

Supplementary Information

**The discovery and optimisation of a novel series of Dyrk1B kinase inhibitors to explore a
MEK resistance hypothesis**

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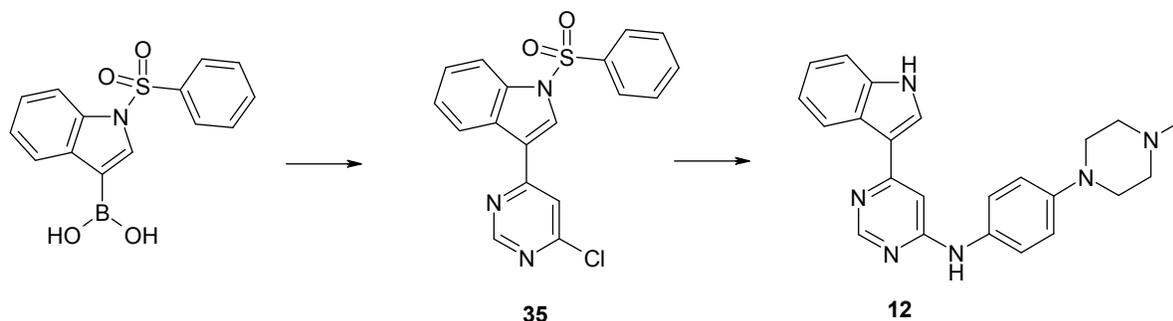
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1. Experimental methods and synthesis details

Chemistry All reactions were performed under inert conditions (nitrogen) unless otherwise stated. Temperatures are given in degrees Celsius ($^{\circ}\text{C}$); operations were carried out at room or ambient temperature, that is, at a temperature in the range of 18 to 25°C . All solvents and reagents were purchased from commercial sources and used without further purification. For coupling reactions, all solvents were dried and degassed prior to reaction. Reactions performed under microwave irradiation utilized either a Biotage Initiator or CEM Discover Microwave. Upon work up, organic solvents were typically dried prior to concentration with anhydrous MgSO_4 or Na_2SO_4 . Flash silica chromatography was typically performed on an Isco Companion, using Silicycle silica gel, 230-400 mesh 40-63 μm cartridges, Grace Resolv silica cartridges or Isolute Flash Si or Si II cartridges. Reverse phase chromatography was performed using a Waters XBridge Prep C18 OBD column, 5 μm silica, 19 mm diameter, 100 mm length), using decreasingly polar mixtures of either water (containing 1% NH_3) and acetonitrile, or water (containing 0.1% formic acid) and acetonitrile, as eluents. Analytical LC-MS was performed on a Waters 2790 LC with a 996 PDA and 2000 amu ZQ Single Quadrupole Mass Spectrometer using a Phenomenex Gemini 50 x 2.1mm 5 μm C18 column, or UPLC was performed on an Waters Acquity Binary Solvent Manager with Acquity PDA and an SQD mass spectrometer using a 50 x 2.1 mm 1.7 μm BEH column from Waters, and purities were measured by UV absorption at 254 nm or TIC and are $\geq 95\%$ unless otherwise stated. NMR spectra were recorded on a Bruker Av400 or Bruker DRX400 spectrometer at 400 MHz in d_6 -DMSO at 303K unless

otherwise indicated. ^1H NMR spectra are reported as chemical shifts in parts per million (ppm) relative to an internal solvent reference. Yields are given for illustration only and are not necessarily those which can be obtained by diligent process development; preparations were repeated if more material was required.



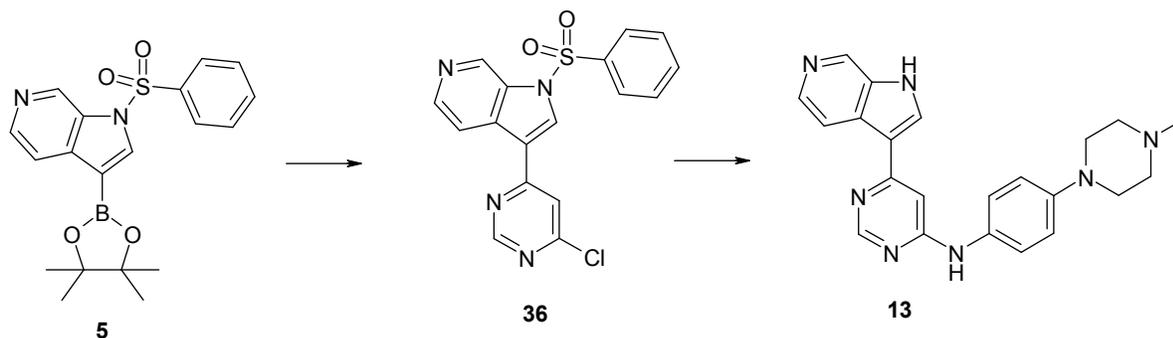
3-(6-Chloropyrimidin-4-yl)-1-(phenylsulfonyl)-1H-indole (35)

Tetrakis(triphenylphosphine)palladium(0) (0.96 g, 0.8 mmol) was added in one portion to 4,6-dichloropyrimidine (3.7 g, 24.9 mmol), 1-(phenylsulfonyl)-1H-indol-3-ylboronic acid (5.0 g, 16.6 mmol) and 2M sodium carbonate (33.2 ml, 66.4 mmol) in dioxane (80 ml) at 25°C under nitrogen. The resulting mixture was stirred at 80°C for 1 hour. The reaction mixture was allowed to cool and then quenched with water (750 ml), the precipitate was collected by filtration, washed with water and dried under vacuum to afford crude product as a green solid. The crude solid was triturated with diethyl ether to give a solid which was collected by filtration and dried under vacuum to give **35** as a cream solid (2.6 g, 43%). ^1H NMR δ 7.41 (1H, td), 7.46 (1H, ddd), 7.61 - 7.68 (2H, m), 7.71 - 7.77 (1H, m), 8.03 (1H, d), 8.09 - 8.15 (2H, m), 8.49 (1H, d), 8.58 (1H, dd), 9.06 (1H, s), 9.09 (1H, d); m/z (ES+) (M+H)⁺ = 369.95; HPLC tR = 3.54 min.

6-(1H-Indol-3-yl)-N-(4-(4-methylpiperazin-1-yl)phenyl)pyrimidin-4-amine (12)

3-(6-Chloropyrimidin-4-yl)-1-(phenylsulfonyl)-1H-indole **35** (200 mg, 0.5 mmol), 4-(4-methylpiperazin-1-yl)aniline (269 mg, 1.4 mmol) and 4-methylbenzenesulfonic acid hydrate (411 mg, 2.2 mmol) were dissolved in NMP (20 ml) and sealed into a microwave tube. The reaction was heated to 200°C for 2 hours then cooled to room temperature. The reaction mixture was diluted with ethyl acetate (100 ml), and washed sequentially with saturated aq. Na_2CO_3 (100 ml), water (2 x 100 ml) and saturated brine (100 ml). The organic layer was dried, filtered and

evaporated to afford crude product which was purified by crystallisation from DCM to afford **12** as a white solid (104 mg, 50%). ^1H NMR δ 2.23 (3H, s), 2.44 - 2.49 (4H, m), 3.05 - 3.14 (4H, m), 6.94 (2H, d), 7.11 (1H, d), 7.13 - 7.24 (2H, m), 7.44 - 7.56 (3H, m), 8.07 (1H, d), 8.21 (1H, dd), 8.54 (1H, s), 9.18 (1H, s), 11.64 (1H, br s); HRMS Found: ESI^+ m/z , 385.21335 ($\text{M}+\text{H}$) $^+$ expected: 385.20624; HPLC t_R = 2.84 min.

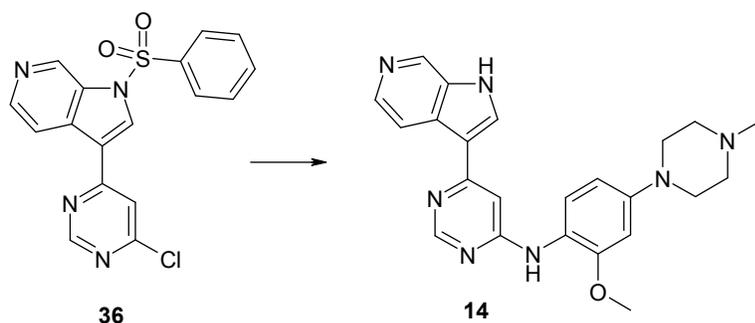


3-(6-Chloropyrimidin-4-yl)-1-(phenylsulfonyl)-1H-pyrrolo[2,3-c]pyridine (**36**)

The procedure described above for compound **35** was repeated using 4,6-dichloropyrimidine and 1-(phenylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-c]pyridine **5** to afford compound **36** as a pink solid in 37% yield. ^1H NMR δ 7.72 (2H, t), 7.83 (1H, tt), 8.26 (2H, dd), 8.53 (1H, dd), 8.56 (1H, d), 8.58 (1H, d), 9.16 (1H, d), 9.31 (1H, s), 9.36 (1H, d) m/z (ES^+), ($\text{M}+\text{H}$) $^+$ = 370.99; HPLC t_R = 2.47 min.

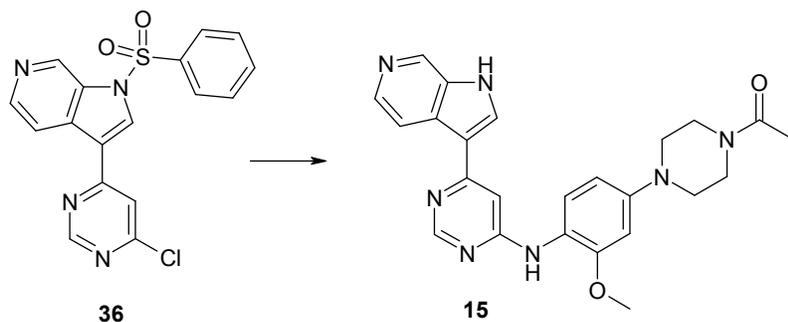
N-(4-(4-Methylpiperazin-1-yl)phenyl)-6-(1H-pyrrolo[2,3-c]pyridin-3-yl)pyrimidin-4-amine (**13**)

The procedure described above for compound **12** was repeated using compound **36** and 4-(4-methylpiperazin-1-yl)aniline to afford compound **13** as a yellow solid in 33% yield. ^1H NMR δ 2.23 (3H, s), 2.47 (4H, t), 3.10 (4H, t), 6.94 (2H, d), 7.11 (1H, d), 7.51 (2H, d), 8.12 (1H, dd), 8.26 (1H, d), 8.29 (1H, s), 8.56 (1H, d), 8.83 (1H, d), 9.23 (1H, s), 12.11 (1H, s); HRMS Found: ESI^+ m/z , 386.20828 ($\text{M}+\text{H}$) $^+$ expected: 386.20149; HPLC t_R = 1.87 min.



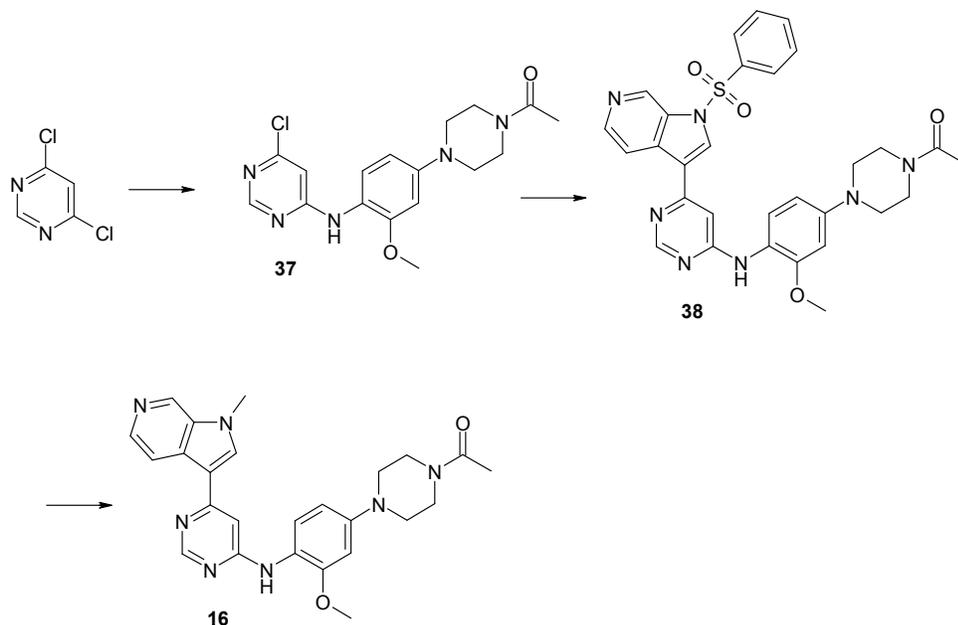
***N*-(2-Methoxy-4-(4-methylpiperazin-1-yl)phenyl)-6-(1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)pyrimidin-4-amine (14)**

The procedure described above for compound **13** was repeated using compound **36** and 2-methoxy-4-(4-methylpiperazin-1-yl)aniline to afford compound **14** as a yellow solid in 26% yield. ¹H NMR 2.23 (3H, s), 2.44 – 2.48 (4H, m), 3.11 – 3.16 (4H, m), 3.83 (3H, s), 6.51 (1H, dd), 6.65 (1H, d), 7.13 (1H, s), 7.69 (1H, d), 8.12 (1H, dd), 8.2 – 8.25 (2H, m), 8.45 – 8.5 (2H, m), 8.80 (1H, d), 12.06 (1H, br s); HRMS Found: ESI⁺ m/z, 416.21887 (M+H)⁺ expected: 416.21206; HPLC tR = 1.58 min.



