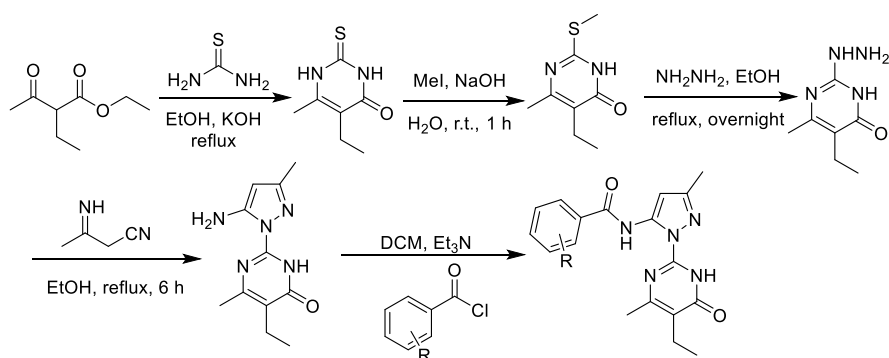


Supplemental Methods



Synthetic Methods. General Procedure for Synthesis of MMV6766477 analogs

All tested compounds have purity of >95% as judged by HPLC analysis (UV detection at 210 nm). Chemical shifts δ are in ppm, and spectra are referenced using the residual solvent peak. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), double doublet (dd), quintet (quin), multiplet (m), broad signal (br s). Mass spectra (m/z) were recorded on an Agilent LC-MS 1100 or 1290 Infinity using ESI ionization. All chemicals were used as received unless otherwise noted.

Step 1

Ethyl 2-ethyl-3-oxobutanoate (1.6 mL, 10 mmol) was added to a stirred suspension of thiourea (766 mg, 10 mmol) and KOH (677 mg, 12 mmol) in EtOH (20 mL). The solution was refluxed for 5 h and completion of the reaction was confirmed by LCMS. The solid formed was collected by filtration and then dissolved in H_2O . The solution was then acidified with 1N HCl to pH 1 to give white precipitate of 5-ethyl-6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one that was filtered and dried under vacuum (1.39 g, 82% yield). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.29 (s, 1H), 12.06 (s, 1H), 2.24 (q, $J = 8.0$ Hz, 2H), 2.11 (s, 3H), 0.92 (t, $J = 8.0$ Hz, 3H). ESI-MS (m/z): 171.1 $[\text{M}+\text{H}]^+$.

Step 2

NaOH (176 mg, 4.4 mmol) was added to a suspension of 5-ethyl-6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (685 mg, 4.0 mmol) in H₂O (12 mL) and the resulting mixture was allowed to stir to clarity. Then, the solution was cooled in an ice bath, and iodomethane (0.28 mL, 4.4 mmol) was added dropwise to the solution. The reaction was warmed to room temperature and continued to stir for 1 h. The solution was then acidified with 1N HCl to pH 7, and the precipitate formed was filtered, washed with H₂O and dried under vacuum to yield 5-ethyl-6-methyl-2-(methylthio)pyrimidin-4(3H)-one as a white solid (677 mg, 92% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.44 (s, 1H), 2.44 (s, 3H), 2.35 (q, *J* = 8.0 Hz, 2H), 2.20 (s, 3H), 0.96 (t, *J* = 8.0 Hz, 3H). ESI-MS (*m/z*): 185.1 [M+H]⁺.

Step 3

5-Ethyl-6-methyl-2-(methylthio)pyrimidin-4(3H)-one (619 mg, 3.3 mmol), hydrazine monohydrate (0.24 mL, 5 mmol), and EtOH (4 mL) were combined in a microwave tube and heated at 100 °C for 12 h. Then, the solution was filtered and the solid was washed with EtOH and dried overnight. The filtrate was condensed to give a purple oil, and EtOH and EtOAc were added to precipitate out any remaining product. Some solid formed and was filtered and dried overnight. The samples were combined to give 5-ethyl-2-hydrazineyl-6-methylpyrimidin-4(3H)-one as a light purple solid (352 mg, 62% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.26 (q, *J* = 8.0 Hz, 2H), 2.05 (s, 3H), 0.91 (t, *J* = 8.0 Hz, 3H). ESI-MS (*m/z*): 169.1 [M+H]⁺.

