Supplementary data

Structure-activity relationship study of acridine analogs as haspin and DYRK2 kinase inhibitors

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***In vitro* kinase inhibition assay profile.**

Compound **1** was screened against a panel of 270 kinases at 10 μM at Carna Biosciences (http://www.carnabio.com/english/). The percent inhibitions for each kinase are listed in Table S1 with those demonstrating > 90% inhibition highlighted in red. Also, an interaction map for **1** is shown in Figure S1. The kinase tree was generated using percent inhibition values verses controls and the 'TreeSpot' kinome data visualization tool available as a web-based application (http://www.kinomescan.com/login.aspx). Only kinases with percent control values < 30% are displayed. One kinase (PKCB2, an isoform of PKCB) that meets this criterion is not displayed in Figure S1. Although haspin was not available in the original profile, it was subsequently found to give 100% inhibition of haspin activity in the Carna Bioscience assay at 10 μM and it has therefore been added to Figure S1 for comparison. The kinase dendrogram was adapted by KINOME*scan* and is reproduced with permission from *Science* (http://www.sciencemag.org/) and Cell Signaling Technology, Inc. (http://www.cellsignal.com/).

Table S1.

Kinase	% Inhibition **1** 10 μM
ABL	47
ABL(T315I)	64
ACK (TNK2)	37
ALK	41
ARG	26
AXL	55
BLK	72
BMX	29
BRK	33
BTK	64
CSK	57
CTK	48
DDR1	4
DDR2	54
EGFR	47
EGFR(L858R)	40
EGFR(T790M)	36
EPHA1	64
EPHA2	33
EPHA3	0
EPHA4	0
EPHA5	30
EPHA6	32
EPHA7	28

EPHA8	67
EPHB1	0
EPHB2	0
EPHB3	32
EPHB4	46
FAK	26
FER	34
FES	11
FGFR1	62
FGFR2	43
FGFR3	48
FGFR3(K650E)	73
FGFR3(K650M)	84
FGFR4	71
FGR	34
FLT1	33
FLT3	81
FLT4	72
FMS	37
FRK	39
FYN	51
HCK	58
HER2	9
HER4	28
IGF1R	39
INSR	52
IRR (INSRR)	47
ITK	28
JAK1	17
JAK2	32
JAK3	57
KDR (VEGFR2)	37
KIT	56
KIT(T670I)	67
KIT(V560G)	49
LCK	45
LTK	75
LYNa	46
LYNb	46
MER	63
MET	59
MET(Y1235D)	75
MUSK	32
PDGFRa	81
PDGFRa(T674I)	80
PDGFRb	56
PYK2	46
RET	51

RON	65
ROS	90
SRC	26
SRM	52
SYK	48
TEC	31
TIE2	52
TNK1	46
TRKA	50
TRKB	94
TRKC	68
TXK	32
TYK2	28
TYRO3	0
YES	56
ZAP70	65
AKT1	31
AKT2	37
AKT3	26
AMPKa1/b1/g1	56
AMPKa2/b1/g1	41
AurA	63
AurB-INCENP	40
AurC	43
BMPR1A	56
BRAF	8
BRAF(V600E)	7
BRSK1	22
BRSK2	0
CaMK1a	13
CaMK1d	15
CaMK2a	8
CaMK2b	18
CaMK2g	7
CaMK2d	34
CaMK4	14
CDC2-CycB1	69
CDC8-Dbf4	9
CDK2-CycA	56
CDK3-CycE1	55
CDK4-CycD4	26
CDK5-p25	61
CDK6-CycD3	0
CDK7-CycH-MAT1	45
CDK9-CycT1	46
CGK2 (PRKG2)	67
CHK1	24
CHK2	22

