Supporting Information

Discovery of Potent Benzolactam IRAK4 Inhibitors with Robust *in vivo* Activity

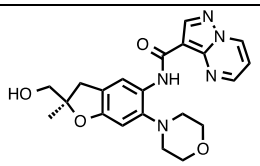
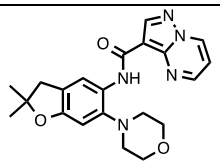
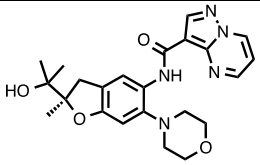
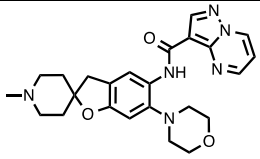
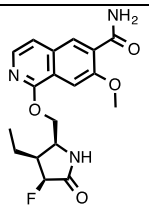
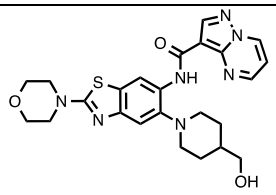
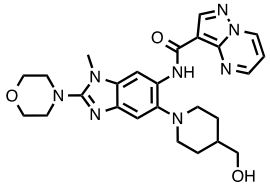
Naomi S. Rajapaksa,[†] Alberto Gobbi,[†] Joy Drobnick,[†] Steven Do,[†] Aleksandr Kolesnikov,[†,¢] Jun Liang,[†] Yongsheng Chen,[‡] Swathi Sujatha-Bhaskar,[†] Zhiyu Huang,[†] Hans Brightbill,[†] Ross Francis,[†] Christine Yu,[†] Edna F. Choo,[†] Kevin DeMent,[†,∇] Yingqing Ran,[†,^] Le An,[†] Claire Emson,[†,○] Jonathan Maher,[†] John Wai,[‡] Brent S. McKenzie,[†] Patrick J. Lupardus,[†,#] Ali A. Zarrin,[†,□] James R. Kiefer,[†] and Marian C. Bryan[†*]

†Genentech, Inc., One DNA Way, South San Francisco, California 94080, USA.

‡ WuXi Apptech, 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai 200131, P. R. China

Present Addresses: ∇K. D.: Takeda Bio Development Center Ltd., 9625 Towne Center Drive, San Diego, California 92121, USA. ○C. E.: Medimmune, Inc., 1 Medimmune Way, Gaithersburg, Maryland 20878, USA. ¢A. K.: 623 38th Ave. San Francisco, CA 94121, USA. ^Y. R.: 836 Polaris Ave., Foster City, CA 94404, USA. #P. J. L.: Synthekine, Inc., 1700 Owens Street, Suite 500, San Francisco, California 94157, USA. □A.Z.: TRex Bio 863 Mitten Rd, Burlingame, CA 94010, USA.

Table S1. Inhibitory potency of dihydrobenzofuran and tool compounds in the IL-6 human whole blood assay

Cmpd[a]	Structure	hWB IL-6 IC_{50} (μM)[b]	MI (%)
4 (DHBF-41)		0.46 ± 0.16 (*n* = 16)	72 ± 7.4
5 (DHBF-23)		10.3 ± 9.4 (*n* = 3)	56 ± 12
DHBF-43		0.65 ± 0.22 (*n* = 9)	84 ± 7
DHBF-44		0.60 ± 0.17 (*n* = 7)	77 ± 9.9
1[c]		0.015 ± 0.005 (*n* = 25)	87 ± 3.5
DHBF-20		0.61 ± 0.064 (*n* = 3)	87 ± 1.4
DHBF-17		1.6 ± 0.26 (*n* = 3)	88 ± 1

[a]Compounds labeled "DHBF-x," indicate the compound number (x) from the referenced publication.[1] [b]Data are the geometric mean of at least two independent experiments ± standard deviation for the number (*n*) of experiments conducted. [c]Compound **1** is from the referenced publication.[2]

Crystallography

IRAK4 protein was expressed in insect cells, purified, and crystallized as previously described. [1] Diffraction data for the complex of IRAK4 and **19** were collected at beamline 22 of the Advanced Photon Source, and the structure was determined by molecular replacement in space group C2 and with unit cell dimensions 140.9Å, 140.2Å, 87.6Å, a=c=90°, and b=123.2°, with four protein molecules in the asymmetric unit. Clear electron density of the inhibitor (Figure S1) was observed in all protein molecules. The structure was refined in BUSTER (Table S2).*

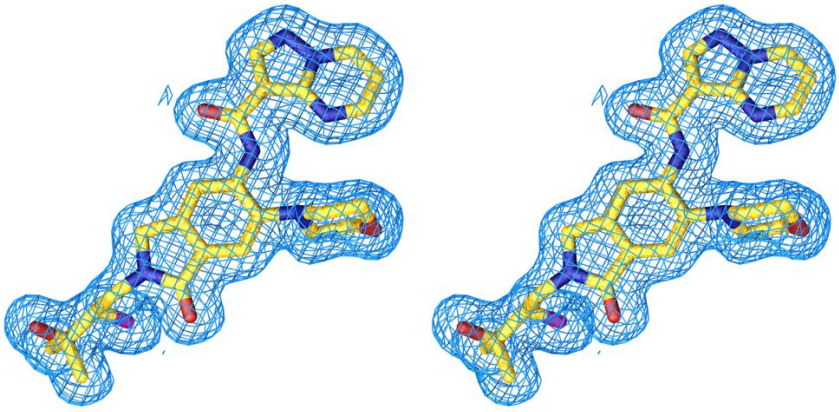


Figure S1: Electron density map of inhibitor 19 in complex with IRAK4. Divergent eye stereo diagram of the simulated annealing composite omit difference electron density map, $(2m|F_o| - D|F_c|) \exp(i\alpha_c)$, of the IRAK4 inhibitor **19**, contoured at 1σ.

* BUSTER-TNT 2.X, Global Phasing Ltd, Sheraton House, Cambridge CB3 0AX, UK Version: 2.11.5.

Table S2: IRAK4 crystallographic data collection and refinement statistics.

PDB code	**6UYA**
Compound #	**19**
Beamline	APS 22ID
Wavelength (Å)	1.0000
Space group	C2
Kinase molecules in ASU	4
Resolution range (Å)	74.1-1.74
Highest resolution bin (Å)	1.83 – 1.74
Redundancy	4.4 (4.3)
Completeness (%)	99.7 (100)
Mean I/σ_I	13.8 (2.4)
R_{merge} (%)	5.4 (54.1)
Refinement	
Resolution range (Å)	25.5 – 1.74
No. reflections (R_{free} set)	144,614 (7,278)
R_{work}, R_{free} (%)	18.2, 20.7
No. non-hydrogen atoms in ASU	9,071
No. water molecules in ASU	887
Rmsd bond lengths (Å)	0.010
Rmsd bond angles (°)	1.03
B-factors (Å^2)	
Protein	31.8
Ligand	20.2
Sulfate	47.8
Water	40.0

Values in parentheses represent data from the highest resolution shell.

Figure S2. Effect of compound **4** and **19** on the proinflammatory cytokines IL-6, TNFα, and IFNα in an R848-induced mouse PD model. A) Plasma concentration upon dosing a nanosuspension of either **4** or **19** in MCT. B) IL-6 levels as determined by MSD upon stimulation of R848 and compound treatment. C) TNFα levels as determined by MSD upon stimulation of R848 and compound treatment. Mouse whole blood TNFα IC_{50} = 234 nM for **19** vs 425 nM for **4**. D) IFNα levels as determined by ELISA upon stimulation of R848 and compound treatment.

