Supporting Information

Discovery and Optimization of a Novel Series of Competitive and CNS Penetrant Protease Activated Receptor 4 (PAR4) Inhibitors

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Experimental Methods

General Synthetic Methods and Instrumentation

All commercially available reagents and reaction solvents were used as received, unless otherwise noted. Reactions were conducted at room temperature (rt, approximately 23 °C) unless otherwise noted. All 1H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 (400 MHz) or Bruker AV-NMR (600 MHz) instrument. Chemical shifts are reported in ppm relative to residual solvent peaks as an internal standard set to δH 7.26 or δC 77.0 ($CDCl_3$) and δH 3.31 or δC 49.0 (CD_3OD). Data are reported as follows: chemical shift, multiplicity (s = singlet, d =doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, and coupling constant (Hz). IR spectra were recorded as thin films and are reported in wavenumbers (cm^{-1}). Low resolution mass spectra were obtained on an Agilent 1200 LCMS with electrospray ionization. High resolution mass spectra were recorded on a Waters Qtof-API-US plus Acquity system. The value Δ is the error in the measurement (in ppm) given by the equation Δ = [(ME − MT)/MT] × 106, where ME is the experimental mass and MT is the theoretical mass. The HRMS results were obtained with ES as the ion source and leucine enkephalin as the reference. Optical rotations were measured on a PerkinElmer-341 polarimeter. Analytical thin layer chromatography was performed on 250 μM silica gel 60 F254 plates. Visualization was accomplished with UV light, and/or the use of ninhydrin, anisaldehyde and ceric ammonium molybdate solutions followed by charring on a hot-plate. Chromatography on silica gel was performed using Silica Gel 60 (230−400 mesh) from Sorbent Technologies. Analytical HPLC was performed on an Agilent 1200 analytical LCMS with UV detection at 214 and 254 nm along with ELSD detection. Solvents for extraction, washing, and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. and were used without purification. All polymer-supported reagents were purchased from Biotage, Inc. Flame-dried (under vacuum) glassware was used for all reactions. All reagents and solvents were commercial grade and

purified prior to use when necessary. High-resolution mass spectrometry (HRMS) data were obtained using a Micromass Q-Tof API-US mass spectrometer.

General Procedure 1: Synthesis of Amides

A solution of HATU (1.1 eq), *N,N*-Diisopropylethylamine (3.0 eq), 3-Bromo-1-Methylpyrazole-5-carboxylic acid (1.0 eq) in DMF (0.25M) was stirred at room temperature for 20 min to which the appropriate amine (1.1 eq) was then added. The reaction mixture was stirred at room temperature for 1 h. Upon completion, the reaction mixture was diluted in H_2O and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to provide the desired product.

General Procedure 2: Synthesis of Phenols

A degassed solution of tetrakis(triphenylphosphine)palladium(0) (5 mol%), cesium carbonate (1.5 eq), the appropriate (5-bromo-2-methyl-pyrazol-3-yl)-[2-phenylmorpholin-4-yl]methanone (1.0 eq), and 5-hydroxy-2-methoxyphenylboronic acid (1.1 eq) in 1,4-Dioxane: H_2O (2:1)(0.2M) was heated to 100°C and let stir for 16 h. The reaction mixture was then cooled, diluted with H_2O, and extracted with DCM (3x). The combined organics were passed through a phase separator, concentrated, and purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to provide the desired product.

General Procedure 3: Synthesis of benzyl ethers

A solution of the appropriate [5-(5-hydroxy-2-methoxy-phenyl)-2-methyl-pyrazol-3-yl]-(2-phenylmorpholin-4-yl)methanone (1.0 eq), Di-tert-butyl azodicarboxylate (1.5 eq), triphenylphosphine (1.3 eq), and the appropriate benzyl alcohol (1.1 eq) in THF (0.2M) was allowed to stir at room

temperature for 16 h. The reaction mixture was concentrated, re-dissolved in MeOH, loaded onto a pre-wetted SCX cartridge, washed with 6 column volumes of MeOH, eluted with 1 column volume 7N NH_3/MeOH, and concentrated to remove triphenylphosphine oxide. The crude material was then purified using a Gilson HPLC system (30 x 50 mm column; H_2O with 0.1% TFA:acetonitrile). Fractions containing the desired product were quenched with saturated $NaHCO_3$, extracted with DCM, and concentrated to liberate the product as the free base.

General Procedure 4: Synthesis of benzyl ethers

A solution of [5-(5-hydroxy-2-methoxy-phenyl)-2-methyl-pyrazol-3-yl]-[(*2S*)-2-phenylmorpholin-4-yl]methanone (1.0 eq), potassium carbonate (1.5 eq), and the appropriate benzyl bromide (1.1 eq) in DMF (0.15M) was heated to 60 °C and stirred for 3 h. The reaction mixture was passed through a syringe filter and the crude material was purified using a Gilson HPLC (30 x 50 mm column, H_2O with 0.1% TFA:acetonitrile). Fractions containing the desired product were quenched with saturated $NaHCO_3$, extracted with DCM, and concentrated to liberate the product as the free base.

(5-bromo-2-methyl-pyrazol-3-yl)-[(2S)-2-phenylmorpholin-4-yl]methanone (12)

This compound was synthesized according to general procedure 1. White solid (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.32 (m, 5H), 6.32 (s, 1H), 4.68-4.48 (m, 2H), 4.16-3.87 (m, 5H), 3.71 (bs, 1H), 3.43-2.89 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 159.7, 138.5, 136.7, 128.8, 126.2, 124.3, 109.2, 78.2, 66.9, 53.7, 48.4, 47.4, 42.2, 38.7. HRMS (TOF, ES+) calc'd for $C_{15}H_{16}BrN_3O_2$ 349.0426; found, 349.0431.

