Supplementary Information

Structure-guided evolution of potent and selective CHK1 inhibitors through scaffold morphing

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Table of Contents

1. Experimental details for preparation and characterization for compounds (*rac*)-3, (*S*)-3, 6, (*R*)-7, (*S*)-7, 9-19, 21, 22, 25-34

(3-(4-(2-(Aminomethyl)morpholino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)-phenyl)methanol (6) A mixture of *tert*-butyl (4-(5-bromo-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)morpholin-2-yl)methylcarbamate **39** (60 mg, 0.11 mmol), $Pd(PPh_3)_4$ (7 mg, 6 mol%), 3-(hydroxymethyl)phenylboronic acid (33 mg, 0.22 mmol) and Na_2CO_3 (30 mg, 0.28 mmol) in DME (2.5 mL) and water (1.0 mL) was heated to 120^{o}C in a microwave reactor for 30 min. The mixture was partitioned between brine (10 mL) and ethyl acetate (2 x 8 mL). The combined organic layers were washed with brine (10 mL), water (10 mL), dried, filtered and concentrated. Preparative TLC, eluting with ethyl acetate (R_f = 0.56), gave *tert*-butyl (4-(5-(3-(hydroxymethyl)phenyl)-7-((2-(trimethylsilyl)-ethoxy)-methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)morpholin-2-yl)methyl-carbamate as a yellow oil (31 mg, 49%). LC-MS (LCT, 6 mins) R_t 5.47 min; *m/z* (ESI) 570 [MH^+]. A mixture of *tert*-butyl (4-(5-(3-(hydroxymethyl)phenyl)-7-((2-(trimethylsilyl)-ethoxy)-methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)morpholin-2-yl)methyl-carbamate (29 mg, 0.05 mmol), 1.0M TBAF/THF (0.4 mL, 0.4 mmol) and ethane-1,2-*di*amine (6 uL) in DMF (1 mL) was stirred at 60 ^{o}C under N_2 for 16 hrs. It was diluted with NaCl solution (8 mL) and extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (10 mL), water (10 mL), dried (Na_2SO_4), filtered and concentrated. Crude oil (12 mg) was obtained (LC-MS (LCT, 6 min) R_t 4.18 min; *m/z* (ESI) 440 [MH^+]). Without further purification, the crude oil was dissolved in a mixture of MeOH (1 mL) and 4M HCl/dioxane (3 mL). The solution was stirred at r.t. for 12 hrs. The solvents were evaporated and the residue was purified on SCX-II acidic resin (1 g) eluting with methanol then 2M ammonia-

methanol. After the basic fractions were combined and evaporated, the crude oil was purified by preparative TLC, eluting with (DCM:MeOH:NH_3/10:1:0.2). Yellow oil (3 mg, 17%) was obtained. ^{1}H NMR (500 MHz, CD_3OD) δ 2.62 – 2.82 (2H, m), 2.85 – 2.94 (1H, m), 3.45 – 3.55 (1H, m), 3.63 – 3.85 (4H, m), 4.12 (1H, m), 4.73 (2H, s), 7.34 (1H, s), 7.35 – 7.40 (1H, d, *J* = 7 Hz), 7.45 – 7.50 (2H, m), 7.54 (1H, s), 8.37 (1H, s); LC-MS (LCT, 6 min) R_t 1.81 min; *m/z* (ESI) 340 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{18}H_{22}N_5O_2$ (M+H) 340.1774, found 340.1769.

(*R*)-(4-(9*H*-Pyrimido[4,5-*b*]indol-4-yl)morpholin-2-yl)methanamine ((*R*)-7) A solution of **48** (0.025 g, 0.123 mmol), (*S*)-benzyl morpholin-2-ylmethylcarbamate[1,2] (0.026 g, 0.104 mmol) and Et_3N (0.20 mL, 1.4 mmol) in DMF (1.5 mL) was heated in a microwave reactor at 120^{o}C for 1 h. The mixture was partitioned between water (15 mL), 1M citric acid (5 mL) and EtOAc (10 mL). The organic extract was dried, filtered and concentrated. Preparative TLC, eluting with EtOAc, gave (*R*)-benzyl (4-(9*H*-pyrimido[4,5-*b*]indol-4-yl)morpholin-2-yl)methylcarbamate (0.011 g, 25%). A mixture of (*R*)-benzyl (4-(9*H*-pyrimido[4,5-*b*]indol-4-yl)morpholin-2-yl)methylcarbamate (0.011 g, HCO_2NH_4 (0.10 g, 1.6 mmol) and 10% Pd on carbon (0.03 g) in MeOH (2 mL) was stirred at rt for 16 h. The mixture was filtered and the filtrate was concentrated. Preparative TLC, eluting with 10% MeOH-DCM, gave (***R*)-7** (0.92 mg, 13%). ^{1}H NMR (500 MHz, CD_3OD) δ 3.10-3.22 (3H, m), 3.41 (1H, ddd, *J* = 15, 12, 3 Hz), 3.92 (1H, ddd, *J* = 12, 12, 3 Hz), 4.00-4.05 (1H, m), 4.13-4.16 (1H, m), 4.23 (1H, br d, *J* = 13 Hz), 4.29 (1H, br d, *J* = 13 Hz), 7.36 (1H, dd, *J* = 8, 8 Hz), 7.49 (1H, dd, *J* = 8, 8 Hz), 7.58 (1H, d, *J* = 8 Hz), 7.79 (1H, d, *J* = 8 Hz), 8.49 (1H, s), 8.55 (1H, br s); LC-MS (LCT, 6 min) R_t = 1.90 min; *m/z* (ESI+) 284 (MH^+). Hi-Res MS (ESI) *m/z* calcd for $C_{15}H_{17}N_5O$ (M+H) 284.1506, found 284.1496.

(*S*)-(4-(9*H*-Pyrimido[4,5-*b*]indol-4-yl)morpholin-2-yl)methanamine ((*S*)-7)

Prepared from **48** and (*R*)-benzyl morpholin-2-ylmethylcarbamate[1,2] to give (**S)-7** (2.3 mg, 21%). ^{1}H NMR (500 MHz; CD_3OD) δ 2.93-2.94 (2H, m), 3.05 (1H, dd, *J* = 13, 3 Hz), 3.35-3.38 (1H, m), 3.83-3.93 (2H, m), 4.10-4.13 (1H, m), 4.19 (1H, br d, *J* = 13 Hz), 4.26 (1H, br d, *J* = 13 Hz), 7.35 (1H, dd, *J* = 8, 8 Hz), 7.47 (1H, dd, *J* = 8, 8 Hz), 7.57 (1H, d, *J* = 8 Hz), 7.79 (1H, d, *J* = 8 Hz), 8.46 (1H, s), 8.57 (1H, br s); LC-MS (LCT, 6 min) R_t = 1.91 min; *m/z* (ESI+) 284 (MH^+). Hi-Res MS (ESI) *m/z* calcd for $C_{15}H_{17}N_5O$ (M+H) 284.1506, found 284.1495.

