Supporting information for

Identification of 3-aminothieno[2,3-*b*]pyridine-2-carboxamides and 4-aminobenzothieno[3,2-*d*]pyrimidines as LIMK1 Inhibitors

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Biological Experimental

To measure LIMK1 activity, 12 nM of LIMK1 enzyme (Upstate Biotechnology, Lake Placid, NY) was incubated with 10 μM cofilin-2 protein substrate and 10 μM ATP in reaction buffer containing 20 mM HEPES pH 7.4, 150 mM NaCl, 10 mM $MgCl_2$, 0.25 mM EGTA, 0.01 % Triton X-100, 0.01 % (w/v) chicken ovalbumine, and 1 mM DTT. Test compounds were diluted in DMSO to 100-fold of the final concentration; robotic pin tool transfer (V&P Scientific, San Diego, CA and MiniTrak IX, Perkin Elmer, Waltham, MA) was used to deliver the compound dilutions into assay buffer prior to addition of the kinase. After incubation for 60 minutes at room temperature the remaining ATP was detected by addition of equal volumes of Kinase-Glo reagent (Promega, Madison, WI) and detection of the luminescence on an EnVision 2103 plate reader (Perkin Elmer). The data was standardized according to positive and negative controls (DMSO without inhibitor and no enzyme, respectively). IC_{50} were calculated by non-linear regression based on a four-parameter logistic model.

Chemistry Experimental

General

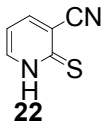
All non-aqueous reactions were performed in oven-dried glassware under an atmosphere of dry nitrogen, unless otherwise specified. Tetrahydrofuran was freshly distilled from sodium/benzophenone under N_2. Dichloromethane was freshly distilled from CaH_2 under N_2. All other solvents were reagent grade. Petroleum ether describes a mixture of hexanes in the bp range 40-60 °C. Analytical thin-layer chromatography was performed on Merck silica gel $60F_{254}$ aluminium-backed plates and were visualised by fluorescence quenching under UV light. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.063mm). All NMR spectra were recorded on a Bruker Avance DRX 300 with the solvents indicated (^{1}H NMR at 300 MHz). Chemical shifts are reported in ppm on the δ scale, referenced to the appropriate solvent peak. LCMS was recorded on a Finnigan LCQ Advantage using a Finnigan

Surveyor PDA Detector. LCMS conditions used to assess purity of compounds were as follows, column: Gemini 3µ C18 20x4.0mm 110A; injection volume: 10 µL; flow rate 1.5 mL/min; gradient: 10-100% of B over 10 min, (solvent A: water; solvent B AcCN, 0.1% formic acid).

Compounds **1**-**20**, **41**, **44**, were purchased from commercial vendors (Chembridge and ChemDiv).

Experimental Procedures

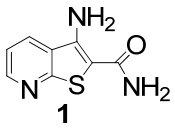
2-Thioxo-1,2-dihydropyridine-3-carbonitrile **22**



22

2-Chloronicotinonitrile **21** (7.2 mmol) and thiourea (7.2 mmol) in ethanol (15 mL) was heated to reflux for 4 h. The solution was allowed to cool and concentrated *in vacuo*. The residue was partitioned between 1N sodium hydroxide solution (20 mL) and ethyl acetate (20 mL). The layers were separated and the aqueous layer acidified to pH 4 with 2N hydrochloric acid. The resulting precipitate was filtered off at the pump, washing with water to yield **22** as a yellow solid (92%). ^{1}H-NMR (DMSO) δ 14.17 (1H, bs), 8.09 (1H, dd, *J* 1.8 and 7.4 Hz), 7.91(1H, dd, *J* 1.8 and 6.2 Hz), 6.84 (1H, dd, *J* 6.2 and 7.4 Hz).

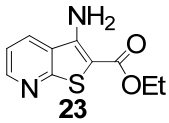
3-Aminothieno[2,3-b]pyridine-2-carboxamide **1**



1

2-Thioxo-1,2-dihydropyridine-3-carbonitrile **22** (15.0 mmol), diisopropylethylamine (15.0 mmol) and 2-bromoacetamide (15.0 mmol) in anhydrous *N*,*N*-dimethylformamide (8 mL) was allowed to stir for 30 min at room temperature. Potassium carbonate (15.0 mmol) was added and the solution was heated to 80 ^{o}C for 20 h. Ice-water was added and the resulting precipitate was filtered off, washing with water to obtain the carboxamide **1** as a pale-yellow solid (71%). LCMS - rt 5.85, M+H 194. ^{1}H-NMR (DMSO) δ 8.61 (1H, dd, *J* 1.6 and 4.6 Hz), 8.41 (1H, dd, *J* 1.6 and 8.1 Hz), 7.40 (1H, dd, *J* 4.6 and 8.1 Hz), 7.16 (4H, bs).

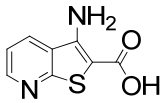
Ethyl 3-aminothieno[2,3-b]pyridine-2-carboxylate **23**



23

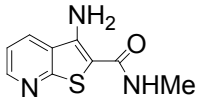
2-Thioxo-1,2-dihydropyridine-3-carbonitrile **22** (15.0 mmol), diisopropylethylamine (15.0 mmol) and ethyl 2-bromoacetate (15.0 mmol) in anhydrous *N*,*N*-dimethylformamide (8 mL) was allowed to stir for 30 min at room temperature. Potassium carbonate (15.0 mmol) was added and the solution was heated to 80 °C for 20 h. Ice-water was added and the resulting precipitate was filtered off, washing with water to obtain the carboxamide **23** as a pale-yellow solid (80%). ^{1}H-NMR (DMSO) δ 8.66 (1H, dd, *J* 1.6 and 4.6 Hz), 8.52 (1H, dd, *J* 1.7 and 8.1 Hz), 7.44 (1H, dd, *J* 4.6 and 8.1 Hz), 4.26 (2H, q, *J* 7.1 Hz), 1.29 (3H, t, *J* 7.1 Hz).

