Supporting Information

N-(1-Benzyl-3,5-dimethyl-1*H*-pyrazol-4-yl)benzamides: Antiproliferative Activity and Effects on mTORC1 and Autophagy

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Table S1. *In Vitro* ADME Properties of Compounds 22 and 23.[a]

Compd	Plasma Stability (% remaining, 6 h/24 h)		Microsomal Stability $t_{1/2}$ (min)		Aqueous Solubility (μM)
	Mouse	Human	Mouse	Human	
22	91/77	99/100	60	34	67.3 ± 5.2
23	88/93	98/100	53	53	166 ± 2

[a] Plasma and microsomal stabilities as well as thermodynamic aqueous solubility were determined in triplicate.

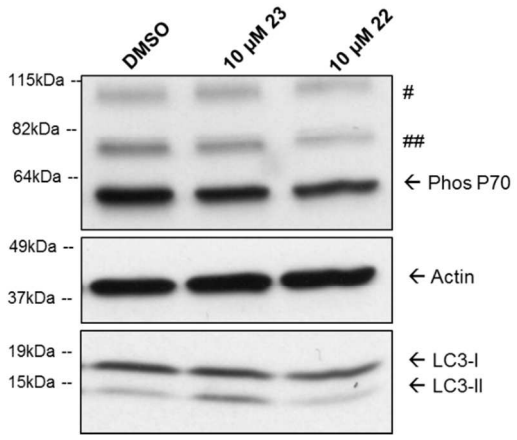


Figure S1. Induction of basal autophagy. ARPE-19 cells were treated for 4 h with DMSO, compound **23** or **22**. Representative Western blot of LC3 and phosphorylated P70 after treatment with **23** or **22** (10 μM). #, non-specific; ##, phosphorylated P85 S6 kinase (an isoform of P70 S6 kinase).

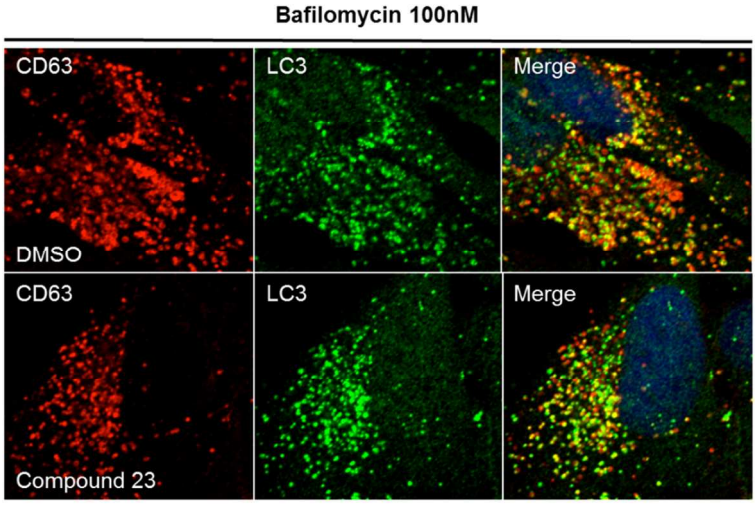


Figure S2. Co-localization between LC3 and CD63 in bafilomycin-treated ARPE-19 cells. LC3/CD63-positive structures corresponded to degradation-deficient autolysosomes.

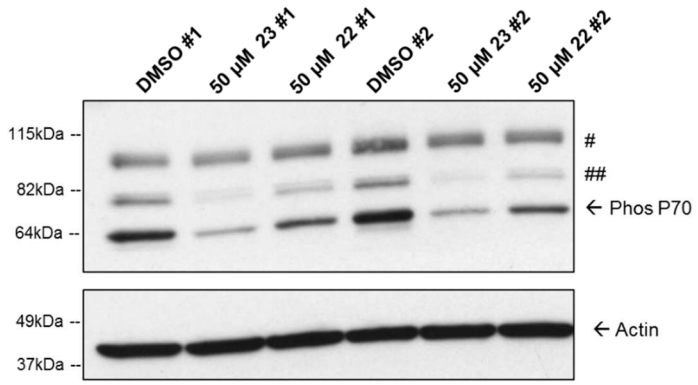


Figure S3. Decreased basal mTORC1 activity. ARPE-19 cells were treated for 4 h with DMSO, compound **23**, or **22** without starvation. Western blot of phosphorylated P70 after treatment with **23** or **22** (50 μM). #, non-specific; ##, phosphorylated P85 S6 kinase (an isoform of P70 S6 kinase).

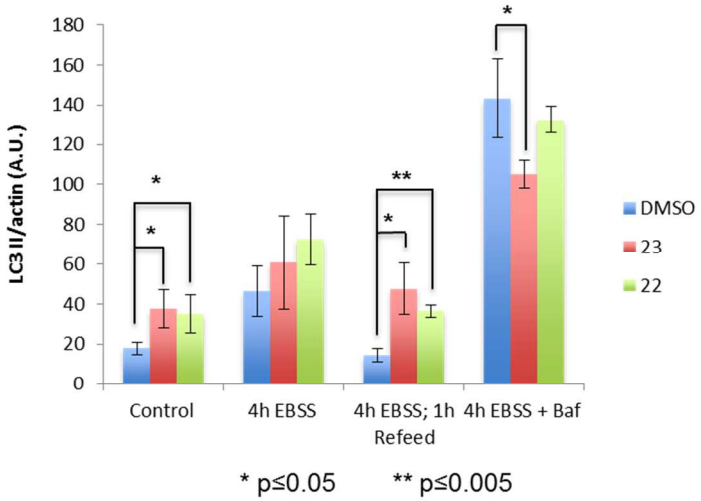


Figure S4. Impaired autophagy flux. The experiments were the same as those presented in Figure 3A. Values for **23**- and **22**-treated samples were expressed as fold induction compared to DMSO-treated control sample.

