Supporting Information

Structural Optimization of a Pyridinylimidazole Scaffold: Shifting the Selectivity from p38α Mitogen-Activated Protein Kinase to c-Jun N-terminal Kinase 3.

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Experimental procedures

General procedure for the synthesis of compounds 15a-l (general procedure B)

In a 3 neck round-bottom flask under anhydrous conditions 2-chloro-4-methylpyridine (**9**) (1 eq) and the appropriate ethyl ester (1 eq) were dissolved in dry THF (2 mL). After cooling the reaction mixture to 0 °C, 2 M Sodium bis(trimethylsilyl)amide (NaHDMS) in dry THF (2.2 eq) was added dropwise and the mixture was stirred at 0 °C for 1.5 to 6 h. After adding H_2O, the aqueous phase was extracted 3 times with DCM or EtOAc and washed with NaCl saturated solution. The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated at reduced pressure. The obtained residue was employed for the next step without further purification or purified by flash column chromatography.

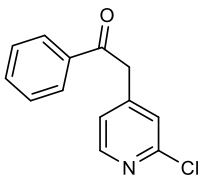
General procedure for the synthesis of compounds 16a-l (general procedure C)

Ethan-1-one intermediates **15a-l** (1 eq) and SeO_2 (1.1 eq) were suspended in 5-10 mL of glacial AcOH and the reaction mixture was stirred at 65 °C for 2 to 3 h. After cooling to rt, the formed solid residue of Se was removed by filtration and the filtrate was diluted with EtOAc and then washed with saturated $NaHCO_3$ solution for 4 times. Finally, the organic phase was washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and concentrated at reduced pressure. The residue was purified by flash column chromatography.

General procedure for the synthesis of compounds 17a-l (general procedure D)

In a pressure vial ethane-1,2-dione derivatives **16a-l** (1 eq) and NH_4OAc (10 eq) were suspended in 3 mL of glacial AcOH and after that a 37% aqueous solution of formaldehyde (1 eq) was added. The reaction vessel was heated in a CEM microwave reactor at 180 °C, with initial power of 200 W, for 2 to 5 min. The mixture was added dropwise to NH_4OH concentrated solution at 0°C. The suspension obtained was extracted 3 times with EtOAc and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The residue was purified by flash column chromatography.

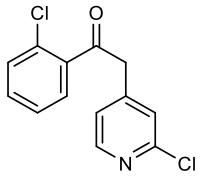
2-(2-Chloropyridin-4-yl)-1-phenylethan-1-one (15a)



The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (1.0 g, 7.84 mmol), ethyl benzoate (1.17 g, 7.84 mmol) and 2 M NaHDMS in dry THF (8.62 mL, 17.25 mmol) (1.5 h). 1.82 g of product were obtained, which were employed for the following step without further purification (100% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 4.56 (s, 2H), 7.33 (dd, *J*

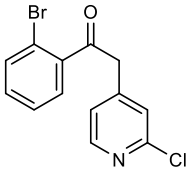
= 5.1, 1.4 Hz, 1H), 7.46 (br. s, 1H), 7.53 - 7.61 (m, 2H), 7.65 - 7.72 (m, 1H), 8.01 - 8.08 (m, 2H), 8.36 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = ppm 43.5, 124.9, 125.7, 128.2, 128.8, 133.6, 136.1, 148.4, 149.4, 150.1, 195.9; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{13}H_{10}ClNO$: 232.0, found: 232.0; *m*/*z* $[M-H]^-$ calcd. for $C_{13}H_{10}ClNO$: 230.0, found: 229.8; HPLC (method 2): t_R = 5.020 min.

1-(2-Chlorophenyl)-2-(2-chloropyridin-4-yl)ethan-1-one (15b)



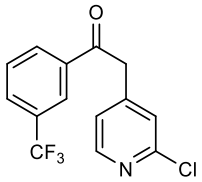
The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (1.4 g, 10.83 mmol), ethyl 2-chlorobenzoate (2.0 g, 10.83 mmol) and 2 M NaHDMS in dry THF (11.9 mL, 23.8 mmol) (5 h). Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 1.96 g of the desired product (65% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.28 (s, 2H), 7.15 (d, *J* = 5.04 Hz, 1H), 7.28 (br. s, 1H, overlapping with the solvent peak), 7.32 - 7.50 (m, 4H), 8.36 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 48.0, 123.7, 125.4, 127.2, 129.3, 130.7, 131.1, 132.6, 138.4, 145.9, 149.6, 151.8, 198.1 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{13}H_9Cl_2NO$: 266.0, found: 266.0; *m*/*z* $[M-H]^-$ calcd. for $C_{13}H_9Cl_2NO$: 264.0, found: 263.8; HPLC (method 2): t_R = 5.960 min.

1-(2-Bromophenyl)-2-(2-chloropyridin-4-yl)ethan-1-one (15c)



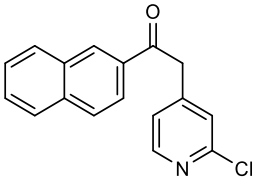
The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (1.1 g, 8.73 mmol), ethyl 2-bromobenzoate (2.0 g, 8.73 mmol) and 2 M NaHDMS in dry THF (9.5 mL, 19.02 mmol) (1.5 h). Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 2.48 g of the desired product (92% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.45 (s, 2H), 7.34 (d, *J* = 4.9 Hz, 1H), 7.45 - 7.48 (m, 2H), 7.49 - 7.60 (m, 1H), 7.70 - 7.77 (m, 1H), 7.80 (dd, *J* = 5.9, 1.6 Hz, 1H), 8.37 (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 48.0, 123.7, 125.4, 127.2, 129.3, 130.7, 131.1, 132.6, 138.4, 145.9, 149.6, 151.8, 198.1 ppm; MS-ESI: *m*/*z* $[M-H]^-$ calcd. for $C_{13}H_9BrClNO$: 308.0, found: 307.8; HPLC (method 2): t_R = 6.130 min.

2-(2-Chloropyridin-4-yl)-1-(3-(trifluoromethyl)phenyl)ethan-1-one (15d)



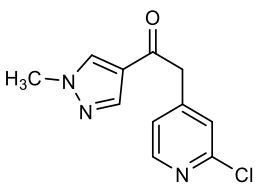
The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (820 mg, 6.42 mmol), ethyl 3-(trifluoromethyl)benzoate (1.4 g, 6.42 mmol) and 2 M NaHDMS in dry THF (7.1 mL, 14.12 mmol) (3 h). 1.82 g of product were obtained, which were employed for the following step without further purification (81% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.33 (s, 2H), 7.14 (dd, *J* = 5.0, 1.5 Hz, 1H), 7.63 - 7.72 (m, 1H), 7.86 - 7.93 (m, 1H), 8.18 (d, *J* = 7.9 Hz, 1H), 8.25 (br. s, 1H), 8.38 ppm (dd, *J* = 5.0, 0.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$), δ = 44.4, 123.8 (q, *J* = 272.5 Hz), 124.0, 125.5 (q, *J* = 3.8 Hz), 125.7, 129.9, 130.5 (q, *J* = 3.5 Hz), 131.7, 132.0 (q, *J* = 32.7 Hz), 136.8, 146.1, 150.1, 152.3, 194.0 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{14}H_9ClF_3NO$: 300.0, found: 300.0; *m*/*z* $[M-H]^-$ calcd. for $C_{14}H_9ClF_3NO$: 297.9, found: 263.8; HPLC (method 2): t_R = 7.240 min.

2-(2-Chloropyridin-4-yl)-1-(naphthalen-2-yl)ethan-1-one (15e)



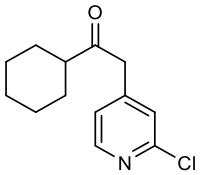
The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (7.0 g, 54.8 mmol), ethyl 3-naphtoate (11.1 g, 54.8 mmol) and 2 M NaHDMS in dry THF (60 mL, 122 mmol) (1.5 h). After work up, the residue was washed with Et_2O and then filtered off, affording 11.5 g of the desired product (73% yield); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 4.70 (s, 2H), 7.38 (d, *J* = 5.1 Hz, 1H), 7.51 (s, 1H), 7.62 - 7.74 (m, 2H), 7.98 - 8.09 (m, 3H), 8.17 (d, *J* = 8.1 Hz, 1H), 8.38 (d, *J* = 5.1 Hz, 1H), 8.81 ppm (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 43.5, 123.6, 124.9, 125.7, 127.0, 127.7, 128.4, 128.8, 129.6, 130.3, 132.1, 133.4, 135.1, 148.5, 149.4, 150.1, 195.9 ppm; MS-ESI: *m*/*z* $[M-H]^-$ calcd. for $C_{17}H_{12}ClNO$: 280.1, found: 280.2; HPLC (method 2): t_R = 7.814 min.

2-(2-Chloropyridin-4-yl)-1-(1-methyl-1*H*-pyrazol-4-yl)ethan-1-one (15f)



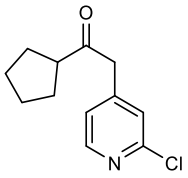
The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (1.65 g, 12.97 mmol), ethyl 1-methyl-1*H*-pyrazole-4-carboxylate (2 g, 12.97 mmol) and 2 M NaHDMS in dry THF (13 mL, 25.94 mmol) (3 h). After work up, the residue was washed with Et_2O and then filtered off, affording 1.2 g of the desired product (39% yield); ^{1}H NMR (250 MHz, DMSO-d_6) δ = 3.89 (s, 3H), 4.22 (s, 2H), 7.31 (d, *J* = 4.9 Hz, 1H), 7.44 (s, 1H), 8.02 (s, 1H), 8.33 (d, *J* = 4.9 Hz, 1H), 8.49 ppm (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 44.8, 122.6, 124.5, 125.3, 134.2, 139.9, 148.1, 149.5, 150.1, 189.4 ppm; MS-ESI: *m*/*z* $[M-H]^-$ calcd. for $C_{11}H_{10}ClN_3O$: 234., found: 234.2; HPLC (method 2): t_R = 1.895 min.

2-(2-Chloropyridin-4-yl)-1-cyclohexylethan-1-one (15g)



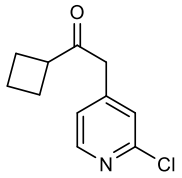
The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (1.63 g, 12.8 mmol), ethyl cyclohexanecarboxylate (2.0 g, 12.8 mmol) and 2 M NaHDMS in dry THF (14.1 mL, 28.2 mmol) (2 h). Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 2.16 g of the desired product (71% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.20 - 1.59 (m, 5H), 1.71 - 2.12 (m, 5H), 2.39 - 2.64 (m, 1H), 3.84 (s, 2H), 7.15 (dd, *J* = 5.0, 1.3 Hz, 1H), 7.27 (s, 1H), 8.41 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$), δ = 25.4, 25.6, 28.3, 45.9, 50.9, 123.6, 125.2, 146.5, 149.5, 151.7, 208.2 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{13}H_{16}ClNO$: 238.1, found: 238.1; *m*/*z* $[M-H]^-$ calcd. for $C_{13}H_{16}ClNO$: 236.1, found: 235.9; HPLC (method 2): t_R = 6.700 min.

