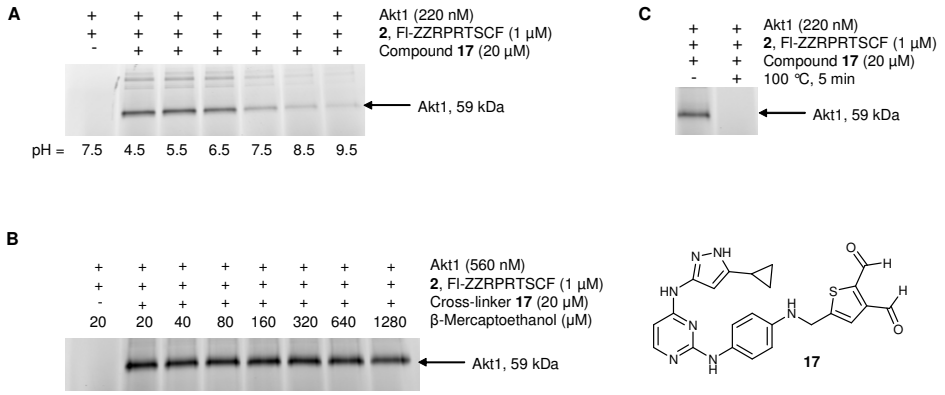
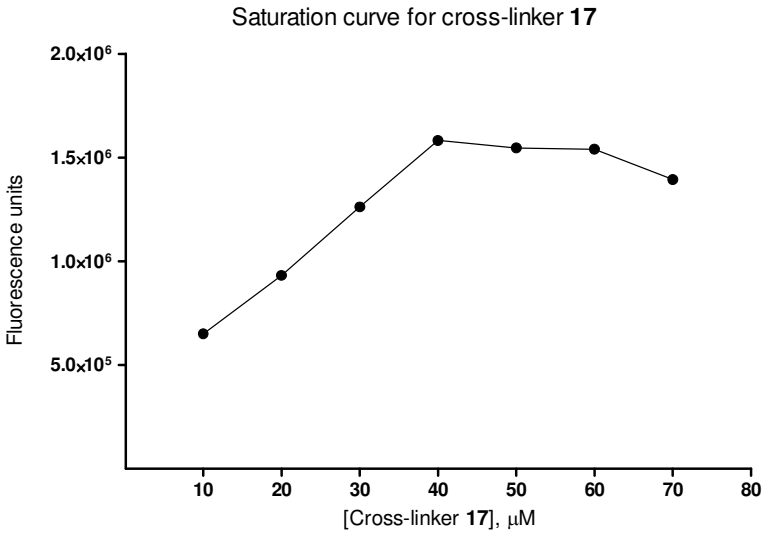
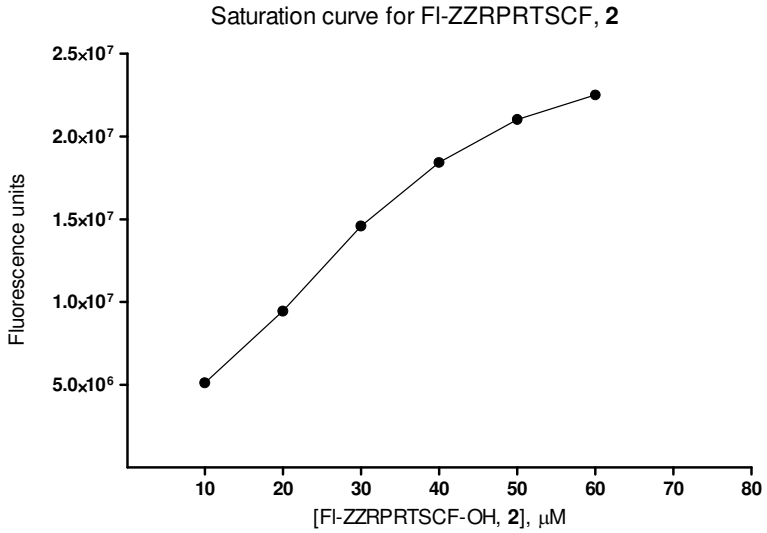


Supplemental Figure 1. (A) Cross-linking of Akt1 and fluorescent substrate peptide **2** with cross-linker **17** at different pH values. Akt1 (220 nM) and fluorescent peptide **2** (1 μM) were treated with cross-linker **17** (20 μM) for 20 min at r.t. at different pH values, followed by SDS-PAGE and in gel scanning fluorescence. (excitation at 480 nM, emission collected with a 520 nM band pass filter) (B) Akt1 (560 nM) and fluorescent peptide **2** (1 μM) were treated with cross-linker **17** (20 μM) for 20 min at r.t. in the presence of increasing concentrations of β-mercaptoethanol, followed by SDS-PAGE and in gel fluorescence scanning. (C) Decomposition of the cross-linked product upon heating. Akt1 (220 nM) and fluorescent peptide **2** (1 μM) were treated with cross-linker **17** (20 μM) for 20 min at r.t., then boiled for 5 min, followed by SDS-PAGE and in gel scanning fluorescence. (excitation at 480 nM, emission collected with a 520 nM band pass filter)



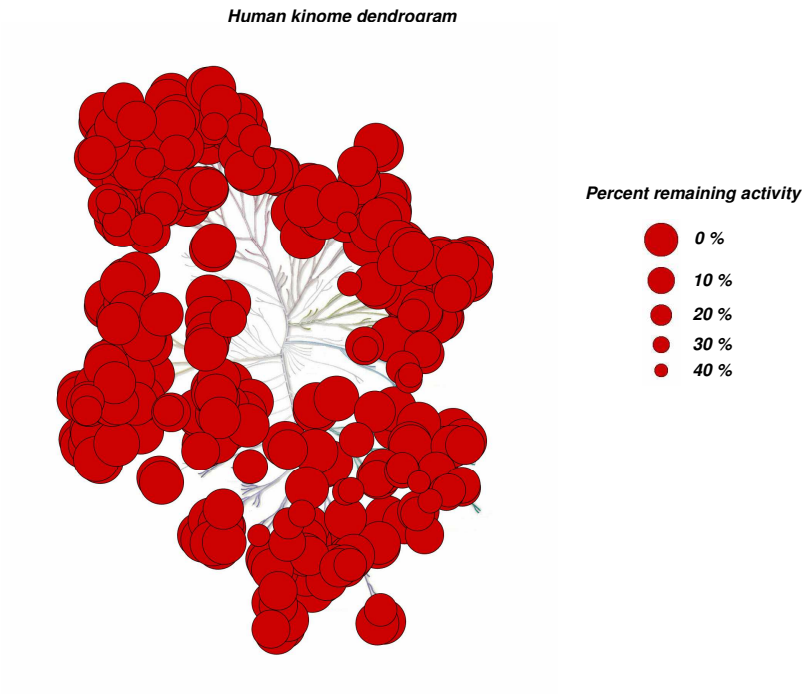
Supplemental Figure 2. Concentration dependence for the crosslinking reaction at different concentrations of cross-linker **17**. Akt1 (220 nM) and fluorescent peptide **2** (1 μM) were treated with indicated concentrations of the cross-linker **17** for 20 min at r.t. at pH 6.5, followed by SDS-PAGE and in gel scanning fluorescence. (excitation at 480 nM, emission collected with a 520 nM band pass filter).