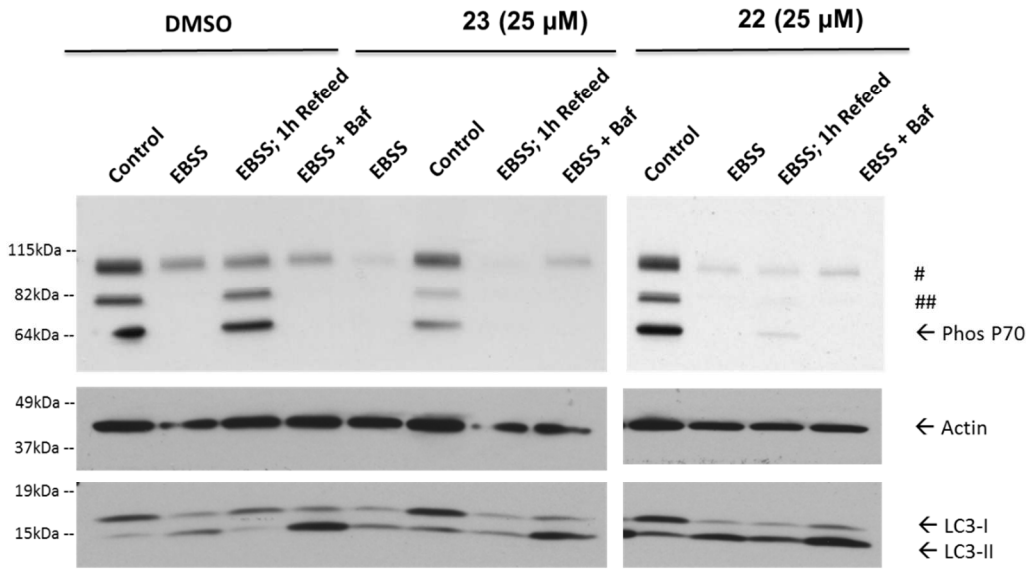


Figure S5. Reduced mTORC1 reactivation and impaired recovery of LC3 turnover under refeed conditions. ARPE-19 cells were treated with DMSO, compound **23** or **22** (25 μM) under starvation (4 h EBSS), starvation/refeed (4 h EBSS followed by 1 h complete medium recovery), and starvation/bafilomycin (4 h EBSS containing 100 nM bafilomycin) conditions (n = 3). Representative Western blot of phosphorylated P70 and LC3. #, non-specific; ##, phosphorylated P85 S6 kinase (an isoform of P70 S6 kinase).

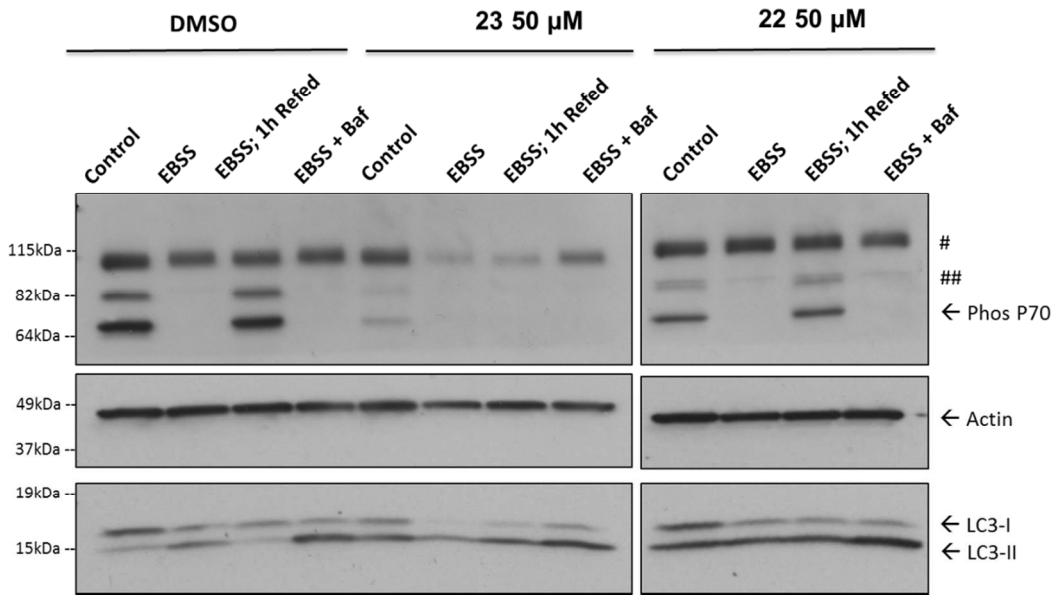


Figure S6. Reduced mTORC1 reactivation and impaired recovery of LC3 turnover under refeed conditions. ARPE-19 cells were treated with DMSO, compound **23** or **22** (50 μM) under starvation (4 h EBSS), starvation/refeed (4 h EBSS followed by 1 h complete medium recovery), and starvation/bafilomycin (4 h EBSS containing 100 nM bafilomycin) conditions (n = 3). Representative Western blot of phosphorylated P70 and LC3. #, non-specific; ##, phosphorylated P85 S6 kinase (an isoform of P70 S6 kinase).

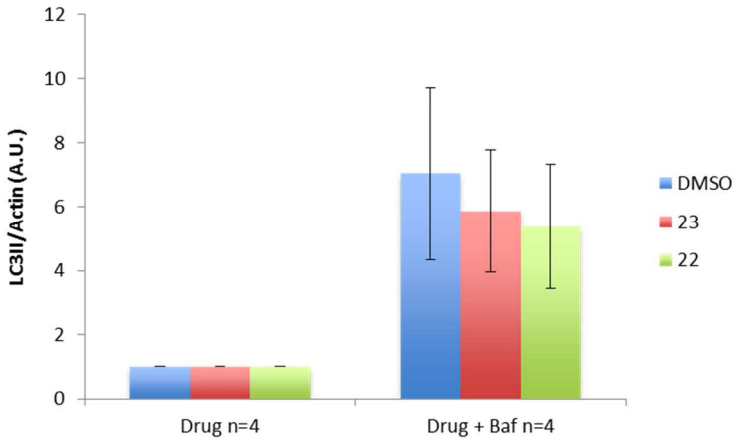
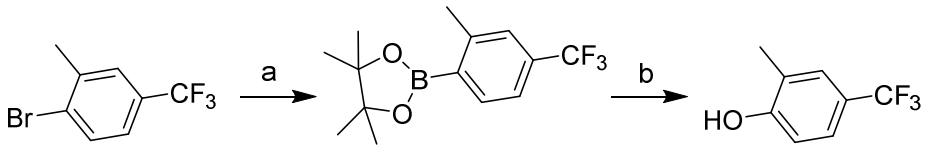


Figure S7. Autophagic flux in full medium. ARPE-19 cells were treated for 4 h with DMSO, compound **23** or **22** (25 μM) in the presence of 100 nM bafilomycin in full medium. No statistically significant reduction in autophagic flux was observed.

Scheme S1[a]



31 **32** **33**

[a] Reagents and conditions: (a) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, DMSO, 80 ºC, 66%; (b) mCPBA, EtOH, H_2O, 77%.

