Identification of novel ERβ ligands

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INTRODUCTION

Estrogen receptor (ER) is a major therapeutic target in the treatment of estrogen-related diseases, such as breast cancer, the most frequently diagnosed life-threatening cancer in women. There is a need to develop potent ER ligands capable of selective targeting of cancer cells without affecting normal cells. Blocking ERα action by antagonists and inhibition of steroidogenic enzymes is standard therapy in the treatment of breast cancer for many years. On the other hand, ERβ isoform usually has anti-proliferative and tumor-suppressive functions, so targeting ERβ with specific agonists represents new promising approach not only in breast cancer therapy, but also prostate. Beside anticancer activity of ERβ agonists, their application is considered in the treatment of depression, anxiety and inflammation. In medicinal chemistry significant research attention has been paid to the synthesis of steroid derivatives and investigation of their biological activity. In order to obtain potent antiproliferative agents triazole ring is often incorporated as a pharmacophore into the steroid skeletion.

MATERIALS AND METHODS

Saccharomyces cerevisiae FY250 strain (MATα, *ura3-52, his32Δ00, leu2Δ1, trp1Δ6*) and plasmid constructs pRF4-6-hERβ LBD-EYFP and pRF4-6-hAR LBD-EYFP for the flurescent cellular sensor used in this study, were generously provided by Dr. Blake Peterson (The University of Kansas). LBD of steroid receptor was expressed in-frame with yellow fluorescent protein (YFP) in *Saccharomyces cerevisiae* (**Figure 1**). Upon ligand-binding induced dimerization, fluorescence resonance energy transfer (FRET) between YFP molecules was analyzed by fluorescence spectroscopy and microscopy.

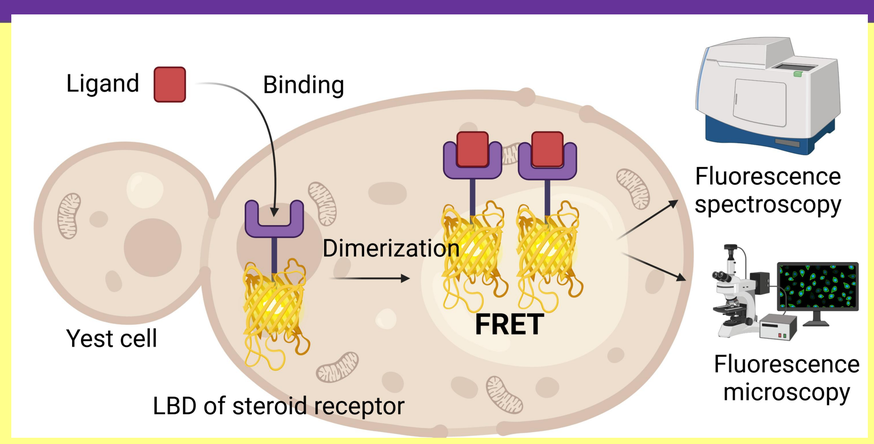

***Figure 1**. FRET-based assay in yeast (Created with BioRender.com).*

AIM OF THE STUDY

The aim of this study was to evaluate binding affinities of novel *N*(2)-substituted D-condensed steroidal triazoles (**Figure 2**) for ligand-binding domains (LBDs) of ERβ and androgen receptor using yeast-based fluorescent assay [1].

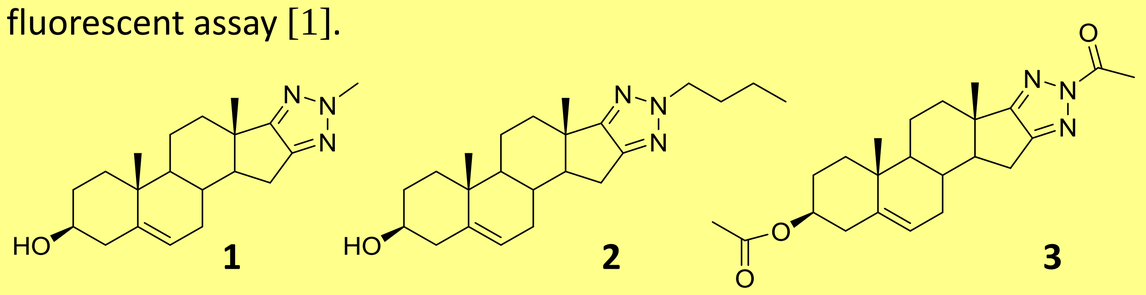

***Figure 2.** Structures of tested N(2)-substituted D-condensed steroidal triazoles.*

RESULTS AND DISCUSSION

We have identified new selective ERβ ligands without androgenic properties, but further experiments are required to determine whether their mechanism of action is agonistic or antagonistic. As can be seen from **Figure 3.** *N*(2)-methyl-3β-hydroxy[1,2,3]triazolo[4',5':16,17]androst-5-ene (**1**) and *N*(2)-acetyl-3β-acetoxy[1,2,3]triazolo[4',5':16,17]androst-5-ene (**3**) displayed high binding affinity for LBD ERβ (**Figure 4**), similar to natural ligand, estrone (E1), while *N*(2)-butyl derivative (**2**) had no estrogenic properties. None of tested compounds showed affinity for AR.

