Supporting Information for:

A Chemical-Genetic Approach to Generate Selective Covalent Inhibitors of Protein Kinases

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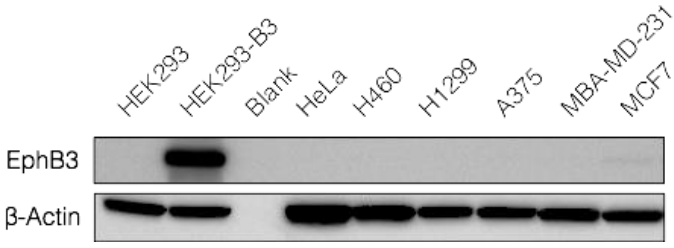


Figure S1. Screening of a number of cell lines using western blot fails to detect expression of endogenous EphB3 protein. HEK293-B3 cells that stably express EphB3 were used as a positive control.

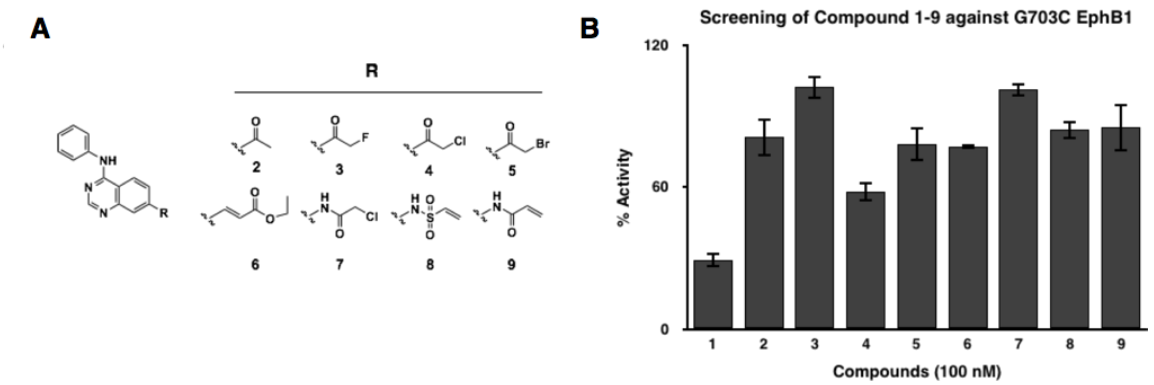


Figure S2. Screening of a panel of electrophilic inhibitors for inhibition against G703C EphB1. (A) Structures of quinazolines **2**-**9**, which contain various electrophiles at position 7. (B) Compounds **1**-**9** were screened at 100 nM for inhibition against G703C EphB1 *in vitro*. Compound **1** caused the most potent inhibition among the nine compounds. The kinase activity was measured in duplicates using the disk assay and normalized to that of the control (DMSO).

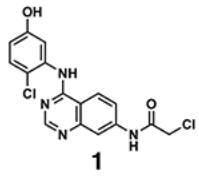
Table S1. Percentage inhibition of a panel of 249 kinases by 100 nM of compound **1**