EXPERIMENTAL METODS

Chemical Synthesis. All commercial reagents were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer) using 2 packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2, whereas 2 packed columns of molecular sieves were used to dry DMF. Solvents were dispensed under argon. Flash chromatography was performed with Ultra Pure silica gel (Silicycle) or with RediSep R_f silica gel columns on a Teledyne ISCO CombiFlash® R_f system using the solvents as indicated. Nuclear magnetic resonance spectra were recorded on a Varian 600 MHz with Me_4Si or signals from residual solvent as the internal standard for ^1H. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), and dd (double doublet). Values given for coupling constants are first order. High resolution mass spectra were recorded on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. Analysis of sample purity was performed on an Agilent 1200 Infinity series HPLC system with a Phenomenex Gemini C18 column (5 μ, 4.6×250 mm). HPLC conditions were as follows: solvent A = water, solvent B = MeCN or MeOH, and flow rate = 2.0 mL/min. Compounds were eluted with a gradient of from 10% to 100% MeCN/water or from 10 to 100% MeOH/water in 15 min. Purity was determined by the absorbance at 254 nm. All tested compounds have a purity of ≥ 95%.

***N*-(1-Benzyl-3,5-dimethyl-1*H*-pyrazol-4-yl)-4-(phenoxymethyl)benzamide (1).** To a solution of 4-(phenoxymethyl)-benzoic acid (55 mg, 0.24 mmol) and EDC (46 mg, 0.24 mmol) in

DMF/CH_2Cl_2 (1:1, 10 mL) was added amine **72a** (40 mg, 0.20 mmol) and the mixture was allowed to stir at rt for 12 h. After the solvents were removed, the residue was diluted with EtOAc (30 mL), H_2O (10 mL) and saturated $NaHCO_3$ (10 mL). After separation, the organic layer was washed with brine (20 mL) and concentrated. The residue was purified by flash column chromatography (0-10% MeOH/CH_2Cl_2) to give compound **1** as a white solid (85 mg, 90%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.90 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.33-7.27 (m, 4H), 7.27-7.21 (m, 2H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.00-6.95 (m, 3H), 5.23 (s, 2H), 5.15 (s, 2H), 2.21 (s, 3H), 2.10 (s, 3H). HRMS (ESI$^+$) $C_{26}H_{26}N_3O_2$ $(M+H)^+$ 412.2020, found 412.2022.

***N*-(1-(4-Carbamoylbenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)-4-(phenoxymethyl)benzamide (2).** A solution of methyl ester **26b** (47 mg, 0.10 mmol) in NH_3/MeOH (~7 N, 5 mL) in a sealed tube was heated at 70 ºC for 16 h. After the solvent was evaporated in vacuo, the residue was purified by flash column chromatography (0-20% MeOH/CH_2Cl_2) to afford compound **2** as a white solid (14 mg, 31%). ^{1}H NMR (DMSO-d_6, 600MHz) δ 9.55 (s, 1H), 7.97 (d, *J* = 7.8 Hz, 2H), 7.92 (s, 1H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.34 (s, 1H), 7.30 (dd, *J* = 7.8, 7.8 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 7.02 (d, *J* = 7.8 Hz, 2H), 6.95 (dd, *J* = 7.5, 7.5 Hz, 1H), 5.29 (s, 2H), 5.20 (s, 2H), 2.06 (s, 3H), 2.04 (s, 3H). HRMS (ESI$^+$) calcd for $C_{27}H_{27}N_4O_3$ $(M+H)^+$ 455.2083, found 455.2087.

Compounds **3** and **4** were prepared from **26c** and **26d**, respectively, in a fashion similar to the one described for compound **2**.

***N*-(1-(3-Carbamoylbenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)-4-(phenoxymethyl)benzamide (3).** White solid, 23 mg, 50% yield. ^{1}H NMR (DMSO-d_6, 600MHz) δ 9.56 (s, 1H), 8.01-7.94 (m, 3H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.73 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.42 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.38 (s, 1H), 7.30 (dd, *J* = 7.5, 7.5Hz, 2H), 7.26 (d, *J* = 7.8 Hz, 1H), 7.02 (d, *J* = 7.8 Hz, 2H), 6.95 (dd, *J* = 7.5, 7.5 Hz, 1H), 5.27 (s, 2H), 5.20 (s, 2H), 2.08 (s, 3H), 2.04 (s, 3H). HRMS (ESI$^+$) calcd for $C_{27}H_{27}N_4O_3$ $(M+H)^+$ 455.2083, found 455.2082.

***N*-(1-(2-Carbamoylbenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)-4-(phenoxymethyl)benzamide (4).** White solid, 13 mg, 29% yield. ^{1}H NMR (DMSO-d_6, 600MHz) δ 9.58 (s, 1H), 8.04 (s, 1H), 7.98 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 7.8 Hz, 2H), 7.55-7.50 (m, 2H), 7.38 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.35-7.27 (m, 3H), 7.02 (d, *J* = 7.8 Hz, 2H), 6.95 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.68 (d, *J* = 7.8 Hz, 1H), 5.41 (s, 2H), 5.20 (s, 2H), 2.04 (s, 3H), 2.04 (s, 3H). HRMS (ESI$^+$) calcd for $C_{27}H_{27}N_4O_3$ $(M+H)^+$ 455.2083, found 455.2081.

3-((3,5-Dimethyl-4-(4-(phenoxymethyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (5). A solution of methyl ester **26c** (47 mg, 0.10 mmol) in $MeNH_2$/EtOH (33 wt. %, 7 mL) in a sealed tube was heated at 70 ºC for 24 h. After the solvent was evaporated in vacuo, the residue was purified by flash column chromatography (0-20% MeOH/CH_2Cl_2) to afford compound **5** as a white solid (30 mg, 64%). ^{1}H NMR (DMSO-d_6, 600MHz) δ 9.56 (s, 1H), 8.47-8.40 (m, 1H), 7.97 (d, *J* = 7.8 Hz, 2H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.69 (s, 1H), 7.56 (d, *J* = 7.8 Hz, 2H), 7.43 (dd, *J* = 7.4, 7.4 Hz, 1H), 7.33-7.24 (m, 3H), 7.02 (d, *J* = 7.8 Hz, 2H), 6.95 (dd, *J* = 7.5, 7.5 Hz, 1H), 5.28 (s, 2H), 5.20 (s, 2H), 2.78 (d, *J* = 4.8 Hz, 3H), 2.07 (s, 3H), 2.04 (s, 3H). HRMS (ESI$^+$) calcd for $C_{28}H_{29}N_4O_3$ $(M+H)^+$ 469.2240, found 469.2240.