Cyano-(4-cyano-2-nitro-phenyl)-acetic acid ethyl ester (43) To a suspension of sodium hydride (0.96 g, 60% in mineral oil, 24 mmol) in DMF (6 mL) at 0°C was added ethylcyanoacetate (2.72 g, 24 mmol) in DMF (2 mL) under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature for 30 minutes then 4-fluoro-3-nitro-benzonitrile **41** (2.05 g, 22.7 mmol) in DMF (5 mL) was added dropwise. 1M HCl and ethyl acetate were added to the reaction mixture after 1 hour. The organic layer was washed with water and brine, then dried over sodium sulphate and concentrated to give a brown oil which was purified by flash column chromatography to give the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (3H, t, *J* = 7 Hz), 4.24 (2H, q, *J* = 7 Hz), 7.10 (2H, br s), 7.33 (1H, dd, *J* = 8, 1 Hz), 7.49 (1H, d, *J* = 1 Hz), 7.62 (1H, d, *J* = 8 Hz), 11.07 (1H, s).

2-Amino-6-cyano-1*H*-indole-3-carboxylic acid ethyl ester (45) Zinc powder (2.40 g) was added portionwise to a solution of **43** (1.15 g, 4.4 mmol) in 10 mL acetic acid at 80°C. The mixture was then heated at 95°C for 30 minutes. The reaction was then cooled to room temperature, filtered and the catalyst rinsed with acetic acid. The filtrate was concentrated to near dryness then neutralized with saturated sodium bicarbonate solution. The product was then isolated by filtration and washing with

ethyl acetate to give the title compound as a tan solid (700 mg, 69%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (3H, t, *J* = 7 Hz), 4.24 (2H, q, *J* = 7 Hz), 4.24 (2H, q, *J* = 7 Hz), 7.10 (2H, br, s), 7.33 (1H, dd, *J* = 8, 1 Hz), 7.49 (1H, d, *J* = 1 Hz), 7.62 (1H, d, *J* = 8 Hz), 11.07 (1H, s); LC-MS (ZQ, 4 min) R_t 1.84 min; *m/z* (ESI-) 228 [M-H].

4-Hydroxy-9*H*-pyrimido[4,5-*b*]indole-7-carbonitrile (47) A solution of 2-amino-6-cyano-1*H*-indole-3-carboxylic acid ethyl ester (750 mg, 3.3 mmol) and ammonium formate (193 mg, 3.3 mmol) in 4 mL formamide was heated to 175°C overnight. The reaction mixture was allowed to cool to room temperature then poured onto water. The resulting precipitate was collected by filtration to give 297 mg of the title compound as a black solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (1H, dd, *J* = 8, 1 Hz), 7.93 (1H, s), 8.10 (1H, d, *J* = 8 Hz), 8.23 (1H, d, *J* = 4 Hz), 12.5 (1H, br s), 12.7 (1H, br s); LC-MS (ZQ, 4 min) R_t 1.12 min; *m/z* (ESI-) 209 [M-H].

4-Chloro-9*H*-pyrimido[4,5-*b*]indole-7-carbonitrile (50) A solution of 4-hydroxy-9*H*-pyrimido[4,5-*b*]indole-7-carbonitrile (215 mg, 1.02 mmol) in 5 mL $POCl_3$ (5 mL) was heated at 100°C for 18 hours. Upon cooling to room temperature the reaction mixture was evaporated to dryness and the residue treated with saturated sodium bicarbonate solution and ethyl acetate. The organic phase was dried over sodium sulphate and concentrated to the title compound as an orange solid. LC-MS (ZQ, 4 min) R_t 1.66 min; *m/z* (ESI-) 227/229 [M-H]. The product was used without further purification.

4-(2-(Aminomethyl)morpholino)-9*H*-pyrimido[4,5-*b*]indole-7-carbonitrile (9) A solution of 4-chloro-9*H*-pyrimido[4,5-*b*]indole-7-carbonitrile (30 mg, 0.131 mmol), *tert*-butyl morpholin-2-ylmethylcarbamate (57 mg, 0.262 mmol) and Et_3N (0.037 mL, 0.262 mmol) in NMP (2 mL) was heated at 140°C in a microwave reactor for 15 min. The reaction mixture was concentrated to dryness and purified by flash

chromatography eluting with 1:4 EtOAc-hexane affording *tert*-butyl (4-(7-cyano-9*H*-pyrimido[4,5-*b*]indol-4-yl)morpholin-2-yl)methylcarbamate as a pale yellow solid (13 mg, 0.032 mmol, 24%). LC-MS (ZQ, 4 min) R_t 1.24 min; *m/z* (ESI+)353, 409 [M+H].

To a solution of *tert*-butyl (4-(7-cyano-9*H*-pyrimido[4,5-*b*]indol-4-yl)morpholin-2-yl)methylcarbamate (5 mg, 0.012 mmol) in DCM (0.2 mL) was added 4M HCl/dioxane (0.021 mL, 0.086 mmol) and the reaction mixture stirred for 1 h. Isolation by SPE on a MP-TsOH cartridge, eluting with 2N NH_3 in MeOH, followed by concentration, gave **9** as a pale yellow solid (3 mg, 78%). ^{1}H NMR (400 MHz, CD_3OD) δ 2.80-2.82 (2H, m), 3.10 (1H, dd, *J* = 11, 13 Hz), 3.35 (2H, s), 3.38-3.45 (1H, m), 3.72-3.78 (1H, m), 3.83 (1H, dt, *J* = 3, 12 Hz), 4.08 (1H, dd, *J* = 2, 12 Hz), 4.21 (1H, d, *J* = 13 Hz), 4.29 (1H, dt, *J* = 2, 13 Hz), 7.62 (1H, dd, *J* = 1, 8 Hz), 7.87-7.89 (2H, m), 8.49 (1H, s); LC-MS (ZQ, 7 min) R_t = 1.38 min; *m/z* (ESI+) 309 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{16}H_{17}N_7O$ (M+H) 309.1458, found 309.1467.

The following compounds were prepared in an analogous fashion:

***N*^{1}-(9*H*-Pyrido[4',3':4,5]pyrrolo[2,3-*d*]pyrimidin-4-yl)ethane-1,2-diamine (10)**

Title compound isolated as a pale yellow powder (5.8 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.09 (2H, t, J = 6 Hz), 3.84 (2H, dd, J = 12, 6 Hz), 8.01 (1H, t, J = 6 Hz), 8.45 – 8.38 (3H, m), 8.46 (1H, s), 8.81 (1H, s); LC-MS (ZQ, 7 min) R_t = 1.11 min; *m/z* (ESI+) 229 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{11}H_{13}N_6$ (M+H) 229.1196, found 229.1196.

