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SUPPLEMENTAL INFORMATION

Synthesis and Application of Light-Switchable Arylazopyrazole Rapamycin Analogs

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1. Synthesis of arylazopyrazole rapamycin analogs.

All chemicals obtained from commercial sources Acros, Alfa Aesar and Fisher Scientific were used without further purification unless stated otherwise. Rapamycin was obtained from Capot Chemical. All reactions were performed in flame dried glassware under nitrogen atmosphere and stirred magnetically unless stated otherwise. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance III 400 MHz or 500 MHz NMR spectrometer with chemical shifts reported relative to either residual $CDCl_3$ (7.26 ppm), d_6-DMSO (2.50 ppm), or CD_3OD (3.30 ppm). High resolution mass spectrometry (HRMS) was performed on a Q-Exactive (Thermo Scientific) mass spectrometer by University of Pittsburgh mass spectrometry facility.

3-(2-(4-(Hydroxymethyl)phenyl)hydrazineylidene)pentane-2,4-dione (2). 4-Amino benzyl alcohol **1** (1.25 g, 10.1 mmol, 1 eq) was dissolved in acetic acid (15 mL) and cooled to 0°C. Concentrated hydrochloric acid (2.3 mL) was added dropwise. Sodium nitrite (840 mg, 12.1 mmol, 1.2 eq) was dissolved in minimal H_2O and added dropwise to the reaction mixture. After 45 minutes, a solution of 2,4-pentanedione (1.35 mL, 13.4 mmol, 1.3 eq), sodium acetate (2.46 mg, 30.9 mmol, 3 eq), ethanol (10 mL) and H_2O (6 mL) was added in portions. After stirring overnight at room temperature, the yellow precipitate was collected. The solid was washed with hexanes, and dried under vacuum to yield the product as a bright yellow solid (1.055 g, 44% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.78 (b, 1 H), 7.43 (s, 4 H), 4.74 (s, 2 H), 2.63 (s, 3 H), 2.52 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.2, 140.9, 139.3, 128.0, 115.9, 63.3, 30.1, 25.1, 21.4; HRMS $(M+H)^+$ calcd for $C_{13}H_{17}ON_4$ $(M+H)^+$ 235.1077, found 235.1093.

(*E*)-(4-((3,5-Dimethyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)methanol (3). A solution of diketone **2** (500 mg, 2.1 mmol, 1 eq) and hydrazine (0.130 mL, 4.2 mmol, 2 eq) in ethanol (10 mL) was stirred at room temperature for 1 hour. The reaction mixture was concentrated in vacuo to give the product as an orange solid (502 mg, 95% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.41 (d, 2 H, J = 8.5 Hz), 7.13 (d, 2 H, J = 8.5 Hz), 4.35 (s, 2 H), 3.29 (q, 1 H, J = 6.5 Hz), 2.26 (t, 1 H, J = 2.5 Hz), 2.20 (s, 6 H), 0.85 (t, 1 H, J = 6.5 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 152.8, 146.7, 143.1, 134.2, 130.8, 128.3, 127.1, 121.4, 115.1, 63.9; HRMS $(M+H)^+$ calcd for $C_{12}H_{15}ON_4$ $(M+H)^+$ 231.1240, found 231.1237.

(*E*)-(4-((1,3,5-Trimethyl-1H-pyrazol-4-yl)diazenyl)phenyl)methanol (4). A solution of diketone **2** (1.055 g, 4.50 mmol, 1 eq) in ethanol (50 mL) was heated to reflux with stirring. Methylhydrazine (4.5 mL, 15 eq) was added to the solution, and the reaction mixture was stirred under reflux for 2.5 hours. The reaction mixture was then concentrated in vacuo to yield the product as an orange solid (952 mg, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 2 H, J = 8 Hz), 7.45 (d, 2 H, J = 8 Hz), 4.76 (s, 2H), 3.78 (s, 3H), 2.58 (s, 3 H), 2.50 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.2, 142.5, 142.0, 138.7, 127.4, 122.0, 65.0, 35.9, 18.4, 13.8, 10.0; HRMS $(M+H)^+$ calcd for $C_{13}H_{17}ON_4$ $(M+H)^+$ 245.1397, found 245.1404.

(*E*)-(4-((3,5-Dimethyl-1-phenyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)methanol (5). A solution of diketone **2** (201 mg, 0.85 mmol, 1 eq) in ethanol (5 mL) was prepared. Phenylhydrazine (100 µL, 0.85 mmol, 1 eq) was added and the reaction mixture was stirred under reflux for 2 hours. The reaction mixture was then concentrated in vacuo and the residue was purified via flash chromatography on silica gel (1% acetone in chloroform) to yield the product as an orange solid (157 mg, 60% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, 2 H, J = 8 Hz), 7.51 (m, 7 H), 4.74 (s, 2H), 2.65 (s, 3 H), 2.59 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.1, 143.9, 142.4, 139.2, 138.9, 136.2, 129.3, 128.2, 127.5, 124.9, 122.1, 64.9, 14.0, 11.4; HRMS $(M+H)^+$ calcd for $C_{18}H_{19}ON_4$ $(M+H)^+$ 307.1553, found 307.1554.

