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Advancing the Utility of Thyrotropin-Releasing Hormone (TRH) as a CNS Agent

Daniel L. De La Cruz and Katalin Prokai-Tatrai

Department of Pharmacology and Neuroscience, UNT Health Science Center at Fort Worth, 3500 Camp Bowie Blvd, Fort Worth, TX 76107

* Corresponding author: (katalin.prokai@unthsc.edu)

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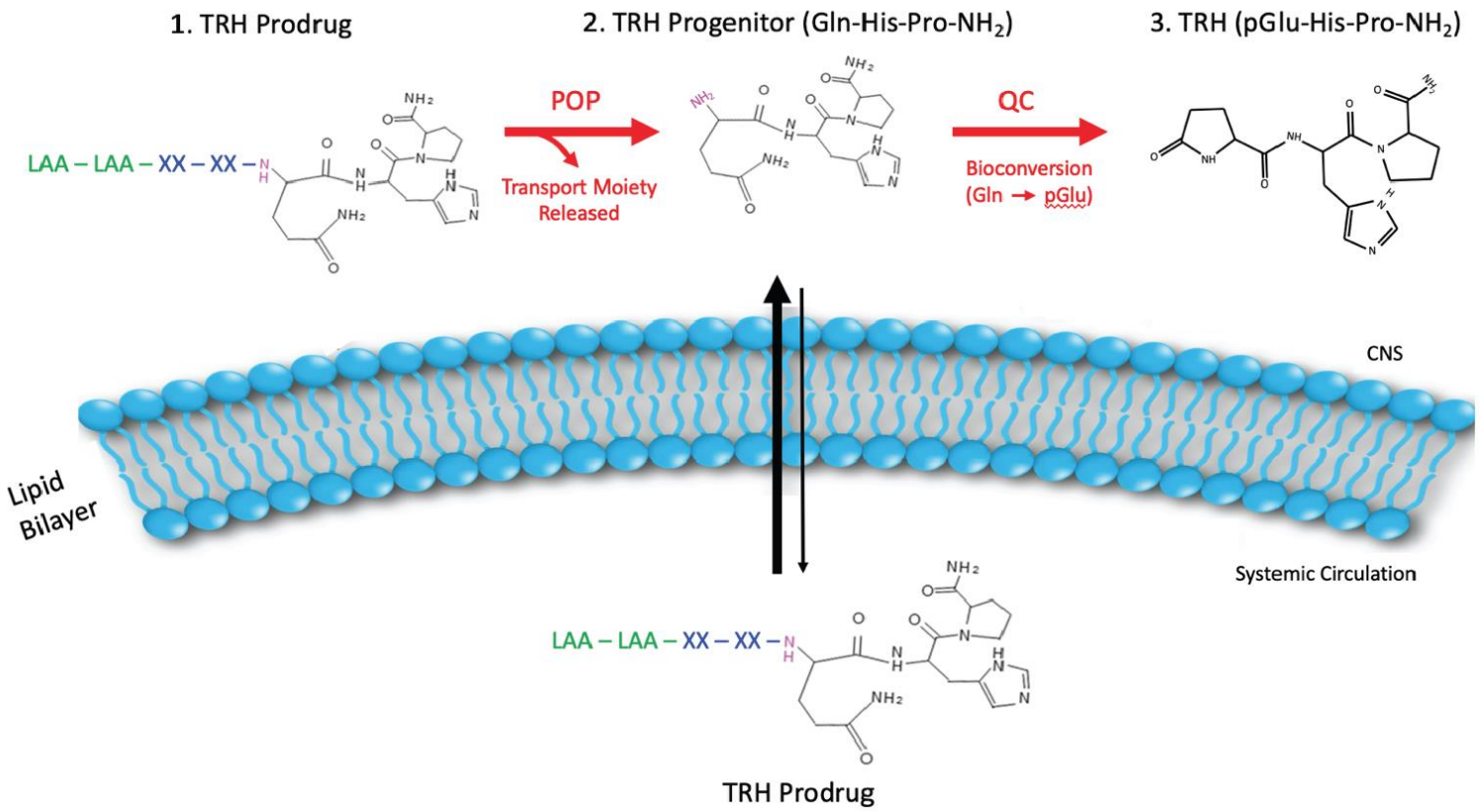
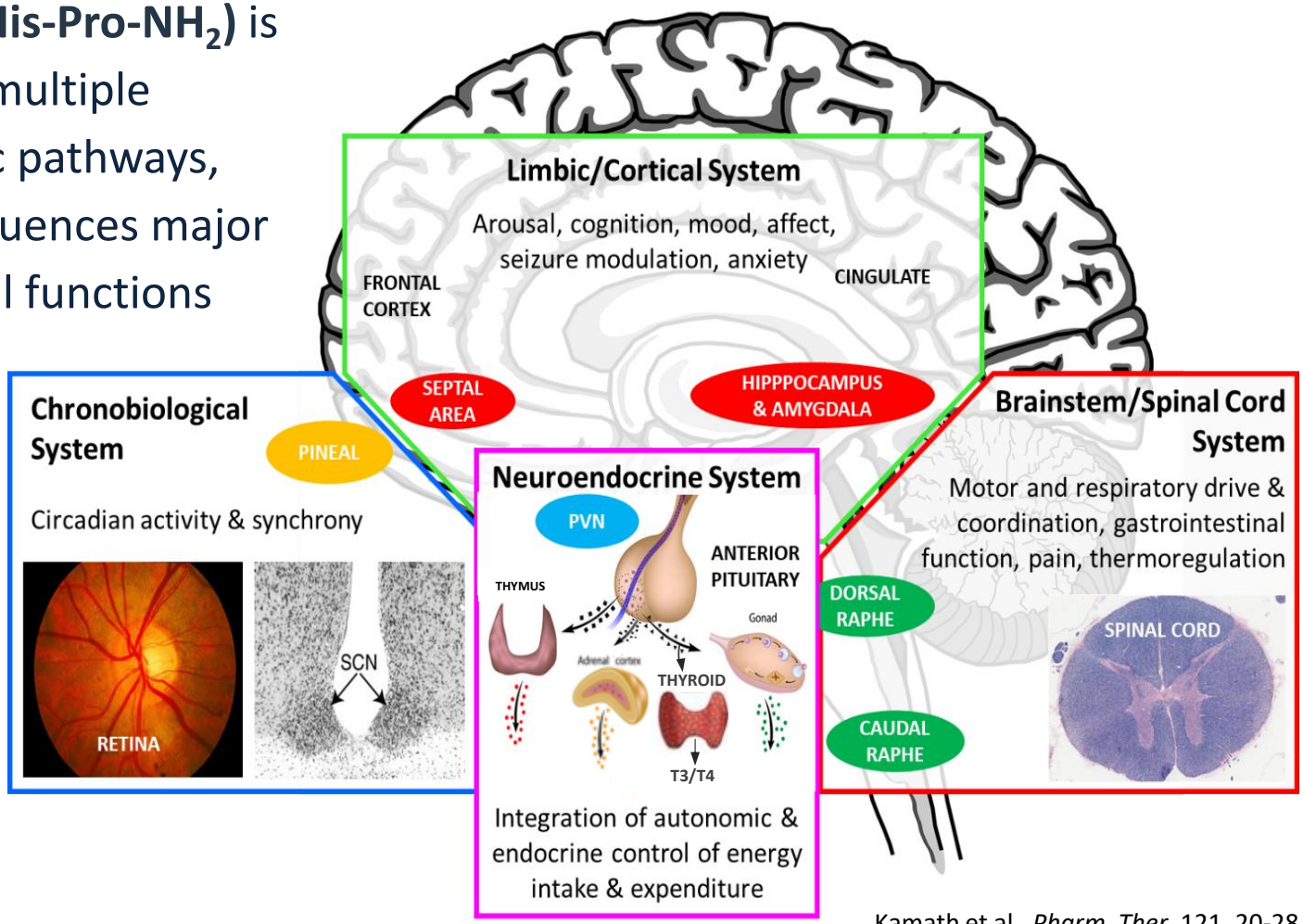