***N*^{1}-(9*H*-Pyrido[4',3':4,5]pyrrolo[2,3-*d*]pyrimidin-4-yl)propane-1,3-diamine (11)**

Title compound isolated as a pale yellow powder (6.1 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.01 – 1.88 (2H, m), 2.87 (2H, t, *J* = 7 Hz), 3.70 (2H, s), 7.80 (1H, s), 8.44 – 8.36 (4H, m), 8.45 (1H, d, *J* = 6 Hz), 8.81 (1H, s); LC-MS (ZQ, 7 min) R_t =

1.19 min; *m/z* (ESI+) 243 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{12}H_{15}N_6$ (M+H) 243.1353, found 243.1353.

1-(9*H*-Pyrido[4',3':4,5]pyrrolo[2,3-*d*]pyrimidin-4-yl)piperidine-4-amine (12) Title compound isolated as a yellow powder (11.2 mg, 43%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.39 (2H, dt, *J* = 12, 9 Hz), 1.83 (2H, d, *J* = 10 Hz), 2.96-3.14 (3H, m), 4.14 (2H, d, *J* = 13 Hz), 7.42 (1H, d, *J* = 5 Hz), 8.21 (2H, d, *J* = 7 Hz), 8.30 (1H, d, *J* = 7 Hz), 8.63 (1H, s); LC-MS (ZQ, 7 min) R_t = 1.28 min; *m/z* (ESI+) 269 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{14}H_{17}N_6$ (M+H) 269.1509, found 269.1513.

(1-(9*H*-Pyrido[4',3':4,5]pyrrolo[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)methan amine (13) Title compound isolated as a pale yellow powder (9.5 mg, 35%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.26-1.47 (2H, m), 1.89 (3H, dd, *J* = 4, 12 Hz), 2.73 (2H, t, *J* = 12 Hz), 3.10-3.23 (2H, m), 4.39 (2H, d, *J* = 13 Hz), 7.65 (1H, d, *J* = 5 Hz), 8.38-8.47 (3H, m), 8.48-8.54 (1H, m), 8.86 (1H, s); LC-MS (ZQ, 7 min) R_t = 1.41 min; *m/z* (ESI+) 283 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{18}H_{22}N_5O_2$ (M+H) 283.1666, found 283.1675.

***N*^{4}-(Piperidin-4-ylmethyl)-*N*^{6}-(pyridin-3-yl)pyrimidine-4,6-diamine (14)** A solution of 3-aminopyridine (114 mg, 1.211 mmol), Sodium *tert*-butoxide (116 mg, 1.211 mmol) and bis(tri-*tert*-butylphosphine)palladium(0) in toluene (0.5 mL) was stirred for 10 min. 4,6-dichloropyrimidine (150 mg, 1.007 mmol) was added and the reaction mixture heated at 80^{o}C for 2 h. The reaction mixture was diluted with MeOH, filtered to remove precipitate and then passed through a PS-Thiol column and concentrated. The residue was triturated with DCM and the combined organic washings were concentrated affording a 3:1 mixture of 6-chloro-*N*-(pyridin-3-yl)pyrimidin-4-amine and 3-aminopyridine (150 mg overall) which was used directly in the next reaction. LC-MS (ZQ, 7 min) R_t = 1.57 min; *m/z* (ESI+) 207 [MH^+].

6-Chloro-*N*-(pyridin-3-yl)pyrimidin-4-amine (150 mg, 75% purity, 0.542 mmol), *tert*-butyl 4-(aminomethyl)piperidine-1-carboxylate (232 mg, 1.083 mmol) and Et_3N (110 mg, 1.083 mmol) were dissolved in NMP (1 mL) and heated at 145°C in a microwave reactor for 15 min. The solution was purified directly by preparative HPLC to give *tert*-butyl 4-((6-(pyridin-3-ylamino)pyrimidin-4-ylamino)methyl)piperidine-1-carboxylate. LC-MS (ZQ, 7 min) R_t = 2.44 min; *m/z* (ESI+) 385 [MH^+]. This was dissolved in DCM (2 mL) and TFA (3 mL) and stirred for 1 h. Isolation by SPE on a MP-TsOH cartridge, washing with MeOH, DCM and MeOH before eluting with 7N NH_3 in MeOH, followed by concentration, gave **14** as a pale yellow solid (4.1 mg, 2.7%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.24-1.36 (2H, m), 1.76-1.91 (3H, m), 2.74 (2H, dt, *J* = 2, 12 Hz), 3.15-3.24 (3H, m), 3.37 (2H, s), 5.84 (1H, s), 7.34 (1H, dd, *J* = 5, 8 Hz), 8.10 (1H, br d, *J* = 9 Hz), 8.12 (1H, s), 8.68 (1H, br d, *J* = 3 Hz). Hi-Res MS (ESI) R_t = 0.80 min *m/z* calcd for $C_{15}H_{19}N_6$ (M+H) 284.1666, found 284.1670.

6-Chloro-*N*-(pyrazin-2-yl)pyrimidin-4-amine (56) A mixture of 2-aminopyrazine **53** (230 mg, 2.42 mmol), sodium *tert*-butoxide (232 mg, 2.42 mmol) and bis(tri-*tert*-butylphosphine)palladium (0) (51 mg, 0.1 mmol) in toluene (2 mL) was de-gassed under a stream of nitrogen over 10 min. 4,6-Dichloropyrimidine (300 mg, 2.01 mmol) was added to the mixture and the reaction was heated at 80°C for 2 h. After cooling the solution was passed through a PS-SH cartridge and the solvent removed *in vacuo*. The residue was triturated with dichloromethane and the resulting solid was collected and dried by vacuum filtration to give 333 mg of the expected product which was used without further purification. LC-MS (ZQ, 7 min) R_t = 1.65 min; *m/z* (ESI+) 208 [MH^+].

***N*-(6-(4-(Aminomethyl)piperidin-1-yl)pyrimidin-4-yl)pyrazin-2-amine (15)** A mixture of 6-chloro-*N*-(pyrazin-2-yl)pyrimidin-4-amine (20 mg, 0.096 mmol), *tert*-

butyl *N*-(4-piperidinylmethyl)carbamate (41 mg, 0.193 mmol) and triethylamine (27 µl, 0.193 mmol) in 1-methyl-2-pyrrolidinone (1 mL) was heated at 140 ^{O}C for 10 min using microwave irradiation. The mixture was concentrated *in vacuo* and the residue purified using preparative HPLC. The purified solid was dissolved in dichloromethane (4 mL) and treated with trifluoroacetic acid (4 mL) over 1 h at room temperature. The solution was applied onto a MP-TsOH SPE cartridge, washed, then eluted with 2N ammonia and concentrated to give 4.4 mg (16%) of the required product. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (3H, m), 1.74 (1H, m), 1.90 (2H, d, *J* = 12 Hz), 2.61 (2H, d, *J* = 7 Hz), 2.95 (2H, m), 4.45 (2H, d, *J* = 11 Hz), 7.17 (1H, d, *J* = 1 Hz), 8.08 (1H, d, *J* = 3 Hz), 8.23 (1H, d, *J* = 1 Hz), 8.29 (1H, dd, *J* = 3, 1 Hz), 8.83 (1H, d, *J* = 1 Hz); LC-MS (ZQ, 7 min) R_t = 1.62 min; *m/z* (ESI+) 286 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{15}H_{20}N_7$ (M+H) 286.1775, found 286.1785.