Step 4

3-Iminobutanenitrile (197 mg, 2.4 mmol) was added to a stirred solution of 5-ethyl-2-hydrazineyl-6-methylpyrimidin-4(3H)-one (344 mg, 2.0 mmol) in EtOH (6 mL). After heating at 100 °C for 6 h, the mixture was cooled to room temperature and concentrated to give a yellow solid. The solid was recrystallized from EtOAc/Hexanes to provide 2-(5-amino-3-methyl-1H-pyrazol-1-yl)-5-ethyl-6-methylpyrimidin-4(3H)-one (**SW223075**) as a yellow solid (820 mg, 88% yield). ¹H NMR (400

MHz, DMSO- d_6) δ 11.39 (br s, 1H), 6.84 (br s, 2H), 5.25 (s, 1H), 2.40 (q, J = 8.0 Hz, 2H), 2.27 (s, 3H), 2.08 (s, 3H), 0.99 (t, J = 8.0 Hz, 3H). ESI-MS (m/z): 234.1 $[M+H]^+$.

Step 5

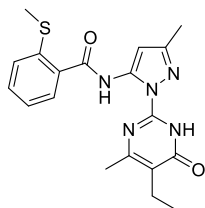
The acid chlorides are either commercially available or prepared from the corresponding carboxylic acid.

Acylation Procedure A: Acid chloride (0.15 mmol) was added to a stirred solution of 2-(5-amino-3-methyl-1H-pyrazol-1-yl)-5-ethyl-6-methylpyrimidin-4(3H)-one (23 mg, 0.1 mmol) in DCM (4 mL) and triethylamine (0.35 mL) at -40 °C. The solution was stirred for 2 h and monitored by LCMS. After the reaction was complete, the reaction was quenched with NaHCO₃ solution, and was allowed to stir for 5 minutes. Then the aqueous phase was extracted with DCM (3 × 10 mL), and washed with 1N HCl (1 × 10 mL) and brine (1 × 10 mL). It was dried with Na₂SO₄ and condensed. The resulting residue was then purified by column on silica gel to give the product.

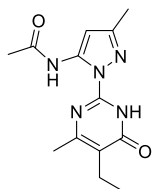
Acylation Procedure B: Acid chloride (0.6 mmol) was added to a stirred solution of 2-(5-amino-3-methyl-1H-pyrazol-1-yl)-5-ethyl-6-methylpyrimidin-4(3H)-one (23 mg, 0.1 mmol) in DCM (4 mL) and triethylamine (55 μ L, 0.4 mmol) at 0 °C. The solution was stirred for 30 minutes at room temperature and monitored by LCMS. After the reaction was complete, the reaction mixture was diluted with water and extracted with DCM (3 × 10 mL). The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. Flash column chromatography of the residue furnished the *N,O*-diacylated product.

To a solution of the above *N,O*-diacylated compound in MeOH (4 mL), solid K₂CO₃ (41 mg, 0.3 mmol) was added, and the resulting mixture was stirred for 30 minutes at room temperature. After that, the reaction was quenched by addition of water. The aqueous layer was extracted with EtOAc (3 × 10 mL), and the combined organic layer was dried over anhydrous Na₂SO₄,

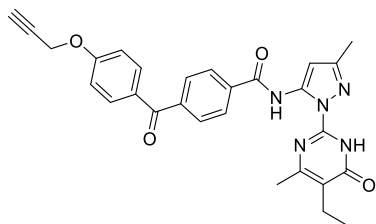
evaporated under reduced pressure and purified by flash column chromatography to give the product.



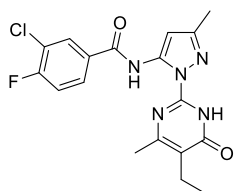
***N*-(1-(5-Ethyl-4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-methyl-1H-pyrazol-5-yl)-2-(methylthio)benzamide (MMV676477).** The target compound (85% yield) was obtained by the general procedure B described above. ^1H NMR (400 MHz, CDCl_3) δ 12.29 (s, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.23 (t, J = 8.0 Hz, 1H), 6.89 (s, 1H), 2.52 (q, J = 8.0 Hz, 2H), 2.48 (s, 3H), 2.28 (s, 3H), 2.26 (s, 3H), 1.10 (t, J = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 164.2, 161.3, 157.5, 153.6, 146.2, 141.3, 140.8, 132.0, 127.9, 126.1, 124.3, 122.9, 98.9, 21.2, 19.2, 16.1, 14.3, 12.9. ESI-MS (m/z): 384.1 $[\text{M}+\text{H}]^+$.



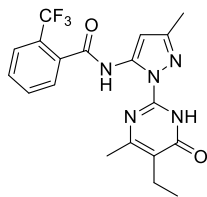
***N*-(1-(5-Ethyl-4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-methyl-1H-pyrazol-5-yl)acetamide (SW223023).** The target compound (57% yield) was obtained by the general procedure A described above. ^1H NMR (400 MHz, CDCl_3) δ 11.62 (s, 1H), 6.68 (s, 1H), 2.51 (q, J = 6.8, 5.9 Hz, 2H), 2.33 (s, 3H), 2.24 (s, 3H), 2.23 (s, 3H), 1.10 (t, J = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 166.4, 161.2, 157.3, 153.1, 140.4, 122.6, 98.5, 24.4, 21.2, 18.8, 14.1, 12.8. ESI-MS (m/z): 276 $[\text{M}+\text{H}]^+$.