2-(2-Chloropyridin-4-yl)-1-cyclopentylethan-1-one (15h)



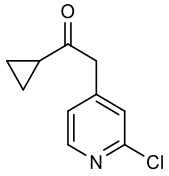
The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (2.7 g, 21.1 mmol), ethyl cyclopentanecarboxylate (2.0 g, 21.1mmol) and 2 M NaHDMS in dry THF (23.2 mL, 46.4 mmol) (3 h). Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 1.77 g of the desired product (38% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.55 - 1.92 (m, 8H), 2.90 - 3.03 (m, 1H), 3.75 (s, 2H), 7.07 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.19 (br. s, 1H), 8.32 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$), δ = 25.9, 28.9, 47.2, 51.6, 123.6, 125.2, 146.5, 149.5, 151.7, 207.5 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{14}ClNO$: 224.1, found: 224.0; *m*/*z* $[M-H]^-$ calcd. for $C_{12}H_{14}ClNO$: 222.1, found: 222.1; HPLC (method 2): t_R = 5.530 min.

2-(2-Chloropyridin-4-yl)-1-cyclobutylethan-1-one (15i)



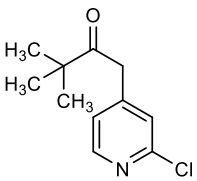
The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (2.7 g, 21.1 mmol), ethyl cyclobutanecarboxylate (2.9 g, 21.1 mmol) and 2 M NaHDMS in dry THF (25.7 mL, 51.5 mmol) (3 h). Purification by flash column chromatography (SiO_2, *n*-hex/EtOAc 95:05 to 80:20) afforded 1.22 g of the desired product (25% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.78 - 2.02 (m, 2H), 2.10 - 2.30 (m, 4H), 3.28 - 3.39 (m, 1H), 3.63 (s, 1H), 7.04 (dd, *J* = 5.1, 1.4 Hz, 1H), 7.16 - 7.20 (m, 1H), 8.30 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ = 17.6, 24.3, 45.5, 45.6, 123.6, 125.2, 146.3, 149.5, 151.7, 206.1 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{11}H_{12}ClNO$: 210.1, found: 210.0; *m*/*z* $[M-H]^-$ calcd. for $C_{11}H_{12}ClNO$: 208.1, found: 207.8; HPLC (method 2): t_R = 4.320 min.

2-(2-Chloropyridin-4-yl)-1-cyclopropylethan-1-one (15j)



The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (3.3 g, 26.28 mmol), ethyl cyclopropanecarboxylate (3 g, 26.28 mmol) and 2 M NaHDMS in dry THF (29 mL, 52.56 mmol) (3 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 100:0 to 60:40) afforded 1.62 g of the desired product (32% yield); ^{1}H NMR (250 MHz, DMSO-d_6) δ = 0.84 - 1.01 (m, 4H), 2.02 - 2.21 (m, 1H), 4.05 (s, 2H), 7.24 (dd, *J* = 5.0, 1.3 Hz, 1H), 7.38 (dd, *J* = 1.4, 0.7 Hz, 1H), 8.32 ppm (dd, *J* = 5.1, 0.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 10.5, 20.4, 47.3, 124.6, 125.3, 147.8, 149.4, 150.1, 205.8 ppm; MS-ESI: *m*/*z* $[M-H]^-$ calcd. for $C_{10}H_{10}ClNO$: 194.0, found: 194.2; HPLC (method 2): t_R = 2.736 min.

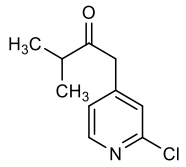
1-(2-Chloropyridin-4-yl)-3,3-dimethylbutan-2-one (15k)



The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (1.96 g, 15.36 mmol), ethyl pivalate (2 g, 15.36 mmol) and 2 M NaHDMS in dry THF (16.9 mL,

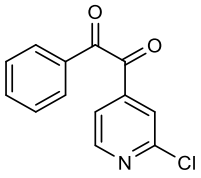
33.8 mmol) (6h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 2.9 g of the desired product (90% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.19 (s, 9H), 2.02 (s, 2H), 7.02 (dd, *J* = 6.6, 3.8 Hz, 1H), 7.13 - 7.15 (m, 1H), 8.28 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$), δ = 26.1, 41.9, 44.8, 123.7, 125.3, 147.1, 149.3, 151.5, 210.3 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{11}H_{14}ClNO$: 212.1, found: 212.2; *m*/*z* $[M-H]^-$ calcd. for $C_{11}H_{14}ClNO$: 210.1, found: 210.1; HPLC (method 2): t_R = 4.870 min.

1-(2-Chloropyridin-4-yl)-3-methylbutan-2-one (15l)



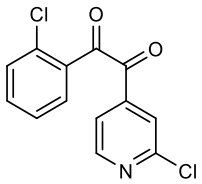
The title compound was synthesized according to general procedure B starting from 2-chloropicoline (**9**) (1.64 g, 12.91 mmol), ethyl isobutyrate (1.5 g, 12.91 mmol) and 2 M NaHDMS in dry THF (14.2 mL, 28.41 mmol) (4h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 2.8 g of the desired product (100% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.15 (d, *J* = 6.9 Hz, 6H), 2.78 (sep, *J* = 6.9 Hz, 1H), 3.75 (s, 2H), 7.05 (dd, *J* = 6.4, 3.8 Hz, 1H), 7.18 - 7.20 (m, 1H), 8.32 ppm (d, *J* = 5.1 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$), δ = 17.8, 41.0, 45.5, 123.4, 125.0, 146.2, 149.3, 151.5, 208.7 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{10}H_{12}ClNO$: 198.1, found: 198.1; *m*/*z* $[M-H]^-$ calcd. for $C_{10}H_{12}ClNO$: 196.1, found: 195.9; HPLC (method 2): t_R = 3.620 min.

1-(2-Chloropyridin-4-yl)-2-phenylethane-1,2-dione (16a)



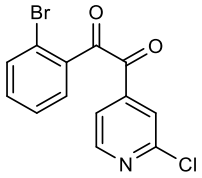
The title compound was synthesized according to general procedure C starting from **15a** (2.0 g, 8.63 mmol) and SeO_2 (1 g, 9.50 mmol) (3h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 948 mg of the desired product (45% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.61 - 7.66 (m, 2H), 7.81 - 7.87 (m, 1H), 7.94 - 7.96 (m, 1H), 8.01 - 8.04 (m, 2H), 8.71 ppm (dd, *J* = 5.0 Hz, 0.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 122.0, 123.3, 129.4, 130.2, 131.8, 135.5, 141.9, 151.3, 151.5, 190.8, 191.3 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{13}H_8ClNO_2$: 246.0, found: 246.1; *m*/*z* $[M-H]^-$ calcd. for $C_{13}H_8ClNO_2$: 244.0, found: 244.0; HPLC (method 2): t_R = 5.790 min.

1-(2-Chlorophenyl)-2-(2-chloropyridin-4-yl)ethane-1,2-dione (16b)



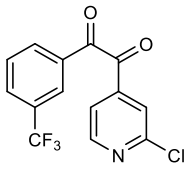
The title compound was synthesized according to general procedure C starting from **15b** (1.85 g, 6.95 mmol) and SeO_2 (848 mg, 7.65 mmol) (3h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 592 mg of the desired product (30% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.44 - 7.25 (m, 2H), 7.58 - 7.65 (m, 1H), 7.75 - 7.79 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.83 - 7.93 (m, 2H), 8.66 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 121.3, 123.8, 127.7, 130.4, 132.0, 133.2, 133.9, 135.2, 141.2, 151.0, 152.9, 188.9, 192.1 ppm; MS-ESI: *m*/*z* $[M+MeOH]^+$ calcd. for $C_{13}H_7Cl_2NO_2$: 312.0, found: 312.0; HPLC (method 2): t_R = 6.690 min.

1-(2-Bromophenyl)-2-(2-chloropyridin-4-yl)ethane-1,2-dione (16c)



The title compound was synthesized according to general procedure C starting from **15c** (2.0 g, 6.44 mmol) and SeO_2 (786 mg, 7.08 mmol) (3 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 643 mg of the desired product (30% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.64 - 7.74 (m, 2H), 7.80 - 7.92 (m, 2H), 7.97 (dd, *J* = 3.7, 1.3 Hz, 1H), 8.05 (br. s, 1H), 8.76 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 121.4, 122.23, 123.5, 128.4, 132.5, 133.6, 134.5, 135.5, 141.5, 151.4, 151.6, 188.3, 191.8 ppm; MS-ESI: *m*/*z* $[M+MeOH]^+$ calcd. for $C_{13}H_7BrClNO_2$: 355.9, found: 356.0; HPLC (method 2): t_R = 6.650 min.

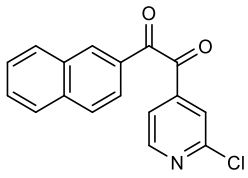
1-(2-Chloropyridin-4-yl)-2-(3-(trifluoromethyl)phenyl)ethane-1,2-dione (16d)



The title compound was synthesized according to general procedure C starting from **15d** (1.4 g, 4.67 mmol) and SeO_2 (570 mg, 5.14 mmol) (3h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 437 mg of the desired product (30% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.69 - 7.78 (m, 2H), 7.86 (dd, *J* = 1.3, 0.8 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 8.30 (br. s, 1H), 8.68 ppm (dd, *J* = 5.1, 0.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$), δ = 121.0,

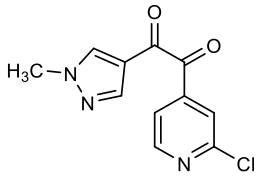
121.2 (q, *J* = 272.0 Hz), 123.7, 126.8 (q, *J* = 3.7 Hz), 129.9, 131.7 (q, *J* = 3.4 Hz), 132.0 (q, *J* = 34.4 Hz), 132.7, 133.3 141.3, 151.2, 153.2, 189.7, 189.8 ppm; MS-ESI: *m*/*z* $[M+MeOH]^+$ calcd. for $C_{14}H_7ClF_3NO_2$: 346.0, found: 346.1; HPLC (method 2): t_R = 7.670 min.

