Supporting Information

"Potent and selective CK2 kinase inhibitors with effects on Wnt pathway signaling *in vivo*."

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Definition of terms:
APC: adenomatous polyposis coli; 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; Xantphos: 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene; DMA: N,N-dimethylacetamide; DMF: N,N-dimethylformamide; DMSO: dimethylsulfoxide; DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; MsCl: methanesulfonyl chloride; NMP: N-methyl pyrrolidin-1-one; *t*-BuXPhos: di-*tert*-butyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine; TFA: 2,2,2-trifluoroacetic acid; GSK-3β: glycogen synthase kinase 3 beta; HipK: homeodomain-interacting protein kinase; ABT: 1-aminobenzotriazole.

Compound characterization data and representative synthetic procedures:

All reagents and solvents used were purchased from commercial sources and were used without further purification. ^{1}H NMR spectra were obtained using a Bruker 300 MHz, 400 MHz, or 500 MHz spectrometer at temperatures ranging from 23 °C to 100 °C; chemical shifts are expressed in parts per million (ppm, δ units) and are referenced to the residual protons in the deuterated solvent used. Coupling constants are given in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br s (broad singlet). The purity of compounds submitted for screening was >95% as determined by UV analysis of liquid chromatography-mass spectroscopy (LCMS) chromatograms at 254 nM and the TAC (Total Absorption Chromatogram) performed with an Agilent 1100 equipped with Waters columns (Atlantis T3, 2.1x50 mm, 3 μm; or Atlantis dC18, 2.1 x 50 mm, 5 μm) eluted with a gradient mixture of water and acetonitrile with either formic acid or ammonium acetate or ammonium hydroxide added as a modifier. Additional evidence of purity was provided by the MS TIC (Total Ion Current) trace in ESI +ve and –ve ion modes Reverse-phase chromatography was performed as specified for individual compounds. Thin layer chromatography was performed using EMD silica gel 60 F254 plates. Column chromatography was performed using SiliCycle SiliaSep preloaded silica gel cartridges on Teledyne ISCO CombiFlash Companion automated purification systems. Unless otherwise indicated, all final compounds were purified to ≥ 95% purity as assessed by analytical HPLC using an Agilent 1100 equipped with Waters columns (Atlantis 11 T3, 2.1x50 mm, 3 μm; or Atlantis dC18, 2.1 x 50 mm, 5 μm) eluted for > 10 minutes with a gradient mixture of water and acetonitrile with either formic acid or ammonium acetate added as a modifier, monitored at wavelengths of 220, 254, and 280 nm.

***N*-[5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-(4-methylpiperazin-1-yl)phenyl]acetamide** (**7a**). A stirred suspension of N-(2-fluoro-5-nitrophenyl)acetamide (**3**, 0.30 g, 1.5 mmol, 1.0 eq), Cs_2CO_3 (0.54 g, 1.65 mmol 1.1 eq) and 1-methylpiperazine (0.185 mL, 1.67 mmol, 1.1 eq) in DMF (3.0 mL) was heated at 80 °C for 3 hrs. The mixture was allowed to cool to 25 °C and concentrated to dryness under reduced pressure. The crude product was purified by silica gel chromatography to give N-(5-nitro-2-(4-methylpiperazin-1-yl)phenyl)acetamide (**4a**, 0.32 g, 1.15 mmol), which was dissolved in MeOH (5 mL) and hydrogenated (balloon) over 5% Pd/C, (0.3g) for 12 hrs at 25 °C. The mixture was filtered through Celite, washed with MeOH and concentrated under reduced pressure to give 0.27 g N-(5-amino-2-(4-methylpiperazin-1-yl)phenyl)acetamide (**5a**) as a white solid. This material was added to a mixture of 5-chloro-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (**6**, 80 mg, 0.34 mmol, 1.1 eq), Cs_2CO_3 (145 mg, 0.45 mmol, 1.5 eq) and (9,9-dimethyl-9H-xanthene-4,5-diyl)bis(diphenylphosphine) (20 mg, 0.03 mmol) in DMA (0.5 mL) and heated at 150 °C for 30 minutes in a microwave under Argon. The reaction mixture was allowed to cool, filtered and concentrated to dryness. The residue was purified by silica gel chromatography (hexanes/EtOAc to EtOAc/MeOH/Et_3N (10:1:0.1)) to afford the title compound (50 mg, 0.11 mmol, 33%). ^{1}H NMR

(300 MHz, MeOH-d_4) δ 8.16 (s, 1H), 8.08 (s, 1H), 7.72 (br d, 1H), 7.22 (d, 1H), 6.02 (s, 1H), 2.92-2.95 (m, 4H), 2.59-2.77 (m, 5H), 2.39 (s, 3H), 2.21 (s, 3H), 0.84-0.98 (m, 2H), 0.68-0.79 (m, 2H); m/z 446; Purity (LCMS): 92%.

The following examples (**7b**, **7f**, **7g**, **7j**, and **7k**) were prepared according to the above procedure:

***N*-(5-(3-Cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-((2-(dimethylamino)ethyl)(methyl)amino)phenyl)acetamide (7b).** Prepared from *N*1,*N*1,*N*2-trimethylethane-1,2-diamine (Aldrich). ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.22 (d, *J*=2.45 Hz, 1H), 8.09 (s, 1H), 7.71 -7.81 (m, 1H), 7.27 (d, *J*=8.67 Hz, 1H), 6.05 (s, 1H), 3.03 (t, *J*=5.93 Hz, 2H), 2.63 - 2.72 (m, 4H), 2.45 (t, *J*=5.93 Hz, 2H), 2.31 (s, 6H), 2.22 (s, 3H), 0.84 - 0.95 (m, 2H), 0.69 - 0.78 (m, 2H); *m/z* 448; Purity (LCMS): 95%.

***N*-(5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-((2-hydroxyethyl)(methyl) amino)phenyl)acetamide** (**7f**). Prepared from 2-(methylamino)ethanol (Aldrich). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.60 (s, 1H), 9.56 (s, 1H), 8.32 (s, 1H), 8.27 (d, *J*=2.26 Hz, 1H), 8.14 (s, 1H), 7.82 (br. s., 1H), 7.20 (d, *J*=8.67 Hz, 1H), 6.05 (s, 1H), 5.25 - 5.32 (m, 1H), 3.51 - 3.64 (m, 2H), 2.73 - 2.83 (m, 2H), 2.67 (s, 3H), 2.55 (br. s., 1H), 2.10 (s, 3H), 0.75 - 0.86 (m, 2H), 0.72 (t, *J*=3.01 Hz, 2H); *m/z* 420; Purity (LCMS): 95%.