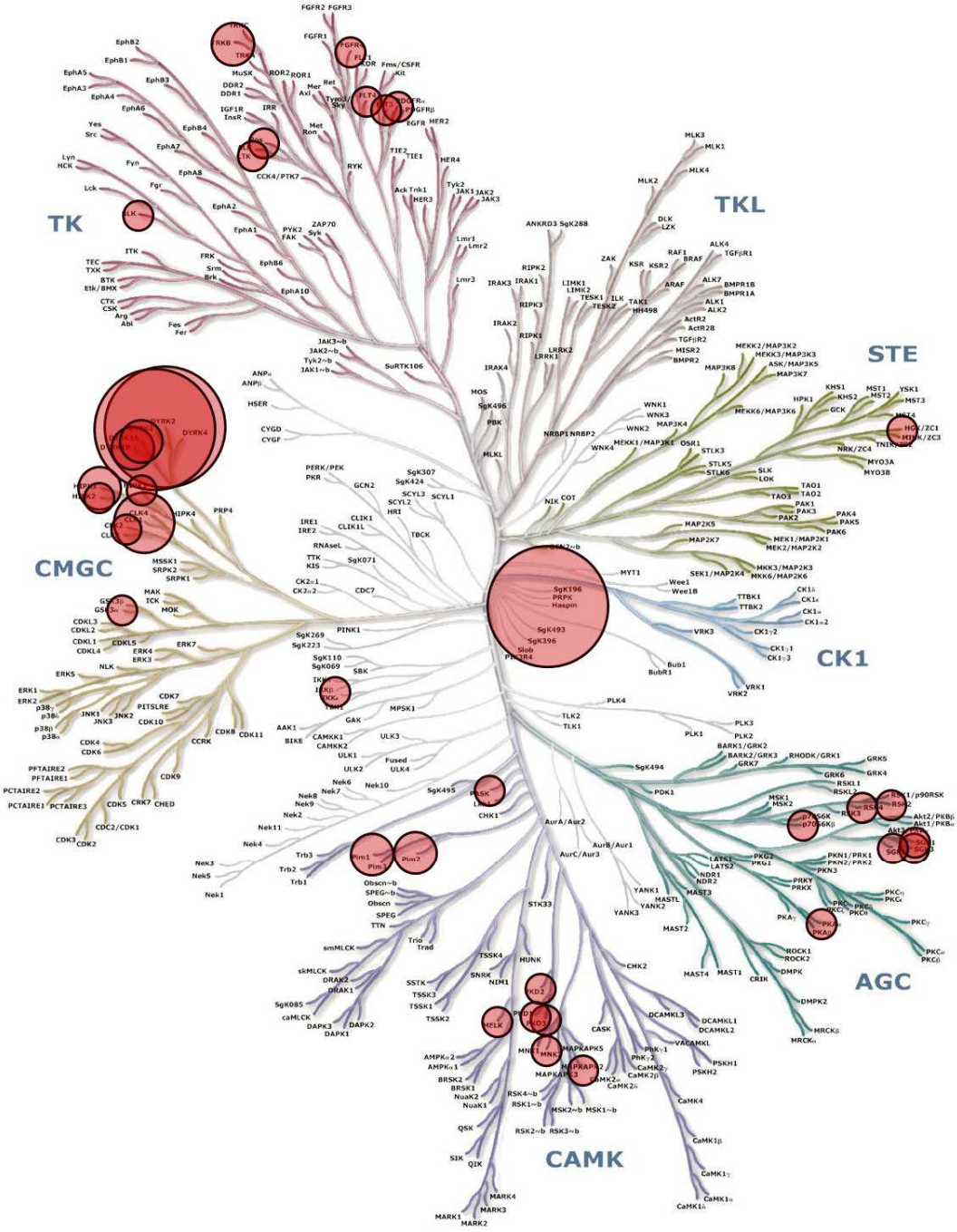
CK1a	16
CK1d	34
CK1e	62
CK1g1	22
CK1g2	28
CK1g3	18
CK2a1/b	49
CLK1	98
CLK2	79
CLK3	56
COT (MAP3K8)	0
CRIK	6
DAPK1	29
DCAMKL2	29
DLK (MAP3K12)	18
DYRK1A	94
DYRK1B	93
DYRK2	100
DYRK3	100
EEF2K	28
Erk1	51
Erk2	43
Erk5	61
GSK3a	79
GSK3b	65
HGK (MAP4K4)	72
HIPK1	92
HIPK2	85
HIPK3	90
IKKa	7
IKKb	75
IKKe	1
IRAK1	25
IRAK4	2
JNK1	27
JNK2	20
JNK3	32
LIMK1	1
LKB1-MO25a-STRADa	0
LOK	0
MAP2K1	24
MAP2K2	11
MAP2K3	48
MAP2K4	0
MAP2K5	0
MAP2K6	65
MAP2K7	4
MAP3K1	0

