Supplementary Information

4-(3-chloro-5-(trifluoromethyl)pyridin-2-yl)-N-(4-methoxypyridin-2-yl)piperazine-1-carbothioamide (ML267), a potent inhibitor of bacterial phosphopantetheinyl transferase that attenuates secondary metabolism and thwarts bacterial growth

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Table of Contents

Supplemental Figure 1: **1** is a confirmed inhibitor of Sfp-PPTase **S2**
Supplemental Figure 2: Controls for Sfp reversibility experiments **S3**
Supplemental Figure 3: Activity of inactive control (**12**) **S4**
Supplemental Figure 4: Sfp, AcpS and human PPTase activity of **55** **S4**
Supplemental Figure 5: Cellular toxicity of screening hit **1** and **55** in HepG2 cells **S5**
Supplemental Figure 6: Survival data for mouse model of Staphylococcal septicemia. **S5**
Supplemental Table 1: minimum bactericidal activity (MBC) of **ML267** (**55**) **S6**
Supplemental Table 2: MIC values for **ML267** (**55**) against numerous MRSA strains **S6**
Supplemental Table 3: Summary of reactive metabolite ID with GSH for **55** and **41** **S7**
Supplemental Figure 7: aqueous stability study of **ML267** (**55**) **S8**
Supplemental methods (Biology) **S9-S13**
General methods (chemistry) and compound characterization **S14-S32**
References **S33**

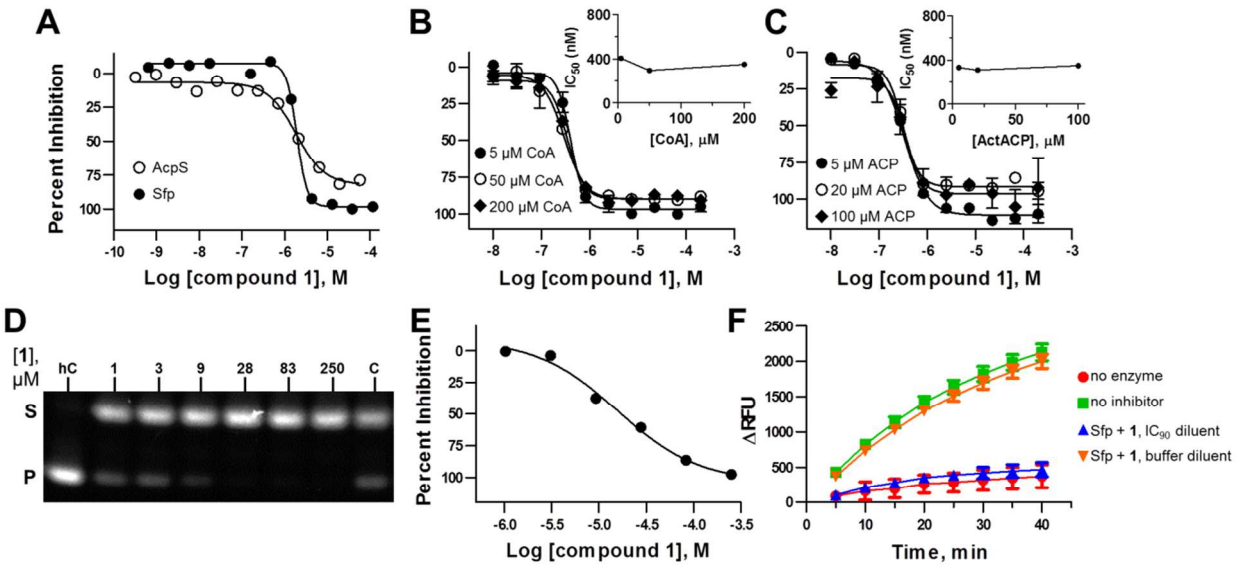


Figure S1. (1) is a confirmed inhibitor of Sfp-PPTase. A) Concentration response curves for the resynthesized sample of **1** in the primary screening assay with AcpS-PPTase (○) and Sfp-PPTase (●) enzymes, where IC_{50} values of 1.8 and 1.9 μM, were observed, respectively. B) Concentration response curves for **1** in assays with varying [CoA] and constant [ACP]. In these conditions, the IC_{50} value was unaffected (inset), indicative of noncompetitive behavior with respect to CoA. C) Concentration response curves for **1** in assays with varying [ACP] and constant [CoA]. In these conditions, the IC_{50} values were unaffected (inset), indicative of noncompetitive behavior with respect to ACP D) Gel image for label-free gel shift assays containing varying concentrations of **1**; hC = holo-CP control, C = no compound control, S = substrate, P = product. E) Densitometry data for the gel in E), which indicate that the compound inhibits Sfp-PPTase in the absence of exogenous labels and with a whole-protein substrate. F) Reaction time courses were collected after rapidly diluting Sfp-PPTase that was incubated with **1** at a concentration sufficient to inhibit the activity 90% (blue). The diluted sample rapidly recovered enzymatic activity (orange), indicating that **1** reversibly inhibits Sfp-PPTase.

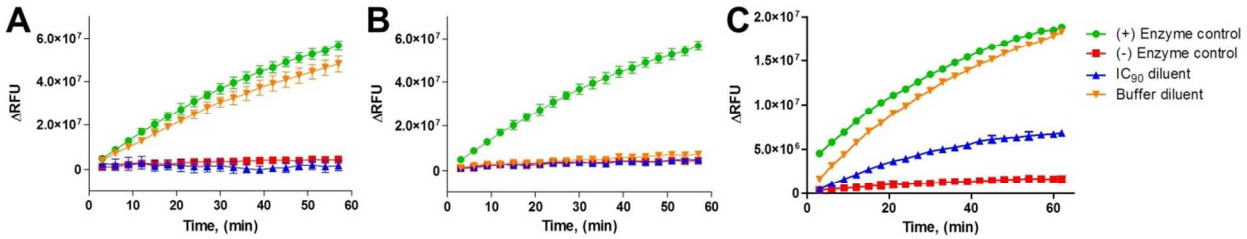


Figure S2. Controls for Sfp reversibility experiments. Reversibility was assessed in rescue-by-dilution experiment and data are shown above. After incubation of the enzyme with test compounds at a concentration exhibiting 90% inhibition (IC_{90}), the samples were diluted 100 fold in reaction buffer, resulting in the reduction of test compound concentration to level that should exhibit 10% inhibition (IC_{10}) for a reversible, stoichiometric inhibitor in rapid equilibrium. The resulting enzyme solutions were immediately assayed and time courses are shown above for A) 3'-phosphoadenosine-5'-monophosphate (PAP), a known reversible inhibitor; B) SCH-202676, a known covalent inhibitor; C) probe compound, **ML267** (**55**). For each plot, traces are shown for the enzyme vehicle-only control (●), no enzyme control (■), enzyme-inhibitor solutions diluted into IC_{90} diluent (▲), and buffer diluent (▼). Restoration of enzymatic activity in the buffer diluted sample (▼) is indicative of reversible behavior.

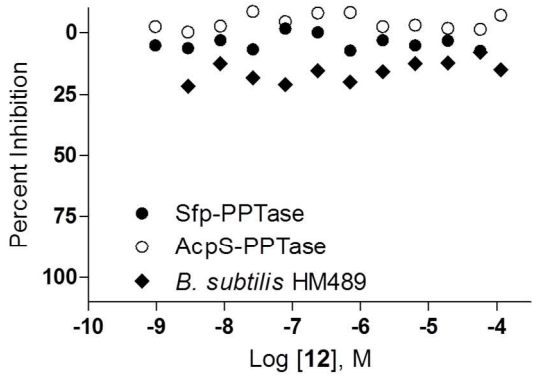


Figure S3. Activity of inactive control (12). Data are presented for the activity of **12** in biochemical assay with Sfp-PPTase (●), AcpS-PPTase (○), and microbial susceptibility testing with B. subtilis HM489 (♦).

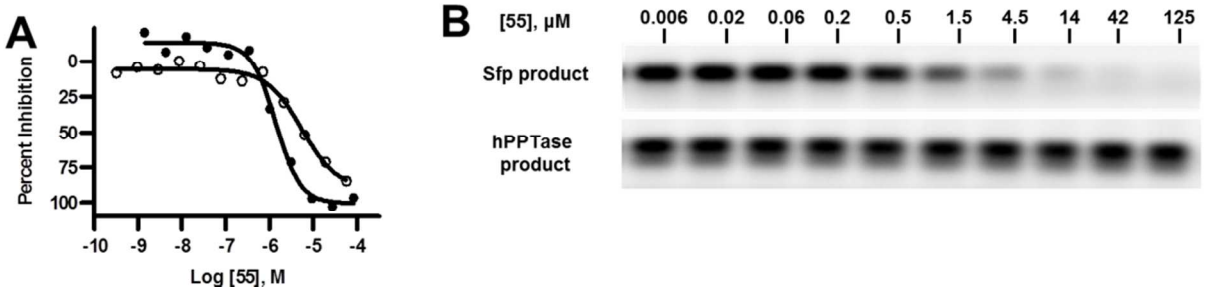


Figure S4. Sfp, AcpS and human PPTase activity of 55. A) Concentration response curves for **55** tested in the HTS assay with AcpS-PPTase (○) and Sfp-PPTase (●) enzymes, where IC_{50} values of 0.29 and 8.1 µM, were observed, respectively. B) Fluorescent images from gel-based assays for **55** testing with Sfp-PPTase and the human enzyme. The compound exhibited no inhibition of the human enzyme, demonstrating a high selectivity for the bacterial enzyme.

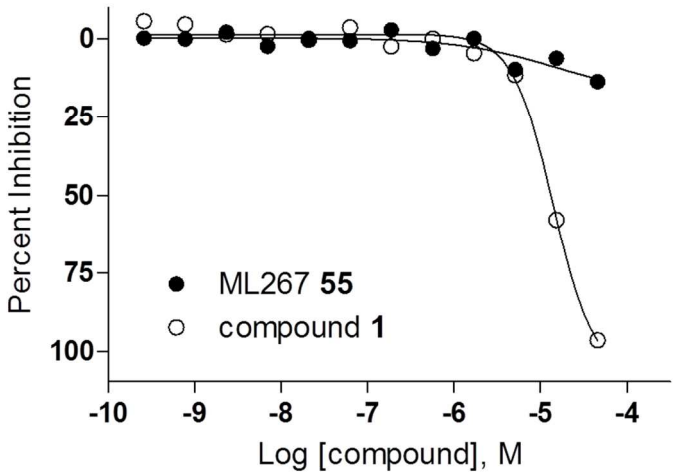


Figure S5. Human cellular toxicity assessment of compound 1 and 55 in HepG2 cells. Human cellular toxicity was assessed in HepG2 cells using the CellTiter-Glo assay format, and data are shown above for the primary screening hit **1** (○) and **55** (●).

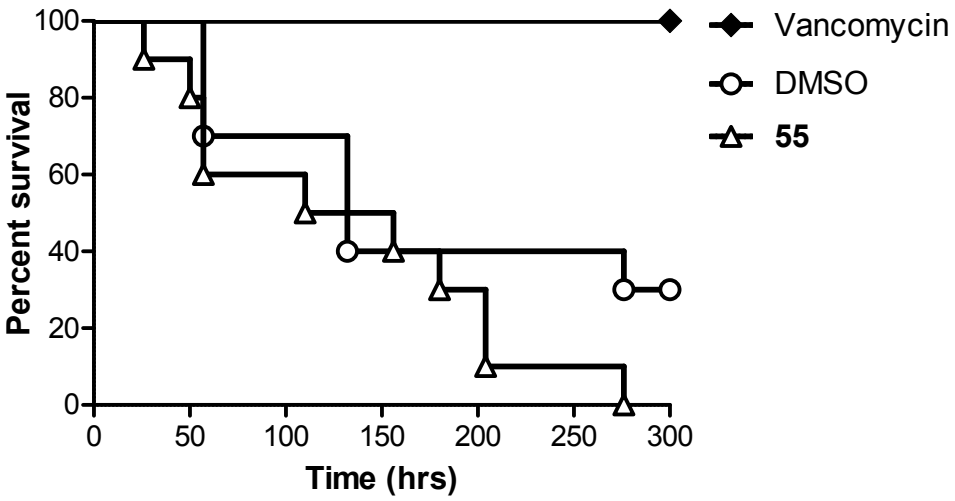


Figure S6. Survival data for mouse model of Staphylococcal septicemia. Survival data are presented for the cohorts that received treatment with **55** (Δ, 30 mpk), vancomycin (♦, 50 mpk) or vehicle (○).

Table S1. Minimum bactericidal activity of **ML267** (**55**).

