Supporting Information

"Structure and property based design of pyrazolo[1,5-a]pyrimidine inhibitors of CK2 kinase with activity in vivo."

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Compound characterization data and representative synthetic procedures:

5-(1-Acetylindolin-6-ylamino)-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.66 - 0.73 (m, 2 H), 0.76 - 0.84 (m, 2 H), 2.17 (s, 3 H), 2.54 - 2.57 (m, 1 H), 3.10 (t, 2 H), 4.11 (t, 2 H), 6.04 (s, 1 H), 7.18 (d, 1 H), 7.84 (d, 1 H), 8.07 (s, 1 H), 8.19 (s, 1 H), 8.34 (s, 1 H), 9.65 (s, 1 H); m/z 374.

N-[2-Chloro-5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]phenyl]acetamide (7a). ^{1}H NMR (400 MHz, DMSO-d_6); δ 0.67 (m, 2 H), 0.83 (m, 2 H), 2.07 (s, 3 H), 2.59 (m, 1 H), 6.02 (s, 1 H), 7.42 (d, 1 H), 7.83 (m, 1 H), 7.96 (d, 1 H), 8.26 (s, 1 H), 8.40 (s, 1 H), 9.48 (s, 1 H), 9.85 (s, 1H); m/z 382.

N-(5-(3-Cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-methylphenyl)acetamide (7b). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.67 - 0.76 (m, 2 H), 0.76 - 0.84 (m, 2 H), 2.07 (s, 3 H), 2.17 (s, 3 H), 2.56 - 2.63 (m, 1 H), 6.01 (s, 1 H), 7.16 (d, 1 H), 7.65 (s, 2 H), 8.22 (s, 1 H), 8.35 (s, 1 H), 9.26 (s, 1 H), 9.61 (s, 1 H); m/z 362.

N-[5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-isopropyl-phenyl]acetamide (7c). A mixture of N-(5-amino-2-isopropylphenyl)acetamide (132 mg, 0.68 mmol), potassium fluoride (20 mg, 0.34 mmol), 5-chloro-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (80 mg, 0.34 mmol) and NMP (0.2 mL) was heated at 140 °C for 7h under Ar. The mixture was allowed to cool to 25 °C and water (10 mL) was added. The resulting brown solid was collected, washed with 10% MeOH in CH_2Cl_2 (3 mL) followed by MeOH (2 mL) to give the title compound (69 mg, 52%) as an off white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.63 - 0.76 (m, 2 H), 0.76 - 0.87 (m, 2 H), 1.10 - 1.19 (m, 6 H), 2.01 - 2.09 (m, 3 H), 2.60 (br. s., 1 H), 3.04 - 3.16 (m, 1H), 6.01 (s, 1 H), 7.26 (d, 1 H), 7.53 (s, 1 H), 7.72 (d, 1 H), 8.18 (s, 1 H), 8.34 (s, 1 H), 9.31 (s, 1 H), 9.60 (s, 1 H); m/z 390.

N-[5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-cyclopropyl-phenyl]acetamide (7d). A suspension of N-(5-amino-2-cyclopropylphenyl)acetamide (0.078 g, 0.41 mmol), potassium fluoride (0.020 g, 0.34 mmol), and 5-chloro-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (0.08 g, 0.34 mmol) in NMP (0.1 mL) was heated at 140 °C for 16 h. The mixture was allowed to cool to 25 °C, diluted with water (5 mL) and the resulting precipitate was collected. The solid was dissolved in 10% MeOH in CH_2Cl_2 (20 mL) and the resulting solution was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was suspended in EtOAc (1 mL), heated, collected by filtration, washed with MeOH (1 mL) and dried to give the title compound as an off-white solid (0.030 g, 22 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.54 - 0.63 (m, 2 H), 0.67 - 0.77 (m, 2 H), 0.77 - 0.86 (m, 2 H), 0.87- 0.93 (m, 2 H), 1.94 (d, 1 H), 2.10 (s, 3 H), 2.60 (d, 1 H), 6.02 (s, 1 H), 6.92 (d, 1 H), 7.68 (s, 2 H), 8.19 (s, 1 H), 8.34 (s, 1 H), 9.31 (br. s., 1 H), 9.62 (s, 1 H); m/z 388.