MAP3K2	0
MAP3K3	10
MAP3K4	0
MAP3K5	7
MAP4K2 (GCK)	55
MAPKAPK2	71
MAPKAPK3	61
MAPKAPK5	59
MARK1	32
MARK2	25
MARK3	28
MARK4	36
MELK	84
MGC42105 (NIM1)	25
MINK	36
MLK1 (MAP3K9)	35
MLK2 (MAP3K10)	66
MLK3 (MAP3K11)	63
MNK1	43
MNK2	78
MRCKa	24
MRCKb	39
MSK1 N-term (RPS6KA5)	37
MSK2 N-term (RPS6KA4)	39
MSSK1 (SRPK3)	53
MST1	37
MST2	5
MST3	35
MST4	41
NDR1	40
NEK1	59
NEK2	30
NEK6	63
NEK7	38
NEK9	50
NuaK1	0
p38a	56
p38b	46
p38d	14
p38g	24
p70S6K (RPS6KB1)	70
p70S6Kb (RPS6KB2)	78
PAK1	0
PAK2	0
PAK3	54
PAK5 (PAK7)	34
PAK6	8
PASK	71

PBK	63
PDHK2	26
PDHK4	14
PDK1	19
PEK (EIF2AK3)	16
PGK (PRKG1)	36
PHKG1	56
PHKG2	32
PIM1	95
PIM2	91
PKACa (PRKACA)	74
PKCa	7
PKCb1	9
PKCb2	19
PKCd	28
PKCe	23
PKCg	25
PKCh	15
PKCi	42
PKCq	26
PKCz	55
PKD1	85
PKD2	87
PKD3	72
PKN1	34
PKR (EIF2AK2)	15
PLK1	35
PLK2	10
PLK3	52
PLK4	50
PRKX	61
RAF1	15
ROCK1	6
ROCK2	12
RSK1 N-term (RPS6KA1)	67
RSK2 N-term (RPS6KA3)	74
RSK3 N-term (RPS6KA2)	58
RSK4 N-term (RPS6KA6)	74
SGK	77
SGK2	76
SGK3	76
skMLCK	33
SLK	28
SRPK1	30
SRPK2	0
TAK1-TAB1 (MAP3K7)	17
TAOK2	12
TBK1	32

TSSK1	17
TSSK2	60
TTK (MPS1)	11
WEE1	4
WNK1	0
PIK3CA/PIK3R1	38

Figure S1.




Percent Control
0%
0.1%
0.1-1%
1-5%
5-10%
10-30%

TR-FRET haspin assay

Kinase reactions were performed in 50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, 0.01% Brij-35 using Proxiplate 384 Plus white assay plates (PerkinElmer). MBP-Haspin at 0.17 nM (0.05 nM enzyme final) and 0.33 μM biotinylated H3(1-21) peptide (0.1 μM peptide final, at the K_m) in a volume of 3 μL kinase buffer were added to 2 μL solutions of compound. The kinase reaction was initiated by addition of 5 μL of 400 μM ATP per reaction (200 μM ATP final, near K_m). The reaction was incubated for 10 minutes at room temperature. Reaction was terminated by addition of 10 μL 50 mM EDTA, 2 nM Europium labeled anti-Histone H3T3ph antibody, 40 nM Streptavidin-APC. After a two hour incubation at room temperature, TR-FRET measurements were performed using a PHERAstar HTS microplate reader (BMG Labtech, Offenberg, Germany), and were expressed as ratios of acceptor fluorescence at 665 nm over donor fluorescence at 620 nm.

DYRK2 inhibitor assay

Test compounds in 2.5 μL (0.001-67 μM final concentration) were incubated in 25 μL in the presence of 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 10 μM ATP (K_M value), trace amounts of radioactively labeled γ^{33}P-ATP (50 nM, PerkinElmer), 10 nM GST-DYRK2 enzyme (Carna Biosciences, Japan) and 150 μM biotin-Woodtide peptide substrate (biotin-KKISGRLSPIMTEQ-NH_2, Abgent, at K_M value) at room temperature. Reactions were stopped after 10 min by addition of 30 mM EDTA followed by spotting 10 μL of the reaction mix on to P81 phosphocellulose filter (Whatman). P81 filters were washed three times for 10 min in 0.75% phosphoric acid to remove free γ^{33}P-ATP and then air-dried. γ^{33}P-ATP incorporation was measured using a MicroBeta liquid scintillation counter (PerkinElmer). Background level of ^{33}P incorporation was defined from control reaction lacking peptide.