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The new thienopyrimidine-based allosteric regulator of luteinizing hormone receptor and its unexpected inverse agonist effects in the study of blood testosterone level in male rat

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The new thienopyrimidine-based allosteric regulator of lutheinizing hormone receptor and its unexpected inverse agonist effects in the study of blood testosterone level in male rat

Administration of the new allosteric regulator of LH receptor TPX51

Co-administration of TPX51 with LHR agonists hCG or TP03

60 min after treatment with TPX51

- ✓ Basal plasma **testosterone** level ↑
- ✓ Basal ***Star*** and ***LHR*** genes expression in a rat testes ↑

120-300 min after treatment with TPX51

- ✓ Basal plasma and intratesticular **testosterone** level ↓
- ✓ Basal ***Star*** and ***LHR*** genes expression in a rat testes ↓

180 min after treatment with TPX51 of LHR-agonist stimulated rats

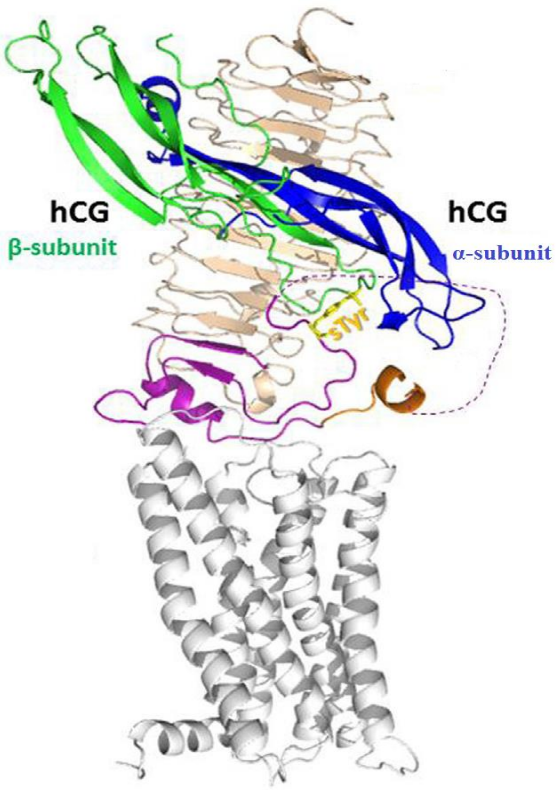
- ✓ Stimulated levels of plasma and intratesticular **testosterone** ↓
- ✓ Stimulated expression of ***Star*** gene in a rat testes ↓

Abstract:

Development of low-molecular-weight allosteric modulators of the luteinizing hormone receptor (LHR) is one of the primary goals of endocrinology. For this, the most promising approach is the use of thieno-[2,3-d]-pyrimidine derivatives. The aim of the work was to investigate the effects of new thieno-[2,3-d]-pyrimidine-based compound, 5-amino-4(3((bis(dimethylamino)methylene)amino)phenyl-N-(tert-butyl)-2-(methylthio)thieno[2,3-d]-pyrimidine-6-carboxamide (TPX51) (12 mg/kg, i.p.), and its combined effects with human chorionic gonadotropin (hCG) (20 IU/rat, s.c.) or thieno-[2,3-d]-pyrimidine-based LHR-agonist TP03 (12 mg/kg, i.p.) on both blood and intratesticular testosterone levels and testicular expression of the genes encoding cholesterol-transporting protein StAR and LHR in rats. 60 min after TPX51 treatment, blood and intratesticular testosterone levels and the *Star* and *Lhr* expression were increased. However, 120-300 min after TPX51 treatment, blood and intratesticular testosterone levels was dramatically decreased as compared with control. Additionally, the expression of *Star* was reduced. Co-administration of TPX51 with hCG or TP03 (180 min after treatment) resulted in decrease in LHR-agonist-induced stimulation of blood and intratesticular testosterone levels and the *Star* expression in the rat testes, and the effect of TPX51 was more pronounced in the case of gonadotropin. Thus, at an early stage the TPX51 functions as an LHR-agonist, stimulating the testicular steroidogenesis, but at a later time it possess the properties of a potent inverse agonist, reducing both basal and LHR-agonists-stimulated steroidogenesis, which may be due to the biodegradation of TPX51 into its derivatives.

