Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 433–443
Bioorganic & Medicinal Chemistry

Modifications of the pyroglutamic acid and histidine residues in thyrotropin-releasing hormone (TRH) yield analogs with selectivity for TRH receptor type 2 over type 1

Navneet Kaur,[a,†] Vikramdeep Monga,[a] Xinping Lu,[b] Marvin C. Gershengorn[b] and Rahul Jain[a,*]

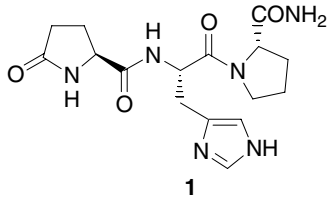
[a]*Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar, Punjab 160062, India*
[b]*Clinical Endocrinology Branch, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA*

Received 28 August 2006; revised 19 September 2006; accepted 21 September 2006
Available online 10 October 2006

Abstract—Thyrotropin-releasing hormone (TRH) analogs in which the N-1(τ) or the C-2 position of the imidazole ring of the histidine residue is substituted with various alkyl groups and the L-pyroglutamic acid (pGlu) is replaced with the L-pyro-2-aminoadipic acid (pAad) or (*R*)- and (*S*)-3-oxocyclopentane-1-carboxylic acid (Ocp) were synthesized and studied as agonists for TRH receptor subtype 1 (TRH-R1) and subtype 2 (TRH-R2). We observed that several analogs were selective agonists of TRH-R2 showing relatively less or no activation of TRH-R1. For example, the most selective agonist of the series **13**, in which pGlu is replaced with the pAad and histidine residue is substituted at the N-1 position with an isopropyl group, was found to activate TRH-R2 with a potency (EC_{50} = 1.9 μM) but did not activate TRH-R1 (potency > 100 μM); that is, exhibited >51-fold greater selectivity for TRH-R2 versus TRH-R1. Analog **8**, in which pGlu is replaced with pAad and histidine is substituted at the N-1(τ) position with a methyl group, exhibited a binding affinity (K_i = 0.0032 μM) to TRH-R1 that is similar to that of [Nτ(1)-Me-His]-TRH and displayed potent activation of TRH-R1 and TRH-R2 (EC_{50} = 0.0049 and 0.0024 μM, respectively). None of the analogs in which pGlu is replaced with the bioisosteric (*R*)- and (*S*)-(Ocp) and the imidazole ring is substituted at the N-1(τ) or C-2 position were found to bind or activate either TRH-R1 or TRH-R2 at the highest test dose of 100 μM.

1. Introduction

Thyrotropin-releasing hormone (**1**, TRH) (Fig. 1) is a unique orally active tripeptide. The discovery and chemical characterization of TRH by Schally and coworkers[1] and Guillemin and coworkers[2] provided confirmation for the founding principles of neuroendocrinology. TRH is synthesized in the hypothalamus, acts in the anterior pituitary and control levels of thyrotropin (thyroid-stimulating hormone, TSH) and prolactin. TRH plays a central role in regulating the pituitary–thyroid axis by stimulating thyrotropin release and its de novo synthesis. In addition to its hormonal effects, TRH is also found in many other tissues and is involved in a wide variety of physiological activities. Most notable is its broad spectrum of stimulatory actions within the CNS.[3] TRH is implicated as a cognition enhancer and could be found useful in the treatment of brain and spinal injury and several CNS disorders, including Alzheimer's disease, motor neuron disease (MND), Parkinson's disease, stroke, and seizure activity.[4–6] The clinical utility of TRH, however, is severely curtailed due to factors such as its short half life, possibly due to rapid enzymatic degradation, its



1

Figure 1. Structure of the thyrotropin-releasing hormone (TRH).

Keywords: TRH; Peptide synthesis; TRH-R1; TRH-R2; CNS activity; Hormonal activity.
* Corresponding author. Tel.: +91 0172 221 4682; fax: +91 0172 221 4692; e-mail: rahuljain@niper.ac.in
† Present address: Jubilant Chemsys, Noida, India.

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.09.045

hydrophilic character and ineffectiveness in penetrating blood–brain barrier (BBB), and manifestations of endocrine side-effects at doses required for CNS activities.

TRH binds within the seven-transmembrane helical region of its receptors, which are members of the rhodopsin/β-adrengeric receptor subfamily of G-protein coupled receptors (GPCRs) and primarily activate the phosphatidylinositol-calcium-protein kinase C transduction pathway.[7] At present, identity of two TRH receptor subtypes is known. The first TRH receptor (TRH-R1) was cloned from a mouse and several other species, including rat and human.[8–10] In 1998, a second subtype of TRH receptor (TRH-R2) was identified in several species;[11–14] however, TRH-R2 has not been identified in humans.[15] The two subtypes of TRH receptor from the same species reveal approximately 51% similarity.[16] A comparison of TRH-R1 and TRH-R2 has been made regarding their expression and tissue distribution in rodents. Both receptor subtypes were found to exhibit a distinct distribution pattern. TRH-R1 is highly expressed in the anterior pituitary and exhibits a limited mRNA expression pattern in other regions of the CNS. This indicates that TRH-R1 selective analogs may produce its specific hormonal activity (TSH-releasing effects). In contrast, TRH-R2 is highly expressed in brain and spinal cord, but is not detectable in the pituitary.[16] The specific expression of TRH-R2 in areas that are important for the transmission of somatosensory signals and CNS functions indicates that this receptor subtype may be of importance in the CNS. These observations indicate that TRH-R2 specific analog may possibly elicit CNS selective biological responses of TRH. Therefore, TRH analogs selective for TRH-R1 or TRH-R2 have a significant role in study of these receptors in mammalian physiology.

2. Results and discussion

All three residues in TRH contribute to its binding to TRH receptors. Key residues, pGlu and His, account for a large part of TRH binding energy and affinity.[17] Thus, any modification of these important residues or their replacement with carefully chosen counterparts may influence TRH receptor binding and activation profiles. The search for synthetic analogs of TRH which exhibit selective affinities and activation for the two known receptor subtypes has generated a large number of analogs. We found that retention of the carbonyl but replacement of the ring NH by a methylene group of pGlu provides an analog whose binding affinity and activation potency were about 100-fold less than those of TRH.[18] In continuation of this study, we have also demonstrated that replacement of the pGlu with other hydrogen-bond donating moieties in TRH does not abolish binding to or activation of TRH-R1.[19] More recently, we observed that replacement of pGlu with (*R*)-3-oxocyclopentane-1-carboxylic acid led to a TRH analog (also called (*R*)-desaza-TRH) with lower binding affinity than TRH but with greater ability to activate TRH-R1 and TRH-R2.[20] Similarly, we have observed that modulation of histidine residue in TRH by placement of a hydrophobic bulky group at the N-1(τ) or the C-2 position of the imidazole ring results in discovery of first selective agonist at TRH receptor subtype.[21,22] These studies indicate that independent change of pGlu and His residues in TRH profoundly alters its receptor binding affinity and signal transducing potency. In continuation of our efforts to synthesize TRH analogs wherein pGlu and histidine residues were replaced or modified, we synthesized analogs by simultaneous modifications at the pGlu and histidine residues to get additional insights into the structure activity relationship of TRH analogs. We replaced pGlu with (*R*/*S*)-3-oxocyclopentane-1-carboxylic acids (Ocp) or L-pyro-2-aminoadipic acid (pAad) and modulation of the histidine residue was achieved by placement of an alkyl group at either the N-1(τ) or the C-2 position of the imidazole ring (Fig. 2). This report describes synthesis, receptor binding, and activation studies of four new series of TRH analogs (Series **1**–**4**, Fig. 3).

