Supporting information for

Synthesis, Biological Evaluation and Structure-Activity Relationships of a Novel Class of Apurinic/Apyrimidinic Endonuclease 1 Inhibitors

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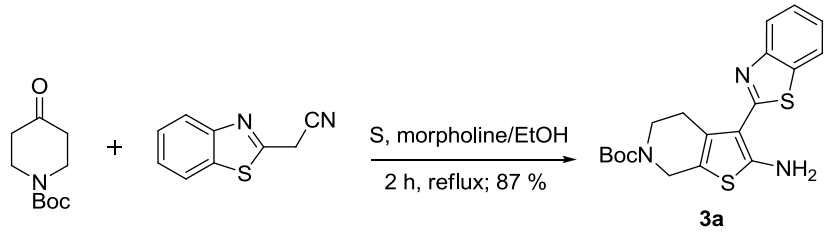
General Methods (Chemistry). Unless otherwise stated, all reactions were carried out under an atmosphere of dry argon or nitrogen in dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while room temperature (rt) is noted as 25 ^{o}C. All solvents were of anhydrous quality purchased from Sigma Aldrich Chemical Co. and used as received. Commercially available starting materials and reagents were purchased from Sigma Aldrich and were used as received. Analytical thin layer chromatography (TLC) was performed with Sigma Aldrich TLC plates (5 x 20 cm, 60 Å, 250 µm). Visualization was accomplished by irradiation under a 254 nm UV lamp. Chromatography on silica gel was performed using forced flow (liquid) of the indicated solvent system on Biotage KP-Sil pre-packed cartridges and using the Biotage SP-1 automated chromatography system. ^{1}H- and ^{13}C NMR spectra were recorded on a Varian Inova 400 MHz spectrometer. Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CDCl_3$ 7.26 ppm, 77.00 ppm, DMSO-d_6 2.49 ppm, 39.51 ppm for ^{1}H, ^{13}C respectively). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, brs = broad singlet, m = multiplet), coupling constants, and number of protons. Low resolution mass spectra (electrospray ionization) were acquired on an Agilent Technologies 6130 quadrupole spectrometer coupled to the HPLC system. High resolution mass spectral data was collected in-house using an Agilent 6210 time-of-flight mass spectrometer, also coupled to an Agilent Technologies 1200 series HPLC system. If needed, products were purified via a Waters semi-preparative HPLC equipped with a Phenomenex Luna® C18 reverse phase (5 micron, 30 x 75 mm) column having a flow rate of 45 mL/min. The mobile phase was a mixture of acetonitrile (0.025% TFA) and H_2O (0.05% TFA), and the temperature was maintained at 50 ^{o}C.
Samples were analyzed for purity on an Agilent 1200 series LC/MS equipped with a Luna® C18 reverse phase (3 micron, 3 x 75 mm) column having a flow rate of 0.8-1.0 mL/min over a 7-minute gradient and a 8.5 minute run time. Purity of final compounds was determined to be >95%, using a 3 µL injection with quantitation by AUC at 220 and 254 nm (Agilent Diode Array Detector).

General procedure for the synthesis of 3,6-disubstituted-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-amine (Procedure A): A mixture of appropriate ketone (1 mmol, 1 eq), substituted acetonitrile (1 mmol, 1 eq), sulfur (1 mmol, 1 eq) in ethanol (0.2 molar reaction concentration) was added morpholine (1 mmol, 1 eq) and stirred under reflux for 0.5 to 1 h. Excess solvent was removed under diminished pressure. The product was purified on a Biotage® silica gel column or by recrystallization.
General procedure for the acylation (Procedure B): A mixture of appropriate amine (1 mmol, 1 eq) and hunig's base (2 mmol, 2 eq) in dichloromethane (0.2 molar reaction concentration) was added acetyl chloride/substituted acid chlorides (1.5 mmol, 1.5 eq) at 0 ^{o}C and then stirred at room temperature for 1 h. Excess solvent was removed under diminished pressure and the residue was purified on a Biotage® silica gel column. Elution with 10-20 % ethyl acetate in hexanes gave the pure products.

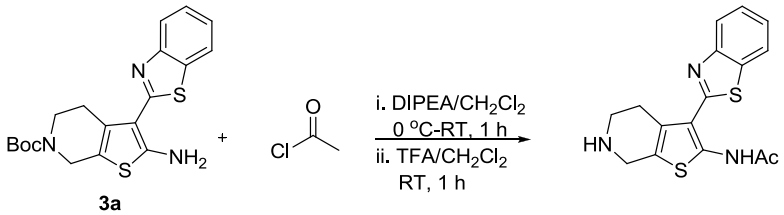
General Procedure for the deprotection of Boc (Procedure C): The boc-protected amine (2.328 mmol) in dichloromethane (15 mL) was added TFA (5 mL) and stirred at room temperature for 1 h. Excess solvent was evaporated, neutralized with 10 % sodium bicarbonate and the product was collected by filtration or purified on a preparative HPLC.

General Procedure for reductive amination (Procedure D): A mixture appropriate amine (1 mmol, 1 eq) and acetone/appropriate carbonyl compound (10 mmol, 10 eq) in a mixture of MeOH/THF (4/2 mL, 0.2 molar reaction concentration) was added sodium cyanoborohydride (3 mmol, 3 eq) and few drops of acetic acid. The reaction mixture was stirred at room temperature for 6-10 h. Volatiles were removed and the crude solid was purified on a preparative HPLC.



3a

Synthesis of t-butyl 2-amino-3-(benzo[d]thiazol-2-yl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate: This compound was prepared following **Procedure A**. A mixture of 1-boc-4-piperidone (1.5 g, 7.53 mmol, 1 eq), 2-(benzo[d]thiazol-2-yl)acetonitrile (1.312 g, 7.53 mmol, 1 eq), sulfur (0.241 g, 7.53 mmol, 1 eq) in EtOH (30 mL) was added morpholine (0.66 mL, 7.53 mmol, 1 eq) and stirred under reflux for 2 h. Excess solvent was removed under diminished pressure. The product was purified on a Biotage® silica gel column. Elution with 5-40% ethyl acetate in hexanes gave the product. Yield: 87 % (2.55 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (s, 9 H), 2.84 (m, 2 H), 3.66 (t, J = 5.4 Hz, 2 H), 4.36 (s, 2 H), 7.31 (t, J = 7.6 Hz, 1 H), 7.45 (t, J = 7.6 Hz, 1 H), 7.89 (d, J = 8.0 Hz, 1 H), 8.00 (d, J = 7.8 Hz, 1 H) and 8.14 (s, 2 H); HRMS (ESI) m/z $(M+H)^+$ calcd. for $C_{19}H_{22}N_3O_2S_2$, 388.1148; found 388.1153.

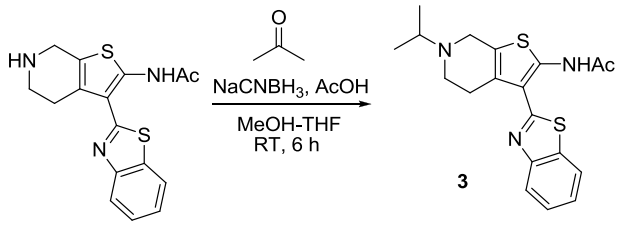


3a

General procedure for acetylation: This compound was prepared following **Procedure B**. A mixture of tert-butyl 2-amino-3-(benzo[d]thiazol-2-yl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (1.6 g, 4.13 mmol, 1 eq) and hunig's base (1.4 mL, 8.26 mmol, 2 eq) in dichloromethane (25 mL) was added acetyl chloride (0.35 mL, 4.95 mmol, 1.5 eq) at 0 ^{o}C and then stirred at room temperature for 1 h. Excess solvent was removed under diminished pressure and the residue was purified on a Biotage® silica gel column. Elution with 10% ethyl acetate in hexanes gave the product. Yield: 91 % (1.62 g).

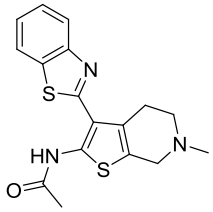
The above product (1 g, 2.328 mmol) in dichloromethane (15 mL) was added TFA (5 mL) and stirred at room temperature for 1 h (**Procedure C**). Excess solvent was evaporated, neutralized with 10 % sodium bicarbonate and the precipitate was collected by filtration. The product was dried to get a yellow solid. Yield: 99 % (0.76 g).

LC-MS: rt (min) = 2.95 (4.5 min run); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.30 (s, 3 H), 2.78 (m, 2 H), 3.02 (t, J = 5.6 Hz, 2 H), 3.80 (s, 2 H), 7.41 - 7.48 (m, 1 H), 7.53 - 7.60 (m, 1 H) and 8.11 (t, J = 7.8 Hz, 2 H); HRMS (ESI) m/z $(M+H)^+$ calcd. for $C_{16}H_{16}N_3OS_2$, 330.0729; found 330.0736.

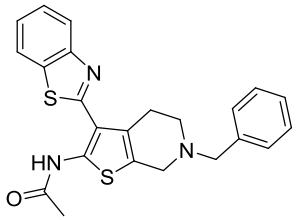


Synthesis of *N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (3): This compound was prepared following Procedure D. *N*-(3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (0.1 g, 0.304 mmol, 1 eq) and acetone (0.22 mL, 3.04 mmol, 10 eq) in a mixture of MeOH/THF (4/2 mL) was added sodium cyanoborohydride (0.057 g, 0.911 mmol, 3 eq) and few drops of acetic acid. The reaction mixture was stirred at room temperature for 6 h. Volatiles were removed and the crude solid was purified on a preparative HPLC. Yield: 75 % (0.11 g).

