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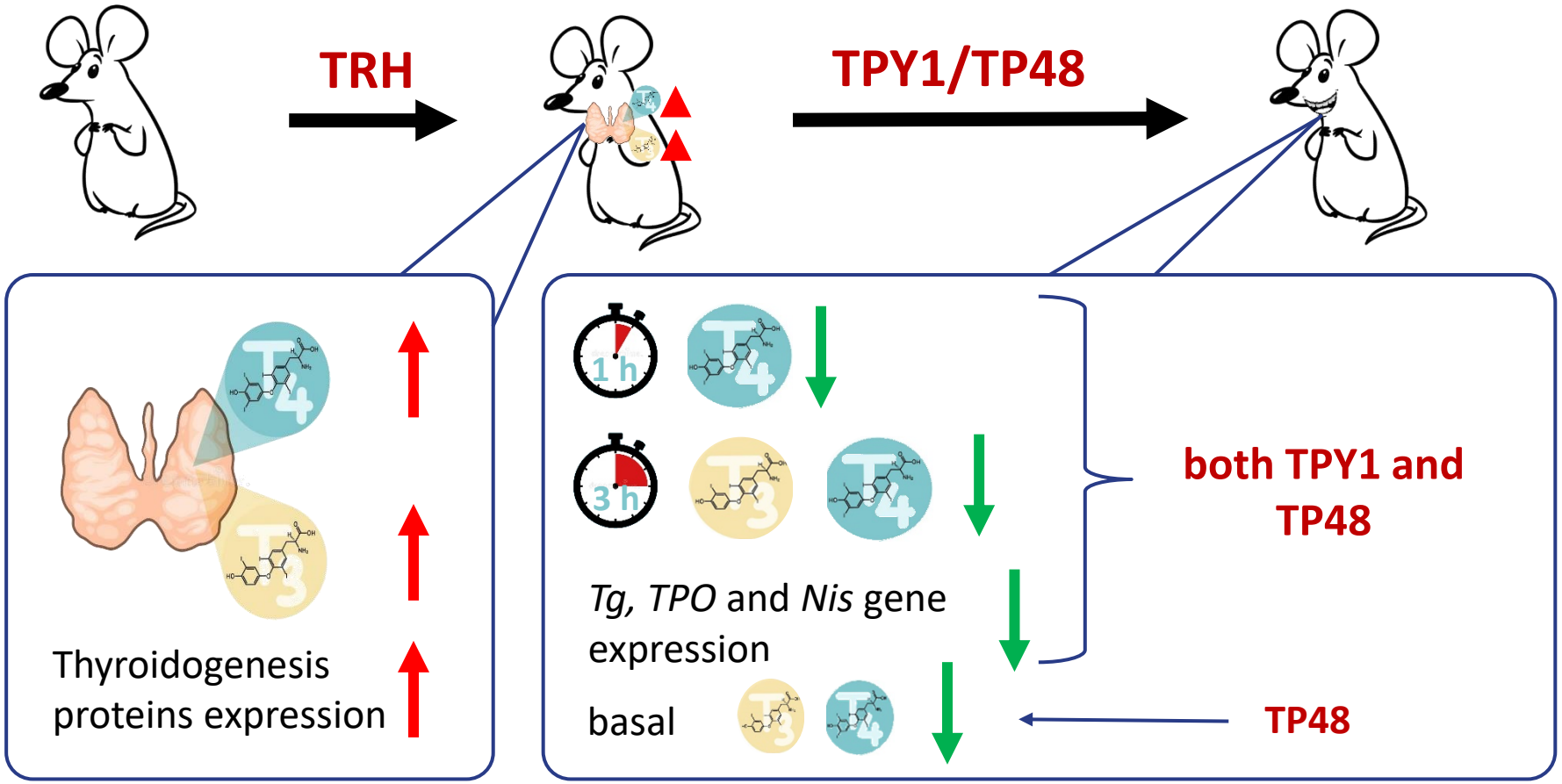
The development of ligands of the thyrotropin receptor transmembrane allosteric site with the activity of antagonists and inverse agonists

Ekaterina Fokina [1,*], Andrey Bakhtyukov [1], Kira Derkach [1], Viktor Sorokoumov [1, 2], Lev Klys [1], Alexander Shpakov [1]

[1] I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Saint Petersburg;
[2] Saint Petersburg State University, Saint Petersburg.