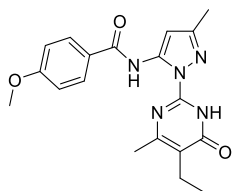
***N*-(1-(5-Ethyl-4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-methyl-1H-pyrazol-5-yl)-4-(4-(prop-2-yn-1-yloxy)benzoyl)benzamide (SW223022).** The target compound (60% yield) was obtained by the general procedure B described above. ^1H NMR (400 MHz, CDCl_3) δ 12.59 (s, 1H), 10.27 (br s, 1H), 8.11 (d, $J = 8.0$ Hz, 2H), 7.90 (d, $J = 8.0$ Hz, 2H), 7.85 (d, $J = 12.0$ Hz, 2H), 7.07 (d, $J = 12.0$ Hz, 2H), 6.89 (s, 1H), 4.80 (d, $J = 2.4$ Hz, 2H), 2.60-2.54 (m, 3H), 2.41 (s, 3H), 2.32 (s, 3H), 1.13 (t, $J = 8.0$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 194.5, 162.7, 161.6, 160.9, 157.0, 153.7, 146.2, 141.9, 140.7, 136.1, 132.7, 130.2, 127.4, 123.3, 114.8, 99.1, 77.6, 76.3, 56.1, 21.4, 19.1, 14.4, 12.9. ESI-MS (m/z): 496.1 $[\text{M}+\text{H}]^+$.



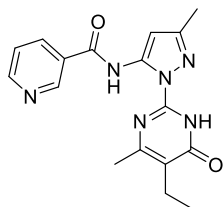
3-Chloro-*N*-(1-(5-ethyl-4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-methyl-1H-pyrazol-5-yl)-4-fluorobenzamide (SW223041). The target compound (88% yield) was obtained by the general procedure B described above. ^1H NMR (400 MHz, CDCl_3) δ 12.54 (s, 1H), 8.05 (dd, $J = 8.0, 4.0$ Hz, 1H), 7.97-7.91 (m, 1H), 7.21 (t, $J = 8.0$ Hz, 1H), 6.82 (s, 1H), 2.56 (q, $J = 8.0$ Hz, 2H), 2.43 (s, 3H), 2.30 (s, 3H), 1.12 (t, $J = 8.0$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 162.1, 161.2, 159.6, 157.2, 153.6, 140.4, 130.6 (d), 129.8, 128.1 (d), 123.2, 122.2, 122.0, 117.6, 117.3, 98.9, 21.4, 19.0, 14.3, 12.9. ESI-MS (m/z): 390.1 $[\text{M}+\text{H}]^+$.



***N*-(1-(5-Ethyl-4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-methyl-1H-pyrazol-5-yl)-2-(trifluoromethyl)benzamide (SW223073).** The target compound (91% yield) was obtained by the general procedure B described above. ^1H NMR (400 MHz, CDCl_3) δ 12.11 (s, 1H), 7.84-7.80(m, 1H), 7.74-7.64 (m, 3H), 6.89 (s, 1H), 2.50 (q, J = 8.0 Hz, 2H), 2.31 (s, 3H), 2.07 (s, 3H), 1.08 (t, J = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 163.6, 161.1, 157.3, 153.4, 145.9, 140.0, 134.9, 132.2, 131.0, 128.4, 127.0 (q), 124.8, 122.8, 122.9, 122.0, 99.3, 20.9, 18.9, 14.2, 12.8. ESI-MS (m/z): 406.1 $[\text{M}+\text{H}]^+$.

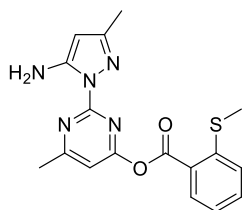


***N*-(1-(5-Ethyl-4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-methyl-1H-pyrazol-5-yl)-4-methoxybenzamide (SW223072).** The target compound (85% yield) was obtained by the general procedure B described above. ^1H NMR (400 MHz, CDCl_3) δ 12.32 (s, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.00 (d, J = 8.0 Hz, 2H), 6.83 (s, 1H), 3.90 (s, 3H), 2.56 (q, J = 8.0 Hz, 2H), 2.42 (s, 3H), 2.29 (s, 3H), 1.13 (t, J = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 163.2, 161.1, 157.2, 153.7, 146.2, 141.2, 129.4, 125.7, 123.0, 114.2, 98.5, 55.7, 21.3, 19.0, 14.3, 12.9. ESI-MS (m/z): 368.1 $[\text{M}+\text{H}]^+$.