N-(5-(3-Cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-4-fluoro-2-methylphenyl)acetamide (7e). ^{1}H NMR (400 MHz, DMSO-d_6); δ 0.67-0.71 (m, 2 H) 0.76-0.79 (m, 2 H) 2.04 (s, 3 H) 2.18 (s, 3 H) 2.53 - 2.64 (m, 1 H) 6.03 (s, 1 H) 7.15 (d, 1 H) 7.87 (d, 1 H) 8.23 (s, 1 H) 8.33 (s, 1 H) 9.28 (s, 1 H) 9.34 (s, 1 H); m/z 380.

N-[5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-cyclopropyl-phenyl]acetamide (7f).

A mixture of 5-chloro-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (130 mg, 0.56 mmol), N-(5-amino-2-cyclopropyl-4-fluorophenyl)-N-methylacetamide (124 mg, 0.56 mmol), $Pd_2(dba)_3$ (26 mg, 0.03 mmol), 9,9-dimethyl-9H-xanthene-4,5-diyl)bis(diphenylphosphine) (32.2 mg, 0.06 mmol) and cesium carbonate (363 mg, 1.11 mmol) in DMA (0.5 mL) was heated at 150 °C under microwave irradiation for 30 min. The mixture was allowed to cool to 25 °C, diluted with MeOH (1 mL) and filtered. The filtrate was concentrated under reduced pressure to afford a residue which was chromatographed on silica. Further chromatography using reversed-phase HPLC afforded the title compound (20 mg, 9 %) as an off white solid. ^{1}H NMR (300 MHz, MeOD) δ 0.61 - 0.70 (m, 2 H), 0.70 - 0.78 (m, 2 H), 0.87 - 0.95 (m, 2 H), 0.95 - 1.04 (m, 2 H), 1.91 - 2.02 (m, 1 H), 2.20 (s, 3 H), 2.64 - 2.70 (m, 1 H), 6.11 (s, 1 H), 6.84 (d, 1 H), 8.11 (s,1 H), 8.23 (d, 1 H); m/z 406.

N-(5-(3-Cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-methylpyridin-3-yl)acetamide (7g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.73 (d, 2 H), 0.83 (d, 2 H), 2.11 (s, 3 H), 2.38 (s, 3 H), 2.57 (br. s., 1H) 6.01 (s, 1 H), 8.13 (s, 1 H), 8.29 (s, 1 H), 8.37 (s, 1 H), 8.88 (br. s., 1 H), 9.44 (s, 1 H), 9.76 (s, 1 H); m/z 363.

N-[2-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-5-methyl-4-pyridyl]acetamide (7h). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.73 (d, 2 H), 0.85 (d, 2 H), 2.13 (s, 3H), 2.18 (s, 3H), 2.62 (br. s., 1 H), 7.36 (s, 1 H), 7.98 (s, 1 H), 8.09 (s, 1 H), 8.35 (s, 1 H), 8.38 (s, 1 H), 9.38 (s, 1 H), 10.11 (s, 1 H); m/z 363.

N-[5-[[3-cyano-7-(cyclobutylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-methyl-phenyl]acetamide (7i). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.73-1.77 (m, 2H), 2.08 (s, 3 H), 2.16-2.20 (m, 5 H), 2.35 (br, s., 2H), 4.01-4.03 (m, 1 H), 5.66 (s, 1 H), 7.17 (s, 1 H), 7.62 (s, 2 H), 8.06 (s, 1 H), 8.36 (s, 1 H), 9.25 (br. s., 1 H), 9.47 (s, 1 H); m/z 376.

N-[5-[[3-cyano-7-(cyclobutylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-4-fluoro-2-methyl-phenyl]acetamide (7j). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.74 (br s, 2 H), 2.04 (s, 3 H), 2.17-2.22 (m, 5H), 2.35 (br s, 2H), 4.00-4.03 (m, 1 H), 5.71 (br s, 1H), 7.17 (s, 1 H), 7.90 (s, 1 H), 8.12 (s, 1 H), 8.36 (s, 1 H), 9.16 (s, 1 H), 9.35 (s, 1 H); m/z 394.

N-(5-(3-Cyano-7-(1-methyl-1H-pyrazol-3-ylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-methylphenyl)acetamide (7k). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.07 (s, 3 H), 2.17 (s, 3 H), 3.85 (s, 3 H), 6.23 (d, 1 H), 7.04 (s, 1 H), 7.17 (d, 1 H), 7.66-7.68 (m, 3 H), 8.45 (s, 1 H), 9.28 (s, 1 H), 9.74 (s, 1 H), 10.12 (s, 1H); m/z 402.

