

## Brief Articles

### Thyrotropin-Releasing Hormone (TRH) Analogues That Exhibit Selectivity to TRH Receptor Subtype 2

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Thyrotropin-releasing hormone (TRH) analogues in which the C-2 position of the imidazole ring of the centrally placed histidine residue is substituted with various alkyl groups were synthesized and studied as agonists for TRH receptor subtype 1 (TRH-R1) and subtype 2 (TRH-R2). Several analogues were found to be selective agonists for TRH-R2 exhibiting no activation of TRH-R1. For example, analogue 4 ( $R = n\text{-C}_3\text{H}_7$ ) was found to activate TRH-R2 with a potency ( $\text{EC}_{50}$ ) of  $0.41\text{ }\mu\text{M}$  but did not activate TRH-R1 (potency  $> 100\text{ }\mu\text{M}$ ). This study describes the first discovery of TRH-R2-specific agonists and provides impetus to design predominately CNS-effective TRH peptides.

#### Introduction

Thyrotropin-releasing hormone (TRH, L-pGlu-L-His-L-ProNH<sub>2</sub>), a simple tripeptide amide, is the first neuropeptide whose chemical structure was elucidated.<sup>1,2</sup> TRH is synthesized in the hypothalamus and acts in the anterior pituitary to control levels of TSH (thyrotropin, thyroid stimulating hormone) and prolactin. This peptide is the first hypothalamic releasing factor characterized, establishing the fundamental proof for the existence of neuroendocrine regulation of pituitary functions by hypothalamic neuronal structures.<sup>3,4</sup> TRH plays a central role in regulating the pituitary-thyroid axis by stimulating TSH release and de novo synthesis in all mammalian species. The peptide also acts as an equally potent stimulator of prolactin secretion and synthesis. Though the neuroendocrine action of TRH on the anterior pituitary (PIT) dominated the earlier research, it was discovered that the effects of TRH are not limited to the anterior pituitary but that TRH exerts profound effects on the central nervous system (CNS).<sup>5</sup> Thus, TRH has been shown to (a) alter neuronal excitability, (b) enhance transmitter release and turnover, (c) increase CNS arousal, (d) increase blood pressure, body temperature and respiration rate, (e) alter body water and food intake, (f) enhance locomotor activity, and (g) produce antinociception. Interestingly, the potential therapeutic applications of TRH that have attracted the most attention are not based on its endocrine properties but on its broad spectrum of stimulatory actions within the CNS. These CNS-mediated effects provides the rationale for the use of TRH in the treatment of brain and spinal injury and certain CNS disorders, including Alzheimer's disease and motor neuron disease (MND). Research efforts toward development of potent TRH analogues with selective CNS

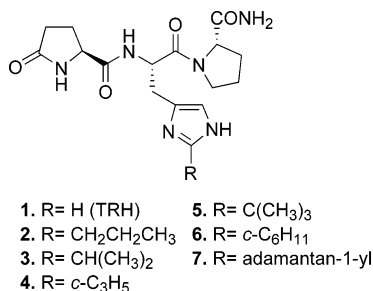
activity have not been successful. Although, analogues with potent CNS activity were synthesized, none were completely devoid of endocrine side effects. Moreover, in common with other potential drugs that are peptides, the therapeutic efficacy of TRH is compromised by its instability and hydrophilic nature. Thus, the high-dosage regimes needed to obtain neuropharmacological effects often result in adverse side effects arising from the endocrine actions of TRH.

