

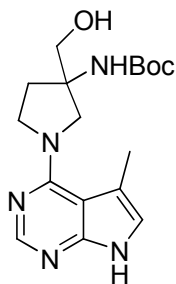
Supporting Information

The Design of Selective, ATP-Competitive Inhibitors of Akt

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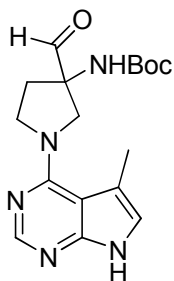
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I. Synthesis of racemic aniline analog intermediates**A1**

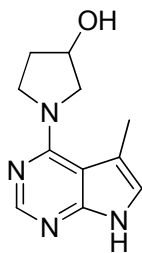
Synthesis of *tert*-butyl 3-(hydroxymethyl)-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-ylcarbamate (**A1**). A solution of 4-chloro-5-methyl-7H-pyrrolo[2,3-d]pyrimidine (**15**, 1.51 g; 9.25 mmol) in 2-propanol (16 mL) was treated with DIPEA (5 mL; 27.25 mmol) followed by (3-hydroxymethyl-pyrrolidin-3-yl)-carbamic acid *tert*-

butyl ester (**16**, 2.00 g; 9.25 mmol, see Tomita et al., J. Med. Chem. 2002, 45, 5564). The reaction mixture was stirred at 80 °C overnight. The reaction mixture was then concentrated, treated with ethyl acetate (100 mL), and filtered. The solids were rinsed with ethyl acetate (2 x 75 mL) and dried to provide *tert*-butyl 3-(hydroxymethyl)-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-ylcarbamate (**A1**). Yield: 2.31 g, 72 %. MS^+ (M+1)⁺: 348.1; t_R (LCMS standard): 1.1 min.

**17**

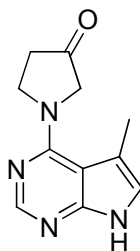
Synthesis of *tert*-butyl 3-formyl-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-ylcarbamate (**17**). A solution of *tert*-butyl 3-(hydroxymethyl)-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-ylcarbamate (**A1**) (1.40 g; 4.0 mmol) in DMSO (19 mL) was cooled to 0 °C, treated with triethylamine (1.65 mL; 12.04 mmol), and stirred at 0 °C for 10 min. The reaction mixture was then treated with a solution of SO₃-pyridine complex (1.90 g; 12.04 mmol) in DMSO (6 mL). The mixture was stirred at 25 °C for 2 h., treated with ethyl acetate (300 mL), and washed in with brine (75 mL), H₂O (75 mL), 5 % aq NaHCO₃ (75 mL) and sat aq. CuSO₄ (25 mL). The organic phase was collected, dried over Na₂SO₄, filtered, and concentrated to provide *tert*-butyl 3-formyl-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-ylcarbamate (**17**). Yield: 1.29 g, 93 %. MS^+ (M+1)⁺: 346.1; t_R (LCMS standard): 1.1 min.

II. Synthesis of racemic amide analog intermediates

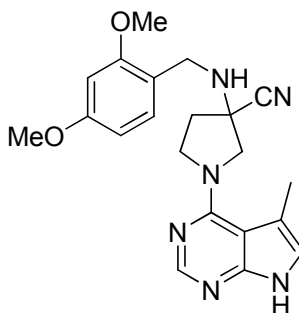
**A2**

Step 1. Synthesis of 1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-ol (**A2**). A solution of 4-chloro-5-methyl-7H-pyrrolo[2,3-d]pyrimidine (9.87 g; 57.5 mmol) in 2-propanol (100 mL) was treated with diisopropylethyl amine (16 mL; 86.3 mmol) followed by 3-pyrrolidinol (5.26 g; 60.4 mmol). The reaction mixture was stirred at 80 °C overnight. The reaction mixture was concentrated and the resultant slurry was treated with ethyl acetate (40 mL). The precipitate was collected by filtration, rinsed with ethyl

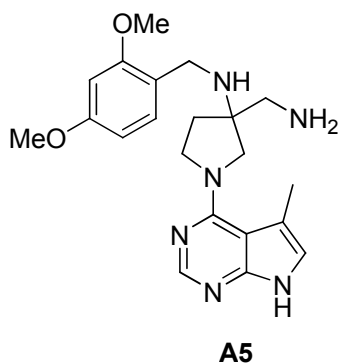
acetate (2 x 75 mL) and dried to provide 1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-ol (**A2**). Yield: 10.6 g, 84%. MS^+ (M+1) $^+$: 219.1; t_R (LCMS polar): 0.5 min.