Abstract: Thyrotropin-releasing hormone (TRH) is a small tripeptide having the sequence pGlu-His-Pro-NH_2. It was initially discovered and studied for its role within multiple neuroendocrine pathways, yet TRH also influences broad neurological effects throughout the CNS by acting as a neurotransmitter and neuromodulator, mediating effects on feeding behavior, thermogenesis, locomotor activation, and autonomic regulation. TRH's ability to alter brain chemistry, behavior, and physiology implies great potential in treating neurological and psychological disorders, however, pharmacological applications have been largely unrealized due to peripheral exposure and brain delivery shortcomings following systemic administration of the peptide. Therefore, our laboratory has focused on developing a medicinal chemistry-based prodrug approach to overcome these limitations. Our TRH prodrugs are designed to exhibit favorable physiochemical properties to increase brain permeability and subsequent bioactivation by the preferentially brain expressed enzymes, prolyl oligopeptidase and glutaminyl cyclase. TRH's ability to increase acetylcholine release is well documented and its neurochemical assessment has been utilized in determining the CNS delivery of prodrug-derived TRH. Our laboratory's recent discovery of the first functional TRH antagonist (pGlu-βGlu-Pro-NH_2) and its allosteric binding site was also substantiated with neuropharmacological tests that assessed the attenuation of TRH's central cholinergic actions, which should prove useful in the development and validation of future prodrug designs. Altogether, these advancements establish not only the first TRH prodrug capable of delivering the metabolically liable TRH to the brain, but also a mechanistic elucidation of various TRH receptor-binding modalities.

Keywords: TRH; acetylcholine; antagonist; allosteric binding, brain delivery; docking; microdialysis; prodrug.

Introduction

TRH (pGlu-His-Pro-NH_2) is involved in multiple homeostatic pathways, where it influences major physiological functions



Kamath et al. *Pharm. Ther*. 121, 20-28 (2009).

TRH as a CNS Agent

Human Health Significance

CNS modulatory actions:

- Neurotransmitter (NT)
- Neuromodulator of other NTs

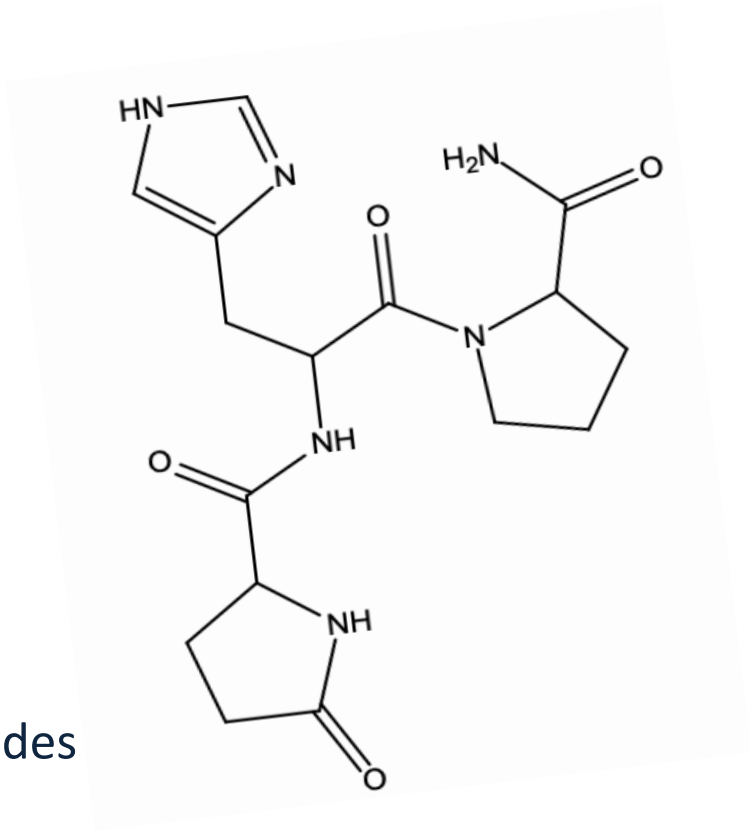
  e.g., acetylcholine release

- Activation of sympathetic nervous system

TRH (pGlu-His-Pro-NH_2)

TRH Brain Delivery

- Clinically relevant central effects
- Biomarkers of peripheral impact
- Poor drug-like properties
- Characteristics typical of small peptides



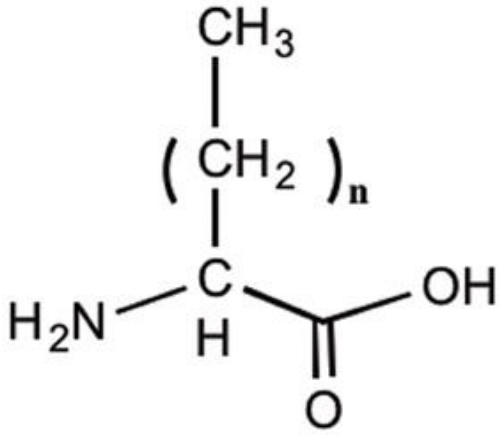
Development of a Novel CNS-delivery Method