ABL1	54	CLK2	-5	GSK3β	4	MEK3	10	PIM-1-KINASE	12	SPHK1	-2
AKT1	0	CLK3	2	HASPIN	-1	MELK	1	PIM2	1	SPHK2	18
AKT2	1	CRAF	6	HCK	23	MER	-24	PIM3	0	SRC	18
AKT3	1	CSK	8	HIPK1	-4	MET	0	PKA	-1	SRMS	-4
ALK	0	DAPK1	-1	HIPK2	-1	MKNK1	1	PKACB	-4	SRPK1	-1
AMP-A1B1G1	-2	DAPK3	0	HIPK3	-1	MNK2	0	PKCα	5	SRPK2	2
AMP-A2B1G1	1	DCAMKL2	1	HIPK4	1	MRCKα	0	PKCβ1	2	STK16	14
ARG	27	DDR1	20	IGF1R	2	MRCKβ	0	PKCβ2	6	STK25	-5
ARK5	5	DDR2	3	IKKα	1	MSK1	4	PKCη	8	SYK	-14
AURORA-A	6	DYRK1A	-5	IKKβ	4	MSK2	3	PKCγ	13	TAK1	0
AURORA-B	8	DYRK1B	0	IKKε	4	MSSK1	4	PKCι	1	TAOK2	1
AURORA-C	4	DYRK2	5	INSR	1	MST1	1	PKCθ	5	TAOK3	2
AXL	2	DYRK3	1	IRAK1	2	MST2	3	PKCζ	-7	TBK1	0
BLK	7	DYRK4	-6	IRAK4	1	MST3	2	PKN1	0	TEC	-22
BMX	-2	EGFR	51	IRR	-14	MST4	-3	PKN2	-2	TIE2	9
BRAF	16	EPH-A1	51	ITK	-15	MUSK	1	PLK1	4	TNIK	1
BRK	38	EPH-A2	13	JAK1	1	NDR2	1	PLK3	0	TNK1	-1
BRSK1	1	EPH-A3	15	JAK2	1	NDRG1	-5	PLK4	-2	TNK2	-6
BRSK2	-1	EPH-A4	36	JAK3	-2	NEK1	-5	PRAK	-18	TRKA	1
BTK	7	EPH-A5	17	JNK1	-3	NEK2	0	PRKACA	2	TRKB	1
CAMK1A	1	EPH-A8	11	JNK2	-8	NEK6	-1	PRKD1	5	TRKC	-7
CAMK1D	8	EPH-B1	12	JNK3	-7	NEK7	0	PRKD2	3	TSSK1	-2
CAMK2A	5	EPH-B2	36	KDR	50	NEK9	1	PRKD3	-2	TSSK2	1
CAMK2B	0	EPH-B3	98	KIT	11	p38α	5	PRKG1	2	TTK	1
CAMK2D	-1	EPH-B4	6	LATS1	6	p38β	3	PRKG2	5	TXK	-5
CAMK2G	5	ERB-B2	-11	LATS2	2	p38δ	1	PRKX	4	TYK2	0
CAMK4	5	ERB-B4	10	LCK	2	p38γ	0	PTK5	3	TYRO3	2
CDK1	-2	FAK	17	LOK	2	P70S6K1	1	PYK2	4	YES	27
CDK2	-1	FER	-3	LRRK2^{G2019S}	3	P70S6K2	-16	RET	67	ZAP70	0
CDK2-CYCLINE	0	FES	6	LTK	-9	PAK1	2	RIPK2	77		
CDK3-CYCLINE	1	FGFR1	3	LYNA	1	PAK2	0	ROCK1	-3		
CDK4-CYCLIND	3	FGFR2	3	LYNB	5	PAK2	0	ROCK1	-3		
CDK5	-1	FGFR2	3	LYNB	5	PAK4	0	RON	1		
CDK5-P25	3	FGFR4	0	MAP4K4	3	PAK5	0	ROS	2		
CDK5-P25	3	FGR	8	MAP4K5	-9	PAK6	1	RSK1	2		
CDK9-CYCLINT1	-3	FLT-1	33	MAPK1	4	PAR-1B-α	2	RSK2	1		
CHEK1	2	FLT-3	2	MAPK3	1	PASK	1	RSK3	1		
CHEK2	2	FLT-4	28	MAPKAPK-2	1	PDGFRα	6	RSK4	-1		
CK1	6	FMS	10	MAPKAPK-3	3	PDGFRβ	4	SGK1	-2		
CK1-ε	4	FRAP1	-5	MARK1	2	PDK1	11	SGK2	-2		
CK1-γ1	-7	FYN	-1	MARK3	3	PERK	1	SGK3	0		
CK1-γ2	13	GRK6	6	MARK4	-1	PHK-γ1	11	SIK	1		
CK1-γ3	-7	GRK7	-4	MEK1	2	PHK-γ2	-4	SLK	0		
CLK1	2	GSK3α	6	MEK2	1	PI3Kα	37	SNF1LK2	1		

Table S2. IC_{50} values of different 7-chloroacetamidoquinazolines against variants of ABL and FGFR4. *All IC_{50} values were determined using the disk kinase assay.*