Supplemental Figure 3. Concentration dependence for the crosslinking reaction at different concentrations of Fl-ZZRPRTSCF, **2**. Akt1 (220 nM) and different concentrations of fluorescent peptide **2** were treated with the cross-linker **17** (20 μM) for 20 min at r.t., at pH 6.5, followed by SDS-PAGE and in gel scanning fluorescence. (excitation at 480 nM, emission collected with a 520 nM band pass filter).

Materials. Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Sodium cyanoborohydride, butyl lithium, thiophene-2,3-dicarboxaldehyde, ethyleneglycol, N-hydroxysuccinimide, oxalylchloride, N,N-diisopropyl-N-ethylamine, 2-aminoethanol, β-mercaptoethanol (BME), trifluoroacetic acid (TFA). Anhydrous, low-amine N,N-dimethylformaide (DMF) was purchased from EM science. Recombinant AKT1 was purchased from Calbiochem. Fluorescent peptide substrates were prepared as described earlier.[1] Typhoon 9400 was used for all direct in-gel scanning fluorescent measurements (490 nm excitation band, 520 emission filter)

Kinase assays and binding constant measurements for compounds 3 and 4. All measurements were performed as described earlier.[2]



Supplemental Figure 4. Small molecule-kinase interaction map for compound **3** that was screened against a panel of 359 human kinases. Kinases found to bind are marked with red circles, where larger circles indicate higher-affinity binding. The kinase dendrogram was adapted and is reproduced with permission from *Science* (http://www.sciencemag.org/) and Cell Signaling Technology, Inc. (http://www.cellsignal.com/).

Supplemental Table 1. Kinase binding interactions for compounds **3** and **4** with 402 kinases.

Kinase	Compound **3,** % remaining kinase activity compared to control	Compound **4**, % remaining kinase activity compared to control
AAK1	0.5	0.65
ABL1	4.8	3.2
ABL1(E255K)	12	10
ABL1(F317I)	40	40
ABL1(F317L)	21	21
ABL1(H396P)	4.8	2.8
ABL1(M351T)	3.8	2.1
ABL1(Q252H)	3.5	1.5
ABL1(T315I)	0	0
ABL1(Y253F)	4	2.6
ABL2	2.6	0.5
ACVR1	0.1	0
ACVR1B	0.8	0.8
ACVR2A	0.65	0.85
ACVR2B	1.9	1.4
ACVRL1	3.4	1.6
ADCK3	12	85
ADCK4	2.6	17
AKT1	100	100
AKT2	94	100
AKT3	100	100

ALK	0.45	0.15
AMPK-alpha1	0.75	0.15
AMPK-alpha2	0.55	0.6
ANKK1	1.4	0.15
ARK5	0.55	0.45
ASK1	4.5	68
ASK2	5.1	15
AURKA	0.7	1.5
AURKB	0.45	0
AURKC	0.25	0.65
AXL	0.2	0
BIKE	0.2	0.4
BLK	0.3	0.05
BMPR1A	2.4	1
BMPR1B	0	0.05
BMPR2	0	0.55
BMX	8.2	12
BRAF	47	88
BRAF(V600E)	50	99
BRK	13	27
BRSK1	2.3	1.9
BRSK2	4.1	0.7
BTK	0.65	9.3
CAMK1	0.55	2.4
CAMK1D	2.8	6.8

CAMK1G	15	35
CAMK2A	4	7.4
CAMK2B	4	9
CAMK2D	7.7	7.4
CAMK2G	15	18
CAMK4	100	49
CAMKK1	1.2	6.4
CAMKK2	4.4	1.4
CDC2L1	2.8	28
CDC2L2	3.4	40
CDK11	7.4	96
CDK2	0	0
CDK3	0	0
CDK5	0	0.65
CDK7	0	0.1
CDK8	18	88
CDK9	0.2	0.8
CDKL2	0.85	0.35
CDKL3	0.2	0
CDKL5	0	0.15
CHEK1	6.6	0
CHEK2	3.6	2.9
CIT	1.9	3.3
CLK1	0.5	0.2
CLK2	0.2	0.35

CLK3	3.5	2.2
CLK4	0.85	2.4
CSF1R	4.2	0.1
CSK	20	65
CSNK1A1L	39	0.95
CSNK1D	10	0.05
CSNK1E	6.2	0.7
CSNK1G1	28	22
CSNK1G2	14	0.4
CSNK1G3	22	3.2
CSNK2A1	2	1.6
CSNK2A2	3	0.8
CTK	3.9	2.6
DAPK1	0.45	0.75
DAPK2	1.8	5.4
DAPK3	0.6	0.9
DCAMKL1	3.2	0.3
DCAMKL2	0.15	0.35
DCAMKL3	0.2	0.15
DDR1	0.45	2
DDR2	1.8	2.5
DLK	2.7	2.1
DMPK	1.8	0.85
DMPK2	7.7	1.4
DRAK1	0.05	0.05

DRAK2	0.4	0.3
DYRK1A	0.1	0.15
DYRK1B	0.3	0.3
DYRK2	0.35	0.7
EGFR	16	47
EGFR(E746-A750del)	18	9.6
EGFR(G719C)	16	45
EGFR(G719S)	22	50
EGFR(L747-E749del, A750P)	10	7.5
EGFR(L747-S752del, P753S)	20	22
EGFR(L747-T751del,Sins)	15	16
EGFR(L858R)	15	18
EGFR(L858R,T790M)	12	9.2
EGFR(L861Q)	13	25
EGFR(S752-I759del)	19	29
EPHA1	1.2	3.4
EPHA2	1	9.2
EPHA3	0.05	1.8
EPHA4	4.6	12
EPHA5	0.05	1.8
EPHA6	0.45	1.3
EPHA7	0.1	0.15
EPHA8	20	46
EPHB1	0	0.25
EPHB2	5	14

EPHB3	6.7	7.9
EPHB4	1.1	5
EPHB6	0.25	0.7
ERBB2	76	100
ERBB3	5.2	88
ERBB4	30	59
ERK1	0	5
ERK2	0	4.8
ERK3	0.2	23
ERK4	0.3	29
ERK5	0	3.6
ERK8	0.1	0.1
ERN1	0.35	1.6
FAK	0.2	0.5
FER	2.1	0.25
FES	13	3.6
FGFR1	0.25	0.05
FGFR2	0.8	0.7
FGFR3	0.95	1.4
FGFR3(G697C)	1.2	0.25
FGFR4	5	0.85
FGR	2	1.1
FLT1	0.25	0.1
FLT3	0.1	0
FLT3(D835H)	0	0