The following compounds were prepared in an analogous fashion:

5-(6-(4-(Aminomethyl)piperidin-1-yl)pyrimidin-4-ylamino)pyrazine-2-carbonitrile (16) Title compound isolated as a cream powder (33.5 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16-1.26 (2H, m), 1.88 (2H, d, *J* = 12 Hz), 1.98 (1H, br s), 2.73 (2H, t, *J* = 6 Hz), 3.09 (2H, t, *J* = 12 Hz), 3.59 (1H, s), 3.65-3.75 (2H, m), 7.16 (1H, s), 8.17 (3H, br s), 8.47 (1H, s), 8.88 (2H, m), 11.91 (1H, br s); LC-MS (ZQ, 7 min) R_t = 2.08 min; *m/z* (ESI+) 311 [MH^+], (ESI-) 309 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{15}H_{19}N_8$ (M+H) 311.1727, found 311.1736.

5-(6-(4-Aminopiperidin-1-yl)pyrimidin-4-ylamino)pyrazine-2-carbonitrile (17) Title compound isolated as a yellow powder (20.6 mg, 38%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (2H, m), 1.80 (2H, br d, *J* = 13 Hz), 2.91-3.05 (3H, m), 4.21 (2H, br d, *J* = 13 Hz), 7.16 (1H, s), 8.33 (1H, s), 8.82 (1H, d, *J* = 1 Hz), 9.00 (1H, d, *J* = 1

Hz); LC-MS (ZQ, 7 min) R_t = 1.84 min; *m/z* (ESI+) 297 [MH^+], (ESI-) 295 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{14}H_{17}N_8$ (M+H) 297.1571, found 297.1579.

5-(6-(2-Aminoethylamino)pyrimidin-4-ylamino)pyrazine-2-carbonitrile (18) Title compound isolated as a yellow powder (14.0 mg, 32%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.89 (2H, t, *J* = 6 Hz), 3.45 (2H, br s), 7.05 (1H, br s), 7.84 (1H, br s), 8.27 (1H, s), 8.40 (1H, br s), 8.78 (1H, d, *J* = 1 Hz), 8.88 (1H, br s); LC-MS (ZQ, 7 min) R_t = 1.53 min; *m/z* (ESI+) 257 [MH^+], (ESI-) 255 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{11}H_{13}N_8$ (M+H) 257.1258, found 257.1266.

5-(6-(3-Aminopropylamino)pyrimidin-4-ylamino)pyrazine-2-carbonitrile (19) Title compound isolated as a yellow powder (17.6 mg, 38%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.51 (2H, quin, *J* = 7 Hz), 2.53 (3H, m), 3.10 (3H, br s), 6.79 (1H, br s), 7.44 (1H, br s), 8.00 (1H, s), 8.19 (1H, br s), 8.53 (1H, s), 8.63 (1H, br s); LC-MS (ZQ, 7 min) R_t = 1.74 min; *m/z* (ESI+) 271 [MH^+], (ESI-) 269 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{12}H_{15}N_8$ (M+H) 271.1414, found 271.1423.

5-(6-(3-Hydroxypropylamino)pyrimidin-4-ylamino)pyrazine-2-carbonitrile (21) Title compound isolated as a white powder (6 mg, 26%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.67 (2H, dt, *J* = 7, 7 Hz), 3.30 (2H, *obscured by H_2O peak*), 3.47 (2H, t, *J* = 7 Hz), 4.46 (1H, br s), 6.97 (1H, br s), 7.41 (1H, br s), 8.23 (1H, s), 8.76 (1H, s), 8.88 (1H, br s), 10.61 (1H, br s); LC-MS (LCT, 6 min) R_t 2.03 min; *m/z* (ESI+) 272 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{12}H_{13}N_7O$ (M+H) 272.1254, found 272.1256.

5-(6-((Tetrahydro-2*H*-pyran-4-yl)methylamino)pyrimidin-4-ylamino)pyrazine-2-carbonitrile (22) MeCN (0.2ml) was added to 5-(6-chloropyrimidin-4-ylamino)pyrazine-2-carbonitrile (20 mg, 0.086 mmol), 4-(aminomethyl)tetrahydropyran (18 mg, 2 eq.), and triethylamine (0.02 ml, 1.5 eq.) in a 0.2-0.5ml Biotage microwave vial,which was then sealed and heated to 145°C for 30

mins. Upon cooling the volatiles were removed and the crude was taken up in a mixture of DCM (89%), MeOH (10%), 0.88 s.g. NH_3 (1%) which was added to a conditioned Trikonex column. After eluting with the same mixture, the appropriate band was isolated and the pure desired compound was recovered as a yellow powder (11 mg, 41%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15-1.24 (2H, m), 1.60 (2H, d, *J* = 13 Hz), 1.73-1.83 (1H, m), 3.15-3.28 (4H, m), 3.84 (2H, dd, *J* = 3, 11 Hz), 7.00 (1H, br s), 7.50 (1H, br s), 8.22 (1H, s), 8.75 (1H, s), 8.86 (1H, br s), 10.59 (1H, br s); LC-MS (LCT, 6 min) R_t 2.79 min; *m/z* (ESI+) 312 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{15}H_{18}N_7O$ (M+H) 312.1567, found 312.1574.

5-(1-(Piperidin-4-ylmethyl)-1*H*-imidazo[4,5-*c*]pyridin-6-ylamino)pyrazine-2-carbonitrile (25) The title compound was prepared using methods analogous to those described in the synthesis of **24**. Title compound isolated as a yellow powder (10 mg, 11%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21-1.41 (2H, m), 1.52-1.59 (2H, m), 2.02 (1H, m), 2.54-2.61 (3H, m), 3.04-3.11 (2H, m), 4.16 (1H, m), 8.16 (1H, s), 8.34 (1H, s), 8.40 (1H, s), 8.77 (1H, d, *J* =1 Hz), 8.80 (1H, d, *J* = 1 Hz), 10.87 (1H, br s); LC-MS (ZQ, 7 min) R_t = 1.47 min; *m/z* (ESI+) 335 [MH^+], (ESI-) 333 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{17}H_{19}N_8$ (M+H) 335.1727, found 335.1744.

