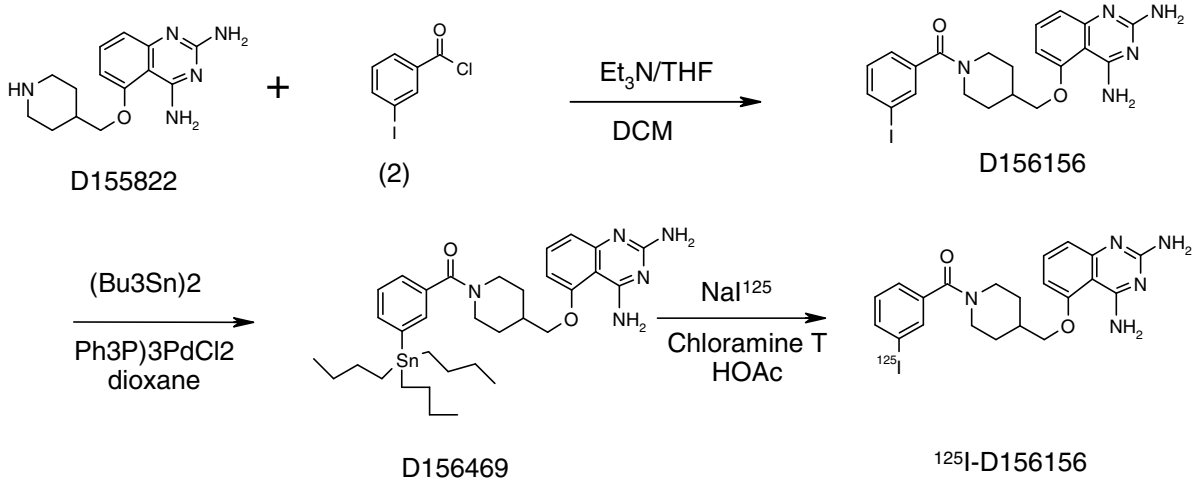
SUPPLEMENTARY TEXT

Materials and Methods

Synthesis of C5-substituted quinazolines. The C5-substituted quinazolines were synthesized according to previously published methods [14].



Preparation of D156156. A mixture of 5-(piperidin-4-ylmethoxy)-quinazoline-2,4-diamine (D155822) (60 mg, 0.22 mmol) [Thurmond, 2007 #75] and 3-iodobenzoyl chloride (58 mg, 0.22 mmol) in THF (2 mL) and DCM (2 mL) was added triethylamine (110 □L, 0.77 mmol). The reaction mixture was stirred at room temperature for 20 hours, then concentrated *in vacuo*. Residue diluted with water, basified with 1N NaOH solution, filtered, washed with water and dried *in vacuo*. The residue was purified by flash chromatography using silica gel and 5-10% MeOH/DCM gradient was used for elution to provide (3-iodophenyl)-[4-(2,4-diamino-quinazolin-5-yloxymethyl)-piperidin-1-yl]-methanone as an off white solid (70 mg; 63% yield). ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.82 (d, *J* = 7.6 Hz, 1H), 7.73 (s, 1H), 7.40 (d, *J* = 6.4 Hz, 1H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 7.18 (br s, 2H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 8.0 Hz, 1H), 5.94 (br s, 2H), 4.49 (m, 1H), 4.03 (d, *J* = 6.4 Hz, 2H), 3.57 (m,1H), 3.11 (m, 1H), 2.82 (m, 1H), 2.21 (m, 1H), 1.87 (m, 1H), 1.75 (m, 1H), 1.32 (m, 2H). MS m/z (ESI) 505 (M+H)+. HPLC purity (Area % = 97.6%).

Preparation of D156469. To a solution of compound D156156 (200 mg, 0.43 mmol) and bis-tributyl tin (280 mg, 0.48 mmol) in dioxane (5.0 mL) was added (Ph3P)2PdCl2 (140 mg, 0.02 mmol) at room temperature. The reaction mixture was stirred and degassed with Argon for 3 min, then heated at 90 ^{o}C for 42 h. The reaction mixture was cooled to room temperature, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using 5-15% methanol in ethyl acetate followed by prep TLC using 5% methanol in dichloromethane to provide D159469 (72 mg 27% yield) as an off white solid. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.5 (d, *J* = 7.2 Hz, 1H), 7.28-7.48 (m, 4H), 7.27 (br s, 2H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.6 (d, *J* = 8.0 Hz, 1H), 6.13 (br s, 2H), 4.53 (m, 1H), 4.05 (d, *J* = 6.4 Hz, 2H), 3.637 (m,1H), 3.08 (m, 1H), 2.85 (m, 1H), 2.23 (m, 1H),