FLT3(D835Y)	0.35	0.35
FLT3(ITD)	0	0.2
FLT3(K663Q)	0.4	0.45
FLT3(N841I)	0.3	0.2
FLT4	0	0
FRK	3.8	9.2
FYN	1	0.85
GAK	0.5	3.6
GCN2(Kin.Dom.2,S808G)	0.2	0.1
GRK1	2	0.85
GRK4	0	0
GRK7	0.3	0.2
GSK3A	0	0
GSK3B	0.05	0.05
HCK	1.8	0.5
HIPK1	0.1	0.55
HIPK2	0.2	0.45
HIPK3	0.35	0.2
HIPK4	0	0
HPK1	2.1	0.85
HUNK	23	5
ICK	0.05	0.45
IGF1R	0.1	0.5
IKK-alpha	0.55	4.2
IKK-beta	1.4	44

IKK-epsilon	3.4	1.9
INSR	0.4	1.9
INSRR	0.25	0.4
IRAK1	0.35	0.1
IRAK3	0.55	2.6
ITK	0.05	0.2
JAK1(JH1domain-catalytic)	0.65	3.4
JAK1(JH2domain-pseudokinase)	0	0
JAK2(JH1domain-catalytic)	0.55	1.6
JAK3(JH1domain-catalytic)	0	0.75
JNK1	3.3	68
JNK2	3.8	92
JNK3	3.8	23
KIT	0.55	0
KIT(D816V)	0.7	0.45
KIT(L576P)	0.15	0
KIT(V559D)	0.1	0
KIT(V559D,T670I)	6.4	0.1
KIT(V559D,V654A)	16	2.2
LATS1	4	3
LATS2	3.9	1.7
LCK	0.25	0.35
LIMK1	1.7	1.4
LIMK2	3.8	10
LKB1	0	0

LOK	0.25	0.05
LTK	11	3.6
LYN	8.4	4.1
LZK	0.3	0.3
MAK	0.45	20
MAP3K1	28	0
MAP3K15	5.4	11
MAP3K2	3.4	0.7
MAP3K3	0.1	0.1
MAP3K4	36	9.3
MAP4K2	0.4	0.3
MAP4K3	1.4	1.2
MAP4K4	1	0.45
MAP4K5	0.5	0.15
MAPKAPK2	46	15
MAPKAPK5	0.1	0
MARK1	8.2	0.45
MARK2	1.6	0.85
MARK3	0	0
MARK4	2.6	0
MAST1	0	0
MEK1	0.1	0.2
MEK2	0.1	0.2
MEK3	0.45	0.75
MEK4	0.3	37

MEK6	0.4	18
MELK	9.6	0
MERTK	0	0
MET	0.25	0.35
MET(M1250T)	0.1	0.05
MET(Y1235D)	0.55	1.6
MINK	0	0
MKNK1	2.7	2.8
MKNK2	7	14
MLCK	0.05	0
MLK1	0.1	0.05
MLK2	6.4	1.2
MLK3	1.4	0.15
MRCKA	43	49
MRCKB	39	19
MST1	1.4	1.2
MST1R	69	54
MST2	3.2	0.2
MST3	0.15	0.4
MST4	0.15	0.1
MUSK	0.4	0.55
MYLK	6	8.2
MYLK2	0.2	0.2
MYO3A	1.4	1.3
MYO3B	9.4	1.8

NDR1	2	3.6
NDR2	9.2	24
NEK1	1.6	9.6
NEK2	8.2	7.2
NEK5	0.65	0.85
NEK6	0	0
NEK7	0.3	0.1
NEK9	0.25	0.15
NIM1	17	2.4
NLK	4.3	33
OSR1	2.6	9.2
p38-alpha	82	100
p38-beta	19	100
p38-delta	0.7	100
p38-gamma	14	100
PAK1	3.6	19
PAK2	16	38
PAK3	3	2.4
PAK4	0.8	2.8
PAK6	1.9	6.2
PAK7	0.2	0.25
PCTK1	0.25	0.15
PCTK2	0	1.6
PCTK3	0	0.35
PDGFRA	14	0.35

PDGFRB	0.05	0
PDPK1	12	17
PFTAIRE2	0.2	3.4
PFTK1	0	2.3
PHKG1	3.6	3.5
PHKG2	0.5	2.3
PIK3C2B	46	100
PIK3C2G	12	97
PIK3CA	58	98
PIK3CA(C420R)	82	100
PIK3CA(E542K)	99	100
PIK3CA(E545A)	83	100
PIK3CA(E545K)	92	100
PIK3CA(H1047L)	65	96
PIK3CA(H1047Y)	77	100
PIK3CA(M1043I)	100	98
PIK3CA(Q546K)	88	100
PIK3CB	64	100
PIK3CD	33	87
PIK3CG	8.6	69
PIK4CB	0.15	1.8
PIM1	49	100
PIM2	12	100
PIM3	45	100
PIP5K1A	5.3	0

PIP5K2B	0.75	1.2
PKAC-alpha	40	26
PKAC-beta	28	26
PKMYT1	81	52
PKN1	2	2
PKN2	8	3.8
PLK1	4.8	3
PLK2	0.35	0.25
PLK3	0.95	5.2
PLK4	0.7	0.4
PRKCD	14	4.7
PRKCE	9.3	1.5
PRKCH	10	8.6
PRKCQ	11	26
PRKD1	0.85	0.55
PRKD2	0.05	0
PRKD3	0.05	0
PRKG1	11	3.8
PRKG2	16	9.2
PRKR	0.45	2
PRKX	30	3.6
PRP4	2.4	7.5
PYK2	1.5	1
QSK	7.9	2.9
RAF1	100	100

RET	0	0
RET(M918T)	0	0
RET(V804L)	0	0
RET(V804M)	0	0
RIOK1	0.4	2.2
RIOK2	0.7	0.45
RIOK3	0.3	0.45
RIPK1	0.3	1.8
RIPK2	4.2	12
RIPK4	0.05	0.05
ROCK1	4.4	4.6
ROCK2	13	13
ROS1	0.75	1.2
RPS6KA1(Kin.Dom.1-N-terminal)	0.4	0.3
RPS6KA1(Kin.Dom.2-C-terminal)	0.95	35
RPS6KA2(Kin.Dom.1-N-terminal)	1	0.75
RPS6KA2(Kin.Dom.2-C-terminal)	0.35	4.6
RPS6KA3(Kin.Dom.1-N-terminal)	7.4	4.4
RPS6KA4(Kin.Dom.1-N-terminal)	2.4	0.45
RPS6KA4(Kin.Dom.2-C-terminal)	0.1	0.25
RPS6KA5(Kin.Dom.1-N-terminal)	2.3	3
RPS6KA5(Kin.Dom.2-C-terminal)	5.1	14
RPS6KA6(Kin.Dom.1-N-terminal)	3.9	3.5
RPS6KA6(Kin.Dom.2-C-terminal)	2	42
SBK1	0	0

