked (depth to deep zone) chondrocyte loss and/or collagen disruption, 1 or 2 small tarsals surfaces have full thickness loss of cartilage.

5 = Severe = Severe diffuse loss of toluidine blue staining with multifocal severe (depth to tide mark) chondrocyte loss and/or collagen disruption affecting more than 2 cartilage surfaces.

### **Ankle Bone Resorption**

0 = Normal

0.5 = Very minimal = Minimal resorption affects only marginal zones and affects only a few joints.

1 = Minimal = Small areas of resorption, not readily apparent on low magnification, rare osteoclasts.

2 = Mild = More numerous areas of resorption, not readily apparent on low magnification, osteoclasts more numerous, <1/4 of tibia or tarsals at marginal zones resorbed.

3 = Moderate = Obvious resorption of medullary trabecular and cortical bone without full thickness defects in cortex, loss of some medullary trabecular, lesion apparent on low magnification, osteoclasts more numerous, 1/4 to 1/3 of tibia or tarsals affected at marginal zones.

4 = Marked = Full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts, 1/2 to 3/4 of tibia or tarsals affected at marginal zones.

5 = Severe = Full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts, >3/4 of tibia or tarsals affected at marginal zones, severe distortion of overall architecture.

### **Periosteal New Bone Formation**

Studies that go beyond the acute inflammatory stage often show varying degrees of periosteal new bone formation. In order to convey the degree of periosteal new bone formation the following scores are applied based on the distribution of periosteal bone proliferation and a measurement of width of periosteal new bone formation at the widest location.

0 = Normal, no periosteal proliferation.

0.5 = Minimal focal or multifocal proliferation, measures less than 127  $\mu\text{m}$  width (1–2 units) at any location.

1 = Minimal multifocal proliferation, width at any location measures 127–252  $\mu\text{m}$  (3–4 units).

2 = Mild multifocal on tarsals, diffuse in some locations, width at any location 253–441  $\mu\text{m}$  (5–7 units).

3 = Moderate multifocal on tarsals, diffuse in most other locations, width at any location measures 442–630  $\mu\text{m}$  (8–10 units).

4 = Marked multifocal on tarsals, diffuse at most other locations, width at any location measures 630–819  $\mu\text{m}$  (11–13 units).

5 = Severe, multifocal on tarsals, diffuse at most other locations, width at any location measures >819  $\mu\text{m}$  (>13 units).

## DiscoverX Primary Screen Data for 52

COMPOUND	52	COMPOUND	52
GENE SYMBOL	% control at 10 $\mu$ M	GENE SYMBOL	% control at 10 $\mu$ M
AAK1	89	MAP3K4	66
ABL1(E255K)-phosphorylated	98	MAP4K2	95
ABL1(F317I)-nonphosphorylated	76	MAP4K3	87
ABL1(F317I)-phosphorylated	98	MAP4K4	100
ABL1(F317L)-nonphosphorylated	72	MAP4K5	87
ABL1(F317L)-phosphorylated	92	MAPKAPK2	60
ABL1(H396P)-nonphosphorylated	96	MAPKAPK5	90
ABL1(H396P)-phosphorylated	75	MARK1	64
ABL1(M351T)-phosphorylated	92	MARK2	79
ABL1(Q252H)-nonphosphorylated	99	MARK3	100
ABL1(Q252H)-phosphorylated	100	MARK4	82
ABL1(T315I)-nonphosphorylated	97	MAST1	100
ABL1(T315I)-phosphorylated	83	MEK1	82
ABL1(Y253F)-phosphorylated	78	MEK2	77
ABL1-nonphosphorylated	78	MEK3	83
ABL1-phosphorylated	63	MEK4	100
ABL2	100	MEK5	92
ACVR1	100	MEK6	73
ACVR1B	78	MELK	59
ACVR2A	100	MERTK	100
ACVR2B	89	MET	93
ACVRL1	82	MET(M1250T)	99
ADCK3	86	MET(Y1235D)	96
ADCK4	100	MINK	72
AKT1	95	MKK7	92
AKT2	84	MKNK1	90
AKT3	90	MKNK2	92

ALK	100	MLCK	87
ALK(C1156Y)	100	MLK1	71
ALK(L1196M)	85	MLK2	42
AMPK-alpha1	93	MLK3	85
AMPK-alpha2	97	MRCKA	90
ANKK1	65	MRCKB	100
ARK5	100	MST1	100
ASK1	57	MST1R	100
ASK2	100	MST2	64
AURKA	100	MST3	100
AURKB	71	MST4	82
AURKC	100	MTOR	60
AXL	84	MUSK	85
BIKE	100	MYLK	83
BLK	58	MYLK2	87
BMPR1A	83	MYLK4	85
BMPR1B	79	MYO3A	92
BMPR2	88	MYO3B	100
BMX	87	NDR1	72
BRAF	83	NDR2	99
BRAF(V600E)	99	NEK1	100
BRK	100	NEK10	100
BRSK1	92	NEK11	79
BRSK2	91	NEK2	100
BTK	90	NEK3	72
BUB1	100	NEK4	98
CAMK1	100	NEK5	67
CAMK1D	99	NEK6	97
CAMK1G	96	NEK7	97
CAMK2A	100	NEK9	86

