Discovery of a Novel Series of Potent and Selective Alkynylthiazole-Derived PI3Kγ Inhibitors

Upul K. Bandarage*, Alex M. Aronov, Jingrong Cao, Jon H. Come, Kevin M. Cottrell, Robert J. Davies, Simon Giroux, Marc Jacobs, Sudipta Mahajan, David Messersmith, Cameron S. Moody, Rebecca Swett, Jinwang Xu.

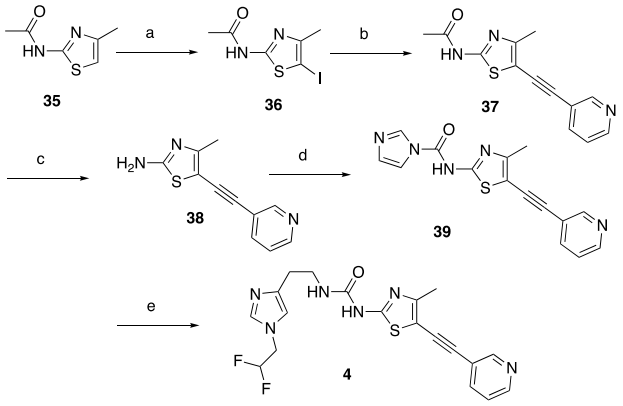
Vertex Pharmaceuticals Incorporated, 50 Northern Avenue, Boston, Massachusetts 02210, United States

Content

EXPERIMENTAL SECTION

All commercially available reagents and anhydrous solvents were used without further purification. Purity assessment for final compounds based on analytical HPLC: column, 4.6 x 50 mm Waters YMC Pro-C18 column, 5 μM, 120Å. Mobile phases are as follows: A, water with 0.2% formic acid; B, acetonitrile with 0.2% formic acid; gradient, 10-90% B in 3 min with 5 min run time. The flow rate is 1.5 mL/min. All compounds were ≥ 95% purity. Mass samples were analyzed on a Waters/MicroMass ZQ, ZMD, Quattro LC, or

Quatro II mass spectrometer operated in a single MS mode with electrospray ionization. Samples were introduced into the mass spectrometer using flow injection (FIA) or chromatography. The mobile phase for all mass analysis consisted of acetonitrile-water mixtures with either 0.2% formic acid or ammonium formate. High-resolution mass measurements were performed on a Thermo Q Exactive mass spectrometer with a heated electrospray source operated in positive ion mode. 1H NMR spectra were recorded either using a Bruker Avance 400 (400 MHz) or a Bruker Avance II-300 (300 MHz) instrument and are quoted in ppm relative to a tetramethylsilane internal standard, or by referencing on the chemical shift of the deuterated solvent. The column chromatography was performed using Teledyne ISCO RediSep Normal Phase (35-70 microns) or RediSep Gold Normal Phase (25-40 microns) silica flash columns using a Teledyne ISCO Combiflash Companion or Combiflash Rf purification system. Preparative reversed phase chromatography was carried out using a Gilson 215 liquid handler coupled to a UV-Vis 156 Gilson detector, an Agilent Zorbax SB-C18 column, 21.2 x 100 mm, a linear gradient from 10-90% acetonitrile in water over 10 min (0.1% TFA); the flow rate was 20 mL/min. Microwave-assisted reactions were performed using a CEM Discover S-Class microwave instrument (single mode microwave reactor) with 48-position autosampler. Temperature was monitored during microwave reactions by a vertically sensored infrared temperature sensor, which comes as a standard feature of the CEM Discover S-Class system.



Reagents and conditions. (a) NIS, DCM, r.t., 10 min, 66% (b) 3-ethynylpyridine, $Pd(PPh_3)_4$, CuI, Et_3N, dioxane, r.t., 1.5 h, 77% (c) hydrazine hydrate, THF, 85 °C, 6 h, 77% (d) CDI, DMF, 70 °C, 2 h, 92% (e) 2-(1-(2,2-difluoroethyl)-1*H*-imidazol-4-yl)ethan-1-amine, Et_3N, THF, r.t., 20 h, 58%.

***N*-(5-Iodo-4-methyl-thiazol-2-yl)acetamide (36)**

To a stirred solution of *N*-(4-methylthiazol-2-yl)acetamide, **35** (5.2 g, 33.33 mmol) in acetonitrile (100 mL) was added NIS (8.98 g, 39.95 mmol) in small portions at room temperature. The resultant suspension was stirred at room temperature for 10 min. The precipitate was collected by filtration and the precipitate was washed with cold CH_3CN (25 mL), dried in high vacuum for 6 h to afford compound **36** (6.2 g, 66%) as off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 2.57 (s, 3H), 2.12 (s, 3H). mass spectrum (ESI) m/z 283.0 $[M + H]^+$ ($C_6H_7IN_2OS$) requires 282.9.

***N*-(4-Methyl-5-(pyridin-3-ylethynyl)thiazol-2-yl)acetamide (37)**

Compound **36** (500 mg, 1.77 mmol) and 3-ethynylpyridine (228 mg, 2.22 mmol) were stirred in THF (5 mL). After purging the solution with N_2, CuI (34 mg, 0.177 mmol) and bis(triphenylphosphine)palladium(II) chloride (124 mg, 0.177 mmol) were added, followed by the addition of Et_3N (538 mg, 5.32 mmol). The reaction mixture was stirred at RT for 1.5 h. The reaction mixture was filtered and the volatiles were removed under reduced pressure to produce compound **37** (300 mg, 77%): ^{1}HNMR (DMSO-d_6) δ 12.43 (s, 1H), 8.74 (s, 1H), 8.57 (d, J = 2.9 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.46 (dd, J = 4.8, 7.9 Hz, 1H), 2.41 (s, 3H), and 2.16 (s, 3H); mass spectrum (ESI) m/z $[M + H]^+$ 258.1 ($C_{13}H_{11}N_3OS$) requires 258.2.

4-Methyl-5-(pyridin-3-ylethynyl)thiazol-2-amine (38)

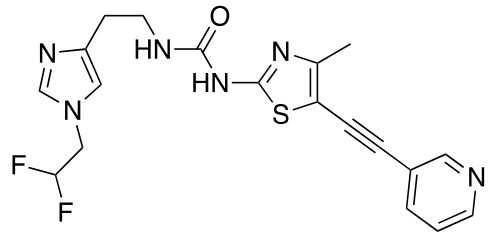
The compound **37**(400 mg, 1.56 mmol) was stirred in hydrazine hydrate at 70 °C for 20 minutes and water (5 mL) was added. The resulting precipitate was filtered, washed with water and dried under high vacuum to afford compound **38** (260 mg, 77%). mass spectrum (ESI) m/z $[M + H]^+$ 216.3 ($C_{11}H_9N_3S$) requires 216.1.

***N*-(4-Methyl-5-(pyridin-3- ylethynyl)thiazol-2-yl)- 1H-imidazole- 1-carboxamide (39)**

Compound **38** (91 mg, 0.423 mmol), 1,1'carbonyldiimidazole (103 mg, 0.634 mmol), and

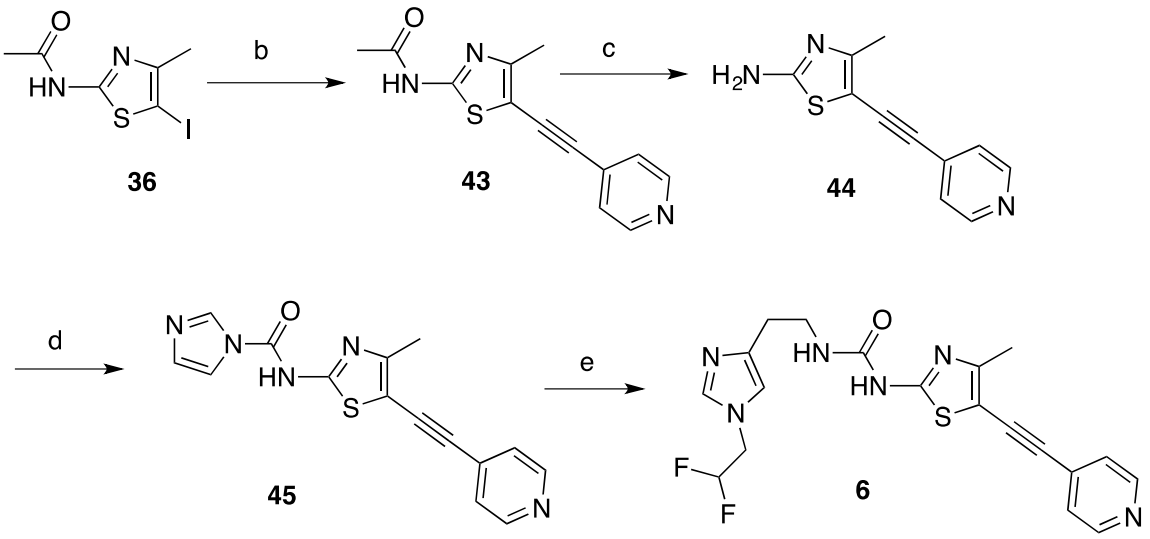
triethylamine (85.6 mg, 0.845 mmol) were stirred in DMF (2.1 mL) at 70 °C for 2 h. MTBE was added and the resulting precipitate collected by filtration to produce *N*-(4-methyl-5-(pyridin-3- ylethynyl)thiazol-2-yl)- 1H-imidazole- 1-carboxamide, **39** (120 mg, 92%).