2.1. Chemistry

3-Oxocyclopentane-1-carboxylic acid was prepared in four steps from 4-vinylcyclohexene following previously published procedure.[23] Resolution of the racemic carboxylic acid with brucine hydrate readily afforded (1*R*)- and (1*S*)-3-oxocyclopentane-1-carboxylic

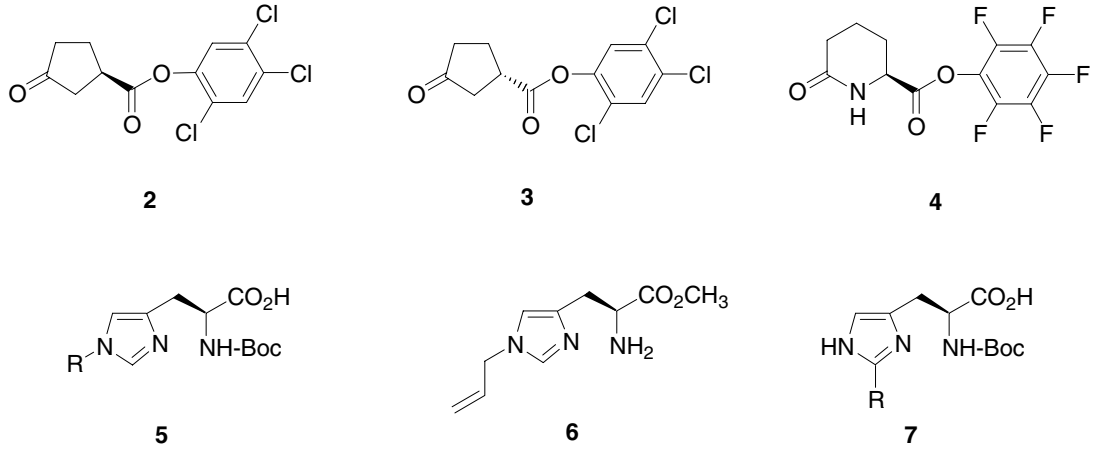


Figure 2. Structures of various building blocks used for the synthesis of TRH analogs.

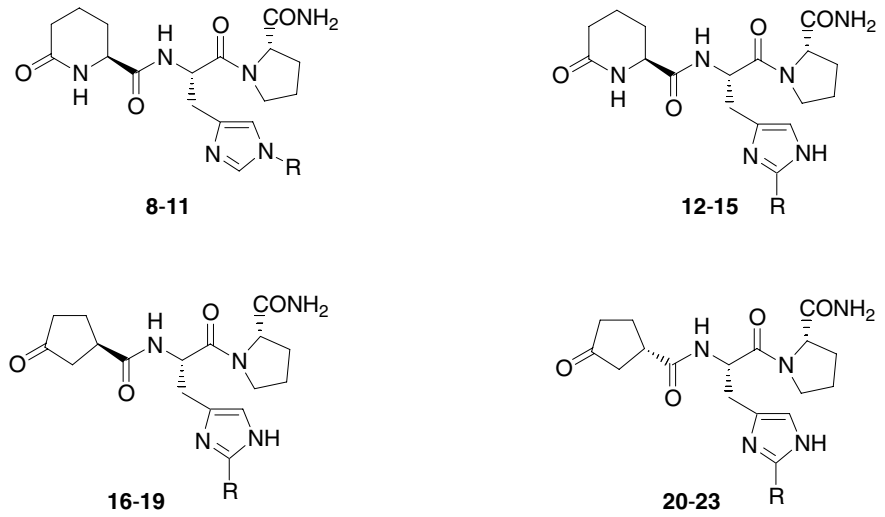
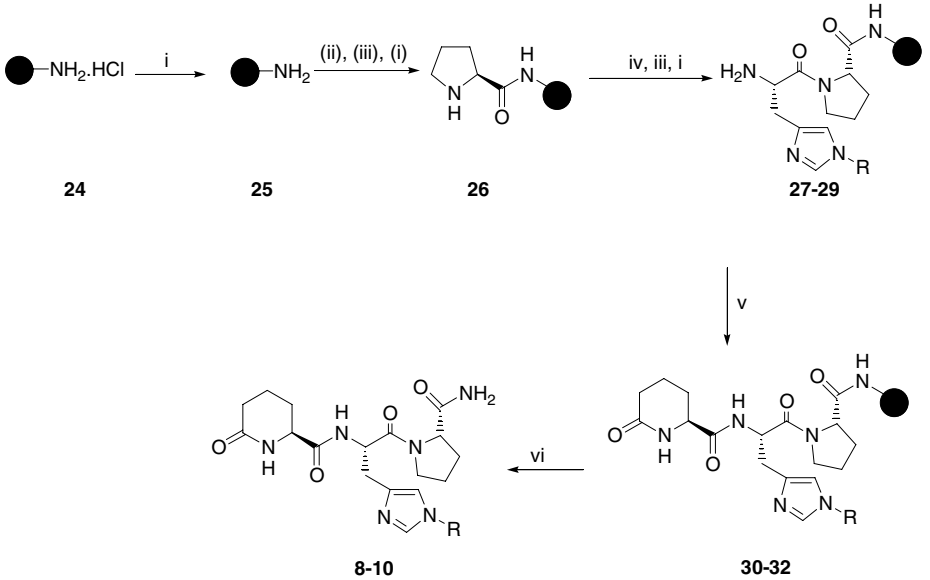


Figure 3. General structures of the synthesized TRH analogs.

acids[24,25] and were converted to their respective 2,4,5-trichlorophenyl active esters **2**–**3** (Fig. 2) upon reaction with 2,4,5-trichlorophenol in the presence of DCC in DCM at −10 °C for 5 h.[19] At the same time, L-pyro-2-aminoadipic acid (L-pAad) was synthesized in three steps from commercially available *N*-ε-benzyloxycarbonyl-L-lysine [H-L-Lys(Z)-OH] and converted to its activated pentafluoroester derivative **4** upon reaction with pentafluorophenol in the presence of DCC in DCM at −10 °C for 4 h as described.[26] On the other hand, *N*-α-Boc-1(τ)-alkyl-L-histidines **5** (Fig. 2) were synthesized in one step from commercially available *N*-α-Boc-L-histidine with NaH at −15 °C for 30 min in anhydrous DMF, followed by addition of the appropriate commercially available alkyl halides at −5 °C for 4 h as described.[27] While, 1-allyl-L-histidine methyl ester **6** was synthesized in four overall steps from L-histidine methyl ester dihydrochloride as described.[22] Finally, 2-alkyl-L-histidines were synthesized from commercial L-histidine methyl ester dihydrochloride in three steps as described[28,29] and converted to *N*-α-Boc-2-alkyl-L-histidines **7** (Fig. 2).[30]

Synthesis of tripeptides, L-pAad-(1-alkyl)-L-His-L-Pro-NH_2 **8**–**10**, was achieved on solid support (Scheme 1). All peptides were assembled on a CS BIO (San Carlos, CA; model no. CS 136) automated peptide synthesizer. Briefly, 4-methylbenzhydrylamine-functionalized, 1% cross-linked polystyrene resin (MBHA·HCl, **24**) was neutralized with DIEA (10% solution in DCM), and subsequently coupled with preformed TBTU activated ester of Boc-L-proline in DMF for 90 min to produce protected amino acid-linked resin. Deprotection of the *tert*-Boc group with TFA (20% solution in DCM) followed by neutralization with DIEA (10% solution in DCM) provided free amino acid-linked resin **26**. This was subsequently submitted to further coupling and deprotection cycles with the *N*-α-Boc-1(τ)-alkyl-L-histi-



Scheme 1. Reagents and conditions: (i) 10% DIEA, DCM, 5 min; (ii) Boc-L-Pro-OH, TBTU, 10% DIEA, 90 min; (iii) 20% TFA, 20 min; (iv) Boc-(1-alkyl)-L-His-OH (**5**), TBTU, 10% DIEA, 90 min; (v) **4**, DMF, 12 h, rt; (vi) TFMSA, EDT, thioanisole, TFA, 2 h, rt.

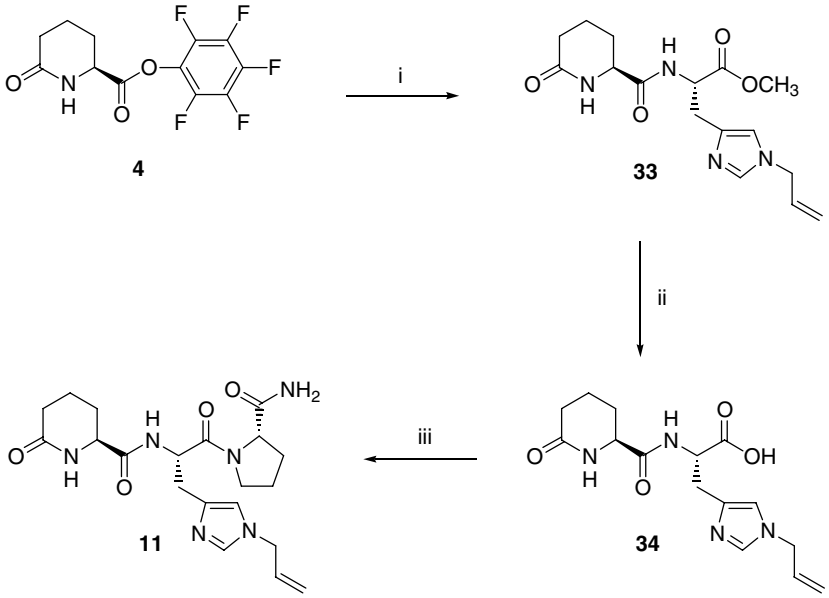
dines **5** and finally with **4** in DMF for 12 h at ambient temperature to afford peptide resins **30**–**32**. The intermediate coupling steps were monitored by Kaiser's test, which showed negative results after each coupling step. Cleavage from the resin support was affected using trifluoromethanesulfonic acid (TFMSA) in TFA in the presence of thioanisole and 1,2-ethanedithiol (EDT) as scavengers for 2 h at ambient temperature to afford peptide amides **8**–**10** (Scheme 1).