LC-MS: rt (min) = 4.42; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.33 (d, J = 6.3 Hz, 6 H), 2.36 (s, 3 H), 3.29 - 3.55 (m, 2 H), 3.65 – 3.74 (m, 1 H), 4.55 – 4.61 (m, 2 H), 4.72 (s, 2 H), 7.49 (t, J = 7.6 Hz, 1 H), 7.60 (t, J = 7.7 Hz, 1 H), 8.16 (dd, J = 8.0 and 3.7 Hz, 2 H) and 12.06 (s, 1H); HRMS (ESI) m/z $(M+H)^+$ calcd. for $C_{19}H_{22}N_3OS_2$, 372.1199; found 372.1199.

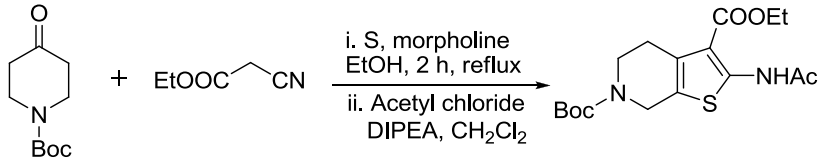


***N*-(3-(benzo[d]thiazol-2-yl)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (6):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 4.24; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.35 (s, 3 H), 2.98 (s, 3 H), 3.21 - 3.29 (m, 2 H), 3.49 – 3.65 (m, 2 H), 4.45 (s, 2 H), 7.50 (t, J = 7.6 Hz, 1 H), 7.61 (t, J = 7.6 Hz, 1 H), 8.19 (m, 2 H), and 12.51 (s, 1 H); HRMS (ESI) m/z $(M+Na)^+$ calcd. for $C_{17}H_{17}N_3OS_2Na$, 366.0705; found 366.0702.

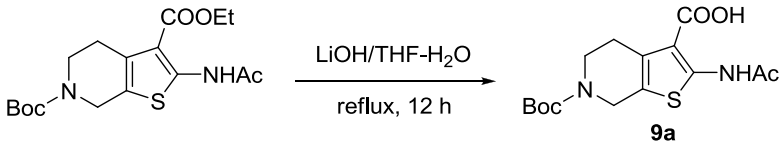


***N*-(3-(benzo[d]thiazol-2-yl)-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (7):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 4.88; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.32 (s, 3 H), 2.84 (t, J = 5.5 Hz, 2 H),

2.89 – 2.95 (m, 2 H), 3.57 (s, 2 H), 3.71 (s, 2 H), 7.25 - 7.32 (m, 1 H), 7.33 - 7.41 (m, 4 H), 7.46 (t, *J* = 7.8 Hz, 1 H), 7.58 (t, *J* = 7.8 Hz, 1 H), 8.14 (d, *J* = 8.4 Hz, 2 H) and 12.59 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{23}H_{22}N_3OS_2$, 420.1199; found 420.1207.

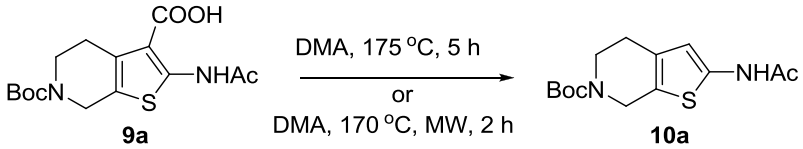


Synthesis of 6-tert-butyl 3-ethyl 2-acetamido-4,5-dihydrothieno[2,3-c]pyridine-3,6(7H)-dicarboxylate: This compound was prepared following the general **procedure A** (Yield after recrystallization from ethanol, 84%) (Reference 1) and **Procedure B** (Yield after purification on a Biotage® silica gel column with 10 % ethyl acetate in hexanes, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (t, *J* = 7.1 Hz, 3 H), 1.41 (s, 9 H), 2.24 (s, 3 H), 2.73 - 2.81 (m, 2 H), 3.55 (t, *J* = 5.3 Hz, 2 H), 4.28 (q, *J* = 7.1 Hz, 2 H), 4.43 (s, 2 H) and 10.94 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{17}H_{25}N_2O_5S$, 369.1479; found 369.1477.



9a

Synthesis of 2-acetamido-6-(tert-butoxycarbonyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic acid: A solution of 6-tert-butyl 3-ethyl 2-acetamido-4,5-dihydrothieno[2,3-c]pyridine-3,6(7H)-dicarboxylate (1 mmol, 1 eq) in THF (4 mL) was added a solution of lithium hydroxide in water (1 mL) and refluxed for 12 h. Reaction mixture was neutralized with 10 % HCl and extracted with ethyl acetate. The organic layer was washed with water and brine, and then the organic layer was dried over magnesium sulfate. After evaporation of the solvent, the crude product was purified on a Biotage® silica gel column. Elution with 40 % ethyl acetate (contains 0.05 % acetic acid) in hexanes. Yield 65 %. ^{1}H NMR (400 MHz, DMSO-d_6); δ 1.41 (s, 9 H), 2.22 (s, 3 H), 2.74 – 2.79 (m, 2 H), 3.54 (t, *J* = 5.5 Hz, 2 H), 4.42 (s, 2 H), 11.14 (s, 1 H) and 13.17 (brs, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{15}H_{21}N_2O_5S$, 341.1166; found 341.1167.

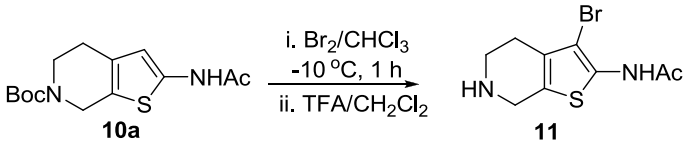


9a **10a**

Synthesis of tert-butyl 2-acetamido-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (10a). *Method I:* A solution of 2-acetamido-6-(*t*-butoxycarbonyl)-4,5,6,7-tetrahydro- thieno[2,3-c]pyridine-3-carboxylic acid (1.5 g, 4.41 mmol) in dimethylacetamide (9 mL) was heated in MW for 2 h at 170 °C. The product was extracted with ethyl acetate. The organic layer was washed with water and brine and dried over sodium sulfate. The crude residue obtained after evaporation of the solvent was purified on a Biotage® silica gel column. Elution with 50 % ethyl acetate in hexanes gave the pure product. Yield: 77 % (1.306 g).

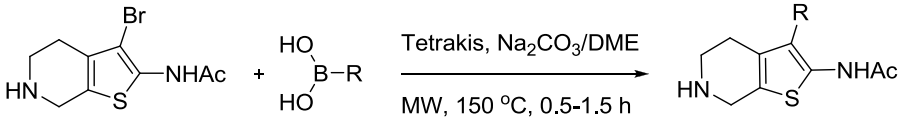
Method II: A solution 2-acetamido-6-(tert-butoxycarbonyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic acid (6 g, 17.63 mmol) in DMA (35 mL) was stirred at 175 °C

for 5 h in an open round bottomed flask. The reaction was then worked up and purified as described for *Method I*. Yield: 65 % (3.4 g).
LC-MS: rt (min) = 5.18; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (s, 9 H), 2.03 (s, 3 H), 2.50 - 2.54 (m, 2 H), 3.55 (t, *J* = 5.7 Hz, 2 H), 4.41 (s, 2 H), 6.33 (s, 1 H) and 10.99 (s, 1 H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 22.5, 24.8, 28.0, 79.1, 109.9, 121.9, 129.9, 137.7, 153.9 and 166.1.



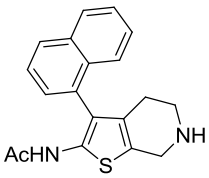
10a **11**

Synthesis of tert-butyl 2-acetamido-3-bromo-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (11): A solution of tert-butyl 2-acetamido-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (1.98 g, 6.68 mmol, 1eq) in chloroform (67 mL) was added bromine (0.344 mL, 6.68 mmol, 1 eq) in chloroform (10 mL) at -10 °C over a period of 1 h (faster addition removes the Boc group). After completion of the addition, the reaction mixture was diluted with chloroform and the organic layer was washed with sodium bicarbonate solution and brine. The organic layer was dried over sodium sulfate and concentrated. The crude product was purified on a Biotage® silica gel column. Elution with 25 % ethyl acetate in hexanes gave the pure product. Yield: 70 % (1.76 g).
LC-MS: rt (min) = 3.60 (4.5 min run); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9 H), 1.60 (brs, 1 H), 2.26 (s, 3 H), 2.53 – 2.59 (m, 2 H), 3.67 – 3.72 (m, 2 H), 4.53 (s, 2 H) and 7.8 (s, 1H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{14}H_{20}BrN_2O_3S$, 375.0373; found 375.0375. The above product was deprotected with TFA using general procedure C to get the compound (**11**). LC-MS: rt (min) = 2.64; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.13 (s, 3 H), 2.40 - 2.49 (m, 2 H), 3.12 (t, *J* = 5.7 Hz, 2 H), 3.94 (s, 2 H) and 10.32 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_9H_{12}BrN_2OS$, 274.9898; found 274.9855.



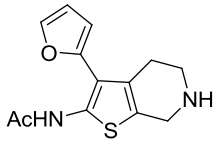
11 **11-20**

General procedure for Suzuki coupling: A mixture of *N*-(3-bromo-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (0.05g, 0.182 mmol, 1 eq), appropriate boronic acid or pinacol ester (0.273 mmol, 1.5 eq) and a 2M solution of sodium carbonate (2-4 eq) in dimethoxy ethane (2 mL) was bubbled with argon for 5 minutes. Tetrakis (5-10 mol %) was then added and heated in microwave at 150 °C for 0.5-1.5 h. The solvent was evaporated in a blow down unit. The residue was re-dissolved in 2 mL DMF, filtered through thiol cartridge and purified in a preparative HPLC.

