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 MgCl₂, 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). IC₅₀ 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 N₂. Dichloromethane was freshly distilled from CaH₂ under N₂. 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 60F₂₅₄ 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 (¹H 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

![2-Thioxo-1,2-dihydropyridine-3-carbonitrile 22](image)

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

![3-Aminothieno[2,3-b]pyridine-2-carboxamide 1](image)

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 °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
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%). 1H-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%). 1H-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

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 (MgSO4) and concentrated in vacuo to give the amide 24 as a pale-yellow solid (70%). LCMS – rt 8.08, M+H 312. 1H-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

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 \textit{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

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 °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) \(\delta\) 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

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 °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\(_3\)) \(\delta\) 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
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[1,3]oxazine-2,4-dione 32

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

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
1H-Benzod[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-benzod[1,3]oxazine-2,4-dione
pale-brown solid (81%). 1H-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-benzod[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 (MgSO₄)
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%). 1H-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

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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%). 1H-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

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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%). 1H-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-Hydroxybenzothiophene-2-carboxamide 40

![Structure of 3-Hydroxybenzothiophene-2-carboxamide 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) $\delta$ 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

![Structure of 4-Amino-6-azabenzothieno[3,2-d]pyrimidine 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) $\delta$ 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) $\delta$ 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
A stirred mixture of ethyl 3-aminobenzothiophene-2-carboxylate 28 (3.62 mmol) in formamide (5 mL) was heated to 150 °C. Formamidine acetate (3.62 mmol) was added and the mixture heated at 150 °C for 45 min. The addition of formamidine acetate (3.62 mmol) was repeated every 45 min 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 54 as a tanned solid (95%). LCMS- rt 5.20, M+H 203. ¹H-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

A mixture of 3H-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 °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. ¹H-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 °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. ¹H-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

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 °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$_3$) $\delta$ 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) $\delta$ 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

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) $\delta$ 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
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

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

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$_3$) $\delta$ 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

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) $\delta$ 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) $\delta$ 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-5H-pyrimido[5,4-b]indole 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-5H-pyrimido[5,4-b]indole 61 as a tanned solid (60%). LCMS- rt 2.42, M+H 185. $^1$H-NMR (DMSO) $\delta$ 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

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) $\delta$ 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

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\begin{align*}
\text{NH}_2 & \quad \text{CO}_2\text{Me} \\
\end{align*}
\]

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 (MgSO₄) 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 °C for 24 h. Ice-water was added and the mixture extracted with ethyl acetate (2 x 10 mL). The organic layer was dried (MgSO₄) 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%). ¹H-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

\[
\begin{align*}
\text{OH} & \quad \text{N} \\
\end{align*}
\]

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 °C. Formamidine acetate (1.88 mmol) was added and the mixture heated at 150 °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 °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 5-methyl-5H-pyrimido[5,4-b]indol-4-ol 66 as a brown solid (67%). LCMS- rt 5.07, M+H 200. ¹H-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-5H-pyrimido[5,4-b]indole 67

\[
\begin{align*}
\text{NH}_2 & \quad \text{N} \\
\end{align*}
\]
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 °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) \(\delta\) 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 °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-5H-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

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) \(\delta\) 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

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 °C 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
A mixture of 2,6-nicotinic acid (4.4 mmol), phenylboronic acid (4.4 mmol), potassium carbonate (15.5 mmol) and PdCl₂(PPh₃)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 (MgSO₄) and concentrated in vacuo to obtain the acid 79 as a solid (70%). ¹H-NMR (CDCl₃) 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

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 (5 ml) and NH₄OH 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%). ¹H-NMR (CDCl₃) 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 (MgSO₄) 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%). ¹H-NMR (CDCl₃) 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

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

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 ºC 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

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 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 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
2-Aminothieno[2,3-b]pyridine-3-carbonitrile 45 (0.29 mmol) and trimethylorthoformate (1.16 mmol) heated to 130 °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 °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%). ^1H-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

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 °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.