N-(5-(3-Cyano-7-(1-methyl-1H-imidazol-4-ylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-methylphenyl)acetamide (7l). To a microwave vial containing N-(5-amino-2-methylphenyl)acetamide (0.096 g, 0.58 mmol), 5-chloro-7-(1-methyl-1H-imidazol-4-ylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (0.08 g, 0.29 mmol), sodium tert-butoxide (0.093 g, 0.96 mmol) and 2-dicyclohexylphosphino-2',4',6'-tri-iso-propyl-1,1'-biphenyl (0.014 g, 0.03 mmol) and $Pd_2(dba)_3$ (0.013 g, 0.01 mmol) was added DMA (1.8 ml). The vial was flushed with argon, and the mixture was heated at 100 °C under microwave irradiation 50 min. The reaction mixture was allowed to cool to 25 °C and filtered through Celite. The filtrate was diluted with EtOAc (5 mL), washed with brine (5 mL), dried over Na2SO4, filtered and concentrated under reduced pressure to give an oil which was chromatographed on silica to give the title compound (0.015 g, 13%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.04 (s, 3H), 2.13 (s, 3H), 3.66 (s, 3H), 6.62 (s, 1H), 7.04 (s, 1H), 7.14 (d, 1H), 7.59 (d, 1H), 7.68 (d, 1H), 8.41 (s, 1H), 9.25 (s, 1H), 9.63 (s, 1H), 9.78 (s, 1H); m/z 402.

5-Chloro-7-(1-(2-hydroxyethyl)-1H-pyrazol-3-ylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (9a). A mixture of 5,7-dichloropyrazolo[1,5-a]pyrimidine-3-carbonitrile (213 mg, 1.0 mmol), N-ethyl-N-isopropylpropan-2-amine (0.52 mL, 3.0 mmol) 2-(3-amino-1H-pyrazol-1-yl)ethanol (127 mg, 1.0 mmol) in ethanol (20 mL) was stirred at 25 °C overnight. The mixture was concentrated under reduced pressure and diluted with water. The resulting precipitate was collected, washed with water and dried to give the title compound (211 mg, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.77 (t, 2 H), 4.17 (t, 2 H), 4.93 (br s, 1 H), 6.31 (s, 1 H), 7.59 (s, 1 H), 7.74 (s, 1H), 8.79 (s, 1 H), 11.19 (s, 1 H); m/z 304.

5-chloro-7-(1-(2-hydroxyethyl)-1H-imidazol-4-ylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (9b). A solution of 2-(4-nitro-1H-imidazol-1-yl)ethanol (797 mg, 5.07 mmol) in MeOH (100 mL) was hydrogenated (Pd/C) under H_2 (1 atm) at 50°C. The mixture was filtered and concentrated under vacuum to give a dark oil that was dissolved in EtOH (20 mL). To this solution was added 5,7-dichloropyrazolo[1,5-a]pyrimidine-3-

carbonitrile (900 mg, 4.22 mmol) and TEA (1.76 mL, 12.6 mmol) and the mixture was stirred at 25°C overnight. The resulting grey solid was collected, washed with water and dried to afford the title compound (770 mg, 60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.68 (q, 2 H), 4.04 (t, 2 H), 5.00 (t, 1 H), 7.19 (d, 1 H), 7.42 (s, 1 H), 7.66 (s, 1 H), 8.77 (s, 1 H), 10.99 (s, 1 H); m/z 304.

N-(5-(3-cyano-7-(1-(2-hydroxyethyl)-1H-pyrazol-3-ylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-methylphenyl)acetamide (10a). A mixture of 5-chloro-7-(1-(2-hydroxyethyl)-1H-pyrazol-3-ylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (211 mg, 0.69 mmol) and N-(5-amino-2-methylphenyl)acetamide (228 mg, 1.39 mmol) in NMP (1 mL) was heated at 140°C for 5h. The solvent was removed under reduced pressure and the residual material was chromatographed on silica to give the title compound (148 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (s, 3 H), 2.17 (s, 3 H), 3.78 (q, 2 H), 3.98-4.17 (m, 2 H), 4.89 (t, 1 H), 6.21 (d, 1 H), 7.06 (s, 1 H), 7.16 (d, 1 H), 7.59 - 7.72 (m, 3 H), 8.43 (s, 1 H), 9.25 (s, 1 H), 9.70 (s, 1 H), 10.11 (s, 1 H); m/z 432.