Keywords: Thieno-[2,3-d]-pyrimidine; Allosteric regulator, Luteinizing Hormone Receptor, Testosterone, Rat

Introduction



- The Luteinizing hormone receptor (LHR) plays an important role in the functioning of the male and female reproductive systems, and therefore is an important target for the search for pharmacological drugs
- In addition to the orthosteric site for binding of native ligands (Human chorionic gonadotropin and LH), the LHR contains an allosteric site located in the transmembrane domain
- The most promising regulators of the LHR allosteric site is the thienopyrimidine derivatives

Introduction

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Article

Comparative Study of the Steroidogenic Effects of Human Chorionic Gonadotropin and Thieno[2,3-D]pyrimidine-Based Allosteric Agonist of Luteinizing Hormone Receptor in Young Adult, Aging and Diabetic Male Rats

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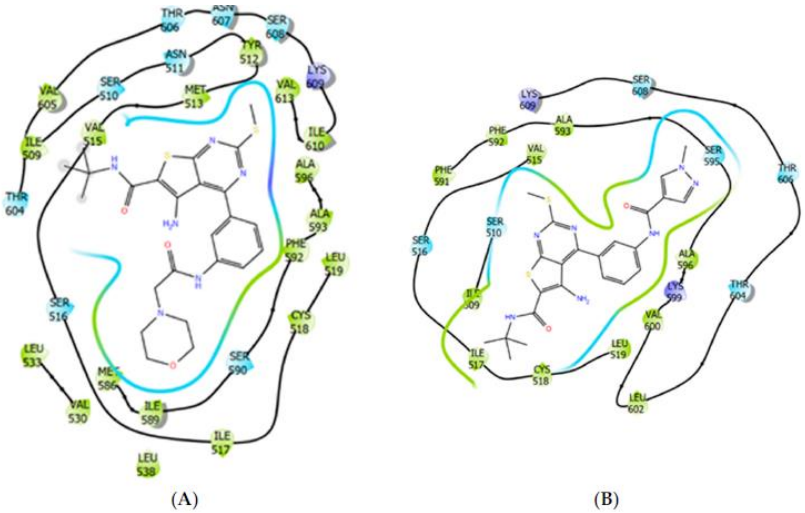


Figure 8. The molecular docking of the thienopyrimidine derivatives Org 43553 (reference compound) (A) and TP4/2 (testing compound) (B) into the allosteric site of rat LHCGR. Amino acid residues with the hydrophobic (in green), positively charged (in violet) or polar groups (in blue) are shown. The same colors are used to indicate contacts between the allosteric agonist and the surface of the LHCGR allosteric site.

- There are several LHR-stimulating allosteric agonists were developed and investigated by our research group last years.
- Now we continue the searching of most effective and selective allosteric modulators of LHR.
- The developed of new thienopyrimidine-based allosteric modulators important not only for medical sciences, but also for a better understanding of the mechanisms of allosteric regulation of the LHR and downstream signaling pathways.