1-(2-Chloropyridin-4-yl)-2-(naphthalen-2-yl)ethane-1,2-dione (16e)



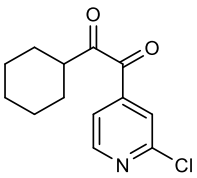
The title compound was synthesized according to general procedure C starting from **15e** (500 mg, 1.77 mmol) and SeO_2 (216 mg, 1.95 mmol) (1.5 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 75:25) afforded 250 mg of the desired product (48% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.54 - 7.72 (m, 2H), 7.75 (dd, *J* = 5.1, 1.4 Hz, 1H), 7.84 - 8.03 (m, 4H), 8.08 (dd, *J* = 8.6, 1.7 Hz, 1H), 8.42 (br. s, 1H), 8.63 ppm (dd, *J* = 5.0, 0.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 121.0, 123.5, 123.6, 127.4, 128.0, 129.3, 129.5, 130.0, 132.2, 134.0, 136.6, 141.7, 151.1, 153.0, 191.1, 191.7 ppm; HPLC (method 2): t_R = 8.316 min.

1-(2-Chloropyridin-4-yl)-2-(1-methyl-1*H*-pyrazol-4-yl)ethane-1,2-dione (16f)



The title compound was synthesized according to general procedure C starting from **15f** (974 mg, 4.13 mmol) and SeO_2 (500 mg, 4.54 mmol) (1.5 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 50:50) afforded 362 mg of the desired product (35% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 3.98 (s, 3H), 7.74-7.81 (m, 1H), 7.89 (br. s, 1H), 8.09 (br. s, 1H), 8.13 (s, 1H), 8.59 ppm (dd, *J* = 5.1, 0.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 39.5, 119.0, 121.5, 124.1, 135.4, 141.7, 142.2, 150.8, 152.7, 182.3, 189.0 ppm.

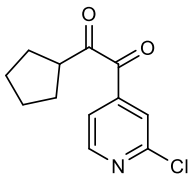
1-(2-Chloropyridin-4-yl)-2-cyclohexylethane-1,2-dione (16g)



The title compound was synthesized according to general procedure C starting from **15g** (2.0 g, 8.41 mmol) and SeO_2 (1.2 g, 9.25 mmol) (2 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 820 mg of the desired product (38% yield); ^{1}H NMR (300 MHz,

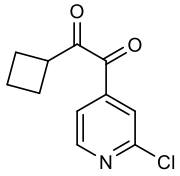
$CDCl_3$) δ = 1.12 - 1.48 (m, 5H), 1.68 - 1.95 (m, 5H), 3.10-3.23 (m, 1H), 7.68 (dd, *J* = 5.1, 1.4 Hz, 1H), 7.71 - 7.84 (m, 1H), 8.61 ppm (d, *J* = 5.1 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 25.2, 25.6, 27.2, 45.3, 121.2, 123.8, 141.5, 150.9, 152.9, 190.1, 203.1 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd for $C_{13}H_{14}ClNO_2$: 252.1, found: 252.0; *m*/*z* $[M-H]^-$ calcd. for $C_{13}H_{14}ClNO_2$: 250.1, found: 249.9; HPLC (method 2): t_R = 7.690 min.

1-(2-Chloropyridin-4-yl)-2-cyclopentylethane-1,2-dione (16h)



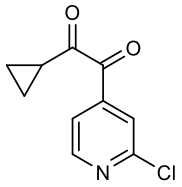
The title compound was synthesized according to general procedure C starting from **15h** (1.65 g, 7.37 mmol) and SeO_2 (900 mg, 8.11 mmol) (2 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 834 mg of the desired product (48% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.59 - 1.99 (m, 8H), 3.57 - 3.77 (m, 1H), 7.72 (dd, *J* = 5.1, 1.4 Hz, 1H), 7.83 (s, 1H), 8.61 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 26.0, 28.2, 46.3, 121.3, 123.9, 141.6, 150.9, 152.8, 189.6, 202.1 ppm; MS-ESI: *m*/*z* $[M-H]^-$ calcd. for $C_{12}H_{12}ClNO_2$: 236.1, found: 236.0; HPLC (method 2): t_R = 6.690 min.

1-(2-Chloropyridin-4-yl)-2-cyclobutylethane-1,2-dione (16i)



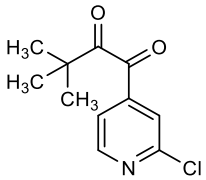
The title compound was synthesized according to general procedure C starting from **15i** (1.10 g, 5.24 mmol) and SeO_2 (640 mg, 5.77 mmol) (1.5 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 605 mg of the desired product (52% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.90 - 2.20 (m, 2H), 2.26 - 2.43 (m, 4H), 3.92 - 4.04 (m, 1H), 7.76 (dd, *J* = 5.1, 1.4 Hz, 1H), 7.87 (br. s, 1H), 8.65 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 17.9, 23.8, 40.9, 121.1, 123.7, 141.2, 150.5, 152.5, 188.4, 199.7 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd for $C_{11}H_{10}ClNO_2$: 224.0, found: 224.1; *m*/*z* $[M-H]^-$ calcd. for $C_{11}H_{10}ClNO_2$: 222.0, found: 222.0; HPLC (method 2): t_R = 5.560 min.

1-(2-Chloropyridin-4-yl)-2-cyclopropylethane-1,2-dione (16j)



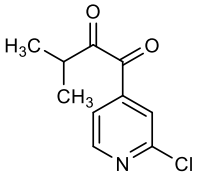
The title compound was synthesized according to general procedure C starting from **15j** (1.0 g, 5.11 mmol) and SeO_2 (620 mg, 5.62 mmol) (1.5 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 75:25) afforded 690 mg of the desired product (64% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.20 - 1.40 (m, 4H), 2.64 - 2.77 (m, 1H), 7.77 (dd, *J* = 5.1, 1.4 Hz, 1H), 7.85 - 7.93 (m, 1H), 8.60 ppm (dd, *J* = 5.1, 0.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 14.2, 17.7, 121.7, 124.3, 141.3, 150.8, 152.7, 187.9, 199.3 ppm; HPLC (method 2): t_R = 3.488 min.

1-(2-Chloropyridin-4-yl)-3,3-dimethylbutane-1,2-dione (16k)



The title compound was synthesized according to general procedure C starting from **15k** (2.6 g, 12.28 mmol) and SeO_2 (1.5 g, 13.51 mmol) (5 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 1.28 g of the desired product (46% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.31 (s, 9H), 7.55 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.65 - 7.69 (m, 1H), 8.61 ppm (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 25.9, 42.8, 120.7, 123.2, 141.7, 151.1, 153.0, 191.9, 207.9 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{11}H_{12}ClNO_2$: 226.1, found: 226.1; *m*/*z* $[M-H]^-$ calcd. for $C_{11}H_{12}ClNO_2$: 224.1, found: 223.9; HPLC (method 2): t_R = 7.920 min.

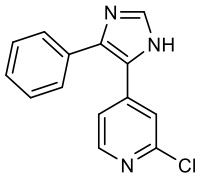
1-(2-Chloropyridin-4-yl)-3-methylbutane-1,2-dione (16l)



The title compound was synthesized according to general procedure C starting from **15l** (2.3 g, 11.64 mmol) and SeO_2 (1.4 g, 12.80 mmol) (5 h); Purification by flash column chromatography (SiO_2, *n*-hexane/EtOAc 90:10 to 80:20) afforded 854 mg of the desired product (35% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.21 (d, *J* = 6.9 Hz, 6H), 3.43 (sep, *J* = 6.9 Hz, 1H), 7.70 (dd, *J* = 5.1, 1.4 Hz, 1H), 7.80-7.83 (dd, *J* = 1.3 Hz, 0.8 Hz, 1H), 8.62 ppm (dd, *J* = 5.0 Hz, 0.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 16.8, 35.9, 121.2, 123.8, 141.5, 150.9, 152.9, 189.8, 203.6 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for

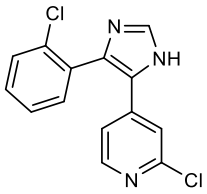
$C_{10}H_{10}ClNO_2$: 212.0, found: 212.1; *m/z* $[M-H]^-$ calcd. for $C_{10}H_{10}ClNO_2$: 210.0, found: 210.0; HPLC (method 2): t_R = 5.050 min.

2-Chloro-4-(4-phenyl-1*H*-imidazol-5-yl)pyridine (17a)



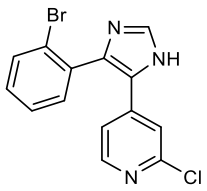
The title compound was synthesized according to general procedure D starting from **16a** (840 mg, 3.41 mmol), NH_4OAc (2.6 g, 34.1 mmol), and formaldehyde 37% aqueous solution (277 µL, 3.41 mmol) (2 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 90:10) afforded 349 mg of the desired product (36% yield); ^1H NMR (300 MHz, DMSO-d_6) δ = 7.41 (d, *J* = 5.0 Hz, 1H), 7.45 - 7.52 (m, 6H), 7.90 (br. s, 1H), 8.24 (d, *J* = 5.1 Hz, 1H), 12.81 ppm (br. s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6), δ = 119.5, 120.0, 128.6, 128.9, 130.3, 130.4, 131.7, 136.5, 146.0, 149.7, 150.6, 150.6 ppm; MS-ESI: *m/z* $[M+H]^+$ calcd. for $C_{14}H_{10}ClN_3$: 256.1, found: 256.0; *m/z* $[M-H]^-$ calcd. for $C_{14}H_{10}ClN_3$: 254.1, found: 253.8; HPLC (method 2): t_R = 4.270 min.

2-Chloro-4-(4-(2-chlorophenyl)-1*H*-imidazol-5-yl)pyridine (17b)



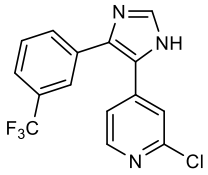
The title compound was synthesized according to general procedure D starting from **16b** (500 mg, 1.78 mmol), NH_4OAc (1.37 g, 17.8 mmol), and formaldehyde 37% aqueous solution (145 µL,1.78 mmol) (3 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 97:03 to 90:10) afforded 293 mg of the desired product (57% yield); ^1H NMR (300 MHz, DMSO-d_6), δ = 7.20 (br. s, 1H), 7.35 (br. s, 1H), 7.45 - 7.72 (m, 4H), 7.96 (br. s, 1H), 8.15 - 8.28 (m, 1H), 12.92 ppm (br. s, 1H); MS-ESI: *m/z* $[M+H]^+$ calcd. for $C_{14}H_9Cl_2N_3$: 290.0, found: 290.0; *m/z* $[M-H]^-$ calcd. for $C_{14}H_9Cl_2N_3$: 288.0, found: 287.8; HPLC (method 2): t_R = 4.880 min.