(*E*)-(4-((3,5-Dimethyl-1-(naphthalen-1-yl)-1H-pyrazol-4-yl)diazenyl)phenyl)methanol (6). A solution of diketone **2** (200 mg, 0.85 mmol, 1 eq) and napthylhydrazine (170 mg, 1.07 mmol, 1.25 eq) in ethanol (10 mL) was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo and purified via flash chromatography on silica gel (1% acetone in chloroform) to yield the product as an orange solid (50 mg, 17% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, 1 H, J = 9 Hz), 7.98 (d, 1 H, J = 9 Hz), 7.87 (d, 2 H, J = 8 Hz), 7.55 (m, 7 H), 4.77 (s, 2 H), 2.68 (s, 3 H), 2.45 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 152.8, 143.5, 141.1, 134.6, 130.0, 128.1, 127.5, 126.7, 125.5, 122.1, 114.3, 109.4, 78.1, 63.5, 25.1, 14.1, 12.5, 9.2; HRMS $(M+H)^+$ calcd for $C_{22}H_{21}ON_4$ $(M+H)^+$ 357.1710, found 357.1728.

(*E*)-(4-((1-Butyl-3,5-dimethyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)methanol (7). A suspension of arylazopyrazole **3** (100 mg, 0.44 mmol, 1 eq) and potassium hydroxide (24 mg, 0.44 mmol, 1 eq) in dry acetonitrile (6 mL) was created. Butyl iodide (55 µL, 0.48 mmol, 1.1 eq) was added and heated under reflux overnight. The reaction mixture was concentrated in vacuo and the residue was purified via flash chromatography on silica gel (gradient 0-30% ethyl acetate in dichloromethane) to yield the product as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, 2 H, J = 8.5 Hz), 7.47 (d, 2 H, J = 8.5 Hz), 4.78 (s, 2 H), 4.05 (t, 2 H, J = 7 Hz), 2.60 (s, 3 H), 2.52 (s, 3 H), 1.83 (p, 2 H, J = 7 Hz), 1.59 (s, 1 H), 1.41 (hex, 2 H, J = 7 Hz), 0.98 (t, 3 H, J = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.3, 142.4, 141.9, 138.4, 135.0, 127.5 ,121.9, 65.1, 48.9, 32.1, 19.9, 14.0, 13.7, 9.9; HRMS $(M+H)^+$ calcd for $C_{16}H_{23}ON_4$ $(M+H)^+$ 287.1879, found 287.1866.

(*E*)-1-(4-((4-(Hydroxymethyl)phenyl)diazenyl)-3,5-dimethyl-1*H*-pyrazol-1-yl)hexan-1-one (8). A suspension of arylazopyrazole **3** (100 mg, 0.44 mmol, 1 eq) and potassium hydroxide (24 mg, 0.44 mmol, 1 eq) in dry acetonitrile (8 mL) was created. Bromohexane (71 mg, 0.44 mmol, 1

eq) was added and the reaction mixture was heated to reflux overnight. The reaction mixture was concentrated in vacuo and the residue was purified via flash chromatography on silica gel (gradient 0-20% ethyl acetate in dichloromethane) to yield the product as an orange oil (57 mg, 40%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, 2 H, J= 8.5 Hz), 7.48 (d, 2 H, J = 8.5 Hz), 4.78 (s, 2 H), 4.04 (t, 2 H, J = 6.5 Hz), 2.61 (s, 3 H), 2.53 (s, 3 H), 1.73 (p, 2 H, J = 6.5 Hz), 1.35 (m, 6 H), 0.92 (t, 3 H, J = 6.5 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 153.3, 142.4, 141.9, 138.4, 135.0, 127.5, 121.9, 65.1, 49.1, 31.4, 30.0, 26.4, 22.5, 14.0, 9.9; HRMS $(M+H)^+$ calcd for $C_{68}H_{100}O_{15}N_5$ $(M+H)^+$ 1226.7225, found 1226.7210.