***N*-[5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]-2-[3-(dimethylamino)propyl-methyl-amino]phenyl]acetamide (7g).** Prepared from *N*1,*N*1,*N*3-trimethylpropane-1,3-diamine (Acros Organics). ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.05 - 8.17 (m, 2H), 7.87 (br. s., 1H), 7.33 (d, *J*=8.85 Hz, 1H), 6.02 (s, 1H), 3.05 - 3.22 (m, 4H), 2.85 (s, 6H), 2.76 (s, 3H), 2.63 - 2.72 (m, 1H), 2.25 (s, 3H), 1.81 - 1.99 (m, 2H), 0.88 - 1.00 (m, 2H), 0.69 - 0.84 (m, 2H); *m/z* 462; Purity (LCMS): 99%.

(*S*)-*N*-(5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-(3-(dimethylamino)pyrrolidin-1-yl)phenyl)acetamide (**7j**). Prepared from (*S*)-*N*, *N*-dimethylpyrrolidin-3-amine (Aldrich). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.77 (br. s., 1H), 9.59 (s, 1H), 9.01 (s, 1H), 8.33 (s, 1H), 8.16 (s, 1H), 7.80 (br. s., 1H), 7.11 (s, 1H), 5.99 (s, 1H), 3.97 (br. s., 1H), 3.10-3.30 (m, 4H) 2.85-2.89 (m, 6H), 2.50-2.53 (m, 1H), 2.33 (br. s., 1H), 2.11 (br. s., 4H), 0.80 (br. s., 2H), 0.72 (br. s., 2H); *m/z* 460; Purity (LCMS): 99%.

(*R*)-*N*-(5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-(3-(dimethylamino)piperidin-1-yl)phenyl)acetamide (**7k**). Prepared from (*R*)-*N*,*N*-dimethylpiperidin-3-amine. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.03 - 8.16 (m, 2H), 7.85 (br. s., 1H), 7.25 (d, *J*=8.85 Hz, 1H), 6.02 (s, 1H), 3.55 (d, *J*=1.32 Hz, 1H), 3.06 - 3.23 (m, 1H), 2.98 (br. s., 7H), 2.85 (br. s., 2H), 2.59 - 2.74 (m, 1H), 2.25(s, 3H), 2.10 (br. s, 1H), 1.88 (d, *J*=8.10 Hz, 3H), 0.88 - 1.00 (m, 2H), 0.69 - 0.80 (m, 2H); *m/z* 475; Purity (LCMS): 99%.

***N*-(5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-((2-(diethylamino)ethyl)(methyl)amino)phenyl)acetamide** (**7c**). Prepared from *N*1, *N*1-diethyl-*N*2-methylethane-1, 2-diamine (Aldrich) according to the procedure for **7a** but with the following modification. A suspension of *N*-(5-amino-2-((2-(diethylamino)ethyl)(methyl)amino)phenyl)acetamide (**5c**, 0.11 g, 0.41 mmol), potassium fluoride (0.020 g, 0.34 mmol), and 5-chloro-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (**6**, 0.08 g, 0.34 mmol) in NMP (0.1 mL) was heated under microwave irradiation at 140 °C for 7 hrs. The mixture was diluted with water (2 mL) treated with K_2CO_3 (0.5g) and the resulting precipitate was collected. The filtrate was extracted with EtOAc to obtain additional product and the organic extract was combined with the previously collected solid. The solution was dried over Na_2SO_4, filtered and concentrated under reduced pressure and chromatographed on silica (hexanes/EtOAc to EtOAc/MeOH/Et_3N (10:1:0.1)). The resulting residue was triturated with Et_2O (1 mL) and EtOAc (1 mL), collected, washed with EtOAc (1 mL) and dried to afford the title compound as a pale pink solid (72 mg, 44%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.61 (br. s, 2H), 8.31 (s, 1H), 8.19 (s, 1H) 8.13 (s, 1H), 7.84 (br d, 1H), 7.24 (d, 1H), 6.04 (s, 1H), 2.78 (br t, 2H), 2.40-2.60 (m, 7H), 2.63 (s, 3H), 2.09 (s, 3H), 0.96 (t, 6H), 0.65-0.85 (m, 4H); *m/z* 476; Purity (LCMS): 99%.

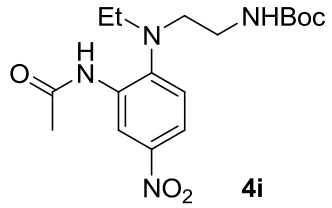
***N*-(5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-(methyl(2-morpholinoethyl)amino)phenyl)acetamide** (**7d**). Compound **7f** (0.33 g, 0.78 mmol) was dissolved in pyridine (2.0 mL) and the solution was cooled to 0 °C. Methanesulfonyl chloride (0.14 mL, 1.88 mmol) was added and the mixture was stirred at 0 °C for 0.5 hr then 25 °C for 1 hr. Water (20 mL) was added and the mixture was ex-

tracted with EtOAc (3 x 20 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was chromatographed on silica gel (hexanes/EtOAc) to give 2-((2-acetamido-4-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)(methyl)amino)ethyl methanesulfonate (0.35 g, 89 %) as a sticky colorless oil. This material was dissolved in acetonitrile (2.0 mL), treated with morpholine (0.18 g, 2.0 mmol) and stirred at 65 °C for 18hrs. The mixture was concentrated to dryness under reduced pressure and chromatographed on silica gel (hexanes/EtOAc) to give the title compound. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.20 (br. s, 1H), 8.08 (s, 1H), 7.77 (br. d, *J*=7.72 Hz, 1H), 7.20-7.37 (m, 1H), 6.03 (s, 1H), 3.63-3.82 (m, 4H), 3.09 (br. t, *J*=6.40 Hz, 2H), 2.59-2.70 (m, 4H), 2.47 (br. s, 6H), 2.25 (s, 3H), 0.83-1.00 (m, 2H), 0.65-0.78 (m, 2H); *m/z* 499; Purity (LCMS): 92%.

