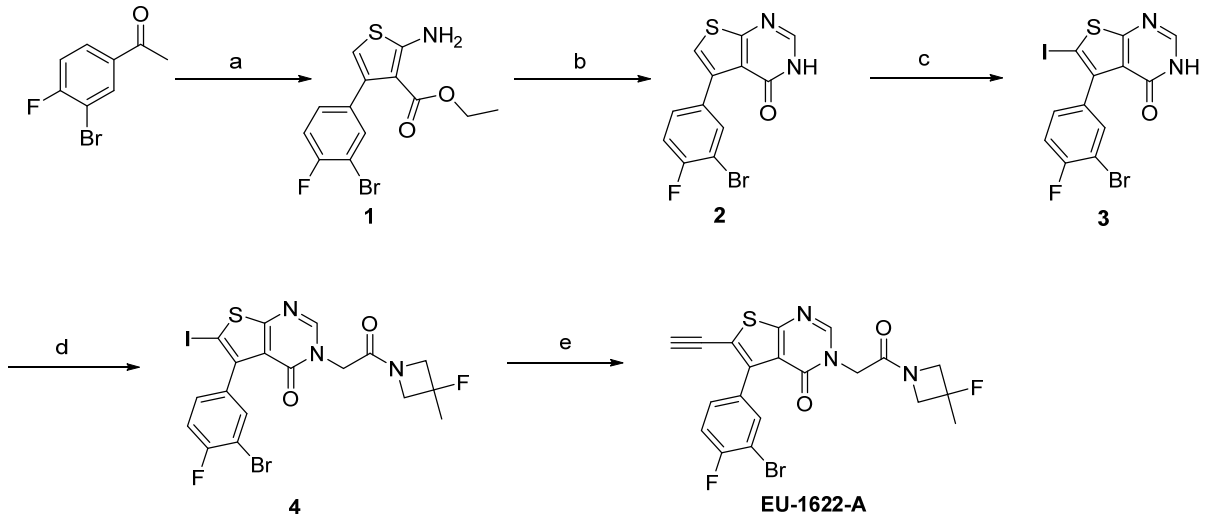
Supplementary Methods

Chemicals and Synthesis of EU-1622-240

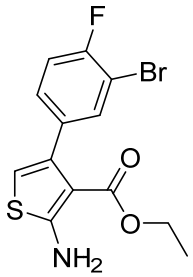
D,L-2-Amino-5-phosphonovalerate (DL-APV, CAS# 76326-31-3) and 7-Cl-kynurenate (CAS# 18000-24-3) were obtained from Tocris (Minneapolis, MN). 2-(Hydroxypropyl)-β-cyclodextrin (CAS# 128446-35-5) was obtained from Acros Organics or ChemCenter. Compound **1** was obtained from Asinex (BAS 08756685, Winston-Salem, NC), Compound **5** was from Chembridge Corporation (7788789, San Diego, CA), Compound **6** was from Princeton Biomolecular Research (OSSK 548281, Princeton, NJ), Compound **4** was from Enamine (T5399350, Monmouth Junction, NJ); the purity for all commercial compounds was >90%. All other chemicals were obtained from Sigma (St. Louis, MO). **EU1622-240** (Compound **2**)**,** Compound **7**, Compound **8**, Compound **9**, and Compound **10** were synthesized as described below.

Synthetic pathway for making EU-1622 Analogs

Scheme 1: Synthesis of Analogs Compound **1**, Compound **2**, Compound **3**, Compound **4**, Compound **5**, and **EU-1622-240**.

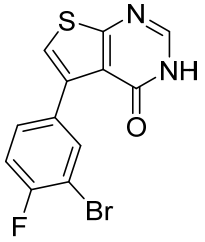


Reagents and conditions: (a) $TiCl_4$ (1M solution in DCM), ethyl cyanoacetate, pyridine, DCM, 0 ºC – rt, o/n; (b) Et_2NH, sulfur, THF, rt, 2h; (c) $HCONH_2$, reflux, 16h; (d) K_2CO_3, KI, acetone, 80 ºC, 16 h; (e) 1. Trimethylsilylacetylene, Et_3N, CuI, $PdCl_2(PPh_3)_2$, THF, 60°C. 2. TBAF, THF, 0°C, 30 min.