(3-(2,5-dimethoxyphenyl)-1-methyl-1H-pyrazol-5-yl)(2-phenylmorpholino)methanone (9)

This compound was synthesized according to general procedure 2. White solid (88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 3.1 Hz, 1H), 7.35 (d, *J* = 19.3 Hz, 7H), 6.90 (d, *J* = 6.5 Hz, 2H), 6.85

(dd, J = 8.9, 3.1 Hz, 1H), 4.56 – 4.49 (m, 1H), 4.06 (s, 3H), 3.82 (d, J = 7.2 Hz, 6H), 3.21 (t, J = 102.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.23, 153.99, 151.17, 146.61, 128.72, 128.49, 126.12, 122.30, 115.07, 113.14, 113.13, 108.46, 78.30, 77.48, 77.16, 76.84, 67.00, 56.36, 55.97, 38.48. LC-MS [m/z + H] = 332.0.

[5-(5-hydroxy-2-methoxy-phenyl)-2-methyl-pyrazol-3-yl]-[(2S)-2-phenylmorpholin-4-yl]methanone (15)

This compound was synthesized according to general procedure 2. White solid (88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69-7.52 (m, 1H), 7.49-7.29 (m, 5H), 6.89-6.77 (m, 3H), 4.82-4.42 (m, 2H), 4.36-3.93 (bs, 5H), 3.91-3.62 (bs, 4H), 3.58-2.85 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2, 151.0, 150.0, 146.5, 132.3, 132.2, 132.1, 132.0, 128.7, 128.6, 128.5, 126.1, 122.1, 115.8, 115.1, 113.3, 108.4, 78.2, 77.4, 67.0, 56.3, 38.4. HRMS (TOF, ES+) calc'd for $C_{22}H_{23}N_3O_4$, 393.1689; found, 393.1700.

2-[[4-methoxy-3-[1-methyl-5-[(*2S*)-2-phenylmorpholine-4-carbonyl]pyrazol-3-yl]phenoxy]methyl]benzonitrile ((*S*)-17i)

This compound was synthesized according to general procedure 4. Orange Oil (60% yield). $[\alpha]^{25}{}_D$ = -46.1 (c = 1.95, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73-7.68 (m, 3H), 7.62 (td, J = 7.6 Hz, 1.1 Hz, 1H), 7.44-7.29 (m, 6H), 6.97-6.90 (m, 3H), 5.28 (s, 2H), 4.88-4.43 (m, 2H), 4.07 (bs, 5H), 3.83 (bs, 4H), 3.60-2.86 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2, 152.5, 151.8, 146.4, 141.1, 138.6, 135.1, 133.1, 132.9, 128.7, 128.6, 128.5, 128.4, 126.1, 122.5, 117.3, 115.6, 114.9, 112.9, 111.2, 108.5, 78.3, 68.4, 67.0, 56.3, 54.0, 48.4, 42.1, 38.5. HRMS (TOF, ES+) calc'd for $C_{30}H_{28}N_4O_4$, 508.2111; found, 508.2116.

2-[[4-methoxy-3-[1-methyl-5-[(*2R*)-2-phenylmorpholine-4-carbonyl]pyrazol-3-yl]phenoxy]methyl]benzonitrile ((*R*)-17i)

This compound was synthesized according to general procedure 4. Orange Oil (80% yield). $[\alpha]^{25}{}_{D}$ =+54.5 (c = 2.57, CHCl3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73-7.68 (m, 3H), 7.62 (td, *J* = 7.6 Hz, 1.1 Hz, 1H), 7.44-7.29 (m, 6H), 6.97-6.90 (m, 3H), 5.28 (s, 2H), 4.88-4.43 (m, 2H), 4.07 (bs, 5H), 3.83 (bs, 4H), 3.60-2.86 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2, 152.5, 151.8, 146.4, 141.1, 138.6, 135.1, 133.1, 132.9, 128.7, 128.6, 128.5, 128.4, 126.1, 122.5, 117.3, 115.6, 114.9, 112.9, 111.2, 108.5, 78.3, 68.4, 67.0, 56.3, 54.0, 48.4, 42.1, 38.5. HRMS (TOF, ES+) calc'd for $C_{30}H_{28}N_4O_4$, 508.2111; found, 508.2116.

Synthesis of [5-[2-methoxy-5-[[2-(trifluoromethoxy)phenyl]methoxy]phenyl]-2-methyl-pyrazol-3-yl]-[(*2S*)-2-phenylmorpholin-4-yl]methanone ((*S*)-17l)

This compound was synthesized according to general procedure 4. Orange oil (62% yield). $[\alpha]^{25}{}_{D}$ = -47.6 (*c* = 2.23, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, *J* = 7.5, 1.52 Hz, 2H) 7.51-7.26 (m, 8H), 6.91 (d, *J* = 2.5 Hz, 3H), 5.17 (s, 2H), 4.85-4.42 (m, 2H), 4.07 (bs, 5H), 3.83 (bs, 4H), 3.6-2.85 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2, 152.8, 151.6, 146.8, 146.5, 135.1, 130.4, 130.3, 130.2, 129.6, 129.2, 128.7, 128.5, 127.1, 127.0, 126.1, 122.5, 120.7 (q, J_{CF} = 256 Hz), 120.6, 120.5, 115.5, 114.9, 112.9, 108.5, 78.2, 67.0, 65.1, 64.4, 56.3, 38.5. HRMS (TOF, ES+) calc'd for $C_{30}H_{28}F_3N_3O_5$, 567.1981; found, 567.1989.

