Supporting Information

Discovery of GSK2656157: An Optimized PERK Inhibitor Selected for Preclinical Development

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PERK assays. The PERK enzyme assay and PERK autophosphorylation assay in A549 cells were carried out as previously described.[1]

Protein crystallography. Methods to crystallize and solve the structure of **6** complexed with PERK were identical to those previously described.[1] The crystallographic data and final refinement statistics are summarized in Table 1. Reported interatomic distances between heavy atoms were measured with COOT or PyMol. The deposited coordinate file (PDB accession code = 4M7I) has a MolProbity[5] score of 1.70 (98%) and clash score of 8.89 (96%). Structure figures were generated with PyMol (www.schrodinger.com).

Table S1. X-ray Data and Refinement Statistics

X-ray Data	Compound **6** w/PERK
Cell dimensions Å Cell angles in degrees	101.60 101.60 158.03 90 90 120
Space group	$P6_122$
Resolution Å	50-2.34
Unique reflections	20,810
Completeness % (last shell)	99.9 (100.0)
I/s (last shell)	44.5 (6.9)
Rsym[a] (last shell)	5.0 (40.8)
Refinement Statistics	
Resolution Å	2.34
Rfactor[b] %	20.7
Rfree %	28.2
RMSD[c] bond lengths Å	0.007
RMSD bond angles in degrees	1.01
Total of non-hydrogen atoms	2135
Ligand	31
Protein	1979
Water	125

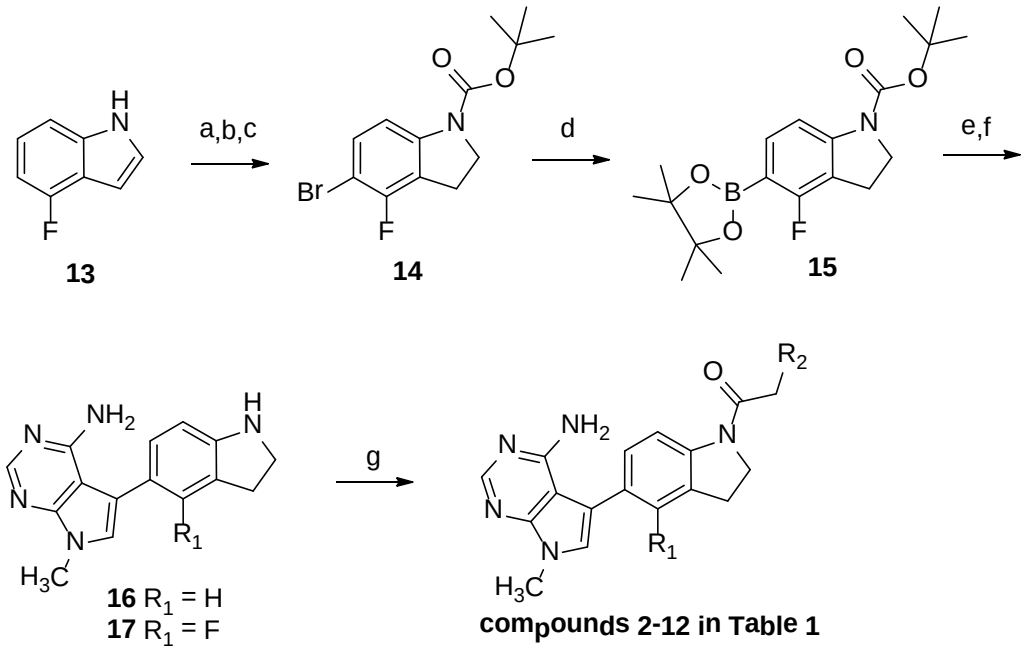
[a]Rsym = ∑ | Iavg – Ii | / ∑Ii
[b]Rfactor = ∑ |Fp – F calc | /∑Fp, where Fp and Fcalc are observed and calculated structure factors. Rfree is calculated from a randomly chosen 3.25% of reflections that are never used in refinement and Rfactor is calculated from the remaining 96.75% of reflections.
[c]RMSD is the root mean square deviation from ideal geometry

DMPK Methods. All studies were conducted after review by the Institutional Animal Care and Use Committee at GSK and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals. Pharmacokinetics were studied in male Sprague-Dawley rats, male beagle dogs and/or male CD-1 mice following single intravenous and/or oral administration. Absolute oral bioavailability was estimated using a cross-over study design (n=2 or 3), unless otherwise indicated. Blood samples were assayed using protein precipitation followed by LC/MS/MS analysis, and the resulting concentration-time data were analyzed by non-compartmental methods (WinNonlin Professional, v4.1). Concentration-dependent inhibition of cytochrome P450 isozyme assays were performed at Cyprotex Discovery Ltd (Macclesfield, UK) or CellzDirect (currently Life Technolgies, Grand Island, NY, USA). Assays were conducted using proprietary screening techniques based on a previously reported method employing human liver microsomes as the test matrix.[3] The following substrates (and isoforms) were employed: phenacetin (1A2), diclofenac (2C9), S-mephenytoin (2C19), bufuralol (or dextromethorphan) (2D6), paclitaxel (2C8), midazolam and nifedipine (3A4). Test compound (6-12 concentrations from 0.01 to 30 uM, ≤1% (v/v) DMSO final) was incubated with human liver microsomes (0.1 – 0.5 mg/mL depending on the reaction) and NADPH (1 mM) in the presence of the substrate (at a concentration approximating the Michaelis-Menten constant) for 5 - 60 min (depending on the reaction) at 37°C in 90-100 mM potassium phosphate buffer, pH 7.4. Reactions were terminated by the addition of a stop solution. The samples were then centrifuged, and the supernatants combined in cassettes of up to 4 for the simultaneous analysis of the probe metabolites and internal standard by LC-MS/MS. Internal standard was added to the final sample prior to analysis. A decrease in the formation of the metabolites

compared to vehicle control was used to calculate percentage control activity. Percentage control activity versus drug concentration data were fitted to estimate IC50 values (the test compound concentration which produced 50% inhibition).

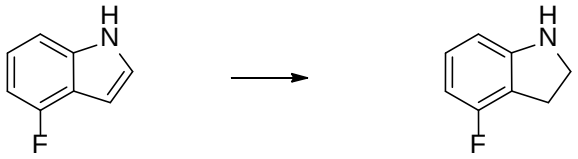
Chemistry. Unless otherwise noted, commercially available materials were used without further purification. Bromo-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine and compound **16** (General Scheme) were prepared as previously described.[1] Air or moisture sensitive reactions were carried out under a nitrogen or argon atmosphere. Anhydrous solvents were obtained from Sigma-Aldrich and used as received. Flash chromatography was performed using silica gel under standard techniques or with silica gel cartridges on an Analogix® flash chromatography instrument. NMR spectra were recorded on a Bruker 400 MHz spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm) relative to an internal solvent reference. Coupling constants (J) are recorded in Hertz. LC-MS analyses were performed on a Sciex or Agilent open access instrument. Analytical HPLC data was recorded with an analytical C18 column eluting with an acetonitrile/water (+0.1% TFA) gradient over 4 minutes. Compounds were detected by UV (254, 214 and 333 nm). All final compounds with reported biological data were determined to be >95% purity based on LC-MS, NMR and analytical HPLC data unless otherwise noted.

