ECMC-P 2025 Conference

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Activity, pharmacokinetics, and tissue distribution of 5-amino-N-tert-butyl-2-(methylsulfanyl)-4-(3-(nicotinamido)phenyl)thieno[2,3-d]pyrimidine-6-carboxamide (TP03), an allosteric agonist of the luteinizing hormone receptor

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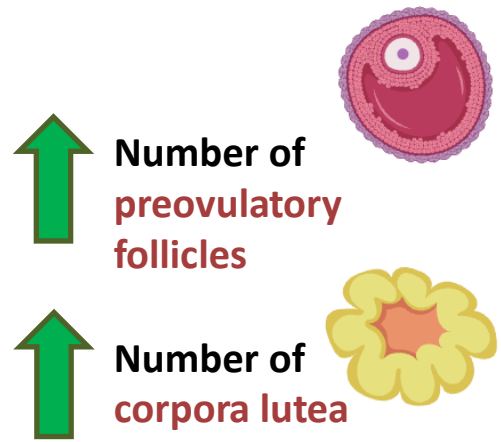
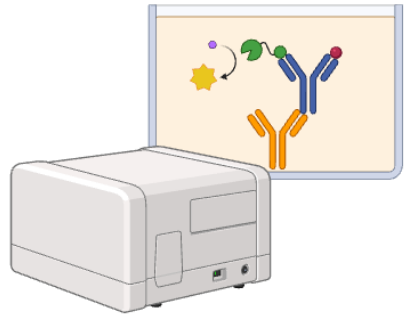


Administration of the new allosteric agonist of LH receptor, TP03



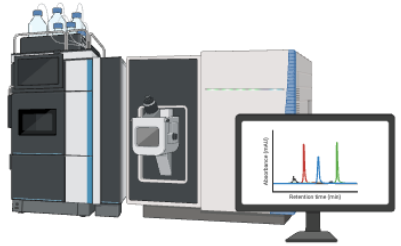
Blood plasma estradiol level

Blood plasma progesterone level

Rapid absorption of TP03 in blood plasma

Cmax = 3530 ng/mL
Tmax = 15 min

Long half-life in bloodstream (2.94 h)
Elimination constant 0.24 h^{-1}



Rapid accumulation in ovaries and liver (1 h after administration)

Subsequent redistribution from ovaries to liver

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Abstract:
Gonadotropin preparations with luteinizing hormone (LH) activity are widely used to stimulate ovulation, but they have a number of side effects. Low-molecular allosteric LH receptor agonists, including our developed compound TP03, may be an alternative. TP03 was obtained with the acylation of 5-amino-4-(3-aminophenyl)-N-(tert-butyl)-2-(methylthio)thieno[2,3-d]pyrimidine-6-carboxamide, and, according to high-resolution mass spectrometry, this MW was 515.1304 (the calculated MW for $[M+Na^+]$ was 515.1294). Being a hydrophobic compound, TP03 is able to penetrate the transmembrane tunnel of the LH receptor, interacting with the transmembrane allosteric site. The aim of this study was to investigate the effect of TP03 on ovarian steroidogenesis and ovulation in immature female rats, as well as to investigate its pharmacokinetics in blood plasma and its distribution in ovaries and liver. Immature female rats stimulated with Follimag were administered TP03 (15 mg/kg, i.p. in DMSO), after which the levels of estradiol and progesterone in blood and the number of preovulatory follicles and corpora lutea in ovaries were assessed for 24 h. Using reversed-phase HPLC with tandem mass spectrometry, TP03 levels were measured in blood plasma, ovaries and liver. TP03 increased blood progesterone levels and, after 16–24 hours, led to the formation of corpora lutea in ovaries. The maximum plasma concentration of TP03 was 3530 ng/mL, and the time to reach maximum concentration was 15 min, indicating rapid absorption of TP03. The elimination constant, characterizing the rate of TP03 elimination, was 0.24 h^{-1}, and the half-life of TP03 was 2.94 h. Thus, TP03 is a long-lived compound in the bloodstream. Rapid accumulation of TP03 in ovaries and liver was demonstrated 1 hour after administration. Thus, the high efficacy of TP03 as an ovulation inducer is largely due to its rapid absorption in ovaries and long half-life in the bloodstream.