Synthesis of [5-[2-methoxy-5-[[2-(trifluoromethoxy)phenyl]methoxy]phenyl]-2-methyl-pyrazol-3-yl]-[(*2R*)-2-phenylmorpholin-4-yl]methanone ((*R*)-17l)

This compound was synthesized according to general procedure 4. Orange oil (62% yield). $[\alpha]^{25}{}_{D}$ = +46.7 (*c* = 2.24, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, *J* = 7.5, 1.5 Hz, 2H) 7.51-7.26 (m, 8H), 6.91 (d, *J* = 2.5 Hz, 3H), 5.17 (s, 2H), 4.85-4.42 (m, 2H), 4.07 (bs, 5H), 3.83 (bs, 4H), 3.6-2.85 (m,

2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2, 152.8, 151.6, 146.8, 146.5, 135.1, 130.4, 130.3, 130.2, 129.6, 129.2, 128.7, 128.5, 127.1, 127.0, 126.1, 122.5, 120.7 (q, J_{CF} = 256 Hz), 120.6, 120.5, 115.5, 114.9, 112.9, 108.5, 78.2, 67.0, 65.1, 64.4, 56.3, 38.5. HRMS (TOF, ES+) calc'd for $C_{30}H_{28}F_3N_3O_5$, 567.1981; found, 567.1989.

4-[[4-methoxy-3-[1-methyl-5-[(*2S*)-2-phenylmorpholine-4-carbonyl]pyrazol-3-yl]phenoxy]methyl]pyridine-3-carbonitrile ((*S*)-19a)

This compound was synthesized according to general procedure 4. Clear oil (4-25% yield). $[\alpha]^{23}{}_D$ = -32.5 (*c* = 0.82, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.81 (d, *J* = 5.2 Hz, 1H), 7.72 (d, *J* = 5.1 Hz, 1H), 7.66 (s, 1H), 7.47-7.29 (bs, 5H), 6.92 (d, *J* = 5.1 Hz, 3H), 5.29 (s, 2H), 4.82-4.42 (m, 2H), 4.06 (bs, 5H), 3.83 (bs, 4H), 3.58-2.87 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.9, 161.2, 153.4, 152.9, 152.1, 151.9, 150.3, 146.2, 128.8, 128.5, 126.2, 122.8, 121.9, 115.5, 115.1, 114.8, 113.0, 108.5, 108.0, 78.3, 67.1, 67.0, 56.3, 55.7, 38.5, 32.0, 29.8, 25.0, 17.2. HRMS (TOF, ES+) calc'd for $C_{29}H_{27}N_5O_4$, 509.2063; found, 509.2068.

4-[[4-methoxy-3-[1-methyl-5-[(*2R*)-2-phenylmorpholine-4-carbonyl]pyrazol-3-yl]phenoxy]methyl]pyridine-3-carbonitrile ((*R*)-19a)

This compound was synthesized according to general procedure 4. Clear oil (6-28% yield). $[\alpha]^{23}{}_D$ = +29.4 (*c* = 0.92, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.81 (d, *J* = 5.2 Hz, 1H), 7.72 (d, *J* = 5.1 Hz, 1H), 7.66 (s, 1H), 7.47-7.29 (bs, 5H), 6.92 (d, *J* = 5.1 Hz, 3H), 5.29 (s, 2H), 4.82-4.42 (m, 2H), 4.06 (bs, 5H), 3.83 (bs, 4H), 3.58-2.87 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.9, 161.2, 153.4, 152.9, 152.1, 151.9, 150.3, 146.2, 128.8, 128.5, 126.2, 122.8, 121.9, 115.5, 115.1, 114.8, 113.0, 108.5, 108.0, 78.3, 67.1, 67.0, 56.3, 55.7, 38.5, 32.0, 29.8, 25.0, 17.2. HRMS (TOF, ES+) calc'd for $C_{29}H_{27}N_5O_4$, 509.2063; found, 509.2068.

[5-[2-methoxy-5-[(3-methyl-4-pyridyl)methoxy]phenyl]-2-methyl-pyrazol-3-yl]-((*2S*)-2-phenylmorpholin-4-yl)methanone ((*S*)-19b)

This compound was synthesized according to general procedure 3. Clear oil (47% yield). $[\alpha]^{25}{}_{D}$ = -59.4 (*c* = 1.18, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 11.5 Hz, 2H), 7.64 (s, 1H), 7.47 (d, *J* = 4.8 Hz, 1H), 7.44-7.29 (bs, 5H), 6.92 (s, 3H), 5.07 (s, 2H), 4.84-4.42 (m, 2H), 4.07 (bs, 5H), 3.84 (bs, 4H), 3.60-2.85 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2, 152.7, 151.7, 150.8, 148.0, 146.4, 144.4, 138.7, 135.2, 130.8, 128.8, 128.5, 126.1, 122.5, 121.7, 115.7, 114.4, 113.0, 108.5, 78.3, 67.5, 67.0, 56.3, 54.0, 48.4, 47.6, 42.1, 38.5, 15.8. HRMS (TOF, ES+) calc'd for $C_{29}H_{30}N_4O_4$, 498.2267; found, 498.2277.

Synthesis of [5-[2-methoxy-5-[(3-methyl-4-pyridyl)methoxy]phenyl]-2-methyl-pyrazol-3-yl]-((*2R*)-2-phenylmorpholin-4-yl)methanone ((*R*)-19b)

This compound was synthesized according to general procedure 3. Clear oil (32% yield). $[\alpha]^{24}{}_{D}$ = +51.2 (*c* = 2.07, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 11.5 Hz, 2H), 7.64 (s, 1H), 7.47 (d, *J* = 4.8 Hz, 1H), 7.44-7.29 (bs, 5H), 6.92 (s, 3H), 5.07 (s, 2H), 4.84-4.42 (m, 2H), 4.07 (bs, 5H), 3.84 (bs, 4H), 3.60-2.85 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2, 152.7, 151.7, 150.8, 148.0, 146.4, 144.4, 138.7, 135.2, 130.8, 128.8, 128.5, 126.1, 122.5, 121.7, 115.7, 114.4, 113.0, 108.5, 78.3, 67.5, 67.0, 56.3, 38.5, 15.8. HRMS (TOF, ES+) calc'd for $C_{29}H_{30}N_4O_4$, 498.2267; found, 498.2277.