3-Aminothieno[2,3-b]pyridine-2-carboxylic acid



Ethyl 3-aminothieno[2,3-b]pyridine-2-carboxylate **23** (0.26 mmol) in dioxane (5 mL) and 2N sodium hydroxide solution (5 mL) was heated at 70 °C for 4 h. The solution was evaporated to half the original volume, and then acidified with 10% citric acid solution. The precipitate was filtered off, washing with water to obtain the 3-aminothieno[2,3-b]pyridine-2-carboxylic acid as a solid (95%). ^{1}H-NMR (DMSO) δ 8.43 (1H, dd, *J* 1.5 and 4.2 Hz), 8.11 (1H, dd, *J* 1.8 and 8.1 Hz), 7.25 (1H, dd, *J* 4.5 and 8.1 Hz), 6.43 (2H, bs).

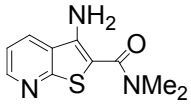
3-Amino-*N*-methylthieno[2,3-b]pyridine-2-carboxamide **24**



24

3-Aminothieno[2,3-b]pyridine-2-carboxylic acid (0.15 mmol), diisopropylethylamine (0.9 mmol), HBTU (0.22 mmol) in *N*,*N*-dimethylformamide (1 mL) was allowed to stir for 5 min. Methylamine hydrochloride (0.36 mmol) was added and the solution stirred for 6 h in a sealed vessel. Ice-water was added and precipitate that formed was filtered off washing with water to afford a solid. The solid was taken up in ethyl acetate (10 mL) and washed with 2N sodium hydroxide solution (5 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give the amide **24** as a pale-yellow solid (70%). LCMS – rt 8.08, M+H 312. ^{1}H-NMR (DMSO) δ 8.60 (1H, dd, *J* 1.5 and 4.5 Hz), 8.40 (1H, dd, *J* 1.8 and 8.1 Hz), 7.59-7.62 (1H, m), 7.42 (1H, dd, *J* 4.5 and 8.1 Hz), 7.10 (2H, bs), 2.73 (3H, d, *J* 4.5 Hz).

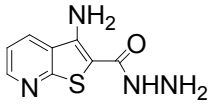
3-Amino-*N*,*N*-dimethylthieno[2,3-b]pyridine-2-carboxamide **25**



25

3-Aminothieno[2,3-b]pyridine-2-carboxylic acid (0.15 mmol), diisopropylethylamine (0.9 mmol), HBTU (0.22 mmol) in *N*,*N*-dimethylformamide (1 mL) was allowed to stir for 5 min. *N*,*N*-Dimethylamine hydrochloride (0.36 mmol) was added and the solution stirred for 1.5 h in a sealed vessel. Ice-water was added and the solution was extracted in ethyl acetate (10 mL) and washed with 2N sodium hydroxide solution (5 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give the amide **25** as a pale-yellow solid (85%). LCMS – rt 6.15, M+H 222.

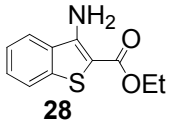
3-Aminothieno[2,3-b]pyridine-2-carbohydrazide **26**



26

Ethyl 3-aminothieno[2,3-b]pyridine-2-carboxylate **23** (0.29 mmol) and hydrazine hydrate (2.3 mmol) in dioxane was stirred at 90 ^{o}C for 20 h. Ice-water was added and the precipitate was filtered off, washing with water to give the hydrazide **26** as a solid (58%). ^{1}H-NMR (DMSO) δ 8.98 (1H, bs), 8.60 (1H, dd, *J* 1.5 and 4.5 Hz), 8.40 (1H, dd, *J* 1.8 and 8.1 Hz), 7.40 (1H, dd, *J* 4.5 and 8.1 Hz), 7.10 (2H, bs), 4.4 (2H, bs).

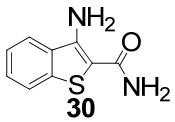
Ethyl 3-aminobenzo[b]thiophene-2-carboxylate **28**



28

2-Fluorobenzonitrile **27** (8.25 mmol), diisopropylethylamine (8.25 mmol) and ethyl 2-mercaptoacetate (8.25 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) was allowed to stir for 30 min at room temperature. Potassium carbonate (8.25 mmol) was added and the solution was heated to 80 ^{o}C for 20 h. Ice-water was added and the resulting precipitate was filtered off, washing with water to obtain the carboxylate as a white solid (92%). LCMS- rt 7.43, M+H 222. ^{1}H-NMR ($CDCl_3$) δ 7.43 (1H, d, *J* 8.1 Hz), 7.65 (1H, d, *J* 8.1), 7.49-7.44 (1H, m), 7.39-7.34 (1H, m), 4.36 (2H, q, *J* 7.1 Hz), 1.40 (3H, t, *J* 7.1 Hz).

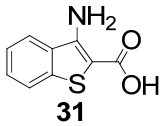
3-Aminobenzo[b]thiophene-2-carboxamide **30**



30

2-Thiolbenzonitrile **29** (5.2 mmol), chloroacetamide (5.2 mmol), triethylamine (10.4 mmol) in dimethylsulfoxide (4 mL) was allowed to stir at 60 °C for 20 h. Ice-water was added and the resulting precipitate was filtered off, washing with water to obtain the carboxamide **30** as a white solid (55%). ^{1}H-NMR (DMSO) δ 8.03 (1H, d, *J* 8.0 Hz), 7.81 (1H, d, *J* 8.9 Hz), 7.45 (1H, t, *J* 8.0 Hz), 7.39 (1H, t, *J* 8.0 Hz), 7.07 (4H, bs).

