Supplementary file

Discovery of a broad spectrum antiproliferative agent with selectivity for DDR1 kinase: Cell line-based assay, kinase panel, and molecular docking and toxicity studies.

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1- ¹H NMR chart of compound **KST9046**.

2- High resolution Mass Spectrometry (HRMS) peak of compound **KST9046**.

3- NCI biological evaluation data of compound **KST9046** (NCS: 777737):

National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC : 777737 / 1			Experiment ID : 1402NS22					Test Type : 08				Units : Molar			
Report Date : February 26, 2014			Test Date : February 03, 2014					QNS :				MC :			
COMI : KKRE9046 (134652)			Stain Reagent : SRB Dual-Pass Related					SSPL : 0YXX							
Log10 Concentration															
Panel/Cell Line	Time														
	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GISD	TGI	LC50
Leukemia															
CCRF-CEM	0.524	2.249	2.223	2.106	1.917	0.510	1.251	98	92	81	-3	42	2.33E-6	-	> 1.00E-4
HL-60(TB)	0.770	2.228	2.307	2.085	2.125	0.518	1.415	105	90	93	-33	44	2.20E-6	-	> 1.00E-4
K-562	0.244	1.382	1.365	1.332	1.183	0.274	0.497	98	95	82	3	22	2.52E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.622	2.053	1.980	1.871	1.738	0.364	1.162	95	87	78	-42	38	1.72E-6	-	> 1.00E-4
RPMI-8226	0.780	2.025	2.022	1.946	1.557	0.423	1.089	100	94	62	-46	25	1.30E-6	-	> 1.00E-4
SR	0.276	0.750	0.770	0.763	0.663	0.207	0.464	104	103	82	-25	40	1.98E-6	-	> 1.00E-4
Non-Small Cell Lung Cancer															
A549(ATCC)	0.433	1.568	1.552	1.587	1.534	0.410	0.569	99	102	97	-5	12	2.88E-6	-	> 1.00E-4
HOP-62	0.874	1.796	1.712	1.662	1.684	0.616	0.676	91	85	88	-30	-23	2.10E-6	5.60E-6	> 1.00E-4
HOP-92	1.040	1.585	1.520	1.521	1.446	0.890	0.998	88	88	74	-14	-4	1.88E-6	6.88E-6	> 1.00E-4
NCH-H226	1.503	2.951	2.905	2.930	2.901	1.448	1.156	97	99	97	-4	-23	2.91E-6	9.19E-6	> 1.00E-4
NCH-H23	0.583	1.351	1.319	1.411	1.471	0.444	0.842	96	108	116	-24	34	2.96E-6	-	> 1.00E-4
NCH-H322M	0.892	1.955	1.893	1.915	1.943	0.796	1.388	94	96	99	-11	47	2.79E-6	-	> 1.00E-4
NCH-H460	0.262	1.764	1.763	1.802	1.698	0.210	0.411	100	103	96	-20	10	2.48E-6	-	> 1.00E-4
NCH-H522	0.889	1.546	1.454	1.513	1.427	0.364	0.507	86	95	82	-59	-43	1.68E-6	3.81E-6	-
Colon Cancer															
COLO 205	0.580	1.669	1.665	1.601	1.630	0.094	0.982	100	94	96	-84	37	1.81E-6	-	> 1.00E-4
HCO-2998	0.863	2.143	1.999	2.071	2.341	0.923	1.344	89	94	116	5	38	3.90E-6	> 1.00E-4	> 1.00E-4
HCT-116	0.300	2.098	2.117	2.033	1.841	0.302	0.551	101	96	86	-	14	2.61E-6	> 1.00E-4	> 1.00E-4
HCT-15	0.309	1.685	1.658	1.563	1.505	0.336	0.658	98	91	87	2	25	2.72E-6	> 1.00E-4	> 1.00E-4
HT29	0.261	1.162	1.173	1.157	1.111	0.260	0.509	101	99	94	-1	28	2.93E-6	-	> 1.00E-4
KM12	0.583	2.358	2.235	2.301	2.192	0.144	0.902	93	97	91	-74	19	1.77E-6	-	> 1.00E-4
SW-620	0.240	1.212	1.183	1.185	1.140	0.233	0.525	97	97	93	-3	29	2.79E-6	-	> 1.00E-4
CNS Cancer															
SF-268	0.553	1.622	1.587	1.548	1.505	0.469	0.741	97	93	89	-15	18	2.37E-6	-	> 1.00E-4
SF-295	0.941	2.840	2.826	2.684	2.723	0.663	1.027	99	92	94	-30	5	2.27E-6	-	> 1.00E-4
SF-539	0.912	2.413	2.397	2.270	2.348	0.622	1.310	99	90	96	-32	26	2.28E-6	-	> 1.00E-4
