Supporting Information

Mass spectrometry-based selectivity profiling reveals a highly selective MELK inhibitor that causes delayed mitotic entry in cells

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Running title: MELK inhibition causes delayed mitotic entry

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Supplemental Figures
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Supplemental Table
Table S1 – Mass spectrometry data (provided as separate excel file)
Figure S1. Full competition MIB/MS selectivity profiles for 1 μM 8a, HTH, and OTS. MDA-MB-468 cells were treated with 1μM 8a, HTH, or OTS, and competition MIB/MS was completed as in Fig. 2A. All 235 protein kinases that were quantified by MS are displayed. Results are from the experiment shown in Fig. 2A.
Figure S2. Full competition MIB/MS selectivity profiles for 10, 100, and 1000 nM 8a. MDA-MB-468 cells were treated with 10, 100, or 1000 nM 8a, and competition MIB/MS was completed as in Fig. 2A. All 235 protein kinases that were quantified by MS are displayed. The results shown here are indicative of one experiment.
Figure S3. Full competition MIB/MS selectivity profiles for 1, 3, and 10 μM 8a. MDA-MB-468 cells were treated with 10, 100, or 1000 nM 8a, and competition MIB/MS was completed as in Fig. 2A. All 221 protein kinases that were quantified by MS are displayed. Results are from the experiment shown in Fig. 2C.
Figure S4. 8a has no detectable affinity for STK11 or MAP2K4 at 3 µM. The Eurofins DiscoverX cell-free assay platform was used to determine the binding affinity of 8a for STK11, MAP2K4, and MELK. Results shown here are indicative of two biological replicates. The binding constant (Kd) displayed in each plot is the average of the two replicates.
Figure S5. Aurora A and B are minimally inhibited by 8a in cell-free kinase assays. The Thermo SelectScreen kinase assay service was used to determine the percent inhibition of Aurora A and B upon exposure to 8a at concentrations ranging from 5 nM to 100 μM. Results shown are representative of two independent experiments.
Figure S6. Treatment with 8a causes decreased viability in HeLaS3 cells. A, HeLaS3 cells were treated with DMSO or 8a at concentrations ranging from 1 nM to 30 μM. Cells were exposed to 8a for 72h before viability was measured using a resazurin assay. Two biological replicates were completed, and representative results are displayed here. B, asynchronous HeLaS3 cells were treated with DMSO or 0.5, 1, or 3 μM 8a for 1, 2, 4, 8, 12, or 24h. Cells were harvested and immunoblotted with the indicated antibodies. The split in the center of the image indicates that the 1, 2, and 4h samples were immunoblotted separately from the 8, 12, and 24h samples, due to space constraints. Three biological replicates were completed. Data displayed here are representative results. C, asynchronous HeLaS3, U2OS, MDA-MB-231, and MDA-MB-468 cells were harvested and immunoblotted with the indicated antibodies. The displayed results are representative of three biological replicates.
Figure S7. Additional replicates of live-cell imaging experiment measuring G2 and M phase lengths. Asynchronous U2OS cells harboring fluorescent PCNA were treated with DMSO, 1 μM, or 3 μM 8a and G2 and M phase lengths were measured as described in Fig 7. A and B. Three independent experiments were completed, and representative results are shown in Fig. 7. Curves for G2 length (top), M length (middle), and quantified cell cycle phase lengths (bottom) for additional replicates are shown here.