TRH initiates its effects by interacting with receptors on the surface of cells. Receptors for TRH belongs to the rhodopsin/ $\beta$ -adrenergic receptor subfamily of seven transmembrane (TM)-spanning, G protein-coupled receptors (GPCRs).<sup>6</sup> The first TRH receptor (TRH-R1) was cloned from a mouse pituitary tumor, and then orthologous receptors were cloned from different species, including rat, chicken, *Catostomus commersoni*, and human.<sup>7–9</sup> Recently, a second subtype of TRH receptor (TRH-R2) was identified in rats, mouse, and *Catostomus commersoni*.<sup>10–13</sup> Amino acid sequences of the two subtypes of TRH receptor from the same species reveal a 51% overall identity.<sup>14</sup> Subsequent comparison of TRH-R1 and TRH-R2 has been made regarding their tissue distribution. TRH-R1 is highly expressed in the anterior pituitary and is mainly involved in the signaling of TRH within neuroendocrine brain regions, the autonomic nervous system, and the visceral brainstem regions. TRH-R1 exhibits only a very limited mRNA expression pattern in other regions of the CNS. In contrast, TRH-R2 is strongly expressed in rat brain and spinal cord, but is not detectable in the pituitary.<sup>14</sup> The specific expression of TRH-R2 in brain areas that are important for the transmission of somatosensory signals and higher CNS functions indicates that in the CNS this receptor subtype may be of major functional importance. Therefore, we believe that a therapeutically useful CNS selective TRH analogue (mediated by TRH-R2) should exert weak or no TSH-releasing effects (mediated by TRH-R1). However, analogues reported to date exhibit

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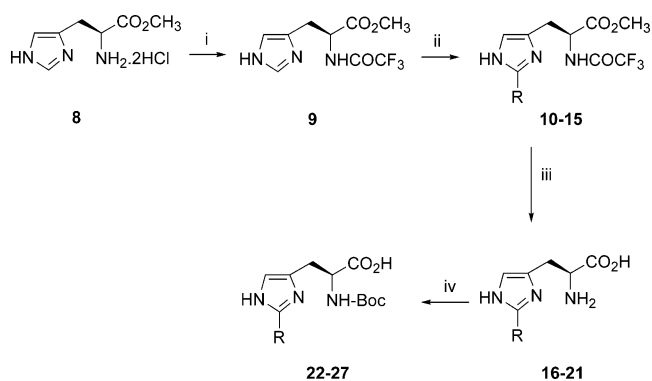
**Figure 1.**

strong TSH-releasing effects while at the same time produce “nonendocrine” CNS effects.<sup>15</sup> Therefore, if analogues were developed with high selectivity for TRH-R2 receptor subtype, they could probably demonstrate more specific and selective profiles of CNS effects.

All three residues in TRH contribute to its binding to TRH receptors.<sup>16</sup> Removal of key amino acids, pGlu by proteolytic pyroglutamyl aminopeptidase II, and histidine by the histidyl-proline iminopeptidase have restricted the therapeutic utility of this important hormonal/CNS peptide.<sup>17</sup> With a paradigm shift in peptide research, it is now established that synthetic and modified  $\alpha$ -amino acids play a significant role in the area of peptide-based drug design and discovery research. These important building blocks are being extensively incorporated into bioactive peptides to restrict their conformational flexibility, enhance proteolytic stability, improve drug-like properties, and most importantly increase selectivity. Upon the basis of these observations, our current research efforts are in the direction of synthesizing novel TRH analogues with particular emphasis on the replacement of the pGlu and His residues with their modified counterparts and scaffolds.<sup>18,19</sup> In continuation of our research program, we report herein synthesis and receptor binding studies for a set of six TRH analogues where the central histidine residue is modified by incorporating an alkyl group at the C-2 position of the imidazole ring (Figure 1). Hydrophobic alkyl substituents, such as propyl, isopropyl, *tert*-butyl, cyclobutyl, cyclohexyl, and an adamantyl group, were selected for this initial study. In addition, to observe the effect of their placement on receptor subtype selectivity, the choice of these groups was also made upon the assumption that their incorporation may slightly reduce the hydrophilicity of the synthesized peptides thereby making them amenable for drug development.