SNB-19	0.652	1.929	1.890	1.878	1.811	0.692	0.649	97	96	91	3	-	2.91E-6	7.42E-6	> 1.00E-4
SNB-75	0.876	1.620	1.539	1.488	1.530	0.362	0.283	89	82	88	-59	-68	1.81E-6	3.98E-6	8.73E-6
U251	0.556	2.009	2.020	2.036	2.027	0.583	0.824	101	102	101	2	18	3.28E-6	> 1.00E-4	> 1.00E-4
Melanoma															
LOX IMVI	0.321	1.634	1.649	1.651	1.729	0.273	0.708	101	101	107	-15	29	2.93E-6	-	> 1.00E-4
MALME-3M	0.834	1.444	1.392	1.411	1.441	0.583	0.835	91	95	99	-30	-	2.41E-6	-	> 1.00E-4
M14	0.417	1.417	1.369	1.349	1.317	0.363	0.690	95	93	90	-13	27	2.45E-6	-	> 1.00E-4
MDA-MB-435	0.588	2.201	2.174	2.055	1.690	0.360	0.574	98	91	68	-39	-2	1.48E-6	4.34E-6	> 1.00E-4
SK-MEL-2	1.168	2.146	2.117	2.131	2.132	0.481	1.050	97	98	99	-69	-10	2.04E-6	4.23E-6	-
SK-MEL-28	0.727	1.962	1.956	1.856	1.793	0.584	1.158	100	91	86	-20	35	2.20E-6	-	> 1.00E-4
SK-MEL-5	0.892	3.183	3.125	3.129	2.914	0.020	0.877	97	98	88	-98	-2	1.61E-6	2.98E-6	-
UACC-257	0.999	1.866	1.819	1.835	1.893	0.657	1.162	95	96	103	-34	19	2.44E-6	-	> 1.00E-4
UACC-62	1.037	2.743	2.706	2.675	2.488	0.298	1.002	98	96	85	-71	-3	1.68E-6	3.50E-6	-
Ovarian Cancer															
IGROV1	0.558	1.825	1.830	1.824	1.850	0.427	0.578	100	100	102	-23	2	2.59E-6	-	> 1.00E-4
OVCAR-3	0.411	1.055	1.076	1.158	0.940	0.205	0.335	103	116	82	-50	-18	1.75E-6	4.17E-6	-
OVCAR-4	0.662	1.226	1.236	1.174	1.196	0.565	0.418	102	91	95	-15	-37	2.56E-6	7.34E-6	> 1.00E-4
OVCAR-5	0.725	1.834	1.790	1.641	1.653	0.795	1.165	96	83	84	6	40	2.72E-6	> 1.00E-4	> 1.00E-4
OVCAR-8	0.535	1.803	1.779	1.782	1.775	0.478	0.517	98	98	98	-11	-3	2.76E-6	7.98E-6	> 1.00E-4
NOVADR-RES	0.550	1.407	1.410	1.441	1.467	0.475	0.526	100	104	107	-14	-4	2.97E-6	7.71E-6	> 1.00E-4
SK-OV-3	1.162	2.147	2.147	2.090	2.135	0.853	1.035	100	94	99	-27	-11	2.45E-6	6.13E-6	> 1.00E-4
Renal Cancer															
786-O	0.679	2.455	2.451	2.340	2.198	0.875	0.752	100	94	86	11	4	3.00E-6	> 1.00E-4	> 1.00E-4
A498	1.329	1.981	1.946	1.881	1.804	1.049	1.086	95	85	73	-21	-18	1.75E-6	5.96E-6	> 1.00E-4
ACHN	0.437	1.510	1.503	1.448	1.422	0.394	0.619	99	94	92	-10	17	2.58E-6	-	> 1.00E-4
CAKI-1	0.611	2.343	2.241	2.268	2.276	0.616	1.056	94	96	96	26	26	3.02E-6	> 1.00E-4	> 1.00E-4
SN12C	1.400	3.056	3.105	3.111	3.004	1.130	1.919	103	103	97	-19	31	2.53E-6	-	> 1.00E-4
TK-10	0.968	1.826	1.785	1.799	1.771	0.870	0.921	95	97	94	-10	-5	2.63E-6	7.98E-6	> 1.00E-4
UO-31	0.694	1.974	1.824	1.849	1.721	0.628	0.899	88	90	80	-10	16	2.17E-6	-	> 1.00E-4
Prostate Cancer															
PC-3	0.583	2.296	2.270	2.201	1.989	0.522	0.840	98	94	82	-10	15	2.22E-6	-	> 1.00E-4
DU-145	0.320	1.189	1.157	1.102	1.208	0.351	0.478	96	90	102	4	18	3.38E-6	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.443	1.998	1.875	1.773	1.765	0.311	0.494	92	86	85	-30	3	2.02E-6	-	> 1.00E-4
MDA-MB-231/ATCC	0.670	1.692	1.670	1.684	1.605	0.596	0.669	98	99	92	-11	-	2.54E-6	-	> 1.00E-4
HS 578T	1.217	2.189	2.128	2.149	2.179	0.998	1.044	94	96	99	-18	-14	2.62E-6	7.80E-6	> 1.00E-4
BT-549	1.381	2.473	2.450	2.375	2.329	1.202	1.337	98	91	87	-13	-3	2.34E-6	7.41E-6	> 1.00E-4
T-47D	0.810	1.384	1.366	1.284	1.272	0.444	0.649	97	83	81	-45	-20	1.75E-6	4.37E-6	> 1.00E-4
MDA-MB-468	0.875	1.672	1.651	1.681	1.583	0.719	0.884	97	101	89	-18	1	2.31E-6	-	> 1.00E-4