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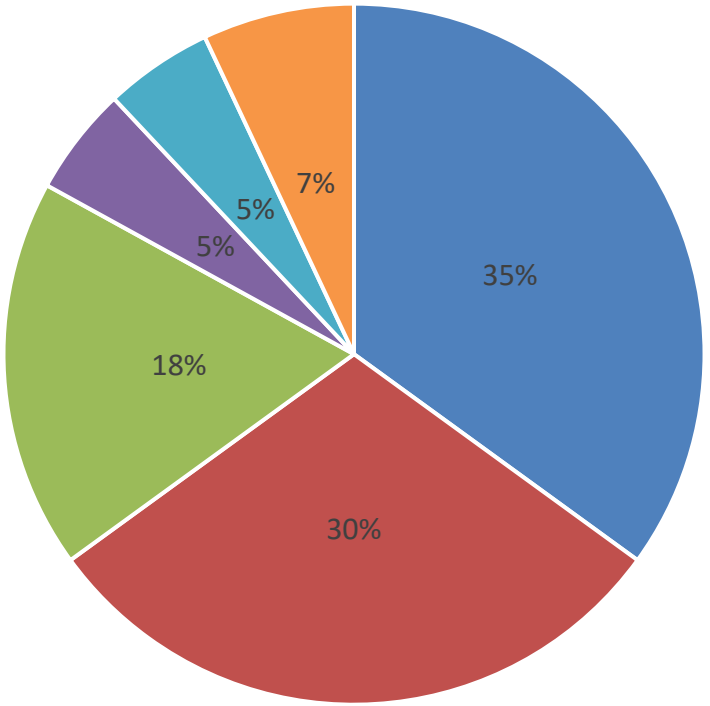
Keywords: allosteric agonist; luteinizing hormone receptor; ovaries; ovulation; pharmacokinetics; progesterone

Introduction

- According to the World Health Organization, infertility is **one of the biggest health issues** affecting **millions of people** of reproductive age
- Globally **one in six people** experience infertility in their lifetime
- Searching for the **new infertility treatment strategies** becomes more and more relevant



Causes of female infertility

- Endocrine diseases
- Obstruction of the fallopian tubes
- Diseases of the uterus
- Psychological factors
- Immunological factors
- Other

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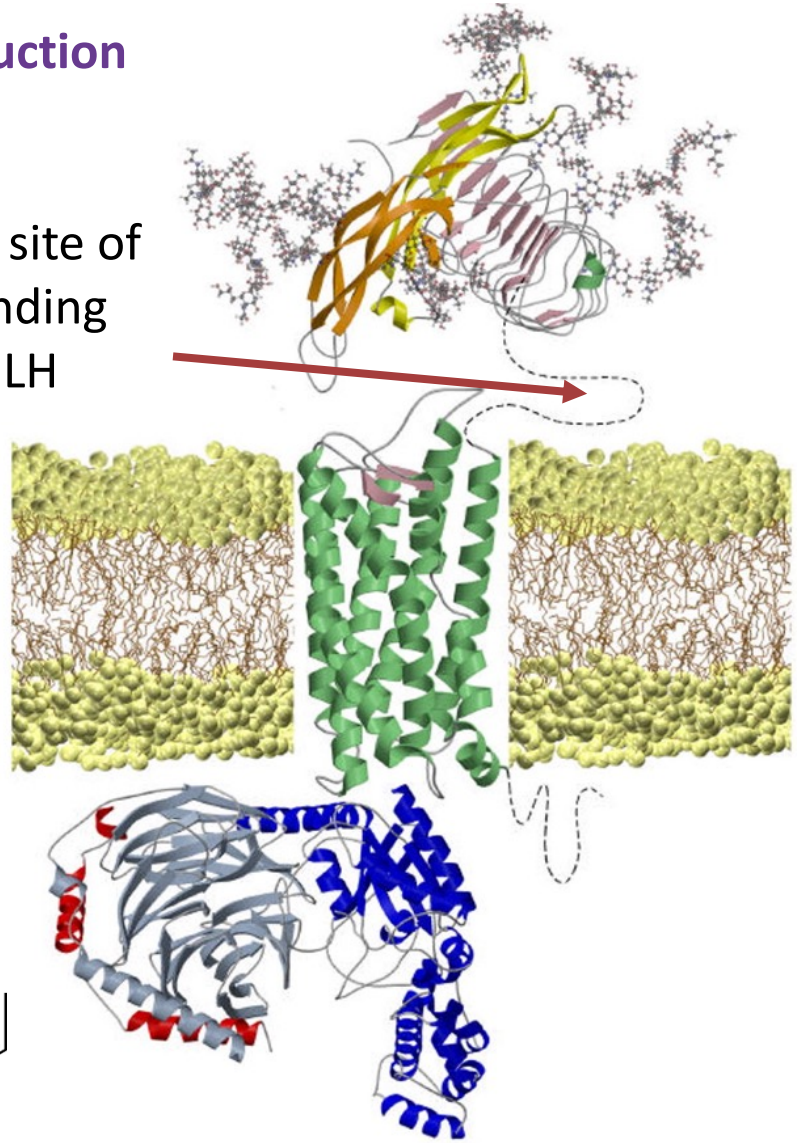
Introduction