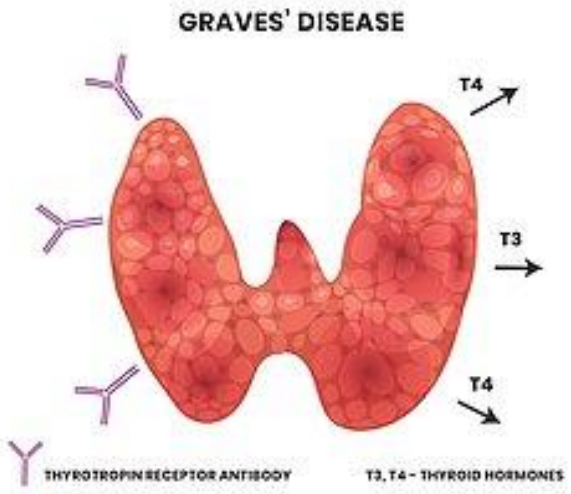
* Corresponding author: fokina-katrina@yandex.ru

The development of ligands of the thyrotropin receptor transmembrane allosteric site with the activity of antagonists and inverse agonists



Abstract:
Thyroid-stimulating hormone (TSH) is the main regulator of thyroid hormones production. The increased activity of TSH receptors due to mutations or interaction with the stimulating TSH receptor-autoantibodies leads to oncological and autoimmune thyroid diseases. Currently, no effective drugs have been developed to prevent TSH receptor hyperactivation. One of the approaches is the use of low-molecular-weight antagonists and inverse agonists of the transmembrane allosteric site of TSH receptor, in particular, thieno[2,3d]pyrimidine derivatives (TPs). The aim of the study was to explore the new TPs with such activity: 5-amino-*N*-(*tert*-butyl)-4-(4-iodophenyl-)-2-(methylthio)thieno[2,3d]pyrimidine-6-carboxamide (**TP48**) and 5-amino-*N*-(*tert*-butyl)-4-(4-(3-methoxyprop-1yn-1-yl)phenyl)-2-(methylthio)thieno[2,3d]-pyrimidine-6-carboxamide (**TPY1**). When **TP48** and **TPY1** (15 mg/kg, i.p.) were administered to male Wistar rats, thyroliberin-induced production of thyroxine and triiodothyronine was reduced. **TP48** and **TPY1** also weakened the stimulating effects of thyroliberin on the expression of the *Tg*, *TPO*, and *Dio2* genes, which encode the enzymes responsible for thyroid hormone synthesis, such as thyroglobulin, thyroid peroxidase and D2-deiodinase, and unexpectedly reduced expression of the *Tshr* gene encoding TSH receptor. **TP48** also decreased basal thyroid hormone levels in control rats. Thus, new allosteric antagonists (**TPY1**) and inverse agonists (**TP48**) of TSH receptor have been developed, which can become prototypes for the creation of drugs to treat hyperthyroidism and thyroid cancer.
Keywords: thyroid-stimulating hormone; thyrotropin receptor; thienopyrimidine; allosteric antagonist; allosteric inverse agonist; thyroid gland