Methyl arylazopyrazole rapamycin (14). A solution of benzyl alcohol **4** (100 mg, 0.40 mmol, 1 eq) and *N,N'*-disuccinimidyl carbonate, DSC (152 mg, 0.60 mmol, 1.5 eq) in dry acetonitrile (3 mL) under nitrogen was prepared. Triethylamine (170 µL, 1.21 mmol, 3 eq) was added and the reaction mixture was stirred for 4 hours. The mixture was concentrated in vacuo, and the residue was purified via flash chromatography on silica gel (gradient 15-40% ethyl acetate in dichloromethane) to yield NHS carbonate **9** as an orange solid (106 mg, 68% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, 2 H, J = 6.9 Hz), 7.45 (d, 2 H, J = 8.7 Hz), 5.27 (s, 2H), 3.75 (2, 3H), 2.80 (s, 4H), 2.54 (s, 3 H), 2.46 (s, 3 H). A solution of NHS carbonate **9** (35 mg, 0.091 mmol, 1.3 eq), rapamycin (63 mg, 0.068 mmol, 1 eq), and DMAP (44 mg, 0.27 mmol, 4 eq) in dry DCM (1 mL) under nitrogen was prepared. The reaction mixture was stirred at room temperature overnight, concentrated in vacuo, and the residue was purified via flash chromatography on silica gel (gradient 20-40% ethyl acetate in DCM) to yield the product as a yellow solid (17 mg, 21% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.77 (d, 2 H, J = 8.5 Hz), 7.49 (d, 2 H, J = 8.5 Hz), 6.48 (dd, 1 H, J = 12, 10 Hz), 6.30 (dd, 1 H, J = 12, 9 Hz), 6.21 (d, 1 H, J = 9 Hz), 6.12 (d, 1 H J = 10 Hz), 5.47 (dd, 1 H, J = 11, 9.5 Hz), 5.22 (m, 3 H), 5.11 (m, 2 H), 4.51 (m, 1 H), 4.16 (m, 2 H), 4.01 (d, 1 H, J = 3 Hz), 3.78 (s, 3 H), 3.39 (s, 2 H), 3.26 (s, 3 H), 3.11 (s, 3 H), 2.81 (d, 1 H, J = 7 Hz), 2.67 (m, 4 H), 2.47 (s, 3 H), 2.31 (m, 2 H), 2.16 (m, 2 H), 1.91 (d, 1 H, J = 6.5 Hz), 1.82 (s, 2 H), 1.71 (s, 3 H), 1.64 (m, 2 H), 1.53 (m, 1 H), 1.48 (q, 2 H, J = 6.5 Hz), 1.33 (m, 1 H), 1.21 (m, 2 H), 1.09 (d, 2 H, J = 6.5 Hz), 1.01 (d, 2 H, J = 7 Hz), 0.98 (d, 2 H, J = 7 Hz), 0.90 (d, 2 H, J = 7 Hz), 0.83 (d, 2 H, J = 6.5 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 207.3, 198.4, 169.7, 167.8, 139.4, 136.9, 134.6, 132.7, 130.9, 129.1, 128.8, 127.7, 126.4, 121.4, 120.2, 99.3, 85.9, 83.4, 80.9, 76.2, 74.1, 68.6, 67.2, 56.9, 54.9, 51.3, 45.9, 44.1, 40.4, 40.1, 40.0, 38.5, 35.8, 35.1, 34.7, 34.3, 33.7, 32.6, 31.3, 30.0, 29.3, 26.6, 26.5, 24.8, 24.7, 22.3, 20.7, 20.4, 19.6, 14.7, 14.5, 14.2, 13.0, 12.4, 9.6, 8.4; HRMS $(M+H)^+$ calcd for $C_{65}H_{94}O_{15}N_5$ $(M+H)^+$ 1184.6741, found 1184.6750.

***n*-Butyl arylazopyrazole rapamycin (15)**. A solution of arylazopyrazole **7** (50 mg, 0.18 mmol, 1 eq), DSC (67 mg, 0.27 mmol, 1.5 eq), and 4-dimethylaminopyridine, DMAP (5 mg, cat.) in dry acetonitrile (3 mL) was prepared. *N,N*-Diisopropylethylamine, DIPEA (0.09 mL, 0.54 mmol, 3 eq) was added and the reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was concentrated in vacuo and purified via flash chromatography on silica gel (gradient 0-25% ethyl acetate in dichloromethane) to yield **10** as an orange oil (73 mg, 97% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, 2 H, J = 8.5 Hz), 7.59 (d, 2 H, J = 8.5 Hz), 5.20 (s, 2 H), 4.05 (t, 2 H, J = 6.5 Hz), 2.61 (s, 3 H), 2.52 (s, 3 H), 1.86 (p, 2 H, J = 6.5 Hz), 1.39 (p, 2 H, J = 6.5 Hz), 1.29 (m, 4 H), 1.02 (t, 3 H, J = 6.5 Hz). A solution of arylazopyrazole **10** (73 mg, 0.17 mmol, 4 eq), rapamycin (39 mg, 0.04 mmol, 1 eq), and 9-azajulolidine (37 mg, 0.21 mmol, 5 eq) in dry

dichloromethane (3 mL) was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and the residue was purified via flash chromatography on silica gel (gradient 0-30% ethyl acetate in dichloromethane) to yield the product as a yellow solid (21 mg, 40% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.79 (d, 2 H, J = 8.5 Hz), 7.45 (d, 2 H, J = 8.5 Hz), 6.45 (dd, 1 H, J = 12, 10 Hz), 6.33 (dd, 1 H, J = 12, 9 Hz), 6.19 (d, 1 H, J = 9 Hz), 6.10 (d, 1 H, J = 10 Hz), 5.51 (m, 1 H), 5.26 (m, 2 H), 5.17 (m, 1 H), 4.63 (t, 1 H, J = 3 Hz), 4.51 (m, 3 H), 4.04 (m, 2 H), 4.00 (d, 1 H, J = 6.5 Hz), 3.71 (d, 1 H, J = 6.5 Hz), 3.59 (d, 1 H, J = 7.5 Hz), 3.48 (m, 2 H), 3.41 (s, 2 H), 3.29 (s, 3 H), 3.15 (s, 3 H), 2.98 (b, 1 H), 2.87 (d, 1 H, J = 7 Hz), 2.71 (d, 1 H, J = 3 Hz), 2.60 (s, 3 H), 2.49 (s, 3 H), 2.32 (m, 1 H), 2.08 (m, 2 H), 1.91 (t, 1 H, J = 6.5 Hz), 1.84 (m, 2 H), 1.73 (m, 2 H), 1.54 (m, 1 H), 1.47 (q, 2 H, J = 7 Hz), 1.40 (m, 2 H), 1.38 (s, 2 H), 1.11 (m, 2 H), 1.09 (d, 2 H, J = 7 Hz), 1.02 (m, 6 H), 0.97 (d, 2 H, J = 6.5 Hz), 0.89 (m, 5 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.2, 169.2, 166.8, 154.8, 153.7, 142.4, 140.2, 138.6, 136.2, 136.1, 135.1, 133.7, 130.2, 129.6, 129.0, 128.9, 128.8, 126.7, 126.4, 121.9, 121.8, 120.5, 98.5, 84.8, 84.4, 80.9, 80.4, 75.5, 69.1, 67.2, 59.4, 57.6, 55.9, 55.8, 53.4, 51.3, 48.9, 46.6, 44.2, 41.4, 40.7, 40.2, 38.9, 38.3, 35.8, 35.7, 35.2, 34.7, 34.5, 33.7, 33.2, 32.1, 32.0, 29.7, 29.0, 27.3, 27.1, 26.9, 25.2, 24.3, 22.7, 21.7, 21.6, 20.7, 20.6, 19.9 19.8, 16.4, 16.3, 16.1, 16.0, 15.9, 15.8, 14.1, 13.9, 13.8, 13.7, 13.6, 13.1, 11.4, 10.2, 10.0, 9.9; HRMS $(M+H)^+$ calcd for $C_{68}H_{100}O_{15}N_5$ $(M+H)^+$ 1226.7210, found 1226.7225.

