Development of the small-molecule HTR-81: a cancer targeting, anti-proliferative compound

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Background

The mitochondrial protein caseinolytic protease P (ClpP) is a mitochondrial protease that is involved in mitochondrial protein quality control[1].

When ClpP is hyperactivated by ClpP agonists (i.e. TR-81), ClpP inhibits mitochondrial transcription, disrupts mitochondrial metabolism, and impedes cancer cell growth and proliferation through degrading specific mitochondrial proteins[2].

Due to the high potency of the TR compounds, these small molecules are being pursued as anti-cancer drugs, but targeting the drug to the cancer cells is a concern[3].

Objectives

- Develop a small-molecule drug that activates ClpP and targets cancer cells specifically
- Create a bifunctional molecule that utilizes extracellular HSP90 to target Clp activator of cancer cells
- Investigate dose and kinetic properties of TR-81 and HTR-81 in cancer cell model

Methods

SUM159 and MDA-MB-231 human triple negative breast cancer cell lines were used in these studies.

The small molecules TR-81 and HTR-81 were used at varying concentrations and studied by:

- EDC Coupling Reaction enabled the creation of HTR-81 followed by HPLC to purify the product
- Thin Layer Chromatography, LC-MS, and NMR to confirm the occurrence of reaction and validate the creation of HTR-81
- Immunoblotting to assess TR-81 and HTR-81's effects on downstream targets related to cellular and mitochondrial stress.
- Dose response curve assessing the response of cancer cell lines to TR-81 and HTR-81 dosage.

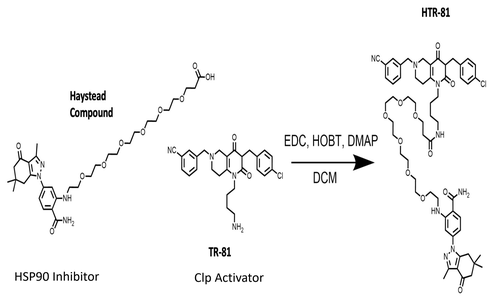


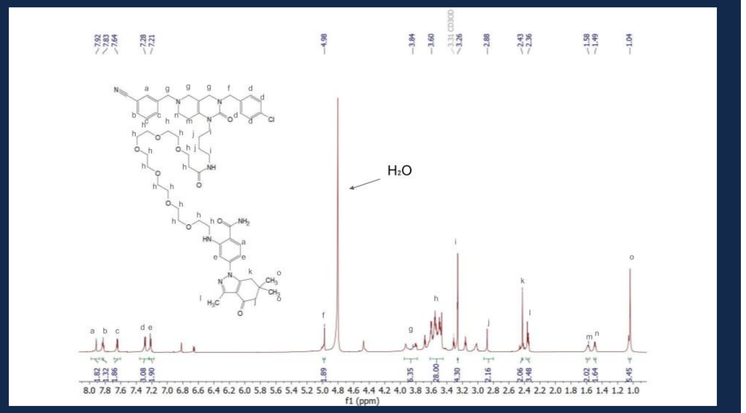
Figure 1: HTR-81 EDC Coupling Reaction
Carbodiimide chemistry conjugation performed at RT, the Haystead compound and TR-81 compound were allowed to react for 3 hrs to produce the HTR-81 product

Results

Figure 2: Proton NMR Confirms HTR-81 Synthesis

HTR-81 dissolved in deuterated methanol. Product of 95% purity confirmed.