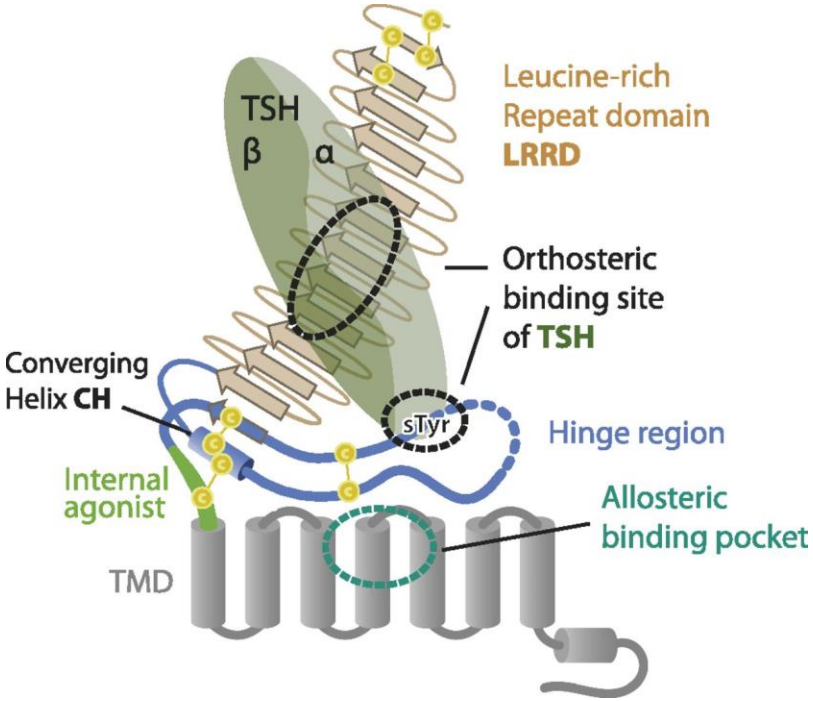
Introduction



Grave`s disease pathogenesis

Autoimmune hyperthyroidism is a widespread disease of the thyroid gland. The molecular cause of autoimmune hyperthyroidism is the production of stimulating antibodies to the thyroid-stimulating hormone (TSH) receptor. This leads to the activation of the synthesis and secretion of thyroxine (T4) and triiodothyronine (T3). For the treatment of autoimmune hyperthyroidism, pharmacological drugs, surgical methods, and radioactive iodine therapy can be used. However, all these approaches have serious limitations and can lead to severe complications. One of them may be the creation of antagonists of the TSH receptor, which can prevent its activation by stimulating antibodies.

Introduction



TSH receptor structure [1]

1. Marcinkowski P., et al. Thyrotropin Receptor: Allosteric Modulators Illuminate Intramolecular Signaling Mechanisms at the Interface of Ecto- and Transmembrane Domain // Mol Pharmacol. – 2019. – T. 96, № 4. – C. 452-462.

Of greatest interest are low-molecular-weight compounds that are able to penetrate into the transmembrane domain of the TSH receptor and specifically bind to the allosteric site located in it. This site controls signal transduction from the extracellular TSH-binding domain to the effector proteins. Blocking the allosteric site interferes with the signaling generated by TSH or stimulatory antibodies and prevents the development of hyperthyroidism.

We and other authors have shown that thieno[2,3-d]-pyrimidine derivatives are suitable candidates for the role of ligands for the transmembrane allosteric site of the TSH receptor

Introduction

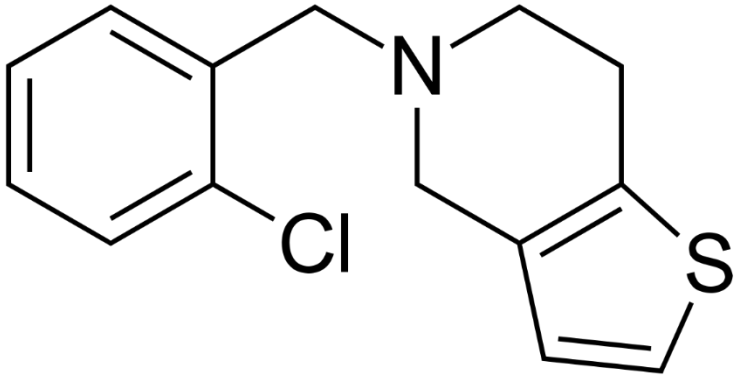
We divided rats into six groups (n=5 for each)

- **Control group**
  Got physiological saline instead of treatment
- **TRH group**
  Got intranasal thyroliberin at a dose of 100 µg/rat
- **TPY1 group**
  Got TPY1 at a dose of 25 mg/kg
- **TP48 group**
  Got TP48 at a dose of 25 mg/kg
- **TPY1+TRH**
  Got TRH at a dose of 100 µg/rat 20 min after 25 mg/kg of TPY1
- **TP48+TRH**
  Got TRH at a dose of 100 µg/rat 20 min after 25 mg/kg of TP48

