Allosteric Inhibitors, Crystallography and Comparative Analysis Reveal Network of Coordinated Movement Across Human Herpesvirus Proteases

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Supporting Information

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Supplemental Table 1

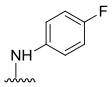
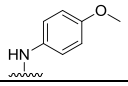
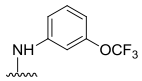
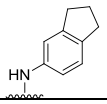
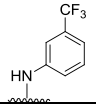
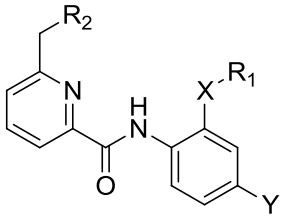
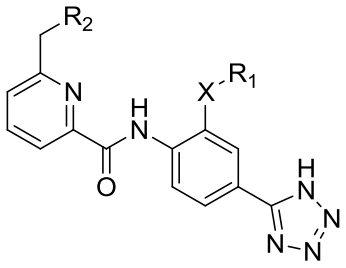
X-R^1	KSHV Pr IC_{50} (μM)	Compound
	5.4 ± 0.6	1
	2.6 ± 0.2	2
	4.7 ± 3	3
	2.8 ± 3	4
	3.7 ± 1.3	5
	2.5 ± 4	6
	0.61 ± 0.11	7
	0.85 ± 0.13	8

Table S1. Structure activity relationships of R^1 position. Varying the substitutions of the R^1 position shows that the pocket accommodates modes substitutions well, with larger, fused systems such as indane **5** being deleterious to activity. The more hydrophobic connectivity and substitutions, as in **7** and **8**, modestly improve potency. IC_{50} values are reported from replicates of two or three experiments plus or minus one standard deviation of the fitted values.



Supplemental Table 2

X-R^1	R^2	KSHV Pr IC_{50} (μM)	Compound
G	A	13.1 ± 1.1	11
G	B	6.6 ± 0.6	12
G	C	6.6 ± 0.4	13
G	D	3.6 ± 0.2	14
G	E	5.7 ± 0.3	15
G	F	8.5 ± 0.7	16
H	A	1.4 ± 0.1	17
H	B	2.8 ± 0.3	18
H	D	1.7 ± 0.2	19
H	E	1.5 ± 0.1	20
H	F	1.3 ± 0.2	21
I	A	1.1 ± 0.2	22
I	B	0.85 ± 0.1	23
I	C	1.3 ± 0.1	24
I	D	1.1 ± 0.1	25
I	F	0.73 ± 0.1	26



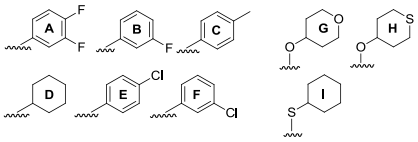


Table S2. Structure activity relationships while varying both X-R^1 and R^2 on the tetrazole containing compounds. Modest substitutions at R^2 were well tolerated. The nature of the connectivity and hydrophobicity of R^1 drives potency into the nanomolar range for compounds 21 and 24. IC_{50} values are reported from replicates of two or three experiments plus or minus one standard deviation of the fitted values.

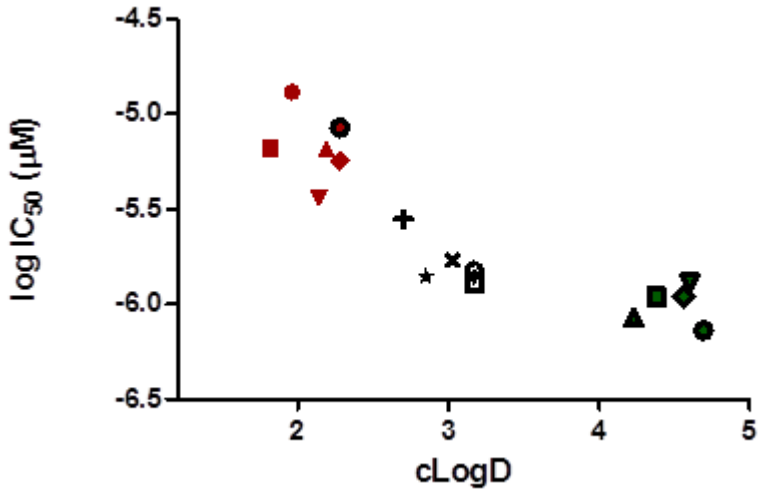


Figure S1. Compounds from table S2 are plotted with cLogD vs. log IC_{50} (μM). The R^2 groups are colored as follows, thio-cyclohexyl is green, ether-thiopyran, white and the tetrahydropyran ether is red. The R^1 substitutions contribution to the cLogD clearly influences potency.

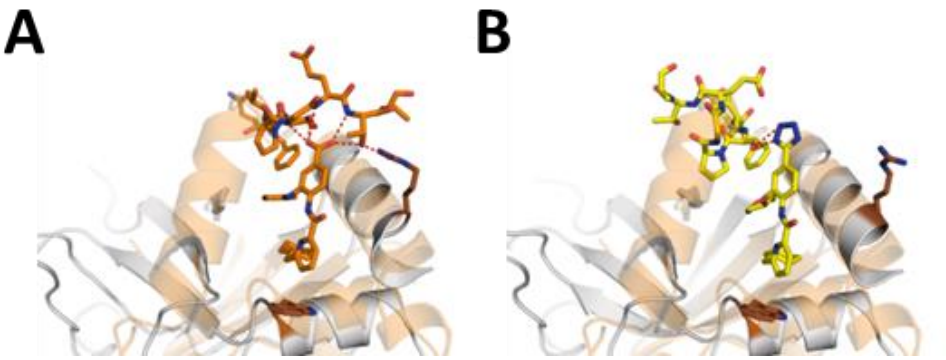


Figure S2. Distinct C-terminal conformations are identified in this study. A. The carboxylic acid containing compounds form an intricate hydrogen bonding network with the C-terminal backbone residues 193-195 and another network through the carboxylic acid of the small molecules, T195 and R82. This positioning of the residues represents a significant conformational change as compared to the full-length wt dimeric protein, where they are helical (peach). B. The tetrazole compound forms one two hydrogen bonds to the C-terminal residues in the co-crystal structure. The trajectory of the truncated residues is similar to that of the wt-dimeric protein (peach)

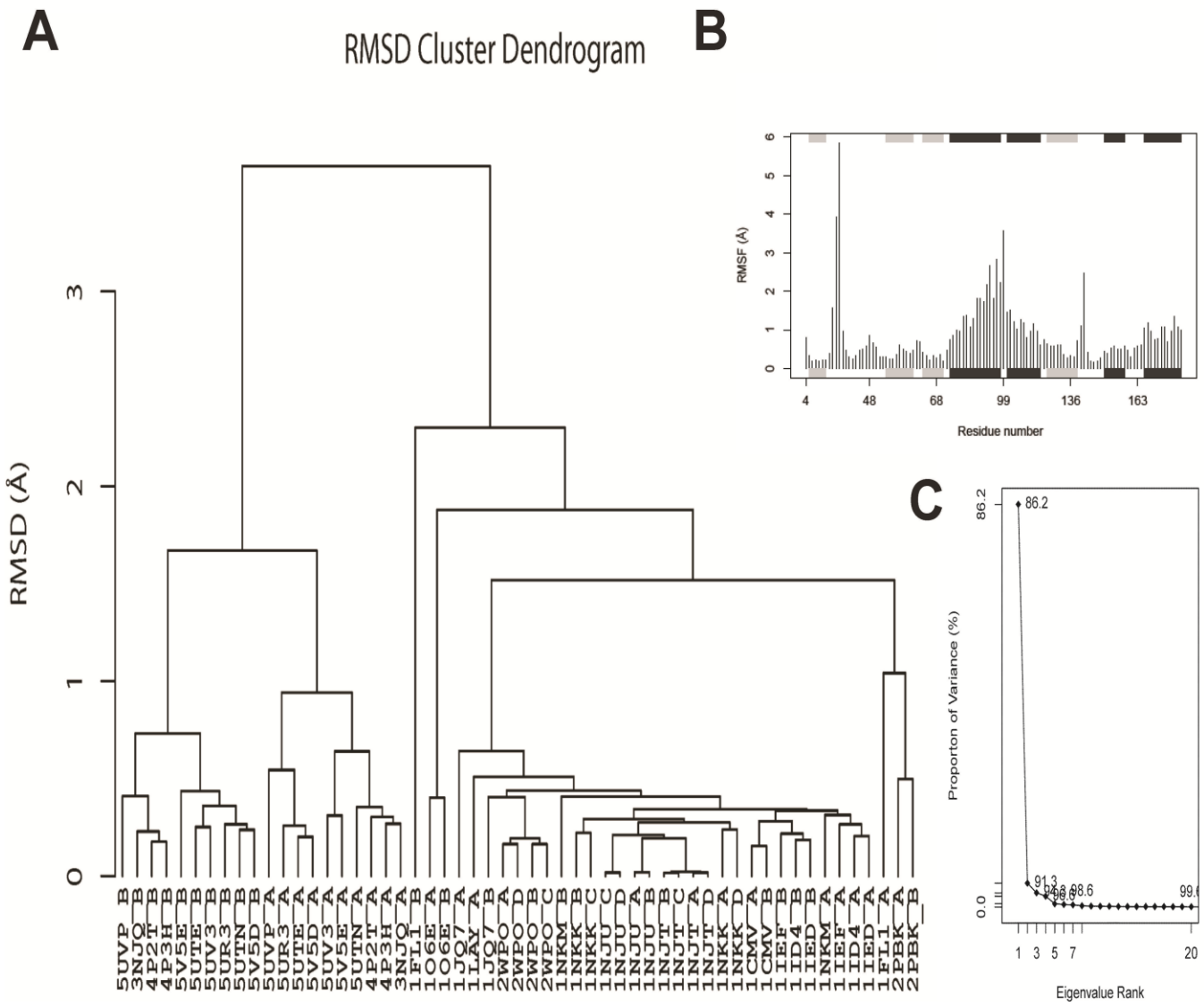


Figure S3. Comparison of congener bound structure across the herpesvirus family. **A**. The RMSD of the structures compared in this study cluster in a way that largely recapitulates phylogeny. **B**. The RMSF deviations of the 24 structures compared show several distinct regions of major fluctuation. **C**. Principal component analysis shows that the majority of the variance is captured in PC1, which contains the regions with the highest RMSF variability.

Biochemical, Biophysical and Biological Experimental Procedures

Protein expression and purification

The T195V variant of the wt-KSHV Pr was made using the following primers.

CTA TAG CTT TTG CCA TTA AGA CCT CCA GGG GAC TGA CGA AAT

ATT TCG TCA GTC CCC TGG AGG TCT TAA TGG CAA AAG CTA TAG

KSHV wt and T195V proteases were made or maintained on the pET-16b Vector (Novagen) and transformed into Rosetta 2 BL21(DE3) pLysS cells (Promega) for protein expression. Overnight 1 mL cultures containing appropriate antibiotics were grown at 37 ºC in shaking tubes. 50 mL cultures were inoculated and grown overnight and then used for inoculation of 1L cultures, all in shaking vessels. The 1L cultures were grown at 37 °C until they reached an OD_{600} of 0.45. The temperature was then dropped to 30 ºC for approximately 30 minutes until the OD_{600} reached 0.6. IPTG was added to a final concentration of 1mM and expression was allowed for 5 hours at 30 °C. Cells were pelleted and resuspended in a lysis buffer containing 50 mM potassium phosphate, pH 7.8, 25mM KCl, 1mM EDTA and 1mM beta-meracaptoethanol. Cells were lysed under sonication and the pellet removed. The supernatant was taken to 4ºC and streptomycin sulfate was added slowly to a final solution of 1% (w:v), from a 5% stock solution. The solution was stirred for 45 min. at 4 ºC. The solution was centrifuged at 12K for 25 minutes and the pellet was discarded. The solution was taken to 4 ºC and ammonium sulfate was added slowly, with stirring, to a final concentration of 268 g/L. The solution was stirred overnight at 4 ºC. The mixture was centrifuged at 12K for 25 minutes and the supernatant was discarded. The resulting pellet was resuspended in 50 mM potassium phosphate, pH 7.8, 25mM KCl, 1mM EDTA and 1mM beta-meracaptoethanol, 0.5M ammonium sulfate and subjected to butyl sepharose column chromatography, with an elution buffer lacking ammonium sulfate. The active fractions were dialyzed

into 50 mM Tris pH 8.0, 0.1 mM EDTA, 1mM beta-mercaptoethanol and subjected to a Mono Q column with an elution buffer as above and containing 0.5 M KCl. Pooled active fractions were dialyzed into 25 mM potassium phosphate, pH 8.0, 150 mM KCl, 0.1mM EDTA and 1mM beta-meracaptoethanol and subjected to size exclusion chromatography over an S75 preparatory column.

Comparative analysis

The Bio3D package was installed in R and the various analyses were performed as described in the user guide, demo and tutorials for the package, found here http://thegrantlab.org/bio3d/index.php. [1]

Kinetics assays

Kinetics assays were performed on a BioTek H4 instrument and using our YtBuQA-ACC substrate. Briefly, for full time-course experiments, protease was diluted into assay buffer containing 25 mM potassium phosphate, 150 mM KCl and 0.1 mM EDTA at pH 8.0 to a working concentration of 0.325 μM. Enzyme was plated prior to compound addition and plates were shaken for 10 seconds prior to initiation of the reaction by the addition of substrate to start the reactions. The progress curves were then followed for two hours. For IC_{50} value determination, enzyme was similarly diluted into the assay buffer above and plated into Corning round bottom 96-well plates, 96 μl per well, at 0.400 μM concentrations for the tetrazole series and 1.0 μM for the carboxylic acid series. Compounds were serially diluted from 5, 10 or 20 mM stock solutions in DMSO down to appropriate concentrations prior to addition onto the plate such that 2 μl of added compound yielded the desired working concentration in each well. Compounds were assayed in duplicate or triplicate. After compound addition, the plates were incubated at 30 °C for 1 hour and then moved to the plate reader. Substrate was added (2 μl) from DMSO stocks to a working concentration of 10 μM to start the reaction and the reactions were monitored by measuring the increase in fluorescence intensity. Initial velocity values were used to calculate relative activity versus DMSO control wells. Data from IC_{50} experiments and full progress curves for

determination of k_{obs} and K_i were fit using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. IC_{50} data were fit to the equation:

$$\textit{Initial Velocity} = 100 / (1 + ([\textit{inhibitor concentration}] / IC_{50})^N)$$

to initial velocity data from individual experiments normalized to the response in the absence of inhibitor (100%), IC_{50} is the concentration that inhibits the response half-maximally and N is the Hill slope. Values reported are from two to three replicates.[2]

X-ray Crystallography

As previously reported, KSHV Pr Δ196 solutions for crystallography were diluted to 7 mg/mL in 100 mM KCl, 0.07 mM EDTA, 16.5 mM KP_i (pH 8.0), and 0.66 mM DTT. The various inhibitors were added to a final concentration of 1 mM and incubated at 30 °C for 30 min. The protein/inhibitor solution was added in a 1:1 ratio to the reservoir solution, and crystals were grown at 17 °C with the hanging drop vapor diffusion method. Data were collected at Lawrence Berkeley National Laboratory Advanced Light Source beamline 8.3.1 using a crystal flash-cooled to 100 K in mother liquor with 12% glycerol as the cryoprotectant.

