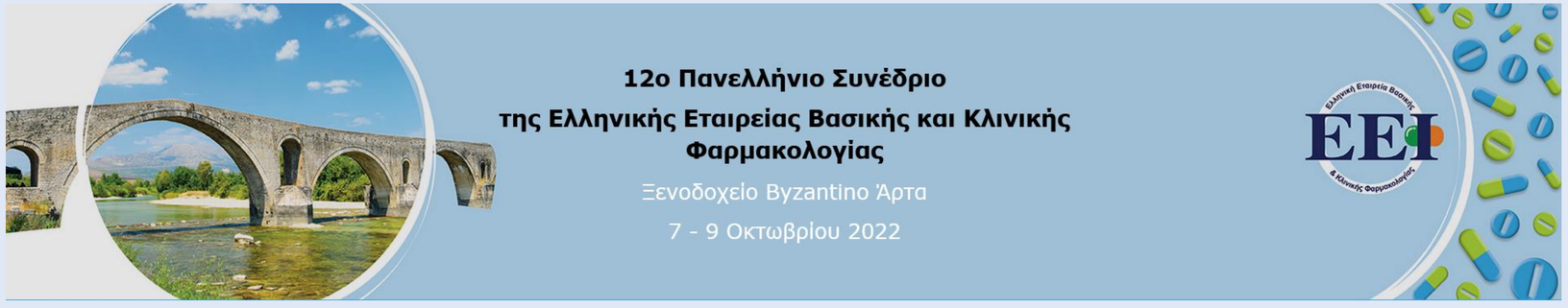

Antiproliferative effects of a novel GnRH analogue conjugated with mitoxantrone

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INTRODUCTION

The gonadotropin-releasing hormone (GnRH) plays a key role in the function of the reproductive system through its interaction with its receptor (GnRH-R) as it regulates the circadian secretion of follicle-stimulating hormone (FSH) & luteinizing hormone (LH), which in turn affect steroidal sex hormone production[1,2]. However, GnRH seems to have a direct action on certain cancer types as GnRH-R is also highly expressed in various types of breast and genital tumor cells, including ovarian ones. Previous studies have shown that GnRH analogues (such as leuprolide) exert antitumor effects on ovarian and other cancer cells, through their interaction with the GnRH-R expressed in these cells[3,4].

AIM

In this study we aimed to develop novel GnRH analogues with antitumor properties, by conjugating an analogue of leuprolide, with the cytotoxic agent, mitoxantrone, thus creating the conjugate Con7. Con7 is anticipated to release mitoxantrone into cancer cells after its binding to GnRH-R and subsequent internalization of the GnRH-R/Con7 complex. The release of mitoxantrone from con7 will be achieved with the use of the thioredoxin reductase system. This system expressed in cancer cells decomposes the con7 conjugate into its free peptide and the cytotoxic drug mitoxantrone.

METHODS

To create the con7 conjugate we chemically modified the GnRH analogue, leuprolide, and conjugated it with mitoxantrone. Mitoxantrone is an already known cytotoxic compound, that is used to treat certain types of cancer. We conjugated mitoxantrone with the modified leuprolide, to achieve targeted therapy. In order to examine the antiproliferative effects of con7, we used the MTT assay[5]. The MTT assay is a colorimetric assay, which determines cell metabolic activity, an indicator of cell proliferation. We incubated the ovarian cancer cell line, SK-OV-3, with con7 at different concentrations for 1-4 days, in order to determine its antiproliferative activity.

