Discovery and characterization of 1*H*-pyrazol-5-yl-2-phenylacetamides as novel, non-urea containing GIRK1/2 potassium channel activators

Joshua M. Wieting[†], Anish K. Vadukoot[‡], Swagat Sharma[‡], Kristopher K. Abney[∫], Thomas M. Bridges[∞], J. Scott Daniels[ʃ], Ryan D. Morrison[ʃ], Kevin Wickman[∇], C. David Weaver[§,Δ], and Corey R. Hopkins[‡,*]

[†]Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University, Nashville, TN 37232, [‡]Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198, [∫]Department of Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN 37208, [∞]Vanderbilt Center for Neuroscience Drug Discovery, Nashville, TN 37232, [ʃ]Sano Informed Prescribing, Franklin, TN 37067, [∇]Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455, [§]Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232, [Δ]Vanderbilt Institute of Chemical Biology, Nashville, TN 37232

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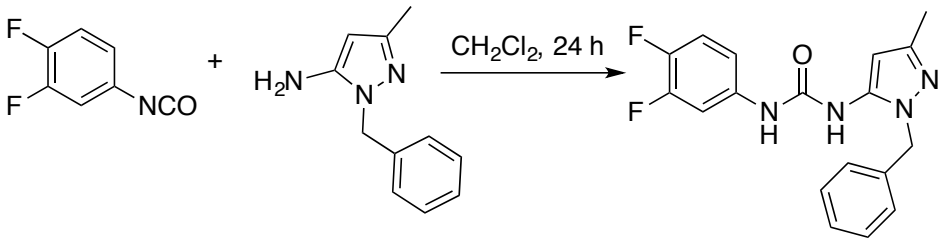
General Experimental. All NMR spectra were recorded on a 400 MHz AMX, AV-400 or Avance III HD 500 MHz Bruker NMR spectrometer. ^{1}H and ^{13}C chemical shifts are reported in δ values in ppm downfield with the deuterated solvent as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), integration, coupling constant (Hz). Low resolution mass spectra were obtained on an Agilent 6120 or 6150 with ESI source. Method A: MS parameters were as follows: fragmentor: 70, capillary voltage: 3000 V, nebulizer pressure: 30 psig, drying gas flow: 13 L/min, drying gas temperature: 350 ^{o}C. Samples were introduced via an Agilent 1290 UHPLC comprised of a G4220A binary pump, G4226A ALS, G1316C TCC, and G4212A DAD with ULD flow cell. UV absorption was generally observed at 215 nm and 254 nm with a 4 nm bandwidth. Column: Waters Acquity BEH C18, 1.0 x 50 mm, 1.7 um. Gradient conditions: 5% to 95% CH_3CN in H_2O (0.1% TFA) over 1.4 min, hold at 95% CH3CN for 0.1 min, 0.5 mL/min, 55 ^{o}C. Method B: MS parameters were as follows: fragmentor: 100, capillary voltage: 3000 V, nebulizer pressure: 60 psig, drying gas flow: 12 L/min, drying gas temperature: 350 ^{o}C. Samples were introduced via an Agilent 1260 HPLC comprised of a degasser, G7111A quaternary pump, G4237A ALS, G7116A TCC, G1314 VWD with a ULD flow cell. UV absorption was generally observed at 215 nm and 254 nm with a 4 nm bandwidth. Column: Poroshell 120, SB-C18, 4.6 x 75 mm, 2.7 mm. Gradient conditions: 5% to 80% CH_3CN in H_2O (0.1% TFA) from 0 – 4 min, 80 to 95% CH_3CN in H_2O (0.1% TFA) from 4 – 6 min, hold at 95% CH_3CN from 6 – 8 min, 95% CH_3CN for 0.1 min, 1.5 mL/min, 45 ^{o}C. High resolution mass spectra were obtained on a Waters Micromass® Q-TOF with ESI source. Solvents for

extraction, washing and chromatography were HPLC grade. All reagents were purchased from commercial sources and were used without purification.

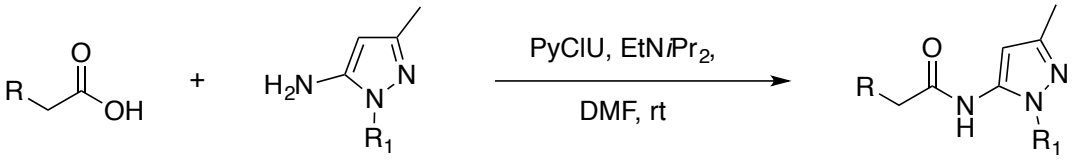
Chemical Synthesis:



1-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-3-(3,4-difluorophenyl)urea (1): A 1 dram vial equipped with a magnetic stir bar and a screw cap vial was charged with the appropriate 1-benzyl-3-methyl-1*H*-pyrazol-5-amine (0.10 mmol, 1 equivalent), CH_2Cl_2 (0.4 mL), 3,4-Difluorophenyl isocyanate (12 μL, 0.10 mmol, 1 equivalent) and the reaction mixture was stirred at ambient temperature for 24 hours. Solvent was removed and the resulting solid was washed with diethyl ether to yield the corresponding urea product.

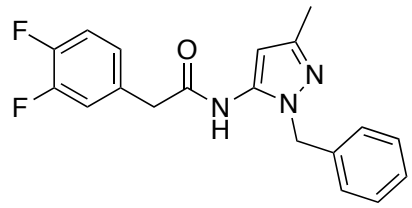
Analytical LCMS: single peak (254 nm), R_T = 1.025 min; MS (ESI^+) *m/z* 343.4 $[M + H]^+$

General Procedure for Amide Couplings:



A one dram vial equipped with magnetic stir bar and screw cap vial was charged with the appropriate phenyl acetic acid derivative (0.10 mmol), chlorodipyrrolidinocarbenium hexafluorophosphate (PyClU) (33 mg, 0.10 mmol), DMF (0.3 mL), Hunig's Base (0.1 mL, 0.57 mmol) and was stirred for approximately 5 minutes. The appropriate 5-Amino pyrazole (0.10 mmol) was added and the reaction stirred until LCMS analysis indicated significant consumption of the starting materials (10 mins-18 hours). The crude reaction

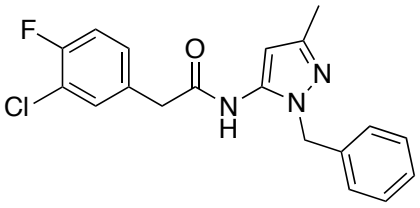
mixture was purified via mass-directed acidic reverse phase preparative HPLC to yield the corresponding amide products. This general procedure used for the entire library of compounds. Representative lead compounds and their characterization data are shown below.



***N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-2-(3,4-difluorophenyl)acetamide (7a):**

Following the general procedure above, compound (**7a**) was obtained (58.2 mg; 57% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.960 min; MS (ESI^+) *m/z* = 342.2 $[M + H]^+$.

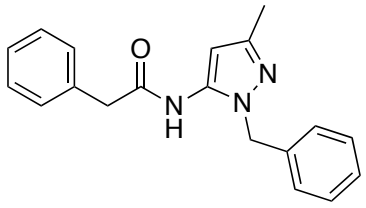


***N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-2-(3-chloro-4-fluorophenyl)acetamide (7b):**

Following the general procedure above, compound (**7b**) was obtained (47.3 mg; 49% yield).

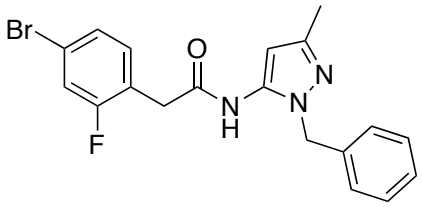
Analytical LCMS (Method A): single peak (254 nm), R_T = 0.993 min; MS (ESI^+) *m/z* = 358.2 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 10.13 (s, 1H), 7.48 (d, J = 6.09 Hz, 1H),

7.20 - 7.39 (m, 4H), 7.03 (d, *J* = 7.00 Hz, 2H), 6.05 (s, 1H), 5.14 (s, 2H), 3.66 (s, 2H), 2.07 - 2.12 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.3, 157.1, 155.2, 146.3, 137.5, 136.5, 133.3, 131.1, 129.8, 128.3, 127.2, 127.0, 119.1, 116.7, 98.7, 50.8, 40.7, 13.7.



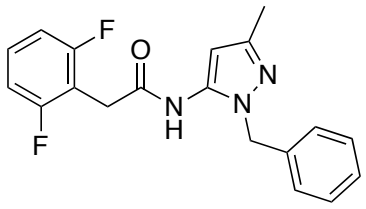
***N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-2-phenylacetamide (7c):** Following the general procedure above, compound (**7c**) was obtained (23.2 mg; 76% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.895 min; MS (ESI$^+$) *m/z* = 306.4 $[M + H]^+$.