Introduction

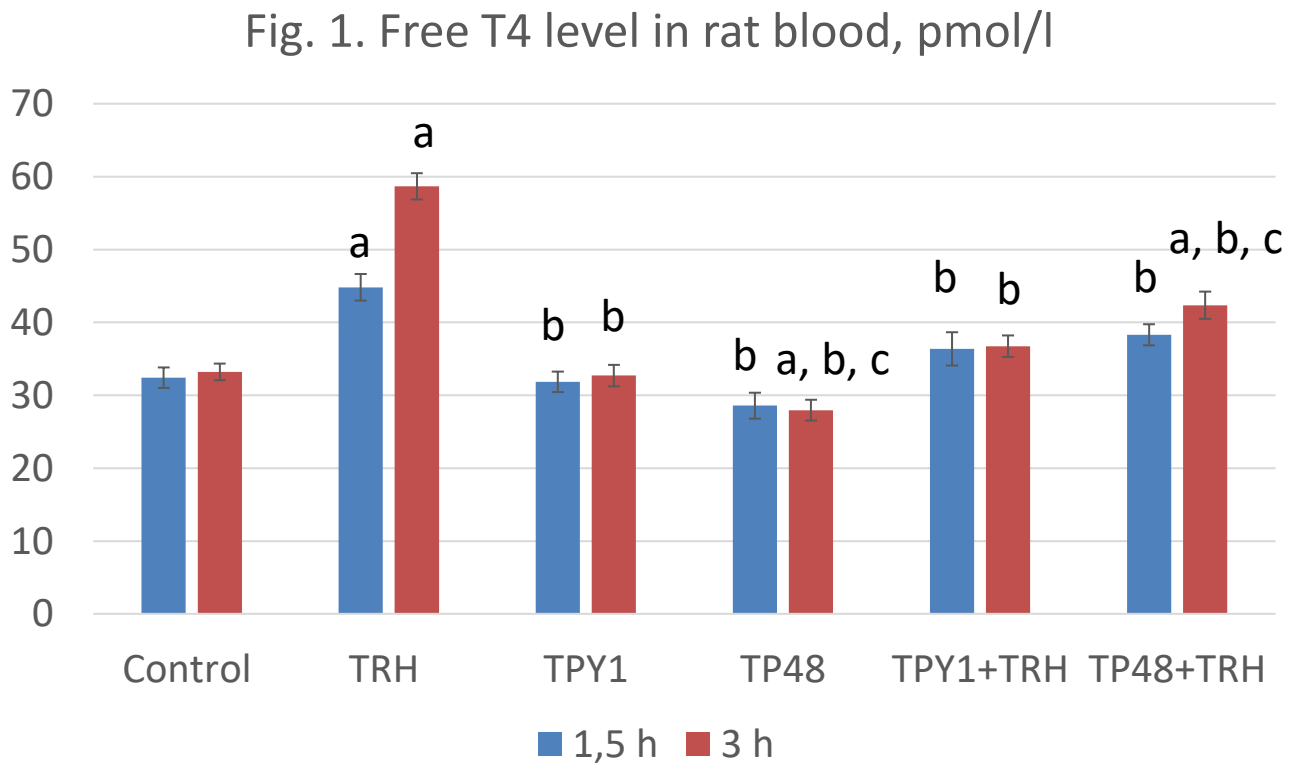
So, we evaluated basal, induced (with TRH) and affected by thienopyrimidine levels of thyroid hormones (T3, T4) and expression of thyroidogenesis proteins.

Time points were 1,5 hour and 3 hours.