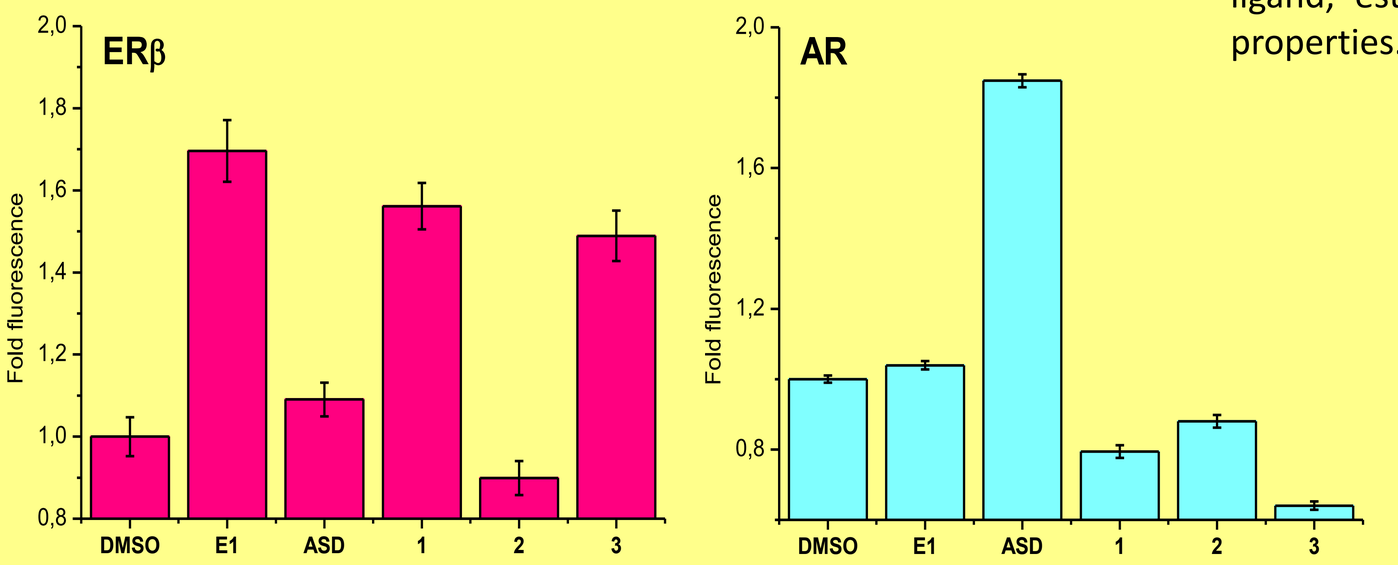

***Figure 3**. Relative binding affinities expressed as fold fluorescence change between ligand-treated and control cells in the absence of ligand expressing ERβ LBD-YFP and AR LBD-YFP; control ligands: E1-estrone, ASD-androstenedione.*

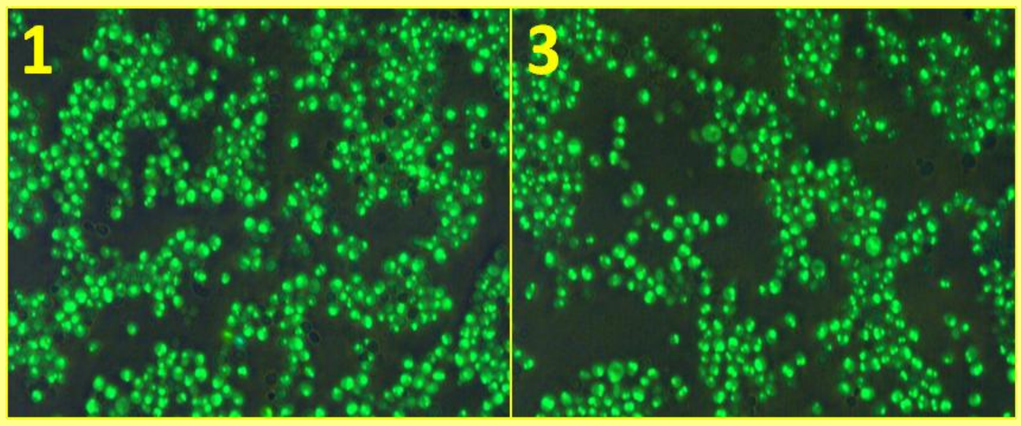

***Figure 4**. Fluorescence micrographs of recombinant yeast cells expressing ERβ LBD-YFP treated with steroidal triazoles **1** and **3**.*

CONCLUSION

Having in mind the broad therapeutic potential of specific ERβ ligands, our findings indicate that steroid derivatives containing triazole are promising bioactive compounds in the field of anticancer agents.

REFERENCES

[1] Bekić, Sofija S., Maja A. Marinović, Edward T. Petri, Marija N. Sakač, Andrea R. Nikolić, Vesna V. Kojić, and Andjelka S. Ćelić. "Identification of D-seco modified steroid derivatives with affinity for estrogen receptor α and β isoforms using a non-transcriptional fluorescent cell assay in yeast." *Steroids* 130 (2018): 22-30.

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