With the exception of structure **1**, diffraction images were processed using MOSFLM2 and the CCP4 suite, operated through the Elves scripts. The resulting structure models were refined over multiple rounds of restrained refinement and isotropic B-factor minimization with Phenix and Coot. For compound **1**, the diffraction images were processed using Xia2 and the CCP4 suite and the resulting structure was refined as above.

For room temperature structure determination, we harvested large (200-300μm), single crystals of KSHV Pr bound to compounds **1** and **4**. The crystals were mounted in standard cryoloops and were sealed inside polyester capillaries (MicroRT, Mitegen) along with a small plug of mother liquor in order to prevent crystal dehydration. X-ray diffraction data were collected from these crystals at the Advanced

Light Source on beamline 8.3.1, which was equipped with an ADSC Quantum315 CCD detector. Crystals were maintained at 280K throughout the course of data collection. In order to mitigate radiation damage at non-cryogenic temperatures, we attempted to distribute the X-ray dose through the entire crystal volume by matching the X-ray beam size to the smallest dimension of the crystal, and also by translating the crystal along its longest dimension during data collection.

Raw diffraction images from room temperature X-ray data sets were processed with Xia2, using XDS for indexing and integration and XSCALE for scaling and merging. Initial phases were calculated using PHASER, with a previously-determined KSHV Pr structure (3NJQ) as a search model. Following molecular replacement, iterative model-building and atomic refinement was performed. Model building was performed using COOT, and refinement of atomic positions and B-factors was performed using phenix.refine with TLS parameterization, a riding hydrogen model, and automatic weight optimization.

Beamline 8.3.1 at the Advanced Light Source is operated by the University of California Office of the President, Multicampus Research Programs and Initiatives grant MR-15-328599 and Program for Breakthrough Biomedical Research, which is partially funded by the Sandler Foundation. Additional support comes from National Institutes of Health (GM105404, GM073210, GM082250, GM094625), National Science Foundation (1330685), Plexxikon Inc. and the M.D. Anderson Cancer Center. The Advanced Light Source (Berkeley, CA), a national user facility operated by Lawrence Berkeley National Laboratory on behalf of the US Department of Energy under contract number DE-AC02-05CH11231, Office of Basic Energy Sciences, through the Integrated Diffraction Analysis Technologies program, supported by the US Department of Energy Office of Biological and Environmental Research. Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by

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Conditions for crystallography were as follows:

Compound **1**: 0.1M Sodium acetate pH 7.8, 0.88M NaH_2PO_4/1.32M K_2HPO_4, 0.2M KCl

Compound **2**: 0.3M Imidazole, pH 8.0, 0.4M, NaH_2PO_4/1.6M, K_2HPO_4, 0.2M NaCl

Compound **3**: 0.4M, NaH_2PO_4/1.6M, K_2HPO_4, 0.2M NaCl

Compound **4**: 0.1M Imidazole pH 8.0, 0.34M NaH_2PO_4/1.36M K_2HPO_4, 0.2MKCl

Compound **14**: 0.1M Sodium acetate pH 5.5, 0.8M NaH_2PO_4/1.2M K_2HPO_4

	5V5D	**5UVP**
resolution range (A)	47.05-2.10 (2.22-2.10)	73.1 - 1.94 (2.009 - 1.94)
space group	I222	I222
unit cell	69.06, 94.09, 119.68, 90, 90, 90	66.6041, 92.5732, 119.152, 90, 90, 90
observed reflections	95321 (13730)	849949 (83061)
unique reflections	20558 (2956)	27698 (2762)
multiplicity	4.6 (4.6)	30.7 (29.9)
completeness	89.3% (92.0%)	99 % (100%)
I/sig(I)	13.76 (2.44)	24.79 (1.01)
Wilson B factor	28.28	29.37
CC(1/2)	99.8 (78.4)	95.1% (59.0)
R-work	0.1744 (0.2410)	0.1948 (0.2874)
R-free	0.2106 (0.2690)	0.2171 (0.3413)
protein residues	377	380
solvent molecules	107	39.18
Ramachandran favored	98.37%	96%
Ramachandran outliers	0.00%	1.3

Clashscore	0.65	1.41

	5V5E	**5UTE**
resolution range (A)	31.16-2.30 (2.42-2.30)	73.47 - 2.05 (2.123 - 2.05)
space group	I222	I222
unit cell	73.40, 96.84, 119.08, 90, 90, 90	68.3298 93.3282 119.147 90 90 90
observed reflections	69361 (9943)	373979 (25860)
unique reflections	18809 (2636)	24318 (2383)
multiplicity	3.7 (3.8)	15.4 (10.7)
completeness	97.7% (97.0%)	100% (100%)
I/sig(I)	9.31 (1.71)	8.97 (1.52)
Wilson B factor	43.7	22.32
CC(1/2)	99.6 (55.0)	0.312 (0.739)
R-work	0.1986 (0.3117)	0.1828 (0.2492)
R-free	0.2304 (0.3360)	0.2359 (0.3406)
protein residues	369	375
solvent molecules	43	31.31
Ramachandran favored	96.10%	98%
Ramachandran outliers	0.00%	0
Clashscore	1.36	14.29

	5UV3	**5UR3**
resolution range (A)	59.5 - 1.95 (2.02 - 1.95)	54.55 - 1.8 (1.864 - 1.8)
space group	I222	I222
unit cell	71.3263 95.8283 118.997 90 90 90	67.4 92.86 118.93 90 90 90
observed reflections	397549 (24962)	252316 (14218)
unique reflections	30117 (2956)	34879 (3414)
multiplicity	13.2 (8.4)	7.2 (4.2)
completeness	100% (100%)	100% (100%)
I/sig(I)	10.93 (1.69)	22.67 (2.88)
Wilson B factor	24.89	20.77
CC(1/2)	0.543 (0.71)	0.999 (0.84)
R-work	0.2015 (0.2830)	0.1797 (0.2388)

R-free	0.2366 (0.3372)	0.2225 (0.2786)
protein residues	380	382
solvent molecules	40.23	30.78
Ramachandran favored	96%	97%
Ramachandran outliers	1.1	0
Clashscore	11.8	5.14
	5UTN	
resolution range (A)	56.71 - 1.8 (1.864 - 1.8)	
space group	I222	
unit cell	70.3563 95.8011 119.253 90 90 90	
observed reflections	538571 (36746)	
unique reflections	37695 (3705)	
multiplicity	14.3 (9.9)	
completeness	100% (100%)	
I/sig(I)	24.64 (2.39)	
Wilson B factor	24.12	
CC(1/2)	0.433 (0.778)	
R-work	0.1983 (0.2447)	
R-free	0.2295 (0.2972)	
protein residues	378	
solvent molecules	39.84	
Ramachandran favored	95%	
Ramachandran outliers	1.3	
Clashscore	12.75	

Viral re-infection assay

Media refers to DMEM High glucose + 10% FBS + 100 µg/mL Streptomycin sulfate + 100 units/mL Penicillin 'G' unless otherwise stated. Both iSLK (uninfected) and iSLK-219 (latently infected) cells were plated in 24-well sterile tissue culture-treated plates with lids (Costar 3526) at $1x10^4$ cells/well and $4.5x10^4$ cells/well, respectively, in 0.5 mL media per well. Cells were allowed to adhere overnight in a temperature, humidity, and atmosphere controlled incubator at 37 degrees Celsius, 5% CO_2, relative humidity >60%. After overnight incubation iSLK-219 cells should be approximately 80% confluent. Induction media was prepared by the addition of 3 µg/mL doxycycline and 1 mM sodium butyrate (no

puromycin is included in this media). Test compounds were added to induction media to a final concentration of 0.4% DMSO for all compound concentrations tested. This concentration of DMSO is non-cytotoxic. Induction of the lytic cycle in the presence or absence of compound was allowed to take place for 48 hours. Four hundred microliters of spent media from iSLK-219 cells was collected and filtered using a sterile 0.45 μm spin filter (Spin-X Centrifuge Tube Filter 0.45 μm Cellulose Acetate RNase/DNase free; Costar 8162) to remove cells while maintaining virus. Spent media was diluted 1:1 into media supplemented with 12 μg/mL Polybrene transfection reagent (Millipore) to arrive at 0.8 mL virus-containing media with 6 μg/mL Polybrene. Media from the iSLK (uninfected) cells was discarded and replaced with virus-containing media. Cells were spinoculated at 2000 rpm for 1 hr at RT. Cells were examined under a microscope before and after spinoculation to identify wells that may have dried during centrifugation. Immediately after centrifugation 1.0 mL warm media + 6 μg/mL Polybrene is added to each well and plate is allowed to incubate for an additional hour at 37 degrees Celsius. Media is then replaced with 0.5 mL fresh warm media. Newly infected cells were allowed to incubate for 48 hours to allow GFP signal to develop. After 48 hours, these cells are trypsinized, spun down, and re-suspended in flow cytometry media (PBS, Ca2+ and Mg2+ free with 0.04% EDTA, 2% FBS). GFP positive cells were counted using a FACScalibur (BD Biosciences) with 10,000 cells counted per sample. Percent reinfection was computed as the number of GFP positive cells in a given sample divided by the number of GFP positive cells in the DMSO control times 100 and measurements were made in triplicate.[3]

Sytox red assay

Cells were treated with compound as described for the viral replication assay but in the absence of any induction reagents (doxycycline and sodium butyrate). After 48 hours treatment cells were removed from the plate using Enzyme Free Cell Dissociation Buffer (Gibco), pelleted, and resuspended in flow cytometry buffer (PBS, Ca^{2+} and Mg^{2+} free with 0.04% EDTA, 2% FBS) supplemented with Sytox red

dye. The remainder of the assay was performed according to the manufacturer instructions and measurements were made using a FACScalibur (BD Biosciences) instrument. Data were analyzed using FloJo software v. 10.0.6.

MTS assay

MTS assays were performed in the same way that Sytox red assay was performed except in 96-well plates and according to the MTS instructions (Promega). Cells per well was optimized and 1000 cells per well seeding was found to be optimal. Absorbance was averaged across triplicates and the values reported are the mean $\pm$ standard deviation.

General Chemistry Methods

Reagents and solvents were purchased from Sigma- Aldrich, Acros, Ark Pharm or AK Scientific and used as received unless otherwise indicated. Flash column chromatography was carried out using a Biotage Isolera Four system and SiliaSep silica gel cartridges from Silicycle. Hydrogenation reactions were carried out in ThalesNano H-Cube reactor using 30 mm 10% Pd/C catalyst cartridges unless otherwise stated. ^{1}H NMR spectra were recorded on a Varian INOVA-400 400MHz spectrometer or Bruker 500 MHz spectrometer. Chemical shifts are reported in δ units (ppm) relative to residual solvent peak. Coupling constants (J) are reported in hertz (Hz). Characterization data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet), coupling constants, number of protons, mass to charge ratio. LCMS analyses were performed on a Waters Micromass ZQ/Waters 2795 Separation Module/Waters 2996 Photodiode Array Detector/Waters 2424 Evaporative Light Scattering Detector system. All final compounds were tested at or above 95% purity unless otherwise stated.

Method A for hydrogenation

A methanolic solution of the nitro compound was passed through 10 % Pd/C catalyst at a rate of 1 mL/min in the H-Cube reactor under atmospheric pressure and ambient temperature until the reaction was judged complete by LCMS. The reaction mixture was concentrated under reduced pressure to obtain the crude amine that was used without further purification.

Method B for amide coupling

To a solution of 6-(cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride (1 equiv.) in dichloromethane stirring at 0 ºC , was added 1-chloro-*N,N*,2-trimethylpropenylamine (1.1 equiv.). After 10 min, were added the amine (1 equiv.), and *N,N*-diisopropylethylamine (2 equiv.). The mixture was stirred at ambient temperature until the reaction was judged complete by LCMS. The reaction mixture was then diluted with dicholoromethane, washed with saturated sodium bicarbonate solution, water and brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure and purified by flash column chromatography to obtain the product.

Method C for ester hydrolysis

To a solution of the ester (1 equiv.) in a mixture of methanol/water, was added 1M aqueous solution of lithium hydroxide (2 equiv.). The mixture was stirred at 65 ºC until the reaction was judged complete by LCMS. The reaction mixture was concentrated in vacuo to remove methanol and adjusted to ~ pH 4 with 1N hydrochloric acid. The precipitate formed was collected and purified by flash column chromatography to obtain the product.

Methyl 6-(cyclohexylmethyl)pyridine-2-carboxylate

To a suspension of methyl-6-bromopyridine-2-carboxylate (0.5 g, 2.3 mmol) and [1,3-Bis(diphenylphosphino)propane]nickel(II) chloride (0.063 g, 0.1 mmol) stirring in dichloromethane (15 mL) under argon, was added 0.5 M solution of (cyclohexylmethyl)zinc bromide in tetrahydrofuran (9.258 ml, 4.6 mmol). After stirring at ambient temperature for 90 min, added saturated ammonium

chloride (15 mL) and separated the layers. The organic layer was washed with water (15 mL) and brine (15 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure and purified by flash column chromatography (25% ethyl acetate/hexanes) to obtain 0.5 g (93%) of the title compound as pale yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.9 (d, *J* = 7.7 Hz, 1H), 7.67 (t, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 7.7 Hz, 1H), 3.95 (s, 3H), 2.73 (d, *J* = 7.3 Hz, 2H), 1.73-1.78 (m, 1H), 1.58-1.65 (m, 4H), 1.09-1.21 (m, 4H), 0.91-1.01 (m, 2H); LCMS m/z 234 (MH+).

6-(Cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride

To a solution of methyl 6-(cyclohexylmethyl)pyridine-2-carboxylate (0.16 g, 0.68 mmol) in 2:1 mixture of methanol/water (6 mL), was added 1M aqueous solution of lithium hydroxide (1.36 mL, 1.36 mmol) and stirred at 45 ºC for 3 h. The reaction mixture was concentrated in vacuo to remove methanol and adjusted to pH 2 with 1N hydrochloric acid. The mixture was concentrated azeotropically with toluene to dryness to obtain 0.19 g of the title compound as brown oil and hydrochloride salt that was used without further purification. LCMS m/z 219.9 (MH+).

Methyl 4-nitro-3-(phenylamino)benzoate

To solution of methyl-3-fluoro-4-nitrobenzoate (0.1 g, 0.5 mmol) in *N,N*-dimethylformamide (3 mL), were added aniline (0.047 g, 0.5 mmol) and potassium carbonate (0.14 g, 1.0 mmol). The mixture was stirred at 90 ^{o}C for 60 h. The reaction mixture was then diluted with ethyl acetate (10 mL), washed with water (10 mL) and brine (10 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure and purified by flash column chromatography (10% ethyl acetate/hexanes) to obtain 40 mg (29%) of methyl 4-nitro-3-(phenylamino)benzoate as a bright orange solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.24 (d, *J* = 8.9 Hz, 1H), 7.91 (d, *J* = 1.6 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 1H), 7.33-7.36 (m, 1H), 7.25-7.28 (m, 3H), 3.87 (s, 3H); LCMS m/z 273.1 (MH+).

