Supplemental information for:

Broad-spectrum allosteric inhibition of herpesvirus proteases

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Short title: Allosteric inhibition of HHV proteases
Figure S1. Dynamic light scattering of non-ionic compounds. Buffer and DMSO alone (a), compound 4 (b), and compound 6 (c) show no aggregation. Compound 7 (d) exhibits large ~ 100 nm radius aggregates. All compounds were at 1.56 μM concentration in 2% DMSO.
Figure S2. Combined chemical shift perturbations for $^{13}$C-$^1$H HSQC Ile spectra. The combined chemical shift perturbations for KSHV (a), CMV (b), and HSV-2 (c) proteases for all isoleucines in their respective truncated constructs. The dotted line indicates a cutoff for significant perturbation.
Figure S3. $^{15}$N-HSQC spectra for truncated KSHV protease with inhibitors. Spectra for KSHVΔ196 protease in the absence of inhibitor (black), presence of compound 2 (red), or presence of compound 3 (cyan) are shown. The aromatic hot spot residue tryptophan 109 (W109) is highlighted.
Figure S4. HSV-2 Pr D213 $^{13}$C-$^1$H HSQC isoleucine assignments. Single point mutations from isoleucine to valine for each isoleucine in the truncated HSV-2 protease construct were made and their spectra recorded (a-h), allowing assignment of all peaks. Resonances labeled with a prime sign indicate minor conformations.
Figure S5. CMV Pr D221 $^{13}$C-$^1$H HSQC isoleucine assignments. A single point mutation, I96V, was introduced the spectrum recorded, allowing assignment of both I61 and I96 peak resonances.
Figure S6. Overlay of monomer A and monomer B. Monomer A and B largely overlap, however the C-terminal residues of monomer A (green) differ in position from those of monomer B (purple), resulting in distinct contact surfaces with the small molecule inhibitor.
Figure S7. Symmetry-mate bridging molecule perturbs inhibitor binding to monomer A. The crystal structure of compound 2 bound to monomer A (green) is shown in the presence of a symmetry-mate bridging molecule (dark grey).
Figure S8. Overlay of PLOP-minimized model and crystal structure. The PLOP-minimized model (grey and green) is shown overlayed with the crystal structure (blue-grey). Relatively little structural change occurs with minimization, however a critical interaction between inhibitor and R82 is observed.
Figure S9. DD2 inhibition of KSHV Pr R82Q. The potency of DD2 is reduced against KSHV Pr R82Q relative to WT. This supports a model where DD2 interacts with R82.
Table S1. Data collection and refinement statistics for compound

|                      | Compound 2-KSHV Pr Δ196 |
|----------------------|--------------------------|
| Wavelength (Å)       | 1                        |
| Resolution range (Å) | 74.75 - 1.45 (1.481 - 1.43) |
| Space group          | 1 2 2 2                  |
| Unit cell            | 69.12 95.892 119.342 90 90 90 |
| Total reflections    | 748120 (84478)           |
| Unique reflections   | 73379 (10614)            |
| Multiplicity         | 10.2 (8.0)               |
| Completeness (%)     | 100.0 (100.0)            |
| Mean I/sigma(I)      | 13.1 (2.1)               |
| Wilson B-factor      | 20.23                    |
| R-merge              | 0.096 (0.919)            |
| R-meas               | 0.104                    |
| CC1/2                | 0.998 (0.857)            |
| CC*                  | 0.824 (0.917)            |
| R-work               | 0.1729 (0.3457)          |
| R-free               | 0.1978 (0.3466)          |
| Number of non-hydrogen atoms | 3404          |
| macromolecules       | 2973                     |
| ligands              | 112                      |
| water                | 319                      |
| Protein residues     | 375                      |
| RMS(bonds)           | 0.012                    |
| RMS(angles)          | 1.42                     |
| Ramachandran favored (%) | 98                      |
| Ramachandran outliers (%) | 0                      |
| Clashscore           | 3.75                     |
| Average B-factor     | 38.30                    |
| macromolecules       | 38.40                    |
| ligands              | 23.80                    |
| solvent              | 42.00                    |

