Use of fluorescent yeast-based biosensors for evaluation of the binding affinities of new steroid hormone and bile acid derivatives for select steroid receptors

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INTRODUCTION

Steroid hormones regulate essential physiological processes, including reproductive functions, immune system, carbohydrate metabolism, mineral balance, etc. Their action is mediated through steroid receptors that belong to superfamily of nuclear receptors, class of ligand-activated transcription factors. In addition to their physiological role, steroid hormones may also be involved in the development of hormone-dependent cancers (prostate, breast), leading causes of death among men and women worldwide. Proliferation of these cells is influenced by the levels of steroid hormones, so effective treatment strategies are focused on blocking steroid hormones activity targeting steroid receptors and preventing binding of endogenous hormones (**Figure 1**). Identification of compounds that modulate the activity of androgen (AR) or estrogen receptors (ER) is one of the major goals in the design of new treatments of hormone-dependent cancers. Similarly, glucocorticoid receptor (GR) ligands are used to treat autoimmune and inflammatory diseases, but their long-term use is often associated with numerous undesirable side effects and drug resistance, so great effort has been made to find new modulators.

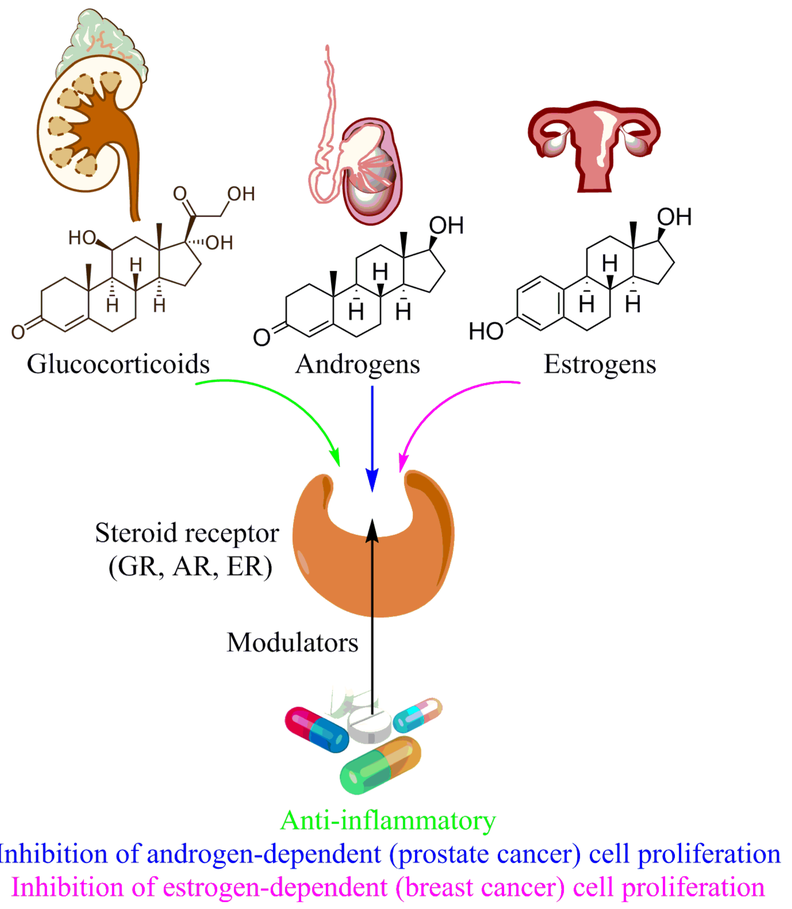

***Figure 1**. Clinical significance of modulation of steroid receptor activity.*

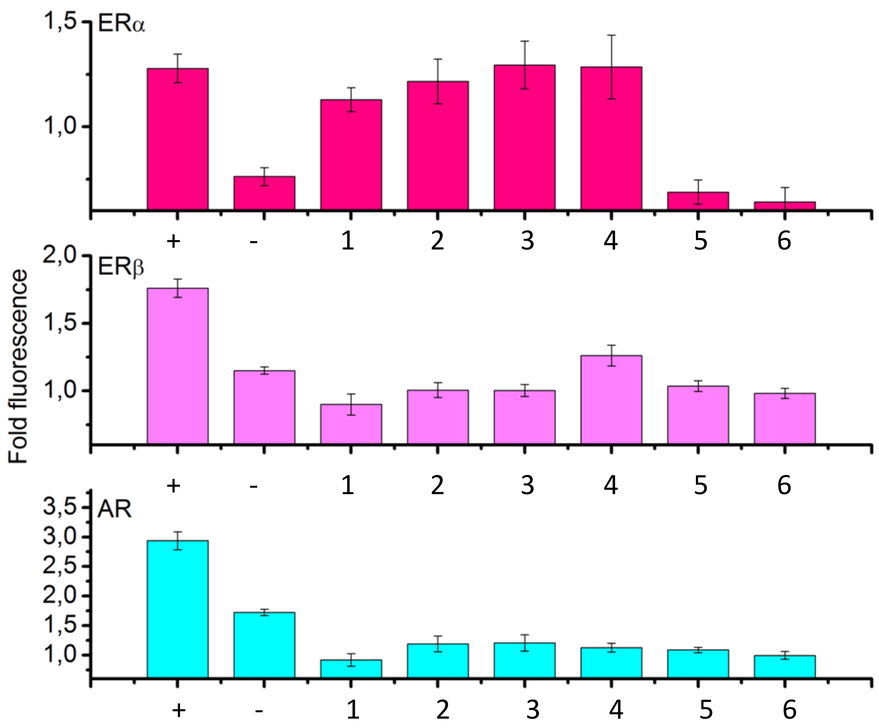

***Figure 4**. Relative binding affinities* expressed as fold fluorescence change between ligand-treated and control cells in the absence of ligand expressing ERα LBD-YFP, ERβ LBD-YFP, AR LBD-YFP and GR LBD-YFP.