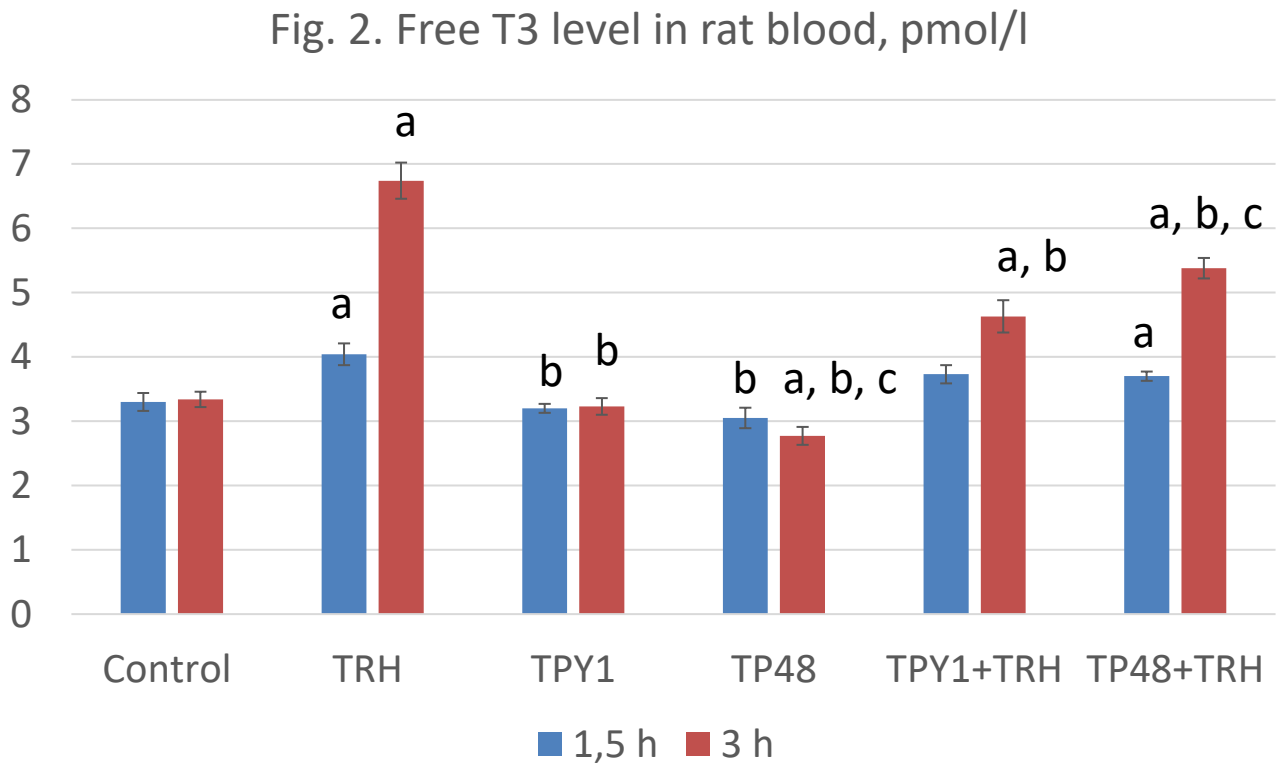
Thienopyridines structure formula

Results and discussion



Differences with the control ([a]), with the TRH group ([b]), between the TPY1 and TP48 groups ([c]), and between the TPY1+TRH and TP48+TRH groups ([d]) are statistically significant at $P < 0.05$