1-(4-(4-(6-(1*H*-Pyrrolo[2,3-*c*]pyridin-3-yl)pyrimidin-4-ylamino)-3-methoxyphenyl)piperazin-1-yl)ethanone (15)

The procedure described above for compound **13** was repeated using compound **36** and 1-[4-(4-amino-3-methoxyphenyl)piperazin-1-yl]ethanone to afford compound **15** as a yellow solid in 51% yield. ¹H NMR δ 2.06 (3H, s), 3.12 (2H, t), 3.18 (2H, t), 3.61 (4H, q), 3.86 (3H, s), 6.55 (1H, dd), 6.71 (1H, d), 7.19 (1H, s), 7.77 (1H, d), 8.15 (1H, dd), 8.25 (1H, d), 8.26 (1H, s), 8.51 (1H, d), 8.54 (1H, s), 8.82 (1H, d); HRMS Found: ESI⁺ m/z, 444.21425 (M+H)⁺ expected: 444.20697; HPLC tR = 1.49 min.



1-(4-(4-(6-Chloropyrimidin-4-ylamino)-3-methoxyphenyl)piperazin-1-yl)ethanone (37)

N,N-Diisopropylethylamine (0.43 ml, 2.5 mmol) was added in one portion to 4,6-dichloropyrimidine (0.27 g, 1.80 mmol) and 1-(4-(4-amino-3-methoxyphenyl)piperazin-1-yl)ethanone (0.41 g, 1.63 mmol) in ethanol (10 ml) at 25°C under nitrogen. The resulting solution was stirred at 60°C for 18 hours. The reaction mixture was evaporated to dryness and dissolved in DCM and was purified by flash silica chromatography, elution gradient 0 to 3% methanol in DCM. Pure fractions were evaporated to dryness to afford **37** as a white solid after ether trituration (0.53 g, 90%). ¹H NMR δ 2.05 (3H, s), 3.14 (2H, t), 3.20 (2H, t), 3.56 - 3.63 (4H, m), 3.80 (3H, s), 6.49 - 6.57 (2H, m), 6.68 (1H, d), 7.38 (1H, s), 8.31 (1H, s), 9.04 (1H, s); m/z (ES+), (M+H)+ = 362.41; HPLC tR = 1.60 min.

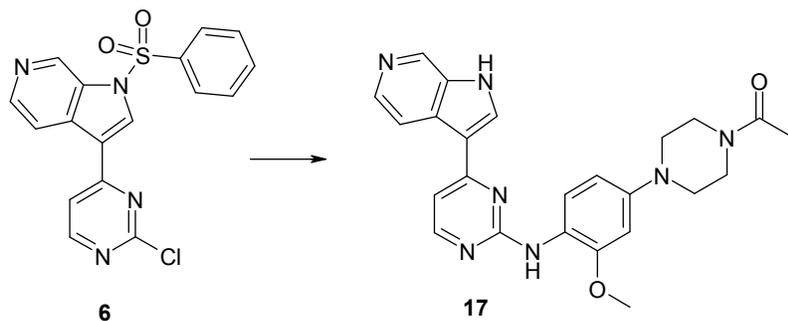
1-(4-(3-Methoxy-4-(6-(1-(phenylsulfonyl)-1H-pyrrolo[2,3-c]pyridin-3-yl)pyrimidin-4-ylamino)phenyl)piperazin-1-yl)ethanone (38)

Tetrakis(triphenylphosphine)palladium(0) (80 mg, 0.06 mmol) was added in one portion to 1-(phenylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-c]pyridine **36** (422 mg, 1.1 mmol), 1-(4-(4-(6-chloropyrimidin-4-ylamino)-3-methoxyphenyl)piperazin-1-yl)ethanone **37** (250 mg, 0.69 mmol) and sodium carbonate (1.0 ml, 2.1 mmol) in dioxane (6 ml) at 25°C under nitrogen. The resulting suspension was stirred at 80°C for 2.5 hours. The reaction mixture was allowed to cool and diluted with ethyl acetate and water. The organic layer was collected and the aqueous layer was extracted with ethyl acetate. The combined organic extracts

were purified by ion exchange chromatography, using an 20g SCX-2 column. The desired product was eluted from the column using 7M ammonia in methanol and product fractions were evaporated to dryness to afford crude product which was purified by flash silica chromatography, elution gradient 0 to 5% methanol in DCM to afford **38** as a green solid (235 mg, 58 %). m/z (ES+), (M+H)+ = 584.08; HPLC tR = 2.19 min.

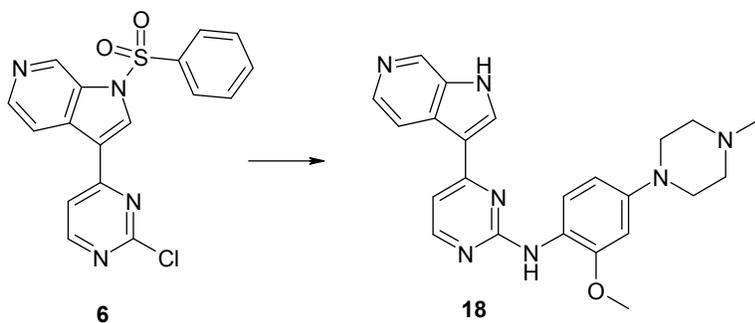
1-(4-(3-Methoxy-4-(6-(1-methyl-1H-pyrrolo[2,3-c]pyridin-3-yl)pyrimidin-4-ylamino)phenyl)piperazin-1-yl)ethanone (16)

Sodium methoxide (54 mg, 1 mmol) was added in one portion to 1-(4-(3-methoxy-4-(6-(1-phenylsulfonyl)-1H-pyrrolo[2,3-c]pyridin-3-yl)pyrimidin-4-ylamino)phenyl)piperazin-1-yl)ethanone **38** (235 mg, 0.4 mmol) in THF (4 ml) at 25°C under nitrogen. The resulting suspension was stirred at 25°C for 10 minutes. Methanol (2.5 ml) was added to improve solubility and the reaction stirred for 18 hours at room temperature. Methyl Iodide (60 μ L, 0.9 mmol) was added in one portion and the solution was stirred at 25°C for a 4 hours. The reaction was concentrated and the residue purified by ion exchange chromatography, using an SCX (10g) column. The desired product was eluted from the column using 7M ammonia in methanol and product fractions were evaporated to dryness to afford crude product. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 μ silica, 21 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% ammonia) and acetonitrile as eluents. Fractions containing the desired compound were evaporated to dryness and triturated with DCM to afford **16** as a yellow solid. (44 mg, 24%). ¹H NMR δ 2.07 (3H, s), 3.08 - 3.21 (4H, m), 3.56 - 3.64 (4H, m), 3.86 (3H, s), 3.99 (3H, s), 6.56 (1H, dd), 6.72 (1H, d), 7.13 (1H, s), 7.73 (1H, d), 8.15 (1H, d), 8.27 (1H, s), 8.30 (1H, d), 8.50 (1H, s), 8.56 (1H, s), 8.92 (1H, s); HRMS Found: ESI⁺ m/z, 458.22919 (M+H)⁺ expected: 458.22262; HPLC tR = 1.63 min.



1-(4-(4-(4-(1*H*-Pyrrolo[2,3-*c*]pyridin-3-yl)pyrimidin-2-ylamino)-3-methoxyphenyl)piperazin-1-yl)ethanone (17)

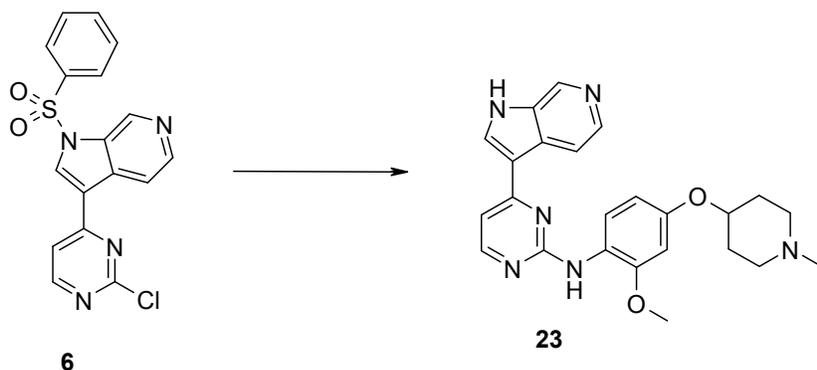
Dioxane (2.5 ml) was added to 3-(2-chloropyrimidin-4-yl)-1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*c*]pyridine **6** (100 mg, 0.3 mmol), 1-(4-(4-amino-3-methoxyphenyl)piperazin-1-yl)ethanone (168 mg, 0.7 mmol), 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthene (19 mg, 0.03 mmol), palladium(II) acetate (4.84 mg, 0.02 mmol) and cesium carbonate (351 mg, 1.1 mmol) at 25°C and sealed into a microwave tube. The reaction was heated to 150°C for 1 hour in the microwave reactor and cooled to room temperature. The reaction mixture was diluted with water and the reaction mixture was acidified with 2M HCl. The crude product was purified by ion exchange chromatography, using a 5g SCX-3 column. The desired product was eluted from the column using 7M ammonia in methanol and product fractions were evaporated to dryness to afford crude product. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5μ silica, 21 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% ammonia) and acetonitrile as eluents. Fractions containing the desired compound were evaporated to dryness to afford **17** as a brown solid (23 mg, 19%). ¹H NMR δ 2.07 (3H, s), 3.09 - 3.21 (4H, m), 3.62 (4H, q), 3.83 (3H, s), 6.59 (1H, dd), 6.73 (1H, d), 7.21 (1H, d), 7.76 (1H, d), 7.96 (1H, s), 8.16 (1H, d), 8.24 (1H, d), 8.28 (1H, d), 8.47 (1H, s), 8.80 (1H, d), 12.17 (1H, s); HRMS Found: ESI⁺ m/z, 444.21346 (M+H)⁺ expected: 444.20697; HPLC tR = 1.65 min.



***N*-(2-Methoxy-4-(4-methylpiperazin-1-yl)phenyl)-4-(1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)pyrimidin-2-amine (18)**

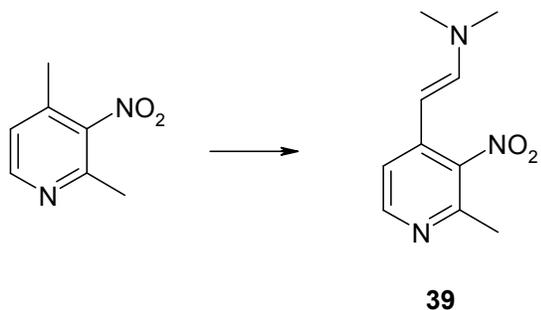
The procedure described above for compound **17** was repeated using compound **6** and 2-methoxy-4-(4-methylpiperazin-1-yl)aniline to afford compound **18** as a black solid in 16% yield. ¹H NMR δ 2.26 (3H, s), 2.48 - 2.52 (4H, m), 3.18 (4H, t), 3.82 (3H, s), 6.56 (1H, dd), 6.69 (1H,

d), 7.20 (1H, dd), 7.73 (1H, t), 7.96 (1H, s), 8.16 (1H, dd), 8.23 (1H, d), 8.28 (1H, dd), 8.47 (1H, s), 8.81 (1H, d), 12.17 (1H, s); HRMS Found: ESI⁺ m/z, 416.21875 (M+H)⁺ expected: 416.21206; HPLC tR= 1.68 min.