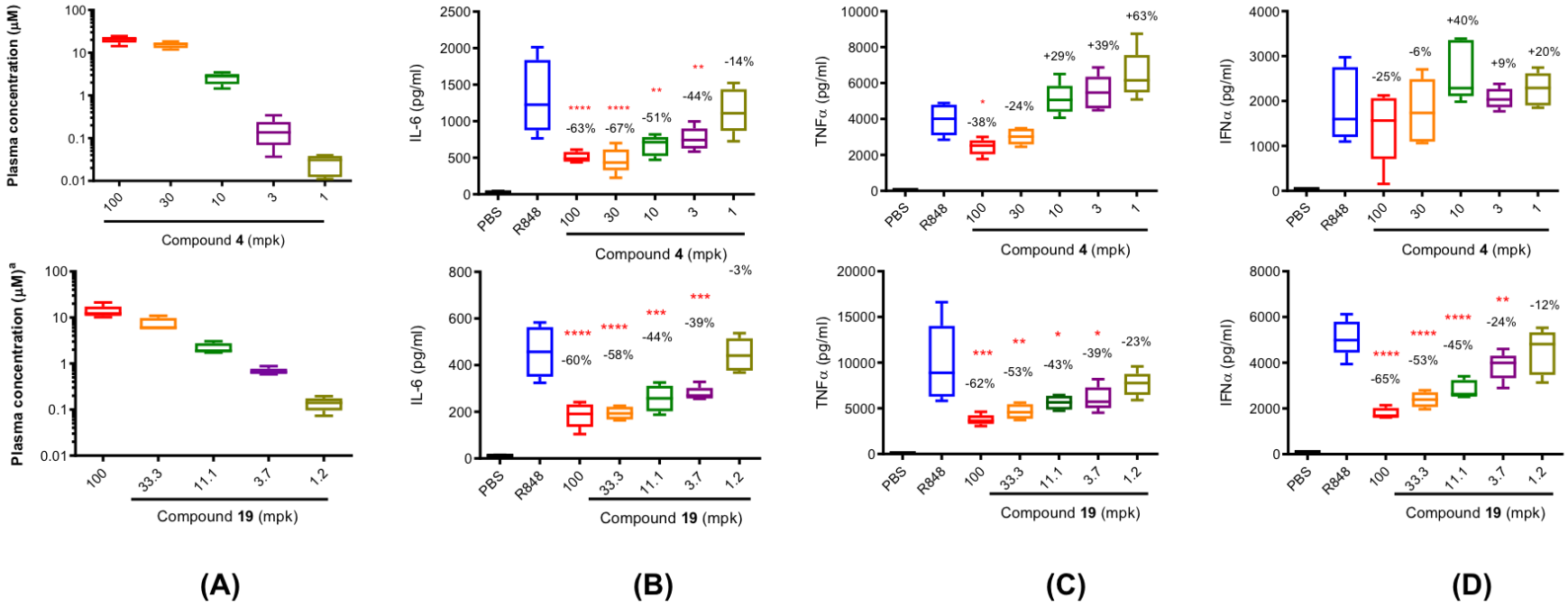


Table S3. Percent inhibition of indicated kinases at 1 μM compound **19.**

Kinase[a]	**% inhibition**
ACVR1B	-1
ACVR2B	12.5
AKT1	0.5
AKT2	7.5
ALK2	8
ARK5	23.5
ASK1	3.5
Abl	9.8
Aurora_A	20.5
Aurora_B	21.3
Axl	17
B-Raf	-0.5
BMPR1A	7
BTK	42
Blk	37.5
Bmx	33.3
BrSK1	3
Brk	10.5
CAMKK1	3.5
CAMKK2	8.5
CDK1/cyclinB	2.5
CDK2/cyclinA	3
CDK5/p25	9
CDK7/cyclinH	4
CDK8/cyclinC	13.5
CDK9/cyclinT1	2.5
CHK1	3
CHK2	13
CK1_alpha1	-0.5
CK1_delta	1.5
CK1_epsilon1	5.5
CK1_gamma1	-3.5
CK1_gamma2	5
CK2_alpha1	3.5
CLK1	7.5
CLK2	20.3
CLK3	10
CLK4	47.8
CSF1R	25.3
CSK	22
CaMKI	-5.5
CaMKII_beta	-5.5
CaMKI_delta	2
CamKII_alpha	2.5
CamKIV	-6
Cot	3
DAPK1	5.5
DCAMKL2	0.5
DDR1	-2
DMPK	2.1
DNA-PK	9
DRAK1	11.5
DYRK1A	2
DYRK3	2
DYRK4	-5
EGFR	6.8
EGFR(T790M,L858R)	13
ERK2	0.5
EphA1	20.8
EphA3	4.5
EphA7	20
EphA8	13
EphB1	23
EphB3	0
ErbB2	1.5
ErbB4	5
FAK	19.5
FGFR1	5.5
FGFR3	1.5
FGFR4	-2
Fes	19
Fgr	53
Flt1	-1
Flt3	90.8
Flt4	13
Frk	5.5
GRK2	-3
GRK3	-1
GRK5	-2
GRK6	3.5
GSK3_alpha	15.5
GSK3_beta	7.8
HIPK1	0
HIPK2	4.5
HIPK4	2.5
HPK1	13.3
Hyl	-4
IGF1R	11.5
IKK_alpha	8.5
IKK_beta	1.5
IKK_epsilon	-0.5
IRAK1	64.3
IRAK4	101.3
IRR	18
ITK	21
InsR	34.5
JAK1	83.5
JAK2	80.5
JAK3	28
JNK1_alpha1	-1.5
JNK2	2.5
JNK3	10.5
KDR	17
KHS1	36.5
Kit	3
LIMK1	-4.5
LRRK2	91.4
LTK	45
Lck	41.3
Lyn	55.3
MAP4K4	86.3
MAPKAPK2	9.5
MAPKAPK3	6
MARK1	-1
MARK3	8.5
MEK1	20.5
MEK3	12
MEKK2	3.5
MELK	12.5
MKK6	-5.5
MKNK1	-6
MKNK2	6.5
MLK1	14
MLK2	7.5
MRCK_alpha	1.5
MSK1	-2
MSSK1	2.5
MST1	-1
MST2	7

MST3	5.5
MST4	2
MYLK(smMLCK)	12
MYLK3(caMLCK)	4.5
Mer	28
Met	-2
Mink1	80.5
MuSK	13
NEK1	11
NEK4	10
NEK6	7
NEK9	-2.5
NLK	7
PAK1	9
PAK3	2
PAK4	5
PAK6	7
PASK	-6.5
PDGFR_alpha	40.5
PDK1(direct)	4.5
PI3K-A	21.5
PI3K-G	46.5
PIM1	1.5
PKA	5
PKC_alpha	11
PKC_beta1	4
PKC_delta	12.5
PKC_epsilon	13
PKC_eta	8
PKC_theta	1
PKC_zeta	6

PKD1	7.5
PKG1_alpha	1
PLK1	-2
PLK2	-3.5
PLK3	-3
PRAK	-3.5
PRK1	0.5
PRKAA1	5.5
PhK_gamma1	18.5
PhK_gamma2	-3
PrKX	-2.5
RAF1(Y340D,Y341D)	-1.5
RIPK2	22.5
ROCK1	5
ROCK2	6
RSK1	7.5
RSK2	15
RSK3	9
Ret	31.5
Ron	2.5
Ros	38.5
Rse	18
SGK1	4
SGK2	5.5
SGK3	-0.5
SIK2	12
SLK	4
SPHK1	-11
SRPK1	1.5
STK16	15

STK33	3.5
Src	23.8
Srm	16
Syk	9
TAK1-TAB1	74.5
TAO1	2
TBK1	5.5
TEC	0.5
TGFBR1	0.5
TNK2	37.5
TSSK1	5
TTK	13
TXK	34
TYK2	53.5
Tie2	6.5
TrkA	71
TrkB	86
WEE1	5.5
WNK2	2.5
YSK1	5
Yes	41.5
ZAK	5.5
ZAP-70	-6.5
ZIPK	3.5
eEF-2K	0
mTOR	0
p38_alpha(direct)	4
p38_beta	4
p38_delta	5.5
p38_gamma	0.5
p70S6K	1

[a]ATP concentration at Km_{app}. Data show inhibition of single replicates.

Table S4. IC_{50} determination of those kinases inhibited by compound **19** at >80% when assayed at 1 μM.

Kinase	ATP concentration	IC_{50} (μM)
Flt3	Km app	0.177
JAK1	Km app	0.282
JAK2	Km app	0.486
LRRK2	Km app	0.198
MAP4K4	Km app	0.68
Mink1	Km app	0.879
TAK1-TAB1	NA	0.966
TrkA	Km app	0.313
TrkB	Km app	0.259

Assay Conditions

LogD$_{7.4}$ Experimental Procedure: Methods for determining LogD have been previously described. [3]

Kinetic Solubility Assay: Methods for measuring compound kinetic solubility have previously been described. [4]

IRAK4 Biochemical Potency: Methods for assessing inhibition of human IRAK4 catalytic activity have been previously described. [5]

R848-Induced IL-6 Production in Human Whole Blood
Test compounds (stock solutions in DMSO) were dispensed into a 96-well plate (17.5 µL/well). Human whole blood was diluted with serum-free RPMI 1640 medium at a ratio of 1:0.6 and added (140 µL/well). Plates were incubated for 90 min at 37 °C, 5% CO_2. R848 (Invivogen cat # tlrl-r848, 1 mg/mL stock) was diluted to 12.5 µM with RPMI. The R848 stock (17.5 µL/well) or just media for control wells was added (final volume was 175 µL/well) and incubated for 3.5 h. After treatment, the plates were sealed and centrifuged at 3000 rpm for 2 min. Plasma from each well was transferred to human IL-6 MSD plates (25 µL) and to storage plates (60 µL).

Liver Microsome Stability[6]
Liver microsomal stability assay was performed on a BioCel 1200 liquid handling workstation (Agilent Technologies, Santa Clara, CA). Compounds (1.0 µM) were incubated for 5 min at 37 ^{o}C in 100 µL of a reaction mixture containing 100 mM phosphate buffer (pH 7.4) and 0.5 mg/mL liver microsomes and 1 mM NADPH. At different time intervals (0, 20, 40 and 60 min), aliquots of 20 µL of reaction mixtures were taken out and mixed with 4-volumes of acetonitrile (ACN) containing 0.1 µM propranolol as the internal standard to stop metabolic reaction. The samples were then centrifuged at 3250xg for 40 min to remove precipitated protein. The supernatants were subsequently transferred to a new 96-well plate and diluted 2-fold using deionized water, and were then subjected to LC-MS/MS analysis using an ABI Sciex 5500 QTRAP® mass spectrometer (Applied Biosystems, Foster City, CA) coupled with a Agilent 1260 HPLC (Agilent Technologies, Santa Clara, CA). Percent of remaining was calculated using peak area ratio of test compound to the internal standard at different time points relative to the control (T=0 min).

Hepatocyte Stability[7]
Cryopreserved human hepatocytes from a 10 donor pool were quickly thawed at 37 °C, suspended in prewarmed In VitroGRO™ HT Medium, and then centrifuged at 100×g at room temperature for 10 min. The supernatants were discarded, and cells were resuspended in 5 mL DMEM medium. Cell viability in suspension was counted on a Hepatometer ® Vision (Lonza, NC), and viable cells were then adjusted to 1.0×10^6 cells/mL in DMEM. Drugs were first diluted to 2 µM with DMEM medium, and then aliquots of 125 µL of drug-containing medium were transferred into triplicate wells of 96-well non-coated plates. Incubation was initiated by the addition of 125 µL of hepatocyte suspension to yield a total incubation volume of 250 µL. Final concentration of each drug was 1 µM, and final cell density was 0.5×10^6 cells/mL. Incubations were conducted in a humidified incubator at 37 °C. Aliquots of 50 µL incubation medium were

taken out at different time intervals (0, 60, 120 and 180 min), and immediately mixed with 100 µL of ice-cold acetonitrile containing 50 nM propranolol (internal standard). Samples were then centrifuged at 3000 x g for 5 min, and 80 µL of supernatant was taken out and diluted with 160 µL of water prior to LC/MS-MS analysis using an ABI Sciex 5500 QTRAP® mass spectrometer (Applied Biosystems, Foster City, CA) coupled with a Agilent 1260 HPLC (Agilent Technologies, Santa Clara, CA).

Kinase selectivity

Compounds were profiled at 1 µM for inhibition of various kinases with the SelectScreen Kinase Profiling Services platform (Thermo Fisher Scientific). IC_{50} determination was performed using the Z'-Lyte Kinase Biochemical Assay (Thermo Fisher Scientific) with the concentration of ATP denoted in Table S4.

Plasma Protein Binding Assay[8]

Plasma protein binding experiments were performed in triplicate (n=3) using a Single-Use RED Plate by following the standard protocol. Initially, individual drugs were spiked to plasma (pH 7.4) to achieve a final concentration of 5 µM, and then 300 µL of drug-plasma mixtures were transferred to the donor wells of the RED plate which was pre-loaded with 500 µL phosphate buffer saline (133 mM) on the receiver wells. The RED plate was sealed with a gas permeable membrane and placed in a shaking incubator (450 rpm, VWR Symphony^{TM}) for 6 hr at 37ºC with 5% CO_2. At the end of incubation, aliquots of 30 µL samples were taken out of the RED device and matrix equalized with an equal volume of plasma or buffer, and samples were then immediately quenched with ice cold acetonitrile (sample:acetonitrile 1:3) containing either propranolol or labetalol as an internal standard. After shaking for 15 min at 500 rpm on a Thermo Scientific Compact Digital MicroPlate Shaker, all samples were then subjected to centrifugation at 3700 rpm for 15 min (Beckman Coulter Allegra X 12R) to remove plasma protein. Subsequently, supernatants were collected and then diluted with an equal volume of water prior to LC-MS/MS analysis.