4-(4-(2-Bromophenyl)-1*H*-imidazol-5-yl)-2-chloropyridine (17c)



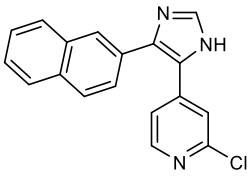
The title compound was synthesized according to general procedure D starting from **16c** (541 mg, 1.67 mmol), NH_4OAc (1.28 g, 16.7 mmol), and formaldehyde 37% aqueous solution (136 µL, 1.67 mmol) (3 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 97:03 to 90:10) afforded 392 mg of the desired product (70% yield); ^{1}H NMR (300 MHz, DMSO-d_6), δ = 7.10 - 7.65 (m, 5H), 7.82 - 7.95 (m, 2H), 8.21 (br. s, 1H), 12.85 ppm (br. s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6), δ = 118.4, 118.9, 124.0, 128.3, 128.9, 131.5, 131.9, 132.46, 132.53, 133.1, 136.5, 145.5, 149.6, 150.7 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{14}H_9BrClN_3$: 334.0, found: 334.0; *m*/*z* $[M-H]^-$ calcd. for $C_{14}H_9BrClN_3$: 332.0, found: 331.8; HPLC (method 2): t_R = 5.070 min.

2-Chloro-4-(4-(3-(trifluoromethyl)phenyl)-1*H*-imidazol-5-yl)pyridine (17d)



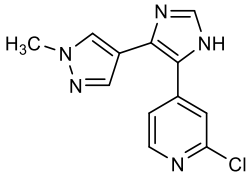
The title compound was synthesized according to general procedure D starting from **16d** (437 mg, 1.39 mmol), NH_4OAc (1.07 g, 13.9 mmol), and formaldehyde 37% aqueous solution (113 µL, 1.39 mmol) (2 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 90:10) afforded 175 mg of the desired product (39% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.32 (dd, *J* = 5.2, 1.5 Hz, 1H), 7.51 - 7.74 (m, 4H), 7.76 (br. s, 1H), 8.08 (br. s, 1H), 8.30 ppm (d, *J* = 5.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$), δ = 119.9, 121.6, 123.4 (q, *J* = 272.5 Hz), 124.9 (q, *J* = 3.7 Hz), 125.5 (q, *J* = 3.7 Hz), 129.5, 130.1, 131.0, 131.3, 131.4 (q, *J* = 32.9 Hz), 131.5, 136.1, 142.9, 149.4, 151.8 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{15}H_9ClF_3N_3$: 324.0, found: 323.9; *m*/*z* $[M-H]^-$ calcd. for $C_{15}H_9ClF_3N_3$: 322.0, found: 321.8; HPLC (method 2): t_R = 6.960 min.

2-Chloro-4-(4-(naphthalen-2-yl)-1*H*-imidazol-5-yl)pyridine (17e)



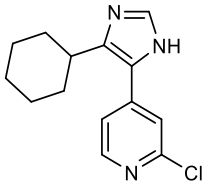
The title compound was synthesized according to general procedure D starting from **16e** (250 mg, 0.84 mmol), NH_4OAc (651 mg, 8.45 mmol), and formaldehyde 37% aqueous solution (69 µL, 0.84 mmol) (5 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 95:05) afforded 125 mg of the desired product (48% yield); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.41 (d, *J* = 4.5 Hz, 1H), 7.50 - 7.64 (m, 4H), 7.89 - 8.05 (m, 4H), 8.08 (s, 1H), 8.17 - 8.30 (m, 1H), 12.96 ppm (br. s., 1H); MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{18}H_{12}ClN_3$: 306.1, found: 306.4; *m*/*z* $[M-H]^-$ calcd. for $C_{18}H_{12}ClN_3$: 304.1, found: 304.4; HPLC (method 2): t_R = 7.144 min.

2-Chloro-4-(4-(1-methyl-1*H*-pyrazol-4-yl)-1*H*-imidazol-5-yl)pyridine (17f)



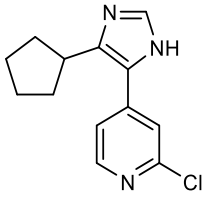
The title compound was synthesized according to general procedure D starting from **16f** (362 mg, 1.45 mmol), NH_4OAc (1.12 g, 14.5 mmol), and formaldehyde 37% aqueous solution (118 µL, 1.45 mmol) (5 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 95:05) afforded 140 mg of the desired product (37% yield); ^{1}H NMR (250 MHz, DMSO-d_6) δ = 3.91 (s, 3H), 7.46 - 7.60 (m, 1 H), 7.64 (br. s, 2H), 7.83 (br. s, 1H), 7.99 (br. s, 1H), 8.27 (d, *J* = 5.1 Hz, 1H), 12.59 ppm (br. s., 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 38.7, 110.4, 119.1, 119.6, 122.6, 130.0, 131.6, 136.2, 138.1, 146.2, 149.8, 150.7 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{10}ClN_5$: 260.1, found: 260.0; *m*/*z* $[M-H]^-$ calcd. for $C_{12}H_{10}ClN_5$: 258.1, found: 258.0; HPLC (method 2): t_R = 1.584 min.

2-Chloro-4-(4-cyclohexyl-1*H*-imidazol-5-yl)pyridine (17g)



The title compound was synthesized according to general procedure D starting from **16g** (700 mg, 2.78 mmol), NH_4OAc (2.14 g, 27.8 mmol), and formaldehyde 37% aqueous solution (226 µL, 2.78 mmol) (3 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 97:03 to 90:10) afforded 269 mg of the desired product (37% yield); ^{1}H NMR (300 MHz, DMSO-d_6), δ = 1.20 - 1.60 (m, 5H), 1.65 - 1.82 (m, 5H), 2.94 - 3.07 (m, 1H), 7.54 (d, *J* = 4.5 Hz, 1H), 7.60 (br. s, 1H), 7.69 (br. s, 1H), 8.34 (d, *J* = 5.0 Hz, 1H), 12.28 ppm (br. s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6), δ = 25.4, 25.9, 32.1, 34.7, 119.5, 120.0, 130.5, 135.3, 136.1, 146.6, 149.9, 150.8 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{14}H_{16}ClN_3$: 262.1, found: 262.1; *m*/*z* $[M-H]^-$ calcd. for $C_{14}H_{16}ClN_3$: 260.1, found: 259.9; HPLC (method 2): t_R = 5.050 min.

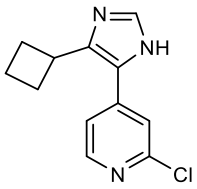
2-Chloro-4-(4-cyclopentyl-1*H*-imidazol-5-yl)pyridine (17h)



The title compound was synthesized according to general procedure D starting from **16h** (700 mg, 2.94 mmol), NH_4OAc (2.24 g, 29.4 mmol), and formaldehyde 37% aqueous solution (239 µL, 2.94 mmol) (3 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 97:03 to 90:10) afforded 326

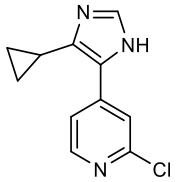
mg of the desired product (45% yield); ^{1}H NMR (300 MHz, DMSO-d_6), δ = 1.64 - 1.80 (m, 4H), 1.95 - 2.10 (m, 2H), 3.40-3.47 (m, 1H), 7.58 (d, *J* = 5.0 Hz, 1H), 7.62 (br. s, 1H), 7.70 (s, 1H), 8.33 (d, *J* = 5.1 Hz, 1H), 12.27 ppm (br. s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6), δ = 24.8, 32.6, 35.7, 119.6, 119.9, 131.2, 134.3, 135.3, 146.3, 149.7, 150.5 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{13}H_{14}ClN_3$: 248.1, found: 247.9; *m*/*z* $[M-H]^-$ calcd. for $C_{13}H_{14}ClN_3$: 246.1, found: 245.8; HPLC (method 2): t_R = 3.270 min.

2-Chloro-4-(4-cyclobutyl-1*H*-imidazol-5-yl)pyridine (17i)



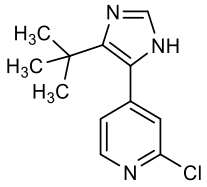
The title compound was synthesized according to general procedure D starting from **16i** (500 mg, 2.24 mmol), NH_4OAc (1.71 g, 22.4 mmol), and formaldehyde 37% aqueous solution (181 µL, 2.24 mmol) (3 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 97:03 to 90:10) afforded 255 mg of the desired product (49% yield); ^{1}H NMR (300 MHz, DMSO-d_6), δ = 0.98 - 1.17 (m, 2H), 1.35 - 1.70 (m, 4H), 2.95 - 3.10 (m, 1H), 6.50 - 7.00 (m, 3H), 7.40 - 7.60 (m, 1H), 11.35 – 11.85 ppm (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6), δ = 17.4, 28.2, 30.8, 119.2, 119.5, 130.4, 134.5, 135.4, 145.6, 149.6, 150.5 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{12}ClN_3$: 234.1, found: 234.0; *m*/*z* $[M-H]^-$ calcd. for $C_{12}H_{12}ClN_3$: 232.1, found: 231.8; HPLC (method 2): t_R = 2.750 min.

2-Chloro-4-(4-cyclopropyl-1*H*-imidazol-5-yl)pyridine (17j)



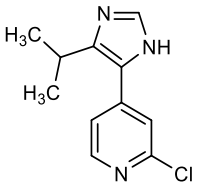
The title compound was synthesized according to general procedure D starting from **16j** (500 mg, 2.38 mmol), NH_4OAc (1.83 g, 23.8 mmol), and formaldehyde 37% aqueous solution (194 µL, 2.38 mmol) (5 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 95:05) afforded 350 mg of the desired product (67% yield); ^{1}H NMR (250 MHz, DMSO-d_6) δ = 0.68 - 0.82 (m, 2H), 0.93 - 1.11 (m, 2H), 2.03 - 2.20 (m, 1H), 7.64 (s, 1H), 7.79 (br. s., 2H), 8.27 - 8.42 (m, 1H), 12.23 ppm (br. s., 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 6.8, 7.3, 119.0, 119.3, 132.5, 132.7, 134.6, 146.1, 149.8, 150.7 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{11}H_{10}ClN_3$: 220.1, found: 220.2; *m*/*z* $[M-H]^-$ calcd. for $C_{11}H_{10}ClN_3$: 218.2, found: 304.4; HPLC (method 2): t_R = 1.760 min.

4-(4-(*Tert*-butyl)-1*H*-imidazol-5-yl)-2-chloropyridine (17k)



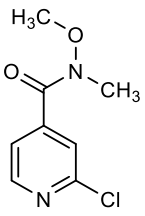
The title compound was synthesized according to general procedure D starting from **16k** (1.1 g, 4.89 mmol), NH_4OAc (3.77 g, 48.9 mmol), and formaldehyde 37% aqueous solution (397 μL, 4.89 mmol) (5 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 97:03 to 90:10) afforded 283 mg of the desired product (24% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.26 (s, 9H), 7.44 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.47 (br. s, 1H), 7.63 (s, 1H), 8.37 (d, *J* = 4.9 Hz, 1H), 12.04 ppm (br. s., 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 30.6, 31.2, 124.2, 124.5, 131.7, 134.3, 136.6, 149.0, 149.3, 149.8 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{12}H_{14}ClN_3$: 236.1, found: 236.2; *m*/*z* $[M-H]^-$ calcd. for $C_{12}H_{14}ClN_3$: 234.1, found: 234.0; HPLC (method 2): t_R = 2.470 min.