Reaction of **4** with 1-allyl-L-histidine methyl ester (**6**) at 4 °C for 36 h in anhydrous EtOAc gave dipeptide methyl ester **33** in good yield (Scheme 2). Basic hydrolysis of the latter dipeptide **33** with methanolic 0.5 N NaOH solution at 0 °C for 30 min followed by neutralization of the resultant peptide salt with Dowex ion-exchange resin (50× 2-200, H^+ form) produced dipeptide carboxylic acid **34**. Coupling of the latter dipeptide **34** with commercially available L-prolinamide in the presence of 1,3-diisopropylcarbodiimide (DIC) and *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) in anhydrous DMF at 4 °C for 36 h produced tripeptide **11** (Scheme 2).

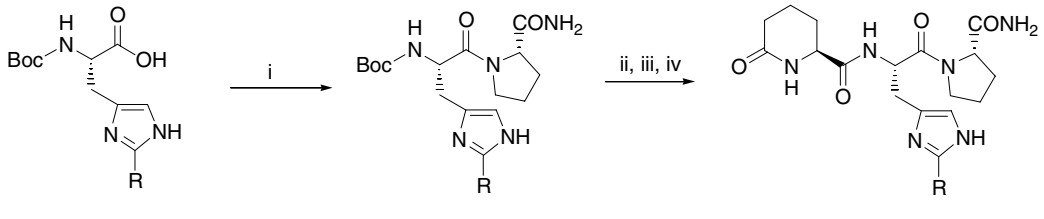
The solution phase peptide synthesis of the intermediate dipeptides **35**–**38** was accomplished by the reaction of the commercial L-prolinamide with *N*-α-Boc-2-alkyl-L-histidines **7** in the presence of HONB and DIC at 4 °C for 36 h. Subsequently, cleavage of the *N*-α-Boc group with TFA (10% solution in DCM) at ambient temperature for 30 min 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_3 in MeOH for 10 min at ambient temperature. Complete removal of the solvent followed by coupling of the free dipeptides with **4** in DMF at 4 °C for 36 h produced TRH analogs **12**–**15** (Scheme 3). Finally, coupling reaction of the aforementioned free dipeptides obtained from **35**–**38** with **2** or **3** in DMF at 4 °C for 36 h produced TRH analogs **16**–**23** (Scheme 4).

2.2. Receptor binding and activation studies at TRH-R1 and TRH-R2

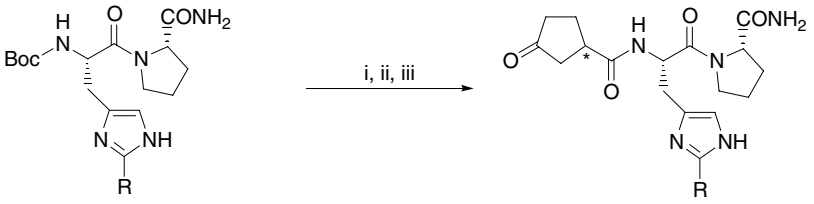
TRH analogs **8**–**23** were examined for their affinity for TRH-R1 and TRH-R2 and their ability to serve as agonists for the receptors.[31] Affinities, reported as K_i (μM) values, were determined by measuring the concentration of the analog required to compete with 2 nM [^{3}H][Nτ(1)-Me-His]-TRH for binding to the receptor. [Nτ(1)-Me-His]-TRH binds to TRH-R1 and TRH-R2 with affinities higher than TRH. The agonist behavior of the analogs was tested in HEK 293EM cells stably expressing TRH-R1 or TRH-R2 by incubating the cells with various doses of the analogs as described.[20,32] The extent



Scheme 2. Reagents and conditions: (i) 1-allyl-L-His-OMe (**6**), EtOAc, 4 °C, 36 h, (ii) 0.5 N NaOH, MeOH, 0 °C, 30 min, Dowex ion-exchange (50× 2-200, H+ form); (iii) L-ProNH_2, DIC, HONB, DMF, 4 °C, 36 h.



Scheme 3. Reagents and conditions: (i) L-ProNH_2, HONB, DIC, DMF, 4 °C, 36 h; (ii) 10% TFA, 30 min, rt; (iii) 7 N NH_3/MeOH, 10 min, rt; (iv) **4**, DMF, 4 °C, 36 h.



Scheme 4. Reagents and conditions: (i) 10% TFA, 30 min, rt; (ii) 7 N NH_3/MeOH, 10 min, rt; (iii) **2** or **3**, DMF, 4 °C, 36 h.

of agonist behavior was then determined by measuring signaling through a reporter gene and the data are reported as EC_{50} (μM) values (Table 1).

None of the analogs **16**–**23**, where pGlu was replaced with either (*R*)- or (*S*)-(Ocp) and histidine was substituted at the N-1(τ) or the C-2 positions, bound with high affinity (K_i) to both TRH-R1 and TRH-R2, and were inactive at both TRH receptors (Table 1).

TRH analogs **8**–**11**, in which pGlu was replaced with pAad and His was substituted at N-1(τ) position, produced interesting results (Table 1). Analogs **10** and **11** of the series exhibited binding affinities (K_i) to TRH-R1 and TRH-R2 that were lower than that of TRH. Analog **8** (R = L-pAad, $R_1 = CH_3$, $R_2 = H$) exhibited binding affinities ($K_i = 0.0032$ μM and $K_i = 0.0049$ μM at TRH-R1 and TRH-R2, respectively) that were modestly higher than that of TRH and comparable to that of [Nτ(1)-Me-His]-TRH, which is the most potent TRH analog found previously. Similarly, analog **9** (R = L-pAad, $R_1 = C_2H_5$, $R_2 = H$) displayed high binding affinities ($K_i = 0.011$ μM and $K_i = 0.014$ μM at TRH-R1 and TRH-R2, respectively). Analogs **8** and **9** also exhibited high potencies at both receptors while showing modest 2- to 5-fold selectivity for TRH-R2 versus TRH-R1.

Finally, analogs **12**–**15**, in which pGlu was replaced with pAad and histidine was substituted with a bulky hydrophobic alkyl group at the C-2 position, exhibited a definite preference as an agonist for TRH-R2 over TRH-R1. The placement of an isopropyl group at the C-2 position led to the most selective analog **13** [$R = CH(CH_3)_2$] that exhibited >51-fold selectivity for TRH-R2 ($EC_{50} = 1.9$ μM) versus TRH-R1 while displaying full agonist activity at TRH-R2. Placement of an *n*-propyl group led to analog **12** ($R = CH_2CH_2CH_3$) that exhibited agonist potency [$EC_{50} = 0.029$ μM] that is about 10-fold lower than that of TRH ($EC_{50} = 0.003$ μM), but 13-fold selectivity for TRH-R2 versus TRH-R1. Placement of a *tert*-butyl group at the C-2 position of imidazole ring (analog **14**) resulted in >30-fold selectivity to TRH-R2 ($EC_{50} = 3.3$ μM) versus TRH-R1 (Table 1). These results indicate a possible link between modification at the pGlu and substitution at the N-1 position of His to a modification at the C-2 position of the central His as important factors in the discovery of peptides that exhibit high specificity as agonist at TRH receptors.