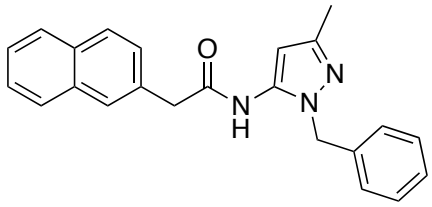
***N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-2-(4-bromo-2-fluorophenyl)acetamide (7d):** Following the general procedure above, compound (**7d**) was obtained (10.9 mg; 27% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 1.016 min; MS (ESI$^+$) *m/z* = 404.0 $[M + H]^+$.



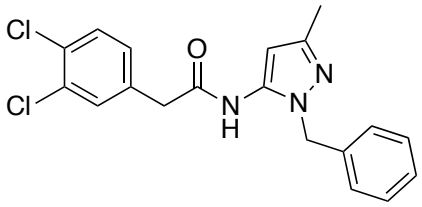
***N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-2-(2,6-difluorophenyl)acetamide (7e):** Following the general procedure above, compound (**7e**) was obtained (20.0 mg; 59% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.906 min; MS (ESI^+) *m/z* = 342.2 $[M + H]^+$.



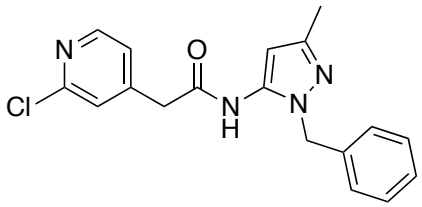
***N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-2-(naphthalen-2-yl)acetamide (7f):** Following the general procedure above, compound (**7f**) was obtained. (75.0 mg; 79%)

Analytical LCMS (Method B): single peak (254 nm), R_T = 4.315 min; MS (ESI^+) *m/z* = 356.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 10.20 (s, 1H), 7.95 – 7.82 (m, 3H), 7.79 (s, 1H), 7.50 (tt, *J* = 7.0, 5.2 Hz, 2H), 7.42 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.25 – 7.18 (m, 3H), 7.04 – 6.98 (m, 2H), 6.07 (s, 1H), 5.14 (s, 2H), 3.82 (s, 2H), 2.09 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.36, 146.76, 137.96, 137.07, 133.68, 133.44, 132.33, 128.77, 128.25, 127.97, 127.90, 127.66, 127.57, 126.63, 126.15, 99.18, 51.33, 42.83, 14.22.



N-(1-benzyl-3-methyl-1H-pyrazol-5-yl)-2-(3,4-dichlorophenyl)acetamide (7g):

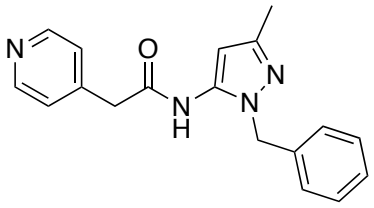
Following the general procedure above, compound (**7g**) was obtained. (72.2 mg; 73%) Analytical LCMS (Method B): single peak (254 nm), R_T = 4.443 min; MS (ESI^+) *m/z* = 374.0 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 10.15 (s, 1H), 7.65 – 7.50 (m, 2H), 7.40 – 7.20 (m, 4H), 7.12 – 6.96 (m, 2H), 6.06 (s, 1H), 5.14 (s, 2H), 3.68 (s, 2H), 2.09 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.60, 146.79, 137.96, 137.04, 136.90, 131.70, 131.22, 130.85, 130.10, 129.85, 128.80, 127.69, 127.49, 99.20, 51.33, 41.36, 14.21.



***N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-2-(2-chloropyridin-4-yl)acetamide (7h):**

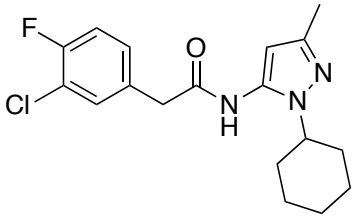
Following the general procedure above, compound (**7h**) was obtained. (68.4 mg; 75%) Analytical LCMS (Method B): single peak (254 nm), R_T = 3.506 min; MS (ESI^+) *m/z* = 341.0 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 10.23 (s, 1H), 8.33 (d, J = 5.1 Hz, 1H), 7.43 (s, 1H), 7.35 – 7.20 (m, 5H), 7.12 – 6.95 (m, 2H), 6.07 (s, 1H), 5.17 (s, 2H), 3.76 (s, 2H), 2.10 (S, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.66, 150.67, 150.13, 148.76,

146.84, 137.98, 136.81, 128.83, 127.72, 127.47, 125.37, 124.56, 99.21, 51.33, 41.25, 31.17, 14.21.



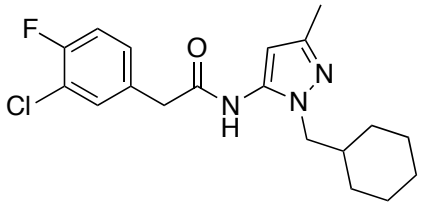
N-(1-benzyl-3-methyl-1H-pyrazol-5-yl)-2-(pyridin-4-yl)acetamide (7i): Following the general procedure above, compound (**7i**) was obtained. (55.0 mg; 67%)

Analytical LCMS (Method B): single peak (254 nm), R_T = 2.294 min; MS (ESI^+) *m/z* = 307.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.56 – 8.42 (m, 2H), 7.39 – 7.18 (m, 5H), 7.10 – 6.98 (m, 2H), 6.07 (s, 1H), 5.16 (s, 2H), 3.70 (s, 2H), 2.10 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.13, 149.94, 146.80, 144.75, 137.99, 136.92, 128.84, 127.70, 127.47, 124.99, 99.18, 51.34, 41.80, 14.21.



2-(3-chloro-4-fluorophenyl)-*N*-(1-cyclohexyl-3-methyl-1*H*-pyrazol-5-yl)acetamide (8a): Following the general procedure above, compound (**8a**) was isolated as a colorless oil (15.3 mg, 44% yield).

Analytical LCMS (Method AB: single peak (254 nm), R_T = 4.326 min; MS (ESI^+) *m/z* = 350.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) The compound exists as a 4:1 ratio of amide rotamers. Signals corresponding to the major rotamer: δ 7.38 (d, *J* = 6.6 Hz, 1H), 7.21–7.06 (m, 2H), 5.96 (s, 1H), 3.64 (s, 2H), 3.62–3.51 (m, 1H), 2.20 (s, 3H), 1.93–1.58 (m, 7H), 1.32–1.10 (m, 3H), Signals corresponding to the minor rotamer: δ 5.88 (s, 1H), 3.45 (s, 2H), 3.93–3.78 (m, 1H), 2.26 (s, 3H); ^{13}C NMR (101 MHz, CDCl3) only the chemical shifts of the major amide rotamer are reported ™ 14.0, 25.0, 25.7, 32.5, 42.4, 57.0, 100.0, 117.2 (J_{CF} = 21 Hz), 121.7 (J_{CF} = 18 Hz), 128.0 (J_{CF} = 7 Hz), 131.2 (J_{CF} = 4 Hz), 131.3, 147.2, 157.6 (J_{CF} = 250 Hz), 168.7; HRMS (ESI^+): calculated for $C_{18}H_{21}ClFN_3O$, 349.1357; found 350.1435 $[M + H]^+$.

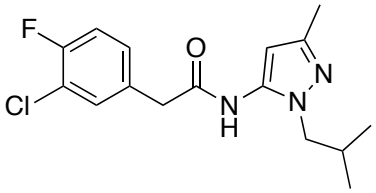


2-(3-chloro-4-fluorophenyl)-*N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (8b): Following the general procedure above, compound (**8b**) was isolated as a white wax (72.5 mg; 76% yield).

Analytical LCMS (Method B): single peak (254 nm), R_T = 4.522 min; MS (ESI^+) *m/z* = 364.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) The compound exists as a 5:1 ratio of amide rotamers. Signals corresponding to the major rotamer: δ 7.36 (d, *J* = 6.6 Hz, 1H), 7.212–7.09 (m, 2H), 6.04 (s, 1H), 3.63 (s, 2H), 3.50 (d, *J* = 7.4 Hz, 1H), 2.18 (s, 3H), 1.72–1.54 (m, 4H), 1.52–1.35 (m, 2H), 1.19–0.99 (m, 3H), 0.94–0.54 (m, 2H), Signals

corresponding to the minor rotamer: δ 5.89 (s, 1H), 3.44 (s, 2H), 3.58 (d, *J* = 7.4 Hz, 2H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, CDCl3) only the chemical shifts of the major amide rotamer are reported δ 13.9, 25.5, 26.0, 30.6, 38.4, 42.5, 54.3, 99.0, 117.2 (J_{CF} = 21 Hz), 121.7 (J_{CF} = 18 Hz), 129.1 (J_{CF} = 7 Hz), 131.1 (J_{CF} = 4 Hz), 131.4, 134.9, 147.4, 157.6 (J_{CF} = 250 Hz), 168.0;

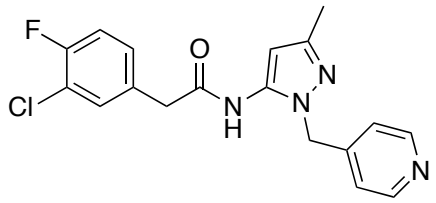
HRMS (ESI$^+$): calculated for $C_{19}H_{23}ClFN_3O$, 363.1514; found 364.1592 [M + H]$^+$.