Results and discussion



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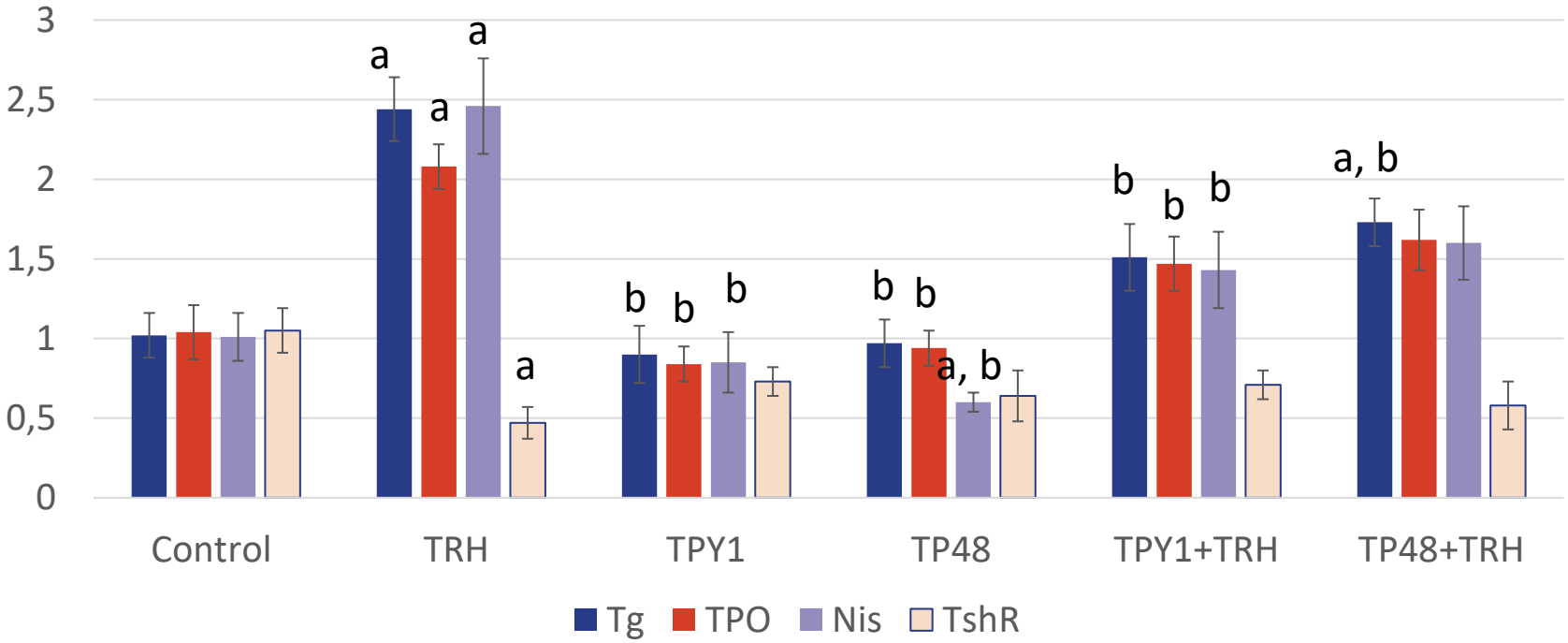
Results and discussion

Fig. 3. Thyroidogenesis proteins expression



Differences with the control ([a]), with the TRH group ([b]), between the TPY1 and TP48 groups ([c]), and between the TPY1+TRH and TP48+TRH groups ([d]) are statistically significant at $P < 0.05$

Results and discussion

After intranasal administration of **TRH** to rats, which stimulates the synthesis and secretion of TSH, an increase in the concentration of thyroid hormones in the blood was detected as early as 1.5 h, and the stimulating effect of TRH was increased after 3 h. It was shown that 1.5 h after TRH treatment, **both thieno[2,3-d]-pyrimidines** significantly reduced only the stimulating effect of this releasing factor on the fT4 level, while after 3 h they reduced the levels of all forms of T4 and T3.

TPY1 did not affect the baseline levels of thyroid hormones, while TP48 after incubation for 3.5 h significantly reduced the baseline levels of fT4 and fT3 as compared to the control group.

TRH treatment increased the expression of genes for thyroglobulin (*Tg*), thyroperoxidase (*TPO*) and Na^+/I^--cotransporter (*Nis*). Pretreatment of rats with **TPY1 and TP48** reduced TRH-induced stimulation of the *Tg*, *TPO* and *Nis* gene expression, and TPY1 was more effective than TP48.

Conclusions

Based on this, **TPY1** can be classified as a neutral antagonist, which distinguishes it from **TP48**, which is an inverse agonist that reduces not only stimulated but and baseline levels of T4 and T3.

Thus, we have developed a new thieno[2.3-d]-pyrimidine derivative TPY1 with the activity of a neutral antagonist of the TSH receptor which reduced TRH-stimulated levels of thyroid hormones and the expression of thyroidogenic genes in rats. Unlike the compound TP48, TPY1 did not reduce the baseline levels of thyroid hormones and the expression of thyroidogenic genes, which **reduces the risks of developing hypothyroidism** when using it. The compound TPY1 can be a prototype of drugs for normalizing T4 and T3 levels in autoimmune hyperthyroidism and Graves' ophthalmopathy.

Acknowledgments

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