**A3**

Step 2. Synthesis of 1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-one (**A3**). A solution of 1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-ol (**A2**) (10.6 g; 48.5 mmol) in DMSO (22.2 mL) was cooled to 0°C and treated with triethylamine (22.2 mL; 124 mmol). After stirring at 0 °C for 10 min., SO₃-Pyridine complex (10.62 g; 67.9 mmol) was added in 3 portions over 20 min, and the mixture was stirred at 25 °C overnight. The reaction was treated with chloroform (40 mL), and the resultant precipitate was collected by filtration and rinsed with chloroform to provide 1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-one (**A3**). Yield: (7.0 g, 67 %). MS^+ (M+1) $^+$: 217.1; t_R (LCMS standard): 1.0 min.

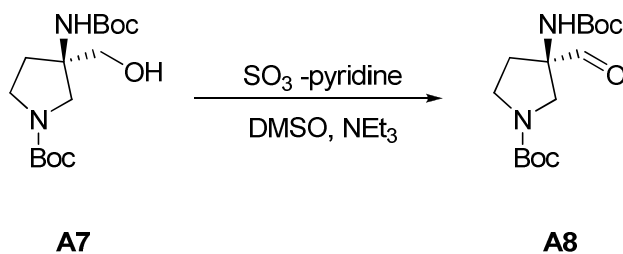
**A4**

Step 3. Synthesis of 3-(2,4-dimethoxybenzylamino)-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidine-3-carbonitrile (**A4**). A mixture of 1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-one (**A3**) (5.68 g; 26.3 mmol), trimethylsilyl cyanide (1.65 mL; 30.8 mmol) and ZnCl₂ (473 mg; 3.47 mmol) was stirred at 25 °C for 15 min. A solution of 2,4-dimethoxybenzylamine (4.4 mL; 29 mmol) in MeOH (130 mL) was then added dropwise, and the mixture was heated to 80 °C and stirred. After 24 hours the precipitate was collected by filtration, rinsed with ethyl acetate (2 x 100 mL), and dried to provide 3-(2,4-dimethoxybenzylamino)-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidine-3-carbonitrile (**A4**). Yield: 7.5 g, 73 %. MS^+ (M+1) $^+$: 393.3; t_R (LCMS standard): 1.7 min.



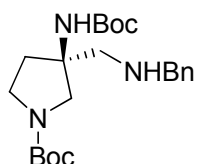
Step 4. Synthesis of **3-(aminomethyl)-N-(2,4-dimethoxybenzyl)-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-amine (A5)**. Lithium aluminum hydride (1.0 M in THF, 9.0 mL, 9.0 mmol) was added to a solution of 3-(2,4-dimethoxybenzylamino)-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidine-3-carbonitrile (**A4**) (3.0 g; 7.6 mmol) in DCM (40 mL), and the solution was stirred at 25 °C for 16 h. The mixture was treated with 1N NaOH (50 mL), and the resultant organic phase was collected. The aqueous phase was extracted with ethyl acetate (3 x 150 mL), and the combined organic phases were washed with brine (75 mL), dried over Na₂SO₄, filtered, and concentrated to provide 3-(aminomethyl)-N-(2,4-dimethoxybenzyl)-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-amine (**A5**). Yield: 3.0 g, 99 %. MS⁺ (M+1)⁺: 397.3; t_R (LCMS standard): 0.4 min.

III. Synthesis of non-racemic amide analog intermediates



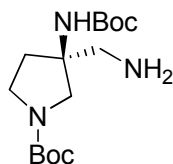
Step 1. Synthesis of **(R)-tert-butyl 3-(tert-butoxycarbonylamino)-3-formylpyrrolidine-1-carboxylate (A8)**. A solution of a SO₃-pyridine complex (63.0g, 396 mol) in 340 mL DMSO under a nitrogen atmosphere was cooled in a salt-packed ice bath and treated with triethylamine (57 mL, 409 mmol). The resultant slurry was then treated drop-wise with a solution of (R)-tert-butyl 3-(tert-butoxycarbonylamino)-3-(hydroxymethyl)pyrrolidine-1-carboxylate (**A7**) (42.0g, 132 mmol, for the preparation of this intermediate, see WO 2008012635), in DMSO (170 mL) while maintaining an internal temperature below 25°C. The reaction mixture was slowly warmed to 25 °C and