Minimum Inhibitory Concentration (μg/mL)			
compound\Strain	B. subtilis HM489	S. aureus 6538	S. aureus BAA-1717
ML267	1.5 ± 0.29	3.1 ± 0.57	4.3 ± 0.81
Minimum Bactericidal Concentration (μg/mL)			
ML267	3.4	13.6	3.4

Table S2. MIC values for **ML267** (**55**) against a variety of clinical strains of MRSA.[a]

Strain	**MSSA BK4546**	**MRSA USA500-2273**	**MRSA USA300-19321**	**MRSA USA300-FPR3757**	**MRSA USA500-2104**	**MRSA USA500-2736**	**MRSA USA400-MW2**	**MRSA USA300-LAC**	**MRSA USA300-19321**	**MRSA USA300-18807**
Cmpd 55 MIC (μM)	3.3	4	2.5	1.2	1.6	1.5	1.8	2.1	2.5	1.9

[a] The experiments were conducted at the anti-infectives core at NYU.

Table S3. Summary of reactive metabolite identification with GSH for **55** and **41**. All experiments were conducted at Pharmaron Inc.[a]

Test Substance	GSH Adducts			
	Peak No.	**R.T. (min)**	**Expected (*m/z*)**	**Mass shift**
Cmpd. 55	n/a	n/a	n/a	n/a
Cmpd. 41	n/a	n/a	n/a	n/a
Nefazodone	N1	5.49	792.0	16
	N2	5.58	805.6	32

[a] A 50 μM solution of compound **55** or **41** were incubated with human liver microsomes (1 mg/mL) for 60 minutes at 37 ^{o}C in the presence of NADPH (1 mM) and glutathione (GSH) (5 mM). The reaction was quenched with 150 μL of TCA (10%), vortexed and centrifuged for 10 min at 10,000 g (4 ^{o}C) and purified via solid-phase extraction with an Oasis HLB SPE cartridge (3 cc, 60 mg). The methanol fraction was dried by N_2 at 35 ^{o}C and reconstituted with 100 μL of water-methanol (v/v, 5:95) mixture. The resulted solution was analyzed by LC-MS/MS. (Waters ACQUITY UPLC, API 4000 Qtrap). Conclusion: No GSH adducts were noted with either **55** or **41**.

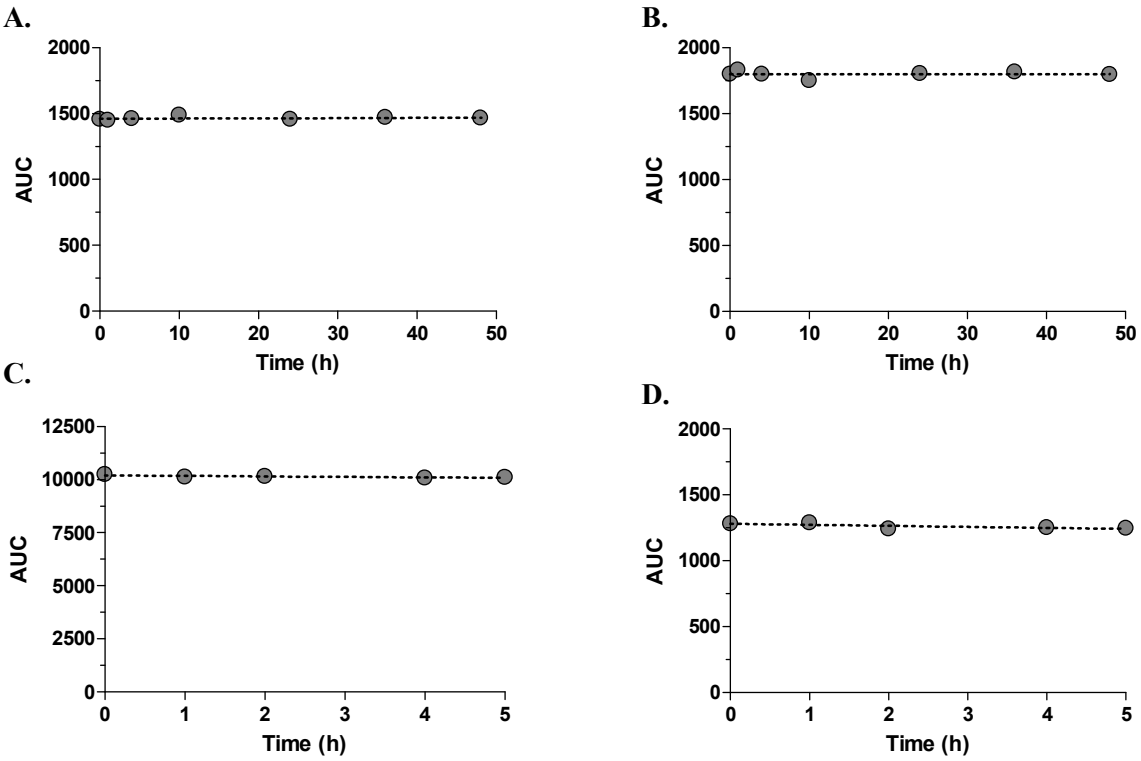


Figure S7. Stability of **55** in DPBS (pH 7.4) buffer (*Panel A*) and PPTase buffer at room temperature over 48 h (*Panel B*). Stability of **55** at pH 2 (*Panel C*) and pH 9 at room temperature over 5 h (*Panel D*).

.

SUPPLEMENTAL METHODS

Label-free gel assay for phosphopantetheinylation

Test compound (0.5 μL) dissolved in DMSO was added to 1.33X enzyme solution (15 μL) containing 66 nM Sfp, 66 mM HEPES•Na pH 7.6, 13.3 mM $MgCl_2$, 0.0133% NP40, and 1.33 mg/mL BSA. These solutions were incubated at room temperature for 10 minutes, at which point the phosphopantetheinylation reaction was initiated by the addition of 5X substrate solution (4 μL) containing 50 μM *apo*-ACP and 50 μM coenzyme A in 10 mM HEPES•Na pH 7.6. After incubation at room temperature for 30 minutes, quench/load solution (5 μL) containing 50 mM EDTA pH 8.0, 50% glycerol and 0.005% phenol red was added. The samples were electrophoretically separated on a discontinuous polyacrylamide gel using the Laemmli buffers sans SDS, with urea (2 M final) included in the resolving gel (15% total acrylamide/bisacrylamide concentration).

After separation, the gels were fixed (50% MeOH, 7% AcOH, 30 min), washed thrice with deionized water (200 mL, 5 min. per wash), and stained with Sypro Ruby® according to the manufacturer's recommendations. Imaging was accomplished with a Bio-Rad ChemiDoc™ XRS Gel Imager using standard ethidium bromide settings. Protein bands were quantified via densitometry using the ImageJ software package.

Mechanism of inhibition experiments: HPLC assay for phosphopantethienylation

Enzyme Reaction. Enzyme reactions were conducted in a 25 μL total assay volume in 384-well Greiner polypropylene plates. DMSO solution of test compound (0.5 μL) was added to a 1.25X solution of Sfp (20 μL, 37.5 nM) prepared in 1.25X HPLC assay buffer (62.5 mM MES-Na, 12.5 mM $MgCl_2$, 0.0125% NP40, pH 6.0). After a 10 minute incubation at room temperature, reaction was initiated by the addition of 5.5X substrate solution (containing variable concentrations of CoA and ActACP in 10 mM HEPES-Na, pH 7.5). Following a 30 minute incubation, reactions were quenched by the addition of 50 mM EDTA, pH 8.0 (25 μL), and the plate was heat sealed.

HPLC Separation. Portions of the quenched enzyme reactions (25 μL) were analyzed with an Agilent 1200 instrument fitted with a multiwell plate-compatible injector and diode array detector using a Jupiter C_4 column (part number 00B-416-60, 50 x 2 mm length containing 5 μm particles, 300 Å pores, Phenomonex, Torrance, CA, USA), using a constant flow rate of 0.4 mL/min. Mobile phases (Buffer A: H_2O with 0.1% v/v TFA; Buffer B: 95% acetonitrile with 0.1% v/v TFA) were prepared from HPLC grade solvents. The separation was accomplished as follows: after equilibration with 5% Buffer B, the samples were injected, and the mobile phase was ramped up to 50% Buffer B over 0.5 minutes, followed by an isocratic flow at this composition for 1.5 minutes. Separation of the *apo*- and *holo*- forms of ActACP was then achieved with a linear gradient from 50% to 70% Buffer B over 2 minutes. The mobile phase was then ramped to 95% Buffer B in 0.5 minutes, the column was washed by a 1 minute isocratic flow, and then adjusted to starting conditions with a 0.5 minute linear gradient to 5% Buffer B. Effective binding of the next sample required the inclusion of an end-run equilibration under this condition for 2 minutes. With this program, the two forms of the protein exhibited retention times of 4.7 and 5.3 minutes for the *holo*- and *apo*- forms of ActACP, respectively, with a typical

baseline resolution between the peaks of 0.3 minutes. The absorbance trace recorded at 210 nm was baseline corrected, integrated, and the peak areas for *holo*-ActACP were normalized to positive and negative controls.

Biochemical Reversibility Experiments

A DMSO solution of test compounds at a concentration 500 times their respective IC_{50} (1 μL) were added to a 1.66 μM solution of Sfp (49 μL, dissolved in assay buffer (66 mM HEPES-Na, 13 mM MgCl2, 0.13% BSA, 0.013% NP40) and equilibrated at room temperature for 30 minutes. This solution was then serial diluted 10-fold twice in assay buffer containing 2% DMSO. The resulting solution (37.5 μL, 100-fold total dilution) was added to individual wells of a 384-well plate. Assay reactions were immediately initiated by the addition of a 4-X substrate solution (12.5 μL, containing 50 μM FiTC-YbbR peptide and 100 μM Rhodamine CoA dissolved in 10 mM HEPES-Na, pH 7.5), and the reaction time course was monitored in an Envision multilabel plate reader using the standard fluorescein settings.

Microbial susceptibility testing.

Innoculum preparation. *Bacillus subtilis* strains were maintained on lysogeny broth (LB) solidified by the addition of 1.5% w/v agar. Single colonies were used to inoculate cation-adjusted mueller hinton II broth (2 mL) and shaken overnight at 30º C. In the morning, this culture (100 μL) was used to seed a fresh LB medium (10 mL) and was shaken at 30º C until the culture OD600 reached 0.5. This culture was diluted 1:100 in fresh cation-adjusted mueller hinton II broth to provide the inoculum below.

Bacterial susceptibility. Cation-adjusted mueller hinton II broth (2 μL) was dispensed into wells of a sterile white 1536-well plate. Test compounds (23 nL) prepared as serial dilutions in DMSO were added to the plate by pintool transfer. Innoculum (2 μL) was added; the plates were covered with a vented Kalypsys assay lid and incubated at 30 ºC. After 5 h, Bac-Titer Glo (4 μL; Promega Corp, Madison, WI) was added to the plates. They were incubated 10 minutes at room temperature, and then the luminescence was detected in a ViewLux multimodal plate reader.

HepG2 cytotoxicity counterscreen

Test compounds' toxicity was assessed by measuring cellular ATP content using a luciferase-coupled ATP quantitation assay (CellTiter-Glo; Promega, Madison, WI). In this assay, luminescent signal is proportional to amount of ATP, and thus to the number of metabolically competent cells. Briefly, HepG2 cells were dispensed at 2,000 cells/5 μL/well in tissue-culture treated 1,536-well white/solid bottom assay plates (Greiner Bio-One North America, Monroe, NC) using a Flying Reagent Dispenser (Aurora Discovery, Carlsbad, CA). Cells were incubated at 37 °C for 6 hr to allow for cell attachment, followed by addition of compounds via pin tool (Kalypsys, San Diego, CA). After compound addition, plates were incubated for 48 hr at 37 °C. At the end of the incubation period, 5 μL of CellTiter-Glo™ reagent was added, plates were incubated at room temperature in the dark for 30 min, and the luminescence intensity of each well was determined using a ViewLux plate reader (PerkinElmer, Shelton, CT). The positive

control was 92 μM and 41 μM of tetra-*N*-Octylammonium bromide, and the negative control was DMSO.