Prodrug Delivery Model

- Blood-brain barrier permeability
- Metabolic stability for CNS transport from the circulation
- Prodrug metabolism directed to the site-of-action by preferentially brain-expressed enzymes

Prodrug Approach

Medicinal Chemistry-Driven Approach



LAA

- Creation of inert prodrug compounds
- Bioreversible chemical modification(s)
- Conjugation of “promoiety(ies)” to the agent/ analogue
- Brain-targeting approach: bioactivation of prodrug occurs via brain enzymes

e.g., increasing lipophilicity via α-lipoamino acids (LAAs)

TRH Progenitor Sequence (pGlu-His-Pro-NH_2)

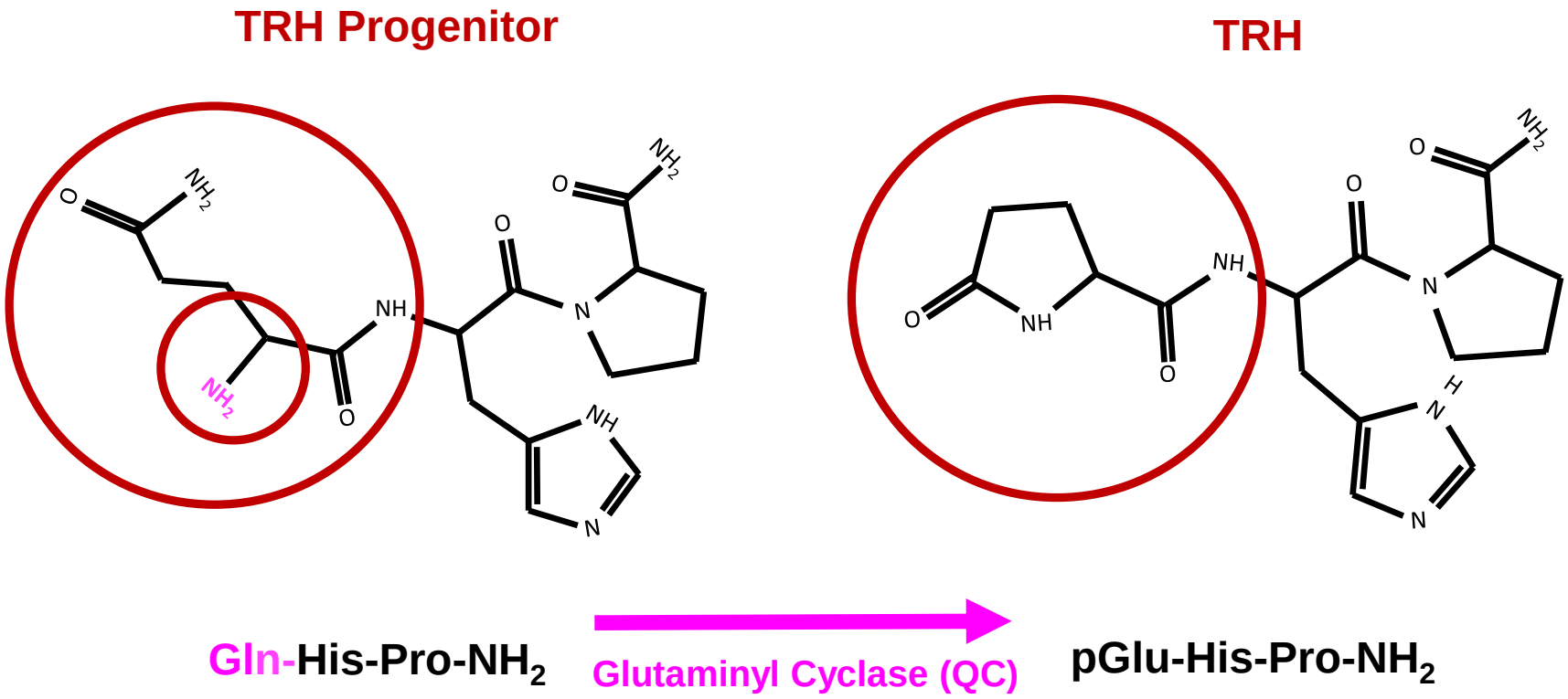
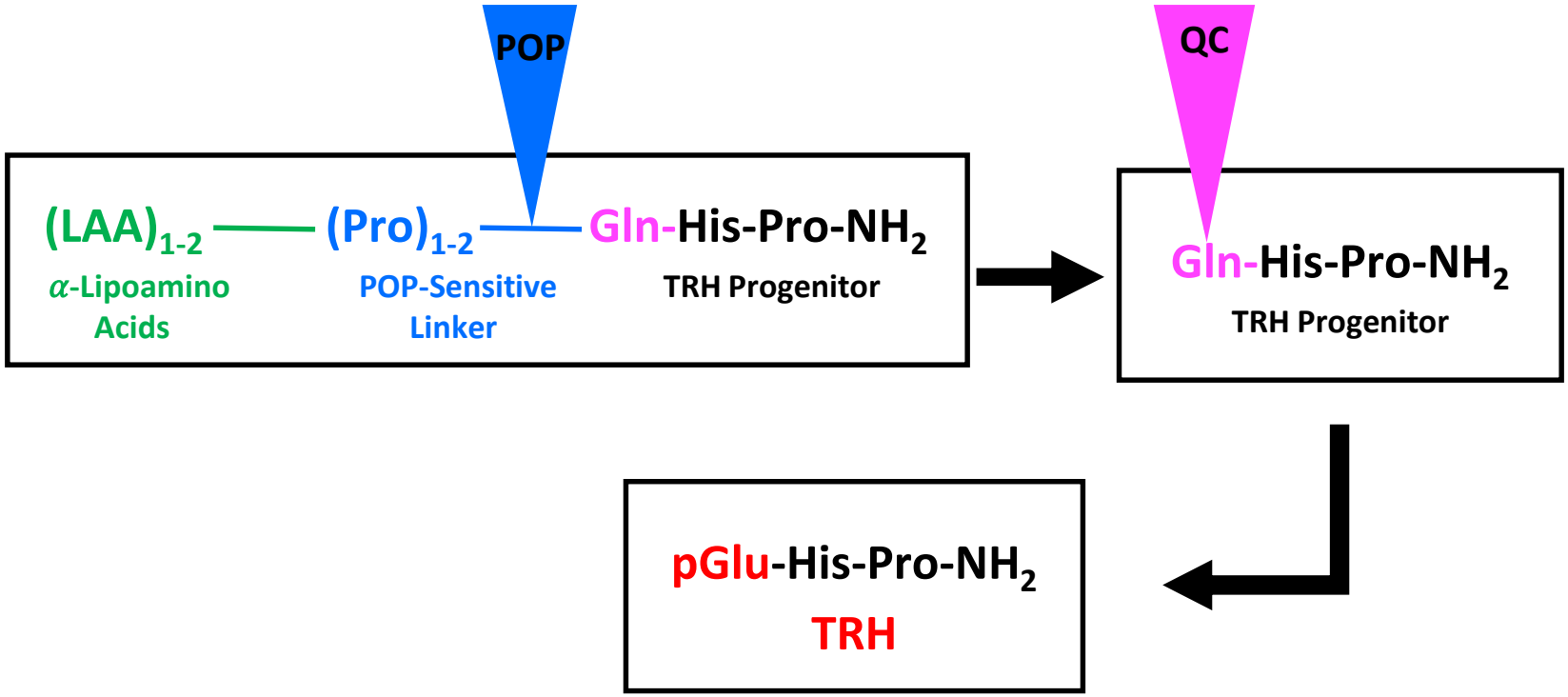