***n*-Hexyl arylazopyrazole rapamycin (16)**. A solution of arylazopyrazole **8** (55 mg, 0.16 mmol, 1 eq), DSC (53 mg, 0.21 mmol, 1.3 eq), and DMAP (5 mg, cat.) in dry acetonitrile (3 mL) was created. DIPEA (0.1 mL, 0.32 mmol, 2 eq) was added and the reaction mixture was stirred for 1.5 hours. The reaction mixture was concentrated in vacuo and the residue was purified via flash chromatography on silica gel (gradient 0-15% ethyl acetate in dichloromethane) to yield **11** as an orange oil (64 mg, 85% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, 2 H, J = 8.5 Hz), 7.41 (d, 2 H, J = 8.5 Hz), 5.29 (s, 2 H), 3.95 (t, 2 H, J = 7 Hz), 2.78 (s, 4 H), 2.52 (s, 3 H), 2.43 (s, 3 H), 1.77 (m, 2 H), 1.26 (m, 6 H), 0.81 (m, 3 H). A solution of arylazopyrazole **11** (110 mg, 0.21 mmol, 3 eq), rapamycin (64 mg, 0.07 mmol, 1 eq), and 9-azajulolidine (61 mg, 0.35 mmol, 5 eq) in dry dichloromethane (3 mL) was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and the residue was purified via flash chromatography on silica gel (gradient 0-25% ethyl acetate in DCM) to yield the product as a yellow solid (8.3 mg, 9% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (d, 2 H, J = 8.5 Hz), 7.52 (d, 2 H, J = 8.5 Hz), 6.44 (dd, 1 H, J = 12, 10 Hz), 6.27 (dd, 1 H, J = 12, 9 Hz), 6.17 (d, 1 H, J = 10 Hz), 6.11 (d, 1 H, J = 9 Hz), 5.49 (m, 2 H), 5.22 (m, 2 H), 5.10 (m, 2 H), 4.52 (m, 1 H), 4.19 (d, 1 H, J = 3 Hz), 4.11 (t, 2 H, J = 6.5 Hz), 4.01 (d, 1 H, J = 7 Hz), 3.71 (d, 1 H, J = 6.5 Hz), 3.59 (t, 1 H, J = 7 Hz), 3.47 (m, 1 H), 3.42 (s, 3 H), 3.27 (s, 3 H), 3.15 (s, 3 H), 2.89 (d, 1 H, J = 7 Hz), 2.61 (s, 3 H), 2.51 (s, 3 H), 2.32 (m, 1 H), 2.29 (d, 1 H, J = 6.5 Hz), 2.19 (m, 1 H), 2.10 (m, 3 H), 1.97 (m, 1 H), 1.88 (s, 3 H), 1.78 (m, 2 H), 1.61 (m, 2 H), 1.50 (p, 2 H, J = 7 Hz), 1.44 (m, 6 H), 1.22 (m, 2 H), 1.10 (d, 2 H, J = 6.5 Hz), 1.01 (d, 2 H, J = 7 Hz), 0.99 (d, 2 H, J = 7 Hz), 0.91 (m, 4 H), 0.82 (d, 2 H, J = 6.5 Hz) ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.2, 169.2, 166.8, 154.8, 153.7, 142.4, 140.2, 138.6, 136.2, 136.1, 135.5, 135.1, 133.7, 130.2, 129.6, 129.0, 128.9, 126.7, 126.4, 121.9, 120.5, 98.5, 84.8, 84.4, 80.9, 80.4, 75.5, 69.1, 67.1, 59.4, 57.6, 55.9, 55.8, 53.4, 51.3, 49.1, 46.6, 44.2, 41.4, 40.7, 40.2, 38.9, 38.2, 35.8, 35.2, 34.7, 34.5, 33.7, 33.2, 32.8, 31.6, 31.4, 31.3, 30.0, 29.9, 29.7, 29.6, 29.0, 27.3, 27.1, 26.9, 26.4, 26.2, 25.3, 22.7, 22.5, 21.5, 21.0, 20.7, 20.6, 18.8, 16.4, 16.2, 16.1, 15.9, 15.8, 14.1,

13.9, 13.8, 11.4, 10.2, 10.1, 9.9; HRMS $(M+H)^+$ calcd for $C_{74}H_{98}O_{15}N_5$ $(M+H)^+$ 1254.7523, found 1254.7505.