Synthesis of [5-[5-[(3-chloro-4-pyridyl)methoxy]-2-methoxy-phenyl]-2-methyl-pyrazol-3-yl]-[(*2S*)-2-phenylmorpholin-4-yl]methanone ((*S*)-19d)

This compound was synthesized according to general procedure 3. Orange oil (78% yield). $[\alpha]^{24}{}_{D}$ = -37.8 (*c* = 2.58, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.50 (d, *J* = 4.9 Hz, 1H), 7.65 (s, 1H), 7.59 (d, *J* = 4.9 Hz, 1H), 7.49-7.28 (bs, 5H), 6.91 (s, 3H), 5.17 (s, 2H), 4.84-4.42 (m, 2H), 4.07 (bs, 5H), 3.83 (bs, 4H), 3.60-2.82 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2, 154.3, 152.2, 151.9, 149.1, 148.2, 146.3, 144.4, 132.3, 132.2, 132.0, 129.7, 128.7, 128.5, 126.1, 122.7, 122.4, 115.3, 114.8,

112.9, 108.5, 78.3, 67.0, 66.5, 56.3, 38.5, 28.3, 28.0. HRMS (TOF, ES+) calc'd for $C_{28}H_{27}ClN_4O_4$, 518.1733; found, 518.1721.

Synthesis of 5-[5-[(3-chloro-4-pyridyl)methoxy]-2-methoxy-phenyl]-2-methyl-pyrazol-3-yl]-[(*2R*)-2-phenylmorpholin-4-yl]methanone ((*R*)-19d)

This compound was synthesized according to general procedure 3. Orange oil (76% yield). $[\alpha]^{24}{}_{D}$ = +39.6 (*c* = 2.51, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.50 (d, *J* = 4.9 Hz, 1H), 7.65 (s, 1H), 7.59 (d, *J* = 4.9 Hz, 1H), 7.49-7.28 (bs, 5H), 6.91 (s, 3H), 5.17 (s, 2H), 4.84-4.42 (m, 2H), 4.07 (bs, 5H), 3.83 (bs, 4H), 3.60-2.82 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2, 154.3, 152.2, 151.9, 149.1, 148.2, 146.3, 144.4, 132.3, 132.2, 132.0, 129.7, 128.7, 128.5, 126.1, 122.7, 122.4, 115.3, 114.8, 112.9, 108.5, 78.3, 67.0, 66.5, 56.3, 38.5, 28.3, 28.0. HRMS (TOF, ES+) calc'd for $C_{28}H_{27}ClN_4O_4$, 518.1733; found, 518.1721.

3-bromo-6-chloro-[1,2,4]triazolo[4,3-*b*]pyridazine

A solution of 6-chloro-[1,2,4]triazolo[4,3-B]pyridazine (1.0 eq), *N*-Bromosuccinimide (1.1 eq) in DMF (0.25M) was heated to reflux and was stirred for 16 h. The reaction mixture was then cooled to room temperature. While stirring, an aqueous solution of saturated sodium bicarbonate was added drop wise. The layers were separated and the aqueous layer was extracted with EtOAc (3x). The combined organics were dried over $MgSO_4$, filtered, concentrated and purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to provide the desired product as a white solid (62-72% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 9.6 Hz, 1H), 7.20 (d, *J* = 9.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 150.8, 144.6, 126.5, 125.0, 123.3. HRMS (TOF, ES+) calc'd for $C_5H_2BrClN_4$, 231.9151; found, 231.9151.

(*2S*)-4-(3-bromo-[1,2,4]triazolo[4,3-*b*]pyridazin-6-yl)-2-phenyl-morpholine

A solution of potassium carbonate (1.5 eq), *2S*-phenylmorpholine (1.1 eq), and 3-bromo-6-chloro-[1,2,4]triazolo[4,3-b]pyridazine (1.0 eq) in DMF (0.1M) was heated to 60 °C and stirred for 4 h. The reaction mixture was then diluted in H_2O, extracted with $CHCl_3$/IPA (3:1), passed through a phase separator, and concentrated. The crude material was purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to provide the titled compound as a white solid (72% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 10.1 Hz, 1H), 7.47-7.33 (m, 5H), 6.99 (d, *J* = 10.1 Hz, 1H), 4.65 (dd, *J* = 10.6, 2.5 Hz, 1H), 4.26-4.14 (m, 2H), 4.09 (d, *J* = 13.5 Hz, 1H), 3.92 (td, *J* = 11.8, 2.7 Hz, 1H), 3.35 (dt, *J* = 12.6, 3.5 Hz, 1H), 3.11 (dd, *J* = 13.1, 10.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 155.4, 144.3, 138.9, 128.8, 128.6, 126.4, 125.1, 125.0, 124.2, 114.0, 113.8, 77.8, 66.4, 52.3, 45.7. HRMS (TOF, ES+) calc'd for $C_{15}H_{14}BrN_5O$, 359.0382; found, 259.0385.

(*2R*)-4-(3-bromo-[1,2,4]triazolo[4,3-*b*]pyridazin-6-yl)-2-phenyl-morpholine

A solution of potassium carbonate (1.5 eq), *2R*-phenylmorpholine (1.1 eq), and 3-bromo-6-chloro-[1,2,4]triazolo[4,3-b]pyridazine (1.0 eq) in DMF (0.1M) was heated to 60 °C and stirred for 4 h. The reaction mixture was diluted in H_2O, extracted with $CHCl_3$/IPA (3:1), passed through a phase separator, and concentrated. The crude material was purified via flash chromatography (Teledyne ISCO system, silica gel column, hexanes:EtOAc) to provide the desired product as a white solid (68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 10.1 Hz, 1H), 7.47-7.33 (m, 5H), 6.99 (d, *J* = 10.1 Hz, 1H), 4.65 (dd, *J* = 10.6, 2.5 Hz, 1H), 4.26-4.14 (m, 2H), 4.09 (d, *J* = 13.5 Hz, 1H), 3.92 (td, *J* = 11.8, 2.7 Hz, 1H), 3.35 (dt, *J* = 12.6, 3.5 Hz, 1H), 3.11 (dd, *J* = 13.1, 10.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 155.4, 144.3, 138.9, 128.8, 128.6, 126.4, 125.1, 125.0, 124.2, 114.0, 113.8, 77.8, 66.4, 52.3, 45.7. HRMS (TOF, ES+) calc'd for $C_{15}H_{14}BrN_5O$, 359.0382; found, 259.0385.