Figure 1. TRH progenitor has a **free amino terminus** that is converted to TRH by QC.

Novel Prodrug Design

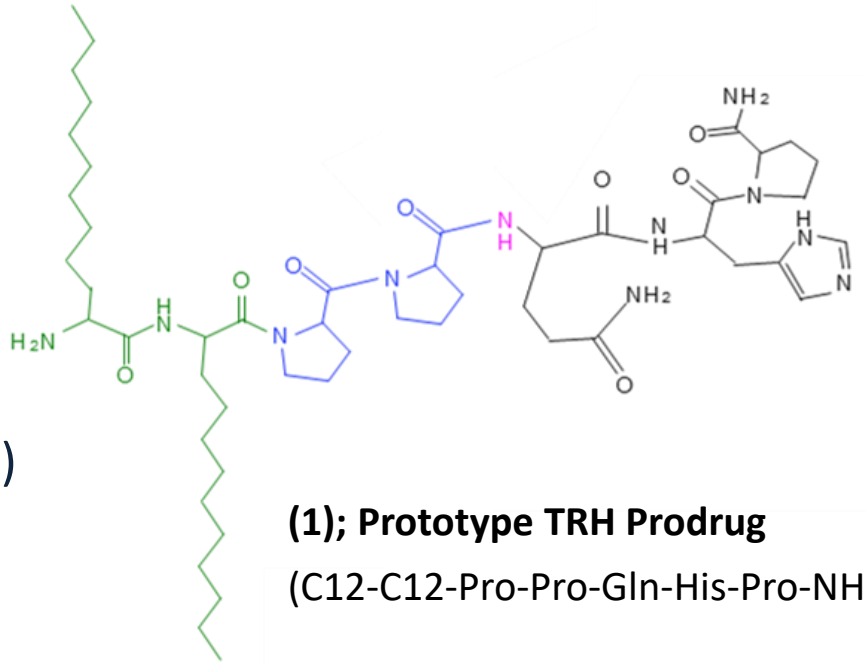


Scheme 1. Schematic illustration of our novel prodrug design concept with biotransformation to TRH within the brain by prolyl oligopeptidase (POP) and QC.

Constructed Prodrug Structure

Components of Prodrug (1)

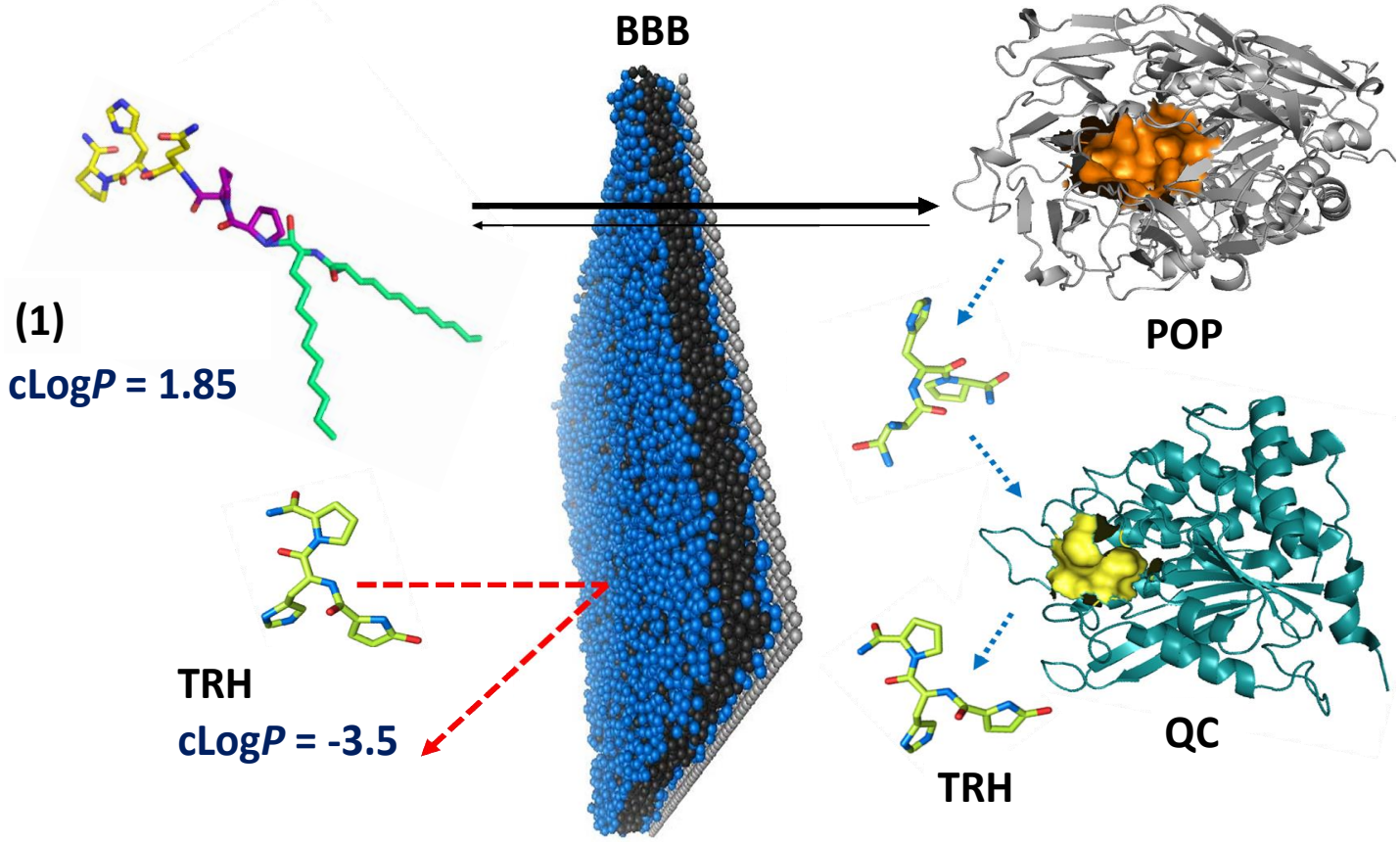
- Progenitor Gln-His-Pro-NH_2 (black)
- Extended from the progenitor sequence's N-terminus (pink)
- (Pro-Pro) POP-sensitive linker (blue)
- 2-aminododecanoyl (C12) LAA residues (green)



(1); Prototype TRH Prodrug

(C12-C12-Pro-Pro-Gln-His-Pro-NH_2)

Bioconversion of Prodrug (1)



Scheme 2. Schematic illustration of (**1**), lead TRH prodrug, with biotransformation to TRH within the brain.