CAMK2B	100	NIK	96
CAMK2D	100	NIM1	87
CAMK2G	100	NLK	90
CAMK4	100	OSR1	100
CAMKK1	100	p38-alpha	80
CAMKK2	100	p38-beta	98
CASK	87	p38-delta	100
CDC2L1	76	p38-gamma	100
CDC2L2	85	PAK1	93
CDC2L5	79	PAK2	91
CDK11	81	PAK3	71
CDK2	80	PAK4	92
CDK3	89	PAK6	96
CDK4-cyclinD1	84	PAK7	85
CDK4-cyclinD3	97	PCTK1	85
CDK5	100	PCTK2	97
CDK7	94	PCTK3	96
CDK8	88	PDGFRA	100
CDK9	97	PDGFRB	90
CDKL1	100	PDPK1	46
CDKL2	79	PFCDPK1(P.falciparum)	94
CDKL3	100	PFPK5(P.falciparum)	95
CDKL5	100	PFTAIRE2	89
CHEK1	95	PFTK1	100
CHEK2	68	PHKG1	92
CIT	96	PHKG2	100
CLK1	100	PIK3C2B	21
CLK2	96	PIK3C2G	9.8
CLK3	83	PIK3CA	8.9
CLK4	86	PIK3CA(C420R)	8.7



CSF1R	74	PIK3CA(E542K)	9.6
CSF1R-autoinhibited	85	PIK3CA(E545A)	7
CSK	91	PIK3CA(E545K)	5.8
CSNK1A1	74	PIK3CA(H1047L)	0
CSNK1A1L	88	PIK3CA(H1047Y)	3.9
CSNK1D	83	PIK3CA(I800L)	17
CSNK1E	69	PIK3CA(M1043I)	1
CSNK1G1	26	PIK3CA(Q546K)	8.1
CSNK1G2	22	PIK3CB	0
CSNK1G3	16	PIK3CD	0
CSNK2A1	72	PIK3CG	0
CSNK2A2	94	PIK4CB	9
CTK	100	PIM1	82
DAPK1	81	PIM2	75
DAPK2	100	PIM3	76
DAPK3	100	PIP5K1A	96
DCAMKL1	94	PIP5K1C	5
DCAMKL2	100	PIP5K2B	100
DCAMKL3	100	PIP5K2C	32
DDR1	100	PKAC-alpha	59
DDR2	97	PKAC-beta	74
DLK	84	PKMYT1	100
DMPK	84	PKN1	74
DMPK2	94	PKN2	98
DRAK1	86	PKNB(M.tuberculosis)	100
DRAK2	78	PLK1	100
DYRK1A	89	PLK2	79
DYRK1B	89	PLK3	81
DYRK2	85	PLK4	69
EGFR	73	PRKCD	95

EGFR(E746-A750del)	76	PRKCE	98
EGFR(G719C)	65	PRKCH	94
EGFR(G719S)	74	PRKCI	69
EGFR(L747-E749del, A750P)	67	PRKCQ	93
EGFR(L747-S752del, P753S)	71	PRKD1	100
EGFR(L747-T751del,Sins)	80	PRKD2	100
EGFR(L858R)	74	PRKD3	100
EGFR(L858R,T790M)	86	PRKG1	97
EGFR(L861Q)	87	PRKG2	85
EGFR(S752-I759del)	100	PRKR	100
EGFR(T790M)	97	PRKX	87
EIF2AK1	99	PRP4	89
EPHA1	71	PYK2	64
EPHA2	68	QSK	84
EPHA3	88	RAF1	73
EPHA4	84	RET	86
EPHA5	100	RET(M918T)	86
EPHA6	86	RET(V804L)	80
EPHA7	86	RET(V804M)	91
EPHA8	100	RIOK1	94
EPHB1	80	RIOK2	32
EPHB2	86	RIOK3	91
EPHB3	82	RIPK1	76
EPHB4	66	RIPK2	100
EPHB6	96	RIPK4	100
ERBB2	100	RIPK5	92
ERK2	100	ROCK1	100
ERK3	100	ROCK2	100
ERK4	94	ROS1	72
ERK5	93	RPS6KA4(Kin.Dom.1-N-terminal)	80