N-[5-[[3-cyano-7-[[1-(2-hydroxyethyl)imidazol-4-yl]amino]pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-methylphenyl]acetamide (10b). A mixture of 5-chloro-7-(1-(2-hydroxyethyl)-1H-imidazol-4-ylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (1.22 g, 4.02 mmol) and N-(5-amino-2-methylphenyl)acetamide (1.32 g, 8.03 mmol) in NMP (9 mL) was heated at 140 °C for 5 hr. The mixture was allowed to cool to 25°C, concentrated to dryness and chromatographed on silica to give the title compound (485 mg, 28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (s, 3 H), 2.16 (s, 3 H), 3.65 (q, 2H), 4.00 (q, 2H), 4.95 (q, 1H), 6.67 (s, 1 H), 7.11 (s, 1 H), 7.14 (d, 1 H), 7.61 (s, 1 H), 7.64 (s, 1 H), 7.70 (d, 1H), 8.42 (s, 1 H), 9.26 (s, 1 H), 9.64 (s, 1 H), 9.77 (s, 1 H); m/z. 432.

N-(5-(3-cyano-7-(1-(2-(dimethylamino)ethyl)-1H-pyrazol-3-ylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-methylphenyl)acetamide (11a). A mixture of 2-(3-(5-(3-acetamido-4-methylphenylamino)-3-cyanopyrazolo[1,5-a]pyrimidin-7-ylamino)-1H-pyrazol-1-yl)ethyl 4-methylbenzenesulfonate (46.1 mg, 0.07 mmol) and dimethylamine (63.1 mg, 1.40 mmol, 2M in THF, 2 ml) was stirred at 25°C for 4h. The solvent was removed in vacuo and the crude material was purified by reversed phase semi-preparative HPLC (acetonitrile/water/0.1% TFA) to afford the desired material (22 mg, 59%); m/z 459. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.08 (s, 3 H), 2.18 (s, 3 H), 2.77 (d, 6 H), 3.63 (d, 2 H), 4.51 (t, 2 H), 6.32 (d, 1 H), 7.13 - 7.31 (m, 2 H), 7.64 (s, 2 H), 7.81 (d, 1 H), 8.45 (s, 1 H), 9.31 (s, 1 H), 9.82 (br s, 2 H), 10.26 (s, 1 H).

The following examples were prepared using the method above:

N-[5-[[3-cyano-7-[[1-(2-pyrrolidin-1-ylethyl)pyrazol-3-yl]amino]pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-methyl-phenyl]acetamide (11b). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.73 - 1.90 (m, 2 H), 2.01 (br s, 2 H), 2.08 (s, 3 H), 2.19 (s, 3 H), 2.99 (d, 2 H), 3.49 (br s, 2 H), 3.68 (d, 2 H), 4.46 (t, 2 H), 6.35 (d, 1 H), 7.13 (s, 1 H), 7.21 (d, 1 H), 7.62 (s, 2 H), 7.80 (d, 1 H), 8.47 (s, 1 H), 9.29 (s, 1 H), 9.68 (s, 1 H), 10.31 (s, 1 H); m/z 485.

N-[5-[[3-cyano-7-[[1-(2-morpholinoethyl)pyrazol-3-yl]amino]pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-methyl-phenyl]acetamide (11c). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.08 (s, 3 H), 2.19 (s, 3 H), 3.00 (br s, 2H), 3.63 (br s, 4 H), 3.97 (br s, 2 H), 4.51 (br s, 2 H), 6.34 (s, 1 H), 7.09 (s, 1 H), 7.20 (d, 1 H), 7.63 (s, 2 H), 7.80 (d, 1 H), 8.47 (s, 1 H), 9.28 (s, 1 H), 9.66 (s, 1 H), 10.31 (br s, 1 H); m/z 501.

N-[5-[[3-cyano-7-[[1-(2-dimethylaminoethyl)imidazol-4-yl]amino]pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-methyl-phenyl]acetamide (11d). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (s, 3 H), 2.16 (s, 3 H), 2.19 (s, 6 H), 2.58 (t, 2 H), 4.07 (t, 2 H) , 6.61 (s, 1 H), 7.11-7.17 (m, 2 H), 7.62-7.65 (m, 2 H), 7.69 (d, 1 H), 8.42 (s, 1 H), 9.26 (s, 1 H), 9.63 (s, 1 H); m/z 459.