3. Conclusions

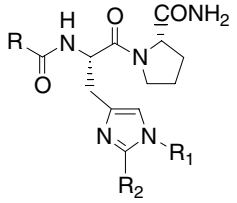
In the present study, we synthesized TRH analogs in which the pGlu residue of the native peptide was replaced with non-proteinogenic residues such as (*R*)- and (*S*)-3-oxocyclopentane-1-carboxylic acids and L-pyro-2-aminoadipic acid, and modified the central histidine by placement of alkyl groups of various sizes at the N-1(τ) or C-2 position of the imidazole ring. The resulting peptides exhibited interesting properties especially in regard to selectivity for TRH-R2 over TRH-R1. For example, analogs **12**–**14** were found to be full agonist at TRH-R2 without activation of TRH-R1; these compounds, however, bind with very low affinity to both TRH-R2 and TRH-R1. These findings are likely related to our observations that TRH-R2 exhibits greater potencies versus binding affinities for some of the other analogs than TRH-R1. Although we do not understand the mechanism(s) that underlie these observations, we think they may be related to the particular conformation of unliganded TRH-R2, which exhibits a very high constitutive (basal) signaling activity, versus that of TRH-R1, which exhibits low constitutive signaling. While, analog **8** displayed binding affinity that is similar to that of [Nτ(1)-Me-His]-TRH at TRH-R1 and TRH-R2. These results support our earlier observation that substitution of the pGlu with selected hydrogen-bond donating moieties in the TRH allows the peptides to efficiently bind and activate both TRH receptors and modulation of His is important for high selectivity at TRH receptors. In conclusion, the TRH peptide can be modified at both pGlu and histidine with non-proteinogenic counterparts to obtain analogs which display high binding affinity and specificity. These newly synthesized analogs containing modified residues could be considered as important research tools possibly due to their increased stability and hydrophobicity to discover additional TRH peptides that selectively and potently bind and activate TRH receptor subtypes and help in understanding the hormonal and CNS mechanisms of action of TRH.

4. Experimental

4.1. Synthesis

Amino acids and resin used during this study were purchased from Novabiochem (Germany) or ChemImpex International (Wood Dale, IL, USA). All other chemicals were purchased from Aldrich Chemical Ltd (Milwaukee, WI, USA). Solvents used for the peptide synthesis and

Table 1. Binding affinities K_i (μM) and signaling (activation) potencies EC_{50} (μM) produced by TRH analogs **8**–**23** for TRH-R1 and TRH-R2



Compound	R	R_1	R_2	K_i[a] (μM)		EC_{50}[b] (μM)		
				TRH-R1	TRH-R2	TRH-R1	TRH-R2	Fold selectivity (TRH-R2)
8	L-pAad	CH_3	H	0.0032 (0.0025–0.0043)	0.013 (0.0090–0.018)	0.0049 (0.0023–0.011)	0.0024 (0.0012–0.0047)	2
9	L-pAad	C_2H_5	H	0.011 (0.009–0.013)	0.034 (0.030–0.038)	0.014 (0.010–0.017)	0.0028 (0.0019–0.0038)	5
10	L-pAad	$CH_2C_6H_5$	H	>100	>100	17 (8.1–38)	2.0 (1.5–2.8)	8.5
11	L-pAad	$CH_2CH{=}CH_2$	H	7.5 (5.1–11.3)	21 (10.3–38.2)	5.1 (3.2–10.1)	0.34 (0.18–0.60)	15
12	L-pAad	H	C_3H_7	0.40 (0.31–0.63)	0.29 (0.22–0.39)	0.38 (0.28–0.51)	0.029 (0.018–0.040)	13
13	L-pAad	H	$CH(CH_3)_2$	>100	>100	>100	1.9 (0.87–2.56)	>51
14	L-pAad	H	$C(CH_3)_3$	>100	>100	>100	3.3 (1.7–6.3)	>30
15	L-pAad	H	*c*-C_6H_{11}	>100	>100	>100	>100	—
16	(*R*)-Ocp	H	C_3H_7	>100	>100	>100	>100	—
17	(*R*)-Ocp	H	$CH(CH_3)_2$	>100	>100	>100	>100	—
18	(*R*)-Ocp	H	$C(CH_3)_3$	>100	>100	>100	>100	—
19	(*R*)-Ocp	H	*c*-C_6H_{11}	>100	>100	>100	>100	—
20	(*S*)-Ocp	H	C_3H_7	>100	>100	>100	>100	—
21	(*S*)-Ocp	H	$CH(CH_3)_2$	>100	>100	>100	>100	—
22	(*S*)-Ocp	H	$C(CH_3)_3$	>100	>100	>100	>100	—
23	(*S*)-Ocp	H	*c*-C_6H_{11}	>100	>100	>100	>100	—
TRH				0.02	0.01	0.003	0.003	—
[Nτ(1)-Me-His]-TRH				0.003	—	0.0005	—	—

All data are means (95% confidence limits) of 9 doses of analogs assayed in duplicate determinations in three experiments.

[a] For binding, cells expressing TRH-R1 or TRH-R2 were incubated with 1 nM [^{3}H]N(1)-Me-His-TRH in the absence or presence of various doses of unlabeled TRH analogs for 1 h at 37 °C.

[b] For signaling, cells expressing TRH-R1 or TRH-R2 and a CREB-luciferase reporter were incubated with various doses of TRH analogs for 6 h at 37 °C, and luciferase activity was measured. Experiments were performed with intact HEK293EM cells.

purification were acquired from commercial sources, were of analytical or HPLC grade and used without further purification unless otherwise stated. Peptides were routinely checked for their purity on pre-coated silica gel G_{254} TLC plates (Merck) and the spots were visualized under UV spectrophotometer and then by exposure to iodine vapors. Column chromatographic purification was carried out on Merck silica gel (230–400 mesh) or neutral alumina. ^{1}H NMR spectra were recorded on a 300 MHz Bruker FT–NMR (Avance DPX 300) spectrometer using tetramethylsilane (TMS) as internal standard and the chemical shifts are reported in δ units. Mass spectra were recorded on a HRMS (Finnigan Mat LCQ spectrometer) (APCI/ESI). Elemental analyses were recorded on an Elementar Vario EL spectrometer. All final peptides were checked for their homogeneity on a Shimadzu LS10AT HPLC system using a Merck Lichrospher® 100 RP-18 (10 μm) column. The samples were analyzed using a solvent system of CH_3CN/H_2O/TFA (95:5:0.05%) at a flow rate of 1 mL/min. Following abbreviations were used: pAad, L-pyro-2-aminoadipic acid; His, histidine; Im, imidazole; $ProNH_2$; prolinamide; DCC, 1,3-dicyclohexylcarbodiimide; DCM, dichloromethane; DIC, 1,3-diisopropylcarbodiimide; DIU, 1,3-diisopropylurea; DMF, *N*,*N*′-dimethylformamide; EDT, 1,2-ethanedithiol; EtOAc, ethyl acetate; pGlu, L-pyroglutamic acid; HONB, *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide; DIEA, *N*,*N*′-diisopropylethylamine; MBHA, 4-methylbenzhydrylamine resin; MeOH, methanol; Ocp, 3-oxo-1-cyclopentanecarboxylic acid; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid.

4.2. Synthesis of 2,4,5-trichloro-1-[(1*R*)- and (1*S*)-3-oxo-1-cyclopentylcarbonyloxy]benzenes (2–3)

The racemic 3-oxo-1-cyclopentanecarboxylic acid was prepared from 1-vinylcyclohexene in four steps following reported procedure.[23] Resolution of racemic carboxylic acid with brucine hydrate readily afforded (1*R*)- and (1*S*)-3-oxo-1-cyclopentanecarboxylic acids as described (Fig. 2).[24,25] The latter compounds were converted to their 2,4,5-trichlorophenyl esters **2**–**3** by reaction with 2,4,5-trichlorophenol in the presence of DCC in DCM at ambient temperature as discussed.[19]

4.3. Synthesis of 2,3,4,5,6-pentafluoro-(2*S*)-6-oxohexahydro-2-pyridine carboxylate (4)

The title amino acid was prepared in three steps from *N*-ε-Cbz-L-lysine and subsequently converted to its pentafluorophenyl ester **4** by reaction with pentafluorophenol in the presence of DCC in EtOAc at ambient temperature as described.[26]

4.4. Synthesis of *N*-α-(*tert*-butoxycarbonyl)-1-alkyl-L-histidines (5) and 1-allyl-L-histidine methyl ester (6)

N-α-Boc-1-alkyl-L-histidine derivatives **5** in which alkyl group is represented by a methyl or an ethyl or a benzyl were synthesized using the one-pot reaction of commercially available *N*-α-Boc-L-histidine with alkyl halides in NaH at −15 °C in the presence of CH_3CN as described.[27] On the other hand, 1-allyl-L-histidine methyl ester **6** was synthesized in five-steps from L-histidine methyl ester as described.[22]