2-(3-chloro-4-fluorophenyl)-*N*-(1-isobutyl-3-methyl-1*H*-pyrazol-5-yl)acetamide (8c): Following the general procedure above, compound (**8c**) was isolated as a clear yellow oil (10.8 mg, 33% yield).

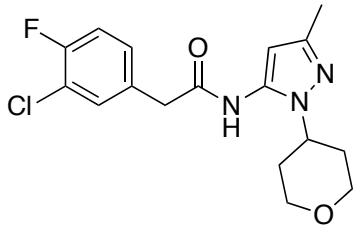
Analytical LCMS (Method A): single peak (254 nm), R_T = 0.943 min; MS (ESI$^+$) *m/z* = 324.2 [M + H]$^+$. ^{1}H NMR (400 MHz, $CDCl_3$) The compound exists as a 5:1 ratio of amide rotamers. Signals corresponding to the major rotamer: δ 7.37 (d, *J* = 6.6 Hz, 1H), 7.21–7.09 (m, 2H), 6.04 (s, 1H), 3.65 (s, 2H), 3.50 (d, *J* = 7.4 Hz, 1H), 2.19 (s, 3H), 2.03–1.90 (m, 1H), 0.74 (d, *J* = 6.6 Hz, 6H), Signals corresponding to the minor rotamer: δ 5.90 (s, 1H), 3.45 (s, 2H), 3.56 (d, *J* = 7.4 Hz, 2H), 2.26 (s, 3H), 0.84 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, CDCl3) only the chemical shifts of the major amide rotamer are reported δ 13.9, 19.8, 29.3, 42.5, 55.4, 99.4, 117.3 (J_{CF} = 21 Hz), 121.8 (J_{CF} = 18 Hz), 129.1 (J_{CF} = 7 Hz), 131.0 (J_{CF} = 4 Hz), 131.4, 134.6, 147.4, 157.7 (J_{CF} = 250 Hz), 168.0;

HRMS (ESI$^+$): calculated for $C_{16}H_{19}ClFN_3O$, 323.1201; found 324.1279 $[M + H]^+$.



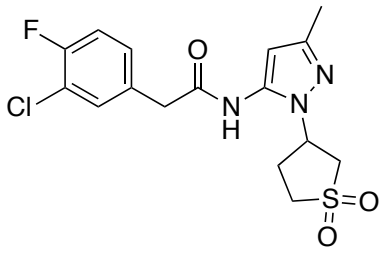
2-(3-chloro-4-fluorophenyl)-*N*-(3-methyl-1-(pyridin-4-ylmethyl)-1*H*-pyrazol-5-yl)acetamide (8d): Following the general procedure above, compound (**8d**) was obtained (11.5 mg; 32% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.710 min; MS (ESI$^+$) *m/z* = 359.3 $[M + H]^+$.



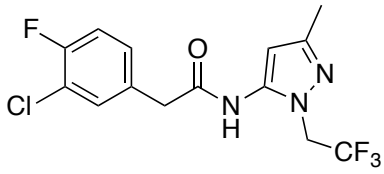
2-(3-chloro-4-fluorophenyl)-*N*-(3-methyl-1-(tetrahydro-2H-pyran-4-yl)-1*H*-pyrazol-5-yl)acetamide (8e): Following the general procedure above, compound (**8e**) was obtained (8.2 mg; 23% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.859 min; MS (ESI$^+$) *m/z* = 352.3 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 9.98 (s, 1H), 7.52 - 7.60 (m, 1H), 7.39 (d, J = 9.13 Hz, 2H), 5.94 (s, 1H), 4.06 - 4.13 (m, 1H), 3.92 (dd, J = 3.96, 11.26 Hz, 2H), 3.69 (s, 2H), 3.22 - 3.31 (m, 2H), 2.10 (s, 3H), 1.93 (dd, J = 4.26, 12.17 Hz, 2H), 1.64 (d, J = 12.17 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.3, 145.7, 135.1, 133.6, 131.1, 130.0, 129.9, 116.8, 99.1, 66.3, 59.7, 54.9, 52.3, 40.8, 32.4, 20.8, 14.1.



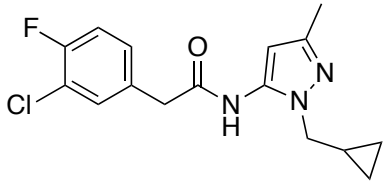
2-(3-chloro-4-fluorophenyl)-*N*-(1-(1,1-dioxidotetrahydrothiophen-3-yl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (8f): Following the general procedure above, compound (**8f**) was obtained (10.0 mg; 26% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.868 min; MS (ESI^+) *m/z* = 386.2 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 10.12 (s, 1H), 7.49 - 7.58 (m, 1H), 7.37 (d, *J* = 9.13 Hz, 2H), 6.01 (s, 1H), 5.09 (s, 1H), 3.73 (s, 2H), 3.54 - 3.61 (m, 1H), 3.44 - 3.52 (m, 1H), 3.30 (s, 1H), 3.13 - 3.22 (m, 1H), 2.46 (t, *J* = 7.61 Hz, 2H), 2.12 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.9, 147.3, 136.4, 133.2, 131.4, 130.1, 130.0, 116.7, 116.6, 99.1, 54.5, 51.6, 51.0, 40.5, 29.0, 13.9.



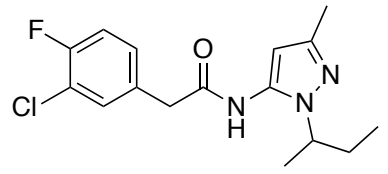
2-(3-chloro-4-fluorophenyl)-*N*-(3-methyl-1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl)acetamide (8g): Following the general procedure above, compound (**8g**) was obtained (10.8 mg; 31% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.982 min; MS (ESI^+) *m/z* = 350.2 $[M + H]^+$.



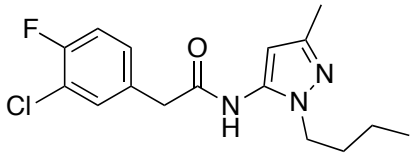
2-(3-chloro-4-fluorophenyl)-*N*-(1-(cyclopropylmethyl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (8h): Following the general procedure above, compound (**8h**) was obtained (11.2 mg; 35% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.908 min; MS (ESI^+) *m/z* = 322.2 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.49 - 7.59 (m, 1H), 7.27 - 7.43 (m, 2H), 5.98 (s, 1H), 3.73 (d, J = 6.70 Hz, 2H), 3.69 (s, 2H), 2.08 (s, 3H), 1.06 - 1.14 (m, 1H), 0.36 - 0.45 (m, 2H), 0.21 (q, J = 4.77 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.4, 145.5, 135.6, 133.4, 131.1, 129.9, 129.8, 119.1, 116.7, 116.6, 98.6, 51.5, 40.8, 13.7, 11.2, 3.4.



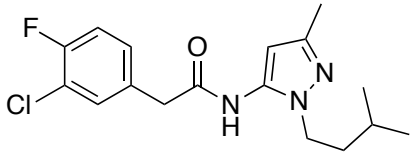
***N*-(1-(sec-butyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(3-chloro-4-fluorophenyl)acetamide (8i):** Following the general procedure above, compound (**8i**) was obtained (6.4 mg; 20% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.951 min; MS (ESI^+) *m/z* = 324.3 $[M + H]^+$.



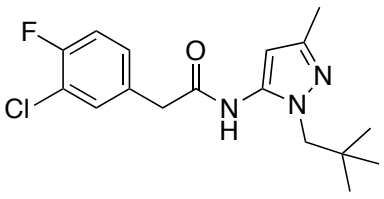
***N*-(1-butyl-3-methyl-1*H*-pyrazol-5-yl)-2-(3-chloro-4-fluorophenyl)acetamide (8j):** Following the general procedure above, compound (**8j**) was obtained (11.7 mg; 36% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.890; MS (ESI^+) *m/z* = 324.4 $[M + H]^+$.



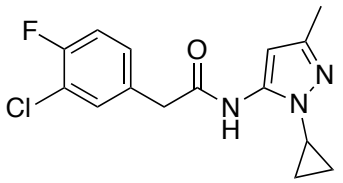
2-(3-chloro-4-fluorophenyl)-*N*-(1-isopentyl-3-methyl-1*H*-pyrazol-5-yl)acetamide (8k): Following the general procedure above, compound (**8k**) was obtained (14.4 mg; 43% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.940; MS (ESI^+) m/z 338.2 $[M + H]^+$.