Methyl 4-amino-3-(phenylamino)benzoate

A solution of methyl 4-nitro-3-(phenylamino)benzoate (0.034 g, 0.1 mmol) in methanol (50 mL) was subjected to conditions described in Method A to obtain ~ 30 mg of methyl 4-amino-3-(phenylamino)benzoate as colorless oil that was used without further purification in the next reaction. LCMS m/z 243.1 (MH+).

Methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-(phenylamino)benzoate

To a solution of 6-(cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride (0.034 g, 0.12 mmol)) in dichloromethane (2 mL) stirring at 0 °C , was added 1-chloro-*N,N*,2-trimethylpropenylamine (0.017 mL, 0.13 mmol). After 10 min, were added methyl 4-amino-3-(phenylamino)benzoate (0.03 g, 0.12 mmol), and *N,N*-diisopropylethylamine (0.042 mL, 0.24 mmol). The mixture was subjected to conditions described in Method B and purified by flash column chromatography (40% ethyl acetate/hexanes) obtain 29 mg (50%) of methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-(phenylamino)benzoate as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.83 (s, 1H), 8.47 (d, *J* = 8.5 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 7.99 (d, *J* = 1.6 Hz, 1H), 7.91 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.75 (t, *J* = 7.7 Hz, 1H), 7.19-7.25 (m, 3H), 6.79-6.87 (m, 3H), 3.88 (s, 3H), 2.55 (d, *J* = 7.1 Hz, 2H), 1.52-1.63 (m, 6H), 1.10-1.12 (m, 3H), 0.87-0.93 (m, 2H); LCMS m/z 444.3 (MH+).

4-[6-(Cyclohexylmethyl)pyridine-2-amido]-3-(phenylamino)benzoic acid (1)

To a solution of methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-(phenylamino)benzoate (0.021 mg, 0.047 mmol) in 2:1 mixture of methanol/water (3 mL), was added 1M aqueous solution of lithium hydroxide (0.094 mL, 0.094 mmol). The mixture was stirred at 45 °C until the reaction was judged complete by LCMS. The reaction mixture was concentrated in vacuo to remove methanol and adjusted to pH 7 with 1N hydrochloric acid. The mixture was then extracted with ethyl acetate (3 x 10 mL). The organic extracts were washed with water (10 mL) and brine (10 mL). The organic layer was dried over magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography

(40% ethyl acetate/hexanes) to obtain 10 mg (49%) of the title compound as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.01 (s, 1H), 8.46 (d, *J* = 8.5 Hz, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 8.05 (d, *J* = 2 Hz, 1H), 7.94 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.84 (t, *J* = 7.7 Hz, 1H), 7.20-7.30 (m, 2H), 6.84-6.89 (m, 4H), 2.64 (d, *J* = 7.1 Hz, 2H), 1.54-1.64 (m, 4H), 1.12-1.14 (m, 3H), 0.90-0.93 (m, 2H); HRMS m/z expected: 430.2125, found: 430.2121 (MH+; $C_{26}H_{28}O_3N_3$).

Methyl 3-[(4-fluorophenyl)amino]-4-nitrobenzoate

To solution of methyl-3-fluoro-4-nitrobenzoate (0.1 g, 0.5 mmol) in dimethylsulfoxide (1 mL), were added 4-fluoroaniline (0.048 mL, 0.5 mmol) and *N,N*-diisopropylethylamine (0.175 mL, 1.0 mmol). The mixture was stirred at 100 ^{o}C until the reaction was judged complete by LCMS. The mixture was cooled and then poured in ice/water mixture. The precipitate formed was filtered and dried to obtain 115 mg (79%) of the title compound as a bright orange solid that was used without further purification. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.36 (s, 1H), 8.24 (d, *J* = 8.8 Hz, 1H), 7.72 (s, 1H), 7.34-7.36 (m, 1H), 7.23-7.26 (m, 2H), 7.12-7.16 (m, 2H), 3.87 (s, 3H); LCMS m/z 291.0 (MH+).

Methyl 4-amino-3-[(4-fluorophenyl)amino]benzoate

A solution of methyl 3-[(4-fluorophenyl)amino]-4-nitrobenzoate (0.025 g, 0.086 mmol) in methanol (15 mL) was subjected to conditions described in Method A to obtain ~ 17 mg of the title compound as colorless oil that was used without further purification. LCMS m/z 261.0 (MH+).

Methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-[(4-fluorophenyl)amino]benzoate

To a solution of 6-(cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride (0.014 g, 0.065 mmol)) in dichloromethane (1 mL) stirring at 0 °C , was added 1-chloro-N,N,2-trimethylpropenylamine (0.008 mL, 0.072 mmol). After 25 min, were added methyl 4-amino-3-[(4-fluorophenyl)amino]benzoate (0.017 g, 0.065 mmol), and N,N-diisopropylethylamine (0.023 mL, 0.13 mmol). The mixture was subjected to conditions described in Method B and purified by flash column chromatography (20% ethyl

acetate/hexanes) to obtain 18 mg (60%) of methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-[(4-fluorophenyl)amino]benzoate as a yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.77 (s, 1H), 8.42 (d, *J* = 8.4 Hz, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.88-7.91(m, 2H), 7.73-7.76 (m, 1H), 7.22-7.29 (m, 1H), 6.90-6.94 (m, 2H), 6.75-6.79 (m, 2H), 5.66 (s, 1H), 3.88 (s, 3H), 2.55 (d, *J* = 7.1 Hz, 2H), 1.53-1.64 (m, 4H), 1.11-1.20 (m, 7H); LCMS m/z 462.2 (MH+).

4-[6-(Cyclohexylmethyl)pyridine-2-amido]-3-[(4-fluorophenyl)amino]benzoic acid (2)

To a solution of methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-[(4-fluorophenyl)amino]benzoate (0.018 mg, 0.036 mmol) in 2:1 mixture of methanol/water (3 mL), was added 1M aqueous solution of lithium hydroxide (0.094 mL, 0.094 mmol). The mixture was stirred at 65 °C until the reaction was judged complete by LCMS. The reaction mixture was subjected to conditions described in Method C and purified by flash column chromatography (25% ethyl acetate/hexanes/0.5% formic acid) to obtain 4 mg (23%) of the title compound as a pale yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 (d, *J* = 8 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.72-7.74 (m, 2H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 6.69-6.73 (m, 2H), 6.54-6.58 (m, 2H), 2.35 (d, *J* = 7.1 Hz, 2H), 1.33-1.43 (m, 7H), 0.94 (bs, 2H); HRMS m/z expected: 448.2031, found: 430.2027 (MH+; $C_{26}H_{27}O_3N_3F$)..

Methyl 3-[(4-methoxyphenyl)amino]-4-nitrobenzoate

To solution of methyl-3-fluoro-4-nitrobenzoate (0.1 g, 0.5 mmol) in dimethylsulfoxide (1 mL), were added 4-methoxyaniline (0.062 mg, 0.5 mmol) and *N,N*-diisopropylethylamine (0.174 mL, 1.0 mmol). The mixture was stirred at 120 ºC until the reaction was judged complete by LCMS analysis. The reaction mixture was then diluted with ethyl acetate (10 mL), washed with saturated ammonium chloride (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure and purified by flash column chromatography (10% ethyl acetate/hexanes) to obtain 115 mg (76%) of methyl 3-[(4-methoxyphenyl)amino]-4-

nitrobenzoate as a red solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.23 (d, *J* = 8.5 Hz, 1H), 7.69 (s, 1H), 7.27-7.36 (m, 1H), 7.19 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H); LCMS m/z 303.0 (MH+).

Methyl 4-amino-3-[(4-methoxyphenyl)amino]benzoate

A solution of methyl 3-[(4-methoxyphenyl)amino]-4-nitrobenzoate (0.022 g, 0.07 mmol) in methanol (10 mL) was subjected to conditions described in Method A to obtain ~ 20 mg of the title compound as a brown oil that was used without further purification. LCMS m/z 273.1 (MH+).

Methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-[(4-methoxyphenyl)amino]benzoate

To a solution of 6-(cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride (0.016 g, 0.07 mmol)) in dichloromethane (1 mL) stirring at 0 °C , was added 1-chloro-*N,N*,2-trimethylpropenylamine (0.011 mL, 0.08 mmol). After 30 min, were added methyl 4-amino-3-[(4-methoxyphenyl)amino]benzoate (0.02 g, 0.07 mmol), and *N,N*-diisopropylethylamine (0.026 mL, 0.14 mmol). The mixture was subjected to conditions described in Method B and purified by flash column chromatography (10% ethyl acetate/hexanes) to obtain 17 mg (49%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.72 (s, 1H), 8.33 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.82-7.87 (m, 2H), 7.74 (t, *J* = 7.7 Hz, 1H), 7.22-7.24 (m, 1H), 6.78-6.84 (m, 4H), 5.66 (s, 1H), 3.86 (s, 3H), 3.74 (s, 3H), 2.58 (d, *J* = 7.1 Hz, 2H), 1.54-1.68 (m, 4H), 1.11-1.15 (m, 4H) 0.91 (d, *J* = 11.6 Hz, 1H); LCMS m/z 474.2 (MH+).

4-[6-(Cyclohexylmethyl)pyridine-2-amido]-3-[(4-methoxyphenyl)amino]benzoic acid (3)

To a solution of methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-[(4-methoxyphenyl)amino]benzoate (0.017 mg, 0.036 mmol) in 5:1 mixture of methanol/water (2 mL), was added 1M aqueous solution of lithium hydroxide (0.072 mL, 0.072 mmol). The mixture was stirred at 65 °C until the reaction was judged complete by LCMS. The reaction mixture was subjected to

conditions described in Method C and purified by flash column chromatography (5% methanol/dichloromethane/0.5% formic acid) to obtain 5 mg (30%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.75 (s, 1H), 8.37 (d, *J* = 8.4 Hz, 1H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.87-7.91 (m, 2H), 7.75 (t, *J* = 7.7 Hz, 1H), 7.23-7.25 (m, 1H), 6.79-6.85 (m, 4H), 3.75 (s, 3H), 2.58 (d, *J* = 7.1 Hz, 2H), 1.55-1.67 (m, 6H), 1.12-1.14 (m, 3H), 0.87-0.88 (m, 2H); HRMS m/z expected: 460.2231, found: 460.2228 (MH+; $C_{27}H_{30}O_4N_3$).

Methyl 4-nitro-3-{[3-(trifluoromethoxy)phenyl]amino}benzoate

To solution of methyl-3-fluoro-4-nitrobenzoate (0.1 g, 0.5 mmol) in dimethylsulfoxide (1 mL), was added 3-(trifluoromethoxy)aniline (0.067 mL, 0.5 mmol) and N,N-diisopropylethylamine (0.174 mL, 1.0 mmol). The mixture was stirred at 100 ºC for 18 h. The reaction mixture was then diluted with ethyl acetate (10 mL), washed with saturated ammonium chloride (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried over magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography (15% ethyl acetate/hexanes) to obtain 34 mg (19%) of the title compound as a bright orange solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.41 (s, 1H), 8.24-8.27 (m, 1H), 7.98 (s, 1H), 7.43-7.45 (m, 1H), 7.20 (d, *J* = 8 Hz, 1H), 7.15 (s, 1H), 7.08-7.10 (m, 1H), 3.89 (s, 3H); LCMS m/z 357.0 (MH+).

Methyl 4-amino-3-{[3-(trifluoromethoxy)phenyl]amino}benzoate

A solution of a methyl 4-nitro-3-{[3-(trifluoromethoxy)phenyl]amino}benzoate (0.025 g, 0.07 mmol) in methanol (10 mL) was subjected to conditions described in Method A to obtain ~ 22 mg of the title compound as a reddish-brown oil that was used without further purification. LCMS m/z 327.0 (MH+).

Methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-{[3-(trifluoromethoxy)phenyl]amino}benzoate

To a solution of 6-(cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride (0.014 g, 0.064 mmol)) in dichloromethane (1 mL) stirring at 0 °C , was added 1-chloro-N,N,2-trimethylpropenylamine (0.009 mL, 0.071 mmol). After 20 min, methyl 4-amino-3-{[3-(trifluoromethoxy)phenyl]amino}benzoate (0.021 g, 0.064 mmol), and *N,N*-diisopropylethylamine (0.022 mL, 0.12 mmol) were added. The mixture purified by flash column chromatography (15% ethyl acetate/hexanes) to obtain 13 mg (38%) of the title compound as an orange oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.79 (s, 1H), 8.45 (d, *J* = 8.5 Hz, 1H), 7.94-8.06 (m, 3H), 7.75 (t, *J* = 7.7 Hz, 1H), 7.18-7.22 (m, 2H), 6.69 (d, *J* = 8.3 Hz, 2H), 6.62 (s, 1H), 5.90 (s, 1H), 3.89 (s, 3H), 2.53 (d, *J* = 7 Hz, 2H), 1.52-1.60 (m, 8H), 1.11 (bs, 2H) 0.88 (d, *J* = 9.2 Hz, 1H); LCMS m/z 528.1 (MH+).

4-[6-(Cyclohexylmethyl)pyridine-2-amido]-3-{[3-(trifluoromethoxy)phenyl]amino}benzoic acid (4)

To a solution of methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-{[3-(trifluoromethoxy)phenyl]amino}benzoate (0.013 mg, 0.0256 mmol) in 5:1 mixture of methanol/water (3 mL), was added 1M aqueous solution of lithium hydroxide (0.05 mL, 0.05 mmol). The mixture was stirred at 70 ºC until the reaction was judged complete by LCMS. The reaction mixture was subjected to conditions described in Method C and purified by flash column chromatography (35% ethyl acetate/hexanes/0.5% formic acid) to obtain 10 mg (79%) of the title compound as a beige colored solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.84 (s, 1H), 8.51 (d, *J* = 8.5 Hz, 1H), 8.00-8.06 (m, 3H), 7.75 (t, *J* = 7.7 Hz, 1H), 7.18-7.22 (m, 2H), 6.69-6.71 (m, 2H), 6.63 (s, 1H), 5.89 (s, 1H), 2.53 (d, *J* = 7 Hz, 2H), 1.52-1.62 (m, 4H), 1.24-1.27 (m, 3H), 1.10-1.11 (m, 2H), 0.86-0.89 (m, 2H), ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.12, 162.25, 160.24, 149.93, 148.37, 148.19, 138.87, 138.09, 131.31, 127.97, 127.61, 127.16, 121.76, 119.77, 119.43, 113.49, 110.90, 106.82, 45.11, 37.83, 32.73, 26.35, 25.89. HRMS m/z expected: 514.1948, found: 430.2121 (MH+; $C_{27}H_{27}O_4N_3F$).