4-methoxy-3-[6-[(*2S*)-2-phenylmorpholin-4-yl]-[1,2,4]triazolo[4,3-b]pyridazin-3-yl]phenol

A degassed solution of tetrakis(triphenylphosphine)palladium(0) (7 mol%), cesium carbonate (1.5 eq), 5-hydroxy-2-methoxyphenylboronic acid (1.1 eq), and (*2S*)-4-(3-bromo-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-2-phenyl-morpholine (1.0 eq) in 1,4-Dioxane/H_2O (2:1, 0.1M) was heated to 100°C and let stir for 5 h. The reaction mixture was diluted with H_2O and extracted with DCM (3x). The combined organics were then passed through a phase separator and concentrated. The crude material was purified via flash chromatography (Teledyne ISCO system, silica gel column, DCM:MeOH) to provide the desired product as a white solid (54% yield). $[\alpha]^{24}{}_D$ = +27.7 (*c* = 1.01, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 10.0 Hz, 1H), 7.63 (d, *J* = 2.8 Hz, 1H), 7.38-7.27 (m, 6H), 7.02 (dd, *J* = 8.9, 2.8 Hz, 1H), 6.94 (d, *J* = 10.1 Hz, 1H), 6.84 (d, *J* = 8.9 Hz, 1H), 4.55 (dd, *J* = 10.5, 1.9 Hz, 1H), 4.12-3.99 (m, 2H), 3.92 (d, *J* = 13.1 Hz, 1H), 3.78 (td, *J* = 11.7, 2.0 Hz, 1H), 3.67 (s, 3H), 3.18 (td, *J* = 12.5, 3.3 Hz, 1H), 2.94 (dd, *J* = 12.8, 10.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 154.7, 151.5, 151.1, 147.8, 142.9, 139.1, 128.7, 128.4, 126.2, 124.4, 119.6, 118.9, 115.4, 114.0, 112.7, 77.3, 66.4, 56.2, 52.7, 45.9. HRMS (TOF, ES+) calc'd for $C_{22}H_{21}N_5O_3$, 403.1644; found, 403.1648.

4-methoxy-3-[6-[(*2R*)-2-phenylmorpholin-4-yl]-[1,2,4]triazolo[4,3-b]pyridazin-3-yl]phenol

A degassed solution of tetrakis(triphenylphosphine)palladium(0) (7 mol%), cesium carbonate (1.5 eq), 5-hydroxy-2-methoxyphenylboronic acid (1.1 eq), and (*2R*)-4-(3-bromo-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-2-phenyl-morpholine (1.0 eq) in 1,4-Dioxane/H_2O (2:1, 0.1M) was heated to 100°C and let stir for 5 h. The reaction mixture was diluted with H_2O and extracted with DCM (3x). The combined organics were then passed through a phase separator and concentrated. The crude material was purified via flash chromatography (Teledyne ISCO system, silica gel column, DCM:MeOH) to provide the desired product as a white solid (53% yield). $[\alpha]^{24}{}_D$ = -26.3 (*c* = 0.565, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 10.0 Hz, 1H), 7.63 (d, *J* = 2.8 Hz, 1H), 7.38-7.27 (m, 6H), 7.02 (dd, *J* = 8.9, 2.8 Hz, 1H), 6.94 (d, *J* = 10.1 Hz, 1H), 6.84 (d, *J* = 8.9 Hz, 1H), 4.55 (dd, *J* = 10.5, 1.9 Hz, 1H), 4.12-3.99 (m, 2H), 3.92 (d, *J* = 13.1 Hz, 1H), 3.78 (td, *J* = 11.7, 2.0 Hz, 1H), 3.67 (s, 3H), 3.18 (td, *J* = 12.5, 3.3 Hz, 1H), 2.94 (dd, *J* =

12.8, 10.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 154.7, 151.5, 151.1, 147.8, 142.9, 139.1, 128.7, 128.4, 126.2, 124.4, 119.6, 118.9, 115.4, 114.0, 112.7, 77.3, 66.4, 56.2, 52.7, 45.9. HRMS (TOF, ES+) calc'd for $C_{22}H_{21}N_5O_3$, 403.1644; found, 403.1648.

2-[[4-methoxy-3-[6-[(*2S*)-2-phenylmorpholin-4-yl]-[1,2,4]triazolo[4,3-b**]pyridazin-3-yl]phenoxy]methyl]benzonitrile ((*S*)-21)**

A solution of potassium carbonate (1.5 eq), 2-cyanobenzyl bromide (1.1 eq), 4-methoxy-3-[6-[(*2S*)-2-phenylmorpholin-4-yl]-[1,2,4]triazolo[4,3-b]pyridazin-3-yl]phenol (1 eq), in DMF (0.2M) was heated to 65 °C and stirred for 16 h. The reaction mixture was passed through a syringe filter and crude material was then purified using a Gilson HPLC system (30 x 50 mm column; H_2O with 0.1% TFA:acetonitrile). Fractions containing the desired product were quenched with saturated $NaHCO_3$, extracted with DCM, and concentrated to liberate the product as the free base (40% yield). $[\alpha]^{24}{}_D$ = +19.7 (*c* = 3.6, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 10.4 Hz, 1H), 7.71-7.60 (m, 3H), 7.45 (dd, *J* = 7.3, 1.6 Hz, 1H), 7.41 (d, *J* = 3.1 Hz, 1H), 7.39-7.28 (m, 5H), 7.17 (dd, *J* = 9.0, 3.1 Hz, 1H), 6.99 (t, *J* = 10.1 Hz, 2H), 5.22 (s, 2H), 4.62 (dd, *J* = 10.6, 2.6 Hz, 1H), 4.16 (dd, *J* = 11.5, 2.2 Hz, 1H), 4.07 (dt, *J* = 14.0, 2.0 Hz, 1H), 3.96 (d, *J* = 13.3 Hz, 1H), 3.86 (td, *J* = 11.9, 2.7 Hz, 1H), 3.79 (s, 3H), 3.24 (td, *J* = 12.5, 3.6 Hz, 1H), 3.23 (dd, *J* = 10.6, 2.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 154.8, 153.2, 151.9, 146.8, 143.1, 140.7, 139.1, 133.2, 133.0, 128.7, 128.7, 128.6, 128.4, 126.2, 125.1, 118.4, 118.4, 117.2, 116.6, 113.3, 112.7, 111.4, 77.6, 77.4, 68.7, 66.5, 56.4, 52.7, 46.0. HRMS (TOF, ES+) calc'd for $C_{30}H_{26}N_6O_3$, 518.2066; found, 518.2076.