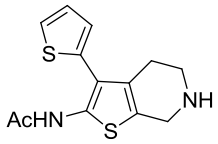


N-(3-(naphthalen-1-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (12): LC-MS: rt (min) = 3.79; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.91 (s, 3 H), 2.13 - 2.34 (m, 2 H), 3.30 – 3.38 (m, 2 H), 4.31 - 4.39 (m, 2 H), 7.33 (d, *J* = 6.1 Hz, 1 H), 7.40 - 7.51 (m,

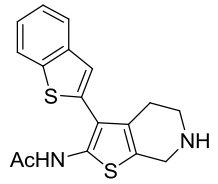
2 H), 7.53 - 7.64 (m, 2 H), 8.02 – 8.04 (m, 2 H) and 10.06 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{19}H_{20}N_2OS$, 323.1213; found 323.1215.



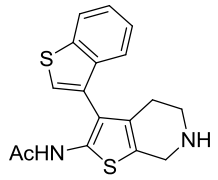
***N*-(3-(furan-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (13):** LC-MS: rt (min) = 3.07; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.16 (s, 3 H), 2.78 - 2.86 (m, 2 H), 3.39 – 3.45 (m, 2 H), 4.28 (s, 2 H), 6.58 - 6.66 (m, 2 H), 7.76 – 7.85 (m, 1 H), and 10.26 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{13}H_{15}N_2O_2S$, 263.0849; found 263.0849.



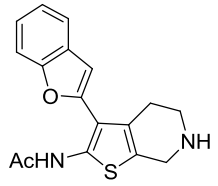
***N*-(3-(thiophen-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (14):** LC-MS: rt (min) = 3.14; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (s, 3 H), 2.63 - 2.72 (m, 2 H), 3.01 – 3.30 (m, 3 H), 4.23 - 4.33 (m, 2 H), 7.04 - 7.12 (m, 1 H), 7.17 - 7.25 (m, 1 H), 7.64 - 7.71 (m, 1 H) and 10.24 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{13}H_{15}N_2OS_2$, 279.062; found 279.0618.



***N*-(3-(benzo[b]thiophen-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (15):** LC-MS: rt (min) = 3.94; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.07 (s, 3 H), 2.72 – 2.76 (m, 2 H), 3.32 - 3.43 (m, 2 H), 4.30 (s, 2 H), 7-37 - 7.41 (m, 3 H), 7.86 - 7.93 (m, 1 H), 7.98 - 8.05 (m, 1 H) and 10.48 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{17}H_{17}N_2OS_2$, 329.0777; found 329.0767.

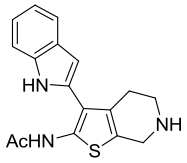


***N*-(3-(benzo[b]thiophen-3-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (16):** LC-MS: rt (min) = 3.79; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.95 (s, 3 H), 2.32 – 2.46 (m, 2 H), 3.35 - 3.41 (m, 2 H), 4.32 (s, 2 H), 7.25 - 7.35 (m, 1 H), 7.36 - 7.47 (m, 2 H), 7.73 (s, 1H), 8.01 - 8.13 (m, 1 H) and 10.23 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{17}H_{17}N_2OS_2$, 329.0777; found 329.0773.

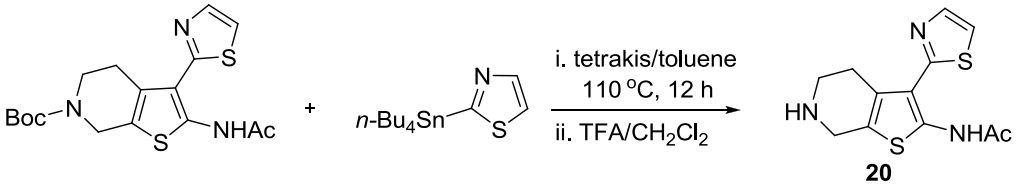


***N*-(3-(benzofuran-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (17):** LC-MS: rt (min) = 3.89; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.15 (s, 3 H), 2.85 - 2.94 (m,

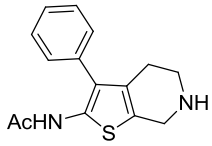
2 H), 3.38 - 3.47 (m, 2 H), 4.31 (s, 2 H), 7.04 (s, 1 H), 7.31 (ddd, *J* = 12.7, 7.6 and 1.2 Hz, 2 H), 7.62 (d, *J* = 7.8 Hz, 1 H), 7.68 (d, *J* = 7.2 Hz, 1 H) and 10.57 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{17}H_{17}N_2O_2S$, 313.1005; found 313.1006.



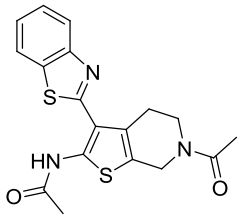
***N*-(3-(1H-indol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (18):** LC-MS: rt (min) = 3.77; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.08 (s, 3 H), 2.72 - 2.80 (m, 2 H), 3.34 - 3.43 (m, 2 H), 4.31 (s, 2 H), 6.43 - 6.48 (m, 1 H), 6.99 - 7.05 (m, 1 H), 7.08 - 7.15 (m, 1 H), 7.38 - 7.43 (m, 1 H), 7.54 - 7.59 (m, 1 H), 10.39 (m, 1 H) and 11.19 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{17}H_{17}N_3OS$, 312.1165; found 312.1165.



***N*-(3-(thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (20):** A mixture of t-butyl 2-acetamido-3-bromo-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (0.1 g, 0.27 mmol, 1 eq) and 2-(tributylstannyl)thiazole (0.084 mL, 0.27 mmol, 1 eq) in toluene (2 ml) was bubbled with argon for 5 minutes. Tetrakis (0.031 g, 0.027 mmol, 0.1 eq) was then added and stirred for 12 h at 110 °C. Excess solvent solvent was removed and the residue was dissolved in DCM and treated with TFA. The crude product was finally purified on a preparative HPLC. LC-MS: rt (min) = 3.35; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3 H), 3.03 - 3.11 (m, 2 H), 3.07 (t, *J* = 5.7 Hz, 2 H), 4.49 (t, *J* = 5.7 Hz, 2 H), 7.85 (d, *J* = 3.3 Hz, 1 H), 8.06 (d, *J* = 3.3 Hz, 1 H) and 12.34 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{12}H_{14}N_3OS_2$, 280.0573; found 280.0573.

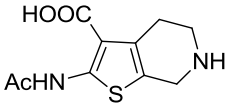


***N*-(3-phenyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (22):** LC-MS: rt (min) = 3.25; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.01 (s, 3 H), 2.53 - 2.59 (m, 2 H), 3.31 – 3.36 (m, 2 H), 4.29 (s, 2 H), 7.26 (d, *J* = 7.0 Hz, 2 H), 7.38 - 7.42 (m, 1 H), 7.45 – 7.51 (m, 2 H) and 10.10 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{16}H_{17}N_2OS$, 273.1056; found 273.1056.

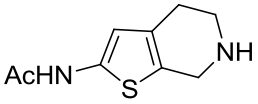


***N*-(6-acetyl-3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (8):** This compound was prepared by acetylation of *N*-(3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide with acetyl chloride. LC-MS: rt (min) = 5.87; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.15 (s, 3 H), 2.33 (s, 3 H), 2.89 –

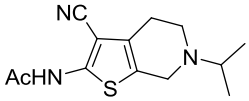
2.95 (m, 2 H), 3.01 - 3.06 (m, 2 H), 4.63 (s, 2 H), 7.47 (t, *J* = 7.5 Hz, 1 H), 7.59 (t, *J* = 7.6 Hz, 1 H), 8.16 (dd, *J* = 7.9 and 3.8 Hz, 2 H) and 12.53 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{18}H_{18}N_3O_2S_2$, 372.0835; found 372.0826.



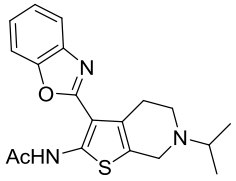
2-Acetamido-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylic acid (9): This compound was prepared by de-protecting **9a** using procedure C. LC-MS: rt (min) = 3.25; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.25 (s, 3 H), 2.99 (t, *J* = 5.5 Hz, 2 H), 3.33 - 3.40 (m, 2 H), 4.24 (s, 2 H), 11.19 (s, 1 H) and 13.44 (brs, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{10}H_{13}N_2O_3S$, 241.0641; found 241.0640.



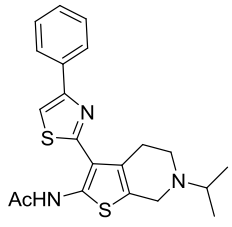
***N*-(4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (10):** This compound was prepared by de-protecting **10a** using procedure C. LC-MS: rt (min) = 1.33; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.05 (s, 3 H), 2.76 (t, *J* = 5.8 Hz, 2 H), 3.35 (t, *J* = 6.2 Hz, 2 H), 4.22 (s, 2 H), 6.40 (s, 1 H) and 11.19 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_9H_{13}N_2OS$, 197.0743; found 197.0744.



***N*-(3-cyano-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (21):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 2.78; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (d, *J* = 5.5 Hz, 6 H), 2.22 (s, 3 H), 2.88 - 2.95 (m, 2 H), 3.39 – 3.45 (m, 1H), 3.63 - 3.80 (m, 2 H), 4.31 – 4.47 (m, 2 H), and 11.93 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{13}H_{18}N_3OS$, 264.1165; found 264.1165.