The following example was prepared using the method above:

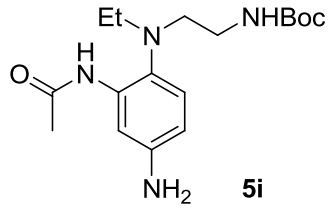
***N*-(5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)-2-((2-(3,3-difluoroazetidin-1-yl)ethyl)(methyl)amino)phenyl)acetamide** (**7e**). Prepared from 3,3-difluoroazetidine. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.11 (s, 1H) 7.99 (d, *J*=4.33 Hz, 1H), 7.69 (br. s., 1 H), 7.06 - 7.24 (m, 1H), 5.85 - 5.99 (m, 1H), 3.83 - 4.05 (m, 1H), 3.42 - 3.81 (m, 4H), 3.40 -3.45 (m, 1H), 3.14 (br. s., 2H), 2.64 (s 3H), 2.49 - 2.58 (m, 1H), 2.11 (s, 3H), 0.71 - 0.87 (m, 2H), 0.53 - 0.70 (m, 2H); *m/z* 496; Purity (LCMS): 96%.

***N*-(2-((2-aminoethyl)(ethyl)amino)-5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)acetamide** (**7i**).



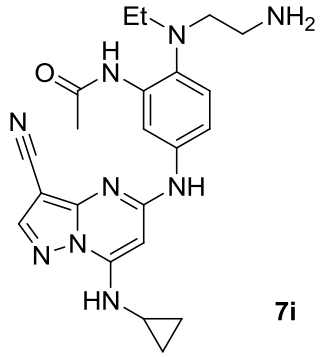
4i

A mixture of *N*-(2-fluoro-5-nitrophenyl)acetamide (**3**, 1.0 g, 5.05 mmol), Cs_2CO_3 (3.62 g, 11.1 mmol), *tert*-butyl 2-(ethylamino)ethylcarbamate hydrochloride (1.28 g, 5.55 mmol) in DMF (20 mL) was stirred at 80 °C for 20 hrs. The mixture was allowed to cool to 25 °C and concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL), washed with saturated aqueous NaCl solution, dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The resulting material was chromatographed on silica (hexanes/EtOAc) to give *tert*-butyl 2-((2-acetamido-4-nitrophenyl)(ethyl)amino)ethylcarbamate (**4i**) as a yellow solid (0.8 g, 44%); *m/z* 367; Purity (LCMS): 92%.



5i

A mixture of *tert*-butyl 2-((2-acetamido-4-nitrophenyl)(ethyl)amino)ethylcarbamate (**4i**, 0.8g, 2.18 mmol) and Pd/C (10% by weight, 0.116 g) in MeOH (5 mL) was degassed and flushed with H_2 (balloon) and stirred at 25 °C overnight. The mixture was filtered through a pad of diatomaceous earth (Celite) and the pad was washed with MeOH (10 mL). The filtrate was concentrated to dryness under reduced pressure to give *tert*-butyl 2-((2-acetamido-4-aminophenyl)(ethyl)amino) ethylcarbamate (0.63 g, 86% yield) as an oil; *m/z* 337; Purity (LCMS): 96%.



7i

A mixture of 5-chloro-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (**6**, 0.050 g, 0.21 mmol), *tert*-butyl 2-((2-acetamido-4-aminophenyl)(ethyl)amino)ethylcarbamate (**5i**, 0.072 g, 0.21 mmol), di-*tert*-butyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine (0.018 g, 0.04 mmol), Cs_2CO_3 (0.209 g, 0.64 mmol) and $Pd_2(dba)_3$ (0.020 g, 0.02 mmol) in anhydrous DMA (0.5 ml) was purged and flushed with argon. 1,4-dioxane (2 mL) was added via syringe and the resulting suspension was heated at 130 °C under microwave irradiation for 30 min. The reaction mixture was allowed to cool to 25 °C, filtered through diatomaceous earth (Celite) with the aid of CH_2Cl_2 and MeOH and concentrated to dryness *in vacuo*. The resulting solid (50 mg, 43%, *m/z* 534) was chromatographed on silica (CH_2Cl_2/EtOAc), dissolved in CH_2Cl_2 (0.5 mL) and treated with TFA (0.5 mL) at 25 °C for 2 hrs. The mixture was concentrated to dryness *in vacuo* to give N-(2-((2-aminoethyl)(ethyl)amino)-5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)acetamide as a TFA salt (**7i**, 37 mg, 72%); ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.11 (s, 1H), 7.96 (d, 1H), 7.76 - 7.88 (m, 1H), 7.24 (d, 1H), 6.01 (s, 1H), 3.38 (t, 2H), 2.99 - 3.10 (m, 4H), 2.62 - 2.75 (m, 1H), 2.22 (s, 3H), 1.05 (t, 3H), 0.88 - 1.00 (m, 2H), 0.71 - 0.81 (m, 2H); *m/z* 434; Purity (LCMS): 97%.

The following examples were prepared using the method above (**7h**, **7l**, **7m**, **7n**, **7o**, **7p** and **7q**):

***N*-(2-((2-aminoethyl)(methyl)amino)-5-(3-cyano-7-(cyclopropylamino) pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)acetamide** (**7h**). Prepared from *tert*-butyl 2-(methylamino)ethylcarbamate. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.30 (s, 1H), 8.08 (s, 1H), 7.80 (d, 1H), 7.23 (d, 1H), 5.99 (s, 1H), 3.34-3.35 (m, 2H), 3.02-3.10 (m, 2H), 2.67-2.70 (m, 4H), 2.21(s, 3H), 0.90 (m, 2H), 0.74 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.5, 157.0, 151.0, 148.2, 145.0, 137.6, 135.8, 134.1, 120.7, 114.9, 114.5, 111.5, 76.2, 60.7, 38.3, 24.3, 23.3, 6.5; *m/z* 420; HRMS: 420.2255 (calcd), 420.2271 (found); Purity (LCMS): 99%.

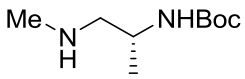
***N*-[2-[(*3S*)-3-aminopyrrolidin-1-yl]-5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]phenyl]acetamide** (**7l**). Prepared from (3*S*)-(-)-3-(*tert*-butoxycarbonylamino)pyrrolidine hydrochloride. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.01 (s, 1H), 7.84-8.02 (m, 1H), 7.53-7.78 (m, 1H), 7.14 (br. d, *J*=8.67 Hz, 1H), 5.97 (s, 1H), 3.85-4.13 (m, 1H), 3.34-3.53 (m, 1H), 3.34-3.43 (m, 1H), 3.24 (br. dd, *J*=2.26, 10.55 Hz, 1H), 2.94-3.11 (m, 1H), 2.64 (tt, *J*=3.53, 6.83 Hz, 1H), 2.49 (dtd, *J*=4.90, 8.43, 13.47 Hz, 1H), 2.22 (s, 3H), 1.96-2.12 (m, 1H), 0.80-0.97 (m, 2H), 0.63-0.77 (m, 2H); *m/z* 432; Purity (LCMS): 98%.