1-(2-(1-(2,2-Difluoroethyl)- 1*H* imidazol- 4-yl)ethyl)-3-(4-methyl-5-(2-(pyridin-3-yl)ethynyl)thiazol-2-yl)urea (4)



A solution of compound **39** (25 mg, 0.08 mmol) and 2-(1-(2,2-difluoroethyl)-1H-imidazol-4-yl)ethanamine (80 mg, 0.32 mmol) and Et_3N (0.112 mL) in DCM (0.4 mL) was stirred for 20 h at RT. The reaction mixture was filtered, treated with cold methyl *t*-butylether and filtered once more. The filtrate was concentrated under reduced pressure and purified by medium pressure silica gel chromatography (0-10% MeOH/DCM) to afford compound **4** (19.5 mg**,** 58%) ^{1}H NMR (DMSO-d_6): 10.84 (s, 1H), 8.71 (d, J = 1.5 Hz, 1H), 8.55 (dd, J =1.6, 4.9 Hz, 1H), 8.18 (s, 1H), 7.94 (dt, J = 7.9, 2.5 Hz, 1H), 7.44 (dd, J = 4.9, 7.9 Hz, 1H), 7.20 (s, 1H), 6.72 (s, 1H), 6.64 - 6.37 (m, 1H), 4.60 (dt, J = 2.9, 15.9 Hz, 2H), 3.40 (m, 2H), 2.73 (t, J =6.8 Hz, 2H), 2.35 (s, 3H). HRMS: m/z calcd for $C_{19}H_{18}F_2N_6OS$ $[M^+ + 1]^+$ 417.1231; found 417.1313.

Scheme 3. Synthesis of Compound **6**



Reagents and conditions. (a) 4-ethynylpyridine, $Pd(PPh_3)_4$, CuI, Et_3N, dioxane, 80 °C, 45 min, 66% (b) $NH_2NH_2.H_2O$, THF, 100 °C, 6 h, 76.5% (c) CDI, DMF, 65 °C, 1 h, 87% (d) 2-(1-(2,2-difluoroethyl)-1*H*-imidazol-4-yl)ethan-1-amine, Et_3N, THF, r.t., 1 h, 44%.

Step 1*: N*-(4-Methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)acetamide (43)

A solution of *N*-(4-iodo-5-methyl-thiazol-2-yl)acetamide, **36** (4 g, 14.18 mmol), 4 -ethynylpyridine (1.609 g, 15.60 mmol) Et_3N (4 mL, 28.70 mmol) in 1,4-dioxane (10 was purged with N_2 (5 min) and $Pd(PPh_3)_4$ (819.3 mg, 0.71 mmol) and CuI (135.0 mg, 0.71 mmol) were added. The solution was heated at 85 °C for 45 min and the solvent was evaporated under reduced pressure. Water (50 mL) was added and the suspension was stirred at RT 10 min to form a brown precipitate and the precipitate was filtered and washed with water (3x25 mL) and then DCM (3x25 mL) until a brownish yellow precipitate was formed. The product was dried in high vacuum to afford compound **43** (2.4 g, 66%) ^{1}HNMR (300 MHz, DMSO) δ 12.45 (s, 1H), 8.60 (d, J = 5.2 Hz, 2H), 7.49 (d, J = 8.0 Hz, 2H), 2.45 (s, 3H), 2.17 (s, 3H); mass spectrum (ESI) m/z $[M + H]^+$ 258.3 ($C_{13}H_{11}N_3OS$) requires 258.1.

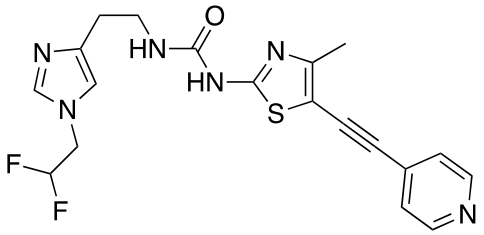
Step 2: 4-Methyl-5-(pyridin-4-ylethynyl)thiazol-2-amine (44)

Hydrazine monohydrate (25 mL, 50.52 mmol) was added to a stirred suspension of compound **43** (5 g, 19.43 mmol) in THF (75 mL) and the solution was heated at 100 °C for 6 h. The brown color reaction mixture was cooled to RT and concentrated under reduced pressure to give brownish yellow residue which was dissolved in warm methanol (50 mL) and filtered. The filtrate was concentered under reduced pressure to afford a brownish solid and the solid was decanted with ether and the ether layer was discarded. The solid was collected and dried in high vacuum for 4 h to afford compound **44** (3.2 g, 76.5% as a yellowish brown solid. ^{1}H NMR (300 MHz, CD_3OD) δ 8.48 (d, J = 6 Hz, 2H), 7.40 (d, J = 6.0 Hz, 2H), 2.89 (s, 3H). mass spectrum (ESI) m/z $[M + H]^+$ 216.1 ($C_{11}H_9N_3S$) requires 216.1.

Step 3: *N*-[4-Methyl-5-[2-(4-pyridyl)ethynyl]thiazol-2-yl]imidazole-1-carboxamide (45)

A mixture of **44** (100 mg, 0.46 mmol) and di(imidazol-1-yl)methanone (75.32 mg, 0.4645 mmol) in anhydrous DCM (5 mL) was heated to reflux (65 °C) in for 1 h and cooled to room temperature . The solvent was evaporated under reduced pressure and anhydrous ether was added and the solid was filtered. The brown solid was washed with ether (10 mL) and dried in high vacuum for 2 h to afford compound **45** (126 mg, 87%).

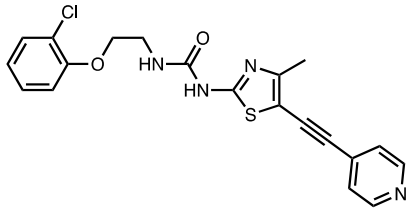
Step 4: 1-(2-(1-(2,2-Difluoroethyl)-1*H*-imidazol-4-yl)ethyl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (6).



A mixture of **45** (50 mg, 0.1616 mmol), 2-[1-(2,2-difluoroethyl)imidazol-4-yl]ethanamine (33.97 mg, 0.19 mmol) and DIPEA (30 µL) in THF (1 mL) was stirred at RT for 1 h and the solvent was evaporated under reduced pressure. The crude product was purified by reversed-phase chromatography (Column: C18. Gradient: 0-100 % MeCN in water with 0.1 % HCl) to afford compound **6.HCl** (36 mg, 44.3%) as a yellow powder. (CD_3OD): 9.00 (s, 1H), 8.77 (d, J= 5.4 Hz, 2H), 8.06 (d, J = 5.5 Hz, 2H), 7.53 (s,1H), 6.1 (t, J = 51 Hz,

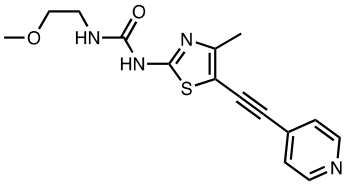
1H), 4.73 (t, *J* = 15 Hz, 2H), 3.58 (m, 2H), 3.00 (m, 2H), 2.50 (s, 3H). HRMS: m/z calcd for $C_{19}H_{18}F_2N_6OS$ [M^++1] 417.1309; found, 417.1304.

1-(2-(2-Chlorophenoxy)ethyl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (7)



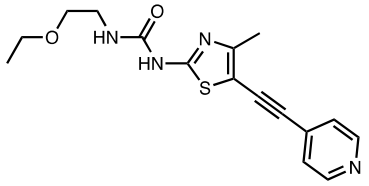
A solution of compound **45** (25 mg, 0.08 mmol) and 2-(2-chlorophenoxy)ethan-1-amine (29 mg, 0.17 mmol) and DIPEA (0.45 mL) in THF (1 mL) was stirred at room temperature for 20 h and the reaction mixture was filtered. The crude product was purified by reversed-phase chromatography (Column: C18. Gradient: 0 -100 % MeCN in water with 0.1 % HCl) to afford compound **7.HCl** (30 mg, 40.5%) as a yellow powder. ^{1}H NMR (300 MHz, MeOD) δ 8.77 (d, *J* = 5.4 Hz, 2H), 8.06 (d, *J* = 5.5 Hz, 2H), 7.53 (s, 1H), 6.10 (t, *J* = 51 Hz, 1H), 4.73 (t, *J* =15 Hz, 2H), 3.58 (m, 2H), 3.00 (m, 2H), 2.50 (s, 3H). HRMS: m/z calcd for $C_{20}H_{17}ClN_4O_2S$ [M^+ + 1]; 413.0833 found, 413.0839.