SgK085	0	0.4
SgK110	2.9	2.2
SIK	4.3	4.2
SIK2	8	7
SLK	0.2	0.1
SNARK	0.35	0.55
SRC	0.1	0.1
SRMS	0.35	6.8
SRPK1	0.4	0
SRPK2	1.4	1.4
SRPK3	0.3	0.15
STK16	0.25	1.4
STK33	2.4	0.85
STK35	9.8	3.2
STK36	2.5	13
STK39	0.6	2.1
SYK	0.6	0.15
TAK1	0.3	0.2
TAO1	0	3.1
TAOK1	0.1	0.25
TAOK3	0.1	0.3
TBK1	2.7	2.4
TEC	28	93
TESK1	36	24
TGFBR1	0.5	0.5

TGFBR2	0.15	0.2
TIE1	0.6	0.3
TIE2	1.2	0.05
TLK1	0.05	0.35
TLK2	0	0.2
TNIK	0.2	0.05
TNK1	0	0.05
TNK2	6.6	1.4
TNNI3K	34	17
TRKA	0.1	0.35
TRKB	0	0.1
TRKC	0	0.1
TSSK1B	31	9.1
TTK	0.55	34
TXK	5	4.6
TYK2(JH1domain-catalytic)	0	2.9
TYK2(JH2domain-pseudokinase)	0.9	0.7
TYRO3	22	25
ULK1	0.1	0
ULK2	0.25	0.15
ULK3	0	0
VEGFR2	1.2	0
WEE1	8.4	3.4
WEE2	26	5.8
YANK2	25	4

YANK3	24	3.4
YES	2.3	0.7
YSK1	0.25	1.5
YSK4	0.05	0
ZAK	5.2	2
ZAP70	8.2	3.2

Supplemental Table 2. Representative dissociation constants (K_d) for 40 representative kinases that displayed binding interactions with the kinase inhibitor **3.**

Kinase	K_d, nM	Kinase	K_d, nM
ABL1	834	FYN	241
ABL1(T315I)	96	HCK	4990
AKT1	>40000	IGF1R	1480
AURKA	87.5	JAK2(Kin.Dom.2)	93.2
AURKB	199	JNK2	90.3
AURKC	81.8	JNK3	26.1
BRAF(V600E)	>40000	KIT	852
BTK	3890	KIT(D816V)	102
CDK2	4.62	LCK	870
CDK5	27.6	p38-alpha	11800
EGFR	7830	PDPK1	5220
EPHA3	112	PIM2	1910
ERBB2	>40000	RAF1	>40000
ERBB4	8540	RET	152
ERK2	49.7	SRC	495
FGFR2	294	SYK	200
FGFR3	507	TGFBR1	324
FLT3	41.4	TIE2	825
FLT3(D835H)	20.6	TTK	153
FLT4	211	VEGFR2	2680

~5Å

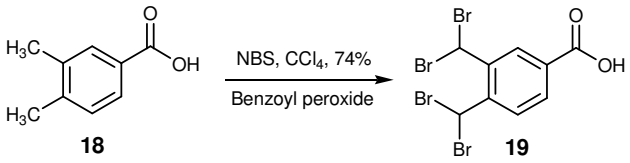
Crystallization and structure determination of c-Src bound to compound 4. Chicken c-Src kinase domain was expressed and purified as described before.[3] The c-Src•compound **4** complex was formed in a solution of 250 μM kinase domain, 375 μM compound **4**, 5% DMSO, 20 mM Tris (pH 7.5), 100 mM NaCl, 1 mM DTT. Crystals were grown overnight at 20ºC using the hanging drop vapor diffusion method (1 μl protein + 1 μl mother liquor) in a mother liquor of 0.1 M MES (pH 6.5), 10% glycerol, 25 mM ammonium acetate, 50 mM sodium acetate. Crystals were cryoprotected in mother liquor and 25 % glycerol, frozen, and stored in liquid nitrogen. Diffraction data were collected at the Advanced Light Source (Lawrence Berkeley National Laboratory) beamline 8.3.1. Data were processed in space group P1 by using DENZO and Scalepack.[4] The structure was solved by molecular replacement by using the kinase domain of human c-Src (PDB code 2SRC)[5] (residues 260–520) without the C helix (residues 298–310) and the activation loop (residues 400–425) as the template in Phaser,[6,3] through the ccp4i interface.[7,4] The structure was built in Coot[8,5], and refinement of the structure was straightforward and was conducted by using CNS 1.2. [9,10,6, 7]

Supplemental Table 3.. Data Collection and Refinement Statistics

Data Collection	
Space Group	P1
Unit Cell	a = 63.7 Å, b = 74.5 Å, c = 84.7 Å
	$\alpha = 89.1°$, $\beta = 89.9°$, $\gamma = 78.4°$
# Molecules/asymmetric unit	4
X-ray source	ALS 8.2.1
Wavelength (Å)	1.00
Resolution (Å)	50-2.35
I/σ_I	10.0 (4.1)
Completeness (%)	92.8 (93.9)
R_{sym} (%)	6.3 (21.2)
Model Refinement	
Resolution (Å)	2.35
# of reflections R_{work}/R_{free}	48108 / 2572
R_{work}/R_{free}	23.4 / 26.6
Rms deviation from ideality in bond length (Å)	0.006
Rms deviation from ideality in angles (°)	1.3
Number of protein atoms in model	8539
Number of drug atoms in model	116
Number of water molecules in model	220

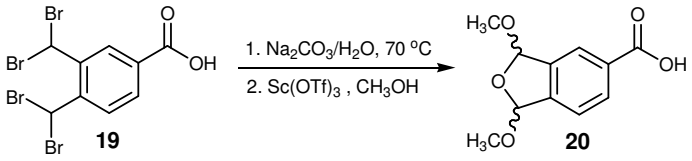
Numbers in parentheses refer to the outer resolution shell (2.35 Å – 2.43 Å)

General protocol for cross-linking reactions with AKT1. His_6-tagged human Akt1, 1 μM peptide **2**, and 20 μM cross-linker were incubated in 30 μL of 25 mM HEPES (pH= 6.5), 150 mM NaCl, 2 mM $MgCl_2$, 20 μM BME for 20 min at rt. After 20 min, 6 μL of 6x loading buffer was added to quench the reactions. The sample mixtures (10 μL) were resolved by 12% SDS-PAGE, followed by SDS-PAGE and direct in gel scanning fluorescence measurements (490 nm excitation band, 520 nm emission band).