General Synthetic Scheme



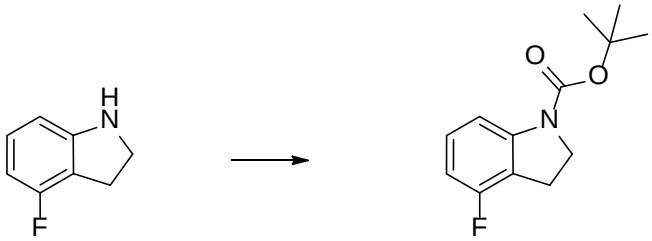
(a) $NaBH_3CN$, HOAc, 89%; (b) Boc_2O, DMAP, CH_3CN, 95%; (c) NBS, DCM, 99%; (d) bis(pinacolato)diboron, KOAc, $PdCl_2(dppf)$; (e) 5-Bromo-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine,[1] K_3PO_4, $(t\text{-}Bu)_3PHBF_4$, $Pd_2(dba)_3$, 80°C; (f) HCl, dioxane; (g) heteroaryl acetic acid, coupling reagent

Compound Preparation

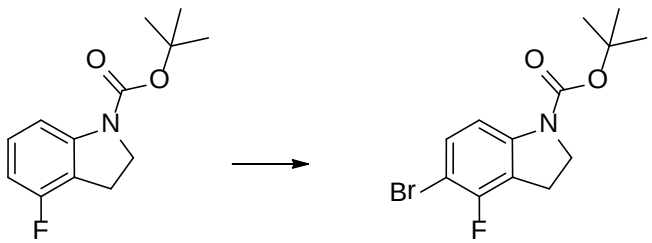


Sodium cyanoborohydride (71.2 g, 1133 mmol) was added portion-wise to an ice cooled solution of 4-fluoroindole (49.41 g, 366 mmol) in acetic acid (0.471 L). The reaction was allowed to warm to room temperature and stir for 1 hour. The reaction mixture was cooled in an ice bath and neutralized with 50% wt/wt NaOH (0.443 L), keeping the internal temperature <15 °C. Water (500 mL) was added, and the mixture was extracted with ether (2 x 500 mL).

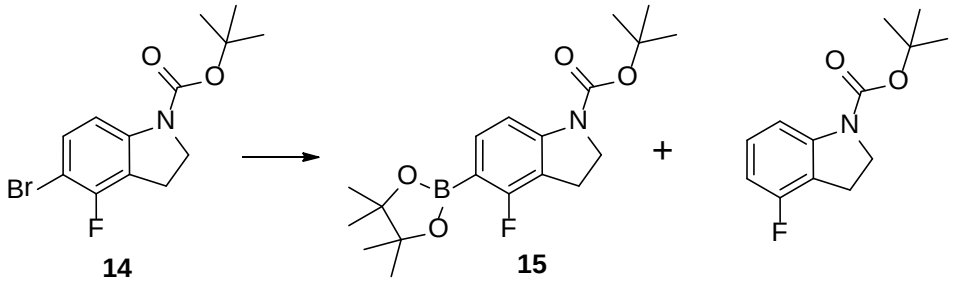
The combined ether extracts was dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel column eluting with EtOAc and hexanes (5% to 33%) and the pure product fractions were combined and concentrated to dryness to give 4-fluoro-2,3-dihydro-1H-indole (44.55 g, 89 % yield) as an oil. LC-MS (ES) m/z = 138.3 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ 6.85 - 6.97 (m, 1H) 6.30 (dd, *J* = 8.5, 5.2 Hz, 2H) 5.78 (br s, 1H) 3.47 (td, *J* = 8.7, 1.9 Hz, 2H) 2.94 (t, *J* = 8.7 Hz, 2H).



A 1 L round-bottomed flask was charged with 4-fluoro-2,3-dihydro-1H-indole (21.51 g, 157 mmol), Boc_2O (36.4 mL, 157 mmol), DIPEA (54.8 mL, 314 mmol), DMAP (0.192 g, 1.568 mmol) and $CHCl_3$ (300 mL) to give a yellow solution that was stirred for 4 days. The reaction mixture was diluted with 0.1 M HCl (250 mL) and extracted with $CHCl_3$ (400 mL). The $CHCl_3$ layer was dried over Na_2SO_4, filtered through pad of silica gel (1 x1 inch), and concentrated to dryness to afford 1,1-dimethylethyl 4-fluoro-2,3-dihydro-1H-indole-1-carboxylate (35.36 g, 95 % yield). LC-MS (ES) m/z = 182.2 $[M+H-tBu]^+$. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ 7.27-7.59 (br s, 1H) 7.12 - 7.26 (m, 1H) 6.76 (t, *J* = 8.8 Hz, 1H) 3.96 (t, *J* = 8.7 Hz, 2H) 3.08 (t, *J* = 8.7 Hz, 2H) 1.51 (s, 9H).



N-bromosuccinimide (33 g, 185 mmol) was added to a stirring solution of 1,1-dimethylethyl 4-fluoro-2,3-dihydro-1H-indole-1-carboxylate (35.36 g, 149 mmol) in DCM (105 mL) at room temperature. After 2 hours, the reaction mixture was diluted with water (100 mL) and saturated aqueous Na_2CO_3 (100 mL). The whole was extracted with DCM (200 mL), and the organic layer dried over Na_2SO_4, filtered through pad of silica gel (2x2 inch), and concentrated to dryness to obtain 1,1-dimethylethyl 5-bromo-4-fluoro-2,3-dihydro-1H-indole-1-carboxylate as an off-white solid (47.9 g, 99 % yield). LC-MS (ES) m/z = 260.1, 262.1 $[M+H-tBu]^+$. ^1H NMR (400 MHz, *DMSO-d_6*) δ 7.36-7.50 (m, 2H) 3.98 (t, *J* = 8.7 Hz, 2H) 3.12 (t, *J* = 8.7 Hz, 2H) 1.50 (s, 9H).



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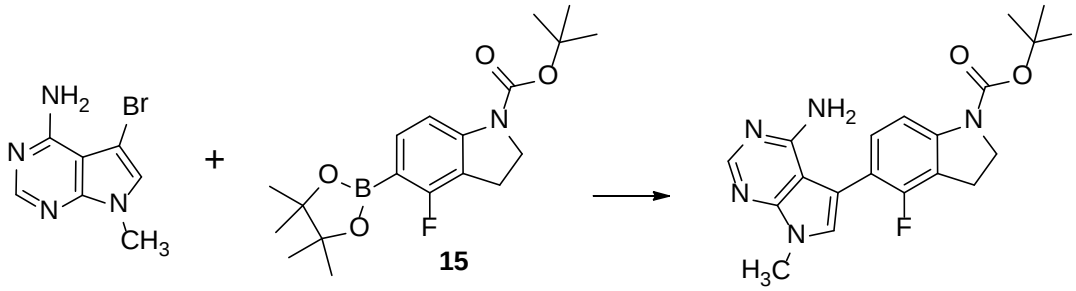
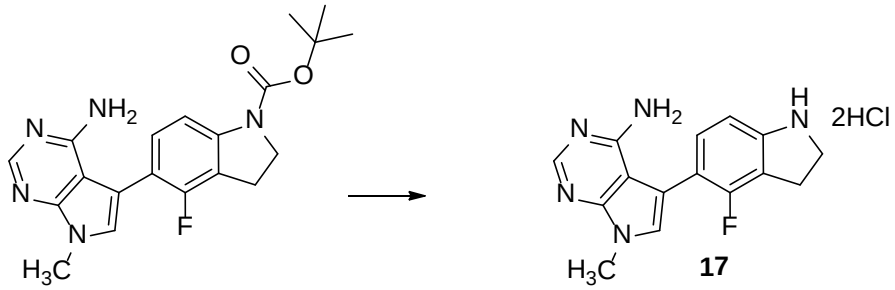
A 5L RB flask was charged with 1,1-dimethylethyl 5-bromo-4-fluoro-2,3-dihydro-1H-indole-1-carboxylate (170.84 g, 540 mmol), bis(pinacolato)diboron (206 g, 811 mmol), potassium acetate (159 g, 1621 mmol) and dioxane (1700 mL) to give a yellow suspension. The mixture was stirred and purged with argon for 2 minutes, then PdCl2(dppf)-CH_2Cl_2 adduct (5 g, 6.12 mmol) was added to the reaction mixture, which was then stirred at 80 °C overnight. The cooled reaction mixture was diluted with water (1L) and saturated aqueous $NaHCO_3$ (800 mL), then extracted with EtOAc (3 x 1L). The combined EtOAc extracts was washed with water, dried