4.5. Synthesis of *N*-α-(*tert*-butoxycarbonyl)-2-alkyl-L-histidines (7)

2-Alkyl-L-histidines required for the synthesis of tripeptides were synthesized in three convenient steps from L-histidine methyl ester and converted to *N*-α-Boc-2-alkyl-L-histidines **7** as described.[28–30] The key step of the synthesis proceeds through a homolytic free radical reaction which offers a unique procedure for C–C bond formation at the most electron-deficient C-2 position of the imidazole ring in the acidic reaction medium.[33,34]

4.6. General method for the synthesis of (2*S*)-1-{(2*S*)-3-(1-alkyl-1*H*-4-imidazolyl)-2-[(2*S*)-6-oxohexahydro-2-pyridinylcarboxamido]propanoyl}azolane-2-carboxamides [L-pAad-(1-alkyl)-L-His-L-Pro-NH_2] (8–10)

4-Methylbenzhydrylamine (MBHA·HCl) resin (**24**, 500 mg, 0.31 mmol) was charged into the reaction vessel and the requisite amino acids (0.93 mmol) were loaded sequentially into the amino acid vessels of the peptide synthesizer. The MBHA·HCl resin **24** was neutralized with DIEA (10% solution in DCM) for 5 min and then washed with DMF (2× 10 mL) and once with DCM (10 mL). The reaction of the first amino acid Boc-L-Pro-OH was done in the presence of coupling reagent TBTU and DIEA (10% solution in DCM) for 90 min. After the completion of coupling step, the *tert*-Boc group was cleaved with TFA (20% solution in DCM) for 20 min. The amino acid linked resin was again neutralized with DIEA (10% solution in DCM) for 5 min and washed with DMF (2× 10 mL) and once with DCM (10 mL) to afford **26**. Reaction of **26** with preformed TBTU esters of Boc-1-alkyl-L-histidines **5** for 90 min in DMF followed by removal of *tert*-Boc group with TFA (20% solution in DCM) and finally neutralization with DIEA (10% in DCM) afforded dipeptides **27**–**29**. The latter dipeptides **27**–**29** upon coupling with **4** in DMF for 12 h at ambient temperature produced desired peptide resin **30**–**32**. All coupling reactions were monitored quantitatively by Kaiser's test for completion.

After the successful completion of synthesis, the peptides were cleaved from the solid support using TFMSA. Accordingly, dry peptide-linked resin **30**–**32** was taken in two-necked round-bottomed flask equipped with a drying tube and a rubber septum. EDT (0.5 mL), thioanisole (1.0 mL), and TFA (10 mL) were added to the reaction vessel through a septum and reaction mixture was stirred for 10 min at 4 °C. TFMSA (1.0 mL) was then added and reaction mixture was stirred at ambient temperature for 2 h. The crude peptide was separated from solid support by filtration and resin was washed with TFA (3× 4 mL). Solvent was removed under reduced pressure and residue was neutralized with saturated ammonium bicarbonate solution. The non-polar impurities were removed by extracting the aqueous layer with diethyl ether (3× 10 mL). The aqueous layer was evapo-

rated under reduced pressure to afford crude peptide, which upon purification using column chromatography over neutral alumina using $CHCl_3/CH_3OH$ (4:1) as eluant produced tripeptides **8**–**10**.

4.6.1. (2*S*)-1-{(2*S*)-3-(1-Methyl-1*H*-4-imidazolyl)-2-[(2*S*)-6-oxohexahydro-2-pyridinylcarboxamido]propanoyl}azolane-2-carboxamide [L-pAad-(1-methyl)-L-His-L-Pro-NH_2] (8). Mp 117–119 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.71 (s, 1H, 2-Ar–H), 7.11 (s, 1H, 5-Ar–H), 4.51 (m, 1H, α-CH), 4.40 (m, 1H, α-CH), 4.36 (m, 1H, α-CH), 3.29 (s, 3H, N–CH_3), 3.06 (m, 2H, Im-CH_2), 2.30 (m, 2H, CH_2), 2.07 (m, 4H, 2× CH_2),1.98–0.87 (m, 6H, 3× CH_2); MS (ESI): *m*/*z* 391 (M+1); Anal. Calcd for $C_{18}H_{26}N_6O_4$ (390.4): C, 55.37; H, 5.5; N, 21.52. Found: C, 55.62; H, 6.94; N, 21.85; R_f = 0.62 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 4.54 min, purity: 97.5%.

4.6.2. (2*S*)-1-{(2*S*)-3-(1-Ethyl-1*H*-4-imidazolyl)-2-[(2*S*)-6-oxohexahydro-2-pyridinylcarboxamido]propanoyl}azolane-2-carboxamide [L-pAad-(1-ethyl)-L-His-L-Pro-NH_2] (9). Mp 107–109 °C (dec.);^{1}H NMR (CD_3OD): δ 7.87 (s, 1H, 2-Ar–H), 7.36 (s, 1H, 5-Ar–H), 4.95 (m, 1H, α-CH), 4.41 (m, 1H, α-CH), 4.18 (q, 2H, N–CH_2, J = 7.3 Hz), 4.05 (m, 1H, α-CH), 3.81 (m, 2H, CH_2), 3.03 (m, 2H, Im-CH_2), 2.32-1.89 (m, 6H, 3× CH_2), 1.47 (t, 3H, CH_3, J = 7.2 Hz), 1.41-0.90 (m, 4H, 2× CH_2); MS (ESI): *m*/*z* 405 (M+1); Anal. Calcd for $C_{19}H_{28}N_6O_4$ (404.2): C, 56.42; H, 6.98; N, 20.78. Found: C, 56.49; H, 7.23; N, 20.59; R_f = 0.57 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 6.24 min, purity: 96.5%.

4.6.3. (2*S*)-1-{(2*S*)-3-(1-Benzyl-1*H*-4-imidazolyl)-2-[(2*S*)-6-oxohexahydro-2-pyridinylcarboxamido]propanoyl}azolane-2-carboxamide [L-pAad-(1-benzyl)-L-His-L-Pro-NH_2] (10). Mp 120–122 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.65 (s, 1H, 2-Ar–H), 7.30 (m, 5H, Ar–H), 7.02 (s, 1H, 5-Ar–H), 5.18 (s, 2H, CH_2), 4.39 (m, 1H, α-CH), 4.07 (m, 1H, α-CH), 3.75 (m, 1H, α-CH), 2.94 (m, 2H, Im-CH_2), 2.37 (m, 2H, CH_2), 2.25 (m, 4H, 2× CH_2), 1.88–0.87 (m, 6H, 3× CH_2); MS (ESI): *m*/*z* 467 (M+1); Anal. Calcd for $C_{24}H_{30}N_6O_4$ (466.2): C, 61.79; H, 6.48; N, 18.01. Found: C, 61.95; H, 6.17; N, 18.37; R_f = 0.62 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 6.77 min, purity: 98.0%.

4.7. Synthesis of methyl (2*S*)-3-(1-allyl-1*H*-4-imidazolyl)-2-[(2*S*)-6-oxohexahydro-2-pyridinylcarboxamido]propanoate [L-pAad-(1-allyl)-L-His-OMe] (33)

2,3,4,5,6-Pentafluoro-(2*S*)-6-oxohexahydro-2-pyridine carboxylate (**4**, 1 mmol) was added dropwise to 1-allyl-L-histidine methyl ester (1 mmol) in EtOAc (50 mL) and reaction mixture was stirred at 4 °C for 36 h. Complete removal of the solvent under reduced pressure gave crude product. Column chromatographic purification on silica gel (230–400 mesh) using EtOAc/CH_3OH (94:6) as eluant provided dipeptide methyl ester **33**.

Yield: 75%; mp. 73–74 °C (dec.); ^{1}H NMR (CD_3OD) δ 7.94 (s, 1H, 2-Ar–H), 6.89 (s, 1H, 5-Ar–H), 5.98 (m, 1H, CH), 5.18 (m, 2H, CH_2), 4.68 (m, 2H, N–CH_2), 4.58 (m, 1H, α-CH), 4.02 (m, 1H, α-CH), 3.70 (s, 3H, OCH_3), 3.06 (m, 2H, Im-CH_2), 2.01–1.704 (m, 6H, 3× CH_2); MS (ESI): *m*/*z* 335 (M+1); Anal. Calcd for $C_{15}H_{20}N_4O_4$ (334.2): C, 56.24; H, 6.29; N, 17.49. Found: C, 56.39; H, 6.54; N, 17.88.