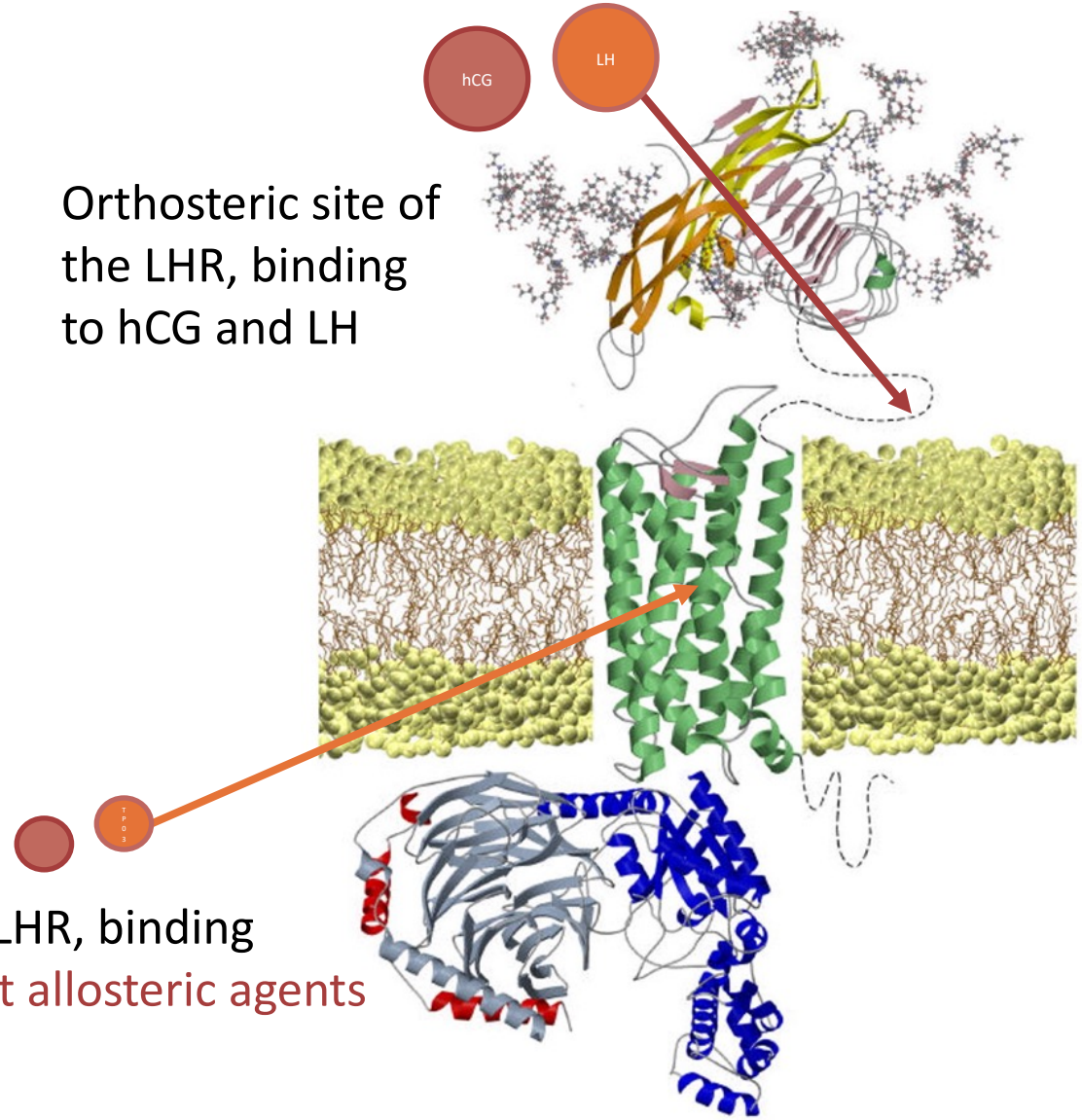
Orthosteric site of the LHR, binding to hCG and LH