1.89 (m, 1H), 1.75 (m, 1H), 1.42 (m, 6H), 1.32 (m, 2H), 1.27 (m, 6H), 1.06 (m, 6H), 0.83 (m, 9H). MS *m/z* (ESI) 667$(M+H)^+$. HPLC purity (Area Percent = 96.0%).

Preparation of ^{125}I-D156156. Added 30uL of 2% acetic acid in methanol to 21 uL (10.1 mCi, 5.05 nmol) of aqueous NaI125. Added 15uL (30ug, 45 nmol) of the stannane D156469 in methanol/DMF (1:1) followed by a 20uL solution of chloramine T (200 ug, 712 nmol) of 10ug/uL solution in methanol were added. The reaction mixture was incubated for 3 minutes at room temperature and then 20uL (200ug, 1.05 μmoles) of aqueous sodium metabisulfite was added. The reaction mixture was injected on to a preparative HPLC column (MacMod: ACE 300SB, C18, 5um, 300A, 4.6 mm x 250 mm) using 0.4 mL of initial HPLC eluent, 0.1% TFA in water. The samples were eluted using 20-70% eluent B over 50min, (eluent A 0.1% TFA in water, eluent B 1% TFA in ACN). Four 0.85mL were collected, added 0.2 mL water and sample was re-injected. Two fractions ~6mCi were collected. A sample of the product co-elutes with authentic cold standard, D156156. A solution made with 400 mg sucrose, 7.6 mL water and 0.4 mL ethanol was used as a medium for freeze drying. QC check of a sample analyzed on a Jupiter 7.5 cm C4 column, indicated < 0.1% free iodine and > 98.1% radiochemical purity. Specific radioactivity 2000 Ci/ mmol. Five 230uCi samples were shipped on cold pack for biological evaluations. Radioiodination was performed at GE Healthcare, Bio-Science Division, Woburn, MA.

Real-time PCR analysis of cellular SMN mRNA. D156844 and TSA (Trichostatin A, Sigma, T-8552) were formulated in DMSO and added to NSC34 cell culture for a final concentration of 500 nM/50 nM and 100 nM, respectively. Final concentration of DMSO was 0.5%. The cells were incubated for 18 hours and then harvested for RNA isolation using RNeasy Mini Kit (Qiagen) according to manufactures protocol. RNA quality and quantity was measured on an Agilent 2100 Bioanalyzer (Agilent Technologies). cDNA synthesis was performed using High Capacity cDNA Archive kit (Applied Biosystems), according to manufactures protocol. Gene expression analysis was performed using TaqMan Assays-on-Demand Gene Expression Products (Applied Biosystems) according to manufactures protocol (SMN assay # Mm00488315_m1). Each assay was run in triplicate and the data was normalized against an internal housekeeping gene, B-actin (ACTB assay # 4352933E). The relative difference in expression was calculated using the equation $2e(-\Delta C_T)$. Statistical comparisons between groups in the gene expression studies, was done by using Students t-test, an associated probability of <5% was considered significant.

ProtoArray® Human Protein Microarrays. All clones used to generate the human protein collection were fully sequenced and subcloned into the expression vector, pDEST™20 (Invitrogen). These clones were used to express proteins in Sf9 insect cells as N-terminal GST-fusions using the Bac-to-Bac® Baculovirus Expression System (Invitrogen). Insect cell lysates were loaded directly into 96-well plates containing glutathione resin (GE Healthcare). After washing, purified proteins were eluted under conditions designed to obtain native proteins, then stored at -80°C. Protein arrays were printed on 1x3 inch modified glass slides using a 48 pin contact arrayer (OmniGrid, Genomics Solutions) at 4°C. Protein arrays were stored at -20°C until use.