over Na_2SO_4, filtered through pad of silica gel and concentrated. The residue was chromatographed on a 5 x 6 inch silica gel plug eluting with 5-15% EtOAc/hexanes to give 1,1-dimethylethyl 4-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydro-1H-indole-1-carboxylate **15** (256 g) which was 76% pure by NMR and 78% pure by LC-MS. The difficult to remove impurity was the reduction byproduct 1,1-dimethylethyl 4-fluoro-2,3-dihydro-1H-indole-1-carboxylate, which is inert to the next step. Therefore, the boronate ester **15** was used as is. LC-MS (ES) m/z = 308.3 $[M+H\text{-}tBu]^+$. ^1H NMR (400 MHz, *DMSO-d_6*) δ 7.35-7.60 (m, 2H) 3.85 - 4.04 (m, 2H) 3.04 (t, *J* = 8.7 Hz, 3H) 1.50 (s, 9H) 1.28 (s, 6H) 1.17 (s, 6H).



A 3L RB flask was charged with 5-bromo-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (53 g, 233 mmol), 1,1-dimethylethyl 4-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydro-1H-indole-1-carboxylate (76% pure, 123 g, 257 mmol), K_3PO_4 (tribasic, 99 g, 467 mmol), dioxane (800 mL) and water (267 mL) to give a yellow suspension at room temperature under argon. The reaction was stirred and purged with argon for 5 minutes. $(t\text{-}Bu)_3PHBF_4$ (3.39 g, 11.67 mmol) and $Pd_2(dba)_3$ (5.34 g, 5.84 mmol) were added to the reaction mixture, which was heated to 80 °C for 2 hours. After cooling to room temperature, the precipitated solids were collected by filtration and washed water and then with ether. The solids were dissolved in

$CHCl_3$/10% MeOH. The solution was stirred with Si-thiol (3g) and MP-TMT (Biotage) for 2 hours to remove residual palladium. The solution was filtered through pad of Celite and concentrated to give 1,1-dimethylethyl 5-(4-amino-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-4-fluoro-2,3-dihydro-1H-indole-1-carboxylate (60.7 g, 68 %). LC-MS (ES) m/z = 384.2 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*, *denotes minor rotamer peak) δ *8.32, 8.14 (s, 1H) 7.45-7.68 (br s, 1H) 7.24 (s, 1H) 7.18 (t, *J* = 8.0 Hz, 1H) 5.88-6.15 (br s, 2H) 4.02 (t, *J* = 8.7 Hz, 2H) 3.74 (s, 3H) 3.14 (t, *J* = 8.6 Hz, 2H) 1.52 (s, 9H).



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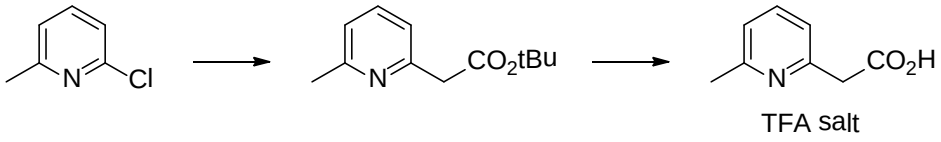
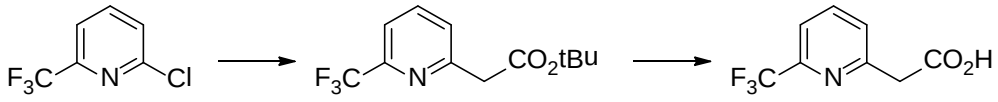
4N HCl in dioxane (800 mL) was added to stirring suspension of 1,1-dimethylethyl 5-(4-amino-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-4-fluoro-2,3-dihydro-1H-indole-1-carboxylate (55.5 g, 145 mmol) in dioxane (100 mL) at room temperature. After stirring overnight, the reaction was filtered and the collected solid was washed with ether and dried to afford (4-fluoro-2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (49.6 g, 94%). LC-MS (ES) m/z = 284.2 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.50 (s, 1H) 7.58 (s, 1H) 7.14 (t, *J* = 7.8 Hz, 1H) 6.72-6.84 (m, 1H) 3.85 (s, 3H) 3.66 (t, *J* = 8.2 Hz, 2H) 3.13 (t, 2H). NHs were too broad to detect.



Pyridyl acetic acid esters were prepared by the method of Biscoe and Buchwald.[4] A stirring solution of 2-chloro-6-methylpyridine (40.28 g, 316 mmol) and1,1-dimethylethyl acetate (55.0 g, 474 mmol) in toluene (600 mL) was purged with N_2 gas for 10 minutes , and then chloro(2-di-t-butylphosphino-2',4',6'-tri-i-propyl-1,1'-biphenyl)[2-(2-aminoethyl)phenyl]palladium(II) (2.168 g, 3.16 mmol) was added. N_2 gas was purged for an additional 10 minutes. The reaction was cooled in ice bath and LHMDS (1M in toluene, 947 mL, 947 mmol) was added dropwise over 1 hour. After the addition the reaction was stirred at room temperature for 3 hours then was quenched with 800mL of saturated aqueous NH_4Cl. The mixture was extracted with EtOAc (3 X 400 mL), and the combined organics was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residual oil was purified with three 400g silica gel cartridges preconditioned with hexane, eluting with 0 to 25% EtOAc in hexane. The fractions containing product were combined from all three runs and loaded onto another 400g silica gel cartridge column using the same conditions to afford 1,1-dimethylethyl (6-methyl-2-pyridinyl)acetate (44.36 g, 68 %) as a light yellow oil. LC-MS (ES) m/z = 208.2 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 7.63 (t, J = 7.71 Hz, 1H), 7.11 (t, J = 7.07 Hz, 2H), 3.67 (s, 2H), 2.43 (s, 3H), 1.40 (s, 9H).

TFA (165 mL, 2147 mmol) was added drop wise to a stirring solution of 1,1-dimethylethyl (6-methyl-2-pyridinyl)acetate (44.5 g, 215 mmol) and triethylsilane (86 mL, 537 mmol) in DCM (300 mL) at room temperature. After addition, the mixture was allowed to stir overnight. To drive the reaction to completion, and additional 2mL of TFA was added and the reaction was

stirred 5 hours. The reaction was then concentrated in vacuo on a rotary evaporator with water bath set to 60°C. Diethyl ether (300mL) was added to the residual oil, which caused an exothermic precipitation of product. The mixture was allowed to cool and the solid was isolated by filtration and washed with diethyl ether and dried to give (6-methyl-2-pyridinyl)acetic acid TFA salt (53.7 g, 94 %) as a white solid. LC-MS (ES) m/z = 152.3 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.16 (t, 1H), 7.58 (d, J = 7.83 Hz, 2H), 3.98 (s, 2H), 2.63 (s, 3H). Microanalysis calculated for $C_8H_9NO_2$•TFA: C: 45.29, H: 3.80, N: 5.28; found: 45.31, H: 3.56, N: 5.27.