1-(2-Methoxyethyl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (8)



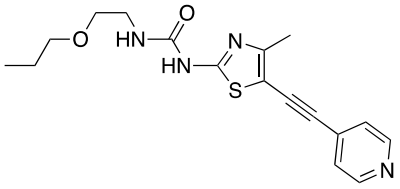
A mixture of **45** (50 mg, 0.1616 mmol), 2-methoxyethan-1-amine (18 mg, 0.24 mmol) and DIPEA (140 µL) in THF (1 mL) was stirred at RT for 1 h and the solvent was evaporated under reduced pressure. The crude product was purified by reversed-phase chromatography (Column: C18. Gradient: 0-100 % MeCN in water with 0.1 % HCl) to afford compound **8. HCl** (35 mg, 60%) as a yellow powder. ^{1}HNMR (CD_3OD): 8.82 (d, 2H), 7.98 (d, 2H), 3.42-3.39 (m, 2H), 3.34-3.30 (m, 2H), 3.28 (s, 3H), 2.43 (s, 3H). HRMS: m/z calcd for $C_{15}H_{16}N_4O_2S$ [M^+ + 1] 317.1065; found 317.1068.

1-(2-Ethoxyethyl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (9)



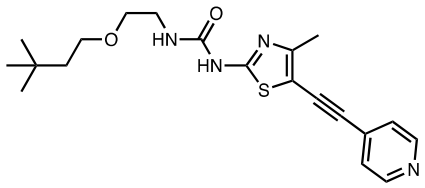
Compound **9** was synthesized in a manner similar to compound **8** using **45** and 2-ethoxyethan-1-amine to afford **9.HCl** (29 mg, 48%). ^{1}HNMR (CD_3OD: 8.77 (s, 2H), 8.08 (s, 2H), 3.58 - 3.51 (m, 4H), 3.44 (t, J = 6 Hz, 2H), 2.52 (s, 3H), 1.21 (t, J = 6.0 Hz, 3H). HRMS: m/z calcd for $C_{16}H_{18}N_4O_2S$ [M^+ + 1] 331.1229; found 331.1222.

1-(4-Methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)-3-(2-propoxyethyl)urea (10)



Compound **10** was synthesized in a manner similar to compound **8** using **45** and 2-propoxyethan-1-amine to afford **10.HCl** (17 mg, 27%). ^{1}HNMR (DMSO-d_6): 11.0 (brs, 1H), 8.73 (d, J = 3.0 Hz, 2H), 7.81 (d, J = 3.0 Hz, 2H), 6.80 (brs, 1H), 3.46 - 3.30 (m, 4H), 2.41 (s, 3H), 2.38 (m, 2H), 1.56 - 1.49 (m, 2H), 0.88 (t, J = 9.0 Hz, 3H). HRMS: m/z calcd for $C_{17}H_{20}N_4O_2S$ [M^+ + 1] 345.1386; found 345.1388.

1-(2-(3,3-Dimethylbutoxy)ethyl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (11)

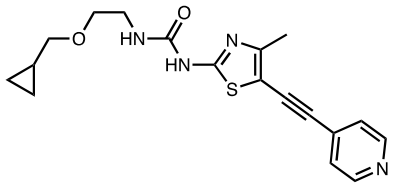


Compound **11** was synthesized in a manner similar to compound **8** using **45** and 2-propoxyethan-1-amine to afford compound **11.HCl** (4.3mg,

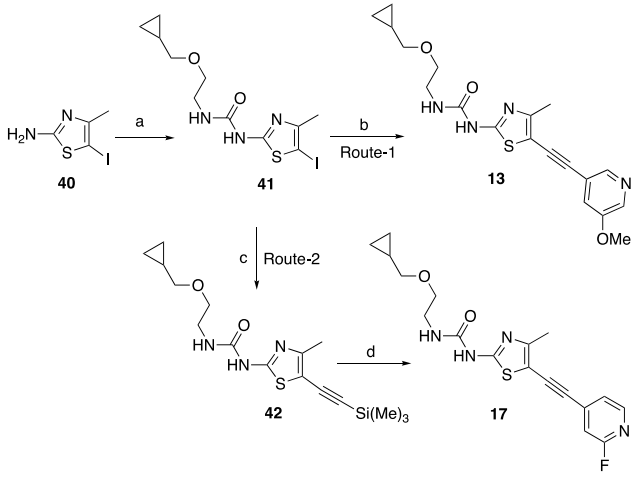
6%). ^{1}HNMR (DMSO-d_6):

^{1}H NMR (400 MHz, DMSO) δ 11.12 (s, 1H), 8.81 (brs, 2H), 7.98 - 7.86 (m, 2H), 6.91 (t, J = 5.7 Hz, 1H), 3.52 - 3.37 (m, 4H), 3.30 (q, J = 5.5 Hz, 2H), 2.43 (s, 3H), 1.45 (t, J = 7.4 Hz, 2H), 0.89 (s, 9H). HRMS: m/z calcd for $C_{20}H_{26}N_4O_2S$ [M^+ + 1] 387.1847; found 387.1849

1-(2-(Cyclopropylmethoxy)ethyl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (12)



Compound **12** was synthesized in a manner similar to compound **8** using **45** and 2- to 2-(cyclopropylmethoxy)ethan-1-amine to afford **12.HCl** (20 mg, 31%). ^{1}HNMR (DMSO-*d*6): 11.2 (brs, 1H), 8.78 (d, J = 6.0 Hz, 2H), 7.91 (d, J = 6.0 Hz, 2H), 6.91(brm, 1H) 3.48 - 3.44 (m, 2H), 3.34 - 3.25 (m, 4H), 2.42 (s, 3H), 1.00 - 0.97 (m, 1H), 0.47- 0.43 (m, 2H), 0.17 - 0.20 (m, 2H). HRMS: m/z calcd for $C_{18}H_{20}N_4O_2S$ [M^+ + 1] 357.1378; found 357.1380.



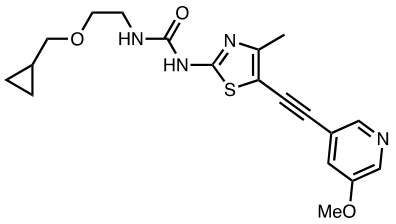
1-(Cyclopropylmethyl)-3-(5-iodo-4-methylthiazol-2-yl)urea (41)

To a stirred solution of iodo-4-methyl-thiazol-2-amine **40** (3.46 g, 14.41 mmol) in DCM (72 mL) was added CDI (2.57 g, 15.85 mmol) and the solution was heated at 60 °C for 2 h. The reaction mixture was cooled and 2-(cyclopropylmethoxy)ethanamine (2.5 g, 21.62 mmol). The solution was stirred at RT for 2 h. The reaction mixture was washed with water, the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by ISCO eluting with ethyl acetate /hexanes to afford compound **41** (2.5 g, 45%). ^{1}HNMR (DMSO-*d6*): 10.56 (s, 1H), 6.55 - 6.65 (m, 1H), 3.48 - 3.44 (m, 2H), 3.34 - 3.25 (m, 4H), 2.42 (s, 3H), 1.00 - 0.97 (m, 1H), 0.47 - 0.43 (m, 2H), 0.17 - 0.20 (m, 2H)**.** mass spectrum (ESI) m/z $[M + H]^+$ 282.5 ($C_{11}H_{16}IN_3O_2S$) requires 282.0.

1-(Cyclopropylmethyl)-3-(5-ethynyl-4-methylthiazol-2-yl)urea (42)

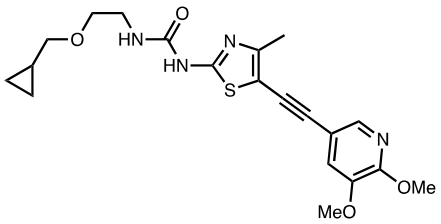
A solution of **41** (740 mg, 1.941 mmol), ethynyl(trimethyl)silane (591.0 mg, 850.4 µL, 6.017 mmol) , Et_3N (0.406 mL) in THF (1 mL) was purged with N_2 (5 min)$Pd(PPh_3)_4$ and (224 mg, 0.19 mmol), CuI (74 mg, 0.39 mmol were added. The solution was warmed to 65 °C and TBAF in THF (0.2 mL)) dropwise. The solution was stirred at 65 °C for 1 h and the solvent was evaporated under reduced pressure. The crude product was purified by ISCO eluting with (EtOAc/hexanes 1:1) to afford compound 1-(cyclopropylmethyl)-3-(4-methyl-5-((trimethylsilyl)ethynyl)thiazol-2-yl)urea (396 mg, 58%) Mass spectrum (ESI) m/z $[M + H]^+$ = 352.4. mass spectrum (ESI) m/z $[M + H]^+$ 352.4 ($C_{16}H_{25}N_3O_2SSi$) requires 352.2.