Small molecule target identification microarray assay. Protein microarrays were blocked for 1 hour in Tris buffer (50 mM Tris-HCl pH 7.5, 5 mM $MgSO_4$, 0.1% v/v Tween20) with low-speed orbital shaking. A solution of 100 nM ^{125}I-D156156 tracer with 1 nM ^{125}I-Streptavidin (GE Healthcare, Amersham) in 50 mM Tris-HCl pH 7.5, 5 mM $MgSO_4$, 0.1% v/v Tween20 was applied to the surface of the microarrays and allowed to incubate under a coverslip (Hyperslip^{TM}) at 4°C for 90 minutes. Following incubation, arrays were washed three times with 50 mM Tris-HCl pH 7.5, 5 mM $MgSO_4$, 0.1% v/v Tween20 buffer using 28 ml per wash. After washing, arrays were placed into a slide holder and spun for 2 minutes at 2000 rpm in a plate centrifuge. Dried arrays were exposed to a phosphorimager screen, images were acquired using a Perkin-Elmer Phosphorimager, data were extracted with microarray data acquisition software (Genepix, Molecular Devices), and analyzed using data analysis software (Prospector, Invitrogen).

Small molecule competition binding microarray assay. Protein microarrays were assayed as described above with probing solutions comprised of 100 nM ^{125}I-D156156 mixed with 10 nM ^{125}I-streptavidin for positional mapping, in the presence or absence of 10 μM unlabeled competitor compound. Unlabeled competitor molecules included D156156, D156115, D156844, staurosporine, and tertbutylquinone. All assays were carried out in duplicate.

Computational docking of D156844 into human DcpS. The 1.90Å structure of human DcpS in complex with m7GpppG (PDB ID:1ST0) was used for our ligand docking studies [15]. To prepare the DcpS protein structure for computational docking studies, the Tripos force field implemented in Sybyl7.2 (Tripos Inc., St. Louis, MO) was used to fix protein charges ("Kollman All"), and define hydrogens and their bond orientations [42,43]. The active site definition was made with a radius of 6.5 Å around the bound substrate m7GpppG in the closed conformation of the DcpS active site [15]. Six water molecules engaged in hydrogen bonds with active site residues (waters 5, 52, 58, 110, 231, and 307 in PDB ID IST0) were included in the definition of the active site to set up the docking protocols in the FlexX environment. The 2D structure of D156844 was converted into energy minimized 3D conformations using the CONCORD module implemented in Sybyl.7.2 [44]. A fragment-based flexible ligand docking approach was then carried out with the FlexX module (BioSolveIT GmbH, St. Augustin, Germany) using the prepared protein model and thirty different D156844 conformers with varying conformational energy and scores were obtained [45-49]. All of the lowest energy conformers revealed similar binding poses to DcpS. The qunazoline moeity of D156844 was found to engage in a strong hydrogen bond network with Glu185, Pro204, Asp205 and Leu206. In some of the low energetic conformers a water mediated interaction was observed with the ternary nitrogen of the saturated ring which is on the other side of the ligand.

Generation of labeled cap structure and in vitro decapping assays. RNA corresponding to the pcDNA3 (Invitrogen) polylinker (pcP) was transcribed in vitro by SP6 polymerase according to the manufacturer (Promega) from a PCR-generated template using T7 and SP6 promoter primers. Cap labeling of the RNA was carried out with the vaccinia virus capping enzyme in the presence of [α-^{32}P] GTP and S-adenosyl-

methionine (SAM) as previously described [57] to label the α-phosphate (relative to the terminal guanosine; m^7G*pppN-). Labeled cap structure was generated by treating the cap-labeled RNA with 1 unit nuclease P1 (Sigma) to degrade the RNA leaving the cap structure containing the pyrophosphate linkage, intact as previously described [18]. Decapping assays were carried out with 5 ng Flag-tagged recombinant DcpS protein or 20μg K562 cell extract as indicated and 20 nM unlabeld cap structure (purchased from New England Biolab) spiked with radioactive cap structure in decapping buffer (10 mM Tris-HCl pH 7.5, 100 mM KOAc, 2 mM $Mg(OAc)_2$, 2 mM DTT) for 30 seconds at room temperature. Decapping reactions were terminated with the addition of 1.7N formic acid. An aliquot of each reaction was spotted onto polyethylenimine (PEI) cellulose thin-layer chromatography (TLC) plates (Sigma) that were pre-run in water, air dried, and then developed with 0.45 M $(NH_4)_2SO_4$ at room temperature [20]. The TLC plates were air dried and exposed to PhosphorImager for quantification of radioactive spots, performed using the Molecular Dynamics Phosphorimager (Storm860) using ImageQuant-5 software.