Phenyl arylazopyrazole rapamycin (17). A solution of benzyl alcohol **5** (61 mg, 0.20 mmol, 1 eq) and DSC (120 mg, 0.47 mmol, 2.4 eq) in dry acetonitrile (2 mL) was prepared. Triethylamine (0.2 mL, 2.72 mmol, 13 eq) was added and the reaction mixture was stirred at room temperature for 20 hours, then concentrated in vacuo. The residue was purified via flash chromatography on silica gel (1% acetone in chloroform) to yield NHS carbonate **12** as an orange solid (56 mg, 63% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, 2 H, J = 8.5 Hz), 7.49 (d, 2 H, J = 8.5 Hz), 7.38 (m, 5 H), 5.18 (s, 2 H), 2.83 (s, 3 H), 2.65 (s, 3 H), 2.58 (s, 4 H). A solution of NHS carbonate **12** (56 mg, 0.12 mmol, 3 eq), rapamycin (38 mg, 0.04 mmol, 1 eq), and DMAP (25 mg, 0.22 mmol, 5.5 eq) in dry dichloromethane (1 mL) under nitrogen was prepared and stirred overnight. The reaction mixture was concentrated in vacuo, then the residue was purified via flash chromatography on silica gel (30% ethyl acetate in dichloromethane) to yield **17** as an orange solid (5 mg, 10% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.73 (d, 2 H, J = 8.5 Hz), 7.48 (m, 7 H), 6.40 (dd, 1 H, J = 12, 10 Hz), 6.22 (dd, 1 H, J = 12 Hz, 9 Hz), 6.14 (d, 1 H, J = 10 Hz), 6.06 (d, 1 H, J = 9 Hz), 5.39 (dd, 1 H, J = 14, 10 Hz), 5.17 (m, 3 H), 5.03 (m, 2 H), 4.41 (m, 1 H), 4.14 (m, 2 H), 4.11 (d, 1 H, J = 7 Hz), 4.02 (m, 1 H), 3.92 (d, 1 H, J = 3 Hz), 3.61 (d, 1 H, J = 6.5 Hz), 3.50 (d, 1 H, J = 6.5 Hz), 3.37 (s, 3 H), 3.29 (s, 3 H), 3.21 (s, 3 H), 3.05 (s, 3 H), 2.72 (d, 1 H, J = 6.5 Hz), 2.59 (s, 3 H), 2.48 (s, 3 H), 2.41 (dd, 1 H, J = 12, 6 Hz), 2.20 (m, 1 H), 2.09 (m, 2 H), 1.81 (d, 1 H, J = 7 Hz), 1.78 (s, 3 H), 1.64 (m, 4 H), 1.45 (m, 2 H), 1.29 (m, 3 H), 1.11 (m, 1 H), 0.98 (d, 2 H, J = 6.5 Hz), 0.91 (d, 2 H, J = 6 Hz), 0.85 (d, 2 H, J = 7 Hz), 0.81 (d, 2 H, J = 7 Hz), 0.77 (d, 2 H, J = 6.5 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 207.3, 198.4, 169.7, 167.8, 153.4, 144.4, 139.4, 138.7, 137.8, 137.6, 132.7, 130.9, 129.1, 128.9, 127.7, 125.1, 124.9, 121.6, 120.3, 99.3, 85.9, 83.4, 80.9, 80.5, 76.2, 74.1, 68.5, 57.0, 56.5, 54.9, 51.3, 45.9, 44.1, 40.4, 40.0, 38.5, 35.8, 35.1, 34.3, 33.7, 32.6, 31.3, 30.8, 30.0, 29.3, 26.4, 24.7, 22.3, 20.7, 20.4, 14.7, 14.6, 14.2, 13.0, 12.6, 12.4, 10.1, 9.8, 9.5; HRMS $(M+H)^+$ calcd for $C_{70}H_{96}O_{15}N_5$ $(M+H)^+$ 1246.6897, found 1246.6929.

Naphthyl arylazopyrazole rapamycin (18). A solution of benzyl alcohol **6** (69 mg, 0.19 mmol, 1 eq) and DSC (74 mg, 0.29 mmol, 1.5 eq) in dry acetonitrile (3 mL). Triethylamine (0.08 mL, 0.6 mmol, 3 eq) was added and the reaction mixture was stirred overnight. The reaction mixture was concentrated in vacuo and the residue was purified via flash chromatography on silica gel (gradient 0-5% ethyl acetate in dichloromethane) to yield NHS carbonate **13** as an orange solid (90 mg, 96% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, 1 H, J = 8.5 Hz), 7.95 (d, 1 H, J = 8.5 Hz), 7.85 (d, 2 H, J = 8 Hz), 7.50 (m, 7 H), 5.38 (s, 2 H), 2.66 (s, 4 H), 2.59 (s, 3 H), 2.42 (s, 3 H). A solution of the NHS carbonate **13** (90 mg, 0.18 mmol, 3 eq), DMAP (40 mg, 0.33 mmol, 5.5 eq) and rapamycin (50 mg, 0.06 mmol, 1 eq) in dry dichloromethane (3 mL) under nitrogen was prepared. The reaction was stirred at room temperature for 2 days, then concentrated in vacuo. The residue was purified via flash chromatography on silica gel (gradient 35-50% ethyl acetate in dichloromethane) to yield the product as an orange solid (9 mg, 13% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (d, 1 H, J = 9 Hz), 8.11 (d, 1 H, J = 9 Hz), 7.86 (m, 2 H), 7.60 (m, 8 H), 7.38 (d, 1 H, J = 8.5 Hz), 6.48 (dd, 1 H, J = 14, 10 Hz), 6.32 (dd, 1 H, J = 14, 9 Hz), 6.10 (m, 2 H), 5.51 (m, 2 H), 5.38 (t, 1 H, J = 6 Hz), 5.26 (m, 3 H), 5.12 (m, 2 H), 4.62 (m, 1 H), 4.29 (m, 2 H), 4.05 (d, 1 H, J = 6.5 Hz), 3.73 (d, 1 H, J = 8 Hz), 3.58 (d, 1 H, J = 6.5 Hz), 3.49 (m, 5 H), 3.17 (s, 3 H), 3.05