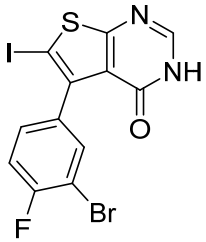


Compound 1: ethyl 2-amino-4-(3-bromo-4-fluorophenyl)thiophene-3-carboxylate: Compound **1** was prepared similar as previously described[57]. 1-(3-bromo-4-fluorophenyl)ethan-1-one, **1** (2.4 g, 11.2 mmol, 1.0 eq) and ethyl cyanoacetate (1.4 mL, 13.4 mmol, 1.2 eq) were dissolved in DCM (40 mL) and cooled to 0ºC. $TiCl_4$ (1.0 M solution in DCM, 22.3 mL. 22.3 mmol, 2.0 eq) was added dropwise, and the resulting mixture was stirred for 30 minutes. Dry pyridine (0.7 mL, 8.9 mmol, 0.8 eq) was added dropwise, the ice bath was then removed, and the resulting mixture was stirred for 1 h. A second aliquot of pyridine (2.2 mL, 26.8 mmol, 2.4 eq) was then added dropwise, and the reaction mixture was stirred overnight at room temperature. The resulting mixture was poured onto 3.0 M HCl solution (aq), and the organic layer was separated. The aqueous layer was extracted twice with DCM, and the combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to produce an oil. The oil was dissolved in THF (10 mL), and sulfur (0.5 g, 14.5 mmol, 1.3 eq) was added, followed by the dropwise addition of diethylamine (4.4 mL, 42.4 mmol, 3.8 eq). The solution was stirred for 2 h at room temperature. The mixture was diluted in diethyl ether and washed with water (2X) and brine (1X), and the organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude mixture was purified via trituration in hexanes to yield the title compound (2.1 g, 67% over 2 steps) as a off-white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.35 (m, 1H), 7.11 – 7.03 (m, 2H), 6.02 (s, 2H), 3.95 (brs, 2H), 2.40 (q, *J* = 7.5 Hz,

2H), 1.08 (t, *J* = 7.5 Hz, 3H), 0.88 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.59, 161.34, 158.14 (d, *J* = 246.5 Hz), 135.66 (d, *J* = 4.1 Hz), 134.81, 132.88, 130.29 (d, *J* = 7.0 Hz), 126.01, 115.36 (d, *J* = 22.1 Hz), 107.87 (d, *J* = 21.0 Hz), 106.45, 59.40, 21.11, 16.41, 13.79. ^{19}F NMR (565 MHz, $CDCl_3$) δ -110.8. HRMS: [APCI+] $[M+H]^+$ calc. for $C_{15}H_{16}O_2N^{79}BrF^{32}S$, 372.00637, observed, 372.0065.



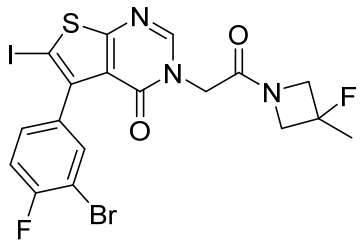
5-(3-bromo-4-fluorophenyl)thieno[2,3-d]pyrimidin-4(3H)-one (**2**). Thiophene analogue **1** (1.2g, 3.7mmol) and formamide (2.64mL, 66.6mmol) were combined in a flask equipped with a condenser and heated to 180 °C overnight (ca. 16 hours). Afterward, the mixture was allowed to cool to room temperature, and the resulting mixture was poured over ice, rinsing the flask with water (3x5 mL). The resulting mixture was stirred for 30 minutes. The resulting precipitate was filtered, washed with water (5x10 mL) and diethyl ether (3x10 mL) to afford the title compound as a brown solid (905 mg, 2.78 mmol, 75%). The product was carried forward without further purification.



5-(3-bromo-4-fluorophenyl)-6-iodothieno[2,3-d]pyrimidin-4(3H)-one (**3**). The following protocol was adapted from the literature.[61] A 100 mL round bottom flask equipped with a stir bar was charged in order with AcOH (10 mL), water (2 mL), H_2SO_4 (0.57 mL, 10.26 mmol), **17b** (3300.00 mg, 10.15 mmol), H_5IO_6 (2313.33 mg, 10.15 mmol), and I_2 (3091.06 mg, 12.18 mmol) under ambient air. The mixture was then heated to 75 °C for 3 hours. Afterwards, the mixture was allowed to cool to rt, and the precipitate was filtered and washed with 5:1

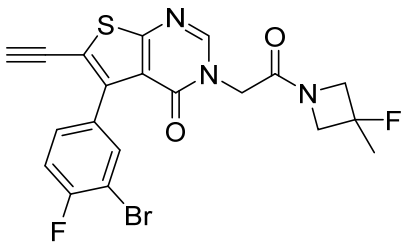
acetic acid:water (3x10 mL) followed by diethyl ether (5x20 mL). The solid was then dried on the filter frit for an additional 2 hours at room temperature to afford the title compound as a beige solid (4540 mg, 10.065 mmol, 99%).

