An internally quenched peptide as a new model substrate for rhomboid intramembrane proteases

Elena Arutyunova, Zhenze Jiang, Jian Yang, Ayodeji N. Kulepa, Howard S. Young, Steven Verhelst, Anthony J. O'Donoghue and M. Joanne Lemieux

Supplementary material

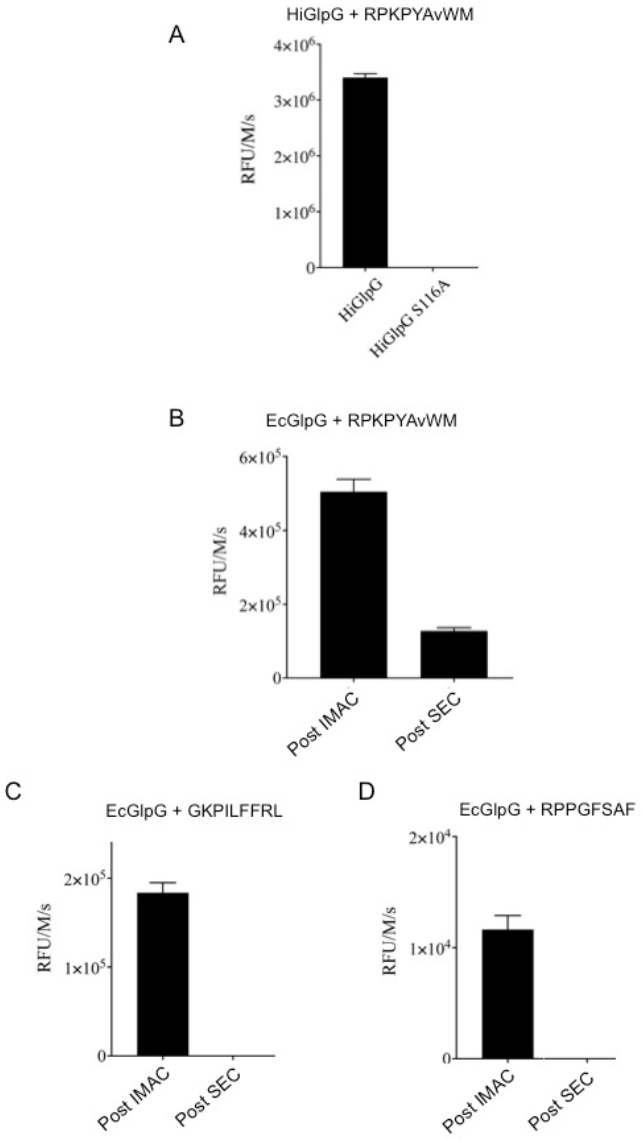

Figure S1. A. The S116A inactive mutant of HiGlpG is unable to cleave the internally quenched peptide, RPKPYAvWM. B,C,D. Assessment of the internally quenched peptide substrate cleavage reveals that compared to post immobilized metal affinity chromatography (IMAC) there is a loss of hydrolysis after size exclusion chromatography (SEC). RFU: relative fluorescence units. The data presented as mean (n=3) ± SE.