Validated by TLC, LCMS, and NMR



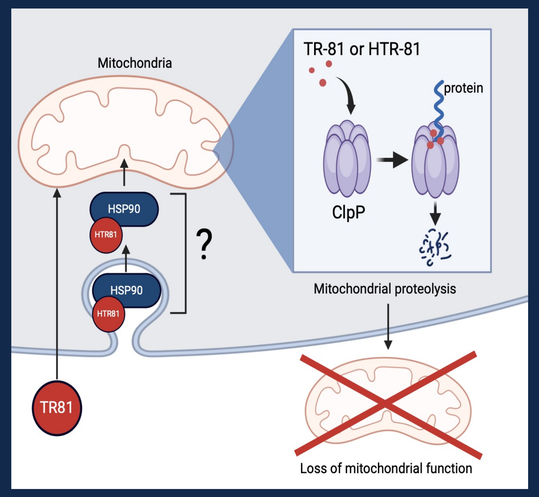


Figure 3:
Model for application of TR-81

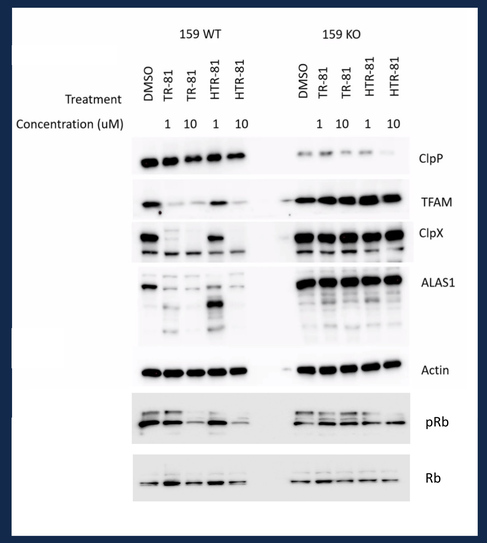
HSP90 serves as specific binding site for HTR-81 enabling targeted endocytosis of the compound to the mitochondria.

Objective of determining if TR-81 and HTR-81 serve as ClpP agonists leading to degradation of mitochondrial proteins and loss of mitochondrial function.

Figure 4:
Characterization of the Effect of TR-81 and HTR-81 on Cancer Cell Lines

Analysis of indicated proteins in response to treatment of TR-81 and HTR-81 for 24 hours in WT and ClpP KO SUM 159 lines.

Proteins depleted after TR-81 and HTR-81 treatment measured by Western Blotting



Results Continued

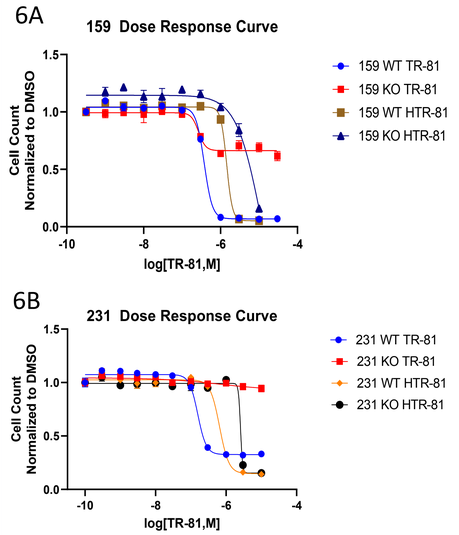


Figure 6: Triple Negative Breast Cancer Cell Growth is Inhibited by TR-81 and HTR-81

A: Comparison of drug sensitivity in SUM 159 cell line treated with HTR-81 and TR-81 in log10 (n=2 replicates) for 3 days.

B: Comparison of drug sensitivity in MDA-MB-231 cell lines treated with HTR-81 and TR-81 in log10 (n=2 replicates) for 3 days.

Discussion

- Chemical reaction generated the HTR-81 product as confirmed by proton NMR
- TR and HTR -81 compounds induce mitochondrial stress causing the degradation of TFAM, ClpX, and ALAS1 (Clp substrate proteins)
- HTR-81 is a dual acting small molecule drug that acts regardless of ClpP presence
- Future Directions: Determine if HTR-81 is getting into the mitochondria by targeting and binding to extracellular HSP90

References

1. Daglish, S. C. D., et al. (2023) *MDPI.*
2. Graves, P. R., et al (2022) *ACS Chemical Biology.*
3. Fennell, E. M. J., et al. (2022) *Pharmacology Research and Perspectives.*
4. Howe, M. K., et. al, (2014) *Chem Biol.*

Acknowledgements

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