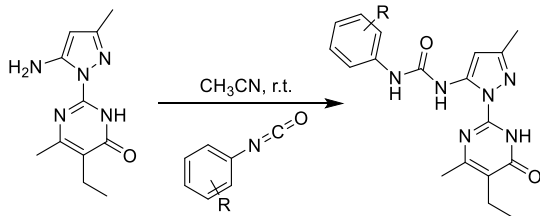
***N*-(1-(5-Ethyl-4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-methyl-1H-pyrazol-5-yl)**

nicotinamide (SW223074). The target compound (80% yield) was obtained by the general procedure B described above. ^1H NMR (400 MHz, CDCl_3) δ 12.61 (s, 1H), 10.27 (br s, 1H), 9.22 (d, $J = 1.2$ Hz, 1H), 8.84 (dd, $J = 4.8, 1.6$ Hz, 1H), 8.33 (td, $J = 8.0, 1.6$ Hz, 1H), 7.51 (dd, $J = 7.6, 4.8$ Hz, 1H), 6.87 (s, 1H), 2.57 (q, $J = 8.0$ Hz, 2H), 2.43 (s, 3H), 2.32 (s, 3H), 1.13 (t, $J = 8.0$ Hz, 3H); ^{13}C NMR (101 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$) δ 161.5, 153.3, 153.0, 152.9, 147.9, 140.1, 135.9, 129.4, 124.1, 99.0, 21.3, 18.9, 14.0, 12.8. ESI-MS (m/z): 339.2 $[\text{M}+\text{H}]^+$.

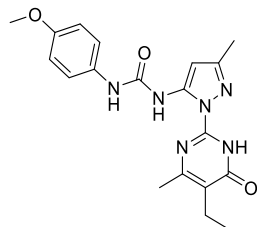


2-(5-Amino-3-methyl-1H-pyrazol-1-yl)-6-methylpyrimidin-4-yl 2-(methylthio)benzoate

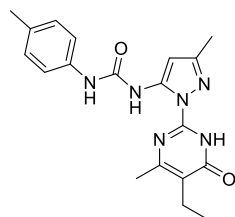
(SW233010). The target compound (41% yield) was obtained by the similar procedure A described above. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.21 (dd, $J = 7.9, 1.5$ Hz, 1H), 7.73-7.69 (m, 1H), 7.49 (d, $J = 8.2$ Hz, 1H), 7.36-7.32 (m, 1H), 7.18 (s, 1H), 6.71 (s, 2H), 5.25 (s, 1H), 2.56 (s, 3H), 2.48 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 172.4, 166.0, 162.8, 157.4, 151.8, 151.2, 145.3, 134.9, 132.5, 125.6, 124.4, 124.0, 108.4, 88.7, 24.2, 15.2, 14.4. ESI-MS (m/z): 356 $[\text{M}+\text{H}]^+$.



To a suspension of 2-(5-amino-3-methyl-1H-pyrazol-1-yl)-5-ethyl-6-methylpyrimidin-4(3H)-one (23 mg, 0.1 mmol) in CH₃CN (5 mL), isocyanate (0.15 mmol) was added. The mixture was stirred at room temperature and monitored by LCMS. After the reaction was complete, the solution was condensed and resulting residue was purified by flash chromatography on silica gel to provide the product.



1-(1-(5-Ethyl-4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-methyl-1H-pyrazol-5-yl)-3-(4-methoxyphenyl)urea (SW223102). The target compound (64% yield) was obtained by the general procedure described above. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.55 (s, 1H), 10.06 (s, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 6.55 (s, 1H), 3.75-3.55 (m, 5H), 2.42 (s, 3H), 2.23 (s, 3H), 1.04 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 155.4, 152.0, 150.7, 149.7, 142.3, 132.6, 121.2, 120.2, 118.9, 114.4, 114.4, 96.8, 55.6, 19.9, 18.5, 14.1, 13.0. ESI-MS (*m/z*): 383 [M+H]⁺.



1-(1-(5-Ethyl-4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-methyl-1H-pyrazol-5-yl)-3-(p-tolyl)urea (SW223101). The target compound (66% yield) was obtained by the general procedure described above. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.57 (s, 1H), 10.23 (s, 1H), 7.43 (d, *J* = 8.2 Hz, 2H), 7.27 (d, *J* = 3.3 Hz, 1H), 7.09 (d, *J* = 8.1 Hz, 2H), 6.58 (s, 1H), 2.51 (q, *J* = 7.3 Hz, 2H),

2.45 (s, 3H), 2.24 (s, 6H), 1.04 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 151.8, 150.6, 149.5, 142.0, 138.6, 137.1, 131.9, 129.9, 129.6, 129.6, 129.0, 119.4, 118.5, 96.8, 21.2, 20.8, 18.5, 14.2, 13.1. ESI-MS (m/z): 367 $[\text{M}+\text{H}]^+$.