Evaluation the Efficacy of Various Hydrophobic Degrons for PROTAC-Mediated Degradation of the Androgen Receptor

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Abstract

- The androgen receptor (AR) pathway is a major contributor to prostate cancer (PC) & tumor growth. Therapeutic strategies & drugs attempt to disrupt this pathway to slow or stop tumor growth. Typically, an AR antagonist is used, which binds to the AR as an inhibitor.
- Targeted protein degradation is a rapidly growing area in drug design & has been suggested as another treatment strategy for cancers.[1] One method of targeted protein degradation is hydrophobic tagging.[2]
- One strategy uses heterobifunctional molecules known as **PRO**teolysis **TA**rgeting **C**himeras (PROTACs). These consist of a ligand for a protein of interest (POI) on one end, connected by a linker to a group known to induce degradation, also known as a degron.[1]
- Our research aims: treat PC cells with PROTACs to selectively degrade the AR by attaching various hydrophobic moieties, measuring their relative effectiveness as degrons. If these are effective degrons, a chemical library of degrons can be established.

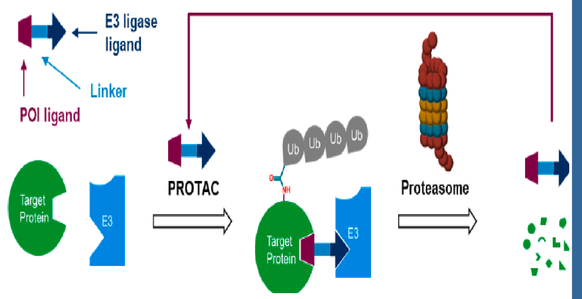

Figure 1. PROTAC mode of action, using an E3 ligase.[6]

Compound Design

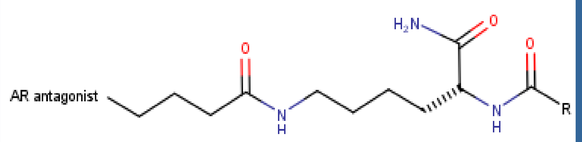

Figure 2. Generic structure of the AR PROTAC

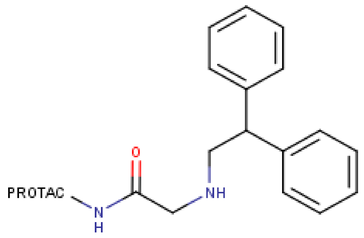

Figure 3. C1-1A; Diphenylethylene Peptoid Moiety

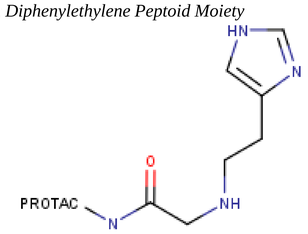

Figure 4. C1-1B; Histidine Peptoid Moiety

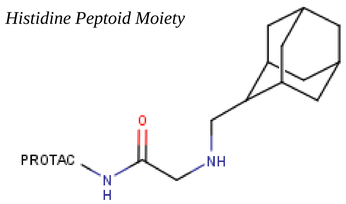

Figure 5. C1-6A; Adamantyl Peptoid Moiety

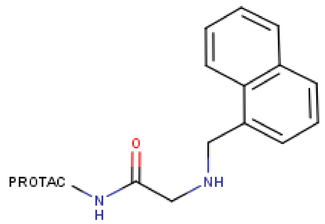

Figure 6. C1-6B; Naphthalene Peptoid Moiety

Synthesis and Characterization

Synthesis

- Each peptoid was synthesized using a solid-phase peptoid synthesis procedure[3], using Rink-amide Resin.
- After an unsuccessful BOC-anhydride protection reaction for some of the bulkier compounds (C1-6A, C1-6B) 4-dimethylaminopyridine (DMAP) was used as a catalyst.
- Preparative high-performance liquid chromatography (H_2O/ACN)
- The AR antagonist was synthesized and purified through column chromatography[4].

Characterization

- Analytical high-performance liquid chromatography was used to confirm the presence of the synthesized compounds. (H_2O/ACN)
- Mass spectrometry was also used to confirm that the proper compounds had been synthesized by finding the weight of the molecular ion.

Further Research

- Link the AR antagonist and hydrophobic moieties
- More batches of PROTACs are being synthesized and purified
- Various linkers can be tested.[5]
- LNCaP, an AR-positive prostate cancer cell line, will be used to test these compounds in vitro
- If testing is successful, the Ubiquitin-Proteasome system can be analyzed to determine the mechanism of degradation
- NMR spectra will be obtained.

Discussion

- PROTACs and hydrophobic tagging have shown separate successes in the past.
- These hydrophobic moieties and the AR antagonist have been successfully synthesized.
- The compounds are relatively small, meaning they could be druggable.
- If these compounds are effective at degrading the androgen receptor, this can then be applied to other cancers and diseases that rely on a protein-mediated pathway.
- If these drug treatments are successful, a chemical library can be established

References

1. Paiva, S., Crews, C. M. “Targeted protein degradation: elements of PROTAC design.” *Current Opinion in Chemical Biology*. **2019**, 50, pp. 111-1119.
2. Kelesa, T. K., et al. “Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins.” *Nature Chemical Biology*. **2011**, 7, pp. 538-543
3. Park, S., Kwon, Y. “Facile solid-phase parallel synthesis of linear and cyclic peptoids for comparative studies of biological activity.” *ACS Comb. Sci.* **2015**, 17, pp. 196-201.
4. Teutsch, G., et al. “Non-steroidal antiandrogens: synthesis and biological profile of high-affinity ligands for the androgen receptor.” *J. Steroid Biochem. Molec Biol*. **1994**, 48, 1, pp. 111-119.
5. Cyrus, K., et al. “Impact of linker length on activity of PROTACs.” *Mol Biosyst*. **2011**, 7, 2.
6. Burkoth TS, Fafarman AT, Charych DH, Connolly MD, Zuckermann RN. Incorporation of unprotected heterocyclic side chains into peptoid oligomers via solid-phase submonomer synthesis. J Am Chem Soc. 2003;125(29):8841–5.