4.8. Synthesis of (2*S*)-3-(1-allyl-1*H*-4-imidazolyl)-2-[(2*S*)-6-oxohexahydro-2-pyridinylcarboxamido]propanoic acid [L-pAad-(1-allyl)-L-His-OH] (34)

L-pAad-(1-allyl)-L-His-OMe (**33**, 1 mmol) was dissolved in a mixture of CH_3OH (50 mL) and 0.5 N NaOH (40 mL). The solution was stirred at 0 °C for 30 min, and water (35 mL) was added to the solution. The pH of the solution was adjusted to 6.0 with Dowex (50× 2-200, H^+ form) ion-exchange resin. The resin was removed by filtration, and the filtrate was evaporated under reduced pressure to afford L-pAad-(1-allyl)-L-His-OH **34**.

Yield: 76%; mp. 100–102 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.30 (s, 1H, 2-Ar–H), 6.82 (s, 1H, 5-Ar–H), 5.95 (m, 1H, CH_2), 5.30 (m, 2H, CH_2), 4.68 (m, 2H, N–CH_2), 4.05 (m, 1H, α-CH), 3.30 (m, 1H, α-CH), 3.20 (m, 2H, Im-CH_2), 2.3–1.8 (m, 6H, 3× CH_2); MS (ESI): *m*/*z* 321 (M+1); Anal. Calcd for $C_{15}H_{20}N_4O_4$ (320.3): C, 56.24; H, 6.29; N, 17.49. Found: C, 56.39; H, 6.54; N, 17.88.

4.9. Synthesis of (2*S*)-1-{(2*S*)-3-(1-allyl-1*H*-4-imidazolyl)-2-[(2*S*)-6-oxohexahydro-2-pyridinylcarboxamido]propanoyl}azolane-2-carboxamide [L-pAad-(1-allyl)-L-His-L-Pro-NH_2] (11)

Dipeptide acid (**34**, 1 mmol) was dissolved in DMF (25 mL), and HONB (1 mmol) was added to the solution followed by addition of DIC (1 mmol) under cooling at 4 °C. Stirring of the reaction mixture continued for another 5 min at 4 °C. L-ProNH_2 (1 mmol) was then added in one portion. The reaction mixture was stirred for additional 36 h at 4 °C. Solvent was evaporated under reduced pressure to afford crude product. Flash column chromatography using $CHCl_3/CH_3OH$ (4:1) as the solvent system produced **11**.

Yield: 60%; mp 109–110 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.54 (s, 1H, 2-Ar–H), 6.97 (s, 1H, 5-Ar–H), 6.01 (m, 1H, CH), 5.24 (m, 2H, CH_2), 4.89 (m, 2H, N–CH_2), 4.39 (m, 1H, α-CH), 4.01 (m, 1H, α-CH), 3.79 (m, 1H, α-CH), 2.94 (m, 2H, Im-CH_2), 2.30–1.27 (m, 12H, 6× CH_2); MS (APCI): *m*/*z* 417 (M+1); Anal. Calcd for $C_{20}H_{28}N_6O_4$ (416.2): C, 56.70; H, 6.51; N, 20.88. Found: C, 57.05; H, 6.22; N, 21.07; R_f = 0.58 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 4.35 min, purity: 97.0%.

4.10. General method for the synthesis of (2*S*)-1-[(2*S*)-2-(2*S*)-6-oxohexahydro-2-pyridinylcarboxamido]-3-(2-alkyl-1 *H*-imidazolyl)propanoyl]azalone-2-carboxamides [L-pAad- (2-alkyl)-L-His-L-Pro-NH_2] (12–15)

Protected dipeptide[21] (**35**–**38**, 0.73 mmol) was treated with a 10% solution of TFA in DCM (15 mL) at ambient temperature for 30 min to cleave the *tert*-Boc group. Solvent was evaporated and the dipeptide salt was neu-

tralized by stirring with a solution of 7 N NH_3 in CH_3OH (10 mL) for 10 min at ambient temperature. Solvent was removed to generate free base. A solution of free peptide in DMF (4 mL) was cooled to 4 °C, and **4** (0.81 mmol) was added and the reaction mixture stirred at 4 °C for 36 h. The solvent was removed under reduced pressure and the resulting residue purified by flash column chromatography using $CH_3OH/CHCl_3$ (4:1) as eluant.

4.10.1. (2*S*)-1-[(2*S*)-2-(2*S*)-6-Oxohexahydro-2-pyridinylcarboxamido]-3-(2-propyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamide [L-pAad-(2-propyl)-L-His-L-Pro-NH_2] (12). Yield: 64%; mp. 87–89 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.34 (br s, 1H, NH), 7.32 (s, 1H, 5-Ar–H), 7.17 (br s, 1H, NH), 4.94 (m, 1H, α-CH), 4.48 (m, 1H, α-CH), 3.81 (m, 1H, α-CH), 3.49 (m, 2H, CH_2), 3.20 (m, 2H, Im-CH_2), 2.90 (m, 2H, CH_2), 2.33 (t, 2H, CH_2, J = 7.1 Hz), 2.03 (m, 10H, 5× CH_2), 0.98 (t, 3H, CH_3, J = 7.4 Hz); MS (APCI): *m*/*z* 419 (M+1); Anal. Calcd for $C_{20}H_{30}N_6O_4$ (418.2): C, 57.40; H, 7.23; N, 20.08. Found: C, 57.68; H, 7.55; N, 20.37; R_f = 0.63 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 4.20 min, purity: 98.0%.

4.10.2. (2*S*)-1-[(2*S*)-2-(2*S*)-6-Oxohexahydro-2-pyridinylcarboxamido]-3-(2-isopropyl-1*H*-imidazolyl)propanoyl]-azalone-2-carboxamide [L-pAad-(2-isopropyl)-L-His-L-Pro-NH_2] (13). Yield: 71%; mp. 95–97 °C (dec.);^{1}H NMR (CD_3OD): δ 7.22 (s, 1H, 5-Ar–H), 4.99 (m, 1H, α-CH), 4.47 (m, 1H, α-CH), 4.04 (m, 1H, α-CH), 3.45 (m, 2H, Im-CH_2), 3.18 (m, 4H, 2× CH_2), 2.01 (m, 1H, CH), 2.33–1.73 (m, 8H, 4× CH_2), 1.39 (d, 6H, 2× CH_3, J = 6.5 Hz); MS (APCI): *m*/*z* 419 (M+1); Anal. Calcd for $C_{20}H_{30}N_6O_4$ (418.2): C, 57.40; H, 7.23; N, 20.08. Found: C, 57.49; H, 7.46; N, 19.89; R_f = 0.63 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 5.40 min, purity: 96.0%.

4.10.3. (2*S*)-1-[(2*S*)-2-(2*S*)-6-Oxohexahydro-2-pyridinylcarboxamido]-3-(2-*tert*-butyl-1*H*-imidazolyl)propanoyl]-azalone-2-carboxamide [L-pAad-(2-*tert*-butyl)-L-His-L-Pro-NH_2] (14). Yield: 72%; mp. 101–103 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.14 (s, 1H, 5-Ar–H), 4.86 (m, 1H, α-CH), 4.42 (m, 1H, α-CH), 4.21 (m, 1H, α-CH), 3.35 (m, 2H, CH_2), 3.22 (m, 2H, Im-CH_2), 2.27–1.94 (m, 10H, 5× CH_2), 1.45 (s, 9H, 3× CH_3); MS (APCI): *m*/*z* 433 (M+1); Anal. Calcd for $C_{21}H_{32}N_6O_4$ (432.3): C, 58.32; H, 7.46; N, 19.43. Found: C, 58.65; H, 7.99; N, 19.16; R_f = 0.71 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 5.90 min, purity: 96.0%.

4.10.4. (2*S*)-1-[(2*S*)-2-(2*S*)-6-Oxohexahydro-2-pyridinylcarboxamido]-3-(2-cyclohexyl-1*H*-imidazolyl)propanoyl]-azalone-2-carboxamide [L-pAad-(2-cyclohexyl)-L-His-L-Pro-NH_2] (15). Yield: 75%; mp. 109–111 °C (dec.); ^{1}H NMR (CD_3OD): δ 6.91 (s, 1H, 5-Ar–H), 4.34 (m, 1H, α-CH), 4.11 (m, 1H, α-CH), 3.97 (m, 1H, α-CH), 3.52 (m, 2H, CH_2), 3.41 (m, 2H, Im-CH_2), 2.80 (m, 1H, CH), 2.44 (m, 4H, 2× CH_2), 2.23–1.95 (m, 4H, 2× CH_2), 1.82–0.81 (m, 10H, 5× CH_2); MS (APCI): *m*/*z* 459 (M+1); Anal. Calcd for $C_{23}H_{34}N_6O_4$ (458.6): C, 60.24; H, 7.47; N, 18.33. Found: C, 60.44; H, 7.20; N, 18.53; R_f = 0.70 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 6.10 min, purity: 97.0%.