1-(2-(Cyclopropylmethoxy)ethyl)-3-(5-((5-methoxypyridin-3-yl)ethynyl)-4-methylthiazol-2-yl)urea (13)



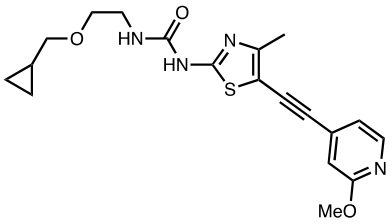
A solution of 3-ethynyl-5-methoxypyridine (26.20 mg, 0.197 mmol), **42** (50 mg, 0.13 mmol), Et_3N (0.055 mL) in THF (3 mL) was purged with N_2 (5 min) and Pd $(PPh_4)_3$ (23 mg, 0.02 mmol) and CuI (8 mg, 0.32 mmol) were added. The solution was stirred at 65 ^{0}C for 1 h and the solvent was evaporated under reduced pressure. The crude product was purified by reversed-phase chromatography (Column: C18. Gradient: 0-100 % MeCN in water with 0.1 % HCl) to afford compound **13.HCl** (23 mg, 41.4%). ^{1}HNMR (DMSO-d_6): δ 10.79 (brs, 1H), 8.40 (brs, 2H), 7.60 (s, 1H), 6.76 (s, 1H), 3.87 (s, 3H), 3.48 - 3.45 (m, 2H), 3.32 - 3.24 (m, 4H), 2.36 (s, 3H), 1.00 (m, 1H), 0.48 - 0.45 (m, 2H), 0.19-0.17 (m, 2H). HRMS: m/z calcd for $C_{19}H_{22}N_4O_3S$ [M^+ + 1] 387.1485; found 387.1485.

1-(2-(Cyclopropylmethoxy)ethyl)-3-(5-((5,6-dimethoxypyridin-3-yl)ethynyl)-4-methylthiazol-**2-yl)urea (14)**



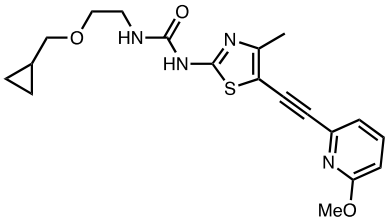
Compound **14** was synthesized in a manner similar to compound **13** using **41** and 2-methoxy-4-((trimethylsilyl)ethynyl)pyridine to afford **14.** 0.5 methane sulfonic acid salt **(**21.4 mg, 40%). ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.67 (s, 1H), 7.89 (d, J = 1.8 Hz, 1H), 7.40 (d, J = 1.8 Hz, 1H), 6.67 - 6.65 (m, 1H), 3.89 (s, 3H), 3.82 (s, 3H), 3.46 (t, J = 5.5 Hz, 2H), 3.34 - 3.23 (m, 4H), 2.33 (s, 3H), 1.10 - 0.84 (m, 1H), 0.57 - 0.36 (m, 2H), 0.30 - 0.09 (m, 2H). HRMS: m/z calcd for $C_{20}H_{24}N_4O_4S$ [M^+ + 1] 417.1590 found 417.1591.

1-(2-(Cyclopropylmethoxy)ethyl)-3-(5-((2-methoxypyridin-4-yl)ethynyl)-4-methylthiazol-2-yl)urea (15)



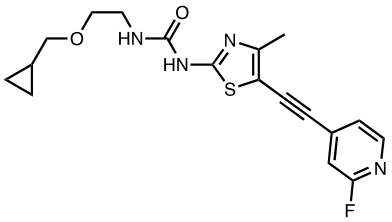
Compound **15** was synthesized in a manner similar to compound **13** using **41** and 2-methoxy-4-((trimethylsilyl)ethynyl)pyridine to afford **15.HCl** (25 mg, 52%).^{1}H NMR (300 MHz, DMSO-d_6) δ 10.79 (s, 1H), 8.17 (d, 1H), 7.05 (dd, 1H), 6.91 (s, 1H), 6.70 (t, 1H), 3.86 (s, 3H), 3.46 (t, 2H), 3.29 (m, 4H), 2.35 (s, 3H), 0.99 (m, 1H), 0.46 (m, 2H), 0.18 (m, 2H). HRMS: m/z calcd for $C_{19}H_{22}N_4O_3S$ [M^+ + 1] 387.1485; found 387.1484.

(2-(Cyclopropylmethoxy)ethyl)-3-(5-((6-methoxypyridin-2-yl)ethynyl)-4-methylthiazol-2-yl)urea (16)



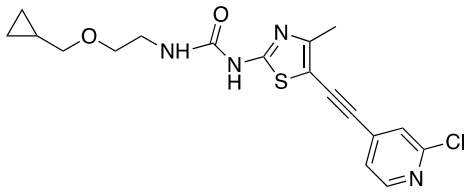
Compound **16** was synthesized in a manner similar to compound **17** using **42** and 2-bromo-6-methoxypyridine to afford **16.HCl** (16 mg, 27%). ^{1}HNMR (DMSO-d_6): δ 10.81 (brs, 1H), 7.72 (dd, J = 8.3, 7.4 Hz, 1H), 7.21 (d, J = 7.2 Hz, 1H), 6.83 (d, J = 8.3 Hz, 1H), 6.67-6.66 (m, 1H), 3.87 (s, 3H), 3.46 (t, J = 5.5 Hz, 2H), 3.37 - 3.28 (m, 2H), 3.26 (d, J = 6.8 Hz, 2H), 2.35 (s, 3H), 1.10 - 0.90 (m, 1H), 0.65 - 0.37 (m, 2H), 0.25 - 0.08 (m, 2H). HRMS: m/z calcd for $C_{19}H_{22}N_4O_3S$ [M^+ + 1] 387.1484; found 387.1485.

1-(2-(Cyclopropylmethoxy)ethyl)-3-(5-((2-fluoropyridin-4-yl)ethynyl)-4-methylthiazol-2-yl)urea (17)



To a solution of compound **42** (50 mg, 0.142 mmol), 2-fluoro-4-iodo-pyridine (32 mg, 0.143 mmol) and Et_3N (0.025 mL) in THF (1 mL) were added $Pd(PPh_3)_4$ (16 mg, 0.016 mmol) and CuI (6 mg, 0.03 mmol) were added and purged with N_2. The solution was heated at 65 °C and a solution of TBAF (1M in THF, 0.3 mL) was added dropwise. The solution was stirred at 65 °C for 1h. The solvent was evaporated under reduced pressure. The crude product was purified by ISCO eluting with EtOAc/hexanes (1:1) and the pure product was dissolved in CH_3CN (0.2 mL) and 2N HCl (0.1 mL) was added. The mixture was lyophilized to afford to afford **17** (29.4 mg, 50.3%) as yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.90 (m, 1H), 8.25 (d, J = 5.2 Hz, 1H), 7.44 (d, J = 5.2 Hz, 1H), 7.33 (s, 1H), 6.75 (m, 1H), 3.46 (t, J = 5.5 Hz, 2H), 3.32 - 3.25 (m, 4H), 2.34 (s, 3H), 1.13 - 0.86 (m, 1H), 0.55 - 0.40 (m, 2H), 0.19 - 0.19 (m, 2H). HRMS: m/z calcd for $C_{18}H_{19}FN_4O_2S$ [M^+ + 1] 375.1289; found 375.1292.

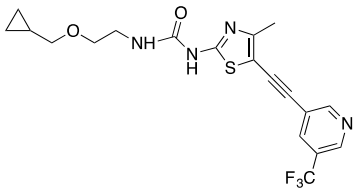
1-(5-((2-Chloropyridin-4-yl)ethynyl)-4-methylthiazol-2-yl)-3-(2 (cyclopropylmethoxy)ethyl)urea (18)



Compound **18** was synthesized in a manner similar to compound **17** using **42** and 2-fluoro-4-iodo-pyridine to afford **18.HCl** (20 mg, 33%) ^{1}HNMR (DMSO-d_6): 10.77 (s,1H), 9.01 (s,1H), 8.93 (s, 1H), 8.42 (s,1H), 6.68 (t,1H), 3.46 (m, 2H), 3.27 (m, 4H), 2.38 (s, 3H), 1.00 (m, 1H), 0.47 (m, 2H), 0.19 (m, 2H). HRMS: m/z calcd for $C_{18}H_{19}ClN_4O_2S$ [M^+ + 1]

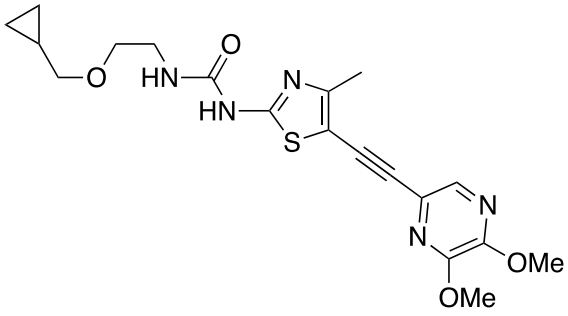
391.0990; found 391.0992.