## Results and Discussion

**Chemistry.** 2-Alkyl-L-histidines **16–21** required for the preparation of *N*- $\alpha$ -Boc-2-alkyl-L-histidines **22–27** were synthesized in three convenient steps (Scheme 1).<sup>20,21</sup> Thus, commercially available L-histidine methyl ester dihydrochloride (**8**) upon reaction with trifluoroacetic anhydride for 24 h at ambient temperature provided *N*- $\alpha$ -trifluoroacetyl-L-histidine methyl ester (**9**). The latter compound **9** upon reaction with various commercially available alkylcarboxylic acids in the presence of 10% H<sub>2</sub>SO<sub>4</sub>, catalytic silver nitrate, and ammonium persulfate at 70–80 °C for 15 min provided the *N*- $\alpha$ -trifluoroacetyl-2-alkyl-L-histidine methyl esters **10–15**. The reaction proceeds through a free radical

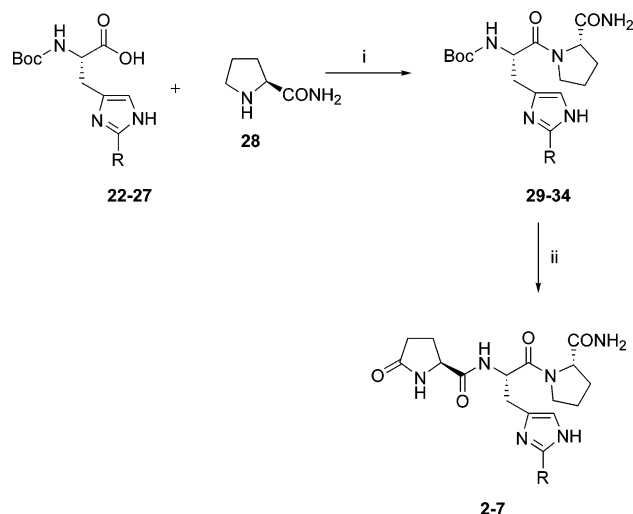
**Scheme 1**<sup>a</sup>

<sup>a</sup> Reagents and conditions: (i) (CF<sub>3</sub>CO)<sub>2</sub>O, rt, 24 h; (ii) RCO<sub>2</sub>H, AgNO<sub>3</sub>, 10% H<sub>2</sub>SO<sub>4</sub>, (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, 70–80 °C, 15 min; (iii) 6N HCl, reflux, Dowex ion-exchange resin (50  $\times$  2–200, H<sup>+</sup> form); (iv) a. Boc<sub>2</sub>O, 4 N NaOH, dioxane–H<sub>2</sub>O (2:1), rt, 24 h; b. MeOH, 12 h; c. KHSO<sub>4</sub>, pH = 3.75.

mechanism and offers an unparalleled procedure for functionalization of the electron-deficient imidazole ring.<sup>22,23</sup> Reaction involves nucleophilic addition of an alkyl radical (generated by the silver-catalyzed oxidative decarboxylation of alkylcarboxylic acid with ammonium persulfate) to a protonated imidazole ring followed by rearomatization leading to direct and selective imidazole C-2 alkylation. Complete deprotection of **10–15** by refluxing a solution of them in 6 N HCl and evaporation of the acidic hydrolysis solution under reduced pressure produced amino acid dihydrochloride salts. The free amino acids **16–21** were obtained by elution with 25% NH<sub>4</sub>OH solution from Dowex ion-exchange resin (50  $\times$  2–200, H<sup>+</sup> form) column. Compounds **16–21** upon reaction with di-*tert*-butyl dicarbonate in the presence of aqueous 4 N NaOH solution at ambient temperature in dioxane–water (2:1) mixture for 24 h followed by stirring with MeOH for 12 h led to the formation of *N*- $\alpha$ -Boc-2-alkyl-L-histidines **22–27**, suitable for the synthesis of TRH analogues.<sup>24</sup> At the same time, L-pyroglutamic acid (L-pGlu) upon reaction with 2,4,5-trichlorophenol in the presence of 1,3-dicyclohexylcarbodiimide (DCC) in DMF for 12 h gave 2,4,5-trichlorophenyl active ester (L-pGlu-OTcp) in excellent yields as described earlier.<sup>19</sup>