stirred for 3 h. The reaction mixture was cooled to 0 °C and treated with pre-chilled ethyl acetate (1.2 L). The mixture was treated with brine (420 mL), and the resultant organic phase was collected and washed with brine (420 mL), water (420 mL), sat. NaHCO₃ (2 x 420 mL), and saturated aq. CuSO₄ (2x 175 mL). The organic phase was dried over MgSO₄, filtered, and concentrated to provide (R)-tert-butyl-3-(tert-butoxycarbonyl)-3-formylpyrrolidine-1-carboxylate as a white solid. Yield: 39.4g, 95%. ¹H NMR (400 MHz, DMSO-d₆) δ ppm 9.41 (s, 1H), 7.90 (m, 1H), 3.63 (m, 1H), 3.40 - 3.20 (5H), 1.83 - 2.08 (m, 2H), 1.39 (s, 18H).



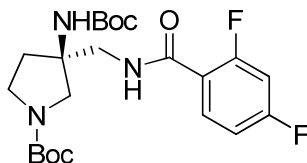
A9

Step 2. Synthesis of **(S)-tert-butyl 3-((benzylamino)methyl)-3-(tert-butoxycarbonyl)pyrrolidine-1-carboxylate (A9)**. A mixture of (R)-tert-butyl-3-(tert-butoxycarbonyl)-3-formylpyrrolidine-1-carboxylate (A8) (38.0g, 121 mmol), benzyl amine (13.2 mL, 121 mmol) and 3A molecular sieves (16 g) in anhydrous MeOH (500 mL) was stirred at 25°C under nitrogen for 18 h. Sodium borohydride (6.9 g, 182 mmol) was added in 3 portions, and the reaction mixture was stirred for 3 h. The mixture was concentrated, and the residue was treated with ethyl acetate (300 mL). The resultant organic phase was washed three times with 1N NaOH (40 mL), and the combined organic phases were dried over Na₂SO₄ filtered, and concentrated. The resultant residue was passed through a plug of silica eluting with 100% EtOAc to provide (S)-tert-butyl 3-((benzylamino)methyl)-3-(tert-butoxycarbonyl)pyrrolidine-1-carboxylate (A9) as a colorless residue. Yield: 48.6g, 99%. ¹H NMR (400 MHz, methanol-d₄) δ ppm 7.30 - 7.28 (m, 5H), 4.86 (s, 2H), 3.77 - 3.53 (m, 2H), 3.38-3.28 (m, 2H), 2.8 (dd, 2H), 2.2-1.8 (m, 2H), 1.43 (m, 18H).

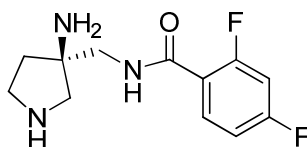


A10

Step 3. Synthesis of **(S)-tert-butyl 3-(aminomethyl)-3-(tert-butoxycarbonyl)pyrrolidine-1-carboxylate (A10)**. A solution of (S)-tert-butyl 3-((benzylamino)methyl)-3-(tert-butoxycarbonyl) pyrrolidine-1-carboxylate (A9) (48.0 g, 118 mmol) in THF (140 mL) and MeOH (570 mL) was treated with 10% palladium on carbon (15 g, 50% wet). The reaction mixture was then hydrogenated for 18h (40 psi, H₂ (g)). The mixture was filtered through Celite and the solids washed with ethyl acetate. The combined filtrates and washings were concentrated to provide (S)-tert-butyl 3-(aminomethyl)- 3-(tert-butoxycarbonyl)pyrrolidine-1-carboxylate (A10) as a white solid. Yield: 35.5g, 95% yield. APCI MS+ (M+1) = 316.5. ¹H NMR (400 MHz, methanol-d₄) δ ppm 3.7-3.5 (m, 2H), 3.4-3.2 (m, 3H), 3.0-2.7 (m, 1H), 2.2-1.85 (m, 2H), 1.45 (m, 18H).