Compound	ABL		Fold selectivity
	WT	G321C	
1	690	140	4.9
4	>10,000	1223	>8.0
10	530	14	37

Compound	FGFR4		Fold selectivity
	WT	G556C	
1	1,700	77	22
4	>10,000	522	19
10	>10,000	163	>61



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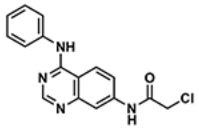

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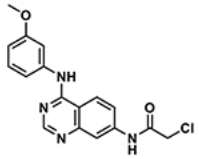

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Table S3. Crystallographic data collection and refinement statistics.

	EphB1 Compound 1	EphB1 G703C Compound 1
PDB entry	5MJA	5MJB
Data collection		
Space group	*P* 31 2 1	*P* 31 2 1
Cell dimensions		
a, b, c (Å)	102.1, 102.1, 157.14	102.2, 102.2, 157.6
α, β, γ (°)	90, 90, 120	90, 90, 120
Resolution (Å)	2.14 (2.20—2.14)	2.23 (2.29—2.23)
R_{merge}	0.108 (2.164)	0.106 (1.707)
$I/\sigma I$	16.2 (1.5)	10.2 (1.6)
$CC_{1/2}$	1.000 (0.620)	0.997 (0.622)
Completeness (%)	100.0 (100.0)	100.0 (100.0)
Multiplicity	19.7 (19.3)	9.7 (10.1)
Refinement		
Resolution (Å)	76.89—2.14	58.84—2.23
No. reflections	1035318 (74250)	454865 (34943)
No unique reflections	52668 (3844)	47109 (3466)
R_{work} / R_{free}	0.200 / 0.236	0.199/0.214
R.m.s deviations		
Bond lengths (Å)	0.010	0.010
Bond angles (º)	1.04	1.05

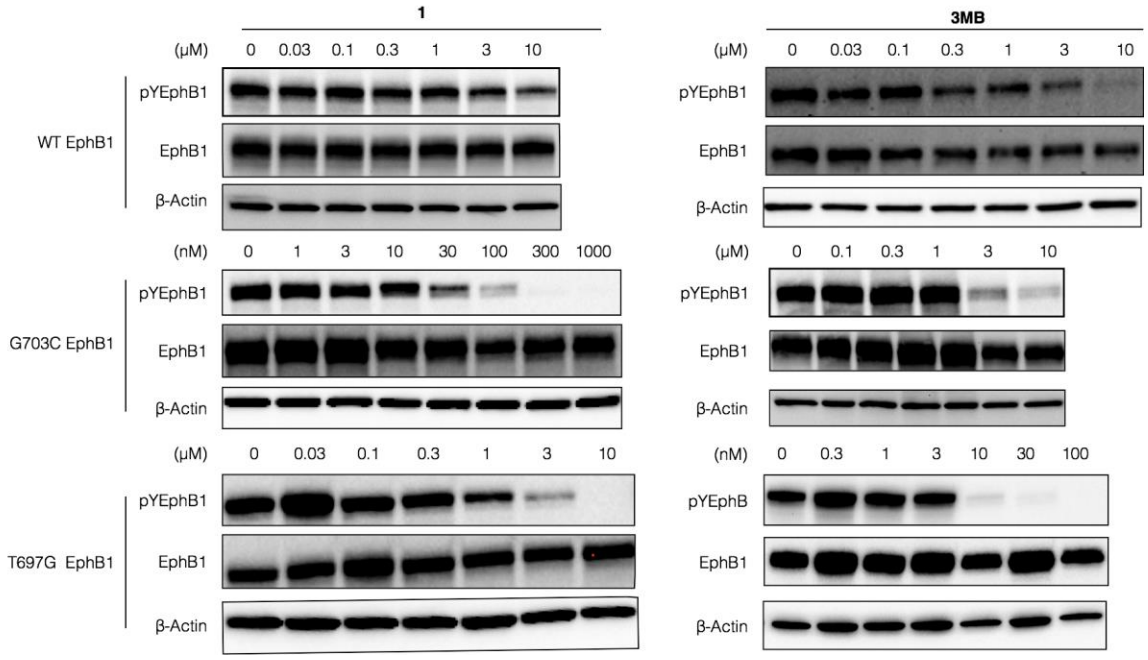

Figure S3. Inhibition of EphB1 variants by compound **1** and 3MB-PP1 in cells. Cell lysates were resolved by SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted with different antibodies as indicated.