N-[5-[[3-cyano-7-[[1-(2-pyrrolidin-1-ylethyl)imidazol-4-yl]amino]pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-methyl-phenyl]acetamide (11e). A solution of 2-(4-(5-(3-acetamido-4-methylphenylamino)-3-cyanopyrazolo[1,5-a]pyrimidin-7-ylamino)-1H-imidazol-1-yl)ethyl 4-methylbenzenesulfonate (50 mg, 0.09 mmol) and pyrrolidine (0.140 mL, 1.71 mmol) in acetonitrile (1 mL) and NMP (0.5 mL) was stirred at 25 °C overnight. The precipitate was collected, washed with CH_2Cl_2 and dried to afford the title compound (33 mg, 80% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.68-1.69 (m, 4 H), 1.86-1.95 (m, 2 H), 2.06 (s, 3 H), 2.16 (s, 3H), 2.76 (t, 2 H), 4.09 (t, 2 H), 6.63 (s, 1 H), 7.11-7.17 (m, 2 H), 7.62-7.66 (m, 2 H), 7.69 (d, 1 H), 8.42 (s, 1 H), 9.25 (s, 1 H), 9.63 (s, 2 H); m/z 485.

N-[5-[[3-cyano-7-[[1-(2-morpholinoethyl)imidazol-4-yl]amino]pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-methyl-phenyl]acetamide (11f). A solution of 2-(4-(5-(3-acetamido-4-methylphenylamino)-3-cyanopyrazolo[1,5-a]pyrimidin-7-ylamino)-1H-imidazol-1-yl)ethyl 4-methylbenzenesulfonate (50 mg, 0.09 mmol) and morpholine (0.148 mL, 1.71 mmol) in acetonitrile (1 mL) and NMP (0.5 mL) was stirred at 25 °C overnight. The mixture was concentrated under reduced pressure and chromatographed on silica to afford the title compound (27 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (s, 3 H), 2.16 (s, 3 H), 2.39-2.48 (m, 4 H), 2.64 (t, 2 H), 3.54-3.61 (m, 4 H), 4.10 (t, 2 H), 6.66 (s, 1 H), 7.10-7.18 (m, 2 H), 7.61-7.66 (m, 2 H), 7.68 (br. s., 1 H), 8.42 (s, 1 H), 9.25 (s, 1 H) , 9.63 (s, 1 H), 9.77 (br. s., 1 H); m/z 501.

N-(5-(3-cyano-7-(oxetan-3-ylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-chlorophenyl) acetamide (12a). A mixture of 5-chloro-7-(oxetan-3-ylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (100 mg, 0.40 mmol), N-(5-amino-2-chlorophenyl)acetamide (81 mg, 0.44 mmol), $Pd_2(dba)_3$ (18 mg, 0.02 mmol), Xantphos (23 mg, 0.04 mmol) and Cs_2CO_3 (144 mg, 0.44 mmol) in DMA (2 mL) was heated at 150 °C under microwave irradiation for 60 minutes. The reaction mixture was allowed to cool to 25 °C, filtered through Celite and concentrated under reduced pressure. The residue was purified by reversed-phase HPLC to give the title compound (7 mg, 4 %) as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.11 (s, 3 H), 4.68 - 4.93 (m, 4 H), 5.53 (s, 1 H), 7.44 (d, 1H), 7.95 (d, 1 H), 8.43 (s, 1 H), 9.47 (s, 1 H), 9.77 (s, 1 H); m/z 398.

The following examples were prepared using the method above:

N-(5-(3-cyano-7-(oxetan-3-ylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-methylphenyl) acetamide (12b). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.07 (s, 3 H), 2.17 (s, 3 H), 4.63-4.92 (m, 4 H), 5.49 (s, 1 H), 7.15 (d, 1 H), 7.48-7.71 (m, 2 H), 8.39 (s, 1 H), 8.56 (d, 1 H), 9.24 (s, 1 H), 9.52 (s, 1 H); m/z 378.

N-[5-[[3-cyano-7-(oxetan-3-ylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-cyclopropyl-phenyl]acetamide (12c). A microwave vial containing a mixture of N-(5-amino-2-cyclopropylphenyl)acetamide (0.152 g, 0.80 mmol), $Pd_2(dba)_3$ (0.037 g, 0.04 mmol), 5-chloro-7-(oxetan-3-ylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (0.20 g, 0.80 mmol), di-*tert*-butyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine (0.034 g, 0.08 mmol) and Cs_2CO_3 (0.52 g, 1.60 mmol) in anhydrous DMA (1.0 mL) was purged with N_2 and capped. The resulting mixture was heated at 145 °C under microwave irradiation for 30 minutes, allowed to cool to 25 °C and filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica to give a solid which was washed with warm EtOAc to afford the title compound (0.028 g, 9 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.58-0.59 (m, 2H), 0.89-0.91 (m, 2H), 2.11 (s, 3 H) 4.75 - 4.82 (m, 4 H) 5.50 (s, 1 H) 6.92 (d, 1 H) 7.63-7.65 (m, 2 H) 8.40 (s, 1 H), 8.57 (s, 1H), 9.32 (br s, 1 H) 9.54 (s, 1 H); m/z 404.