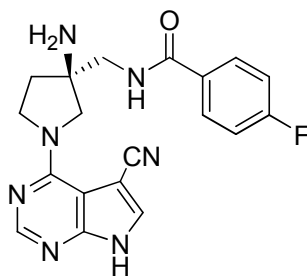
**A11**

Step 4. Synthesis of **(S)-tert-butyl 3-(tert-butoxycarbonyl)-3-((2,4-difluorobenzamido)methyl)pyrrolidine-1-carboxylate (A11)**. A solution of (S)-tert-butyl 3-(aminomethyl)-3-(tert-butoxycarbonyl)pyrrolidine-1-carboxylate (**A10**) (36 g, 110 mmol) in THF (384 mL) and DMF (1 mL) was cooled to 0 °C and treated with 2,4-difluorobenzoyl chloride. The resulting mixture was stirred at 0 °C for 10 min., and *N,N*-diisopropylethylamine (23.87 mL, 137 mmol) was added over 10 min. The reaction mixture was then stirred at 20 °C overnight. The mixture was treated with water (250 mL) and extracted with ethyl acetate (3 x 200 mL). The combined organic extracts were washed with water (1x 100 mL) and brine (1x 100 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resultant white foam was purified by silica gel chromatography (300 g, 0-100% ethyl acetate in hexanes) to provide (S)-tert-butyl 3-(tert-butoxycarbonyl)-3-((2,4-difluorobenzamido)methyl)pyrrolidine-1-carboxylate (**A11**) as a white solid. Yield: 48.8g, 93%. TLC R_f = 0.25 (1:1 ethyl acetate / hexanes). APCI MS+ (M+1) = 490.1.

**A12**

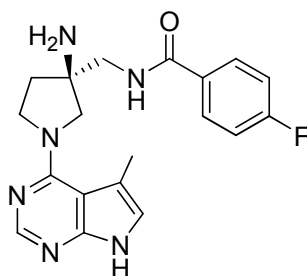
Step 5: **Synthesis of (R)-N-((3-aminopyrrolidin-3-yl)methyl)-2,4-difluorobenzamide (A12)**. Acetyl chloride (76 mL, 1071 mmol) was added to anhydrous methanol (300 mL) with stirring at 0°C. After 30 min., a solution of (S)-tert-butyl 3-(tert-butoxycarbonyl)-3-((2,4-difluorobenzamido)methyl)pyrrolidine-1-carboxylate (**A11**) (48.8 g, 107 mmol) in methanol (170 mL) was added drop-wise, and the reaction mixture was warmed to 20°C and stirred for 2 h. The mixture was then concentrated under reduced pressure to provide (R)-N-((3-aminopyrrolidin-3-yl)methyl)-2,4-difluorobenzamide (**A12**) as a white solid which was used without further purification. Yield: 35 g, 99%. TLC R_f = 0.1 (1:1 ethyl acetate / hexanes).

IV. Synthesis of analogs 31-41



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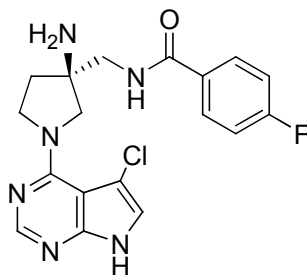
Synthesis of **(S)-N-((3-amino-1-(5-cyano-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-4-fluorobenzamide (31)**. Compound **31** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-fluorobenzoyl chloride in step 4 in place of 2,4-difluorobenzoyl chloride, and using 4-chloro-7H-pyrrolo[2,3-d]pyrimidine-5-carbonitrile as a coupling partner in place of 4-chloro-5-ethyl-7H-pyrrolo[2,3-d]pyrimidine. ^1H NMR (DMSO- d_6) δ ppm 8.53 (t, $J = 5.7$ Hz., 1H), 8.22 (s, 1H), 8.19 (s, 1H), 7.95 (dd, $J = 8.8, 5.7$ Hz., 2H), 7.30 (dd, $J = 8.8, 8.8$ Hz., 2H), 3.93-3.90 (m, 1H), 3.71 (d, $J = 11.4$ Hz., 1H), 3.57 (d, $J = 11.4$ Hz., 1H), 3.47 (d, $J = 5.7$ Hz., 2H), 3.48-3.46 (m, 1H), 2.05-1.83 (m, 4H) ppm.



32

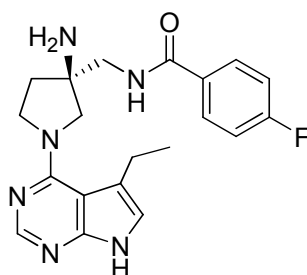
Synthesis of **(S)-N-((3-amino-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-4-fluorobenzamide (32)**. Compound **32** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-fluorobenzoyl chloride in step 4 in place of 2,4-difluorobenzoyl chloride, and using 4-chloro-5-methyl-7H-pyrrolo[2,3-d]pyrimidine as a coupling partner in place of 4-chloro-5-ethyl-7H-pyrrolo[2,3-d]pyrimidine. ^1H NMR (DMSO- d_6) δ ppm 11.3 (bs, 1H), 8.56 (t, $J = 5.7$ Hz., 1H), 8.04 (s, 1H), 7.94 (dd, $J = 8.8, 5.7$ Hz., 2H), 7.30 (dd, $J = 8.8, 8.8$ Hz., 2H), 6.93 (s, 1H), 3.88-3.82 (m, 1H), 3.75-3.71 (m, 1H), 3.68 (d, $J = 10.9$ Hz., 1H), 3.47 (m, 3H), 2.31 (s, 3H), 1.95-1.73 (m, 4H) ppm.