- Luteinizing hormone receptor (LHR) is one of the main regulators of the reproductive system
- That makes LHR a fundamentally important and perspective target for the new pharmaceutical agents
- The most commonly used drugs, including human chorionic gonadotropin (hCG) and recombinant LH, bind to the orthosteric site, located at the extracellular domain of the LHR
- Modern treatment protocols use high dosages of hCG or LH, which leads to the hyperstimulation and subsequent desensitization of LHR, while low dosages have been proven ineffective

Introduction

- All existing problems encourage scientists to search for alternative approaches to the treatment of reproductive diseases
- Using another binding site, the allosteric site, located at the transmembrane domain of the LHR, may be the solution of the problems
- The effective candidates for binding to the allosteric site of the LHR may be low-molecular-weight thienopyrimidine derivatives, such as TP03, developed in our laboratory



Orthosteric site of the LHR, binding to hCG and LH

Allosteric site of the LHR, binding low-molecular-weight allosteric agents

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Introduction

Comparison of Steroidogenic and Ovulation-Inducing Effects of Orthosteric and Allosteric Agonists of Luteinizing Hormone/Chorionic Gonadotropin Receptor in Immature Female Rats

by Kira V. Derkach [1], Ivan A. Lebedev [1], Irina Yu. Morina [1,*], Andrey A. Bakhtyukov [1], Alena S. Pechalnova [1], Viktor N. Sorokoumov [1,2], Veronica S. Kuznetsova [1], Irina V. Romanova [1] and Alexander O. Shpakov [1,*]

The Effects of Separate and Combined Treatment of Male Rats with Type 2 Diabetes with Metformin and Orthosteric and Allosteric Agonists of Luteinizing Hormone Receptor on Steroidogenesis and Spermatogenesis

by Andrey A. Bakhtyukov [1], Kira V. Derkach [1], Viktor N. Sorokoumov [1,2], Anna M. Stepochkina [1], Irina V. Romanova [1], Irina Yu. Morina [1], Irina O. Zakharova [1], Liubov V. Bayunova [1] and Alexander O. Shpakov [1,*]