AIM

Biosensors developed in yeast cells are attractive research area in biomedicine because they allow for detection of molecules with various structures and biological activities, economically and simply, without use of harmful radioactive materials. Our goal was to apply a fluorescent biosensor in yeast for identification of new steroid receptor ligands, following previously described procedures [1-5]. In the focus of this research was evaluation of the binding affinities of steroid hormone and bile acid derivatives. This biosensor is based on the expression of the ligand binding domain (LBD) of ERα, ERβ, AR or GR fused with yellow fluorescent protein (YFP) in *Saccharomyces cerevisiae*. After ligand binding-induced dimerization of steroid receptor, fluorescence resonance energy transfer (FRET) between two YFP molecules occurs causing an increase in fluorescence (**Figure 2).**

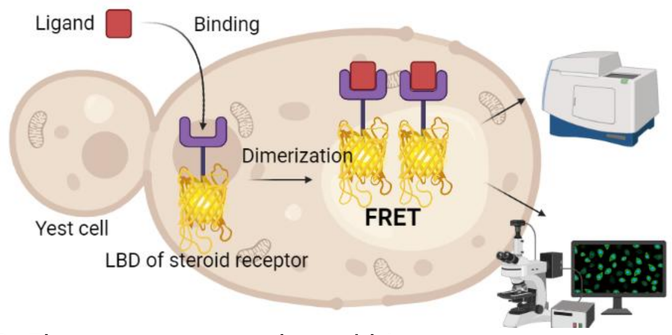

***Figure 2**. Fluorescent yeast-based biosensor.*

MATERIAL AND METHODS

Lithium acetate/polyethylene glycol transformation of yeast cells.

Growth to saturation in selective media (supplemented with 2% raffinose) at 28°C.

Dilution of saturated yeast cells and incubation until log phase of growth.

Galactose (2%) induction of protein expression and addition of tested compounds dissolved in DMSO.

Incubation for additional 14-16h at 25°C and fluorimetric detection

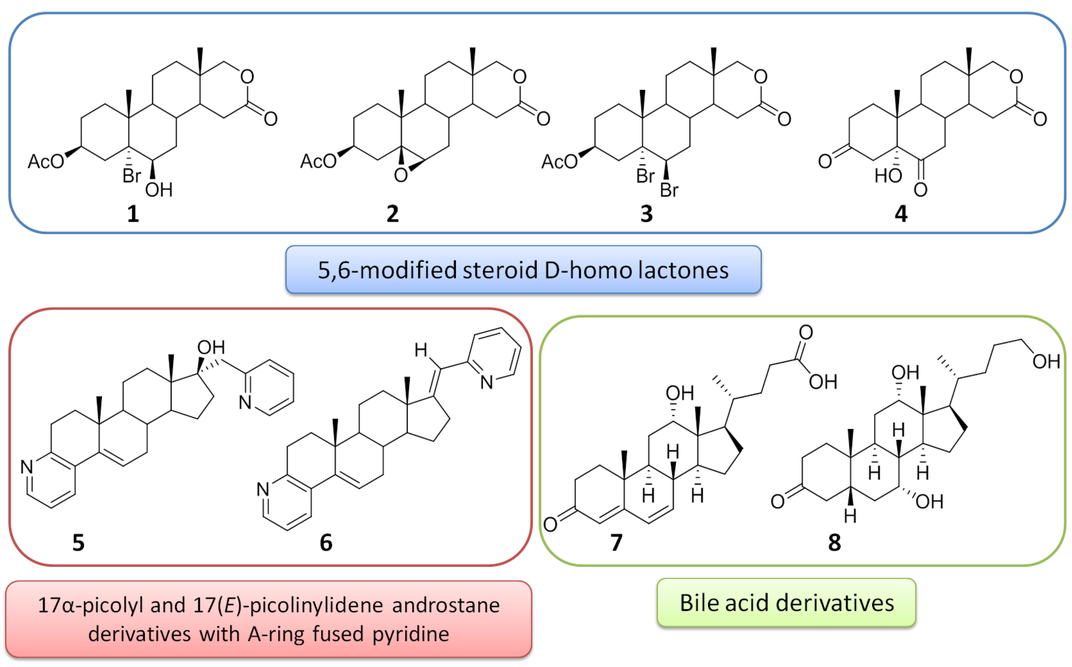

***Figure 3**. Classes of tested compounds.*

RESULTS AND DISCUSSION

As can be seen from **Figure 4.** none of tested compounds showed affinity for AR. Among tested 5,6-modified steroid D-homo lactones, all compounds displayed high affinity for ERα, similar to natural ligand, estradiol, while binding of derivative **4** for ERβ was weak [4]. Binding of compounds **1**-**3** for ERβ was not detected. Our results also showed that 17α-picolyl and 17(*E*)-picolinylidene androstane derivatives with A-ring fused pyridine have negligible estrogenicity and androgenicity in a fluorescent yeast-based assay [3]. On the other hand, binding affinity of bile acid derivatives, dienone **7** and C-24 alcohol **8** for GR was moderate. To elucidate mechanisms of action for these compounds, additional experiments are necessary, and to better understand the molecular interactions within the ligand-binding pocket of the receptor, molecular docking analysis can be conducted.

CONCLUSION

Yeast-based fluorescent biosensors used in this work have proven to be very useful for *in vitro* screening of novel anti-cancer and anti-inflammatory drug candidates, as well as for elimination of compounds that do not deserve further attention and resources due to their lack of desired bioactivities.

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