To a stirred solution of tert-butyl acetate (1.01 mL, 7.5 mmol), 2-chloro-6-(trifluoromethyl)pyridine (908 mg, 5.00 mmol), and chloro(2-di-t-butylphosphino-2',4',6'-tri-i-propyl-1,1'-biphenyl)[2-(2-aminoethyl)phenyl]palladium(II) (343 mg, 0.50 mmol) in toluene (10 mL) at 0 °C in a 100-mL RBF under N_2 was added a solution (pre-cooled to 0 °C) of LHMDS (1M in toluene) (15 mL, 15 mmol). After 30 minutes, the mixture was allowed to warm to room temperature and stir overnight. The mixture was poured into saturated aqueous NH_4Cl and water (1:1, 40 mL), and extracted with ethyl acetate (3 x 100 mL). The combined organics were dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography (0-25% EtOAc in hexanes, 90g silica gel column) to afford 1,1-dimethylethyl [6-(trifluoromethyl)-2-pyridinyl]acetate (701 mg, 54 % yield) as a pale yellow oil. LC-MS (ES) m/z =

206 $[M+H\text{-}t\text{Bu}]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 1.41 (s, 9H), 3.88 (s, 2H), 7.61 - 7.71 (m, 1H), 7.77 - 7.85 (m, 1H), 8.02 - 8.11 (m, 1H).

To a solution of 1,1-dimethylethyl [6-(trifluoromethyl)-2-pyridinyl]acetate (698 mg, 2.7 mmol), triethylsilane (1.07 mL, 6.7 mmol) in DCM (10 mL) was added TFA (2.68 mL, 34.7 mmol) drops wise via syringe. The reaction was stirred overnight at room temperature, then was concentrated to afford [6-(trifluoromethyl)-2-pyridinyl]acetic acid (535 mg, 98 % yield) as a yellow oil which solidified on standing. LC-MS (ES) m/z = 206 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.89 (s, 2H), 7.70 (d, J = 7.83 Hz, 1H), 7.81 (d, J = 7.58 Hz, 1H), 7.97 - 8.16 (m, 1H), 12.26 - 12.88 (m, 1H). Microanalysis calculated for $C_8H_6F_3NO_2$: C: 46.84, H: 2.95, N: 6.83, F: 27.78; found: C: 46.13, H: 2.99, N: 6.48, F: 27.52.

7-methyl-5-[1-(2-pyridinylacetyl)-2,3-dihydro-1H-indol-5-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine (2)

A solution of 5-(2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (150 mg, 0.44 mmol), 2-pyridinylacetic acid•HCl (77 mg, 0.44 mmol), HATU (169 mg, 0.44 mmol), and DIEA (0.39 mL, 2.2 mmol) was stirred at room temperature overnight. The mixture was poured into water (10 mL) and stirred for 30 minutes. The resulting suspension was filtered, the collected solid was washed with water and then dried to afford 7-methyl-5-[1-(2-pyridinylacetyl)-2,3-dihydro-1H-indol-5-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**2**) (149 mg, 88 %) as a white solid. LC-MS(ES) m/z = 385 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.52 (d, J =

4.29 Hz, 1H) 8.09 - 8.20 (m, 2H) 7.72 - 7.84 (m, 1H) 7.39 (d, J = 7.83 Hz, 1H) 7.16 - 7.35 (m, 4H) 5.80 - 6.27 (br s, 2H) 4.27 (t, J = 8.34 Hz, 2H) 4.05 (s, 2H) 3.74 (s, 3H) 3.23 (t, 2H).

7-methyl-5-[1-(3-pyridinylacetyl)-2,3-dihydro-1H-indol-5-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine (3)

A solution of 5-(2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (150 mg, 0.44 mmol), 3-pyridinylacetic acid•HCl (77 mg, 0.44 mmol), HATU (169 mg, 0.44 mmol), and DIEA (0.39 mL, 2.2 mmol) was stirred at room temperature overnight. The mixture was poured into water (10 mL) and stirred for 30 minutes. The resulting suspension was filtered, the collected solid was washed with water and then dried to afford 7-methyl-5-[1-(3-pyridinylacetyl)-2,3-dihydro-1H-indol-5-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**3**) (170 mg, 95 % yield) as a white solid. LC-MS (ES) m/z = 385 [M+H]+. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.41 - 8.59 (m, 2H) 8.07 - 8.18 (m, 2H) 7.72 (d, J=7.83 Hz, 1H) 7.30 - 7.45 (m, 2H) 7.16 - 7.29 (m, 2H) 5.79 - 6.28 (br s, 2H) 4.28 (t, J = 8.34 Hz, 2H) 3.94 (s, 2H) 3.73 (s, 3H) 3.26 (t, 2H).

7-methyl-5-[1-(4-pyridinylacetyl)-2,3-dihydro-1H-indol-5-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine (4)

A solution of 5-(2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (150 mg, 0.44 mmol), 4-pyridinylacetic acid•HCl (77 mg, 0.44 mmol), HATU (169 mg, 0.44 mmol), and DIEA (0.39 mL, 2.2 mmol) was stirred at room temperature overnight. The mixture was poured into water (10 mL) and stirred for 30 minutes. The resulting suspension was

filtered, the collected solid was washed with water and then dried to afford 7-methyl-5-[1-(4-pyridinylacetyl)-2,3-dihydro-1H-indol-5-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**4**) (160 mg, 94 %) as a white solid. LC-MS (ES) m/z = 385 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.47 - 8.59 (m, 2H) 8.07 - 8.21 (m, 2 H) 7.29 - 7.40 (m, 3H) 7.19 - 7.29 (m, 2H) 5.85 - 6.33 (br s, 2H) 4.24 (t, J = 8.46 Hz, 2H) 3.96 (s, 2H) 3.74 (s, 3H) 3.24 (t, J = 8.21 Hz, 2H).

7-methyl-5-{1-[(6-methyl-2-pyridinyl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7H-pyrrolo[2,3-d]pyrimidin-4-amine (5)

A solution of 5-(2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (150 mg, 0.44 mmol), (6-methyl-2-pyridinyl)acetic acid•TFA (118 mg, 0.44 mmol), HATU (169 mg, 0.44 mmol), and DIEA (0.39 mL, 2.2 mmol) in DMF (3 mL) was stirred overnight at room temperature. Water (15 mL) was added to the reaction mixture, which was then stirred 30 minutes. The mixture was extracted with ethyl acetate: methanol (ca. 1% methanol, 3 x 30 mL) and the combined organics were dried over sodium sulfate, filtered and concentrated. The residue was adsorbed onto silica and purified by flash chromatography (0-10% MeOH in EtOAc, 12g silica column) to afford 7-methyl-5-{1-[(6-methyl-2-pyridinyl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**5**) (167 mg, 95 %) as an off-white solid. LC-MS (ES) m/z = 399 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.18 (s, 1H) 8.12 (d, J=8.08 Hz, 1H) 7.68 (t, J=7.58 Hz, 1H) 7.27 - 7.34 (m, 2H) 7.23 (d, J = 8.34 Hz, 1H), 7.17 (t, J = 7.33 Hz, 2H) 6.01 - 6.41 (br s, 2H) 4.29 (t, J = 8.46 Hz, 2H) 4.00 (s, 2H) 3.75 (s, 3H) 3.23 (t, J = 8.34 Hz, 2H) 2.46 (s, 3H).