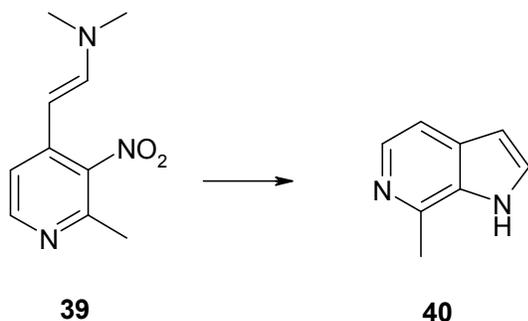
***N*-[2-methoxy-4-[(1-methyl-4-piperidyl)oxy]phenyl]-4-(1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)pyrimidin-2-amine (**23**)**

2-Methoxy-4-[(1-methyl-4-piperidyl)oxy]aniline (75 mg, 0.32 mmol, obtained as described in WO2008003958) was added to a solution of 1-(benzenesulfonyl)-3-(2-chloropyrimidin-4-yl)pyrrolo[2,3-*c*]pyridine **6** (90 mg, 0.24 mol) in 4-methylpentanol (7 mL). *p*-Toluenesulphonic acid (93 mg, 0.49 mmol) was added, then the mixture was stirred at 130 °C for 5 hours. Sodium hydroxide (39 mg, 0.97 mmol) was added then the mixture was stirred at 100 °C for 15 minutes. The reaction mixture was cooled to 25 °C, then diluted with methanol and purified by ion exchange chromatography, using an SCX-2 column. The desired product was eluted from the column using 2M ammonia in methanol and evaporated to dryness. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 μ silica, 21 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% formic acid) and acetonitrile as eluents. Fractions containing the desired compound were loaded onto an SCX-2 column. The product was eluted from the column using 2M ammonia in methanol and evaporated to dryness to afford **23** as a pale yellow solid (26 mg, 25%). ¹H NMR δ 1.68 – 1.72 (2H, m), 1.94 – 1.98 (2H, m), 2.22 – 2.28 (5H, m), 2.65 - 2.69 (2H, m), 3.81 (3H, s), 4.38 – 4.43 (1H, m), 6.60 - 6.63 (1H, m), 6.68 - 6.69 (1H, m), 7.22 (1H, d), 7.73 - 7.76 (1H, m), 8.01 (1H, s), 8.14 (1H, d), 8.22 (1H, d), 8.29 (1H, d), 8.48 (1H, s), 8.81 (1H, s), 12.18 (1H, s). MS: ESI⁺ m/z, 431; HPLC tR = 0.75 min.



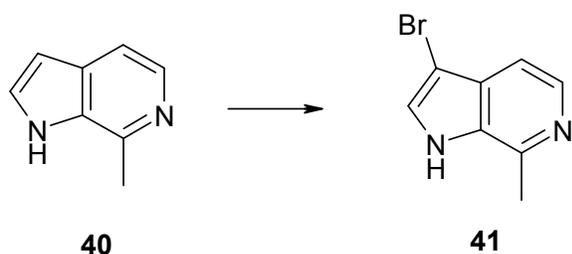
(E)-N,N-Dimethyl-2-(2-methyl-3-nitropyridin-4-yl)ethenamine (39)

1,1-Dimethoxy-*N,N*-dimethylmethanamine (3.6 ml, 27 mmol) was added in one portion to 2,4-dimethyl-3-nitropyridine (2 g, 13 mmol) in DMF (6.5 ml) at 25°C under nitrogen. The resulting solution was stirred at 100°C for 18 hours. The reaction mixture was evaporated to dryness and the crude product was purified by flash silica chromatography, elution gradient 10 to 60% ethyl acetate in isohexane. Pure fractions were evaporated to dryness to afford **39** as an orange solid (1.2 g, 45 %). ¹H NMR (CDCl₃) δ 2.45 (3H, s), 2.92 (6H, s), 4.82 (1H, d), 7.06 (1H, d), 7.11 (1H, d), 8.15 (1H, d); m/z (ES⁺), (M+H)⁺ = 208.07; HPLC tR = 2.53 min.



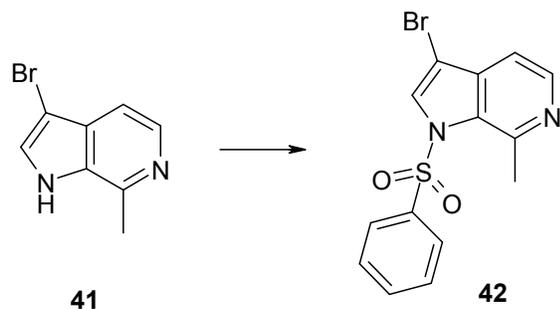
7-Methyl-1H-pyrrolo[2,3-c]pyridine (40)

(*E*)-*N,N*-Dimethyl-2-(2-methyl-3-nitropyridin-4-yl)ethenamine **39** (3 g, 14.5 mmol) and 10% palladium/carbon (1 g, 0.94 mmol) in ethanol (213 ml) was stirred under an atmosphere of hydrogen at 1 atm and 25°C for 3 hours. The reaction mixture was filtered through dicalite and evaporated to dryness. The crude product was purified by flash silica chromatography, elution gradient 5 to 15% methanol in DCM. Pure fractions were evaporated to dryness to afford **40** as a beige solid (0.92 g, 48%). ¹H NMR δ 2.66 (3H, s), 6.48 (1H, d), 7.35 (1H, d), 7.54 (1H, s), 7.94 (1H, d), 11.55 (1H, s); m/z (ES⁺), (M+MeCN)⁺ = 176.10; HPLC tR = 1.70 min.



3-Bromo-7-methyl-1H-pyrrolo[2,3-c]pyridine (**41**)

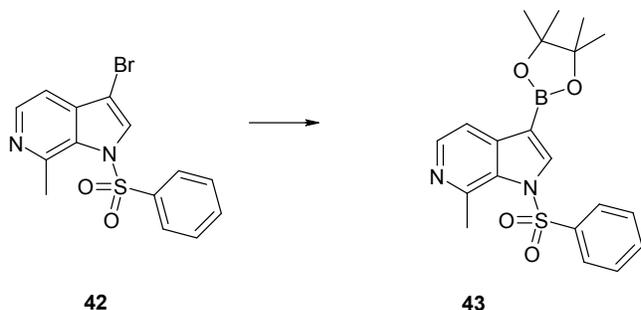
N-Bromosuccinimide (1.2 g, 6.9 mmol) was added in one portion to 7-methyl-1H-pyrrolo[2,3-c]pyridine **40** (912 mg, 6.9 mmol) in acetonitrile (36.5 ml) at 25°C. The resulting suspension was stirred at 25°C for 2.5 hours. The reaction mixture was quenched with water, extracted with ethyl acetate, the organic layers were combined and were washed with saturated brine and dried, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% methanol in DCM. Pure fractions were evaporated to dryness to afford **41** as a cream solid (1.1 g, 78%). ¹H NMR δ 2.67 (3H, s), 7.23 (1H, d), 7.78 (1H, d), 8.07 (1H, d), 11.99 (1H, s); *m/z* (ES⁺), (M+H)⁺ = 212.93; HPLC tR = 2.43 min.



1-(Benzenesulfonyl)-3-bromo-7-methylpyrrolo[2,3-c]pyridine (**42**)

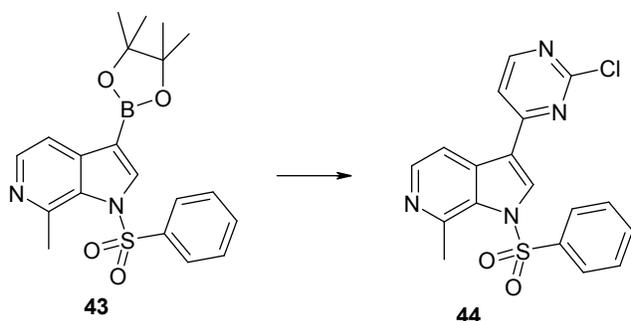
Sodium hydride (0.28 g, 5.9 mmol) was added in one portion to 3-bromo-7-methyl-1H-pyrrolo[2,3-c]pyridine **42** (1.1 g, 5.4 mmol) in THF (53 ml) at 25°C under nitrogen. The resulting mixture was stirred at 25°C for 15 minutes and then benzenesulfonyl chloride (0.72 ml, 5.6 mmol) was added dropwise over 30 minutes at 25°C, the resulting solution was stirred at 25°C for 80 minutes. The reaction was incomplete and further sodium hydride (100 mg) was added in one portion and the mixture was stirred at 25°C for a further 30 minutes. The reaction was incomplete and further sodium hydride (100 mg) was added in one portion and the mixture was stirred at 25°C for a further 45 minutes. The reaction mixture was quenched with saturated aq. NH₄Cl (5 ml), and partitioned between ethyl acetate and water. The organic layer was separated and aqueous layer extracted with ethyl acetate, the combined organics were washed with

saturated brine, dried, filtered and evaporated to give crude product. The crude product was purified by flash silica chromatograph, elution gradient 0 to 20% ethyl acetate in DCM. Pure fractions were evaporated to dryness to afford **42** as a beige solid (0.89 g, 47%). ¹H NMR δ 2.72 (3H, s), 7.40 (1H, d), 7.66 (2H, t), 7.78 (1H, t), 7.90 (2H, d), 8.36 (1H, d), 8.39 (1H, s); m/z (ES+), (M+H)+ = 352.87; HPLC tR = 3.10 min.



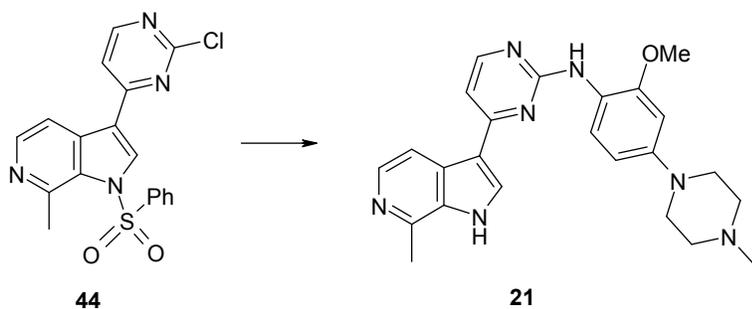
1-(Benzenesulfonyl)-7-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrrolo[2,3-c]pyridine (43)

PdCl₂(dppf) (186 mg, 0.25 mmol) was added in one portion to 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (773 mg, 3 mmol), 3-bromo-7-methyl-1-(phenylsulfonyl)-1H-pyrrolo[2,3-c]pyridine **42** (891 mg, 2.5 mmol) and potassium acetate (847 mg, 8.6 mmol) in dimethoxyethane (16 ml) at 25°C under nitrogen. The resulting mixture was stirred at 80°C for 18 hours. The reaction mixture was partitioned between ethyl acetate and water, shaken and filtered through dicalite. The ethyl acetate layer was separated and the aqueous layer was extracted with ethyl acetate, the combined organic layers were washed with saturated brine and dried and filtered. The solution was evaporated to dryness to give a dark oil which was triturated with isohexane and diethyl ether. The filtrate was evaporated to dryness to give **43** (1 g) as a dark oil which was used without further purification. ¹H NMR δ 1.41 (12H, s), 2.67 (3H, s), 7.69 - 7.76 (3H, m), 7.84 (1H, t), 7.93 - 7.97 (2H, m), 8.29 (1H, s), 8.33 (1H, d); m/z (ES+), (M+H)+ = 399.03; HPLC tR = 2.16 min.



3-(2-Chloropyrimidin-4-yl)-7-methyl-1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*c*]pyridine (**44**)

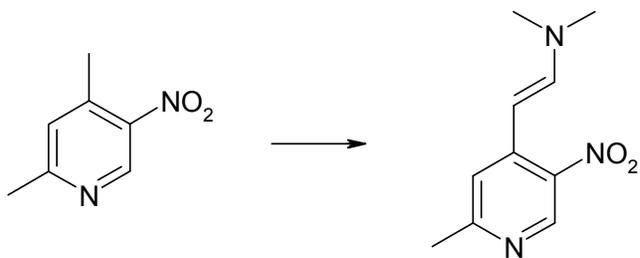
2M Aq. sodium carbonate (5.1 ml, 10.1 mmol) was added in one portion to 2,4-dichloropyrimidine (0.76 g, 5.1 mmol), 7-methyl-1-(phenylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrrolo[2,3-*c*]pyridine **43** (1.01 g, 2.54 mmol) and Tetrakis(triphenylphosphine)palladium(0) (0.12 g, 0.1 mmol) in dimethoxyethane (20 ml) at 25°C under nitrogen. The resulting suspension was stirred at 60°C for 2 hours. The reaction mixture was partitioned between ethyl acetate and water. Combined organic extracts were washed with saturated brine, dried, filtered and evaporated to give crude product. The crude product was purified by flash silica chromatography, elution gradient 20 to 70% ethyl acetate in isohexane. Pure fractions were evaporated to dryness and the residue triturated with diethyl ether to give a solid which was collected by filtration and dried under vacuum to give **44** as a white solid (0.61 g, 63%). ¹H NMR δ 2.70 (3H, s), 7.69 (2H, t), 7.81 (1H, t), 7.98 (2H, d), 8.36 (2H, d), 8.41 (1H, d), 8.83 (1H, d), 9.21 (1H, s); m/z (ES+), (M+H)⁺ = 384.99; HPLC tR = 3.14 min.