2-[[4-methoxy-3-[6-[(*2R*)-2-phenylmorpholin-4-yl]-[1,2,4]triazolo[4,3-b]pyridazin-3-yl]phenoxy]methyl]benzonitrile ((*R*)-21)

A solution of potassium carbonate (1.5 eq), 2-cyanobenzyl bromide (1.1 eq), 4-methoxy-3-[6-[(*2R*)-2-phenylmorpholin-4-yl]-[1,2,4]triazolo[4,3-b]pyridazin-3-yl]phenol (30.mg, 0.07000mmol) in DMF

(0.2M) was heated to 65 °C and stirred for 16 h. The reaction mixture was passed through a syringe filter and crude material was then purified using a Gilson HPLC system (30 x 50 mm column; H_2O with 0.1% TFA:acetonitrile). Fractions containing the desired product were quenched with saturated $NaHCO_3$, extracted with DCM, and concentrated to liberate the product as the free base (35% yield). $[\alpha]^{24}{}_D$ = -15.5 (*c* = 0.47, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 10.4 Hz, 1H), 7.71-7.60 (m, 3H), 7.45 (dd, *J* = 7.3, 1.6 Hz, 1H), 7.41 (d, *J* = 3.1 Hz, 1H), 7.39-7.28 (m, 5H), 7.17 (dd, *J* = 9.0, 3.1 Hz, 1H), 6.99 (t, *J* = 10.1 Hz, 2H), 5.22 (s, 2H), 4.62 (dd, *J* = 10.6, 2.6 Hz, 1H), 4.16 (dd, *J* = 11.5, 2.2 Hz, 1H), 4.07 (dt, *J* = 14.0, 2.0 Hz, 1H), 3.96 (d, *J* = 13.3 Hz, 1H), 3.86 (td, *J* = 11.9, 2.7 Hz, 1H), 3.79 (s, 3H), 3.24 (td, *J* = 12.5, 3.6 Hz, 1H), 3.23 (dd, *J* = 10.6, 2.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 154.8, 153.2, 151.9, 146.8, 143.1, 140.7, 139.1, 133.2, 133.0, 128.7, 128.7, 128.6, 128.4, 126.2, 125.1, 118.4, 118.4, 117.2, 116.6, 113.3, 112.7, 111.4, 77.6, 77.4, 68.7, 66.5, 56.4, 52.7, 46.0. HRMS (TOF, ES+) calc'd for $C_{30}H_{26}N_6O_3$, 518.2066; found, 518.2076.

Computational Methods

PAR4 structure preparation:

The PAR4 structure for modeling was generated using the RosettaCM comparative modeling protocol using experimentally-determined structures of PARs 1 and 2 (PDB ID: 3VW7 and 5NDD, respectively)[1]. Briefly, RosettaCM takes the user-defined aligned sequences from proteins with high sequence similarity to the PAR4 sequence, superimposes residues accordingly, then undergoes residue insertions using both PDB-derived fragments and provided templates, followed by Monte Carlo-based backbone and sidechain perturbations and optimized using energy-based minimization. Clustering of the final models was used to obtain an "ensemble-like" representation to be carried forward into docking studies.

Ligand preparation:

Ligands were all prepared using the Biology and Chemistry Library (BCL)[2] for conformer generation and parameter files were created using the molfile_to_params.py script within Rosetta.

Ligand docking:

We docked compounds into the protein models using the RosettaLigand protocol[3, 4]. This contains low-resolution sampling for initial pocket placement with coarse-grained sampling, high-resolution sampling of the ligand, protein sidechains and protein backbone based on Monte Carlo simulations with acceptance based on Metropolis criteria. This is followed by a final minimization to obtain a local minimum and report a final score for that protein-ligand conformation. All compounds were docked using the wider sampling strategy below (specifically the inclusion of the "Transform" mover, which is a low-resolution sampling step. Further inspection on specific binding mode of known compounds used an alignment of the query compound to the predicted pose, the low-resolution Transform sampling step was eliminated, and we performed only high-resolution sampling and final minimization.

Analysis of the output conformations from docking was performed by looking at the score variation across a number of poses from different starting structures, clustering of these outputs, and visual inspection using previous knowledge of known binders to PARs.

RosettaCM, ligand preparation and docking were performed using the scripts provided below.