N-[5-[[3-cyano-7-(oxetan-3-ylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-4-fluoro-2-methyl-phenyl]acetamide (12d). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.03 (s, 3 H) 2.18 (s, 3 H) 4.64 - 4.90 (m, 4 H) 5.55 (s, 1 H) 7.15 (d, 1 H) 7.88 (d, 1 H) 8.39 (s, 1 H) 9.22 (br s, 1 H) 9.35 (s, 1 H); m/z 396.

N-[5-[[3-cyano-7-(oxetan-3-ylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-cyclopropyl-4-fluoro-phenyl]acetamide (12e). A microwave vial containing a mixture of N-(5-amino-2-cyclopropyl-4-fluorophenyl)acetamide (100 mg, 0.48 mmol), 5-chloro-7-(oxetan-3-ylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (132 mg, 0.53 mmol), Cs_2CO_3 (469 mg, 1.44 mmol), $Pd_2(dba)_3$ (44 mg, 0.05 mmol) and di-*tert*-butyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine (41 mg, 0.10 mmol) in 1,4-dioxane (4 mL) was purged with N_2 and capped. The resulting mixture was heated at 145 °C under microwave irradiation for 30 minutes. The mixture was allowed to cool to 25 °C, concentrated under reduced pressure and the residue was chromatographed on silica to give the title compound (48 mg, 24%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.60-0.67 (m, 2 H), 0.89-0.93 (m, 2 H), 1.93-2.02 (m, 1 H), 2.05 (s, 3 H), 4.73-4.80 (m, 4 H), 5.56 (s, 1 H), 6.81 (d, 1 H), 7.92 (d, 1 H), 8.39 (s, 1 H), 8.61 (br. s., 1 H), 9.20 (s, 1 H), 9.45 (s, 1 H); m/z 422.

Definition of terms:
PI3K: Phosphatidylinositol 3-kinase; AKT: Protein Kinase B; NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells; Wnt: a hybrid of Int and Wg (wingless) in *Drosophila*; CYP: Cytochrome p450 isoenzyme; hERG; human *Ether-à-go-go* Related Gene; MDR: permeability glycoprotein, abbreviated as P-gp or Pgp; dba; dibenzylidene acetone, DMA; dimethylacetamide, DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene, GSK-3β: glycogen synthase kinase 3 beta, HipK2: homeodomain-interacting protein kinase 2; CDK2: Cyclin dependent kinase 2.

Biological Assays

CK2 in vitro mobility shift assay:

Activity of N-terminal 6 X His-tagged recombinant human full length Casein Kinase 2 alpha subunit (CK2) was determined in-vitro using a Caliper LabChip^{TM} mobility shift assay on a Caliper LC3000 reader (Caliper Life Sciences Inc, Hopkinton, MA) which measures the fluorescence of phosphorylated and unphosphorylated "CK2tide" (BODIPY-FL-RRRDDDSDDD-CONH2, Intonation Technologies Inc., Boston, MA) and calculates a ratiometric (i.e., ratio of product formed to total product and substrate remaining) value to determine percent turnover. CK2 (48.7kDa, Cat. # 14-445, Upstate/Millipore, Billerica, MA) was expressed in Sf21 insect cells with typical yield >70% purity. Phosphorylation of the CK2tide in the presence and absence of compound of interest was determined. 5 µl of enzyme/substrate/adenosine triphosphate (ATP) mix consisting of 10.5 nM CK2, 3.6 µM CK2tide, 180mM NaCl and 62.4 µM ATP in 1.2x buffer was pre-incubated with 2 µl of compound for 20 minutes at 25 °C. Reactions were initiated with 5 µl of Metal mix consisting of 24 mM $MgCl_2$ in 1.2x buffer and incubated at 25 °C for 90 minutes. Reactions were stopped by the addition of 5 µl of termination buffer consisting of 100 mM HEPES, 121 mM ethylenediamine tetraacetic acid, 0.8% Coatin Reagent 3 (Caliper, MA), and 0.01% Tween. Phosphorylated and unphosphorylated substrate was detected by a Caliper LC3000 reader (Caliper, MA) in the presence of separation buffer consisting of 100 mM HEPES, 16 mM ethylenediamine tetraacetic acid, 0.1% Coatin Reagent 3 (Caliper, MA), 0.015% Brij-35, 5% DMSO, and 5.6 mM $MgCl_2$. The separation conditions used by the Caliper LC3000 were -1.7 PSI, -500 V upstream voltage, -2000 V downstream voltage, 0.2 second sample duration sampling (sip), 55 second post duration sampling (sip), 10% laser strength. The values for percent inhibition of CK2 enzyme activity were plotted as a function of the compound concentration and the IC_{50} values were determined.