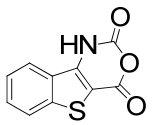
3-Aminobenzo[b]thiophene-2-carboxylic acid **31**



31

Ethyl 3-aminobenzo[b]thiophene-2-carboxylate **28** (0.26 mmol) in ethanol (5 mL) and 2N sodium hydroxide solution (5 mL) was heated at 70 °C for 4 h. Ethanol was evaporated and the aqueous acidified to obtain 3-aminobenzo[b]thiophene-2-carboxylic acid **31** as a white solid (58%). ^{1}H-NMR (DMSO) δ 8.07 (2H, d, *J* 8.0 Hz), 7.79 (1H, d, *J* 8.0 Hz), 7.47 (1H, t, *J* 7.0 Hz), 7.37 (1H, t, *J* 7.0 Hz).

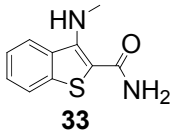
1H-benzo[d]thiophene[1,3]oxazine-2,4-dione **32**



32

3-Aminobenzo[b]thiophene-2-carboxylic acid **31** (1.45 mmol) and triphosgene (2.2 mmol) in dry dioxane (5 mL) was refluxed for 20 h. Ice-water was added to the mixture and the precipitate that formed was filtered off, washing with water to obtain **32** as a white solid (88%). ^{1}H-NMR (DMSO) δ 12.82 (1H, bs), 8.33 (1H, d, *J* 8.1 Hz), 8.12 (1H, d, *J* 8.2 Hz), 7.68 (1H, dt, *J* 1.2 and 7.1 Hz), 7.57 (1H, dt, *J* 1.0 and 8.2 Hz).

3-(Methylamino)benzo[b]thiophene-2-carboxamide **33**

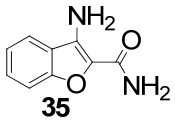


33

1H-Benzo[d]thiophene[1,3]oxazine-2,4-dione **32** (0.23 mmol) and sodium hydride (0.24 mmol) in *N*,*N*-dimethylacetamide (1.5 mL) was allowed to stir for 5 min at 20 °C. Iodomethane (0.46 mmol) and the mixture was allowed to stir for 20 h. 10% Citric acid solution was then added and the precipitate that formed was filtered off, washing with water to give *N*-methyl-benzo[d]thiophene[1,3]oxazine-2,4-dione pale-brown solid (81%). ^{1}H-NMR (DMSO) δ 8.47 (1H, d, J 8.6 Hz), 8.18 (1H, d, J 8.0 Hz), 7.69 (1H, t, J 7.2 Hz), 7.59 (1H, t, J 8.4 Hz), 3.92 (3H, s).

N-Methyl-benzo[d]thiophene[1,3]oxazine-2,4-dione (0.15 mmol) in ammonium hydroxide (1 mL) and dioxane (1 mL) was heated under microwave irradiation (250W) at 100 °C for 2 h. The mixture was then diluted with brine (5 mL) and extracted with ethyl acetate (5 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified by column chromatography gradient eluting from 10% ethyl acetate to 50% ethyl acetate to afford **33** as a pale-yellow solid (40%). ^{1}H-NMR (DMSO) δ 8.22-8.19 (1H, m), 7.73-7.69 (1H, m), 7.46-7.41 (1H, m), 7.37-7.31 (1H, m), 5.60 (2H, bs), 3.37 (1H, s).

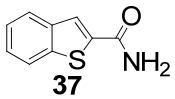
3-Aminobenzofuran-2-carboxamide **35**



35

2-Hydroxybenzonitrile **34** (1.68 mmol), 2-bromoacetamide (1.70 mmol) and potassium carbonate (3.36 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) was allowed to stir at 80 °C for 20 h. Ice-water was added and the resulting precipitate was filtered off, washing with water to obtain the benzofuran **35** as a white solid (51%).^{1}H-NMR (DMSO) δ 7.72 (1H, d, *J* 7.6 Hz), 7.63 (1H, t, *J* 7.8 Hz), 7.5-7.35 (2H, m), 7.12-7.05 (2H, m), 4.65 (2H, s).

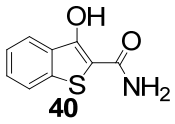
Benzo[b]thiophene-2-carboxamide **37**



37

Benzo[b]thiophene-2-carboxylic acid **36** (0.56 mmol), triethylamine (1.12 mmol), HBTU (0.78 mmol) in *N*,*N*-dimethylformamide (1 mL) was allowed to stir for 5 min. Concentrated ammonium hydroxide (1 mL) was added and the solution stirred for 30 min. Ice-water was added and precipitate that formed was filtered off washing with water to afford the amide **37** as a white solid (61%). ^{1}H-NMR (DMSO) δ 8.18 (1H, bs), 8.05 (1H, s), 8.01-7.96 (1H, m), 7.92-7.87 (1H, m), 7.58 (1H, bs), 7.47-7.38 (2H, m).

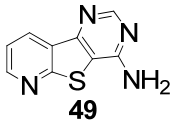
3-Hydroxybenzo[b]thiophene-2-carboxamide **40**



40

Methyl thiosalicylate **38** (2.4 mmol), 2-bromoacetamide (2.6 mmol) and potassium carbonate (2.6 mmol) in dimethylsulfoxide (3 mL) was allowed to stir at 20 °C for 20 h. Ice-water was added and the precipitate that formed was filtered off and dried in a vacuum oven. The solid was then dissolved in dioxane (4 mL) and potassium *tert*-butoxide (4.8 mmol) was added. The solution was allowed to stir at 60 °C for 20 h. An ice-cold solution of 10% citric acid solution was added and precipitate that formed was filtered off, washing with water to obtain the benzothiophene **40** as a white solid (60%). ^{1}H-NMR (DMSO) δ 12.3 (1H, bs), 7.95-7.89 (2H, m), 7.75 (2H, bs), 7.52-7.39 (2H, m).