5-(3-bromo-4-fluorophenyl)-3-(2-(3-fluoro-3-methylazetidin-1-yl)-2-oxoethyl)-6-iodothieno[2,3-d]pyrimidin-4(3H)-one



A 100 mL round bottom flask equipped with a stir bar was charged with 5-(3-bromo-4-fluorophenyl)-6-iodothieno[2,3-d]pyrimidin-4(3H)-one (451.5mg, 0.1mmol), 2-bromo-1-(3-fluoro-3-methyl-azetidin-1-yl)ethanone (33.61 mg, 0.16 mmol), POTASSIUM CARBONATE (41.46 mg, 0.3 mmol), Acetone (1.3 mL), and potassium iodide (23.14 mg, 0.14 mmol) under ambient air. The mixture was then heated to 60°C overnight. Afterwards, the mixture was filtered (with methanol and DCM). The filtrate was then adsorbed onto celite and dried *in vacuo*. Flash chromatography (10-75% ethyl acetate in hexanes) afforded 5-(3-bromo-4-fluorophenyl)-3-(2-(3-fluoro-3-methylazetidin-1-yl)-2-oxoethyl)-6-iodothieno[2,3-d]pyrimidin-4(3H)-one (527.581 mg, 0.9091 mmol, 90.7% yield) as a white solid.TLC (2:3 ethyl acetate:hexanes): Rf = 0.28. Visualized with UV. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.33 (d, J = 3.5 Hz, 1H), 7.55 (dd, J = 6.5, 2.1 Hz, 1H), 7.29 – 7.25 (m, 2H), 7.22 (t, J = 8.4 Hz, 1H), 4.68 (d, J = 15.6 Hz, 1H), 4.54 – 4.41 (m, 2H), 4.32 (dd, J = 16.9, 9.7 Hz, 1H), 4.06 (dd, J = 17.3, 11.2 Hz, 1H), 1.70 – 1.63 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.5 (d, J_{C-F} = 3.1 Hz), 159.9, 158.3, 154.8, 147.9, 141.1,

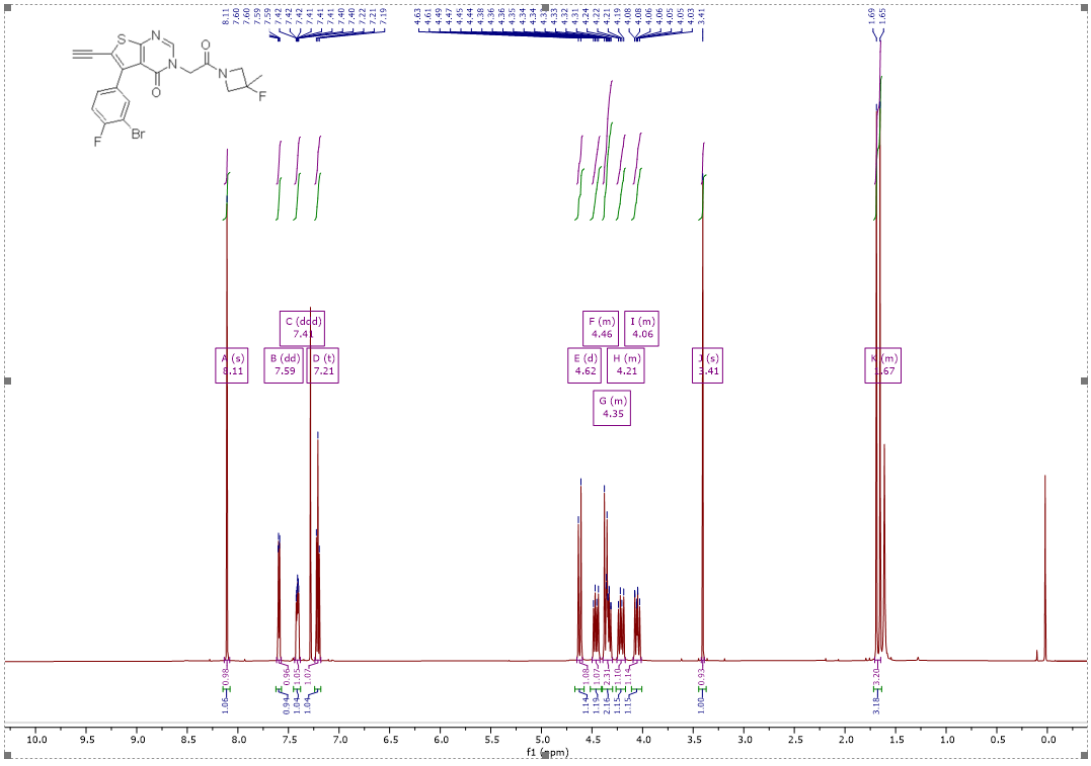
135.3, 132.72(d, J_{C-F} = 4.2 Hz), 131.0 (d, J_{C-F} = 7.3 Hz), 121.8, 116.2, 116.0, 108.7, 108.6, 90.7, 89.3, 79.7, 62.1 (d, J_{C-F} = 28.6 Hz), 60.8 (d, J_{C-F} = 28.1 Hz), 45.11, 22.96 (d, J_{C-F} = 25.4 Hz). ^{19}F NMR (565 MHz, $CDCl_3$) δ -107.20, -141.17. HRMS: [APCI$^+$] [M+H]$^+$ calc. for $C_{18}H_{14}O_2N_3{}^{79}BrF_2{}^{127}I{}^{32}S$, 579.90034, observed, 579.89974.