MDCK Permeability Assay

Madin-Darby Kidney cells (MDCKI) were obtained from the National Institutes of Health, (Bethesda, MD). Cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 5 µg/mL plasmocin before seeding on Millipore Millicell-24 well plates at 2.5 x 10^5 cells/mL and allowed to grow for 5 days. Prior to the permeability experiment cell monolayers were equilibrated in transport buffer (Hank's Balanced Salt Solution with 10 mM Hepes, pH 7.4) for 20 minutes at 37°C with 5% CO2 and 95% relative humidity. Test compound dose solutions were prepared at 10 µM in transport buffer containing the monolayer integrity marker lucifer yellow (100 µM). The dose solutions were added to the donor chambers and transport buffer was added to all receiver chambers. The permeability was examined in the apical to basolateral (A:B) and basolateral to apical (B:A) directions. The receiver chambers were sampled at 60, 120, and 180 min and were replenished with fresh transport buffer. Lucifer yellow was measured using a fluorescence plate reader (ex: 425 nm; em: 530 nm) and compound concentrations in the donor and receiving compartments were determined by LC-MS/MS analysis. The apparent permeability (P_{app}) in the A:B and B:A directions, was calculated as follows:

$P_{app} = (dQ/dt)•(1/AC_0)$,

Where: dQ/dt = rate of compound appearance in the receiver compartment; A = surface area of the insert; and C_0 = initial substrate concentration at time 0 min.

PK/PD Model Methods:

Mice:

6-8 weeks old female C57BL/6 mice were purchased from Jackson labs and housed at Genentech under specific pathogen free conditions. All animal procedures were conducted under a protocol approved by the Institutional Animal Care and Use Committee at Genentech, and were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

R848-Induced IFNα/IL-6/TNFα PK/PD model:

R848 was purchased from Invivogen, San Diego and resuspended with endotoxin-free water. 1 hour prior to R848 administration, IRAK4 small molecule inhibitor in MCT nanosuspension or vehicle alone was given orally (PO). At 0 hour, 5µg R848 or PBS was dosed intravenously. 1h post R848 administration, mice were sacrificed and blood were collected by terminal cardiac puncture under anesthesia for analysis of cytokines and PK of the compounds. INFα was assayed by ELISA and IL-6/TNFα were assayed by MSD.

Synthetic Procedures

All chemicals were purchased from commercial suppliers and used as received. ^{1}H NMR spectra were recorded on Bruker Avance 400 or 500 spectrometers. Chemical shifts are expressed in δ ppm referenced to an internal standard, tetramethylsilane (δ = 0 ppm). Abbreviations used in describing peak signals are br = broad signal, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. All final compounds were purified to have purity higher than 95% by reverse-phase high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), or normal-phase silica gel flash chromatography.

The purity was assessed by reverse-phase HPLC with an isocratic gradient of 5−95% acetonitrile in water (with either acid or base modifier) and monitored by diode array ultraviolet detection at 254 nm. Low-resolution mass spectra were recorded on a liquid chromatography−mass spectrometer in electrospray positive (ES +) mode. High-resolution mass spectrometry (HRMS) experiments were performed on a Dionex LC Ultimate3000 coupled with a ThermoScientific Q Exactive orbitrap mass spectrometer using electrospray ionization (ESI) as the ionization source and a Phenomenex XB-C18, 1.7 mm, 50 mm × 2.1 mm column with a 0.7 mL/min flow rate at 40 °C for LC separation. Solvent A was 0.1% formic acid (FA) in water, and solvent B was 0.1% FA in acetonitrile. The gradient consisted of 2−98% solvent B over 7 min and held at 98% B for 1.5 min following equilibration for 1.0 min. The LC was monitored by UV absorbance at 220 and 254 nm. MS full scans with 10 000 resolution were applied to all experiments.

Compounds from Table S1 (**4**, **5**, **DHBF-43**, **DHBF-44**, **DHBF-20**, **DHBF-17**) were prepared and purified to higher than 95% purity by reverse-phase high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), or normal-phase silica gel flash chromatography according to the procedures previously described.[4] Compounds **1**, **4** and **5** were prepared as previously reported.[1-2]

Compound 6. *N*-(2,2-Dimethyl-6-morpholino-1-oxo-2,3-dihydro-1*H*-inden-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

***N*-(2,3-Dihydro-1H-inden-5-yl)acetamide.** To a stirred solution of 2,3-dihydro-1*H*-inden-5-ylamine (15.0 g, 113 mmol) and triethylamine (17.1 g, 169 mmol) in dichloromethane (300 mL) was added acetyl chloride (26.5 g, 338 mmol) dropwise. The mixture was stirred at 26 °C for 2.5 h, quenched with methanol (20 mL) and water (100 mL), and extracted with dichloromethane (150 mL x 3). The combined organic phase was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (20-45% ethyl acetate in petroleum ether) to afford *N*-(2,3-dihydro-1*H*-inden-5-yl)acetamide (19.0 g, 96 %) as a light brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.43 (s, 1H), 7.17 - 7.12 (m, 2H), 2.89-2.83 (m, 4H), 2.14 (s, 3H), 2.06 (m, 2H).

***N*-(6-Bromoindan-5-yl)acetamide.** To a stirred solution of *N*-(2,3-dihydro-1*H*-inden-5-yl)acetamide (19.0 g, 108 mmol) in acetic acid (350 mL) was added bromine (6.83 mL, 133 mmol) in acetic acid (3 mL) at 0 °C over a period of 20 min. The mixture was stirred at 26 °C for 1 h under a nitrogen atmosphere, and diluted with water until no more precipitate formed. The precipitate was collected, washed with water,

and dried under vacuum to give *N*-(6-bromoindan-5-yl)acetamide (27.0 g, 98%) as a light yellow solid. LCMS (ESI): *m/z* = 255.8 $[M+H]^+$.

***N*-(6-Bromo-1-oxo-indan-5-yl)acetamide.** To a stirred solution of *N*-(6-bromoindan-5-yl)acetamide (27.0 g, 106 mmol) in acetic acid (300 mL) was added dropwise chromium trioxide (44.0 g, 440 mmol) in 50% aqueous acetic acid (34 mL) at 50 °C and stirred for another 20 min. Then it was cooled to 0 °C when the reaction was quenched with 2-propanol (10 mL). The solvent was removed in vacuo, and the residue was diluted with water (100 mL) and extracted with ethyl acetate (100 mL x 3). The combined organic phase was washed with 0.5 M aqueous NaOH (50 mL) and brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by flash column chromatography (0-4% methanol in dichloromethane) to afford *N*-(6-bromo-1-oxo-indan-5-yl)acetamide (13.1 g, 46%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.94 (s, 1H), 7.91 (br s, 1H), 3.11 (t, *J* = 6.0 Hz, 2H), 2.71 (t, *J* = 6.0 Hz, 2H), 2.30 (s, 3H).

5-Amino-6-bromo-indan-1-one. A mixture of *N*-(6-bromo-1-oxo-indan-5-yl)acetamide (13.1 g, 48.9 mmol) and 6M aqueous hydrochloric acid (260 mL, 1.56 mol) was stirred at 100 °C for 1 h under a nitrogen atmosphere. The solution was cooled to 0 °C and adjusted to pH = 8 with a 10 M aqueous sodium hydroxide solution. The precipitate formed was collected, washed with water, and dried under vacuum to afford 5-amino-6-bromo-indan-1-one (10.8 g, 98%) as a light brown powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 6.74 (s, 1H), 4.68 (s, 2H), 2.98 (t, *J* = 6.0 Hz, 2H), 2.64 (t, *J* = 6.0 Hz, 2H).

6-Bromo-5-nitro-indan-1-one. To a suspension of 5-amino-6-bromo-indan-1-one (4.0 g, 17.7 mmol) in 20% aqueous tetrafluoroboric acid (16 mL) at 0 °C was added 4 M aqueous sodium nitrite (1.9 g, 27.3 mmol) drop wise over a period of 5 min. The mixture was stirred for 50 min after the addition was completed. The resulting foamy suspension was added portion wise to a vigorously stirred mixture of copper (5.3 g, 83.2 mmol) and sodium nitrite (16.3 g, 237 mmol) in water (32 mL) at 26 °C over a period of 30 min. During the addition, excessive foaming was broken up by the addition of small amounts of diethyl ether. After the mixture was stirred for a further 50 min, it was filtered through Celite pad and washed with ethyl acetate (300 mL). The organic phase was separated, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (10-20% ethyl acetate in petroleum ether) to afford 6-bromo-5-nitro-indan-1-one (2.1 g, 47 %) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.85 (s, 1H), 3.21 (t, *J* = 6.0 Hz, 2H), 2.83 (t, *J* = 6.0 Hz, 2H).

6-Bromo-2,2-dimethyl-5-nitro-indan-1-one. To a mixture of 6-bromo-5-nitro-indan-1-one (1.2 g, 4.5 mmol) and iodomethane (1.4 mL, 22.9 mmol) in *N*, *N*-dimethylformamide (40 mL) was added 60% sodium hydride mineral oil (544 mg, 13.6 mmol) in batches at 0 °C. The mixture was stirred at 0 °C for 10 minutes, quenched by saturated ammonium chloride solution (10 mL) and extracted with ethyl acetate (100 mL x 3). The combined organic phase was dried over sodium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (2-5% ethyl acetate in petroleum ether) to afford 6-bromo-2,2-dimethyl-5-nitro-indan-1-one (450 mg, 35%) as a yellow solid. LCMS (ESI): *m/z* = 284.1 $[M+H]^+$.

2,2-Dimethyl-6-morpholino-5-nitro-indan-1-one. To a stirred solution of 6-bromo-2,2-dimethyl-5-nitro-indan-1-one (450 mg, 1.6 mmol) in dimethyl sulfoxide (18 mL) was added morpholine (276 mg, 3.2 mmol) and *N*,*N*-diisopropylethylamine (614 mg, 4.8 mmol). The mixture was stirred at 110 ^{o}C for 16 h, diluted with water (50 mL), and extracted with ethyl acetate (100 mL x 3). The combined organic phase was washed with brine (30 mL), dried over sodium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (10% ethyl acetate in petroleum ether) to give the mixture of 2,2-dimethyl-6-morpholino-5-nitro-indan-1-one and 6-bromo-2,2-dimethyl-5-morpholino-indan-1-one (340 mg, 74%) as a yellow solid which was used directly for next step. LCMS (ESI): *m/z* = 290.9 $[M+H]^+$.

5-Amino-2,2-dimethyl-6-morpholino-indan-1-one. To a stirred mixture of 2,2-dimethyl-6-morpholino-5-nitro-indan-1-one and 6-bromo-2,2-dimethyl-5-morpholino-indan-1-one (340 mg) in ethanol (25 mL) and water (5 mL) was added iron (327 mg, 5.9 mmol) and ammonium chloride (313 mg, 5.9 mmol). The mixture was stirred at 80 ^{o}C for 2 h under nitrogen atmosphere. The reaction mixture was filtered and concentrated. The residue was purified by flash column chromatography (20-60% ethyl acetate in petroleum ether) to afford 5-amino-2,2-dimethyl-6-morpholino-indan-1-one (190 mg, 62%) as light a yellow solid. LCMS (ESI): *m/z* = 261.0 $[M+H]^+$.