4-Amino-6-azabenzothieno[3,2-d]pyrimidine **49**

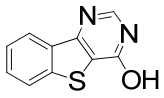


49

2-Thioxo-1,2-dihydropyridine-3-carbonitrile **22** (15 mmol), diisopropylethylamine (15 mmol) and 2-bromoacetonitrile (15 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) was allowed to stir for 30 min at room temperature. Potassium carbonate (15 mmol) was added and the solution was heated to 80 °C for 20 h. Ice-water was added and the resulting precipitate was filtered off, washing with water to obtain the carboxylate as a white solid to give the 2-aminothieno[2,3-b]pyridine-3-carbonitrile **45** (75%). ^{1}H-NMR (DMSO) δ 8.69 (1H, dd, J 1.6 and 4.6 Hz), 8.48 (1H, dd, *J* 1.7 and 8.2 Hz), 7.50 (1H, dd, *J* 4.6 and 8.2 Hz), 7.30 (2h, bs).

A stirred mixture of 2-aminothieno[2,3-b]pyridine-3-carbonitrile **45** (3.0 mmol) in formamide (10 mL) was heated to 150 °C. Formamidine acetate (3.0 mmol) was added and the mixture heated at 150 °C for 45 min. The addition of formamidine acetate (3.0 mmol) was repeated every 1 h for 6 h. Ice-water was added and the precipitate that was formed was filtered off, washed with water, and dried in a vacuum oven to obtain the pyrimidine **49** as a pale brown solid (80%). ^{1}H-NMR (DMSO) δ 8.80 (1H, dd, *J* 1.8 and 4.5 Hz), 8.61 (1H, dd, *J* 1.8 Hz and 8.1 Hz), 8.54 (1H, s), 7.64 (2H, bs), 7.61 (1H, dd, *J* 4.5 and 8.1 Hz).

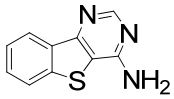
4-Hydroxybenzothieno[3,2-d]pyrimidine **54**



54

A stirred mixture of ethyl 3-aminobenzo[*b*]thiophene-2-carboxylate **28** (3.62 mmol) in formamide (5 mL) was heated to 150 ^{o}C. Formamidine acetate (3.62 mmol) was added and the mixture heated at 150 ^{o}C for 45 min. The addition of formamidine acetate (3.62 mmol) was repeated every 45 min for 6h. Ice-water was added and the precipitate that was formed was filtered off, washed with water, and dried in a vacuum oven to obtain the pyrimidine **54** as a tanned solid (95%). LCMS- rt 5.20, M+H 203. ^{1}H-NMR (DMSO) δ 8.33 (1H, s), 8.26-8.21 (1H, m), 8.16-8.12 (1H, m), 7.69-7.56 (1H, m).

4-Aminobenzothieno[3,2-d]pyrimidine **56**

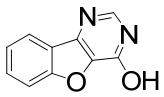


56

A mixture of 3*H*-benzothieno[3,2-*d*]pyrimid-4-one **54** (2.48 mmol) and *N*,*N*-dimethylformamide (0.025 mmol) in phosphorous oxychloride (6 mL) was allowed to stir at 90 ^{o}C for 20 h. The phosphorous oxychloride was removed *in vacuo*. Ice-water was added and the solution neutralized by portionwise addition of solid sodium hydrogen carbonate. The resulting precipitate was filtered off, washed with water and dried in a vacuum oven to give the 4-chlorobenzothieno[3,2-*d*]pyrimidine as a tanned solid (86%). LCMS- rt 7.73, M+H 221. ^{1}H-NMR (DMSO) δ 9.17 (1H, s), 8.48 (1H, d, *J* 7.8 Hz), 8.29 (1H, d, *J* 8.1 Hz), 7.84 (1H, dt, *J* 7.2 and 1.2 Hz), 7.71 (1H, dt, *J* 7.2 Hz and 0.9).

A suspension of 4-chlorobenzothieno[3,2-*d*]pyrimidine (0.45 mmol) in concentrated ammonium hydroxide (1 mL) and dimethylsulfoxide (3 mL) was heated to 100 ^{o}C under microwave irradiation (250 W) for 2 h. Ice-water was added and the precipitate that resulted was filtered off, washed with water, and dried in a vacuum oven to obtain the 4-amino pyrimidine **56** as a pale-yellow solid (95%). LCMS- rt 4.08, M+H 402. ^{1}H-NMR (DMSO) δ 8.51 (1H,s), 8.30 (1H, s, *J* 7.2 Hz), 8.12 (1H, s, *J* 8.1 Hz), 7.68-7.51 (4H, m).

4-Hydroxybenzofuro[3,2-d]pyrimidine **55**



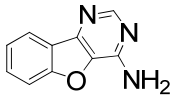
55

2-Hydroxybenzonitrile **34** (4.2 mmol), ethyl 2-bromoacetate (4.2 mmol) and potassium carbonate (8.4 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) was allowed to stir at 80 ^{o}C for 20 h. Ice-water was

added and the resulting precipitate was filtered off, washing with water to obtain ethyl 3-aminobenzofuran-2-carboxylate **53** as a white solid (81%). ^{1}H-NMR ($CDCl_3$) δ 7.60-7.57 (1H, m), 7.47-7.44 (2H, m), 7.27-7.22 (1H, m), 4.44 (2H, q, *J* 7.1 Hz), 1.44 (3H, t, *J* 7.1 Hz).

A stirred mixture of ethyl 3-aminobenzofuran-2-carboxylate **53** (2.66 mmol) in formamide (4 mL) was heated to 150 °C. Formamidine acetate (2.66 mmol) was added and the mixture heated at 150 °C for 45 min. The addition of formamidine acetate (2.66 mmol) was repeated every 45 min for 4 h and left to stir for 20 h at 150 °C. Ice-water was added and the precipitate that was formed was filtered off, washed with water, and dried in a vacuum oven to obtain the 4-hydroxybenzofuro[3,2-d]pyrimidine **55** as a tanned solid (85%). ^{1}H-NMR (DMSO) δ 8.22 (1H, s), 8.04-8.01 (1H, m), 7.83-7.80 (1H, m), 7.69-7.63 (1H, m), 7.51-7.46 (1H, m).