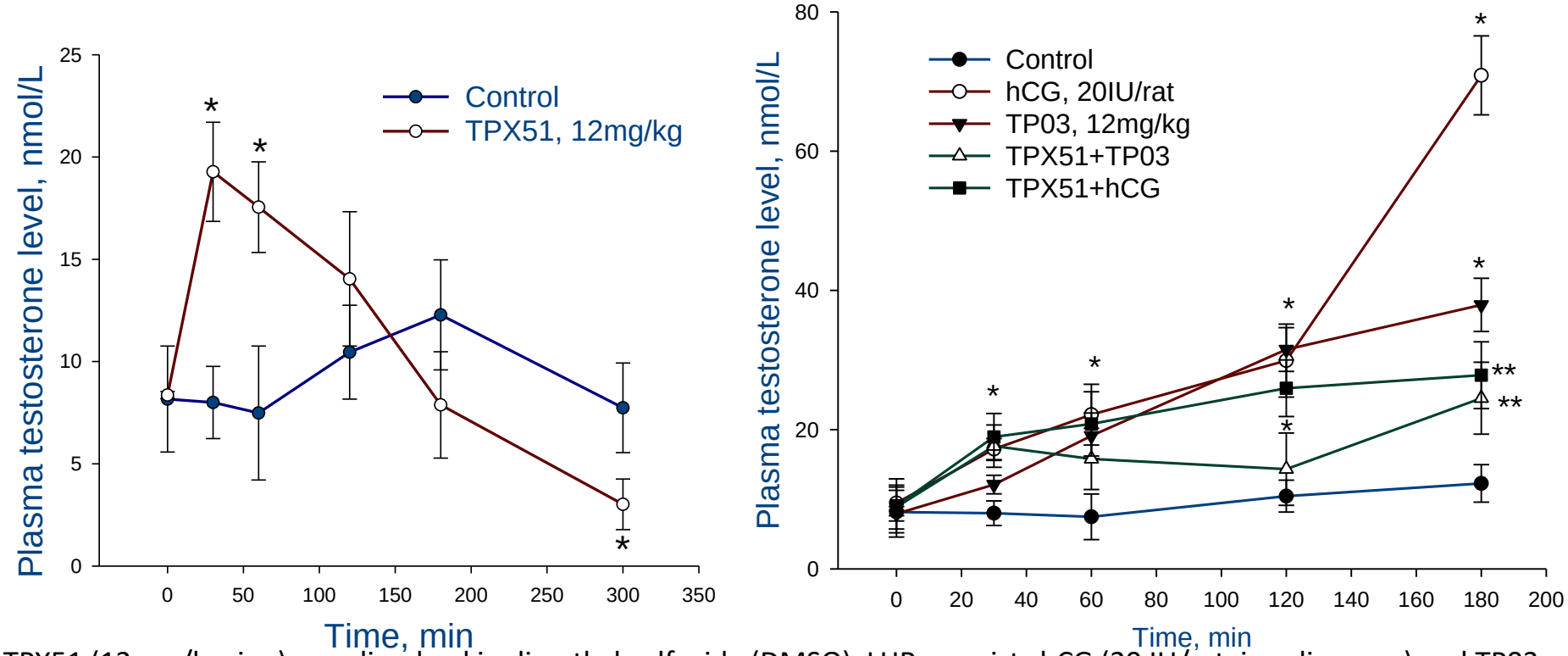
Bakhtyukov, A.A.; et al. Comparative Study of the Steroidogenic Effects of Human Chorionic Gonadotropin and Thieno[2,3-D]pyrimidine-Based Allosteric Agonist of Luteinizing Hormone Receptor in Young Adult, Aging and Diabetic Male Rats. *Int. J. Mol. Sci.* **2020**, *21*, 7493. https://doi.org/10.3390/ijms21207493

Introduction

The aim of our work was to invistegate the new thienopyrimidine-based compound 5-amino-4-(3((bis-(dimethylamino)-methylene)-amino)-phenyl-N-(tert-butyl)-2-(methylthio)thieno-[2,3-d]-pyrimidine-6-carboxamide (TPX51), and its combined effects with human chorionic gonadotropin (hCG) or thieno-[2,3-d]-pyrimidine-based LHR-agonist TP03 on both blood and intratesticular testosterone levels and testicular expression of the genes encoding cholesterol-transporting protein StAR and LHR in male rats.

Results and discussion

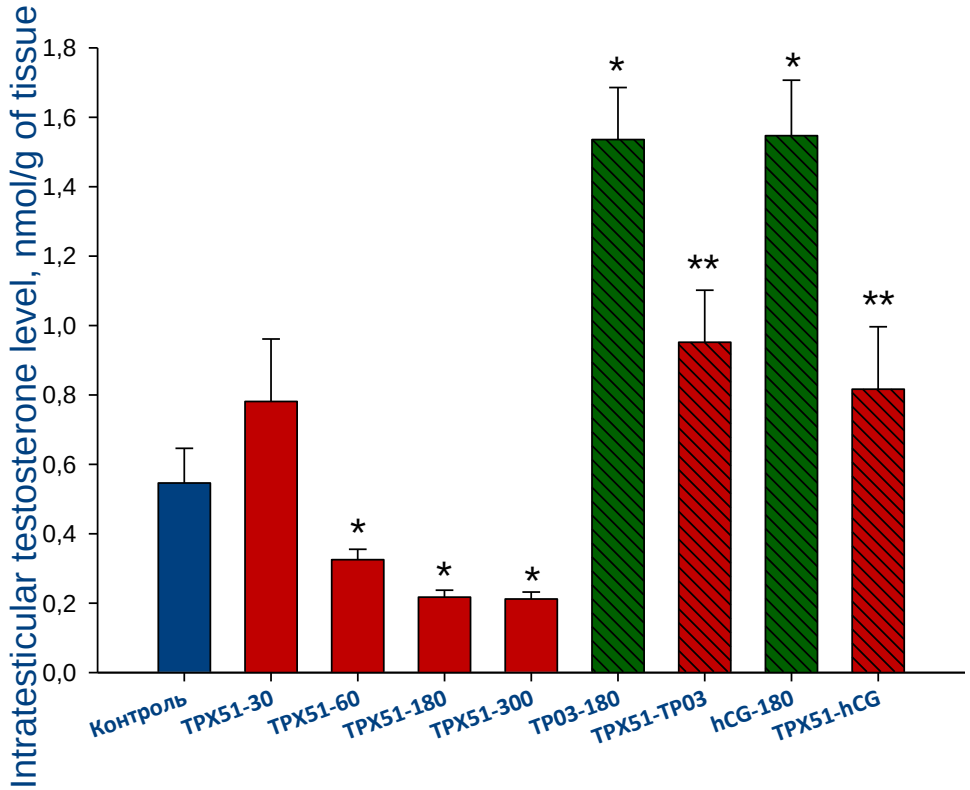
Figure 1. Effect of TPX51 administration on basal (A) and LHR-agonist-stimulated (B) testosterone level in blood plasma of male rats