18 **19**

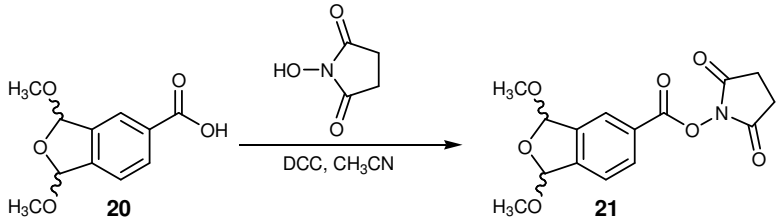
Synthesis of compound 19. Compound **19** was synthesized according to the previously reported method.[11] 3,4-dimethylbenzoic acid (11.76 g, 78 mmol), N-Bromosuccinimide (57 g, 322 mmol) and benzoyl peroxide (1.8 g, 7.4 mmol) were refluxed in carbon tetrachloride (170 mL) for 16h. Solids were filtered and washed with Et_2O (2×150 mL) followed by benzene (2×150 mL). Organic filtrates were evaporated, and dissolved in 10% Na_2CO_3 (200 mL). Aqueous phase was washed with CH_2Cl_2 (2×100 mL), acidified to pH 1 with HCl and extracted with EtOAc (2×200 mL). Organic layer was washed with brine (200 mL), dried with $MgSO_4$ and concentrated *in vacuo* affording tetrabromoacid **19** (27.3 g, 74% yield).



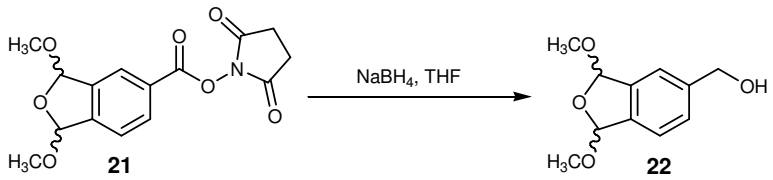
19 **20**

Synthesis of compound 20. Tetrabromoacid **19** (26g, 56 mmol) was dissolved in 10 % Na_2CO_3 (200 mL) and kept at 70 °C for 4h. Reaction mixture was cooled down to 0 °C and acidified with HCl to pH 1 and extracted with EtOAc (2×200 mL). Organic layer washed with brine (200mL),

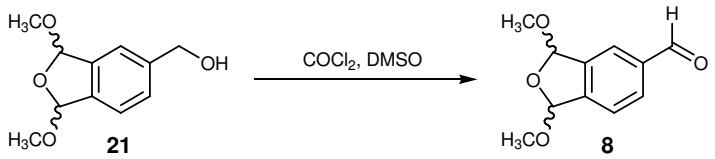
dried with $MgSO_4$ and concentrated *in vacuo*. Yellow solids were dissolved in 150 mL CH_3OH and treated with $Sc(OTf)_3$ (1g, 2 mmol) at r.t. overnight. Resulting white solids were filtered affording acid **20** (4.3g) as a 1:1 mixture of isomers, 34% combined yield over two steps. ^1H NMR (400 MHz, DMSO) δ 13.12 (s, 1H), 7.99 (d, 1H, *J* = 8Hz), 7.89 (s, 1H), 7.49 (d, 1H, *J* = 8Hz), 6.05 (s, 2H), 3.31 (s, 3H), 3.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 167.4, 143.2, 139.5, 132.9, 131.4, 124.7, 124.0, 105.2, 105.1, 54.8, 54.7. MS calculated for $C_{11}H_{12}O_5$ 224.07, found 193.05 (- OCH_3)



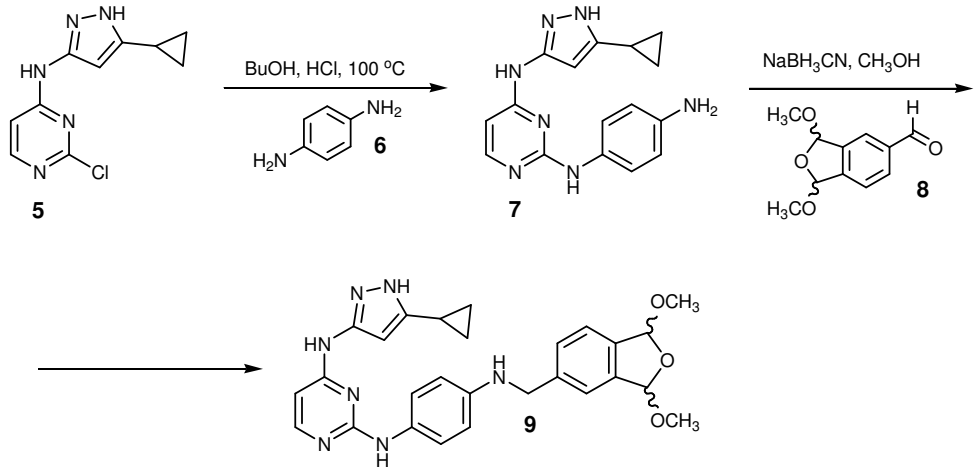
Synthesis of compound 21. Solution of acid **20** (2g, 8.9 mmol) in CH_3CN (40 mL) was treated with N-hydroxysuccinimide (1.03g, 8.9 mmol) followed by DCC (1.84g, 8.9 mmol). Reaction mixture was stirred for 1h at room temperature and filtered. Filtrates were concentrated *in vacuo*, dissolved in EtOAc (10 mL) and purified (Hexanes : EtOAc = 100:0 → 0:100) affording succinimide ester **21** (2.66g, 93% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, 1H, *J* = 8Hz), 8.13 (s, 1H), 7.51 (d, 1H, *J* = 8Hz), 6.05 (s, 1H), 6.03 (s, 1H), 3.42 (s, 3H), 3.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 161.5, 145.1, 139.7, 132.3, 127.0, 125.9, 124.0, 105.2, 105.1, 54.9, 54.8, 25.8. MS calculated for $C_{15}H_{15}NO_7$ 321.08, found 290.06 (- OCH_3)



Synthesis of compound 22. Solution of succinimide ester **21** (762 mg, 2.37 mmol) in THF (30 mL) was treated with solid $NaBH_4$ (0.45 g, 11.85 mmol) and stirred at ambient temperature overnight and then refluxed for 1h. Reaction mixture was quenched with 1N NaOH (20 mL) and refluxed for 1h. Reacton mixture was diluted with water (30 mL) and EtOAc (30 mL). Aqueous phase was extracted with the additional amount of EtOAc (30 mL), combined organic extracts were washed with brine (50 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo* and purified (Hexanes : EtOAc = 100:0 → 25:75) affording alcohol **22** (0.255 g, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.30 (m, 3H), 6.02 (s, 1H), 5.99 (s, 1H), 4.66 (s, 2H), 3.42 (s, 3H), 3.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.3, 139.0, 137.8, 128.6, 123.3, 121.4, 105.59, 105.57, 64.9, 54.6, 54.3. MS calculated for $C_{11}H_{14}O_4$ 210.09, found 179.07 (- OCH_3).