Prokai-Tatrai et al. *Pharmaceutics*, *11*.7: 349 (2019).

Microdialysis – ACh, a Biomarker of TRH Release

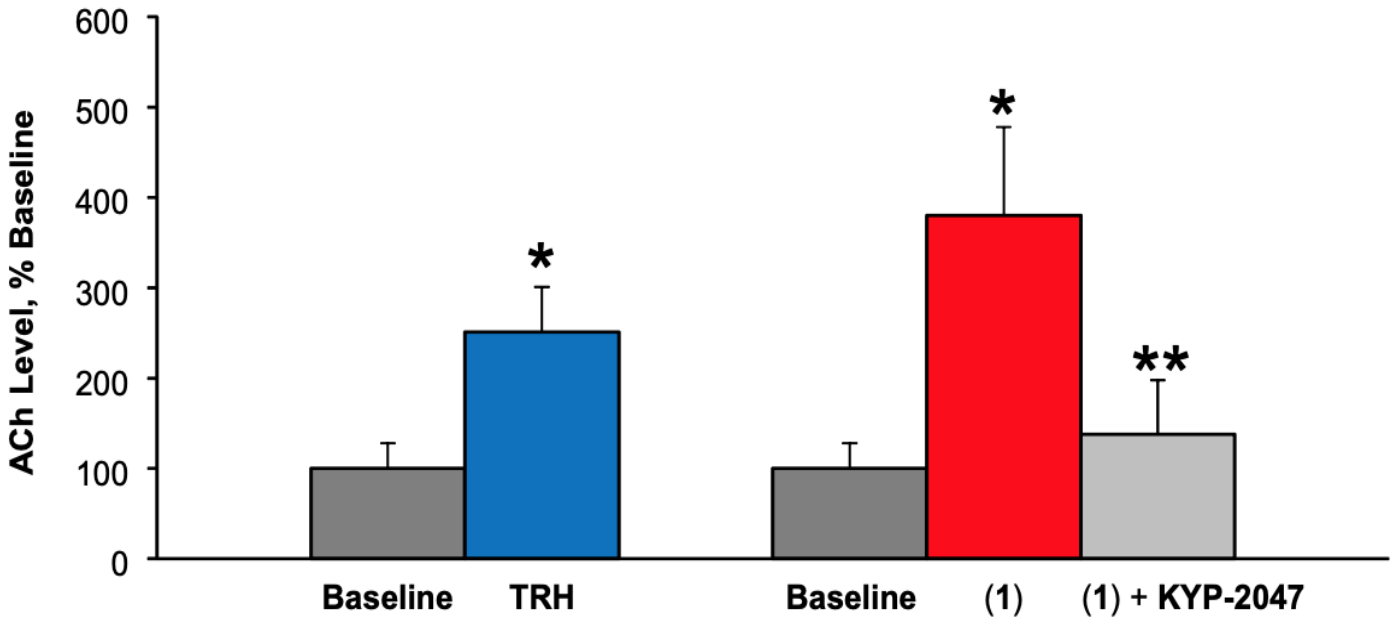


Figure 2. In rat frontal cortex, baseline was taken as 100% of steady-state ACh concentrations for each animal before perfusion with test compounds and compared to the measured ACh increase. *Indicates statistically significant difference ($p<0.05$) from baseline level. **Indicates statistically significant difference ($p<0.05$) from prodrug perfusion, but no difference from the baseline.

Prokai-Tatrai et al. *Pharmaceutics. 11.*7: 349 (2019).

Metabolic Stability Studies

In vitro prodrug stability in male CD-1 mouse serum and brain homogenate

Table 1. Test compounds were used at 10 µM concentration, while KYP-2047 (a POP inhibitor) was used at 50 µM. Data represent averages, n = 3, errors are standard deviations.

Test compound	$t_{1/2}$ in Serum	$t_{1/2}$ in 20% (w/v) Brain Homogenate
TRH	7 ± 4 min	4 ± 1 min
(1)	100 ± 7 min	47 ± 6 min
(1) + KYP-2047	115 ± 13 min	>24 h

Prokai-Tatrai et al. *Pharmaceutics*, *11*.7: 349 (2019).

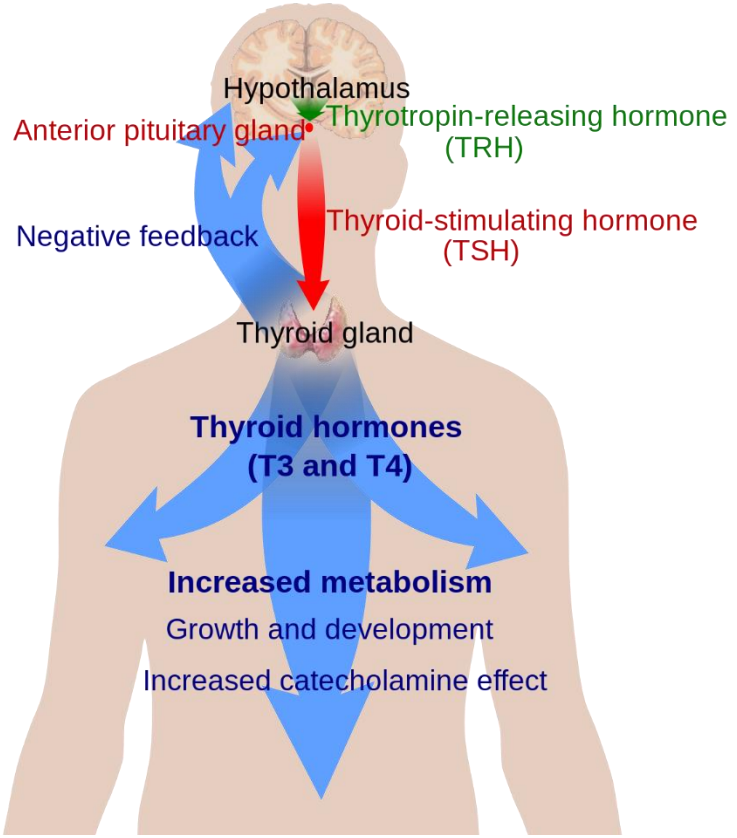
Neuroendocrine Therapeutic Safety Assessment

TRH Challenge*

Hypothalamic-Pituitary-Thyroid Axis

- Single bolus s.c. injection of TRH given to CD-1 mice induces TSH release
- LC-MS/MS method developed to quantify T3/T4 serum concentrations

Thyroid System



*Greeley et al. *Endoc. Res. Comm*. 9, 169-177 (1982).