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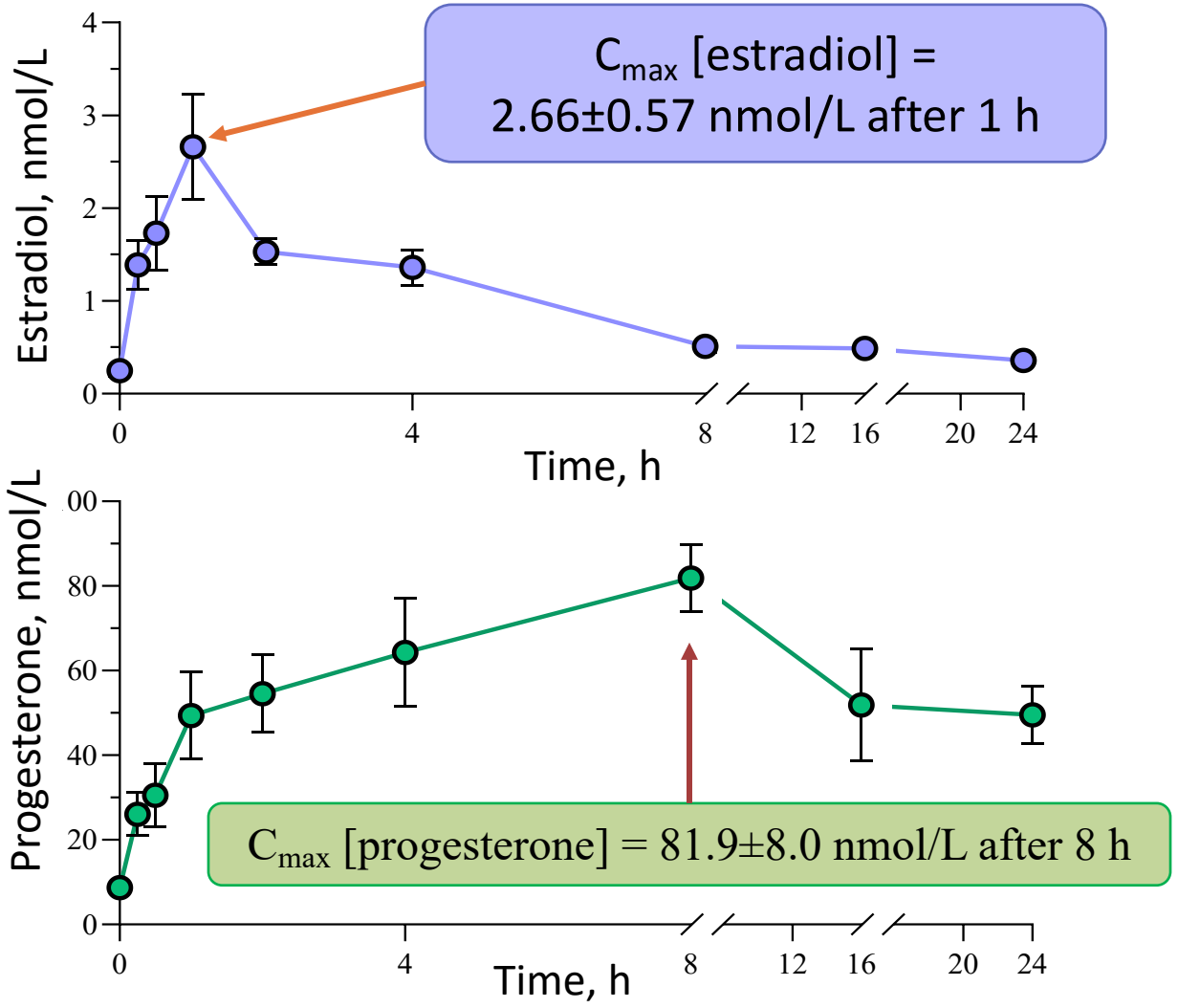
- Our research group has developed and estimated the effectiveness of several allosteric agonists on testicular and ovarian steroidogenesis in rats
- Although we have proved their positive effect on steroidogenesis in rats, the pharmacokinetics of these compounds remained unclear
- The aim of this study was to investigate the effect of TP03 on ovarian steroidogenesis and ovulation in immature female rats, as well as to investigate its pharmacokinetics in blood plasma and its distribution in ovaries and liver