4-Aminobenzofuro[3,2-d]pyrimidine **57**

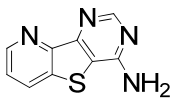


57

A mixture of 4-hydroxybenzofuro[3,2-d]pyrimidine **55** (1.8 mmol) and *N*,*N*-dimethylformamide (0.018 mmol) in phosphorous oxychloride (6 mL) was allowed to stir at 90 °C for 20 h. The phosphorous oxychloride was removed *in vacuo*. Ice-water was added and the solution neutralized by portionwise addition of solid sodium hydrogen carbonate. The resulting precipitate was filtered off, washed with water and dried in a vacuum oven to give the 4-chlorobenzofuro[3,2-d]pyrimidine as a tanned solid (95%). ^{1}H-NMR (DMSO) δ 9.05 (1H, s), 8.29-8.25 (1H, m), 7.99-7.97 (1H, m), 7.90-7.85 (1H, m), 7.64-7.59 (1H, m).

A suspension of 4-chlorobenzofuro[3,2-d]pyrimidine (0.29 mmol) in concentrated ammonium hydroxide (0.5 mL) and dimethylsulfoxide (2 mL) was heated to 100 °C under microwave irradiation (250 W) for 2 h. Ice-water was added and the precipitate that resulted was filtered off, washed with water, and dried in a vacuum oven to obtain the 4-aminobenzofuro[3,2-d]pyrimidine **57** as a yellow solid (65%). LCMS – rt 5.38, M+H 186.

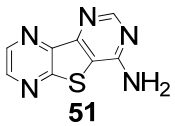
4-Amino-9-azabenzothieno[3,2-d]pyrimidine **50**



50

4-Amino-9-azabenzothieno[3,2-d]pyrimidine **50** was made following the protocol of 4-aminobenzothieno[3,2-d]pyrimidine **56**, starting from commercially available 3-chloropicolinonitrile.

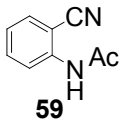
4-Amino-6,9-diazabenzothieno[3,2-d]pyrimidine **51**



51

4-Amino-6,9-diazabenzothieno[3,2-d]pyrimidine **51** was made following the protocol of 4-aminobenzothieno[3,2-d]pyrimidine **56**, starting from commercially available 3-chloropyrazine-2-carbonitrile.

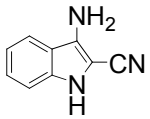
N-(2-cyanophenyl)acetamide **59**



59

A mixture of anthranilamide **58** (42.3 mmol), acetic anhydride (42.3 mmol) and dimethylaminopyridine (2.1 mmol) in dichloromethane (50 mL) was allowed to stir for 20 h at room temperature. Petroleum ether was added to the reaction mixture. The precipitate that formed was filtered off, washing with petroleum ether to obtain the acetamide **59** as a white solid (88%). LCMS- rt 3.76, M+H 376. ^{1}H-NMR ($CDCl_3$) δ 8.35 (1H, d, *J* 8.7 Hz), 7.75 (1H, bs), 7.60-7.55 (2H, m), 7.19-7.13 (1H, m), 2.26 (3H, s).

3-Amino-1H-indole-2-carbonitrile **60**

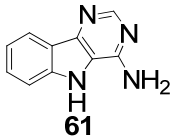


60

A mixture of *N*-(2-cyanophenyl)acetamide **59** (28 mmol), chloroacetonitrile (28 mmol) and potassium *tert*-butoxide (28 mmol) in *N*,*N*-dimethylformamide (20 mL) was allowed to stir for 20 h at room temperature. Ice-water was added to the reaction mixture. The mixture was extracted with ethyl acetate (2 x 30 mL). The organic layer was dried ($MgSO_4$) and the organic layer was concentrated *in vacuo* to obtain an oil. The oil was subjected to silica chromatography gradient eluting with 100% petroleum ether to 60% ethyl acetate /petroleum ether to obtain 1-acetyl-3-amino-1H-indole-2-carbonitrile as a pale orange solid (54%). LCMS- rt 5.62, M+H 200. ^{1}H-NMR (DMSO) δ 8.09 (1H, d, *J* 8.7 Hz), 7.93 (1H, d, *J* 8.0 Hz), 7.51 (1H, t, *J* 8.4 Hz), 7.31 (1H, t, *J* 7.9 Hz), 6.69 (2H, bs), 2.71 (3H, s).

A mixture of 1-acetyl-3-amino-1H-indole-2-carbonitrile (13.6 mmol), and potassium carbonate (27.2 mmol) in a solution of water (20 mL) and ethanol (20 mL) was allowed to reflux for 4 h. Ice-water was added to the reaction mixture. The mixture was extracted with ethyl acetate (2 x 40 mL). The organic layer was dried ($MgSO_4$) and the organic layer was concentrated *in vacuo* to obtain an oil. The oil was subjected to silica chromatography gradient eluting with 100% dichloromethane to 5% methanol /dichloromethane to obtain 3-amino-1H-indole-2-carbonitrile **60** as a white solid (24%). LCMS- rt 5.62, M+H 158. ^{1}H-NMR (DMSO) δ 10.63 (1h, bs), 7.71 (1H, d, *J* 8.1 Hz), 7.25-7.14, (2H, m), 6.96-6.90 (1H, m), 5.65 (2H, bs).