4- Results of the kinase panel activity % of compound **KST9046** over 347 enzymes at 10µM.

Kinase	% Enzyme Activity (relative to DMSO controls)	
	Data 1	Data 2
ABL1	100.12	99.99
ABL2/ARG	93.03	92.82
ACK1	99.17	95.67
AKT1	98.99	98.88
AKT2	97.50	96.00
AKT3	100.94	99.98
ALK	100.46	96.95
ALK1/ACVRL1	109.07	103.99
ALK2/ACVR1	104.74	107.02
ALK3/BMPR1A	107.05	106.71
ALK4/ACVR1B	102.18	100.48
ALK5/TGFBR1	100.03	90.92
ALK6/BMPR1B	100.83	99.84
ARAF	58.27	53.18
ARK5/NUAK1	100.45	93.72
ASK1/MAP3K5	89.82	87.52
Aurora A	90.92	85.97
Aurora B	104.70	104.44
Aurora C	109.23	105.56
AXL	96.47	93.65
BLK	101.70	100.41
BMPR2	108.88	106.73
BMX/ETK	101.71	98.26
BRAF	87.25	84.47
BRK	95.23	94.59
BRSK1	105.11	98.33
BRSK2	97.99	93.04
BTk	100.98	100.89
c-Kit	103.48	100.45
c-MER	100.04	98.13
c-MET	97.05	96.68
c-Src	106.52	101.19
CAMK1a	102.65	102.07
CAMK1b	100.94	98.98
CAMK1d	97.04	95.92
CAMK1g	105.36	101.71
CAMK2a	108.93	108.07
CAMK2b	107.95	107.37
CAMK2d	103.33	103.00
CAMK2g	107.78	104.41