Statistics for the highest-resolution shell are shown in parentheses.
Table S2. Data collection and refinement statistics for compound 3

|                                | Compound 3-KSHV Pr Δ I96 |
|--------------------------------|--------------------------|
| Wavelength (Å…)                | 1                        |
| Resolution range (Å…)          | 74.75 - 2.15 (2.165 - 2.09) |
| Space group                    | I 2 2 2                  |
| Unit cell                      | 69.737 95.947 119.245 90 90 90 |
| Total reflections              | 150488 (11776)           |
| Unique reflections             | 22808 (2491)             |
| Multiplicity                   | 6.6 (4.7)                |
| Completeness (%)               | 93.7 (72.6)              |
| Mean I/sigma(I)                | 18.1 (1.7)               |
| Wilson B-factor                | 29.3                     |
| R-merge                        | 0.118 (0.708)            |
| R-meas                         | 0.140 (0.903)            |
| CC1/2                          | 0.665 (0.577)            |
| CC*                            | 0.894 (0.855)            |
| R-work                         | 0.1843 (0.2683)          |
| R-free                         | 0.2460 (0.3552)          |
| Number of non-hydrogen atoms   | 3336                     |
| macromolecules                 | 3033                     |
| ligands                        | 112                      |
| water                          | 191                      |
| Protein residues               | 382                      |
| RMS(bonds)                     | 0.003                    |
| RMS(angles)                    | 0.79                     |
| Ramachandran favored (%)       | 97                       |
| Ramachandran outliers (%)      | 0.26                     |
| Clashscore                     | 1.28                     |
| Average B-factor               | 42.70                    |
| macromolecules                 | 43.50                    |
| ligands                        | 28.60                    |
| solvent                        | 38.10                    |

Statistics for the highest-resolution shell are shown in parentheses.
Scheme 1:

\[
\begin{align*}
2M + I & \rightleftharpoons D + S + I \rightleftharpoons DS + I \rightarrow D + P \\
M + MI & \rightleftharpoons DI \rightleftharpoons DS + I \rightarrow DI + P
\end{align*}
\]

Supplemental equations:

Equation 1 describes the linear relationship between dependent variable \(E_0\) and independent variable \(\sqrt{k_{\text{exp}}}\) in the presence of inhibitor used for Zhang-Poorman analysis:

\[
\frac{E_0}{\sqrt{k_{\text{exp}}}} = \sqrt{k_{\text{exp}}} \left( \frac{K_M}{k_{\text{cat}}} + \frac{\sqrt{K_d K_M}}{k_{\text{cat}} + k'_{\text{cat}}} \frac{I}{K_c} \right) + \sqrt{k_{\text{exp}}} \left( \frac{K_d K_M}{4 k_{\text{cat}}} + \frac{k'_{\text{cat}}}{k_{\text{cat}}} \frac{I}{K_c} \right) (1)
\]

In the absence of inhibitor equation 1 simplifies to equation 2 below.

\[
\frac{E_0}{\sqrt{k_{\text{exp}}}} = \sqrt{k_{\text{exp}}} \left( \frac{K_M}{k_{\text{cat}}} \right) + \sqrt{k_{\text{exp}}} 
\]

The slope of each line is equal to \(\frac{K_M}{k_{\text{cat}} + k'_{\text{cat}}} \frac{I}{K_c}\) where \(K_c = D\cdot I/\text{DI}\) is the competitive inhibition equilibrium constant and \(K'_c = D\cdot S\cdot I/\text{DSI}\) is the noncompetitive equilibrium constant (D, I, DI, DS, and DSI are the molar concentrations of dimer, inhibitor, dimer-inhibitor, dimer-substrate, and dimer-substrate-inhibitor, respectively). The noncompetitive rate constant, \(k'_{\text{cat}}\), is the rate of conversion of dimer-substrate-inhibitor complex to dimer-inhibitor plus product. Dissociative inhibition (dimer disruption) results in an increasing intercept with increasing inhibitor
concentration. Purely dissoassociative inhibition results in a constant slope while mixed-type inhibition results in variable slope with varying inhibitor concentration.