5-(3*H*-Imidazo[4,5-*c*]pyridin-6-ylamino)-3-(piperidin-4-ylmethoxy)pyrazine-2-carbonitrile (26) The title compound was prepared using methods analogous to those described in the synthesis of **24**. Title compound isolated as a yellow powder (2.1 mg, 2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35-1.44 (2H, m), 1.82 (2H, d, *J* = 12 Hz), 1.98-2.08 (1H, m), 2.64-2.70 (2H, m), 3.13 (2H, d, *J* = 12 Hz), 4.37 (2H, d, *J* = 6 Hz), 8.19 (1H, br s), 8.31 (1H, br s), 8.36 (1H, s), 8.75 (1H, d, *J* = 1 Hz), 10.85 (1H, br s); LC-MS (ZQ, 7 min) R_t = 1.50 min; *m/z* (ESI+) 351 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{17}H_{19}N_8O$ (M+H) 351.1676, found 351.1683.

5-(Isoquinolin-3-ylamino)pyrazine-2-carbonitrile (27) Title compound isolated as a pale yellow powder (2.0 mg, 3%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52-7.56 (1H, m), 7.75-7.71 (1H, m), 7.91 (1H, d, *J* = 8 Hz), 8.08 (1H, d, *J* = 8 Hz), 8.50 (1H, s), 8.72 (1H, d, *J* = 1 Hz), 8.82 (1H, d, *J* = 1 Hz), 9.21 (1H, s), 11.03 (1H, s); LC-MS (ZQ, 7 min) R_t = 2.77 min; *m/z* (ESI+) 248 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{14}H_{10}N_5$ (M+H) 248.0931, found 248.0930.

5-(Isoquinolin-3-ylamino)-3-(piperidin-4-ylmethoxy)pyrazine-2-carbonitrile (28) Title compound isolated as a pale yellow powder (10.3 mg, 15%).^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22-1.32 (2H, m), 1.75 (2H, br d, *J* = 13 Hz), 1.93-2.03 (1H, m), 2.47-2.53 (2H, m, *partially obscured by DMSO*), 2.99 (2H, br d, *J* = 12 Hz), 4.83 (2H, d, *J* = 7 Hz), 7.54-7.58 (1H, m), 7.74-7.78 (1H, m), 7.84 (1H, d, *J* = 8 Hz), 8.09 (1H, d, *J* = 8 Hz), 8.24 (1H, s), 8.48 (1H, s), 9.22 (1H, s); LC-MS (ZQ, 7 min) R_t = 2.38 min; *m/z* (ESI+) 361 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{20}H_{21}N_6O$ (M+H) 361.1771, found 361.1788.

5-(8-Chloroisoquinolin-3-ylamino)pyrazine-2-carbonitrile (29) Title compound isolated as a yellow powder (12.0 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71-7.64 (2H m), 7.92 (1H, d, *J* = 8 Hz), 8.57 (1H, s), 8.72 (1H, d, *J* = 2 Hz), 8.84 (1H, d, *J* = 2 Hz), 9.40 (1H, s), 11.20 (1H, br s); LC-MS (ZQ, 7 min) R_t = 3.04 min; *m/z* (ESI+) 282 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{14}H_9ClN_5$ (M+H) 282.0541, found 282.0550.

5-((8-Chloroisoquinolin-3-yl)amino)-3-(piperidin-4-ylmethoxy)pyrazine-2-carbonitrile (30) Title compound isolated as a pale yellow powder (3.7 mg, 3%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25-1.36 (3H, m), 1.73-1.80 (2H, m), 1.95-2.07 (2H, m), 2.99-3.05 (2H, m), 4.44 (2H, d, *J* = 6 Hz), 7.65-7.68 (1H, m), 7.71-7.76 (1H, m), 7.85 (1H, m), 8.31 (1H, s), 8.46 (2H, s), 8.50 (1H, s), 9.44 (1H, br s); LC-MS (ZQ, 7

min) Rt = 3.23 min; *m/z* (ESI+) 395 [MH^+], (ESI-) 393 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{20}H_{20}ClN_6O$ (M+H) 395.1382, found 395.1400.

5-(Isoquinolin-3-ylamino)-3-(piperidin-3-yloxy)pyrazine-2-carbonitrile (31) Title compound isolated as a pale yellow powder (3.8 mg, 26%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.53-1.71 (2H, m), 1.74-1.81 (1H, m), 2.20-2.26 (1H, m), 2.4-2.58 (2H, m, *partly obscured by DMSO*), 2.67-2.73 (1H, m), 2.83-2.89 (1H, m), 5.07-5.13 (1H, m), 7.54 (1H, t, J = 7.0 Hz), 7.75 (1H, t, J = 7.0 Hz), 7.92 (1H, d, J = 8.0 Hz), 8.08 (1H, d, J = 8.0 Hz), 8.19 (1H, s), 8.48 (1H, s), 9.20 (1H, s), 11.05 (1H, br s); LC-MS (ZQ, 7 min) R_t = 2.55 min; *m/z* (ESI+) 347 [MH^+], (ESI-) 345 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{19}H_{19}N_6O$ (M+H) 347.1615, found 347.1628.

5-(8-Chloroisoquinolin-3-ylamino)-3-(piperidin-3-yloxy)pyrazine-2-carbonitrile (32) Title compound isolated as a pale brown powder (2.3 mg, 2.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.24 (1H, m), 1.38 (1H, m), 1.53-1.62 (1H, m), 1.75-1.84 (1H, m), 2.19-2.27 (1H, m), 2.69-2.76 (1H, m), 2.83-2.92 (1H, m), 5.12 (2H, m), 7.67-7.70 (1H, m), 7.72-7.77 (1H, m), 7.96 (1H, m), 8.23 (1H, m), 8.36 (1H, s), 8.57 (1H, s), 9.41 (1H, s), 11.30 (1H, br s); LC-MS (ZQ, 7 min) R_t = 2.91 min; *m/z* (ESI+) 381 [MH^+], (ESI-) 379 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{19}H_{18}ClN_6O$ (M+H) 381.1225, found 381.1229.

5-(8-Chloroisoquinolin-3-ylamino)-3-(2-(dimethylamino)ethoxy)pyrazine-2-carbonitrile (33) Title compound isolated as a pale yellow powder (2.2 mg, 2.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (6H, s), 2.78 (2H, t, *J* = 6 Hz), 4.68 (2H, t, *J* = 6 Hz), 7.66-7.75 (2H, m), 7.91 (1H, d, *J* = 8 Hz), 8.25 (1H, s), 8.55 (1H, s), 9.40 (1H, s), 11.25 (1H, br s); LC-MS (ZQ, 7 min) R_t = 3.09 min; *m/z* (ESI+) 369 [MH^+]. Hi-Res MS (ESI) *m/z* calcd for $C_{18}H_{18}ClN_6O$ (M+H) 369.1225, found 369.1235.

3-(1-(Dimethylamino)propan-2-yloxy)-5-(isoquinolin-3-ylamino)pyrazine-2-carbonitrile (34) Title compound isolated as a pale yellow powder (5.4 mg, 8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (3H, d, *J* = 6 Hz), 2.21 (6H, s), 2.32-2.34 (1H, m), 2.63-2.67 (1H, m), 5.49-5.53 (1H, m), 7.54-7.58 (1H, m), 7.73-7.77 (1H, m), 7.84 (1H, d, *J* = 8 Hz), 8.09 (1H, d, *J* = 8 Hz), 8.23 (1H, s), 8.45 (1H, s), 9.22 (1H, s); LC-MS (ZQ, 7 min) R_t 2.93 min; *m/z* (ESI-) 347 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{19}H_{18}ClN_6O$ (M+H) 349.1771, found 349.1774.