1-(2-(Cyclopropylmethoxy)ethyl)-3-(4-methyl-5-((5-(trifluoromethyl)pyridin-3-yl)ethynyl)thiazol-2-yl)urea (19)



Compound **19** was synthesized in a manner similar to compound **17** using **42** and 3-iodo-5-(trifluoromethyl)pyridine to afford **19.HCl** (30 mg, 50%). ^{1}HNMR (DMSO-d_6): δ 10.77 (s, 1H), 9.01(s, 1H), 8.93 (s, 1H), 8.42 (s, 1H), 6.70 - 6.68 (m, 1H), 3.47 (m, 2H), 3.27 (m, 4H), 2.40 (s, 3H), 1.01 (m, 1H), 0.52 - 0.43 (m, 2H), 0.19 (m, 2H). HRMS: m/z calcd for $C_{19}H_{19}F_3N_4O_2S$ [M^+ + 1] 425.1260; found 425.1253.

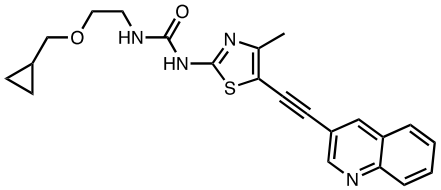
1-(2-(Cyclopropylmethoxy)ethyl)-3-(5-((5,6-dimethoxypyrazin-2-yl)ethynyl)-4-methylthiazol-2-yl)urea (20)



Compound **20** was synthesized in a manner similar to compound **17** using **42** and 5-bromo-2,3-dimethoxypyrazine to afford **20 HCl**. (13 mg, 20%). ^{1}HNMR (DMSO-d_6): δ 10.73 (s, 1H), 7.96 (s, 1H), 6.75-6.65 (m, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 3.46 (t, J = 5.5 Hz, 2H), 3.39 - 3.29 (m, 2H), 3.26 (d, J = 6.8 Hz, 2H), 2.33 (s, 3H), 0.97 (m, 1H), 0.54 - 0.37 (m, 2H), 0.24 - 0.10 (m, 2H). HRMS: m/z calcd for $C_{19}H_{23}F_3N_5O_4S$ [M^+ + 1] 418.1543; found 418.1540.

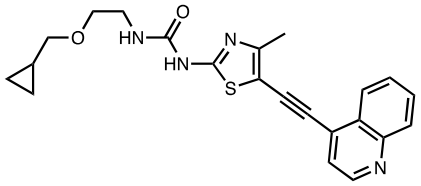
1-(2-(Cyclopropylmethoxy)ethyl)-3-(4-methyl-5-(quinolin-3-ylethynyl)thiazol-2-

yl)urea (21)



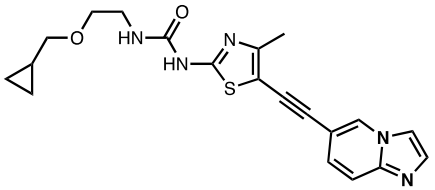
Compound **21** was synthesized in a manner similar to compound **13** using **42** and 3-bromoquinoline to afford **21** (09 mg, 15.5%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.87 (m, 1H), 9.05 (d, J = 2.0 Hz, 1H), 8.69 (d, J = 1.8 Hz, 1H), 8.06 (dd, J = 12.2, 8.1 Hz, 2H), 7.85 (t, J = 7.1 Hz, 1H), 7.71 (t, J = 7.4 Hz, 1H), 6.83 (t, J = 5.4 Hz, 1H), 3.47 (t, J = 5.5 Hz, 2H), 3.42-3.25(m, 4H), 2.41 (s, 3H), 1.12 - 0.85 (m, 1H), 0.58 - 0.35 (m, 2H), 0.33 - 0.05 (m, 2H). HRMS: m/z calcd for $C_{22}H_{22}N_4O_2S$ [M^+ + 1] 407.1536; found 407.153.

1-(2-(Cyclopropylmethoxy)ethyl)-3-(4-methyl-5-(quinolin-4-ylethynyl)thiazol-2-yl)urea (22)



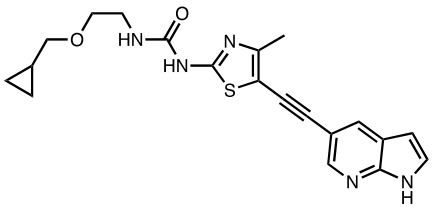
Compound **22** was synthesized in a manner similar to compound **17** using **42** and 4-iodoquinoline to afford **22** (09 mg, 15.5%),^{1}H NMR (300 MHz, DMSO-d_6) δ 11.12 (s, 1H), 9.08 (d, J = 5.2 Hz, 1H), 8.37 (d, J = 8.2 Hz, 1H), 8.24 (d, J = 8.4 Hz, 1H), 8.04 (t, J = 7.1 Hz, 1H), 7.96 (d, J = 5.1 Hz, 1H), 7.95 - 7.85 (m, 1H), 6.92 (t, J = 5.4 Hz, 1H), 3.48 (t, J = 5.5 Hz, 2H), 3.35-3.26 (m, 4H), 2.53 (s, 3H), 1.08 - 0.91 (m, 1H), 0.52 - 0.37 (m, 2H), 0.25 - 0.12 (m, 2H). mass spectrum (ESI) m/z [M + H]$^+$ 407.1 ($C_{22}H_{22}N_4O_2S$) requires 407.5.

1-(2-(Cyclopropylmethoxy)ethyl)-3-(5-(imidazo[1,2-*a*]pyridin-6-ylethynyl)-4-methylthiazol-2-yl)urea (23)



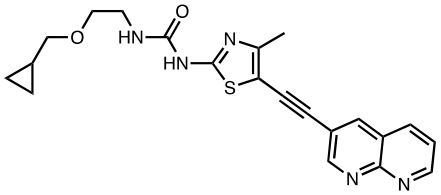
Compound **23** was synthesized in a manner similar to compound **17** using **42** and 6-iodoimidazo[1,2-*a*]pyridine to afford **23.HCl** (12.3mg, 20%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.86 (s, 1H), 9.23 (s, 1H), 8.28-8.25 (m, 2H), 7.99 (s, 2H), 6.81 (t, J = 5.4 Hz, 1H), 3.46 - 3.11 (m, 6H), 2.32 (s, 3H), 1.01 - 0.96 (m, 1H), 0.58 - 0.30 (m, 2H), 0.29 - 0.11 (m, 2H). HRMS: m/z calcd for $C_{20}H_{21}N_5O_2S$ [M^++1] 396.1489; found 396.1487.

1-(5-((1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)ethynyl)-4-methylthiazol-2-yl)-3-(2-(cyclopropylmethoxy)ethyl)urea (24).



Compound **24** was synthesized in a manner similar to compound **17** using **42** and 5-iodo-1*H*-pyrrolo[2,3-*b*]pyridine to afford **24.HCl** (16 mg, 26%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.94 (s, 1H), 10.5 (brs, 1H), 8.17 (s, 1H), 7.55 (d, J = 3.3 Hz, 1H), 6.74 (s, 1H), 6.52 (s, 1H), 3.46 (t, J = 5.5 Hz, 2H), 3.39 - 3.15 (m, 5H), 2.31 (s, 3H), 0.97 (m, 1H), 0.58 - 0.36 (m, 2H), 0.29 - 0.13 (m, 2H). HRMS: m/z calcd for $C_{20}H_{21}N_5O_2S$ [M^++1] 396.1491; found 396.1489.

