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Rational design, synthesis and evaluation of mitoxantrone conjugated with mutated Gonadotropin Releasing Hormone (GnRH) for the treatment of hormone-dependent cancer

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Introduction

Gonadotropin-releasing hormone (GnRH) or luteinizing hormone-releasing hormone (LHRH), has a significant role in fertility by initiating the regulation of gonadal axial, (1, 2). Several GnRH peptide analogues have been clinically used for the treatment not only to address fertility issues but also to treat hormone-dependent cancers, as GnRH receptors (GnRHR) are overexpressed in cancer cells. A promising strategy for selective immunosuppression of cancer cells, includes the development of delivery systems based on GnRH analogues conjugated with anti-cancer drugs to target the GnRHR on cancer cells (3). This targeted approach is advantageous due to the higher expression of GnRH receptors compared to healthy cells, allowing for more precise and effective treatment (2). In this study, we designed a modified mitoxantrone molecule (immunosuppressing agent) conjugated with an altered GnRH peptide *via* a disulfide bond (3). This conjugation aims to facilitate the *in situ* release of mitoxantrone through the reduction of the disulfide bond by the thioredoxin system.

Experimental

General synthetic route:

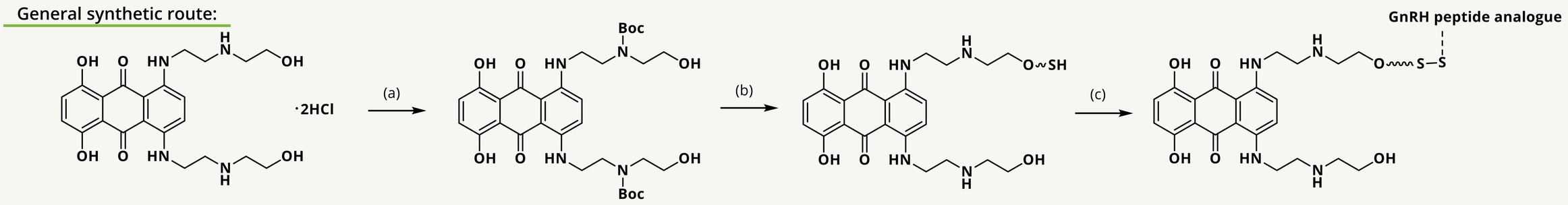
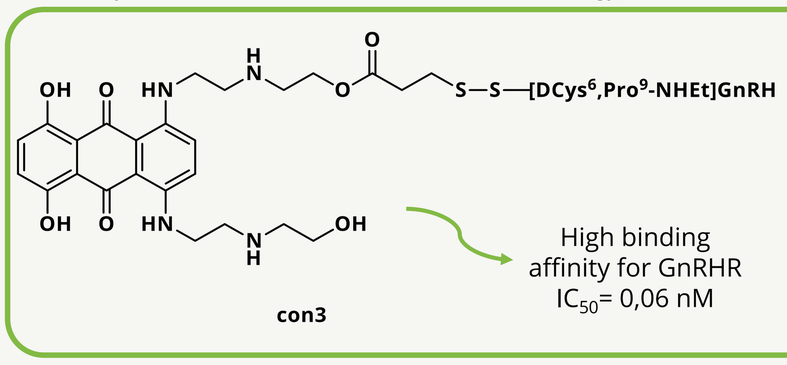


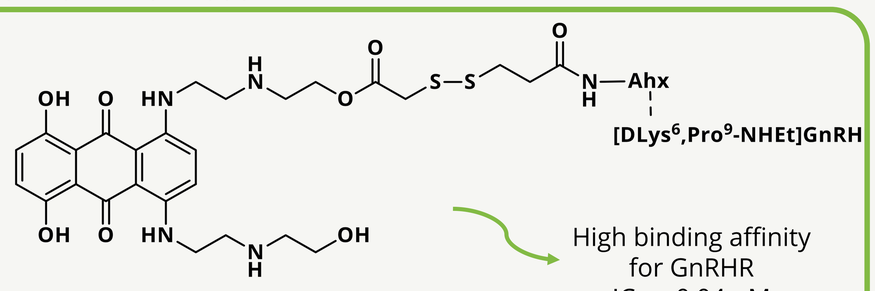
Fig.1: General synthetic route of conjugates. **(a)** Protection of aliphatic secondary amines: Boc_2O, anhydrous MeOH/THF, TEA, from 0 °C for 1 h to RT for 2 h; **(b)** i) Modified Steglich esterification on one aliphatic hydroxyl group: 3-(2-pyridyldithio)propanoic acid (con3) or $TrtSCH_2CO_2H$ (con7), DMAP, HOBt, DIC, CH_2Cl_2, 0 °C, 24 h; ii) Removal of protecting groups: TFA, TES, CH_2Cl_2, RT, 1.5 h; **(c)** Synthesis of conjugates: GnRH peptides analogues (synthesised in solid phase using CLTR-Cl or Ethyl Indole AM resin *via* Fmoc/ tBu methodology), MeOH, RT, 24 h (con3) or 6 h (con7) (3).

Structure of synthesised conjugates



con3

High binding affinity for GnRHR IC_{50}= 0,06 nM



con7

High binding affinity for GnRHR IC_{50}= 0,04 nM

Identification of synthesised conjugates by ESI-MS and 1D/ 2D NMR studies.