Membrane damage assessment assay

Assessments of membrane activity were performed in *B. subtilis* HM168 similarly to the protocol of Singh,[1] using the BacLight™ nucleic acid staining system (Invitrogen Corp, Carlsbad, CA, USA). The organism was maintained on LB medium solidified by the addition of bactoagar to 1.5% w/v, and all liquid culturing was performed in 250 mL baffled glass fernbach flasks containing 25 mL of LB medium. Bacterial cultures were grown from single colonies in LB medium at 30 ºC with shaking at 300 rpm. When the culture reached an OD of 0.5, the flask was chilled on wet ice for 10 min, and then the cells collected by centrifugation at 10,000 x g for 5 min. The cells were resuspended in sterile 0.85% NaCl (4 mL) and concentrated by centrifugation at 10,000 x g for 5 min. The cells were resuspended gently in 0.85% NaCl and adjusted to an OD value of 0.20. Cell suspension (50 μL) was added to 1.5 mL tubes containing a solution of test compounds dissolved in PBS (50 μL). The tubes were incubated at room temperature for 15 minutes, after which 11 μL of a 10 X BacLight™ solution (18 μL each of the Syto9 and propidium iodide solutions per mL deionized H_2O) was added to the bacterial suspensions, and the cells were allowed to stain 5 minutes at room temperature. The bacterial suspensions were then used to prepare slides and were imaged on a Nikon Eclipse Ti inverted microscope with a 40 X objective using standard FITC and TRITC optical settings for Syto9 and propidium iodide, respectively.

Assay for surfactin biosynthetic capacity of *B. subtilis*

Innoculum preparation. *Bacillus subtilis* OKB105 was streaked for single colonies on trypticase soy agar from a frozen stock of vegetative cells and grown for 12 h at 30 ºC. A single colony was used to inoculate a seed culture of 10 mL cation-adjusted Mueller Hinton Broth-II (CA-MHB-II) in a 250 mL Fernbach flask, and the culture shaken in an Innova 42R incubator shaker possessing a 1 inch orbit (30 ºC at 300 RPM). When an OD_{600} of ~1.0 was reached, the cells were collected by centrifugation (10 min at 4000 x g), resuspended in CA-MHB-II (10 mL) and again pelleted by centrifugation (4000 x g for 10 min). The cells were resuspended in 1.5 mL CA-MHB-II medium, adjusted to an OD_{600} of 5, and used immediately as an innoculum for the cultures below.

Bacterial culture. 250 mL Fernbach flasks containing CA-MHB-II medium (25 mL) were prepared and the medium supplemented with Antifoam A (12.5 μL, 1% v/v solution; final concentration 0.0005% v/v) and test compound to the stated concentration by the addition of 100 X DMSO solution (250 μL, final DMSO concentration 1%). The flasks were innoculated with 250 μL of bacterial cells prepared as above to give a starting OD_{600} = 0.05, and they were incubated with shaking (30 ºC, 300 RPM). Samples (1 mL) were collected every 2 hours, their OD_{600} determined, and processed to determine the concentration of surfactin (*vide infra*).

Sample processing. 800 μL of bacterial culture was transferred to a 1.5 mL eppendorf tube and the cells pelleted by centrifugation at 17000 x g for 5 min. 700 μL of the resulting supernatant was flash frozen by transfer to 96w- deep well polypropylene plates (Bio-Rad, Hercules, CA) maintained on crushed dry ice throughout the experiment. After sample collection was complete, the plates were stored at -25 ºC.

Plates were subsequently thawed in a 37 ºC water bath and 300 µL of sample transferred into a new deep-well plate. Sample recovery standards (300 µL) were included in remaining rows of the plate. These plates were covered with aluminum seals, frozen on dry ice, the seals pierced with a 96 well- pin array (V & P Scientific, San Diego, CA) the plates equilibrated to -80 ºC and subsequently lyophilized overnight. The resulting powders were resuspended in 300 µL surfactin recovery solution (80 % v/v acetonitrile + 1 % v/v acetic acid). The plates were floated in a sonicating water bath for 30 minutes, and 200 µL of the resulting suspension was transferred to wells of a 0.2 µm Acroprep® filter plate (product number 5045, Pall Corporation, Port Washington, NY) that had been prewashed with 200 µL of surfactin recovery solution. The filtrate recovery plates were sealed and stored at 4 ºC until analysis.

Determination of surfactin concentration. Surfactin concentration was determined with a quantitative LC-MS method using an Agilent 1290 Infinity UHPLC system fitted with 6140 quadrupole MS detector. Separations were performed on a 50 x 2.0 mm Jupiter C4 column (00B-416-60, Phenomenex Inc, Torrance, CA), and MS detector settings included a drying gas flow of 11 L/min at a temperature of 350 ºC, nebulizer pressure of 45 psig, and capacitor set to 3000v. The MS operated in positive ion mode and was set to spend 50% cycle time in single ion mode (SIM) focused on a M/Z of 1036.5; and the balance of cycle time scanning M/Z ranging from 300 to 1050. Mobile phases consisted of water + 0.05% v/v TFA (A) and acetonitrile + 0.025% v/v TFA (B). Following equilibration of the column under conditions of 50% B at a flow rate of 0.7 mL/min, 20 µL of sample was injected. The sample was then eluted with a 4 min linear gradient from these starting conditions to 90% B. This was followed by a 1 min linear gradient to 100% B, followed by a 0.2 min linear gradient back to 90% B. The solvent system was then stepped to 50% B to prepare the instrument for the next sample.

Data processing was conducted with Agilent Chemstations. The SIM trace centered on surfactin C (exact mass: 1035.68, [M+H]: 1036.68), the most abundant isomer, was analyzed for total ion count of the peak detected by the integrator between 1.5 and 3 minutes, and unknown sample concentrations were calculated from a standard curve prepared from a commercial sample of surfactin (S3523, Sigma Aldrich, St. Louis, MO).

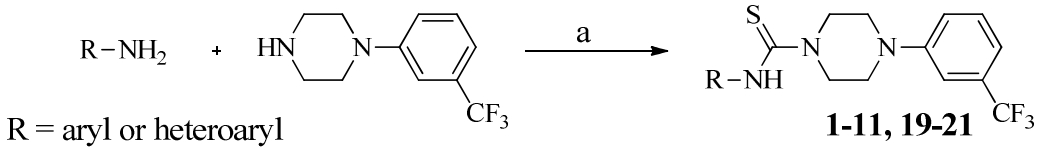
Mouse model of S. aureus septicemia.

This experiment was conducted by the Anti-infectives Screening Core, NYU School of Medicine according to protocols approved by the NYU School of Medicine Institutional Animal Care and use Committee. Female Swiss Webster mice (30 animals, 5 weeks old) were infected intravenously with 1 x 10^7 CFU of S. aureus BK2395 (PFGE USA500) . The mice were divided into three cohorts of 10 animals each that received treatments at 4, 24, and 48 hours post infection with Compound **55** (30 mpk), vancomycin (50 mpk) or vehicle. Animals were monitored three times daily for 14 days, and the time of death recorded, which was indicated in this experiment as the point at which significant morbidity occurred (weight loss, ruffled fur, paralysis and inability to acquire food or water.

Compound stability study protocol.

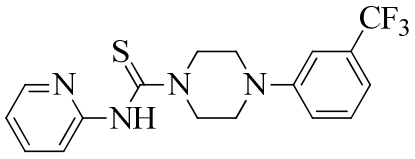
A 10 mM solution of **55** in DMSO (10 μL) was dissolved in appropriate buffer (190 μL) and injected (15 μL) to an analytical LC/MS (7 injections over 48 h for A and B and 5 injections over 5 h for C and D). The stability of the compound was monitored by plotting area under the curve (AUC) vs time

General Methods for Chemistry. All air or moisture sensitive reactions were performed under positive pressure of nitrogen with oven-dried glassware. Anhydrous solvents such as dichloromethane, *N,N*-dimethylforamide (DMF), acetonitrile, methanol and triethylamine were purchased from Sigma-Aldrich. Preparative purification was performed on a Waters semi-preparative HPLC system. The column used was a Phenomenex Luna C18 (5 micron, 30 x 75 mm) at a flow rate of 45 mL/min. The mobile phase consisted of acetonitrile and water (each containing 0.1% trifluoroacetic acid). A gradient of 10% to 50% acetonitrile over 8 minutes was used during the purification. Fraction collection was triggered by UV detection (220 nm). Analytical analysis was performed on an Agilent LC/MS (Agilent Technologies, Santa Clara, CA). Method 1: A 7 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with an 8 minute run time at a flow rate of 1 mL/min. A Phenomenex Luna C18 column (3 micron, 3 x 75 mm) was used at a temperature of 50° C. Method 2: A 3 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with a 4.5 minute run time at a flow rate of 1 mL/min. A Phenomenex Gemini Phenyl column (3 micron, 3 x 100 mm) was used at a temperature of 50° C. Purity determination was performed using an Agilent Diode Array Detector for both Method 1 and Method 2. Mass determination was performed using an Agilent 6130 mass spectrometer with electrospray ionization in the positive mode. ^{1}H NMR spectra were recorded on Varian 400 MHz spectrometers. Chemical shifts are reported in ppm with undeuterated solvent (DMSO-d_6 at 2.49 ppm) as internal standard for DMSO-d_6 solutions. All of the analogs tested in the biological assays have purity greater than 95%, based on both analytical methods**.** *High resolution mass* spectrometry was recorded on Agilent 6210 Time-of-Flight LC/MS system. Confirmation of molecular formula was accomplished using electrospray ionization in the positive mode with the Agilent Masshunter software (version B.02).



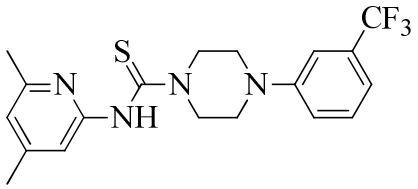
R = aryl or heteroaryl **1-11, 19-21**

<u>General Procedure A</u>. Preparation of compounds 1-11, 19-21: A mixture of the substituted amine (0.124 g, 1.0 mmol) and 1,1'-thiocarbonyldiimidazole (0.187 g, 1.05 mmol) in dichloromethane (2 mL) was stirred for 15 min at room temperature. 1-(3-chloro-5-(trifluoromethyl)pyridin-2-yl)piperazine (0.292 g, 1.1 mmol) was added to the clear yellow solution, and the reaction mixture was stirred at 40 ^{o}C for 1 h. The solvent was evaporated and the crude product was taken up in 2 mL DMSO and purified via reverse phase chromatography to afford compounds **1-11, 19-21** as TFA salts.

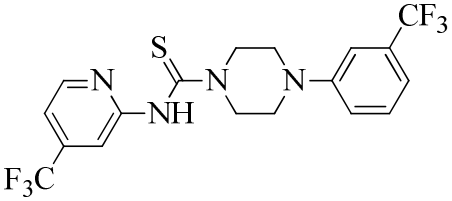


***N*-(pyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (2).** LC-MS Retention Time: (Method 1, 7 min) = 4.989 min and (Method 2, 3 min) = 3.237 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.91 (brs, 1H), 8.29 (dd, *J* = 4.5, 2.1 Hz, 1H), 7.98 –7.35 (m,

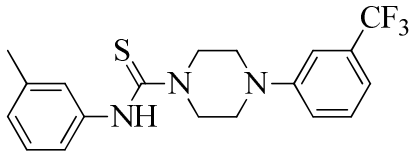
3H), 7.32 – 6.80 (m, 4H), 4.06 (dd, *J* = 6.7, 3.8 Hz, 4H), 3.41 – 3.33 (m, 4H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{17}H_{18}F_3N_4S$, 367.1199; found 367.1205.



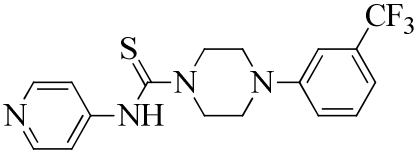
***N*-(4,6-dimethylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (3).** LC-MS Retention Time: (Method 1, 7 min) = 5.099 min and (Method 2, 3 min) = 3.31 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (t, *J* = 8.0 Hz, 1H), 7.25 – 7.14 (m, 3H), 7.06 (d, *J* = 7.6 Hz, 1H), 6.91 (s, 1H), 4.02 (t, *J* = 4.9 Hz, 4H), 2.50 – 2.43 (m, 4H), 2.39 (s, 3H), 2.28 (s, 3H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{19}H_{22}F_3N_4S$, 395.1512; found 395.1518.



4-(3-(Trifluoromethyl)phenyl)-*N*-(4-(trifluoromethyl)pyridin-2-yl)piperazine-1-carbothioamide TFA (4). LC-MS Retention Time: (Method 1, 7 min) = 6.698 min and (Method 2, 3 min) = 3.939 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (brs, 1H), 8.55 (d, *J* = 5.2 Hz, 1H), 8.02 (d, *J* = 1.8 Hz, 1H), 7.52 – 7.33 (m, 2H), 7.28 – 7.14 (m, 2H), 7.09 (d, *J* = 7.9 Hz, 1H), 4.07 (t, *J* = 5.1 Hz, 4H), 3.40 (dd, *J* = 6.3, 4.1 Hz, 4H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{19}H_{21}F_3N_3S$, 435.1073; found 435.1076.