2-Chloro-4-(4-isopropyl-1*H*-imidazol-5-yl)pyridine (17l)



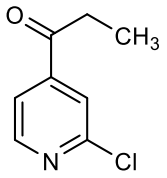
The title compound was synthesized according to general procedure D starting from **16l** (750 mg, 4.89 mmol), NH_4OAc (2.74 g, 35.5 mmol), and formaldehyde 37% aqueous solution (289 μL, 3.55 mmol) (3 min); Purification by flash column chromatography (SiO_2, DCM/EtOH 97:03 to 90:10) afforded 135 mg of the desired product (17% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.35 (d, *J* = 7.0 Hz, 6H), 3.44 (sep, *J* = 7.0 Hz, 1H), 7.51 (dd, *J* = 5.2, 1.5 Hz, 1H), 7.56 - 7.65 (m, 1H), 7.69 (s, 1H), 8.36 ppm (d, *J* = 5.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6), δ = 21.9, 24.6, 119.4, 119.8, 129.9, 135.1, 136.6, 146.3, 149.6, 150.5 ppm.; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_{11}H_{12}ClN_3$: 222.1, found: 222.2; *m*/*z* $[M-H]^-$ calcd. for $C_{11}H_{12}ClN_3$: 220.1, found: 220.0; HPLC (method 2): t_R = 2.080 min.

2-Chloro-*N*-methoxy-*N*-methylisonicotinamide (20)[1]



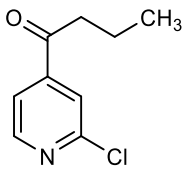
The title compound was synthesized as previously reported[1] and the analytical data were in agreement with the reported ones.

1-(2-Chloropyridin-4-yl)propan-1-one (22)



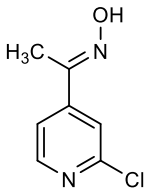
A solution of Weinreb amide **20** (10 g 49.84 mmol) in dry THF (60 mL) was cooled to -10 °C and after that a 2M solution of ethylmagnesium bromide in dry THF (15.6 mL, 64.8 mmol), previously diluted wit 30 mL of dry THF, was added dropwise. After completion of the addition the reaction mixture was stirred at the same temperature for 3 h. After letting the mixture heat to rt, 200 mL of NH_4Cl saturated solution were added and the mixture was stirred for 10 min. The 2 phases formed were separated and the aqueous phase was further extracted 3 times with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. Finally, the residue was purified by flash column chromatography (SiO_2, *n*-hexane/EtOAc 85:15 to 70:30 affording 5.82 g of the desired compound (69% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.24 (t, *J* = 7.2 Hz, 3H), 2.99 (q, *J* = 7.2 Hz, 2H), 7.66 (dd, *J* = 5.0, 1.5 Hz, 1H), 7.76 (dd, *J* = 1.4, 0.7 Hz, 1H), 8.57 ppm (dd, *J* = 5.1, 0.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 7.6, 32.4, 119.8, 122.3, 145.5, 150.8, 152.8, 198.5 ppm; HPLC (method 2): t_R = 3.651 min.

1-(2-Chloropyridin-4-yl)butan-1-one (23)



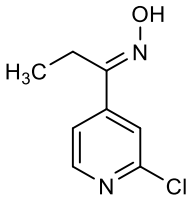
Under Argon atmosphere, Mg turnings (5.5 g, 100 mmol) were suspended in dry THF (40 mL) and subsequently 1-bromopropane (6.15 g, 50 mmol) was added and the mixture was stirred at rt for 60 min. The residual Mg was let decanting and the surnatant was added dropwise to a solution of compound **20** (5.0 g, 25 mmol) in dry THF (20 mL) under Argon atmosphere, previously cooled at -10 °C and the mixture was stirred at the same t for 3 h. After heating to rt, NH_4Cl saturated solution (100 mL) was added to the reaction. The 2 phases formed were separated and the aqueous phase was further extracted for 2 times with EtOAc. The combined organic layers were then dried over anhydrous Na_2SO_4 and the solvent was evaporated at reduced pressure. Finally, the residue was purified by flash column chromatography (SiO_2, *n*-hexane/EtoAc 8:2 to 7:3) affording 3.4 g of the desired compound (75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.99 (t, *J* = 7.4 Hz, 3H), 1.65 - 1.88 (m, 2H), 2.92 (t, *J* = 7.2 Hz, 2H), 7.64 (dd, *J* = 5.1, 1.4 Hz, 1H), 7.68 - 7.79 (m, 1H), 8.54 ppm (dd, *J* = 5.1, 0.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 13.6, 17.1, 40.9, 119.8, 122.3, 145.8, 150.8, 152.9, 198.1 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_9H_{10}ClNO$: 184.0, found: 184.0; *m*/*z* $[M-H]^-$ calcd. for $C_9H_{10}ClNO$: 182.0, found: 181.9; HPLC (method 2): t_R = 3.390 min.

1-(2-Chloropyridin-4-yl)ethan-1-one oxime (24)



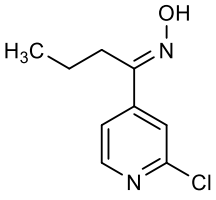
To a solution of hydroxylamine hydrochloride (1.61 g, 23.14 mmol) in a mixture of H_2O/MeOH (50 mL, 1:1), 5 mL of 20% NaOH solution were added. After cooling the reaction mixture at 0 °C, 4-acetyl-2-chloropyridine (**21**, 3.0 g, 19.28 mmol) was added in one portion and the reaction mixture was stirred for 2 h at the same temperature. The obtained precipitate was filtered off, washed with cold H_2O and dried, affording 2.96 g of the desired compound which was used for the following step without further purification (90% yield); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.15 (s, 3H), 7.63 (d, J = 5.1 Hz, 1H), 7.65 (s, 1H), 8.40 (d, J = 5.3 Hz, 1H), 11.93 ppm (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 10.9, 119.2, 120.2, 147.6, 150.0, 150.8,150.9; MS-FAB: m/z $[M+H]^+$ calcd. for $C_9H_{10}ClNO$: 171.0, found: 171.1; HPLC (method 1): t_R = 3.690 min.

1-(2-Chloropyridin-4-yl)ethan-1-one oxime (25)[2]



The title compound was synthesized as previously reported and analytical data were in agreement with the reported ones.[2]

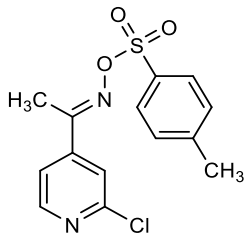
1-(2-Chloropyridin-4-yl)butan-1-one oxime (26)



Compound **23** (3.38 g, 18.43 mmol) was dissolved in MeOH (20 mL) and, after cooling the reaction to 0 °C, a mixture of hydroxylamine hydrochloride (1.41 g, 20.27 mmol) in H_2O (10 mL) and 20% NaOH (10 mL) was added dropwise and the reaction mixture was letting slowly heating to rt and stirred for 1 h. After removing the MeOH at reduced pressure, H_2O was added, and the aqueous phase was extracted 3 times with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. Finally, the residue was purified by flash column chromatography (SiO_2, *n*-hexane/EtOAc 80:20) affording 3.1 g of the desired product (85% yield); ^{1}H NMR (300 MHz, $CDCl_3$)

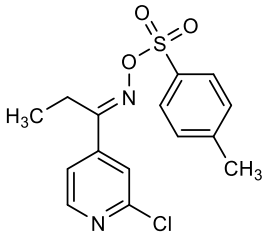
δ = 0.99 (t, *J* = 7.4 Hz, 3H), 1.50 - 1.67 (m, 2H), 2.66 - 2.82 (m, 2H), 7.46 (dd, *J* = 5.3, 1.6 Hz, 1H), 7.56 (dd, *J* = 1.5, 0.6 Hz, 1H), 8.40 (dd, *J* = 5.3, 0.6 Hz, 1H), 9.70 ppm (br. s., 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.1, 19.6, 27.2, 119.3, 121.3, 146.8, 149.7, 152.0, 156.5 ppm; MS-ESI: *m*/*z* $[M+H]^+$ calcd. for $C_9H_{11}ClN_2O$: 199.1, found: 199.1; *m*/*z* $[M-H]^-$ calcd. for $C_9H_{11}ClN_2O$: 197.1, found: 196.9; HPLC (method 2): t_R = 5.451 min.

1-(2-Chloropyridin-4-yl)ethan-1-one *O*-tosyl oxime (27)



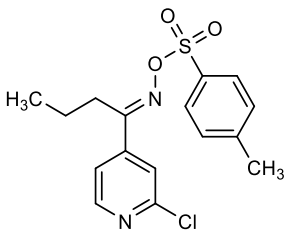
To a solution of 1-(2-chloropyridin-4-yl)ethan-1-one oxime (**24**, 2.94 g, 17.23 mmol) in dry pyridine (15 mL) *p*-toluenesulfonyl chloride (3.94 g, 48.28 mmol) was added and the mixture was stirred at rt for 24 h. 100 mL of ice-cold H_2O were added and the mixture was stirred for 3 h. Finally the precipitate formed was filtered off, washed with cold H_2O and dried, obtaining 4.11 g of the desired product (74% yield); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.35 (s, 3H), 2.41 (s, 3H), 7.50 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 5.3 Hz, 1H), 7.66 (s, 1H), 7.92 (d, *J* = 7.8 Hz, 2H), 8.50 (d, *J* = 5.1 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ = 13.7, 21.1, 120.1, 121.7, 128.5, 130.1, 131.5), 144.1, 145.8, 150.6, 151.0, 162.1; MS-FAB: *m*/*z* $[M+H]^+$ calcd. for $C_{14}H_{13}ClN_2O_3S$: 325.1, found: 325.1; HPLC (method 1): t_R = 6.930 min.

1-(2-Chloropyridin-4-yl)propan-1-one *O*-tosyl oxime (28)[2]



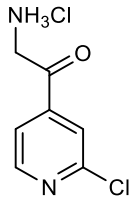
The title compound was synthesized as previously reported and analytical data were in agreement with the published ones.[2]

1-(2-Chloropyridin-4-yl)butan-1-one *O*-tosyl oxime (29)



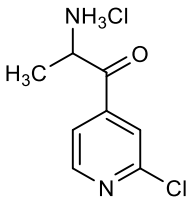
To a solution of 1-(2-chloropyridin-4-yl)butan-1-one oxime (**26**, 3.09 g, 15.58 mmol) in dry pyridine (20 mL) *p*-toluenesulfonyl chloride (4.45 g, 23.38 mmol) was added and the mixture was stirred at rt for 48 h. 100 mL H_2O were added and the aqueous phase was extracted 3 times with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated at reduced pressure, affording 4.4 g of the desired product which was used for the following step without further purification (80% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.80 (t, *J* = 7.4 Hz, 3H), 1.30 - 1.47 (m, 2H), 2.40 (s, 3H), 2.75 - 2.87 (m, 2H), 7.45 - 7.53 (m, 2H), 7.56 (dd, *J* = 5.2, 1.5 Hz, 1H), 7.62 - 7.67 (m, 1H), 7.85 - 7.95 (m, 2H), 8.50 ppm (dd, *J* = 5.2, 0.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 13.4, 19.2, 21.1, 28.4, 120.6, 121.8, 128.5, 130.1, 131.4, 143.3, 145.8, 150.8, 151.2, 165.3 ppm; HPLC (method 2): t_R = 8.430 min.