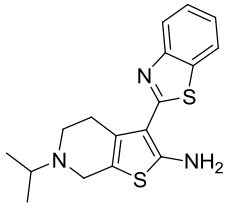


***N*-(3-(benzo[d]oxazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (23):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 4.24; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 (d, *J* = 6.5 Hz, 6 H), 2.32 (s, 3 H), 2.76 - 2.82 (m, 2 H), 2.84 - 2.95 (m, 1 H), 2.96 - 3.05 (m, 2 H), 3.61 (s, 2 H), 7.38 - 7.46 (m, 2 H), 7.73 - 7.78 (m, 1 H), 7.79 - 7.84 (m, 1 H) and 11.65 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{19}H_{22}N_3O_2S$, 356.1427; found 356.1430.

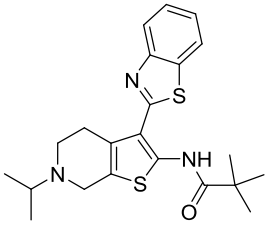


***N*-(6-isopropyl-3-(4-phenylthiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (24):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 4.72; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (d, *J* = 5.3 Hz, 6 H), 2.33 (s, 3 H),

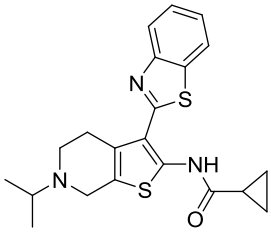
3.13 - 3.22 (m, 1 H), 3.24 - 3.32 (m, 2 H), 3.64 – 3.89 (m, 2 H), 4.40 – 4.55 (m, 2 H), 7.39 - 7.47 (m, 1 H), 7.53 (t, *J* = 7.6 Hz, 2 H), 8.03 (d, *J* = 7.4 Hz, 2 H), 8.24 (s, 1 H) and 12.42 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{21}H_{24}N_3OS_2$, 398.1355; found 398.1361.



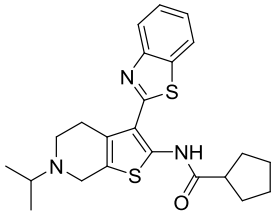
3-(Benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-amine (25): This compound was prepared following Procedures A, C and D. LC-MS: rt (min) = 4.88; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (d, *J* = 6.3 Hz, 6 H), 3.11 (t, *J* = 5.4 Hz, 2 H), 3.43 - 3.52 (m, 2 H), 3.57 (m, 1 H), 4.23 (s, 2 H), 7.35 (t, *J* = 7.5 Hz, 1 H), 7.49 (t, *J* = 7.6 Hz, 1 H), 7.92 (d, *J* = 8.0 Hz, 1 H), 8.04 (d, *J* = 7.8 Hz, 1 H) and 9.26 (brs, 2 H), HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{17}H_{20}N_3S_2$, 330.1093; found 330.1100.



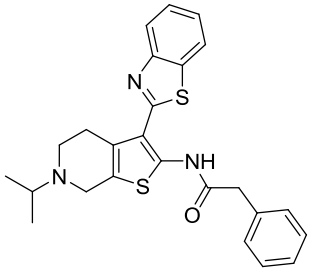
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)pivalamide (26):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 4.82; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 - 1.41 (m, 15 H), 3.21 - 3.31 (m, 1 H), 3.38 - 3.48 (m, 2 H), 3.67 - 3.94 (m, 2 H), 4.40 - 4.61 (m, 2 H), 7.52 (t, *J* = 7.6 Hz, 1 H), 7.64 (t, *J* = 7.7 Hz, 1 H), 8.01 (d, *J* = 8.0 Hz, 1 H), 8.22 (d, *J* = 7.8 Hz, 1 H) and 13.24 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{22}H_{28}N_3OS_2$, 414.1668; found 414.1677.



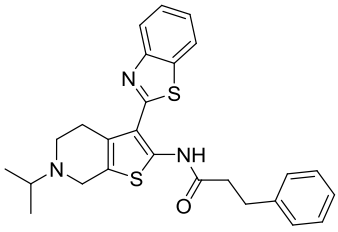
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)cyclopropanecarboxamide (27):** This compound was prepared following Procedures A-D. LC-MS: rt (min) =4.82 ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.98 - 1.06 (m, 4 H), 1.35 (brs, 6 H), 2.11 - 2.19 (m, 1 H), 3.16 - 3.29 (m, 1 H), 3.36 - 3.46 (m, 2 H), 3.64 - 3.92 (m, 2 H), 4.36 - 4.60 (m, 2 H), 7.51 (t, *J* = 7.5 Hz, 1 H), 7.62 (t, *J* = 7.6 Hz, 1 H), 8.21 (t, *J* = 8.2 Hz, 2 H) and 12.84 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{21}H_{24}N_3OS_2$, 398.1355; found 398.1358.



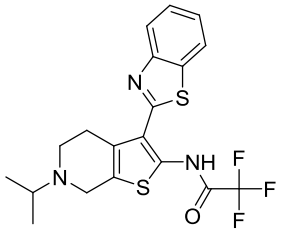
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)cyclopentanecarboxamide (28):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 5.31; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (d, *J* = 5.7 Hz, 6 H), 1.64 - 1.79 (m, 4 H), 1.79 - 1.90 (m, 2 H), 2.03 - 2.13 (m, 2 H), 3.13 (quin, *J* = 8.1 Hz, 1 H), 3.21 - 3.30 (m, 1 H), 3.36 - 3.44 (m, 2 H), 3.66 - 3.93 (m, 2 H), 4.39 - 4.61 (m, 2 H), 7.51 (t, *J* = 7.5 Hz, 1 H), 7.63 (t, *J* = 7.6 Hz, 1 H), 8.09 - 8.24 (m, 2 H) and 12.81 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{23}H_{28}N_3OS_2$, 426.1668; found 426.1678.



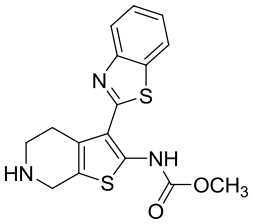
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-2-phenylacetamide (29):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 5.15; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (d, *J* = 5.3 Hz, 6 H), 3.17 - 3.25 (m, 1 H), 3.26 - 3.39 (m, 2 H), 3.66 - 3.91 (m, 2 H), 4.04 (s, 2 H), 4.37 - 4.60 (m, 2 H), 7.32 - 7.40 (m, 1 H), 7.40 - 7.52 (m, 5 H), 7.61 (t, *J* = 7.6 Hz, 1 H), 7.91 (d, *J* = 8.2 Hz, 1 H), 8.16 (d, *J* = 8.0 Hz, 1 H) and 12.52 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{25}H_{26}N_3OS_2$, 448.1512; found 448.1521.



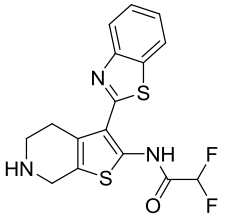
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-3-phenylpropanamide (30):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 5.35; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (d, *J* = 5.7 Hz, 6 H), 2.95 - 3.01 (m, 2 H), 3.02 - 3.09 (m, 2 H), 3.16 - 3.27 (m, 1 H), 3.37 - 3.46 (m, 2 H), 3.67 - 3.92 (m, 2 H), 4.35 - 4.60 (m, 2 H), 7.11 - 7.20 (m, 1 H), 7.22 - 7.36 (m, 4 H), 7.50 (t, *J* = 7.6 Hz, 1 H), 7.61 (t, *J* = 7.6 Hz, 1 H), 8.17 (dd, *J* = 16.0 and 8.0 Hz, 2 H) and 12.59 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{26}H_{28}N_3OS_2$, 462.1668; found 462.1661.



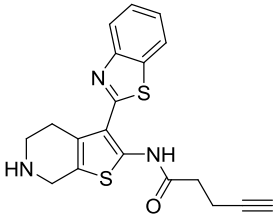
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-2,2,2-trifluoroacetamide (31):** This compound was prepared following Procedure A followed by EDC coupling with TFA and then Procedures C-D. LC-MS: rt (min) = 5.18; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (dd, *J* = 6.3 and 3.3 Hz, 6 H), 3.23 - 3.446 (m, 3 H, merged in water peak), 3.55 - 3.88 (m, 2 H), 4.41 – 4.58 (m, 2 H), 7.38 - 7.55 (m, 2 H) and 7.76 - 8.25 (m, 2 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{19}H_{19}F_3N_3OS_2$, 426.0916; found 426.0926.



Methyl 3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-ylcarbamate (32): This compound was prepared following Procedures A-C. LC-MS: rt (min) = 4.24; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.14 - 3.22 (m, 2 H), 3.46 - 3.54 (m, 2 H), 3.83 (s, 3 H), 4.36 (s, 2 H), 7.49 (t, *J* = 7.5 Hz, 1 H), 7.59 (t, *J* = 7.6 Hz, 1 H), 8.05 (d, *J* = 8.0 Hz, 1 H), 8.18 (d, *J* = 8.0 Hz, 1 H), 9.29 (brs, 1 H) and 11.88 (brs, 1H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{16}H_{16}N_3O_2S_2$, 346.0678; found 346.0683.

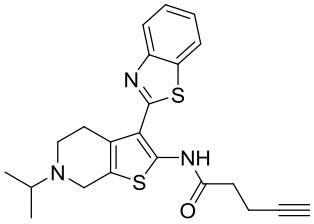


***N*-(3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-2,2-difluoro-acetamide (33):** This compound was prepared following Procedure A followed by EDC coupling with difluoroacetic acid and then Procedures C. LC-MS: rt (min) = 4.69; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.18 - 3.28 (m, 2 H), 3.55 (t, *J* = 5.8 Hz, 2 H), 4.41 (s, 2 H), 7.53 (t, *J* = 7.4 Hz, 1 H), 7.63 (t, *J* = 7.5 Hz, 1 H), 8.02 (d, *J* = 8.0 Hz, 1 H), 8.22 (d, *J* = 7.8 Hz, 1 H) and 13.64 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{16}H_{14}F_2N_3OS_2$, 366.0541; found 366.0546.