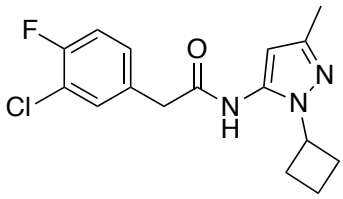
2-(3-chloro-4-fluorophenyl)-*N*-(3-methyl-1-neopentyl-1*H*-pyrazol-5-yl)acetamide (8l): Following the general procedure above, compound (**8l**) was obtained (1.6 mg; 5% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 1.008 min; MS (ESI^+) *m/z* = 338.4 $[M + H]^+$.



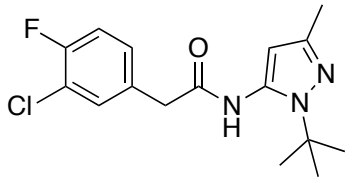
2-(3-chloro-4-fluorophenyl)-*N*-(1-cyclopropyl-3-methyl-1*H*-pyrazol-5-yl)acetamide (8m): Following the general procedure above, compound (**8m**) was obtained (4.5 mg; 15% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.870 min; MS (ESI^+) *m/z* = 308.3 $[M + H]^+$.



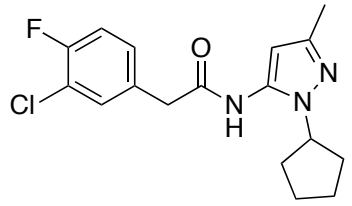
2-(3-chloro-4-fluorophenyl)-*N*-(1-cyclobutyl-3-methyl-1*H*-pyrazol-5-yl)acetamide (8n): Following the general procedure above, compound (**8n**) was obtained (2.5 mg; 8% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.940 min; MS (ESI^+) *m/z* = 322.4 $[M + H]^+$.



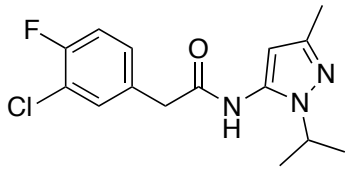
***N*-(1-(tert-butyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(3-chloro-4-fluorophenyl)acetamide (8o):** Following the general procedure above, compound (**8o**) was obtained (4.6 mg; 14% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.850 min; MS (ESI^+) *m/z* = 324.2 $[M + H]^+$.



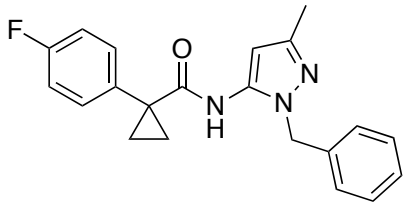
2-(3-chloro-4-fluorophenyl)-*N*-(1-cyclopentyl-3-methyl-1*H*-pyrazol-5-yl)acetamide (8p): Following the general procedure above, compound (**8p**) was obtained (5.2 mg; 16% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.963 min; MS (ESI^+) *m/z* = 336.3 $[M + H]^+$.



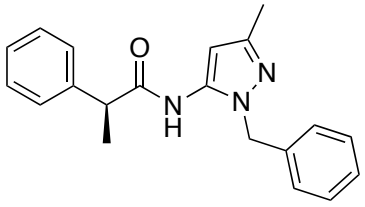
2-(3-chloro-4-fluorophenyl)-*N*-(1-isopropyl-3-methyl-1*H*-pyrazol-5-yl)acetamide (8q): Following the general procedure above, compound (**27**) was obtained (11.4 mg; 37% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.815 min; MS (ESI^+) *m/z* = 310.3 $[M + H]^+$.



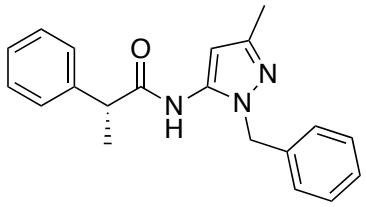
***N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-1-(4-fluorophenyl)cyclopropane-1-carboxamide (9a):** Following the general procedure above, compound (**9a**) was obtained (3.3 mg; 9% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 1.012 min; MS (ESI^+) *m/z* = 350.2 $[M + H]^+$.



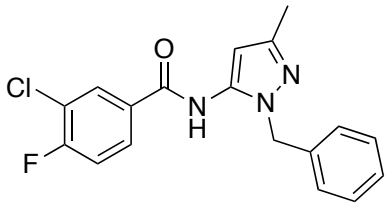
(*S*)-*N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-2-phenylpropanamide (9b): Following the general procedure above, compound (**9b**) was obtained (12.2 mg; 38% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.985 min; MS (ESI^+) *m/z* = 320.4 $[M + H]^+$.



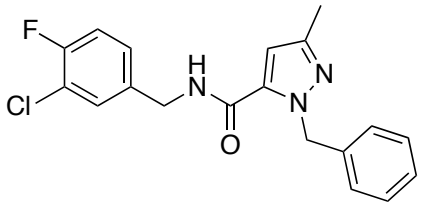
(*R*)-*N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-2-phenylpropanamide (9c): Following the general procedure above, compound (**9c**) was obtained (14.4 mg; 45% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.987 min; MS (ESI^+) *m/z* = 320.5 $[M + H]^+$.



***N*-(1-benzyl-3-methyl-1*H*-pyrazol-5-yl)-3-chloro-4-fluorobenzamide (9d):** Following the general procedure above, compound (**9d**) was obtained (12.2 mg; 36% yield).

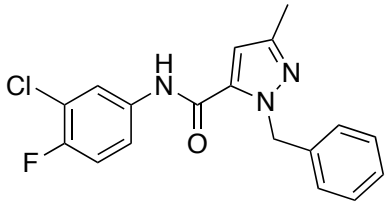
Analytical LCMS (Method A): single peak (254 nm), R_T = 1.017 min; MS (ESI^+) *m/z* = 344.3 $[M + H]^+$.



1-benzyl-*N*-(3-chloro-4-fluorobenzyl)-3-methyl-1*H*-pyrazole-5-carboxamide (9e):

Following the general procedure above, compound (**9e**) was obtained (27.5 mg; 77% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 1.031 min; MS (ESI^+) *m/z* = 358.2 $[M + H]^+$.

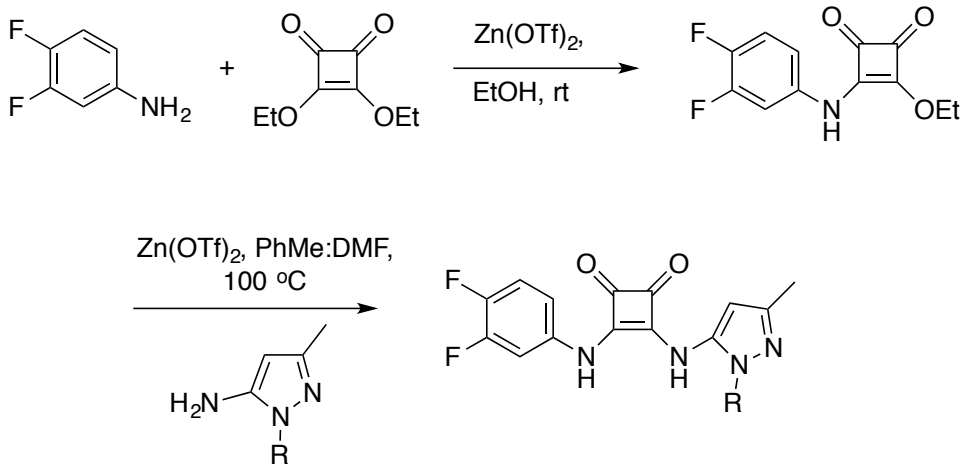


1-benzyl-*N*-(3-chloro-4-fluorophenyl)-3-methyl-1*H*-pyrazole-5-carboxamide (9f):

Following the general procedure above, compound (**9f**) was obtained (30.8 mg; 90% yield).

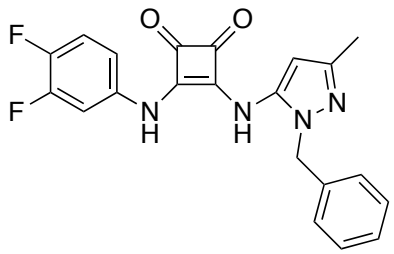
Analytical LCMS (Method A): single peak (254 nm), R_T = 1.141 min; MS (ESI^+) *m/z* = 344.4 $[M + H]^+$.