2-Amino-1-(2-chloropyridin-4-yl)ethan-1-one hydrochloride (30)



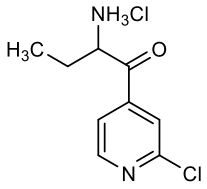
Under Argon atmosphere potassium (241 mg, 6.16 mmol) was added portionwise to 20 mL of absolute EtOH. After complete dissolution the reaction mixture was cooled to 0 °C and a solution of 1-(2-chloropyridin-4-yl)ethan-1-one *O*-tosyl oxime (**27**, 2 g, 6.16 mmol) in absolute EtOH (100 mL) was slowly added dropwise. After completion of the addition the reaction mixture was stirred for 1 h at rt and after that 150 mL of dry Et_2O were added and the mixture was stirred at rt for 16 h. The white precipitate formed was filtered and washed twice with 50 mL Et_2O. HCl was bubbled in the combined organic layer and the white precipitate formed was filtered off, washed with Et_2O and dried. The solid obtained was dissolved in concentrated HCl (25 mL) and stirred at 55 °C for 50 h. After concentrating the mixture at reduced pressure warm MeOH was added and the product was then precipitated by adding Et_2O. The red solid formed was finally filtered off and dried giving 210 mg which were use in the following step without further purification (17% yield); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 45.4, 120.6, 122.3, 143.1, 151.4, 151.5, 192.1 ppm; MS-FAB: *m*/*z* $[M+H]^+$ calcd. for $C_7H_8Cl_2N_2O$: 171.0, found: 171.1; HPLC (method 2): t_R = 1.145 min.

2-Amino-1-(2-chloropyridin-4-yl)propan-1-one hydrochloride (31)



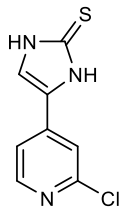
Under Argon atmosphere potassium (1.18 g, 30.14 mmol) was added portionwise to 50 mL of absolute EtOH. After complete dissolution the reaction mixture was cooled to 0 °C and a solution of 1-(2-chloropyridin-4-yl)propan-1-one *O*-tosyl oxime (**28**, 10.21 g, 30.14 mmol) in absolute EtOH (200 mL) was added dropwise. After completion of the addition the reaction mixture was stirred for 1 h at 0 °C and after that 100 mL of dry Et_2O were added and the mixture was stirred at rt for 16 h. The white precipitate formed was filtered and the filtrate was concentrated at reduced pressure. The oily residue obtained was dissolved in concentrated HCl (30 mL) and stirred at 55 °C for 1 h. Finally, after evaporating the solvent at reduced pressure, the residue was treated with acetone and the white precipitate formed was filtered off and washed with cold acetone, giving 3.34 g of the desired compound as a hydrochloride salt (50% yield); HPLC (method 2): t_R = 1.258 min.

2-Amino-1-(2-chloropyridin-4-yl)butan-1-one hydrochloride (32)



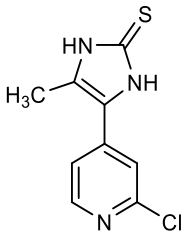
The title compound was synthesized following the same procedure as compound **31** starting from potassium (718 mg, 18.37 mmol) and 1-(2-chloropyridin-4-yl)butan-1-one *O*-tosyl oxime (**29**, 4.32 g, 12.25 mmol) affording 1.7 g of the desired product as a hydrochloride salt which was directly used for the following step (60% yield).

4-(2-Chloropyridin-4-yl)-1,3-dihydro-2*H*-imidazole-2-thione (33)



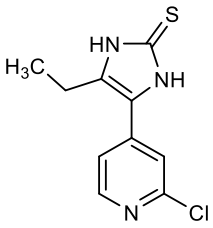
Compound **30** (3.54 g, 17.1 mmol) was dissolved in MeOH (90 mL) and after that KSCN (8.25 g, 84.9 mmol) was added. The reaction mixture was heated to reflux temperature and stirred for 4 h. The yellow precipitate formed was filtered off, washed with H_2O and dried *in vacuo* obtaining 1.51 g of the desired product which was used for the following step without further purification (43% yield); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.66 (d, *J* = 5.1 Hz, 1H), 7.83 (s, 1H), 7.87 (s, 1H) 8.36 (d, 5.3 Hz, 1H), 12.51 (s, 1H), 13.82 ppm (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 117.0, 117.3, 117.7, 125.3, 138.6, 150.3, 151.2, 163.5 ppm; MS-FAB: *m*/*z* $[M+H]^+$ calcd. for $C_8H_6ClN_3S$: 212.0, found: 212.2; HPLC (method 2): t_R=2.270 min.

4-(2-Chloropyridin-4-yl)-5-methyl-1,3-dihydro-2*H*-imidazole-2-thione (34)[2]



Compound **31** (5.5 g, 21.36 mmol) was dissolved in MeOH (50 mL) and after that KSCN (6.23 g, 97.18 mmol) was added. The reaction mixture was heated to reflux temperature and stirred for 3 h. After cooling down, the white precipitate formed was filtered off, washed with cold MeOH and cold H_2O and dried, obtaining 3.42 g of the desired compound (71% yield); Analytical data were in agreement with the previously reported ones.[2]

4-(2-Chloropyridin-4-yl)-5-ethyl-1,3-dihydro-2*H*-imidazole-2-thione (35)



The title compound was synthesized following the same procedure as compound **34** starting from **32** (1.65 g, 6.08 mmol) and KSCN (1.77 g, 18.23 mmol), giving 850 mg of the desired product (60% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.17 (t, *J* = 7.5 Hz, 3H), 2.66 (q, *J* = 7.5 Hz, 2H), 7.44 (dd, *J* = 5.4, 1.6 Hz, 1H), 7.51 - 7.60 (m, 1H), 8.38 (dd, *J* = 5.4, 0.4 Hz, 1H), 12.35 - 12.51 ppm (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 13.0, 17.9, 118.8, 119.4, 119.7, 132.4, 139.1, 150.2, 151.1, 161.8 ppm; MS-ESI: *m*/*z* [M-H]$^-$ calcd for $C_{10}H_{10}ClN_3S$: 238.0, found: 237.9; HPLC (method 2): t_R=2.698 min.

Structure determination of compound 49

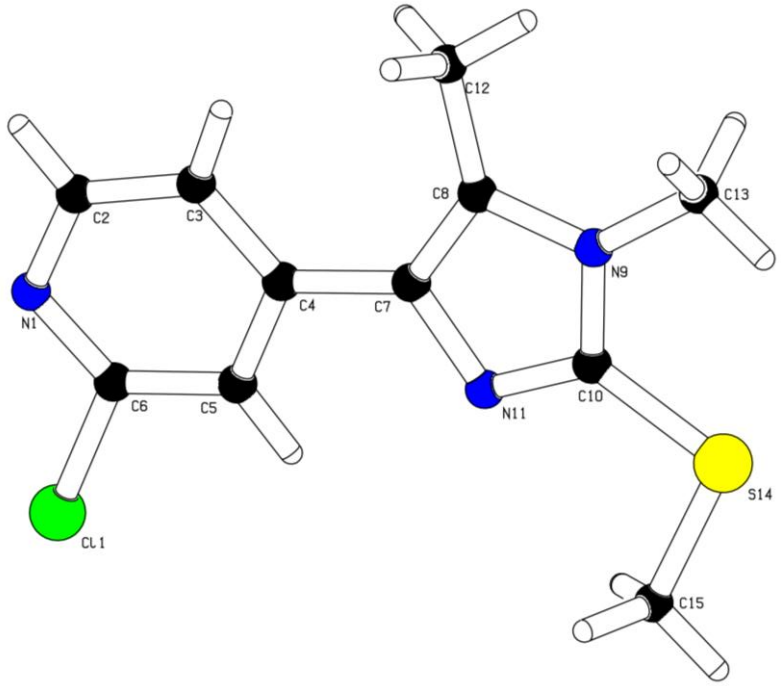


Figure S1. Crystal structure of compound **49**. The structure confirms the substitution of the imidazole-N atom distal from the pyridine ring. Diffraction data were collected at 193 K with a STOE IPDS-2T diffractometer with Mo Kα radiation. Data processing was performed using the STOE-software. The structure was solved using Direct methods (SIR-2004) and refined with SHELXL-2014. Data for atomic coordinates, thermal parameters and reflections can be obtained from the Cambridge Crystallographic Data Centre under the CCDC Nr. 1824231.

Table S1. Data collection and refinement statistics

Data collection	
Space group	P-1 (triclinic Nr.2)
Cell dimensions	determinate from 5730 reflections with 2.7°< θ < 28.2°
a,b,c (Å)	8.2302(8), 8.2302(8), 9.1962(9)
α, β, γ (°)	100.036(7), 98.894(8), 111.423(7)
V(Å³), z	586.65(9), 2
Crystal size (mm³)	0.04 x 0.16 x 0.24 (colorless plate)
Range of Measurement	$2° \leq \theta \leq 28°$, $-10 \leq h \leq 10$ $-11 \leq k \leq 11$ $-12 \leq l \leq 12$
No. of reflections:	
Measured	6001
Unique	2814 (R_{int} = 0.0482)
Observed (|F|/σ(F) > 4.0)	1976

Table S1. continued

Refinement	
Nr. of parameters	148
wR2	0.1069
R1(observed), R(all)	0.0391, 0.0612
Goodness of Fit	0.971
Max. deviation of parameters	0.001 * e.s.d.
Max. Peak final diff. Fourier synthesis (e $Å^{-3}$)	0.22,-0.25