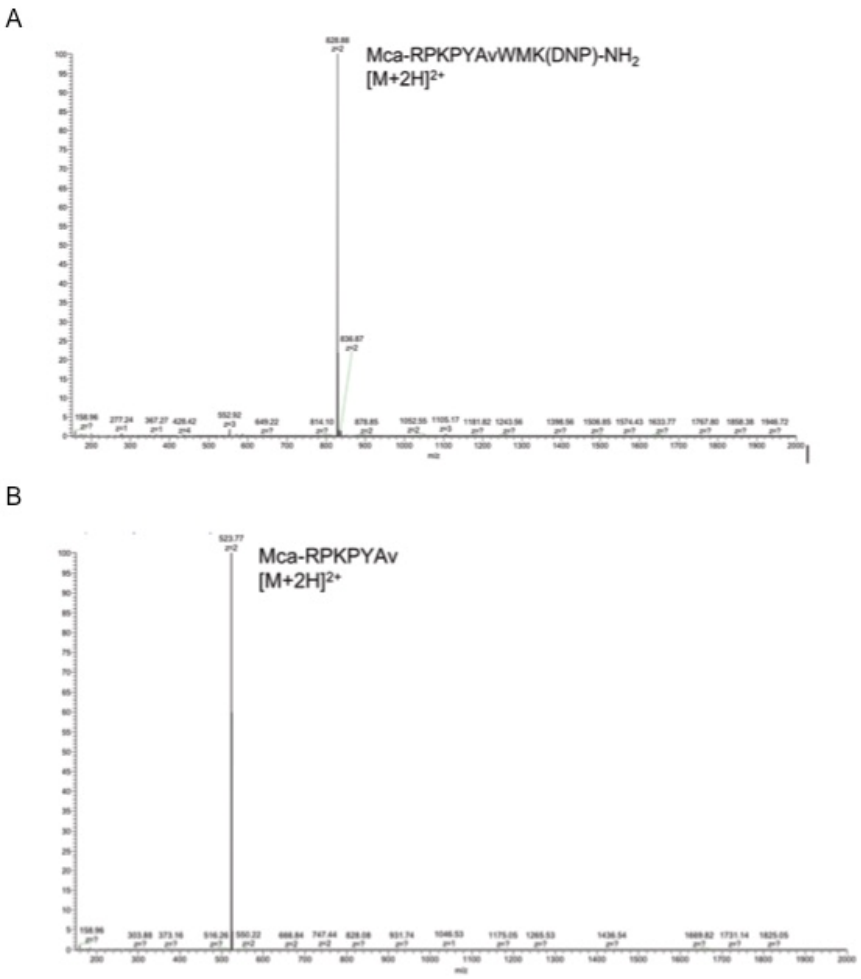

Figure S2. Mass spectrometry was used to identify the cleavage site within the RPKPYAvWM substrate. After 4 hours incubation of HiGlpG with the substrate, we discovered that the full length Mca-RPKPYAvWMK(DNP) substrate that eluted between minute 20 and 21 was no longer detectable and a single cleavage product corresponding to RPKPYAv was found to elute at minute 14.

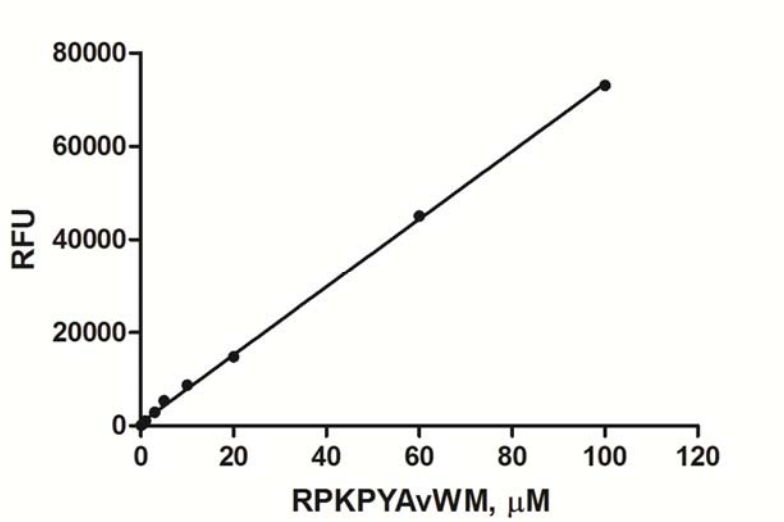

Figure S3. The proportionality between the concentration of substrate used in the kinetic assay and the fluorescence emitted.

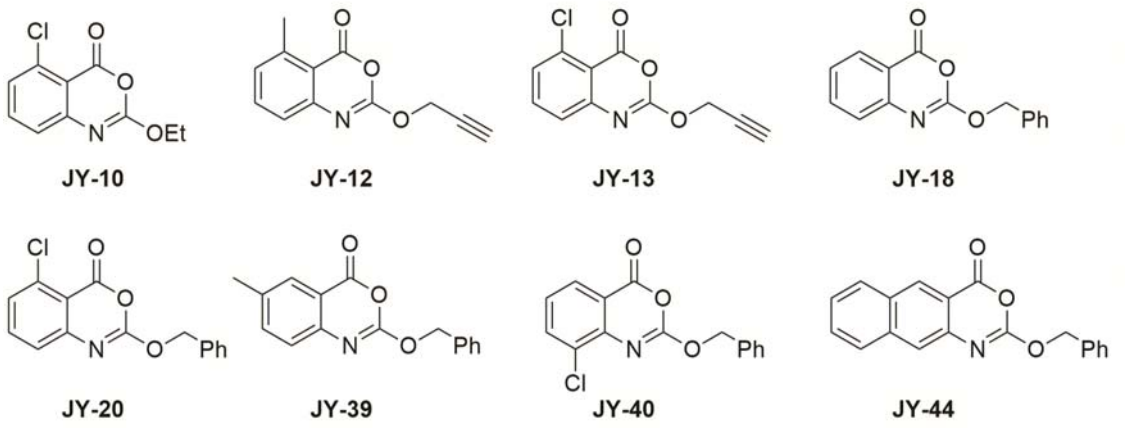

Figure S4. Chemical structures of benoxazin-4-ones inhibitors used in this study.