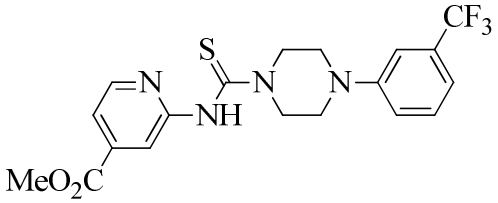


***N*-(*m*-tolyl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (5).** LC-MS Retention Time: (Method 1, 7 min) = 6.523 min and (Method 2, 3 min) = 3.84 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.34 (s, 1H), 7.44 (td, *J* = 8.0, 1.0 Hz, 1H), 7.28 – 7.05 (m, 6H), 6.97 – 6.89 (m, 1H), 4.08 – 4.01 (m, 4H), 3.41 – 3.31 (m, 4H), 2.28 (s, 3H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{19}H_{21}F_3N_3S$, 380.1403; found 380.1401.

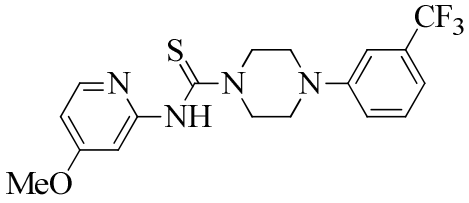


***N*-(pyridin-4-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (6).** LC-MS Retention Time: (Method 1, 7 min) = 4.737 min and (Method 2, 3 min) = 2.981 min; ^{1}H

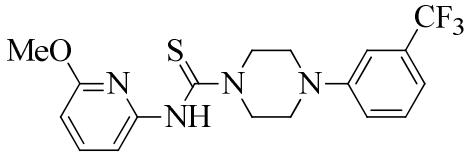
NMR (400 MHz, DMSO-d_6): δ 8.61 – 8.51 (m, 2H), 7.83 – 7.73 (m, 2H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.28 – 7.08 (m, 3H), 4.11 (t, *J* = 5.0 Hz, 4H), 3.44 (t, *J* = 5.3 Hz, 4H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{17}H_{18}F_3N_4S$, 367.1199; found 367.1198.



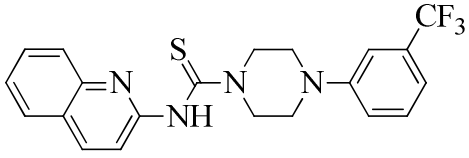
Methyl 2-(4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamido)isonicotinate TFA (7). LC-MS Retention Time: (Method 1, 7 min) = 6.108 min and (Method 2, 3 min) = 3.618 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.20 (s, 1H), 8.48 (d, *J* = 5.1 Hz, 1H), 8.17 (s, 1H), 7.53 – 7.37 (m, 2H), 7.28 – 7.17 (m, 2H), 7.12 – 7.03 (m, 1H), 4.13 – 4.03 (m, 4H), 3.89 (s, 3H), 3.39 (t, *J* = 5.3 Hz, 4H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{19}H_{20}F_3N_4O_2S$, 425.1254; found 425.1246.



***N*-(4-methoxypyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (8).** LC-MS Retention Time: (Method 1, 7 min) = 4.961 min and (Method 2, 3 min) = 3.201 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, *J* = 6.4 Hz, 1H), 7.52 – 7.40 (m, 1H), 7.28 – 7.05 (m, 4H), 6.85 (s, 1H), 4.08 (t, *J* = 5.2 Hz, 4H), 3.89 (s, 3H), 3.39 (t, *J* = 5.3 Hz, 4H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{18}H_{20}F_3N_4OS$, 397.1304; found 397.1309.

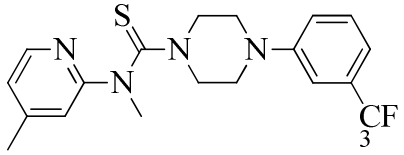


***N*-(6-methoxypyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (9).** LC-MS Retention Time: (Method 1, 7 min) = 5.419 min and (Method 2, 3 min) = 3.383 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.75 (s, 1H), 8.04 (dd, *J* = 3.1, 0.7 Hz, 1H), 7.57 – 7.35 (m, 2H), 7.29 – 7.14 (m, 3H), 7.12 – 7.05 (m, 1H), 4.11 – 4.02 (m, 4H), 3.82 (s, 3H), 3.38 – 3.38 (m, 4H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{18}H_{20}F_3N_4OS$, 397.1304; found 397.1304.

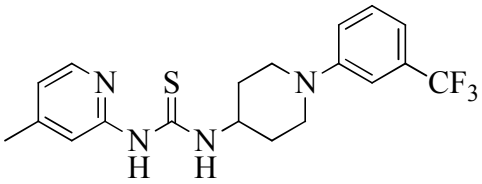


***N*-(quinolin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (10).** LC-MS Retention Time: (Method 1, 7 min) = 6.065 min and (Method 2, 3 min) = 3.584 min; ^{1}H

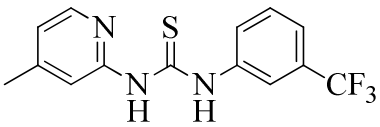
NMR (400 MHz, DMSO-d_6): δ 8.58 (d, *J* = 8.2 Hz, 1H), 7.90 – 7.75 (m, 2H), 7.74 – 7.59 (m, 2H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.29 – 7.22 (m, 1H), 7.21 (s, 1H), 7.11 – 7.00 (m, 2H), 4.41 – 4.26 (m, 4H), 3.41 - 3.36 (m, 4H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{21}H_{20}F_3N_4S$, 417.1355; found 417.1351.



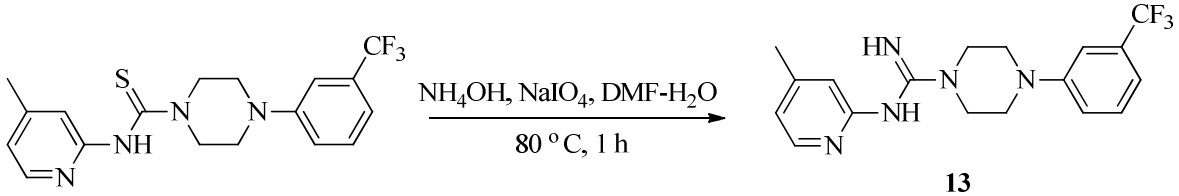
***N*-methyl-N-(4-methylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (19).** LC-MS Retention Time: (Method 1, 7 min) = 5.488 min and (Method 2, 3 min) = 3.305 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, *J* = 5.0 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.23 – 7.04 (m, 3H), 6.87 (d, *J* = 4.9 Hz, 1H), 6.76 (s, 1H), 3.89 – 3.80 (m, 4H), 3.40 (s, 3H), 3.34 – 3.27 (m, 4H), 2.29 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{22}F_3N_4S$, 395.1512; found 395.1522.



1-(4-Methylpyridin-2-yl)-3-(1-(3-(trifluoromethyl)phenyl)piperidin-4-yl)thiourea TFA (20). LC-MS Retention Time: (Method 1, 7 min) = 6.492 min and (Method 2, 3 min) = 3.907 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.97 (d, *J* = 7.6 Hz, 1H), 10.50 (s, 1H), 8.08 (d, *J* = 5.3 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.31 – 7.18 (m, 2H), 7.07 (dd, *J* = 7.6, 1.4 Hz, 1H), 6.99 (d, *J* = 1.4 Hz, 1H), 6.92 – 6.85 (m, 1H), 4.41 (ddq, *J* = 13.4, 9.1, 4.0 Hz, 1H), 3.66 (dt, *J* = 13.1, 4.3 Hz, 2H), 3.09 (ddd, *J* = 13.0, 10.0, 3.0 Hz, 2H), 2.28 (s, 3H), 2.13 (dq, *J* = 13.0, 3.7 Hz, 2H), 1.67 (dtd, J = 13.2, 9.7, 3.7 Hz, 2H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{22}F_3N_4S$, 395.1512; found 395.1510.

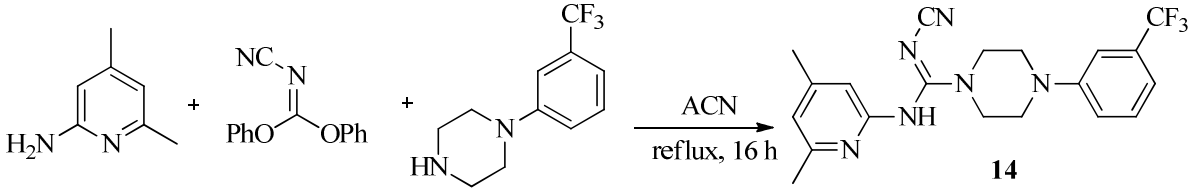


1-(4-Methylpyridin-2-yl)-3-(3-(trifluoromethyl)phenyl)thiourea TFA (21). LC-MS Retention Time: (Method 1, 7 min) = 6.343 min and (Method 2, 3 min) = 3.708 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 10.25 (s, 1H), 8.23 (d, *J* = 5.3 Hz, 1H), 7.80 – 7.73 (m, 1H), 7.69 – 7.47 (m, 3H), 7.10 (s, 1H), 7.04 – 6.97 (m, 1H), 2.33 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{14}H_{13}F_3N_3S$, 312.0777; found 312.0789.

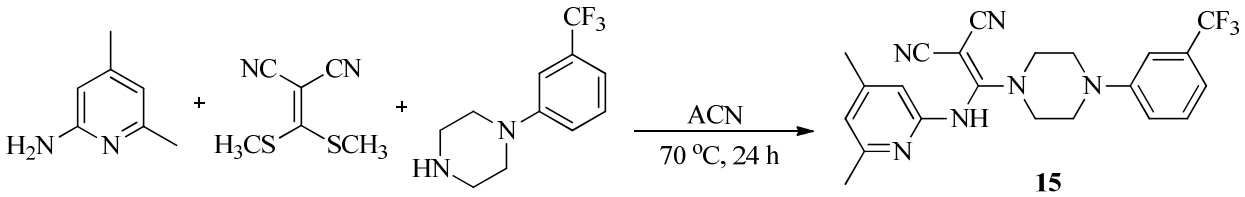


13

Synthesis of *N*-(4-methylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carboximidamide TFA (13) (adopted from a literature method[3]). A mixture of *N*-(4-methylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide (0.075 g, 0.197 mmol) and ammonium hydroxide (0.38 mL, 2.96 mmol, 15 eq) in DMF (1 mL) was added sodium periodate (0.046 g, 0.217 mmol, 1.1 eq) in water (0.5 mL). The reaction mixture was stirred at 80 °C for 1 h. After completion of the reaction, the crude product was purified via reversed phase chromatography to give N-(4-methylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carboximidamide as TFA salt. LC-MS Retention Time: (Method 1, 7 min) = 4.854 min and (Method 2, 3 min) = 2.324 min; HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{18}H_{21}F_3N_5$, 366.1800; found 366.1811.

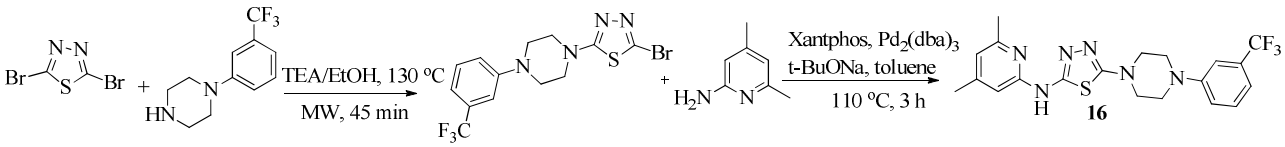


Synthesis of *N*'-cyano-*N*-(4,6-dimethylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)-piperazine-1-carboximidamide TFA (14) (adopted from a reported protocol[4]). A mixture of 4,6-dimethylpyridin-2-amine (0.2 g, 1.637 mmol, 1eq) and diphenyl cyanocarbonimidate (0.390 g, 1.637 mmol, 1 eq) in acetonitrile (5 mL) was refluxed for 4 h. 1-(3-(Trifluoromethyl)phenyl)piperazine (0.62 mL, 3.27 mmol, 2 eq) was then added and heated to reflux for 12 h. The crude solid obtained after evaporating off acetonitrile was taken up in DMSO and purified via reversed phase chromatography to give N'-cyano-N-(4,6-dimethylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carboximid-amide as TFA salt. LC-MS Retention Time: (Method 1, 7 min) = 4.922 min and (Method 2, 3 min) = 2.992 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.44 (t, J = 8.0 Hz, 1H), 7.29 – 7.20 (m, 3H), 7.13 – 7.07 (m, 1H), 6.98 – 6.93 (m, 1H), 3.99 – 3.92 (m, 4H), 3.56 – 3.46 (m, 4H), 2.70 (s, 3H), 2.33 (s, 3H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{20}H_{22}F_3N_6$, 403.1853; found 403.1847.