5-(8-Chloroisoquinolin-3-ylamino)-3-(1-(dimethylamino)propan-2-yloxy)pyrazine-2-carbonitrile (*rac*-(3)) Title compound isolated as a pale yellow powder (1.8 mg, 2.6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (3H, d, *J* = 6 Hz), 2.20 (6H, s), 2.52-2.56 (1H, m), 2.63-2.68 (1H, m), 5.47-5.51 (1H, m), 7.66-7.68 (1H, m), 7.70-7.74 (1H, m), 7.84 (1H, d, *J* = 8 Hz), 8.25 (1H, s), 8.50 (1H, s), 9.40 (1H, s); LC-MS (ZQ, 7 min) R_t 3.28 min; *m/z* (ESI-) 381 & 383 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{19}H_{21}ClN_6O$ (M+H) 383.1382, found 383.1380.

(*S*)-5-(8-Chloroisoquinolin-3-ylamino)-3-(1-(dimethylamino)propan-2-yloxy)pyrazine-2-carbonitrile ((*S*)-3) Title compound isolated as a pale yellow powder (8.0 mg, 8%).^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (3H, d, *J* = 6 Hz), 2.20 (6H, s), 2.50-2.56 (1H, m, *partly obscured by DMSO*), 2.63-2.68 (1H, m), 5.47-5.52 (1H, m), 7.66-7.68 (1H, m), 7.71-7.75 (1H, m), 7.85 (1H, d, *J* = 8 Hz), 8.24 (1H, s), 8.50 (1H, s), 9.41 (1H, s); LC-MS (ZQ, 7 min) Rt = 3.28 min; *m/z* (ESI-) 381 & 383 [M-H]. Hi-Res MS (ESI) *m/z* calcd for $C_{19}H_{20}ClN_6O$ (M+H) 383.1382, found 383.1392.

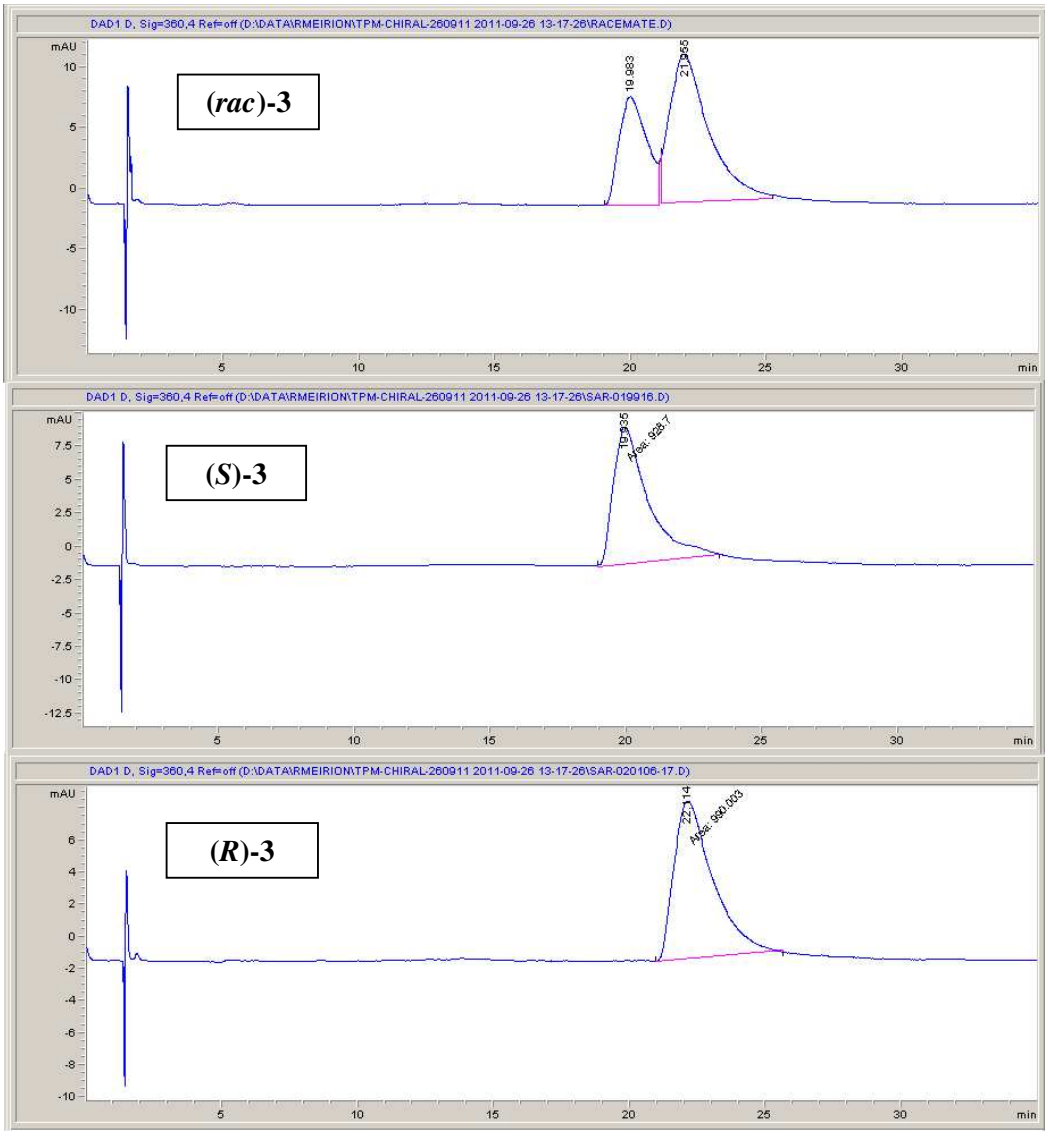
***tert*-Butyl 4-((6-bromo-1*H*-imidazo[4,5-*c*]pyridin-1-yl)methyl)piperidine-1-carboxylate (60)** The title compound was prepared using methods analogous to those described in synthesis of **62** replacing 4-methoxybenzylamine with tert-butyl

4-(aminomethyl)piperidine-1-carboxylate. LC-MS (ZQ, 4 min) Rt = 1.91 min; *m/z* (ESI+) 395 & 397 [MH^+].