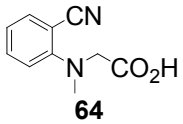
4-Amino-5*H*-pyrimido[5,4-*b*]indole **61**



61

A stirred mixture of 3-amino-1H-indole-2-carbonitrile **60** (2.66 mmol) in formamide (4 mL) was heated to 150 °C. Formamidine acetate (2.66 mmol) was added and the mixture heated at 150 °C for 45 min. The addition of formamidine acetate (2.66 mmol) was repeated every 45 min for 4 h and left to stir for 20 h at 150 °C. Ice-water was added and the precipitate that was formed was filtered off, washed with water, and dried in a vacuum oven to obtain the 4-amino-5*H*-pyrimido[5,4-*b*]indole **61** as a tanned solid (60%). LCMS- rt 2.42, M+H 185. ^{1}H-NMR (DMSO) δ 10.94 (1H, bs), 8.28 (1H, s), 8.05 (1H, d, *J* 7.9 Hz), 7.61 (1H, d, *J* 8.3 Hz), 7.49 (1H, t, *J* 7.0 Hz), 7.20 (1H, t, *J* 7.1 Hz), 6.89 (2H, bs).

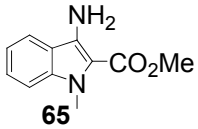
2-((2-Cyanophenyl)(methyl)amino)acetic acid **64**



64

2-Fluoro-benzonitrile (4.13 mmol), sarcosine (4.54 mmol), potassium carbonate (10.0 mmol), and copper acetate (0.41 mmol) in dimethylsulfoxide (4 mL) was allowed to stir at 140 °C for 20 h. Ice-water is added, followed by ethyl acetate (10 mL) and the layers were separated. The aqueous layer is then acidified to pH 2 with concentrated hydrochloric acid. The aqueous layer is then extracted with ethyl acetate (2 x 10 mL). The organic layer is dried ($MgSO_4$) and concentrated *in vacuo* to afford the aniline **64** as an orange oil (89%). LCMS- rt 5.38, M+H 191. ^{1}H-NMR (DMSO) δ 12.70 (1H, bs), 7.44 (1H, dd, *J* 6.0 and 1.5 Hz), 7.50-7.44 (1H, m), 6.96 (1H, d, *J* 8.7 Hz), 6.89-6.84 (1H, m), 4.19 (2H, s), 3.06 (3H, s).

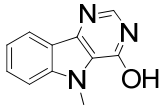
Methyl 3-amino-1-methyl-1H-indole-2-carboxylate **65**



65

2-((2-Cyanophenyl)(methyl)amino)acetic acid **64** (3.42 mmol) was heated to reflux in thionyl chloride (10 mL) for 30 min. The solution was cooled and evaporated to dryness *in vacuo*. Anhydrous methanol (10 mL) was added to the resulting residue and allowed the solution was allowed to sit for 5 min. The methanol was then removed *in vacuo* and the residue dissolved in ethyl acetate (20 mL). The organic solution was then washed with 10% sodium hydrogen carbonate solution (20 mL), dried ($MgSO_4$) and concentrated under vacuum to afford an oily residue. The residue was dissolved in *N*,*N*-dimethylformamide (5 mL) and potassium carbonate (3.42 mmol) was added. This mixture was then heated at 60 ^oC for 24 h. Ice-water was added and the mixture extracted with ethyl acetate (2 x 10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was subjected to column chromatography eluting with 100% petroleum ether to 40% ethyl acetate/petroleum ether to afford the indole **65** as an oil (53%). ^{1}H-NMR (DMSO) δ 7.81-7.77 (1H, m), 7.31-7.28 (2H, m), 6.95-6.90 (1H, m), 5.88 (2H, bs), 3.81 (3H, s), 3.76 (3H, s).

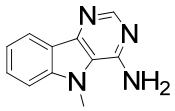
5-Methyl-5H-pyrimido[5,4-b]indol-4-ol **66**



66

A stirred mixture of methyl 3-amino-1-methyl-1H-indole-2-carboxylate **65** (1.88 mmol) in formamide (6 mL) was heated to 150 ^oC. Formamidine acetate (1.88 mmol) was added and the mixture heated at 150 ^oC for 45 min. The addition of formamidine acetate (1.88 mmol) was repeated every 45 min for 4 h and left to stir for 20 h at 150 ^oC. Ice-water was added and the precipitate that was formed was filtered off, washed with water, and dried in a vacuum oven to obtain the 5-methyl-5H-pyrimido[5,4-b]indol-4-ol **66** as a brown solid (67%). LCMS- rt 5.07, M+H 200. ^{1}H-NMR (DMSO) δ 12.35 (1H, bs), 8.02-7.99 (1H, m), 7.96 (1H, s), 7.67-7.64 (1H, m), 7.56-7.50 (1H, m), 7.29-7.24 (1H, m), 4.15 (3H, s).

4-Amino5-methyl-5*H*-pyrimido[5,4-*b*]indole **67**

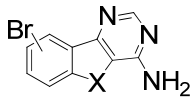


67

A mixture of 5-methyl-5H-pyrimido[5,4-b]indol-4-ol **66** (1.0 mmol) and *N*,*N*-dimethylformamide (0.001 mmol) in phosphorous oxychloride (6 mL) was allowed to stir at 90 ^{o}C for 20 h. The phosphorous oxychloride was removed *in vacuo*. Ice-water was added and the solution neutralized by portionwise addition of solid sodium hydrogen carbonate. The resulting precipitate was filtered off, washed with water and dried in a vacuum oven to give 4-chloro-5-methyl-5H-pyrimido[5,4-b]indole (92%). LCMS- rt 6.50, M+H 218. ^{1}H-NMR (DMSO) δ 8.81 (1H, s), 8.28-8.25 (1H, m), 7.85-7.77 (2H, m), 7.43-7.40 (1H, m), 4.18 (3H, s).

A suspension of 4-chloro-5-methyl-5H-pyrimido[5,4-b]indole (0.45 mmol) in concentrated ammonium hydroxide (1 mL) and dimethylsulfoxide (3 mL) was heated to 150 ^{o}C in a sealed pressure vessel for 20 h. Ice-water was added and the precipitate that resulted was filtered off, washed with water, and dried in a vacuum oven to obtain the 4-amino5-methyl-5*H*-pyrimido[5,4-*b*]indole **67** as a pale-yellow solid (90%). LCMS- rt 5.52, M+H 199.