Biomarker of TRH Endocrine Activation – T3/T4 Serum Levels

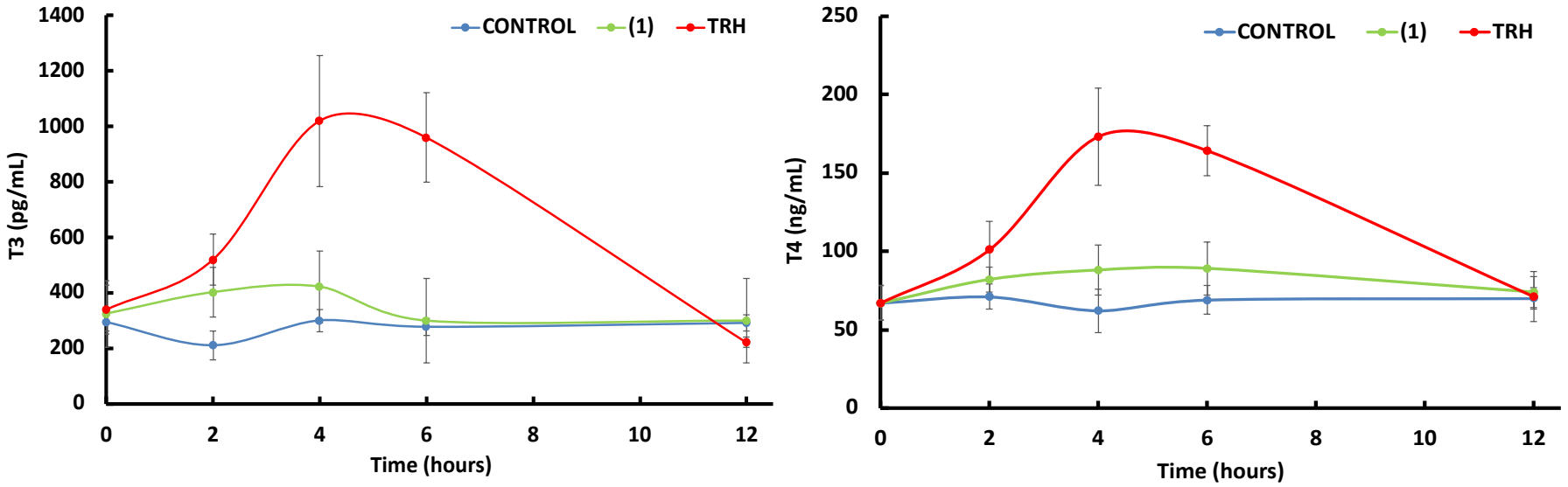


Figure 3. TRH challenge in male CD-1 mouse serum with equimolar dosage TRH vs (1) (s.c. administration, 15 μM/kg body weight). Data shown as AVE ± SDEV, n=4.