CAMK4	91.78	91.36
CAMKK1	111.14	109.43
CAMKK2	80.47	75.55
CDC7/DBF4	102.16	102.09
CDK1/cyclin A	101.74	98.09
CDK1/cyclin B	100.65	97.77
CDK1/cyclin E	97.05	93.72
CDK14/cyclin Y (PFTK1)	93.89	92.72
CDK16/cyclin Y (PCTAIRE)	96.30	95.25
CDK2/cyclin A	98.31	97.57
CDK2/Cyclin A1	96.33	96.29
CDK2/cyclin E	96.78	93.55
CDK2/cyclin O	88.94	84.97
CDK3/cyclin E	104.34	103.95
CDK4/cyclin D1	102.92	100.84
CDK4/cyclin D3	99.49	99.39
CDK5/p25	105.78	105.68
CDK5/p35	98.57	97.92
CDK6/cyclin D1	99.23	98.80
CDK6/cyclin D3	103.22	103.05
CDK7/cyclin H	104.59	103.08
CDK9/cyclin K	110.86	106.16
CDK9/cyclin T1	91.74	90.65
CHK1	94.26	91.69
CHK2	94.91	91.07
CK1a1	96.48	96.16
CK1d	106.61	106.09
CK1epsilon	93.57	93.11
CK1g1	99.77	98.49
CK1g2	94.37	91.99
CK1g3	111.12	108.95
CK2a	107.22	103.57
CK2a2	103.55	93.63
CLK1	96.42	96.05
CLK2	98.76	95.86
CLK3	105.71	104.46
CLK4	85.28	79.36
COT1/MAP3K8	96.40	95.04
CSK	102.14	100.03
CTK/MATK	93.20	88.63
DAPK1	99.13	93.56
DAPK2	107.80	106.46
DCAMKL1	107.43	94.25
DCAMKL2	96.24	95.61
DDR1	26.10	22.37
DDR2	95.22	93.82
DLK/MAP3K12	118.20	110.78

DMPK	99.55	98.96
DMPK2	101.99	101.66
DRAK1/STK17A	99.80	94.70
DYRK1/DYRK1A	92.05	89.43
DYRK1B	97.20	94.37
DYRK2	101.14	96.60
DYRK3	111.26	106.53
DYRK4	102.36	101.91
EGFR	97.70	94.20
EPHA1	108.32	105.60
EPHA2	103.97	102.52
EPHA3	98.84	97.34
EPHA4	95.88	93.36
EPHA5	102.64	102.61
EPHA6	80.14	79.09
EPHA7	90.58	90.51
EPHA8	104.11	102.95
EPHB1	101.43	97.92
EPHB2	99.39	98.97
EPHB3	95.21	93.62
EPHB4	100.28	95.13
ERBB2/HER2	81.14	81.00
ERBB4/HER4	103.53	102.66
ERK1	100.24	96.82
ERK2/MAPK1	104.45	103.14
ERK5/MAPK7	96.67	94.91
ERK7/MAPK15	93.74	93.26
FAK/PTK2	104.47	104.37
FER	108.36	107.37
FES/FPS	106.48	102.27
FGFR1	96.78	92.87
FGFR2	101.05	99.62
FGFR3	105.43	105.22
FGFR4	90.54	88.55
FGR	97.93	97.43
FLT1/VEGFR1	95.24	94.96
FLT3	52.31	49.96
FLT4/VEGFR3	94.83	93.70
FMS	98.10	95.32
FRK/PTK5	99.06	98.87
FYN	95.74	95.62
GCK/MAP4K2	99.31	96.66
GLK/MAP4K3	89.55	86.31
GRK1	97.06	93.90
GRK2	100.80	98.83
GRK3	103.83	103.13
GRK4	97.62	96.92