RESULTS

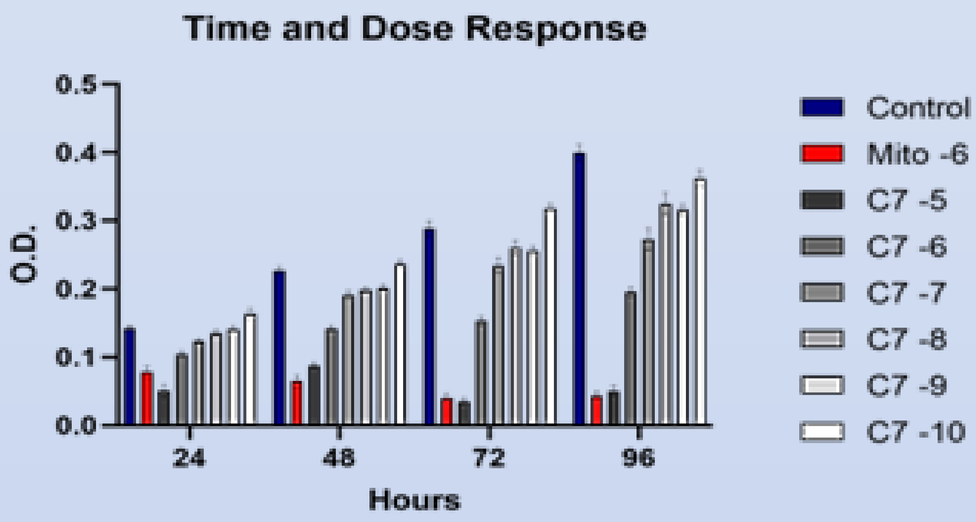

***Figure 1**: Proliferation of SK-OV-3 cells at different days*

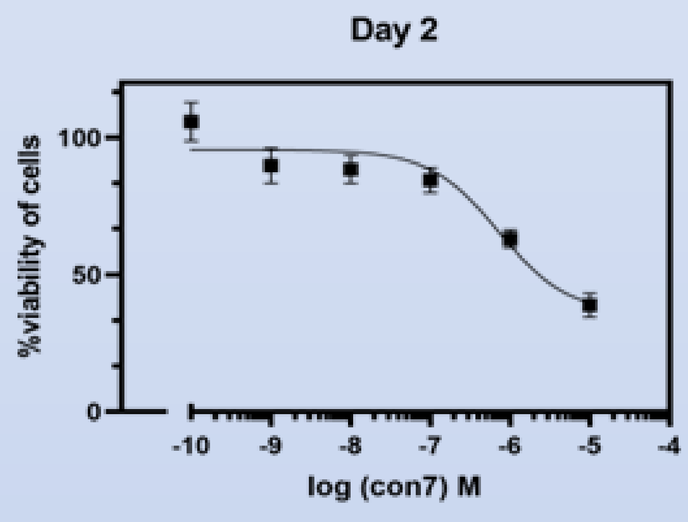

Figure 2**: Antiproliferative effects of increasing concentrations of con7 on **day 2

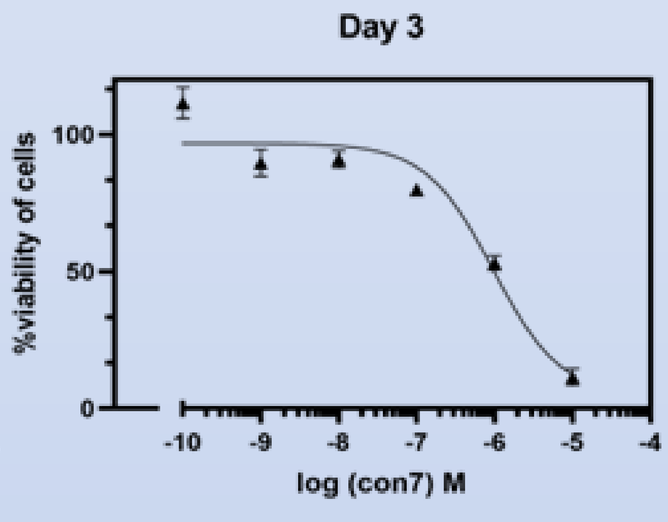

Figure 3**: Antiproliferative effects of increasing concentrations of con7 on **day 3

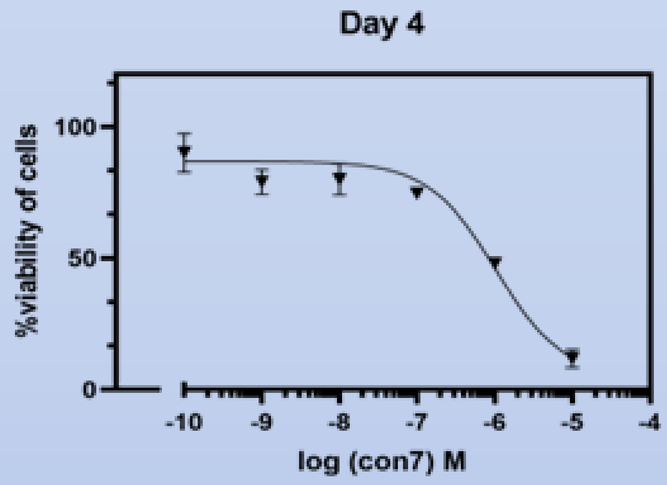

Figure 4**: Antiproliferative effects of increasing concentrations of con7 on **day 4

We tested the ability of con7 to inhibit the proliferation of ovarian cancer SK-OV-3 cells. The results have shown that the proliferation of SK-OV-3 cells was inhibited by Con7 in a time-dependent manner (1-4 days) (Figure 1). Interestingly, Con7 inhibited the proliferation of SK-OV-3 cells in a dose-dependent manner (Figures 2-4). The antiproliferative potency of con7 after exposure of SK-OV-3 to peptide for 2,3 and 4 days, was 1.4 μM, 0.72 μM, 0.97 μM, and 0,83 μM, respectively.

CONCLUSIONS

- Proliferation of SK-OV-3 cells was inhibited by Con7 in a time and dose-dependent manner.
- Con7 could set the basis in developing novel cytotoxic agents specifically targeting GnRH-R expressing tumors

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