N-(2-Methoxy-4-(4-methylpiperazin-1-yl)phenyl)-4-(7-methyl-1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)pyrimidin-2-amine (**21**)

3-(2-Chloropyrimidin-4-yl)-7-methyl-1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*c*]pyridine **44** (150 mg, 0.4 mmol), 2-methoxy-4-(4-methylpiperazin-1-yl)aniline (216 mg, 0.97 mmol) and *p*-toluenesulfonic acid (285 mg, 1.5 mmol) were suspended in NMP (4 ml) and sealed into a microwave tube. The reaction was heated to 150°C for 90 minutes in the microwave reactor and cooled to room temperature. The reaction was diluted with methanol and purified by ion exchange chromatography, using an SCX-2 column (20 g). The desired product was eluted from the column using 7M ammonia in methanol and evaporated to dryness. The crude product was purified by flash silica chromatography, elution gradient 0 to 6% 7N ammonia in methanol in DCM. Pure fractions were evaporated to dryness to **21** as a solid (131 mg, 78%). ¹H NMR δ 2.25 (3H, s), 2.51 (4H, s), 2.69 (3H, s), 3.16 (4H, s), 3.82 (3H, s), 6.54 (1H, d), 6.68 (1H, s), 7.21 (1H,

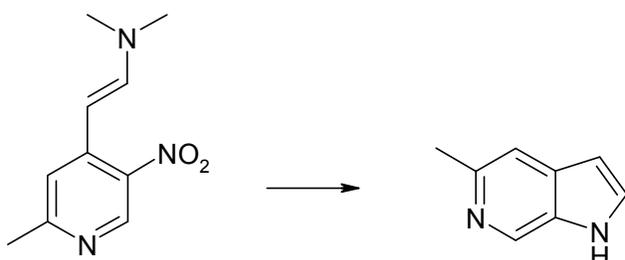
d), 7.73 (1H, d), 7.92 (1H, s), 8.03 (1H, s), 8.08 (1H, s), 8.27 (1H, d), 8.43 (1H, s), 12.16 (1H, s); HRMS Found: ESI⁺ m/z, 430.23434 (M+H)⁺ expected: 430.22771; HPLC tR = 2.86 min.



45

(E)-N,N-Dimethyl-2-(2-methyl-5-nitropyridin-4-yl)ethenamine (45)

1,1-dimethoxy-*N,N*-dimethylmethanamine (9 ml, 67 mmol) was added in one portion to 2,4-dimethyl-5-nitropyridine (5 g, 33 mmol) in DMF (16 ml) at 25°C under nitrogen. The resulting solution was stirred at 100°C for 2 hours. The reaction mixture was evaporated to dryness and the crude product was purified by flash silica chromatography, elution gradient 20 to 80% ethyl acetate in isohexane. Pure fractions were evaporated to dryness to afford **45** as a red solid (4.9 g, 72 %). ¹H NMR (CDCl₃) δ 2.48 (3H, s), 3.01 (6H, s), 5.97 (1H, d), 7.10 (1H, s), 7.27 (1H, d), 8.93 (1H, s); m/z (ES⁺), (M+H)⁺ = 208.22; HPLC tR = 2.35 min.

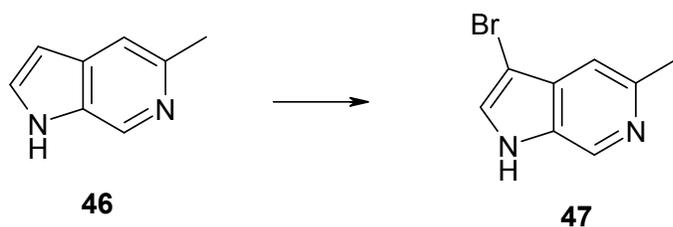


45

46

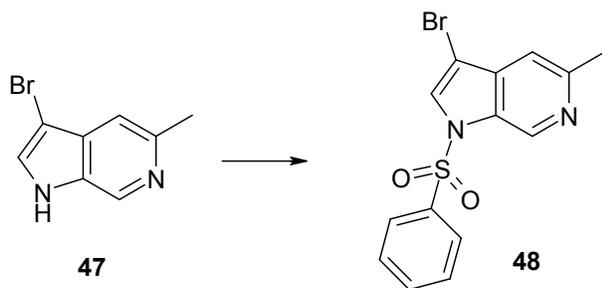
5-Methyl-1H-pyrrolo[2,3-c]pyridine (46)

(*E*)-*N,N*-Dimethyl-2-(2-methyl-5-nitropyridin-4-yl)ethenamine **45** (2.24 g, 10.81 mmol) and 10% palladium/carbon (0.75 g, 0.7 mmol) in ethanol (159 ml) was stirred under an atmosphere of hydrogen at 25°C for 2.5 hours. The reaction mixture was filtered through dicalite and evaporated to dryness. The crude product was purified by flash silica chromatography, elution gradient 0 to 15% methanol in DCM. Pure fractions were evaporated to dryness to afford **46** as a beige solid (0.82 g, 58%). ¹H NMR δ 2.49 (3H, s), 6.37 - 6.40 (1H, m), 7.34 (1H, s), 7.52 (1H, t), 8.61 (1H, s), 11.38 (1H, s); m/z (ES⁺), (M+MeCN)⁺ = 173.88; HPLC tR = 1.99 min.



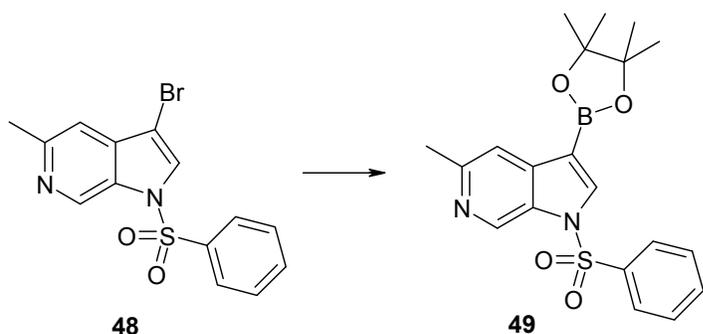
3-Bromo-5-methyl-1*H*-pyrrolo[2,3-*c*]pyridine (**47**)

N-Bromosuccinimide (1.1 g, 6.2 mmol) was added in one portion to 5-methyl-1*H*-pyrrolo[2,3-*c*]pyridine **46** (820 mg, 6.2 mmol) in acetonitrile (33 ml) at 25°C. The resulting solution was stirred at 25°C for 3.5 hours. The reaction mixture was quenched with water, extracted with ethyl acetate (3 x 100 ml), the organic layer was dried, filtered and evaporated to afford crude solid. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% methanol in DCM. Pure fractions were evaporated to dryness to afford **47** as a cream solid (1.2 g, 92%). ¹H NMR δ 2.54 (3H, s), 7.25 (1H, s), 7.77 (1H, s), 8.66 (1H, d), 11.89 (1H, s); *m/z* (ES⁺), (M+H)⁺ = 212.93; HPLC tR = 2.60 min.



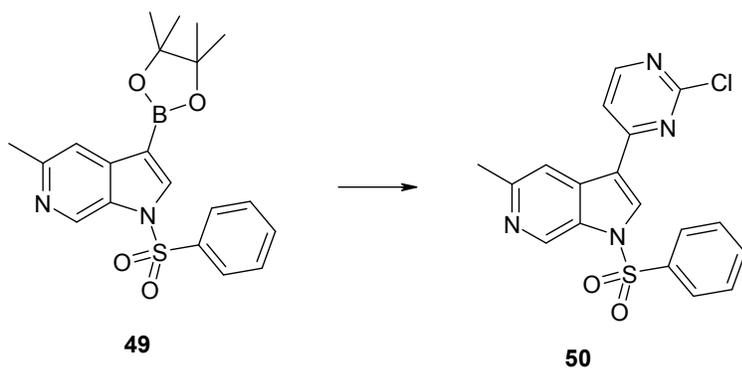
1-(Benzenesulfonyl)-3-bromo-5-methyl-pyrrolo[2,3-*c*]pyridine (**48**)

Sodium hydride (0.3 g, 6.3 mmol) was added in one portion to 3-bromo-5-methyl-1*H*-pyrrolo[2,3-*c*]pyridine **47** (1.2 g, 5.7 mmol) in THF (56 ml) at 25°C under nitrogen. The resulting mixture was stirred at 25°C for 15 minutes and then benzenesulfonyl chloride (0.76 ml, 6 mmol) was added dropwise over 10 minutes, the resulting solution was stirred at 25°C for 2.5 hours. The reaction mixture was quenched with saturated aq. NH₄Cl (5 ml), diluted with water (20 ml), and extracted with ethyl acetate. The organic layer was separated, dried, filtered and evaporated to give crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 20% ethyl acetate in DCM. Pure fractions were evaporated to dryness to afford **48** as a beige solid (1.8 g, 91%). ¹H NMR δ 2.56 (3H, s), 7.37 (1H, s), 7.64 (2H, t), 7.75 (1H, t), 8.11 (2H, d), 8.36 (1H, s), 9.13 (1H, d); *m/z* (ES⁺), (M+H)⁺ = 352.87; HPLC tR = 3.14 min.



5-Methyl-1-(phenylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-c]pyridine (49)

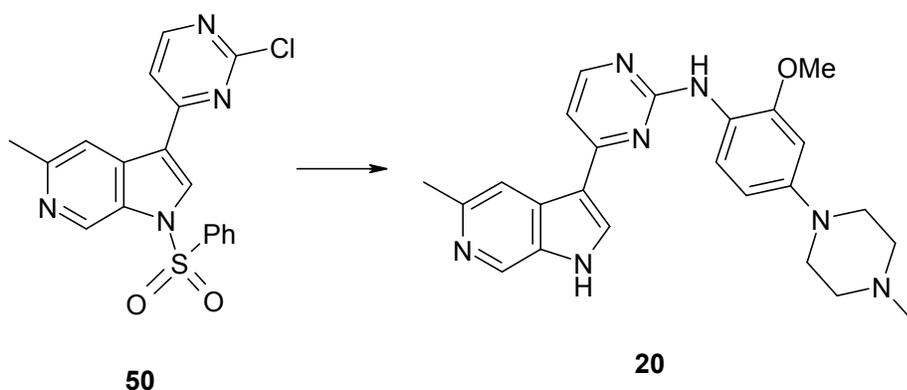
PdCl₂(dppf) (0.38 g, 0.5 mmol) was added in one portion to 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (1.6 g, 6.2 mmol), 3-bromo-5-methyl-1-(phenylsulfonyl)-1H-pyrrolo[2,3-c]pyridine **48** (1.8 g, 5.2 mmol) and potassium acetate (1.7 g, 17.6 mmol) in DME (32 ml) at 25°C under nitrogen. The resulting mixture was stirred at 80°C for 18 hours. The reaction mixture was partitioned between ethyl acetate and water, shaken and filtered through dicalite. The ethyl acetate layer was separated, washed with saturated brine and dried, filtered and evaporated to dryness to give an oil which was triturated with diethyl ether and isohexane, to give a solid which was collected by filtration and dried under vacuum to give **49** as a beige solid (1.6 g, 79%). ¹H NMR δ 1.32 (12H, s), 2.54 (3H, s), 7.56 (1H, s), 7.64 (2H, t), 7.75 (1H, t), 8.13 (1H, s), 8.16 (1H, d), 8.18 (1H, s), 9.09 (1H, d); m/z (ES⁺), (M+H)⁺ = 399.06; HPLC tR = 2.17 min.