3-((3,5-Dimethyl-4-(4-(phenoxymethyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*,*N*-dimethylbenzamide (6). To a solution of carboxylic acid **27** (40 mg, 0.088 mmol), HBTU (40 mg, 0.11 mmol) and Et_3N (25 μL, 0.18 mmol) in DMF/CH_2Cl_2 (1:1, 10 mL) was added dimethylamine (0.24 μL, 0.26 mmol) and the mixture was allowed to stir at rt for 12 h. After the solvents were removed, the residue was diluted with EtOAc (30 mL) and H_2O (10 mL). After separation, the organic layer was washed with brine (20 mL) and concentrated. The residue was purified by flash column chromatography (0-15% MeOH/CH_2Cl_2) to give compound **6** as a white solid (40 mg, 94%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.92 (d, *J* =7.8 Hz, 2H), 7.67 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 2H), 7.34-7.25 (m, 4H), 7.15-7.11 (m, 2H), 7.00-6.95 (m, 3H), 5.21 (s, 2H), 5.13 (s, 2H), 3.06 (s, 3H), 2.92 (s, 3H), 2.16 (s, 3H), 2.04 (s, 3H). HRMS (ESI$^+$) calcd for $C_{29}H_{31}N_4O_3$ $(M+H)^+$ 483.2396, found 483.2410.

Compounds **7-10** were prepared from carboxylic acid **27** via a HBTU-mediated amide formation in a fashion similar to the one described for compound **6**.

3-((3,5-Dimethyl-4-(4-(phenoxymethyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-ethylbenzamide (7). White solid, 28% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.93 (d, *J* =8.4 Hz, 2H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.52 (s, 1H), 7.36 (dd, *J* =7.5, 7.5 Hz, 1H), 7.32-7.28 (m, 2H), 7.27 (s, 1H), 7.24 (d, *J* = 7.8 Hz, 1H), 7.00-6.95 (m, 3H), 6.55-6.50 (m, 1H), 5.24 (s, 2H), 5.15 (s, 2H), 3.50-3.43 (m, 2H), 2.18 (s, 3H), 2.05 (s, 3H), 1.22 (t, *J* = 7.2 Hz, 3H). HRMS (ESI$^+$) calcd for $C_{29}H_{31}N_4O_3$ $(M+H)^+$ 483.2396, found 483.2395.

3-((3,5-Dimethyl-4-(4-(phenoxymethyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-isopropylbenzamide (8). White solid, 93% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.91 (d, *J* =7.8 Hz, 2H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.40-7.36 (m, 2H), 7.30 (dd, *J* =8.1, 8.1 Hz, 2H), 7.24-7.22 (m, 2H), 7.00-6.96 (m, 3H), 6.09-6.05 (m, 1H), 5.28 (s, 2H), 5.16 (s, 2H), 4.32-4.24 (m, 1H), 2.21 (s, 3H), 2.10 (s, 3H), 1.25 (d, *J* = 6.6 Hz, 6H). HRMS (ESI$^+$) calcd for $C_{30}H_{33}N_4O_3$ $(M+H)^+$ 497.2553, found 497.2558.

3-((3,5-Dimethyl-4-(4-(phenoxymethyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-phenylbenzamide (9). White solid, 49% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.09 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 2H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* =7.8 Hz, 2H), 7.56 (d, *J* = 7.8, Hz, 2H), 7.44 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.38 (s, 1H), 7.37-7.28 (m, 6H), 7.14 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.00-6.95 (m, 3H), 5.31 (s, 2H), 5.16 (s, 2H), 2.21 (s, 3H), 2.11 (s, 3H). HRMS (ESI$^+$) calcd for $C_{33}H_{31}N_4O_3$ $(M+H)^+$ 531.2396, found 531.2392.

3-((4-(4-((Cyclohexyloxy)methyl)benzamido)-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (10). White solid, 9.3 mg, 17% yield. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.52 (s, 1H), 8.46-8.40 (m, 1H), 7.93 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.68 (s, 1H), 7.45-7.40 (m, 3H), 7.26 (d, *J* = 7.8 Hz, 1H), 5.27 (s, 2H), 4.56 (s, 2H), 3.38-3.33 (m, 1H), 2.77 (d, *J* = 4.8 Hz, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.91-1.84 (m ,2H), 1.70-1.64 (m, 2H), 1.33-1.20 (m, 6H). HRMS (ESI$^+$) calcd for $C_{28}H_{35}N_4O_3$ $(M+H)^+$ 475.2709, found 475.2707.

3-((3,5-Dimethyl-4-(4-((*o*-tolyloxy)methyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (11). To a solution of compound **30** (30 mg, 0.073 mmol) in DMF (5 mL)

was added Cs_2CO_3 (36 mg, 0.11 mmol) and *o*-cresol (9 mg, 0.080 mmol) and the reaction mixture was allowed to stir at 50 ºC for 14 h. After cooled to rt, the solution was diluted with saturated NH_4Cl and extracted with EtOAc. The organic phase was washed with water, brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (0-15% MeOH/CH_2Cl_2) to afford **11** as a white solid (20 mg, 57%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.91 (d, *J* = 7.8 Hz, 2H), 7.74 (d, *J* = 7.2 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.40 (dd, *J* = 8.1, 8.1 Hz, 1H), 7.30-7.27 (m, 2H), 7.15 (s, 1H), 7.09 (d, *J* = 7.8 Hz, 2H), 6.87 (d, *J* = 7.8 Hz, 2H), 6.59-6.53 (m, 1H), 5.29 (s, 2H), 5.13 (s, 2H), 3.00 (d, *J* = 4.8 Hz, 3H), 2.29 (s, 3H), 2.21 (s, 3H), 2.09 (s, 3H). HRMS (ESI$^+$) calcd for $C_{29}H_{31}N_4O_3$ $(M+H)^+$ 483.2396, found 483.2396.

Compounds **12-23** were prepared from intermediate **30** via a displacement reaction by a phenol in a fashion similar to the one described for compound **11**.

3-((3,5-Dimethyl-4-(4-((*m*-tolyloxy)methyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (12). White solid, 25 mg, 71% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.93 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.60 (s, 1H), 7.54 (d, *J* = 7.8 Hz, 2H), 7.36 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.26-7.23 (m, 1H), 7.20-7.15 (m, 2H), 6.82-6.78 (m, 2H), 6.78-6.72 (m, 2H), 5.24 (s, 2H), 5.13 (s, 2H), 2.96 (d, *J* = 4.8 Hz, 3H), 2.33 (s, 3H), 2.17 (s, 3H), 2.04 (s, 3H). HRMS (ESI$^+$) calcd for $C_{29}H_{31}N_4O_3$ $(M+H)^+$ 483.2396, found 483.2393.