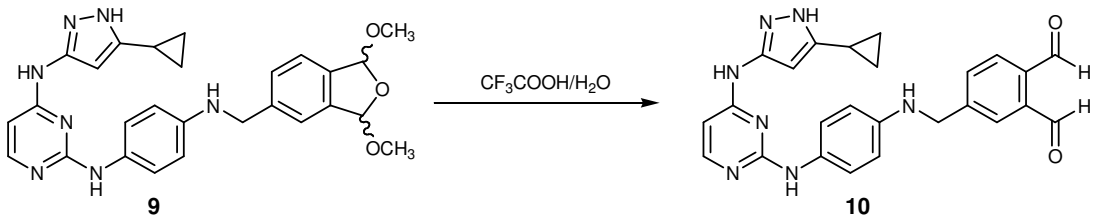


Synthesis of aldehyde 8. Solution of DMSO (0.8g, 10 mmol) in CH_2Cl_2 (50 mL) was cooled down to -78 °C and treated dropwise with $(COCl)_2$ (0.655g, 5.15 mmol). Stirring continued for 10 min at -78 °C, followed by dropwise addition of solution of alcohol **21** (0.5g, 2.4 mmol) in CH_2Cl_2 (20 mL). After 15 min NEt_3 (0.784g, 7.6 mmol) was added to the reaction mixture and cooling was removed. After 30 min of stirring, reaction mixture was quenched with saturated sodium bicarbonate (40 mL). Organic layers were separated, aqueous extracted with CH_2Cl_2 (30 mL), dried with $MgSO_4$, filtered, evaporated and purified (Hex:EtOAc = 100:0 → 40:60). Aldehyde **8** (330 mg, 62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H), 7.93 (d, 1H, J = 7.6 Hz), 7.88 (s, 1H), 7.52 (d, 1H, J = 7.6 Hz), 6.06 (s, 2H), 3.46 (s, 3H), 3.44 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.5, 144.5, 139.8, 138.2, 131.5, 124.8, 124.0, 105.3, 105.2, 55.0, 54.8. MS calculated for $C_{11}H_{12}O_4$ 208.09, found 177.16 (- OCH_3).



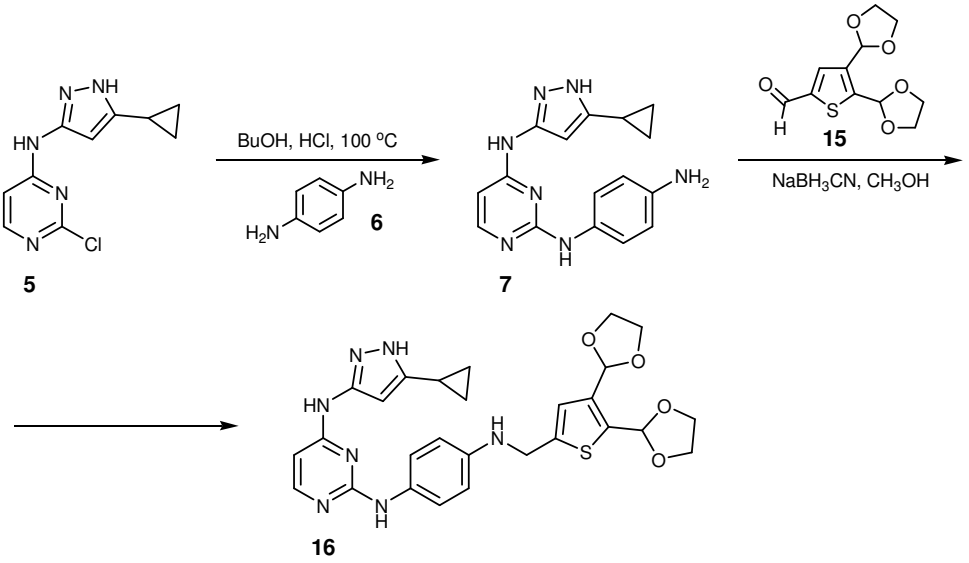
Synthesis of compound 9. (a) Synthesis of intermediate **7**. Pyrimidine monochloride **5** (1.6 g, 6.8 mmol) and *p*-phenylenediamine **6** (0.74 g, 6.8 mmol) were dissolved in BuOH (60 mL) followed by the addition of concentrated HCl (0.1 mL). Resulting mixture was kept at 100 °C overnight. Purple precipitates were collected by filtration, washed with 20 mL of BuOH and dried at 50 °C under vacuum, affording amine **7** (1.5 g, 73% yield) which was used in the next step without further purification. MS calculated for $C_{16}H_{17}N_7$ 307.15, found 308.17.

(b) Synthesis of compound **9**. Amine **7** (0.06 g, 195 µmol) and aldehyde **8** (0.035g, 168 µmol) were dissolved in CH_3OH (5mL) and treated with solid $NaBH_3CN$ (0.01g, 168 µmol) and stirred at ambient temperature overnight. Reaction mixture was evaporated and purified (CH_2Cl_2 : CH_3OH = 100:0 → 92:8 (1% NEt_3)). Compound **9** (0.064g, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (bs, 1H), 7.80 (m, 1H), 7.67 (bs, 1H), 7.32 (m, 3H), 7.14 (m, 2H), 6.49 (s, 1H), 6.47 (s, 1H), 6.20 (m, 2H), 4.25 (m, 2H), 1.76 (m, 1H), 0.85 (m, 2H), 0.58 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.4, 160.1, 155.6, 144.9, 142.1, 139.1, 137.5, 129.5, 129.2, 129.1, 124.0, 123.3, 121.8, 113.4, 106.7, 105.7, 97.4, 54.9, 54.6, 46.8, 8.9, 8.2. MS calculated for $C_{27}H_{29}N_7O_3$ 499.23, found 500.14 (M^+).



9 10

Synthesis of cross-linker 10. Dimethoxyacetal **9** (0.054g, 0.108 mmol) was treated with H_2O (2 mL) and CF_3COOH (2 mL). Resulting mixture was stirred 1h at ambient temperature and evaporated. HPLC purification CH_3CN/H_2O (0.1% TFA) followed by lyophilization gave cross-linker **10** (0.024g, 51% yield). ^{1}H NMR (400 MHz, DMSO) δ 12.35 (bs, 1H), 11.06 (bs, 1H), 10.47 (s, 1H), 10.40 (s, 1H), 10.25 (bs, 1H), 7.94 (m, 2H), 7.83 (s, 2H), 7.08 (bs, 3H), 6.61 (bs, 3H), 6.30 (bs, 1H), 2.46 (bs, 2H), 1.13 (4H), 0.88 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.7, 193.4, 160.0, 159.6, 159.3, 154.0, 148.0, 146.8, 137.1, 135.8, 132.8, 131.5, 128.7, 126.5, 119.0, 116.0, 113.1, 46.3, 9.2, 8.6. MS calculated for $C_{25}H_{23}N_7O_2$ 453.19, found 454.11.