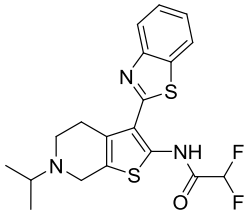


***N*-(3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)pent-4-ynamide (34):** This compound was prepared following Procedure A-C. LC-MS: rt (min)

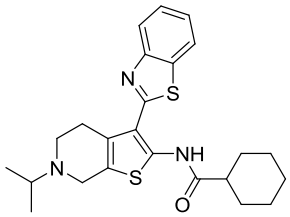
= 4.58; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 - 2.71 (m, 2 H), 2.82 - 2.90 (m, 3 H), 3.14 - 3.23 (m, 2 H), 3.49 – 3.55 (m, 2 H), 4.36 (s, 2 H), 7.50 (t, *J* = 7.7 Hz, 1 H), 7.62 (t, *J* = 7.7 Hz, 1 H), 8.19 (dd, *J* = 8.1 and 2.5 Hz, 2 H) and 12.64 (s, 1 H); HRMS (ESI) *m*/*z* $(M+Na)^+$ calcd. for $C_{19}H_{17}N_3OS_2Na$, 390.0705; found 390.0707.



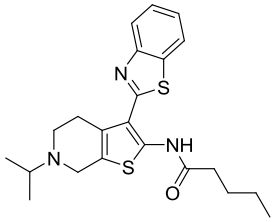
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)pent-4-ynamide (35):** This compound was prepared following Procedure A-D. LC-MS: rt (min) = 4.76; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (d, *J* = 5.4 Hz, 6 H), 2.60 (td, *J* = 7.0 and 2.4 Hz, 2 H), 2.83 - 2.95 (m, 3 H), 3.17 - 3.28 (m, 1 H), 3.67 - 3.91 (m, 2 H), 4.40 - 4.63 (m, 2 H), 7.51 (t, *J* = 7.6 Hz, 1 H), 7.62 (t, *J* = 7.6 Hz, 1 H), 8.20 (dd, *J* = 8.0 and 3.7 Hz, 2 H) and 12.63 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{22}H_{24}N_3OS_2$, 410.1355; found 410.1355.



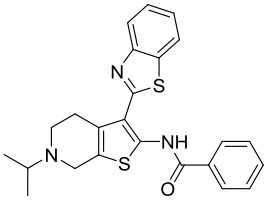
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-2,2-difluoroacetamide(36):** This compound was prepared following Procedure A followed by EDC coupling with difluoroacetic acid and then Procedures C-D. LC-MS: rt (min) = 4.87; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (d, *J* = 5.3 Hz, 6 H), 3.20 - 3.29 (m, 1 H), 3.65 - 3.95 (m, 2 H), 4.47 - 4.61 (m, 2 H), 7.50 - 7.69 (m, 2 H), 8.00 - 8.29 (m, 2 H) and 13.63 (m, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{19}H_{20}N_3OS_2$, 408.1010; found 408.1010.



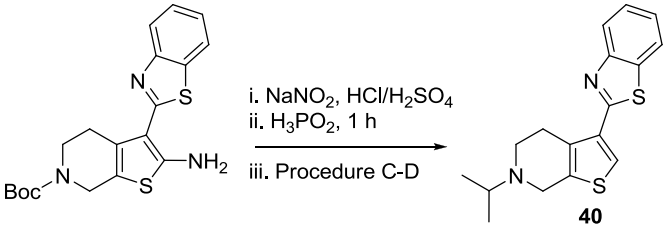
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)cyclohexanecarboxamide (37):** This compound was prepared following Procedure A-D. LC-MS: rt (min) = 5.51; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 - 1.62 (m, 12 H), 1.74 - 1.87 (m, 2 H), 2.04 - 2.10 (m, 2 H), 2.58 - 2.67 (m, 1 H), 3.26 - 3.34 (m, 3 H), 3.52 - 3.85 (m, 2 H), 4.02 - 4.45 (m, 2 H), 7.50 (t, *J* = 7.5 Hz, 1 H), 7.63 (t, *J* = 7.6 Hz, 1 H), 8.01 - 8.23 (m, 2 H) and 12.89 (s, 1 H); HRMS (ESI) m/z $(M+H)^+$ calcd. for $C_{24}H_{30}N_3OS_2$, 440.1825; found 440.1823.



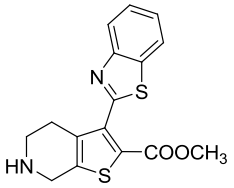
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)pentanamide (38):** This compound was prepared following Procedure A-D. LC-MS: rt (min) = 5.19; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.95 (t, *J* = 7.3 Hz, 3 H), 1.29 - 1.49 (m, 8 H), 1.66 - 1.80 (m, 2 H), 2.65 (t, *J* = 7.5 Hz, 2 H), 3.17 - 3.28 (m, 1 H), 3.35 - 3.49 (m, 2 H), 3.68 - 3.93 (m, 2 H), 4.37 - 4.59 (m, 2 H), 7.51 (t, *J* = 7.5 Hz, 1 H), 7.63 (t, *J* = 7.6 Hz, 1 H), 8.03 - 8.32 (m, 2 H) and 12.67 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{22}H_{28}N_3OS_2$, 414.1668; found 414.1675.



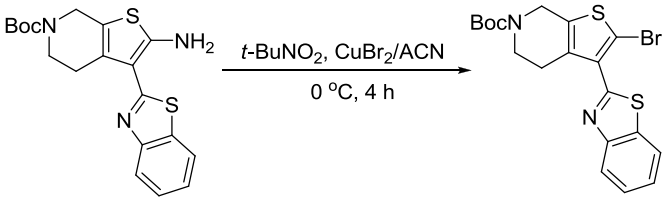
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)benzamide (39):** This compound was prepared following Procedure A-D. LC-MS: rt (min) = 5.27; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (d, *J* = 5.3 Hz, 6 H), 3.35 - 3.51 (m, 3 H), 3.69 - 3.97 (m, 2 H), 4.40 - 4.69 (m, 2 H), 7.55 (t, *J* = 7.6 Hz, 1 H), 7.68 (t, *J* = 7.6 Hz, 1 H), 7.74 - 7.82 (m, 3 H), 8.12 - 8.27 (m, 4 H) and 13.92 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{24}H_{24}N_3OS_2$, 434.1355; found 434.1355.



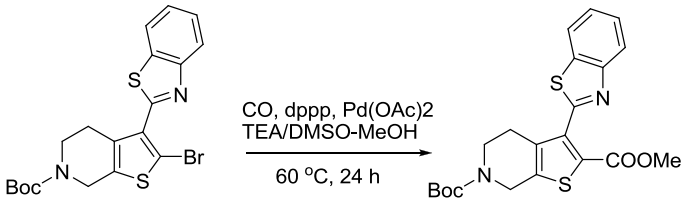
2-(4,5,6,7-Tetrahydrothieno[2,3-c]pyridin-3-yl)benzo[d]thiazole (40): t-Butyl 2-amino-3-(benzo[d]thiazol-2-yl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (1 g, 2.58 mmol, 1 eq) and hydrochloric acid (0.16 mL, 5.16 mmol, 2 eq) in sulfuric acid (2 mL) was slowly added sodium nitrite (0.27 g, 3.9 mmol, 1.5 eq) at 0 ^{o}C. After stirring for 1 h at 0 ^{o}C, phosphinic acid (1.70 g, 25.8 mmol, 10 eq) was added and stirred further an hour. The reaction mixture was slowly neutralized with saturated bicarbonate and the product was collected by filtration. This crude product was converted into compound **40** following Procedures C-D and further purified in a preparative HPLC. LC-MS: rt (min) = 1.33; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (d, *J* = 5.5 Hz, 6 H), 3. 49 - 3.58 (m, 1 H), 3.67 - 3.88 (m, 2 H), 4.47 - 4.72 (m, 2 H), 7.48 (t, *J* = 7.6 Hz, 1 H), 7.55 (t, *J* = 7.6 Hz, 1 H), 8.03 (d, *J* = 8.0 Hz, 1 H), 8.15 (d, *J* = 7.8 Hz, 1 H) and 8.38 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{17}H_{19}N_2S_2$, 315.0984; found 315.0986.



Methyl 3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-2-carboxylate (41): This compound was prepared from **41a** following Procedure C. LC-MS: rt (min) = 3.72; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.73 -2.81 (m, 2 H), 3.38 - 3.41 (m, 2 H), 3.74 (s, 3 H), 4.51 (s, 2 H), 7.51 - 7.64 (m, 2 H), 8.10 (d, J = 7.8 Hz, 1 H), 8.21 (d, J = 7.8 Hz, 1 H) and 9.17 (br, 1 H); HRMS (ESI) m/z $(M+H)^+$ calcd. for $C_{16}H_{15}N_2O_2S_2$, 331.0569; found 331.0575.



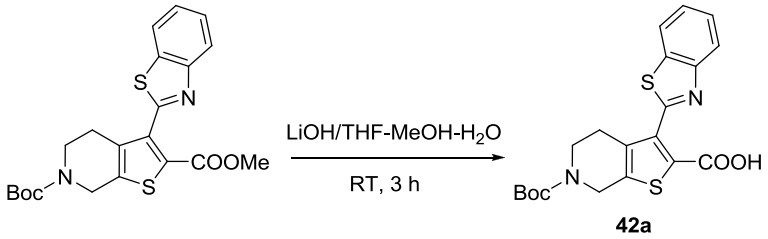
A suspension of copper(II) bromide (4.64 g, 20.77 mmol, 1.4 eq) in acetonitrile (100 mL) was added tert-butyl nitrite (2.6 mL, 19.3 mmol, 1.3 eq) slowly and then tert-butyl 2-amino-3-(benzo[d]thiazol-2-yl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (5.75 g, 14.8 mmol, 1 eq) was added portion wise at 0 °C. The reaction mixture was warmed to room temperature with stirring over 4 h. The execss solvent was removed under diminished pressure and the crude residue was purified on a Biotage® silica gel column. Elution with 5% ethyl acetate in hexanes gave the product. Yield: 54 % (3.62 g). LC-MS: rt (min) = 4.13 (4.5 min run); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (s, 9 H), 2.87 - 2.92 (m, 2 H), 3.57 - 3.63 (m, 2 H), 4.56 (s, 2 H), 7.48 - 7.63 (m, 2 H) and 8.07 - 8.22 (m, 2 H); HRMS (ESI) m/z $(M+H)^+$ calcd. for $C_{19}H_{20}BrN_2O_2S_2$, 451.0144; found 451.0159.