**Supplemental derivation 1:**

In our Zhang-Poorman analysis with both DD2 and compound 2, increasing inhibitor concentration resulted in decreasing slope. This indicates that the derivative of the slope term with respect to inhibitor concentration (a) must be less than zero (b). To investigate whether this could inform mode of binding we solved this inequality.

\[
\frac{\partial}{\partial I} \left( \frac{K_M \left(1 + \frac{I}{K_c}\right)}{k_{cat} + k'_{cat} \frac{I}{K_c'}} \right) = \frac{K_M K'_{c} \left(k_{cat}K'_{c} - K_c k'_{cat}\right)}{K_c(ik'_{cat} + k_{cat}K'_{c})^2} \quad (a)
\]

\[
\frac{K_M K'_{c} \left(k_{cat}K'_{c} - K_c k'_{cat}\right)}{K_c(ik'_{cat} + k_{cat}K'_{c})^2} < 0 \quad (b)
\]

Solving (b) while assuming all constants greater than 0, it follows that:

\[0 < K'_{c} < \frac{K_c k'_{cat}}{k_{cat}} \quad \text{and} \quad \frac{K'_{c}}{K_c} < \frac{k'_{cat}}{k_{cat}} \quad (c)\]

Since inhibition takes place we assume \(k'_{cat} < k_{cat}\), therefore in combination with (c):

\[\frac{K'_{c}}{K_c} < \frac{k'_{cat}}{k_{cat}} < 1 \quad \text{and} \quad K'_{c} < K_c \quad (d)\]

Since \(K'_{c}\) is less than \(K_c\), apparent binding of inhibitor to the dimer-substrate-inhibitor complex is tighter than binding of inhibitor to the dimer alone. Without additional knowledge of \(k'_{cat}, K_c, or\)
The inequality \( K'_c / K_c < (k'_{cat} / k_{cat}) \) is uninformative, though necessarily true for the above interpretation.

**Supplemental Methods:**

**Site-Directed Mutagenesis**

All DNA plasmids were produced by mutagenesis of the template sequences using a QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent). The resulting PCR-reaction mixtures were first transformed into XL-10 Gold Ultracompetent Cells (Agilent). Cultures containing the XL-10 transformed cells were grown overnight in Luria Broth containing carbenicillin (100 mg/mL) and miniprepped using Qiagen Miniprep Kits. DNA sequences of the mutated plasmids were verified via DNA sequencing prior to re-transformation into Rosetta2 (DE3) pLysS Competent Cells (Novagen/EMD Millipore). Primers (Integrated DNA Technologies, Inc.) encoding the isoleucine to valine mutations are listed in Table S1, below.

**Table S2: Ile-to-Val site-directed mutagenesis primers**

| Primers                      | CMV Pr D221                        | HSV-2 Pr D213                        |
|-----------------------------|------------------------------------|-------------------------------------|
| **Ile61Val**                | 5'-CGCTCCCGCTCAACGTAACCACGACGAC-3' | 5'-GGGCGGTGACGTAGACGGGACCCGCC-3'    |
|                            | 5'-GTGGTGTTGACGGGACCCGCC-3'       | 5'-GGCCACGTAGACGGGACCCGCC-3'       |
| **Ile96Val**                | 5'-CCAGGTTTCTGGAGGTTACGCGCGGT-3'  | 5'-AGAACCCTGCGCCGTCAACGTAGACCAC-3' |
|                            | 5'-AGCCACGTAGACGGGACCCGCC-3'     | 5'-GTGGTGTTGACGGGACCCGCC-3'       |
| **Ile21Val**                | 5'-GGGCGGTGACGTAGACGGGACCCGCC-3' | 5'-GGCCACGTAGACGGGACCCGCC-3'       |
|                            | 5'-GGCCACGTAGACGGGACCCGCC-3'     | 5'-GTGGTGTTGACGGGACCCGCC-3'       |
| **Ile57Val**                | 5'-AGAACCCTGCGCCGTCAACGTAGACCAC-3' | 5'-GTGGTGTTGACGGGACCCGCC-3'     |
|                            | 5'-GTGGTGTTGACGGGACCCGCC-3'     | 5'-GTGGTGTTGACGGGACCCGCC-3'       |
| **Ile86Val**                | 5'-TTTTTTGCGGCGGTGCGGAGTTAC-3'  | 5'-CTGCACGACGCACCAGCCCAAAAA-3'    |
|                            | 5'-CTGCACGACGCACCAGCCCAAAAA-3'  | 5'-CTGCACGACGCACCAGCCCAAAAA-3'    |
Crystallographic data processing

Diffraction images were processed using MOSFLM\textsuperscript{2} and the CCP4 suite\textsuperscript{3} (specifically SCALA/Truncate), operated through the Elves scripts.\textsuperscript{4} The resulting structure was solved by molecular replacement with Phaser\textsuperscript{5} using PDB 3NJQ as the template search model. The resulting structure model was a dimer in an asymmetric unit and was subjected to multiple rounds of restrained refinement and isotropic $B$-factor minimization with Phenix\textsuperscript{6} and Coot.\textsuperscript{7} “Riding” hydrogens were included during refinement.