***N*-(2,2-dimethyl-6-morpholino-1-oxo-2,3-dihydro-1H-inden-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide.** A mixture of 5-amino-2,2-dimethyl-6-morpholino-indan-1-one (50 mg, 0.2 mmol) and pyrazolo[1,5-*a*]pyrimidine-3-carbonyl chloride (52 mg, 0.3 mmol) in pyridine (5 mL) was stirred at 28 ^{o}C for 20 h and concentrated. The residue was purified by flash column chromatography (eluting 0-1% methanol in dichloromethane) followed by prep-TLC (100% ethyl acetate). The crude product was further triturated with methanol to afford *N*-(2,2-dimethyl-6-morpholino-1-oxo-indan-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (54 mg, 68%) as a white solid. 1H NMR (400 MHz, DMSO-d_6): δ 10.94 (s, 1H), 9.40 (d, *J* = 6.8 Hz, 1H), 8.98 (d, *J* = 4.4 Hz, 1H), 8.75 (s, 1H), 8.68 (s, 1H), 7.53 (s, 1H), 7.38 (dd, *J* = 6.8, 4.4 Hz, 1H), 3.91 - 3.85 (m, 4H), 2.98 (s, 2H), 2.91-2.86 (m, 4H), 1.14 (s, 6H). Target exact mass 405.18 (C22H23N5O3); LCMS (ESI): *m/z* = 406.1 $[M+H]^+$.

Compounds 7, 8: Enantiomers of *N*-[1-Hydroxy-2,2-dimethyl-6-morpholino-indan-5-yl]pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

5-Amino-2,2-dimethyl-6-morpholino-indan-1-ol. To a stirred solution of 5-amino-2,2-dimethyl-6-morpholino-indan-1-one (150 mg, 0.6 mmol) in tetrahydrofuran (9 mL) and methanol (3 mL) was added sodium borohydride (65 mg, 1.7 mmol) at 0 ^{o}C. The mixture was stirred under nitrogen atmosphere at 0 ^{o}C for 10 min, then at 28 ^{o}C for 8.5 h. The reaction mixture was quenched with water (15 mL) and extracted with dichloromethane (50 mL x 3). The combined organic phase was dried over sodium sulfate, filtered and concentrated to afford crude 5-amino-2,2-dimethyl-6-morpholino-indan-1-ol (150 mg, 99%) as a light brown solid, which was used for the next step directly without further purification. LCMS (ESI): *m/z* = 263.1 $[M+H]^+$.

***N*-(1-Hydroxy-2,2-dimethyl-6-morpholino-2,3-dihydro-1H-inden-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide.** A mixture of 5-amino-2,2-dimethyl-6-morpholino-indan-1-ol (150 mg, 0.6 mmol) and pyrazolo[1,5-*a*]pyrimidine-3-carbonyl chloride (135 mg, 0.7 mmol) in pyridine (15 mL) was stirred at 28 ^{o}C for 18 h and concentrated. The residue was purified by flash

column chromatography (0-2% methanol in dichloromethane) to afford *N*-(1-hydroxy-2,2-dimethyl-6-morpholino-indan-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (220 mg, 94%) as a light yellow solid. LCMS (ESI): *m/z* = 408.1 $[M+H]^+$.

N-(1-hydroxy-2,2-dimethyl-6-morpholino-indan-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (220 mg, 0.5 mmol) was resolved by chiral SFC (Column: chiralpak AD-3 100*4.6 mm ID, 3 um; mobile phase: A: CO_2, B: ethanol (0.05% DEA); gradient: from 5% to 40% of B; flow rate, 2.8 ml/min; column temperature 40C.) to afford enantiomers: **7** (59.4 mg, 27%; RT = 4.667 min) and **8** (56.6 mg, 26%; RT = 4.955 min) as light yellow solids with absolute stereochemistry undetermined.

Compound 7 (Peak 1): ^{1}H NMR (400 MHz, CD_3OD) δ 9.13 (dd, *J* = 7.2, 1.6 Hz, 1H), 8.93 (dd, *J* = 4.0, 1.6 Hz, 1H), 8.66 (s, 1H), 8.31 (s, 1H), 7.31 - 7.27 (m, 2H), 4.61 (s, 1H), 3.99 - 3.95 (m, 4H), 2.95 - 2.90 (m, 4H), 2.77 (d, *J* = 15.2 Hz, 1H), 2.64 (d, *J* = 15.2 Hz, 1H), 1.18 (s, 3H), 1.03 (s, 3H). Target exact mass 407.2 (C22H25N5O3); LCMS LCMS (ESI): *m/z* = 408.2 $[M+H]^+$.

Compound 8 (Peak 2): ^{1}H NMR (400 MHz, CD_3OD) δ 9.13 (dd, *J* = 7.2, 1.6 Hz, 1H), 8.94 (dd, *J* = 4.0, 1.6 Hz, 1H), 8.67 (s, 1H), 8.32 (s, 1H), 7.32 - 7.28 (m, 2H), 4.61 (s, 1H), 3.99 - 3.95 (m, 4H), 2.95 - 2.90 (m, 4H), 2.77 (d, *J* = 15.6 Hz, 1H), 2.64 (d, *J* = 15.2 Hz, 1H), 1.18 (s, 3H), 1.03 (s, 3H). Target exact mass 407.2 (C22H25N5O3); LCMS (ESI): *m/z* = 408.2 $[M+H]^+$.

Compound 9. *N*-(2-methyl-6-morpholino-1-oxoisoindolin-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

6-Chloro-2-methyl-5-nitroisoindolin-1-one. To a solution of methyl 2-(bromomethyl)-5-chloro-4-nitrobenzoate (prepared following protocol from WO 2013/079505) (500 mg, 1.6 mmol) in methanol (16 mL) was added triethylamine (1.9 mmol,1.2 equiv) and methylamine (33% in ethanol, 1.94 mmol, 1.2 equiv). The reaction mixture was heated at 70 °C for 4 h, cooled to ambient temperature, and diluted with isopropyl acetate and 1N HCl. The layers were separated and the aqueous layer was extracted with isopropyl acetate (2x). The combined organic layers were dried over sodium sulfate and carried on without further purification. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.00 (s, 1H), 7.90 (s, 1H), 4.45 (s, 2H), 3.24 (s, 3H).

2-Methyl-6-morpholino-5-nitroisoindolin-1-one. A solution of 6-chloro-2-methyl-5-nitroisoindolin-1-one (186 mg, 0.82 mmol), morpholine (0.086 mL, 0.98 mmol, 1.2 equiv), diisopropylamine (0.29 mL, 1.6 mmol, 2.0 equiv) in DMSO (1 mL) was heated at 90 °C for 18 h. The reaction mixture was cooled to ambient temperature, diluted with water and extracted with isopropyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and absorbed onto celite to be purified by silica gel chromatography (0% to 100% isopropyl acetate in heptanes) to afford 2-methyl-6-morpholino-5-nitroisoindolin-1-one (286 mg) as an orange foam. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.78 (s, 1H), 7.63 (s, 1H), 4.39 (s, 2H), 3.89 – 3.78 (m, 4H), 3.23 (s, 3H), 3.13 – 3.04 (m, 4H). MS (ESI): *m/z* = 278.0 $[M+1]^+$.

5-Amino-2-methyl-6-morpholinoisoindolin-1-one. 2-Methyl-6-morpholino-5-nitroisoindolin-1-one (228 mg, 0.82 mmol) was brought up in ethanol (0.16 M) and water (0.16 M) and treated with tin(II) chloride dehydrate (624 mg, 3.28 mmul, 4.0 equiv) and heated to 65°C for 5 h.

Dichloromethane (11 mL) and 2M NaOH (aq) (5.5 mL) were added and the mixture was passed through a hydrophobic frit. The solvent was removed *in vacuo* to give the desired product as an orange solid. The crude material was carried on without further purification. MS (ESI): *m/z* = 248.1 $[M+1]^+$.

***N*-(2-Methyl-6-morpholino-1-oxoisoindolin-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide.** A solution of 5-amino-2-methyl-6-morpholino-isoindolin-1-one (203 mg, 0.82 mmol), pyrazolo[1,5-a]pyrimidine-3-carbonyl chloride (224 mg, 1.2 mmol, 1.5 equiv), 4-dimethylaminopyridine (20 mg, 0.164 mmol, 0.2 equiv), and diisopropylethylamine (0.43 mL, 2.5 mmol, 3.0 equiv) in DCE (4 mL) was stirred at ambient temperature for 18 h. The reaction was concentrated under reduced pressure and purified by reverse phase HPLC to afford *N*-(2-methyl-6-morpholino-1-oxoisoindolin-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (17%). ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.88 (s, 1H), 9.40 (dd, J = 7.0, 1.6 Hz, 1H), 8.99 (dd, J = 4.2, 1.6 Hz, 1H), 8.74 (d, J = 4.6 Hz, 2H), 7.56 (s, 1H), 7.38 (dd, J = 7.0, 4.2 Hz, 1H), 4.45 (s, 2H), 3.94 – 3.84 (m, 4H), 3.07 (s, 3H), 2.97 – 2.86 (m, 4H). Target exact mass 407.2 (C20H20N6O3); MS (ESI): *m/z* = 393.1 $[M+1]^+$.

Compound 10. *N*-(2-Methyl-7-morpholino-1-oxo-1,2,3,4-tetrahydroisoquinolin-6-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

Ethyl 2-methyl-5-morpholino-4-nitro-benzoate. A mixture of ethyl 5-fluoro-2-methyl-4-nitro-benzoate (1.4 g, 6.16 mmol) and morpholine (2.15 g, 24.65 mmol) was stirred at 110 ^{o}C for 2 h. The reaction mixture was concentrated to dryness and purified by silica gel column chromatography (eluting gradient 10-50% ethyl acetate: petroleum ether) to afford ethyl 2-methyl-5-morpholino-4-nitro-benzoate (1.6 g, 88 % yield) as a red oil.

Ethyl 5-morpholino-4-nitro-2-(2-oxoethyl)benzoate. To a solution of ethyl 2-methyl-5-morpholino- 4-nitro-benzoate (0.5 g, 1.70 mmol) in *N*,*N*- dimethylformamide (5 mL) was added Bredereck's reagent (0.3 g, 1.70 mmol). The mixture was at 140 ^{o}C for 3 h, cooled to room temperature, concentrated to dryness and purified by silica gel column chromatography (eluting gradient 10-50% ethyl acetate: petroleum ether) to afford ethyl 5-morpholino-4-nitro-2-(2-oxoethyl)benzoate (0.2 g, 36% yield) as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 7.80 (s, 1H), 7.61 (s, 1H), 4.37 (q, J = 6.8 Hz, 2H), 4.08 (s, 2H), 3.85 (t, J = 4.4 Hz, 4H), 3.10 (t, J = 4.4 Hz, 4H), 1.40 (t, J = 7.2 Hz, 3H).