***N*-[2-[(*3R*)-3-aminopyrrolidin-1-yl]-5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]phenyl]acetamide** (**7m**). Prepared from (3*R*)-(+)-3-(*tert*-butoxycarbonylamino)pyrrolidine. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.01 (s, 1H), 7.84-8.02 (m, 1H), 7.53-7.78 (m, 1H), 7.14 (br. d, *J*=8.67 Hz, 1H), 5.97 (s, 1H), 3.85-4.13 (m, 1H), 3.34-3.53 (m, 1H), 3.34-3.43 (m, 1H), 3.24 (br. dd, *J*=2.26, 10.55 Hz, 1H), 2.94-3.11 (m, 1H), 2.64 (tt, *J*=3.53, 6.83 Hz, 1H), 2.49 (dtd, *J*=4.90, 8.43, 13.47 Hz, 1H), 2.22 (s, 3H), 1.96-2.12 (m, 1H), 0.80-0.97 (m, 2H), 0.63-0.77 (m, 2H); *m/z* 432; Purity (LCMS): 96%.

***N*-[2-[(*3S*)-3-amino-1-piperidyl]-5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]phenyl]acetamide** (**7n**). Prepared from (*S*)-(-)-3-*tert*-butoxycarbonylaminopiperidine. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.42 (br. s, 2H), 7.95-8.20 (m, 2H), 7.83 (br. d, *J*=6.97 Hz, 1H), 7.19 (d, *J*=8.67 Hz, 1H), 6.01 (s, 1H), 3.53-3.59 (m, 1H), 3.21-3.26 (m, 1H), 2.80-3.01 (m, 3H), 2.58-2.72 (m, 1H), 2.23 (s, 3H), 1.68-2.13 (m, 4H), 0.88-0.92 (m, 2H), 0.73-0.75 (m, 2H); *m/z* 446; Purity (LCMS): 98%.

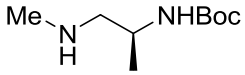
***N*-[2-[(*3R*)-3-amino-1-piperidyl]-5-[[3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-yl]amino]phenyl]acetamide** (**7o**). Prepared from (*R*)-(+)-3-*tert*-butoxycarbonylaminopiperidine. ^{1}H NMR (300

MHz, MeOH-d_4) δ 8.42 (br. s, 2H), 7.95-8.20 (m, 2H), 7.83 (br. d, *J*=6.97 Hz, 1H), 7.19 (d, *J*=8.67 Hz, 1H), 6.01 (s, 1H), 3.53-3.59 (m, 1H), 3.21-3.26 (m, 1H), 2.80-3.01 (m, 3H), 2.58-2.72 (m, 1H), 2.23 (s, 3H), 1.68-2.13 (m, 4H), 0.88-0.92 (m, 2H), 0.73-0.75 (m, 2H); *m/z* 446; Purity (LCMS): 97%.

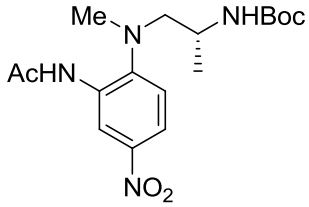


(*R*)-*tert*-butyl 1-(methylamino)propan-2-ylcarbamate. A solution of (*R*)-(+)-2-(tert-butoxycarbonylamino)-1-propanol (0.75 g, 4.28 mmol) in CH_2Cl_2 (5 mL) was cooled to 0 °C. Triethylamine (0.776 mL, 5.56 mmol) and methanesulfonyl chloride (0.40 mL, 5.14 mmol) were added and the mixture was stirred at 0 °C for 40 min. The mixture was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The resulting white solid was dissolved in methanamine (5.35 mL, 42.8 mmol, 8 M in EtOH) and the mixture was heated at 85 °C under microwave irradiation for 40 min. The mixture was concentrated to dryness under reduced pressure and the residue was chromatographed on silica to give (*R*)-*tert*-butyl 1-(methylamino)propan-2-ylcarbamate (0.25 g, 31 %) as an oil. ^{1}H NMR (300 MHz, MeOH-d_4) δ 3.66 - 3.83 (m, 1 H), 2.47 - 2.62 (m, 2 H), 2.38 (s, 3 H), 1.46 (s, 9 H), 1.10 - 1.15 (m, 3 H); *m/z* 189.

The following example was prepared using the above method:

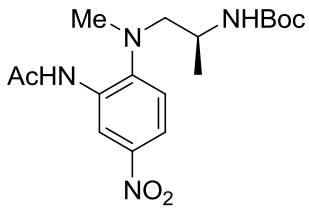


(*S*)-*tert*-butyl 1-(methylamino)propan-2-ylcarbamate. Prepared from (*S*)-(-)-2-(*tert*-butoxycarbonylamino)-1-propanol. ^{1}H NMR (300 MHz, MeOH-d_4) δ 3.66 - 3.79 (m, 1 H), 2.49 - 2.57 (m, 2 H), 2.37 (s, 3 H), 1.40 - 1.49 (m, 9 H); *m/z* 189.

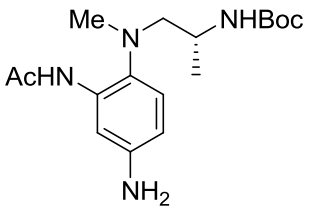


(*R*)-*tert-butyl* 1-((2-acetamido-4-nitrophenyl)(methyl)amino)propan-2-ylcarbamate. *N*-(2-fluoro-5-nitrophenyl)acetamide (0.25 g, 1.27 mmol) and Cs_2CO_3 (0.41 g, 1.27 mmol) were added to a solution of (*R*)-*tert*-butyl 1-(methylamino)propan-2-yl carbamate (0.240 g, 1.27 mmol) in DMF (2 mL) and the mixture was stirred at 60 °C for 3h. The reaction was allowed to cool to 25 °C, and concentrated to dryness under reduced pressure. The residue was dissolved in EtOAc (10 mL), washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The resulting material was chromatographed on silica (EtOAc/hexanes) to give (*R*)-*tert*-butyl 1-((2-acetamido-4-nitrophenyl)(methyl)amino)propan-2-ylcarbamate (0.250 g, 53.5 %) as an oil. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.77 (d, 1 H), 7.99 (dd, 1 H), 7.29 (d, 1 H), 3.79 - 3.93 (m, 1 H), 3.11 (d, 2 H), 2.89 (s, 3 H), 2.27 (s, 3 H), 1.40 (s, 10 H), 1.08 (d, 3 H); *m/z* 367.