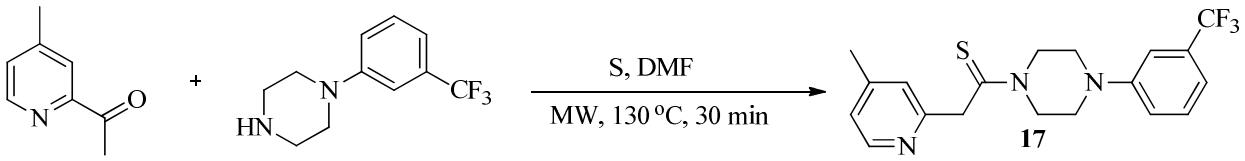
Synthesis of 2-(((4,6-dimethylpyridin-2-yl)amino)(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)methyl-ene)malononitrile TFA (15) (adopted from a reported method[5]). A mixture of 1-(3-(trifluoromethyl)phenyl)piperazine (0.46 mL, 2.45 mmol, 1.5 eq) and 2-(bis(methylthio)methylene)malononitrile (0.279 g, 1.64 mmol, 1 eq) in acetonitrile (5 mL) was stirred at 70 °C for 1 h. To the clear solution was added 4,6-dimethylpyridin-2-amine (0.2 g, 1.64 mmol, 1 eq) and refluxed for 24 h. The crude solid obtained after evaporating off acetonitrile was taken up in DMSO and purified via reversed phase chromatography to give 2-(((4,6-dimethylpyridin-2-yl)amino)(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)methyl-ene)malononitrile as TFA salt. LC-MS Retention Time: (Method 1, 7 min) = 4.995 min and (Method 2, 3 min) = 2.994 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (brs, 2H), 7.50 – 7.40 (m, 1H), 7.28 – 7.17 (m, 3H), 7.15 – 7.00 (m, 2H), 4.14 – 4.05 (m, 4H), 3.47 (t, J = 5.3 Hz, 4H),

2.66 (s, 3H), 2.38 (s, 3H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{22}H_{22}F_3N_6$, 427.1853; found 427.1857.

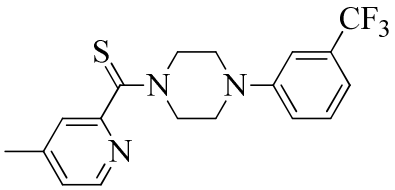


Synthesis of *N*-(4,6-dimethylpyridin-2-yl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1,3,4-thiadiazol-2-amine TFA (16). A mixture of 2,5-dibromo-1,3,4-thiadiazole (0.900 g, 3.69 mmol, 1 eq), 1-(3-(trifluoromethyl)phenyl)piperazine (0.73 mL, 3.87 mmol, 1.05 eq) and NEt_3 (1.03 mL, 7.38 mmol, 2 eq) in ethanol (8 mL) was heated under microwave at 130 °C for 45 min. The crude product obtained after concentration was purified on a biotage flash system eluting with 20 % ethyl acetate in hexanes to get 2-bromo-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1,3,4-thiadiazole (Yield 1.4 g, 96 %).

A mixture of 2-bromo-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1,3,4-thiadiazole (0.2 g, 0.509 mmol, 1eq), 4,6-dimethylpyridin-2-amine (0.093 g, 0.763 mmol, 1.5 eq), sodium *t*-butoxide (0.098 g, 1.02 mmol, 2 eq) and xantphos (0.029 g, 0.051 mmol, 10 mol %) in toluene (3 mL) was degassed with argon for 5 minutes. $Pd_2(dba)_3$ (0.047 g, 0.051 mmol, 10 mol%) was then added and stirred at 110 °C for 3 h. The solvent was evaporated by blowing air and the crude product was dissolved in DMF then stirred with palladium scavenger for 30 min. The solution was filtered through a thiol cartridge and finally purified on a preparative HPLC to furnish pure *N*-(4,6-dimethylpyridin-2-yl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-1,3,4-thiadiazol-2-amine as a TFA salt. . LC-MS Retention Time: (Method 1, 7 min) = 5.492 min and (Method 2, 3 min) = 2.986 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.33 – 7.21 (m, 2H), 7.16 – 7.07 (m, 1H), 6.64 – 6.56 (m, 2H), 3.54 – 3.51 (m, 4H), 3.45 – 3.36 (m, 4H), 2.39 (s, 3H), 2.22 (s, 3H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{20}H_{22}F_3N_6S$, 435.1573; found 435.1590.

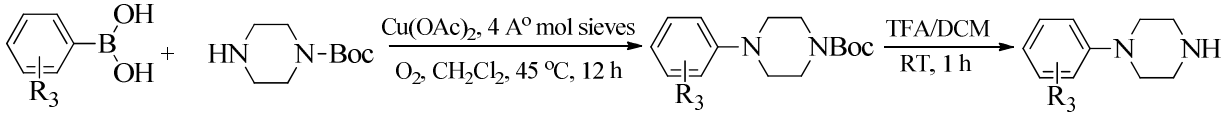


Synthesis of 2-(4-methylpyridin-2-yl)-1-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethanethione TFA (17) (<u>adopted from a reported method[6]</u>). A mixture of 1-(4-methylpyridin-2-yl)ethanone (0.2 g, 1.480 mmol, 1eq), 1-(3-(trifluoromethyl)phenyl)piperazine (0.417 mL, 2.220 mmol, 1.5 eq) and sulfur (0.059 g, 1.850 mmol, 1.25 eq) in DMF (4 mL) was heated under microwave irradiation at 130 °C for 30 minutes. The reaction mixture was filtered through a syringe filter and purified on a preparative HPLC to furnish 2-(4-methylpyridin-2-yl)-1-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethanethione as a TFA salt. LC-MS Retention Time: (Method 1, 7 min) = 4.719 min and (Method 2, 3 min) = 2.945 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (t, J = 5.2 Hz, 1H), 7.49 – 7.40 (m, 3H), 7.29 – 6.95 (m, 3H), 4.49 (s, 2H), 4.36 – 4.34 (m, 4H), 4.08 – 4.12 (m, 4H), 2.42 (s, 3H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{19}H_{21}F_3N_3S$, 380.1403; found 380.1407.



(4-Methylpyridin-2-yl)(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)methanethione TFA (18). This compound was prepared following the above same procedure used for compound **17** starting from 4-methylpicolinaldehyde (0.20 g, 1.651 mmol, 1 eq), 1-(3-(trifluoromethyl)phenyl)piperazine (0.465 mL, 2.48 mmol, 1.5 eq) and sulfur (0.066 g, 2.06 mmol, 1.25 eq) in DMF solvent. LC-MS Retention Time: (Method 1, 7 min) = 5.831 min and (Method 2, 3 min) = 2.635 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (t, J = 5.0 Hz, 1H), 7.47 – 7.34 (m, 2H), 7.26 – 7.05 (m, 4H), 4.44 – 4.36 (m, 2H), 3.65 – 3.50 (m, 4H), 3.33 – 3.25 (m, 2H), 2.35 (d, *J* = 4.2 Hz, 3H); HRMS (ESI) *m/z*; $(M+H)^+$ calcd. for $C_{18}H_{19}F_3N_3S$, 366.1246; found 366.1255.

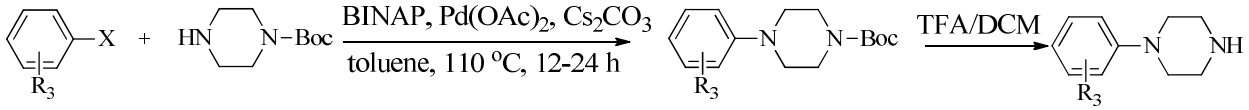
General procedures for the preparation of aryl piperazines.



R_3 = 3-OCF_3, 3-SO_2Me, 3-OMe-5-CF_3, 3-CF_3-4-Cl,

General Procedure (modified from a reported method[7]): A mixture of arylboronic acid (3.2 mmol, 2 eq), copper (II) acetate (0.029 g, 0.16 mmol, 10 mol %) and 4 Å molecular sieves (0.2 g) in dichloromethane (4 mL) was stirred for 5 minutes at room temperature. *t*-Butyl piperazine-1-carboxylate (0.3 g, 1.61 mmol, 1 eq) was then added, followed by bubbling with oxygen for 5 minutes. The vial was then sealed and filled with oxygen. The mixture was stirred overnight at 45 ^{o}C. After completion of the reaction, the reaction mixture was filtered through a metal scavenger cartridge (2 times to remove the copper completely). The crude products were then deprotected with TFA/dichloromethane as described in step D, and the products were either purified by HPLC or used directly in the next step.

General Procedure for Boc Deprotection (Step D): *Tert*-butyl 4-arlylpiperazine-1-carboxylate (1 mmol, 1 eq) in dichloromethane (5 mL) was added trifluoroacetic acid (2 mL) and stirred at room temperature for 1 h. The reaction mixture was concentrated and dried under high vacuum. The product was used as such in the next step.

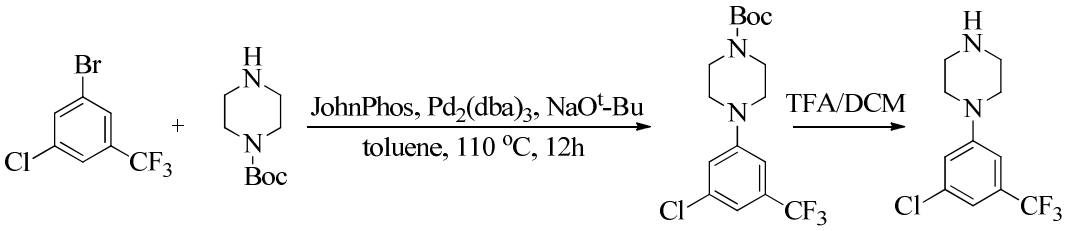


X = Br or I

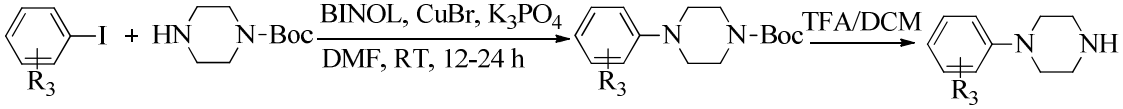
R_3 = 3,5-CF_3, 2-CF_3-4-Cl,

General Procedure (procedure adopted from a reported method[8]): A mixture of aryl bromide/aryl iodide (1 mmol, 1 eq), t-butyl piperazine-1-carboxylate (1.3 mmol, 1.3 eq), Cs_2CO_3

(1.5 mmol, 1.5 eq), BINAP (10 mol%) and $Pd(OAc)_2$ (5 mol%) in toluene (3 mL) was bubbled with argon for 5 minutes. The vial was capped and stirred at 110 ^oC for 12 – 24 h. After completion of the reaction, the solvent was evaporated. The crude solid was dissolved in methanol/methylene chloride and stirred with a metal scavenger and filtered through celite. The crude products were purified on biotage® flash chromatography or reversed-phase HPLC and then deprotected with TFA as described in step D.

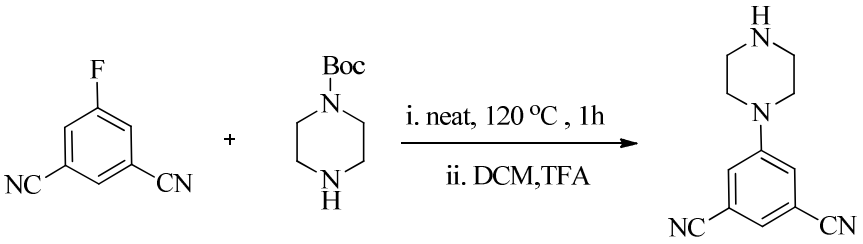


General Procedure (procedure adopted from a reported method[9]): A mixture of aryl bromide (1 mmol, 1 eq), t-butyl piperazine-1-carboxylate (1.3 mmol, 1.3 eq), Na-O^{t}Bu (1.5 mmol, 1.5 eq), JohnPhos (10 mol%) and $Pd_{2(}dba)_3$ (5 mol%) in toluene (3 mL) was bubbled with argon for 5 minutes. The vial was capped and stirred at 110 ^oC for 12 h. After completion of the reaction, the solvent was evaporated. The crude solid was dissolved in methanol/methylene chloride and stirred with a metal scavenger and filtered through celite. The crude product was purified on biotage® flash chromatography eluting with 25 % ethyl acetate in hexanes. The product was then deprotected with TFA using step D.