TRH Antagonist – Microdialysis

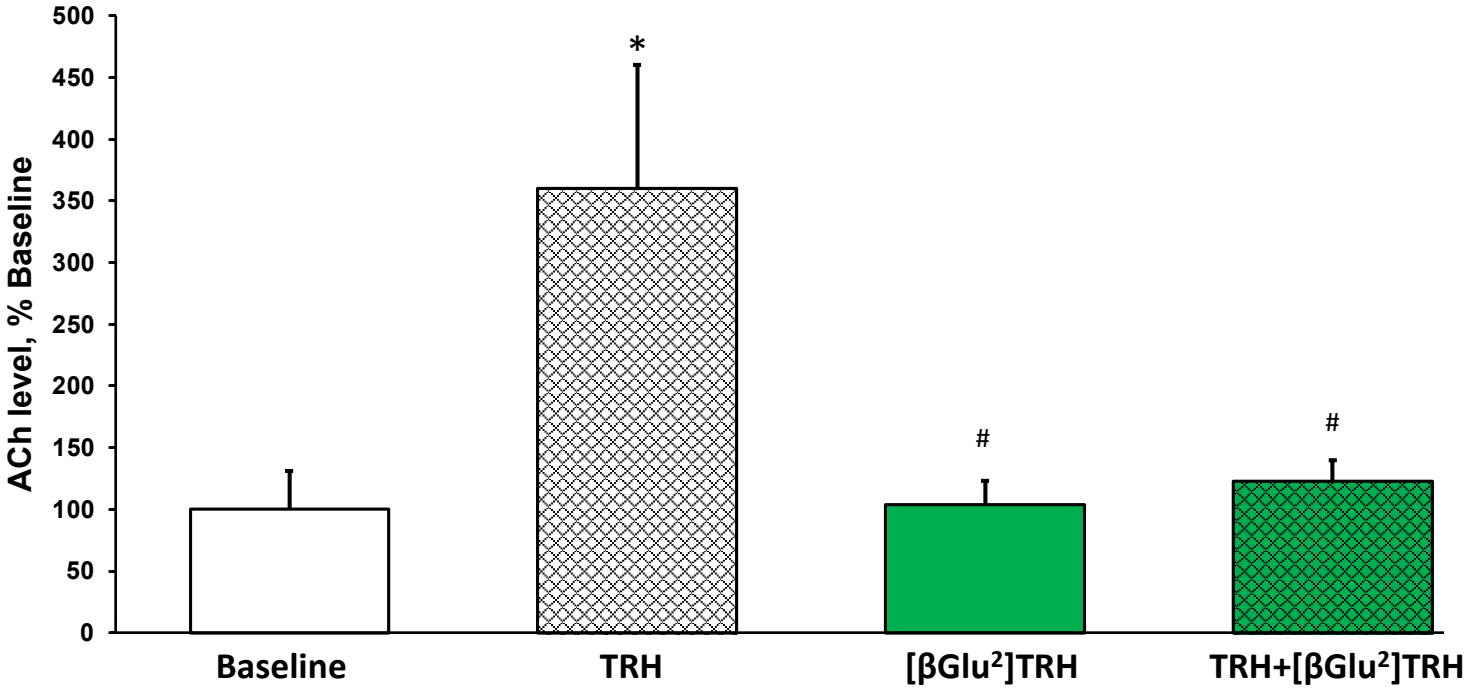


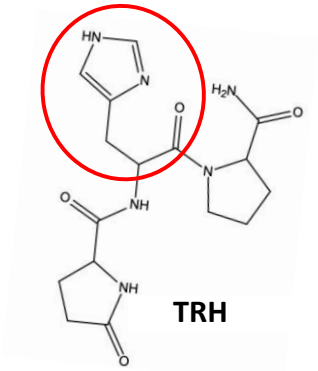
Figure 4. Percent changes in hippocampal ACh levels upon perfusing test agents alone or together with an equimolar concentration of TRH (3 µM, each in aCSF containing 2 µM neostigmine). The steady-state baseline ACh level was measured in the microdialysates collected for 2 h in 20-min fractions after 1 h of the initiation of the experiment, then perfusion switched to solutions containing test agents. ACh levels after the test agents' perfusions were expressed as % changes 3–6 fractions after equilibration compared with the steady state baseline ACh levels taken as 100%. ANOVA followed by post hoc Tukey's tests ($p < 0.05$, $n = 4$): * Indicates statistically significant difference from baseline control, # Indicates statistically significant difference from TRH alone.

Prokai-Tatrai et al. *Int. J. Mol. Sci.* 2021, 22, 6230.

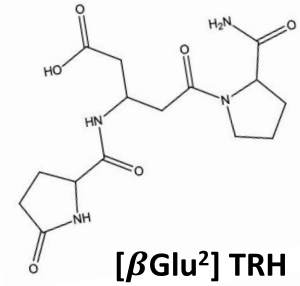
Human TRH Receptor (hTRH-R) Homology Model

hTRH-R Binding Modalities

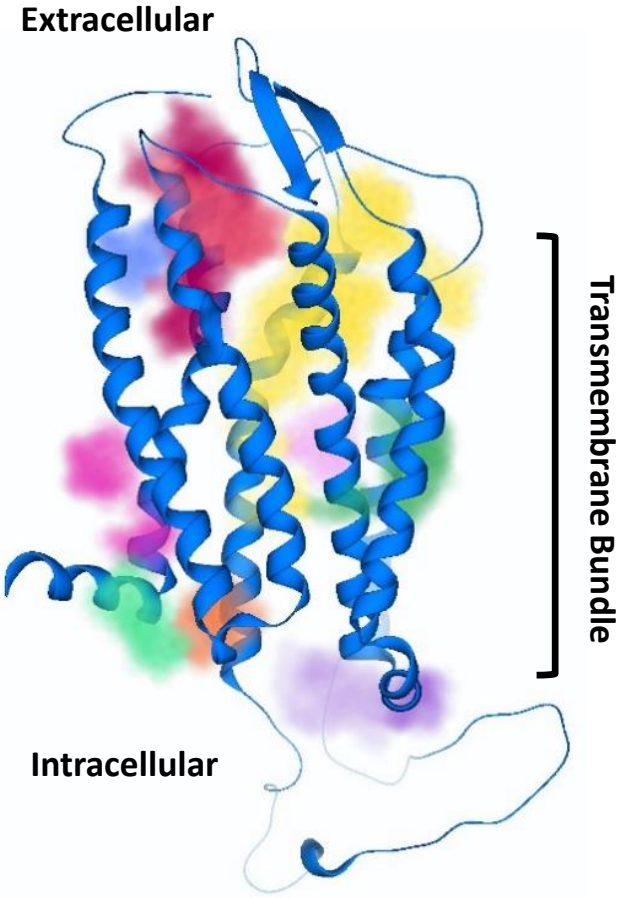
- Model validation using TRH and analogues with [His^2] substitutions
- Binding pockets explored
- Sequential TRH binding: surface recognition then ligand internalization to the transmembrane domain



TRH



[βGlu^2] TRH



De La Cruz et al. *Molecules*, *26*: 5397 (2021).