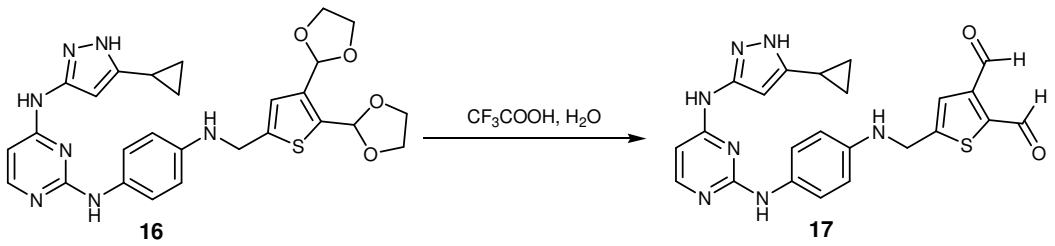


5 6 7 15 16

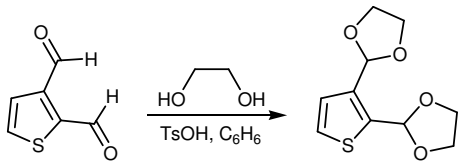
Synthesis of compound 16. (a) Synthesis of intermediate **7**. Pyrimidine monochloride **5** (1.6 g, 6.8 mmol) and *p*-phenylenediamine **6** (0.74 g, 6.8 mmol) were dissolved in BuOH (60 mL) followed by the addition of concentrated HCl (0.1 mL). Resulting mixture was kept at 100 °C overnight. Purple precipitates were collected by filtration, washed with 20 mL of BuOH and dried

at 50 °C under vacuum, affording amine **7** (1.5 g, 73% yield) which was used in the next step without further purification. MS calculated for $C_{16}H_{17}N_7$ 307.15, found 308.17.

(b) Synthesis of compound **16**. Amine **7** (0.06 g, 195 µmol) and aldehyde **15** (0.048g, 187 µmol) were dissolved in CH_3OH (5mL) and treated with solid $NaBH_3CN$ (0.01g, 168 µmol) and stirred at ambient temperature overnight. Mixture was evaporated and purified (CH_2Cl_2 : CH_3OH - 20:1 (1% NEt_3)). Compound **16** (0.064g, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (bs, 1H), 7.78 (m, 2H), 7.17 (m, 2H), 6.90 (s, 1H), 6.51 (m, 2H), 6.15 (m, 1H), 5.73 (m, 1H), 4.27 (s, 2H), 3.99 (m, 4H), 3.87 (m, 4H), 1.74 (m, 1H), 0.80 (m, 2H), 0.56 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2, 155.6, 144.3, 143.9, 143.6, 139.1, 138.9, 137.5, 136.1, 130.2, 125.0, 124.3, 123.7, 113.6, 99.1, 98.6, 97.5, 65.5, 65.4, 46.5, 9.0, 8.3. MS calculated for $C_{27}H_{29}N_7O_4S$ 547.2, found 548.07.

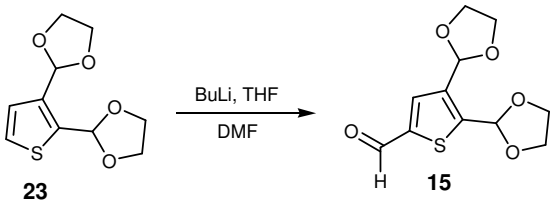


Synthesis of cross-linker 17. Compound **16** (0.035g, 64 µmol) was dissolved in a 1:1 mixture of CH_3CN-H_2O (3 mL) and treated with trifluoroacetic acid (10 µL, 2 eq.). Reaction mixture was stirred overnight and purified *via* HPLC CH_3CN/H_2O (0.1% TFA) followed by lyophilization to afford cross-linker **17** (13 mg, 45% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.94 (bs, 1H), 10.38 (s, 1H), 10.34 (s, 1H), 9.54 (bs, 1H), 8.73 (bs, 1H), 7.81 (m, 1H), 7.53 (s, 1H), 7.31 (s, 1H), 7.21 (s, 1H), 6.54 (s, 1H), 6.52 (s, 1H), 4.49 (s, 2H), 1.76 (bs, 1H), 0.83 (m, 2H), 0.59 (m, 2H). MS calculated for $C_{23}H_{21}N_7O_2S$ 459.15, found 459.99.



14 **23**

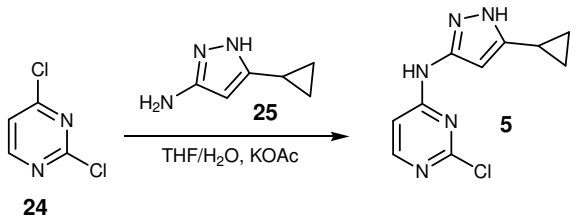
Synthesis of compound 23. 2,3-Bis(1,3-dioxolan-2-yl)thiophene **23** was synthesized according to the previously published procedure.[12] 2,3-Thiophene dicarboxaldehyde **14** (5.35g, 38 mmol), p-toluenesulfonic acid monohydrate (0.1 g,) and ethylene glycol (11.73 g, 190 mmol) were dissolved in benzene (80 mL). Resulting mixture was refluxed for 2h and water was collected in a Dean-Stark trap. Reaction mixture was washed with 5% Na_2CO_3 (100 mL), aqueous layer was extracted once with EtOAc (100 mL), dried with $MgSO_4$, filtered and evaporated. Purification of the resulting oily residue (Hexanes/EtOAc) afforded 2,3-Bis(1,3-dioxolan-2-yl)thiophene **23** (7g, 85% yield).



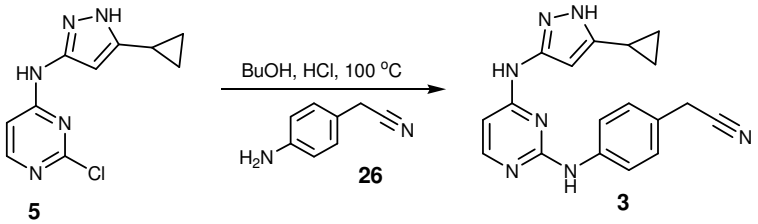
23 **15**

Synthesis of compound 15. 2,3-Bis(1,3-dioxolan-2-yl)thiophene **23** (2.6 g, 11.40 mmol) in THF (50 mL) was cooled down to -78 °C and treated dropwise with BuLi (12.5 mmol). Temperature was raised to 0 °C and reaction mixture was stirred for additional 30 min. After 30 min of stirring, DMF was added (12.5 mmol, 0.971 mL). Cooling was removed and the reaciton mixture stirred at ambient temperature for 30 min followed by quenching with H_2O (50 mL). Layers were separated, and aqueous layer extracted with Et_2O (100 mL). Organic extracts were dried with $MgSO_4$, filtered and evaporated. Purification of the resulting residue (Hexanes/EtOAc) afforded aldehyde **15** (2.2 g, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 7.74 (s, 1H), 6.32 (s, 1H), 5.98 (s, 1H), 4.10-4.05 (m, 4H), 4.05-3.95 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.4,

150.5, 142.2, 138.8, 136.1, 98.8, 98.6, 65.8, 65.5. MS calculated for $C_{11}H_{12}O_5S$ 256.04, found 257.03.