Analog synthesis

$^1$H NMR spectra were recorded on a Varian INOVA-400 400 MHz spectrometer. Chemical shifts are reported in $\delta$ units (ppm) relative to TMS as an internal standard. Coupling constants ($J$) are reported in hertz (Hz). $^{13}$C NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer equipped with a QCI CyroProbe. All other reagents and solvents were purchased from Sigma-Aldrich and used as received. Air and/or moisture sensitive reactions were carried...
out under an argon atmosphere in oven-dried glassware using anhydrous solvents from commercial suppliers. Air and/or moisture sensitive reagents were transferred via syringe or cannula and were introduced into reaction vessels through rubber septa. Solvent removal was accomplished with a rotary evaporator at ca. 10-50 Torr. Column chromatography was carried out using a Biotage SP1 flash chromatography system and silica gel cartridges from Biotage. Analytical TLC plates from EM Science (Silica Gel 60 F254) were employed for TLC analyses. Hydrogenation reactions were carried out with a ThalesNano H-Cube hydrogenator.

All synthesized analogs were judged to be of 95% or higher purity based on analytical LC/MS analysis. LC/MS analyses were performed on a Waters Micromass ZQ/Waters 2795 Separation Module/Waters 2996 Photodiode Array Detector system controlled by MassLynx 4.0 software. Separations were carried out on an X Terra® MS C18 5μm 4.6x50mm column at ambient temperature using a mobile phase of water-acetonitrile containing 0.05% trifluoroacetic acid. Gradient elution was employed wherein the acetonitrile-water ratio was increased linearly from 5 to 95% acetonitrile over 2.5 minutes, then maintained at 95% acetonitrile for 1.5 min., and then decreased to 5% acetonitrile over 0.5 min, and maintained at 5% acetonitrile for 0.5 min. Compound purity was determined by integrating peak areas of the liquid chromatogram, monitored at 254 nm. Compound 2 was a cream-colored powder. Compounds 1 and 3-7 were white powders.

N-(2-Benzyl-4-carbamoyl-phenyl)-6-(cyclohexylmethyl)pyridine-2-carboxamide (4) 3-Benzyl-4-[[6-(cyclohexylmethyl)pyridine-2-carbonyl]amino]benzoic acid (DD2, 16 mg, 0.037 mmol), di-tert-butyl dicarbonate (11 mg, 0.0048 mmol), ammonium bicarbonate (4 mg, 0.048 mmol) and pyridine (0.5 ml, 0.0048 mmol) in acetone (1 mL) were stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure, diluted with water and
extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over magnesium sulfate and concentrated under reduced pressure. The crude material thus obtained was purified by flash column chromatography (30% ethyl acetate-hexanes and 5% methanol-dichloromethane) to obtain the title compound in 63% yield. \( ^1 \)H NMR (CDCl\(_3\)) \( \delta \) 10.35 (s, 1H), 8.53 (d, \( J = 6 \) Hz, 1H), 8.06 (d, \( J = 6 \) Hz, 1H), 7.83 (s, 1H), 7.73-7.76 (m, 2H), 7.20-7.28 (m, 6H), 4.18 (s, 2H), 2.64 (d, \( J = 6 \) Hz, 2H), 1.60-1.68 (m, 5H), 0.93-0.98 (m, 2H); LCMS (ESI) \( m/z \) 428 (MH\(^+\)). \( ^{13} \)C NMR (125.73 MHz, DMSO-\( d_6 \)) \( \delta \) 164.7, 159.1, 157.3, 145.6, 136.1, 135.8, 135.5, 127.8, 127.7, 127.5, 125.9, 125.6, 124.5, 124.0, 123.8, 118.2, 116.9, 29.8, 23.3, 23.0