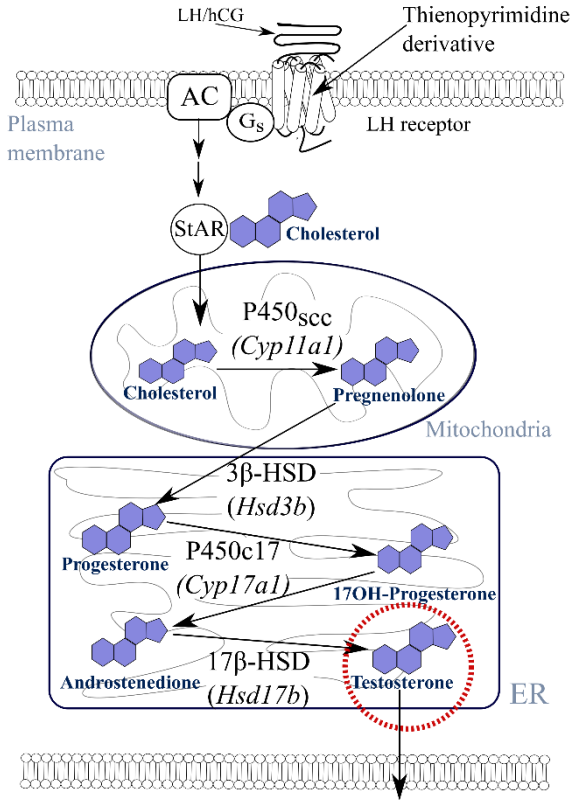


TPX51 (12 mg/kg, i.p.) was dissolved in dimethyl sulfoxide (DMSO). LHR-agonists hCG (20 IU/rat, in saline, s.c.) and TP03 (12 mg/kg, in DMSO, i.p.) were administrated 30 min before TPX51. Control group was administrated with DMSO, i.p. The blood samples was collected after 30, 60, 120, 180 and 300 min after TPX51 (or DMSO) administration. * - differences between control and other groups are statistically significant at $P < 0.05$; ** - differences between groups TP03 or hCG and TPX51+TP03 or TPX51+hCG are statistically significant at $P < 0.05$. M ± SEM. n = 5.

Figure 2. Effect of TPX51 administration on basal and LHR-agonist-stimulated intratesticular testosterone level of male rats