2. Chiral HPLC details for compounds (*rac*)-3, (*S*)-3, (*R*)-3

LC-MS CHROMASOLV solvents or alternative eluent modifiers were purchased from Sigma Aldrich (Poole, UK) unless otherwise stated. 1.5µL standard injections of the sample were made onto an Agilent Ultron ES-OVM (150 x 4.6mm, Agilent Technologies, Santa Clara, USA). Solvents were degassed on a 1200 series degasser (Agilent, Santa Clara, USA). Chromatographic separation at room temperature was carried out using a 1200 Series HPLC (Agilent, Santa Clara, USA) over a 35 minute isocratic elution at 85% PBS / 15% Acetonitrile at a flow rate of 1.5mL/min (PBS = 6.85mM sodium chloride, 0.5mM phosphate buffer, 0.135mM potassium chloride). UV-Vis spectra were acquired at 360nm on a 1200 Series diode array detector (Agilent, Santa Clara, USA). Raw data was processed using Agilent Chemstation software (version B.03.01). Analysis of **(*S*)-3** and **(*R*)-3** indicated enantiomeric excess of greater than 95%.

Compound	Peak 1 (t_R) (min)	Peak 2 (t_R) (min)	ee
(*rac*)-3	19.98	21.96	N/A
(*S*)-3	19.94	----	> 95%
(*R*)-3	----	22.11	> 95%



3. Experimental details for CHK1 and CHK2 enzyme inhibition assays

Measurement of Inhibition of CHK1 Kinase

CHK1 kinase function was measured in a DELFIA® assay in order to monitor phosphorylation of a CDC25C peptide using a specific phospho antibody. The

enzyme reaction was carried out in polypropylene plates (Greiner) using a reaction mix (25 μL) containing full length CHK1 enzyme, prepared in house, and peptide mix (CHK1, 1nM; Biotin-KKKVSRSGLYRSPSMPENLNRPR, 1 μM or 15 μL), ATP (30 μM or 5 μL) and either DMSO (2.5%) or test compound (5 μL) diluted to a give a range of concentrations (from 0 to 100 μM in 2.5% DMSO, final concentrations) in assay buffer (40 mM Tris, 40 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT and 0.1% Tween 20). The reaction mixture was incubated for 30 min at r.t. and then stopped by the addition of buffer (125 μL) containing 40 mM EDTA, 0.05% Tween 20, 0.1% BSA in TBS (10x concentrate, Sigma). An aliquot (100 μL) of the stopped reaction mixture was transferred to a black neutravidin-coated plate (Perbio) and incubated for 1 h on a shaker (Titertek, Flow Laboratories) at r.t. The plates were washed four times with wash buffer (25 mM Tris (pH 8), 150 mM NaCl, and 0.1% Tween 20) (WellWash4, Thermo Life Sciences) and incubated for 1 h as before with an antibody mixture (100 μL) consisting of anti-phospho CDC25C (1.25 nM, Cell Signalling Technology-9528) and europium-labelled anti-rabbit IgG (0.3 μg/mL, AD0105, PerkinElmer Life Sciences) diluted in DELFIA assay buffer (PerkinElmer Life Sciences). The plates were washed a further four times with wash buffer before the addition of enhancement solution (100 μL/well, PerkinElmer Life Sciences). The plate was read on a $Victor_2$ 1420 multilabel counter (Perkin Elmer Life Sciences) using a time-resolved measurement mode reading fluorescence at 615 nm. The concentration of test compound required toinhibit enzyme activity by 50% was calculated (IC_{50}).

Measurement of Inhibition of CHK2 Kinase

CHK2 kinase activity was measured in a DELFIA® assay that monitors phosphorylation of a CDC25C peptide using a specific phospho antibody. The

enzyme reaction was carried out in 96-well polypropylene plates (Greiner). The reaction mix (total volume 25 μL) contained enzyme and peptide mix (15 μL) (containing full length CHK2, prepared in-house, 1 nM; Biotin-KKKVSRSGLYRSPSMPENLNRPR, 1 μM), ATP (30 μM, 5 μL) and either DMSO (2.5%) or test compound (5 μL) diluted to a give a range of concentrations (0-100 μM in 2.5% DMSO, final concentrations) in assay buffer (40 mM HEPES (pH7.4), 40 mM KCl, 2 mM $MgCl_2$, 10 mM DTT and 0.02% Tween 20). The reaction mixture was incubated for 30 min at r.t. and stopped by the addition of buffer (125 μL) containing 40 mM EDTA, 0.05% Tween 20, 0.1% BSA in TBS (10x concentrate, Sigma). An aliquot (100 μL) of the reaction mix was transferred to a black neutravidin-coated 96-well plate (Perbio) and incubated for 1 h on a shaker (Titertek, Flow Laboratories) at r.t. The plates were washed four times with wash buffer (25 mM Tris (pH 8), 150 mM NaCl and 0.1% Tween 20) (WellWash4, Thermo Life Sciences) and incubated for 1 h as before with antibody mix (100 μL) consisting of anti-phospho CDC25C (diluted 1/4000 equivalent to 0.35 nM-1.25 nM, #9528, Cell Signalling Technology) and europium-labelled anti-rabbit IgG, (0.3 μg/mL, AD0105, PerkinElmer Life Sciences) diluted in DELFIA assay buffer (PerkinElmer Life Sciences). The plates were washed a further four times with wash buffer before the addition of enhancement solution (100 μL/well, PerkinElmer Life Sciences). The plate was read on a $Victor_2$ 1420 multilabel counter (PerkinElmer Life Sciences) using a time-resolved measurement mode reading fluorescence at 615 nm. The concentration of test compound required to inhibit enzyme activity by 50% was calculated (IC_{50}).

4. Summary of X-ray crystal structure determinations of CHK1 in complex with 2, 4, 6, 8, 20, (*R*)-3

The crystallography described here was carried out as previously reported[2] with one difference: the search model for molecular replacement was PDB 2wmw (with waters and ligands removed from this structure). Supplementary Table 1 shows the data collection and refinement statistics for the structures described herein.