Western Blotting

HCT-116 and DLD-1 HA-myr-AKT1 human colon cancer cells, were used to measure pAKT^{S129} and total AKT. HCT-116 and DLD-1 AKT1 cells were plated in RPMI/DMEM respectively, supplemented with 10% FBS, 1% L-Glu and allowed to adhere for 16-24hrs at 37 °C in 6 well plates. CK2 inhibitor compounds were screened using a concentration response in cells for 3 and 24hrs at 37 °C, 5% CO_2. After incubation, protein lysates were made for Western blotting. Cells were washed with PBS, lysed with lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate,1 mM Na_3VO_4,1 µg/ml leupeptin and complete protease inhibitor cocktail [Roche]), scraped, transferred to a centrifuge tube, placed on ice for 20 minutes, and gently vortexed to completely lyse the cells. The lysate was clarified by centrifugation and the supernatent protein quantified by using as standard BCA assay protocol (Pierce), and stored at -80 °C. Western blots of the lysates were used to detect pAKT^{S129} using an in-house engineered rabbit polycolonal antibody and Total AKT antibody from Cell Signaling Technology (Cat# 9272). Total protein (20-40 ug) was separated on a 10% Bis-Tris Novex gel and transferred to a nitrocellulous membrane. Membranes were blocked in 10% nonfat milk in PBS-T (PBS-Tween20 0.1%) and then probed with primary antibody diluted in 5% nonfat milk in PBS-T overnight at 4 degrees C with shaking, the blots were washed in PBS-T, and incubated with Horseradish peroxidase (HRP)-tagged secondary antibody diluted in 5% nonfat milk in PBS-T for 1 hour at room temperature. Proteins were detected with ECL reagent (Pierce SuperSignal Dura ECL).

HCT-116 72hr Alamar Blue cell proliferation assay.

This fluorometric assay determines the effect CK2 inhibitors have on cancer cell growth. The Alamar Blue assay incorporates a fluorometric growth indicator based on detection of metabolic activity. Specifically, the system incorporates an oxidation-reduction (REDOX) indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. A decrease in fluorescence is indicative of cell death. GI_{50}s (Growth Inhibition Conc. 50%) are calculated for each compound using a HCT-116 cells on Day 0 fluorescence read as the Min and Day 3 DMSO vehicle control fluorescence as Max. Percent Net Growth for each compound concentration is calculated and the GI_{50} reported. HCT-116 cells were seeded at 2500 cells/well in Costar Flat bottomed 96 well plates (Black wall/clear bottom) in 90 µL of phenol-red free RPMI 1640 supplemented with 10% FBS / 1% L-Glu and incubated overnight in 37 °C, 5% CO_2. Compound plates were then treated with 10 µL of 10X compound (9 pt dose response, triplicates across plates) and incubated for 72hrs at 37 °C, 5% CO_2. The Alamar Blue assay was them performed by adding 10 µL of reagent to the compound treated cell plates and incubating for 4hrs at 37 °C, 5% CO_2. Fluorescence was measured at 535 nm (excitation) and 590 nm (emission) using a Tecan Ultra plate reader. Percent Net Growth relative to a Day 0 cell only Alamar Blue plate read was calculated for the 72 hr assay plates and the GI_{50} calculated.

Aqueous Solubility
A known amount of sample is incubated in a 0.1 M phosphate buffer (500 µL, pH 7.4) with stirring for 24 hours at 25°C. The sample is then centrifuged at 3800 rpm for 30 min and analyzed by HPLC/UV and LC/MS/MS. The peak area of the sample is determined and quantitated against that of the standard (test compound dissolved in DMSO). The solubility of the sample is calculated according to the following equation: Solubility= [(peak area of sample)/(peak area of standard)]x100. The upper limit for the measurement is approximately 1000 µM.