Library Synthesis of Squareamide Compounds:



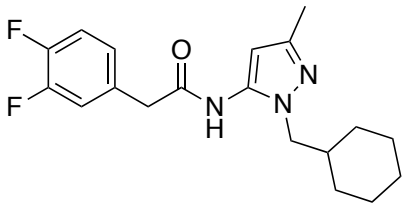
Adapting a procedure similar to Taylor et al (Reference: Tostami, A.; Colin, A.; Li, X. Y.; Chudzinski, M. G.; Lough, A. J.; Taylor, M. S. J. Org. Chem., 2010, 75, 3983–3992). To a stirred solution of 3,4-diethoxycyclobut-3-ene-1,2- dione (0.88 mL, 6.0 mmol, 1.2 equiv) and zinc trifluoromethanesulfonate (181 mg, 0.5 mmol, 0.10 equivalents) in ethanol (15 mL) at room temperature was added 3,4-difluoroaniline (645 mg, 5.0 mmol, 1.0 equiv). After the solution stirred for 4 h at room temperature a white precipitate formed and this solid was collected via filtration. The solid was further washed with cold ethanol (2 x 2 mL yielding 3-((3,4-difluorophenyl)amino)-4-ethoxycyclobut-3-ene-1,2-dione as a white solid. A 1 dram vial equipped with a magnetic stir bar and a screw cap vial was charged with the appropriate 5-amino pyrazole (0.22 mmol, 1.1 equivalents), 3-((3,4-difluorophenyl)amino)-4-ethoxycyclobut-3-ene-1,2-dione (51 mg, 1.0 equivalent, 0.20 mmol), zinc trifluoromethanesulfonate (15 mg, 0.04 mmol, 0.20 equivalents), toluene (0.9 mL), DMF (0.1 mL), and the reaction mixture was heated to 100 °C for 24 hours. The reaction mixture was diluted with water (2 mL) and the mixture was extracted with 3:1 Chloroform:Isopropanol (4 x 3 mL). The organic layers were passed through a phase

separator and solvent was removed. The crude reaction mixture was purified via mass-directed acidic reverse phase preparative HPLC to yield the corresponding squareamide products.



3-((1-benzyl-3-methyl-1*H*-pyrazol-5-yl)amino)-4-((3,4-difluorophenyl)amino)cyclobut-3-ene-1,2-dione (9g): Following the general procedure above, compound (**9g**) was obtained (15.7 mg; 20% yield).

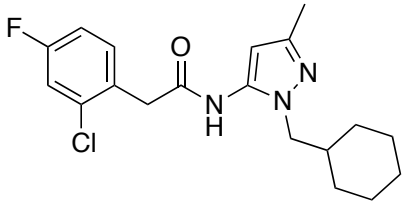
Analytical LCMS (Method A): single peak (254 nm), R_T = 0.942 min; MS (ESI^+) *m/z* = 395.2 $[M + H]^+$.



***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(3,4-difluorophenyl)acetamide (10a):** Following the general procedure above, compound (**10a**) was obtained (3.2 mg; 9% yield).

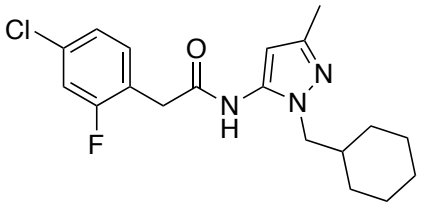
Analytical LCMS (Method B): single peak (254 nm), R_T = 4.342 min; MS (ESI^+) *m/z* = 348.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.46 – 7.35 (m, 2H), 7.21

– 7.14 (m, 1H), 5.96 (s, 1H), 3.65 (d, J = 11.8 Hz, 4H), 2.08 (s, 3H), 1.68 – 1.52 (m, 5H), 1.40 – 1.32 (m, 2H), 1.07 (h, J = 9.8, 9.4 Hz, 3H), 0.76 (t, J = 12.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.81, 145.94, 136.63, 133.83, 126.50, 126.45, 126.42, 118.57, 118.44, 117.81, 117.67, 99.04, 53.65, 41.73, 38.26, 30.29, 26.33, 25.59, 14.21. HRMS (ESI$^+$) calculated for $C_{19}H_{23}F_2N_3O$, 347.1809; found 348.1887 $[M + H]^+$.



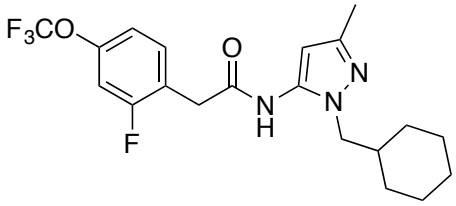
2-(2-chloro-4-fluorophenyl)-*N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (10b): Following the general procedure above, compound (**10b**) was obtained (4.3 mg, 12% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.949 min; MS (ESI$^+$) *m/z* = 364.4 $[M + H]^+$.



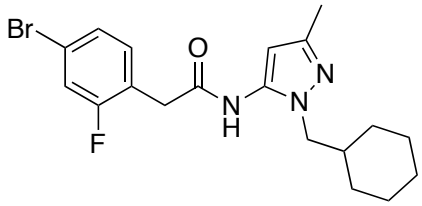
2-(4-chloro-2-fluorophenyl)-*N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (10c): Following the general procedure above, compound (**10c**) was obtained. (252.0 mg, 67%)

Analytical LCMS (Method B): single peak (254 nm), R_T = 4.584 min; MS (ESI^+) *m/z* = 364.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.47 – 7.39 (m, 2H), 7.28 (dd, *J* = 8.3, 2.1 Hz, 1H), 5.95 (s, 1H), 3.74 (s, 2H), 3.71 (d, *J* = 7.2 Hz, 2H), 2.08 (s, 3H), 1.70 (ddp, *J* = 11.2, 7.2, 3.4 Hz, 1H), 1.66 – 1.55 (m, 3H), 1.47 – 1.39 (m, 2H), 1.12 (h, *J* = 10.1, 9.3 Hz, 3H), 0.85 (qd, *J* = 11.7, 3.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.00, 161.98, 160.00, 145.94, 136.72, 133.70, 133.66, 132.81, 132.73, 124.96, 124.93, 122.52, 122.39, 116.29, 116.08, 53.63, 38.31, 35.52, 30.35, 26.39, 25.65, 14.24.



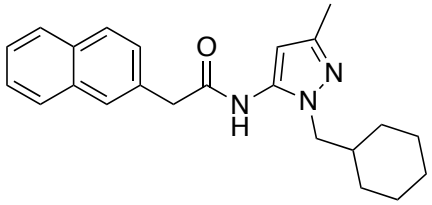
***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(2-fluoro-4-(trifluoromethoxy)phenyl)acetamide (10d):** Following the general procedure above, compound (**10d**) was obtained (5.3 mg, 13% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 1.014 min; MS (ESI^+) *m/z* = 414.3 $[M + H]^+$.



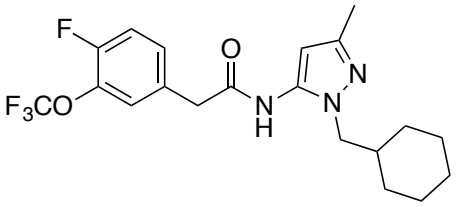
2-(4-bromo-2-fluorophenyl)-*N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (10e): Following the general procedure above, compound (**10e**) was obtained (5.8 mg, 14% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.994 min; MS (ESI^+) *m/z* = 410.2 $[M + H]^+$.



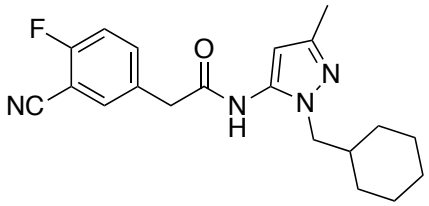
***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(naphthalen-2-yl)acetamide (10f):** Following the general procedure above, compound (**10f**) was obtained. (30.0 mg, 75%)

Analytical LCMS (Method B): single peak (254 nm), R_T = 4.618 min; MS (ESI^+) *m/z* = 362.3 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.95 – 7.82 (m, 4H), 7.50 (pd, *J* = 7.0, 1.5 Hz, 3H), 5.96 (s, 1H), 3.81 (s, 2H), 3.61 (d, *J* = 7.2 Hz, 2H), 2.07 (s, 3H), 1.56 (ddp, *J* = 11.0, 7.5, 3.7 Hz, 1H), 1.50 – 1.40 (m, 3H), 1.33 – 1.22 (m, 2H), 1.02 – 0.80 (m, 3H), 0.69 – 0.56 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.38, 145.90, 136.77, 133.82, 133.48, 132.39, 128.29, 127.98, 127.93, 127.87, 126.64, 126.14, 99.07, 53.64, 43.10, 38.23, 30.19, 26.17, 25.51, 14.23.



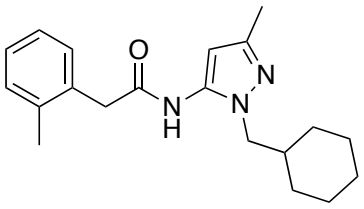
***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(4-fluoro-3-(trifluoromethoxy)phenyl)acetamide (10g):** Following the general procedure above, compound (**10g**) was obtained. (32.0 mg, 76.1%)

Analytical LCMS (Method B): single peak (254 nm), R_T = 4.755 min; MS (ESI^+) *m/z* = 414.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 9.96 (s, 1H), 7.54 – 7.46 (m, 2H), 7.41 (ddd, *J* = 8.6, 4.7, 2.1 Hz, 1H), 5.96 (s, 1H), 3.71 (s, 2H), 3.65 (d, *J* = 7.2 Hz, 2H), 2.08 (s, 3H), 1.73 – 1.50 (m, 4H), 1.37 (d, *J* = 12.3 Hz, 2H), 1.07 (h, *J* = 11.6, 11.0 Hz, 3H), 0.81 – 0.66 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.77, 152.03, 145.98, 136.63, 133.99, 130.64, 124.88, 117.88, 117.73, 98.99, 53.62, 41.52, 38.29, 30.28, 26.29, 25.58, 14.22.