5-{4-fluoro-1-[(6-methyl-2-pyridinyl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (6)

A 2 L round-bottomed flask was charged with 5-(4-fluoro-2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (47.7 g, 134 mmol), HATU (61.1 g, 161 mmol) and DMF (500 mL) to give a yellow solution at room temperature. The reaction was stirred and cooled to 0 ˚C, and DIEA (108 mL, 616 mmol) was added to the reaction mixture. After 10 min, (6-methyl-2-pyridinyl)acetic acid•TFA (38.9 g, 147 mmol) was added portion-wise to the reaction mixture over 1 hour, keeping the temperature below 5 ˚C. The reaction was allowed to warm to room temperature and stir for 2 hours. The mixture was poured into 2.5 L of ice cold water. The yellow solid was filtered off, washed with water, and then dissolved in $CHCl_3$/10% MeOH. The mixture was treated with MP-TMT (1g) and Si-thiol silica gel (11g) and Darco G-60-100 (5g) at 50 ˚C overnight to remove residual palladium. The hot reaction mixture was filtered, concentrated, and the residue was chromatographed on silica gel (5 x 200g cartridges) eluting with a gradient of 75-100% A/B (A = 10% MeOH/EtOAc, B = EtOAc). Fractions containing pure product were concentrated to dryness, and the solid was stirred with 600 mL of iPrOH and heated to reflux. After 1 hour, the mixture was cooled to room temperature and the soldi was collected and washed with iPrOH followed by hexanes, and dried in a vacuum oven at 70 °C. NMR showed the presence of 1% 3H-[1,2,3]triazolo[4,5-b]pyridin-3-ol (HATU byproduct), which was removed by stirring with 1N NaOH for 1 hour, filtering, and washing the solid with 300 mL of 1N NaOH, and then thoroughly with water (2 L). The solid was dried in a vacuum oven for 3 days to give pure 5-{4-fluoro-1-[(6-methyl-2-pyridinyl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**6**) (37.7 g, 67%) as an off-white solid. LC-MS (ES) m/z = 417.2 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO*-d_6) δ 8.10 - 8.18 (m, 1H) 7.92 (d, *J* = 8.3 Hz, 1H) 7.66 (t, *J* = 7.6 Hz, 1H) 7.26 (s, 1H) 7.11 - 7.24 (m,

3H) 6.03 (br s, 2H) 4.35 (t, *J* = 8.5 Hz, 2H) 4.00 (s, 2H) 3.74 (s, 3H) 3.24 (t, *J* = 8.3 Hz, 2H) 2.45 (s, 3H). ^{13}C NMR (100 MHz, *DMSO-d*$_6$) δ 169.0 157.8 157.6 156.0 (d, $^{1}J_{FC}$ = 242.2 Hz) 155.0 152.1 150.7 145.0 (d, $^{3}J_{FC}$ = 7.3 Hz) 137.3 131.3 (d, $^{3}J_{FC}$ = 2.2 Hz) 125.7 121.6 121.6 119.31 (d, $^{2}J_{FC}$ = 23.4 Hz) 117.5 (d, $^{2}J_{FC}$ = 15.3 Hz) 112.7 107.5 101.3 49.2 45.5 31.2 24.5 24.5. ^{19}F NMR (376 MHz, decoupled, *DMSO-d*$_6$) δ -121.4. Microanalysis calculated for $C_{23}H_{21}FN_6O \bullet H_2O$: C: 63.58, H: 5.34, N: 19.34; found: C: 64.09, H: 5.16, N: 19.59.

7-methyl-5-(1-{[6-(trifluoromethyl)-2-pyridinyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (7)

A solution of 5-(2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (150 mg, 0.44 mmol), [6-(trifluoromethyl)-2-pyridinyl]acetic acid (91 mg, 0.44 mmol), HATU (169 mg, 0.44 mmol), and DIEA (0.31 mL, 1.8 mmol) in DMF (3 mL) was stirred overnight at room temperature. The reaction mixture was poured into water (10 mL) and stirred for 30 minutes. The resulting precipitate was collected by filtration, dried under vacuum for an hour, then adsorbed onto silica and purified by flash chromatography (0-10% MeOH in EtOAc) to afford 7-methyl-5-(1-{[6-(trifluoromethyl)-2-pyridinyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**7**) (80 mg, 40%) as a beige solid. LC-MS (ES) m/z = 453 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ 8.15 (s, 1H) 8.05 - 8.13 (m, 2H) 7.83 (d, *J* = 7.58 Hz, 1H) 7.71 (d, *J* = 7.83 Hz, 1H) 7.34 (s, 1H) 7.26 (s, 1H) 7.23 (d, *J* = 8.34 Hz, 1H), 5.85 - 6.26 (br s, 2H) 4.31 (t, *J* = 8.46 Hz, 2H) 4.21 (s, 2H) 3.74 (s, 3H) 3.26 (t, *J* = 8.34 Hz, 2H).

5-(4-fluoro-1-{[6-(trifluoromethyl)-2-pyridinyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (8)

To a suspension of 5-(4-fluoro-2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (200 mg, 0.56 mmol) and HATU (235 mg, 0.62 mmol) in DMF (2 mL) at room temperature was added DIEA (412 uL, 2.36 mmol) in one portion. To this mixture was added [6-(trifluoromethyl)-2-pyridinyl]acetic acid (179 mg, 0.87 mmol) portion-wise over a 1 hour period. After stirring 30 minutes, the mixture was poured into 20 mL of ice cold water, which gave a suspension that was filtered. The cake was washed with water and dried, then chromatographed on a 40 g silica gel cartridge using gradient elution of 1% A in CHCl3 to 65% A in CHCl3 (A was a mixture of 3200/800/80 $CHCl_3$/MeOH/NH_4OH). The combined fractions containing product were concentrated and purified by further by chromatography on a 60 g silica gel cartridge with a gradient elution of 1% to 75% A in EtOAc (A was a mixture of 20% MeOH in EtOAc). The combined fractions containing the pure product were concentrated, and the residue was dissolved in 12 mL of 10% MeOH in DCM and concentrated in vacuo to a suspension (about 1 mL). This mixture was diluted with 12 mL of MTBE, concentrated to half volume, and diluted with another 10 mL of MTBE. The suspension was filtered, and the solid was washed with MTBE (2x 4 mL) and hexane (3x 4 mL), and dried under vacuum at 65 °C for 18 hours to afford 5-(4-fluoro-1-{[6-(trifluoromethyl)-2-pyridinyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**8**) (205 mg, 78%) as a white solid. LC-MS (ES) m/z = 471 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ, 8.14 (s, 1H) 8.10 (t, *J* = 7.8 Hz, 1H) 7.89 (d, *J* = 8.1 Hz, 1H) 7.83 (d, *J* = 7.6 Hz, 1H) 7.71 (d, *J* = 7.8 Hz, 1H) 7.28 (s, 1H) 7.20 (t, *J* = 8.1 Hz, 1H) 5.90 - 6.19 (br s, 2H) 4.38 (t, *J* = 8.6 Hz, 2H) 4.22 (s, 2H) 3.74 (s, 3H) 3.28 (t, *J* = 8.5 Hz, 2H).

7-methyl-5-{1-[(3-methyl-1H-pyrazol-1-yl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7H-pyrrolo[2,3-d]pyrimidin-4-amine (9)

To a mixture of 5-(2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine •2HCl (150 mg, 0.44 mmol) and (3-methyl-1H-pyrazol-1-yl)acetic acid (62 mg, 0.44 mmol) in THF (3 mL) was added DIPEA (0.23 mL, 1.3 mmol) drop wise. The mixture was cooled in an ice bath and T3P® (1-propanephosphonic acid cyclic anhydride), 50% in ethylacetate (~1.68M) (0.317 mL, 0.532 mmol) was then added drop wise. After stirring 30 minutes, the ice bath was removed and the mixture was allowed to warm to room temperature and stir 2 hours. The mixture was diluted with water (~5 mL) and basified to pH 7-8 with 0.5M NaOH. The precipitated solid was collected, and the wet cake was suspended in ethanol (~5mL) with heating for 10 minutes then cooled. The solid was collected and washed with ethanol, followed by hexanes and was dried to give 7-methyl-5-{1-[(3-methyl-1H-pyrazol-1-yl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**9**) (114 mg, 63 %) as a light gray powder. LC-MS(ES) m/z = 388.3 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.15 (s, 1H) 8.07 (d, *J* = 8.3 Hz, 1H) 7.59 (d, *J* = 2.3 Hz, 1H) 7.34 (s, 1H) 7.22 - 7.29 (m, 2H) 6.07 (d, *J* = 2.0 Hz, 1H) 5.94 - 6.27 (br s, 2H) 5.16 (s, 2H) 4.22 (t, *J* = 8.3 Hz, 2H) 3.73 (s, 3H) 3.26 (t, *J* = 8.3 Hz, 2H) 2.17 (s, 3H).