3-((3,5-Dimethyl-4-(4-((*p*-tolyloxy)methyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (13). White solid, 13 mg, 37% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.95 (d, *J*

= 7.8 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.64 (s, 1H), 7.56 (d, *J* = 7.8 Hz, 2H), 7.36 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.26-7.23 (m, 1H), 7.20-7.17 (m, 2H), 7.14 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.90 (dd, *J* = 7.8,7.8 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.80-6.75 (m, 1H), 5.23 (s, 2H), 5.16 (s, 2H), 2.95 (d, *J* = 4.2 Hz, 3H), 2.31 (s, 3H), 2.16 (s, 3H), 2.04 (s, 3H). HRMS (ESI^+) calcd for $C_{29}H_{31}N_4O_3$ $(M+H)^+$ 483.2396, found 483.2398.

3-((4-(4-((2-Methoxyphenoxy)methyl)benzamido)-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (14). White solid, 22 mg, 60% Yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.91 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.44 (s, 1H), 7.38 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.28-7.26 (m, 1H), 7.16 (s, 1H), 6.98-6.92 (m, 2H), 6.89-6.83 (m, 2H), 6.68-6.62 (m, 1H), 5.26 (s, 2H), 5.24 (s, 2H), 3.91 (s, 3H), 2.98 (d, *J* = 4.8 Hz, 3H), 2.19 (s, 3H), 2.06 (s, 3H). HRMS (ESI^+) calcd for $C_{29}H_{31}N_4O_4$ $(M+H)^+$ 499.2345, found 499.2348.

3-((4-(4-((3-Methoxyphenoxy)methyl)benzamido)-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (15). White solid, 26 mg, 71% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.94 (d, *J* = 8.4 Hz, 2H), 7.75 (s, 1H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.34 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.24 (d, *J* = 7.2 Hz, 1H), 7.21-7.16 (m, 2H), 6.86-6.81 (m, 1H), 6.56 (d, *J* = 8.4 Hz, 1H), 6.55-6.52 (m, 2H), 5.21 (s, 2H), 5.12 (s, 2H), 3.78 (s, 3H), 2.94 (d, *J* = 4.8 Hz, 3H), 2.14 (s, 3H), 2.02 (s, 3H). HRMS (ESI^+) calcd for $C_{29}H_{31}N_4O_4$ $(M+H)^+$ 499.2345, found 499.2350.

3-((4-(4-((4-Methoxyphenoxy)methyl)benzamido)-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (16). White solid, 28 mg, 77% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.92 (d, *J* = 7.2 Hz, 2H), 7.74 (d, *J* = 7.2 Hz, 1H), 7.56 (d, *J* = 7.8 Hz, 2H), 7.40 (dd, *J* = 7.8, 7.8 Hz, 1H),

7.31-7.27 (m, 2H), 7.15 (s, 1H), 6.91 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 7.8 Hz, 2H), 6.58-6.53 (m, 1H), 5.29 (s, 2H), 5.11 (s, 2H), 3.77 (s, 3H), 3.00 (d, *J* = 4.2 Hz, 3H), 2.21 (s, 3H), 2.09 (s, 3H). HRMS (ESI^+) calcd for $C_{29}H_{31}N_4O_4$ $(M+H)^+$ 499.2345, found 499.2349.

3-((4-(4-((2-Chlorophenoxy)methyl)benzamido)-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (17). White solid, 15 mg, 41% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.94 (d, *J* = 8.4 Hz, 2H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 8.4 Hz, 2H), 7.42-7.36 (m, 3H), 7.29-7.26 (m, 1H), 7.20 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.16 (s, 1H), 6.97-6.91 (m, 2H), 6.63-6.58 (m, 1H), 5.28 (s, 2H), 5.24 (s, 2H), 2.99 (d, *J* = 4.8 Hz, 3H), 2.20 (s, 3H), 2.08 (s, 3H). HRMS (ESI^+) calcd for $C_{28}H_{28}ClN_4O_3$ $(M+H)^+$ 503.1850, found 503.1846.

3-((4-(4-((3-Chlorophenoxy)methyl)benzamido)-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (18). White solid, 36 mg, 95% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.94 (d, *J* = 7.8 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.41 (s, 1H), 7.39 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.28-7.25 (m, 1H), 7.21 (dd, *J* = 8.4, 8.4 Hz, 1H), 7.17 (s, 1H), 7.00-6.95 (m, 2H), 6.88-6.84 (m, 1H), 6.64-6.57 (m, 1H), 5.27 (s, 2H), 5.14 (s, 2H), 2.99 (d, *J* = 4.8 Hz, 3H), 2.20 (s, 3H), 2.07 (s, 3H). HRMS (ESI^+) calcd for $C_{28}H_{28}ClN_4O_3$ $(M+H)^+$ 503.1850, found 503.1857.

3-((4-(4-((4-Chlorophenoxy)methyl)benzamido)-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (19). White solid, 30 mg, 82% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.93 (d, *J* = 7.8 Hz, 2H), 7.73 (d, *J* = 7.2 Hz, 1H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.39 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.34 (s, 1H), 7.10-7.22 (m, 3H), 7.17 (s, 1H), 6.90 (d, *J* = 7.8 Hz, 2H), 6.60-6.54 (m, 1H), 5.29

(s, 2H), 5.13 (s, 2H), 2.99 (d, *J* = 4.8 Hz, 3H), 2.20 (s, 3H), 2.08 (s, 3H). HRMS (ESI^+) calcd for $C_{28}H_{28}ClN_4O_3$ $(M+H)^+$ 503.1850, found 503.1858.

3-((3,5-Dimethyl-4-(4-((2-(trifluoromethyl)phenoxy)methyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (20). White solid, 31 mg, 79% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.96 (d, *J* = 8.4 Hz, 2H), 7.82 (s, 1H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.48 (dd, *J* = 8.1, 8.1 Hz, 1H), 7.33 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.23 (d, *J* = 7.2 Hz, 1H), 7.18 (s, 1H), 7.06-7.00 (m, 2H), 6.89-6.83 (m, 1H), 5.24 (s, 2H), 5.20 (s, 2H), 2.93 (d, *J* = 4.8 Hz, 3H), 2.14 (s, 3H), 2.02 (s, 3H). HRMS (ESI^+) calcd for $C_{29}H_{28}F_3N_4O_3$ $(M+H)^+$ 537.2114, found 537.2108.

3-((3,5-Dimethyl-4-(4-((3-(trifluoromethyl)phenoxy)methyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (21). White solid, 17 mg, 43% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.96 (d, *J* = 7.2 Hz, 2H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.60-7.53 (m, 3H), 7.41 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.37 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.28-7.21 (m, 3H), 7.18 (s, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 6.73-6.68 (m, 1H), 5.25 (s, 2H), 5.18 (s, 2H), 2.97 (d, *J* = 4.8 Hz, 3H), 2.18 (s, 3H), 2.05 (s, 3H). HRMS (ESI^+) calcd for $C_{29}H_{28}F_3N_4O_3$ $(M+H)^+$ 537.2114, found 537.2112.