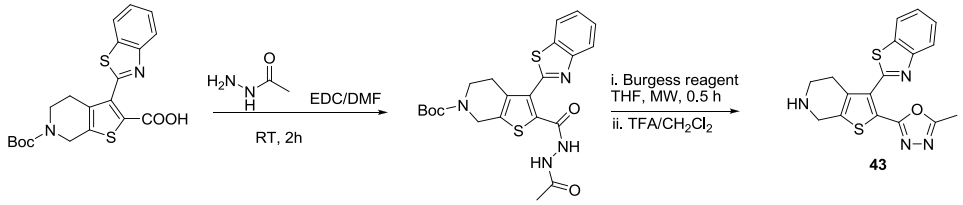
41a

Synthesis of 6-tert-butyl 2-methyl 3-(benzo[d]thiazol-2-yl)-4,5-dihydrothieno[2,3-c]pyridine-2,6(7H)-dicarboxylate (41a): A mixture of tert-butyl 3-(benzo[d]thiazol-2-yl)-2-bromo-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (1.25 g, 2.77 mmol, 1 eq) 1,3-bis(diphenylphosphino)propane (0.23 g, 0.55 mmol, 0.2 eq) and TEA (1.5 mL, 11.1 mmol, 4 eq). in MeOH (20 mL) and DMSO (20 mL) was bubbled with argon for 10 minutes. Palladium(II) acetate (0.12 g, 0.55 mmol, 0.2 eq) was then added and the reaction mixture was saturated with CO and stirred for 24 h with a supply of CO(g) (ballon) at 60 °C. The crude reaction mixture was extracted with ethyl acetate. The organic layer was washed with water, ammonium chloride, and brine, and dried over sodium sulfate. The crude residue obtained after evaporation of the solvent was purified on a Biotage® silica gel column. Elution with 10% ethyl acetate in hexanes gave the

product. Yield: 69 % (0.823 g). LC-MS: rt (min) = 3.39 (4.5 min run); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 - 1.50 (s, 9 H), 3.55 - 3.62 (m, 2 H), 3.67 - 3.74 (m, 5 H), 4.69 (s, 2 H), 7.47 - 7.62 (m, 2 H) and 8.05 - 8.22 (m, 2 H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{21}H_{23}N_2O_4S_2$, 431.1094; found 431.1107.



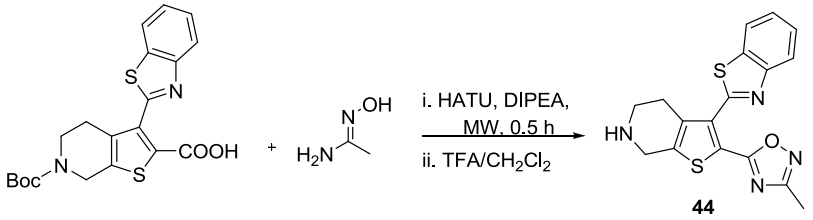
3-(benzo[d]thiazol-2-yl)-6-(t-butoxycarbonyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-2-carboxylic acid (42a): A solution of 6-tert-butyl 2-methyl 3-(benzo[d]thiazol-2-yl)-4,5-dihydrothieno[2,3-c]pyridine-2,6(7H)-dicarboxylate (1.3 g, 3.02 mmol, 1 eq) in THF/MeOH (3/1, 18 mL) was added lithium hydroxide (0.362 g, 15.1 mmol, 5 eq) in water (2 mL) and stirred at room temperature for 3 h. The reaction mixture was neutralized with 5 % HCl and extracted with ethyl acetate. The organic layer was subsequently washed with water, brine and dried over sodium sulfate. The crude product obtained after evaporation of the solvent was dried under vacuum which is sufficiently pure.



2-(3-(Benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-5-methyl-1,3,4-oxadiazole (43): A mixture of 3-(benzo[d]thiazol-2-yl)-6-(tert-butoxycarbonyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-2-carboxylic acid (0.182 g, 0.44 mmol, 1 eq), acetylhydrazide (0.039 g, 0.52 mmol, 1 eq), EDC (0.25 g, 1.31 mmol, 3 eq) and DMAP (0.027 g, 0.22 mmol, 0.5 eq) in DMF (1.5 mL) was stirred at room temperature for 2 h. The reaction mixture was extracted with ethyl acetate and washed with water and brine. The organic layer was dried on sodium sulfate and concentrated under reduced pressure. The crude product was further dried under vacuum.

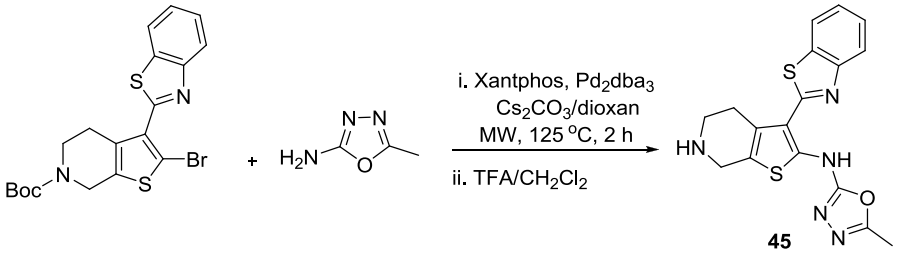
A mixture of the crude tert-butyl 2-(2-acetylhydrazinecarbonyl)-3-(benzo[d]thiazol-2-yl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (0.2 g, 0.42 mmol, 1 eq) and , Burgess reagent (0.15 g, 0.64 mmol, 1.5 eq) in THF (2 mL) was heated in microwave for 30 min at 140 ^{o}C. The product was purified in a preparative HPLC, deprotected with TFA following procedure D and again purified in a preparative HPLC.

LC-MS: rt (min) = 3.52; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.44 (s, 3 H), 2.95 (t, *J* = 5.6 Hz, 2 H), 3.41 - 3.51 (m, 2 H), 4.55 (s, 2 H), 7.49 - 7.65 (m, 2 H), 8.10 (d, *J* = 8.0 Hz, 1 H), 8.15 - 8.24 (m, 1 H) and 9.34 (brs, 1 H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{17}H_{15}N_4OS_2$, 355.0682; found 355.0690.



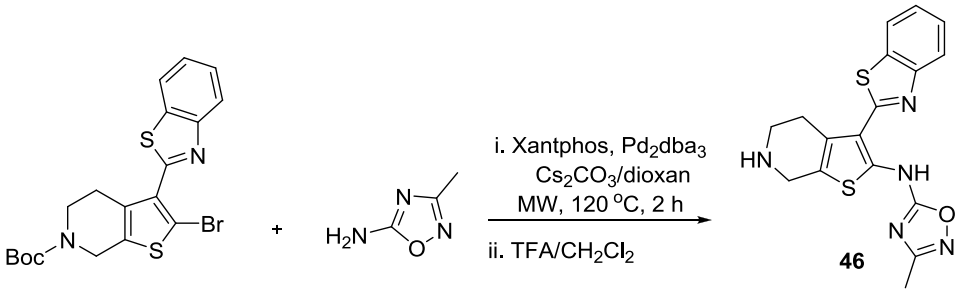
5-(3-(Benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-3-methyl-1,2,4-oxadiazole (44): A mixture of 3-(benzo[d]thiazol-2-yl)-6-(tert-butoxycarbonyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-2-carboxylic acid (0.162 g, 0.39 mmol, 1 eq), (Z)-*N*'-hydroxyacetimidamide (0.036 g, 0.48 mmol, 1.2 eq), HATU (0.163 g, 0.43 mmol, 1.1 eq) and DIPEA (0.18 mL, 1.03 mmol, 2.6 eq) in DMF (2 ml) was heated in the MW for 50 ^{o}C then 15 min then another 15 min at 150 ^{o}C. The product was purified in a preparative HPLC, de-protected with TFA following procedure D and again purified in a preparative HPLC.

LC-MS: rt (min) = 3.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.30 (s, 3 H), 2.83 - 2.95 (m, 2 H), 3.34 - 3.44 (m, 2 H), 4.51 (s, 2 H), 7.55 - 7.64 (m, 2 H), 8.12 (d, J = 8.0 Hz, 1 H) and 8.23 (d, J = 8.0 Hz, 1 H); HRMS (ESI) m/z $(M+H)^+$ calcd. for $C_{17}H_{15}N_4OS_2$, 355.0682; found 355.0682.