2-Methyl-7-morpholino-6-nitro-isoquinolin-1-one. To a solution of methylamine (135 mg, 4.34 mmol) in *N*,*N*-dimethylformamide (5 mL) was added ethyl 5-morpholino-4-nitro-2-(2-oxoethyl)benzoate (200 mg, 0.62 mmol) and the reaction was irradiated under microwave conditions at 140 ^{o}C for 30 min. The reaction mixture was concentrated to dryness and purified by preparatory TLC (eluent 50% ethyl acetate: petroleum ether) to afford 2-methyl-7-morpholino-6-nitro-isoquinolin-1-one (60 mg, 33% yield) as a red oil. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.83 (s, 1H), 7.09 (d, J = 7.6 Hz, 1H), 6.47 (d, J = 7.6 Hz, 1H), 3.86 (t, J = 4.4 Hz, 4H), 3.63 (s, 3H), 3.12 (t, J = 4.4 Hz, 4H).

6-Amino-2-methyl-7-morpholino-3,4-dihydroisoquinolin-1-one. To a solution of 2-methyl-7-morpholino-6-nitro-isoquinolin-1-one (150 mg, 0.52 mmol) in methanol (5 mL) was added 10 %

palladium on carbon (55 mg, 0.05 mmol). The reaction mixture was stirred at 65 ^{o}C for 1 h under hydrogen gas (15 psi), filtered and the filtrate was concentrated to obtain 6-amino-2-methyl-7-morpholino-3,4-dihydroisoquinolin-1-one (120 mg, 88% yield) as a white solid. The crude was used directly without further purification. LCMS (ESI): m/z = 262.0 $[M+H]^+$.

***N*-(2-Methyl-7-morpholino-1-oxo-1,2,3,4-tetrahydroisoquinolin-6-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide.** To a solution of pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (82 mg, 0.51 mmol) and 6-amino-2-methyl-7-morpholino-3,4-dihydroisoquinolin-1-one (120 mg, 0.46 mmol) in pyridine (6 mL) was added phosphorus oxychloride (0.21 mL, 2.3 mmol) and the reaction was stirred at 25 ^{o}C for 2 h. The reaction mixture was quenched with aqueous 10% sodium bicarbonate (5 ml) solution at 0 °C and extracted with ethyl acetate (10 mL * 2). The organic phases were isolated, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by reverse phase chromatography (acetonitrile 45-75% 0.05% ammonia in water) to give *N*-(2-methyl-7- morpholino-1-oxo-3,4- dihydroisoquinolin-6-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (17 mg, 9% yield) as a white solid. ^{1}HNMR (400 MHz, $CDCl_3$) δ 10.86 (s, 1H), 8.87 - 8.70 (m, 3H), 8.56 (s, 1H), 7.98 (s, 1H), 7.12 (s, 1H), 4.03 - 3.95 (m, 4H), 3.59 - 3.56 (m, 2H), 3.16 (s, 3H), 3.04 - 2.99 (m, 6H). Target exact mass 406.18 (C21H22N6O3); LCMS (ESI): m/z = 407.0 $[M+H]^+$.

Compound 11. *N*-(2-methyl-7-morpholino-1-oxo-1,2-dihydroisoquinolin-6-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

Following the procedures described for Compound **10**, and substituting the hydrogenation step with Fe/NH_4Cl reduction, *N*-(2-methyl-7-morpholino-1-oxo-6-isoquinolyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (150 mg, 64%) was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.91 (s, 1H), 8.90 - 8.80 (m, 4H), 8.24 (s, 1H), 7.12 (dd, *J* = 7.2, 4.0 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 6.53 (d, *J* = 7.2 Hz, 1H), 4.07 - 3.98 (m, 4H), 3.60 (s, 3H), 3.13 - 3.02 (m, 4H). Target exact mass 404.18 (C21H20N6O3); LCMS (ESI): *m/z* = 405.2 $[M+H]^+$.

Compound 12. *N*-[2,2-Dimethyl-6-(morpholin-4-yl)-1,1-dioxo-2,3- dihydro-benzothiophen-5-yl]pyrazolo[1,5-a]pyrimidine-3-carboxamide

Methyl 4-chloro-2-fluoro-5-nitro-benzoate. To a mixture of 4-chloro-2-fluoro-5-nitro-benzoic acid (5.06 g, 23.1 mmol) in methyl alcohol (15 mL) was added drop wise sulfurous dichloride (10 mL). The mixture was stirred at 90 ^{o}C for 16 h. The mixture was concentrated to afford methyl 4-chloro-2-fluoro-5-nitro-benzoate (5.2 g, 92%) as a white solid, which was used directly to next step without further purification. MS (ESI): m/z =234.1$[M+1]^+$.

4-Chloro-2-methylsulfanyl-5-nitro-benzoate. A mixture of methyl 4-chloro-2-fluoro-5-nitro-benzoate (800 mg, 3.43 mmol) in tetrahydrofuran (20 mL) was added sodium methanethiolate (20% wt in water, 1.2 g, 3.43 mmol). The mixture was stirred at 25 ^{o}C for 4 h. Water and ethyl acetate (30 mL) were added. The organic layer was separated, dried over sodium sulfate and concentrated to afford methyl 4-chloro-2-methylsulfanyl-5-nitro-benzoate (800 mg) as a yellow solid, which was used directly to next step without further purification. MS (ESI): m/z =262.0$[M+1]^+$.

Methyl 2-methylsulfanyl-4-morpholino-5-nitro-benzoate. A mixture of methyl 4-chloro-2-methylsulfanyl-5-nitro-benzoate (600 mg, 2.29 mmol) in morpholine (4 g, 46 mmol) was stirred at 60 ^{o}C overnight. The mixture was concentrated and the residue was purified by silica gel chromatography using ethyl acetate:petroleum ether (1:4) as eluting solvents to afford methyl 2-methylsulfanyl-4-morpholino-5-nitro-benzoate (506 mg, 70%) as yellow solid. MS (ESI): m/z =313.0$[M+1]^{+}$.

Methyl 2-methylsulfonyl-4-morpholino-5-nitro-benzoate. To a mixture of methyl 2-methylsulfanyl-4-morpholino-5-nitro-benzoate (506 mg, 1.62 mmol) in methyl alcohol (10 mL) and water (10 mL) was added oxone (1.08 g, 6.48 mmol). The mixture was stirred at 50 ^{o}C for 5 h. Ethyl acetate (30 mL) and water was added and the organic layer was separated, dried over sodium sulfate and concentrated to afford methyl 2-methylsulfonyl-4-morpholino-5-nitro-benzoate (478 mg) as a yellow solid, which was used directly to next step without further purification. MS (ESI): m/z =345.0$[M+1]^{+}$.

6-Morpholino-5-nitro-1,1-dioxo-benzothiophen-3-one. A mixture of methyl 2-methylsulfonyl-4-morpholino-5-nitro-benzoate (574 mg, 1.67 mmol) in *N*,*N*-dimethylformamide (15 mL) was treated with sodium hydride (60% wt with mineral oil, 100 mg, 2.5 mmol) at 0 ^{o}C. The mixture was stirred at 25 ^{o}C for 4 h. Water was added slowly and the pH was adjusted to 3 using 1N HCl. The aqueous layer was extracted with ethyl acetate (70 mL). The organic layer was concentrated to afford 6-morpholino-5-nitro-1,1-dioxo-benzothiophen-3-one (560 mg) as a yellow solid, which was used directly to next step without further purification. MS (ESI): m/z =313.0$[M+1]^{+}$.

2,2-Dimethyl-6-morpholino-5-nitro-1,1-dioxo-benzothiophen-3-one. A mixture of 6-morpholino-5-nitro-1,1-dioxo-benzothiophen-3-one (560 mg, 1.79 mmol) iodomethane (1.27 g, 8.97 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.36 g, 8.97 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred at 40 ^{o}C in a sealed tube for 16 h. Water was added and the aqueous layer was extracted with ethyl acetate (70 mL). The organic layer was washed with brine, dried over sodium sulfate and concentrated. The residue was purified by silica gel chromatography using ethyl acetate:petroleum ether (1:5) as eluting solvents to afford 2,2-dimethyl-6-morpholino-5-nitro-1,1-dioxo-benzothiophen-3-one (250 mg, 33%) as yellow solid. MS (ESI): m/z =341.1 $[M+1]^{+}$.

2,2-Dimethyl-6-morpholino-5-nitro-1,1-dioxo-3H-benzothiophen-3-ol. A mixture of 2,2-dimethyl-6-morpholino-5-nitro-1,1-dioxo-benzothiophen-3-one (200 mg, 0.47 mmol) in methyl alcohol (30 mL) was treated with sodium borohydride (53.6 mg, 1.41 mmol) The mixture was stirred at 25 ^{o}C for 3 h. The mixture was quenched with water and ethyl acetate (30 mL) was added. The organic layer was separated, dried over sodium sulfate and concentrated to afford 2,2-dimethyl-6-morpholino-5-nitro-1,1-dioxo-3H-benzothiophen-3-ol (150 mg) as a brown solid, which was used directly to next step without further purification. MS (ESI): m/z =343.1 $[M+1]^{+}$.

2,2-Dimethyl-6-morpholino-5-nitro-3H-benzothiophene 1,1-dioxide. A mixture of 2,2-dimethyl-6-morpholino-5-nitro-1,1-dioxo-3H-benzothiophen-3-ol (150 mg, 0.44 mmol) triethylsilane (2 mL, 12.52 mmol) in trifluoroacetic acid (8 mL, 108 mmol) was stirred at 50 ^{o}C for 16 h. The mixture was concentrated and purified by silica gel chromatography using ethyl acetate:petroleum ether (1:5 to 1:3) as eluting solvents to afford 2,2-dimethyl-6-morpholino-5-

nitro-3H-benzothiophene 1,1-dioxide (88 mg, 62%) as a yellow solid. MS (ESI): m/z =327.0 $[M+1]^+$.

2,2-Dimethyl-6-morpholino-1,1-dioxo-3H-benzothiophen-5-amine. A mixture of 2,2-dimethyl-6-morpholino-5-nitro-3H-benzothiophene 1,1-dioxide (78 mg, 0.24 mmol) and 10 wt% palladium on carbon (20 mg, 0.24 mmol) in methyl alcohol (20 mL) was stirred at 25 °C under hydrogen atmosphere for 1h. The reaction was filtered through celite and concentrated under reduced pressure to afford 2,2-dimethyl-6-morpholino-1,1-dioxo-3H-benzothiophen-5-amine (60 mg) as a pink solid, which was used directly to next step without further purification. MS (ESI): m/z =297.2 $[M+1]^+$.