Results and discussion





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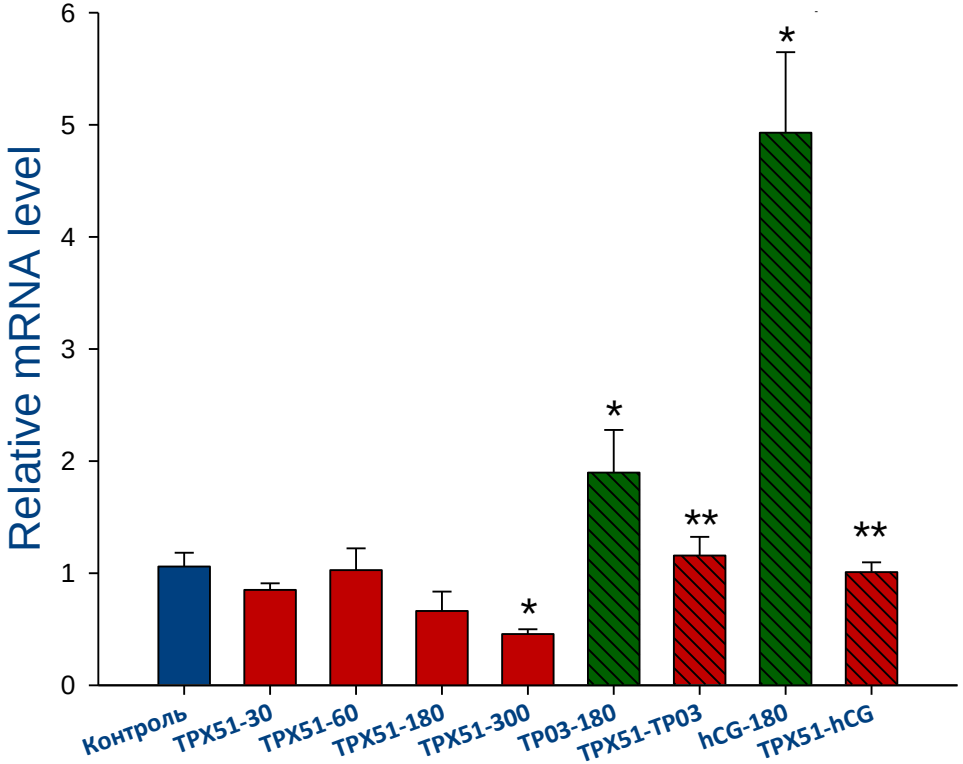
* - differences between control and other groups are statistically significant at $P < 0.05$; ** - differences between groups TP03 or hCG and TPX51+TP03 or TPX51+hCG are statistically significant at $P < 0.05$. M ± SEM. n = 5.

Figure 3. Relative mRNA level of the *Star* gene in rat testes after administration of TPX51, TP03 or hCG and their combined treatment