DcpS gene design, protein expression, purification, crystallization and ligand-bound X-ray crystal structure determination. Starting with the reported crystal structures of human and mouse DcpS enzymes [15,16,21], we used the comparative sequence viewer of the synthetic gene design software, Gene Composer™ [50], to design a new N-terminally deleted form of human DcpS missing the first 37 amino acids (Δ37) which could not previously be visualized in reported DcpS structures [15,16,21]. The Gene Composer™ software was also used to design codon optimized gene for expression of the Δ37DcpS enzyme in *E. coli*. The gene design settings were based on the use of codon frequency table (**Supplementary Table 2**) for highly-expressed *E. coli* proteins with a codon threshold cutoff of 2.0% (codons used at a frequency of less than 2% are not included in the gene design). In addition, all cryptic Shine-Dalgarno translation starts were eliminated as well as any large RNA secondary structures through codon engineering (**Supplementary Data**). The Gene Composer designed synthetic gene for Δ37DcpS was purchased from DNA2.0 (San Diego, CA) and then subcloned into an arabinose inducible expression vector (pBAD-ara) as an N-terminal in-frame fusion to a His6-Smt3 protein (a ubiquitin-like Smt protein with an N-terminal hexa-histine tag) [51] The His6-Smt3-Δ37hDcpS fusion protein was expressed in *E. coli* TOP10 (Invitrogen) cells cultures at 37°C in 1 liter of 2xYT medium inoculated with 10 ml of an overnight culture. When the culture reached an OD_{600} of ~0.7, the arabinose inducer was added to a final concentration of 0.1% w/v and protein expression was allowed to occur for 3 hrs at 37°C. Cells were harvested by centrifugation at 4°C and the final cell pellet was frozen at -80oC until used for protein purification.

A 13.6 g pellet from a 1 liter E. coli culture was resuspended in lysis buffer (20 mM Tris-HCl pH 8.0, 100 mM L-arginine, 1% v/v NP-40, 0.1 mg Lysozyme / g of cell paste, and 20 Units Benzonase. Cells were lysed at 4°C via nitrogen cavitation following pressurization at 2300 psi for 20 minutes and rapid extrusion to atmospheric pressure. Cell lysate was clarified by centrifugation at 4.2K rpm 4°C for 45 min. The cell supernatant was the passed over a 5ml HisTrap (GE Healthcare) nickel charged immobilized metal chelate affinity column and eluted with a linear gradient of imidazole.

Protein samples were loaded to the column which had been pre-equilibrated in a run buffer of 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.5 mM TCEP, 100 mM Arginine, 50 mM imidazole. Proteins were eluted in the same run buffer with a linear gradient of imidazole up to 500 mM imidazole. The peak fractions containing SMT3- Δ37hDcpS were pooled and cleaved overnight at 4°C with 0.5 ug/ml final concentration of His6-Ulp1 protease [51]. The His6-Ulp1 digestion mix was diluted 2x with run buffer and passed back over a HisTrap nickel column. The purified Δ37hDcpS (N-terminal Ser followed by DcpS residues Ala38-Ser337) was collected in the flow through since the liberated His6-Smt3 and His6-Ulp1 remained bound to the column. The flow through Δ37hDcpS material was then concentrated to 7.3 mg/ml (measured by UV absorbance) and set up in crystallization trials with 1mM final concentration of C5-quinazoline compounds (D156844, D157493, and D153429) which were added to the protein solution as a 10 mM stock dissolved in water.

Sitting drop vapor diffusion crystallization trials were set up versus optimizations from published DcpS conditions [21], as well as the sparse matrix Wizard-III crystallization screen (Emerald BioSystems, Bainbridge Island, WA). Multiple crystals of Δ37hDcpS-DG156844 grew at room temperature from Wizard-III condition #4 containing 20% w/v PEG 3350, 200 mM NH_3-formate. Ligand bound DcpS co-crystals were harvested from crystallization drops using nylon cryoloops, and quickly dipped into a cryoprotectant solution containing 80% v/v mother liquor and 20% v/v ethylene glycol, before being flash frozen in liquid nitrogen. X-ray diffraction data for cryopreserved crystals were collected on a Saturn4 CCD mounted on a Rigaku 007-HF X-ray generator. One degree oscillations were collected for one minute each over 180 degrees. Data were processed and scaled with HKL2000 [52]. The X-ray structure was solved by molecular replacement with MOLREP [53] using PDB file 1STO [15]. The initial R-factor and R-free were 0.29 and 0.36 respectively. The model was further refined using REFMAC 5.0 [54]. Electron density was visually inspected for presence of bound ligands which were unambiguously placed into the model structure. The protein-ligand model was further rebuilt and refined in consecutive cycles using COOT and REFMAC 5.0 [54,55]. Final R-factor and R-free were 0.208 and 0.273 respectively to a resolution of 2.3 Å, see **Table 2**. Some figures were made with CCP4MG [56].