PAR4 structure preparation

```
<path to Rosetta>/main/source/bin/rosetta_scripts.default.linuxgccrelease
@ flags.txt
```

flags.txt

```
# i/o
-in:file:fasta par4.fasta
-parser:protocol rosetta_cm.xml
-restore_talaris_behavior

#membrane
-in:file:spanfile par4.span
-membrane:no_interpolate_Mpair
-membrane:Menv_penalties
-rg_reweight 0.1

# relax options
-relax:minimize_bond_angles
-relax:minimize_bond_lengths
-relax:jump_move true
-default_max_cycles 200
-relax:min_type lbfgs_armijo_nonmonotone
-score:weights membrane_highres_Menv_smooth.wts
-use_bicubic_interpolation
-hybridize:stage1_probability 1.0
-sog_upper_bound 15

-linmem_ig 10
```

par4.fasta

rosetta_cm.xml

```
<ROSETTASCRIPTS>
    <TASKOPERATIONS>
    </TASKOPERATIONS>
    <SCOREFXNS>
        <ScoreFunction name="stage1" weights="stage1_membrane.wts"
symmetric="0">
            <Reweight scoretype="atom_pair_constraint" weight="1"/>
        </ScoreFunction>
        <ScoreFunction name="stage2" weights="stage2_membrane.wts"
symmetric="0">
            <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
        </ScoreFunction>
        <ScoreFunction name="fullatom" weights="stage3_rlx_membrane.wts"
symmetric="0">
            <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
        </ScoreFunction>
        <ScoreFunction name="membrane"
weights="membrane_highres_Menv_smooth" symmetric="0">
            <Reweight scoretype="cart_bonded" weight="0.5"/>
            <Reweight scoretype="pro_close" weight="0"/>
        </ScoreFunction>
    </SCOREFXNS>
    <FILTERS>
    </FILTERS>
    <MOVERS>
        <Hybridize name="hybridize" stage1_scorefxn="stage1"
stage2_scorefxn="stage2" fa_scorefxn="fullatom" batch="1"
stage1_increase_cycles="1.0" stage2_increase_cycles="1.0"
linmin_only="1" realign_domains="0" disulf_file="disulf.txt"
add_hetatm="1">
            <Template pdb="par4_threaded_5ndd.pdb" cst_file="NONE"
weight="1.000"/>
            <Template pdb="par4_threaded_3vw7.pdb" cst_file="NONE"
weight="1.000"/>
        </Hybridize>
        <ClearConstraintsMover name="clearconstraints"/>
    </MOVERS>
    <APPLY_TO_POSE>
    </APPLY_TO_POSE>
    <PROTOCOLS>
        <Add mover="hybridize"/>
        <Add mover="clearconstraints"/>
    </PROTOCOLS>
    <OUTPUT scorefxn="membrane"/>
</ROSETTASCRIPTS>
```

par4.span

```
TM region prediction for target.topo predicted using OCTOPUS
7 303
antiparallel
n2c
12 32 12 32
46 67 47 67
81 111 81 111
124 144 124 144
178 198 178 198
216 236 216 236
253 273 253 273
```

par4.disulfide

```
82 161
```

converted_alignment.aln

```
## par4_threaded_3vw7
#
scores_from_program: 0
0 ----DSSRALLLGWVPTRLVPALYGLVLVVGLPANGLALWVLATQA-
PRLPSTMLLMNLAAADLLLALALPPRIAYHLRGQRWPFGEAACRLATAALYGHMYGSVLLLAAVSLDRYLALV
HPLRARALRGRRLALGLCMAAWLMAAALALPLTLQRQTFRLARSDRVLCHDALPLDAQASHWQPAFTCLALLG
CFLPLLAMLLCYGATLHTLAASGR---RYGHALRLTAVVLASAVAFFVPSNLLLLLHYSDPSP-
SAWGNLYGAYVPSLALSTLNSCVDPFIYYYVSAEF--------
0 -----
DASGYLTSSWLTLFVPSVYTGVFVVSLPLNIMAIVVFILKMKVKKPAVVYMLHLATADVLFVSVLPFKISYYF
SGSDWQFGSELCRFVTAAFYCNMYASILLMTVISIDRFLAVVYPMRTLG-RAS----
FTCLAIWALAIAGVVPLLLKEQTIQVPGLGITTCHDVLSETLLEGYYAYYFSAFSAVFFFVPLIISTVCYVSI
IRCLSSSAANRSKKSRALFLSAAVFCIFIICFGPTNVLLIAHYSFLSHTSTTEAAYFAYLLCVCVSSISCCID
PLIYYYASSEC--------
--
## par4_threaded_5ndd
#
scores_from_program: 0
0 ----DSSRALLLGWVPTRLVPALYGLVLVVGLPANGLALWVLATQA-
PRLPSTMLLMNLAAADLLLALALPPRIAYHLRGQRWPFGEAACRLATAALYGHMYGSVLLLAAVSLDRYLALV
HPLRARALRGRRLALGLCMAAWLMAAALALPLTLQRQTFRLARSDRVLCHDALPLDAQASHWQPAFTCLALLG
CFLPLLAMLLCYGATLHTLAASGR---RYGHALRLTAVVLASAVAFFVPSNLLLLLHYSDPSP-
SAWGNLYGAYVPSLALSTLNSCVDPFIYYYVSAEF--------
0
FSVDEFSASVLTGKLTTVFLPIVYTIVFVVALPSNGMALWVFLFRTKKKAPAVIYMANLALADLLSVIWFPLK
IAYHIHGNNWIYGEALCNVLIGFFYANMYCSILFLTCLSVQRAWEIVNPMGHSR-
KKANIAIGISLAIWLLILLVTIPLYVVKQTIFIPALQITTCHDVLPEQLLVGDMFNYFLSLAIGVFLFPAFLT
ASAYVLMIRALENSEK---KRKRAIKLAVTVAAMYLICFTPSNLLLVVHYFLIKS-
QGQSHVYALYIVALCLSTLNSCIDPFVYYFVSHDFRDHAKNAL
```

Ligand preparation protocol

```
# This script uses an SDF of each ligand as the initial input

# Sanity check using BCL
bcl.exe molecule:Filter -add_h -neutralize -defined_atom_types -3d -
input_filenames ${NAME}_ligand.sdf -output_matched
${NAME}_ligand.CLEANED.sdf -output_unmatched ${NAME}_ligand.UNCLEANED.sdf
-message_level Debug

# Generate conformers using BCL
bcl.exe molecule:ConformerGenerator -rotamer_library cod -top_models 100
-ensemble_filenames ${NAME}_ligand.CLEANED.sdf -conformers_single_file
${NAME}_ligand.CLEANED.conf.sdf -conformation_comparer
'Dihedral(method=Max)' 30 -max_iterations 1000;

# Generate parameters file
python2.7 <path to
Rosetta>/main/source/scripts/python/public/molfile_to_params.py -n
${NAME} -p ${NAME} --mm-as-virt --long-names --conformers-in-one-file
${NAME}_ligand.CLEANED.conf.sdf --chain X
```

