Overcoming the Challenges of Human HSP60 and ERK5 Inhibition In Cancer Through Heterobifunctional Degraders and Improved Analogs

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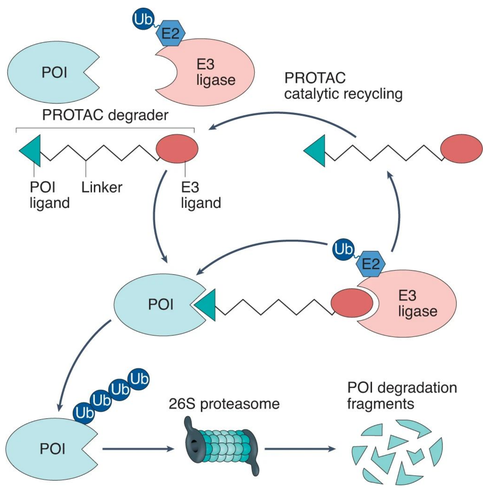
Overview of HSP60, ERK5, and Heterobifunctional Degraders

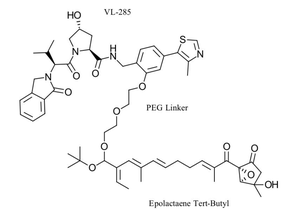
- Extracellular Regulated Kinase 5 (ERK5) is a Kinase at the end of the 4th and recently discovered Mitogen Activated Protein Kinase (MAPK) Pathway Responsible for cellular processes such as increased proliferation
- It has been found to be upregulated in many different cancer types, and upregulation typically correlates with worse prognosis
- Genetic Knockdown of ERK5 has shown to have anticancer effects
- Heat Shock Protein 60 kDa (HSP60) is a chaperone protein responsible for refolding misfolded proteins and has been shown to be upregulated in certain cancers
- Genetic knockdown of HSP60 had been shown to have anticancer effects
- Proteolysis Targeting Chimeras (PROTAC), are molecules that are capable of hijacking the E3 Ubiquitin Ligase system by simultaneously binding an E3 ligase and a target protein, promoting polyubiquitination and degradation of the target protein.
- The target protein ligand and the E3 Ligase ligand are connected by a Polyethylene Glycol (PEG) linker.
- PROTAC molecules allow complete degradation of proteins as compared to typical Small Molecule Inhibitors (SMI) that inhibit the active site exclusively. This allows PROTAC molecules to remove both the active and scaffolding functions of proteins.
- PROTACS have also been shown to bypass drug resistance, as well as promote great specificity as compared to their SMI counterparts.
- The PROTAC molecules presented use Mizoribine and Epolactaene Tert-Butyl Ester as protein ligands, and both use VL-285, a ligand for the Von Hippel-Lindau (VHL) E3 Ligase.

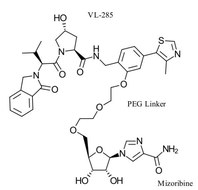


HSP60-PROTAC Design (Mizoribine and Epolactaene)

- Epolactaene Tert-Butyl Ester (ETB) is a bioactive molecule isolated from the fungal strain *Peniciilium* sp. BM 1689-P that has both anticancer and HSP60 inhibiting properties.
- Contrary to MB, ETB inhibits HSP60 chaperoning activity allosterically via binding Cys442 residues.
- Interestingly, ATPase activity is not inhibited, but chaperone activity is drastically reduced
- Incorporation of ETB into a PROTAC allows for degradation of HSP60, increasing potency while preventing ATP hydrolysis simultaneously.
- The PEG linker was added at the ester group of the molecule, as the catalytic activity of ETB is predicted to be located at or near the alpha, beta-unsaturated ketone. The PEG was also added here to reduce steric hindrance from the PEG and VL-285 on ETB binding