Methyl 3-[(2,3-dihydro-1H-inden-5-yl)amino]-4-nitrobenzoate

To a solution of methyl-3-fluoro-4-nitrobenzoate (0.1 g, 0.5 mmol) in dimethylsulfoxide (1 mL), was added 5-aminoindan (0.067 g, 0.5 mmol) and N,N-diisopropylethylamine (0.175 ml, 1.0 mmol) and stirred at 120 ^{o}C for an hour, The reaction mixture was then diluted with ethyl acetate (10 mL), washed with saturated ammonium chloride (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried over magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography (15% ethyl acetate/hexanes) to obtain 100 mg (64%) of the title compound as a bright orange solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.44 (s, 1H), 8.23 (d, *J* = 8.9 Hz, 1H), 7.84 (s, 1H), 7.27-7.30 (m, 2H), 7.12 (s, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 3.86 (s, 3H), 2.93 (d, *J* = 7.4 Hz, 4H), 2.13 (q, *J* = 7.4 Hz, 2H); LCMS m/z 313.1 (MH+).

Methyl 4-amino-3-[(2,3-dihydro-1H-inden-5-yl)amino]benzoate

A solution of a methyl 3-[(2,3-dihydro-1H-inden-5-yl)amino]-4-nitrobenzoate (0.027 g, 0.086 mmol) in methanol (20 mL) was subjected to conditions described in Method A to obtain ~ 17 mg of the title compound as a colorless oil that was used without further purification. LCMS m/z 283.1 (MH+).

Methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-[(2,3-dihydro-1H-inden-5-yl)amino]benzoate

To a solution of 6-(cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride (0.013 g, 0.06 mmol)) in dichloromethane (1 mL) stirring at 0 °C , was added 1-chloro-N,N,2-trimethylpropenylamine (0.009 mL, 0.066 mmol). After 20 min, were added methyl 4-amino-3-[(2,3-dihydro-1H-inden-5-yl)amino]benzoate (0.017 g, 0.06 mmol), and N,N-diisopropylethylamine (0.021 mL, 0.12 mmol). The mixture purified by flash column chromatography (35% ethyl acetate/hexanes) to obtain 23 mg (79%) of the title compound as a yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.84 (s, 1H), 8.44 (dd, *J* = 8.5, 1.7 Hz, 1H), 8.05 (d, *J* = 7.7 Hz, 1H), 7.96 (s, 1H), 7.86-7.88 (m, 1H), 7.73 (t, *J* = 7.7 Hz, 1H), 7.22 (d, *J* = 7.7 Hz, 1H), 7.05 (d, *J* = 7.7 Hz, 1H), 6.62-6.67 (m, 2H), 5.52 (s, 1H), 3.86 (s, 3H), 2.79 (t, *J* =

7.2 Hz, 4H), 2.55 (d, *J* = 7.2 Hz, 2H), 1.96-2.05 (m, 2H), 1.53-1.60 (m, 7H), 1.10 (bs, 2H) 0.87-0.92 (m, 2H); LCMS m/z 484.2 (MH+).

4-[6-(Cyclohexylmethyl)pyridine-2-amido]-3-[(2,3-dihydro-1H-inden-5-yl)amino]benzoic acid (5)

To a solution of methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-[(2,3-dihydro-1H-inden-5-yl)amino]benzoate (0.017 mg, 0.035 mmol) in 5:1 mixture of methanol/water (3 mL), was added 1M aqueous solution of lithium hydroxide (0.07 mL, 0.07 mmol). The mixture was stirred at 70 ºC until the reaction was judged complete by LCMS. The reaction mixture was subjected to conditions described in Method C and purified by flash column chromatography (50% ethyl acetate/hexanes/0.5% formic acid) to obtain 5 mg (31%) of the title compound as a beige colored solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.88 (s, 1H), 8.49 (d, *J* = 8.6 Hz, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.99 (s, 1H), 7.93 (dd, *J* = 8.5, 2 Hz, 1H), 7.75 (t, *J* = 7.7 Hz, 1H), 7.22-7.24 (m, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 6.63-6.69 (m, 2H), 2.80 (t, *J* = 7.3 Hz, 4H), 2.56 (d, *J* = 7.2 Hz, 2H), 2.02 (t, *J* = 7.3 Hz, 2H), 1.56-1.65 (m, 6H), 1.24 (bs, 4H), 0.86-0.84-0.87 (m, 1H); HRMS m/z expected: 470.2438, found: 470.2431 (MH+; $C_{29}H_{32}O_3N_3$).

Methyl 3-[(3-trifluoromethylphenyl)amino]-4-nitrobenzoate

To solution of methyl-3-fluoro-4-nitrobenzoate (0.1 g, 0.5 mmol) in dimethylsulfoxide (1 mL), was added 3-(trifluoromethyl)aniline (0.062 mL, 0.5 mmol) and N,N-diisopropylethylamine (0.174 mL, 1.0 mmol). The mixture was stirred at 100 ºC until the reaction was judged complete by LCMS. The reaction mixture was then diluted with ethyl acetate (10 mL), washed with saturated ammonium chloride (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried over magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography (15% ethyl acetate/hexanes) to obtain 56 mg (33%) of the title compound as a bright orange solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.43 (bs, 1H), 8.26 (dd, *J* = 8.8, 1.2 Hz, 1H), 7.91 (s, 1H), 7.43-7.57 (m, 4H), 3.88 (s, 3H); LCMS m/z 341.1(MH+).

Methyl 4-amino-3-{[3-(trifluoromethyl)phenyl]amino}benzoate

A solution of a methyl 4-nitro-3-{[3-(trifluoromethyl)phenyl]amino}benzoate (0.025 g, 0.07 mmol) in methanol (20 mL) was subjected to conditions described in Method A to obtain ~ 21 mg of the title compound as a reddish-brown oil that was used without further purification. LCMS m/z 311.1 (MH+).

Methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-{[3-(trifluoromethyl)phenyl]amino}benzoate

To a solution of 6-(cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride (0.015 g, 0.068 mmol)) in dichloromethane (1 mL) stirring at 0 °C , was added 1-chloro-*N,N*,2-trimethylpropenylamine (0.01 mL, 0.074 mmol). After 20 min, were added methyl 4-amino-3-{[3-(trifluoromethyl)phenyl]amino}benzoate (0.021 g, 0.068 mmol), and *N,N*-diisopropylethylamine (0.024 mL, 0.13 mmol). The mixture purified by flash column chromatography (20% ethyl acetate/hexanes) to obtain 21 mg (61%) of the title compound as an orange solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.82 (bs, 1H), 8.46 (d, *J* = 8.4 Hz, 1H), 7.95-8.07 (m, 2H), 7.75 (t, *J* = 7.7 Hz, 1H), 7.21-7.31 (m, 2H), 7.04-7.09 (m, 2H), 6.90 (d, *J* = 7.9 Hz, 1H), 5.91 (s, 1H), 3.89 (s, 3H), 2.52 (d, *J* = 7 Hz, 2H), 1.50-1.59 (m, 5H), 1.09 (bs, 2H) 0.86 (d, *J* = 10.6 Hz, 2H); LCMS m/z 512.1 (MH+).

4-[6-(Cyclohexylmethyl)pyridine-2-amido]-3-{[3-(trifluoromethyl)phenyl]amino}benzoic acid (6)

To a solution of methyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-{[3-(trifluoromethyl)phenyl]amino}benzoate (0.021 mg, 0.04 mmol) in 5:1 mixture of methanol/water (3 mL), was added 1M aqueous solution of lithium hydroxide (0.08 mL, 0.08 mmol). The mixture was stirred at 70 ºC until the reaction was judged complete by LCMS. The reaction mixture was subjected to conditions described in Method C and purified by flash column chromatography (35% ethyl acetate/hexanes/0.5% formic acid) to obtain 13 mg (64%) of the title compound as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d* with few drops of CD_3OD) δ 8.47 (d, *J* = 8.4 Hz, 1H), 7.90-7.97 (m, 3H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.16-7.20 (m, 2H), 6.96-6.99 (m, 2H), 6.83 (d, *J* = 8.1 Hz, 1H), 2.42 (d, *J*

= 7 Hz, 2H), 1.41-1.53 (m, 6H), 1.01-1.03 (m, 2H), 0.76-0.81 (m, 2H), ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.09, 162.28, 160.25, 148.31, 147.15, 138.92, 138.28, 131.39, 130.86, 130.69, 130.37, 128.07, 127.62, 127.17, 119.81, 119.49, 118.08, 115.51, 111.09, 45.08, 37.88, 32.69, 26.30, 25.89.; HRMS m/z expected: 498.1999, found: 498.1995 (MH+; $C_{27}H_{27}O_3N_3F_3$).

***tert*-Butyl 3-fluoro-4-nitrobenzoate**

To a solution of 3-fluoro-4-nitrobenzoic acid (0.5 g, 2.7 mmol) in *N,N'*-dimethylformamide (5 mL), were added tert-butanol (1.3 ml, 13.5 mmol), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.52 g, 2.7 mmol) and 4-dimethylaminopyridine (0.033 g, 0.3 mmol). After stirring at room temperature for 8 h, the reaction mixture was diluted with ethyl acetate, washed with water, saturated ammonium chloride solution and brine. The organic layer was dried over magnesium sulfate and purified by flash chromatography (15% ethyl acetate/hexanes) to obtain 220 mg (34%) of the title compound as a cream-colored solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.04-8.08 (m, 1H), 7.84-7.79 (m, 2H), 1.60 (s, 9H).

***tert*-Butyl 3-(cyclohexyloxy)-4-nitrobenzoate**

To a solution of sodium hydride (0.008 g, 0.3 mmol) in THF, was added cyclohexanol (0.3 ml, 2.8 mmol). After 5 minutes, was added *tert*-butyl 3-fluoro-4-nitrobenzoate (0.075 g, 0.3 mmol) and stirred at room temperature for an hour. The reaction mixture was diluted with ethyl acetate, washed with saturated ammonium chloride solution, water and brine. The organic layer was dried over magnesium sulfate and purified by flash chromatography (5% EtOAC/hexanes) to obtain 90 mg (90%) of *tert*-butyl 3-(cyclohexyloxy)-4-nitrobenzoate as a pale yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.68-7.73 (m, 2H), 7.54 (dt, *J* = 8.5, 1.2 Hz, 1H), 4.49-4.54 (m, 1H), 1.90-1.95 (m, 2H), 1.75-1.80 (m, 2H), 1.63-1.68 (m, 2H), 1.49-1.54 (m, 11H), 1.37-1.41 (m, 2H); LCMS m/z 344.1 (MNa+).

***tert*-Butyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-(cyclohexyloxy)benzoate**

A solution of a *tert*-butyl 3-(cyclohexyloxy)-4-nitrobenzoate (0.033 g, 0.1 mmol) in methanol (15 mL) was subjected to conditions described in Method A to obtain 26 mg of crude *tert*-butyl 4-amino-3-(cyclohexyloxy)benzoate as a colorless oil that was used without further purification.

To a solution of 6-(cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride (0.02 g, 0.09 mmol)) in dichloromethane (1 mL) stirring at 0 ^{o}C , was added 1-chloro-*N,N*,2-trimethylpropenylamine (0.013 mL, 0.098 mmol). After 20 min, were added *tert*-butyl 4-amino-3-(cyclohexyloxy)benzoate (0.026 mg, 0.09 mmol), and *N,N*-diisopropylethylamine (0.031 mL, 0.18 mmol). The mixture purified by flash column chromatography (15% ethyl acetate/hexanes) to obtain 37 mg (84%) of *tert*-butyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-(cyclohexyloxy)benzoate as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.01 (s, 1H), 8.67 (d, *J* = 8.6 Hz, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.77 (t, *J* = 7.7 Hz, 1H), 7.58-7.64 (m, 2H), 7.25-7.27 (m, 1H), 4.46-4.50 (m, 1H), 2.73 (d, *J* = 7.2 Hz, 2H), 2.03-2.06 (m, 2H), 1.85-1.88 (m, 3H), 1.56-1.73 (m, 13H), 1.42-1.47 (m, 4H), 1.18-1.24 (m, 5H), 1.00-1.06 (m, 3H); LCMS m/z 493.3 (MH+).

4-[6-(Cyclohexylmethyl)pyridine-2-amido]-3-(cyclohexyloxy)benzoic acid (7)

To a mixture of *tert*-butyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-(cyclohexyloxy)benzoate (0.036 g, 0.07 mmol,) in 5:1 mixture of dioxane/water (1 mL), was added 4M solution of hydrochloric acid in dioxane (0.5 mL) and catalytic amount of triethylsilane (0.002 mL). After stirring at room temperature for 60 hours, the reaction mixture was concentrated down to dryness, washed with hexanes and dried to obtain 26 mg (75%) of the title compound as a hydrochloride salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.57 (d, *J* = 8.2 Hz, 1H), 7.96-8.01 (m, 2H), 7.59-7.61 (m, 2H), 7.51 (d, *J* = 7 Hz, 1H), 4.60-4.64 (m, 1H), 2.72 (d, *J* = 7.2 Hz, 2H), 1.95-1.99 (m, 2H), 1.77-1.79 (m, 3H), 1.44-1.65 (m, 10H), 1.12-1.17 (m, 3H), 0.98-1.03 (m, 2H), ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.37, 162.12, 160.38, 148.80, 146.05, 138.96, 132.72, 127.80, 126.33, 123.35, 120.00, 118.20, 114.37, 76.18, 45.54, 38.48,

32.98, 31.57, 26.46, 26.03, 25.66, 23.22; HRMS m/z expected: 437.2421, found: 437.2428 (MH+; $C_{26}H_{33}O_4N_2$).

tert-Butyl 4-nitro-3-(phenylsulfanyl)benzoate

To a solution of *tert*-butyl 3-fluoro-4-nitrobenzoate (0.05 g, 0.2 mmol) in dimethylsulfoxide (1 mL), was added thiophenol (0.021 ml, 0.2 mmol) and *N,N*-diisopropylethylamine (0.072 ml, 0.4 mmol). After stirring at room temperature for 3 h, the mixture was diluted with ethyl acetate, washed with saturated ammonium chloride solution, water and brine. The organic layer was dried over magnesium sulfate and purified by flash chromatography (10% ethyl acetate/hexanes) twice to obtain 64 mg of *tert*-butyl 4-nitro-3-(phenylsulfanyl)benzoate as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.22 (d, *J* = 8.6 Hz, 1H), 7.72-7.75 (m, 1H), 7.58-7.60 (m, 2H), 7.49-7.51 (m, 3H), 7.44 (s, 1H), 1.42(m, 9H); LCMS m/z 354.1 (MNa+).

tert-Butyl 4-amino-3-(phenylsulfanyl)benzoate

To a refluxing solution of *tert*-butyl 4-nitro-3-(phenylsulfanyl)benzoate (0.058, 0.18 mmol) and ammonium chloride (0.094 g, 1.8 mmol) in 2:1 mixture of ethanol/water (6 mL), was added iron (0.029 g, 0.5 mmol) in portions over a period of 30 min. After refluxing for 45 minutes, dichloromethane (20 mL) was added to the reaction mixture. The organic layer was washed with brine, dried over magnesium sulfate and purified by flash chromatography (20% ethyl acetate/hexanes) to obtain 46 mg of the title compound as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.09 (s, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.75 (s, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 7.21-7.23 (m, 1H), 7.14-7.16 (m, 1H), 7.07-7.09 (m, 2H), 1.56 (s, 9H); LCMS m/z 302.1 (MH+).