3-(2-Chloropyrimidin-4-yl)-5-methyl-1-(phenylsulfonyl)-1H-pyrrolo[2,3-c]pyridine (50)

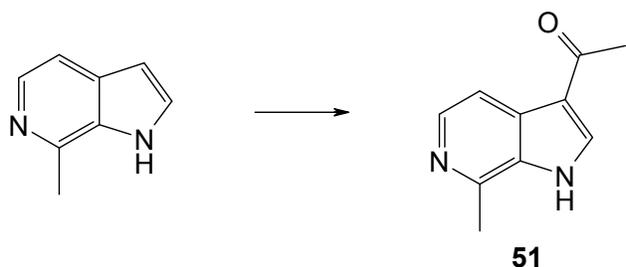
Tetrakis(triphenylphosphine)palladium(0) (0.058 g, 0.05 mmol) was added in one portion to a degassed mixture of 2,4-dichloropyrimidine (0.37 g, 2.5 mmol), 5-methyl-1-(phenylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-c]pyridine **49** (0.5 g, 1.3 mmol)

and 2M aq. sodium carbonate (2.5 ml, 5 mmol) in dimethoxyethane (10 ml) at 25°C under nitrogen. The resulting suspension was stirred at 60°C for 2 hours. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was collected, dried, filtered and evaporated to dryness to give crude product. The crude product was purified by flash silica chromatography, elution gradient 10 to 60% ethyl acetate in isohexane. Pure fractions were evaporated to dryness to afford **50** (0.35 g, 73%) as a white solid. ¹H NMR δ 2.60 (3H, s), 7.66 (2H, t), 7.77 (1H, t), 8.16 - 8.22 (3H, m), 8.26 (1H, d), 8.80 (1H, d), 9.14 (1H, s), 9.17 (1H, d); m/z (ES+), (M+H)⁺ = 384.99; HPLC tR = 3.18 min.



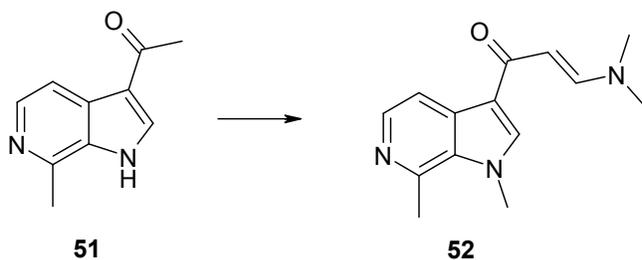
***N*-(2-Methoxy-4-(4-methylpiperazin-1-yl)phenyl)-4-(5-methyl-1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)pyrimidin-2-amine (**20**)**

3-(2-Chloropyrimidin-4-yl)-5-methyl-1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*c*]pyridine **50** (150 mg, 0.4 mmol), 2-methoxy-4-(4-methylpiperazin-1-yl)aniline (216 mg, 0.97 mmol) and p-toluenesulfonic acid (285 mg, 1.5 mmol) were suspended in NMP (4 ml) and sealed into a microwave tube. The reaction was heated to 150°C for 90 minutes in the microwave reactor and cooled to room temperature. The reaction was diluted with methanol and purified by ion exchange chromatography, using an SCX-2 column. The desired product was eluted from the column using 7M ammonia in methanol and evaporated to dryness. The crude product was purified by flash silica chromatography, elution gradient 0 to 5% 7N ammonia in methanol in DCM. Pure fractions were evaporated to dryness to afford **20** as a beige solid (135 mg, 81%). ¹H NMR δ 2.25 (3H, s), 2.49 (3H, s), 2.51 (4H, s), 3.18 (4H, s), 3.80 (3H, s), 6.57 (1H, d), 6.69 (1H, s), 7.17 (1H, d), 7.66 (1H, d), 7.92 (1H, s), 8.03 (1H, s), 8.25 (1H, d), 8.40 (1H, s), 8.65 (1H, s), 12.00 (1H, s); HRMS Found: ESI⁺ m/z, 430.23416 (M+H)⁺ expected: 430.22771; HPLC tR = 2.91 min.



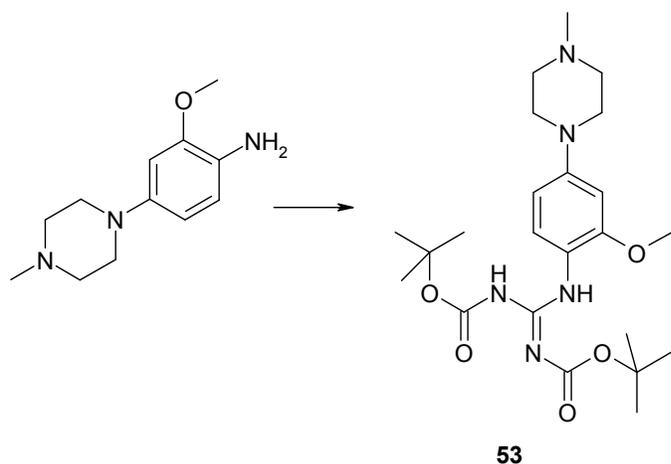
1-(7-Methyl-1H-pyrrolo[2,3-c]pyridin-3-yl)ethanone (51)

Aluminum trichloride (4.6 g, 34.6 mmol) was ground in a pestle and mortar and was added in one portion to 7-methyl-1H-pyrrolo[2,3-c]pyridine (916 mg, 6.9 mmol) in DCM (124 ml). The resulting mixture was stirred for 1 hour at 25°C and then acetyl chloride (2.5 ml, 34.6 mmol) was added dropwise over 15 minutes. The resulting mixture was stirred for 18 hours, then cooled to 0°C and quenched with dropwise addition of methanol (25 mL). The mixture was evaporated to dryness and then dissolved in water and added to an SCX-2 cartridge (100 g), washed with methanol and eluted with 2N ammonia in methanol. The solution was evaporated to dryness to give **51** which was used without further purification (1.1 g, >100%); m/z (ES+), (M+H)⁺ = 205.99; HPLC tR = 0.77 min.



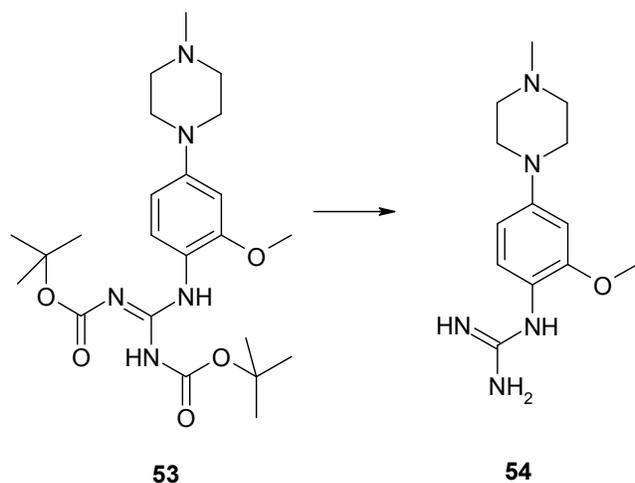
(E)-1-(1,7-Dimethyl-1H-pyrrolo[2,3-c]pyridin-3-yl)-3-(dimethylamino)prop-2-en-1-one (51)

1,1-Dimethoxy-N,N-dimethylmethanamine (14.3 ml, 106.7 mmol) was added in one portion to crude **51** (1.24 g, 7.1 mmol) in DMF (21 ml) at 25°C. The resulting mixture was stirred at 100°C for 21 hours. The reaction mixture was allowed to cool and then evaporated to dryness. The crude product was purified by flash silica chromatography, elution gradient 0 to 5% methanol in DCM. Pure fractions were evaporated to dryness to afford **52** as an orange solid (0.63 g, 36%). ¹H NMR δ 2.89 (3H, s), 2.99 (6H, s), 4.12 (3H, s), 5.69 (1H, d), 7.56 (1H, d), 8.02 (2H, s), 8.24 (1H, s); m/z (ES+), (M+H)⁺ = 244.19; HPLC tR = 1.65 min.



(E)-Tert-butyl (tert-butoxycarbonylamino)(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)methylenecarbamate (53)

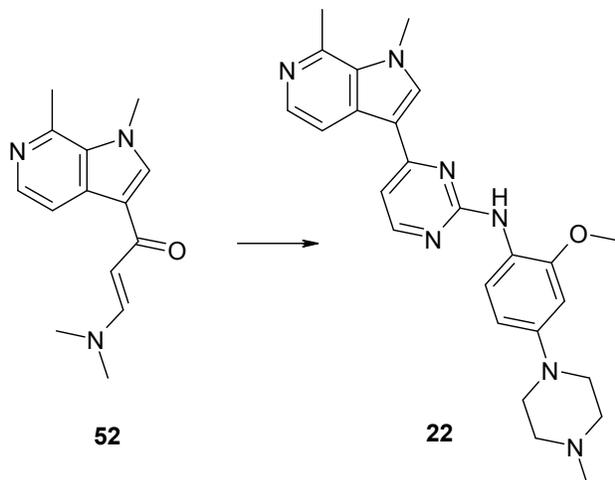
2-Methoxy-4-(4-methylpiperazin-1-yl)aniline (504 mg, 2.3 mmol) was added in one portion to (*Z*)-tert-butyl (1*H*-pyrazol-1-yl)methylenedicarbamate (589 mg, 1.9 mmol) in acetonitrile (9.8 ml) at 25°C. The resulting solution was stirred at 25°C for 24 hours. The reaction mixture was evaporated to dryness and redissolved in ethyl acetate and water, the organic layer collected and washed sequentially with water and saturated brine. The organic layer was dried, filtered and evaporated to afford crude product **53** (880 mg, 100%) which was used without further purification. *m/z* (ES+) (M+H)⁺ = 464.42; HPLC tR = 3.78 min.



1-(2-Methoxy-4-(4-methylpiperazin-1-yl)phenyl)guanidine (54)

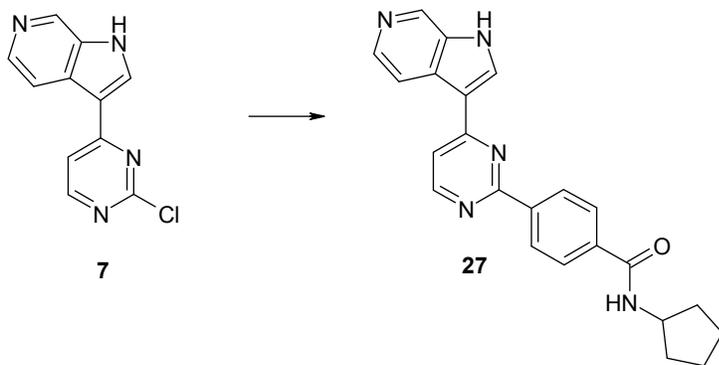
(*Z*)-Tert-butyl (tert-butoxycarbonylamino)(2-methoxy-4-(4-methylpiperazin-1-yl)phenylamino)methylenecarbamate (880 mg, 1.9 mmol) was added portionwise to 5M HCl in isopropanol (8.7 ml) at 25°C. The resulting mixture was stirred at 80°C for 5 hours. The reaction

mixture was diluted with methanol (40 ml), then diethyl ether (80 ml). The resulting precipitate was collected by filtration, washed with diethyl ether and dried under vacuum to afford the title compound as a purple di-HCl solid **54** (581 mg, 91%) which was used without further purification. $^1\text{H NMR}$ δ 2.79 (3H, d), 3.07 - 3.28 (4H, m), 3.48 (2H, d), 3.82 (3H, s), 3.85 - 3.94 (2H, m), 6.59 (2H, dd), 6.73 (1H, d), 7.06 (1H, d), 7.27 (3H, s), 9.22 (1H, s), 11.53 (1H, s); m/z (ES+) (M+H) $^+$ = 264.42; HPLC tR = 1.61 min.



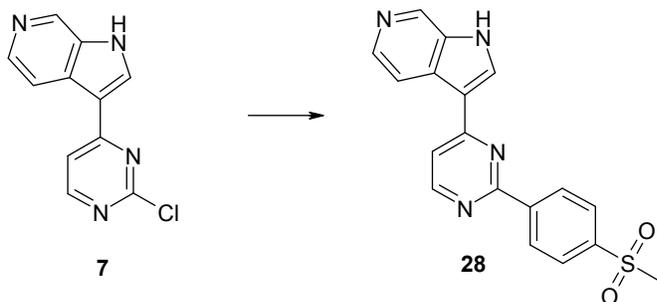
4-(1,7-Dimethyl-1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)-*N*-(2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)pyrimidin-2-amine (22)

2-*Tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diaza-phosphorine (2.4 ml, 8.3 mmol) was added in one portion to **52** (288 mg, 1.2 mmol) and **54** (1.2 g, 3.6 mmol) in DMA (9.4 ml) at 25°C under nitrogen. The resulting mixture was stirred at 120°C for 24 hours. The reaction mixture was evaporated and partitioned between ethyl acetate and water, the organic layer was collected and washed sequentially with water and saturated brine. The organic layer was dried, filtered and evaporated to afford crude product. The crude solid was triturated with methanol to give a solid which was collected by filtration and dried under vacuum to give **22** as a pale yellow solid (150 mg, 29%). $^1\text{H NMR}$ δ 2.25 (3H, s), 2.47 - 2.54 (4H, m), 2.92 (3H, s), 3.16 (4H, t), 3.81 (3H, s), 4.16 (3H, s), 6.54 (1H, dd), 6.67 (1H, d), 7.08 (1H, d), 7.71 (1H, d), 7.93 (1H, s), 7.99 (1H, d), 8.13 (1H, d), 8.26 (1H, d), 8.35 (1H, s); HRMS Found: ESI $^+$ m/z , 444.24982 (M+H) $^+$ expected: 444.24336; HPLC tR = 1.81 min.