**N-[2-Benzyl-4-(4H-1,2,4-triazol-3-yl)phenyl]-6-(cyclohexylmethyl)pyridine-2-carboxamide (5)** N-(2-Benzyl-4-carbamoyl-phenyl)-6-(cyclohexylmethyl)pyridine-2-carboxamide (4, 10 mg, 0.023 mmol) and acetic acid (0.01 mL) in N,N-dimethylformamide dimethyl acetal (0.100 mL) were heated to 80°C for 30 min. The reaction mixture was concentrated under reduced pressure and diluted with AcOH (0.1 mL). Hydrazine hydrate (0.01 mL) was added and the mixture was heated to 110°C for 45 min. The reaction mixture was then diluted with ethyl acetate and washed with saturated sodium bicarbonate, water and brine. The ethyl acetate layer was dried over magnesium sulfate and concentrated under reduced pressure. The crude material thus obtained was purified by flash column chromatography (40% ethyl acetate-hexanes) to obtain the title compound in 85% yield. \( ^1 \)H NMR (CDCl\(_3\)) \( \delta \) 10.39 (s, 1H), 8.55 (d, \( J = 6 \) Hz, 1H), 8.13-8.23 (m, 4H), 7.82 (t, \( J = 6 \) Hz, 1H), 7.17-7.29 (m, 6H), 4.19 (s, 2H), 2.65 (d, \( J = 6 \) Hz, 2H), 1.61-1.68 (m, 5H), 1.16-1.24 (m, 4H), 0.93-1.02 (m, 2H); LCMS (ESI) \( m/z \) 452 (MH\(^+\)). \( ^{13} \)C NMR (125.73 MHz, DMSO-\( d_6 \)) \( \delta \) 161.7, 160.6, 159.9, 151.9, 148.4, 144.5, 139.0, 138.4, 131.7, 131.4, 128.6,
128.3, 128.2, 127.7, 127.0, 126.6, 126.4, 125.1, 124.8, 123.5, 122.0, 119.4, 44.8, 37.8, 37.0, 32.5, 26.0, 25.6

**N-(2-Benzyl-4-cyanophenyl)-6-(cyclohexylmethyl)pyridine-2-carboxamide (7)**

N-(2-Benzyl-4-carbamoyl-phenyl)-6-(cyclohexylmethyl)pyridine-2-carboxamide (4, 20 mg, 0.047 mmol), trifluoroacetic anhydride (0.026 mL, 0.19 mmol) and pyridine (0.022 ml, 0.28 mmol) in 1,4-dioxane (1 mL) were stirred at room temperature for 18 h. The reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic extract was dried over magnesium sulfate and concentrated under reduced pressure. The crude material thus obtained was purified by flash column chromatography (20% ethyl acetate-hexanes) to afford the title compound in 89% yield. 

$^1$H NMR (CDCl$_3$) $\delta$ 10.43 (s, 1H), 8.63 (d, $J = 6$ Hz, 1H), 8.06 (d, $J = 6$ Hz, 1H), 7.77 (t, $J = 6$ Hz, 1H), 7.63 (dd, $J = 6$, 3 Hz, 1H), 7.50 (s, 1H), 7.23-7.34 (m, 6H), 4.14 (s, 2H), 2.64 (d, $J = 6$ Hz, 2H), 1.63-1.70 (m, 5H), 1.15-1.20 (m, 4H), 0.95-0.98 (m, 2H); LCMS (ESI) $m/z$ 410 (MH+). 

$^{13}$C NMR (125.73 MHz, DMSO- $d_6$) $\delta$ 161.9, 160.0, 147.8, 140.0, 138.5, 137.9, 134.6, 131.8, 131.7, 128.7, 128.2, 127.4, 126.7, 121.6, 119.7, 118.9, 106.4, 44.8, 37.8, 36.4, 32.5, 26.0, 25.6