ERK8	100	RPS6KA4(Kin.Dom.2-C-terminal)	84
ERN1	85	RPS6KA5(Kin.Dom.1-N-terminal)	83
FAK	89	RPS6KA5(Kin.Dom.2-C-terminal)	100
FER	100	RSK1(Kin.Dom.1-N-terminal)	100
FES	100	RSK1(Kin.Dom.2-C-terminal)	97
FGFR1	90	RSK2(Kin.Dom.1-N-terminal)	100
FGFR2	100	RSK2(Kin.Dom.2-C-terminal)	100
FGFR3	90	RSK3(Kin.Dom.1-N-terminal)	100
FGFR3(G697C)	64	RSK3(Kin.Dom.2-C-terminal)	96
FGFR4	100	RSK4(Kin.Dom.1-N-terminal)	82
FGR	89	RSK4(Kin.Dom.2-C-terminal)	100
FLT1	82	S6K1	84
FLT3	97	SBK1	86
FLT3(D835H)	75	SGK	100
FLT3(D835Y)	65	SgK110	99
FLT3(ITD)	100	SGK2	100
FLT3(K663Q)	83	SGK3	62
FLT3(N841I)	50	SIK	100
FLT3(R834Q)	88	SIK2	100
FLT3-autoinhibited	100	SLK	84
FLT4	85	SNARK	100
FRK	93	SNRK	89
FYN	100	SRC	81
GAK	97	SRMS	89
GCN2(Kin.Dom.2,S808G)	74	SRPK1	65
GRK1	94	SRPK2	73
GRK4	70	SRPK3	85
GRK7	87	STK16	95
GSK3A	100	STK33	64
GSK3B	87	STK35	90

HASPIN	100	STK36	94
HCK	100	STK39	84
HIPK1	93	SYK	100
HIPK2	100	TAK1	97
HIPK3	95	TAOK1	100
HIPK4	100	TAOK2	100
HPK1	64	TAOK3	89
HUNK	100	TBK1	83
ICK	100	TEC	97
IGF1R	100	TESK1	68
IKK-alpha	80	TGFBR1	100
IKK-beta	100	TGFBR2	70
IKK-epsilon	100	TIE1	92
INSR	100	TIE2	94
INSRR	72	TLK1	91
IRAK1	100	TLK2	89
IRAK3	86	TNIK	80
IRAK4	100	TNK1	92
ITK	100	TNK2	91
JAK1(JH1domain-catalytic)	99	TNNI3K	100
JAK1(JH2domain-pseudokinase)	77	TRKA	100
JAK2(JH1domain-catalytic)	100	TRKB	95
JAK3(JH1domain-catalytic)	100	TRKC	98
JNK1	93	TRPM6	65
JNK2	84	TSSK1B	64
JNK3	99	TTK	95
KIT	93	TXK	70
KIT(A829P)	85	TYK2(JH1domain-catalytic)	100
KIT(D816H)	80	TYK2(JH2domain-pseudokinase)	100
KIT(D816V)	73	TYRO3	97

<b>KIT(L576P)</b>	<b>100</b>	<b>ULK1</b>	<b>100</b>
<b>KIT(V559D)</b>	<b>100</b>	<b>ULK2</b>	<b>97</b>
<b>KIT(V559D,T670I)</b>	<b>74</b>	<b>ULK3</b>	<b>100</b>
<b>KIT(V559D,V654A)</b>	<b>79</b>	<b>VEGFR2</b>	<b>89</b>
<b>KIT-autoinhibited</b>	<b>95</b>	<b>VRK2</b>	<b>72</b>
<b>LATS1</b>	<b>93</b>	<b>WEE1</b>	<b>97</b>
<b>LATS2</b>	<b>100</b>	<b>WEE2</b>	<b>92</b>
<b>LCK</b>	<b>76</b>	<b>WNK1</b>	<b>99</b>
<b>LIMK1</b>	<b>93</b>	<b>WNK3</b>	<b>100</b>
<b>LIMK2</b>	<b>84</b>	<b>YANK1</b>	<b>100</b>
<b>LKB1</b>	<b>72</b>	<b>YANK2</b>	<b>80</b>
<b>LOK 94</b>	<b>100</b>	<b>YANK3</b>	<b>95</b>
<b>LRRK2</b>	<b>98</b>	<b>YES</b>	<b>90</b>
<b>LRRK2(G2019S)</b>	<b>84</b>	<b>YSK1</b>	<b>100</b>
<b>LTK</b>	<b>100</b>	<b>YSK4</b>	<b>72</b>
<b>LYN</b>	<b>88</b>	<b>ZAK</b>	<b>72</b>
<b>LZK</b>	<b>78</b>	<b>ZAP70</b>	<b>88</b>
<b>MAK</b>	<b>99</b>		
<b>MAP3K1</b>	<b>100</b>		
<b>MAP3K15</b>	<b>100</b>		
<b>MAP3K2</b>	<b>100</b>		
<b>MAP3K3</b>	<b>90</b>		

**KinomeScan Selectivity Scores for 52**

<b>Compound</b>	<b>Selectivity Score Type</b>	<b>Number of Hits</b>	<b>Number of Non-Mutant Kinases</b>	<b>Screening Concentration</b>	<b>Selectivity Score</b>
<b>52</b>	<b>S(35)</b>	<b>13</b>	<b>395</b>	<b>10000</b>	<b>0.033</b>
<b>52</b>	<b>S(10)</b>	<b>7</b>	<b>395</b>	<b>10000</b>	<b>0.018</b>
<b>52</b>	<b>S(1)</b>	<b>3</b>	<b>395</b>	<b>10000</b>	<b>0.008</b>