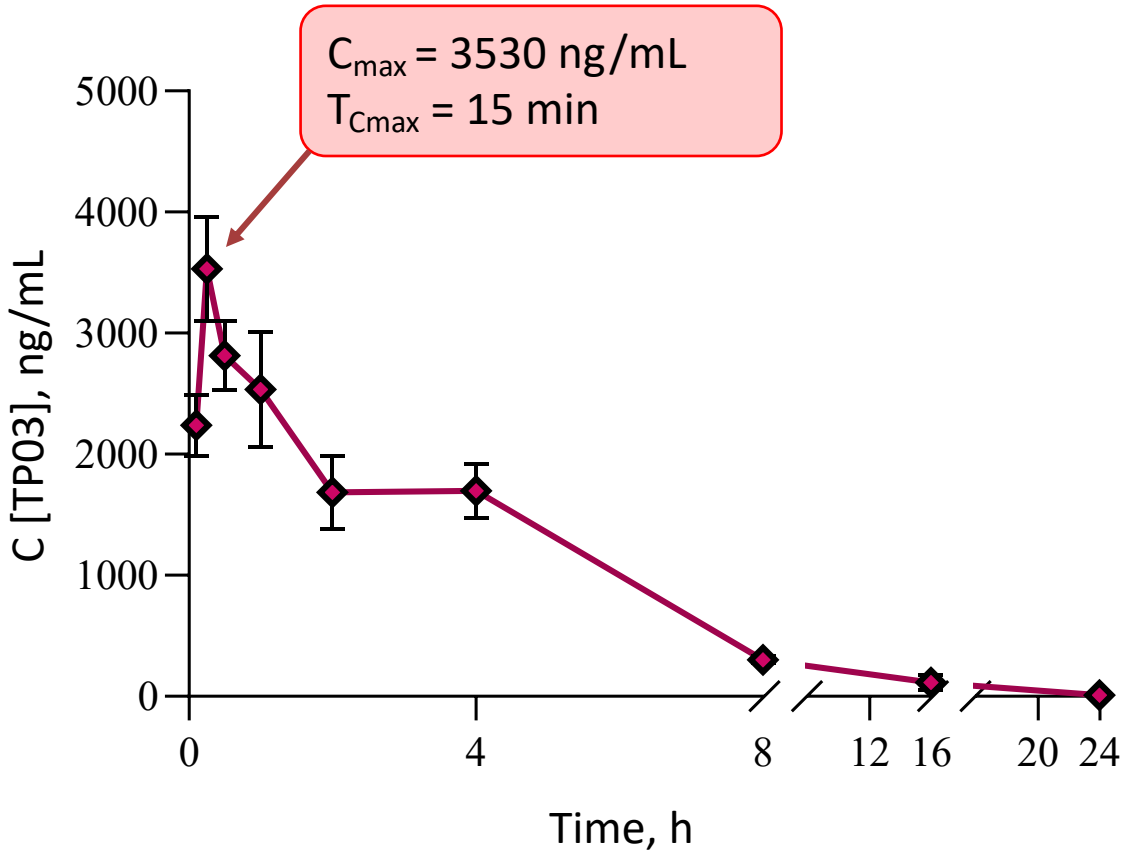
Results and discussion

- Administration of TP03 (15 mg/kg, i.p.) to immature female rats pretreated with Follimag led to an increase of blood plasma hormone levels
- The level of estradiol reached the maximum concentration 1 h after the administration of TP03, then the level of estradiol decreased rapidly
- Dinamic of progesterone concentration was different and the level of progesterone reached the maximum concentration 8 h after administration of TP03 with subsequent slow decrease



Results and discussion

- The maximum concentration of TP03 in blood plasma was 3530 ng/mL and the time to reach that concentration was 15 min, which points to its rapid absorption
- The elimination constant characterizing the rate of TP03 elimination from the body was 0.24 h^{-1}
- The period of elimination the half of the TP03 was 3.11 h
- Average retention time in the body was 4.29 h
- All of the above showed that TP03 is a long-living compound in the bloodstream