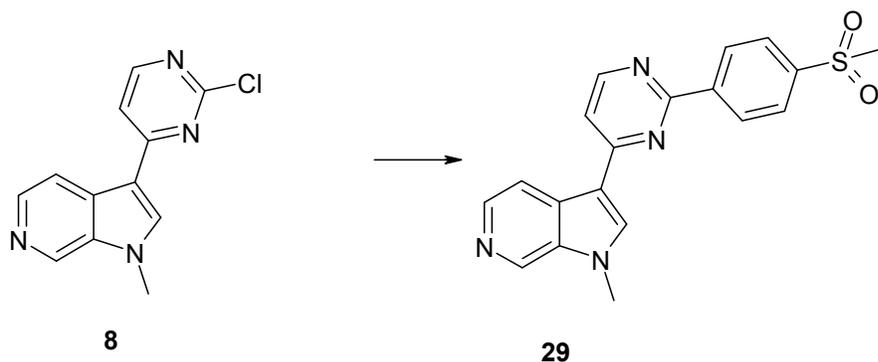
4-(4-(1*H*-Pyrrolo[2,3-*c*]pyridin-3-yl)pyrimidin-2-yl)-*N*-cyclopentylbenzamide (**27**)

3-(2-Chloropyrimidin-4-yl)-1*H*-pyrrolo[2,3-*c*]pyridine **7** (50 mg, 0.22 mmol), *N*-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide (68 mg, 0.22 mmol) and 2*N* sodium carbonate (0.33 ml, 0.65 mmol) were suspended in dioxane (0.9 ml), water (0.3 ml), DMF (0.6 ml), ethanol (0.6 ml) and purged with nitrogen. Bis(triphenylphosphine)palladium(II) chloride (27 mg, 0.04 mmol) was added and the mixture sealed into a microwave tube. The reaction was heated to 130°C for 40 minutes in the microwave reactor and cooled to room temperature. The mixture was concentrated and the crude product was purified by ion exchange chromatography, using an SCX column. The desired product was eluted from the column using 7*M* ammonia in methanol and pure fractions were evaporated to dryness to afford crude product which was purified by preparative HPLC (Sunfire C18 column, 5 μ silica, 19 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% ammonia) and acetonitrile as eluents. Fractions containing the desired compound were evaporated to dryness to afford **27** as a white solid (18 mg, 22%). ¹H NMR 1.51 – 1.66 (4*H*, m), 1.68 – 1.80 (2*H*, m), 1.88 – 2.00 (2*H*, m), 4.23 – 4.33 (1*H*, m), 8.05 (2*H*, d), 8.40 – 8.51 (4*H*, m), 8.73 (1*H*, s), 8.95 (1*H*, d), 9.80 (1*H*, s), 9.85 (1*H*, s), 9.92 (1*H*, s), 13.50 (1*H*, br s); HRMS Found: ESI⁺ *m/z*, 384.18140 (*M*+*H*)⁺ expected: 384.17461; HPLC tR= 1.25 min.



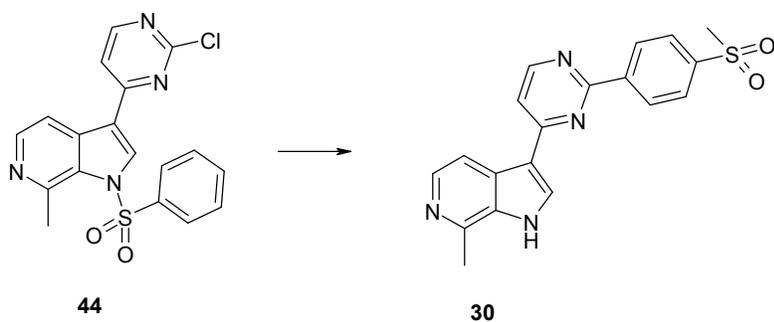
3-(2-(4-(Methylsulfonyl)phenyl)pyrimidin-4-yl)-1*H*-pyrrolo[2,3-*c*]pyridine (**28**)

The procedure described above for compound **27** was repeated using compound **7** and 4-(methylsulfonyl)phenylboronic acid to afford compound **28** as a yellow solid in 24% yield. ^1H NMR δ 3.31 (3H, s), 7.97 (1H, d), 8.16 (2H, d), 8.37 (1H, d), 8.51 (1H, dd), 8.72 - 8.78 (3H, m), 8.86 (1H, d), 8.89 (1H, d), 12.44 (1H, s); HRMS Found: ESI^+ m/z , 351.09076 ($\text{M}+\text{H}$) $^+$ expected: 351.08375; HPLC t_R = 2.49 min.



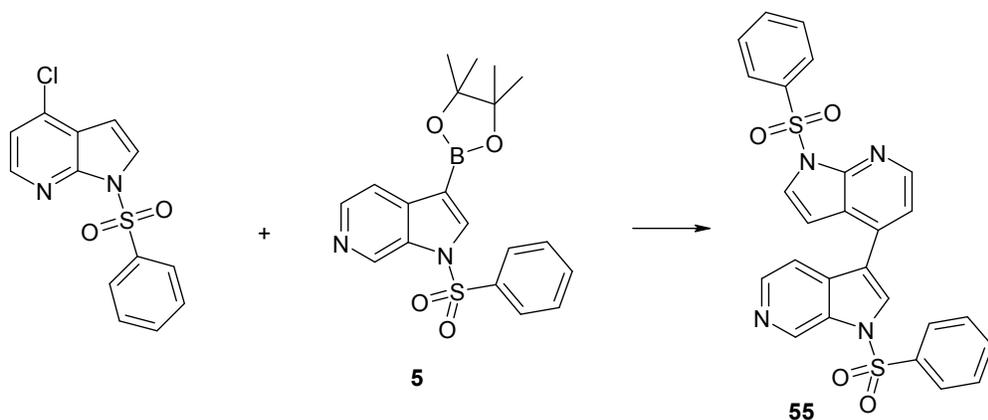
1-Methyl-3-(2-(4-(methylsulfonyl)phenyl)pyrimidin-4-yl)-1H-pyrrolo[2,3-c]pyridine (29)

2M Aq. sodium carbonate (0.5 ml, 1 mmol) was added to 4-(methylsulfonyl)phenylboronic acid (75 mg, 0.37 mmol), compound **8** (70 mg, 0.25 mmol) and bis(triphenylphosphine)palladium(II) chloride (9 mg, 0.01 mmol) in DME (2 ml) and sealed into a microwave tube. The reaction was heated to 130°C for 40 minutes in the microwave reactor and cooled to room temperature. 2M aq. sodium carbonate (0.5 ml) was added to the reaction and sealed into a microwave tube. The reaction was heated to 140°C for 40 minutes in the microwave reactor and cooled to room temperature. The reaction mixture was diluted with ethyl acetate and washed with water, the organic layer was collected and purified by ion exchange chromatography, using a 5g SCX-3 column. The desired product was eluted from the column using 7M ammonia in methanol. The precipitate formed after leaving for 1 hour was collected by filtration, washed with methanol then diethyl ether and dried to afford **29** as a yellow solid (47 mg, 52%) ^1H NMR δ 3.30 (3H, s), 4.05 (3H, s), 7.88 (1H, d), 8.15 (2H, d), 8.41 (1H, d), 8.48 (1H, dd), 8.71 - 8.77 (3H, m), 8.86 (1H, d), 9.00 (1H, d); HRMS Found: ESI^+ m/z , 365.10660 ($\text{M}+\text{H}$) $^+$ expected: 365.09940; HPLC t_R = 1.83 min.



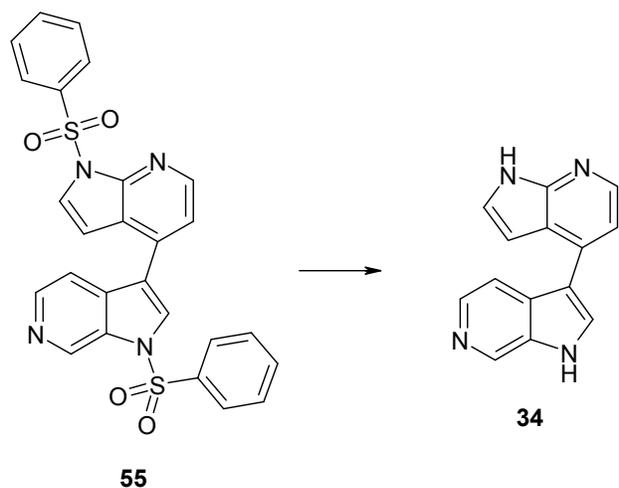
7-Methyl-3-(2-(4-(methylsulfonyl)phenyl)pyrimidin-4-yl)-1H-pyrrolo[2,3-c]pyridine (**30**)

Sodium carbonate (390 μ l, 0.78 mmol) was added in one portion to compound **44** (100 mg, 0.26 mmol), 4-(methylsulfonyl)phenylboronic acid (78 mg, 0.39 mmol), bis(triphenylphosphine)palladium(II) chloride (9.1 mg, 0.01 mmol) in DME (2.2 ml) and sealed into a microwave tube. The reaction was heated to 130°C for 40 minutes in the microwave reactor and cooled to room temperature. 2M aqueous sodium carbonate (0.5 ml) was added to the reaction and sealed into a microwave tube. The reaction was heated to 140°C for 40 minutes in the microwave reactor and cooled to room temperature. The reaction mixture was diluted with ethyl acetate and washed with water, the organic layer collected and purified by ion exchange chromatography, using a 5g SCX-3 column. The desired product was eluted from the column using 7M ammonia in methanol and then purified by HPLC (Waters XBridge Prep C18 OBD column, 5 μ silica, 21 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH₃) and acetonitrile as eluents to afford **44** as a white solid (30 mg, 31 %) ¹H NMR δ 2.74 (3H, s), 3.27 (3H, s), 7.98 (1H, d), 8.16 (2H, d), 8.23 (1H, d), 8.35 (1H, d), 8.72 (1H, s), 8.75 (2H, d), 8.85 (1H, d), 12.41 (1H, s); HRMS Found: ESI⁺ m/z, 365.10611 (M+H)⁺ expected: 365.09940; HPLC tR = 0.96 min.



1-(Phenylsulfonyl)-3-(1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-1H-pyrrolo[2,3-c]pyridine (55)

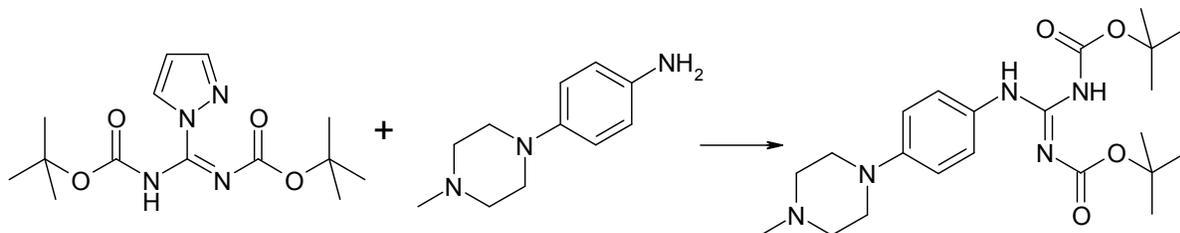
4-Chloro-1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridine (434 mg, 1.5 mmol), compound **5** (570 mg, 1.5 mmol) and *tetrakis*(triphenylphosphine)palladium(0) (69 mg, 0.06 mmol) were suspended in dioxane (12 mL) and sealed into a microwave tube with 2N aqueous sodium carbonate (3 mL, 5.9 mmol). The reaction was heated to 140°C for 30 minutes in the microwave reactor and cooled to room temperature. The reaction mixture was evaporated to dryness, diluted with ethyl acetate and water, shaken and filtered. The organic layer was collected and the aqueous layer extracted with ethyl acetate. The combined organic layers were dried, filtered and purified by flash silica chromatography elution gradient 50 to 100% isohexane in ethyl acetate. Pure fractions were evaporated to dryness to afford **55** as a white solid (485 mg, 64 %) ¹H NMR δ 9.34 (d, 1H), 8.53 (s, 1H), 8.48 (dd, 2H), 8.27 - 8.21 (m, 2H), 8.20 - 8.14 (m, 2H), 8.03 (d, 1H), 7.79 - 7.70 (m, 3H), 7.70 - 7.61 (m, 4H), 7.57 (d, 1H), 6.90 (d, 1H); m/z (ES+) (M+H)⁺ = 515.08; HPLC tR= 2.68 min.