(m, 1 H), 2.89 (s, 3 H), 2.89 (m, 1 H), 2.62 (s, 2 H), 2.45 (m, 1 H), 2.30 (m, 1 H), 2.17 (m, 1 H), 2.02 (m, 2 H), 1.91 (m, 1 H), 1.81 (m, 2 H), 1.77 (m, 1 H), 1.59 (m, 4 H), 1.48 (m, 1 H), 1.31 (m, 1 H), 1.17 (m, 2 H), 1.09 (m, 1 H), 0.98 (d, 2 H, 7 Hz), 0.92 (d, 2 H, 6.5 Hz), 0.81 (m, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.2, 169.2, 166.8, 154.8, 153.6, 144.2, 141.1, 140.2, 136.7, 136.1, 135.5, 135.2, 134.3, 133.7, 130.3, 130.2, 129.9, 129.6, 129.1, 128.9, 128.2, 127.6, 126.8, 126.7, 126.4, 125.3, 125.1, 122.9, 122.1, 120.6, 98.5, 84.8, 84.4, 80.9, 80.5, 69.1, 67.2, 59.4, 57.6, 55.9, 55.8, 53.4, 51.3, 46.6, 44.2, 41.5, 40.7, 40.2, 38.9, 38.3, 36.1, 35.8, 35.2, 34.7, 33.2, 32.8, 31.6, 31.3, 29.7, 29.1, 27.3,27.1, 26.9, 25.3, 22.7, 21.5, 20.7, 20.6, 18.8, 16.4, 16.3, 16.1, 15.9, 15.8, 14.1, 14.0, 13.8, 13.1, 12.9, 11.4, 10.7, 10.3, 10.1; HRMS $(M+H)^+$ calcd for $C_{74}H_{98}O_{15}N_5$ $(M+H)^+$ 1296.7054, found 1296.7084.

2. Photochemical characterization of AAP analogs.

PSS determination. 4 mM solutions of each rapalog **14**-**18** were prepared using 20% D_2O in d_6-DMSO (we were unable to generate solutions with higher D_2O concentration due to the limited solubility of rapamycin). Each solution was irradiated in the dark for one hour using a benchtop VWR dual UV transilluminator set to 365 nm (7 mW). Each compound was characterized by ^{1}H NMR calibrated to d_6-DMSO to calculate *cis*:*trans* ratio by relative integrations of 2H phenyl peaks. This provided the PSS for the *cis*-isomer. The solutions were then irradiated with a 530 nm LED (262 mW) for one hour in a dark room, followed by ^{1}H NMR analysis to determine the *trans*-isomer PSS. Light intensity measurements were performed using a ThorLabs power and energy meter console (PM200) with sensor (S170C).

Thermal stability. To determine the thermal stability of the *cis*-isomer, the samples were irradiated with the 365 nm light source same as above, then maintained at 37 ºC for one week, with ^{1}H NMR characterization taken at various time points. The percentage *cis*-isomer remaining was calculated and plotted in GraphPad Prism 7 and fitted with a one-phase decay equation to determine the $t_{1/2}$.

Photocycling. To characterize the robustness of photocycling for **14**, a 4 mM solution was prepared in 20% D_2O in d_6-DMSO and irradiated with 365 nm light for one hour, then characterized by ^{1}H NMR as described above. The sample was then irradiated with 530 nm light for an hour, followed by ^{1}H NMR analysis. This process was repeated for eight total photoswitching steps (or four cycles). The ^{1}H NMR signals (ppm) used for the NMR determination of photoswitching and thermal stability are listed in Table S2.

3. Biological evaluation of arylazopyrazole rapamycin analogs.

Rapamycin (and photoswitchable analogs) stocks were generated at 5 mM in sterile-filtered DMSO. Working solutions of 1 mM were prepared in sterile-filtered DMSO and stored at –20 ºC. Live Cell Imaging Solution (LCIS) was purchased from Molecular Probes/Invitrogen. Chemically competent bacterial cells (Top10) were prepared in-house. HEK293T cells were obtained from ATCC and monitored every three months to ensure the absence of mycoplasma contamination. The 530 nm LED was obtained from Mouser Electronics (LUMILEDS LXML-PM01-0100).

Cloning of DNA constructs. All cloning was performed using Top10 cells (Invitrogen). DreamTaq Green DNA polymerase (Thermo) was used for PCR amplification, while Phusion DNA polymerase (Thermo) was used for site directed mutagenesis.

Site directed mutagenesis was performed using CLuc-FKBP as the template to generate CLuc-FKBP-Q53F (Primers P1 and P2) and CLuc-FKBP-Q53W (Primers P3 and P4) mutations in FKBP. Both mutations were validated by Sanger sequencing at Genewiz using their BGHR sequencing primer.

