Supporting Information

Bis-Aryl Urea Derivatives as Potent and Selective Lim Kinase Inhibitors

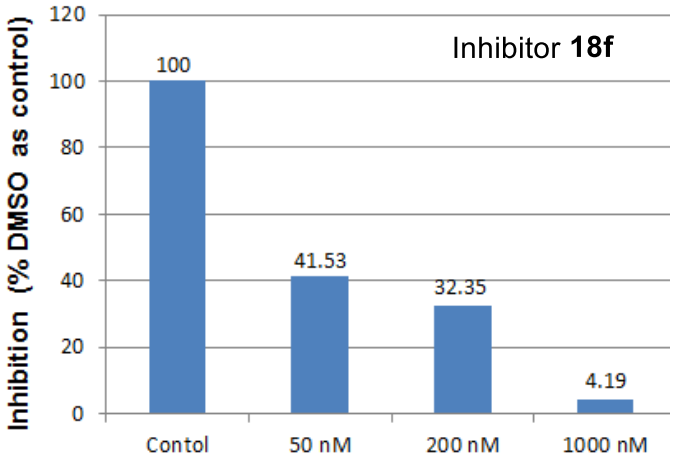
Yan Yin[†ø], Ke Zheng[†], Nibal Eid[‡¥], Shannon Howard[‡¥], Ji-Hak Jeong[Δ], Fei Yi[£], Jia Guo[£], Chul Min Park[†§], Mathieu Bibian[†], Weilin Wu[Δ], Pamela Hernandez[‡¥], HaJeung Park[∞], Yuntao Wu[£], Jun-Li Luo[Δ], Philip V. LoGrasso[‡¥]*, and Yangbo Feng[†]*

[†]Medicinal Chemistry; [‡]Discovery Biology; [∞]Crystallography/Modeling Facility, Translational Research Institute, [¥]Department of Molecular Therapeutics, and [Δ]Department of Cancer Biology, The Scripps Research Institute, Scripps Florida, 130 Scripps Way, #2A1, Jupiter, FL 33458; [ø]School of Chemical and Environmental Engineering, Shanghai Institute of Technology, 100 Hai Quan Rd., Shanghai, 201418, P. R. China; [£]National Center for Biodefense and Infectious Diseases, School of System Biology, George Mason University, Manassas, VA 20110.

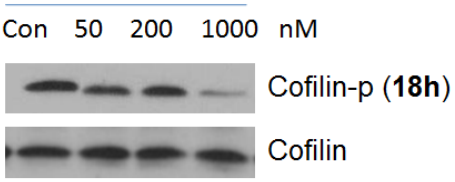
Corresponding authors: Yangbo Feng: yfeng@scripps.edu, 561-228-2201
Philip V. LoGrasso: lograsso@scripps.edu, 561-228-2230

Present address: [§]C.M.P: Korea Research Institute of Chemical Technology (KRICT), 141 Gajeongro, Yuseong, Korea 305-600.

1, Western blot assay data in PC-3 cells for 18f and 18h.



Suppl-Figure 1. Western blot cofilin phosphorylation assays for inhibitor **18f**.



Suppl-Figure 2. Western blot cofilin phosphorylation assays for inhibitor **18h**.

2, in vitro and in vivo PK protocols.