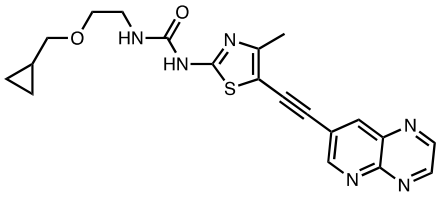
1-(5-((1,8-Naphthyridin-3-yl)ethynyl)-4-methylthiazol-2-yl)-3-(2-(cyclopropylmethoxy)ethyl)urea (25)



Compound **25** was synthesized in a manner similar to compound **13** using **42** and 3-bromo-1,8-naphthyridine to afford **25.HCl** (5.5 mg, 08.6%). ^{1}H MR (300 MHz, DMSO-d_6) δ 10.83 (m, 1H), 9.25-9.18 (brm, 2H), 8.72 (s, 1H), 8.52 (d, J = 8.2 Hz, 1H), 7.76 (m, 1H), 6.78 (t,

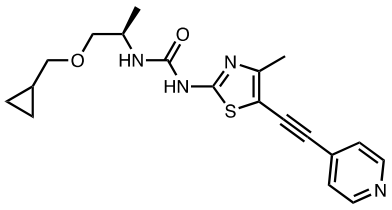
J = 5.3 Hz, 1H), 3.47 (t, *J* = 5.5 Hz, 2H), 3.48-3.25 (m, 4H), 2.41 (s, 3H), 1.12 - 0.90 (m, 1H), 0.55 - 0.39 (m, 2H), 0.19-0.16 (m, 2H). HRMS: m/z calcd for $C_{21}H_{21}N_5O_2S$ [M^++1] 408.1489; found 408.1493.

1-(2-(Cyclopropylmethoxy)ethyl)-3-(4-methyl-5-(pyrido[2,3-*b*]pyrazin-7-ylethynyl)thiazol-2-yl)urea (26)



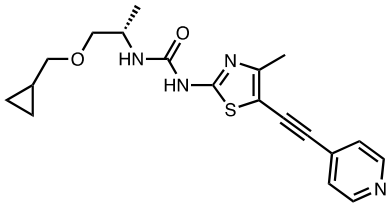
Compound **26** was synthesized in a manner similar to compound **17** using **42** and 7-bromopyrido[2,3-*b*]pyrazine afford **26.HCl** (11 mg, 19%).^{1}H NMR (300 MHz, DMSO-d_6) δ 10.74 (brs, 1H), 9.05-8.99 (m, 1H), 8.52 (s, 1H), 7.79 - 7.25 (m, 2H), 6.64 (s, 1H), 3.34 - 3.25 (m, 2H), 3.16-3.07 (m, 4H), 2.25 (s, 3H), 0.94 - 0.66 (m, 1H), 0.39 - 0.23 (m, 2H), 0.08 - 0.02 (m, 2H); Mass spectrum (ESI) m/z [M + H]$^+$ = 410.4. HRMS: m/z calcd for $C_{20}H_{20}N_6O_2S$ [M^++1] 409.1441; found 409.1441

(*R*)-1-(1-(Cyclopropylmethoxy)propan-2-yl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (27)



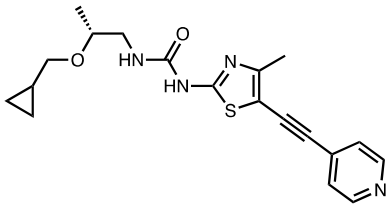
Compound **27** was synthesized in a manner similar to compound **6** using **45** and (*R*)-1-(cyclopropylmethoxy)propan-2-amine to afford **27.HCl** (23 mg, 31.6%).^{1}H NMR (300 MHz, CD_3OD) δ 8.74 (d, *J* = 9.0 Hz, 2H), 8.04 (d, *J* = 6.0 Hz, 2H), 4.01-3.97 (m, 1H), 3.49-3.45 (m, 2H), 3.33- 3.30 (m, 2H), 2.49 (s, 3H), 1.22 (d, *J* = 6.0 Hz, 3H), 1.06-1.04 (m, 1H), 0.53-0.49 (m, 2H), 0.20-0.23 (m, 2H). HRMS: m/z calcd for $C_{19}H_{22}N_4O_2S$ [M^++1] 371.1538; found 371.1536

(*S*)-1-(1-(Cyclopropylmethoxy)propan-2-yl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (28)



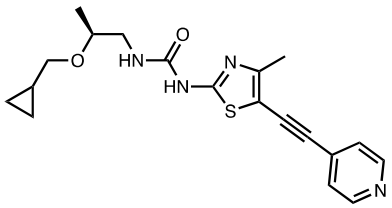
Compound **28** was synthesized in a manner similar to compound **6** using **45** and *(S)*-1-(cyclopropylmethoxy)propan-2-amine to afford **27.HCl** (27 mg, 39%)**.** ^{1}H NMR (300 MHz, CD_3OD) δ 8.74 (s, 2H), 8.04 (s, 2H), 4.01-3.97 (m, 1H), 3.49-3.45(m, 2H), 3.33-3.30 (m, 2H), 2.49 (s, 3H), 1.22 (d, *J* = 6.0 Hz, 3H), 1.06-1.04 (m, 1H), 0.53-0.49 (m, 2H), 0.20-0.23 (m, 2H). HRMS: m/z calcd for $C_{19}H_{22}N_4O_2S$ [M^++1] 371.1536; found 371.1542.

***R*)-1-(2-(Cyclopropylmethoxy)propyl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (29)**



Compound **29** was synthesized in a manner similar to compound **6** using **45** and (*R*)-2-(cyclopropylmethoxy)propan-1-amine to afford **29.HCl** (2.5 mg, 04%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.75 (d, *J* = 9 Hz, 2H), 8.03 (d, *J* = 9 Hz, 2H), 3.66-3.61 (m, 1H), 3.42-3.31 (m, 3H), 3.21-3.15(m, 1H), 2.49 (s, 3H), 1.16 (d, *J* = 6.0 Hz, 3H), 1.07-0.98 (m, 1H), 0.50-0.48 (m, 2H), 0.22-0.19 (m, 2H). HRMS: m/z calcd for $C_{19}H_{22}N_4O_2S$ [M^++1] 371.1536; found 371.1536.

(*S*)-1-(2-(Cyclopropylmethoxy)propyl)-3-(4-methyl-5-(pyridin-4-ylethynyl)thiazol-2-yl)urea (30)

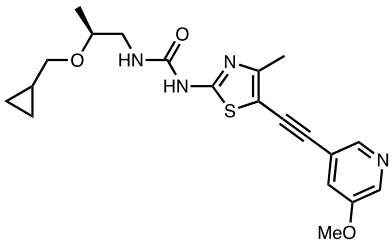


Compound **30** was synthesized in a manner similar to compound **6** using **45** and (*S*)-2-(cyclopropylmethoxy)propan-1-amine to afford **30.HCl** (31 mg, 46%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.75 (d, *J* = 9 Hz, 2H), 8.03 (d, *J* = 9 Hz, 2H), 3.66 -3.61 (m, 1H), 3.42-3.31 (m, 3H), 3.21-3.15 (m, 1H), 2.49 (s, 3H), 1.16 (d, *J*= 6.0 Hz, 3H), 1.07-0.98 (m, 1H), 0.50-0.48 (m, 2H), 0.22-0.19 (m, 2H). mass spectrum (ESI) m/z $[M + H]^+$ = 371.4. HRMS: m/z calcd for $C_{19}H_{22}N_4O_2S$ $[M^+ + 1]$ 371.1536; found 371.1534.

1-[(2*S*)-2-(Cyclopropylmethoxy)propyl]-3-(5-iodo-4-methyl-thiazol-2-yl)urea

To a stirred solution of *N*- (5-iodo-4-methyl-thiazol-2-yl)imidazole-1-carboxamide (1.39 g, 4.152 mmol), (2*S*)-2-(cyclopropylmethoxy)propan-1-amine (590 mg, 4.567 mmol)bin THF (50 mL) was added DIEA (3.6 mL) and the reaction mixture was stirred at RT for 18 h. The reaction mixture was washed with water, the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by ISCO eluting with ethyl acetate /hexanes to afford compound 1-[(2*S*)-2-(cyclopropylmethoxy)propyl]-3-(5-iodo-4-methyl-thiazol-2-yl)urea (0.4 g, 24.4%); mass spectrum (ESI) m/z $[M + H]^+$ 396.2 ($C_{12}H_8N_3OS$) requires 396.0

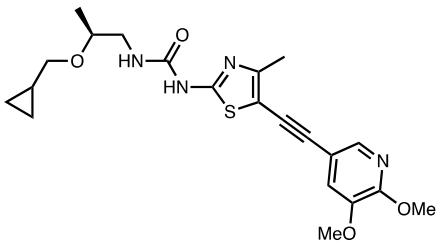
(*S*)-1-(2-(Cyclopropylmethoxy)propyl)-3-(5-((5-methoxypyridin-3-yl)ethynyl)-4-methylthiazol-2-yl)urea (31)



Compound **31** was synthesized in a manner similar to compound **13** using 1-[(2*S*)-2-

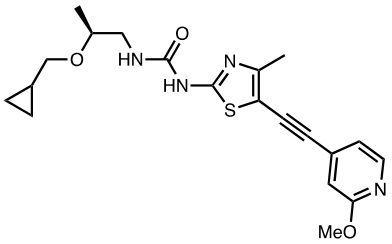
(cyclopropylmethoxy)propyl]-3-(5-iodo-4-methyl-thiazol-2-yl)urea and 2-methoxy-5-((trimethylsilyl)ethynyl)pyridine to afford **31.HCl** (0.6.5 mg, 17.5%). ^{1}H NMR (300 MHz, MeOD) δ 7.83 -7.35 (m, 3H), 3.91(s, 3H), 3.75 - 3.51 (m,1H), 3.47 - 3.25 (m, 3H), 3.17-3.13 (m, 1H), 2.39 (s, 3H), 1.15 (d, *J* = 6.2 Hz, 3H), 1.10 -0.92 (m, 1H), 0.61 -0.42 (m, 2H), 0.34 - 0.13 (m, 2H). HRMS: m/z calcd for $C_{20}H_{24}N_4O_3S$ [M^++1] 401.1642; found 401.1641.