Human TRH Receptor (hTRH-R) Homology Model

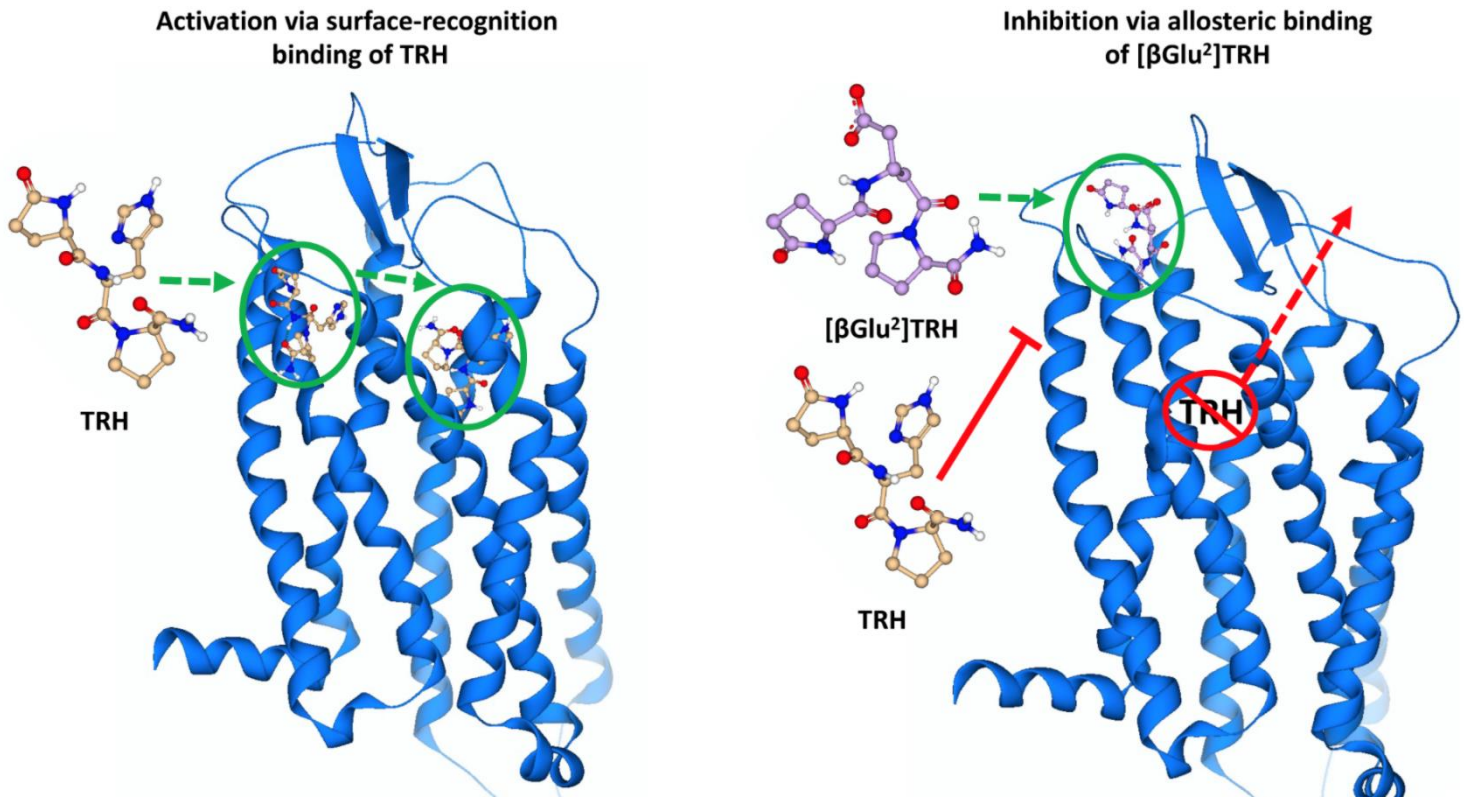


Figure 5. Docked TRH poses within its sequential binding sites of the hTRH-R homology model (left), and the allosteric binding of [βGlu²]TRH, the first TRH functional antagonist (right).

De La Cruz et al. *Molecules*, *26*: 5397 (2021).

Allosteric hTRH-R Binding Studies with TRH and Its Analogues

Table 2. Binding affinities of TRH (Figure 1a) and TRH-like peptides (Figure 1b-e) within the proposed allosteric binding site of the hTRH-R homology model. K_d denotes dissociation constant.

Ligand	Binding Affinity (ΔG, kcal/mol)	K_d
TRH (1a)	– [1]	– [1]
[βGlu^2]TRH (1b)	–8.8 ± 2.5	nM
[Glu^2]TRH (1c)	–6.1 ± 2.5	nM–μM
[β-homoGlu^2]TRH (1d)	–5.6 ± 2.5	μM–mM
[Asp^2]TRH (1e)	–5.4 ± 2.5	μM–mM

[1] N/A (no binding pose could be generated).

De La Cruz et al. *Molecules*, *26*: 5397 (2021).

hTRH-R Binding Study with TRH vs Prodrug (1)

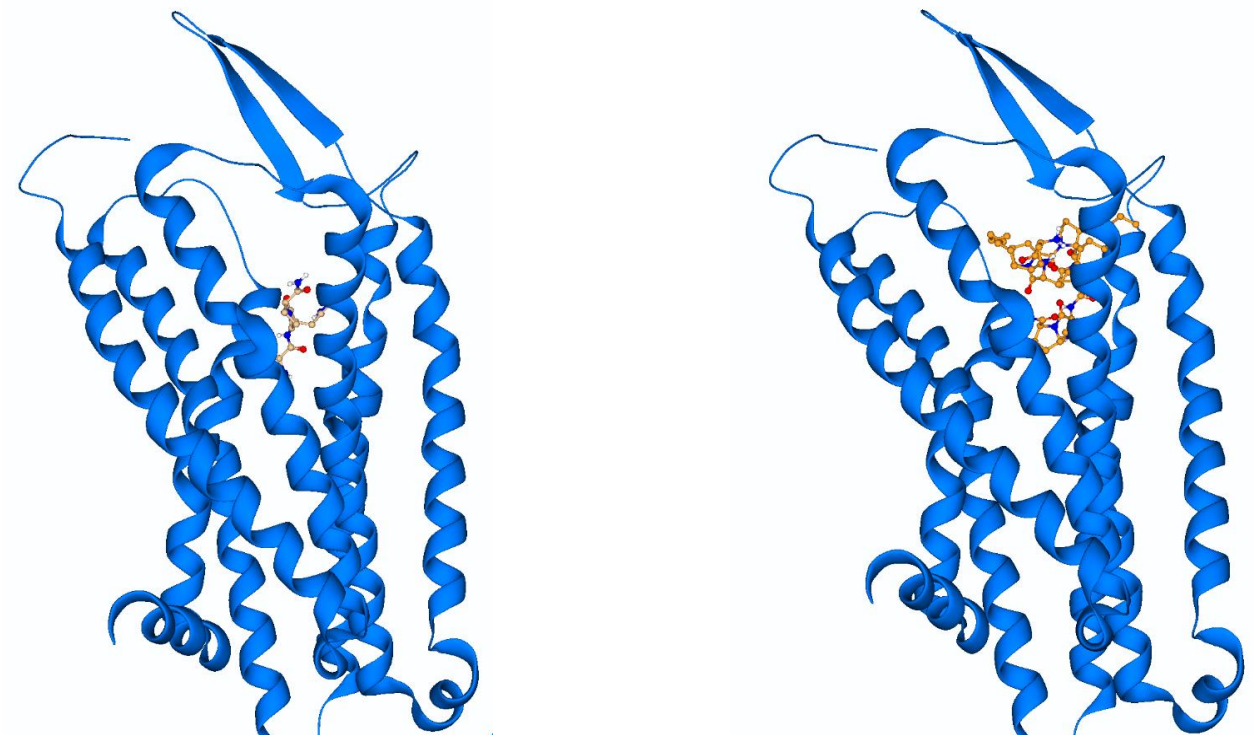


Figure 6. Docked ligand poses within the active site of the hTRH-R homology model, using TRH (-8.1 kcal/mol) and prodrug (**1**) (8.8 kcal/mol), on the left and right, respectively. A positive binding affinity for (**1**), indicates a non-fit due to intermolecular clashes.

De La Cruz et al. *Molecules*, *26*: 5397 (2021).

Conclusions

- First TRH prodrug (1), C12-C12-Pro-Pro-Gln-His-Pro-NH_2, shown to be capable of delivering the metabolically highly liable TRH into the brain
- Prodrug delivery was validated by utilizing TRH's cholinergic action via neurochemical and neuropharmacological tests
- Using the HPT axis, a therapeutic safety assessment of (1) revealed no increase in serum T3 and T4 level upon prodrug administration
- Discovery and characterization of [βGlu^2]TRH as the first functional TRH antagonist
- Validation and mechanistic elucidation of various TRH receptor-binding modalities

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