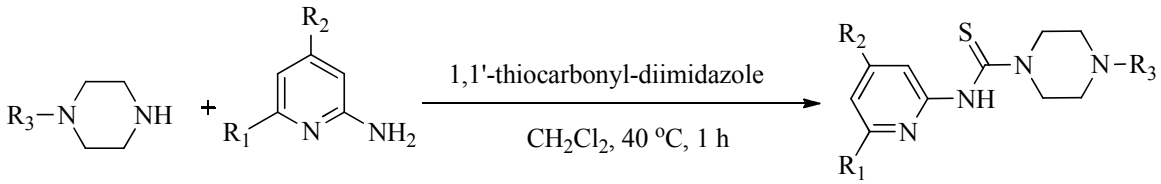
R_3 = 2-Cl-4-CF_3, 3,4,5-Cl, 2,3,4-Cl.

General Procedure (procedure adopted from a reported method[10]): A mixture of aryl iodide (1 mmol, 1 eq), tert-butyl piperazine-1-carboxylate (1.5 mmol, 1.5 eq), potassium phosphate (2 mmol, 2 eq), 1,1'-binaphthyl-2,2'-diol (0.2 mmol, 20 mol%) and copper(I)bromide (0.2 mmol, 20 mol%) in DMF (4 mL) was stirred at room temperature under nitrogen atmosphere for 12 h. The product was extracted with dichloromethane. The organic layer was successively washed with water, 1% HCl and brine, and dried with sodium sulfate. The crude product was purified on a biotage flash system eluting with 10 % ethyl acetate in hexanes. The product was deprotected with TFA as described in step D.

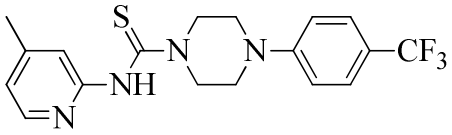


Synthesis of 5-(piperazin-1-yl)isophthalonitrile: A mixture of 5-fluoroisophthalonitrile (0.5 g, 3.42 mmol, 1eq) and tert-butyl piperazine-1-carboxylate (1.275 g, 6.84 mmol, 2 eq) heated neat

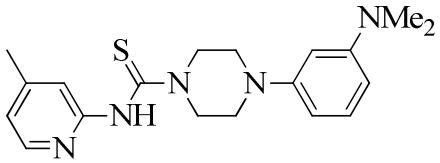
at 120 ^{o}C for 1 h. The crude product was dissolved in CH_2Cl_2 and treated with TFA as described in step D. The crude product obtained after evaporation of the solvent was purified in HPLC.



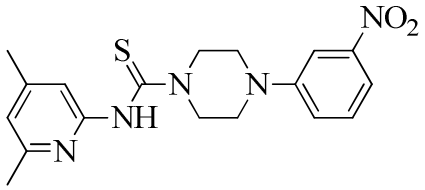
Synthesis of compounds 22 -56: These compounds are prepared from various aryl piperazines and 2-amino-4,6-substituted pyridines following the **General procedure A**.



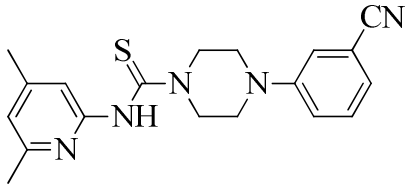
***N*-(4-methylpyridin-2-yl)-4-(4-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (23).** LC-MS Retention Time: (Method 1, 7 min) = 4.998 min and (Method 2, 3 min) = 3.23 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, J = 5.4 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.43 (s, 1H), 7.15 – 6.97 (m, 3H), 4.06 (t, *J* = 5.2 Hz, 4H), 3.50 – 3.41 (m, 4H), 2.34 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{20}F_3N_4S$, 381.1355; found 381.1352.



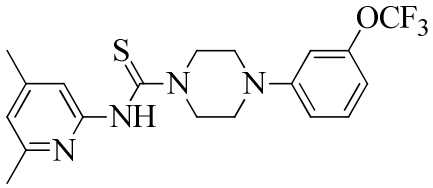
4-(3-(Dimethylamino)phenyl)-N-(4-methylpyridin-2-yl)piperazine-1-carbothioamide TFA (24). LC-MS Retention Time: (Method 1, 7 min) = 3.221 min and (Method 2, 3 min) = 2.524 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (brs, 1H), 7.92 (s, 1H), 7.15 –6.67 (m, 3H), 6.47 – 6.25 (m, 3H), 4.03 (dd, *J* = 6.2, 3.9 Hz, 4H), 3.31 (dd, *J* = 6.6, 3.5 Hz, 4H), 2.91 (s, 6H), 2.33 (s, 3H); HRMS (ESI) m/z; (M+Na)+ calcd. for $C_{19}H_{25}N_5NaS$, 378.1723; found 378.1723.



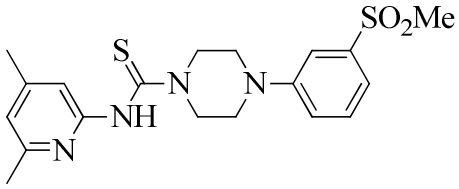
***N*-(2,4-dimethylpyridin-2-yl)-4-(3-nitrophenyl)piperazine-1-carbothioamide TFA (25).** LC-MS Retention Time: (Method 1, 7 min) = 4.559 min and (Method 2, 3 min) = 3.067 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 (t, *J* = 2.4 Hz, 1H), 7.60 (dt, *J* = 8.1, 2.0 Hz, 1H), 7.50 (td, *J* = 8.2, 2.0 Hz, 1H), 7.40 (dt, *J* = 8.5, 2.3 Hz, 1H), 7.25 (s, 1H), 6.91 (s, 1H), 4.13 – 4.01 (m, 4H), 3.41 -3.34 (m, 4H), 2.41 (s, 3H), 2.30 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{22}N_5O_2S$, 372.1489; found 372.1502.



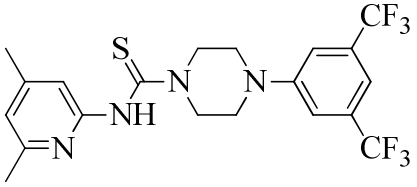
4-(3-Cyanophenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (26). LC-MS Retention Time: (Method 1, 7 min) = 4.372 min and (Method 2, 3 min) = 2.803 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 – 7.38 (m, 1H), 7.37 – 7.34 (m, 1H), 7.32 – 7.24 (m, 2H), 7.18 (dd, *J* = 7.5, 1.2 Hz, 1H), 6.94 (s, 1H), 4.04 (t, *J* = 5.1 Hz, 4H), 3.41- 3.39 (m, mH), 2.42 (s, 3H), 2.31 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{22}N_5S$, 352.1590; found 352.1590.



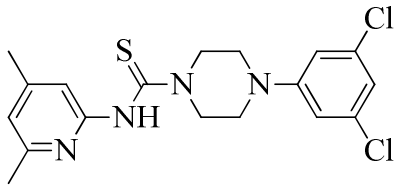
***N*-(4,6-dimethylpyridin-2-yl)-4-(3-(trifluoromethoxy)phenyl)piperazine-1-carbothioamide TFA (27).** LC-MS Retention Time: (Method 1, 7 min) = 5.161 min and (Method 2, 3 min) = 3.097 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30 (td, *J* = 8.3, 2.3 Hz, 1H), 7.20 (s, 1H), 6.93 (dt, *J* = 8.8, 2.5 Hz, 1H), 6.85 – 6.77 (m, 2H), 6.72 – 6.66 (m, 1H), 3.99 (m, 4H), 3.43 -3.39 (m, 4H), 2.35 (s, 3H), 2.23 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{22}F_3N_4OS$, 411.1461; found 411.1479.



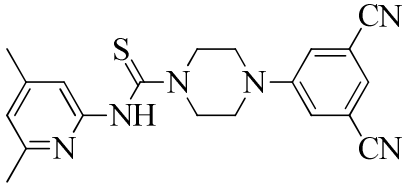
***N*-(4,6-dimethylpyridin-2-yl)-4-(3-(methylsulfonyl)phenyl)piperazine-1-carbothioamide TFA (28).** LC-MS Retention Time: (Method 1, 7 min) = 3.894 min and (Method 2, 3 min) = 2.639 min; ^{1}H NMR (400 MHz, DMSO-d*6*) δ 7.52 – 7.44 (m, 1H), 7.44 – 7.32 (m, 2H), 7.31 – 7.20 (m, 2H), 6.89 (s, 1H), 4.06 (m, 4H), 3.57 – 3.42 (m, 4H), 3.19 (s, 3H), 2.41 (s, 3H), 2.29 (s, 3H); HRMS (ESI) m/z; (M+Na)+ calcd. for $C_{19}H_{24}N_4NaO_2S_2$, 427.1233; found 427.1228.



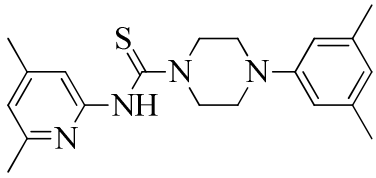
4-(3,5-Bis(trifluoromethyl)phenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (29). LC-MS Retention Time: (Method 1, 7 min) = 5.537 min and (Method 2, 3 min) = 3.263 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.46 (s, 2H), 7.31 (s, 1H), 7.25 (s, 1H), 6.86 (s, 1H), 4.09 – 4.01 (m, 4H), 3.52 (dd, *J* = 6.5, 4.1 Hz, 4H), 2.40 (s, 3H), 2.28 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{20}H_{21}F_6N_4S$, 463.1386; found 463.1401.



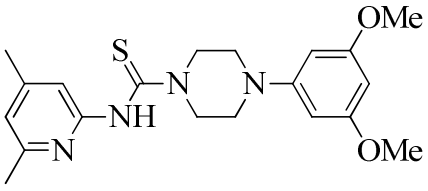
4-(3,5-Dichlorophenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (31). LC-MS Retention Time: (Method 1, 7 min) = 5.283 min and (Method 2, 3 min) = 2.95 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.21 (d, *J* = 8.3 Hz, 1H), 6.94 (d, *J* = 1.8 Hz, 2H), 6.87 (t, *J* = 1.7 Hz, 1H), 6.80 (s, 1H), 4.00 (t, *J* = 5.3 Hz, 4H), 3.47 – 3.39 (m, 4H), 2.37 (s, 3H), 2.26 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{20}H_{21}Cl_2N_4S$, 463.1386; found 463.1401.



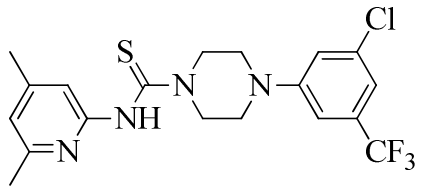
4-(3,5-Dicyanophenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (32). LC-MS Retention Time: (Method 1, 7 min) = 4.351 min and (Method 2, 3 min) = 3.048 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (brs, 1H), 7.67 (m, 3H), 7.25 (s, 1H), 6.79 (s, 1H), 4.01 (s, 4H), 3.34 -.3.30 (m, 4H), 2.37 (s, 3H), 2.25(s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{20}H_{26}N_6S$, 377.1543; found 377.1553.



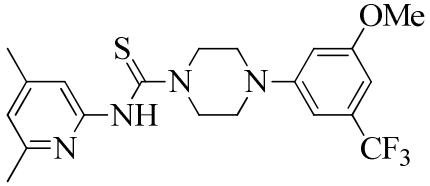
4-(3,5-Dimethylphenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (33). LC-MS Retention Time: (Method 1, 7 min) = 4.739 min and (Method 2, 3 min) = 3.143 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.24 (s, 1H), 6.94 (s, 1H), 6.56 - 6.47 (m, 3H), 4.04 - 4.01 (m, 4H), 3.31 -3.21 (m, 4H), 2.43 (s, 3H), 2.32 (s, 3H), 2.21 (s, 6H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{20}H_{27}N_4S$, 355.1951; found 355.1962.



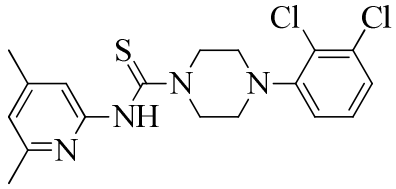
4-(3,5-Dimethoxyphenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (34). LC-MS Retention Time: (Method 1, 7 min) = 4.394 min and (Method 2, 3 min) = 3.065 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26 (d, *J* = 1.8 Hz, 1H), 6.99 (s, 1H), 6.10 (d, *J* = 2.1 Hz, 2H), 6.00 (q, *J* = 2.0 Hz, 1H), 4.04 – 4.02 (m, 4H), 3.71 (s, 6H), 3.31 – 3.21 (m, 4H), 2.44 (s, 3H), 2.33 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{20}H_{27}N_4O_2S$, 387.1849; found 387.1867.



