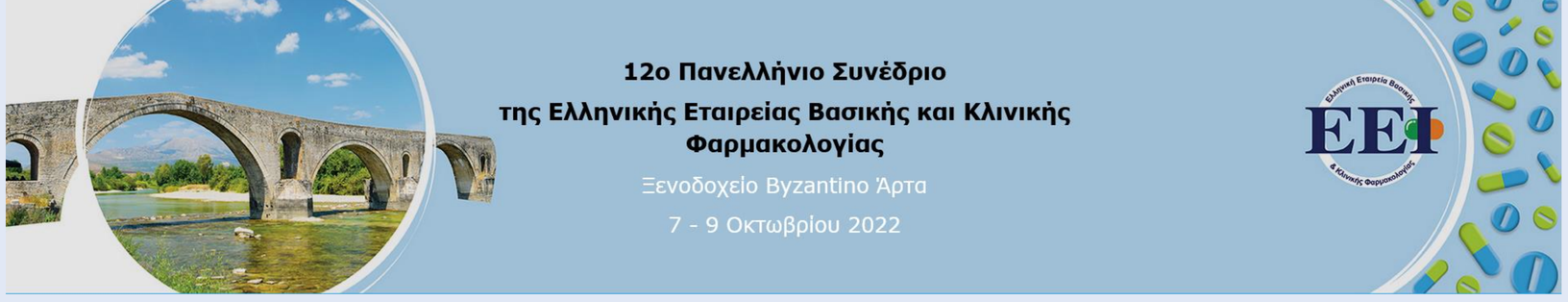

Pharmacological properties of novel GnRH analogues conjugated with anthraquinones

Christos Markatos[1] , Vlasios Karageorgos[1] , Georgia Biniari[2] , Michalis Deiktakis[3] , Maria Venihaki[3] , Theodore Tselios[2] , George Liapakis[1]

[1] Department of Pharmacology, School of Medicine, University of Crete, Heraklion, Greece

[2] Department of Chemistry, University of Patras, 26504 Rion, Greece

[3] Department of Clinical Chemistry, School of Medicine, University of Crete, Heraklion, Greece

INTRODUCTION

Gonadotropin releasing hormone (GnRH) is a hypothalamic decapeptide able to bind specific receptors on pituitary gonadotrope cells and modulate the synthesis and secretion of gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In turn, these hormones regulate gonadal steroidogenesis and gametogenesis[1,2]. The receptor (GnRH-R) for GnRH, in addition to its essential role in the function of the reproductive system is also highly expressed in different types of cancer cells. Previous studies have shown that GnRH analogues exert antiproliferative actions in various cancer cells, through their interaction with the GnRH-R expressed in these cells[3,4].

AIM

In the present study we aimed to design, synthesize and pharmacologically characterize novel GnRH analogues conjugated with anthraquinones or their derivative, mitoxantrone (con1-con8). Several (or all) of these analogues (and especially those containing mitoxantrone) are anticipated to have cytotoxic properties by releasing into cancer cells the anthraquinone group after their interaction with the GnRH-R expressed in these cells and their subsequent internalization in complex with the receptor.

METHODS

We created the con1-con8 analogues by modifying the GnRH analogue, leuprolide and conjugated it with anthraquinones or their derivative, mitoxantrone. Leuprolide is an already known agonist of GnRH receptor. To evaluate the pharmacological properties of GnRH analogues we determined their binding affinities in competition radioligand binding studies using membrane homogenates from HEK 293 cells stably expressing the GnRH-R, and the [^{125}I]-DTyr^{6}-His^{5}-GnRH as radioligand[5]. Data were analyzed by nonlinear regression analysis and apparent binding affinities (IC_{50} values) were obtained by fitting the data to a one-site competition model.

