NMR - Restrained Structural Elucidation of Mitoxantrone Conjugates with GnRH Peptide Analogues

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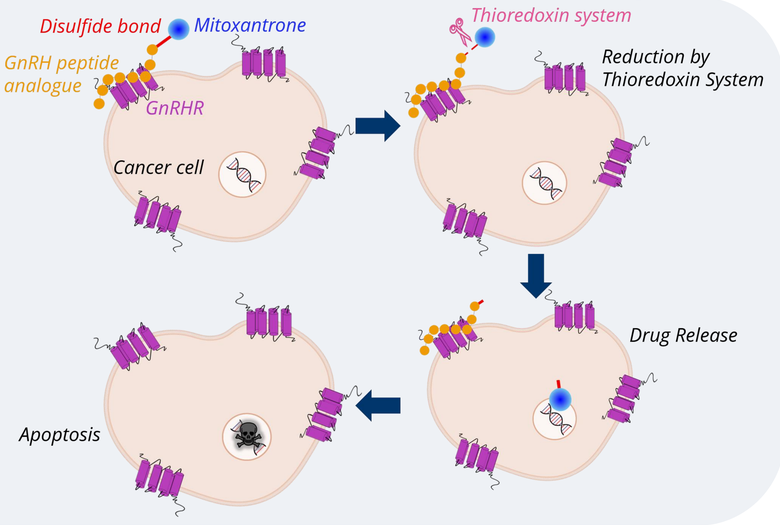
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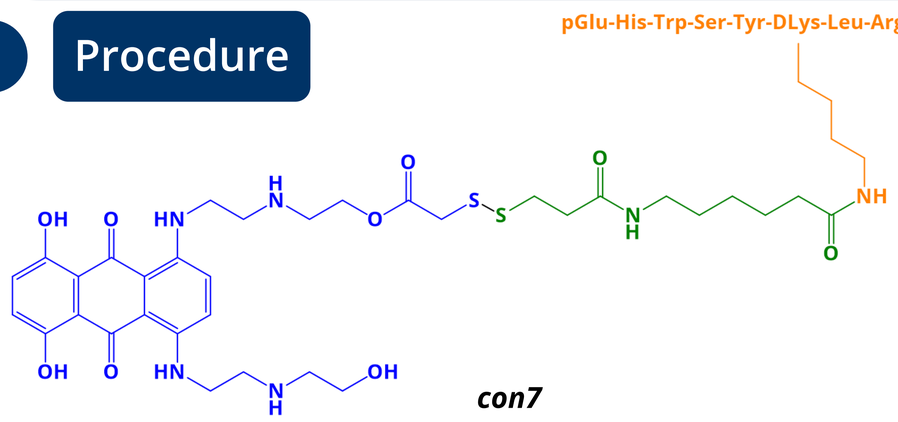
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Introduction

Gonadotropin-releasing hormone receptors (GnRHRs) are overexpressed in several hormone-dependent cancers, making them promising targets for selective drug delivery[1,2]. GnRH peptide–drug conjugates exploit GnRHR-mediated targeting to preferentially deliver cytotoxic agents to cancer cells[2]. **GnRH–mitoxantrone conjugates** have demonstrated selective **anticancer activity** against endometrial and ovarian cancer cells[3-5]. The cytotoxic molecule is linked to the peptide through a disulfide bond, enabling thioredoxin-mediated cleavage and drug release. However, their **structural features** and peptide–drug interactions involved in receptor recognition remain insufficiently understood. Therefore, these GnRH–mitoxantrone conjugates incorporating peptide analogues derived from the GnRH agonist Leuprolide were structurally characterized to elucidate their conformational properties, drug orientation, disulfide-bond accessibility, and implications for GnRHR recognition[6].