Thermal shift assay by nanoDSF

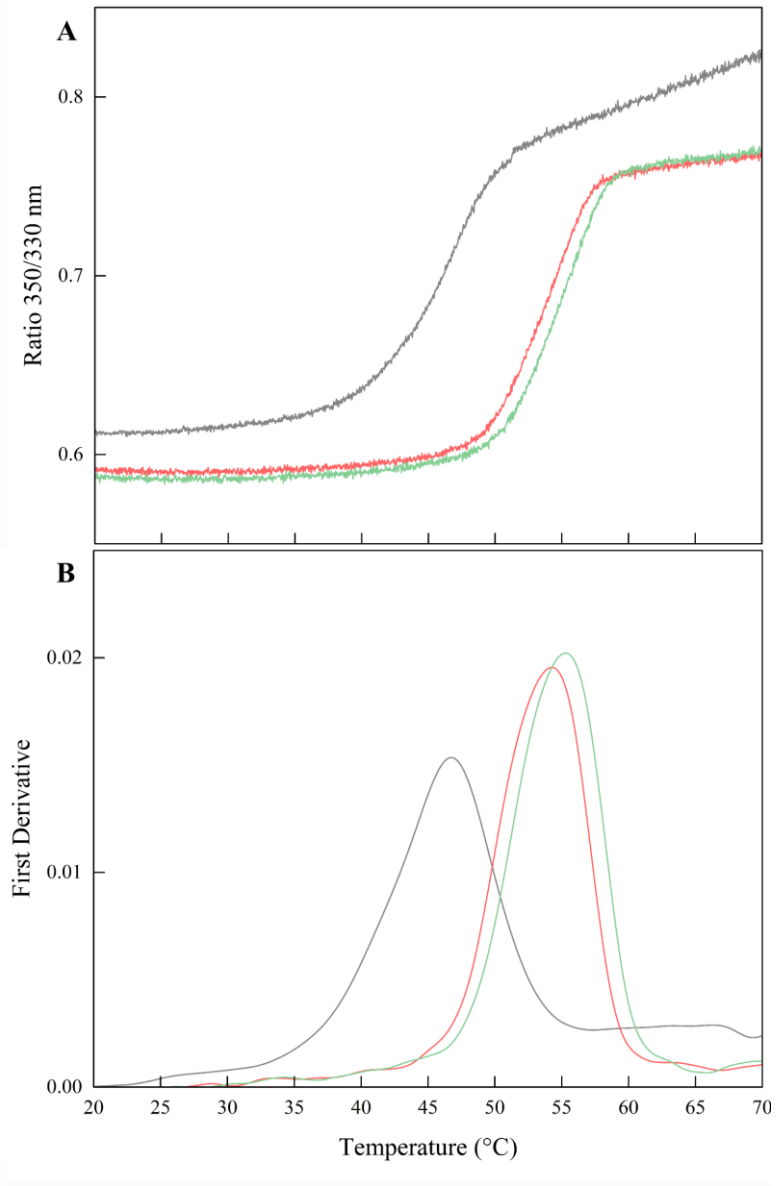


Figure S2. Effects of ligand binding on JNK3 thermal stability determined by nanoDSF. a) Intrinsic fluorescence intensity ratio of tryptophans and tyrosines (350/330 nm) plotted as a function of temperature; b) First derivative analysis used to deduce the melting temperature (T_m). The gray curve corresponds to JNK3, whereas the light red and light green curves correspond to the JNK3-**38** and JNK3-**44** complexes, respectively.

Experimental procedure

Samples were prepared using the protein buffer 50 mM HEPES pH 7, 100 mM NaCl, 2 mM $MgCl_2$, 10 mM β-mercaptoethanol. Compounds **38** and **44** (stock concentration of 10 mM in DMSO) were diluted in protein buffer to 100 µM and then added to the protein sample. The final protein and inhibitor

concentrations were 5 μM and 50 μM, respectively. 10 μL of each sample were loaded into capillary glass tubes and measured in triplicates in a single nanoDSF experiment using a Prometheus NT.48 instrument (NanoTemper Tecnhologies, Munich). All loaded capillaries were heated from 20 °C to 70 °C with a rate of 1 °C/min. Changes in the intrinsic fluorescence of tryptophans and tyrosines were monitored as the ratio of the emission at the wavelengths of 350 and 330 nm as a function of temperature. First derivative analysis of the resulting melting curves allowed the determination of the melting temperature (T_m).

Structure determination of JNK3 in complex with compounds 38 and 44

Crystallization of JNK3 and the inhibitor complexes

JNK3 crystals were obtained by adapting the experimental procedure described by Lange *et al*.[3] Pure protein in 50 mM HEPES pH 7, 100 mM NaCl, 2 mM $MgCl_2$, 10 mM β-mercaptoethanol buffer was initially mixed with 1 mM AMP-PCP (β,γ-Methyleneadenosine 5′-triphosphate disodium salt from Sigma-Aldrich), 0.4 mM Zwittergent 3-14 (Calbiochem) and 10% ethylene glycol and incubated on ice for 30 min. Crystals were grown at 20 °C using the sitting drop vapor diffusion method in a reservoir solution of 0.1 M Bis-Tris pH 5.5, 0.2 M NaCl and 29% (v/v) polyethylene glycol 3350. To improve the crystal quality, a final JNK3 concentration of 2.5 mg/mL was used together with microseeding. After one week, AMP-PCP containing crystals were incubated with the inhibitors over 36 h by gradually exchanging the crystal drop solution with reservoir solution supplemented with 10 mM of compounds **38** or **44**. The crystals were then stepwise cryo-protected by incubation in reservoir solution containing 10 mM of the respective compound and 15% (v/v) glycerol and flash frozen in liquid nitrogen prior to synchrotron data collection. For structural comparison, crystals containing AMP-PCP were also cryo-protected with 15% (v/v) glycerol and flash frozen for data collection

Data collection and structure determination

Diffraction data were collected at 100 K and a wavelength of 1 Å at the Swiss Light Source (PSI, Villigen, Switzerland) beamline X06DA using the Pilatus 2M-F detector. Data processing was performed using the XDS-software[4] and initial phases were obtained by molecular replacement using MOLREP[5] and a search model derived from a reported complex structure (PDB ID 4X21)[3]. The individual JNK3 complexes belonged to different space groups, which resulted in a different number of copies of ligand-bound JNK3 in the individual crystal asymmetric units. For JNK3 bound to compound **38** and AMP-PCP, chain A was used for further structural analysis, due to the lower overall B-factors and the high structural similarity to the other copies (Figure S4 for JNK3-**38** superposed copies). All ligands could be placed unambiguously in the difference electron density map in all JNK3 chains. Structural refinement was carried out by alternating cycles of model building in Coot[6] and restrained reciprocal refinement including transition-libration-screw (TLS) parameterization in REFMAC5[7]. After the final refinement step, ligand molecules were removed from the model and an unbiased difference omit map was produced in PHENIX[8] using simulated annealing. Data collection and refinement statistics were obtained (Table S2) and the simulated annealing omit difference maps were calculated (Figure S5). Structural figures were prepared using PyMOL (The PyMOL Molecular Graphics System, Version 1.8.4.1 Schrödringer, LLC).

Table S2. Data collection and refinement statistics.

	JNK3-AMP-PCP	JNK3-**38**	JNK3-**44**
PDB ID	6EQ9	6EMH	6EKD
Data collection*			
Space group	$P2_12_12$	$P2_12_12_1$	$C222_1$
Cell dimensions			
a, *b*, *c* (Å)	156.73, 110.49, 43.95	88.56, 114.26, 157.8	81.51, 124.82, 68.89
α, *β*, *γ* (°)	90, 90, 90	90, 90, 90	90, 90, 90
JNK3 monomer/ASU	2	4	1
Resolution (Å)	47.23-1.83 (1.94-1.83)	48.01-1.76 (1.81-1.76)	48.49-2.10 (2.15-2.10)
Measured reflections	784898 (108776)	4164005 (296660)	540357 (39002)
Unique reflections	68590 (10906)	157963 (11494)	20913 (1538)
Completeness (%)	98.8 (95.9)	99.9 (98.9)	100 (99.9)
Redundancy	11.4 (9.97)	26.4 (25.8)	25.8 (25.4)
CC1/2 (%)	99.9 (66.2)	100 (63.2)	100 (55.6)
$I/\sigma(I)$	18.1 (1.63)	22.98 (1.4)	26.4 (1.61)
Wilson *B*-factor (Å^2)	29.75	30.84	43.79
Refinement			
Resolution (Å)	47.23-1.83	48.01-1.76	48.49-2.10
R_{work}/R_{free}	20.62/25.18	21.81/26.85**	20.56/25.90
Number of atoms			
Protein chain a/b/c/d	2812/2606	2826/2826/2700/2665	2558
Water	391	861	104
Ligand***	62	100	27
B-factors (Å^2)			
Protein chain a/b/c/d	34.68/39.57	38.5/40.7/44.2/46.4	52.55
Water	41.26	43.6	48.46
Ligand***	47.85	37.7	46.48
R.m.s. deviations			
Bond lengths (Å)	0.015	0.014	0.015
Bond angles (°)	1.439	1.515	1.513

*Values in parentheses correspond to the highest resolution shell.

**Pseudo translational symmetry is present.

***AMP-PCP and compounds **38** and **44**.

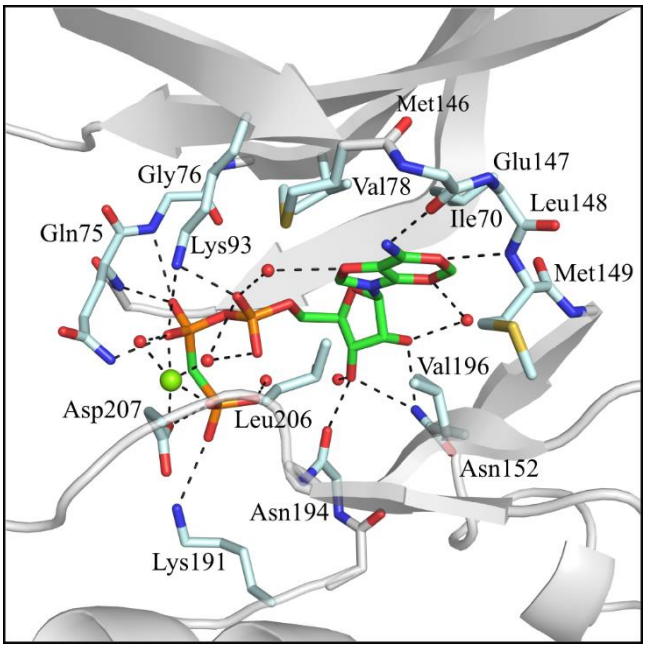


Figure S3. Binding mode of the non-hydrolyzable ATP analogue, AMP-PCP, to JNK3. For clarity, only the chain A present in the crystal asymmetric unit is shown. AMP-PCP is shown in stick display with the adenine and ribose groups highlighted in green and the three phosphate groups in orange. Amino acids contributing to key interactions are shown as sticks in light blue and are labeled. A magnesium ion interacting with the AMP-PCP molecule is displayed as a green sphere, H_2O molecules are shown as red spheres, and hydrogen bonds as black dashed lines. The structure of unphosphorylated JNK3 in complex with an ATP analogue was first reported in 1998[9], and the structure reported here in complex with AMP-PCP is substantially identical, with an overall RMSD of 0.853 Å.