3-((3,5-Dimethyl-4-(4-((4-(trifluoromethyl)phenoxy)methyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (22). White solid, 7.0 mg, 18% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.94 (d, *J* = 8.4 Hz, 2H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 4H), 7.40 (dd, *J* = 8.1, 8.1 Hz, 1H), 7.32 (s, 1H), 7.28 (d, *J* = 7.2 Hz, 1H), 7.17 (s, 1H), 7.04 (d, *J* = 9.0 Hz, 2H),

6.58-6.53 (m, 1H), 5.29 (s, 2H), 5.20 (s, 2H), 3.00 (d, *J* = 4.8 Hz, 3H), 2.21 (s, 3H), 2.09 (s, 3H). HRMS (ESI^+) calcd for $C_{29}H_{28}F_3N_4O_3$ $(M+H)^+$ 537.2114, found 537.2119.

3-((3,5-Dimethyl-4-(4-((2-methyl-4-(trifluoromethyl)phenoxy)methyl)benzamido)-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (23). White solid, 14 mg, 16% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.96 (d, *J* = 7.8 Hz, 2H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 7.2 Hz, 2H), 7.52 (s, 1H), 7.44-7.40 (m, 2H), 7.37 (d, *J* = 7.5, 7.5 Hz, 1H), 7.29-7.26 (m, 1H), 7.17 (s, 1H), 6.89 (d, *J* = 9.0 Hz, 1H), 6.67 (d, *J* = 3.6 Hz, 1H), 5.26 (s, 2H), 5.21 (s, 2H), 2.97 (d, *J* = 4.8 Hz, 3H), 2.34 (s, 3H), 2.18 (s, 3H), 2.06 (s, 3H). HRMS (ESI^+) calcd for $C_{30}H_{30}F_3N_4O_3$ $(M+H)^+$ 551.2270, found 551.2265.

1-Benzyl-3,5-dimethyl-1*H*-pyrazol-4-amine (25a). To a solution of 3,5-dimethyl-4-nitropyrazole (**24**, 1.41 g, 10 mmol) in DMF (30 mL) were added benzyl bromide (2.05 g, 12 mmol) and Cs_2CO_3 (6.52 g, 20 mmol) and the mixture was allowed to stir at rt for 24 h. The reaction was quenched with water (50 mL) and the mixture was extracted with EtOAc. The organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. After purification by flash column chromatography (0-50% EtOAc/hexanes), 1-benzyl-3,5-dimethyl-4-nitro-1*H*-pyrazole was obtained as a white solid (1.39 g, 60%). ^1H NMR ($CDCl_3$, 600 MHz) δ 7.37-7.28 (m, 3H), 7.16-7.12 (m, 2H), 5.26 (s, 2H), 2.56 (s, 3H), 2.55 (s, 3H). HRMS (ESI^+) calcd for $C_{12}H_{14}N_3O_2$ $(M+H)^+$ 232.1081, found 232.1079.

This nitro compound (1.39 g, 6.0 mmol) and $NiCl_2 \cdot 6H_2O$ (2.85 g, 12 mmol) were then dissolved in MeOH (70 mL). $NaBH_4$ (907 mg, 24 mmol) was slowly added to the above solution and the

mixture was allowed to stir at rt for 3 h. The reaction was quenched with saturated NH_4Cl (50 mL) and the mixture was extracted with EtOAc. The organic phase was washed with water and brine, dried over anhydrous K_2CO_3, and concentrated in vacuo. The residue was purified by flash column chromatography (0-60% EtOAc/hexanes) to afford compound **25a** as a brown oil (1.00 g, 50% over two steps). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.32-7.25 (m, 2H), 7.24-7.20 (m, 1H), 7.07-7.03 (m, 2H), 5.16 (s, 2H), 2.20 (s, 3H), 2.04 (s, 3H). HRMS (ESI^+) $C_{12}H_{16}N_3$ $(M+H)^+$ 202.1339, found 202.1342.

Intermediates **25b-d** were prepared with methyl 4-(bromomethyl)benzoate, methyl 3-(bromomethyl)benzoate, and methyl 2-(bromomethyl)benzoate, respectively, in a fashion similar to the one described for intermediate **25a**.

Methyl 4-((4-Amino-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)benzoate (25b). Brown oil, 788 mg, 91% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.96 (d, J = 7.8 Hz, 2H), 7.08 (d, J = 7.8 Hz, 2H), 5.21 (s, 2H), 3.9 (s, 3H), 2.20 (s, 3H), 2.03 (s, 3H). HRMS (ESI^+) calcd for $C_{14}H_{18}N_3O_2$ $(M+H)^+$ 260.1394, found 260.1395.

Methyl 3-((4-Amino-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)benzoate (25c). Brown oil, 683 mg, 81% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.92 (d, J = 7.8 Hz, 1H), 7.80 (s, 1H), 7.36 (dd, J = 7.8, 7.8 Hz, 1H), 7.19 (d, J = 7.2 Hz, 1H), 5.20 (s, 2H), 3.90 (s, 3H), 2.20 (s, 3H), 2.05 (s, 3H). HRMS (ESI^+) calcd for $C_{14}H_{18}N_3O_2$ $(M+H)^+$ 260.1394, found 260.1394.

Methyl 2-((4-Amino-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)benzoate (25d). Brown oil, 776 mg, 89% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.00 (d, *J* = 8.4 Hz, 1H), 7.38 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.29 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 5.61 (s, 2H), 3.93 (s, 3H), 2.22 (s, 3H), 2.03 (s, 3H). HRMS (ESI^+) calcd for $C_{14}H_{18}N_3O_2$ $(M+H)^+$ 260.1394, found 260.1396.

Compounds **26b-d** were prepared from intermediates **25b-d**, respectively, via an EDC-mediated amide formation in a fashion similar to the one described for compound **1**.

Methyl 4-((3,5-Dimethyl-4-(4-(phenoxymethyl)benzamido)-1*H*-pyrazol-1-yl)methyl)benzoate (26b). White solid, 300 mg, 64% yield. ^{1}H NMR (DMSO-d_6, 600MHz) δ 9.56 (s, 1H), 7.97 (d, *J* = 8.4 Hz, 2H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.83 (s, 1H), 7.56 (d, *J* = 7.8 Hz, 2H), 7.51 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.30 (dd, *J* = 7.8, 7.8 Hz, 2H), 7.02 (d, *J* = 7.8 Hz, 2H), 6.95 (dd, *J* = 7.5, 7.5 Hz, 1H), 5.32 (s, 2H), 5.20 (s, 2H), 3.85 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H). HRMS (ESI^+) calcd for $C_{28}H_{28}N_3O_4$ $(M+H)^+$ 470.2080, found 470.2083.