***N*-[2,2-Dimethyl-6-(morpholin-4-yl)-1,1-dioxo-2,3-dihydro-benzothiophen-5-yl]pyrazolo[1,5- a]pyrimidine-3-carboxamide.** A mixture of 2,2-dimethyl-6-morpholino-1,1-dioxo-3H-benzothiophen-5-amine (50 mg, 0.17 mmol), pyrazolo[1,5-a]pyrimidine-3-carbonyl chloride (153 mg, 0.84 mmol), and potassium carbonate (880 mg, 1.69 mmol) in toluene (10 mL) was stirred at 110 °C for 14 h. Ethyl acetate (10 mL) and water were added. The organic layer was separated, dried over sodium sulfate and concentrated. The residue was purified by preparative HPLC (Gilson 281, Xbridge 21.2*250mm c18, 10um; A: acetonitrile 25-55%; B: 10 M ammonium bicarbonate in water) to afford *N*-(2,2-dimethyl-6-morpholino-1,1-dioxo-3H-benzothiophen-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (30 mg, 40%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 9.42 (dd, *J* = 1.6, 7.2 Hz, 1H), 9.00 (dd, *J* = 1.6, 4.4 Hz, 1H), 8.76 (s, 1H), 8.63 (s, 1H), 7.70 (s, 1H), 7.39 (dd, *J* = 4.4, 7.2 Hz, 1H), 3.88-3.90 (m, 4H), 3.15 (s, 2H), 2.92-2.95 (m, 4H), 1.38 (s, 6H). Target exact mass 441.15 ($C_{21}H_{23}N_5O_4S$); MS (ESI): m/z =442.1 $[M+1]^+$.

Compounds 13 & 14. *N*-(2,2-dimethyl-6-morpholino-1-oxido-2,3-dihydrobenzo[*b*]thiophen-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

1-(2,4-Difluorophenyl)-2-methylpropan-2-ol. To a solution of methyl 2-(2,4-difluorophenyl)acetate (38.0 g, 204 mmol) in anhydrous tetrahydrofuran (300 mL) was added methylmagnesium bromide (3 M in ethyl ether; 204 mL, 612 mmol) dropwise at -78 °C. The reaction was warmed to room temperature and stirred for 30 min. It was quenched with saturated aqueous ammonium chloride (200 mL) at 0 °C and extracted with ethyl acetate (300 mL x2). The combined organic phase was washed with brine (100 mL x2), dried over sodium sulfate and concentrated. The residue was purified by flash chromatography (eluting 0-10% ethyl acetate in petroleum ether) to afford 1-(2,4-difluorophenyl)-2-methyl-propan-2-ol (38 g, 100%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 - 7.17 (m, 1H), 6.85 - 6.75 (m, 2H), 2.77 (s, 2H), 1.23 (s, 6H).

1-(2,4-Difluorophenyl)-2-methylpropan-2-yl carbamimidothioate. To a mixture of thiourea (6.13 g, 80.6 mmol) in trifluoroacetic acid (100 mL, 53.7 mmol) was added 1-(2,4-difluorophenyl)-2-methyl-propan-2-ol (10.0 g, 53.7 mmol) and stirred at 75 °C for 6 h under nitrogen. The mixture was taken up in ethyl acetate (150 mL x2) and washed with water (100 mL x2), brine (80 mL x2), dried over sodium sulfate and concentrated. The resulting white solid of crude 2-[2-(2,4-difluorophenyl)-1,1-dimethyl-ethyl]isothiourea (11.3 g, 86%) was used directly without further purification. LCMS (ESI): *m/z* = 244.9 $[M+H]^+$.

6-Fluoro-2,2-dimethyl-2,3-dihydrobenzo[b]thiophene. To a mixture of 2-[2-(2,4-difluorophenyl)-1,1-dimethyl-ethyl]isothiourea (6.32 g, 25.9 mmol) in dimethyl sulfoxide (50 mL) was added sodium hydroxide (4.14 g, 103 mmol) and stirred at 90 ^{o}C for 4 h. The reaction was diluted with water (80 mL) and extracted with ethyl acetate (150 mL x2). The combined organic phase was washed with water (80 mL x2), brine (50 mL), dried over sodium sulfate and concentrated. The residue was purified by flash chromatography (eluting 0-1% ethyl acetate in petroleum ether) to afford 6-fluoro-2,2-dimethyl-3H-benzothiophene (2.65 g, 56%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 - 7.02 (m, 1H), 6.85 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.67 (dt, *J* = 6.4, 2.4 Hz, 1H), 3.04 (s, 2H), 1.55 (s, 6H).

6-Fluoro-2,2-dimethyl-5-nitro-2,3-dihydrobenzo[b]thiophene. To a solution of 6-fluoro-2,2-dimethyl-3H-benzothiophene (2.0 g, 10.97 mmol) in acetic acid (20 mL) was added nitric acid (0.29 mL, 16.46 mmol) at 0 ^{o}C and stirred at 0 to 25 ^{o}C for 0.5 h. The reaction was diluted with water (30 mL) and extracted with dichloromethane (80 mL x2). The combined organic phase was washed with saturated aq. sodium bicarbonate (30 mL x2), brine (30 mL), dried over sodium sulfate and concentrated. The residue was purified by prep-TLC (eluting 10% ethyl acetate in petroleum ether) to afford 6-fluoro-2,2-dimethyl-5-nitro-3H-benzothiophene (240 mg, 10%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 7.6 Hz, 1H), 7.01 (d, *J* = 10.8 Hz, 1H), 3.14 (s, 2H), 1.59 (s, 6H).

4-(2,2-Dimethyl-5-nitro-2,3-dihydrobenzo[b]thiophen-6-yl)morpholine. To a mixture of 6-fluoro-2,2-dimethyl-5-nitro-3H-benzothiophene (240 mg, 1.06 mmol) in acetonitrile (10mL) was added morpholine (920 mg, 10.6 mmol) and *N*,*N*-diisopropylethylamine (1.36 g, 10.6 mmol). The mixture was stirred at 75 ^{o}C for 3 h and concentrated. The residue was purified by prep-TLC (eluting 10% ethyl acetate in petroleum ether) to afford 4-(2,2-dimethyl-5-nitro-3H-benzothiophen-6-yl)morpholine (270 mg, 87%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 6.89 (s, 1H), 3.86 - 3.83 (m, 4H), 3.09 (s, 2H), 3.04 - 3.01 (m, 4H), 1.58 (s, 6H). LCMS (ESI): *m/z* = 294.9 $[M+H]^+$.

2,2-Dimethyl-6-morpholino-5-nitro-2,3-dihydrobenzo[b]thiophene 1-oxide. To a mixture of 4-(2,2-dimethyl-5-nitro-3H-benzothiophen-6-yl)morpholine (270 mg, 0.92 mmol) in dichloromethane (8 mL) was added 3-chloroperoxybenzoic acid (186 mg, 0.92 mmol) and stirred at 25 ^{o}C for 2 h. The reaction was quenched with water (20 mL) and extracted with dichloromethane (30 mL x2). The combined organic phase was washed with water (20 mL x2) and brine (10 mL), dried over sodium sulfate and concentrated. The residue was purified by prep-TLC (eluting 50% ethyl acetate in petroleum ether) to afford 2,2-dimethyl-6-morpholino-5-nitro-3H-benzothiophene 1-oxide (75 mg, 26%) as a yellow oil. LCMS (ESI): *m/z* = 310.9 $[M+H]^+$.

5-Amino-2,2-dimethyl-6-morpholino-2,3-dihydrobenzo[b]thiophene 1-oxide. To a solution of 2,2-dimethyl-6-morpholino-5-nitro-3H-benzothiophene 1-oxide (90.0 mg, 0.29 mmol) in water (2 mL) and ethanol (10 mL) was added ammonium chloride (77.6 mg, 1.45 mmol) and iron (81.0 mg, 1.45 mmol). The reaction was stirred at 80 ^{o}C for 2 h and filtered and concentrated. The residue was diluted with water (15 mL) and extracted with ethyl acetate (20 mL x2). The combined organic phase was washed with brine (15 mL x2), dried over sodium sulfate and concentrated. The residue was purified by flash chromatography (eluting 0-10% methyl alcohol in dichloromethane) to

afford 5-amino-2,2-dimethyl-6-morpholino-2,3-dihydrobenzo[*b*]thiophene 1-oxide (70 mg, 86%) as a white solid. LCMS (ESI): *m/z* = 280.9 $[M+H]^+$.

_N_-(2,2-dimethyl-6-morpholino-1-oxido-2,3-dihydrobenzo[b]thiophen-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide

To a solution of 2,2-dimethyl-6-morpholino-1-oxo-3H-benzothiophen-5-amine (70.0 mg, 0.25 mmol) in pyridine (3 mL) was added pyrazolo[1,5-*a*]pyrimidine-3-carbonyl chloride (54.4 mg, 0.30 mmol) and stirred at 50 ºC for 2 h. The reaction was concentrated and diluted with water (10 mL), and extracted with ethyl acetate (30 mL x2).The combined organic phase was washed with brine (10 mL x2), dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography (eluting 50% ethyl acetate in petroleum ether) to afford N-(2,2-dimethyl-6-morpholino-1-oxo-3H-benzothiophen-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (50 mg, 47%) as a yellow solid. LCMS (ESI): *m/z* = 426.0 $[M+H]^+$.

The mixture was resolved by chiral SFC (Column: chiralpak AD-3 100*4.6 mm ID, 3 um; mobile phase: A: CO_2, B: methanol (0.05% DEA); gradient: from 5% to 40% of B; flow rate, 2.8 ml/min; column temperature 40C) to afford *N*-(2,2-dimethyl-6-morpholino-1-oxido-2,3-dihydrobenzo[b]thiophen-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (20 mg, 40%; RT = 3.916 min) and (19 mg, 38%; RT = 4.990 min) as yellow solids with absolute stereochemistry undetermined.

Compound 13, Peak 1: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.83 (s, 1H), 8.86 (dd, *J* = 7.2, 2.0 Hz, 1H), 8.82 (dd, *J* = 4.4, 1.6 Hz, 1H), 8.80 (s, 1H), 8.74 (s, 1H), 7.68 (s, 1H), 7.12 (dd, *J* = 7.2, 4.4 Hz, 1H), 4.00 (t, *J* = 4.4 Hz, 4H), 3.55 (d, *J* = 16.0 Hz, 1H), 3.05 - 2.96 (m, 5H), 1.55 (s, 3H), 1.35 (s, 3H). Target exact mass 425.15 (C21H23N5O3S); LCMS (ESI): *m/z* = 426.0 $[M+H]^+$.