5-(3-bromo-4-fluorophenyl)-6-ethynyl-3-(2-(3-fluoro-3-methylazetidin-1-yl)-2-oxoethyl)thieno[2,3-d]pyrimidin-4(3H)-one (**EU-1622-240**). A 20 mL vial equipped with a stir bar was charged with (970.00 mg, 1.67 mmol), triethylamine (1.40 mL, 10.03 mmol), trimethylsilylacetylene (1.17 mL, 8.36 mmol), and THF (8 mL). The mixture was then degassed by bubbling argon into the solution for 30 minutes. Afterwards, CuI (25.47 mg, 0.13 mmol) and $Pd(PPh_3)_2Cl_2$ (58.68 mg, 0.08 mmol) were added, and the vial was crimp sealed and heated to 60 °C for 18 hours. Afterwards, the mixture was diluted in ethyl acetate, filtered over celite, and concentrated to afford the TMS-protected product as a red oil. The TMS-protected product was then dissolved in THF (5 mL) and cooled to 0 °C in a brine ice bath. TBAF (3.34 mL, 3.34 mmol) (1M in THF) was then added dropwise, and the resulting mixture was stirred for 30 minutes. Afterwards, the mixture was diluted in ethyl acetate (50 mL), washed with saturated sodium bicarbonate solution, dried over anhydrous sodium sulfate, filtered, and concentrated to afford a red oil. Flash chromatography (0-10% methanol in dichloromethane) followed by prep-HPLC (MeCN in water) and lyophilization afforded the title compound. Beige solid (306 mg, 0.6398 mmol, 38.265 % yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.74 – 7.70 (m, 1H), 7.46 – 7.42 (m, 1H), 7.17 (td, J = 8.4, 0.8 Hz,

1H), 4.61 (d, *J* = 15.4 Hz, 1H), 4.44 (dd, *J* = 18.8, 9.4 Hz, 1H), 4.34 (dd, *J* = 15.2, 5.9 Hz, 1H), 4.31 (ddd, *J* = 17.0, 9.6, 0.0 Hz, 1H), 4.19 (td, *J* = 19.9, 11.2 Hz, 1H), 4.03 (dd, *J* = 11.2, 6.1 Hz, 1H), 3.39 (s, 1H), 1.65 (d, *J* = 21.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.8 (d, $J_{C\text{-}F}$ = 3.1 Hz), 164.1, 159.1 (d, $J_{C\text{-}F}$ = 248.9 Hz), 156.5, 148.5, 140.8, 135.4, 131.2 (d, $J_{C\text{-}F}$ = 7.3 Hz), 130.7 (d, $J_{C\text{-}F}$ = 3.9 Hz), 120.8, 117.5, 115.7 (d, $J_{C\text{-}F}$ = 22.6 Hz), 108.3 (d, $J_{C\text{-}F}$ = 21.2 Hz), 90.0 (d, $J_{C\text{-}F}$ = 205.6 Hz), 86.0, 75.5, 62.3 (d, $J_{C\text{-}F}$ = 28.6 Hz), 60.7 (d, $J_{C\text{-}F}$ = 28.1 Hz), 44.8, 23.0 (d, $J_{C\text{-}F}$ = 25.5 Hz). ^{19}F NMR (565 MHz, $CDCl_3$) δ -107.7, -141.3. HRMS: [APCI+] $[M+H]^+$ calc. for $C_{20}H_{15}O_2N_3{}^{79}BrF_2{}^{32}S$, 478.00309, observed, 478.00408.

EU-1622-240 ^{1}H NMR Spectrum



EU-1622-240 ^{13}C NMR Spectrum