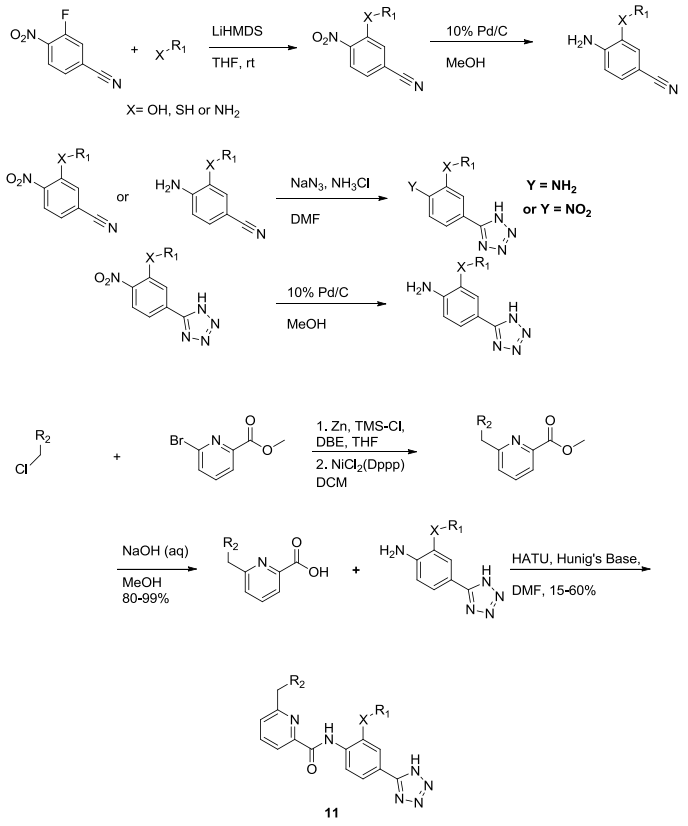
tert-Butyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-(phenylsulfanyl)benzoate

To a solution of 6-(cyclohexylmethyl)pyridine-2-carboxylic acid hydrochloride (0.015 g, 0.07 mmol)) in dichloromethane (1 mL) stirring at 0 ^{o}C , was added 1-chloro-*N,N*,2-trimethylpropenylamine (0.01 mL, 0.075 mmol). After 20 min, were added *tert*-butyl 4-amino-3-(phenylsulfanyl)benzoate (0.02 mg, 0.07 mmol), and *N,N*-diisopropylethylamine (0.024 mL, 0.14 mmol). The mixture purified by flash column chromatography (20% ethyl acetate/hexanes) to obtain 12 mg (36%) of the title compound as an orange colored oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.56 (s, 1H), 8.84 (d, *J* = 8.7 Hz, 1H), 8.28 (s, 1H), 8.02-8.11 (m, 3H), 7.73 (t, *J* = 7.7 Hz, 1H), 7.18-7.22 (m, 4H), 7.10-7.12 (m, 1H), 2.64 (d, *J* = 7.1 Hz, 2H), 1.58-1.67 (m, 12H), 1.13-1.26 (m, 6H), 0.87-0.97 (m, 2H); LCMS m/z 503.3 (MH+).

4-[6-(Cyclohexylmethyl)pyridine-2-amido]-3-(phenylsulfanyl)benzoic acid (8)

To a mixture of *tert*-butyl 4-[6-(cyclohexylmethyl)pyridine-2-amido]-3-(phenylsulfanyl)benzoate (0.012 g, 0.02 mmol,) in 5:1 mixture of dioxane/water (0.5 mL), was added 4M solution of hydrochloric acid in dioxane (0.25 mL) and catalytic amount of triethylsilane (0.001 mL). After stirring at room temperature for 72 hours, the reaction mixture was concentrated down to dryness, washed with hexanes and dried to obtain 10 mg (87%) of the title compound as a hydrochloride salt. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.65 (s, 1H), 8.91 (d, *J* = 8.6 Hz, 1H), 8.41 (s, 1H), 8.20 (dd, *J* = 8.8, 1.6 Hz, 1H), 8.04 (d, *J* = 7.7 Hz, 1H), 7.75 (t, *J* = 7.7 Hz, 1H), 7.25-7.26 (m, 1H), 7.21 (d, *J* = 4.2 Hz, 4H), 7.10-7.14 (m, 1H), 2.66 (d, *J* = 7.1 Hz, 2H), 1.80-1.83 (m, 1H), 1.66-1.69 (m, 4H), 1.14-1.26 (m, 4H), 0.95-1.00 (m, 2H); HRMS m/z expected: 447.1737, found: 447.1731 (MH+; $C_{26}H_{27}O_3N_2S$).

The following scheme and procedures were used for the tetrazole analogs.



Scheme S1. Synthesis of tetrazole analogs.

General Method D

In a 50 mL round-bottomed flask, tetrahydrofuran (18.06 ml), an appropriate coupling partner (1.0 equiv.), and lithium bis(trimethylsilyl)-amide, 1.0M solution in tetrahydrofuran (6.020 ml, 6.0 mmol, 1.0 equiv.) were stirred for twenty minutes. 3-fluoro-4-nitrobenzonitrile (1.000 g, 6.0 mmol, 1.0 equiv.) was added and the reaction was stirred for 4 hours followed by slow addition of water. The organics were extracted thrice from brine with dichloromethane, dried over $MgSO_4$ and purified using flash column chromatography (0-20% ethyl acetate in hexane, unless otherwise noted).

4-nitro-3-((tetrahydro-2H-pyran-4-yl)oxy)benzonitrile

Prepared using general method D tetrahydro-2H-pyran-4-ol (0.615 g, 6.0 mmol, 1.0 equiv. Red solid, yield 0.5 g (33.5%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.83 (d, *J* = 8.2 Hz, 1H), 7.35 (d, *J* = 1.5 Hz,

1H), 7.33 (dd, *J* = 8.2, 1.5 Hz, 1H), 4.78 – 4.68 (m, 1H), 4.00 – 3.89 (m, 2H), 3.68 – 3.58 (m, 2H), 2.11 – 1.99 (m, 2H), 1.91 – 1.80 (m, 2H).

3-(cyclohexylthio)-4-nitrobenzonitrile

Synthesized using general method D and cyclohexanethiol (0.700 g, 6.0 mmol, 1.0 equiv). Orange solid, yield 1.1 g (61.2%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.17 (t, *J* = 8.3, 3.8 Hz, 1H), 7.73 (d, *J* = 8.2 Hz, 1H), 7.52 (t, *J* = 8.4 Hz, 1H), 3.49 – 3.25 (m, 1H), 2.16 – 1.96 (m, 2H), 1.86 (dq, *J* = 10.3, 5.4, 4.6 Hz, 3H), 1.77 – 1.19 (m, 9H).

4-nitro-3-((tetrahydro-2H-thiopyran-4-yl)oxy)benzonitrile

Synthesized using general method D with tetrahydro-2H-thiopyran-4-ol (0.700 g, 5.9 mmol, 1.0 equiv). Yellow solid, yield 0.605 g (38.7%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.84 (d, *J* = 8.1 Hz, 1H), 7.41 – 7.27 (m, 2H), 4.70 – 4.52 (m, 1H), 2.97 (ddd, *J* = 13.0, 8.5, 3.8 Hz, 2H), 2.53 (ddd, *J* = 14.0, 7.0, 3.8 Hz, 2H), 2.26 – 1.98 (m, 4H).

4-amino-3-((tetrahydro-2H-pyran-4-yl)oxy)benzonitrile

To a solution of 4-nitro-3-((tetrahydro-2H-pyran-4-yl)oxy)benzonitrile (0.500 g, 2.0 mmol, 1.0 equiv.) in 20 ml methanol was added palladium, 10% on carbon (0.050 g, 0.5 mmol, 0.2 equiv.) in a 50 ml flask. The mixture was stirred under a hydrogen balloon overnight. Upon completion, the mixture was filtered over a pad of Celite, dried under vacuum and carried forward through the next reaction without further purification.

4-amino-3-(cyclohexylthio)benzonitrile

To a solution of 3-(cyclohexylthio)-4-nitrobenzonitrile (300 mg, 1.1 mmol, 1.0 equiv.) in EtOH/water was added ammonium chloride (0.484 g, 9.8 mmol, 10.8 equiv.), and the mixture was heated to reflux. Iron (0.152 g, 2.7 mmol, 3.0 equiv.) was added in portions over a period of 30 minutes. The reaction

was refluxed for an additional 45 minutes and then cooled. Dichloromethane was added and the organics extracted thrice. Washed the dichloromethane extracts with brine, dried over $MgSO_4$ and concentrated to yield an orange solid that was used without further purification.

General procedure E

To a solution of an appropriate benzonitrile (1.0 equiv.) and sodium azide (371 mg, 5.7 mmol, 5.9 equiv.) in *N,N*-Dimethylformamide (3 ml) was added ammonium chloride (281 mg, 5.7 mmol, 5.9 equiv.). The reaction mixture was heated to 120 ^{o}C in a sealed vessel with stirring overnight. The reaction was quenched with water and taken to pH 4.0 with HCl. The aqueous media was extracted with dichloromethane thrice and the organics combined and dried over $MgSO_4$ and under vacuum. The material was re-dissolved in Methanol/Dichloromethane, adhered to silica gel and subjected to column chromatography using a 0-10% Methanol:Dichloromethane gradient unless otherwise noted.

2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline

Prepared using general procedure E and 4-amino-3-((tetrahydro-2H-pyran-4-yl)oxy)benzonitrile (210 mg, 1.0 mmol, 1.0 equiv.). White solid, yield 0.213 g (29.7%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.49 (d, J = 1.9 Hz, 1H), 7.39 (dd, J = 8.2, 1.9 Hz, 1H), 6.84 (d, J = 8.2 Hz, 1H), 4.60 (tt, J = 8.2, 4.0 Hz, 1H), 4.06 – 3.92 (m, 2H), 3.59 (ddd, J = 11.8, 8.9, 3.0 Hz, 2H), 2.16 – 2.02 (m, 2H), 1.89 – 1.71 (m, 2H).

2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)aniline

Prepared using general procedure E and 4-amino-3-(cyclohexylthio)benzonitrile (0.275 g, 1.2 mmol, 1.0 equiv.). The desired material was isolated as a red-solid/oilish material. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 (d, J = 2.1 Hz, 1H), 8.07 (s, 1H), 7.86 (dd, J = 8.4, 2.1 Hz, 1H), 6.76 (d, J = 8.4 Hz, 1H), 1.91 – 1.81 (m, 3H), 1.71 – 1.62 (m, 2H), 1.36 – 1.19 (m, 2H), 1.24 – 1.09 (m, 3H). LCMS m/z 276.00 (MH+).

5-(4-nitro-3-((tetrahydro-2H-thiopyran-4-yl)oxy)phenyl)-1H-tetrazole

Prepared using general method E and 4-nitro-3-((tetrahydro-2H-thiopyran-4-yl)oxy)benzonitrile (0.200 g, 0.8 mmol, 1.0 equiv.). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.93 – 7.85 (m, 2H), 7.76 (ddd, *J* = 8.6, 1.7, 0.9 Hz, 1H), 4.78 (q, *J* = 4.8 Hz, 1H), 3.00 – 2.93 (m, 2H), 2.62 – 2.44 (m, 3H), 2.24 – 2.08 (m, 5H).

2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline

Prepared using general method A and 5-(4-nitro-3-((tetrahydro-2H-thiopyran-4-yl)oxy)phenyl)-1H-tetrazole (0.110 g, 0.4 mmol, 1.0 equiv.) Yield 0.88 g (88.6%), brown oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.61 (d, *J* = 1.8 Hz, 1H), 7.51 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 3.66 (tt, *J* = 9.3, 3.6 Hz, 1H), 2.86 – 2.72 (m, 2H), 2.69 – 2.53 (m, 2H), 2.22 – 2.12 (m, 2H), 1.77 – 1.63 (m, 2H).

General Procedure F

In a 100 mL round-bottomed flask, dichloromethane (30 ml), an appropriate aryl or alkyl zinc(II) halide (2.0 equiv.), methyl 6-bromopicolinate (1.0 equiv.) and dichloro(1,3-bis(diphenylphosphino)propane)nickel (0.01 equiv.) were dissolved. The reaction mixture was stirred at room temperature for two hours and then diluted with acidified brine (pH 3.8). The organics were washed thrice with this solution, collected, dried over $MgSO_4$, and adhered to silica under vacuum. The desired product was obtained using flash chromatography using a 5-20% Ethyl Acetate:Hexanes gradient unless otherwise noted.

Methyl 6-(4-chlorobenzyl)picolinate

Prepared using general procedure F and (4-chlorobenzyl)zinc(II) bromide (0.500 M, 5.503 ml, 2.8 mmol, 2.0 equiv.). Clear oil, yield 115.4 mg (32%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.97 – 7.87

(m, 1H), 7.67 (td, *J* = 7.8, 1.4 Hz, 1H), 7.41 – 7.10 (m, 5H), 4.21 (s, 2H), 3.97 (d, *J* = 1.4 Hz, 3H). LCMS m/z 261.70 (MH+).

Methyl 6-(3-chlorobenzyl)picolinate

Prepared using general procedure F and (3-chlorobenzyl)zinc(II) bromide (0.500 M, 5.503 ml, 2.8 mmol, 2.0 equiv.). Clear oil, yield 102.0 mg (28.3%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.99 – 7.92 (m, 1H), 7.70 (t, *J* = 7.8 Hz, 1H), 7.25 – 7.08 (m, 5H), 4.22 (s, 2H), 3.98 (s, 3H). LCMS m/z 261.70 (MH+).

Methyl 6-(4-methylbenzyl)picolinate

Prepared using general procedure F and (4-methylbenzyl)zinc(II) chloride (0.500 M, 5.503 ml, 2.8 mmol, 2.0 equiv). Clear oil, yield 104 mg, (31.3%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.94 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.71 – 7.60 (m, 1H), 7.28 – 7.07 (m, 5H), 4.23 (s, 2H), 4.04 – 3.93 (m, 3H), 2.32 (d, *J* = 7.9 Hz, 3H). LCMS m/z 241.29 (MH+).

Methyl 6-(3,4-difluorobenzyl)picolinate

Prepared using general procedure F and (3,4-difluorobenzyl)zinc(II) bromide (0.500 M, 5.503 ml, 2.8 mmol, 2.0 equiv). Yellowish oil, used in subsequent reaction without purification. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.96 (d, *J* = 7.7 Hz, 1H), 7.76 – 7.68 (m, 1H), 7.22 (d, *J* = 7.7 Hz, 1H), 7.10 – 6.98 (m, 3H), 4.19 (s, 2H), 3.97 (s, 3H). LCMS m/z 263.24 (MH+).

Methyl 6-(3-fluorobenzyl)picolinate

Prepared using general procedure F and (3-fluorobenzyl)zinc(II) chloride (0.500 M, 5.503 ml, 2.8 mmol, 2.0 equiv.). Used in subsequent reaction without purification. Orange oil, used without further characterization. LCMS m/z 245.25 (MH+).

General Procedure G

An appropriate picolinate was taken in methanol/water and 3.1 equivalents NaOH (1M) were added and stirred at room temperature for overnight. The reaction mixture was then concentrated, adjusted to pH4 with 1M HCl and extracted with EtOAc. The organics were washed thrice with brine and dried over $MgSO_4$. The resultant picolinic acids were used in the subsequent reactions with no further purification.