The following example was prepared using the above method:

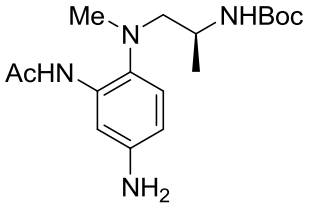


(*S*)-*tert-butyl* 1-((2-acetamido-4-nitrophenyl)(methyl)amino)propan-2-ylcarbamate. Prepared from (*S*)-*tert*-butyl 1-(methylamino)propan-2-yl carbamate. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.77 (d, 1 H), 7.99 (dd, 1 H), 7.29 (d, 1H), 3.82-3.89 (m, 1H), 3.10-3.12 (m, 2 H), 2.89 (s, 3 H), 2.27 (s, 3 H), 1.40 (s, 9 H), 1.08 (d, 3 H); *m/z* 367.

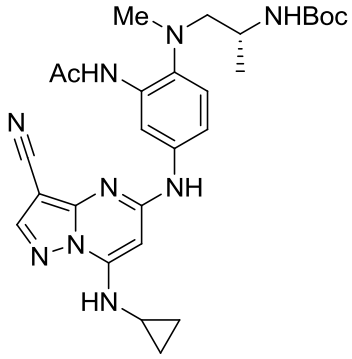


(*R*)-*tert*-butyl 1-((2-acetamido-4-aminophenyl)(methyl)amino)propan-2-ylcarbamate. A mixture of (*R*)-*tert*-butyl 1-((2-acetamido-4-nitrophenyl)(methyl)amino)propan-2-ylcarbamate (0.250 g, 0.68 mmol) and Pd/C (Degussa type, 0.036 g, 0.03 mmol) in MeOH (5 mL) was stirred under H_2 (balloon) at 25 °C for 2h. The mixture was filtered through diatomaceous earth (Celite) and the filtrate was concentrated under reduced pressure to give (*R*)-*tert*-butyl 1-((2-acetamido-4-aminophenyl)(methyl)amino) propan-2-ylcarbamate (0.210 g, 91 %) as an oil. ^{1}H NMR (300 MHz, MeOH -d_4) δ 7.71 (br. s., 1 H), 7.05 (d, *J*=8.48 Hz, 1 H), 6.47 (dd, *J*=8.48, 2.64 Hz, 1 H), 2.68 - 2.88 (m, 2 H), 2.59 (s, 3 H), 2.21 (s, 3 H), 1.43 (s, 9 H), 1.07 (d, *J*=6.59 Hz, 3 H); *m/z* 337.

The following example was prepared using the above method:

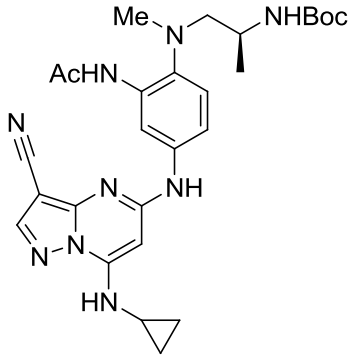


(*S*)-*tert*-butyl 1-((2-acetamido-4-aminophenyl)(methyl)amino)propan-2-yl carbamate. Prepared from (*S*)-*tert*-butyl 1-((2-acetamido-4-nitrophenyl)(methyl)amino)propan-2-yl carbamate. . ^{1}H NMR (300 MHz, MeOH -d_4) 7.71 (br. s., 1 H), 7.05 (d, *J*=8.48 Hz, 1 H), 6.47 (dd, *J*=8.38, 2.54 Hz, 1 H), 3.58-3.62 (m, 1 H), 2.67 - 2.90 (m, 2 H), 2.59 (s, 3 H), 2.21 (s, 3 H), 1.43 (s, 9 H), 1.07 (d, *J*=6.59 Hz, 3 H); *m/z* 337.

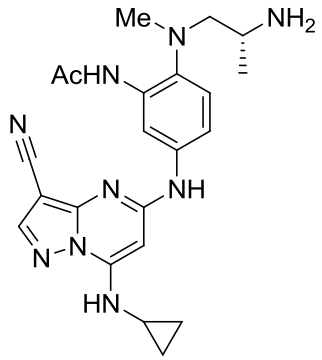


(*R*)-*tert*-butyl 1-((2-acetamido-4-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)(methyl)amino)propan-2-ylcarbamate. A suspension of (*R*)-*tert*-butyl 1-((2-acetamido-4-aminophenyl)(methyl)amino) propan-2-ylcarbamate (138 mg, 0.41 mmol), 5-chloro-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidine-3-carbonitrile (80 mg, 0.34 mmol), potassium fluoride (19.9 mg, 0.34 mmol) in NMP (0.4 mL) was heated at 140 °C for 24 h under N_2. The mixture was allowed to cool to 25 °C and diluted with water (10 mL). The solid that formed was collected by filtration, dissolved in MeOH/CH_2Cl_2 (20 mL, 1:10), dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The resulting solid was chromatographed on silica (EtOAc/hexanes) to give (*R*)-*tert*-butyl 1-((2-acetamido-4-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)(methyl)amino)propan-2-ylcarbamate (40.0 mg, 21.9 %) as an oil; *m/z* 534.

The following example was prepared using the above method:

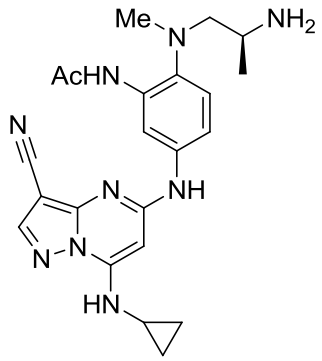


(*S*)-*tert*-butyl 1-((2-acetamido-4-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)(methyl)amino)propan-2-ylcarbamate. Prepared from (*S*)-*tert*-butyl 1-((2-acetamido-4-aminophenyl)(methyl)amino) propan-2-ylcarbamate; *m/z* 534.