GRK5	95.92	91.94
GRK6	100.41	98.86
GRK7	98.21	96.81
GSK3a	95.11	94.32
GSK3b	105.81	102.14
Haspin	75.34	73.79
HCK	95.11	91.74
HGK/MAP4K4	88.74	87.76
HIPK1	97.60	97.02
HIPK2	96.27	93.90
HIPK3	111.46	109.76
HIPK4	89.05	87.47
HPK1/MAP4K1	104.12	104.09
IGF1R	95.74	94.64
IKKa/CHUK	98.39	97.00
IKKb/IKBKB	93.91	92.75
IKKe/IKBKE	106.32	106.15
IR	99.50	98.80
IRAK1	98.37	97.87
IRAK4	104.16	102.54
IRR/INSRR	97.41	97.19
ITK	98.23	97.93
JAK1	100.05	97.05
JAK2	103.54	101.01
JAK3	93.86	88.56
JNK1	103.86	102.16
JNK2	95.38	94.40
JNK3	105.45	103.82
KDR/VEGFR2	100.28	99.08
KHS/MAP4K5	99.82	99.26
LATS1	101.02	97.67
LATS2	88.71	88.56
LCK	91.94	88.69
LCK2/ICK	103.52	102.58
LIMK1	59.75	59.36
LIMK2	90.34	87.80
LKB1	102.81	102.68
LOK/STK10	92.99	91.89
LRRK2	86.37	86.30
LYN	92.24	85.50
LYN B	92.04	90.33
MAPKAPK2	106.93	106.50
MAPKAPK3	101.76	100.82
MAPKAPK5/PRAK	96.87	96.67
MARK1	99.61	98.93
MARK2/PAR-1Ba	93.35	89.08
MARK3	101.19	99.63

MARK4	97.78	97.31
MEK1	98.28	93.60
MEK2	95.11	92.18
MEK3	100.66	98.74
MEKK1	101.54	97.65
MEKK2	111.16	109.95
MEKK3	98.25	97.97
MELK	83.79	81.49
MINK/MINK1	104.29	94.81
MKK4	105.06	104.59
MKK6	118.03	116.97
MKK7	99.98	99.42
MLCK/MYLK	91.69	87.70
MLCK2/MYLK2	103.17	102.86
MLK1/MAP3K9	88.28	87.22
MLK2/MAP3K10	50.42	42.74
MLK3/MAP3K11	76.70	75.74
MNK1	94.12	93.01
MNK2	96.45	96.15
MRCKa/CDC42BPA	103.04	101.44
MRCKb/CDC42BPB	103.78	103.10
MSK1/RPS6KA5	102.47	101.40
MSK2/RPS6KA4	110.25	104.23
MSSK1/STK23	102.64	99.10
MST1/STK4	94.81	89.73
MST2/STK3	107.26	107.05
MST3/STK24	77.62	76.42
MST4	91.44	89.59
MUSK	106.73	105.16
MYLK3	105.25	104.12
MYO3b	97.67	96.91
NEK1	89.35	86.83
NEK11	93.85	91.34
NEK2	101.43	100.58
NEK3	95.42	89.43
NEK4	92.73	92.38
NEK5	96.35	104.08
NEK6	106.06	106.04
NEK7	94.53	93.23
NEK9	91.82	90.91
NIM1	99.63	98.11
NLK	96.52	94.99
OSR1/OXSR1	104.28	101.52
P38a/MAPK14	93.16	88.17
P38b/MAPK11	104.02	100.77
P38d/MAPK13	105.95	103.61
P38g	101.55	100.79