6-(3-chlorobenzyl)picolinic acid

Prepared using general procedure G and methyl 6-(3-chlorobenzyl)picolinate (0.100 g, 0.4 mmol, 1.0 equiv.) was taken in White solid, yield 0.094g (99.3%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 (d, *J* = 7.7 Hz, 1H), 7.86 (t, *J* = 7.7 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.30 – 7.18 (m, 3H), 7.18 – 7.08 (m, 1H), 4.19 (s, 2H). LCMS m/z 247.68 (MH+).

6-(4-chlorobenzyl)picolinic acid

Prepared using general procedure G and methyl 6-(4-chlorobenzyl)picolinate (0.115 g, 0.4 mmol, 1.0 equiv.) White solid, yield 93.0 mg (85.4%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.66 (s, 1H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.83 (t, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.30 – 7.22 (m, 2H), 7.19 – 7.11 (m, 2H), 4.18 (s, 2H). LCMS m/z 247.68 (MH+).

6-(4-methylbenzyl)picolinic acid

Prepared using general procedure G and methyl 6-(4-methylbenzyl)picolinate (0.300 g, 1.0 mmol). Orange oil, quantitative yield. Carried forward without further characterization. LCMS m/z 227.26 (MH+).

6-(3-fluorobenzyl)picolinic acid

Prepared using general procedure G and methyl 6-(3-fluorobenzyl)picolinate (0.300 g, 1.2 mmol, 1.0 equiv.). Reddish oil, yield 62.0 mg (21.9%). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.06 (d, *J* = 7.8 Hz, 1H), 7.84 (t, *J* = 7.7 Hz, 1H), 7.68 (dd, *J* = 11.5, 7.8 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 2H), 7.28 (td, *J* = 7.2, 4.1 Hz, 1H), 7.09 (dd, *J* = 11.6, 8.8 Hz, 0H), 7.01 (d, *J* = 7.7 Hz, 1H), 6.94 (t, *J* = 8.7 Hz, 2H), 4.21 (s, 2H). ^{19}F NMR (376 MHz, Chloroform-d) δ -113.11 (q, *J* = 8.4 Hz), -113.62 (q, *J* = 7.7, 6.7 Hz). LCMS m/z 231.22 (MH+).

6-(3,4-difluorobenzyl)picolinic acid

Prepared using general procedure G and methyl 6-(3,4-difluorobenzyl)picolinate (0.300 g, 1.2 mmol, 1.0 equiv.). Brown oil, yield 88.0 mg (31%). Carried forward without further characterization. LCMS m/z 249.22 (MH+).

6-(cyclohexyloxy)picolinic acid

In a flame dried 10 ml sealed vessel, lithium bis(trimethylsilyl)-amide, 1.0M solution in tetrahydrofuran (0.780 ml, 0.8 mmol, 2.2 equiv.), cyclohexanol (0.044 ml, 0.4 mmol, 1.2 equiv.), and 6-fluoropicolinic acid (0.050 g, 0.4 mmol, 1.0 equiv.) were dissolved in anhydrous dioxane. The reaction was stirred at 120 ºC overnight. The reaction was quenched with the slow addition of water and acidified to pH 4.0. The aqueous media was extracted 3X with dichloromethane and the organics combined, dried and subjected to column chromatography using a 0-10% Methanol:Dichloromethane gradient. Yellow oil, yield 46 mg (58.7%). Carried forward without further characterization.

General Procedure H

The picolinic acid and aniline derivatives were dissolved to make stock solutions in *N,N*-Dimethylformamide between 0.3 and 0.9 M in concentration. The reactants were stirred with 1.1 eq. HATU and 2.0 equivalents of *N,N*-Diisopropylethylamine in 300 *N,N*-Dimethylformamide until LC-MS indicated completion. The mixture was then directly injected and onto a preparatory reverse phase

column and purified using the specified conditions. Each compound was lyophilized to yield the solid material listed.

6-(3,4-difluorobenzyl)-N-(2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (11)

Synthesized using general procedure H, 6-(3,4-difluorobenzyl)picolinic acid (18 µmol, 1.0 equiv.) and 2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (18 µmol, 1.0 equiv). The material was isolated using a 55-75% Methanol:Water (0.05 % formic acid) gradient. White powder, 5.9 mg (67.9%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (s, 1H), 8.55 (dd, J = 8.4, 1.6 Hz, 1H), 8.15 – 7.98 (m, 2H), 7.76 (s, 1H), 7.66 (dt, J = 8.4, 1.7 Hz, 1H), 7.60 (dt, J = 7.3, 1.6 Hz, 1H), 7.44 – 7.29 (m, 2H), 7.18 – 7.09 (m, 1H), 6.51 (s, 1H), 4.81 (dt, J = 7.7, 4.1 Hz, 1H), 4.23 (s, 2H), 3.97 – 3.86 (m, 2H), 3.60 – 3.49 (m, 2H), 2.06 (d, J = 13.3 Hz, 2H), 1.77 – 1.65 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.59, 161.54, 159.48, 149.36, 146.32, 139.85, 137.28, 128.89, 127.33, 125.94, 120.53, 119.83, 119.40, 118.16, 118.03, 112.08, 73.26, 64.64, 42.75, 32.02. ^{19}F NMR (376 MHz, DMSO-d_6) δ -138.81 – -139.28 (m), -142.18 – -142.51 (m). HRMS m/z expected: 493.1794, found: 493.1790 (MH+; $C_{25}H_{23}O_3N_6F_2$).

6-(3-fluorobenzyl)-N-(2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (12)

Synthesized using general procedure H, 6-(3-fluorobenzyl)picolinic acid (24 µmol, 1.0 equiv.) and 2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (24 µmol, 1.0 equiv.). The material was isolated using a 75-95% Methanol:Water (0.05 % formic acid) gradient method. The desired fractions were lyophilized to yield a yellow solid powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.66 (d, J = 8.4 Hz, 1H), 8.11 – 8.01 (m, 2H), 7.81 (s, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.65 (d, J = 7.3 Hz, 1H), 7.41 – 7.29 (m, 1H), 7.17 (d, J = 8.3 Hz, 2H), 7.06 (td, J = 8.6, 2.4 Hz, 1H), 4.86 (tt, J = 8.0, 3.9

Hz, 1H), 4.27 (s, 2H), 4.00 – 3.91 (m, 2H), 3.62 – 3.53 (m, 2H), 2.15 – 2.05 (m, 2H), 1.81 – 1.70 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.77, 159.67, 149.07, 146.46, 142.31, 139.84, 130.97, 130.45, 127.52, 125.29, 121.39, 120.56, 119.48, 115.98, 115.81, 113.88, 113.71, 112.36, 73.48, 64.66, 43.43, 31.94. HRMS m/z expected: 475.1888, found: 475.1886 (MH+; $C_{25}H_{24}O_3N_6F$).

6-(4-methylbenzyl)-N-(2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (13)

Synthesized using general procedure H, 6-(4-methylbenzyl)picolinic acid (13 µmol, 1.0 equiv.) and 2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (13 µmol, 1.0 equiv.). The material was isolated using 60-90% Methanol:Water (0.05% formic acid) gradient. The desired fractions were lyophilized to yield a white solid powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.87 (s, 1H), 8.67 (d, J = 8.4 Hz, 1H), 8.03 (dt, J = 15.1, 7.5 Hz, 2H), 7.81 (d, J = 1.7 Hz, 1H), 7.72 (dd, J = 8.3, 1.6 Hz, 1H), 7.59 (d, J = 7.4 Hz, 1H), 7.22 (d, J = 7.7 Hz, 2H), 7.12 (d, J = 7.7 Hz, 2H), 4.92 – 4.83 (m, 1H), 4.19 (s, 2H), 4.02 – 3.93 (m, 2H), 3.63 – 3.54 (m, 2H), 2.16 – 2.06 (m, 2H), 1.83 – 1.73 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.86, 160.64, 148.89, 146.46, 139.63, 136.49, 135.97, 130.62, 129.66, 129.07, 127.33, 120.62, 120.30, 119.45, 112.43, 73.54, 64.70, 43.60, 31.96. HRMS m/z expected: 471.2139, found: 471.2134(MH+; $C_{26}H_{27}O_3N_6$).

6-(cyclohexylmethyl)-N-(2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (14)

Synthesized using general procedure H, 6-(cyclohexylmethyl)pyridine-2-carboxylic acid (23 µmol, 1.0 equiv.) and 2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (23 µmol, 1.0 equiv.). The material was isolated using 50-85% Methanol:Water (0.05% formic acid) gradient. White powder, 5.2 mg (48.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.70 (dd, J = 8.5, 2.0 Hz, 1H), 8.07 – 7.96 (m, 2H), 7.83 (s, 1H), 7.73 (dd, J = 8.4, 1.9 Hz, 1H), 7.55 (dd, J = 7.2, 1.7 Hz, 1H), 4.90 (dt, J = 8.3, 4.1

Hz, 1H), 4.02 – 3.92 (m, 2H), 3.67 – 3.56 (m, 2H), 2.76 (d, *J* = 7.2 Hz, 2H), 2.20 – 2.10 (m, 2H), 1.86 – 1.73 (m, 2H), 1.70 – 1.56 (m, 6H), 1.25 – 1.10 (m, 4H), 1.09 – 0.95 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.97, 160.44, 156.23, 148.83, 146.35, 139.00, 130.59, 127.81, 121.27, 120.59, 119.93, 119.26, 112.35, 73.43, 64.72, 45.56, 38.54, 32.97, 32.06, 26.44, 26.00. HRMS m/z expected: 463.2452, found: 463.2447(MH+; $C_{25}H_{31}O_3N_6$).

6-(4-chlorobenzyl)-N-(2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (15)

Synthesized using general procedure H, 6-(4-chlorobenzyl)picolinic acid (24 μmol, 1.0 equiv.) and 2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (24 μmol, 1.0 equiv.). The material was isolated using 60-90% Methanol:Water (0.05 % formic acid) gradient. Yellow solid, 5.9 mg (50.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.66 (d, *J* = 8.4 Hz, 1H), 8.15 – 7.99 (m, *J* = 6.9 Hz, 2H), 7.81 (s, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.62 (d, *J* = 7.3 Hz, 1H), 7.36 (q, *J* = 8.1 Hz, 4H), 4.91 – 4.82 (m, 1H), 4.24 (s, 2H), 4.00 – 3.91 (m, 2H), 3.61 – 3.52 (m, 2H), 2.14 – 2.04 (m, 2H), 1.80 – 1.69 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.81, 159.87, 156.02, 149.05, 146.47, 139.82, 138.59, 131.67, 131.01, 130.63, 129.05, 127.51, 120.64, 120.53, 119.49, 112.45, 73.51, 64.67, 43.07, 31.93. HRMS m/z expected: 491.1593, found: 491.1588 (MH+; $C_{25}H_{24}O_3N_6Cl$).

6-(3-chlorobenzyl)-N-(2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (16)

Synthesized using general procedure H, 6-(3-chlorobenzyl)picolinic acid (23 μmol, 1.0 equiv.) and 2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (23 μmol, 1.0 equiv.). The material was isolated using 60-90% Methanol:Water (0.05 % formic acid) gradient. White solid, yield 2.5 mg (21.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.65 (d, *J* = 8.4 Hz, 1H), 8.11 – 8.02 (m, 2H), 7.80 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.66 (d, *J* = 7.2 Hz, 1H), 7.41 (s, 1H), 7.40 – 7.32 (m, 1H), 7.30 (d, *J* = 7.7 Hz, 2H), 4.91 – 4.82 (m, 1H), 4.26 (s, 2H), 4.00 – 3.92 (m, 2H), 3.62 – 3.53 (m, 2H), 2.15 –

2.06 (m, 2H), 1.81 – 1.71 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.75, 159.61, 149.11, 146.45, 142.04, 139.88, 133.66, 130.95, 130.35, 128.94, 127.89, 127.54, 127.00, 120.60, 120.51, 119.47, 112.33, 73.45, 64.67, 43.33, 31.95. HRMS m/z expected: 491.1593, found: 491.1589 (MH+; $C_{25}H_{24}O_3N_6Cl$).

6-(3,4-difluorobenzyl)-N-(2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (17)

Synthesized using general procedure H, 6-(3,4-difluorobenzyl)picolinic acid (18 μmol, 1.0 equiv.) and 2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (18 μmol, 1.0 equiv.). The material was isolated using a 60-90% Methanol:Water (0.05 % formic acid) gradient method. Yellow solid, yield 2.2 mg (23.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.88 (s, 1H), 8.73 (d, J = 8.4 Hz, 1H), 8.21 – 8.11 (m, 2H), 7.87 (s, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 7.4 Hz, 1H), 7.54 – 7.43 (m, 2H), 7.29 – 7.22 (m, 1H), 4.37 (s, 2H), 3.09 – 2.98 (m, 2H), 2.77 – 2.70 (m, 2H), 2.39 – 2.29 (m, 2H), 2.14 – 2.04 (m, 2H). HRMS m/z expected: 509.1566, found: 509.1562 (MH+; $C_{25}H_{23}O_2N_6F_2S$).

6-(3-fluorobenzyl)-N-(2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (18)

Synthesized using general procedure H, 6-(3-fluorobenzyl)picolinic acid (24 μmol, 1.0 equiv.) and 2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (24 μmol, 1.0 equiv.). The material was isolated using a 60-90% Methanol:Water (0.05 % formic acid) gradient method. Yellow solid, yield 3.2 mg (25.4%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.76 (d, J = 8.4 Hz, 1H), 8.25 – 8.11 (m, 2H), 7.88 (d, J = 1.9 Hz, 1H), 7.81 (dd, J = 8.5, 1.8 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.51 – 7.43 (m, 1H), 7.32 – 7.24 (m, 2H), 7.16 (td, J = 8.9, 2.2 Hz, 1H), 4.39 (s, 2H), 3.11 – 3.01 (m, 2H), 2.81 – 2.70 (m, 2H), 2.42 – 2.31 (m, 2H), 2.19 – 2.06 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.82, 159.79, 149.07, 146.39, 142.38, 139.85, 131.07, 131.01, 130.63, 127.53, 125.38, 121.19, 120.63, 119.57,

116.04, 115.87, 113.91, 113.75, 112.43, 74.85, 73.07, 43.46, 40.49, 40.32, 40.15, 39.99, 39.82, 39.65, 39.49, 32.53, 25.12.HRMS m/z expected: 491.1660 found: 491.1656 (MH+; $C_{25}H_{24}O_2N_6FS$).