Ligand docking protocol

```
<path to Rosetta>/main/source/bin/rosetta_scripts.linuxgccrelease \
        @ flags.txt \
        -parser:protocol ligand_docking.xml \
        -s "model${N}.pdb ${LIG}.pdb " \
        -extra_res_fa ${LIG}.params \
        -out:file:silent model${N}_${LIG}.out \
        -out:file:scorefile model${N}_${LIG}.sc \
```

flags.txt

```
-nstruct 200
-ex1
-ex2
-ignore_zero_occupancy
-overwrite

-mistakes:restore_pre_talaris_2013_behavior
-score:analytic_etable_evaluation true
```

ligand_docking.xml

```
<ROSETTASCRIPTS>
    <SCOREFXNS>
        <ScoreFunction name="ligand_soft_rep" weights="ligand_soft_rep">
            <Reweight scoretype="fa_elec" weight="0.42"/>
            <Reweight scoretype="hbond_bb_sc" weight="1.3"/>
            <Reweight scoretype="hbond_sc" weight="1.3"/>
            <Reweight scoretype="rama" weight="0.2"/>
        </ScoreFunction>

        <ScoreFunction name="hard_rep" weights="ligand">
            <Reweight scoretype="fa_intra_rep" weight="0.004"/>
            <Reweight scoretype="fa_elec" weight="0.42"/>
            <Reweight scoretype="hbond_bb_sc" weight="1.3"/>
            <Reweight scoretype="hbond_sc" weight="1.3"/>
            <Reweight scoretype="rama" weight="0.2"/>
        </ScoreFunction>
    </SCOREFXNS>
    <LIGAND_AREAS>
        <LigandArea name="docking_sidechain" chain="X" cutoff="6.0"
add_nbr_radius="true" all_atom_mode="true" minimize_ligand="10"/>
        <LigandArea name="final_sidechain" chain="X" cutoff="6.0"
add_nbr_radius="true" all_atom_mode="true"/>
        <LigandArea name="final_backbone" chain="X" cutoff="7.0"
add_nbr_radius="false" all_atom_mode="true" Calpha_restraints="0.3"/>
    </LIGAND_AREAS>
    <INTERFACE_BUILDERS>
        <InterfaceBuilder name="side_chain_for_docking"
ligand_areas="docking_sidechain"/>
        <InterfaceBuilder name="side_chain_for_final"
ligand_areas="final_sidechain"/>
        <InterfaceBuilder name="backbone" ligand_areas="final_backbone"
extension_window="3"/>
    </INTERFACE_BUILDERS>
     <MOVEMAP_BUILDERS>
        <MoveMapBuilder name="docking" sc_interface="side_chain_for_docking"
minimize_water="true"/>
        <MoveMapBuilder name="final" sc_interface="side_chain_for_final"
bb_interface="backbone" minimize_water="true"/>
    </MOVEMAP_BUILDERS>
    <SCORINGGRIDS ligand_chain="X" width="30.0">
        <ClassicGrid grid_name="vdw" weight="1.0"/>
    </SCORINGGRIDS>

     CONTINUE ON NEXT PAGE…
```

```
    <MOVERS>
        <Transform name="transform" chain="X" box_size="5.0"
move_distance="0.1" angle="5.0" cycles="500" repeats="1" temperature="5"
initial_perturb="3.0" />
        <HighResDocker name="high_res_docker" cycles="6"
repack_every_Nth="3" scorefxn="ligand_soft_rep" movemap_builder="docking"/>
        <FinalMinimizer name="final" scorefxn="hard_rep"
movemap_builder="final"/>
        <InterfaceScoreCalculator name="add_scores" chains="X"
scorefxn="hard_rep" compute_grid_scores="0"/>
        <ParsedProtocol name="low_res_dock">
            <Add mover_name="transform"/>
        </ParsedProtocol>
        <ParsedProtocol name="high_res_dock">
            <Add mover_name="high_res_docker"/>
            <Add mover_name="final"/>
        </ParsedProtocol>
        <ParsedProtocol name="reporting">
            <Add mover_name="add_scores"/>
        </ParsedProtocol>
    </MOVERS>
    <PROTOCOLS>
        <Add mover_name="low_res_dock"/>
        <Add mover_name="high_res_dock"/>
        <Add mover_name="reporting"/>
    </PROTOCOLS>
</ROSETTASCRIPTS>
```


1. Song Y, DiMaio F, Wang RY, Kim D, Miles C, Brunette T, et al. High-resolution comparative modeling with RosettaCM. Structure. 2013;21(10):1735-42. doi: 10.1016/j.str.2013.08.005. PubMed PMID: 24035711; PubMed Central PMCID: PMCPMC3811137.

2. Kothiwale S, Mendenhall JL, Meiler J. BCL::Conf: small molecule conformational sampling using a knowledge based rotamer library. J Cheminform. 2015;7:47. doi: 10.1186/s13321-015-0095-1. PubMed PMID: 26473018; PubMed Central PMCID: PMCPMC4607025.

3. Meiler J, Baker D. ROSETTALIGAND: protein-small molecule docking with full side-chain flexibility. Proteins. 2006;65(3):538-48. doi: 10.1002/prot.21086. PubMed PMID: 16972285.

4. Combs SA, Deluca SL, Deluca SH, Lemmon GH, Nannemann DP, Nguyen ED, et al. Small-molecule ligand docking into comparative models with Rosetta. Nat Protoc. 2013;8(7):1277-98. doi: 10.1038/nprot.2013.074. PubMed PMID: 23744289; PubMed Central PMCID: PMCPMC5750396.