(*R*)-*N*-(2-((2-aminopropyl)(methyl)amino)-5-(3-cyano-7-(cyclopropylamino) pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)acetamide (7p). A solution of (*R*)-*tert*-butyl 1-((2-acetamido-4-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)(methyl)amino)propan-2-ylcarbamate (0.043 g, 0.08 mmol) in TFA (0.5 mL) and CH_2Cl_2 (0.5 mL) was stirred at 25 °C for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by semi-preparative HPLC to give (*R*)-*N*-(2-((2-aminopropyl)(methyl)amino)-5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)acetamide (0.018 g, 40.8 %) as a trifluoroacetic acid (TFA) salt; ^{1}H NMR (300 MHz, MEOH-d_4) δ 8.11 (s, 1H), 7.94 (d, *J*=2.64 Hz, 1H), 7.74 - 7.83 (m, 1H), 7.24 (d, *J*=8.85 Hz, 1H), 6.00 (s, 1H), 3.36 - 3.47 (m, 2H), 2.88 - 3.00(m, 1H), 2.63 - 2.74 (m, 4H), 2.23 (s, 3H), 1.33 (d, *J*=6.40 Hz, 3H), 0.88 - 0.99 (m, 2H), 0.72 - 0.81 (m, 2H); *m/z* 434. Purity (LCMS): 95%. Post-purification enantiomeric purity was assessed by HPLC using a Chiralpak AD column (4.6 x 250 mm, 10µ) with a 70:30 gradient of hexane/1:1 MeOH:iPrOH and 0.1% diethylamine as the mobile phase (flow rate = 1.0 mL/min) monitored at a wavelength of 220 nm. The product had a retention time of 5.4 minutes, >98% ee.

The following example was prepared using the above method:



(*S*)-*N*-(2-((2-aminopropyl)(methyl)amino)-5-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)acetamide (**7q**). Prepared from (*S*)-*tert*-butyl 1-((2-acetamido-4-(3-cyano-7-

(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)(methyl)amino)propan-2-ylcarbamate. ^{1}H NMR (300 MHz, MEOH-d_4) δ 8.11 (s, 1H), 7.94 (d, *J*=2.64 Hz, 1H), 7.74 - 7.83 (m, 1H), 7.24 (d, *J*=8.85 Hz, 1H), 6.00 (s, 1H), 3.36 - 3.47 (m, 2H), 2.88 - 3.00(m, 1H), 2.63 - 2.74 (m, 4H), 2.23 (s, 3H), 1.33 (d, *J*=6.40 Hz, 3H), 0.88 - 0.99 (m, 2H), 0.72 - 0.81 (m, 2H); *m/z* 434. Purity (LCMS): 95%. Post-purification enantiomeric purity was assessed by HPLC using a Chiralpak AD column (4.6 x 250 mm, 10μ) with a 70:30 gradient of hexane/1:1 MeOH:iPrOH and 0.1% diethylamine as the mobile phase (flow rate = 1.0 mL/min) monitored at a wavelength of 220 nm. The product had a retention time of 4.1 minutes, >98% ee.

***N*-(3-(3-cyano-7-(cyclopropylamino)pyrazolo[1,5-a]pyrimidin-5-ylamino)phenyl)acetamide** (**1**). Prepared from *N*-(3-aminophenyl)acetamide. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92 (s, 1H), 9.66 (s, 1H), 8.34 (s, 1H), 8.21 (s, 1H), 7.69-7.88 (m, 2H), 7.23 (t, J=8.08 Hz, 1H), 7.06 (br. d, J=8.08 Hz, 1H), 6.04 (s, 1H), 2.57 (br. s, 1H), 2.03 (s, 3H), 0.60-0.90 (m, 4H); m/z 348; Purity (LCMS): 95%.

CK2 in vitro mobility shift assay

Activity of N-terminal 6xHis-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. T he 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 16-24 hrs at 37 °C in 6 well plates. CK2 inhibitor compounds were screened using a concentration response in cells for 3 and 24 hrs 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 internal engineered rabbit polycolonal antibody and Total AKT antibody from Cell Signaling Technology (Cat# 9272). Total protein (20-40 μg) 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 ºC 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 hr at room temperature. Proteins were detected with ECL reagent (Pierce SuperSignal Dura ECL).

Protocol for detection of anti-active β-catenin in Wnt3a-expressing L cells

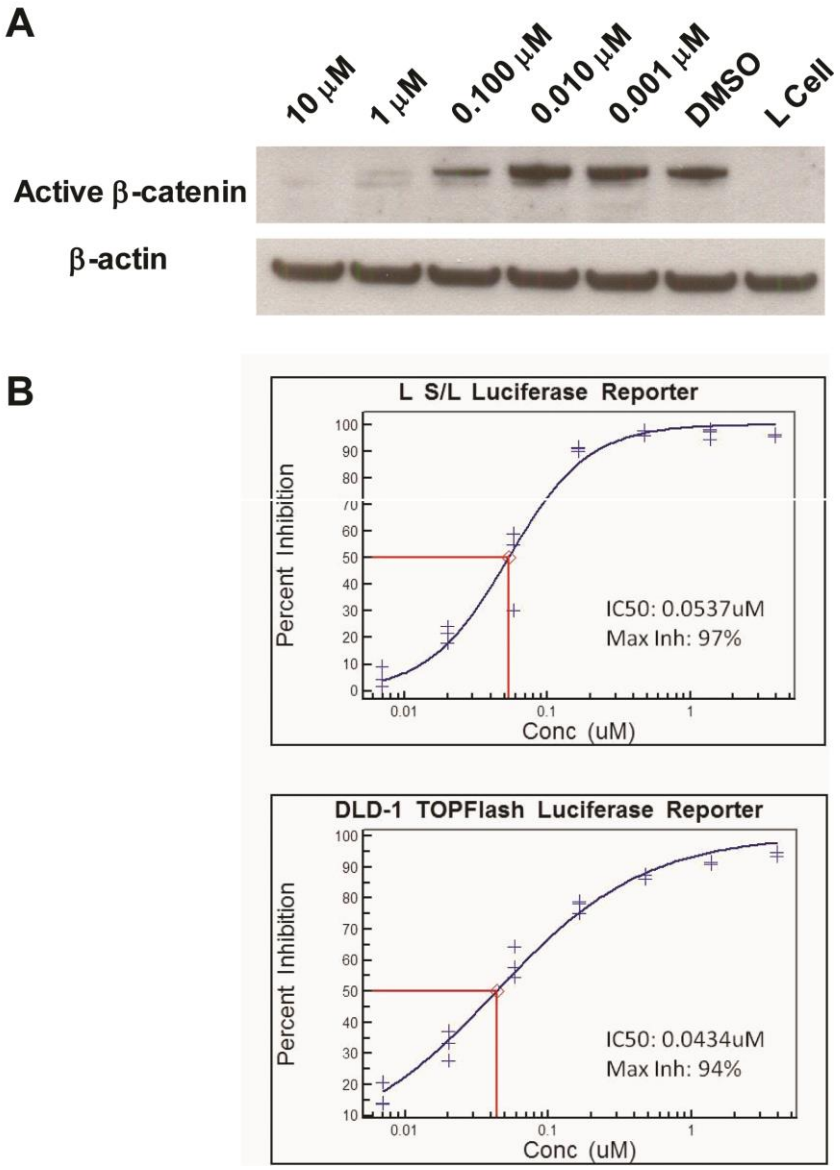
Mouse L-Wnt3a cells (ATCC CRL-2647) constitutively expressing Wnt3a were maintained in DMEM supplemented with 10% FBS, 1% L-Glutamine and 400ug/ml G418. L Cells (ATCC CRL-2648) were maintained in DMEM supplemented with 10% FBS and 1% L-Glutamine. L Wnt3a cells were treated with compound for 1 hr before harvesting lysates for analysis by Western blot. L Cell lysate was used as a negative control for