6-(cyclohexylmethyl)-N-(2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (19)

Synthesized using general procedure H, 6-(cyclohexylmethyl)pyridine-2-carboxylic acid (23 µmol, 1.0 equiv.) and 2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (23 µmol, 1.0 equiv.). Yellowish solid, yield 2.7 mg (24.5%). ^{1}H NMR (500 MHz, DMSO-d_6) 1H NMR (500 MHz, DMSO-d6) δ 10.99 (s, 1H), 8.77 (d, *J* = 8.4 Hz, 1H), 8.16 – 8.06 (m, 2H), 7.88 (s, 1H), 7.80 (d, *J* = 8.5 Hz, 1H), 7.64 (d, *J* = 7.3 Hz, 1H), 3.07 – 2.98 (m, 2H), 2.88 (d, *J* = 7.3 Hz, 2H), 2.84 – 2.74 (m, 2H), 2.45 – 2.36 (m, 2H), 2.18 – 2.08 (m, 2H), 1.99 – 1.86 (m, 1H), 1.80 – 1.66 (m, 6H), 1.37 – 1.22 (m, 4H), 1.19 – 1.08 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.00, 160.53, 148.84, 146.28, 139.00, 130.59, 127.82, 121.45, 120.59, 119.99, 119.35, 112.39, 74.98, 73.08, 45.60, 38.57, 32.97, 32.76, 26.45, 26.06, 25.35. HRMS m/z expected: 479.2224, found: 479.2219 (MH+; $C_{25}H_{31}O_2N_6S$).

6-(4-chlorobenzyl)-N-(2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (20)

Synthesized using general procedure H, 6-(4-chlorobenzyl)picolinic acid (23 µmol, 1.0 equiv.) and 2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (23 µmol, 1.0 equiv.). Yellow solid, yield 4.0 mg (35.1%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.91 (s, 1H), 8.75 (d, *J* = 8.4 Hz, 1H), 8.16 (dt, *J* = 15.0, 7.5 Hz, 2H), 7.88 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.70 (d, *J* = 7.5 Hz, 1H), 7.47 (q, *J* = 8.3 Hz, 4H), 4.37 (s, 2H), 3.10 – 3.01 (m, 3H), 2.79 – 2.70 (m, 2H), 2.39 – 2.30 (m, 2H), 2.16 – 2.05 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.80, 159.97, 149.08, 146.37, 139.82, 138.61, 131.67, 131.06, 130.55, 129.08, 127.51, 120.57, 119.55, 112.44, 74.84, 43.09, 32.53, 25.12. HRMS m/z expected: 507.1364, found: 507.1360 (MH+; $C_{25}H_{24}O_2N_6ClS$).

6-(3-chlorobenzyl)-N-(2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (21)

Synthesized using general procedure H, 6-(3-chlorobenzyl)picolinic acid (23 μmol, 1.0 equiv.) and 2-((tetrahydro-2H-thiopyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (29 ul, 0.0 mmol, 1.1 equiv.) ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.76 (d, J = 8.4 Hz, 1H), 8.25 – 8.11 (m, 2H), 7.88 (d, J = 1.9 Hz, 1H), 7.81 (dd, J = 8.5, 1.8 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.51 – 7.43 (m, 1H), 7.32 – 7.24 (m, 2H), 7.16 (td, J = 8.9, 2.2 Hz, 1H), 4.39 (s, 2H), 3.05 (ddd, J = 12.5, 8.5, 3.0 Hz, 2H), 2.75 (ddd, J = 13.7, 8.4, 3.1 Hz, 2H), 2.42 – 2.31 (m, 2H), 2.12 (ddd, J = 13.4, 8.0, 3.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.77, 159.71, 149.11, 146.37, 142.05, 139.88, 133.68, 130.98, 130.44, 129.01, 127.96, 127.55, 127.02, 121.63, 120.64, 120.56, 119.55, 112.39, 74.85, 43.36, 32.55, 25.15. HRMS m/z expected: 507.1364, found: 507.1360 (MH+; $C_{25}H_{24}O_2N_6ClS$).

N-(2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)phenyl)-6-(3,4-difluorobenzyl)picolinamide (22)

Synthesized using general procedure H, 6-(3,4-difluorobenzyl)picolinic acid (25 μmol, 1.0 equiv.) and 2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)aniline (25 μmol, 1.0 equiv.). White solid powder, yield 3.2 mg (22.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 8.67 (d, J = 8.6 Hz, 1H), 8.22 (d, J = 2.0 Hz, 1H), 8.13 – 7.99 (m, 3H), 7.66 (dd, J = 6.8, 2.0 Hz, 1H), 7.48 – 7.39 (m, 1H), 7.41 – 7.33 (m, 1H), 7.20 (s, 1H), 6.51 (s, 1H), 4.24 (s, 2H), 3.08 – 2.98 (m, 1H), 1.91 – 1.82 (m, 2H), 1.63 (d, J = 12.7 Hz, 2H), 1.50 – 1.42 (m, 1H), 1.36 – 1.12 (m, 5H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.52, 161.80, 159.43, 158.00, 150.79, 149.27, 140.46, 139.84, 137.30, 134.87, 128.28, 127.43, 126.04, 125.41, 122.49, 120.66, 119.50, 118.30, 118.17, 118.08, 117.94, 48.61, 42.78, 33.41, 25.74, 25.46. HRMS m/z expected: 507.1773, found: 507.1769 (MH+; $C_{25}H_{24}O_2N_6F_2S$).

N-(2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)phenyl)-6-(3-fluorobenzyl)picolinamide (23)

Synthesized using general procedure H, 6-(3-fluorobenzyl)picolinic acid (25 μmol, 1.0 equiv.) and 2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)aniline (25 μmol, 1.0 equiv.). White solid, yield 2.3 mg (18%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 8.71 (d, J = 9.0 Hz, 1H), 8.24 (d, J = 2.2 Hz, 1H), 8.14 – 8.00 (m, 3H), 7.71 – 7.63 (m, 1H), 7.40 – 7.30 (m, 1H), 7.25 – 7.17 (m, 2H), 7.10 – 7.00 (m, 1H), 6.50 (bs, 1H), 4.25 (s, 2H), 3.07 (ddt, J = 10.5, 7.3, 3.6 Hz, 1H), 1.92 – 1.83 (m, 2H), 1.67 – 1.59 (m, 2H), 1.46 (d, J = 11.9 Hz, 1H), 1.40 – 1.26 (m, 2H), 1.27 – 1.04 (m, 3H). HRMS m/z expected: 489.1867, found: 489.1867 (MH+; $C_{26}H_{26}ON_6FS$).

N-(2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)phenyl)-6-(4-methylbenzyl)picolinamide (24)

Synthesized using general procedure H, 6-(4-methylbenzyl)picolinic acid (23 μmol, 1.0 equiv.) and 2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)aniline (23 μmol, 1.0 equiv.). White solid, yield 3.4 mg (30.3%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.51 (s, 1H), 8.72 (d, J = 8.5 Hz, 1H), 8.25 (d, J = 1.9 Hz, 1H), 8.12 – 7.99 (m, 4H), 7.62 (d, J = 7.3 Hz, 1H), 7.28 (d, J = 7.7 Hz, 2H), 7.12 (d, J = 7.6 Hz, 2H), 4.19 (s, 2H), 3.15 – 3.05 (m, 1H), 2.25 (s, 3H), 1.96 – 1.88 (m, 2H), 1.75 – 1.62 (m, 2H), 1.55 – 1.45 (m, 1H), 1.48 – 1.31 (m, 2H), 1.29 – 1.17 (m, 2H), 1.21 – 1.08 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 163.54, 161.94, 160.57, 149.05, 139.65, 136.58, 135.95, 135.05, 129.64, 129.16, 128.44, 127.27, 122.56, 120.37, 119.43, 73.10, 48.65, 43.72, 33.39, 25.71, 25.52, 21.09. HRMS m/z expected: 485.2118, found: 485.2116 (MH+; $C_{27}H_{29}ON_6S$).

6-(cyclohexylmethyl)-N-[2-cyclohexylsulfanyl-4-(1H-tetrazol-5-yl)phenyl]pyridine-2-carboxamide (25)

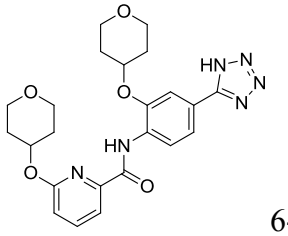
Synthesized using general procedure H, 6-(cyclohexylmethyl)pyridine-2-carboxylic acid (23 μmol, 1.0 equiv.) and 2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)aniline (23 μmol, 1.0 equiv.). Yield 4.1 mg (37.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.56 (s, 1H), 8.76 (d, J = 8.6 Hz, 1H), 8.26 (d, J = 2.1 Hz, 1H), 8.14 – 8.06 (m, 1H), 8.05 – 7.95 (m, 2H), 7.54 (dd, J = 7.2, 1.6 Hz, 1H), 3.19 – 3.07 (m, 1H), 2.76 (d, J = 7.2 Hz, 2H), 1.97 – 1.88 (m, 4H), 1.76 – 0.96 (m, 17H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.25, 160.37,

148.78, 141.81, 139.04, 135.30, 128.87, 127.85, 122.87, 122.21, 120.09, 119.41, 99.04, 48.79, 45.55, 38.25, 33.38, 33.08, 26.45, 26.12, 25.69, 25.62.

6-(3-chlorobenzyl)-N-(2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (26)

Synthesized using general procedure H, 6-(3-chlorobenzyl)picolinic acid acid (23 μmol, 1.0 equiv.) and 2-(cyclohexylthio)-4-(1H-tetrazol-5-yl)aniline acid (23 μmol, 1.0 equiv.). Yield 3.7 mg (32.1%), white powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.59 (s, 1H), 8.84 (d, J = 8.6 Hz, 1H), 8.35 (d, J = 1.9 Hz, 1H), 8.25 – 8.12 (m, 3H), 7.84 – 7.76 (m, 1H), 7.56 (s, 1H), 7.49 – 7.37 (m, 4H), 4.36 (s, 2H), 3.24 – 3.14 (m, 1H), 2.03 – 1.94 (m, 2H), 1.80 – 1.69 (m, 2H), 1.63 – 1.53 (m, 1H), 1.53 – 1.38 (m, 2H), 1.37 – 1.26 (m, 2H), 1.29 – 1.13 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.93, 159.56, 149.11, 142.08, 141.47, 139.90, 135.23, 133.65, 130.90, 129.05, 128.74, 128.01, 127.60, 126.99, 122.85, 120.71, 119.54, 48.70, 43.41, 33.43, 25.73, 25.49 HRMS m/z expected: 477.2431, found: 477.2428 (MH+; $C_{26}H_{33}ON_6S$).

6-((tetrahydro-2H-pyran-4-yl)oxy)-N-(2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)phenyl)picolinamide (27)



6-((tetrahydro-2H-pyran-4-yl)oxy)picolinic acid (0.043 g, 0.2 mmol, 1.0 equiv.), 2-((tetrahydro-2H-pyran-4-yl)oxy)-4-(1H-tetrazol-5-yl)aniline (0.050 g, 0.2 mmol, 1.0 equiv), HATU (0.073 g, 0.2 mmol, 1.0 equiv.), and N,N-diisopropylethylamine (0.067 ml, 0.4 mmol, 2.0 equiv.) were stirred in DMF for 45 minutes. The reaction mixture was diluted with dichloromethane and the organics were washed thrice with pH 4 (HCl) brine. The organics were dried over $MgSO_4$ and concentrated. The product was isolated via reverse phase chromatography using a 75-85% Methanol:H_2O (0.1% TFA) gradient. Yield 0.025 g (28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 8.64 (d, J = 8.9 Hz,

1H), 8.00 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 8.7 Hz, 2H), 7.73 (d, *J* = 9.1 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 1H), 5.45 – 5.40 (m, 1H), 4.88 – 4.83 (m, 1H), 3.99 – 3.94 (m, 2H), 3.91 – 3.86 (m, 2H), 3.61 – 3.56 (m, 4H), 2.21 – 2.16 (m, 2H), 2.10 – 2.05 (m, 2H), 1.78 – 1.73 (m, 4H). HRMS m/z expected: 467.2037, found: 467.2035 (MH+; $C_{23}H_{27}O_5N_6$).This compound had no measurable effect at KSHV protease in our activity based assay.

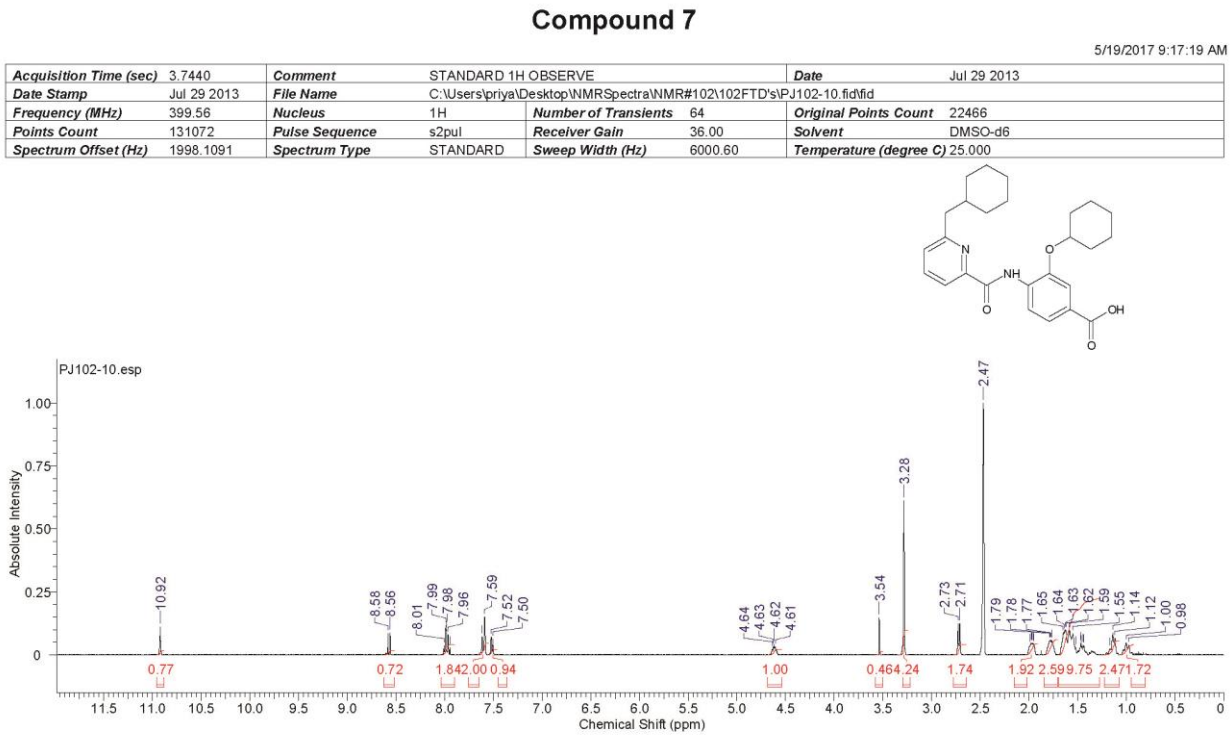


Figure S4. Representative ^{1}H Spectrum of carboxylic acid series (Compound 7) from this study.