7-methyl-5-{1-[(5-methyl-1H-pyrazol-1-yl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10)

A solution of 5-(2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (150 mg, 0.44 mmol), (5-methyl-1H-pyrazol-1-yl)acetic acid (62 mg, 0.44 mmol), HATU (169 mg, 0.44 mmol), and DIEA (0.31 mL, 1.8 mmol) was stirred at room temperature for 3 days. The resulting suspension was poured into water (10 mL) and stirred for 30 minutes. The mixture was poured into 10 mL water and 20 mL of brine, and and extracted with ethyl acetate (3 x 80 mL). The combined organics were dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography (0-10% MeOH in EtOAc, 12g silica gel column) to afford 7-methyl-5-{1-[(5-methyl-1H-pyrazol-1-yl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**10**) (143.5 mg, 84 %) as a white solid. LC-MS(ES) m/z = 388 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.16 (s, 1H) 8.07 (d, *J* = 8.08 Hz, 1H) 7.35 (d, *J* = 4.29 Hz, 2H) 7.16 - 7.30 (m, 2H) 6.08 (s, 1H) 5.91-6.35 (br s, 2H) 5.22 (s, 2H) 4.29 (t, *J* = 8.34 Hz, 2H) 3.74 (s, 3H) 3.28 (t, *J* = 8.34 Hz, 2 H) 2.24 (s, 3H).

5-{1-[(3,5-dimethyl-1H-pyrazol-1-yl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11)

To a mixture of 5-(2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine •2HCl (175 mg, 0.52 mmol) and (3,5-dimethyl-1H-pyrazol-1-yl)acetic acid (80 mg, 0.52 mmol) in DMF (3 mL) was added DIPEA (0.27 mL, 1.6 mmol) dropwise. The mixture was cooled in an ice bath, and T3P® (1-propanephosphonic acid cyclic anhydride), 50% in ethylacetate (~1.68M) (0.370 mL, 0.621 mmol) was then added drop wise. After stirring 30 minutes, the ice bath was removed and the mixture was allowed to warm to room temperature and stir 2hours. The mixture was diluted with water (~5 mL) and basified to pH 7-8 with 0.5M NaOH. Methanol was

added to give a clear solution. This solution was loaded onto a reversed phase C18 SF25-55g Analogix cartridge and the product purified by eluting with a gradient of 30-95% methanol-water. The combined fractions containing the pure product was evaporated and azeotroped with acetonitrile followed by benzene to give a solid. The solid was triturated with acetonitrile (~4 mL), filtered, and washed with acetonitrile to afford 5-{1-[(3,5-dimethyl-1H-pyrazol-1-yl)acetyl]-2,3-dihydro-1H-indol-5-yl}-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**11**) (90 mg, 41 %) as a white solid after drying under vacuum. LCMS (ES) m/z = 402.4 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.15 (s, 1H) 8.07 (d, *J* = 8.1 Hz, 1H) 7.34 (s, 1H) 7.20 - 7.29 (m, 2H) 6.08 (br s, 2H) 5.86 (s, 1H) 5.09 (s, 2H) 4.26 (t, *J* = 8.3 Hz, 2 H) 3.74 (s, 3H) 3.27 (t, *J* = 8.3 Hz, 2H) 2.17 (s, 3H) 2.10 (s, 3H).

5-{1-[(3,5-dimethyl-1H-pyrazol-1-yl)acetyl]-4-fluoro-2,3-dihydro-1H-indol-5-yl}-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (12)

To a suspension of 5-(4-fluoro-2,3-dihydro-1H-indol-5-yl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine•2HCl (200 mg, 0.56 mmol) and HATU (235 mg, 0.62 mmol) in DMF (2 mL) at room temperature was added DIEA (314 uL, 1.80 mmol) in one portion. To this mixture was added (3,5-dimethyl-1H-pyrazol-1-yl)acetic acid (87 mg, 0.56 mmol, 1 equiv) portion-wise over a 1 h period. After 30 minutes the reaction was incomplete, so an additional 18 mg of the acid was added. After 1 hour, the mixture was poured into 20 mL of ice cold water to give a suspension, which was filtered. The collected solid was dried and then dissolved in 10% MeOH in DCM and absorbed onto a dry load silica cartridge. Purification on a 40 g silica gel cartridge using gradient elution of 1% A in $CHCl_3$ to 65% A in $CHCl_3$ (A was a mixture of 3200/800/80

$CHCl_3$/MeOH/NH_4OH) gave partially purified product, which was then re-chromatographed on a 60 g silica gel cartridge using gradient elution of 1% A in EtOAc 100% A (A was a mixture of 20% MeOH in EtOAc). The combined product fractions were concentrated in vacuo and slurried in 12 mL of MTBE. The product was filtered, washed with MTBE (2x 4 mL) and hexane (3x 4 mL), and dried under vacuum at 65 °C for 18 hours to afford 5-{1-[(3,5-dimethyl-1H-pyrazol-1-yl)acetyl]-4-fluoro-2,3-dihydro-1H-indol-5-yl}-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**12**) (98 mg) as a white solid. LC-MS (ES) m/z = 420 $[M+H]^+$. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.14 (s, 1H) 7.87 (d, *J* = 8.1 Hz, 1H) 7.28 (s, 1H) 7.21 (t, *J* = 8.1 Hz, 1H) 5.93 - 6.17 (br s, 2H) 5.86 (s, 1H) 5.11 (s, 2H) 4.34 (t, *J* = 8.3 Hz, 2H) 3.74 (s, 3H) 3.29 (t, *J* = 8.3 Hz, 2H) 2.16 (s, 3H) 2.10 (s, 3H).

Kinase selectivity profiling of compound 8. Data for compound **8** (10 µM, n = 2) was obtained from Reaction Biology Corp. (Malvern, PA, www.reactionbiology.com). Reactions were carried out at 10 µM ATP. Raw data indicates residual % enzyme activity (relative to DMSO controls) after compound treatment. Selectivity data for **6** and a description of the GCN2 assay was recently reported.[5]

Table S2. Kinase profile data for compound 8.