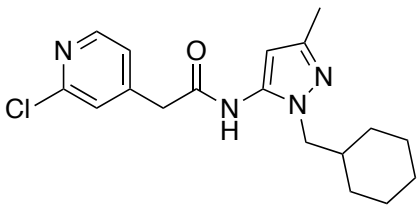
2-(3-cyano-4-fluorophenyl)-*N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (10h): Following the general procedure above, compound (**10h**) was obtained. (25.0 mg, 69%).

Analytical LCMS (Method B): single peak (254 nm), R_T = 4.135 min; MS (ESI^+) *m/z* = 355.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.86 (dd, *J* = 6.3, 2.2 Hz, 1H), 7.74 (ddd, *J* = 8.0, 5.3, 2.3 Hz, 1H), 7.53 (t, *J* = 9.1 Hz, 1H), 5.96 (s, 1H), 3.74 (s, 2H), 3.66 (d, *J* = 7.2 Hz, 2H), 2.08 (s, 3H), 1.68 – 1.52 (m, 4H), 1.38 (d, *J* = 12.8 Hz, 2H), 1.07 (q, *J* = 9.7, 9.0 Hz, 3H), 0.84 – 0.69 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.54, 145.98, 137.58, 137.50, 136.60, 134.65, 133.87, 133.85, 117.06, 116.91, 114.41, 100.34, 100.22, 98.95, 53.62, 41.12, 38.30, 30.32, 26.33, 25.61, 14.22. HRMS (ESI^+) calculated for $C_{20}H_{23}FN_4O$, 354.1856; found, 355.1934 $[M + H]^+$.



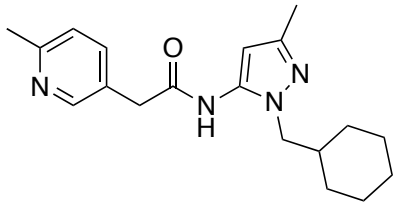
***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(o-tolyl)acetamide (10i):** Following the general procedure above, compound (**10i**) was obtained (10.3 mg, 32% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.944 min; MS (ESI^+) *m/z* = 326.4 $[M + H]^+$.



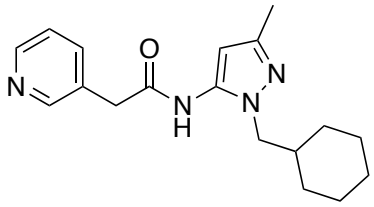
2-(2-chloropyridin-4-yl)-*N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (10j): Following the general procedure above, compound (**10j**) was obtained. (47.0 mg, 75%).

Analytical LCMS (Method B): single peak (254 nm), R_T = 3.858 min; MS (ESI^+) *m/z* = 347.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.38 (d, *J* = 5.0 Hz, 1H), 7.49 (s, 1H), 7.40 – 7.34 (m, 1H), 5.98 (s, 1H), 3.76 (s, 2H), 3.67 (d, *J* = 7.3 Hz, 2H), 2.08 (s, 3H), 1.71 – 1.52 (m, 4H), 1.39 (d, *J* = 12.8 Hz, 2H), 1.09 (t, *J* = 9.0 Hz, 3H), 0.79 (tt, *J* = 11.9, 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.61, 150.74, 150.23, 148.91, 146.01, 136.50, 125.23, 124.54, 98.98, 53.65, 41.54, 38.31, 30.32, 26.33, 25.62, 14.22.



***N*-(1-cyclohexyl-3-methyl-1*H*-pyrazol-5-yl)-2-(6-methylpyridin-3-yl)acetamide (10k):** Following the general procedure above, compound (**10k**) was obtained (15.0 mg, 62%).

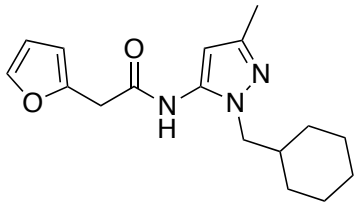
Analytical LCMS (Method B): single peak (254 nm), R_T = 2.756 min; MS (ESI^+) *m/z* = 327.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.39 (d, *J* = 2.3 Hz, 1H), 7.61 (dd, *J* = 8.0, 2.3 Hz, 1H), 7.22 (d, *J* = 7.9 Hz, 1H), 5.95 (s, 1H), 3.64 (d, *J* = 7.8 Hz, 4H), 2.44 (s, 3H), 2.08 (s, 3H), 1.69 – 1.53 (m, 4H), 1.36 (d, *J* = 12.8 Hz, 2H), 1.06 (q, *J* = 9.5, 8.7 Hz, 3H), 0.77 (t, *J* = 12.1 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.09, 156.61, 149.70, 145.91, 137.27, 136.68, 128.71, 123.12, 99.02, 53.64, 38.26, 30.28, 26.33, 25.60, 24.09, 14.23.



***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(pyridin-3-yl)acetamide (10l) :**

Following the general procedure above, compound (**10l**) was obtained (25.0 mg. 78%).

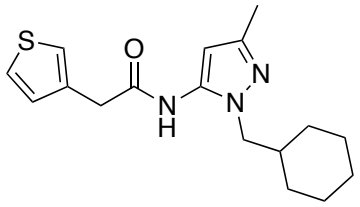
Analytical LCMS (Method B): single peak (254 nm), R_T = 2.797 min; MS (ESI^+) *m/z* = 313.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 10.00 (s, 1H), 8.54 (d, *J* = 2.2 Hz, 1H), 8.48 (dd, *J* = 4.8, 1.7 Hz, 1H), 7.74 (dt, *J* = 7.9, 2.0 Hz, 1H), 7.37 (dd, *J* = 7.8, 4.8 Hz, 1H), 5.97 (s, 1H), 3.71 (s, 2H), 3.67 (d, *J* = 7.2 Hz, 2H), 2.08 (s, 3H), 1.71 – 1.50 (m, 4H), 1.38 (d, *J* = 12.9 Hz, 2H), 1.08 (t, *J* = 9.3 Hz, 3H), 0.79 (qd, *J* = 10.7, 9.7, 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.86, 150.54, 148.38, 145.94, 137.09, 136.68, 131.84, 123.88, 98.95, 53.63, 38.28, 30.30, 26.34, 25.61, 14.23.



***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(furan-2-yl)acetamide (10m):**

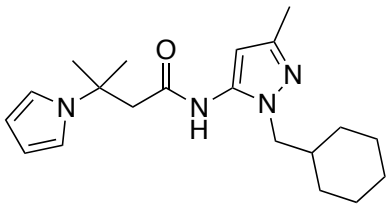
Following the general procedure above, compound (**10m**) was obtained (4.3 mg; 14% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.803 min; MS (ESI^+) *m/z* = 302.4 $[M + H]^+$.



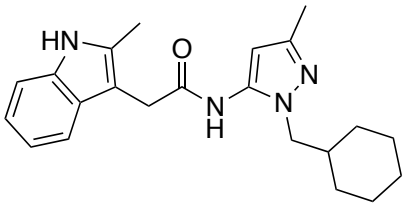
***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(thiophen-3-yl)acetamide (10n):** Following the general procedure above, compound (**10n**) was obtained (3.9 mg; 12% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.857 min; MS (ESI^+) *m/z* = 318.3 $[M + H]^+$.



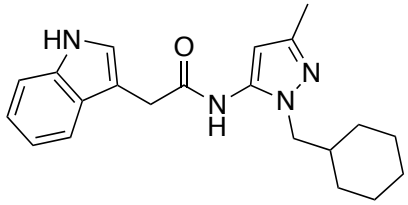
***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-3-methyl-3-(1H-pyrrol-1-yl)butanamide (10o):** Following the general procedure above, compound (**10o**) was obtained (10.0 mg; 29% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 1.016 min; MS (ESI^+) *m/z* = 343.5 $[M + H]^+$.



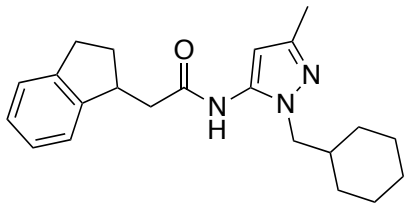
***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(2-methyl-1H-indol-3-yl)acetamide (10p):** Following the general procedure above, compound (**10p**) was obtained (7.9 mg; 22% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.986 min; MS (ESI^+) *m/z* = 365.5 $[M + H]^+$.