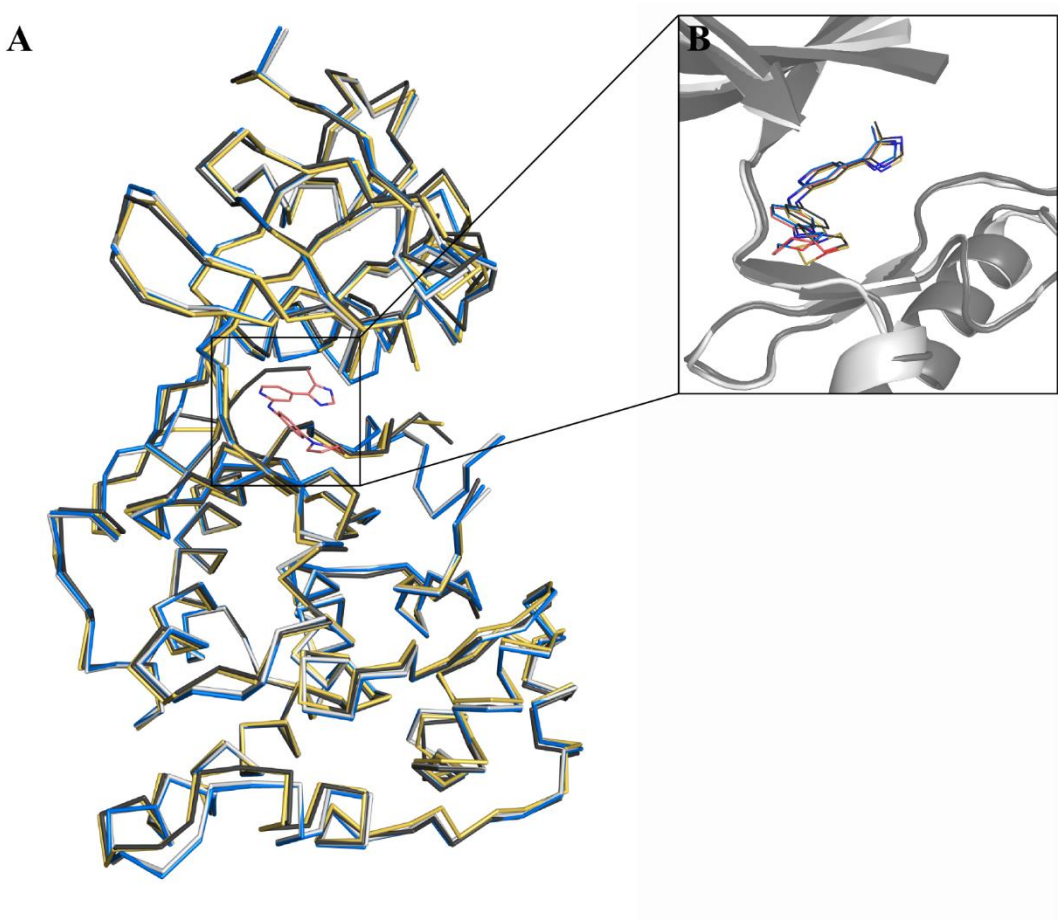


Figure S4. Overlay of the four JNK3-**38** copies present in the crystal asymmetric unit. Superposition of the copies was performed using the "align" function in PyMOL; a) Overview of the four protein chains shown in ribbon display (A: light gray, B: blue, C: dark gray and D: yellow) and the compound bound to chain A (light red); b) Close-up view of the binding pocket of chains A (light grey) and C (dark grey) and superposition of the four compounds bound to each copy in the asymmetric unit. Compounds are shown in the same colors as the protein chains displayed in a). Orientation and placement of all compounds is almost identical.

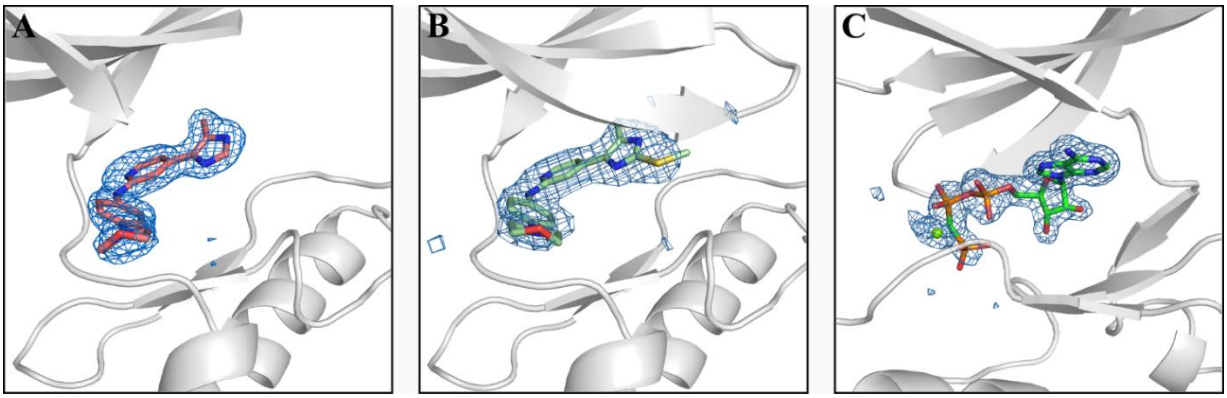


Figure S5. Binding of compounds **38** and **44** and AMP-PCP to JNK3. Simulated annealing omit difference electron density maps were countered at 3.0 σ and are displayed within a radius of 5 Å around the ligand; a) compound **38** bound to chain A of the crystal asymmetric unit; b) Compound **44**; c) AMP-PCP bound to chain A of the crystal asymmetric unit.

In vitro metabolic stability of compound 44

time [min]	Sum of metabolites [%]	Metabolic degradation [%]
0	0.00	100.00
10	1.06	96.62
20	1.97	94.49
30	2.70	91.63
60	4.28	88.65
120	6.71	86.13
180	8.10	82.59
240	8.91	80.61

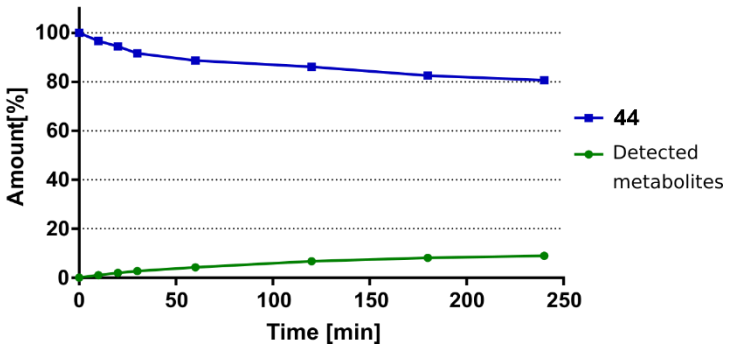


Figure S6. Results of metabolic stability assay; A) table reporting percentage of residual compound and formed metabolites at different time points; B) plot of obtained results;

Experimental procedure

Pooled adult male and female human liver microsomes (HLMs) were purchased from Merck (Schnelldorf, Germany). The incubations of HLMs were performed in the presence of an NADPH-regenerating system consisting of 5 mM glucose-6-phosphate, 5 U/mL glucose-6-phosphate dehydrogenase, 1 mM $NADP^+$, and 4 mM $MgCl_2$-hexahydrate. All solutions were made in 0.1 M Tris buffer (pH 7.4 at 37 °C). After addition of the HLMs to the NADPH-regenerating system the mix was pre-incubated at 37 °C for 5 min and 750 rpm in a shaker. The final concentration of microsomal protein content was 1 mg/mL. Compound **44** was pre-incubated separately under the same conditions.

The reaction was started by adding compound **44** to the NADPH-regenerating system and the incubation mix was subsequently split into 50 µL aliquots. The incubations were stopped at selected time points (0, 10, 20, 30, 60, 120, 180, 240 min) by the addition of 100 µL ice-cold acetonitrile spiked with internal standard with a concentration of 33 µM. After vortexing, the samples were centrifuged (19800 rcf at 4 °C, 20 min) and the supernatant was subjected to LC-MS analysis. In order to ensure that the decrease of compound concentration was exclusively due to metabolic degradation heat inactivated HLMs were used as control. All incubations were conducted in triplicates. None of the incubations exceeded the limit of 1% organic solvent.

Kinase selectivity screening of 44.

Compound **44** was tested at Cerep, Eurofins (Celle L'Evescault, France) in the ExpresS Diversity Kinase Panel against 45 selected human kinases at a concentration of 10 µM.

Table S3. Inhibition of selected kinases (n=2).

#	kinase name	kinase family[a]	mean inhibition (%)
1	Abl	TK	19.3
2	Akt1 (PKBalpha)	AGC	-2.9
3	Aurora-A	other	40.6
4	CaMK2alpha	CAMK	-5.7
5	CDK1	CAMGC	62.0
6	CDK2	CAMGC	66.4
7	CHK1	CAMK	12.4
8	CHK2	CAMK	-5.4
9	c-Raf (Raf-1)	TKL	64.1
10	EGFR	TK	56.6
11	EPHA2	TK	17.1
12	EPHA3	TK	-6.3
13	EphB4	TK	18.2
14	ERK2 (MAPK1)	CMGC	5.3
15	FGFR	TK	26.2
16	FGFR2	TK	44.9
17	FGFR3	TK	70.4
18	GSK3beta	CMGC	5.7
19	HGK (MAP4K4)	STE	77.9
20	IKKalpha	Other	48.8
21	IR	RTK	-1.8
22	IRAK4	TKL	45.8
23	JAK3	TK	65.5
24	JNK1	CMGC	88.6
25	KDR (VEGFR2)	TK	95.1

Table S3. continued.

#	Kinase Name	kinase family*	mean inhibition (%)
26	LCK	TK	15.8
27	MAPKAPK2	CAMK	-1.9
28	MARK1	CAMK	20.1
29	Met	TK	28.2
30	MNK2	CAMK	94.9
31	NEK2	Other	20.0
32	PAK2	STE	1.4
33	PAK4	STE	-3.9
34	PDK1	AGC	41.3
35	PIM2	CAMK	-4.8
36	PKA	AGC	5.0
37	PKCbeta	AGC	1.3
38	PLK1	Other	6.3
39	ROCK1	AGC	-4.2
40	SAPK2A (p38alpha)	CMGC	11.4
41	SGK1	AGC	34.8
42	SIK	CAMK	21.6
43	SRC	TK	12.5
44	TAO2	STE	2.1
45	TRKA	TK	10.1

[a]AGC: containing PKA, PKG and PKC families; CAMK: calcium/calmoduline-dependent protein kinases; CK1: casein kinase 1-like; CMGC: containing CDK, MAPK, GSK3 and CLK families; TK: tyrosine kinase; TKL: tyrosine kinase-like; STE: homologs of yeast sterile 7, sterile 11, sterile 20 kinases.

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