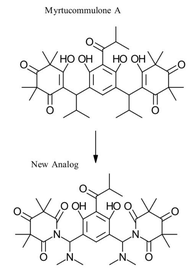
- Mizoribine (MB) is a bioactive imidazole nucleoside Isolated from *Eupenicllium brefeldianum.* It is an immunosuppressant used in renal transplants, as well as a variety of renal autoimmune diseases
- It possesses anticancer activity, and inhibits Human HSP60 via inhibition of ATP hydrolysis
- The incorporation of MB into a PROTAC aims to address two major problems with typical HSP60 Inhibition: concentration required for inhibition and reduction of the immunosuppressive effect.
- Use of PROTAC should allow for a more potent chemical effect (degradation vs inhibition) as well as greater target specificity for cytosolic/mitochondrial HSP60 vs its Nuclear off targets.
- The PEG linker was added to the 5' hydroxyl group on the ribose, due to studies predicting that the catalytic activity of MB occurs near the amine group. This location allows for the least amount of steric hindrance from the PEG linker and VL-285 on mb binding

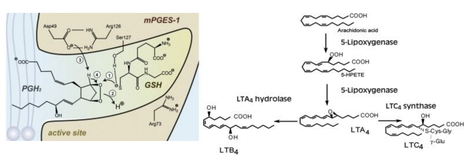




Myrtucommulone A analog

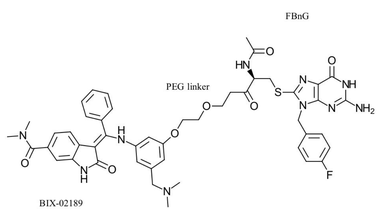
- Myrtucommulone A (MC) is a bioactive molecule Isolated from *Myrtus Communis* that has antibacterial, antioxidant, anti-inflammatory, and anticancer properties.
- MC is a bit of a different case from the other two molecules, as while it has potent anticancer and HSP60 inhibiting properties, it has major off-targets that cause the compound to cause unwanted immune responses
- The molecule hinders arachidonic acid metabolism via inhibition of microsomal prostaglandin E2 synthase-1 (mPGES-1) and 5-Lipoxygenase (5-LOX)
- Addition of nitrogens aims to accomplish two tasks in reducing off targets
- The first is to reduce mPGES-1 affinity by removing carbon bound deprotonation sites, as well as altering the 2 sterically adjacent hydroxyl groups
- mPGES-1 mechanism of action involves carbon bound deprotonation, as well as O-O bond breakage from 2 sterically adjacent oxygens
- The second is to reduce 5-LOX affinity by removing the double bond conjugated to the ketone, which is similar to the main target group of 5-LOX, albeit in a lipid structure
- Another enzymatic function of 5-LOX is to add O2 directly to the edge of a double bond to begin lipoxygenation, which is fully prevented by the nitrogen addition
- The exact mechanism of action for 5-LOX is not known, but the biosynthetic pathway is shown





ERK5 AUTAC Design

- ERK5 Is a special case, as PROTAC molecules have been developed for ERK5, and have shown no anticancer activity.
- Furthermore, typical small molecule inhibitors of ERK5 have been shown to be paradoxical activators in cells, leading to kinase activation by said molecules instead of inhibition
- In response to this we have designed a heterobifunctional degrader using the autophagy-targeting chimera (AUTAC) methodology instead
- This hijacks another cellular degradation mechanism, known as autophagy, which is responsible for degrading larger cellular objects, such as organelles, but can be used to degrade proteins as well.
- This AUTAC molecule incorporates BIX-02189, a potent ERK5 inhibitor, a PEG linker, and FBnG as an autophagy recruiting ligand
- The PEG was added onto the benzene ring to reduce steric hindrance from the PEG Linker and FBnG on BIX-02189 binding



Future Directions

- Computational analysis of drug binding affinity
- Testing of binding affinity via *in vitro* studies
- Testing of compounds in cancer cell lines
- A common problem among PEG chimeric molecules is potency is heavily correlated to PEG length, further screening and testing would have to be done to determine the optimal PEG length