Bromo substituted 4-aminobenzothieno[3,2-d]pyrimidines **68-71** and 4-amino-5-methyl-benzoindolo[3,2-d]pyrimidine **72**



68 X = S, 9-Br
69 X = S, 8-Br
70 X = S, 7-Br
71 X = S, 6-Br
72 X = NMe, 7-Br

9-Bromo-4-aminobenzothieno[3,2-d]pyrimidine **68** was made following the protocol of 4-aminobenzothieno[3,2-d]pyrimidine **56**, starting from commercially available 2-bromo-6-fluorobenzonitrile.

8-Bromo-4-aminobenzothieno[3,2-d]pyrimidine **69** was made following the protocol of 4-aminobenzothieno[3,2-d]pyrimidine **56**, starting from commercially available 5-bromo-2-fluorobenzonitrile.

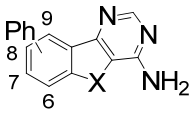
7-Bromo-4-aminobenzothieno[3,2-d]pyrimidine **70** was made following the protocol of 4-aminobenzothieno[3,2-d]pyrimidine **56**, starting from commercially available 4-bromo-2-fluorobenzonitrile.

LCMS- rt 5.50, M+H 280. ^{1}H-NMR (DMSO) δ 8.51 (1H, s), 8.47 (1H, d, *J* 1.7 Hz), 8.18 (1H, d, *J* 8.4 Hz), 7.70 (1H, dd, *J* 8.5 and 1.8 Hz), 7.60 (1H, bs).

6-Bromo-4-aminobenzothieno[3,2-d]pyrimidine **71** was made following the protocol of 4-aminobenzothieno[3,2-d]pyrimidine **56**, starting from 3-bromo-2-fluorobenzonitrile.
3-Bromo-2-fluorobenzonitrile can be made from commercially available 3-bromo-2-fluorobenzaldehyde, via a phosphorous chloride dehydration of the oxime intermediate.

4-Amino-7-bromo-5-methyl-pyrimido[5,4-*b*]indole **72** was made following the protocol of 4-amino-5-methyl-pyrimido[5,4-*b*]indole **67**, starting from commercially available 4-bromo-2-fluoro-benzonitrile.

Phenyl substituted 4-aminobenzothieno[3,2-d]pyrimidines **73-76** and 4-amino-5-methyl-benzoindolo[3,2-d]pyrimidine **77**

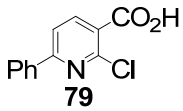


73 X = S, 9-Ph
74 X = S, 8-Ph
75 X = S, 7-Ph
76 X = S, 6-Ph
77 X = NMe, 7-Ph

A mixture of 7-bromo-aminobenzothieno[3,2-*d*]pyrimidine **70** (0.36 mmol), potassium carbonate (0.89 mmol), phenylboronic acid (0.4 mmol), tetrabutylammonium bromide (0.036 mmol) and $PdCl_2(PPh_3)_4$ (0.036 mmol) in a solution of dioxane (4 mL) and water (1 mL) were heated for 120 ^oC under microwave irradiation for 2h. 10% Citric acid solution (10 mL) was added and the precipitate that resulted was filtered off, washed with water, and dried in a vacuum oven. The solid was dissolved in a dilute methanol/tetrahydrofuran mixture with warming, and filtered. The filtrate was concentrated to dryness *in vacuo*. The resulting residue is triturated with diethylether, and filtered off to give **75** as a tanned solid (70%). Alternatively the crude residue is applied to column chromatography gradient eluting with 100% dichloromethane to 15% methanol dichloromethane. LCMS – rt 7.53, M+H 278. ^{1}H-NMR (DMSO) δ 8.52 (1H, s), 8.46-8.45 (1H, m), 8.33 (1H, d, J 8.8 Hz), 7.88-7.81 (3H, m), 7.54-7.41 (5H, m).

Compounds **73**, **74**, **76** and **77** were made in the analogous manner to that described for the above **75**, from compounds **68**, **69**, **71** and **72** respectively.

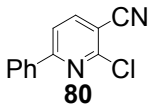
2-Chloro-6-phenylnicotinic acid **79**



79

A mixture of 2,6-nicotinic acid (4.4 mmol), phenylboronic acid (4.4 mmol), potassium carbonate (15.5 mmol) and $PdCl_2(PPh_3)_2$ (5 mol %) in a solution of dimethoxyethane (5 mL), ethanol (5 mL) and water (5 mL) was allowed to reflux for 4 h. The mixture was partitioned between ethyl acetate and water. The layers were then separated. The aqueous layer was then acidified with 2N hydrochloric acid solution to pH 5 and extracted with ethyl acetate (2 x 20 ml). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to obtain the acid **79** as a solid (70%). ^{1}H-NMR ($CDCl_3$) δ 8.13 (1H, d, *J* 8.0 Hz), 8.09-8.05 (2H, m), 7.99 (1H, d, *J* 8.0 Hz), 7.53-7.47 (3H, m).

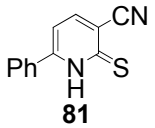
2-Chloro-6-phenylnicotinonitrile **80**



80

A mixture of 2-chloro-6-phenylnicotinic acid **79** in thionyl chloride was allowed to reflux for 1 h. The reaction mixture was concentrated to dryness *in vacuo*. The residue was dissolved in dioxane (5ml) and NH_4OH solution was added. Ice-water was added to the reaction mixture. The precipitate that formed was filtered off, washing with water and dried in a vacuum oven to obtain 2-chloro-6-phenylnicotinamide as a white solid (90%). ^{1}H-NMR ($CDCl_3$) δ 8.07-7.94 (5H, m), 7.73 (1H, bs), 7.54-7.48 (3H, m). A mixture of 2-chloro-6-phenylnicotinamide in acetic anhydride was allowed to reflux for 2 h. The reaction mixture was concentrated to dryness *in vacuo*. Ice-water was added to the reaction mixture. The mixture was partitioned between ethyl acetate and 10% sodium hydrogen carbonate solution. The layers were then separated. The organic layer was dried ($MgSO_4$) and the organic layer was concentrated *in vacuo* to obtain an oil. The oil was subjected to silica chromatography gradient eluting with 10% ethyl acetate /petroleum ether to 50% ethyl acetate /petroleum ether to obtain the nitrile **80** as a white solid (49%). ^{1}H-NMR ($CDCl_3$) δ 8.05-8.00 (2H, m), 7.99 (1H, d, *J* 8.4 Hz), 7.76 (1H, d, *J* 8.1 Hz), 7.51-7.49 (3H, m).