Lipophilicity, octanol (pH 7.4)
Measurement of octanol-water partition coefficient (Log *D*) is based on the shake-flask principle. Test compound is added to octanol (435 µL), stirred for 5 min, diluted with 10 mM sodium phosphate buffer (pH adjusted to 7.4) and mixed through inversion for 5 h at 25 ºC. The mixture is centrifuged for 30 min at 3000 RPM and the octanol layer is separated. The concentration of test compound in the octanol and aqueous layers is determined by measuring the integrated sample peak area using HPLC/UV and LCMS/MS and logD calculated using the equation: log D = log [(concentration in octanol/volume of octanol)/(concentration in buffer/volume of buffer)].

Plasma Protein Binding
Protein binding is determined using rapid equilibrium dialysis to separate free from bound compound. The amount of compound in plasma (10 µM initial concentration) and in dialysis buffer (pH 7.4 phosphate buffer) is measured by LC-MS/MS after equilibration at 37°C in a dialysis chamber. Free fraction (Fu) is calculated for each sample from the equation Fu = [free] / {[free]+[bound]} = [rcvr] / [donor]. Percent Protein Binding for each sample is calculated from the Free fraction (Fu) from the equation: %Bound = 100 * (1-Fu).

hERG binding assay
As described in: Bridgland-Taylor, M.H.; Hargreaves, A.C.; Easter, A.; Orme, A.; Henthorn, D.C.; Ding, M.; Davis, A.M.; Small, B.G.; Heapy, C.G.; Abi-Gerges, N.; Persson, F.; Jacobson, I.; Sullivan, M.; Albertson, N.; Hammond, T.G.; Sullivan, E.; Valentin, J.-P.; Pollard, C.E. "Optimisation and validation of a medium-throughput electrophysiology-based hERG assay using IonWorks™ HT" *Journal of Pharmacological and Toxicological Methods*, Volume 54, **2006**, Pages 189-199, http://dx.doi.org/10.1016/j.vascn.2006.02.003.

Protein Crystallization
Recombinant human CK2 was expressed and purified from *E. coli* according to literature methods.[1] Apo crystals were grown by vapor diffusion at room temperature in 4 µL hanging drops containing equal parts of protein and reservoir solution. Optimized reservoirs contained a range of 22-26% PEG 6K, 200 mM ammonium sulfate, and 100 mM MES, pH 6.5. Compound was solubilized in DMSO to 100 mM stock and then diluted 1:20 into reservoir solution. Apo crystals were soaked overnight in this 5 mM solution, equilibrated over the same reservoir supplemented with 5% DMSO. The soaked crystals were frozen in a cryo solution containing 8 µL reservoir, 2 µL of ethylene glycol, and 0.5 µL of the compound stock solution.

Structure Solution and Refinement
Diffraction data were obtained in house from an FRE+ generator outfitted with a CCD detector. Data were integrated and scaled using d*Trek software.[2] The structure was determined by molecular replacement using AMoRe and refined using Refmac[3] with rebuilding in Coot (Emsley P, et al. 2010).[4] Waters were added to the structure using Buster[5] and removed after visual inspection of the electron density. Final refinement statistics converged with Rwork = 0.221 and Rfree = 0.269. The final density surrounding the inhibitor was excellent.

Coordinates
The coordinates and structures factors have been deposited in the protein data bank under accession code 3U4U.

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United Kingdom: Global Phasing Ltd.

Table 1. Crystallographic and Refinement Statistics

Space Group	P212121
Unit Cell	α=β=γ=90, α= 49.5, β=61.9 Å, χ=116.1 Å
Mosaicity	1.88
Wavelength	1.54 Å
Resolution	2.2 Å
Unique reflections	17185
Multiplicity	5.2 (3.5)
Completeness (%)	91.6 (59.4)
I/σI	10.3 (3.4)
R_{merge}[a] (%)	0.084 (0.312)
R_{work}[b]/R_{free}[c] (%)	0.221 / 0.269
Residues	326
Waters	154
Average B factor	
Protein	53.6
Inhibitor	51.1
Water	57.6
Ramachandran	
Most favored	87.9%
Allowed	11.8%
Generously allowed	0.3%
Disallowed	0.0%

[a]R_{merge} = Σ|I-<I>|/ΣI, where I is the integrated intensity of a given reflection and <I> is the average intensity of multiple observations of symmetry-related reflections. [b]R_{work} = Σ|Fo-Fc|/ΣFo), where Fo and Fc are observed and calculated structure factors. [c]R_{free} was calculated from a 5% subset of reflections that were excluded from the refinement. Parentheses indicate highest resolution shell.