Results and discussion

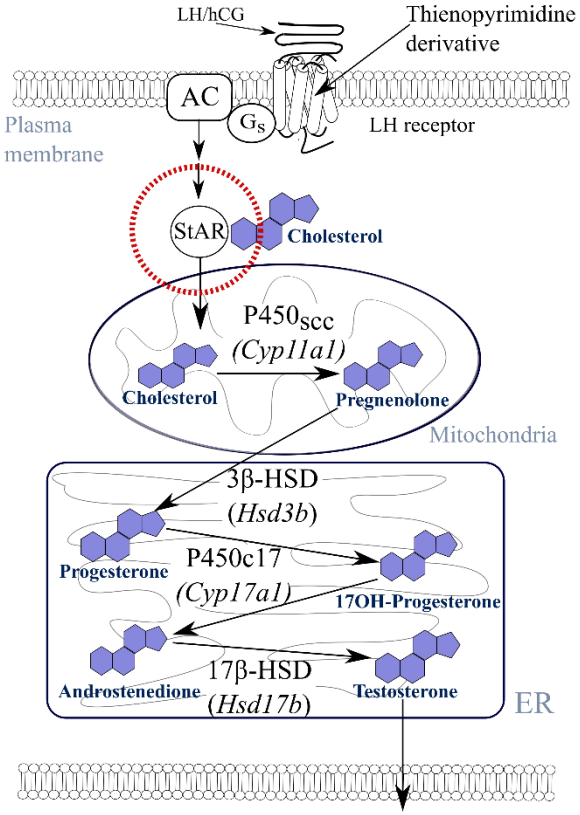


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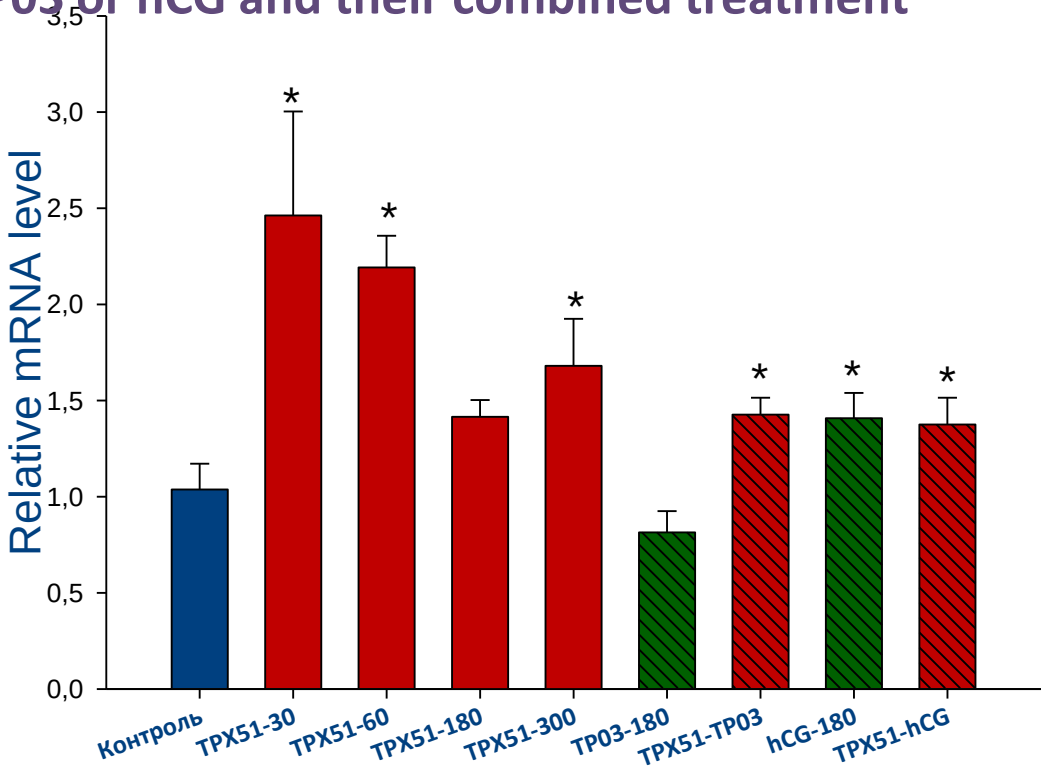
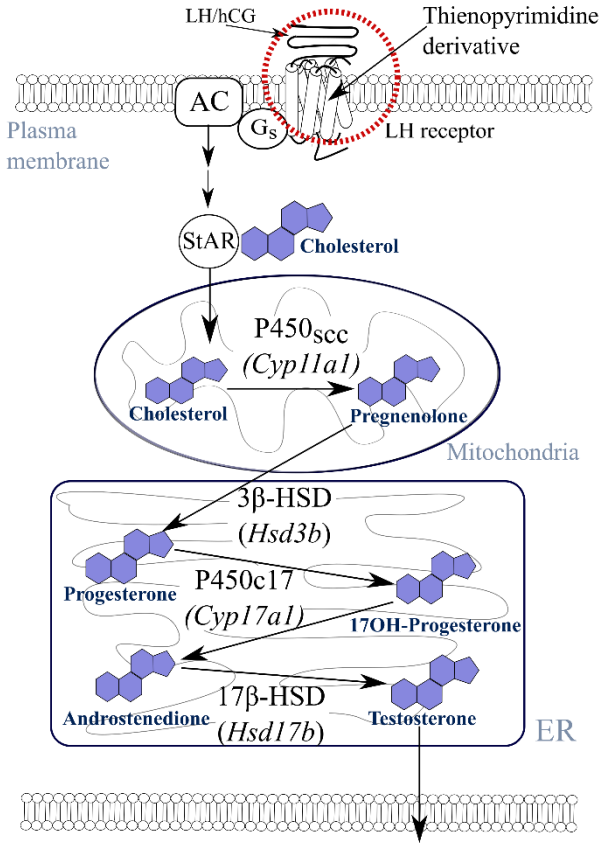
Figure 4. Figure 3. Relative mRNA level of the *LHR* gene in rat testes after administration of TPX51, TP03 or hCG and their combined treatment



Results and discussion



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Conclusions

- The new thienopyrimidine derivatives (TPX51) an early stage (60 min after administration) acts as an LHR-agonist, stimulating the testicular steroidogenesis, but at a later time (>120 min after administration) it possess the properties of a potent inverse agonist, reducing both basal and LHR-agonists-stimulated steroidogenesis.

- This unexpected effects of TPX51 may be due to the biotransformation or biodegradation of TPX51 into its derivatives with the properties of an inverse LHR-agonist.

- Further study of the TPX51 and its derivatives properties will allow a better understanding of the role of function groups at the thienopyrimidine structure when its interacting with the LHR allosteric site.

Acknowledgments

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