To generate CLuc-iFKBP, a Gibson assembly strategy was utilized. CLuc was PCR amplified from CLuc-FKBP using primers P5 and P6, while the iFKBP sequence (a truncated form of FKBP, residues 22 – 108) was PCR amplified from CLuc-FKBP using primers P7 and P8. CLuc-FKBP was linearized with BamHI and NotI to generate the backbone. The backbone, CLuc insert, and iFKBP insert were Gibson assembled, and verified by Sanger sequencing at Genewiz using their BGHR sequencing primer.

For maps of all plasmids used, see **Figure S2**.

Split luciferase reporter. HEK293T cells were plated at 200,000 cells/well in a 6-well clear bottom plate (Greiner) and grown at 37 ºC/5% CO_2. At ~80% confluence, cells were co-transfected with CLuc-FKBP (wild-type, Q53F, Q53W or iFKBP) and FRB-NLuc (666 ng FKBP and 1333 ng FRB, 2000 ng total per well) using Lipofectamine 2000 following the manufacturer's protocol. The media on the cells was replaced with 2 mL of DMEM supplemented with 10% FBS (minus antibiotics), then the transfection mix (all 500 µL) was added to the cells and incubated overnight. Two wells were transfected identically in order to obtain enough cells for a single assay. After ~18 hours of transfection, the media was removed, the cells were lifted with 0.5 mL of TrypLe and transferred to a 15 mL conical tube, then TrypLe was inactivated by the addition of 9.5 mL of media. Cells were pelleted at 1,000 *g* for 10 minutes at room temperature. The media was removed and the cells were resuspended in 1 mL of LCIS. Cells were counted using a hemocytometer in the presence of Trypan Blue to avoid counting dead cells (combination of two wells typically yielded 1.5 – 1.8 million cells). Subsequently, cells were plated in a white, clear bottom 96-well plate at 10,000 cells per well in 90 µL of LCIS.

To prepare the compound solutions, 1 mM stocks of the five analogs were diluted to 0.1 mM in milliQ water (10 µL of stock into 90 µL). Two identical 0.1 mM solutions were prepared and

transferred to a clear, glass ½ dram vial. One vial was irradiated using a UV transilluminator set at 365 nm for 10 minutes, while the other vial was irradiated with the 530 nm LED (output set to 700 mA) for two minutes. In order to obtain final concentrations of 100 and 25 nM once added to cells, a 10X solution (1 μM and 0.25 μM) was prepared for each of the five analogs for both the *cis*- (365 nm) and *trans*-isomers (530 nm). To each well of the 96-well plate containing cells expressing the reporter, 10 μL of compound (either rapamycin, AAP-analog, or DMSO) was added in triplicate and placed in the incubator for two and a half hours. Afterwards, 90 μL of BrightGlo reagent was added to each well and incubated for two minutes, then luminescence was measured on a Tecan M1000. Raw luminescence values were normalized such that DMSO equaled 1. Average fold-change values with error bars representing standard deviation of triplicates are reported.

For experiments with photoswitching in cells, HEK293T cells were transfected the same as above. Cells were counted and plated in a white, 96-well plate just as above. For the turn-on experiment: analog **14** was prepared at 250 nM (maintained in the dark), then 10 μL of this solution was added to the cells in 90 μL LCIS (final concentration of 25 nM). Cells were incubated with compound for one hour in the dark, then a subset of wells were irradiated with 365 nm (UV transilluminator) for either 30, 60, or 120 seconds. After an additional 1.5-hour incubation, luciferase substrates were added and luminescence was measured. For the turn-off experiment: analog **14** was pre-irradiated at 100 μM for 10 minutes with 365 nm light, then diluted to 250 nM. To the cells in 90 μL of LCIS, 10 μL of compound was added and incubated for an hour. Then, a subset of wells was irradiated with a 530 nm LED using a foil mask to prevent undesired irradiation of neighboring wells. After another 90-minute incubation, luciferase substrates were added and luminescence was measured. Normalization to DMSO was performed as described above.

4. Supplemental Tables and Figures:

Table S1. List of primers used to generate the new plasmid constructs.

Primer	Sequence (5' → 3')
P1	aagtttatgctaggcaag**TTC**gaggtgatccgaggctgg
P2	ccagcctcggatcacctc**GAA**cttgcctagcataaactt
P3	aagtttatgctaggcaag**TGG**gaggtgatccgaggctgg
P4	ccagcctcggatcacctc**CCA**cttgcctagcataaactt
P5	ttggtaccgagctcggatccactagtccagtgtggtggaa
P6	gtgtagtgcaccacgcaggtgccccgggacgcgtacgaga
P7	tctcgtacgcgtcccggggcacctgcgtggtgcactacac
P8	ctctagactcgagcggccgccactgtgctggatatctgca

Table S2. ^{1}H NMR signals (ppm) used for the NMR-determination of photoswitching and thermal stability.

compound	*cis* isomer	*trans* isomer
14	6.95-7.05	7.40-7.50
15	6.90-7.00	7.50-7.60
16	6.90-7.00	7.40-7.50
17	7.00-7.10	7.65-7.75
18	7.20-7.30	8.05-8.15