Methyl 3-((3,5-Dimethyl-4-(4-(phenoxymethyl)benzamido)-1H-pyrazol-1-yl)methyl)benzoate (26c). White solid, 200 mg, 40% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.94 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 8.4 Hz, 2H), 7.86 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.40 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.32-7.28 (m, 3H), 7.24-7.20 (m, 1H), 7.00-6.95 (m, 3H), 5.27 (s, 2H), 5.15 (s, 2H), 3.91 (s, 3H), 2.21 (s, 3H), 2.12 (s, 3H). HRMS (ESI^+) calcd for $C_{28}H_{28}N_3O_4$ $(M+H)^+$ 470.2080, found 470.2075.

Methyl 2-((3,5-Dimethyl-4-(4-(phenoxymethyl)benzamido)-1*H*-pyrazol-1-yl)methyl)benzoate (26d). White solid, 776 mg, 89% yield. ^{1}H NMR (DMSO-d_6, 600MHz) δ 9.61 (s, 1H), 7.99 (d, *J* = 7.8 Hz, 2H), 7.93 (d, *J* = 7.2 Hz, 1H), 7.60-7.52 (m, 3H), 7.42 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.30 (dd, *J* = 7.8, 7.8 Hz, 2H), 7.02 (d, *J* = 7.8 Hz, 2H), 6.95 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.55 (d, *J* = 7.2 Hz, 1H), 5.58 (s, 2H), 5.20 (s, 2H), 3.89 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H). HRMS (ESI$^+$) calcd for $C_{28}H_{28}N_3O_4$ $(M+H)^+$ 470.2080, found 470.2081.

3-((3,5-Dimethyl-4-(4-(phenoxymethyl)benzamido)-1*H*-pyrazol-1-yl)methyl)benzoic Acid (27). To a solution of methyl ester **26c** (40 mg, 0.086 mmol) in H_2O/MeOH (1:1, 20 mL) was added NaOH (7 mg, 0.17 mmol) and the mixture was allowed to stir at rt for 12 h. After the MeOH was evaporated in vacuo, the residue was acidified with 1N HCl to pH = 2 and the mixture was extracted with EtOAc. The organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to afford compound **27** as a white solid (23 mg, 59%). ^{1}H NMR (DMSO-d_6, 600MHz) δ13.01 (s, 1H), 9.56 (s, 1H), 7.97 (d, *J* = 8.4 Hz, 2H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.78 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.49 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.30 (dd, *J* = 7.5, 7.5Hz, 2H), 7.02 (d, *J* = 7.8 Hz, 2H), 6.95 (dd, *J* = 7.5, 7.5 Hz, 1H), 5.31 (s, 2H), 5.20 (s, 2H), 2.07 (s, 3H), 2.04 (s, 3H). HRMS (ESI$^+$) calcd for $C_{27}H_{26}N_3O_4$ $(M+H)^+$ 456.1923, found 456.1925.

3-((3,5-Dimethyl-4-nitro-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (28). To a solution of 3,5-dimethyl-4-nitropyrazole (**24**, 2.0 g, 14.2 mmol) in DMF (100 mL) were added methyl 3-(bromomethyl)benzoate (3.90 g, 17.0 mmol) and Cs_2CO_3 (9.23 g, 28.3 mmol) and the mixture was allowed to stir at rt for 12 h. The reaction was quenched with water (50 mL) and the mixture

was extracted with EtOAc. The organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was then dissolved in $MeNH_2$/EtOH (33 wt. %, 25 mL) in a sealed tube and heated at 70 ºC for 24 h. After the solvent was evaporated in vacuo, the residue was purified by flash column chromatography (0-20% MeOH/CH_2Cl_2) to afford compound **28** as a light yellow solid (1.45 g, 36% over two steps). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.68-7.62 (m, 2H), 7.42 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 1H), 6.14 (bs, 1H), 5.29 (s, 2H), 3.01 (d, *J* = 4.2 Hz, 3H), 2.57 (s, 3H), 2.54 (s, 3H). HRMS (ESI^+) calcd for $C_{14}H_{17}N_4O_3$ $(M+H)^+$ 289.1295, found 289.1291.

3-((4-Amino-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (29). Compound **29** was prepared from intermediate **28** via a $NaBH_4$/$NiCl_2$-mediated reduction in a fashion similar to the one described for compound **25a**. Yellow oil, 900 mg, 72% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.63 (d, *J* = 7.8 Hz, 1H), 7.49 (s, 1H), 7.33 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 6.32 (bs, 1H), 5.17 (s, 2H), 2.97 (d, *J* = 4.8 Hz, 3H), 2.19 (s, 3H), 2.04 (s, 3H). HRMS (ESI^+) calcd for $C_{14}H_{19}N_4O$ $(M+H)^+$ 259.1553, found 259.1549.

3-((4-(4-(Chloromethyl)benzamido)-3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)-*N*-methylbenzamide (30). To a solution of amine **29** (900 mg, 3.49 mmol) and Et_3N (1.00 mL, 7.00 mmol) in anhydrous CH_2Cl_2 (100 mL) was added 4-(chloromethyl)benzoyl chloride (790 mg, 4.19 mmol) and the mixture was allowed to stir at rt for 12 h. After the solvents were removed, the residue was diluted with EtOAc (50 mL) and H_2O (50 mL). After separation, the organic layer was washed with brine (20 mL) and concentrated. The residue was purified by flash column chromatography (0-15% MeOH/CH_2Cl_2) to give compound **30** as a white solid

(1.10 g, 61%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.92 (d, *J* = 7.8 Hz, 2H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.60 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 2H), 7.37 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.29-7.25 (m, 1H), 7.19 (s, 1H), 6.75 (bs, 1H), 5.25 (s, 2H), 4.64 (s, 2H), 2.96 (d, *J* = 4.2 Hz, 3H), 2.17 (s, 3H), 2.05 (s, 3H). HRMS (ESI^+) calcd for $C_{22}H_{24}ClN_4O_2$ $(M+H)^+$ 411.1582, found 411.1580.

4,4,5,5-Tetramethyl-2-(2-methyl-4-(trifluoromethyl)phenyl)-1,3,2-dioxaborolane (32). A flask charged with Pd(dppf)Cl_2 (44 mg, 0.06 mmol), KOAc (589 mg, 6.0 mmol), and bis(pinacolato)diboron (559 mg, 2.2 mmol) was flushed with nitrogen. DMSO (10 mL) and 4-bromo-3-methylbenzotrifluoride (**31**, 280 mg, 2.0 mmol) were then added. After being stirred at 80 ºC for 24 h, the reaction mixture was extracted with EtOAc. The organic layer was washed with water and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated in vacuo and the residue was purified by flash column chromatography (0-50% EtOAc/hexanes) to give compound **32** as a colorless oil (380 mg, 66%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.84 (d, *J* = 7.8 Hz, 1H), 7.42-7.37 (m, 2H), 2.58 (s, 3H), 1.35 (s, 12H).