Supplementary Table 1. Data collection and refinement statistics

PDB Code	**2ym3**	**2ym4**	**2ym5**	**2ym6**	**2ym7**	**2ym8**
Compound	**2**	**4**	**6**	**8**	**20**	**(*R*)-3**
Spacegroup	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Lattice constants						
a (Å)	45.05	44.81	44.91	45.06	44.94	44.77
b (Å)	65.79	65.9	65.76	65.93	65.71	65.47
c (Å)	58.14	57.92	58.08	58.15	57.94	57.93
β (°)	94.25	94.18	93.89	93.96	94.14	95.38
Data collection						
Resolution range (Å)	43.50-2.01	44.69-2.35	43.50-2.03	44.95-2.01	36.73-1.81	15.31-2.07
(Highest resolution shell)	(2.12-2.01)	(2.48-2.35)	(2.14-2.03)	(2.11-2.01)	(1.91-1.81)	(2.18-2.07)
Unique reflections	21735 (3215)	12868 (1944)	19321 (3007)	22144 (3278)	29617 (4390)	18722 (2374)
Completeness (%)	95.7 (97.5)	91.3 (95.3)	88.3 (93.6)	97.0 (99.4)	96.7 (99.3)	91.9 (80.1)
Multiplicity	2.4 (2.3)	2.6 (2.5)	2.6 (2.3)	2.6 (2.4)	2.5 (2.3)	2.1 (2.1)
*R*merge (%)	6.6 (48.1)	10.8 (52.2)	9.8 (46.1)	6.9 (41.0)	4.9 (48.9)	7.7 (33.9)
I/σ(I)	8.7 (1.6)	6.1 (1.5)	5.3 (1.3)	8.8 (1.8)	12.2 (1.6)	8.0 (2.3)
Mean(I/σ(I))	9.6 (2.0)	6.2 (1.7)	5.4 (1.9)	8.6 (2.0)	12.4 (1.9)	7.8 (2.3)
Mosaicity (°)	0.700	1.010	0.780	0.740	0.570	1.040
Refinement						
Resolution range (Å)	24.54-2.01	36.99-2.35	36.7-2.03	37.14-2.01	34.24-1.81	15.31-2.07
No. of amino acids	247	251	248	253	248	246
No. of water molecules	181	82	204	183	247	121
No. of ethylene glycol molecules	5	3	1	5	4	9
No. of ligand molecules	1	1	1	1	2	1
*R*factor (%)	17.81	20.41	18.52	18.02	17.22	18.53
*R*free[a] (%)	20.27	24.55	21.60	22.08	20.27	22.59
R.m.s. deviations						
bond lengths (Å)	0.003	0.003	0.006	0.007	0.006	0.004
bond angles (°)	0.698	0.773	0.912	1.009	1.005	0.824
Ramachandran plot						
Favoured (%)	97.1	95.9	97.5	97.2	97.5	97.5
Generously allowed (%)	2.5	3.7	2.1	2.4	2.1	2.1
Forbidden (%)	0.4	0.4	0.4	0.4	0.4	0.4

[a] The Free *R* factor, *R*free, was computed using 5% of the data assigned randomly and is the same for all six structures. The wavelength used for data collection was 1.5418 Å.

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2. Matthews, T. P.; Klair, S.; Burns, S.; Boxall, K.; Cherry, M.; Fisher, M.; Westwood, I. M.; Walton, M. I.; McHardy, T.; Cheung, K.-M. J.; Van Montfort, R.; Williams, D.; Aherne, G. W.; Garrett, M. D.; Reader, J.; Collins, I. Identification of Inhibitors of Checkpoint Kinase 1 through Template Screening. *J. Med. Chem.* **2009**, 52, 4810-4819.

5. Kinase inhibitory profile of (*R*)-3

Kinase inhibitory profile of (*R*)-3 at 10 μM[a]

Kinase	Activity remaining[b]	Kinase	Activity remaining[b]
Abl(h)	101	KDR(h)	89
Aurora-A(h)	82	Lck(h)	57
BTK(h)	95	Lyn(h)	47
CaMKIIβ(h)	40	MAPK1(h)	77
CDK1/cyclinB(h)	77	MAPK2(h)	97
CDK2/cyclinA(h)	87	Met(h)	16
CHK1(h)	2	MINK(h)	112
CHK2(h)	33	MST2(h)	6
cKit(h)	103	mTOR(h)	104
cSRC(h)	89	p70S6K(h)	19
EGFR(h)	133	PDGFRα(h)	118
EphA3(h)	60	PDK1(h)	57
EphB4(h)	103	Pim-1(h)	37
ErbB4(h)	106	PKA(h)	100
FGFR1(h)	83	PKBα(h)	95
FGFR2(h)	84	PKBβ(h)	78
FGFR3(h)	88	PKBγ(h)	85
Flt1(h)	64	PKCα(h)	89
Flt3(h)	6	Ret(h)	2
Flt4(h)	31	ROCK-I(h)	86
GSK3β(h)	78	ROCK-II(h)	86
IR(h)	102	Rsk1(h)	-1
IRAK4(h)	13	SGK(h)	34
JAK2(h)	85	Tie2(h)	67
JAK3(h)	137	TrkA(h)	8

[a] Determined in a radiometric assay format.

[b] % enzyme activity remaining relative to control with no inhibitor. Determined at 10 μM concentration of **(*R*)-3** with [ATP] ~ $K_{m,ATP}$ for each kinase.

Kinase inhibitory profile of (*R*)-3 at 1 μM[a]

Kinase	Activity remaining[b]	Kinase	Activity remaining[b]
ABL	104	MKK1	66
AMPK	32	MKK2	59
ASK1	98 [c]	MKK6	149 [c]
Aurora A	106	MLK1	93
Aurora B	74	MLK3	80
BRK	104	MNK1	100
BRSK1	7	MNK2	99
BRSK2	16	MPSK1	93
BTK	93	MSK1	51
CAMK1	101	MST2	30
CAMKKb	97	MST4	62
CDK2-Cyclin A	105	NEK2a	87
CHK1	5	NEK6	80
CHK2	61	NUAK1	20
CK1	61	OSR1	107
CK2	77	p38a MAPK	94
CLK2	70	p38b MAPK	95
CSK	87	p38d MAPK	90
DAPK1	83	p38g MAPK	99
DYRK1A	90	PAK2	83
DYRK2	101	PAK4	75
DYRK3	85	PAK5	85
EF2K	81	PAK6	96
EIF2AK3	92	PDK1	91
EPH-A2	74	PHK	73
EPH-A4	67	PIM1	85
EPH-B1	114	PIM2	95
EPH-B2	103	PIM3	63
EPH-B3	83	PKA	96
EPH-B4	96	PKBa	106
ERK1	83	PKBb	87
ERK2	96	PKCa	108
ERK8	88	PKCz	115
FGF-R1	94	PKCγ	95
GCK	80	PKD1	79
GSK3b	90	PLK1	102
HER4	94	PRAK	98
HIPK1	103	PRK2	84
HIPK2	99	RIPK2	94
HIPK3	83	ROCK 2	103
IGF-1R	89	RSK1	6
IKKb	91	RSK2	18
IKKe	80	S6K1	57
IR	97	SGK1	75
IRAK1	10	SmMLCK	94
IRAK4	52	Src	107
IRR	87	SRPK1	92
JAK2	92	STK33	53 [c]
JNK1	98	SYK	75
JNK2	98	TAK1	70
JNK3	100	TAO1	85
Lck	80	TBK1	80
LKB1	89	TESK1	101

MAPKAP-K2	121	TIE2	83
MAPKAP-K3	93	TLK1	104
MARK1	95	TrkA	38
MARK2	91	TSSK1	93
MARK3	92	TTBK1	102
MARK4	91	TTK	78
MEKK1	101	VEG-FR	80
MELK	76	YES1	101
MINK1	69	ZAP70	114

[a] Determined in a radiometric assay format.

[b] % enzyme activity remaining relative to control with no inhibitor. Determined at 1 μM concentration of **(*R*)-3** with [ATP] ~ $K_{m,ATP}$ for each kinase unless stated.

[c] The calculated K_m in these assays results in a poor signal to noise ratio and so is assayed above $K_{m\ ATP}$