(*S*)-1-(2-(cyclopropylmethoxy)propyl)-3-(5-((5,6-dimethoxypyridin-3-yl)ethynyl)-4-methylthiazol-2-yl)urea (32)



Compound **32** was synthesized in a manner similar to compound **13** using 1-[(2*S*)-2-(cyclopropylmethoxy)propyl]-3-(5-iodo-4-methyl-thiazol-2-yl)urea and 2,3-dimethoxy-5-((trimethylsilyl)ethynyl)pyridine to afford **32.HCl** (19.5 mg, 49.3%), ^{1}H NMR (300 MHz, MeOD) δ 7.83 (d, *J* = 1.8 Hz, 1H), 7.28 (d, *J* = 1.8 Hz, 1H), 3.96 (s, 3H), 3.86 (s, 3H), 3.64 -3.61 (m, 1H), 3.47 - 3.26 (m, 3H), 3.23 -3.03 (m, 1H), 2.37 (s, 3H), 1.15 (d, *J* = 6.2 Hz, 3H), 1.11 - 0.94 (m, 1H), 0.53-0.50 (m, 2H), 0.24-0.19 (m, 2H). HRMS: m/z calcd for $C_{21}H_{26}N_4O_4S$ [M^++1] 431.1745; found 431.1747.

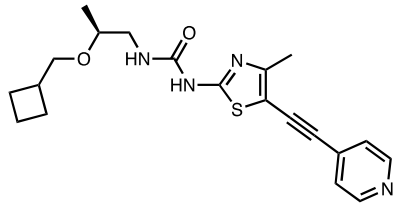
***(S)*-1-(2-(Cyclopropylmethoxy)propyl)-3-(5-((2-methoxypyridin-4-yl)ethynyl)-4-methylthiazol-2-yl)urea (33)**



Compound **33** was synthesized in a manner similar to compound **13** using 1-[(2*S*)-2-(cyclopropylmethoxy)propyl]-3-(5-iodo-4-methyl-thiazol-2-yl)urea and 2-methoxy-4-

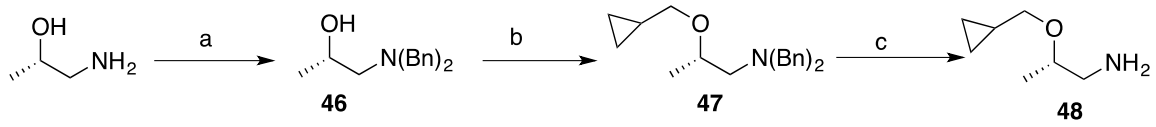
((trimethylsilyl)ethynyl)pyridine to afford (*S*)-2-(cyclopropylmethoxy)propan-1-amine **33.HCl** (6.5 mg, 15%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, *J* = 5.3 Hz, 1H), 6.91 (d, *J* = 5.3 Hz, 1H), 6.80 (s, 1H), 3.95 (s, 3H), 3.74 -3.50 (m, 2H), 3.34 -3.18 (m, 3H), 2.45 (s, 3H), 1.20 (d, *J* = 6.0 Hz, 3H), 1.04 -1.09 (m, 1H), 0.65 - 0.41 (m, 2H), 0.24 - 0.19 (m, 2H). HRMS: m/z calcd for $C_{20}H_{24}N_4O_3S$ [M^++1] 401.1642; found 401.1637.

(*S*)-1-(2-(Cyclobutylmethoxy)propyl)-3-(5-((2-methoxypyridin-4-yl)ethynyl)-4-methylthiazol-2-yl)urea (34)



Compound **34** was synthesized in a manner similar to compound **6** using **47** and (*S*)-2-(cyclobutylmethoxy)propan-1-amine to afford **34.HCl** (17 mg, 24.5%). ^{1}HNMR (300 MHz, d6-DMSO); ^{1}H NMR δ 11.11 (s, 1H), 8.90 (brs, 2H). 7.90 (brs, 2H), 6.83 (s, 1H), 3.46 - 3.10 (m, 6H), 2.40 (s, 3H), 1.98 - 1.96 (m, 6H), 1.06 (d, *J* = 3.0 Hz, 3H). HRMS: m/z calcd for $C_{20}H_{24}N_4O_2S$ [M^++1] 385.1692; found 385.1693.

The synthesis of (2*S*)-2-(cyclopropylmethoxy)propan-1-amine (48)



Step 1

To a stirred solution of (2*S*)-1-aminopropan-2-ol (2.5 g, 33.28 mmol) and benzaldehyde (6.76 mL, 66.56 mmol) in MeOH (22.5 mL) and HOAc (2.5 mL) was added $NaCNBH_3$ (2.614 g, 20.42 mL, 41.60 mmol) was slowly at room temperature. The resulting white suspension was heated at 65 °C for 1h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. Water (100 mL) was added and the aqueous phase was extracted with EtOAc (3x 50 mL). The organic layers

were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by ISCO eluting with EtOAc/hexanes (0-50%)) to afford compound **46** (4.6 g, 54%) as a clear oil. ^{1}HNMR (300 MHz, $CDCl_3$); δ 7.40 - 7.25 (m, 10H), 3.43 & 3.86 (ABq, *J* = 15 Hz, 4H), 3.24 (s, 1H), 2.43 (d, *J*= 6 Hz, 2H), 1.07 (d, *J* = 6 Hz, 3H); mass spectrum (ESI) m/z 256.4 $[M + H]^+$ ($C_{17}H_{21}NO$) requires 256.2.

Step 2

To a stirred solution of compound **46** (2.25 g, 8.81 mmol) in anhydrous DMF (10 mL) was added NaH (845.6 mg, 35.24 mmol) portionwise at room temperature under nitrogen. The resulting suspension was stirred 30 min at RT and then heated at 75 °C for 8 h. The reaction mixture was cooled to room temperature and poured into water (50 mL) and sat $NaHCO_3$ (25 mL) was added. The aqueous layer was extracted with ether/EtOAc (2:1, 2 times), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by ISCO eluting with EtOAc/hexanes (0-10%)) to afford compound **47** (2.2 g, 81%) as an oil. ^{1}HNMR (300 MHz, DMSO-*d6*); δ 7.36 - 7.20 (m, 10H), 3.56 (q, *J* = 14 Hz, 4H), 3.25- 3.15 (m, 2H), 2.50 - 2.44 (m, 1H), 2.46-2.44 (m, 1H), 2.31- 2.25 (m, 1H), 0.98 (d, *J* = 6 Hz, 3H), 0.93 - 0.90 (m, 1H), 0.44 - 0.39 (m, 2H), 0.13 - 0.08 (m, 2H); mass spectrum (ESI) m/z 310.5 $[M + H]^+$ ($C_{21}H_{27}NO$) requires 310.2.

Step 3

A solution containing ammonium formate (2.5 g, 38.8 mmol) and compound **47** (2 g, 6.46 mmol) in methanol (50 mL) was purged with N_2 (5 min) and Pd on C (wet, Degussa, 935 mg, 4.4 mmol) was added at room temperature. The solution was heated at 65 °C for 1 h. The reaction mixture was cooled and filtered over celite pad. The filtrate was concentrated under reduced pressure to afford compound **48** (720 mg, 86%) a pale yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$); δ 3.39 - 3.35 (m, 1H), 3.30 - 3.17 (m, 2H), 2.59 (brs, 2H), 0.97 (d, *J* = 6 Hz, 3H), 0.94 - 0.92 (m, 1H), 0.46 - 0.41 (m, 2H), 0.16 - 0.12 (m, 2H).