**N-[2-Benzyl-4-(1H-1,2,3,4-tetrazol-5-yl)phenyl]-6-(cyclohexylmethyl)pyridine-2-carboxamide (2)**

N-(2-Benzyl-4-cyanophenyl)-6-(cyclohexylmethyl)pyridine-2-carboxamide (7, 14 mg, 0.034 mmol), sodium azide (9 mg, 0.13 mmol), zinc bromide (8 mg, 0.034 mmol) in a 1:2 mixture of 2-propanol/water (0.9 mL) were heated to reflux for 60 h. The reaction mixture was cooled, concentrated under reduced pressure, diluted with water and extracted with ethyl acetate. The organic extract was dried over magnesium sulfate and concentrated under reduced pressure. The crude material thus obtained was purified by flash column chromatography to
afford the title compound in 60% yield. $^1$H NMR (DMSO-$d_6$) $\delta$ 10.34 (s, 1H), 8.37 (d, $J = 9$ Hz, 1H), 8.05 (s, 1H), 7.93-7.99 (m, 3H), 7.48-7.50 (m, 1H), 7.19-7.27 (m, 5H), 4.20 (s, 2H), 2.67 (d, $J = 6$ Hz, 2H), 1.71-1.75 (m, 1H), 1.54-1.61 (m, 4H), 1.11-1.44 (m, 4H), 0.94-0.96 (m, 2H); LCMS (ESI) $m/z$ 453 (MH$^+$). $^{13}$C NMR (125.73 MHz, DMSO-$d_6$) $\delta$ 162.3, 160.3, 148.6, 139.0, 138.8, 132.2, 129.8, 129.1, 128.7, 127.6, 127.0, 126.4, 122.64, 119.9, 45.2, 38.2, 37.3, 32.9, 26.4, 26.0.

N-[2-Benzyl-4-(methylsulfonylcarbamoyl)phenyl]-6-(cyclohexylmethyl)pyridine-2-carboxamide (3) 3-benzyl-4-[(6-(cyclohexylmethyl)pyridine-2-carbonyl]amino]benzoic acid (DD2, 15mg, 0.035 mmol) and 1,1'-carbonyldiimidazole (11 mg, 0.07 mmol) in tetrahydrofuran (1.0 mL) were stirred at room temperature for an hour. Methanesulfonamide (5 mg, 0.053 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.008 mL, 0.053 mmol) were added and the mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with ethyl acetate and washed with 1 N HCl, water and brine. The organic extract was dried over magnesium sulfate and concentrated under reduced pressure. The crude material thus obtained was purified by flash column chromatography (40% ethyl acetate-hexanes) to afford the title compound in 34% yield. $^1$H NMR (CDCl$_3$) $\delta$ 10.45 (s, 1H), 8.64 (d, $J = 6$ Hz, 1H), 8.55 (s, 1H), 8.07 (d, $J = 6$ Hz, 1H), 7.75-7.82 (m, 4H), 7.20-7.31 (m, 5H), 4.18 (s, 2H), 3.43 (s, 3H), 2.64 (d, $J = 6$ Hz, 2H), 1.91-1.95 (m, 1H), 1.55-1.72 (m, 4H), 1.16-1.24 (m, 4H), 0.96-0.98 (m, 2H); LCMS (ESI) $m/z$ 506 (MH$^+$).

Methyl 3-benzyl-4-[6-(cyclohexylmethyl)pyridine-2-amido]benzoate (6) Oxalyl chloride (0.040 mL) was added to 3-benzyl-4-[(6-(cyclohexylmethyl)pyridine-2-carbonyl]amino]benzoic acid (DD2, 5.2 mg, 0.012 mmol) in anhydrous methanol (0.4 mL) and stirred at room temperature for 20 h. The reaction mixture was concentrated under reduced pressure and the
residue thus obtained was purified by flash column chromatography (5% ethyl acetate-hexanes to obtain the title compound in 57% yield. $^1$H NMR (CDCl$_3$) $\delta$ 10.35 (s, 1H), 8.55 (d, $J = 6$ Hz, 1H), 7.98-8.06 (m, 2H), 7.75 (t, $J = 6$ Hz, 1H), 7.20-7.30 (m, 7H), 4.17 (s, 2H), 3.90 (s, 3H), 2.63 (d, $J = 6$ Hz, 2H), 1.56-1.70 (m, 6H), 1.16-1.24 (m, 3H), 0.95-0.98 (m, 2H); LCMS (ESI) m/z 443 (MH$^+$). $^{13}$C NMR (125.73 MHz, DMSO-$d_6$) $\delta$ 166.4, 162.9, 162.5, 160.5, 148.3, 140.4, 138.9, 132.2, 131.4, 129.1, 128.6, 127.8, 127.1, 125.7, 121.5, 120.0, 52.6, 45.2, 38.3, 37.1, 32.9, 26.4, 26.0.

**Dynamic light scattering**

Inhibitors were diluted from concentrated DMSO stocks to buffer with a final DMSO concentration of 2%. Measurements were made on a DynaPro MS/X equipped with a 55 mM laser at 826.6 nm. The integration time was 100 s (10x10 sec) and laser power was 100% unless reduction in laser power was necessary to avoid saturating the detector. The detector angle was 90 degrees. Measurements were performed at room temperature (25 degrees Celsius).
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