S8



33

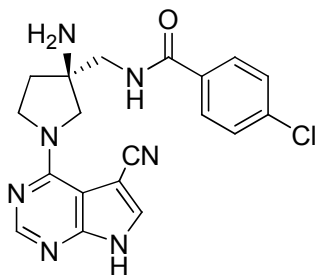
Synthesis of **(S)-N-((3-amino-1-(5-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-4-fluorobenzamide (33)**. Compound **33** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-fluorobenzoyl chloride in step 4 in place of 2,4-difluorobenzoyl chloride, and using 4,5-dichloro-7H-pyrrolo[2,3-d]pyrimidine as a coupling partner in place of 4-chloro-5-ethyl-7H-pyrrolo[2,3-d]pyrimidine. MS⁺ (M+1)⁺: 389.1; t_R (LCMS polar): 2.3 min. ¹H NMR (500 MHz, methanol-d₄) δ ppm 1.97-2.02 (1H, m), 2.14-2.20 (1H, m), 3.64 (2H, d), 3.77 (1H, d), 4.01 (1H, d), 4.03-4.11 (2H, m), 7.18-7.22 (3H, m), 7.89-7.92 (2H, q), 8.10 (1H, s) ppm



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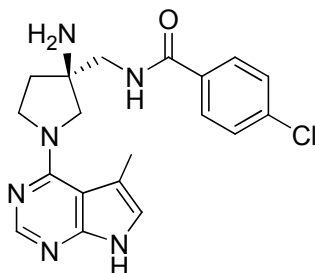
Synthesis of **(S)-N-((3-amino-1-(5-ethyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-4-fluorobenzamide (34)**. Compound **34** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-fluorobenzoyl chloride in step 4 in place of 2,4-difluorobenzoyl chloride. APCI MS⁺ (M+1) = 383.3. ¹H NMR (DMSO-d₆) δ ppm 11.3 (bs, 1H), 8.57 (t, *J* = 5.9 Hz., 1H), 8.04 (s, 1H), 7.95 (dd, *J* = 8.8, 5.7 Hz., 2H), 7.31 (dd, *J* = 8.8, 8.8 Hz., 2H), 6.90 (s, 1H), 3.91-3.86 (m, 1H), 3.73-3.71 (m, 1H), 3.70 (d, *J* = 10.3 Hz., 1H), 3.46-3.34 (m, 3H), 2.75 (q, *J* = 7.3 Hz., 2H), 1.97-1.71 (m, 4H), 1.18 (t, *J* = 7.3 Hz., 3H) ppm.

S9



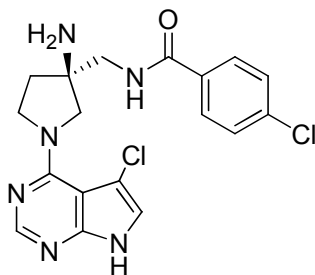
35

Synthesis of **(S)-N-((3-amino-1-(5-cyano-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-4-chlorobenzamide (35)**. Compound **35** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-chlorobenzoyl chloride in step 4 in place of 2,4-difluorobenzoyl chloride, and using 4-chloro-7H-pyrrolo[2,3-d]pyrimidine-5-carbonitrile as a coupling partner in place of 4-chloro-5-ethyl-7H-pyrrolo[2,3-d]pyrimidine. APCI MS⁺ (M+1) = 396.4. ¹H NMR (500 MHz, methanol-d₄) δ ppm 8.21 (s, 1 H), 7.98 (s, 1 H), 7.86 (d, *J*=8.29 Hz, 2 H), 7.49 (d, *J*=6.74 Hz, 2 H), 4.14 (m, 2 H), 4.03 (d, *J*=11.40 Hz, 1 H), 3.86 (d, *J*=11.92 Hz, 1 H), 3.70 (s, 2 H), 2.29 (m, 1 H), 2.13 (m, 1 H)