Kinase	Data 1	Data 2	Avg
ABL1	16.05	14.9	15.47
ABL2/ARG	24.15	20.95	22.55
ACK1	1.55	0.6	1.07
AKT1	92.69	95.19	93.94
AKT2	96.63	102.79	99.71
AKT3	98.88	92.57	95.73
ALK	81.8	76.62	79.21
ALK1/ACVRL1	99.26	99.35	99.31
ALK2/ACVR1	93.2	93.63	93.41

ALK4/ACVR1B	95.08	91.48	93.28
ALK5/TGFBR1	97.7	92.93	95.32
ARAF	31.39	22.88	27.13
ARK5/NUAK1	56.95	68.26	62.61
ASK1/MAP3K5	103.16	104.39	103.77
Aurora A	30	48.94	39.47
Aurora B	8.79	10.21	9.5
Aurora C	41.91	46.18	44.05
AXL	12.62	11.55	12.09
BLK	52.9	51.11	52.01
BMX/ETK	30.73	29.92	30.33
BRAF	53	47.82	50.41
BRK	3.55	5.53	4.54
BRSK1	102.98	104.72	103.85
BRSK2	99.34	102.09	100.71
BTK	44.22	40.58	42.4
CAMK1a	48.37	46.82	47.59
CAMK1b	61.17	63	62.08
CAMK1d	49.36	45.24	47.3
CAMK1g	76.89	77.26	77.08
CAMK2a	117.41	111.28	114.35
CAMK2b	94.34	89.79	92.06
CAMK2d	72.65	71.05	71.85
CAMK2g	88.78	89.06	88.92
CAMK4	87.79	88.01	87.9
CAMKK1	58.31	50.49	54.4
CAMKK2	90	87.89	88.95
CDK1/cyclin A	103.88	101.72	102.8
CDK1/cyclin B	73.4	81.04	77.22
CDK2/cyclin A	112.22	96.62	104.42
CDK2/cyclin E	85.81	84.82	85.31
CDK3/cyclin E	102.45	96.95	99.7
CDK4/cyclin D1	111.59	109.49	110.54
CDK4/cyclin D3	116.22	109.34	112.78
CDK5/p25	91.59	91.03	91.31
CDK5/p35	100.38	99.15	99.77
CDK6/cyclin D1	104.53	100.8	102.67
CDK6/cyclin D3	95.05	109.14	102.1

CDK7/cyclin H	104.78	103.81	104.29
CDK9/cyclin K	85.74	84.14	84.94
CDK9/cyclin T1	104.63	92.91	98.77
CHK1	108.37	114.24	111.31
CHK2	68.2	68.83	68.52
CK1a1	99.7	94.11	96.9
CK1d	91.7	101.18	96.44
CK1epsilon	7.36	6.73	7.04
CK1g1	87.36	90.39	88.88
CK1g2	68.91	71.78	70.34
CK1g3	96.91	90.33	93.62
CK2a	116.07	105.63	110.85
CK2a2	98.32	88.24	93.28
c-Kit	11.17	11.1	11.13
CLK1	101.31	104.51	102.91
CLK2	107.54	108.07	107.81
CLK3	99.23	92.23	95.73
CLK4	103.33	105.16	104.25
c-MER	0.92	0.84	0.88
c-MET	37.46	30.96	34.21
COT1/MAP3K8	88.41	100.85	94.63
CSK	19.76	20.72	20.24
c-Src	72.04	72.07	72.06
CTK/MATK	122.18	120.16	121.17
DAPK1	94.51	82.77	88.64
DAPK2	94.07	89.71	91.89
DCAMKL2	100.07	96.28	98.18
DDR2	13.97	10.84	12.4
DMPK	91.02	84.96	87.99
DRAK1/STK17A	99.04	97.35	98.19
DYRK1/DYRK1A	96.3	94.12	95.21
DYRK1B	92.47	86.07	89.27
DYRK2	103.1	96.05	99.58
DYRK3	107.17	97.87	102.52
DYRK4	100.16	97.27	98.71
EGFR	101.47	95.44	98.45
EPHA1	94.92	85.98	90.45
EPHA2	75.75	70.64	73.2

EPHA3	95.77	92.32	94.04
EPHA4	69.12	64.04	66.58
EPHA5	89.86	86.01	87.93
EPHA6	7.34	7.75	7.55
EPHA7	35.53	33.54	34.53
EPHA8	91.94	86.41	89.17
EPHB1	90.61	89.63	90.12
EPHB2	92.59	86.95	89.77
EPHB3	63.88	59.71	61.79
EPHB4	71.72	64.81	68.26
ERBB2/HER2	78.01	75.92	76.97
ERBB4/HER4	109.48	94.28	101.88
ERK1	135.62	125.49	130.55
ERK2/MAPK1	107.28	91.93	99.61
FAK/PTK2	105.47	99.07	102.27
FER	48.64	46.32	47.48
FES/FPS	74.78	68.22	71.5
FGFR1	21.83	21.03	21.43
FGFR2	37.41	33.03	35.22
FGFR3	43.38	42.28	42.83
FGFR4	61	58.36	59.68
FGR	48.33	45	46.67
FLT1/VEGFR1	82.96	70.59	76.77
FLT3	19.82	19.39	19.6
FLT4/VEGFR3	38.75	36.59	37.67
FMS	20.6	19.75	20.17
FRK/PTK5	56.33	48.94	52.64
FYN	88.8	89.45	89.12
GCK/MAP4K2	17.77	16.76	17.26
GRK2	103.25	92.9	98.08
GRK3	107.4	103.53	105.47
GRK4	102.88	100.46	101.67
GRK5	113.32	106.17	109.74
GRK6	92.15	93.55	92.85
GRK7	106.05	96.86	101.45
GSK3a	96.36	96.36	96.36
GSK3b	105.76	100.57	103.16
Haspin	79.13	76.14	77.63

HCK	52.2	47.16	49.68
HGK/MAP4K4	90.36	109.65	100.01
HIPK1	64.98	55.03	60
HIPK2	27.67	23.79	25.73
HIPK3	58.7	54.99	56.84
HIPK4	31.12	34.05	32.59
IGF1R	92.24	86.48	89.36
IKKa/CHUK	117.22	115.19	116.2
IKKb/IKBKB	92.24	92.22	92.23
IKKe/IKBKE	3.79	3.67	3.73
IR	18.4	22.03	20.21
IRAK1	1.91	2.26	2.09
IRAK4	158.02	132.45	145.23
IRR/INSRR	26.6	21.87	24.24
ITK	92.52	83.65	88.08
JAK1	97.2	95.51	96.35
JAK2	89.75	80.34	85.04
JAK3	109.89	105.18	107.53
JNK1	108.8	103.4	106.1
JNK2	94.81	82.31	88.56
JNK3	117.76	99.71	108.74
KDR/VEGFR2	57.25	60.55	58.9
KHS/MAP4K5	1.04	0.93	0.99
LCK	14.77	14.38	14.58
LIMK1	75.44	66.17	70.81
LKB1	98.01	99.43	98.72
LOK/STK10	19.81	16.66	18.23
LRRK2	32.35	29.55	30.95
LYN	42.27	44.4	43.33
LYN B	76.96	72.11	74.54
MAPKAPK2	89.45	82.34	85.89
MAPKAPK3	107.53	110.72	109.13
MAPKAPK5/PRAK	83.68	82.91	83.3
MARK1	101.65	94.24	97.95
MARK2/PAR-1Ba	108.29	109.84	109.06
MARK3	97.81	99.28	98.54
MARK4	100.3	99.81	100.06
MEK1	91.79	92.94	92.36