The C-terminal to N-terminal solution phase peptide synthesis of the intermediate dipeptides **29–34** was accomplished by the reaction of commercial L-prolinamide (**28**) with *N*- $\alpha$ -Boc-2-alkyl-L-histidines **22–27** in the presence of coupling reagents *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) and 1,3-diisopropylcarbodiimide (DIC) at 4 °C for 36 h. Subsequently, cleavage of the *N*- $\alpha$ -Boc group with TFA (40% solution in DCM) produced dipeptide trifluoroacetate salts, which were not isolated and immediately subjected to next reaction. The free dipeptides were generated in situ by reaction of the latter compounds with 7 N NH<sub>3</sub> in MeOH. Complete removal of the solvent followed by coupling of the free dipeptides with L-pGlu-OTcp in DMF at 4 °C for 36 h produced TRH analogues **2–7** in satisfactory yields (Scheme 2).

**Pharmacology at TRH-R1 and TRH-R2.** Synthesized TRH analogues **2–7** were examined for their affinity for TRH-R1 and TRH-R2 and their ability to serve as agonists for the receptor.<sup>25</sup> Affinities, reported as *K<sub>i</sub>* ( $\mu$ M) values, were determined by measuring the

Scheme 2<sup>a</sup>

<sup>a</sup> Reagents and conditions: (i) HONB, DIC, DMF, 4 °C, 36 h; (ii) a. 40% TFA, DCM, rt, 30 min; b. 7 N NH<sub>3</sub>/MeOH, 10 min; c. L-pGlu-OTcp, DMF, 4 °C, 36 h.

concentration of the analogue required to compete with [N<sup>ε</sup>(1-Me-His)-TRH] for the binding of the receptor. [N<sup>ε</sup>(1-Me-His)-TRH] is known to bind to TRH-R1 and TRH-R2 with affinities approximately 7–10 times higher than TRH. The agonist behavior of the analogues was tested in HEK 293EM cells stably expressing TRH-R1 or TRH-R2 by incubating the cells with various doses of the analogues being examined. The extent of agonist behavior was then determined by measuring signaling through a reporter gene, and the data are reported as EC<sub>50</sub> (μM) values.

None of the 2-alkyl-L-histidine substituted TRH analogues **2–7** bound with high affinity (*K<sub>i</sub>*) to TRH-R1 or TRH-R2 (Table 1). Analogues **2–6**, however, displayed full agonist activity at TRH-R2 but only analogue **2** activated TRH-R1 whereas analogues **3–6** did not activate TRH-R1. Analogue **2** (R = CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>) was a potent agonist for TRH-R2 (EC<sub>50</sub> = 0.024 μM) with >12-fold selectivity for TRH-R2 over TRH-R1. Analogue **4**

(R = *c*-C<sub>3</sub>H<sub>5</sub>) exhibited the highest potency (EC<sub>50</sub> = 0.41 μM) for TRH-R2 of the analogues that did not activate TRH-R1. Since analogues **3**, **5**, and **6** did not activate TRH-R1, they are specific agonists of TRH-R2 also. In contrast, analogue **7** (R = adamantan-1-yl) was inactive at both TRH receptors. These results are highly satisfying and represent the first report of the discovery of TRH analogues that exhibit high selectivity as agonists for TRH-R2. As discussed above, TRH-R2 is predominantly and selectively expressed in brain areas that are important for the higher central nervous system functions, whereas TRH-R1 is known to mainly mediate endocrine functions. Therefore, high agonist selectivity produced by compound **2–6** is of considerable significance and beneficial in search of additional analogues that will not only be CNS-selective but more active than TRH.

## Conclusions

In the present study we have attempted to determine the effects of TRH peptides synthesized through modification at the imidazole ring of the histidine residue on receptor subtype specificity. For the first time, specific TRH agonists toward TRH-R2 receptor at low micromolar concentrations are described. These compounds hold great promise, and we hope that their optimization will lead to additional new synthetic peptides with even greater specificity for TRH-R2. Exploration of alkyl groups of variable sizes at other feasible position(s) on the imidazole ring of TRH and their effect on the receptor subtype selectivity is currently underway in our laboratory.