3-(1H-Pyrrolo[2,3-b]pyridin-4-yl)-1H-pyrrolo[2,3-c]pyridine (34)

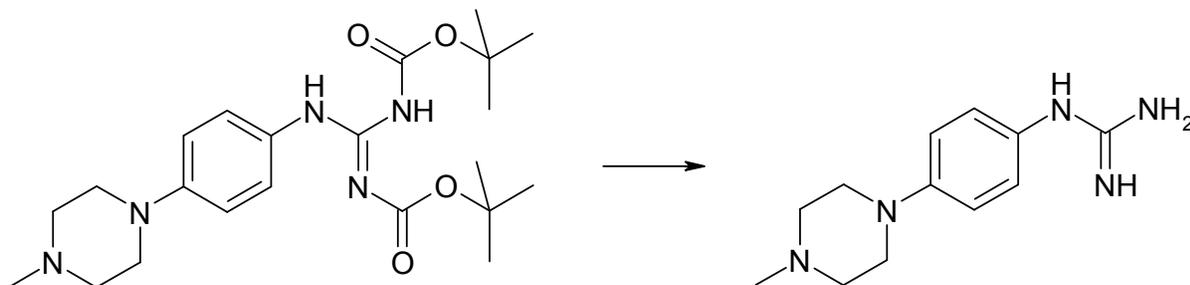
Compound **55** (139 mg, 0.27 mmol) and 2N aqueous sodium carbonate (4.0 mL, 7.1 mmol) were suspended in dioxane (3 mL) and sealed into a microwave tube. The reaction was heated to 140°C for 160 minutes in the microwave reactor and cooled to room temperature. The reaction mixture was evaporated and the crude mixture was taken up in dichloromethane and methanol, filtered and evaporated. The crude product was purified by flash silica chromatography, elution gradient 5 to 10% methanolic ammonia in dichloromethane to afford **34** as a yellow solid (47 mg, 74 %) ¹H NMR δ 12.13 (s, 1H), 11.72 (s, 1H), 8.88 (s, 1H), 8.27 (d, 1H), 8.23 (d, 1H), 8.19

(d, 1H), 7.83 (d, 1H), 7.51 (dd, 1H), 7.36 (d, 1H), 6.69 (dd, 1H); HRMS Found: ESI⁺ m/z, 235.09766 (M+H)⁺ expected: 235.09055; HPLC tR= 0.48 min.



(Z)-tert-Butyl (tert-butoxycarbonylamino)(4-(4-methylpiperazin-1-yl)phenylamino)methylenecarbamate

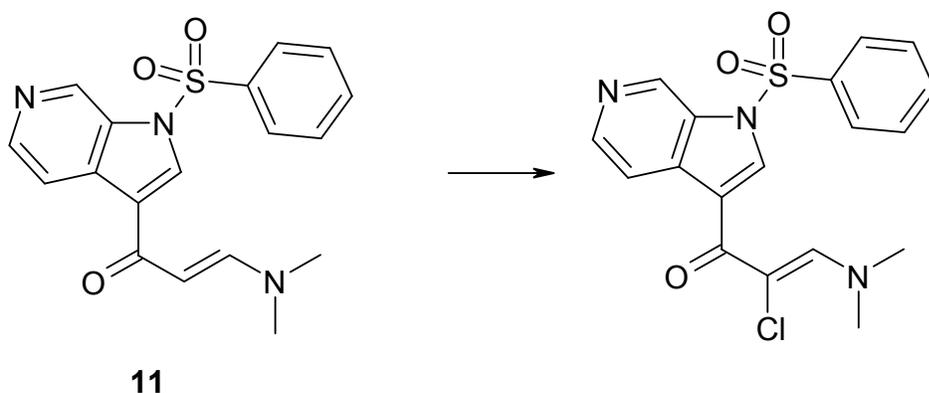
4-(4-Methylpiperazino)aniline (2.22 g, 11.6 mmol) was added in one portion to (Z)-tert-butyl (1H-pyrazol-1-yl)methylenedicarbamate (3 g, 9.7 mmol) in acetonitrile (50 mL) at 25°C. The resulting solution was stirred at 25°C for 1.5 hours. The reaction mixture was evaporated to dryness and redissolved in ethyl acetate/H₂O, the organic layer collected and washed sequentially with water and saturated brine. The organic layer was dried, filtered and evaporated to afford the desired product (3.94 g, 94%) which was used without further purification.



1-(4-(4-methylpiperazin-1-yl)phenyl)guanidine

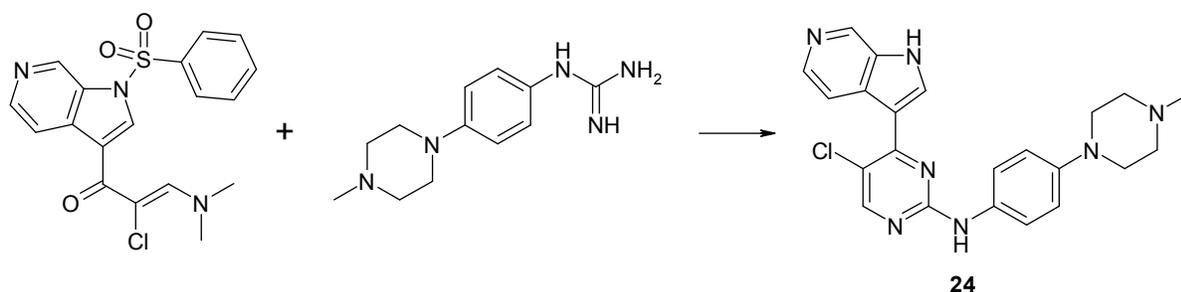
(Z)-tert-Butyl (tert-butoxycarbonylamino)(4-(4-methylpiperazin-1-yl)phenylamino)methylenecarbamate (3.74 g, 8.63 mmol) was added portionwise to 5M HCl in isopropanol (40 ml) at 25°C. The resulting mixture was stirred at 80°C for 20 minutes. The reaction mixture was diluted with methanol (40 mL), then ether (80 mL). The precipitate was collected by filtration, washed with ether and dried under vacuum to afford product which was a mixture of product and mono-BOC protected species. Therefore the solid was added to 5M HCl in isopropanol (200 mL) and the resulting mixture was stirred at 80°C for 2 hours. The reaction

mixture was allowed to cool and then diluted with ether (300 mL). The precipitate was collected by filtration, washed with ether and dried under vacuum at 60°C to afford the title compound as a purple solid (2.72 g, >100%), which was used without further purification. ¹H NMR δ 2.77 (3H, s), 3.06 – 3.2 (4H, m), 3.41 – 3.5 (2H, m), 3.76 – 3.84 (2H, m), 7.05 (2H, d), 7.11 (2H, d), 7.35 (3H, br s), 9.78 (1H, s), 11.30 (1H, br s).



(Z)-2-Chloro-3-(dimethylamino)-1-(1-(phenylsulfonyl)-1H-pyrrolo[2,3-c]pyridin-3-yl)prop-2-en-1-one

Benzyltrimethylammonium dichloroiodate (255 mg, 0.73 mmol) was added in one portion to (*E*)-3-(dimethylamino)-1-(1-(phenylsulfonyl)-1H-pyrrolo[2,3-c]pyridin-3-yl)prop-2-en-1-one **11** (200 mg, 0.56 mmol) in DCM (2 mL) and methanol (1 mL). The resulting mixture was stirred for 90 minutes. The reaction mixture was diluted with DCM (20 mL), and washed sequentially with saturated aq. NaHCO₃ (20 mL) and saturated aqueous sodium thiosulfate (20 mL). The organic layer was evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% methanol in DCM. Pure fractions were evaporated to dryness to afford the title compound (160 mg, 73 %) as a yellow solid. ¹H NMR δ 3.23 (6H, s), 7.61 - 7.68 (3H, m), 7.70 (1H, dd), 7.73 - 7.80 (1H, m), 8.20 (2H, dd), 8.39 (1H, s), 8.43 (1H, d), 9.26 (1H, d). m/z (ES+) (M+H)⁺ = 390.31; HPLC t_R = 1.97 min.



5-Chloro-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-4-(1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)pyrimidin-2-amine (24)

(*Z*)-2-Chloro-3-(dimethylamino)-1-(1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*c*]pyridin-3-yl)prop-2-en-1-one (80 mg, 0.21 mmol), 1-(4-(4-methylpiperazin-1-yl)phenyl)guanidine (144 mg, 0.62 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.18 mL, 1.23 mmol) were dissolved in DMA (4 mL) and sealed into a microwave tube. The reaction was heated to 100°C for 4 hours in the microwave reactor and cooled to room temperature. The reaction mixture was diluted with ethyl acetate (25 mL), and washed sequentially with water (2 x 25 mL) and saturated brine (2 x 25 mL). The organic layer was dried, filtered and evaporated to afford crude product. The crude product was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.1% NH₃) and acetonitrile as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (6 mg, 7%) as a white solid. ¹H NMR δ 2.24 (3H, s), 2.48 (4H, dd), 3.10 (4H, dd), 6.93 (2H, d), 7.55 (2H, d), 8.20 (1H, d), 8.41 (1H, s), 8.43 (1H, s), 8.67 (1H, s), 8.86 (1H, s), 9.39 (1H, s), 12.30 (1H, s). *m/z* (ES-) (M-H)⁻ = 428.42; HPLC t_R = 1.95 min.

2. Biological Assays

In Vitro DYRK1b Kinase Assay

The assay uses a nonradiometric Caliper mobility shift technology (Card *et al.* J. Biomol. Screen, 2009, 14: 31-42) to determine to the ability of test compounds to inhibit phosphorylation of a peptide substrate by recombinant DYRK1b. Test compounds were prepared as 10 mM stock solutions in 100% DMSO and diluted in DMSO as required to give a range of final concentrations. Aliquots (1% DMSO final concentration) of each compound dilution were dispensed into a well of a Greiner 384 well low volume white polystyrene plate (Greiner Cat. No

784075) using Echo acoustic dispensing. A 12 μ l enzyme reaction composed of 1.5 μ M FITC-labelled peptide (FITC-Asp-His-Thr-Gly-Phe-Leu-Thr-Glu-Tyr-Val-Ala-Thr-Arg-NH₂), 30 μ M ATP, 1.5 nM Dyrk1b enzyme (Invitrogen Cat. No PV4650), 1mM DTT, 20 mM MgCl₂, 50 mM HEPES pH7.5 and 0.004% Triton X-100 was incubated at room temperature for 60 minutes. Each reaction was stopped by the addition of 10 μ l of a mixture of 100 mM HEPES pH7.5, 0.03% Brij-35, 40 mM EDTA, 5% DMSO and 0.2% Coating reagent (CaliperLS). The resultant stopped enzyme reactions were analysed using the LabChip 3000 Drug Discovery System (Caliper) according to the manufacturer's instructions.

In Vitro IGFR and CDK2 Kinase Assays

The inhibitory activity of compound against the kinases CDK2 and IGF1R was determined with Caliper off-chip incubation mobility shift assays, using a microfluidic chip to measure the conversion of a fluorescent labeled peptide to a phosphorylated product. Custom peptide substrates with a fluorescent tag, sequence specific for each enzyme, were obtained from Cambridge Research Biochemicals (Cleveland, U.K.). Enzymes, peptide substrates, and ATP were incubated in the presence of compound diluted in DMSO (1% v/v DMSO assay final), in white Greiner 384-well low volume plates, in a total reaction volume of 12 μ L. Enzymes and peptides were added separately to the compound plates and were incubated at room temperature, and the kinase reaction was then quenched with the addition of a stop buffer (comprising 100 mM HEPES (pH 7.5), 5% DMSO, 88 mM EDTA, 0.22% Caliper coating reagent no. 3, 0.033% Brij-35 solution). Stopped assay plates were then read using the Perkin Elmer LabChip® EZ Reader.

The complex of recombinant full-length human cdk2 containing a C-terminal His6-tag and recombinant full-length human cyclin E containing an N-terminal GST-tag (expressed by baculovirus in Sf21 insect cells), was obtained from Millipore. The kinase reaction [90 μ M (Km) ATP, 1nM enzyme, 1.5 μ M substrate] in phosphorylation buffer [50mM HEPES (pH 7.5), 5mM MgCl₂, 1mM DTT, 0.05% CHAPSO] was quenched after a 60 minute incubation.