active β-catenin. Anti active β-catenin (anti-ABC clone 8E7, Millipore 05-665) was used at 1:2000 and anti beta-actin (Abcam ab6276) was used at 1:10,000.

L S/L TOPFlash Luciferase Reporter Assay

Mouse L-Wnt3a cells were purchased from the American Type Culture Collection (ATCC, CRL-2647) and cultured as recommended by the vendor. Mouse fibroblast L S/L Super TOPFlash cells were obtained from R. Nusse's lab (Stanford University). Conditioned media (CM) containing Wnt3a ligand was produced from L-Wnt3a cells (http://www.stanford.edu/~rnusse/wntwindow.html.). Mouse L S/L Super TOPFlash cells were plated in DMEM supplemented with 2% FBS, 1% L-Glu and allowed to adhere for 16-24 h at 37 ^{o}C. The following day, cells were stimulated with the addition of 7 mL Wnt3a conditioned media, dosed with compounds of interest using a Labcyte Echo® Liquid Handler (7 point dilution starting with 10 μM, final well volume 30 mL) and incubated for 24 h at 37 ^{o}C. Following compound treatment, 30 μL of Steady-Glo Luciferase reagent (Promega) was added into each well except wells for "Min" control. The plates were incubated at room temperature for 15 min in the dark. The luciferase signal was read using a Tecan Ultra and the IC_{50} was calculated using an Activity Base template (DMSO control as "Max", no Luciferase reagent as "Min").

Figure S1 Depletion of β-catenin in Wnt3a expressing mouse L cells by compound **7h** (**A**);
Inhibition of L S/L TOPflash luciferase (Wnt3a stimulated context) reporter activity and DLD-1 (constitutively active Wnt context) TOPflash luciferase activity by **7h** (**B**).



DLD-1, SW620 and HCT-116 72hr Alamar Blue CRC cell proliferation assays

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 CRC 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 72 hrs 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 4 hrs 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 hrs 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)] x 100. 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 hrs 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.

Kinase Selectivity Profiling

Cpd	Hipk1 IC_{50} (µM)	Hipk2 IC_{50} (µM)	Hipk3 IC_{50} (µM)	Hipk4 IC_{50} (µM)	Dyrk1a IC_{50} (µM)	Dyrk1b IC_{50} (µM)	Dyrk2 IC_{50} (µM)	Dyrk3 IC_{50} (µM)	Dyrk4 IC_{50} (µM)	Dapk2 IC_{50} (µM)	Dapk3 IC_{50} (µM)
7h	1.3	0.12	0.45	0.11	0.44	1.3	0.027	0.043	0.41	0.008	0.018

Crystallization and structure determination

Recombinant human CK2 was expressed and purified from *E. coli* according to literature methods.[1,2] Apo crystals of CK2 were grown using hanging drop vapor diffusion. The reservoir solution contained 22-26% PEG 6000 (*w/v*), 0.2 M ammonium sulfate and 0.1 M MES (pH 6.5). Drops were set up with 2 µL protein solu-

tion and 2 µL reservoir solution. Trays were incubated at 20 ºC, and crystals appeared after 1 week and reached the final size after 2 weeks. Crystals were soaked in reservoir solution containing 1 mM of compound and 1% DMSO for 24 hrs at 20 °C. Crystals were transferred to a soaking solution containing 20% ethylene glycol, and were vitrified in liquid nitrogen. X-ray diffraction data was collected at cryogenic temperature using synchrotron radiation at the Advanced Photon Source, supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-06CH11357 or using an in house FRE+ generator outfitted with a CCD detector. Diffraction data was processed with XDS[3] and scaled using AIMLESS[4], as implemented in the autoPROC routines from Global Phasing[5]. The structure was solved by molecular replacement using MOLREP[6]. Protein and inhibitor were modeled into the electron density using Coot[7], and the model was refined using autoBUSTER[8] (**Table S1**). Atomic coordinates and structure factors have been deposited in the Protein Data Bank (**5H8B**, **5H8G** and **5H8E**).

Surface Plasmon Resonance

All Surface Plasmon Resonance (SPR) experiments were conducted on a Biacore T200 (GE Healthcare) at 18 °C using 20 mM Tris (pH 7.4), 0.2 M NaCl, 1 mM TCEP, 0.005% Tween-20, 1% DMSO as a running buffer. His-tagged CK2 (residues 6-335) was immobilized by a standard His-capture, amine-couple method using 20 mM Hepes (pH 7.4), 0.2 M NaCl, 1 mM TCEP, 0.005% Tween-20 as an immobilization buffer[10]. Compounds were screened using a 5-injection single-cycle kinetics method with 170, 250 or 270 sec injection time for each concentration followed by a 50 or 116.7 min dissociation period at flow rate 50 µL/min. Sensorgrams were double-referenced to a blank reference flow cell and a buffer blank. Sensorgrams were analyzed with a 1:1 binding model using the Biacore T200 Evaluation Software v2.0 (GE Healthcare).