Compound 14, Peak 2: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.83 (s, 1H), 8.85 (dd, *J* = 6.8, 2.0 Hz, 1H), 8.81 (dd, *J* = 4.0, 1.6 Hz, 1H), 8.79 (s, 1H), 8.73 (s, 1H), 7.67 (s, 1H), 7.12 (dd, *J* = 6.8, 4.0 Hz, 1H), 3.99 (t, *J* = 4.4 Hz, 4H), 3.55 (d, *J* = 16.0 Hz, 1H), 3.03 - 2.94 (m, 5H), 1.54 (s, 3H), 1.34 (s, 3H). Target exact mass 425.15 (C21H23N5O3S); LCMS (ESI): *m/z* = 426.0 $[M+H]^+$.

Compound 15. _N_-(2-methyl-6-morpholino-1,1-dioxo-3H-1,2-benzothiazol-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide

6-Chloro-1,1-dioxo-1,2-benzothiazol-3-one. A mixture of methyl 4-chloro-2-(chlorosulfonyl)benzoate (500 mg, 1.86 mmol) in tetrahydrofuran (10 mL) was added ammonium hydroxide (2 mL, 28%). The mixture was stirred at room temperature for 1 h. The mixture was concentrated to afford 6-chloro-1,1-dioxo-1,2-benzothiazol-3-one (400 mg, crude) as a white solid, which was used directly to next step without further purification. MS (ESI): m/z =218.1 $[M+1]^+$.

6-Chloro-2-methyl-1,1-dioxo-1,2-benzothiazol-3-one. A mixture of 6-chloro-1,1-dioxo-1,2-benzothiazol-3-one (400 mg, 1.84 mmol), iodomethane (391 mg, 2.76 mmol) and cesium carbonate (898 mg, 2.76 mmol) in *N*,*N*-dimethylformamide (6 mL) was stirred at 80 ºC in a sealed tube overnight. Water was added. The mixture was extracted with ethyl acetate (20 mL). The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford 6-

chloro-2-methyl-1,1-dioxo-1,2-benzothiazol-3-one (340 mg, crude) as a brown solid, which was used directly to next step without further purification. MS (ESI): m/z =232.1 $[M+1]^+$.

6-Chloro-2-methyl-5-nitro-1,1-dioxo-1,2-benzothiazol-3-one. A mixture of 6-chloro-2-methyl-1,1-dioxo-1,2-benzothiazol-3-one (500 mg, 2.16 mmol) in fuming sulfuric acid (6 mL) was added drop wise fuming nitric acid (2 mL). The mixture was stirred at 70 ^oC overnight. After cooling down to room temperature, the mixture was poured into ice water. The aqueous phase was extracted with ethyl acetate (20 mL) twice. The organic layers were separated, combined, dried over sodium sulfate and concentrated. The residue was purified by silica gel chromatography using ethyl acetate: petroleum ether (1:5) as eluting solvents to afford 6-chloro-2-methyl-5-nitro-1,1-dioxo-1,2-benzothiazol-3-one (52 mg, 9%) as a white solid. ^1H NMR (400 MHz, CDCl3) δ 8.44 (s, 1H), 8.14 (s, 1H), 3.32 (s, 3H).

2-Methyl-6-morpholino-5-nitro-1,1-dioxo-1,2-benzothiazol-3-one. A mixture of 6-chloro-2-methyl-5-nitro-1,1-dioxo-1,2-benzothiazol-3-one (38 mg, 0.14 mmol) in acetonitrile (3 mL) was added morphine (20 mg, 0.21 mmol). The mixture was stirred at room temperature for 2 h. Water was added. The aqueous phase was extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated to afford 2-methyl-6-morpholino-5-nitro-1,1-dioxo-1,2-benzothiazol-3-one (43 mg, 96%) as a yellow solid. MS (ESI): m/z =328.1 $[M+1]^+$.

2-Methyl-6-morpholino-1,1-dioxo-3H-1,2-benzothiazol-5-amine. A mixture of 2-methyl-6-morpholino-5-nitro-1,1-dioxo-1,2-benzothiazol-3-one (43 mg, 0.13 mmol) in tetrahydrofuran (8 mL) was added drop wise borane-tetrahydrofuran complex (1 M in tetrhydrofuran, 3 mL, 3mmol). The mixture was stirred at 65 ^oC overnight. Methanol was added. The mixture was stirred at room temperature for 30 min. The mixture was concentrated to afford 2-methyl-6-morpholino-1,1-dioxo-3H-1,2-benzothiazol-5-amine (35 mg, crude) as a colorless oil, which was used directly to next step without further purification. MS (ESI): m/z =284.1 $[M+1]^+$.

***N*-(2-Methyl-6-morpholino-1,1-dioxo-3H-1,2-benzothiazol-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide.** A mixture of potassium carbonate (30 mg, 0.21 mmol), 2-methyl-6-morpholino-1,1-dioxo-3H-1,2-benzothiazol-5-amine (35 mg, 0.12 mmol) in toluene (10 mL) was added pyrazolo[1,5-a]pyrimidine-3-carbonyl chloride. The mixture was stirred at 110 ^oC for 4 h. The mixture was concentrated and purified by purified by preparative HPLC (Waters, XBridge C18, 4.6×150mm, 3.5μm, A: acetonitrile, 30%-45%; B: 10 mM ammonium hydrogen carbonate in water) to afford *N*-(2-methyl-6-morpholino-1,1-dioxo-3H-1,2-benzothiazol-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (15 mg, 28%) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H), 9.41 (dd, *J* = 1.2, 6.8 Hz, 1H), 9.00 (dd, *J* = 1.2, 4.0 Hz, 1H), 8.76 (s, 1H), 8.71 (s, 1H), 7.40 (dd, *J* = 4.0, 6.8 Hz, 1H), 4.39 (s, 2H), 3.91-3.88 (m, 4H), 2.95-2.93 (m, 4H), 2.81 (s, 3H). Target exact mass 428.13 (C19H20N6O4S); LCMS (ESI): *m/z* = 429.2 $[M+H]^+$.

Compound 20. (*R*)-*N*-(2-(2-Fluoro-3-hydroxy-3-methylbutyl)-6-morpholino-1,1-dioxo-3H-1,2-benzothiazol-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide. This compound was made in a manner analogous to **15**, replacing ammonium hydroxide with (*R*)-4-amino-3-fluoro-2-methylbutan-2-ol.[9] ^1H NMR (400 MHz, $CDCl_3$-d_6) δ 10.88 (s, 1H), 8.89-8.81 (m, 4H), 7.66 (s, 1H), 7.15 (dd, *J* = 7.2, 4.4 Hz, 1H), 4.72-4.58 (m, 2H), 4.44-4.40 (m, 1H), 4.03 (t, J=4.4 Hz, 4H), 3.92-3.80 (m, 1H), 3.48-3.42 (m, 1H), 3.01 (t, J=4.4 Hz, 4H), 1.35 (s, 6H)

Target exact mass 518.18 (C23H27FN6O5S); LCMS (ESI): *m/z* = 519.2 $[M+H]^+$.

The following compounds were prepared according to the general procedure described for compound **9**:

Compound 16. ***N*-(6-Morpholino-1-oxo-2-((tetrahydro-2*H*-pyran-4-yl)methyl)isoindolin-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide.** This compound was made in a manner analogous to **9**, replacing methylamine with (tetrahydro-2*H*-pyran-4-yl)methanamine. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.92 (s, 1 H), 8.87 - 8.81 (m, 4H), 7.73 (s, 1H), 7.12 (dd, *J* = 6.8, 4.4 Hz, 1H), 4.40 (s, 2H), 4.02 - 3.97 (m, 6H), 3.50 (d, *J* = 7.6 Hz, 2H), 3.40 - 3.35 (m, 2 H), 2.99 (t, *J* = 4.4 Hz, 4H), 2.04 - 2.01 (m, 1H), 1.49 - 1.41 (m, 4H). Target exact mass 476.22 (C25H28N6O4); LCMS (ESI): *m/z* = 477.2 $[M+H]^+$.

Compound 17. *N*-(2-(3-Methoxy-3-methylbutyl)-6-morpholino-1-oxoisoindolin-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide. This compound was made in a manner analogous to **9**, replacing methylamine with 3-methoxy-3-methylbutan-1-amine. ^{1}H NMR (400 MHz, CDCl3) δ 10.92 (s, 1H), 8.88 - 8.82 (m, 4H), 7.72 (s, 1H), 7.12 (dd, J = 6.8, 4.0 Hz, 1H), 4.40 (s, 2H), 4.02 (t, J = 4.4 Hz, 4H), 3.71 - 3.67 (m, 2H), 3.25 (s, 3H), 3.01 (t, J = 4.4 Hz, 4H), 1.87 - 1.83 (m, 4H), 1.25 (s, 6H). Target exact mass 478.232849 (C25H30N6O4); LCMS (ESI): *m/z* = 479.2 $[M+H]^+$.

Compound 18. *N*-[2-(3-Hydroxy-3-methyl-butyl)-6-morpholino-1-oxo-isoindolin-5-yl]pyrazolo[1,5-a]pyrimidine-3-carboxamide. This compound was made in a manner analogous to **9**, replacing methylamine with 4-amino-2-methylbutan-2-ol. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.89 (s, 1H), 9.41 (dd, J = 7.0, 1.6 Hz, 1H), 9.00 (dd, J = 4.2, 1.6 Hz, 1H), 8.75 (d, J = 2.4 Hz, 2H), 7.56 (s, 1H), 7.39 (dd, J = 7.0, 4.2 Hz, 1H), 4.46 (s, 2H), 4.34 (s, 1H), 3.94 – 3.86 (m, 4H), 3.64 – 3.54 (m, 2H), 2.96 – 2.87 (m, 4H), 1.73 – 1.63 (m, 2H), 1.15 (s, 6H). Target exact mass 464.22 (C24H28N6O4); LCMS (ESI) m/z: 465.2 [M+H]+.

Compound 19. (R)-*N*-(2-(2-Fluoro-3-hydroxy-3-methylbutyl)-6-morpholino-1-oxoisoindolin-5-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide. (44 mg, 32%) This compound was made in a manner analogous to **9**, replacing methylamine with (*R*)-4-amino-3-fluoro-2-methylbutan-2-ol[9]. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.90 (s, 1H), 9.41 (dd, J = 7.0, 1.6 Hz, 1H), 9.00 (dd, J = 4.2, 1.6 Hz, 1H), 8.76 (d, J = 2.4 Hz, 2H), 7.59 (s, 1H), 7.39 (dd, J = 7.0, 4.2 Hz, 1H), 4.90 (s, 1H), 4.55 (s, 3H), 4.05 – 3.85 (m, 5H), 3.70 (td, J = 15.9, 9.3 Hz, 1H), 2.99 – 2.85 (m, 4H), 1.24 – 1.12 (m, 6H). Target exact mass 482.21 (C24H27FN6O4); MS (ESI): *m/z* = 483.2 $[M+1]^+$.