2-Methyl-4-(trifluoromethyl)phenol (33). To a solution of compound **32** (380 mg, 1.33 mmol) in EtOH/H_2O (2:1, 6 mL) was added mCPBA (252 mg, 1.46 mmol) and the mixture was allowed to stir at rt for 12 h. After the solvents were removed, the residue was diluted with EtOAc (20 mL), H_2O (10 mL) and saturated $NaHCO_3$ (10 mL). After separation, the organic layer was washed with brine (20 mL) and concentrated. The residue was purified by flash column chromatography (0-50% EtOAc/hexanes) to give phenol **33** as a white solid (180 mg, 77%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.55 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 1H), 2.10 (s, 3H). HRMS (ESI^-) calcd for $C_8H_6F_3O$ $(M-H)^-$ 175.0376, found 175.0378.

Cell Viability Assay. MIA PaCa-2 cells (CRL-1420, ATCC) were maintained in DMEM media supplemented with 10% FBS, 2.5% HS, 1% GlutaMAX, 1% sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin. ARPE-19 cells were maintained in DMEM:F12 media supplemented with 10% FBS, 1% GlutaMAX, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cell culture media and additives were purchased from Gibco. Cells were plated in 96-well plates at 2.5×10^5 cells per well in growth media. One microliter of a 3-fold serially diluted compound solution (9 doses) in DMSO (Sigma) starting at 20 mM (100 μM final concentration) was added to each well. The final volume in each well was 200 μL, yielding a final DMSO concentration of 0.5%. Control wells contained 0.5% DMSO (100% viability) or 25% DMSO (background) and all reactions were done in triplicate. The plate was incubated for 72 h at 37 °C in a 5% CO_2/95% air humidified atmosphere.

Measurement of cell viability was carried out using a modified method of Mosmann based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma). MTT solution was prepared fresh at 1 mg/mL in serum-free and phenol red-free RPMI 1640 media. After 200 μL of MTT solution was added to each well, the plate was incubated as described above for 3 h. The MTT solution was removed, and the formazan crystals were solubilized with 200 μL of isopropanol. The plate was read on a SpectraMax i3 spectrophotometer (Molecular Devices) at 570 nm for formazan and 690 nm for background subtraction. EC_{50} values were calculated by fitting the data in GraphPad Prism software.

Plasma Stability Assay and Microsomal Stability Assay. These assays were performed as described previously.[1]

Thermodynamic Solubility Assay. The aqueous solubility of a test compound was determined in DPBS (pH 7.4) under thermodynamic solubility conditions. Briefly, a saturated solution was made by adding DPBS to the solid compound. The mixture was shaken at 200 rpm for 48 h in a MaxQ 6000 orbital shaker at ambient temperature to allow equilibrium between the solid and dissolved compound. The suspension was then filtered through a 0.45 μm PVDF syringe filter and the filtrate was collected for analysis using UV spectrometry (SpectraMax M5e, Molecular Devices) at $\lambda = 240$ nm.

Antibodies. The following rabbit polyclonal antibodies were used: LC3 (Sigma-Aldrich, L7543), anti-Phospho-p70 S6 kinase, anti-p70 S6 kinase, anti-phospho-4E-BP1, anti-4E-BP1 (Cell Signaling Technology, 9205, 2708, 9451, 9452). The following monoclonal antibodies were used: CD63 clone H5C6, Actin Ab-5 Clone C4 (BD Biosciences, 556019, 612656), Alexa Fluor 568-conjugated goat anti-mouse IgG, Alexa Fluor 488-conjugated goat anti-rabbit IgG (Life Technologies, A-21422, and A-11008), HRP-conjugated anti-mouse or anti-rabbit IgG (Cell Signaling Technology, 7076 and 7074).

Cell Line Cultures and Treatments. ARPE-19 cells (CRL-2302, American Type Culture Collection) were grown at 37 ºC in a 1:1 mixture of DMEM and Ham's F12 media supplemented with 10% fetal bovine serum (Invitrogen), 2 mM Glutamax™, 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco) in a humidified 5% CO_2 atmosphere. For compound treatment

experiments, cells were incubated for the indicated period of time at 37 ºC in medium containing one of the following reagents: DMSO (Sigma-Aldrich), 10 μM, 25 μM, or 50 μM of compound **67** or **66**, and 100 nM bafilomycin (Sigma-Aldrich). For starvation experiments, cells were washed three times in Hank's balanced salt solution (Invitrogen) and incubated for 4 h at 37 ºC in Earle's balanced salt solution (Starvation media) (Sigma-Aldrich).

Immunofluorescence Confocal Microscopy. Cells grown on glass coverslips were washed with PBS and fixed in cold methanol/acetone (1:1 vol/vol) at –20 ºC for 15 min. After fixation, cells were washed with PBS and then incubated with the indicated primary antibodies in IF buffer (PBS containing 10% fetal bovine serum and 0.1% (wt/vol) saponin) for 1 h at room temperature. Cells were washed three times with PBS and incubated with the corresponding secondary antibodies conjugated to Alexa Fluor 568-conjugated goat anti-mouse IgG or Alexa Fluor 488-conjugated goat anti-rabbit IgG in IF buffer for 30 min at room temperature. PBS washed coverslips were mounted onto glass slides with Prolong Diamond antifade with Dapi (Life Technologies).

Electrophoresis and Immunoblotting. Cells were washed with ice-cold PBS, resuspended in lysis buffer (25 mM Hepes-KOH, pH 7.4, 250 mM NaCl, 1% Triton X-100 (wt/vol) supplemented with protease and phosphatase inhibitors cocktail, and lysed by passing the samples 10 times through a 25 gauge needle. Cell lysates were centrifuged at 16,000 x g for 15 min at 4 ºC, and the soluble fractions were collected. Samples were analyzed by SDS-PAGE (4–20% gradient gels, Invitrogen, EC61385BOX) under reducing conditions and transferred to nitrocellulose. Membranes were immunoblotted using the indicated antibodies. Horseradish

peroxidase-chemiluminiscence was developed by using Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life Sciences, NEL 104001EA).

References

(1). Ai, T.; Wilson, D. J.; More, S. S.; Xie, J.; Chen, L. 5-((3-Amidobenzyl)oxy)nicotinamides as Sirtuin 2 Inhibitors. *J. Med. Chem.* **2016,** *59*, 2928-2941.