Amino Acid Sequence of Smt_Δ37hDcpS (R405):

MSHHHHHHSGEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQG
KEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGSAPVRLPFSGFRLQK
VLRESARDKIIFLHGKVNEASGDGDGEDAVVILEKTPFQVEQVAQLLTGSPELQL
QFSNDIYSTYHLFPPRQLNDVKTTVVYPATEKHLQKYLRQDLRLIRETGDDYRNI
TLPHLESQSLSIQWVYNILDKKAEADRIVFENPDPSDGFVLIPDLKWNQQQLDDL
YLIAICHRRGIRSLRDLTPEHLPLLRNILHQGQEAILQRYRMKGDHLRVYLHYLPS
YYHLHVHFTALGFEAPGSGVERAHLLAEVIENLECDPRHYQQRTLTFALRADDP
LLKLLQEAQQS

Nucleotide Sequence of Smt_Δ37hDcpS (smt Bold, Dcps non-bold)

ATGTCCCACCACCACCACCACCATAGCGGCGAAGTAAAACCGGAAGTGAAGCCGGAGAC
CCACATCAACCTGAAGGTTAGCGACGGTAGCAGCGAAATCTTCTTCAAGATTAAGAAGA
CCACCCCGCTGCGTCGCCTGATGGAAGCATTCGCTAAACGCCAGGGCAAGGAAATGGAT
TCCCTGCGCTTTCTGTACGACGGTATCCGTATTCAGGCAGACCAGACTCCGGAAGATCT
GGACATGGAAGATAACGACATTATCGAAGCACACCGTGAACAAATCGGTGGATCCGCGC
CGGTACGCCTGCCGTTCTCTGGCTTCCGTCTGCAGAAAGTTCTGCGCGAATCTGCGCGT
GATAAAATCATTTTCCTTCACGGCAAAGTCAACGAAGCGTCCGGTGACGGCGACGGCGA
AGATGCAGTTGTAATTCTGGAGAAAACTCCATTCCAGGTAGAACAGGTTGCTCAGCTTC
TGACTGGTTCCCCGGAACTGCAGCTGCAGTTCTCCAATGACATCTATTCCACCTACCAC
CTGTTTCCGCCGCGCCAGCTGAACGACGTGAAAACTACTGTTGTTTACCCGGCAACCGA
GAAGCATCTGCAGAAATACCTGCGTCAGGACCTGCGCCTGATCCGCGAAACTGGCGACG
ACTACCGTAACATCACACTGCCGCACCTGGAAAGCCAGAGCCTGTCCATCCAGTGGGTT
TATAACATCCTGGACAAGAAGGCAGAAGCTGACCGTATAGTTTTCGAAAACCCGGACCC
TTCCGACGGCTTCGTTCTGATCCCGGATCTGAAGTGGAACCAGCAACAGCTGGACGACC
TGTACCTGATCGCAATCTGCCATAGACGCGGCATCCGTAGCCTCCGCGACTTAACTCCG
GAACACCTGCCTCTGCTGCGTAACATCCTGCACCAAGGTCAAGAAGCGATCCTGCAGCG
TTACCGCATGAAAGGTGACCACCTGCGTGTGTACCTGCACTACCTGCCGTCTTACTACC
ACCTGCATGTTCACTTCACCGCTCTGGGTTTCGAAGCACCGGGTTCCGGCGTTGAACGT
GCACACCTGTTAGCTGAAGTTATCGAAAACCTGGAGTGTGACCCGCGCCACTACCAGCA
GCGTACTCTGACCTTCGCTCTGCGCGCAGATGACCCGCTGCTTAAACTGCTCCAGGAAG
CTCAGCAATCT