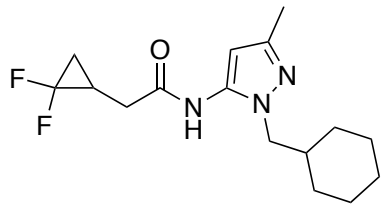
***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(1H-indol-3-yl)acetamide (10q):** Following the general procedure above, compound (**10q**) was obtained (10.5 mg; 30% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.951 min; MS (ESI^+) *m/z* = 351.5 $[M + H]^+$.



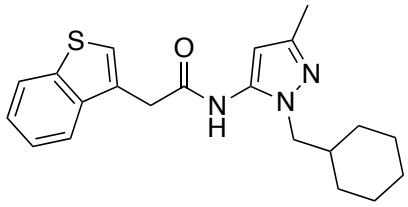
***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(2,3-dihydro-1H-inden-1-yl)acetamide (10r):** Following the general procedure above, compound (**10r**) was obtained (12.4 mg; 48% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 1.081 min; MS (ESI^+) *m/z* = 352.5 $[M + H]^+$.



***N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)-2-(2,2-difluorocyclopropyl)acetamide (10s):** Following the general procedure above, compound (**10s**) was obtained (15.1 mg; 49% yield).

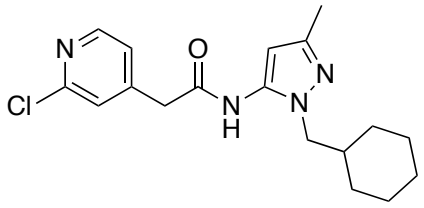
Analytical LCMS (Method A): single peak (254 nm), R_T = 0.898 min; MS (ESI^+) *m/z* = 312.4 $[M + H]^+$.



2-(benzo[b]thiophen-2-yl)-*N*-(1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (10t): Following the general procedure above, compound (**10t**) was obtained (26.0 mg, 68%).

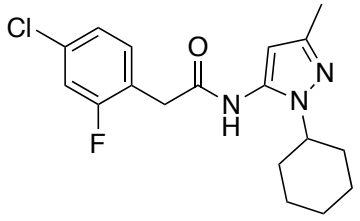
Analytical LCMS (Method B): single peak (254 nm), R_T = 4.596 min; MS (ESI^+) *m/z* = 368.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.03 – 7.97 (m, 1H), 7.91 (dd, *J* = 7.2, 1.8 Hz, 1H), 7.62 (s, 1H), 7.41 (pd, *J* = 7.1, 1.4 Hz, 2H), 5.96 (s, 1H), 3.94 (s, 2H), 3.63 (d, *J* = 7.3 Hz, 2H), 2.08 (s, 3H), 1.67 – 1.46 (m, 4H), 1.36 – 1.26 (m, 2H),

1.02 (t, J = 8.7 Hz, 3H), 0.74 – 0.59 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.48, 145.93, 140.01, 139.06, 136.78, 130.33, 125.46, 124.80, 124.51, 123.38, 122.50, 98.92, 53.66, 38.25, 36.16, 30.22, 26.29, 25.59, 14.23.



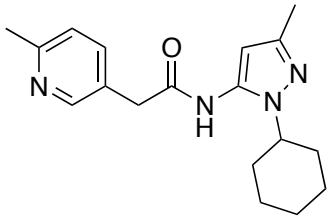
2-(2-chloropyridin-4-yl)-*N*-(1-cyclohexyl-3-methyl-1*H*-pyrazol-5-yl)acetamide (10u): Following the general procedure above, compound (**10u**) was obtained (25.0 mg, 89%).

Analytical LCMS (Method B): single peak (254 nm), R_T = 3.609 min; MS (ESI$^+$) *m/z* = 333.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.38 (d, J = 5.0 Hz, 1H), 7.50 (s, 1H), 7.39 (dd, J = 5.1, 1.4 Hz, 1H), 5.94 (s, 1H), 3.85 (tt, J = 10.1, 5.5 Hz, 1H), 3.78 (s, 2H), 2.09 (s, 3H), 1.80 – 1.58 (m, 8H), 1.20 (dtt, J = 31.0, 12.8, 5.7 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.15, 150.72, 150.22, 149.05, 145.84, 135.09, 125.27, 124.60, 99.37, 55.58, 41.41, 32.86, 25.62, 25.36, 14.40.



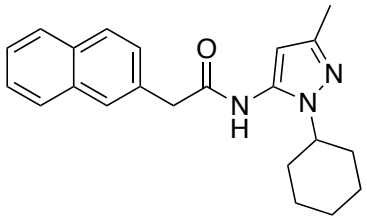
2-(4-chloro-2-fluorophenyl)-*N*-(1-cyclohexyl-3-methyl-1*H*-pyrazol-5-yl)acetamide (10v): Following the general procedure above, compound (**10v**) was obtained (20.0 mg, 68%).

Analytical LCMS (Method B): single peak (254 nm), R_T = 4.376 min; MS (ESI^+) *m/z* = 350.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 9.96 (s, 1H), 7.48 – 7.38 (m, 2H), 7.29 (dd, *J* = 8.2, 2.1 Hz, 1H), 5.91 (s, 1H), 3.93 (tt, *J* = 10.0, 4.7 Hz, 1H), 3.76 (s, 2H), 2.09 (s, 3H), 1.84 – 1.58 (m, 8H), 1.33 – 1.22 (m, 2H), 1.16 (qt, *J* = 12.8, 3.2 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.51, 162.02, 160.04, 145.79, 135.31, 133.71, 132.80, 124.97, 122.50, 116.29, 99.25, 55.52, 35.50, 32.89, 25.66, 14.41.



***N*-(1-cyclohexyl-3-methyl-1*H*-pyrazol-5-yl)-2-(6-methylpyridin-3-yl)acetamide (10w):** Following the general procedure above, compound (**10w**) was obtained (32.0 mg, 62%).

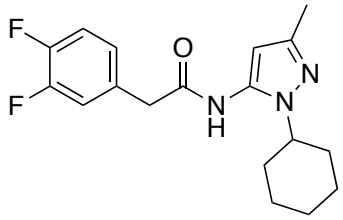
Analytical LCMS (Method B): single peak (254 nm), R_T = 2.480 min; MS (ESI^+) *m/z* = 313.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 9.93 (s, 1H), 8.39 (d, *J* = 2.2 Hz, 1H), 7.62 (dd, *J* = 7.9, 2.3 Hz, 1H), 7.23 (d, *J* = 7.9 Hz, 1H), 5.91 (s, 1H), 5.76 (d, *J* = 1.2 Hz, 1H), 3.82 (tt, *J* = 10.4, 5.4 Hz, 1H), 3.66 (s, 2H), 2.45 (s, 3H), 2.09 (s, 3H), 1.79 – 1.56 (m, 8H), 1.27 – 1.08 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.63, 156.59, 149.72, 145.77, 137.32, 135.31, 128.81, 123.14, 99.40, 55.50, 55.38, 32.83, 25.58, 25.37, 24.09, 14.40.



***N*-(1-cyclohexyl-3-methyl-1*H*-pyrazol-5-yl)-2-(naphthalen-2-yl)acetamide (10x):**

Following the general procedure above, compound (**10x**) was obtained (21.0 mg. 72%).

Analytical LCMS (Method B): single peak (254 nm), R_T = 4.420 min; MS (ESI^+) *m/z* = 348.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.94 – 7.81 (m, 4H), 7.57 – 7.41 (m, 3H), 5.92 (s, 1H), 3.86 – 3.77 (m, 3H), 2.09 (s, 3H), 1.67 (t, *J* = 7.1 Hz, 7H), 1.09 (d, *J* = 9.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.94, 145.75, 135.38, 133.94, 133.48, 132.37, 128.28, 127.97, 127.94, 127.86, 126.66, 126.15, 55.49, 55.39, 42.96, 32.80, 25.51, 25.33, 14.41.

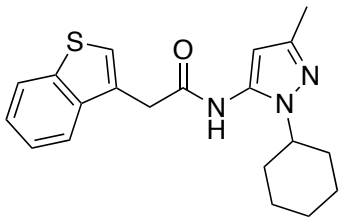


***N*-(1-cyclohexyl-3-methyl-1*H*-pyrazol-5-yl)-2-(3,4-difluorophenyl)acetamide (10y)**:

Following the general procedure above, compound (**10y**) was obtained. (20.0 mg, 71.6%)

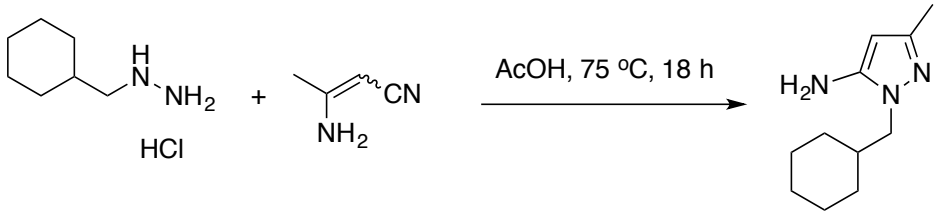
Analytical LCMS (Method B): single peak (254 nm), R_T = 4.137 min; MS (ESI^+) *m/z* = 334.1 $[M + H]^+$. ^1H NMR (499 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.45 – 7.35 (m, 2H), 7.22 – 7.13 (m, 1H), 5.92 (s, 1H), 3.82 (tt, *J* = 10.4, 5.7 Hz, 1H), 3.68 (s, 2H), 2.09 (s, 3H), 1.80

– 1.58 (m, 8H), 1.26 – 1.09 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.36, 145.79, 135.25, 133.90, 126.53, 118.61, 117.80, 99.43, 55.53, 41.58, 32.82, 25.61, 25.36, 14.40.