## Experimental Section

**Receptor Binding Assay.** HEK 293EM cells stably expressing either TRH-R1 or TRH-R2 were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 200 mg/mL hygromycin. For equilibrium binding experiments, HEK 293EM cells stably expressing TRH-R1 or TRH-R2 were seeded into 24-well plates (1.5 × 10<sup>5</sup>/well). After 48 h, cells were incubated at 37 °C for 1 h with [<sup>3</sup>H]N<sup>ε</sup>(1-Me-His)-TRH (MeTRH, 0.1 nM–20 nM) in Hank's Balanced Salt

**Table 1.** Binding Affinities *K<sub>i</sub>* (μM) and Signaling (activation) Potencies EC<sub>50</sub> (μM) Produced by TRH Analogues **2–7** for TRH-R1 and TRH-R2 Receptors

no.	R	<i>K<sub>i</sub></i> (μM) <sup>a</sup>			EC <sub>50</sub> (μM) <sup>b</sup>		
		TRH-R1	TRH-R2	fold select. (TRH-R2)	TRH-R1	TRH-R2	fold select. (TRH-R2)
<b>2</b>	CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	0.32 (0.27–0.37)	0.17 (0.12–0.24)	1.88	0.29 (0.16–0.51)	0.024 (0.011–0.052)	>12
<b>3</b>	CH(CH <sub>3</sub> ) <sub>2</sub>	>100	>100	—	>100	1.5 (0.69–3.2)	>67
<b>4</b>	<i>c</i> -C <sub>3</sub> H <sub>5</sub>	>100	>100	—	>100	0.41 (0.19–0.85)	>244
<b>5</b>	C(CH <sub>3</sub> ) <sub>3</sub>	>100	>100	—	>100	1.3 (0.76–2.2)	>77
<b>6</b>	<i>c</i> -C <sub>6</sub> H <sub>11</sub>	>100	>100	—	>100	1.1 (0.21–5.7)	>91
<b>7</b>	adamantan-1-yl	>100	>100	—	>100	>100	—
TRH	H	0.02 (0.001–0.003)	0.01	2	0.003 (0.002–0.004)	0.003	—
[N <sup>ε</sup> (1-Me-His)-TRH]		0.003	—	—	0.0005	—	—

<sup>a</sup> For binding, cells expressing TRH-R1 or TRH-R2 were incubated with 1 nM [<sup>3</sup>H]N<sup>ε</sup>(1-Me-His)-TRH in the absence or presence of various doses of unlabeled TRH analogues for 1 h at 37 °C. <sup>b</sup> For signaling, cells expressing TRH-R1 or TRH-R2 and a CREB-luciferase reporter were incubated with various doses of TRH analogues for 6 h at 37 °C, and luciferase activity was measured. Experiments were performed with intact HEK. All data are means (95% confidence limits) of nine doses of analogues assayed in duplicate determinations in two experiments.

Solution, pH 7.4, and various doses of TRH analogues. Apparent inhibitory constants ( $K_i$ ) were derived from curves fitted by nonlinear regression analysis and drawn with the PRISM program 3 (GraphPad Software, Inc.) using the formula  $K_i = (IC_{50})/(1 + ([L]/K_d))$  where  $IC_{50}$  is the concentration of unlabeled analogue that half-competes and  $K_d$  is the equilibrium dissociation constant for [ $^3H$ ]N $^{\epsilon}$ (1)-Me-His-TRH.

**Assay of Luciferase Activity.** On the day prior to transfection, the cells were seeded into 24-well plates ( $1.5 \times 10^5$ /well). After 16 h, the media were aspirated and the cells (approximately 50% confluent) were cotransfected with plasmid DNA encoding CREB and CREB-activated luciferase gene (PathDetect CREB trans-Reporting System, Stratagene) using the calcium phosphate method. On the second day, 6 h before the assay, medium containing 10% FBS was changed to medium containing 1% FBS, various concentrations of TRH and TRH analogues were added to the medium. Luciferase activity was measured 24 h after transfection. Cells were washed with phosphate-buffered saline and lysed with 0.2 mL lysis buffer (25 mM Gly-Gly, pH 7.8, 15 mM  $MgSO_4$ , 4 mM EGTA, 1 mM dithiothreitol, 1% Triton X-100). Cell lysates (0.025 mL) were combined automatically with 0.125 mL reaction buffer (25 mM Gly-Gly, pH 7.8, 15 mM  $MgSO_4$ , 4 mM EGTA, 1 mM dithiothreitol, 15 mM  $KH_2PO_4$ , 2 mM ATP) and 0.025 mL luciferin (0.4 mM) in reaction buffer, and the luminescence was measured for 10 s in a TR717 Microplate Luminometer (Tropix, Bedford, MA). The levels of luciferase activity detected by this assay reflect the activation of signaling by TRH.