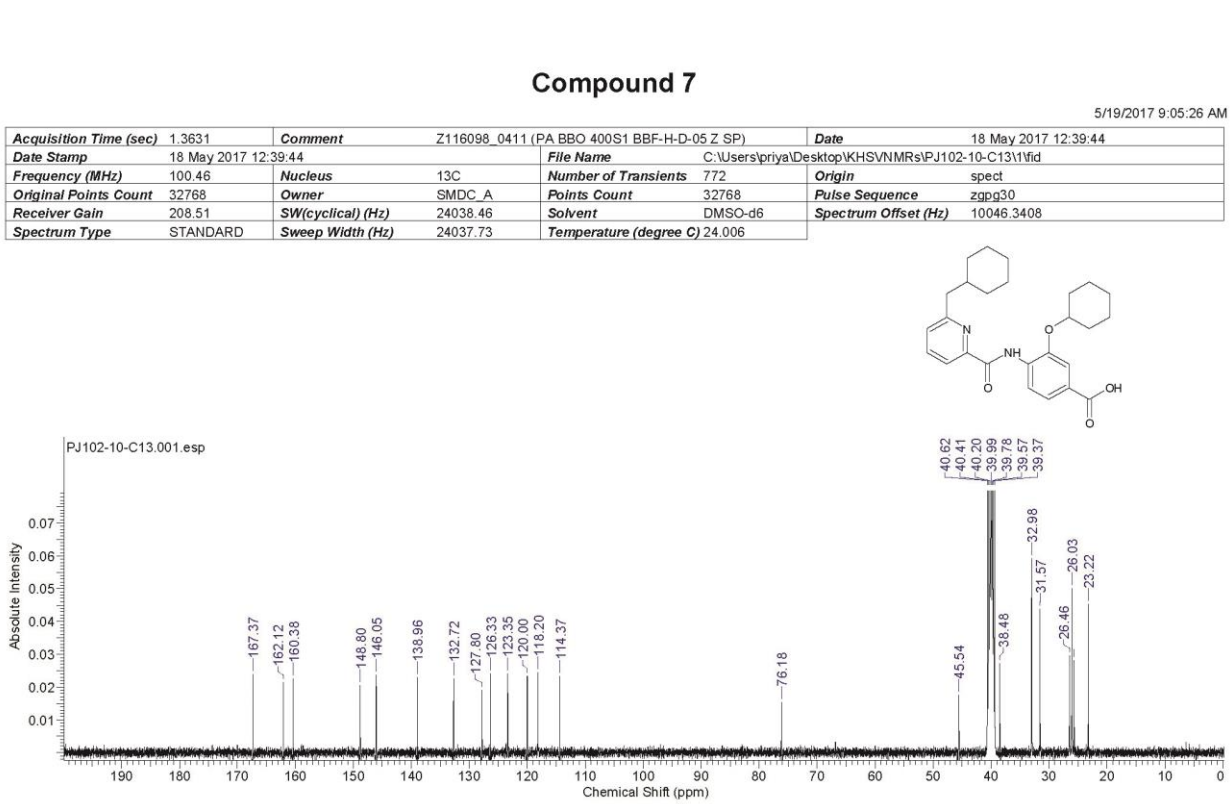


Figure S5. Representative ^{13}C Spectrum of carboxylic acid series (Compound 7) from this study.

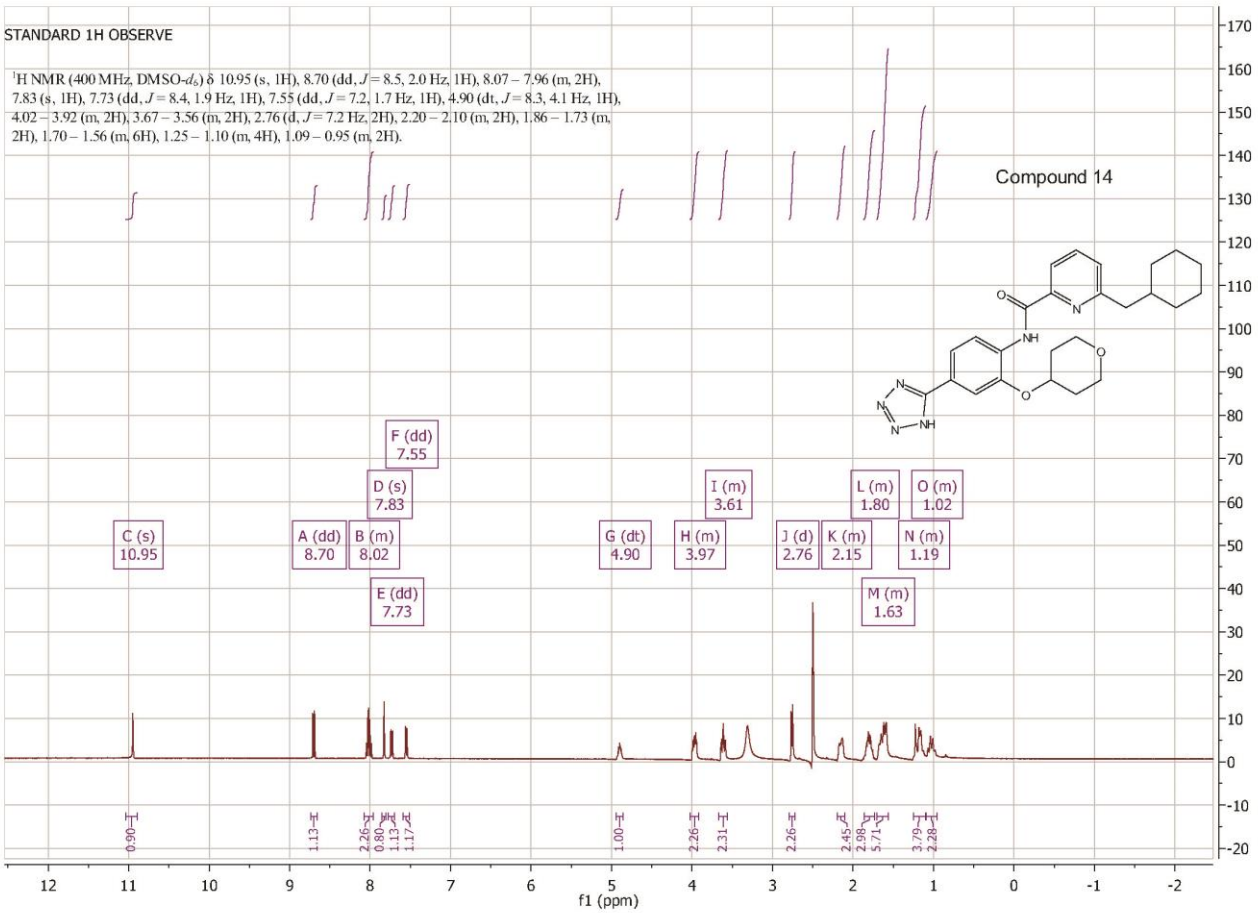


Figure S6. Representative ^{1}H Spectrum of the tetrazole series (Compound 14) from this study.

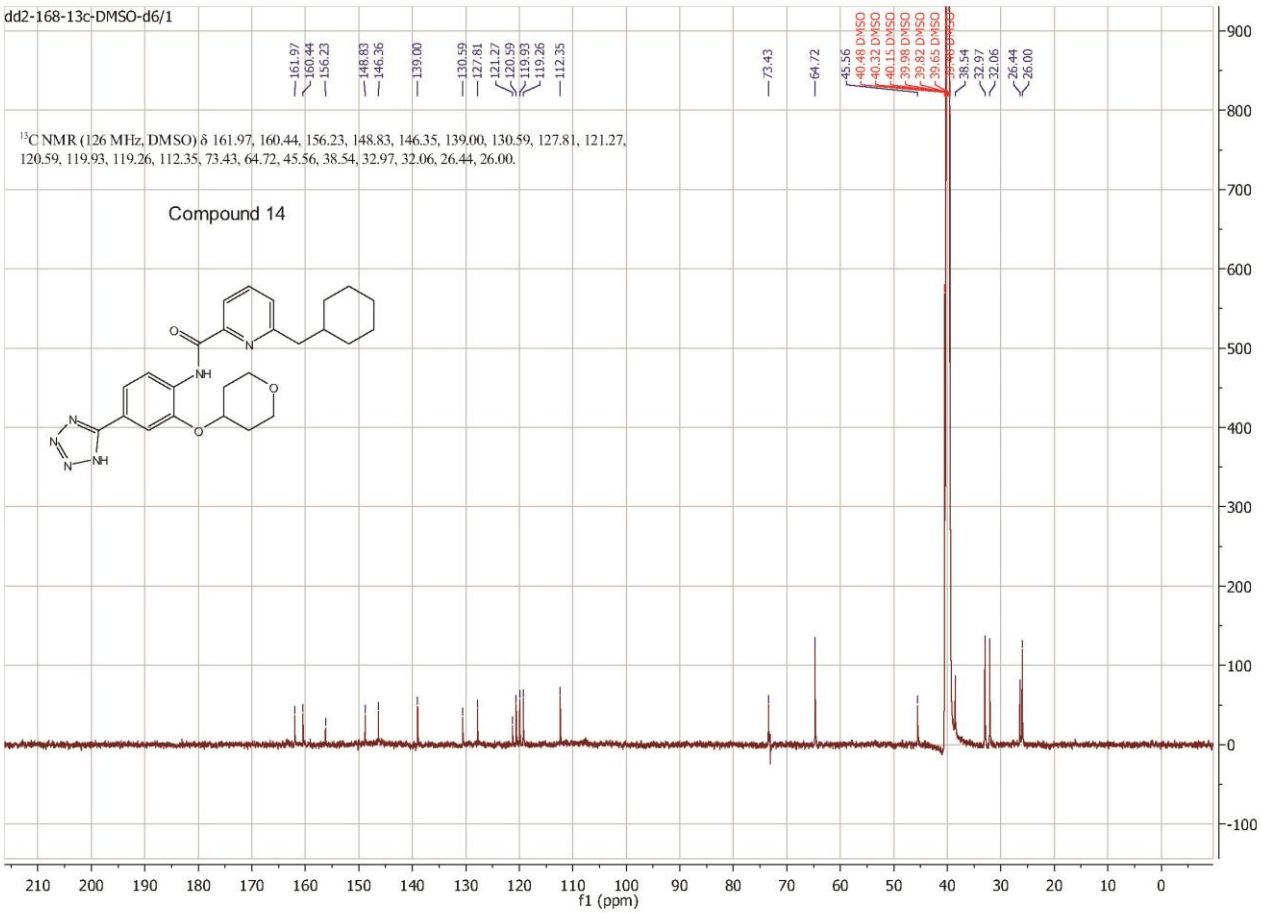


Figure S7. Representative ^{13}C spectrum of the tetrazole series (Compound 14) from this study.

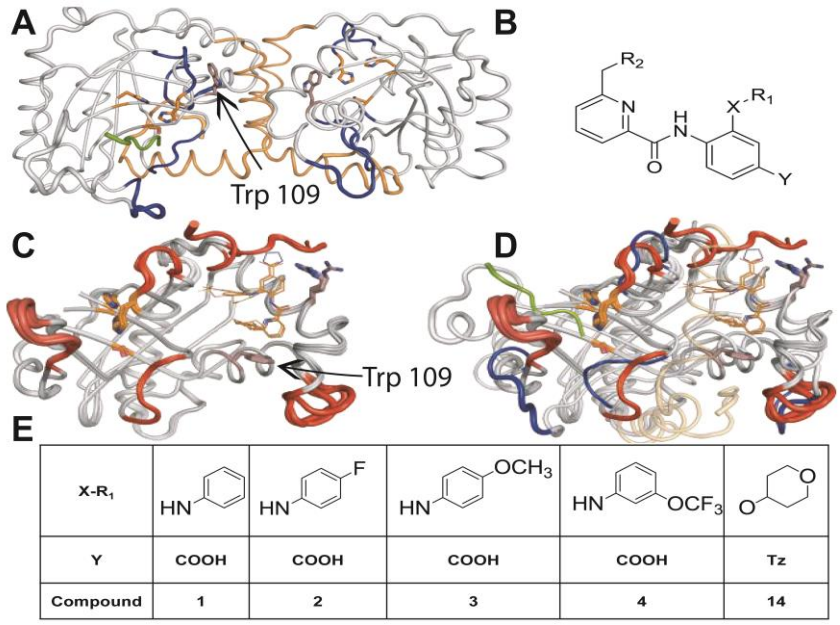


Figure 1. Binding of small molecules to the cryptic binding pocket leads to coordinated rearrangements of distal sites at the protein. A. The KSHV protease dimer (PDB: 2PBK) is shown with the dimer interface helices in orange. Trp109 is shown in brown and blue and is located behind helix 5. The active site residues are shown in orange and the loop regions that adopt distinct conformations in the compound bound monomers are shown in blue. B. The small-molecule scaffold with variable R-group regions is shown, where Y is either COOH or a tetrazole (Tz). C. The cryogenic co-crystal structures solved (PDB codes: 5UR3, 5UVP, 5UV3, 5UTE, 5UTN) in this study are overlaid. The dynamic loop regions are shown in red, scaled to their B-factors and the compounds are shown in orange. D. One monomer from the dimer structure (2PBK) is overlaid with a monomer from this study. Several loops from the monomeric structures, shown in red, are in distinct conformations from those of the dimeric structure shown in blue. E. The R1 groups from compounds that are co-crystallized in this study are shown (benzyl (1), 4-F-Benzyl (2), 4-OCH3-Benzyl (3), 3-OCF3-Benzyl (4), tetrahydropyran (14), R2 is cyclohexyl for each of these compounds.

Comparison of Fits		Comparison of Fits		Comparison of Fits	
Simpler model	Competitive inhibition	Simpler model	Noncompetitive inhibition	Simpler model	Uncompetitive inhibition
Probability it is correct	<0.01%	Probability it is correct	27.31%	Probability it is correct	0.21%
Alternative model	Mixed model inhibition	Alternative model	Mixed model inhibition	Alternative model	Mixed model inhibition
Probability it is correct	>99.99%	Probability it is correct	72.69%	Probability it is correct	99.79%
Ratio of probabilities		Ratio of probabilities	2.66	Ratio of probabilities	473.01
Preferred model	Mixed model inhibition	Preferred model	Mixed model inhibition	Preferred model	Mixed model inhibition
Difference in AICc	21.63	Difference in AICc	1.958	Difference in AICc	12.32

Table S3. Comparison of fits for mode of inhibition with compound 14. Substrate concentrations were varied down a 2-fold dilution series from 200 μM in the presence of varying concentrations of compound 14 or DMSO. Non-linear fitting comparisons were carried out in GraphPad Prism.

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2. Copeland, R. A., In *Evaluation of Enzyme Inhibitors in Drug Discovery*, John Wiley & Sons, Inc.: 2013; pp i-xxxi.
3. (a) Vieira, J.; O'Hearn, P. M., Use of the red fluorescent protein as a marker of Kaposi's sarcoma-associated herpesvirus lytic gene expression. *Virology* **2004,** *325* (2), 225-240; (b) Myoung, J.; Ganem, D., Generation of a doxycycline-inducible KSHV producer cell line of endothelial origin: maintenance of tight latency with efficient reactivation upon induction. *Journal of virological methods* **2011,** *174* (1-2), 12-21; (c) Stürzl, M.; Gaus, D.; Dirks, W. G.; Ganem, D.; Jochmann, R., Kaposi's sarcoma-derived cell line SLK is not of endothelial origin, but is a contaminant from a known renal carcinoma cell line. *International Journal of Cancer* **2013,** *132* (8), 1954-1958.