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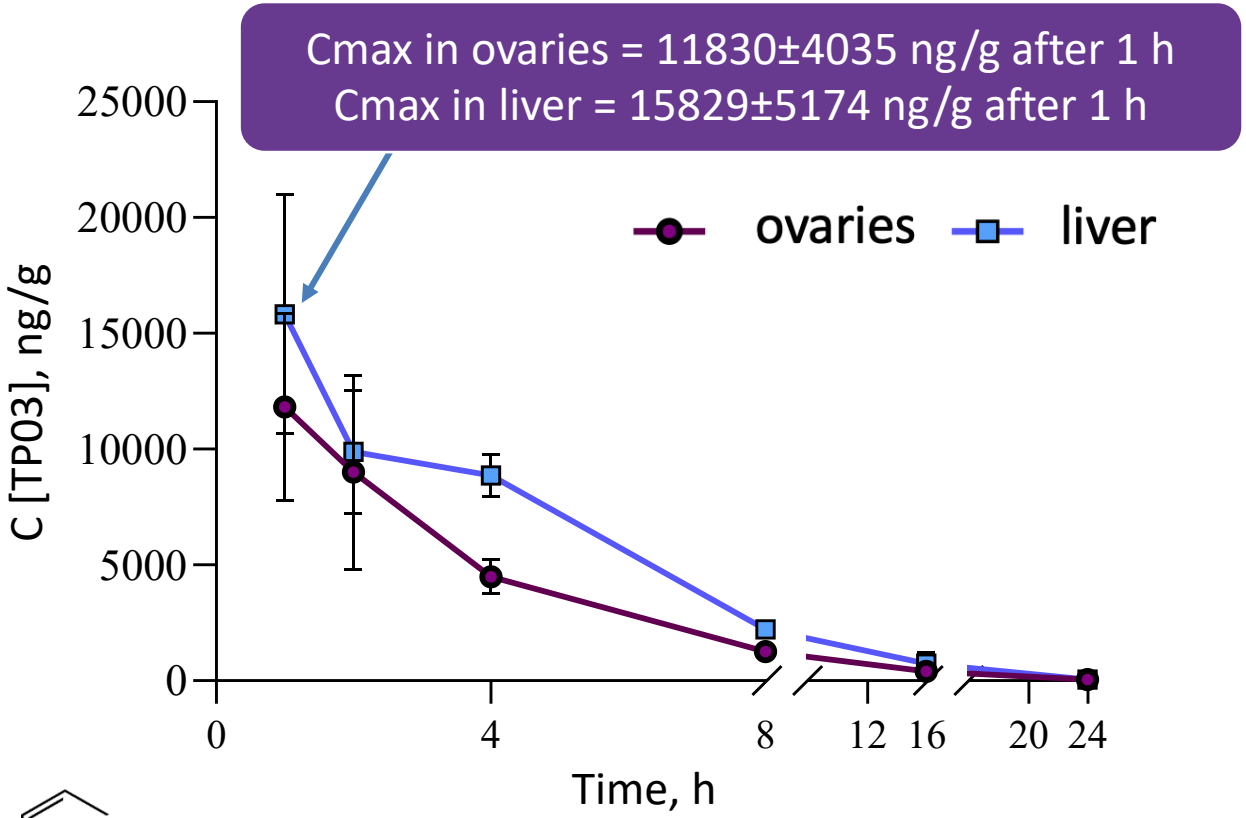
Results and discussion



- TP03 enters ovaries, its main target, and liver, in which most drugs accumulate and degrade
- The absorption was rather fast and TP03 reached the maximum concentrations in tissues 1 h after administration
- Complete elimination of TP03 from the tissues was observed 24h after administration
- The pharmacokinetic profile of TP03 showed an acute peak with long elimination phase with signs of secondary absorption in the liver