Pharmacological evaluation

Cytotoxicity studies

Cytotoxicity studies achieved using **MTT assay**

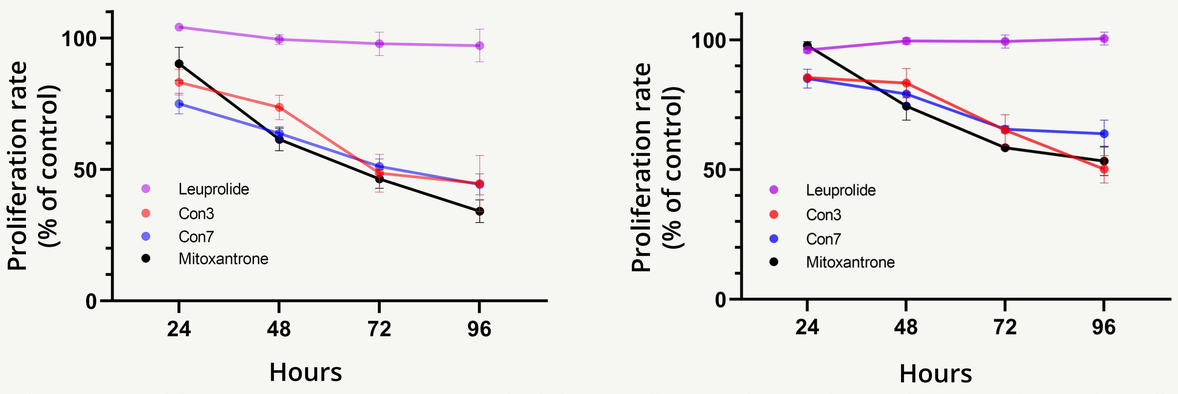


Fig.2: Antiproliferative activity in SKOV-3 and Ishikawa cells over the incubation hours (4 days). The cells were treated with 1μM of con3, con7, mitoxantrone analogue and the commercially available agonist Leuprolide. All analogs decreasing the proliferation rate in a time dependent manner.

- Cytotoxicity studies using MTT assay showed that the proliferation rate decreased in a **dose dependent manner** (data not shown)
- Leuprolide **did not affect** the viability of cells
- Conjugates con3 and con7 reduce the proliferation rate due to **the presence of mitoxantrone analogue**

Inhibition of Thioredoxin System

Cisplatin: Inhibitor of Thioredoxin Reductase ⟶ Thioredoxin system is not functional

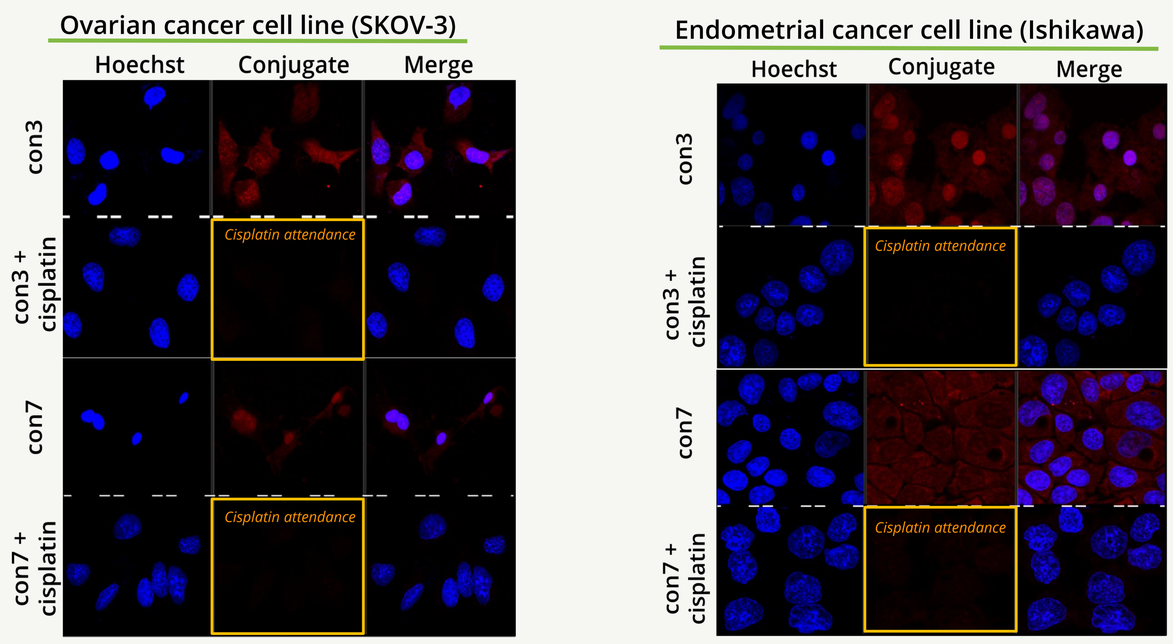


Fig.3: Confocal microscopy images of SKOV-3 and Ishikawa cells treated with con3 or con7 (1μM for 6 h) in the presence or absence of cisplatin. Cells' nuclei staining with Hoechst dye (blue) and the red ones depict the mitoxantrone fluorescence.

- Internalization of mitoxantrone analogue **only** when thioredoxin system is **functional** (active thioredoxin reductase).

Conclusions

The synthesised mitoxantrone-GnRH peptide conjugates were identified by 1D / 2D NMR studies and pharmacologically evaluated using several cancer cell lines. The conjugates con3 and con7 reduce cell proliferation in a time- and dose-dependent manner in SKOV-3 and Ishikawa cell lines. The cytotoxic effect of these conjugates is attributed to the release of the mitoxantrone analogue through the reduction of the disulfide bond by the cancer cells' thioredoxin system. This effect is confirmed by using cisplatin, an inhibitor of the thioredoxin system, which abolishes the intracellular accumulation of mitoxantrone.

References

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