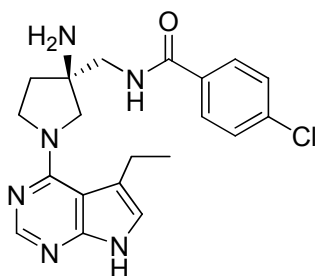


36

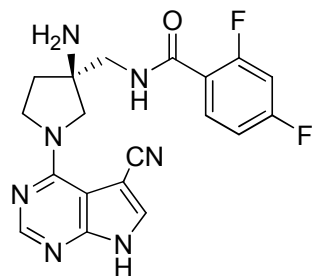
Synthesis of **(S)-N-((3-amino-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-4-chlorobenzamide (36)**. Compound **36** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-chlorobenzoyl chloride in step 4 in place of 2,4-difluorobenzoyl chloride, and using 4-chloro-5-methyl-7H-pyrrolo[2,3-d]pyrimidine as a coupling partner in place of 4-chloro-5-ethyl-7H-pyrrolo[2,3-d]pyrimidine. APCI MS⁺ (M+1) = 385.4. ¹H NMR (500 MHz, methanol-d₄) δ ppm 8.06 (s, 1 H), 7.83 (d, 2 H), 7.49 (d, *J*=8.81 Hz, 2 H), 6.93 (s, 1 H), 3.86 - 4.05 (m, 3 H), 3.60 - 3.70 (m, 3 H), 2.45 (s, 3 H), 2.13 - 2.21 (m, 1 H), 1.96 - 2.03 (m, 1 H)

**37**

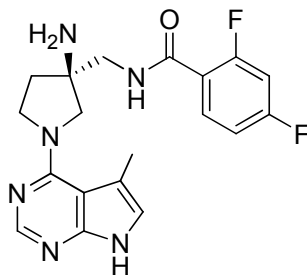
Synthesis of **(S)-N-((3-amino-1-(5-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-4-chlorobenzamide (37)**. Compound **37** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-chlorobenzoyl chloride in step 4 in place of 2,4-difluorobenzoyl chloride, and using 4,5-dichloro-7H-pyrrolo[2,3-d]pyrimidine as a coupling partner in place of 4-chloro-5-ethyl-7H-pyrrolo[2,3-d]pyrimidine. ^1H NMR (500 MHz, methanol- d_4) δ ppm 1.97-1.99 (1H, m), 2.15-2.18 (1H, m), 3.64 (2H, d), 3.76 (1H, d), 4.01 (1H, d), 4.04-4.09 (2H, m), 7.18 (1H, s), 7.48 (1H, s), 7.49 (1H, s), 7.82 (1H, s), 7.84 (1H, s), 8.10 (1H, s) ppm

**38**

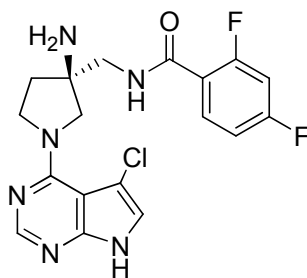
Synthesis of **(S)-N-((3-amino-1-(5-ethyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-4-chlorobenzamide (38)**. Compound **38** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-chlorobenzoyl chloride in step 4 in place of 2,4-difluorobenzoyl chloride. APCI MS+ ($M+1$) = 399.4. ^1H NMR (500 MHz, methanol- d_4) δ ppm 8.05 (s, 1 H), 7.83 (d, $J=8.29$ Hz, 2 H), 7.49 (d, $J=8.29$ Hz, 2 H), 6.93 (s, 1 H), 3.85 - 4.05 (m, 3 H), 3.57 - 3.67 (m, 3 H), 2.79 - 2.91 (m, 2 H), 2.10 - 2.20 (m, 1 H), 1.90 - 1.98 (m, 1 H), 1.28 (t, $J=7.26$ Hz, 3 H)

**39**

Synthesis of **(S)-N-((3-amino-1-(5-cyano-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-2,4-difluorobenzamide (39)**. Compound **39** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-chloro-7H-pyrrolo[2,3-d]pyrimidine-5-carbonitrile as a coupling partner in place of 4-chloro-5-ethyl-7H-pyrrolo[2,3-d]pyrimidine. ¹H NMR (500 MHz, methanol-d₄) δ ppm 8.22 (s, 1 H), 7.99 (s, 1 H), 7.83 (m, 1 H), 7.10 (m, 2 H), 4.15 (m, 2 H), 4.02 (d, *J*=11.92 Hz, 1 H), 3.85 (d, *J*=11.40 Hz, 1 H), 3.70 (q, *J*=13.82 Hz, 2 H), 2.28 (m, 1 H), 2.12 (m, 1 H)