2.1, in vivo Pharmacokinetics

Pharmacokinetics studies were conducted in Sprague Dawley rats. The compound was formulated in a generic formulation at 1 mg/mL (e.g. 10:10:80, DMSO:tween 80: water, v:v:v) and dosed at 0.5 mg/kg intravenous into the femoral vein or 3 mg/kg by oral gavage. Blood was obtained at t=5 min, 15 min, 30 min, 1hr, 2hr, 4hr, 6hr, and 8hr. Blood was collected into EDTA containing tubes and plasma was generated by standard centrifugation methods. All procedures and handling were according to standard operating procedures approved by IAPUC at Scripps Forida. In order to assess in vivo pharmacokinetic parameters an LC-MS/MS bioanalytical method was developed where 25 Tl of plasma was treated with 125 Tl of acetonitrile containing an internal standard in a Millipore Multscreen Slovinter 0.45 micron low binding PTFE hydrophilic filter plate (#MSRLN0450) and allowed to shake at room temperature for five minute. The plate was then centrifuged for 5 minutes at 4000 rpm in a tabletop centrifuge and the filtrate was collected in a polypropylene capture plate. The filtrate (10 Tl) is injected using an Agilent 1200 HPLC equipped with a Thermo Betasil C18 HPLC column 5 T (50×2.1 mm) #70105-052130. Mobile Phase A was water with 0.1% formic acid. Mobile phase B was acetonitrile with 0.1% formic acid. Flow rate was 375 Tl/min using a gradient of 90% A/10% B from, 0-0.5 min, ramped to 5%A95%B at 2 min, held at 5%A95%B until 3.0 min, ramped to 90% A/10% B at 4 min, and held at 90% A10%

B until 7 min. An API Sciex 4000 equipped with a turbo ion spray source was used for all analytical measurement. MRM methods were developed in position ion mode. Peak areas of the analyte ion were measured against the peak areas of the internal standard. Data was fit using WinNonLin using an IV bolus model.

2.2, P450s inhibition

P450s inhibition for four major isoforms are evaluated using a cocktail inhibition assay where the metabolism of specific marker substrate (CYP1A2 phenaceten demethylation to acetaminophen); CYP2C9, tolbutamide hydroxylation to hydrocytolbutamide; CYP2D6, bufuralol hydroxylation to 4'-Hydroxybufuralol; and CYP3A4, midazolam hydroxylation to 1'-Hydroxymidazolam) in the presence or absence of 10 TM probe compound is evaluated. The concentration of each marker substrate is approximately its Km. Conditions were similar to those described by Tesino and Patonay (Testino and Patonay, 2003) except 2C19 was not evaluated as we found that stock solution of the 2C19 probe substrate, omeprazole, had poor stability. Specific inhibitors for each isoform are included in each run to validate the system.

3, Profiling of compound 18b

Inhibitor **18b** was profiled at 1.0 µM against a panel of 61 kinases (Reaction Biology Corporation: (http://www.reactionbiology.com/webapps/site/).

Suppl-Table 1. Profiling data for compound **18b** at 1 µM against 61 kinases.

Kinase	% Activity relative to DMSO control	Kinase	% Activity relative to DMSO control
ABL1	91	LIMK1	9
AKT2	97	LRRK2 (G2019S)	26
ALK	99	MEK1	52
AMPK	81	MLCK/MYLK	80
Aurora A	45	MRCKa/CDC42BPA	104
BRAF	92	mTOR/FRAP1	98
BTK	97	NEK1	78
CAMK2b	97	P38a/MAPK14	91
CDK2/cyclin A	85	p70S6K/RPS6KB1	78
CDK5/p35	95	PAK2	85
CK1d	91	PDGFRb	85
CK2a	62	PIM2	104
c-Kit	82	PKA	51
c-MET	95	PKCa	81
c-Src	87	PKN1/PRK1	75
DAPK1	90	PLK2	103
EGFR	95	RET	38
EPHA3	100	ROCK1	84
ERK2/MAPK1	72	ROCK2	81
FAK/PTK2	79	RSK1	94
FGFR1	96	SGK1	99
FLT3	35	SLK/STK2	75
GSK3b	82	STK16	20
IGF1R	89	STK33	62
IKKa/CHUK	94	TAK1	91
IKKb/IKBKB	90	TAOK1	82
IKKe/IKBKE	85	TBK1	85
JAK3	59	TESK1	102
JNK1	100	TLK1	87
JNK3	109	WEE1	90
KDR/VEGFR2	78		