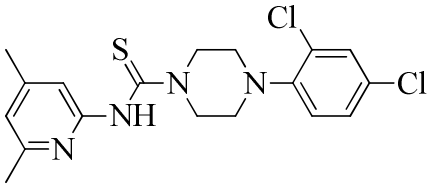
4-(3-Chloro-5-(trifluoromethyl)phenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (35). LC-MS Retention Time: (Method 1, 7 min) = 5.41 min and (Method 2, 3 min) = 3.384 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 – 7.22 (m, 2H), 7.19 – 7.13 (m, 1H), 7.10 (s, 1H), 6.90 (s, 1H), 4.04 (t, *J* = 5.1 Hz, 4H), 3.51 – 3.43 (m, 4H), 2.41 (s, 3H), 2.30 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{21}ClF_3N_4S$, 429.1122; found 429.1131.



***N*-(4,6-dimethylpyridin-2-yl)-4-(3-methoxy-5-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (36).** LC-MS Retention Time: (Method 1, 7 min) = 5.14 min and (Method 2, 3 min) = 3.093 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.24 (d, *J* = 3.2 Hz, 1H), 6.93 – 6.78 (m, 2H), 6.71 (d, *J* = 2.4 Hz, 1H), 6.66 – 6.60 (m, 1H), 4.08 – 4.00 (m, 4H), 3.80 (s, 3H), 3.40 -3.36 (m, 4H), 2.40 (s, 3H), 2.29 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{20}H_{24}F_3N_4OS$, 425.1617; found 425.1618.

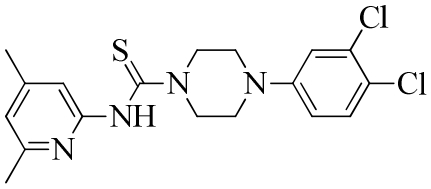


4-(2,3-Dichlorophenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (37). LC-MS Retention Time: (Method 1, 7 min) = 5.096 min and (Method 2, 3 min) = 3.105 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37 – 7.31 (m, 2H), 7.26 – 7.14 (m, 2H), 6.94 (s, 1H), 4.10 – 4.03 (m, 4H), 3.07 (t, J = 4.9 Hz, 4H), 2.43 (s, 3H), 2.32 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{21}Cl_2N_4S$, 395.0858; found 395.0858.

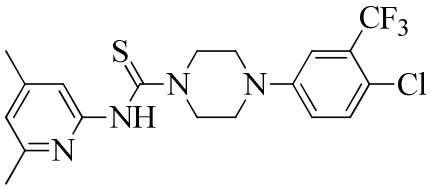


4-(2,4-Dichlorophenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (38). LC-MS Retention Time: (Method 1, 7 min) = 5.222 min and (Method 2, 3 min) = 3.304 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (brs, 1H), 7.58 (d, *J* = 2.5 Hz, 1H), 7.39 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.24 (s, 1H), 7.22 - 7.20 (m, 1H), 6.96 – 6.88 (m, 1H), 4.24 – 3.80 (m, 4H),

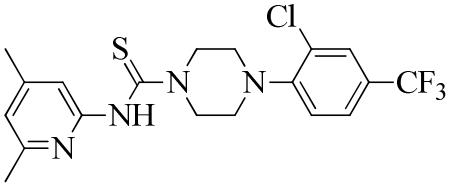
3.16 – 2.91 (m, 4H), 2.43 (s, 3H), 2.30 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{21}Cl_2N_4S$, 395.0866; found 395.0858.



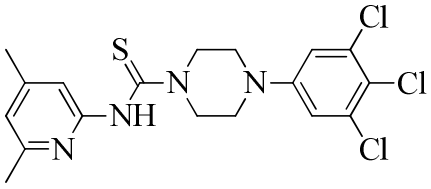
4-(3,4-Dichlorophenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (39). LC-MS Retention Time: (Method 1, 7 min) = 5.129 min and (Method 2, 3 min) = 3.294 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.24 (d, *J* = 6.4 Hz, 1H), 7.16 (d, *J* = 2.8 Hz, 1H), 6.99 – 6.86 (m, 2H), 4.02 (dd, *J* = 7.0, 3.6 Hz, 4H), 3.37 – 3.29 (m, 4H), 2.41 (s, 3H), 2.30 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{21}Cl_2N_4S$, 395.0858; found 395.0862.



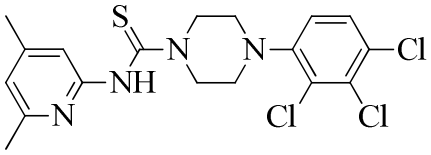
4-(4-Chloro-3-(trifluoromethyl)phenyl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (40). LC-MS Retention Time: (Method 1, 7 min) = 5.241 min and (Method 2, 3 min) = 3.335 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 (t, *J* = 8.3 Hz, 1H), 7.35 – 7.17 (m, 3H), 6.98 (d, *J* = 6.4 Hz, 1H), 4.05 (q, *J* = 4.5 Hz, 4H), 3.40 (q, *J* = 6.2, 5.5 Hz, 4H), 2.49 (s, 3H), 2.33 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{21}ClF_3N_4S$, 429.1122; found 429.1116.



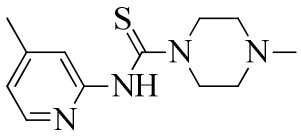
4-(2-Chloro-4-(trifluoromethyl)phenyl)-*N*-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (42). LC-MS Retention Time: (Method 1, 7 min) = 5.314 min and (Method 2, 3 min) = 3.222 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 – 7.78 (m, 1H), 7.72 – 7.63 (m, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.25 (s, 1H), 6.95 (s, 1H), 4.08 (t, *J* = 4.7 Hz, 4H), 3.18 (dd, *J* = 6.5, 3.5 Hz, 4H), 2.44 (s, 3H), 2.32 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{21}ClF_3N_4S$, 429.1122; found 429.1117.



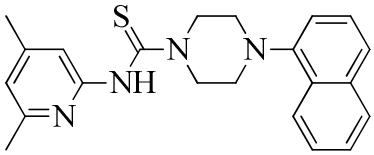
***N*-(4,6-dimethylpyridin-2-yl)-4-(3,4,5-trichlorophenyl)piperazine-1-carbothioamide TFA (43).** LC-MS Retention Time: (Method 1, 7 min) = 5.314 min and (Method 2, 3 min) = 3.222 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25 (d, *J* = 7.3 Hz, 1H), 7.18 (d, *J* = 1.9 Hz, 2H), 6.88 (s, 1H), 4.01 (t, *J* = 5.3 Hz, 4H), 3.37 – 3.4 (m, 4H), 2.40 (s, 3H), 2.29 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{20}Cl_3N_4S$, 431.0441; found 431.0447.



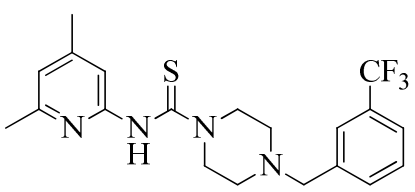
***N*-(4,6-dimethylpyridin-2-yl)-4-(2,3,4-trichlorophenyl)piperazine-1-carbothioamide TFA (44).** LC-MS Retention Time: (Method 1, 7 min) = 5.336 min and (Method 2, 3 min) = 3.235 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (s, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.22 (d, *J* = 8.9 Hz, 1H), 6.78 (s, 1H), 4.02 (d, *J* = 6.4 Hz, 4H), 3.06 (s, 4H), 2.37 (s, 3H), 2.24 (s, 3H).; HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{20}Cl_3N_4S$, 431.0441; found 431.0445.



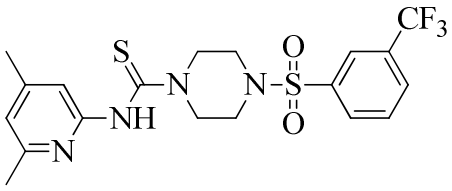
***N*-(4-methylpyridin-2-yl)-4-methylpiperazine-1-carbothioamide TFA (46).** LC-MS Retention Time: (Method 1, 7 min) = 2.266 min and (Method 2, 3 min) = 2.2 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (brs, 1H), 8.14 (d, *J* = 5.4 Hz, 1H), 7.40 (s, 1H), 6.92 (d, *J* = 5.2 Hz, 1H), 3.61 – 2.99 (m, 8H), 2.85 (s, 3H), 2.30 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{19}N_4S$, 252.1351; found 252.1350.



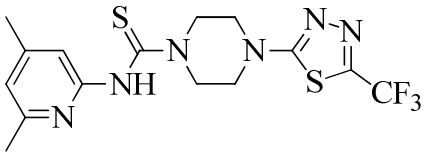
***N*-(4,6-dimethylpyridin-2-yl)-4-(naphthalen-1-yl)piperazine-1-carbothioamide TFA (47).** LC-MS Retention Time: (Method 1, 7 min) = 5.084 min and (Method 2, 3 min) = 3.261 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 – 8.17 (m, 1H), 7.91 (dd, *J* = 7.1, 2.1 Hz, 1H), 7.64 (d, *J* = 8.2 Hz, 1H), 7.58 – 7.49 (m, 2H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.25 (s, 1H), 7.17 (d, *J* = 7.3 Hz, 1H), 6.93 (s, 1H), 4.11 (d, *J* = 59.9 Hz, 4H), 3.15 – 3.11 (m, 4H), 2.44 (s, 3H), 2.32 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{22}H_{25}N_4S$, 377.1794; found 377.1795.



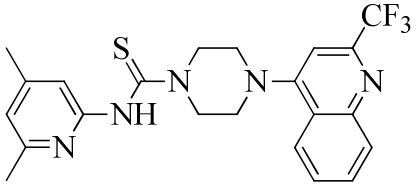
***N*-(4,6-dimethylpyridin-2-yl)-4-(3-(trifluoromethyl)benzyl)piperazine-1-carbothioamide TFA (48).** LC-MS Retention Time: (Method 1, 7 min) = 3.751 min and (Method 2, 3 min) = 2.774 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92– 7.59 (m, 3H), 7.22 (s, 1H), 6.79 (s, 1H), 6.57 (s, 1H), 4.45 (s, 2H), 3.40 – 3.10 (m, 8H), 2.37 (s, 3H), 2.26 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{20}H_{24}F3N4S$, 409.1668; found 409.1674.



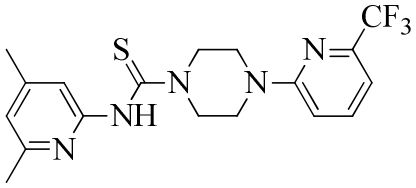
***N*-(4,6-dimethylpyridin-2-yl)-4-((3-(trifluoromethyl)phenyl)sulfonyl)piperazine-1-carbothioamide TFA (49).** LC-MS Retention Time: (Method 1, 7 min) = 4.824 min and (Method 2, 3 min) = 3.225 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 – 8.08 (m, 2H), 8.02 – 7.90 (m, 2H), 7.09 (s, 1H), 6.79 (s, 1H), 4.01 – 3.92 (m, 4H), 3.05 (t, *J* = 4.9 Hz, 4H), 2.27 (s, 3H), 2.23 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{22}F3N4O_2S_2$, 459.1131; found 459.1134.



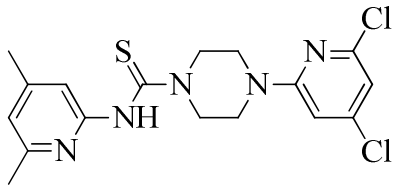
***N*-(4,6-dimethylpyridin-2-yl)-4-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)piperazine-1-carbothioamide TFA (50).** LC-MS Retention Time: (Method 1, 7 min) = 4.222 min and (Method 2, 3 min) = 3.011 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.24 (s, 1H), 6.84 (s, 1H), 4.10 (t, *J* = 5.0 Hz, 4H), 3.77 – 3.67 (m, 4H), 2.40 (m, 3H), 2.28 (m, 4H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{15}H_{18}F3N6S_2$, 403.0987; found 403.0981.