**40**

Synthesis of **(S)-N-((3-amino-1-(5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-2,4-difluorobenzamide (40)**. Compound **40** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4-chloro-5-methyl-7H-pyrrolo[2,3-d]pyrimidine) as a coupling partner in place of 4-chloro-5-ethyl-7H-pyrrolo[2,3-d]pyrimidine. ¹H NMR (500 MHz, methanol-d₄) δ ppm 8.07 (s, 1 H), 7.79 (m, 1 H), 7.11 (m, 2 H), 6.94 (s, 1 H), 4.02 (t, *J*=9.07 Hz, 1 H), 3.93 (m, 2 H), 3.66 (m, 3 H), 2.44 (s, 3 H), 2.18 (m, 1 H), 1.99 (m, 1 H)

**41**

Synthesis of **(S)-N-((3-amino-1-(5-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrrolidin-3-yl)methyl)-2,4-difluorobenzamide (41)**. Compound **41** was prepared by an identical sequence of reactions as was used to prepare compound **42**, but using 4,5-dichloro-7H-pyrrolo[2,3-d]pyrimidine) as a coupling partner in place of 4-chloro-5-ethyl-7H-pyrrolo[2,3-d]pyrimidine. MS⁺ (M+1)⁺: 407.3; t_R (LCMS polar): 2.0 min. ¹H NMR (500 MHz, methanol-d₄) δ ppm 1.92-1.99 (1H, m), 2.10-2.19 (1H, m), 3.61 (2H, s), 3.73 (1H, d), 3.98 (1H, d), 4.03-4.06 (2H, m), 7.05-7.08 (2H, m), 7.16 (1H, s), 7.75-7.76 (1H, m), 8.08 (1H, s) ppm

V. Crystallography

Akt expression, purification and co-crystallization was carried out as described previously (see reference 39). Human PKA-alpha (1-350) was expressed in *E. coli*, with an N-terminal 6x-His tag, followed by a thrombin cleavage site. The protein was purified by two rounds of Ni-affinity purification, before and after cleavage of the 6x-His tag by thrombin. Co-crystals of a ternary complex of hPKA, inhibitor and a 20 amino acid peptide representing residues 5-24 of PKI were obtained by vapor diffusion. The reservoir buffer was 0.1M Tris (pH 8.5), 0.2M LiSO₄ and 25% PEG 3350. The protein solution contained 6mg/ml protein, 50mM Bicine (pH 8.0), 200mM NH₄Acetate, 2mM TCEP, 2mM PMSF, 0.5mM PKI5-24, 2.5mM MgCl₂, 0.5mM inhibitor. Diffraction data to 2.5Å were collected from thin, plate-like crystals, and the structure solved by molecular replacement, using the bovine PKA structure as a reference model. Coordinates and structure factors of all structures have been deposited in the Protein Data Bank with PDB Ids: (Compound **5** – Akt1: 3MV5; Compound **5** – PKA:3MVJ; Compound **42** – Akt1:3MVH).

VI. Pharmacokinetic Studies

Summary Pharmacokinetics of **42** Observed in Preclinical Species.

Species	Route	Dose (mg/kg)	AUC _(0-∞) IV AUC _(0-tlast) PO (ng*hr/mL)	CL _b ¹ (mL/min/kg)	VD _{ss} (L/kg)	T _{1/2} (hr)	C _{max} (ng/mL)	T _{max} (hr)	F ² (%)
Rat	IV ³ (n=3)	2	369 ± 27.9	75.6 ± 5.90	5.73 ± 0.32	1.47 ± 0.07	1080 ± 204	---	---
	PO fast (n=3)	5	1180 ± 648	---	---	---	689 ± 455	0.33 ± 0.14	>100
Dog	IV ³ (n=3)	1.2	1440 ± 464	11.6 ± 4.48	4.80 ± 0.91	4.41 ± 0.82	602 ± 111	---	---
	PO fast (n=3)	2	1280 ± 234	---	---	---	267 ± 31.3	0.42 ± 0.14	54

All data represented in 3 significant figures. Rat PO PK used JVC/PVC rats, only systemic concentrations shown.

$$^1 \text{CL}_b = \text{CL}_p / (\text{B:P})$$

$$^2 \%F = \frac{\text{AUC}(0\text{-tlast})\text{po systemic (ng*hr/ml)}}{\text{AUC}(0\text{-inf})\text{iv systemic (ng*hr/ml)}} * \frac{\text{Dose IV (mg/kg)}}{\text{Dose PO (mg/kg)}}$$

³<2% unchanged parent excreted in the urine (0-24hrs).