2. Description of Biochemical Assays

PI3K Inhibition Assays The compounds of interest were dissolved in DMSO to make 10 μM initial stock solutions. Serial dilutions in DMSO were then made to obtain the final solutions for the assay. Using a Biomek FX from Beckman Coulter, a 1.5 μL aliquot aliquot of DMSO or inhibitor in DMSO was added to each individual well (hereafter, "test well") in a 96 well polystyrene plate [Corning, Costar Item No. 3697]. Using a Titertek Multidrop, 50 μL of ATP Mix [50 mM HEPES (pH 7.5), 3 mM MgCl2, 100 mM NaCl, 2 mM DTT, 1 mM EGTA, 0.03% CHAPS, and ATP (100 μCi/μmol 33P-ATP) (See Table 1 for ATP concentrations, each equal to Km)] was added to each well. To initiate the reaction, 50 μL of Reaction Mixture [50 mM HEPES (pH 7.5), 3 mM MgCl2, 100 mM NaCl, 2 mM DTT, 1 mM EGTA, 0.03% CHAPS, 20 μM PIP2 (phosphatidylinositol(4,5)-bisphosphate diC16 (PI(4,5)P2; Avanti Polar Lipids, Cat. No. 840046P) and PI3K isoform of interest (See Table 1 for isoform concentrations)] was added to each well, followed by incubating the wells for 15 min at room temperature. Table 1 PI3K Isoform PI3Kα PI3Kβ PI3Kγ PI3Kδ Final ATP concentration (equal to Km) 5 μM 27 μM 10 μM 7 μM Final enzyme concentration 4 nM 20 nM 6 nM 6 nM After incubation, the reactions in each well were quenched by addition of 50 μL of stop solution [30% TCA/water, 10 mM ATP]. Each quenched reaction mixture was then transferred to a 96 well glass fiber filter plate [Corning, Costar Item No. 3511]. The plate was vacuum-filtered and washed three times with 150 μL of 5% TCA/water in a modified Bio-Tek Instruments ELX-405 Auto Plate Washer. 50 μL of scintillation fluid was added to each well and the plate read on a Perkin-Elmer TopCountTM NXT liquid scintillation counter to obtain 33P-counts. After removing mean background values for all of the data points, Ki(app) data were calculated from non-linear regression analysis of the initial rate data using the Prism software package (GraphPad Prism, GraphPad Software, San Diego California, USA). The data were fit to the Morrison equation for competitive tight binding Ki as described by Copeland (Reference: RA Copeland, Enzymes, 2nd edition, Wiley, 2000, Equation 9.6). Compound potencies were determined in singlicate. Average Kis are be reported for compounds that have multiple determinations. The Average of the Robust MSRs for PI3K α, β, γ, δ were determined to be 3.2, 3.0, 3.1, and 2.7 respectively.

(Reference :JBiomolScreen_MSR, AMC_Robust_Statistics, Minimum Significant Ratio - A Statistic to Assess Assay Variability)

Table 6

PI3K Isoform	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
Final ATP concentration (μM) (equal to Km)	5	27	10	7
Final enzyme concentration (nm)	4	20	6	6

3. Description of Cellular Assays

Materials

THP-1 were obtained from ATCC. DMEM, GultaMax, Penicillin/Streptomycin, 2-mercaptoethanol, Hepes solution 1M, TrypLE Express, 7.5% BSA, Fraction V were obtained from Invitrogen. Heat Inactivated FBS from ThermoFisher Scientific. Recombinant human MCP-1 from PeproTech; AffiniPure F(ab')2 Fragment Goat Anti-Human IgM from Jackson Immuno Research Labs; CellTiter-Glo luminescent cell viability assay from Promega; Phospho-Akt antibody (S473) AlexaFluor647 conjugated from Cell Signaling Technology; Formaldehyde 37% from Sigma; Methanol from JT Baker; 96-well plates, black with clear bottom and 96 well V bottom polypropylene plates from Costar.

4. Air Pouch Protocol

Male Balb/c mice (8 to 10 weeks) were obtained from Jackson Laboratories and subcutaneous pouches were created on the dorsum by two serial injections of air (5 mL and 3 mL) at 6 and 3 days prior to compound administration. Animals were then treated orally with a single administration of compound **14** prepared as a suspension in 0.2% methylcellulose (MC)/1% sodium lauryl sulfate (SLS) or with vehicle alone at a final dosing volume of 10 mL/kg. One hour after treatment with compound **14**, each mouse received an injection of IL-8 (1 μg) or saline directly into the pouch to

induce chemotaxis of neutrophils. Four hours later, mice were euthanized and cells were extracted from the pouch by lavage. Cells were pelleted, resuspended in 1mL 1X BD lyse/fix buffer, stained with antibodies and then neutrophil numbers were determined by FACS. Cells were stained with an antibody cocktail containing 1:200 anti-mouse CD11bFITC, 1:400 anti-mouse Gr-1 APC and 1:200 Fc block(Cd16/32) for 30 minutes at room temperature. Plates were read on a BD FACS Calibur. % of CD11b/Gr-1 double positive neutrophils was determined via Flow Jo based analysis. Total cell counts were determined using the Guava via count protocol. Plasma and brain tissue were collected for compound concentration analysis. Concentrations were determined using a high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) method.

5. Crystallographic Information.

Methods: PI3K gamma protein was expressed, purified and crystallized as described by Walker *et al.*2 Briefly, PI3K (K1432-L1767) was expressed as a fusion with an uncleavable C-terminal (His)6 tag in *SF21* insect cells. The protein was purified using a combination of metal-affinity (Talon resin), cation-exchange (Poros 20HS resin), and gel filtration chromatography (S-200 resin). The final buffer was 20 mM Tris, 0.5 mM ammonium sulphate, 1% ethylene glycol, 0.02% CHAPS, 5 mM DTT, pH 7.2. Crystals were grown by vapor diffusion using a reservoir solution containing 100 mM Tris buffer, pH 8.5, 25% w/v PEG 3350, 200 mM lithium sulfate, 10 mM DTT and 10 mM EDTA. Inhibitor complexes were prepared by soaking PI3K crystals for 12-24 hours in the reservoir buffer supplemented with 13% w/v glycerol and the inhibitor at a concentration of 1 mM. Prior to data collection the crystals were flash frozen directly in the soaking solution. Xray diffraction data were collected at the 5.0.2 beamline at the Advanced Light Source (ALS) and processed/scaled with Global Phasing autoPROC.3. The starting model was obtained from the RCSB4 (entry 1E7U) whose structure was reported by Walker et al.2 The structure was built with Coot5 and refined using the Global Phasing BUSTER package (Bricogne G., Blanc E., Brandl M., Flensburg C., Keller P., Paciorek W., Roversi P, Sharff A., Smart O.S., Vonrhein C., Womack T.O. (2017). BUSTER version X.Y.Z. Cambridge, United Kingdom: Global Phasing Ltd.

Table 6. Kinase selectivity profile of compound **4, 14, 23, 31** and **32** against a diverse panel of kinases

Kinase	**K_i μM**
CDK2	>4
FLT3	>4
GSK3b	>4
IRAK4	>4
JAK2	>4
JNK3	>4
KDR	>4
MET	>4
PKA	>4
PLK1	>4
ROCK1	>4
SRC	>4
SYK	>4

7. MDCK-MDR1 Permeability Assay

Permeability measurements were conducted according to the Cyprotex protocol using the MDCKMDR1 cell line. Cells between passage numbers 6 -30 were seeded onto a Multiscreen plate at a cell density of 3.4 Å~ 105 cells/cm2 and cultured for 3 days before permeability studies were conducted. The cells in this assay form a cohesive sheet of a single cell layer, filling the surface area of the culture dish, also known as a confluent monolayer, and on day four, the test compound was added to the apical side of the membrane; the transport of the compound across the monolayer was monitored over a time period of 60 min. Test compounds were dissolved in DMSO at a concentration of 10 μM.

The dosing solutions were prepared by diluting the test compounds with assay buffer, pH 7.4, at a final concentration of 5 μM. For the assessment of the apical to basolateral (**"**A−B**"**) permeability, buffer was removed from the apical compartment and replaced with the test compound dosing solution with or without the permeability glycoprotein (P-gp) inhibitor elacridar (2 μM). For the assessment of basolateral to apical (**"**B−A**"**) permeability, buffer was removed from the companion plate and replaced with the test compound dosing solution. Incubations were carried out in duplicates at 37 °C in an atmosphere of 5% CO_2 with a relative humidity of 95%. Each assay included the reference markers propranolol (high permeability) and prazosin (P-gp substrate). After incubation for 60 min, apical and basolateral samples were diluted, and the test compounds were quantified by LC/MS/MS using an 8-point calibration in the range 0.0039 to 3 μM with the appropriate dilution of the samples (receiver dilution factor = 1, donor and C0 dilution factor = 10). The permeability coefficient (Papp) for each compound was calculated from the following equation: Papp = (dQ/dt)/(C0 Å~ S), where dQ/dt is the rate of permeation of the drug across the cells, C0 is the donor compartment concentration at time zero, and S is the area of the cell monolayer. The percent recovery was measured for all incubation conditions. These measurements did not reveal unacceptable compound/plate binding or compound accumulation in the cell monolayer.