RESULTS

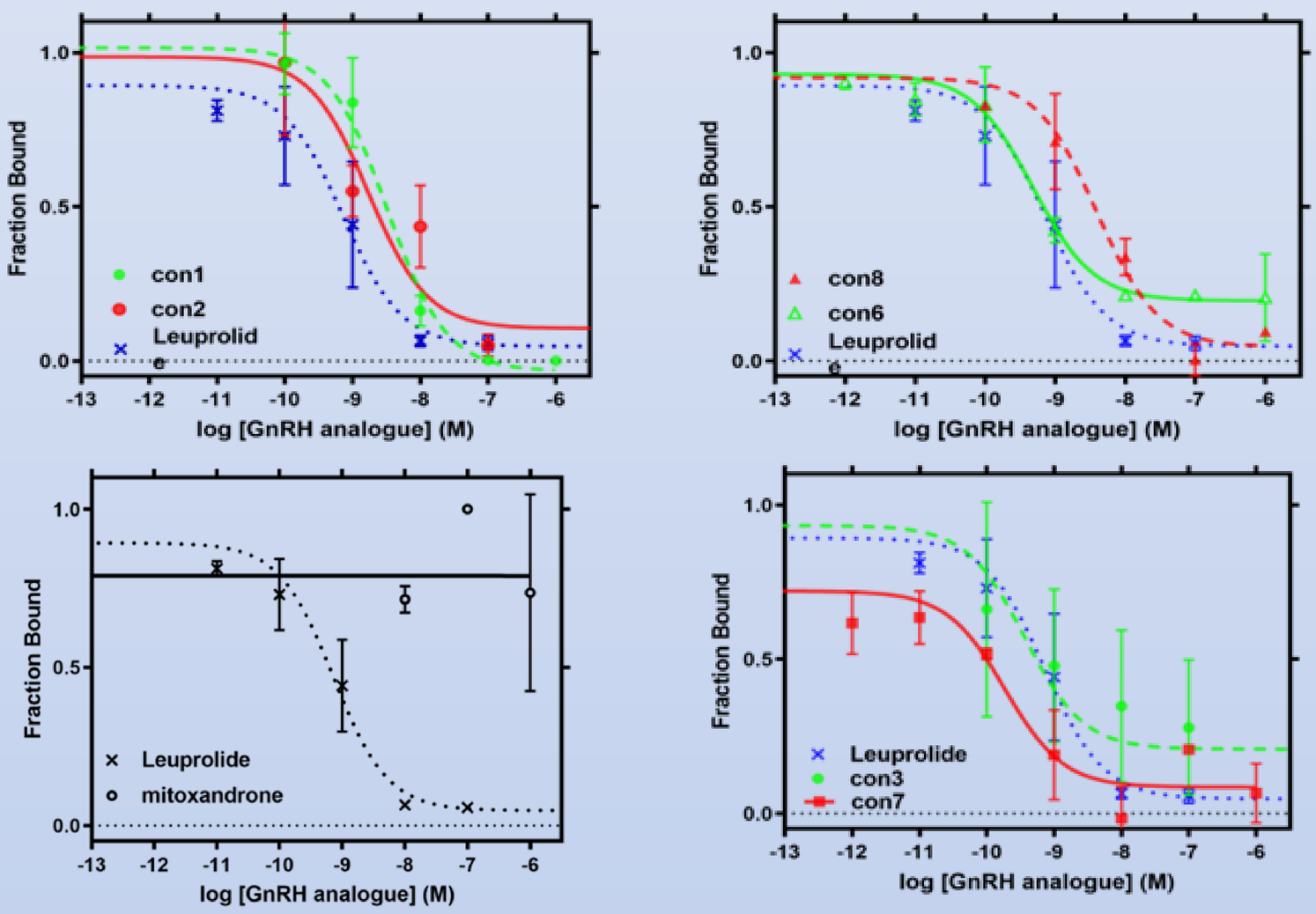

Figure 1: *Radioligand binding assay using increasing concentrations of some of the novel GnRH analogues, compared with leuprolide.*

We pharmacologically evaluated the con1-con8 analogues by testing their ability to inhibit the specific binding of [^{125}I]-DTyr^{6}-His^{5}-GnRH to membranes from HEK 293 cells stably expressing the GnRH-R. All compounds decreased the specific binding of [^{125}I]-DTyr^{6}-His^{5}-GnRH in a dose-response manner, with affinities (0.04-3.5 nM) higher or similar to that of leuprolide (control, 0.6 nM). The compounds with the highest binding affinities were con3 (0.06 nM), con6 (0.07 nM), and con7 (0.04 nM). In contrast to con1-con8 mitoxantrone did nor bind to the GnRH-R.

CONCLUSIONS

1. The GnRH analogues (con1-con8) conjugated with anthraquinones or its derivative, mitoxantrone, bind to GnRH-R with high affinities, similar to or higher than leuprolide. In contrast, mitoxantrone did not bind to the GnRH-R.
2. Con3, and con7, which contain the cytotoxic mitoxantrone, have higher affinities than the other analogues. Additional studies will determine the antiproliferative effects of these analogues.

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CONTACT INFORMATION

George Liapakis. Department of Pharmacology, School of Medicine, University of Crete, Heraklion, Greece, liapakig@uoc.gr