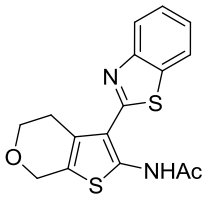
***N*-(3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-5-methyl-1,3,4-oxadiazol-2-amine (45):** A mixture of tert-butyl 3-(benzo[d]thiazol-2-yl)-2-bromo-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (0.2 g, 0.44 mmol, 1 eq), 5-methyl-1,3,4-oxadiazol-2-amine (0.066 g, 0.665 mmol, 1.5 eq), cesium carbonate (0.289 g, 0.89 mmol, 2 eq) and Xantphos (0.026 g, 0.044 mmol, 0.1 eq) in dioxane (3 mL) was bubbled with argon for 5 min and then $Pd_2(dba)_3$ (0.020 g, 0.022 mmol, 0.05 eq) was added and heated in a MW for 2 h at 125 ^{o}C. The reaction mixture was diluted with ethyl acetate and filtered through celite pad and concentrated. The crude product was purified on a Biotage® silica gel column. Elution with 40% ethyl acetate in hexanes gave the product which is further de-protected with TFA following procedure D and purified in a preparative HPLC.

LC-MS: rt (min) = 3.15; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.01 (s, 3 H), 2.94 - 3.03 (m, 2 H), 3.48 - 3.58 (m, 2 H), 4.25 (s, 2 H), 7.59 - 7.71 (m, 2 H), 8.18 (d, J = 8.0 Hz, 1 H), 9.15 - 9.37 (brs, 1 H), 9.77 (d, J = 8.4 Hz, 1 H) and 10.13 (s, 1 H); HRMS (ESI) m/z $(M+H)^+$ calcd. for $C_{17}H_{16}N_5OS_2$, 370.0791; found 370.0797.

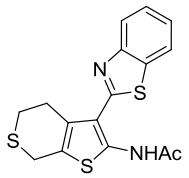


***N*-(3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-3-methyl-1,2,4-oxadiazol-5-amine (46):** A mixture of tert-butyl 3-(benzo[d]thiazol-2-yl)-2-bromo-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (0.15 g, 0.332 mmol, 1 eq), 3-methyl-1,2,4-oxadiazol-5-amine (0.049 g, 0.50 mmol, 1.5 eq), cesium carbonate (0.217 g, 0.66 mmol, 2 eq) and Xantphos (0.019 g, 0.033 mmol, 0.1 eq) in dioxan (3 mL) was bubbled with argon for 5 min and then $Pd_2(dba)_3$ (0.015 g, 0.017 mmol, 0.05 eq) was added and heated in a MW for 2 h at 120 ^oC. The reaction mixture was diluted with ethyl acetate and filtered through celite pad and concentrated. The crude product was purified on a Biotage® silica gel column. Elution with 25% ethyl acetate in hexanes gave the product which is further de-protected with TFA following procedure D and purified in a preparative HPLC.

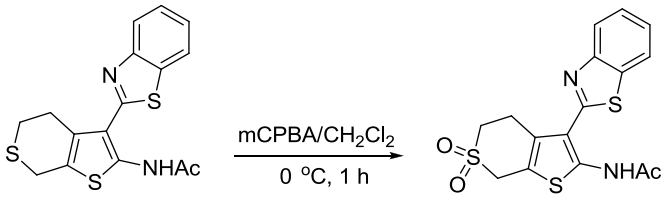
LC-MS: rt (min) = 4.28; ^1H NMR (400 MHz, DMSO-d_6) δ 2.23 (s, 3 H), 3.22 - 3.35 (m, 2 H), 3.39 - 3.49 (m, 2 H), 4.37 (s, 2 H), 7.41 - 7.61 (m, 2 H), 7.95 - 8.16 (m, 2 H) and 9.19 (brs, 1 H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{17}H_{16}N_5OS_2$, 370.0791; found 370.0795.



***N*-(3-(benzo[d]thiazol-2-yl)-5,7-dihydro-4H-thieno[2,3-c]pyran-2-yl)acetamide (47):**This compound was prepared following Procedures A-B. LC-MS: rt (min) = 6.63; ^1H NMR (400 MHz, DMSO-d_6) δ 2.34 (s, 3 H), 2.95 (t, *J* = 5.2 Hz, 2 H), 3.98 (t, *J* = 5.5 Hz, 2 H), 4.71 (s, 2 H), 7.47 (t, *J* = 7.7 Hz, 1 H), 7.59 (t, *J* = 7.6 Hz, 1 H), 8.16 (d, *J* = 8.2 Hz, 2 H) and 12.56 (s, 1 H); HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{16}H_{15}N_2O_2S_2$, 331.0569; found 331.0572.

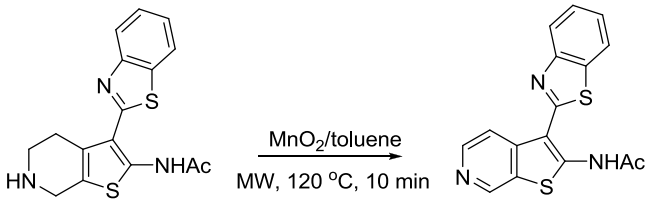


***N*-(3-(benzo[d]thiazol-2-yl)-5,7-dihydro-4H-thieno[2,3-c]thiopyran-2-yl)acetamide (48):** This compound was prepared following Procedures A-B. LC-MS: rt (min) = 7.17; ^1H NMR (400 MHz, DMSO-d_6) δ 2.30 (s, 3 H), 3.02 (m, 2 H), 3.06 - 3.12 (m, 2 H), 3.82 (s, 2 H), 7.47 (t, *J* = 7.6 Hz, 1 H), 7.58 (t, *J* = 7.6 Hz, 1 H), 8.14 (dd, *J* = 8.0 Hz and 2.9 Hz, 2 H) and 12.55 (s, 1 H) ; HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{16}H_{15}N_2OS_3$, 347.0341; found 347.0352.



48 49

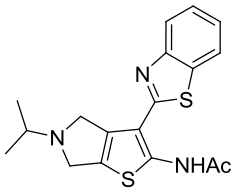
***N*-[3-(1,3-benzothiazol-2-yl)-6,6-dioxido-4,7-dihydro-5H-thieno[2,3-c]thiopyran-2-yl]acetamide (49):** LC-MS: rt (min) = 5.66; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.30 (s, 3 H), 3.42 (t, *J* = 5.9 Hz, 2 H), 3.53 (t, *J* = 6.0 Hz, 2 H), 4.55 (s, 2 H), 7.50 (t, *J* = 7.6 Hz, 1 H), 7.60 (t, *J* = 7.6 Hz, 1 H), 8.18 (dd, *J* = 8.0 and 4.1 Hz, 2 H) and 12.36 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{16}H_{15}N_2O_3S_3$, 379.0239; found 379.0235.



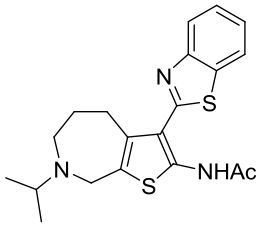
4 50

***N*-(3-(benzo[d]thiazol-2-yl)thieno[2,3-c]pyridin-2-yl)acetamide (50):** A mixture of *N*-(3-(benzo[d]thiazol-2-yl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide (0.1 g, 0.30 mmol, 1 eq) and manganese dioxide (0.132 g, 1.52 mmol, 5 eq) in toluene (4.0 mL) was heated in a MW for 10 min at 120 °C. The crude product obtained after filtration and removal of manganese dioxide was purified in a preparative HPLC.

LC-MS: rt (min) = 4.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.43 (s, 3 H), 7.48 - 7.556 (m, 1 H), 7.60 - 7.67 (m, 1 H), 8.09 - 8.28 (m, 3 H), 8.57 - 8.62 (m, 1 H), 9.19 (s, 1 H) and 13.14 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{16}H_{12}N_3OS_2$, 326.0416; found 326.0420.

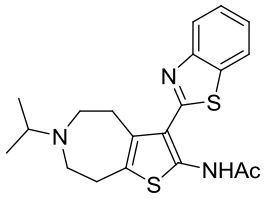


***N*-(3-(benzo[d]thiazol-2-yl)-5-isopropyl-5,6-dihydro-4H-thieno[3,2-c]pyrrol-2-yl)acetamide (51):** This compound was prepared following Procedures A-D. LC-MS: rt (min) = 4.33; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (d, *J* = 6.3 Hz, 6 H), 2.36 (s, 3 H), 3.83 (ddd, *J* = 12.5, 6.2 and 6.0 Hz, 1 H), 4.69 (s, 2 H), 4.84 (s, 2 H), 7.50 (t, *J* = 7.4 Hz, 1 H), 7.61 (t, *J* = 7.3 Hz, 1 H), 8.17 (d, *J* = 8.2 Hz, 2 H) and 12.05 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{18}H_{20}N_3OS_2$, 358.1042; found 358.1054.