6-Phenyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile **81**

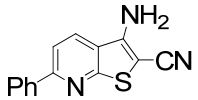


81

A mixture of 2-chloro-6-phenylnicotinonitrile **80** (0.2 mmol) and sodium hydrosulfide (0.2 mmol) in ethanol (3 mL) was allowed to reflux for 2 h. 10% Citric acid solution was added to the reaction mixture.

The precipitate that formed was filtered off, washing with water and dried in a vacuum oven to obtain a bright yellow solid. The solid was suspended between ethyl acetate and 1N sodium hydroxide solution. The layers were then separated. The aqueous layer was then acidified with 2N hydrochloric acid solution to pH 5 and the precipitate that formed was filtered off, washing with water and dried in a vacuum oven to obtain the pyrid-2-thione **81** as a bright yellow solid (60%). ^{1}H-NMR (DMSO) δ 8.06 (1H, d, *J* 7.8 Hz), 7.76-7.73 (2H, m), 7.55-7.52 (2H, m), 7.06 (1H, d, *J* 7.8 Hz).

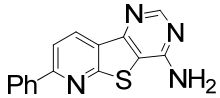
3-Amino-6-phenylthieno[2,3-b]pyridine-2-carbonitrile **82**



82

A mixture of 6-phenyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile **81** (0.7 mmol), diisopropylethylamine (0.7 mmol) and chloroacetonitrile (0.7 mmol) in *N*,*N*-dimethylformamide (3 mL) was allowed to stir for 1 h at room temperature. Ice-water was added to the reaction mixture. The precipitate that formed was filtered off, washing with water and dried in a vacuum oven to obtain a white solid (the solid was a mixture of acyclic and cyclic product). The solid was dissolved in *N*,*N*-dimethylformamide (3 mL) and potassium carbonate (0.7 mmol) was added. The mixture was allowed to stir at 50 ^{o}C for 8 h. Ice-water was added to the reaction mixture. The precipitate that formed was filtered off, washing with water and dried in a vacuum oven to obtain the thieno pyridine **82** as a white solid (68 %). ^{1}H-NMR (DMSO) δ 8.55 (1H, d, *J* 8.7 Hz), 8.17-8.14 (2H, m), 8.11 (1H, d, *J* 8.4 Hz), 7.53-7.50 (3H, m), 7.33 (2H, bs).

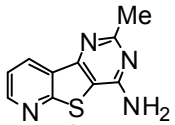
4-Amino-7-phenyl-6-azabenzothieno[3,2-d]pyrimidine **83**



83

A stirred mixture of 3-amino-6-phenylthieno[2,3-b]pyridine-2-carbonitrile **82** (1.88 mmol) in formamide (6 mL) was heated to 150 ^{o}C. Formamidine acetate (1.88 mmol) was added and the mixture heated at 150 ^{o}C for 45 min. The addition of formamidine acetate (1.88 mmol) was repeated every 45 min for 4 h and left to stir for 20 h at 150 ^{o}C. Ice-water was added and the precipitate that was formed was filtered off, washed with water, and dried in a vacuum oven to obtain the 4-amino-7-phenyl-6-azabenzothieno[3,2-d]pyrimidine **83** as a solid (80%). LCMS- rt 5.87, M+H 279. ^{1}H-NMR (DMSO) δ 8.66 (1H, d, *J* 8.1 Hz), 8.52 (1H, s), 8.19-8.15 (3H, m), 7.63 (2H, bs), 7.54-7.52 (2H, m).

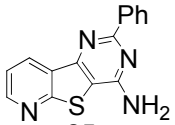
4-Amino-2-methyl-6-azabenzothieno[3,2-d]pyrimidine **84**



84

2-Aminothieno[2,3-b]pyridine-3-carbonitrile **45** (0.29 mmol) and trimethylorthoformate (1.16 mmol) heated to 130 ^{o}C by microwave irradiation (250 W) for 10 min. Water was added and the solid was filtered off and dried *in vacuo*. The solid and ammonium acetate (1.16 mmol) in dioxane (2 mL) was heated to 150 ^{o}C in by microwave irradiation (250 W) for 1 h. Water was added and the solid was then filtered off, washing with water to give a the pyrimidine 84 as a white solid (73%). ^{1}H-NMR (DMSO) δ 8.79 (1H, dd, *J* 1.8 and 4.5 Hz), 8.59 (1H, dd, *J* 1.5 and 7.8 Hz), 7.59 (1H, dd, *J* 4.8 and 8.1 Hz), 7.53 (2H, bs), 2.52 (3H ,s).

4-Amino-2-phenyl-6-azabenzothieno[3,2-d]pyrimidine **85**



85

2-Aminothieno[2,3-b]pyridine-3-carbonitrile **45** (0.35 mmol), potassium butoxide (0.035 mmol) and benzonitrile (1.0 mmol) in dimethylsulfoxide (0.3 mL) was heated to 200 ^{o}C by microwave irradiation (280 W) for 10 min. Water was added and the precipitate that formed was filtered off, washing with water to give the pyrimidine **85** as a solid (90%). LCMS – rt 8.12, M+H 279.