p70S6K/RPS6KB1	98.53	98.27
p70S6Kb/RPS6KB2	100.19	98.18
PAK1	98.77	93.42
PAK2	113.39	112.31
PAK3	105.23	103.95
PAK4	98.60	97.30
PAK5	95.21	93.79
PAK6	108.32	103.77
PASK	81.03	80.68
PBK/TOPK	97.61	97.20
PDGFRa	95.35	92.90
PDGFRb	97.27	96.70
PKD1/PDK1	97.04	96.18
PEAK1	98.41	97.16
PHKg1	90.45	88.66
PHKg2	98.13	97.32
PIM1	89.06	88.93
PIM2	103.70	101.95
PIM3	92.08	90.89
PKA	96.79	94.68
PKAcb	98.46	97.13
PKAcg	100.18	97.71
PKCa	101.69	98.67
PKCb1	101.35	99.72
PKCb2	92.94	90.05
PKCd	107.92	107.10
PKCepsilon	101.94	97.77
PKCeta	106.69	104.56
PKCg	109.85	106.95
PKCiota	100.38	95.89
PKCmu/PRKD1	103.34	101.87
PKCnu/PRKD3	94.75	94.46
PKCtheta	104.77	103.18
PKCzeta	99.96	99.53
PKD2/PRKD2	96.77	96.38
PKG1a	94.40	92.89
PKG1b	78.22	73.54
PKG2/PRKG2	97.94	96.96
PKN1/PRK1	103.61	101.04
PKN2/PRK2	99.93	92.49
PKN3/PRK3	96.63	96.20
PLK1	95.95	93.21
PLK2	106.69	101.40
PLK3	99.93	96.20
PLK4/SAK	101.64	101.53
PRKX	107.09	103.60
PYK2	93.47	88.55

RAF1	76.20	73.07
RET	92.39	88.75
RIPK2	126.38	119.18
RIPK3	104.71	97.85
RIPK5	90.42	89.55
ROCK1	97.44	96.73
ROCK2	82.77	77.87
RON/MST1R	100.00	96.23
ROS/ROS1	113.88	107.66
RSK1	101.27	98.55
RSK2	103.37	102.89
RSK3	96.98	96.60
RSK4	97.66	97.64
SGK1	105.22	105.09
SGK2	101.87	100.12
SGK3/SGKL	98.69	97.77
SIK1	95.57	92.81
SIK2	89.70	89.32
SIK3	92.96	91.52
SLK/STK2	92.11	89.83
SNARK/NUAK2	110.74	108.53
SRMS	94.36	93.85
SRPK1	91.82	91.74
SRPK2	98.41	98.34
SSTK/TSSK6	102.92	97.52
STK16	92.97	92.29
STK22D/TSSK1	101.08	100.03
STK25/YSK1	110.90	110.05
STK32B/YANK2	105.13	103.11
STK32C/YANK3	109.14	107.20
STK33	101.31	97.78
STK38/NDR1	83.60	80.37
STK38L/NDR2	99.57	98.57
STK39/STLK3	94.91	92.76
SYK	108.36	102.34
TAK1	104.26	102.92
TAOK1	97.06	96.02
TAOK2/TAO1	101.88	101.28
TAOK3/JIK	108.96	100.85
TBK1	93.04	87.88
TEC	100.64	100.16
TESK1	100.40	100.09
TGFBR2	110.49	110.23
TIE2/TEK	90.28	90.13
TLK1	101.79	101.77
TLK2	107.03	106.33
TNIK	87.77	86.49

TNK1	94.42	94.32
TRKA	98.02	97.42
TRKB	102.28	100.88
TRKC	91.26	84.37
TSSK2	100.01	99.07
TSSK3/STK22C	118.82	117.97
TTBK1	110.91	101.99
TTBK2	99.73	97.15
TXK	84.65	82.37
TYK1/LTK	92.48	91.02
TYK2	102.46	100.45
TYRO3/SKY	99.22	94.03
ULK1	102.14	101.64
ULK2	102.09	102.05
ULK3	103.84	101.92
VRK1	97.45	97.29
VRK2	100.43	95.86
WEE1	105.21	103.90
WNK1	109.58	105.57
WNK2	97.13	96.43
WNK3	102.34	101.60
YES/YES1	90.18	85.83
ZAK/MLTK	96.89	96.18
ZAP70	101.58	101.51
ZIPK/DAPK3	104.23	104.14