Table S1. Crystallographic statistics

Compound	**2**	**7h**	**7b**
Space Group	$P4_32_12$	$P4_32_12$	$P2_12_12_1$
Unit cell dimensions (Å)	a=b=126.62, c=124.62; α=β=γ=90°	a=b=126.92, c=124.57; α=β=γ=90°	a=47.81, b=78.81, c=81.87; α=β=γ=90°
Wavelength (Å)	1.00000	0.97650	1.54178
Resolution (Å)	24.60-2.55	24.60-2.15	21.22-2.00
Number of reflections	486,625	405,159	85,633
Unique reflections	33,689	55,872	20,991
Multiplicity	14.4 (14.5)	7.3 (7.2)	4.1 (3.9)
Completeness (%)	99.8 (99.1)	99.8 (98.9)	97.9 (77.6)
<I/σI>	15.8 (3.9)	18.6 (3.9)	14.3 (5.2)
R_{merge}[a] (%)	0.242 (0.984)	0.099 (0.594)	0.091 (0.286)
$CC_{1/2}$[9]	0.997 (0.927)	0.999 (0.862)	0.995 (0.895)
R_{work}[b] / R_{free}[c] (%)	19.96 / 23.21	16.48 / 19.61	15.89 / 19.82
Wilson B-factor (Å^2)	31.1	25.9	13.4
Average B-factor All atoms (Å^2)	33.0	32.0	19.0
Total Number of Atoms	6,052	6,287	3,050
Ramachandran Parameters			
Favored (%)	96.4	97.6	97.5
Allowed (%)	99.7	100.0	99.8

[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|F_o-F_c|/ΣF_o), where F_o and F_c are observed and calculated structure factors. [c] R_{free} was calculated from a 5% subset of reflections that were excluded from the refinement. Brackets indicate highest resolution shell.

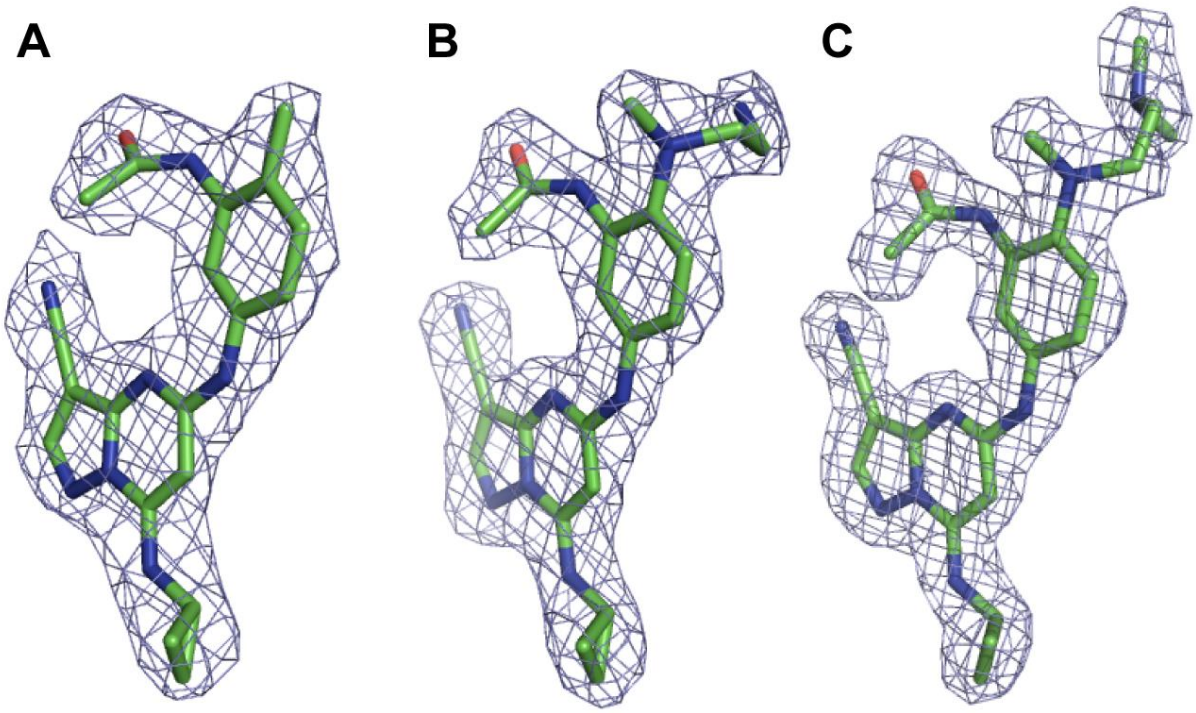


Figure S2. Electron density maps for liganded CK2 complexes. Compounds (**A**) **2**; (**B**) **7h**; and (**C**) **7b**. The compounds are shown as stick models with green carbon atoms, blue nitrogen atoms, red oxygen atoms and yellow sulfur atoms. The final 2Fo-Fc electron density map surrounding the inhibitor is shown as blue mesh (contoured at 1σ).

In vivo tumor xenograft studies

Female Ncr nude (nu/nu genotype; Taconic) mice were housed in individually ventilated cages with 4-5 animals per cage. Experiments were conducted on 6- to 12-week-old animals in full accordance with AstraZeneca animal welfare protocols which are consistent with The American Chemical Society Publications rules and ethical guidelines. Human tumor xenografts were implanted by subcutaneous injection with 0.1 ml of the tumor cell suspension ($3x10^7$ cells/mouse) on the flank. Animals were randomized into treatment groups (n = 8-12 per group) when tumor volumes reached a predefined size (~200-600 mm^3, depending on the study). A solution of compound **7h** was prepared in a 50:50 mixture of 7.5% SEBCD and 5% dextrose at pH 4-5 and administered as a bolus intraperitoneal (IP) injection.

For pharmacodynamic (PD) studies animals bearing established SW620, DLD-1 Topflash or HCT-116 tumor xenografts received vehicle or compound **7h** as a single i.p. bolus dose. At different time points after dosing, animals (n=2-3 per group) were sacrificed and tumor and plasma were harvested for pharmacokinetic and pharmacodynamic analysis. *Ex vivo* Luciferase activity was determined using the Promega Bright-Glo-Luciferase assay kit. Levels of the active form of β-catenin were assessed with Millipore's Anti-Active-β-Catenin (anti-ABC), clone 8E7 mouse monoclonal antibody, which specifically recognizes the dephosphorylated form of beta-catenin at residues Ser37 or Thr41.

In chronic dose studies with compound **7h** in HCT-116 tumor bearing animals, tumor volume was determined twice weekly by measuring the two longest perpendicular axes in the x/y plane of each xenograft tumor to the nearest 0.1 mm with a digital vernier caliper. Tumor volumes were calculated according to the standard equation: volume = $xy^2/2$, and the data were plotted using the geometric mean for each group versus time. Statistical analysis was carried out using a Student's one-tailed t test.

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