IGF-IR human recombinant kinase domain (residues 959 to end) containing an N-terminal His6-tag (expressed by baculovirus in Sf21 insect cells) was obtained from Millipore. The kinase

reaction [7 μ M (below K_m) ATP, 15 nM enzyme, 1.5 μ M substrate] in phosphorylation buffer [50 mM MOPS (pH 6.5), 5 mM $MnCl_2$, 1 mM DTT, 0.004% Triton X-100] was quenched after a 80 minute incubation.

In Vitro Live:Dead Phenotypic Assay

The viability of cells was determined using CellTraceTM calcein green AM and propidium iodide (Molecular probes) stains. Briefly, A375 (ATCC, CRL-1619) cells were seeded in a 96 well plate (at a density to allow for logarithmic growth during the 72 hour assay) and incubated overnight at 37°C, 5% CO₂. For compound experiments, cells were then treated with 1 μ M AZD6244 or 0.1% DMSO in the presence or absence of 1.33 μ M of DYRK1b inhibitors, 19, 31 or 33 for 72 hours. For siRNA experiments, 10nM of DYRK1b or universal scrambled control duplexes were transfected into cells using Lipofectamine 2000 (Invitrogen) during cell seeding. After 24 hours incubation with siRNA duplexes, the cells were treated for a further 72 hours with 1 μ M AZD6244 or 0.1% (v/v) DMSO. After 72 hours, Calcein green AM dye diluted in DMSO and propidium iodide dye diluted in PBS were added to the cells (final concentrations of 0.5 μ M and 2 μ g/ml respectively) and incubated for 30 minutes 37°C, 5% CO₂. The number of live (calcein green AM positive) and dead (propidium iodide positive) cells were counted using an Acumen ExplorerTM. DYRK1b siGENOME Smart Pool siRNAs and Universal scrambled non-targeted control siRNA were purchased from Dharmacon (M-004806 and D001206 respectively). Knockdown of DYRK1b was evaluated by Western blotting using DYRK1b (Cell Signaling Technology, #2703) and GAPDH antibodies (Advanced immunochemicals inc).

In Vitro pS421 DYRK1b Cellular Assay

Cryopreserved DYRK_cmyc transfected Cos-1 cells at 1.17×10^5 /ml were plated out into black 384-well flat bottom plates using a MultidropTM combi dispenser from Thermo Scientific (40 μ l/well). Plates were then incubated overnight at 37°C with 95% humidity in 5% CO₂. For the ELISA, Greiner all-black high-bind 384 well plates (# 781077) were coated with 20 μ l of 9B11 cMyc capture antibody at 1.33 μ g/ml in PBS and incubated overnight at 4°C. The next day, plates were washed 3 times on a Tecan Powerwasher with PBS- 0.5% Tween and blocked by addition of 40 μ l of 3% Bovine Serum Albumin in PBS followed by incubation at room temperature for 2 hours. Plates were then washed just prior to addition of cell lysates. On Day 2,

compounds were added to the cells via acoustic dispensing with an Echo[®]555 liquid handler (Labcyte) and incubated 5 hours at 37°C with 5% CO₂. Medium was then aspirated and 40µl cell lysis buffer (25mM Tris-HCl, 3mM EDTA, 3mM EGTA, 50mM sodium Fluoride, 2mM Sodium orthovanadate, 0.27M Sucrose, 10mM β-glycerophosphate, 5mM Sodium pyrophosphate, 0.5% Triton X-100) was added to all wells using a Multidrop[™] combi. After 10-30 min incubation at room temperature, 20µl of cell lysates was transferred to the ELISA plates using the Plate Mate Plus. The ELISA plates were then incubated at room temperature for 2 hours prior to being washed with PBS-0.5% Tween and incubated with 20µl per well of Phospho-Ser421 polyclonal antibody diluted 1/7000 in 3% BSA in PBS and incubated at room temperature for 90 minutes. After washing, 20µl of HRP-labelled Goat anti-rabbit secondary antibody at a dilution of 1/2000 in 3% BSA in PBS was added to all wells. Following 60 minutes incubation at room temperature, plates were washed & QuantaBlu substrate working solution was prepared according to the manufacturer's instructions and 20 µl added to all wells. After 60mins, the reaction was stopped by addition of 20µl of Stop solution. Fluorescence intensity was measured on a Tecan Safire plate reader using an excitation and emission wavelength of 325 nm 420 nm respectively.

Cells and Reagents

The Cos-1 monkey kidney (ATCC[®] CCL-70[™]) cell line was routinely maintained in Phenol Red-free DMEM supplemented with 5% heat-inactivated fetal bovine serum (Hyclone, # SH30068), and 2mM L-glutamine. Cells were grown under standard culture conditions at 37°C with 95% humidity in 5% CO₂.

A cell bank of Cos-1 cells transfected with an in-house human full length Dyrk1b construct (pCDNA3.1 Dyrk1b, C-terminally c-Myc tagged) was prepared.

Phospho-specific antibodies were raised using a peptide epitope encompassing the P-Ser412 site (ac-CGEPAAARIpSPLGALQHG-nh2) and rabbit polyclonal antibodies were generated using standard procedures (Antibodies: A Laboratory Manual, Harlow and Lane (eds), Cold Spring Harbor Laboratory Press, 1988).

QuantaBlu Fluorogenic Peroxidase Substrate kit was purchased from Thermo Scientific (# 15169). Phenol Red-free DMEM (#31053), L-glutamine (# 25030), lipofectamine 2000 (#116680027), OptiMEM (#31985), were purchased from Life technologies. 9B11 cMyc capture antibody (# 2276), HRP-labelled Goat anti-rabbit antibody (#7074) were purchased from Cell Signalling.

3. Kinase Selectivity

Compounds were screened in a suite of kinase assays at a single concentration of 1 μ M at Millipore (<http://www.millipore.com/>). Data below shows percent inhibition.

Kinase	Compound 19	Compound 31	Compound 33
Abl	21	18	-5
ALK	0	12	6
ALK4	4	10	-9
AMPK α 2	38	11	3
Arg	13	18	3
ARK5	51	35	3
Aurora-B	85	14	6
Aurora-C	55	23	10
Axl	22	-4	9
Blk	15	8	8
Bmx	4	-6	5
BRK	-4	6	-17
BTK	1	15	-10
CaMKI	27	31	15
CaMKII β	48	57	26
CDK1/cyclinB	13	11	-6
CDK2/cyclinA	20	16	-10
CDK6/cyclinD3	4	-2	-12
CDK7/cyclinH/MAT1	8	16	5
CDK9/cyclin T1	34	2	-7
CHK1	28	-13	-7
CK1 γ 1	50	18	5
CK2 α 2	2	2	5
cKit	9	4	-13

c-RAF	-16	16	-4
cSRC	1	-6	-10
DDR2	1	2	7
DMPK	11	22	-4
DRAK1	95	91	41
DYRK2	85	50	5
eEF-2K	-7	2	-10
EGFR	2	13	-3
EGFR(T790M,L858R)	49	93	17
EphA5	5	-3	2
EphB1	8	6	-3
EphB4	-2	13	-5
ErbB4	1	32	6
FAK	36	22	10
Fer	0	4	5
Fes	41	2	4
FGFR1	47	-1	1
FGFR1(V561M)	46	51	12
FGFR2	17	-1	2
FGFR3	45	75	6
FGFR4	-4	-4	-4
Flt1	98	43	8
Flt3	95	95	34
Fms	16	9	-5
Fyn	26	18	15
GSK3 α	10	3	1
GSK3 β	14	7	1
IGF-1R	18	47	7
IGF-1R, activated	-1	2	-2
IKK β	15	10	8
IR	-4	-1	0
IR, activated	13	21	17
IRAK4	48	67	16
IRR	23	30	6
JAK2	16	22	0
JAK3	14	8	-8
JNK1 α 1	-6	7	-11
KDR	88	70	4
Lck	29	44	23

Lck activated	32	42	15
LKB1	-1	5	6
LOK	87	69	8
Lyn	14	11	14
MAPKAP-K2	0	31	39
MARK1	80	29	1
MEK1	-4	16	-1
Met	5	6	12
MINK	87	44	10
MKK7 β	-11	-2	6
MLCK	66	77	13
MLK1	95	92	27
Mnk2	90	29	-2
MSK1	-2	7	-1
MST1	7	9	-1
mTOR	8	11	3
NEK2	0	2	5
p70S6K	5	-9	9
PAK2	-1	-2	25
PAK4	11	-17	2
PAK5	2	0	2
PAR-1B α	75	24	7
PDGFR β	-4	32	-3
PKD1	12	0	-6
PI3 Kinase (p110a/p85a)	9	4	24
PI3 Kinase (p110b/p85a)	1	13	5
PI3 Kinase (p110d/p85a)	1	8	8
PI3 Kinase (p120g)	3	-7	2
PI3KC2g	42	85	6
Pim-2	31	15	-8
PIP4K2a	0	16	-4
PIP5K1a	1	8	4
PIP5K1g	17	10	7
PKA	5	1	-2
PKB α	-6	-1	0
PKB β	-24	-25	-30
PKC α	15	2	0
PKC ϵ	5	8	4
PKC θ	-7	-12	-10

PKG1 α	9	74	-11
Plk1	-6	-19	0
PRAK	39	-7	-1
Ret	45	62	-2
Ret (V804L)	65	62	0
Ret(V804M)	41	26	3
ROCK-I	45	-4	-7
ROCK-II	70	18	13
Ron	15	19	9
Ros	-5	-7	3
Rse	-4	-2	6
Rsk1	39	58	20
SAPK2a	-8	-8	-11
SGK	-10	10	-10
Src(1-530)	8	13	9
SRPK1	17	9	4
TAK1	32	-2	-1
TBK1	74	74	19
TGFBR1	12	13	2
TrkA	63	97	1
ULK2	15	16	4
Yes	8	17	4

4. Crystallography Protocols

Both wild type human TTK(514-795) and IGF(980-1286) proteins were produced in Sf9 insect cells as GST fusion protein with thrombin cleavage site. After affinity purification and cleavage of the GST tag, a gel filtration step was performed and the resulting protein concentrated to 10 and 7.5 mg/ml, respectively. Apo crystals were prepared using the vapour diffusion technique by drops equilibrated against 18% PEG3350, 5 v/v% ethanol and 0.1M sodium citrate pH 6.0 in the case of TTK, and 20% PEG3350, 0.2M magnesium chloride and 0.1M PCTP pH 7.5 for IGF. Protein-ligand complex crystals were generated by soaking apo-crystals with 2-5 mM of the compound (dissolved in DMSO at 100 mM) and ethylene glycol was used to flash-freeze crystals in liquid nitrogen prior to data collection. Diffraction data were collected at the European Synchrotron Radiation Facility for TTK, and on an in-house radiation source for IGF and subsequently processed using XDS¹ and scala² and structures solved using the CCP4 software

suite.³ Crystallographic models were completed in Coot⁴ and refined with Buster.⁵ Crystallographic data and refinement parameters are summarised in the Table below.

	TTK	IGF
PDB accession code	4D2S	4D2R
Data collection statistics		
Cell: space group	C222 ₁	P2 ₁ 2 ₁ 2 ₁
a b c [Å]	108.01, 113.35,	41.154, 66.486,
α β γ [Å]	71.03	121.593
	90, 90, 90	90, 90, 90
Resolution [Å]	2.5 (2.5-2.7) [†]	2.1 (2.1-2.15) [†]
Unique reflections	13748 (1145) [†]	19300 (1228) [†]
Multiplicity	4.2 (3.9) [†]	3.6 (3.13) [†]
Completeness [%]	88.8 (68.0) [†]	95.8 (84.5) [†]
R _{sym} [%]	6.7 (48.0) [†]	8.01 (34.0) [†]
Mean(I)/sd	18.6 (4.2) [†]	8.31 (2.23) [†]
Refinement statistics		
Number of reflections (working /test)	13748 / 689	19257 / 984
R/R _{free} [%]	21.3/24.9	18.11/22.1
Number of atoms:		
protein	1903	2348
water	2	221
heterogen	32	254
Deviation from ideal geometry	0.009	0.010
bond lengths [Å]	1.05	1.03
bond angles [°]		
Ramachandran plot		
most favoured region	95.85	97.63
allowed region	3.73	2.37
disallowed region	0.41	0

[†]Data in parentheses refer to the highest resolution shell.

Table – Crystallographic data collection and refinement summary

¹ Kabsch, W.; XDS Acta Cryst. **2010** D66, 125-132.

² Evans, P.R.; Scaling and assessment of data quality. *Acta Cryst.* **2006**, *D62*, 72-82.

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