1 Procedure



Study Overview

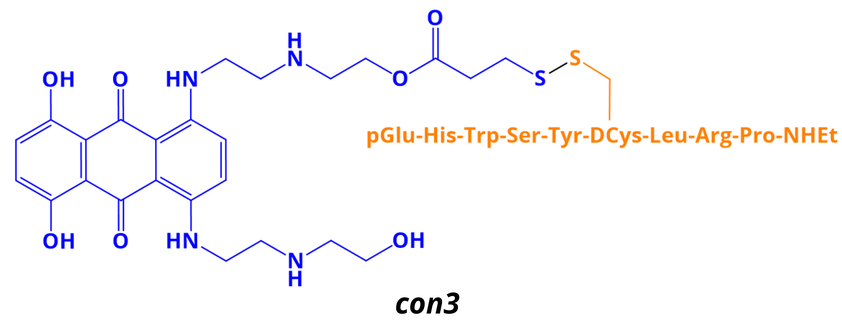
A) Synthesis of conjugates

B) ^{1}H and ^{13}C Assignment

C) NOESY spatial proximities estimation

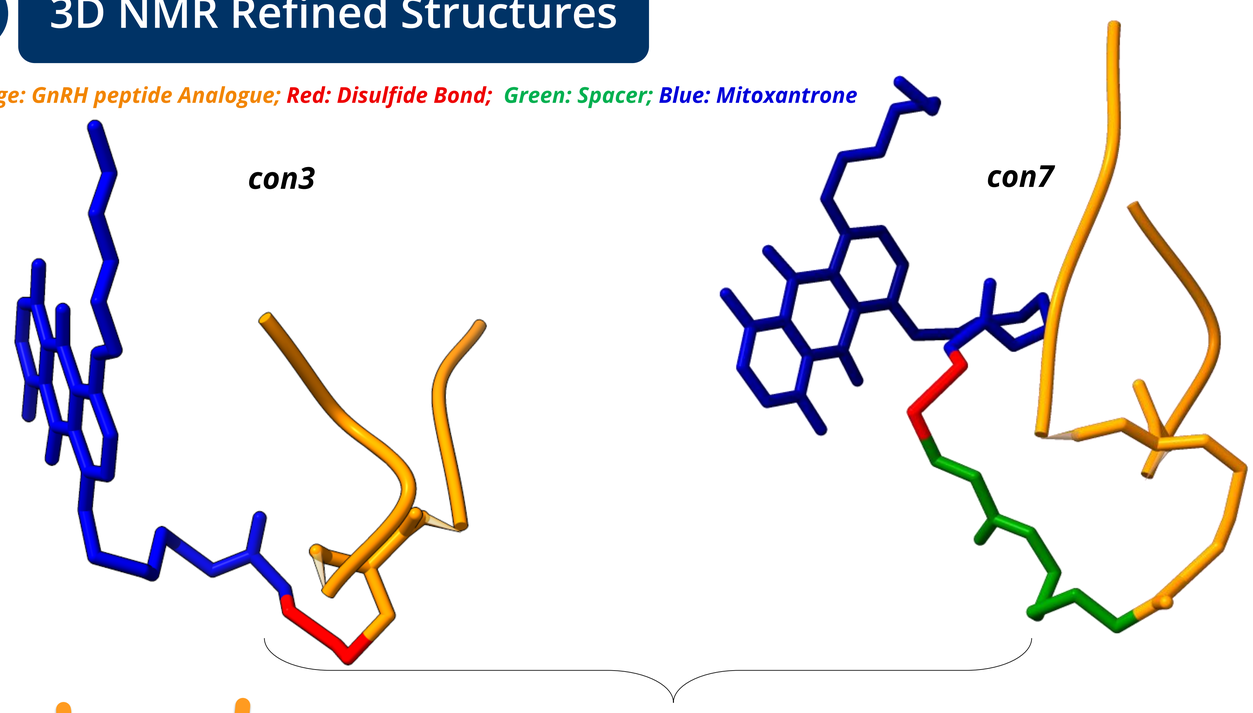
D) NMR-restrained MD refinement

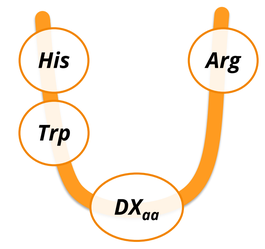
E) Docking studies



2 3D NMR Refined Structures

Orange: GnRH peptide Analogue; Red: Disulfide Bond; Green: Spacer; Blue: Mitoxantrone