4.11. Synthesis of (2*S*)-1-[(2*S*)-2-(1*R*)-3-oxocyclopentylcarboxamido]-3-(2-alkyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamides [(1*R*)-Ocp-(2-alkyl)-L-His-L-Pro-NH_2] (16–19) and (2*S*)-1-[(2*S*)-2-(1*S*)-3-oxocyclopentylcarboxamido]-3-(2-alkyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamides [(1*S*)-3-Ocp-(2-alkyl)-L-His-L-Pro-NH_2] (20–23)

Protected dipeptide[21] (**35**–**38**, 0.73 mmol) was treated with TFA (10% solution) in DCM (15 mL) at ambient temperature for 30 min to cleave the *tert*-Boc group. Solvent was evaporated and the dipeptide salt was neutralized with a solution of 7N NH_3 in CH_3OH (10 mL) for 10 min at ambient temperature, and solvent was removed under reduced pressure to generate free base. A solution of free peptide in DMF (4 mL) was cooled to 4 °C, and 2,4,5-trichloro-1-[(1*S*)-3-oxo-1-cyclopentylcarbonyloxy]benzene (**2**) or 2,4,5-trichloro-1-[(1*R*)-3-oxo-1-cyclopentylcarbonyloxy]benzene (**3**) (0.81 mmol) was added. The reaction mixture was stirred at 4 °C for 36 h. The solvent was removed under reduced pressure and the resulting residue purified by column chromatography using $CH_3OH/CHCl_3$ (4:1) as eluant to produce **16**–**23**.

4.11.1. (2*S*)-1-[(2*S*)-2-(1*R*)-3-Oxocyclopentylcarboxamido]-3-(2-propyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamide [(1*R*)-Ocp-(2-propyl)-L-His-L-Pro-NH_2] (16). Yield: 64%; mp 110–112 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.31 (s, 1H, 5-Ar–H), 4.96 (m, 1H, α-CH), 4.2 (m, 1H, α-CH), 4.24 (m, 1H, α-CH), 3.50 (m, 2H, Im-CH_2), 2.90 (t, 2H, CH_2, J = 7.0 Hz), 2.32–1.92 (m, 12H, 6× CH_2), 1.94 (m, 2H, CH_2), 0.97 (t, 3H, CH_3, J = 7.1 Hz); MS (APCI): *m*/*z* 404 (M+1); Anal. Calcd for $C_{20}H_{29}N_5O_4$ (403.2): C, 59.54; H, 7.24; N, 17.36. Found: C, 59.83; H, 7.49; N, 17.39; R_f = 0.75 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 4.55 min, purity: 95.0%.

4.11.2. (2*S*)-1-[(2*S*)-2-(1*R*)-3-Oxocyclopentylcarboxamido]-3-(2-isopropyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamide [(1*R*)-Ocp-(2-isopropyl)-L-His-L-Pro-NH_2] (17). Yield: 71%; 120–121 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.24 (s, 1H, 5-Ar–H), 4.97 (m, 1H, α-CH), 4.48 (m, 1H, α-CH), 3.58 (m, 2H, Im-CH_2), 3.08 (m, 1H, α-CH), 2.22 (m, 1H, CH), 2.30–1.92 (m, 12H, 6× CH_2), 1.39 (d, 6H, 2× CH_3, J = 6.4 Hz); MS (APCI): *m*/*z* 404 (M+1); Anal. Calcd for $C_{20}H_{29}N_5O_4$ (403.2): C, 59.54; H, 7.24; N, 17.36. Found: C, 59.68; H, 6.96; N, 17.74; R_f = 0.73 [CH_3OH/25% $NH_4OH/CHCl_3$ (20:2:78)]; HPLC: t_R = 5.10 min, purity: 97.0%.

4.11.3. (2*S*)-1-[(2*S*)-2-(1*R*)-3-Oxocyclopentylcarboxamido]-3-(2-*tert*-butyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamide [(1*R*)-Ocp-(2-*tert*-butyl)-L-His-L-Pro-NH_2] (18). Yield: 87%; 122–124 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.24 (s, 1H, 5-Ar–H), 5.02 (m, 1H, α-CH), 4.47 (m, 1H, α-CH), 3.46 (m, 2H, Im-CH_2), 3.12 (m, 1H, α-CH), 2.35–1.94 (m, 12H, 6× CH_2), 1.46 (s, 9H, 3× CH_3); MS (APCI): *m*/*z* 418 (M+1); Anal. Calcd for $C_{21}H_{31}N_5O_4$ (417.2): C, 60.41; H, 7.48; N, 16.77.

Found: C, 60.92; H, 7.80; N, 16.33; R_f = 0.72 [CH_3OH/25% NH_4OH/$CHCl_3$ (20:2:78)]; HPLC: t_R = 5.50 min, purity: 97.0%.

4.11.4. (2*S*)-1-[(2*S*)-2-(1*R*)-3-Oxocyclopentylcarboxamido]-3-(2-cyclohexyl-1*H*-imidazolyl)propanoyl]azalone-2- carboxamide [(1*R*)-Ocp-(2-cyclohexyl)-L-His-L-Pro-NH_2] (19). Yield: 71%; 127–129 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.13 (s, 1H, 5-Ar–H), 4.92 (m, 1H, α-CH), 4.54 (m, 1H, α-CH), 3.42 (m, 2H, Im-CH_2), 3.33 (m, 1H, α-CH), 2.87 (m, 1H, CH), 2.21–1.94 (m, 12H, 6× CH_2), 1.46–1.31 (m, 10H, 5× CH_2); MS (APCI): *m*/*z* 444 (M+1); Anal. Calcd for $C_{23}H_{33}N_5O_4$ (443.3): C, 62.28; H, 7.50; N, 15.79. Found: C, 62.49; H, 7.87; N, 15.97; R_f = 0.75 [CH_3OH/25% NH_4OH/$CHCl_3$ (20:2:78)]; HPLC: t_R = 5.90 min, purity: 97.0%.

4.11.5. (2*S*)-1-[(2*S*)-2-(1*S*)-3-Oxocyclopentylcarboxamido]-3-(2-propyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamide [(1*S*)-Ocp-(2-propyl)-L-His-L-Pro-NH_2] (20). Yield: 74%; 114–116 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.32 (s, 1H, 5-Ar–H), 4.93 (m, 1H, α-CH), 4.52 (m, 1H, α-CH), 4.12 (m, 1H, α-CH), 3.45 (m, 2H, Im-CH_2), 2.91 (t, 2H, CH_2, J = 7.0 Hz), 2.32–1.92 (m, 12H, 6× CH_2), 1.94 (m, 2H, CH_2), 0.94 (t, 3H, CH_3, J = 7.1 Hz); MS (APCI): *m*/*z* 404 (M+1); Anal. Calcd for $C_{20}H_{29}N_5O_4$ (403.2): C, 59.54; H, 7.24; N, 17.36. Found: C, 59.31; H, 7.12; N, 17.59; R_f = 0.75 [CH_3OH/25% NH_4OH/$CHCl_3$ (20:2:78)]; HPLC: t_R = 4.70 min, purity: 98.0%.

4.11.6. (2*S*)-1-[(2*S*)-2-(1*S*)-3-Oxocyclopentylcarboxamido]-3-(2-isopropyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamide [(1*S*)-Ocp-(2-isopropyl)-L-His-L-Pro-NH_2] (21). Yield: 63%; 110–112 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.26 (s, 1H, 5-Ar–H), 4.91 (m, 1H, α-CH), 4.39 (m, 1H, α-CH), 3.56 (m, 2H, Im-CH_2), 3.13 (m, 1H, α-CH), 2.27 (m, 1H, CH), 2.31–1.90 (m, 12H, 6× CH_2), 1.42 (d, 6H, 2× CH_3, J = 6.4 Hz); MS (APCI): *m*/*z* 404 (M+1); Anal. Calcd for $C_{20}H_{29}N_5O_4$ (403.2): C, 59.54; H, 7.24; N, 17.36. Found: C, 59.78; H, 7.39; N, 17.63; R_f = 0.71 [CH_3OH/25% NH_4OH/$CHCl_3$ (20:2:78)]; HPLC: t_R = 5.25 min, purity: 96.0%.