Plasmid construction

All constructs for bacterial expression and transient transfection were prepared by using standard molecular cloning techniques while all retroviral constructs were generated using the Gateway system (Life Technologies).[1] All mutations were generated by standard QuikChange Lighting Site-directed Mutagenesis protocol (Agilent). Details on cloning information and sequence will be made available upon request. EphB3 and EphB1 catalytic domain were created as described previously.[2]

FGFR4 and Abl kinase assays

FGFR4 and Abl kinase activity were determined following the same protocols as EphB1 except that different peptide substrates were used. Poly (4:1 Glu, Tyr) was used as the substrate in FGFR4 kinase assays and Abl-tide (EAIYAAPFAKKK) was used for Abl.[3, 4]

Generation of stable cell line

Briefly, AmphoPack-293 cells (Clontech) were cultured in 10% FBS DMEM under 37 °C and 5% CO2 conditions. The cells were seeded at 4.0 x 10^6 and transfected after 16 hours with retroviral expression vectors by conventional calcium phosphate procedures. At 24 hours post-transfection, polybrene (4 mg/mL) was added, and the medium was filtered through 0.45 μm syringe filters (cellulose, VWR) and subsequently used to infect HEK293 cells. This process was repeated every 8 hours for 2 days. After viral infection, the cells were cultured in 10% FBS/DMEM for 8 hours followed by selection with G418 at 400 μg/mL or puromycin 4 μg/mL (Calbiochem) until control plate shows no sign of viable cells.

Chemical Synthesis

Compounds **1**-**9** were synthesized as previously described.[2] Compound **10** (2-chloro-*N*-(4-((3-methoxyphenyl)amino)quinazolin-7-yl)acetamide) was synthesized as in a similar manner as described below.

To a solution of N^4-(3-methoxyphenyl)quinazoline-4,7-diamine (100 mg, 0.38 mmol) in 1M NaOH and acetonitrile (NaOH/ACN = 1:1, 2 ml) was added 2-chloroacetyl chloride (85 mg, 0.75 mmol). Then the reaction mixture was stirred at 0 ^oC for about 0.5 h. Then the reaction mixture was diluted by saturated aqueous $NaHCO_3$ solution and extracted by EtOAc three times. The combined organic phases were washed with brine and dried over anhydrous sodium sulfate, concentrated and purified by silica gel chromatography

(2–5% MeOH gradient in CH_2Cl_2) to afford the desired compound as a pale yellow solid (40 mg, 31% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.74 (s, 1H), 9.66 (s, 1H), 8.56 (s, 1H), 8.51 (d, *J* = 9.1 Hz, 1H), 8.12 (d, *J* = 2.1 Hz, 1H), 7.73 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.55 (t, *J* = 2.1 Hz, 1H), 7.50–7.46 (m, 1H), 7.29 (t, *J* = 8.2 Hz, 1H), 6.71 (dd, *J* = 8.2, 2.1 Hz, 1H), 4.36 (s, 2H), 3.78 (s, 3H). ^{13}C NMR (125 MHz, dmso) δ 165.40, 159.35, 157.25, 155.05, 150.74, 142.27, 140.40, 129.16, 123.99, 118.70, 115.23, 114.37, 111.41, 108.86, 107.99, 55.07, 43.64. LC-MS (m/z): $[M+H]^+$ calculated for $C_{17}H_{15}ClN_4O_2$ 343.1; found 343.1.

References:

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3. Liu, X., Kung, A., Malinoski, B., Prakash, G. K. S., and Zhang, C. (2015) Development of Alkyne-Containing Pyrazolopyrimidines To Overcome Drug Resistance of Bcr-Abl Kinase, *Journal of Medicinal Chemistry 58*, 9228-9237.
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