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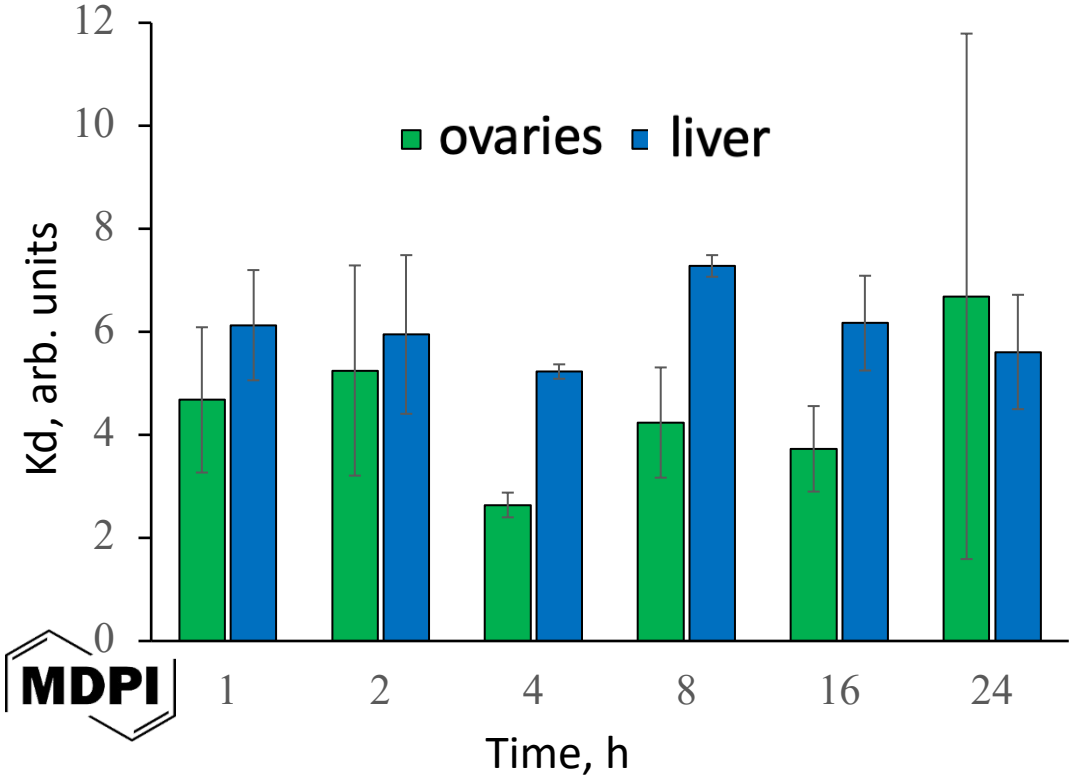
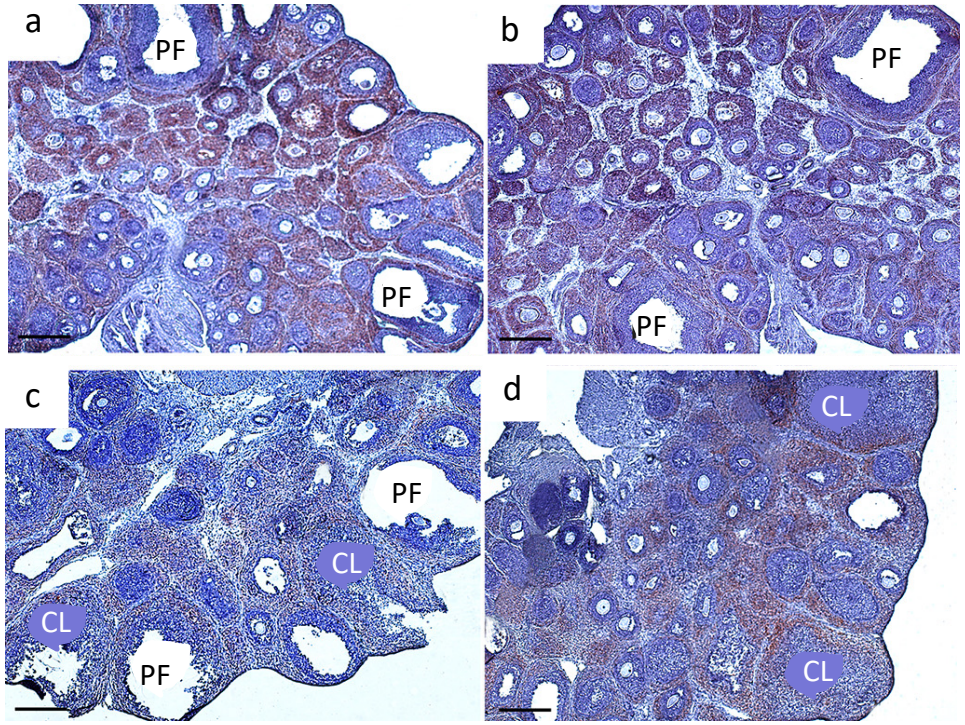
Results and discussion

- Distribution coefficients (Kd) of compound TP03 between blood plasma and tissues at different time points. The coefficients are calculated using the formula tissue [Ct]/blood plasma [Ct] and are expressed in arbitrary units.





- Cross-sections of the ovaries. Section thickness was 10 µm, scale bar 300 µm. PF – preovulatory follicles, CL – corpora lutea
- a – rats 64 h after treatment with Follimag; b – rats 72 h after treatment with Follimag; c – rats 16 h after treatment with TP03 and 64 h after treatment with Follimag; d – rats 24 h after treatment with TP03 and 72 h after treatment with Follimag

Conclusions

- TP03 reaches its maximum concentration in the blood in 15 minutes, which indicates its rapid absorption
- The observed pharmacokinetic profile of TP03 demonstrates a relatively long half-life of TP03 in the bloodstream
- TP03 increases the levels of estradiol and progesterone leading to the formation of corpora lutea 16 and 24 hours after administration
- The obtained results demonstrate the effectiveness of TP03 as an allosteric agonist of the luteinizing hormone receptor and can be used for the further development of pharmaceutical drugs aimed at correcting disorders of the reproductive system

Acknowledgments

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