Plasma and Microsomal Protein Binding (fraction unbound, f_u) and RBC Partitioning (C_b/C_p) for **42**.

Species	Mouse	Rat	Dog	Human
Plasma Protein Binding @ 1 μM (f_u)	0.296	0.330	0.324	0.261
Plasma Protein Binding @ 10 μM (f_u)	0.274	0.305	0.273	0.239
Microsomal Protein Binding @ 1 μM (f_u)	ND	0.667	0.65	0.805
Blood Partitioning (C_b/C_p) @ 1 μM	1.2	1.2	1.3	1.0

ND = Not determined.

VII. Pharmacodynamic Studies

Levels of phosphorylated S6 and Akt were used as pharmacodynamic (PD) measures of Akt/p70S6K1 inhibition. S6 is a substrate of p70S6K1 following stimulation of the PI3K pathway and, upon Akt/p70S6K1 inhibition, the levels of phospho-S6 decrease in a dose responsive manner. Conversely, the blockade of Akt/p70S6K1 also results in the loss of an Akt feedback loop (the phosphorylation of IRS regulatory proteins by p70S6K1), which results in the hyperphosphorylation of Akt on T308 and S473. As Akt and p70S6K1 are both components of the IGF-1R/PI3K/Akt/mTOR signaling and feedback pathways, pharmacodynamic changes in phospho-S6 and phospho-Akt directly reflect the net effects of dual Akt/p70S6K1 blockade.

To define the in vivo potency of **42** in the PC3 xenograft model, oral administration of 25, 75, and 100 mg/kg **42** was performed with blood and tumor sampling over time. Immunoblot analysis of detergent-soluble extracts derived from PC3 tumors showed a significant reduction of S6 phosphorylation, and hyperphosphorylation of Akt upon treatment at doses that produced significant tumor growth inhibition. Plasma drug concentrations peaked rapidly after oral administration of doses between 25-100 mg/kg ($T_{max} = 0.5h$). Peak PD responses of phospho-S6 and phospho-Akt were observed at approximately 2-4h and 1h post-administration of **42**, respectively. The time-course of PD marker response was well described by a PK/PD model at doses that ranged from no efficacy (25 mg/kg) to maximal efficacy (100 mg/kg). Free IC_{50} and EC_{50} values were estimated for phospho-S6 reduction (110 nM) and Akt hyperphosphorylation (216 nM), respectively. These values corresponded well to the cellular IC_{50} for **42** in U87 cells measuring p-GSK-3 α (310 nM). A clear PK/PD relationship for **42** in the PC3 xenograft model was established after single dose oral administration.

In Vivo Pharmacological Properties of **42**

Oral dose (mg/kg)	PC3 tumor growth inhibition ² (bidx10)	Single dose PK and PD ¹								
		C _{max} (ng/ml) [free]	AUC (ng*hr/ml) [free]	C _{ave} ³ (nM) [free]	phospho-S6 reduction			phospho-Akt induction		
					PD _{max} ⁴ (%)	AUIC ⁵ (%*hr)	I _{ave} ⁶ (%)	PD _{max} ⁴ (%)	AUEC ^e (%*hr)	E _{ave} ⁶ (%)
100	75%	3049 [903]	10698 [3167]	1113 [329]	81	1152	48	425	6813	284
75	51%	1996 [591]	5746 [1701]	598 [177]	75	822	34	408	5879	245
25	25%	358 [106]	435 [129]	45 [13]	35	207	9	302	4352	181

¹Studies to describe the PK/PD relationship for **42** were performed in male SCID/Beige mice bearing subcutaneous PC3 prostate carcinoma xenografts. Once tumors reached about ~300mm³ in size, **42** was formulated in 0.5% methylcellulose vehicle and administered orally to 3 mice per dose group. Plasma and tumors were harvested over time, tumor lysates prepared, and the levels of phospho S6 reduction and phospho Akt induction were evaluated by immunoblot.

²Tumor growth inhibition values were determined in separate studies following 10 twice daily doses (n=7-15 mice per dose group). TGI values at 75 and 100 mg/kg represent mean of 2 experiments.

³C_{ave} is the average plasma concentration over the 24h period following a single dose.

⁴PD_{max} following a single dose based on model predictions

⁵AUIC and AUEC - area under the inhibitory/effect curve over a 24h period following a single dose, based on model predictions.

⁶I_{ave} and E_{ave} - average inhibition/effect over a 24h period following a single dose, based on model predictions.