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**Supporting Information Available:** Detailed experimental procedures and spectral data for all synthesized compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Guillemin, R. Hormones Secreted by the Brain. Isolation, Molecular Structure and Synthesis of the First Hypophysiotropic Hypothalamic Hormone (to be Discovered) TRF (Thyrotropin-Releasing Factor). *Science* **1970**, *68*, 64–67.
- (2) Schally, A. V.; Redding, T. W.; Bowers, C. Y.; Barret, J. F. Isolation of Porcine Thyrotropin-Releasing Hormone. *J. Biol. Chem.* **1969**, *244*, 4077–4088.
- (3) Boler, J.; Enzmann, F.; Folkers, K.; Bowers, C. Y.; Schally, A. V. The Identity of Chemical and Hormonal Properties of the Thyrotropin-Releasing Hormone and Pyroglutamyl-Histidyl-Prolinamide. *Biochem. Biophys. Res. Commun.* **1969**, *37*, 705–710.
- (4) Burgus, R.; Dunn, J. F.; Desiderio, D.; Guillemin, R. Derives Polypeptidiques de Syntheses Doues d'activite Hypophysiotropic TRF. *C. R. Acad. Sci. (Paris)* **1969**, *269*, 1870–1877.
- (5) Metcalf, G.; Jackson, I. M. D., Eds., Thyrotropin-Releasing Hormone: Biomedical Significance. *Ann. N. Y. Acad. Sci.* **1989**, *553*, 1–631.
- (6) Straub, R. E.; French, G. C.; Joho, R. H.; Gershengorn, M. C. Expression Cloning of a cDNA Encoding the Mouse Pituitary Thyrotropin-Releasing Hormone Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9514–9518.
- (7) de la Pena, P.; Delgado, L. M.; del Camino, D.; Barros, F. Two Isoforms of the Thyrotropin-Releasing Hormone Receptor Gen-