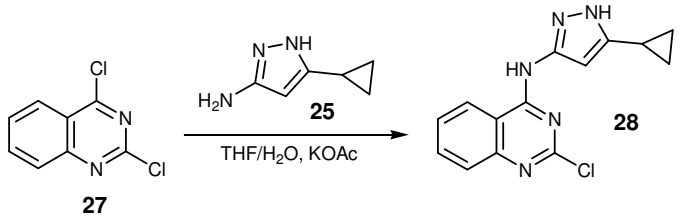


Synthesis of compound 5. 2,4-dichloropyrimidine **24** (3.2 g, 21.6 mmol) and 5-cyclopropyl-2H-pyrazol-3-ylamine **25** (2.65g, 21.5 mmol) were dissolved in a 1:1 mixture of THF and H_2O (140 mL) and treated with KOAc (30 eq., 64 g) and kept at 55 °C for 48h. Layers were separated, organic layer was evaporated, dissolved in CH_2Cl_2 (30 mL) and kept at -20 °C for 3h. Precipitated pyrimidine chloride **5** was collected by filtration. Filtrates were evaporated and dissolved in CH_2Cl_2 (25 mL) and kept at -20 °C for another 3h and filtered. Combined solids were dissolved in $CHCl_3$: CH_3OH 10:1 (25 mL) and purified ($CHCl_3$/CH_3OH), affording compound **5** (46% yield, 2.32g). ^{1}H NMR (400 MHz, DMSO) δ 12.14 (s, 1H), 10.23 (s, 1H), 8.10 (s, 1H), 1.84 (m, 1H), 0.88 (m, 2H), 0.64 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 161.4, 160.7, 160.0, 153.9, 148.6, 147.8, 146.8, 8.4, 8.2. MS calculated for $C_{10}H_{10}ClN_5$ 235.06 (M^+), found 236.15 (M^+).

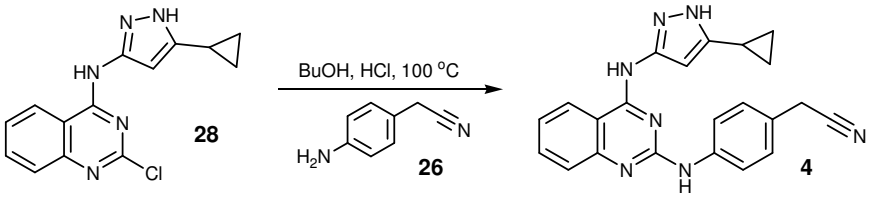


Synthesis of compound 3. Pyrimidine monochloride **5** (0.2 g, 0.85 mmol) and p-aminobenzonitrile **26** (0.112 g, 0.85 mmol) were dissolved in BuOH (8 mL) followed by the addition of concentrated HCl (0.1 mL). Resulting reaction mixture was kept at 100 °C overnight. Solid precipitates were collected by filtration washed with BuOH (8 mL) and dried under vacuum affording compound **3** (0.23 g, 86% yield). ^{1}H NMR (400 MHz, DMSO) δ 11.46 (s, 1H), 10.98 (s, 1H), 7.96 (m, 1H), 7.48 (bs, 2H), 7.37 (d, 2H, J = 8 Hz), 6.49 (bs, 1H), 6.01 (bs, 1H), 4.03 (s,

2H), 1.82 (m, 1H), 0.89 (m, 2H), 0.52 (bs, 2H). ^{13}C NMR (100 MHz, DMSO) δ 160.3, 153.2, 147.6, 145.8, 143.2, 136.5, 129.4, 124.4, 119.8, 100.1, 94.1, 22.6, 8.8, 7.4. MS calculated for $C_{18}H_{17}N_7$ 331.15, found 332.11.



Synthesis of compound 28. Solution of pyrazoloamine **25** (0.267 g, 2.17 mmol) and 2,4-dichloroquinazoline **27** (0.432 g, 2.17 mmol) in EtOH (10 mL) was treated with EtN(i-Pr)$_2$ (3 eq., 1.13 mL) and stirred overnight. Resulting white precipitate was collected by filtration and dried, affording compound **28** (0.476 g, 76% yield). ^{1}H NMR (400 MHz, DMSO) δ 12.31 (bs, 1H), 10.74 (s, 1H), 8.58 (d, 1H, *J* = 8 Hz), 7.79 (dd, 1H, *J* = 8Hz, 7.2 Hz), 7.65 (d, 1H, *J* = 8.4 Hz), 7.52 (dd, 1H, *J* = 7.6 Hz, 7.6 Hz), 3.31 (bs, 1H), 1.91 (m, 1H), 0.92 (m, 2H), 0.69 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 159.2, 157.0, 151.4, 147.3, 146.4, 134.6, 127.4, 127.2, 124.3, 114.1, 96.0, 8.4, 7.6. MS calculated for $C_{14}H_{12}ClN_5$ 285.08, found 286.03.



Synthesis of compound 4. Quinazoline monochloride **28** (0.2 g, 0.7 mmol) and p-aminobenzonitrile **26** (0.093 g, 0.7 mmol) were dissolved in BuOH (8 mL) followed by the addition of concentrated HCl (0.1 mL). Resulting reaction mixture was kept at 100 °C overnight. Solid precipitates were collected by filtration washed with BuOH (8 mL) and dried under vacuum affording compound **4** (0.197 g, 74% yield). ^{1}H NMR (400 MHz, DMSO) δ 11.43 (s, 1H), 10.81 (s, 1H), 8.60 (d, 1H, *J* = 8 Hz), 7.83 (dd, 1H, *J* = 8 Hz, 7.6 Hz), 7.57 (d, 1H, *J* = 8.4 Hz), 7.53 (d, 2H, *J* = 8.4 Hz), 7.44 (dd, 1H, *J* = 7.6 Hz, 8 Hz), 7.38 (d, 2H, *J* = 8.4 Hz), 6.14 (bs, 1H), 4.04 (s,

2H), 1.82 (m, 1H), 0.93 (m, 2H), 0.54 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 159.7, 159.4, 158.8, 152.6, 146.9, 146.2, 136.7, 136.2, 129.4, 125.6, 125.2, 125.0, 119.8, 118.2, 111.0, 96.2, 22.7, 8.5, 7.5. LRMS calculated for $C_{22}H_{19}N_7$ 381.17, found 382.08.

Full list of sixteen or more authors for the following references in the main text is included:

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