Compound 21. *N*-[6-[4-(2,2-Difluoroethyl)piperazin-1-yl]-2-[(2R)-2-fluoro-3-hydroxy-3-methyl-butyl]-1-oxo-isoindolin-5-yl]pyrazolo[1,5-a]pyrimidine-3-carboxamide. This compound was made in a manner analogous to **9**, replacing morpholine with 1-(2,2-difluoroethyl)piperazine. ^{1}H NMR (400 MHz, CDCl3) δ 10.82 (s, 1H), 8.90 - 8.75 (m, 4H), 7.73 (s, 1H), 7.14 (s, 1H), 6.14 - 5.83 (m, 1H), 4.65 - 4.42 (m, 3H), 4.28 - 4.10 (m, 1H), 3.75 - 3.60 (m, 1H), 3.12 - 2.84 (m, 9H), 2.48 - 2.40 (m, 1H), 1.34 (s, 6H). Target exact mass 545.24 (C26H30F3N7O3); LCMS (ESI): m/z = 546.2 [M+H]+.

Compound 22. **(*R*)-*N*-(6-(4-(Azetidin-1-yl)piperidin-1-yl)-2-(2-fluoro-3-hydroxy-3-methylbutyl)-1-oxoisoindolin-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide.** This compound was made in a manner analogous to **9**, replacing morpholine with 4-(azetidin-1-yl)piperidine. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.99 (s, 1H), 9.42 - 9.40 (d, *J* = 5.6 Hz, 1H), 8.93-8.92 (m, 1H), 8.76-8.75 (m, 1H), 7.56 (s, 1H), 7.47-7.44 (m, 1H), 4.90 (s, 1H), 4.58-4.37 (m, 3H), 4.01- 3.6 (m, 2H), 3.14 – 3.12 (m, 4H), 2.99 – 2.96 (m, 2H), 2.77-2.72 (m, 2H), 2.2- 2.1 (m, 1H), 2.00 – 1.96 (m, 2H), 1.82 – 1.79 (m, 2H), 1.62 – 1.59 (m, 2H), 1.18-1.17 (m, 6H). Target exact mass 535.27 (C28H34FN7O3); LCMS (ESI): *m/z* = 536.2 $[M+H]^+$.

Compound 23. (*R*)-*N*-(6-(4-(3,3-difluoroazetidin-1-yl)piperidin-1-yl)-2-(2-fluoro-3-hydroxy-3-methylbutyl)-1-oxoisoindolin-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide. This compound was made in a manner analogous to **9**, replacing morpholine with 4-(3,3-difluoroazetidin-1-yl)piperidine. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.97 (s, 1H), 9.42 - 9.40 (m, 1H), 8.89-8.88 (m, 1H), 8.76-8.75 (m, 1H), 7.56 (s, 1H), 7.45-7.43 (m, 1H), 4.90 (s, 1H), 4.58-4.52 (m, 3H), 4.00- 3.87 (m, 1H), 3.70 – 3.60 (m, 5H), 3.01 – 2.98 (m, 2H), 2.79 – 2.74 (m, 2H), 1.88-1.72 (m, 2H), 1.69 – 1.64 (m, 2H), 1.18-1.17 (m, 6H). Target exact mass 571.25 (C28H32F3N7O3); LCMS (ESI): *m/z* = 572.1 $[M+H]^+$.

Compound 24. **(*R*)-*N*-(2-(2-fluoro-3-hydroxy-3-methylbutyl)-6-(4-(oxetan-3-yl)piperazin-1-yl)-1-oxoisoindolin-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide.** This compound was made in a manner analogous to **9**, replacing morpholine with 1-(oxetan-3-yl)piperazine. ^{1}H NMR (400 MHz, CD_3OD) δ 9.17 - 9.16 (m, 1H), 8.98-8.94 (m, 1H), 8.78 (s, 1H), 8.72 (s, 1H), 7.76 (s, 1H), 7.33 (dd, *J* = 7.2, 4.4 Hz, 1H), 4.77 - 4.74 (m, 2H), 4.68-4.45 (m, 5H), 4.17 - 4.07 (m, 1H), 3.78 - 3.69 (m, 2H), 3.06 (t, *J* = 4.4 Hz, 4H), 2.70 (br s, 4H), 1.30 (s, 6H). Target exact mass 537.25 (C27H32FN7O4); LCMS (ESI): *m/z* = 538.1 $[M+H]^+$.

Compound 25. **(*R*)-*N*-(6-(4-(3,3-difluorocyclobutyl)piperazin-1-yl)-2-(2-fluoro-3-hydroxy-3-methylbutyl)-1-oxoisoindolin-5-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide**. This compound was made in a manner analogous to **9**, replacing morpholine with 1-(3,3-difluorocyclobutyl)piperazine. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.82 (s, 1H), 8.89-8.78 (m, 4H), 7.73 (s, 1H), 7.17-1.14 (m, 1H), 4.64-4.45 (m, 3H), 4.25-4.12 (m, 1H), 3.69-3.67 (m, 1H), 3.03 (m, 4H), 2.77-2.38 (m, 8H), 1.34-1.33 (m, 6H). Target exact mass 571.25 (C28H32F3N7O3); LCMS (ESI): *m/z* = 572.1 $[M+H]^+$.

Table S5. Abbreviations

AB	Apical-to-basolateral permeability
ACN	Acetonitrile
$CDCl_3$	Deuterated chloroform
$cpKa_{MB}$	Calculated most basic pKa
CpG	TLR agonist deoxycytidyl deoxyguanosine oligodinucleotides
DHBF	Dihydrobenzofuran
DMSO	Dimethylsulfoxide
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
FA	Formic acid
Hep	Predicted hepatic clearance in hepatocytes
HPLC	High performance liquid chromatography
HRMS	High-resolution mass spectrometry
HWB	Human whole blood
IFNα	Interferon alpha
IFNγ	Interferon gamma
IL-1R	Interleukin-1 receptor
IL-6	Interleukin-6
IRAK4	Interleukin-1 receptor associated kinase 4
LCMS	Liquid chromatography-mass spectrometry
LLE	Lipophilic ligand efficiency
LM	Predicted hepatic clearance in liver microsomes
MCT	0.5% methyl-cellulose/0.2% tween-80
MDCK	Madin-Darby canine kidney cells
MI	Maximal inhibition
NaOH	Sodium hydroxide
PBS	Phosphate-buffered saline
PD	Pharmacodynamic
PK	Pharmacokinetic
R848	TLR agonist resiquimod
SFC	Supercritical fluid chromatography
SLE	Systemic lupus erythematosus
TLC	Thin-layer chromatography
TLR	Toll-like receptor
TNFα	Tumor necrosis factor alpha

References

1. Bryan, M. C.; Drobnick, J.; Gobbi, A.; Kolesnikov, A.; Chen, Y.; Rajapaksa, N.; Ndubaku, C.; Feng, J.; Chang, W.; Francis, R.; Yu, C.; Choo, E. F.; DeMent, K.; Ran, Y.; An, L.; Emson, C.; Huang, Z.; Sujatha-Bhaskar, S.; Brightbill, H.; DiPasquale, A.; Maher, J.; Wai, J.; McKenzie, B. S.; Lupardus, P. J.; Zarrin, A. A.; Kiefer, J. R. Development of Potent and Selective Pyrazolopyrimidine IRAK4 Inhibitors. *J. Med. Chem*. **2019**, *62*, 6223-6240.
2. Lee, K. L.; Ambler, C. M.; Anderson, D. R.; Boscoe, B. P.; Bree, A. G.; Brodfuehrer, J. I.; Chang, J. S.; Choi, C.; Chung, S.; Curran, K. J.; Day, J. E.; Dehnhardt, C. M.; Dower, K.; Drozda, S. E.; Frisbie, R. K.; Gavrin, L. K.; Goldberg, J. A.; Han, S.; Hegen, M.; Hepworth, D.; Hope, H. R.; Kamtekar, S.; Kilty, I. C.; Lee, A.; Lin, L.-L.; Lovering, F. E.; Lowe, M. D.; Mathias, J. P.; Morgan, H. M.; Murphy, E. A.; Papaioannou, N.; Patny, A.; Pierce, B. S.; Rao, V. R.; Saiah, E.; Samardjiev, I. J.; Samas, B. M.; Shen, M. W. H.; Shin, J. H.; Soutter, H. H.; Strohbach, J. W.; Symanowicz, P. T.; Thomason, J. R.; Trzupek, J. D.; Vargas, R.; Vincent, F.; Yan, J.; Zapf, C. W.; Wright, S. W. Discovery of clinical candidate 1-{[(2S,3S,4S)-3-ethyl-4-fluoro-5-oxopyrrolidin2-yl]methoxy}-7-methoxyisoquinoline-6-carboxamide (PF06650833), a potent, selective inhibitor of interleukin-1 receptor associated kinase 4 (IRAK4), by fragment-based drug design. *J. Med. Chem.* **2017**, *60* (13), 5521–5542.
3. Lin, B.; Pease, J. H. A novel method for high throughput lipophilicity determination by microscale shake flask and liquid chromatography tandem mass spectrometry. *Comb. Chem. High Throughput Screen.* **2013**, *16*, 817–825.
4. Lin, B.; Pease, J. H. A high throughput solubility assay for drug discovery using microscale shake-flask and rapid UHPLC-UV-CLND quantification. *J. Pharm. Biomed. Anal.* **2016**, *122*, 126–140.
5. Bryan, M. C.; Gobbi, A.; Kiefer, J. R.; Kolesnikov, A.; Olivero, A.; Drobnick, J.; Liang, J.; Rajapaksa, N.; Ndubaku, C. I.; Feng, J. Pyrazolo[1,5a]pyrimidine derivatives as IRAK4 Modulators. WO2017108723, June 29, 2017.
6. Williamson, B.; Wilson, C.; Dagnell, G.; Riley, R. J. Harmonized high throughput microsomal stability assay. *J. Pharmacol. Toxicol. Methods* **2017**, *84*, 31–36.
7. Wong, S.; Doshi, U.; Vuong, P.; Liu, N.; Tay, S.; Le, H.; Kosaka, M.; Kenny, J. R.; Li, A. P.; Yan, Z. Utility of pooled cryopreserved human enterocytes as an in vitro model for assessing intestinal clearance and drug-drug interactions. *Drug Metab. Lett.* **2018**, *12*, 3–13.
8. Chen, Y.-C.; Kenny, J. R.; Wright, M.; Hop, C. E. C. A.; Yan, Z. Improving confidence in the determination of free fraction for highly bound drugs using bidirectional equilibrium dialysis. *J. Pharm. Sci.* **2019**, *108* (3), 1296–1302.
9. Bhide, R. S.; Duncia, J. V.; Hynes, J.; Nair, S.; Pitts, W. J.; Kumar, S. R.; Gardner, D. S.; Murugesan, N.; Paidi, V. R.; Santella, J. B.; Sistla, R.; Wu, H. Heteroaryl substituted pyridyl compounds useful as kinase modulators. WO2014074675, May 5, 2014.