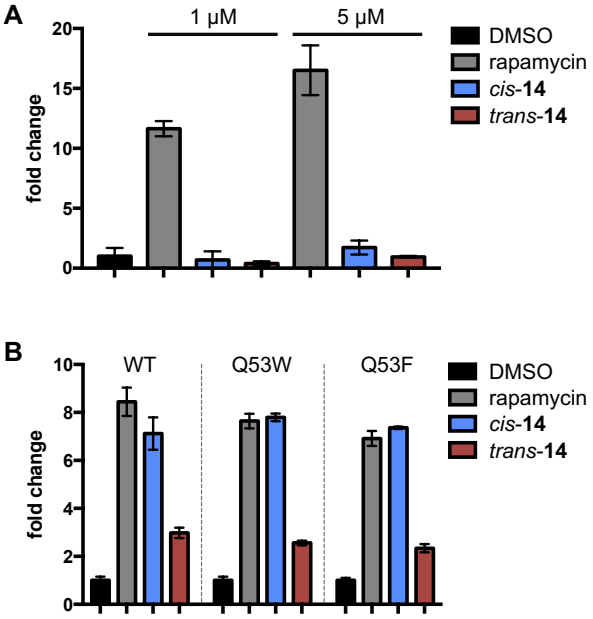

Figure S1. Split luciferase variants were used for testing **14**. a) HEK293T cells expressing the split luciferase reporter (with CLuc-iFKBP and FRB-NLuc) were treated with pre-formed *cis*- or *trans*-isomers of **14** at 1 and 5 µM for 2.5 hours, then luciferase substrates were added and luminescence was measured. b) Either CLuc-FKBP, CLuc-FKBP-Q53F, or CLuc-FKBP-Q53W were co-expressed with FRB-NLuc, and treated with 25 nM of pre-formed *cis*- and *trans*-**14**. Black bars represent the DMSO control and grey bars represent rapamycin. Raw values were normalized to DMSO and error bars represent standard deviation of three replicates.

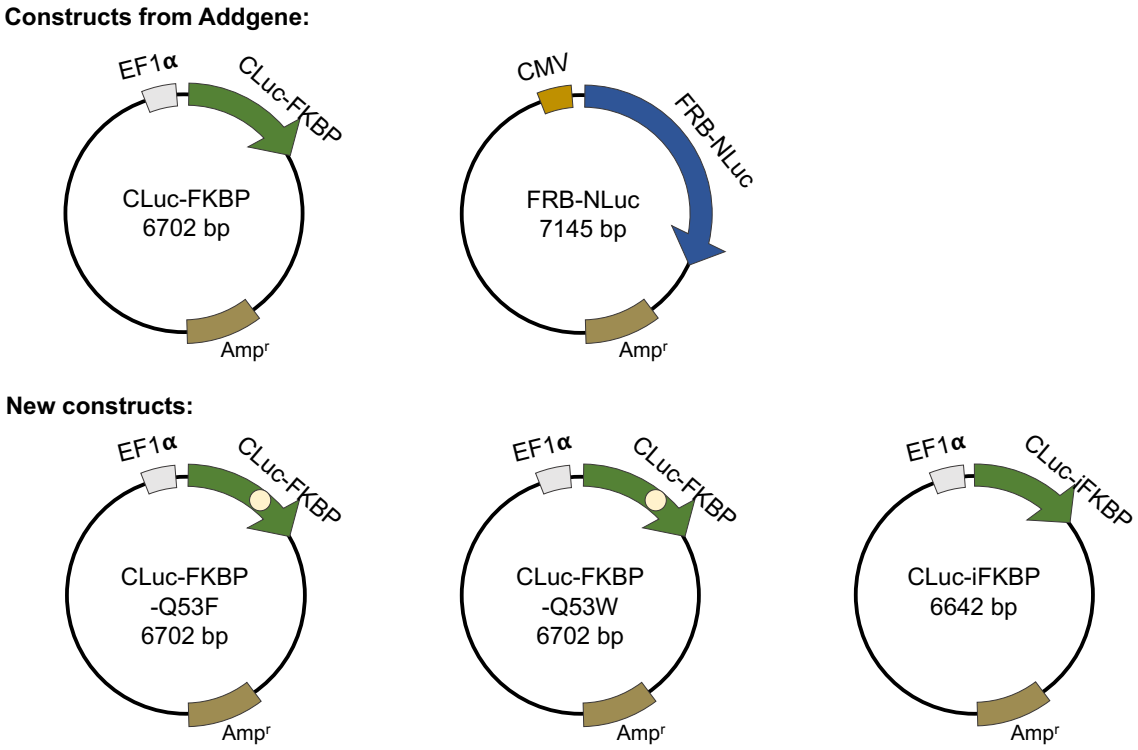

Figure S2. Plasmid maps of all constructs used in this study. CLuc-FKBP and FRB-NLuc were purchased from Addgene (#31184 and #31181). The remaining plasmids were assembled as described in the methods section.

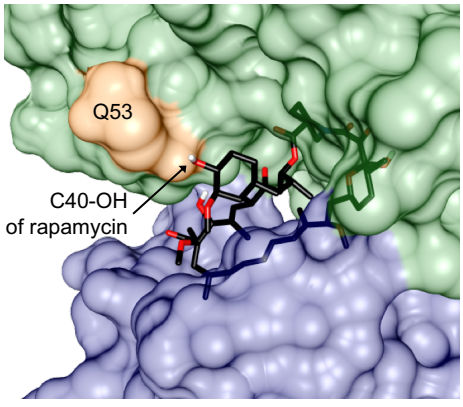

Figure S3. Residue Q53 (indicated in orange) of FKBP was mutated to phenylalanine and tryptophan since it is proximal to the C40 position of rapamycin where the AAP moiety was attached (PDB: 1FAP). FKBP is indicated in green and FRB is in blue.