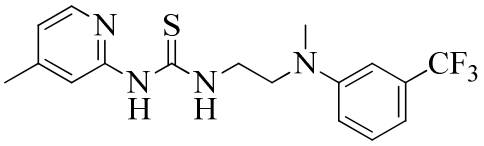
***N*-(4,6-dimethylpyridin-2-yl)-4-(2-(trifluoromethyl)quinolin-4-yl)piperazine-1-carbothioamide TFA (51).** LC-MS Retention Time: (Method 1, 7 min) = 4.833 min and (Method 2, 3 min) = 3.192 min; ^{1}H NMR (400 MHz, DMSO-d*6*) δ 8.21 (dd, *J* = 8.6, 2.8 Hz, 1H), 8.09 (dd, *J* = 8.6, 2.8 Hz, 1H), 7.86 (t, *J* = 7.6 Hz, 1H), 7.72 (t, *J* = 7.6 Hz, 1H), 7.36 – 7.22 (m, 2H), 6.95 (d, *J* = 8.9 Hz, 1H), 4.22 (d, *J* = 5.4 Hz, 4H), 3.34 – 3.31 (m, 4H), 2.44 (s, 3H), 2.32 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{22}H_{23}F3N5S$, 446.1621; found 446.1628.



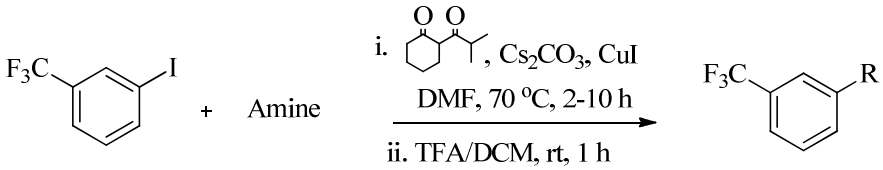
***N*-(4,6-dimethylpyridin-2-yl)-4-(6-(trifluoromethyl)pyridin-2-yl)piperazine-1-carbothioamide TFA (52).** LC-MS Retention Time: (Method 1, 7 min) = 4.863 min and (Method 2, 3 min) = 3.204 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (t, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 6.6 Hz, 1H), 7.20 – 7.03 (m, 2H), 6.98 (d, *J* = 6.5 Hz, 1H), 4.05 (dd, *J* = 6.4, 3.6 Hz, 4H), 3.82 – 3.63 (m, mH), 2.43 (s, 3H), 2.32 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{21}F3N5S$, 396.1464; found 396.1463.



4-(4,6-Dichloropyridin-2-yl)-N-(4,6-dimethylpyridin-2-yl)piperazine-1-carbothioamide TFA (56). LC-MS Retention Time: (Method 1, 7 min) = 4.368 min and (Method 2, 3 min) = 2.932 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (brs, 1H), 7.24 (s, 1H), 6.93 – 6.78 (m, 3H), 4.00 (d, *J* = 6.1 Hz, 4H), 3.59 (dt, *J* = 7.9, 3.6 Hz, 4H), 2.38 (s, 3H), 2.26 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{17}H_{20}Cl_2N_5S$, 396.0811; found 396.0814.



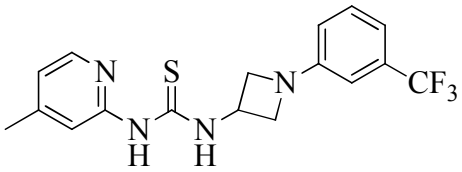
1-(2-(Methyl(3-(trifluoromethyl)phenyl)amino)ethyl)-3-(4-methylpyridin-2-yl)thiourea TFA (57). LC-MS Retention Time: (Method 1, 7 min) = 6.366 min and (Method 2, 3 min) = 3.85 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.77 (t, J = 5.6 Hz, 1H), 10.50 (s, 1H), 7.93 (dd, J = 5.3, 0.7 Hz, 1H), 7.33 (ddd, *J* = 8.6, 7.6, 1.0 Hz, 1H), 7.14 – 7.02 (m, 2H), 6.94 (dt, *J* = 1.5, 0.8 Hz, 1H), 6.90 – 6.82 (m, 2H), 3.86 – 3.76 (m, 2H), 3.66 (t, *J* = 6.8 Hz, 2H), 3.00 (s, 3H), 2.25 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{17}H_{20}F_3N_4S$, 369.1355; found 369.1359.



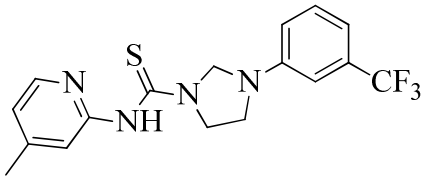
<u>General procedure E (procedure adopted from a reported method[11])</u>: A mixture of 1-iodo-3-(trifluoromethyl)benzene (0.265 mL, 1.838 mmol, 1 eq), amine (2.76 mmol, 1.5 eq), copper(I) iodide (0.018 g, 0.092 mmol, 5 mol%) and cesium carbonate (1.20 g, 3.68 mmol, 2 eq) in DMF (7 mL) was degassed for 5 min and then 2-isobutyrylcyclohexanone (0.062 g, 0.368 mmol, 20 mol%) was added and stirred at 70 °C for 2-10 h. The product was extracted with ethyl acetate, successively washed with water, 1% HCl and brine. The organic layer was dried with sodium

sulfate and the crude product was purified on a biotage flash system eluting with 20% ethyl acetate in hexanes. The product was deprotected with TFA following the step D (above).

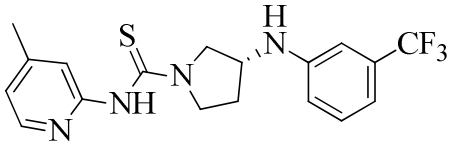
Synthesis of compounds 57-67: These compounds were prepared following the **General procedure A**.



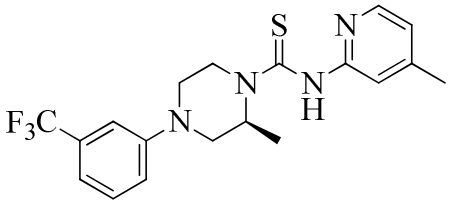
1-(4-Methylpyridin-2-yl)-3-(1-(3-(trifluoromethyl)phenyl)azetidin-3-yl)thiourea (59). LC-MS Retention Time: (Method 1, 7 min) = 4.755 min and (Method 2, 3 min) = 3.141 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, *J* = 5.4 Hz, 1H), 7.25 (t, *J* = 7.9 Hz, 1H), 7.13 (d, *J* = 5.5 Hz, 1H), 7.00 – 6.87 (m, 2H), 6.81 (d, *J* = 7.7 Hz, 1H), 6.47 (s, 1H), 4.45 -4.51 (m, 1H), 3.73 - 3.70 (m, 4H), 2.35 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{17}H_{18}F_3N_4S$, 367.1209; found 367.1214.



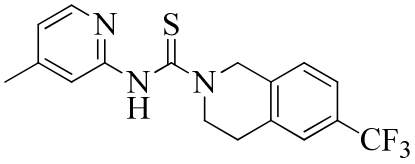
***N*-(4-methylpyridin-2-yl)-3-(3-(trifluoromethyl)phenyl)imidazolidine-1-carbothioamide TFA (60).** LC-MS Retention Time: (Method 1, 7 min) = 5.004 min and (Method 2, 3 min) = 3.288 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (brs, 1H), 8.20 (d, *J* = 5.2 Hz, 1H), 7.75 (d, *J* = 6.8 Hz, 1H), 7.52 – 7.41 (m, 1H), 7.10 (ddd, *J* = 7.5, 1.8, 0.9 Hz, 1H), 7.05 – 6.96 (m, 2H), 6.94 (t, *J* = 2.0 Hz, 1H), 5.03 (s, 2H), 4.05 (t, *J* = 6.7 Hz, 2H), 3.70 (t, *J* = 6.7 Hz, 2H), 2.32 (d, *J* = 0.7 Hz, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{17}H_{18}F_3N_4S$, 367.1209; found 367.1199.



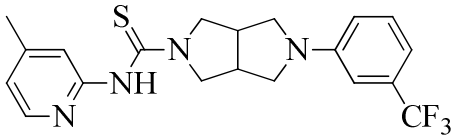
(*R*)-*N*-(4-methylpyridin-2-yl)-3-((3-(trifluoromethyl)phenyl)amino)pyrrolidine-1-carbothioamide TFA (61). LC-MS Retention Time: (Method 1, 7 min) = 4.833 min and (Method 2, 3 min) = 2.988 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67 (brs, 1H), 8.25 (d, *J* = 5.5 Hz, 1H), 7.69 (s, 1H), 7.31 (ddd, *J* = 8.9, 7.5, 1.1 Hz, 1H), 7.14 – 7.05 (m, 1H), 6.92 – 6.78 (m, 3H), 4.80 (brs, 1H), 4.30 – 3.44 (m, 6H), 2.36 (s, 3H), 1.97 -1.91 (m, 1H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{18}H_{20}F_3N_4S$, 381.1355; found 381.1367.



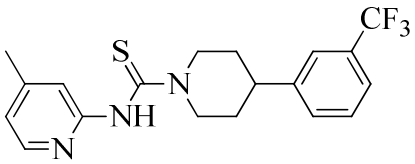
(*S*)-2-methyl-N-(4-methylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperazine-1-carbothioamide TFA (62). LC-MS Retention Time: (Method 1, 7 min) = 5.158 min and (Method 2, 3 min) = 3.366 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 – 7.71 (m, 1H), 7.44 (dq, J = 18.0, 10.1, 9.0 Hz, 2H), 7.32 – 7.09 (m, 3H), 7.11 – 7.02 (m, 1H), 6.80 – 6.64 (m, 1H), 3.97 – 3.67 (m, 4H), 3.23 – 2.72 (m, 3H), 2.33 (s, 3H), 1.29 (dd, J = 12.2, 6.6 Hz, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{22}F_3N_4S$, 395.1512; found 395.1523.



***N*-(4-methylpyridin-2-yl)-6-(trifluoromethyl)-3,4-dihydroisoquinoline-2(1H)-carbothioamide TFA (64).** LC-MS Retention Time: (Method 1, 7 min) = 4.783 min and (Method 2, 3 min) = 3.158 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.67 – 7.51 (m, 2H), 7.43 (d, *J* = 8.5 Hz, 2H), 6.95 (s, 1H), 5.11 (s, 2H), 4.10 - 4.07 (m, 2H), 3.06 – 3.01 (m, 2H), 2.31 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{17}H_{17}F_3N_3S$, 352.1090; found 352.1092.

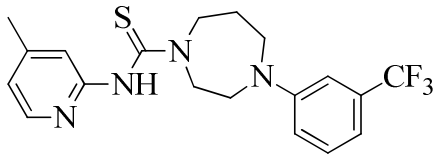


***N*-(4-methylpyridin-2-yl)-5-(3-(trifluoromethyl)phenyl)hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carbothioamide TFA (65).** LC-MS Retention Time: (Method 1, 7 min) = 5.12 min and (Method 2, 3 min) = 3.112 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (brs, 1H), 8.22 (d, *J* = 5.4 Hz, 1H), 7.69 (s, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.08 – 7.02 (m, 1H), 6.93 (d, *J* = 7.6 Hz, 1H), 6.87 – 6.81 (m, 1H), 6.77 (t, *J* = 2.0 Hz, 1H), 4.01 (dd, *J* = 12.8, 6.8 Hz, 2H), 3.69 (dd, *J* = 12.5, 4.0 Hz, 2H), 3.54 (dd, *J* = 9.9, 7.2 Hz, 2H), 3.34 – 3.11 (m, 4H), 2.35 (s, 3H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{20}H_{22}F_3N_4S$, 407.1512; found 407.1528.



***N*-(4-methylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)piperidine-1-carbothioamide TFA (66).** LC-MS Retention Time: (Method 1, 7 min) = 5.003 min and (Method 2, 3 min) = 3.056 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, **J** = 5.4 Hz, 1H), 7.66 – 7.51 (m, 4H), 7.39 (s,

1H), 7.05 – 6.93 (m, 1H), 3.39 – 2.94 (m, 4H), 2.33 (s, 3H), 2.02 – 1.63 (m, 5H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{21}F_3N_3S$, 380.1403; found 380.1414.



***N*-(4-methylpyridin-2-yl)-4-(3-(trifluoromethyl)phenyl)-1,4-diazepane-1-carbothioamide TFA (67).** LC-MS Retention Time: (Method 1, 7 min) = 4.937 min and (Method 2, 3 min) = 2.282 min; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60 (brs, 1H), 8.16 (d, *J* = 5.4 Hz, 1H), 7.35 (dd, *J* = 8.7, 7.5 Hz, 1H), 7.14 – 6.72 (m, 5H), 4.08 - 3.83 (m, 4H), 3.77 (t, *J* = 5.4 Hz, 2H), 3.61 (t, *J* = 6.0 Hz, 2H), 2.28 (s, 3H), 2.10 – 1.95 (m, 2H); HRMS (ESI) m/z; (M+H)+ calcd. for $C_{19}H_{21}F_3N_4S$, 395.1512; found 395.1514.

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