MEK2	100.72	91.29	96.01
MEKK2	3.36	2.41	2.89
MEKK3	9.34	9.07	9.2
MELK	121.7	126.56	124.13
MINK/MINK1	110.16	104.35	107.26
MKK6	104.81	112.91	108.86
MLCK/MYLK	96.89	87.85	92.37
MLCK2/MYLK2	6.63	7.26	6.94
MLK1/MAP3K9	2.2	4.18	3.19
MLK2/MAP3K10	0.6	-0.88	-0.14
MLK3/MAP3K11	1.63	2.7	2.16
MNK1	67.17	64.36	65.77
MNK2	38.92	37.08	38
MRCKa/CDC42BPA	103.65	101.11	102.38
MRCKb/CDC42BPB	89.74	97.14	93.44
MSK1/RPS6KA5	16.96	19.8	18.38
MSK2/RPS6KA4	74.94	80.51	77.72
MSSK1/STK23	87.59	93.59	90.59
MST1/STK4	13.23	12.29	12.76
MST2/STK3	32.66	32.83	32.74
MST3/STK24	121.44	134.85	128.15
MST4	154.76	148.5	151.63
MUSK	88.62	87.73	88.17
MYO3b	50.31	51.26	50.78
NEK1	15.73	15.89	15.81
NEK11	1.13	1.44	1.28
NEK2	83.52	88.21	85.86
NEK3	86.92	80.67	83.8
NEK4	14.08	14.67	14.37
NEK6	78.22	73.69	75.96
NEK7	10.69	7.88	9.29
NEK9	52.25	53.16	52.7
NIK/MAP3K14	103.76	117.59	110.67
NLK	94.92	95.59	95.25
OSR1/OXSR1	61.14	59.62	60.38
P38a/MAPK14	104.5	95.9	100.2
P38b/MAPK11	110.73	101.18	105.96
P38d/MAPK13	67.96	67.46	67.71

P38g	95.74	95.76	95.75
p70S6K/RPS6KB1	75.74	72.22	73.98
p70S6Kb/RPS6KB2	93.44	94.38	93.91
PAK1	76.67	84.71	80.69
PAK2	76.49	69.33	72.91
PAK3	97.97	90.21	94.09
PAK4	102.39	95.56	98.97
PAK5	102.57	97.11	99.84
PAK6	99.88	102.94	101.41
PASK	54.94	51.56	53.25
PBK/TOPK	96.48	95.6	96.04
PDGFRa	86.72	101.04	93.88
PDGFRb	52.39	48.9	50.64
PDK1/PDPK1	112.84	104.36	108.6
PHKg1	101.67	98.19	99.93
PHKg2	104.47	103.04	103.76
PIM1	98.19	99.23	98.71
PIM2	110.25	104.66	107.46
PIM3	117.82	109.96	113.89
PKA	97.08	95.25	96.17
PKAcg	93.65	92.59	93.12
PKCa	131.37	133.09	132.23
PKCb1	103.38	108.4	105.89
PKCb2	107.97	119.8	113.88
PKCd	78.84	75.91	77.37
PKCepsilon	90.34	103.05	96.69
PKCeta	101.57	108.34	104.95
PKCg	87.68	91.27	89.47
PKCiota	90.21	90.11	90.16
PKCmu/PRKD1	62.94	63.53	63.23
PKCnu/PRKD3	48.04	46.02	47.03
PKCtheta	110.66	109.2	109.93
PKCzeta	102.34	100.93	101.63
PKD2/PRKD2	50.1	47.79	48.94
PKG1a	92.85	99.74	96.3
PKG1b	92.76	96.31	94.53
PKG2/PRKG2	114.66	124.56	119.61
PKN1/PRK1	90.38	92.59	91.48

PKN2/PRK2	71.21	74.1	72.65
PLK1	98.79	94.43	96.61
PLK2	48.74	48.4	48.57
PLK3	91.82	86.84	89.33
PRKX	74.43	78.9	76.66
PYK2	82.16	90.52	86.34
RAF1	30.4	30.4	30.4
RET	23.47	17.86	20.67
RIPK2	81.17	94.94	88.06
RIPK5	95.51	107.19	101.35
ROCK1	89.12	92.62	90.87
ROCK2	13.61	11.43	12.52
RON/MST1R	66.32	67.36	66.84
ROS/ROS1	92.85	96.71	94.78
RSK1	44.61	50.03	47.32
RSK2	76.67	78.55	77.61
RSK3	73.03	82.27	77.65
RSK4	100.52	96.31	98.42
SGK1	91.07	88.45	89.76
SGK2	74.32	84.37	79.34
SGK3/SGKL	102.17	103	102.59
SIK2	107	98.04	102.52
SLK/STK2	50.52	50.04	50.28
SNARK/NUAK2	89.57	111	100.29
SRMS	105.51	106.33	105.92
SRPK1	94.05	98.3	96.18
SRPK2	108.41	103.16	105.79
STK16	88.36	89.95	89.16
STK22D/TSSK1	102.64	100.1	101.37
STK25/YSK1	101.82	101.91	101.86
STK33	129.34	88.38	108.86
STK38/NDR1	91.83	92.22	92.03
STK39/STLK3	33.67	42.16	37.91
SYK	104.88	102.83	103.85
TAK1	24.69	26.47	25.58
TAOK1	9.74	10.2	9.97
TAOK2/TAO1	39.3	34.49	36.9
TAOK3/JIK	41.19	33.91	37.55

TBK1	54.77	47.8	51.28
TEC	94.75	99.8	97.27
TGFBR2	91.81	72.48	82.14
TIE2/TEK	14.86	14.02	14.44
TLK2	104.84	91.38	98.11
TRKA	4.52	4.8	4.66
TRKB	14.17	14.12	14.15
TRKC	7.43	7.69	7.56
TSSK2	95.74	87.37	91.55
TTK	72.7	71.17	71.93
TXK	42.6	40.31	41.46
TYK1/LTK	78.61	81.77	80.19
TYK2	88.51	84.78	86.65
TYRO3/SKY	31.71	25.03	28.37
ULK1	15.44	16.77	16.1
ULK2	42.66	44.5	43.58
ULK3	38.69	37.48	38.08
VRK1	98.3	92.07	95.19
WEE1	88.96	77.84	83.4
WNK2	6.97	8.38	7.68
WNK3	13.62	18.21	15.91
YES/YES1	15.9	13.23	14.56
ZAK/MLTK	48.48	45.91	47.19
ZAP70	89.03	86.26	87.64
ZIPK/DAPK3	63.63	61.11	62.37

References

1. Axten, J. M.; Medina, J. R.; Feng, Y.; Shu, A.; Romeril, S.P.; Grant, S.W.; Li, W.H.H.; Heerding, D.A.; Minthorn, E.; Mencken, T.; Atkins, C.; Liu, Q.; Rabindran, S.; Kumar, R.; Hong, X.; Goetz, A.; Stanley, T.; Taylor, J. D.; Sigethy, S.D.; Tomberlin, G.H.; Hassell, A.M.; Kahler, K.M.; Shewchuk, L.M.; Gampe, R.T. Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-

pyrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) *J. Med. Chem.* **2012**, 55, 7193-7207.

2. Davis, I.W., Leaver-Fay, A., Chen, V.B., Block, J.N., Kapral G.J., Wang, X., Murray, L.W., Arendall, W.B., Snoeyink, J., Richardson, J.S., and Richardson, D.C. Molprobity: All-atom contacts and structure validation for proteins and nucleic acids. *Nuc. Acids Res.* **2007**, *35*, W375-83.

3. Bourrie, M, Meunier, V, Berger, Y and Fabre, B. Cytochrome P450 Isoform Inhibitors as a Tool for the Investigation of Metabolic Reactions Catalyzed by Human Liver Microsomes. *J. Pharmacol. Exp. Ther.,* **1996**, *277*, 321-332.

4. Biscoe, M. R.; Buchwald, S. L. *Organic Lett.* **2009**, *11*, 1773–1775.

5. Atkins, C.; Liu, Q.; Minthorn, E.; Zhang, S.; Figueroa, D.J.; Moss, K.; Stanley, T.B.; Sanders, B.; Goetz, A.; Gaul, N.; Choudhry, A.E.; Alsaid, H.; Jucker, B.M.; Axten, J.M.; Kumar, R. Characterization of a novel PERK kinase inhibitor with anti-tumor and anti-angiogenic activity. *Cancer Res.* **2013***, 73,* 1993-2002.