4.11.7. (2*S*)-1-[(2*S*)-2-(1*S*)-3-Oxocyclopentylcarboxamido]-3-(2-*tert*-butyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamide [(1*S*)-Ocp-(2-*tert*-butyl)-L-His-L-Pro-NH_2] (22). Yield: 75%; 120–122 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.22 (s, 1H, 5-Ar–H), 4.93 (m, 1H, α-CH), 4.52 (m, 1H, α-CH), 3.54 (m, 2H, Im-CH_2), 3.27 (m, 1H, α-CH), 2.29–1.84 (m, 12H, 6× CH_2), 1.42 (s, 9H, 3× CH_3); MS (APCI): *m*/*z* 418 (M+1); Anal. Calcd for $C_{21}H_{31}N_5O_4$ (417.2): C, 60.41; H, 7.48; N, 16.77. Found: C, 60.71; H, 7.74; N, 16.98; R_f = 0.74 [CH_3OH/25% NH_4OH/$CHCl_3$ (20:2:78)]; HPLC: t_R = 5.65 min, purity: 97.0%.

4.11.8. (2*S*)-1-[(2*S*)-2-(1*S*)-3-Oxocyclopentylcarboxamido]-3-(2-cyclohexyl-1*H*-imidazolyl)propanoyl]azalone-2-carboxamide [(1*S*)-Ocp-(2-cyclohexyl)-L-His-L-Pro-NH_2] (23). Yield: 75%; 124–125 °C (dec.); ^{1}H NMR (CD_3OD): δ 7.18 (s, 1H, 5-Ar–H), 4.89 (m, 1H, α-CH), 4.62 (m, 1H, α-CH), 3.39 (m, 2H, Im-CH_2), 3.27 (m, 1H, α-CH), 2.95 (m, 1H, CH), 2.24–1.92 (m, 12H, 6× CH_2), 1.39–1.32 (m, 10H, 5× CH_2); MS (APCI): *m*/*z* 444 (M+1); Anal. Calcd for $C_{23}H_{33}N_5O_4$ (443.3): C, 62.28; H, 7.50; N, 15.79. Found: C, 62.10; H, 7.37; N, 15.65; R_f = 0.75 [CH_3OH/25% NH_4OH/$CHCl_3$ (20:2:78)]; HPLC: t_R = 6.35 min, purity: 97.0%.

Acknowledgments

The major part of this research project was supported by the Council of Scientific and Industrial Research (CSIR, Grant No. 01(1861)/03/EMR-II), India. Navneet Kaur thanks the University Grants Commission (UGC), India, for the award of Senior Research Fellowship. Vikramdeep Monga is a recipient of young investigator research award (SR/FTP/CS-79/2005) from the Department of Science and Technology (DST), India.

References and notes

1. Boler, J.; Enzmann, F.; Folkers, K.; Bowers, C. Y.; Schally, A. V. *Biochem. Biophys. Res. Commun.* **1969**, *37*, 705.
2. Burgus, R.; Dunn, J. F.; Desiderio, D.; Guillemin, R. *C. R. Acad. Sci. (Paris)* **1969**, *269*, 1870.
3. Prokai, L. *Prog. Drug Res.* **2002**, *59*, 134.
4. Griffiths, E. C. *Clin. Sci.* **1987**, *73*, 449.
5. Kelly, J. A. *Essays Biochem.* **1995**, *30*, 133.
6. Yamamoto, M.; Sudoh, K.; Sasamata, M. *Eur. J. Pharmacol.* **1991**, *192*, 165.
7. Straub, R. E.; French, G. C.; Joho, R. H.; Gershengorn, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9514.
8. de la Pena, P.; Delgado, L. M.; del Camino, D.; Barros, F. *J. Biol. Chem.* **1992**, *267*, 25703.
9. Duthie, S. M.; Taylor, P. L.; Anderson, L.; Cook, J.; Eidne, K. A. *Mol. Cell. Endocrinol.* **1993**, *95*, R11.
10. Sun, Y. M.; Millar, R. P.; Ho, H.; Gershengorn, M. C.; Illing, N. *Endocrinology* **1998**, *139*, 3390.
11. Cao, J.; O'Donnell, D.; Vu, H.; Payza, K.; Pou, C.; Godbout, C.; Jakob, A.; Pelletier, M.; Lembo, P.; Ahmad, S.; Walker, P. *J. Biol. Chem.* **1998**, *273*, 32281.
12. Harder, S.; Lu, X.; Wang, W.; Buck, F.; Gershengorn, M. C.; Bruhn, T. O. *Endocrinology* **2001**, *142*, 1188.
13. Itadani, H.; Nakamura, T.; Itoh, J.; Iwaasa, H.; Kanatani, A.; Borkowski, J.; Ihara, M.; Ohta, M. *Biochem. Biophys. Res. Commun.* **1998**, *250*, 68.
14. O'Dowd, B. F.; Lee, D. K.; Huang, W.; Nguyen, T.; Cheng, R.; Liu, Y.; Wang, B.; Gershengorn, M. C.; George, S. R. *Mol. Endocrinol.* **2000**, *14*, 183.
15. Matre, V.; Karlsen, H. E.; Wright, M. S.; Lundell, I.; Fjeldheim, A. K.; Gabrielsen, O. S.; Larhammar, D.; Gautvik, K. M. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 179.
16. Sun, Y.; Lu, X.; Gershengorn, M. C. *J. Mol. Endocrinol.* **2003**, *30*, 87.
17. Perlman, J. H.; Laakkonen, L. J.; Guarnieri, F.; Osman, R.; Gershengorn, M. C. *Biochemistry* **1996**, *35*, 7643.
18. Perlman, J. H.; Colson, A.-O.; Jain, R.; Czyzewski, B.; Cohen, L. A.; Osman, R.; Gershengorn, M. C. *Biochemistry* **1997**, *36*, 15670.
19. Jain, R.; Singh, J.; Perlman, J. H.; Gershengorn, M. C. *Bioorg. Med. Chem.* **2002**, *10*, 189.
20. Engel, S.; Neumann, S.; Kaur, N.; Monga, V.; Jain, R.; Northup, J.; Gershengorn, M. C. *J. Biol. Chem.* **2006**, *281*, 13103.

21. Kaur, N.; Lu, X.; Gershengorn, M. C.; Jain, R. *J. Med. Chem.* **2005**, *48*, 6162.
22. Kaur, N.; Monga, V.; Josan, J. S.; Lu, X.; Gershengorn, M. C.; Jain, R. *Bioorg. Med. Chem.* **2006**, *14*, 5981.
23. Stephani, R. A.; Rowe, W. B.; Gass, J. D.; Meister, A. *Biochemistry* **1972**, *11*, 4094.
24. Toki, K. *Bull. Chem. Soc. Jpn.* **1958**, *31*, 333.
25. Curry, K.; Peet, M. J.; Magnuson, D. S. K.; McLennan, H. *J. Med. Chem.* **1988**, *31*, 864.
26. Szirtes, T.; Kisfaludy, L.; Palosi, E.; Szporny, L. *J. Med. Chem.* **1986**, *29*, 1654.
27. Kaur, N.; Monga, V.; Jain, R. *Tetrahedron Lett.* **2004**, *45*, 6883.
28. Jain, R.; El-Kadi, N.; King, M. M.; Cohen, L. A. *Tetrahedron* **1997**, *53*, 2365.
29. Jain, R.; Cohen, L. A.; King, M. M. *Tetrahedron* **1997**, *53*, 4539.
30. van Batenburg, O. D.; Kerling, K. E. T. *Int. J. Peptide Protein Res.* **1976**, *8*, 1.
31. Perlman, J. H.; Thaw, C. N.; Laakkonen, L.; Bowers, C. Y.; Osman, R.; Gershengorn, M. C. *J. Biol. Chem.* **1994**, *269*, 1610.
32. Lu, X.; Huang, W.; Worthington, S.; Drabik, P.; Osman, R.; Gershengorn, M. C. *Mol. Pharmacol.* **2004**, *66*, 1192.
33. Minisci, F. *Synthesis* **1973**, 1.
34. Minisci, F.; Vismara, E.; Fontana, F. *Heterocycles* **1989**, *28*, 489.