- ated by Alternative Splicing have Indistinguishable Functional Properties. *J. Biol. Chem.* **1992**, *267*, 25703–25708.
- (8) Duthie, S. M.; Taylor, P. L.; Anderson, L.; Cook, J.; Eidne, K. A. Cloning and Functional Characterization of the Human TRH Receptor. *Mol. Cell. Endocrinol.* **1993**, *95*, R11–R15.
  - (9) Sun, Y. M.; Millar, R. P.; Ho, H.; Gershengorn, M. C.; Illing, N. Cloning and Characterization of the Chicken Thyrotropin-Releasing Hormone Receptor. *Endocrinology* **1998**, *139*, 3390–3398.
  - (10) Cao, J.; O'Donnell, D.; Vu, H.; Payza, K.; Pou, C.; Godbout, C.; Jakob, A.; Pelletier, M.; Lembo, P.; Ahmad, S.; Walker, P. Cloning and Characterization of a cDNA Encoding a Novel Subtype of Rat Thyrotropin-Releasing Hormone Receptor. *J. Biol. Chem.* **1998**, *273*, 32281–32287.
  - (11) Harder, S.; Lu, X.; Wang, W.; Buck, F.; Gershengorn, M. C.; Bruhn, T. O. Regulator of G Protein Signaling 4 Suppresses Basal and Thyrotropin-Releasing Hormone (TRH)-Stimulated Signaling by Two Mouse TRH Receptors, TRH-R(1) and TRH-R(2). *Endocrinology* **2001**, *142*, 1188–1194.
  - (12) Itadani, H.; Nakamura, T.; Itoh, J.; Iwaasa, H.; Kanatani, A.; Borkowski, J.; Ihara, M.; Ohta, M. Cloning and Characterization of a New Subtype of Thyrotropin-Releasing Hormone Receptors. *Biochem. Biophys. Res. Commun.* **1998**, *250*, 68–71.
  - (13) O'Dowd, B. F.; Lee, D. K.; Huang, W.; Nguyen, T.; Cheng, R.; Liu, Y.; Wang, B.; Gershengorn, M. C.; George, S. R. TRH-R2 Exhibits Similar Binding and Acute Signaling but Distinct Regulation and Anatomic Distribution Compared with TRH-R1. *Mol. Endocrinol.* **2000**, *14*, 183–193.
  - (14) Sun, Y.; Lu, X.; Gershengorn, M. C. Thyrotropin-Releasing Hormone Receptors-Similarities and Differences. *J. Mol. Endocrinol.* **2003**, *30*, 87–97.
  - (15) Prokai, L. Central Nervous System Effects of Thyrotropin-Releasing Hormone and its Analogues: Opportunities and Perspectives for Drug Discovery and Development. *Prog. Drug Res.* **2002**, *59*, 134–169.
  - (16) Perlman, J. H.; Laakkonen, L. J.; Guarnieri, F.; Osman, R.; Gershengorn, M. C. A Refined Model of the Thyrotropin-Releasing Hormone (TRH) Receptor Binding Pocket. Experimental Analysis and Energy Minimization of the Complex between TRH and TRH Receptor. *Biochemistry* **1996**, *35*, 7643–7650.
  - (17) Griffiths, E. C.; Kelly, J. A.; Ashcroft, A.; Ward, D. J.; Robson, B. Comparative Metabolism and Conformation of TRH and its Analogues. *Ann. N. Y. Acad. Sci.* **1989**, *553*, 217–231.
  - (18) Perlman, J. H.; Colson, A. O.; Jain, R.; Czyzewski, B.; Cohen, L. A.; Osman, R.; Gershengorn, M. C. Role of the Extracellular Loops of the Thyrotropin-Releasing Hormone Receptor: Evidence for an Initial Interaction with Thyrotropin-Releasing Hormone. *Biochemistry* **1997**, *36*, 15670–15676.
  - (19) Jain, R.; Singh, J.; Perlman, J. H.; Gershengorn, M. C. Synthesis and Biology of New Thyrotropin-Releasing Hormone (TRH) Analogues. *Bioorg. Med. Chem.* **2002**, *10*, 189–194.
  - (20) Jain, R.; El-Kadi, N.; King, M. M.; Cohen, L. A. Regiospecific Alkylation of Histidines and Histamines at C-2. *Tetrahedron* **1997**, *53*, 2365–2370.
  - (21) Jain, R.; Cohen, L. A.; King, M. M. Synthesis of Novel Ring-Substituted Histidines and Histamines. *Tetrahedron* **1997**, *53*, 4539–4548.
  - (22) Minisci, F. Novel Applications of Free-Radical Reactions in Preparative Organic Chemistry. *Synthesis* **1973**, 1–24.
  - (23) Minisci, F.; Vismara, E.; Fontana, F. Recent Developments of Free-Radical Substitutions of Heteroaromatic Bases. *Heterocycles* **1989**, *28*, 489–519.
  - (24) van Batenburg, O. D.; Kerling, K. E. T. An Improved Synthesis of *tert*-Butyloxycarbonyl-L-histidine. *Int. J. Pept. Protein Res.* **1976**, *8*, 1–2.
  - (25) Perlman, J. H.; Thaw, C. N.; Laakkonen, L.; Bowers, C. Y.; Osman, R.; Gershengorn, M. C. Hydrogen Bonding Interaction of Thyrotropin-Releasing Hormone (TRH) with Transmembrane Tyrosine 106 of the TRH Receptor. *J. Biol. Chem.* **1994**, *269*, 1610–1613.

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