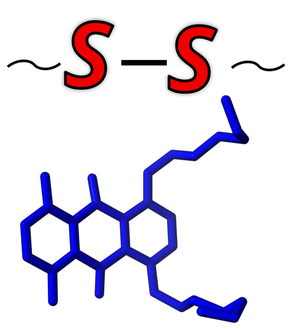


U-shaped peptide backbone

DX_{aa}: DCys[6] or DLys[6], maintain a compact bend conformation

Receptor recognition and binding residues exposed

His[2], Trp[3] and Arg[8] remain solvent-exposed



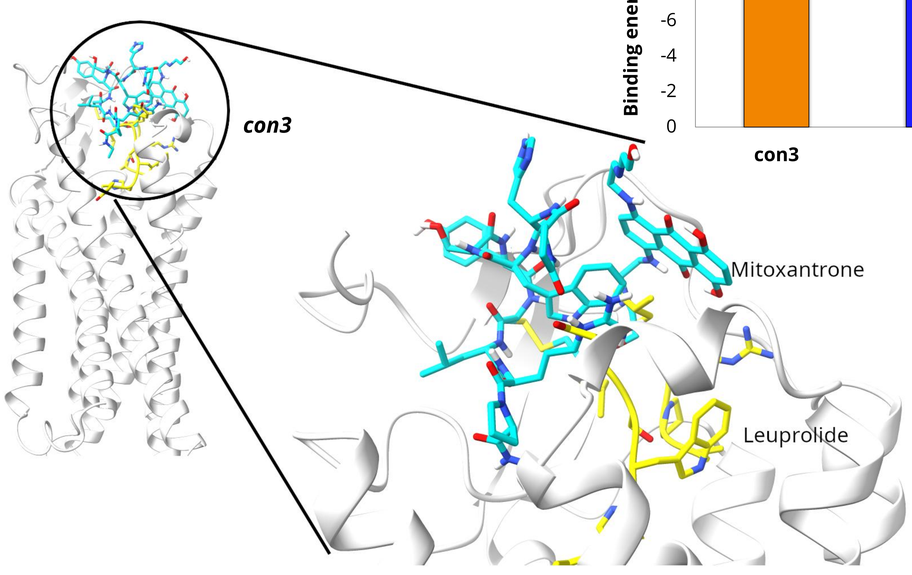
Accessible disulfide bond

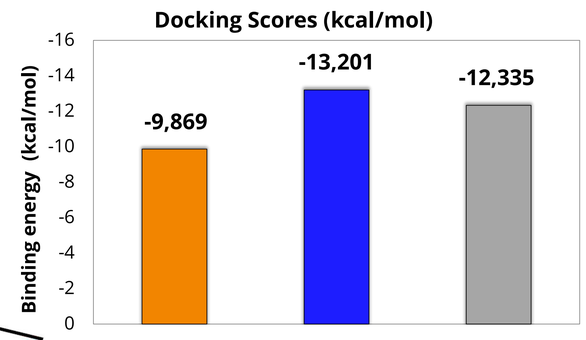
Disulfide bond is solvent-exposed and available for reduction

Mitoxantrone analogue away from the peptide backbone

The mitoxantrone moiety adopts an extended orientation away from the peptide backbone.

3 Docking Studies





Con7 showed a more favorable docking score than Leuprolide, while con3 displayed a less favorable score. The mitoxantrone analogue did not show interactions with GnRHR.

Both conjugates adopted **binding modes** compatible with **GnRHR recognition and binding**, indicating that receptor interactions are primarily mediated by the **peptide moiety**.

4 Conclusions

- ✓ *Con3* and *con7* adopt compact, bent conformations characterized by a U-shaped peptide backbone, consistently supported by NMR spectroscopy and MD simulations.
- ✓ The different substitution at position 6, DCys in *con3* and DLys in *con7*, has a limited influence on the peptide conformation.
- ✓ Key GnRHR recognition residues (His[2], Trp[3] and Arg[8]) remain solvent-exposed and accessible for receptor recognition in both conjugates.
- ✓ The disulfide bond is solvent-exposed and accessible for reduction by thioredoxin system.
- ✓ Docking of the NMR-refined structures supports their compatibility with GnRHR recognition and provides a structural basis for the previously reported biological activity[3].

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References

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