Discovery of Novel Natural Products that Inhibit eIF4E Protein-Protein Interactions

-Yihao Zhuang Mar 9th, 2021

Advisor: **Amanda L. Garner, PhD.**
Co-advisor: **Ashootosh Tripathi, PhD.**
Committee members: **David H. Sherman, PhD. and Roland Kersten, PhD.**

Castration-resistant prostate cancer (CRPC) is the second-most leading cause of cancer-related deaths in American males with 32,000 deaths are reported annually. Although current therapeutics for CRPC including abiraterone, enzalutamide, and epalutamide have led to life-extending advances for the treatment of CRPC, the disease remains uniformly fatal. Recent evidence suggests that these new 2nd generation therapeutics have remodeled the phenotypic landscape of CRPC, giving a rise to AR-low CRPC. AR-low CRPC is characterized by low to absent AR and represents up to 23% of end-stage prostate cancer patients. Therefore, new therapeutics must be developed to treat this emerging lethal AR-low CRPC subset. Dysregulation of mRNA translation is a hallmark of tumorigenesis and tumor progression across various cancer types. In particular, the 4EBP1/eIF4G-eIF4E axis is a major regulator of translation initiation and driver of oncogenesis. A recent study has revealed a new link between AR signaling and translation initiation. It showed that increased eIF4F complex formation is required for AR-low prostate cancer initiation and enhanced cell proliferation *in vivo*, indicating that this disease may be vulnerable to eIF4E inhibition.[14] Combined, these findings suggest that therapeutic disruption of the eIF4E-eIF4G protein-protein interaction (PPI) may be an effective approach for the treatment of AR-low CRPC.

Toward this goal, the Garner Laboratory completed a high-throughput screening (HTS) campaign using the previously described PPI catalytic enzyme-linked click chemistry assay (PPI cat-ELCCA) to identify natural products that inhibit eIF4E PPIs. In this study, I aim to isolate and characterize novel eIF4E PPI inhibitors from the top four strains and characterize these NPs using biochemical and cell-based assays. I re-grew all 16 strains in 1-Liter scale to confirm their activities and reproducibility. Out of the 16 strains, 06282-1I, YNYX-265C, 86930-1I, and 87797-1N showed clear dose-dependent inhibitory activity against the eIF4E-4E BP1 PPI and were selected for further analyses. Using traditional bioactivity guided natural product discovery strategy, I isolated undecylprodigiosin from the NPE (natural product extract) of 06282-1I, which is a red pigment and shows an IC_{50} >250μM against eIF4E PPI. Even though potential antitumor activities of prodigiosins have been reported, they have a wide range of reported bioactivities, which dampens enthusiasm for developing this scaffold for targeting eIF4E PPIs. Furthermore, I also isolated and characterized izumiphenazine A and izumiphenazine B from 06282-1I NPE. Even though they both show IC_{50} ~8 μM against eIF4E PPI in Cat ELCCA, they are not active in CETSA and cap-pull down assays. As a result, they are not selected for further optimization. Deconvolution of YNYX265C, 86930-1I, and 87797-1N are ongoing and some HPLC fractions of these extracts show promising activities against eIF4E PPI. These fractions will be further purified and characterized using mass spectrometry and NMR. Once the most promising NP (natural product) from these strains is selected, I will elucidate and confirm the biosynthetic pathway of this NP. We will then use the precursor-mutasynthesis approach guided by the biosynthetic pathway to synthesize multiple analogues. These analogues will be tested in both biochemical and cellular assays to investigate the structure-activity relationship (SAR).

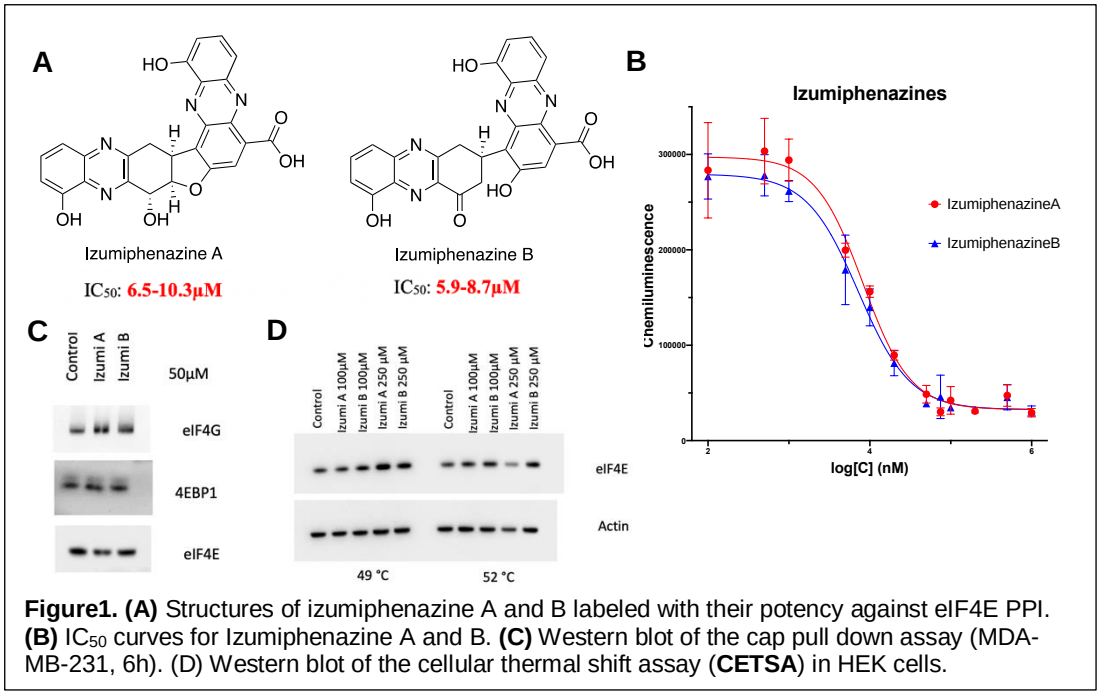

Figure1. (A) Structures of izumiphenazine A and B labeled with their potency against eIF4E PPI. **(B)** IC_{50} curves for Izumiphenazine A and B. **(C)** Western blot of the cap pull down assay (MDA-MB-231, 6h). (D) Western blot of the cellular thermal shift assay (**CETSA**) in HEK cells.