2-(benzo[b]thiophen-2-yl)-N-(1-cyclohexyl-3-methyl-1H-pyrazol-5-yl)acetamide (10z): Following the general procedure above, compound (**10z**) was obtained (30.0 mg, 75%).

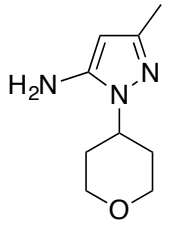
Analytical LCMS (Method B): single peak (254 nm), R_T = 4.378 min; MS (ESI$^+$) *m/z* = 354.1 [M + H]$^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.02 – 7.98 (m, 1H), 7.93 – 7.88 (m, 1H), 7.61 (s, 1H), 7.41 (dtd, *J* = 17.7, 7.2, 1.3 Hz, 2H), 5.92 (s, 1H), 3.95 (s, 2H), 3.81 (tt, *J* = 10.2, 5.5 Hz, 1H), 2.09 (s, 3H), 1.73 – 1.62 (m, 7H), 1.57 (d, *J* = 5.0 Hz, 1H), 1.13 – 1.01 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.07, 145.77, 140.01, 139.07, 135.34, 130.43, 125.41, 124.81, 124.53, 123.39, 122.45, 99.49, 55.51, 36.05, 32.82, 25.52, 25.34, 14.41.



1-(cyclohexylmethyl)-3-methyl-1*H*-pyrazol-5-amine. A 10-20 mL Biotage microwave vial was equipped with a magnetic stir bar and charged with

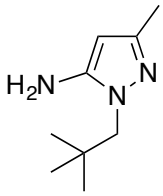
(cyclohexylmethyl)hydrazine•HCl (3.00 g, 1.0 equivalents, 18.2 mmols), 3-aminocrotononitrile (1.50 g, 1.0 equivalents, 18.2 mmols), ethanol (15 mL), acetic acid (1.04 mL, 1.0 equivalents, 18.2 mmols), and this mixture was heated to 75 ºC for 18 hours. The reaction mixture was cooled to ambient temperature and the solvent was removed. The crude reaction mixture was purified via acidic reverse phase preparative HPLC to yield 1-(cyclohexylmethyl)-3-methyl-1H-pyrazol-5-amine as a white solid (1.89 g, 9.8 mmols, 54% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.648 min; MS (ESI^+) *m/z* = 194.4 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 5.32 (s, 1H), 3.66 (d, *J* = 7.3 Hz, 2H), 3.38 (br s, 2H), 2.15 (s, 3H), 1.91–1.81 (m, 1H), 1.75–1.62 (m, 5H), 1.26–1.12 (m, 3H), 1.02–0.90 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 13.9, 25.7, 26.4, 30.9, 38.6, 53.3, 90.9, 144.73, 147.06.



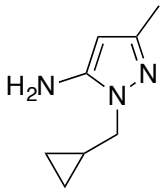
3-methyl-1-(tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-5-amine: Following the general procedure above, compound was obtained (41.7 mg; 23% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.250 min; MS (ESI^+) *m/z* = 182.6 $[M + H]^+$.



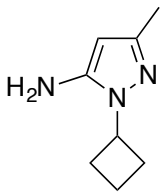
3-methyl-1-neopentyl-1*H*-pyrazol-5-amine: Following the general procedure above, compound was obtained (56.1 mg; 34% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.356 min; MS (ESI^+) *m/z* = 168.2 $[M + H]^+$.



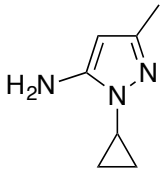
1-(cyclopropylmethyl)-3-methyl-1*H*-pyrazol-5-amine: Following the general procedure above, compound was obtained (31.9 mg; 21% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.312 min; MS (ESI^+) *m/z* = 152.2 $[M + H]^+$.



1-cyclobutyl-3-methyl-1*H*-pyrazol-5-amine: Following the general procedure above, compound was obtained (111.0 mg; 24% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.219 min; MS (ESI^+) *m/z* = 152.2 $[M + H]^+$.



1-cyclopropyl-3-methyl-1*H*-pyrazol-5-amine: Following the general procedure above, compound was obtained (62.8 mg; 15% yield).

Analytical LCMS (Method A): single peak (254 nm), R_T = 0.184 min; MS (ESI^+) *m/z* = 138.2 $[M + H]^+$.

Materials and Methods for *in vitro* Pharmacology

Thallium flux assays were preformed essentially as previously described (Ref. 9). Briefly, HEK-293 cells expressing either GIRK1 and GIRK2 or GIRK1 and GIRK4 were cultured in α-MEM (Corning, Corning, NY) containing 10% (v/v) fetal bovine serum (Thermo Fisher Scientific, Waltham, MA) plus 1x glutagro (Corning, Corning, NY) (referred to hereafter as cell culture medium) at 37 C^o in a humidified 5% CO_2 atmosphere. Cells at ~90% confluence were dislodged from the tissue culture vessel using TrypLE Express (Thermo Fisher Scientific, Waltham, MA) and plated at a density of 20,000 cells/well in 20 µl/well cell culture medium in 384-well, clear-bottom, black-walled, BD PureCoat Amine plates (Corning, Corning, NY) and incubated over night at 37 C^o in a humidified 5% CO_2 atmosphere. On the day of assay the medium was removed from the plates and replaced with 20 µl/well of a solution containing assay buffer (Hanks Buffered Saline Solution (Thermo Fisher Scientific, Waltham, MA) plus 10 mM HEPES (Thermo Fisher Scientific, Waltham, MA)-NaOH, pH 7.2), 1 µM Thallos (TEFlabs, Austin, TX), 0.5% DMSO and 0.036% Pluronic F-127 (Sigma-Aldrich, St. Louis, MO). Cell plates containing Thallos solution were incubated 1 hour at room temperature. Following incubation the Thallos-containing solution was replaced with 20 µl/well assay buffer. The Thallos-loaded cell plates were transferred to a Panoptic kinetic imaging plate reader (WaveFront Biosciences, Franklin, TN). Images acquired at 1 Hz, 480/40 nm excitation and 538/40 nm emission were collected for 10 seconds after which time 20 µl/well of assay buffer containing test compounds at 2-fold over their final concentrations were added. Imaging continued for four minutes at which time 10 µl/well of a solution containing 125 mM

NaHCO3, 1.8 mM $CaSO_4$, 1 mM $MgSO_4$, 5 mM glucose, and 2 mM Tl_2SO_4, 10 mM HEPES-NaOH pH 7.2 was added and images were collected for an additional 2 minutes. To quantify test compound effects on GIRK activity, the initial slopes of the thallium-evoked changes in fluorescence were fit to a four-parameter logistic equation using the Excel (Microsoft, Redmond, WA) plugin XLfit (IDBS, Guildford, UK) to obtain potency and efficacy values. Efficacies are relative to a maximally effective concentration of **1**, our most potent and effective activator which shows low selectivity between GIRK1/2 and GIRK1/4 channels. Ten-point concentration series from 30 μM to 1.5 nM were generated using an Echo liquid handler (Labcyte, San Jose, CA). Final DMSO concentration, 0.24% (v/v), in the assay was constant across all compound concentrations. Unless otherwise indicated, all buffer salts were obtained from Sigma-Aldrich, St. Louis, MO.

***In Vitro* DMPK Methods:**

For the *in vitro* DMPK methods, please see: *ACS Chem. Neurosci.* **2016**, *7*, 1192-1200.

***In vivo* DMPK Methods:**

Mouse brain distribution studies were performed using group housed (12 hr light/dark cycle) adult male C57Bl/6 mice (*n* = 3-4; Jackson Laboratory, Bar Harbor, ME) with access to food and water *ad libitum*. Compounds were formulated as solutions in ethanol/PEG400/DMSO (16-18%/64-72%/10-20%, v/v/v) and administered via intraperitoneal (IP) injection as a cassette dose (1 mg/kg per compound; 0.5 mL/kg dose volume). After 0.25 hr, mice were sacrificed, and blood (subsequently plasma) and whole brain samples were obtained and prepared for bioanalysis by LC-MS/MS essentially as previously described (Wenthur *et al.*, 2013). The studies were approved by the Vanderbilt University Animal Care and Use Committee and used procedures conforming to the guidelines set forth in the National Research Council *Guide for the Care and Use of Laboratory Animals*.

References

J. Med. Chem. **2013**, *56*, 5208–5212.