***N*-(3-(benzo[d]thiazol-2-yl)-7-isopropyl-5,6,7,8-tetrahydro-4H-thieno[2,3-c]azepin-2-yl)acetamide (54):** This compound was prepared following Procedures A-D (A 50 %

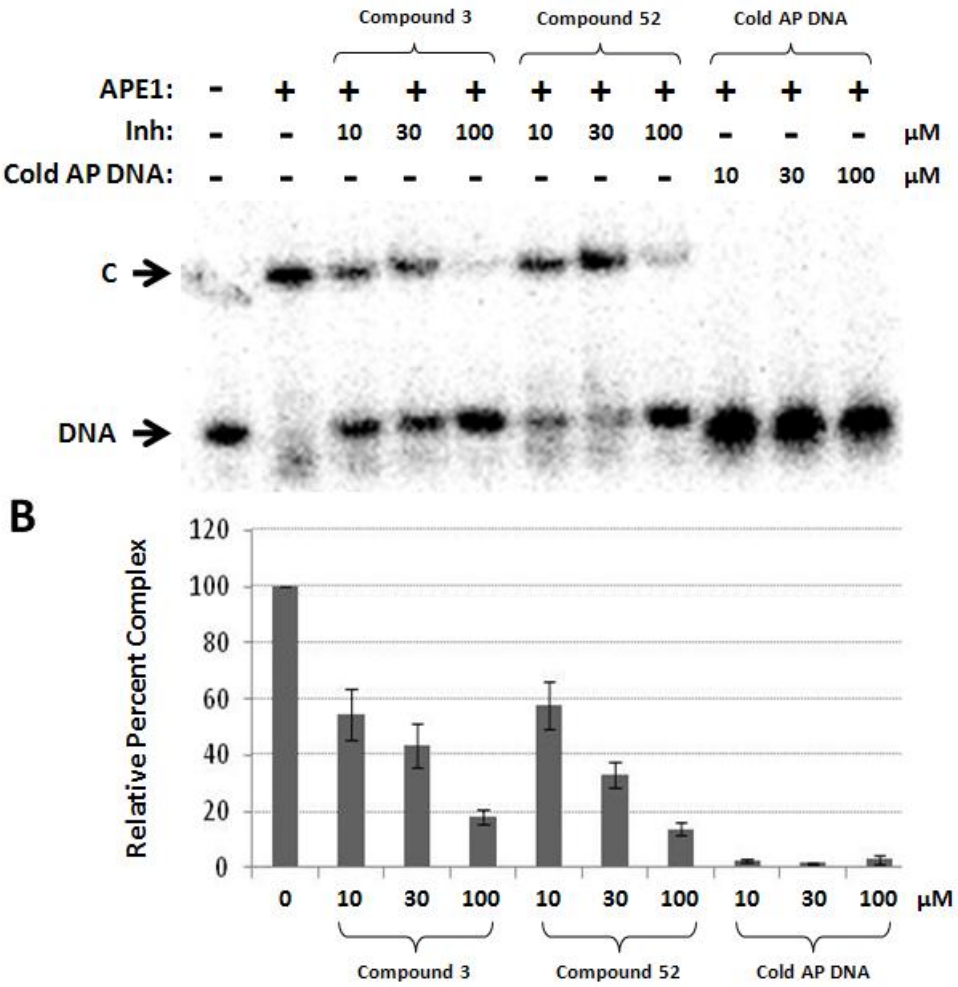
mixture of **54** and **55** which were separated in HPLC after Procedure D) . LC-MS: rt (min) = 4.50; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.01 (d, *J* = 6.3 Hz, 6 H), 1.69 - 1.78 (m, 2 H), 2.20 (s, 3 H), 2.82 (t, *J* = 5.7 Hz, 1 H), 2.96 - 3.42 (m, 4 H), 3.75 (s, 2 H), 7.47 (t, *J* = 7.5 Hz, 1 H), 7.54 - 7.58 (m, 1 H), 8.10 - 8.17 (m, 2 H) and 11.82 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ $C_{20}H_{24}N_3OS_2$, 386.1355; found 386.1372.



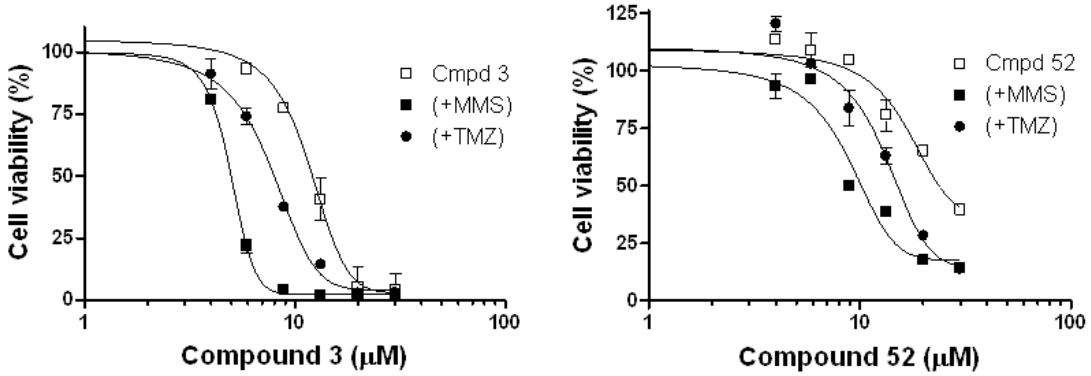
***N*-(3-(benzo[d]thiazol-2-yl)-6-isopropyl-5,6,7,8-tetrahydro-4H-thieno[3,2-d]azepin-2-yl)acetamide (55):** LC-MS: rt (min) = 4.38; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.98 (d, *J* = 6.5 Hz, 6 H), 2.19 (s, 3 H), 2.65 - 2.74 (m, 4 H), 2.79 - 2.86 (m, 2 H), 2.92 - 3.04 (m, 3 H), 7.47 (t, *J* = 7.5 Hz, 1 H), 7.52 - 7.63 (m, 1 H), 8.13 (t, *J* = 7.1 Hz, 2 H) and 11.70 (s, 1 H); HRMS (ESI) *m*/*z* $(M+H)^+$ calcd. for $C_{20}H_{24}N_3OS_2$, 386.1355; found 386.1360.

Reference:

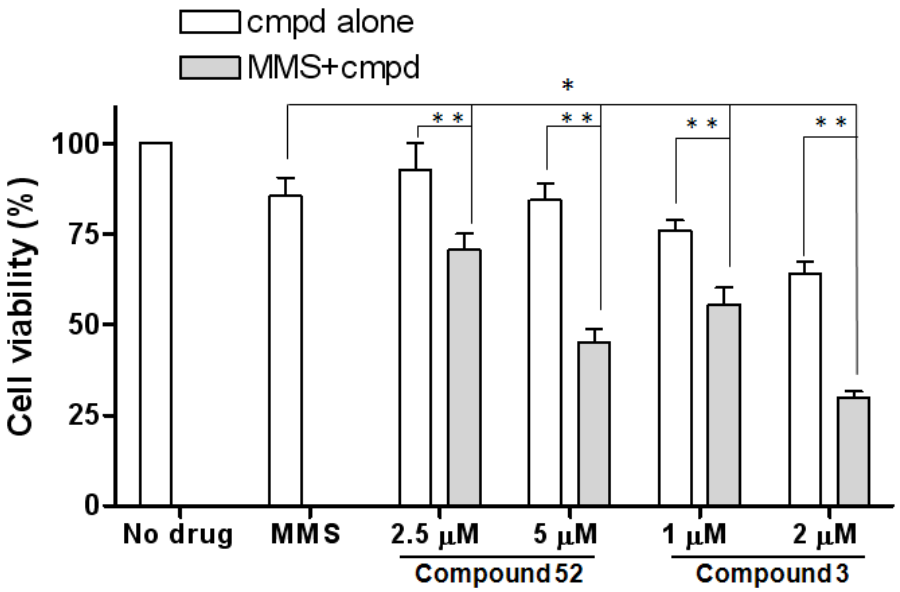
1) Andersen, H. S.; Olsen, O.H.; Iversen, L. F.; Sørensen, A. L. P.; Mortensen, S. B.; Christensen, M. S.; Branner, S.; Hansen, T. K.; Lau, J. F.; Jeppesen L.; Moran, E. J.; Su, J.; Bakir, F.; Judge, L.; Shahbaz, M.; Collins, T.; Vo, T.; Newman, M. J.; Ripka, W. C.; Møller, N. P. H. *J. Med. Chem.*, **2002**, *45*, 4443–4459.



Supplementary Figure 1. Binding of APE1 to inhibitor or substrate DNA. (**A**) Representative EMSA. One hundred fmol of radiolabeled abasic DNA substrate (10 nM) was incubated without inhibitor ("-"), with the indicated inhibitor (Inh), or with cold abasic (AP) DNA substrate (10, 30 or 100 µM) in binding buffer for 5 min on ice. Ten ng of APE1 (28 nM) was then added where indicated and incubated for an additional 5 min on ice. The samples were analyzed on an 8% non-denaturing polyacrylamide gel and subjected to phosphoimager analysis to determine percent of radiolabeled DNA in complex (C) as detailed in Materials and Methods. (**B**) Relative complex formation. Shown is the average and standard deviation of five independent experimental data points, all relative to the APE1 control (without inhibitor or cold AP DNA).



Supplementary Figure 2. Semi-log plot of Figure 6**.** (see figure legend in manuscript for assay details)



Supplementary Figure 3. HeLa cell survival assay. HeLa cells were exposed to No drug or MMS alone for 1 hour (0.4 mM). Then compound **3** (1 µM and 2 µM) and compound **52** (2.5 µM and 5 µM) were added to the cells for 8 hours as indicated. After compound treatment cells were in fresh media incubated for 56 hours at 37°C in 5% CO_2 (for details see below). Plotted is the average and standard deviation of the percent cell survival relative to the control for 6 independent data points. The assay was done in sixplicates. The *p* values were determined for an *n* using the Student's t test where 3, 52 and MMS is compared with **3** and **52** alone (** p <0.0001) and MMS alone (* p< 0.0001).

Methods: The cell survival assay of HeLa cells was based on accurate counts of viable cells on Acumen Explorer eX3 (TTP LabTech) plate reader. Briefly, cells were seeded in 96-well plate at 1000 cells/well (clear bottom black, Greiner) and allow cells to attach for 3 hrs at 37°C in 5% CO_2. Then cells exposed to no drug or 0.4 mM of MMS alone for 1 hour, then replaced with medium containing compound with appropriate concentrations; **3** and **52** alone, or a combination of MMS plus compound **3** and **52** relative to a non-exposed control and incubated for 8 hours. After compounds treatment cells were washed once and replaced with fresh media and incubated for 56 hrs. Cells were fixed with 4% paraformaldehyde for 20 min washed once with PBS and permeabilized with 0.2% Triton X-100 then stained with Hoechst 33342 (0.56 µg/mL final). Nuclei in each well were imaged and enumerated by Acumen at 405 nm and 488 nm emission.